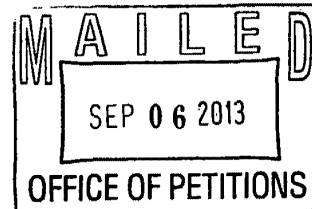




UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

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



In re Patent No. 7,584,857
Issued: September 8, 2009
Application No. 11/499,953
Filed: August 7, 2006
Attorney Docket No. : 118153-02106

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:
:
:

NOTICE

This is a notice regarding your renewed request for acceptance of a fee deficiency submission under 37 CFR 1.28, filed July 15, 2013.

The Office no longer investigates or rejects original or reissue patent 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**.

Status as a small entity has been removed. Accordingly, any future fees paid in this patent must be paid at the undiscounted rate.

Inquiries related to this communication should be directed to the undersigned at (571) 272-3204.

/SDB/

Sherry D. Brinkley
Paralegal Specialist
Office of Petitions

Docket No.: 118153-02106
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Bohm, et al.

Patent No.: 7,584,857

Issued: September 8, 2009

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

**AMENDED NOTIFICATION OF LOSS OF ENTITLEMENT TO SMALL ENTITY
STATUS AND CORRECTION OF ERROR IN SMALL ENTITY STATUS**

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

Dear Madam:

In response to communication dated June 26, 2013 (the "Letter"), Owner hereby submit an Amended Notification of Loss of Entitlement to Small Entity Status and Correction of Error in Small Entity Status. Owner respectfully submit the present submission addresses all the issues raised in the Letter. Accordingly prompt entry hereof is respectfully requested.

In accordance with 37 C.F.R. §§ 1.27(g)(2) and 1.28(c), Owner of the above-identified patent 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. Owner 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.

09/06/2013 CKHLOK 00000019 504876 7504057
01 FC:1599 1025.00 DA

1

07/17/2013 DALLEN 00000023 504876 7504057
01 FC:1599 1025.00 DA

Adjustment date: 09/06/2013 CKHLOK
07/17/2013 DALLEN 00000023 504876 7504057
01 FC:1599 1025.00 CR

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Bohm, et al.

Patent No.: 7,584,857

Issued: September 8, 2009

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Patent No.: 7,584,857

Docket No.: 118153-02106

Please charge our Deposit Account No. 50-4876, under Order No. 118153-02106 from which the undersigned is authorized to draw, in the amount of \$1,025 for the total deficiency owed.

Fee Type	Current Undiscounted Fee	Small Entity Fee Paid	Deficiency Owed
Extension of time Fee, Response within third month	\$1,400	\$555	\$845 (Paid on 5/16/2013)
Trial and Appeal Fee, Notice of Appeal	\$800	\$270	\$530 (Paid on 5/16/2013)
Post-Allowance Fee, Utility issue	\$1,780	\$755	\$1,025 (Paid on 5/16/2013)
Maintenance Fee due at 3.5 years	\$1,600	\$575	\$1,025

Dated: July 15, 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:	16318272
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	METHOD AND APPARATUS FOR SORTING PARTICLES
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	86738
Filer:	David Burns/Pegah Karimi
Filer Authorized By:	David Burns
Attorney Docket Number:	118153-02106
Receipt Date:	15-JUL-2013
Filing Date:	07-AUG-2006
Time Stamp:	16:23:38
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no				
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Notification of loss of entitlement to small entity status	_Amended_Notification_of_Loss_of_Small_Entity_Status_1.pdf	45348 bbee9a3f5af859161d99785c75adef28b718e7d23	no	2
Warnings:					
Information:					

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.

Docket No.: 118153-02106
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Bohm, et al.

Patent No.: 7,584,857

Issued: September 8, 2009

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

**AMENDED NOTIFICATION OF LOSS OF ENTITLEMENT TO SMALL ENTITY
STATUS AND CORRECTION OF ERROR IN SMALL ENTITY STATUS**

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

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In accordance with 37 C.F.R. §§ 1.27(g)(2) and 1.28(c), Owner of the above-identified patent 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. Owner 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.

07/17/2013 DALLEN 00000023 504876 7584857
01 FC:1599 1025.00 DA

Patent No.: 7,584,857

Docket No.: 118153-02106

Please charge our Deposit Account No. 50-4876, under Order No. 118153-02106 from which the undersigned is authorized to draw, in the amount of \$1,025 for the total deficiency owed.

Fee Type	Current Undiscounted Fee	Small Entity Fee Paid	Deficiency Owed
Extension of time Fee, Response within third month	\$1,400	\$555	\$845 (Paid on 5/16/2013)
Trial and Appeal Fee, Notice of Appeal	\$800	\$270	\$530 (Paid on 5/16/2013)
Post-Allowance Fee, Utility issue	\$1,780	\$755	\$1,025 (Paid on 5/16/2013)
Maintenance Fee due at 3.5 years	\$1,600	\$575	\$1,025

Dated: July 15, 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



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
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JUN 26 2013

OFFICE OF PETITIONS

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

In re Patent No. 7,584,857
Issued: September 8, 2009
Application No. 11/499,953

Filed: August 8, 2006

Attorney Docket No.: 118153-02106

NOTICE

The above-identified application was referred to the Office of Petitions in response to a communication filed May 16, 2013, requesting acceptance of a fee deficiency submission under 37 CFR 1.28.

The request is DISMISSED.

While applicant requests acceptance of a fee deficiency, the communication does not comply with the requirements of 37 CFR 1.28. Specifically, 37 CFR 1.28(c)(2)(ii), which states:

(ii) *Itemization of the deficiency payment.* An itemization of the total deficiency payment is required.

The itemization must include the following information:

- (A) Each particular type of fee that was erroneously paid as a small entity, (e.g., basic statutory filing fee, two-month extension of time fee) along with the current fee amount for a non-small entity;
- (B) The small entity fee actually paid, and when. This will permit the Office to differentiate, for example, between two one-month extension of time fees erroneously paid as a small entity but on different dates;
- (C) The deficiency owed amount (for each fee erroneously paid); and
- (D) The total deficiency payment owed, which is the sum or total of the individual deficiency owed amounts set forth in paragraph (c)(2)(ii)(C) of this section.

In this case, there is no detailed breakdown of **all** the deficient fees. The communication merely list a deficiency payment owed for an extension of time, notice of appeal and a

utility issue fee; however, it is noted that the 3 ½ year maintenance fee was also paid as a small entity. Consequently, **a fee deficiency cannot be accepted at this time**. This communication is being treated under 1.27(g)(3) as a notification of a loss of entitlement to small entity status.

Applicant is given **ONE MONTH** to submit a complete listing of all the fees erroneously paid as a small entity and the balance of the fee deficiency to avoid the return of the present fee deficiency paper. This time period is not extendable under 37 CFR 1.136(a).

Further correspondence with respect to this matter should be delivered through one of the following mediums:

By mail: Mail Stop PETITIONS
 Commissioner for Patents
 Post Office Box 1450
 Alexandria, VA 22313-1450

By hand: Customer Service Window
 Mail Stop Petitions
 Randolph Building
 401 Dulany Street
 Alexandria, VA 22314

By fax: (571) 273-8300
 ATTN: Office of Petitions

By Internet: EFS-Web¹

Any questions concerning this matter may be directed to the undersigned at (571) 272-3204.

/SDB/

Sherry D. Brinkley
Petitions Examiner
Office of Petitions

¹ www.uspto.gov/ebc/efs_help.html (for help using EFS-Web call the Patent Electronic Business Center at (866) 217-9197)

Docket No.: 118153-02106
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Bohm, et al.

Patent No.: 7,584,857

Issued: September 8, 2009

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

**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), Owner of the above-identified patent 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. Owner 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.

Patent No.: 7,584,857

Docket No.: 118153-02106

Please charge our Deposit Account No. 50-4876, under Order No. 118153-02106 from which the undersigned is authorized to draw, in the amount of \$2,400 for the total deficiency owed.

Fee Type	Current Undiscounted Fee	Small Entity Fee Paid	Deficiency Owed
Extension of time Fee, Response within third month	\$1,400	\$555	\$845
Trial and Appeal Fee, Notice of Appeal	\$800	\$270	\$530
Post-Allowance Fee, Utility issue	\$1,780	\$755	\$1,025

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:	15793300
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	METHOD AND APPARATUS FOR SORTING PARTICLES
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	86738
Filer:	David Burns/Pegah Karimi
Filer Authorized By:	David Burns
Attorney Docket Number:	118153-02106
Receipt Date:	16-MAY-2013
Filing Date:	07-AUG-2006
Time Stamp:	12:33:39
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no				
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Notification_of_Loss_of_Small_Entity_Status_1.pdf	43370 f53510a60953f5fc1bf6f1c430e2230c1f629599	no	2
Warnings:					
Information:					

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New Applications Under 35 U.S.C. 111

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

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

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New International Application Filed with the USPTO as a Receiving Office

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Docket No.: 118153-02106
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Bohm, et al.

Patent No.: 7,584,857 11499953

Issued: September 8, 2009

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06/06/2013 DALLEN 00000007 504076 11499953
01 FC:1461 2400.00 DA

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Docket No.: 118153-02106

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Dated: May 16, 2013

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David R. Burns

Registration No.: 46,590

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Boston, Massachusetts 02110

(617) 449-6500

(617) 607-9200 (Fax)

Attorney/Agent For Applicant



UNITED STATES PATENT AND TRADEMARK OFFICE

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

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

Mail Date: 04/21/2010

Applicant	: Sebastian Bohm	: DECISION ON REQUEST FOR
Patent Number	: 7584857	: RECALCULATION of PATENT
Issue Date	: 09/08/2009	: TERM ADJUSTMENT IN VIEW
Application No	: 11/499,953	: OF WYETH AND NOTICE OF INTENT TO
Filed	: 08/07/2006	: ISSUE CERTIFICATE OF CORRECTION
		:

The Request for Recalculation is **GRANTED** to the extent indicated.

The patent term adjustment has been determined to be **0** days. The USPTO will *sua sponte* issue a certificate of correction reflecting the amount of PTA days determined by the recalculation.

Prior to the issuance of the certificate of correction, the USPTO will afford patentee an opportunity to be heard and request reconsideration. Accordingly, patentee has **one month or thirty (30) days**, whichever is longer, to file a request for reconsideration of this patent term adjustment calculation. See 35 U.S.C. 154(b)(3)(B)(ii) and 37 CFR 1.322(a)(4). No extensions of time will be granted under 37 CFR 1.136.

Patentee should use document code PET.OP if electronically filing a request for reconsideration of this patent term adjustment calculation. The patentee must also include the information required by 37 CFR 1.705(b)(2) and the fee required by 37 CFR 1.18(e). If patentee does not file a timely request for reconsideration of this patent term adjustment calculation including the information required by 37 CFR 1.705(b)(2) and the fee required by 37 CFR 1.18(e), the USPTO will issue a certificate of correction reflecting the PTA determination noted above.

Patentee should be aware that in order to preserve the right to review in the United States District Court for the District of Columbia of the USPTO patent term adjustment determination, patentee must ensure that he or she also take the steps required under 35 U.S.C. 154(b)(4)(A) in a timely manner. Nothing in the request for recalculation should be construed as providing an alternative time frame for commencing a civil action under 35 U.S.C. 154(b)(4)(A).

Any questions concerning this decision should be directed to the Office of Patent Legal Administration at 571-272-7702.

REQUEST FOR RECALCULATION OF PATENT TERM ADJUSTMENT IN VIEW OF *WYETH**

Attorney Docket
Number: 118153-02106

Patent Number: 7,584,857

Filing Date
(or 371(b) or (f) Date): August 7, 2006

Issue Date: September 8, 2009

First Named
Inventor: John R. Gilbert et al

Title: METHOD AND APPARATUS FOR SORTING PARTICLES

PATENTEE HEREBY REQUESTS RECALCULATION OF THE PATENT TERM ADJUSTMENT (PTA) UNDER 35 USC 154(b) INDICATED ON THE ABOVE-IDENTIFIED PATENT. THE PATENTEE'S SOLE BASIS FOR REQUESTING THE RECALCULATION IS THE USPTO'S PRE-*WYETH* INTERPRETATION OF 35 U.S.C. 154(b)(2)(A).

Note: This form is only for requesting a recalculation of PTA for patents issued before March 2, 2010, if the sole basis for requesting the recalculation is the USPTO's pre-*Wyeth* interpretation of 35 U.S.C. 154(b)(2)(A). See Instruction Sheet on page 2 for more information.

Patentees are reminded that to preserve the right to review in the United States District Court for the District of Columbia of the USPTO's patent term adjustment determination, a patentee must ensure that he or she also takes the steps required under 35 U.S.C. 154(b)(3) and (b)(4) and 37 CFR 1.705 in a timely manner.

**Wyeth v. Kappos*, No. 2009-1120 (Fed. Cir., Jan. 7, 2010).

Signature /David R. Burns/

Date February 12, 2010

Name
(Print/Typed) David R. Burns

Registration Number 46,590

Note: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.



*Total of _____ forms are submitted.

The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 hours 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.

**Instruction Sheet for:
REQUEST FOR RECALCULATION OF PATENT TERM ADJUSTMENT
IN VIEW OF *WYETH****
(Not to be Submitted to the USPTO)

This form is only for requesting a recalculation of PTA for patents issued before March 2, 2010, if the sole basis for requesting the recalculation is the USPTO's pre-*Wyeth* interpretation of 35 U.S.C. 154(b)(2)(A).

This form must be filed within 180 days of the day the patent was granted, with the following exception:

Patentees who received a decision from the USPTO under the USPTO's pre-*Wyeth* interpretation of 35 U.S.C. 154(b)(2)(A) may file a request for reconsideration of that decision if such a request for reconsideration is filed within **two months** of the date of the decision (37 CFR 1.181(f)). If the patentee's sole basis for requesting reconsideration of the decision is the USPTO's pre-*Wyeth* interpretation of 35 U.S.C. 154(b)(2)(A), the request for reconsideration need only state that reconsideration is being requested in view of *Wyeth* (this form may be used for this purpose if it is filed within **two months** of the date of the decision from the USPTO).

Do not use this form if the application has been allowed, but not yet issued as a patent.

- 1. For patents issued before March 2, 2010:** A request for reconsideration under 37 CFR 1.705(d) and the fee set forth in 37 CFR 1.18(e) are not required, provided that the patentee's sole basis for requesting recalculation of the PTA in the patent is the USPTO's pre-*Wyeth* interpretation of 35 U.S.C. 154(b)(2)(A) and this form is filed within 180 days of the day the patent was granted.
- 2. For patents issued on or after March 2, 2010 (do not use this form):** Patentees seeking a revised PTA in a patent issued on or after March 2, 2010, must file a request for reconsideration under 37 CFR 1.705(d) that complies with the requirements of 37 CFR 1.705(b)(1) and (b)(2) within two months of the day the patent issued.

For more information, see "Notice Concerning Calculation of the Patent Term Adjustment With Respect to the Overlapping Delay Provision of 35 U.S.C. 154(b)(2)(A)" available on the USPTO Web site at <http://www.uspto.gov/patents/law/notices/2010.jsp>.

**Wyeth v. Kappos*, No. 2009-1120 (Fed. Cir., Jan. 7, 2010).

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.

Electronic Acknowledgement Receipt	
EFS ID:	7003114
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	METHOD AND APPARATUS FOR SORTING PARTICLES
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	86738
Filer:	David Burns
Filer Authorized By:	
Attorney Docket Number:	118153-02106
Receipt Date:	12-FEB-2010
Filing Date:	07-AUG-2006
Time Stamp:	16:19:23
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no				
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for PTA recalculation in view of Wyeth	Request_for_Recalculation_of_PTA.pdf	150339 6546f7ad50c5f39ba7e2faab50d589659365eabf	no	3
Warnings:					
Information:					

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.



UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	PATENT NUMBER	GROUP ART UNIT	FILE WRAPPER LOCATION
11/499,953	7584857	3653	9200



Correspondence Address/Fee Address Change

The following fields have been set to Customer Number 86738 on 09/15/2009

- Correspondence Address
- Maintenance Fee Address
- Power of Attorney Address

The address of record for Customer Number 86738 is:

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



UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/499,953	09/08/2009	7584857	118153-02106	3579

86738 7590 08/19/2009
MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

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

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

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

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

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

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

Sebastian Bohm, Inverness, UNITED KINGDOM;
John R. Gilbert, Brookline, MA;
Manish Deshpande, Canton, MA;



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
 United States Patent and Trademark Office
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 Alexandria, Virginia 22313-1450
 www.uspto.gov



Bib Data Sheet

CONFIRMATION NO. 3579

SERIAL NUMBER 11/499,953	FILING OR 371(c) DATE 08/07/2006 RULE	CLASS 209	GROUP ART UNIT 3653	ATTORNEY DOCKET NO. 118153-02106	
APPLICANTS Sebastian Bohm, Inverness, UNITED KINGDOM; John R. Gilbert, Brookline, MA; Manish Deshpande, Canton, MA;					
** CONTINUING DATA ***** This application is a CON of 10/940,143 09/13/2004 PAT 7,104,405 which is a DIV of 10/179,488 06/24/2002 PAT 6,808,075 which claims benefit of 60/373,256 04/17/2002					
** FOREIGN APPLICATIONS *****					
IF REQUIRED, FOREIGN FILING LICENSE GRANTED** SMALL ENTITY ** ** 08/22/2006					
Foreign Priority claimed ☐ yes ☐ no 35 USC 119 (a-d) conditions ☐ yes ☐ no ☐ Met after Allowance Verified and Acknowledged _____ Examiner's Signature Initials		STATE OR COUNTRY UNITED KINGDOM	SHEETS DRAWING 5	TOTAL CLAIMS 1	INDEPENDENT CLAIMS 1
ADDRESS 86738					
TITLE METHOD AND APPARATUS FOR SORTING PARTICLES					
FILING FEE RECEIVED 1210	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:		☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit		

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)

959 7590 05/08/2009

LAHIVE & COCKFIELD, LLP
 FLOOR 30, SUITE 3000
 ONE POST OFFICE SQUARE
 BOSTON, MA 02109

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.

11/499,953 08/07/2006 Sebastian Bohm 118153-02106 3579

TITLE OF INVENTION: METHOD AND APPARATUS FOR SORTING PARTICLES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE

nonprovisional YES \$755 \$300 \$0 \$1064 08/10/2009

EXAMINER	ART UNIT	CLASS-SUBCLASS

HAGEMAN, MARK 3653 209-155000

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 _____

3 _____

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

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

(A) NAME OF ASSIGNEE

(B) RESIDENCE (CITY and STATE OR COUNTRY)

Cytonome/ST, LLC

Boston, Massachusetts

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

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

- ☒ Issue Fee
☒ Publication Fee (No small entity discount permitted)
☒ Advance Order - # of Copies **Three (3)**

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)

- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

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 July 31, 2009

Typed or printed name David R. Burns

Registration No. 46,590

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.

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.

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.

CHANGE OF CORRESPONDENCE ADDRESS *Application*

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

Application Number	11/499,953
Filing Date	August 7, 2006
First Named Inventor	Sebastian Bohm
Art Unit	3653
Examiner Name	M. Hageman
Attorney Docket Number	118153-02106

Please change the Correspondence Address for the above-identified patent application to:

☒ The address associated with
Customer Number:

86738

OR

☐ Firm or
Individual Name

Address

City

State

Zip

Country

Telephone

Email

This form cannot be used to change the data associated with a Customer Number. To change the data associated with an existing Customer Number use "Request for Customer Number Data Change" (PTO/SB/124).

I am the:

- ☐ Applicant/Inventor
- ☐ Assignee of record of the entire interest.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).
- ☒ Attorney or agent of record. Registration Number 46,590.
- ☐ Registered practitioner named in the application transmittal letter in an application without an executed oath or declaration. See 37 CFR 1.33(a)(1). Registration Number _____.

Signature
/David R. Burns/

Typed or Printed
Name David R. Burns

Date July 31, 2009

Telephone (617) 449-6500

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 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.

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.

Electronic Patent Application Fee Transmittal				
Application Number:		11499953		
Filing Date:		07-Aug-2006		
Title of Invention:		METHOD AND APPARATUS FOR SORTING PARTICLES		
First Named Inventor/Applicant Name:		Sebastian Bohm		
Filer:		David Burns/Lauren Margolis		
Attorney Docket Number:		TGZ-021DVCN		
Filed as Small Entity				
Utility under 35 USC 111(a) Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Publ. Fee- early, voluntary, or normal	1504	1	300	300
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl issue fee	2501	1	755	755

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Printed copy of patent - no color	8001	3	3	9
Total in USD (\$)				1064

Electronic Acknowledgement Receipt	
EFS ID:	5808556
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	METHOD AND APPARATUS FOR SORTING PARTICLES
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	00959
Filer:	David Burns/Lauren Margolis
Filer Authorized By:	David Burns
Attorney Docket Number:	TGZ-021DVCN
Receipt Date:	31-JUL-2009
Filing Date:	07-AUG-2006
Time Stamp:	16:10:55
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$1064
RAM confirmation Number	2267
Deposit Account	504876
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

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_02106_IssueFee.pdf	113101 3f048d18c96dc7bad56376d4bee385e6cbbdfcfc	no	1

Warnings:

Information:

2	Change of Address	118153_02106_ChngCorrespAddress.pdf	308269 adbb84ea695ebb1fbca2dd45af607ff1e98fb	no	2

Warnings:

Information:

3	Fee Worksheet (PTO-875)	fee-info.pdf	33861 95a3ed7ce17b80e20b458d7e28b9290a4fc6d5f9	no	2

Warnings:

Information:

Total Files Size (in bytes): 455231

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.



UNITED STATES PATENT AND TRADEMARK OFFICE

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

959 7590 05/08/2009

LAHIVE & COCKFIELD, LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER

HAGEMAN, MARK

ART UNIT

PAPER NUMBER

3653

DATE MAILED: 05/08/2009

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/499,953	08/07/2006	Sebastian Bohm	TGZ-021DVCN	3579

TITLE OF INVENTION: METHOD AND APPARATUS FOR SORTING PARTICLES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	\$755	\$300	\$0	\$1055	08/10/2009

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 SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

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

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959 7590 05/08/2009

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

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/499,953	08/07/2006	Sebastian Bohm	TGZ-021DVCN	3579

TITLE OF INVENTION: METHOD AND APPARATUS FOR SORTING PARTICLES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	\$755	\$300	\$0	\$1055	08/10/2009

EXAMINER	ART UNIT	CLASS-SUBCLASS
HAGEMAN, MARK	3653	209-155000

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 _____

2 _____

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

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

(A) NAME OF ASSIGNEE

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

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

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

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

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

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

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

- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

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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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/499,953	08/07/2006	Sebastian Bohm	TGZ-021DVCN	3579
959	7590	05/08/2009	EXAMINER	
LAHIVE & COCKFIELD, LLP FLOOR 30, SUITE 3000 ONE POST OFFICE SQUARE BOSTON, MA 02109			HAGEMAN, MARK	
			ART UNIT	PAPER NUMBER
			3653	
DATE MAILED: 05/08/2009				

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 0 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 0 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.

Notice of Allowability	Application No.	Applicant(s)	
	11/499,953	BOHM ET AL.	
	Examiner	Art Unit	
	Mark Hageman	3653	

-- 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 the amendment after final dated 4-29-2009.

2. ☒ The allowed claim(s) is/are 15-17, 19-21, 23-27, 30 and 31.

3. ☐ 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 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.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
 (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892) 2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948) 3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date <u>4-24-2009</u> 4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material	5. ☐ Notice of Informal Patent Application 6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____. 7. ☐ Examiner's Amendment/Comment 8. ☐ Examiner's Statement of Reasons for Allowance 9. ☐ Other _____.

	/Patrick H. Mackey/ Supervisory Patent Examiner, Art Unit 3653

Search Notes 	Application/Control No. 11499953	Applicant(s)/Patent Under Reexamination BOHM ET AL.
	Examiner Mark Hageman	Art Unit 3653

SEARCHED			
Class	Subclass	Date	Examiner
209	155,552,576,906,644,207 updated	5/5/2009	MCH

SEARCH NOTES		
Search Notes	Date	Examiner
EAST see attached	5/5/2009	Mark Hageman
PLUS search (claims and spec)	1/31/2008	Mark Hageman
Reviewed examiner Dillon's search	1/31/2008	Mark Hageman
FWD/BWD cited documents	5/5/2009	MCH

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner
	Text see EAST	5/5/2009	MCH



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BIB DATA SHEET

CONFIRMATION NO. 3579

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.		
11/499,953	08/07/2006	209	3653	TGZ-021DVCN		
APPLICANTS Sebastian Bohm, Inverness, UNITED KINGDOM; John R. Gilbert, Brookline, MA; Manish Deshpande, Canton, MA; ** CONTINUING DATA ***** This application is a CON of 10/940,143 09/13/2004 PAT 7,104,405 which is a DIV of 10/179,488 06/24/2002 PAT 6,808,075 which claims benefit of 60/373,256 04/17/2002 ** FOREIGN APPLICATIONS ***** ** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** ** SMALL ENTITY ** 08/22/2006						
Foreign Priority claimed ☐ Yes ☒ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged <u>/MARK HAGEMAN/</u> Examiner's Signature		☐ Met after Allowance MCH Initials	STATE OR COUNTRY UNITED KINGDOM	SHEETS DRAWINGS 5	TOTAL CLAIMS 1	INDEPENDENT CLAIMS 1
ADDRESS LAHIVE & COCKFIELD, LLP FLOOR 30, SUITE 3000 ONE POST OFFICE SQUARE BOSTON, MA 02109 UNITED STATES						
TITLE Method and apparatus for sorting particles						
FILING FEE RECEIVED 910	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:			☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit		

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1243	209/906,155,552,576,644,207.ccls.	US-PGPUB; USPAT	OR	ON	2009/05/05 12:37
L2	0	(bohm and desphande).in.	US-PGPUB; USPAT	OR	ON	2009/05/05 12:41
L3	23	(bohm and deshpane).in.	US-PGPUB; USPAT	OR	ON	2009/05/05 12:42
L4	26	((sorting) and (channel) and (outlet) and (reservoir)).clm.	US-PGPUB; USPAT	OR	ON	2009/05/05 12:51
L5	2	((sorting) and (channel) and (substrate) and (actuator) and (buffer)).clm.	US-PGPUB; USPAT	OR	ON	2009/05/05 12:52
L6	13	((sorting) and (channel) and (substrate) and (buffer)).clm.	US-PGPUB; USPAT	OR	ON	2009/05/05 12:52
S1	1	"20030027225"	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:46
S2	21	("3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6432630" "6597438").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:46
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S4	3	("4572664" "6657730" "7075652").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:47
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S6	4	("6273553" "6540895" "6592821" "6877528").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:48
S7	2	("7104405" "6808075").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:57
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S10	19	("20050036920" "20050092658" "20050092662" "20050128479" "5837200" "5971355" "6100541" "6303885" "6438279" "6593749" "6692952" "6802489" "6808075" "6808683" "6838056" "6849459" "6877528" "6878271" "6883957").PN. OR ("7220594").URPN.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:03
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S13	55	tyvoll.in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:31
S14	46	(cytonome).as.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:37
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S19	8	S17 S18	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 11:18
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S21	50	(sebastian and bohm).in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 12:15
S22	1	11/499953	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:22
S23	1	S22 and modif\$5	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:26
S24	1	"4175662".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:30
S25	1	"3984307".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:31
S26	1	"4756427".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:32
S27	1	"5837200".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:33
S28	1	S22 and meniscus	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:53
S29	1	S22 and membrane	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:55

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S37	212313	"137".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:08
S38	16365	"406".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:08
S39	639608	S32 S33 S34 S35 S36 S37 S38	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:08
S40	17278	(microfluid\$4 micro-fluid\$4 (micro adj fluid \$4))	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:09
S41	4366305	(sort\$4 separat\$4 classif\$5)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:10
S42	2875396	(particles cells molecules drops droplets)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:11
S43	1568581	(pump valve)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:11
S44	896240	(actuator deflect\$4)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:12
S45	269472	(bubble meniscus (gas adj filled))	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:12
S46	1144	S40 and S41 and S42 and S43 and S44 and S45	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:12
S47	675	S46 and S39	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:13

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S51	46	(cytonome).as.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16
S52	126	("20010051334" "20020172621" "20030027225" "3289687" "3508654" "3560754" "3984307" "4147621" "4175662" "4233029" "4271119" "4302313" "4426451" "4439526" "4526276" "4554427" "4676274" "4756427" "4908112" "4936465" "4939081" "5030002" "5065978" "5092972" "5101978" "5275787" "5395588" "5429734" "5529465" "5571410" "5637496" "5730187" "5757482" "5777649" "5779868" "5783446" "5789045" "5795727" "5800690" "5837200" "5842787" "5858195" "5858804" "5880071" "5922591" "5957579" "5958202" "5971158" "5976336" "5979664" "5998212" "6001229" "6001231" "6010607" "6010608" "6033546" "6042709" "6048734" "6057111" "6057149" "6062261" "6062681" "6068751" "6080295" "6086825" "6090251" "6102530" "6106685" "6110332" "6110343" "6120666" "6130098" "6132685" "6136212" "6143152" "6145247" "6150119" "6152181" "6165417" "6167910" "6186660" "6192768" "6193471" "6196525" "6197595" "6213151" "6214556" "6221226" "6221654" "6221677" "6231737" "6251343" "6271038" "6273553" "6274089" "6280967" "6284113" "6290909" "6296020" "6306590" "6342142" "6360775" "6376181" "6399396" "6409900" "6416642" "6420143" "6432630" "6455280" "6482652" "6494230" "6524790" "6540895" "6561224" "6567023" "6569674" "6592821" "6597438" "6648015" "6803194" "6877528").PN. OR ("6808075" "6877528" "6976590" "7069943" "7104405" "7157274" "7179423" "7211442").URPN.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16

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5/ 5/ 2009 1:33:51 PM

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Issue Classification 	Application/Control No. 11499953	Applicant(s)/Patent Under Reexamination BOHM ET AL.
	Examiner Mark Hageman	Art Unit 3653

ORIGINAL						INTERNATIONAL CLASSIFICATION												
CLASS		SUBCLASS				CLAIMED					NON-CLAIMED							
209		155				B	0	3	B	5 / 00 (2006.01.01)								
CROSS REFERENCE(S)						B	0	7	C	5 / 00 (2006.01.01)								
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)																	
209	576	906																

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
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/Mark Hageman/ Examiner.Art Unit 3653 (Assistant Examiner)	5-5-2009 (Date)	Total Claims Allowed: 13	
/PATRICK H MACKEY/ Supervisory Patent Examiner.Art Unit 3653 (Primary Examiner)	05/06/2009 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

<i>Index of Claims</i> 	Application/Control No. 11499953	Applicant(s)/Patent Under Reexamination BOHM ET AL.
	Examiner Mark Hageman	Art Unit 3653

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

☐ Claims renumbered in the same order as presented by applicant		☐ CPA		☒ T.D.		☐ R.1.47			
CLAIM		DATE							
Final	Original	05/18/2007	01/31/2008	11/04/2008	05/05/2009				
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OK TO ENTER: /MH/

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: April 29, 2009

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

Docket No.: TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Boehm *et al.*

Application No.: 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3653

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

Examiner: M. Hageman

AMENDMENT AFTER FINAL ACTION UNDER 37 C.F.R. 1.116

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

Dear Madam:

INTRODUCTORY COMMENTS

In response to the Office Action dated November 7, 2008 (Paper No. 20081104), finally rejecting claims 2-14, 28 and 29, the response period of which is extended to May 7, 2009 by the accompanying Request for Extension of Time, please amend the above-identified U.S. patent application as follows:

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	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BOHM
				Art Unit	3653
				Examiner Name	M. Hageman
Sheet	1	of	2	Attorney Docket Number	TGZ-021DVCN

[illegible][illegible]

Examiner Signature	/Mark Hageman/	Date Considered	05/05/2009

*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. ¹ Applicant's unique citation designation number (optional). ² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English language Translation is attached.

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

Dated: April 24, 2009

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

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				Application Number	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BOHM
				Art Unit	3653
				Examiner Name	M. Hageman
				Attorney Docket Number	TGZ-021DVCN
Sheet	2	of	2		

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
/MH/	C1	Chinese Office Action for Application No. 03814212.0, dated February 20, 2009	√

Examiner Signature	/Mark Hageman/	Date Considered	05/05/2009

*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.

¹Applicant's unique citation designation number (optional). ²Applicant is to place a check mark here if English language Translation is attached.

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

Dated: April 29, 2009
Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Boehm *et al.*

Application No.: 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3653

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

Examiner: M. Hageman

AMENDMENT AFTER FINAL ACTION UNDER 37 C.F.R. 1.116

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

Dear Madam:

INTRODUCTORY COMMENTS

In response to the Office Action dated November 7, 2008 (Paper No. 20081104), finally rejecting claims 2-14, 28 and 29, the response period of which is extended to May 7, 2009 by the accompanying Request for Extension of Time, please amend the above-identified U.S. patent application as follows:

AMENDMENTS TO THE CLAIMS

Please cancel claims 2-14, 28 and 29 without prejudice, as set forth in the following listing of claims, which will replace all prior versions, and listings, of claims in the present application

1-14. (Canceled).

15. (Previously Presented) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, and
a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having a first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including a sealed gas-filled reservoir disposed in the substrate in communication with the associated sorting channel and an actuator for increasing an internal pressure of the gas-filled reservoir to selectively modify fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic into the first outlet of the associated sorting channel.

16. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir.

17. (Previously Presented) The particle sorting system of claim 16, wherein each actuator selectively deflects the movable membrane to increase pressure in the gas-filled reservoir to modify said fluid flow.

18. (Canceled).

19. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further comprises a buffer for limiting said modification of the fluid flow to a region of the associated sorting channel along the sorting module and preventing flow disturbance in other regions of the associated sorting channel.

20. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further includes a side channel connecting the gas-filled reservoir to the associated sorting channel.

21. (Previously Presented) A particle sorting system, comprising:

a plurality of substantially parallel sorting channels formed on a substrate, each sorting channel capable of conveying a stream of particles in a carrier fluid,

a plurality of actuators arranged external to the substrate, each actuator associated with a sorting channel formed on the substrate, for selectively creating a flow disturbance within the associated sorting channel to deflect a selected particle in the stream of particles from the stream of particles; and

a plurality of buffers, each buffer associated with an actuator for absorbing the flow disturbance in the associated sorting channel, wherein each buffer comprises a gas-filled reservoir in fluid communication with the associated sorting channel.

22. (Canceled).

23. (Previously Presented) The particle sorting system of claim 21, wherein each sorting channel includes a gas-filled reservoir in fluid communication therewith, and the actuator increases an internal pressure of the gas-filled reservoir to create said flow disturbance.

24. (Previously Presented) The particle sorting system of claim 23, wherein each gas-filled reservoir includes a flexible membrane forming at least a portion of a side wall of the gas-filled reservoir, and the actuator deflects the flexible membrane to increase said internal pressure.

25. (Previously Presented) A particle sorting system, comprising:

a plurality of substantially parallel sorting channels formed on a substrate, and

a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having a first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including a gas-filled reservoir formed in the substrate having a movable wall disposed in communication with the associated sorting channel for selectively modifying fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic into the first outlet of the associated sorting channel.

26. (Previously Presented) The particle sorting system of claim 25, wherein the movable wall comprises a flexible membrane.

27. (Previously Presented) The particle sorting system of claim 25, wherein each sorting module further includes an associated actuator for selectively moving the movable wall to increase an internal pressure of the gas-filled reservoir to selectively modify the fluid flow through the associated sorting channel.

28-29. (Canceled).

30. (Previously Presented) The particle sorting system of claim 23, wherein each buffer is arranged opposite the gas-filled reservoir in the flow channel.

31. (Previously Presented) The particle sorting system of claim 27, wherein the actuator selectively contacts and depresses the movable wall to increase an internal pressure of the gas-filled reservoir.

REMARKS

The foregoing amendment cancels claims 2-14, 28 and 29 without prejudice to filing the same or similar claims in one or more subsequent patent applications. Pending in the application are claims 15-17, 19-21, 23-27, 30 and 31 of which claims 15, 21 and 25 are independent. The following comments address all stated grounds for rejection and place the presently pending claims, as identified above, in condition for allowance.

No new matter is added.

Amendment and/or cancellation of the claims is not to be construed as an acquiescence to any of the objections/rejections set forth in the instant Office Action, and was done solely to expedite prosecution of the application.

35 U.S.C. §112 Rejection

Regarding the rejection of claim 29 under 35 U.S.C. §112, first paragraph for failing to comply with the written description, Applicants respectfully disagree. However, to expedite prosecution of the application Applicants cancel claim 29 without prejudice. Accordingly, Applicants contend that the rejection of claim 29 under 35 U.S.C. §112, first paragraph is moot. Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claim 29 under 35 U.S.C. §112, first paragraph.

35 U.S.C. 103 Rejections

Applicants thank the Examiner for the close review of the claims and for the reconsideration and removal of the previous rejection. In the Office Action, the Examiner rejects claims 2-14, 28 and 29 under 35 U.S.C. 103(a) as being obvious over the Diessel reference (U.S. Patent Number 5,837,200) in view of the Chou reference (U.S. Patent Number 7,258,774).

Applicants contend that claims 2-14, 28 and 29 are patentable over Diessel and Chou, either alone or in combination. Nonetheless, to expedite prosecution of the application

Applicants cancel claims 2-14, 28 and 29 without prejudice. Accordingly, Applicants contend that the rejection of claims 2-14, 28 and 29 under 35 U.S.C. §103(a) is moot. Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 2-14, 28 and 29 under 35 U.S.C. §103(c) and pass the application to allowance.

CONCLUSION

In view of the above amendment, Applicant contends the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned attorney at (617) 227-7400.

If any additional fee is due with this statement, please charge our Deposit Account No. 12-0080, under Order No. TGZ-021DVCN, from which the undersigned is authorized to draw.

Dated: April 29, 2009

Respectfully submitted,

Electronic signature: /David R. Burns/
David R. Burns
Registration No.: 46,590
LAHIVE & COCKFIELD, LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 227-7400
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2009 <i>(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)</i>		Docket Number (Optional) TGZ-021DVCN																									
Application Number 11/499,953-Conf. #3579		Filed August 7, 2006																									
For METHOD AND APPARATUS FOR SORTING PARTICLES																											
Art Unit 3653		Examiner M. Hageman																									
This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application. The requested extension and fee are as follows (check time period desired and enter the appropriate fee below): <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 40%;"></th> <th style="width: 15%; text-align: center;"><u>Fee</u></th> <th style="width: 15%; text-align: center;"><u>Small Entity Fee</u></th> <th style="width: 30%;"></th> </tr> </thead> <tbody> <tr> <td>☐ One month (37 CFR 1.17(a)(1))</td> <td style="text-align: center;">\$130</td> <td style="text-align: center;">\$65</td> <td>\$ _____</td> </tr> <tr> <td>☐ Two months (37 CFR 1.17(a)(2))</td> <td style="text-align: center;">\$490</td> <td style="text-align: center;">\$245</td> <td>\$ _____</td> </tr> <tr> <td>☒ Three months (37 CFR 1.17(a)(3))</td> <td style="text-align: center;">\$1110</td> <td style="text-align: center;">\$555</td> <td>\$ 555.00</td> </tr> <tr> <td>☐ Four months (37 CFR 1.17(a)(4))</td> <td style="text-align: center;">\$1730</td> <td style="text-align: center;">\$865</td> <td>\$ _____</td> </tr> <tr> <td>☐ Five months (37 CFR 1.17(a)(5))</td> <td style="text-align: center;">\$2350</td> <td style="text-align: center;">\$1175</td> <td>\$ _____</td> </tr> </tbody> </table> ☒ Applicant claims small entity status. See 37 CFR 1.27. ☐ A check in the amount of the fee is enclosed. ☐ Payment by credit card. Form PTO-2038 is attached. ☒ The Director has already been authorized to charge fees in this application to a Deposit Account. ☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number <u>12-0080</u>. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. I am the ☐ applicant/inventor. ☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96). ☒ attorney or agent of record. Registration Number <u>46,590</u> ☐ attorney or agent under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____ _____ /David R. Burns/ Signature _____ David R. Burns Typed or printed name _____ April 29, 2009 Date _____ (617) 994-0890 Telephone Number NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below. ☐ Total of <u>1</u> forms are submitted.					<u>Fee</u>	<u>Small Entity Fee</u>		☐ One month (37 CFR 1.17(a)(1))	\$130	\$65	\$ _____	☐ Two months (37 CFR 1.17(a)(2))	\$490	\$245	\$ _____	☒ Three months (37 CFR 1.17(a)(3))	\$1110	\$555	\$ 555.00	☐ Four months (37 CFR 1.17(a)(4))	\$1730	\$865	\$ _____	☐ Five months (37 CFR 1.17(a)(5))	\$2350	\$1175	\$ _____
	<u>Fee</u>	<u>Small Entity Fee</u>																									
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☐ Four months (37 CFR 1.17(a)(4))	\$1730	\$865	\$ _____																								
☐ Five months (37 CFR 1.17(a)(5))	\$2350	\$1175	\$ _____																								

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: April 29, 2009

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

NOTICE OF APPEAL FROM THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES		Docket Number (Optional) TGZ-021DVCN
In re Application of Sebastian BOHM <i>et al.</i>		
Application Number 11/499,953-Conf. #3579		Filed August 7, 2006
For METHOD AND APPARATUS FOR SORTING PARTICLES		
Art Unit 3653		Examiner M. Hageman

Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) \$ 540.00

☒ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by half, and the resulting fee is: \$ 270.00

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

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

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

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 12-0080.

☒ A petition for an extension of time under 37 CFR 1.136(a) (PTO/SB/22) is enclosed.

