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| APPLICATION NO. | ISSUE DATE | PATENT NO. | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|------------|------------|---------------------|------------------|
| 13/527,331 | 04/21/2015 | 9011797 | 118153-01205 | 1757 |

86738 7590 04/01/2015
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

John Richard Gilbert, Brookline, MA;
Sebastian Böhm, Iwerness, UNITED KINGDOM;
Manish Deshpande, Canton, MA;

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PTO/SB/08b (01-08)
Approved for use through 07/31/2008. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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| | | | | | |
|---|---|----|---|--------------------------|---------------------------|
| Substitute for form 1449/PTO | | | | Complete if Known | |
| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary) | | | | Application Number | 13/527,331 Confirm # 1757 |
| | | | | Filing Date | June 19, 2012 |
| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 2 | of | 3 | Attorney Docket Number | 118153-01205 |

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|--|-------|-----------------|-----------------------|----------------|---------|
| | A45 * | US 2006-0278288 | 12-14-2006 | Gilbert et al. | |
| | A46 * | US 2012-0015442 | 12-14-2006 | Gilbert et al. | 01-2012 |
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| FOREIGN PATENT DOCUMENTS | | | | | | |
|--------------------------|--------------------------|---|-----------------------------------|---|--|------------------|
| Examiner Initials* | Cite No. ¹ | Foreign Patent Document | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear | T ⁶ |
| | | Country Code ³ -Number ⁴ -Kind Code ⁵ (if known) | | | | |
| | B1 | WO 91/15750 | 10-17-1991 | Carri Med. Ltd. | | |
| | B2 | WO 98/10267 | 03-12-1998 | Technical University of Denmark | | |
| | B3 | WO 98/07069 | 02-19-1998 | The Regents of the University of Michigan | | |
| | B4 | WO 00/70080 | 11-23-2000 | Caliper Technologies Corp. | | |
| | B5 | EP A 0 177 718 | 04-16-1986 | Partec AG | | Abstract only |
| | B6 | EP A 0 745 682 | 12-04-1996 | Bayer AG | | Abstract only |
| | | | | | | |
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| NON PATENT LITERATURE DOCUMENTS | | | |
|---------------------------------|--------------------------|---|----------------|
| Examiner Initials* | Cite No. ¹ | Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published. | T ² |
| | C1 | International Search Report issued in International Application No. PCT/US2003/12368, dated August 21, 2003 | |
| | C2 | International Preliminary Examination Report issued in International Application No. PCT/US2003/12368, dated August 25, 2004 | |
| | C3 | International Search Report issued in International Application No. PCT/US2003/12360, dated October 3, 2003 | |

PTO/SB/08b (01-08)

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| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 1 | of | 3 | Attorney Docket Number | 118153-01205 |

| U.S. PATENT DOCUMENTS | | | | | |
|-----------------------|--------------------------|--|--------------------------------|--|---|
| Examiner Initials* | Cite No. ¹ | Document Number Number-Kind Code ² (if known) | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear |
| | A1 * | US 3,289,687 | 12-06-1966 | Dunaway | |
| | A2 * | US 3,370,538 | 02-27-1968 | Iliacs | |
| | A3 * | US 3,508,654 O4 | 02-01-1970 | Glaetli | |
| | A4 * | US 3,560,754 | 02-02-1971 | Kamentsky | |
| | A5 * | US 3,791,517 | 02-12-1974 | Friedman | |
| | A6 * | US 3,827,555 | 08-06-1974 | Kamnetsky | |
| | A7 * | US 3,984,307 | 10-05-1976 | Kamnetsky | |
| | A8 * | US 4,153,855 | 05-08-1979 | Feingold | |
| | A9 * | US 4,175,662 | 11-27-1979 | Zold | |
| | A10 * | US 4,426,451 | 01-17-1984 | Columbus | |
| | A11 * | US 4,581,624 | 04-08-1986 | O'Connor | |
| | A12 * | US 4,636,149 | 01-13-1987 | Brown | |
| | A13 * | US 4,676,274 | 06-30-1987 | Brown | |
| | A14 * | US 4,756,427 | 07-12-1988 | Gohde et al. | |
| | A15 * | US 4,908,112 | 03-13-1990 | Pace | |
| | A16 * | US 5,065,978 | 11-19-1991 | Albarda et al. | |
| | A17 * | US 5,092,972 | 03-03-1992 | Ghowzi | |
| | A18 * | US 5,101,978 | 04-07-1992 | Marcus | |
| | A19 * | US 5,265,327 | 11-30-1993 | Faris et al. | |
| | A20 * | US 5,777,649 | 07-07-1998 8 | Otsuka et al. | |
| | A21 * | US 5,789,045 | 08-04-1998 | Wapner et al. | |
| | A22 * | US 5,837,200 | 11-17-1998 | Diessel et al. | |
| | A23 * | US 5,876,187 | 03-02-1999 | Afromowitz et al. | |
| | A24 * | US 5,988,522 | 11-23-1999 | Glezer et al. | |
| | A25 * | US 6,033,191 | 03-07-2000 | Kamper et al. | |
| | A26 * | US 6,048,328 | 04-11-2000 | Haller et al. | |
| | A27 * | US 6,048,734 | 04-11-2000 | Burns et al. | |
| | A28 * | US 6,062,681 | 05-16-2000 | Field et al. | |
| | A29 * | US 6,102,530 | 08-15-2000 | Kim et al. | |
| | A30 * | US 6,152,181 | 11-28-2000 | Wapner et al. | |
| | A31 * | US 6,196,525 | 03-06-2001 | Ganan-Calvo | |
| | A32 * | US 6,203,291 | 03-20-2001 | Stemme et al. | |
| | A33 * | US 6,221,654 | 04-24-2001 | Quake et al. | |
| | A34 * | US 6,273,553 | 08-14-2001 | Kim et al. | |
| | A35 * | US 6,360,775 | 03-26-2002 | Barth et al. | |
| | A36 * | US 6,432,630 | 08-13-2002 | Blankenstein | |
| | A37 * | US 6,561,224 | 05-13-2003 | Cho | |
| | A38 * | US 6,592,821 | 07-15-2003 | Wada et al. | |
| | A39 * | US 5,597,438 | 07-22-2003 | Cabuz et al. 6597438 | |
| | A40 * | US 6,629,820 | 10-07-2003 | Korselsen | |
| | A41 * | US 6,802,342 | 10-12-2004 | Fernandes et al. | |
| | A42 * | US 6,877,528 | 04-12-2005 | Gilbert et al. | |
| | A43 * | US 7,069,943 | 07-04-2006 | Gilbert et al. | |
| | A44 * | US 8,210,209 | 07-03-2012 | Gilbert et al. | |

ME1 14000191v.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /P.H./

PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
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Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

| |
|--------------------|
| (Depositor's name) |
| (Signature) |
| (Date) |

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John R. Gilbert | 118153-01205 | 1757 |

TITLE OF INVENTION: MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL

| APPLN. TYPE | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|----------------|---------------|---------------|---------------------|----------------------|------------------|------------|
| nonprovisional | UNDISCOUNTED | \$960.00 | | | \$960.00 | 03/23/2015 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|------------|----------|----------------|
| P. S. Hyun | 1772 | |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
- | | |
|---|-------------------------|
| 1 | McCarter & English, LLP |
| 2 | David R. Burns |
| 3 | |

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

CYTONOME/ST, LLC

Boston, Massachusetts

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number 50-4876 (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____/David R. Burns/

Date March 23, 2015

Typed or printed name David R. Burns

Registration No. 46,590

Electronic Patent Application Fee Transmittal

| | | | | |
|---|---|-----------------|---------------|-----------------------------|
| Application Number: | 13527331 | | | |
| Filing Date: | 19-Jun-2012 | | | |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| First Named Inventor/Applicant Name: | John Richard Gilbert | | | |
| Filer: | David Burns/Brenda Kowalczyk | | | |
| Attorney Docket Number: | 118153-01205 | | | |
| Filed as Large Entity | | | | |
| Filing Fees for Utility under 35 USC 111(a) | | | | |
| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
| Basic Filing: | | | | |
| Pages: | | | | |
| Claims: | | | | |
| Miscellaneous-Filing: | | | | |
| Petition: | | | | |
| Patent-Appeals-and-Interference: | | | | |
| Post-Allowance-and-Post-Issuance: | | | | |
| Utility Appl Issue Fee | 1501 | 1 | 960 | 960 |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
|--------------------|----------|----------|--------|----------------------|
| Extension-of-Time: | | | | |
| Miscellaneous: | | | | |
| Total in USD (\$) | | | | 960 |

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 21841367 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John Richard Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns |
| Filer Authorized By: | |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 23-MAR-2015 |
| Filing Date: | 19-JUN-2012 |
| Time Stamp: | 12:53:55 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|--|-----------------|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | \$960 |
| RAM confirmation Number | 10937 |
| Deposit Account | 504876 |
| Authorized User | |

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|-----------------|-----------------------------|---------------------------|--|------------------|------------------|
| 1 | Issue Fee Payment (PTO-85B) | Issue_Fee_Transmittal.pdf | 33203 | no | 1 |
| | | | 8642ebc550d40e2310c7e56073877be80943cdb9 | | |

Warnings:

Information:

| | | | | | |
|---|----------------------|--------------|---|----|---|
| 2 | Fee Worksheet (SB06) | fee-info.pdf | 31119 | no | 2 |
| | | | 71cbb391523b64453a90563e1e55ecfce08e6d1 | | |

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Information:

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



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NOTICE OF ALLOWANCE AND FEE(S) DUE

86738 7590 12/23/2014
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Boston, MA 02110

EXAMINER

HYUN, PAUL SANG HWA

ART UNIT

PAPER NUMBER

1772

DATE MAILED: 12/23/2014

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
|-----------------|-------------|----------------------|---------------------|------------------|

13/527,331

06/19/2012

John Richard Gilbert

118153-01205

1757

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|-------------|---------------|---------------|---------------------|----------------------|------------------|----------|
|-------------|---------------|---------------|---------------------|----------------------|------------------|----------|

nonprovisional

UNDISCOUNTED

\$960

\$0

\$0

\$960

03/23/2015

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

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P.O. Box 1450
Alexandria, Virginia 22313-1450
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| |
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| (Depositor's name) |
| (Signature) |
| (Date) |

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 | 1757 |

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|----------------|---------------|---------------|---------------------|----------------------|------------------|------------|
| nonprovisional | UNDISCOUNTED | \$960 | \$0 | \$0 | \$960 | 03/23/2015 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|---------------------|----------|----------------|
| HYUN, PAUL SANG HWA | 1772 | 422-504000 |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

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- 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (**Please first reapply any previously paid issue fee shown above**)

- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 | 1757 |

86738 7590 12/23/2014
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

EXAMINER

HYUN, PAUL SANG HWA

| ART UNIT | PAPER NUMBER |
|----------|--------------|
|----------|--------------|

1772

DATE MAILED: 12/23/2014

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

| | | | |
|--|--------------------------------------|---------------------------------------|--|
| <i>Examiner-Initiated Interview Summary</i> | Application No. 13/527,331 | Applicant(s) GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | |

All participants (applicant, applicant's representative, PTO personnel):

(1) PAUL HYUN. (3) ____.

(2) David Burns. (4) ____.

Date of Interview: 11 December 2014.

Type: ☒ Telephonic ☐ Video Conference
 ☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
 If Yes, brief description: ____.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☐Others
 (For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 40,41,47 and 49.

Identification of prior art discussed: Otsuka et al. (US 5,777,649).

Substance of Interview
 (For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The examiner indicated that the application would be allowable if claim 47 is amended to recite that the flow channel comprises a width and a length that exceeds the width and that the first reservoir dampens or absorbs a pressure pulse propagated across the width of the channel. Applicant authorized the examiner to amend claim 47 via examiner's amendment. Applicant also authorized the examiner to cancel claim 41 and make minor changes to dependent claims 40 and 49.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

| | |
|--|--|
| /PAUL HYUN/ Primary Examiner, Art Unit 1772 | |
|--|--|

| | | | |
|-------------------------------|--------------------------------------|---------------------------------------|--|
| Notice of Allowability | Application No. 13/527,331 | Applicant(s) GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | AIA (First Inventor to File) Status No |

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to RCE filed on 17 November 2014.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are 21, 23-30, 39,40,42-45 and 47-50. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|--|--|
| 1. <input type="checkbox"/> Notice of References Cited (PTO-892) | 5. <input checked="" type="checkbox"/> Examiner's Amendment/Comment |
| 2. <input type="checkbox"/> Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____ | 6. <input checked="" type="checkbox"/> Examiner's Statement of Reasons for Allowance |
| 3. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit of Biological Material | 7. <input type="checkbox"/> Other _____. |
| 4. <input checked="" type="checkbox"/> Interview Summary (PTO-413), Paper No./Mail Date <u>20141211</u> . | |

/PAUL HYUN/
Primary Examiner, Art Unit 1772

Art Unit: 1772

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on November 17, 2014 has been entered.

Claims 21, 23-30, 39-45 and 47-50 are pending. Applicant amended claims 21, 23-25, 30, 39-42 and 47, and added new claim 50.

The double patenting rejections and the rejections under 35 U.S.C. section 112 set forth in the previous Office action have been withdrawn in light of the amendment.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with David Burns on December 11, 2014.

The application has been amended as follows:

Claim 40 (Currently Amended) A method comprising:

providing the microfluidic device of claim 47;

flowing a sample through the microfluidic flow channel;

propagating [a] the pressure pulse across the width of the flow channel [in the crosswise direction]; and

dampening or absorbing the pressure pulse using the first reservoir in fluid communication with the flow channel.

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Claim 41 (Cancelled)

Claim 47 (Currently Amended) A microfluidic device adapted to facilitate analysis or processing of a sample, the microfluidic device comprising:

a microfluidic flow channel formed in a substrate, the flow channel having a width and a length that exceeds the width; and

a first reservoir in fluid communication with the flow channel adapted for dampening or absorbing a pressure pulse propagated across the width of the flow channel, wherein the first reservoir is otherwise sealed from an exterior environment.

Claim 49 (Currently Amended) The device of claim 47, further comprising an actuator or pressurizer operatively associated with flow channel and adapted for generating [a] the pressure pulse across the width of the flow channel [in a crosswise direction].

Allowable Subject Matter

Claims 21, 23-30, 39, 40, 42-45 and 47-50 are allowed.

The following is an examiner's statement of reasons for allowance:

Otsuka et al. (US 5,777,649) disclose an ink jet printing head (see Fig. 1), said head comprising: a channel 9 formed in a substrate 4 (see Fig. 1), the channel having a width and a length exceeding the width; and

a sealed first reservoir 13 in fluid communication with the flow channel 9 (see Fig. 3), said first reservoir 13 including resilient properties for dampening or absorbing a pressure pulse propagated across the flow channel in a crosswise direction (see lines 35-39, col. 6).

However, Otsuka et al. do not disclose:

- 1) a method for sorting particles as recited in claim 42; or
- 2) a reservoir that dampens or absorbs a pressure pulse propagated across the width of a channel as recited in claim 47.

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With respect to claim 42, the device disclosed by Otsuka et al. is a ink jet printing head. Consequently, there is no motivation to apply the method disclosed by Otsuka et al. to particle sorting.

With respect to claim 47, reservoir 13 disclosed by Otsuka et al. is configured to absorb pressure propagated along the length of channel 9 only. Based on the disclosure, there is no motivation to relocate the reservoir such that it absorbs pressure propagated along the width of channel 9.


Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to PAUL HYUN whose telephone number is (571)272-8559. The examiner can normally be reached on M-F 10 AM-6:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, In Suk Bullock can be reached on (571) 272-5954. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.


/PAUL HYUN/
Primary Examiner, Art Unit 1772

| | | | |
|---|--|--|--|
| Issue Classification  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | |


| CPC | | | | | | |
|--------|--|------|---|--------|------|------------|
| Symbol | | | | | Type | Version |
| B01L | | 3 | / | 502738 | F | 2013-01-01 |
| B01L | | 3 | / | 502761 | I | 2013-01-01 |
| B01L | | 3 | / | 502776 | A | 2013-01-01 |
| B01L | | 2200 | / | 0636 | A | 2013-01-01 |
| B01L | | 2200 | / | 0647 | A | 2013-01-01 |
| B01L | | 2300 | / | 0816 | A | 2013-01-01 |
| B01L | | 2300 | / | 0864 | A | 2013-01-01 |
| B01L | | 2300 | / | 089 | A | 2013-01-01 |
| B01L | | 2400 | / | 0415 | A | 2013-01-01 |
| B01L | | 2400 | / | 06 | A | 2013-01-01 |
| B01L | | 2400 | / | 0688 | A | 2013-01-01 |
| B01L | | 2400 | / | 082 | A | 2013-01-01 |
| B07C | | 5 | / | 34 | I | 2013-01-01 |
| F16K | | 99 | / | 0001 | I | 2013-01-01 |
| F16K | | 99 | / | 0017 | I | 2013-01-01 |
| F16K | | 99 | / | 0019 | I | 2013-01-01 |
| F16K | | 99 | / | 0034 | I | 2013-01-01 |
| F16K | | 99 | / | 0036 | I | 2013-01-01 |
| F16K | | 99 | / | 004 | I | 2013-01-01 |
| F16K | | 99 | / | 0046 | I | 2013-01-01 |
| F16K | | 99 | / | 0048 | I | 2013-01-01 |
| F16K | | 2099 | / | 0074 | A | 2013-01-01 |
| F16K | | 2099 | / | 008 | A | 2013-01-01 |
| F16K | | 2099 | / | 0084 | A | 2013-01-01 |
| G01N | | 2015 | / | 149 | A | 2013-01-01 |

| CPC Combination Sets | | | | |
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| Symbol | Type | Set | Ranking | Version |
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|---|------------|------------------------------------|-------------------|
| NONE | | Total Claims Allowed: 19 | |
| (Assistant Examiner) | (Date) | | |
| /PAUL HYUN/ Primary Examiner.Art Unit 1772 | 12/11/2014 | O.G. Print Claim(s) | O.G. Print Figure |
| (Primary Examiner) | (Date) | 1 | 16 |


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| Issue Classification  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | |

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|---|--|--|-----------------------------|
| NONE <div> <div>(Assistant Examiner)</div> <div>(Date)</div> </div> | | Total Claims Allowed: 19 | |
| /PAUL HYUN/ Primary Examiner.Art Unit 1772 <div> <div>(Primary Examiner)</div> <div>(Date)</div> </div> | | O.G. Print Claim(s) 1 | O.G. Print Figure 16 |

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|---|--|--|
| Issue Classification  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

| <input type="checkbox"/> Claims renumbered in the same order as presented by applicant | | | | | | | | | | | | | | | |
|--|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|-------|----------|
| <input type="checkbox"/> CPA | | | | | | | | | | | | | | | |
| <input type="checkbox"/> T.D. | | | | | | | | | | | | | | | |
| <input type="checkbox"/> R.1.47 | | | | | | | | | | | | | | | |
| Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original |
| - | 1 | - | 17 | - | 33 | 13 | 49 | | | | | | | | |
| - | 2 | - | 18 | - | 34 | 15 | 50 | | | | | | | | |
| - | 3 | - | 19 | - | 35 | | | | | | | | | | |
| - | 4 | - | 20 | - | 36 | | | | | | | | | | |
| - | 5 | 14 | 21 | - | 37 | | | | | | | | | | |
| - | 6 | - | 22 | - | 38 | | | | | | | | | | |
| - | 7 | 19 | 23 | 17 | 39 | | | | | | | | | | |
| - | 8 | 16 | 24 | 18 | 40 | | | | | | | | | | |
| - | 9 | 7 | 25 | - | 41 | | | | | | | | | | |
| - | 10 | 8 | 26 | 1 | 42 | | | | | | | | | | |
| - | 11 | 9 | 27 | 2 | 43 | | | | | | | | | | |
| - | 12 | 10 | 28 | 3 | 44 | | | | | | | | | | |
| - | 13 | 11 | 29 | 4 | 45 | | | | | | | | | | |
| - | 14 | 12 | 30 | - | 46 | | | | | | | | | | |
| - | 15 | - | 31 | 5 | 47 | | | | | | | | | | |
| - | 16 | - | 32 | 6 | 48 | | | | | | | | | | |

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|---|------------|------------------------------|-------------------|
| NONE | | Total Claims Allowed: | |
| (Assistant Examiner) | (Date) | 19 | |
| /PAUL HYUN/ Primary Examiner.Art Unit 1772 | 12/11/2014 | O.G. Print Claim(s) | O.G. Print Figure |
| (Primary Examiner) | (Date) | 1 | 16 |

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| Search Notes  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

| CPC- SEARCHED | | |
|----------------|------------|----------|
| Symbol | Date | Examiner |
| B01L 2200/0636 | 12/11/2014 | PSH |
| B01L 2300/0864 | 12/11/2014 | PSH |
| B01L2400/082 | 12/11/2014 | PSH |
| B01L 3/502761 | 12/11/2014 | PSH |
| G01N 2015/149 | 12/11/2014 | PSH |

| CPC COMBINATION SETS - SEARCHED | | |
|---------------------------------|------|----------|
| Symbol | Date | Examiner |
| | | |

| US CLASSIFICATION SEARCHED | | | |
|----------------------------|----------|------------|----------|
| Class | Subclass | Date | Examiner |
| 422 | 502-505 | 12/11/2014 | PSH |
| 251 | 61.1 | 12/11/2014 | PSH |
| 436 | 177 | 12/11/2014 | PSH |

| SEARCH NOTES | | |
|---|-----------|----------|
| Search Notes | Date | Examiner |
| inventor name search | 12/8/2014 | PSH |
| text search | 12/8/2014 | PSH |
| forward and backward searched relevant references | 12/8/2014 | PSH |

| INTERFERENCE SEARCH | | | |
|-------------------------|-----------------------------|------------|----------|
| US Class/ CPC Symbol | US Subclass / CPC Group | Date | Examiner |
| | see search history printout | 12/11/2014 | PSH |

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| | /PAUL HYUN/ Primary Examiner.Art Unit 1772 |
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EAST Search History

EAST Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|-------|------|--|------------------------|------------------|---------|------------------|
| S44 | 30 | deshpande.in. and pressure adj pulse with absorb\$4 | US-PGPUB; USPAT | OR | ON | 2014/12/08 14:35 |
| S45 | 32 | (bohm gilbert deshpande).in. and pressure adj pulse with absorb\$4 | US-PGPUB; USPAT | OR | ON | 2014/12/08 14:35 |
| S46 | 15 | (bohm gilbert deshpande).in. and pressure adj pulse with absorb\$4 | USPAT | OR | ON | 2014/12/08 14:39 |
| S47 | 14 | (bohm gilbert deshpande).in. and pressure adj pulse with absorb\$4 and sort\$4 near4 particle | USPAT | OR | ON | 2014/12/08 14:39 |
| S48 | 36 | ("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6432630" "6597438").PN. OR ("6808075").URPN. | US-PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:40 |
| S49 | 36 | ("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6273553" "6432630" "6540895" "6592821" "6597438" "6877528").PN. OR ("7104405").URPN. | US-PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:40 |
| S50 | 36 | ("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6432630" "6597438").PN. OR ("6808075").URPN. | US-PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:40 |
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| S52 | 32 | ("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5783446" "5837200" "5976336" "5998212" "6120666" "6145247" "6221654" "6432630" "6524790" "6592821" "6597438").PN. OR ("6976590").URPN. | US-PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:41 |

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| S53 | 57 | ("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "4939081" "5030002" "5101978" "5275787" "5395588" "5637496" "5783446" "5795727" "5837200" "5976336" "5979664" "5998212" "6057111" "6120666" "6145247" "6214556" "6221654" "6280967" "6432630" "6455280" "6482652" "6524790" "6540895" "6592821" "6597438" "6803194" "6877528").PN. OR ("7157274").URPN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:41 |
| S54 | 11 | ("20020124896" "20040050866" "20050183995" "5837200" "6103199" "6838056" "7157274" "7220594" "7476363" "7569788").PN. OR ("8123044").URPN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:41 |
| S55 | 110 | ("20020005354" "20020058332" "20020093641" "20030027225" "20040037739" "20040180426" "20040233424" "20060170912" "20060177348" "20070065808" "20080070311" "20100032350" "20100133151" "20110005978" "20120012508" "20120258488" "3289687" "3506654" "3508654" "3508655" "3560754" "3791517" "3827555" "3984307" "3984621" "4147621" "4175662" "4318483" "4361400" "4365719" "4426451" "4526276" "4554427" "4572664" "4676274" "4756427" "4908112" "4936465" "4939081" "5030002" "5065978" "5092972" "5101978" "5193688" "5275787" "5395588" "5637496" "5777649" "5783446" "5789045" "5795727" "5837200" "5885470" "5932100" "5976336" "5979664" "5998212" "6046056" "6048734" "6057111" "6062681" "6102530" "6120666" "6145247" "6152181" "6196525" "6214556" "6221654" "6273553" "6280967" "6318970" "6360775" "6431212" "6432630" "6454862" "6455280" "6482652" "6524790" "6540895" "6561224" "6592821" "6597438" "6657730" "6739576" "6803194" "6808075" "6821484" "6877528" "6976590" "7024281" "7075652" "7104405" "7157274" "7157275" "7220594" "7258774" "7303727" "7389879" "7452725" "7497334" "7569788" "7584857" "7820427" "7963399" "8123044" "8252604").PN. OR ("8408399").URPN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:42 |
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| S58 | 36 | ("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6273553" "6432630" "6540895" "6592821" "6597438" "6877528").PN. OR ("7104405").URPN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:42 |
| S59 | 48 | ("20060278288" "3289687" "3370538" "3508654" "3560754" "3791517" "3827555" "3984307" "4153855" "4175662" "4426451" "4581624" "4636149" "4676274" "4756427" "4908112" "5065978" "5092972" "5101978" "5265327" "5777649" "5789045" "5837200" "5876187" "5988522" "6033191" "6048328" "6048734" "6062681" "6102530" "6152181" "6196525" "6203291" "6221654" "6273553" "6360775" "6432630" "6561224" "6592821" "6597438" "6629820" "6802342" "6877528" "7069943" "8210209").PN. OR ("8623295").URPN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:42 |
| S60 | 57 | ("20020093641" "20030027225" "3508654" "3560754" "3791517" "3827555" | US- PGPUB; | OR | OFF | 2014/12/08 14:43 |

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| S61 | 100 | ("20020005354" "20020058332" "20020093641" "20030027225" "20040180426" "20060177348" "20070065808" "20100032350" "20100133151" "20110005978" "20120006727" "20120012508" "3289687" "3506654" "3508654" "3508655" "3560754" "3791517" "3827555" "3984307" "3984621" "4147621" "4175662" "4318483" "4361400" "4365719" "4426451" "4526276" "4554427" "4572664" "4676274" "4756427" "4908112" "4936465" "4939081" "5030002" "5065978" "5092972" "5101978" "5193688" "5275787" "5395588" "5637496" "5777649" "5783446" "5789045" "5795727" "5837200" "5885470" "5932100" "5976336" "5979664" "5998212" "6046056" "6048734" "6057111" "6062681" "6102530" "6120666" "6145247" "6152181" "6196525" "6214556" "6221654" "6273553" "6280967" "6360775" "6432630" "6454862" "6455280" "6482652" "6524790" "6540895" "6561224" "6592821" "6597438" "6657730" "6803194" "6808075" "6821484" "6877528" "6976590" "7075652" "7104405" "7157274" "7157275" "7220594" "7258774" "7389879" "7452725" "7497334" "7569788" "7584857" "7820427" "7963399").PN. OR ("8567608").URPN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:43 |
| S62 | 106 | ("20020005354" "20020058332" "20020093641" "20030027225" "20040037739" "20040180426" "20040233424" "20060177348" "20070065808" "20100032350" "20100133151" "20110005978" "20120006727" "3289687" "3506654" "3508654" "3508655" "3560754" "3791517" "3827555" "3984307" "3984621" "4147621" "4175662" "4318483" "4361400" "4365719" "4426451" "4526276" "4554427" "4572664" "4676274" "4756427" "4908112" "4936465" "4939081" | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:43 |

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| S63 | 11 | ("20020124896" "20040050866" "20050183995" "5837200" "6103199" "6808075" "6838056" "7157274" "7220594" "7476363" "7569788").PN. OR ("8863962").URPN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 14:43 |
| S64 | 1 | (S48 S49 S50 S51 S52 S53 S54 S55 S56 S57 S58 S59 S60 S61 S62 S63) and microfluidic and reservoir with (bubble air gas) with pressure with (dampen\$4 absorb\$4 attenuat\$4) | US- PGPUB; USPAT | OR | ON | 2014/12/08 14:46 |
| S65 | 3 | microfluidic and reservoir with (bubble air gas) with pressure with (dampen\$4 absorb\$4 attenuat\$4) | US- PGPUB; USPAT | OR | ON | 2014/12/08 14:46 |
| S66 | 172 | microfluidic and (bubble air gas) with pressure with (dampen\$4 absorb\$4 attenuat\$4) | US- PGPUB; USPAT | OR | ON | 2014/12/08 14:59 |
| S67 | 72 | microfluidic and (bubble air gas) with pressure near3 (dampen\$4 absorb\$4 attenuat\$4) | US- PGPUB; USPAT | OR | ON | 2014/12/08 14:59 |
| S68 | 45 | microfluidic and (bubble air gas) with pressure near3 (dampen\$4 absorb\$4 attenuat\$4) not S45 | US- PGPUB; USPAT | OR | ON | 2014/12/08 15:02 |
| S69 | 39 | microfluidic and (bubble air gas) with pressure near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US- PGPUB; USPAT | OR | ON | 2014/12/08 15:03 |
| S70 | 2466 | channel and (bubble air gas) with pressure near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US- PGPUB; USPAT | OR | ON | 2014/12/08 15:04 |
| S71 | 2622 | channel and (bubble air gas) with (pulse pressure) near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US- PGPUB; USPAT | OR | ON | 2014/12/08 15:04 |
| S72 | 271 | channel with (capillary microfluidic micron micrometer) and (bubble air gas) with (pulse pressure) near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US- PGPUB; USPAT | OR | ON | 2014/12/08 15:05 |
| S73 | 210 | channel with (bubble air gas) with (pulse pressure) near3 (dampen\$4 absorb\$4 | US- PGPUB; | OR | ON | 2014/12/08 15:16 |

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|-----|------|--|------------------------|----|-----|------------------|
| | | attenuat\$4) not (bohm gilbert deshpande).in. | USPAT | | | |
| S74 | 4 | channel with (bubble air gas) near2 (reservoir pocket) with (pulse pressure) near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/08 15:16 |
| S75 | 4 | channel with (bubble air gas) near2 (reservoir pocket) with (pulse pressure) near3 (mitigat\$4 lessen\$4 dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/08 15:34 |
| S76 | 51 | channel with (bubble air gas) near2 (pulse pressure) near3 (mitigat\$4 lessen\$4 dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/08 15:35 |
| S77 | 209 | ink adj jet and (bubble air gas) near2 (pulse pressure) near3 (mitigat\$4 lessen\$4 dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/08 16:14 |
| S78 | 101 | ink adj jet and (bubble air gas) near2 (reservoir chamber) with (pulse pressure) near3 (mitigat\$4 lessen\$4 dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/08 16:14 |
| S79 | 39 | ("3930260" "4404566" "4591873" "4905019" "5021809" "5030973" "5162817").PN. OR ("5777649").URPN. | US-PGPUB; USPAT; USOCR | OR | OFF | 2014/12/08 16:17 |
| S80 | 1 | ("6808075").PN. | USPAT; USOCR | OR | OFF | 2014/12/11 16:37 |
| S81 | 31 | (bohm gilbert deshpande).in. and pressure adj pulse with absorb\$4 and sort\$4 near4 particle | US-PGPUB; USPAT | OR | ON | 2014/12/11 18:38 |
| S82 | 9 | (bohm gilbert deshpande).in. and bubble adj valve.ti. | US-PGPUB; USPAT | OR | ON | 2014/12/11 18:39 |
| S83 | 1 | "20120261013" | US-PGPUB; USPAT | OR | ON | 2014/12/11 18:40 |
| S84 | 4177 | (B01L2200/0636 B01L2300/0864 B01L2400/082 B01L3/502761 G01N2015/149).cpc. | US-PGPUB; USPAT | OR | ON | 2014/12/11 18:44 |
| S85 | 0 | S83 and channel with (bubble air gas membrane elastic elastomer\$4 flexible) with (pulse pressure) near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/11 18:50 |
| S86 | 3917 | ((436/177) or (422/504) or (251/61.1)).OCLS. | USPAT; USOCR | OR | OFF | 2014/12/11 18:53 |
| S87 | 2 | S86 and channel with (bubble air gas membrane elastic elastomer\$4 flexible) with (pulse pressure) near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohm gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/11 18:53 |
| S88 | 5102 | ((436/177) or (422/502-505) or (251/61.1)).OCLS. | USPAT; USOCR | OR | OFF | 2014/12/11 19:07 |
| S89 | 5882 | ((436/177) or (422/502-505) or (251/61.1)).OCLS. | US-PGPUB; USPAT | OR | OFF | 2014/12/11 19:07 |
| S90 | 4177 | (B01L2200/0636 B01L2300/0864 B01L2400/082 B01L3/502761 G01N2015/149).cpc. | US-PGPUB; USPAT | OR | ON | 2014/12/11 19:08 |

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|-----|---|--|-----------------|----|----|------------------|
| S91 | 7 | (S90 S89) and channel with (bubble air gas membrane elastic elastomer\$4 flexible) with (pulse pressure) near3 (dampen\$4 absorb\$4 attenuat\$4) not (bohms gilbert deshpande).in. | US-PGPUB; USPAT | OR | ON | 2014/12/11 19:08 |
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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 | 1757 |

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MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

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HYUN, PAUL SANG HWA

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12/15/2014

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@mccarter.com

| | | | |
|---|--------------------------------------|---------------------------------------|--|
| <i>Applicant-Initiated Interview Summary</i> | Application No. 13/527,331 | Applicant(s) GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | |

All participants (applicant, applicant's representative, PTO personnel):

(1) PAUL HYUN. (3) ____.

(2) David Burns. (4) ____.

Date of Interview: 09 December 2014.

Type: ☒ Telephonic ☐ Video Conference
 ☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
 If Yes, brief description: ____.

Issues Discussed ☐101 ☐112 ☒102 ☐103 ☐Others
 (For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 47.

Identification of prior art discussed: Otsuka et al. (US 5,777,649).

Substance of Interview
 (For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The interview was initiated by Applicant to discuss the patentability of claim 47 filed on November 17, 2014 with respect to the disclosure of Otsuka et al. Despite the amendment, the examiner maintained that Fig. 3 of Otsuka et al. anticipates claim 47.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

| | |
|--|--|
| /PAUL HYUN/ Primary Examiner, Art Unit 1772 | |
|--|--|

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews

Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL (Submitted Only via EFS-Web)

| | | | | | | | |
|----------------------|-----------------|-------------|------------|-------------------------------|--------------|----------|------|
| Application Number | 13527331 | Filing Date | 2012-06-19 | Docket Number (if applicable) | 118153-01205 | Art Unit | 1772 |
| First Named Inventor | John R. Gilbert | | | Examiner Name | P. S. Hyun | | |

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.

Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

☐ Other _____

☒ Enclosed

☒ Amendment/Reply

☐ Information Disclosure Statement (IDS)

☐ Affidavit(s)/ Declaration(s)

☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to
Deposit Account No 50-4876

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

| Signature of Registered U.S. Patent Practitioner | | | |
|--|------------------|---------------------|------------|
| Signature | /David R. Burns/ | Date (YYYY-MM-DD) | 2014-11-17 |
| Name | David R. Burns | Registration Number | 46590 |

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

| | | | |
|--|--|--|--|
| PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) | | Docket Number (Optional) 118153-01205 | |
| Application Number 13/527,331 | | Filed June 19, 2012 | |
| For MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| Art Unit 1772 | | Examiner P. S. Hyun | |

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

| | <u>Fee</u> | <u>Small Entity Fee</u> | <u>Micro Entity Fee</u> | |
|--|------------|-------------------------|-------------------------|-----------|
| <input type="checkbox"/> One month (37 CFR 1.17(a)(1)) | \$200 | \$100 | \$50 | \$ _____ |
| <input checked="" type="checkbox"/> Two months (37 CFR 1.17(a)(2)) | \$600 | \$300 | \$150 | \$ 600.00 |
| <input type="checkbox"/> Three months (37 CFR 1.17(a)(3)) | \$1,400 | \$700 | \$350 | \$ _____ |
| <input type="checkbox"/> Four months (37 CFR 1.17(a)(4)) | \$2,200 | \$1,100 | \$550 | \$ _____ |
| <input type="checkbox"/> Five months (37 CFR 1.17(a)(5)) | \$3,000 | \$1,500 | \$750 | \$ _____ |

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☒ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-4876.

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96).

☒ attorney or agent of record. Registration number 46,590.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

| | |
|--|---|
| <u>/David R. Burns/</u> Signature | <u>November 17, 2014</u> Date |
| <u>David R. Burns</u> Typed or printed name | <u>(617) 449-6500</u> Telephone Number |

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

| |
|---|
| <input type="checkbox"/> * Total of <u>1</u> forms are submitted. |
|---|

Electronic Patent Application Fee Transmittal

| | | | | |
|--|---|-----------------|---------------|-----------------------------|
| Application Number: | 13527331 | | | |
| Filing Date: | 19-Jun-2012 | | | |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| First Named Inventor/Applicant Name: | John Richard Gilbert | | | |
| Filer: | David Burns/Brenda Kowalczyk | | | |
| Attorney Docket Number: | 118153-01205 | | | |
| Filed as Large Entity | | | | |
| Utility under 35 USC 111(a) Filing Fees | | | | |
| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
| Basic Filing: | | | | |
| Pages: | | | | |
| Claims: | | | | |
| Miscellaneous-Filing: | | | | |
| Petition: | | | | |
| Patent-Appeals-and-Interference: | | | | |
| Post-Allowance-and-Post-Issuance: | | | | |
| Extension-of-Time: | | | | |
| Extension - 2 months with \$0 paid | 1252 | 1 | 600 | 0038 ⁶⁰⁰ |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
|-----------------------------------|----------|----------|--------|----------------------|
| Miscellaneous: | | | | |
| Request for Continued Examination | 1801 | 1 | 1200 | 1200 |
| Total in USD (\$) | | | | 1800 |

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 20719028 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John Richard Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns/Brenda Kowalczyk |
| Filer Authorized By: | David Burns |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 17-NOV-2014 |
| Filing Date: | 19-JUN-2012 |
| Time Stamp: | 17:06:43 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|--|-----------------|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | \$ 1800 |
| RAM confirmation Number | 4128 |
| Deposit Account | 504876 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

| File Listing: | | | | | |
|------------------------------|---|--|---|------------------|------------------|
| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
| 1 | | Response_with_RCE_17Nov14.pdf | 43797 f301e4e299a014083df6898c931763f57d3a2e12 | yes | 9 |
| | Multipart Description/PDF files in .zip description | | | | |
| | Document Description | | Start | End | |
| | Amendment Copy Claims/Response to Suggested Claims | | 1 | 1 | |
| | Claims | | 2 | 5 | |
| | Applicant Arguments/Remarks Made in an Amendment | | 6 | 9 | |
| Warnings: | | | | | |
| Information: | | | | | |
| 2 | Request for Continued Examination (RCE) | Request_for_Continued_Examination_Fillable_PDF.PDF | 790962 96f8e33d6b62ca2998d672dea58330334f32eaae | no | 3 |
| Warnings: | | | | | |
| Information: | | | | | |
| 3 | Extension of Time | Two_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a.pdf | 23306 f52c974fc0f44d48b7c376592b91258153acc9f60 | no | 1 |
| Warnings: | | | | | |
| Information: | | | | | |
| 4 | Fee Worksheet (SB06) | fee-info.pdf | 32874 d65fbb4603d4491bf007dc88ec7faac26cf3ffcc73 | no | 2 |
| Warnings: | | | | | |
| Information: | | | | | |
| Total Files Size (in bytes): | | | 890939 | | |

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John R. Gilbert et al.

Application No.: 13/527,331

Confirmation No.: 1757

Filed: June 19, 2012

Art Unit: 1772

For: MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING FLUID
FLOW THROUGH A MICROCHANNEL

Examiner: Hyun, Paul S.

**AMENDMENT ACCOMPANYING A REQUEST FOR CONTINUED EXAMINATION
IN RESPONSE TO FINAL OFFICE ACTION AND ADVISORY ACTION**

MS - RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

This response to the Final Office Action dated June 17, 2014 and the Advisory Action dated October 14, 2014, the due date of which is extended to November 17, 2014 by the accompanying Request for a Two-Month Extension of Time, accompanies a Request for Continued Examination. Applicants respectfully request that the Examiner consider the amendments presented herein for the above-identified U.S. patent application as follows:

Proposed Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions of the claims and listings of the claims in the application:

1-20. (Cancelled)

21. (Currently Amended) The device of ~~claim 20~~ claim 47, further comprising a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.

22. (Cancelled)

23. (Currently Amended) The method of claim 40, wherein the pressure pulse is used to sort one or more particles suspended within the sample ~~control or otherwise affect the flow of the sample through the flow channel~~.

24. (Currently Amended) The device of ~~claim 20~~ claim 47, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles suspended within the sample into a selected branch of the branched portion.

25. (Currently Amended) The device of ~~claim 20~~ claim 48, wherein the resilient properties of the first reservoir include a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.

26. (Previously Presented) The device of claim 25, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.

27. (Previously Presented) The device of claim 26, wherein the fluid filled region includes a portion of the first reservoir.

28. (Previously Presented) The device of claim 26, wherein the fluid filled region includes a side channel connecting the first reservoir to the flow channel.

29. (Previously Presented) The device of claim 26, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.

30. (Currently Amended) The device of ~~claim 20~~ claim 48, wherein the resilient properties of the first reservoir include a resilient wall for facilitating dampening or absorbing of the pressure pulse.

31. (Cancelled)

32. (Cancelled)

33. (Cancelled)

34. (Cancelled)

35. (Cancelled)

36. (Cancelled)

37. (Cancelled)

38. (Cancelled)

39. (Currently Amended) The device of ~~claim 20~~ claim 47, wherein the first reservoir is adjacent to an aperture in a sidewall of the flow channel.

40. (Currently Amended) A method comprising:
providing the microfluidic device of ~~claim 20~~ claim 47;
flowing a sample through the microfluidic flow channel;
propagating a pressure pulse across the flow channel in the crosswise direction; and
dampening or absorbing the pressure pulse using the first reservoir in fluid
communication with the flow channel.
41. (Currently Amended) The device of ~~claim 20~~ claim 47, further comprising an actuator or
pressurizer operatively associated with flow channel and adapted for generating the pressure
pulse across the flow channel in the crosswise direction.
42. (Currently Amended) A method for facilitating analysis or processing of a sample, the
method comprising:
flowing a sample through a microfluidic flow channel formed in a substrate;
propagating a pressure pulse across the microfluidic flow channel in a crosswise
direction; and
dampening or absorbing the pressure pulse using a first reservoir in fluid communication
with the microfluidic flow channel,
wherein the pressure pulse is used to sort one or more particles suspended within the
sample.
43. (Previously Presented) The method of claim 42, wherein the pressure pulse is used to
selectively control or otherwise affect the flow of the sample through the flow channel.
44. (Previously Presented) The method of claim 42, wherein the first reservoir includes a gas
pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure
pulse.
45. (Previously Presented) The method of claim 42, wherein the first reservoir includes a
resilient wall for facilitating dampening or absorbing of the pressure pulse.

46. (Cancelled)

47. (Currently Amended) A microfluidic device adapted to facilitate analysis or processing of a sample, the microfluidic device comprising:

a microfluidic flow channel formed in a substrate; and

a ~~sealed~~ first reservoir in fluid communication with the flow channel adapted for dampening or absorbing [[the]] a pressure pulse propagated across the flow channel, wherein the first reservoir is otherwise sealed from an exterior environment.

48. (Previously Presented) The device of claim 47, wherein the first reservoir includes resilient properties which enable the dampening or absorbing the pressure.

49. (Previously Presented) The device of claim 47, further comprising an actuator or pressurizer operatively associated with flow channel and adapted for generating a pressure pulse across the flow channel in a crosswise direction.

50. (New) The device of claim 21, wherein the second reservoir includes a gas pocket, wherein compression of the gas facilitates the generating of the pressure pulse.

REMARKS

The present response is intended to fully address the objections and rejections noted in the Final Office Action mailed June 17, 2014 and the Advisory Action mailed October 14, 2014, and is believed to place the subject application in condition for allowance. Thus, favorable reconsideration and prompt allowance of the subject application is respectfully requested. Applicants do not acquiesce to any portion of the Office Action not particularly addressed herein.

Claims 20, 21, 23 – 30 and 39 – 49 were pending. Proposed amendments to independent claims 42 and 47 have been presented; the dependencies of claims 21, 24, 25, 30, 39 – 41 have been amended to now depend from claim 47, directly or indirectly; claim 23 has been amended to recite that the “pressure pulse is used to sort one or more particles suspended within the sample”; claim 50 has been added to reinstate erroneously cancelled claim 31 (now indirectly dependent from claim 47); and claims 20 and 46 have been cancelled. Upon entry of the amendments, claims 21, 23 – 30 and 39 – 50 will be pending

Applicants respectfully submit that no new matter is introduced by way of the foregoing amendments/additions to the claims. Thus, Applicants respectfully request entry and substantive consideration of the foregoing amendments to the claims.

Request for Examiner Interview:

Applicants representatives respectfully request a telephonic interview with the Examiner prior to consideration of the foregoing amendments and following arguments.

Allowable Subject Matter:

Applicants acknowledge the Examiner’s indication that claim 46 contains allowable subject matter. Applicants have amended independent claim 42 to include the recitation of claim 46, thereby placing claims 42 and its dependent claims 43 – 45 in condition for allowance.

Section 112 Rejections:

Claims 47 – 49 were rejected under 35 USC § 112, 2nd paragraph, for allegedly being indefinite. Applicants have amended claim 47 to clarify that the first reservoir is in fluid communication with the flow channel and is *otherwise sealed from the exterior environment*. This clarifying amendment is supported by the specification as a whole and particularly in view of FIGS. 16 – 17C and the corresponding disclosure. Further, this amendment addresses the concern raised in the Advisory Action regarding a liquid that contributes to the sealing of the first reservoir. Thus, Applicants respectfully request that the Examiner withdraw this rejection.

Anticipation and/or Obviousness Rejections:

I. *Claims 20, 21, 23 – 30 and 39 – 41:*

In the Final Office Action, claims 20, 21, 23 – 30 and 39 – 45 were rejected under 35 USC § 102(b) as allegedly anticipated by or, in the alternative, under 35 USC § 103(a) as allegedly rendered obvious over US Patent No. 5,777,649 to Otsuka *et al.* (“Otsuka”). Applicants respectfully traverse.

Claim 20 has been cancelled. Claims 21, 23 – 30 and 39 – 41 have been amended to depend from claim 47, directly or indirectly. Applicants address the patentability of claim 47 below.

II. *Claims 42-46:*

As noted above, independent claim 42 has been amended to incorporate the allowable subject matter of claim 46, thereby placing claims 42 – 45 in condition for allowance.

III. *Claims 47 – 49 and also Claims 21, 23 – 30, 39 – 41 and 50:*

Independent claim 47, which is directed towards a microfluidic device adapted to facilitate analysis or processing of a sample, recites the microfluidic device includes a microfluidic flow channel formed in a substrate; and a first reservoir in fluid communication with the flow channel adapted for dampening or absorbing the pressure pulse propagated across the flow channel, wherein the first reservoir is otherwise sealed from the exterior environment.

Applicants respectfully submit that Otsuka fails to disclose, teach or suggest a first reservoir which is adapted for dampening or absorbing the pressure pulse propagated across the flow channel and which is otherwise sealed from the exterior environment,” as recited in claim 47. Rather, the buffer chamber in Otsuka is specifically designed to allow gas transmission and is therefore not sealed from the exterior environment. Item 12 of Otsuka is explicitly identified as a “gas *transmitting* sealing member” (see col. 6, lines 31 – 33). Indeed, this gas transmission aspect of the buffer chamber is the entire thrust of Otsuka’s invention (see e.g., col. 6, line 28 – col. 9, line 26, and independent claims 1 and 8).

Applicants further respectfully submit that claim 47 is patentable over Otsuka because Otsuka includes no motivation for sealing its buffer chamber against gas transmission.

Claims 48 and 49 and now also claims 21, 23 – 30, 39 – 41 and new claim 50 depend, directly or indirectly, from claim 47, and therefore these claims are patentably distinguishable over Otsuka for at least the same reasons noted with respect thereto. Moreover, Applicants respectfully submit that the claims recite additional limitations that are not disclosed nor made obvious by Otsuka. Thus, Applicants respectfully request reconsideration and withdrawal of the outstanding obviousness rejections of claims 21, 23 – 30 and 39 – 50 over Otsuka.

Finally, with respect to US 2002/0166585 to O’Connor, which is cited by the Examiner in the “Conclusion” section of the Final Office Action (p.7), Applicants note that O’Connor fails to disclose “a first reservoir in fluid communication with the flow channel ...” as recited in amended claim 47.

Double Patenting:

Claims 20, 24, 30, 39, 41 and 47 – 49 were rejected on the grounds of nonstatutory double patenting as being unpatentable over claim 16 of US 6,808,075. Claims 20, 21, 25, 39 and 41 were rejected on the grounds of nonstatutory double patenting as being unpatentable over claims 9 and 10 of US 6,877,528. Claims 20, 24 and 41 were rejected on the grounds of nonstatutory double patenting as being unpatentable over claim 2 of US 7,569,788. Claims 20, 24 and 41 were rejected on the grounds of nonstatutory double patenting as being unpatentable

over claim 4 of US 7,584,857. Claims 20, 21, 24-30 and 41 were rejected on the grounds of nonstatutory double patenting as being unpatentable over claims 1-9 of US 8,623,295.

Applicants defer the filing of any terminal disclaimers until after identification of further allowable subject matter in the subject application.

CONCLUSION

In view of the foregoing, Applicants submit that each of the Examiner's concerns has been fully and completely addressed. Consequently, Applicants believe the claims are in condition for allowance and solicit both reconsideration of the subject application and its swift passage. If, however, the Office believes that any unresolved issues exist or if, in the opinion of the Office, a telephone conference would expedite passing the present application to issuance, the Office is invited to call the undersigned attorney at (617) 449-6500 so that appropriate arrangements can be made for resolving such issues as expeditiously as possible.

Applicant believes no additional fees are due with this response. However, if additional fees are due, please charge our Deposit Account No. 50-4876, under Order No. 118153-01205 from which the undersigned is authorized to draw.

Dated: November 17, 2014

Respectfully submitted,

Electronic signature: /David R. Burns/
David R. Burns
Registration No.: 46,590
MCCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110
(617) 449-6500
(617) 607-9200 (Fax)
Attorney/Agent For Applicant

| | | | |
|---|---|----------------------------------|---------------------------------------|
| PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875 | Application or Docket Number 13/527,331 | Filing Date 06/19/2012 | <input type="checkbox"/> To be Mailed |
|---|---|----------------------------------|---------------------------------------|

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

(Column 1)

(Column 2)

| FOR | NUMBER FILED | NUMBER EXTRA | RATE (\$) | FEE (\$) |
|--|---|--------------|-----------|----------|
| <input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | |
| <input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | |
| <input type="checkbox"/> EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X \$ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X \$ = | |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | |

APPLICATION AS AMENDED – PART II

(Column 1)

(Column 2)

(Column 3)

| AMENDMENT | 11/17/2014 | CLAIMS REMAINING AFTER AMENDMENT | MINUS | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) |
|-----------------|--|----------------------------------|-------|------------------------------------|---------------|-----------|---------------------|
| | Total (37 CFR 1.16(i)) | * 20 | Minus | ** 21 | = 0 | X \$80 = | 0 |
| | Independent (37 CFR 1.16(h)) | * 2 | Minus | *** 4 | = 0 | X \$420 = | 0 |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| TOTAL ADD'L FEE | | | | | | 0 | |

(Column 1)

(Column 2)

(Column 3)

| AMENDMENT | CLAIMS REMAINING AFTER AMENDMENT | MINUS | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) |
|-----------------|--|-------|------------------------------------|---------------|-----------|---------------------|
| | Total (37 CFR 1.16(i)) | * | Minus | ** | X \$ = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | X \$ = | |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | |
| TOTAL ADD'L FEE | | | | | | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/KATISCHA WANZER/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 | 1757 |

86738 7590 10/14/2014
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

| |
|----------|
| EXAMINER |
|----------|

HYUN, PAUL SANG HWA

| | |
|----------|--------------|
| ART UNIT | PAPER NUMBER |
|----------|--------------|

1772

| | |
|-------------------|---------------|
| NOTIFICATION DATE | DELIVERY MODE |
|-------------------|---------------|

10/14/2014

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@mccarter.com

| | | | |
|---|--------------------------------------|---------------------------------------|--|
| Advisory Action Before the Filing of an Appeal Brief | Application No. 13/527,331 | Applicant(s) GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | AIA (First Inventor to File) Status No |

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 17 September 2014 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

NO NOTICE OF APPEAL FILED

1. ☒ The reply was filed after a final rejection. No Notice of Appeal has been filed. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance;
 (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114 if this is a utility or plant application. Note that RCEs are not permitted in design applications. The reply must be filed within one of the following time periods:

a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.

b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action; or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

c) ☐ A prior Advisory Action was mailed more than 3 months after the mailing date of the final rejection in response to a first after-final reply filed within 2 months of the mailing date of the final rejection. The current period for reply expires _____ months from the mailing date of the prior Advisory Action or SIX MONTHS from the mailing date of the final rejection, whichever is earlier.

Examiner Note: If box 1 is checked, check either box (a), (b) or (c). ONLY CHECK BOX (b) WHEN THIS ADVISORY ACTION IS THE FIRST RESPONSE TO APPLICANT'S FIRST AFTER-FINAL REPLY WHICH WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. ONLY CHECK BOX (c) IN THE LIMITED SITUATION SET FORTH UNDER BOX (c). See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) or (c) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☒ The proposed amendments filed after a final rejection, but prior to the date of filing a brief, will not be entered because

a) ☒ They raise new issues that would require further consideration and/or search (see NOTE below);

b) ☐ They raise the issue of new matter (see NOTE below);

c) ☒ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

NOTE: See Continuation Sheet. (See 37 CFR 1.116 and 41.33(a)).

4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).

5. ☐ Applicant's reply has overcome the following rejection(s): _____.

6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

7. ☒ For purposes of appeal, the proposed amendment(s): (a) ☒ will not be entered, or (b) ☐ will be entered, and an explanation of how the new or amended claims would be rejected is provided below or appended.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

9. ☐ The affidavit or other evidence filed after final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).

10. ☐ The affidavit or other evidence filed after the date of filing the Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing of good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).

11. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

12. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because:
See Continuation Sheet.

13. ☐ Note the attached Information *Disclosure Statement(s)*. (PTO/SB/08) Paper No(s). _____

14. ☐ Other: _____.

STATUS OF CLAIMS

15. The status of the claim(s) is (or will be) as follows:

Claim(s) allowed: _____.

Claim(s) objected to: _____.

Claim(s) rejected: _____.

Claim(s) withdrawn from consideration: _____.

/PAUL HYUN/
 Primary Examiner, Art Unit 1772

Continuation of 3. NOTE: The proposed amendment changes the dependency of claims 21, 24, 25-30 and 39-41, which changes the scope of said claims. The claims were previously dependent on claim 20, which is different in scope than claim 47, the claim from which claims 21, 24, 25-30 and 39-41 previously depended. In addition, the proposed amendment adds new claim 50.

Continuation of 11. does NOT place the application in condition for allowance because: Applicant's arguments are not persuasive.

As a preliminary matter, the double patenting rejection does extend to US 6,808,075. The ambiguity of the rejection stems from an inadvertent "copy and paste" error. The rejection should read, "Claims 20, 24, 30, 39, 41 and 47-49 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 16 of U.S. Patent No. 6,808,075 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because claim 16 of US 6,808,075 B2 anticipates claims 20, 24, 30, 39, 41 and 47-49 of the instant application." The examiner apologizes for the confusion.

With respect to the 35 U.S.C. section 112 rejection, the rejection is maintained despite Applicant's argument. Applicant argues that claim 47 is not indefinite because the specification refers to the limitation "sealed" with respect to the external environment. The argument is not persuasive. Because the specification does not define the limitation "sealed", it is subject to broadest reasonable interpretation, albeit the broadest reasonable interpretation is based on the disclosure of the specification. In this case, the specification is silent regarding whether the limitation "sealed" refers solely to the external environment. [0016] merely discloses that the reservoir is "sealed" by bonding a plate to the flexible membrane. Furthermore, the "sealing" disclosed in [0016] is conditioned on the presence of liquid in the flow channel that forms a meniscus with the first reservoir. Absent claim 47 specifying that the microfluidic flow channel comprises a liquid that contributes to the sealing of the first reservoir, the examiner maintains that the rejection is based on a reasonable interpretation of the limitation "sealed".

With respect to Applicant's argument that claims 47-49 should have been substantively considered on the merits and addressed, the examiner maintains that a rejection based on prior art is impossible given the contradicting nature of claim 47. However, the subject matter of claims 47-49 was addressed in the "conclusion" section of the Office action. Even though the claims were not rejected under 35 U.S.C. section 102/103, the examiner maintains that the claims were properly considered on their merits.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John R. Gilbert et al.

Application No.: 13/527,331

Confirmation No.: 1757

Filed: June 19, 2012

Art Unit: 1772

For: MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING FLUID
FLOW THROUGH A MICROCHANNEL

Examiner: Hyun, Paul S.

AMENDMENT IN RESPONSE TO FINAL OFFICE ACTION

MS After Final
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Final Office Action dated June 17, 2014, Applicants respectfully request entry of this amendment and response, and reconsideration of the above-referenced patent application in view of the following:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

Do not enter

/Paul Hyun/

10/07/2014

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John R. Gilbert et al.

Application No.: 13/527,331

Confirmation No.: 1757

Filed: June 19, 2012

Art Unit: 1772

For: MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING FLUID
FLOW THROUGH A MICROCHANNEL

Examiner: Hyun, Paul S.

AMENDMENT IN RESPONSE TO FINAL OFFICE ACTION

MS After Final
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Final Office Action dated June 17, 2014, Applicants respectfully request entry of this amendment and response, and reconsideration of the above-referenced patent application in view of the following:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions of the claims and listings of the claims in the application:

1-20. (Canceled)

21. (Currently Amended) The device of ~~claim 20~~ claim 47, further comprising a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.

22. (Cancelled)

23. (Previously Presented) The method of claim 40, wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.

24. (Currently Amended) The device of ~~claim 20~~ claim 47, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles suspended within the sample into a selected branch of the branched portion.

25. (Currently Amended) The device of ~~claim 20~~ claim 47, wherein the resilient properties of the first reservoir include a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.

26. (Previously Presented) The device of claim 25, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.

27. (Previously Presented) The device of claim 26, wherein the fluid filled region includes a portion of the first reservoir.

28. (Previously Presented) The device of claim 26, wherein the fluid filled region includes a side channel connecting the first reservoir to the flow channel.

29. (Previously Presented) The device of claim 26, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.

30. (Currently Amended) The device of ~~claim 20~~ claim 48, wherein the resilient properties of the first reservoir include a resilient wall for facilitating dampening or absorbing of the pressure pulse.

31. (Cancelled)

32. (Cancelled)

33. (Cancelled)

34. (Cancelled)

35. (Cancelled)

36. (Cancelled)

37. (Cancelled)

38. (Cancelled)

39. (Currently Amended) The device of ~~claim 20~~ claim 47, wherein the first reservoir is adjacent to an aperture in a sidewall of the flow channel.

40. (Currently Amended) A method comprising:
providing the microfluidic device of ~~claim 20~~ claim 47;
flowing a sample through the microfluidic flow channel;
propagating a pressure pulse across the flow channel in the crosswise direction; and
dampening or absorbing the pressure pulse using the first reservoir in fluid
communication with the flow channel.
41. (Currently Amended) The device of ~~claim 20~~ claim 47, further comprising an actuator or
pressurizer operatively associated with flow channel and adapted for generating the pressure
pulse across the flow channel in the crosswise direction.
42. (Currently Amended) A method for facilitating analysis or processing of a sample, the
method comprising:
flowing a sample through a microfluidic flow channel formed in a substrate;
propagating a pressure pulse across the microfluidic flow channel in a crosswise
direction; and
dampening or absorbing the pressure pulse using a first reservoir in fluid communication
with the microfluidic flow channel,
wherein the pressure pulse is used to sort one or more particles suspended within the
sample.
43. (Previously Presented) The method of claim 42, wherein the pressure pulse is used to
selectively control or otherwise affect the flow of the sample through the flow channel.
44. (Previously Presented) The method of claim 42, wherein the first reservoir includes a gas
pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure
pulse.
45. (Previously Presented) The method of claim 42, wherein the first reservoir includes a
resilient wall for facilitating dampening or absorbing of the pressure pulse.

46. (Cancelled)

47. (Currently Amended) A microfluidic device adapted to facilitate analysis or processing of a sample, the microfluidic device comprising:

a microfluidic flow channel formed in a substrate; and

a sealed first reservoir in fluid communication with the flow channel adapted for dampening or absorbing a pressure pulse propagated across the flow channel.

48. (Previously Presented) The device of claim 47, wherein the first reservoir includes resilient properties which enable the dampening or absorbing the pressure.

49. (Previously Presented) The device of claim 47, further comprising an actuator or pressurizer operatively associated with flow channel and adapted for generating a pressure pulse across the flow channel in a crosswise direction.

50. (New) The device of claim 21, wherein the second reservoir includes a gas pocket, wherein compression of the gas facilitates the generating of the pressure pulse.

REMARKS

The present response is intended to fully address the objections and rejections noted in the outstanding Final Office Action mailed June 17, 2014 (the “Final Office Action”), and is believed to place the subject application in condition for allowance. Thus, favorable reconsideration and prompt allowance of the subject application is respectfully requested. Applicants do not acquiesce to any portion of the Office Action not particularly addressed herein.

Claims 20, 21, 23–30 and 39–49 were pending of which claims 20, 42 and 47 were in independent form. By way of the present response, independent claim 20 is hereby canceled. In accordance therewith, the dependencies of dependent claims 21, 24, 25, 30, 39–41 are amended to depend from claim 47. Also by way of the present response, independent claim 42 is hereby amended to incorporate allowable subject matter from dependent claim 46. In accordance therewith, dependent claim 46 is cancelled, without prejudice. The claims are also hereby amended to address an antecedent basis issue with respect to independent claim 47 and to reintroduce subject matter from previously cancelled claim 31 as new dependent claim 50 (indirectly depending from claim 47). Upon entry of the amendments, claims 21, 23–30 and 39–49 will be pending of which claims 42 and 47 are in independent form.

Applicants respectfully submit that no new matter is introduced by way of the foregoing amendments/additions to the claims. Thus, Applicants respectfully request entry and substantive consideration of the foregoing amendments to the claims.

I. Allowable Subject Matter:

Applicants acknowledge and thank the Examiner for the indication that claim 46 contains allowable subject matter. As noted above, Applicants hereby amend independent claim 42 to include the recitation of claim 46, thereby placing independent claim 42 and dependent claims 43–45 (depending directly or indirectly from claim 42) in condition for allowance.

II. Section 112 Rejections:

a. The Section 112 Rejections to Claims 47-49 are Improper:

Claims 47–49 were rejected under 35 USC § 112, 2nd paragraph, for allegedly being indefinite. Applicants respectfully traverse.

In particular, Applicants respectfully submit that the Examiner failed to construe the term “sealed” in view of the specification as required under MPEP 2111. In this case, the examiner’s interpretation of the term “sealed” is inconsistent with the specification and should not have been applied. See, e.g., *In re Zletz*, 893 F.2d 319, 321, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989).

The specification clearly informs the intended meaning of the term “sealed.” See, e.g., paragraph [0011] of the specification stating that “the microfluidic system includes a microchannel and a sealed, gas-filled reservoir positioned adjacent to and connected to the microchannel.” See also paragraph [0016] of the specification stating that “The aperture defines a reservoir adjacent to the microchannel, wherein the meniscus forms an interface between the microchannel and the reservoir. The flexible membrane is bonded to the second plate to seal the reservoir.” In this context, a person of ordinary skill in the art would clearly understand the term “sealed” as referring to the reservoir being otherwise sealed from an external environment, i.e., not having any additional outlets/inlets (other than with the microchannel).

Thus, as informed by the specification, the reservoir being sealed is not in contradiction to the reservoir being in fluid communication with the microchannel. Accordingly, Applicants respectfully request that the Examiner withdraw the outstanding Section 112 rejections to claims 47-49.

*b. Claims 47-49 Should Be Substantively Considered and Addressed
Irrespective of the Section 112 Rejections thereto:*

Applicants also note that, notwithstanding the Section 112 rejections to claims 47-49 it was improper for the Examiner to fail to consider the claims on their merits. See, e.g., MPEP 2173.06, “when the examiner determines that a claim term or phrase renders the claim indefinite,

the examiner should make a rejection based on indefiniteness under 35 U.S.C. 112(b) or pre-AIA 35 U.S.C. 112, second paragraph, *as well as a rejection(s) in view of the prior art under 35 U.S.C. 102 or 103 that renders the prior art applicable based on the examiner's interpretation of the claim*” (emphasis added). Thus, Applicants request that the Examiner reconsider and address claims 47–49 on the merits irrespective of the disposition of the Section 112 rejections thereto.

On the merits, Applicants reiterate that Otsuka fails to disclose, teach or suggest a sealed first reservoir for dampening or absorbing a pressure pulse, as recited in claim 47. Rather, the buffer chamber in Otsuka is specifically designed to allow gas transmission and is therefore not sealed. Indeed, this gas transmission aspect of the buffer chamber is the entire thrust of Otsuka's invention (see e.g., col. 6, line 28 – col. 9, line 26, and independent claims 1 and 8). O'Connor, likewise fails to disclose, teach or suggest “a sealed first reservoir in fluid communication with the flow channel adapted for dampening or absorbing a pressure pulse propagated across the flow channel,” as recited in amended claim 47.

Although no formal art-based rejections to claims 47-49 were presented in the Office Action, Applicants submit that these claims patentably distinguish over the art of record and respectfully request that the Examiner so find or provide a formal rejection.

III. Anticipation and/or Obviousness Rejections:

a. Claims 20, 21, 23 – 30 and 39 – 41:

In the Final Office Action, claims 20, 21, 23 – 30 and 39 – 45 were rejected under 35 USC § 102(b) as allegedly anticipated by or, in the alternative, under 35 USC § 103(a) as allegedly rendered obvious over US Patent No. 5,777,649 to Otsuka *et al.* (“Otsuka”). Applicants respectfully traverse.

As noted above, claim 20 is hereby cancelled and claims 21, 23–30 and 39–41 are amended to depend from claim 47. Thus, dependent claims 21, 23–30 and 39–41 each depend directly or indirectly from claim 47 and therefor patentably distinguish over the applied

references for at least the same reasons noted above with respect thereto for at least the same reasons noted with respect thereto.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the outstanding art-based rejections to claims 20, 21, 23–30 and 39–41 over Otsuka.

b. Claims 42-46:

As noted above, independent claim 42 is hereby amended to incorporate the allowable subject matter of claim 46, thereby placing claims 42 – 45 in condition for allowance.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the outstanding art-based rejections to claims 42-46 over Otsuka.

Double Patenting:

Claims 20, 21, 24 – 30, 41 and 47 – 49 were rejected on the grounds of nonstatutory double patenting over US 6,877,528, US 7,569,788, US 7,584,857 and/or US 8,623,295. Applicants are uncertain if the Examiner's double patenting rejection extends to US 6,808,075 (see page 3, lines 19-22 of the Office Action) and respectfully request that the Examiner clarify this rejection. Applicants defer the filing of any terminal disclaimers until after identification of allowable subject matter in the subject application.

CONCLUSION

In view of the foregoing, Applicants submit that each of the Examiner's concerns has been fully and completely addressed. Consequently, Applicants believe the claims are in condition for allowance and solicit both reconsideration of the subject application and its swift passage. If, however, the Office believes that any unresolved issues exist or if, in the opinion of the Office, a telephone conference would expedite passing the present application to issuance, the Office is invited to call the undersigned attorney at (617) 449-6500 so that appropriate arrangements can be made for resolving such issues as expeditiously as possible.

Applicants believe no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 50-4876, under Order No. 118153-01205 from which the undersigned is authorized to draw.

Dated: September 17, 2014

Respectfully submitted,

By: /Gabriel R. W. Goldman/
Gabriel R. W. Goldman
Registration No.: 61,343
MCCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110
(617) 449-6500
(617) 607-9200 (Fax)
Attorney/Agent For Applicant

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 20170630 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John Richard Gilbert |
| Customer Number: | 86738 |
| Filer: | Gabriel R.W. Goldman |
| Filer Authorized By: | |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 17-SEP-2014 |
| Filing Date: | 19-JUN-2012 |
| Time Stamp: | 19:24:29 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|------------------------|----|
| Submitted with Payment | no |
|------------------------|----|

File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|-----------------|----------------------|---------------------------|---|------------------|------------------|
| 1 | | 118153_01205_Response.pdf | 58114 27cbc1b44585a2762b4b75b10d7599c136d13de7 | yes | 10 |

| | Multipart Description/PDF files in .zip description | | |
|--|---|-------|-----|
| | Document Description | Start | End |
| | Response After Final Action | 1 | 1 |
| | Claims | 2 | 5 |
| | Applicant Arguments/Remarks Made in an Amendment | 6 | 10 |
| Warnings: | | | |
| Information: | | | |
| Total Files Size (in bytes): | | 58114 | |
| <p>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> | | | |

| | | | |
|---|---|----------------------------------|---------------------------------------|
| PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875 | Application or Docket Number 13/527,331 | Filing Date 06/19/2012 | <input type="checkbox"/> To be Mailed |
|---|---|----------------------------------|---------------------------------------|

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

| FOR | NUMBER FILED | NUMBER EXTRA | RATE (\$) | FEE (\$) |
|--|---|--------------|-----------|----------|
| <input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | |
| <input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (l), or (m)) | N/A | N/A | N/A | |
| <input type="checkbox"/> EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X \$ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X \$ = | |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | |

APPLICATION AS AMENDED – PART II

| AMENDMENT | (Column 1) | (Column 2) | (Column 3) | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) |
|-----------------|--|----------------------------------|------------|------------------------------------|---------------|-----------|---------------------|
| | 02/24/2014 | CLAIMS REMAINING AFTER AMENDMENT | | | | | |
| | Total (37 CFR 1.16(i)) | * 21 | Minus | ** 21 | = 0 | X \$80 = | 0 |
| | Independent (37 CFR 1.16(h)) | * 4 | Minus | ***4 | = 0 | X \$420 = | 0 |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| TOTAL ADD'L FEE | | | | | | 0 | |
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| | 09/17/2014 | CLAIMS REMAINING AFTER AMENDMENT | | | | | |
| | Total (37 CFR 1.16(i)) | * 20 | Minus | ** 21 | = 0 | X \$80 = | 0 |
| | Independent (37 CFR 1.16(h)) | * 2 | Minus | *** 4 | = 0 | X \$420 = | 0 |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| TOTAL ADD'L FEE | | | | | | 0 | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/GLENN BURNS JR/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 | 1757 |

86738 7590 06/17/2014
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

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| EXAMINER |
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HYUN, PAUL SANG HWA

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| ART UNIT | PAPER NUMBER |
|----------|--------------|

1772

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|-------------------|---------------|
| NOTIFICATION DATE | DELIVERY MODE |
|-------------------|---------------|

06/17/2014

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@mccarter.com

| | | | |
|------------------------------|--------------------------------------|---------------------------------------|--|
| Office Action Summary | Application No. 13/527,331 | Applicant(s) GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | AIA (First Inventor to File) Status No |

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 24 February 2014.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

- 5) ☒ Claim(s) 20,21,23-30 and 39-49 is/are pending in the application.
5a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 6) ☐ Claim(s) _____ is/are allowed.
- 7) ☒ Claim(s) 20,21,23-30,39-45 and 47-49 is/are rejected.
- 8) ☒ Claim(s) 46 is/are objected to.
- 9) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some** c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 3) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ |
| 2) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date <u>08/21/12</u> | 4) <input type="checkbox"/> Other: _____ |

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DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Response to Amendment

The amendment filed on February 24, 2014 is acknowledged. Claims 20, 21, 23-30 and 39-49 are pending. Applicant amended claims 20, 21, 23-30 and 40, and added new claims 41-49.

The claim objection and the rejections under 35 U.S.C. section 112 set forth in the previous Office action have been withdrawn in light of the amendment.

With respect to the double patenting rejections set forth in the Office action, Applicant indicated the intent to defer the filing of any terminal disclaimer until allowable subject matter has been identified by the examiner. That said, all applicable rejections (subject to change due to amendments) will be maintained until Applicant files a terminal disclaimer.

Information Disclosure Statement

EP A 0 177 718 and EP A 0 745 682 cited in the information disclosure statement (IDS) submitted on August 21, 2013 were previously not considered by the examiner because the IDS does not include a concise explanation of the relevance of each patent listed that is not in the English language. According to Applicant, only the abstracts of the references were intended to be considered by the examiner. That said, with respect to EP A 0 177 718 and EP A 0 745 682, only the abstracts are being considered.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645

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(Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit <http://www.uspto.gov/forms/>. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to <http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp>.

Claims **20, 21, 25, 39 and 41** are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 9, 9, 9, 9 and 10 of U.S. Patent No. 6,877,528 B2, respectively. Although the claims at issue are not identical, they are not patentably distinct from each other because claims 9, 9, 9, 9 and 10 of US 6,877,528 B2 anticipate claims 20, 21, 25, 39 and 41 of the instant application, respectively.

Claims **20, 24, 30, 39, 41 and 47-49** are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 16 of U.S. Patent No. 6,808,075 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because claim 16 of US 6,877,528 B2 anticipates claims 20, 21, 25, 39, 41 and 47-49 of the instant application.

Claims **20, 24 and 41** are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 2 of U.S. Patent No. 7,569,788 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because claim 2 of US 7,569,788 B2 anticipates claims 20, 24 and 41 of the instant application.

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Claims **20, 24 and 41** are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 4 of U.S. Patent No. 7,584,857 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because claim 4 of US 7,584,857 B2 anticipates claims 20, 24 and 41 of the instant application.

Claims **20, 21, 24-30 and 41** are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 1, 3-9 and 2 of U.S. Patent No. 8,623,295 B2, respectively. Although the claims at issue are not identical, they are not patentably distinct from each other because claims 1, 1, 3-9 and 2 of US 8,623,295 B2 anticipate claims 20, 21, 24-30 and 41 of the instant application, respectively.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims **47-49** are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 47 recites that the first reservoir is sealed, yet it is in fluid communication with the flow channel. The claim is contradictory. Consequently, claims 47-49 will not be examined on the merits. However, attention is directed to the "conclusion" section of the Office action set forth below, which cites prior art that is relevant to claims 47-49.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims **20, 21, 23-30 and 39-45** are rejected under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Otsuka et al. (US 5,777,649).

With respect to claim 20, Otsuka et al. disclose an ink jet printing head (see Fig. 1), said head comprising:

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a channel 9 formed in a substrate 4 (see Fig. 1); and

a first reservoir 13 in fluid communication with the flow channel 9, said first reservoir 13 including resilient properties for dampening or absorbing a pressure pulse propagated across the flow channel in a crosswise direction (see Fig. 1 and lines 35-39, col. 6). It should be noted that the limitation "crosswise direction" is relative. Absent a reference to which the claimed "crosswise direction" can be compared (e.g. fluid flowing in the channel), the direction of the arrows illustrated in Figure 1 of Otsuka et al. is deemed to satisfy the limitation "crosswise direction". Moreover, flow channel 9 has two directional components with respect to flow; flow from branch 8 and flow from channel 81. Thus, the direction of the arrows illustrated in Figure 1 of Otsuka et al. is deemed to satisfy the limitation "crosswise direction" with respect to the flow from branch 8.

While the reference does not disclose that channel 9 is microfluidic, given that channel 9 is part of an ink jet printing head, it appears that it is microfluidic. In the alternative, it would have been obvious to one of ordinary skill in the art to render channel 9 microfluidic given the nature of its intended use (i.e. dispense micro-volumes of ink).

With respect to claim 21, the device further comprises a second reservoir 6 for generating the pressure pulse based on a change in volume or pressure in the second reservoir (see Fig. 1 and Abstract).

With respect to claim 24, the flow channel 9 includes a branched portion that divides the channel into two branches 8 and 13, wherein the pressure pulse is capable of sorting one or more particles suspended within a liquid situated in the flow channel 9 into branch 13 (see arrow in Figure 1).

With respect to claim 25, the first reservoir 13 comprises a gas pocket, the compression of the gas pocket facilitating the dampening of the pressure pulse (see lines 35-40, col. 6).

With respect to claims 26-29, the first reservoir 13 further comprises a fluid-filled region (i.e. side channel of reduced dimensions) connecting channel 9 with the interior of reservoir 13 (see Fig. 2). The fluid-filled region prevents the gas in the first reservoir 13 from leaking into the flow channel 9 (see Fig. 1).

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With respect to claim 30, the first reservoir 13 comprises a gas that forms a resilient wall (see lines 35-40, col. 6). A liquid/gas interface is fully within the scope of a “resilient wall” as disclosed by the specification (see disclosure pertaining to “virtual wall”).

With respect to claim 39, the first reservoir 13 is adjacent to an aperture in a sidewall of channel 9 (see Fig. 2).

With respect to claims 40 and 42, Otsuka et al. disclose a method, the method comprising the steps of:

providing the device disclosed above;

flowing a sample through flow channel 9 (via passage 8, see Fig. 1);

propagating a pressure pulse across the flow channel 9 in a crosswise direction with respect to the flow from passage 8 using the second reservoir 6 (see Abstract); and

using the first reservoir 13 to dampen the pressure pulse generated by the second reservoir 6 (see lines 35-43, col. 6).

With respect to claims 23 and 43, the pressure pulse generated by the second reservoir 6 is used to affect the flow of the sample through the flow channel 9 (see arrows in Figure 1).

With respect to claim 44, the first reservoir 13 comprises a gas pocket, as discussed above.

With respect to claim 45, the first reservoir 13 comprises a gas that forms a resilient wall (see lines 35-40, col. 6). A liquid/gas interface is fully within the scope of a “resilient wall” as disclosed by the specification (see disclosure pertaining to “virtual wall”).

Allowable Subject Matter

Claim 46 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The following is a statement of reasons for the indication of allowable subject matter:

Otsuka et al. disclose the method recited in claim 42, as discussed above. However, the method disclosed by Otsuka et al. is unrelated to particle sorting. Consequently, there is no motivation to apply the method disclosed by Otsuka et al. to particle sorting.

Response to Arguments

The amendment necessitated the new grounds of rejection set forth in the Office action. Most of Applicant's arguments are deemed to be addressed in the new grounds of rejection. Applicant's arguments not addressed in the body of the rejections are addressed below.

With respect to claims 22 and 31-37, Applicant argues that the claims are patentable because Otsuka et al. do not disclose "an actuator or pressurizer operatively associated with the second reservoir". This argument is not persuasive because the claims do not recite an actuator or a pressurizer. The claims merely recite a reservoir, which is taught by Otsuka et al., as discussed in the rejections above. The rejections explicitly identify bubble 6 as the second reservoir. The examiner maintains that bubble 6 disclosed by Otsuka et al. is fully within the scope of the "second reservoir" as defined in the specification and claims.

With respect to claim 24, Applicant argues that the claims are patentable because the device disclosed by Otsuka et al. is not capable of sorting particles. This argument is not persuasive. As elaborated in the rejection above, the device disclosed by Otsuka et al. comprises a branched portion dividing the flow channel into two branches 8 and 13. The pressure pulse produced by bubble 6 propagates along a direction indicated by the arrow illustrated in Figure 1. The examiner maintains that this pressure pulse is capable of directing a particle into branch 13. This is sufficient to anticipate the intended use limitation recited in the claim.

Conclusion

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

1) O'Connor et al. (US 2002/0166585 A1) disclose a microfluidic device comprising a flow channel 106 formed in a substrate 103; and

a sealed first reservoir 105 for dampening or absorbing a pressure pulse propagated across the flow channel (compare Figs. 1A and 1C).

2) Otsuka et al. (US 5,777,649) disclose an ink jet printing head (see Fig. 1), said head comprising:

a channel 9 formed in a substrate 4 (see Fig. 1); and

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a first reservoir 13 in fluid communication with the flow channel 9, said first reservoir 13 for dampening or absorbing a pressure pulse propagated across the flow channel (see Fig. 1 and lines 35-39, col. 6).

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to PAUL HYUN whose telephone number is (571)272-8559. The examiner can normally be reached on M-F 10 AM-6:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, In Suk Bullock can be reached on (571) 272-5954. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/PAUL HYUN/
Primary Examiner, Art Unit 1772

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|-----------------------------------|---------------------------------------|--|-------------|
| Notice of References Cited | Application/Control No. 13/527,331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | Page 1 of 1 |

U.S. PATENT DOCUMENTS

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | Classification |
|---|---|--|-----------------|-----------------|----------------|
| * | A | US-2002/0166585 A1 | 11-2002 | O'Connor et al. | 137/87.01 |
| | B | US- | | | |
| | C | US- | | | |
| | D | US- | | | |
| | E | US- | | | |
| | F | US- | | | |
| | G | US- | | | |
| | H | US- | | | |
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| | M | US- | | | |


FOREIGN PATENT DOCUMENTS

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Country | Name | Classification |
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NON-PATENT DOCUMENTS

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
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| | U | |
| | V | |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

| | | |
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| Search Notes  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

| CPC- SEARCHED | | |
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| Symbol | Date | Examiner |
| | | |


| CPC COMBINATION SETS - SEARCHED | | |
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| Symbol | Date | Examiner |
| | | |

| US CLASSIFICATION SEARCHED | | | |
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| Class | Subclass | Date | Examiner |
| | | | |

| SEARCH NOTES | | |
|---|----------|----------|
| Search Notes | Date | Examiner |
| inventor name search | 6/3/2014 | PSH |
| text search | 6/3/2014 | PSH |
| forward and backward searched relevant references | 6/3/2014 | PSH |


| INTERFERENCE SEARCH | | | |
|-------------------------|-------------------------|------|----------|
| US Class/ CPC Symbol | US Subclass / CPC Group | Date | Examiner |
| | | | |

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| | /PAUL HYUN/ Primary Examiner.Art Unit 1772 |
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| <i>Index of Claims</i>  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

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|---|-----------------|---|-------------------|---|---------------------|---|-----------------|
| ✓ | Rejected | - | Cancelled | N | Non-Elected | A | Appeal |
| = | Allowed | ÷ | Restricted | I | Interference | O | Objected |

| <input type="checkbox"/> Claims renumbered in the same order as presented by applicant | | | | <input type="checkbox"/> CPA | | | | <input type="checkbox"/> T.D. | | | | <input type="checkbox"/> R.1.47 | | | |
|--|----------|------------|------------|------------------------------|--|--|--|-------------------------------|--|--|--|---------------------------------|--|--|--|
| CLAIM | | DATE | | | | | | | | | | | | | |
| Final | Original | 10/21/2013 | 06/11/2014 | | | | | | | | | | | | |
| | 1 | - | - | | | | | | | | | | | | |
| | 2 | - | - | | | | | | | | | | | | |
| | 3 | - | - | | | | | | | | | | | | |
| | 4 | - | - | | | | | | | | | | | | |
| | 5 | - | - | | | | | | | | | | | | |
| | 6 | - | - | | | | | | | | | | | | |
| | 7 | - | - | | | | | | | | | | | | |
| | 8 | - | - | | | | | | | | | | | | |
| | 9 | - | - | | | | | | | | | | | | |
| | 10 | - | - | | | | | | | | | | | | |
| | 11 | - | - | | | | | | | | | | | | |
| | 12 | - | - | | | | | | | | | | | | |
| | 13 | - | - | | | | | | | | | | | | |
| | 14 | - | - | | | | | | | | | | | | |
| | 15 | - | - | | | | | | | | | | | | |
| | 16 | - | - | | | | | | | | | | | | |
| | 17 | - | - | | | | | | | | | | | | |
| | 18 | - | - | | | | | | | | | | | | |
| | 19 | - | - | | | | | | | | | | | | |
| | 20 | ✓ | ✓ | | | | | | | | | | | | |
| | 21 | ✓ | ✓ | | | | | | | | | | | | |
| | 22 | ✓ | - | | | | | | | | | | | | |
| | 23 | ✓ | ✓ | | | | | | | | | | | | |
| | 24 | ✓ | ✓ | | | | | | | | | | | | |
| | 25 | ✓ | ✓ | | | | | | | | | | | | |
| | 26 | ✓ | ✓ | | | | | | | | | | | | |
| | 27 | ✓ | ✓ | | | | | | | | | | | | |
| | 28 | ✓ | ✓ | | | | | | | | | | | | |
| | 29 | ✓ | ✓ | | | | | | | | | | | | |
| | 30 | ✓ | ✓ | | | | | | | | | | | | |
| | 31 | ✓ | - | | | | | | | | | | | | |
| | 32 | ✓ | - | | | | | | | | | | | | |
| | 33 | ✓ | - | | | | | | | | | | | | |
| | 34 | ✓ | - | | | | | | | | | | | | |
| | 35 | ✓ | - | | | | | | | | | | | | |
| | 36 | ✓ | - | | | | | | | | | | | | |

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|---|--|--|
| <p align="center"><i>Index of Claims</i></p>  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

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|---|-----------------|---|-------------------|---|---------------------|---|-----------------|
| ✓ | Rejected | - | Cancelled | N | Non-Elected | A | Appeal |
| = | Allowed | ÷ | Restricted | I | Interference | O | Objected |

| <input type="checkbox"/> Claims renumbered in the same order as presented by applicant | | | | <input type="checkbox"/> CPA | | <input type="checkbox"/> T.D. | | <input type="checkbox"/> R.1.47 | |
|---|----------|------------|------------|-------------------------------------|--|--------------------------------------|--|--|--|
| CLAIM | | DATE | | | | | | | |
| Final | Original | 10/21/2013 | 06/11/2014 | | | | | | |
| | 37 | ✓ | - | | | | | | |
| | 38 | ✓ | - | | | | | | |
| | 39 | ✓ | ✓ | | | | | | |
| | 40 | ✓ | ✓ | | | | | | |
| | 41 | | ✓ | | | | | | |
| | 42 | | ✓ | | | | | | |
| | 43 | | ✓ | | | | | | |
| | 44 | | ✓ | | | | | | |
| | 45 | | ✓ | | | | | | |
| | 46 | | O | | | | | | |
| | 47 | | ✓ | | | | | | |
| | 48 | | ✓ | | | | | | |
| | 49 | | ✓ | | | | | | |

EAST Search History

EAST Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|-------|------|---|----------------------------------|------------------|---------|---------------------|
| S1 | 2 | ((("6877528") or ("7069943")).PN. | USPAT; USOCR | OR | OFF | 2014/06/03 13:35 |
| S2 | 105 | (gilbert bohm deshpane).in. and bubble and reservoir and meniscus | US- PGPUB; USPAT | OR | ON | 2014/06/03 15:07 |
| S3 | 49 | (gilbert bohm deshpane).in. and bubble and reservoir and meniscus and microfluidic | US- PGPUB; USPAT | OR | ON | 2014/06/03 15:07 |
| S4 | 49 | (gilbert bohm deshpane).in. and bubble and reservoir and meniscus and microfluidic and channel | US- PGPUB; USPAT | OR | ON | 2014/06/03 15:07 |
| S5 | 49 | (gilbert bohm deshpane).in. and bubble and reservoir and meniscus and microfluidic and channel and pressure | US- PGPUB; USPAT | OR | ON | 2014/06/03 15:08 |
| S6 | 20 | (gilbert bohm deshpane).in. and bubble and reservoir and meniscus and microfluidic and channel and pressure | USPAT | OR | ON | 2014/06/03 15:08 |
| S7 | 52 | first adj mentioned adj semiconduct\$4.clm. | US- PGPUB; USPAT | OR | ON | 2014/06/05 11:38 |
| S8 | 1 | ("20090127589").PN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/06/05 12:40 |
| S9 | 1 | ("5915583").PN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/06/05 14:40 |
| S10 | 1 | ("6878346").PN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/06/05 14:43 |
| S11 | 30 | friswell.in. | USPAT | OR | OFF | 2014/06/05 14:44 |
| S12 | 1 | ("7111501").PN. | USPAT; USOCR | OR | OFF | 2014/06/05 14:56 |
| S13 | 1 | ("20030058450").PN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/06/06 11:09 |
| S14 | 1 | ("8372340").PN. | US- PGPUB; USPAT; USOCR | OR | OFF | 2014/06/07 19:12 |
| S15 | 3700 | (ching lee richardson).in. and integrated.ti. | US- PGPUB; USPAT | OR | ON | 2014/06/08 14:10 |
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| S16 | 352 | (ching lee richardson).in. and integrated.ti. and vacuum | US-PGPUB; USPAT | OR | ON | 2014/06/08 14:10 |
| S17 | 63 | (ching lee richardson).in. and integrated.ti. and vacuum.clm. | US-PGPUB; USPAT | OR | ON | 2014/06/08 14:10 |
| S18 | 56 | ("20020093641" "20030027225" "3508654" "3560754" "3791517" "3827555" "3984307" "4147621" "4175662" "4318483" "4361400" "4365719" "4526276" "4572664" "4756427" "4936465" "4939081" "5030002" "5101978" "5275787" "5395588" "5637496" "5783446" "5795727" "5837200" "5976336" "5979664" "5998212" "6057111" "6120666" "6145247" "6214556" "6221654" "6273553" "6280967" "6432630" "6454862" "6455280" "6482652" "6524790" "6540895" "6561224" "6592821" "6597438" "6657730" "6803194" "6877528" "7075652" "7220594" "7258774").PN. OR ("7569788").URPN. | US-PGPUB; USPAT; USOCR | OR | OFF | 2014/06/10 17:13 |
| S19 | 781 | sort\$4 near3 (cell particle) and (meniscus bubble) with flow | US-PGPUB; USPAT | OR | ON | 2014/06/10 17:14 |
| S20 | 254 | sort\$4 near3 (cell particle) and (meniscus bubble) near2 flow | US-PGPUB; USPAT | OR | ON | 2014/06/10 17:15 |
| S21 | 41 | sort\$4 near3 (cell particle) same (meniscus bubble) near2 flow | US-PGPUB; USPAT | OR | ON | 2014/06/10 17:15 |
| S22 | 36 | sort\$4 near3 (cell particle) same (meniscus bubble) adj valve | US-PGPUB; USPAT | OR | ON | 2014/06/10 17:21 |
| S23 | 3346 | sort\$4 near3 (cell particle) and deform\$4 | US-PGPUB; USPAT | OR | ON | 2014/06/10 17:25 |
| S24 | 840 | sort\$4 near3 (cell particle) and deform\$4 and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 17:25 |
| S25 | 1 | ("5777649").PN. | USPAT; USOCR | OR | OFF | 2014/06/10 17:41 |
| S26 | 1 | ("3791517").PN. | USPAT; USOCR | OR | OFF | 2014/06/10 18:06 |
| S27 | 60 | ("3560754" "3710933").PN. OR ("3791517").URPN. | US-PGPUB; USPAT; USOCR | OR | OFF | 2014/06/10 18:07 |
| S28 | 23 | (cytomet\$5 sort\$4 near3 (cell particle)).ti. and microfluidic and (absorb\$4 dampen\$4) near2 pressure | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:12 |
| S29 | 8 | (cytomet\$5 sort\$4 near3 (cell particle)).ti. and microfluidic and (absorb\$4 dampen\$4) near2 pressure not (dashpande bohm).in. | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:13 |
| S30 | 1 | (cytomet\$5 sort\$4 near3 (cell particle)).ti. and microfluidic and (absorb\$4 dampen\$4) near2 pressure not (deshpande bohm).in. | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:13 |

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|-----|-------|--|-----------------|----|----|------------------|
| S31 | 86 | microfluidic and (absorb\$4 dampen\$4) near2 pressure not (deshpande bohmi).in. | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:13 |
| S32 | 29 | elastomer\$4 and deform\$4 and microfluidic and control adj channel with temperature | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:17 |
| S33 | 3 | elastomer\$4 and deform\$4 and unger.in. and control adj channel with temperature | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:18 |
| S34 | 21 | elastomer\$4 and deform\$4 and microfluidic and control adj channel with heat\$4 | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:19 |
| S35 | 19182 | elastomer\$4 and deform\$4 and temperature with pressure | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:20 |
| S36 | 711 | elastomer\$4 and deform\$4 and temperature near3 pressure and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:20 |
| S37 | 93 | elastomer\$4 and deform\$4 with valve and temperature near3 pressure and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:38 |
| S38 | 141 | elastomer\$4 and deform\$4 with valve and volume with valve and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 18:42 |
| S39 | 125 | (membrane elastomer\$4) with deform\$4 and volume with valve same (heat\$4 temperature) and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 19:05 |
| S40 | 13 | (membrane elastomer\$4) with deform\$4 and volume with valve with (heat\$4 temperature) and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 19:05 |
| S41 | 289 | (membrane elastomer\$4) with deform\$4 and volume with (heat\$4 temperature) and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 19:06 |
| S42 | 75 | o'connor.in. and microfluidic | US-PGPUB; USPAT | OR | ON | 2014/06/10 19:10 |

6/ 11/ 2014 12:46:37 PM
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PTO/SB/08b (01-08)

Approved for use through 07/31/2008. OMB 0651-0031

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| | | | | | |
|--|---|----|---|-------------------------------|---------------------------|
| Substitute for form 1449/PTO | | | | Complete if Known | |
| INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i> | | | | Application Number | 13/527,331 Confirm # 1757 |
| | | | | Filing Date | June 19, 2012 |
| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 1 | of | 3 | Attorney Docket Number | 118153-01205 |

| U.S. PATENT DOCUMENTS | | | | | |
|-----------------------|--------------------------|---|--------------------------------|--|---|
| Examiner Initials* | Cite No. ¹ | Document Number | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear |
| | | Number-Kind Code ² (if known) | | | |
| | A1 * | US 3,289,687 | 12-06-1966 | Dunaway | |
| | A2 * | US 3,370,538 | 02-27-1968 | Iliacs | |
| | A3 * | US 3,508,654 | 02-01-1970 | Glaetli | |
| | A4 * | US 3,560,754 | 02-02-1971 | Kamentsky | |
| | A5 * | US 3,791,517 | 02-12-1974 | Friedman | |
| | A6 * | US 3,827,555 | 08-06-1974 | Kamnetsky | |
| | A7 * | US 3,984,307 | 10-05-1976 | Kamnetsky | |
| | A8 * | US 4,153,855 | 05-08-1979 | Feingold | |
| | A9 * | US 4,175,662 | 11-27-1979 | Zold | |
| | A10 * | US 4,426,451 | 01-17-1984 | Columbus | |
| | A11 * | US 4,581,624 | 04-08-1986 | O'Connor | |
| | A12 * | US 4,636,149 | 01-13-1987 | Brown | |
| | A13 * | US 4,676,274 | 06-30-1987 | Brown | |
| | A14 * | US 4,756,427 | 07-12-1988 | Gohde et al. | |
| | A15 * | US 4,908,112 | 03-13-1990 | Pace | |
| | A16 * | US 5,065,978 | 11-19-1991 | Albarda et al. | |
| | A17 * | US 5,092,972 | 03-03-1992 | Ghowzi | |
| | A18 * | US 5,101,978 | 04-07-1992 | Marcus | |
| | A19 * | US 5,265,327 | 11-30-1993 | Faris et al. | |
| | A20 * | US 5,777,649 | 07-07-1993 | Otsuka et al. | |
| | A21 * | US 5,789,045 | 08-04-1998 | Wapner et al. | |
| | A22 * | US 5,837,200 | 11-17-1998 | Diessel et al. | |
| | A23 * | US 5,876,187 | 03-02-1999 | Afromowitz et al. | |
| | A24 * | US 5,988,522 | 11-23-1999 | Glezer et al. | |
| | A25 * | US 6,033,191 | 03-07-2000 | Kamper et al. | |
| | A26 * | US 6,048,328 | 04-11-2000 | Haller et al. | |
| | A27 * | US 6,048,734 | 04-11-2000 | Burns et al. | |
| | A28 * | US 6,062,681 | 05-16-2000 | Field et al. | |
| | A29 * | US 6,102,530 | 08-15-2000 | Kim et al. | |
| | A30 * | US 6,152,181 | 11-28-2000 | Wapner et al. | |
| | A31 * | US 6,196,525 | 03-06-2001 | Ganan-Calvo | |
| | A32 * | US 6,203,291 | 03-20-2001 | Stemme et al. | |
| | A33 * | US 6,221,654 | 04-24-2001 | Quake et al. | |
| | A34 * | US 6,273,553 | 08-14-2001 | Kim et al. | |
| | A35 * | US 6,360,775 | 03-26-2002 | Barth et al. | |
| | A36 * | US 6,432,630 | 08-13-2002 | Blankenstein | |
| | A37 * | US 6,561,224 | 05-13-2003 | Cho | |
| | A38 * | US 6,592,821 | 07-15-2003 | Wada et al. | |
| | A39 * | US 5,597,438 | 07-22-2003 | Cabuz et al. | |
| | A40 * | US 6,629,820 | 10-07-2003 | Korselsen | |
| | A41 * | US 6,802,342 | 10-12-2004 | Fernandes et al. | |
| | A42 * | US 6,877,528 | 04-12-2005 | Gilbert et al. | |
| | A43 * | US 7,069,943 | 07-04-2006 | Gilbert et al. | |
| | A44 * | US 8,210,209 | 07-03-2012 | Gilbert et al. | |

PTO/SB/08b (01-08)

Approved for use through 07/31/2008. OMB 0651-0031

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| Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i> | | | | Complete if Known | |
| | | | | Application Number | 13/527,331 Confirm # 1757 |
| | | | | Filing Date | June 19, 2012 |
| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 2 | of | 3 | Attorney Docket Number | 118153-01205 |

| | | | | | |
|--|-------|-----------------|------------|----------------|--|
| | A45 * | US 2006-0278288 | 12-14-2006 | Gilbert et al. | |
| | A46 * | US 2012-0015442 | 12-14-2006 | Gilbert et al. | |
| | | | | | |
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| FOREIGN PATENT DOCUMENTS | | | | | | |
|--------------------------|-----------------------|---|--------------------------------|---|---|----------------|
| Examiner Initials* | Cite No. ¹ | Foreign Patent Document | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear | T ⁶ |
| | | Country Code ³ -Number ⁴ -Kind Code ⁵ (if known) | | | | |
| | B1 | WO 91/15750 | 10-17-1991 | Carri Med. Ltd. | | |
| | B2 | WO 98/10267 | 03-12-1998 | Technical University of Denmark | | |
| | B3 | WO 98/07069 | 02-19-1998 | The Regents of the University of Michigan | | |
| | B4 | WO 00/70080 | 11-23-2000 | Caliper Technologies Corp. | | |
| | B5 | EP A 0 177 718 | 04-16-1986 | Partec AG | | Abstract only |
| | B6 | EP A 0 745 682 | 12-04-1996 | Bayer AG | | Abstract only |
| | | | | | | |
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| NON PATENT LITERATURE DOCUMENTS | | | |
|---------------------------------|--------------------------|---|----------------|
| Examiner Initials* | Cite No. ¹ | Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published. | T ² |
| | C1 | International Search Report issued in International Application No. PCT/US2003/12368, dated August 21, 2003 | |
| | C2 | International Preliminary Examination Report issued in International Application No. PCT/US2003/12368, dated August 25, 2004 | |
| | C3 | International Search Report issued in International Application No. PCT/US2003/12360, dated October 3, 2003 | |

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|-----------------------|-------------|--------------------|------------|
| Examiner Signature | /Paul Hyun/ | Date Considered | 06/03/2014 |
|-----------------------|-------------|--------------------|------------|

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. ¹ Applicant's unique citation designation number (optional). ² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English language Translation is attached.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: August 21, 2012

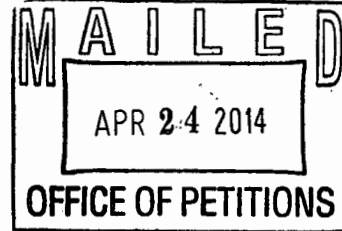
Electronic Signature for David B. Burns: /David B. Burns/



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MCCARTER & ENGLISH, LLP BOSTON
265 FRANKLIN STREET
BOSTON MA 02110



In re Application: :
Gilbert et al. :
Application No. 13/527,331 : **NOTICE**
Filed: June 19, 2012 :
Attorney Docket No. 118153-01205 :

This is a notice regarding your request for acceptance of a fee deficiency submission under 37 CFR 1.28 filed May 16, 2013.

On September 1, 1998, the Court of Appeals for the Federal Circuit held that 37 CFR 1.28(c) is the sole provision governing the time for correction of the erroneous payment of the issue fee as a small entity. See DH Technology v. Synergystex International, Inc. 154 F.3d 1333, 47 USPQ2d 1865 (Fed. Cir. Sept. 1, 1998).

The Office no longer investigates or rejects original or reissue applications under 37 CFR 1.56. **1098 Off. Gaz. Pat. Office 502 (January 3, 1989).** Therefore, nothing in this Notice is intended to imply that an investigation was done.

Your fee deficiency submission under 37 CFR 1.28 is hereby **ACCEPTED**.

This application is no longer entitled to small entity status. Accordingly, all future fees paid in this application must be paid at the large entity rate.

Telephone inquiries concerning this decision should be directed to the undersigned at (571) 272-7751.

/Joan Olszewski/
Joan Olszewski
Office of Petitions

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: February 24, 2014 Signature: /David R. Burns/
(David R. Burns)

Docket No.: 118153-01205
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John R. Gilbert et al.

Application No.: 13/527,331

Confirmation No.: 1757

Filed: June 19, 2012

Art Unit: 1772

For: MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING FLUID
FLOW THROUGH A MICROCHANNEL

Examiner: Hyun, Paul S.

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION
UNDER 37 C.F.R. 1.111

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

INTRODUCTORY COMMENTS

In response to the Office Action dated October 23, 2013, the deadline of which is extended to February 24, 2014 by way of a Request for Extension of Time (one-month) and by virtue of February 23, 2014 falling on a Sunday, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions of the claims and listings of the claims in the application:

1-19. (Canceled)

20. (Currently Amended) A microfluidic device adapted to facilitate analysis or processing of a sample, the microfluidic device comprising:

a microfluidic flow channel formed in a substrate; and
a first reservoir in fluid communication with the flow channel, the first reservoir including resilient properties and adapted for dampening or absorbing a pressure pulse propagated across the flow channel in a crosswise direction.

21. (Currently Amended) The system device of claim 20, further comprising a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.

22. (Canceled)

23. (Currently Amended) The method system of claim 40, [[20,]] wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.

24. (Currently Amended) The system device of claim 20, [[23,]] wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles suspended within the sample into a selected branch of the branched portion.

25. (Currently Amended) The system device of claim 20, wherein the resilient properties of the first reservoir include[[s]] a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.

26. (Currently Amended) The ~~system~~ device of claim 25, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.

27. (Currently Amended) The ~~system~~ device of claim 26, wherein the fluid filled region includes a portion of the first reservoir.

28. (Currently Amended) The ~~system~~ device of claim 26, wherein the fluid filled region includes a side channel connecting the first reservoir to the flow channel.

29. (Currently Amended) The ~~system~~ device of claim 26, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.

30. (Currently Amended) The ~~system~~ device of claim 20, wherein the resilient properties of the first reservoir include[[s]] a resilient wall for facilitating dampening or absorbing of the pressure pulse.

31-38. (Canceled)

39. (Previously Presented) The device of claim 20, wherein the first reservoir is adjacent to an aperture in a sidewall of the flow channel.

40. (Currently Amended) A method comprising:
providing the microfluidic device of claim 20;
flowing a sample through the microfluidic flow channel;
propagating a pressure pulse across the flow channel in the crosswise direction; and
dampening or absorbing the pressure pulse using the first reservoir in fluid communication with the flow channel.

41. (New) The device of claim 20 further comprising an actuator or pressurizer operatively associated with flow channel and adapted for generating the pressure pulse across the flow channel in the crosswise direction.

42. (New) A method for facilitating analysis or processing of a sample, the method comprising:
- flowing a sample through a microfluidic flow channel formed in a substrate;
 - propagating a pressure pulse across the microfluidic flow channel in a crosswise direction; and
 - dampening or absorbing the pressure pulse using a first reservoir in fluid communication with the microfluidic flow channel.
43. (New) The method of claim 42, wherein the pressure pulse is used to selectively control or otherwise affect the flow of the sample through the flow channel.
44. (New) The method of claim 42, wherein the first reservoir includes a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.
45. (New) The method of claim 42, wherein the first reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.
46. (New) The method of claim 42, wherein the pressure pulse is used to sort one or more particles suspended within the sample.
47. (New) A microfluidic device adapted to facilitate analysis or processing of a sample, the microfluidic device comprising:
- a microfluidic flow channel formed in a substrate; and
 - a sealed first reservoir in fluid communication with the flow channel adapted for dampening or absorbing the pressure pulse propagated across the flow channel.
48. (New) The device of claim 47, wherein the first reservoir includes resilient properties which enable the dampening or absorbing the pressure.

49. (New) The device of claim 47, further comprising an actuator or pressurizer operatively associated with flow channel and adapted for generating a pressure pulse across the flow channel in a crosswise direction.

REMARKS

The present response is intended to fully address the objections and rejections noted in the outstanding Office Action mailed October 23, 2013 (the “Office Action”), and is believed to place the subject application in condition for allowance. Thus, favorable reconsideration and prompt allowance of the subject application is respectfully requested. Applicants do not acquiesce to any portion of the Office Action not particularly addressed herein.

Claim Amendments:

As of the mailing of the Office Action, claims 20-40 were pending of which claim 20 was in independent form. By way of the present response, Applicants amend claims 20, 21, 23-30, and 40, introduce new claims 41-49, of which claims 42 and 47 are in independent form, and cancel claims 22 and 31-38, without prejudice. In particular, claims 20 and 40 are amended to recite a pressure pulse propagated “across the flow channel in a crosswise direction.” Moreover, claim 20 is amended to specify that the first reservoir includes resilient properties for dampening or absorbing the pressure pulse propagated across the flow channel. Claims 21 and 23-30 are amended to correct antecedent basis in view of claim 20 reciting a “device” and not a “system”. Claims 23 and 24 are also amended to correct dependencies. In particular, claim 23 is amended to depend from method claim 40 and claim 24 is amended to depend from claim 20.

Finally, new claims 41-49 are introduced. New claim 41 depends from claim 20 and further recites “an actuator or pressurizer operatively associated with flow channel and adapted for generating the pressure pulse across the flow channel in the crosswise direction.” New independent claim 42 recites “[a] method for facilitating analysis or processing of a sample, the method comprising: flowing a sample through a microfluidic flow channel formed in a substrate; propagating a pressure pulse across the microfluidic flow channel in a crosswise direction; and dampening or absorbing the pressure pulse using a first reservoir in fluid communication with the microfluidic flow channel.” New claims 43-46 depend from new claim 42 and include subject matter similar to subject matter recited in claims 23, 25, 30 and 24, respectively. New independent claim 47 is directed towards “[a] microfluidic device adapted to facilitate analysis or

processing of a sample, the microfluidic device comprising a microfluidic flow channel formed in a substrate; and a sealed first reservoir in fluid communication with the flow channel adapted for dampening or absorbing the pressure pulse propagated across the flow channel.” New claims 48 and 49 depend from new claim 47 and include subject matter similar to subject matter recited in claims 20 and 41, respectively.

