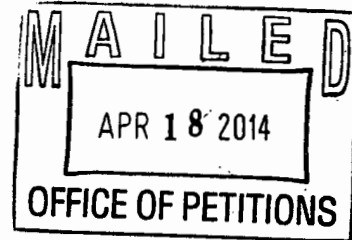




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MCCARTER & ENGLISH, LLP BOSTON
265 FRANKLIN STREET
BOSTON MA 02110



In re Patent No. 8,623,295 :
Issued: January 7, 2014 :
Application No. 13/245,331 : NOTICE
Filed: September 26, 2011 :
Attorney Docket No. 118153-01204 :

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 patent is no longer entitled to small entity status. Accordingly, all future fees paid in this patent 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



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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/245,331	01/07/2014	8623295	118153-01204	5808

86738 7590 12/18/2013
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 49 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, Inverness, UNITED KINGDOM;
Manish Deshpande, Canton, MA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

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

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	13/245,331, Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	3753
				Examiner Name	Craig M. Schneider
Sheet	1	of	3	Attorney Docket Number	118153-01204

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	Kamentsky et al.	
	A7*	US 3,984,307	10-05-1976	Kamentsky et al.	
	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 5,101,978	
	A19*	US 5,265,327	11-30-1993	Faris et al.	
	A20*	US 5,777,649	07-07-1998	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.	

Change(s) applied
to document,
/M.H.E./
9/21/2013

ME1 12896706v.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
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571) 273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

MCCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110

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.

Certificate of Mailing or Transmission

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.
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13/245,331	09/26/2011	John R. Gilbert	118153-01204	5808
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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
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nonprovisional	UNDISCOUNTED	\$1,780.00	\$300.00		\$2,080.00	11/29/2013
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EXAMINER	ART UNIT	CLASS-SUBCLASS
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P. S. Hyun	1772	422-502
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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

CYTONOME/ST, LLC

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

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 asserts 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 form 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: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature	/David R. Burns/	Date	November 27, 2013
Typed or printed name	David R. Burns	Registration No.	46,590

Electronic Patent Application Fee Transmittal

Application Number:	13245331			
Filing Date:	26-Sep-2011			
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			
Attorney Docket Number:	118153-01204			
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:				
Utility Appl Issue Fee	1501	1	1780	1780
Publ. Fee- Early, Voluntary, or Normal	1504	1	300	300 0005

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				2080

Electronic Acknowledgement Receipt

EFS ID:	17523747
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	27-NOV-2013
Filing Date:	26-SEP-2011
Time Stamp:	14:40:39
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 2080
RAM confirmation Number	2847
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	Issue Fee Payment (PTO-85B)	118153_01204_Fee_Transmittal.PDF	18583 68d13b498dc3360722e7d9401df537a7cdf e56f5	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32200 9eafe1a11ee6f0bbca9d96d3a9cf4d166749 fa76	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			50783		
<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>					



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NOTICE OF ALLOWANCE AND FEE(S) DUE

86738 7590 08/28/2013
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

EXAMINER

HYUN, PAUL SANG HWA

ART UNIT

PAPER NUMBER

1772

DATE MAILED: 08/28/2013

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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13/245,331

09/26/2011

John Richard Gilbert

118153-01204

5808

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
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nonprovisional

SMALL

\$890

\$300

\$0

\$1190

11/29/2013

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

**Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
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Alexandria, Virginia 22313-1450
or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

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.

86738 7590 08/28/2013
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

Certificate of Mailing or Transmission

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.
13/245,331	09/26/2011	John Richard Gilbert	118153-01204	5808

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	SMALL	\$890	\$300	\$0	\$1190	11/29/2013

EXAMINER	ART UNIT	CLASS-SUBCLASS
HYUN, PAUL SANG HWA	1772	422-503000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). <input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached. <input type="checkbox"/> "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. <div> 1 _____ 2 _____ 3 _____ </div>
---	---

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: <input type="checkbox"/> Issue Fee <input type="checkbox"/> Publication Fee (No small entity discount permitted) <input type="checkbox"/> Advance Order - # of Copies _____	4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above) <input type="checkbox"/> A check is enclosed. <input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached. <input type="checkbox"/> The Director is hereby authorized to charge the required fee(s), any deficiency, or credit 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

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

☐ Applicant asserting small entity status. See 37 CFR 1.27

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.

☐ Applicant changing to regular undiscounted fee 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: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____

This collection of information 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.

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UNITED STATES DEPARTMENT OF COMMERCE
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Address: COMMISSIONER FOR PATENTS
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/245,331	09/26/2011	John Richard Gilbert	118153-01204	5808

86738 7590 08/28/2013
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

EXAMINER

HYUN, PAUL SANG HWA

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

1772

DATE MAILED: 08/28/2013

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

The Patent Term Adjustment to date is 49 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 49 day(s).

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 Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

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.

Notice of Allowability	Application No. 13/245,331	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 amendment filed on 11 July 2013.
☐ 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 20, 21, 23-37 and 49. 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)

<ol style="list-style-type: none"> 1. <input type="checkbox"/> Notice of References Cited (PTO-892) 2. <input type="checkbox"/> Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____ 3. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit of Biological Material 4. <input checked="" type="checkbox"/> Interview Summary (PTO-413), Paper No./Mail Date <u>20130821</u>. 	<ol style="list-style-type: none"> 5. <input checked="" type="checkbox"/> Examiner's Amendment/Comment 6. <input checked="" type="checkbox"/> Examiner's Statement of Reasons for Allowance 7. <input type="checkbox"/> Other _____.
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/Paul S Hyun/
Examiner, Art Unit 1772

DETAILED ACTION

The amendment filed on July 11, 2013 is acknowledged. Claims 20, 21, 23-37, 48 and 49 remain pending. Claims 27, 28, 33-35 and 38 were indicated as having allowable subject matter. Applicant amended claim 20 to incorporate the allowable subject matter of previously pending claim 38. Applicant also amended claims 21, 23, 30 and 37.

The claim objection and the rejection 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 August 22, 2013.

The application has been amended as follows:

Claim 48 (Cancelled)

Claim 49 (Currently Amended) The system of claim **[48] 20**, wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure.

Allowable Subject Matter

Claims 20, 21, 23-37 and 49 are allowed.

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

Unger et al. (EP 1 065 378 A2) disclose a microfluidic device comprising a flow channel 30 and a first reservoir 32 operatively associated with the flow channel 30, said reservoir capable of dampening or absorbing a pressure pulse propagated across the flow channel (see Fig. 23B, [0144] and [0230] which disclose that the reservoir can expand or retract upon application of pressure).

However, Unger et al. do not disclose an arrangement in which a flow channel is delimited by a pair of opposing apertures, each aperture leading to a reservoir, wherein one of the reservoirs is configured to change volume or generate pressure and the other reservoir is configured to absorb or dampen said pressure, as recited in independent claim 20.

Moreover, based on the disclosure of Unger et al., there is no motivation to modify the device to arrive at the claimed invention.

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 Monday-Friday 10AM-6:30PM.

Art Unit: 1772

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 S Hyun/
Examiner, Art Unit 1772

Search Notes 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.
	Examiner PAUL HYUN	Art Unit 1772

CPC- SEARCHED		
Symbol	Date	Examiner


CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

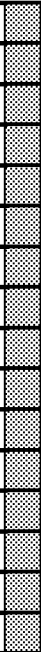
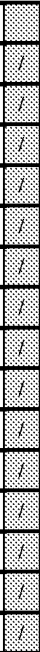
US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
422	502-504,5095	8/22/2013	PSH



SEARCH NOTES		
Search Notes	Date	Examiner
inventor name search	8/22/2013	PSH

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	see search history	8/22/2013	PSH


/PAUL HYUN/ Examiner.Art Unit 1772	
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Issue Classification 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.	
	Examiner PAUL HYUN	Art Unit 1772	

CPC			
Symbol		Type	Version
			

CPC Combination Sets				
Symbol	Type	Set	Ranking	Version
				
				

		Total Claims Allowed:	
		18	
(Assistant Examiner)	(Date)	O.G. Print Claim(s)	O.G. Print Figure
/PAUL HYUN/ Examiner.Art Unit 1772	08/22/2013	1	16
(Primary Examiner)	(Date)		

Issue Classification 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.
	Examiner PAUL HYUN	Art Unit 1772

<input checked="" 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	13	33	18	49								
-	2	-	18	14	34	-	50								
-	3	-	19	15	35	-	51								
-	4	1	20	16	36										
-	5	2	21	17	37										
-	6	-	22	-	38										
-	7	3	23	-	39										
-	8	4	24	-	40										
-	9	5	25	-	41										
-	10	6	26	-	42										
-	11	7	27	-	43										
-	12	8	28	-	44										
-	13	9	29	-	45										
-	14	10	30	-	46										
-	15	11	31	-	47										
-	16	12	32	-	48										

(Assistant Examiner) (Date)		Total Claims Allowed: 18	
/PAUL HYUN/ Examiner.Art Unit 1772 (Primary Examiner)		08/22/2013 (Date)	O.G. Print Claim(s) 1 O.G. Print Figure 16

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	3387	microfluidic and sort\$4 near2 cell	US-PGPUB; USPAT	OR	ON	2013/08/22 15:10
L2	55	microfluidic and sort\$4 near2 cell.ti.	US-PGPUB; USPAT	OR	ON	2013/08/22 15:13
L3	14	microfluidic and sort\$4 near2 cell.ti.	USPAT	OR	ON	2013/08/22 15:14
L4	321	microfluidic.clm. same channel same pressure	US-PGPUB	OR	ON	2013/08/22 15:29
L5	3	microfluidic.clm. same channel same pressure with (volume pulse) same (cell particle)	US-PGPUB	OR	ON	2013/08/22 15:44
L6	25515	(gilbert bohm deshpande)"in." and microfluidic and pulse and aperture and (cell particle)	US-PGPUB	OR	ON	2013/08/22 15:53
L7	91704	(gilbert bohm deshpande)"in." and microfluidic and pulse and aperture and (cell particle)	US-PGPUB; USPAT	OR	ON	2013/08/22 15:53
L8	48	(gilbert bohm deshpande).in. and microfluidic and pulse and aperture and (cell particle)	US-PGPUB; USPAT	OR	ON	2013/08/22 15:53
L9	18	(gilbert bohm deshpande).in. and microfluidic and pulse and aperture and (cell particle)	USPAT	OR	ON	2013/08/22 15:56
L10	3372	(422/502,503,504,509).CCLS.	US-PGPUB; USPAT	OR	OFF	2013/08/22 16:05
L11	1061	l10 and microfluidic and pressure	US-PGPUB; USPAT	OR	ON	2013/08/22 16:05
L12	47	l10 and microfluidic and pressure near3 pulse	US-PGPUB; USPAT	OR	ON	2013/08/22 16:05
L13	18	l10 and microfluidic and pressure near4 flow near4 (redirect\$4 direction)	US-PGPUB; USPAT	OR	ON	2013/08/22 16:06
L14	3567	(422/502,503,504,505,509).CCLS.	US-PGPUB; USPAT	OR	OFF	2013/08/22 16:11
L15	52	l14 and microfluidic and pressure near3 pulse	US-PGPUB; USPAT	OR	ON	2013/08/22 16:11
L16	5	l14 and microfluidic and pressure near3 pulse not l12	US-PGPUB; USPAT	OR	ON	2013/08/22 16:11
L17	10	l14 and microfluidic and pressure near3	US-	OR	ON	2013/08/22

		(absorb\$4 damp\$4) not l12	PGPUB; USPAT			16:13
L18	10	l14 and microfluidic and pressure near3 (absorb\$4 damp\$4)	US- PGPUB; USPAT	OR	ON	2013/08/22 16:13
L19	4	l14 and microfluidic and pressure near3 (absorb\$4 damp\$4) and sort\$4	US- PGPUB; USPAT	OR	ON	2013/08/22 16:14
L20	66	microfluidic and pressure near3 (absorb\$4 damp\$4) and sort\$4	US- PGPUB; USPAT	OR	ON	2013/08/22 16:15

8/ 22/ 2013 4:15:32 PM
C:\ Users\ phyun\ Documents\ EAST\ Workspaces\ 13,245,331.wsp

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: July 11, 2013

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

Docket No.: 118153-01204
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
John R. Gilbert *et al.*

Application No.: 13/245,331

Confirmation No.: 5808

Filed: September 23, 2011

Art Unit: 1772

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

Examiner: Hyun, Paul Sang Hwa

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

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION

Dear Madam:

In response to the non-final Office Action dated April 25, 2013 (Paper No. 201304017), the response due date of which is July 25, 2013. Applicants submits the following amendments and remarks in the above-referenced U.S. patent application:

Amendments to the Claims beginning at page 2 of this paper.

Remarks beginning at page 5 of this paper.

AMENDMENTS TO THE CLAIMS

Please amend the claims, without prejudice, as follows:

- 1-19. (Canceled)
20. (Currently Amended) A microfluidic system comprising:
- a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel;
- a first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel; and
- 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;
- wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow channel relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.
21. (Currently Amended) The system of claim 20 ~~48~~ 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.
22. (Canceled)
23. (Currently Amended) The system of claim 20, ~~22~~, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.
24. (Previously Presented) The system 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.
25. (Previously Presented) The system of claim 24, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.

26. (Previously Presented) The system of claim 25, wherein the fluid filled region includes a portion of the first reservoir.
27. (Previously Presented) The system of claim 25, wherein the fluid filled region includes a side channel connecting the first reservoir to the flow channel.
28. (Previously Presented) The system of claim 25, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
29. (Previously Presented) The system of claim 20, wherein the first reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.
30. (Currently Amended) The system of claim 20, ~~48~~, wherein the second reservoir includes a gas pocket, wherein compression of the gas facilitates the generating of the pressure pulse.
31. (Previously Presented) The system of claim 30 further comprising a fluid filled region interfacing with the flow channel and the gas pocket.
32. (Previously Presented) The system of claim 31, wherein the fluid filled region includes a portion of the second reservoir.
33. (Previously Presented) The system of claim 31, wherein the fluid filled region includes a side channel connecting the second reservoir to the flow channel.
34. (Previously Presented) The system of claim 31, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
35. (Previously Presented) The system of claim 31, wherein the fluid filled region forms a meniscus with the gas pocket.
36. (Previously Presented) The system of claim 35, ~~34~~, wherein the change in volume or pressure in the second reservoir deflects the meniscus into the flow channel.
37. (Currently Amended) The system of claim 20, ~~48~~, wherein the second reservoir includes a deflectable flexible membrane wherein deflection of the membrane increases the pressure in the second reservoir.
- 38-47. (Canceled)

- 48. (Previously Presented) The system 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.
- 49. (Previously Presented) The system of claim 48, wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure.
- 50-51. (Canceled).

REMARKS

The present response is intended to fully address the objections and rejections noted in the outstanding Office Action mailed April 25, 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.

I. Allowable Subject Matter:

As a preliminary matter, Applicants would like to thank the Examiner for his indication of allowable subject matter with respect to claims 27, 28, 33-35 and 38.

II. Claim Amendments:

As of the mailing of the Office Action, claims 20-51 were pending of which claims 39-47 and 50 were withdrawn. Claim 20 is hereby amended to incorporate therein the limitations recited in allowable claim 38 including all limitations recited in intervening claim 48. Claims 21, 23, 30 and 37 are amended to depend from claim 20. Claim 36 is hereby amended to depend from claim 35. Claims 22, 38-47 and 50-52 are hereby canceled without prejudice. Applicants respectfully submit that no new matter is introduced by way of the foregoing amendments to the claims. Thus, Applicants respectfully request prompt entry thereof and substantive consideration of claims 20, 21, 23-37, 48 and 49 as presented herein of which claim 20 is in independent form.

III. Claim Objections:

In the Office Action, the Examiner objected to claim 20 for missing the conjunction "and" after the semicolon in line 4. Claim 20 is hereby amended to include the conjunction

“and.” Accordingly, Applicants respectfully request reconsideration and withdrawal of the objection to claim 20.

IV. Section 112 Rejections:

In the Office Action, claim 22 was rejected under 35 U.S.C. 112, fourth paragraph for allegedly failing to further limit the claim from which it depended. Also in the Office Action, claims 36 and 51 were rejected under 35 U.S.C. 112, second paragraph as allegedly indefinite. In particular, the Examiner noted that there was insufficient antecedent basis for the term “the meniscus” in claim 36 and that the system of claim 51 improperly depended from a method claim. Applicants hereby address the Examiners outstanding Section 112 rejections to dependent claims 22, 36 and 51 by cancelling, without prejudice, claims 22 and 51 and amending claim 36 to depend from claim 35 thereby ensuring proper antecedent basis. In view of such amendments to the claims, Applicants respectfully request reconsideration and withdrawal of the outstanding Section 112 rejections to claims 22, 36 and 51.

V. Art-Based Rejections:

In the Office Action, claims 20-26, 29-32, 37, 48 and 49 were rejected as allegedly anticipated by EP Application No. 1065378 to Unger *et al.* (“Unger”). While applicants do not necessarily agree with the Examiner’s grounds for rejection, in the interests of furthering prosecution and advancing the subject application toward allowance, Applicants hereby amend claim 20 to incorporate therein the limitations recited in allowable claim 38 including all limitations recited in intervening claim 48. Thus, Applicants respectfully submit that claim 20 and all claims depending therefrom patentably distinguish over Unger and are in condition for allowance and respectfully request the Examiner so find. Reconsideration and withdrawal of the outstanding art-based rejections is respectfully requested.

CONCLUSION

In view of the foregoing, Applicants submit that each of the Examiner's concerns has been fully and completely addressed, and that none of the claims presented herein are anticipated by or obvious over the prior art of record. 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.

The Examiner is authorized to charge any fees, including extra claim fees and extension of time fees, for example, charge any deficiency, and/or credit any overpayment to Deposit Account No. 50-4876 under Order No. 118153-01204

Dated: July 11, 2013

Respectfully submitted,

By: /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 Applicants

Electronic Acknowledgement Receipt

EFS ID:	16290376
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	11-JUL-2013
Filing Date:	26-SEP-2011
Time Stamp:	19:15:13
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		118153_01204_Amendment. PDF	29154 8f3e6e162c4c7d29ef0d5479974a531915619bb9	yes	7

	Multipart Description/PDF files in .zip description		
	Document Description	Start	End
	Amendment Copy Claims/Response to Suggested Claims	1	1
	Claims	2	4
	Applicant Arguments/Remarks Made in an Amendment	5	7
Warnings:			
Information:			
Total Files Size (in bytes):		29154	
<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 13/245,331	Filing Date 09/26/2011	<input type="checkbox"/> To be Mailed
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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	07/11/2013	CLAIMS REMAINING AFTER AMENDMENT	Minus	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	* 19	Minus	** 32	= 0	x \$40 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	x \$210 =	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
/DORRETTA BROOKS/

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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

Application No.: 13/245,331

Confirmation No.: 5808

Filed: September 26, 2011

Art Unit: 1772

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

Examiner: P. S. Hyun

**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. 118153-01204 from which the undersigned is authorized to draw, in the amount of \$1,666 for the total deficiency owed.

Fee Type	Current Undiscounted Fee	Small Entity Fee Paid	Deficiency Owed
Basic filing Fee, Utility	\$280	\$95	\$185
Utility Search Fee	\$600	\$310	\$290
Utility Examination Fee	\$720	\$125	\$595
Claims in excess of 20 Fee	\$640	\$240	\$400
Claims in excess of 20 Fee	\$320	\$124	\$196

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:	15797111
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	16-MAY-2013
Filing Date:	26-SEP-2011
Time Stamp:	15:50:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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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	44029 73bc2de1c147f7be913db11aeb3d1ff33418c9de	no	2

Warnings:

Information:

0038

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: 04/17/2014

CKHLOK	SALE	#00000007	Mailroom Dt:	05/16/2013	504876	13245331
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/245,331	09/26/2011	John Richard Gilbert	118153-01204	5808

86738 7590 04/25/2013
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

EXAMINER

HYUN, PAUL SANG HWA

ART UNIT	PAPER NUMBER
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1772

NOTIFICATION DATE	DELIVERY MODE
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04/25/2013

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

Office Action Summary	Application No. 13/245,331	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 April 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) 20-51 is/are pending in the application.
 5a) Of the above claim(s) 39-47 and 50 is/are withdrawn from consideration.
- 6) ☐ Claim(s) _____ is/are allowed.
- 7) ☒ Claim(s) 20-26, 29-32, 36, 37, 48, 49 and 51 is/are rejected.
- 8) ☒ Claim(s) 27, 28, 33-35 and 38 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 26 September 2011 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.

Interim copies:

- a) ☐ All b) ☐ Some c) ☐ None of the: Interim copies of the priority documents have been 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/08)
Paper No(s)/Mail Date <u>01/31/12; 08/21/12</u> | 4) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Election/Restrictions

Applicant's election without traverse of claims 20-38, 48, 49 and 51 in the reply filed on April 12, 2013 is acknowledged. Claims 39-47 and 50 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on January 31, 2012 and August 21, 2012 are being considered by the examiner.

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(d):

(d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), 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.

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 **22** is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), fourth paragraph, for failing to further limit the claim from which it depends.

Claim 22 does not further limit claim 20 because the claim merely specifies the purpose for which the pressure pulse is used (i.e. intended use) and the pressure pulse is not even part of the claimed invention.

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.

Claims **36 and 51** 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 36 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 35. The claim will be examined accordingly.

Claim 51 is a system claim that is dependent on a method claim. Due to the indefiniteness, the claim will not be examined on the merits.

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 **20-26, 29-32, 37, 48 and 49** are rejected under 35 U.S.C. 102(b) as being anticipated by Unger et al. (EP 1 065 378 A2).

With respect to claim 20, Unger et al. disclose a microfluidic device comprising a flow channel 30 and a first reservoir 32 operatively associated with the flow channel 30, said reservoir capable of dampening or absorbing a pressure pulse propagated across the flow channel (see Fig. 23B, [0144] and [0230] which disclose that the reservoir can expand or retract upon application of pressure).

With respect to claim 22, the claim does not further limit claim 20, as discussed above.

With respect to claim 23, the flow channel branches into different channels (see Fig. 30), each of said branches capable of receiving particles suspended in a medium.

With respect to claim 24, the reservoir comprises a gas pocket, the compression of said gas causing the retraction of the reservoir (see [0230]).

With respect to claims 25 and 26, the first reservoir comprises a fluid filled region interfacing the gas pocket and the flow channel. It should be noted that the limitation “fluid filled region” merely connotes a space that is filled by a fluid, which includes gas. That said, any arbitrary space inside the reservoir that interfaces the flow channel can be deemed to be within the scope of the claimed “fluid filled region”.

With respect to claim 29, the reservoir is bounded by a resilient elastomeric wall (see Fig. 24B).

With respect to claim 48, the device comprises a plurality of reservoirs (i.e. second, third, etc.) operatively associated with flow channel 30, wherein the reservoirs are capable of generating a pressure pulse (see [0144] describing that the reservoirs can function as peristaltic pumps).

With respect to claims 21 and 30, the second reservoir is connected to a source of pressure for altering the volume of the reservoir (see [0103] and [0109]).

With respect to claims 31 and 32, the second reservoir comprises a fluid filled region interfacing the gas pocket and the flow channel. It should be noted that the limitation “fluid filled region” merely connotes a space that is filled by a fluid, which includes gas. That said, any arbitrary space inside the second reservoir that interfaces the flow channel can be deemed to be within the scope of the claimed “fluid filled region”.

With respect to claim 37, the second reservoir is bounded by a resilient elastomeric wall (see Fig. 24B). Moreover, it is apparent that the deflection of said elastomeric wall inwardly increases the pressure inside the second reservoir (due to the fact that the volume of the second reservoir decreases).

With respect to claim 49, the change in volume of/pressure inside the second reservoir is transient (see [0144]).

Conclusion

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

O'Connor (US 4,581,624) discloses a microfluidic device comprising a flow channel 14 formed in a substrate (see Fig. 9); and

a first reservoir 42 operatively associated with the flow channel 14, said reservoir capable of dampening a pressure pulse propagated across the flow channel.

Allowable Subject Matter

Claims 27, 28, 33-35 and 38 are 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. Moreover, claim 36 would be allowable if rewritten to overcome the rejection(s) under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), 2nd paragraph, set forth in this Office action and to include 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:

Unger et al. disclose a microfluidic device, as discussed above. However, Unger et al. do not disclose:

1) a channel connecting the flow channel to the reservoir as recited in claims 27, 33 and 38;

2) that the fluid filled region prevents the gas in the reservoir from leaking into the flow channel as recited in claims 28 and 34; or

Art Unit: 1772

3) that the fluid filled region forms a meniscus with the gas pocket as recited in claims 35 and 36.

Moreover, based on the disclosure of Unger et al., there is no motivation to modify the device to arrive at the claimed invention.

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 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 S Hyun/
Examiner, Art Unit 1772

Notice of References Cited	Application/Control No. 13/245,331	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-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	EP 1 065 378 A2	01-2001		Unger et al.	
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	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.

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	13/245,331, Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	3753
				Examiner Name	Craig M. Schneider
Sheet	1	of	3	Attorney Docket Number	118153-01204

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	Kamentsky et al.	
	A7*	US 3,984,307	10-05-1976	Kamentsky et al.	
	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 ,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	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.	

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	13/245,331, Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	3753
				Examiner Name	Craig M. Schneider
Sheet	2	of	3	Attorney Docket Number	118153-01204

	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 6,597,438	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 2006-0278288 A1	12-14-2006	Gilbert et al.	

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 00/070080	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

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 30, 2003		
	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		

ME1 12896706v.1

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

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				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Application Number	13/245,331, Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	3753
				Examiner Name	Craig M. Schneider
Sheet	3	of	3	Attorney Docket Number	118153-01204

		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	

Examiner Signature	/Paul Hyun/	Date Considered	04/22/2013
---------------------------	-------------	------------------------	------------

*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.

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Dated: January 31, 2012

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

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 <i>(Use as many sheets as necessary)</i>				Application Number	13/245,331 Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	1772
				Examiner Name	Paul Sang Hwa Hyun
Sheet	1	of	1	Attorney Docket Number	118153-01204

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 8,210,209	07-03-2012	Gilbert et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ³ -Number ⁴ -Kind Code ⁵ (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	T ⁶

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


Examiner Signature	/Paul Hyun/	Date Considered	04/22/2013
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*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.

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Dated: August 21, 2012

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

Search Notes 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.
	Examiner PAUL HYUN	Art Unit 1772

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
422	537	4/22/2013	PSH

SEARCH NOTES		
Search Notes	Date	Examiner
inventor name search	4/17/2013	PSH
IDS	4/17/2013	PSH
text search	4/17/2013	PSH
forward and backward search	4/17/2013	PSH

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

/PAUL HYUN/ 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
L2	171	("3936029" "4203128" "4293373").PN. OR ("4581624").URPN.	US-PGPUB; USPAT; USOCR	OR	OFF	2013/04/22 11:23
L3	40	l2 and pressure and (deflect\$4 deform\$4) near2 (wall membrane)	US-PGPUB; USPAT	OR	ON	2013/04/22 11:37
L4	35	l2 and pressure and (deflect\$4 deform\$4) near2 (wall membrane) and channel	US-PGPUB; USPAT	OR	ON	2013/04/22 11:48
L6	17	unger.in. and elastomer\$4 and valve and pump.ti.	USPAT	OR	ON	2013/04/22 12:55
L7	218	("4119368" "4153855" "5085562" "5096388" "5164558" "5171132" "5224843" "5259737" "5265327" "5336062" "5375979" "5376252" "5529465" "5659171" "5681024" "5705018" "5759014" "5836750" "5876187" "6007309").PN. OR ("6408878").URPN.	US-PGPUB; USPAT; USOCR	OR	OFF	2013/04/22 12:56
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L18	2	hyun.xa. and control adj channel and membrane and microfluidic	USPAT	OR	ON	2013/04/22 13:07
L19	329	(422/537).OCLS.	US-PGPUB; USPAT	OR	OFF	2013/04/22 13:09
L20	883212	"137"/	US-PGPUB; USPAT	OR	ON	2013/04/22 13:09
L21	145820	("137").CLAS.	US-PGPUB; USPAT	OR	OFF	2013/04/22 13:09
L22	194	(l21 l19) and (elastomer\$4 membrane) near3 (deflect\$4)	USPAT	OR	ON	2013/04/22 13:10
L23	123	(l21 l19) and (elastomer\$4 membrane) near3 (deflect\$4) and channel	USPAT	OR	ON	2013/04/22 13:10
L24	250	(l21 l19) and (elastomer\$4 membrane) near3 (deflect\$4) and channel	US-PGPUB; USPAT	OR	ON	2013/04/22 13:11
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S1	8	(gilbert bohms deshpande).in. and bubble adj. valve.ti.	US-PGPUB; USPAT	OR	ON	2013/04/17 11:14
S2	598	control adj channel and microfluidic and flow adj channel	US-PGPUB; USPAT	OR	ON	2013/04/17 11:49


S4	461	control adj channel and microfluidic and flow adj channel and elastomer\$4 and valve and pump	US-PGPUB; USPAT	OR	ON	2013/04/17 11:56
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4/ 22/ 2013 1:12:05 PM
C:\ Users\ phyun\ Documents\ EAST\ Workspaces\ 13,245,331.wsp

<i>Index of Claims</i> 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.
	Examiner PAUL HYUN	Art Unit 1772

✓	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	01/08/2013	04/22/2013						
	1	-	-						
	2	-	-						
	3	-	-						
	4	-	-						
	5	-	-						
	6	-	-						
	7	-	-						
	8	-	-						
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	19	-	-						
	20	÷	✓						
	21	÷	✓						
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	32	÷	✓						
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	34	÷	O						
	35	÷	O						
	36	÷	✓						

<i>Index of Claims</i> 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.
	Examiner PAUL HYUN	Art Unit 1772

✓	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	01/08/2013	04/22/2013						
	37	÷	✓						
	38	÷	O						
	39	÷	N						
	40	÷	N						
	41	÷	N						
	42	÷	N						
	43	÷	N						
	44	÷	N						
	45	÷	N						
	46	÷	N						
	47	÷	N						
	48	÷	✓						
	49	÷	✓						
	50	÷	N						
	51	÷	✓						

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Dated: April 12, 2013
Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: 118153-01204
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
John R. Gilbert *et al.*

Application No.: 13/245,331

Confirmation No.: 5808

Filed: September 26, 2011

Art Unit: 1772

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

Examiner: Hyun, Paul Sang Hwal

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

RESPONSE TO RESTRICTION REQUIREMENT

Dear Madam:

In response to the Restriction Requirement mailed January 14, 2013, the response due date of which is extended to April 14, 2013 by the accompanying Petition for a Two Month Extension of Time, Applicant respectfully requests reconsideration of the above-referenced U.S. patent application in view of the following:

Remarks begin on page 2 of this paper.

REMARKS

The Examiner identified two groups of claims on page 2 of the Restriction Requirement, and characterized them as follows:

Group I: Claims 20-38, 48, 49 and 51, drawn to a microfluidic system;

Group II: Claims 39-47 and 50, drawn to a method of processing liquid sample using a microfluidic system.

With respect to the restriction required by the Examiner, Applicants elect, *without traverse*, the following invention:

Group I: Claims 20-38, 48, 49 and 51, drawn to a microfluidic system.

Applicants hereby elect the claims of Group I, without traverse.

SUMMARY

Applicants respectfully submit that the above-identified application is in condition for allowance. If a telephone conversation with Applicants' attorney would expedite prosecution of the above-identified application, the Examiner is urged to call Applicants' Attorney at (617) 449-6500.

The Director is hereby authorized to charge \$600.00 under 37 C.F.R. § 1.17(a)(2) to our Deposit Account No. 50-4876, with reference to our Docket No. 118153-01204. The Director is also 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, with reference to our Docket No. 118153-01204.

Respectfully submitted,

Dated: April 12, 2013

Electronic Signature: /David R. Burns/
David R. Burns
Registration No.: 46,590
McCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110
Tel: (617) 449-6500
Fax: (617) 607-9200
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.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)		Docket Number (Optional) 118153-01204																															
Application Number 13/245,331		Filed September 26, 2011																															
For MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL																																	
Art Unit 1772; Confirmation No. 5808		Examiner Hyun, Paul Sang Hwa																															
<p>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.</p> <p>The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):</p> <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;"></th> <th style="text-align: center;"><u>Fee</u></th> <th style="text-align: center;"><u>Small Entity Fee</u></th> <th style="text-align: center;"><u>Micro Entity Fee</u></th> <th style="text-align: center;"></th> </tr> </thead> <tbody> <tr> <td><input type="checkbox"/> One month (37 CFR 1.17(a)(1))</td> <td style="text-align: center;">\$200</td> <td style="text-align: center;">\$100</td> <td style="text-align: center;">\$50</td> <td style="text-align: center;">\$ _____</td> </tr> <tr> <td><input checked="" type="checkbox"/> Two months (37 CFR 1.17(a)(2))</td> <td style="text-align: center;">\$600</td> <td style="text-align: center;">\$300</td> <td style="text-align: center;">\$150</td> <td style="text-align: center;">\$ 600.00</td> </tr> <tr> <td><input type="checkbox"/> Three months (37 CFR 1.17(a)(3))</td> <td style="text-align: center;">\$1,400</td> <td style="text-align: center;">\$700</td> <td style="text-align: center;">\$350</td> <td style="text-align: center;">\$ _____</td> </tr> <tr> <td><input type="checkbox"/> Four months (37 CFR 1.17(a)(4))</td> <td style="text-align: center;">\$2,200</td> <td style="text-align: center;">\$1,100</td> <td style="text-align: center;">\$550</td> <td style="text-align: center;">\$ _____</td> </tr> <tr> <td><input type="checkbox"/> Five months (37 CFR 1.17(a)(5))</td> <td style="text-align: center;">\$3,000</td> <td style="text-align: center;">\$1,500</td> <td style="text-align: center;">\$750</td> <td style="text-align: center;">\$ _____</td> </tr> </tbody> </table> <p><input type="checkbox"/> Applicant asserts small entity status. See 37 CFR 1.27.</p> <p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> A check in the amount of the fee is enclosed.</p> <p><input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.</p> <p><input checked="" type="checkbox"/> The Director has already been authorized to charge fees in this application to a Deposit Account.</p> <p><input checked="" type="checkbox"/> The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number <u>50-4876</u>.</p> <p><input checked="" type="checkbox"/> Payment made via EFS-Web.</p> <p>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.</p> <p>I am the</p> <p><input type="checkbox"/> applicant/inventor.</p> <p><input type="checkbox"/> assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96).</p> <p><input checked="" type="checkbox"/> attorney or agent of record. Registration number <u>46,590</u>.</p> <p><input type="checkbox"/> attorney or agent acting under 37 CFR 1.34. Registration number _____.</p> <div style="display: flex; justify-content: space-between; margin-top: 20px;"> <div style="width: 45%;"> <p style="text-align: center;">_____ /David R. Burns/ Signature</p> <p style="text-align: center;">_____ David R. Burns Typed or printed name</p> </div> <div style="width: 45%;"> <p style="text-align: center;">_____ April 12, 2013 Date</p> <p style="text-align: center;">_____ (617) 449-6500 Telephone Number</p> </div> </div> <p>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*.</p>					<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	\$ _____
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<input type="checkbox"/> *Total of <u>1</u> forms are submitted.																																	

Electronic Patent Application Fee Transmittal

Application Number:	13245331			
Filing Date:	26-Sep-2011			
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:	118153-01204			
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	0064 ⁶⁰⁰

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				600

Electronic Acknowledgement Receipt

EFS ID:	15501269
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	12-APR-2013
Filing Date:	26-SEP-2011
Time Stamp:	16:09:27
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$600
RAM confirmation Number	3106
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 1.17 (Patent application and reexamination processing fees)

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

0066

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Election / Restriction Filed	118153_01204_Response_to_R estriction_Requirement.PDF	11545 36fe29fa952fc4f2efdb9447cb8292af26707cac	no	2
Warnings:					
Information:					
2	Extension of Time	118153_01204_Petition_for_Ex tension_of_Time.PDF	13362 4875733fcc1fc39753e8ad5ddcd7ae921ff01587	no	1
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30973 540a8c2631bf5e2f1d146b364cbe1a47dc981bb7	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			55880		
<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>					



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/245,331	09/26/2011	John Richard Gilbert	118153-01204	5808

86738 7590 01/14/2013
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

EXAMINER

HYUN, PAUL SANG HWA

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

1772

NOTIFICATION DATE	DELIVERY MODE
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01/14/2013

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

Office Action Summary	Application No. 13/245,331	Applicant(s) GILBERT ET AL.	
	Examiner PAUL HYUN	Art Unit 1772	

-- 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 1 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 05 December 2012.
- 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) 20-51 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) ____ is/are rejected.
- 8) ☐ Claim(s) ____ is/are objected to.
- 9) ☒ Claim(s) 20-51 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).
- a) ☐ All b) ☐ Some * c) ☐ None of:
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 type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date ____. | 4) <input type="checkbox"/> Other: ____. |

DETAILED ACTION

The preliminary amendment filed by Applicant on December 5, 2012 is acknowledged.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

- I. Claims 20-38, 48, 49 and 51, drawn to a microfluidic system, classified in class 422, subclass 503.
- II. Claims 39-47 and 50, drawn to a method of processing liquid sample using a microfluidic system, classified in class 436, subclass 180.

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

Inventions I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case the microfluidic system of Group I can be used to conduct a materially different process. Specifically, the microfluidic system can be used to conduct an optically detectable assay without applying a pressure pulse within the device.

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 inventions recite mutually exclusive subject matter and as a result, they are classified in different classes and subclasses. Consequently, a serious burden would be imposed on the examiner if the claims are not restricted.

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.

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.

A telephone call was made to David Burns on January 7, 2013 to request an oral election to the above restriction requirement, but did not result in an election being made.

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 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.

Art Unit: 1772

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 S Hyun/
Examiner, Art Unit 1772



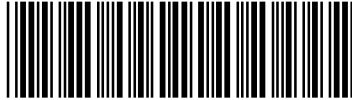
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BIB DATA SHEET


CONFIRMATION NO. 5808

SERIAL NUMBER 13/245,331	FILING or 371(c) DATE 09/26/2011 RULE	CLASS 422	GROUP ART UNIT 1772	ATTORNEY DOCKET NO. 118153-01204	
APPLICANTS John Richard Gilbert, Brookline, MA; Sebastian Böhm, Inverness, UNITED KINGDOM; Manish Deshpande, Canton, MA; ** CONTINUING DATA ***** This application is a CON of 11/433,781 05/12/2006 PAT 8,210,209 which is a CON of 11/021,251 12/21/2004 PAT 7,069,943 which is a CON of 10/179,586 06/24/2002 PAT 6,877,528 which claims benefit of 60/373,256 04/17/2002 ** FOREIGN APPLICATIONS ***** ** IF REQUIRED, FOREIGN FILING LICENSE GRANTED *** SMALL ENTITY ** 10/11/2011					
Foreign Priority claimed <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No 35 USC 119(a-d) conditions met <input type="checkbox"/> Yes <input type="checkbox"/> No Verified and /PAUL SANG HWA HYUN/ Acknowledged Examiner's Signature	<input type="checkbox"/> Met after Allowance Initials	STATE OR COUNTRY MA	SHEETS DRAWINGS 8	TOTAL CLAIMS 28	INDEPENDENT CLAIMS 2
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 894	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		

<i>Index of Claims</i> 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.
	Examiner PAUL HYUN	Art Unit 1772

✓	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	01/08/2013							
	1	-							
	2	-							
	3	-							
	4	-							
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<p align="center"><i>Index of Claims</i></p> 	Application/Control No. 13245331	Applicant(s)/Patent Under Reexamination GILBERT ET AL.
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✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

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CLAIM		DATE							
Final	Original	01/08/2013							
	37	÷							
	38	÷							
	39	÷							
	40	÷							
	41	÷							
	42	÷							
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	48	÷							
	49	÷							
	50	÷							
	51	÷							

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: December 5, 2012

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

Docket No.: 118153-01204
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
John R. Gilbert *et al.*

Application No.: 13/245,331

Confirmation No.: 5808

Filed: September 23, 2011

Art Unit: 1772

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

Examiner: Hyun, Paul Sang Hwa

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

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the claims begin on page 2.

Remarks begin on page 5.

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: December 5, 2012

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

Docket No.: 118153-01204
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Examiner: Hyun, Paul Sang Hwa

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

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the claims begin on page 2.

Remarks begin on page 5.

AMENDMENTS TO THE CLAIMS

Please amend the claims, without prejudice, as follows:

- 1-19. (Canceled)
20. (Currently Amended) A microfluidic system comprising:
- a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel;
- ~~a first reservoir operatively associated with the flow channel; and~~
- a first ~~second~~ reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel, resulting from a change in volume or pressure in the first reservoir.
21. (Currently Amended) The system of claim 48 ~~20~~ further comprising an actuator or pressurizer operatively associated with the second ~~first~~ reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second ~~first~~ reservoir.
22. (Previously Presented) The system of claim 20, wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.
23. (Previously Presented) The system of claim 22, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.
24. (Currently Amended) The system of claim 20, wherein the first ~~second~~ reservoir includes a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.
25. (Previously Presented) The system of claim 24, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.
26. (Currently Amended) The system of claim 25, wherein the fluid filled region includes a portion of the first ~~second~~ reservoir.

27. (Currently Amended) The system of claim 25, wherein the fluid filled region includes a side channel connecting the first ~~second~~ reservoir to the flow channel.
28. (Previously Presented) The system of claim 25, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
29. (Currently Amended) The system of claim 20, wherein the first ~~second~~ reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.
30. (Currently Amended) The system of claim 48, ~~20~~, wherein the second ~~first~~ reservoir includes a gas pocket, wherein compression of the gas facilitates the generating of the pressure pulse.
31. (Previously Presented) The system of claim 30 further comprising a fluid filled region interfacing with the flow channel and the gas pocket.
32. (Currently Amended) The system of claim 31, wherein the fluid filled region includes a portion of the second ~~first~~ reservoir.
33. (Currently Amended) The system of claim 31, wherein the fluid filled region includes a side channel connecting the second ~~first~~ reservoir to the flow channel.
34. (Previously Presented) The system of claim 31, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
35. (Previously Presented) The system of claim 31, wherein the fluid filled region forms a meniscus with the gas pocket.
36. (Currently Amended) The system of claim 31, wherein the change in volume or pressure in the second ~~first~~ reservoir deflects the meniscus into the flow channel.
37. (Currently Amended) The system of claim 48, ~~20~~, wherein the second ~~first~~ reservoir includes a deflectable flexible membrane wherein deflection of the membrane increases the pressure in the second ~~first~~ reservoir.
38. (Currently Amended) The system of claim 48, ~~20~~, wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow channel relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.