WARNING: INFORMATION ON THIS FORM MAY BECOME PUBLIC. CREDIT CARD INFORMATION SHOULD NOT BE INCLUDED ON THIS FORM. PROVIDE CREDIT CARD INFORMATION AND AUTHORIZATION ON PTO-2038.

I am the

☐ applicant /inventor.

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

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

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

/David R. Burns/
Signature

David R. Burns
Typed or printed name

(617) 994-0890
Telephone number

April 29, 2009
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of 1 forms are submitted.

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Dated: April 29, 2009

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

Electronic Patent Application Fee Transmittal				
Application Number:		11499953		
Filing Date:		07-Aug-2006		
Title of Invention:		Method and apparatus for sorting particles		
First Named Inventor/Applicant Name:		Sebastian Bohm		
Filer:		David Burns/Lauren Margolis		
Attorney Docket Number:		TGZ-021DVCN		
Filed as Small 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:				
Notice of appeal	2401	1	270	270
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension - 3 months with \$0 paid	2253	1	555	555
Miscellaneous:				
Total in USD (\$)				825

Electronic Acknowledgement Receipt	
EFS ID:	5243332
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	Method and apparatus for sorting particles
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	00959
Filer:	David Burns/Lauren Margolis
Filer Authorized By:	David Burns
Attorney Docket Number:	TGZ-021DVCN
Receipt Date:	29-APR-2009
Filing Date:	07-AUG-2006
Time Stamp:	16:13:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$825
RAM confirmation Number	2469
Deposit Account	120080
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

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	Amendment After Final	TGZ_021DVCN_Response.pdf	50130	no	7
			2fd67f7c41fb8c03ddfa201714f600d66ecc3919		

Warnings:

Information:

2	Extension of Time	TGZ_021DVCN_ExtensionofTime.pdf	31123	no	1
			3d23aab1ed1dfe8f6df392e018d4395893e13187		

Warnings:

Information:

3	Notice of Appeal Filed	TGZ_021DVCN_NoticeofAppeal.pdf	23895	no	1
			8f51e9a60651aabbec606b7ae965de6055f56afa5		

Warnings:

Information:

4	Fee Worksheet (PTO-875)	fee-info.pdf	32062	no	2
			39dae455fdb206c8f870505b58dc3f75fa22eb39		

Warnings:

Information:

Total Files Size (in bytes): 137210

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 11/499,953		Filing Date 08/07/2006		☐ To be Mailed					
APPLICATION AS FILED – PART I														
(Column 1)			(Column 2)			SMALL ENTITY ☒		OR		OTHER THAN SMALL ENTITY				
FOR		NUMBER FILED		NUMBER EXTRA		RATE (\$)		FEE (\$)						
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A		N/A		N/A								
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A		N/A		N/A								
☐ 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 \$ =				OR				
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 =		*		X \$ =				OR				
☐ 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).												
☐ 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				TOTAL				
APPLICATION AS AMENDED – PART II														
(Column 1)			(Column 2)			(Column 3)			SMALL ENTITY		OR		OTHER THAN SMALL ENTITY	
AMENDMENT	04/29/2009		CLAIMS REMAINING AFTER AMENDMENT				HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA		RATE (\$)		ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))		* 13		Minus		** 20		= 0		X \$26 =		0	
	Independent (37 CFR 1.16(h))		* 3		Minus		*** 3		= 0		X \$110 =		0	
	☐ Application Size Fee (37 CFR 1.16(s))													
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))													
											TOTAL ADD'L FEE		0	
AMENDMENT	Total (37 CFR 1.16(i))		*		Minus		**		=		X \$ =		=	
	Independent (37 CFR 1.16(h))		*		Minus		***		=		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.						Legal Instrument Examiner: /MARY E. EVANS/								
** 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.														

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.

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.

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	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BOHM
				Art Unit	3653
				Examiner Name	M. Hageman
				Attorney Docket Number	TGZ-021DVCN
Sheet	1	of	2		

[illegible][illegible]

Examiner Signature		Date Considered	

*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. ¹ Applicant's unique citation designation number (optional). ² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English language Translation is attached.

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

Dated: April 24, 2009

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

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	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BOHM
				Art Unit	3653
				Examiner Name	M. Hageman
Sheet	2	of	2	Attorney Docket Number	TGZ-021DVCN

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	Chinese Office Action for Application No. 03814212.0, dated February 20, 2009	√

Examiner Signature		Date Considered	

*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.

¹Applicant's unique citation designation number (optional). ²Applicant is to place a check mark here if English language Translation is attached.

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

Dated: April 24, 2009
Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Boehm *et al.*

Application No.: 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3653

For: METHOD AND APPARATUS FOR
SORTING PARTICLES

Examiner: M. Hageman

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Madam:

In accordance with 37 CFR 1.97, Applicants hereby make of record the following additional documents. A PTO Form SB/08 and a full copy of each of the documents required under 37 CFR 1.98(a)(2) accompany this statement.

Applicants have become aware of the following documents, cited in a Chinese Office Action issued February 20, 2009, during the prosecution of Application No. 03814212.0, which corresponds to the above referenced application, and in accordance with 37 CFR 1.97(d) and (e)(1), hereby submit these documents for the Examiner's consideration. These documents are cited on the enclosed PTO Form SB/08, and a copy of an English Translation of the Office Action provided by the Chinese Associate and of each document required under 37 CFR 1.98(a)(2) cited thereon are enclosed as well.

Please note that Applicants' attorney does not include in this Supplemental Information Disclosure Statement references US 3,508,654 and US 5,837,200 cited in the corresponding Office Action. These references were previously submitted in an Information Disclosure Statement to the U.S. Patent Office in this case on January 3, 2007.

865707-1

This statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any document herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

This Supplemental Information Disclosure Statement is filed after the mailing date of a Final Office Action but before payment of the Issue Fee (37 CFR 1.97(d)). Applicants hereby petition that the Supplemental Information Disclosure Statement be considered.

I hereby certify, pursuant to 37 CFR 1.97(e)(1), that each item of information contained in this Supplemental Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this Supplemental Information Disclosure Statement.

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. 12-0080, under Order No. TGZ-021DVCN.

Dated: April 24, 2009

Respectfully submitted,

DRB/mch

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Attorney/Agent For Applicant

865707-1

Electronic Acknowledgement Receipt	
EFS ID:	5204936
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	Method and apparatus for sorting particles
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	00959
Filer:	David Burns/Lauren Margolis
Filer Authorized By:	David Burns
Attorney Docket Number:	TGZ-021DVCN
Receipt Date:	24-APR-2009
Filing Date:	07-AUG-2006
Time Stamp:	16:15:56
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no				
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Filed (SB/08)	TGZ_021DVCN_SB08.pdf	48842 a6e83627ad28c63f4e3420b5d02adadc250d8c0f	no	2
Warnings:					
Information:					

This is not an USPTO supplied IDS fillable form					
2	Transmittal Letter	TGZ_021DVCN_SIDS.pdf	29380 ca4839c2fc80169417bc5a8cfe11e8d0fc16a67a	no	2
Warnings:					
Information:					
3	NPL Documents	TGZ_021DVCN_CIOA038142120.pdf	177988 b5d4487c98af745bba2b53ba0d6c1db3c407faae	no	3
Warnings:					
Information:					
Total Files Size (in bytes):			256210		
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. <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. <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. <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.					



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/499,953	08/07/2006	Sebastian Bohm	TGZ-021DVCN	3579
959 7590 11/07/2008 LAHIVE & COCKFIELD, LLP FLOOR 30, SUITE 3000 ONE POST OFFICE SQUARE BOSTON, MA 02109			EXAMINER HAGEMAN, MARK	
			ART UNIT 3653	PAPER NUMBER
			MAIL DATE 11/07/2008	DELIVERY MODE PAPER

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

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

Office Action Summary	Application No.	Applicant(s)	
	11/499,953	BOHM ET AL.	
	Examiner	Art Unit	
	Mark Hageman	3653	

-- 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 25 August 2008.

2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.

3) ☐ 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

4) ☒ Claim(s) 2-17, 19-21 and 23-31 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5) ☒ Claim(s) 15-17, 19-21, 23-27, 30 and 31 is/are allowed.

6) ☒ Claim(s) 2-14, 28 and 29 is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ 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).

11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

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) ☐ Notice of References Cited (PTO-892) 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date <u>7-25-2008, 8-25-2008</u> .	4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ 5) ☐ Notice of Informal Patent Application 6) ☐ Other: _____.

DETAILED ACTION

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claim 29 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 29 is a negative limitation (see MPEP 2173.05(i)) but the specifications lacks positive recitation of the lack of valves in the first and second outlets. The specification is silent regarding such valves but does not expressly preclude them.

Claim Rejections - 35 USC § 103

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

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

Claims 2-14, 28 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Diessel in view of US 7,258,774 to Chou. Diessel discloses a particle sorting system (abstract), comprising: a plurality of substantially parallel sorting

channels (4) formed on a substrate, a plurality of sensors (9, 10), each sensor associated with one of said sorting channels for detecting and classifying particles in the associated sorting channel based on a first predetermined characteristic (c2 lines 45+); a plurality of sorting modules (figures 1 and 7 and c3 lines 55+), each sorting module associated with one of said sorting channels, for distributing particles having the first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel (c2 lines 40+), each said sorting module including an actuator (12) controlled by one of said plurality of sensors, and particles not having the predetermined characteristic flow into the second outlet undisturbed (20) an aggregation region (c2 lines 49+) for aggregating particles having the predetermined characteristic from a plurality of the first outlets of the sorting channels, at least one secondary sorting channel (37, 38 figure 7) in series with the plurality of substantially parallel sorting channels for collecting selected particles having the first predetermined characteristic from a plurality of first outlets and separating particles in the secondary sorting channel based on a second predetermined characteristic, wherein the second predetermined characteristic is different from the first predetermined characteristic (c5 lines 57+), an inlet channel (3 figure 1) that branches to supply a plurality of the substantially parallel sorting channels with a stream of particles to be sorted.

Diessel does not disclose the actuator to selectively apply a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet. wherein the actuator is arranged external to the substrate

and in communication with the associated sorting channel, wherein each sorting module further includes a gas-filled reservoir external to and in communication with the microchannel interior, wherein a fluid meniscus forms an interface between the gas-filled reservoir and the interior of the associated sorting channel, wherein each actuator selectively increases an internal pressure of the associated gas-filled reservoir to create the pressure pulse in the associated sorting channel, wherein each sorting module further comprises a side passage connecting the associated sorting channel with the gas-filled reservoir, wherein the gas-filled reservoir includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir, wherein the associated actuator selectively deflects the movable membrane to generate the pressure pulse, wherein each sorting module further comprises a buffer for absorbing the pressure pulse, wherein the plurality of sorting channels are formed by grooves etched in a first plate, and the gas-filled reservoirs are formed by a second plate bonded to the first plate, the second plate including a plurality of apertures defining the gas-filled reservoirs, a flexible membrane bonded to the second plate to seal at least one of the gas-filled reservoirs and defining at least a portion of a movable wall of at least one of the gas-filled reservoirs.

Chou discloses an actuator (figures 7b 7h, 31a and 31b, c13 lines 6+, and c31 lines 50+) to selectively apply a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet, wherein the actuator is arranged external to the substrate and in communication with the associated sorting channel (c14 lines 15+), wherein each sorting module further

includes a gas-filled reservoir (c14 lines 15+ and 32) external to and in communication with the microchannel interior, wherein a fluid meniscus (interface of channel 30 and membrane 25) forms an interface between the gas-filled reservoir and the interior of the associated sorting channel, wherein each actuator selectively increases an internal pressure of the associated gas-filled reservoir to create the pressure pulse in the associated sorting channel (c 13 lines 8+), wherein each sorting module further comprises a side passage (32 where it intersects 30 see figure 7h) connecting the associated sorting channel with the gas-filled reservoir, wherein the gas-filled reservoir includes a movable membrane (25) forming at least a portion of a side wall of the gas-filled reservoir, wherein the associated actuator selectively deflects the movable membrane (c13 lines 8+ and figure 7h) to generate the pressure pulse, wherein each sorting module further comprises a buffer (2910 and figures 29a and 29b) for absorbing the pressure pulse, wherein the plurality of sorting channels are formed by grooves etched in a first plate, and the gas-filled reservoirs are formed by a second plate bonded to the first plate, the second plate including a plurality of apertures defining the gas-filled reservoirs, a flexible membrane bonded to the second plate to seal at least one of the gas-filled reservoirs and defining at least a portion of a movable wall of at least one of the gas-filled reservoirs (see figures 1-7 and c5 lines 25+) for the purpose of providing valves that are ideally suited for microfluidic metering and fluid control (c15 lines 13+).

It would have been obvious to one of ordinary skill in the art at the time of the applicant's invention to have modified Diessel to include the actuator system, as taught by Chou, as it would have been obvious to one of ordinary skill in the art to substitute

one type of actuating system for another known type to achieve the predictable result of a valve system ideally suited for microfluidic metering and fluid control.

Allowable Subject Matter

4. Claims 15-17, 19-21, 23-27, 30, and 31 are allowed.
5. The following is a statement of reasons for the indication of allowable subject matter: Relative to claims 15, 25 and the claims which depend therefrom the prior art does not anticipate or render obvious the gas filled reservoir "disposed in the substrate..." in combination with the other elements of the claims. Relative to claim 21 and those that depend therefrom the prior art does not anticipate or render obvious the fluid communication between the gas-filled reservoir and the channel in combination with the other elements of the claims.

Response to Arguments

6. Applicant's arguments filed 8-25-2008 have been fully considered but they are not persuasive. Applicant has argued that the functioning of the Chou device fails to show the "pressure pulse" as claimed. Examiner disagrees and contends that both the closing of the valves and the reversing of flow in Chou generate pressure pulses that serve to deflect selected particles. Examiner contends that in Chou particles naturally flow into the waste ("while particles not having the predetermined characteristics flow into the second outlet undisturbed") and that when desired material is detected the valve and the pump are both utilized (both exerting a pressure pulse) to reverse the flow

and change the path in order to sort the desired particle into the collection pool.

Examiner maintains that although this function may be somewhat different than that of the applicant's device that it still reads on the claim limitations which do not preclude such functioning. The claim does not set forth a transient time of the pressure pulse nor preclude a temporary reversal of flow to effect sorting.

Relative to the various dependant claims examiner maintains that Diessel in view of Chou, as set forth above, recites the various limitations.

Conclusion

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

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

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark Hageman whose telephone number is (571) 272-3027. The examiner can normally be reached on M-F 7:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Patrick Mackey can be reached on (571) 272-6916. 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.

/Patrick H. Mackey/
Supervisory Patent Examiner, Art
Unit 3653

MCH

<i>Search Notes</i> 	Application/Control No. 11499953	Applicant(s)/Patent Under Reexamination BOHM ET AL.
	Examiner Mark Hageman	Art Unit 3653

SEARCHED			
Class	Subclass	Date	Examiner
209	155,552,576,906,644,207	11/4/2008	MCH

SEARCH NOTES		
Search Notes	Date	Examiner
EAST see attached	11/4/2008	Mark Hageman
PLUS search (claims and spec)	1/31/2008	Mark Hageman
Reviewed examiner Dillon's search	1/31/2008	Mark Hageman

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	19	("3791517" "3827555" "4318483" "4365719" "4939081" "5637496" "5783446" "5795727" "5979664" "5998212" "6057111" "6214556" "6280967" "20020093641" "6454862" "6455280" "6482652" "6524790" "6803194").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/11/04 09:36
L2	1	11/499953	US-PGPUB; USPAT; USOCR	OR	ON	2008/11/04 09:58
L3	1	2 and (free valve)	US-PGPUB; USPAT; USOCR	OR	ON	2008/11/04 09:58
L4	60	hageman.xa.	US-PGPUB; USPAT; USOCR	OR	ON	2008/11/04 10:23
L5	1688	209/906,155,552,576,644,207.ccls.	US-PGPUB; USPAT; USOCR	OR	ON	2008/11/04 10:24
L6	1229	209/906,155,552,576,644,207.ccls.	US-PGPUB; USPAT	OR	ON	2008/11/04 10:24
L7	22	6 and (microfluid\$4 micro-fluid\$4)	US-PGPUB; USPAT	OR	ON	2008/11/04 10:25
S1	1	"20030027225"	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:46
S2	21	("3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6432630" "6597438").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:46
S3	22	S1 S2	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:46
S4	3	("4572664" "6657730" "7075652").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:47
S5	25	S3 S4	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:47
S6	4	("6273553" "6540895" "6592821" "6877528").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:48
S7	2	("7104405" "6808075").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 08:57

S8	27	("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6273553" "6432630" "6540895" "6592821" "6597438" "6877528").PN. OR ("6808075" "7104405").URPN.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 09:05
S9	1	10/763112	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 09:21
S10	19	("20050036920" "20050092658" "20050092662" "20050128479" "5837200" "5971355" "6100541" "6303885" "6438279" "6593749" "6692952" "6802489" "6808075" "6808683" "6838056" "6849459" "6877528" "6878271" "6883957").PN. OR ("7220594").URPN.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:03
S11	27	("20030027225" "3508654" "3560754" "3984307" "4147621" "4175662" "4526276" "4554427" "4756427" "4936465" "5030002" "5101978" "5275787" "5395588" "5837200" "5976336" "6120666" "6145247" "6221654" "6273553" "6432630" "6540895" "6592821" "6597438" "6877528").PN. OR ("6808075" "7104405").URPN.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:30
S12	27	S11 S8	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:30
S13	55	tyvoll.in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:31
S14	46	(cytonome).as.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:37
S15	20	("3289687" "4426451" "4676274" "4908112" "5065978" "5092972" "5777649" "5789045" "6048734" "6062681" "6102530" "6152181" "6196525" "6273553" "6360775" "6561224").PN. OR ("6877528").URPN.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:52
S16	126	("20010051334" "20020172621" "20030027225" "3289687" "3508654" "3560754" "3984307" "4147621" "4175662" "4233029" "4271119" "4302313" "4426451" "4439526" "4526276" "4554427" "4676274" "4756427" "4908112" "4936465" "4939081" "5030002" "5065978" "5092972" "5101978" "5275787" "5395588" "5429734" "5529465" "5571410" "5637496" "5730187" "5757482" "5777649" "5779868" "5783446" "5789045" "5795727" "5800690" "5837200" "5842787"	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 10:57

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S17	2	("3721255" "6497252").pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 11:18
S18	6	"20020127736"	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 11:18
S19	8	S17 S18	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 11:18
S20	1170	bohm.in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 12:14
S21	50	(sebastian and bohm).in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 12:15
S22	1	11/499953	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:22
S23	1	S22 and modif\$5	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:26
S24	1	"4175662".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:30
S25	1	"3984307".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:31

S26	1	"4756427".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:32
S27	1	"5837200".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:33
S28	1	S22 and meniscus	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:53
S29	1	S22 and membrane	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:55
S30	6461	wada.in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 14:35
S31	30	("3560754" "3984307" "4009782" "4175662" "4338024" "4361400" "4756427" "4778593" "4786165" "4887721").PN. OR ("5101978").URPN.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:02
S32	75073	"209".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:06
S33	170733	"435".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:07
S34	39837	"436".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:07
S35	100872	"422".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:07
S36	69789	"204".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:07
S37	212313	"137".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:08
S38	16365	"406".clas.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:08
S39	639608	S32 S33 S34 S35 S36 S37 S38	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:08
S40	17278	(microfluid\$4 micro-fluid\$4 (micro adj fluid \$4))	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:09
S41	4366305	(sort\$4 separat\$4 classif\$5)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:10
S42	2875396	(particles cells molecules drops droplets)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:11
S43	1568581	(pump valve)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:11

S44	896240	(actuator deflect\$4)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:12
S45	269472	(bubble meniscus (gas adj filled))	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:12
S46	1144	S40 and S41 and S42 and S43 and S44 and S45	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:12
S47	675	S46 and S39	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:13
S48	1	"20030027225"	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16
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S50	22	S48 S49	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16
S51	46	(cytonome).as.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16
S52	126	("20010051334" "20020172621" "20030027225" "3289687" "3508654" "3560754" "3984307" "4147621" "4175662" "4233029" "4271119" "4302313" "4426451" "4439526" "4526276" "4554427" "4676274" "4756427" "4908112" "4936465" "4939081" "5030002" "5065978" "5092972" "5101978" "5275787" "5395588" "5429734" "5529465" "5571410" "5637496" "5730187" "5757482" "5777649" "5779868" "5783446" "5789045" "5795727" "5800690" "5837200" "5842787" "5858195" "5858804" "5880071" "5922591" "5957579" "5958202" "5971158" "5976336" "5979664" "5998212" "6001229" "6001231" "6010607" "6010608" "6033546" "6042709" "6048734" "6057111" "6057149" "6062261" "6062681" "6068751" "6080295" "6086825" "6090251" "6102530" "6106685" "6110332" "6110343" "6120666" "6130098" "6132685" "6136212" "6143152" "6145247" "6150119" "6152181" "6165417" "6167910" "6186660" "6192768" "6193471" "6196525" "6197595" "6213151" "6214556" "6221226" "6221654" "6221677" "6231737" "6251343" "6271038" "6273553" "6274089" "6280967" "6284113" "6290909"	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16

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S53	50	(sebastian and bohm).in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16
S54	642	S47 not (S53 S52 S51 S50)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 08:16
S55	50	(US-20010024625-\$ or US-20020130448-\$ or US-20020148621-\$ or US-20030000386- \$ or US-20030198523-\$ or US- 20050076646-\$ or US-20050081529-\$ or US-20050150379-\$ or US-20060248897-\$ or US-20060266679-\$ or US-20060278410- \$ or US-20060278736-\$ or US- 20070006571-\$ or US-20070022976-\$ or US-20070059159-\$ or US-20070113561-\$). did. or (US-3682451-\$ or US-3724173-\$ or US-3761064-\$ or US-3761065-\$ or US- 3832121-\$ or US-3832985-\$ or US- 3987132-\$ or US-4157160-\$ or US- 4278132-\$ or US-4282926-\$ or US- 4427074-\$ or US-4689063-\$ or US- 4756427-\$ or US-4860547-\$ or US- 5259403-\$ or US-5372011-\$ or US- 5447203-\$ or US-5590544-\$ or US- 5669946-\$ or US-6143221-\$ or US- 6155430-\$ or US-6257875-\$ or US- 6481357-\$ or US-6808075-\$ or US- 7157046-\$ or US-7294173-\$).did. or (US- 7302795-\$ or US-3592205-\$ or US- 3677029-\$ or US-3686825-\$ or US- 3696929-\$ or US-3710551-\$ or US- 3746322-\$ or US-3751883-\$).did.	US-PGPUB; USPAT	OR	ON	2008/02/01 09:32
S56	50	(US-20010024625-\$ or US-20020130448-\$ or US-20020148621-\$ or US-20030000386- \$ or US-20030198523-\$ or US- 20050076646-\$ or US-20050081529-\$ or US-20050150379-\$ or US-20060248897-\$ or US-20060266679-\$ or US-20060278410- \$ or US-20060278736-\$ or US- 20070006571-\$ or US-20070022976-\$ or US-20070059159-\$ or US-20070113561-\$). did. or (US-3682451-\$ or US-3724173-\$ or US-3761064-\$ or US-3761065-\$ or US- 3832121-\$ or US-3832985-\$ or US- 3987132-\$ or US-4157160-\$ or US- 4278132-\$ or US-4282926-\$ or US- 4427074-\$ or US-4689063-\$ or US- 4756427-\$ or US-4860547-\$ or US- 5259403-\$ or US-5372011-\$ or US- 5447203-\$ or US-5590544-\$ or US- 5669946-\$ or US-6143221-\$ or US-	US-PGPUB; USPAT	OR	ON	2008/02/01 09:32

		6155430-\$ or US-6257875-\$ or US-6481357-\$ or US-6808075-\$ or US-7157046-\$ or US-7294173-\$).did. or (US-7302795-\$ or US-3592205-\$ or US-3677029-\$ or US-3686825-\$ or US-3696929-\$ or US-3710551-\$ or US-3746322-\$ or US-3751883-\$).did.				
S57	50	S55 S56	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 09:32
S58	49	(US-20030198523-\$ or US-20030234210-\$ or US-20040161772-\$ or US-20050092658-\$ or US-20050183995-\$ or US-20060266679-\$ or US-20070065808-\$ or US-20050199252-\$).did. or (US-6808075-\$ or US-6976590-\$ or US-7104405-\$ or US-7157274-\$ or US-4394907-\$ or US-4352430-\$ or US-4407415-\$ or US-5209355-\$ or US-5256880-\$ or US-5017497-\$ or US-4549659-\$ or US-4756427-\$ or US-4858771-\$ or US-4321134-\$ or US-4361400-\$ or US-4444317-\$ or US-4580684-\$ or US-4606636-\$ or US-4626343-\$ or US-4936465-\$ or US-5699724-\$ or US-5733592-\$ or US-5775890-\$ or US-6287832-\$ or US-6376843-\$ or US-6475753-\$).did. or (US-7104403-\$ or US-5394705-\$ or US-4284496-\$ or US-4284495-\$ or US-4361238-\$ or US-4892258-\$ or US-5309773-\$ or US-5317162-\$ or US-5379949-\$ or US-5482216-\$ or US-5520290-\$ or US-5524746-\$ or US-5676256-\$ or US-5958481-\$ or US-5974901-\$).did.	US-PGPUB; USPAT	OR	ON	2008/02/01 09:33
S59	95	S57 S58	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 09:33
S60	82	S59 not (S54 S53 S52 S51 S50)	US-PGPUB; USPAT; USOCR	OR	ON	2008/02/01 09:33
S61	1	"6432630".pn.	US-PGPUB; USPAT; USOCR	OR	ON	2008/11/04 09:31

11/ 4/ 2008 10:35:37 AM

C:\Documents and Settings\mhageman\My Documents\EAST\Workspaces\ 11 cases\ 11_499953.wsp

<i>Index of Claims</i> 	Application/Control No. 11499953	Applicant(s)/Patent Under Reexamination BOHM ET AL.
	Examiner Mark Hageman	Art Unit 3653

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

☐ Claims renumbered in the same order as presented by applicant		☐ CPA		☒ T.D.		☐ R.1.47			
CLAIM		DATE							
Final	Original	05/18/2007	01/31/2008	11/04/2008					
	1	-							
	2	✓	✓	✓					
	3	✓	✓	✓					
	4	✓	✓	✓					
	5	✓	✓	✓					
	6	✓	✓	✓					
	7	✓	✓	✓					
	8	✓	✓	✓					
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	27		✓	=					
	28		✓						
	29			✓					
	30			=					
	31			=					



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 (Use as many sheets as necessary)				Complete if Known	
				Application Number	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BOHM
				Art Unit	3653
				Examiner Name	M. Hageman
Sheet	1	of	2	Attorney Docket Number	TGZ-021DVCN

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-3,791,517	02-12-1974	Friedman	
	A2*	US-3,827,555	08-06-1974	Kamentsky et al.	
	A3*	US-4,318,483	03-09-1982	Lombardo et al.	
	A4*	US-4,365,719	12-28-1982	Kelly et al.	
	A5*	US-4,939,081	07-03-1990	Figdor et al.	
	A6*	US-5,637,496	06-10-1997	Thaler et al.	
	A7*	US-5,783,446	07-21-1998	Saul et al.	
	A8*	US-5,795,727	08-18-1998	Bierre et al.	
	A9*	US-5,979,664	11-09-1999	Brodeur	
	A10*	US-5,998,212	12-07-1999	Corio et al.	
	A11*	US-6,057,111	05-02-2000	Deiss et al.	
	A12*	US-6,214,556	04-10-2001	Olek et al.	
	A13*	US-6,280,967	08-28-2001	Ransom, John et al.	
	A14*	US-20020093641	07-18-2002	Ortyn et al.	
	A15*	US-6,454,862	09-24-2002	Yoshida et al.	
	A16*	US-6,455,280	09-24-2002	Edwards et al.	
	A17*	US-6,482,652	11-19-2002	Furlong et al.	
	A18*	US-6,524,790-B1	02-25-2003	Kopf-Sill et al.	
	A19*	US-6,803,194	10-12-2004	Keck 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-98/10267-A1	03-12-1998	Technical University of Denmark		
	B2	WO-00/70080-A1	11-23-2000	Caliper Technologies Corp.		

Examiner Signature	/Mark Hageman/	Date Considered	11/04/2008

*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 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.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /MH/

Receipt date: 07/25/2008

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Substitute for form 1449/PTO		Complete if Known			
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)		Application Number	11/499,953-Conf. #3579		
		Filing Date	August 7, 2006		
		First Named Inventor	Sebastian BOHM		
		Art Unit	3653		
		Examiner Name	M. Hageman		
Sheet	2	of	2	Attorney Docket Number	TGZ-021DVCN

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	Australian Office Action for Application No. 2003228630, dated February 11, 2008	

Examiner Signature	/Mark Hageman/	Date Considered	11/04/2008

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¹Applicant's unique citation designation number (optional). ²Applicant is to place a check mark here if English language Translation is attached.

Express Mail Label No. EM 067556237 US Dated: July 25, 2008

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Cytonome/ST, LLC Exhibit 2013

NanoCollect Biomedical, Inc. v. Cytonome/ST, LLC, IPR2020-00550

Page 94 of 406

Receipt date: 08/25/2008

PTO/SB/08b (08-08)

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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
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				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BOHM
				Art Unit	3653
				Examiner Name	M. Hageman
Sheet	1	of	1	Attorney Docket Number	TGZ-021DVCN

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

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

Examiner Signature		Date Considered	

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NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
/MH/	C1	Chinese Office Action for Application No. 03825178.7, dated June 27, 2008	

Examiner Signature	/Mark Hageman/	Date Considered	11/04/2008

*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.

¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).	
Dated: August 25, 2008	Electronic Signature for David R. Burns: /David R. Burns/

Application Number 	Application/Control No. 11/499,953	Applicant(s)/Patent under Reexamination BOHM ET AL.	
Document Code - DISQ		Internal Document – DO NOT MAIL	

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 8/12/08	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:
BRIAN

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Dated: August 25, 2008
Electronic Signature for David R. Burns: /David R. Burns/

Docket No.: TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Boehm *et al.*

Application No.: 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3653

For: METHOD AND APPARATUS FOR
SORTING PARTICLES

Examiner: M. Hageman

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

In accordance with 37 CFR 1.97, Applicants hereby make of record the following additional documents. A PTO Form SB/08 and a full copy of each of the documents required under 37 CFR 1.98(a)(2) accompany this statement.

Applicants have become aware of a Chinese Office Action issued June 27, 2008, during the prosecution of Application No. 03825178.7, which corresponds to the above referenced application, and in accordance with 37 CFR 1.97(c)(1) and (e)(1), hereby submit this document for the Examiner's consideration. This document is cited on the enclosed PTO Form SB/08, and a copy of the Office Action required under 37 CFR 1.98(a)(2) cited thereon is enclosed.

This statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any document herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

This Supplemental Information Disclosure Statement is filed more than three months after the U.S. filing date, and after the mailing date of the first Office Action on the merits, but before the mailing date of a Final Office Action or Notice of Allowance (37 CFR 1.97(c)).

I hereby certify, pursuant to 37 CFR 1.97(e)(1), that each item of information contained in this Supplemental Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this Supplemental Information Disclosure Statement.

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. 12-0080, under Order No. TGZ-021DVCN.

Dated: August 25, 2008

Respectfully submitted,

DRB/mch

Electronic signature: /David R. Burns/
David R. Burns
Registration No.: 46,590
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One Post Office Square
Boston, Massachusetts 02109-2127
(617) 227-7400
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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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	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BOHM
				Art Unit	3653
				Examiner Name	M. Hageman
Sheet	1	of	1	Attorney Docket Number	TGZ-021DVCN

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

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

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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. 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.

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	Chinese Office Action for Application No. 03825178.7, dated June 27, 2008	

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Dated: August 25, 2008	Electronic Signature for David R. Burns: /David R. Burns/

Electronic Acknowledgement Receipt	
EFS ID:	3822603
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	Method and apparatus for sorting particles
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	00959
Filer:	David Burns/Lauren Margolis
Filer Authorized By:	David Burns
Attorney Docket Number:	TGZ-021DVCN
Receipt Date:	25-AUG-2008
Filing Date:	07-AUG-2006
Time Stamp:	11:59:46
Application Type:	Utility under 35 USC 111(a)

Payment information:

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File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement Letter	Information_Disclosure_State ment.pdf	28567 fcdcafe93d548c51f28f57acd903c0811b146183	no	2
Warnings:					
Information:					

2	Information Disclosure Statement (IDS) Filed (SB/08)	SB08.pdf	33427 39a23069239bf121fe320f16299fb219079010c	no	1
Warnings:					
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3	NPL Documents	Chinese_Office_Action.pdf	3184228 eb91ec91455fb614d81b38770606cdfb636a94615	no	15
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Dated: August 12, 2008
Electronic Signature for John S. Curran: /John S. Curran/

Docket No. TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Böhm *et al.*

Application No. 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3653

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

Examiner: M. Hageman

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION

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

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated February 12, 2008 (Paper Number 20080131), the response period of which extended to August 12, 2008 by the accompanying Request for Extension of Time, please amend the above-identified U.S. patent application as follows:

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

Remarks/Arguments begin on page 7 of this paper.

AMENDMENTS TO THE CLAIMS

Please cancel claim 22, amend claims 2, 4, 15, 21, 25 and 28 and add claims 28-31 as set forth in the following listing of claims, which will replace all prior versions, and listings, of claims in the application.

1. (Canceled).
2. (Currently Amended) A particle sorting system, comprising:
 - a plurality of substantially parallel sorting channels formed on a substrate,
 - a plurality of sensors, each sensor associated with one of said sorting channels for detecting and classifying particles in the associated sorting channel based on a first predetermined characteristic;
 - a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having the first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including an actuator controlled by one of said plurality of sensors for selectively applying a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet, while particles not having the predetermined characteristic flow into the second outlet undisturbed.
3. ((Previously Presented) The particle sorting system of claim 2, wherein the actuator is arranged external to the substrate and in communication with the associated sorting channel.
4. (Currently Amended) The particle sorting system of claim 3, wherein each sorting module further includes a gas-filled reservoir external to and in communication with ~~the an~~ interior of the microchannel interior, wherein a fluid meniscus forms an interface between the gas-filled reservoir and the interior of the associated sorting channel.
5. (Previously Presented) The particle sorting system of claim 4, wherein each actuator selectively increases an internal pressure of the associated gas-filled reservoir to create the pressure pulse in the associated sorting channel.
6. (Previously Presented) The particle sorting system of claim 4, wherein each sorting module further comprises a side passage connecting the associated sorting channel with the gas-filled reservoir.

7. (Previously Presented) The particle sorting system of claim 4, wherein the gas-filled reservoir includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir, wherein the associated actuator selectively deflects the movable membrane to generate the pressure pulse.

8. (Previously Presented) The particle sorting system of claim 2, wherein each sorting module further comprises a buffer for absorbing the pressure pulse.

9. (Previously Presented) The particle sorting system of claim 2, further comprising an aggregation region for aggregating particles having the predetermined characteristic from a plurality of the first outlets of the sorting channels.

10. (Previously Presented) The particle sorting system of claim 2, further comprising:
at least one secondary sorting channel in series with the plurality of substantially parallel sorting channels for collecting selected particles having the first predetermined characteristic from a plurality of first outlets and separating particles in the secondary sorting channel based on a second predetermined characteristic.

11. (Previously Presented) The particle sorting system of claim 10 wherein the second predetermined characteristic is different from the first predetermined characteristic.

12. (Previously Presented) The particle sorting system of claim 4, wherein the plurality of sorting channels are formed by grooves etched in a first plate, and the gas-filled reservoirs are formed by a second plate bonded to the first plate, the second plate including a plurality of apertures defining the gas-filled reservoirs.

13. (Previously Presented) The particle sorting system of claim 12, further comprising:
a flexible membrane bonded to the second plate to seal at least one of the gas-filled reservoirs and defining at least a portion of a movable wall of at least one of the gas-filled reservoirs.

14. (Previously Presented) The particle sorting system of claim 2, further comprising an inlet channel that branches to supply a plurality of the substantially parallel sorting channels with a stream of particles to be sorted.

15. (Currently Amended) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, and
a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having a first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including a sealed gas-filled reservoir disposed in the substrate in communication with the associated sorting channel and an actuator for increasing an internal pressure of the gas-filled reservoir to selectively modify fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic into the first outlet of the associated sorting channel.
16. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir.
17. (Previously Presented) The particle sorting system of claim 16, wherein each actuator selectively deflects the movable membrane to increase pressure in the gas-filled reservoir to modify said fluid flow.
18. (Canceled).
19. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further comprises a buffer for limiting said modification of the fluid flow to a region of the associated sorting channel along the sorting module and preventing flow disturbance in other regions of the associated sorting channel.
20. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further includes a side channel connecting the gas-filled reservoir to the associated sorting channel.
21. (Currently Amended) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, each sorting channel capable of conveying a stream of particles in a carrier fluid,
a plurality of actuators arranged external to the substrate, each actuator associated with a sorting channel formed on the substrate, for selectively creating a flow disturbance within the

associated sorting channel to deflect a selected particle in the stream of particles from the stream of particles; and

a plurality of buffers, each buffer associated with an actuator for absorbing the flow disturbance in the associated sorting channel, wherein each buffer comprises a gas-filled reservoir in fluid communication with the associated sorting channel.

22. Canceled

23. (Previously Presented) The particle sorting system of claim 21, wherein each sorting channel includes a gas-filled reservoir in fluid communication therewith, and the actuator increases an internal pressure of the gas-filled reservoir to create said flow disturbance.

24. (Previously Presented) The particle sorting system of claim 23, wherein each gas-filled reservoir includes a flexible membrane forming at least a portion of a side wall of the gas-filled reservoir, and the actuator deflects the flexible membrane to increase said internal pressure.

25. (Previously Presented) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, and
a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having a first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including a gas-filled reservoir formed in the substrate having a movable wall disposed in communication with the associated sorting channel for selectively modifying fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic into the first outlet of the associated sorting channel.

26. (Previously Presented) The particle sorting system of claim 25, wherein the movable wall comprises a flexible membrane.

27. (Previously Presented) The particle sorting system of claim 25, wherein each sorting module further includes an associated actuator for selectively moving the movable wall to increase an internal pressure of the gas-filled reservoir to selectively modify the fluid flow through the associated sorting channel.

28. (Currently Amended) A particle sorting system, comprising:

a plurality of substantially parallel sorting channels formed on a substrate,
a plurality of sensors, each sensor associated with one of said sorting channels for detecting and classifying particles in the associated sorting channel based on a first predetermined characteristic;

a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having the first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including an actuator arranged external to the substrate and in communication with the associated sorting channel, the actuator controlled by one of said plurality of sensors for selectively applying a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet, while particles not having the predetermined characteristic flow into the second outlet undisturbed.

29. (NEW) The particle sorting system of claim 2, wherein the first outlet and the second outlet are open and free of valves.

30. (NEW) The particle sorting system of claim 23, wherein each buffer is arranged opposite the gas-filled reservoir in the flow channel.

31. (NEW) The particle sorting system of claim 27, wherein the actuator selectively contacts and depresses the movable wall to increase an internal pressure of the gas-filled reservoir.

REMARKS

The foregoing amendment cancels claim 22, amends claims 2, 4, 15, 21, 25 and 28 and adds dependent claims 28-31. Pending in the application are claims 2-17, 19-21 and 23-28, of which claims 2, 15, 21, 25 and 28 are independent. The following comments address all stated grounds for rejection and place the presently pending claims, as identified above, in condition for allowance.

Claims 2 and 28 are amended to specify that particles not having the predetermined characteristic flow into the second outlet undisturbed, as set forth throughout the specification as originally filed, at least, for example, on page 6, lines 5-7.

Claim 4 is amended for purposes of clarity, namely to change the phrase “the microchannel interior” to ---an interior of the microchannel---.

Claims 15 and 25 are amended to specify that the gas-filled reservoir of each actuator is formed in the substrate, as shown in Figures 1-4 and 7.

Claim 21 is amended to include the subject matter of canceled claim 22.

New dependent claims 29-31 are added to more fully capture the instant invention. Claim 29 depends from claim 2 and further specifies that the first outlet and the second outlet are open and free of valves, and show in the Figures and described in the specification as originally filed.

Claim 30 depends from claim 23 and specifies that each buffer is arranged opposite the gas-filled reservoir in the flow channel, as shown in Figures 1-4 and described at least on page 4, lines 24-26.

Claim 31 depends on claim 27, and specifies that the actuator selectively contacts and depresses the movable wall to increase an internal pressure of the gas-filled reservoir, as shown in Figure 7 and described on page 16, lines 23-25. *No new matter is added.*

Amendment and/or cancellation of the claims is not to be construed as an acquiescence to any of the objections/rejections set forth in the instant Office Action, and was done solely to expedite prosecution of the application. Applicant reserves the right to pursue the claims as originally filed, or similar claims, in this or one or more subsequent patent applications.

Double Patenting Rejections

Applicants thank the Examiner for the close review of the claims. In the Office Action, the Examiner rejects claims 2-17 and 19-28 on the ground of non-statutory obviousness-type double patenting as being unpatentable over co-owned U.S. Patent Number 7,104,405 in view of U.S. Patent Number 6,808,075. Applicants respectfully disagree and submit that the presently pending claims are different and distinguish patentably from claims 1-4 of U.S. Patent Number 7,104,405 and claims 1-22 of U.S. Patent Number 6,808,075.

However, to expedite prosecution, Applicants include herewith a Terminal Disclaimer to overcome the Double Patenting Rejection.