Applicants respectfully submit that no new matter is introduced by way of the foregoing amendments/additions to the claims. Thus, Applicants respectfully request prompt entry and substantive consideration of the foregoing amendments to the claims.

Information Disclosure Statement:

In the Office Action, the Examiner concluded that the information disclosure statement filed August 21, 2012 fails to comply with 37 CFR 1.98(a)(3) because it did not include a concise explanation of the relevance of a pair of references (EP A 0 745 682 and EP 0 1777 718) which were allegedly not in the English language. Applicants note, however, that each of the references were cited with respect to the “Abstract only,” which is in English. Thus, consideration of the Abstract portion of the references is respectfully requested.

Double Patenting:

Claims 20-23, 25-28, 30-34 and 38 were rejected on the grounds of nonstatutory double patenting over US 6,877,528 and claims 20, 23, 25-28 and 30 were rejected on the grounds of nonstatutory double patenting over US 7,069,943. Applicants respectfully request that the Examiner clarify which claims of US 6,877,528 and of US 7,069,943 are being cited in the double patenting rejection. Applicants defer the filing of any terminal disclaimers until after identification of allowable subject matter in the subject application.

Claim Objections:

Claim 20 was objected to because of an informality of missing the conjunction “and.” Applicants hereby amend claim 20 to correct the informality. Applicants respectfully request the Examiner withdraw this objection.

Section 112 Rejections:

Claim 23 was rejected under 35 USC § 112, 4th paragraph, for allegedly failing to further limit the subject matter of the claim upon which it depends. As noted above, Applicants hereby amend claim 23 to depend from method claim 40. Thus, Applicants respectfully request the Examiner withdraw this rejection.

Claim 37 was rejected under 35 USC § 112, 2nd paragraph, for improper antecedent basis for the term “the meniscus.” As noted above, Applicants hereby amend claim 37 to depend from claim 36, thereby providing proper antecedent basis for the term “the meniscus.” Thus, Applicants respectfully request the Examiner withdraw this rejection.

Anticipation Rejections:

I. Friedman:

In the Office Action, claims 20-24 and 39 were rejected under 35 USC § 102(b) as allegedly anticipated by U.S. Patent No. 3,791,517 to Friedman (“Friedman”). Applicants respectfully traverse.

In particular, with respect to amended independent claim 20, Applicants respectfully submit that Friedman fails to disclose, teach or suggest a first reservoir which includes “resilient properties for dampening or absorbing a pressure pulse,” as recited in the claim (Applicants note that the requirement that the first reservoir include resilient properties is a structural requirement which must be substantively addressed by the Examiner). Indeed, control ports 72 and 74 and associated fluid pathways in Friedman are not disclosed as having resilient properties. Moreover, Friedman discloses using a vacuum and/or a pressurizer only in the context of

generating a pressure wave and not in the context of dampening or absorbing a pressure wave. A vacuum, such as taught in Friedman does not exhibit resilient properties and does not dampen or absorb a pressure wave. For example, in the context of simultaneously applying positive and negative pressure from opposite sides as hypothesized by the Examiner, the pressure wave would be continuously generated from both sides and not dampened or absorbed.

Moreover, Friedman teaches away from any application that would either dampen or absorb a generated pressure wave or allow for throughput of a pressure wave (such as hypothesized by the Examiner). This is because the pressure wave in Friedman is specifically adapted for creating and maintaining turbulence in the flow channel, i.e., so as to make use of the wall effect. See, e.g., col. 3, lines 35-65. This is at direct contrast with the subject application where, e.g. the resilient properties of the first reservoir enable dampening/absorbing of a pressure pulse thereby preventing/mitigating turbulence in the flow channel. Accordingly, not only would it not have been obvious but it would have been counter-intuitive for someone of ordinary skill in the art, based on the teachings in Friedman, to include any dampening/absorbing of the generated pressure wave, for example using a reservoir with resilient properties.

Turning to claims 21-24 and 39, Applicants note that each of the claims depends directly or indirectly from claim 20 and therefore patentably distinguishes over Friedman for at least the same reasons noted with respect thereto.

Thus, in view of the above remarks, Applicants respectfully request reconsideration and withdrawal of the outstanding anticipation rejections to claims 20-24 and 39 over Friedman.

II. Otsuka:

In the Office Action, claims 20-40 were rejected under 35 USC § 102(b) as allegedly anticipated by U.S. Patent No. 5,777,649 to Otsuka *et al.* (“Otsuka”). Applicants respectfully traverse.

In particular, with respect to amended independent claim 20, Applicants respectfully submit that Otsuka fails to disclose, teach or suggest a first reservoir which is structurally

capable of “dampening or absorbing a pressure pulse propagated across the flow channel in a crosswise direction,” as recited in the claim.

Otsuka discloses ink passages 5 communicating with a common liquid chamber 9 (see FIG. 22 and col. 2, lines 10-12). A buffering chamber 13 opens to the common liquid chamber 9 at a position facing each ink passage 5 (nozzle 81) (see FIG. 1 and col. 6, lines 39-42). Indeed, the buffer chamber in Otsuka is located at the far end of the common liquid chamber 9 from the nozzles 81 and is therefore specifically positioned to absorb a “backward pressure wave” (parallel with the ink passage), which is a bi-product of ink vibration for example due to the ink ejection elements. See, e.g., the arrows in FIG. 1 and col. 4, line 65 - col. 5 line 7. Otsuka does not, however, structurally address dampening a pressure pulse generated across a flow channel in a crosswise direction (transverse to the flow). Indeed, none of the elements disclosed in Otsuka are structurally capable of absorbing a pressure pulse propagated across a flow channel. Thus, at least because Otsuka fails to disclose a reservoir structurally capable of absorbing a pressure pulse propagated across a flow channel, Otsuka fails to anticipate claim 20.

Turning to claims 21-40, Applicants note that each of the claims depends directly or indirectly from claim 20 and therefore patentably distinguishes over Otsuka for at least the same reasons noted with respect thereto.

Thus, in view of the above remarks, Applicants respectfully request reconsideration and withdrawal of the outstanding anticipation rejections to claims 20-40 over Otsuka.

Obviousness Rejections:

I. Friedman:

In the Office Action, claim 40 was rejected under 35 USC § 103(a) as allegedly obvious over Friedman. Applicants respectfully traverse.

In the Office Action, the Examiner acknowledged that the method in Friedman differs from the method of claim 40 in that Friedman fails to explicitly disclose dampening or absorbing

a pressure pulse. See Page 8 of the Office Action. In an effort to address this shortcoming the Examiner reasoned that since Friedman discloses that the first reservoir can create a vacuum (col. 9, lines 25-30), it would have been obvious to one of ordinary skill in the art to create a vacuum at the first reservoir 74 while applying positive pressure using the second reservoir 72 to create faster switching responses of fluid 29 flowing through the flow channel 66.” Id.

The Examiner’s reasoning, however, is flawed. In particular, as noted above, Friedman discloses using a vacuum and/or a pressurizer only in the context of generating a pressure wave and not in the context of dampening or absorbing a pressure wave. A vacuum, such as taught in Friedman does not dampen or absorb a pressure wave. For example, in the context of simultaneously applying positive and negative pressure from opposite sides as hypothesized by the Examiner, the pressure wave would be continuously generated from both sides and not dampened or absorbed.

Moreover, as noted above, Friedman teaches away from any application that would either dampen or absorb the generated pressure wave or allow for throughput of a pressure wave (such as hypothesized by the Examiner). This is because the pressure wave in Friedman is specifically adapted for creating and maintaining turbulence in the flow channel, i.e., so as to make use of the wall effect. See, e.g., col. 3, lines 35-65. Therefore, not only would it not have been obvious but it would have been counter-intuitive for someone of ordinary skill in the art, based on the teachings in Friedman, to include any dampening/absorbing of the generated pressure wave.

Thus, in view of the above remarks, Applicants respectfully request reconsideration and withdrawal of the outstanding obviousness rejection to claims 40 over Friedman.

II. Otsuka:

In the Office Action, claims 20-40 were rejected under 35 USC § 103(a) as allegedly obvious over Otsuka. Applicants respectfully traverse.

Turning first to claim 20, as noted above, Otsuka fails to disclose, teach or suggest a first reservoir which is structurally capable of “dampening or absorbing a pressure pulse propagated across the flow channel in a crosswise direction,” as recited in the claim.

Indeed, as noted above, the buffer chamber 13 in Otsuka is located at the far end of the common liquid chamber 9 from the nozzles 81 and is specifically positioned to absorb a “backward pressure wave” (parallel with the ink passage), which is a bi-product of ink vibration for example due to the ink ejection elements. See, e.g., the arrows in FIG. 1 and col. 4, line 65 – col. 5, line 7. Otsuka does not, however, structurally address dampening a pressure pulse generated across a flow channel in a crosswise direction (crosswise to the flow). Indeed, none of the elements disclosed in Otsuka are structurally capable of absorbing a pressure pulse propagated across a flow channel.

Moreover, there is no motivation to modify the system in Otsuka to include any structure related to a pressure pulse across a flow channel. Indeed, there are no teachings or suggestions in Otsuka that it would at all be beneficial to address a pressure pulse propagated across a flow channel. Indeed, the central problem addressed by the buffer chamber in Otsuka is that a “backward pressure wave” (parallel with the ink passage) can result in improper ink ejection. A crosswise pressure pulse (crosswise to the ink passage) would not cause similar problems with respect to a downstream ejection of the ink. Thus, Otsuka provides no motivation for dampening such a pressure pulse.

Turning to claims 21-40, Applicants note that each of the claims depends directly or indirectly from claim 20 and therefore patentably distinguishes over Otsuka for at least the same reasons noted with respect thereto. Moreover, Applicants respectfully submit that the claims recite additional limitations that are not disclosed nor made obvious by Otsuka. For example, Otsuka fails to teach or make obvious each of the following elements/limitations:

“a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse,” as recited in claim 21, (note, it is unclear where in the Abstract or Figure 1 a second reservoir is taught which is adapted for generating the pressure wave; notably, according to the Abstract, the pressure wave appears to be generated by an ejection energy

generating element, e.g., a heat generating resistor 3 of Figure 1, which is not disclosed as being a reservoir or having any association with a reservoir);

“an actuator or pressurizer operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir,” as recited in claim 22, (note, the ejection energy generating element, e.g., heat generating resistor 3 in Otsuka is not associated with a reservoir and, moreover, the pressure wave in Otsuka is generated by heat and not by varying the volume or pressure in a reservoir);

using a pressure pulse “to control or otherwise affect the flow of the sample through the flow channel,” as recited in claim 23, (note, that claim 23 now depends from method claim 40 and therefore the functional limitations should be substantively addressed);

“a branched portion, wherein the pressure pulse is used to sort one or more particles suspended within the sample into a selected branch of the branched portion,” as recited in claim 24, (note that it is unclear where in Figure 1 a branched portion is taught, moreover, Otsuka does not disclose, teach or suggest that the pressure wave due to the vibration of the ink is capable of sorting one or more particles into a selected branch);

“a resilient wall for facilitating dampening or absorbing of the pressure pulse,” as recited in claim 30, (note, a gas pocket is not a “wall”);

any of the elements/limitation of claims 31-37 relating to the “second reservoir” (since as noted above, Otsuka fails to disclose teach or suggest a second reservoir); and

“propagating a pressure pulse across the flow channel,” as recited in claim 40, (as noted above, the pressure wave in Otsuka is not generated across the ink passage).

Thus, in view of the above remarks, Applicants respectfully request reconsideration and withdrawal of the outstanding obviousness rejections to claims 20-40 over Otsuka.

III. *Combination of Friedman and Otsuka:*

While not specifically raised as a ground for rejection, Applicants note that the combination of Friedman and Otsuka would not make obvious amended claim 20. In particular, as noted above, Friedman teaches away from any application that would either dampen or absorb the generated pressure wave or allow for throughput of a pressure wave (such as hypothesized by the Examiner). This is because the pressure wave in Friedman is specifically adapted for creating and maintaining turbulence in the flow channel, i.e., so as to make use of the wall effect. See, e.g., col. 3, lines 35-65. Thus, not only would there have been no motivation for a person of ordinary skill in the art to combine the system in Friedman with the buffering chamber in Otsuka, but Friedman, in fact, teaches away from including any dampening/absorbing of the generated pressure wave.

Moreover, as noted above as noted above, there is no motivation to modify the system in Otsuka to include any structure related to a pressure pulse across a flow channel. Indeed, there are no teachings or suggestions in Otsuka that it would at all be beneficial to address a pressure pulse propagated across a flow channel. Indeed, the central problem addressed by the buffer chamber in Otsuka is that a “backward pressure wave” (parallel with the ink passage) can result in improper ink ejection. A crosswise pressure pulse (crosswise to the ink passage) would not cause similar problems with respect to a downstream ejection of the ink. Thus, Otsuka provides no motivation for dampening such a pressure pulse.

Thus, in view of the above remarks, Applicants respectfully submit that claims 20-40 are also patentable over a combination of Friedman and Otsuka, and it is respectfully requested that the Examiner so find.

New Claims:

As noted above, new claims 41-49 are hereby presented the patentability of which over the cited references of Friedman and Otsuka, alone or in combination, is addressed herein.

I. *New Claim 41:*

New claim 41 depends from claim 20 and further recites “an actuator or pressurizer operatively associated with flow channel and adapted for generating the pressure pulse across the flow channel in the crosswise direction.”

Since new claim 41 depends directly or indirectly from claim 20 Applicants respectfully submit that claim 41 patentably distinguishes over Friedman and Otsuka, alone or in combination, for at least the same reasons noted with respect to claim 20.

Moreover Applicants respectfully submit that claim 41 recites additional limitations that are not disclosed nor made obvious by the combination of Friedman and Otsuka. In particular, Applicants note that Otsuka fails to disclose, teach or suggest an actuator or pressurizer which is structurally capable of generating a pressure pulse across the flow channel in the crosswise direction,” as recited in the claim.

Indeed, Otsuka does not concern or involve actively generating a pressure pulse across a flow channel (crosswise to a flow), e.g., to control or otherwise effect flow of a sample. Rather, the pressure wave disclosed in Otsuka is a “backward pressure wave” (parallel with the ink passage), which is a by-product of ink vibration due to the ink ejection elements. See, e.g., col. 4, line 65 – col. 5, line 7.

Moreover, there is no motivation to modify the system in Otsuka to include an actuator or pressurizer for generating a pressure pulse across a flow channel. Indeed, there are no teachings or suggestions in Otsuka that it would at all be beneficial to generate a pressure pulse across a flow channel.

Furthermore, it would be improper for the Examiner, to rely on the teachings in Friedman in an attempt to address/overcome the above noted deficiencies with respect to Otsuka. In particular, Otsuka does not relate using a pressure wave to divert a flow-path such as taught in Friedman and therefore there would be no motivation to combine the vacuum control ports 72 and 74 from Friedman with the system in Otsuka.

Moreover, Friedman directly teaches away from such a combination or any application that would either dampen or absorb the generated pressure wave. This is because the pressure wave in Friedman is specifically adapted for creating and maintaining turbulence in the flow channel, i.e. so as to make use of the wall effect. See e.g., col. 3, lines 35-65.

Thus, for at least the foregoing reasons, Applicants respectfully submit that new claim 41 is patentable over Friedman and Otsuka, alone or in combination, and it is respectfully requested that the Examiner so find.

II. *New Claims 42-46:*

New independent claim 42, is directed towards a method for facilitating analysis or processing of a sample the method including steps of flowing a sample through a microfluidic flow channel formed in a substrate; propagating a pressure pulse across the microfluidic flow channel in a crosswise direction to control or otherwise affect the flow of the sample through the flow channel; and dampening or absorbing the pressure pulse using a first reservoir in fluid communication with the microfluidic flow channel.

Applicants respectfully submit that new claim 42 is patentable over Friedman at least because, as noted above, Friedman fails to disclose, teach or suggest “dampening or absorbing the pressure pulse.” Rather, Friedman teaches away from any application that would either dampen or absorb the generated pressure wave or allow for throughput of a pressure wave (such as hypothesized by the Examiner). This is because the pressure wave in Friedman is specifically adapted for creating and maintaining turbulence in the flow channel, i.e. so as to make use of the wall effect. See e.g., col. 3, lines 35-65. Therefore, not only would it not have been obvious but it would have been counter-intuitive for someone of ordinary skill in the art, based on the teachings in Friedman, to include any dampening/absorbing of the generated pressure wave.

Applicants further respectfully submit that new claim 42 is patentable over Otsuka at least because, as noted above, Otsuka fails to disclose, teach or suggest propagating a pressure pulse across a flow channel (crosswise to the flow), e.g., to control or otherwise effect the flow of the sample. Rather, the pressure wave disclosed in Otsuka is a “backward pressure wave”

(parallel with the ink passage), which is a bi-product of ink vibration due to the ink ejection elements. See, e.g., col. 4, line 65 – col. 5, line 7. Moreover, there is no motivation to modify the system in Otsuka to include generating a pressure pulse across a flow channel. Indeed, Otsuka does not relate using a pressure wave to divert a flow-path (such, e.g., as taught in Friedman) and therefore there would be no benefit in Otsuka to generating a pressure pulse across a flow channel.

Applicants further respectfully submit that new claim 42 is patentable over the combination of Friedman and Otsuka, at least because Friedman teaches away from including any dampening/absorption, and Otsuka includes no motivation for propagating a pressure pulse across a flow channel.

Applicants note that new claims 43-46 depend directly or indirectly from new claim 42 and therefore patentably distinguishes over Friedman and Otsuka, alone or in combination, for at least the same reasons noted with respect thereto.

Thus, for at least the foregoing reasons, Applicants respectfully submit that new claims 42-46 are patentable over Friedman and Otsuka, alone or in combination, and it is respectfully requested that the Examiner so find.

III. *New Claims 47- 49:*

New independent claim 47, which is directed towards a microfluidic device adapted to facilitate analysis or processing of a sample, recites that the microfluidic device includes a microfluidic flow channel formed in a substrate; and a sealed first reservoir in fluid communication with the flow channel adapted for dampening or absorbing the pressure pulse propagated across the flow channel.

Applicants respectfully submit that new claim 47 is patentable over Friedman at least because, Friedman fails to disclose, teach or suggest a sealed first reservoir for dampening or absorbing the pressure pulse. Indeed, Applicants note that the vents in Friedman would have no capability of absorbing/dampening a pressure pulse (indeed, the Examiner's rejection of

previously pending claim 20 relied on hypothetical embodiments where the vents were open). Moreover, Friedman teaches away from any application that would either dampen or absorb the generated pressure wave or allow for throughput of a pressure wave (such as hypothesized by the Examiner). This is because the pressure wave in Friedman is specifically adapted for creating and maintaining turbulence in the flow channel, i.e. so as to make use of the wall effect. See e.g., col. 3, lines 35-65. Therefore, not only would it not have been obvious but it would have been counter-intuitive for someone of ordinary skill in the art, based on the teachings in Friedman, to include any dampening/absorbing of the generated pressure wave.

Applicants further respectfully submit that new claim 47 is patentable over Otsuka at least because, Otsuka also fails to disclose, teach or suggest a sealed first reservoir for dampening or absorbing a pressure pulse. Rather, the buffer chamber in Otsuka is specifically designed to allow gas transmission and is therefore not sealed. Indeed, this gas transmission aspect of the buffer chamber is the entire thrust of Otsuka's invention (see e.g., col. 6, line 28 – col. 9, line 26, and independent claims 1 and 8).

Applicants further respectfully submit that new claim 47 is patentable over the combination of Friedman and Otsuka, at least because Friedman teaches away from including any dampening/absorption, and Otsuka includes no motivation for including structure for dampening a pressure pulse across a flow channel.

Applicants note that new claims 48 and 49 depend directly or indirectly from claim 47 and therefore patentably distinguish over Friedman and Otsuka, alone or in combination, for at least the same reasons noted with respect thereto.

Thus, for at least the foregoing reasons, Applicants respectfully submit that new claims 47-49 are patentable over Friedman and Otsuka, alone or in combination, and it is respectfully requested that the Examiner so find.

CONCLUSION

In view of the foregoing, Applicants submit that each of the Examiner's concerns have been fully and completely addressed. Consequently, Applicants believe the claims are condition for allowance and solicit both reconsideration of the subject application and its swift passage. If, however, the Office believes that any unresolved issues exist or if, in the opinion of the Office, a telephone conference would expedite passing the present application to issue, the Office is invited to call the undersigned attorney at (617) 449-6500 so that appropriate arrangements can be made for resolving such issues as expeditiously as possible. Applicants authorize the Office to charge Deposit Account No. 50-4876, under Attorney Docket No. 118153-01205 from which the undersigned is authorized to draw, for any fees due pursuant to the present response.

Dated: February 24, 2014

Respectfully submitted,

Electronic signature: /David R. Burns/
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Registration No.: 46,590
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Boston, Massachusetts 02110
(617) 449-6500
(617) 607-9200 (Fax)
Attorney/Agent For Applicants

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| | | | |
|---|--|--|--|
| PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) | | Docket Number (Optional) 118153-01205 | |
| Application Number 13/527,331 | | Filed June 19, 2012 | |
| For MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| Art Unit 1772 | | Examiner P. S. Hyun | |

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.
The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

| | <u>Fee</u> | <u>Small Entity Fee</u> | <u>Micro Entity Fee</u> | |
|---|------------|-------------------------|-------------------------|-----------|
| <input checked="" type="checkbox"/> One month (37 CFR 1.17(a)(1)) | \$200 | \$100 | \$50 | \$ 200.00 |
| <input type="checkbox"/> Two months (37 CFR 1.17(a)(2)) | \$600 | \$300 | \$150 | \$ |
| <input type="checkbox"/> Three months (37 CFR 1.17(a)(3)) | \$1,400 | \$700 | \$350 | \$ |
| <input type="checkbox"/> Four months (37 CFR 1.17(a)(4)) | \$2,200 | \$1,100 | \$550 | \$ |
| <input type="checkbox"/> Five months (37 CFR 1.17(a)(5)) | \$3,000 | \$1,500 | \$750 | \$ |

☐ Applicant asserts small entity status. See 37 CFR 1.27.
☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.
☐ A check in the amount of the fee is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☐ The Director has already been authorized to charge fees in this application to a Deposit Account.
☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-4876.
☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.
☐ assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96).
☒ attorney or agent of record. Registration number 46,590.
☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

| | |
|--|---|
| <u>/David R. Burns/</u> Signature | <u>February 24, 2014</u> Date |
| <u>David R. Burns</u> Typed or printed name | <u>(617) 449-6500</u> Telephone Number |

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

| |
|--|
| <input type="checkbox"/> *Total of <u>1</u> forms are submitted. |
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Electronic Patent Application Fee Transmittal

| | | | | |
|--|---|-----------------|---------------|-----------------------------|
| Application Number: | 13527331 | | | |
| Filing Date: | 19-Jun-2012 | | | |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| First Named Inventor/Applicant Name: | John Richard Gilbert | | | |
| Filer: | David Burns/Brenda Kowalczyk | | | |
| Attorney Docket Number: | 118153-01205 | | | |
| Filed as Large Entity | | | | |
| Utility under 35 USC 111(a) Filing Fees | | | | |
| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
| Basic Filing: | | | | |
| Pages: | | | | |
| Claims: | | | | |
| Independent claims in excess of 3 | 1201 | 1 | 420 | 420 |
| Miscellaneous-Filing: | | | | |
| Petition: | | | | |
| Patent-Appeals-and-Interference: | | | | |
| Post-Allowance-and-Post-Issuance: | | | | |
| Extension-of-Time: | 0111 | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
|-----------------------------------|----------|----------|--------|----------------------|
| Extension - 1 month with \$0 paid | 1251 | 1 | 200 | 200 |
| Miscellaneous: | | | | |
| Total in USD (\$) | | | | 620 |

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 18283310 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John Richard Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns |
| Filer Authorized By: | |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 24-FEB-2014 |
| Filing Date: | 19-JUN-2012 |
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| Deposit Account | 504876 |
| Authorized User | |

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| File Listing: | | | | | |
|--|---|--|---|------------------|------------------|
| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
| 1 | | Response_to_OA.pdf | 80410 e2f2caf519af9e214bf9a4ce8caa783c7a605c6f | yes | 19 |
| | Multipart Description/PDF files in .zip description | | | | |
| | Document Description | | Start | End | |
| | Amendment Copy Claims/Response to Suggested Claims | | 1 | 1 | |
| | Claims | | 2 | 5 | |
| | Applicant Arguments/Remarks Made in an Amendment | | 6 | 19 | |
| Warnings: | | | | | |
| Information: | | | | | |
| 2 | Extension of Time | One_Month_Request_for_Extension_of_Time_Under_37_CFR_1136a.pdf | 21876 6f5acec12783b9be6bcf5ced0099e92ae39f4e6e | no | 1 |
| Warnings: | | | | | |
| Information: | | | | | |
| 3 | Fee Worksheet (SB06) | fee-info.pdf | 32841 9d257f9dbded1d036c237f4081a42315a9dc9fc4 | no | 2 |
| Warnings: | | | | | |
| Information: | | | | | |
| Total Files Size (in bytes): | | | 135127 | | |
| <p>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> | | | | | |

| | | | |
|---|---|----------------------------------|---------------------------------------|
| PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875 | Application or Docket Number 13/527,331 | Filing Date 06/19/2012 | <input type="checkbox"/> To be Mailed |
|---|---|----------------------------------|---------------------------------------|

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

(Column 1)

(Column 2)

| FOR | NUMBER FILED | NUMBER EXTRA | RATE (\$) | FEE (\$) |
|--|---|--------------|-----------|----------|
| <input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | |
| <input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | |
| <input type="checkbox"/> EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X \$ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X \$ = | |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | |

APPLICATION AS AMENDED – PART II

(Column 1)

(Column 2)

(Column 3)

| | 02/24/2014 | CLAIMS REMAINING AFTER AMENDMENT | Minus | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) |
|------------------|--|----------------------------------|-------|------------------------------------|---------------|-----------------|---------------------|
| AMENDMENT | Total (37 CFR 1.16(i)) | * 21 | Minus | ** 21 | = 0 | X \$80 = | 0 |
| | Independent (37 CFR 1.16(h)) | * 4 | Minus | ***4 | = 0 | X \$420 = | 0 |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | | TOTAL ADD'L FEE | 0 |

(Column 1)

(Column 2)

(Column 3)

| | | CLAIMS REMAINING AFTER AMENDMENT | Minus | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) |
|------------------|--|----------------------------------|-------|------------------------------------|---------------|-----------------|---------------------|
| AMENDMENT | Total (37 CFR 1.16(i)) | * | Minus | ** | = | X \$ = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | X \$ = | |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | | TOTAL ADD'L FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/ANGELONA JONES/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 | 1757 |

86738 7590 10/23/2013
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

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| EXAMINER |
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HYUN, PAUL SANG HWA

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| ART UNIT | PAPER NUMBER |
|----------|--------------|

1772

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| MAIL DATE | DELIVERY MODE |
|-----------|---------------|

10/23/2013

PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

| | | | |
|------------------------------|--------------------------------------|---------------------------------------|--|
| Office Action Summary | Application No. 13/527,331 | Applicant(s) GILBERT ET AL. | |
| | Examiner PAUL HYUN | Art Unit 1772 | AIA (First Inventor to File) Status No |

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 12 August 2013.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 5) ☒ Claim(s) 20-40 is/are pending in the application.
5a) Of the above claim(s) ____ is/are withdrawn from consideration.
- 6) ☐ Claim(s) ____ is/are allowed.
- 7) ☒ Claim(s) 20-40 is/are rejected.
- 8) ☐ Claim(s) ____ is/are objected to.
- 9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☒ The drawing(s) filed on 19 June 2012 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some * c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 3) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s)/Mail Date. ____. |
| 2) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date <u>08/21/12</u> . | 4) <input type="checkbox"/> Other: ____. |

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

The response filed on August 12, 2013 cancelling all claims and presenting new claims 20-40 is acknowledged.

Information Disclosure Statement

The information disclosure statement filed August 21, 2012 fails to comply with 37 CFR 1.98(a)(3) because it does not include a concise explanation of the relevance, as it is presently understood by the individual designated in 37 CFR 1.56(c) most knowledgeable about the content of the information, of each patent listed that is not in the English language. It has been placed in the application file, but the information referred to therein has not been considered.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d

1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit <http://www.uspto.gov/forms/>. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to <http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp>.

Claims **20-23, 25-28, 30-34 and 38** are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 22, 22, 22, 22, 26, 26, 26, 26, 24, 25, 25, 25, 25 and 25 of U.S. Patent No. 6,877,528 B2, respectively. Although the claims at issue are not identical, they are not patentably distinct from each other because claims 22, 22, 22, 22, 26, 26, 26, 26, 24, 25, 25, 25, 25 and 25 of US

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6,877,528 B2 anticipate claims 20-23, 25-28, 30-34 and 38 of the instant application, respectively.

While the claims of US 6,877,528 B2 do not explicitly recite a substrate, it is evident that the device recited in the claims comprises a substrate.

Claims **20, 23, 25-28 and 30** are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 5, 5, 5, 5, 5, 5 and 11 of U.S. Patent No. 7,069,943 B2, respectively. Although the claims at issue are not identical, they are not patentably distinct from each other because claims 5, 5, 5, 5, 5, 5 and 11 of US 7,069,943 B2 anticipate claims 20, 23, 25-28 and 30 of the instant application, respectively.

While the claims of US 7,069,943 B2 do not explicitly recite a substrate, it is evident that the device recited in the claims comprises a substrate.

Claim Objections

Claim 20 is objected to because of the following informalities:

The word “and” should be inserted after the semicolon in line 4 of the claim.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112 (pre-AIA), fourth paragraph:

Subject to the [fifth paragraph of 35 U.S.C. 112 (pre-AIA)], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claim **23** is rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject

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matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends.

Claim 23 merely recites a method of using the device of claim 20 without further limiting a structure of said device. Applicant may cancel the claim(s), amend the claim(s) to place the claim(s) in proper dependent form, rewrite the claim(s) in independent form, or present a sufficient showing that the dependent claim(s) complies with the statutory requirements.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(b):

(B) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim **37** is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 37 recites the limitation "the meniscus". There is insufficient antecedent basis for this limitation in the claim. It appears that Applicant intended the claim to be dependent on claim 36. The claim will be examined accordingly.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims **20-24 and 39** are rejected under 35 U.S.C. 102(b) as being anticipated by Friedman (US 3,791,517).

With respect to claim 20, Friedman discloses a microfluidic (see lines 47-52, col. 2) device, said device comprising:

a microfluidic flow channel 66;

a first reservoir 74 in fluid communication with the flow channel 66; and

a second reservoir 72 opposite the first reservoir 74;

wherein said first reservoir 74 is capable of dampening or absorbing a pressure pulse propagated across the flow channel (see Fig. 2 and lines 15-30, col. 9 which discloses that reservoir 74 can exhaust positive pressure using vacuum).

With respect to claim 21, the device further comprises a second reservoir 72 for generating the pressure pulse, as discussed above.

With respect to claim 22, the second reservoir 72 comprises a pump 80 that generates the pressure pulse (see Fig. 2).

With respect to claim 23, the claim does not further limit claim 20, as discussed above.

With respect to claim 24, flow channel 66 branches into different channels 64 and 70 (see Fig. 2), each branch capable of receiving particles suspended in a medium.

With respect to claim 39, the first reservoir 74 is adjacent to an aperture in a sidewall of channel 66 (see Fig. 2).

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to

consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claim **40** is rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Friedman.

Friedman further discloses a method comprising the steps of:
providing the device disclosed above;
flowing a sample through the flow channel 66 (see Fig. 2); and
propagating a pressure pulse across the flow channel 66 using the second reservoir 72 (see lines 15-30, col. 9).

The method disclosed by Friedman differs from the claimed method in that Friedman does not explicitly disclose that the first reservoir 74 dampens or absorbs the pressure pulse generated by reservoir 72.

However, Friedman discloses that the first reservoir can create a vacuum (see lines 25-30, col. 9). In light of the disclosure, it would have been obvious to one of ordinary skill in the art to create a vacuum at the first reservoir 74 while applying positive pressure using the second reservoir 72 to create faster switching responses of fluid 29 flowing through the flow channel 66.

Claims **20-40** are rejected under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Otsuka et al. (US 5,777,649).

Otsuka et al. disclose an ink jet printing head (see Fig. 1), said head comprising:

a channel 9; and

a first reservoir 13 in fluid communication with the flow channel 9, said first reservoir 13 capable of dampening or absorbing a pressure pulse propagated across the flow channel (see Fig. 1).

While the reference does not disclose that channel 9 is microfluidic, given that channel 9 is part of an ink jet printing head, it appears that it is microfluidic. In the alternative, it would have been obvious to one of ordinary skill in the art to render channel 9 microfluidic given the nature of its intended use (i.e. dispense micro-volumes of ink).

With respect to claim 21, the device further comprises a second reservoir 6 for generating the pressure pulse (see Fig. 1 and Abstract).

With respect to claim 22, the second reservoir 6 comprises a pressurizer in the form of a resistor 3 that generates the pressure pulse (see Fig. 1).

With respect to claim 23, the claim does not further limit claim 20, as discussed above.

With respect to claim 24, flow channel 9 comprises a branched portion 8, wherein the pressure pulse is capable of diverting flow of fluid into the branched portion 8 (see Fig. 1).

With respect to claim 25, the first reservoir 13 comprises a gas pocket (see lines 35-40, col. 6).

With respect to claims 26-29, the first reservoir 13 further comprises a fluid-filled region (i.e. side channel of reduced dimensions) connecting channel 9 with the interior

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of reservoir 13 (see Fig. 2). The fluid-filled region prevents the gas in the first reservoir 13 from leaking into the flow channel 9 (see Fig. 1).

With respect to claim 30, the first reservoir 13 comprises a gas that forms a resilient wall (see lines 35-40, col. 6)

With respect to claim 31, the second reservoir 6 comprises a gas pocket (see lines 17-20, col. 6).

With respect to claims 32-35, the second reservoir 6 further comprises a fluid-filled region 81 (side channel) connecting channel 9 with the interior of the second reservoir 6 (see Fig. 1). The fluid-filled region 81 prevents the gas in the second reservoir 6 from leaking into the flow channel 9 (see Fig. 1).

With respect to claim 36, the fluid-filled region 81 forms a meniscus with the gas pocket 6 (see Fig. 1).

With respect to claim 37, a change in volume or pressure in the second reservoir 6 would deflect the meniscus into the flow channel 9, assuming that the change in volume or pressure is large enough (see Fig. 1).

With respect to claim 38, the first reservoir 13 is not a through-channel (see Fig. 1).

With respect to claim 39, the first reservoir 13 is adjacent to an aperture in a sidewall of channel 9 (see Fig. 2).

With respect to claim 40, Otsuka et al. disclose a method, the method comprising the steps of:

providing the device disclosed above;

flowing a sample through flow channel 9 (via passage 8, see Fig. 1);
propagating a pressure pulse across the flow channel 9 using the second
reservoir 6 (see Abstract); and
using the first reservoir 13 to dampen the pressure pulse generated by the
second reservoir 6 (see lines 35-43, col. 6).

Any inquiry concerning this communication or earlier communications from the
examiner should be directed to PAUL HYUN whose telephone number is (571)272-
8559. The examiner can normally be reached on Monday-Friday 10AM-6:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's
supervisor, In Suk Bullock can be reached on (571)-272-5954. The fax phone number
for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the
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Application/Control Number: 13/527,331
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/Paul S Hyun/
Examiner, Art Unit 1772

PTO/SB/08b (01-08)

Approved for use through 07/31/2008. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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| Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i> | | | | Complete if Known | |
| | | | | Application Number | 13/527,331 Confirm # 1757 |
| | | | | Filing Date | June 19, 2012 |
| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 1 | of | 3 | Attorney Docket Number | 118153-01205 |

| U.S. PATENT DOCUMENTS | | | | | |
|-----------------------|--------------------------|---|--------------------------------|--|---|
| Examiner Initials* | Cite No. ¹ | Document Number | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear |
| | | Number-Kind Code ² (if known) | | | |
| | A1 * | US 3,289,687 | 12-06-1966 | Dunaway | |
| | A2 * | US 3,370,538 | 02-27-1968 | Iliacs | |
| | A3 * | US 3,508,654 | 02-01-1970 | Glaetli | |
| | A4 * | US 3,560,754 | 02-02-1971 | Kamentsky | |
| | A5 * | US 3,791,517 | 02-12-1974 | Friedman | |
| | A6 * | US 3,827,555 | 08-06-1974 | Kamnetsky | |
| | A7 * | US 3,984,307 | 10-05-1976 | Kamnetsky | |
| | A8 * | US 4,153,855 | 05-08-1979 | Feingold | |
| | A9 * | US 4,175,662 | 11-27-1979 | Zold | |
| | A10 * | US 4,426,451 | 01-17-1984 | Columbus | |
| | A11 * | US 4,581,624 | 04-08-1986 | O'Connor | |
| | A12 * | US 4,636,149 | 01-13-1987 | Brown | |
| | A13 * | US 4,676,274 | 06-30-1987 | Brown | |
| | A14 * | US 4,756,427 | 07-12-1988 | Gohde et al. | |
| | A15 * | US 4,908,112 | 03-13-1990 | Pace | |
| | A16 * | US 5,065,978 | 11-19-1991 | Albarda et al. | |
| | A17 * | US 5,092,972 | 03-03-1992 | Ghowzi | |
| | A18 * | US 5,101,978 | 04-07-1992 | Marcus | |
| | A19 * | US 5,265,327 | 11-30-1993 | Faris et al. | |
| | A20 * | US 5,777,649 | 07-07-1993 | Otsuka et al. | |
| | A21 * | US 5,789,045 | 08-04-1998 | Wapner et al. | |
| | A22 * | US 5,837,200 | 11-17-1998 | Diessel et al. | |
| | A23 * | US 5,876,187 | 03-02-1999 | Afromowitz et al. | |
| | A24 * | US 5,988,522 | 11-23-1999 | Glezer et al. | |
| | A25 * | US 6,033,191 | 03-07-2000 | Kamper et al. | |
| | A26 * | US 6,048,328 | 04-11-2000 | Haller et al. | |
| | A27 * | US 6,048,734 | 04-11-2000 | Burns et al. | |
| | A28 * | US 6,062,681 | 05-16-2000 | Field et al. | |
| | A29 * | US 6,102,530 | 08-15-2000 | Kim et al. | |
| | A30 * | US 6,152,181 | 11-28-2000 | Wapner et al. | |
| | A31 * | US 6,196,525 | 03-06-2001 | Ganan-Calvo | |
| | A32 * | US 6,203,291 | 03-20-2001 | Stemme et al. | |
| | A33 * | US 6,221,654 | 04-24-2001 | Quake et al. | |
| | A34 * | US 6,273,553 | 08-14-2001 | Kim et al. | |
| | A35 * | US 6,360,775 | 03-26-2002 | Barth et al. | |
| | A36 * | US 6,432,630 | 08-13-2002 | Blankenstein | |
| | A37 * | US 6,561,224 | 05-13-2003 | Cho | |
| | A38 * | US 6,592,821 | 07-15-2003 | Wada et al. | |
| | A39 * | US 5,597,438 | 07-22-2003 | Cabuz et al. | |
| | A40 * | US 6,629,820 | 10-07-2003 | Korselsen | |
| | A41 * | US 6,802,342 | 10-12-2004 | Fernandes et al. | |
| | A42 * | US 6,877,528 | 04-12-2005 | Gilbert et al. | |
| | A43 * | US 7,069,943 | 07-04-2006 | Gilbert et al. | |
| | A44 * | US 8,210,209 | 07-03-2012 | Gilbert et al. | |

PTO/SB/08b (01-08)

Approved for use through 07/31/2008. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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| Substitute for form 1449/PTO | | | | Complete if Known | |
| INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary) | | | | Application Number | 13/527,331 Confirm # 1757 |
| | | | | Filing Date | June 19, 2012 |
| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 2 | of | 3 | Attorney Docket Number | 118153-01205 |

| | | | | | |
|--|-------|-----------------|------------|----------------|--|
| | A45 * | US 2006-0278288 | 12-14-2006 | Gilbert et al. | |
| | A46 * | US 2012-0015442 | 12-14-2006 | Gilbert et al. | |
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| FOREIGN PATENT DOCUMENTS | | | | | | |
|--------------------------|-----------------------|---|--------------------------------|---|---|----------------|
| Examiner Initials* | Cite No. ¹ | Foreign Patent Document | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear | T ⁶ |
| | | Country Code ³ -Number ⁴ -Kind Code ⁵ (if known) | | | | |
| | B1 | WO 91/15750 | 10-17-1991 | Carri Med. Ltd. | | |
| | B2 | WO 98/10267 | 03-12-1998 | Technical University of Denmark | | |
| | B3 | WO 98/07069 | 02-19-1998 | The Regents of the University of Michigan | | |
| | B4 | WO 00/70080 | 11-23-2000 | Caliper Technologies Corp. | | |
| | B5 | EP A 0 177 718 | 04-16-1986 | Partec AG | | Abstract only |
| | B6 | EP A 0 745 682 | 12-04-1996 | Bayer AG | | Abstract only |
| | | | | | | |
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| NON PATENT LITERATURE DOCUMENTS | | | |
|---------------------------------|-----------------------|---|----------------|
| Examiner Initials* | Cite No. ¹ | Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published. | T ² |
| | C1 | International Search Report issued in International Application No. PCT/US2003/12368, dated August 21, 2003 | |
| | C2 | International Preliminary Examination Report issued in International Application No. PCT/US2003/12368, dated August 25, 2004 | |
| | C3 | International Search Report issued in International Application No. PCT/US2003/12360, dated October 3, 2003 | |

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[illegible]

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|-----------------------|-------------|--------------------|------------|
| Examiner Signature | /Paul Hyun/ | Date Considered | 10/21/2013 |
|-----------------------|-------------|--------------------|------------|

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. ¹ Applicant's unique citation designation number (optional). ² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English language Translation is attached.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: August 21, 2012

Electronic Signature for David B. Burns: /David B. Burns/




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BIB DATA SHEET


CONFIRMATION NO. 1757

| SERIAL NUMBER | FILING or 371(c) DATE | CLASS | GROUP ART UNIT | ATTORNEY DOCKET NO. | |
|---|---|-------------------------------|---|---------------------------|--------------------------------|
| 13/527,331 | 06/19/2012 | 422 | 1772 | 118153-01205 | |
| RULE | | | | | |
| APPLICANTS John Richard Gilbert, Brookline, MA; Sebastian Böhm, Iwerness, UNITED KINGDOM; Manish Deshpande, Canton, MA; | | | | | |
| ** CONTINUING DATA ***** This application is a CON of 11/433,781 05/12/2006 PAT 8210209 which is a CON of 11/021,251 12/21/2004 PAT 7069943 which is a CON of 10/179,586 06/24/2002 PAT 6877528 which claims benefit of 60/373,256 04/17/2002 | | | | | |
| ** FOREIGN APPLICATIONS ***** | | | | | |
| ** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 07/03/2012 | | | | | |
| Foreign Priority claimed <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No 35 USC 119(a-d) conditions met <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No Verified and /CRAIG M SCHNEIDER/ Acknowledged Examiner's Signature | <input type="checkbox"/> Met after Allowance Initials | STATE OR COUNTRY MA | SHEETS DRAWINGS 8 | TOTAL CLAIMS 19 | INDEPENDENT CLAIMS 5 |
| ADDRESS MCCARTER & ENGLISH, LLP BOSTON 265 Franklin Street Boston, MA 02110 UNITED STATES | | | | | |
| TITLE MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | | | |
| FILING FEE RECEIVED 860 | FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following: | | <input type="checkbox"/> All Fees <input type="checkbox"/> 1.16 Fees (Filing) <input type="checkbox"/> 1.17 Fees (Processing Ext. of time) <input type="checkbox"/> 1.18 Fees (Issue) <input type="checkbox"/> Other _____ <input type="checkbox"/> Credit | | |

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| <i>Index of Claims</i>  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

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|---|-----------------|---|-------------------|---|---------------------|---|-----------------|
| ✓ | Rejected | - | Cancelled | N | Non-Elected | A | Appeal |
| = | Allowed | ÷ | Restricted | I | Interference | O | Objected |

| <input type="checkbox"/> Claims renumbered in the same order as presented by applicant | | | <input type="checkbox"/> CPA | | | <input type="checkbox"/> T.D. | | | <input type="checkbox"/> R.1.47 | | |
|--|----------|------------|------------------------------|--|--|-------------------------------|--|--|---------------------------------|--|--|
| CLAIM | | DATE | | | | | | | | | |
| Final | Original | 10/21/2013 | | | | | | | | | |
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| | 28 | ✓ | | | | | | | | | |
| | 29 | ✓ | | | | | | | | | |
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| <p><i>Index of Claims</i></p>  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

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|---|-----------------|---|-------------------|---|---------------------|---|-----------------|
| ✓ | Rejected | - | Cancelled | N | Non-Elected | A | Appeal |
| = | Allowed | ÷ | Restricted | I | Interference | O | Objected |

| <input type="checkbox"/> Claims renumbered in the same order as presented by applicant | | <input type="checkbox"/> CPA | | <input type="checkbox"/> T.D. | | <input type="checkbox"/> R.1.47 | | | |
|--|----------|------------------------------|--|-------------------------------|--|---------------------------------|--|--|--|
| CLAIM | | DATE | | | | | | | |
| Final | Original | 10/21/2013 | | | | | | | |
| | 37 | ✓ | | | | | | | |
| | 38 | ✓ | | | | | | | |
| | 39 | ✓ | | | | | | | |
| | 40 | ✓ | | | | | | | |


EAST Search History

EAST Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|-------|------|--|-----------------|------------------|---------|------------------|
| S52 | 2 | channel.clm. same (absorb\$4 dampen\$4) same reservoir same pulse | US-PGPUB; USPAT | OR | ON | 2013/10/21 07:41 |
| S53 | 30 | channel.clm. same (absorb\$4 dampen\$4) same reservoir same (pressure pulse) | US-PGPUB; USPAT | OR | ON | 2013/10/21 07:44 |
| S54 | 2 | (gilbert bohm deshpande).in. and channel.clm. same (absorb\$4 dampen\$4) same reservoir same (pressure pulse) | US-PGPUB; USPAT | OR | ON | 2013/10/21 07:44 |
| S56 | 1159 | unger.in. | EPO | OR | ON | 2013/10/21 09:13 |
| S57 | 1 | (gilbert bohm deshpande).in. and method.clm. same channel same (absorb\$4 dampen\$4) same reservoir same (pressure pulse) | US-PGPUB; USPAT | OR | ON | 2013/10/21 09:49 |
| S58 | 3952 | unger.in. elastomeric adj valve | US-PGPUB; USPAT | OR | ON | 2013/10/21 09:51 |
| S59 | 3812 | unger.in. elastomeric adj valve and pressure | US-PGPUB; USPAT | OR | ON | 2013/10/21 09:51 |
| S60 | 2638 | unger.in. elastomeric adj valve and pressure and channel and substrate | US-PGPUB; USPAT | OR | ON | 2013/10/21 09:51 |
| S61 | 131 | unger.in. and elastomeric adj valve and pressure and channel and substrate | US-PGPUB; USPAT | OR | ON | 2013/10/21 09:51 |
| S62 | 1 | (gilbert bohm deshpande).in. and channel.clm. same (absorb\$4 dampen\$4) same reservoir same (pressure pulse) same communication | US-PGPUB; USPAT | OR | ON | 2013/10/21 10:02 |
| S63 | 38 | (gilbert bohm deshpande).in. and channel and (absorb\$4 dampen\$4) same reservoir same (pressure pulse) | US-PGPUB; USPAT | OR | ON | 2013/10/21 10:05 |
| S64 | 1 | ("4581624").PN. | USPAT; USOCR | OR | OFF | 2013/10/21 10:06 |
| S65 | 46 | ("20060278288" "20120015442" "3289687" "3370538" "3508654" "3560754" "3791517" "3827555" "3984307" "4153855" "4175662" "4426451" "4581624" "4636149" "4676274" "4756427" "4908112" "5065978" "5092972" "5101978" "5265327" "5777649" "5789045" "5837200" "5876187" "5988522" "6033191" "6048328" "6048734" "6062681" "6102530" "6152181" "6196525" "6203291" "6221654" "6273553" "6360775" "6432630" "6561224" "6592821" "6597438" "6629820" | US-PGPUB; USPAT | OR | ON | 2013/10/21 10:54 |

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|-----|----|---|-----------------|----|----|------------------|
| | | "6802342" "6877528" "7069943" "8210209").PN. | | | | |
| S66 | 39 | S65 and pressure | US-PGPUB; USPAT | OR | ON | 2013/10/21 11:04 |
| S67 | 35 | S65 and pressure and volume | US-PGPUB; USPAT | OR | ON | 2013/10/21 11:05 |
| S68 | 23 | S65 and pressure and volume and (elastomer\$4 flexible resilient membrane elastic) | US-PGPUB; USPAT | OR | ON | 2013/10/21 11:05 |
| S69 | 16 | S65 and pressure and volume and bubble | US-PGPUB; USPAT | OR | ON | 2013/10/21 11:08 |
| S70 | 14 | S65 and volume and bubble with pressure | US-PGPUB; USPAT | OR | ON | 2013/10/21 12:02 |
| S71 | 6 | S65 and volume with bubble with pressure | US-PGPUB; USPAT | OR | ON | 2013/10/21 12:02 |
| S72 | 5 | S65 and bubble with pressure with (dampen\$4 absorb\$4) and channel | US-PGPUB; USPAT | OR | ON | 2013/10/21 12:03 |
| S73 | 32 | (gilbert bohm deshpane).in. and meniscus.clm. | US-PGPUB; USPAT | OR | ON | 2013/10/21 13:06 |
| S74 | 7 | (gilbert bohm deshpane).in. and meniscus.clm. same gas | US-PGPUB; USPAT | OR | ON | 2013/10/21 13:07 |
| S75 | 0 | (gilbert bohm deshpane).in. and meniscus.clm. same gas and "communication.clm" | US-PGPUB; USPAT | OR | ON | 2013/10/21 13:08 |
| S76 | 6 | (gilbert bohm deshpane).in. and meniscus.clm. same gas and communication.clm. | US-PGPUB; USPAT | OR | ON | 2013/10/21 13:08 |
| S77 | 4 | (gilbert bohm deshpane).in. and meniscus.clm. same gas and communication.clm. with reservoir with channel | US-PGPUB; USPAT | OR | ON | 2013/10/21 13:08 |
| S78 | 12 | S65 and meniscus | US-PGPUB; USPAT | OR | ON | 2013/10/21 13:39 |

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| Search Notes  | Application/Control No. 13527331 | Applicant(s)/Patent Under Reexamination GILBERT ET AL. |
| | Examiner PAUL HYUN | Art Unit 1772 |

| CPC- SEARCHED | | |
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| Symbol | Date | Examiner |
| | | |

| CPC COMBINATION SETS - SEARCHED | | |
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| Symbol | Date | Examiner |
| | | |

| US CLASSIFICATION SEARCHED | | | |
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| Class | Subclass | Date | Examiner |
| | | | |

| SEARCH NOTES | | |
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| Search Notes | Date | Examiner |
| inventor name search | 10/21/2013 | PSH |
| IDS | 10/21/2013 | PSH |
| imported prior art of parent application | 10/21/2013 | PSH |
| text search | 10/21/2013 | PSH |
| forward and backward searched relevant references | 10/21/2013 | PSH |

| INTERFERENCE SEARCH | | | |
|-------------------------|-------------------------|------|----------|
| US Class/ CPC Symbol | US Subclass / CPC Group | Date | Examiner |
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| /PAUL HYUN/ Examiner.Art Unit 1772 | |
|---------------------------------------|--|

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: August 12, 2013

Signature: /David R. Burns/
(David R. Burns)

Docket No.: 118153-01205
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John R. Gilbert et al.

Application No.: 13/527,331

Confirmation No.: 1757

Filed: June 19, 2012

Art Unit: 3753

For: MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING FLUID
FLOW THROUGH A MICROCHANNEL

Examiner: C. M. Schneider

AMENDMENTS AND RESPONSE TO RESTRICTION REQUIREMENT

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

This communication is responsive to the Restriction Requirement set forth in the Office Action, mailed June 11, 2013 (the "Restriction Requirement"), the response due date of which is extended to August 12, 2013 by way of the accompanying Petition for Extension of Time (one month) and by way of August 11, 2013 falling on a Sunday.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

- 1-19. (Canceled)
20. (New) A microfluidic device adapted to facilitate analysis or processing of a sample, the microfluidic device comprising:

a microfluidic flow channel formed in a substrate;

a first reservoir in fluid communication with the flow channel and adapted for dampening or absorbing an induced pressure pulse propagated across the flow channel.
21. (New) The device of claim 20, further comprising a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.
22. (New) The device of claim 21 further comprising an actuator or pressurizer operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir.
23. (New) The device of claim 20, wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.
24. (New) The device of claim 23, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles suspended within the sample into a selected branch of the branched portion.
25. (New) The device of claim 20, wherein the first reservoir includes a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.
26. (New) The device of claim 25, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.
27. (New) The device of claim 26, wherein the fluid filled region includes a portion of the first reservoir.

28. (New) The device of claim 26, wherein the fluid filled region includes a side channel connecting the first reservoir to the flow channel.
29. (New) The device of claim 26, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
30. (New) The device of claim 20, wherein the first reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.
31. (New) The device of claim 21, wherein the second reservoir includes a gas pocket, wherein compression of the gas facilitates the generating of the pressure pulse.
32. (New) The device of claim 31 further comprising a fluid filled region interfacing with the flow channel and the gas pocket.
33. (New) The device of claim 32, wherein the fluid filled region includes a portion of the second reservoir.
34. (New) The device of claim 32, wherein the fluid filled region includes a side channel connecting the second reservoir to the flow channel.
35. (New) The device of claim 32, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
36. (New) The device of claim 32, wherein the fluid filled region forms a meniscus with the gas pocket.
37. (New) The device of claim 32, wherein the change in volume or pressure in the second reservoir deflects the meniscus into the flow channel.
38. (New) The device of claim 20, wherein the first reservoir is not a through-channel.
39. (New) The device of claim 20, wherein the first reservoir is adjacent to an aperture in a sidewall of the flow channel.
40. (New) A method comprising:
providing the microfluidic device of claim 20;

flowing a sample through the microfluidic flow channel;
propagating an induced pressure pulse across the flow channel; and
dampening or absorbing the pressure pulse using the first reservoir in fluid communication with the flow channel.

REMARKS

Responsive to the Restriction Requirement, applicants hereby elect the invention of Group I drawn to a microfluidic device, classified in class 137, subclass 827.

Further responsive to the Restriction Requirement, Applicants hereby cancel, without prejudice, claims 1-19 and add new claims 20-40. No new matter is introduced by way of the foregoing amendments. Applicants contend that new device claims 20-39 fall within the scope of Group I and therefore respectfully request substantive consideration thereof. Applicants further note that new method claim 40 incorporates the device of claim 20 and thus that there is no serious search or examination burden should new method claim 40 be examined herein or in the alternative that new method claim 40 be considered for rejoinder under MPEP 821.04(b) based on the allowability of related device claims 20-39.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-4876, under Order No. 118153-01205.

Dated: August 12, 2013

Respectfully submitted,

By /David R. Burns/
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Boston, Massachusetts 02110
(617) 449-6500
(617) 607-9200 (Fax)
Attorney/Agent For Applicant

Electronic Patent Application Fee Transmittal

| | | | | |
|--|---|-----------------|---------------|-----------------------------|
| Application Number: | 13527331 | | | |
| Filing Date: | 19-Jun-2012 | | | |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| First Named Inventor/Applicant Name: | John Richard Gilbert | | | |
| Filer: | David Burns/Margarita Reben | | | |
| Attorney Docket Number: | 118153-01205 | | | |
| Filed as Large Entity | | | | |
| Utility under 35 USC 111(a) Filing Fees | | | | |
| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
| Basic Filing: | | | | |
| Pages: | | | | |
| Claims: | | | | |
| Claims in Excess of 20 | 1202 | 1 | 80 | 80 |
| Miscellaneous-Filing: | | | | |
| Petition: | | | | |
| Patent-Appeals-and-Interference: | | | | |
| Post-Allowance-and-Post-Issuance: | | | | |
| Extension-of-Time: | 0143 | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
|-----------------------------------|----------|----------|--------|----------------------|
| Extension - 1 month with \$0 paid | 1251 | 1 | 200 | 200 |
| Miscellaneous: | | | | |
| Total in USD (\$) | | | | 280 |

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 16563074 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John Richard Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns/Gabriel Goldman |
| Filer Authorized By: | David Burns |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 12-AUG-2013 |
| Filing Date: | 19-JUN-2012 |
| Time Stamp: | 15:52:12 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|--|-----------------|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | \$ 280 |
| RAM confirmation Number | 2665 |
| Deposit Account | 504876 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

0145

| File Listing: | | | | | |
|---|---|----------------------------|---|------------------|------------------|
| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
| 1 | Extension of Time | 118153_01205_EOT.PDF | 13315 4f9668bb73764114c38fa55dc55ec1ce7a08f1b0 | no | 1 |
| Warnings: | | | | | |
| Information: | | | | | |
| 2 | Amendment/Req. Reconsideration-After Non-Final Reject | 118153_01205_Amendment.PDF | 18862 f9e478263c002da9a78ebc79d89a0e4340eea5e4 | no | 5 |
| Warnings: | | | | | |
| Information: | | | | | |
| 3 | Fee Worksheet (SB06) | fee-info.pdf | 32608 341fc0cdef9154568d485fb41d00a98ebb5ca5fc | no | 2 |
| Warnings: | | | | | |
| Information: | | | | | |
| Total Files Size (in bytes): | | | 64785 | | |
| <p>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> | | | | | |

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

| | | | |
|--|--|--|--|
| PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) | | Docket Number (Optional) 118153-01205 | |
| Application Number 13/527,331 | | Filed June 19, 2012 | |
| For MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| Art Unit 3753 | | Examiner J. C. Rodriguez | |

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application. The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

| | <u>Fee</u> | <u>Small Entity Fee</u> | <u>Micro Entity Fee</u> | |
|---|------------|-------------------------|-------------------------|-----------|
| <input checked="" type="checkbox"/> One month (37 CFR 1.17(a)(1)) | \$200 | \$100 | \$50 | \$ 200.00 |
| <input type="checkbox"/> Two months (37 CFR 1.17(a)(2)) | \$600 | \$300 | \$150 | \$ _____ |
| <input type="checkbox"/> Three months (37 CFR 1.17(a)(3)) | \$1,400 | \$700 | \$350 | \$ _____ |
| <input type="checkbox"/> Four months (37 CFR 1.17(a)(4)) | \$2,200 | \$1,100 | \$550 | \$ _____ |
| <input type="checkbox"/> Five months (37 CFR 1.17(a)(5)) | \$3,000 | \$1,500 | \$750 | \$ _____ |

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☒ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-4876.

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96).

☒ attorney or agent of record. Registration number 46,590.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/David R. Burns/
Signature

David R. Burns
Typed or printed name

August 12, 2013
Date

(617) 449-6500
Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of 1 forms are submitted.

| | | | |
|---|---|----------------------------------|---------------------------------------|
| PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875 | Application or Docket Number 13/527,331 | Filing Date 06/19/2012 | <input type="checkbox"/> To be Mailed |
|---|---|----------------------------------|---------------------------------------|

 ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

| FOR | NUMBER FILED | NUMBER EXTRA | RATE (\$) | FEE (\$) |
|--|---|--------------|-----------|----------|
| <input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | |
| <input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | |
| <input type="checkbox"/> EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X \$ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X \$ = | |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | |

APPLICATION AS AMENDED – PART II

| AMENDMENT | (Column 1) | (Column 2) | (Column 3) | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) |
|-----------------|--|----------------------------------|------------|------------------------------------|---------------|-----------|---------------------|
| | 08/12/2013 | CLAIMS REMAINING AFTER AMENDMENT | | | | | |
| | Total (37 CFR 1.16(i)) | * 21 | Minus | ** 20 | = 1 | X \$80 = | 80 |
| | Independent (37 CFR 1.16(h)) | * 1 | Minus | ***5 | = 0 | X \$420 = | 0 |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| TOTAL ADD'L FEE | | | | | | | 80 |

| AMENDMENT | (Column 1) | (Column 2) | (Column 3) | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) |
|-----------------|--|----------------------------------|------------|------------------------------------|---------------|-----------|---------------------|
| | | CLAIMS REMAINING AFTER AMENDMENT | | | | | |
| | Total (37 CFR 1.16(i)) | * | Minus | ** | = | X \$ = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | X \$ = | |
| | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| TOTAL ADD'L FEE | | | | | | | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

 LIE
/YUVANNA CHAPHIV/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 | 1757 |

86738 7590 06/11/2013
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

| |
|----------|
| EXAMINER |
|----------|

SCHNEIDER, CRAIG M

| | |
|----------|--------------|
| ART UNIT | PAPER NUMBER |
|----------|--------------|

3753

| | |
|-------------------|---------------|
| NOTIFICATION DATE | DELIVERY MODE |
|-------------------|---------------|

06/11/2013

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@mccarter.com

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:
 - I. Claims 1-14, drawn to a microfluidic device, classified in class 137, subclass 827.
 - II. Claims 15-17, drawn to a method of varying an electrical resistance in a microchannel, classified in class 137, subclass 1.
 - III. Claims 18 and 19, drawn to an electrophoretic system, classified in class 204, subclass 194.

The inventions are distinct, each from the other because of the following reasons:

Inventions II and I are related as process and apparatus for its practice. The inventions are distinct if it can be shown that either: (1) the process as claimed can be practiced by another and materially different apparatus or by hand, or (2) the apparatus as claimed can be used to practice another and materially different process. (MPEP § 806.05(e)). In this case the method of invention II can be performed by a plunger operated actuator that when depressed pushes the air to deform the meniscus (no diaphragm structure being used).

2. Inventions I and III are related as subcombinations disclosed as usable together in a single combination. The subcombinations are distinct if they do not overlap in scope and are not obvious variants, and if it is shown that at least one subcombination

Art Unit: 3753

is separately usable. In the instant case, subcombination I has separate utility such as controlling the flow of the fluid in a non-voltage device. See MPEP § 806.05(d).

The examiner has required restriction between subcombinations usable together. Where applicant elects a subcombination and claims thereto are subsequently found allowable, any claim(s) depending from or otherwise requiring all the limitations of the allowable subcombination will be examined for patentability in accordance with 37 CFR 1.104. See MPEP § 821.04(a). Applicant is advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

3. Inventions II and III are related as subcombinations disclosed as usable together in a single combination. The subcombinations are distinct if they do not overlap in scope and are not obvious variants, and if it is shown that at least one subcombination is separately usable. In the instant case, subcombination II has separate utility such as varying electrical resistance in a device that does not utilize electrophoresis. See MPEP § 806.05(d).

The examiner has required restriction between subcombinations usable together. Where applicant elects a subcombination and claims thereto are subsequently found allowable, any claim(s) depending from or otherwise requiring all the limitations of the allowable subcombination will be examined for patentability in accordance with 37 CFR 1.104. See MPEP § 821.04(a). Applicant is advised that if any claim presented in a

Art Unit: 3753

continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

4. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because at least the following reason(s) apply:

The claims would require searching for more than one invention.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Art Unit: 3753

5. Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to CRAIG SCHNEIDER whose telephone number is (571)272-3607. The examiner can normally be reached on M-F 8:00 -4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Stephen Hepperle can be reached on (571) 272-4913. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Art Unit: 3753

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Craig M Schneider/
Primary Examiner, Art Unit 3753
June 3, 2013

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John R. Gilbert et al.

Application No.: 13/527,331

Confirmation No.: 1757

Filed: June 19, 2012

Art Unit: 3753

For: MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING FLUID
FLOW THROUGH A MICROCHANNEL

Examiner: C. M. Schneider

**NOTIFICATION OF LOSS OF ENTITLEMENT TO SMALL ENTITY STATUS AND
CORRECTION OF ERROR IN SMALL ENTITY STATUS**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

In accordance with 37 C.F.R. §§ 1.27(g)(2) and 1.28(c), Applicant hereby notifies the Office of a loss of entitlement to small entity status and respectfully requests excuse of an error in which certain fees were paid as a small entity subsequent to such loss of entitlement to small entity status. Applicant asserts that its status as a small entity was established in good faith, and fees paid as a small entity were paid in good faith, and through error and without deceptive intent the Office was not notified of the loss of entitlement to small entity status. Per 37 C.F.R. § 1.28(c)(2), below please find an itemization of the deficiency in fees owed resulting from the previous erroneous payment of fees paid as a small entity.

Please charge our Deposit Account No. 50-4876, under Order No. 118153-01205 from which the undersigned is authorized to draw, in the amount of \$1,660 for the total deficiency owed.

| Fee Type | Current Undiscounted Fee | Small Entity Fee Paid | Deficiency Owed |
|---|---------------------------------|------------------------------|------------------------|
| Independent claims in excess of three Fee | \$840 | \$250 | \$590 |
| Basic filing Fee, Utility | \$280 | \$95 | \$185 |
| Utility Search Fee | \$600 | \$310 | \$290 |
| Utility Examination Fee | \$720 | \$125 | \$595 |

Dated: May 16, 2013

Respectfully submitted,

Electronic signature: /David R. Burns/
David R. Burns

Registration No.: 46,590
MCCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110
(617) 449-6500
(617) 607-9200 (Fax)
Attorney/Agent For Applicant

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 15797352 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John Richard Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns/Pegah Karimi |
| Filer Authorized By: | David Burns |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 16-MAY-2013 |
| Filing Date: | 19-JUN-2012 |
| Time Stamp: | 16:01:24 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|------------------------|----|
| Submitted with Payment | no |
|------------------------|----|

File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|-----------------|-------------------------------|---|---|------------------|------------------|
| 1 | Miscellaneous Incoming Letter | Notification_of_Loss_of_Small_ Entity_Status_1.pdf | 44222 1d74bb47a76235e999bc3c69bc06fd49bc40d3b5 | no | 2 |

Warnings:

Information:

0158

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 04/23/2014

| | | | | | | |
|--------|------|-----------|--------------|------------|----------|----------|
| CKHLOK | SALE | #00000003 | Mailroom Dt: | 05/16/2013 | 504876 | 13527331 |
| | | 01 | FC : 1461 | | 1,660.00 | DA |



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|--------------------|-----------------------|-----------------------|------------------------|
| 13/527,331 | 06/19/2012 | John Richard Gilbert | 118153-01205 |

CONFIRMATION NO. 1757

PUBLICATION NOTICE



OC000000057149692

86738
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

Title:MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL

Publication No.US-2012-0261013-A1

Publication Date:10/18/2012

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <http://pair.uspto.gov/>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

| | | | | | |
|--|---|----|---|--------------------------|---------------------------|
| Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i> | | | | Complete if Known | |
| | | | | Application Number | 13/527,331 Confirm # 1757 |
| | | | | Filing Date | June 19, 2012 |
| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 1 | of | 3 | Attorney Docket Number | 118153-01205 |

| U.S. PATENT DOCUMENTS | | | | | |
|-----------------------|--------------------------|---|--------------------------------|--|---|
| Examiner Initials* | Cite No. ¹ | Document Number | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear |
| | | Number-Kind Code ² (if known) | | | |
| | A1 * | US 3,289,687 | 12-06-1966 | Dunaway | |
| | A2 * | US 3,370,538 | 02-27-1968 | Iliacs | |
| | A3 * | US 3,508,654 | 02-01-1970 | Glaetli | |
| | A4 * | US 3,560,754 | 02-02-1971 | Kamentsky | |
| | A5 * | US 3,791,517 | 02-12-1974 | Friedman | |
| | A6 * | US 3,827,555 | 08-06-1974 | Kamnetsky | |
| | A7 * | US 3,984,307 | 10-05-1976 | Kamnetsky | |
| | A8 * | US 4,153,855 | 05-08-1979 | Feingold | |
| | A9 * | US 4,175,662 | 11-27-1979 | Zold | |
| | A10 * | US 4,426,451 | 01-17-1984 | Columbus | |
| | A11 * | US 4,581,624 | 04-08-1986 | O'Connor | |
| | A12 * | US 4,636,149 | 01-13-1987 | Brown | |
| | A13 * | US 4,676,274 | 06-30-1987 | Brown | |
| | A14 * | US 4,756,427 | 07-12-1988 | Gohde et al. | |
| | A15 * | US 4,908,112 | 03-13-1990 | Pace | |
| | A16 * | US 5,065,978 | 11-19-1991 | Albarda et al. | |
| | A17 * | US 5,092,972 | 03-03-1992 | Ghowasi | |
| | A18 * | US 5,101,978 | 04-07-1992 | Marcus | |
| | A19 * | US 5,265,327 | 11-30-1993 | Faris et al. | |
| | A20 * | US 5,777,649 | 07-07-1993 | Otsuka et al. | |
| | A21 * | US 5,789,045 | 08-04-1998 | Wapner et al. | |
| | A22 * | US 5,837,200 | 11-17-1998 | Diessel et al. | |
| | A23 * | US 5,876,187 | 03-02-1999 | Afromowitz et al. | |
| | A24 * | US 5,988,522 | 11-23-1999 | Glezer et al. | |
| | A25 * | US 6,033,191 | 03-07-2000 | Kamper et al. | |
| | A26 * | US 6,048,328 | 04-11-2000 | Haller et al. | |
| | A27 * | US 6,048,734 | 04-11-2000 | Burns et al. | |
| | A28 * | US 6,062,681 | 05-16-2000 | Field et al. | |
| | A29 * | US 6,102,530 | 08-15-2000 | Kim et al. | |
| | A30 * | US 6,152,181 | 11-28-2000 | Wapner et al. | |
| | A31 * | US 6,196,525 | 03-06-2001 | Ganan-Calvo | |
| | A32 * | US 6,203,291 | 03-20-2001 | Stemme et al. | |
| | A33 * | US 6,221,654 | 04-24-2001 | Quake et al. | |
| | A34 * | US 6,273,553 | 08-14-2001 | Kim et al. | |
| | A35 * | US 6,360,775 | 03-26-2002 | Barth et al. | |
| | A36 * | US 6,432,630 | 08-13-2002 | Blankenstein | |
| | A37 * | US 6,561,224 | 05-13-2003 | Cho | |
| | A38 * | US 6,592,821 | 07-15-2003 | Wada et al. | |
| | A39 * | US 5,597,438 | 07-22-2003 | Cabuz et al. | |
| | A40 * | US 6,629,820 | 10-07-2003 | Korselsen | |
| | A41 * | US 6,802,342 | 10-12-2004 | Fernandes et al. | |
| | A42 * | US 6,877,528 | 04-12-2005 | Gilbert et al. | |
| | A43 * | US 7,069,943 | 07-04-2006 | Gilbert et al. | |
| | A44 * | US 8,210,209 | 07-03-2012 | Gilbert et al. | |

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

| | | | | | |
|--|---|----|---|-------------------------------|---------------------------|
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| | | | | Filing Date | June 19, 2012 |
| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 2 | of | 3 | Attorney Docket Number | 118153-01205 |

| | | | | | |
|--|-------|-----------------|------------|----------------|--|
| | A45 * | US 2006-0278288 | 12-14-2006 | Gilbert et al. | |
| | A46 * | US 2012-0015442 | 12-14-2006 | Gilbert et al. | |
| | | | | | |
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| FOREIGN PATENT DOCUMENTS | | | | | | |
|--------------------------|--------------------------|---|-----------------------------------|---|--|------------------|
| Examiner Initials* | Cite No. ¹ | Foreign Patent Document | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear | T ⁶ |
| | | Country Code ³ -Number ⁴ -Kind Code ⁵ (if known) | | | | |
| | B1 | WO 91/15750 | 10-17-1991 | Carri Med. Ltd. | | |
| | B2 | WO 98/10267 | 03-12-1998 | Technical University of Denmark | | |
| | B3 | WO 98/07069 | 02-19-1998 | The Regents of the University of Michigan | | |
| | B4 | WO 00/70080 | 11-23-2000 | Caliper Technologies Corp. | | |
| | B5 | EP A 0 177 718 | 04-16-1986 | Partec AG | | Abstract only |
| | B6 | EP A 0 745 682 | 12-04-1996 | Bayer AG | | Abstract only |
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| NON PATENT LITERATURE DOCUMENTS | | | |
|---------------------------------|--------------------------|---|----------------|
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| | C1 | International Search Report issued in International Application No. PCT/US2003/12368, dated August 21, 2003 | |
| | C2 | International Preliminary Examination Report issued in International Application No. PCT/US2003/12368, dated August 25, 2004 | |
| | C3 | International Search Report issued in International Application No. PCT/US2003/12360, dated October 3, 2003 | |

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| | | | | Application Number | 13/527,331 Confirm # 1757 |
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| | | | | First Named Inventor | John Richard Gilbert |
| | | | | Art Unit | 2853 |
| | | | | Examiner Name | Not Yet Assigned |
| Sheet | 3 | of | 3 | Attorney Docket Number | 118153-01205 |

| | | |
|-----|--|--|
| C4 | International Preliminary Examination Report issued in International Application No. PCT/US2003/12360, dated July 21, 2004 | |
| C5 | International Search Report issued in International Application No. PCT/US2003/12359, dated September 2, 2003 | |
| C6 | International Preliminary Examination Report issued in International Application No. PCT/US2003/12359, dated September 5, 2004 | |
| C7 | Supplementary European Search Report issued in European Application No. 03726391.0-2204, dated October 6, 2009 | |
| C8 | Communication pursuant to Article 94(3) EPC issued in European Application No. 03726391.0-2204, dated April 12, 2010 | |
| C9 | Search Information Statement issued in Australian Application No. 2010200179, dated March 21, 2011 | |
| C10 | Examiner's Report issued in Australian Application No. 2010200179, dated March 22, 2011 | |
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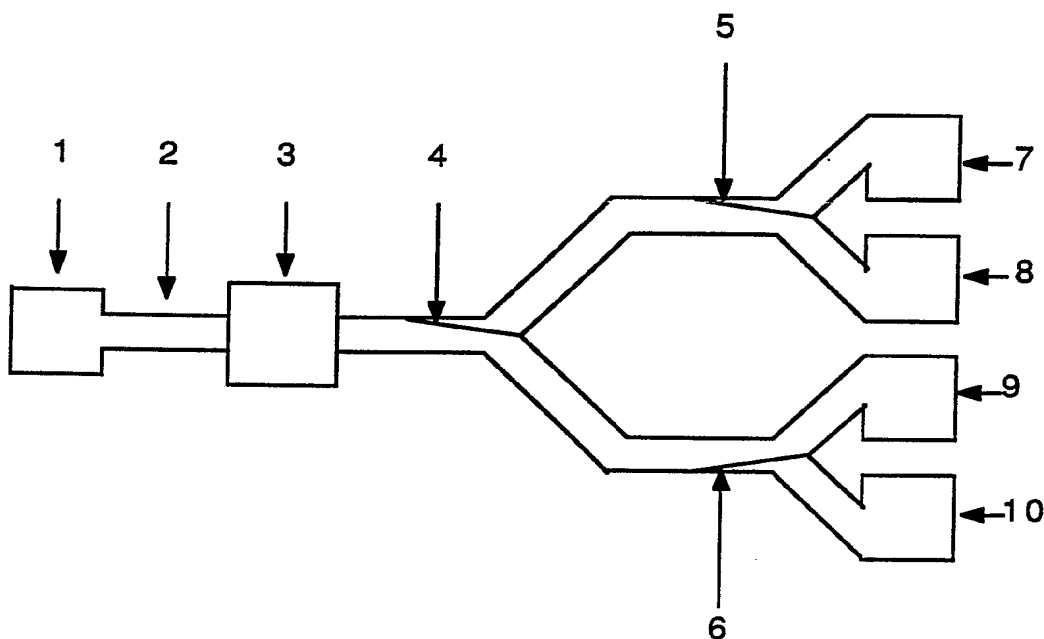
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|-----------|---|
| (51) International Patent Classification 5 : G01N 15/14, 15/02, B07C 5/342 | A1 | (11) International Publication Number: WO 91/15750 (43) International Publication Date: 17 October 1991 (17.10.91) |
| (21) International Application Number: PCT/GB91/00542 (22) International Filing Date: 8 April 1991 (08.04.91) (30) Priority data: 9008044.1 9 April 1990 (09.04.90) GB (71) Applicant (for all designated States except US): CARRI-MED LTD. [GB/GB]; Carri-Med House, Glebelands Centre, Vincent Lane, Dorking, Surrey RH4 3YX (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : KAYE, Paul, Henry [GB/GB]; TRACEY, Mark, Christopher [GB/GB]; Hatfield Polytechnic, College Lane, Hatfield, Herts. AL10 9AB (GB). | | (74) Agent: MAURY, Richard, Philip; Sommerville & Rush-ton, 11 Holywell Hill, St. Albans, Herts. AL1 1EZ (GB). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB, GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i> |

(54) Title: MICROFABRICATED DEVICE FOR BIOLOGICAL CELL SORTING



(57) Abstract

Apparatus for sorting cells into spatially separate sub-groups, comprising a microfabricated moveable structure (4, 5, 6) for directing cells between distinct spatial locations.

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Microfabricated Device for Biological Cell Sorting

This invention relates to the sorting of biological cells into spatially separate sub groups. The criteria
5 for sorting may be applied to measurements obtained by any technique or techniques suitable for microfabricated or electronic implementation on a substrate.

Microfabrication and microelectronic techniques offer a
10 number of actual and potential measurement techniques that may be applied to characterize biological cells. Examples of these are microrheological measurements as disclosed in International Patent Application No. PCT/GB91/00289 and morphological measurements. When
15 suitably implemented such techniques can be applied on a cell by cell basis.

When characterizing a population of cells it can be of interest to workers to be able to isolate a sub
20 population whose measured parameters lie within certain bounds. This could enable, for example, the culturing of a cell line from sorted cells possessing a certain property or properties of interest.

25 We have discovered that recent developments in

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microfabrication, namely the development of controlled, deflectable, microbeams capable of deflections in the order of a few micrometres, may be used to provide the component parts of microfabricated cell sorters.

- 5 Relevant microelectromechanical structures, and their formation by selective CVD techniques, are disclosed in: "Selective Chemical Vapor Deposition of Tungsten for Microelectromechanical Structures" by N.C. MACDONALD et al., Sensors and Actuators, 20 (1989) 123-133. The
- 10 so-called "microtweezers" are activated by control voltages applied to electrodes.

- According to the current invention such a machine is implemented by utilising a microfabricated moveable
- 15 structure, preferably microfabricated beams, to direct cells between distinct spatial locations.

- A particular implementation of the invention is shown in the drawing, which is a topological plan view of a
- 20 substrate on which a cell sorter is formed. In this example the cells to be sorted, whose diameters may be only a few micrometres, are fed sequentially from an entry port 1, via a guiding structure 2, past a generalised sensing device or zone 3. Depending upon the
- 25 result of the measurement and its interpretation the deflectable beams 4, 5, 6, are set into appropriate states to direct the cell into a particular destination,

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for example, a hole 7, 8, 9 or 10 etched through the substrate. In the example shown the destination is structure 9. The beams, along with associated bifurcations can be extended to a tree structure of 'n' levels thereby yielding 2^n possible sorting sub ranges.

The absolute lengths of the channels are as short as possible while maintaining compatibility with interconnecting structures. The shafts 1, 3, 7-10 may be fabricated by selective etching as disclosed in the copending International Patent Application referred to above; the etched substrate is closed by a glass/silica cover, thereby forming the channels as tunnels.

Typical dimensions of the entry port 1 are 500 μ m or less square; of the sensing zone about 100 μ m square; of the channels about 5 to 10 μ m in width and depth; of the holes (shafts) 7 - 10 about 500 μ m or less square; and of the beams, which are typically of constant rectangular section, about 150 μ m long by 3 μ m square. The overall dimensions of the device could be 1cm square.

The beams are activated by selective control voltages applied as signals for control circuitry (not shown) operated, for example, under the control of a microprocessor or other computer. The computer is

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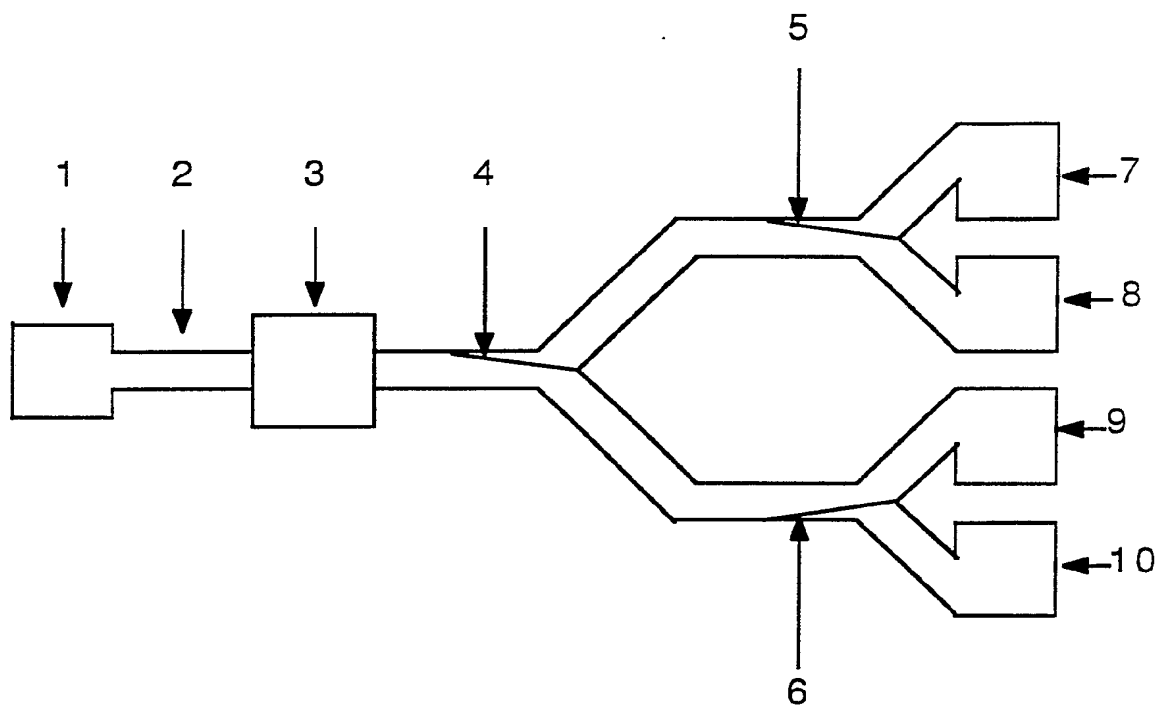
programmed to control the beams' deflection in response to the desired cell-sorting procedural steps.

Claims:

1. Apparatus for sorting cells into spatially separate sub-groups, comprising a microfabricated moveable structure (4, 5, 6) for directing cells between distinct spatial locations.
2. Method for sorting cells into spatially separate sub-groups, comprising directing the cells into distinct spatial locations using a microfabricated moveable structure.

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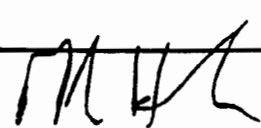
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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 91/00542

| | | |
|---|---|-------------------------------------|
| I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ | | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | | |
| Int.Cl. 5 | G01N15/14 ; G01N15/02 ; B07C5/34 | |
| II. FIELDS SEARCHED ¹ | | |
| Minimum Documentation Searched ² | | |
| Classification System | Classification Symbol | |
| Int.Cl. 5 | G01N ; B07C ; B01 | |
| Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ³ | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁴ | | |
| Category ⁵ | Citation of Document ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| A | US.A.4 676 274 (JAMES F BROWN) June 30, 1987 see column 9, line 22 - line 56 see column 12, line 7 - line 60; figures 12,18 --- | 1,2 |
| A | REVIEW OF SCIENTIFIC INSTRUMENTS. vol. 51, no. 1, January 1980, NEW YORK US pages 111 - 115; P LENZ: 'Mechanical cell separator' see page 111 - page 113 --- | 1,2 |
| A | PROCEEDINGS OF THE IEEE. vol. 70, no. 5, May 1982, NEW YORK US pages 420 - 457; K E PETERSEN: 'Silicon as a mechanical material' see page 434 - page 435 see page 442 - page 443 see page 446 - page 449 --- | 1,2 |
| -/-- | | |
| ⁶ Special categories of cited documents : ¹⁰ ¹⁴ "A" document defining the general state of the art which is not considered to be of particular relevance ¹⁵ "E" earlier document but published on or after the international filing date ¹⁶ "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) ¹⁷ "O" document referring to an oral disclosure, use, exhibition or other means ¹⁸ "P" document published prior to the international filing date but later than the priority date claimed ¹⁹ "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention ²⁰ "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step ²¹ "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. ²² "&" document member of the same patent family | | |
| IV. CERTIFICATION | | |
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| 12 JULY 1991 | 30.07.91 | |
| International Searching Authority | Signature of Authorized Officer | |
| EUROPEAN PATENT OFFICE | HODSON C.M.T.  | |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) | | |
|--|--|-----------------------|
| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
| A | EP,A,177 718 (PARTEC AG) April 16, 1986 --- | |

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB9100542
SA 46699

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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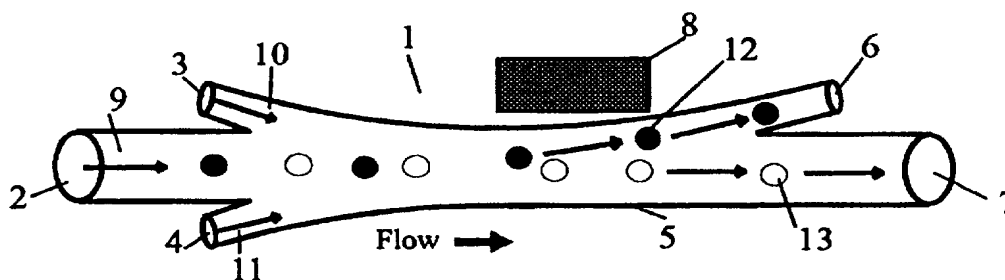
| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| US-A-4676274 | 30-06-87 | EP-A- 0293519 | 07-12-88 |
| EP-A-177718 | 16-04-86 | CA-A- 1256825 | 04-07-89 |
| | | JP-A- 61137062 | 24-06-86 |
| | | US-A- 4756427 | 12-07-88 |

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| (51) International Patent Classification ⁶ : G01N 15/14, 33/543, B03C 1/035 | | A1 | (11) International Publication Number: WO 98/10267 |
| | | | (43) International Publication Date: 12 March 1998 (12.03.98) |
| (21) International Application Number: PCT/DK97/00368 | | | (81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, ES, FI, FI (Utility model), GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). |
| (22) International Filing Date: 4 September 1997 (04.09.97) | | | |
| (30) Priority Data: 0953/96 4 September 1996 (04.09.96) DK 0150/97 10 February 1997 (10.02.97) DK | | | |
| (71) Applicant (for all designated States except US): TECHNICAL UNIVERSITY OF DENMARK [DK/DK]; DTU - Building 101 A, DK-2800 Lyngby (DK). | | | |
| (72) Inventor; and (75) Inventor/Applicant (for US only): BLANKENSTEIN, Gert [DE/DE]; Wiesinger Weg 5, D-20253 Hamburg (DE). | | | |
| (74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annae Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK). | | | Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |

(54) Title: A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS



(57) Abstract

A micro flow system is provided for separating particles, comprising a microfabricated member having a flow channel (5) defined therein for guiding a flow of a fluid containing the particles through the flow channel, first inlet means (2) positioned at one end of the flow channel for entering the fluid into the flow channel, first outlet means (7) positioned at the other end of the flow channel for discharging the fluid from the flow channel, the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross section of the flow channel, the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field. Further, a micro flow system is provided for analysing components of a fluid comprising a microfabricated member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.

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A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS

FIELD OF THE INVENTION

5 The present invention relates to methods and apparatuses for detection, separation, sorting, and analysis of particles, such as cells, cell organelles, beads, molecules, such as Deoxyribonucleic acid (DNA), proteins, etc. in a fluid. In particular, the invention relates to particle separation by using different forces such as magnetic, electrophoretic, hydrodynamic and/or gravitational forces, e.g. for utilisation in flow cytometry, light microscopy, electrophoretic separation, magnetophoresis,
10 etc.

BACKGROUND OF THE INVENTION

Flow cytometry is a well known technique that is used for high throughput measurements of
15 optical and/or electrical characteristics of microscopic biological samples. Flow cytometry instruments analyse and isolate cells and organelles with particular physical, biochemical, and immunological properties.

Traditionally, cell sorting by flow cytometry (fluorescence activated cell sorting) has been the
20 method of choice for isolation of specific cell populations by surface markers. However, cell sorting by flow cytometry suffers from several drawbacks, especially high dilution of desired cell sample, low speed and sterility problems. Furthermore, the equipment is very costly with high operation and maintenance cost, making the technique available only to a limited number of laboratories.

25 During the last few years, isolation of cells by antibody-coupled magnetic beads and carriers has been developed into a reliable tool for the isolation and characterisation of cell populations. Immunomagnetic cell separation, e.g. as commercially introduced by Dynal A/S and Miltenyi Biotec, has become an established method for cell analysis in clinical diagnostics. Due to the relatively low prize, this method is attractive in flow cytometry, especially in sorting of rare
30 cellular events. For example, sorting of fetal cells contained in maternal blood sample provides a non-invasive alternative to prenatal diagnostic procedures, such as amniocentesis of chorionic villus sampling. Another rare event scenario is the detection of low concentration of cancer cells which has an important role in diagnosis of minimal residual disease and evaluation of appropriate therapies. Another medical application for cell sorting systems is the diagnosis of
35 bacterial and viral diseases.

Although this method offers relatively inexpensive approach to sort rare cellular event, it adds considerable time onto the overall rare event detection and it does not offer the multiparameter

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analysis readily available with flow cytometry techniques. Existing techniques for magnetic separation are suffering from the low purity of the sorted cell fraction and the low recovery rate of the sorted cells. In most systems several washing steps have to be implemented into the separation procedure which then causes cell losses. Additionally small subpopulation of labelled
5 cells cannot be directly isolated by existing magnetic separation techniques.

A good overview about fluorescence activated cell sorting procedures and magnetic activated cell sorting is given in Melamed et. al., "Flow Cytometry and Sorting, (Ed. Melamed et. al., Wiley & Sons Inc., 1990).

10

SUMMARY OF THE INVENTION

Advances in microfabrication and microfluidic technologies continue to fuel further investigation into the miniaturisation of bioanalytical instruments and biochemical assays in general. The
15 present invention relates to development of a low cost non-invasive diagnostic test method and devices for carrying out such tests that include measuring, monitoring, sorting and analysing samples containing particles, such as organic cells, microbeads, cell organelles and macromolecules such as DNA. The present invention provides a cheap, fast and reliable method and devices for handling, sorting and analysis of such particles.

20

Separation may be performed according to various physical properties, such as fluorescent properties or other optical properties, magnetic properties, density, electrical properties, etc. According to an important aspect of the invention, particle separation is performed by aligning the particles in one row of particles in a micro flow channel so that particles can be treated
25 individually.

Thus, it is an object of the present invention to provide a micro flow system and a method of particle separation having an improved efficiency of particle separation compared to the prior art.

30 It is another object of the present invention to provide a micro flow system and a method for particle separation in which cell lysis is minimised.

It is yet another object of the present invention to provide an improved method for preparation of fluids containing particles for separation and analysis of the particles.

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It is a still further object of the present invention to provide a micro flow system and a method for simultaneous separation of particles into a plurality of groups of particles.

It is a still further object of the present invention to provide a micro flow system including facilities for pre-treatment and/or post-treatment of a sample.

It is a still further objective of the invention is develop a system for separation and analysis of fetal cells in whole maternal blood samples using an integrated automated micro flow system. The system is designed by downscaling and combining different methods for handling, manipulation and analysis of biochemical samples. Thus, prenatal diagnostics by analysis of fetal cells separated from a whole maternal blood sample is an area, which can benefit from advances in miniaturisation.

It is another objective of the invention is develop a system for separation and analysis of cancer cells from a sample containing cancer cells and healthy cells using an integrated automated micro flow system. The system is also designed by downscaling and combining different methods for handling, manipulation and analysis of biochemical samples. Thus, cancer diagnostics by analysis of cancer cells separated from healthy cells is also an area which can benefit from advances in miniaturisation.

According to a first aspect of the invention the above and other objects are fulfilled by a micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

first outlet means positioned at the other end of the flow channel for discharging the fluid from the flow channel,

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field.

According to a second aspect of the invention the above and other objects are fulfilled by a method of separating particles, comprising the steps of

guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel,

5 positioning the flow channel in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field and thereby separated from the fluid.

10 According to a third aspect of the invention the above and other objects are fulfilled by a micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

15

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

20 first and second outlet means positioned at the other end of the flow channel for discharging of fluid from the flow channel,

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

25 monitoring means positioned at the flow channel for monitoring parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to a monitored parameter,

control means for controlling passage of fluid through the first and the second outlet means,

30 respectively, in response to the output signal of the monitoring means whereby particles may be separated according to values of a parameter monitored by the monitoring means.

According to a fourth aspect of the invention the above and other objects are fulfilled by a method of separating particles, comprising the steps of

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guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel, the flow channel having first and second outlet means for discharging of fluid from the flow channel,

monitoring parameters of a particle residing within a measurement volume within the flow channel and

- 5 controlling passage of fluid through the first and the second outlet means, respectively, in response to a monitored parameter value whereby particles may be separated according to values of a monitored parameter.

10 According to a preferred embodiment of the invention, a method of separating fetal cells from maternal cells, comprising the steps of selective magnetically staining of fetal cells in a fluid containing fetal and maternal cells, guiding a flow of the fluid containing the fetal cells through a flow channel in such a way that one fetal cell at the time passes a cross-section of the flow channel, positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained fetal cells residing in the flow
15 channel are deflected in the direction of the magnetic field.

Further a method is provided for separating cancer cells from other cells, comprising the steps of selective magnetically staining of cancer cells in a fluid containing cancer and other cells, guiding a flow of the fluid containing the cancer cells through a flow channel in such a way that one
20 cancer cell at the time passes a cross-section of the flow channel, positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained cancer cells residing in the flow channel are deflected in the direction of the magnetic field.

- 25 The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may comprise living cells, chromosomes, organelles, beads, biomolecules, such as Deoxyribonucleic acid (DNA), proteins, etc.

30 Preferably, the flow through the flow channel is a laminar flow so that flow of particles are predictable and easy to control, e.g. with a flow of guiding buffers.

When the flow is laminar, the stream of particles can be positioned as desired within the flow channel, e.g. by controlling flow velocities of the fluid containing particles at the particle inlet of the member and flow velocities of guiding buffers at corresponding inlets.

35

Preferably, the flow channel is small for the flow through the channel to have a low Reynolds number, e.g. in the range of 0.01-500, such as 0.05-50, preferably 0.1-25. Thereby, inertial effects, which causes turbulence and secondary flows are negligible, viscous effects dominate the

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dynamics, and mixing is caused only by diffusion. Flow of the sample, which is the fluid containing particles and guiding buffers can be laminated in guided layers through the channel and displacement of particles in the channel is only caused by the external force applied. The Reynolds number referred to is based on the hydraulic diameter of the flow channel, the flow velocity in the axial direction and the fluid density and viscosity, $Re = \rho D_h v / \mu$ where the hydraulic diameter D_h is defined as four times the cross-sectional area divided by the wetted perimeter.

The illustrated flow channels of the micro flow system have a width ranging from 0.1 to 0.55 mm, preferably ranging from 0.1 to 0.4 mm, in particular ranging from 0.1 to 0.2 mm, and a depth ranging from 0.04 to 0.2 mm, preferably ranging from 0.04 to 0.1. With respect to the lowest cross-sectional area of the flow channel, it is preferred that this area is in the range of 0.004 to 0.11 mm², in particular in the range of 0.004 to 0.02 mm².

It is believed that any length of the flow channel within the range of 0.1 to 20 mm, preferably 1.0 to 3.5 mm, would lead to satisfactory results.

Preferably, the system is operating with total volumetric flow rates of 0.1 up to 200 μ l/min, which gives a flow velocity of 15 mm/min up to 180 mm/min. The average residence time of a particle inside the flow channel, which corresponds to a separation time ranging from 0.1 to 6 sec. The residence time of the sample is defined by the total volumetric flow rate of the system.

The micro flow system may comprise flow rate adjustment means for adjustment of the time the particles reside in the flow channel.

Preferably, the fluid channel is sized so that for efficient separation, particles are displaced 10 - 30 μ m in the flow channel. Thereby, the particle may only be exposed to a field for a very short period of time and thus, continuous separation of particles may be facilitated.

In order to collect the particles, which are deflected in the flow channel, the micro flow system may further comprise second outlet means for discharging particles having been deflected in the flow channel.

The micro flow system may comprise second inlet means for entering a first guiding buffer into the flow channel together with the fluid containing particles. When the flow is laminar, the two fluids flow through the flow channel in parallel abutting each other along a small area extending along a longitudinal axis of the flow channel whereby the cross-section and the path through the flow channel of the flow of the fluid containing particles may be controlled by the first guiding buffer flow. Further, particles in the fluid containing particles may be deflected into the guiding

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buffer fluid when the two fluids pass the field essentially perpendicular to the longitudinal axis of the flow channel. Furthermore, two (or even more) outlets may be provided at the down stream end of the flow channel for discharging the guiding buffer now containing separated particles and fluid substantially without particles susceptible to the field essentially perpendicular the flow channel, correspondingly.

The micro flow system may further comprise third inlet means for entering a second guiding buffer for improved control of the path of particle flow through the flow channel. By adjustment of the flow velocities of the guiding buffers and the fluid containing particles, the flow within the flow channel of fluid containing particles may be controlled to flow within an essentially cylindrical shaped domain with a longitudinal axis extending substantially parallel to a longitudinal axis of the flow channel and further the position within the flow channel and the diameter of the flow cylinder may be controlled by corresponding adjustments of the volumetric ratio between the flow rate of the fluid containing particles and the flow rate of the guiding buffers.

It is possible to control the cross-sectional area of the domain containing the sample to be a little larger than the cross-sectional area of the particles by adjusting the volumetric flow rates of the sample and of the one or two guiding buffers in such a way that the particles contained in the sample are aligned in a single row of particles. This is a very important feature since it enables individual treatment of each particle and it leads to a sensitive method of sorting particles according to their susceptibility to a field. A sample flow layer thickness less than 1 μm may be achieved.

Preferably, the channel depth is small enough, e.g. below 50 μm , to allow observation of the particles flowing through the channel by a microscope. In an important embodiment of the present invention, the micro flow system comprises a cover, e.g. a transparent or translucent cover, for covering the flow channel. When the cover is transparent or translucent, it will be possible to observe events in the flow channel, e.g. passage of a stained or coloured particle or cell.

The member with the flow channel may be produced from any suitable material, such as silicon, polymers, such as Plexiglas, Teflon, etc., glass, ceramics, metals, such as copper, alumina, stainless steel, etc., etc.

The channel may be provided in the member by any suitable manufacturing process, such as milling, etching, etc.

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In a preferred embodiment of the invention, the member is a silicon chip manufactured utilising photolithography and the channel is etched into the silicon chip.

5 The field may be a magnetic field, an electric field, a gravity field, etc., and any combination of such fields.

A magnetic field may be generated by permanent magnets, such as rare earth magnets, such as samarium-germanium magnets, a mixture of ferromagnetic powder and epoxy, etc., etc., electromagnets, e.g., in silicon integrated electromagnets, etc. The magnets are preferably
10 positioned adjacent to the flow channel so that the magnetic field is substantially perpendicular to a longitudinal axis of the flow channel.

In a preferred embodiment of the invention, the magnets are positioned in and glued to rectangular slots that are etched into a silicon chip. The slots are located adjacent to the
15 separation flow channel. In the example shown in Fig. 1, a permanent magnet or an electromagnet can be received by slots in the micro flow system. The slots are, e.g., 0.5 mm wide, 0.5 mm long and 0.2 mm deep. For generation of a magnetic field, a solid magnetic block, i.e. rare earth magnet can be glued into the slot. Alternatively, a mixture of ferromagnetic powder and epoxy can be injected into the slots to produce a high magnetic field gradient.

20

The strength of the magnetic field inside the micro flow system may be adjustable. If an electromagnet is used for generation of the magnetic field, the magnitude of the field may be varied by varying the amplitude of the voltage input to the electromagnet. If a permanent magnet generated the magnetic field, the magnitude of the field may be varied by varying the distance
25 between the magnet and the flow channel of the micro flow system.

As already mentioned, the net displacement of a particle in the micro flow system depends on the force applied to it by the field. This can be utilised for separation of a first group of particles of various types in a fluid into a plurality of set of particles; each set comprising a specific type of
30 particles. A micro flow system with e.g. five separation outlets may be used to separate a fluid containing particles into five sets of particles, each set comprising particles that are influenced by the field with a force of a specific magnitude, in the following denoted particles with a specific F-value. Particles with a low F-value are only deflected by a small amount by the field and are discharged from the flow channel through a corresponding outlet port. Particle deflection is
35 increased with increasing F-values whereby such particles are discharged from the flow channel through the corresponding other outlets.

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The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may be magnetically stained to facilitate separation in a magnetic field.

In the present context, the term staining is to be understood in a broad sense. The term is intended to cover any way of marking a particle thereby facilitating detection of the particle. For example a cell may be stained with a fluorescent substance, such as acridin orange, methylene blue, etc, facilitating detection of the stained particles by a fluorescence detector, or, a particle is said to be magnetically stained when it is coupled to a magnetic microbead. The microbead may for example carry a monoclonal or polyclonal antibody on its surface for coupling to an antigene of a cell to be separated utilizing a magnetic field.

In the case where particles have to be detected in a flow channel by optical means, such particles are preferably stained with a chromophoric reagent, or, a fluorescent probe.

An electric field may be generated by electrodes, such as metal electrodes, such as gold electrodes, etc. The electrode may be positioned inside the flow channel, e.g. to introduce electrophoretic forces, e.g. for separation of charged molecules in the fluid, or outside the flow channel e.g. to introduce dielectrophoretic forces, e.g. for separation of particles contained in the flow according to the susceptibility of the particles to the field. Preferably, the electrodes are positioned in such a way that the electric field is essentially perpendicular to a longitudinal axis of the flow channel.

The electric field may be a high frequency field, e.g. a 5 MHz field generated by electrodes positioned inside the flow channel. Living cells positioned in an electric field will be polarized and will be influenced by the field and thus, an alternating field may be used to separate living cells from other particles.

The field generated across the flow channel may be utilised for immobilisation of particles whereby particles may be held in substantially fixed positions within the flow channel for a specific period, e.g. as outlined in Fig. 6, allowing chemical reactions with the particles to take place and/or kinetic measurements on the particles to be performed and/or to bring the particles into contact with different chemical substances or for separating the particles from the sample. The particles may undergo a washing step before removal or reduction of the field redisperses them.

According to a fifth aspect of the invention the above and other objects are fulfilled by a micro flow system for separating particles, comprising a member having

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a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

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field generating means positioned proximate to the other end of the flow channel for generating a field substantially along a longitudinal axis of the flow channel whereby the particles are drawn by the field along the channel and distributed according to their susceptibility to the field and their mobility.

10

For example, means for generating a magnetic field may be situated at the closed end of a micro flow channel, which at the other end has at least one inlet for entering a sample containing magnetic labelled macromolecules, i.e. ribonucleic acid or proteins. The sample is entered into the channel where the particles are drawn by the magnetic field along the channel and, as by electrophoresis, the particles will be distributed according to their susceptibility to the magnetic field and their mobility. The generated magnetic field is removed after a predetermined time interval and the distribution of particles can then be observed.

15

According to another embodiment of the invention, the flow through the sort outlet is not continuous but only allowed by a controlling means, e.g. a valve, when a particle with the desired characteristics is detected by a detection means. The particles are sorted using hydrodynamic forces in the sense that the flow is diverged from the ordinary outlet to the sort outlet only when it contains a particle that fulfils certain criteria. The concentration of sorted particles in the flow out of the sort outlet will consequently be high. This is especially an advantage for sample flow with rare occurrence of particles that are searched for. The detection means can be e.g. optical detection means or magnetic detection means e.g. a Hall sensor or means for detecting e.g. electrical or other properties of the particles. The detection means can in an alternative embodiment be used for counting of particles with the desired characteristics as a separate function or in connection with any of the other embodiments described herein.

25

30

In yet another embodiment, the field strength is adjustable, e.g. by adjusting the voltage supplied to an electromagnet or to a set of electrodes or by adjusting the distance from a permanent magnet to the flow channel. Particles are in a first operation mode entrapped inside the flow channel by the field at high intensity while at the same time the sort outlet is closed. In a second operation mode, the field is reduced and the sort outlet is open in such a way that the entrapped particles are redispersed and moved out of the sort outlet. Particles that are rare in the sample can by switching between these two operational modes be sorted out in a highly concentrated form. An example of this embodiment is outlined in Fig. 6.

35

In a further interesting embodiment, the micro flow system according to the invention involves facilities for performing pre-treatment and/or post-treatment of the fluid comprising the particles. These possibilities are outlined in Figs. 5(f), 7 and 10. As an example, the particles may be treated with a reagent before entering the flow channel, e.g. undergo magnetic or chromophoric staining. Post-treatment may comprise means for collecting or concentrating the deflected particles or means for contacting the deflected particles with one or more reagent(s).

By one possible combination of the pre-treatment and the post-treatment facilities, cells may undergo magnetic staining before entering the flow channel, and after separation the staining may be removed by treatment of the stained cells with a suitable reagent.

It is an important advantage of the present invention that a micro flow system is provided that operates continuously with no requirement for operator intervention.

It is another advantage of the present invention that separation may be performed in one step.

It is still another advantage of the present invention that the particles can be separated in a continuous flow without substantially interfering with the flow itself and that separated particles may be collected at corresponding separated outlets of the flow channel without having to interrupt the flow in the flow channel.

It is another important advantage of the invention that the particles contained in the sample by the adjustment of the flow rate of one or more guiding buffers can be lined up in one row such that the particles can be analysed and sorted individually. This results in a sorting system with the highest sensitivity to the susceptibility of the single particle to the field applied to the sorting channel and a sorting system with the highest resolution of the detection means of the characteristics exhibited by the particles.

It is yet another advantage of the present invention that the micro flow system is easily integrated into other continuous flow systems, such as flow cytometers, flow injection analysis systems, etc.

It is a further advantage of the present invention that particles may be separated into a plurality of groups of particles, e.g. different subpopulations of cells, based on different susceptibility to the field generated across the flow channel of the different groups of particles. This may be obtained by using a multiple outlet micro flow system as outlined in Fig. 5(c).

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It is a still further advantage of the present invention that the micro flow system allows observation of particles in the flow channel using a microscope.

It is a still further advantage of the invention that a closed system is provided allowing
5 biohazardous samples, such as samples containing pathogens, to be entered into the system without contaminating the laboratory environment and without causing hazard for operators working with pathogen biomaterials.

It is a still further advantage of the invention that a system with a low shear stress in the flow is
10 provided allowing a gentle treatment of biological samples, e.g. fragile living cells, especially when two guiding buffers are introduced in the channel.

It is a still further advantage of the invention that a high concentration of the sorted particles can be obtained even from samples with rare occurrence of particles that are searched and sorted for.

15

According to an important aspect of the invention, a new system for immunomagnetic cell separation and manipulation is provided that utilises a silicon based micro fabricated flow chip. The system combines the advantage of flow cytometry and immunomagnetic separation technique. The flow chip will be an important component of a portable micro system for cell
20 sorting and analysis. The flow chip is designed for rapid immunomagnetic cell separation nearly without any pressure drop. Its simple and cheap fabrication and versatile sorting and detection properties offer an alternative to conventional cell separation systems.

It is an advantage of the invention that a micro flow system is provided that is cheap, easy to
25 operate, versatile, simple and portable and that offers the possibility of automation.