39. (Currently Amended) A method comprising:
- flowing a sample having one or more particles suspended in a suspension medium through a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of the sample;
- ~~propagating~~ ~~generating~~ a pressure pulse ~~across~~ ~~by changing a volume or pressure in a first reservoir operatively associated with the flow channel;~~ and
- dampening or absorbing the pressure pulse using a first ~~second~~ reservoir operatively associated with the flow channel.
40. (Previously Presented) The method of claim 39, wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.
41. (Previously Presented) The method of claim 40, wherein the pressure pulse is used to sort one or more particles into a selected branch of a branched portion of the flow channel.
42. (Currently Amended) The method of claim 39, wherein the dampening or absorbing of the pressure pulse is facilitated by compression of gas in a gas pocket in the first ~~second~~ reservoir.
43. (Currently Amended) The method of claim 39, wherein the dampening or absorbing of the pressure pulse is facilitated by a flexible wall of the first reservoir. ~~second membrane~~.
44. (Currently Amended) The method of claim 50, 39, wherein the generating the pressure pulse is facilitated by compression of gas in a gas pocket in the second ~~first~~ reservoir.
45. (Currently Amended) The method of claim 42, 39, wherein a fluid filled region interfaces with the flow channel and the gas pocket. ~~wherein the fluid filled region forms a meniscus with the gas pocket.~~
46. (Currently Amended) The method of claim 44; ~~39~~:
- wherein a fluid filled region interfaces with the flow channel and the gas pocket;
- wherein the fluid filled region forms a meniscus with the gas pocket; and
- wherein the change in volume or pressure in the second ~~first~~ reservoir deflects the meniscus into the flow channel.

47. (Currently Amended) The method of claim 50, ~~39~~, wherein the generating the pressure pulse includes deflecting a deflectable flexible membrane to increase the pressure in the second ~~first~~ reservoir.
48. (New) The system 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.
49. (New) The system of claim 48, wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure.
50. (New) The method of claim 39, further comprising generating the pressure pulse by changing a volume or pressure in a second reservoir operatively associated with the flow channel.
51. (New) The system of claim 50, wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure.

REMARKS

By way of the present preliminary amendment, claims 20, 21, 24, 26, 27, 29, 30, 32, 33, 36-39, and 42-47 are amended and new claims 48-51 are introduced. More particularly, Applicants hereby broaden independent claims 20 and 39 by removing from each subject matter relating to the fluid reservoir for generating the pressure pulse. The removed subject matter is incorporated into new dependent claims 48 and 50, respectively. In accordance with the amendments to claims 20 and 39, dependent claims 21 30, 37 and 44-47 are amended to ensure proper antecedent basis. Also in accordance with the amendments to claims 20 and 39, the claims are universally amended to swap the terms “first reservoir” and “second reservoir.” Finally, new claims 49 and 51 are introduced incorporating subject matter from paragraph [0074] of the specification. No new matter is introduced by way of the foregoing amendments.

Applicants respectfully request prompt entry and substantive consideration of the claim amendments presented herein prior to examination on the merits. After the claim amendments presented herein are entered, claims 20-49 will be pending.

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. 118153-01204.

Dated: December 5, 2012

Respectfully submitted,

Electronic signature for : /David R. Burns/
David R. Burns
Registration No. 46,590
MCCARTER & ENGLISH, LLP
265 Franklin Street
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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: December 5, 2012

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

Docket No.: 118153-01204
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
John R. Gilbert *et al.*

Application No.: 13/245,331

Confirmation No.: 5808

Filed: September 23, 2011

Art Unit: 1772

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

Examiner: Hyun, Paul Sang Hwa

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

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the claims begin on page 2.

Remarks begin on page 5.

AMENDMENTS TO THE CLAIMS

Please amend the claims, without prejudice, as follows:

- 1-19. (Canceled)
20. (Currently Amended) A microfluidic system comprising:
- a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel;
- ~~a first reservoir operatively associated with the flow channel; and~~
- a first ~~second~~ reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel, resulting from a change in volume or pressure in the first reservoir.
21. (Currently Amended) The system of claim 48 ~~20~~ further comprising an actuator or pressurizer operatively associated with the second ~~first~~ reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second ~~first~~ reservoir.
22. (Previously Presented) The system of claim 20, wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.
23. (Previously Presented) The system of claim 22, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.
24. (Currently Amended) The system of claim 20, wherein the first ~~second~~ reservoir includes a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.
25. (Previously Presented) The system of claim 24, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.
26. (Currently Amended) The system of claim 25, wherein the fluid filled region includes a portion of the first ~~second~~ reservoir.

27. (Currently Amended) The system of claim 25, wherein the fluid filled region includes a side channel connecting the first ~~second~~ reservoir to the flow channel.
28. (Previously Presented) The system of claim 25, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
29. (Currently Amended) The system of claim 20, wherein the first ~~second~~ reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.
30. (Currently Amended) The system of claim 48, ~~20~~, wherein the second ~~first~~ reservoir includes a gas pocket, wherein compression of the gas facilitates the generating of the pressure pulse.
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37. (Currently Amended) The system of claim 48, ~~20~~, wherein the second ~~first~~ reservoir includes a deflectable flexible membrane wherein deflection of the membrane increases the pressure in the second ~~first~~ reservoir.
38. (Currently Amended) The system of claim 48, ~~20~~, wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow channel relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.

39. (Currently Amended) A method comprising:
- flowing a sample having one or more particles suspended in a suspension medium through a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of the sample;
- ~~propagating~~ ~~generating~~ a pressure pulse ~~across~~ ~~by changing a volume or pressure in a first reservoir~~ ~~operatively associated with the flow channel~~; and
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44. (Currently Amended) The method of claim 50, 39, wherein the generating the pressure pulse is facilitated by compression of gas in a gas pocket in the second ~~first~~ reservoir.
45. (Currently Amended) The method of claim 42, 39, wherein a fluid filled region interfaces with the flow channel and the gas pocket. ~~wherein the fluid filled region forms a meniscus with the gas pocket.~~
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47. (Currently Amended) The method of claim 50, ~~39~~, wherein the generating the pressure pulse includes deflecting a deflectable flexible membrane to increase the pressure in the second ~~first~~ reservoir.
48. (New) The system 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.
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REMARKS

By way of the present preliminary amendment, claims 20, 21, 24, 26, 27, 29, 30, 32, 33, 36-39, and 42-47 are amended and new claims 48-51 are introduced. More particularly, Applicants hereby broaden independent claims 20 and 39 by removing from each subject matter relating to the fluid reservoir for generating the pressure pulse. The removed subject matter is incorporated into new dependent claims 48 and 50, respectively. In accordance with the amendments to claims 20 and 39, dependent claims 21 30, 37 and 44-47 are amended to ensure proper antecedent basis. Also in accordance with the amendments to claims 20 and 39, the claims are universally amended to swap the terms “first reservoir” and “second reservoir.” Finally, new claims 49 and 51 are introduced incorporating subject matter from paragraph [0074] of the specification. No new matter is introduced by way of the foregoing amendments.

Applicants respectfully request prompt entry and substantive consideration of the claim amendments presented herein prior to examination on the merits. After the claim amendments presented herein are entered, claims 20-49 will be pending.

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. 118153-01204.

Dated: December 5, 2012

Respectfully submitted,

Electronic signature for : /David R. Burns/
David R. Burns
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Attorney for Applicants

AMENDMENTS TO THE CLAIMS

Please amend the claims, without prejudice, as follows:

- 1-19. (Canceled)
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21. (Currently Amended) The system of claim ~~48~~ 20 further comprising an actuator or pressurizer operatively associated with the second ~~first~~ reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second ~~first~~ reservoir.
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- ~~propagating~~ ~~generating~~ a pressure pulse ~~across~~ ~~by changing a volume or pressure in a first reservoir~~ ~~operatively associated with the flow channel~~; and
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Dated: December 5, 2012

Respectfully submitted,

Electronic signature for : /David R. Burns/
David R. Burns
Registration No. 46,590
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(617) 607-9200 (Fax)
Attorney for Applicants

Electronic Acknowledgement Receipt

EFS ID:	14390414
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	05-DEC-2012
Filing Date:	26-SEP-2011
Time Stamp:	16:45:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Preliminary Amendment	118153-01204_Preliminary_Amendmentdoc_1.PDF	43681 75555048a0d7796b0e265d158bf3e15fa64810a4	no	6

Warnings:

Information:

0095

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.

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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875					Application or Docket Number 13/245,331		Filing Date 09/26/2011		<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))										
* 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	12/05/2012	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	* 32	Minus	** 28	= 4	X \$31 =	124	OR	X \$ =	
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X \$125 =	0	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))										
TOTAL ADD'L FEE						124	OR	TOTAL ADD'L FEE		
(Column 1)			(Column 2)			SMALL ENTITY OR		OTHER THAN SMALL ENTITY		
AMENDMENT	Total (37 CFR 1.16(i))	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$ =		X \$ =		
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$ =		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							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:
/ADRIANE WINSTON/

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.

Document code: WFEE

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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	13/245,331 Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	1772
				Examiner Name	Paul Sang Hwa Hyun
Sheet	1	of	1	Attorney Docket Number	118153-01204

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 8,210,209	07-03-2012	Gilbert et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ³ -Number ⁴ -Kind Code ⁵ (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	T ⁶

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

Examiner Signature		Date Considered	
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*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.

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Dated: August 21, 2012
Electronic Signature for David R. Burns: /David R. Burns/

Electronic Acknowledgement Receipt

EFS ID:	13551184
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	21-AUG-2012
Filing Date:	26-SEP-2011
Time Stamp:	20:35:56
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	118153_01204_IDS.pdf	14301 1ead1f61ab4aae0e2e42fb54ed2a7ac725d6be80	no	3

Warnings:

Information:

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2	Information Disclosure Statement (IDS) Form (SB08)	118153_01204_SB08.pdf	17157 42bf0042392577e7022ad648b83d382c7d489e3d	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
Total Files Size (in bytes):				31458	
<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>					

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Dated: August 21, 2012
Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: 118153-01204
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
John Richard Gilbert *et al.*

Application No. 13/245,331

Confirmation No.: 5808

Filed: September 26, 2011

Art Unit: 1772

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

Examiner: Paul Sang Hwa Hyun

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (IDS)

Dear Sir:

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
13/527,331	06-19-2012	N/A	Pending	118153-01205

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, to the best of Applicants’ knowledge, before the mailing of a first Office Action on the merits, under 37 C.F.R. § 1.97(b)(3). 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. 118153-01204.

Dated: August 21, 2012

Respectfully submitted,

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

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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	13/245,331, Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	3753
				Examiner Name	Craig M. Schneider
Sheet	1	of	3	Attorney Docket Number	118153-01204

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	Kamentsky et al.	
	A7*	US 3,984,307	10-05-1976	Kamentsky et al.	
	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 ,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	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.	

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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	13/245,331, Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	3753
				Examiner Name	Craig M. Schneider
Sheet	2	of	3	Attorney Docket Number	118153-01204

	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 6,597,438	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 2006-0278288 A1	12-14-2006	Gilbert et al.	

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 00/070080	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

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 30, 2003	
	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	

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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	13/245,331, Confirm # 5808
				Filing Date	September 26, 2011
				First Named Inventor	John Richard Gilbert
				Art Unit	3753
				Examiner Name	Craig M. Schneider
Sheet	3	of	3	Attorney Docket Number	118153-01204

		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	

Examiner Signature		Date Considered	
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*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.

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Dated: January 31, 2012

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



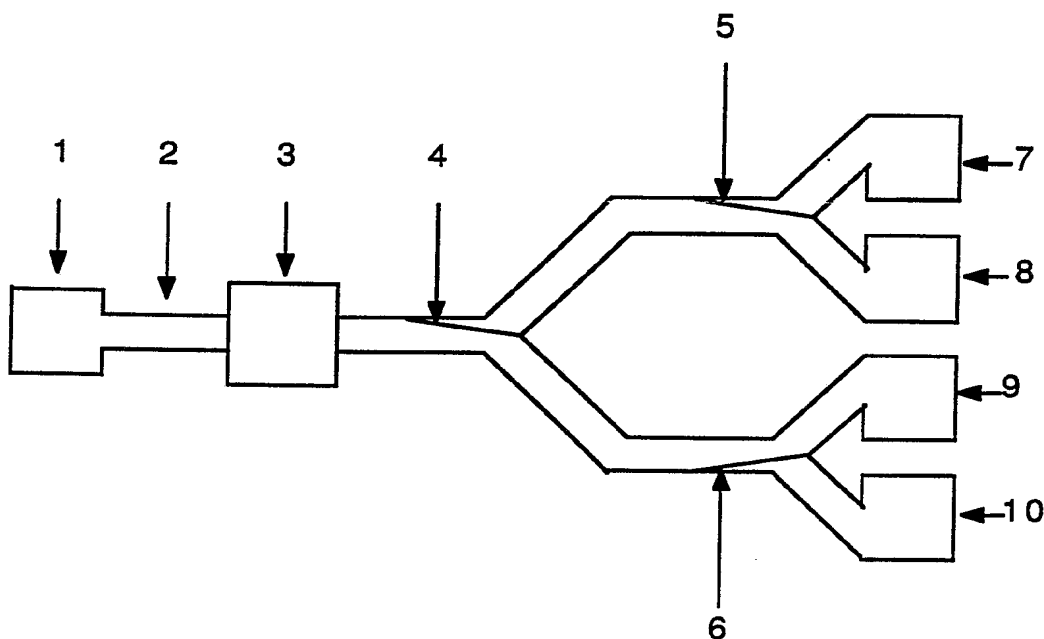
PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

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.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
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BF	Burkina Faso	GB	United Kingdom	MW	Malawi
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DE	Germany	MC	Monaco	US	United States of America
DK	Denmark				

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PCT/GB91/00542

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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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4

programmed to control the beams' deflection in response to the desired cell-sorting procedural steps.

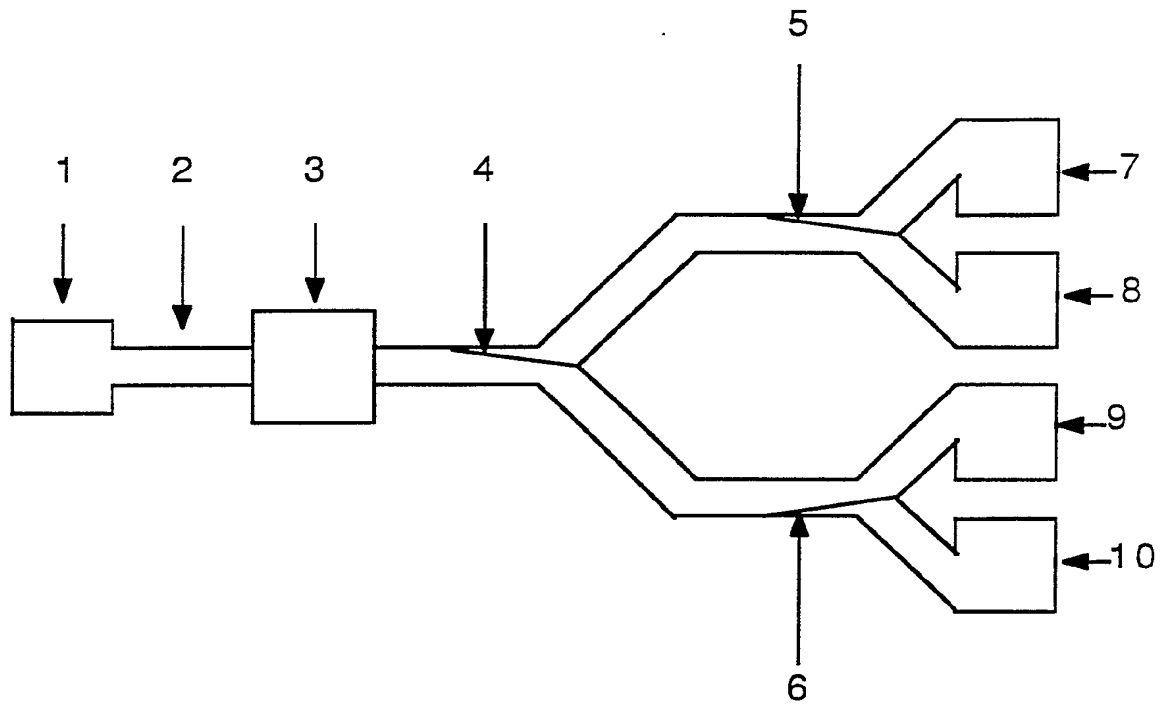
Claims:

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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PCT/GB91/00542

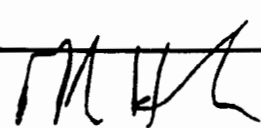
1/1.



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 ⁷ "A" document defining the general state of the art which is not considered to be of particular relevance ⁸ "E" earlier document but published on or after the international filing date ⁹ "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) ¹⁰ "O" document referring to an oral disclosure, use, exhibition or other means ¹¹ "P" document published prior to the international filing date but later than the priority date claimed ¹² "I" 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 ¹³ "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step ¹⁴ "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. ¹⁵ "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
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
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

12/07/91

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



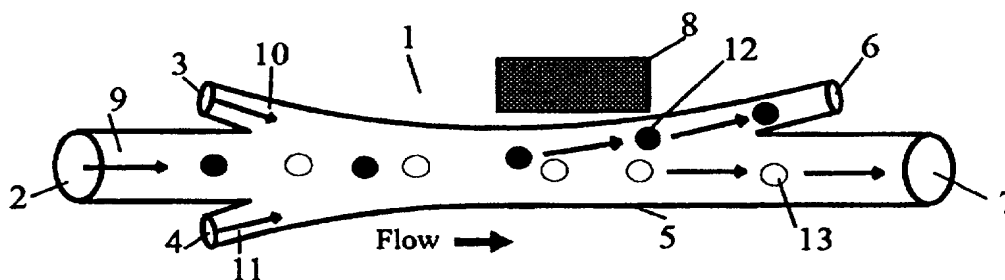
PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(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.

35

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

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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,

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,

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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
5 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
10 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.
15

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
20 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
25 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.

30 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
35 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.

5 It is a still further advantage of the invention that a closed system is provided allowing 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.

10 It is a still further advantage of the invention that a system with a low shear stress in the flow is provided allowing a gentle treatment of biological samples, e.g. fragile living cells, especially when two guiding buffers are introduced in the channel.

15 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.

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.

25 It is an advantage of the invention that a micro flow system is provided that is cheap, easy to 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.

35 The continuous separation system (CSS) allows efficient enrichment as well as depletion of 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.

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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, where 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

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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.

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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.

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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.

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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.

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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).

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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

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,

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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

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

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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 whereto 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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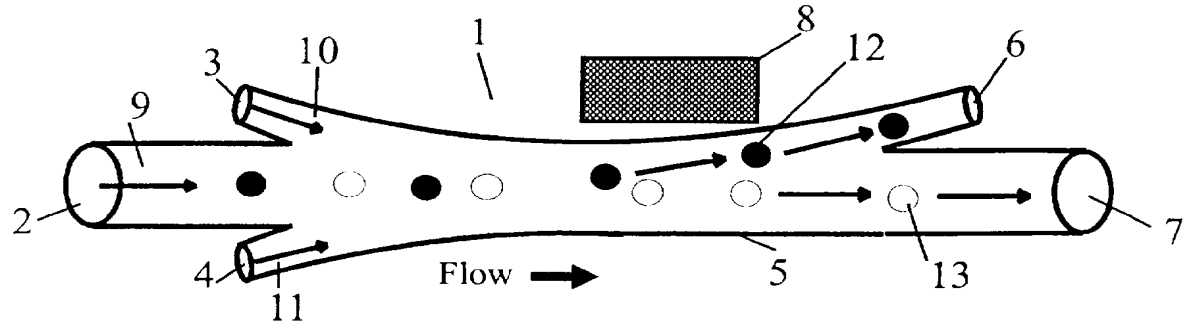


Fig. 1

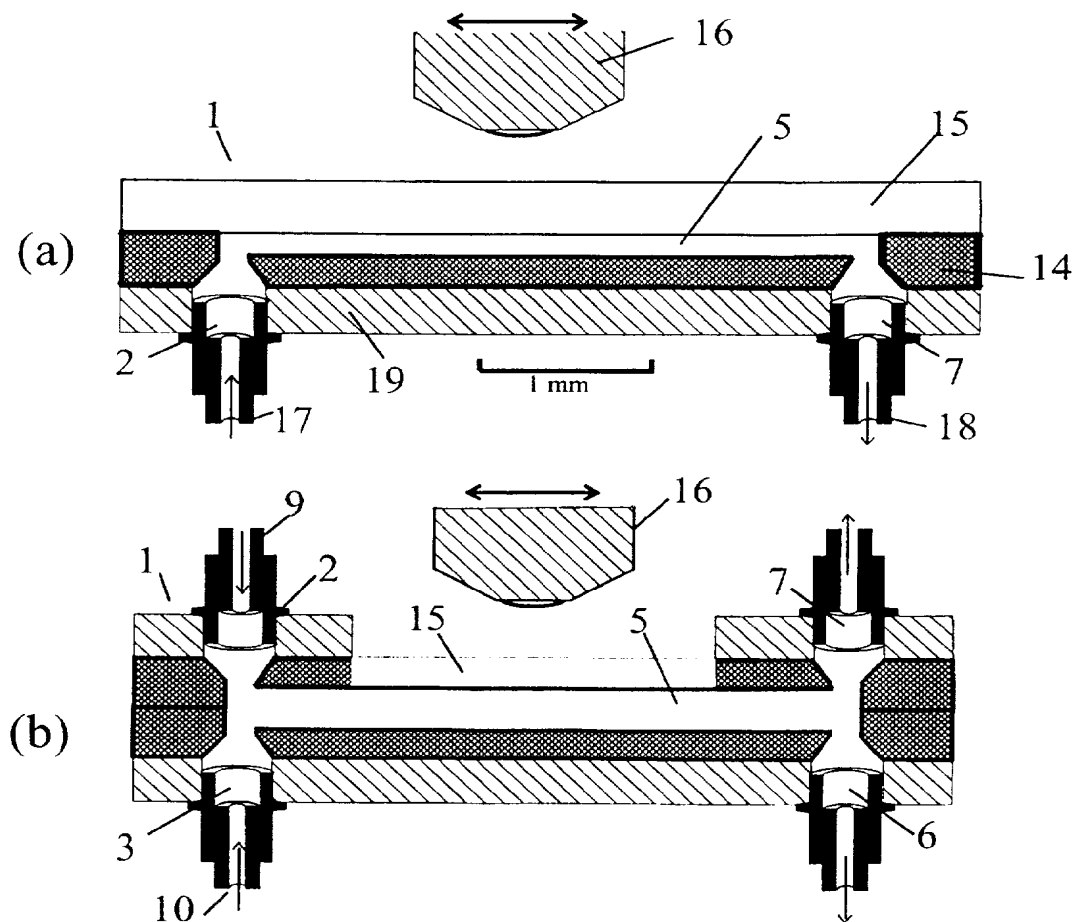


Fig. 2

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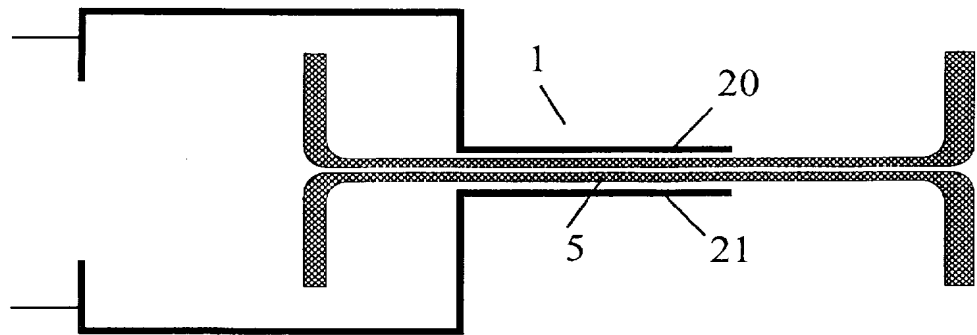


Fig. 3

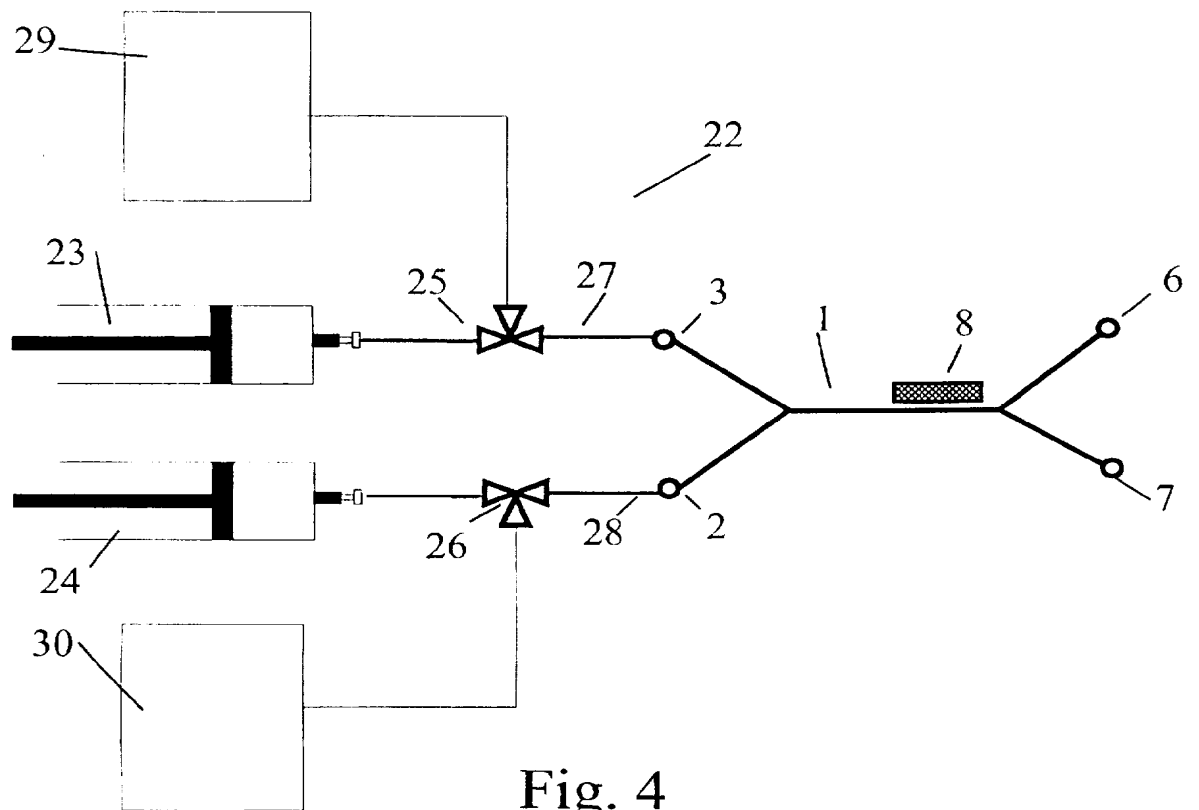


Fig. 4

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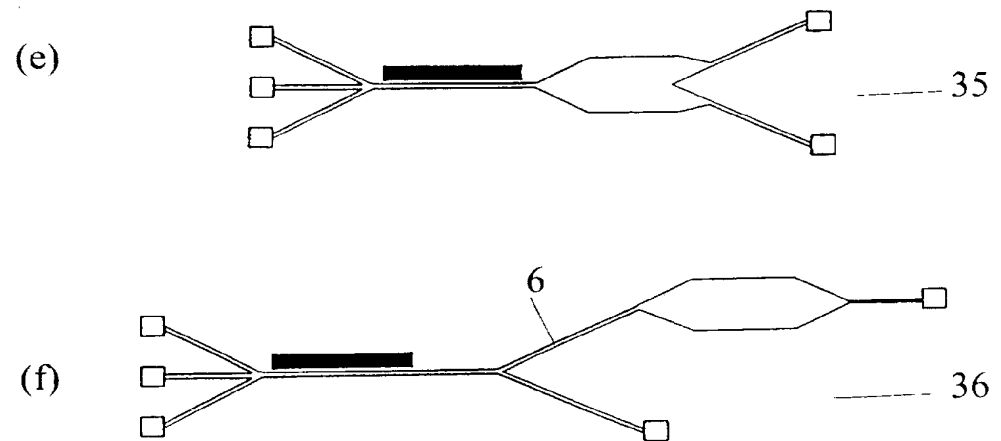
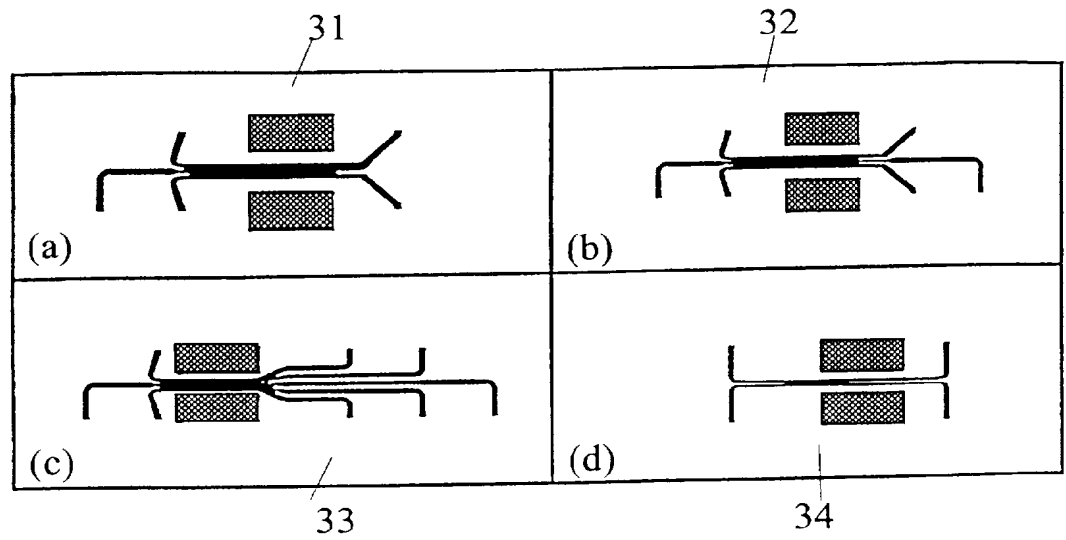


Fig. 5

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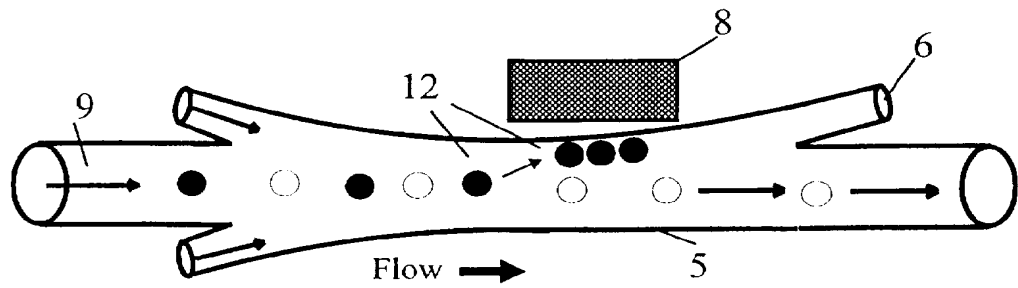


Fig. 6

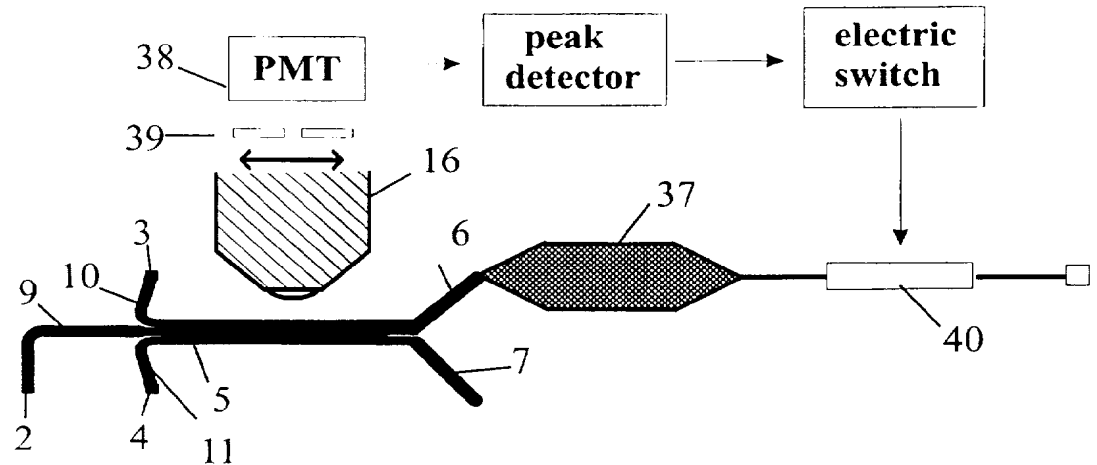


Fig. 7

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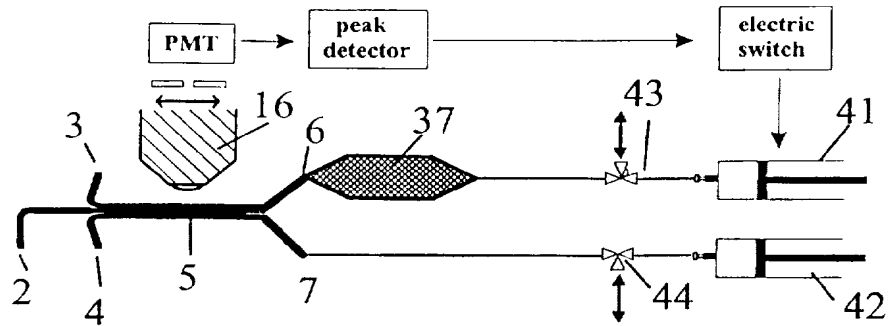


Fig. 8

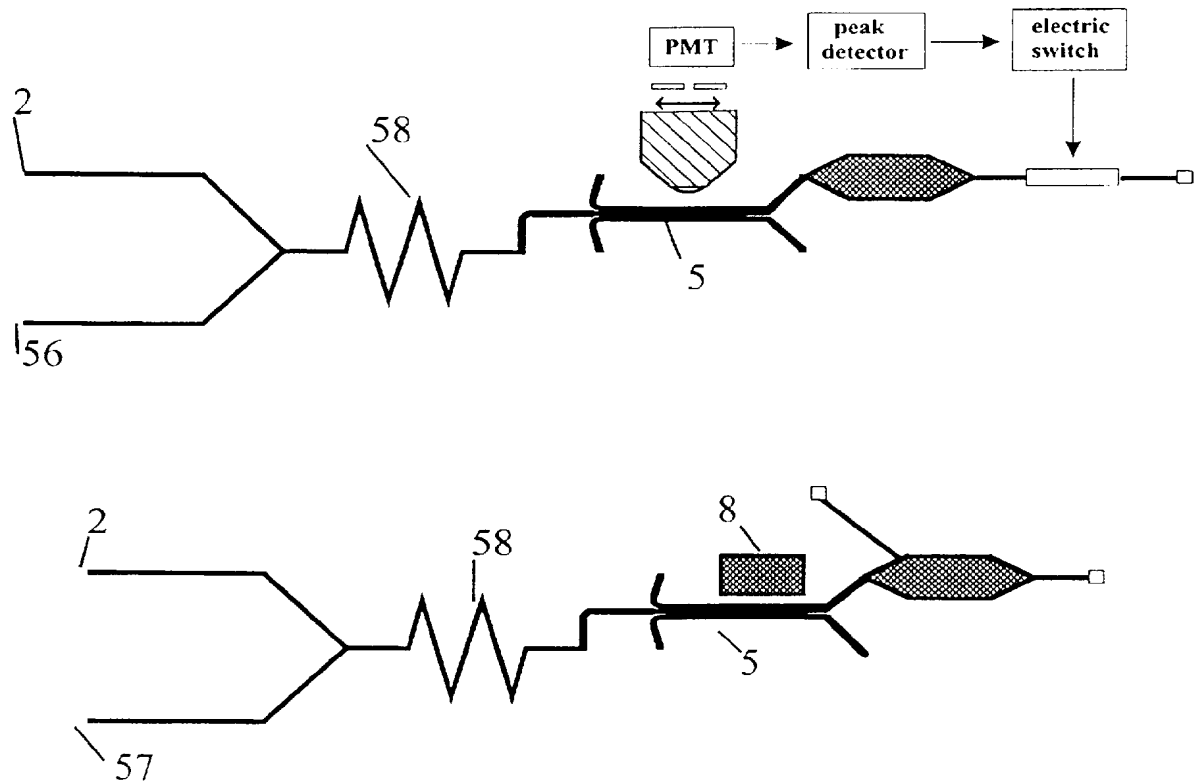


Fig. 10

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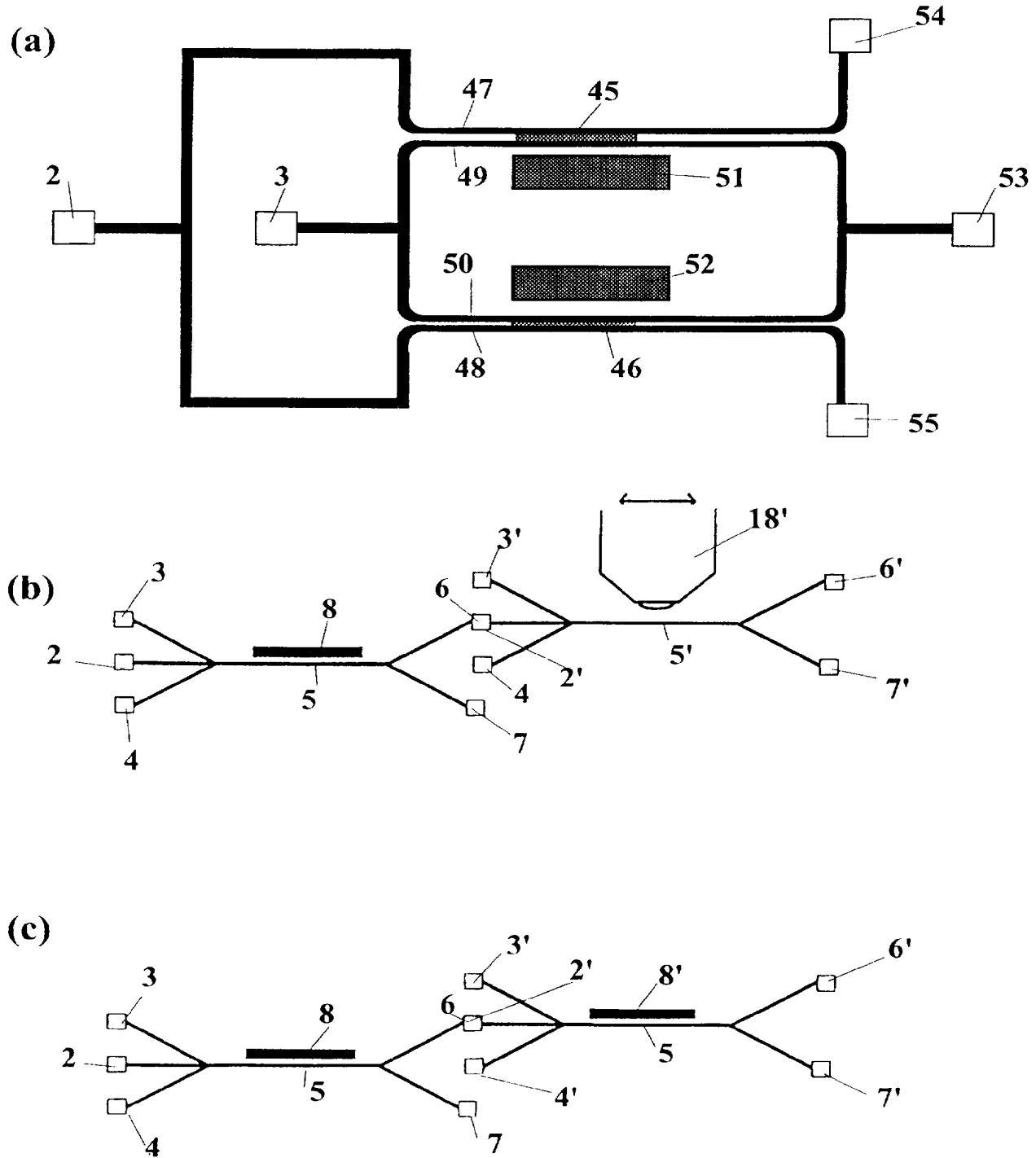


Fig. 9

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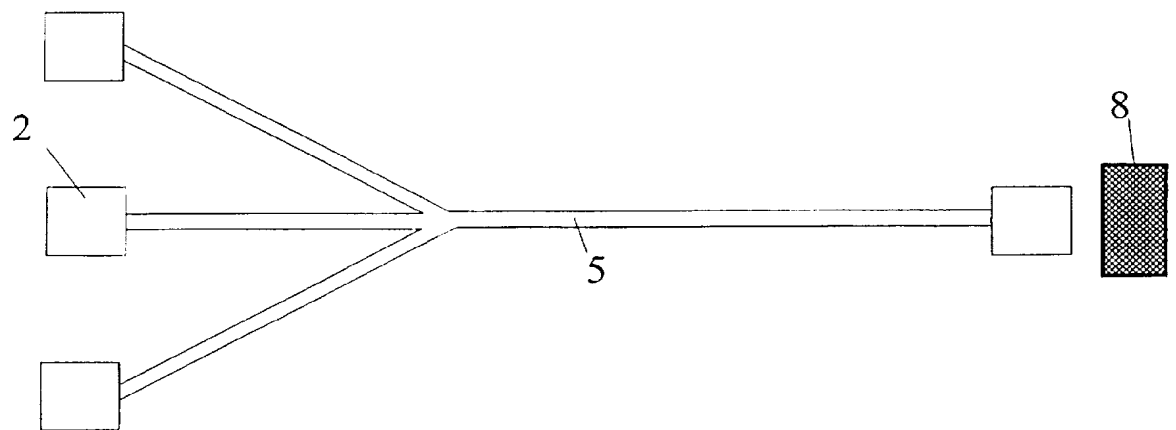
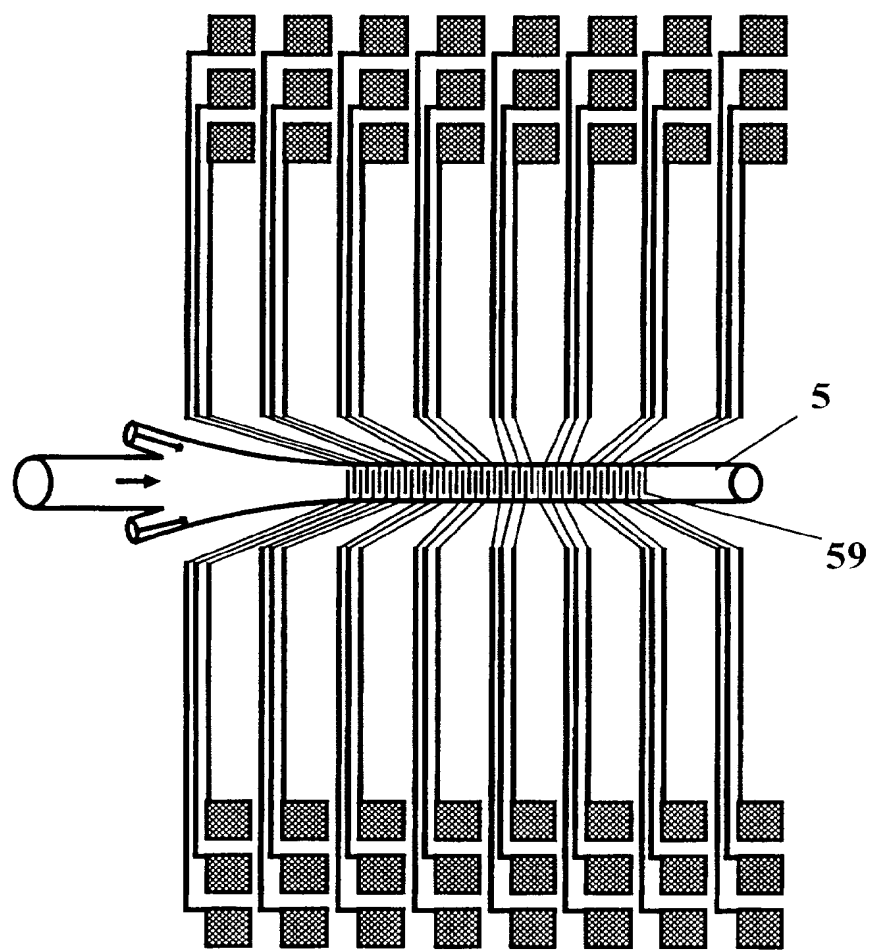