35 U.S.C. §112 Rejections

Regarding the rejection of claims 4-7, 12 and 12 under 35 U.S.C. §112, second paragraph for being indefinite, Applicants have amended claim 4 to rectify the lack of antecedent basis for the microchannel interior, and request that the 35 U.S.C. §112, second paragraph rejection be reconsidered and withdrawn.

35 U.S.C. §103 Rejections

Applicants thank the Examiner for the close review of the claims and for the reconsideration and removal of the previous rejection. In the Office Action, the Examiner rejects claims 2-17 and 19-28 under 35 U.S.C. 103(a) as being obvious over the Diessel reference (U.S. Patent Number 5,837,200) in view of the Chou reference (U.S. Patent Number 7,258,774). As recognized by the Examiner, the Diessel reference lacks a sorting system that employs a pressure pulse, as recited in independent claims claim 2 and 28, and/or a gas-filled reservoir for selectively deflecting a selected particle, as recited in independent claims 15 and 25. In addition, as recognized by the Examiner, the Diessel reference also fails to disclose a buffer/actuator arrangement in a sorting system, as set forth in independent claim 21.

According to the Examiner, the Chou reference, in Figures 7B, 7H, 31A and 31B discloses an external actuator to selectively apply a pressure pulse to deflect a particle having a predetermined characteristic, a gas-filled reservoir, a fluid meniscus, a side passage, a movable membrane, a buffer and other features of the invention. The Examiner concludes that it would be obvious to include the actuator system taught by Chou in the system of Diessel. According to the Examiner, the combination of the Chou reference and the Diessel reference renders claims 2-17 and 19-28 unpatentable.

Applicants respectfully disagree and submit that the claims distinguish over the cited references. Even in combination, the references fail to disclose the subject matter of claims 2-17 and 19-28. Moreover, motivation to combine the references, as required under 35 U.S.C. 103, is lacking.

The Chou reference does not disclose the claimed actuator for selectively deflecting selected particles from a stream of particles. The Chou reference is directed to a microfluidic device comprises pumps, valves, and fluid oscillation dampers. The Chou reference employs valves to direct entities to either a waste pool or a collection pool. In one embodiment shown in Figures 31a and 31b, the Chou reference employs a pump to reverse a flow of materials through an open collection valve 3018A. The Chou reference does not rely on a pressure *pulse* that selectively deflects a single particle at a time, but rather, a pump that changes the whole flow of the *entire* material in the channel for an extended period of time “until the material is again detected by the detector”, as set forth in column 31, lines 55-65-column 32, line 3. The Chou reference relies on “changing pressure applied to flow channel inlets and outlets”, rather than a pressure pulse, which is momentary and directly applied to a single selected particle, as recited in claims 2 and 28.

Figure 31a of Chou illustrates the use of valves 3018 to selectively block outlets 3026 and 3030, but does not employ a positive pressure pulse applied to a particle.

As set forth on column 14, lines 15+ (cited by the Examiner), the Chou reference relies on air pressure to actuate a membrane 25 that separates two flow channels. The membrane 25

selectively pinches off flow F passing through a flow channel or allows the flow channel to remain open. In contrast, the flow channels in the claimed invention remain open, relying on a pressure pulse applied to a single selected particle to control fluid flow. The embodiments of Figures 7A-7H in Chou merely show a valve that operates by selectively blocking a channel, rather than a sorting actuator, and are therefore inapplicable to the claimed invention.

In addition, Chou fails to disclose that particles not having a predetermined characteristic flow into a second outlet undisturbed, as also set forth in claims 2 and 28. In Chou, particles flow to both the waste channel and collection channel in the absence of a blocked valve.

Moreover, Chou fails to disclose sealed gas-filled reservoir, particularly a sealed gas-filled reservoir disposed in the same substrate as an associated sorting channel, as set forth in independent claims 15 and 25. As set forth in column 14, line 15+, an external solenoid valve may be used to apply pressure to a flow channel inlet or outlet. However, Chou does not disclose a reservoir formed in a substrate, rather than a distant pressure source. There is no indication that the source of the pressure in Chou comprise a sealed, gas-filled reservoir, or that the increased pressure is used to selectively modify fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic. Rather, the external solenoid valve of Chou is used merely to induce the flow of fluid through a channel, rather than effect a sorting process.

Regarding independent claim 21, the Chou reference also does not disclose a sorting system that creates a flow disturbance within an associated sorting channel to deflect a selected. Chou also fails to disclose a buffer for absorbing a flow disturbance comprising a gas-filled reservoir in fluid communication with the associated sorting channel. Even if the damper in Chou could be considered a buffer, the “damper is separated from the fluid flow channel by a thin membrane” (see column 27, lines 23-38 and Figures 26-28). Therefore, the damper of Chou must be sealed *from* the flow channel, in contrast to the claimed invention, which required fluid communication between the channel and buffer.

The dependent claims recite additional patentable features not disclosed in the cited references. For example, claims 3 specifies that the actuator is arranged external to the substrate

and in communication with the associated sorting channel, a feature not disclosed the cited references, as described above.

Claims 4-6, 18 and 23 recite a gas-filled reservoir in communication with a sorting channel for sorting particles, a feature not disclosed in the cited references. No gas-filled reservoir is shown or described in Chou.

Claims 7, 13, 16, 17 and 24 recite a movable membrane forming a side wall of a reservoir, which an actuator deflects to generate a pressure pulse to deflect a particle. The membrane 25 of Chou does not generate a pressure pulse, nor does the membrane 25 form a side wall of a reservoir.

Claims 8, 19 and 22 recite a buffer for absorbing a pressure pulse used to selectively deflect a particle, a feature not disclosed in the cited references, as described above.

Claim 9 recites an aggregation region for aggregating particles having the predetermined characteristic from a plurality of the first outlets of the sorting channels, a feature also not disclosed in the cited references.

Claims 10-11 recite a secondary sorting channel for receiving sorted particles and performing a secondary sorting operation, also not disclosed in the cited references.

Claims 12-13 specify that the system comprises a plurality of plates stacked together, a feature also not disclosed in the cited references.

New Claims

New dependent claims 29-31 also recite patentable features not disclosed in the cited references. For example, claim 29, which depends from claim 2, specifies that the first outlet and the second outlet are open and free of valves, in clear contrast to the Chou reference, which relies on waste valves and collection valves 3018 to control fluid flow.

Claim 30 depends from claim 23 and specifies that each buffer is arranged opposite the gas-filled reservoir in the flow channel. In Chou, the flow channels are flat and have no features opposite the damper areas.

Claim 31 depends from claim 27 and specifies that the actuator selectively contacts and depresses the movable wall to increase an internal pressure of the gas-filled reservoir. Chou lacks an actuator that directly contacts a membrane.

For at least these reasons, Applicants submit that the pending claims distinguish patentably over the combination of the Diessel reference and the Chou reference, and request that the obviousness-type rejection be reconsidered and withdrawn.

CONCLUSION

In view of the above amendment and remarks, Applicants believe the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned attorney at (617) 227-7400.

If any additional fee is due with this statement, please charge our Deposit Account No. 12-0080, under Order No. TGZ-021DVCN, from which the undersigned is authorized to draw

Dated: August 12, 2008

Respectfully submitted,

Electronic Signature: /John S. Curran/
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2008 <i>(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)</i>		Docket Number (Optional) TGZ-021DVCN																									
Application Number 11/499,953-Conf. #3579		Filed August 7, 2006																									
For METHOD AND APPARATUS FOR SORTING PARTICLES																											
Art Unit 3653		Examiner M. Hageman																									
This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application. The requested extension and fee are as follows (check time period desired and enter the appropriate fee below): <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 40%;"></th> <th style="width: 15%; text-align: center;"><u>Fee</u></th> <th style="width: 15%; text-align: center;"><u>Small Entity Fee</u></th> <th style="width: 30%;"></th> </tr> </thead> <tbody> <tr> <td>☐ One month (37 CFR 1.17(a)(1))</td> <td style="text-align: center;">\$120</td> <td style="text-align: center;">\$60</td> <td>\$ _____</td> </tr> <tr> <td>☐ Two months (37 CFR 1.17(a)(2))</td> <td style="text-align: center;">\$460</td> <td style="text-align: center;">\$230</td> <td>\$ _____</td> </tr> <tr> <td>☒ Three months (37 CFR 1.17(a)(3))</td> <td style="text-align: center;">\$1050</td> <td style="text-align: center;">\$525</td> <td>\$ 525.00</td> </tr> <tr> <td>☐ Four months (37 CFR 1.17(a)(4))</td> <td style="text-align: center;">\$1640</td> <td style="text-align: center;">\$820</td> <td>\$ _____</td> </tr> <tr> <td>☐ Five months (37 CFR 1.17(a)(5))</td> <td style="text-align: center;">\$2230</td> <td style="text-align: center;">\$1115</td> <td>\$ _____</td> </tr> </tbody> </table> ☒ Applicant claims small entity status. See 37 CFR 1.27. ☐ A check in the amount of the fee is enclosed. ☐ Payment by credit card. Form PTO-2038 is attached. ☒ The Director has already been authorized to charge fees in this application to a Deposit Account. ☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 12-0080 . I have enclosed a duplicate copy of this sheet. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. I am the ☐ applicant/inventor. ☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96). ☒ attorney or agent of record. Registration Number 50,445 ☐ attorney or agent under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____ _____ /John S. Curran/ Signature _____ John S. Curran Typed or printed name _____ August 12, 2008 Date _____ (617) 994-0854 Telephone Number NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below. ☐ Total of 1 forms are submitted.					<u>Fee</u>	<u>Small Entity Fee</u>		☐ One month (37 CFR 1.17(a)(1))	\$120	\$60	\$ _____	☐ Two months (37 CFR 1.17(a)(2))	\$460	\$230	\$ _____	☒ Three months (37 CFR 1.17(a)(3))	\$1050	\$525	\$ 525.00	☐ Four months (37 CFR 1.17(a)(4))	\$1640	\$820	\$ _____	☐ Five months (37 CFR 1.17(a)(5))	\$2230	\$1115	\$ _____
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Dated: August 12, 2008

Electronic Signature for John S. Curran: /John S. Curran/

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TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) TGZ-021DVCN
In re Application of: Sebastian BOHM <i>et al.</i> Application No.: 11/499,953-Conf. #3579 Filed: August 7, 2006 For: METHOD AND APPARATUS FOR SORTING PARTICLES The owner*, <u>Cytonome, Inc.</u>, of <u>100</u> percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior prior patent No. 6,808,075 and pending prior patent No. 7,104,405 as the term of said patent is defined in 35 U.S.C. 154 and 173, and as the term of said prior patents is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patents are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns. In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 and 173 of the prior patents, "as the term of said prior patents are presently shortened by any terminal disclaimer," in the event that said prior patents later: - expires for failure to pay a maintenance fee; - is held unenforceable; - is found invalid by a court of competent jurisdiction; - is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321; - has all claims canceled by a reexamination certificate; - its reissued; or - is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer. Check either box 1 or 2 below, if appropriate. 1. ☐ For submissions on behalf of a business/organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the business/organization. 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. 2. ☒ The undersigned is an attorney or agent of record. Reg. No. <u>50,445</u> <u>/John S. Curran/</u> Signature <u>John S. Curran</u> Typed or printed name <u>August 12, 2008</u> Date <u>(617) 994-0890</u> Telephone Number ☒ Terminal disclaimer fee under 37 CFR 1.20(d) is included. 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. *Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner). Form PTO/SB/96 may be used for making this statement. See MPEP § 324.	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4). Dated: August 12, 2008 Electronic Signature for John S. Curran: <u>/John S. Curran/</u>	
Error! Unknown document property name. Error! Unknown document property name.	

Electronic Patent Application Fee Transmittal				
Application Number:		11499953		
Filing Date:		07-Aug-2006		
Title of Invention:		Method and apparatus for sorting particles		
First Named Inventor/Applicant Name:		Sebastian Bohm		
Filer:		John S. Curran/Lauren Margolis		
Attorney Docket Number:		TGZ-021DVCN		
Filed as Small Entity				
Utility Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Claims in excess of 20	2202	2	25	50
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Statutory disclaimer	2814	1	65	65

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Extension - 3 months with \$0 paid	2253	1	525	525
Miscellaneous:				
Total in USD (\$)				640

Electronic Acknowledgement Receipt

EFS ID:	3765641
Application Number:	11499953
International Application Number:	
Confirmation Number:	3579
Title of Invention:	Method and apparatus for sorting particles
First Named Inventor/Applicant Name:	Sebastian Bohm
Customer Number:	959
Filer:	John S. Curran
Filer Authorized By:	
Attorney Docket Number:	TGZ-021DVCN
Receipt Date:	12-AUG-2008
Filing Date:	07-AUG-2006
Time Stamp:	15:45:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 640
RAM confirmation Number	1131
Deposit Account	120080
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (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	Amendment - After Non-Final Rejection	Response.pdf	84277	no	13
			eb08ed974869e92613c9971976a9efea47cc967d		

Warnings:**Information:**

2	Extension of Time	Petition_for_Extension_of_Ti me.pdf	31189	no	1
			e8667b268c8fedb56317c2e76a9385c973cc9e6cd		

Warnings:**Information:**

3	Terminal Disclaimer Filed	Terminal_Disclaimer.pdf	24848	no	1
			31712679ffe7e423df238c22361183080d3f25f		

Warnings:**Information:**

4	Fee Worksheet (PTO-06)	fee-info.pdf	8436	no	2
			179a1baebd65091c4d9d9b35679f2c71fe43bfb6		

Warnings:**Information:**

Total Files Size (in bytes):	148750

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 11/499,953		Filing Date 08/07/2006		☐ To be Mailed							
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				N/A						
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A		N/A		N/A				N/A						
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A		N/A		N/A				N/A						
TOTAL CLAIMS (37 CFR 1.16(i))		minus 20 =		*		X \$ =				X \$ =						
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 =		*		X \$ =				X \$ =						
☐ 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).														
☐ 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				TOTAL						
APPLICATION AS AMENDED – PART II																
(Column 1)			(Column 2)			(Column 3)			SMALL ENTITY		OR		OTHER THAN SMALL ENTITY			
AMENDMENT	08/12/2008		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA		RATE (\$)		ADDITIONAL FEE (\$)		RATE (\$)		ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))		* 28		Minus ** 26		= 2		X \$25 =		50		OR X \$ =			
	Independent (37 CFR 1.16(h))		* 5		Minus *** 5		= 0		X \$105 =		0		OR X \$ =			
	☐ Application Size Fee (37 CFR 1.16(s))															
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))															
						TOTAL ADD'L FEE		50		OR		TOTAL ADD'L FEE				
AMENDMENT	Total (37 CFR 1.16(i))		*		Minus **		=		X \$ =		OR X \$ =		X \$ =		OR X \$ =	
	Independent (37 CFR 1.16(h))		*		Minus ***		=		X \$ =		OR X \$ =		X \$ =		OR X \$ =	
	☐ Application Size Fee (37 CFR 1.16(s))															
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))															
						TOTAL ADD'L FEE				OR		TOTAL ADD'L FEE				
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.										Legal Instrument Examiner: /MARY A. HOLMES/						

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.



07-28-08

TH

PTO/SB/21 (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 displays a valid OMB control number.

TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	11/499,953-Conf. #3579
	Filing Date	August 7, 2006
	First Named Inventor	Sebastian BOHM
	Art Unit	3653
	Examiner Name	M. Hageman
Total Number of Pages in This Submission	Attorney Docket Number	TGZ-021DVCN

ENCLOSURES (Check all that apply)

☒ Fee Transmittal Form ☐ Fee Attached ☐ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☒ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please Identify below): Return Receipt Postcard PTO form SB/08 Copies of Three (3) References
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	LAHIVE & COCKFIELD, LLP		
Signature			
Printed name	David R. Burns		
Date	July 25, 2008	Reg. No.	46,590

Express Mail Label No. EM 067556237 US Dated: July 25, 2008



PTO/SB/17 (10-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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

Effective on 12/08/2004. Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).		Complete if Known	
FEE TRANSMITTAL For FY 2008		Application Number	11/499,953-Conf. #3579
		Filing Date	August 7, 2006
		First Named Inventor	Sebastian BOHM
		Examiner Name	M. Hageman
		Art Unit	3653
☒ Applicant claims small entity status. See 37 CFR 1.27	Attorney Docket No.	TGZ-021DVCN	
TOTAL AMOUNT OF PAYMENT	(\$) 180.00		

METHOD OF PAYMENT (check all that apply)	
☐ Check	☐ Credit Card
☐ Money Order	☐ None
☐ Other (please identify): _____	
☒ Deposit Account	Deposit Account Number: 12-0080
Deposit Account Name: Lahive & Cockfield, LLP	
For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)	
☒ Charge fee(s) indicated below	☐ Charge fee(s) indicated below, except for the filing fee
☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17	☒ Credit any overpayments

FEE CALCULATION							
1. BASIC FILING, SEARCH, AND EXAMINATION FEES							
	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fees Paid (\$)
Utility	310	155	510	255	210	105	
Design	210	105	100	50	130	65	
Plant	210	105	310	155	160	80	
Reissue	310	155	510	255	620	310	
Provisional	210	105	0	0	0	0	
2. EXCESS CLAIM FEES							
						Small Entity	
						Fee (\$)	Fee (\$)
Fee Description							
Each claim over 20 (including Reissues)						50	25
Each independent claim over 3 (including Reissues)						210	105
Multiple dependent claims						370	185
Total Claims		Extra Claims	Fee (\$)	Fee Paid (\$)	Multiple Dependent Claims		
- =		x	=		Fee (\$)	Fee Paid (\$)	
HP = highest number of total claims paid for, if greater than 20.							
Indep. Claims		Extra Claims	Fee (\$)	Fee Paid (\$)			
- =		x	=				
HP = highest number of independent claims paid for, if greater than 3.							
3. APPLICATION SIZE FEE							
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$260 (\$130 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).							
Total Sheets		Extra Sheets	Number of each additional 50 or fraction thereof		Fee (\$)	Fee Paid (\$)	
- 100 =		/50 =	(round up to a whole number) x		=		
4. OTHER FEE(S)							
Non-English Specification, \$130 fee (no small entity discount)						Fees Paid (\$)	
Other (e.g., late filing surcharge): 1806 Submission of an Information Disclosure Statement						180.00	

SUBMITTED BY			
Signature	<i>David R Burns</i>	Registration No. (Attorney/Agent)	46,590
Name (Print/Type)	David R. Burns	Telephone	(617) 994-0890
		Date	July 25, 2008

Express Mail Label No. EM 067556237 US Dated: July 25, 2008



PTO/SB/17 (10-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL For FY 2008

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$)
180.00

Complete if Known

Application Number	11/499,953-Conf. #3579
Filing Date	August 7, 2006
First Named Inventor	Sebastian BOHM
Examiner Name	M. Hageman
Art Unit	3653
Attorney Docket No.	TGZ-021DVCN

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 12-0080 Deposit Account Name: Lahive & Cockfield, LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, except for the filing fee

☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	
Utility	310	155	510	255	210	105	
Design	210	105	100	50	130	65	
Plant	210	105	310	155	160	80	
Reissue	310	155	510	255	620	310	
Provisional	210	105	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee (\$)	Small Entity Fee (\$)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	210	105
Multiple dependent claims	370	185

Total Claims	Extra Claims	Fee (\$)	Fee Paid (\$)	Multiple Dependent Claims
				Fee (\$)
				Fee Paid (\$)

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee (\$)	Fee Paid (\$)

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$260 (\$130 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).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)

4. OTHER FEE(S)

Non-English Specification, \$130 fee (no small entity discount)

Other (e.g., late filing surcharge): 1806 Submission of an Information Disclosure Statement 180.00

SUBMITTED BY

Signature	David R. Burns	Registration No. (Attorney/Agent)	46,590	Telephone	(617) 994-0890
Name (Print/Type)	David R. Burns	Date	July 25, 2008		

Express Mail Label No. EM 067556237 US Dated: July 25, 2008



Express Mail Label No. EM 067556237 US Dated: July 25, 2008

Docket No.: TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Boehm *et al.*

Application No.: 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3653

For: METHOD AND APPARATUS FOR
SORTING PARTICLES

Examiner: M. Hageman

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

In accordance with 37 CFR 1.97, Applicants hereby make of record the following additional documents. A PTO Form SB/08 and a full copy of each of the documents required under 37 CFR 1.98(a)(2) accompany this statement.

Applicants have become aware of the following documents, cited in an Australian Office Action issued February 11, 2008, during the prosecution of Application No. 2003228630, which corresponds to the above referenced application, and in accordance with 37 CFR 1.97(c) and (e)(1) or (b)(3), hereby submit these documents for the Examiner's consideration. These documents are cited on the enclosed PTO Form SB/08, and a copy of the Office Action and of each document required under 37 CFR 1.98(a)(2) cited thereon are enclosed as well.

Applicants have not submitted copies of each cited U.S. patent and U.S. patent application in accordance with 37 CFR 1.98(a)(2)(ii). Applicants submit herewith copies of foreign patents and non-patent references in accordance with 37 CFR 1.98(a)(2).

07/28/2008 CCHAU1 00000033 120080 11499953
01 FC:1806 180.00 DA

The Examiner is also advised that the following commonly owned patents and applications contain subject matter that may be related to the present application.

Application No.:	10/179488	Filing date:	06-24-2002
Application No.:	10/329008	Filing date:	12-23-2002
Application No.:	11/101038	Filing date:	04-06-2002
Application No.:	10/940143	Filing date:	09-13-2004
Application No.:	10/664587	Filing date:	09-16-2003
Application No.:	11/603444	Filing date:	11-22-06

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 herein, but will be made available upon request.

This statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any document herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

This Supplemental Information Disclosure Statement is filed more than three months after the U.S. filing date, and after the mailing date of the first Office Action on the merits, but before the mailing date of a Final Office Action or Notice of Allowance (37 CFR 1.97(c)).

Application No.: 11/499,953

Docket No.: TGZ-021DVCN

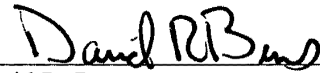
Please charge our Deposit Account No. 12-0080 in the amount of \$180.00 covering the fee set forth in 37 CFR 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. TGZ-021DVCN.

Dated: July 25, 2008

DRB/mch

Respectfully submitted,

By



David R. Burns

Registration No.: 46,590

LAHIVE & COCKFIELD, LLP

One Post Office Square

Boston, Massachusetts 02109-2127

(617) 227-7400

(617) 742-4214 (Fax)

Attorney/Agent For Applicant



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 (Use as many sheets as necessary)		Complete if Known			
		Application Number	11/499,953-Conf. #3579		
		Filing Date	August 7, 2006		
		First Named Inventor	Sebastian BOHM		
		Art Unit	3653		
		Examiner Name	M. Hageman		
Sheet	1	of	2	Attorney Docket Number	TGZ-021DVCN

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-3,791,517	02-12-1974	Friedman	
	A2*	US-3,827,555	08-06-1974	Kamentsky et al.	
	A3*	US-4,318,483	03-09-1982	Lombardo et al.	
	A4*	US-4,365,719	12-28-1982	Kelly et al.	
	A5*	US-4,939,081	07-03-1990	Figdor et al.	
	A6*	US-5,637,496	06-10-1997	Thaler et al.	
	A7*	US-5,783,446	07-21-1998	Saul et al.	
	A8*	US-5,795,727	08-18-1998	Bierre et al.	
	A9*	US-5,979,664	11-09-1999	Brodeur	
	A10*	US-5,998,212	12-07-1999	Corio et al.	
	A11*	US-6,057,111	05-02-2000	Deiss et al.	
	A12*	US-6,214,556	04-10-2001	Olek et al.	
	A13*	US-6,280,967	08-28-2001	Ransom, John et al.	
	A14*	US-20020093641	07-18-2002	Ortyn et al.	
	A15*	US-6,454,862	09-24-2002	Yoshida et al.	
	A16*	US-6,455,280	09-24-2002	Edwards et al.	
	A17*	US-6,482,652	11-19-2002	Furlong et al.	
	A18*	US-6,524,790-B1	02-25-2003	Kopf-Sill et al.	
	A19*	US-6,803,194	10-12-2004	Keck et al.	

FOREIGN PATENT DOCUMENTS						
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	B1	WO-98/10267-A1	03-12-1998	Technical University of Denmark		
	B2	WO-00/70080-A1	11-23-2000	Caliper Technologies Corp.		

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(21) International Application Number: PCT/DK97/00368 (22) International Filing Date: 4 September 1997 (04.09.97) (30) Priority Data: 0953/96 4 September 1996 (04.09.96) DK 0150/97 10 February 1997 (10.02.97) DK (71) Applicant (for all designated States except US): TECHNICAL UNIVERSITY OF DENMARK [DK/DK]; DTU - Building 101 A, DK-2800 Lyngby (DK). (72) Inventor; and (75) Inventor/Applicant (for US only): BLANKENSTEIN, Gert [DE/DE]; Wiesinger Weg 5, D-20253 Hamburg (DE). (74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annae Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).		(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, ES, FI, FI (Utility model), GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS			
(57) Abstract A micro flow system is provided for separating particles, comprising a microfabricated member having a flow channel (5) defined therein for guiding a flow of a fluid containing the particles through the flow channel, first inlet means (2) positioned at one end of the flow channel for entering the fluid into the flow channel, first outlet means (7) positioned at the other end of the flow channel for discharging the fluid from the flow channel, the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross section of the flow channel, the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field. Further, a micro flow system is provided for analysing components of a fluid comprising a microfabricated member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.			

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A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS

FIELD OF THE INVENTION

5 The present invention relates to methods and apparatuses for detection, separation, sorting, and analysis of particles, such as cells, cell organelles, beads, molecules, such as Deoxyribonucleic acid (DNA), proteins, etc. in a fluid. In particular, the invention relates to particle separation by using different forces such as magnetic, electrophoretic, hydrodynamic and/or gravitational forces, e.g. for utilisation in flow cytometry, light microscopy, electrophoretic separation, magnetophoresis,
10 etc.

BACKGROUND OF THE INVENTION

Flow cytometry is a well known technique that is used for high throughput measurements of
15 optical and/or electrical characteristics of microscopic biological samples. Flow cytometry instruments analyse and isolate cells and organelles with particular physical, biochemical, and immunological properties.

Traditionally, cell sorting by flow cytometry (fluorescence activated cell sorting) has been the
20 method of choice for isolation of specific cell populations by surface markers. However, cell sorting by flow cytometry suffers from several drawbacks, especially high dilution of desired cell sample, low speed and sterility problems. Furthermore, the equipment is very costly with high operation and maintenance cost, making the technique available only to a limited number of laboratories.

25 During the last few years, isolation of cells by antibody-coupled magnetic beads and carriers has been developed into a reliable tool for the isolation and characterisation of cell populations. Immunomagnetic cell separation, e.g. as commercially introduced by Dynal A/S and Miltenyi Biotec, has become an established method for cell analysis in clinical diagnostics. Due to the relatively low prize, this method is attractive in flow cytometry, especially in sorting of rare
30 cellular events. For example, sorting of fetal cells contained in maternal blood sample provides a non-invasive alternative to prenatal diagnostic procedures, such as amniocentesis of chorionic villus sampling. Another rare event scenario is the detection of low concentration of cancer cells which has an important role in diagnosis of minimal residual disease and evaluation of appropriate therapies. Another medical application for cell sorting systems is the diagnosis of
35 bacterial and viral diseases.

Although this method offers relatively inexpensive approach to sort rare cellular event, it adds considerable time onto the overall rare event detection and it does not offer the multiparameter

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analysis readily available with flow cytometry techniques. Existing techniques for magnetic separation are suffering from the low purity of the sorted cell fraction and the low recovery rate of the sorted cells. In most systems several washing steps have to be implemented into the separation procedure which then causes cell losses. Additionally small subpopulation of labelled
5 cells cannot be directly isolated by existing magnetic separation techniques.

A good overview about fluorescence activated cell sorting procedures and magnetic activated cell sorting is given in Melamed et. al., "Flow Cytometry and Sorting. (Ed. Melamed et. al., Wiley & Sons Inc., 1990).

10

SUMMARY OF THE INVENTION

Advances in microfabrication and microfluidic technologies continue to fuel further investigation into the miniaturisation of bioanalytical instruments and biochemical assays in general. The
15 present invention relates to development of a low cost non-invasive diagnostic test method and devices for carrying out such tests that include measuring, monitoring, sorting and analysing samples containing particles, such as organic cells, microbeads, cell organells 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.

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Separation may be performed according to various physical properties, such as fluorescent properties or other optical properties, magnetic properties, density, electrical properties, etc. According to an important aspect of the invention, particle separation is performed by aligning the particles in one row of particles in a micro flow channel so that particles can be treated
25 individually.

Thus, it is an object of the present invention to provide a micro flow system and a method of particle separation having an improved efficiency of particle separation compared to the prior art.

30 It is another object of the present invention to provide a micro flow system and a method for particle separation in which cell lysis is minimised.

It is yet another object of the present invention to provide an improved method for preparation of fluids containing particles for separation and analysis of the particles.

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It is a still further object of the present invention to provide a micro flow system and a method for simultaneous separation of particles into a plurality of groups of particles.

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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.

5 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.

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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.

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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

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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,

first outlet means positioned at the other end of the flow channel for discharging the fluid from the flow channel,

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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,

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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,

first and second outlet means positioned at the other end of the flow channel for discharging of fluid from the flow channel,

20 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. 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.

30 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,

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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 channel are deflected in the direction of the magnetic field.

- 15 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 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.

- 20 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.

- 25 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.

- 30 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, $Re = \rho Dh / \mu$ where the hydraulic diameter Dh 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 antigen 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.

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.

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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.

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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.

It is yet another advantage of the present invention that the micro flow system is easily
30 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.

$$10 \quad F = N \cdot S \cdot \mu_B \cdot \text{grad } B$$

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

$$G = (\rho_{\text{particle}} - \rho_{\text{liquid}}) g \frac{\pi}{6} d^3$$

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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

$$U(t) = U_{\infty} (1 - e^{-t/U_{\infty}})$$

10 For a particle submerged in water with a diameter of $30 \mu\text{m}$ and a density of 1.2 times the density of water the settling velocity is $9 \times 10^{-5} \text{ m/s}$. The particle will reach 90 % of this velocity after 2.1×10^{-5} seconds why the transient phase can be neglected. It will take the particle 0.33 seconds to travel a distance of $30 \mu\text{m}$, which makes the method reasonable to employ for separation purposes.

15 While instrumentation in chemistry and biochemistry has become more automated in recent years, the preparation of samples remains a highly laboratory intensive task. The demand is increasing for high throughput, easier to use cost effective analytical devices and assays. Creating this opportunity is e.g. the world-wide effort to sequence the Human Genome, resulting in the
 20 development of new DNA diagnostics and therapeutics. Another important trend is the minimisation of health care costs and hospital admissions by testing patients and monitoring therapeutic use in less expensive environments, the so-called point-of-care analysis.

Micro flow devices containing arrays of nucleic acid hybridisation sites, known as genosensors, are
 25 being developed for a variety of uses in genomic analysis. A great deal of the overall genosensor development effort involves optimisation of experimental conditions in the actual use of genosensors.

Another embodiment of the invention is dealing with a low-tech form of genosensor and
 30 immunosensor technology, involving arrays of oligonucleotides on a microchip, which can be used to define optimal operating conditions and to develop applications of hybridisation arrays in genome mapping and sequencing. The genosensor array is placed in a micro flow channel system allowing an operation in a flow-through mode. Thus several steps of microliquid handling, e.g. washing and staining steps, reagent addition, can be integrated as an automated routine

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procedure. Additionally, micro flow devices containing arrays of antibody/antigen sites, known as immunosensors, can be designed in the same way. The system could be used for combinatorial screening (high-throughput screening) and pharmacokinetic studies.

- 5 According to a sixth aspect of the invention the above and other objects are fulfilled by a micro flow system for analysing components of a fluid, comprising a member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised
10 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
15 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.

- In an embodiment of the invention, the member comprises a plurality of flow channels arranged
20 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
25 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.

- According to a eighth aspect of the invention, a method of forming assay sites comprising
30 immobilised reagents in a flow channel is provided, the method comprising the steps of

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
5 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
10 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
15 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
20 molecular biology are combined.

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BRIEF DESCRIPTION OF THE DRAWINGS

Exemplary embodiments of the invention will now be described with reference to the accompanying drawings in which

5

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
35 field generating means comprising a magnet 8 is located adjacent to the flow channel 5 and 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

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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

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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]

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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-way control valves 25, 26 and capillary tubings 27, 28. The capillary tubings 27, 28 are used for
 20 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)-

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(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.

The sorting apparatus was designed to achieve a minimal dilution of the separated sample
5 fraction. Hydrodynamic separation of particles can be performed due to the optical, electrical, magnetic and/or other properties of the particle-containing sample.

An example of an optical and mechanical arrangement of the apparatus based on fluorescence
10 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

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channel 5 to the syringe pumps 41, 42, respectively, into a position where the 3-way valves 43, 44 open for flow between the syringe pumps 41, 42 and a waste container (not shown) and where the stepper motors driving the syringe pumps 41, 42 are operated in the reverse direction of the normal operation direction to empty the syringe pumps 41, 42.

5

Fig. 9(a) shows two flow channels 45, 46 operating in parallel. The sample containing particles enters the flow channels 45, 46 through inlet ports 47, 48, respectively. The guiding buffer enters the flow channels through the inlet ports 49, 50, respectively. In the flow channels 45, 46, particles susceptible to the magnetic field generated by magnets 51, 52, respectively, are deflected from the sample containing particles into the corresponding guiding buffer and flow thereafter through the sort outlet 53. The remaining part of the sample leave the flow channels 45, 46 through the waste outlets 54, 55, respectively. Separation is increased by using a plurality of flow channels coupled in parallel.

15 Fig. 9(b) and (c) shows examples of combinations of micro flow systems for magnetic, hydrodynamic or gravitational separation. In Fig. 9(b), particles are first separated from a sample in a magnetic separation channel, where after the sorted particles are subjected to a hydrodynamic separation due to the optical properties of the particles. Thus, it is possible to analyse and separate particles from a sample based on both optical and magnetic properties of the particles or to another combination of properties or characteristics. In Fig. 9(c), two magnetic separation channels are coupled in series in order to obtain a highly purified product.

Fig. 10 illustrates examples of micro flow systems having means for automated labelling of particles with fluorescence or magnetic probes. The system may be combined with post-treatment means for removal of the probes or for other treatment of the sorted particles. The system contains a micro flow system containing channels 56, 57 for addition of liquids to the sample, e.g. reagents for cell lysis or staining, a channel 58 for incubation and cultivation or storage of the sample for further processing and a separation channel 5. A sample is introduced into the micro flow system via an inlet 2 and one or more reagents can be added continuously to the sample, which is transported into the incubation channel 58. A simple micro flow structure was constructed for sample pre-treatment. Preferably, the flow rates are managed by computer-controlled syringe pumps. The incubation period between mixing and analysis of the sample is given by the volumetric flow rate of the syringe pumps and the cross-sectional area and length of the incubation channel.

35

Fig. 11 shows a micro flow system for magnetic separation of macromolecules, i.e. ribonucleic acid or proteins from a sample. Magnetic beads labelled with a fluorescence dye and a probe, specific for i.e. DNA are added to the sample which is then incubated. This sample is entered via inlet

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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
5 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
10 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
15 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,
20 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
25 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
30 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
35 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.

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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

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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

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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.

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Table 2 Separation efficiencies

Paramagnetic Bead	Size μm	Separation Efficiency [%] ¹		
		A)	B)	C)
Polysciences				
25 % iron-oxide	1-10	>99	>99	95
50 % iron-oxide	1-10	>99	>99	96.5
Paesel + Lorei				
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 $\mu\text{l}/\text{min}$, B)= 50 $\mu\text{l}/\text{min}$, C)= 100 $\mu\text{l}/\text{min}$

EXAMPLE 2

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 Biotech was used. JURKAT cells were suspended in 1% PBS/BSA to a concentration of $10^7/\text{ml}$. Biotin-conjugated CD4 magnetic microbeads were 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, 50 $\mu\text{l}/\text{min}$) were performed. The same experiments were performed using magnetically unstained cells.

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 1.5×10^8 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.

15

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.

35

Nucleated red blood cells are found in maternal blood in a concentration of 10 to 1000 per ml of all nucleated cells. Bianchi has shown (D.W. Bianchi, Journal of Pediatrics, 1995, 127, 6, p. 847-856) that it is possible to use such cells for genetic screening in prenatal diagnosis. The cell surface

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marker CD71+ for example, is an appropriate marker to select such cells from maternal blood. Test results demonstrates that magnetic activated cell sorting is powerful enrichment system for sorting and isolating fetal nucleated blood cells from maternal blood. For this the magnetic activated cell micro technology as described in this invention is used. Fetal cells are distinguished
5 and separated from maternal blood by the use of a specific surface marker (CD71) which is only present on the cell membrane of fetal nucleated blood cells. By selectively attaching a magnetic antibody probe to CD71, a magnetic probe is attached substantially exclusively to fetal cells.

EXAMPLE 5

10

This example concerns depletion of magnetically labelled CD45 positive cells (maternal leukocytes) from a maternal blood sample spiked with cord blood. A flow chip described in Fig. 1 was used in a system as described in Fig. 4. In this experiment a 1:3 spike (fetal/maternal, v/v) was used to demonstrate the performance of the magnetic separation. Heparin was used as an
15 anti-coagulant. The nucleated cells were labelled with CD45 coated magnetic 0.1 μ micro particles (Immunicom, U.S.A.), using a monoclonal antibody against CD45 as the first layer. The cell suspension was collected at both outlets 6 and 7 (see Fig. 1). For testing the sorting efficiency, parts of both the collected fractions were analysed on microscope slides. The results showed that most of the cells, more than 95%, collected at the sort outlet 6 were CD45 positive.

20

EXAMPLE 6

Fluorescence activated cell sorting using the device described in Fig. 7. First results have shown an enrichment factor of more than 300, which indicates that the employed device is a powerful
25 tool for enrichment of rare cellular events.

EXAMPLE 7

An example is given for the embodiment of the invention as described in Fig. 12 for the use of
30 multiple sensor array technology for sensing of a group of analytes in one step. For this purpose, the biosensing components such as antigens or antibodies can be loaded into a specific assay of the flow channel and immobilised there.

Magnetic particles carrying an antigen probe are immobilised on the surface of the micro flow
35 channel by magnetic means. The immobilisation of each probe is exactly specified to a site by switching on a specific electro-magnet.

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After loading the surface with different groups of antigen probes, the test solution is guided through the flow channel 5 of the microchip. If the sample contains an antibody, which is complementary to one of the different antibodies, it will bind to that specific site where this antibody is immobilised. In a third step, the sample solution has to be removed, and a liquid
5 containing a secondary antibody against the FC region of the first antibody is guided through the micro flow channel. The secondary antibody is coupled to a fluorescence dye allowing the identification of a specific assay site where the antibodies have 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.

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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.

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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.

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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,

20 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,

25 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.

30. A micro flow system for separating particles, comprising a member having

30 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,

35 field generating means positioned proximate to the other end of the flow channel for generating a field substantially along a longitudinal axis of the flow channel whereby the particles are drawn by the field along the channel and distributed according to their susceptibility to the field and their mobility.

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31. A micro flow system for analysing components of a fluid, comprising a member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.

32. A micro flow system according to claim 31, further comprising field generating means positioned proximate to at least some of the assay sites for generation of a field proximate to the corresponding assay site whereby reagents residing in the flow channel and being susceptible to the field when generated at a selected assay site are attracted to and immobilised at the selected assay site, or, are rejected from the selected assay site.

33. A micro flow system according to claim 31 or 32, wherein the member comprises a plurality of flow channels arranged in parallel or in series and each of which has assay sites whereby the fluid containing particles is brought into contact with a large number of assay sites.

34. A method of separating particles, comprising the steps of
guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel,

positioning the flow channel in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field and thereby separated from the fluid.

35. A method of separating fetal cells from maternal cells, comprising the steps of
selective magnetically staining of fetal cells in a fluid containing fetal and maternal cells,
guiding a flow of the fluid containing the fetal cells through a flow channel in such a way that one fetal cell at the time passes a cross-section of the flow channel,
positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained fetal cells residing in the flow channel are deflected in the direction of the magnetic field.

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36. A method of separating cancer cells from other cells, comprising the steps of
- selective magnetically staining of cancer cells in a fluid containing cancer and other cells,
- 5 guiding a flow of the fluid containing the cancer cells through a flow channel in such a way that one cancer cell at the time passes a cross-section of the flow channel,
- positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained cancer cells residing in the flow
- 10 channel are deflected in the direction of the magnetic field.
37. A method of separating particles, comprising the steps of
- guiding a flow of a fluid containing the particles through a flow channel in such a way that one
- 15 particle at the time passes a cross-section of the flow channel, the flow channel having first and second outlet means for discharging of fluid from the flow channel,
- monitoring parameters of a particle residing within a measurement volume within the flow channel and
- 20 controlling passage of fluid through the first and the second outlet means, respectively, in response to a monitored parameter value whereby particles may be separated according to values of a monitored parameter.
- 25 38. A method of analysing components of a fluid, comprising the steps of entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, the channel having a plurality of assay sites, each of which comprises immobilised reagents whereby the fluid can be analyzed for a plurality of components while residing in the channel.
- 30 39. A method of forming assay sites comprising immobilised reagents in a flow channel, comprising the steps of
- preparing selected surfaces of the assay sites for immobilisation of selected reagents,
- 35 dispensing a selected reagent proximate to a corresponding selected assay site, and

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generating a field proximate to the selected site whereby the reagent is attracted towards and brought into contact with the surface of the selected assay site by the field generated and is immobilised upon contact with the surface.