A miniaturised flow channel system is provided that utilises the advantageous fluid behaviour in micro systems. The invented system operates continuously. Instead of holding back the magnetisable particles in the separation unit, the particles are deflected into the direction of the
30 magnetic field while passing it continuously. By splitting the fluid flow into two or more outlets, the deflection of the particles can be used for separation of particles into different sets of particles, each of which leaves the flow channel through a specific outlet.

The continuous separation system (CSS) allows efficient enrichment as well as depletion of
35 labelled sample contents of interest. The CSS is designed to fit under a microscope allowing parallel detection of the optical properties of the sample and the control of separation of particles.

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An advantage of the geometry of the invented separation flow channel is that a magnetised or electrically charged particle has to be moved only over a distance of 10 - 30 μm to be separated from the fluid containing particles.

5 Furthermore, the invention enables isolation of multiple cell or particle subpopulations from a single sample at the same time. The magnitude and direction of the force F on a magnetisable particle, e.g. a magnetically labelled cell, is dependent on the magnitude of the magnetic field and the number of magnetic moments inducible on a labelled cell.

10 $F = N \cdot S \cdot \mu_B \cdot \text{grad } B$

where S is the number of Bohr magnetons (μ_B) per particle and N is the number of particles per cell.

15 Beads with small S are moving a less distance in lateral direction in relation to the flow through the flow channel than beads with a higher S value. This can be used to separate subpopulation of cells labelled with different magnetisable beads: By splitting the flow channel in various outlet channels cells can be distinguish and separated due to their individual F values.

20 The drag force on a spherical particle can be found from the particle Reynolds number, based on particle diameter, particle velocity relative to the fluid and fluid viscosity and density. In a flow with a Reynolds number less than 100, the drag force D on the particle can be found from a modified version of Stokes law

$$D = 3\pi\mu U d \left(1 + \frac{3}{16} \text{Re}\right)^{1/2}$$

25 where μ denotes the viscosity of the liquid, U is the relative velocity of the particle and d is the diameter. The numerical value of the parenthesis on the right hand side of the above formula is close to unity for Reynolds numbers less than one why it in that case can be omitted. The magnitude of the drag force on the particles, the force applied to the particle by the field, the distance the particle needs to be moved and the time available for the separation are all important aspects to be considered when a separation process and the device for carrying it out is
30 designed.

An example is given for separation by gravitational means. The effective gravitational force G defined as the gravitational force minus the buoyancy force is found as

$$G = (\rho_{\text{particle}} - \rho_{\text{liquid}}) g \frac{\pi}{6} d^3$$

where g is the gravitational constant. For simplicity, a Reynolds number for the particle of less than one is assumed why the drag force D is given in a simple form. These two forces, D and G , are equal when the maximum velocity, the settling velocity U_{∞} has been reached. This velocity is
5 found as

$$U_{\infty} = \frac{(\rho_{\text{particle}} - \rho_{\text{liquid}})g d^2}{18\mu}$$

The velocity to a given time t can be found as

$$U(t) = U_{\infty}(1 - e^{-\frac{g}{U_{\infty}}t})$$

10 For a particle submerged in water with a diameter of $30 \mu\text{m}$ and a density of 1.2 times the density of water the settling velocity is $9 \times 10^{-5} \text{ m/s}$. The particle will reach 90 % of this velocity after 2.1×10^{-5} seconds why the transient phase can be neglected. It will take the particle 0.33 seconds to travel a distance of $30 \mu\text{m}$, which makes the method reasonable to employ for separation purposes.

15

While instrumentation in chemistry and biochemistry has become more automated in recent years, the preparation of samples remains a highly laboratory intensive task. The demand is increasing for high throughput, easier to use cost effective analytical devices and assays. Creating this opportunity is e.g. the world-wide effort to sequence the Human Genome, resulting in the
20 development of new DNA diagnostics and therapeutics. Another important trend is the minimisation of health care costs and hospital admissions by testing patients and monitoring therapeutic use in less expensive environments, the so-called point-of-care analysis.

Micro flow devices containing arrays of nucleic acid hybridisation sites, known as genosensors, are
25 being developed for a variety of uses in genomic analysis. A great deal of the overall genosensor development effort involves optimisation of experimental conditions in the actual use of genosensors.

Another embodiment of the invention is dealing with a low-tech form of genosensor and
30 immunosensor technology, involving arrays of oligonucleotides on a microchip, which can be used to define optimal operating conditions and to develop applications of hybridisation arrays in genome mapping and sequencing. The genosensor array is placed in a micro flow channel system allowing an operation in a flow-through mode. Thus several steps of microliquid handling, e.g. washing and staining steps, reagent addition, can be integrated as an automated routine

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procedure. Additionally, micro flow devices containing arrays of antibody/antigen sites, known as immunosensors, can be designed in the same way. The system could be used for combinatorial screening (high-throughput screening) and pharmacokinetic studies.

5 According to a sixth aspect of the invention the above and other objects are fulfilled by a micro flow system for analysing components of a fluid, comprising a member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.

The system may further comprise field generating means positioned proximate to at least some of the assay sites for generation of a field proximate to the corresponding assay site whereby reagents residing in the flow channel and being susceptible to the field when generated at a selected assay site are attracted to and immobilised at the selected assay site, or, are rejected from the selected assay site.

15 In an embodiment of the invention, the member comprises a plurality of flow channels arranged in parallel or in series and each of which has assay sites whereby the fluid containing particles is brought into contact with a large number of assay sites.

According to a seventh aspect of the invention, a method of analysing components of a fluid is provided, comprising the steps of entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, the channel having a plurality of assay sites, each of which comprises immobilised reagents whereby the fluid can be analyzed for a plurality of components while residing in the channel.

25 According to a eighth aspect of the invention, a method of forming assay sites comprising immobilised reagents in a flow channel is provided, the method comprising the steps of

30 preparing selected surfaces of the assay sites for immobilisation of selected reagents,

dispensing a selected reagent proximate to a corresponding selected assay site, and

35

generating a field proximate to the selected site whereby the reagent is attracted towards and brought into contact with the surface of the selected assay site by the field generated and is immobilised upon contact with the surface.

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Thus, the micro flow system of the previous section with a flow channel with assay sites may further comprise field generating means positioned proximate to at least some of the areas adapted to comprise immobilised reagents, each field generating means generating a field proximate to the corresponding area whereby reagents entering the flow channel and being susceptible to the field generated at the area are attracted to and immobilised at the area or are rejected from the area. Alternatively, the width of the channel of the micro flow system can be extended to accommodate a two-dimensional grid of areas to comprise immobilised reagents with fields generating means positioned proximate to at least some of these areas. In another embodiment the micro flow system for analysing a sample with a large number of reagents simultaneously may consist of an array comprising a number of parallel channels each with a plurality of areas adapted to comprise immobilised reagents located in the flow channels and further comprising field generating means to generate a field proximate to the areas whereby reagents being susceptible to the field are immobilised at the area. The field generating means may be e.g. permanent magnets, electrodes or electromagnets.

The devices with assay sites enable rapid manipulation, detection, and analysis of macromolecules, particles and cells in biologic or chemical samples in that a plurality of tests can be performed on the same microchip. According to the invention, micro flow systems and molecular biology are combined.

BRIEF DESCRIPTION OF THE DRAWINGS

Exemplary embodiments of the invention will now be described with reference to the accompanying drawings in which

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Fig. 1 illustrates the operation of particle separation according to the present invention,

Fig. 2 shows a cross-sectional view of a separation flow channel according to the present invention. (a) shows the main embodiment and (b) shows a cross-sectional view of a separation
10 flow channel for gravitational separation,

Fig. 3 shows a micro flow system with electrodes as field generating means,

Fig. 4 shows a flow diagram of a magnetic particle separation apparatus according to the present
15 invention,

Fig. 5 shows flow diagrams of various embodiments of the present invention. (a)-(d) show embodiments with various numbers of inlets and outlets, and (e) shows an embodiment with an enlarged separation chamber, and (f) shows an embodiment with an enlarged chamber for
20 collecting separated particles,

Fig. 6 illustrates entrapment of magnetic particles in a flow channel,

Fig. 7 shows a flow diagram for optical detection and hydrodynamic separation using a blocking
25 valve,

Fig. 8 shows a flow diagram for optical detection and hydrodynamic separation using syringe pumps,

Fig. 9 shows a flow diagram of two flow channels coupled in parallel (a) and in sequence (b) and
30 (c),

Fig. 10 illustrates the principle of introducing a pre-treatment facility in the member comprising the micro flow system, here further combined with a post-treatment facility or a hydrodynamic
35 separation facility,

Fig. 11 shows a flow channel for magnetophoresis,

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Fig. 12 shows a flow channel having a serial array of assay sites equipped with electrodes to immobilise probes,

Fig. 13 shows a flow channel having a serial array of assay sites equipped with magnets to
5 immobilise probes,

Fig. 14 shows a flow channel having a two-dimensional array of assay sites equipped with magnets to immobilise probes,

10 Fig. 15(a) and (b) shows two devices each comprising a parallel array of micro flow channels each of which contains an assay site,

Fig. 16 illustrates the preparation of a micro flow system,

15 Fig. 17 shows diagrams from the magnetic separation described in Example 3, and

Fig. 18 is a flow chart illustrating a process for separating fetal cells from a maternal blood sample by combining different separation methods as described in Example 4.

20 DETAILED DESCRIPTION OF THE DRAWINGS

According to a preferred embodiment of the invention, magnetically stained particles, e.g. cells labelled immunologically with magnetic particles, such as antibody-coupled magnetic beads, are separated from non-magnetic particles, i.e. non-labelled cells, by exposing the particles to a
25 magnetic field generated with a permanent or an electromagnet. Positive or negative selection methods may be employed. By positive cell separation, cells of a specific cell type are separated and isolated from a heterogeneous mixture of cells.

Fig. 1 illustrates the principle of the separation method according to the invention. A micro flow
30 system 1 is shown having three inlet and two outlet ports. The sample 9 containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation flow channel 5 of the micro flow system 1 by two guiding buffers 10 and 11, each of which enters the separation flow channel through inlet ports 3 and 4, respectively. A field generating means comprising a magnet 8 is located adjacent to the flow channel 5 and
35 generates a magnetic field across the flow channel 5. When the sample 9 containing particles passes the magnetic field, magnetically stained particles 12 are drawn into the guiding buffer 10 and leave the flow channel 5 together with the guiding buffer 10 through the sort outlet 6 while

non-labelled cells 13 which are not influenced by the magnetic force remain in the fluid 9 leaving the flow channel 5 through the waste outlet 7.

Due to the small channel dimensions, the flow is laminar with negligible influence of inertial forces. Mixing of the sample flow and the guiding buffers is not detectable since the contact area is small and the contact time is reduced to a subsecond range. The thickness of the sample flow can be precisely adjusted by variation of the flow rate of the two guiding buffers. This enables the adjustment and optimisation of the magnetic micro flow system for various cell types and sizes. The volume flow of the sample and the two guiding buffers are adjusted so that the particles in the sample are lined up into a single stream of particles.

The magnetic field in the micro flow channel operates as an extremely sensitive filter for magnetic particles, e.g. cells. Cells labelled with superparamagnetic beads (e.g. MACS, Dynal) are magnetised and attracted by the magnetic field whereby the flow of magnetised particles is deflected into the sort outlet. The short residence time of the fluids in the flow channel and the low Reynolds numbers of the flow in the flow channel minimise the influence of gravity compared to the influence of the magnetic force.

Fig. 2 shows a cross-sectional view of two variants of the micro flow system 1 manufactured utilising semiconductor technology. The micro flow system may be manufactured in any suitable material such as polymers, glass, semiconductors, such as silicium, germanium, gallium arsenate, etc., etc.

The first micro flow system (a) shown is a 3-layer sandwich. The central layer 14 is a silicon wafer having a flow channel 5 etched into it. The silicon wafer 14 is covered with a transparent plate 15, such as a glass plate, having a thickness of, e.g., 0.16 mm. Fluids inside the flow channel 5 may be observed through the glass plate 15, e.g. utilising a microscope 16 (detection means). The fluid inlet 2 and outlet 7 are connected to tubings 17, 18, e.g. fused silica capillary or Teflon tubings, for entering fluids into or discharging fluids from the flow channel 5. Buffer inlets 3 and 4 and the outlet 6 for the separated particles are not shown. The bottom plate 19, e.g. made of plastic, facilitates mounting of the tubings 17, 18.

A modified version (b) of the micro channel system for separation was designed with gravitation as the force field, thus sorting particles due to their density and/or diffusion constant, the latter mainly being controlled by the shape and size of the particles. The system is during operation positioned with the flow plane substantially perpendicular to the direction of the force of gravity. As illustrated in Fig. 2(b), this embodiment of a micro flow system 1 has a sample inlet port 2 and an outlet port 7 located above the micro channel 5 and a buffer inlet port 3 and an outlet port 6

located below the micro channel 5. The sample containing particles 9 enters the separation flow channel through inlet port 2, and a guiding buffer 10 enters the separation flow channel 5 through inlet port 3. In this way, two laminated layers of fluid extending along the horizontal plane are created continuously flowing through the separation flow channel 5 of the micro flow system 1. Particles move from the particle containing layer to the guiding buffer layer by sedimentation. When the sample containing particles 9 passes the flow channel 5, particles with certain density and size properties are drawn into the guiding buffer 10 by the gravitational force and leave the flow channel 5 together with the guiding buffer 10 through the outlet port 6 while particles which are less susceptible to the gravitational field remain in the sample 9 leaving the flow channel 5 through the waste outlet 7. The vertical displacement of a specific particle in the sample is given by its density and diffusion constant and the contact time of the sample layer with the guiding buffer layer. The contact time is defined by the total flow rate of the fluids passing through the micro systems 1 and the length of the micro channel 5. The system can be adjusted such that a desirable or appropriate specimen can be withdrawn and separated from the sample flow due to their density and/or diffusion properties by adjusting the volumetric flow rates of the guiding buffer and particle containing sample.

Alternatively, the micro flow system may comprise two further inlet ports for entering a second and a third guiding buffer into the micro channel 5, where the two further inlet ports are positioned above the micro channel, one on each side of the sample inlet port 2. The flow rates of the sample and the second and third guiding buffers may be adjusted so that the particles contained in the sample are lined up in a single line.

Characteristic features of an exemplary embodiment of a micro flow system according to the invention, e.g. as shown in Figs. 1 and 2, is shown in Table 1.

Table 1 Characteristics, micro flow system

| | | |
|----|-----------------------------|--|
| 30 | <u>Manufacturing method</u> | Material: Silicium Oxide, SiO ₂ |
| | | Photo-lithography |
| | | Wet-chemical etching |
| 35 | <u>Flow Channel</u> | |
| | Cross sectional area | 0.1 - 0.55 mm width x 0.04 - 0.2 mm depth |
| | Length | 1.0 - 200 mm |
| | Total flow rate [µl/min] | 1 - 200 |
| | Flow velocity [mm/min] | 15 - 180 |
| | Reynolds number | 0.1 - 20 |
| | Separation time | 0.1 sec - 6.0 sec [2 µl/min] |

Magnet

Permanent Magnet

Rare Earth Samarium-Germanium 0.5 x 0.5 x 0.2 mm

Electromagnet

5 Holding Magnet 25 mm 12 V D.C. RS

Fig. 3 shows a micro flow system 1 utilising electrodes 20, 21 to generate an electric field across the flow channel 5. The electrodes 20, 21 may introduce dielectrophoretic or electrophoretic forces
10 utilised for particle separation. For electrophoretic separation to take place, gold electrodes may be positioned at the inside of the walls of the flow channel 5. By applying a voltage across the electrodes, an electrical field is generated substantially perpendicular to a longitudinal axis of the flow channel. The electrical field cause deflection of charged particles or molecules in the flow channel 5 whereby electrically charged particles can be deflected away from the sample
15 containing particles flowing in the micro flow channel and into a guiding buffer also flowing in the flow channel and abutting the sample containing particles in the micro flow channel.

Fig. 4 shows a micro flow apparatus 22 including a micro flow system 1 as shown in Figs. 1 and 2. The micro flow system 1 has two inlets 2, 3 and two outlets 6,7, two syringe pumps 23, 24, two 3-
20 way control valves 25,26 and capillary tubings 27, 28. The capillary tubings 27, 28 are used for interconnecting the two syringe pumps 23, 24 with the inlets 3, 2, respectively, of the micro flow system 1.

Conventional syringe pumps with means, e.g. stepping-motors (not shown), to move the pistons at
25 a predetermined speed have been utilised for generating a continuous flow of the guiding buffer through the inlet tube 27 and a continuous flow of the sample through the inlet tube 28. The system can be operated in a first loading mode where the two 3-way control valves 25, 26 open for flow between the syringe pumps 23, 24 and the buffer reservoir 29 and the sample reservoir 30, respectively, and the syringe pumps 23, 24 are loaded with buffer and sample from the reservoirs
30 29, 30, respectively. In a consecutive second operational mode the two 3-way valves 25, 26 open for flow between the syringe pumps 23, 24 and the capillary tubing 27 to the buffer inlet 3 and the capillary tubing 28 to the sample inlet 2 of the micro flow system 1, respectively. The syringe pumps are in this second operational mode controlled to generate a predetermined volumetric flow rate through the micro flow system 1.

35

Fig. 5 illustrates various micro flow systems 31, 32, 33, 34, 35, and 36 having flow channels of different geometries, illustrating different embodiments of the invention. Micro flow systems with two or three inlet ports and two, three or five outlet ports, respectively, are shown in Figs. 5(a)-

(d). The system shown in 5(a) with inlet ports for sample and two guiding buffers, respectively, and sort outlet port and waste outlet port is similar to the system shown in Fig. 1. Figs. 5(b) and (c) show systems with multiple outlet ports, three and five, respectively, whereto particles can be sorted and leave the flow channel through according to their susceptibility to the applied field. A simple system with two inlet and two outlet ports are shown in Fig. 5(d) similar to the one in Fig. 2(b) that is used for gravitational sorting. A micro flow system with a separation channel equipped with a magnet where the width of the separation channel is enlarged before the bifurcation in a sort outlet and a waste outlet is shown in Fig. 5(e). According to the behaviour of liquids in a flow channel, the size of the cross-sectional area occupied by the sample flow is proportional to the width of the separation channel. According to this, the transversal distance between two particles A and B is increased proportional to the increase of the width of the separation channel. A larger distance between particles, which are to be separated, yields a higher selectivity of the mechanical separation. Fig. 5(f) shows a micro flow system where the width of the outlet channel 6 is increased to form a chamber where the sorted particles are collected for further processing or analysis, e.g. detection, staining, destaining or cultivation.

Fig. 6 illustrates a system in which particles are entrapped inside the micro flow channel 5 for a desired period using the electromagnet-equipped apparatus. In this case, the magnetic field is adjusted so that magnetic particles 12 are drawn to the inner wall of the micro flow channel 5 close to the electromagnet 8. Upon removal of the current to the electromagnet 8 the particles 12 are redispersed and are rapidly moved to the sorting outlet port 6. This 2-step sorting procedure is an alternative to the continuous sorting procedure that is particularly useful in sorting of extremely rare events where dilution of the sorted cell fraction could be a problem. The sorting outlet port 6 may be closed when the current to the electromagnet 8 is turned on and is open when the current to the electromagnet 8 is turned off. The figure shows magnetic particles 12 in the process of being withdrawn from a continuous sample flow 9. The magnetic particles 12 are attracted by the magnetic field and withdrawn from the sample flow 9 by precipitation at the inner wall of the micro flow channel 5 proximate to the electromagnet 8. When the current supplied to the electromagnet 8 is turned off, the magnetic particles 12 are released into the flow again. The separation flow channel may not have a sort outlet, instead a buffer may enter the micro flow channel 5 after the sample and the entrapped particles may be released by removing the current supplied to the electromagnet 8.

Fig. 7 illustrates another embodiment of the present invention for separation of particles fulfilling certain criteria from the sample 9 by hydrodynamic force. The apparatus comprises a 2-way valve 40 and a micro flow system having a separation flow channel 5 with three inlets 2, 3, 4, two outlets 6, 7 and a collecting chamber 37. The sample 9 containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation

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flow channel 5 of the micro flow system by two guiding buffers 10 and 11, each of which enters the separation flow channel 5 through inlet ports 3 and 4, correspondingly. The sample 9 is monitored utilising a microscope objective 16. The apparatus has control means 38 for controlling the two-way valve 40. The control means comprises monitoring means having an optical detection means, e.g. a photomultiplier system (PMT), a CCD camera/chip or a photo diode, optically connected to the microscope objective 16. The objective 16 is focused on the measuring volume, which is located inside the flow channel 5. The size of the measuring volume is defined by a pinhole or slit 39 positioned in front of the optical detector and by the magnification of the objective 16. The 2-way valve 40, e.g. a piezoelectric drop-on-demand ink-jet printing valve, is connecting the collecting chamber 37 to the sort outlet 6. The flow restriction of the waste outlet channel 7 is much higher than the flow restriction of the sort outlet channel. This can be achieved by attaching a flow restrictor (not shown) to the waste outlet channel 7. Thus, if the 2-way valve 40 is open the sample 9 containing particles is deflected towards the sort outlet 6. The collecting chamber 37 is used to collect and capture the sorted particles for post-analysis. Other particles continue to flow out through the waste outlet 7.

Particles are physically separated using hydrodynamic forces according to optical measurements on each particle. The photomultiplier (PMT) signal generated when a particle resides in the measuring volume is transmitted to a pulse-height analyser also comprised within the control means 38. A selection circuit provides an activating signal whenever a specific particle exhibits photometric properties of a predetermined type. If the PMT signal for a specific particle indicates that the particle is of a specific type an actuation pulse is produced. The valve 40 opens at the actuation pulse, causing the liquid containing the specific particle to flow through the sort outlet 6 and to be captured inside the collecting chamber 37. The duration of the actuation pulse is made sufficiently long for the desired particle to be transported into the collecting chamber 37. For light excitation several sources can be used, e.g. laser, tungsten lamp, photo diode. For bundling of the light, a fibre optic cable, a photo lens, an objective or a light microscope can be used. Various optical detection methods, e.g. fluorescence, absorbency, can be used.

The micro flow system may be positioned on a movable table so that the micro flow system may be moved into selected positions relative to the microscope whereby an appropriate volume of the micro flow channel may be moved into the measurement volume of the apparatus.

During or after sorting, the captured sample can be analysed again, using e.g. a microscope. When the valve 40 is closed, particles are entrapped inside the collecting chamber 37 and can be observed for a desired period. A desired liquid or reagent for washing, cultivation or staining of particles or cells may be entered into the collecting chamber 37. After the separation process, the

particles may be withdrawn by flushing the collecting chamber 37 with an appropriate buffer entering the micro flow system through one of the inlets 2, 3, 4.

5 The sorting apparatus was designed to achieve a minimal dilution of the separated sample fraction. Hydrodynamic separation of particles can be performed due to the optical, electrical, magnetic and/or other properties of the particle-containing sample.

10 An example of an optical and mechanical arrangement of the apparatus based on fluorescence detection is illustrated schematically in Fig. 7. The sample 9, e.g. particle suspension, is guided through the separation channel 5 by two guiding buffers 10, 11 so that the particles contained in the sample 9 are lined up in a single stream flowing in a plane perpendicular to the optical axis of the objective 16. The flow is illuminated with a mercury arc lamp passing excitation filters for e.g. fluorescein measurement. A dichroic mirror reflects the excitation light to the sorting chip via e.g. a 20x microscope objective 16. The fluorescence light emission is collected by the same objective 16
15 passing a dichroic mirror. Behind the mirror, a slit 39 works as field stop limiting the detection area to a small stripe. Each particle passing the objective 16 is generating a short signal from the photomultiplier that is optically connected to the objective 16. The photomultiplier signal is amplified and transmitted to a peak detector.

20 The actuation frequency of the valve 40 used in this device is 1500 Hz which corresponds to a minimal actuation time of 0.6 msec.

Fig. 8 illustrates an alternative embodiment of the separation apparatus shown in Fig. 7 with a separation flow channel 5 having three inlets 2, 3, 4 and two outlets 6, 7. The sample containing
25 particles enters the separation flow channel 5 through the centre inlet port 2 and two guiding buffers enters the channel 5 through the other two inlet ports 3, 4, respectively. Flow speed adjustment means comprising stepper motor driven syringe pumps 41, 42 are connected to the two outlet ports 6, 7, respectively. The syringe pumps 41, 42 suck the sample and buffer via inlet 2, 3 and 4, respectively, through the separation flow channel 5. The cells are monitored at the
30 optical axis of the microscope objective 16 and flow to the separation junction. The guiding buffers and the sample containing unselected cells flow out into the waste outlet syringe pump 42. If a specific cell has optical properties causing an actuation pulse, the stepper motor of the pump 41 at the sort outlet is actuated and the stepper motor of the pump 42 at the waste outlet is stopped causing the liquids to flow to the sort outlet 6. The period the pump 41 at the sort outlet is
35 switched on, respectively the pump 42 at the waste outlet is switched off is made sufficiently long to ensure that the desired cell has entered into the collecting chamber 37. When one of or both syringe pumps 41, 42 after some operation time need to be emptied, the 3-way valves 43, 44 are switched from their normal operation position where they open for flow from the separation flow

channel 5 to the syringe pumps 41, 42, respectively, into a position where the 3-way valves 43, 44 open for flow between the syringe pumps 41, 42 and a waste container (not shown) and where the stepper motors driving the syringe pumps 41, 42 are operated in the reverse direction of the normal operation direction to empty the syringe pumps 41, 42.

5

Fig. 9(a) shows two flow channels 45, 46 operating in parallel. The sample containing particles enters the flow channels 45, 46 through inlet ports 47, 48, respectively. The guiding buffer enters the flow channels through the inlet ports 49, 50, respectively. In the flow channels 45, 46, particles susceptible to the magnetic field generated by magnets 51, 52, respectively, are deflected from the sample containing particles into the corresponding guiding buffer and flow thereafter through the sort outlet 53. The remaining part of the sample leave the flow channels 45, 46 through the waste outlets 54, 55, respectively. Separation is increased by using a plurality of flow channels coupled in parallel.

15 Fig. 9(b) and (c) shows examples of combinations of micro flow systems for magnetic, hydrodynamic or gravitational separation. In Fig. 9(b), particles are first separated from a sample in a magnetic separation channel, where after the sorted particles are subjected to a hydrodynamic separation due to the optical properties of the particles. Thus, it is possible to analyse and separate particles from a sample based on both optical and magnetic properties of the particles or to another combination of properties or characteristics. In Fig. 9(c), two magnetic separation channels are coupled in series in order to obtain a highly purified product.

Fig. 10 illustrates examples of micro flow systems having means for automated labelling of particles with fluorescence or magnetic probes. The system may be combined with post-treatment means for removal of the probes or for other treatment of the sorted particles. The system contains a micro flow system containing channels 56, 57 for addition of liquids to the sample, e.g. reagents for cell lysis or staining, a channel 58 for incubation and cultivation or storage of the sample for further processing and a separation channel 5. A sample is introduced into the micro flow system via an inlet 2 and one or more reagents can be added continuously to the sample, which is transported into the incubation channel 58. A simple micro flow structure was constructed for sample pre-treatment. Preferably, the flow rates are managed by computer-controlled syringe pumps. The incubation period between mixing and analysis of the sample is given by the volumetric flow rate of the syringe pumps and the cross-sectional area and length of the incubation channel.

35

Fig. 11 shows a micro flow system for magnetic separation of macromolecules, i.e. ribonucleic acid or proteins from a sample. Magnetic beads labelled with a fluorescence dye and a probe, specific for i.e. DNA are added to the sample which is then incubated. This sample is entered via inlet

port 2 into the separation chamber 5 and the particles are drawn by the field generated by the magnet 8 along the separation channel 5 due to their mobility. After a defined period, the magnetic field is removed and the fluorescence banding can be observed under a microscope. By running standards of known size, it is possible to calibrate the system and to separate particles of e.g. DNA due to their size and shape, similar to electrophoresis.

Fig. 12(a) with details in Fig. 12(b) shows a serial sensor array. A micro flow channel 5 has a plurality of assay sites, each equipped with field generating means 59 that may be individually turned on and off. The flow channel 5 shown has rectangular electrodes 59 positioned in small grooves at the bottom wall of the flow channel 5. A voltage can be applied selectively to each electrode 59. Various probes, receptors, indicators, etc. may be attracted to and immobilised at selected electrodes 59 by applying a voltage to the selected electrodes 59 while a fluid containing the corresponding probes, receptors, indicators, etc. resides in the flow channel 5. Preparation of the multiple assay sites may be accomplished by sequentially loading each assay site with a specific probe. Voltage is applied to one or more specific electrodes in the micro flow channel 5, and a fluid containing a specific probe, reagent or indicator, etc. is entered into the micro flow channel 5 where the probes etc. will be attracted to the electrodes to 59 which the voltage is applied. Subsequently, the voltage is turned off. Then, a voltage is applied to the next electrode 59 and the next fluid containing a specific probe etc. is entered into the micro flow channel 5. Thus, various assay sites each containing a specific probe, reagent, or indicator can be created. Antibodies, fluorescence molecules, DNA, RNA and protein dyes are examples of probes.

As an alternative to the electrodes 59, magnetic force can be selectively applied to the assay sites with an array of electromagnets positioned at or near the surface of the micro flow channel 5 to immobilise probes etc. that have magnetic properties to desired assay sites. Alternatively, a photoactivation process can be used for covalent coupling of molecules or particles to the surface of the channel 5 at the assay site.

One example of a probe is DNA, which has an overall negative charge, drawn to the electrode 59 surface by a positive bias, another example is DNA-coated magnetic particles that are drawn to the surface of micro flow channel 5 by magnetic means.

By modification or coating of the surface of the micro flow 5 channel and/or the electrodes 59 or magnets, specific chemical and mechanical properties can be created. To increase the binding forces of the probe, the surface may be coated with a specific layer or matrix, e.g. a polymer such as urethane or a reactive chemical group. When the current to the selected electrode or electromagnet is switched off the probe remains on the surface e.g. by absorption. Thus, an encapsulation or immobilization of the molecule is achieved.

Thus, a field generated at selected assay sites across the flow channel 5 may be utilized for immobilization of particles, such as biomolecules, whereby these may be held in substantially fixed positions within the flow channel permanently or for a specific period of time allowing
5 chemical reactions between the particles and an entered reagent to take place and/or kinetic measurements on the particles to be performed and/or the particles to be brought into contact with different chemical substances. For analysis of the reactions in the micro flow system, optical detection means, e.g. a microscope, may be used.

- 10 It is an important advantage of the device that a number of assays can be performed in a single device. During operation of the device, various processing steps, such as e.g. washing steps, and reagent addition, etc., may be performed.

- 15 Fig. 13 shows a micro flow device with a flow channel 5 and with a serial array of assay sites and permanent magnets 8 positioned on a separate cartridge 60. A second cartridge 61 has a flow channel 5 with an inlet 2 and an outlet 7. The cartridge 60 carrying the magnets 8 can be positioned exactly below the second cartridge 61 so the magnets 8 are accurately positioned below the assay sites in the flow channel 5 as shown in the figure below cartridge 61.

- 20 Probes to be immobilised at a specific assay site utilising a magnetic field, as described in Fig. 12 or Fig. 13, may be positioned at the desired assay site by a method comprising the steps of positioning a defined volume of the liquid containing the magnetic probe or reagent using e.g. inkjet based dispenser technology, within a specific volume of the flow channel 5 right over one of the permanent magnets 8 for immobilization of the magnetic probe or reagent in an assay site at
25 the surface of the flow channel 5. The method may be repeated for various probes to be immobilised at various assay sites, respectively. After the immobilization, the cartridge 61 containing the flow channel 5 is covered by a transparent cartridge 15, e.g. a glass plate, allowing the assay site array with the probes inside the micro flow channel 5 to be observed. An analysis with the assay site array is performed by introducing a sample through inlet 2 into the micro flow
30 channel 5 where it passes the array of assay sites and leaves the micro flow channel 5 through outlet 7. An objective 16, optically connected to an optical detector, e.g. a fluorescence microscope, may be focused on the array in the micro flow channel 5 to monitor the chemical reactions at the assay sites.

- 35 Fig. 14 shows another embodiment of the invention comprising a cartridge 62 with a micro flow channel containing assay sites 63 arranged in a two-dimensional array, a cartridge 64 with permanent magnets 8 and a transparent cartridge (not shown) to cover the cartridge 62 with the micro flow channel. The assay sites 63 are formed as small grooves at the surface of the bottom

wall of the micro flow channel. The dimensions of the cartridges 62, 64 and the position of the assay sites 63 and the magnets 8 are the same, so if cartridge 62 is placed over cartridge 64 as shown in Fig. 14(b), the magnets 8 are located under the assay sites 63.

5 The embodiments shown in Fig. 12, 13 and 14 may be utilised for hybridisation of DNA as described below. A magnetic carrier including a DNA probe may be immobilised at a specific assay site as described previously. In this way, an array of assay sites is created in a micro flow channel wherein each assay site contains a different DNA probe. Thereafter, a sample containing target molecules is entered into the micro flow channel preferably until the sample has filled the
10 micro flow channel. After the target molecule has been hybridised to a DNA of a specific assay site, a solution of reporter probes, e.g. fluorescence probes, is entered into the flow channel where it binds on the assay site carrying the hybridised DNA. By using a fluorescence detector, e.g. a photomultiplier, focused on the different assay sites the reaction at each assay site can be monitored. By removing the magnetic field on a specific assay site the magnetic material
15 including the DNA probe can be removed, so the process can be reversed. Thus a renewable array of assay sites can be created and wash processes can be implemented in the operation of the array of assay sites.

Fig. 15(a) shows a device according to the invention for performing a multiple assay analysis in a
20 micro flow system by using a parallel array of assay sites. The system comprises an array of parallel micro flow channels 65 each of which contains one assay site with a specific probe immobilised using e.g. an electrical or magnetic field or by photoactivation as described previously. For example, a cartridge containing a permanent magnet (not shown) can be positioned below the parallel micro flow channels 65 in whereby magnetic probes can be
25 immobilized in the micro flow system using dispensing technology. In this way, a plurality of assay sites may be created in the parallel flow channel 65 array allowing a simultaneous analysis of a sample with a plurality of probes or reagents defined by the number of parallel micro flow channels 65.

30 The micro flow system consists of two parallel flow channels 66, 67 which are connected via a number of parallel micro flow channels 65 each containing an assay site. An injection flow channel 66 has an inlet 2 and is connected to an outlet 68 via a blocking valve 69, and the waste flow channel 67 is connected to a waste outlet 70 via a blocking valve 71. By blocking one of the two outlets 68, 70 with the blocking valves 69, 71, respectively, it is possible to guide the injected
35 flow through the array of channels 65 containing assay sites or through the injection channel outlet 68, respectively. The flow through all channels 65 containing assay sites is merged into the waste channel 67 and is leaving the system via the waste outlet 70. During passage of the

channels 65 containing the assay sites, the sample comes into contact with sensing probes, which are immobilized at the assay sites. Chemical reactions may be detected as described for Fig. 12.

In Fig. 15(b) an alternative embodiment of an array of parallel channels 65 containing assay sites is shown. The micro flow system has three inlet ports 2, 3, 4 to enter different liquids into the micro flow system. By connecting outlet port 68 to a flow restrictor, only one blocking valve 71 is needed to operate the system. If the blocking valve 71 between the channel array 65 and the waste outlet 70 is closed, the channel array 65 is blocked and the flow from inlet 2, 3, 4 will pass the injection channel 66 and leave the micro flow system via outlet 68. When the blocking valve 71 is open, the liquid introduced in the injection channel 66 will flow into the sensor channel array 65 because of the higher flow restriction at outlet 68 compared to waste outlet 70.

Fig. 16 shows a micro flow system manufactured as a 3-layer sandwich. The central layer is a silicon wafer having a flow channel etched into it. The silicon wafer is covered with a transparent plate, such as a glass plate, having a thickness of, e.g., 0.16 mm. Fluids inside the flow channel may be monitored through the glass plate, e.g. utilising a microscope or other optical detection means. The fluid inlet and outlet are connected to tubings, e.g. fused silica capillary or Teflon tubings, for entering fluids into or discharging fluids from the flow channel. Buffer inlets and the outlet for the sorted particles are not shown. The bottom plate, e.g. made of plastic, facilitates mounting of the tubings.

Figs. 16(1) to (5) illustrates the following description of the manufacturing and preparation of a micro flow system. A separation flow channel was designed to fit into a system comprising a bonded silicon/glass sandwich. The micro channels were etched into a silicon wafer covered with a boron glass plate having a thickness of 0.2 mm allowing monitoring of the micro channels, using i.e. a microscope. The separation flow channel was fabricated on a 4", 350 μ m, <100> silicon wafer. A 1.5 μ m layer of SiO₂ was applied to the surface of the silicon wafer and was patterned with a mask containing the channel layout. A 2.6 μ m layer of photoresist was spun on top of the SiO₂ and patterned with a mask defining intermediate holes. The two-step mask consisting of a SiO₂ mask and a photoresist mask was used for etching a two level structure with vertical walls by reactive ion etching (RIE) in a SF₆:O₂ plasma. The holes were initially etched to a depth of 22 μ m and then etched deeper together with the channels, which were etched to depths in the range from 40 μ m to 100 μ m. A layer of 1.8 μ m SiO₂ was patterned with a mask for inlets and outlets on the back of the silicon wafer. The etching was carried out in KOH at 80°C and was stopped when all the intermediate holes were clearly visible from the back. Finally, a glass wafer was anodically bonded to the silicon wafer. The micro channels were designed for volumetric flow rates of 0.1 to 200 μ l/min with a mean flow speed of maximum 100 (mm/min).

The separation flow channel may be provided with one or two permanent or electromagnets in three different ways:

- (a) Rare earth Samarium-Cobalt block magnets of 1.0 x 1.0 x 0.5 mm (Goudsmit, Netherlands) may be glued with silicon rubber into the opening slot of the separation flow channel.
- 5 (b) Rare earth (Sr) magnetic powder (Tropag, Hamburg, Germany) can be mixed with epoxy 1:1 (v/v) and this magnetic paste may be glued into the opening slot of the separation flow channel yielding a thick film magnetic layer of 1.0 x 1.0 x 0.5 mm.
- (c) Ferrite steel wool may be glued with silicon rubber into the opening slot of the separation flow channel. A high magnetic field gradient can then be induced inside the opening slots by applying
- 10 an external magnetic field, e.g. by an electromagnet (Goudsmit, Netherlands) positioned proximate to the separation flow channel.

EXAMPLE 1

- 15 A micro flow system with a layout as sketched in Fig. 5(d) with two inlets and two outlets has been tested utilising it for separation of various magnetisable particles. The test conditions are listed below.

| | | |
|----|------------------------------|------------------------------|
| | Particle concentration | 10 ⁷ particles/ml |
| 20 | Total flow rate | 25 µl/min |
| | Length flow chip | 3.5 mm |
| | Channel width | 250 µm |
| | Channel depth | 60 µm |
| | Separation time | 2.4 sec |
| 25 | Desired particle deflection: | 10 µm |

The separation efficiency (enrichment rate) E and depletion rate 1/E are defined by

$$E = \frac{\frac{\% \text{ positive particles after separation}}{\% \text{ negative particles after separation}}}{\frac{\% \text{ positive particles before separation}}{\% \text{ negative particles before separation}}}$$

- 30 For separation of various paramagnetic standard beads of different sizes and paramagnetic field strength, the results are shown in the Table 2.

Table 2 Separation efficiencies

| | Paramagnetic Bead | Size µm | Separation Efficiency [%] ¹ | | |
|----|--------------------------|------------|--|------|------|
| | | | A) | B) | C) |
| 5 | Polysciences | | | | |
| | 25 % iron-oxide | 1-10 | >99 | >99 | 95 |
| | 50 % iron-oxide | 1-10 | >99 | >99 | 96.5 |
| | Paesel + Lorei | | | | |
| 10 | Magnetic Affinity | 0.5-1.5 | >99 | >99 | 97.5 |
| | Boehringer | | | | |
| | Streptavidin Magnetic | 1 | 90.5 | 88.7 | 89.5 |
| | Dynal | | | | |
| | Magnetic Mass Dyal M-450 | 1-10 | 98.0 | >99 | 96.5 |
| 15 | | | | | |

¹ total flow rates: A)= 10 µl/min, B)= 50 µl/min, C)= 100 µl/min

EXAMPLE 2

20 Further, the micro flow system used in Example 1 has also been tested by utilising it for separation of Human T-lymphocytes (JURKAT cells). Magnetically stained and unstained JURKAT cells were used to form a heterogeneous cell sample. For magnetic staining of the cells, a CD4-magnetic surface marker from Miltenyi Biotec was used. JURKAT cells were suspended in 1% PBS/BSA to a concentration of 10^7 /ml. Biotin-conjugated CD4 magnetic microbeads were
25 added at 2 µl stock/ 10^7 cells following the manufacturer instruction.

The magnetically stained cells (10^7 cells/ml) flowed through the microchip for 10 min. and fluids were collected at the two outlets. Three experiments at different flow rates (5, 25, 50µl/min) were performed. The same experiments were performed using magnetically unstained cells.

30

An aliquot of the collected samples was analysed under a microscope and the particles were counted using a Neubauer microscopy chamber. For each experiment 1 µl sample was analysed:

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| Run | flow rate [μl/min] | cells [%] at Sort outlet |
|-----|----------------------------|-----------------------------|
| 5 | Negative (unstained cells) | |
| | 5 | <0.1 |
| | 25 | <0.1 |
| | 50 | <0.1 |
| | Control ¹ | |
| 10 | 5 | n.n. |
| | 25 | n.n. |
| | 50 | n.n. |
| | Positive (stained cells) | |
| | 5 | 95.5 |
| 15 | 25 | 92.8 |
| | 50 | 80.5 |
| | Control ¹ | |
| | 5 | n.n. |
| | 25 | n.n. |
| 20 | 50 | n.n. |

¹ using the micro flow system without an integrated magnet

EXAMPLE 3

25

The system employed for separation of magnetisable particles from a sample is shown in Fig. 4. It comprises two syringe infusion pumps (Harvard Apparatus, Southnatic, Az) that provides constant flow rates of 0.1 to 100 μl/min using a 0.5 ml micro syringe (Hamilton, Bonaduz, Switzerland), a separation flow channel of silicon for the separation of the magnetisable particles, and a collecting unit for collecting of the sorted particles. Two 3-way microvalves (Lee, Parameter AB, Sweden) were integrated into the apparatus for sterile solution handling. All components were interconnected with fused silica capillaries (340 μm id., Supelco, U.S.A.). The SFC was placed under an inverted microscope (Axiovert 100, Zeiss, Germany) for visualisation of the separation procedure. All micro channels and tubing were deactivated by silanisation as described in Blankenstein, G. Scampavia L, Branebjerg J, Larsen UD, Ruzicka J (1996): Flow switch for analyte injection and cell/particle sorting in Analytical Methods and Instrumentation, μTAS '96 conference, 17-22 November 1996, Basel. A FACScan with 488 nm argon laser excitation and collection of forward and side scatter and fluorescence of fluorescein were used (Becton Dickinson,

Mountain View, CA) for all experiments. Results were collected and analysed using the FACScan research software (Becton Dickinson).

Results on the use of a separation flow channel equipped with a permanent magnet optimised for Dynal beads are shown in Fig. 17. A bead suspension of $1-5 \times 10^6$ particles/ml containing a mixture of non labelled magnetic Dynal particles (d: $4.5 \mu\text{m}$, M-450) and fluorescence calibration beads (d: $3.2 \mu\text{m}$, Dako A/S, Glostrup, Denmark) have been separated. About 1 ml of the non-magnetic, non-deflected fraction was collected at the waste outlet and analysed by flow cytometry (B). To enumerate the positive and negative fractions, two windows were set for the statistic evaluation. Before separation, the sample contained 38.3 % fluorescence particles and 55.8 % magnetic particles, respectively (a). After sorting by the described system almost all magnetic particles were found in the sorted fraction collected from the sort outlet (b) and non-magnetic particles were found in the negative fraction (c) collected from the waste outlet, respectively. Under optimised conditions, an enrichment rate of 350 was achievable.

EXAMPLE 4

This example concerns enrichment of fetal cells in a sample for magnetic activated cell sorting. A combination of the embodiment of the invention as shown in Figs. 7 and 10 (upper), optical cytometry, and Figs. 4 and 10 (lower), magnetic cell separation, provides a powerful apparatus for efficient enrichment of fetal cells in a sample.

The process for increasing the concentration of fetal cell in maternal blood samples involves the following steps (see Fig. 18): (i) A first selection step for removal of the majority of the maternal blood cells based upon their volume, size and density; (ii) A second sorting step for isolation of the fetal blood cells from the remaining maternal blood cells based on immuno-fluorescent separation using a device as described in Fig. 7 and/or based on immuno-magnetic separation using a device as described in Fig. 4. In the examples shown in Fig. 9(b), the magnetic blood sample is first separated in a magnetic separation chamber, followed by a separation due to optical properties of the sample, or two magnetic separations are performed one after the other, see Fig. 9(c), in order to obtain a highly purified product.

An example of sorting of particles of very low concentration from a sample of maternal blood in a non-invasive prenatal screening test is presented in the following paragraph.

Nucleated red blood cells are found in maternal blood in a concentration of 10 to 1000 per ml of all nucleated cells. Bianchi has shown (D.W. Bianchi, Journal of Pediatrics, 1995, 127, 6, p. 847-856) that it is possible to use such cells for genetic screening in prenatal diagnosis. The cell surface

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marker CD71+ for example, is an appropriate marker to select such cells from maternal blood. Test results demonstrates that magnetic activated cell sorting is powerful enrichment system for sorting and isolating fetal nucleated blood cells from maternal blood. For this the magnetic activated cell micro technology as described in this invention is used. Fetal cells are distinguished
5 and separated from maternal blood by the use of a specific surface marker (CD71) which is only present on the cell membrane of fetal nucleated blood cells. By selectively attaching a magnetic antibody probe to CD71, a magnetic probe is attached substantially exclusively to fetal cells.

EXAMPLE 5

10

This example concerns depletion of magnetically labelled CD45 positive cells (maternal leukocytes) from a maternal blood sample spiked with cord blood. A flow chip described in Fig. 1 was used in a system as described in Fig. 4. In this experiment a 1:3 spike (fetal/maternal, v/v) was used to demonstrate the performance of the magnetic separation. Heparin was used as an
15 anti-coagulant. The nucleated cells were labelled with CD45 coated magnetic 0.1 μ micro particles (Immunicon, U.S.A.), using a monoclonal antibody against CD45 as the first layer. The cell suspension was collected at both outlets 6 and 7 (see Fig. 1). For testing the sorting efficiency, parts of both the collected fractions were analysed on microscope slides. The results showed that most of the cells, more than 95%, collected at the sort outlet 6 were CD45 positive.

20

EXAMPLE 6

Fluorescence activated cell sorting using the device described in Fig. 7. First results have shown an enrichment factor of more than 300, which indicates that the employed device is a powerful
25 tool for enrichment of rare cellular events.

EXAMPLE 7

An example is given for the embodiment of the invention as described in Fig. 12 for the use of
30 multiple sensor array technology for sensing of a group of analytes in one step. For this purpose, the biosensing components such as antigens or antibodies can be loaded into a specific assay of the flow channel and immobilised there.

Magnetic particles carrying an antigen probe are immobilised on the surface of the micro flow
35 channel by magnetic means. The immobilisation of each probe is exactly specified to a site by switching on a specific electro-magnet.

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After loading the surface with different groups of antigen probes, the test solution is guided through the flow channel 5 of the microchip. If the sample contains an antibody, which is complementary to one of the different antibodies, it will bind to that specific site where this antibody is immobilised. In a third step, the sample solution has to be removed, and a liquid
5 containing a secondary antibody against the FC region of the first antibody is guided through the micro flow channel. The secondary antibody is coupled to a fluorescence dye allowing the identification of a specific assay site where the antibodies has been binded. The device can be used for rapid screening of blood samples, e.g. for identification of bacteria or virus in blood samples having a micro flow channel with virus/bacteria specific antigen probes.

10

CLAIMS

1. A micro flow system for separating particles, comprising a member having
5 a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

first inlet means positioned at one end of the flow channel for entering the fluid into the flow
10 channel,

first outlet means positioned at the other end of the flow channel for discharging the fluid from the flow channel,

15 the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field
20 across the flow channel are deflected in the direction of the field.
2. A micro flow system according to claim 1, wherein the member further comprises field generating means positioned proximate to the flow channel for generating a field substantially perpendicular to a longitudinal axis of the flow channel.
25
3. A micro flow system according to claim 1 or 2, further comprising monitoring means positioned at the flow channel for monitoring parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to a monitored parameter.
30
4. A micro flow system according to claim 3, wherein the monitoring means comprise optical detection means for monitoring optical parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to an optical parameter.
35
5. A micro flow system according to claim 3 or 4, wherein the monitoring means comprise a Hall sensor for measurement of a magnetic parameter of a magnetic particle within a specific volume of the flow channel.

6. A micro flow system according to any of claims 3-5, further comprising field generating control means for controlling the strength of the field generated by the field generating means in response to the output signal of the monitoring means whereby particles are separated according to values of a parameter monitored by the monitoring means.
7. A micro flow system according to any of the preceding claims, wherein the Reynolds number of the flow of the fluid containing the particles through the channel is in the range of 0.01-500, preferably in the range of 0.05-50, in particular in the range of 0.1-25.
8. A micro flow system according to any of claims 1-6, wherein the lowest cross-sectional area of the flow channel is in the range of 0.004-0.11 mm².
9. A micro flow system according to any of the preceding claims, further comprising second outlet means for discharging particles having been deflected in the flow channel.
10. A micro flow system according to any of the preceding claims, wherein the field generating means comprises a magnet.
11. A micro flow system according to claim 10, wherein the field generating means further comprise ferrite members positioned at the flow channel for focussing of a magnetic field.
12. A micro flow system according to any of the preceding claims, wherein the field generating means comprises an electrode.
13. A micro flow system according to any of the preceding claims, wherein positions in relation to the flow channel of the field generating means are adjustable for adjustment of the strength of the field across the flow channel.
14. A micro flow system according to any of the preceding claims, further comprising flow speed adjustment means for adjustment of the time the particles reside in the flow channel.
15. A micro flow system according to any of the preceding claims, further comprising a cover for covering the flow channel.
16. A micro flow system according to claim 15, wherein the cover is a transparent or translucent cover allowing optical monitoring of the flow channel.

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17. A micro flow system according to any of the preceding claims, further comprising second inlet means for entering a first guiding buffer, the cross-section and the path through the flow channel of the flow of the fluid containing particles being controlled by the first guiding buffer flow.
- 5 18. A micro flow system according to claim 17, further comprising third inlet means for entering a second guiding buffer, the cross-section and the path through the flow channel of the flow of the fluid containing particles being controlled by the first and second guiding buffer flows that surround the flow of the fluid containing particles.
- 10 19. A micro flow system according to claim 18, wherein the width and the position of the flow of fluid containing particles is controlled by adjustment of the volumetric ratio between the fluid flow rate and the flow rate of the guiding buffers.
20. A micro flow system according to any of the preceding claims, wherein the deflected particles
15 comprise living cells.
21. A micro flow system according to any of the preceding claims, wherein the deflected particles comprise beads, microspheres, chromosomes, organelles, biomolecules, or proteins.
- 20 22. A micro flow system according to any of the preceding claims, wherein the deflected particles have been magnetically, chromophorically, or fluorescently stained.
23. A micro flow system according to any of the preceding claims, comprising a plurality of outlets for discharging of particles separated according to their different susceptibility to the field across
25 the flow channel.
24. A micro flow system according to any of the preceding claims, wherein the member further comprises pre-treatment and/or post-treatment facilities.
- 30 25. A micro flow system according to claim 24, wherein the pre-treatment facilities comprise incubation means for preparing or pre-reacting the fluid comprising the particles.
26. A micro flow system according to claim 24 or 25, wherein the pre-treatment facilities comprise means for magnetic, fluorescent, or chromophoric staining.
35
27. A micro flow system according to claim 24, wherein the post-treatment facilities comprise means for collecting or concentrating the deflected particles.

28. A micro flow system according to claim 24, wherein the post-treatment facilities comprise means for bringing the deflected particles into contact with one or more reagent(s).

29. A micro flow system for separating particles, comprising a member having

5

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

10

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

first and second outlet means positioned at the other end of the flow channel for discharging of fluid from the flow channel,

15

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

monitoring means positioned at the flow channel for monitoring parameters of a particle residing within a measurement volume within the flow channel and providing an output signal

20

corresponding to a monitored parameter,

control means for controlling passage of fluid through the first and the second outlet means, respectively, in response to the output signal of the monitoring means whereby particles may be separated according to values of a parameter monitored by the monitoring means.

25

30. A micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

30

inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

field generating means positioned proximate to the other end of the flow channel for generating a field substantially along a longitudinal axis of the flow channel whereby the particles are drawn by the field along the channel and distributed according to their susceptibility to the field and their mobility.

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31. A micro flow system for analysing components of a fluid, comprising a member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.

32. A micro flow system according to claim 31, further comprising field generating means positioned proximate to at least some of the assay sites for generation of a field proximate to the corresponding assay site whereby reagents residing in the flow channel and being susceptible to the field when generated at a selected assay site are attracted to and immobilised at the selected assay site, or, are rejected from the selected assay site.

33. A micro flow system according to claim 31 or 32, wherein the member comprises a plurality of flow channels arranged in parallel or in series and each of which has assay sites whereby the fluid containing particles is brought into contact with a large number of assay sites.

34. A method of separating particles, comprising the steps of
guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel,
positioning the flow channel in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field and thereby separated from the fluid.

35. A method of separating fetal cells from maternal cells, comprising the steps of
selective magnetically staining of fetal cells in a fluid containing fetal and maternal cells,
guiding a flow of the fluid containing the fetal cells through a flow channel in such a way that one fetal cell at the time passes a cross-section of the flow channel,
positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained fetal cells residing in the flow channel are deflected in the direction of the magnetic field.

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36. A method of separating cancer cells from other cells, comprising the steps of
- selective magnetically staining of cancer cells in a fluid containing cancer and other cells,
- 5 guiding a flow of the fluid containing the cancer cells through a flow channel in such a way that one cancer cell at the time passes a cross-section of the flow channel,
- positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained cancer cells residing in the flow
- 10 channel are deflected in the direction of the magnetic field.
37. A method of separating particles, comprising the steps of
- guiding a flow of a fluid containing the particles through a flow channel in such a way that one
- 15 particle at the time passes a cross-section of the flow channel, the flow channel having first and second outlet means for discharging of fluid from the flow channel,
- monitoring parameters of a particle residing within a measurement volume within the flow channel and
- 20 controlling passage of fluid through the first and the second outlet means, respectively, in response to a monitored parameter value whereby particles may be separated according to values of a monitored parameter.
- 25 38. A method of analysing components of a fluid, comprising the steps of entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, the channel having a plurality of assay sites, each of which comprises immobilised reagents whereby the fluid can be analyzed for a plurality of components while residing in the channel.
- 30 39. A method of forming assay sites comprising immobilised reagents in a flow channel, comprising the steps of
- preparing selected surfaces of the assay sites for immobilisation of selected reagents,
- 35 dispensing a selected reagent proximate to a corresponding selected assay site, and

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generating a field proximate to the selected site whereby the reagent is attracted towards and brought into contact with the surface of the selected assay site by the field generated and is immobilised upon contact with the surface.

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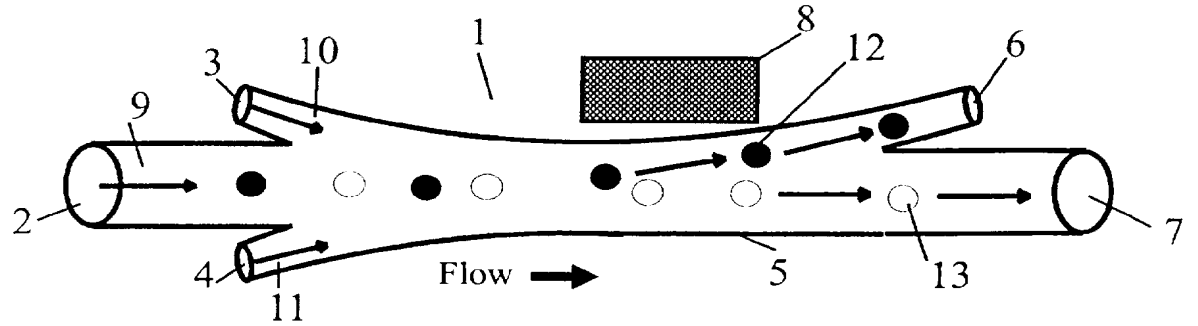


Fig. 1

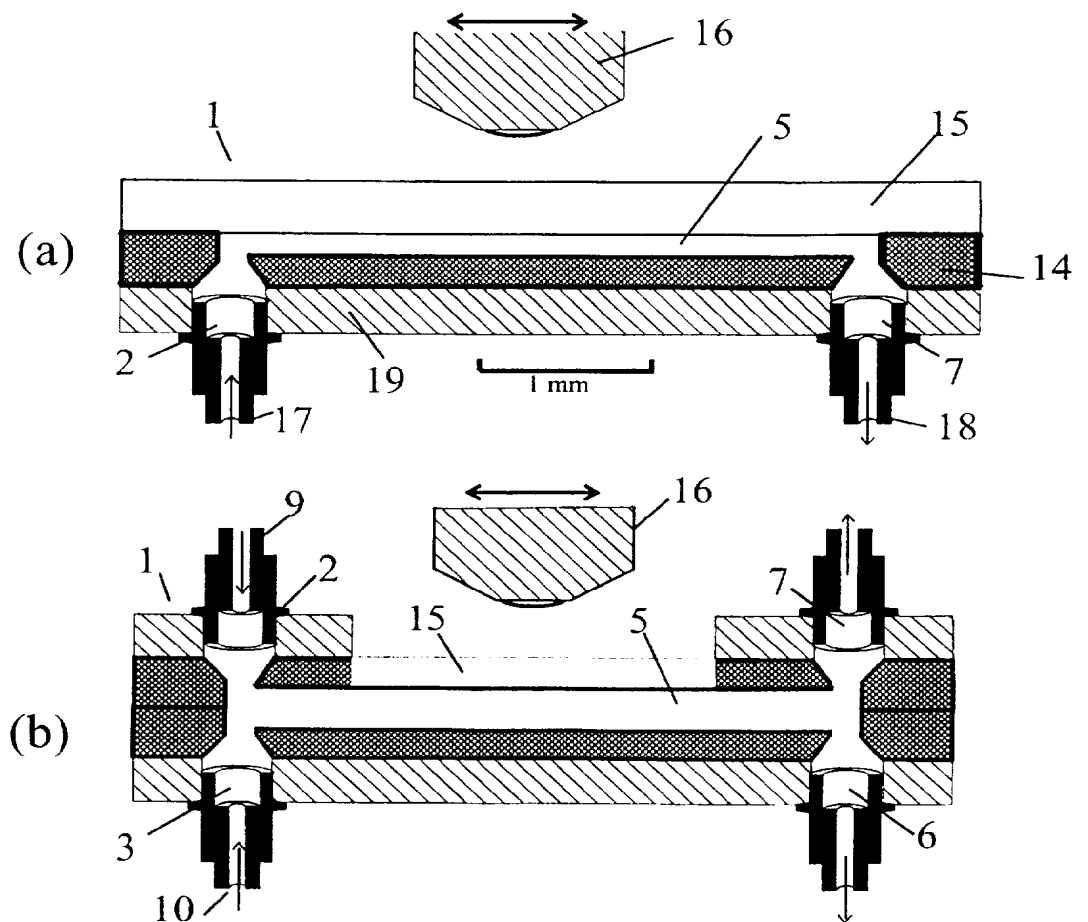


Fig. 2

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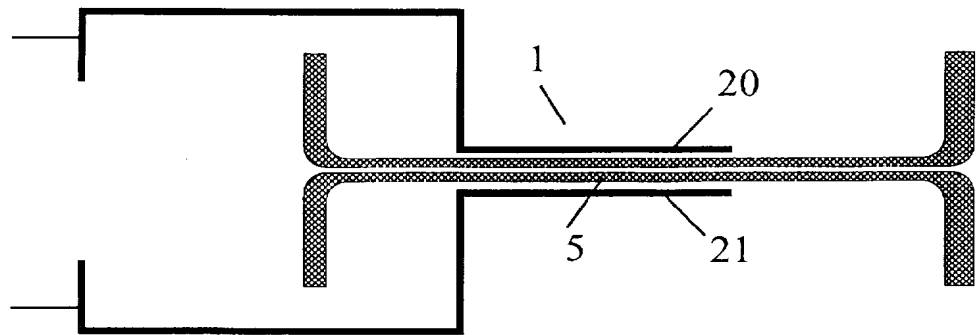


Fig. 3

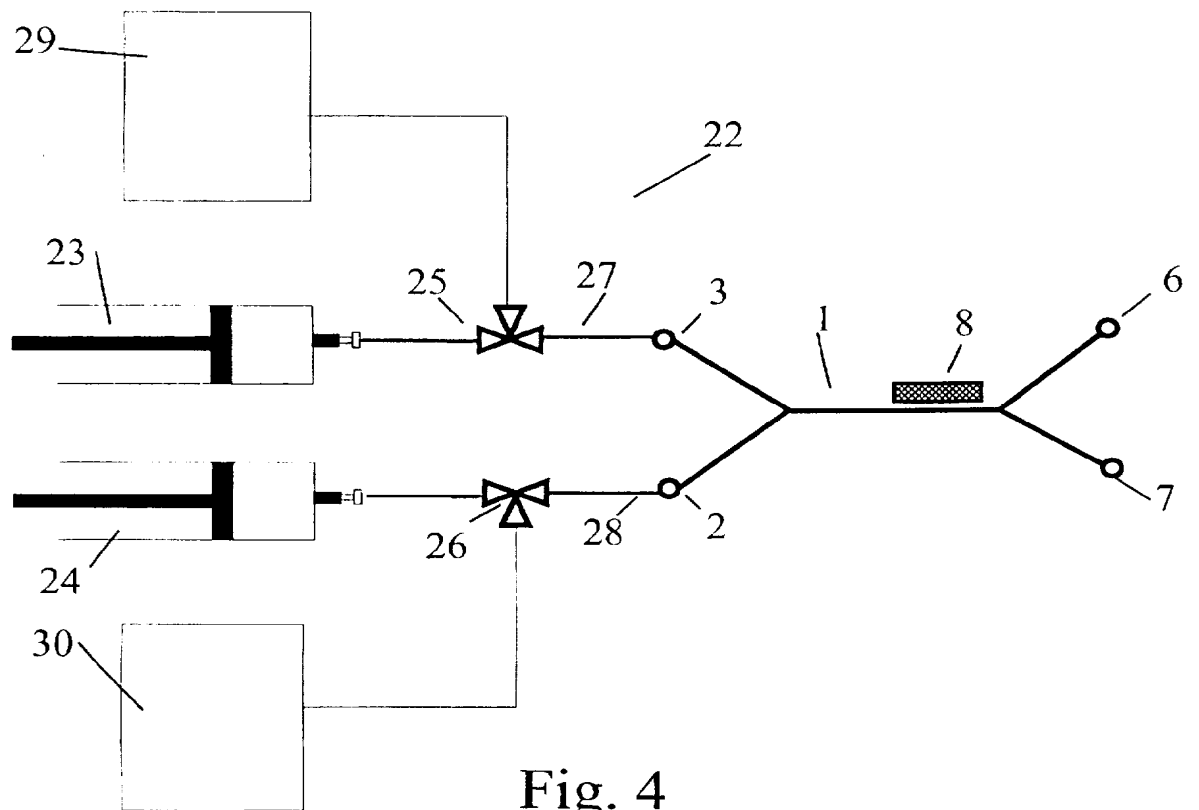


Fig. 4

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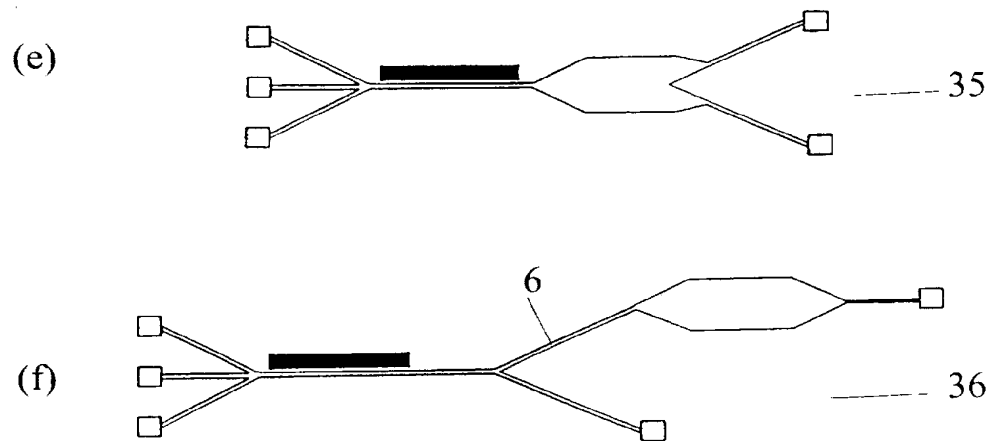
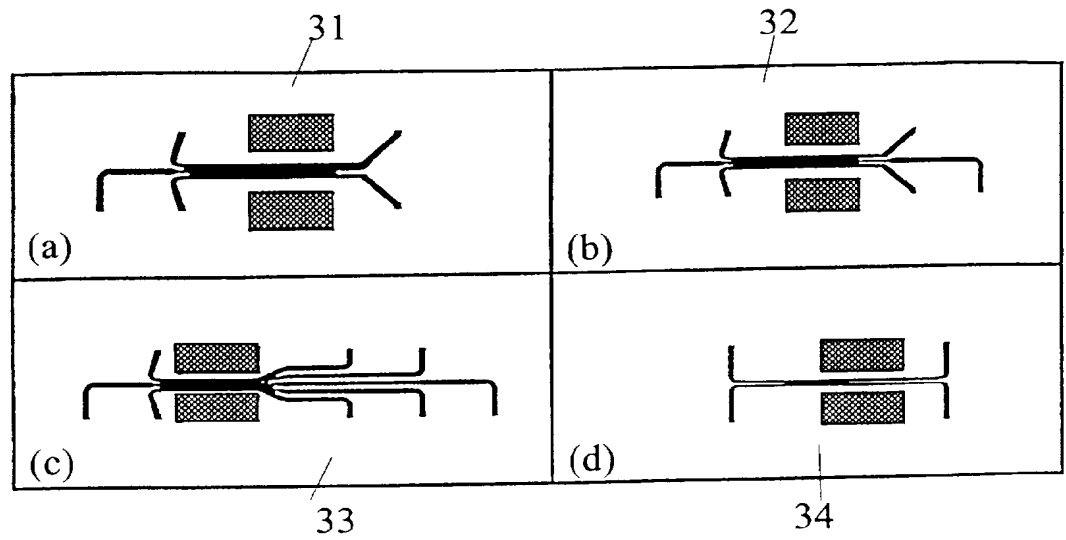


Fig. 5

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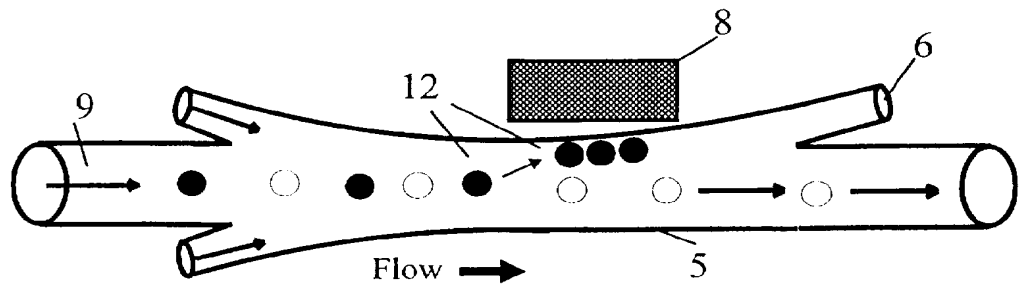


Fig. 6

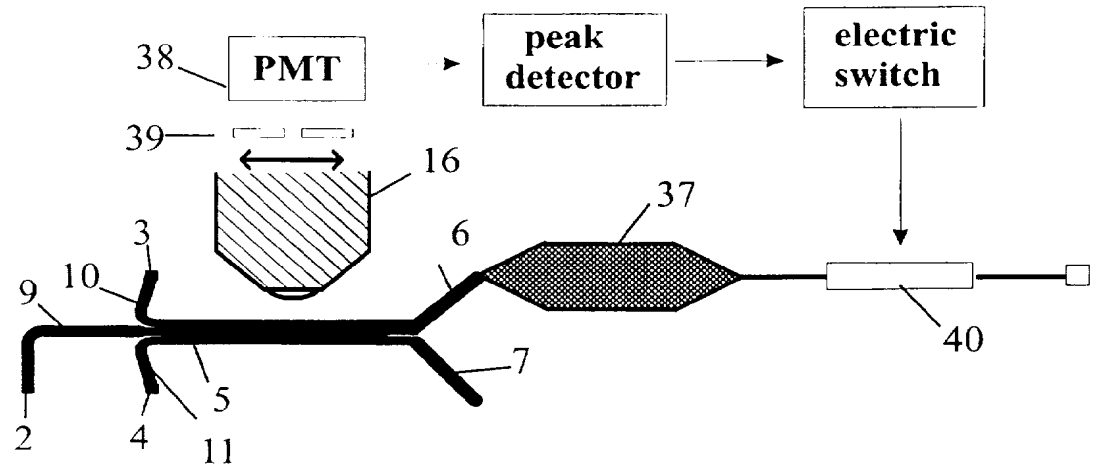


Fig. 7

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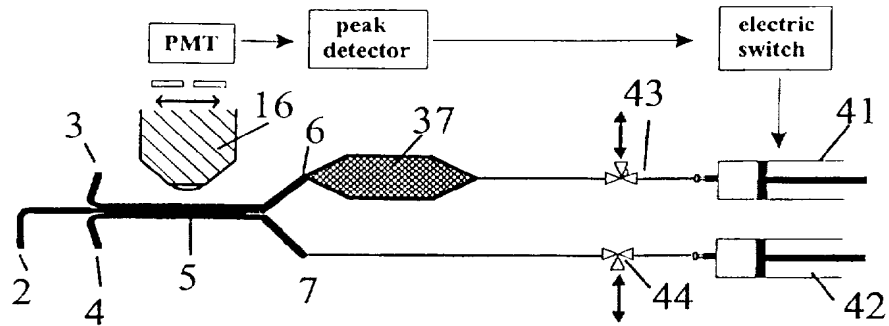


Fig. 8

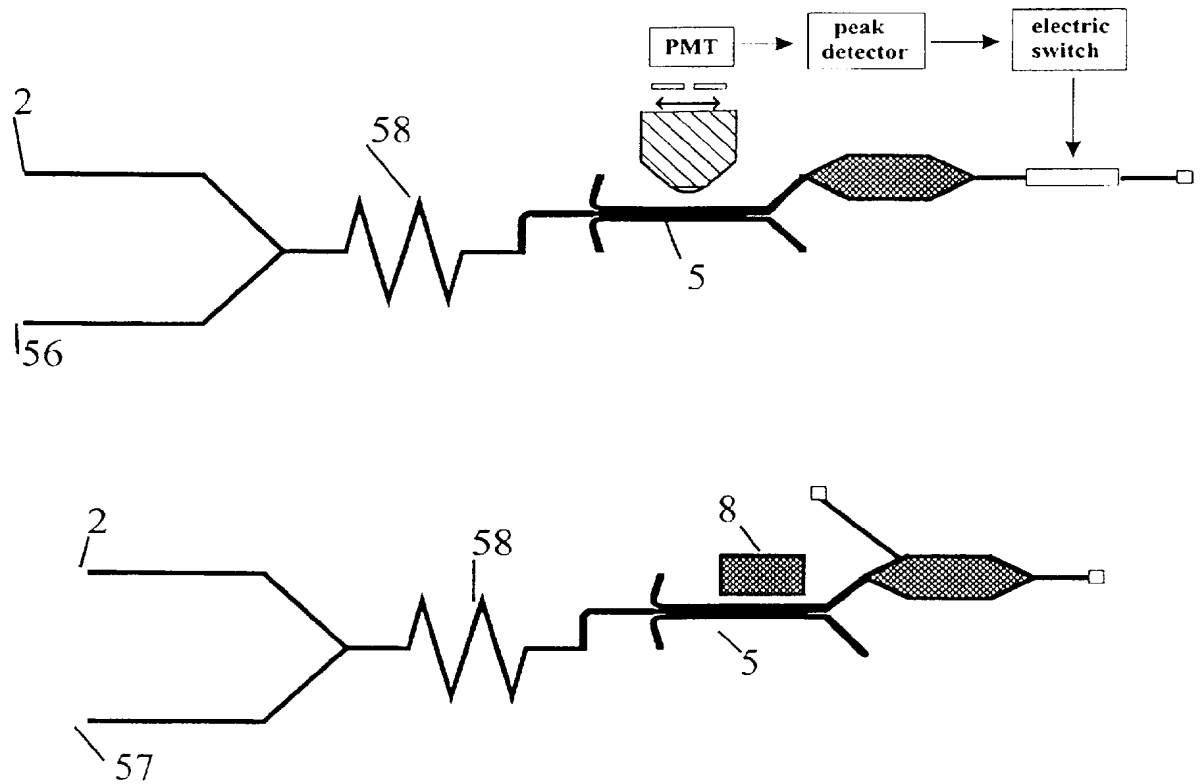


Fig. 10

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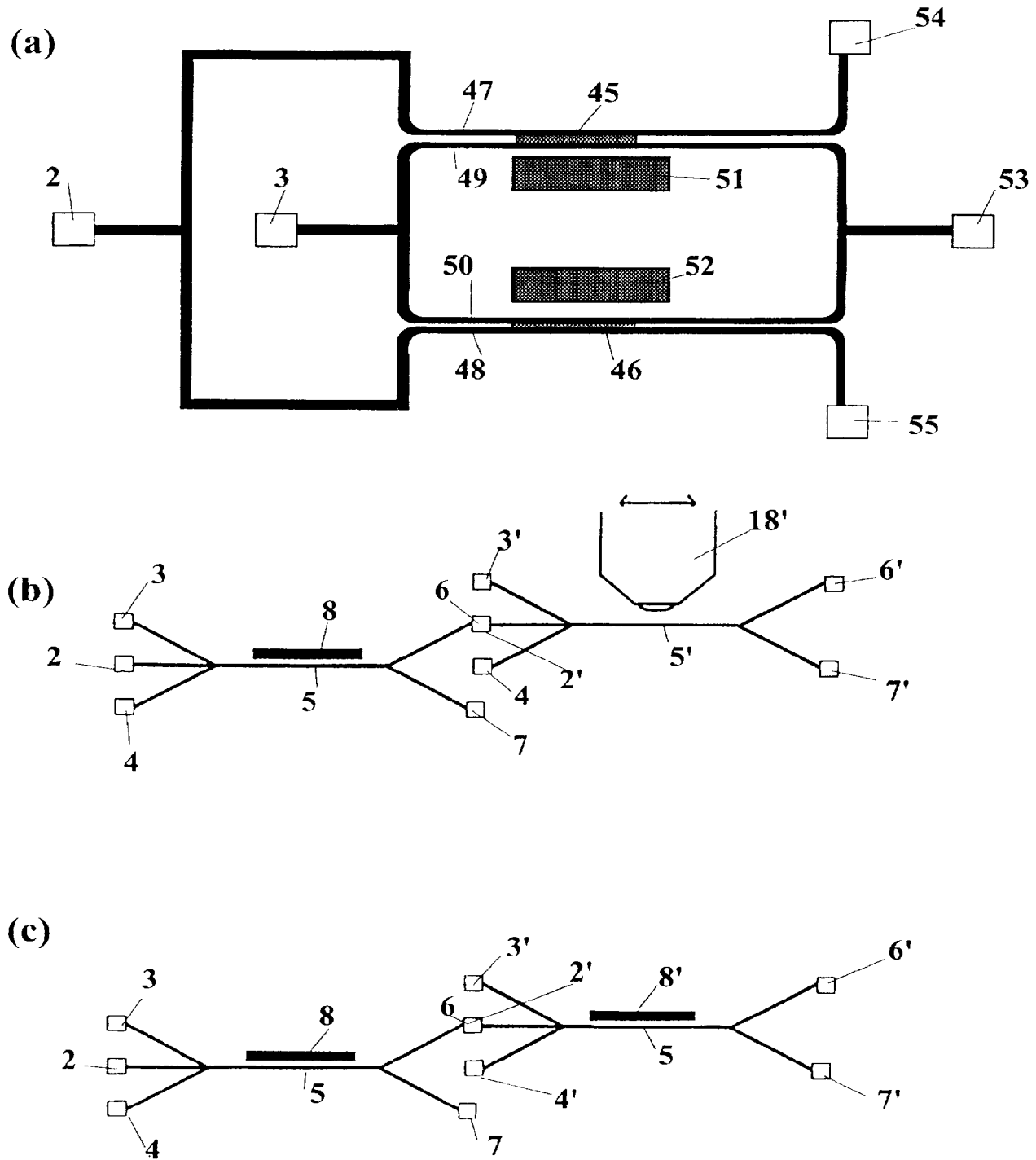


Fig. 9

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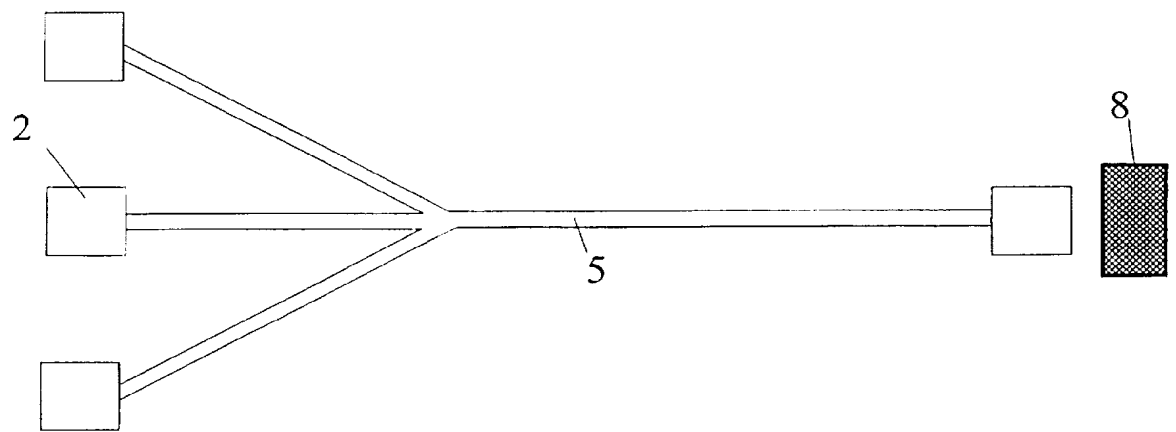
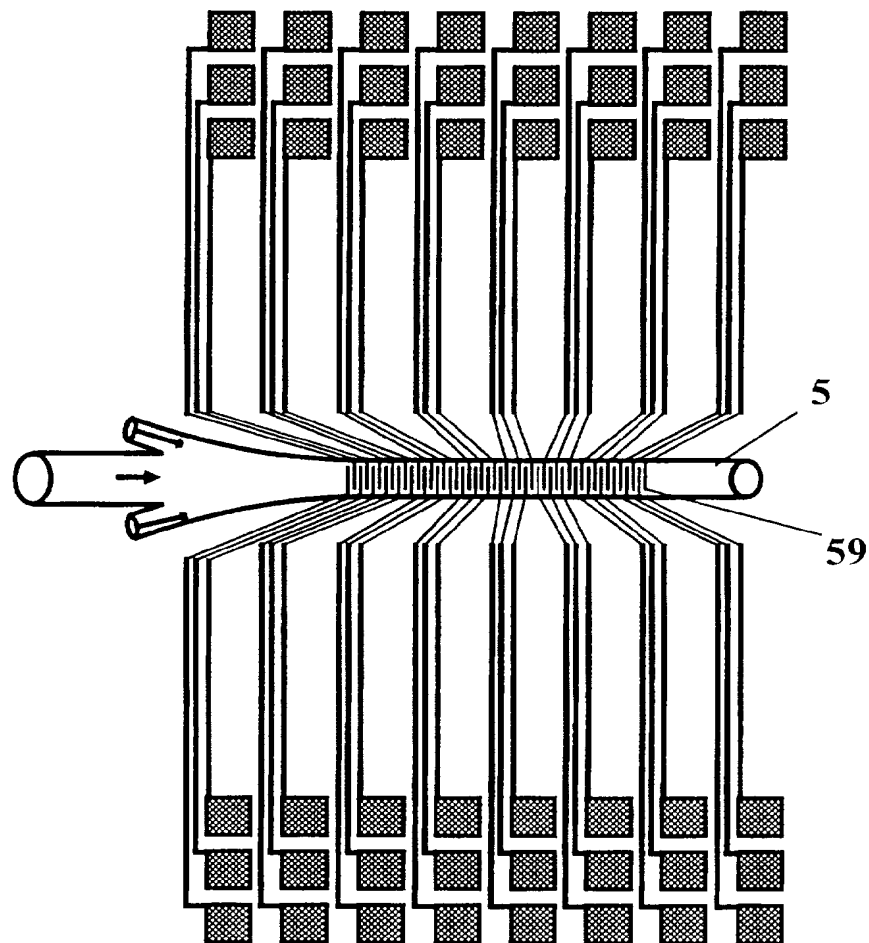


Fig. 11

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(a)



(b)

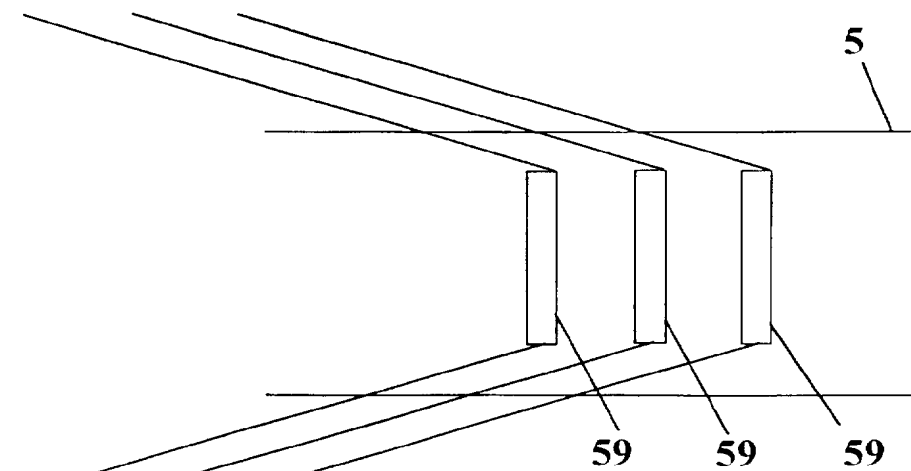


Fig. 12

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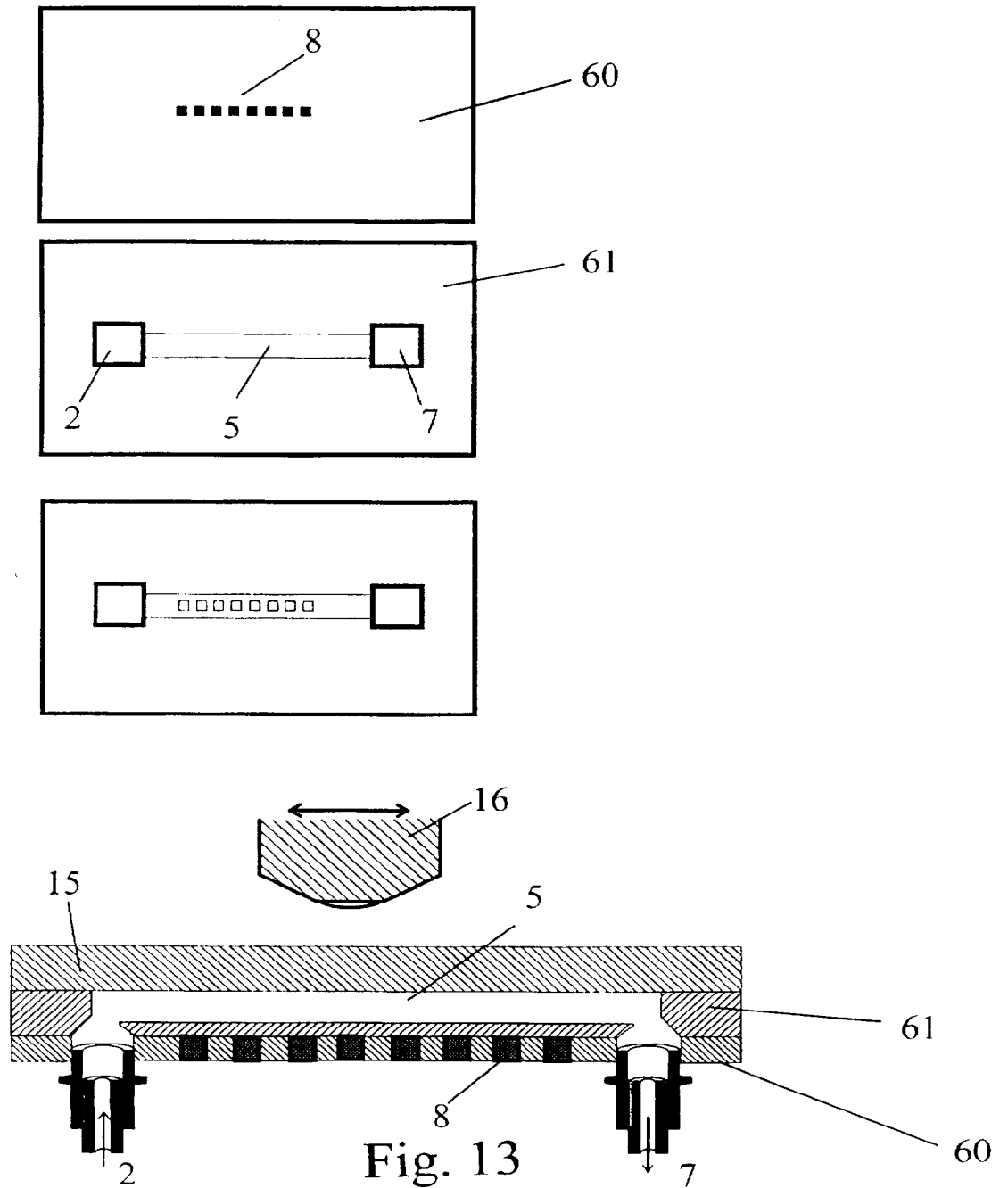
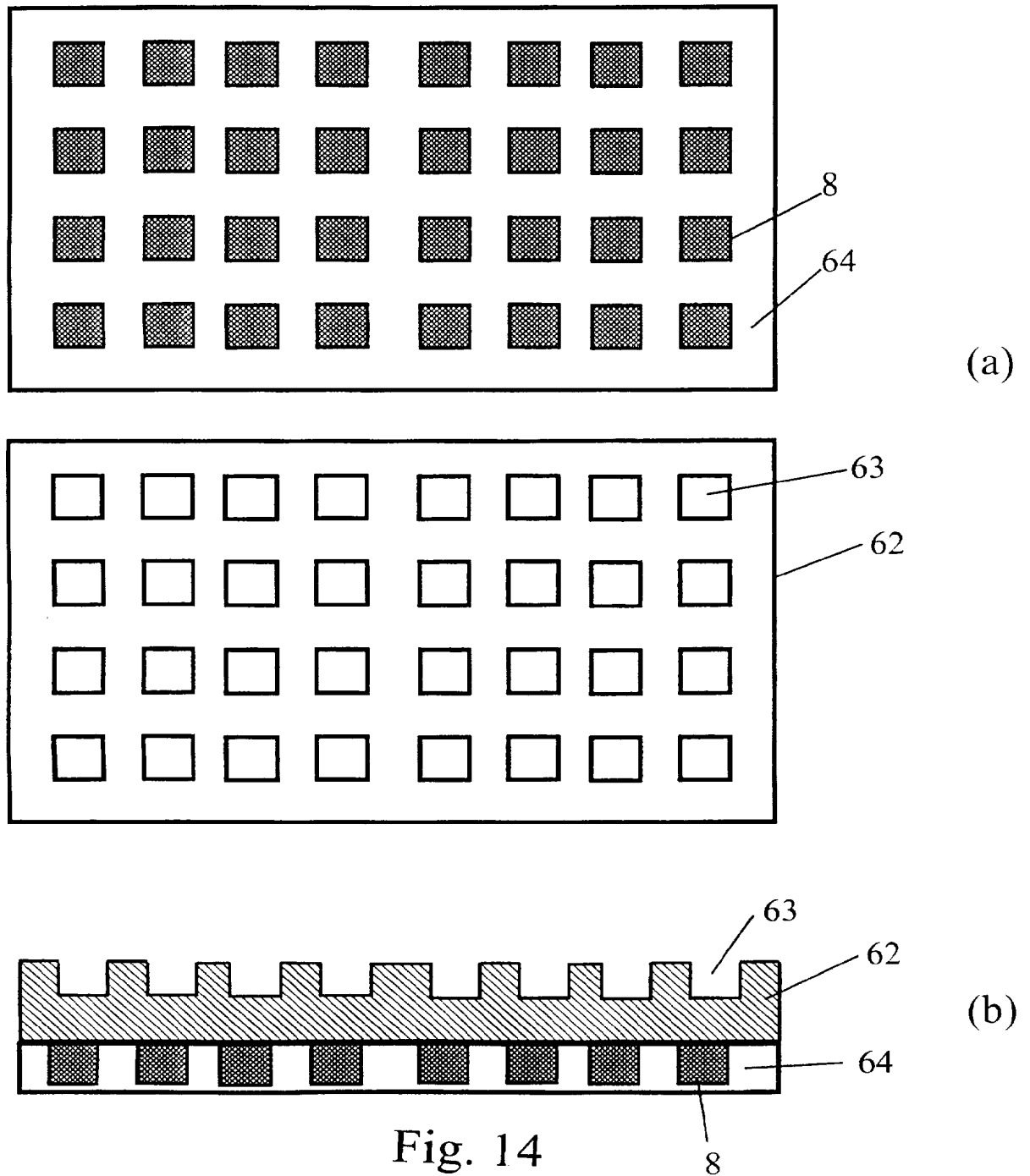


Fig. 13

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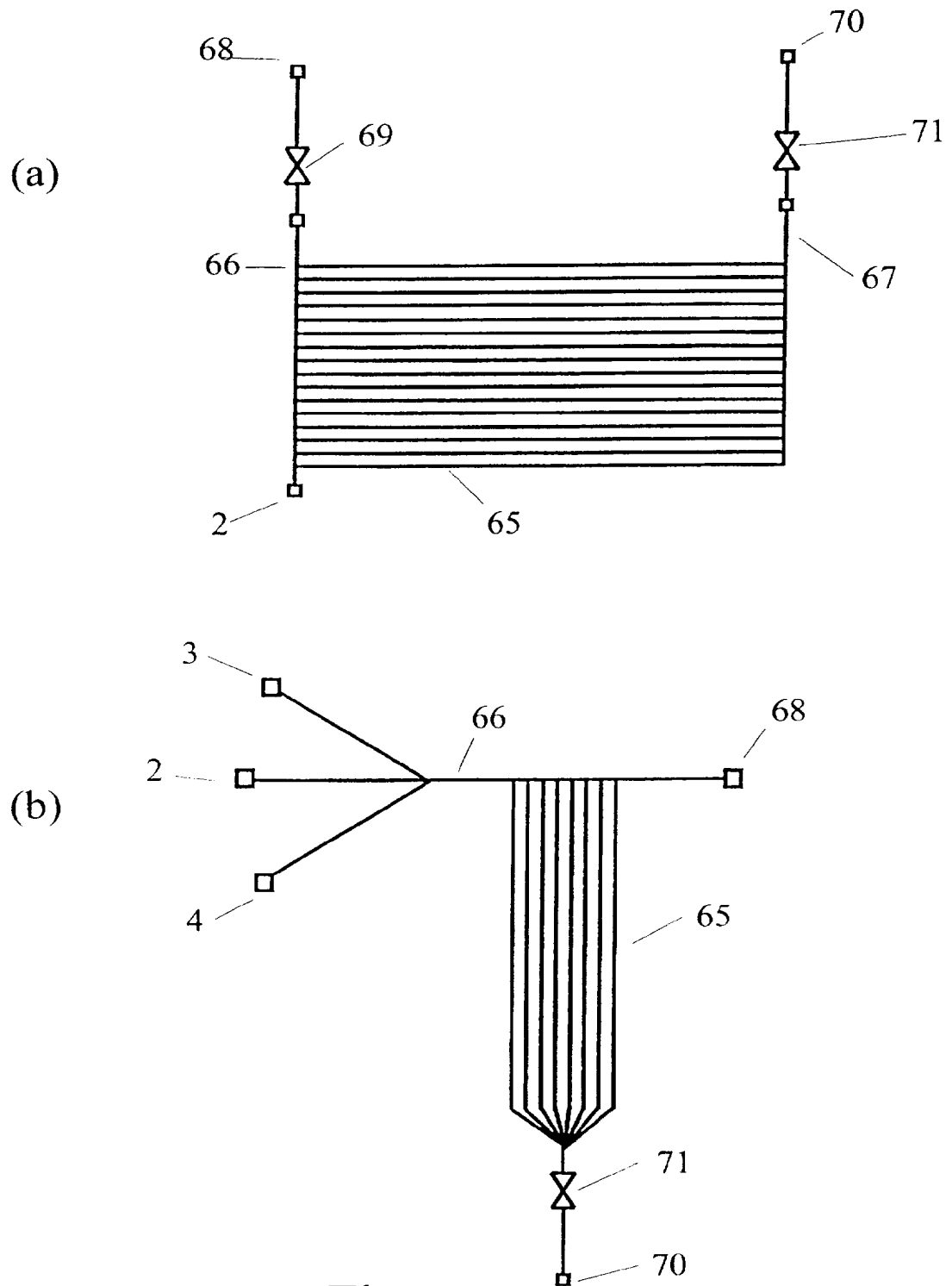


Fig. 15

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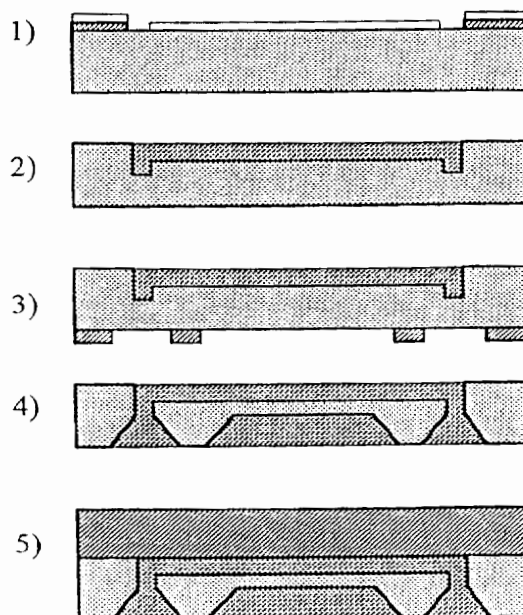


Fig. 16

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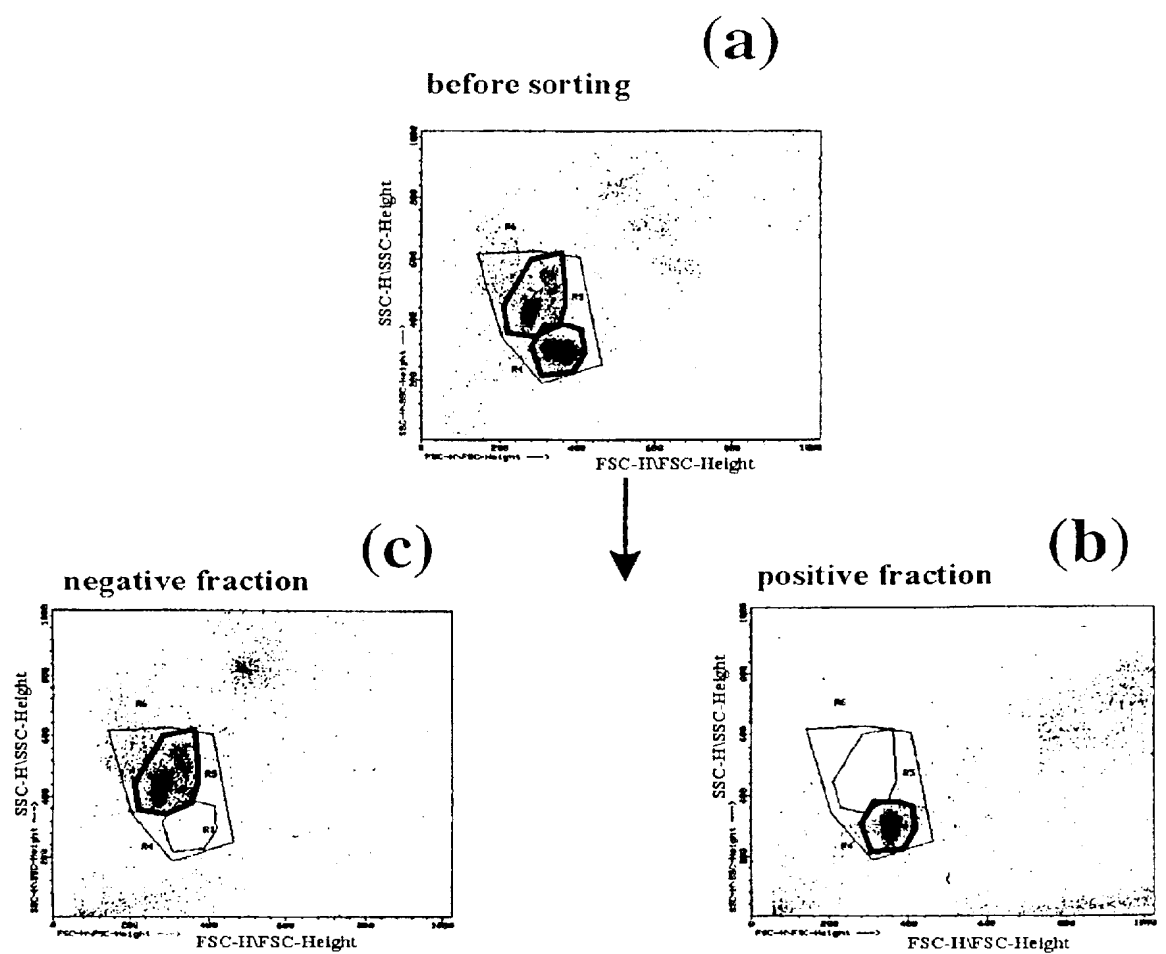


Fig. 17

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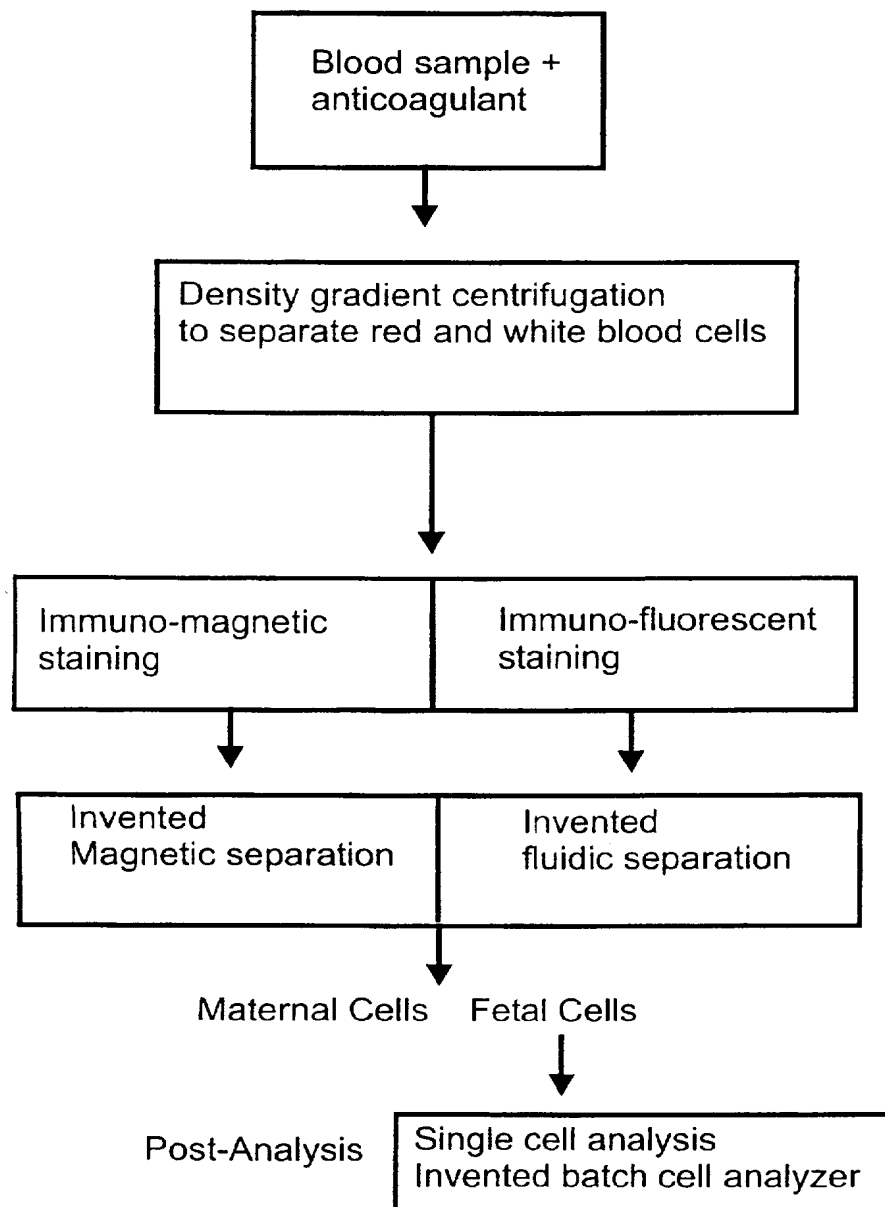


Fig. 18

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 97/00368

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N15/14 G01N33/543 B03C1/035

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N B03C B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|--|
| Y | <p>AHN C H ET AL: "A FULLY INTEGRATED MICROMACHINED MAGNETIC PARTICLE MANIPULATOR AND SEPARATOR" PROCEEDING OF THE WORKSHOP ON MICRO ELECTRO MECHANICAL SYSTEMS (MEM, OISO, JAN. 25 - 28, 1994, no. WORKSHOP 7, 25 January 1994, INSTITUTE OF ELECTRICAL AND ELECTRONICS ENGINEERS, pages 91-96, XP000528399 see the whole document</p> <p>---</p> <p>-/--</p> | <p>1-4,9, 10,12, 17, 20-27, 29,30, 34,35</p> |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex

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Date of the actual completion of the international search

12 December 1997

Date of mailing of the international search report

- 7. 01. 98

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Authorized officer

Brison, O

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/DK 97/00368

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|--|---|
| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| Y | US 3 827 555 A (KAMENSKY L ET AL) 6 August 1974 see the whole document | 1-4,9, 10,12, 17, 20-27, 29,30,34 |
| X | --- | 37 |
| Y | US 4 279 345 A (ALLRED JOHN C) 21 July 1981 see the whole document | 12 |
| Y | --- | |
| Y | WO 96 09409 A (MILTENYI BIOTECH INC) 28 March 1996 see page 5, line 10 - page 8, line 20 | 35 |
| Y | --- | |
| Y | US 4 910 148 A (SORENSEN OTTO ET AL) 20 March 1990 see the whole document | 36 |
| Y | --- | |
| Y | US 3 560 754 A (KAMENSKY LOUIS A) 2 February 1971 see the whole document | 36 |
| X | --- | |
| X | WO 94 15193 A (SIENNA BIOTECH INC) 7 July 1994 see abstract | 38 |
| X | --- | |
| X | WO 93 22058 A (UNIV PENNSYLVANIA) 11 November 1993 see page 11, line 1-14 | 39 |
| A | --- | |
| A | US 5 053 344 A (ZBOROWSKI MACIEJ ET AL) 1 October 1991 see abstract | 1 |
| A | --- | |
| A | US 5 483 469 A (VAN DEN ENGH GER ET AL) 9 January 1996 see the whole document | 1 |
| | ----- | |

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PCT/DK 97/00368

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|--|------------------|-------------------------|------------------|
| US 3827555 A | 06-08-74 | US 3984307 A | 05-10-76 |
| US 4279345 A | 21-07-81 | NONE | |
| WO 9609409 A | 28-03-96 | AU 3593595 A | 09-04-96 |
| | | CA 2200294 A | 28-03-96 |
| | | EP 0778899 A | 18-06-97 |
| US 4910148 A | 20-03-90 | NONE | |
| US 3560754 A | 02-02-71 | BE 689813 A | 02-05-67 |
| | | CH 446769 A | |
| | | DE 1598634 A | 15-04-71 |
| | | FR 1501326 A | 29-01-68 |
| | | GB 1101063 A | |
| | | NL 6615120 A | 18-05-67 |
| | | SE 333264 B | 08-03-71 |
| WO 9415193 A | 07-07-94 | AU 670489 B | 18-07-96 |
| | | AU 5873494 A | 19-07-94 |
| | | CA 2151060 A | 07-07-94 |
| | | CN 1092866 A | 28-09-94 |
| | | EP 0676045 A | 11-10-95 |
| | | JP 8505231 T | 04-06-96 |
| | | US 5589401 A | 31-12-96 |
| WO 9322058 A | 11-11-93 | US 5304487 A | 19-04-94 |
| | | US 5296375 A | 22-03-94 |
| | | AT 155711 T | 15-08-97 |
| | | AT 140025 T | 15-07-96 |
| | | AT 140880 T | 15-08-96 |
| | | AU 677780 B | 08-05-97 |
| | | AU 4222393 A | 29-11-93 |
| | | AU 680195 B | 24-07-97 |
| | | AU 4222593 A | 29-11-93 |
| | | AU 677781 B | 08-05-97 |
| | | AU 4222693 A | 29-11-93 |
| | | AU 4222793 A | 29-11-93 |
| | | AU 677197 B | 17-04-97 |
| | | AU 4223593 A | 29-11-93 |

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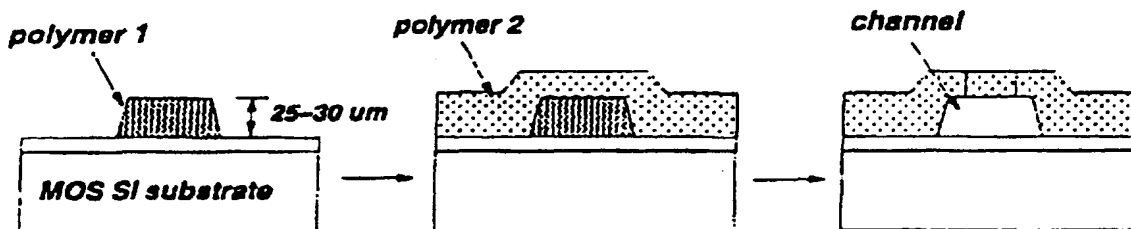
| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO 9322058 A | | CA 2134474 A | 11-11-93 |
| | | CA 2134475 A | 11-11-93 |
| | | CA 2134476 A | 11-11-93 |
| | | CA 2134478 A | 11-11-93 |
| | | DE 69303483 D | 08-08-96 |
| | | DE 69303483 T | 06-02-97 |
| | | DE 69303898 D | 05-09-96 |
| | | DE 69303898 T | 20-02-97 |
| | | DE 69312483 D | 04-09-97 |
| | | EP 0637996 A | 15-02-95 |
| | | EP 0637997 A | 15-02-95 |
| | | EP 0639223 A | 22-02-95 |
| | | EP 0637998 A | 15-02-95 |
| | | EP 0637999 A | 15-02-95 |
| | | HK 16897 A | 13-02-97 |
| | | JP 7506430 T | 13-07-95 |
| | | JP 7506431 T | 13-07-95 |
| | | JP 7506256 T | 13-07-95 |
| | | JP 7506257 T | 13-07-95 |
| | | JP 7506258 T | 13-07-95 |
| | | WO 9322053 A | 11-11-93 |
| | | WO 9322054 A | 11-11-93 |
| | | WO 9322421 A | 11-11-93 |
| | | WO 9322055 A | 11-11-93 |
| | | US 5635358 A | 03-06-97 |
| | | US 5427946 A | 27-06-95 |
| | | US 5498392 A | 12-03-96 |
| | | US 5587128 A | 24-12-96 |
| | | US 5486335 A | 23-01-96 |
| | | US 5637469 A | 10-06-97 |
| ----- | | | |
| US 5053344 A | 01-10-91 | NONE | |
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| US 5483469 A | 09-01-96 | NONE | |
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(54) Title: POLYMER-BASED MICROMACHINING TECHNOLOGY FOR MICROFLUIDIC DEVICES



Simplified fabrication process for plastic channels.