Fig. 11

(a)



(b)

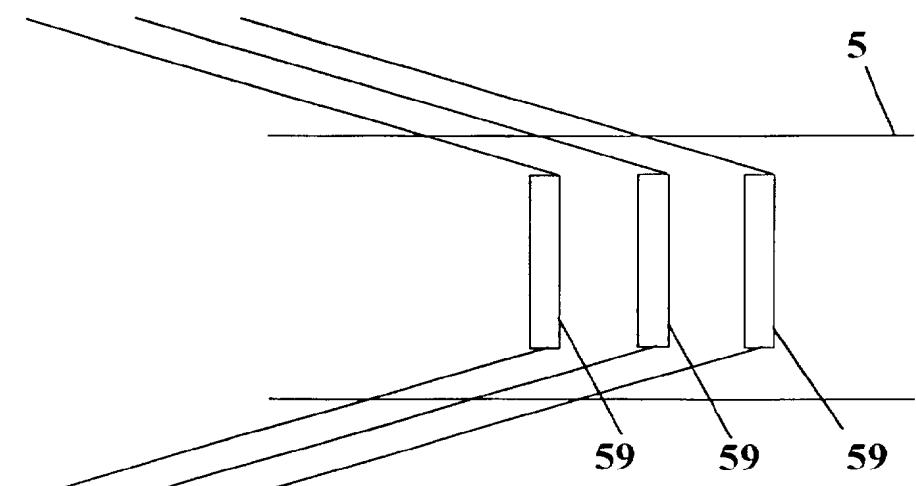


Fig. 12

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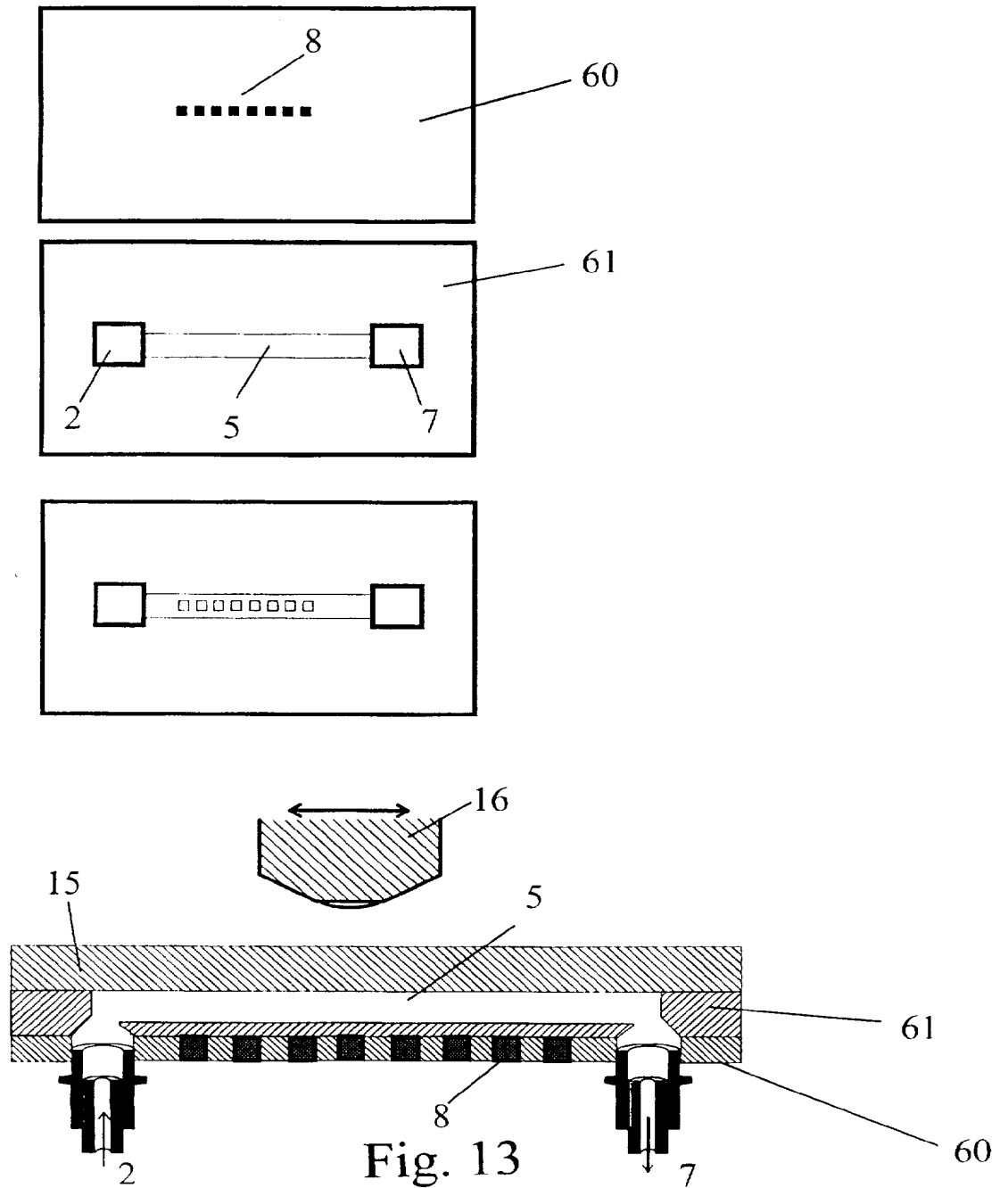
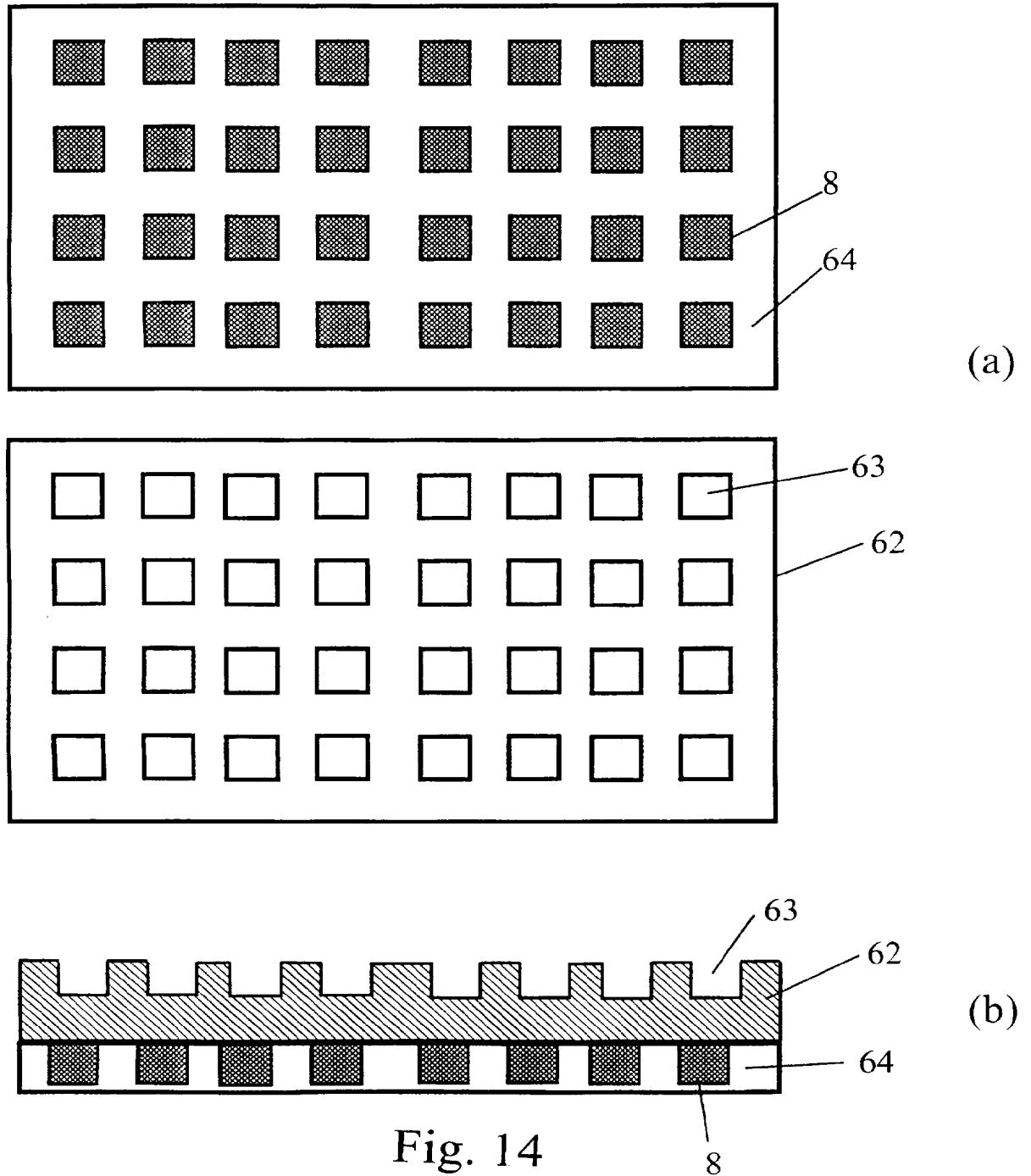


Fig. 13

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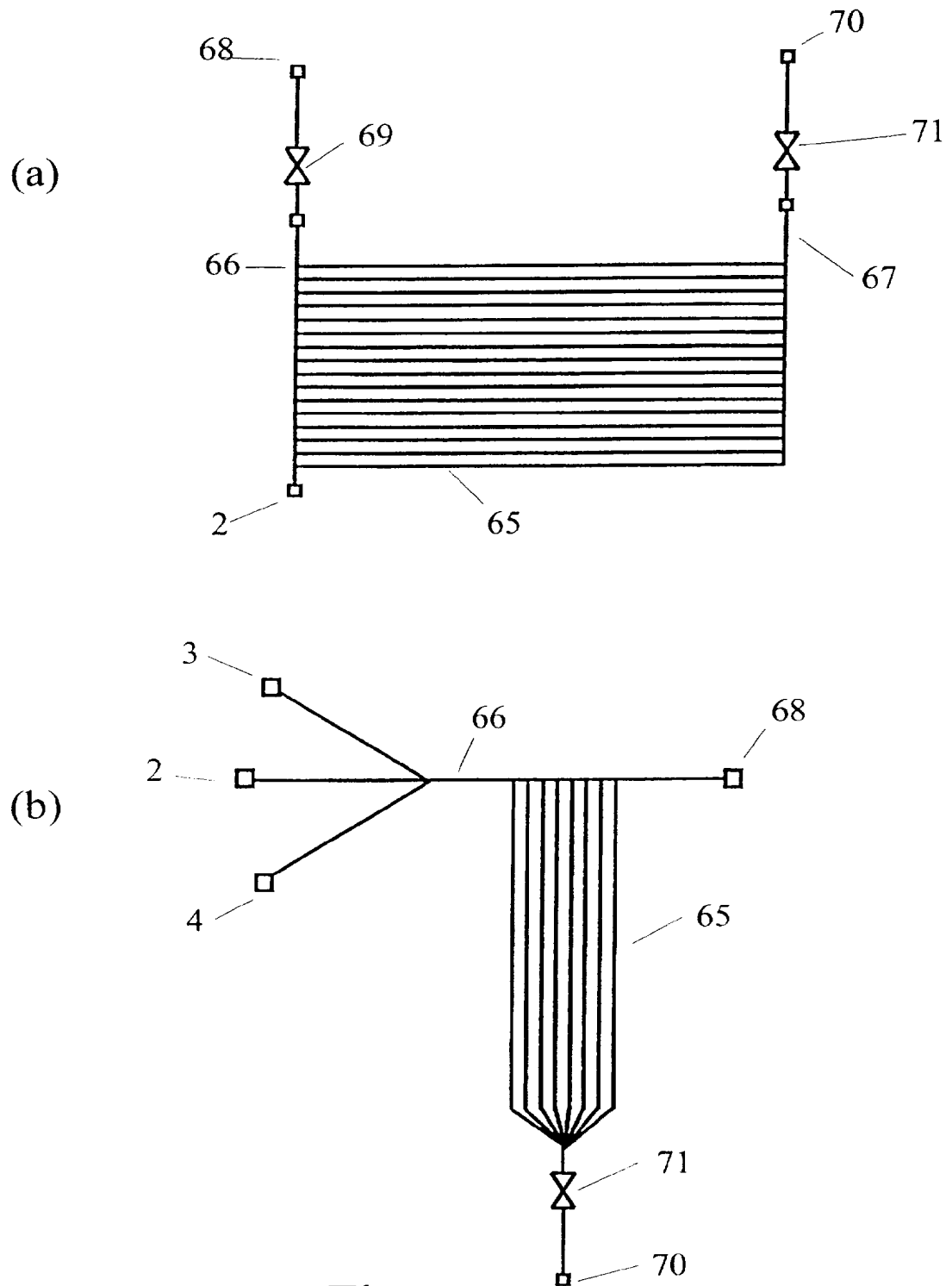


Fig. 15

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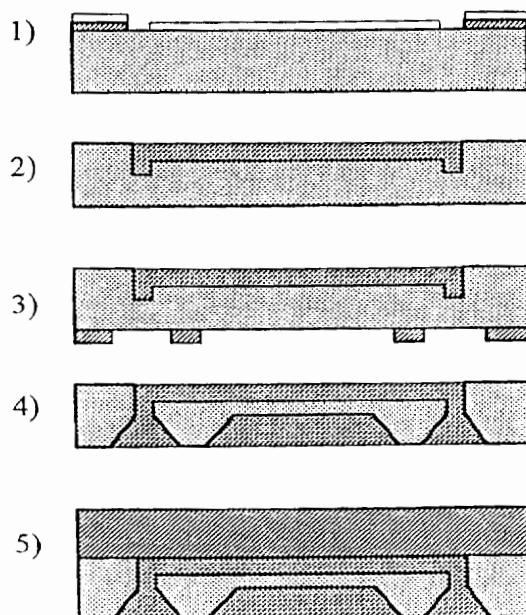


Fig. 16

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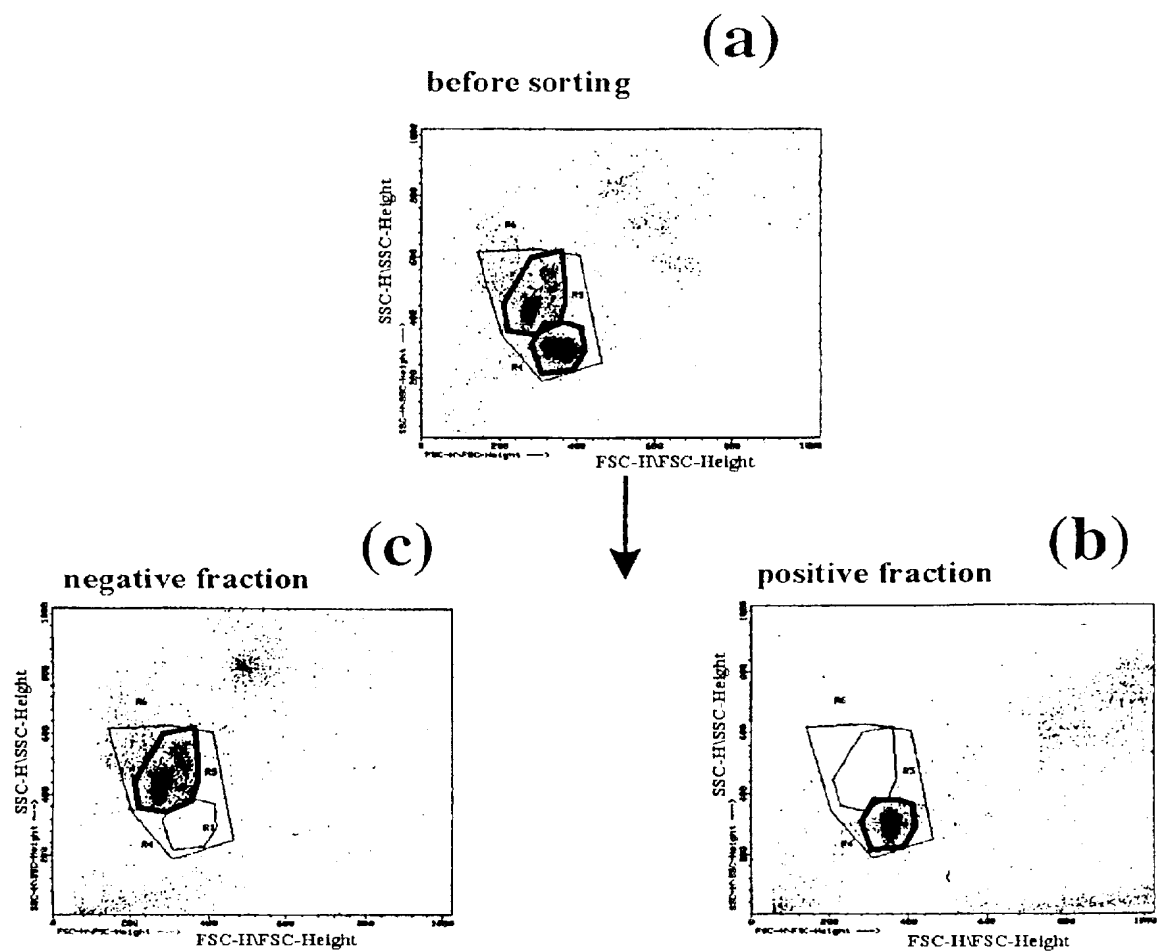


Fig. 17

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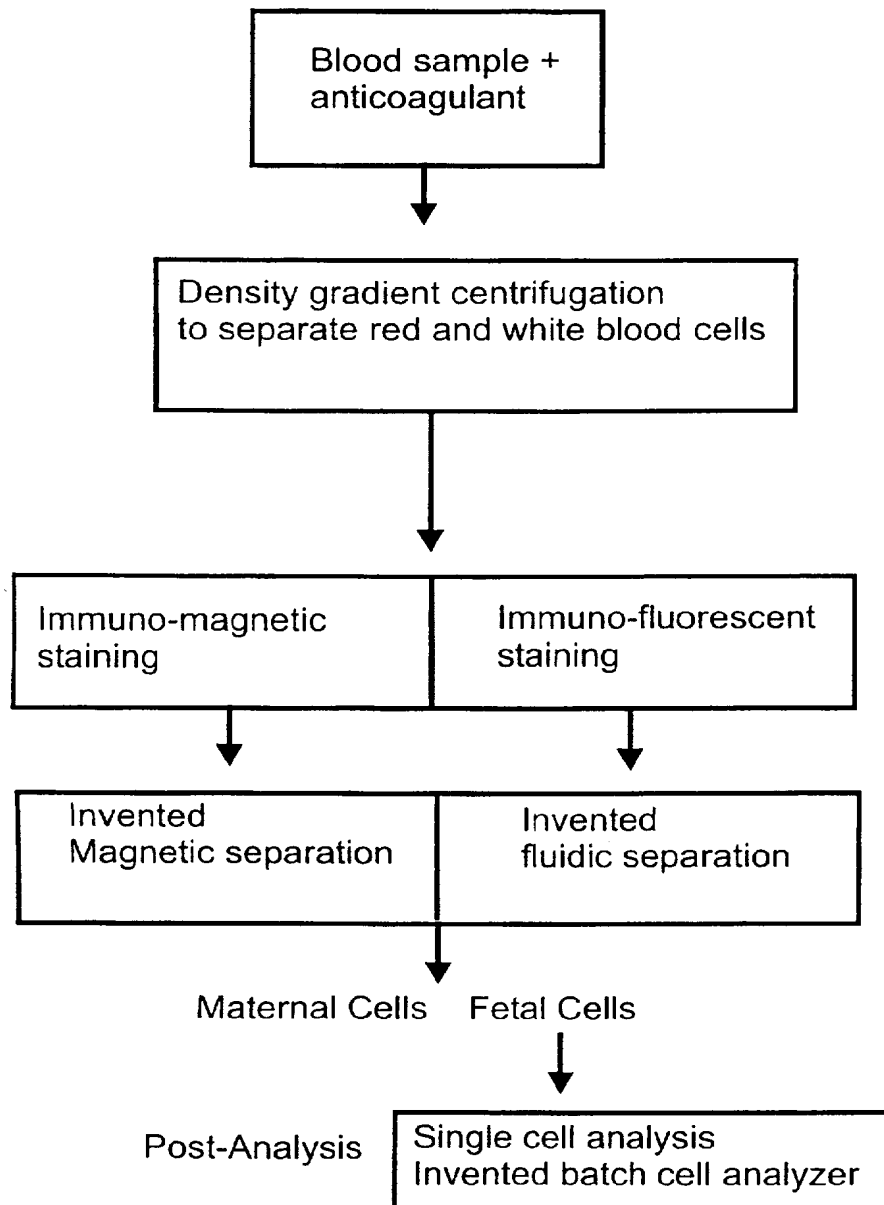


Fig. 18

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>

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INTERNATIONAL SEARCH REPORT

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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	---	
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A	US 5 483 469 A (VAN DEN ENGH GER ET AL) 9 January 1996 see the whole document	1

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PCT/DK 97/00368

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Information on patent family members

International Application No
PCT/DK 97/00368

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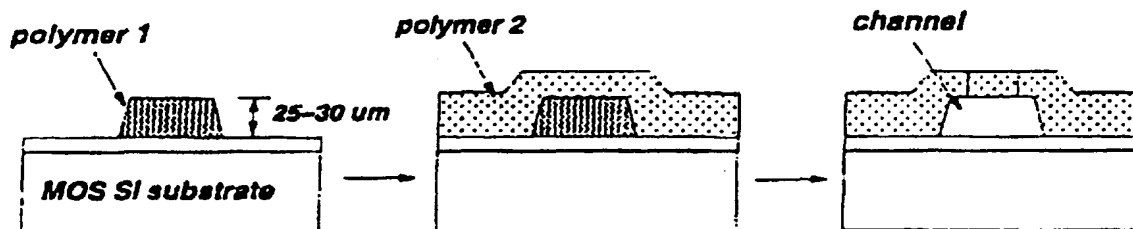
US 5483469 A	09-01-96	NONE	



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US97/14054		(74) Agents: CARROLL, Peter, G. et al.; Medlen & Carroll, LLP, Suite 2200, 220 Montgomery Street, San Francisco, CA 94104 (US).	
(22) International Filing Date: 11 August 1997 (11.08.97)			
(30) Priority Data: 60/023,393 12 August 1996 (12.08.96) US 08/807,184 6 August 1997 (06.08.97) US		(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(60) Parent Application or Grant (63) Related by Continuation US Not furnished (CIP) Filed on Not furnished		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>	
(71) Applicant (for all designated States except US): THE RE- GENTS OF THE UNIVERSITY OF MICHIGAN [US/US]; Wolverine Tower, Room 2071, 3003 South State Street, Ann Arbor, MI 48109-1280 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only): MASTRANGELO, Car- los, H. [US/US]; 5844 Creekview Court, Ann Arbor, MI 48105 (US). MAN, Piu, F. [CA/US]; Suite 109, 536 S. Forest Avenue, Ann Arbor, MI 48104 (US). WEBSTER, James, R. [US/US]; 9319 Harbor Cove Circle #312, Whit- more Lake, MI 48189 (US).			

(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.

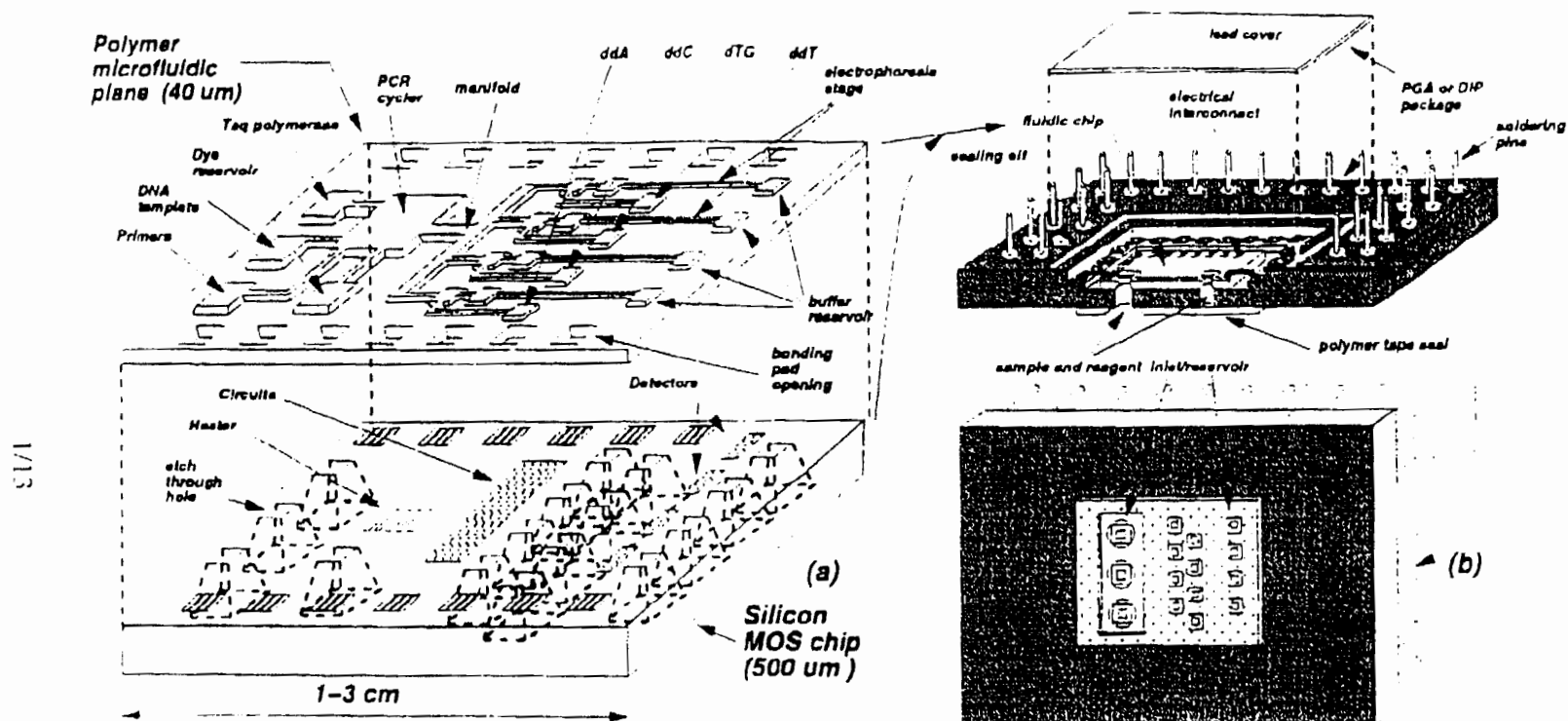


Figure 1: (a) The polymer fluid channels and reservoirs are constructed by deposition of plastics on top of a silicon MOS control chip. Etch-through holes are formed in the silicon substrate to serve as funnel-like inlets and reservoirs for the sample, reagents and buffer solutions. (b) Fluidic chip 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

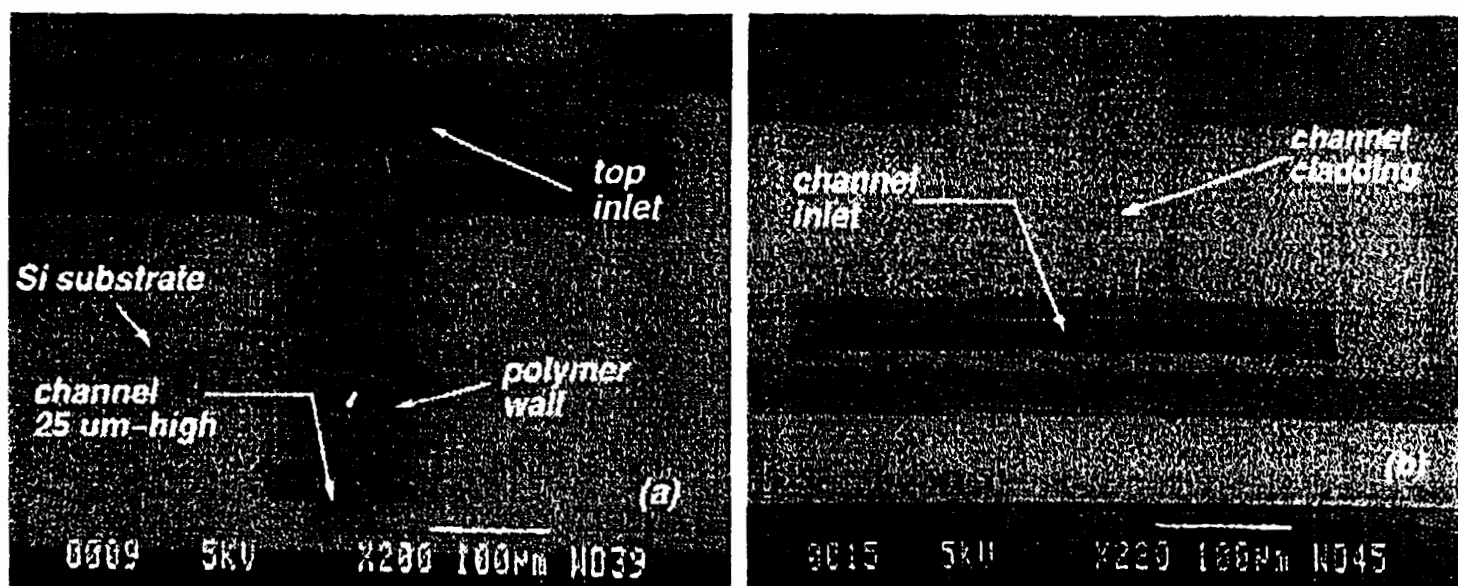


Figure 2: Top view and cross-section (a) of example polymer channel with front side inlet (b) formed on top of a regular Si substrate. Channel heights can be adjusted between 0.5-100 μm .

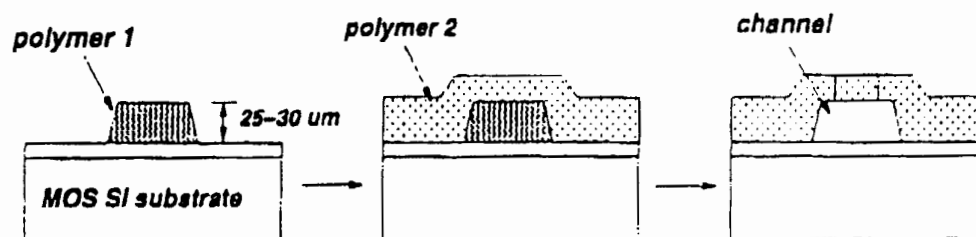


Figure 3: Simplified fabrication process for plastic channels.

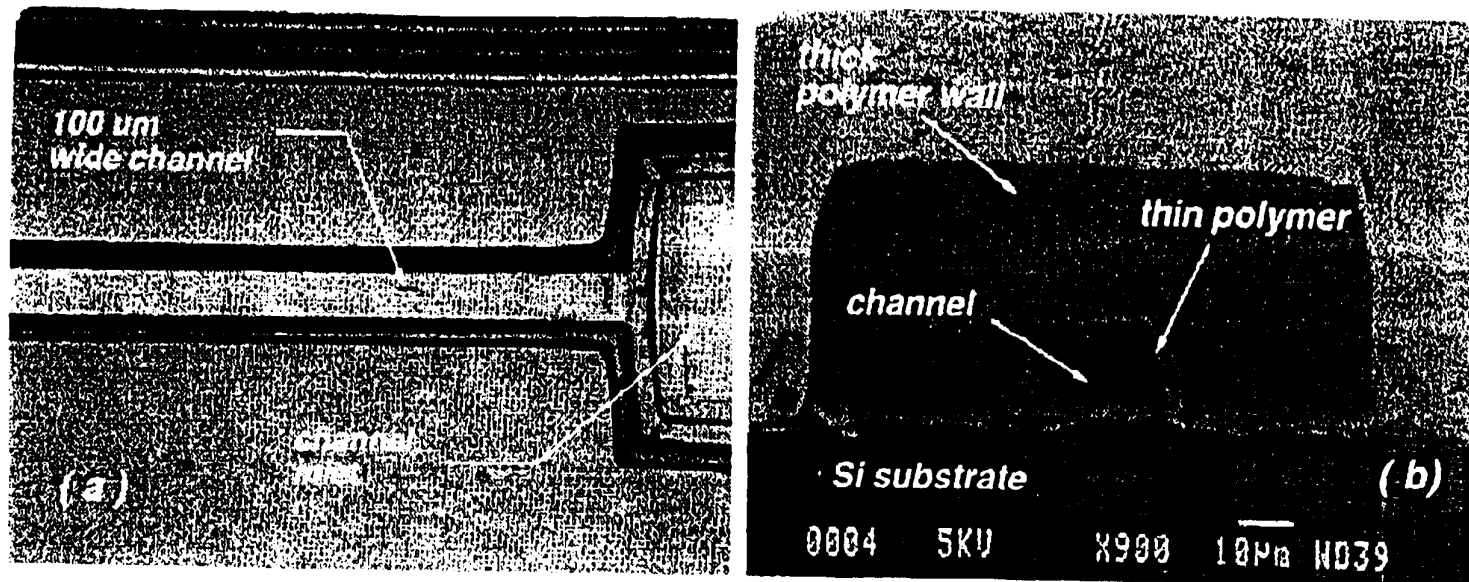


Figure 4: (a) Top view of example three-polymer channel 100 μm wide and 25 μm -thick with front side inlet. (b) closeup cross-section showing the three-polymer construction of the channels.

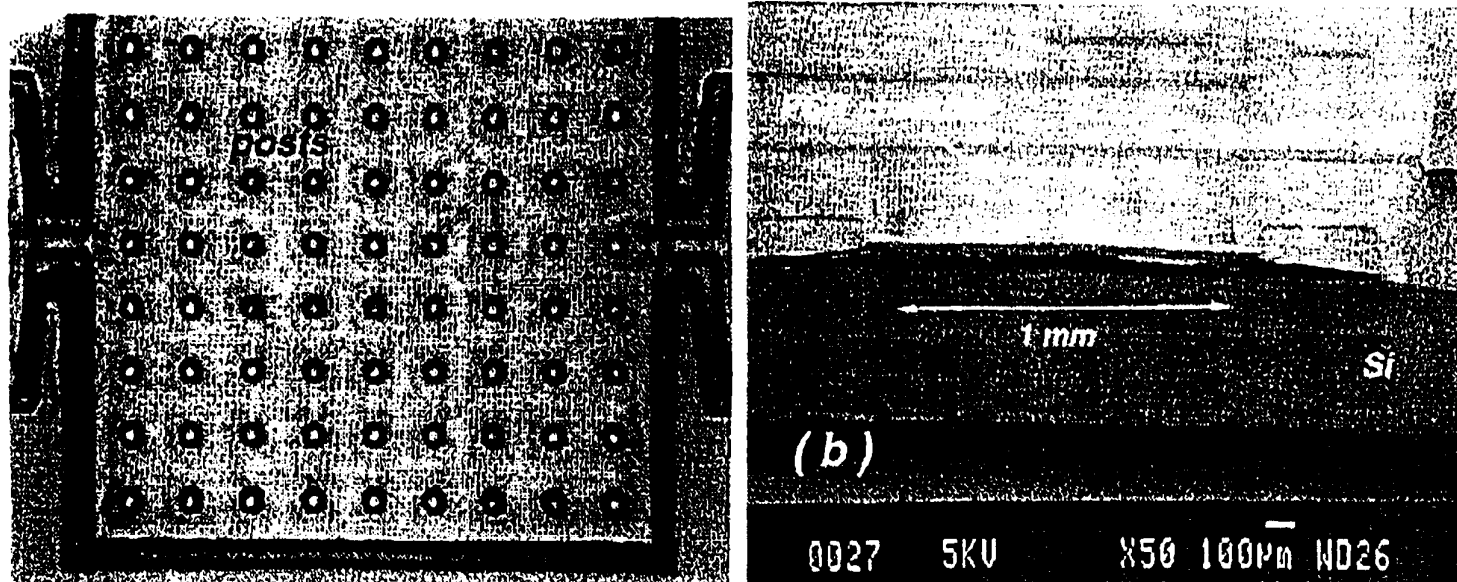


Figure 5: (a) Top view of 1 mm² thermal cycling chamber (b) cross-section of 25 µm-high, 1 mm² polymer PCR chamber fabricated on top of a silicon wafer.

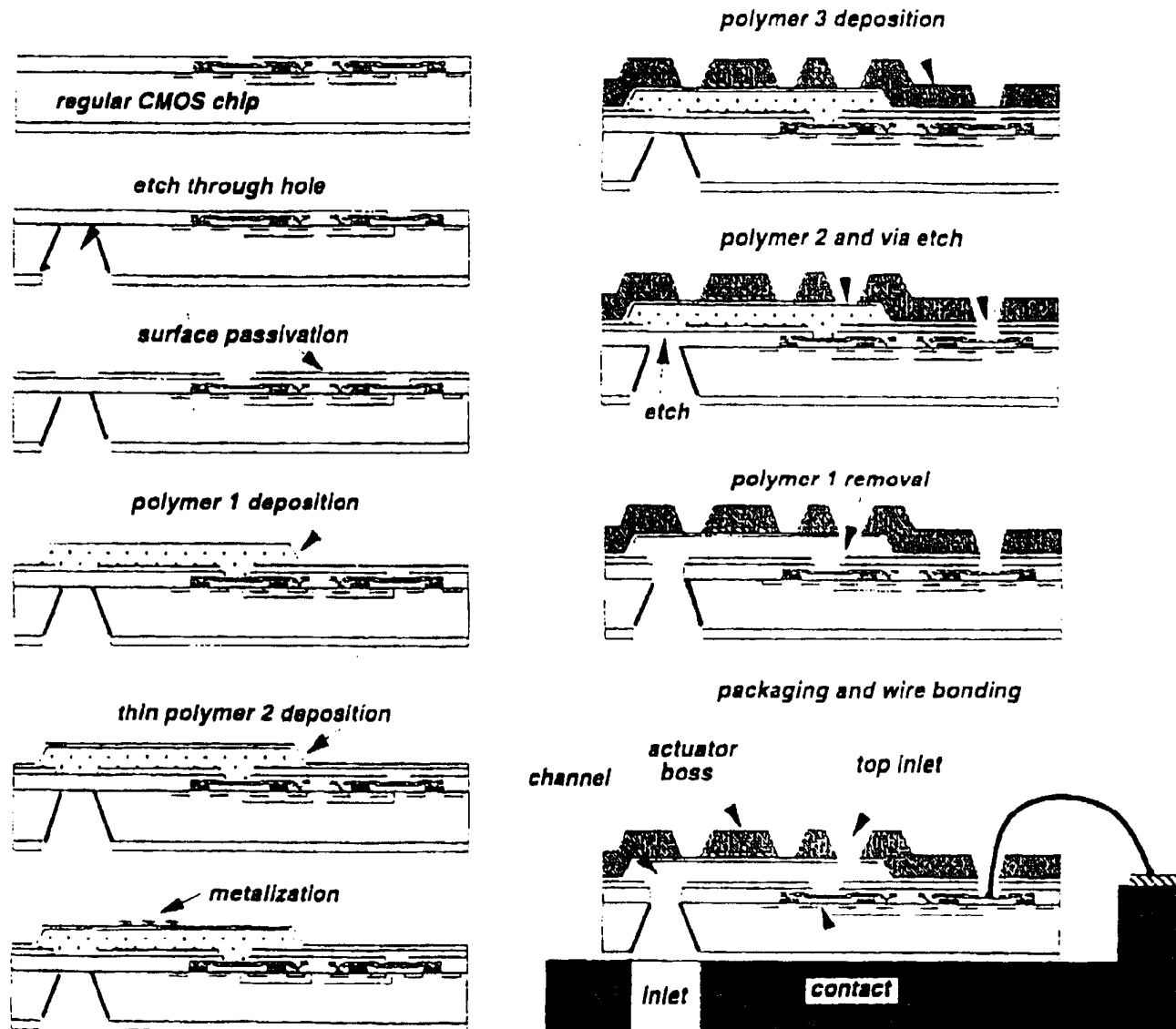


Figure 6: Simplified 3-level polymer process with embedded metalization. This process requires four lithographic masks and is capable of constructing all the microfluidic parts shown in this proposal. The figure shows circuits, backside and front inlets, actuators, channels, and bonding wires.