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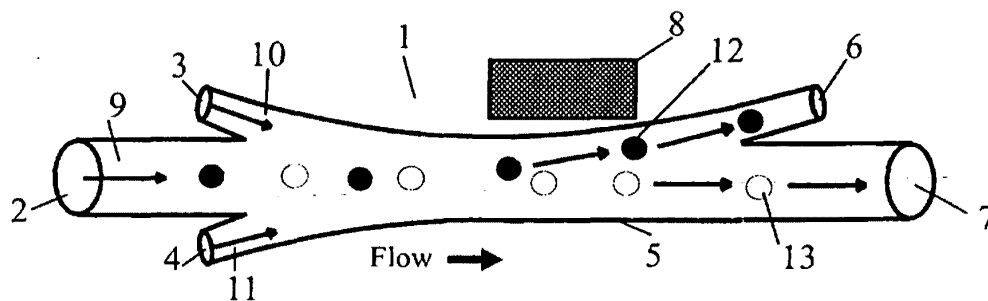


Fig. 1

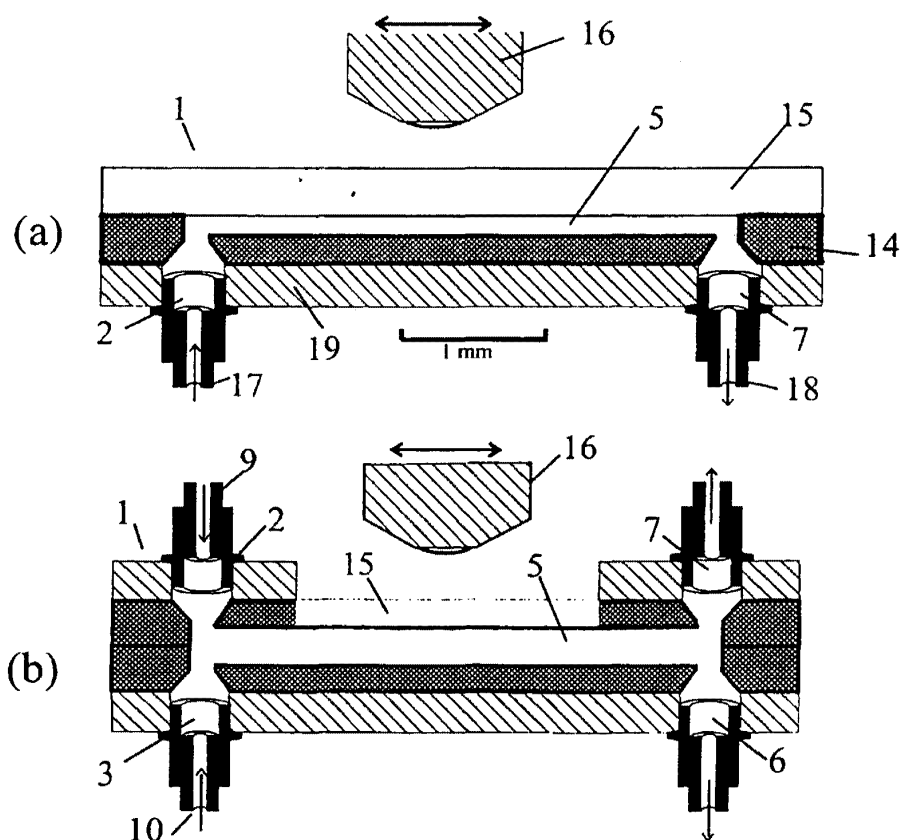


Fig. 2

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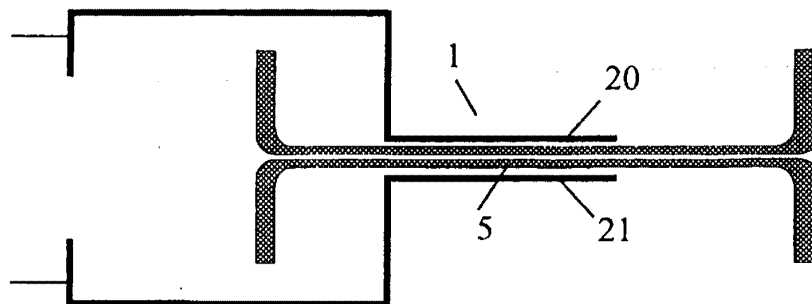


Fig. 3

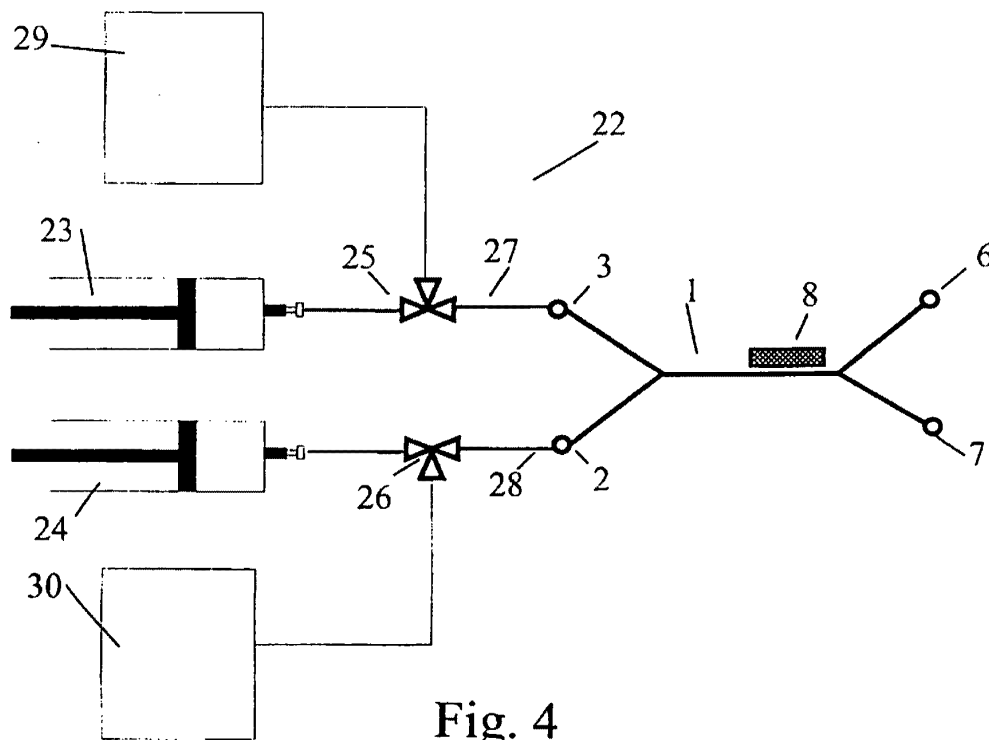


Fig. 4

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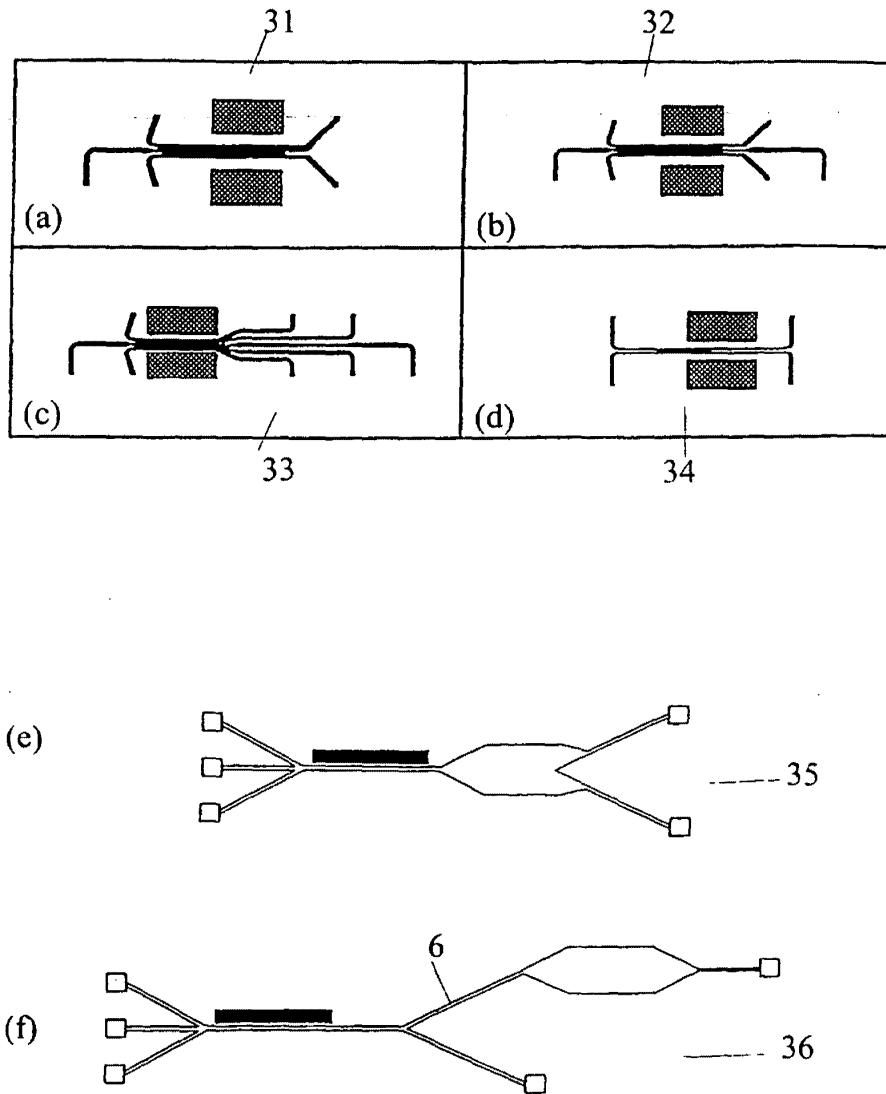


Fig. 5

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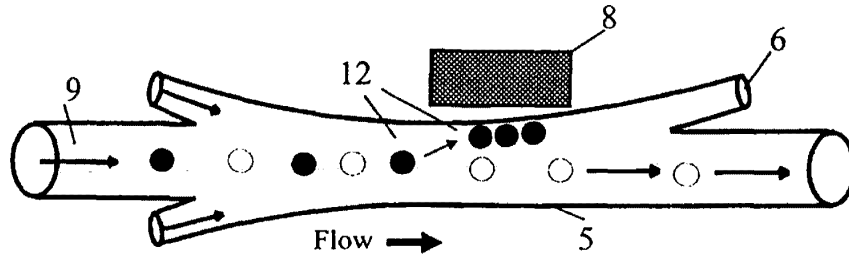


Fig. 6

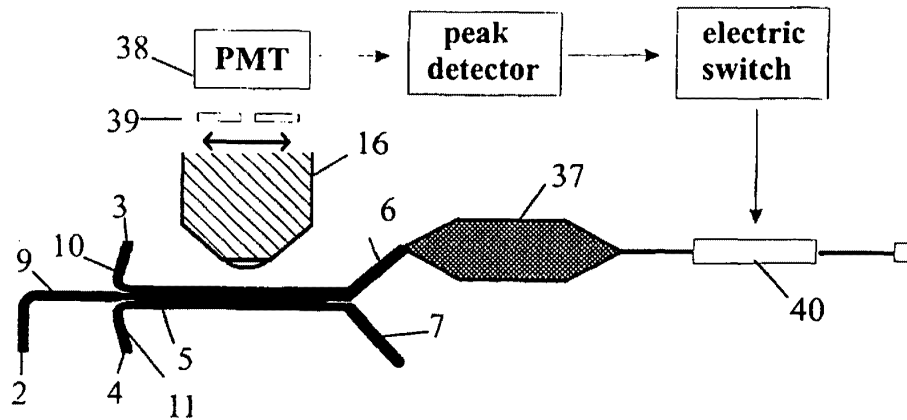


Fig. 7

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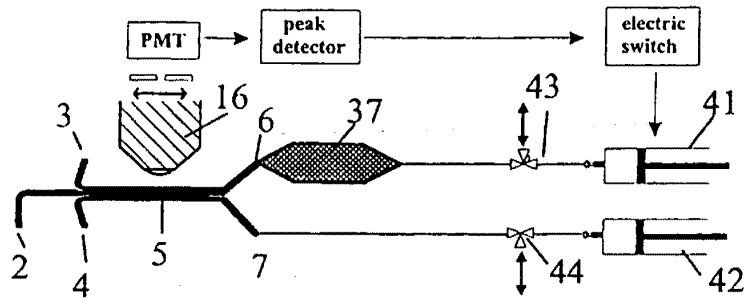


Fig. 8

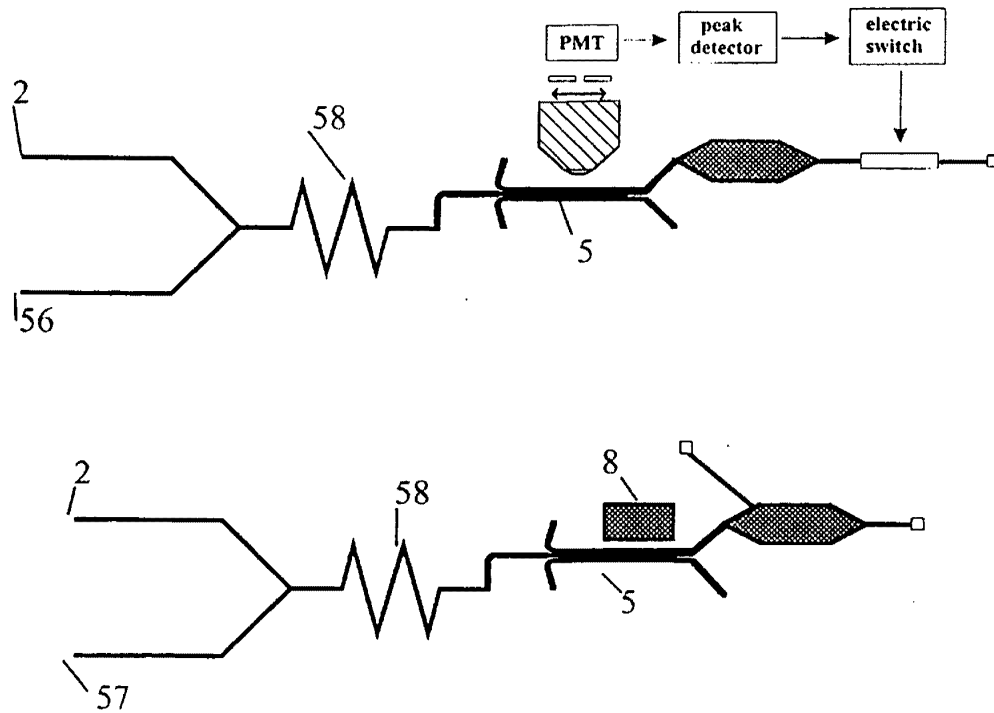


Fig. 10

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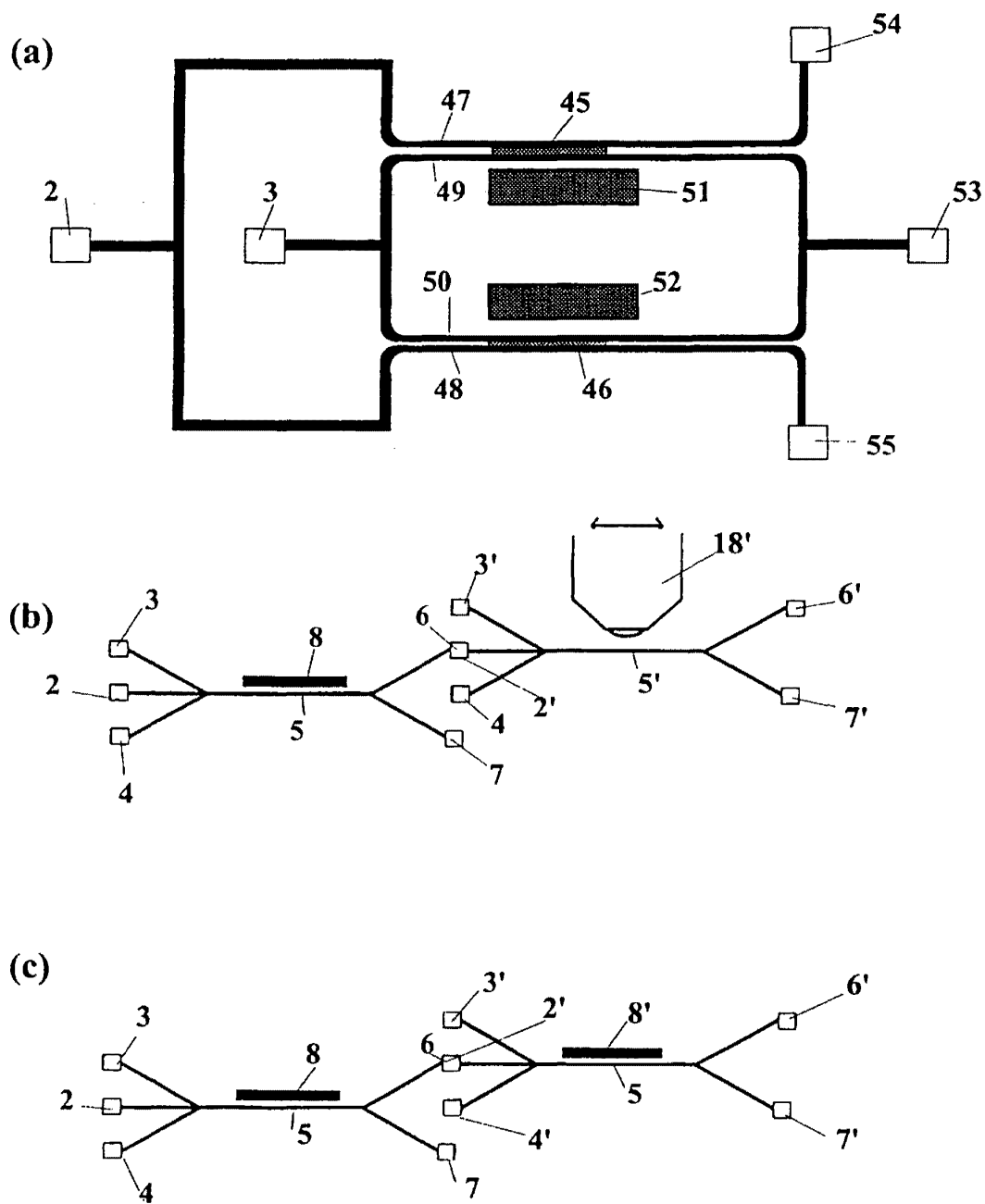


Fig. 9

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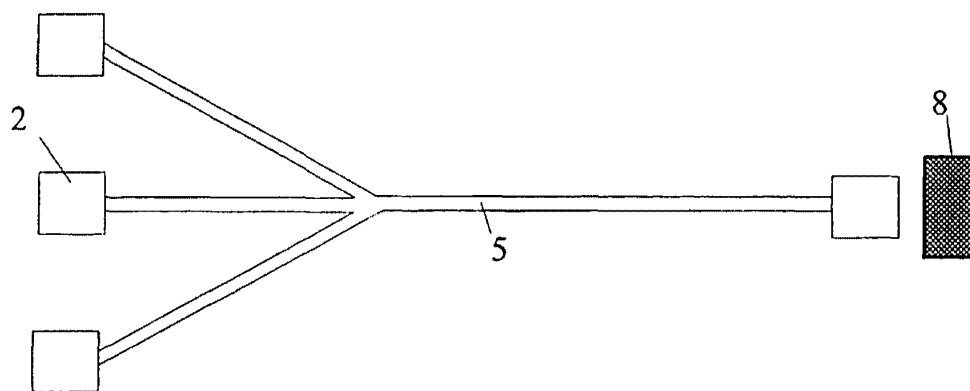


Fig. 11

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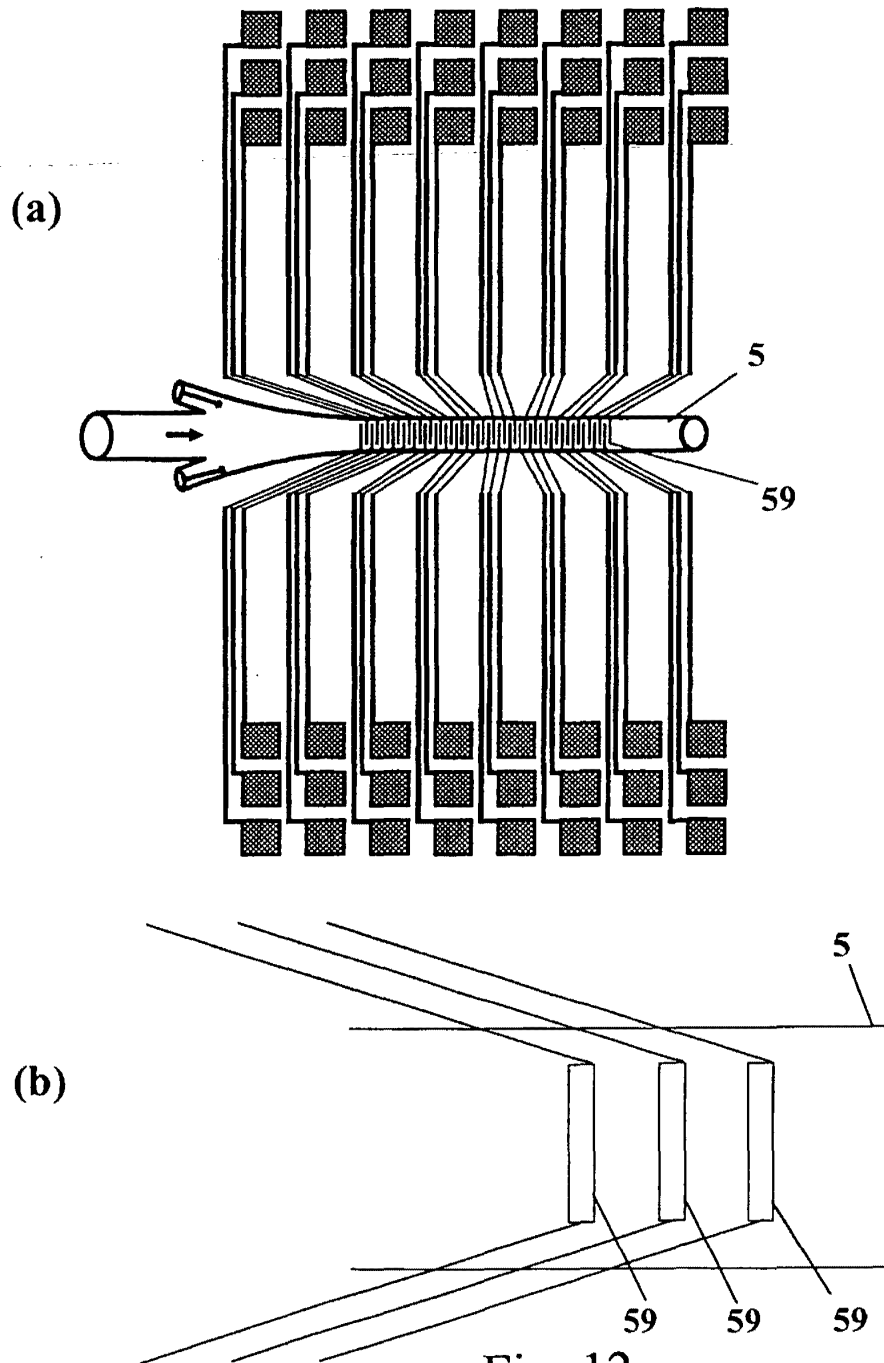
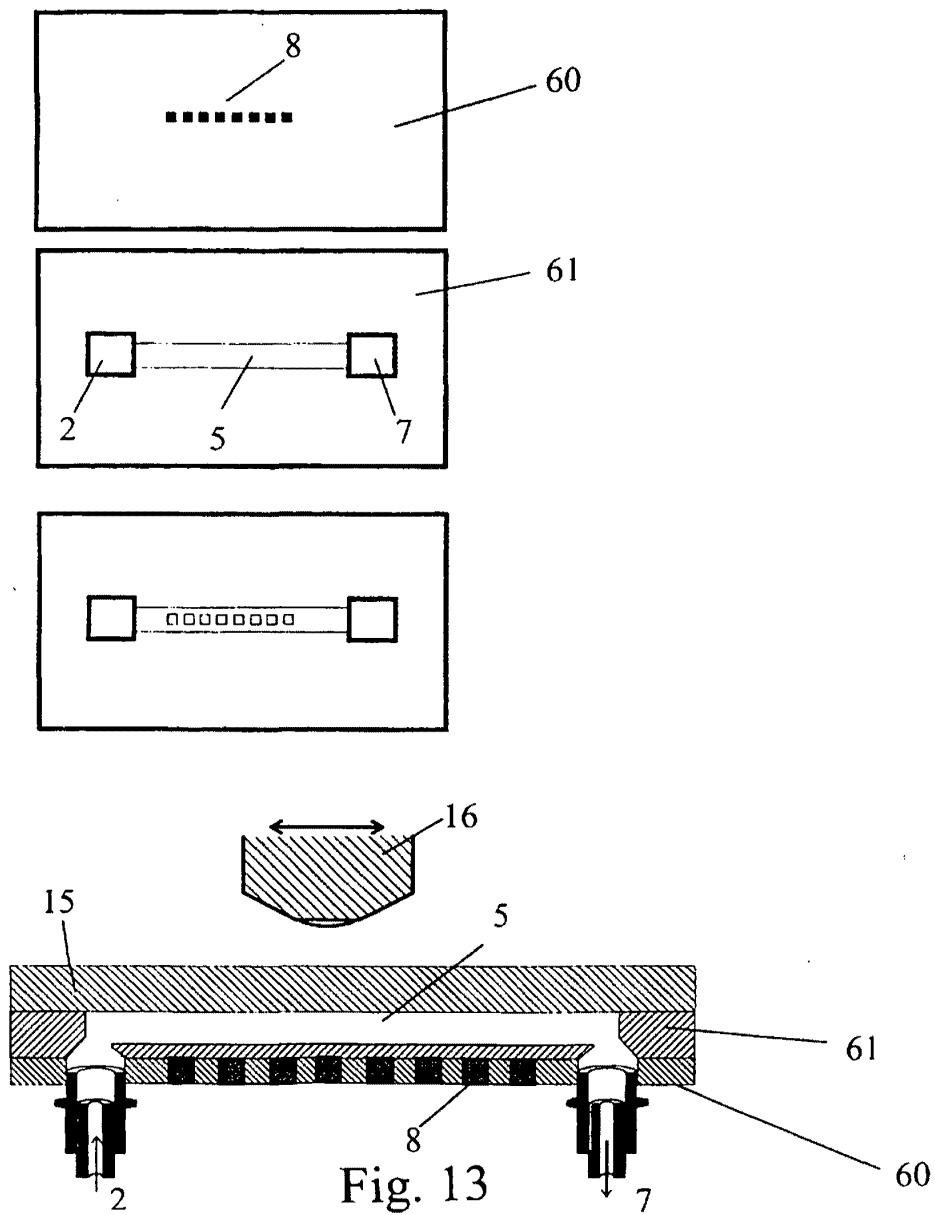


Fig. 12

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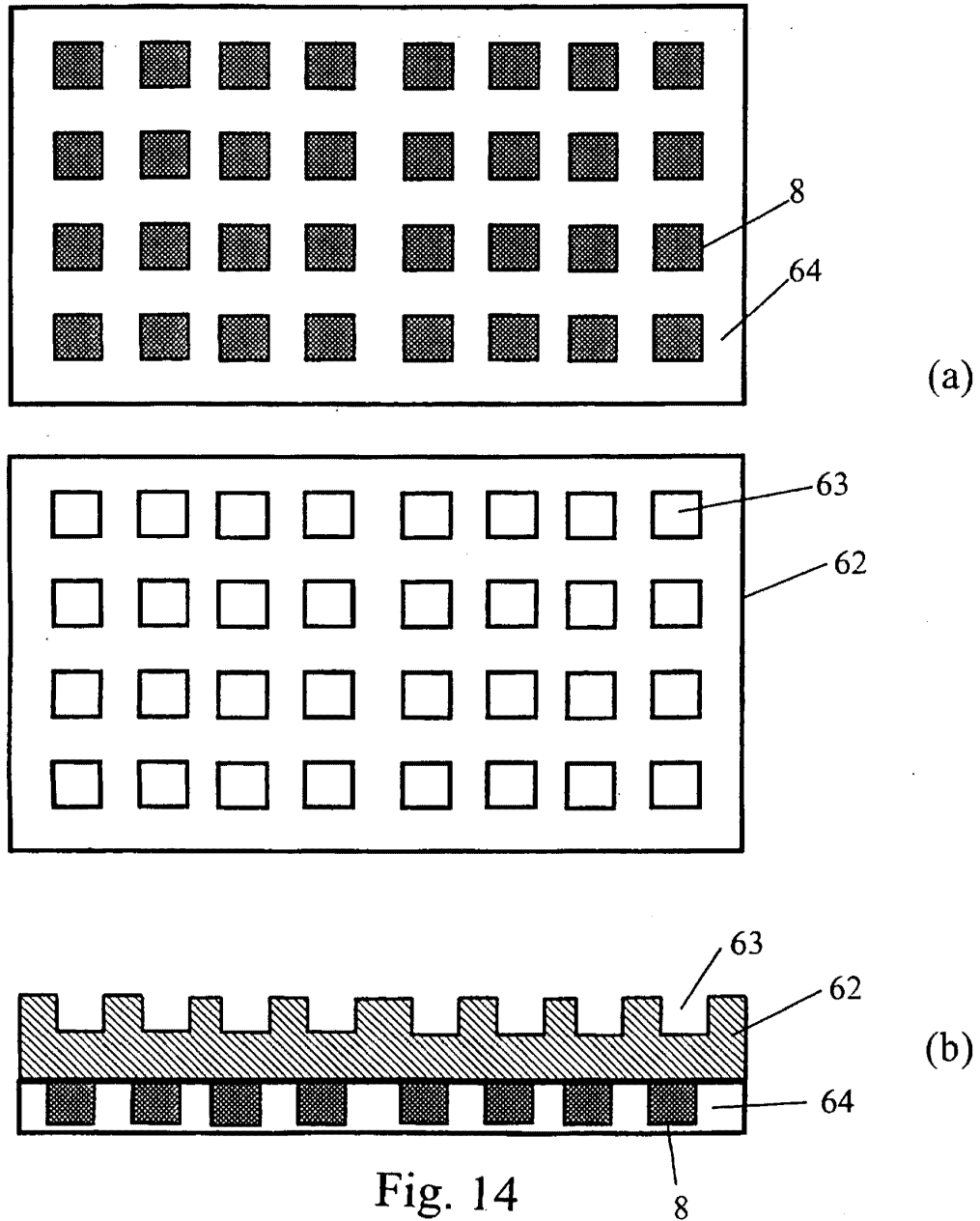
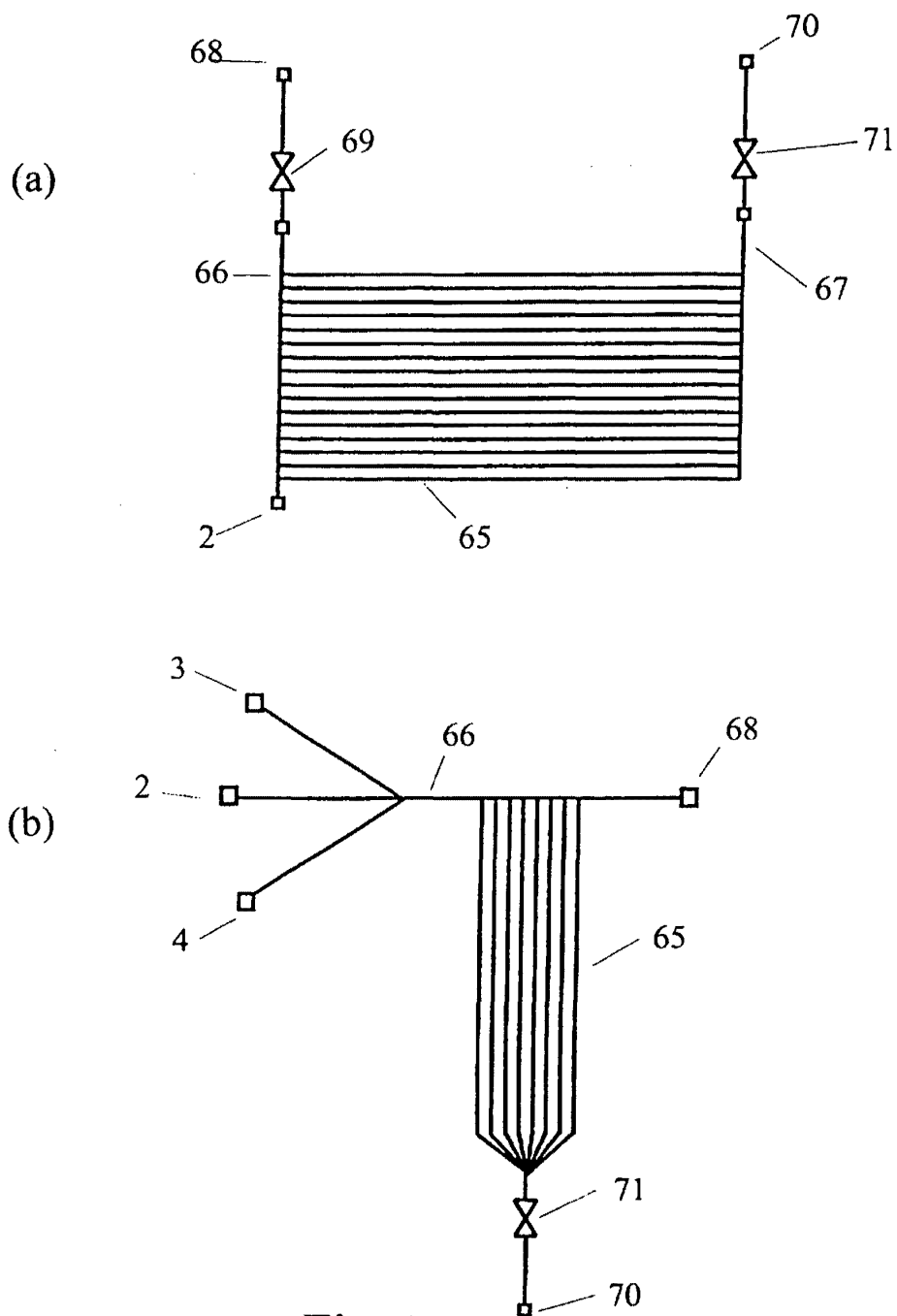


Fig. 14

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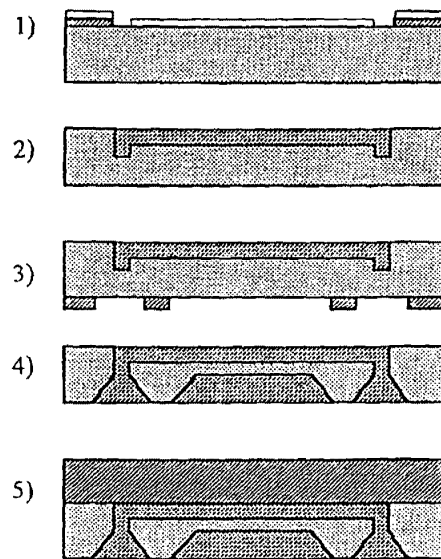


Fig. 16

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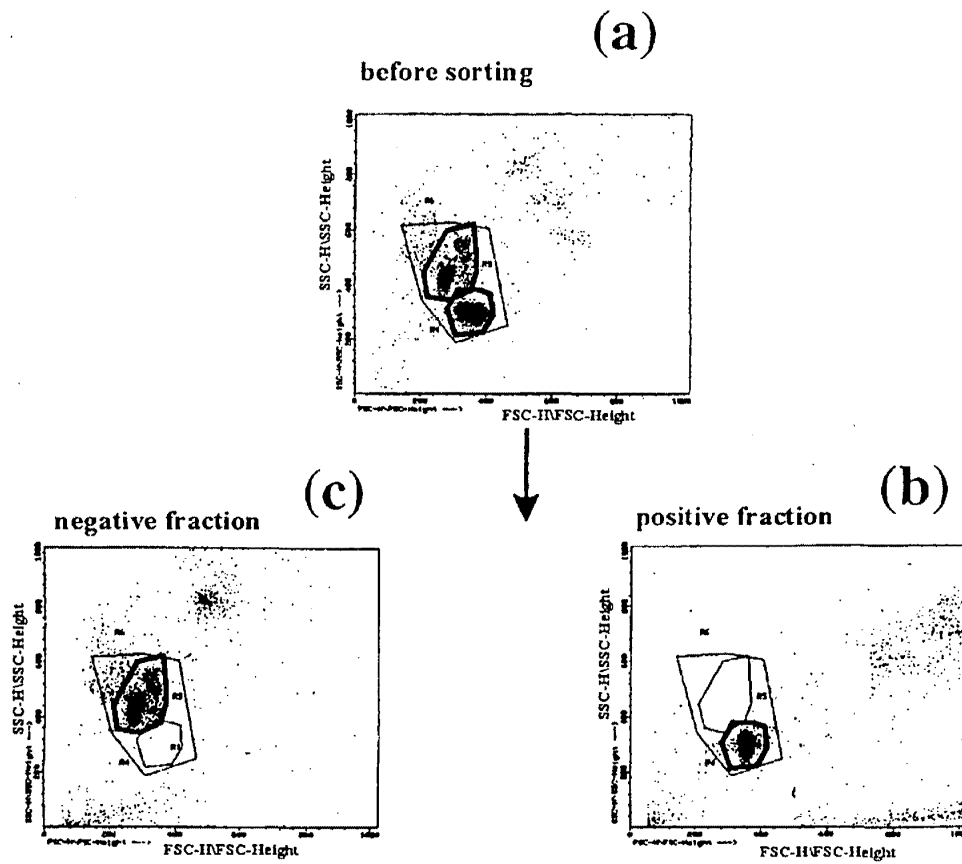


Fig. 17

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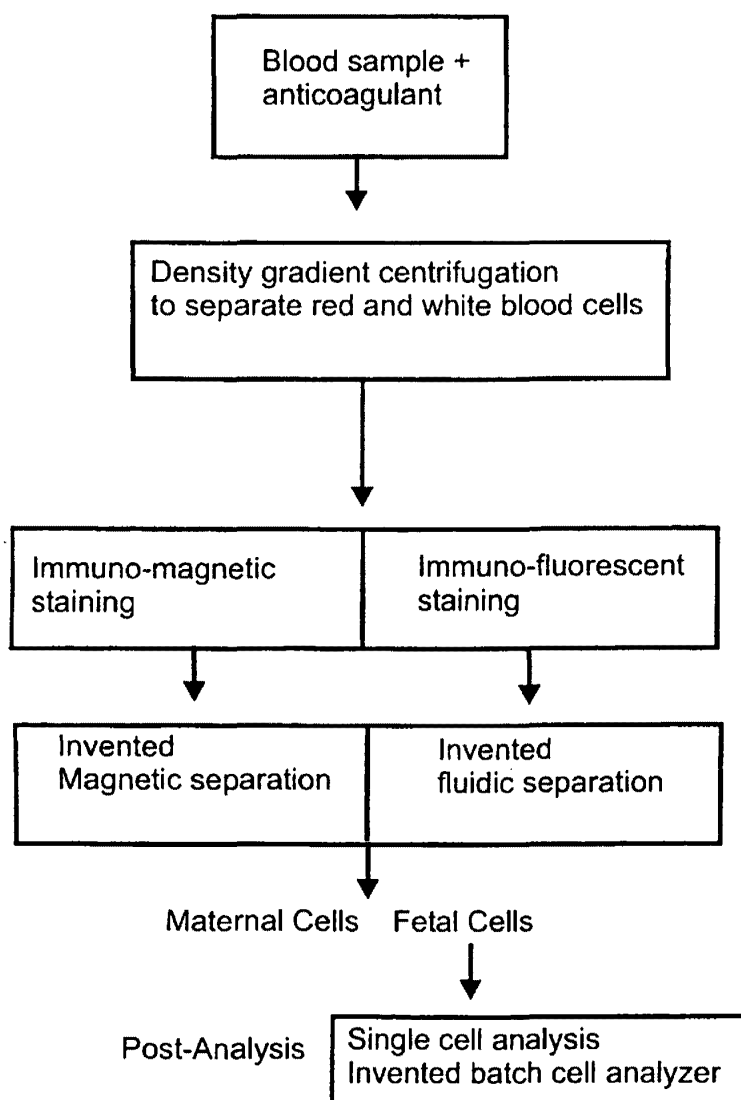


Fig. 18

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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		
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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	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 --- -/-	1-4, 9, 10, 12, 17, 20-27, 29, 30, 34, 35
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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	1-4, 9, 10, 12, 17, 20-27, 29, 30, 34
X	see the whole document	37
Y	US 4 279 345 A (ALLRED JOHN C) 21 July 1981	12
Y	WO 96 09409 A (MILTENYI BIOTECH INC) 28 March 1996	35
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X	see the whole document	
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A	see page 11, line 1-14	
A	US 5 053 344 A (ZBOROWSKI MACIEJ ET AL) 1 October 1991	1
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A	US 5 483 469 A (VAN DEN ENGH GER ET AL) 9 January 1996	1
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Information on patent family members

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Cytonome/ST, LLC Exhibit 2013

NanoCollect Biomedical, Inc. v. Cytonome/ST, LLC, IPR2020-00550

Page 191 of 406

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/00	A1	(11) International Publication Number: WO 00/70080 (43) International Publication Date: 23 November 2000 (23.11.00)
(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 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.		

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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.,

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

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

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

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

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 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.

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 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 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 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.

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

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

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.

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 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.

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 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 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 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 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

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.

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.

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.

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

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

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

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.