(57) Abstract

The present invention relates to polymer-based micro-electro-mechanical system (MEMS) technology suitable for the fabrication of integrated microfluidic systems, particularly medical and chemical diagnostics system, ink-jet printer head, as well as any devices that requires liquid- or gas-filled cavities for operation. The integrated microfluidic systems may consist of pumps, valves, channels, reservoirs cavities, reaction chambers, mixers, heaters, fluidic interconnects, diffusers, nozzles, and other microfluidic components on top of a regular circuit substrate. This technology is vastly superior than any alternatives available such as glass-based, polysilicon-based MEMS technology as well as hybrid "circuit board" technology because of its simple construction low cost, low temperature processing, and its ability to integrate any electronic circuitry easily along with the fluidic parts.

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POLYMER-BASED MICROMACHINING TECHNOLOGY FOR MICROFLUIDIC DEVICES

This invention was made with U.S. government support under grant NIH-R01-H601044 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to compounds and methods for micromachining, and in particular, polymer-based technology for fabricating microfluidic devices.

BACKGROUND

Microfluidic systems are highly useful in medical diagnostics, environmental monitorings, biological food testing, chemical sensing and analysis. Current efforts on the fabrication of microfluidic systems and fluidic technologies have been mainly focused on individual component development. Individual components such as pumps [See M. Esashi *et al.* "Normally Close Microvalve and Micropump Fabricated on a Silicon Wafer," in *International Workshop on Micro Electromechanical Systems (MEMS 89)*, pp. 29-34 (1989); R. Zengerie *et al.*, "A Micro Membrane Pump with Electrostatic Actuation," in *International Workshop on Micro Electromechanical Systems (MEMS 92)*, pp. 19-24 (1992); W. Zang and C.H. Ahn, "A Bi-directional Magnetic Micropump on a Silicon Wafer," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '96)*, pp. 94-97 (1996)], valves [See T. Ohnstein *et al.*, "Micromachined Silicon Valve," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '90)*, pp.95-97 (1990); J.G. Smith, "Piezoelectric Micropump with Three Valves Working Peristaltically," *Sensors and Actuators*, Vol. A21-23, pp. 203-206 (1990); Y.-C Lin *et al.*, "Characteristics of a Polyamide Microvalve," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '96)*, pp. 113-116 (1996)], fluidic channels [J. Pfahler *et al.*, "Liquid Transport in Micron and Submicron Channels," *Sensors and Actuators*, Vol. A21-23, pp. 431-434 (1990)], reaction chambers [S. Nakagawa *et al.*, "A Micro Chemical Analyzing System Integrated on a Silicon Wafer," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '90)*, pp. 89-94 (1990)], separation stages [D.J. Harrison *et al.*, "Chemical Analysis and Electrophoresis

Systems Integrated on Glass and Silicon Chips," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '92)*, pp. 110-113 (1992); A. Manz *et al.*, "Integrated Electroosmotic Pumps and Flow Manifolds for Total Chemical Analysis Systems," in *Transducers '91*, pp. 939-941 (1991); A. Manz *et al.*, "Planar Chips Technology for

5 Miniaturization and Integration of Separation Techniques into Monitoring Systems: Capillary Electrophoresis on a Chip," *J. Chromatography*, Vol. 593, pp. 253-258 (1992)] and detection techniques [R.C. Anderson, G.J. Bodgdan and R.J. Lipshutz, "Miniaturized Genetic Analysis System," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '96)*, pp. 258-261 (1996)], have been fabricated using a sundry of technologies. Numerous fluidic

10 propulsional methods have also been developed based on mechanical pumping, pneumatic forces, electrosmosis [D.J. Harrison *et al.*, "Miniaturized Chemical Analysis Systems Based on Electrophoretic Separations and Electrosmotic Pumping," in *Transducers '93*, pp. 403-406 (1993)], dielectrophoresis [H.A. Pohl, *Dielectrophoresis*, Cambridge: Cambridge University Press (1978)], surface tension gradients [M.A. Burns *et al.*, "Microfabricated Structures for

15 Integrated DNA Analysis," *Proc. Natl. Acad. Sci. USA*, 93:5556-5561 (1996); H. Matsumoto and J.E. Colgate, "Preliminary Investigation of Micropumping Based on Electrical Control of Interfacial Tension," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '90)*, pp. 105-110 (1990); G. Beni and M.A. Tenan, "Dynamics of Electrowetting Displays," *J. Appl. Phys.*, Vol. 52, pp. 6011-6015 (1981), bubble generation [L. Lin *et al.*,

20 "Microbubble Powered Actuator," in *Transducers '91*, pp. 1041-1044 (1991)], and evaporation-condensation [T.K. Jun and C.J. Kim, "Microscale Pumping with Traversing Bubbles in Microchannels," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '96)*, pp. 144-147 (1996)].

However, efforts on system integration of components to date have been limited [S.C. Terry *et al.*, "A Gas Chromatographic Air Analyzer Fabricated on a Silicon Wafer," *IEEE Trans. on Electron Devices*, Vol. ED-26, pp. 1880-1886 (1979); A. van der Berg and P. Bergveld, *Micro Total Analysis Systems*, New York: Kluwer (1994)], which mainly include the use of bulk micromachining and surface micromachining technology. Both of them lack

25 simple construction of components and the capability to integrate all of components with

30 electronic circuitry.

Bulk micromachining technology, which includes the use of glass wafer processing, silicon-to-glass wafer bonding, has been commonly used to fabricate individual microfluidic components. In Europe, this glass-bonding technology has also been used to fabricate

microfluidic systems. In these systems, the control electronics are implemented on a hybrid manner external to the system. This system integration method has several problems.

Because these systems rely on bonding of substrates, it is essential that the bonding surfaces create an hermetic seal to prevent the leakage of chemicals and reagents. This poses a great difficulty when interconnection leads are present on the bonding surfaces which prevent the surfaces from being flat. It is well known that it is exceedingly difficult to attain good bonds even using well-characterized techniques such as anodic bonding when steps on the bonding surfaces exceed 0.2 μm . Because of this planarity requirement, these devices require either complex planarization schemes, or the use of diffused leads. Because of these difficulties, only relatively simple fluidic systems can be constructed using this scheme.

Unlike bulk micromachining, surface micromachining technology can be used to fabricate individual fluidic components as well as microfluidic systems with on-chip electronics. In addition, unlike bonded-type devices, hermetic channels can be built in a relatively simple manner using channel walls made of polysilicon [J.R. Webster *et al.*, "Monolithic Capillary Gel Electrophoresis Stage with On-Chip Detector," in *International Conference on Micro Electromechanical Systems (MEMS 96)*, pp. 491-496 (1996)], silicon nitride [C.H. Mastrangelo and R.S. Mullet, "Vacuum-Sealed Silicon Micromachined Incandescent Light Source," in *Int. Electron Devices Meeting (IDEM 89)*, pp. 503-506 (1989)] and silicon dioxide. Surface micromachined channels that are constructed with these thin films have two basic problems. Because the thickness of the films is limited to a few microns, the height of cavities and channels is very small ($< 5 \mu\text{m}$) resulting in sub-pL sample volumes. These exceedingly small sample volumes both strain the requirements for the sensitivity limits of detection schemes and also aggravate the surface adsorption of samples and reagents on the wall which further diminishes the sample concentration. On the fabrication side, due to the strong nature of the silicon based building materials, the formation of long capillaries is difficult to accomplish. This is because the etchants used for the sacrificial etch are relatively slow and in many cases extremely reactive (such as hydrofluoric acid HF). In these structures, it is customary to leave devices in the sacrificial etch solution for many hours. Special passivation layers (primarily silicon nitride SiN) are used to passivate any active electronic devices that must survive the sacrificial etch.

What is needed is micromachining technology that allows for fabrication of channels with a flexibility in cross section and channel length at virtually no loss in system performance. In addition, the technology should be low cost and should not require specialized equipment.

5

SUMMARY OF THE INVENTION

The present invention relates to polymer-based micro-electro-mechanical system (MEMS) technology suitable for the fabrication of integrated microfluidic systems, particularly medical and chemical diagnostics system, ink-jet printer head, as well as any devices that requires liquid- or gas-filled cavities for operation. The integrated microfluidic systems may consist of pumps, valves, channels, reservoirs, cavities, reaction chambers, mixers, heaters, fluidic interconnects, diffusers, nozzles, and other microfluidic components on top of a regular circuit substrate. This technology is vastly superior than any alternatives available such as glass-based, polysilicon-based, MEMS technology as well as hybrid 'circuit board' technology (See e.g. D. VerLee *et al.*, "Fluid Circuit Technology: Integrated Interconnect Technology for Miniature Fluidic Devices," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '96)*, pp. 9-12 (1996); R.C. Anderson *et al.*, "Miniaturized Genetic Analysis System," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '96)*, pp. 258-261 (1996); M. Albin *et al.*, "Micromachining and Microgenetics: Where Are They and Where Do They Work Together," in *International Workshop on Solid-State Sensors and Actuators (Hilton Head '96)*, pp. 253-257 (1996), where plastic have been used because of its high versatility to provide transparent surfaces and benign surfaces].

The present invention contemplates both methods and devices. In one embodiment, the present invention contemplates a method for fabricating microchannels, comprising the steps: a) providing a substrate, said substrate having upper and lower surfaces; b) depositing a first polymer layer on said upper surface of said substrate, said first polymer layer having i) a lower surface contacting said upper surface of said substrate and ii) an upper surface; c) depositing a second polymer layer on said upper surface of said first polymer layer, said second polymer layer having i) a lower surface contacting said upper surface of said first polymer layer and ii) an upper surface; and d) sacrificially etching said first polymer layer under conditions such that said second polymer layer comprises walls of a microchannel.

In another embodiment, the present invention contemplates a method for fabricating microchannels, comprising the steps: a) providing a silicon substrate, said silicon substrate having upper and lower surfaces; b) depositing a first polymer layer on said upper surface of said silicon substrate, said first polymer layer having i) a lower surface contacting said upper surface of said silicon substrate and ii) an upper surface; c) photolithographically defining said first polymer layer under conditions such that said first polymer layer has a desired thickness; d) depositing a second polymer layer on said upper surface of said first polymer layer, said second polymer layer having i) a lower surface contacting said upper surface of said first polymer layer and ii) an upper surface; and e) sacrificially etching said first polymer layer under conditions such that said second polymer layer comprises walls of a microchannel.

It is not intended that the microchannels of the present invention be limited to any specific dimensions. In one embodiment, the desired thickness of said first polymer layer is between 0.5 and 200 μ and this thickness defines the height of the channel.

Of course, the present invention contemplates additional fabrication steps. In one embodiment, the present invention contemplates, prior to step (d) the step of photolithographically defining said second polymer layer. In another embodiment, the present invention contemplates after step (d) the step (e) of etching openings in said walls of said microchannel. In yet another embodiment, the present invention contemplates after step (d) and before step (e) the step of depositing a third polymer layer on said upper surface of said second polymer layer.

It is not intended that the present invention be limited by the particular type of polymers. Polymers include, but are not limited to, photoresist, polyamide, benzocyclobutene, polydimethylsiloxane, vapor-deposited p-xylylene, pTFE, PMMA, p-HMDS, polypropylene, starch-based polymers, epoxy, and acrylics. In one embodiment, polymer pairs are selected wherein the first polymer is soluble in a particular solvent, while the second polymer is not soluble in the same solvent. This permits the use of the polymer pair such that sacrificial etching with the solvent (or a mixture of solvents) removes the first polymer but leaves the second polymer intact to serve as the polymer for the microchannel.

It is not intended that the present invention be limited by the particular barrier layer if used. The barrier layers include, but is not limited to, metal, stack of different metals, polymers, silicon-based materials, and other inorganic materials.

The present invention contemplates numerous arrangements of the various polymer layers. For example, in one embodiment, the present invention contemplates a method for

fabricating microchannels, comprising the steps: a) providing a silicon wafer, said silicon wafer having upper and lower surfaces; b) depositing a first layer of a first polymer on said upper surface of said silicon wafer, said first polymer layer having i) a lower surface contacting said upper surface of said silicon wafer and ii) an upper surface; c) depositing a second polymer layer on said upper surface of said first layer, said second polymer layer having i) a lower surface contacting said upper surface of said first layer and ii) an upper surface; d) photolithographically defining said second polymer layer under conditions such that said second polymer layer has a thickness between 0.5 and 200 μ ; e) depositing a second layer of said first polymer on said upper surface of said second polymer layer, said second layer of said first polymer having i) a lower surface contacting said upper surface of said second polymer layer and ii) an upper surface; f) sacrificially etching said second polymer layer under conditions such that said first and second layers of said first polymer comprise walls of a microchannel, said microchannel having a height defined by said thickness of said second polymer layer of step (d); and g) etching one or more openings in said walls of said microchannel.

This latter embodiment can also contain additional polymers. In one embodiment, the method further comprises after step (e) and before step (f) the step of depositing a layer of a third polymer on said upper surface of said second layer of said first polymer.

Again, the present invention is not limited to particular polymers. In the latter embodiment of the method of the present invention, it is preferred that said first and second layers of said first polymer comprise vapor-deposited p-xylylene.

The present invention is not limited to the nature of number of openings. In one embodiment, first and second openings are etched, said first opening serving as an inlet and said second opening serving as an outlet. In one embodiment, said openings are etched from said lower surface of said silicon wafer into said lower surface of said first layer of said first polymer. In another embodiment, said openings are etched from said upper surface of said layer of said third polymer into said upper surface of said second layer of said first polymer.

The polymer-based micromachining technology proposed here encompasses the combined benefits of the plastic fluidic technologies while capable of integration of active microfluidic and electronic devices on a monolithic package. Hermetically sealed channels can be fabricated with a flexibility in cross section and channel length at virtually no loss in system performance. System integration is realized by the ability to fabricate various fluidic components using the same process on top of standard yet complex MOS control circuitry. In

addition, the polymer technology of the present invention is very low cost and does not require specialized equipment.

The devices of the present invention, once fabricated, have a variety of uses. In one embodiment, the present invention contemplates a polymer microchannel fabricated on a silicon substrate, wherein said polymer microchannel is capable of conveying liquids or gases (or both). The present invention is not limited by the nature of the liquid or gas. In one embodiment, the present invention contemplates a polymer microchannel fabricated on a silicon substrate, said microchannel containing one or more microdroplets. In another embodiment, the present invention contemplates a polymer microchannel fabricated on a silicon substrate, said microchannel connected to a reaction chamber, said reaction chamber containing biological reactants.

It is not intended that the present invention be limited by the nature of the flow-directing means used to convey fluids through the polymer channels of the present invention. In one embodiment, the present invention contemplates a device comprising a polymer channel, said polymer channel comprising one or more electrodes positioned such that they will be in contact with a liquid sample placed in said channel. The present invention also contemplates a method of conveying liquid in a microchannel, comprising introducing a liquid into an embodiment of such a device. In one embodiment, two electrodes are employed and a potential is applied between the two electrodes. At both ends of the electrodes, hydrolysis takes place and a bubble is generated. The gas bubble continues to grow as the electrodes continue pumping electrical charges to the fluid. The expanded bubble creates a pressure differential between the two sides of the liquid drop which eventually is large enough to push the liquid forward and move it through the polymer channel.

DEFINITIONS

The following definitions are provided for the terms used herein:

The term "biological reactions" is used herein to mean reactions involving biomolecules (and biological reactants) such as enzymes (*e.g.*, polymerases, nucleases, etc.) and nucleic acids (both RNA and DNA). Biological samples are those containing biomolecules, such proteins, lipids, nucleic acids. The sample may be from a microorganism (*e.g.*, bacterial culture) or from an animal, including humans (*e.g.* blood, urine, etc.). Alternatively, the sample may have been subject to purification (*e.g.* extraction) or other treatment. Biological reactions require some degree of biocompatibility with the device. That

is to say, the reactions ideally should not be substantially inhibited by the characteristics or nature of the device components. To this end, in one embodiment, the polymer-based technology of the present invention contemplates polymer-based microchannels comprising biocompatible polymers that do not inhibit biological reactions.

5 "Chemical reactions" means reactions involving chemical reactants, such as inorganic compounds. Again, it is desired that the reactions should not be substantially inhibited by the characteristics or nature of the device components. To this end, in one embodiment, the polymer-based technology of the present invention contemplates polymer-based microchannels comprising polymers that do not inhibit chemical reactions.

10 "Channels" are pathways through a medium (*e.g.*, silicon) that allow for movement of liquids and gasses. Channels thus can connect other components, *i.e.*, keep components "in liquid communication." "Microdroplet transport channels" are channels configured (in microns) so as to accommodate "microdroplets." While it is not intended that the present invention be limited by precise dimensions of the channels or precise volumes for
15 microdroplets, illustrative ranges for channels and microdroplets are as follows: the channels can be between 0.5 and 200 microns in depth (preferably between 10 and 100 μm) and between 50 and 1000 μm in width (preferably 500 μm), and the volume of the microdroplets can range (calculated from their lengths) between approximately one (1) and (100) nanoliters (more typically between ten and fifty).

20 The term "conveying" is used herein to mean "causing to be moved through" as in the case where a microdroplet is conveyed through a transport channel to a particular point, such as a reaction region. Conveying can be accomplished via flow-directing means.

 "Flow-directing means" is any means by which movement of liquid (*e.g.* a microdroplet) in a particular direction is achieved. A variety of flow-directing means are
25 contemplated, including but not limited to pumps such as the "bubble pump" described herein. A preferred directing means employs a surface-tension-gradient mechanism in which discrete droplets are differentially heated and propelled through etched channels. For continuous flow of liquids, pumps are contemplated.

 "Initiating a reaction" means causing a reaction to take place. Reactions can be
30 initiated by any means (*e.g.*, heat, wavelengths of light, addition of a catalyst, etc.). The present invention contemplates initiating reactions in the channels and reaction chambers fabricated according to the methods of the present invention.

"Liquid barrier" or "moisture barrier" is any structure or treatment process on existing structures that prevents short circuits and/or damage to electronic elements (*e.g.*, prevents the destruction of the aluminum heating elements). In one embodiment of the present invention, the liquid barrier comprises a first silicon oxide layer, a silicon nitride layer, and/or a second silicon oxide layer.

"Nucleic Acid Amplification" involves increasing the concentration of nucleic acid, and in particular, the concentration of a particular piece of nucleic acid. A preferred technique is known as the "polymerase chain reaction." Mullis, *et al.*, U.S. patents Nos. 4,683,195 and 4,683,202, hereby incorporated by reference, describe a method for increasing the concentration of a segment of target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a molar excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence. The two primers are complementary to their respective strands of the double-stranded sequence. The mixture is denatured and then allowed to hybridize. Following hybridization, the primers are extended with polymerase so as to form complementary strands. The steps of denaturation, hybridization, and polymerase extension can be repeated as often as needed to obtain a relatively high concentration of a segment of the desired target sequence. The length of the segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to by the inventors as the "Polymerase Chain Reaction" (hereinafter PCR). Because the desired segment of the target sequence become the dominant sequences (in terms of concentration) in the mixture, they are said to be "PCR-amplified."

DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic of one embodiment of an integrated microfluidic system using the technology of the present invention.

Figure 2 is a photograph of an electron micrograph of a polymer channel made according to the present invention. Figure 2 (a) is a top view and Figure 2 (b) is a cross-section.

Figure 3 is a schematic showing a simplified fabrication method of the present invention.

Figure 4 (a) shows the top view of a microchannel with toptype inlet fabricated using the methods of the present invention. Figure 4 (b) is a closeup electron-micrograph showing the three polymer construction of a channel made according to the methods of the present invention.

5 Figure 5 (a) shows the top view of a thermal cycling chamber constructed using the methods of the present invention. Figure 5(b) shows the cross-section of a similar PCR thermal cycling chamber showing the large volume of the cavity.

Figure 6 is a series of schematics showing the basic process steps of one embodiment of the fabrication method of the present invention.

10 Figure 7 is a schematic showing the simple suction pump.

Figure 8 is a schematic showing the cross-section of a pass-through valve implemented using the polymer based channel technology of the present invention.

Figure 9 is a schematic showing one embodiment of a bubble pump and capillary valve.

15 Figure 10 is a schematic showing liquid movement via the bubble pump and capillary valve.

Figure 11 shows another embodiment of a reaction chamber constructed using the methods of the present invention.

20 Figure 12 shows another embodiment of a thermocompression suction pump fabricated according to the methods of the present invention.

Figure 13 is a schematic showing one embodiment of the fabrication method for the bubble pump and capillary valve.

DESCRIPTION OF THE INVENTION

25 The present invention relates to polymer-based micro-electro-mechanical system (MEMS) technology suitable for the fabrication of integrated microfluidic systems, particularly medical and chemical diagnostics system, ink-jet printer head, as well as any devices that requires liquid-or gas-filled cavities for operation. The integrated microfluidic systems may consist of pumps, valves, channel, reservoirs, cavities, reaction chambers,
30 mixers, beaters, fluidic interconnects, diffusers, nozzles, and other microfluidic components on top of a regular circuit substrate.

Devices fabricated under this invention consist of two planar levels of functionality (See Figure 1). The top level consists of a polymer-based microfluidic plane, 30-50 μ thick

that is constructed on top of a regular silicon substrate. This plane of functionality contains all the microfluidic components of the diagnostic system such as channels, pumps, valves, thermal cycling reactor chambers, reservoirs, and electrophoresis stages. Beneath the microfluidic plane, the control, detection, drive and communication electronics are constructed in a conventional CMOS substrate chip. A series of holes are etched through the silicon substrate using anisotropic etching thus forming sample and reagent inlets and providing access to the microfluidic plane.

Figure 1(b) shows the proposed packaging assembly. First, access holes are ultrasonically drilled through a PGA ceramic package. The two-plane chip is mounted on the package and wire bonds are connected on the lead side for the electrical interconnects. The "fluidic" interconnects of the system are on the opposite side of the chip and hence do not interfere with the electrical connections. The chip cavity is next closed thus isolating the fragile electronic and microfluidic components from the surrounding environment.

Figure 2 shows SEM photographs of 25 μ -high, 3 mm-long polymer channels with frontside inlet. The details of the fabrication process are discussed in the Experimental section.

The proposed technology is suitable for the construction of a wide range of automated analytical instruments hence serving as a general testbench. When properly constructed, the testbench components can be configured to perform many different functions thus in essence forming a "fluidic microprocessor". The present invention contemplates the use of a common fluidic interconnect (*i.e.*, a fluidic bus) to move samples from any reservoir (or fluidic register) to any other, as well as allowing for controlled mixing. The set of mixing operations performed by the fluidic processor can be controlled by an external logic circuit.

The invention of polymer-based micromachining technology is vastly superior than those based on wafer bonding bulk micromachining, polysilicon surface micromachining, and hybrid plastic 'circuit board' technology in the following six categories. (a) The microfluidic plane fabrication is implemented by deposition of low-temperature layers that do not degrade or change the underlying circuit characteristics or its conventional process flow. (b) The fluidic-plane is fabricated using weak chemicals that do not attack the circuit plane in any way. Therefore, complex microfluidic devices can be fabricated using a regular MOS substrate plane containing the control circuitry, and a polymer-based microfluidic plane constructed on top of it. (c) Leak-tight channels are formed due to the conformality of the polymer deposition (a severe problem present in glass bonded devices): (d) Tall channels and

chambers of different heights can be easily fabricated. Unlike silicon-based films, these polymers can be quite thick with a thickness reported as high as 200 μ . Currently we can fabricate channels that range from 0.5 to 100 μ in height. (e) Very long channels can be fabricated with short etching times (also a problem with poly-Si). (f) Polymer channel walls do not have the severe surface adsorption problems observed in silicon and glass walls. (g) Polymer channels are optically transparent and are therefore are much simpler to test. (h) Its process simplicity and its capability of integrating individual fluidic components together make integrated microfluidic system possible. (i) Its batch-processing ability make microfluidic system inexpensive.

The following sections show several fabrication processes of microfluidic components. These microfluidic components and their process flows are presented to demonstrate the feasibility of polymer-based technology to fabricate the microfluidic systems. It is not intended that the present invention be limited by a particular type of fabrication process.

I. Selection of Polymers and Polymer Pairs

As noted above, it is not intended that the present invention be limited by the particular type of polymers. In one embodiment, polymer pairs are selected wherein the first polymer is soluble in a particular solvent, while the second polymer is not soluble in the same solvent. This permits the use of the polymer pair such that sacrificial etching with the solvent removes the first polymer but leaves the second polymer intact to serve as the polymer for the microchannel.

Selection of polymer pairs and solvents can readily be achieved by making reference to known solubilities of particular polymers with particular solvents. An example of such known solubilities is shown in the Table below (where "+" means soluble, "-" means insoluble, "(+)" means soluble at high temperatures). Specifically, illustrative solvents and polymers are given along with their corresponding δ_1 and δ_2 values.

| Illustrative Solubilities and Solubility Parameters of Polymers | | | | | |
|---|------------|-------------------------------------|---|---|--|
| Solvent | | Solubility of the Polymer | | | |
| | δ_1 | Poly(isobutene) $\delta_2 = 7.9$ | Poly(methyl methacrylate) $\delta_2 = 9.1$ | Poly(vinyl acetate) $\delta_2 = 9.4$ | Poly(hexamethylene adipamide) $\delta_2 = 13.6$ |
| Decafluorobutane | 5.2 | - | - | - | - |
| Neopentane | 6.25 | + | - | - | - |
| Hexane | 7.3 | + | - | - | - |
| Diethyl ether | 7.4 | - | - | - | - |
| Cyclohexane | 8.2 | + | - | - | - |
| Carbon tetrachloride | 8.62 | + | + | - | - |
| Benzene | 9.2 | + | + | + | - |
| Chloroform | 9.3 | + | + | + | - |
| Butanone | 9.3 | - | + | + | - |
| Acetone | 9.8 | - | + | + | - |
| Carbon disulfide | 10.0 | - | - | - | - |
| Dioxane | 10.0 | - | + | + | - |
| Dimethyl formamide | 12.1 | - | + | + | (+) |
| <i>m</i> -Cresol | 13.3 | - | + | + | + |
| Formic acid | 13.5 | - | + | - | + |
| Methanol | 14.5 | - | - | - | - |
| Water | 23.4 | - | - | - | - |

The utility of the solubility parameter approach is that δ (solubility parameter) can be calculated for both the polymer and solvent. There are three separate forces which together influence the δ 's numerical value. These include: 1) the number of dispersive forces present; 2) the number of dipole-dipole forces present; and 3) the number of hydrogen bonding forces present. As a first approximation and in the absence of strong interactions such as hydrogen bonding, solubility of a polymer in a solvent can be expected if $\delta_1 - \delta_2$ is less than 1.7 - 2.0, but not if appreciably larger.

Thus, as a general rule of thumb, a polymer is expected to be soluble in a solvent where δ_1 is approximately equal to δ_2 . Of course, it is contemplated that these values need

not be equal. The present invention contemplates using polymers and solvents where the values of δ_1 and δ_2 are within approximately 2.0 units (more preferably within approximately 1.0 unit and most preferably within approximately 0.5 units).

It is not intended that the present invention be limited to the solvents and polymers set forth in the above Table. Other solvents and polymers are contemplated. For example, soluble solutions of poly(styrene) ($\delta_2 = 9.3$), are readily obtained with butanone ($\delta_1 = 9.3$) and dimethylformamide ($\delta_1 = 12.1$), but not with acetone ($\delta_1 = 9.8$). In the liquid acetone, the acetone molecules form dimers through dipole-dipole interactions. In these dimers, the keto groups are shielded by the methyl groups, and so are no longer able to solvate the phenyl groups of the poly(styrene). The addition of the cyclohexane ($\delta_1 = 8.2$) decreases the tendency of the acetone molecules toward association, and thus frees keto groups for solvation. For the same reason, it is also possible to have 40% solutions of poly(styrene) in acetone. Butanone, on the other hand, is "internally diluted" by the additional CH_2 group, and is therefore a solvent over the whole concentration range.

Similar reasoning applies to mixtures of solvents. A solvent with a lower solubility parameter (*i.e.* than that of the polymer) can be combined with a solvent with a higher solubility parameter (*i.e.* than that of the polymer). Poly(acrylonitrile) ($\delta_2 = 12.8$), for example dissolves in both dimethylformamide ($\delta_1 = 12.1$) and malodinitrile ($\delta_1 = 15.1$), but not in a mixture of the two. In some embodiments, the present invention contemplates etching with solvent mixtures.

II. Basic Polymer Process: Polymer Channels

The basic fabrication process begins with a conventional silicon CMOS processed substrate, as shown in Figure 3. In this figure, inlet and outlet are etched from the front side. However, backside inlet and outlet can easily be made by etching holes into the substrate from the backside using an anisotropic etchant such as ethylenediamine pyrazine (EDP), tetramethylammonium hydroxide (TMAH) or even XeF_2 stopping at the passivation oxide or nitride (also see Section III). Other alternatives on passivation layer includes oxide/nitride/oxide and low stress silicon-rich silicon nitride. Next, the microfluidic plane is constructed using the simplified process shown below. First a thick polymer layer is deposited and photolithographically defined onto the substrate with the thickness required for the channel height (0.5-100 μ). Next a second polymer is deposited serving as the walls of the channels. The second polymer is next etched defining the extent of the channel walls. Holes

to polymer 1 can be etched either from the front or through the backside by plasma etching the passivation oxide (see Section III). Polymer 1 is now sacrificially etched leaving a channel behind. In some cases where polymer 2 may react with polymer 1, a third material serves as a barrier layer, is deposited in between polymer 1 and polymer 2. The barrier layer has to be relatively conformal to provide a complete physical contact of polymer 1 from polymer 2.

Figure 4 is an example of a simple polymer channel: Figure 4(a) shows the top view of a 100μ wide, 25μ -high channel with toptype inlet fabricated using this process. Channels as long as 3mm are completely sacrificially released in under 20 minutes time. These channels are optically clear. Figure 4(b) shows a closeup SEM showing the three polymer construction of a 15μ -high channel with 40μ -thick walls.

In this example, photodefinable polyamide is used as polymer 2, vapor-deposited p-xylylene as the thin intermediate barrier material, and thick photoresist as polymer 1. The details of embodiments of the fabrication process are set forth in the Examples. It is not intended that the present invention be limited by the particular type of polymers. Polymers include, but are not limited to, photoresist, polyamide, benzocyclobutene, polydimethylsiloxane, vapor-deposited p-xylylene, pTEE, PMMA, p-HMDS, polypropylene, starch-based polymers, epoxy, and acrylics. In addition, it is not intended that the present invention be limited by the particular barrier layer. The barrier layers include, but is not limited to, metal, stack of different metals, pTFE, polymers, silicon-based materials, and other inorganic materials. Fluidic Interconnects can also easily be fabricated as shown in Example 2 (below).

III. Fabricating Reservoirs, Mixers, Reaction Chambers, Cavities

Reservoirs, mixers, reaction chambers, and any cavities can be formed by proper enlargement of the channels while heaters, detectors, and active circuits reside on the CMOS plane below. Figure 5 shows an example of a reaction chamber. The details of one embodiment of the fabrication process are set forth in the Examples. Figure 5a shows the top view of 1 mm^2 , $25\mu\text{m}$ -high thermal cycling chamber constructed using this process. The dots on the cavity are posts periodically spaced inside the cavity which enhance mixing and stiffen the cavity to prevent it collapse. The cavity is connected at both ends with short channels leading to top inlets and outlets. Figure 5(b) shows the cross-section of a similar PCR thermal cycling chamber showing the large volume of the cavity. Ink-jet printer head

can be fabricated similar to the PCR reactor except that there are arrays of etch holes (circular or square) on the structural polymer. Instead of forming post in the large cavity, internal walls are built so that each etch hole is isolated and is connected to an internal reservoir, which stores ink. Under each isolate cavities, heaters are placed or suspended on tope of the substrate.

IV. Fabricating Actuators

The same process also allows the fabrication of actuators such as valves and pumps. The polymer process must include the following layers: (1) a sacrificial polymer layer for forming cavities and channels; (2) a thick elastic structural polymer layer for forming elastic diaphragms needed in pumps and valves. This thin polymer can also serve as a barrier layer in some cases; (3) a thick structural polymer layer for construction of thick channel walls and bossed diaphragms; and (4) a metal layer that can carry current either embedded between (2) and (3) or on top of layer (3). Figure 6 shows the basic process steps carried out to fabricate the actuator.

The process begins with a conventional CMOS, bipolar, or BiCMOS substrate passivated by a layer of silicon dioxide. Metal lines can be present on the circuit side if desired. The next step in the process consists of the opening of holes on the backside oxide by plasma etching. These holes will provide access to the microfluidic polymer plane. After this step, the circuit substrate is immersed in a silicon rich TMAH solution that anisotropically etches the substrate all the way to the passivation oxide layer. The cavities formed by this etch are bounded by the 111 crystal planes of the substrate. The silicon saturated TMAH solution will not attack any aluminum wires exposed on the surface [U. Schankenberg *et al.*, "TMAHW Etchants for Silicon Micromachining," in *Transducers '91*, pp. 815-818 (1991)].

After rinsing and drying the substrates, a passivation layer is deposited on top of the circuits and subsequently patterned. The passivation layer is needed if the system being built must handle fluids that contain mobile ions such as sodium ions and potassium ions (many biochemical reactions require weak KCl buffer solutions) which can easily diffuse through the oxide passivation of the circuit plane. Several types of passivation layers can be used (or even none). Conventional materials such as sputtered SiN serve the purpose quite well. Polymers can also be used as a barrier to the contaminant diffusion. The barrier layer is next photolithographically defined and etched on the areas leading to the backside inlet and metal contact areas.

Wafers that have reached this state are ready for the construction of the microfluidic plane. First a thick polymer 1 layer is deposited and etched on top of the substrate. This thick polymer serves as a spacer layer that determines the height of channels and microfluidic components. Multiple thicknesses on the same wafer can be obtained through time etching or multiple depositions. The range of thicknesses for this polymer is between 0.5-200 μ . Next a thin layer of polymer (polymer 2) 0.5-2 μ thick is deposited uniformly onto the wafer. Polymer 2 serves as a thin structural layer that is easily deflected, and as a barrier layer in some cases. Its main purpose is for the fabrication of flexible membranes required by the pumps and valves described earlier. Next, a thin layer 1-2 μ of aluminum is evaporated on top of polymer 2 and it is photolithographically defined to form parallel conductors and interconnects used in the fluidic actuators. The process is followed by the deposition and patterning of a thick polymer (polymer 3) that provides structural support for the channels and microfluidic component walls. Next holes are opened on the thin polymer 2 layer leading to bonding pads on the circuit plane and also providing access to polymer 1 if desired from the front side. At this point the remainder passivation oxide that separates polymer 1 from the backside inlets is etched by plasma etching. In the last step of the process, polymer 1 is sacrificially etched thus releasing the channels and fluidic actuators. The chips are next mounted on a ceramic package and the electrical leads are formed by wire bonding. As an example, polymer 1 can be photoresist, polymer 2 can be p-xylylene, and polymer 3 can be polyamide.

Droplets of the samples must be moved to the various parts of the system in order to perform various tests on the fluidic chip. A thermocompression suction pumps that create a pressure differential between the two sides of the liquid drop can be used. A simple suction pump can be constructed as shown in Figure 7. The pump basically consists of a heater and a valve. This device is constructed with an additional thin polymer layer and an embedded metallization inserted on the microfluidic plane. The construction of this type of pump is thus entirely compatible with the polymer-based technology. This pump basically consists of a heater constructed on a polysilicon heater constructed on the MOS plane placed under the polymer channel, and an exhaust valve. The exhaust valve consists of an enlarged version of the channel with a central outlet. On top of the polymer valve, a series of parallel aluminum wires are patterned. The aluminum wires are placed between a thin polymer and a thick one thus forming a boss region and a diaphragm. This valve is magnetically actuated upon application of a transversal magnetic field B (through an external permanent magnet). The

valve either closes or opens depending on the direction of the current I on the parallel conductors. Magnetic actuation was chosen for the valve because the tall channels generated by the polymer-based process have very high pull-in voltages hence rendering electrostatic actuation impractical.

5 The thermocompression pump operates as follows: first, the exhaust valve is opened and the heater is powered up. Next the valve is closed and the heater is turned off thus forming a partial vacuum on the channels as the trapped air cools down. This partial vacuum propels the drop forward until the pressure difference disappears. The cycle is repeated for continuous pumping. Propulsion of the droplet in the reverse direction can also be achieved
10 by expanding the gas rather than contracting it. This type of simple pump can generate pneumatic pressure differences as high as 0.5μ , well above the threshold needed for the onset of motion of droplets in capillaries. If the pneumatic drop motion mechanism is used, *pass-through valves* are needed into the system. These valves basically constrict the channels at various points in the system thus acting as gates that block the passage of liquids. Pass-
15 through valves are necessary to impede the continuous drawing of liquids onto the capillary. This situation occurs due to the fact that when droplets are moved inside the capillary channels, they form a partial vacuum behind them which can in turn move drops on other parts of the system. Pass through valves impede the entrance of these drops onto places where there exist temporary vacuum pressure differences. It is also important to point out that once a channel
20 is blocked, it is necessary to open an exhaust valve (such as those made for the thermocompression pump) attached to the same end as the blocking valve if additional liquid drop motion is desired. The exhaust valve provides an additional inlet that impedes the formation of a vacuum behind the moving drop that would ultimately stop additional drop motion.

25 This brings the important point that pumping of discrete drops can only be accomplished when one of channel ends is open to the environment. This problem has been pointed out by Anderson (*supra*) who has proposed the use of permeable membranes to eliminate this problem. Figure 8 shows the cross-section of a pass-through valve implemented using the polymer based channel. The valve basically consists of a flat round segment of an
30 enlarged channel that is constructed in its collapsed form by the deliberate removal of polymer 1. A patch of polymer 3 (Teflon) is placed under the valve such that polymer 2 does not stick to the surface. The valve is opened or closed by applying a current to the

conductors above the pass-through valve which buckle the diaphragm upward thus opening the valve.

V. Bubble Pumps and Capillary Valves

5 As noted above, it is not intended that the present invention be limited by the nature of the flow-directing means used to convey fluids through the polymer channels of the present invention. In one embodiment, the present invention contemplates a polymer channel comprising one or more electrodes in contact with the liquid sample. A schematic of one embodiment of the bubble pump and capillary valve is shown in Figure 9. The bubble pump
10 comprises a polymer channel with several electrodes (e.g. Ti/Pt electrodes) in contact with the liquid sample. When coupled with the capillary valve, the bubble pump can actuate a certain quantity of fluidic samples along the polymer chip. The capillary valve is essentially a narrow section of a polymer channel. In operation, the fluidic sample is first injected in the inlet reservoir. As soon as the fluid is loaded, it moves in the channel by capillary force.
15 The fluid then passes the narrow section of the channel but stops at the edge where the channel widens again (see Figure 10A). After the fluidic sample is loaded, a potential is applied between two electrodes. At both ends of the electrodes, hydrolysis occurs and bubble is generated. The bubble keeps on growing as the electrodes continue pumping electrical charges to the fluid (Figure 10B). The expanding bubble then creates a pressure differential
20 between the two sides of the liquid drop, which eventually large enough to push the liquid forward (Figure 10C).

The combination of bubble pump and capillary valve is an excellent fluid propelling device. This actuating device does not require any moving parts and is easy to fabricate. In addition, the device produces a well-controlled fluid motion, which depends on the bubble
25 pressure. The bubble pressure is controlled by the amount of charges pumped by the electrodes. Furthermore, the power consumption of the device is minimal.

EXPERIMENTAL

The following examples serve to illustrate certain preferred embodiments and aspects
30 of the present invention and are not to be construed as limiting the scope thereof.

EXAMPLE 1

This example describes one embodiment of the fabrication method of the present invention. More specifically, this example describes one embodiment of the process flow for channels and cavities with frontside inlets and outlets.

- 5 The process starts with a 4 inch silicon wafer. Thereafter, the steps are as follows:
0. (Optional) Deposit 2 μ -thick p-xylylene with primer.
 1. HMDS is spread at 200 rpm for 16s, and spun at 4000rpm for 30s.
 2. Photoresist (AZP4620) is spread at 200rpm for 16s, and spun at 750rpm for
10 50s (about 20 μ -thick). Experiments reveal that this resist does not stick too well with silicon
 substrate without applying HMDS. For some other resist, HMDS may not be applied.
 3. Remove the resist beads which reside on the edge of the wafer using Q-tip
 soaked with acetone. This step is necessary because these beads will glue to the mask plate
 during mask aligning.
 4. Soft baked on a hot plate for 2 mins. at 130°C. Lower temperature with longer
15 bake time should also work.
 5. Patterned and exposed at intensity of 1250 ml/cm².
 6. Developed for 16 mins in AZ312: H2O=1:1.
 7. Hard baked at 130° for 3 mins.
 8. Plasma etching using O₂ at 80W: 250mT for 2 mins. Longer etching time may
20 burn the resist.
 9. Deposit 2 μ m-thick p-xylylene without applying primer. The p-xylylene will
 crack if it is too thin, and resist will crack if primer is applied. Without primer, the adhesion
 between p-xylylene and silicon substrate is not too good.
 10. Plasma etching using O₂ at 80W for 2 mins. This dry etching is necessary to
25 improve the adhesion between parylene and polyamide.
 11. Polyamide (Polyamide 7020 from OCG) is spread at 200rpm for 10s, and spun
 at 2500rpm for 10s (about 40 μ -thick).
 12. Soft bake at 90°C for 2 mins, followed by baking at 110° for 7 mins..
 13. Pattered and exposed at intensity of 350ml/cm².
 - 30 14. Bake at 105° C for 1 mins.
 15. Let it rest for 30 mins.
 16. Develop (QZ2501 from OCG) for 6 mins. Agitate.

17. Rinse (QZ3512 from OCG) for 2 mins. Agitate. If some white residual is observed, put the wafer to the developer.
18. Blow dry.
19. Repeat Step 2 to 6. No HMDS is applied because adhesion between p-xylylene and polyamide is strong enough.
20. Etching p-xylylene: Plasma etching using O_2 at 250W, 250mT for 20 mins.
21. Sacrificial release: Immersed into hot Acetone for 20 mins.
22. Rinse with IPA and water.
23. (Optional) Final Cure 250° for 30 mins. Higher temperature may burn p-xylylene.

EXAMPLE 2

This example describes another embodiment of the fabrication method of the present invention. More specifically, this example describes one embodiment of the process flow for fluidic interconnect. The process starts with a 4 inch silicon wafer. Thereafter the steps are as follows:

1. Repeat Step 11 to 18 from Example 1.
2. Repeat Step 2 to 6 from Example 1.
3. Repeat Step 9 to 22 from Example 1.
4. (Optional) Final Cure 250° for 30 mins. Higher temperature may burn p-xylylene.

EXAMPLE 3

In this example, another embodiment of a reaction chamber is constructed using the methods of the present invention. The fabrication process is same as in Example 1 (above). Figure 11 shows the resulting reaction chamber with one supporting post. The reaction chamber is approximately 1mm long, 1mm wide and 20 μ m high.

EXAMPLE 4

In this example, another embodiment of a thermocompression suction pump is fabricated according to the methods of the present invention. A thermocompression suction pump is fabricated (Figure 12) that is similar to the one described in Figure 7 of the patent. Figure 12 shows a polymer valve of 1mm in diameter with a 30 μ m thick boss of 320 μ m in

diameter. The valve orifice is 80 μm in diameter. A combination of chromium and gold of 50 μm -wide are placed on top of the 4 μm -thick p-xylylene diaphragm. The polymer valve is connected to a stand channel of 100 μm wide.

5

EXAMPLE 5

In this example, one embodiment of the fabrication method for the bubble pump and capillary valve is described. Figure 13 is a schematic showing the embodiment of the fabrication method for the bubble pump and capillary valve.

The process begins with a 100 mm p-type silicon substrate with a 0.7 μm layer of thermally grown silicon dioxide. The hydrolysis electrodes are next formed by evaporating a thin layer of 20 nm of Ti followed by a 30 nm layer of Pt and patterned by liftoff using a 3 μm of Shirley 1827 photoresist and acetone. A uniform thin layer of p-xylylene (Specialty Coating Services, parylene-C) 2 μm -thick is vapor deposited at room temperature on the oxidized substrates. The p-xylylene layer is next roughened with a 100W, 300mT O_2 plasma for 2 minutes to improve the adhesion of the subsequent polymer layer. A 10 μm -thick layer of photosensitive polyamide (OCG Probimide 7020) is next spin-cast at 7000 rpm for 10 sec. serving as a stop. The sample is soft baked at 90°C for 2 min. followed by 110° for 7 min. The soft baked polyamide is next exposed and patterned with a dose of 350mJ/cm² followed by a bath in OCG QZ2501 developer for 5 min and a 1 min. rinse in QZ3512. Next, a second 2 μm layer of p-xylylene is deposited on top of the polyamide and selectively etched on the Pt electrode areas with a 250W, 50mT O_2 plasma for 30 min. After that, the polymer channel is ready to be constructed. First, a 20 μm -thick sacrificial layer of AZP4620 (Hoechst) photoresist is spin cast at 750 rpm for 50 sec. and soft baked at 130° and exposed using a dose of 1750mJ/cm² to define the channel area. A second partial exposure of 250mJ/cm² is performed to define the narrow section of the channel, followed by a 10 min development in AZ 312. After that, the wafer is hard baked at 130°C, followed by a short O_2 plasma descum. A third 2 μm thick layer of p-xylylene is deposited which completely seals the resist layer and prevents the subsequent polyamide solvent from attacking the soft sacrificial resist. The top p-xylylene is next roughened with O_2 plasma and the top structural layer is constructed by spin-casting and patterning a 30 μm thick of photoresist polyamide as described earlier. This is followed by an O_2 plasma descum and a final 1 μm p-xylylene deposition. Access holes to the sacrificial resist are patterned using a 20 μm -thick layer of resist (AZP4620) and etched in a 250 W, 50mT, O_2 plasma for 35 min. that removes the two

p-xylylene layers on top of the sacrificial resist layer and at the same time the three p-xylylene layers on top of the platinum electrodes for electrical contact. The masking resist and the sacrificial resist layer are both removed in a warm bath of acetone for about 50 min, followed by a rinse in isopropyl alcohol and DI water.

5

From the above, it should be clear that the polymer-based technology of the present invention provides a micromachining technology that allows for fabrication of channels with a flexibility in cross section and channel length with virtually no loss in system performance.

10 In addition, the polymer technology of the present invention is low cost and does not require specialized equipment.

CLAIMS

1. A method for fabricating microchannels, comprising the steps:

a) providing a substrate, said substrate having upper and lower surfaces;

5 b) depositing a first polymer layer on said upper surface of said substrate, said first polymer layer having i) a lower surface contacting said upper surface of said substrate and ii) an upper surface;

10 c) depositing a second polymer layer on said upper surface of said first polymer layer, said second polymer layer having i) a lower surface contacting said upper surface of said first polymer layer and ii) an upper surface; and

d) sacrificially etching said first polymer layer under conditions such that said second polymer layer comprises walls of a microchannel.

15 2. The method of Claim 1, wherein said substrate is a silicon substrate.

3. The method of Claim 1, further comprising, prior to step (c) the step of photolithographically defining said first polymer layer under conditions such that said first polymer layer has a desired thickness.

20 4. The method of Claim 3, wherein said desired thickness of said first polymer layer is between 0.5 and 200 μ .

25 5. The method of Claim 1, further comprising, prior to step (d) the step of photolithographically defining said second polymer layer.

6. The method of Claim 1, further comprising, after step (d) the step (e) of etching openings in said walls of said microchannel.

30 7. The method of Claim 6, further comprising after step (d) and before step (e) the step of depositing a third polymer layer on said upper surface of said second polymer layer.

8. The method of Claim 1, wherein said first polymer and said second polymer are selected from the group consisting of photoresist, polyamide, benzocyclobutene,

polydimethylsiloxane, vapor-deposited p-xylylene, pTFE, PMMA, p-HMDS, polypropylene, starch-based polymers, epoxy, and acrylics.

9. A method for fabricating microchannels, comprising the steps:

- 5 a) providing a silicon substrate, said silicon substrate having upper and lower surfaces;
- b) depositing a first polymer layer on said upper surface of said silicon substrate, said first polymer layer having i) a lower surface contacting said upper surface of said silicon substrate and ii) an upper surface;
- 10 c) photolithographically defining said first polymer layer under conditions such that said first polymer layer has a desired thickness;
- d) depositing a second polymer layer on said upper surface of said first polymer layer, said second polymer layer having i) a lower surface contacting said upper surface of said first polymer layer and ii) an upper surface; and
- 15 e) sacrificially etching said first polymer layer under conditions such that said second polymer layer comprises walls of a microchannel.

10. The method of Claim 9, wherein said desired thickness of said first polymer layer of step (c) is between 0.5 and 200 μ .

11. The method of Claim 9, further comprising, prior to step (e) the step of photolithographically defining said second polymer layer.

12. The method of Claim 1, further comprising, after step (e) the step (f) of etching openings in said walls of said microchannel.

13. The method of Claim 12, further comprising after step (e) and before step (f) the step of depositing a third polymer layer on said upper surface of said second polymer layer.

14. The method of Claim 9, wherein said first polymer and said second polymer are selected from the group consisting of photoresist, polyamide, benzocyclobutene, polydimethylsiloxane, vapor-deposited p-xylylene, pTFE, PMMA, p-HMDS, polypropylene, starch-based polymers, epoxy, and acrylics.

15. A method for fabricating microchannels, comprising the steps:

a) providing a silicon wafer, said silicon wafer having upper and lower surfaces;
b) depositing a first layer of a first polymer on said upper surface of said silicon wafer, said first polymer layer having i) a lower surface contacting said upper surface of said silicon wafer and ii) an upper surface;

c) depositing a second polymer layer on said upper surface of said first layer, said second polymer layer having i) a lower surface contacting said upper surface of said first layer and ii) an upper surface;

d) photolithographically defining said second polymer layer under conditions such that said second polymer layer has a thickness between 0.5 and 200 μ ;

e) depositing a second layer of said first polymer on said upper surface of said second polymer layer, said second layer of said first polymer having i) a lower surface contacting said upper surface of said second polymer layer and ii) an upper surface;

f) sacrificially etching said second polymer layer under conditions such that said first and second layers of said first polymer comprise walls of a microchannel, said microchannel having a height defined by said thickness of said second polymer layer of step (d); and

g) etching one or more openings in said walls of said microchannel.

16. The method of Claim 15, further comprising after step (e) and before step (f) the step of depositing a layer of a third polymer on said upper surface of said second layer of said first polymer.

17. The method of Claim 16, wherein said first and second layers of said first polymer comprise vapor-deposited p-xylylene.

18. The method of Claim 17, wherein, at step (g), first and second openings are etched, said first opening serving as an inlet and said second opening serving as an outlet.

19. The method of Claim 18, wherein said openings are etched from said lower surface of said silicon wafer into said lower surface of said first layer of said first polymer.

20. The method of Claim 18, wherein said openings are etched from said upper surface of said layer of said third polymer into said upper surface of said second layer of said first polymer.
- 5 21. A device, comprising a polymer channel comprising one or more electrodes positioned such that they will be in contact with a liquid sample placed in said channel.
22. The device of Claim 21, wherein said polymer channel comprises two electrodes.
- 10 23. A method of conveying liquid in a microchannel, comprising introducing a liquid into the device of Claim 22.
24. The method of Claim 23, wherein a potential is applied between the two electrodes under conditions such that said potential results in the generation of a gas bubble large enough
- 15 to move at least a portion of said liquid in said polymer channel.

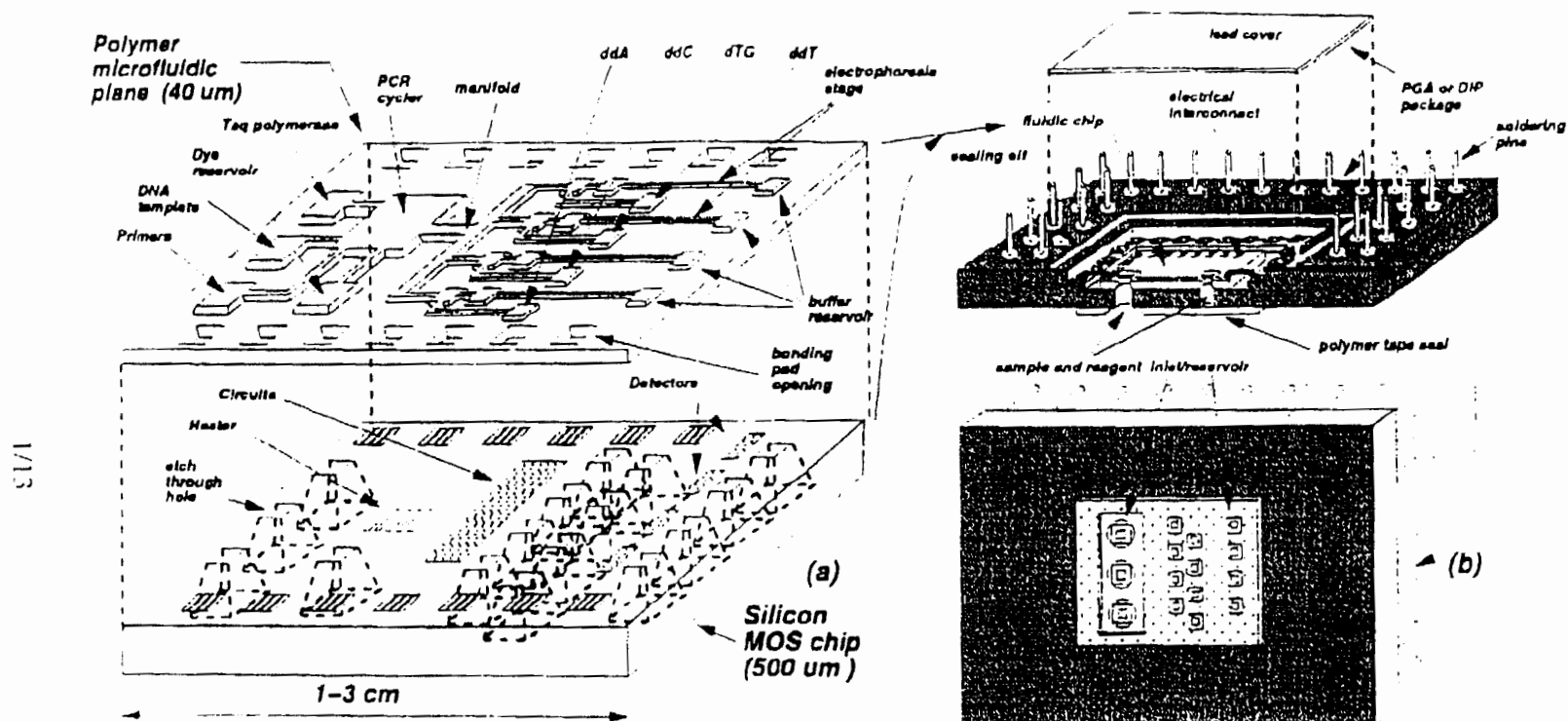


Figure 1: (a) The polymer fluid channels and reservoirs are constructed by deposition of plastics on top of a silicon MOS control chip. Etch-through holes are formed in the silicon substrate to serve as funnel-like inlets and reservoirs for the sample, reagents and buffer solutions. (b) Fluidic chip is mounted on a PGA or MCM package that isolates fluidic and electrical interconnect. Some of the sample inlets are sealed to serve as reagent reservoirs

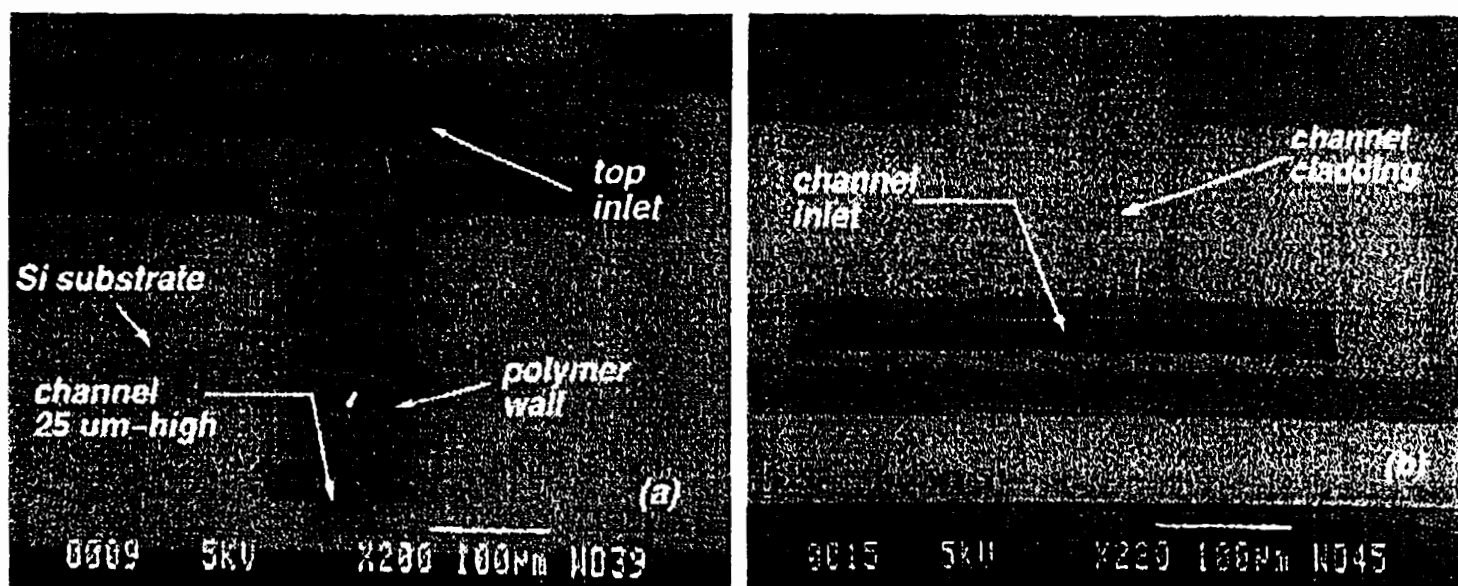


Figure 2: Top view and cross-section (a) of example polymer channel with front side inlet (b) formed on top of a regular Si substrate. Channel heights can be adjusted between 0.5-100 μm .

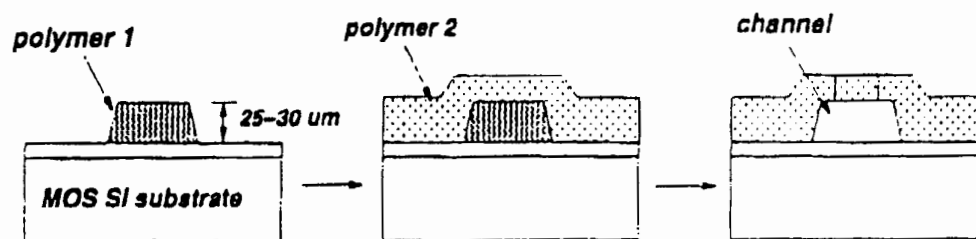


Figure 3: Simplified fabrication process for plastic channels.

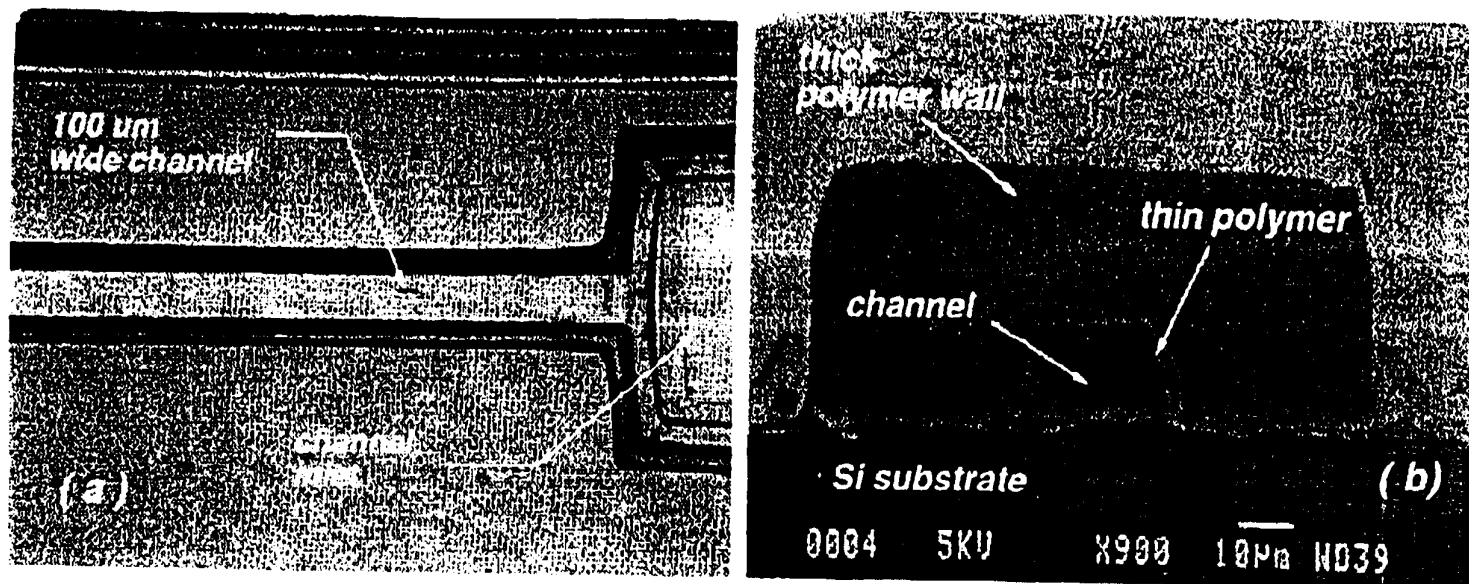


Figure 4: (a) Top view of example three-polymer channel 100 μm wide and 25 μm -thick with front side inlet. (b) closeup cross-section showing the three-polymer construction of the channels.

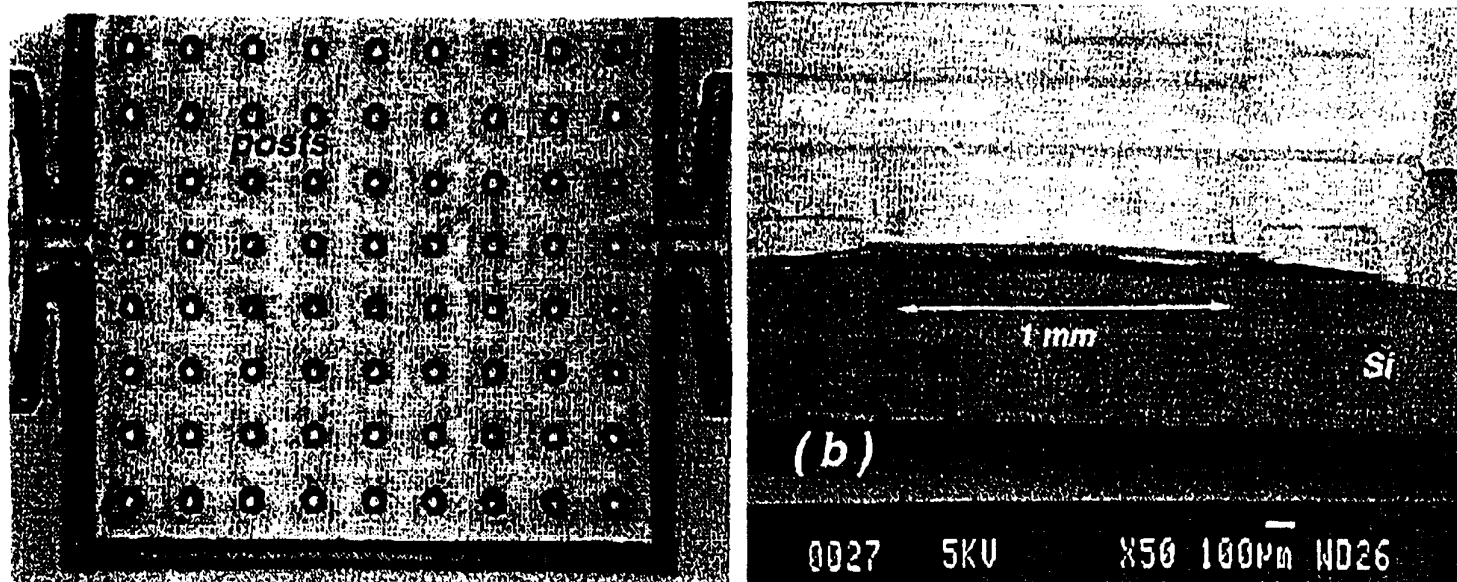


Figure 5: (a) Top view of 1 mm² thermal cycling chamber (b) cross-section of 25 μm-high, 1 mm² polymer PCR chamber fabricated on top of a silicon wafer.

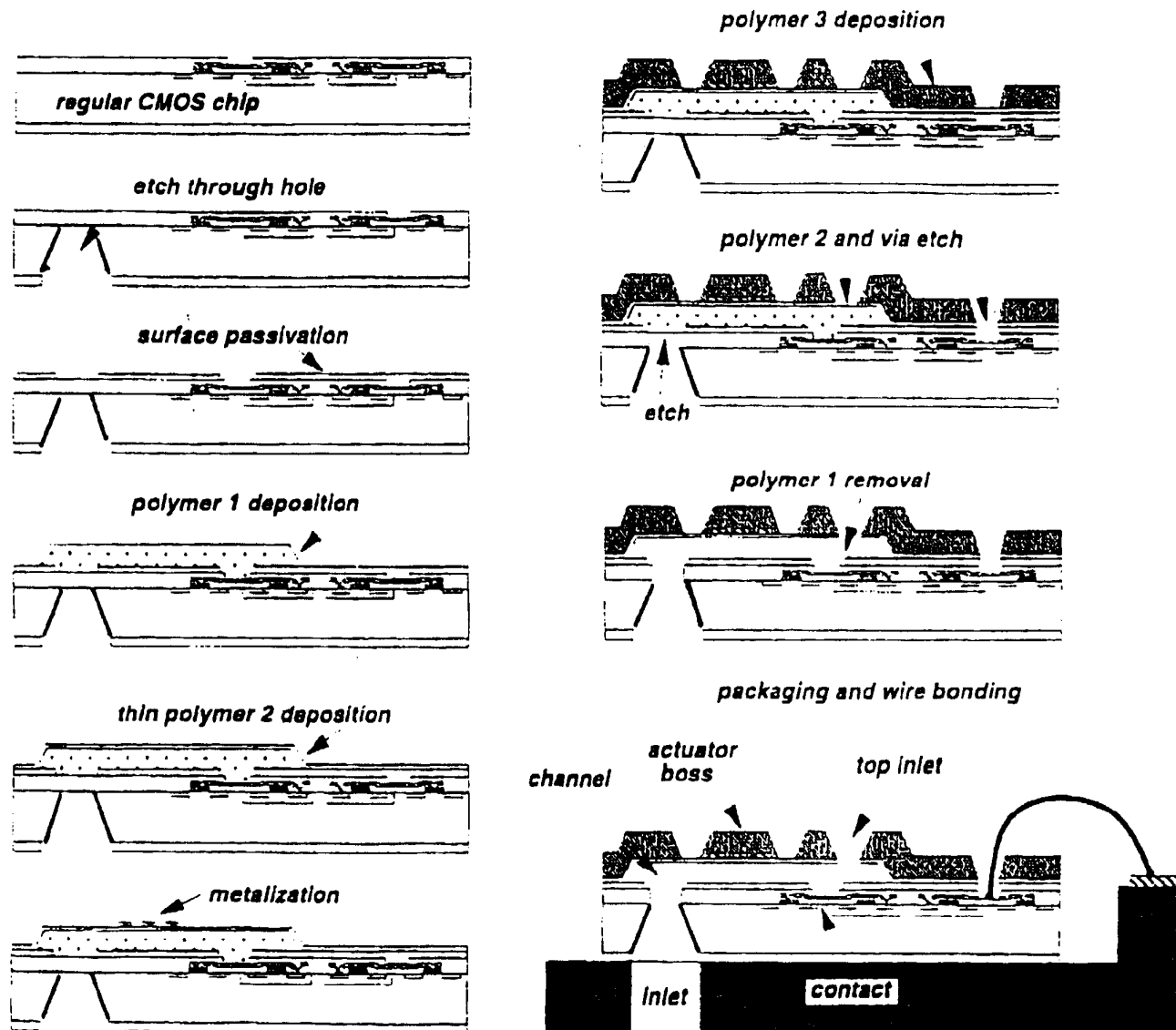


Figure 6: Simplified 3-level polymer process with embedded metalization. This process requires four lithographic masks and is capable of constructing all the microfluidic parts shown in this proposal. The figure shows circuits, backside and front inlets, actuators, channels, and bonding wires.

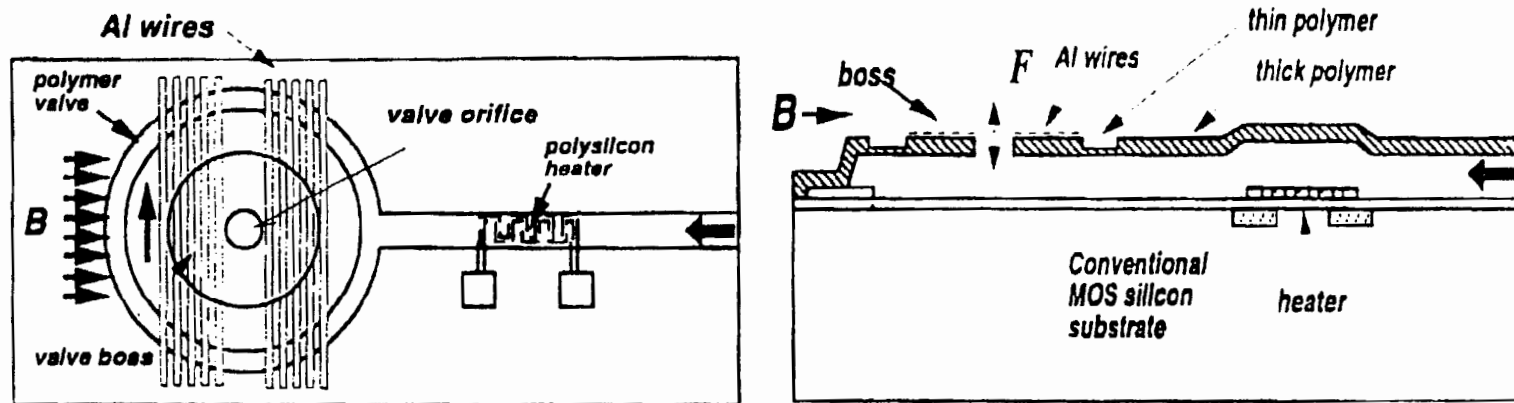


Figure 7: Polymer magnetically activated thermocompression suction pump

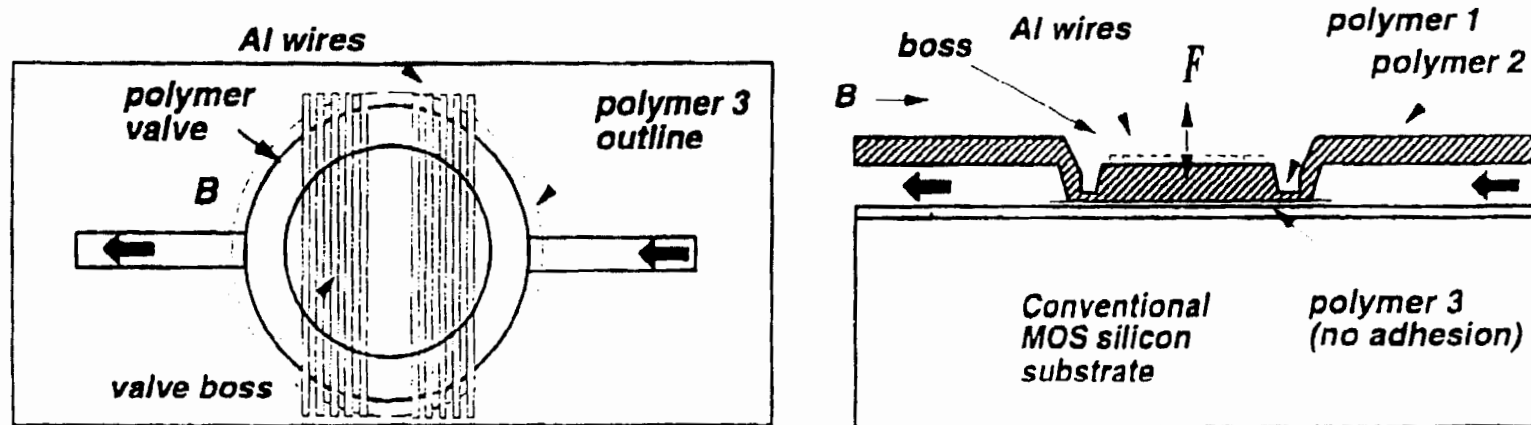


Figure 8: Pass-through valve. The valve is normally closed and opens when the diaphragm is buckled upwards.

FIGURE 9

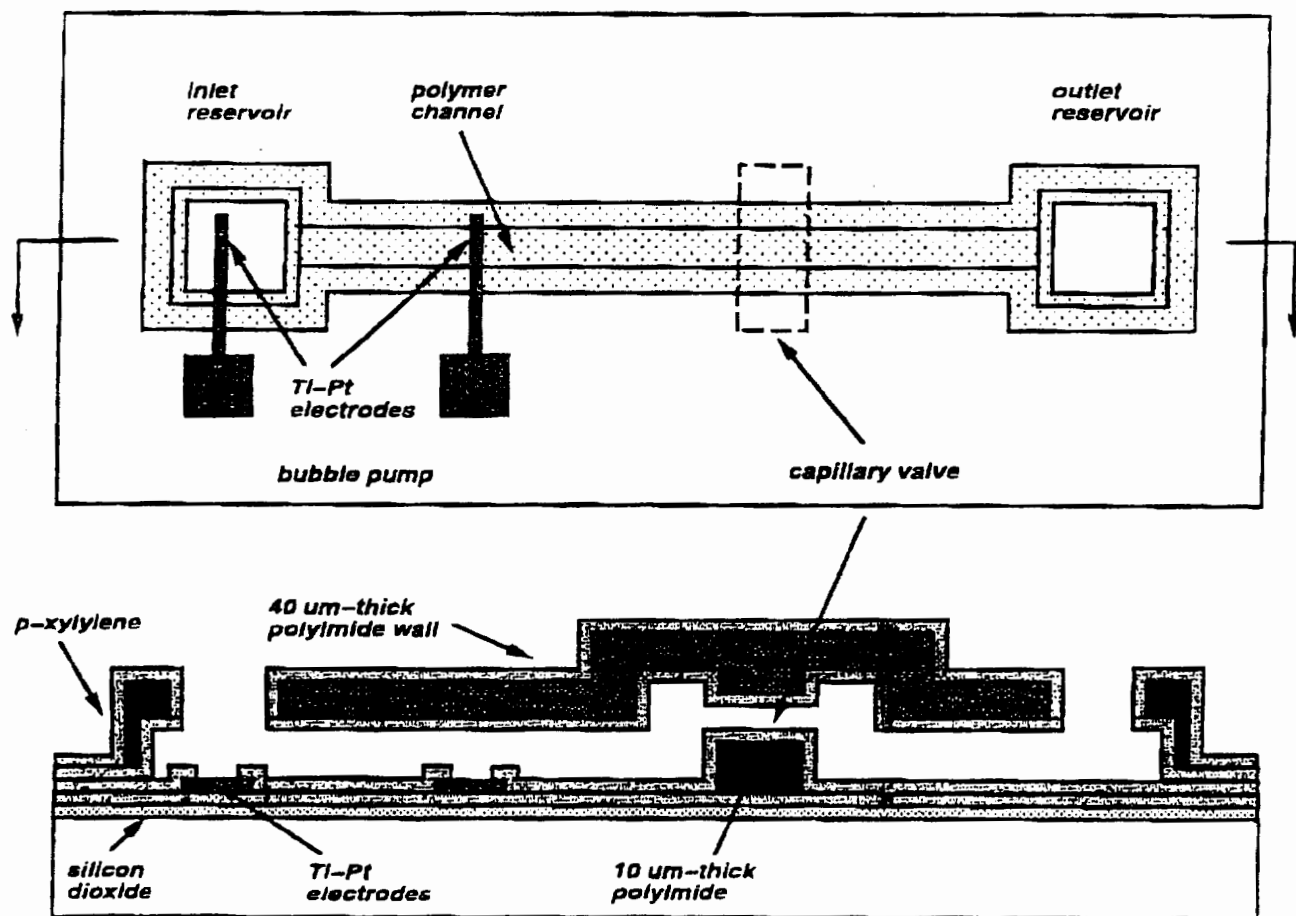


FIGURE 10

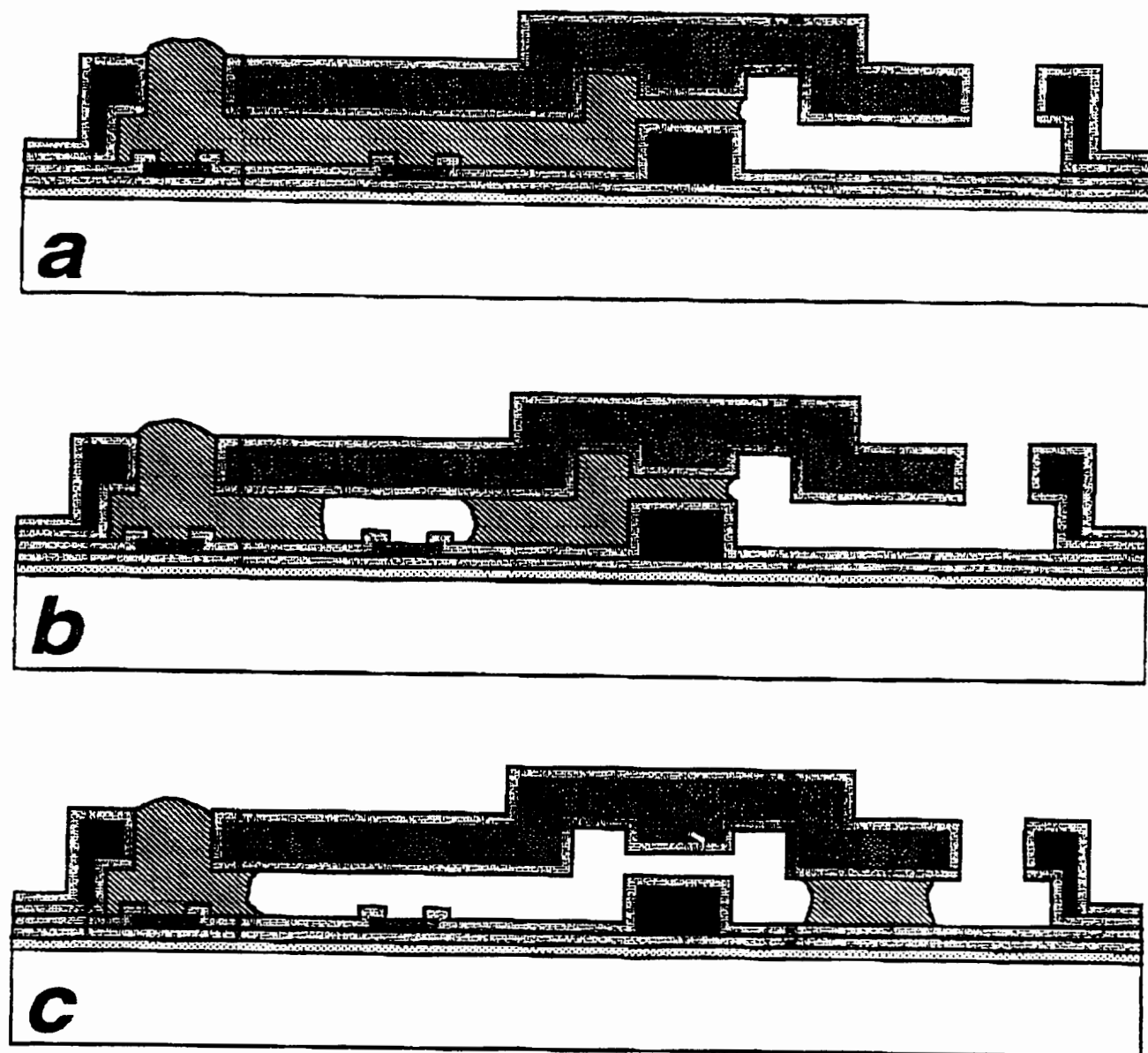
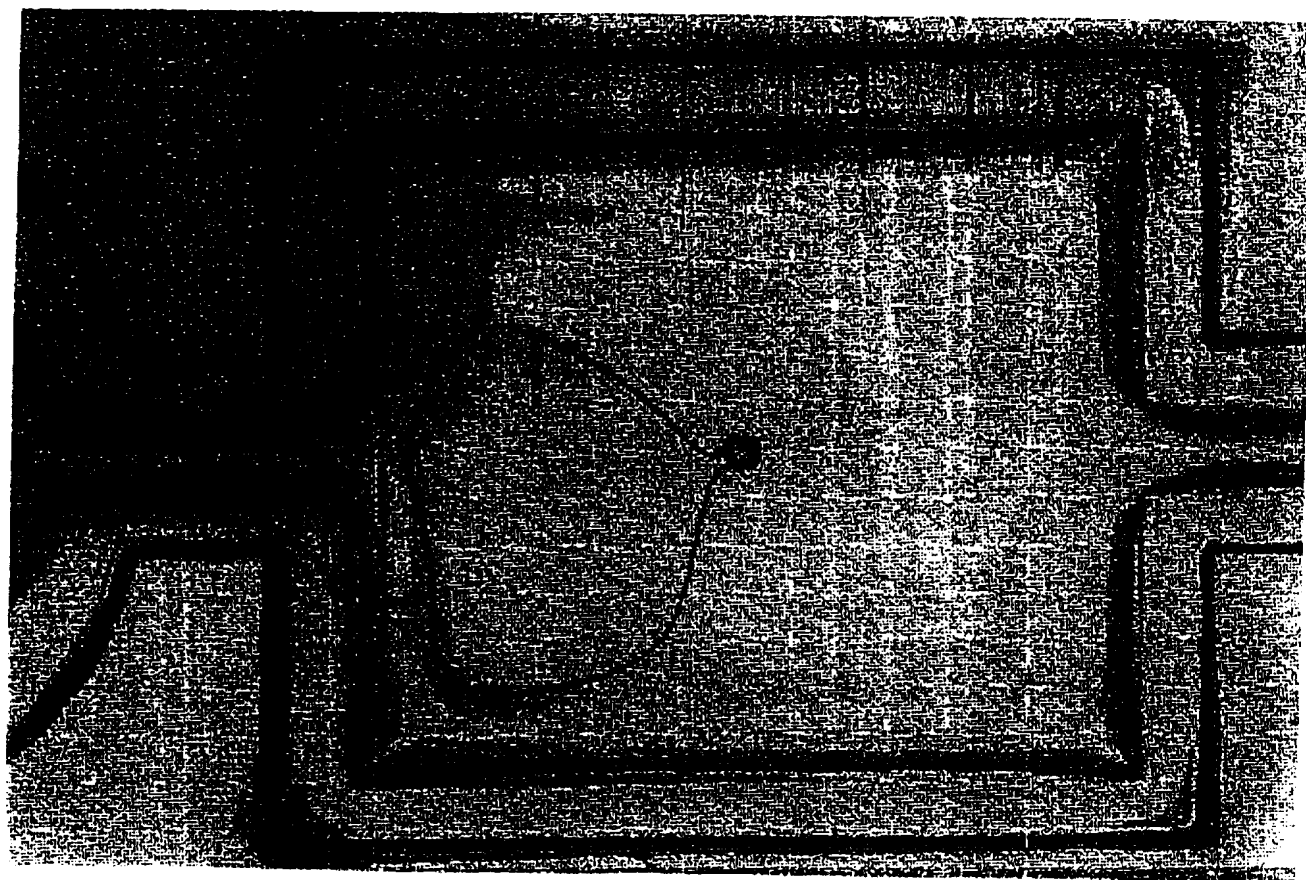


FIGURE 11



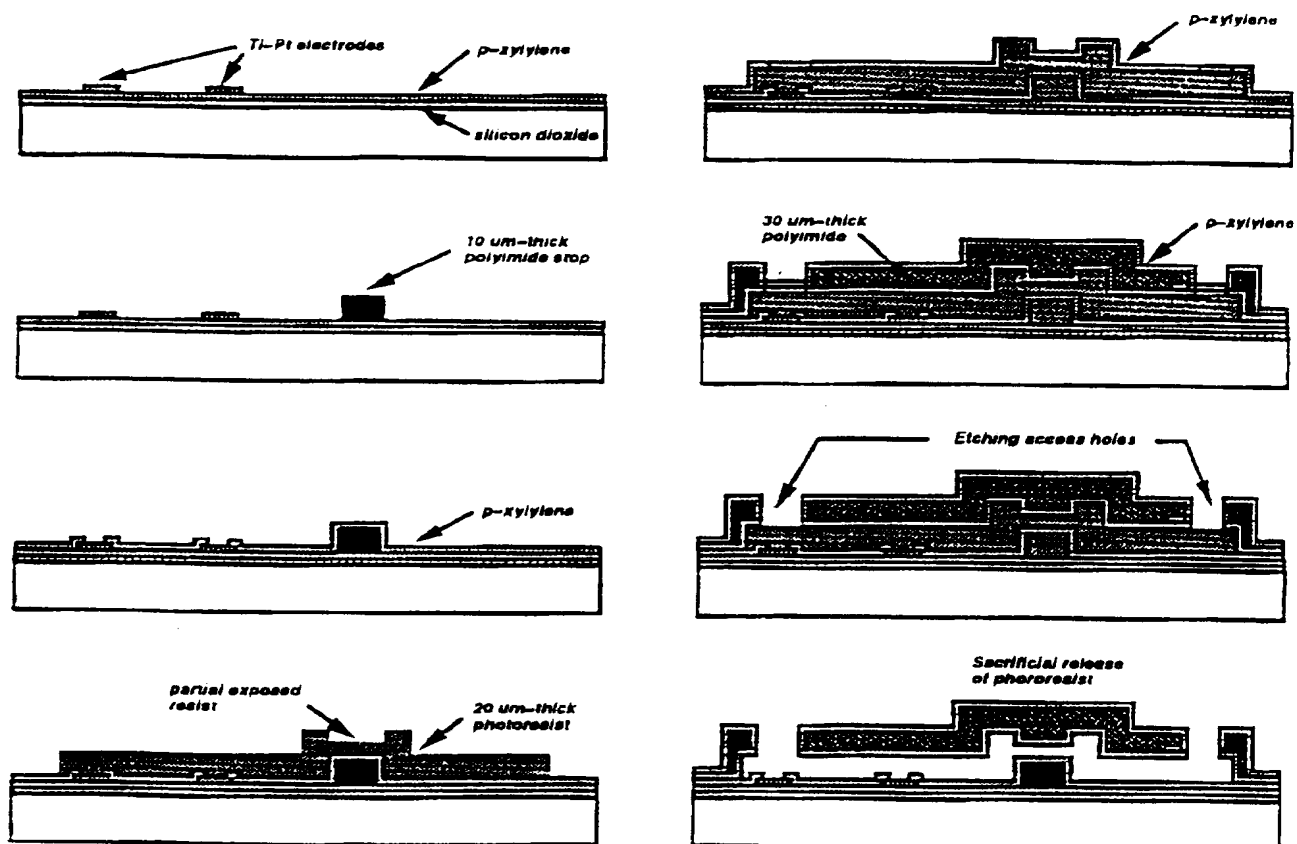
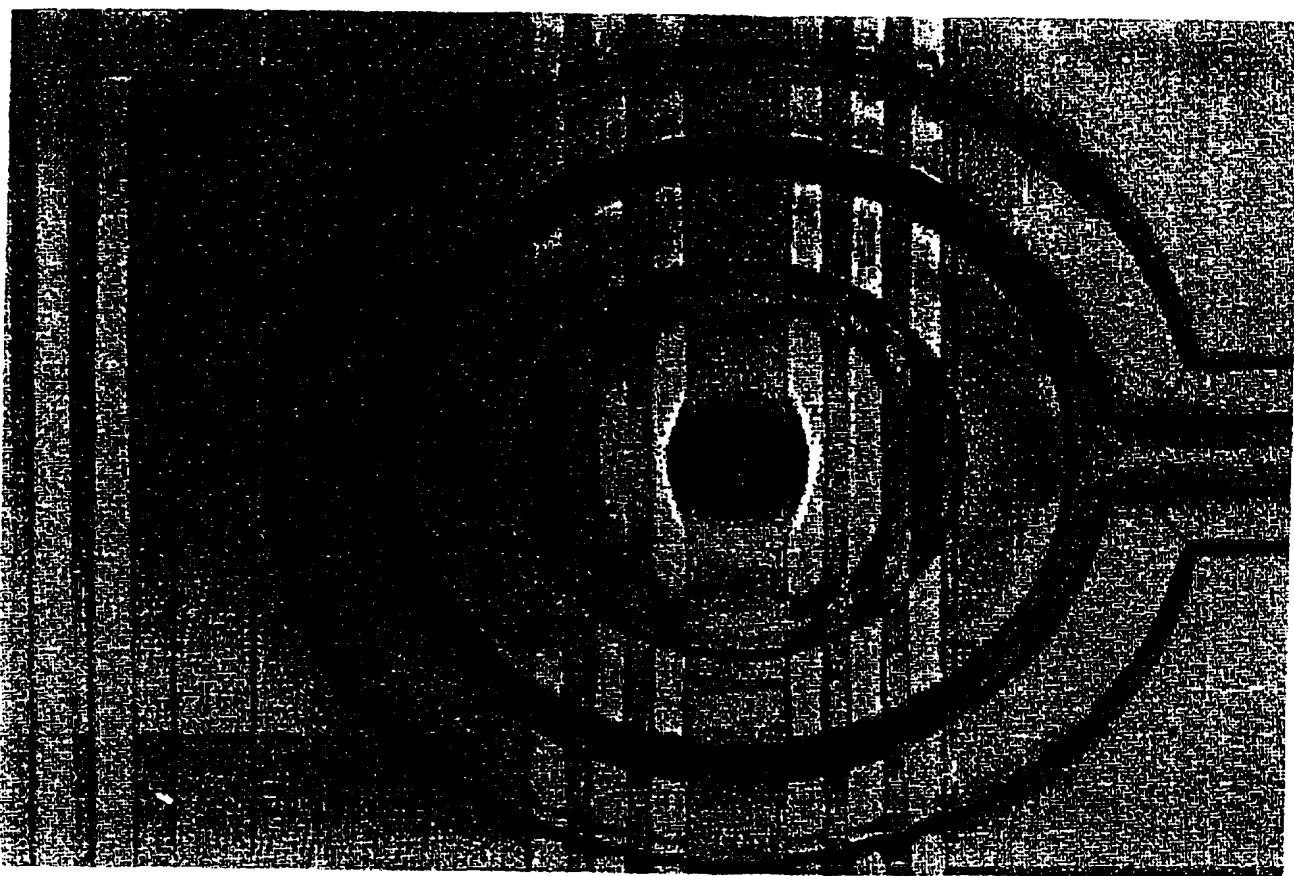


Fig. 13: Process flow of bubble pump and capillary valve

FIGURE 12



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14054

A. CLASSIFICATION OF SUBJECT MATTER

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US CL :430/311, 313, 314, 319, 394

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 430/311, 313, 314, 319, 394

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| Y | US 4,153,855 A (FEINGOLD) 08 May 1979, column 2, lines 6-37 and claim 2. | 1-24 |
| Y | US 5,265,327 A (FARIS ET AL) 30 November 1993, abstract, column 10, line 53 to column 11, line 12 and claims 3 and 4. | 1-24 |

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| (51) International Patent Classification ⁶ : C12Q 1/00 | A1 | (11) International Publication Number: WO 00/70080 (43) International Publication Date: 23 November 2000 (23.11.00) |
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| (54) Title: FOCUSING OF MICROPARTICLES IN MICROFLUIDIC SYSTEMS (57) Abstract <p>Methods and systems for particle focusing to increase assay throughput in microscale systems are provided. The invention includes methods for providing substantially uniform flow velocity to flowing particles in microfluidic devices. Methods of sorting members of particle populations, such as cells and various subcellular components are also provided. Integrated systems in which particles are focused and/or sorted are additionally included.</p> | | |

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5 **FOCUSING OF MICROPARTICLES IN MICROFLUIDIC SYSTEMS**

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CROSS REFERENCE TO RELATED APPLICATIONS

This application is related to and claims priority to and the benefit of provisional application 60/134,472, filed 5/17/99, Wada et al., "Focusing of
15 Microparticles in Microfluidic Systems," pursuant to 35 U.S.C. § 119(e), as well as any other applicable statute or rule. This priority application is incorporated herein in its entirety for all purposes.

BACKGROUND OF THE INVENTION

A variety of cell-based assays are of considerable commercial
20 relevance in screening for modulators of cell-based activity. For example, compounds which affect cell death can have profound biological activities and are desirably screened for in cell-based assays. Cell death has become recognized as a physiological process important in normal development, hormonal regulation of various tissues, and, e.g., in regulation of the receptor repertoires of both T and B
25 lymphocytes. The finding that a pattern of morphological changes is common to many examples of programmed cell death (or PCD) led to the suggestion of a common mechanism, and the term "apoptosis" was defined to include both the morphological features and the mechanism common to such programmed cell death (Kerr et al., Br. J. Cancer 26:239). This concept was extended by the finding that
30 nuclear DNA fragmentation correlates well with apoptotic morphology (Arends et al.,

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Am. J. Pathol. 136:593 (1990)), and the scientific literature contains many examples of PCD accompanied by these features. There are also clear examples of PCD in the absence of apoptotic morphology or DNA fragmentation (Clarke, Anat. Embryol. 181:195 (1990), Martin et al, J. Cell Biol. 106:829 (1988), and Ishigami et al., J.
5 Immunol. 148:360 (1992)).

Cell-based assay systems model relevant biological phenomena, and have generally been widely adopted as screening assays, e.g., when screening for a compound's effect(s) on apoptosis or other biological phenomena. Pioneering technology providing cell- and other particle-based microscale assays are set forth in
10 Parce et al. "High Throughput Screening Assay Systems in Microscale Fluidic Devices" WO 98/00231; in PCT/US00/04522, filed February 22, 2000, entitled "Manipulation of Microparticles In Microfluidic Systems," by Mehta et al.; and in PCT/US00/04486, filed February 22, 2000, entitled "Devices and Systems for Sequencing by Synthesis," by Mehta et al.

15 Other cell-based assays include various methods for the preparative or analytic sorting of different types of cells. For example, cell panning generally involves attaching an appropriate antibody or other cell-specific reagent to a solid support and then exposing the solid support to a heterogeneous cell sample. Cells possessing, e.g., the corresponding membrane-bound antigen will bind to the support,
20 leaving those lacking the appropriate antigenic determinant to be washed away. Other well-known sorting methods include those using fluorescence-activated cell sorters ("FACSs"). FACSs for use in sorting cells and certain subcellular components such as molecules of DNA have been proposed in, e.g., Fu, A.Y. et al. (1999) "A Microfabricated Fluorescence-Activated Cell Sorter," Nat. Biotechnol. 17:1109-1111;
25 Unger, M., et al. (1999) "Single Molecule Fluorescence Observed with Mercury Lamp Illumination," Biotechniques 27:1008-1013; and Chou, H.P. et al. (1999) "A Microfabricated Device for Sizing and Sorting DNA Molecules," Proc. Nat'l. Acad. Sci. 96:11-13. These sorting techniques utilizing generally involve focusing cells or other particles by flow channel geometry.

30 While cell-based assays are generally preferred in certain microscale screening applications, certain of these assays are difficult to adapt to conventional notions of high-throughput or ultra high-throughput screening assay systems. For

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example, one difficulty in flowing assay systems is that, during pressure-based flow of fluids in channels, non-uniform flow velocities are experienced. Faster fluid and material flow is observed in the center of a moving fluid stream than on the edge of a moving fluid stream. This non-uniform flow velocity reduces throughput for flowing assays, because assay runs have to be spaced well apart in the fluid stream to prevent overlap of materials moving at different velocities.

Accordingly, it would be advantageous to provide mechanisms for facilitating cell-based assays, including cell sorting techniques, especially in microscale systems. Additional microscale assays directed at subcellular components, such as nucleic acids would also be desirable. The present invention provides these and other features which will become clear upon consideration of the following.

SUMMARY OF THE INVENTION

The present invention relates to methods of focusing particles in microchannels, e.g., to improve assay throughput, to sort particles, to count particles, or the like. In the methods of the invention, cells and other particles are focused in the center of, to one side of, or in other selected regions of microscale channels, thereby avoiding, e.g., the above noted difficulties inherent in pressure-based flow of particles. Furthermore, the device structures of the present invention are optionally integrated with other microfluidic systems. Other reactions or manipulations involving cells, other particles, or fluids upstream of the detection zone are also optionally performed, e.g., monitoring drug interactions with cells or other particles.

In one aspect, the invention provides methods of providing substantially uniform flow velocity to particles flowing in a first microchannel. In the methods, the particles are optionally flowed in the microchannel, e.g., using pressure-based flow, in which the particles flow with a substantially non-uniform flow velocity. Prior to performing the flowing step, the particles are optionally sampled with at least one capillary element, e.g., by dipping the capillary element into a well containing the particles on a microwell plate and drawing the particles into, e.g., reservoirs, microchannels, or other chambers of the device. The particles (e.g., a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a set of functionalized microbeads, a molecule, a set of molecules, etc.) are optionally focused horizontally and/or vertically in the first microchannel to provide substantially

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uniform flow velocity to the particles in the first microchannel. Particles are optionally focused using one or more fluid direction components (e.g., a fluid pressure force modulator, an electrokinetic force modulator, a capillary force modulator, a fluid wicking element, or the like). Additional options include sorting,
5 detecting or otherwise manipulating the focused particles.

The particles are horizontally focused in the microchannel, e.g., by introducing a low density fluid and a high density fluid into the microchannel, causing the particles to be focused in an intermediate density fluid present between the high density fluid and the low density fluid. The particles are also optionally focused in a
10 top or a bottom portion of the microchannel by introducing a high or a low density fluid into the microchannel with the flowing particles. The particles are vertically or horizontally focused in the microchannel, e.g., by simultaneously introducing fluid flow from two opposing microchannels into the first microchannel during flow of the particles in the first channel. Vertical focusing is also optionally achieved to one side
15 of a microchannel by simultaneously introducing fluid flow from, e.g., a second microchannel into the first microchannel during flow of the particles in the first microchannel.

In another aspect, the invention also provides particle washing or exchange techniques. For example, focused cells or other particles are optionally
20 washed free of diffusible material by introducing a diluent into the first microchannel from at least a second channel and removing the resulting diluted diffused product comprising diluent mixed with the diffusible material through at least a third microchannel.

Alternating arrangements of diluent input and diffused product output
25 channels are also optionally used to further wash the particles. For example, in one aspect the methods of the invention include simultaneously introducing the diluent into the first microchannel from the second microchannel and a fourth microchannel, where the second and fourth microchannel intersect the first microchannel at a common intersection region. Optionally, the methods include sequentially
30 introducing the diluent into the first microchannel from the second microchannel and a fourth microchannel, wherein the second and fourth microchannels intersect the first microchannel at an offset intersection region. The diffused product is typically

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removed through the third microchannel and a fifth microchannel, which third and fifth microchannels intersect the first microchannel at a common intersection region. In further washing steps, the diluent is introduced through sixth and seventh microchannels which intersect the first microchannel at a common intersection. The
5 resulting further diluted diffused product is removed through eighth and ninth microchannels, which intersect the first microchannel at a common intersection. Diluent is optionally introduced into the first microchannel by pressure or electrokinetic flow.

10 In one preferred assay of the invention, the particles are cells and the method includes performing a TUNEL assay or an Annexin-V assay on the cells in the channel to measure apoptosis.

Integrated systems for performing the above methods, including the particle sorting embodiments, are also provided.

An integrated system for providing substantially uniform flow velocity
15 to flowing members of at least one particle population in a microfluidic device optionally includes a body structure that includes at least a first microchannel disposed therein. A first fluid direction component (e.g., a fluid pressure force modulator) is typically coupled to the first microchannel for inducing flow of a fluidic material that includes the members of the at least one particle population in the first
20 microchannel. The first fluid direction component generally induces non-uniform flow. A source of at least one fluidic material is optionally fluidly coupled to the first microchannel. The system also optionally includes at least a second microchannel that intersects the first microchannel for introducing at least one fluid into the first microchannel to horizontally or vertically focus the members of the at least one
25 particle population in the first microchannel. The at least one fluid is optionally introduced using a second fluid direction component that includes one or more of a fluid pressure force modulator, an electrokinetic force modulator, a capillary force modulator, a fluid wicking element, or the like. At least one flow control regulator for regulating flow of the fluidic material or the fluid in the first or second microchannel
30 is also optionally provided. A computer including an instruction set directing simultaneous flow of the members of the at least one particle population in the first microchannel and simultaneous introduction of the at least one fluid from the second

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microchannel into the first microchannel is optionally also operably coupled to a fluid movement system for directing flow of materials in the microchannels.

As a further option, this integrated system additionally includes at least a third microchannel which intersects the first microchannel in an intersection region
5 common to the second microchannel. The flow control regulator of this system optionally further regulates flow of the at least one fluid in the second and the third microchannels. In this embodiment, the computer typically also includes an instruction set for simultaneously flowing fluids from the second and third microchannels into the first microchannel.

10 In particle washing systems, typically, at least fourth and fifth channels which intersect the first microchannel at a common intersection downstream of the second and third microchannels are provided. The computer further includes an instruction set for simultaneously flowing material from the first microchannel into the fourth and fifth microchannels. Sixth and seventh microchannels which intersect
15 the first microchannel at a common intersection downstream of the fourth and fifth microchannels, with the computer further comprising an instruction set for simultaneously flowing material from the sixth and seventh microchannels into the first microchannel are optionally provided. Similarly, eighth and ninth microchannels which intersect the first microchannel at a common intersection downstream of the
20 sixth and seventh microchannels, the computer further including an instruction set for simultaneously flowing material from the first microchannel into the eighth and ninth microchannels are optionally provided.

The integrated system optionally includes sources for any reagent or particle used in the methods noted above, such as one or more sources of terminal
25 deoxynucleotide transferase, one or more sources of one or more fluorescein labeled nucleotides or other labeled polynucleotides, one or more sources of Annexin V, one or more sources of an AnnexinV-biotin conjugate, one or more sources of a DNA dye, one or more sources of Camptothecin, one or more sources of Calcein-AM, one or more sources of a control cell, one or more sources of a test cell, etc.

30 Signal detector(s) mounted proximal to the first microchannel for detecting a detectable signal produced by one or more of the members of the at least one particle population in the microchannel are typically provided in the integrated

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systems of the invention. The detector also optionally includes, e.g., a fluorescent excitation source and a fluorescent emission detection element. Optionally, the computer is operably linked to the signal detector and has an instruction set for converting detected signal information into digital data.

5 The integrated system of the present invention is also optionally used to sort the members of a particle population (e.g., a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a set of functionalized microbeads, a molecule, a set of molecules, or the like). In this embodiment, the integrated system typically additionally includes a third and a fourth microchannel which intersect the
10 first microchannel downstream from the intersection of the second microchannel with the first microchannel. The fourth microchannel also generally intersects the first microchannel downstream from the intersection of the third microchannel with the first microchannel. The flow control regulator of this system optionally further regulates flow of the at least one fluid in the third or the fourth microchannels.
15 Furthermore, the signal detector typically detects a detectable signal produced by a selected member of the particle population between the intersections of the second and the third microchannels with the first microchannel.

 In this particle sorting embodiment, the computer is optionally operably linked to the first or other fluid direction component(s), the flow control
20 regulator, and the signal detector. Additionally, the instruction set typically directs simultaneous introduction of the at least one fluid from the third microchannel into the first microchannel to horizontally or vertically focus the selected member of the particle population such that the selected member is directed into the fourth microchannel in response to the detectable signal produced by the selected member.
25 Optionally, the instruction set further directs simultaneous introduction of the at least one fluid from the third microchannel by activating a heating element (e.g., a Joule heating electrode, a conductively coated microchannel portion, etc.) disposed within the third microchannel or a well that fluidly communicates with the third microchannel.

30 In another embodiment, at least a portion of the first microchannel optionally includes a separation element disposed therein. The separation element optionally includes, e.g., two sides and at least a portion of the separation element is

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typically disposed upstream of the fourth microchannel. In this embodiment, a selected member of the particle population is generally directed to one of the two sides of the separation element and into the fourth microchannel that intersects the first microchannel in response to the detectable signal produced by the selected
5 member.

The integrated system for use in particle sorting also optionally includes a fifth microchannel which intersects the first microchannel in an intersection region common to the second microchannel. In this case, the flow control regulator also typically regulates flow of the at least one fluid in the second and the fifth
10 microchannels, and the computer optionally includes an instruction set for simultaneously flowing fluids from the second and the fifth microchannels into the first microchannel. Similarly, the system also optionally includes a sixth microchannel which intersects the first microchannel in an intersection region common to the third microchannel. In this embodiment, the flow control regulator
15 optionally additionally regulates flow of the at least one fluid in the third and the sixth microchannels. Furthermore, the computer also typically includes an instruction set for flowing fluids from the third and the sixth microchannels into the first microchannel. Optionally, the instruction set directs individual or simultaneous fluid flow from the third and sixth microchannels by individually or simultaneously
20 activating at least one heating element (e.g., a Joule heating electrode, a conductively coated microchannel portion, or the like) disposed within each of the third and sixth microchannels or within at least one well that fluidly communicates with each of the third and sixth microchannels.

Many additional aspects of the invention will be apparent upon review,
25 including uses of the devices and systems of the invention, methods of manufacture of the devices and systems of the invention, kits for practicing the methods of the invention and the like. For example, kits comprising any of the devices or systems set forth above, or elements thereof, in conjunction with packaging materials (e.g., containers, sealable plastic bags, etc.) and instructions for using the devices, e.g., to
30 practice the methods herein, are also contemplated.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a schematic showing focusing of cells in a microscale system by simultaneous flow from side channels into a main channel through which the cells are being flowed. Figure 1B is a photomicrograph of focused labeled cells
5 flowing in a microchannel.