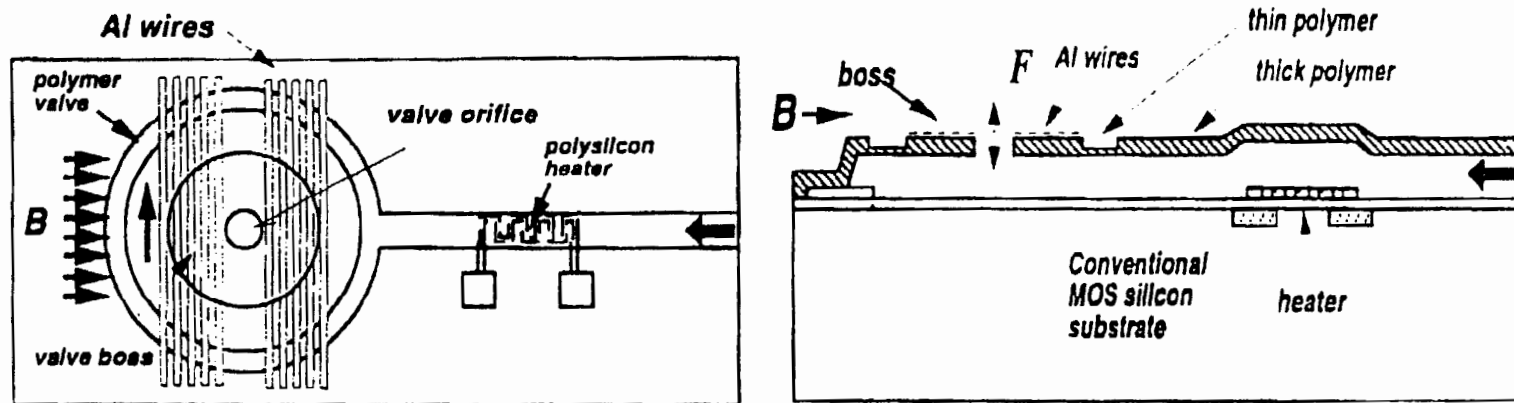


Figure 7: Polymer magnetically activated thermocompression suction pump

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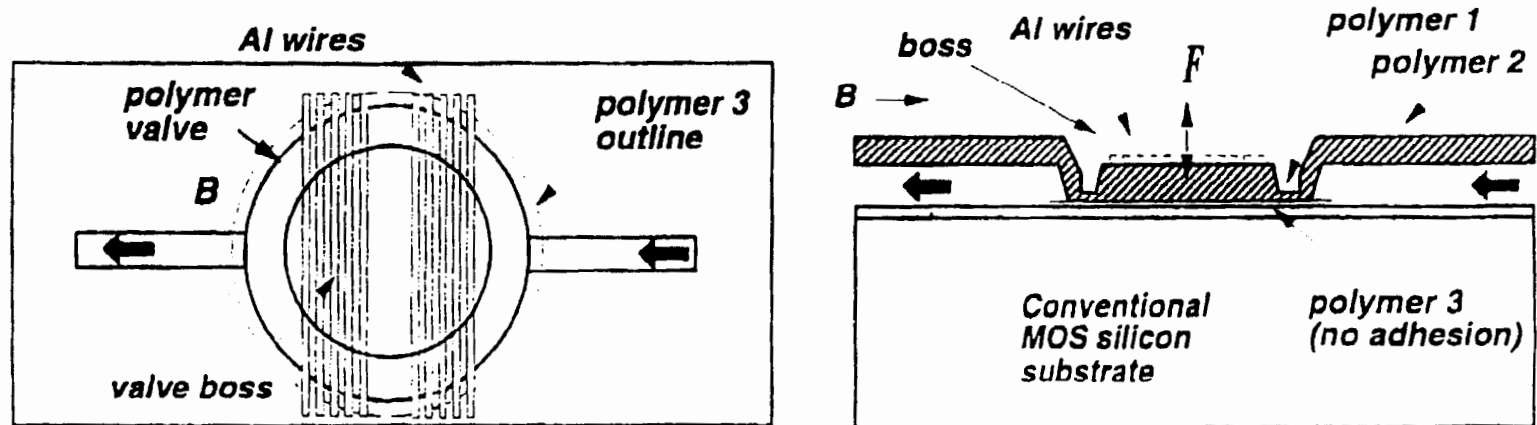


Figure 8: Pass-through valve. The valve is normally closed and opens when the diaphragm is buckled upwards

FIGURE 9

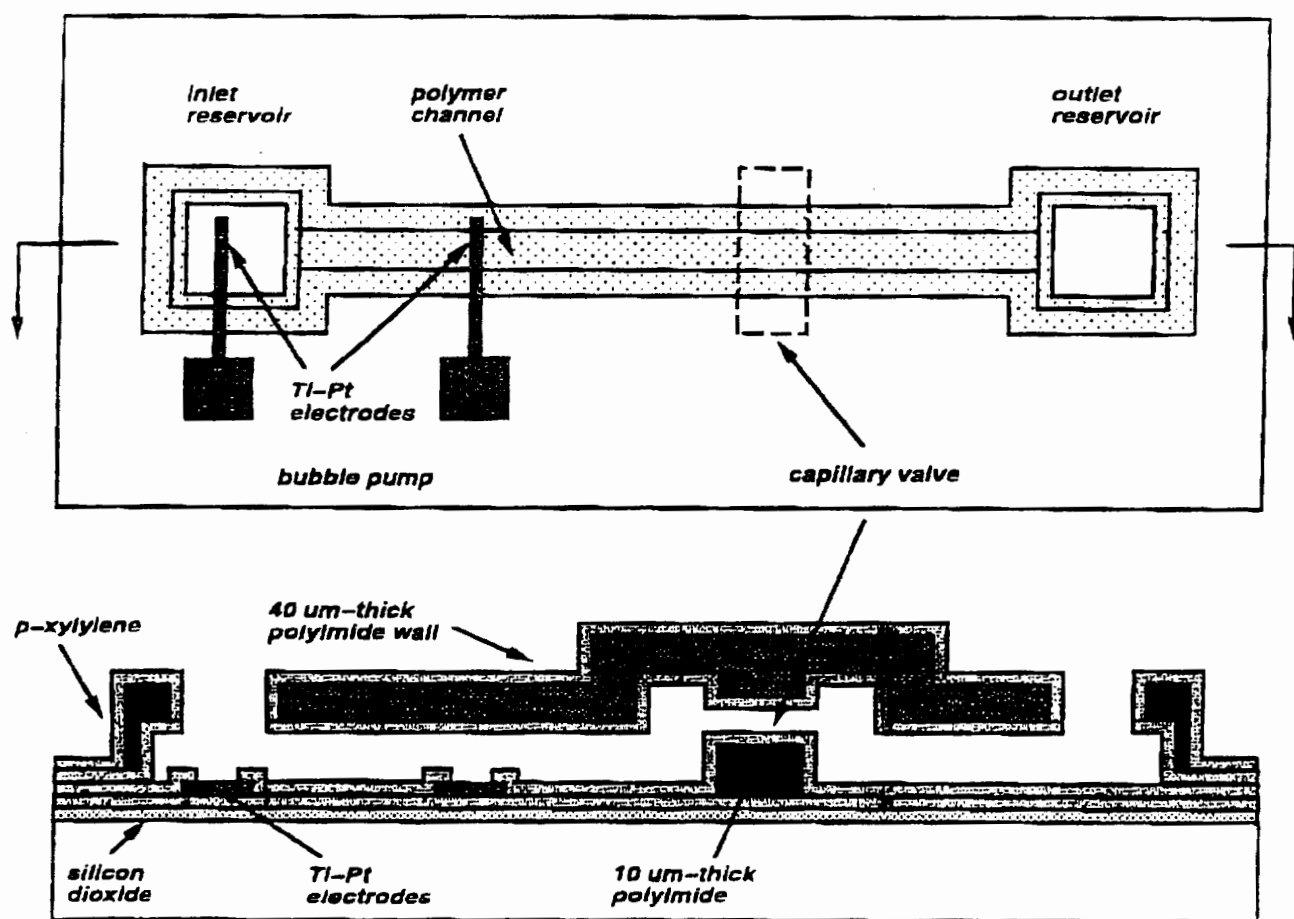


FIGURE 10

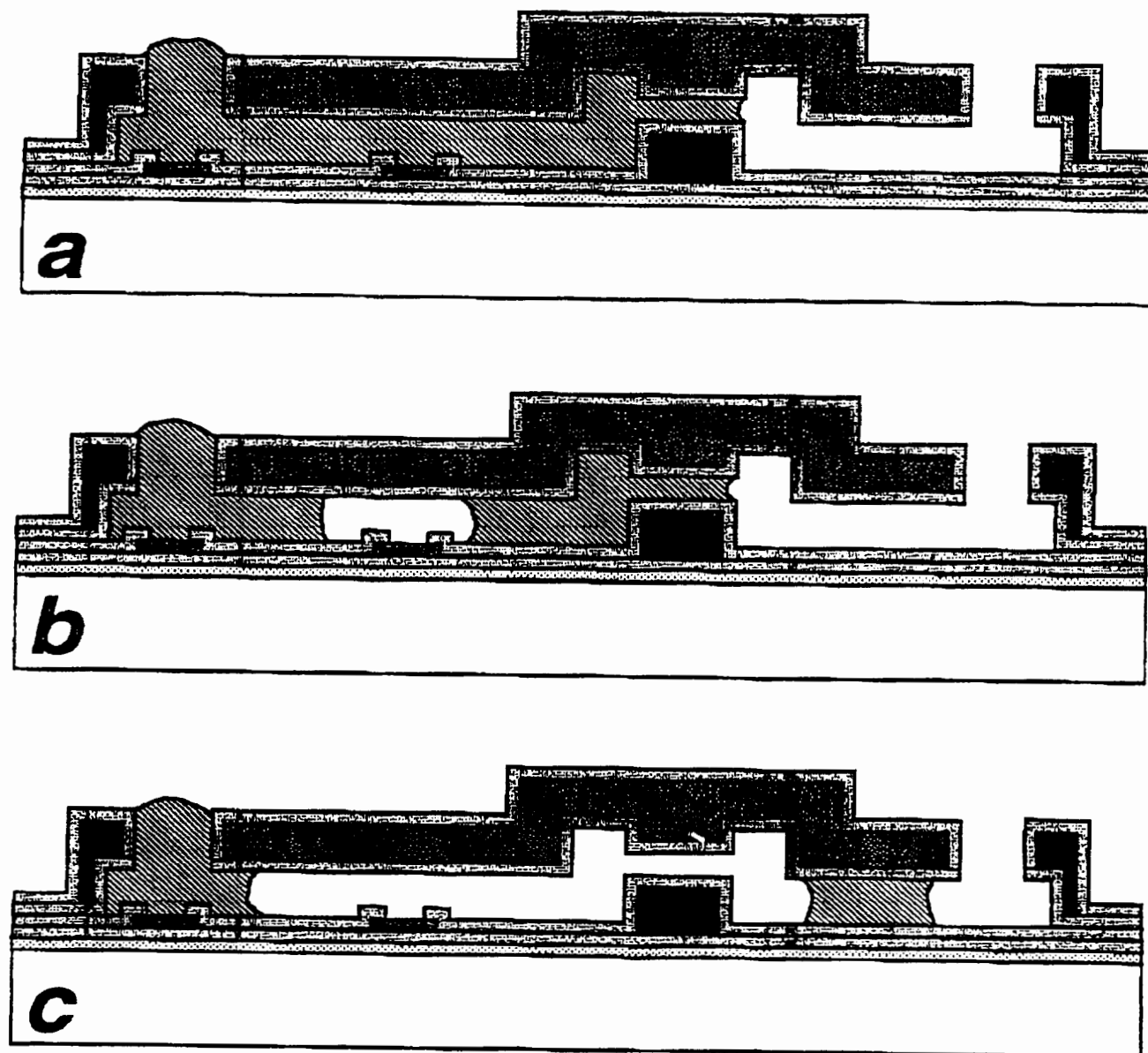
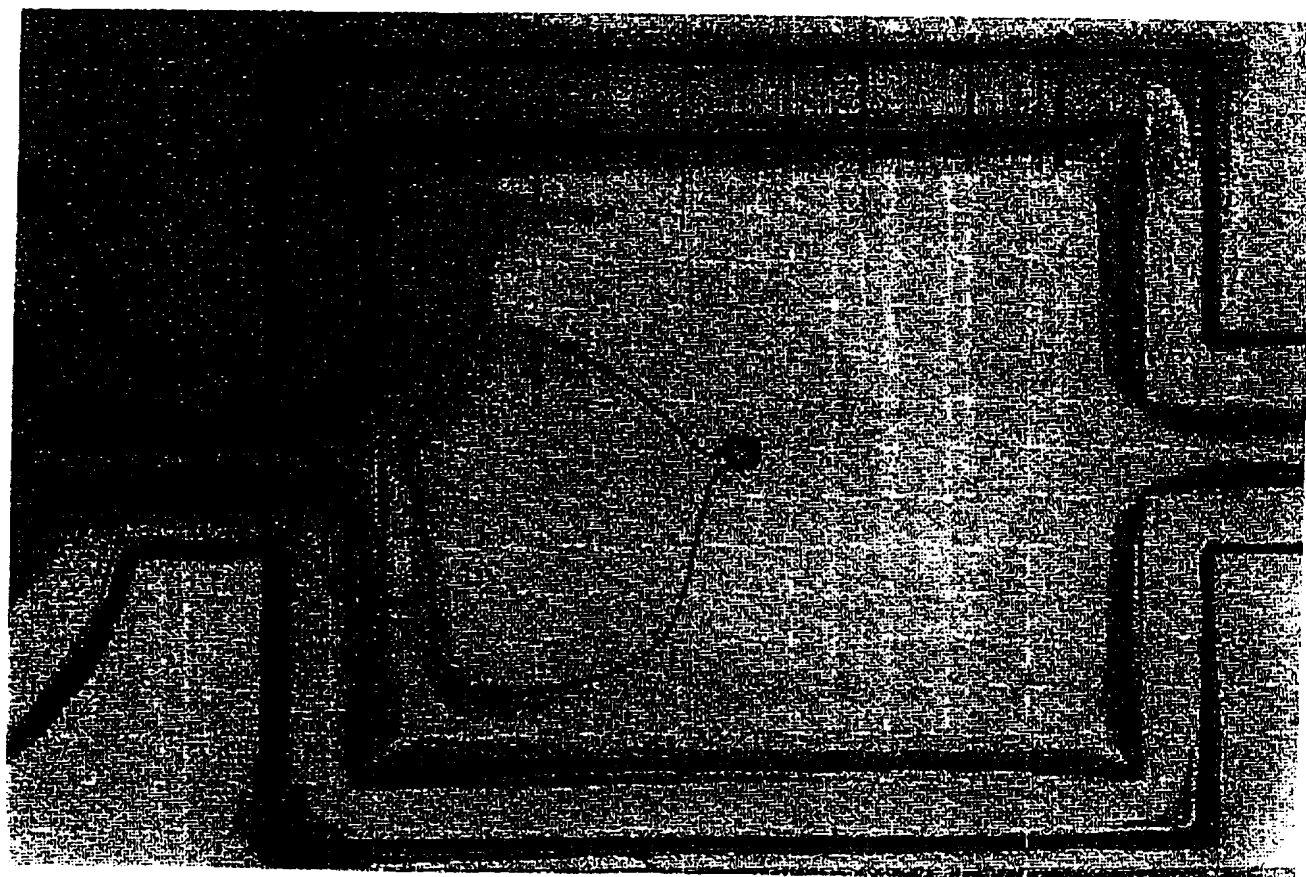


FIGURE 11



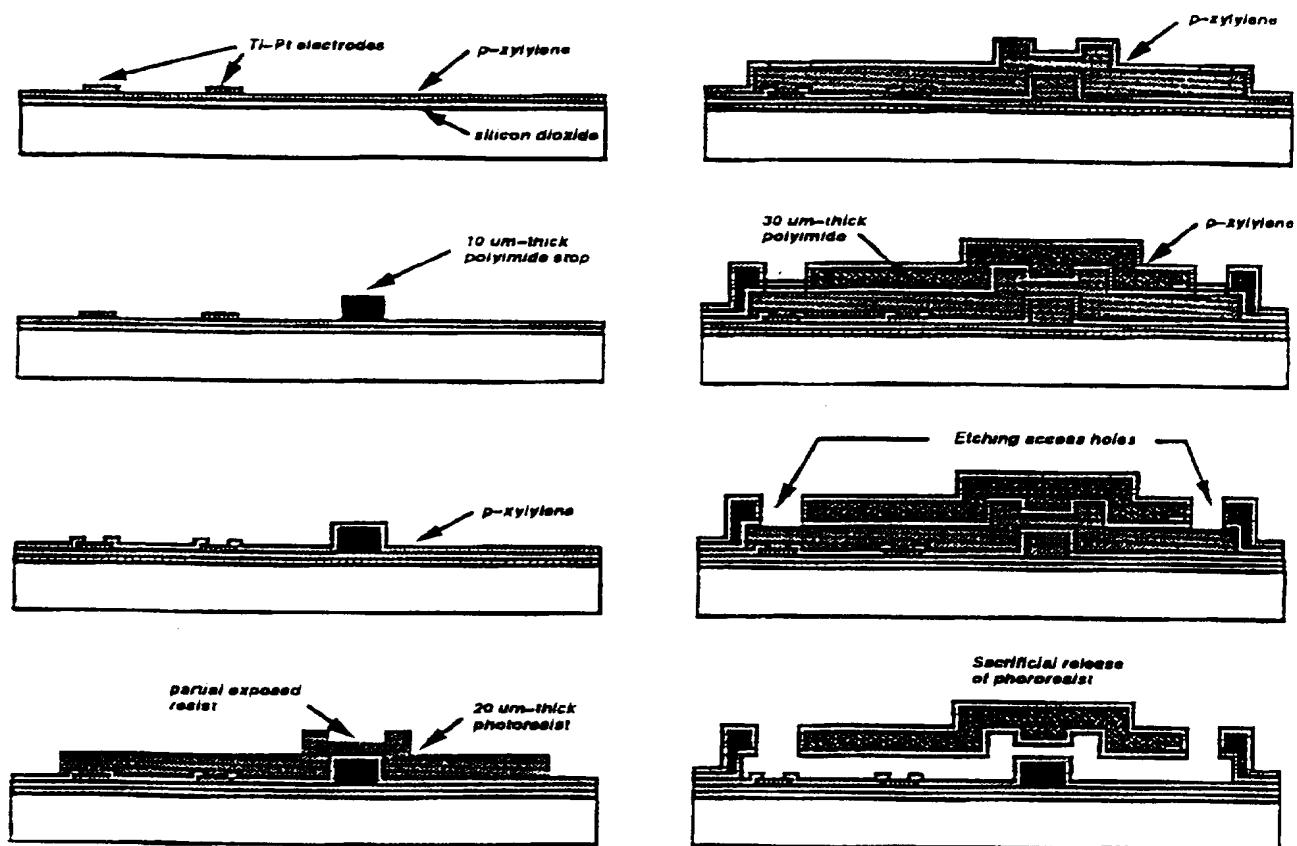
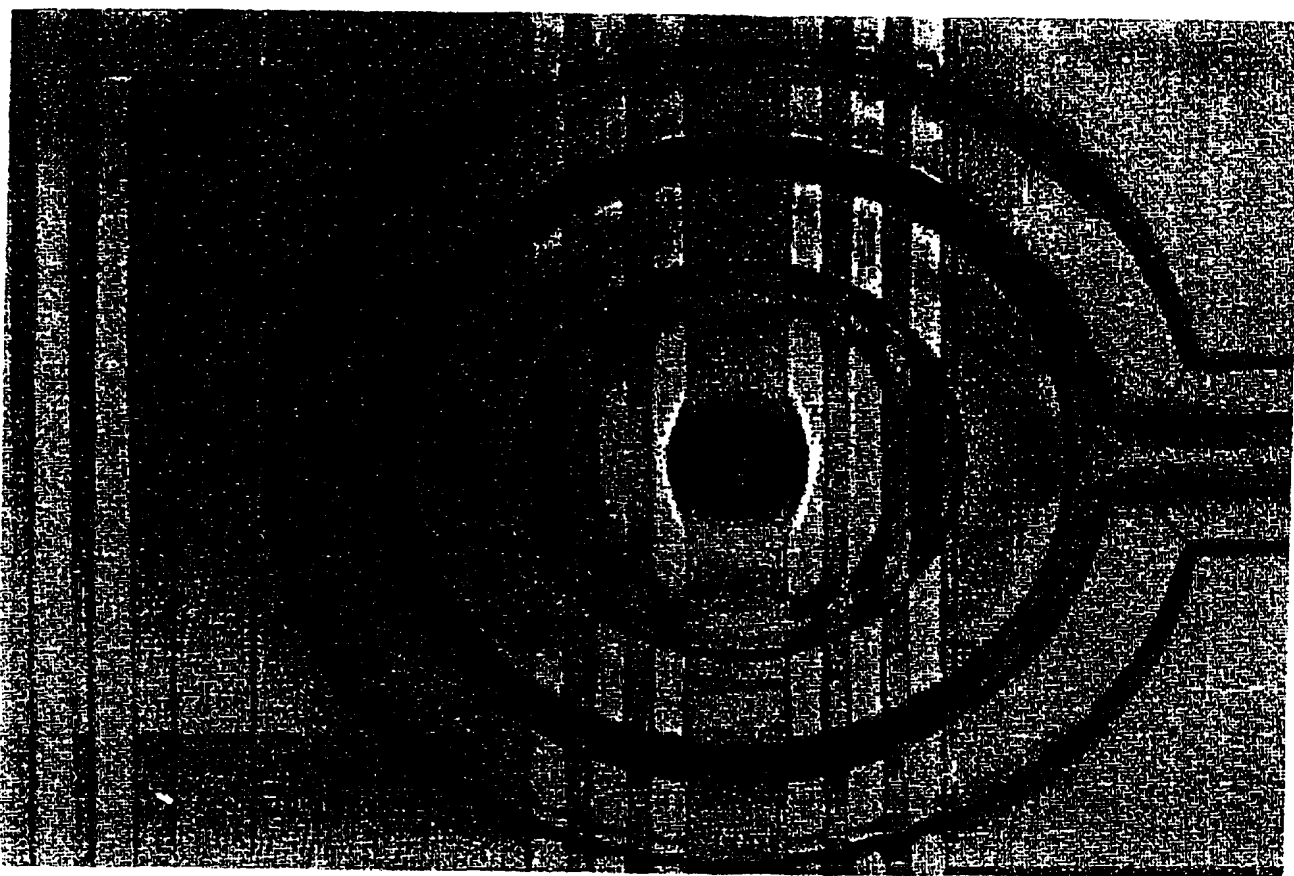


Fig. 13: Process flow of bubble pump and capillary valve

FIGURE 12



INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G03F 7/00

US CL :430/311, 313, 314, 319, 394

According to International Patent Classification (IPC) or to both national classification and IPC

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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
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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



Further documents are listed in the continuation of Box C.



See patent family annex.

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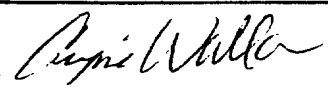
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US00/13294 (22) International Filing Date: 11 May 2000 (11.05.00) (30) Priority Data: 60/134,472 17 May 1999 (17.05.99) US (71) Applicant (for all designated States except US): CALIPER TECHNOLOGIES CORP. [US/US]; 605 Fairchild Drive, Mountain View, CA 94043 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): WADA, H., Garrett [US/US]; 55 Victoria Drive, Atherton, CA 94027 (US). KOPF-SILL, Anne, R. [US/US]; 30 Minoca Road, Portola Valley, CA 94028 (US). ALAJOKI, Marja, Liisa [FI/US]; 2774 Randers Court, Palo Alto, CA 94306 (US). PARCE, J., Wallace [US/US]; 754 Los Robles Avenue, Palo Alto, CA 94306 (US). WANG, Benjamin, N. [US/US]; 474 Ramsell Street, San Francisco, CA 94132 (US). CHOW, Andrea, W. [US/US]; 670 Cuesta Drive, Los Altos, CA 94024 (US). DUBROW, Robert, S. [US/US]; 766 Orange Avenue, San Carlos, CA 94070 (US). (74) Agents: QUINE, Jonathan, Alan; The Law Offices of Jonathan Alan Quine, P.O. Box 458, Alameda, CA 94501 (US) et al.		(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, 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, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(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.

In one preferred assay of the invention, the particles are cells and the
10 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 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 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 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 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, 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 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).

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
15 vertically in the first microchannel such that selected individual members are directed 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 an 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.
- 10 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.

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
25

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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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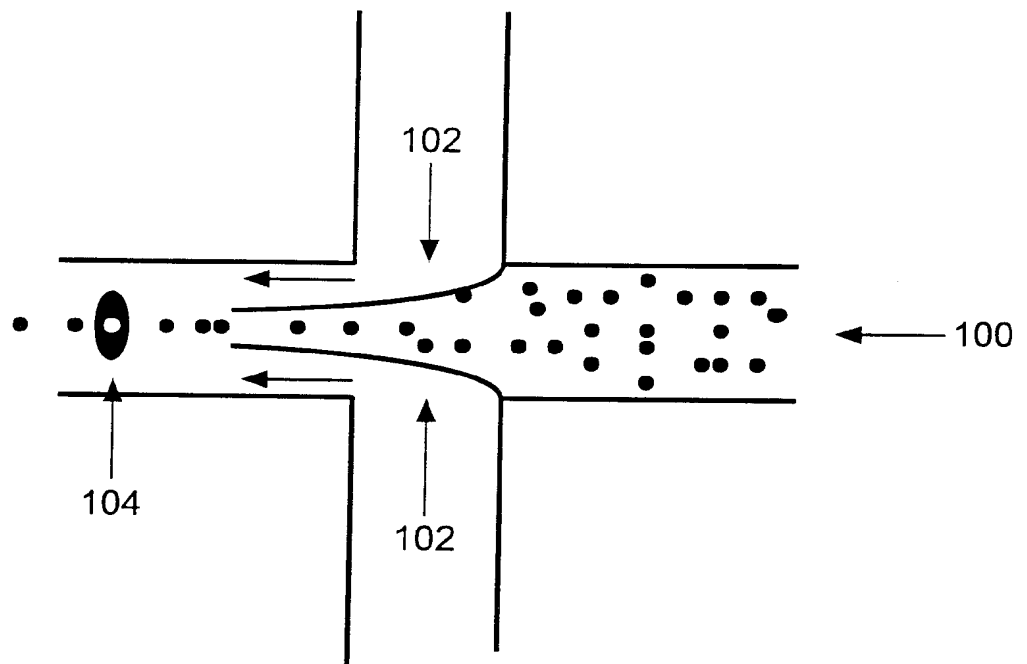


Fig. 1A

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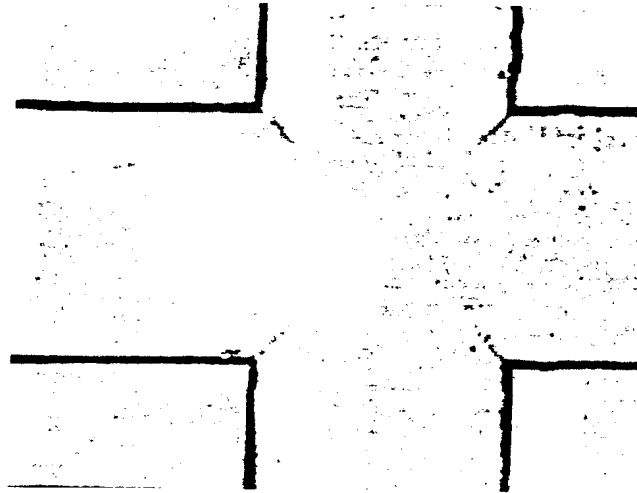


Fig. 1B

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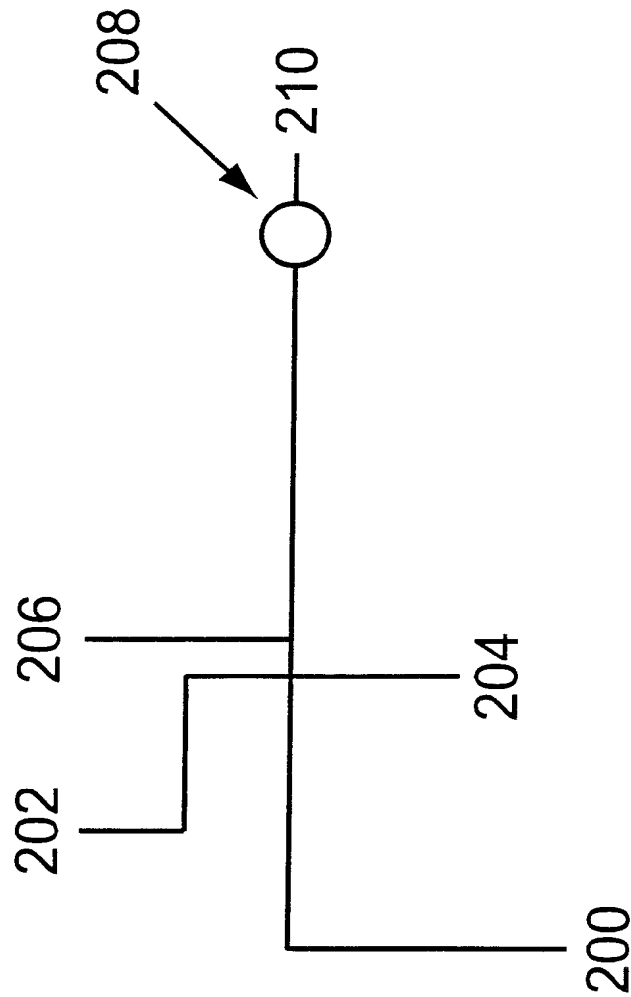


Fig. 2A

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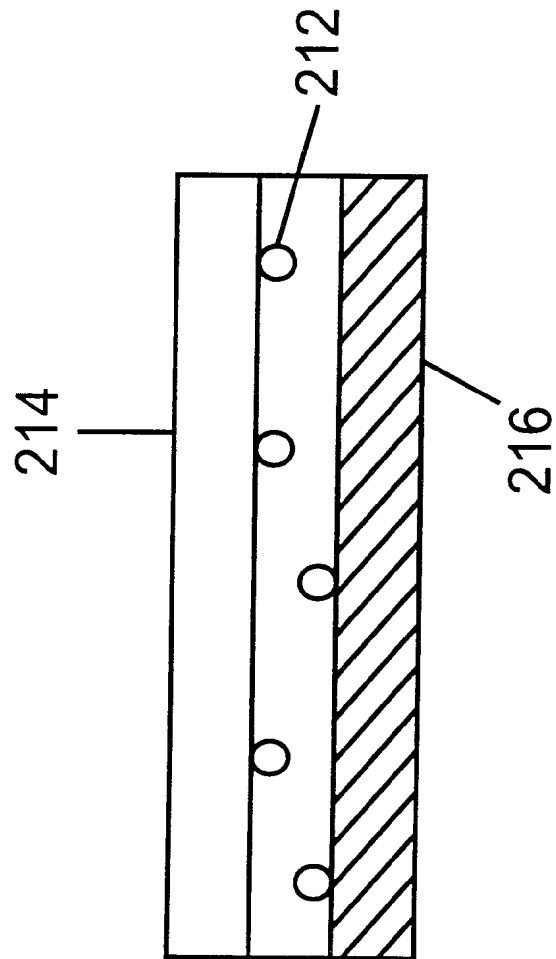


Fig. 2B

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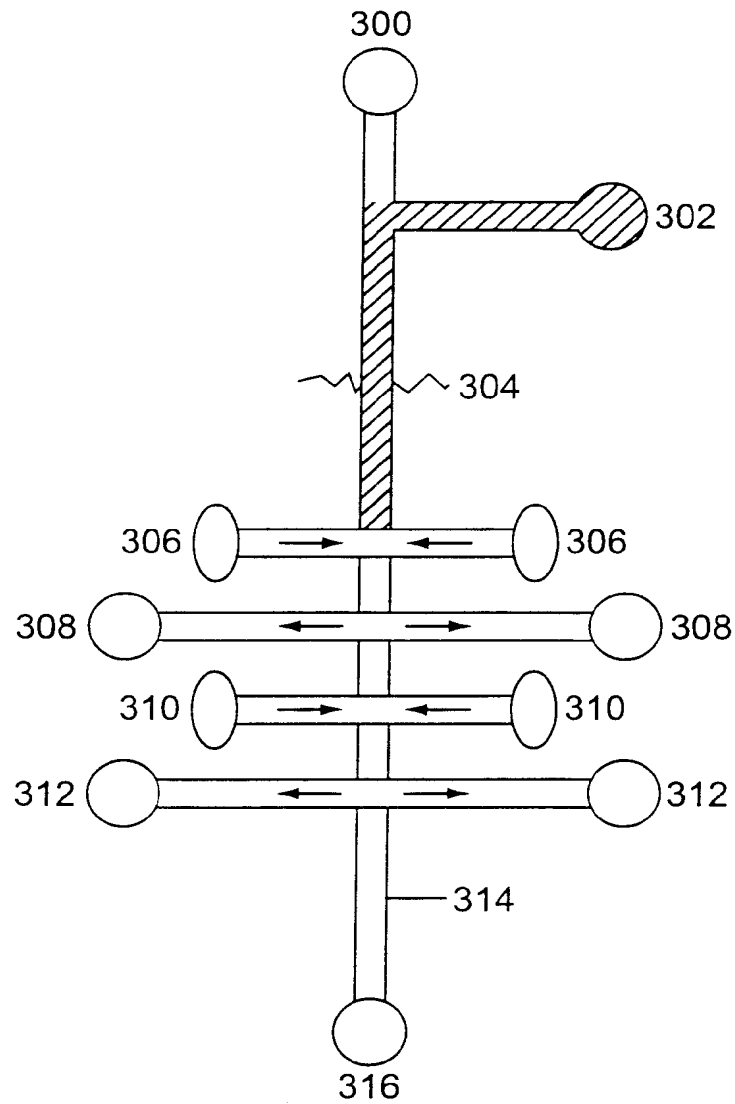


Fig. 3

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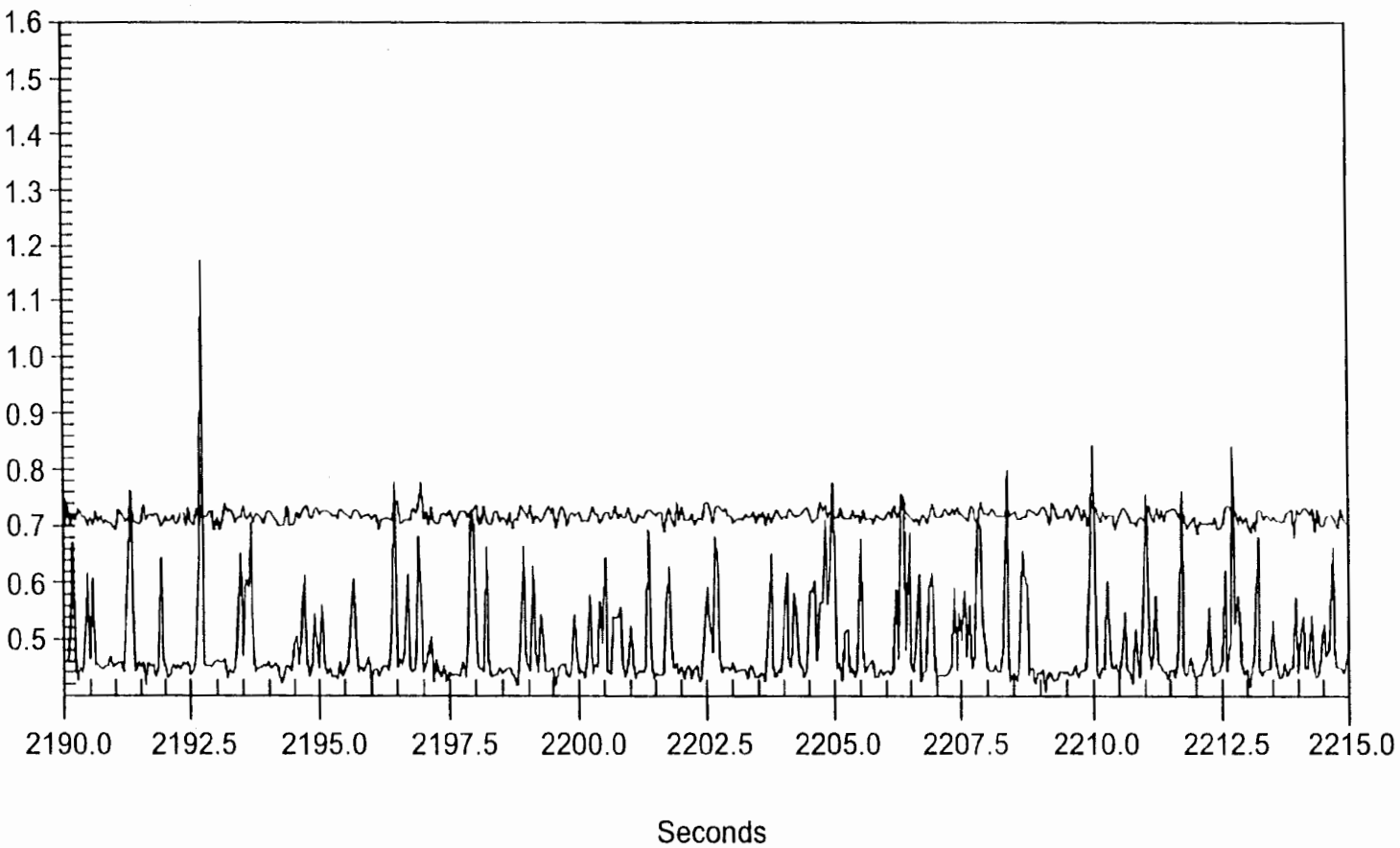


Fig. 4

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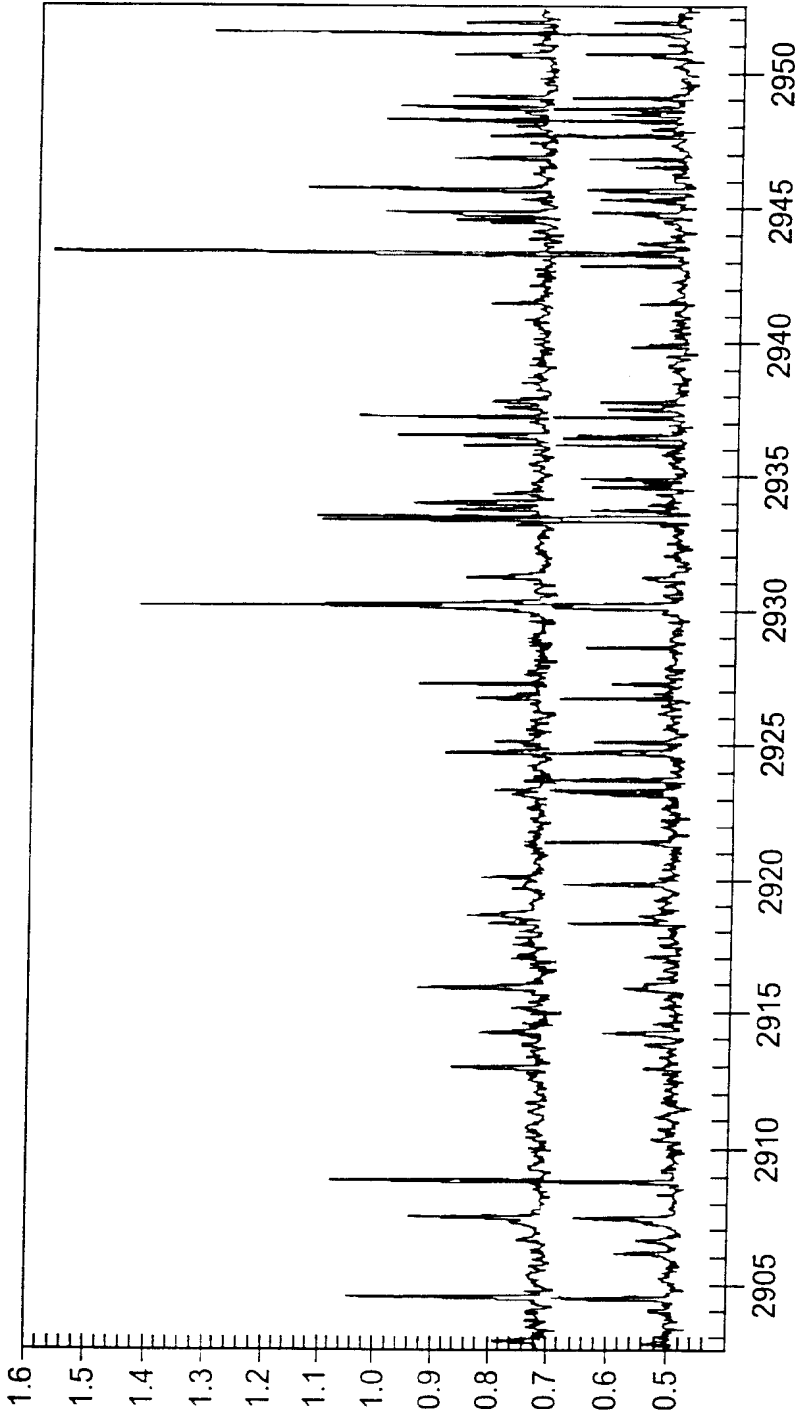


Fig. 5

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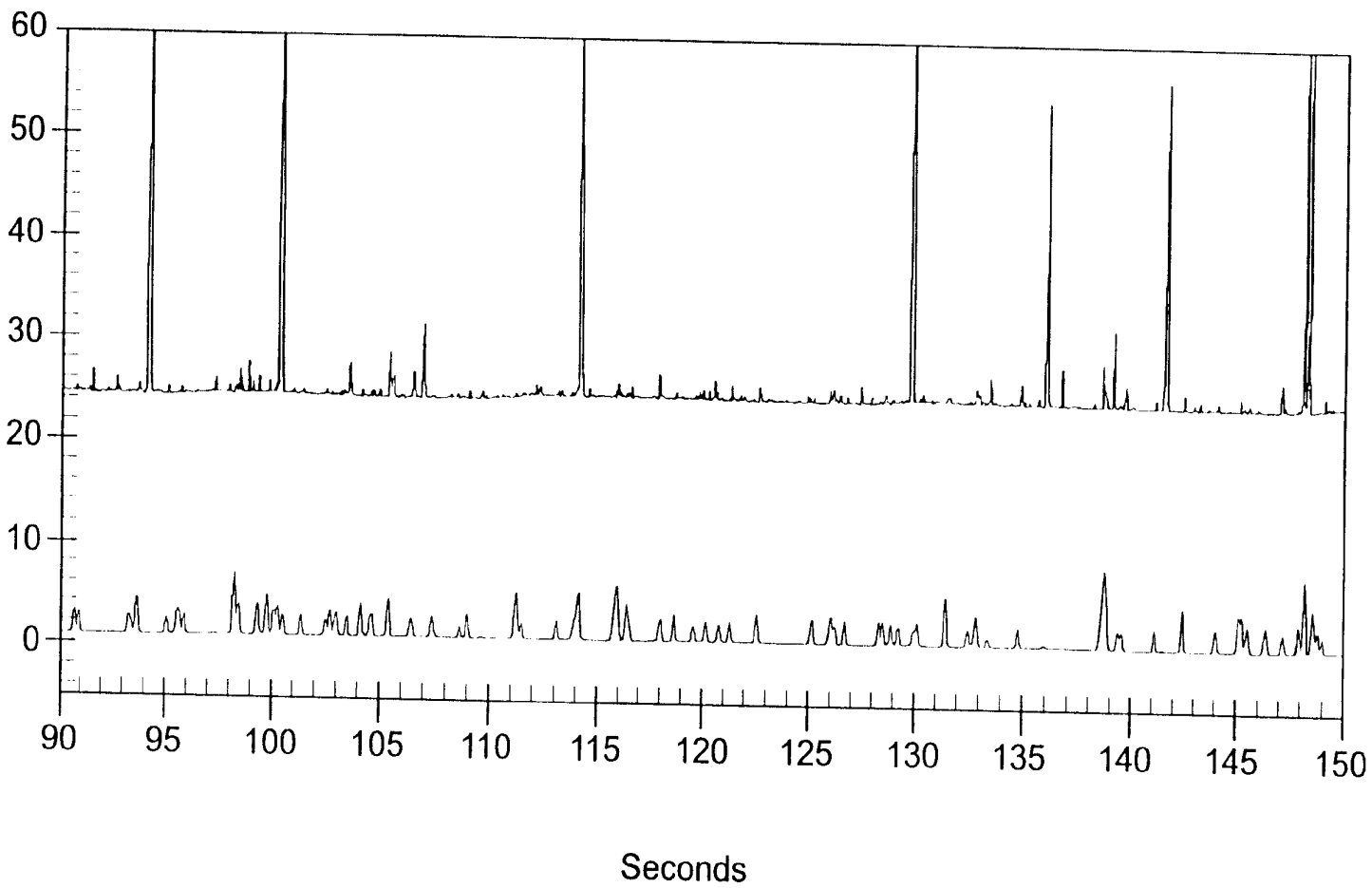