It is also worth noting that horizontal and vertical focusing are optionally both performed to center cells vertically and horizontally in the center of microscale channels. Furthermore, the extent of hydrodynamic focusing, using either or both methods, is typically controlled by a variety of variables including viscosity in pressure driven flows, electrokinetic mobility in electrokinetic flow, fluid density in gravity driven flow, or the like. As will be recognized by those of skill, the enhanced control of focusing, provided by the present invention, allows for a large gamut of flow-based applications, including any particle and/or bulk fluid application that involves small, uniform detection regions.

10 USE OF FOCUSING TO SORT PARTICLES

The separation of fluorochrome-labeled cells with fluorescence-activated cell sorters is well-known in the art, as mentioned above. In brief, the technique generally involves incubating a mixed population cells with a fluorescently-labeled antibody against a specific antigenic determinant displayed on the surfaces of target cells. The suspension of cells is then typically expelled, one cell at a time, from a sample chamber of the device through a small vibrating nozzle that generates microdroplets. Each microdroplet contains a single cell that the FACS assays for the presence or absence of the fluorochrome label using a laser source and a detector. Droplets that fluoresce are electrically charged in proportion to their fluorescence and in turn separated as they pass between charged plates. General references describing FACS include, e.g., Kuby, Immunology (3rd Ed.) W.H. Freeman and Company, New York (1997), Watson, et al., Recombinant DNA (2nd Ed.) W.H. Freeman and Company, New York (1992), and Alberts, et al. Molecular Biology of the Cell (3rd Ed.) Garland Publishing, Inc., New York (1994). Other references relating to flow cytometry include, e.g., Radbruch (Ed.) Flow Cytometry and Cell Sorting, Springer-Verlag, New York (1992), Owens and Loken, Flow Cytometry Principles for Clinical Laboratory Practice, Wiley-Liss, New York (1995), and Jarosqueski and Heller (Eds.) Flow Cytometry Protocols: Methods in Molecular Biology, Vol. 91, Humana Press (1997).

30 The present invention provides microfluidic devices and methods for sorting particles (e.g., fluorescently-labeled particles) that use hydrodynamic flow to

focus and/or sort the particles. The methods for sorting members of a particle population (e.g., a cell, a set of cells, a microbead, a set of microbeads, a functionalized microbead, a set of functionalized microbeads, a molecule, a set of molecules, or the like) typically include flowing the members of a particle population
5 in a first microchannel. The members of the particle population are focused horizontally and/or vertically in the first microchannel such that selected individual members are directed into at least a second microchannel that intersects with the first microchannel.

In one embodiment, a portion of the first microchannel optionally
10 includes at least one separation element disposed therein. The separation element generally includes at least two sides in which at least a portion of the separation element is disposed upstream of the second microchannel. In this embodiment, the members of the particle population are optionally focused horizontally and/or vertically in the first microchannel such that selected individual members are directed
15 to at least one of the at least two sides of the separation element and into, e.g., at least a second microchannel that intersects the first microchannel. In other embodiments, this separation element is omitted.

The focusing steps of the particle sorting methods, described above, optionally include horizontally and/or vertically focusing the members of the particle
20 population in the first microchannel by introducing at least one fluid flow (e.g., a buffer, a high density fluid, a low density fluid, or the like) from at least a third microchannel that intersects with the first microchannel upstream from, e.g., the second microchannel. This is optionally performed by any method for flowing fluid in a microfluidic device, including electrokinetic flow, pressure-based flow, and the
25 like.

For example, methods employing electrostatic forces to transport and focus samples generally involve inducing sample flow in a transport channel by applying a first electrical potential to the channel. Thereafter, a second electrical potential is typically applied to focusing channels that intersect with the transport
30 channel in a cross-configuration to vertically focus the transported sample in the center of the transport channel. This electrokinetic-based system is also optionally utilized to direct or “nudge” flowing samples to effect a sorting function, e.g., by

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 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 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., 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. 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 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 opposing microchannels is typically located downstream from detector **2204** for introducing at least one hydrodynamic flow **2202** so as to direct selected cells **2208**

(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

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,
5 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
10 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
15 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
20 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
25 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
30 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

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

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 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

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

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™),

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

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).

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 SBFI and PBFI, 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-methoxyquinolyl)acetic acid, and N-(6-methoxyquinolyl)acetoethyl ester (Molecular
15 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

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

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

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.

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

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

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

10 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

15 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,

20 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

25 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.

30 Typically, vast libraries of test compounds are separately tested for potential effects on different cellular functions. In preferred aspects, large libraries of

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.

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

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 .

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

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.

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

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
5 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
10 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.
15 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
20 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
25 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
30 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),

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

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 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 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.

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 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 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 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

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.

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 fluoresceinated 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.

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 µg 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 CaCl₂. 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.

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

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.

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.

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.
4. The method of claim 1, comprising focusing the particles using
 - 15 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.

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. 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

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.

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;

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;

at least one source of the at least one fluidic material fluidly coupled to the
5 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;

at least one flow control regulator for regulating flow of the at least one fluidic
10 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.

 32. The integrated system of claim 29, wherein the at least one fluid
20 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.

 33. The integrated system of claim 29, further comprising at least a third microchannel which intersects the first microchannel in an intersection region
25 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.

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
25 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

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

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,

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.

1/30

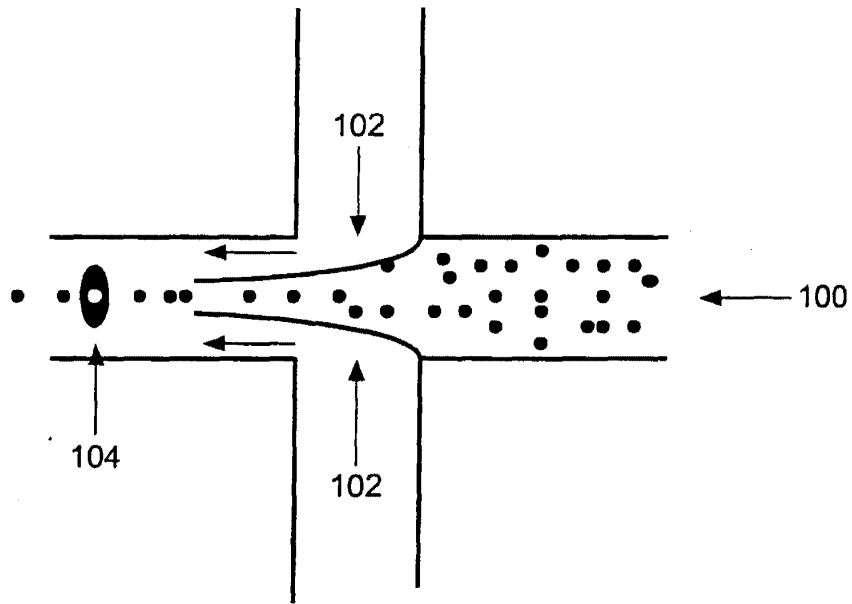


Fig. 1A

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Fig. 1B

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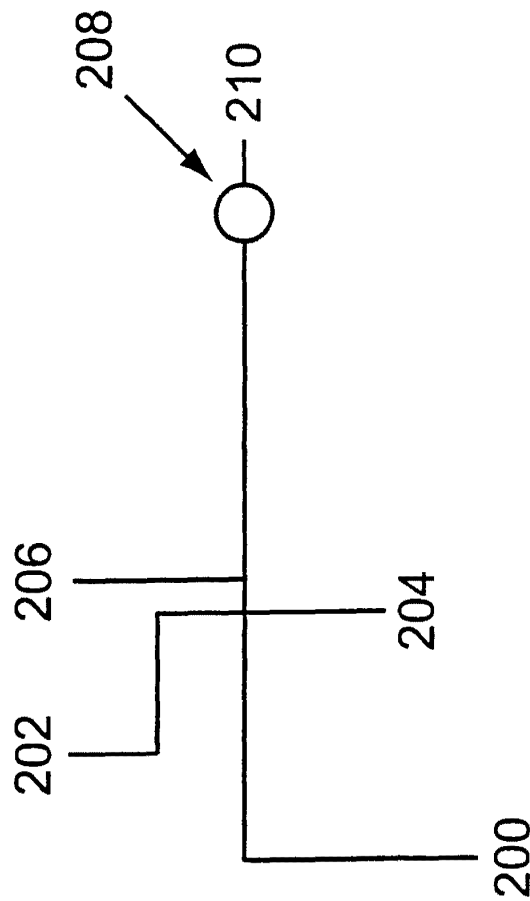


Fig. 2A

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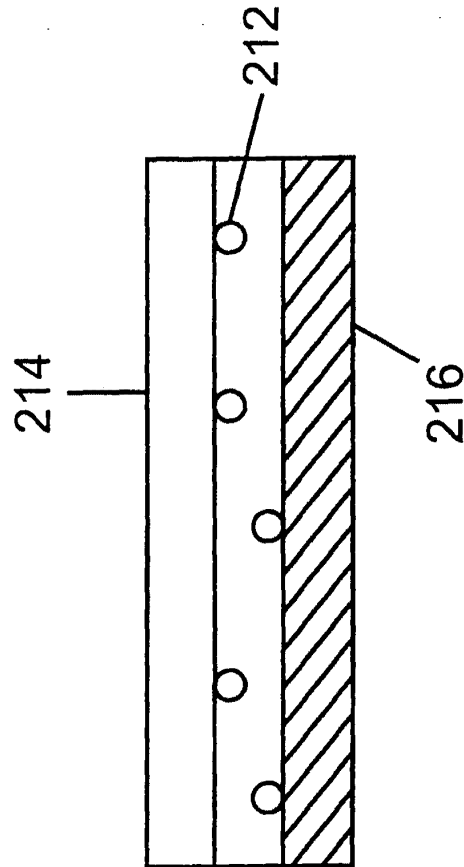


Fig. 2B

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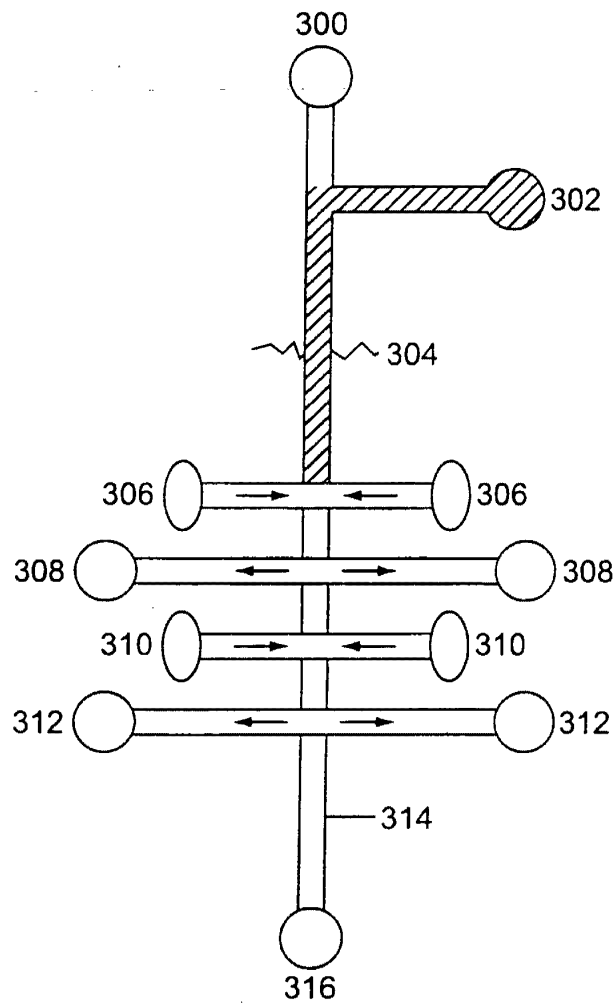


Fig. 3

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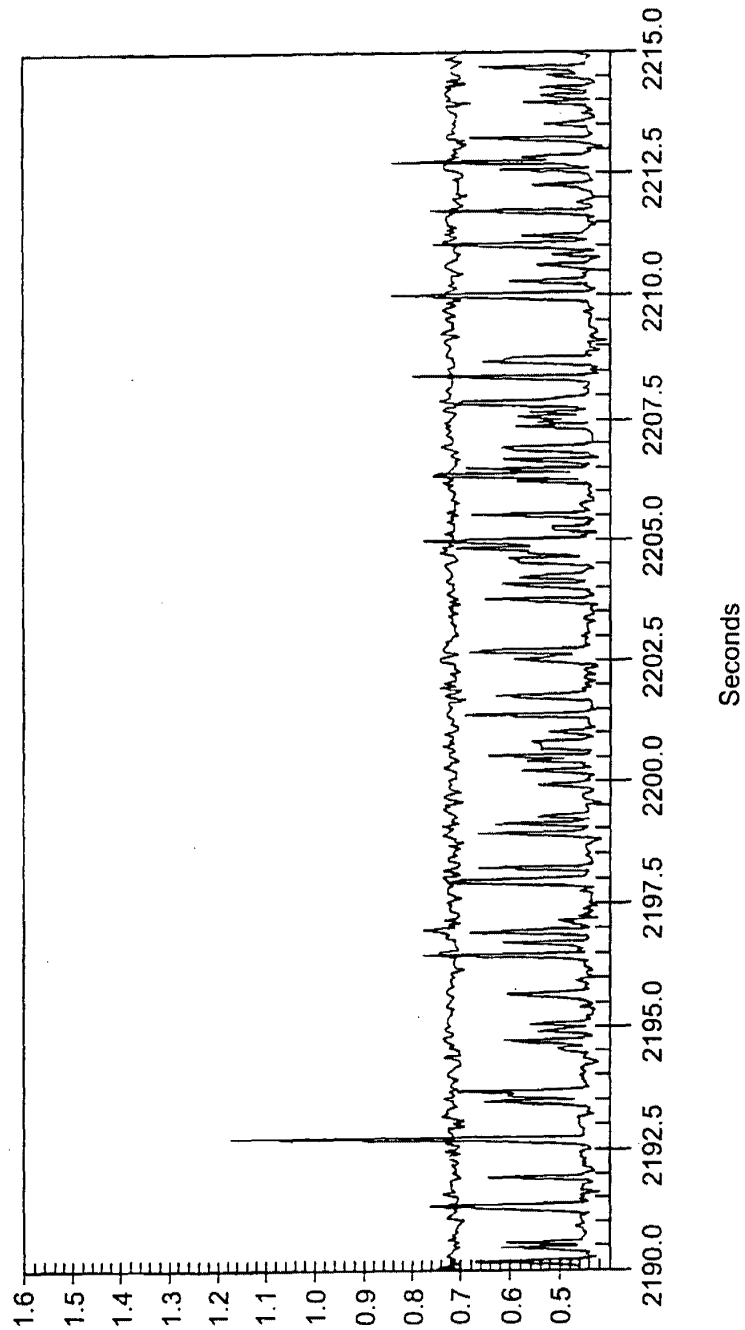


Fig. 4

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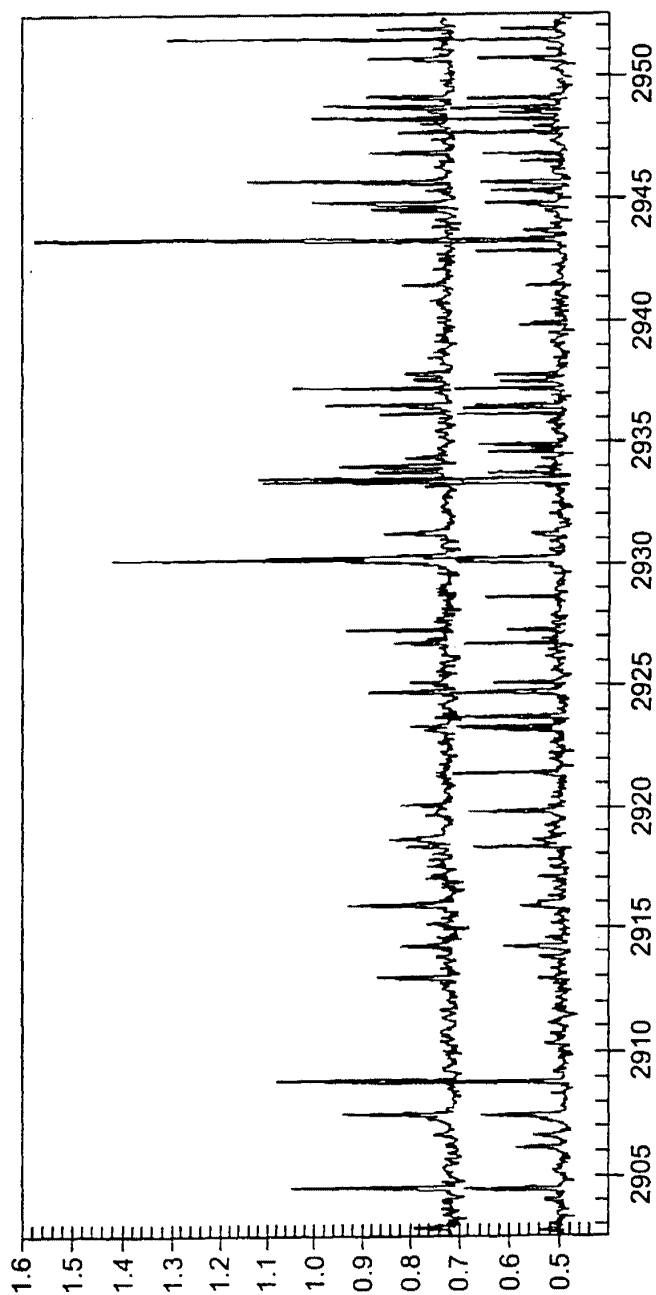


Fig. 5

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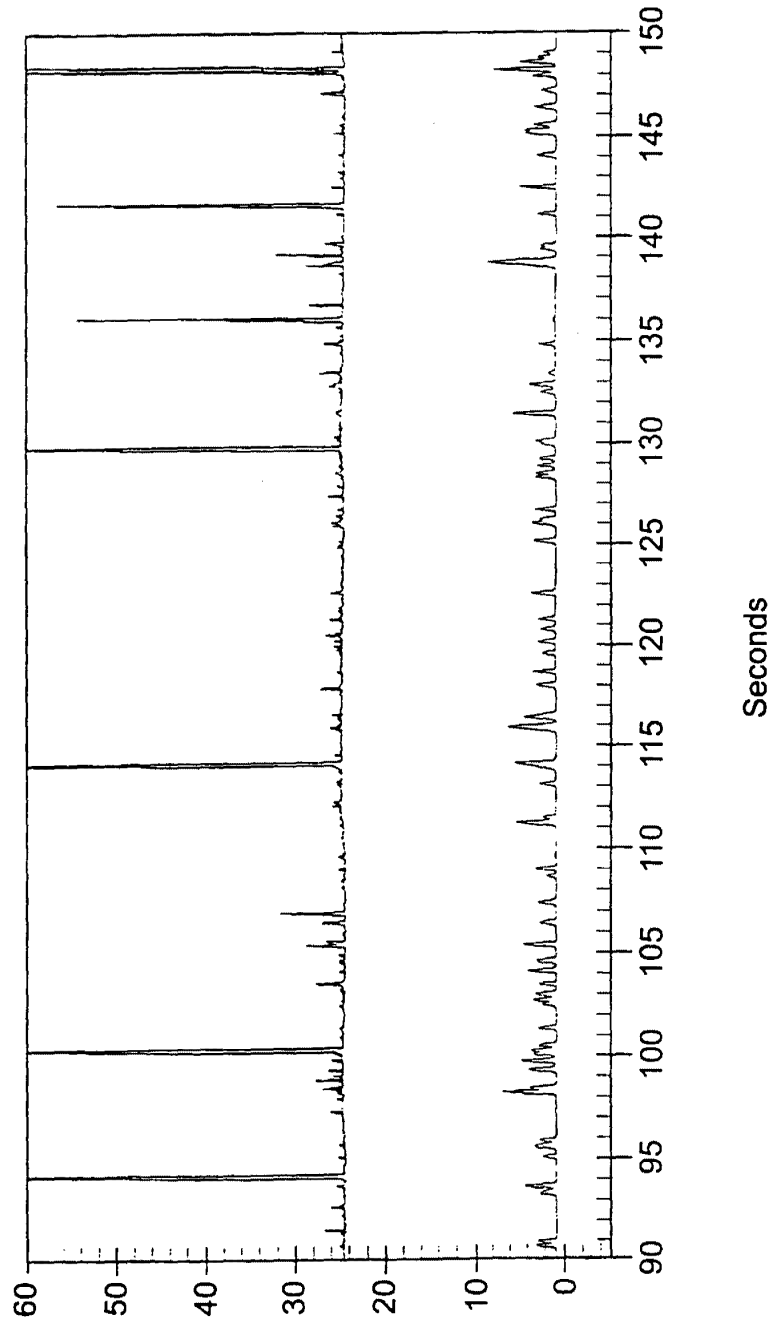


Fig. 6

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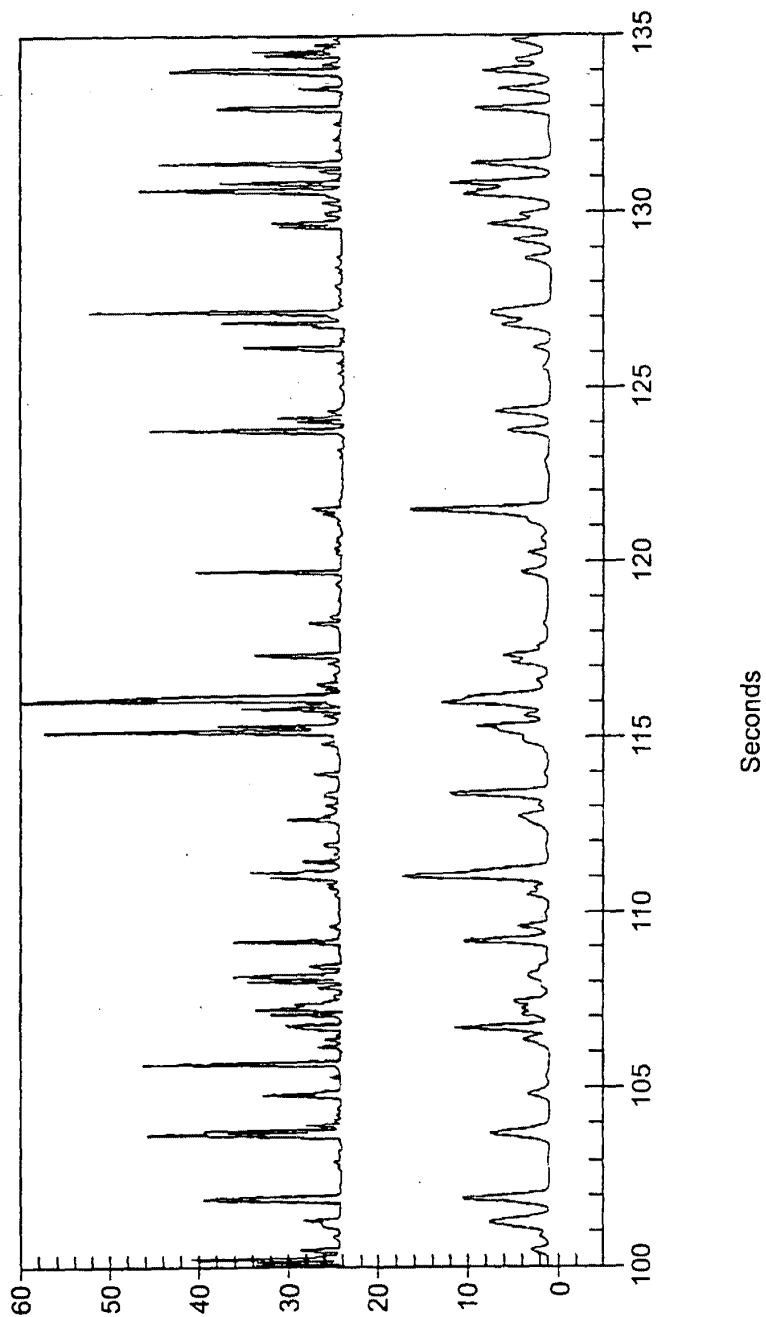


Fig. 7

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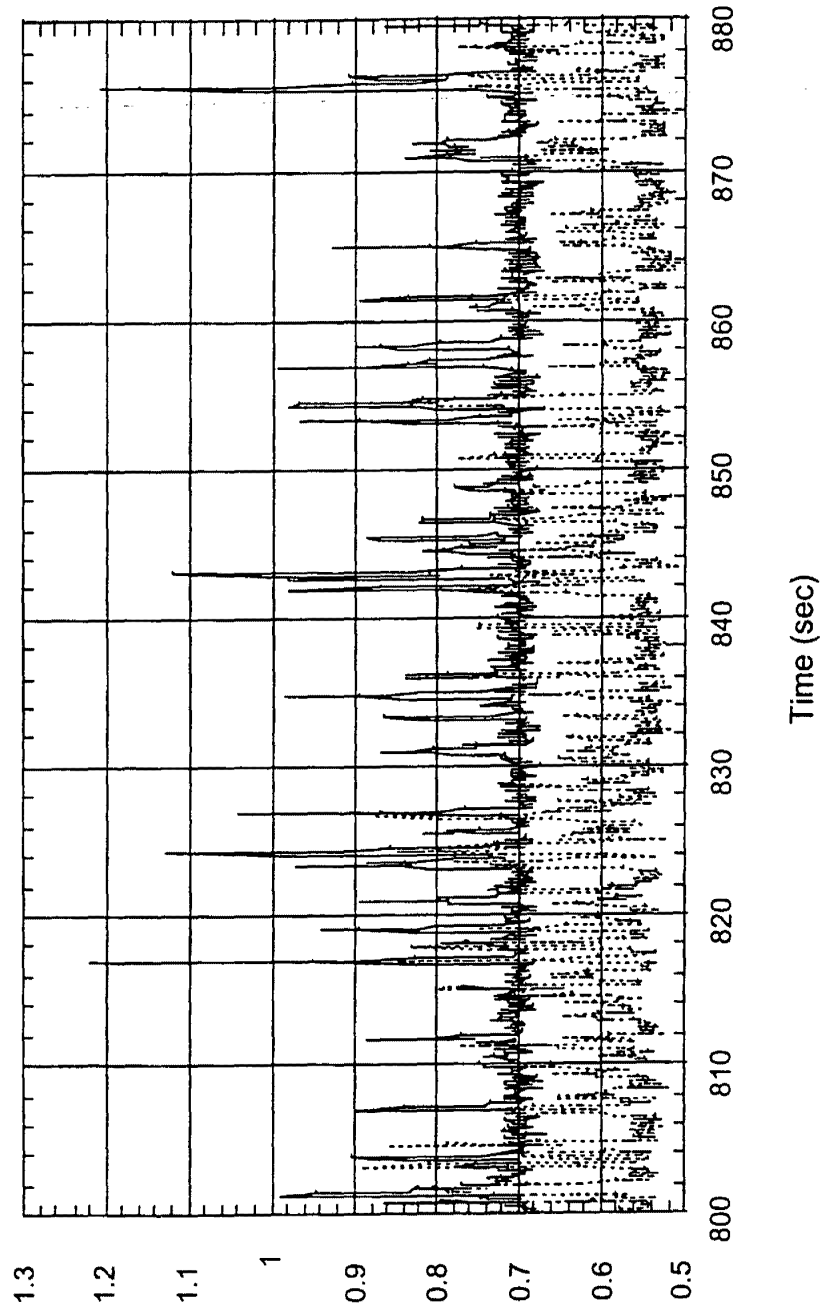


Fig. 8

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11/30

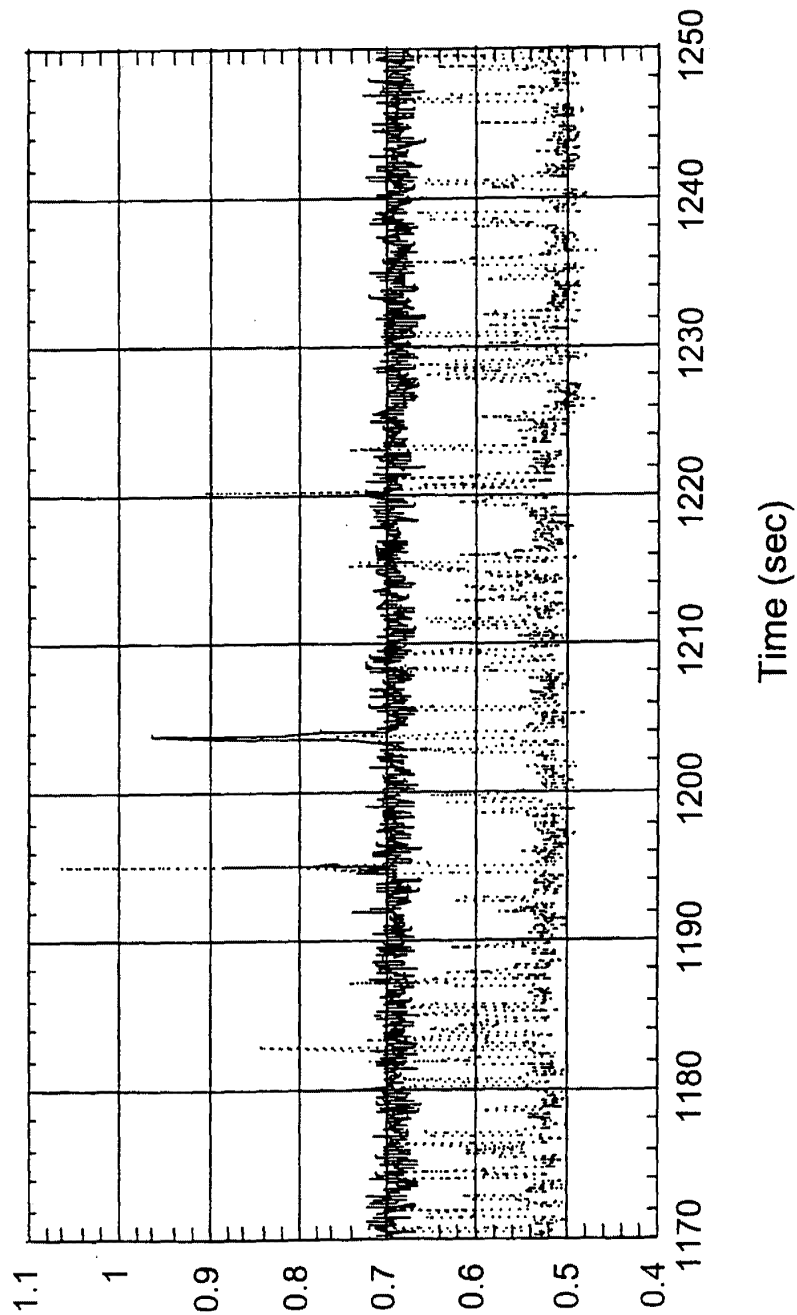


Fig. 9

SUBSTITUTE SHEET (RULE 26)

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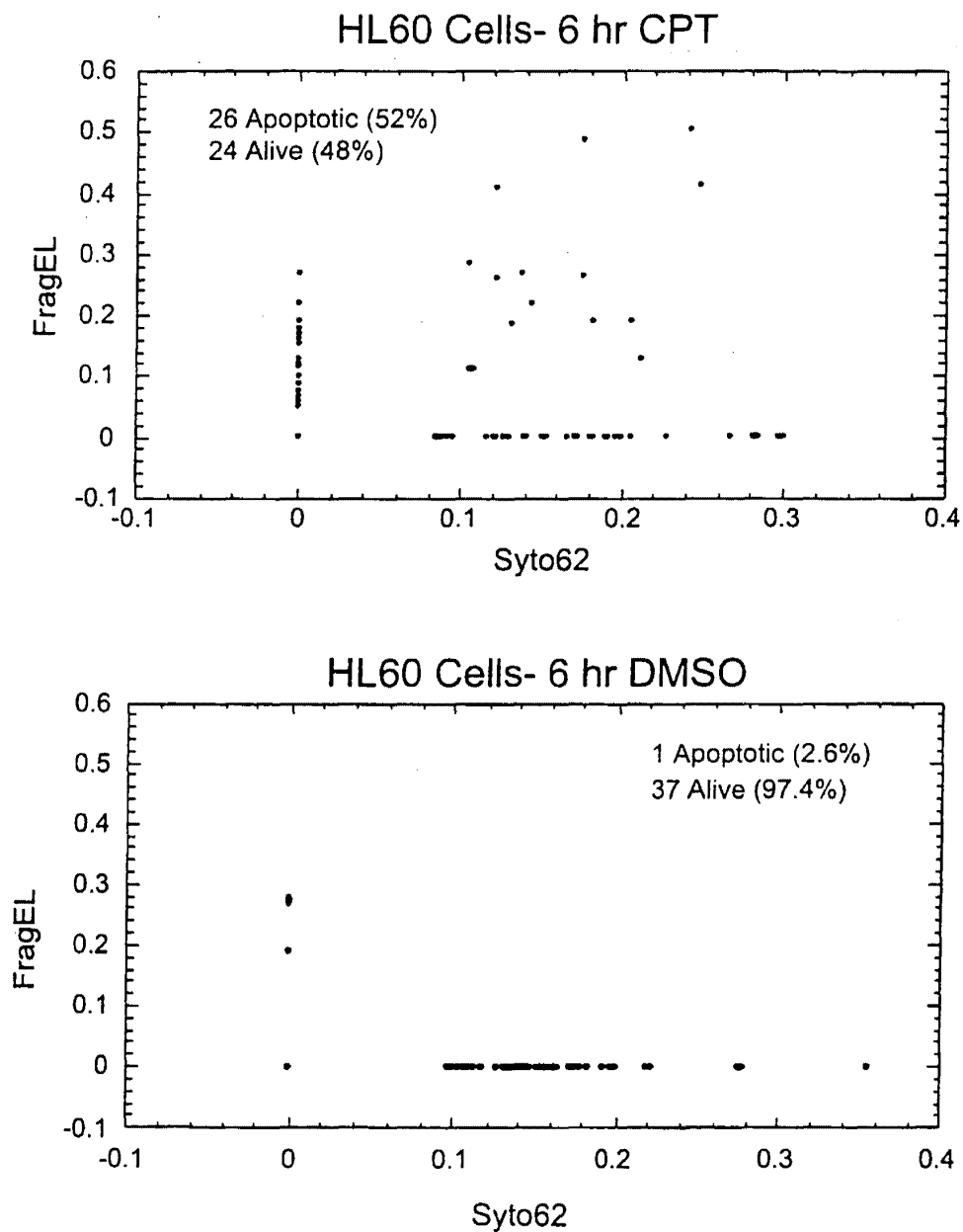
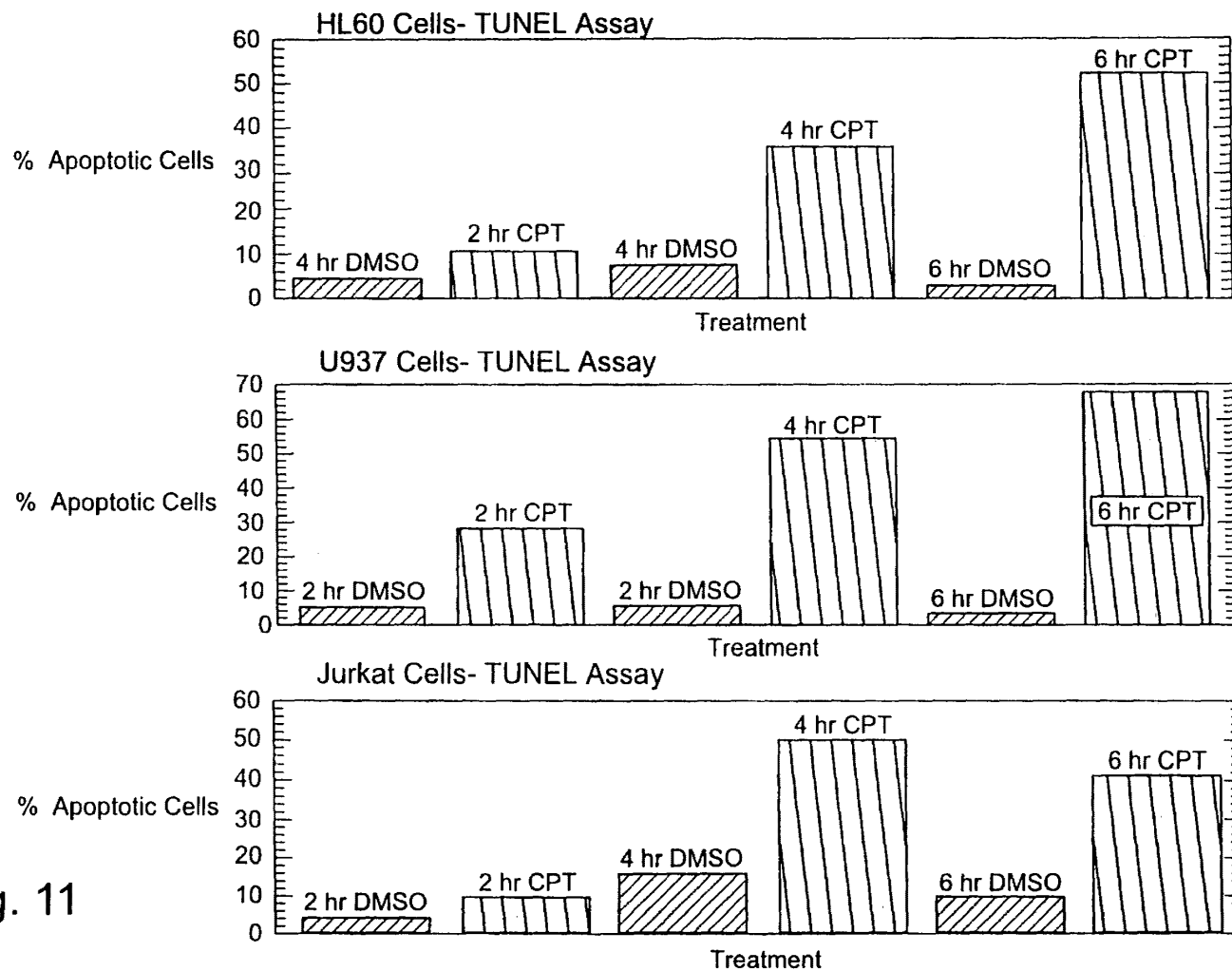


Fig. 10

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Fig. 11



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PCT/US00/13294

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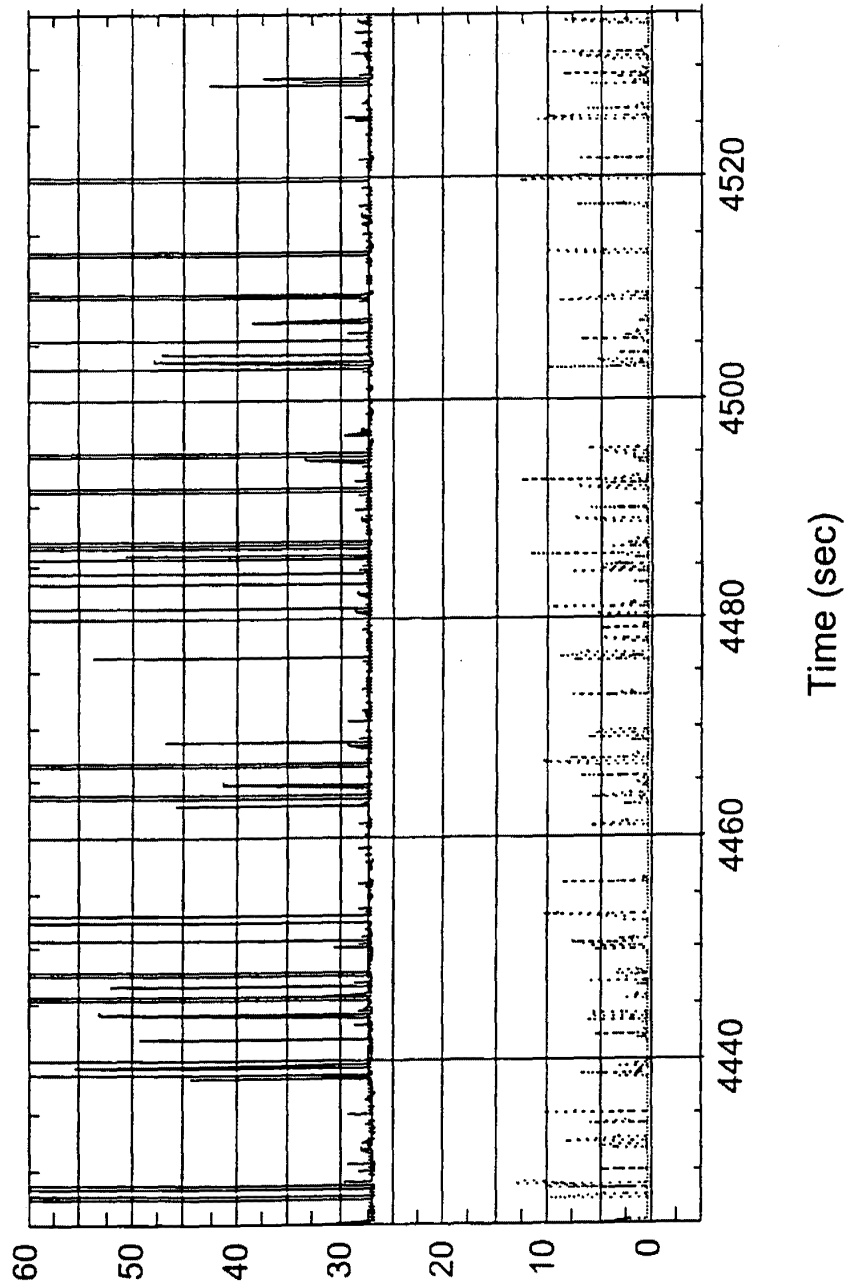