Figure 2A is a schematic of a microfluidic system with a pressure-source (in this case a vacuum source) for achieving fluid movement. Figure 2B shows a cross-sectional view down a channel having high, medium and low fluid density.

10 Figure 3 is a schematic drawing of a microfluidic system adapted to washing reagents from microparticles.

Figure 4 is a data graph illustrating a control analysis, e.g., U937 cells not treated to induce apoptosis. The bottom line corresponds to the SYTO®-62, which indicates the mere presence of cells, whereas the top line corresponds to the
15 fluorescein end labeled nucleic acids.

Figure 5 is a data graph illustrating U937 cells treated with Camptothecin to induce apoptosis. As can be seen, corresponding peaks are seen on both the lower and upper lines, indicating the presence of apoptotic cells.

Figure 6 is a data graph illustrating an analysis of U937 cells that were
20 not treated to induce apoptosis. In this case, the bottom line indicates live cell count (Calcein), while the top line indicates the presence of apoptotic cells (Annexin-V-Cy5). As can be seen, a few apoptotic cells are present within the control experiment.

Figure 7 is a data graph showing an analysis of U937 cells treated with Camptothecin to induce apoptosis. The top trace includes a much greater number of
25 peaks representing apoptotic cells, and particularly as a percentage of total cells in the analysis, e.g., as compared to the lower line.

Figure 8 is a data graph showing results from a TUNEL assay with HL-60 cells treated with Camptothecin for 6 hours to induce apoptosis. The bottom line traces the total cell count. The top line traces apoptotic cells labeled at DNA
30 damage points. In this case, almost all cells had sustained apoptotic DNA damage.

Figure 9 is a data graph showing results from a TUNEL assay. HL-60 cells (not treated for apoptosis) were used as control cells. The bottom trace is for

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SYTO®-62 indicating total cell count. The top trace is for fluorescein labeled nucleotide which signifies the presence of apoptosis related DNA damage.

Figure 10 is a data graph showing results from TUNEL assays from Figures 8 and 9 analyzed using appropriate software. Data is presented as scatter plot
5 format in Figure 10.

Figure 11 is a data graph showing results from TUNEL assays from data represented in bar graph format. Additional data for different time points and two other cell lines is included.

Figure 12 is a data graph showing an Annexin-V assay. HL60 cells
10 were treated with Camptothecin for four hours to induce apoptosis. The bottom trace indicates live cell count (Calcein). The top trace indicates Annexin-V-Cy5 labeling. Cells labeled only for Calcein are live cells and not apoptotic. In this case, almost all cells are apoptotic.

Figure 13 is a data graph showing an Annexin-V assay. HL-60 cells
15 (not treated for apoptosis) are control cells. The bottom trace indicates live cell count. The top trace indicates Annexin-V-Cy5 labeling. Cells labeled with both dyes are apoptotic. Cells labeled only for Annexin-Cy5 are dead cells. In this case, only a few cells were apoptotic.

Figure 14 is a data graph showing data analysis of Figures 12 and 13.
20 Data is presented in scatter plot format in Figure 14.

Figure 15 is a data graph showing data represented in bar graph format.

Figure 16 shows a "T-junction" microchannel configuration for hydrodynamically focusing cells for use in, e.g., flow cytometry.

Figure 17 depicts a chip design used to conduct fluorescence
25 population focusing experiments.

Figure 18 is a histogram showing the distribution of results obtained in a fluorescence intensity population experiment conducted utilizing hydrodynamic focusing.

Figures 19 and 20 are histograms showing the distribution of results
30 obtained in fluorescence intensity population experiments conducted in the absence of hydrodynamic focusing.

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Figure 21 is a microchannel network that is optionally utilized to hydrodynamically focus particles.

Figure 22 is a schematic illustration of a particle sorting configuration utilizing sets of opposing microchannels to focus and/or otherwise direct the flow of the members of, e.g., a cell population to achieve cell sorting.

Figure 23 is a schematic representation of a microchannel configuration that includes one separation element embodiment.

Figure 24 is a schematic depiction of a microchannel configuration that includes Joule heating electrodes for use in particle sorting.

Figures 25A, B, and C are histograms showing the distribution of results obtained in resolution experiments using various microbead concentrations.

Figure 26 is a histogram showing the distribution of results obtained in a resolution experiment using viable cells stained with an intercalating dye.

DETAILED DISCUSSION OF THE INVENTION

The invention relates to particle focusing to improve assay throughput by regularizing flow velocity. As used herein, the term “particle” refers to a discretely describable material element, such as a cell, microbead (e.g., functionalized or non-functionalized), molecule (e.g., a nucleic acid, a polypeptide, etc.), or the like. For example, a particle typically includes, e.g., a soluble or non-soluble component of a fluid mixture, such as a solubilized or suspended molecule, liposome, cell, organelle, bead, or the like. Ordinarily, the particle is a detectable unit; for example, in the case of molecular particles, the particle is detectable, e.g., via a label. Similarly, microbeads and cells are detected, e.g., optically or through an associated label (this detection is also optionally, e.g., optical or via fluorescence).

The following provides details regarding various aspects of the methods of focusing particles, e.g., vertically and/or horizontally within the microscale systems of the invention. It also provides details pertaining to methods of washing cells and to particular assays, such as cell viability screening, which are optionally performed using the methods and devices of the present invention. Devices and integrated systems are also discussed extensively.

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LAMINAR FLOW VERTICAL FOCUSING OF CELLS AND OTHER PARTICLES
IN MICROSCALE SYSTEMS

Microfluidic hydrodynamic focusing is a highly effective technique when used, e.g., in flow cytometry applications, such as the assessment of live/dead cell ratios, the analysis of transfection efficiencies, the sizing of various molecular components (e.g., polynucleotides, polypeptides, etc.), the study of apoptosis, or the like. Fluid flow streams are optionally “pinched” or “focused” into a narrow region of a microchannel to facilitate single particle or narrow streamline detection using a variety of optical detection schemes (discussed further, below). In one embodiment, particle focusing is achieved electrodynamically (see also, Ramsey et al., “Apparatus and Method for Performing Electrodynamic Focusing on a Microchip,” U.S. Pat. No. 5,858,187, issued January 12, 1999). In other preferred embodiments, pressure, Joule heating, and/or other fluid movement methods are used to focus cells or other particles.

As used herein, a “vertically” focused particle stream refers to a particle stream that is substantially focused, pinched, narrowed, or otherwise confined along or proximal to a plane that extends, in one dimension, from the highest to the lowest internal surface of a microchannel (e.g., along the length of a cross-sectional line or axis of the microchannel), and along a selected length of the microchannel, in another dimension, when the microfluidic device is oriented for typical operational usage. A vertically focused particle stream corresponds to a plane that is oriented approximately 90° from a plane in which a horizontally focused particle stream (discussed below) would be oriented.

Vertical focusing is optionally achieved by flowing fluids into a microchannel (e.g., from one or more sides of the microchannel) in which a particle stream is flowing to focus the particles along or proximal to the vertical plane having a dimension that corresponds to a selected vertical line or axis of the microchannel. The level of focusing along or proximal to the vertical plane is optionally varied. Furthermore, as indicated, a particle stream is also optionally vertically focused away from the vertical cross-sectional axis, e.g., to one side of a microchannel. An additional option includes simultaneously horizontally and vertically focusing a particle stream to position the stream within a desired region of a microchannel.

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For example, the measurement of fluorescent signals associated with single cells (or other particles) in microfluidic channels is difficult if the cells or particles are randomly disbursed in the channels. The channels are usually about 100 μm or larger in width to prevent clogging by particle aggregates; however, particles such as cells are typically on the order of 10 μm in diameter. An interrogating light beam is focused on a spot usually much less than the 100 μm width of the channel. As a result, it is useful, e.g., to center particles in the channel, to position particles to one side of a channel, or to otherwise focus flowing particles in a portion of a channel prior to detection to obtain accurate fluorescent readings on all the particles passing through the channel.

Figure 1A shows an example cell (or other particle) analysis chip design for on-chip focusing. The microchannel network geometry includes a cross formed by four microchannels that intersect in a common intersection region. Figure 1B shows a photomicrograph of this microchannel configuration with focused labeled cells flowing in a main or analysis microchannel. Cells or other particles are optionally flowed in the main microchannel and/or focused using various methods, which include the use of electrokinetic forces, pressure gradients, surface tension gradients, gravitational forces, or the like. Furthermore, combinations of these fluid flow/focusing methods are also optionally used. A preferred method of flowing particles in the main microchannel utilizes pressure-based flow.

In the embodiment depicted in Figure 1, cells **100** (or other particles) are typically flowed from one microchannel into the cross-junction and focused by introducing hydrodynamic flows **102** from the two orthogonal microchannels. Non-orthogonal (e.g., opposing or non-opposing) microchannels are also optionally used. For example, as discussed herein, particles are optionally focused with a single focusing microchannel, or alternately, by using a series of offset focusing microchannels to achieve focusing by serial introduction of fluids from the offset channels. As depicted in both panels of Figure 1, cells **100** are optionally constrained to the center of a detection microchannel downstream from the two orthogonal microchannels by hydrodynamic flows **102** introduced from both sides as cells **100** pass through detector **104**. Using these methods, an experiment is optionally conducted which uses, e.g., a small optical detection system on the order of the size of

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the particular cell or other particle being analyzed to derive accurate and precise measurements.

Figure 21 depicts a microchannel network that is optionally used to hydrodynamically focus particles, e.g., cells, microbeads, molecules, or the like in parallel through common detection zone **2100**. For example, five cell suspensions are typically loaded into wells **2102** and flowed towards common detection zone **2100**. Cell buffer solutions are also typically loaded into particular wells (e.g., well **2104**) in the device and flowed towards common detection zone **2100**. As shown, in the region just prior to common detection zone **2100**, each microchannel in which cells are flowed intersects with two buffer flowing microchannels in a cross-configuration to focus the cells as they pass through common detection zone **2100**.

In another embodiment of these methods, a “T-junction” microchannel geometry is optionally used to focus cells or other particles **1600** to one side of detection microchannel **1604**. (FIG. 16). As shown in Figure 16, a “T-junction” typically includes an orthogonal intersection of two microchannels. However, microchannel geometries that include non-orthogonal intersections are also optionally used (e.g., a “Y-junction” or the like). In this embodiment, cells or other particles **1600** are typically flowed from one well **1602** and pinched to one side of detection microchannel **1604** by only one other introduced fluid stream **1606** and the wall of detection microchannel **1604** opposing the “T-junction” as cells or other particles **1600** pass through detector **1608**. This embodiment generally achieves the same effect as the cross-microchannel formats, but with the added advantage of using less space on the microfluidic device due to the use of one focusing channel, rather than two which, in turn, provides for greater parallelism or complexity in chip design, and for more control of the hydrodynamic focusing mechanism.

The focusing of flow to one side of a microchannel also provides a solution to various problems associated with controlling fluid flow under lower pressures (e.g., difficulties regulating fluid direction pumps, etc.). For example, the resistance produced by pinching fluid materials against microchannel walls functions to reduce the velocity of the pinched fluid stream. As a result, pinched fluids are optionally flowed under higher pressures, e.g., for greater control, while achieving comparable low pressure flow velocities. Resistance is also altered, e.g., by varying

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the viscosity of pinched fluid materials, which provides an added level of control over flow rates and the extent of fluid pinching.

FOCUSING CELLS HORIZONTALLY IN A MICROCHANNEL

In high throughput screening applications using cells or microparticles, the throughput is increased if all of the cells or microparticles move at a common velocity. The parabolic nature of pressure-based flow causes cells at the center of a flow stream to move faster than those near the walls of a channel. This phenomenon causes dispersion in the system since cells can move from one sample into another. To focus cells in the center of a channel, high and low density fluid streams are incorporated into fluid flow of the cells or particles. The high density fluid keeps the cells or other microparticles off of the bottom of the channel, while the low density fluid keeps cells or microparticles off of the top of the microchannel, thereby focusing the cells in the center of the fluid stream. Even after diffusion brings the high, low, and middle density fluids into equilibrium, the cells generally remain focused in the center of a microchannel. Optionally, the point in the microchannel in which the high and low density fluids are flowed into the main channel is deeper than other channel regions to improve distribution of the different density fluids.

A “horizontally” focused particle stream, as used herein, refers to a particle stream that is substantially focused, pinched, narrowed, or otherwise confined along or proximal to a plane that extends, in one dimension, from one substantially vertical internal microchannel surface to another (e.g., substantially parallel to the dimension of the lowest internal microchannel surface that extends from one vertical internal surface to another), and along a selected length of the microchannel, in another dimension, when the microfluidic device is oriented for typical operational usage. A horizontally focused particle stream corresponds to a plane that is oriented approximately 90° from a plane in which a vertically focused particle stream (discussed above) would be oriented.

As mentioned, horizontal particle focusing typically involves using focusing fluids having densities that differ relative to the density of a particle stream. For example, higher density fluids are optionally used to substantially prevent the particle stream from contacting the lowest internal surface of the microchannel over a

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particular length. In contrast, a less dense focusing fluid is optionally used to substantially prevent the particle stream from contacting the highest internal surface of the microchannel over a selected length. Additionally, both lower and higher density focusing fluids are optionally flowed simultaneously to substantially prevent the particle stream from contacting either the highest or lowest internal microchannel surfaces. The extent of horizontal focusing achieved is optionally regulated, e.g., by varying the density and/or the volume of focusing fluid flowed into a microchannel. A further option includes simultaneously horizontally and vertically focusing a particle stream to position the stream within a desired region of a microchannel.

Figure 2A is a schematic representation of a microfluidic system that uses vacuum source **210** to achieve fluid movement. In this embodiment, test compounds are typically flowed through capillary channel **200** (capillary channels or elements are discussed in greater detail, below) into a detection microchannel of the device. High density buffer and low density buffer are optionally flowed into the detection microchannel through high density buffer microchannel **202** and low density buffer microchannel **204**, respectively, both of which intersect the detection microchannel upstream from detector **208**. Cell microchannel **206** typically intersects the detection microchannel downstream from the intersections of high density buffer microchannel **202** and low density buffer microchannel **204**, but upstream from detector **208**. Cells in medium density buffer are typically flowed into the detection channel from cell microchannel **206**, becoming focused between the high and low density buffers, and exposed to the test compounds prior to flowing through detector **208**. Figure 2B shows a cross-sectional view down the detection microchannel which includes low density buffer **214**, cells **212**, and high density buffer **216**.

Fluid density-based focusing also optionally entails focusing cells or other particles either at the top or the bottom of a microchannel. For example, a high density fluid is optionally flowed into a microchannel to keep cells or other particles off of the bottom of the microchannel, i.e., focused at the top of the microchannel. Alternatively, a low density fluid is flowed into a microchannel to keep cells or other particles off of the top of the microchannel, i.e., focused on the bottom of the microchannel.

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It is also worth noting that horizontal and vertical focusing are optionally both performed to center cells vertically and horizontally in the center of microscale channels. Furthermore, the extent of hydrodynamic focusing, using either or both methods, is typically controlled by a variety of variables including viscosity in pressure driven flows, electrokinetic mobility in electrokinetic flow, fluid density in gravity driven flow, or the like. As will be recognized by those of skill, the enhanced control of focusing, provided by the present invention, allows for a large gamut of flow-based applications, including any particle and/or bulk fluid application that involves small, uniform detection regions.

10 USE OF FOCUSING TO SORT PARTICLES

The separation of fluorochrome-labeled cells with fluorescence-activated cell sorters is well-known in the art, as mentioned above. In brief, the technique generally involves incubating a mixed population cells with a fluorescently-labeled antibody against a specific antigenic determinant displayed on the surfaces of target cells. The suspension of cells is then typically expelled, one cell at a time, from a sample chamber of the device through a small vibrating nozzle that generates microdroplets. Each microdroplet contains a single cell that the FACS assays for the presence or absence of the fluorochrome label using a laser source and a detector. Droplets that fluoresce are electrically charged in proportion to their fluorescence and in turn separated as they pass between charged plates. General references describing FACS include, e.g., Kuby, Immunology (3rd Ed.) W.H. Freeman and Company, New York (1997), Watson, et al., Recombinant DNA (2nd Ed.) W.H. Freeman and Company, New York (1992), and Alberts, et al. Molecular Biology of the Cell (3rd Ed.) Garland Publishing, Inc., New York (1994). Other references relating to flow cytometry include, e.g., Radbruch (Ed.) Flow Cytometry and Cell Sorting, Springer-Verlag, New York (1992), Owens and Loken, Flow Cytometry Principles for Clinical Laboratory Practice, Wiley-Liss, New York (1995), and Jarosqueski and Heller (Eds.) Flow Cytometry Protocols: Methods in Molecular Biology, Vol. 91, Humana Press (1997).

30 The present invention provides microfluidic devices and methods for sorting particles (e.g., fluorescently-labeled particles) that use hydrodynamic flow to

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focus and/or sort the particles. The methods for sorting members of a particle population (e.g., a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a set of functionalized microbeads, a molecule, a set of molecules, or the like) typically include flowing the members of a particle population
5 in a first microchannel. The members of the particle population are focused horizontally and/or vertically in the first microchannel such that selected individual members are directed into at least a second microchannel that intersects with the first microchannel.

In one embodiment, a portion of the first microchannel optionally
10 includes at least one separation element disposed therein. The separation element generally includes at least two sides in which at least a portion of the separation element is disposed upstream of the second microchannel. In this embodiment, the members of the particle population are optionally focused horizontally and/or vertically in the first microchannel such that selected individual members are directed
15 to at least one of the at least two sides of the separation element and into, e.g., at least a second microchannel that intersects the first microchannel. In other embodiments, this separation element is omitted.

The focusing steps of the particle sorting methods, described above, optionally include horizontally and/or vertically focusing the members of the particle
20 population in the first microchannel by introducing at least one fluid flow (e.g., a buffer, a high density fluid, a low density fluid, or the like) from at least a third microchannel that intersects with the first microchannel upstream from, e.g., the second microchannel. This is optionally performed by any method for flowing fluid in a microfluidic device, including electrokinetic flow, pressure-based flow, and the
25 like.

For example, methods employing electrostatic forces to transport and focus samples generally involve inducing sample flow in a transport channel by applying a first electrical potential to the channel. Thereafter, a second electrical potential is typically applied to focusing channels that intersect with the transport
30 channel in a cross-configuration to vertically focus the transported sample in the center of the transport channel. This electrokinetic-based system is also optionally utilized to direct or “nudge” flowing samples to effect a sorting function, e.g., by

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applying an electrical potential to at least one of the intersecting focusing channels to direct sample flow towards or away from that focusing channel and into, e.g., an additional intersecting channel or into a channel region. That is, electrokinetic force can be applied to “nudge” materials across the width of a first channel at an

5 intersection of the first channel and a second channel, resulting in downstream focusing of materials in the channel. This application of electrokinetic force is optionally electrokinetic, electroosmotic, or both. See also, e.g., Ramsey et al., “Apparatus and Method for Performing Electrodynamic Focusing on a Microchip,” U.S. Pat. No. 5,858,187, issued January 12, 1999. As mentioned, other alternative

10 techniques for inducing the flow of focusing fluids to sort particles according to the methods of the present invention include pressure, hydrostatic, wicking, capillary, and other forces. Fluid direction components based upon these forces are discussed, below.

In another embodiment, the fluid flow is induced by heating (e.g.,

15 Joule heating, etc.) a fluid in the third microchannel. Alternatively, the focusing steps include horizontally and/or vertically focusing the members of the particle population in the first microchannel by introducing at least one fluid flow from at least one of at least a third and at least a fourth microchannel that oppose one another and that intersect with the first microchannel upstream from the second microchannel.

20 Similarly, the fluid flow is optionally introduced by heating (e.g., Joule heating or the like) a fluid in at least one of the third or fourth microchannels. Embodiments that involve heat induced fluid flow are discussed further below.

Figure 22 schematically illustrates one particle sorting configuration utilizing these methods. As shown, cells **2200** are generally flowed in a main

25 microchannel that includes at least two sets of opposing microchannels for focusing and/or otherwise directing the flow of cells **2200** using hydrodynamic flow **2202** (e.g., cell buffer flow). One set of opposing microchannels is typically located, e.g., upstream from detector **2204** for simultaneously introducing hydrodynamic flow **2202** from both microchannels to focus cells **2200**, as described above. A second set of

30 opposing microchannels is typically located downstream from detector **2204** for introducing at least one hydrodynamic flow **2202** so as to direct selected cells **2208**

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(e.g., fluorescently-labeled cells) and non-selected cells **2206** into, in this case, one of two microchannels, each terminating in particular collection wells **2210**.

Figure 23 schematically depicts a microchannel configuration that includes one embodiment of separation element **2310**. In this example, cells **2300** are typically flowed in a microchannel that intersects with another microchannel located upstream from detector **2304**. The upstream intersecting microchannel is optionally used to focus cells **2300** to one side of the microchannel as cells **2300** pass through detector **2304** using hydrodynamic flow **2302**. This illustration also includes a set of opposing microchannels located downstream from the detector for introducing at least one hydrodynamic flow **2302** to direct selected cells **2308** (e.g., fluorescently-labeled cells) and non-selected cells **2306** to either side of separation element **2310** and into, in this case, one of two microchannels, each terminating in particular collection wells **2312**. Separation element **2310** is optionally omitted, with cells **2300** or other particles being directed into destination regions as desired.

The inverse relationship between temperature and viscosity for various buffers, gels, and other materials is also optionally exploited in the present invention to effect particle sorting. For example, upon detection of a desired particle, e.g., a buffer or gel disposed in a downstream side-channel is typically heated to decrease fluid viscosity (i.e., to induce fluid flow) to thus direct particles within the microfluidic device. Although any heating source, functional in the devices of the present invention, is optionally used in these embodiments, Joule heating is a preferred method.

Joule heating is typically produced by flowing current through an electrode or other conductive component positioned within a well, microscale channel, or other cavity within the device. The resulting flow of current into fluid within the well, channel, or cavity results in resistive heating of the fluid. By substantially increasing the current across the channel, rapid temperature changes are induced that are optionally monitored by conductivity. Because nanoliter volumes of fluid have tiny thermal mass, transitions between temperatures are typically extremely short. For example, oscillations between any two temperatures above 0°C and below 100°C in 100 milliseconds have been performed. Thus, the present invention optionally uses power sources that pass electrical current through, e.g., a focusing

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channel region for heating purposes. In exemplary embodiments, fluid passes through a channel of a desired cross-section (e.g., diameter) to enhance thermal transfer of energy from the current to the fluid. The channels are optionally formed on almost any type of substrate material such as, amorphous materials (e.g., glass, plastic, silicon), composites, multi-layered materials, combinations thereof, or the like.

In general, electric current passing through fluid in a channel produces heat by dissipating energy through the electrical resistance of the fluid. Power dissipates as the current passes through the fluid and goes into the fluid as energy as a function of time to heat the fluid. The following well-known mathematical expression generally relates the power dissipated in a fluid ("P") to the electric current passing through the fluid ("I") and the electrical resistance of fluid ("R"):

$$P=I^2R$$

In these embodiments, a portion of the power goes into kinetic energy for moving the fluid through the channel. However, it is also possible to use a selected portion of the power to controllably heat fluid in a channel or selected channel regions, e.g., to further induce fluid movement by reducing fluid viscosity. A channel region suitable for heating is optionally narrower or smaller in cross-section than other channel regions in the channel structure, as a smaller cross-section provides higher resistance in the fluid, which increases the temperature of the fluid as electric current passes through. Alternatively, the electric current is increased across the length of the channel by increased voltage, which also increases the amount of power dissipated into the fluid to correspondingly increase fluid temperature.

To selectively control the temperature of fluid in a region of a channel, a power supply applies voltage and/or current in various ways. For instance, a power supply optionally applies direct current (i.e., DC) or alternating current (AC), which passes through the channel and into a channel region which is, e.g., smaller in cross-section to heat fluid in the region. Alternatively, a power supply applies a pulse or impulse of current and/or voltage, which passes through the channel and into a channel region to heat fluid in the region. Pulse width, shape, and/or intensity are optionally adjusted, e.g., to heat the fluid substantially while moving the fluid. Still further, the power supply optionally applies any combination of DC, AC, and pulse, depending upon the application. In practice, direct application of electric current to

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fluids in the microchannels of the invention results in extremely rapid and easily controlled changes in temperature.

A controller or computer such as a personal computer is generally used to monitor the temperature of the fluid in the region of the channel where the fluid is heated. The controller or computer typically receives current and voltage information from, e.g., the power supply and identifies or detects fluid temperature in the channel region. The controller or computer also typically receives current information from an operably connected detector, e.g., when a selected particle is detected, which triggers the flow of current through, e.g., one or more Joule heating electrodes. Depending upon the desired temperature of fluid in the region, the controller or computer adjusts voltage and/or current to meet the desired fluid temperature.

In certain embodiments, the region which is heated optionally includes a “coil” which is typically in a planar arrangement. Transfer of heat from the coil to a channel through a substrate material is used to heat the focusing fluids, gels or other materials disposed in the channel. Alternatively, the coil itself is optionally the focusing channel. A voltage is applied between regions of the coil to direct current through the fluid for heating purposes. In particular, a power supply provides a voltage differential between regions of the coil. Current flows between the regions and traverses a plurality of coils or coil loops, which are defined by a substrate. The shape and size of the coils typically influences an ability of current to heat the fluid in the coil. As current traverses through the fluid, energy is transferred to the fluid for heating purposes. Cooling coils are also optionally included.

One embodiment of Joule heating-based particle sorting is schematically illustrated in Figure 24. As shown, particles are typically flowed from particle well **2400** into main microchannel **2402**. In the configuration depicted, focusing microchannel **2404** intersects with main microchannel **2402** in a “T-junction” upstream from detector **2408**. Similar to the device represented in Figure 23 (discussed above), focusing flow stream **2406** is optionally used to focus or pinch the particles against the wall of main microchannel **2402** opposing the “T-junction” as the particles flow through detector **2408**. When selected particle **2410** (e.g., a fluorescently-labeled particle) is detected, an electrical signal from detector **2408** typically triggers the flow of current to Joule heating electrodes **2416**, which are

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disposed within wells of branched particle sorting microchannel **2418**. Optionally, branched particle sorting microchannel **2418** includes more than two branches or is a single, unbranched microchannel. Additionally, greater or less than two Joule heating electrodes **2416** are optionally included. Although not shown in Figure 24,
5 conductive coatings are also optionally included in lieu of or in addition to the heating electrodes for effecting the flow of a focusing fluid.

The heat produced by current flowing from Joule heating electrodes **2416** raises the temperature of, e.g., a buffer or gel disposed within branched particle sorting microchannel **2418**, thus reducing hydrodynamic resistance in the buffer or
10 gel. The reduced viscosity, in conjunction with vacuum source(s) that are typically operably connected to the device at non-selected particle collection well **2420** and/or selected particle collection well **2422**, induce or increase flow of the buffer or gel from branched particle sorting microchannel **2418**, which intersects main microchannel **2402** downstream from detector **2408**. The induced flow of buffer or
15 gel into main microchannel **2402** thus deflects or redirects the flow of selected particle **2410** into selected particle collection well **2422**. In this embodiment, non-selected particle **2412** does not trigger Joule heating/redirecting flow and as such, flows unimpeded into non-selected particle collection well **2420**.

Many alternative embodiments that incorporate Joule heating to
20 control fluid viscosity and particle flow are possible. For example, although not shown in Figure 22, one or both microchannels of the second set of opposing microchannels located downstream from detector **2204** optionally include one or more Joule heating electrodes (e.g., 1, 2, 3, 4, 5, or more electrodes) and/or conductive coating portions. One or both of the opposing microchannels also optionally includes
25 a branched channel structure similar to the branched structure depicted in Figure 24, above (i.e., branched particle sorting microchannel **2418**). Optionally, one or both of the opposing microchannels includes greater than two branches, each branch of which optionally includes one or more Joule heating electrode(s). In other embodiments, separation elements, such as those discussed above are optionally included. Preferred
30 buffers, gels, and other materials used the in these heating embodiments have relatively sharp temperature/viscosity transitions in a narrow temperature range and are generally known in the art. Joule heating is described further in, e.g., in

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08/977,528, entitled "Electrical Current for Controlling Fluid Temperatures in Microchannels," filed November 25, 1997 by Calvin Chow, Anne R. Kopf-Sill and J. Wallace Parce; and in PCT/US98/06723, entitled "Closed-Loop Biochemical Analyzers," filed April 3, 1998, by Knapp.

5 There are various other techniques that are also optionally used to heat buffers, gels, and other materials to effect particle focusing. For example, resistive heating typically results from current applied to conductively coated well or microchannel portions, from one or more electrodes directly (e.g., a thermocouple, etc.), or the like. Other heating methods optionally include directing, e.g., light from
10 a laser source through one or more fiber optic cables to, e.g., wells that fluidly communicate with focusing channels. Heat from other external sources is also optionally utilized in the methods described herein.

WASHING CELLS IN MICROFLUIDIC CHANNEL SYSTEMS

In one aspect, the invention provides in-system cell washing capability.
15 This concept is also optionally extended to washing any other particulate sample. For an extensive description of microfluidic systems comprising particles, *see*, U.S. Provisional Patent Application No. 60/128,643, filed April 4, 1999, entitled "Manipulation of microparticles in microfluidic systems," by Mehta et al. In this embodiment, diffusible substances such as antibodies, proteins, salts, drugs, etc. are
20 removed by washing the materials away from the particles in a microscale system.

For this embodiment of the invention, lateral channels are placed downstream of the point at which diffusible substances are present in a central main channel. For example, as illustrated in Figure 3, cells flowed from sample well **300** are optionally incubated with labeled antibody flowed from test compound well **302**
25 in incubation channel region **304**, with lateral channels being placed downstream of incubation channel region **304**. Wash buffer is typically injected simultaneously from both sides of the main channel from lateral wash channels **306**. Although not shown, wash buffer is optionally sequentially introduced into the main channel from lateral wash channels that intersect the main channel in an offset intersection region (i.e., the
30 lateral wash channels are at least somewhat staggered, instead of intersecting directly across from one another). This buffer is then generally removed by flowing the

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diffusible material into another set of lateral wash channels **308**. Further washing is optionally achieved by placing additional lateral wash channels **310** farther downstream from incubation channel region **304** for flowing wash buffer into the main channel and additional wash channels **312** are also optionally included for

5 flowing wash buffer out of the main channel. By repeating wash in-wash out steps, diffusible materials are removed from the main channel. Thereafter, labeled cells are optionally detected in detection zone **314** and subsequently flowed into waste well **316**.

Assuming laminar flow, the cells or other particles remain centered in

10 the main channel and do not flow into the lateral wash channels, preventing clogging of the wash channels. Flow in the main channel, and in the wash channels is optionally performed by electrokinetic or pressure-based flow of materials.

CELL VIABILITY SCREENING

The methods and systems of the present invention are particularly

15 applicable in performing cell viability assays as well as for screening test compounds for their effects on cell viability. Such assays are generally utilized in performing toxicity studies, antibiotic screening methods, or the like, and are particularly suitable for the methods and systems of the present invention. Accordingly, in these aspects, the cellular function specific indicator is an indicator of cell viability.

20 In operation, the suspension of cells typically includes a label. The cells are optionally treated with a second function labeling group that indicates the viability of the cells in the suspension. Specifically, the function label preferentially stains or labels either viable or non-viable cells. A variety of viability indicative dyes are generally commercially available. For example, fluorogenic esterase substrates,

25 such as Calcein AM, BCECF AM and fluorescein diacetate, are optionally loaded into adherent or nonadherent cells, and are suitable indicators of cell viability. Specifically, these esterase substrates measure both esterase activity, which is required to activate the fluorescence of the dye, as well as cell-membrane integrity, which retains the fluorescent materials intracellularly. Other suitable viability

30 indicators include polyfluorinated fluorescein derivatives (i.e., DFFDA, TFFDA, HFFDA and Br₄TFFDA), polar nucleic acid based dyes (i.e., SYTOX Green™),

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dimeric and monomeric cyanine dyes (i.e., TOTO™ and TO-PRO™ series dyes from Molecular Probes), ethidium and propidium dyes (i.e., ethidium bromide, ethidium homodimer and propidium iodide).

Depending upon the viability indicator used, the level of function label
5 is indicative of the number of either viable or non-viable cells, while the level of
reference label is indicative of the number of total cells, e.g., viable and non-viable.
Comparison of the levels of the two labels then provides an indication of the relative
viability of the cells in the suspension, regardless of the number of cells being
detected, e.g., multiple cells, aggregates, or individual cells). In particular, where two
10 cell populations show a similar level of reference label, but one population shows a
lower level of viability indicator, it will be understood that the second population is
less viable, e.g., has more nonviable cells. It will be appreciated that many dyes or
labels described for use as reference labels are also often used as viability labels.
Accordingly, it will generally be desirable to select a reference label that labels both
15 viable and nonviable cellular material, and which is distinguishable from the function
label. Examples of such reference labels include, e.g., lipophilic membrane labels, or
the like.

In performing screening assays, cell suspensions that are exposed to
different test compounds or agents are flowed past the detection point and the relative
20 viability of the cells is determined, as compared to a control. Increases or decreases
in cellular viability indicate that the compound or agent improves or decreases cellular
viability. Such assays are readily employed in identifying antimicrobial, antibiotic or
other viability affecting agents. Similarly, such assays are optionally employed in
screening for effectors of pathways involved in apoptosis or programmed cell death,
25 e.g., ras mediated pathways.

The methods and devices of the present invention are optionally used
to perform the cell viability assays described herein. In one embodiment, e.g., cells
exposed to selected test compounds are washed free of excess staining dyes or the like
before being focused horizontally and/or vertically as they flow past a detection point
30 to obtain accurate fluorescent readings (e.g., derived from function and/or reference
labels). Thereafter, cells (e.g., viable and non-viable cells) are optionally sorted

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similarly using hydrodynamic flow. These embodiments are depicted, e.g., in Figures 22 and 23, which are discussed further above.

ELIMINATING ADSORPTION OF MATERIALS DURING FLOW

Adhesion of materials during pressure-based flow using prior art methods can be problematic, because the flow velocity at the channel wall is low. This low flow velocity increases the time that a material remains in position proximal to a given region of the microscale channel. This increased proximity to a single region leads to formation of strong interactions between the channel region and the material. Thus, one particular advantage of the present method is that focusing of materials in the center of microchannels inhibits adsorption of the materials to the walls of microscale channels and other microscale elements during flow of the materials.

ASSAY SYSTEMS

As noted above, the methods and systems of the present invention are useful in assaying for virtually any cellular function, provided that either the function or a result of the function is independently detectable. In biological applications, and particularly pharmaceutical research, a number of specific types of assays are generally used as screening models for the identification of potential drug candidates, or "lead compounds." The assay types most frequently used in these screening operations generally include transport assays, binding assays, viability assays, and expression assays.

Transport

In a first aspect, the methods and systems of the present invention are used in assaying cellular transport functions, i.e., ion flux, and intracellular pH regulation. In particular, cellular transport channels have been generally shown to be responsive to important cellular events, e.g., receptor mediated cell activation, and the like. For example, G-protein coupled receptors have been shown to directly or indirectly activate or inactivate ion channels in the plasma membrane or endosomal membranes of cells, thereby altering their ion permeability and thus effecting the excitability of the membrane and intracellular ion concentrations. See, Hille, Ionic Channels of Excitable Membranes, Sinauer Assoc. (1984).

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In accordance with this aspect of the present invention, therefore, the function specific label comprises an indicator of the level of a particular intracellular species. In particularly preferred aspects, the intracellular species is an ionic species, such as Ca^{2+} , Na^+ , K^+ , Cl^- , or H^+ (e.g., for pH measurements). A variety of

5 intracellular indicator compounds are commercially available for these ionic species (e.g., from Molecular Probes, Eugene OR). For example, commonly used calcium indicators include analogs of BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid), such as Fura-2, Fluo-2 and Indo-1, which produce shifts in the

10 fluorescent excitation or emission maxima upon binding calcium, and Fluo-3 and Calcium Green-2, which produce increases in fluorescence intensity upon binding calcium. See also, U.S. Patent No. 5,516,911. Sodium and potassium sensitive dyes include SBF1 and PBF1, respectively (also commercially available from Molecular Probes). Examples of commercially available chloride sensitive indicators include 6-methoxy-N-(sulfopropyl)quinolinium (SPQ), N-(sulfopropyl)acridinium (SPA), N-(6-

15 methoxyquinolyl)acetic acid, and N-(6-methoxyquinolyl)acetoethyl ester (Molecular Probes, Inc.), all of which are generally quenched in the presence of chloride ions.

In a related aspect, the function specific indicator is an intracellular pH indicator compound. Specifically, intracellular pH changes have been found to be associated with biologically and pharmaceutically important cellular events, including

20 cell proliferation, apoptosis, fertilization, malignancy, ion transport, drug resistance, lysosomal storage disorders, and Alzheimer's disease. A variety of indicator compounds are commercially available to indicate the intracellular pH of cells, and are readily applicable to the present invention as indicators of cellular function. Examples of these pH indicators include, e.g., SNARFL, SNARF, BCECF, and

25 HPTS, available from Molecular Probes, Inc.

In operation, a suspension of cells that is to be assayed is flowed along a channel. The cells include a reference label as described above, e.g., SYTO® dyes available from Molecular Probes. The cells are also treated with an intracellular indicator of the level of the species for which relative transport levels are to be

30 determined, and which indicator is distinguishable from the reference label. As a specific example, the cells are optionally stained with, e.g., SYTO®-62 as a reference label. SYTO®-62 is a red nucleic acid dye that is generally excited by light at

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approximately 655 nm, and which emits light at approximately 675 nm. The cells are also optionally treated with an intracellular calcium indicator, e.g., Fluo-3, also available from Molecular Probes, which is excited at 488 nm and emits at approximately 530 nm. The two labels are easily distinguishable based upon their
5 differing fluorescent emission maxima.

At a point in the channel, the cells are illuminated with a broad spectrum of light, e.g., light that encompasses the excitation maxima of both the SYTO®-62 and Fluo-3 labels. Emitted fluorescence is then passed through optical filtering systems that separate and separately detect the SYTO®-62 fluorescence and
10 the Fluo-3 fluorescence. The levels of fluorescence from each dye are then compared. For example, the comparison optionally includes plotting the level of reference label versus the level of function label. Over the course of the assay, a number of separate data points are gathered that represent different cells or groups of cells that are detected. These are plotted and the slope of the resulting line is calculated. Changes
15 in this slope are indicative of changes in the level of the function that is being assayed.

Binding

I. Generally

In an alternate aspect, the methods and systems of the present
20 invention are used in assaying cellular binding functions, such as ligand-receptor binding, nucleic acid hybridization, antigen/antibody binding, cell-cell interactions, and the like. As with transport functions, cellular binding functions are often necessary precursors to a variety of cellular functions. Specifically, many biological responses are often triggered and/or controlled by the binding of a receptor to its
25 ligand. For example, interaction of growth factors, i.e., EGF, FGF, PDGF, etc., with their receptors stimulates a wide variety of biological responses including, e.g., cell proliferation and differentiation, activation of mediating enzymes, stimulation of messenger turnover, alterations in ion fluxes, activation of enzymes, changes in cell shape and the alteration in genetic expression levels. Accordingly, control of the
30 interaction of the receptor and its ligand may offer control of the biological responses caused by that interaction. As noted, included within these biological functions controlled by binding reactions are many transport functions, e.g., G-protein linked

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receptor activation, as set forth above. Accordingly, these binding functions may be detected by detecting the downstream event for which binding is a precursor, e.g., enhanced or decreased transport function, expression of receptor linked reporter label, protein translocation, or by detecting actual binding of cells with a binding agent, e.g.,
5 a ligand, nucleic acid or the like, through the inclusion in the ligand of a binding indicator, e.g., fluorescent resonance energy transfer dyes (FRET), molecular beacons, etc. For example, in the case of cell-cell interactions, detection of binding may be accomplished by labeling the cells' surfaces with both elements of appropriate FRET dyes, e.g., energy donor and energy acceptor. Upon cell-cell binding, these
10 elements are placed in sufficient proximity for energy transfer, allowing their detection.

Alternatively, fluorescence polarization detection methods are used to detect binding of relatively small molecules, e.g., ligands, antibodies, etc., to relatively large structures, e.g., cells. Fluorescence polarization assays for use in
15 microfluidic systems are generally described in Provisional U.S. Application No. 60/088,650, filed June 8, 1998, incorporated herein by reference.

A variety of other detection/labeling mechanisms are also available for detecting binding of one molecule, e.g., a ligand or antibody, to another molecule, e.g., a cell surface receptor. For example, a number of labeling materials change their
20 fluorescent properties upon binding to hydrophobic sites on proteins, e.g., cell surface proteins. Such labels include, e.g., 8-amino-1-naphthalene sulfonate (ANS), 2-p-toluidinylnaphthalene-6-sulfonate (TNS) and the like. Alternatively, detectable enzyme labels are utilized that cause precipitation of fluorescent products on solid phases, i.e., cell surfaces are optionally used as function indicators of binding. For
25 example, alkaline phosphatase substrates that yield fluorescent precipitates are optionally employed in conjunction with alkaline phosphatase conjugates of cell binding components. Such substrates are generally available from Molecular Probes, Inc., and are described in, e.g., U.S. 5,316,906 and U.S. 5,443,986.

II. Cell Rolling Assays

30 In a related but alternative aspect, the present invention provides methods, devices and systems for use in performing in vitro cell rolling assays. In particular, it has been reported that several classes of cell adhesion molecules

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participate in a wide range of important physiological functions, including wound healing, inflammation and cancer metastasis. Some examples of these molecules include selectins and integrins which mediate the rolling and subsequent immobilization of white blood cells along the endothelial lining of blood vessel, thus
 5 allowing them to migrate out of the blood vessel and toward the target tissue. Cell rolling assays are designed to mimic *in vitro* the rolling phenomenon *in vivo*, to create a more effective model for use in screening potential effectors of that phenomenon. See, e.g., Lawrence et al., J. Immunol., (1993) 151:6338-6346 and Brunk et al., Biophys. J. (1997) 72:2820-2833.

10 Generally, the assay is performed by flowing a suspension of cells over a surface upon which ligands are immobilized, and observing the numbers of firmly attached and/or rolling cells on that surface as well as the velocity of the rolling cells. The present invention employs the microfluidic systems described herein, in the performance of these assay types. In particular, as described in greater detail below,
 15 the cell suspension bearing an appropriate reference label is introduced into a channel in which an appropriate ligand of interest is immobilized on the inner surface.

Immobilization of ligands on the interior surface of channels is optionally accomplished by covalently attaching the ligands to the surface or by adsorbing the ligands on the surface. Covalent attachment of ligands to surfaces of
 20 solid substrates has been described in the art. See, e.g., Sundberg, et al., J. Am. Chem. Soc. (1995) 117:12050-57.

In accordance with the present invention, the cell suspension is flowed through the channel, i.e., using pressure flow, as described in greater detail below, and the number of cells that are rolling over or firmly attached to the interior surface of
 25 the channel is monitored using an appropriate detection system. Alternatively, cells are pulsed through the channel to facilitate their monitoring. Typically, such systems employ a video imaging system that images and identifies the cells as they enter the imaged area, and tracks the cells path through the field, determining their relative velocity. Alternatively, point detection systems, e.g., as described herein, are used
 30 which detect cells at two separate points in the channel, and determine their relative velocity. In the latter case, it is generally desirable to provide the cells in suspension that is sufficiently dilute so as to permit correlation between the two detectors.

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Alternatively, cells may be coded with mixtures of different, distinguishable labels to permit the correlation among cells between points. Such coded cells optionally include wide varieties of different labels, or alternatively, include a set of two, three, four, five, six, seven or eight different labels at varying relative levels, where the
5 profile of the relative levels of labels identifies the different cells.

In screening assays, the test compounds are introduced into a channel, e.g., via an external sample accessing capillary (e.g., an electrokinetic injector or other capillary element) where they contact the suspension of cells. The cell suspension is then assayed for rolling or firmly attached cells, and the effect of the test compound,
10 if any, on the cell rolling or binding is determined as compared to the control, e.g., in the absence of the test compound.

III. Expression

In a further aspect, the methods and systems of the present invention are used to assay cellular expression functions, and particularly, for the effect of test
15 compounds on such cellular expression. Such assays are generally utilized in screening for effectors of given biological processes, which effectors target those functions at the gene expression level. In accordance with the present invention, therefore, the function label is indicative of the level of gene expression, for a particular gene of interest.

Gene expression levels are typically assayed by quantifying the level
20 of gene product from the gene of interest, e.g., the amount of protein produced by the cells. Alternate methods of gene expression analysis examine the amount of RNA transcribed from the gene of interest. Typically, such assays involve the use of a nucleic acid hybridization assay to identify a pattern of RNA transcription following
25 an activating event.

The methods and systems of the present invention are readily applied to such expression analyses. In particular, in accordance with the present invention, the function label is typically provided that is expressed by the cells during the expression function. For example, chimeric reporter systems are optionally employed
30 as function labels or indicators of gene expression. Chimeric reporter systems typically incorporate a heterogeneous reporter system integrated into the coding sequence of the gene of interest. The expression of the gene of interest is then

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accompanied by the expression of the reporter, which is then detected. For example, a receptor may be a fusion between the product of the gene of interest and heterologous protein, e.g., an enzyme whose activity is readily assayable, or an otherwise detectable protein, e.g., luciferase, aequorin, green fluorescent protein (GFP), β -galactosidase, alkaline phosphatase, or the like. The expressed reporter is then detected and compared with the level of reference label, to provide a quantitative determination of expression levels on a per cell basis. Expression of gene products to a detectable level can require varying amounts of time, e.g., several minutes to hours. Accordingly, the assay time is varied to allow such expression. As noted herein, such variation is generally accomplished by slowing the flow rates of the cell suspension through a channel and/or lengthening the channel.

Alternatively, the function label is provided as an element of a binding molecule that specifically associates with the downstream indicator of gene expression, e.g., an expressed protein, wherein the binding of the binding molecule (bearing the function label) to the gene product of interest produces a detectable property within the cell, e.g., as described with reference to the binding assays, above. The assay methods are then carried out in the same manner as described with reference to the binding functions, described above. In the case of expressed proteins, the binding molecule optionally includes an antibody specific for the gene product, or a specific binding partner, where the expressed protein is a member of a binding pair, e.g., a receptor or ligand.

Because gene expression assays typically require much longer incubation times than other assay types described herein, modified methods are optionally employed. For example, in one aspect, cells are flowed through the channel of the system, and preferably, through multiple parallel channels of the system, and contacted with surfaces of the channel(s) that cause them to adhere. Test compounds are then introduced into the channel(s) and allowed to flow over the adhered cells, e.g., for from 5 to 60 minutes. Any effects of these test compounds on the level of function label, and therefore, gene expression, is determined in the channel(s), and compared to the level of reference label. The comparison then allows quantification of the level of expression on a per cell basis. Optionally, the reaction channel is provided such that the travel time of the cells from the point of test

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compound addition to detection is sufficient to permit appropriate expression analysis. In certain aspects, tortuous channels, e.g., serpentine channels, and the like, are used to extend channel lengths to expand the travel time. Alternatively or additionally, flow rates are substantially reduced to increase this travel time.

5 SCREENING ASSAYS

As noted repeatedly above, the assays described herein are particularly useful in performing high-throughput screening assays. As used herein, the term "test compound" refers to the collection of compounds that are to be screened for their ability to affect a particular biochemical system. Test compounds optionally include a wide variety of different compounds, including chemical compounds, mixtures of chemical compounds, e.g., polysaccharides, small organic or inorganic molecules, biological macromolecules, e.g., peptides, proteins, nucleic acids, or an extract made from biological materials such as bacteria, plants, fungi, or animal cells or tissues, naturally occurring or synthetic compositions. Depending upon the particular embodiment being practiced, the test compounds may be provided, e.g., injected, free in solution, or may be attached to a carrier, or a solid support, e.g., beads. A number of suitable solid supports may be employed for immobilization of the test compounds. Examples of suitable solid supports include agarose, cellulose, dextran (commercially available as, e.g., Sephadex®, Sepharose®, etc.), carboxymethyl cellulose, polystyrene, polyethylene glycol (PEG), filter paper, nitrocellulose, ion exchange resins, plastic films, glass beads, polyaminemethylvinylether maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. Additionally, for the methods and apparatuses described herein, test compounds may be screened individually, or in groups. Group screening is particularly useful where hit rates for effective test compounds are expected to be low such that one would not expect more than one positive result for a given group. Alternatively, such group screening is optionally used where the effects of different test compounds are differentially detected in a single system, e.g., through electrophoretic separation of the effects, or differential labeling which enables separate detection.

Typically, vast libraries of test compounds are separately tested for potential effects on different cellular functions. In preferred aspects, large libraries of

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chemical compounds prepared using combinatorial synthesis techniques are typically employed as test compounds in high-throughput screening applications, to identify any such compounds that may have pharmacologically beneficial activities. In optional preferred aspects, test compounds include large libraries of naturally occurring materials or compounds, libraries of genetic material, protein fragments, and the like.

In general, the test compounds are separately introduced into the assay systems described herein. The relative level of a particular cellular function is then assessed in the presence of the test compound, and this relative level of function is then compared to a control system, which lacks an introduced test compound. Increases or decreases in relative cellular function are indicative that the test compound is an enhancer or an inhibitor of the particular cellular function, respectively.

OVERALL SYSTEMS

As noted above, the present invention also provides systems and devices used to practice the above-described methods. The system includes a channel along which is flowed a suspension of cells or other particles, and cell focusing means as noted above (laminar flow focusing channels, density gradient layers, or both). A source of different test compounds is optionally linked to the channel, for introducing the different test compounds into the channel whereupon they are contacted with, e.g., the cells. One or more detectors are also provided in sensory communication with the channel, e.g., for detecting and quantifying both the level of reference label and the level of function label present on the cells. As used herein, the phrase "sensory communication" refers to orientation of the detector such that it is capable of obtaining an appropriate signal from the point of interest. In the case of optical detectors, sensory communication provides a detector oriented such that it is capable of receiving an optical signal from a channel of a microfluidic device. Such detection is optionally direct, or includes an intervening optical pathway, e.g., lenses, fiber optics, etc. In the case of chemical detectors, such sensory communication typically requires a sensor component disposed in contact with the fluid material within the channel.

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The detector(s) is/are operably linked to a processor, e.g., a computer, for recording the detected signals, and providing a report of relative activity of the cells that are being assayed. The computer also typically includes appropriate programming for determining whether one assay, e.g., a first screening assay, shows
5 greater or lesser cellular function than another assay, e.g., a control.

Detector systems optionally includes one or more different detector elements, and are selected to detect relevant labels present in the cells. For example, in the case of cells that include reference and function labels that are fluorescent, the detector typically includes a dual wavelength fluorescent detector. This detector
10 typically includes a light source. Appropriate light sources typically vary depending upon the type of detection being employed. For example, in some cases broad spectrum illumination is desirable while in other cases, a narrower spectrum illumination is desired. Typically, the light source is a coherent light source, such as a laser, or laser diode, although other light sources, such as LEDs, lamps or other
15 available light sources are also optionally employed. In the case of a fluorescent detector, excitation light, e.g., light of appropriate wavelength to excite labels, from the light source is directed at an analysis channel, e.g., disposed in a microfluidic device, via an optical train that includes optional lens, beam splitters, and objective lenses

20 In some instances, electrokinetic material transport systems are used to direct one or more of the flow of cell suspensions, the injection of test compounds, and other material movement parameters. In such cases, the overall system used in performing the assay will typically include an appropriate controller and interface for controlling such electrokinetic material transport. Typically, such transport systems
25 include one or more electrical power supplies that are operably coupled to the termini of the channels in a microfluidic device, e.g., as described in greater detail below. The connection of the power supply(ies) with the channels is typically accomplished via electrodes placed into reservoirs at the termini of the channels, which electrodes are coupled to the power supply(ies). The power supply(ies) then deliver(s)
30 appropriate voltage levels to the various electrodes to yield a desired flow pattern within the channels of the device, e.g., flowing the cell suspension and periodically injecting a test compound. The power supply is typically linked to an appropriately

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programmed computer which directs the application of voltages in accordance with a user selected flow profile.

ASSAY DEVICES

Microfluidic devices and assay components which are optionally adapted to the present invention are described in various PCT applications and issued U.S. Patents, such as, U.S. Patent Nos. 5,699,157 (J. Wallace Parce) issued 12/16/97, 5,779,868 (J. Wallace Parce et al.) issued 07/14/98, 5,800,690 (Calvin Y.H. Chow et al.) issued 09/01/98, 5,842,787 (Anne R. Kopf-Sill et al.) issued 12/01/98, 5,852,495 (J. Wallace Parce) issued 12/22/98, 5,869,004 (J. Wallace Parce et al.) issued 02/09/99, 5,876,675 (Colin B. Kennedy) issued 03/02/99, 5,880,071 (J. Wallace Parce et al.) issued 03/09/99, 5,882,465 (Richard J. McReynolds) issued 03/16/99, 5,885,470 (J. Wallace Parce et al.) issued 03/23/99, 5,942,443 (J. Wallace Parce et al.) issued 08/24/99, 5,948,227 (Robert S. Dubrow) issued 09/07/99, 5,955,028 (Calvin Y.H. Chow) issued 09/21/99, 5,957,579 (Anne R. Kopf-Sill et al.) issued 09/28/99, 5,958,203 (J. Wallace Parce et al.) issued 09/28/99, 5,958,694 (Theo T. Nikiforov) issued 09/28/99, and 5,959,291 (Morten J. Jensen) issued 09/28/99; and published PCT applications, such as, WO 98/00231, WO 98/00705, WO 98/00707, WO 98/02728, WO 98/05424, WO 98/22811, WO 98/45481, WO 98/45929, WO 98/46438, and WO 98/49548, WO 98/55852, WO 98/56505, WO 98/56956, WO 99/00649, WO 99/10735, WO 99/12016, WO 99/16162, WO 99/19056, WO 99/19516, WO 99/29497, WO 99/31495, WO 99/34205, WO 99/43432, and WO 99/44217, which are all incorporated herein by reference.

As noted above, the assays of the present invention are carried out within fluidic channels, along which the cell suspensions and/or other particles are flowed. In some cases, the channels may simply be present in a capillary tube, e.g., a glass, fused silica, quartz or plastic capillary. The capillary channel is fluidly coupled to a source of the suspension of cells or other particles, which are then flowed along the capillary channel. In particularly preferred aspects, the channel is integrated into the body structure of a microfluidic device. As used herein, the term "microfluidic" generally refers to one or more fluid passages, chambers or conduits which have at least one internal cross-sectional dimension, e.g., depth, width, length, diameter, etc., that is less than 500 μm , and typically between about 0.1 μm and about 500 μm .

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In the devices of the present invention, the microscale channels or chambers preferably have at least one cross-sectional dimension between about 0.1 μm and 200 μm , more preferably between about 0.1 μm and 100 μm , and often between about 0.1 μm and 50 μm . Accordingly, the microfluidic devices or systems prepared in accordance with the present invention typically include at least one microscale channel, usually at least two intersecting microscale channels, and often, three or more intersecting channels disposed within a single body structure. Channel intersections may exist in a number of formats, including cross intersections, "Y" or "T" intersections, or any number of other structures, whereby two channels are in fluid communication.

The body structure of the microfluidic devices, described herein, typically comprises an aggregation of two or more separate layers which when appropriately mated or joined together, form the microfluidic device of the invention, e.g., containing the channels and/or chambers described herein. Typically, the microfluidic devices described herein will comprise a top portion, a bottom portion, and an interior portion, wherein the interior portion substantially defines the channels and chambers of the device.

In preferred aspects, the bottom portion of the device comprises a solid substrate that is substantially planar in structure, and which has at least one substantially flat upper surface. A variety of substrate materials may be employed as the bottom portion. Typically, because the devices are microfabricated, substrate materials will be selected based upon their compatibility with known microfabrication techniques, e.g., photolithography, wet chemical etching, laser ablation, air abrasion techniques, injection molding, embossing, and other techniques. The substrate materials are also generally selected for their compatibility with the full range of conditions to which the microfluidic devices are typically exposed, including extremes of pH, temperature, salt concentration, and application of electric fields. Accordingly, in some preferred aspects, the substrate material optionally includes materials normally associated with the semiconductor industry in which such microfabrication techniques are regularly employed, including, e.g., silica based substrates, such as glass, quartz, silicon or polysilicon, as well as other substrate materials, such as gallium arsenide and the like. In the case of semiconductive materials, it will often be desirable to provide an insulating coating or layer, e.g., silicon oxide, over the substrate material, and

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particularly in those applications where electric fields are to be applied to the device or its contents.

In additional preferred aspects, the substrate materials will comprise polymeric materials, e.g., plastics, such as polymethylmethacrylate (PMMA),
5 polycarbonate, polytetrafluoroethylene (TEFLON™), polyvinylchloride (PVC), polydimethylsiloxane (PDMS), polysulfone, polystyrene, polymethylpentene, polypropylene, polyethylene, polyvinylidene fluoride, ABS (acrylonitrile-butadiene-styrene copolymer), and the like. Such polymeric substrates are readily manufactured using available microfabrication techniques, as described above, or from
10 microfabricated masters, using well known molding techniques, such as injection molding, embossing or stamping, or by polymerizing the polymeric precursor material within the mold. See, e.g., U.S. Pat. No. 5,512,131. Such polymeric substrate materials are preferred for their ease of manufacture, low cost and disposability, as well as their general inertness to most extreme reaction conditions. Again, these polymeric materials
15 optionally include treated surfaces, e.g., derivatized or coated surfaces, to enhance their utility in the microfluidic system, e.g., provide enhanced fluid direction, e.g., as described in U.S. Patent Application Serial No. 08/843,212, filed April 14, 1997, and which is incorporated herein by reference in its entirety for all purposes.

The channels and/or chambers of the microfluidic devices are typically
20 fabricated into the upper surface of the bottom substrate or portion of the device, as microscale grooves or indentations, using the above described microfabrication techniques. The top portion or substrate also comprises a first planar surface, and a second surface opposite the first planar surface. In the microfluidic devices prepared in accordance with certain aspects of the methods described herein, the top portion also
25 includes a plurality of apertures, holes or ports disposed therethrough, e.g., from the first planar surface to the second surface opposite the first planar surface.

The first planar surface of the top substrate is then mated, e.g., placed into contact with, and bonded to the planar surface of the bottom substrate, covering and sealing the grooves and/or indentations in the surface of the bottom substrate, to form
30 the channels and/or chambers (i.e., the interior portion) of the device at the interface of these two components. Bonding of substrates is typically carried out by any of a number of different methods, e.g., thermal bonding, solvent bonding, ultrasonic welding, and the like.

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The holes in the top portion of the device are oriented such that they are in communication with at least one of the channels and/or chambers formed in the interior portion of the device from the grooves or indentations in the bottom substrate. In the completed device, these holes function as reservoirs for facilitating fluid or material introduction into the channels or chambers of the interior portion of the device, as well as providing ports at which electrodes may be placed into contact with fluids within the device, allowing application of electric fields along the channels of the device to control and direct fluid transport within the device. In many embodiments, extensions are provided over these reservoirs to allow for increased fluid volumes, permitting longer running assays, and better controlling fluid flow parameters, e.g., hydrostatic pressures. Examples of methods and apparatuses for providing such extensions are described in, e.g., Dubrow et al., "Microfluidic Devices and Systems Incorporating Cover Layers," WO 99/43432, published September 2, 1999, which are incorporated herein by reference in their entirety for all purposes. These devices are optionally coupled to other sample introduction ports, e.g., one or more pipettor or capillary elements (e.g., 1, 2, 3, 4, 6, 8, 10, 12, or more elements) which serially introduce multiple samples, e.g., from the wells of a microwell plate. Thus, in some embodiments, both reservoirs in the upper surface and external capillary elements are present in a single device. Alternatively, the devices include only one or more capillary elements for sample or other material introduction.

The sources of reagents, samples (e.g., cells, microbeads, DNA or other molecules, etc.), buffers, and other materials are optionally fluidly coupled to the microchannels in any of a variety of ways. In particular, those systems comprising sources of materials set forth in Knapp et al., "Closed Loop Biochemical Analyzers" (WO 98/45481; PCT/US98/06723) and U.S. Pat. No. 5,942,443, issued August 24, 1999, entitled "High Throughput Screening Assay Systems in Microscale Fluidic Devices" to J. Wallace Parce et al. and, e.g., in PCT/US00/04522, filed February 22, 2000, entitled "Manipulation of Microparticles In Microfluidic Systems," by Mehta et al. are applicable.

In these systems, one or more capillary or pipettor elements (i.e., an element that includes, e.g., a channel in which components are optionally moved from a source to a microscale element such as a second channel or reservoir) is temporarily

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or permanently coupled to a source of material. The source is optionally internal or external to a microfluidic device that includes the pipettor or capillary element. Example sources include microwell plates, membranes or other solid substrates comprising lyophilized components, wells or reservoirs in the body of the microscale device itself and others. In one preferred embodiment, one or more capillary elements are used to draw cell or other samples from microwell plates into the body structure of the device. The channel disposed in the capillary element typically includes a diameter in the range of about 1 μm to about 100 μm , more preferably in the range of about 25 μm to about 75 μm , e.g., about 50 μm in diameter. These devices are typically included as part of an automated or integrated system which further enhances device throughput. Integrated systems are discussed further above and in the references cited herein.

In many embodiments, the microfluidic devices will include an optical detection window disposed across one or more channels and/or chambers of the device. Optical detection windows are typically transparent such that they are capable of transmitting an optical signal from the channel/chamber over which they are disposed. Optical detection windows are optionally merely a region of a transparent cover layer, e.g., where the cover layer is glass or quartz, or a transparent polymer material, e.g., PMMA, polycarbonate, etc. Alternatively, where opaque substrates are used in manufacturing the devices, transparent detection windows fabricated from the above materials are optionally separately manufactured into the device.

In many aspects, it is desirable to provide the interior surfaces of the channels with an appropriate treatment to prevent the adhesion of cells or other particles to that surface. For example, in the case of glass or other highly charged channel surfaces, some cell types often have a tendency to stick to the channel surfaces, interfering with the flowing of cells through the channels. For example, in the case of mammalian cell-based assays, many mammalian cell types are particularly adherent to certain types of surfaces, e.g., glass and some plastics. Accordingly, in some embodiments, it is desirable to treat or coat the interior surfaces of the channels to prevent cell adhesion. A variety of surface treatments are optionally employed to accomplish this goal. For example, charge masking coatings such as polyols (e.g., polyvinylalcohol (PVA)), polyethyleneimine (PEI), polyethylene glycol (PEG),

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polyacrylamides (e.g., polyacrylamide, polymethylacrylamide, polydimethacrylamide, and the like), carbohydrates such as polysucrose (Ficoll™), polyglucose (dextran and cellulose), and polytetrafluoroethylene (Teflon™), or the like. Alternatively, covalent surface treatments are also optionally used to prevent surface adhesion of cells, such as silanization (e.g., using dimethyl or dichlorosilane) of glass or plastic surfaces. Other surface treatments are generally described, with reference to device fabrication techniques, above.

The flowing of the suspension of cells or other particles along one or more channels of the devices described herein is optionally carried out by a number of mechanisms, including pressure based-flow, electrokinetic flow, or other mechanisms or combinations of mechanisms. In a first preferred aspect, a pressure differential is used to flow the suspension of cells along a channel. Application of a pressure differential along a channel is carried out by a number of means. For example, in a simple passive aspect, the cell suspension is deposited in a reservoir at one end of a channel and at a sufficient volume or depth, that the cell suspension creates a hydrostatic pressure differential along the length of the channel, e.g., by virtue of its having greater depth than a reservoir at an opposite terminus of the channel. The hydrostatic pressure then causes the cell suspension to flow along the length of the channel. Typically, the reservoir volume is quite large in comparison to the volume or flow through rate of the channel, e.g., 10 μ l reservoirs, vs. 1000 μ m² channel cross-section. As such, over the time course of the assay, the flow rate of the cell suspension will remain substantially constant, as the volume of the reservoir, and thus, the hydrostatic pressure changes very slowly. Applied pressure is then readily varied to yield different cell suspension flow rates through the channel. In screening applications, varying the flow rate of the cell suspension is optionally used to vary the incubation time of the cells with the test compound. In particular, by slowing the cells flow rate along the channel, one can effectively lengthen the amount of time between introduction of test compounds and detection of their effects. Alternatively, channel lengths, detection points, or test compound introduction points are varied in fabrication of the devices, to vary incubation times.

In many applications, it may be desirable to provide relatively precise control of the flow rate of the cell suspension and/or other particles, e.g., to precisely control incubation times, or the like. As such, in many preferred aspects, flow systems

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that are more active than hydrostatic pressure driven systems are employed. For example, the cell suspension is optionally flowed by applying a pressure differential across the length of a channel. For example, a pressure source (positive or negative) is applied at the cell suspension reservoir at one end of a channel, and the applied pressure
5 forces the suspension through the channel. The pressure source is optionally pneumatic, e.g., a pressurized gas, or alternatively is a positive displacement mechanism, i.e., a plunger fitted into a cell suspension reservoir, for forcing the cell suspension through the analysis channel. Alternatively, a vacuum source is applied to a reservoir at the opposite end of the channel to draw the suspension through the channel. Pressure or
10 vacuum sources are optionally supplied external to the device or system, e.g., external vacuum or pressure pumps sealably fitted to the inlet or outlet of a channel, or they are internal to the device, e.g., microfabricated pumps integrated into the device and operably linked to a channel. Examples of microfabricated pumps have been widely described in the art. See, e.g., published International Application No. WO 97/02357.

15 In alternate aspects, other flow systems are employed in transporting or focusing the cellular suspension and/or other particles in a channel. In one embodiment, cells are initially flowed under pressure, but focused using alternative fluid direction components, such as an electrokinetic force modulator. While electrokinetic forces typically produce substantially uniform fluid flow in
20 microchannels, they tend to disrupt cellular membranes. Thus, the use of electrokinetic motive forces is typically limited to focusing pressure-based cellular flows, which as discussed have non-uniform flow velocities.

Electrokinetic transport systems typically utilize electric fields applied along the length of channels that have a surface potential or charge associated
25 therewith. When fluid is introduced into the channel, the charged groups on the inner surface of the channel ionize, creating locally concentrated levels of ions near the fluid surface interface. Under an electric field, this charged sheath migrates toward the cathode or anode (depending upon whether the sheath comprises positive or negative ions) and pulls the encompassed fluid along with it, resulting in bulk fluid
30 flow. This flow of fluid is generally termed electroosmotic flow. Where the fluid includes a cell or other particle suspension, those components are also pulled along. A more detailed description of controlled electrokinetic material transport systems in

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microfluidic systems is described in published International Patent Application No. WO 96/04547, which is incorporated herein by reference.

Hydrostatic, wicking and capillary forces are also optionally used to provide for fluid flow. See, e.g., "Method and Apparatus for Continuous Liquid Flow in Microscale Channels Using Pressure Injection, Wicking and Electrokinetic Injection," by Alajoki et al., Attorney Docket Number 017646-007100, filed February 5, 1999.

In alternative aspects, flow of the cell suspension and/or other particles is driven by inertial forces. In particular, channels are optionally disposed in a substrate that has the conformation of a rotor, with channels extending radially outward from the center of the rotor. The cell suspension is deposited in a reservoir that is located at the interior portion of the rotor and is fluidly connected to the channels. During rotation of the rotor, the centripetal force on the cell suspension forces the cell suspension through the channels, outward toward the edge of the rotor. Multiple channels are typically provided in the rotor to perform multiple different analyses. Detection of the function and reference labels is then carried out by placing a detector under the spinning rotor and detecting the signal as a channel passes over the detector. Examples of rotor systems have been previously described for performing a number of different assay types. See, e.g., Published International Application No. WO 95/02189. Test compound reservoirs are optionally provided in the rotor, in fluid communication with one or more channels, such that the rotation of the rotor also forces the test compounds into the one or more channels.

Although illustrated in the figures herein as a single channel and accessing capillary, it will be readily appreciated that these aspects may be provided as multiple parallel channels and accessing capillaries (discussed above), in order to substantially increase the throughput of the system. Specifically, single body structures are optionally provided with multiple parallel channels coupled to multiple sample accessing capillaries that are positioned to sample multiple samples at a time from sample libraries, e.g., multiwell plates. As such, these capillaries are generally spaced at regular distances that correspond with the spacing of wells in multiwell plates, e.g., 9 mm centers for 96 well plates, 4.5 mm for 384 well plates, and 2.25 mm for 1536 well plates.

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The present invention is further illustrated with reference to the following non-limiting examples.

EXAMPLES

TUNEL ASSAY AND ANNEXIN-V ASSAY FOR APOPTOSIS DETECTION

5 A TUNEL assay (TdT (Terminal deoxynucleotidyl transferase)-mediated dUTP Nick End Labeling) was used to measure cellular apoptosis in accordance with the above-described methods and systems. In a TUNEL Assay, damaged DNA is labeled with fluorescinated nucleotides. Terminal deoxynucleotidyl transferase (TdT) binds to exposed 3' ends of DNA fragments generated in response
10 to apoptotic signals and catalyzes the addition of fluorescein-labeled deoxynucleotides. U937 cells were treated with Camptothecin to induce apoptosis. The cells were then harvested and fixed with 4% formaldehyde and stored in 80% ethanol at +4°C until labeling was performed.

The cells were rehydrated in TBS buffer. They were then treated with 20
15 µg/ml of proteinase K for 5 minutes and resuspended in equilibration buffer. The cells were incubated in a working TdT labeling reaction mixture from Oncogene Research Products containing TdT enzyme and FragEL TdT labeling reaction mix. The cells were resuspended in TBS and then counterstained with a DNA dye (SYTO®-62 DNA dye at 1 µM for 10 minutes RT) to obtain a total cell count.

20 After washing the cells in TBS, they were resuspended in Hanks' Balanced Salt Solution with 10% OPTIPREP at 5×10^6 /ml and loaded in a microfluidic device having the channel geometry shown in Figure 1A, for analysis. Fluorescence was detected using 488nm excitation, while emission was read at 525 nm and 680 nm.

Figure 4 illustrates a control analysis, e.g., U937 cells not treated to induce
25 apoptosis. The bottom line corresponds to the SYTO®-62, which indicates the mere presence of cells, whereas the top line corresponds to the fluorescein end labeled nucleic acids. Apoptotic cells would yield a peak on both the upper and lower lines, indicating the presence of an apoptotic cell. As can be seen, there are substantially no discernible peaks in the upper line, indicating a lack of apoptosis in the control. Figure 5 represents U937 cells
30 treated with Camptothecin to induce apoptosis. As can be seen, corresponding peaks are seen on both the lower and upper lines, indicating the presence of apoptotic cells.

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In an Annexin-V assay, change in the outer membrane of apoptotic cells is detected. A membrane component, phosphatidylserine (PS) is translocated to the outer layer. Annexin-V binds to PS and is conjugated to biotin to allow secondary reactions to fluorescently labeled Streptavidin for detection. U937 cells were treated with Camptothecin to induce apoptosis. The cells were harvested and washed in phosphate buffered saline (PBS). Cells were incubated with Annexin-V-Biotin (Boehringer Mannheim) in HEPES buffer, and then incubated with 5 μ g streptavidin-Cy5 (Amersham) and 1 mM of Calcein-AM dye in HEPES buffer. The HEPES buffer solution contained 10mM HEPES, pH 7.4; 140 mM NaCl; and a 5 mM CaCl_2 . The cells were washed and resuspended in HEPES buffer with 8.5% sucrose and loaded in a microfluidic device for analysis. Fluorescence was detected using excitation at 488 nm and 635 nm, and emission was read at 525 nm and 682 nm.

Figure 6 indicates analysis of U937 cells that were not treated to induce apoptosis. In this case, the bottom line indicates live cell count (Calcein), while the top line indicates the presence of apoptotic cells (Annexin-V-Cy5). As can be seen, a few apoptotic cells are present within the control experiment. Figure 7, on the other hand, shows an analysis of the same cells treated with Camptothecin to induce apoptosis. As can be seen, the top trace includes a much greater number of peaks representing apoptotic cells, and particularly as a percentage of total cells in the analysis, e.g., as compared to the lower line.

Figure 8 shows results from a TUNEL assay with HL-60 cells treated with Camptothecin for 6 hours to induce apoptosis. The bottom line traces the total cell count. The top line traces apoptotic cells labeled at DNA damage points. In this case, almost all cells had sustained apoptotic DNA damage.

Figure 9 shows results from a TUNEL assay. HL-60 cells (not treated for apoptosis) were used as control cells. The bottom trace is for SYTO®-62 indicating total cell count. The top trace is for Fluorescein labeled nucleotide which signifies the presence of apoptosis related DNA damage.

Figures 10 and 11 shows results from TUNEL assays from Figures 8 and 9 analyzed using appropriate software. Data is presented as scatter plot format in Figure 10. In Figure 11, data is represented in bar graph format. Additional data for different time points and two other cell lines is included.

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Figure 12 shows and Annexin-V assay. HL60 cells were treated with Camptothecin for four hours to induce apoptosis. The bottom trace indicates live cell count (Calcein). The top trace indicates Annexin-V-Cy5 labeling. Cells labeled only for Calcein are live cells and not apoptotic. In this case, almost all cells are apoptotic.

Figure 13 shows an Annexin-V assay. HL-60 cells (not treated for apoptosis) are control cells. The bottom trace indicates live cell count. The top trace indicates Annexin-V-Cy5 labeling. Cells labeled with both dyes are apoptotic. Cells labeled only for Annexin-CY5 are dead cells. In this case, only a few cells were apoptotic.

Figures 14 and 15 show data analysis of figures 12 and 13. Data is presented in scatter plot format in Figure 14. In Figure 15, data is represented in bar graph format. Additional data for different time points and two other cell lines is included.

15 FLUORESCENCE POPULATION AND CONCENTRATION EXPERIMENTS

Figure 17 depicts a chip employing a channel geometry that was used to conduct fluorescence population experiments to confirm the feasibility of the device as a microfluidic flow cytometer. The experiment entailed comparing microfluidic hydrodynamic focusing data obtained by flowing fluorescently-labeled calibration beads (Quantum 26 Fluorescein Microbeads Standards B-111698 from Flow Cytometry Standards Corp.) through the microfluidic flow cytometer (both focused and unfocused) with data provided in a certificate of calibration that accompanied the beads. Flow Cytometry Standards Corp. obtained the calibration data by flowing the same type of bead through a traditional flow cytometer.

Figure 18 is a histogram showing the distribution of results obtained in the fluorescence intensity population experiment conducted utilizing hydrodynamic focusing in the microfluidic flow cytometer. Figures 19 and 20 are histograms showing the distribution of results obtained in the fluorescence intensity population experiment conducted, in the same microfluidic flow cytometer, in the absence of hydrodynamic focusing. As shown, the focused beads resolve into a more uniform fluorescence intensity population than the unfocused beads which are more widely

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distributed. Furthermore, the focused beads are comparable to results achievable using traditional flow cytometers, which typically include a coefficient of variation (%CV) of about 6%, according to the calibration data provided by Flow Cytometry Standards Corp. The calculated %CV for the focused beads in the microfluidic system was about 6.8%, whereas that calculated for the unfocused beads was only about 28.8%. In general, an acceptable %CV is less than 15%, which further demonstrates the feasibility of the device, e.g., as a flow cytometer.

Figures 25A, B, and C are histograms showing the distribution of results obtained in resolution experiments using various microbead to buffer concentrations, namely, 1:1, 1:3, and 1:7, respectively. The experiments were conducted using an Agilent bioanalyzer microfluidic system to determine whether microbead doublet populations could be resolved. As shown, even at the highest microbead concentration, where the largest population of doublets was expected, significant resolution of the two populations was observed with a %CV of about 6.7%. (FIG. 25A). Similar %CVs were obtained at the two other concentrations. To further confirm the capabilities of the devices and methods of the present invention an additional resolution experiment was conducted using viable THP-1 cells stained with SYTO®-62 intercalating dye. As shown in Figure 26, significant resolution was achieved with a %CV comparable to those obtained using traditional flow cytometric devices.

The discussion above is generally applicable to the aspects and embodiments of the invention described herein.

Moreover, modifications are optionally made to the methods and devices described herein without departing from the spirit and scope of the invention as claimed, and the invention is optionally put to a number of different uses including the following:

The use of a microfluidic system containing at least a first substrate and having a first channel and a second channel intersecting the first channel, at least one of the channels having at least one cross-sectional dimension in a range from 0.1 to 500 μm , in order to test the effect of each of a plurality of test compounds on a biochemical system comprising one or more focused cells or particles.

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The use of a microfluidic system as described herein, wherein a biochemical system flows through one of said channels substantially continuously, providing for, e.g., sequential testing of a plurality of test compounds.

5 The use of a microfluidic device as described herein to modulate reactions within microchannels or microchambers.

The use of electrokinetic injection in a microfluidic device as described herein to modulate or achieve flow in the channels.

10 The use of a combination of wicks, electrokinetic injection and pressure based flow elements in a microfluidic device as described herein to modulate, focus, or achieve flow of materials, e.g., in the channels of the device.

An assay utilizing a use of any one of the microfluidic systems or substrates described herein.

15 While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and apparatus described above may be used in various combinations. All publications, patent applications and other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual
20 publication or patent document were individually so denoted.

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WHAT IS CLAIMED IS:

1. A method of providing substantially uniform flow velocity to particles flowing in a first microchannel, the method comprising:
 5 flowing the particles in the first microchannel, wherein the particles comprise a non-uniform flow velocity; and,
 focusing the particles horizontally or vertically in the first microchannel, thereby providing substantially uniform flow velocity to the particles flowing in the first microchannel.
- 10 2. The method of claim 1, further comprising sampling the particles with at least one capillary element prior to performing the flowing step.
3. The method of claim 1, comprising flowing the particles using pressure-based flow.
- 15 4. The method of claim 1, comprising focusing the particles using one or more fluid direction component comprising one or more of: a fluid pressure force modulator, an electrokinetic force modulator, a capillary force modulator, or a fluid wicking element.
5. The method of claim 1, wherein the focusing step further comprises focusing the particles horizontally and vertically in the first microchannel.
- 20 6. The method of claim 1, comprising focusing the particles vertically or horizontally in the first microchannel by simultaneously introducing fluid flow from two opposing microchannels into the first microchannel.
7. The method of claim 1, comprising focusing the particles horizontally in the microchannel by introducing a low density fluid and a high density
 25 fluid into the microchannel, wherein the particles are focused in an intermediate density fluid present between the high density fluid and the low density fluid.

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8. The method of claim 1, comprising focusing the particles horizontally or vertically in the first microchannel by simultaneously introducing at least one fluid flow from at least a second microchannel that comprises at least one intersection with the first microchannel.
- 5 9. The method of claim 1, further comprising sorting the particles.
- 10 10. The method of claim 9, wherein the particles comprise one or more of: a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a set of functionalized microbeads, a molecule, or a set of molecules.
11. The method of claim 1, further comprising washing a diffusible material from the particles by introducing a diluent into the first microchannel from at least a second microchannel and removing the resulting diluted diffused product comprising diluent mixed with the diffusible material through at least a third microchannel.
- 15 12. The method of claim 11, the method comprising sequentially introducing the diluent into the first microchannel from the second microchannel and a fourth microchannel, wherein the second and fourth microchannels intersect the first microchannel at an offset intersection region.
- 20 13. The method of claim 11, the method comprising simultaneously introducing the diluent into the first microchannel from the second microchannel and a fourth microchannel, wherein the second and fourth microchannel intersect the first microchannel at a common intersection region.
- 25 14. The method of claim 13, the method comprising removing the diffused product through the third microchannel and a fifth microchannel, which third and fifth microchannels intersect the first microchannel at a common intersection region.
15. The method of claim 14, further comprising introducing the diluent through a sixth and seventh microchannel which sixth and seventh intersect the first microchannel at a common intersection and removing the resulting further

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diluted diffused product through an eighth and ninth microchannel, which eighth and ninth microchannel intersect the first microchannel at a common intersection.

16. The method of claim 15, wherein the diluent is introduced into the first microchannel by pressure-based flow.

5 17. The method of claim 15, wherein the diluent is introduced into the first microchannel by electrokinetic flow.

18. The method of claim 1, wherein the particles comprise cells and the method further comprises performing a TUNEL assay or an Annexin-V assay on the cells in the channel to measure apoptosis.

10 19. A method of sorting members of at least one particle population, the method comprising:

flowing the members of the at least one particle population in a first microchannel; and,

15 focusing the members of the at least one particle population horizontally or vertically in the first microchannel such that selected individual members are directed into at least a second microchannel that intersects with the first microchannel, thereby sorting the members of the at least one particle population.

20 20. The method of claim 19, wherein at least a portion of the first microchannel comprises at least one separation element disposed therein, the at least one separation element comprising at least two sides, wherein at least a portion of the at least one separation element is disposed upstream of the at least the second microchannel.

25 21. The method of claim 20, wherein the focusing step further comprises focusing the members of the at least one particle population horizontally or vertically in the first microchannel such that the selected individual members are directed to at least one of the at least two sides of the at least one separation element and into the second microchannel that intersects the first microchannel.

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22. The method of claims 19 or 21, wherein the members of the at least one particle population comprise one or more of: a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a set of functionalized microbeads, a molecule, or a set of molecules.

5 23. The method of claims 19 or 21, wherein the focusing step further comprises horizontally or vertically focusing the members of the at least one particle population in the first microchannel by introducing at least one fluid flow from a third microchannel that intersects with the first microchannel upstream from the second microchannel.

10 24. The method of claim 23, comprising introducing the at least one fluid flow by heating at least one fluid in the third microchannel.

25. The method of claim 24, wherein the heating step comprises Joule heating the at least one fluid.

15 26. The method of claims 19 or 21, wherein the focusing step further comprises horizontally or vertically focusing the members of the at least one particle population in the first microchannel by introducing at least one fluid flow from at least one of at least a third and at least a fourth microchannel that oppose one another and that intersect with the first microchannel upstream from the second microchannel.

20 27. The method of claim 26, comprising introducing the at least one fluid flow by heating at least one fluid in at least one of the third or fourth microchannels.

28. The method of claim 27, wherein the heating step comprises Joule heating the at least one fluid.

25 29. An integrated system for providing substantially uniform flow velocity to flowing members of at least one particle population in a microfluidic device, the system comprising:

a body structure comprising at least a first microchannel disposed therein;

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at least a first fluid direction component coupled to the first microchannel for inducing flow of at least one fluidic material comprising the members of the at least one particle population in the first microchannel;

5 at least one source of the at least one fluidic material fluidly coupled to the first microchannel;

at least a second microchannel which intersects the first microchannel for introducing at least one fluid into the first microchannel to horizontally or vertically focus the members of the at least one particle population in the first microchannel;

10 at least one flow control regulator for regulating flow of the at least one fluidic material or the at least one fluid in the first or the second microchannels; and,

a computer comprising an instruction set directing simultaneous flow of the members of the at least one particle population in the first microchannel and simultaneous introduction of the at least one fluid from the second microchannel into the first microchannel.

15 30. The integrated system of claim 29, wherein the first fluid direction component induces non-uniform flow.

31. The integrated system of claim 29, wherein the first fluid direction component comprises a fluid pressure force modulator.

20 32. The integrated system of claim 29, wherein the at least one fluid is introduced using at least a second fluid direction component comprising one or more of: a fluid pressure force modulator, an electrokinetic force modulator, a capillary force modulator, or a fluid wicking element.

25 33. The integrated system of claim 29, further comprising at least a third microchannel which intersects the first microchannel in an intersection region common to the second microchannel, wherein the at least one flow control regulator further regulates flow of the at least one fluid in the second and the third microchannels, and wherein the computer further comprises an instruction set for simultaneously flowing fluids from the second and third microchannels into the first microchannel.

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34. The integrated system of claims 29 or 33, further comprising at least a fourth and fifth channel which intersect the first microchannel at a common intersection downstream of the second and third microchannels, the computer further comprising an instruction set for simultaneously flowing material from the first
5 microchannel into the fourth and fifth microchannels.

35. The integrated system of claims 34, further comprising sixth and seventh microchannels which intersect the first microchannel at a common intersection downstream of the fourth and fifth microchannels, the computer further comprising an instruction set for simultaneously flowing material from the sixth and
10 seventh microchannels into the first microchannel.

36. The integrated system of claim 35, further comprising eighth and ninth microchannels which intersect the first microchannel at a common intersection downstream of the sixth and seventh microchannels, the computer further comprising an instruction set for simultaneously flowing material from the first microchannel into
15 the eighth and ninth microchannels.

37. The integrated system of claim 29, comprising one or more source of reagent selected from the group of reagents consisting of: terminal deoxynucleotide transferase, one or more fluoresceins labeled nucleotide, Annexin V, an AnnexinV-biotin conjugate, a DNA dye, Camptothecin, Calcein-AM, a control
20 cell, and a test cell.

38. The integrated system of claim 29, further comprising at least one signal detector mounted proximal to the first microchannel for detecting a detectable signal produced by one or more of the members of the at least one particle population in the first microchannel.

25 39. The integrated system of claim 38, further comprising at least a third and at least a fourth microchannel which intersect the first microchannel downstream from the intersection of the second microchannel with the first microchannel, wherein the fourth microchannel intersects the first microchannel

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downstream from the intersection of the third microchannel with the first microchannel.

40. The integrated system of claim 39, wherein the at least one flow control regulator further regulates flow of the at least one fluid in the third or the
5 fourth microchannels.

41. The integrated system of claim 40, wherein the at least one signal detector detects a detectable signal produced by at least one selected member of the at least one particle population between the intersections of the second and the third microchannels with the first microchannel.

10 42. The integrated system of claim 41, wherein the computer is operably linked to the first fluid direction component, the at least one flow control regulator, and the at least one signal detector, wherein the instruction set further directs simultaneous introduction of the at least one fluid from the third microchannel into the first microchannel to horizontally or vertically focus the at least one selected
15 member of the at least one particle population such that the at least one selected member is directed into the fourth microchannel at least in response to the detectable signal produced by the at least one selected member.

43. The integrated system of claim 42, wherein the instruction set further directs simultaneous introduction of the at least one fluid from the third
20 microchannel by activating at least one heating element disposed within the third microchannel or at least one well that fluidly communicates with the third microchannel.

44. The integrated system of claim 43, wherein the at least one heating element comprises at least one Joule heating electrode or at least one a
25 conductively coated microchannel portion.

45. The integrated system of claim 42, wherein at least a portion of the first microchannel comprises at least one separation element disposed therein, the at least one separation element comprising at least two sides, wherein at least a

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portion of the at least one separation element is disposed upstream of the fourth microchannel.

46. The integrated system of claim 45, wherein the at least one selected member of the at least one particle population is directed to at least one of the at least two sides of the at least one separation element and into the fourth microchannel that intersects the first microchannel at least in response to the detectable signal produced by the at least one selected member.

47. The integrated system of claims 38, 42, or 46, wherein the detector comprises a fluorescent excitation source and a fluorescent emission detection element.

48. The integrated system of claim 47, wherein the computer is operably linked to the signal detector and comprises an instruction set for converting detected signal information into digital data.

49. The integrated system of claims 42 or 46, wherein the at least one selected member of the at least one particle population comprises one or more of: a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a molecule, or a set of molecules.

50. The integrated system of claims 42 or 46, further comprising at least a fifth microchannel which intersects the first microchannel in an intersection region common to the second microchannel, wherein the at least one flow control regulator further regulates flow of the at least one fluid in the second and the fifth microchannels, and wherein the computer further comprises an instruction set for simultaneously flowing fluids from the second and the fifth microchannels into the first microchannel.

51. The integrated system of claim 50, further comprising at least a sixth microchannel which intersects the first microchannel in an intersection region common to the third microchannel, wherein the at least one flow control regulator further regulates flow of the at least one fluid in the third and the sixth microchannels,

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and wherein the computer further comprises an instruction set for flowing fluids from the third and the sixth microchannels into the first microchannel.

5 **52.** The integrated system of claim 51, wherein the instruction set directs individual or simultaneous fluid flow from the third and sixth microchannels by individually or simultaneously activating at least one heating element disposed within each of the third and sixth microchannels or within at least one well that fluidly communicates with each of the third and sixth microchannels.

10 **53.** The integrated system of claim 52, wherein the at least one heating element comprises at least one Joule heating electrode or at least one conductively coated microchannel portion.

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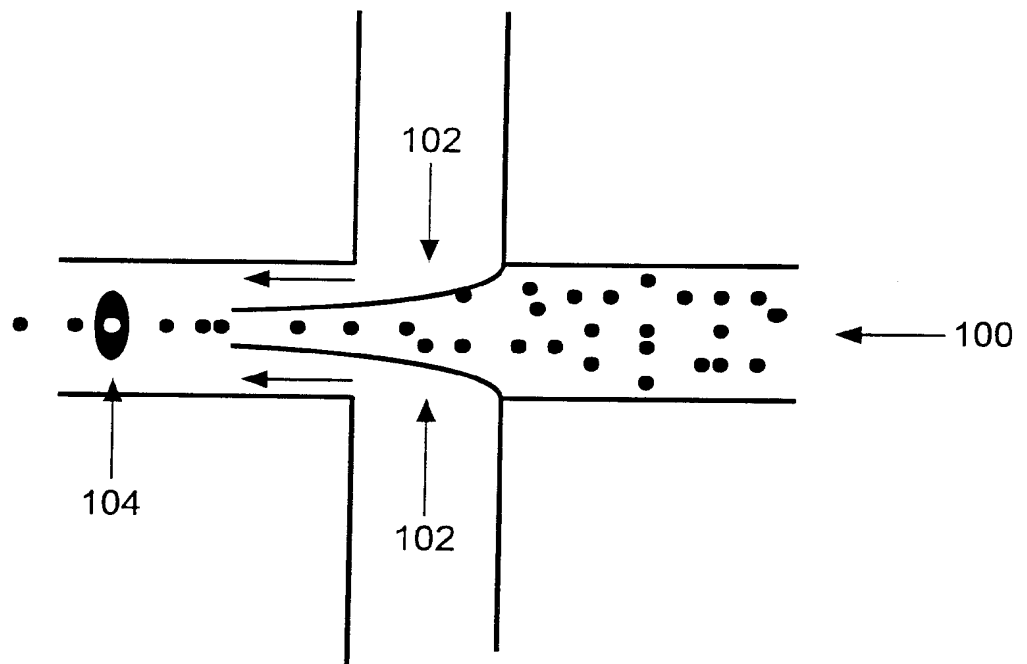


Fig. 1A

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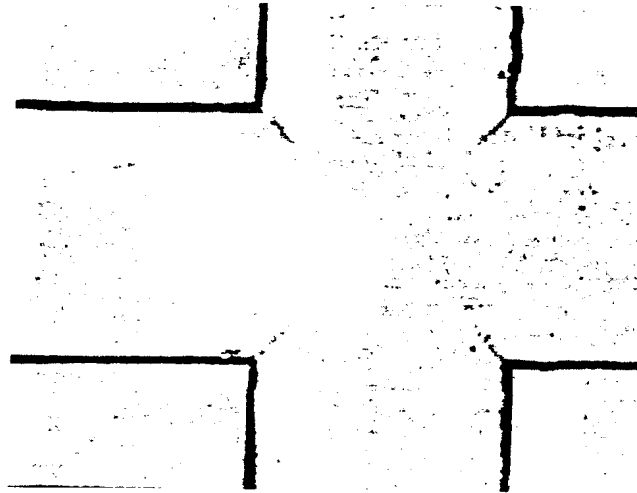


Fig. 1B

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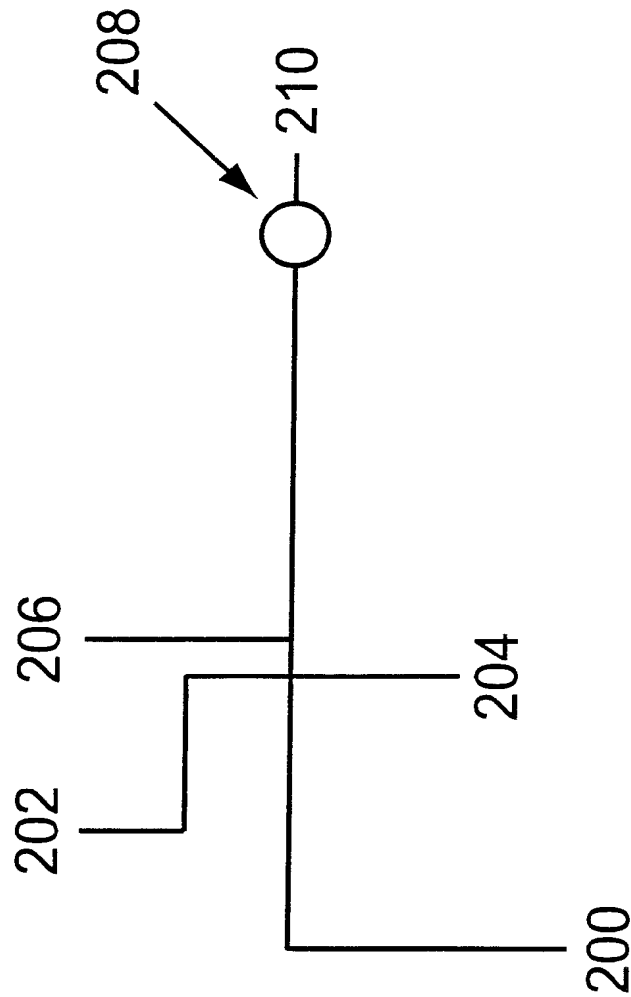


Fig. 2A

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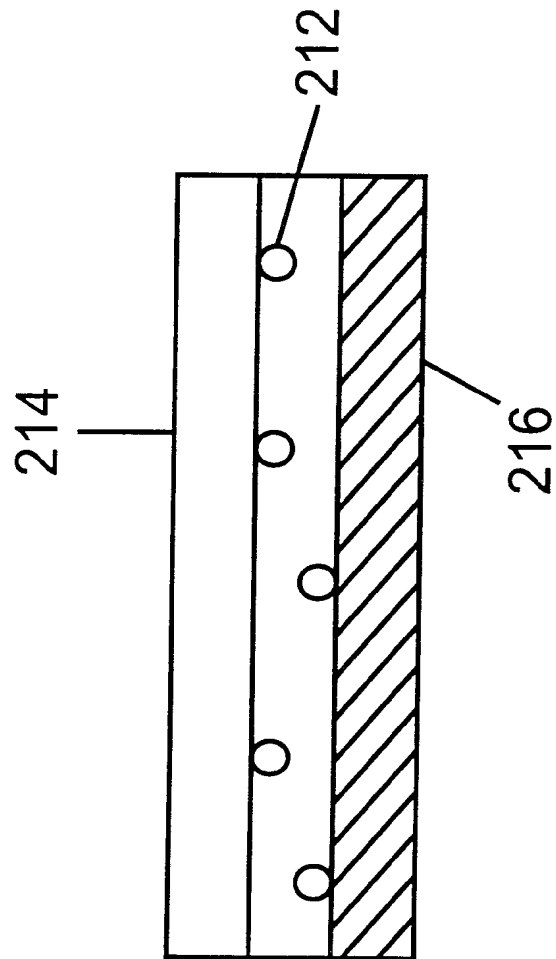


Fig. 2B

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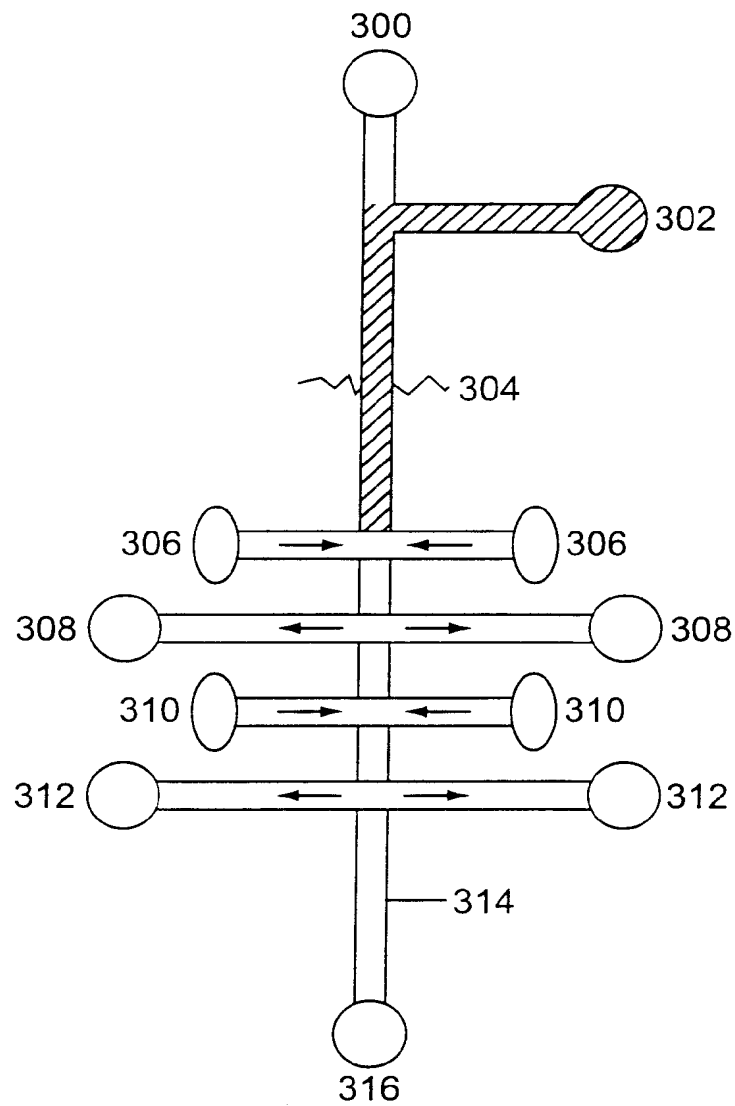


Fig. 3

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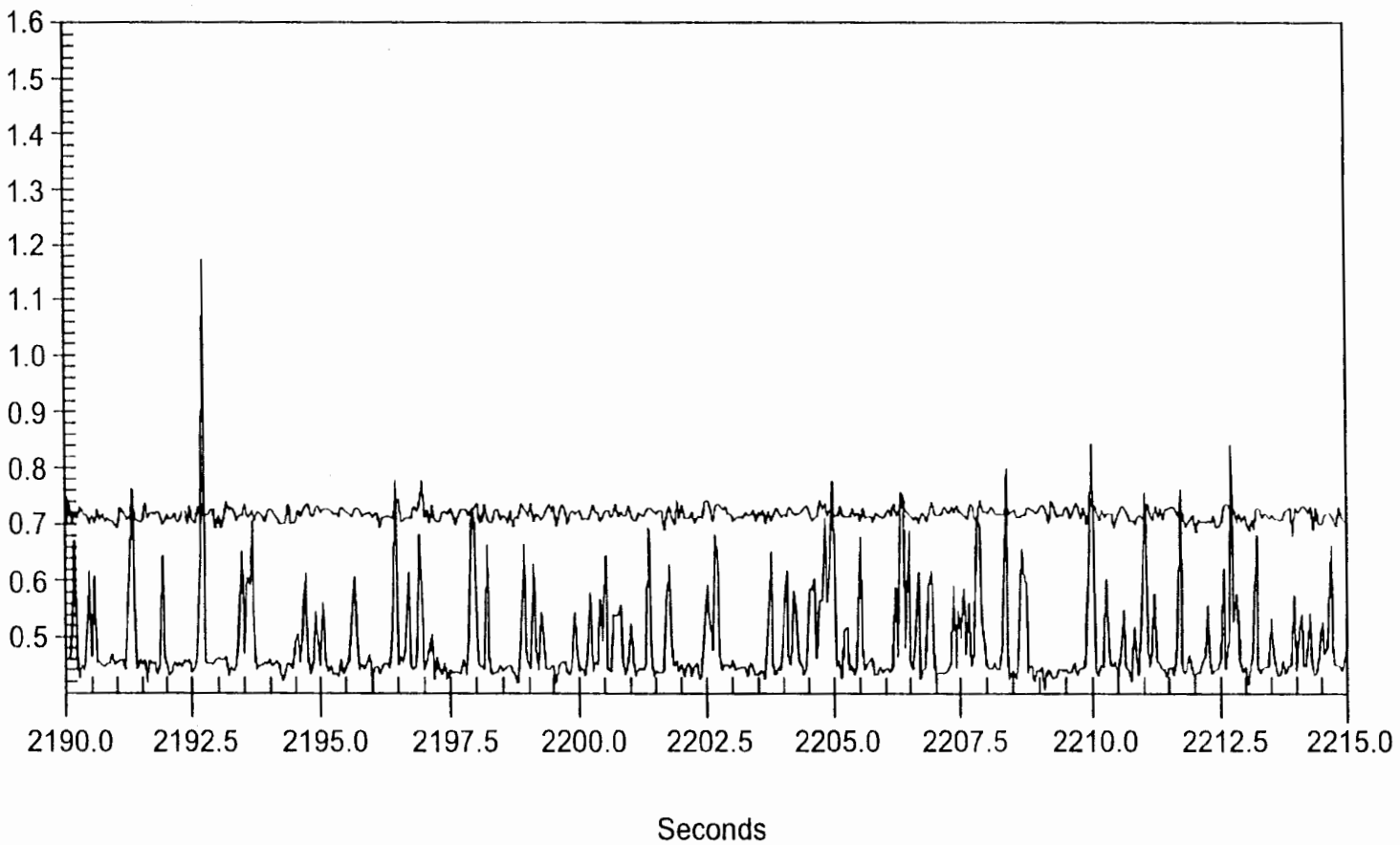


Fig. 4

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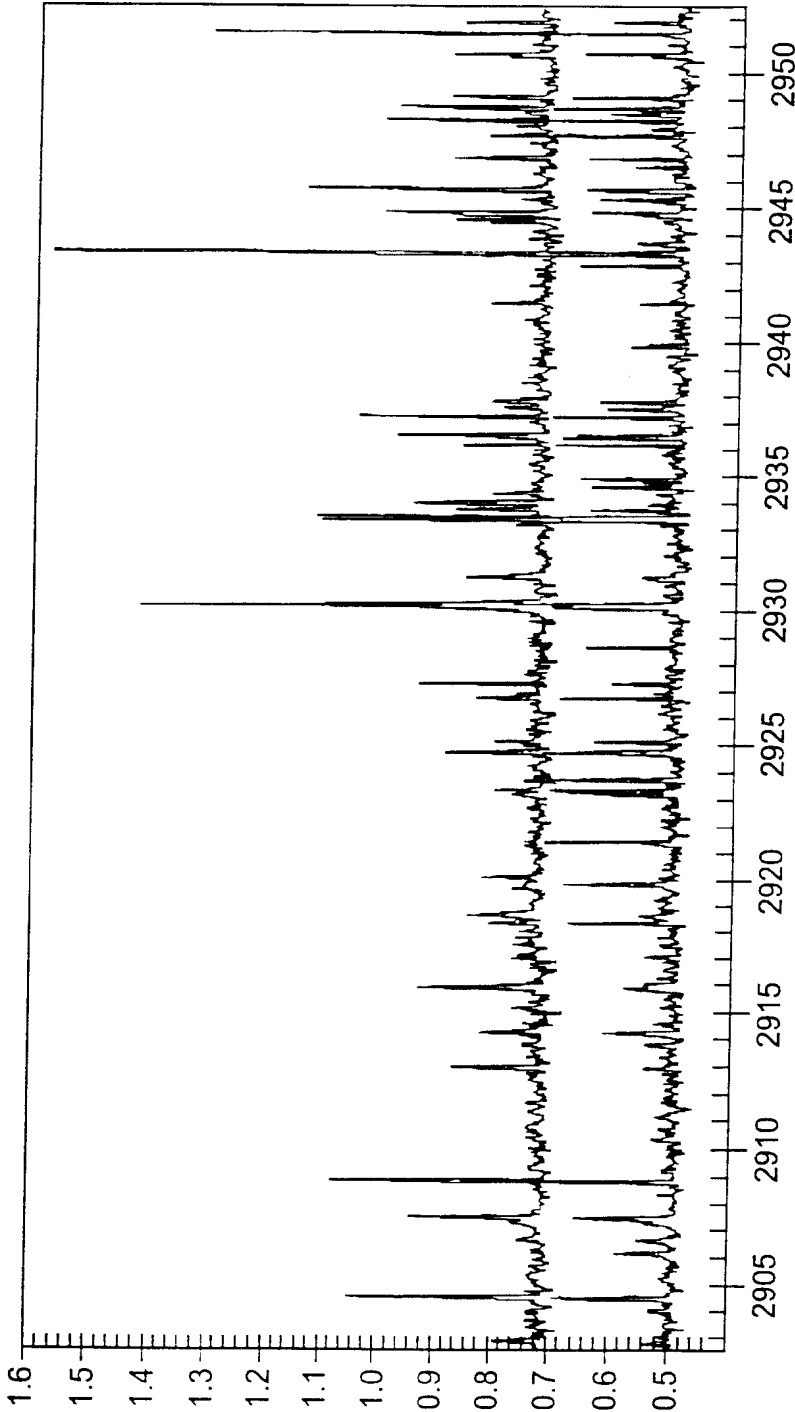


Fig. 5

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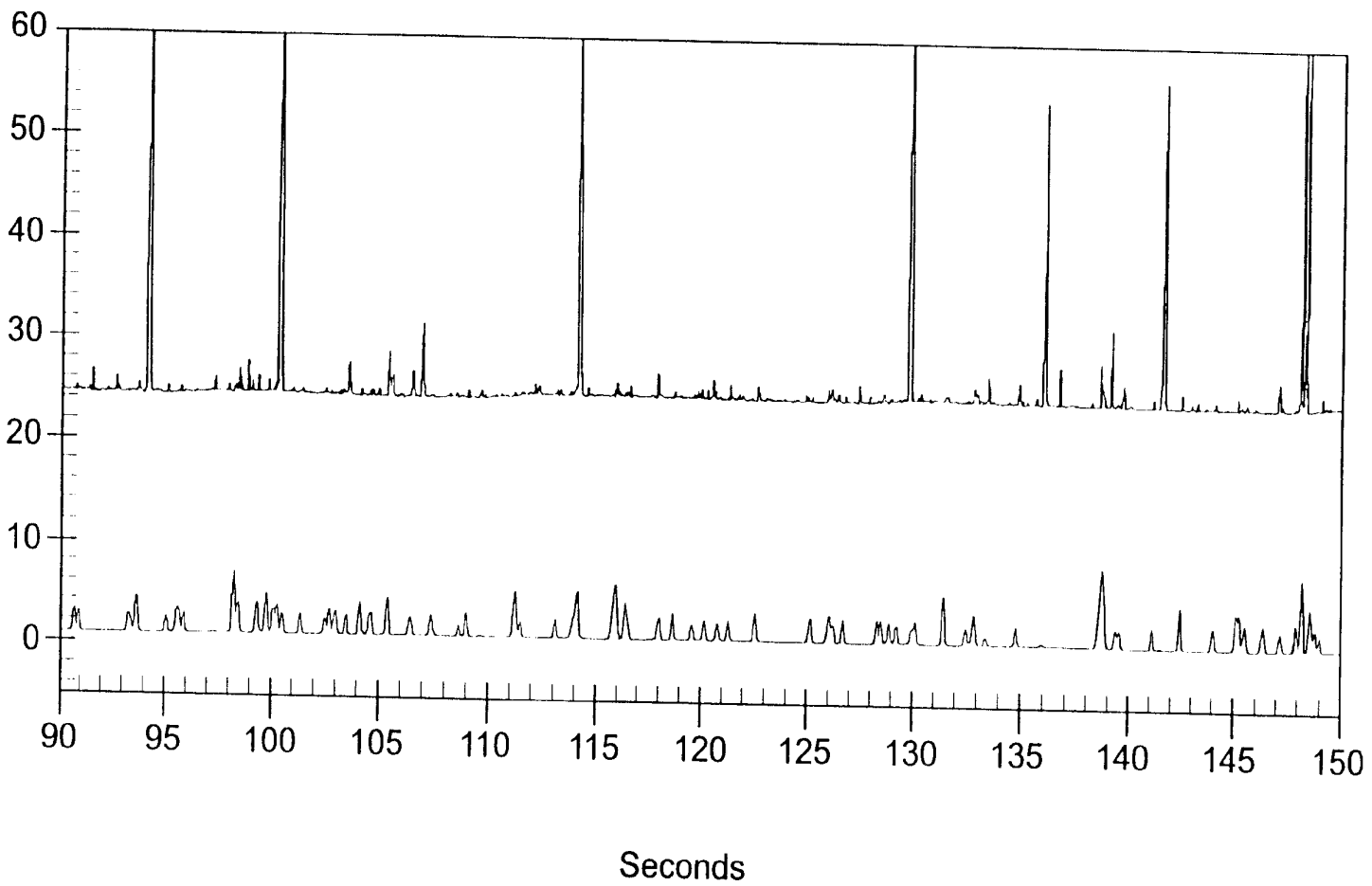