Fig. 6

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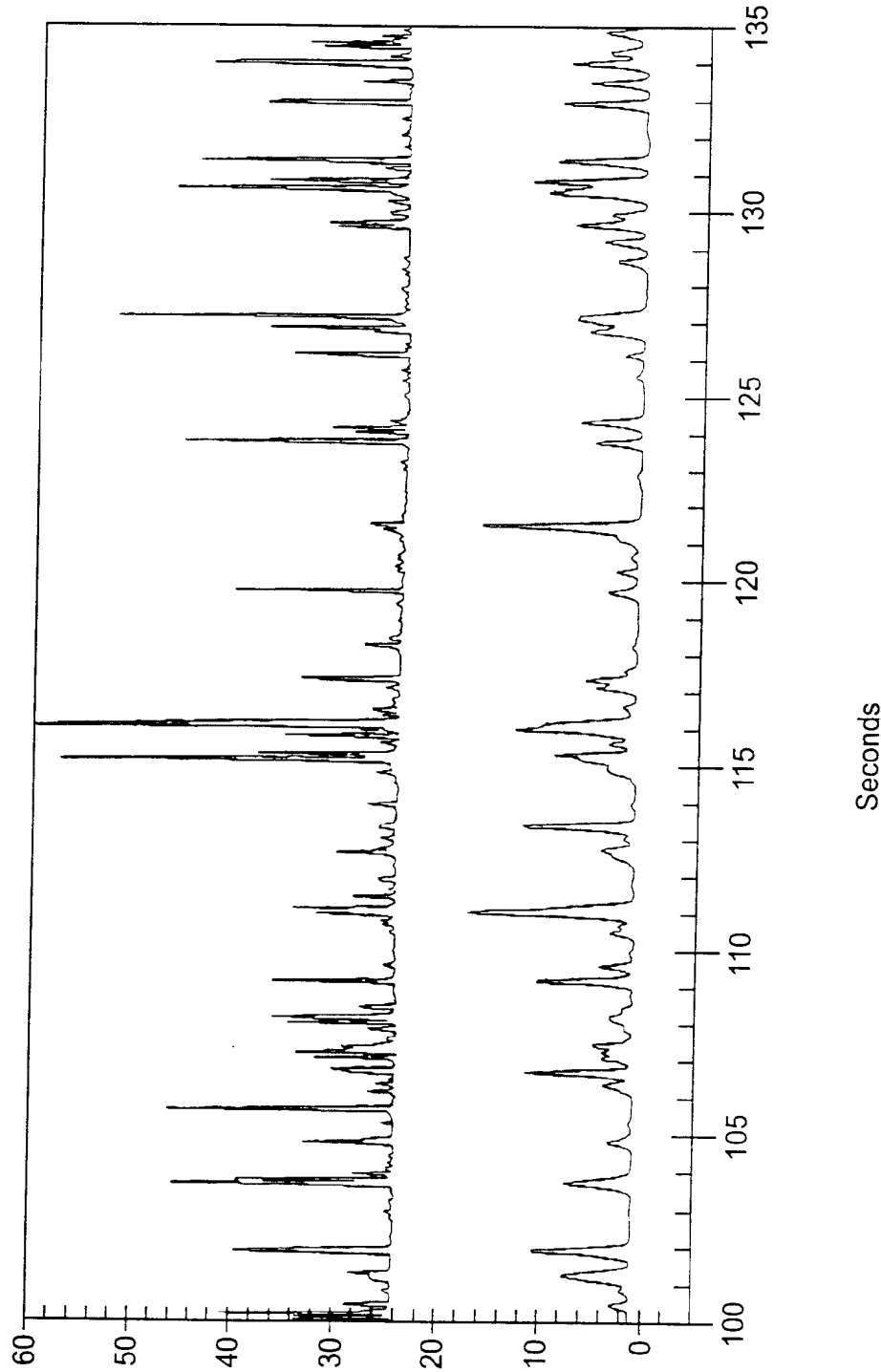


Fig. 7

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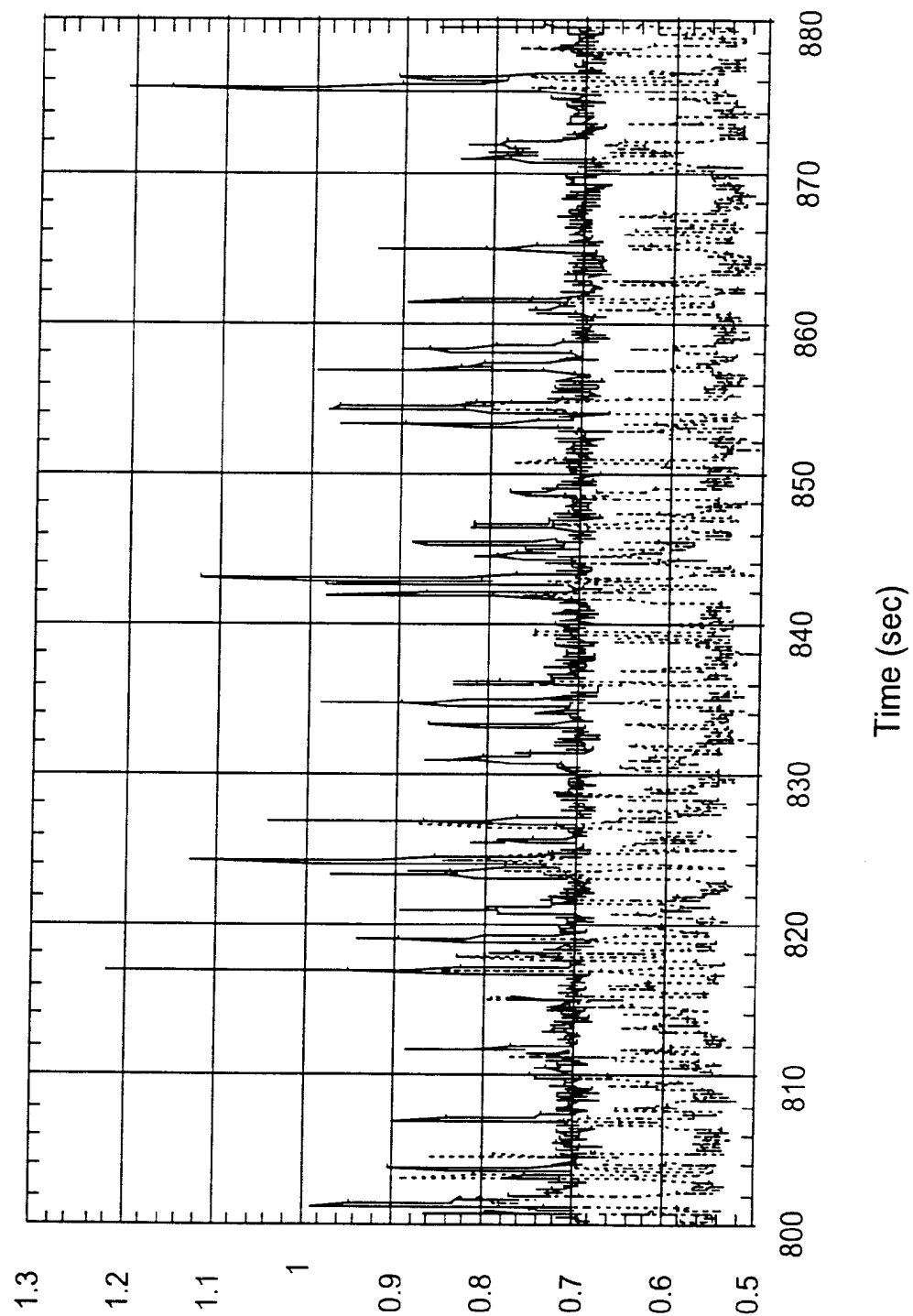


Fig. 8

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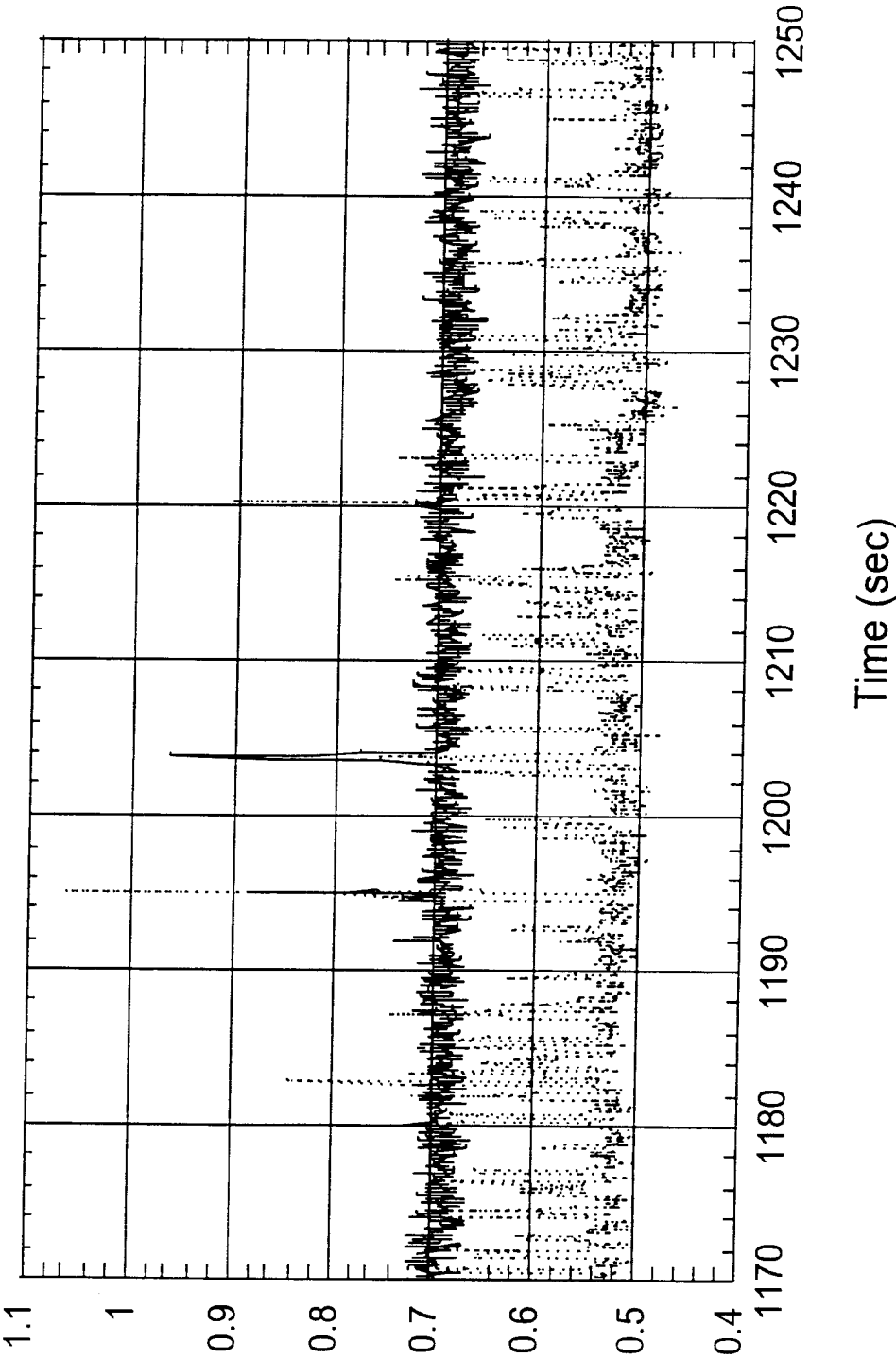


Fig. 9

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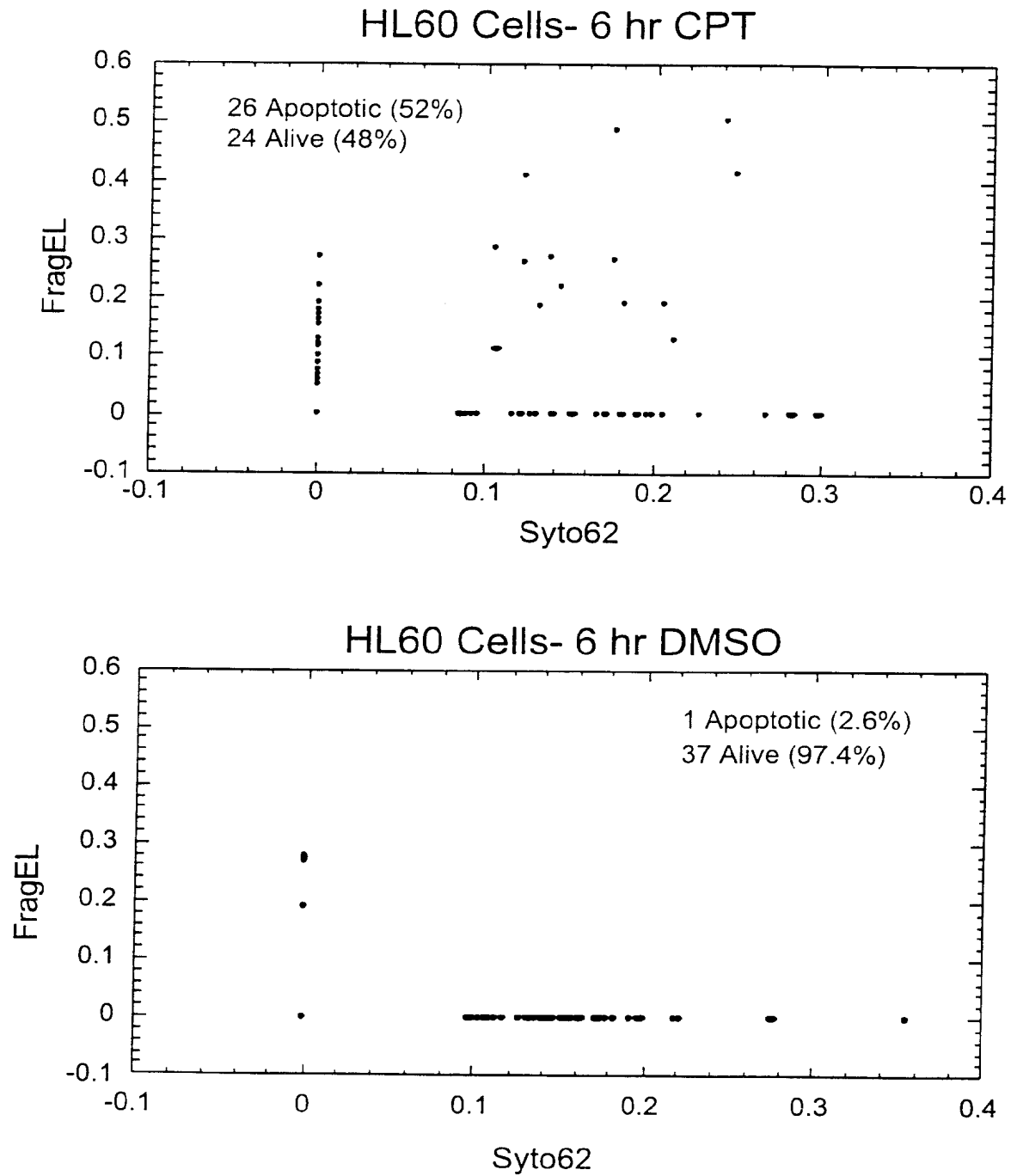


Fig. 10

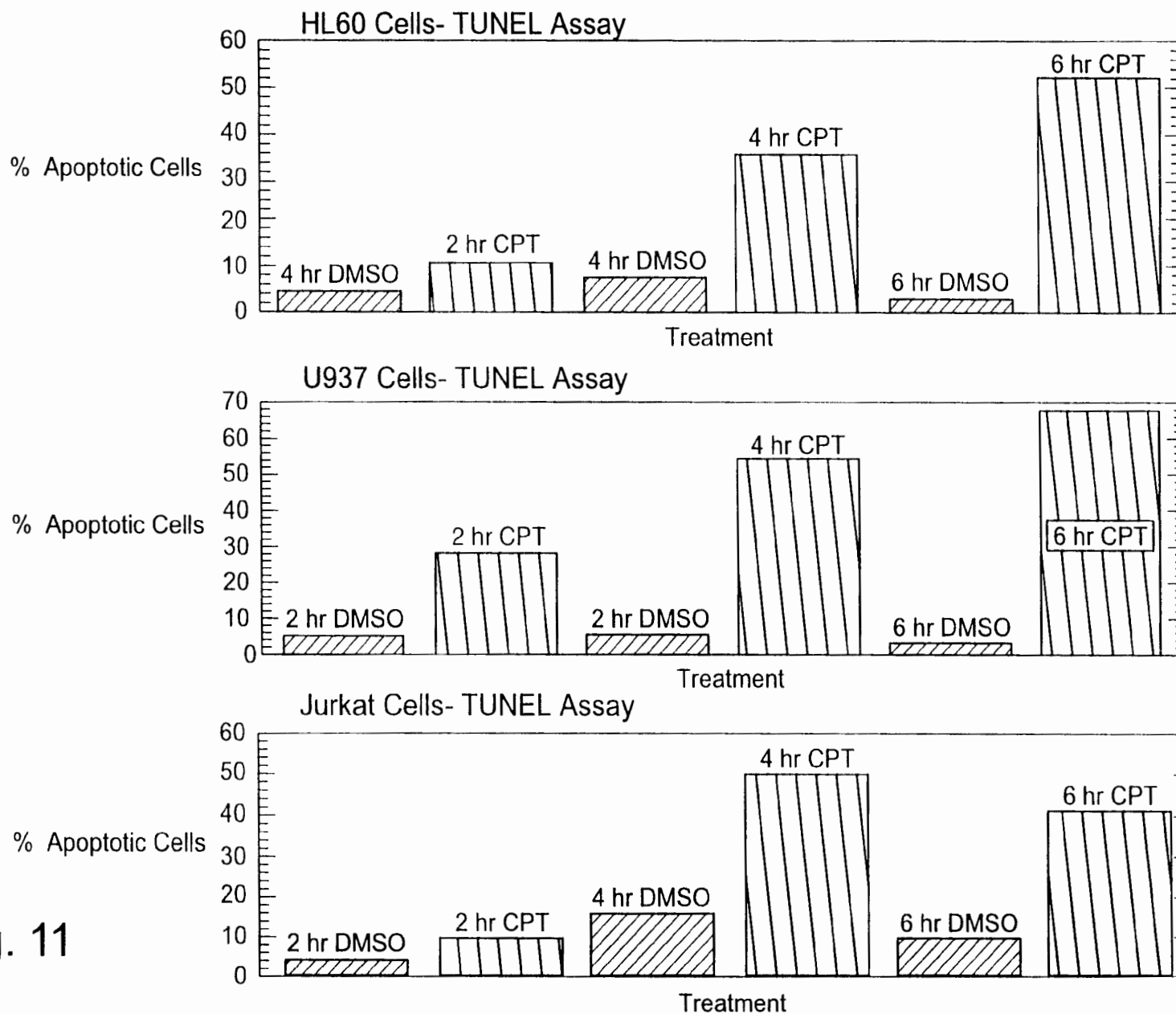
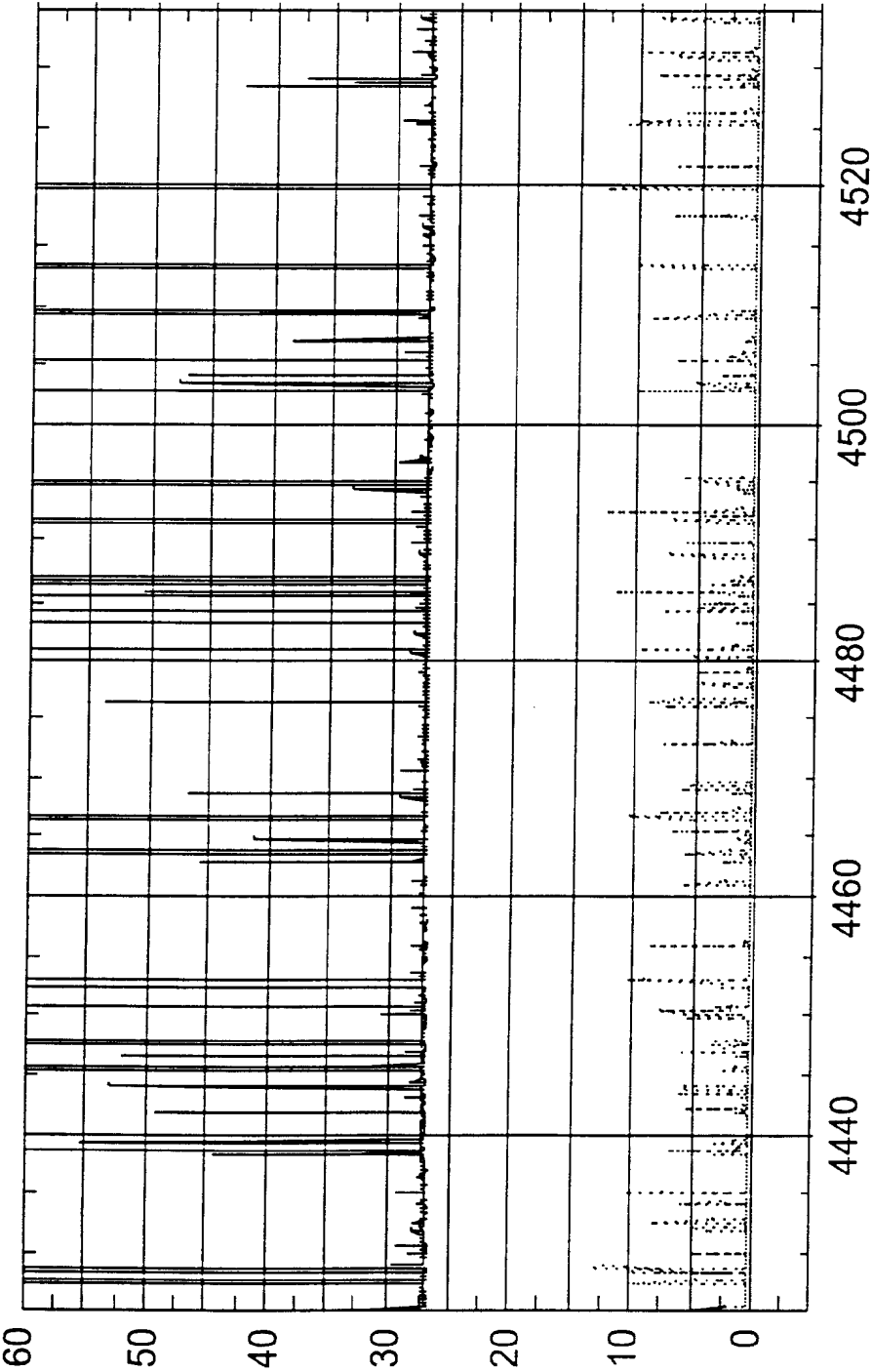


Fig. 11

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Time (sec)
Fig. 12

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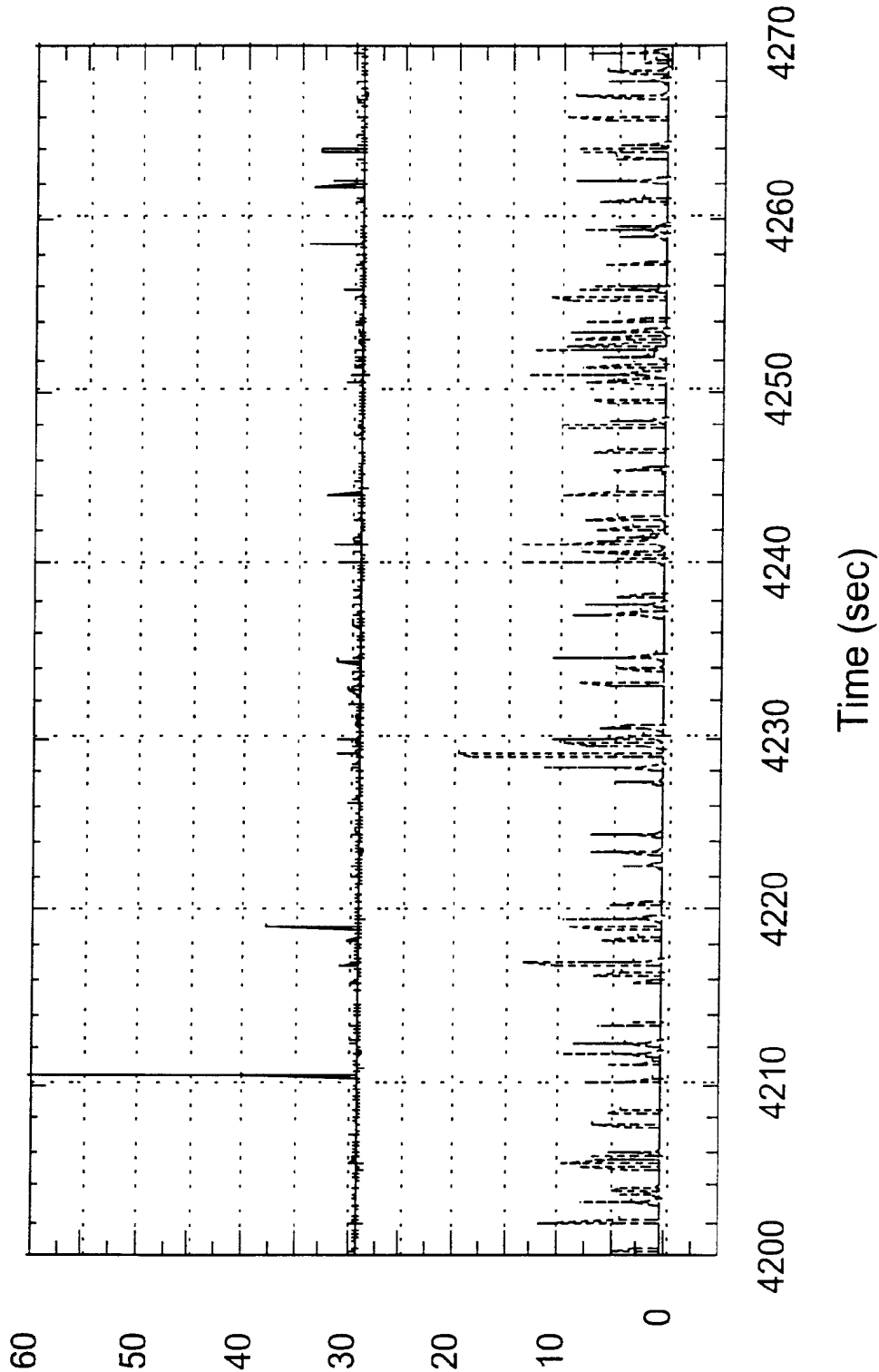
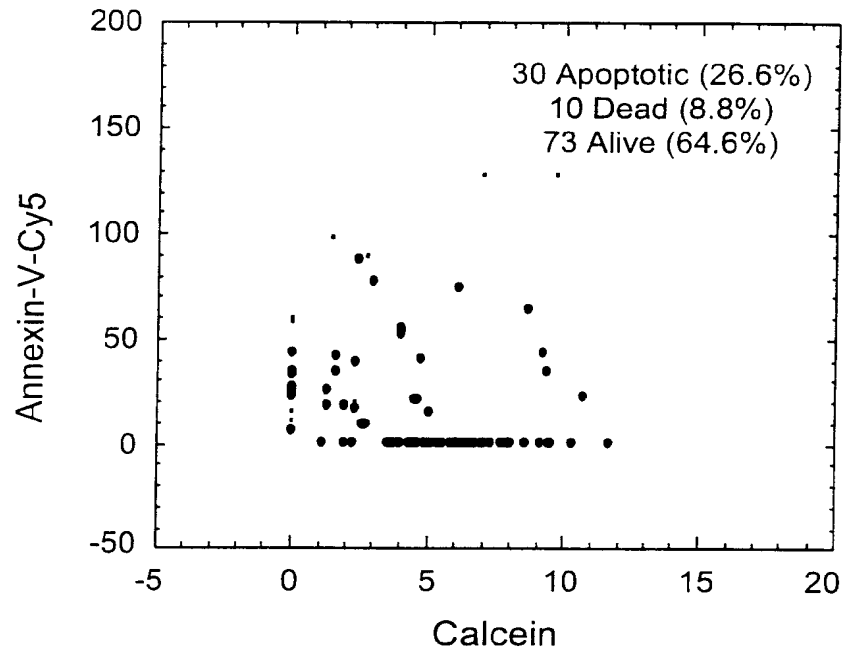


Fig. 13

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HL-60 Cells- 4 hr CPT



HL-60 Cells- 4 hr DMSO

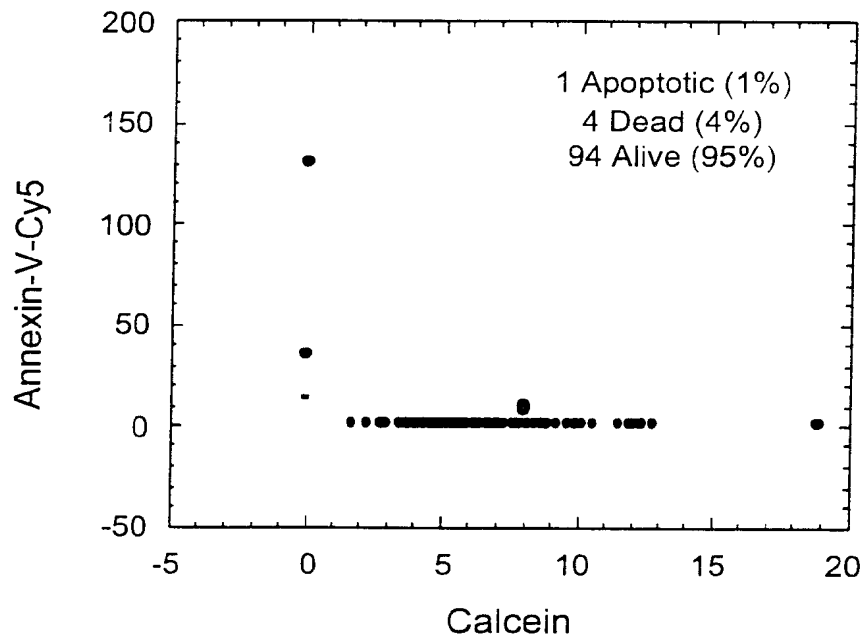


Fig. 14

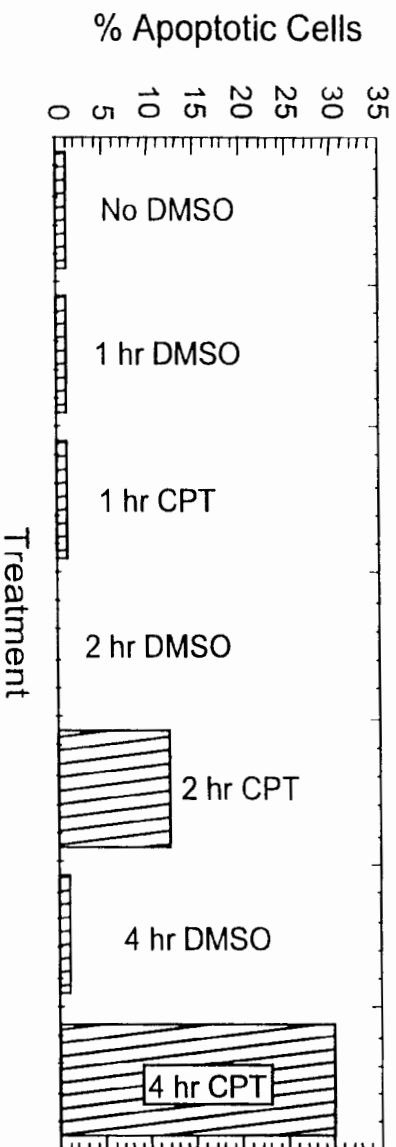
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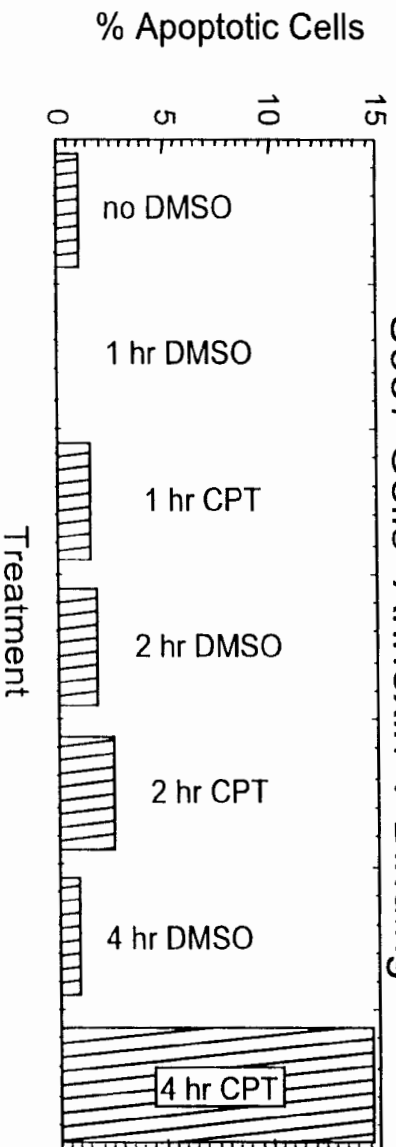
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HL-60 Cells- Annexin-V Binding



U937 Cells- Annexin-V Binding



Jurkat Cells- Annexin-V Binding

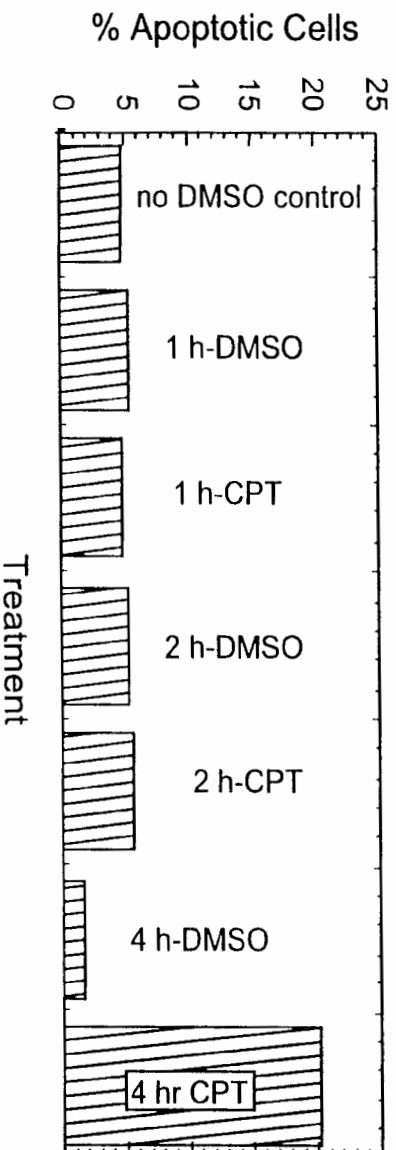


Fig. 15

SUBSTITUTE SHEET (RULE 26)

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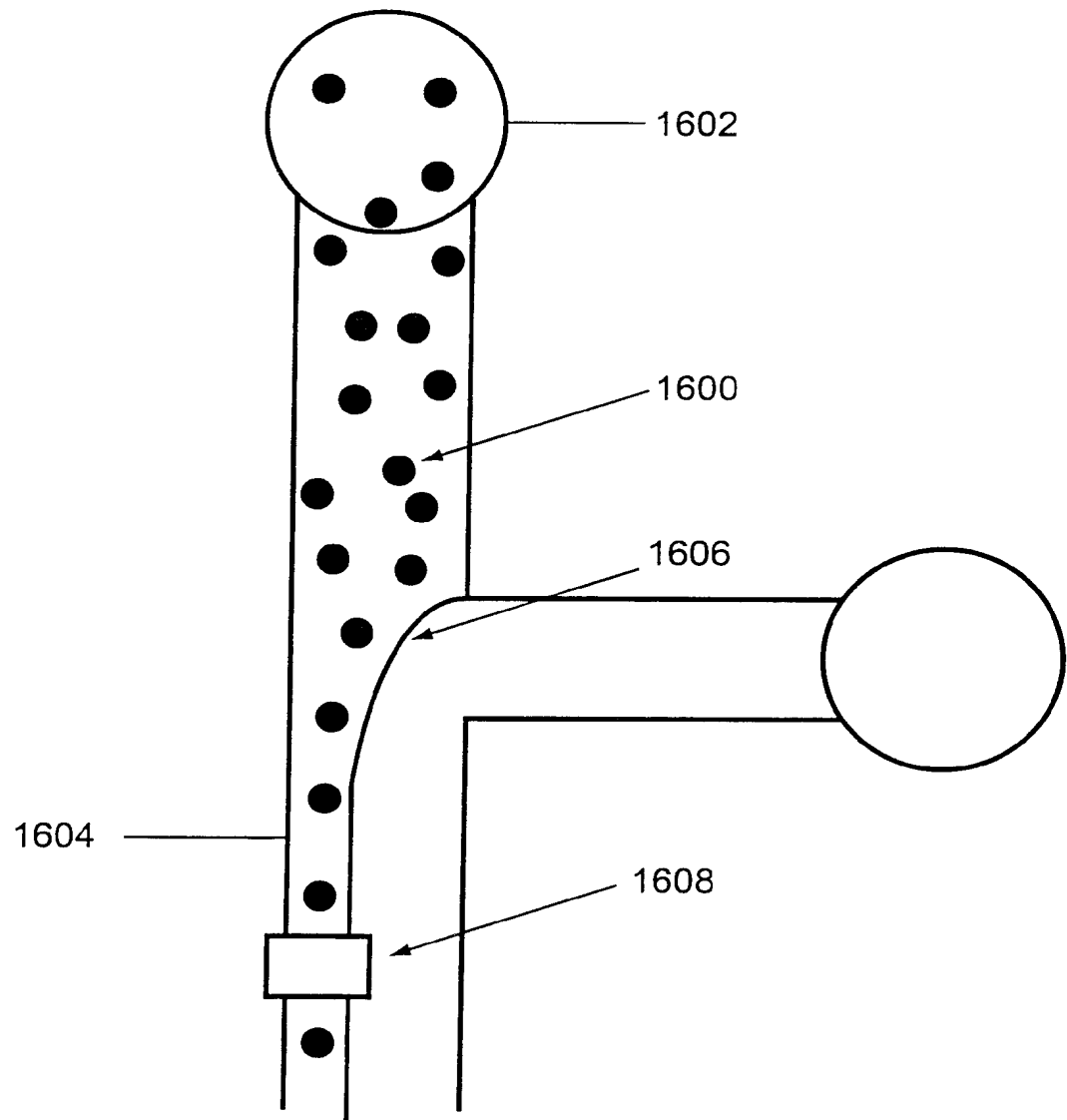


Fig. 16

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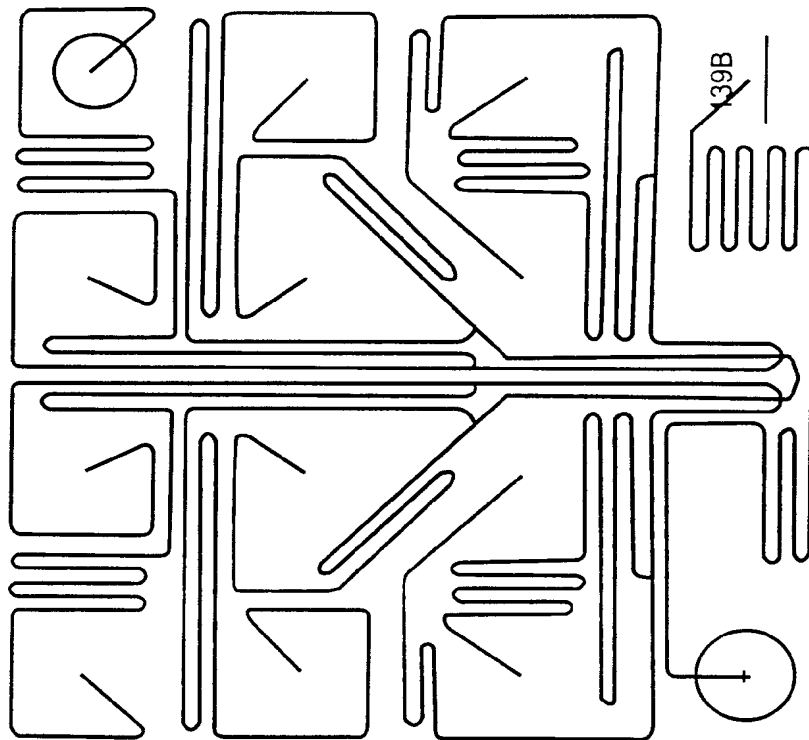


Fig. 17

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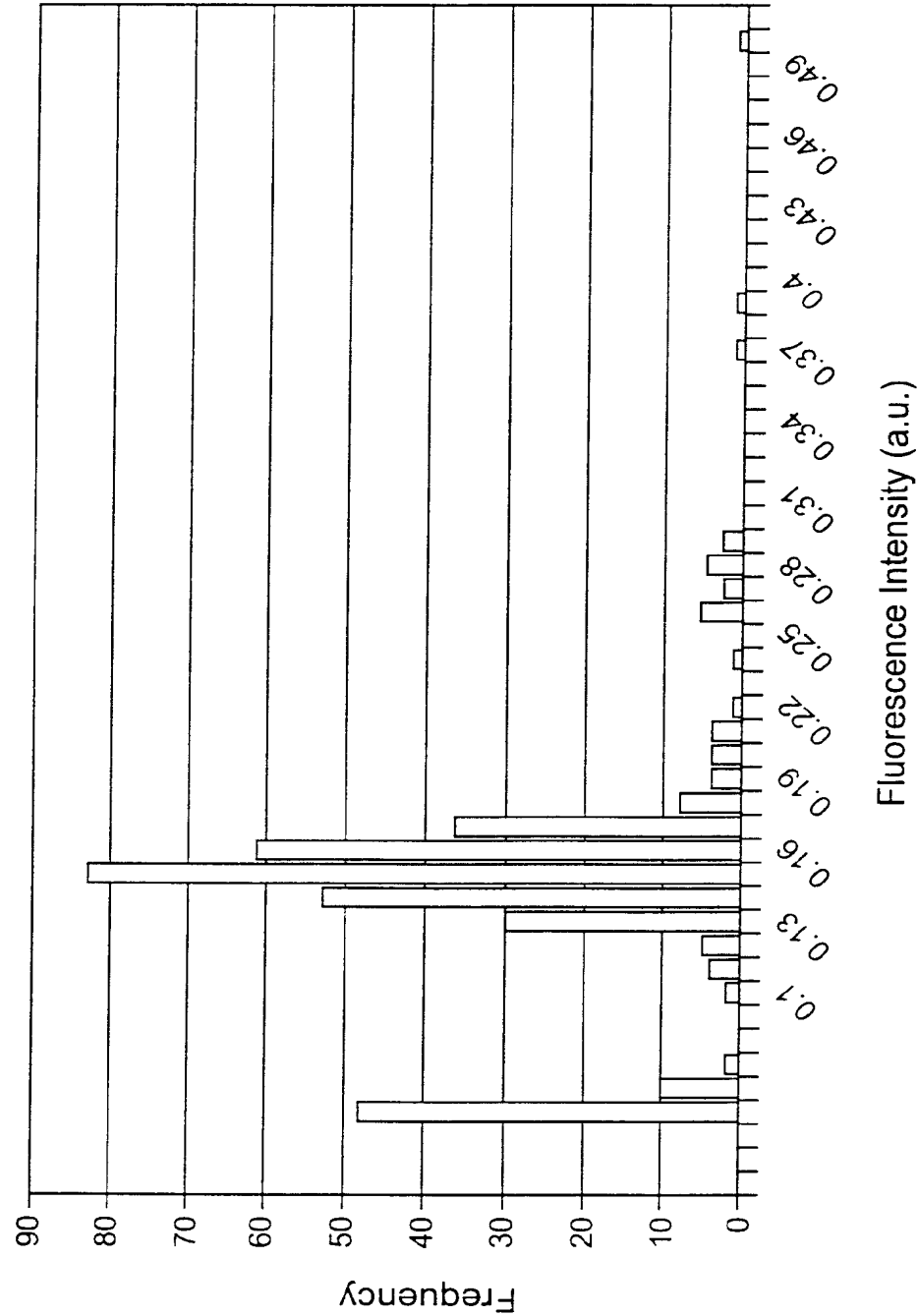