Fig. 12

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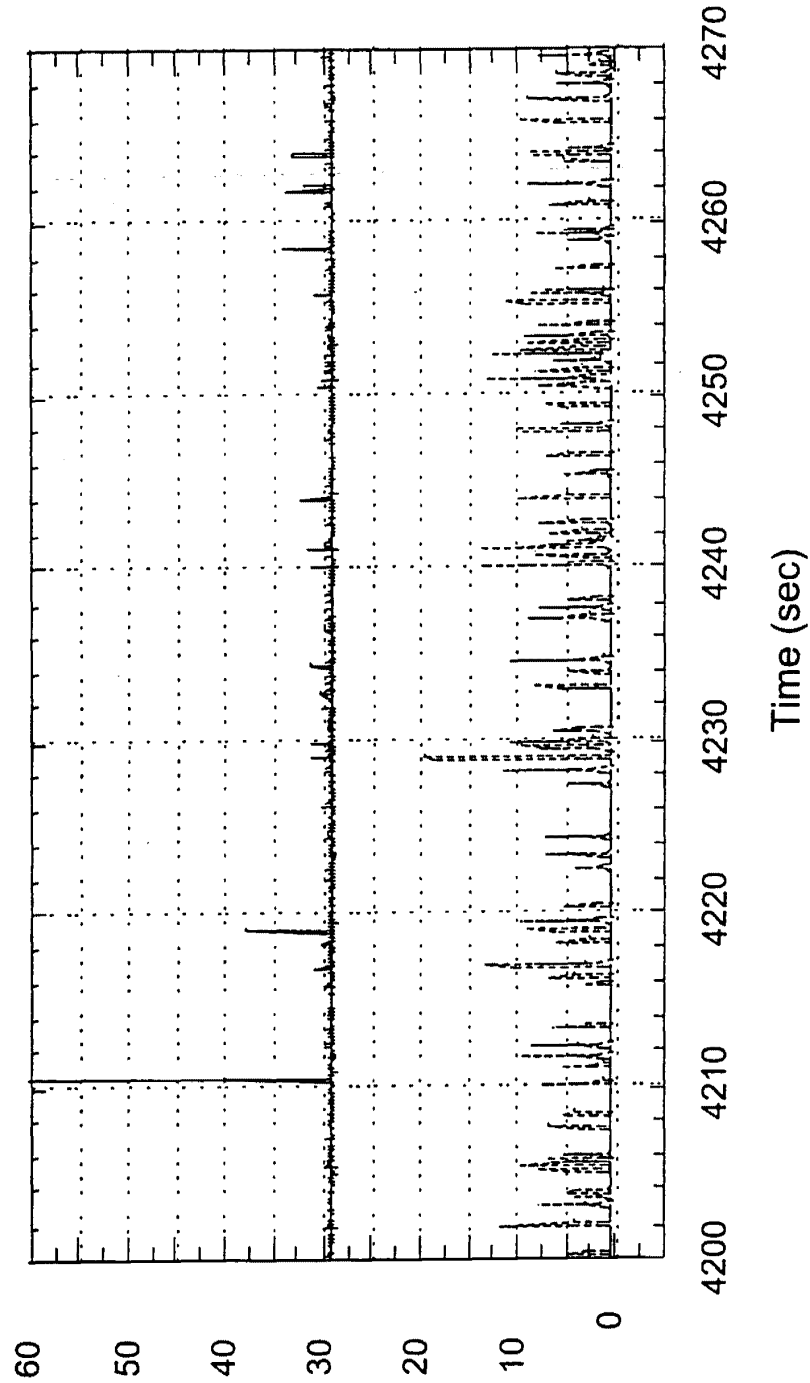
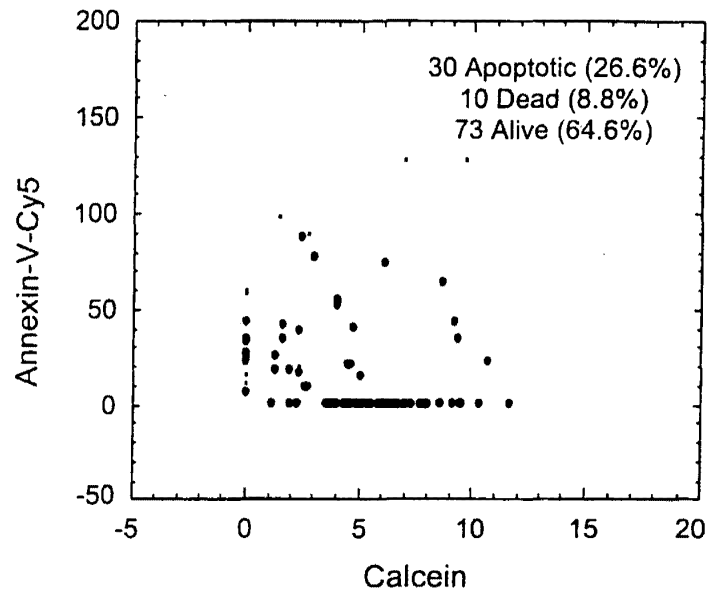


Fig. 13

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HL-60 Cells- 4 hr CPT



HL-60 Cells- 4 hr DMSO

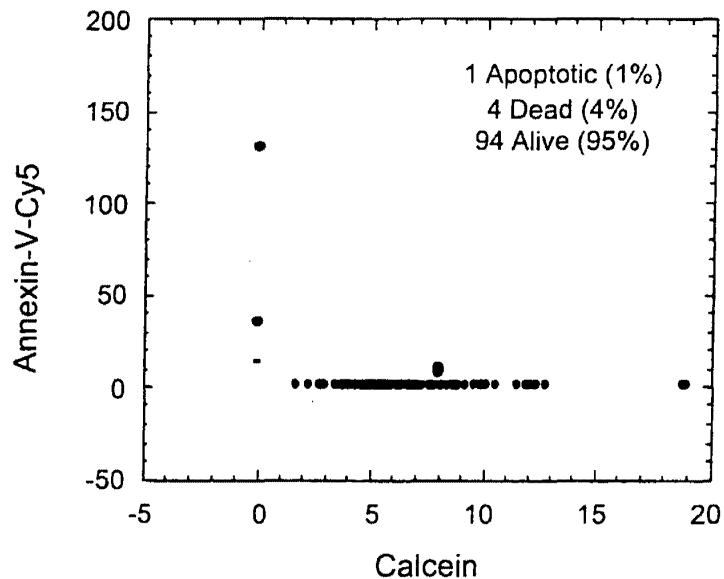


Fig. 14

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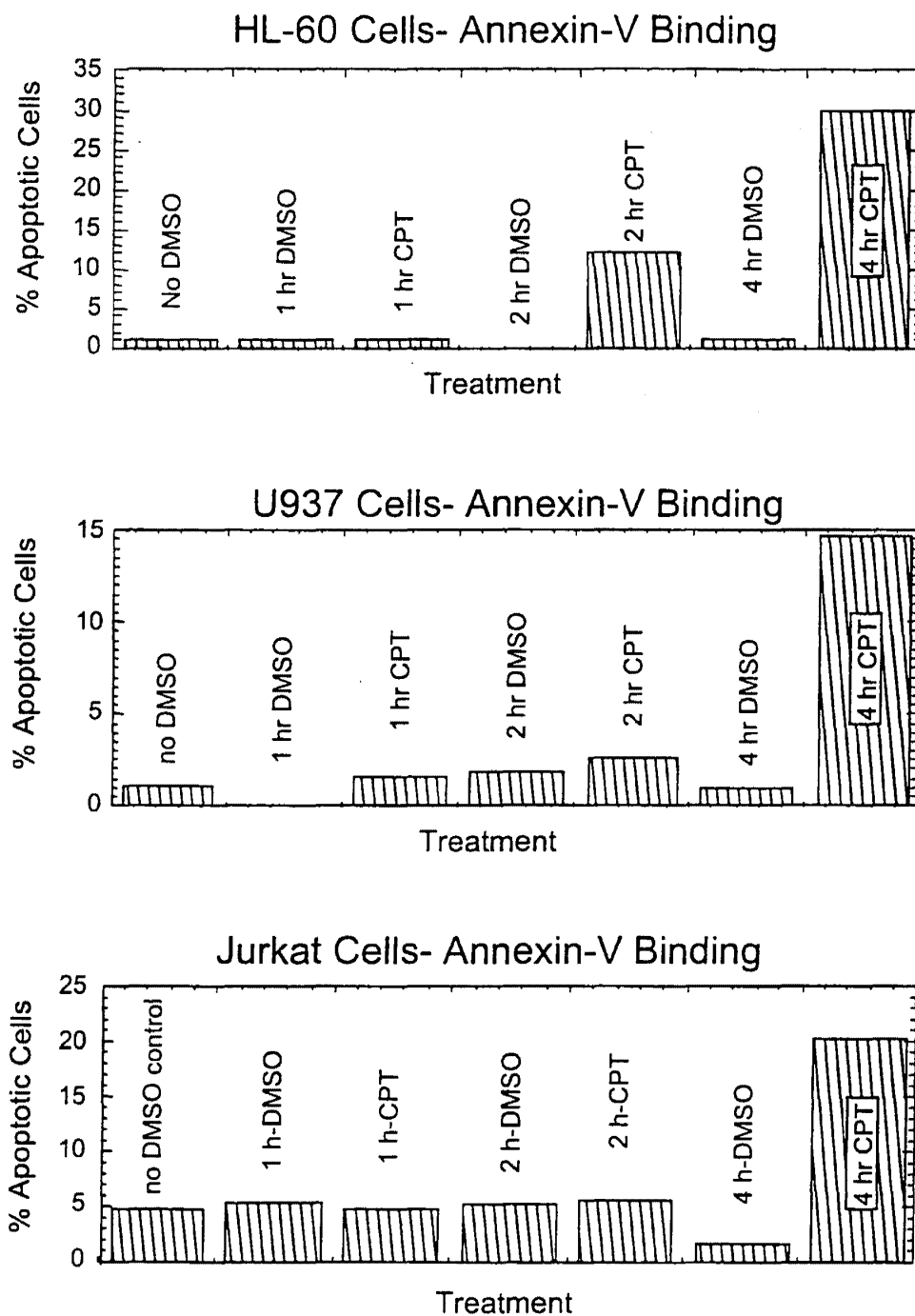


Fig. 15

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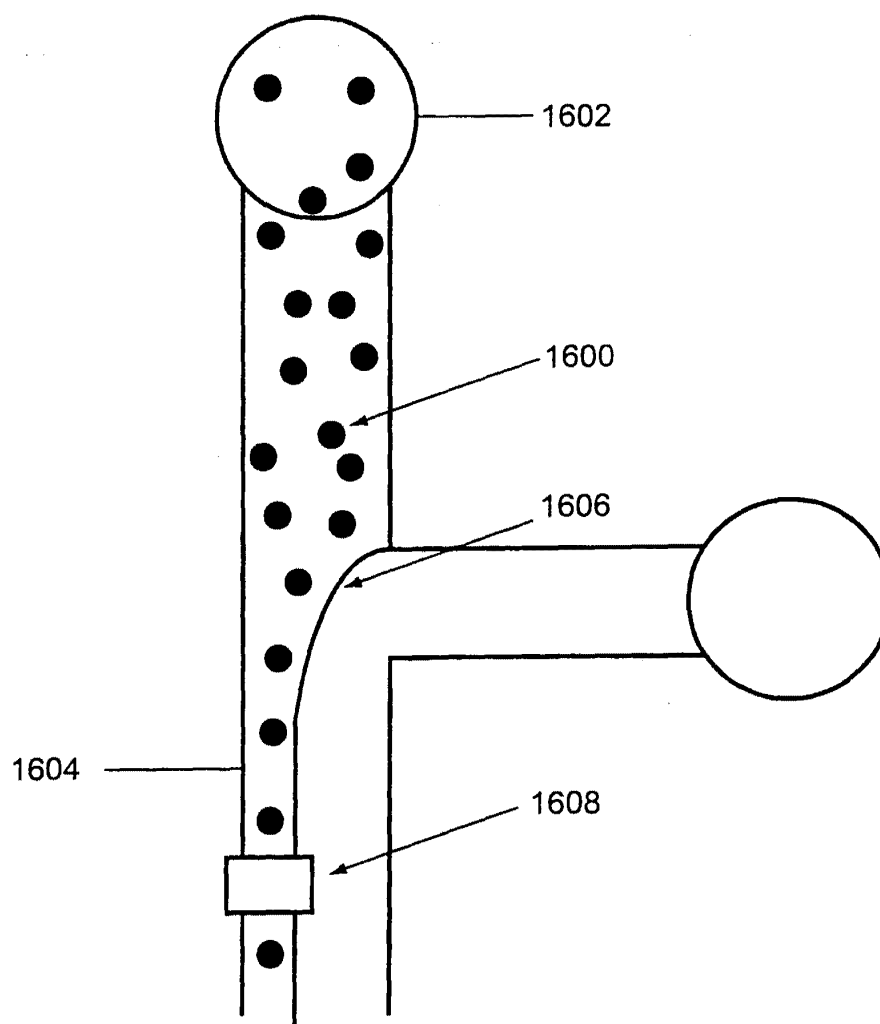


Fig. 16

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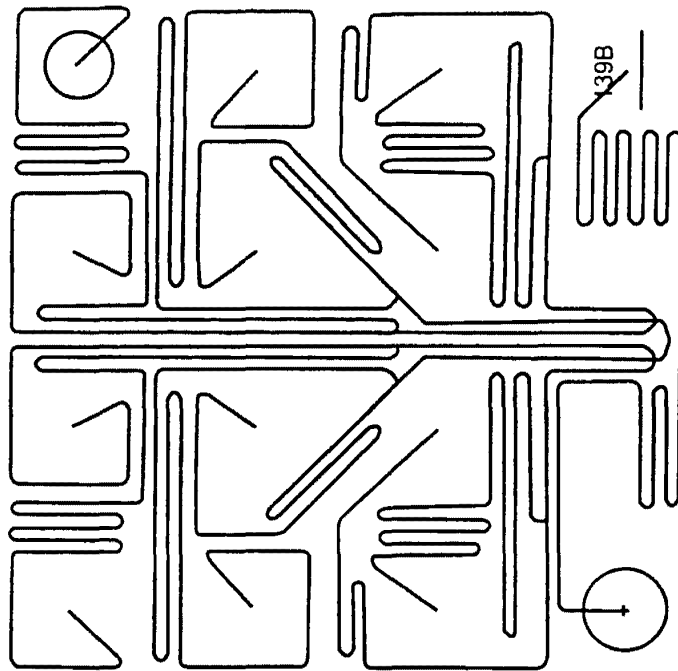


Fig. 17

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19991219004

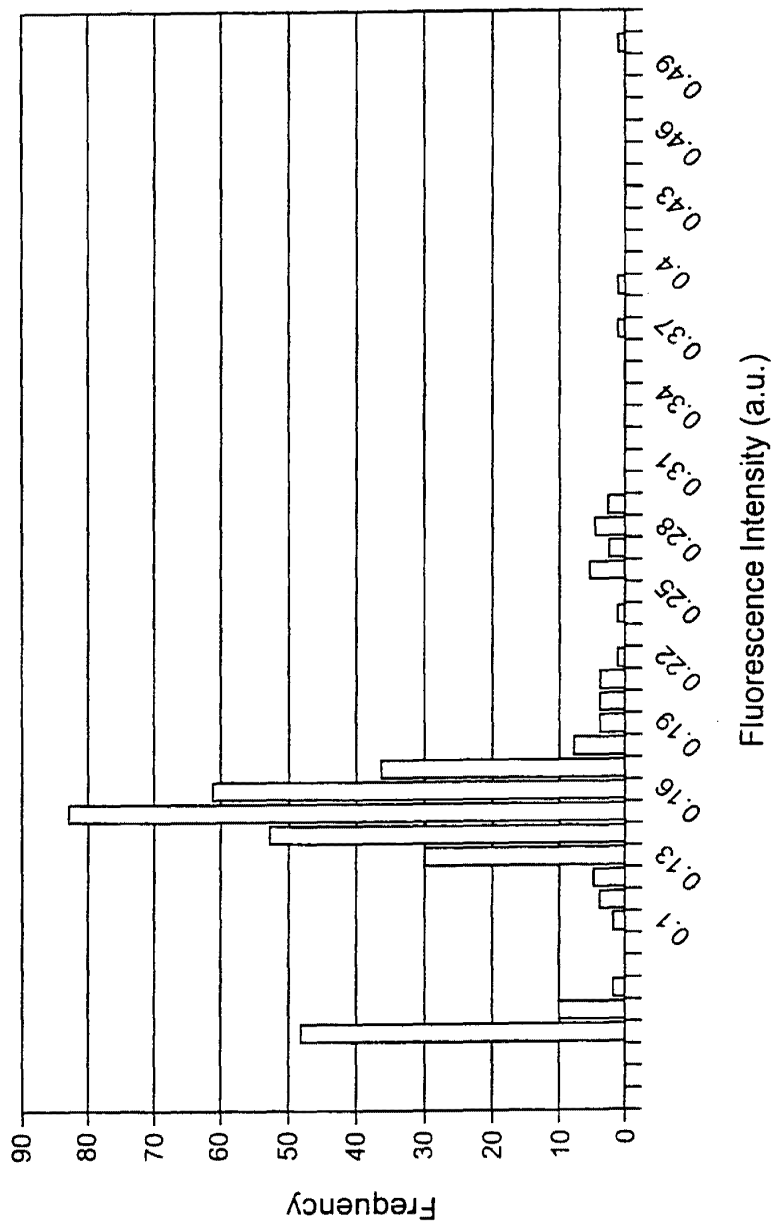


Fig. 18

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19991219003

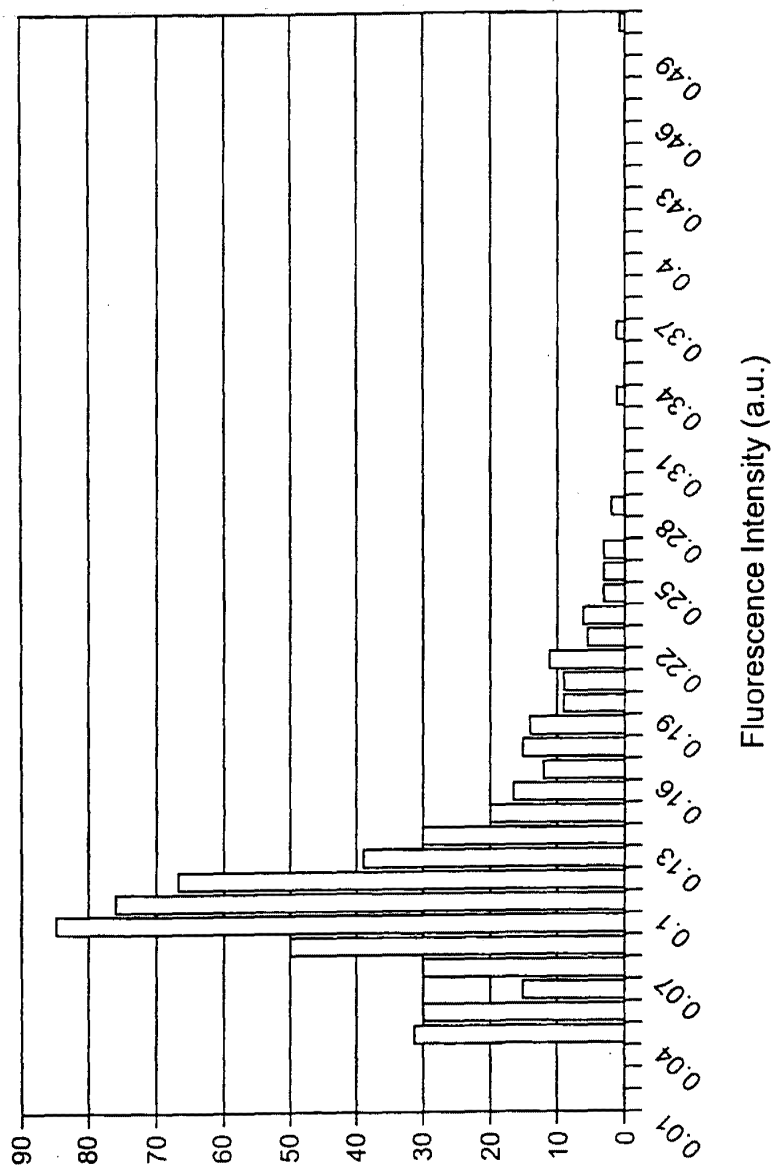


Fig. 19

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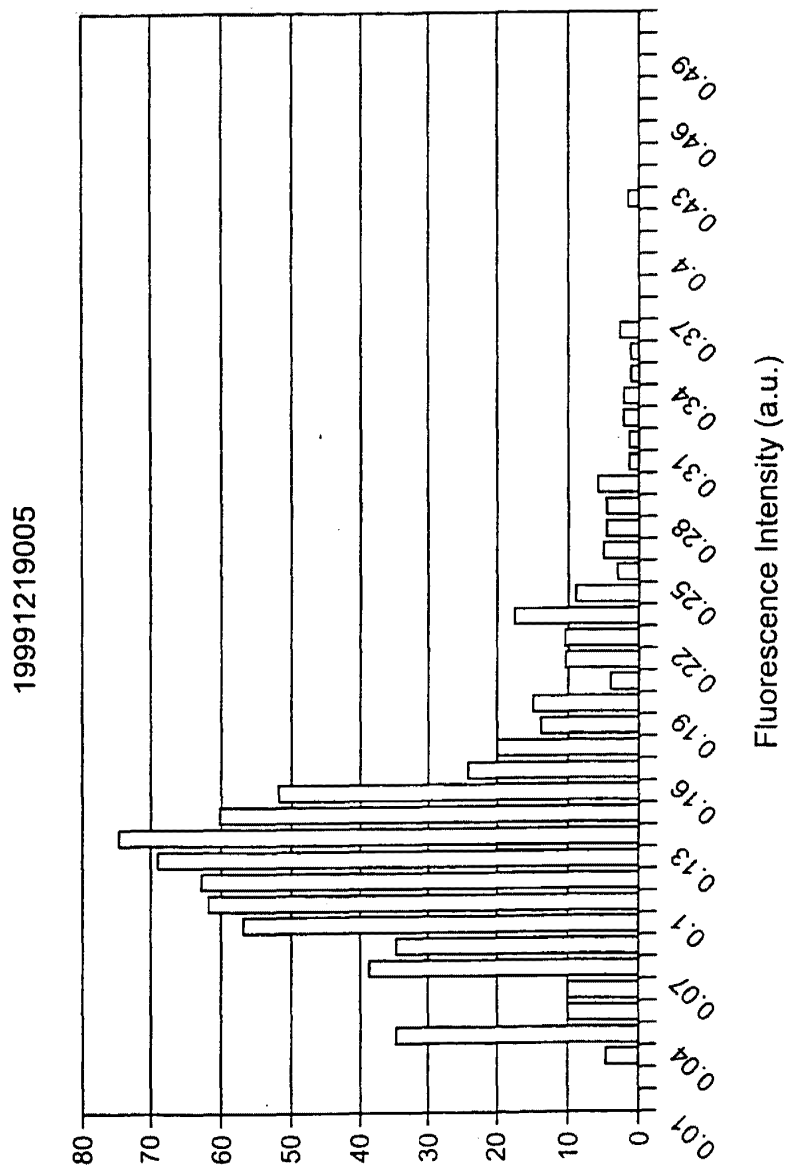


Fig. 20

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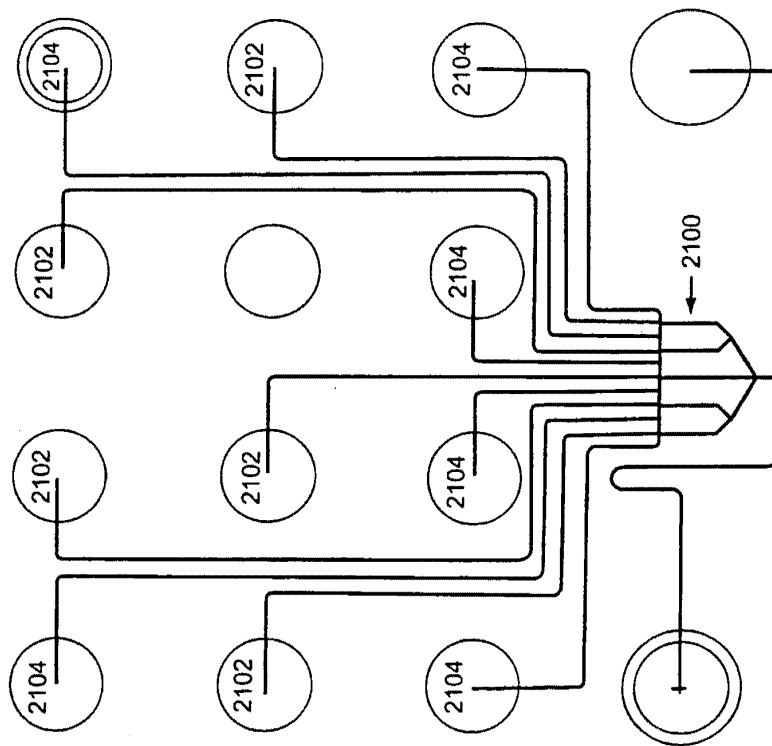


Fig. 21

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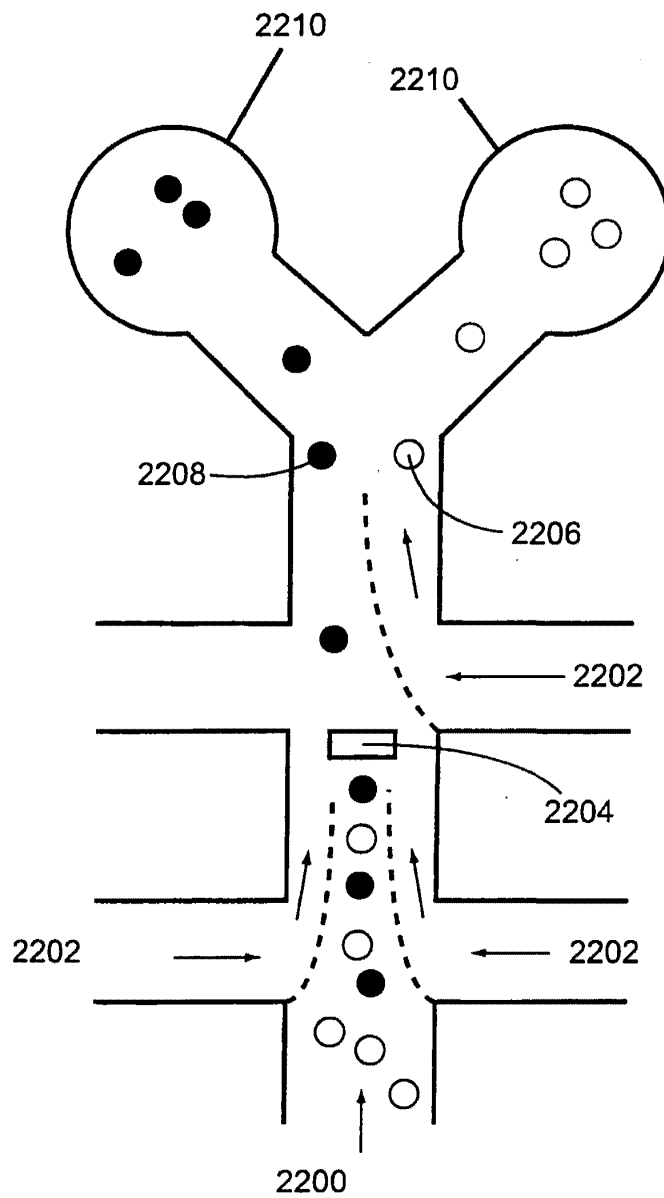


Fig. 22

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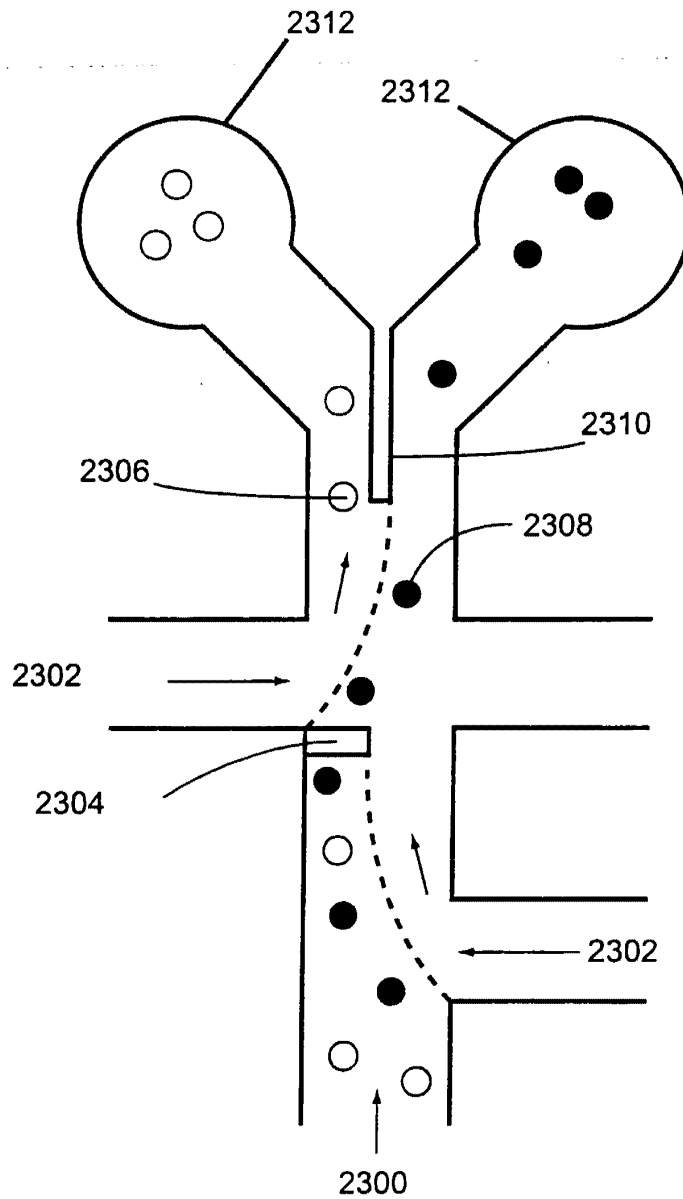


Fig. 23

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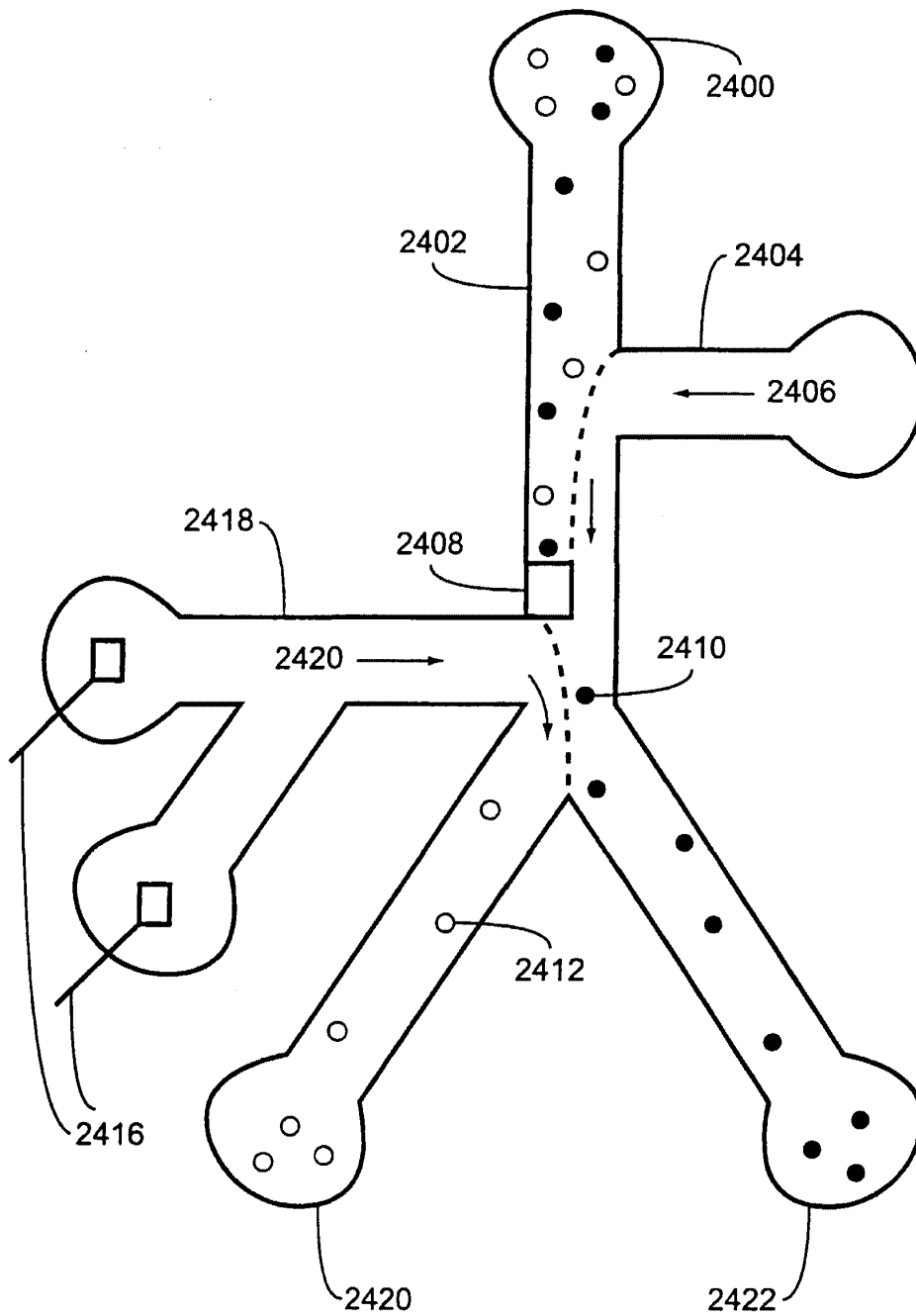


Fig. 24

SUBSTITUTE SHEET (RULE 26)

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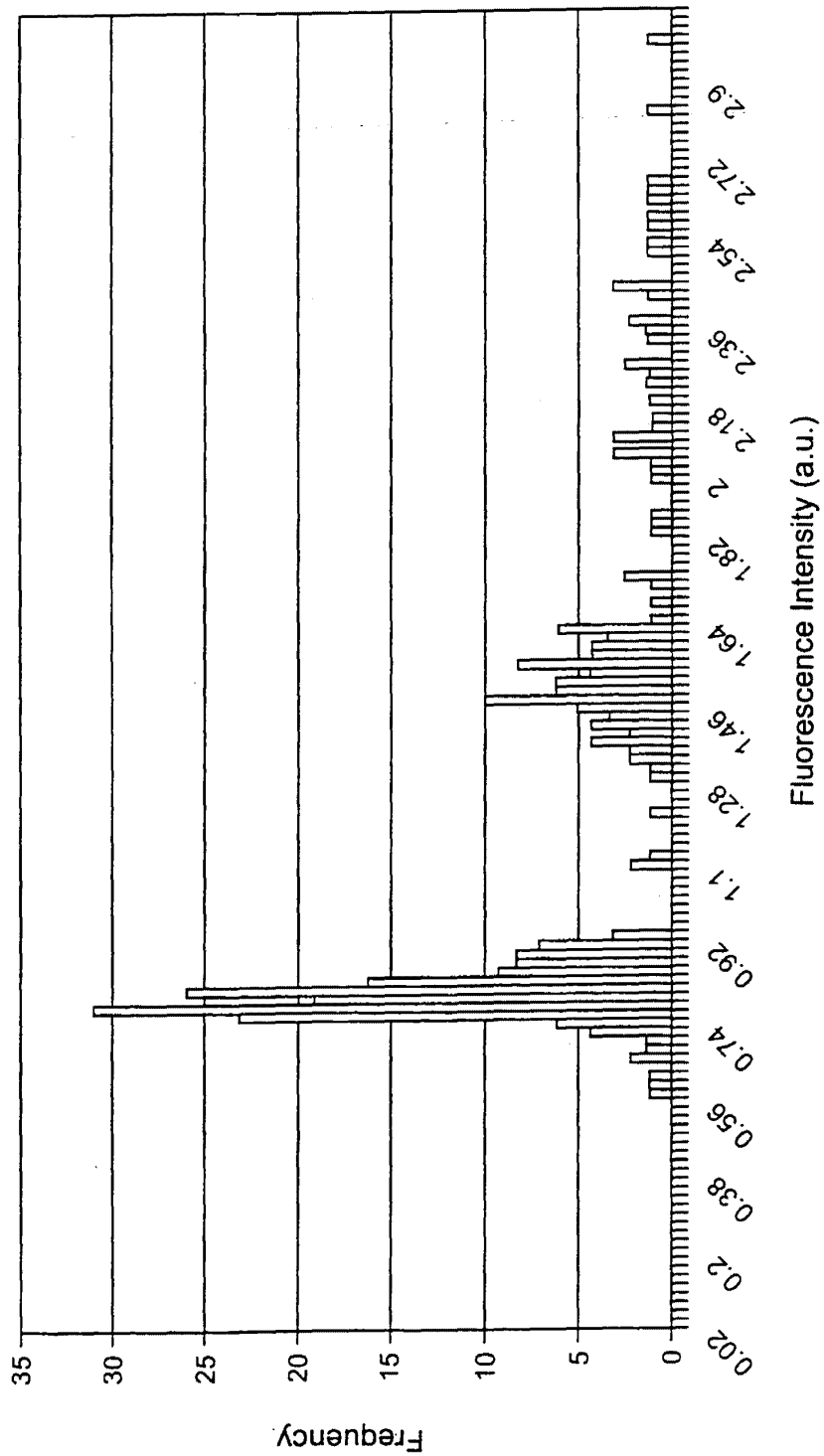


Fig. 25A

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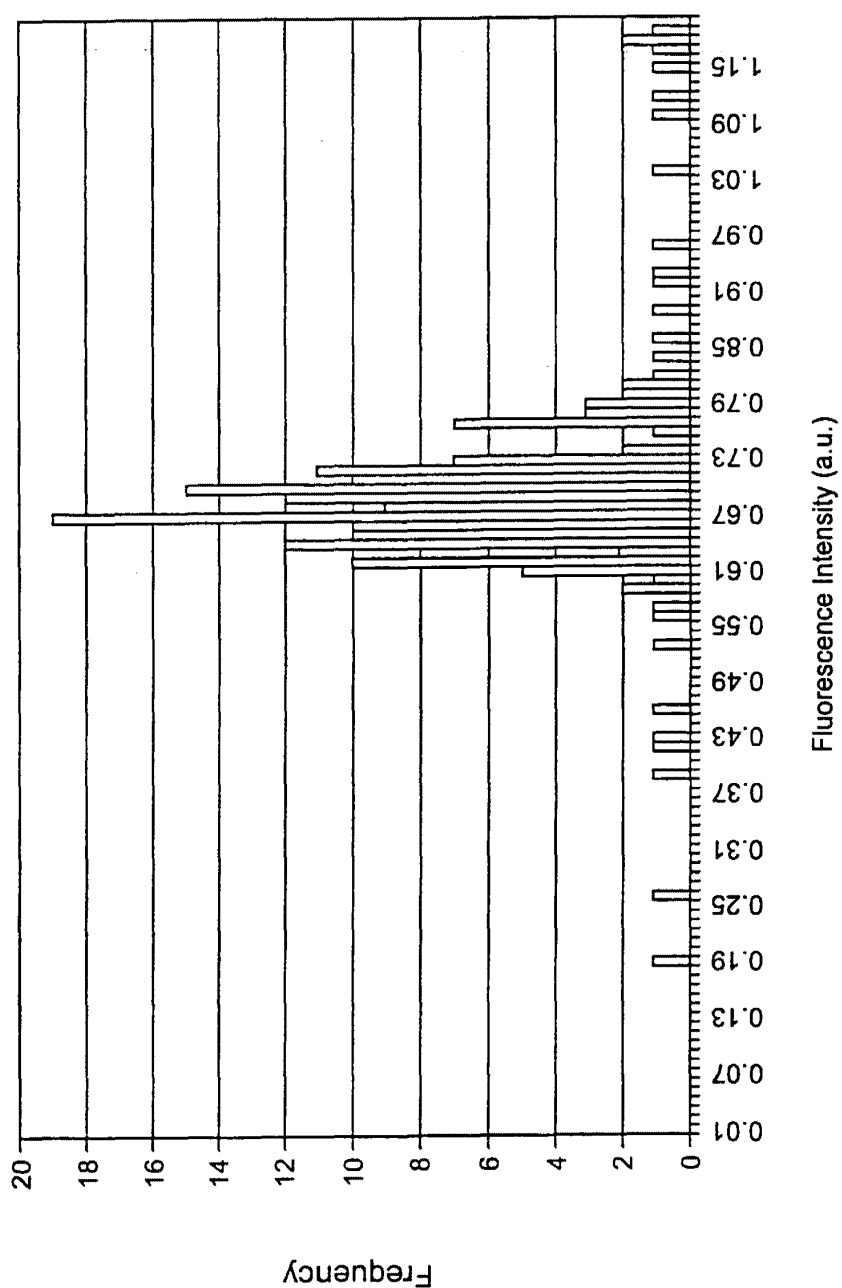