Fig. 6

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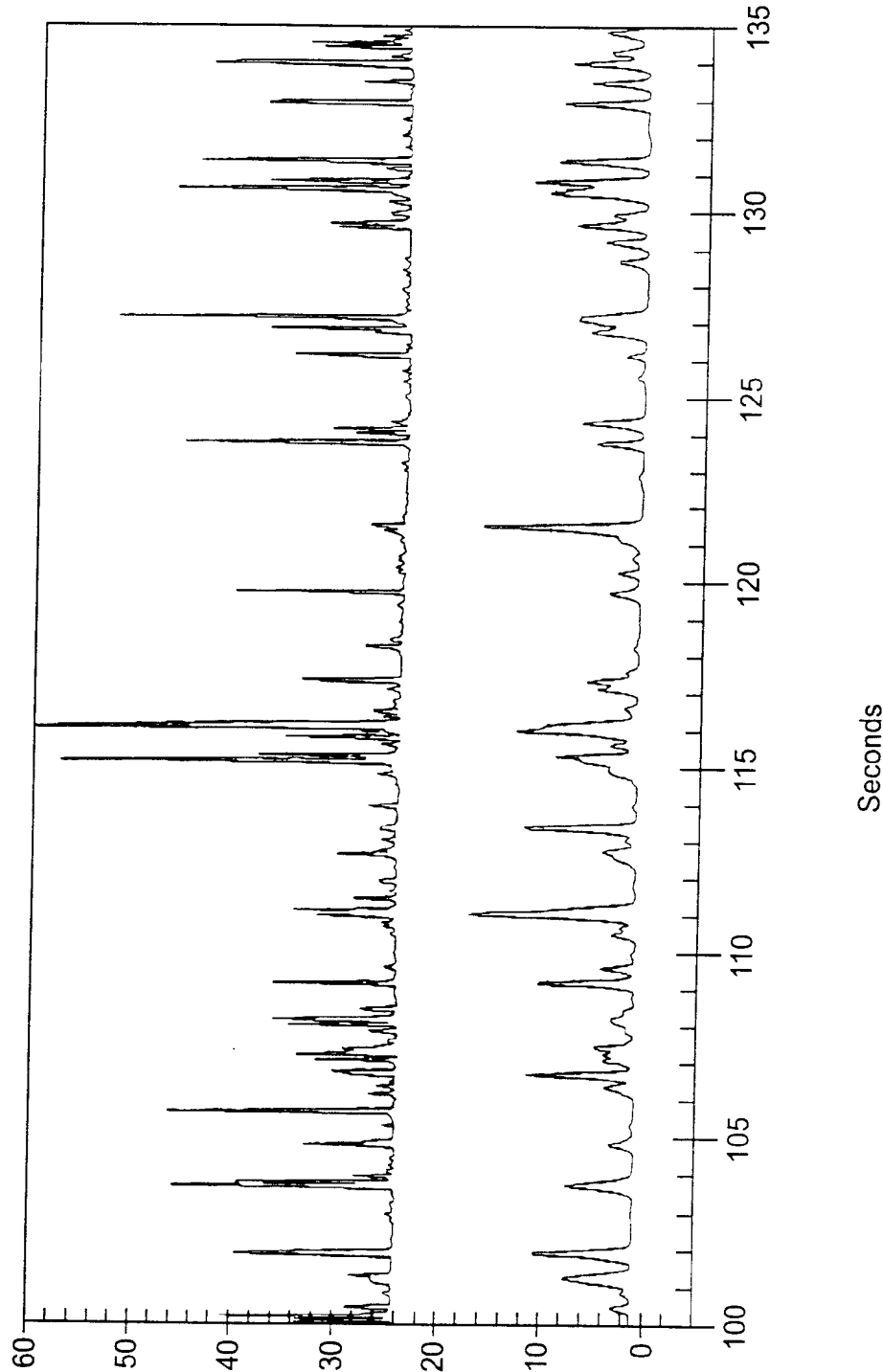


Fig. 7

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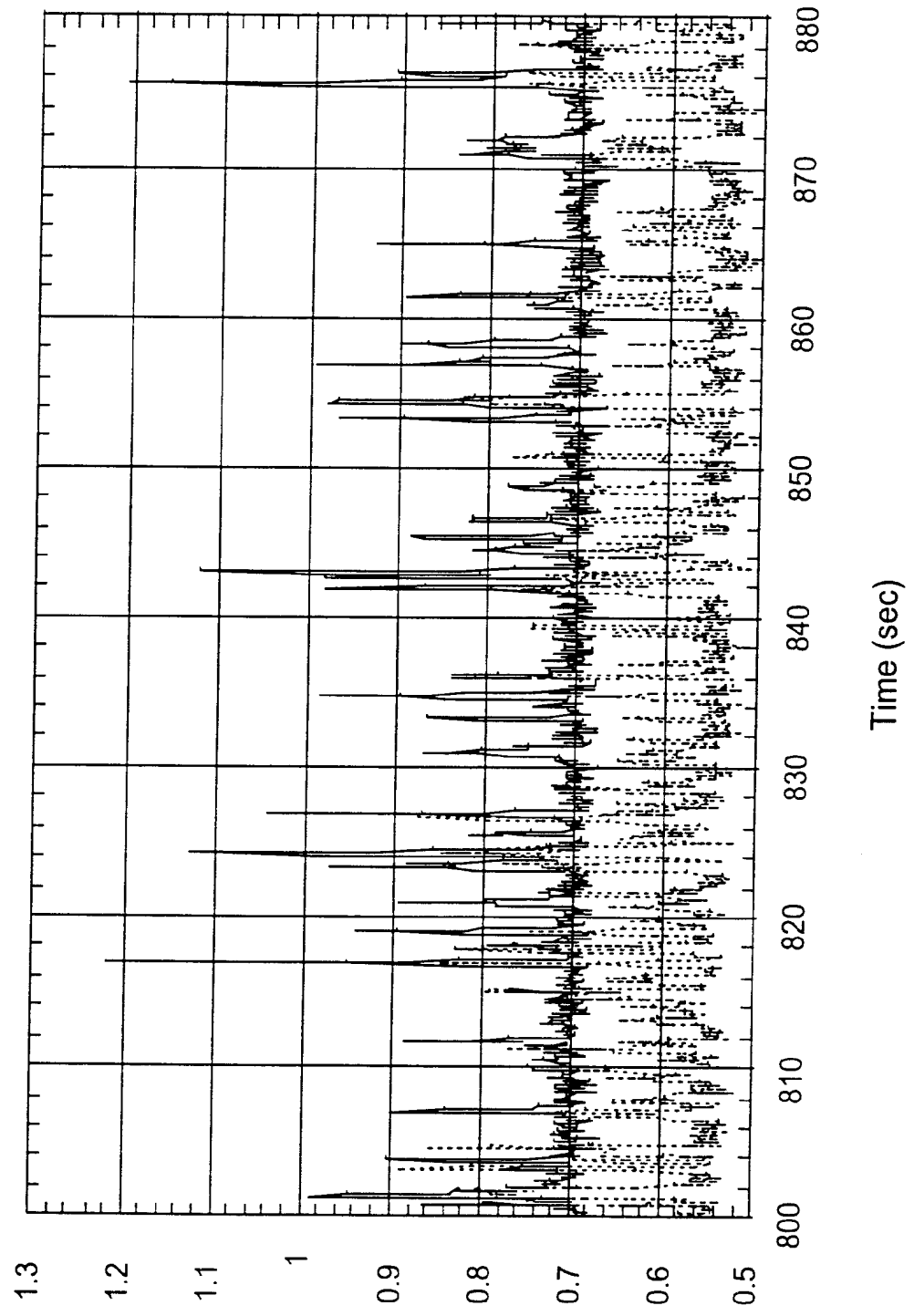


Fig. 8

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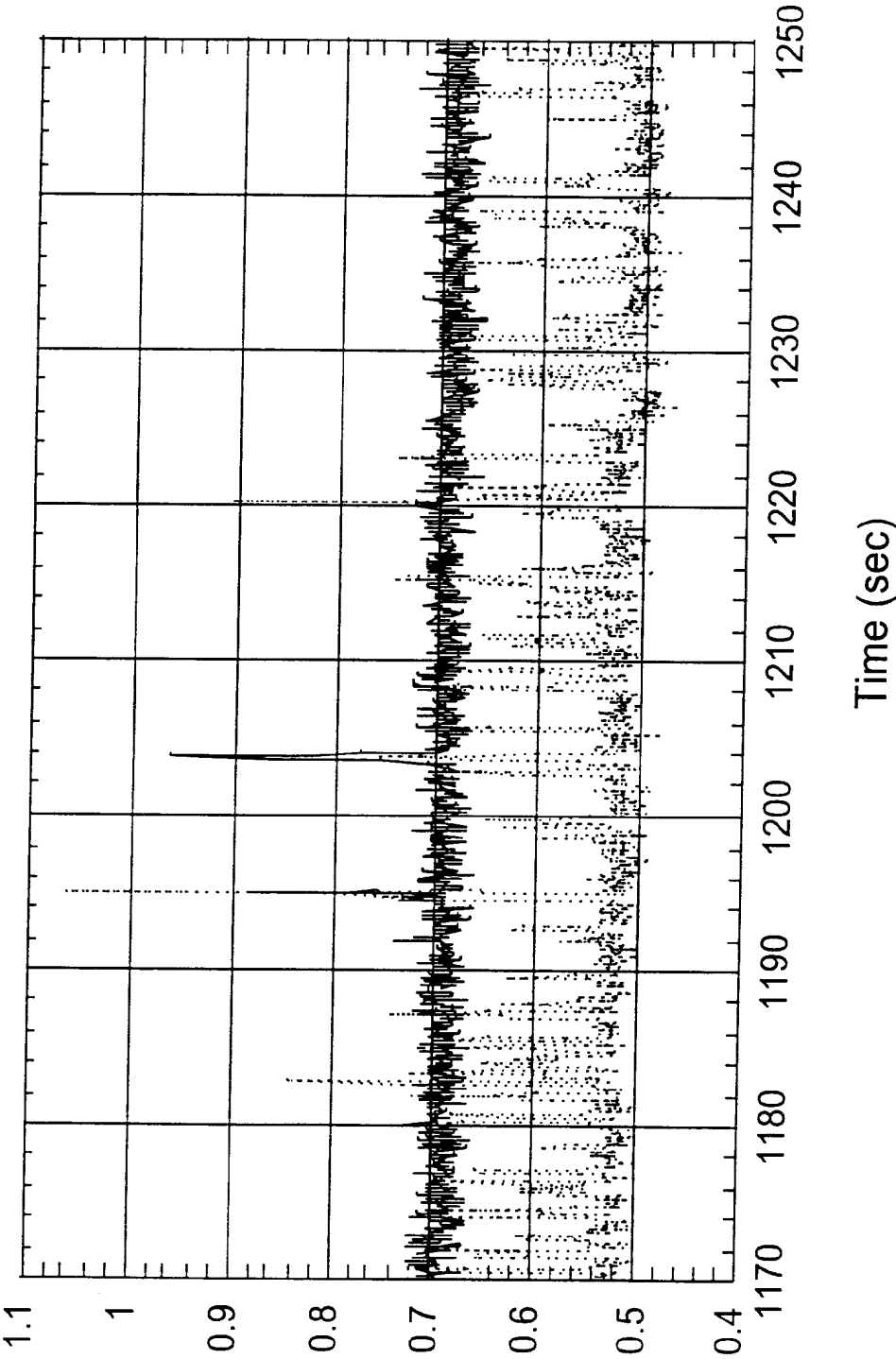


Fig. 9

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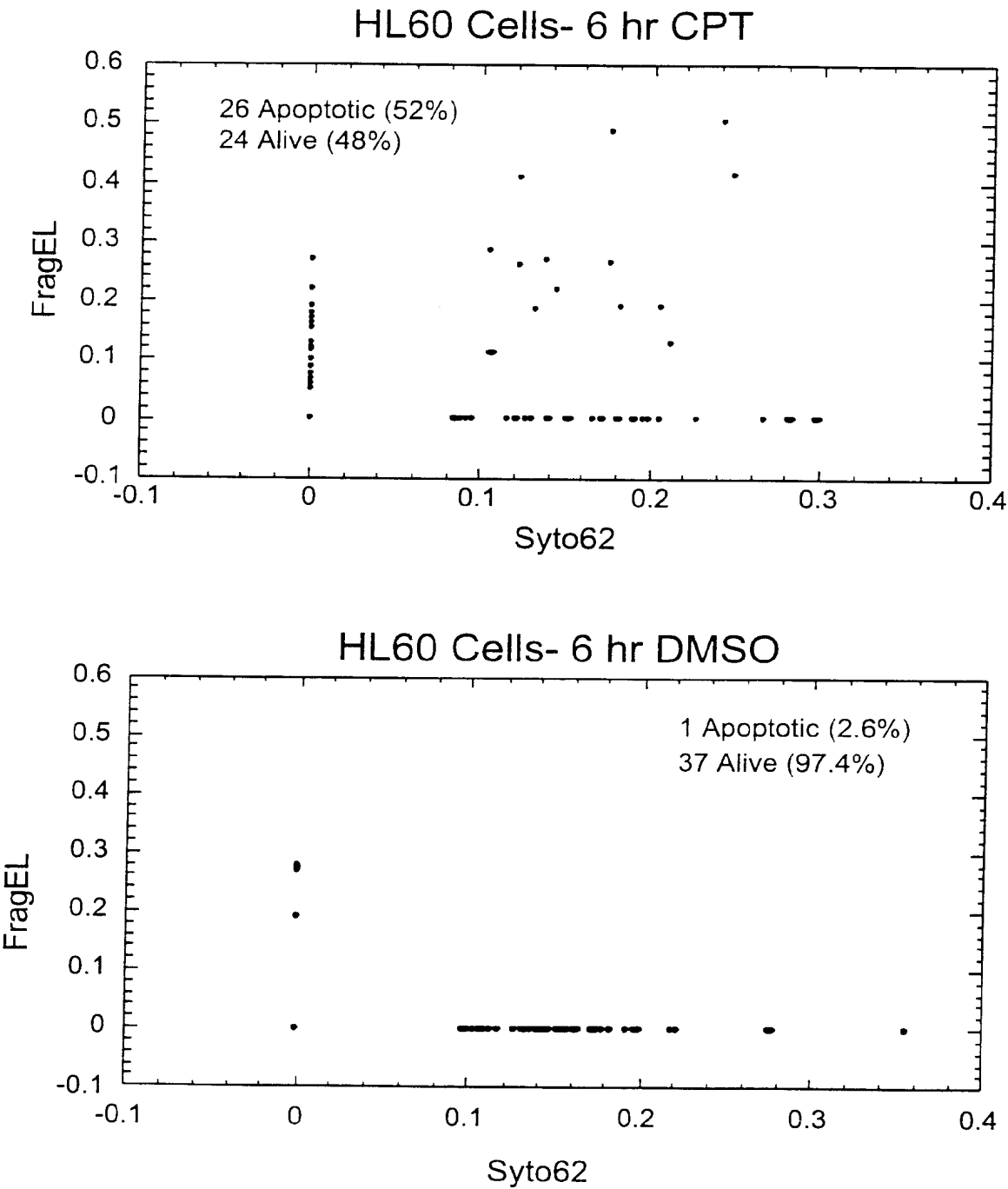


Fig. 10

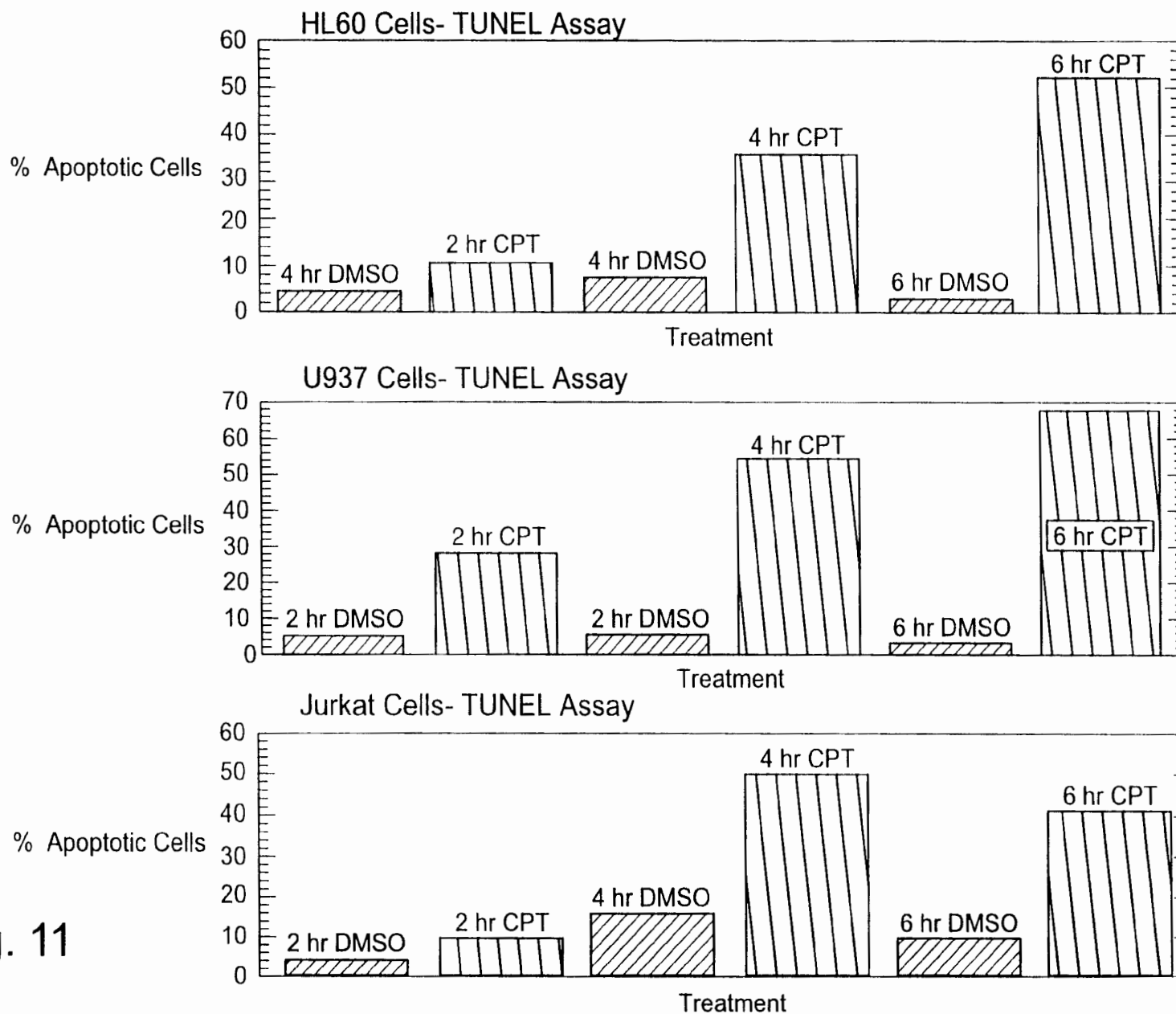
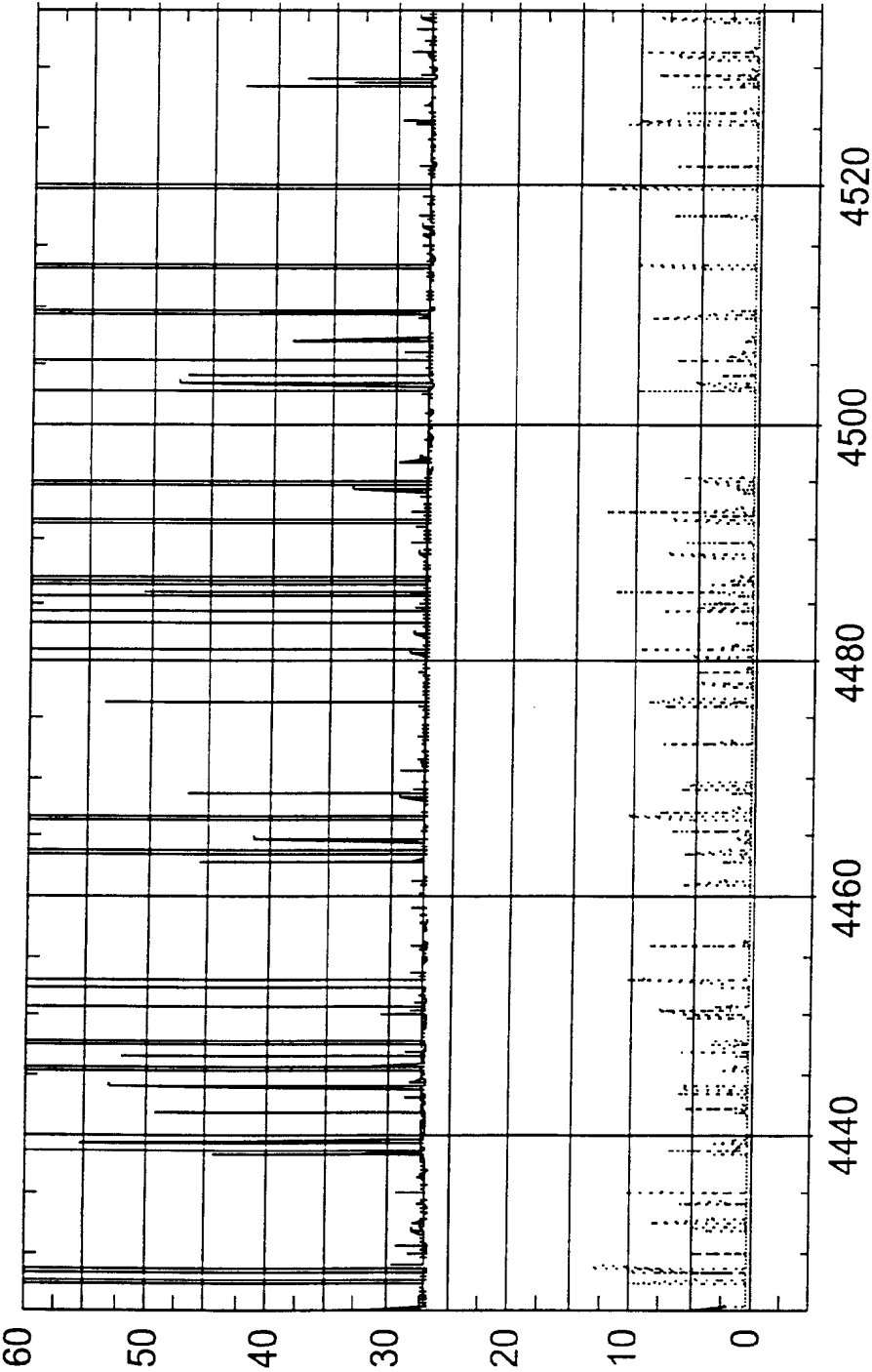


Fig. 11

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Time (sec)

Fig. 12

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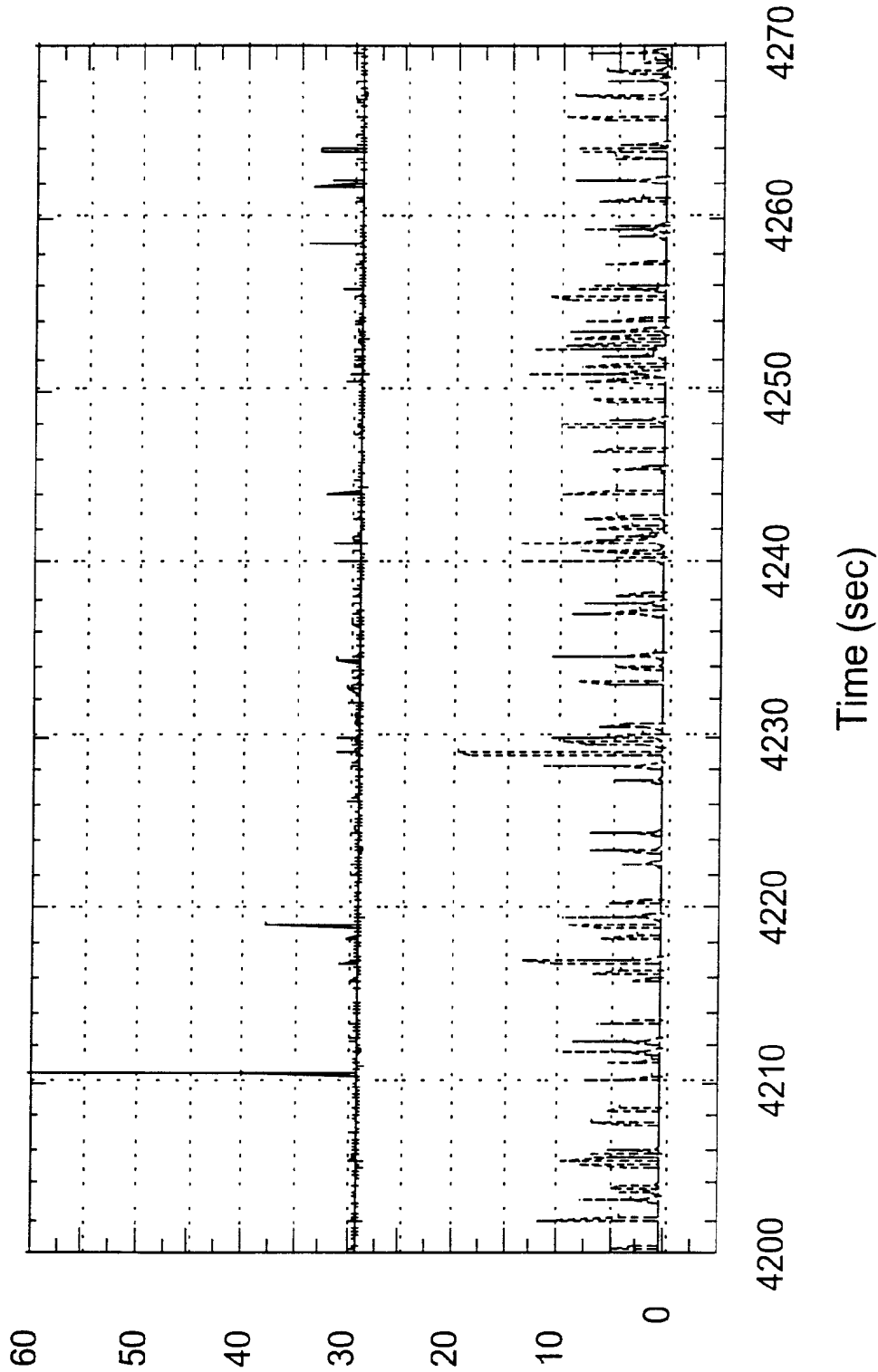
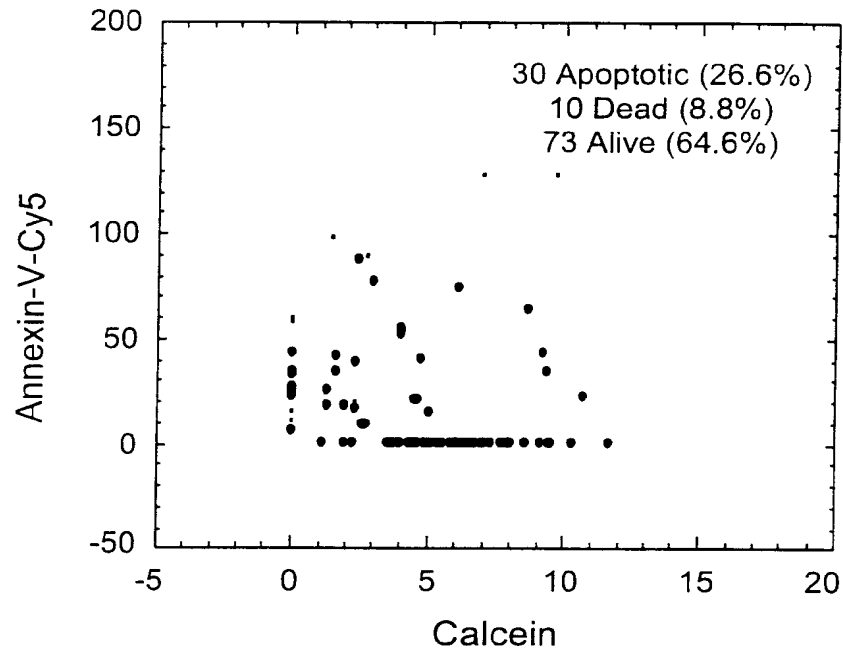


Fig. 13

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HL-60 Cells- 4 hr CPT



HL-60 Cells- 4 hr DMSO

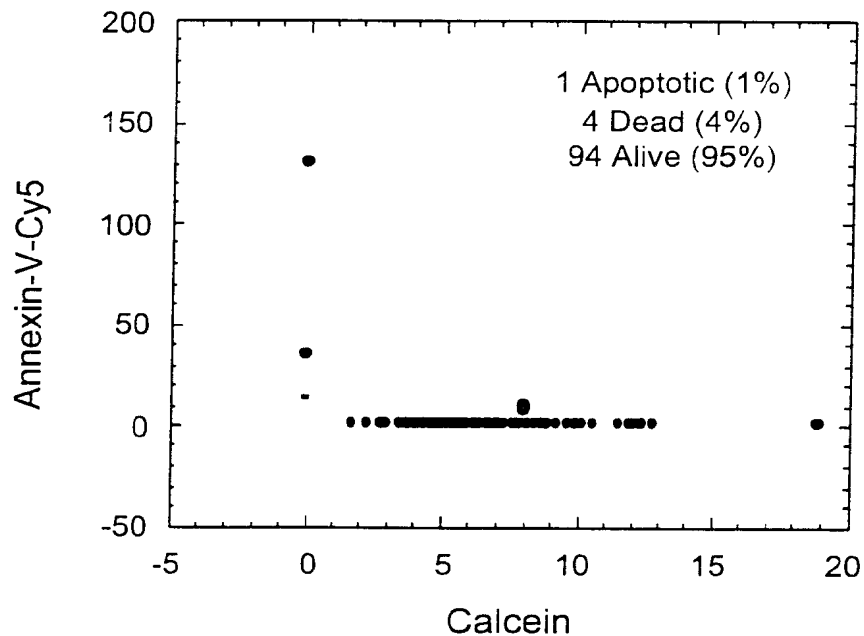


Fig. 14

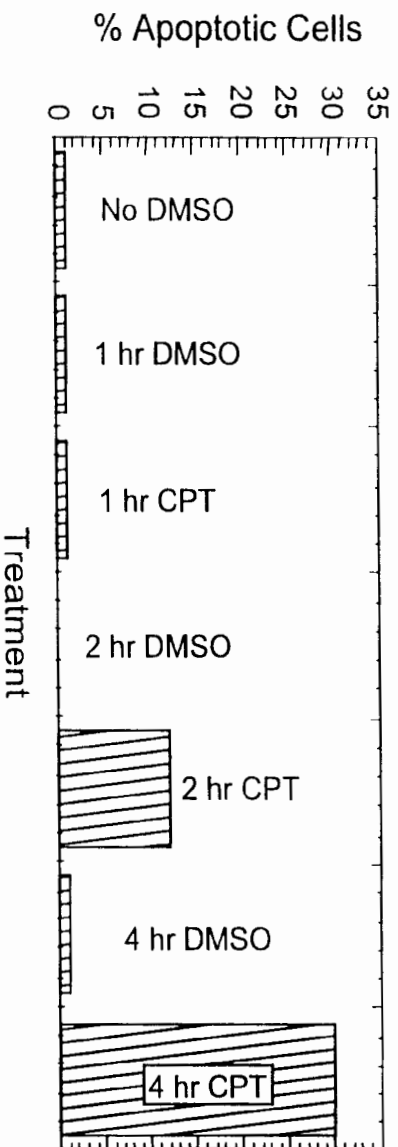
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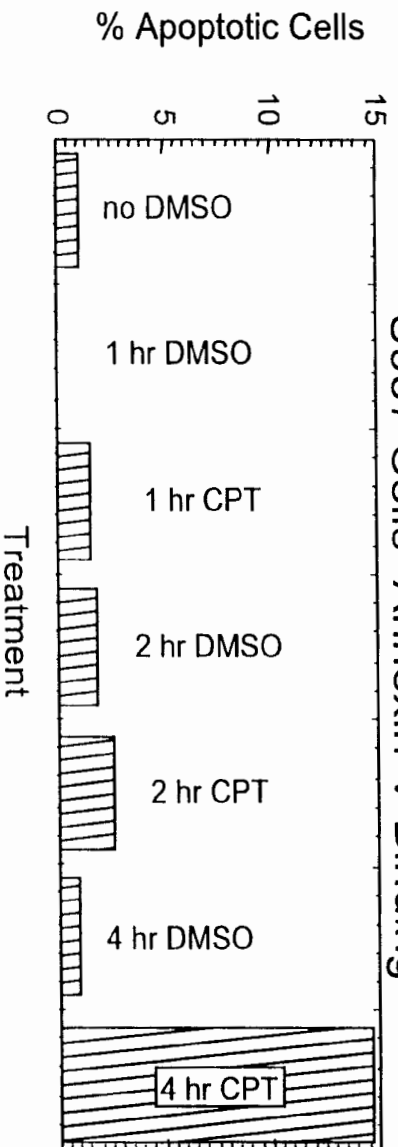
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HL-60 Cells- Annexin-V Binding



U937 Cells- Annexin-V Binding



Jurkat Cells- Annexin-V Binding

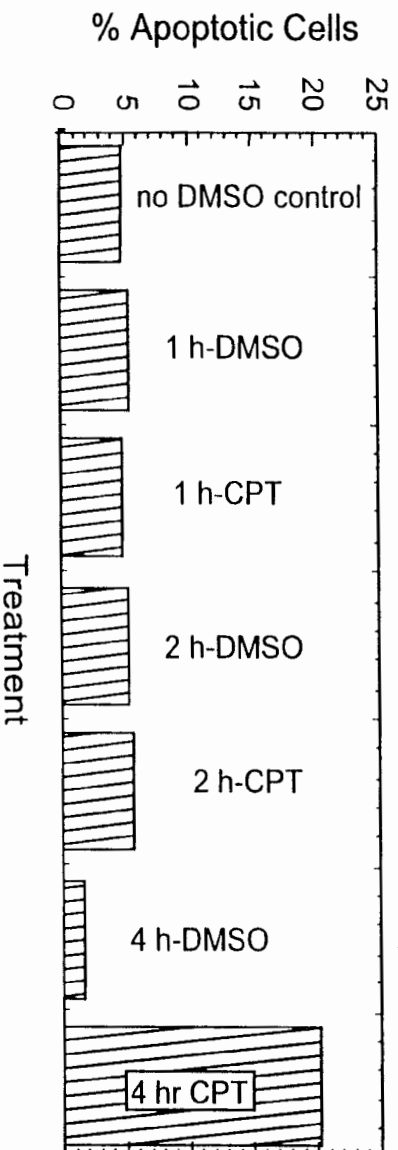


Fig. 15

SUBSTITUTE SHEET (RULE 26)

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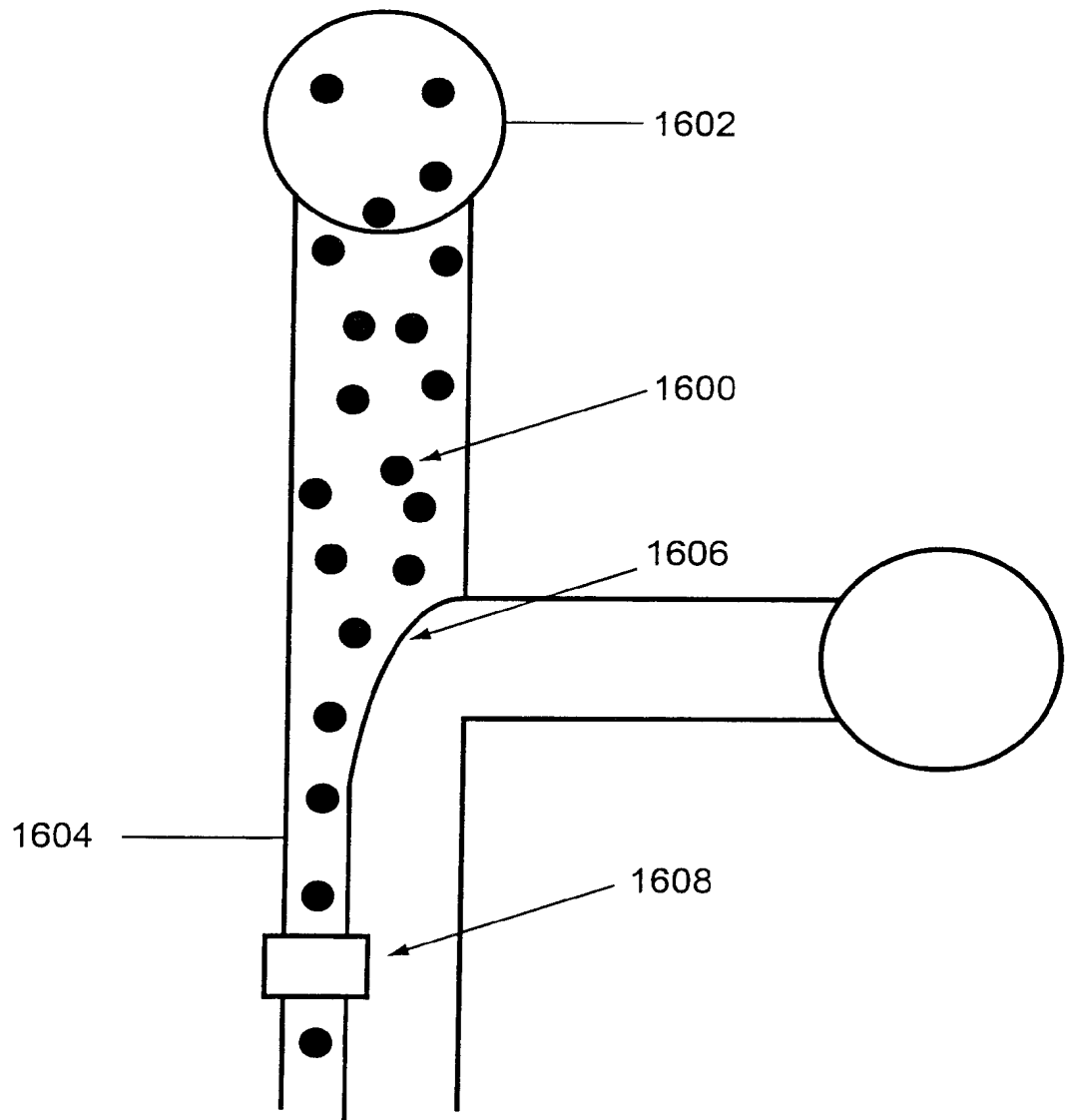


Fig. 16

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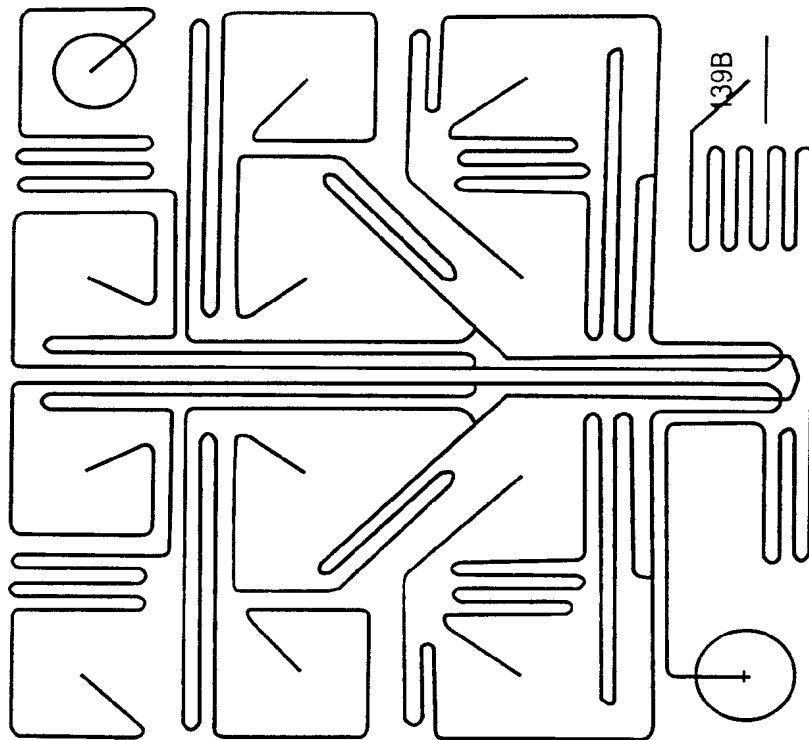


Fig. 17

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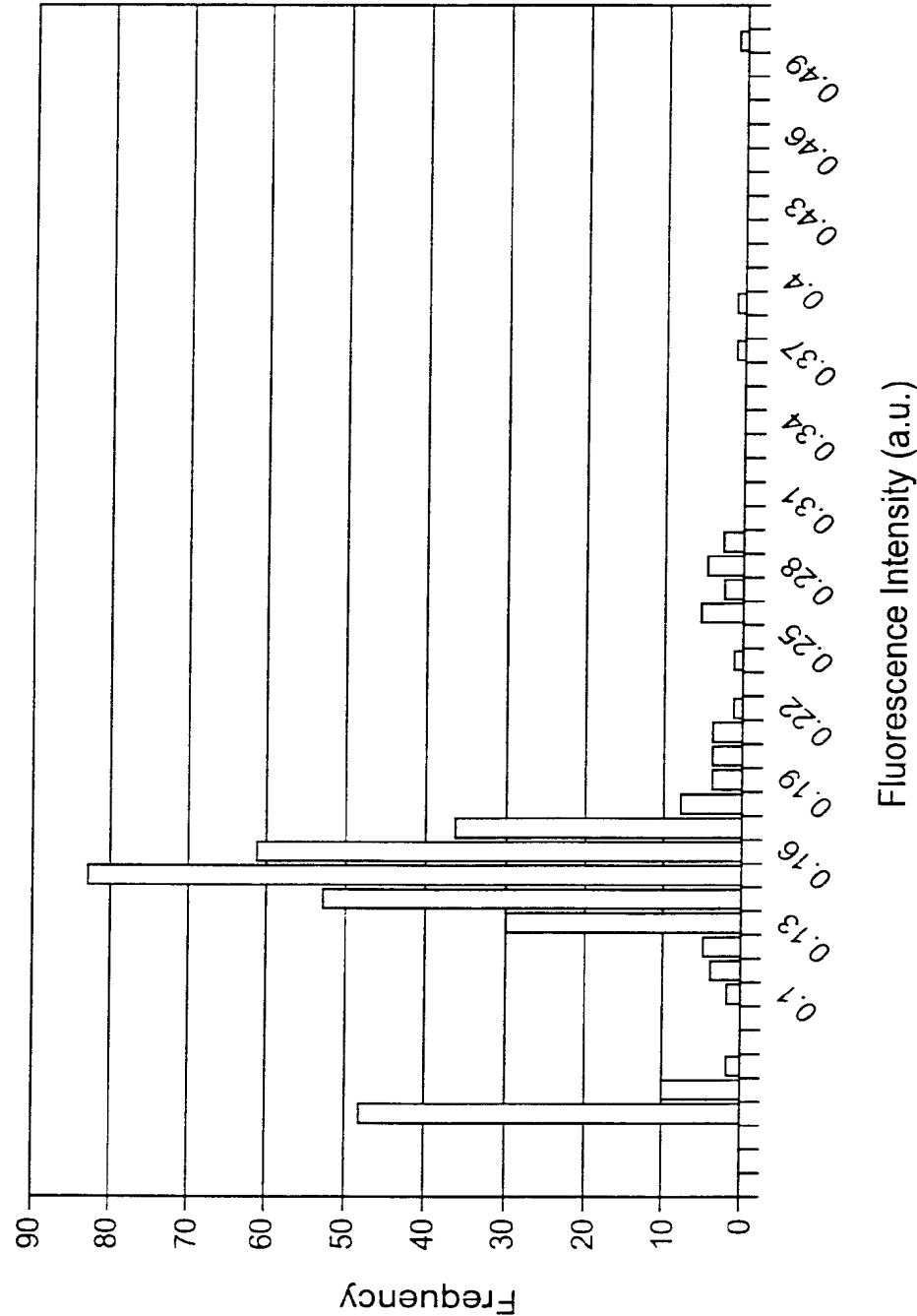


Fig. 18

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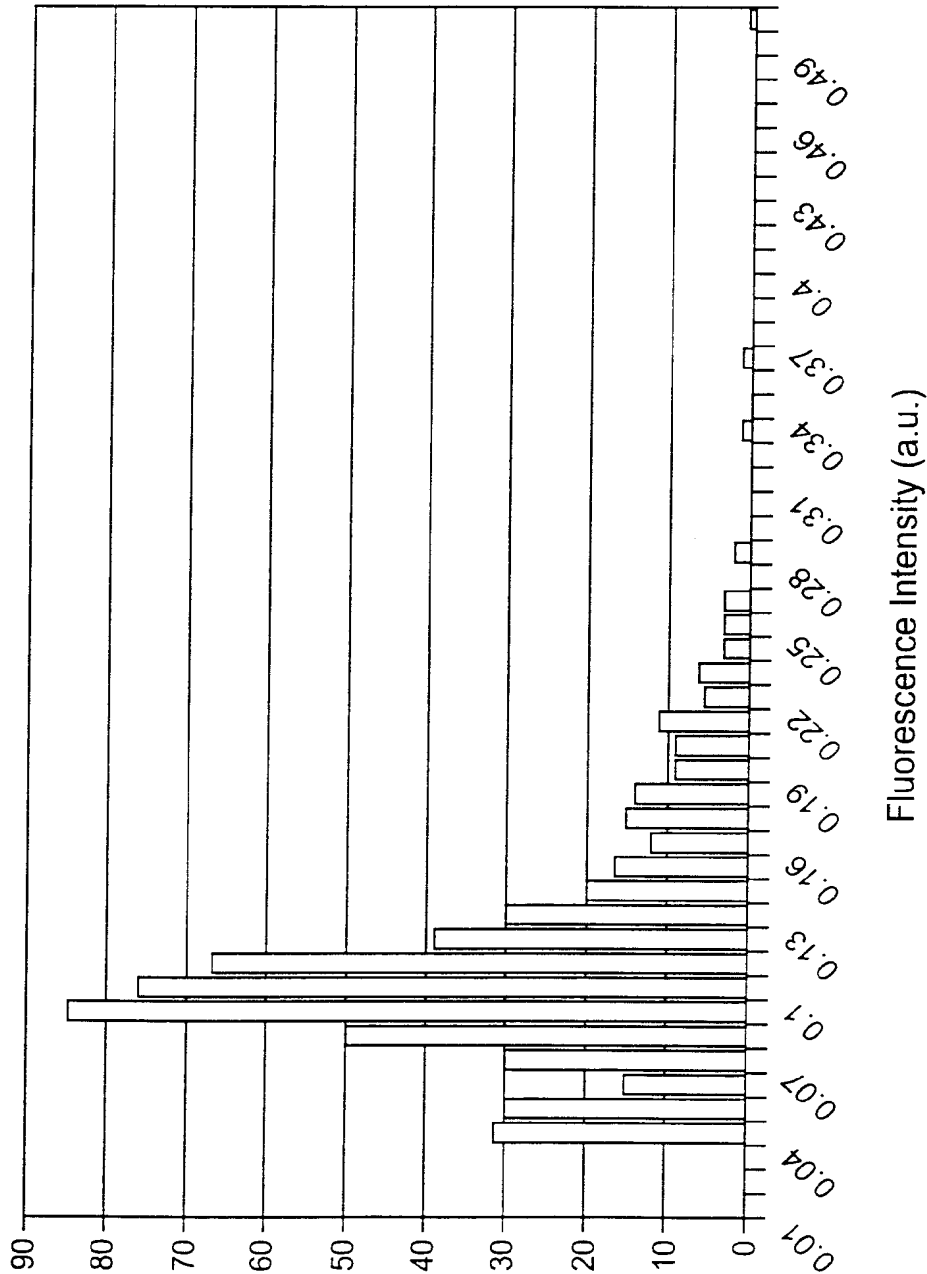


Fig. 19

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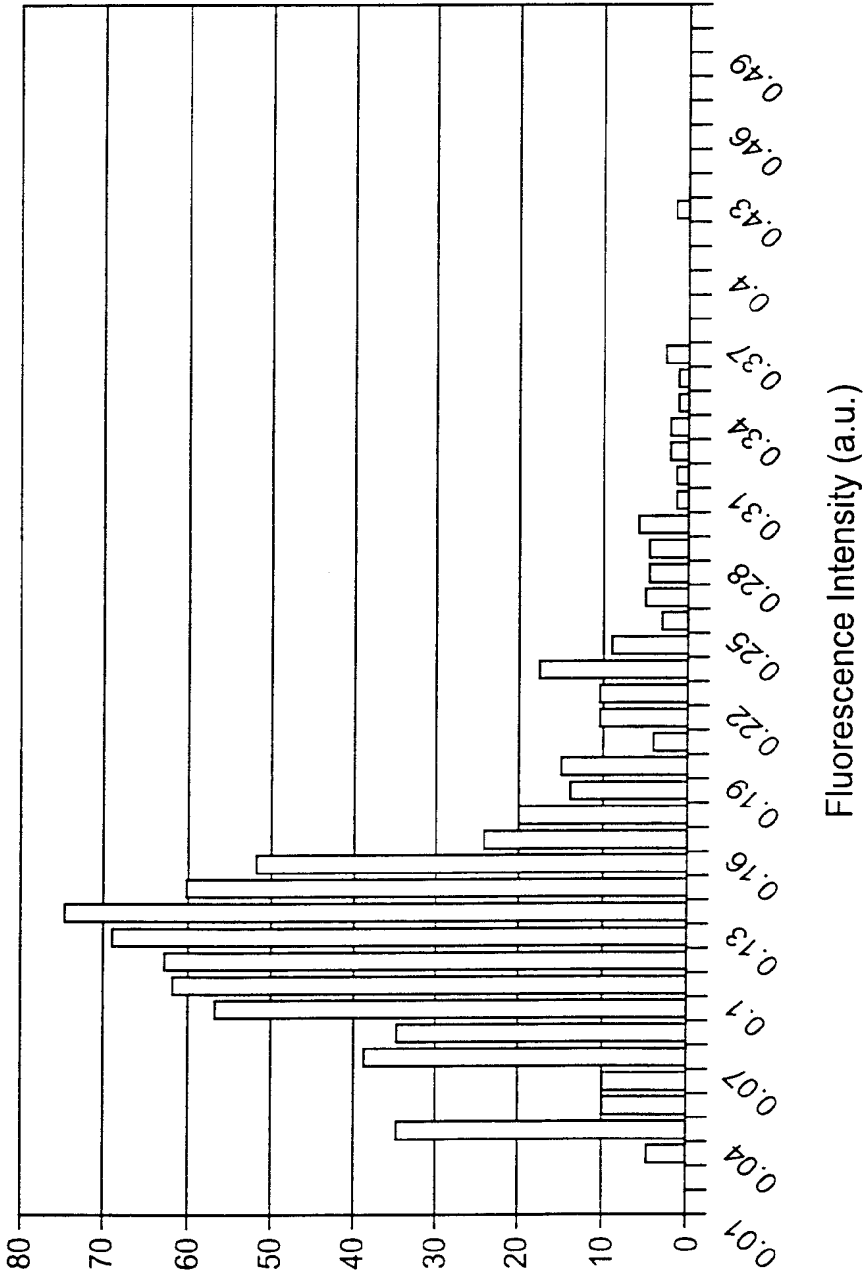


Fig. 20

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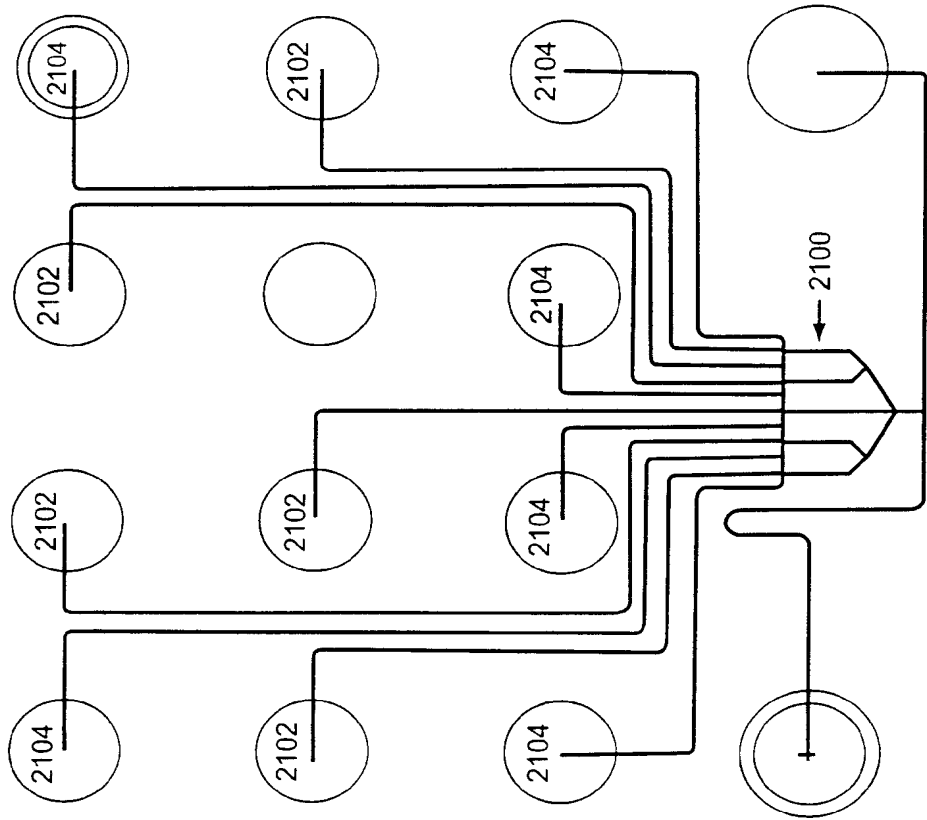


Fig. 21

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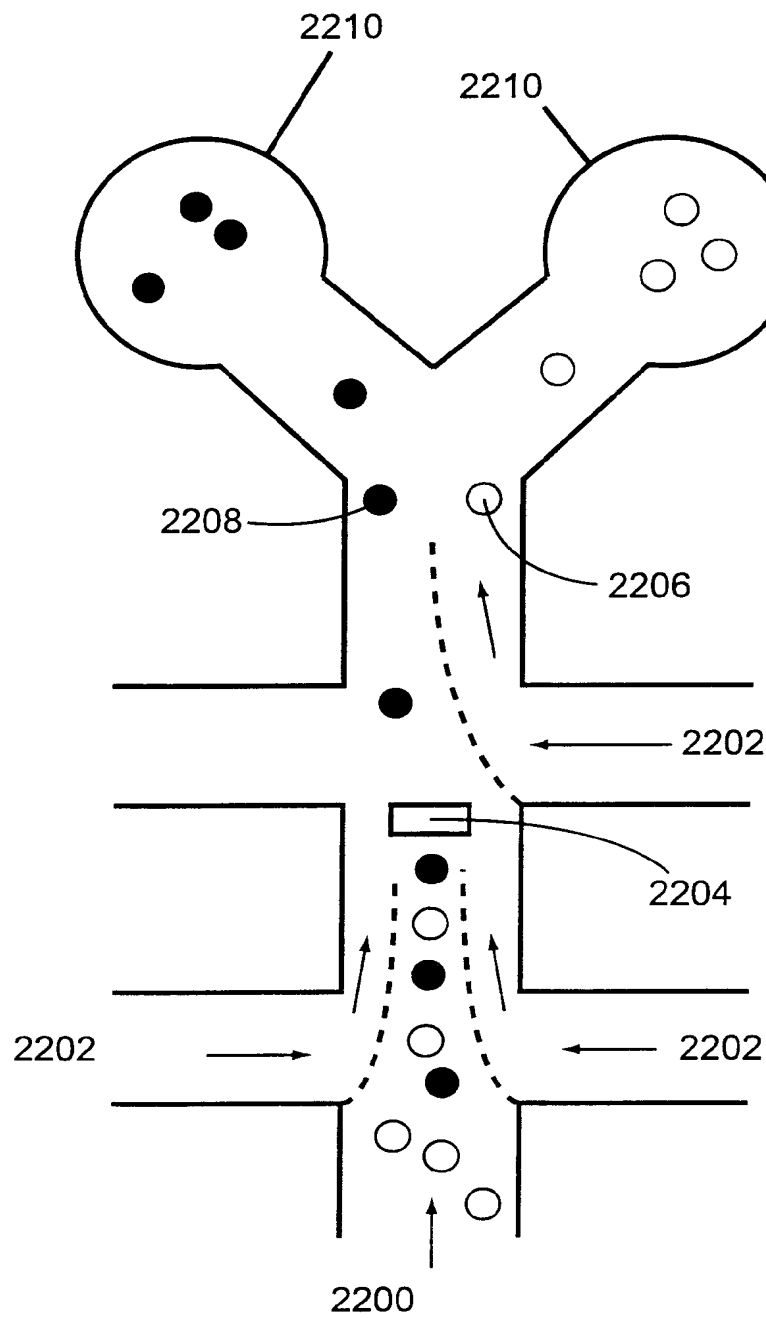


Fig. 22

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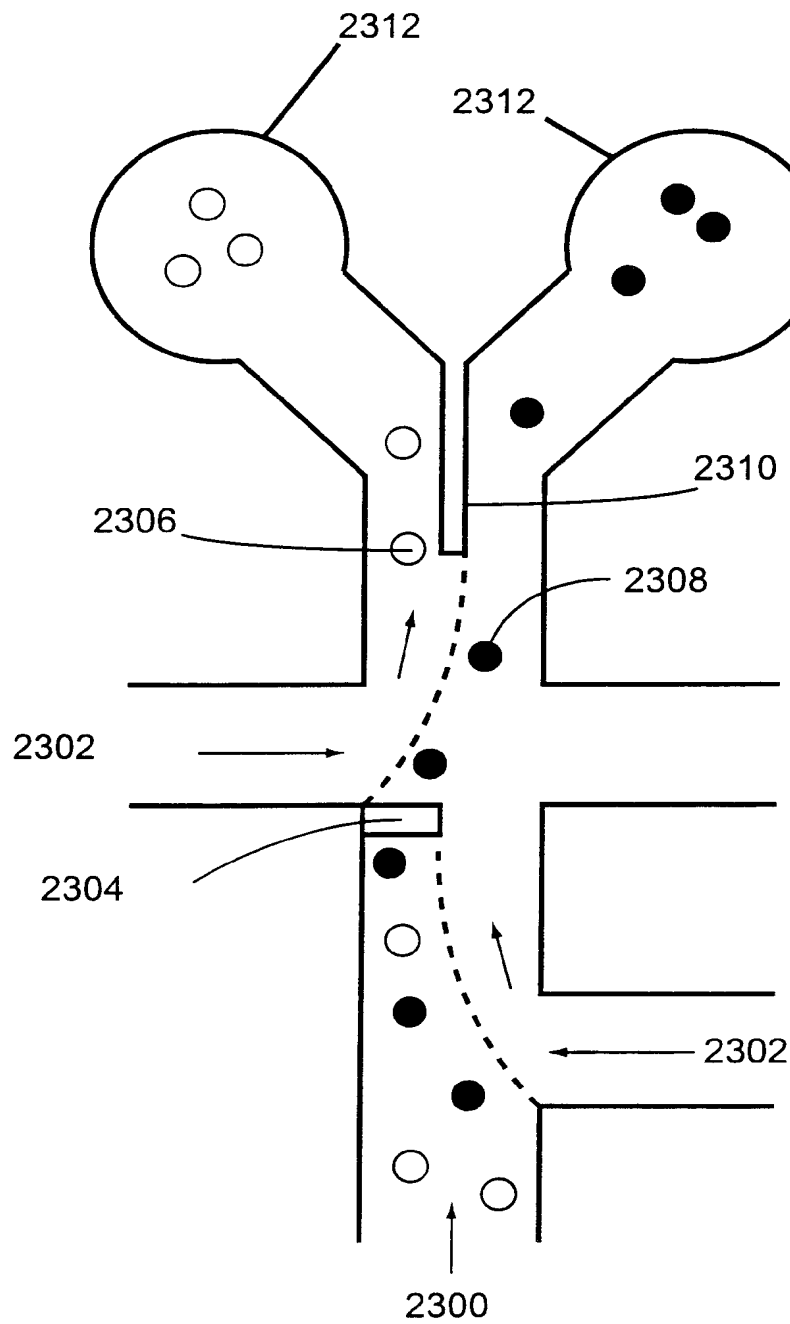


Fig. 23

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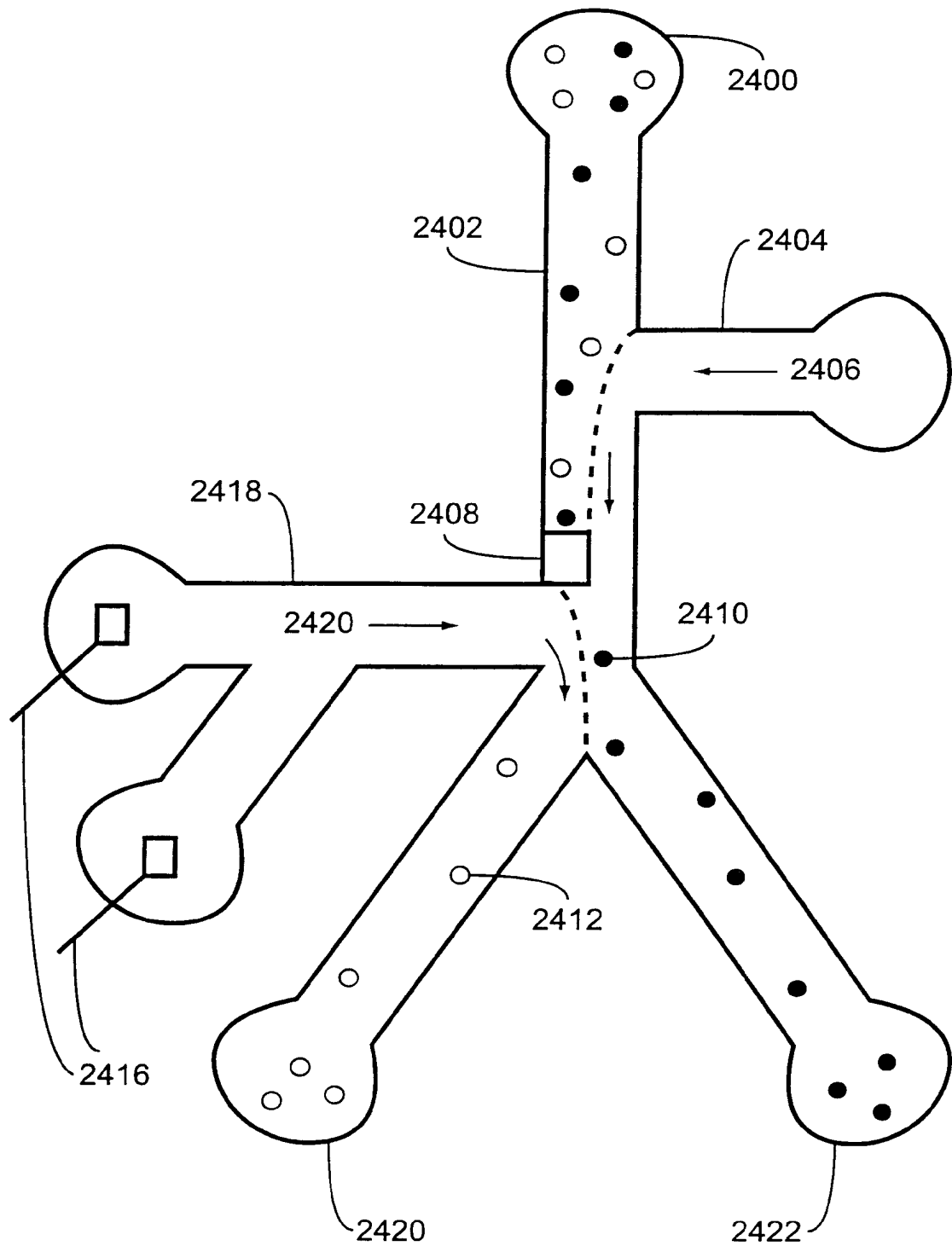


Fig. 24

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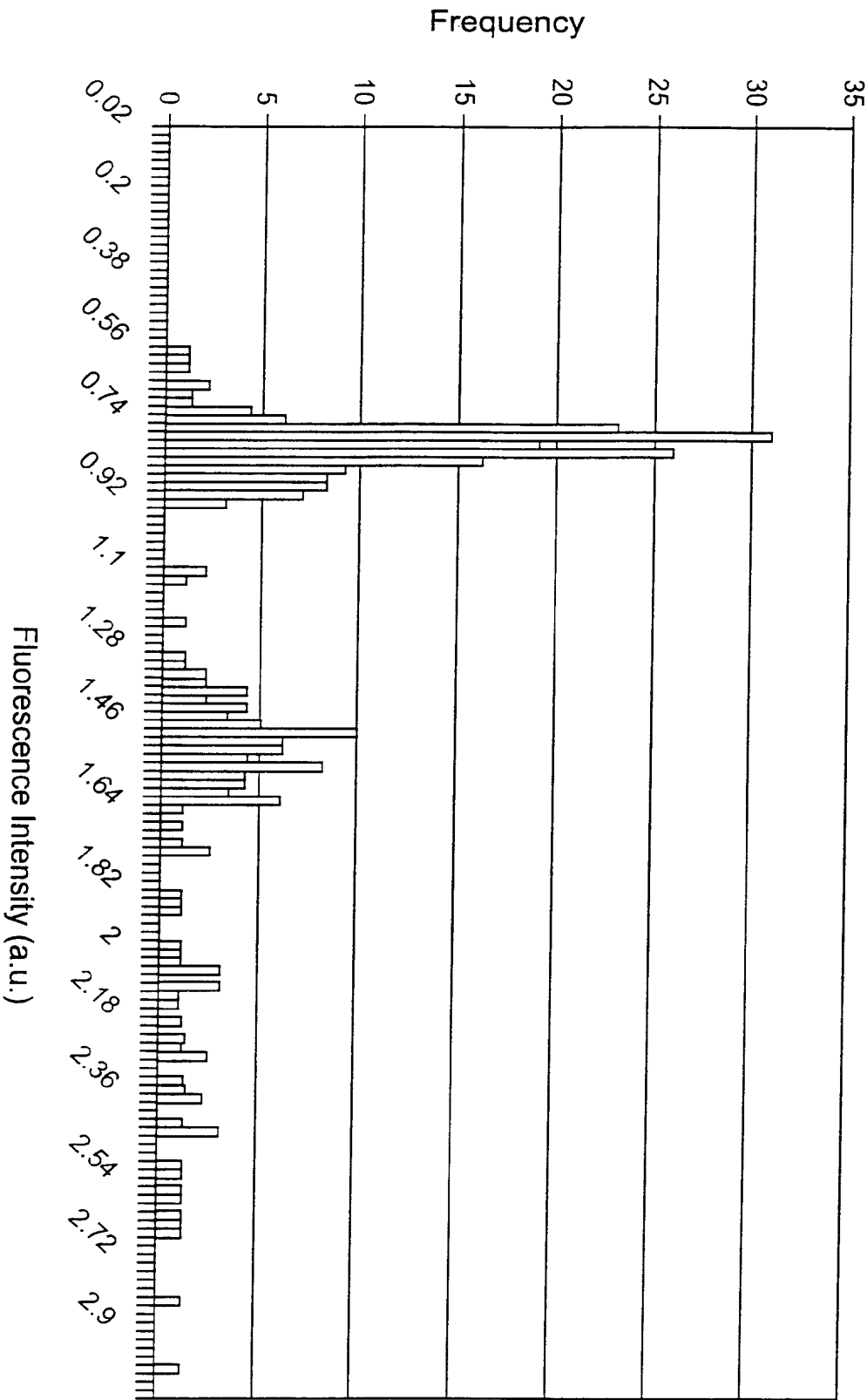


Fig. 25A

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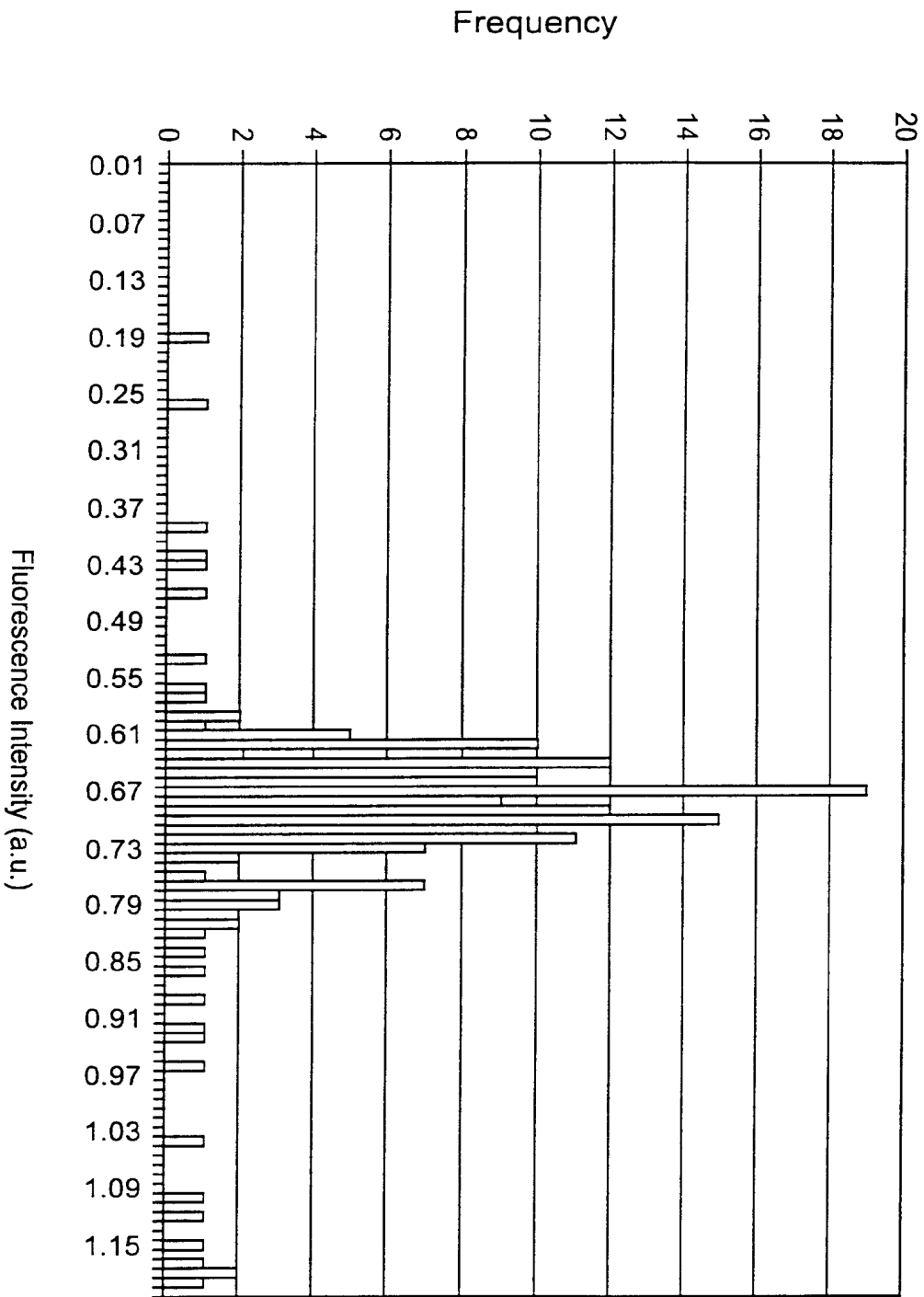


Fig. 25B

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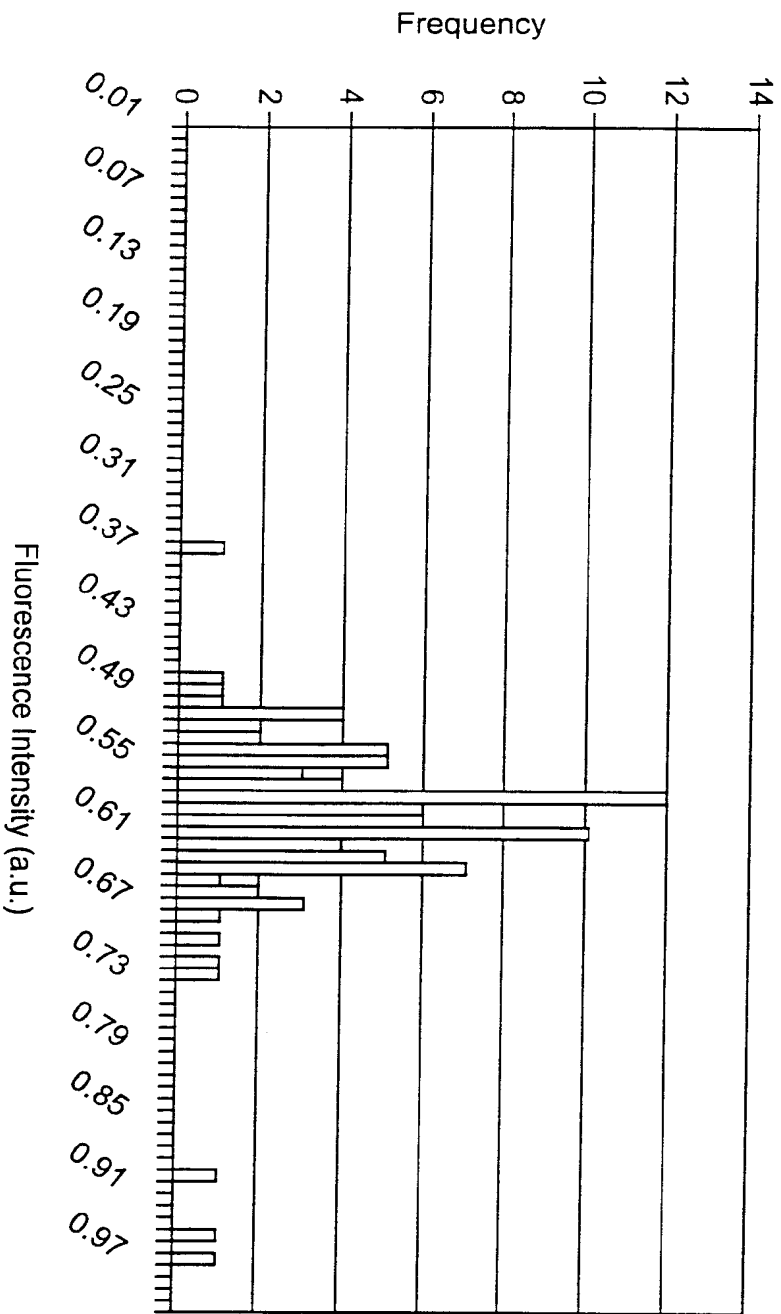


Fig. 25C

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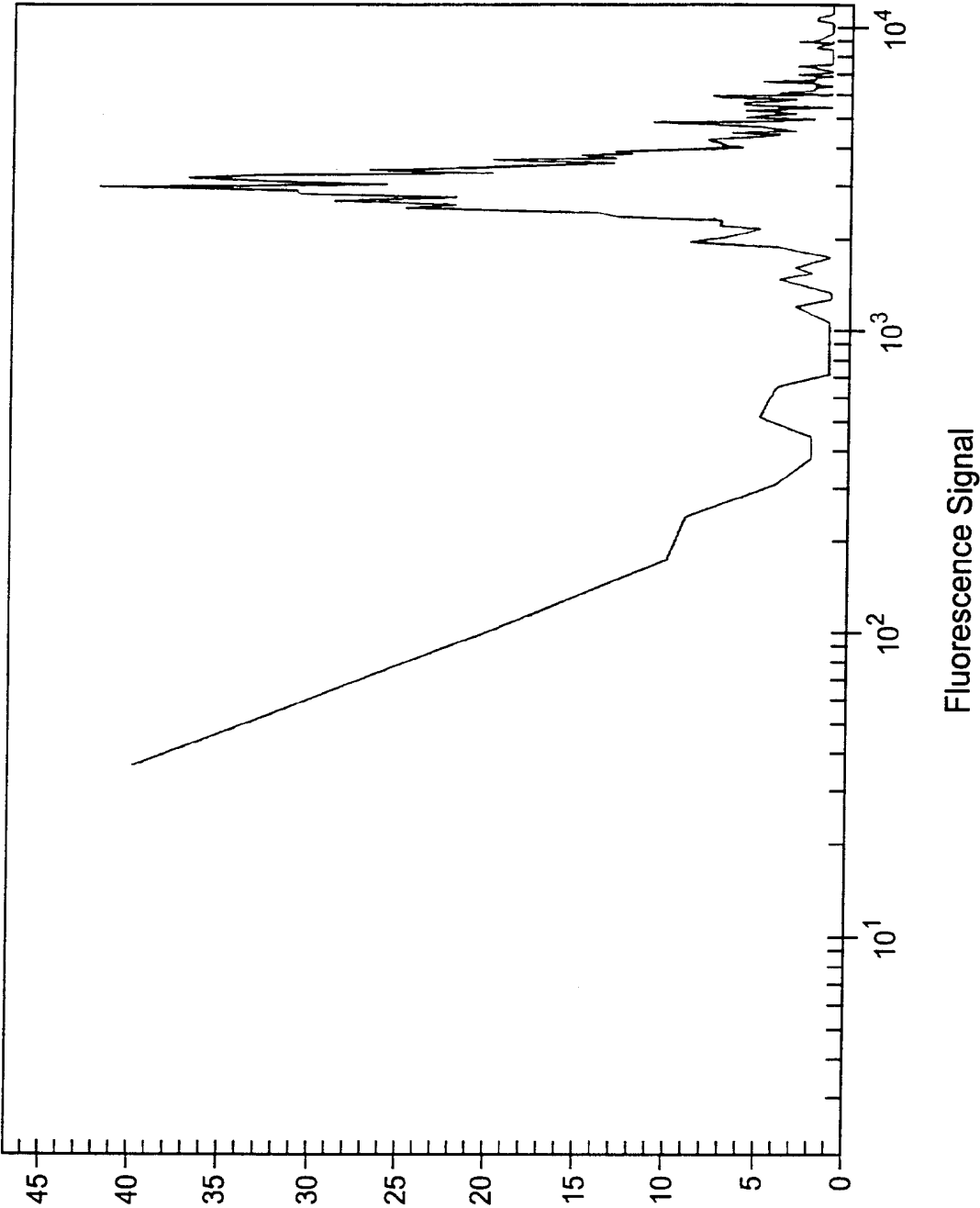


Fig. 26

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/13294

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/00

US CL : 435/4

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 7.2, 7.21, 287.1, 287.3; 204/451, 452, 600, 601, 602, 603, 604

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A, P | US 5,972,710 A (WEIGL et al.) 26 October 1999 (26.10.99), see entire document. | 1-53 |
| Y | WATSON. The early fluidic and optical physics of cytometry. Cytometry. 1999, Vol. 38, pages 2-14, entire document. | 1-53 |
| A | KONONENKO et al. Non-equilibrium integral Doppler anemometric analysis of particle mixtures in a channel flow using an intrinsic hydrodynamic focusing force biased by another force. Journal of Chromatography, 1991, Vol. 553, pages 517-530. | 1-53 |
| A | KESSLER, B.O. Hydrodynamic focusing of motile algal cells. Nature. 17 January 1985, Vol. 313, pages 218-220. | 1-53 |
| Y | US 5,858,187 A (RAMSEY et al.) 12 January 1999, see entire document. | 1-53 |
| A,P | US 6,067,157 A (ALTENDORF) 23 May 2000, see entire document. | 1-53 |
| A,P | US 6,049,380 A (GOODWIN ET AL.) 11 April 2000 see entire document. | 1-53 |
| A | US 5,608,519 A (GOURLEY ET AL.) 04 March 1997 see entire document. | 1-53 |
| A | US 4,793,705 A (SHERA) 27 December 1988, see entire document. | 1-53 |
| Y | KNIGHT et al. Hydrodynamic focusing on a silicon chip: Mixing nanoliters in microseconds. Physical Review Letters. 27 April 1998, Vol.80, No. 17, pages 3863-3866, entire document.. | 1-53 |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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"E" earlier application or patent published on or after the international filing date

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

05 July 2000 (05.07.2000)

Date of mailing of the international search report

15 AUG 2000

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

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Abstract EP0177718

It describes a sorting method and apparatus for automatic sorting of microscopic particles.

In the process, the particles are suspended in a fluid through a closed channel out and pass a measuring point, trigger in the sorted out particles of a signal, and a subsequent branch wave which redirects the stream containing the particles (particle flow) at the junction for the duration of the particle passage.

To implement this procedure appropriate device comprises a supply channel for the stream of particles, one measurement channel, one in the measuring channel arranged measuring pressure wave generator in one of the two is located on the branch leading away channels .

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Europäisches Patentamt
European Patent Office
Office européen des brevets

11 Veröffentlichungsnummer:

0 177 718
A2

12

EUROPÄISCHE PATENTANMELDUNG

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30 Priorität: 11.09.84 CH 4326/84

43 Veröffentlichungstag der Anmeldung:
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64 Benannte Vertragsstaaten:
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D-2000 Hamburg 36(DE)

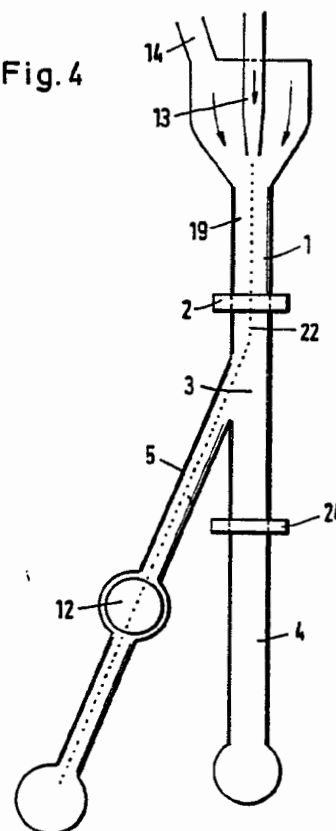
54 Verfahren und Vorrichtung zur Sortierung von mikroskopischen Partikeln.

57 Es wird ein Sortierverfahren sowie eine Vorrichtung für die automatische Sortierung von mikroskopischen Partikeln beschrieben.

Bei dem Verfahren werden die Partikel in einer Flüssigkeit suspendiert durch einen geschlossenen Kanal geführt und passieren dabei eine Messstelle, in der die auszusortierenden Partikel ein Signal auslösen, und eine anschließende Verzweigung wobei durch das Signal in einem der beiden Kanäle hinter der Verzweigung eine Druckwelle erzeugt wird, welche den die Partikel enthaltenden Strom (Partikelstrom) an der Verzweigung für die Dauer des Partikeldurchgangs umlenkt.

Die zur Durchführung dieses Verfahren geeignete Vorrichtung weist einen Zuführkanal für den Partikelstrom, einen Messkanal, eine im Messkanal angeordnete Messstelle, eine Verzweigung des Messkanals in einen Sortierkanal und einen Abfallkanal und einen Druckwellengenerator auf, der in einem der beiden von der Verzweigung wegführenden Kanäle angeordnet ist.

Fig. 4



EP 0 177 718 A2

Verfahren und Vorrichtung zur Sortierung von mikroskopischen Partikeln

Die vorliegende Erfindung betrifft ein Verfahren zum automatischen Sortieren von mikroskopischen Partikeln, bei dem die Partikel in einer Flüssigkeit suspendiert durch einen geschlossenen Kanal geführt werden und dabei eine Messstelle, in der die auszusortierenden Partikel ein Signal auslösen, und eine anschließende Verzweigung passieren, und eine Vorrichtung zur Durchführung des Verfahrens mit einem Zuführkanal für den Partikelstrom, einem Messkanal, einer im Messkanal angeordneten Messstelle, einer Verzweigung des Messkanals in einen Sortierkanal und einen Abfallkanal und einem Druckwellengenerator.

Es gibt mikrophotometrische Verfahren zur Messung morphologischer und biochemischer Eigenschaften von makroskopischen Partikeln, Zellen, Blutzellen, Tumorzellen etc. Diese Verfahren basieren darauf, dass in einem photometrischen Apparat Licht, das von jedem einzelnen in Flüssigkeit suspendierten und durch eine Messstelle, die Durchflussmesskammer, hindurchgesaugten oder -gedrückten Partikel ausgeht, mit Hilfe lichtempfindlicher Detektoren (Photomultiplier, Photozelle) erfasst und entsprechend seiner Intensität und/oder zeitlichen Verteilung gemessen wird. Das Ergebnis solcher Messungen an einzelnen Zellen sind Histogramme (Impulshöhenverteilungen). Derartige Verfahren erlauben die Messung von bis zu einigen tausend Zellen pro Sekunde. Das Hauptanwendungsgebiet ist die Zellbiologie und hier insbesondere die Krebszellforschung sowie die experimentelle oder quantitative Zytologie.

Es wurden zusätzlich Verfahren vorgeschlagen, die Zellen zunächst einzeln zu vermessen bzw. deren biochemische oder morphologische Eigenschaften zu messen und unmittelbar im Anschluss daran diese aufgrund vorwählbarer Kriterien zu sortieren. Solche Sortiervorrichtungen arbeiten z.B. nach folgendem Prinzip: Nach der Erfassung der Partikeleigenschaft innerhalb der Durchflussmesskammer oder in einem feinen freien eine Porenöffnung verlassenden Strahl wird der Suspensionsstrahl mit Hilfe eines Piezo-Transducers in homogene feine Tröpfchen von einigen μm bis zu einigen 10 μm Durchmesser zerlegt. Unter der Annahme, dass sich zu einer bestimmten Zeit nach der photometrischen Messung eine bestimmte auszusortierende Zelle im Suspensionsstrahl unmittelbar vor der Loslösung dieses partikeltragenden Flüssigkeitsanteils als Tröpfchen befindet, wird der Flüssigkeitsstrahl elektrisch geladen. Das bedeutet, dass auch das unmittelbar danach sich loslösende Tröpfchen geladen ist. Die Kette der geladenen und ungeladenen Tröpfchen wird nun an einem elektrostatisch geladenen Elektrodenpaar vorbeigeführt. Hier werden nur die geladenen Tröpfchen in der gewünschten Weise abgelenkt und in einem Gefäß aufgefangen. Es sind Vorrichtungen bekannt, diese Zellen sogleich in Kulturgefäßen zu deponieren, um z.B. Zelllinien zu züchten, die auf jeweils eine aussortierte besondere Zelle zurückgehen. Derartige Verfahren werden z.B. in der Gentechnologie eingesetzt.

Im Unterschied zu diesem "offenen" Sortiersystem wurden auch geschlossene Anordnungen vorgeschlagen (Zöld, oder Wiecek oder Kamensky). Die von Kamensky beschriebene Anordnung benutzt einen Piezo-Transducer zur kurzzeitigen Störung eines Partikelstromes, der, umgeben von teilchenfreier Lösung, eine Messkammer passiert. Jede von einem interessierenden Partikel initiierte Störung des Partikelstromes soll dazu führen, dass das betreffende Partikel in den an sich teilchenfreien Hüllstrom gelangt, von wo es in eine separate Auffangvorrichtung geleitet wird.

Die von Wiecek et al. beschriebene Vorrichtung bedient sich eines kapillaren Kanalsystems mit einer z.B. Y-förmigen Verzweigung, die sich in Flussrichtung der Suspension hinter der Messstelle befindet. In jedem Arm der Y-förmigen Verzweigung befindet sich ein mikroskopisch kleines Luftventil. An Piezo-membranen befestigte Stößel mit kleinen Kolben öffnen und schliessen diese Ventile entsprechend der Sortierentscheidung, die aufgrund jedes einzelnen Messwertes der Zellen getroffen wird. Das Öffnen eines Ventils führt in dem System, das mit Vakuum betrieben wird, zum Einströmen von Luft in den betreffenden Arm des Kanalsystems und damit zu einer Verringerung der Fließgeschwindigkeit. Bei richtiger Wahl der Verzögerungszeit, die zwischen der Messung einer Zelle und dem Öffnen eines Ventils liegt, kann erreicht werden, dass die interessierenden Zellen in den einen Ast und die nichtinteressierenden Zellen in den zweiten Ast des Kapillarsystems fließen. Die Sortiervorrichtung kann mit einem oder zwei gegensinnig arbeitenden Ventilen betrieben werden.

Eine weitere, von Zöld vorgeschlagene, Sortiervorrichtung arbeitet ebenfalls mit einem geschlossenen sich verzweigenden Kapillarsystem, in dem die einzelnen Kanäle durch elektrische Entladung zwischen Mikroelektroden und dadurch bewirkte "Gasbildung" mehr oder weniger verschlossen werden, um die interessierenden Zellen in den jeweils gewünschten Kanal zu lenken.

Bei dem Tröpfchen-Sortierer handelt es sich um ein offenes System, in dem mikroskopisch kleine, die Zellen enthaltende Tröpfchen (Nebetröpfchengröße) im freien Fall an elektrisch geladenen Elektrodenplatten vorbeigeführt werden müssen. Zunächst ist es schwierig, die Zeit festzulegen, die zwischen dem Messen und dem Laden des sobald vom Flüssigkeitsstrom sich lösenden Tröpfchens vergeht. Diese Zeit muss weitgehend empirisch ermittelt werden. Die Qualität des Sortiervorganges (Reinheit der Probe) muss umständlich dadurch ermittelt werden, dass die "sortierten" Zellen erneut gemessen werden.

Ein entscheidender weiterer Nachteil dieser Anordnung besteht darin, dass die mikroskopisch kleinen Tröpfchen zu einem beträchtlichen Teil sich nicht in der gewünschten Weise in das Auffanggefäß bewegen. Aufgrund komplizierter Ladungsverhältnisse der Tröpfchen einerseits und der Geräteteile andererseits, sowie wegen der Kleinheit der Tröpfchen und der Anfälligkeit durch Luftströmungen bewegt sich ein beträchtlicher Anteil der Tröpfchen unkontrolliert in den Raum. Dies ist von grösster Bedeutung, weil die Zellen i.a. mit fluoreszierenden Farbstoffen, die in der Regel mutagen und cancerogen sind, gefärbt sind oder radioaktive Stoffe (z.B. ^3H -Thymidin) enthalten. Das sich bildende Aerosol mit derartig markierten Zellen stellt eine reale Gefahr für das Bedienungspersonal dar, das sich durch Einatmen beträchtlich kontaminieren kann.

Ein weiterer Nachteil solcher offener Sortiersysteme besteht darin, dass sich diese nicht über längere Zeit unter sterilen Bedingungen betreiben lassen. Dies wäre allerdings wichtig, weil die sortierten Zellen häufig das Ausgangsmaterial für neue Zellkulturen sein sollen.

Der Nachteil der von Kamensky vorgeschlagenen geschlossenen Anordnung besteht darin, dass nach jedem einzelnen Sortiervorgang, der je eine Störung des Zentralstrahles, d.h. eine Vermischung des Zentralstrahles mit dem Hüllstrom voraussetzt, eine vergleichsweise lange Zeit vergeht, bis sich der Zentralstrahl mit den Zellen wieder etabliert und stabilisiert hat. Ausserdem ist die Aussonderung interessierender Zellen erheblich vom Zufall abhängig: nicht

alle gewünschten Zellen bewegen sich durch die Piezo-Störung weit genug vom ehem. Zentralstrahl weg, um sicher aus der Peripherie des Flüssigkeitsstromes abgeführt werden zu können. Das System, das seit fast 20 Jahren bekannt ist, konnte nicht realisiert werden, es führt nicht zu brauchbaren Sortiierungsergebnissen.

Die von Zöld vorgeschlagene Anordnung ist nicht geeignet, Zellen mit dem Ziel zu sortieren, diese weiter zu kultivieren. Zellen sind rel. fragil; sie verlieren durch die erforderlichen "Explosionen" ihre Vitalität. Das System ist zudem sehr aufwendig wegen der beträchtlichen Entladungen, die kurzzeitig und mit hoher Frequenz erfolgen sollen. Die sich bildenden Gase müssen zudem schnell abgeführt werden; Gase würden wegen der Elastizität und der Neigung des Systems zu Eigenschwingungen den Sortierablauf stören.

In der Anordnung von Wicorek et al. erweisen sich aus ähnlichen Gründen die eingeführten Luftmengen als nachteilig. Die Anordnung ist dadurch relativ langsam bzw. wegen der Luftelastizität träge und schwinganfällig. Die mikroskopisch kleinen Ventile sind schwer herzustellen, störanfällig und wartungsaufwendig, wenn man bedenkt, dass die Lufttrittsschlitze im geöffneten Zustand nur eine Weite von einigen μm bis wenige $10\ \mu\text{m}$ haben. Der Sortiervorgang erfordert eine immer wieder zu kontrollierende und zu korrigierende Feineinstellung der Weite der Lufttrittöffnungen. Die erforderliche Luftzufuhr erschwert einen sterilen Betrieb der Anordnung.

Der Erfindung liegt die Aufgabe zugrunde, ein Sortierverfahren und eine Vorrichtung bereitzustellen, die nicht mit den Nachteilen bekannter Systeme behaftet sind und in denen insbesondere Partikel in einem geschlossenen System steril, zuverlässig und schonend sortiert werden.

Gemäss der vorliegenden Erfindung wird diese Aufgabe gelöst durch ein Sortierverfahren der eingangs angegebenen Art, das sich dadurch auszeichnet, dass durch das Signal in einem der beiden Kanäle hinter der Verzweigung eine Druckwelle erzeugt wird, welche den die Partikel enthaltenden Strom (Partikelstrom) an der Verzweigung für die Dauer des Partikeldurchgangs umleitet.

Eine Vorrichtung zur Durchführung dieses Verfahrens zeichnet sich nach der Erfindung dadurch aus, dass der Druckwellengenerator in einem der beiden von der Verzweigung wegführenden Kanäle angeordnet ist.

Der Verzweigungswinkel liegt vorzugsweise zwischen 10° und 45° . Er kann aber mehr sein oder auch 0° , d.h. eine Trennwand wird den einzigen Kanal in einem Abfallkanal und einem Sortierkanal trennen.

Die zu sortierenden Partikel werden vorzugsweise in einem Zentralstrahl, der von teilchenfreier Lösung (z.B. Wasser) umgeben ist, einer Messstelle zugeführt. Hier werden sie z.B. mit Hilfe einer mikroskopischen Auflichtanordnung durch ein Mikroskopobjektiv hindurch zur Fluoreszenz angeregt. Es können auch andere Messungen wie Streulichtmessung oder Messung des elektrischen Widerstandes ausgeführt werden. Jedes Messsignal wird entsprechend seiner Amplitude oder der zeitlichen Verteilung (Dauer) mit an sich bekannten Mitteln analysiert (z.B. mit Hilfe eines Vielkanalanalysators oder eines Rechners). Soll ein Partikel aussortiert werden, wird ein normiertes Signal, das auf das betreffende Messsignal zurückgeht, zu einem Hochspannungsimpulsgeber geleitet, der nach einer von der Fliegeschwindigkeit und dem Abstand zwischen der Messstelle und der Kanalverzweigung abhängenden Zeit ein Hochspannungssignal zu einer Piezokristallsäule leitet. Eine solche Säule dehnt sich z.B. bei einer angelegten Gleichspannung bzw. während eines Signals von $1000\ \text{V}$ um 20 bis $40\ \mu\text{m}$ aus. Diese Piezosäule weist eine Verlängerung in

Form eines Stössels auf. Dieser Stössel befindet sich in einer vakuumdichten Buchse in der Messkammer im Bereich eines der beiden abführenden Kapillarkanäle. Bei Anlegen einer Hochspannung bewegt sich der Stössel einige $10\ \mu\text{m}$ in Richtung quer zum Kanal. Der zeitliche Verlauf dieser Bewegung entspricht der Form des Hochspannungssignals. Zwei Effekte können hier ausgenutzt werden:

1. Wenn der Stössel in den Kanal hineinragt, wird der Querschnitt dieses Kanals eingeengt. Es fliesst weniger Flüssigkeit in Richtung des Auffanggefässes, das mit diesem Kanal verbunden ist. Wichtig ist, dass die Dimensionen der beiden Kanalzweige so gewählt werden, dass die Partikel normalerweise, d.h. bei nichtangeregter Piezosäule, in den Abfallkanal fließen. Dies kann auch durch geeignete Wahl der die Fliegeschwindigkeit beeinflussenden Druckverhältnisse (Vakuumverhältnisse) erreicht werden.

In den zweiten Kanal fliesst im Ruhezustand lediglich ein Teil des partikelfreien Hüllstromes. Die durch die Anregung der Piezosäule verursachte Verengung führt dazu, dass je nach Dauer des anliegenden Hochspannungssignals ein Teil der Partikel enthaltenden Flüssigkeitssäule in den Sortierkanal abgelenkt wird. Dies wiederholt sich bei jedem zu sortierenden Partikel bis zu 1000 mal pro Sekunde.

2. Wenn sich der Stössel unter dem Niveau des Kanals befindet, (z.B. in einem von dem Kanal abzweigenden Blindkanal) verursacht die Anregung der Piezosäule eine Druckwelle, die sich in beide Richtungen in dem der Piezosäule zugehörenden Kanal fortsetzt. Diese Druckwelle verursacht eine verzögerungsfreie Ablenkung des die Partikel enthaltenden Zentralstrahles in den Sortierkanal. Damit erfolgt eine sehr präzise und beliebig kurzzeitige Ablenkung des Partikelstromes in den Sortierkanal. Der Stössel ist hierbei dem Abfallkanal zugeordnet.

Von den beiden Effekten ist der zweite der dominierende. Dieser tritt sowohl dann auf, wenn sich das Stösselende unter dem Kanalboden befindet als auch dann, wenn der Stössel in den Kanal hineinreicht.

Der Vorgang lässt sich bis zu 1000 mal pro Sekunde wiederholen, was einer Sortiergeschwindigkeit von 1000 Zellen/Sekunde entspricht.

Es ist auch möglich, diesen Vorgang dadurch zu steuern, dass man statt der spannungsabhängigen Längenausdehnung der Piezosäule oder anderer mechanischen Vorrichtung z.B. eines Schrittmotors genau umgekehrt arbeitet: Von einem hohen Spannungsniveau ausgehend werden kurze -Impulse generiert, die zu einer Verkürzung der Piezosäule führen. Das führt zu kurzzeitigen neg. Druckstössen, wodurch die Zellen in den gewünschten Kanal hineingesaugt werden. Hierzu muss sich der Stössel in dem Sortierkanal befinden. Aus Gründen der Schonung der Zellen und des sterilen Arbeitens ist es aber zweckmässiger, mit Druckwellen zu arbeiten, die von der im Abfallkanal installierten Piezosäule kommen.

Die Vorrichtung der vorliegenden Erfindung besitzt die Vorteile, dass sie ein geschlossenes System aufweist und leicht sterilisierbar ist. Es verursacht keine Kontamination der Umgebung und keine Gefährdung des Personals. Da es keine Gase im System gibt, gibt es auch keine Elastizität. Es ist also schwingunanfällig und weist eine besonders schnelle Sortiergeschwindigkeit auf.

In der Praxis zeigte sich ein bemerkenswerter Vorteil: das System sortiert entweder richtig oder garnicht - damit sind, abgesehen von statistischen Koinzidenzen bei zu grosser Zelldichte, Fehlsortierungen nicht möglich.

Die Wahl der Zeitverzögerung zwischen Messen und Ablenken (Hochspannungssignal) ist sehr einfach, da man im Mikroskopokular die abgelenkten Zellen einzeln zur Einstellung in Form eines Striches sieht. Wird richtig sortiert, ist

der an gleicher Stelle im Bereich der Verzweigung liegende "Umlenkpunkt" der sortierten Zellen deutlich sichtbar an immer der gleichen Stelle. Im Unterschied zu anderen Systemen sind die Kanäle mit 100 bis 500 µm besonders weit -dadurch wenig stör anfällig durch Schmutzpartikel.

Weitere Merkmale, Einzelheiten und Vorteile der Erfindung ergeben sich aus der folgenden Beschreibung einer bevorzugten Ausführungsform der Erfindung.

Der Erfindungsgegenstand wird nun anhand eines in den Zeichnungen dargestellten Ausführungsbeispiels erläutert. Die Zeichnungen zeigen in

Fig. 1 eine Perspektivansicht einer Partikelsortier-
richtung.

Fig. 2 einen Teilschnitt durch eine Partikelsortier-
richtung für die Verwendung von Aufsicht-Fluoreszenz

Fig. 3 einen Teilschnitt durch eine Partikelsortier-
richtung für die Verwendung von Durchlicht (Fluoreszenz,
Absorption, Streulicht).

Fig. 4 eine schematische Aufsicht einer Partikelsortier-
vorrichtung

Fig. 5 & 6 schematische Aufsichten von Teilen von
Partikelsortiervorrichtungen

Fig. 7 einer Seitenschnitt einer Partikelsortier-
richtung

Fig. 8 & 9 schematische Darstellungen von der Sortie-
rung einer Partikel

Fig. 10 eine schematische Darstellung einer Partikel-
sortiervorrichtung mit Vorrats- und Aus-
fuhrflüssigkeitsbehältern

Fig. 11 ein Histogramm von auf der Grundlage von
Unterschiedlichen DNS-Gehalten sortierten DNS Zellen

Das Ausführungsbeispiel wurde speziell für die Sortie-
rung von Zellen mit verschiedenen DNS-Gehalten durch die
Messung biochemischer Eigenschaften von Ehrlich-
Ascites-Tumorzellen der Maus durch die Bestimmung des
DNS-Gehaltes konzipiert. Diese Messung beruht darauf,
dass die zu messenden Zellen nach Anfärbung mit dem
fluoreszierenden Agens Fluoreszenzlicht aussenden, wenn
sie in der Messanordnung mit kurzwelligem Licht angeregt
werden.

Wie aus Fig. 1 und 4 ersichtlich, werden die fluores-
zenzmarkierten Zellen durch einen Zuführkanal (13), der im
allgemeinen aus einem dünnen Röhrchen mit einem Innen-
durchmesser von 100 bis 300 µm besteht, einem Messka-
nal (1) zugeführt. Der Messkanal mit einer Breite von 1 bis
5 mm weist eine zweite Zuführung (14) für teilchenfreies
Wasser (oder eine andere Lösung als Dispersionsmedium
für die Zellen) (4) auf. Wenn in dem Messkanal (1) sowohl
die Zellsuspension durch das Röhrchen (13) als auch die
teilchenfreie Flüssigkeit einfließen, bildet sich ein laminarer
Strom von Zellen, der umgeben ist von zellfreier Lösung.
Dieser Strom ist im Messkanal (1) sehr stabil. Der Messka-
nal hat einen Querschnitt von z.B. 100 µm mal 200 µm, er
ist also flach bei Betrachtung durch ein Messobjektiv (18).
Der Messkanal weist eine Verzweigung (3) in einer Sortier-
kanal (4) und einen Abfallkanal (5) auf. In den Abfallkanal
(5) strömt der Zellstrom, wenn er unbeeinflusst ist. Die aus-
zusortierenden Zellen werden in den Sortierkanal geleitet.

In Flussrichtung kurz vor dieser Verzweigung befindet
sich eine Messstelle (2), die durch das Messobjektiv beo-
achtet wird (Fig. 2,3). Im Bereich dieser Messstelle wer-
den die Zellen, die sich einzeln durch sie hindurchbewe-
gen, zur Fluoreszenz angeregt. Das Fluoreszenzlicht gelangt
durch das Messobjektiv zu einem Photomultiplier (15) zur
Messung der Intensität der jeder einzelnen Zelle zugeordne-
ten Messsignale. Diese Messsignale werden mit elektronis-
chen Mitteln registriert und klassifiziert.

Anstelle der Verwendung nur eines Objektivs für die
Beleuchtung der Messstelle und für die Sammlung des
Fluoreszenzlichtes (Auflichtfluoreszenzmessung) kann auch
ein separates Objektiv für die Sammlung des Fluoreszenz-
lichtes verwendet werden (Durchlichtanordnung).

Am Abfallkanal (5) ist ein Druckwellengenerator in
Form einer Piezosäule (11) angeordnet, die mit einem in
den Kanal einschiebbaren Stößel (12) versehen ist (Fig.
5,7). Die Öffnung, durch die der Stößel (12) in den Kanal
(5) ragt, ist mit einer vakuumdichten Buchse (7) versehen.
Je nachdem, ob die Piezosäule mit einem Signal angeregt
wird oder nicht, ragt der Stößel (12) mehr oder weniger
weit in den Kanal (5). Aufgrund vorgegebener Algorithmen
bzw. an der Auswertelektronik vorgegebener Signalfenster
kann vorgewählt werden, welche Arten von Zellen mit be-
stimmten Eigenschaften aussortiert werden sollen. Immer
dann, wenn eine solche Zelle gemessen wurde, trifft die
Elektronik die Entscheidung, die Piezosäule (11) durchzu-
schalten.

Selbstverständlich wäre es auch möglich, den Druck-
wellengenerator am Sortierkanal (4) anzuordnen. Diese Al-
ternative ist in Fig. 6 gezeigt. Hierzu muss sich der Stößel
(12) der Piezosäule (11) in umgekehrter Richtung bewegen,
so dass mit einer negativen Druckwelle (Sog) gearbeitet
wird.

Zu beachten ist, daß der Flüssigkeitsstrom kontinuier-
lich in beide Kanäle fließt, damit der Laminarfluß erhalten
bleibt. Bei völligem Verschließen des einen Kanals würde
der Laminarstrom aufgelöst und sich der zentrale Partikel-
strom mit dem Hüllstrom vermischen.

Wenn im Falle der Verwendung einer Druckwelle die
Piezosäule kurzzeitig angeregt wird, kommt es zu einer
kurzzeitigen Ablenkung des laminaren Partikelstromes in
den Sortierkanal (4) (Fig. 8 und 9). Mit Hilfe geeigneter
elektronischer Mittel (Zeitsteuerung) wird dafür Sorge getra-
gen, dass die Piezosäule mit der richtigen Verzögerung
durchgeschaltet wird, damit das immer genau dann erfolgt,
wenn sich die zu sortierenden Zellen unmittelbar vor der
Verzweigung befinden. Die Fließgeschwindigkeit des lami-
naren Stromes beträgt bei diesem Ausführungsbeispiel etwa
0,20 bis 2 m/sec je nach angelegtem Druck oder Vakuum
zum Transport der Ströme.