Fig. 18

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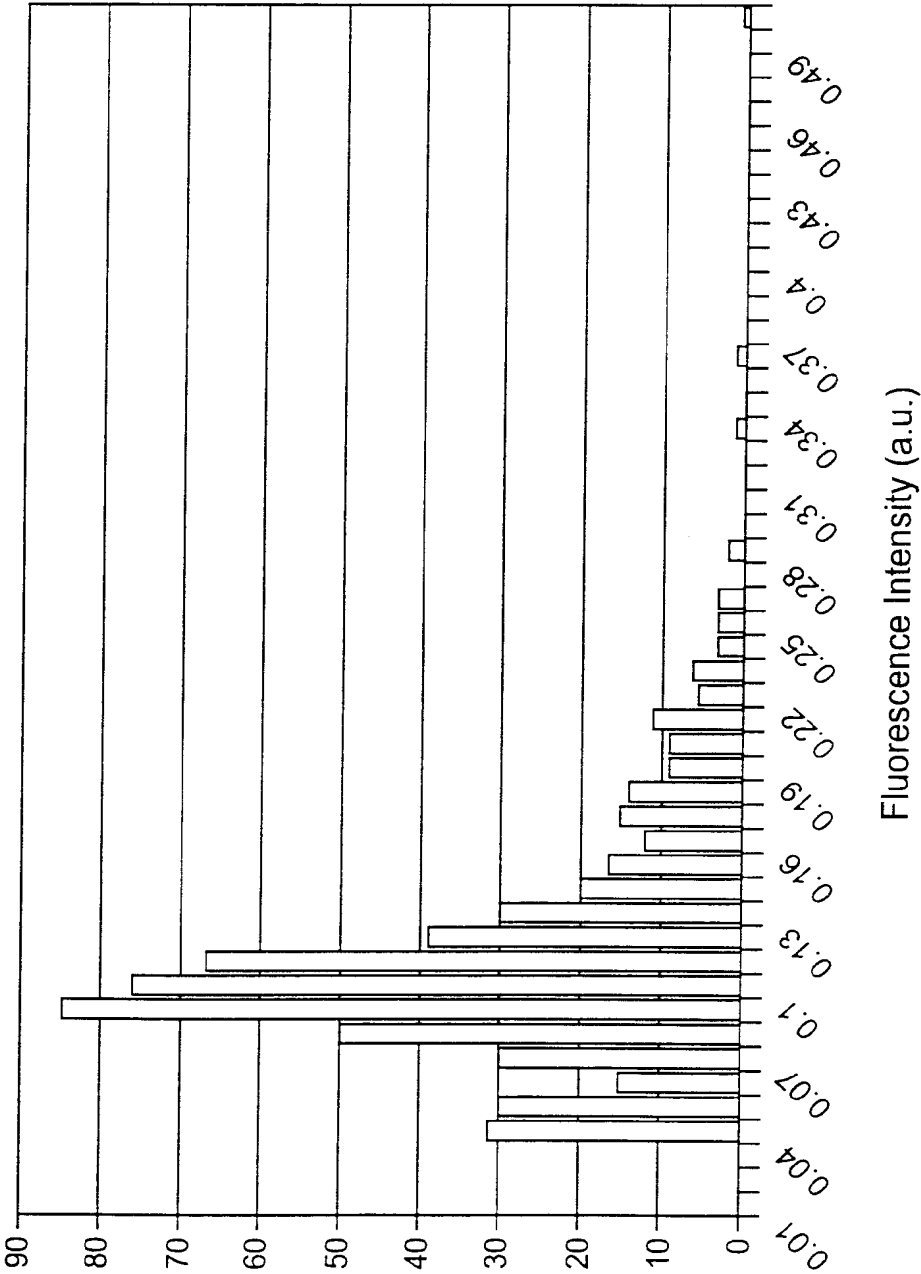


Fig. 19

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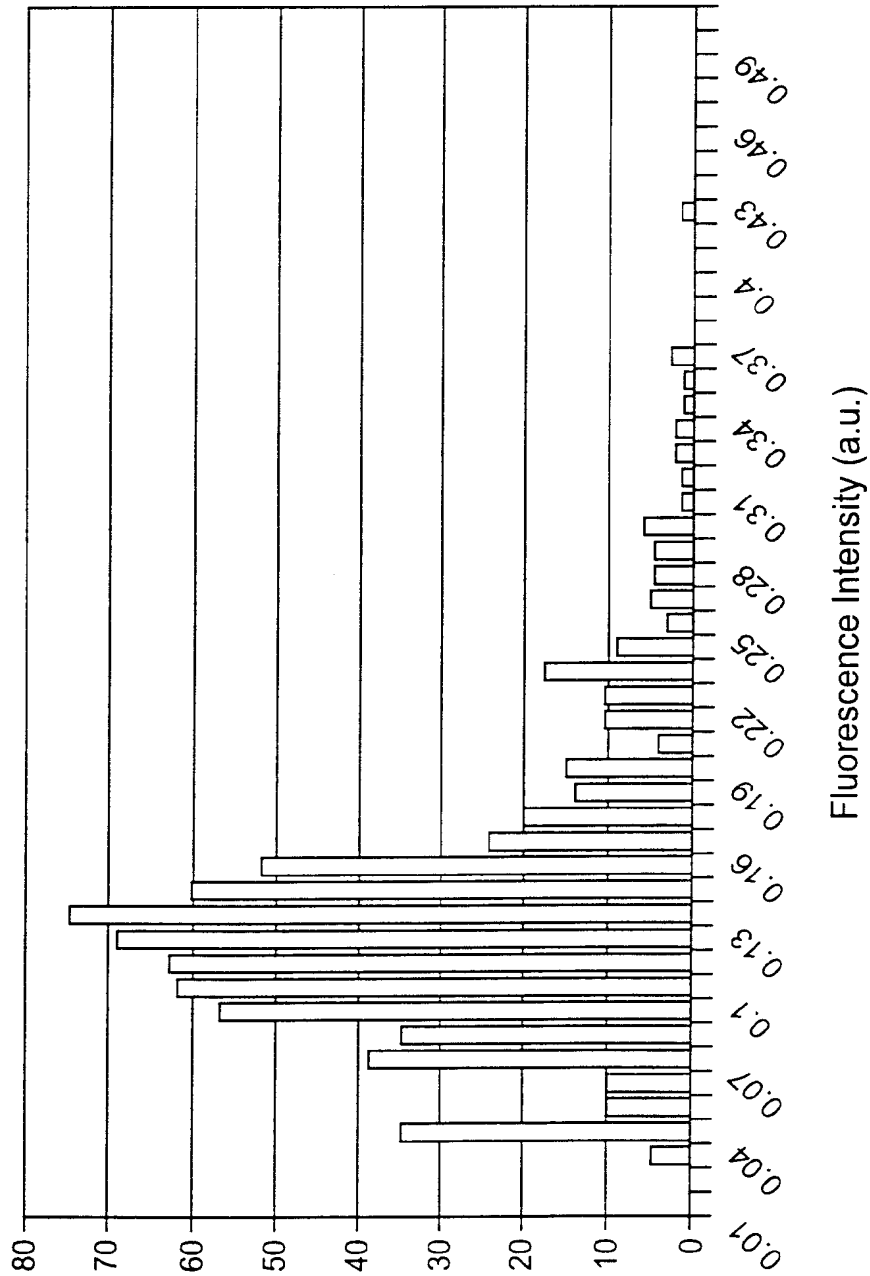


Fig. 20

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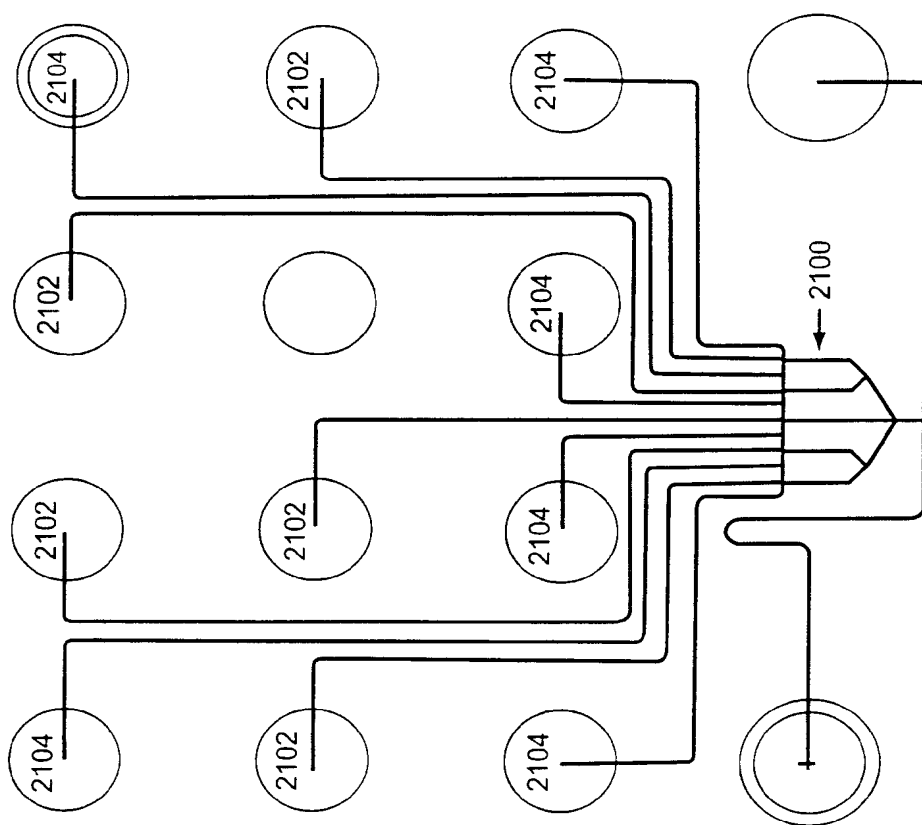


Fig. 21

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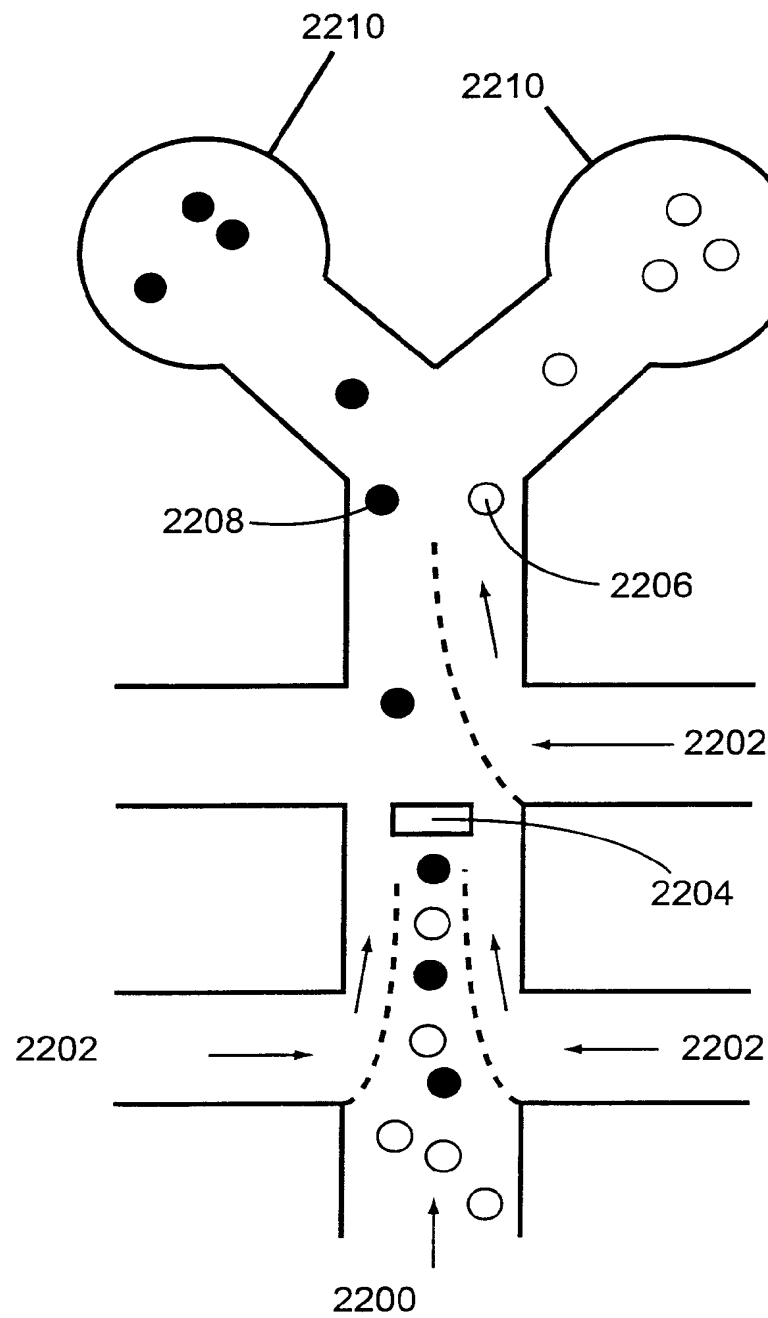


Fig. 22

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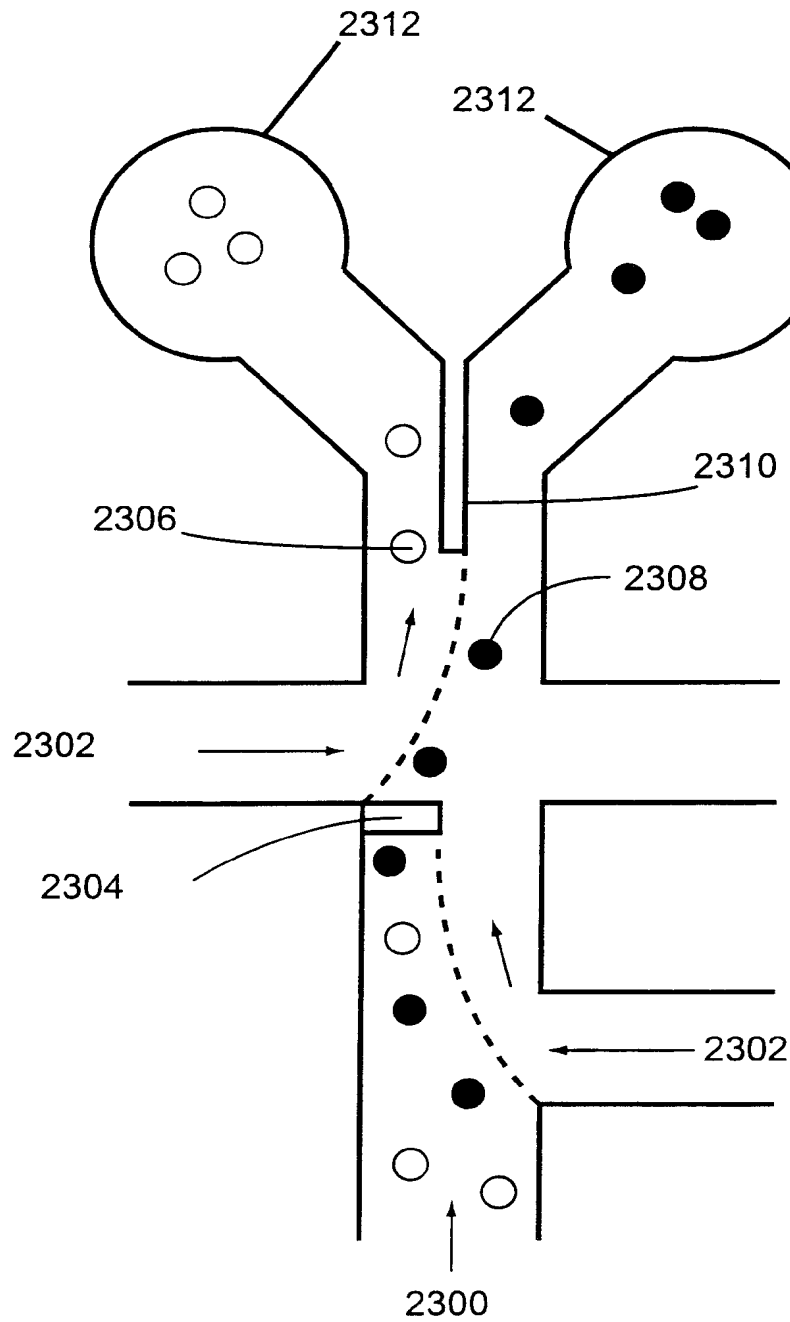


Fig. 23

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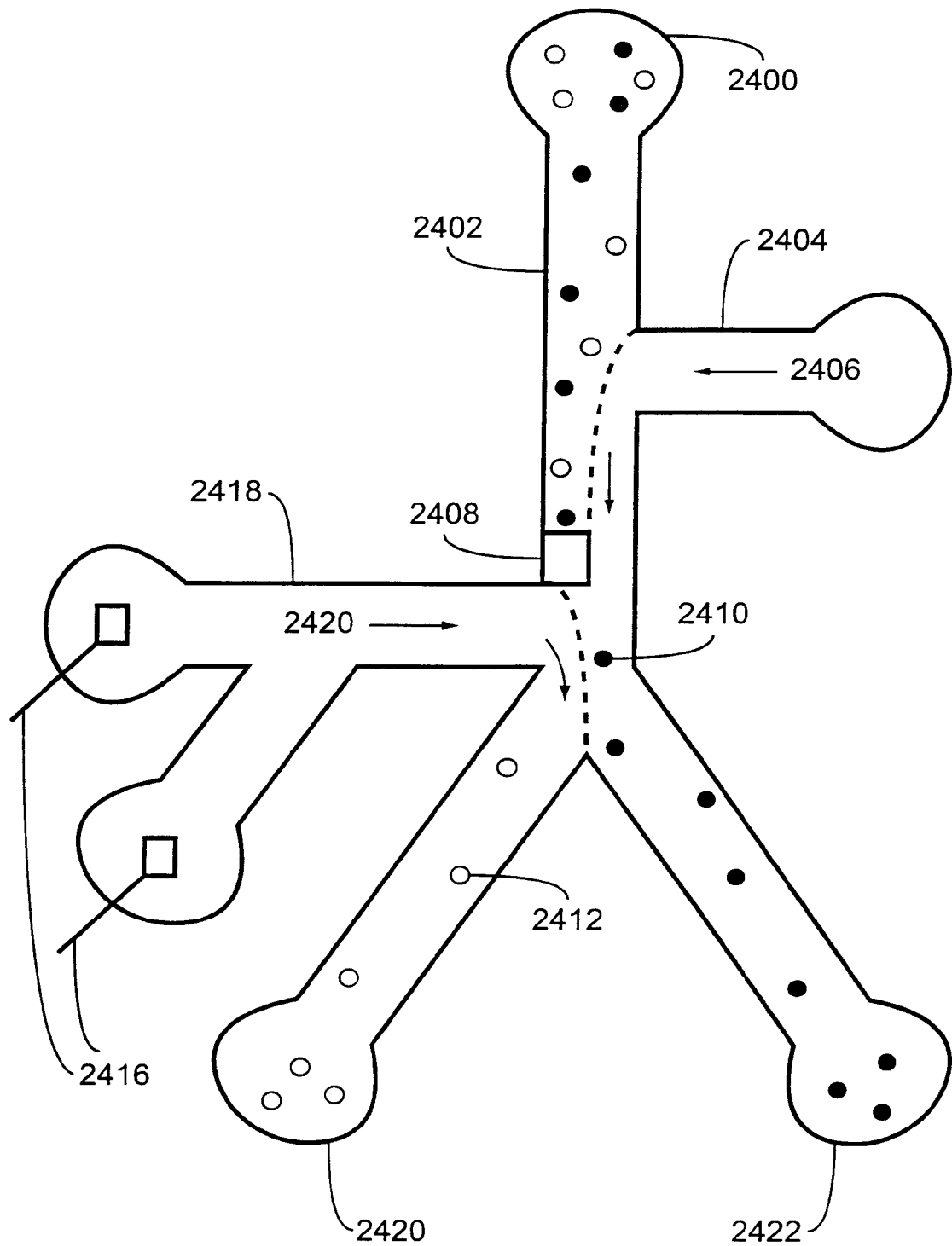


Fig. 24

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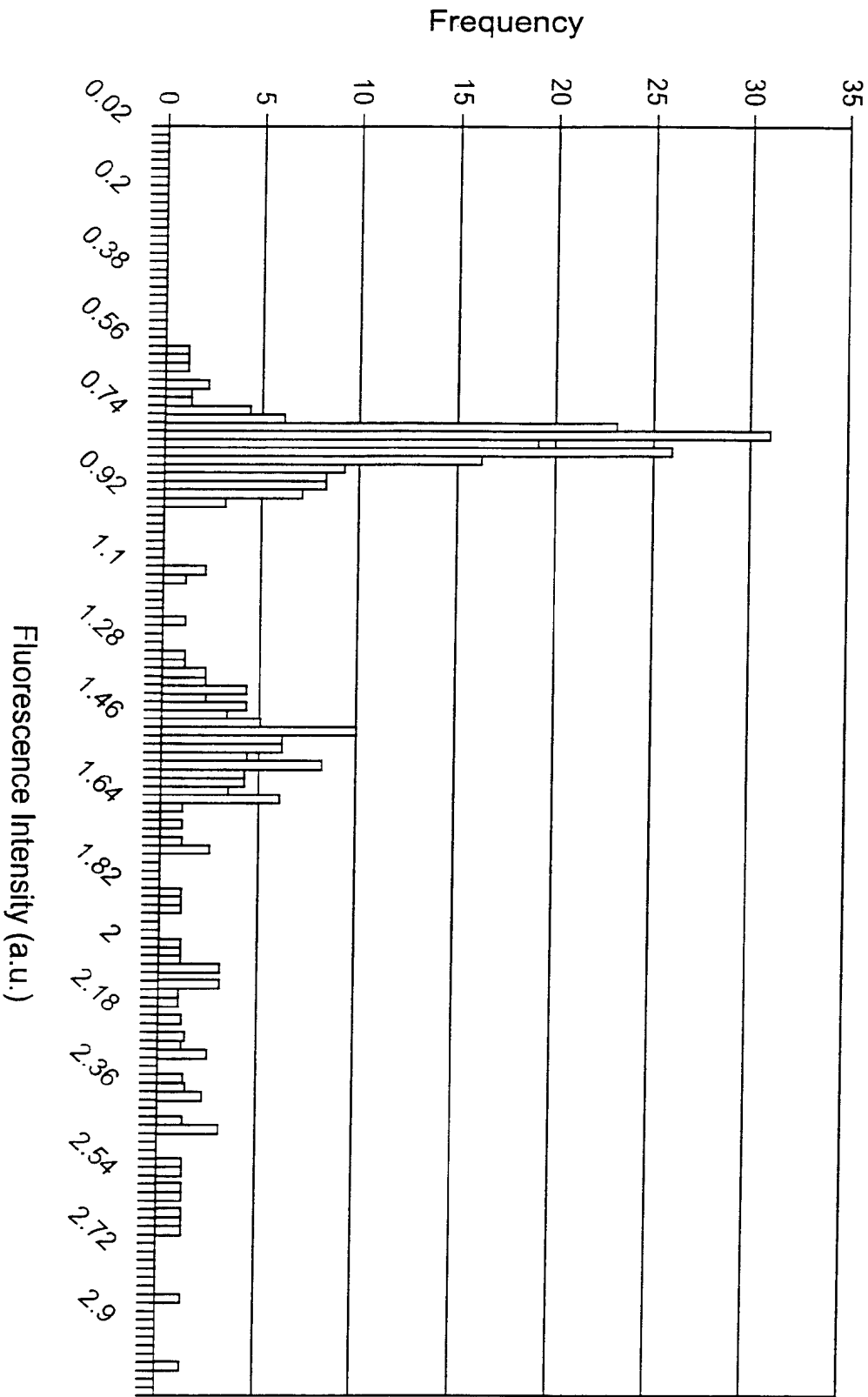


Fig. 25A

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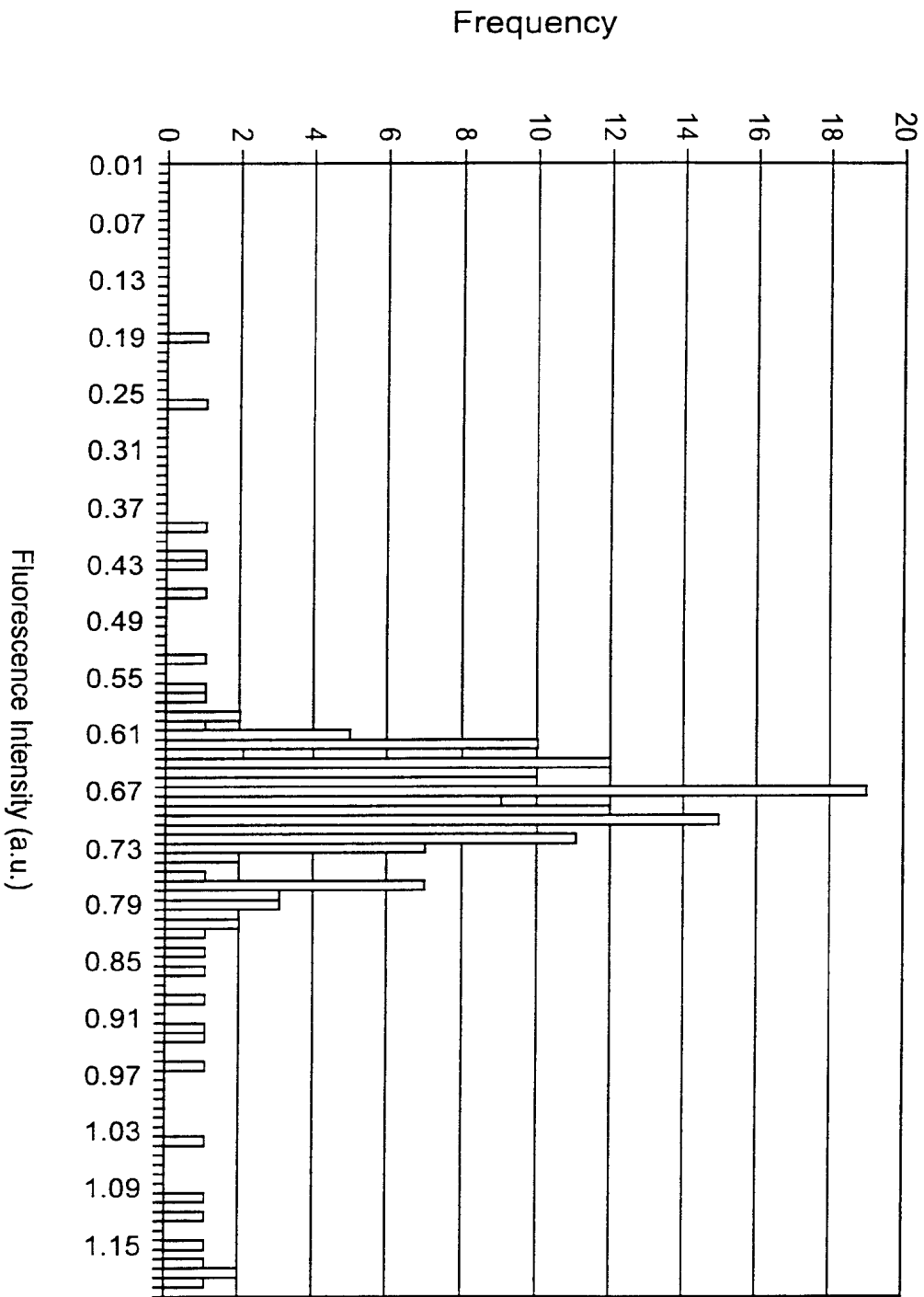


Fig. 25B

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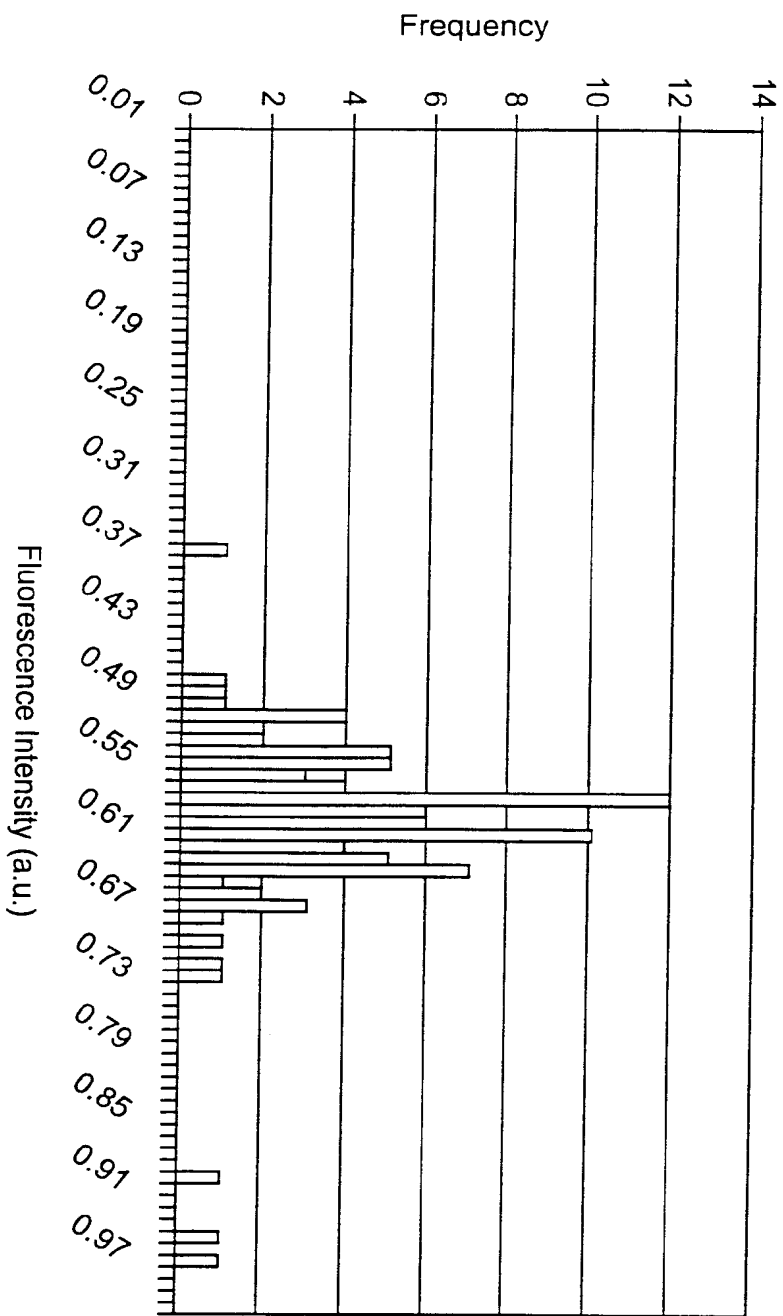


Fig. 25C

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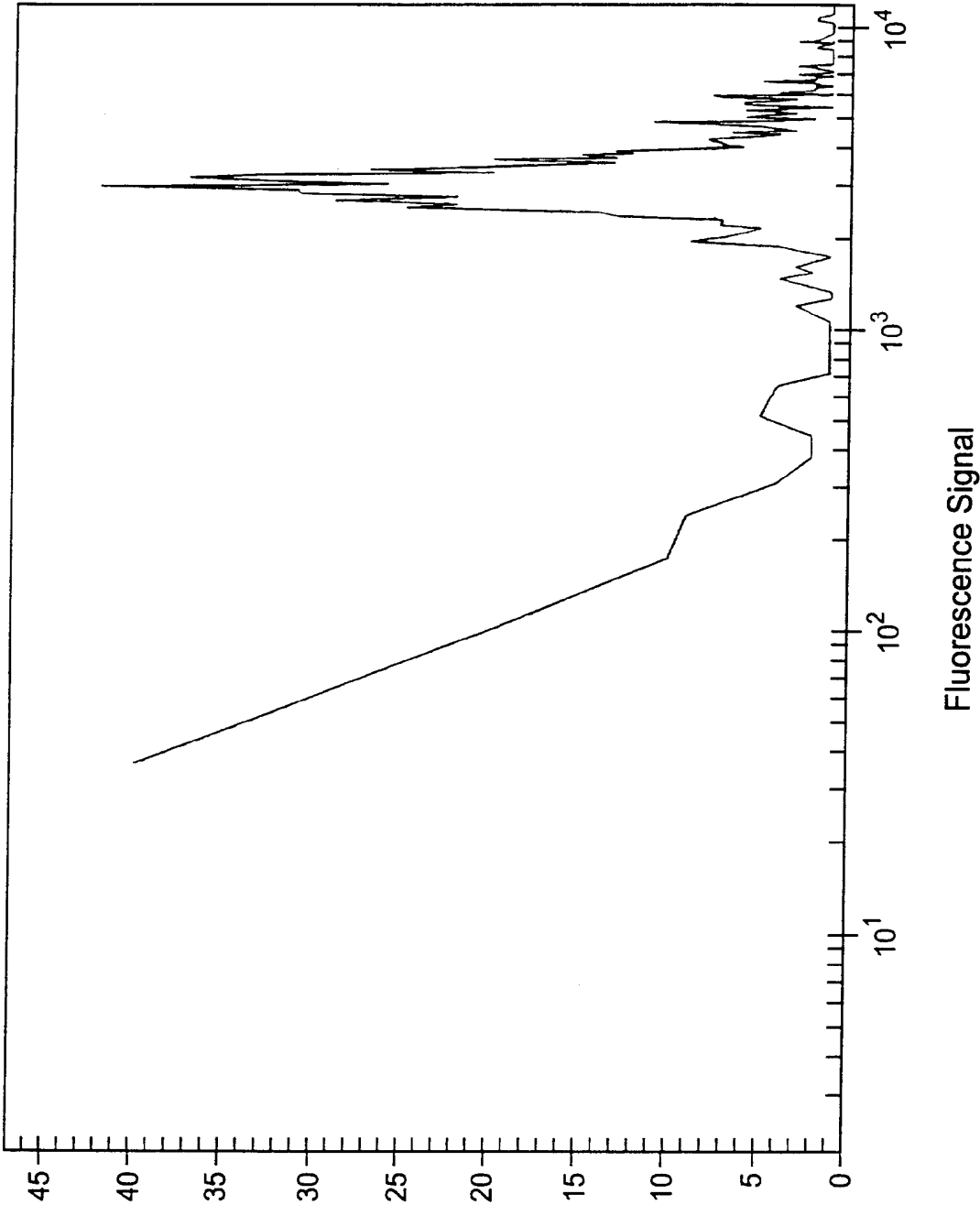


Fig. 26

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.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"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

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 .

(19)



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:
16.04.86 Patentblatt 86/16(64) Benannte Vertragsstaaten:
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Patentanwälte Holländer & Meyer Jungfernstieg 38
D-2000 Hamburg 36(DE)

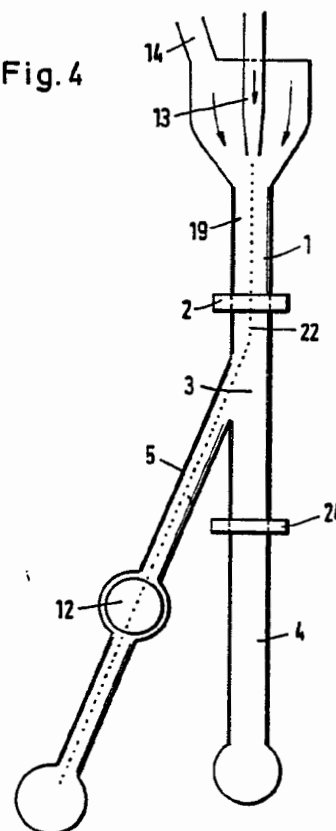
(54) Verfahren und Vorrichtung zur Sortierung von mikroskopischen Partikeln.