Entsprechend der gewünschten Fließgeschwindigkeit
wird die Verzögerungszeit zwischen der Messung der Zel-
leigenschaft und dem Durchschalten der Piezosäule variiert,
sie liegt bei diesem Ausführungsbeispiel zwischen 20 und
200 microsec. Diese Zeit hängt auch davon ab, wie nahe
die Messstelle vor der Verzweigung liegt. Fig. 9 zeigt im
Vergleich zu Fig. 8, wie durch Einschaltung der Piezosäule
eine kurze Flüssigkeitssäule aus dem Abfallkanalstrom in
den Sortierkanal abgeleitet wird.

Im Sortierkanal (11) kann eine weitere Messstelle (24)
angeordnet sein, die durch dasselbe Objektiv beleuchtet
und beobachtet wird (Fig. 4,5). Das bedeutet, dass jede
Zelle, die diese zweite Messstelle passiert, ein zweites
Messsignal liefert. Da in diesen Kanal nur die aussortierten
Zellen fließen sollen, erhält man aus diese Weise die
Messwerte der aussortierten Zellen, ohne

daß die aussortierte Probe in einem zweiten
Meßvorgang erneut gemessen werden muß.

In Fig. 10 ist eine schematische Ansicht einer Partikel-
sortiervorrichtung gezeigt, bei der die einzelnen
Flüssigkeitsbehälter in ihrer Anordnung zur Sortier-
richtung gezeigt sind.

Fig. 11 zeigt das Ergebnis einer Sortierung verschiede-
ner Größenklassen von DNA-Zellen. Fig. 11 oben zeigt die
Anzahl aller Zellen (y-Achse) in ihrer
Größenklassenverteilung (x-Achse). Wenn die

Meßapparatur auf G_1 eingestellt ist, werden die zugehörigen Zellen dieser Größenklasse sortiert, bei Einstellung des Meßfensters auf G_2 die Zahl der diesem Meßwert zugehörigen Zellen.

Die Verteilung der mit dem Fenster G_1 aussortierten Zellen zeigt Fig. 11 Mitte, während die Verteilung der G_2 -Zellen in Fig. 11 unten dargestellt sind.

Als Anwendungsgebiete ergeben sich insbesondere:

1. Sortierung unterschiedlicher Blutzellen, Leukämiezellen,
2. Sortierung unterschiedlicher Zellen aus Gewebekulturen

3. Sortierung unterschiedlicher Tumorzellen aus menschlichen Tumorbiospien, um diese vom Pathologen begutachten zu lassen. Dies ist deshalb von Bedeutung, weil in vielen Fällen nur sehr wenige Tumorzellen in den Proben vorhanden sind und eine vorangehende Anreicherung derselben die Diagnose erleichtert. Besondere Bedeutung hat das im Rahmen der Krebsfrüherkennung bei Reihenuntersuchungen z.B. bei der Vaginalzytologie.

4. Aussortierung bestimmter mit Oberflächenmarkern versehener Zellen nach Markierung mit Antikörpern z.B. zum Zwecke der Züchtung von bestimmten Zellklonen im Rahmen der Gentechnologie.

5. Isolierung bestimmter Heferassen im Gärungsgewerbe und in der Biotechnologie.

6. Sortierung unterschiedlicher mikroskopisch kleiner Partikel in der Industrie, Farbpigmente, pharmazeutische Industrie.

Die Methode ist insbesondere anwendbar bei allen Partikeln im Größenbereich von $1\mu\text{m}$ bis einige $100\mu\text{m}$.

Bezugszeichenliste

- 1 Messkanal
- 2 Messstelle
- 3 Y-Verzweigung
- 4 Sortierkanal
- 5 Abfallkanal
- 6 Ausgang für die sortierten Zellen
- 7 Vakuumdichte Buchse
- 8 Abfall - mit Saugflasche (Pumpe) verbunden
- 11 Piezosäule
- 12 Stößel
- 13 Einlass für Partikel-suspension (Zentralstrahl)
- 14 Zufuhr der Hüllstromlösung (Wasser)
- 15 Photomultiplier
- 16 Dichroitischer Spiegel (Lichtteiler)
- 17 Lichtquelle (UV oder Laser zum Fluoreszenzanregung)
- 18 Mess-Objektiv

- 19 Hülllösung
- 21 Deckglas
- 5 22 Partikelstrom
- 23 Kollektor
- 24 Zweite Messstelle
- 10 25 Wasservorrat
- 26 Zellvorrat
- 15 27 Abfall
- 28 Sortierte Zellen

20 Ansprüche

1. Verfahren zum automatischen Sortieren von mikroskopischen Partikeln, bei dem die Partikel in einer Flüssigkeit suspendiert durch einen geschlossenen Kanal geführt werden und dabei eine Messstelle, in der die auszusortierenden Partikel ein Signal auslösen, und eine anschließende Verzweigung passieren, dadurch gekennzeichnet, dass durch das Signal in einem der beiden Kanäle hinter der Verzweigung eine Druckwelle erzeugt wird, welche den die Partikel enthaltenden Strom (Partikelstrom) an der Verzweigung für die Dauer des Partikeldurchgangs umleitet.

2. Sortierverfahren nach Anspruch 1, dadurch gekennzeichnet, dass die Druckwelle in dem Kanalzweig erzeugt wird, in den der unbeeinflusste Partikelstrom strömt, wodurch der Partikelstrom für die Dauer eines Partikeldurchgangs in den anderen Kanalzweig umgeleitet wird.

3. Sortierverfahren nach Anspruch 1, dadurch gekennzeichnet, dass die Druckwelle in dem Kanalzweig erzeugt wird, durch den die auszusortierenden Partikel strömen.

4. Sortierverfahren nach einem der Ansprüche 1-3, dadurch gekennzeichnet, dass der Partikelstrom in einem partikelfreien Hüllstrom umgehen ist.

5. Sortiervorrichtung zur Durchführung des Verfahrens nach Anspruch 1 mit einem Zuführkanal (13) für den Partikelstrom (22), einem Messkanal (1), einer im Messkanal angeordneten Messstelle (2), einer Verzweigung (3) des Messkanals in einen Sortierkanal (4) und einen Abfallkanal (5) und einem Druckwellengenerator (11,12), dadurch gekennzeichnet, dass der Druckwellengenerator (11) in einem der beiden von der Verzweigung (3) wegführenden Kanäle (4,5) angeordnet ist.

6. Sortiervorrichtung nach Anspruch 5, dadurch gekennzeichnet, dass sie eine Hüllstromzufuhr aufweist, an welcher der Partikelstrom in einen Hüllstrom fließt.

7. Sortiervorrichtung nach einem der Ansprüche 5-6, dadurch gekennzeichnet, dass der Druckwellengenerator im Sortierkanal angeordnet ist.

8. Sortiervorrichtung nach einem der Ansprüche 5-6, dadurch gekennzeichnet, dass der Druckwellengenerator im Abfallkanal angeordnet ist.

9. Sortiervorrichtung nach einem der Ansprüche 5-8, dadurch gekennzeichnet, dass sie eine Messstelle im Sortierkanal aufweist um die Sortierfunktion zu kontrollieren.

10. Sortiervorrichtung nach einem der Ansprüche 5-8, dadurch gekennzeichnet, dass der Druckwellengenerator eine Piezosäule ist.

11. Sortiervorrichtung nach einem der Ansprüche 5-9, dadurch gekennzeichnet, dass der Druckwellengenerator ein Schrittmotor ist.

12. Sortiervorrichtung nach einem der Ansprüche 5-11, dadurch gekennzeichnet, dass sich der Druckwellengenerator ausserhalb des entsprechenden Kanals befindet.

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Fig.1

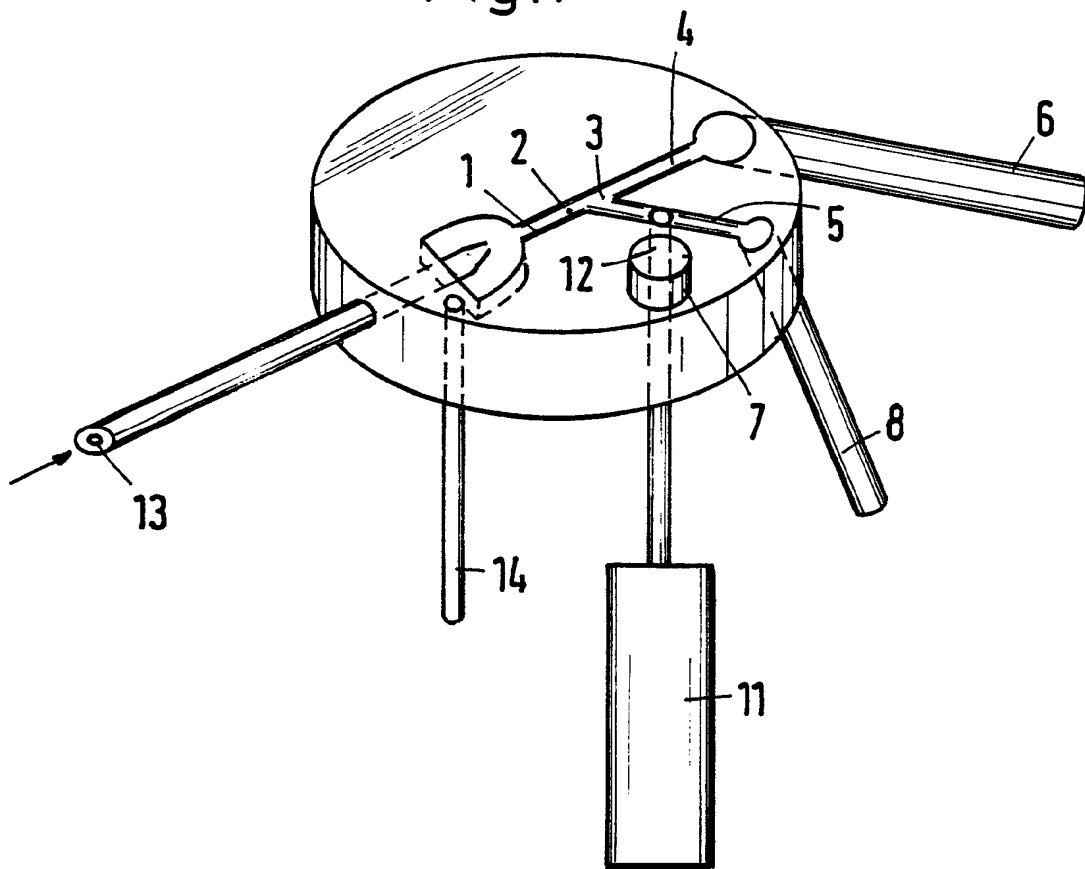


Fig.2

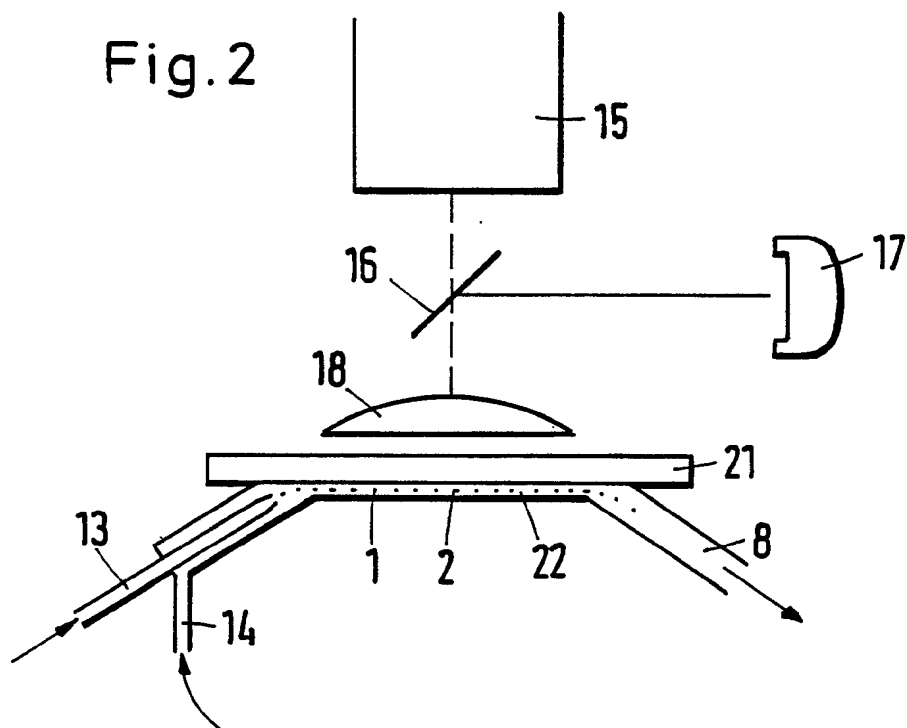


Fig. 3

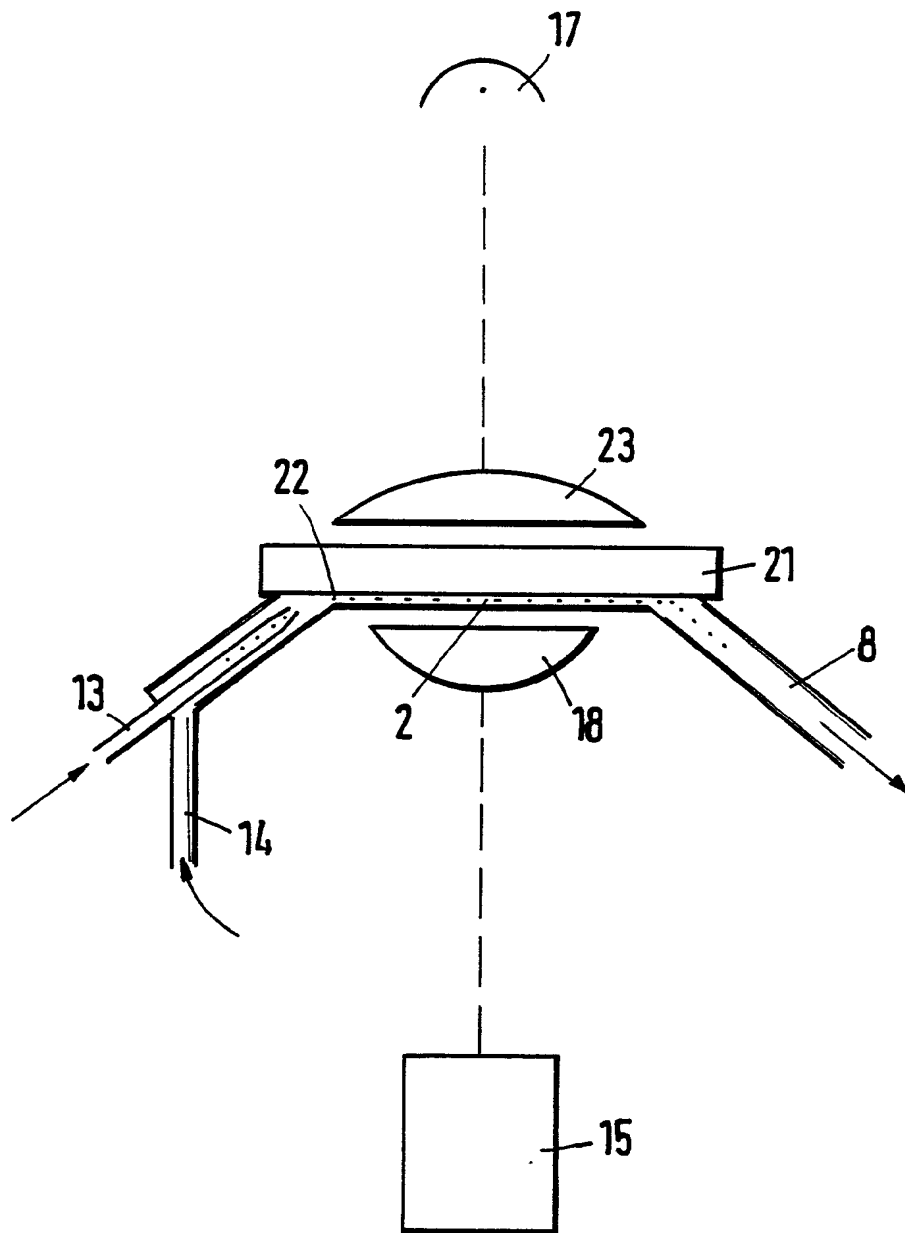


Fig. 4

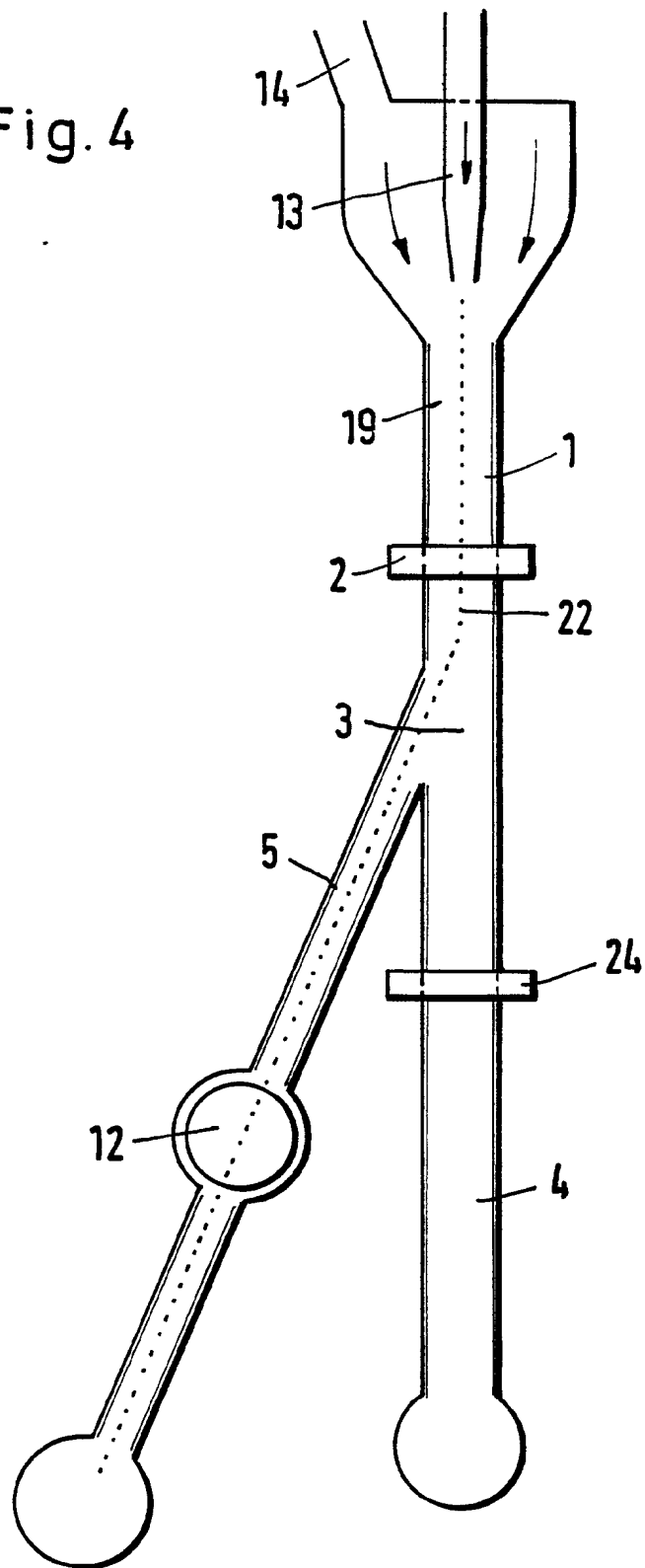


Fig. 5

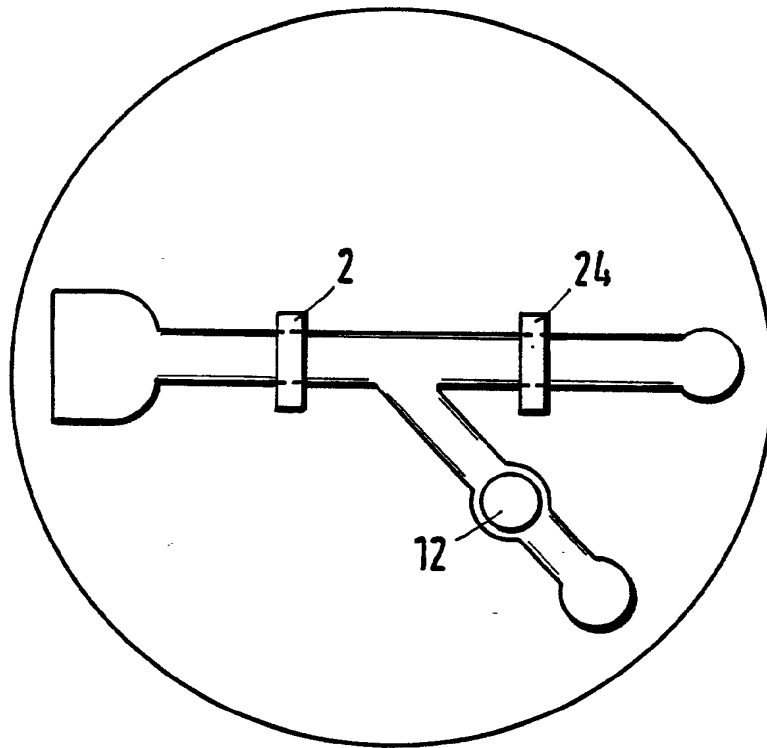
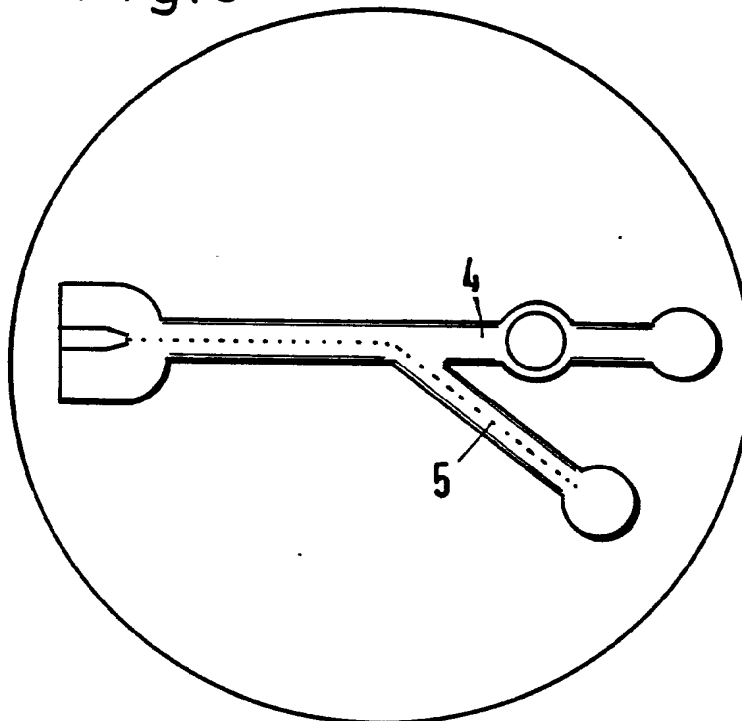


Fig. 6



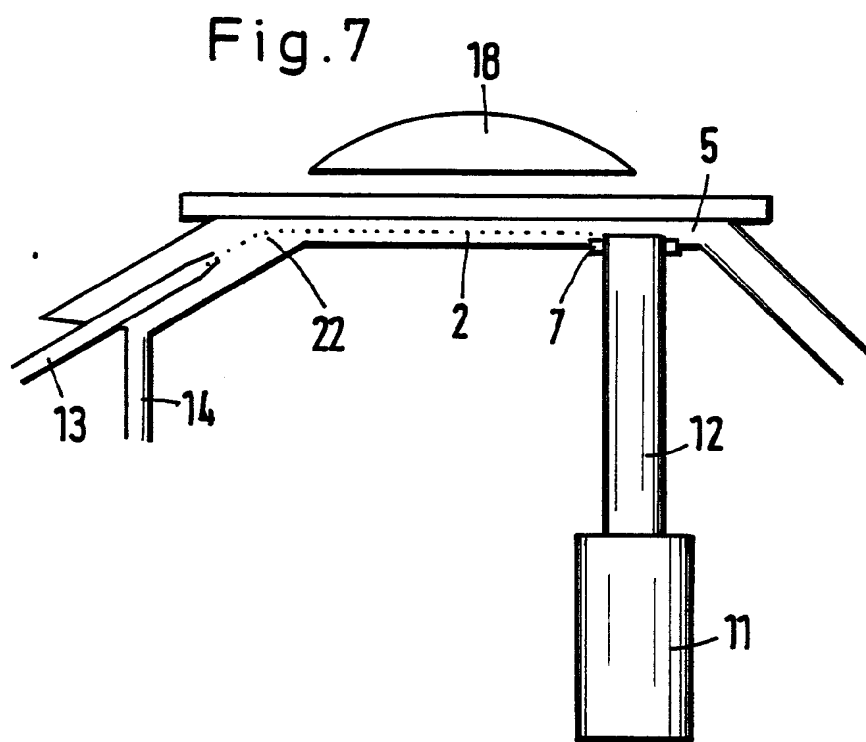


Fig.8

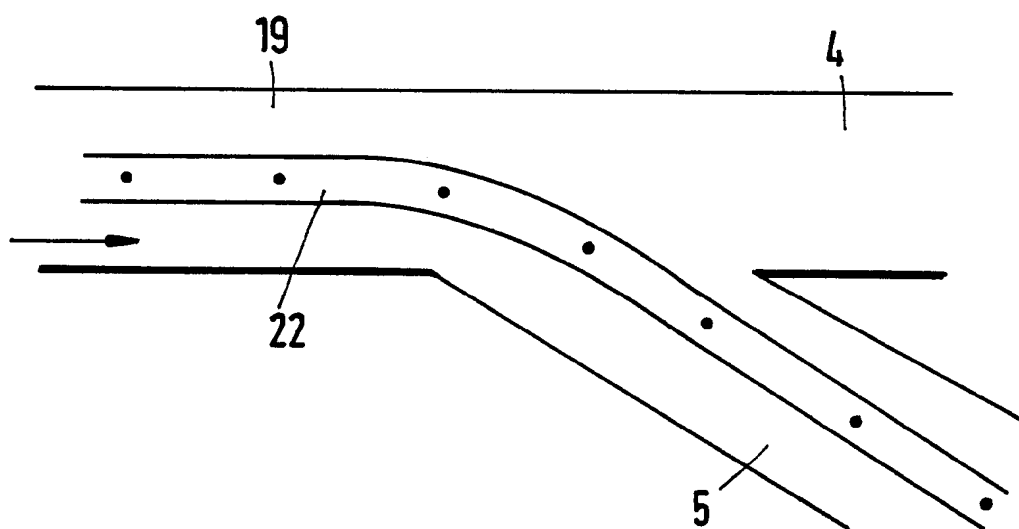


Fig.9

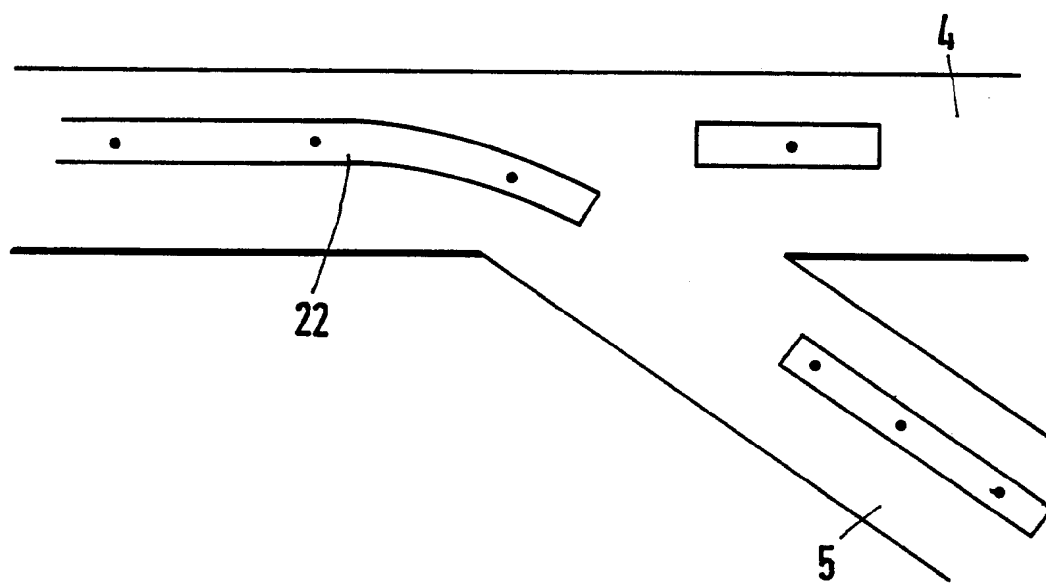


Fig.10

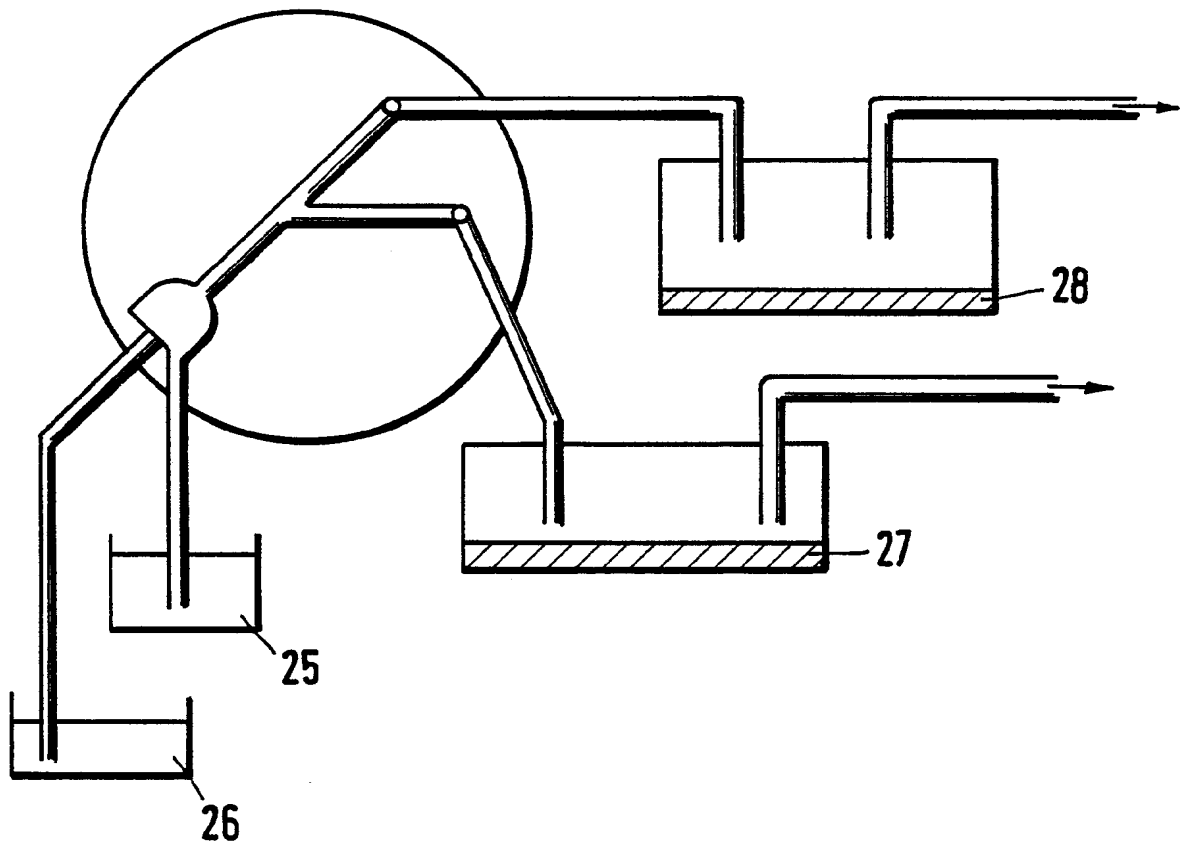
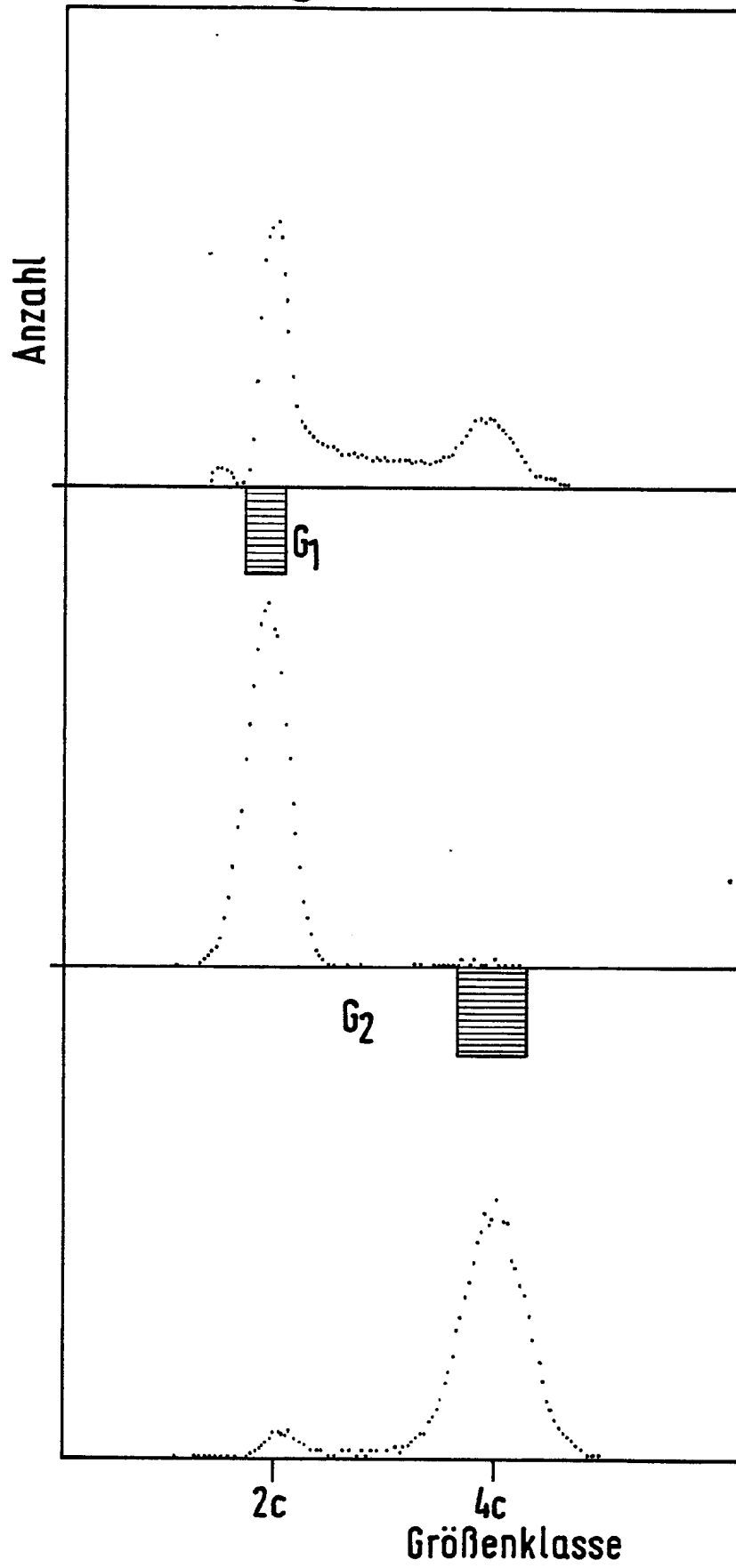


Fig. 11



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Beschreibung

[0001] Die Erfindung betrifft einen Sortierapparat für einzelne biologische Objekte in einer Flüssigkeit zum Einsatz in der Biotechnologie. Der Sortierapparat beruht darauf, dass die Objekte hinsichtlich ihrer physikalischen Eigenschaften direkt oder indirekt mit Hilfe geeigneter Sensoren klassifiziert werden. Die Objekte können zur Klassifizierung auch in signifikanter Weise markiert werden. Ein an einer Weiche angebrachter Sortieraktor kann dann die klassifizierten biologischen Objekte auf räumlich getrennte Auslasskanäle aufteilen. Bei der indirekten Klassifizierung wird der Sortieraktor von dem zugeordneten Sensor in Abhängigkeit von der Signalintensität des Sensors gesteuert. Als Sensoren sind in erster Linie Fluoreszenzsensoren geeignet. Unter "biologischen Objekten" werden im Rahmen der vorliegenden Anmeldung vor allem (lebende) biologische Zellen verstanden.

[0002] Vorrichtungen zur fluoreszenzaktivierten Zellsortierung (FACS) sind kommerziell erhältlich. Bei den Sortierprinzipien unterscheidet man zwischen fluidischen Methoden (EP-A-0 177 718) und tröpfchenorientierten Systemen. Eine solche Vorrichtung ist z. B. in der Patentschrift US-A-3 826 364 beschrieben. Bei beiden Methoden werden die zu untersuchenden Zellen durch eine Hüllflüssigkeit vereinzelt, so dass sie individuell fluoreszenzspektroskopisch oder durch Detektion des Streulichts vermessen werden können.

[0003] Im ersten Fall wird die zu untersuchende Dispersion aus zu untersuchenden Zellen in einem Flüssigkeitsstrahl geführt, der aufgrund eines Fluoreszenzsignals in einen Arm von nicht zu sortierenden und in einen Arm von zu sortierenden Zellströmen aufgespalten wird. Hierbei kann die Strömung durch einen piezoelektrischen Transducer oder einen Ultraschallwandler entsprechend beeinflusst werden.

[0004] Die am meisten verwendete Methode stellt die Sortierung von einzelnen Tröpfchen dar. Bei diesem Prinzip tritt ein Zellstrom durch eine Düse aus. Aufgrund einer definierten mechanischen Schwingung der Düse reißt der Flüssigkeitsstrahl in einer wohl definierten Entfernung unterhalb des Messortes in Tröpfchen auf, die jeweils maximal eine Zelle tragen. In Abhängigkeit von einer Schwellintensität wird ein Sortiervorgang eingeleitet, indem der Flüssigkeitsstrahl über die Hüllflüssigkeit elektrostatisch aufgeladen wird. Ist ein geladenes Tröpfchen mit der auszusortierenden Zelle vorn Flüssigkeitsstrahl abgerissen, so wird der Flüssigkeitsstrahl augenblicklich wieder entladen, so dass die folgenden Tropfen nicht aufgeladen werden. Damit bleibt nur das Tröpfchen mit der zu selektierenden Zelle geladen. Die Tropfen fliegen nun durch das Ablenkfeld eines Kondensators. Das geladene Tröpfchen mit der zu sortierenden Zelle wird entsprechend abgelenkt und landet in einem Sortiergefäß, während die nicht zu sortierenden Tropfen geradeaus in ein anderes Gefäß fliegen.

[0005] Im Gegensatz zum fluidischen Sortierer wer-

den mit dem Tröpfchensortierer höhere Sortierraten erzielt. So ist er in der Lage, pro Tag ca. 10^8 Zellen zu prozessieren. Hierbei ist jedoch damit zu rechnen, dass nur 80 % der zu selektierenden Zellen tatsächlich sortiert werden können. Bei sehr seltenen Ereignissen in einer Zellpopulation sind jedoch höhere Durchsätze wünschenswert von etwa 10^{10} Zellen.

[0006] Aus der WO-A-9 428 119 ist eine Sortiervorrichtung für in Flüssigkeiten suspendierte Zellen bekannt. Die Vorrichtung weist einen von der Flüssigkeit durchströmbaren Hauptkanal auf mit einem Detektor und diesem nachgeordneter Gabelung. Der Detektor klassiert die Partikel in der Flüssigkeit nach ihren physikalischen Eigenschaften, nach denen sie mittels eines Wasserstoßes in eine vorbestimmte Verzweigung des Kanals getrieben werden. Mit der Vorrichtung werden mit Zellen beladene Kunststoffpartikel in einer Größe von ca. 3 mm sortiert. Ein Hinweis auf ein mikrostrukturiertes System von multiparallelen Hauptkanälen, Sortiermodulen, Weichen und zwei Summenauslasskanälen fehlt hier völlig. Mit der Vorrichtung ist die gewünschte Leistungssteigerung der Sortierung nicht möglich. Weiterhin ist ein magnetisches Verfahren bekannt (MACS = **M**agnetic **a**ctivated **c**ell **s**orter), das zur Separation großer Zellpopulationen kommerziell erhältlich ist. Das Verfahren ist beispielsweise beschrieben in "Cytometry" 11 (1990), Seiten 231 bis 238. Bei diesem Sortiervorgang wird die Bindung der relevanten Zellen an magnetische Beads ausgenutzt. Im Separationsschritt werden die in dieser Weise markierten Zellen in der MACS-Säule durch ein inhomogenes Magnetfeld zurückgehalten, während die nicht-markierten Zellen ungehindert die Säule passieren. Hierbei kann jedoch nur zwischen magnetischen und nicht-magnetischen Zellen unterschieden werden.

[0007] Der Erfindung liegt die Aufgabe zugrunde, einzelne biologische Objekte aufgrund einer magnetischen Markierung oder aufgrund einer Klassifizierung durch messtechnisch erfassbare Eigenschaften räumlich zu separieren, wobei der Zelldurchsatz signifikant verbessert werden soll.

[0008] Ausgangspunkt ist dabei eine Sortiervorrichtung mit folgenden prinzipiellen Merkmalen:

- i) Die Flüssigkeit mit den zu sortierenden biologischen Objekten strömt über eine Zuführung durch einen Hauptkanal, an dem eine Weiche mit mindestens zwei Auslaßkanälen A,B angeordnet ist.
- ii) An dem Hauptkanal kann zur Detektierung und Klassifizierung von nicht magnetisch markierten Zellen mindestens ein Sensor angeordnet sein.
- iii) An der Weiche ist ein Sortieraktor angebracht, der die magnetisch markierten, bzw. klassifizierten Zellen anschließend selektiv auf die Auslaßkanäle A,B verteilt.

[0009] Die erfindungsgemäße Lösung der oben angegebenen Aufgabe besteht im Falle von magnetisch markierten Zellen darin,

a) daß sich die Zuführung in ein mikrostrukturiertes System von multiparallelen Hauptkanälen und Sortiermodulen mit jeweils einer Weiche zur Aufteilung in zwei verschiedene Auslaßkanäle A,B verzweigt,

b) daß innerhalb eines Sortiermoduls an jedem Hauptkanal jeweils ein magnetischer Ablenkmodul als Sortieraktor angeordnet ist

c) und daß jeder Auslaßkanal B mit einem Summenkanal für die unselektierten Zellen und jeder Auslaßkanal A mit einem Summenkanal für die selektierten Zellen verbunden ist.

[0010] Eine alternative Lösung, die auch im Falle von nicht magnetisch markierten Objekten zur Anwendung kommen kann und bei der die Objekte ggf. vorher optisch markiert werden, besteht erfindungsgemäß darin,

a) daß sich die Zuführung in ein mikrostrukturiertes System von multiparallelen Hauptkanälen und Sortiermodulen mit jeweils einer Weiche zur Aufteilung in zwei verschiedene Auslaßkanäle A,B verzweigt,

b) daß innerhalb eines Sortiermoduls an jedem Hauptkanal mindestens ein Sensor und an jeder Weiche ein von dem jeweiligen Sensore gesteuerter Sortieraktor angeordnet sind,

c) und daß jeder Auslaßkanal B mit einem Summenkanal für die unselektierten Zellen und jeder Auslaßkanal A mit einem Summenkanal für die selektierten Zellen verbunden ist.

[0011] Vorteilhaft ist an jedem Hauptkanal ein optischer Sensor zur Bestimmung der Zellengröße angeordnet. Zweckmäßig sind dabei die an den Hauptkanälen angeordneten optischen Sensoren über einen Lichtleiter mit einer externen Lichtquelle verbunden. Als optische Sensoren werden vorteilhaft Fluoreszenzsensoren eingesetzt.

[0012] Gemäß einer bevorzugten Ausführungsform ist das mikrostrukturierte System einschließlich der optischen Sensoren und der Sortieraktoren in einen monolithischen Block integriert, wobei der monolithische Block vorzugsweise 200 bis 1000 Sortiermodule enthält und die lichte Weite der Kanäle 5 µm bis 60 µm beträgt.

[0013] Besonders vorteilhaft werden dabei sämtliche Elemente des mikrostrukturierten Systems einschließlich der Steuerelektronik fotolithografisch in einen Silizium-Chip integriert.

[0014] Entsprechend einer apparativen Weiterbildung bzw. Komplettierung sind die Sortiermodule mit einem gemeinsamen, externen Rechner verbunden, der

den Schwellwert bzw. den Maximalwert für die Selektierung der Zellen vorgibt.

[0015] Als Sortieraktoren können zur Steuerung der biologischen Flüssigkeitsströme piezoelektrische, magnetostriktive, fluidische oder ultraschallaktive Bauelemente eingesetzt werden.

[0016] Auf der Grundlage der Erfindung kann mit Hilfe der Methoden aus der Mikrosystemtechnik unter Anwendung des bekannten Prinzips "Messen-Entscheiden-Sortieren" ein Zellsortierapparat realisiert werden, dessen Leistungsfähigkeit gegenüber konventionellen Zellsortierapparaten um mindestens zwei Größenordnungen gesteigert ist, was einen signifikanten Fortschritt bei der Lösung des Problems der Zellsortierung darstellt. So können z.B. innerhalb von 24 h 10^{10} Zellen gegenüber 10^8 Zellen in Geräten prozessiert werden. Aufgrund der Fertigung des Sortierapparates mit Hilfe der Mikrosystemtechnik können bei entsprechend hohen Stückzahlen die Herstellungskosten einer solchen Vorrichtung deutlich gesenkt werden.

[0017] Der erfindungsgemäße Mikrosystem-Sortierapparat kann zum Screening von Transfektionen und Zellbibliotheken, zur Anreicherung von High-Producern bei Pro- und Eukaryonten und ggf. in der Medizintechnik zur Anreicherung von Knochenmarkstammzellen eingesetzt werden.

[0018] Im Folgenden wird die Erfindung an Hand von Zeichnungen und Ausführungsbeispielen näher erläutert.

[0019] Es zeigen:

Fig. 1 schematisch den Aufbau der neuen Sortiervorrichtung

Fig. 2 den prinzipiellen Aufbau eines Sortiermoduls

Fig. 3 a und 3b das Funktionsprinzip eines piezoelektrischen Sortieraktors

Fig. 4 das Funktionsprinzip eines fluidischen Sortieraktors

Fig. 5a und 5b einen Sortieraktor mit elektrostatischem Antrieb

Fig. 6 einen elektromagnetischen Sortieraktor für magnetisch markierte Zellen und

Fig. 7 eine modulare Hintereinanderschaltung von magnetischen und optischen Sortiermodulen

[0020] Bei der in Fig. 1 schematisch dargestellten Sortiervorrichtung wird der zu sortierende, durch die Zuführung 1 strömende Zellstrom 2 über die Hauptkanäle 3 auf einzelne Sortiermodule 4 aufgeteilt, die unabhän-

gig voneinander parallel die jeweiligen Teilströme verarbeiten. Die Hauptkanäle 3 sind dabei als Mikrokanäle mit einem Durchmesser bzw. einer Kanalweite von 7 µm ausgebildet. Nach der Sortierung vereinigen sich die Ströme der positiven und der negativen Kanäle aus jedem Einzelmodul 4 zu einem Summenkanal 5 der positiven und einem Summenkanal 6 der negativen Zellen. Die positiven Zellen werden in dem Auffanggefäß 7, die negativen Zellen in dem Gefäß 8 gesammelt. Durch den multiparallelen Betrieb einer großen Anzahl solcher Einheiten, vorzugsweise > 100, in einem sog. "Sorter-Array" kann eine beträchtliche Steigerung des Durchsatzes erreicht werden.

[0021] Der Aufbau des individuellen Sortiermoduls 4 des Sorter-Arrays ist in Fig. 2 dargestellt. Er umfaßt hier jeweils einen Hauptkanal 3 mit einem Streulichtsensor 9, einem Fluoreszenzsensor 10 und einer stromabwärts von den Sensoren angeordneten Weiche 11 mit einem Sortieraktor 12. Mit dem Streulichtsensor werden die zu sortierenden Zellen nach ihrer geometrischen Größe klassifiziert, um einerseits Störsignale zu unterdrücken und andererseits als Sortierparameter zur Verfügung zu stehen. Als Effekt wird hier die größenabhängige Lichtstreuung der Zellen bei der Wellenlänge des Anregungslichts ausgenutzt. Dieses Prinzip wird auch bei dem konventionellen FACS angewandt. Die Fluoreszenzintensität als Hauptparameter wird von dem optischen Detektor 10 detektiert. Die Detektoren 9 und 10, ggf. in Kombination mit optischen Filtern, sind unter dem Hauptkanal 3 angeordnet. Das Anregungslicht wird von einer außerhalb angeordneten Beleuchtungseinrichtung 13 über Lichtleiter herangeführt.

[0022] Die Detektoren 9 und 10 sind über die Leitungen 14 mit einer Auswerte- und Steuerelektronik 15 verbunden, die über die Leitung 16 mit dem Sortieraktor 12 und über die Leitung 17 mit einem externen Rechner 18 in Verbindung steht. Die gesamte Elektronik 15 besteht aus einem Verstärkungsteil für das Meßsignal z.B. mit Feldeffekttransistoren und einem Steuerteil. Im Steuerteil kommen Speicher, Logikelemente wie z.B. Komparatoren und Module für die Datenkommunikation zum Einsatz. Der Hauptkanal 3 mit der Weiche 11, die optischen Sensoren 9 und 10, der Sortieraktor 12 sowie die Auswerte- und Steuerelektronik 15 sind einschließlich der elektrischen Leitungen 14, 16, 17 auf einem monolithischen Silizium-Chip integriert. Der gesamte Elektronikteil 15 wird dabei als ASIC (anwendungsspezifischer integrierter Schaltkreis) auf dem Si-Chip ausgeführt. Die auf dem Chip integrierten elektrischen Verbindungen 14 versorgen die Steuerelektronik 15 mit den Informationen, um den Sortieraktor 12, z.B. ein Piezoelement, zu betätigen. Die Datenverarbeitung erfolgt parallel in jedem Einzelmodul. Lediglich die Sortierkriterien werden von dem externen Rechner 18 für alle Module gemeinsam vorgegeben. Als Sortierkriterium können hier relative Schwellwerte zugrunde gelegt werden. So bewirkt z.B. der Sortieraktor 12 eine Umleitung der Zellströmung auf den Schenkel bzw. Auslaßkanal 19 der Wei-

che 11, wenn das Meßsignal > 20% und < 80% des als Fluoreszenzmittelwert vorgegebenen Sollwerts ist. Außerhalb des Sortiermoduls 4 ist der abzweigende Schenkel bzw. Auslaßkanal 19 der Weiche 11 mit dem Summenkanal 5 und der geradlinige Schenkel bzw. Auslaßkanal 20 mit dem Summenkanal 6 verbunden (s. Fig. 1).

[0023] Analog zu den bekannten Zellsortierapparaten wird also die Zellströmung bei einer positiven Sortierentscheidung so beeinflusst, daß die zu sortierenden Zellen in den Schenkel bzw. Auslaßkanal 19 und damit in den Summenkanal 5 austreten, während die restlichen Zellen das Modul durch den Auslaßkanal 20 verlassen und dem anderen Summenkanal 6 zugeführt werden (s. auch Fig. 1). Bei einer Parallelschaltung von ca. 1000 Einzelmodulen 4 kann der Durchsatz und damit die Ausbeute bei der Zellsortierung um mehrere Größenordnungen gesteigert werden.

[0024] Für den Sortieraktor, der die Strömung der Zelldispersion in der oben beschriebenen Weise beeinflusst, kommen eine Reihe von Akteurprinzipien in Frage. Zunächst werden an Hand von Fig. 3a und 3b Verfahren beschrieben, die zu einer Verengung des Kanal- bzw. Schenkelquerschnitts 20 führen. Die Figuren 3a und 3b zeigen einen Querschnitt durch das Si-Grundmaterial 22 mit dem Auslaßkanal 20. Oberhalb des Auslaßkanals 20 ist ein kanalverengender Aktor 22 in Form einer Blattfeder angeordnet, der sich in Fig. 3a im entspannten Zustand und in Fig. 3b im aktivierten Zustand befindet. Ein derartiger Aktor kann als Piezoelement ausgeführt werden. Die Verwendung des piezoelektrischen Ablenkungsprinzips für Zellsortierer ist bereits bekannt. Durch Anlegen einer elektrischen Spannung an ein derartiges Element wird die Blattfeder mehr oder weniger durchgebogen. Als Material kommt dafür Polysilizium in Frage, das bereits als Bestandteil eines piezoresistiven Drucksensors beschrieben wurde. Außerdem sind piezoelektrische Aktoren auf der Basis von Lithiumniobat bekannt. Ferner wurden auch Ultraschallgeber für die Beeinflussung von Zellströmen eingesetzt.

[0025] Ein äquivalenter Effekt kann auch durch einen thermomechanischen Aktor erzielt werden. Hierbei wird eine Siliziumfederstruktur ausgewölbt, so daß wiederum die Strömung der Zelldispersion aufgrund einer Kanalverengung beeinflusst werden kann. Außerdem sind Zungen bekannt, die sich unter dem Einfluß eines externen Magnetfeldes durch magnetostruktive Kräfte verbiegen und auf diese Weise den Kanalquerschnitt verengen können.

[0026] Darüberhinaus sollten auch fluidische Elemente, bei denen kleine Flüssigkeitsströmungen große Flüssigkeitsströme steuern, die gestellte Aufgabe lösen können. Ein nach diesem Prinzip arbeitender Sortieraktor ist schematisch in Figur 4 dargestellt. In einem mikrostrukturierten Grundmaterial befinden sich die Kanäle 23 mit den Wandungen 24, die die Flüssigkeitsströme begrenzen. Die Zelldispersion 2 tritt durch eine Eintrittsdüse 25 in das Sortiermodul ein und wird hierbei seitlich

durch einen kleinen Flüssigkeitssteuerstrom 26 beeinflußt. Dieser Steuerstrom, der durch die Einlaßöffnungen 27 beispielsweise von links nach rechts fließt, führt dazu, daß sich der Zellstrom 2 aufgrund des Coanda-Effekts an die rechte Seite anschmiegt und somit das Sortiermodul durch die Austrittsöffnung 28 verläßt. Bei einer entgegengesetzten Flußrichtung des Steuerstroms stellen sich die Verhältnisse seitenverkehrt dar und der Hauptstrom würde das Modul durch die Öffnung 29 verlassen. Die Ausgleichsöffnungen 30 dienen zum Druckausgleich im Modul.

[0027] Weiterhin können Sortieraktoren auf der Basis von schiebergesteuerten Ventilen eingesetzt werden. Ein derartiger elektromechanisch betriebener Sortieraktor ist in den Fig. 5a und 5b dargestellt. Er besteht aus einem Plattenkondensator 31 mit einer feststehenden 32 und einer beweglichen Platte 33. An der beweglichen Platte 33 ist eine Ventilscheibe 34 angebracht, die den Kanalquerschnitt des Auslaßkanals 20 der Weiche 11 verschließen kann. Auf diese Weise kann die Strömung der Zelldispersion in dem Sortiermodul bzw. im Auslaßkanal 20 in Abhängigkeit der angelegten elektrischen Spannung gezielt beeinflußt werden. In Fig. 5a ist der Zustand dargestellt, bei dem die durch den Hauptkanal 3 einfließende Zelldispersion ungestört durch den Auslaßkanal 20 austritt. Im Fall einer positiven Sortierentscheidung wird die Ventilscheibe 34 gem. Fig. 5b in den Kanal 20 eingefahren, so daß die Dispersion in den anderen Auslaßkanal 19 abgelenkt wird.

[0028] Der Vorteil einer Separation von magnetisch markierten Zellen besteht darin, daß im Gegensatz zur Fluoreszenzmarkierung auf eine Sensoreinrichtung verzichtet werden kann. In diesem Sinn wird die magnetische Markierung von einem als Aktor wirkenden Magnetfeld als sortiertechnisch relevante physikalische Größe verwendet. Die zur Ablenkung nötigen Magnetfelder von magnetisch markierten Zellen werden durch planare Magnetspulen, deren Windungen z.B. aus Gold bestehen, realisiert. Auf diesem Prinzip beruht ein bekannter magnetischer Mikromotor, der auf Si-Substrat hergestellt wurde. In Fig. 6 ist ein entsprechendes magnetisches Ablenkmodul schematisch dargestellt. Die Zellen 35 treten in das Modul wieder über den Hauptkanal 3 ein. Entlang der Kanalstruktur sind planar aufgebrachte Magnetspulen 36 angebracht, die ggf. in einer zweiten Ebene über den Kanälen liegen. Mit dieser Anordnung der Spulen 36 wird der Ablenkweg für die Zellen 35 festgelegt, die magnetische Beads als Markierung tragen und damit das Modul durch den Auslaßkanal 19 verlassen. Magnetisch unmarkierte Zellen werden von den Magnetfeldern nicht abgelenkt und passieren daher das Modul durch den anderen Auslaßkanal 20.

[0029] Ein derartiger magnetischer Sortiermodul kann auch mit den oben beschriebenen optischen Sortiermodulen 4 in Fig. 1 kombiniert werden, womit ein zusätzlicher Sortierparameter zur Verfügung steht. Fig. 7 zeigt schematisch eine Anordnung für zwei par-

allel ausgeführte Sortiereinheiten. Eine Einheit besteht hierbei aus einem magnetischen Sortiermodul 37 bzw. 38 (gem. Fig. 6) und jeweils aus zwei über die Auslaßkanäle 39,40 bzw. 41,42 verbundenen optischen Sortiereinheiten 43,44 bzw. 45,46. Mit dieser Anordnung können in dem Summenkanal 47 die magnetisch positiv und Fluoreszenz-positiv gemessenen Zellen, im Summenkanal 48 die magnetisch positiven und Fluoreszenz-negativen, im Summenkanal 49 die magnetisch negativen und Fluoreszenz-positiven und im Summenkanal 50 die magnetisch negativen und Fluoreszenz-negativen Zellen gesammelt werden.

[0030] Falls nur mit magnetisch markierten Zellen gearbeitet wird, kann ein magnetisches Ablenkmodul 37 oder 38 das optische Sortiermodul 4 in Fig. 1 ersetzen. Durch die gezielte Einstellung der Magnetfelder können in einer Kaskadenanordnung von magnetischen Ablenkmodulen die Zellen nach der Anzahl der gebundenen magnetischen Beads differenziert werden.

[0031] Die Dimensionen eines Moduls richten sich nach der Größe der Zellen, die sortiert werden sollen. Der Durchmesser der Kanäle für Bakterien beträgt in der Regel 5 - 10 µm, der für eukaryontische Zellen 30 - 60 µm. Als Grundmaterial für die mikrostrukturierte Sortiervorrichtung dient vorzugsweise Silizium, da sich hier sowohl Kanalstrukturen und optische Detektionselemente (Photodioden) als auch die Steuerelektronik monolithisch integrieren lassen. Hierbei werden die in der Halbleitertechnik üblichen Verfahren der Photolithographie, Ionendiffusion, Aufdampftechniken und naßchemisches Ätzen eingesetzt.

Patentansprüche

1. Sortiervorrichtung für in Flüssigkeiten dispergierte biologische Objekte, bei der die Objekte in einer für die Sortierung signifikanten Weise magnetisch markiert sind, bestehend aus

i) einem mit einer Zuführung (1) verbundenen, von der biologischen Flüssigkeit durchströmten Hauptkanal (3) und einer am Hauptkanal (3) angeordneten Weiche (11) mit mindestens zwei Auslasskanälen (19, 20) und

ii) einem an der Weiche (11) angebrachten Sortieraktor (12), der die magnetisch markierten Zellen selektiv auf einen der beiden Auslasskanäle (19, 20) verteilt,

dadurch gekennzeichnet,

a) **dass** sich die Zuführung (1) in ein mikrostrukturiertes System von multiparallelen Hauptkanälen (3) und Sortiermodulen (4) mit jeweils einer Weiche (11) zur Aufteilung in zwei verschiedene Auslasskanäle (19, 20) ver-

zweigt,

b) **dass** innerhalb eines Sortiermoduls (4) an jedem Hauptkanal (3) ein magnetischer Ablenkmodul (37, 38) als Sortieraktor (12) angeordnet ist und

c) **dass** jeder Auslasskanal (20) mit einem Summenkanal (6) für die unselektierten Zellen und jeder Auslasskanal (19) mit einem Summenkanal (5) für die selektierten Zellen verbunden ist.

2. Sortiervorrichtung für in Flüssigkeiten dispergierte biologische Objekte, bei der die Objekte gegebenenfalls in einer für die Sortierung signifikanten Weise markiert sind, bestehend aus

i) einem mit einer Zuführung (1) verbundenen, von der biologischen Flüssigkeit durchströmten Hauptkanal (3) und einer am Hauptkanal (3) angeordneten Weiche (11) mit mindestens zwei Auslasskanälen (19, 20),

ii) mindestens einem am Hauptkanal (3) angeordneten Sensor (9 oder 10), der die Zellen detektiert und klassifiziert und

iii) einem an der Weiche (11) angebrachten Sortieraktor (12), der die klassifizierten Zellen anschließend selektiv auf einen der beiden Auslasskanäle (19, 20) verteilt,

dadurch gekennzeichnet,

a) **dass** sich die Zuführung (1) in ein mikrostrukturiertes System von multiparallelen Hauptkanälen (3) und Sortiermodulen (4) mit jeweils einer Weiche (11) zur Aufteilung in zwei verschiedene Auslasskanäle (19, 20) verzweigt,

b) **dass** innerhalb eines Sortiermoduls (4) an jedem Hauptkanal (3) mindestens ein Sensor (9 oder 10) und an jeder Weiche (11) ein von dem jeweiligen Sensor gesteuerter Sortieraktor (12) angeordnet ist und

c) **dass** jeder Auslasskanal (20) mit einem Summenkanal (6) für die unselektierten Zellen und jeder Auslasskanal (19) mit einem Summenkanal (5) für die selektierten Zellen verbunden ist.

3. Sortiervorrichtung nach den Ansprüchen 1 und 2, **dadurch gekennzeichnet, dass** an jedem Hauptkanal (3) ein optischer Sensor (9) zur Bestimmung der Zellengröße angeordnet ist.

4. Sortiervorrichtung nach den Ansprüchen 1 bis 3, **dadurch gekennzeichnet, dass** die an den Hauptkanälen (3) angeordneten optischen Sensoren (9 und/oder 10) über Lichtleiter mit einer externen Lichtquelle (13) verbunden sind.

5. Sortiervorrichtung nach den Ansprüchen 1 bis 4, **dadurch gekennzeichnet, dass** mindestens ein optischer Sensor aus einem Fluoreszenzsensor (10) besteht.

6. Sortiervorrichtung nach den Ansprüchen 1 bis 5, **dadurch gekennzeichnet, dass** das mikrostrukturierte System einschließlich der optischen Sensoren (9 und/oder 10), der Sortieraktoren (12) und einer mit den Sensoren (9 und/oder 10) und den Sortieraktoren (12) verbundenen Auswerte- und Steuerelektronik (15) in einen monolithischen Block integriert sind.

7. Sortiervorrichtung nach Anspruch 6, **dadurch gekennzeichnet, dass** der monolithische Block 200 bis 1.000 Sortiermodule (4) enthält und dass die lichte Weite der Kanäle (3, 19, 20) 5 bis 60 µm beträgt.

8. Sortiervorrichtung nach den Ansprüchen 6 und 7, **dadurch gekennzeichnet, dass** sämtliche Elemente des mikrostrukturierten Systems einschließlich der Steuerelektronik (15) fotolithografisch in einen Silizium-Chip integriert sind.

9. Sortiervorrichtung nach den Ansprüchen 1 bis 8, **dadurch gekennzeichnet, dass** die Sortiermodule (4) mit einem gemeinsamen externen Rechner (18) verbunden sind, der den Schwellwert und den Maximalwert für die Selektierung der Zellen vorgibt.

10. Sortiervorrichtung nach den Ansprüchen 1 bis 9, **dadurch gekennzeichnet, dass** als Sortieraktoren (12) zur Steuerung der biologischen Flüssigkeitsströme piezoelektrische, magnetostruktive, fluidische oder ultraschallaktive Bauelemente eingesetzt werden.

Claims

1. Sorting device for biological objects dispersed in liquids, wherein the objects are magnetically labelled in a significant manner for sorting, consisting of

i) a main channel (3) which is connected to a feed inlet (1) and through which the biological liquid flows and a switch unit (11) which is arranged on the main channel (3) and comprises at least two outlet channels (19, 20) and

ii) a sorting actuator (12) which is arranged on the switch unit (11) and which distributes the magnetically labelled cells selectively to one of the two outlet channels (19, 20),

characterized in that

a) the feed inlet (1) branches into a microstructured system of multiparallel main channels (3) and sorting modules (4) each having a switch unit (11) for division into two different outlet channels (19, 20),

b) a sorting module (4) has a magnetic deflection module (37, 38) arranged in it on every main channel (3) as a sorting actuator (12), and

c) every outlet channel (20) is connected to a summing channel (6) for the unselected cells and every outlet channel (19) is connected to a summing channel (5) for the selected cells.

2. Sorting device for biological objects dispersed in liquids, wherein the objects are magnetically labelled in a significant manner for sorting, consisting of

i) a main channel (3) which is connected to a feed inlet (1) and through which the biological liquid flows and a switch unit (11) which is arranged on the main channel (3) and comprises at least two outlet channels (19, 20),

ii) at least one sensor (9 or 10) which is arranged on the main channel (3) and which detects and classifies the cells, and

iii) a sorting actuator (12) which is arranged on the switch unit (11) and which distributes the classified cells selectively to one of the two outlet channels (19, 20),

characterized in that

a) the feed inlet (1) branches into a microstructured system of multiparallel main channels (3) and sorting modules (4) each having a switch unit (11) for division into two different outlet channels (19, 20),

b) inside a sorting module (4) at least one sensor (9 or 10) is arranged on each main channel (3) and a sorting actuator (12) controlled by the sensor in question is arranged on each switch unit (11)

c) every outlet channel (20) is connected to a summing channel (6) for the unselected cells and every outlet channel (19) is connected to a

summing channel (5) for the selected cells.

3. Sorting device according to Claims 1 and 2, **characterized in that** an optical sensor (9) for determining cell size is arranged on each main channel (3).

4. Sorting device according to Claims 1 to 3, **characterized in that** the optical sensors (9 and/or 10) arranged on the main channels (3) are connected via light guides to an external light source (13).

5. Sorting device according to Claims 1 to 4, **characterized in that** at least one optical sensor comprises a fluorescence sensor (10).

6. Sorting device according to Claims 1 to 5, **characterized in that** the microstructured system including the optical sensors (9 and/or 10), the sorting actuators (12) and an evaluation and control electronic system (15) connected to the sensors (9 and/or 10) and the sorting actuators (12) are integrated in a monolithic block.

7. Sorting device according to Claim 6, **characterized in that** the monolithic block contains 200 to 1 000 sorting modules (4) and **in that** the internal diameter of the channels (3, 19, 20) is 5 to 60 μm .

8. Sorting device according to Claims 6 and 7, **characterized in that** all the elements of the microstructured system including the control electronic system (15) are photolithographically integrated in a silicon chip.

9. Sorting device according to Claims 1 to 8, **characterized in that** the sorting modules (4) are connected to a common external computer (18) which presets the threshold value and the maximum value for the selection of the cells.

10. Sorting device according to Claims 1 to 9, **characterized in that** the sorting actuators (12) used for controlling the biological streams of liquid are piezoelectric, magnetostrictive, fluidic or ultrasonic components.

Revendications

1. Dispositif de triage pour des objets biologiques dispersés dans des liquides, dans lequel les objets sont marqués par voie magnétique d'une manière significative pour le triage, constitué par :

i) un canal principal (3) relié à une conduite d'amenée (1) et traversé par le liquide biologique, et par un aiguillage (11) agencé sur le canal principal (3) et comprenant au moins deux

canaux de sortie (19, 20), et
 ii) par un actionneur de triage (12) agencé sur l'aiguillage (11), qui répartit les cellules marquées par voie magnétique sélectivement vers l'un des deux canaux de sortie (19, 20),

caractérisé en ce que

a) la conduite d'amenée (1) se bifurque en un système microstructuré de canaux principaux multiparallèles (3) et de modules de triage (4) avec un aiguillage respectif (11) pour la subdivision en deux différents canaux de sortie (19, 20),

b) un module de déviation magnétique (37, 38) est agencé en tant qu'actionneur de triage (12) à l'intérieur d'un module de triage (4) sur chaque canal principal (3), et

c) chaque canal de sortie (20) est relié à un canal de sommation (6) pour les cellules non sélectionnées et chaque canal de sortie (19) est relié à un canal de sommation (5) pour les cellules sélectionnées.

2. Dispositif de triage pour des objets biologiques dispersés dans des liquides, dans lequel les objets sont marqués le cas échéant d'une manière significative pour le triage, constitué par :

i) un canal principal (3) relié à une conduite d'amenée (1) et traversé par le liquide biologique, et par un aiguillage (11) agencé sur le canal principal (3) et comprenant au moins deux canaux de sortie (19, 20),

ii) au moins un détecteur (9 ou 10) agencé sur le canal principal (3), qui détecte et classe les cellules, et

iii) un actionneur de triage (12) agencé sur l'aiguillage (11), qui répartit sélectivement ensuite les cellules classées vers l'un des deux canaux de sortie (19, 20),

caractérisé en ce que

a) la conduite d'amenée (1) se bifurque en un système microstructuré de canaux principaux multiparallèles (3) et de modules de triage (4) avec un aiguillage respectif (11) pour la subdivision en deux différents canaux de sortie (19, 20),

b) au moins un détecteur (9 ou 10) est agencé à l'intérieur d'un module de triage (4) sur chaque canal principal (3), et un actionneur de triage (12) commandé par le détecteur respectif est agencé sur chaque aiguillage (11), et

c) chaque canal de sortie (20) est relié à un canal de sommation (6) pour les cellules non sélectionnées et chaque canal de sortie (19) est

relié à un canal de sommation (5) pour les cellules sélectionnées.

3. Dispositif de triage selon les revendications 1 et 2, **caractérisé en ce que** sur chaque canal principal (3) est agencé un détecteur optique (9) pour déterminer la taille des cellules.

4. Dispositif de triage selon les revendications 1 à 3, **caractérisé en ce que** les détecteurs optiques (9 et/ou 10) agencés sur les canaux principaux (3) sont reliés à une source de lumière externe (13) via un guide de lumière.

5. Dispositif de triage selon les revendications 1 à 4, **caractérisé en ce qu'**au moins un détecteur optique est constitué par un détecteur de fluorescence (10).

6. Dispositif de triage selon les revendications 1 à 5, **caractérisé en ce que** le système microstructuré, y compris les détecteurs optiques (9 et/ou 10), les actionneurs de triage (12) et une électronique d'évaluation et de commande (15) reliée aux détecteurs (9 et/ou 10) et aux actionneurs de triage (12), sont intégrés dans un bloc monolithique.

7. Dispositif de triage selon la revendication 6, **caractérisé en ce que** le bloc monolithique comprend 200 à 1000 modules de triage (4), et **en ce que** la largeur libre des canaux (3, 19, 20) est de 5 à 60 μm .

8. Dispositif de triage selon les revendications 6 et 7, **caractérisé en ce que** tous les éléments du système microstructuré, y compris l'électronique de commande (15), sont intégrés dans une puce de silicium par voie photolithographique.

9. Dispositif de triage selon les revendications 1 à 8, **caractérisé en ce que** les modules de triage (4) sont reliés à un calculateur externe commun (18) qui prédétermine la valeur seuil et la valeur maximale pour la sélection des cellules.

10. Dispositif de triage selon les revendications 1 à 9, **caractérisé en ce qu'**en tant qu'actionneurs de triage (12) pour commander les courants de liquide biologiques, on utilise des composants piézo-électriques, magnétostrictifs, fluidiques ou activés par ultrasons.

Fig. 1

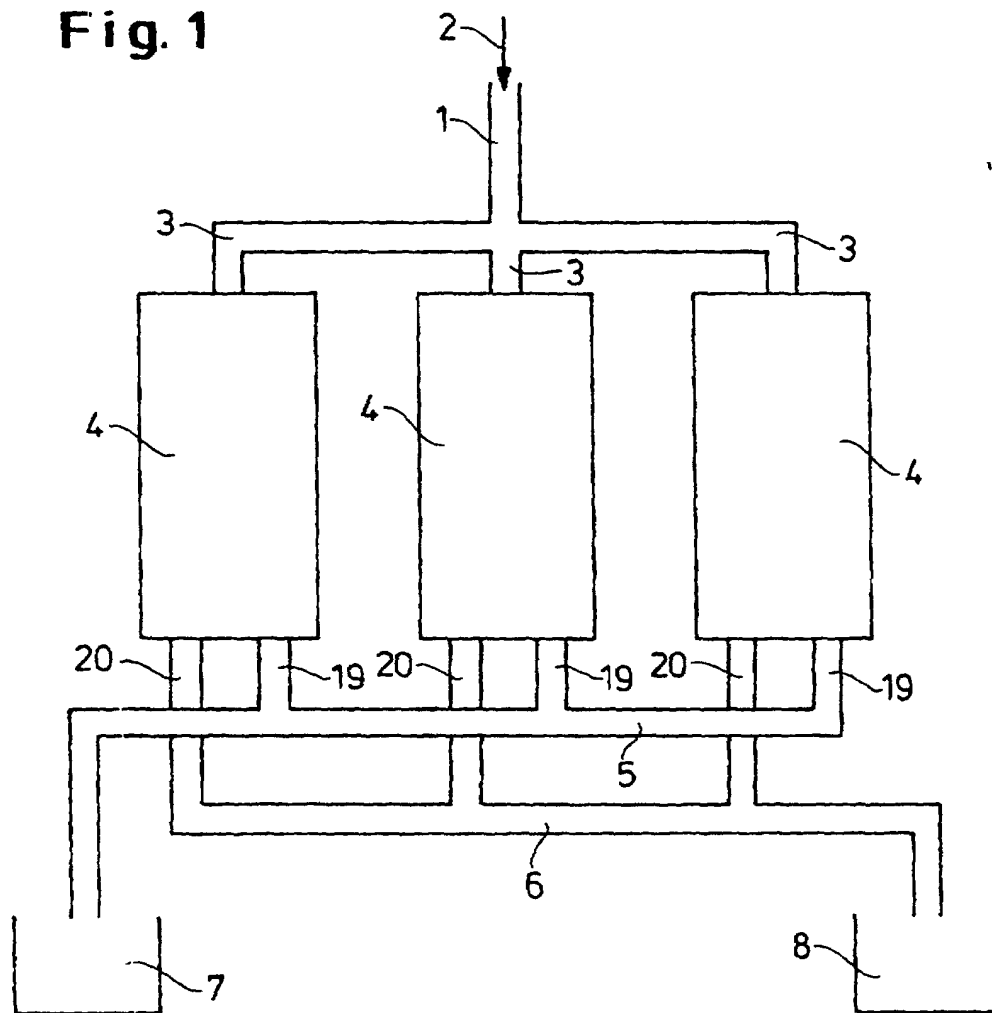


Fig. 2

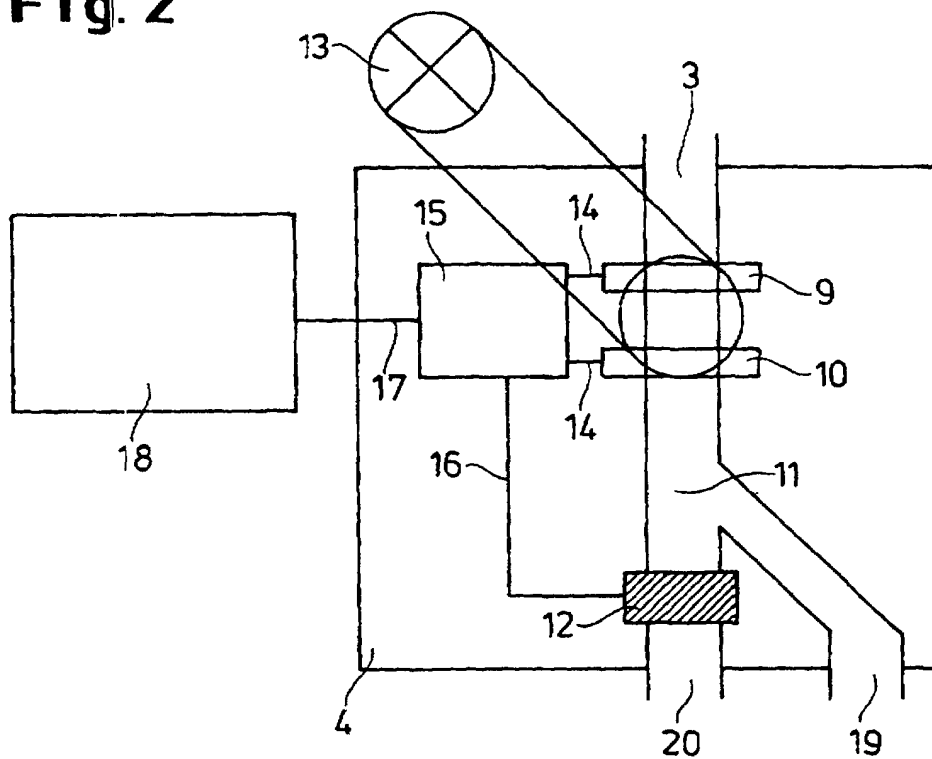


Fig. 3a

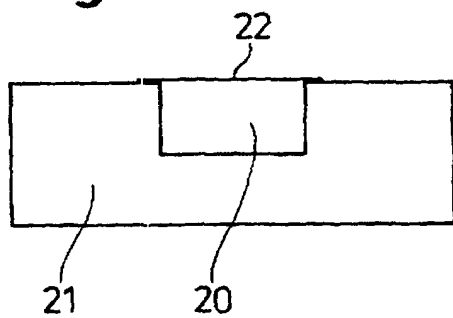


Fig. 3b

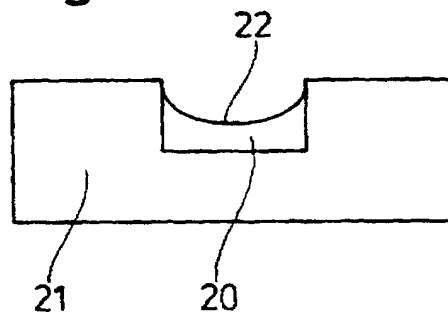


Fig. 4

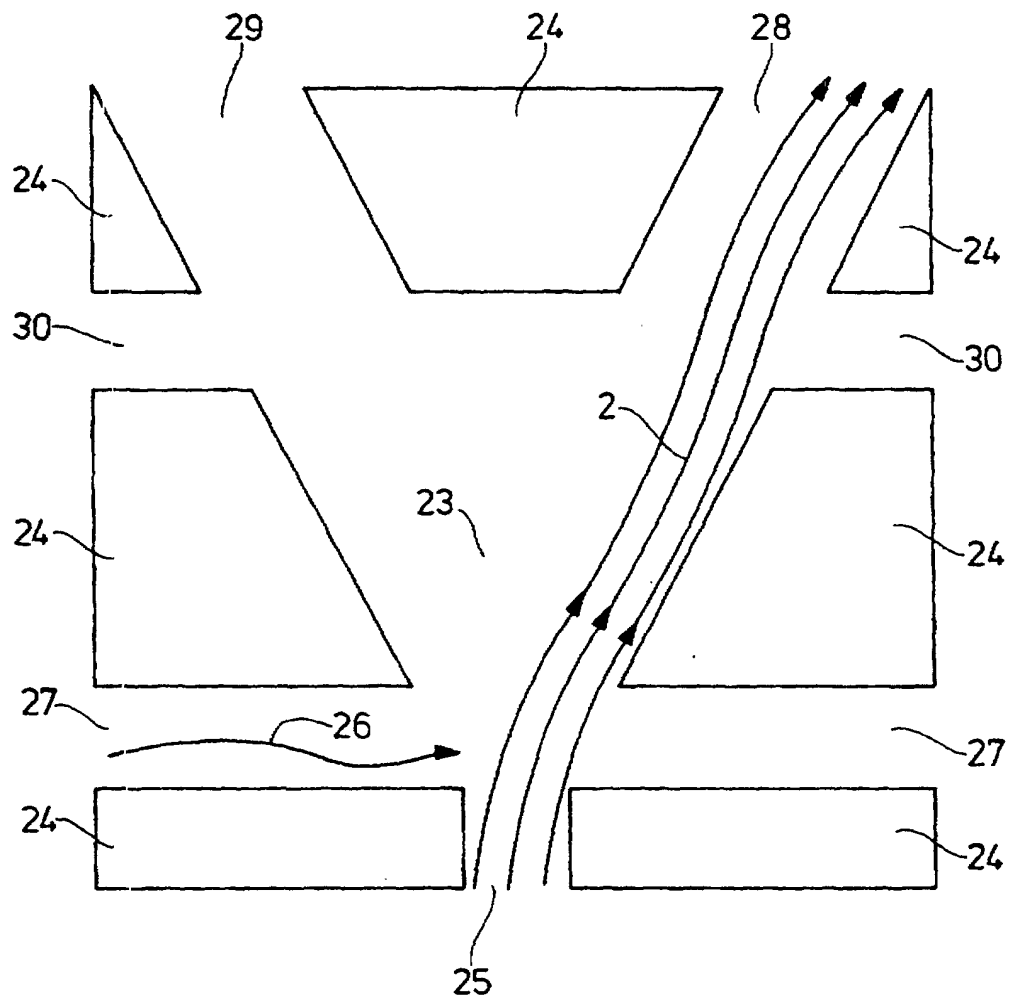


Fig. 5a

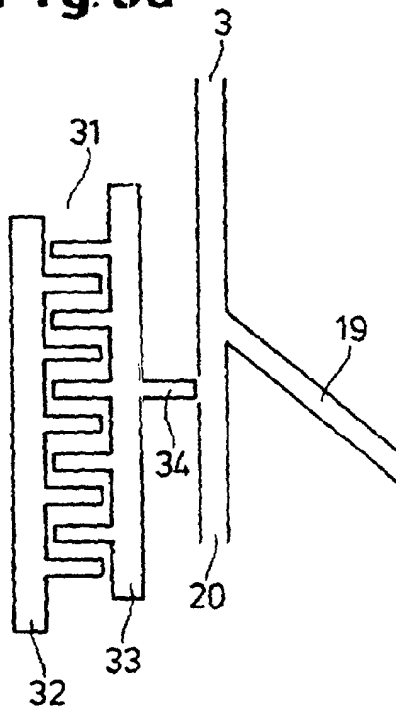


Fig. 5b

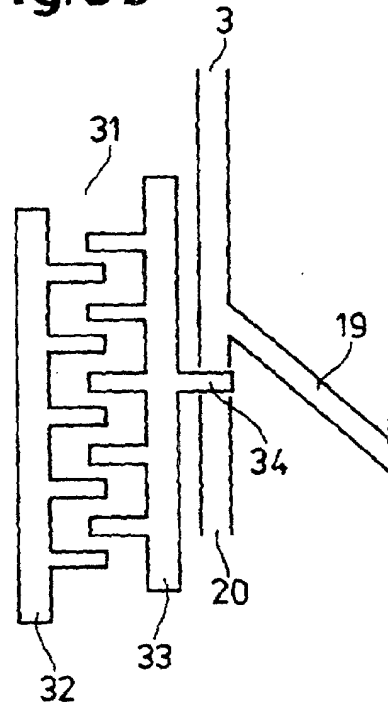


Fig. 6

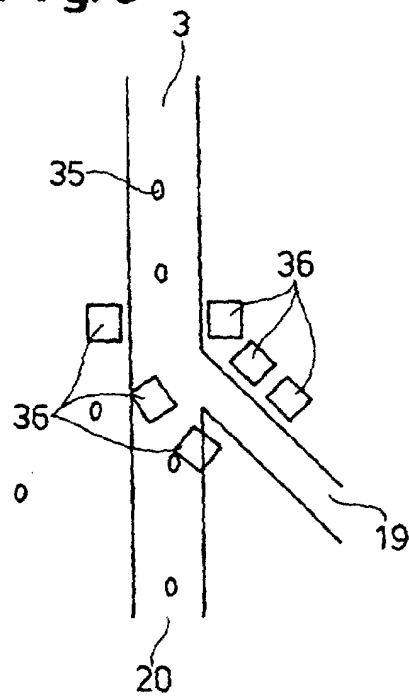
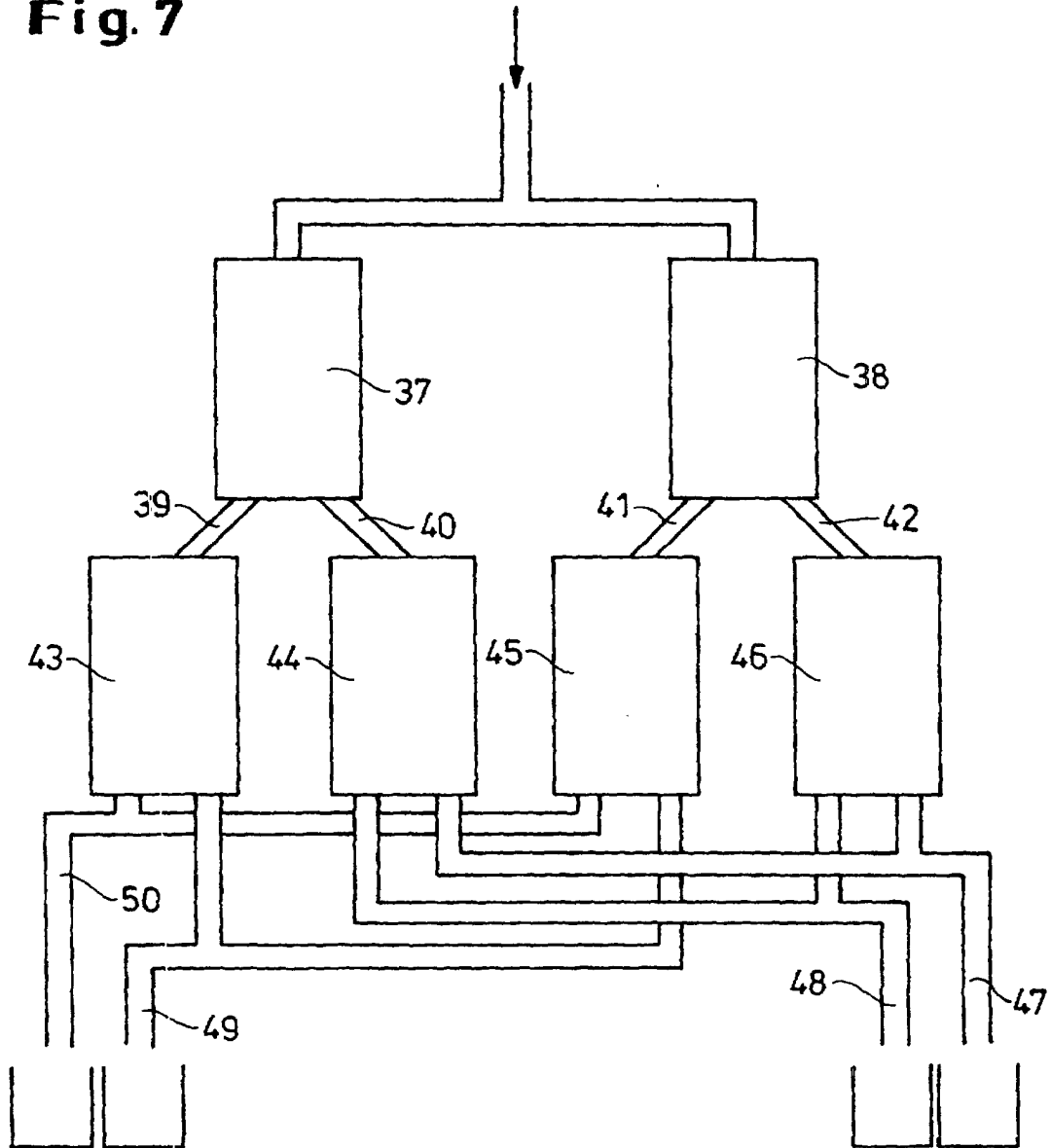
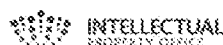


Fig. 7





Espacenet

Bibliographic data: EP0745682 (A1) — 1996-12-04

Sorting apparatus for biological cells and viruses

Inventor(s): DIESSEL EDGAR DR [DE]; WEICHEL WALTER DR [DE] ±

Applicant(s): BAYER AG [DE] ±

Classification:
 - international: *B01L3/00; B03C1/035; C12M1/00; C12M1/42; C12Q1/04; C12Q1/24; C12Q1/70; F15C1/08; G01N33/569; G01N15/14; (IPC1-7): C12Q1/02; C12Q1/24; G01N33/569*

- european: *B01L3/00C6M; B01L3/00C6M16; B03C1/035; F15C1/08*

Application number: EP19960108042 19960521

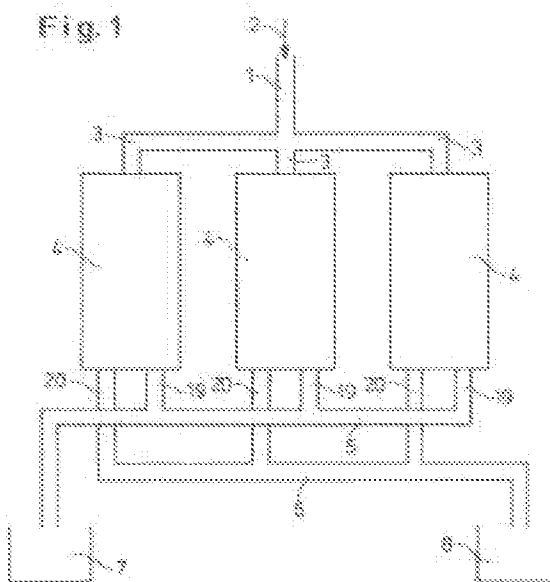
Priority number(s): DE19951020298 19950602

Also published as: EP0745682 (B1) US5837200 (A) JP8332074 (A) DE19520298 (A1)

Abstract of EP0745682 (A1)

Sorting of magnetically marked cells or viruses, dispersed in fluids, in an arrangement which comprises a main channel (3) with diverter (11) having at least two outlet channels (19,20), one of which has a sorting device (12) for selective sepn. of magnetically marked cells. The system includes a number of such modules in parallel, feeding a collection channel for selected cells and a collective channel for unselected channels. At least 1 sensor (9, 10) is provided on each main channel, connected to the sorting device. The sensor is pref. an optical sensor which may be connected to the channel by an optical fibre.

Fig. 1



Last updated: 5.12.2011 Worldwide Database 5.7.31; 92p

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/12368

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B07C 5/34; B03B 5/22

US CL : 209/3.2, 155

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 209/3.1, 3.2, 155, 552, 576, 906

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST (search terms: cell, sorting, microstructure, microfabricated, fluid, sensor, speed, velocity)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | US 3,508,654 A (GLAETTLI) 28 April 1970 (28.04.1970), figures 1-3, column 3, line 23-column 6, line 18, | 1, 2, 4, 5 |
| --- | figure 3, near channel 69. | ----- |
| Y | | 17-19 |
| X | US 5,837,200 A (DIESEL et al) 17 November 1998 (17.11.1998), figures 1 and 2, | 14-16 |
| --- | column 4, line 37-column 5, line 37, | ----- |
| Y | figures 1, 2 and 7. | 7, 8, 13, 20, 24, 25 |
| Y | US 3,560,754 A (KAMENTSKY) 02 February 1971 (02.02.1971), figures 1-2, column 6, lines 11-18. | 3, 15 |
| Y | US 3,984,307 A (KAMENTSKY et al) 05 October 1976 (05.10.1976), column 4, lines 44-68. | 6 |
| Y, E | US 6,597,438 B1 (CABUZ et al) 22 July 2003 (22.07.2003), figures 2-4, 8, column 2, lines 32-42 and column 9, line 55-column 10, line 34. | 7, 8, 13, 20-25 |
| A, P | US 6,432,630 B1 (BLANKENSTEIN) 13 August 2002 (13.08.2002). | |
| A | US 5,976,336 A (DUBROW et al) 02 November 1999 (02.11.1999). | |

| | |
|---|--|
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. | <input type="checkbox"/> See patent family annex. |
| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "B" earlier application or patent published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

| | |
|--|--|
| Date of the actual completion of the international search | Date of mailing of the international search report |
| 11 August 2003 (11.08.2003) | 21 AUG 2003 |
| Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230 | Authorized officer Donald Walsh Telephone No. 703-308-1113 |

INTERNATIONAL SEARCH REPORT

PCT/US03/12368

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| A | US 5,885,470 A (PARCE et al) 23 March 1999 (23.03.1999). | |
| A | US 5,101,978 A (MARCUS) 07 April 1992 (07.04.1992). | |

PATENT COOPERATION TREATY

PCT

REC'D 15 AUG 2004

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | |
|--|---|---|
| Applicant's or agent's file reference TGZ-021CPPC | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) | |
| International application No. PCT/US03/12368 | International filing date (day/month/year) 17 April 2003 (17.04.2003) | Priority date (day/month/year) 17 April 2002 (17.04.2002) |
| International Patent Classification (IPC) or national classification and IPC IPC(7): B07C 5/34; B03B 5/22 and US Cl.: 209/3.2, 155 | | |
| Applicant TERAGENICS, INC. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

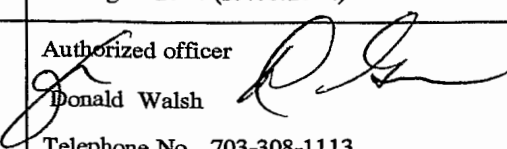
☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the

PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

| | |
|--|--|
| Date of submission of the demand 12 November 2003 (12.11.2003) | Date of completion of this report 25 August 2004 (25.08.2004) |
| Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230 | Authorized officer  Donald Walsh Telephone No. 703-308-1113 |

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US03/12368

I. Basis of the report**1. With regard to the elements of the international application:***

- ☒ the international application as originally filed.
- ☒ the description:
pages 1-22 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.
- ☒ the claims:
pages 23-28, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.
- ☒ the drawings:
pages 1-7, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.
- ☐ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages NONE
- ☐ the claims, Nos. NONE
- ☐ the drawings, sheets/fig NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/12368

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

| | | |
|-------------------------------|--------------------|-----|
| Novelty (N) | Claims <u>1-25</u> | YES |
| | Claims <u>NONE</u> | NO |
| Inventive Step (IS) | Claims <u>1-25</u> | YES |
| | Claims <u>NONE</u> | NO |
| Industrial Applicability (IA) | Claims <u>1-25</u> | YES |
| | Claims <u>NONE</u> | NO |

2. CITATIONS AND EXPLANATIONS

Claims 1-6 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a particle sorting system with sensors for determining particle size and velocity, wherein the sorting actuator modifies a pressure in a sealed chamber to deflect a meniscus formed by a carrier fluid so that particles are deflected into a second outlet.

Claims 7-13, 23 and 24 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a microfabricated particle sorting system with a first sensor for sensing a predetermined characteristic and a second sensor for determining a particle velocity.

Claims 14-22 and 25 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a particle sorting system with primary and secondary sorting channels that carry a flow of particles suspended in a carrier liquid, wherein an enrichment region adjusts a volume of carrier liquid surrounding the particles after the particles having a predetermined characteristic are aggregated and said secondary sorting channels are in series with the plurality of plural primary sorting channels.

Claims 1-25 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in the particle sorting industry.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 October 2003 (30.10.2003)

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(10) International Publication Number
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CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD,
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VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

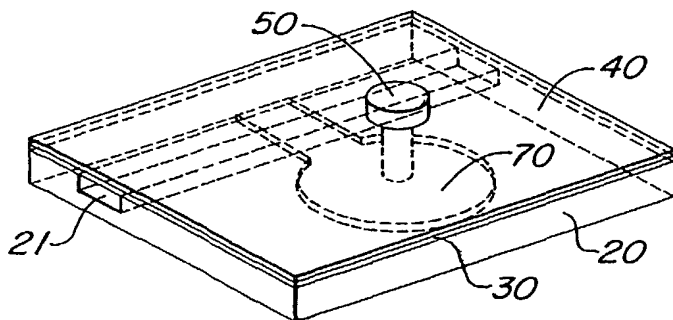
Published:

- with international search report
- before the expiration of the time limit for amending the
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*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID THROUGH A MICROCHANNEL



(57) Abstract: A microfluidic system includes a bub-
ble valve for regulating fluid flow through a microchan-
nel (21). The bubble valve includes a fluid meniscus
interfacing the microchannel (21) interior and an actu-
ator (50) for deflecting the membrane (40) into the mi-
crochannel (21) interior to regulate fluid flow. The ac-
tuator (50) generates a gas bubble in a liquid in the mi-
crochannel (21) when a sufficient pressure is generated
on the membrane (40).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/12360

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : F15B 21/00, F16K 31/02

US CL : 137/806, 807, 827, 830, 831, 833; 204/601

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 137/806, 807, 827, 830, 831, 833; 204/601

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X --- | US, 4,676,274 A (BROWN) 30 June 1987 (30.06.1987), see entire document. | 1-6 and 19 ----- |
| Y | | 7, 8, 12-18 |
| X --- | US 5,092,972 A (GHOWSI) 03 March 1992 (03.03.1992), see entire document. | 23 and 24 ----- |
| Y | | 20-22 |
| X | US 5,065,978 A (ALBARDA et al) 19 November 1991 (19.11.1991), see entire document. | 9-11 |
| Y,E | US 6,561,224 B1 (CHO) 13 May 2003 (13.05.2003) see column 7, lines 4+. | 7, 8, 12-18 |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

25 September 2003 (25.09.2003)

Date of mailing of the international search report

30 OCT 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
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Authorized officer

A. Michael Chambers

Telephone No. 703-308-08

PATENT COOPERATION TREATY

PCT

REC'D 13 SEP 2004

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | | |
|--|--|---|--|
| Applicant's or agent's file reference TGZ-012PC | | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) | |
| International application No. PCT/US03/12360 | International filing date (day/month/year) 17 April 2003 (17.04.2003) | Priority date (day/month/year) 17 April 2002 (17.04.2002) | |
| International Patent Classification (IPC) or national classification and IPC IPC(7): F15B 21/00, F16K 31/02 and US Cl.: 137/806, 807, 827, 830, 831, 833; 204/601 | | | |
| Applicant TERAGENICS, INC. CYTONOME, INC. | | | |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>3</u> sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of ___ sheets.</p> <p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the report</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of report with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the international application</p> | | | |
| Date of submission of the demand 12 November 2003 (12.11.2003) | | Date of completion of this report 21 July 2004 (21.07.2004) | |
| Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230 | | Authorized officer A. Michael Chambers Telephone No. 703-308-0861 Sheila H. Veney Paralegal Specialist Tech. Center 3700 | |

Form PCT/IPEA/409 (cover sheet)(July 1998)

I. Basis of the report1. With regard to the **elements** of the international application:*

- ☒ the international application as originally filed.
- ☒ the description:
pages 1-21 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

- ☒ the claims:
pages 22-25, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

- ☒ the drawings:
pages 1-8, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

- ☐ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE
- ☒ the claims, Nos. NONE
- ☒ the drawings, sheets/fig NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. STATEMENT**

| | | |
|-------------------------------|---|-----|
| Novelty (N) | Claims <u>7,8,12-18,20-22,25 and 26</u> | YES |
| | Claims <u>1-6, 9-11, 19, 23, 24</u> | NO |
| Inventive Step (IS) | Claims <u>NONE</u> | YES |
| | Claims <u>1-26</u> | NO |
| Industrial Applicability (IA) | Claims <u>1-26</u> | YES |
| | Claims <u>NONE</u> | NO |

2. CITATIONS AND EXPLANATIONS

Claims 1-6 and 19 lack novelty under PCT Article 33(2) as being anticipated by Brown.

In Figures 14 and 17 Brown clearly shows a "gas-filled reservoir" responsive to fluid pressure. Actuator recitation is readable on the gas pressure source through inlet 125.

Claims 23 and 24 lack novelty under PCT Article 33(2) as being anticipated by Ghowsi.

Ghowsi clearly show a electrophoretic system including a voltage source.

Claims 9-11 lack novelty under PCT Article 33(2) as being anticipated by Albarda et al.

Albarda clearly shows a diaphragm "actuator" which is discussed in column 7, lines 4+. A number of actuators are disclosed in column 7, lines 35+ including piezoelectric and magnetic.

Claims 7, 8, 12-18 lack an inventive step under PCT Article 33(3) as being obvious over Brown in view of Ghowsi. In Figures 14 and 17 Brown clearly shows a "gas-filled reservoir" responsive to fluid pressure. Actuator recitation is readable on the gas pressure source through inlet 125. Brown shows a "movable membrane" 35 in Figure 2. Ghowsi discloses a number of actuators in column 5, lines 32+ including piezoelectric and magnetic. To modify the microfluidic device of Brown to include a piezoelectric actuator for a microfluidic system, as taught by Ghowsi in order to provide more effective control of the bubble controlled via passage 125 of Brown would not involve an inventive step since Ghowsi shows a a piezoelectric actuator ina is well known to the routineer in the microfluidic valve art to control "bubble" movement. The modified microfluidic "valve" of Brown in view of Ghowsi could be operated by the recited method steps (claims 17-18).

Claims 20-22, 25 and 26 lack an inventive step under PCT Article 33(3) as being obvious over Ghowsi. To operate the microchannel of Ghowsil by the recited method steps would not involve any inventive step and is well known to the routineer in the valve art.

Claims 1-26 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in the microfluidic valve industry.

----- NEW CITATIONS -----

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/12359

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B07C 5/34; B03B 5/22

US CL : 209/3.2, 155

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 209/3.1, 3.2, 155, 552, 576, 906

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST (search terms: fluid, switch, bubble, valve, pressure, pulse, cell, sorting)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | US 5,837,200 A (DIESSEL et al) 17 November 1998 (17.11.1998), figures 1 and 2, column 4, line 37-column 5, line 37, figures 1 and 7. | 14, 15 |
| --- | | ----- |
| Y | | 29-32 |
| X | US 3,508,654 A (GLAETTLI) 28 April 1970 (28.04.1970), figures 1-3, column 3, line 23-column 6, line 18. | 14, 15, 33 |
| Y | US 4,175,662 A (ZOLD) 27 November 1979 (27.11.1979), figure 6. | 29-32 |
| A, P | US 2003/0027225 A1 (WADA et al) 06 February 2003 (06.02.2003). | |
| A | US 5,101,978 A (MARCUS) 07 April 1992 (07.04.1992). | |
| A | US 4,756,427 A (GOHDE et al) 12 July 1988 (12.07.1988). | |
| A | US 3,984,307 A (KAMENSKY et al) 05 October 1976 (05.10.1976). | |

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

| | | | |
|--|---|-----|--|
| * Special categories of cited documents: | | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier application or patent published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

Date of the actual completion of the international search

28 June 2003 (28.06.2003)

Date of mailing of the international search report

02 SEP 2003

Name and mailing address of the ISA/US

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P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

Donald Walsh

Telephone No. 703-308-1113

PATENT COOPERATION TREATY

PCT

| | |
|-------|-------------|
| REC'D | 22 APR 2005 |
| WIPO | PCT |

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | |
|---|--|--|
| Applicant's or agent's file reference TGZ-021PC | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) | |
| International application No. PCT/US03/12359 | International filing date (day/month/year) 17 April 2003 (17.04.2003) | Priority date (day/month/year) 17 April 2002 (17.04.2002) |
| International Patent Classification (IPC) or national classification and IPC IPC(7): B07C 5/34; B03B 5/22 and US Cl.: 209/3.2, 155 | | |
| Applicant TERAGENICS, INC. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

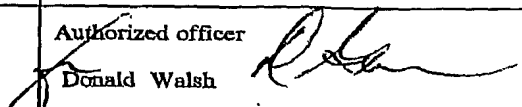
☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the

PCT).

These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

| | |
|--|--|
| Date of submission of the demand 12 November 2003 (12.11.2003) | Date of completion of this report 05 September 2004 (05.09.2004) |
| Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230 | Authorized officer  Donald Walsh Telephone No. 703-308-1113 |

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US03/12359

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.☒ the description:

pages 1-20 as originally filed

pages NONE, filed with the demand

pages NONE, filed with the letter of _____

☒ the claims:

pages _____, as originally filed

pages NONE, as amended (together with any statement) under Article 19

pages NONE, filed with the demand

pages 21-29, filed with the letter of 16 June 2004 (16.06.2004)

☒ the drawings:

pages 1-5, as originally filed

pages NONE, filed with the demand

pages NONE, filed with the letter of _____

☐ the sequence listing part of the description:

pages NONE, as originally filed

pages NONE, filed with the demand

pages NONE, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).☐ the language of publication of the international application (under Rule 48.3(b)).☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.☐ filed together with the international application in computer readable form.☐ furnished subsequently to this Authority in written form.☐ furnished subsequently to this Authority in computer readable form.☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.4. ☐ The amendments have resulted in the cancellation of:☐ the description, pages NONE☐ the claims, Nos. NONE☐ the drawings, sheets/fig NONE5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/12359**V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. STATEMENT**

| | | |
|-------------------------------|--------------------|-----|
| Novelty (N) | Claims <u>1-33</u> | YES |
| | Claims <u>NONE</u> | NO |
| Inventive Step (IS) | Claims <u>1-33</u> | YES |
| | Claims <u>NONE</u> | NO |
| Industrial Applicability (IA) | Claims <u>1-33</u> | YES |
| | Claims <u>NONE</u> | NO |

2. CITATIONS AND EXPLANATIONS

Claims 1-28 and 33 satisfy the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a carrier fluid that forms a meniscus in the side channel to separate the sealed chamber from the carrier fluid and deflects the meniscus when the sensor senses a predetermined characteristic or a buffer chamber positioned adjacent to a duct side channel in combination with an actuator that provides a positive pressure pulse that is absorbed by the buffer.

Claim 32 satisfies the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the aggregation region or summing channel that aggregates particles from the plurality of primary sorting channels and directs said particles to at least one secondary sorting channel.

Claims 29-31 satisfy the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest each of said two outlet channels on each sorting module connected to a separate summing channel based on a predetermined characteristic and at least one secondary sorting module connected to the respective summing channel.

Claims 1-33 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in the object sorting industries.

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 13551247 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John Richard Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns/Ishna Neamatullah |
| Filer Authorized By: | David Burns |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 21-AUG-2012 |
| Filing Date: | 19-JUN-2012 |
| Time Stamp: | 20:54:01 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|------------------------|----|
| Submitted with Payment | no |
|------------------------|----|

File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|-----------------|----------------------|----------------------|---|------------------|------------------|
| 1 | Transmittal Letter | 118153_01205_IDS.pdf | 14223 f8be48ff9b8a08853d7ceff9451e1746b06c8668 | no | 3 |

Warnings:

Information:

0415

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| 2 | Information Disclosure Statement (IDS) Form (SB08) | 118153_01205_SB08.pdf | 35919 | no | 3 |
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| Information: | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| 3 | Foreign Reference | B1.PDF | 282056 | no | 11 |
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| Information: | | | | | |
| 4 | Foreign Reference | B2.PDF | 2630994 | no | 62 |
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| Information: | | | | | |
| 6 | Foreign Reference | B4.PDF | 3586325 | no | 91 |
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| Warnings: | | | | | |
| Information: | | | | | |
| 7 | Foreign Reference | B5.PDF | 608435 | no | 15 |
| | | | 78b88994854af584bb74bc274b2bff52897e16ca | | |
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| Information: | | | | | |
| 8 | Foreign Reference | B6.PDF | 160499 | no | 14 |
| | | | e277323f4e21752b8b670042734039de03ee6311 | | |
| Warnings: | | | | | |
| Information: | | | | | |
| 9 | Non Patent Literature | C1.PDF | 80912 | no | 2 |
| | | | 8ff18195a6ef63efdcdf50043e5cb07734db8824 | | |
| Warnings: | | | | | |
| Information: | | | | | |
| 10 | Non Patent Literature | C2.PDF | 174480 | no | 3 |
| | | | 0e4005ebfc627b22bd47de8556832a6928dfab5d | | |

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|------------------------------|-----------------------|---------|--|----|---|
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| Information: | | | | | |
| 11 | Non Patent Literature | C3.PDF | 112619 | no | 2 |
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| Information: | | | | | |
| 12 | Non Patent Literature | C4.PDF | 186537 | no | 3 |
| | | | c995f06ce476f9dc5ac740d0aa3430fca4073218 | | |
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| Information: | | | | | |
| 13 | Non Patent Literature | C5.PDF | 65189 | no | 1 |
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| Information: | | | | | |
| 16 | Non Patent Literature | C8.PDF | 200668 | no | 6 |
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| Warnings: | | | | | |
| Information: | | | | | |
| 17 | Non Patent Literature | C9.PDF | 52521 | no | 3 |
| | | | fe979b0f27b831f98daaf47ca3c1bb115b4c4255 | | |
| Warnings: | | | | | |
| Information: | | | | | |
| 18 | Non Patent Literature | C10.PDF | 66030 | no | 3 |
| | | | 1947bd559513366049679ad47598bb0ad6fd1d25 | | |
| Warnings: | | | | | |
| Information: | | | | | |
| Total Files Size (in bytes): | | | 10718027 | | |

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: August 21, 2012
Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: 118153-01205
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John Richard Gilbert *et al.*

Application No. 13/527,331

Confirmation No.: 1757

Filed: June 19, 2012

Art Unit: 2853

For: MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING
FLUID FLOW THROUGH A
MICROCHANNEL

Examiner: Not Yet Assigned

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT (IDS)

Dear Sir/Madam:

Pursuant to 37 C.F.R. §§ 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08 form. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), Applicants have not submitted copies of U.S. patents and U.S. patent applications. Applicants submit herewith copies of the cited foreign patents and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

Applicants also wish to bring to the attention of the Examiner the following applications which contain related subject matter:

| U.S. Application / Patent No. | Filing Date | Publication No. | Status | Attorney Docket No. |
|--------------------------------------|--------------------|------------------------|---------------|----------------------------|
| 10/179,586 | 06-24-2002 | 6,877,528 | Granted | 118153-01201 |
| 11/021,251 | 12-21-2004 | 7,069,943 | Granted | 118153-01202 |
| 11/433,781 | 05-12-2006 | 8,210,209 | Granted | 118153-01203 |
| 13/245,331 | 09-26-2011 | 2012-0015442 | Pending | 118153-01204 |

Applicants understand that papers from the prosecution of the above-identified cases may be accessed electronically via PAIR. Accordingly, copies of the foregoing applications or file histories thereof are not provided herewith, but will be made available upon request.

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 C.F.R. § 1.56(a) exists. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication or other information referred to therein is “prior art” for this invention unless specifically designated as such.

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and the Examiner is respectfully requested to consider the cited references.

This Information Disclosure Statement is being filed within three months of the filing date of the above-referenced application under 37 C.F.R. § 1.97(b)(1). Therefore, Applicants believe that no fee is due. Nonetheless, the Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-4876, under Attorney Docket No. 118153-01205.

Dated: August 21, 2012

Respectfully submitted,

Electronic signature: /David R. Burns/
David R. Burns, Esq.
Registration No.: 46,590
McCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110
Tel: (617) 449-6500
Fax: (617) 607-9200
Attorney/Agent For Applicants

| PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875 | | | | | | Application or Docket Number 13/527,331 | |
|--|---|------------------------------------|--------------------|--------------|--------------------|--|--------------------|
| APPLICATION AS FILED - PART I | | | | | | | |
| (Column 1) | | (Column 2) | | SMALL ENTITY | | OTHER THAN SMALL ENTITY | |
| FOR | NUMBER FILED | NUMBER EXTRA | RATE(\$) | FEE(\$) | | RATE(\$) | FEE(\$) |
| BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | 95 | | N/A | |
| SEARCH FEE (37 CFR 1.16(k), (l), or (m)) | N/A | N/A | N/A | 310 | | N/A | |
| EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | 125 | | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(j)) | 19 | minus 20 = * | x 30 | = 0.00 | OR | | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 5 | minus 3 = * | x 125 | = 250 | | | |
| APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | 0.00 | | | |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | 0.00 | | | |
| | | | TOTAL | 780 | | TOTAL | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | | | | | |
| APPLICATION AS AMENDED - PART II | | | | | | | |
| (Column 1) | | (Column 2) | | (Column 3) | | SMALL ENTITY | |
| AMENDMENT A | CLAIMS REMAINING AFTER AMENDMENT | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$) | ADDITIONAL FEE(\$) | | RATE(\$) |
| | Total (37 CFR 1.16(i)) | * | Minus ** | = | x | = | OR |
| | Independent (37 CFR 1.16(h)) | * | Minus *** | = | x | = | OR |
| | Application Size Fee (37 CFR 1.16(s)) | | | | | | OR |
| | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | OR |
| | | | TOTAL ADD'L FEE | | | OR | TOTAL ADD'L FEE |
| (Column 1) | | (Column 2) | | (Column 3) | | SMALL ENTITY | |
| AMENDMENT B | CLAIMS REMAINING AFTER AMENDMENT | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$) | ADDITIONAL FEE(\$) | | RATE(\$) |
| | Total (37 CFR 1.16(i)) | * | Minus ** | = | x | = | OR |
| | Independent (37 CFR 1.16(h)) | * | Minus *** | = | x | = | OR |
| | Application Size Fee (37 CFR 1.16(s)) | | | | | | OR |
| | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | OR |
| | | | TOTAL ADD'L FEE | | | OR | TOTAL ADD'L FEE |
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1. | | | | | | | |



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY. DOCKET NO | TOT CLAIMS | IND CLAIMS |
|-----------------------|--------------------------|-----------------|---------------|-----------------|------------|------------|
| 13/527,331 | 06/19/2012 | 2853 | 780 | 118153-01205 | 19 | 5 |

CONFIRMATION NO. 1757

86738
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

FILING RECEIPT



OC00000005522775

Date Mailed: 07/10/2012

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

John Richard Gilbert, Brookline, MA;
Sebastian Böhm, Iwerness, UNITED KINGDOM;
Manish Deshpande, Canton, MA;

Assignment For Published Patent Application

CYTONOME/ST, LLC, Boston, MA

Power of Attorney: The patent practitioners associated with Customer Number 86738

Domestic Priority data as claimed by applicant

This application is a CON of 11/433,781 05/12/2006 PAT 8210209
which is a CON of 11/021,251 12/21/2004 PAT 7069943
which is a CON of 10/179,586 06/24/2002 PAT 6877528
which claims benefit of 60/373,256 04/17/2002

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.)

If Required, Foreign Filing License Granted: 07/03/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/527,331**

Projected Publication Date: 10/18/2012

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW
THROUGH A MICROCHANNEL

Preliminary Class

346

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

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NOT GRANTED

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www.uspto.gov

| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|--------------------|-----------------------|-----------------------|------------------------|
| 13/527,331 | 06/19/2012 | John R. Gilbert | 118153-01205 |

86738
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

CONFIRMATION NO. 1757
POA ACCEPTANCE LETTER



Date Mailed: 07/10/2012

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 06/19/2012.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/tqlam/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Electronic Acknowledgement Receipt

| | |
|--|---|
| EFS ID: | 13054619 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| <p>06/29/2012 MTEKLEMI 00000017 504876 13527331</p> <p>01 FC:1201 500.00 DA</p> <p>Adjustment date: 07/05/2012 MTEKLEMI</p> <p>06/29/2012 MTEKLEMI 00000017 504876 13527331</p> <p>01 FC:1201 500.00 CR</p> <p>Title of Invention:</p> <p>Adjustment date: 07/05/2012 MTEKLEMI</p> <p>06/20/2012 INTEFSW 00005419 504876 13527331</p> <p>01 FC:1011 380.00 CR</p> <p>02 FC:1111 620.00 CR</p> <p>03 FC:1311 250.00 CR</p> | <p>07/05/2012 MTEKLEMI 00000010 504876 13527331</p> <p>01 FC:4011 95.00 DA</p> <p>02 FC:2111 310.00 DA</p> <p>03 FC:2311 125.00 DA</p> <p>04 FC:2201 250.00 DA</p> <p> MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL</p> |
| First Named Inventor/Applicant Name: | John R. Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns/Gabriel Goldman |
| Filer Authorized By: | David Burns |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 19-JUN-2012 |
| Filing Date: | |
| Time Stamp: | 18:02:20 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|---|-----------------|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | \$1250 |
| RAM confirmation Number | 5419 |
| Deposit Account | 504876 |
| Authorized User | |
| <p>The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:</p> <p>Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)</p> | |

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| | | | | | | | | | |
|---|---|---------------------|--------------|----------------|-----------------|-------|---|------------------------|--|
| UTILITY PATENT APPLICATION TRANSMITTAL <small>(ONLY FOR NEW NONPROVISIONAL APPLICATIONS UNDER 37 CFR 1.53(B))</small> | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%;">Attorney Docket No.</td> <td>118153-01205</td> </tr> <tr> <td>First Inventor</td> <td>John R. Gilbert</td> </tr> <tr> <td>Title</td> <td>MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL</td> </tr> <tr> <td>Express Mail Label No.</td> <td></td> </tr> </table> | Attorney Docket No. | 118153-01205 | First Inventor | John R. Gilbert | Title | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | Express Mail Label No. | |
| Attorney Docket No. | 118153-01205 | | | | | | | | |
| First Inventor | John R. Gilbert | | | | | | | | |
| Title | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | | | | | | |
| Express Mail Label No. | | | | | | | | | |

| | |
|--|---|
| APPLICATION ELEMENTS <small>See MPEP chapter 600 concerning utility patent application contents.</small> | ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450 |
|--|---|

| | |
|--|--|
| <ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> Fee Transmittal Form (e.g., PTO/SB/17) 2. <input checked="" type="checkbox"/> Applicant claims small entity status. <small>See 37 CFR 1.27.</small> 3. <input checked="" type="checkbox"/> Specification [Total Pages <u>23</u>] <small>Both the claims and abstract must start on a new page (For information on the preferred arrangement, see MPEP 608.01(a))</small> 4. <input checked="" type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets <u>8</u>] 5. Oath or Declaration [Total Sheets <u>8</u>] <ol style="list-style-type: none"> a. <input type="checkbox"/> Newly executed (original or copy) b. <input checked="" type="checkbox"/> A copy from a prior application (37 CFR 1.63(d)) <small>(for continuation/divisional with Box 18 completed)</small> <ol style="list-style-type: none"> i. <input type="checkbox"/> DELETION OF INVENTOR(S) <small>Signed statement attached deleting inventor(s) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).</small> 6. <input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76 7. <input type="checkbox"/> CD-ROM or CD-R in duplicate, large table or Computer Program (<i>Appendix</i>) <input type="checkbox"/> Landscape Table on CD 8. Nucleotide and/or Amino Acid Sequence Submission <small>(if applicable, items a. – c. are required)</small> <ol style="list-style-type: none"> a. <input type="checkbox"/> Computer Readable Form (CRF) b. Specification Sequence Listing on: <ol style="list-style-type: none"> i. <input type="checkbox"/> CD-ROM or CD-R (2 copies); or ii. <input type="checkbox"/> Paper c. <input type="checkbox"/> Statements verifying identity of above copies | ACCOMPANYING APPLICATION PARTS <ol style="list-style-type: none"> 9. <input type="checkbox"/> Assignment Papers (cover sheet & document(s)) Name of Assignee <div style="border: 1px solid black; height: 20px; width: 100%;"></div> 10. <input checked="" type="checkbox"/> 37 CFR 3.73(b) Statement <input checked="" type="checkbox"/> Power of Attorney <small>(when there is an assignee)</small> 11. <input type="checkbox"/> English Translation Document (if applicable) 12. <input type="checkbox"/> Information Disclosure Statement (PTO/SB/08 or PTO-1449) <input type="checkbox"/> Copies of citations attached 13. <input checked="" type="checkbox"/> Preliminary Amendment 14. <input type="checkbox"/> Return Receipt Postcard (MPEP 503) <small>(Should be specifically itemized)</small> 15. <input type="checkbox"/> Certified Copy of Priority Document(s) <small>(if foreign priority is claimed)</small> 16. <input type="checkbox"/> Nonpublication Request under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent. 17. <input type="checkbox"/> Other: <div style="border: 1px solid black; height: 40px; width: 100%;"></div> |
|--|--|

| | |
|--|--|
| 18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76: <input checked="" type="checkbox"/> Continuation <input type="checkbox"/> Divisional <input type="checkbox"/> Continuation-in-part (CIP) of prior application No.: <u>11/433781</u> Prior application information: Examiner <u>Craig M. Schneider</u> Art Unit: <u>3753</u> | |
|--|--|

| | |
|---|--|
| 19. CORRESPONDENCE ADDRESS | |
| <input checked="" type="checkbox"/> The address associated with Customer Number: <u>86738</u> | OR <input type="checkbox"/> Correspondence address below |
| Name | |
| Address | |
| City | State |
| Country | Zip Code |
| Telephone | Email |
| Signature | Date |
| /David R. Burns/ | June 19, 2012 |
| Name (Print/Type) | Registration No. (Attorney/Agent) |
| David R. Burns | 46,590 |

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 19, 2012

Electronic Signature for David R. Burns: /David R. Burns/

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| | | | |
|---|---|------------------------|--------------|
| Application Data Sheet 37 CFR 1.76 | | Attorney Docket Number | 118153-01205 |
| | | Application Number | |
| Title of Invention | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | |
| <p>The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.</p> <p>This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.</p> | | | |

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Applicant Information:

| | | | | | | | |
|---|-------------------|---|----|--|--|---|------|
| Applicant 1 | | | | | | Remove | |
| Applicant Authority | | <input checked="" type="radio"/> Inventor | | <input type="radio"/> Legal Representative under 35 U.S.C. 117 | | <input type="radio"/> Party of Interest under 35 U.S.C. 118 | |
| Prefix | Given Name | Middle Name | | Family Name | | Suffix | |
| Mr. | John | R. | | Gilbert | | | |
| Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service | | | | | | | |
| City | Brookline | State/Province | MA | Country of Residence i | | US | |
| Citizenship under 37 CFR 1.41(b) i | | US | | | | | |
| Mailing Address of Applicant: | | | | | | | |
| Address 1 | | 69 Naples Road | | | | | |
| Address 2 | | | | | | | |
| City | Brookline | State/Province | | MA | | | |
| Postal Code | 02446 | Country i | US | | | | |
| Applicant 2 | | | | | | Remove | |
| Applicant Authority | | <input checked="" type="radio"/> Inventor | | <input type="radio"/> Legal Representative under 35 U.S.C. 117 | | <input type="radio"/> Party of Interest under 35 U.S.C. 118 | |
| Prefix | Given Name | Middle Name | | Family Name | | Suffix | |
| Mr. | Sebastian | | | Böhm | | | |
| Residence Information (Select One) <input type="radio"/> US Residency <input checked="" type="radio"/> Non US Residency <input type="radio"/> Active US Military Service | | | | | | | |
| City | Iverness | Country Of Residence i | | UK | | | |
| Citizenship under 37 CFR 1.41(b) i | | DE | | | | | |
| Mailing Address of Applicant: | | | | | | | |
| Address 1 | | 9 Muirfield Court | | | | | |
| Address 2 | | | | | | | |
| City | Iverness | State/Province | | UK | | | |
| Postal Code | IV2 4DP | Country i | UK | | | | |
| Applicant 3 | | | | | | Remove | |
| Applicant Authority | | <input checked="" type="radio"/> Inventor | | <input type="radio"/> Legal Representative under 35 U.S.C. 117 | | <input type="radio"/> Party of Interest under 35 U.S.C. 118 | |
| Prefix | Given Name | Middle Name | | Family Name | | Suffix | |
| Mr. | Manish | | | Deshpande | | | |
| Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service | | | | | | | |
| City | Canton | State/Province | MA | Country of Residence i | | US | 0429 |

| | | | |
|---|---|------------------------|--------------|
| Application Data Sheet 37 CFR 1.76 | | Attorney Docket Number | 118153-01205 |
| | | Application Number | |
| Title of Invention | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | |

| | | | |
|---|----------------------|----------------------|------------------------------------|
| Citizenship under 37 CFR 1.41(b) i | | IN | |
| Mailing Address of Applicant: | | | |
| Address 1 | 34 Apple Blossom Way | | |
| Address 2 | | | |
| City | Canton | State/Province | MA |
| Postal Code | 02021 | Country ⁱ | US |
| All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button. | | | <input type="button" value="Add"/> |

Correspondence Information:

| | | | |
|---|------------------------|--|---|
| Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a). | | | |
| <input type="checkbox"/> An Address is being provided for the correspondence Information of this application. | | | |
| Customer Number | 86738 | | |
| Email Address | docketing@mccarter.com | <input type="button" value="Add Email"/> | <input type="button" value="Remove Email"/> |

Application Information:

| | | | |
|---|---|---|-------------------------------------|
| Title of the Invention | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | |
| Attorney Docket Number | 118153-01205 | Small Entity Status Claimed | <input checked="" type="checkbox"/> |
| Application Type | Nonprovisional | | |
| Subject Matter | Utility | | |
| Suggested Class (if any) | | Sub Class (if any) | |
| Suggested Technology Center (if any) | N/A | | |
| Total Number of Drawing Sheets (if any) | 8 | Suggested Figure for Publication (if any) | |

Publication Information:

| |
|--|
| <input type="checkbox"/> Request Early Publication (Fee required at time of Request 37 CFR 1.219) |
| <input type="checkbox"/> Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing. |

Representative Information:

| | | | |
|---|--|--|---|
| Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing. | | | |
| Please Select One: | <input checked="" type="radio"/> Customer Number | <input type="radio"/> US Patent Practitioner | <input type="radio"/> Limited Recognition (37 CFR 11.9) |

| | | | |
|---|---|------------------------|--------------|
| Application Data Sheet 37 CFR 1.76 | | Attorney Docket Number | 118153-01205 |
| | | Application Number | |
| Title of Invention | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | |
| Customer Number | 86738 | | |

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

| | | | | | |
|---|--------------------|--------------------------|--------------------------|---------------|-------------------------|
| Prior Application Status | Pending | Remove | | | |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) | | |
| | Continuation of | 11433781 | 2006-05-12 | | |
| Prior Application Status | Patented | Remove | | | |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) | Patent Number | Issue Date (YYYY-MM-DD) |
| 11433781 | Continuation of | 11021251 | 2004-12-21 | 7069943B | 2006-07-04 |
| Prior Application Status | Patented | Remove | | | |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) | Patent Number | Issue Date (YYYY-MM-DD) |
| 11021251 | Continuation of | 10179586 | 2002-06-24 | 6877528B | 2005-04-12 |
| Prior Application Status | Pending | Remove | | | |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) | Patent Number | Issue Date (YYYY-MM-DD) |
| 10179586 | non provisional of | 60373256 | 2002-04-17 | | |
| Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button. Add | | | | | |

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

| | | | |
|--|----------------------|---------------------------------|--|
| Remove | | | |
| Application Number | Country ⁱ | Parent Filing Date (YYYY-MM-DD) | Priority Claimed |
| | | | <input type="radio"/> Yes <input type="radio"/> No |
| Additional Foreign Priority Data may be generated within this form by selecting the Add button. Add | | | |

Assignee Information:

Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of Title 37 of the CFR to have an assignment recorded in the Office.

| | |
|--|------------------------|
| Assignee 1 | Remove |
| If the Assignee is an Organization check here. <input checked="" type="checkbox"/> | |
| Organization Name | Cytonome/ST, LLC |

| | | | |
|---|---|------------------------|--------------|
| Application Data Sheet 37 CFR 1.76 | | Attorney Docket Number | 118153-01205 |
| | | Application Number | |
| Title of Invention | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | |

| | | | |
|--|-------------------|----------------|------------------------------------|
| Mailing Address Information: | | | |
| Address 1 | 27 Drydock Avenue | | |
| Address 2 | | | |
| City | Boston | State/Province | MA |
| Country i | | Postal Code | 02210 |
| Phone Number | | Fax Number | |
| Email Address | | | |
| Additional Assignee Data may be generated within this form by selecting the Add button. | | | |
| | | | <input type="button" value="Add"/> |

Signature:

| | | | | | |
|--|------------------|-----------|-------|---------------------|------------|
| A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature. | | | | | |
| Signature | /David R. Burns/ | | | Date (YYYY-MM-DD) | 2012-06-19 |
| First Name | David | Last Name | Burns | Registration Number | 46590 |

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: June 19, 2012

Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: 118153-01205
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
John R. Gilbert *et al.*

Application No.: Not Yet Assigned

Confirmation No.: Not Yet Assigned

Filed: June 19, 2012

Art Unit: Not Yet Assigned

For: **MICROFLUIDIC SYSTEM INCLUDING A
BUBBLE VALVE FOR REGULATING
FLUID FLOW THROUGH A
MICROCHANNEL**

Examiner: Not Yet Assigned

MS Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

FIRST PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2.

Remarks begin on page 3.

AMENDMENTS TO THE SPECIFICATION

Please replace the first paragraph of the application (Page 1, lines 5-11) with the following amended replacement paragraph:

Related Applications

This application is a continuation of U.S. Patent Application Serial No. 11/433,781, entitled “Microfluidic System Including a Bubble Valve for Regulating Fluid Flow Through A Microchannel”, filed on May 12, 2006, which is a continuation of U.S. Patent Application No. 11/021,251 filed December 21, 2004 which, in turn, is a continuation of U.S. Patent Application No. 10/179,586 filed June 24, 2002, which claims priority to U.S. Provisional Patent Application No. 60/373,256 filed April 17, 2002; and is related to U.S. Patent Application No. 10/179,488, entitled “Method and Apparatus for Sorting Particles”, filed June 24, 2002. The contents of all of the above-referenced applications are herein incorporated by reference in their entirety.

REMARKS

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-4876, under Order No. 118153-01205.

Dated: June 19, 2012

Respectfully submitted,

Electronic signature for : /David R. Burns/
David R. Burns
Registration No. 46,590
McCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110
(617) 449-6500
(617) 607-9200 (Fax)
Attorney for Applicants

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(b).

I hereby appoint:



Practitioners associated with the Customer Number:

86738

OR



Practitioner(s) named below (If more than ten patent practitioners are to be named, then a customer number must be used):

| Name | Registration Number | Name | Registration Number |
|------|---------------------|------|---------------------|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

as attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:



The address associated with Customer Number:

86738

OR



Firm or Individual Name

| | | | |
|-----------|-------|-----|--|
| Address | | | |
| City | State | Zip | |
| Country | | | |
| Telephone | Email | | |

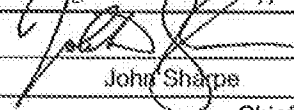
Assignee Name and Address:

Cytonome/ST, LLC
27 Drydock Avenue
Boston, Massachusetts 02210

A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

| | | | |
|-----------|---|-----------|----------------|
| Signature |  | Date | 21 AUGUST 2009 |
| Name | John Sharpe | Telephone | 617-330-5030 |
| Title | Chief Executive Officer | | |

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Cytomone/ST, LLC

Application No./Patent No.: Not yet assigned Filed/Issue Date: June 19, 2012

Entitled: Microfluidic System Including a Bubble Valve for Regulating Fluid Flow Through A Microchannel

Cytomone/ST, LLC, a Limited Liability Company
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest.
(The extent (by percentage) of its ownership interest is _____ %)

in the patent application/patent identified above by virtue of either:

- A. ☐ An assignment from the inventors of the above-referenced application. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

OR

- B. ☒ A chain of title from the inventors, of the parent patent application serial no. 11/021,251, to the current assignee as follows:

1. From: Cytonome, Inc. To: Cytonome/ST, LLC
The document was recorded in the United States Patent and Trademark Office at Reel 023525, Frame 0158, or for which a copy thereof is attached.
2. From: Teragenics, Inc. To: Cytonome, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 018336, Frame 0925, or for which a copy thereof is attached.
3. From: Teragenics, Inc. To: Cytonome, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 017985, Frame 0682, or for which a copy thereof is attached.

☒ Additional documents in the chain of title are listed on a supplemental sheet.

- ☒ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/David R. Burns/
Signature

June 19, 2012
Date

David R. Burns
Printed or Typed Name

(617) 449-6500
Telephone Number

Attorney on Record
Title

(Attorney Docket No. 118153-01205)

STATEMENT UNDER 37 CFR 3.73(b) - Supplemental Sheet

Continuation of chain title from the inventor(s) to the current assignee.

4. From: John R. Gilbert et al. To: Coventor, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 018336 , Frame 0928 , or for which a copy thereof is attached.
5. From: Coventor, Inc. To: Teragencis, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 018336 , Frame 0877 , or for which a copy thereof is attached.
6. From: Coventor, Inc. To: Teragencis, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 018336 , Frame 0844 , or for which a copy thereof is attached.

(Attorney Docket No. 118153-01204)

COPY

Attorney's
Docket
Number CVZ-012

Declaration, Petition and Power of Attorney for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR
REGULATING FLUID FLOW THROUGH A MICROCHANNEL**

the specification of which:

_____ is attached hereto.

X was filed on June 24, 2002 as

X Application Serial No. 10/179,586.

_____ and was amended on _____
(if applicable)

I do not know and do not believe that the subject matter of this application was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date of this application, or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date of this application on an application filed more than twelve months (six months if this application is for a design) before the filing of this application; and I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

CLAIM OF BENEFIT OF EARLIER FOREIGN APPLICATION(S)

I hereby claim priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application(s) for patent or inventor's certificate filed by me on the same subject matter having a filing date before that of the application(s) from which priority is claimed.

Check one:

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

| Country | Application Number | Date of Filing (month,day,year) | Priority Claimed Under 35 USC 119 |
|---------|--------------------|------------------------------------|--|
| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

| |
|--|
| |
| |
| |

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

60/373,256
(Application Serial No.)

April 17, 2002
(Filing Date)

(Application Serial No.)

(Filing Date)

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any earlier United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the earlier application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date(s) of the earlier application(s) and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier application(s), if any, described below, I do not know and do not believe that the same was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date(s) of said earlier application(s), or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date(s) of said earlier application(s) on an application filed more than twelve months (six months if this application is for a design) before the filing of said earlier application(s); and I acknowledge that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

| (Application Serial No.) | (Filing Date) | (Status) (patented,pending,aband.) |
|--------------------------|---------------|---------------------------------------|
|--------------------------|---------------|---------------------------------------|

| (Application Serial No.) | (Filing Date) | (Status) (patented,pending,aband.) |
|--------------------------|---------------|---------------------------------------|
|--------------------------|---------------|---------------------------------------|

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

| | | | |
|------------------------|-----------------|---------------------|---------------------------|
| James E. Cockfield | Reg. No. 19,162 | Maria C. Laccotripe | Limited Recognition |
| Thomas V. Smurzynski | Reg. No. 24,798 | | Under 37 C.F.R. § 10.9(b) |
| Ralph A. Loren | Reg. No. 29,325 | Debra J. Milasincic | Reg. No. 46,931 |
| Giulio A. DeConti, Jr. | Reg. No. 31,503 | David R. Burns | Reg. No. 46,590 |
| Ann Lamport Hammitte | Reg. No. 34,858 | Sean D. Detweiler | Reg. No. 42,482 |
| Elizabeth A. Hanley | Reg. No. 33,505 | Cynthia L. Kanik | Reg. No. 37,320 |
| Amy E. Mandragouras | Reg. No. 36,207 | Theodore R. West | Reg. No. 47,202 |
| Anthony A. Laurentano | Reg. No. 38,220 | Shayne Y. Huff | Reg. No. 44,784 |
| Kevin J. Canning | Reg. No. 35,470 | Hathaway P. Russell | Reg. No. 46,488 |
| Jane E. Remillard | Reg. No. 38,872 | Daniel B. Ko | Reg. No. 47,332 |
| DeAnn F. Smith | Reg. No. 36,683 | John S. Curran | Reg. No. 50,445 |
| Peter C. Lauro | Reg. No. 32,360 | Lisa M. DiRocco | Reg. No. P51,619 |
| Jeanne M. DiGiorgio | Reg. No. 41,710 | Richa Nand | Reg. No. P52,059 |
| Megan E. Williams | Reg. No. 43,270 | | |
| Jeremiah Lynch | Reg. No. 17,425 | | |
| David J. Rikkers | Reg. No. 43,882 | | |

Send Correspondence to: Anthony A. Laurentano, Esq. at Customer Number: **000959** whose address is:

LAHIVE & COCKFIELD, LLP, 28 State Street, Boston, MA 02109

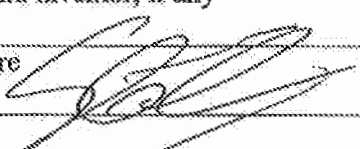
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Anthony A. Laurentano, Esq., (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Declaration, Petition and Power of Attorney for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR
REGULATING FLUID FLOW THROUGH A MICROCHANNEL**

the specification of which:

_____ is attached hereto.

X was filed on June 24, 2002 as

X Application Serial No. 10/179,586.

_____ and was amended on _____
(if applicable)

I do not know and do not believe that the subject matter of this application was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date of this application, or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date of this application on an application filed more than twelve months (six months if this application is for a design) before the filing of this application; and I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

CLAIM OF BENEFIT OF EARLIER FOREIGN APPLICATION(S)

I hereby claim priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application(s) for patent or inventor's certificate filed by me on the same subject matter having a filing date before that of the application(s) from which priority is claimed.

Check one:

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

| Country | Application Number | Date of Filing (month,day,year) | Priority Claimed Under 35 USC 119 |
|---------|--------------------|------------------------------------|--|
| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
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| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| | | | <input type="checkbox"/> Yes <input type="checkbox"/> No |

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

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CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

60/373,256
(Application Serial No.)

April 17, 2002
(Filing Date)

(Application Serial No.)

(Filing Date)

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any earlier United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the earlier application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date(s) of the earlier application(s) and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier application(s), if any, described below, I do not know and do not believe that the same was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date(s) of said earlier application(s), or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date(s) of said earlier application(s) on an application filed more than twelve months (six months if this application is for a design) before the filing of said earlier application(s); and I acknowledge that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

| (Application Serial No.) | (Filing Date) | (Status) (patented,pending,aband.) |
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| (Application Serial No.) | (Filing Date) | (Status) (patented,pending,aband.) |
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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

| | | | |
|------------------------|-----------------|---------------------|---------------------------|
| James E. Cockfield | Reg. No. 19,162 | Maria C. Laccotripe | Limited Recognition |
| Thomas V. Smurzynski | Reg. No. 24,798 | | Under 37 C.F.R. § 10.9(b) |
| Ralph A. Loren | Reg. No. 29,325 | Debra J. Milasincic | Reg. No. 46,931 |
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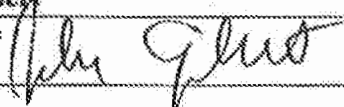
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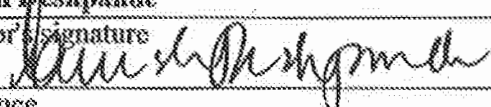
Anthony A. Laurentano, Esq. (617) 227-7400

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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**Microfluidic System Including a Bubble Valve for Regulating
Fluid Flow Through a Microchannel**

Related Applications

5 This application is a continuation of U.S. Patent Application No. 11/021,251 filed December 21, 2004 which, in turn, is a continuation of U.S. Patent Application No. 10/179,586 filed June 24, 2002, which claims priority to U.S. Provisional Patent Application No. 60/373,256 entitled "Microfluidic System Including a Bubble Valve for Regulating Fluid Flow Through a Microchannel" filed April 17, 2002; and is related to U.S. Patent Application
10 No. 10/179,488, entitled "Method and Apparatus for Sorting Particles", filed June 24, 2002. The contents of both applications are herein incorporated by reference.

Field of the Invention

15 The present invention relates to microscale fluid handling devices and systems. More particularly, the present invention relates to a method and system for controlling liquid flow in a microchannel by the introduction of a gas bubble to a microfluidic system.

Background of the Invention

20 In the chemical, biomedical, bioscience and pharmaceutical industries, it has become increasingly desirable to perform large numbers of chemical operations, such as reactions, separations and subsequent detection steps, in a highly parallel fashion. The high throughput synthesis, screening and analysis of (bio)chemical compounds, enables the economic discovery of new drugs and drug candidates, and the implementation of sophisticated medical diagnostic equipment. Of key importance for the improvement of the chemical operations
25 required in these applications are an increased speed, enhanced reproducibility, decreased consumption of expensive samples and reagents, and the reduction of waste materials.

30 Microfluidic devices and systems provide improved methods of performing chemical, biochemical and biological analysis and synthesis. Microfluidic devices and systems allow for the performance of multi-step, multi-species chemical operations in chip-based micro chemical analysis systems. Chip-based microfluidic systems generally comprise conventional 'microfluidic' elements, particularly capable of handling and analyzing chemical and biological specimens. Typically, the term microfluidic in the art refers to systems or devices having a network of processing nodes, chambers and reservoirs connected

by channels, in which the channels have typical cross-sectional dimensions in the range between about 1.0 μm and about 500 μm . In the art, channels having these cross-sectional dimensions are referred to as 'microchannels'.

5 By performing the chemical operations in a microfluidic system, potentially a number of the above-mentioned desirable improvements can be realized. Downscaling dimensions allows for diffusional processes, such as heating, cooling and passive transport of species (diffusional mass-transport), to proceed faster. One example is the thermal processing of liquids, which is typically a required step in chemical synthesis and analysis. In comparison
10 with the heating and cooling of liquids in beakers as performed in a conventional laboratory setting, the thermal processing of liquids is accelerated in a microchannel due to reduced diffusional distances. Another example of the efficiency of microfluidic systems is the mixing of dissolved species in a liquid, a process that is also diffusion limited. Downscaling the typical dimensions of the mixing chamber thereby reduces the typical distance to be
15 overcome by diffusional mass-transport, and consequently results in a reduction of mixing times. Like thermal processing, the mixing of dissolved chemical species, such as reagents, with a sample or precursors for a synthesis step, is an operation that is required in virtually all chemical synthesis and analysis processes. Therefore, the ability to reduce the time involved in mixing provides significant advantages to most chemical synthesis and analysis processes.

20 Another aspect of the reduction of dimensions is the reduction of required volumes of sample, reagents, precursors and other often very expensive chemical substances. Milliliter-sized systems typically require milliliter volumes of these substances, while microliter sized microfluidic systems only require microliters volumes. The ability to perform these
25 processes using smaller volumes results in significant cost savings, allowing the economic operation of chemical synthesis and analysis operations. As a consequence of the reduced volume requirement, the amount of chemical waste produced during the chemical operations is correspondingly reduced.

30 In microfluidic systems, regulation of minute fluid flows through a microchannel is of prime importance, as the processes performed in these systems highly depend on the delivery and movement of various liquids such as sample and reagents. A flow control device may be used to regulate the flow of liquid through a microchannel. Regulation includes control of

flow rate, impeding of flow, switching of flows between various input channels and output channels as well as volumetric dosing.

U.S. Patent Number 6,062,681 describes a bubble valve for a liquid flow channel in which the flow of a liquid is controlled by the generation of a gas bubble in the channel using a heater placed in the liquid. As the heater is activated, a bubble is formed which can be enlarged or reduced in size by increasing or decreasing, respectively, the temperature of the heater. The described system presents a number of disadvantages, namely, the required power to operate the valve and the inherent requirement that liquid in the channel be heated upon passing the valve. Even small increases in liquid temperature, by only a couple of degrees, can have disastrous effects on the highly heat sensitive biochemical substances present in the liquids to be controlled in many microfluidic systems. In addition, the required on-chip electric circuitry for the heater increases the complexity of the described valve and consequently results in unacceptably high costs, particularly if the fluidic system employing the bubble valve only used for a single application.

Other valves in the prior art use electrochemical means to produce a bubble in a liquid.

20 **Summary of the Invention**

The present invention provides a bubble valve for controlling, regulating or varying fluid flow through a microfluidic system. The bubble valve regulates fluid flow through a channel using an externally operated mechanical or pneumatic actuator. The actuator causes a deflection of a fluid meniscus into the interior of the channel to regulate liquid flow. The actuator may mechanically force a gas bubble into a fluid carrying microchannel to inhibit liquid flow or to cause liquid flow by applying a sufficiently high pressure to the meniscus. The bubble valve effectively controls the flow of liquids in microfluidic systems, without heating the fluid and without complex on-chip circuitry.

30 The microfluidic system includes a microchannel and a sealed, gas-filled reservoir positioned adjacent to and connected to the microchannel. The gas filled reservoir has a movable wall and a meniscus formed by a liquid in the microchannel that forms an interface between the reservoir and the microchannel interior. The meniscus may form a portion of the side wall of the microchannel. An external mechanical actuator may be used to deflect the

movable wall of the reservoir. As the movable wall is deflected, the volume of the reservoir decreases and the gas pressure inside the reservoir increases, causing the meniscus to deflect into the microchannel, thereby modifying the cross-sectional area of the microchannel and consequently varying the flow of liquid through the channel. The increased pressure in the reservoir pushes gas from the reservoir into the microchannel. The gas may result in a local gas bubble being forced into the microchannel from the gas-filled reservoir. The resulting gas bubble occupies a portion of the cross-section of the channel, allowing liquid flow through the channel to be effectively controlled by controlling the size of the gas bubble via the external actuator.

10

The meniscus may comprise a virtual wall formed in a side wall of the microchannel. The virtual wall is a meniscus formed by a liquid in the microchannel that fills an aperture formed in the side wall of the microchannel and essentially replaces the removed portion of the side wall without affecting the properties of liquid flow through the channel. A gas bubble can be forced into the channel by applying a gas pressure at the opening using an external pneumatic actuator. The gas pressure forces the meniscus inside the channel, which varies the flow of liquid through the channel interior.

15

According to one embodiment, the microchannel includes a hydrophobic patch spanning the width of the microchannel at the location where the gas bubble is introduced to enhanced on-off switching of the bubble valve. The hydrophobic patch anchors the bubble in a particular location in the microchannel. If the introduced gas bubble covers the whole area of the patch, the bubble is effectively retained by capillary forces and blocks any liquid flow up to a certain pressure difference, depending on the level of hydrophobicity of the patch.

25

Alternatively or in combination with a hydrophobic patch, the microchannel can be locally shaped into a cavity for receiving and anchoring the gas bubble. By providing an appropriate cavity, the bubble can be kept in place during operation, reducing the risk that the gas bubble is carried away with the liquid.

30

According to one aspect of the invention, a microfluidic device is provided. The microfluidic device comprises a microchannel having an interior bounded by a side wall and a valve for regulating the flow of fluid through the microchannel. The valve comprises a gas-filled reservoir, a fluid meniscus interfacing the reservoir and the interior and an actuator for

varying the volume of the reservoir to increase an internal pressure of the reservoir to vary the flow of liquid through the channel.

According to another aspect, a microfluidic device is provided, comprising a first
5 plate having a groove formed therein defining a microchannel, a second plate for enclosing the microchannel and a flexible membrane. The second plate is bonded to the first plate and has an aperture adjacent to the groove sized and dimensioned to form a meniscus when the microchannel is filled with a liquid. The aperture defines a reservoir adjacent to the
microchannel, wherein the meniscus forms an interface between the microchannel and the
10 reservoir. The flexible membrane is bonded to the second plate to seal the reservoir.

According to another aspect, a method of making a bubble valve is provided, the method comprises providing a microchannel having an interior bounded by a side wall, an aperture formed in the side wall and a valve chamber adjacent to the aperture in
15 communication with the interior, filling the microchannel with a liquid to form a meniscus of the liquid in the aperture, whereby the step of filling traps a gas in the valve chamber and providing an actuator for increasing the pressure in the valve chamber to deflect the meniscus into the interior.

20 According to yet another aspect, a method of making a bubble valve is provided. The method comprises providing a microchannel having an interior bounded by a side wall, an aperture formed in the side wall and a valve chamber adjacent to the aperture in communication with the interior, filling the microchannel with a liquid to form a meniscus of the liquid in the aperture and applying and sealing an actuator comprising a chamber to a top
25 surface of the microchannel to form a gas-filled chamber adjacent to the meniscus. The actuator varies the pressure in the gas-filled chamber to deflect the meniscus into the interior, thereby regulating fluid flow.

According to still another aspect, a microfluidic device is provided comprising a
30 microchannel having an interior bounded by a side wall, a bubble valve for creating and injecting a bubble into the microchannel interior to regulate fluid flow through the microchannel and a hydrophobic patch for retaining the bubble in a predetermined position in the microchannel interior.

According to yet another aspect a bubble valve in a particle sorting device for separating particles having a predetermined characteristic from particles not having a predetermined characteristic is provided. The bubble valve comprises a gas-filled reservoir, a side channel in communication with a channel through which a stream of particles in a carrier fluid passes, wherein the carrier fluid forms a meniscus in the side channel adjacent to the gas-filled reservoir and an actuator for deflecting the meniscus to create a pressure pulse to selectively deflect a particle having the predetermined characteristic from the stream of particles.

According to still another aspect, a method of varying an electrical resistance in a microchannel is provided. The method comprises generating a bubble and injecting the bubble into a liquid in the microchannel, whereby the bubble varies the electrical resistance of the microchannel.

According to yet another aspect, an electrophoretic system is provided, comprising an electrokinetically operated microchannel, a sample well for providing an sample to the microchannel, a voltage source and a bubble valve for injecting a bubble into the microchannel to vary the electrical resistance of the microchannel.

According to a final aspect of the invention, an electrokinetic column to column switch is provided, comprising a first electrokinetically operated microchannel, a second electrokinetically operated microchannel in communication with the first electrokinetically operated microchannel and a bubble valve for selectively blocking flow from the first electrokinetically operated microchannel to the second electrokinetically operated microchannel by selectively injecting a bubble into a microchannel.

Brief Description of the Drawings

Figure 1 is a schematic view of a microfluidic system suitable for implementing the illustrative embodiment of the invention.

Figure 2 shows an exploded view of a bubble valve according to an illustrative embodiment of the present invention.

Figure 3 shows an isometric view of the bubble valve of Figure 2.

Figure 4 shows a top view of the bubble valve of Figure 2.

Figures 5a-c are cross-sectional views of the bubble valve of Figure 2 in operation.

Figure 6 shows an exploded view of an alternative embodiment of a bubble valve according to the present invention.

Figure 7 shows a top view of the bubble valve of Figure 6.

5 Figures 8a-c are cross-sectional view of the bubble valve of Figure 6 in operation.

Figure 9 shows an application of the bubble valve of an illustrative embodiment of the present invention in a microchannel.

10 Figure 10 shows a Y-intersection in a microfluidic system of an embodiment of the invention that implements a bubble valve to control liquid flow according to the teachings of the present invention.

Figure 11 shows a Y-intersection in a microfluidic system of another embodiment of the invention that implements a bubble valve to control liquid flow according to the teachings of the present invention.

15 Figures 12a-c shows an electrophoresis system that implements a bubble valve to control electrical current during electrokinetic injection according to the teachings of the present invention.

Figure 13a shows an electrokinetic column-column switch implementing a bubble valve according to the teachings of the present invention.

20 Figure 13b shows an alternate electrokinetic column-column switch implementing a bubble valve according to another embodiment of the present invention.

Figure 14 shows a selective resistance circuit that employs a bubble valve to control electrical current according to the teachings of the present invention.

Figure 15 shows an alternative selective resistance circuit that employs a bubble valve to control electrical current according to the teachings of the present invention.

25 Figure 16 shows a particle sorting system that implements a bubble valve of the present invention to produce fluid impulses to sort particles.

Figures 17a, 17b and 17c illustrate the operation of the particle sorting system of Figure 16.

30 **Detailed Description of the Invention**

The present invention provides an improved bubble valve for controlling fluid flow through a microchannel in a microfluidic system. The invention further provides a method of forming the bubble valve. The bubble valve of the present invention can be applied in numerous microfluidic systems for controlling and switching fluid flows. Examples of

suitable applications include, but are not limited to: flow cytometry, column switching, 2-D separations, cell or particle sorting applications on a chip, regulating pressurized fluid flows including on-off switching, regulating electrokinetic fluid flows and electrokinetically induced processes including on-off switching and electrokinetic sample injection and channel
5 to channel switching.

Figure 1 illustrates a microfluidic system suitable for implementing the illustrative embodiment of the present invention. The illustrative microfluidic system 100 comprises a substrate 101 having one or more microchannels 21 disposed therein. The microchannels
10 transport fluid through the microfluidic system 100 for processing, handling, and/or performing any suitable operation on a liquid sample. As used herein, the term “microfluidic” refers to a system or device for handling, processing, ejecting and/or analyzing a fluid sample including at least one channel having microscale dimensions. The term “channel” as used herein refers to a pathway formed in or through a medium that allows for
15 movement of fluids, such as liquids and gases. The term “microchannel” refers to a channel preferably formed in a microfluidic system or device having cross-sectional dimensions in the range between about 1.0 μm and about 500 μm , preferably between about 25 μm and about 250 μm and most preferably between about 50 μm and about 100 μm . One of ordinary skill in the art will be able to determine an appropriate volume and length of the microchannel.
20 The ranges are intended to include the above-recited values as upper or lower limits. The microchannel can have any selected shape or arrangement, examples of which include a linear or non-linear configuration and a U-shaped configuration. The microfluidic system 100 may comprise any suitable number of microchannels 21 for transporting fluids through the microfluidic system 100.

25 The microfluidic system 100 includes a bubble valve 10, 10' shown in Figures 2-8c for controlling liquid flow through a microchannel of the system. According to the illustrative embodiment, the microchannel is defined by a side wall having any suitable shape enclosing at least a portion of the interior of the channel. The bubble valve may be formed
30 by a gas-filled reservoir positioned adjacent to the microchannel including a meniscus that forms the interface between the reservoir and the microchannel interior. The meniscus may form a portion of the side wall of the microchannel. The bubble valve includes an actuator for modifying the pressure in the reservoir to deflect the meniscus into the channel interior,

thereby modifying the cross-sectional area of the microchannel and consequently varying the flow of liquid through the channel.

According to an alternate embodiment, the bubble valve is formed by a meniscus in a separate side channel that communicates with and intersects a microchannel through which a liquid to be controlled flows. One skilled in the art will recognize that the meniscus can be located at any location relative to the microchannel through which liquid flows.

The gas-filled reservoir may be formed when filling the microchannel having an aperture in a side wall and a reservoir formed adjacent to the aperture. An empty microchannel may be filled with liquid, forming the meniscus in the aperture, which traps the gas that forms the gas bubble and forms a gas pocket in the reservoir adjacent to the meniscus. The creation of the gas pocket on filling provides a sterile gas bubble and reduces contaminants in the system. Alternatively, the air pocket may be created by introducing a gas to the reservoir after filling of the microchannel.

FIG. 2 shows an exploded view of an embodiment of an illustrative bubble valve of the present invention. The microfluidic system may be formed by a plurality of stacked layers. As shown in Figure 2, the illustrative microfluidic system 100 includes a first plate 20 in which a groove defining the microchannel 21 is provided. A hydrophobic patch 22 may be applied to an inner wall of the microchannel 21. A second plate 30 for enclosing the microchannel is bonded to the first plate 20 and includes an aperture 31. A third plate 40 is bonded on top of second plate 30 to close and seal the stacked structure. The aperture 31 of the intermediate second plate 30 defines a void in the system adjacent to the microchannel 21.

FIGS. 3 and 4 illustrates the assembled bubble valve 10. As shown, the stacked first plate 20, second plate 30 and third plate 40 define a closed, gas filled gas reservoir 70, which can be actuated with a displacement actuator 50. According to the illustrative embodiment, the aperture 31 defining the reservoir 70 comprises a main body 31a and a slot 31b extending from the main body 31a. When the microfluidic system 10 is assembled, the slot 31b of the aperture 31 defines a gap in the side wall of the microchannel 21 that provides access to and communicates with the interior of the microchannel 21.

The bubble valve 10 operates to control the flow of liquid through the microchannel 21. A meniscus is formed in the aperture 31, which interfaces with and separates the microchannel interior from the reservoir 70. According to the embodiment shown in Figures 2-8c, the meniscus is formed by a liquid filling the microchannel in the slot 31b. One skilled in the art will recognize that other suitable devices may be used to form the meniscus. The liquid in the slot 31b is retained in the microchannel by capillary forces. The actuator 50 deflects the upper wall of the reservoir, defined by plate 40, which decreases the volume of the reservoir 70. The actuator 50 may comprise any suitable device for deflecting the wall, such as an electromagnetic actuator or a piezoelectric element. The plate 40 may comprises a flexible membrane. The decreased volume consequently increases the pressure of the reservoir 70 and causes the meniscus 80 to deflect into the channel interior to create a constriction in the channel, thereby impeding fluid flow or pushing fluid away from the meniscus. If a sufficient pressure is applied to the meniscus, the actuator generates and enlarges a bubble in the liquid of the microchannel, which blocks fluid flow. The hydrophobic patch 22 provides an anchor for the bubble and retains the bubble at a selected location in the microchannel.

After the microchannel is filled with a liquid 60, the bubble valve 10 is ready for operation. FIG. 5a-c illustrate the operation of the bubble valve according to the teachings of the invention. FIG. 5a shows the bubble valve in an 'open' state. According to the embodiment of Figures 5a-5c, the meniscus between the reservoir and the interior of the microchannel is defined by the meniscus 80, formed by the liquid 60 in the slot 31b in the second plate 30. In the 'open' state, liquid flows freely through the microchannel 21 and the valve does not impose any additional flow resistance in the channel.

The slot 31b may be sized and dimensioned to form a "virtual wall" in the microchannel. As used herein, "virtual wall" refers to the meniscus 80 formed by the first liquid 60 in the aperture formed in the side wall of the microchannel 20, which essentially replaces the removed portion of the side wall without affecting the properties of the microchannel. The meniscus surface can be, although not required, substantially co-planar with the wall of the microchannel in which the meniscus is formed. The word "virtual" is chosen to express the effect that the overall liquid flow through the microchannel 21 of the microfluidic system 100 is not influenced by the virtual wall, i.e. the flow of liquid in the microfluidic system having a virtual wall is substantially identical to the flow of liquid

through an identical microfluidic system in which no virtual wall is present. One of ordinary skill will recognize that the meniscus may be convex or concave, depending on the appropriate system pressure.

5 When the actuator 50 is actuated, the bubble valve 10 switches to a “pinched” state, as shown in Figure 5b, to inhibit fluid flow through the channel interior. In the ‘pinched’ state, the actuator 50 deflects the top of the gas reservoir 70 for a certain fraction, increasing the pressure in the reservoir 70 and forcing the meniscus 80 down into the channel 21. The deflection of the meniscus locally reduces the cross-section of the channel 21 and introduces
10 an additional flow resistance to the liquid flow. The degree of reduction in the liquid flow through the microchannel corresponds to the amplitude, frequency and duration of the displacement of the meniscus 80, which are controllable by the actuator 50. One skilled in the art will recognize that any suitable means for varying the pressure within the reservoir 70 may be used to deflect the meniscus 80, thereby regulating fluid flow.

15

 When the actuator is fully actuated, the bubble valve 10 is switched to a closed state, as illustrated in Figure 5c. As shown, in the closed state, the meniscus 80 deflects fully to form and introduce a gas bubble 81 into the microchannel 21. The gas bubble 81 is retained by the hydrophobic patch 22 formed in the channel wall opposite the slot 31b. As a result,
20 the liquid flow in the channel is substantially blocked. By reducing the pressure on the meniscus 80, the bubble valve 10 can be brought from the ‘closed’ state of Figure 5c via the ‘pinched’ state of Figure 5b back to the ‘open’ state of Figure 5a.

 According to one embodiment, the bubble valve 10 may be used as a check valve for
25 regulating pressure in the microchannel. When the pressure in the microchannel exceeds a maximum breaking pressure, the bubble collapses, opening the valve and allowing fluid to flow through the channel, thereby reducing the pressure in the microchannel. The breaking pressure depends on the hydrophobicity of the hydrophobic patch 22, as well as the geometry of the microchannel.

30

 Alternatively or in combination with the hydrophobic patch 22, the microchannel 21 can be locally shaped into a cavity for receiving and anchoring the gas bubble 81. By providing an appropriate cavity, the bubble can be kept in place during operation, reducing the risk that the gas bubble is carried away with the liquid.

According to the embodiments shown in Figures 2-5c, the actuator 50 is integrated in the microfluidic chip 100. However, one skilled in the art will recognize that an external, reusable actuator may also be used to control formation of a gas bubble in the microchannel.

5

FIG. 6 shows an alternative embodiment of a microfluidic system 100' including a bubble valve 10' having an external actuator 90 according to the teachings of the invention. In the embodiment shown in Figure 6, the microfluidic system 100' includes a first plate 200 including a groove defining the microchannel 210 and a second plate 300 bonded to the first plate 200 for enclosing the microchannel 210 and in which a virtual wall opening 32 is formed. The virtual wall opening 32 is sized and dimensioned to form a "virtual wall" when the microchannel 210 is filled with a liquid.

Upon filling of the microchannel 21 with a liquid, a virtual wall 32a is formed in virtual wall opening 32. As shown in Figures 7, 8a-8c, the microfluidic system further includes an external actuator, illustrated as pressurizer 90, pressed and sealed onto the top of the second plate 300 to form a tight seal. The external pressurizer 90 defines a sealed pressurizing chamber 92 adjacent to the virtual wall 32a. The pressurizer varies the pressure within the pressurizing chamber 92 to control liquid flow through the microchannel 210 by modifying the position of the virtual wall. The pressurizer 90 may include a source of pressurized gas (not shown) and a gas inlet 91 to allow a gas pressure to be applied to the virtual wall 32a in order to move the virtual wall. The pressurizer may alternatively include a flexible wall that deflects to vary the volume of the chamber 92 upon activation of an actuator, such as a piezoelectric element or electromagnetic actuator.

25

FIG. 8a shows a cross-section of the bubble valve 10' of Figures 6 and 7 in the 'open' state. As shown, when the microchannel 210 is filled with liquid 600, a virtual wall 32a, defined by a meniscus 800, is formed within the virtual wall opening 32. The meniscus essentially replaces the absent portion of the side wall of the microchannel and allows liquid to flow through the channel interior unimpeded and uninfluenced by the virtual wall.

30

FIG. 8b depicts the 'pinched' state of the bubble valve, when the pressurizer 90 is activated. As shown, activation of the pressurizer 90 increases the internal pressure within the pressurizing chamber 92. The increased pressure moves the meniscus 800 down the

channel height and into the microchannel interior, thereby regulating liquid flow. The pressurized controls the level of the internal pressure in order to control the amount of deflection of the meniscus and therefore the rate of fluid flow.

5 To switch the valve to a 'closed' state, as shown in Figure 8c, the pressurizer 90 applies a large pressure that is sufficient to form and introduce a gas bubble 810 into the channel 210. The hydrophobic patch 220 retains the gas bubble 810 in place. As a result, the liquid flow in the channel is blocked up to a 'breaking pressure', which depends on the hydrophobicity of hydrophobic patch 220. A higher hydrophobicity results in a larger
10 breaking pressure. By reducing the pressure on the meniscus 800 the bubble valve 10' can be brought from the 'closed' state of Figure 8c via the 'pinched' state of Figure 8b back to the 'open' state of Figure 8a.

FIG. 9 shows an application of a bubble valve 10 for flow regulation in a
15 microchannel 121 according to one embodiment of the invention. During typical operation of a microfluidic system, a pressure difference is applied over the length of a microchannel 121. A bubble valve 10 can be employed to regulate the flow through the microchannel between zero and a maximum flow rate, depending on the applied pressure difference.

20 FIG. 10 shows a portion of a microfluidic system according to an embodiment of the invention forming a Y-intersection comprising two inlet microchannels 121a and 121b and an outlet channel 121c that combines the fluids flowing through the two inlet microchannels. The first microchannel 121a carries a first liquid and the second microchannel 121b carries a second liquid. The microchannels 121a and 121b are each controlled by a corresponding
25 bubble valve, 10a and 10b, respectively, for regulating the combined composition and flow rate through the outlet microchannel 121c. One skilled in the art will recognize that that the number of inlet channels is not limited to two, but is presented here merely as an example.

FIG. 11 shows a Y-intersection of a microfluidic system according to another
30 embodiment of the invention. As shown the Y-intersection comprises an inlet microchannel 221c and two outlet microchannels 221a and 221b for splitting the incoming liquid flow from the inlet microchannel 221c. The flow of each outlet channel is regulated by a corresponding bubble valve 200a and 200b, respectively. The incoming liquid flow from the inlet microchannel 221c can be split between microchannel 221a and microchannel 221b in any

required ratio. One skilled in the art will recognize that that the number of inlet channels is not limited to two, but is presented here merely as an example.

FIG. 12a-12c shows the implementation of an electrophoresis system 110 comprising
 5 five bubble valves, 10a-e of the present invention, arranged with a crossed microchannel configuration. Regulation of the bubble valve 10a regulates the electric current through the associated electrokinetically operated microchannel 115a. The pinching of the liquid in the electrokinetically operated microchannel 115a by a bubble valve will result in an increased electrical resistance in the microchannel 115a. As a result, the migration of charged species
 10 and electro-osmotic flow in the electrokinetically operated microchannel 115a can be regulated. A voltages difference for the injection of sample from a well storing a supply of a sample 125 between the bubble valve 10a and the crossing point 111 of the microchannels and consecutive separation are provided via wells V+ and V0. Electrodes are placed in the V+ and V0 wells and energized with a constant voltage difference during the operation of the
 15 electrophoresis system.

In the injection phase, valves 10a, 10b, 10c and 10d are substantially in the open position, allowing passage of electrical current up to a required level for injection (FIG 12b shows direction of current / sample), while bubble valve 10e is closed. The valves are kept in
 20 this position long enough for the sample to move from the sample well 125 towards and past the central injection crossing 111. Varying the opening ratio of valves 10a, 10b and 10d can be adjusted to confine the sample to a narrow flow through the injection cross 111, as shown in Figure 12c (i.e. 'pinched injection').

25 Immediately after the injection phase, a plug of sample is injected and separated in the separation column 115a (microchannel which runs horizontally in figure) by closing bubble valves 10a, 10d and 10c and opening valves 10b and 10e. Now the total voltage difference is applied longitudinally over the separation channel, resulting in the separation of the constituents in the sample (Figure 12c).

30

FIG. 13a illustrates another application of the bubble valve of the present invention implemented in a column-column switch 130 for electrokinetically transferring a substance from a first electrokinetically operated microchannel 215a to a second electrokinetically operated microchannel 215b. The column-column switch 130 comprises a bubble valve 10b

of the present invention that connects the first electrokinetically operated microchannel 215a to the second electrokinetically operated microchannel 215b. Both electrokinetically operated microchannel 215a and electrokinetically operated microchannel 215b are connected to corresponding wells well 220a-d. In the following example, it is assumed that the substances electrokinetically move from a positive electric potential to ground and that well 120c and well 120b are provided with a positive potential whilst well 120a and well 120d are grounded.

At first, the two electrokinetically operated microchannel 215a and electrokinetically operated microchannel 215b are operated independently and the connecting bubble valve 10b is in the 'closed' state whilst bubble valve 10a and bubble valve 10c are in the 'open' state. To electrokinetically transfer substance from electrokinetically operated microchannel 215a to the electrokinetically operated microchannel 215b, bubble valve 10a and bubble valve 10c are switched to the closed state, and the connecting bubble valve 10b is opened momentarily to allow passage of an amount of substance from the electrokinetically operated microchannel 215a to the electrokinetically operated microchannel 215b. The amount transferred depends directly upon the time bubble valve 10b is opened. After the required amount of substance is transferred, the connecting bubble valve 10b is closed and the bubble valve 10a and bubble valve 10b are opened again.

FIG. 13b illustrates the implementation of another column to column switch 130' to exchange liquid from a first column 215a' selectively into a second column 215b'. The first column and the second column are each crossed by a transfer column 250, operated by a first bubble valve 10a arranged on one end of the transfer column 250 and a second bubble valve 10b arranged on the opposite end of the transfer column 250. The first one of the bubble valves is attached to the first column 215a' and is actuated upon transiently by an external actuator for increasing the pressure within the bubble valve reservoir. Increasing the pressure on the bubble valve will deflect the meniscus 80, inducing a transient flow in the transfer column 250 from the pressurized bubble valve 10a towards the second bubble valve 10b, which comprises a compressible volume of gas. This transient flow effectively transfers a liquid volume from the first column 215a' to the second column 215b'. Upon deactivation of the external actuator, the flow in the transfer column 250 reverses as the compressed gas volume in the second bubble valve 10b exerts a pressure on the liquid in the transfer column 250.

FIG. 14 shows a selective resistance circuit employing a bubble valve of the present invention for selectively including a predefined electrical resistance in an electrokinetic circuit. The circuit 140 comprises an inlet microchannel 321, which splits into two paths.

5 The first path 322 includes a fluidic resistor 240a and a bubble valve 10a, the second parallel path 323 includes a fluidic resistor 240b and a bubble valve 10b. The fluidic resistors 240a-b comprise a channel of appropriate length to results in a certain electrical resistance. The bubble valve 10a and the bubble valve 10b can be switched each to either on to allow fluid flow through the associated microchannel or off to block fluid flow through the associated

10 microchannel. As a result, the overall electrical resistance of the electrokinetic circuit can be switched between four values: infinite (both bubble valves 10a-b are off), the resistance of fluidic resistor 240a (bubble valve 10a on, bubble valve 10b off), the resistance of fluidic resistor 240b (bubble valve 10a off, bubble valve 10b on) and the parallel resistance of fluidic resistor 24a-b (both bubble valves 10a and 10b on).

15

FIG. 15 shows an alternative resistance circuit 150 according to another application of the invention, now for the selective application of a voltage. By opening either the bubble valve 10a or bubble valve 10b, the voltage imposed on an outgoing channel 523 can be selected. Fluidic resistor 245a and 245b function to limit the electric current in either of the

20 two states to a predetermined value.

FIG. 16 illustrates another application of the bubble valve 10 of the present invention in a particle sorting application, wherein the bubble valve is positioned in a side channel that communicates with a channel through which particles in suspension flow. According to one

25 application of the present invention, a particle sorter 160 comprises a closed channel system of capillary size for sorting particles, such as cells. The channel system comprises a first supply duct 162 for introducing a stream of particles and a second supply duct 164 for supplying a carrier liquid. The first supply duct 162 ends in a nozzle, and a stream of particles is introduced into the flow of carrier liquid. The first supply duct 162 and the

30 second supply duct 164 enter a measurement duct 166, which branches into a first branch 172a and a second branch 172b at a branch point 171. A measurement region 182a is defined in the measurement duct 166 and is associated with a detector 182b to sense a predetermined characteristic of particles in the measurement region 182a. Two opposed bubble valves 10a and 10b are positioned in communication with the measurement duct 166 and are spaced

opposite each other. The bubble valves 10a, 10b communicate with the measurement duct 166 through a pair of opposed side passages 174a and 174b, respectively. Liquid is allowed to partly fill these side passages 174a and 174b to form a meniscus 175 therein. An external actuator 176 is also provided for actuating the bubble valves 10a, 10b, which momentarily
 5 causes a flow disturbance in the duct to deflect the flow therein when activated by the actuator 176.

In a suspension introduced by the first supply duct 162, two types of particles can be distinguished, normal particles 180a and particles of interest 180b. The flow rates in both
 10 branches 172a and 172b are adjusted so that the stream of particles normally flows through the second branch 172b. Upon sensing the predetermined characteristic in the particles in the measurement region 182a, the detector 182b raises a signal. The external actuator 176 activates the bubble valves 10a, 10b when signaled by the detector 182b in response to sensing the predetermined characteristic, to create a flow disturbance in the measurement
 15 duct 166 between the sideways passages 174a, 174b, to deflect the particle having the predetermined characteristic so that it flows down the first branch duct 172a rather than the second branch duct 172b. The detector communicates with the actuator 176, so that when the detector 182b senses a predetermined characteristic in a particle, the actuator activates the first bubble valve 10a to cause pressure variations in the reservoir 70 of the first bubble valve.
 20 The activation of the first bubble valves causes a transient pressure variation in the first side passage 174a. The second side passage 174b and the second bubble valve 10b absorb the transient pressure variations in the measurement duct 166 induced via the actuator 176. Basically, the reservoir 70b of the second bubble valve 10b is a chamber having a resilient wall or contains a compressible fluid such as a gas. The resilient properties allow the flow of
 25 liquid from the measurement duct into the second side passage 174b.

Figures 17a-17c illustrate the operation of the particle sorting system 160 of Figure 16. In Figure 17a, the detector raises a signal to activate the actuator. Upon activation of the actuator, the pressure within the reservoir of the first bubble valve 10a is increased, causing a
 30 transient discharge of liquid from the first side passage 174a as indicated by the arrow. The sudden pressure increase caused at this point in the duct causes liquid to flow into the second side passage 174b because of the resilient properties of the reservoir of the second bubble valve 10b. This movement of liquid into the second side passage 174b is indicated with an arrow. Resultingly, as can be seen in the figure, the flow through the duct is deflected causing

the selected particle of interest 178b located between the first side passage 174a and the second side passage 174b to be shifted perpendicular to its flow direction in the normal state. The flow resistances to the measurement duct 166, the first branch 172a and the second branch 172b is chosen so that the preferred direction of the flow to and from the first side passage 174a and the second side passage 174b has an appreciable component perpendicular to the normal flow through the measurement duct 166. This goal can for instance be reached by the first branch 172a and the second branch 172b so that their resistances to flow is large in comparison with the flow resistances of the first side passage 174a and the second side passage 174b.

10

Figure 17b shows the particle sorting system 160 during the relief of the first bubble valve reservoir when the particle of interest 178b has left the volume between the first side passage 174a and the second side passage 174b. The actuator 176 is deactivated, causing the pressure inside the reservoir to return to the normal pressure. During this relief phase there is a negative pressure difference between the two reservoirs of the bubble valves, causing a liquid flow through the first side passage 174a and the second side passage 174b opposite to the liquid flow shown in the previous figure and is indicated by the arrows.

15

Figure 17c shows the particle sorting system 160 after completion of the switching sequence. The pressures inside the reservoirs of the bubble valves has been equalized so the flow through the measurement duct 166 is normalized. As the particle of interest 178b has been displaced radially, it will flow into the first branch 172a as was the objective of the switching operation.

20

According to yet another embodiment, the cross-sectional dimensions of a microchannel including a bubble valve according to the teachings of the present invention may be varied locally to affect the pressure within the microchannel interior. For example, the microchannel may be narrowed or widened at certain locations to increase or decrease the capillary forces acting on a fluid in the microchannel interior. One of ordinary skill in the art will be able to determine a suitable cross-sectional dimension to achieve a desired pressure within the microchannel interior.

25

30

The bubble valve of the present invention may be implemented in a variety of microfluidic devices used for many applications. In a particular application, the bubble valve

is implemented in a flow-cytometer based instrument for sorting or physically separating particles of interest from a sample or for measuring selected physical and chemical characteristics of cells or particles in suspension as they travel past a particular site. The bubble valve may also be employed in devices for sequencing or manipulating DNA, medical
5 diagnostic instruments, devices for drug discovery, chemical analysis and so on.

The present invention provides an improved system and method for regulating fluid flow in a microchannel for a variety of applications. The bubble valve of the present invention is easy to operate and control, simple to manufacture and economical. In addition,
10 the bubble valve does not adversely affect the liquid in the microchannel. The bubble valve effectively controls the flow of liquids in microfluidic systems, without heating the fluid and without complex on-chip circuitry.

The present invention has been described relative to an illustrative embodiment. Since
15 certain changes may be made in the above constructions without departing from the scope of the invention, it is intended that all matter contained in the above description or shown in the accompanying drawings be interpreted as illustrative and not in a limiting sense.

It is also to be understood that the following claims are to cover all generic and
20 specific features of the invention described herein, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween.

Having described the invention, what is claimed as new and protected by Letters
Patent is:

25

What is claimed is:

1. A microfluidic device, comprising:
a microchannel having an interior bounded by a side wall;
- 5 a valve for regulating the flow of fluid through the microchannel, the valve comprising a gas-filled reservoir, a fluid meniscus interfacing the reservoir and the interior and an actuator for varying the volume of the reservoir to increase an internal pressure of the reservoir to vary the flow of liquid through the channel.
- 10 2. The microfluidic device of claim 1, wherein the actuator deflects the meniscus into the microchannel interior to regulate the flow of fluid through the microchannel.
3. The microfluidic device of claim 1, wherein the meniscus comprises a virtual wall formed in an aperture in the side wall of the microchannel when the microchannel is filled
- 15 with a liquid.
4. The microfluidic device of claim 1, wherein the actuator generates a gas bubble in the microchannel.
- 20 5. The microfluidic device of claim 4, further comprising a hydrophobic patch attached to an inner wall of the microchannel for anchoring the gas bubble.
6. The microfluidic device of claim 4, wherein the microchannel defines a cavity for retaining the gas bubble.
- 25 7. The microfluidic device of claim 1, wherein the gas filled reservoir comprises a movable membrane.
8. The microfluidic device of claim 7, wherein the actuator deflects the movable
- 30 membrane to create a bubble in the microchannel interior.
9. A microfluidic device comprising:
a first plate having a groove formed therein defining a microchannel;

a second plate for enclosing the microchannel bonded to the first plate having an aperture adjacent to the groove sized and dimensioned to form a meniscus when the microchannel is filled with a liquid, the aperture defining a reservoir adjacent to the microchannel, wherein the meniscus forms an interface between the microchannel and the

5 reservoir; and

a flexible membrane bonded to the second plate to seal the reservoir.

10. The microfluidic device of claim 9, further comprising an actuator for deflecting the flexible membrane, wherein the deflection of the membrane increases the pressure in the
10 reservoir and deflects the meniscus into the microchannel to regulate liquid flow therethrough.

11. The microfluidic device of claim 9, further comprising a hydrophobic patch attached to the groove.

15

12. The microfluidic device of claim 9, wherein the meniscus forms a bubble in the microchannel to block liquid flow.

13. A method of making a bubble valve, comprising:

20

providing a microchannel having an interior bounded by a side wall, an aperture formed in the side wall and a valve chamber adjacent to the aperture in communication with the interior;

filling the microchannel with a liquid to form a meniscus of the liquid in the aperture, whereby the step of filling traps a gas in the valve chamber;

25

providing an actuator for increasing the pressure in the valve chamber to deflect the meniscus into the interior.

14. The method of claim 13, wherein the step of providing an actuator comprises

applying and sealing an actuator comprising a chamber to a top surface of the

30

microchannel to form a gas-filled chamber adjacent to the meniscus, wherein the actuator varies the pressure in the gas-filled chamber to deflect the meniscus into the interior, thereby regulating fluid flow.

15. A method of varying an electrical resistance in a microchannel, comprising the steps of:

generating a bubble;

injecting the bubble into a liquid in the microchannel, whereby the bubble varies the

5 electrical resistance of the microchannel.

16. The method of claim 15, wherein the step of generating a bubble comprises applying a pressure to a meniscus formed in a side wall of the microchannel, wherein the meniscus is formed by the liquid in the microchannel filling an aperture in the side wall.

10

17. The method of claim 16, wherein the step of applying a pressure comprises increasing the pressure within a sealed reservoir formed adjacent to the aperture.

18. An electrophoretic system, comprising

15 a first electrokinetically operated microchannel;

a sample well for providing a sample to the first electrokinetically operated microchannel microchannel;

a voltage source;

a bubble valve for injecting a bubble into the microchannel to vary the electrical

20 resistance of the microchannel.

19. The electrophoretic system of claim 18, further comprising:

a second electrokinetically operated microchannel in communication with the first electrokinetically operated microchannel, wherein the

25 a bubble valve selectively blocks flow from the first electrokinetically operated microchannel to the second electrokinetically operated microchannel by selectively injecting a bubble into a microchannel.

Abstract

A microfluidic system includes a bubble valve for regulating fluid flow through a microchannel. The bubble valve includes a fluid meniscus interfacing the microchannel interior and an actuator for deflecting the membrane into the
5 microchannel interior to regulate fluid flow. The actuator generates a gas bubble in a liquid in the microchannel when a sufficient pressure is generated on the membrane.

FIG. 1

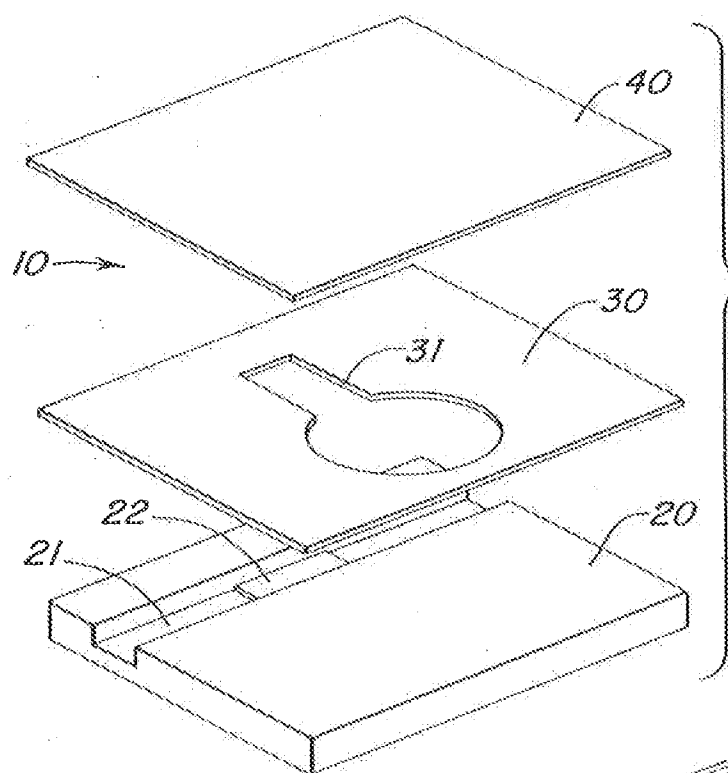
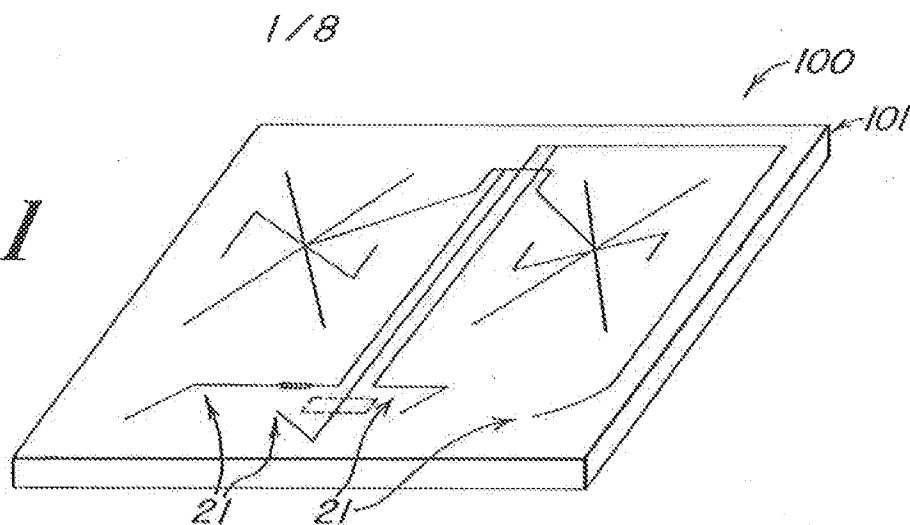


FIG. 2

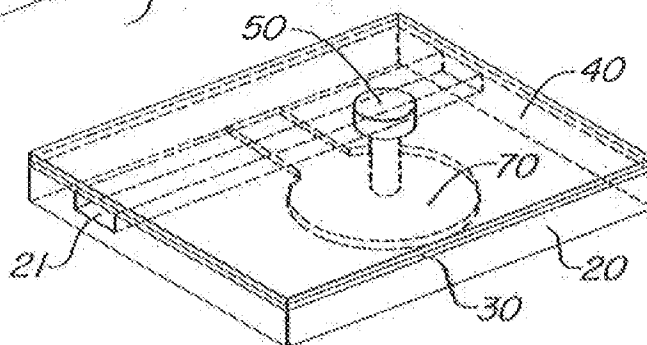
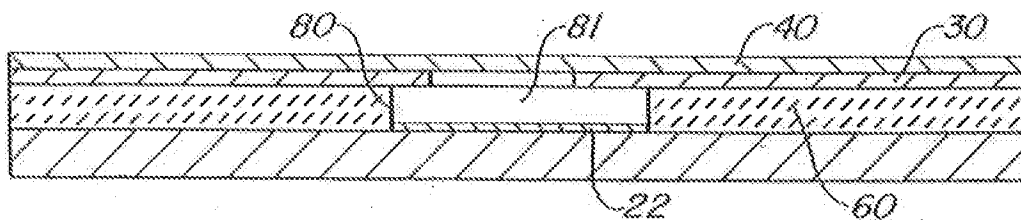
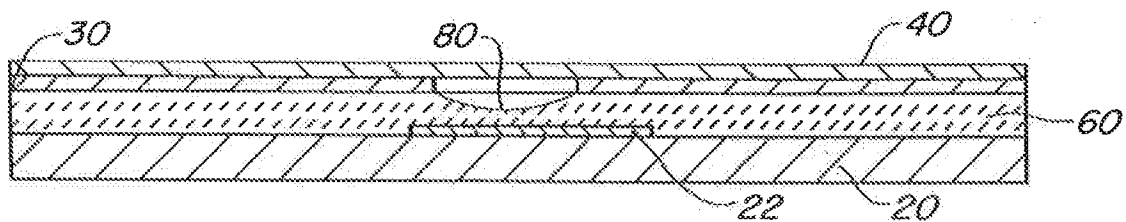
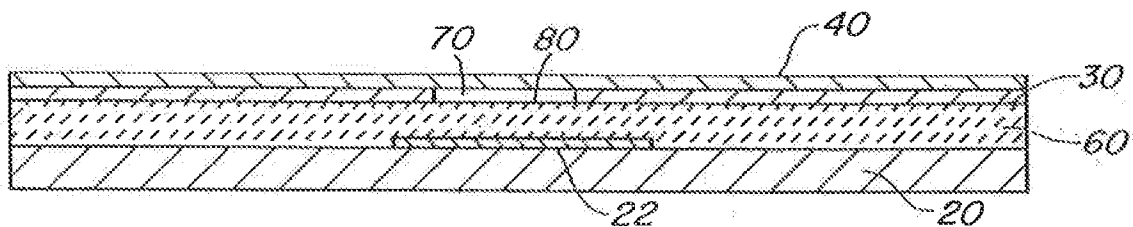
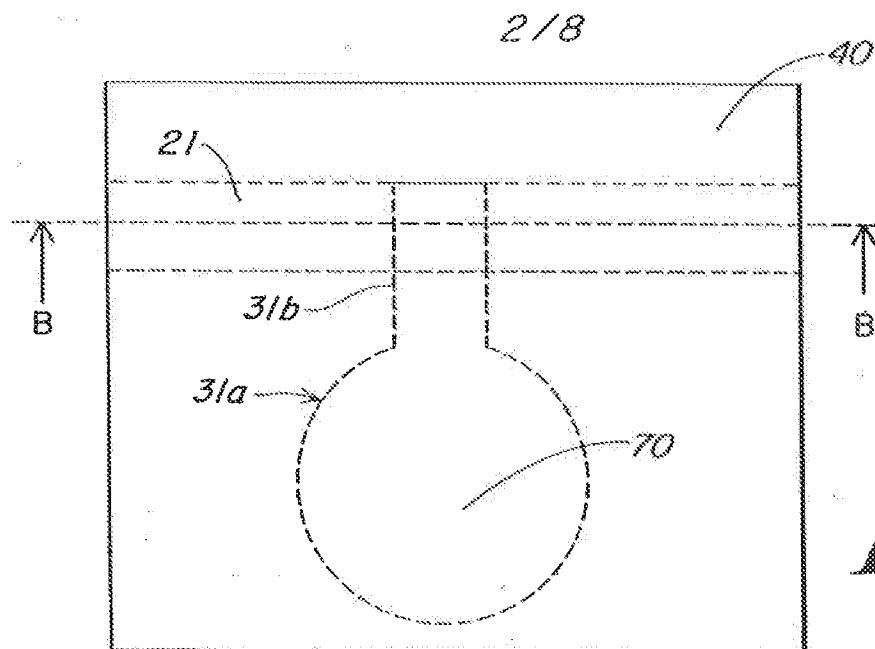


FIG. 3



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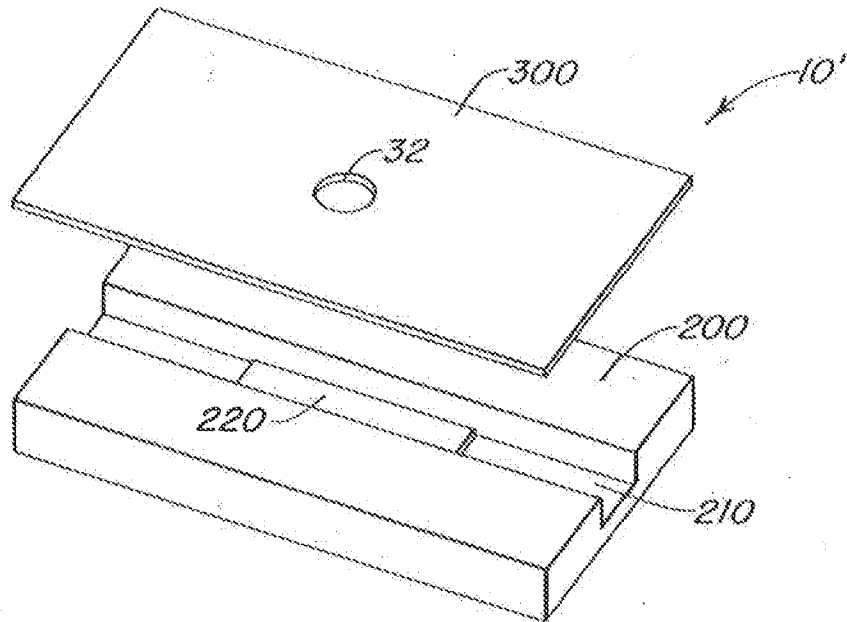


FIG. 6

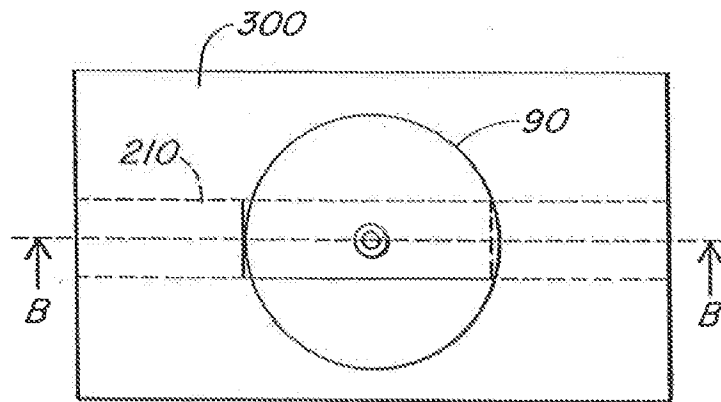


FIG. 7

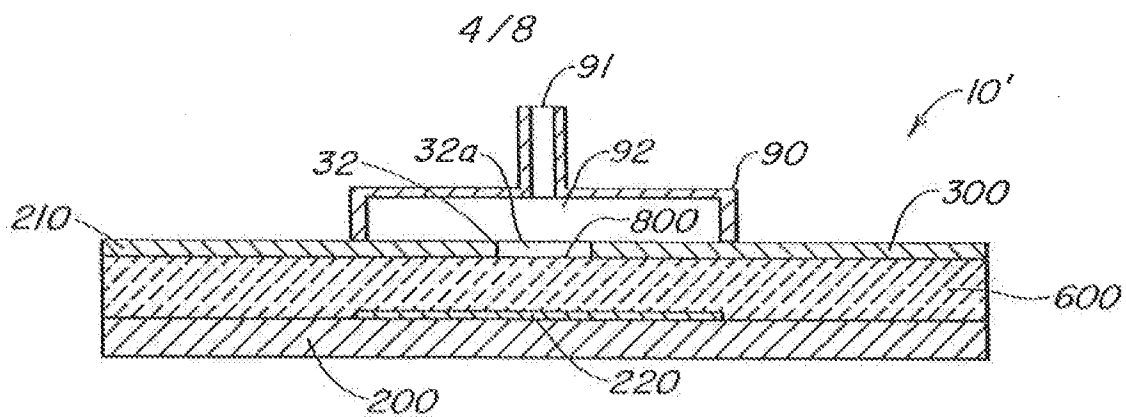


FIG. 8A

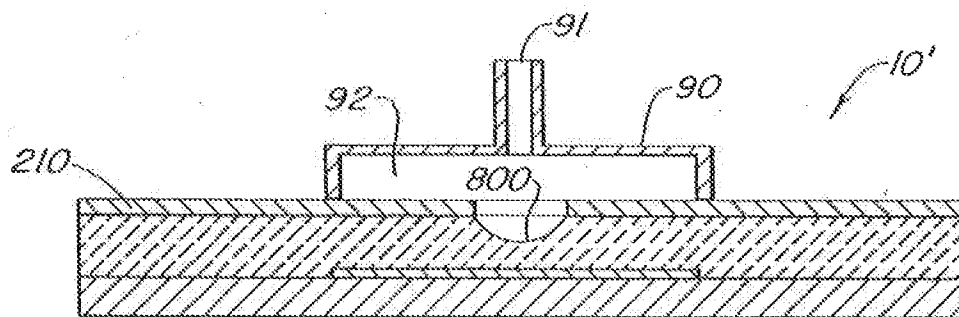


FIG. 8B

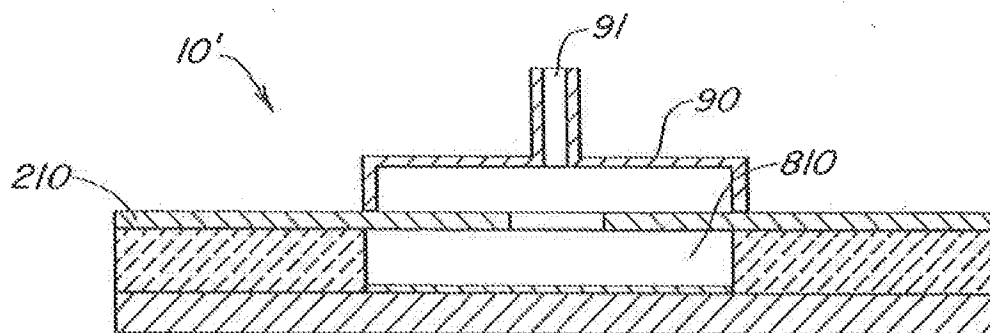


FIG. 8C

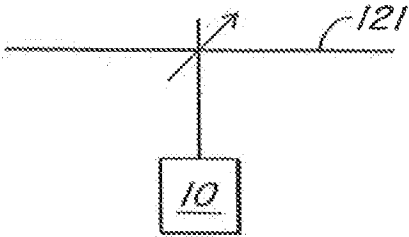


FIG. 9

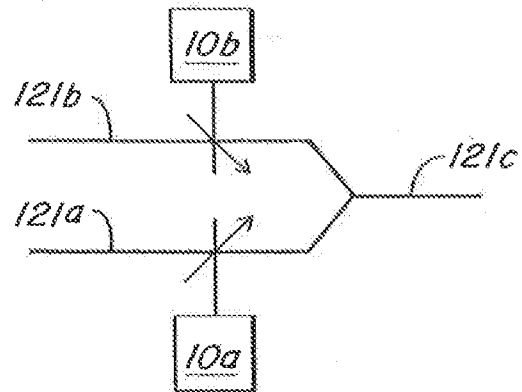


FIG. 10

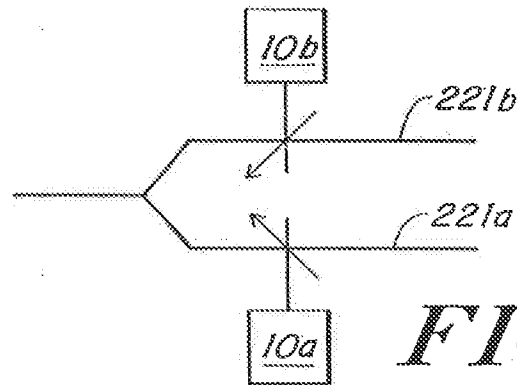


FIG. 11

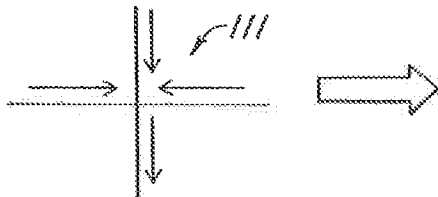


FIG. 12B

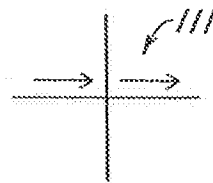


FIG. 12C

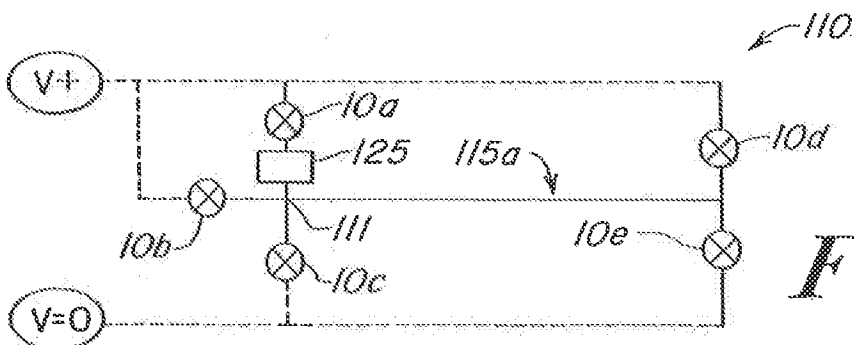


FIG. 12A

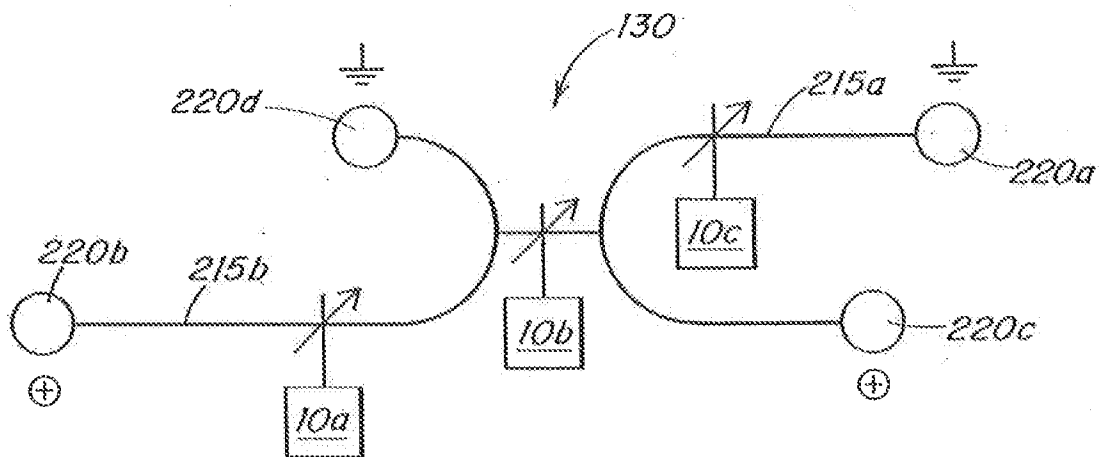


FIG. 13A

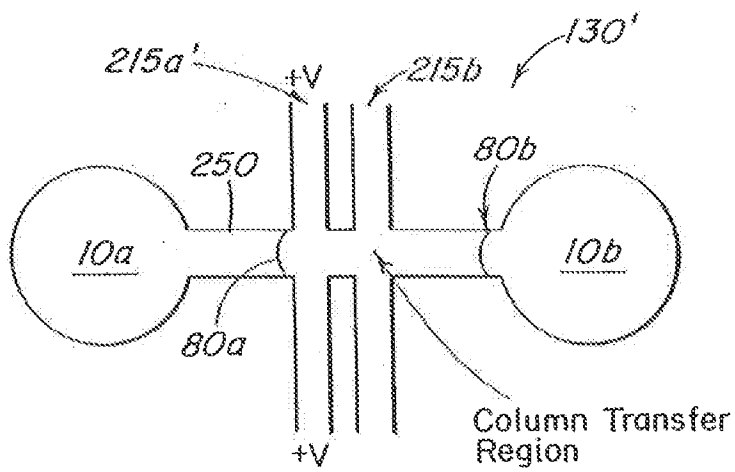


FIG. 13B

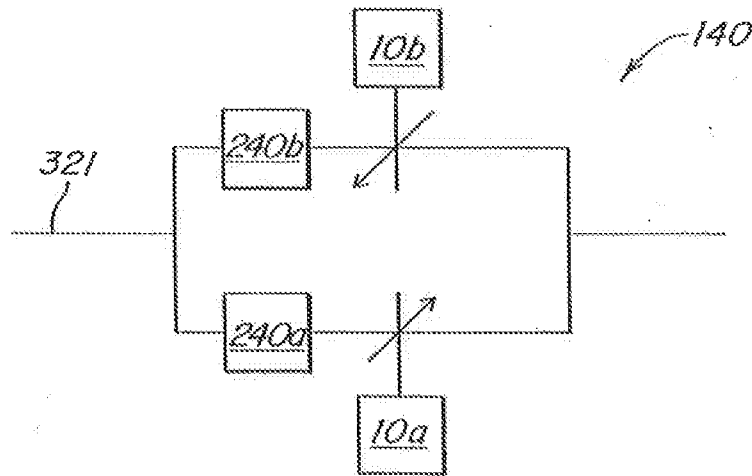


FIG. 14

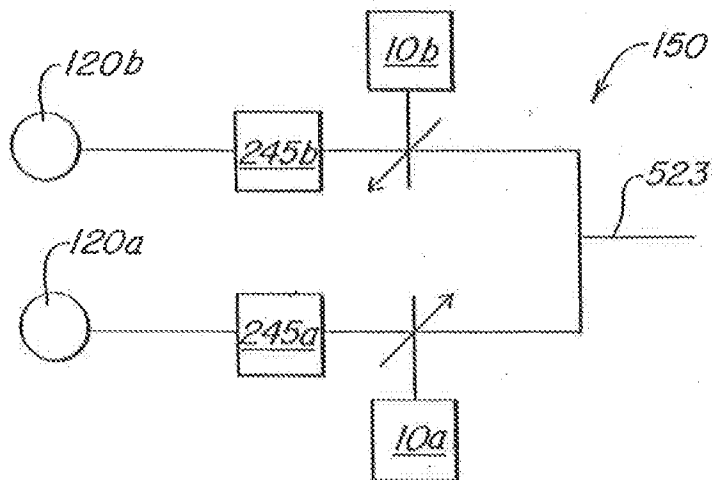


FIG. 15

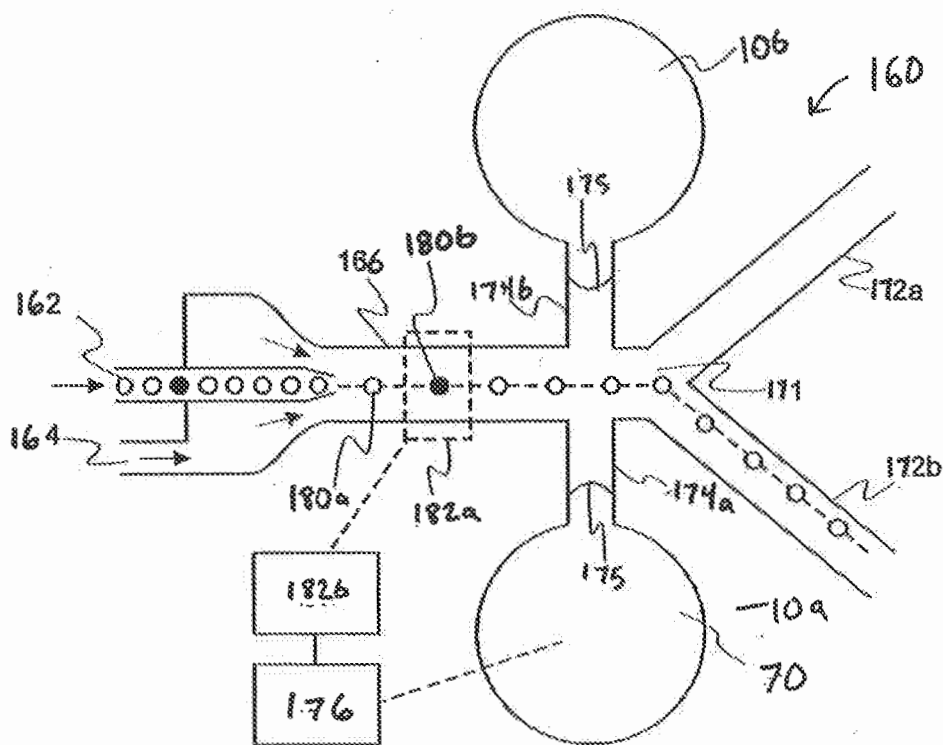


FIG. 16

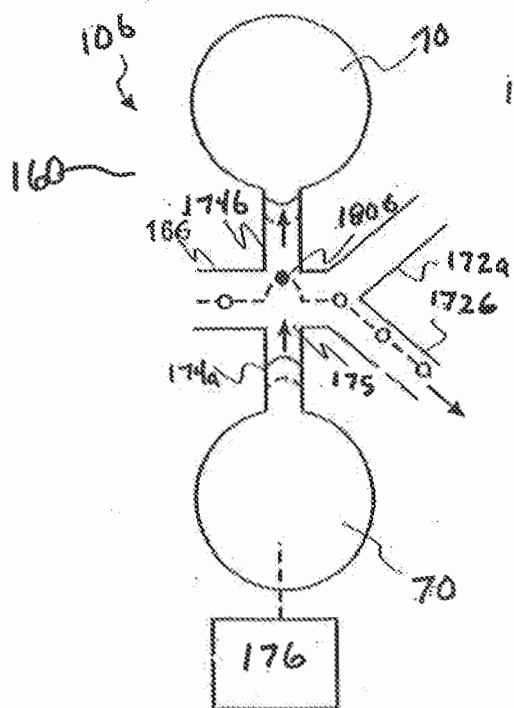


FIG. 17A

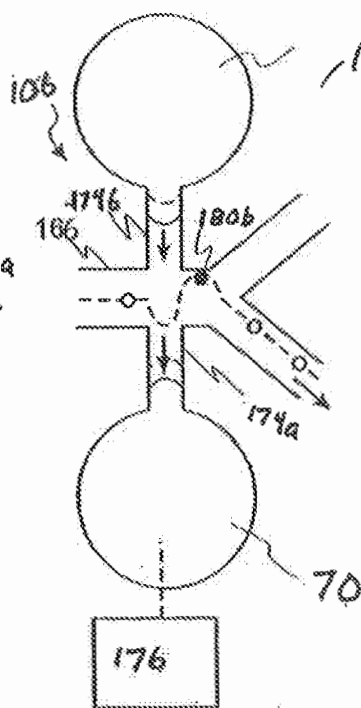


FIG. 17B

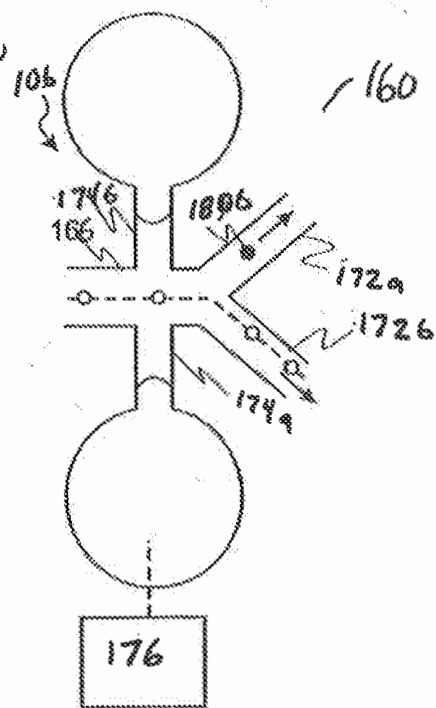


FIG. 17C

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 13052208 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John R. Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns/Gabriel Goldman |
| Filer Authorized By: | David Burns |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 19-JUN-2012 |
| Filing Date: | |
| Time Stamp: | 17:55:15 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|------------------------|----|
| Submitted with Payment | no |
|------------------------|----|

File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|-----------------|--------------------------------|--------------------------------------|---|------------------|------------------|
| 1 | Transmittal of New Application | 118153_01205_Utility_Transmittal.pdf | 21861 57e99867d84164726f8d899757c227a6fdc52fa1 | no | 1 |

Warnings:

Information:

0479

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|------------------------------|---|---|---|-----|----|
| 2 | Application Data Sheet | 118153_01205_Application_Data_Sheet.PDF | 1407707 4d6c3471933cd9ed13ca77cfe7ec875fcb62897e | no | 5 |
| Warnings: | | | | | |
| Information: | | | | | |
| 3 | Preliminary Amendment | 118153_01205_Preliminary_Amendment.pdf | 12372 aa78a6ce9e5f529f706186c3f5016c73d1f01e0d | no | 3 |
| Warnings: | | | | | |
| Information: | | | | | |
| 4 | Power of Attorney | 118153_01205_Copy_of_POA.PDF | 261570 1b12ae6c6a1d4fad90d3506d35bf5a0476b2494 | no | 1 |
| Warnings: | | | | | |
| Information: | | | | | |
| 5 | Assignee showing of ownership per 37 CFR 3.73(b). | 118153_01205_Statement_3_73b.pdf | 15340 dd4137220d6478800829706e317fb2f88f4db80e | no | 2 |
| Warnings: | | | | | |
| Information: | | | | | |
| 6 | Oath or Declaration filed | 118153_01205_Original_Declaration.PDF | 605074 74d4326a7676749972348c9ad342e6a96ac145b5 | no | 8 |
| Warnings: | | | | | |
| Information: | | | | | |
| 7 | | 118153_01205_Application.PDF | 1164455 4e31714ba41931246be9c5841ee21bd34343e880 | yes | 23 |
| | Multipart Description/PDF files in .zip description | | | | |
| | Document Description | | Start | End | |
| | Specification | | 1 | 19 | |
| | Claims | | 20 | 22 | |
| | Abstract | | 23 | 23 | |
| Warnings: | | | | | |
| Information: | | | | | |
| 8 | Drawings-only black and white line drawings | 118153_01205_Drawings.PDF | 356983 599aca08b533f0211f61d2376399a1099718ea69 | no | 8 |
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Patent Application Fee Transmittal

| | | | | |
|--|---|-----------------|---------------|-----------------------------|
| Application Number: | 13527331 | | | |
| Filing Date: | | | | |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL | | | |
| First Named Inventor/Applicant Name: | John R. Gilbert | | | |
| Filer: | David Burns/Gabriel Goldman | | | |
| Attorney Docket Number: | 118153-01205 | | | |
| Filed as Large Entity | | | | |
| Utility under 35 USC 111(a) Filing Fees | | | | |
| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
| Basic Filing: | | | | |
| Utility application filing | 1011 | 1 | 380 | 380 |
| Utility Search Fee | 1111 | 1 | 620 | 620 |
| Utility Examination Fee | 1311 | 1 | 250 | 250 |
| Pages: | | | | |
| Claims: | | | | |
| Miscellaneous-Filing: | | | | |
| Petition: | | | | |
| Patent-Appeals-and-Interference: | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
|-----------------------------------|----------|----------|--------|----------------------|
| Post-Allowance-and-Post-Issuance: | | | | |
| Extension-of-Time: | | | | |
| Miscellaneous: | | | | |
| Total in USD (\$) | | | | 1250 |

Electronic Acknowledgement Receipt

| | |
|---|---|
| EFS ID: | 13054619 |
| Application Number: | 13527331 |
| International Application Number: | |
| Confirmation Number: | 1757 |
| Title of Invention: | MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL |
| First Named Inventor/Applicant Name: | John R. Gilbert |
| Customer Number: | 86738 |
| Filer: | David Burns/Gabriel Goldman |
| Filer Authorized By: | David Burns |
| Attorney Docket Number: | 118153-01205 |
| Receipt Date: | 19-JUN-2012 |
| Filing Date: | |
| Time Stamp: | 18:02:20 |
| Application Type: | Utility under 35 USC 111(a) |

Payment information:

| | |
|--|-----------------|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | \$ 1250 |
| RAM confirmation Number | 5419 |
| Deposit Account | 504876 |
| Authorized User | |

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Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|-----------------|----------------------|--------------|---|------------------|------------------|
| 1 | Fee Worksheet (SB06) | fee-info.pdf | 35272 a049f643a4b22f62de80898d5b3b0e3956e5da30 | no | 2 |

Warnings:**Information:**

| | |
|-------------------------------------|-------|
| Total Files Size (in bytes): | 35272 |
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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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| PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875 | | | | | Application or Docket Number 13/527,331 | | Filing Date 06/19/2012 | | <input type="checkbox"/> To be Mailed | |
|--|---|----------------------------------|------------|------------------------------------|---|---|----------------------------------|-------------------------|---------------------------------------|---------------------|
| APPLICATION AS FILED – PART I | | | | | | | | | | |
| (Column 1) | | | (Column 2) | | | SMALL ENTITY <input checked="" type="checkbox"/> OR | | OTHER THAN SMALL ENTITY | | |
| FOR | NUMBER FILED | NUMBER EXTRA | RATE (\$) | FEE (\$) | OR | RATE (\$) | FEE (\$) | | | |
| <input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | | | N/A | | | | |
| <input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (l), or (m)) | N/A | N/A | N/A | | | N/A | | | | |
| <input type="checkbox"/> EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | | | N/A | | | | |
| TOTAL CLAIMS (37 CFR 1.16(j)) | minus 20 = | * | X \$ | = | OR | X \$ | = | | | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X \$ | = | | X \$ | = | | | |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$250 (\$125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | | | | | | |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | | | | | | |
| | | | TOTAL | | | TOTAL | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | | | | | | | | |
| APPLICATION AS AMENDED – PART II | | | | | | | | | | |
| (Column 1) | | | (Column 2) | | | SMALL ENTITY OR | | OTHER THAN SMALL ENTITY | | |
| AMENDMENT | 06/19/2012 | CLAIMS REMAINING AFTER AMENDMENT | MINUS | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) | OR | RATE (\$) | ADDITIONAL FEE (\$) |
| | Total (37 CFR 1.16(i)) | * 19 | Minus | ** 20 | = 0 | X \$30 = | 0 | OR | X \$ | = |
| | Independent (37 CFR 1.16(h)) | * 14 | Minus | *** 3 | = 11 | X \$125 = | 1375 | OR | X \$ | = |
| <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | | |
| <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | | OR | | |
| | | | | | | TOTAL ADD'L FEE | 1375 | OR | TOTAL ADD'L FEE | |
| (Column 1) | | | (Column 2) | | | SMALL ENTITY OR | | OTHER THAN SMALL ENTITY | | |
| AMENDMENT | | CLAIMS REMAINING AFTER AMENDMENT | MINUS | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE (\$) | ADDITIONAL FEE (\$) | OR | RATE (\$) | ADDITIONAL FEE (\$) |
| | Total (37 CFR 1.16(i)) | * | Minus | ** | = | X \$ | = | OR | X \$ | = |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | X \$ | = | OR | X \$ | = |
| <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | | |
| <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | | OR | | |
| | | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |
| <p>* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.</p> <p>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".</p> <p>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".</p> <p>The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.</p> | | | | | | | | | | |

Legal Instrument Examiner:
/CHRIS A. JONES/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.