(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 Fliessrichtung 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 -Thymin) 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, stör anfällig 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

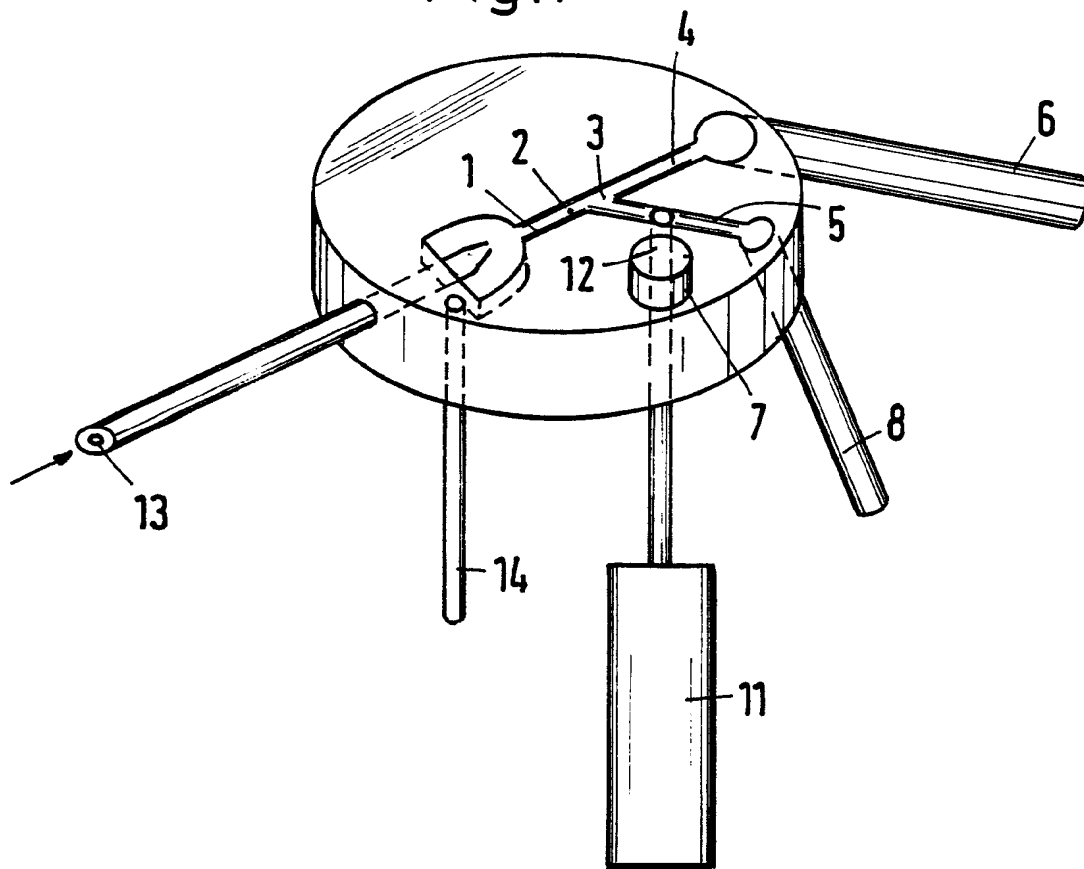


Fig.2

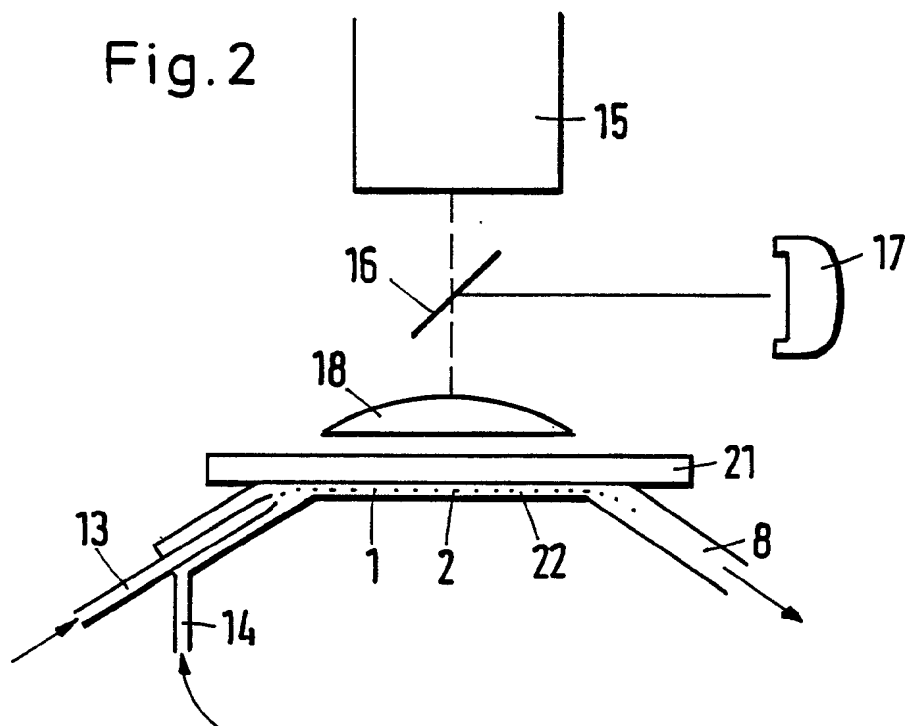


Fig. 3

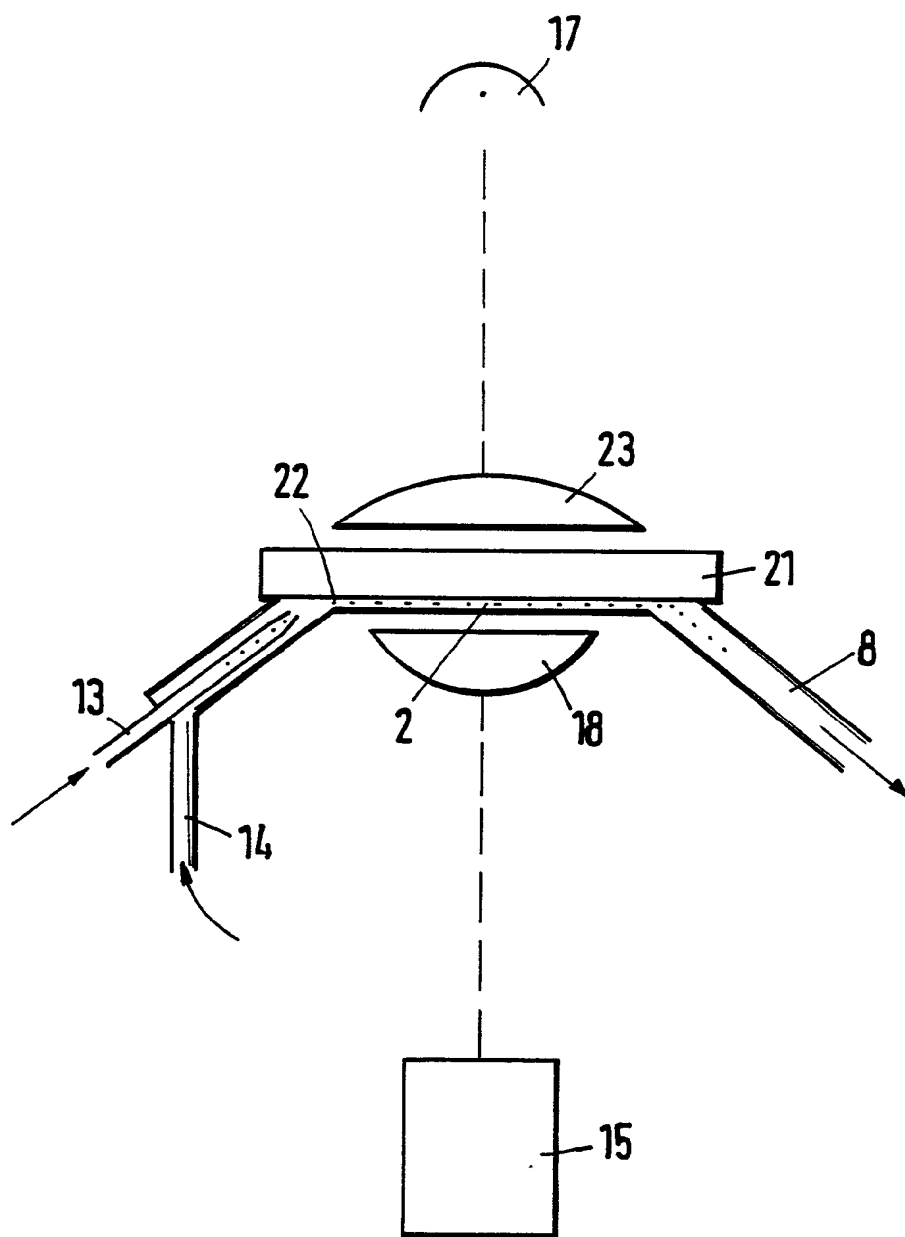


Fig. 4

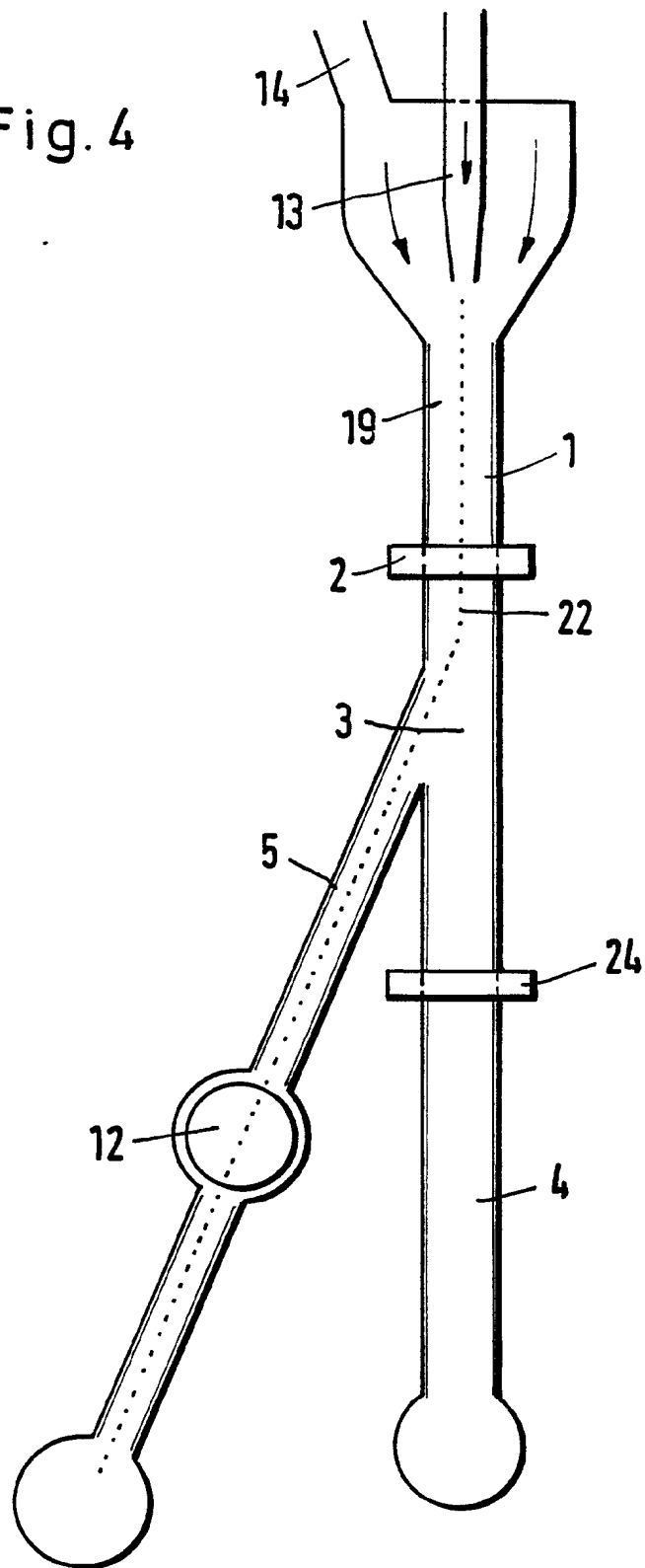


Fig. 5

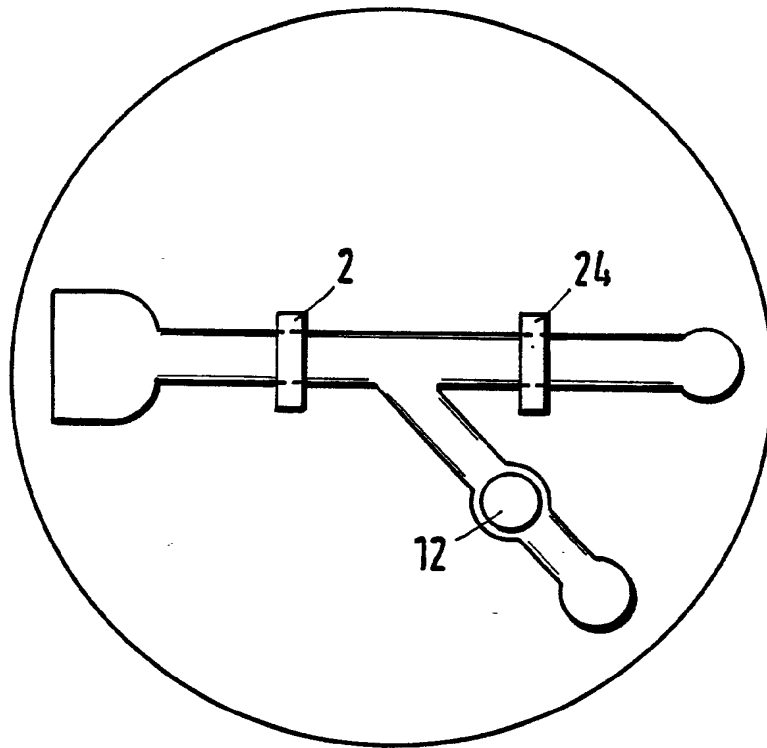
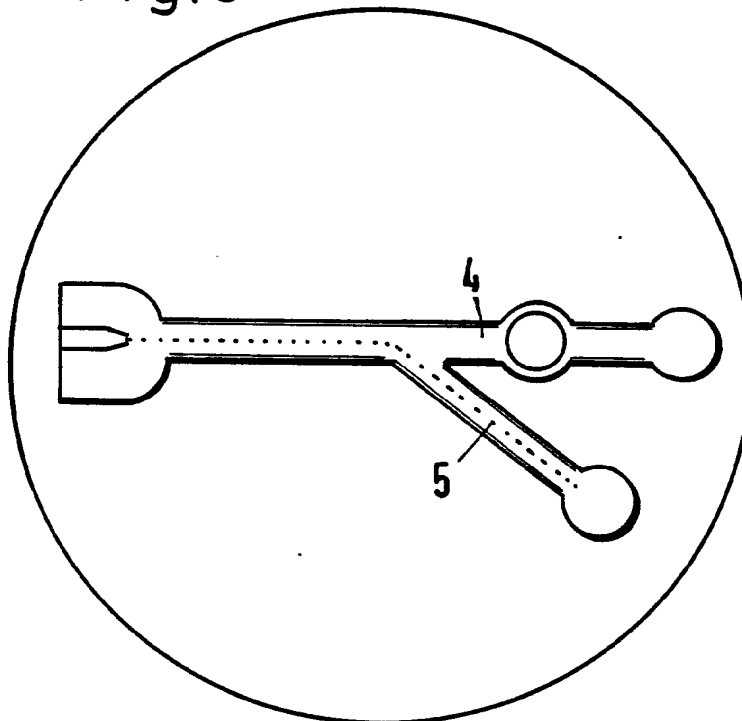


Fig. 6



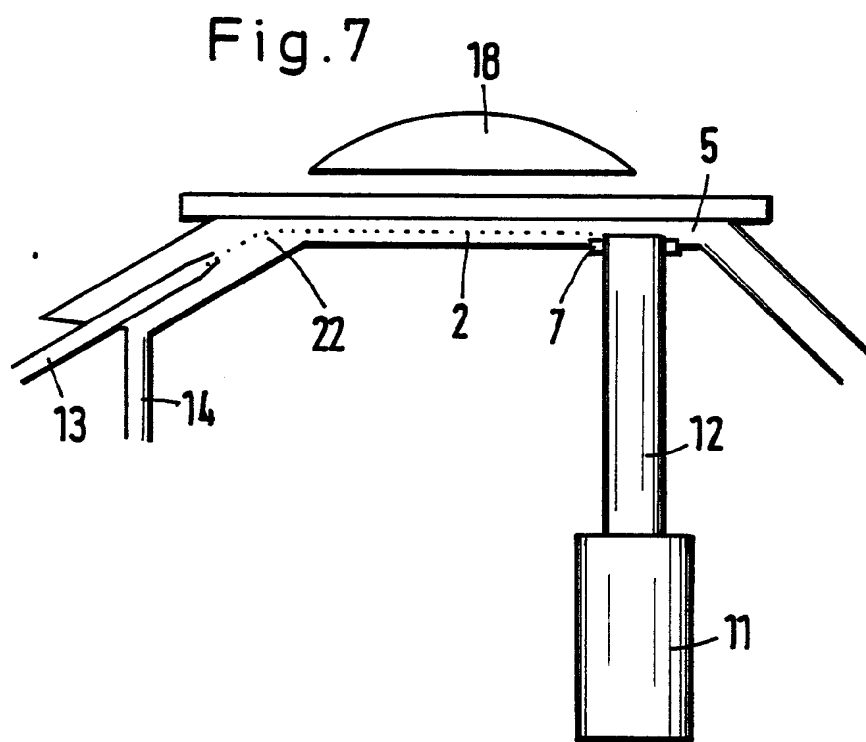


Fig.8

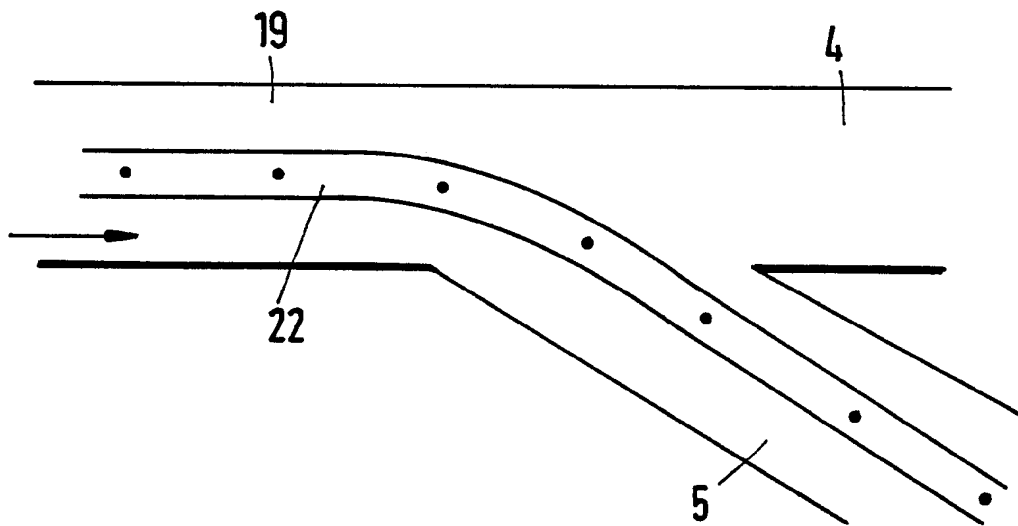


Fig.9

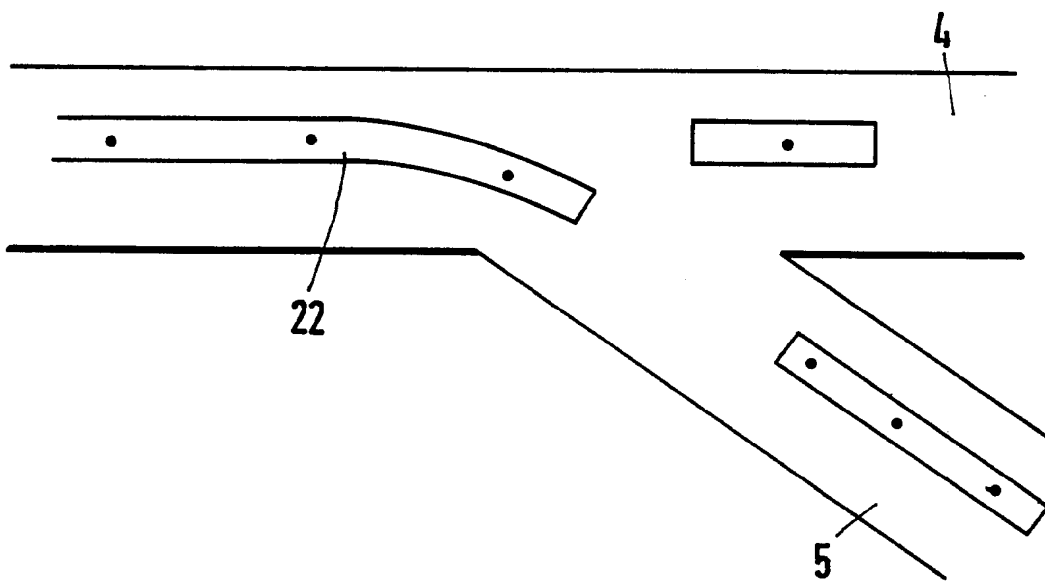


Fig.10

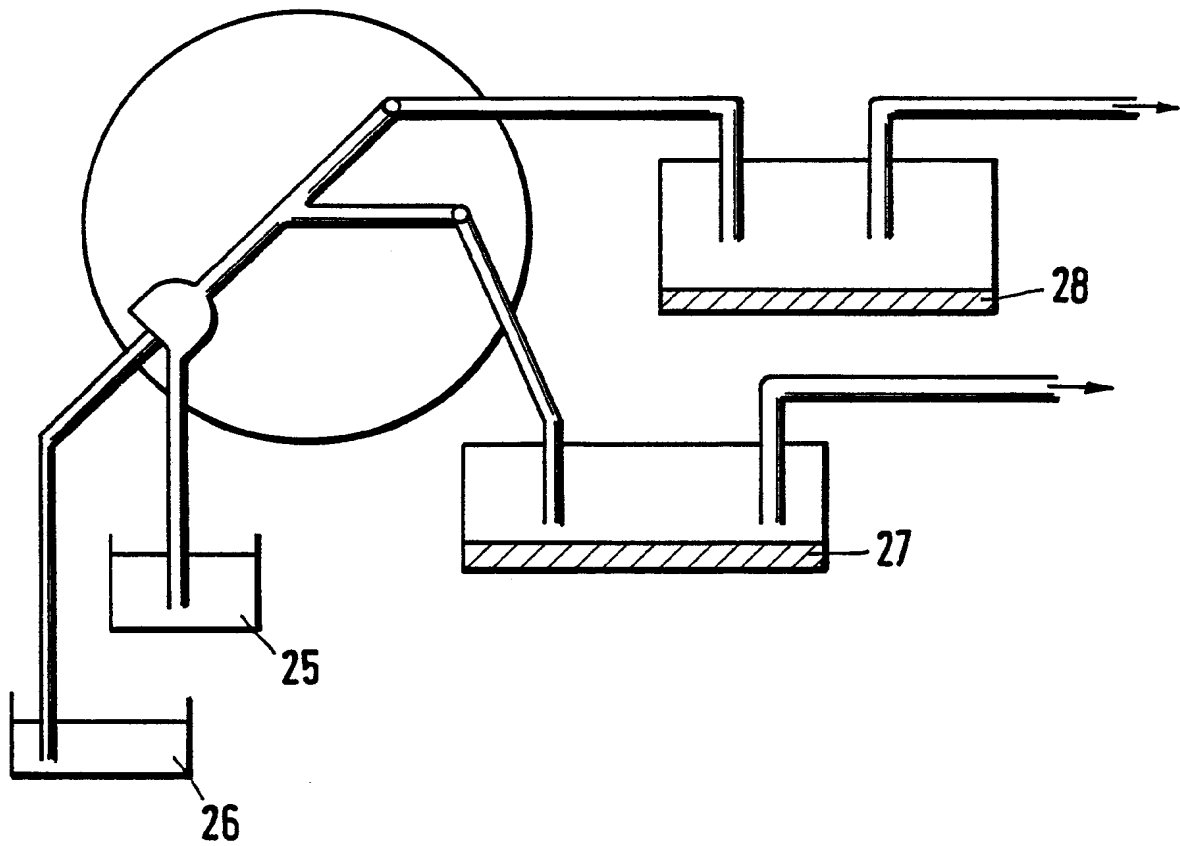
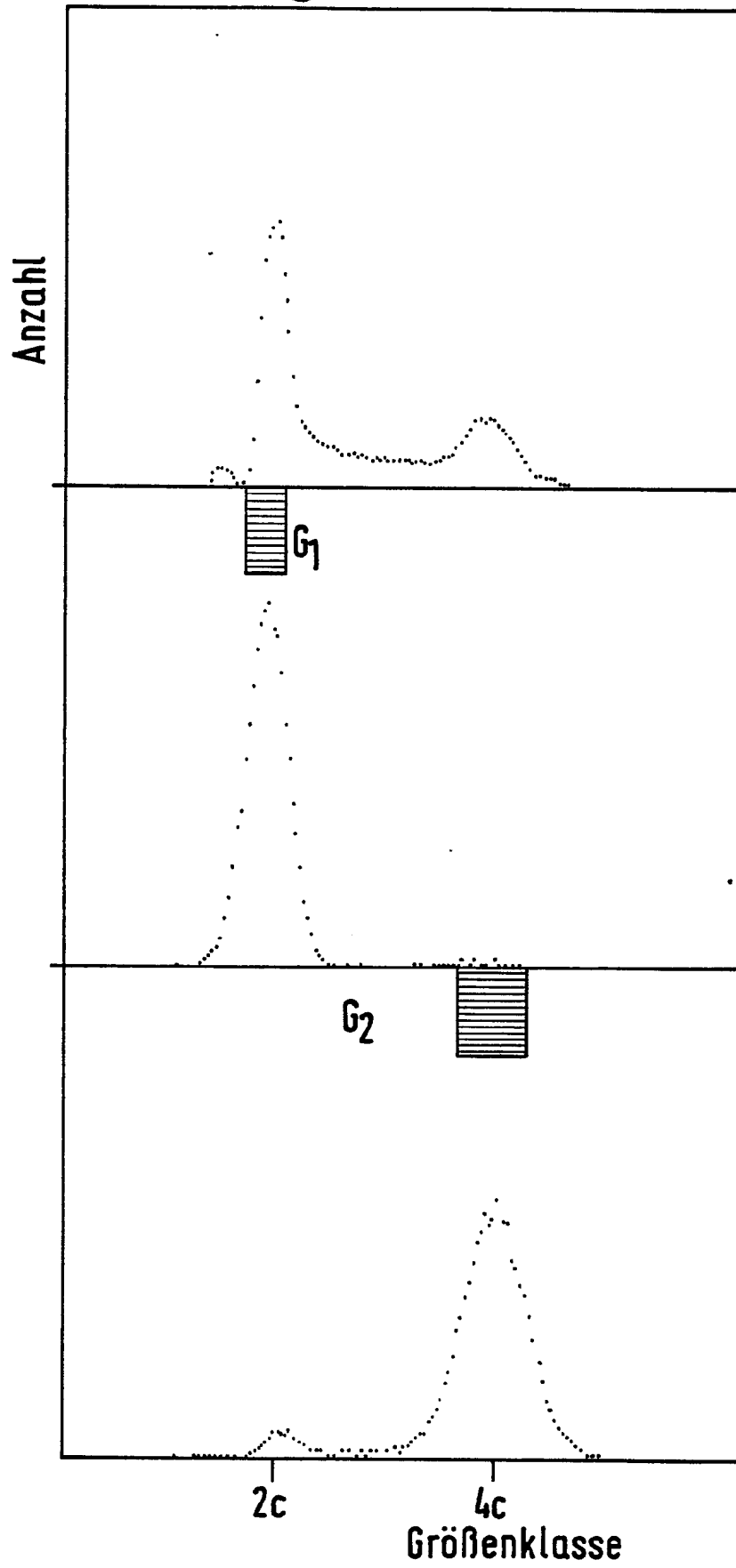


Fig. 11



(19)



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(54) **Sortiervorrichtung für biologische Zellen oder Viren**

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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 werden

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

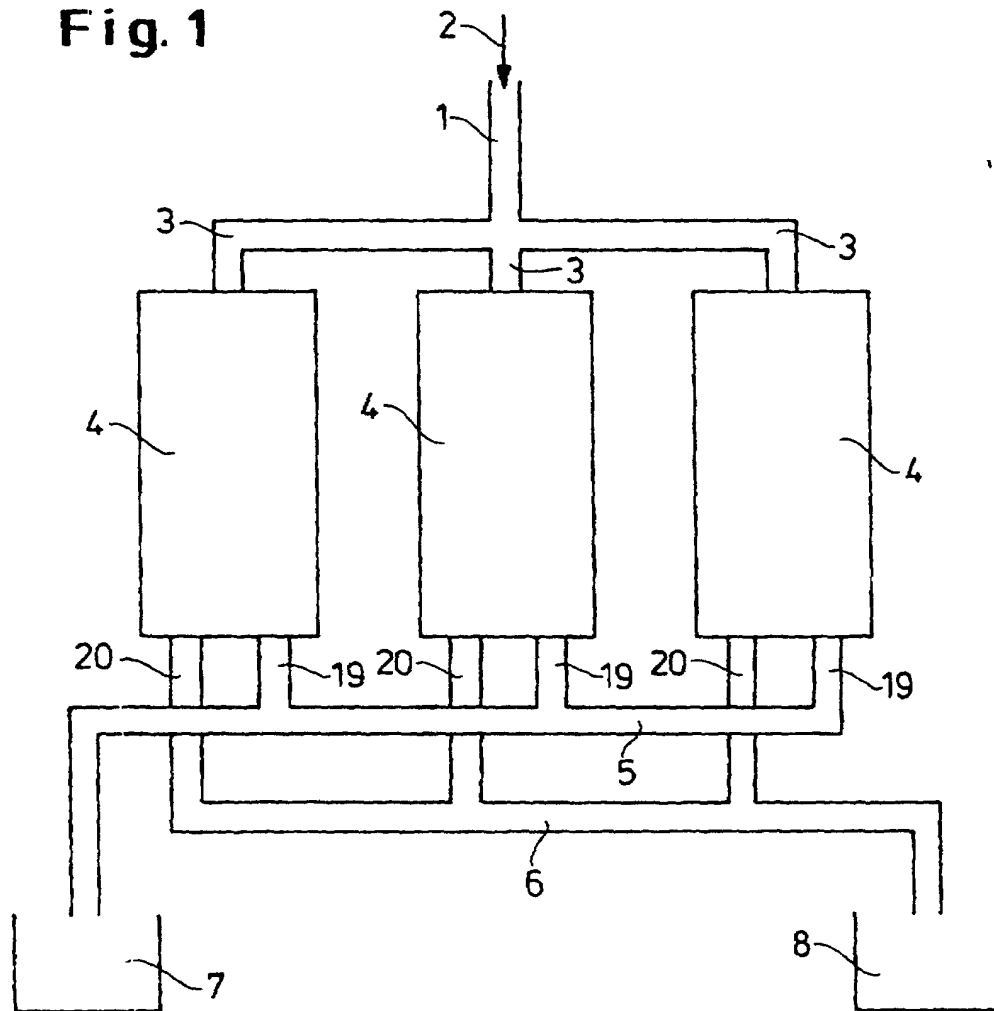


Fig. 2

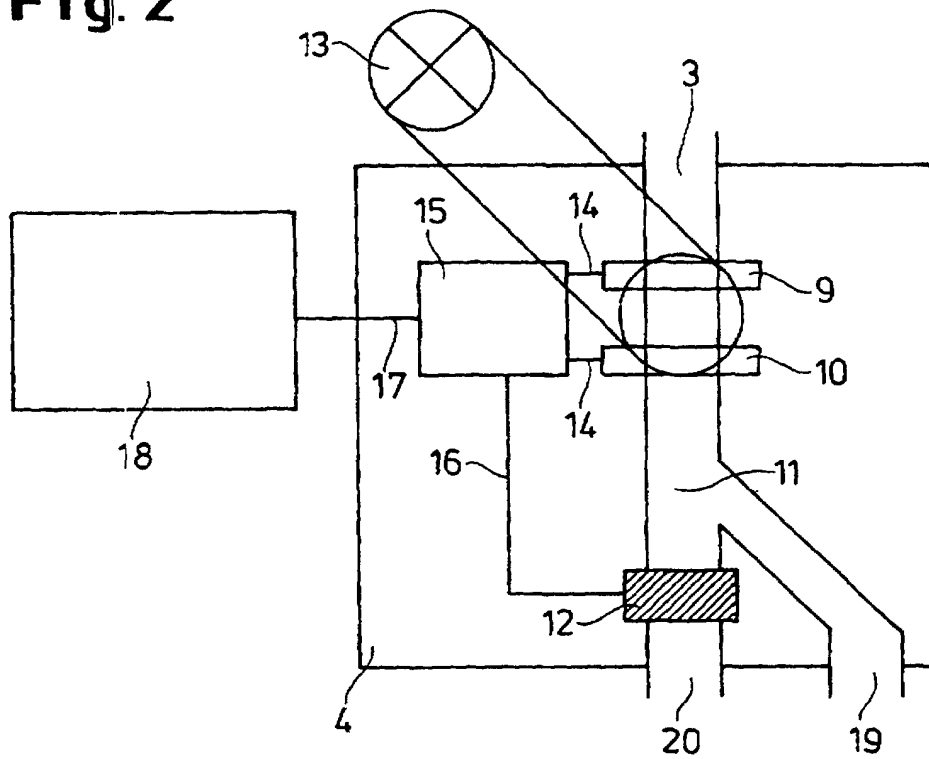


Fig. 3a

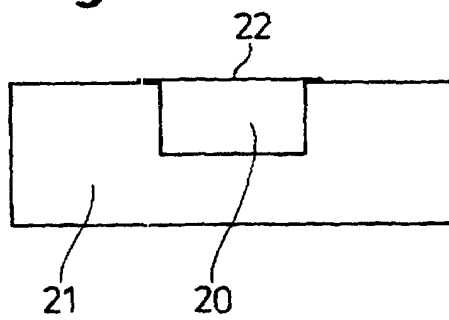


Fig. 3b

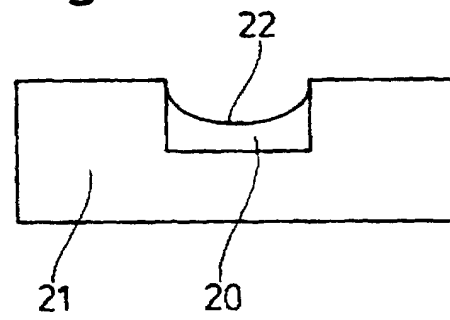


Fig. 4

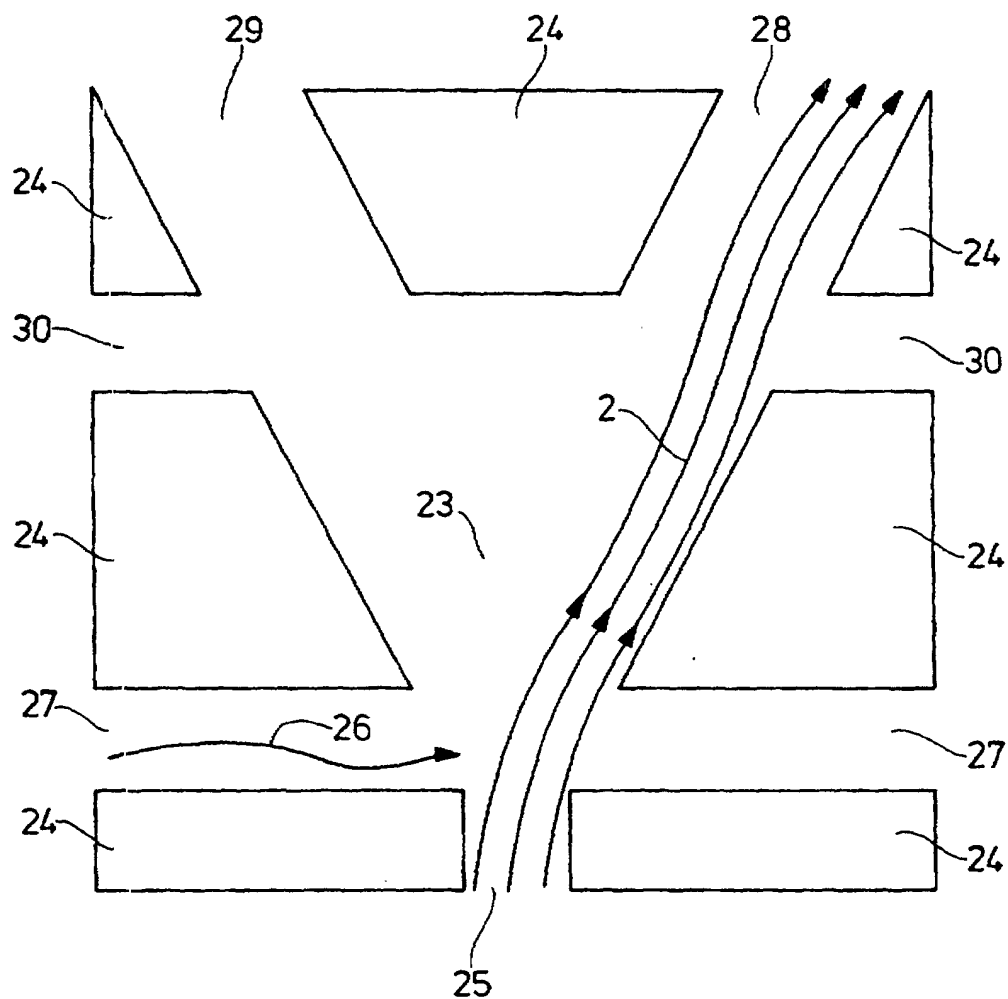


Fig. 5a

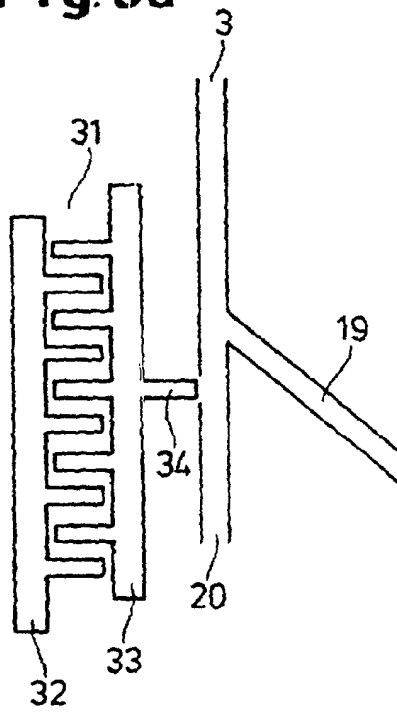


Fig. 5b

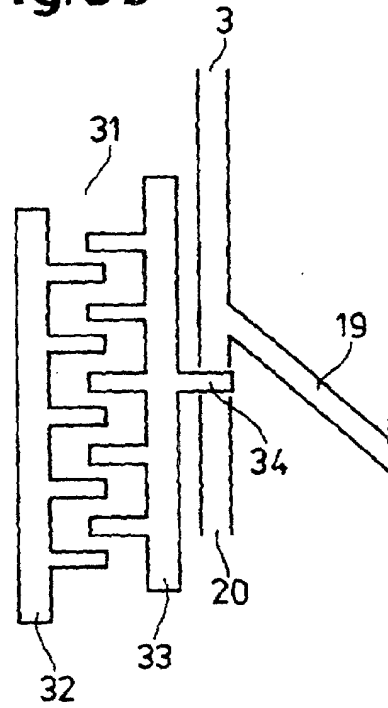


Fig. 6

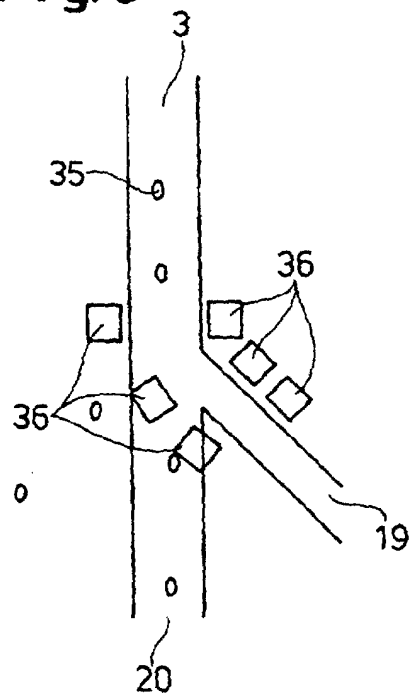
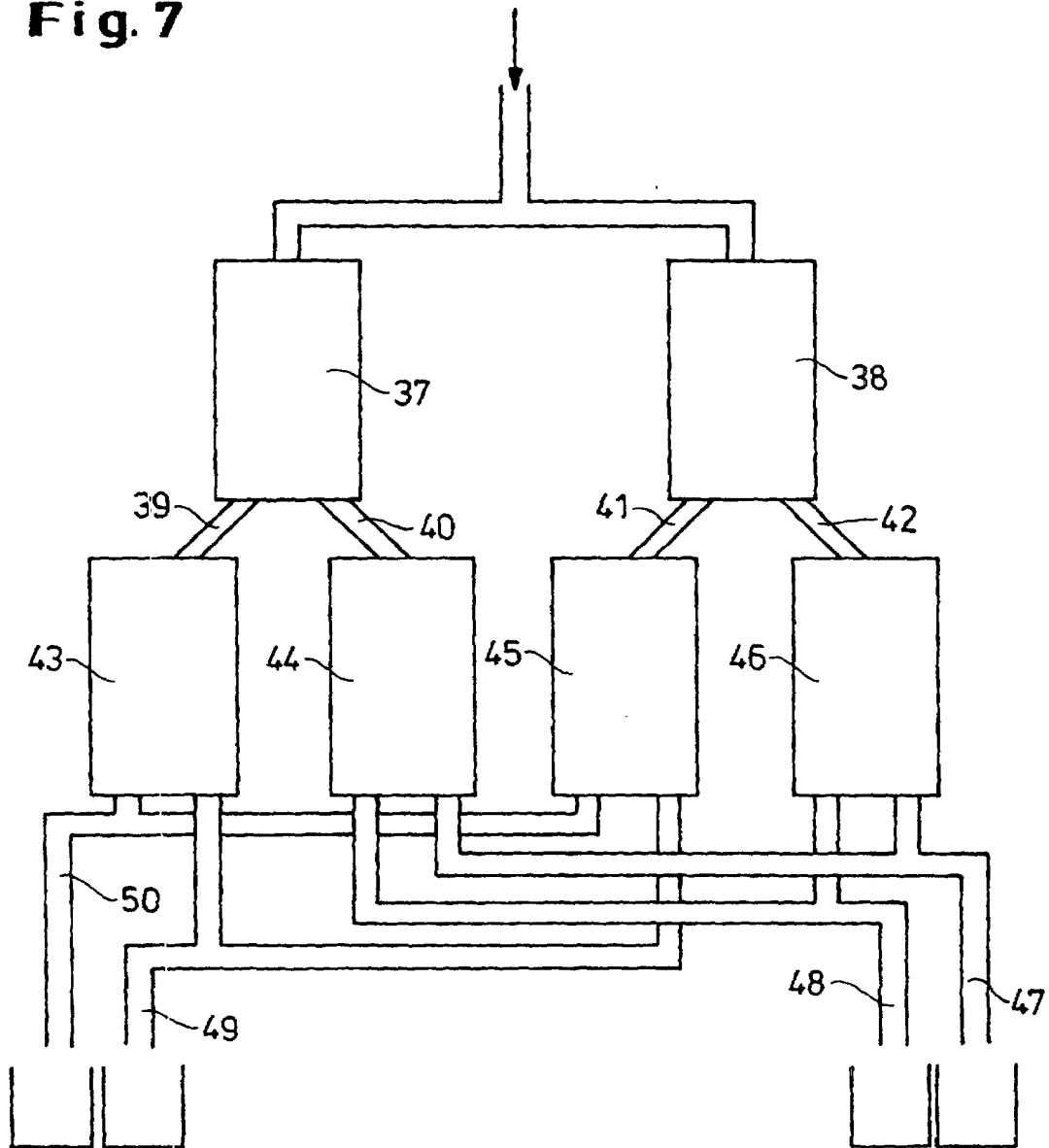
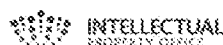


Fig. 7





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

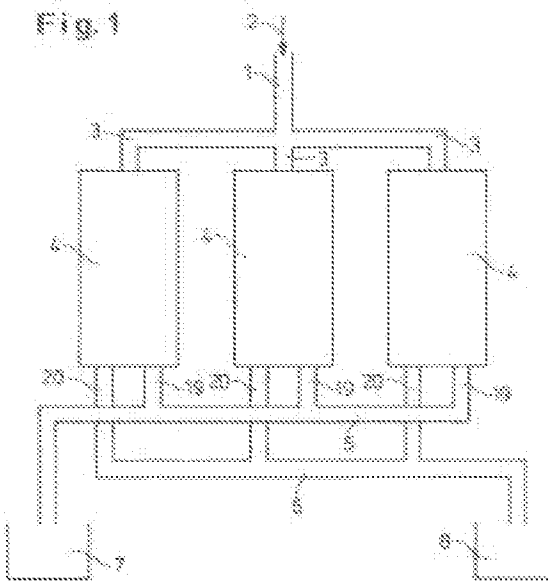
Priority number(s): DE19951020298 19950602

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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



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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.
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"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 11 August 2003 (11.08.2003)	Date of mailing of the international search report 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.

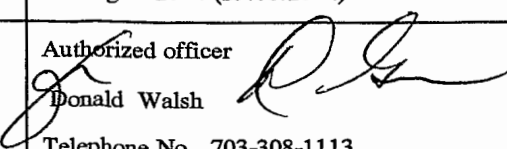
☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the

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

Form PCT/IPEA/409 (cover sheet)(July 1998)

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.

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

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

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.

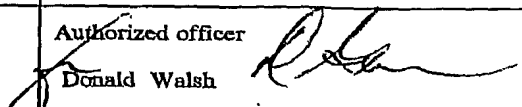
☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the

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:	11965210
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	31-JAN-2012
Filing Date:	26-SEP-2011
Time Stamp:	16:21:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	118153_01204_IDS.pdf	12639 214c1cf9d6c5417cb7bc4c2faa6f193e006150ef	no	2

Warnings:

Information:

0356

2	Information Disclosure Statement (IDS) Form (SB08)	118153_01204_SB_08_Form. pdf	32525	no	3
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Warnings:					
Information:					
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3	Foreign Reference	B1.PDF	282056	no	11
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Warnings:					
Information:					
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Warnings:					
Information:					
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Warnings:					
Information:					
6	Foreign Reference	B4.PDF	3586325	no	91
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Warnings:					
Information:					
7	Foreign Reference	B5.PDF	608435	no	15
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Warnings:					
Information:					
8	Foreign Reference	B6.PDF	160499	no	14
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Warnings:					
Information:					
9	Non Patent Literature	C1.PDF	80912	no	2
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Warnings:					
Information:					
10	Non Patent Literature	C2.PDF	174480	no	3
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Information:					
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12	Non Patent Literature	C4.PDF	186537	no	3
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16	Non Patent Literature	C8.PDF	200668	no	6
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18	Non Patent Literature	C10.PDF	66030	no	3
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Information:					
Total Files Size (in bytes):			10713049		

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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.

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: January 31, 2012
Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: 118153-01204
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
John Richard Gilbert *et al.*

Application No.: 13/245,331

Confirmation No.: 5808

Filed: September 26, 2011

Art Unit: 3753

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

Examiner: Craig M. Schneider

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

INFORMATION DISCLOSURE STATEMENT (IDS)

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.56, 1.97 and 1.98, Applicants hereby respectfully direct the attention of the United States Patent and Trademark Office to the references listed in the attached PTO/SB/08 form. Applicants respectfully request that the information in the references 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. § 1.97, Applicants cite the following commonly owned applications directed to related subject matter:

Application No.	Attorney Docket No.	Filing Date	Status
10/179,586	118153-01201	06-24-2002	Granted
11/021,251	118153-01202	12-21-2004	Granted
11/433,781	118153-01203	05-12-2006	Pending

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. § 1.98(a)(2)(ii), Applicants have not submitted copies of U.S. patents and U.S. patent applications. In accordance with 37 C.F.R. §§ 1.98(a)(2)(ii) and (iv), Applicants submit copies of the cited foreign patent documents and non-patent literature documents.

This Information Disclosure Statement is filed, to the best of Applicants' knowledge, before the mailing of a first action on the merits, under 37 C.F.R. § 1.97(b).

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.

Applicants respectfully submit that this Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and request the Examiner to consider the listed references.

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 Order No. 118153-01204.

Dated: January 31, 2012

Respectfully submitted,

By: /David R. Burns/
David R. Burns
Registration No.: 46,590
MCCARTER & ENGLISH, LLP
265 Franklin Street
Boston, MA 02110
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Attorney/Agent For Applicants



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/245,331	09/26/2011	John Richard Gilbert	118153-01204

CONFIRMATION NO. 5808

86738
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

PUBLICATION NOTICE



OC000000052075160

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

Publication No. US-2012-0015442-A1

Publication Date: 01/19/2012

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



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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
13/245,331	09/26/2011	2853	770	118153-01204	28	2

CONFIRMATION NO. 5808

86738
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

FILING RECEIPT



Date Mailed: 10/13/2011

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, Inverness, 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
which is a CON of 11/021,251 12/21/2004 PAT 7,069,943
which is a CON of 10/179,586 06/24/2002 PAT 6,877,528
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: 10/11/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/245,331**

Projected Publication Date: 01/19/2012

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).

LICENSE FOR FOREIGN FILING UNDER**Title 35, United States Code, Section 184****Title 37, Code of Federal Regulations, 5.11 & 5.15****GRANTED**

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/245,331	09/26/2011	John Richard Gilbert	118153-01204

86738
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

CONFIRMATION NO. 5808
POA ACCEPTANCE LETTER



Date Mailed: 10/13/2011

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 09/26/2011.

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.

/llvuong/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 13/245,331				
APPLICATION AS FILED - PART I										
(Column 1)		(Column 2)		SMALL ENTITY		OR 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))	28	minus 20 = *	8	x 30 =	240					
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2	minus 3 = *		x 125 =	0.00					
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	770		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		OR OTHER THAN SMALL ENTITY		
AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x	=		x	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=		x	=
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE			TOTAL ADD'L FEE	
AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x	=		x	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=		x	=
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE			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.										

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, LLCApplication No./Patent No.: Not yet assigned Filed/Issue Date: September 23, 2011Entitled: 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: 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.

☒ 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

September 26, 2011
Date

David R. Burns
Printed or Typed Name

(617) 449-6500
Telephone Number

Attorney on Record
Title

(Attorney Docket No. 118153-01204)

STATEMENT UNDER 37 CFR 3.73(b) - Supplemental Sheet

Continuation of chain title from the inventor(s) to the current assignee.

4. From: Coventor, Inc. To: Teragenics, 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.
5. From: Teragenics, Inc. To: Cytonome, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 017895 , Frame 0682 , or for which a copy thereof is attached.

(Attorney Docket No. 118153-01204)

**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. 11/433,781 filed May 12, 2006 which, in turn, 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
10 Flow Through a Microchannel” 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. Each of the above applications are incorporated herein by reference in their entirety.

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
25 diagnostic equipment. Of key importance for the improvement of the chemical operations required in these applications are an increased speed, enhanced reproducibility, decreased consumption of expensive samples and reagents, and the reduction of waste materials.

 Microfluidic devices and systems provide improved methods of performing chemical,
30 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 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 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.

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 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.

5 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
10 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
15 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
25 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.

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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.

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.

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.

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.

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.

5 According to another aspect, a microfluidic device is provided, comprising a first 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
10 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.

 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
15 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 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
25 the liquid in the aperture and applying and sealing an actuator comprising a chamber to a top 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.

30 According to still another aspect, a microfluidic device is provided comprising a 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.

5 Figure 7 shows a top view of the bubble valve of Figure 6.

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.

25 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.

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.

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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 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 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 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. 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.

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 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.

5 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.

10 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
15 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 10 of the present invention. The microfluidic system may be formed by a plurality of stacked
20 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
25 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,
30 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.

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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.

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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.

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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
5 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
10 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
15 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.

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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
25 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,
30 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).

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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
5 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
15 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.

Figure 17c shows the particle sorting system 160 after completion of the switching
20 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.

According to yet another embodiment, the cross-sectional dimensions of a
25 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
30 will be able to determine a suitable cross-sectional dimension to achieve a desired pressure within the microchannel interior.

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 microchannel interior to regulate
5 fluid flow. The actuator generates a gas bubble in a liquid in the microchannel when a sufficient pressure is generated on the membrane.

Electronic Patent Application Fee Transmittal

Application Number:				
Filing Date:				
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:	118153-01204			
Filed as Small Entity				
Utility under 35 USC 111(a) Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	95	95
Utility Search Fee	2111	1	310	310
Utility Examination Fee	2311	1	125	125
Pages:				
Claims:				
Claims in excess of 20	2202	8	30	240
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				770

Electronic Acknowledgement Receipt

EFS ID:	11049157
Application Number:	13245331
International Application Number:	
Confirmation Number:	5808
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:	118153-01204
Receipt Date:	26-SEP-2011
Filing Date:	
Time Stamp:	16:13:56
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$770
RAM confirmation Number	2488
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 1.17 (Patent application and reexamination processing fees)

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_01204_Utility_Transmittal.pdf	92587	no	2
			16b3fc8fe6eb1f1ce04f531c5da7c3c9f7da1c5c		
Warnings:					
Information:					
2	Application Data Sheet	118153_01204_ADS.pdf	899292	no	5
			dcfaf9ff769b9ca35f43054a7cf6d5c27e8c25be		
Warnings:					
Information:					
3		118153_01204_Preliminary_Amendment.pdf	39352	yes	5
			a083201882e6f9f97a5f8467f4276c9dccc35bd		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Preliminary Amendment		1	1	
	Claims		2	4	
	Applicant Arguments/Remarks Made in an Amendment		5	5	
Warnings:					
Information:					
4	Drawings-only black and white line drawings	118153_01204_Drawings.pdf	536337	no	8
			2bcdcb36c6a962632a7634d5ec5e42f528f2527d6		
Warnings:					
Information:					
5	Oath or Declaration filed	118153_01204_Copy_of_Declaration.pdf	1025088	no	8
			3dab7ab76431f1ff9d1789fd2e8b583c05913baf		
Warnings:					
Information:					
6	Power of Attorney	118153_01204_Copy_of_POA.pdf	261570	no	1
			1b12ae6c6a1d4fad90d3506d35bf5a0476b2494		
Warnings:					
Information:					
7	Assignee showing of ownership per 37 CFR 3.73(b).	118153_01204_3_73b_Form.pdf	22796	no	2
			0b9ae2fba62229e932f62d200762ebac0083c26b		
					0397

Warnings:					
Information:					
8		118153_01204_Patent_Appln. pdf	131011 <small>1fc18483173743874dc8ab683c9e27fe59e c903</small>	yes	23
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Specification		1	19	
	Claims		20	22	
	Abstract		23	23	
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	37041 <small>28747c72b982238dcfa0116e9bc84489bc0 9eab5</small>	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			3045074		
<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>					

UTILITY
PATENT APPLICATION
TRANSMITTAL

N/A

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

- ## ACCOMPANYING APPLICATION PARTS

9. ☐ **Assignment Papers** (cover sheet & document(s))
- Name of Assignee _____
10. ☒ **37 CFR 3.73(b) Statement** ☒ **Power of Attorney**
(when there is an assignee)
11. ☐ **English Translation Document** (if applicable)
12. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
☐ Copies of citations attached
13. ☒ **Preliminary Amendment**
14. ☐ **Return Receipt Postcard** (MPEP 503)
(Should be specifically itemized)
15. ☐ **Certified Copy of Priority Document(s)**
(if foreign priority is claimed)
16. ☐ **Nonpublication Request** under 35 U.S.C. 122(b)(2)(B)(i).
Applicant must attach form PTO/SB/35 or equivalent.
17. ☒ **Other: Filing Fees**

Art Unit: 3753

☒ The address associated with Customer Number: 86738 **OR** ☐ Correspondence address below

September 26, 2011

(Attorney/Agent)	46,590
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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 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.

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.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	118153-01204
		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

<input type="checkbox"/>	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	
	John	Richard		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	
	Sebastian			Böhm			
Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service							
City	Inverness	State/Province	UK	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	Inverness	State/Province					
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	
	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 0401	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number		118153-01204	
		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		US			
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.					

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	docket@mccarter.com	Add Email	Remove Email

Application Information:

Title of the Invention	MICROFLUIDIC SYSTEM INCLUDING A BUBBLE VALVE FOR REGULATING FLUID FLOW THROUGH A MICROCHANNEL		
Attorney Docket Number	118153-01204	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)			
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	118153-01204
		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	11/433781	2006-05-12
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
11/433781	Continuation of	11/021251	2004-12-21
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
11/021251	Continuation of	10/179586	2002-06-24
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10/179586	non provisional of	60/373256	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 checked="" 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.

Remove	
Assignee 1	
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	118153-01204
		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	US	Postal Code	02210
Phone Number		Fax Number	
Email Address			
Additional Assignee Data may be generated within this form by selecting the Add button.			
			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)	2011-09-26
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.**

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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.
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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: September 26, 2011

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

Docket No.: 118153-01204
(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: September 23, 2011

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

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the claims begin on page 2.

Remarks begin on page 5.

AMENDMENTS TO THE CLAIMS

Please cancel claims 1-19.

Please add new claims 20-47.

1-19. (Canceled)

20. (New) A microfluidic system comprising:

a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel;

a first reservoir operatively associated with the flow channel; and

a second reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse resulting from a change in volume or pressure in the first reservoir.

21. (New) The system of claim 20 further comprising an actuator or pressurizer operatively associated with the first reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the first reservoir.

22. (New) The system of claim 20, wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.

23. (New) The system of claim 22, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.

24. (New) The system of claim 20, wherein the second reservoir includes a gas pocket, wherein compression of the gas facilitates the dampening or absorbing of the pressure pulse.

25. (New) The system of claim 24, further comprising a fluid filled region interfacing with the flow channel and the gas pocket.

26. (New) The system of claim 25, wherein the fluid filled region includes a portion of the second reservoir.

27. (New) The system of claim 25, wherein the fluid filled region includes a side channel connecting the second reservoir to the flow channel.
28. (New) The system of claim 25, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
29. (New) The system of claim 20, wherein the second reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.
30. (New) The system of claim 20, wherein the first reservoir includes a gas pocket, wherein compression of the gas facilitates the generating of the pressure pulse.
31. (New) The system of claim 30 further comprising a fluid filled region interfacing with the flow channel and the gas pocket.
32. (New) The system of claim 31, wherein the fluid filled region includes a portion of the first reservoir.
33. (New) The system of claim 31, wherein the fluid filled region includes a side channel connecting the first reservoir to the flow channel.
34. (New) The system of claim 31, wherein the fluid filled region prevents the gas from the gas pocket from leaking into the flow channel.
35. (New) The system of claim 31, wherein the fluid filled region forms a meniscus with the gas pocket.
36. (New) The system of claim 31, wherein the change in volume or pressure in the first reservoir deflects the meniscus into the flow channel.
37. (New) The system of claim 20, wherein the first reservoir includes a deflectable flexible membrane wherein deflection of the membrane increases the pressure in the first reservoir.
38. (New) The system of claim 20, wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow channel relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.
39. (New) A method comprising:

flowing a sample having one or more particles suspended in a suspension medium through a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of the sample;

generating a pressure pulse by changing a volume or pressure in a first reservoir operatively associated with the flow channel; and

dampening or absorbing the pressure pulse using a second reservoir operatively associated with the flow channel.

40. (New) The method of claim 39, wherein the pressure pulse is used to control or otherwise affect the flow of the sample through the flow channel.
41. (New) The method of claim 40, wherein the pressure pulse is used to sort one or more particles into a selected branch of a branched portion of the flow channel.
42. (New) The method of claim 39, wherein the dampening or absorbing of the pressure pulse is facilitated by compression of gas in a gas pocket in the second reservoir.
43. (New) The method of claim 39, wherein the dampening or absorbing of the pressure pulse is facilitated by a flexible wall of the second membrane.
44. (New) The method of claim 39, wherein the generating the pressure pulse is facilitated by compression of gas in a gas pocket in the first reservoir.
45. (New) The method of claim 39, wherein the fluid filled region forms a meniscus with the gas pocket.
46. (New) The method of claim 39:

wherein a fluid filled region interfaces with the flow channel and the gas pocket;

wherein the fluid filled region forms a meniscus with the gas pocket; and

wherein the change in volume or pressure in the first reservoir deflects the into the flow channel.
47. (New) The method of claim 39, wherein the generating the pressure pulse includes deflecting a deflectable flexible membrane to increase the pressure in the first reservoir.

REMARKS

By way of the present preliminary amendment, originally pending claims 1-19 are cancelled, without prejudice, and new claims 20-47 are added. *Support for new claims 20-47 can be found throughout the specification as originally filed.*

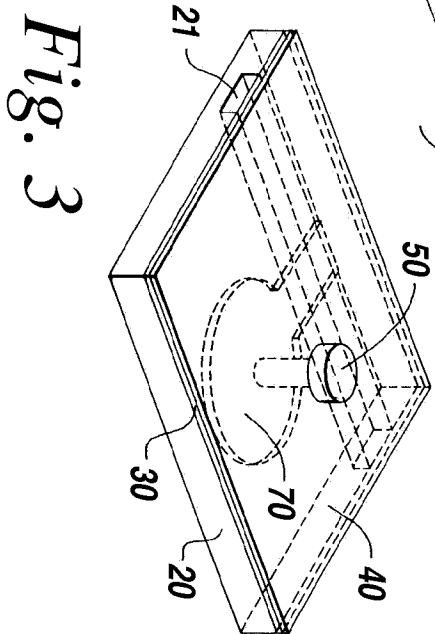
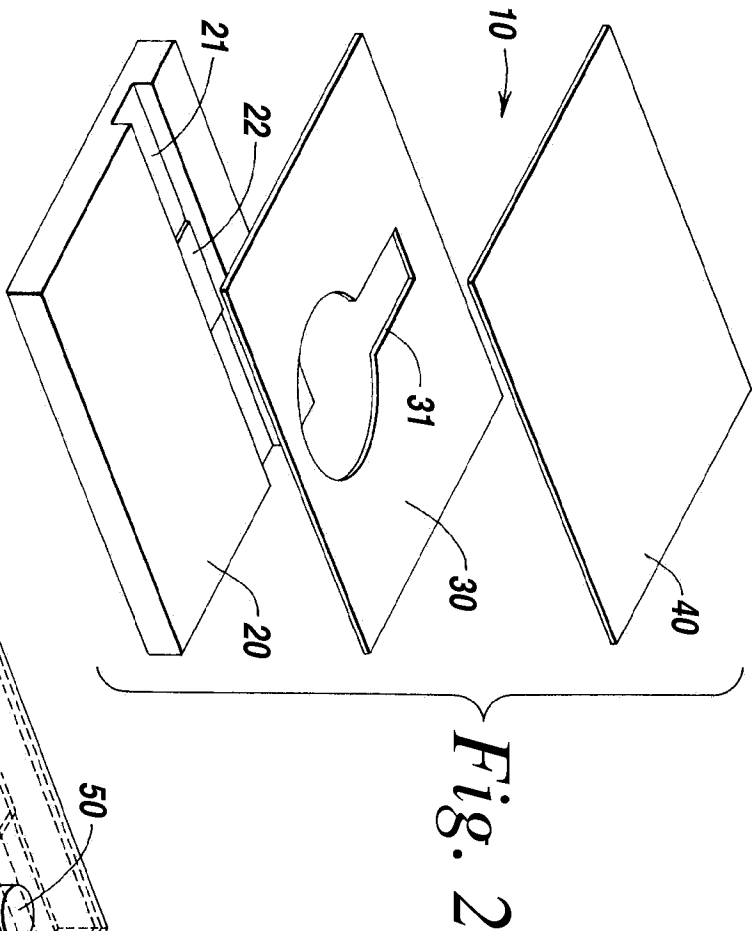
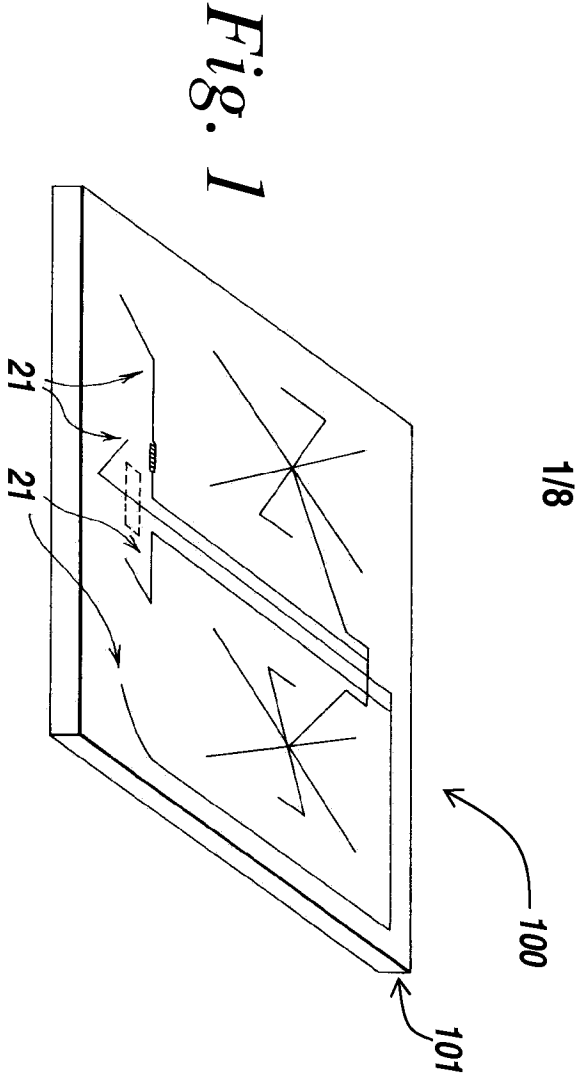
Applicants respectfully request prompt entry and substantive consideration of the claim amendments presented herein prior to examination on the merits. After the claim amendments presented herein are entered, claims 20-47 will be pending.

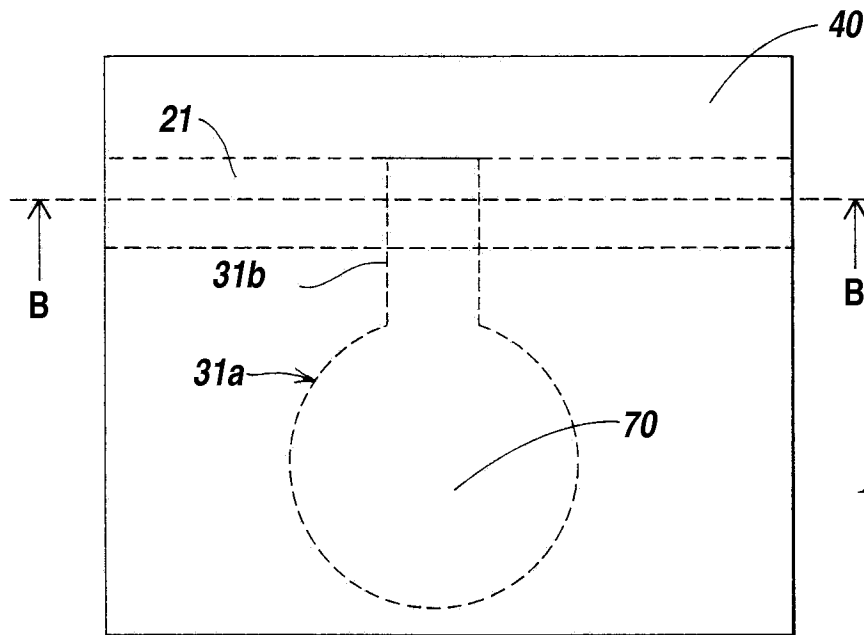
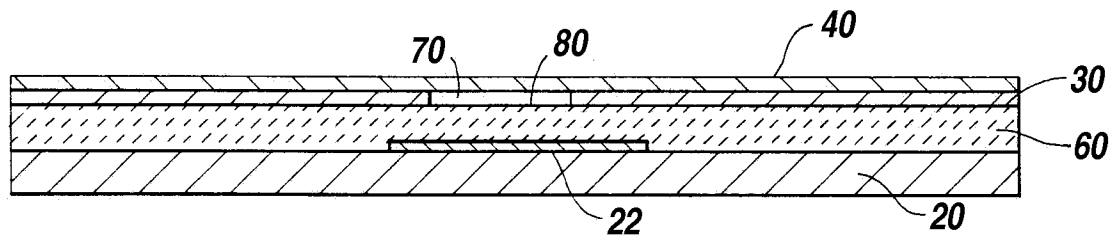
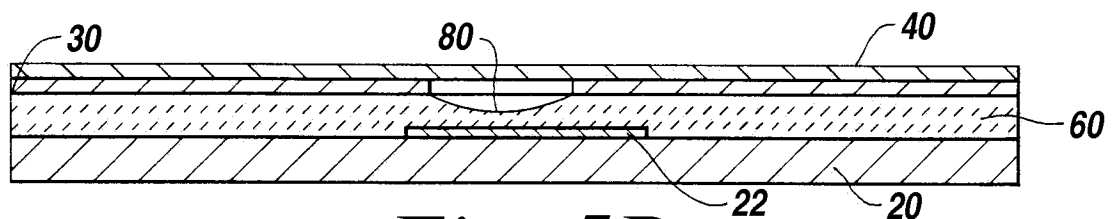
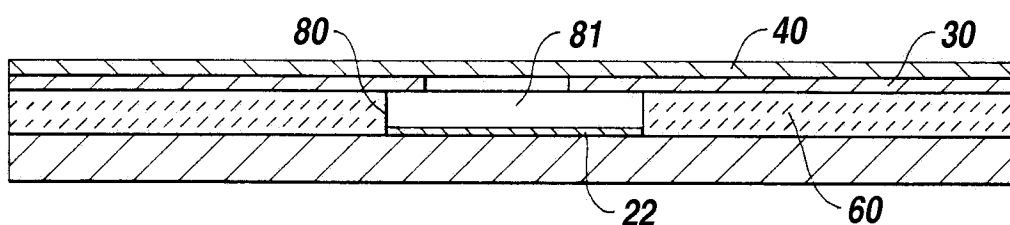
The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-4876, under Order No. 118153-01204.

Dated: September 26, 2011

Respectfully submitted,

Electronic signature for : /David R. Burns/
David R. Burns
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MCCARTER & ENGLISH, LLP
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Boston, Massachusetts 02110
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(617) 607-9200 (Fax)
Attorney for Applicants



*Fig. 4**Fig. 5A**Fig. 5B**Fig. 5C*

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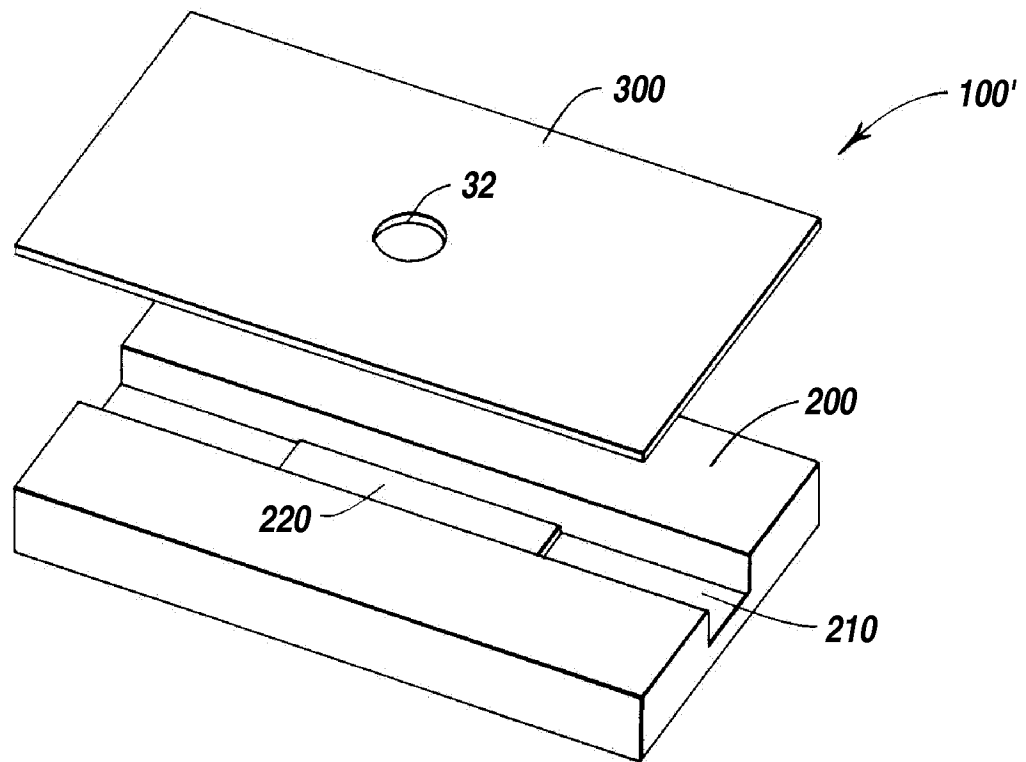


Fig. 6

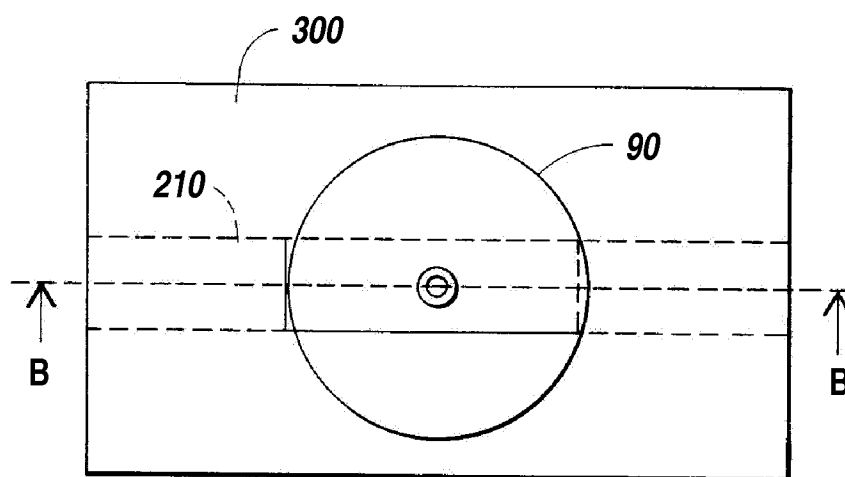


Fig. 7

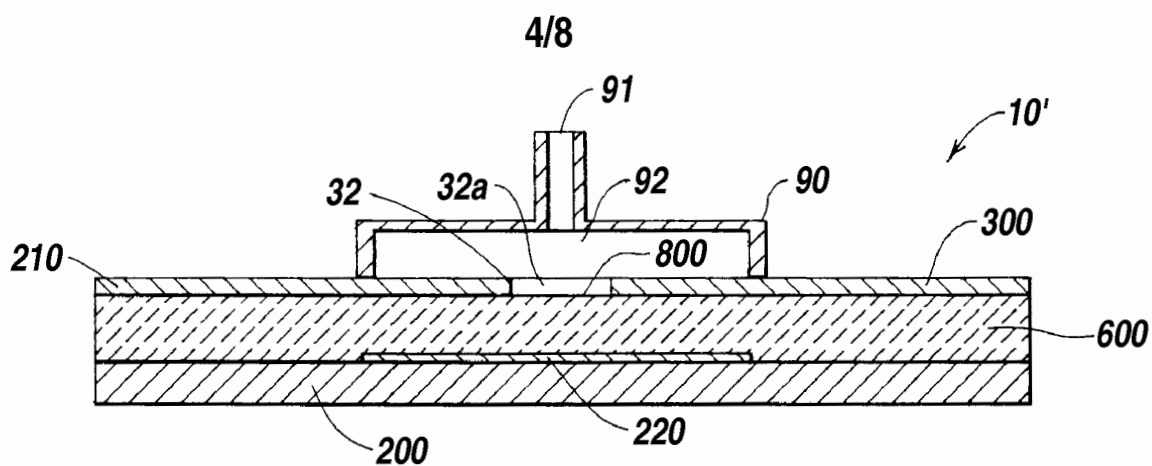


Fig. 8A

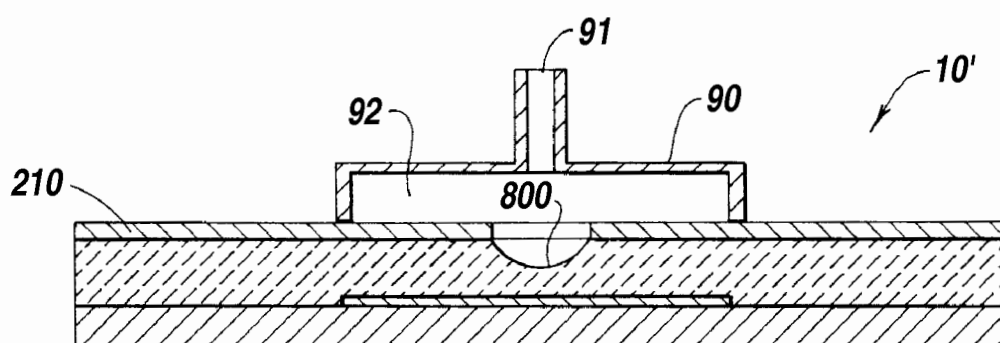


Fig. 8B

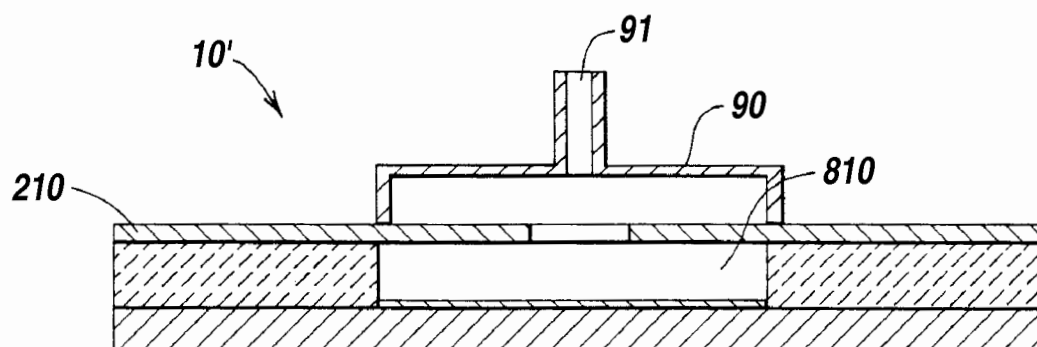


Fig. 8C

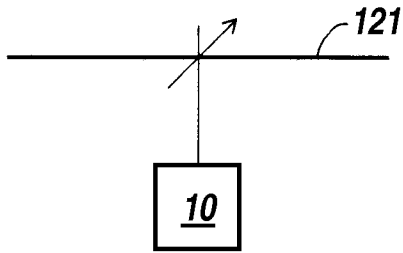


Fig. 9

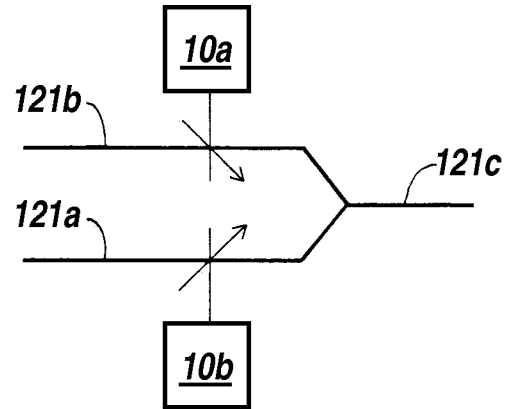


Fig. 10

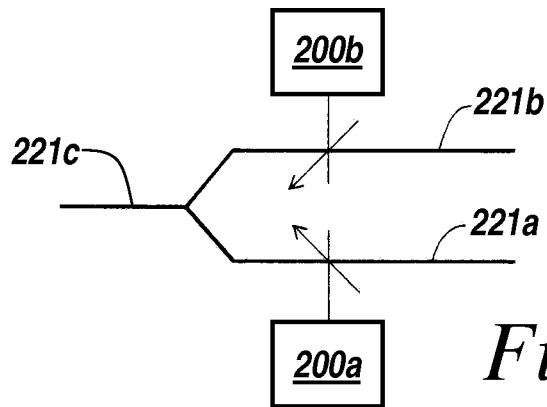


Fig. 11

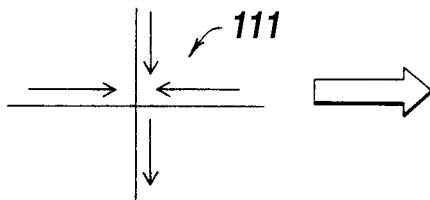


Fig. 12B

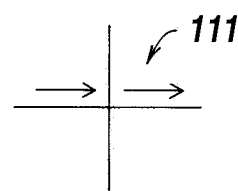


Fig. 12C

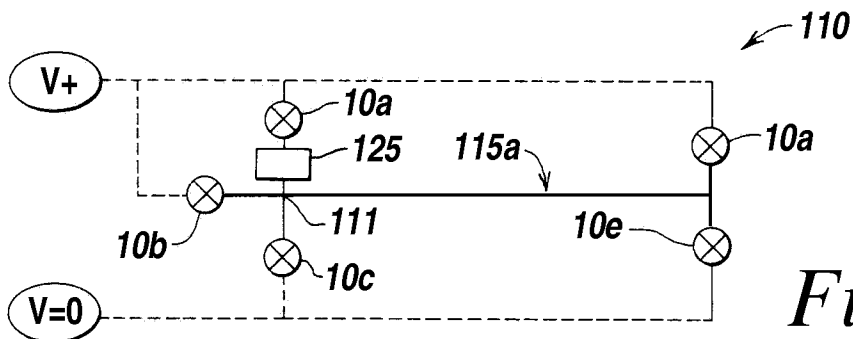


Fig. 12A

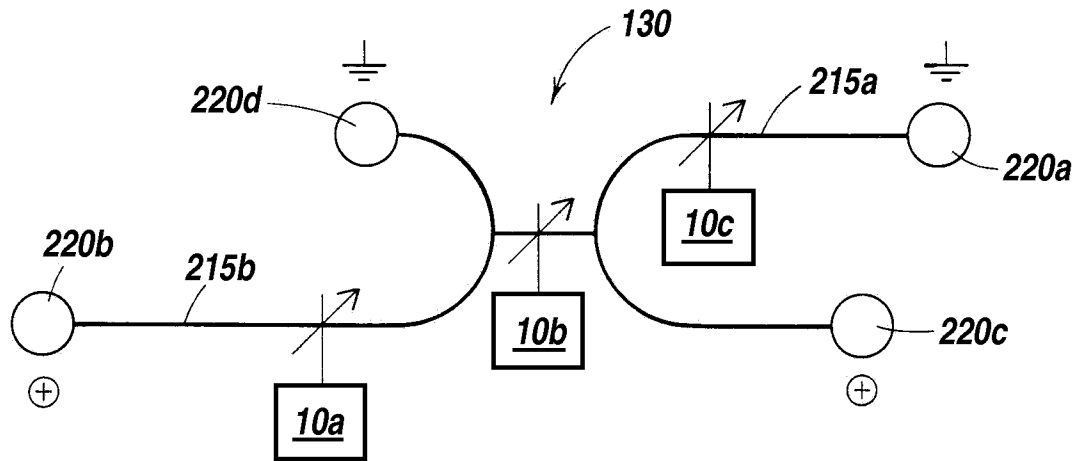


Fig. 13A

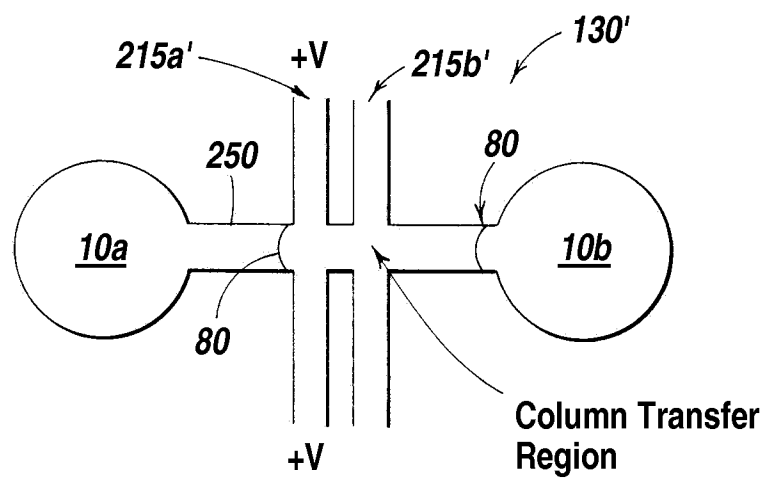
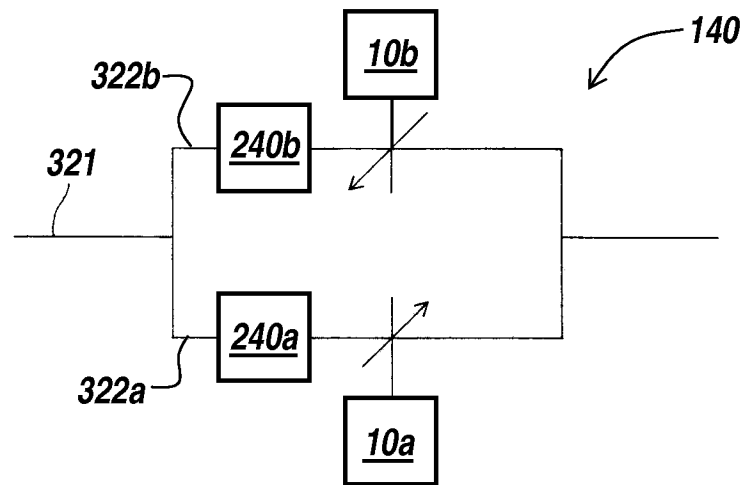
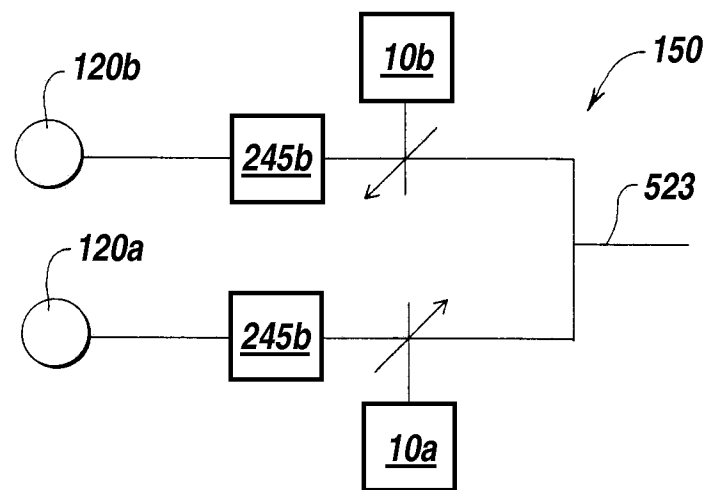
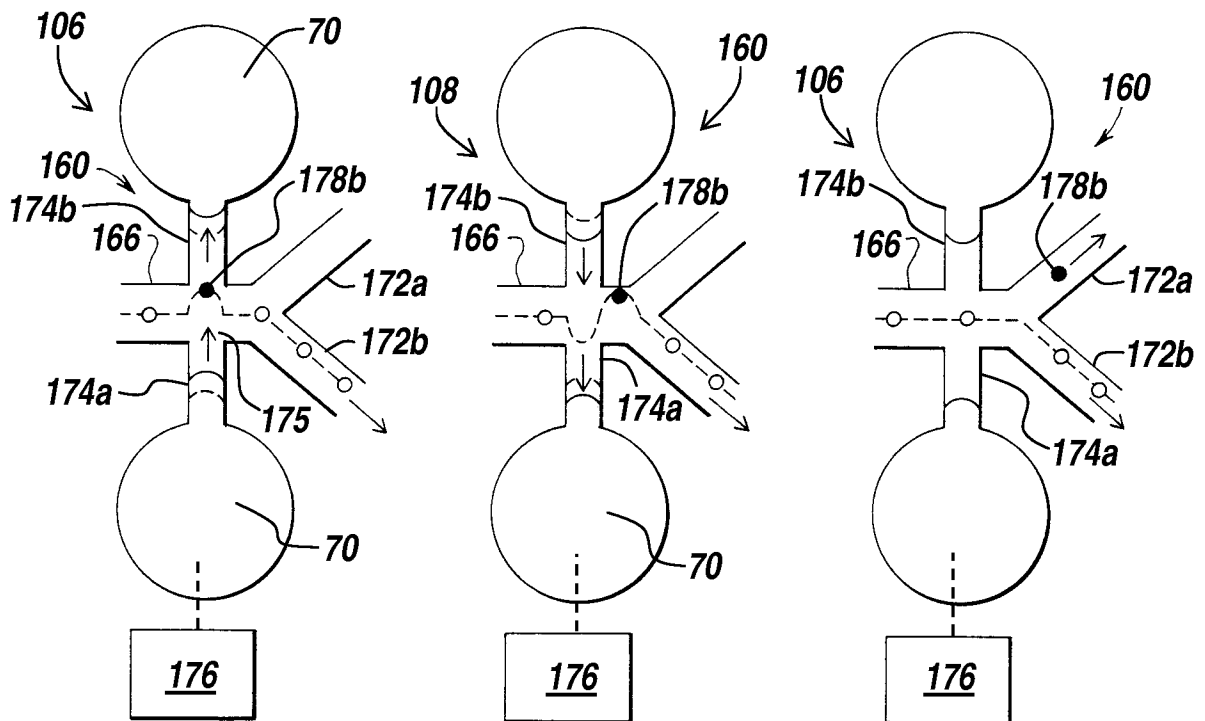


Fig. 13B

*Fig. 14**Fig. 15*



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 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
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

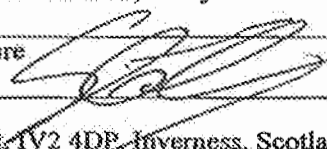
Direct Telephone Calls to:

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.

Full name of sole or first inventor John Richard Gilbert	
Inventor's signature	Date
Residence 69 Naples Road, Brookline, MA 02446	
Citizenship United States	
Post Office Address (if different)	

Full name of second inventor, if any Sebastian Böhm	
Inventor's signature 	Date Oct. 31, 2002
Residence 9 Muirfield Court, IV2 4DP, Inverness, Scotland (UK)	
Citizenship Germany	
Post Office Address (if different)	

Full name of third inventor, if any Manish Deshpande	
Inventor's signature	Date
Residence 34 Apple Blossom Way, Canton, MA 02021	
Citizenship United States	
Post Office Address (if different)	

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
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the specification of which:

_____ is attached hereto.

X was filed on June 24, 2002 as

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_____ and was amended on _____
(if applicable)

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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:

X 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
			_ Yes _ No _
			_ Yes _ No _
			_ Yes _ No _
			_ Yes _ No _
			_ Yes _ No _

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION**

[illegible]

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.

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Ann Lampion 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
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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:

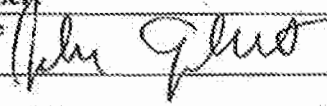
LAHIVE & COCKFIELD, LLP, 28 State Street, Boston, MA 02109

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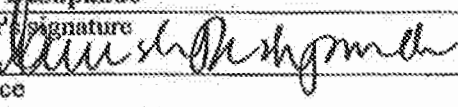
Anthony A. Laurentano, Esq, (617) 227-7400

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.

Full name of sole or first inventor John Richard Gilbert	
Inventor's signature 	Date 10/28/02
Residence 69 Naples Road, Brookline, MA 02446	
Citizenship United States	
Post Office Address (if different)	

Full name of second inventor, if any Sebastian Böhm	
Inventor's signature	Date
Residence 9 Muirfield Court, IV2 4DP, Inverness, Scotland (UK)	
Citizenship Germany	
Post Office Address (if different)	

Full name of third inventor, if any Manish Deshpande	
Inventor's signature 	Date 10/28/02
Residence 34 Apple Blossom Way, Canton, MA 02021	
Citizenship United States	
Post Office Address (if different)	