Fig. 25B

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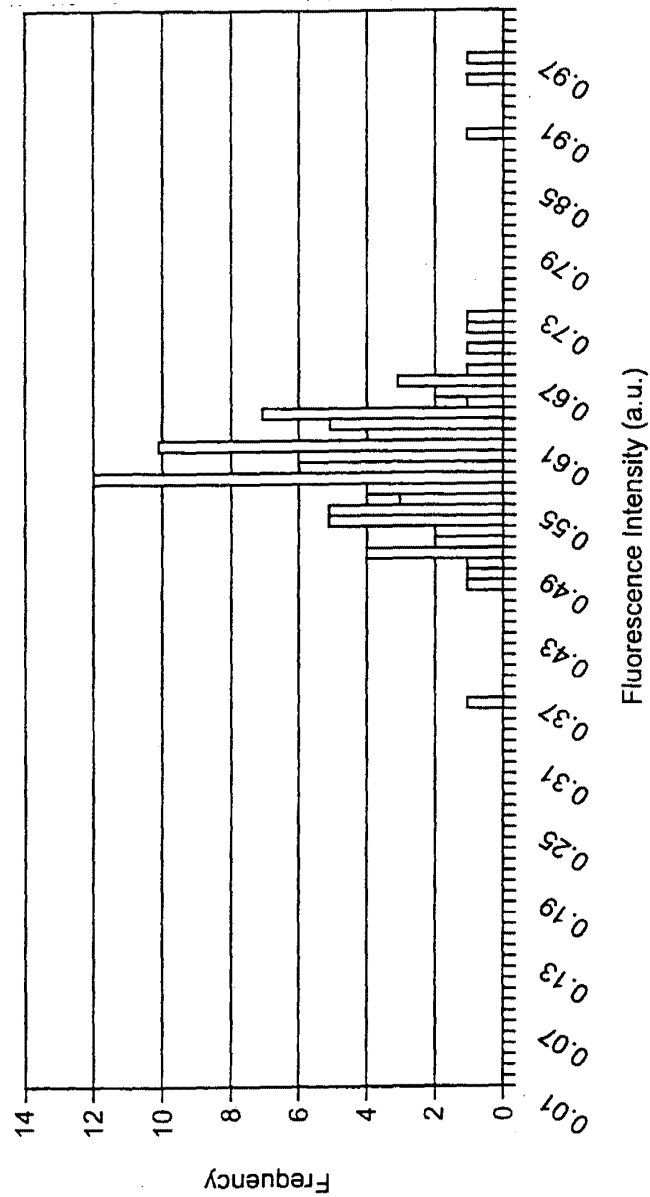


Fig. 25C

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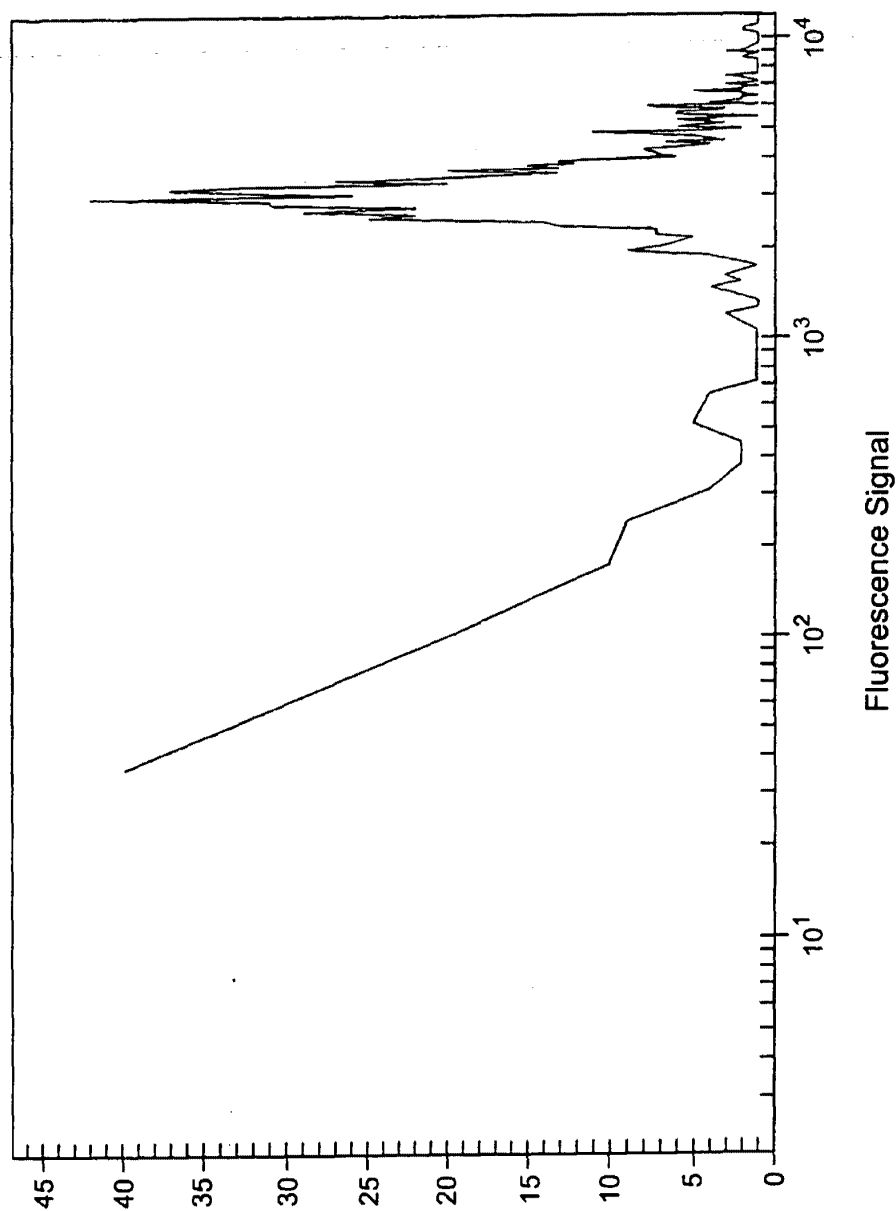


Fig. 26

SUBSTITUTE SHEET (RULE 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: <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"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</td> </tr> <tr> <td>"E" earlier application or patent published on or after the international filing date</td> <td>"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</td> </tr> <tr> <td>"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)</td> <td>"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</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"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	
"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 Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer Long Le Telephone No. (703) 308-0196										

Form PCT/ISA/210 (second sheet) (July 1998)



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/499,953	08/07/2006	Sebastian Bohm	TGZ-021DVCN	3579
959 7590 02/12/2008 LAHIVE & COCKFIELD, LLP ONE POST OFFICE SQUARE BOSTON, MA 02109-2127			EXAMINER HAGEMAN, MARK	
			ART UNIT 3653	PAPER NUMBER
			MAIL DATE 02/12/2008	DELIVERY MODE PAPER

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

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

Office Action Summary	Application No.	Applicant(s)	
	11/499,953	BOHM ET AL.	
	Examiner	Art Unit	
	Mark Hageman	3653	

-- 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 19 November 2007.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ 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

4) ☒ Claim(s) 2-17 and 19-28 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) 2-17 and 19-28 is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ 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).

11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

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) ☒ Notice of References Cited (PTO-892) 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date <u>9-5-2007</u> .	4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ 5) ☐ Notice of Informal Patent Application 6) ☐ Other: _____.

DETAILED ACTION

Double Patenting

1. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

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

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

2. Claims 2-17 and 19-28 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4 of U.S. Patent No.

7,104,405 in view of US 6,808,075. The claims of the current applications constitute combinations of claims elements from the cited patents that would have been obvious to one of ordinary skill in the art. Specifically the 405 patent claims the parallel and serial sorting arrangements while the 075 patent has claims directed to the details of the sensors and actuators.

3. Regarding the applicants request for withdrawal of the double patenting rejection based on the restriction requirement mailed 1-14-2004, examiner notes that a

prohibition of double patenting does not exist for the claims in the current application, see MPEP 804.01.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

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.

5. Claims 4-7 , 12 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

6. Claim 4 recites the limitation "the microchannel interior" in line 3. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 103

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

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

Claims 2-17 and 19-28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Diessel in view of US 7,258,774 to Chou. Diessel discloses a particle sorting system (abstract), comprising: a plurality of substantially parallel sorting channels (4) formed on a substrate, a plurality of sensors (9, 10), each sensor associated with one of said sorting channels for detecting and classifying particles in the

associated sorting channel based on a first predetermined characteristic (c2 lines 45+); a plurality of sorting modules (figures 1 and 7 and c3 lines 55+), each sorting module associated with one of said sorting channels, for distributing particles having the first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel (c2 lines 40+), each said sorting module including an actuator (12) controlled by one of said plurality of sensors, an aggregation region (c2 lines 49+) for aggregating particles having the predetermined characteristic from a plurality of the first outlets of the sorting channels, at least one secondary sorting channel (37, 38 figure 7) in series with the plurality of substantially parallel sorting channels for collecting selected particles having the first predetermined characteristic from a plurality of first outlets and separating particles in the secondary sorting channel based on a second predetermined characteristic, wherein the second predetermined characteristic is different from the first predetermined characteristic (c5 lines 57+), an inlet channel (3 figure 1) that branches to supply a plurality of the substantially parallel sorting channels with a stream of particles to be sorted.

Diessel does not disclose the actuator to selectively apply a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet, wherein the actuator is arranged external to the substrate and in communication with the associated sorting channel, wherein each sorting module further includes a gas-filled reservoir external to and in communication with the microchannel interior, wherein a fluid meniscus forms an interface between the gas-

filled reservoir and the interior of the associated sorting channel, wherein each actuator selectively increases an internal pressure of the associated gas-filled reservoir to create the pressure pulse in the associated sorting channel, wherein each sorting module further comprises a side passage connecting the associated sorting channel with the gas-filled reservoir, wherein the gas-filled reservoir includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir, wherein the associated actuator selectively deflects the movable membrane to generate the pressure pulse, wherein each sorting module further comprises a buffer for absorbing the pressure pulse, wherein the plurality of sorting channels are formed by grooves etched in a first plate, and the gas-filled reservoirs are formed by a second plate bonded to the first plate, the second plate including a plurality of apertures defining the gas-filled reservoirs, a flexible membrane bonded to the second plate to seal at least one of the gas-filled reservoirs and defining at least a portion of a movable wall of at least one of the gas-filled reservoirs.

Chou discloses an actuator (figures 7b 7h, 31a and 31b and c13 lines 6+) to selectively apply a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet, wherein the actuator is arranged external to the substrate and in communication with the associated sorting channel (c14 lines 15+), wherein each sorting module further includes a gas-filled reservoir (c14 lines 15+ and 32) external to and in communication with the microchannel interior, wherein a fluid meniscus (interface of channel 30 and membrane 25) forms an interface between the gas-filled reservoir and the interior of the associated sorting

channel, wherein each actuator selectively increases an internal pressure of the associated gas-filled reservoir to create the pressure pulse in the associated sorting channel (c 13 lines 8+), wherein each sorting module further comprises a side passage (32 where it intersects 30 see figure 7h) connecting the associated sorting channel with the gas- filled reservoir, wherein the gas-filled reservoir includes a movable membrane (25) forming at least a portion of a side wall of the gas-filled reservoir, wherein the associated actuator selectively deflects the movable membrane (c13 lines 8+ and figure 7h) to generate the pressure pulse, wherein each sorting module further comprises a buffer (2910 and figures 29a and 29b) for absorbing the pressure pulse, wherein the plurality of sorting channels are formed by grooves etched in a first plate, and the gas-filled reservoirs are formed by a second plate bonded to the first plate, the second plate including a plurality of apertures defining the gas-filled reservoirs, a flexible membrane bonded to the second plate to seal at least one of the gas-filled reservoirs and defining at least a portion of a movable wall of at least one of the gas-filled reservoirs (see figures 1-7 and c5 lines 25+) for the purpose of providing valves that are ideally suited for microfluidic metering and fluid control (c15 lines 13+).

It would have been obvious to one of ordinary skill in the art at the time of the applicant's invention to have modified Diessel to include the actuator system, as taught by Chou, as it would have been obvious to one of ordinary skill in the art to substitute one type of actuating system for another known type to achieve the predictable result of a valve system ideally suited for microfluidic metering and fluid control.

Response to Arguments

8. Applicant's arguments with respect to claims 2-17 and 19-28 have been considered but are moot in view of the new ground(s) of rejection. Applicant's remarks regarding the double patenting rejection are addressed in the double patenting section at the beginning of this office action.

Conclusion

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

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark Hageman whose telephone number is (571) 272-3027. The examiner can normally be reached on M-F 7:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Patrick Mackey can be reached on (571) 272-6916. 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.

/Patrick H. Mackey/
Supervisory Patent Examiner, Art
Unit 3653

MCH

Notice of References Cited	Application/Control No. 11/499,953		Applicant(s)/Patent Under Reexamination BOHM ET AL.	
	Examiner Mark Hageman		Art Unit 3653	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
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*	B	US-6,221,654	04-2001	Quake et al.	435/287.3
*	C	US-6,561,224	05-2003	Cho, Steven T.	137/831
*	D	US-7,220,594	05-2007	Foster et al.	436/180
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	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

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	P					
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	R					
	S					
	T					

NON-PATENT DOCUMENTS

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

<i>Index of Claims</i> 	Application/Control No. 11499953	Applicant(s)/Patent Under Reexamination BOHM ET AL.
	Examiner Mark Hageman	Art Unit 3653

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

☐ Claims renumbered in the same order as presented by applicant		☐ CPA		☐ T.D.		☐ R.1.47			
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Final	Original	05/18/2007	01/31/2008						
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Search Notes 	Application/Control No. 11499953	Applicant(s)/Patent Under Reexamination BOHM ET AL.
	Examiner Mark Hageman	Art Unit 3653

SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST see attached	2/1/2008	Mark Hageman
PLUS search (claims and spec)	1/31/2008	Mark Hageman
Reviewed examiner Dillon's search	1/31/2008	Mark Hageman

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner

EAST Search History

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S28	1	S22 and meniscus	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:53
S29	1	S22 and membrane	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 13:55
S30	6461	wada.in.	US-PGPUB; USPAT; USOCR	OR	ON	2008/01/31 14:35

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(Based on PTO 04-07 version)

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BÖHM
				Art Unit	3651
				Examiner Name	J. A. Dillon
Sheet	1	of	1	Attorney Docket Number	TGZ-021DVCN

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-4,572,664	02-25-1986	Hanson et al.	
	A2*	US-6,657,730-A1	12-02-2003	Pfau et al.	
	A3*	US-7,075,652	07-11-2006	Sarvazyan et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	Publication Date	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ³
		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)	MM-DD-YYYY			

*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 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 IPW. 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.

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: 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.

¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature	/Mark Hageman/	Date Considered	02/01/2008

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /MH/

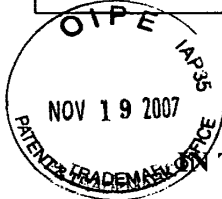
PLUS Search Results for S/N 11499953, Searched Thu Jan 31 09:01:31 EST 2008
The Patent Linguistics Utility System (PLUS) is a USPTO automated search system for U.S. Patents from 1971 to the present PLUS is a query-by-example search system which produces a list of patents that are most closely related linguistically to the application searched. This search was prepared by the staff of the Scientific and Technical Information Center, SIRA.

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PLUS Search Results for S/N 11499953, Searched Thu Jan 31 09:01:37 EST 2008
The Patent Linguistics Utility System (PLUS) is a USPTO automated search system for U.S. Patents from 1971 to the present PLUS is a query-by-example search system which produces a list of patents that are most closely related linguistically to the application searched. This search was prepared by the staff of the Scientific and Technical Information Center, SIRA.

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Docket No. TGZ-021DVCN
(PATENT)

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Böhm *et al.*

Application No. 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3651

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

Examiner: J.A. Dillon, Jr.

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION

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

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated May 18, 2007 (Paper Number 20070514), the response period of which extended to November 19, 2007 by the accompanying Request for Extension of Time and because November 18, 2007 fell on a Sunday, please amend the above-identified U.S. patent application as follows:

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

Remarks/Arguments begin on page 7 of this paper.

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02 FC:2202 75.00 DA

AMENDMENTS TO THE CLAIMS

Please amend claims 3, 15, 17 and 21, add new claims 24-28 and cancel claim 18, as set forth in the following listing of claims, which will replace all prior versions, and listings, of claims in the application.

1. (Canceled).
2. (Previously Presented) A particle sorting system, comprising:
 - a plurality of substantially parallel sorting channels formed on a substrate,
 - a plurality of sensors, each sensor associated with one of said sorting channels for detecting and classifying particles in the associated sorting channel based on a first predetermined characteristic;
 - a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having the first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including an actuator controlled by one of said plurality of sensors for selectively applying a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet.
3. (Currently Amended) The particle sorting system of claim 2, wherein the actuator is arranged external to the substrate and in communication with the associated sorting channel.
4. (Previously Presented) The particle sorting system of claim 3, wherein each sorting module further includes a gas-filled reservoir external to and in communication with the microchannel interior, wherein a fluid meniscus forms an interface between the gas-filled reservoir and the interior of the associated sorting channel.
5. (Previously Presented) The particle sorting system of claim 4, wherein each actuator selectively increases an internal pressure of the associated gas-filled reservoir to create the pressure pulse in the associated sorting channel.
6. (Previously Presented) The particle sorting system of claim 4, wherein each sorting module further comprises a side passage connecting the associated sorting channel with the gas-filled reservoir.

7. (Previously Presented) The particle sorting system of claim 4, wherein the gas-filled reservoir includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir, wherein the associated actuator selectively deflects the movable membrane to generate the pressure pulse.

8. (Previously Presented) The particle sorting system of claim 2, wherein each sorting module further comprises a buffer for absorbing the pressure pulse.

9. (Previously Presented) The particle sorting system of claim 2, further comprising an aggregation region for aggregating particles having the predetermined characteristic from a plurality of the first outlets of the sorting channels.

10. (Previously Presented) The particle sorting system of claim 2, further comprising:
at least one secondary sorting channel in series with the plurality of substantially parallel sorting channels for collecting selected particles having the first predetermined characteristic from a plurality of first outlets and separating particles in the secondary sorting channel based on a second predetermined characteristic.

11. (Previously Presented) The particle sorting system of claim 10 wherein the second predetermined characteristic is different from the first predetermined characteristic.

12. (Previously Presented) The particle sorting system of claim 4, wherein the plurality of sorting channels are formed by grooves etched in a first plate, and the gas-filled reservoirs are formed by a second plate bonded to the first plate, the second plate including a plurality of apertures defining the gas-filled reservoirs.

13. (Previously Presented) The particle sorting system of claim 12, further comprising:
a flexible membrane bonded to the second plate to seal at least one of the gas-filled reservoirs and defining at least a portion of a movable wall of at least one of the gas-filled reservoirs.

14. (Previously Presented) The particle sorting system of claim 2, further comprising an inlet channel that branches to supply a plurality of the substantially parallel sorting channels with a stream of particles to be sorted.

15. (Currently Amended) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, and
a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having a first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including a sealed gas-filled reservoir disposed in communication with the associated sorting channel and an actuator for increasing an internal pressure of the gas-filled reservoir to selectively modify ~~modifying~~ fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic into the first outlet of the associated sorting channel.
16. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir.
17. (Currently Amended) The particle sorting system of claim 16, ~~further comprising a plurality of actuators, each actuator associated with a sorting module,~~ wherein each actuator selectively deflects the movable membrane to increase pressure in the gas-filled reservoir to modify said fluid flow.
18. (Canceled).
19. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further comprises a buffer for limiting said modification of the fluid flow to a region of the associated sorting channel along the sorting module and preventing flow disturbance in other regions of the associated sorting channel.
20. (Previously Presented) The particle sorting system of claim 15, wherein each sorting module further includes a side channel connecting the gas-filled reservoir to the associated sorting channel.
21. (Currently Amended) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, each sorting channel capable of conveying a stream of particles in a carrier fluid,
a plurality of actuators arranged external to the substrate, each actuator associated with a sorting channel formed on the substrate, for selectively creating a flow disturbance within the

associated sorting channel to deflect a selected particle in the stream of particles from the stream of particles; and

a plurality of buffers, each buffer associated with an actuator for absorbing the flow disturbance in the associated sorting channel.

22. (Previously Presented) The particle sorting system of claim 21, wherein each buffer comprises a gas-filled reservoir in fluid communication with the associated sorting channel.

23. (Previously Presented) The particle sorting system of claim 21, wherein each sorting channel includes a gas-filled reservoir in fluid communication therewith, and the actuator increases an internal pressure of the gas-filled reservoir to create said flow disturbance.

24. (Previously Presented) The particle sorting system of claim 23, wherein each gas-filled reservoir includes a flexible membrane forming at least a portion of a side wall of the gas-filled reservoir, and the actuator deflects the flexible membrane to increase said internal pressure.

25. (New) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, and
a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having a first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including a gas-filled reservoir having a movable wall disposed in communication with the associated sorting channel for selectively modifying fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic into the first outlet of the associated sorting channel.

26. (New) The particle sorting system of claim 25, wherein the movable wall comprises a flexible membrane.

27. (New) The particle sorting system of claim 25, wherein each sorting module further includes an associated actuator for selectively moving the movable wall to increase an internal pressure of the gas-filled reservoir to selectively modify the fluid flow through the associated sorting channel.

28. (New) A particle sorting system, comprising:

a plurality of substantially parallel sorting channels formed on a substrate,

a plurality of sensors, each sensor associated with one of said sorting channels for detecting and classifying particles in the associated sorting channel based on a first predetermined characteristic;

a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having the first predetermined characteristic to first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including an actuator arranged external to the substrate and in communication with the associated sorting channel, the actuator controlled by one of said plurality of sensors for selectively applying a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet.

REMARKS

The foregoing amendment amends independent claims 15 and 21 as well as dependent claims 3 and 17 and cancels claim 18. Claims 25-28 are added to more fully capture the instant invention. Pending in the application are claims 2-17 and 19-28, of which claims 2, 15, 21, 25 and 28 are independent. The following comments address all stated grounds for rejection and place the presently pending claims, as identified above, in condition for allowance.

Claim 3 is amended to specify that the actuator is external to the substrate.

Independent claim 15 is amended to specify that the gas-filled reservoir is sealed, as set forth on page 6, lines 20-21, and to recite an actuator for selectively increasing the internal pressure of the gas-filled reservoir, as set forth in claim 18.

Independent claim 21 is amended to specify that the actuators are arranged external to the substrate, as set forth throughout the application as originally filed, at least, for example, on page 11, line 10.

New claim 25 corresponds to original claim 15, and further specifies that each gas-filled reservoir includes a movable wall, as set forth at least, for example, on page 13, line 32-34. New claim 26 depends from claim 25, and further specifies that the movable wall is a flexible membrane. New claim 27 depends from claim 25 and further specifies that each sorting module includes an associated actuator for selectively moving the movable wall to increase an internal pressure of the gas-filled reservoir to selectively modify the fluid flow through the associated sorting channel.

New claim 28 corresponds to the subject matter of claim 3, rewritten in independent form. *No new matter is added.*

Amendment and/or cancellation of the claims is not to be construed as an acquiescence to any of the objections/rejections set forth in the instant Office Action, and was done solely to expedite prosecution of the application. Applicant reserves the right to pursue the claims as originally filed, or similar claims, in this or one or more subsequent patent applications.

Allowable Claims

On page 1 of the Office Action, the Examiner indicates that claims 2-24 are rejected. However, the Examiner fails to provide *any* evidence that claims 2-9 are not patentable. The Examiner only provides reasons for rejecting claims 10-24 under the “Double Patenting Rejection” section and the “35 U.S.C. 103 Rejections” section. The Examiner does not mention claims 2-9 as being rejected, nor does the Examiner provide a prior art against claims 2-9 and/or reason(s) that claims 2-9 are not patentable. As Applicants are entitled, under 35 U.S.C. §102 and 35 U.S.C. §103, to a patent unless the Examiner can make a *prima facie* case of anticipation or non-obviousness, Applicants submit that claims 2-9 are allowable. Further, Applicants understand that if the Examiner issues a subsequent Office Action to detail any rejection of claims 2-9, that Office Action will be a non-final Office Action.

Double Patenting Rejections

Applicants thank the Examiner for the close review of the claims. In the Office Action, the Examiner rejects claims 10-24 on the ground of non-statutory obviousness-type double patenting. However, Applicants submit that the presently pending claims are different and distinguish patentably from claims 1-4 of U.S. Patent Number 7,104,405 and claims 1-22 of U.S. Patent Number 6,808,075, because claims 2-17 and 19-24 correspond to the Claims 29-32 drawn to a sorting system identified by the Examiner as patently distinct Group III in the Restriction Requirement mailed January 14, 2004. Accordingly, Applicants respectfully request the Examiner to withdraw the non-statutory obviousness-type double patenting rejection of claims 10-24.

35. U.S.C. 103 Rejections

In the Office Action, the Examiner rejects claims 10-24 under 35 U.S.C. 103(a) as being obvious over the Diessel reference (U.S. Patent Number 5,837,200) or the Dubrow reference (U.S. Patent Number 5,976,336) in view of either the Shoor reference (U.S. Patent Number 4,526,276) or the Zold reference (U.S. Patent Number 4,936,465) or the Gaelatti reference (U.S. Patent Number 3,508,654). As recognized by the Examiner, the Diessel reference and the Dubrow reference lacks a sorting system that employs a pressure pulse, as recited in claim 2, and/or a gas-filled reservoir for selectively deflecting a selected particle, as recited in claim 15. In addition, as recognized by the Examiner, the Diessel reference and the Dubrow reference also

fail to disclose a buffer/actuator arrangement in a sorting system, as set forth in independent claim 21. According to the Examiner, the Shoor reference, the Zold reference and the Glaettli reference disclose a pressure pulse/buffer actuator arrangement. The Examiner concludes that it would be obvious to modify the devices of Diessel and Dubrow to include a “pressure pulse/buffer actuator arrangement in order to increase system versatility as taught by” Shoor, Zold and Glaettli, rendering claims 10-24 unpatentable. Applicants submit that none of the Shoor, Zold or Glaettli references teaches use of a transient pressure pulse to selectively deflect a selected particle, an associated actuator, or a buffer. To expedite prosecution, Applicants have amended independent claim 15 to specify that the gas-filled reservoir is sealed and that an actuator selectively increases the internal pressure of the gas-filled reservoir, a feature also lacking in the cited references. Independent claim 21 is amended to specify that each actuator is arranged *external* to the substrate, a feature lacking in the cited references

Even in combination, the references fail to disclose the subject matter of claims 10-24. Moreover, motivation to combine the references, as required under 35 U.S.C. 103, is lacking.

The Shoor Reference

The Shoor reference is directed to an apparatus that utilizes gas actuation to sort particles. The apparatus in Shoor relies on at least one electrode “on the inner tube which generates bubbles to block the entrance to the inner tube”. (See column 6, lines 5-6.) As set forth in column 6, lines 55-58, “[e]lectrical impulses are provided to gas generating electrodes 68 which, when activated, cause gas bubbles 80 to occur at the proper time to **block** entrance end 61 of the inner tube. Accordingly, the gas bubbles prevent certain particles, determined in accordance with the information from the analyzer, from entering the inner tube. Further, the occurrence of gas bubbles deflects the flowing particles into annular space 60 between the inner and outer tubes.” (Emphasis added.) Although, claim 2 does not appear rejected under 35 U.S.C. §103, Applicants for the sake of expatiated prosecution, make note that the gas bubbles present a *prolonged* obstruction to block an entrance, rather than a *transient* pressure pulse that deflects a single selected particle, as recited in claim 2.

The Shoor reference also does not disclose a sorting device including a *sealed*, gas-filled chamber and an actuator for selectively increasing an internal pressure of the gas-filled reservoir

to effect sorting, as set forth in independent claim 15. In fact, the Shoor reference teaches away from a gas-filled chamber, as the flow channels 35, 45 are open. In addition, there is no chamber in Shoor that increases in pressure to effect sorting. Rather, as described above, Shoor relies on selective obstruction of an entrance to direct particles.

In addition, the Shoor reference does not employ an external actuator that is *external* to a substrate in which channels are formed, as set forth in claim 21. The use of an external actuator provides significant advantages not seen in the cited references. For example, an external actuator does not get contaminated, is easily replaced, and is reusable for multiple sorting episodes. In contrast, the electrodes 70 and 68 are disposed *within* the flow chamber 20 and therefore exposed to the sheath fluid and particles. The electrodes could not be external to the assembly 15, because the bubbles that selectively block the entrances to the tubes 40 and 41 must be formed within the flow chamber 20, requiring the electrodes to also be formed within the assembly 15. Therefore, Shoor in fact teaches away from an external actuator.

The Zold Reference

The Zold reference also fails to disclose the subject matter of the claimed invention. According to the Examiner, the Zold reference discloses sorting by pressure pulse via a meniscus interface, a buffer and/or a gas-filled reservoir and is therefore combinable with the Diessel reference and the Dubrow reference to render the subject matter of claim 10-24 obvious. However, the Zold reference does not rely on a pressure pulse, i.e., a positive, momentary flow disturbance, to deflect a particle from a stream of cells. Rather, the Zold reference paralyzes flow through an outlet channel 16 or 17 to displace an entire flow stream, rather than a single, selected particle. In Zold, a gas bubble generated between either of two electrode pairs blocks flow through a selected outlet channel to change the overall flow of particles via a suction effect, without applying a pressure pulse to a single particle at a time. The gas bubble created in Zold does not *deflect* a particle from a stream. In Zold, the device returns to steady-state only when the outlet channel becomes unblocked. In contrast, the present invention automatically returns to steady state after application of the momentary deflecting impulse. See column 17, lines 2-40, column 18, lines 47-54 and, column 19, lines 12-60 of Zold. Therefore, the subject matter of claim 2 is different from the subject matter described in the Zold reference.

The Zold reference also does not employ a sealed, gas-filled reservoir, or an actuator that selectively *increases* an internal pressure of a gas-filled reservoir to selectively deflect a particle, as recited in independent claim 15. The cavities 20, 21 of Zold do not increase in pressure at any point. The buffer cavities 20 and 21 are not sealed and are simply “large holes...drilled into the body 1 of the device quasi perpendicularly into its top surface 4”, as set forth in column 20, lines 41-44 and shown in Figures 1 and 2. No meniscus is employed or present in the device of Zold to deflect a particle. Therefore, claims 15-20 are patentable over the prior art.

The Zold reference also does not disclose an external actuator, as recited in claim 21. Rather, similar to the Shoor reference, the Zold reference requires *internal* electrodes 18, 19 that are exposed to the fluid stream. The Zold reference could not operate using external actuators, rendering claims 21-24 patentable over the Zold reference.

The Glaettli Reference

The Glaettli reference is directed to a particle separation apparatus that relies on *suction* to extract a volume of liquid from a channel. The Glaettli reference does not disclose use of a pressure pulse, nor does the Glaettli reference disclose a buffer for absorbing a pressure pulse. The Glaettli device requires that “a sufficiently large volume of fluid is extracted from the junction to include the selected cell and withdraw it sufficiently far from the junction” (see column 2, lines 50-55.) The use of *prolonged* suctioning (negative pressure) is different from the use of a positive, transient pressure pulse to deflect a particle. Although Applicants understand claim 2 is not formally rejected under 35 U.S.C. §103, for the sake of expedited prosecution, Applicants take note that the sorting mechanism of Glaettli is opposite from the claimed means for sorting set forth in claim 2.

The Glaettli reference also does not disclose *sealed*, gas-filled chamber and an actuator for selectively increasing an internal pressure of the gas-filled reservoir to effect sorting, as set forth in independent claim 15. Even if the dead-end 71 of Glaettli could be considered a sealed, gas-filled reservoir, the dead end 71 and trapped gas bubble do not effect sorting, nor does the pressure in the dead end 71 increase. Rather, Glaettli relies on a decrease in pressure in the dead end 71 of the device to prevent cavitation effects, in contrast to the claimed invention.

The Glaettli reference also does not disclose a plurality of actuators arranged external to the substrate for selectively deflecting a selected particle and/or buffers for absorbing the flow disturbance in the associated sorting channel. The piston 59 of Glaettli is disposed *within* a channel 57, in contact with the carrier fluid. In addition, no buffer for absorbing a flow disturbance is provided in Glaettli.

Dependent claims

The dependent claims recite additional patentable features not disclosed in the cited references.

For example, claim 3 specifies that the actuator is arranged external to the substrate and in communication with the associated sorting channel, a feature not disclosed the cited references, as described above.

Claims 4-6, 18 and 23 recite a gas-filled reservoir in communication with a sorting channel for sorting particles, a feature not disclosed in the cited references.

Claims 7, 13, 16 and 17 recite a movable membrane forming a side wall of a reservoir, which an actuator deflects to generate a pressure pulse to deflect a particle.

Claims 8, 19 and 22 recite a buffer for absorbing a pressure pulse used to selectively deflect a particle, a feature not disclosed in the cited references, as described above.

Claim 9 recites an aggregation region for aggregating particles having the predetermined characteristic from a plurality of the first outlets of the sorting channels, a feature also not disclosed in the cited references.

Claims 10-11 recite a secondary sorting channel for receiving sorted particles and performing a secondary sorting operation.

Claims 12-13 specify that the system comprises a plurality of plates stacked together, a feature also not disclosed in the cited references.

New Claims

New claim 24 corresponds to original claim 15, and further specifies that each gas-filled reservoir includes a movable wall, a feature also not disclosed in the cited references, as set forth above. New claim 26 depends from claim 25, and further specifies that the movable wall is a flexible membrane, a feature also not set forth in the cited references. New claim 27 depends from claim 25 and further specifies that each sorting module includes an associated actuator for selectively moving the movable wall to increase an internal pressure of the gas-filled reservoir to selectively modify the fluid flow through the associated sorting channel, a feature also not disclosed in the cited references

New claim 28 is directed to a sorting system that includes an actuator arranged external to the substrate in which the sorting channels are formed, a feature also not disclosed in the cited references, as described above.

Application No.: 11/499,953

Art Unit: 3651

Docket No.: TGZ-021DVCN

CONCLUSION

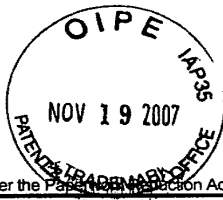
In view of the above amendment and remarks, Applicants believe the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned attorney at (617) 227-7400.

If any additional fee is due with this statement, please charge our Deposit Account No. 12-0080, under Order No. TGZ-021DVCN, from which the undersigned is authorized to draw

Dated: November 19, 2007

Respectfully submitted,

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Boston, Massachusetts 02109
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(617) 742-4214 (Fax)
Attorney For Applicants



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PTO/SB/21 (10-07)

Approved for use through 10/31/2007. OMB 0851-0031

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

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TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	11/499,953-Conf. #3579
	Filing Date	August 7, 2006
	First Named Inventor	Sebastian BÖHM
	Art Unit	3651
	Examiner Name	J. A. Dillon
	Attorney Docket Number	TGZ-021DVCN
Total Number of Pages in This Submission		

ENCLOSURES (Check all that apply)		
☒ Fee Transmittal Form ☐ Fee Attached ☒ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☒ Extension of Time Request ☐ Express Abandonment Request ☐ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please identify below): Return Receipt Postcard
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	LAHIVE & COCKFIELD, LLP		
Signature			
Printed name	David R. Burns		
Date	November 19, 2007	Reg. No.	46,590

Express Mail Label No. EV 956468350 US Dated: November 19, 2007



PTO/SB/17 (10-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004. Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).		Complete if Known	
FEE TRANSMITTAL For FY 2008		Application Number	11/499,953-Conf. #3579
		Filing Date	August 7, 2006
		First Named Inventor	Sebastian BÖHM
		Examiner Name	J. A. Dillon
		Art Unit	3651
☒ Applicant claims small entity status. See 37 CFR 1.27		Attorney Docket No.	TGZ-021DVCN
TOTAL AMOUNT OF PAYMENT		(\$)	810.00

METHOD OF PAYMENT (check all that apply)	
☐ Check	☐ Credit Card
☐ Money Order	☐ None
☐ Other (please identify): _____	
☒ Deposit Account	Deposit Account Number: <u>12-0080</u>
	Deposit Account Name: <u>Lahive & Cockfield, LLP</u>
For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)	
☒ Charge fee(s) indicated below	☐ Charge fee(s) indicated below, except for the filing fee
☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17	☒ Credit any overpayments

FEE CALCULATION							
1. BASIC FILING, SEARCH, AND EXAMINATION FEES							
	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		<u>Small Entity</u>		<u>Small Entity</u>		<u>Small Entity</u>	
Application Type	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fees Paid (\$)
Utility	310	155	510	255	210	105	
Design	210	105	100	50	130	65	
Plant	210	105	310	155	160	80	
Reissue	310	155	510	255	620	310	
Provisional	210	105	0	0	0	0	
2. EXCESS CLAIM FEES							
						Fee (\$)	Small Entity Fee (\$)
Fee Description							
Each claim over 20 (including Reissues)						50	25
Each independent claim over 3 (including Reissues)						210	105
Multiple dependent claims						370	185
Total Claims	Extra Claims	Fee (\$)	Fee Paid (\$)	Multiple Dependent Claims			
<u>26</u>	<u>- 23 = 3</u>	<u>x 25.00 =</u>	<u>75.00</u>	Fee (\$)	Fee Paid (\$)		
HP = highest number of total claims paid for, if greater than 20.							
Indep. Claims	Extra Claims	Fee (\$)	Fee Paid (\$)				
<u>5</u>	<u>- 3 = 2</u>	<u>x 105.00 =</u>	<u>210.00</u>				
HP = highest number of independent claims paid for, if greater than 3.							
3. APPLICATION SIZE FEE							
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$260 (\$130 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).							
Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)			
<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>			
4. OTHER FEE(S)							
Non-English Specification, \$130 fee (no small entity discount)						Fees Paid (\$)	
Other (e.g., late filing surcharge): <u>2253 Extension for response within third month</u>						<u>525.00</u>	

SUBMITTED BY			
Signature	<u>Daniel R. Burns</u>	Registration No. (Attorney/Agent)	<u>46,590</u>
Name (Print/Type)	<u>David R. Burns</u>	Telephone	<u>(617) 994-0890</u>
		Date	<u>November 19, 2007</u>

Express Mail Label No. EV 956468350 US Dated: November 19, 2007



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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2006 (Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)		Docket Number (Optional) TGZ-021DVCN	
Application Number 11/499,953-Conf. #3579		Filed August 7, 2006	
For METHOD AND APPARATUS FOR SORTING PARTICLES			
Art Unit 3651		Examiner J. A. Dillon	
This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.			
The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):			
	<u>Fee</u>	<u>Small Entity Fee</u>	
☐ One month (37 CFR 1.17(a)(1))	\$120	\$60	\$ _____
☐ Two months (37 CFR 1.17(a)(2))	\$460	\$230	\$ _____
☒ Three months (37 CFR 1.17(a)(3))	\$1050	\$525	\$ 525.00
☐ Four months (37 CFR 1.17(a)(4))	\$1640	\$820	\$ _____
☐ Five months (37 CFR 1.17(a)(5))	\$2230	\$1115	\$ _____
☒ Applicant claims small entity status. See 37 CFR 1.27.			
☐ A check in the amount of the fee is enclosed.			
☐ Payment by credit card. Form PTO-2038 is attached.			
☒ The Director has already been authorized to charge fees in this application to a Deposit Account.			
☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 12-0080. I have enclosed a duplicate copy of this sheet.			
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.			
I am the ☐ applicant/inventor.			
☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).			
☒ attorney or agent of record. Registration Number 46,590			
☐ attorney or agent under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____			
_____ Signature		_____ Date	
_____ David R. Burns Typed or printed name		_____ (617) 994-0890 Telephone Number	
NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.			
☐ Total of 1 forms are submitted.			

11/20/2007 FHETEKI1 00000044 120080 11499953
03 FC:2253 525.00 DA

Express Mail Label No. EV 956468350 US Dated: November 19, 2007

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 11/499,953		Filing Date 08/07/2006		☐ To be Mailed	
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		N/A					
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A		N/A					
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A		N/A					
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X \$	=	X \$	=				
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X \$	=	X \$	=				
☐ 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).									
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))										
			TOTAL		TOTAL					
* If the difference in column 1 is less than zero, enter "0" in column 2.										
APPLICATION AS AMENDED – PART II										
(Column 1)			(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY	
AMENDMENT	11/19/2007	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	RATE (\$)	ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	* 26	Minus	** 23	= 3	X \$25 =	75	OR	X \$ =	
	Independent (37 CFR 1.16(h))	* 5	Minus	*** 3	= 2	X \$105 =	210	OR	X \$ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE	285	OR	TOTAL ADD'L FEE	
AMENDMENT	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$ =		OR	X \$ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$ =		OR	X \$ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.										

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.

Legal Instrument Examiner:
/JACQULYN L. WILLIAMS/



UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	PATENT NUMBER	GROUP ART UNIT	FILE WRAPPER LOCATION
11/499,953		3651	36M1

Correspondence Address / Fee Address Change

The following fields have been set to Customer Number 959 on 09/05/2007

- Correspondence Address
- Maintenance Fee Address

The address of record for Customer Number 959 is:

LAHIVE & COCKFIELD, LLP
ONE POST OFFICE SQUARE
BOSTON,MA 02109-2127

09-06-07

ITW
#

PTO/SB/21 (04-07)

Approved for use through 09/30/2007. 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 displays a valid OMB control number.

TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	11/499,953-Conf. #3579
	Filing Date	August 7, 2006
	First Named Inventor	Sebastian BÖHM
	Art Unit	3651
	Examiner Name	J. A. Dillon
Total Number of Pages in This Submission	Attorney Docket Number	TGZ-021DVCN

ENCLOSURES (Check all that apply)		
☒ Fee Transmittal Form ☐ Fee Attached ☐ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☒ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please identify below): Return Receipt Postcard PTO form SB/08 Certificate of Mailing
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	LAHIVE & COCKFIELD, LLP		
Signature	<i>David R. Burns</i>		
Printed name	David R. Burns		
Date	September 5, 2007	Reg. No.	46,590

Express Mail Label No. EV 956474854 US Dated: September 5, 2007



Effective on 12/08/2004. Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818). FEE TRANSMITTAL For FY 2007		Complete if Known	
		Application Number	11/499,953-Conf. #3579
☒ Applicant claims small entity status. See 37 CFR 1.27		Filing Date	August 7, 2006
TOTAL AMOUNT OF PAYMENT (\$) 180.00		First Named Inventor	Sebastian BÖHM
		Examiner Name	J. A. Dillon
		Art Unit	3651
		Attorney Docket No.	TGZ-021DVCN

METHOD OF PAYMENT (check all that apply)	
☐ Check	☐ Credit Card
☐ Money Order	☐ None
☐ Other (please identify): _____	
☒ Deposit Account	Deposit Account Number: <u>12-0080</u> Deposit Account Name: <u>Lahive & Cockfield, LLP</u>
For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)	
☒ Charge fee(s) indicated below	☐ Charge fee(s) indicated below, except for the filing fee
☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17	☒ Credit any overpayments

FEE CALCULATION							
1. BASIC FILING, SEARCH, AND EXAMINATION FEES							
	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fees Paid (\$)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	
2. EXCESS CLAIM FEES							
							Small Entity
							Fee (\$)
Fee Description							Fee (\$)
Each claim over 20 (including Reissues)							50
Each independent claim over 3 (including Reissues)							25
Multiple dependent claims							200
							360
							180
Total Claims							
Extra Claims							
Fee (\$)							
Fee Paid (\$)							
HP = highest number of total claims paid for, if greater than 20.							
Indep. Claims							
Extra Claims							
Fee (\$)							
Fee Paid (\$)							
HP = highest number of independent claims paid for, if greater than 3.							
3. APPLICATION SIZE FEE							
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), 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).							
Total Sheets		Extra Sheets	Number of each additional 50 or fraction thereof		Fee (\$)	Fee Paid (\$)	
- 100 =		/50 =	(round up to a whole number) x				
4. OTHER FEE(S)							
Non-English Specification, \$130 fee (no small entity discount)							
Other (e.g., late filing surcharge): 1806 Submission of an Information Disclosure Statement							180.00

SUBMITTED BY			
Signature	<u>David R. Burns</u>	Registration No. (Attorney/Agent)	46,590
Name (Print/Type)	David R. Burns	Telephone	(617) 994-0890
		Date	September 5, 2007

Express Mail Label No. EV 956474854 US Dated: September 5, 2007



Express Mail Label No. EV 956474854 US Dated: September 5, 2007

Docket No.: TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Böhm *et al.*

Application No.: 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3651

For: METHOD AND APPARATUS FOR
SORTING PARTICLES

Examiner: J. A. Dillon

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

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

In accordance with 37 CFR 1.97, Applicants hereby make of record the following additional documents. A PTO Form SB/08 required under 37 CFR 1.98(a)(2) accompanies this statement.

Applicants have not submitted copies of each cited U.S. patent in accordance with 37 CFR 1.98(a)(2)(ii).

This statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any document herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

09/07/2007 RFEKADU1 00000003 120080 11499953

01 FC:1606 180.00 DA

This Supplemental Information Disclosure Statement is filed more than three months after the U.S. filing date, and after the mailing date of the first Office Action on the merits, but before the mailing date of a Final Office Action or Notice of Allowance (37 CFR 1.97(c)).

Please charge our Deposit Account No. 12-0080 in the amount of \$180.00 covering the fee set forth in 37 CFR 1.17(p). 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. 12-0080, under Order No. TGZ-021DVCN.

Dated: September 5, 2007

DRB/mch

Respectfully submitted,

By David R. Burns
David R. Burns
Registration No.: 46,590
LAHIVE & COCKFIELD, LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 227-7400
(617) 742-4214 (Fax)
Attorney/Agent For Applicant



Used in Lieu of PTO/SB/08A/B
(Based on PTO 04-07 version)

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	11/499,953-Conf. #3579
				Filing Date	August 7, 2006
				First Named Inventor	Sebastian BÖHM
				Art Unit	3651
				Examiner Name	J. A. Dillon
Sheet	1	of	1	Attorney Docket Number	TGZ-021DVCN

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-4,572,664	02-25-1986	Hanson et al.	
	A2*	US-6,657,730-A1	12-02-2003	Pfau et al.	
	A3*	US-7,075,652	07-11-2006	Sarvazyan et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	Publication Date	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ⁸
		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)	MM-DD-YYYY			

*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 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 IPW. 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. ⁸ Applicant is to place a check mark here if English language Translation is attached.

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: 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.

¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature	Date Considered



Application No. (if known): 11/499,953

Attorney Docket No.: TGZ-021DVCN

Certificate of Express Mailing Under 37 CFR 1.10

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail, Airbill No. EV 956474854 US in an envelope addressed to:

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

on September 5, 2007
Date

Signature

David R. Burns

Typed or printed name of person signing Certificate

46,590
Registration Number, if applicable

(617) 994-0890
Telephone Number

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

Return Receipt Postcard
Transmittal (1 page)
Fee Transmittal (1 page, in duplicate)
Supplemental Information Disclosure Statement (2 pages)
PTO form SB/08 (3 References) (1 page)



UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/499,953	08/07/2006	Sebastian Bohm	TGZ-021DVCN	3579
959 7590 05/18/2007 LAHIVE & COCKFIELD, LLP ONE POST OFFICE SQUARE BOSTON, MA 02109-2127			EXAMINER DILLON JR, JOSEPH A	
			ART UNIT	PAPER NUMBER
			3651	
			MAIL DATE	DELIVERY MODE
			05/18/2007	PAPER

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

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

Office Action Summary	Application No. 11/499,953	Applicant(s) BOHM ET AL.	
	Examiner Joseph A. Dillon, Jr.	Art Unit 3651	

-- 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 29 November 2006.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ 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

4) ☒ Claim(s) 2-24 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) 2-24 is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☒ The drawing(s) filed on 07 August 2006 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).

11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

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) ☒ Notice of References Cited (PTO-892)	4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)	5) ☐ Notice of Informal Patent Application.
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date <u>1/8/07</u> .	6) ☐ Other: _____

DETAILED ACTION

Double Patenting

1. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

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

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

2. Claims 10-24 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4 of U.S. Patent No. 7,104,405 and over claims 1-22 of U.S. Patent No. 6,808,075. Although the conflicting claims are not identical, they are not patentably distinct from each other because they fully anticipate the claimed subject matter.

Claim Rejections - 35 USC § 103

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

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

4. Claims 10-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over either Diessel et al. (5,837,200) or Dubrow et al. (5,976,336) in view of either Shoor et al. (4,526,276) or Zold (4,936,465) or Galaettli (3,508,654).

Diessel et al. (5,837,200) and Dubrow et al. (5,976,336) teach(es) a plurality of microchannel sorting on a substrate. Diessel et al. (5,837,200) and Dubrow et al. (5,976,336) lack(s) a pressure pulse/buffer actuator arrangement. Shoor et al. (4,526,276) and Zold (4,936,465) and Galaettli (3,508,654) teach(es) a pressure pulse/buffer actuator arrangement.

It would have been obvious to modify either Diessel et al. (5,837,200) or Dubrow et al. (5,976,336) to provide a pressure pulse/buffer actuator arrangement in order to increase system versatility as taught by Shoor et al. (4,526,276) or Zold (4,936,465) or Galaettli (3,508,654).

Conclusion

5. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.
6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Joseph A. Dillon, Jr. whose telephone number is (571)272-6913. The examiner can normally be reached on 8-5:30, every other Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gene Crawford can be reached on (571)272-6911. 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).


JOE DILLON, JR.
SUMMARY PATENT EXAMINER



PTO/SB/08A/B (09-06)

Approved for use through 03/31/2007. 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	
		Application Number	11/499,953-Conf. #3579
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)		Filing Date	August 7, 2006
		First Named Inventor	Sebastian BÖHM
		Art Unit	3651
		Examiner Name	Not Yet Assigned
		Attorney Docket Number	TGZ-021DVCN
Sheet	1	of	1

U.S. PATENT DOCUMENTS						
Examiner Initials*	Cite No.	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)				
J	A1*	US-3,508,654		04/1970	Glaetli	
	A2*	US-3,560,754		02/1971	Kamentsky	
	A3*	US-3,984,307		10/1976	Kamentsky et al.	
	A4*	US-4,147,621		04-03-1979	Giddings	
	A5*	US-4,175,662		11-27-1979	Zold	
	A6*	US-4,526,276		07-02-1985	Shoor et al.	
	A7*	US-4,554,427		11-19-1985	Flick et al.	
	A8*	US-4,756,427		07-12-1988	Gohde et al.	
	A9*	US-4,936,465		06-26-1990	Zold	
	A10*	US-5,030,002		07-09-1991	North, Jr.	
	A11*	US-5,101,978		04-07-1992	Marcus	
	A12*	US-5,275,787		01-04-1994	Yuguchi et al.	
	A13*	US-5,395,588		03-07-1995	North, Jr. et al.	
	A14*	US-5,837,200		11-17-1998	Diessel et al.	
	A15*	US-5,976,336		11-02-1999	Dubrow et al.	
	A16*	US-6,120,666		09-19-2000	Jacobson et al.	
	A17*	US-6,145,247		11-14-2000	McKinnis	
J	A18*	US-6,221,654-B1		04-24-2001	Quake et al.	
	A19*	US-6,432,630-B1		08-13-2002	Blankenstein	
	A20*	US-20030027225-A1		02-06-2003	Wada et al.	
	A21*	US-6,597,438-B1		07-22-2003	Cabuz 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 ³
		Country Code ⁴ -Number ⁴ -Kind Code ⁴ (if known)					

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. ¹ CITE NO.: Those application(s) which are marked with a 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. ² Applicant's unique citation designation number (optional). ³ See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 601.04. ⁴ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁵ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁶ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁷ Applicant is to place a check mark here if English language Translation is attached.

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: 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.

¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature		Date Considered	5/14/07

Notice of References Cited	Application/Control No. 11/499,953	Applicant(s)/Patent Under Reexamination BOHM ET AL.	
	Examiner Joseph A. Dillon, Jr.	Art Unit 3651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,273,553 B1	08-2001	Kim et al.	347/48
*	B	US-6,540,895 B1	04-2003	Spence et al.	204/450
*	C	US-6,592,821 B1	07-2003	Wada et al.	422/68.1
*	D	US-6,877,528 B2	04-2005	Gilbert et al.	137/827
	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
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	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.

Search Notes

Application/Control No.

11/499,953

Examiner

Joseph A. Dillon, Jr.

Applicant(s)/Patent under
Reexamination

BOHM ET AL.

Art Unit

3651

SEARCHED

Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED

Class	Subclass	Date	Examiner

**SEARCH NOTES
(INCLUDING SEARCH STRATEGY)**

	DATE	EXMR
consulted parent, backward/forward citation, parent & 6,808,075	5/14/2007	JD

Index of Claims



Application/Control No.

11/499,953

Examiner

Joseph A. Dillon, Jr.

Applicant(s)/Patent under Reexamination

BOHM ET AL.

Art Unit

3651

✓	Rejected
=	Allowed

—	(Through numeral) Cancelled
+	Restricted

N	Non-Elected
I	Interference

A	Appeal
O	Objected

Claim		Date											
Final	Original	5/14/07											
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UNITED STATES PATENT AND TRADEMARK OFFICE

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 Alexandria, Virginia 22313-1450
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Bib Data Sheet

CONFIRMATION NO. 3579

SERIAL NUMBER 11/499,953	FILING OR 371(c) DATE 08/07/2006 RULE	CLASS 209	GROUP ART UNIT 3651	ATTORNEY DOCKET NO. TGZ-021DVCN
APPLICANTS Sebastian Bohm, Inverness, UNITED KINGDOM; John R. Gilbert, Brookline, MA; Manish Deshpande, Canton, MA;				
** CONTINUING DATA ***** This application is a CON of 10/940,143 09/13/2004 PAT 7,104,405 which is a DIV of 10/179,488 06/24/2002 PAT 6,808,075 which claims benefit of 60/373,256 04/17/2002				
** FOREIGN APPLICATIONS ***** <i>None</i>				
IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 08/22/2006				
** SMALL ENTITY **				
Foreign Priority claimed ☐ yes ☒ no 35 USC 119 (a-d) conditions met ☐ yes ☒ no ☒ Met after Allowance		STATE OR COUNTRY UNITED KINGDOM	SHEETS DRAWING 5	TOTAL CLAIMS 1
Verified and Acknowledged <u>Examiner's Signature</u> <u>Initials</u>				
ADDRESS 959				
TITLE Method and apparatus for sorting particles				
FILING FEE RECEIVED 575	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:		☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit	



IRW

PTO/SB/21 (09-06)
Approved for use through 03/31/2007. 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 displays a valid OMB control number.

TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	11/499,953-Conf. #3579
	Filing Date	August 7, 2006
	First Named Inventor	Sebastian BÖHM
	Art Unit	3651
	Examiner Name	Not Yet Assigned
Total Number of Pages in This Submission	Attorney Docket Number	TGZ-021DVCN

ENCLOSURES (Check all that apply)		
☐ Fee Transmittal Form ☐ Fee Attached ☐ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☒ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please identify below): Return Receipt Postcard PTO form SB/08 Certificate of Mailing
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	LAHIVE & COCKFIELD, LLP		
Signature			
Printed name	David R. Burns		
Date	January 3, 2007	Reg. No.	46,590

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.	
Dated: January 3, 2007	Signature: (David R. Burns)

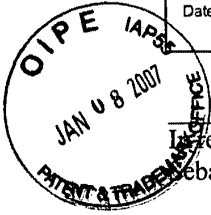
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Dated: January 3, 2007

Signature:

David R. Burns
(David R. Burns)

Docket No.: TGZ-021DVCN
(PATENT)



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Large Patent Application of:
Sebastian Böhm *et al.*

Application No.: 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3651

For: METHOD AND APPARATUS FOR
SORTING PARTICLES

Examiner: Not Yet Assigned

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

INFORMATION DISCLOSURE STATEMENT (IDS)

Dear Sir:

Pursuant to 37 CFR 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. 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.

This Information Disclosure Statement is filed before the mailing date of a first Office Action on the merits as far as is known to the undersigned (37 CFR 1.97(b)(3)).

In accordance with 37 CFR 1.98(a)(2)(ii), Applicants have not submitted copies of U.S. patents and U.S. patent applications.

In accordance with 37 CFR 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 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this

Application No.: 11/499,953

Docket No.: TGZ-021DVCN

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 CFR 1.98 and the Examiner is respectfully requested to consider the listed references.

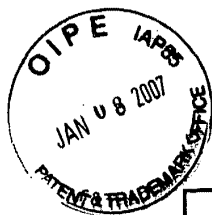
The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. TGZ-021DVCN.

Dated: January 3, 2007

DRB/mch

Respectfully submitted,

By David R. Burns
David R. Burns
Registration No.: 46,590
LAHIVE & COCKFIELD, LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 227-7400
(617) 742-4214 (Fax)
Attorney/Agent For Applicant



PTO/SB/08A/B (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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		Filing Date	August 7, 2006		
		First Named Inventor	Sebastian BÖHM		
		Art Unit	3651		
		Examiner Name	Not Yet Assigned		
Sheet	1	of	1	Attorney Docket Number	TGZ-021DVCN

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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
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	A1*	US-3,508,654	04/1970	Glaetli	
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	A6*	US-4,526,276	07-02-1985	Shoor et al.	
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	A8*	US-4,756,427	07-12-1988	Gohde et al.	
	A9*	US-4,936,465	06-26-1990	Zold	
	A10*	US-5,030,002	07-09-1991	North, Jr.	
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	A14*	US-5,837,200	11-17-1998	Diessel et al.	
	A15*	US-5,976,336	11-02-1999	Dubrow et al.	
	A16*	US-6,120,666	09-19-2000	Jacobson et al.	
	A17*	US-6,145,247	11-14-2000	McKinnis	
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	A20*	US-20030027225-A1	02-06-2003	Wada et al.	
	A21*	US-6,597,438-B1	07-22-2003	Cabuz et al.	

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		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)	MM-DD-YYYY			

*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.

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: 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.

¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature	Date Considered



Application No. (if known): 11/499,953

Attorney Docket No.: TGZ-021DVCN

Certificate of Mailing under 37 CFR 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

MS Amendment
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on January 3, 2007
Date

David R. Burns
Signature

David R. Burns
Typed or printed name of person signing Certificate

46,590
Registration Number, if applicable

(617) 227-7400
Telephone Number

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

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Transmittal (1 page)
Information Disclosure Statement (2 pages)
PTO form SB/08 (21 References) (1 page)



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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/499,953	08/07/2006	Sebastian Bohm	TGZ-021DVCN

CONFIRMATION NO. 3579

00959
LAHIVE & COCKFIELD, LLP
ONE POST OFFICE SQUARE
BOSTON, MA02109-2127

Title: Method and apparatus for sorting particles

Publication No. US-2006-0266679-A1

Publication Date: 11/30/2006

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.

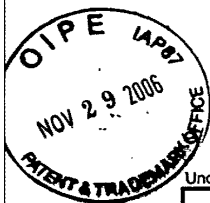
The publication may be accessed through the USPTO's publicly available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

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 703-305-3028.

Pre-Grant Publication Division, 703-605-4283



11-30-06

IFW

PTO/SB/21 (09-06)

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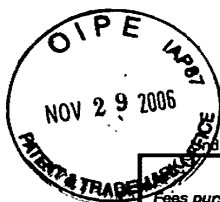
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	11/499,953-Conf. #3579
	Filing Date	August 7, 2006
	First Named Inventor	Sebastian BÖHM
	Art Unit	3651
	Examiner Name	Not Yet Assigned
Total Number of Pages in This Submission	Attorney Docket Number	TGZ-021DVCN

ENCLOSURES (Check all that apply)		
☒ Fee Transmittal Form ☐ Fee Attached ☒ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☐ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please identify below): Return Receipt Postcard
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	LAHIVE & COCKFIELD, LLP		
Signature			
Printed name	David R. Burns		
Date	November 29, 2006	Reg. No.	46,590

Express Mail Label No. EV956492708US Dated: November 29, 2006



PTO/SB/17 (07-06)
Approved for use through 01/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Effective on 12/08/2004. Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).		Complete if Known	
FEE TRANSMITTAL For FY 2006		Application Number	11/499,953-Conf. #3579
		Filing Date	August 7, 2006
		First Named Inventor	Sebastian BÖHM
		Examiner Name	Not Yet Assigned
		Art Unit	3651
☒ Applicant claims small entity status. See 37 CFR 1.27		Attorney Docket No.	TGZ-021DVCN
TOTAL AMOUNT OF PAYMENT		(\$)	75.00

METHOD OF PAYMENT (check all that apply)	
☐ Check	☐ Credit Card
☐ Money Order	☐ None
☐ Other (please identify): _____	
☒ Deposit Account	Deposit Account Number: <u>12-0080</u> Deposit Account Name: <u>Lahive & Cockfield, LLP</u>
For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)	
☒ Charge fee(s) indicated below	☐ Charge fee(s) indicated below, except for the filing fee
☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17	☒ Credit any overpayments

FEE CALCULATION							
1. BASIC FILING, SEARCH, AND EXAMINATION FEES							
	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fee (\$)	Fees Paid (\$)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	
2. EXCESS CLAIM FEES							
							Small Entity
							Fee (\$)
Each claim over 20 (including Reissues)							50
Each independent claim over 3 (including Reissues)							200
Multiple dependent claims							360
							180
Total Claims	Extra Claims	Fee (\$)	Fee Paid (\$)		Multiple Dependent Claims		
23	- 20 = 3	x 25.00 =	75.00		Fee (\$)		Fee Paid (\$)
HP = highest number of total claims paid for, if greater than 20.							
Indep. Claims	Extra Claims	Fee (\$)	Fee Paid (\$)				
3	- 3 = 0	x 100.00 =	0.00				
HP = highest number of independent claims paid for, if greater than 3.							
3. APPLICATION SIZE FEE							
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), 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).							
Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof		Fee (\$)	Fee Paid (\$)		
	- 100 =	/50 (round up to a whole number) x					
4. OTHER FEE(S)							
Non-English Specification, \$130 fee (no small entity discount)							
Other (e.g., late filing surcharge):							

SUBMITTED BY			
Signature	<u>David R Burns</u>	Registration No. (Attorney/Agent)	46,590
Name (Print/Type)	David R. Burns	Telephone	(617) 227-7400
		Date	November 29, 2006

Express Mail Label No. EV956492708US	Dated: November 29, 2006



Express Mail Label No. EV 956492708US
Dated: November 27, 2006

Docket No. TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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

Application No. 11/499,953

Confirmation No.: 3579

Filed: August 7, 2006

Art Unit: 3651

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

Examiner: Not Yet Assigned

FIRST PRELIMINARY AMENDMENT

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

Dear Sir:

INTRODUCTORY COMMENTS

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

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

Remarks/Arguments begin on page 6 of this paper.

12/01/2006 EAREGAY1 00000102 120080 11499953

01 FC:2202 75.00 DA

AMENDMENTS TO THE CLAIMS

Please cancel claim 1 and add new claims 2-24, as set forth in the following listing of claims, which will replace all prior versions, and listings, of claims in the application.

1. (CANCELLED).
2. (NEW) A particle sorting system, comprising:
 - a plurality of substantially parallel sorting channels formed on a substrate,
 - a plurality of sensors, each sensor associated with one of said sorting channels for detecting and classifying particles in the associated sorting channel based on a first predetermined characteristic;
 - a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having the first predetermined characteristic to first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including an actuator controlled by one of said plurality of sensors for selectively applying a pressure pulse to deflect a particle having the first predetermined characteristic in the associated sorting channel into said first outlet.
3. (NEW) The particle sorting system of claim 2, wherein the actuator is arranged external to and in communication with the associated sorting channel.
4. (NEW) The particle sorting system of claim 3, wherein each sorting module further includes a gas-filled reservoir external to and in communication with the microchannel interior, wherein a fluid meniscus forms an interface between the gas-filled reservoir and the interior of the associated sorting channel.
5. (NEW) The particle sorting system of claim 4, wherein each actuator selectively increases an internal pressure of the associated gas-filled reservoir to create the pressure pulse in the associated sorting channel.

6. (NEW) The particle sorting system of claim 4, wherein each sorting module further comprises a side passage connecting the associated sorting channel with the gas-filled reservoir.

7. (NEW) The particle sorting system of claim 4, wherein the gas-filled reservoir includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir, wherein the associated actuator selectively deflects the movable membrane to generate the pressure pulse.

8. (NEW) The particle sorting system of claim 2, wherein each sorting module further comprises a buffer for absorbing the pressure pulse.

9. (NEW) The particle sorting system of claim 2, further comprising an aggregation region for aggregating particles having the predetermined characteristic from a plurality of the first outlets of the sorting channels.

10. (NEW) The particle sorting system of claim 2, further comprising:
at least one secondary sorting channel in series with the plurality of substantially parallel sorting channels for collecting selected particles having the first predetermined characteristic from a plurality of first outlets and separating particles in the secondary sorting channel based on a second predetermined characteristic.

11. (NEW) The particle sorting system of claim 10 wherein the second predetermined characteristic is different from the first predetermined characteristic.

12. (NEW) The particle sorting system of claim 4, wherein the plurality of sorting channels are formed by grooves etched in a first plate, and the gas-filled reservoirs are formed by a second plate bonded to the first plate, the second plate including a plurality of apertures defining the gas-filled reservoirs.

13. (NEW) The particle sorting system of claim 12, further comprising:

a flexible membrane bonded to the second plate to seal at least one of the gas-filled reservoirs and defining at least a portion of a movable wall of at least one of the gas-filled reservoirs.

14. (NEW) The particle sorting system of claim 2, further comprising an inlet channel that branches to supply a plurality of the substantially parallel sorting channels with a stream of particles to be sorted.

15. (NEW) A particle sorting system, comprising:
a plurality of substantially parallel sorting channels formed on a substrate, and
a plurality of sorting modules, each sorting module associated with one of said sorting channels, for distributing particles having a first predetermined characteristic to a first outlet of the associated sorting channel and particles not having the first predetermined characteristic to a second outlet of the associated sorting channel, each said sorting module including a gas-filled reservoir disposed in communication with the associated sorting channel for selectively modifying fluid flow through the associated sorting channel to selectively deflect a particle having the first predetermined characteristic into the first outlet of the associated sorting channel.

16. (NEW) The particle sorting system of claim 15, wherein each sorting module further includes a movable membrane forming at least a portion of a side wall of the gas-filled reservoir.

17. (NEW) The particle sorting system of claim 16, further comprising a plurality of actuators, each actuator associated with a sorting module, wherein each actuator selectively deflects the movable membrane to increase pressure in the gas-filled reservoir to modify said fluid flow.

18. (NEW) The particle sorting system of claim 15, further comprising a plurality of actuators for selectively increasing an internal pressure in the gas-filled reservoir to cause said modification of fluid flow through the associated sorting channel.

19. (NEW) The particle sorting system of claim 15, wherein each sorting module further comprises a buffer for limiting said modification of the fluid flow to a region of the associated sorting channel along the sorting module and preventing flow disturbance in other regions of the associated sorting channel.

20. (NEW) The particle sorting system of claim 15, wherein each sorting module further includes a side channel connecting the gas-filled reservoir to the associated sorting channel.

21. (NEW) A particle sorting system, comprising:

a plurality of substantially parallel sorting channels formed on a substrate, each sorting channel capable of conveying a stream of particles in a carrier fluid,

a plurality of actuators, each actuator associated with a sorting channel formed on the substrate, for selectively creating a flow disturbance within the associated sorting channel to deflect a selected particle in the stream of particles from the stream of particles; and

a plurality of buffers, each buffer associated with an actuator for absorbing the flow disturbance in the associated sorting channel.

22. (NEW) The particle sorting system of claim 21, wherein each buffer comprises a gas-filled reservoir in fluid communication with the associated sorting channel.

23. (NEW) The particle sorting system of claim 21, wherein each sorting channel includes a gas-filled reservoir in fluid communication therewith, and the actuator increases an internal pressure of the gas-filled reservoir to create said flow disturbance.

24. (NEW) The particle sorting system of claim 23, wherein each gas-filled reservoir includes a flexible membrane forming at least a portion of a side wall of the gas-filled reservoir, and the actuator deflects the flexible membrane to increase said internal pressure.

Application No.: 11/499,953

Docket No.: TGZ-021DVCN

REMARKS

Entry of this Preliminary Amendment is respectfully requested.

Dated: November 29, 2006

Respectfully submitted,

By David R Burns
David R. Burns
Registration No.: 46,590
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Boston, Massachusetts 02109
(617) 227-7400
(617) 742-4214 (Fax)
Attorney For Applicants

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PATENT APPLICATION FEE DETERMINATION RECORD					Application or Pocket Number 11/499953	
Substitute for Form PTO-875						
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))						
SEARCH FEE (37 CFR 1.16(k), (l), or (m))						
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))						
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X	=	X	=
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X	=	X	=
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).					
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		TOTAL	
APPLICATION AS AMENDED – PART II						
(Column 1)			(Column 2)		(Column 3)	
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	RATE (\$)
	Total (37 CFR 1.16(i))	Minus	**	=	=	=
	Independent (37 CFR 1.16(h))	Minus	***	=	=	=
	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 (\$)
	Total (37 CFR 1.16(i))	Minus	**	=	=	=
	Independent (37 CFR 1.16(h))	Minus	***	=	=	=
	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 number found in the appropriate box in column 1.						

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

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

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080706

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

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UTILITY PATENT APPLICATION TRANSMITTAL (ONLY FOR NEW NONPROVISIONAL APPLICATIONS UNDER 37 CFR 1.53(B))	Attorney Docket No. TGZ-021DVCN	
	First Inventor Sebastian BÖHM	
	Title	METHOD AND APPARATUS FOR SORTING PARTICLES
	Express Mail Label No. EV682430460US	

APPLICATION ELEMENTS See MPEP chapter 600 concerning utility patent application contents.	ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

1. ☒ Fee Transmittal Form (e.g., PTO/SB/17) (Submit an original and a duplicate for fee processing) 2. ☒ Applicant claims small entity status. See 37 CFR 1.27. 3. ☒ Specification [Total Pages 21] Both the claims and abstract must start on a new page (For information on the preferred arrangement, see MPEP 608.01(a)) 4. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 5] 5. Oath or Declaration [Total Sheets 10] a. ☐ Newly executed (original or copy) b. ☒ A copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional with Box 18 completed) i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b). 6. ☒ Application Data Sheet. See 37 CFR 1.76 7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix) ☐ Landscape Table on CD 8. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. - c. are required) a. Computer Readable Form (CRF) i. ☐ Computer Readable Form (CRF) ii. ☐ Transfer Request (37 CFR 1.821(e)) b. Specification Sequence Listing on: i. ☐ CD-ROM or CD-R (2 copies); or ii. ☐ Paper c. ☐ Statements verifying identity of above copies	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 foreign patent documents, publications, & other information 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: Designation of Record Attorneys (1 page)

18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: **10/940143**

Prior application information: Examiner **Joseph A. Dillon** Art Unit: **3651**

19. CORRESPONDENCE ADDRESS

☒ The address associated with Customer Number: **00959** OR ☐ Correspondence address below

Name	Elizabeth A. Hanley LAHIVE & COCKFIELD, LLP		
Address	28 State Street		
City	Boston	State	MA
Country	US	Zip Code	02109
Telephone	(617) 227-7400		
Email Address			
Signature	David R. Burns		Date
Name (Print/Type)	David R. Burns		Registration No. (Attorney/Agent)
			46,590

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

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

Effective on 12/08/2004. Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818). FEE TRANSMITTAL For FY 2005		Complete if Known <table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td>Application Number</td><td>Not Yet Assigned</td></tr> <tr><td>Filing Date</td><td>Concurrently Herewith</td></tr> <tr><td>First Named Inventor</td><td>Sebastian BÖHM</td></tr> <tr><td>Examiner Name</td><td>Not Yet Assigned</td></tr> <tr><td>Art Unit</td><td>N/A</td></tr> <tr><td>Attorney Docket No.</td><td>TGZ-021DVCN</td></tr> </table>		Application Number	Not Yet Assigned	Filing Date	Concurrently Herewith	First Named Inventor	Sebastian BÖHM	Examiner Name	Not Yet Assigned	Art Unit	N/A	Attorney Docket No.	TGZ-021DVCN
Application Number	Not Yet Assigned														
Filing Date	Concurrently Herewith														
First Named Inventor	Sebastian BÖHM														
Examiner Name	Not Yet Assigned														
Art Unit	N/A														
Attorney Docket No.	TGZ-021DVCN														
☒ Applicant claims small entity status. See 37 CFR 1.27															
TOTAL AMOUNT OF PAYMENT	(\$)	500.00													

METHOD OF PAYMENT (check all that apply)

☐ Check
 ☐ Credit Card
 ☐ Money Order
 ☐ None
 ☐ Other (please identify): _____

☒ Deposit Account
 Deposit Account Number: 12-0080
 Deposit Account Name: Lahive & Cockfield, LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below
☐ Charge fee(s) indicated below, except for the filing fee

☒ Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17
☒ Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	
Utility	300	150	500	250	200	100	500.00
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee (\$)	Small Entity Fee (\$)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee (\$)	=	Fee Paid (\$)		Multiple Dependent Claims
1	- 20 =	x	=			Fee (\$)
						Fee Paid (\$)

Indep. Claims	Extra Claims	Fee (\$)	=	Fee Paid (\$)	
1	- 3 =	x	=		

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), 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).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)
26	- 100 =	/50	(round up to a whole number) x	

4. OTHER FEE(S)

Non-English Specification, \$130 fee (no small entity discount)	
Other (e.g., late filing surcharge):	

SUBMITTED BY

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Name (Print/Type)	David R. Burns		Date August 7, 2006

Express Mail Label No. EV682430460US Dated: August 7, 2006

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UTILITY PATENT APPLICATION TRANSMITTAL (ONLY FOR NEW NONPROVISIONAL APPLICATIONS UNDER 37 CFR 1.53(B))		Attorney Docket No. TGZ-021DVCN
		First Inventor Sebastian BÖHM
Title METHOD AND APPARATUS FOR SORTING PARTICLES		
		Express Mail Label No. EV682430460US

APPLICATION ELEMENTS See MPEP chapter 600 concerning utility patent application contents.	ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	
1. ☒ Fee Transmittal Form (e.g., PTO/SB/17) (Submit an original and a duplicate for fee processing)	ACCOMPANYING APPLICATION PARTS 9. ☐ Assignment Papers (cover sheet & document(s)) Name of Assignee: 10. ☐ 37 CFR 3.73(b) Statement (when there is an assignee) ☐ Power of Attorney 11. ☐ English Translation Document (if applicable) 12. ☐ Information Disclosure Statement (PTO/SB/08 or PTO-1449) ☐ Copies of foreign patent documents, publications, & other information 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: Designation of Record Attorneys (1 page) 	
2. ☒ Applicant claims small entity status. See 37 CFR 1.27.		
3. ☒ Specification [Total Pages <u>21</u>] Both the claims and abstract must start on a new page (For information on the preferred arrangement, see MPEP 608.01(a))		
4. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets <u>5</u>]		
5. Oath or Declaration [Total Sheets <u>10</u>] a. ☐ Newly executed (original or copy) b. ☒ A copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional with Box 18 completed) i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).		
6. ☒ Application Data Sheet. See 37 CFR 1.76		
7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix) ☐ Landscape Table on CD		
8. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. - c. are required) a. Computer Readable Form (CRF) i. ☐ Computer Readable Form (CRF) ii. ☐ Transfer Request (37 CFR 1.821(e)) b. Specification Sequence Listing on: i. ☐ CD-ROM or CD-R (2 copies); or ii. ☐ Paper c. ☐ Statements verifying identity of above copies		
18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76: ☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: <u>10/940143</u> Prior application information: Examiner <u>Joseph A. Dillon</u> Art Unit: <u>3651</u>		
19. CORRESPONDENCE ADDRESS ☒ The address associated with Customer Number: <u>00959</u> OR ☐ Correspondence address below		
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Signature <u>David R Burns</u> Date <u>August 7, 2006</u>		
Name (Print/Type) <u>David R. Burns</u> Registration No. (Attorney/Agent) <u>46,590</u>		

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Effective on 12/08/2004. Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818). FEE TRANSMITTAL For FY 2005		Complete if Known	
☒ Applicant claims small entity status. See 37 CFR 1.27		Application Number	Not Yet Assigned
		Filing Date	Concurrently Herewith
		First Named Inventor	Sebastian BÖHM
		Examiner Name	Not Yet Assigned
		Art Unit	N/A
TOTAL AMOUNT OF PAYMENT	(\$) 500.00	Attorney Docket No.	TGZ-021DVCN

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 12-0080 Deposit Account Name: Lahive & Cockfield, LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

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FEE CALCULATION							
1. BASIC FILING, SEARCH, AND EXAMINATION FEES							
Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	
Utility	300	150	500	250	200	100	500.00
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	
2. EXCESS CLAIM FEES							
Fee Description	Fee (\$)	Small Entity Fee (\$)					
Each claim over 20 (including Reissues)	50	25					
Each independent claim over 3 (including Reissues)	200	100					
Multiple dependent claims	360	180					
Total Claims	Extra Claims	Fee (\$)	Fee Paid (\$)	Multiple Dependent Claims			
1	- 20 =	x	=	Fee (\$)	Fee Paid (\$)		
Indep. Claims	Extra Claims	Fee (\$)	Fee Paid (\$)				
1	- 3 =	x	=				
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4. OTHER FEE(S)							
Non-English Specification, \$130 fee (no small entity discount)				Fees Paid (\$)			
Other (e.g., late filing surcharge):							

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Name (Print/Type)	David R. Burns			Date	August 7, 2006

Express Mail Label No. EV682430460US Dated: August 7, 2006

METHOD AND APPARATUS FOR SORTING PARTICLES

Related Applications

This application claims priority to divisional U.S. Patent Application No. 10/940,143
5 entitled "Method and Apparatus for Sorting Particles" filed September 13, 2004, which is a
divisional of U.S. Patent Application No. 10/179488 entitled "Method and Apparatus for
Sorting Particles" filed June 24, 2002 which claims priority to U.S. Provisional Patent
Application No. 60/373,256 entitled "Microfluidic System Including a Bubble Valve for
Regulating Fluid Flow Through a Microchannel" filed April 17, 2002, the contents of which
10 are herein incorporated by reference.

Field of the invention

The invention relates to a method and apparatus for the sorting of particles in a
suspension, where the input flow path of a sorting module can be split into several output
15 channels. More particular, the invention relates to a particle sorting system in which a
plurality of sorting modules are interconnected as to yield an increased particle throughput.

Background of the invention

In the fields of biotechnology, and especially cytology and drug screening, there is a
20 need for high throughput sorting of particles. Examples of particles that require sorting are
various types of cells, such as blood platelets, white blood cells, tumorous cells, embryonic
cells and the like. These particles are especially of interest in the field of cytology. Other
particles are (macro) molecular species such as proteins, enzymes and poly-nucleotides. This
family of particles is of particular interest in the field of drug screening during the
25 development of new drugs.

Methods and apparatuses for particle sorting are known, and the majority described in
the prior art work in the condition where the particles are suspended in a liquid flowing
through a channel network having at least a branch point downstream and are operated
30 according the detect-decide-deflect principle. The moving particle is first analyzed for a
specific characteristic, such as optical absorption, fluorescent intensity, size etc. Depending
on the outcome of this detection phase, it is decided how the particle will be handled further
with. The outcome of the decision is then applied to deflect the direction of specific particle
towards a predetermined branch of the channel network.

Of importance is the throughput of the sorting apparatus, i.e. how many particles can be sorted per unit of time. Typical sorting rates for sorters employing flows of particle suspension in closed channels are in the range from a few hundred particles per second to thousands of particles per second, for a single sorting unit.

5

An example of a sorting device is described in U.S. 4,175,662, the contents of which are herein incorporated by reference. In the '662 patent, a flow of particles, cells in this case, flows through the center of a straight channel, which branches into two perpendicular channels at a branching point downstream (T-branch). The entering particles are surrounded by a sheath of compatible liquid, keeping the particles confined to the center of the channel. In normal conditions, the flow ratio through the two branches is adjusted so that the particles automatically flow through one of the branches. In a section of the channel a characteristic of the particles is determined using a detector, which can be an optical system (detection phase). The detector raises a signal, which is interpreted. When the detector detects a particle possessing a predetermined characteristic in the decision phase, a deflector is activated for deflecting the particle in a deflection phase. In this case, the deflector comprises an electrode pair, positioned in the branch of the channel where the particles normally flow through in the inactivated state of the deflector. By the application of current pulses, the aqueous liquid is electrolysed, yielding a gas bubble evolving between the electrode pair. As the gas bubble increases in size, the flow rate through this branch is reduced during the evolving phase. After the current pulse is applied, the bubble growth stops and the gas bubble is carried along with the flow. As a result, the flow through the specific branch is momentarily reduced and the particle of interest changes paths and flows down the other branch.

The described device is effective for sorting particles, however one serious drawback is that gas bubbles are created which potentially can accumulate at certain points of the fluidic network or clog flow channels, yielding erroneous sorting. Another drawback is that the generated gasses (mostly oxygen and hydrogen) and ionic species (mostly OH^- and H^+) influence the particles flowing through the branch with the electrode pair. In addition, cells and delicate proteins such as enzymes are very fragile and can be destroyed by the fouling constituents co-generated with the gas bubble. Another drawback is the complexity of the overall sorting apparatus. In particular, the micro electrode construction is very complex to mount and assemble in the small channels of the system. As a result, the cost of a sorting unit is relatively large.

Another example of a particle sorting system of the prior art is disclosed in U.S. 3,984,307, the contents of which are herein incorporated by reference. In the '307 patent, the particles are flowing, confined by a flowing sheath liquid, through the center of a channel. After passing a detector section, the channel branches to two channels under an acute angle (Y-branch). Just before the branching point, an electrically activated transducer is located in the channel for deflecting a specific particle having an appropriate, predetermined characteristic. The transducer described is a piezo actuator or ultrasonic transducer, yielding upon electrical activation a pressure wave in the channel. The generated pressure wave momentarily disturbs the flow in one branch thus deflecting the particle of interest into the other branch

Also in this device, as in the previous discussed device, the deflector is incorporated within the channel system, resulting in relatively large costs of construction. Another drawback is the deflector principle used. The generated pressure waves are not confined to the branching point, but will propagate upstream into the detector section as well as downstream both branches and influence the overall flow through the channel. This is particularly a drawback if sorters of this type are connected either in series or in parallel as to build a sorter system with increased throughput. Pressure waves generated in one sorter can then influence the flows and deflection of particles in neighboring sorter units.

Another disclosed sorter, U.S. 4,756,427, the contents of which are herein incorporated by reference, is analogous to the sorter disclosed the earlier discussed '662. In this case however, the flow in one branch is disturbed by momentarily changing the resistance of the branch. The resistance is changed by changing the height of the branch channel by an external actuator. In the preferred embodiment, this external actuator is a piezo disc glued on top of the channel, causing it to move downwards upon activation.

Although the construction of the sorter described in the '427 patent is less complex as the previous sorter structures, it is still problematic to couple multiple sorter modules of the described type together to increase the sorting rate. This is, as in the sorter described in '307 because of the generated pressure waves causing interference with other sorter modules.

Another particle sorting device is described in U.S. Patent Number 5,837,200, the contents of which are herein incorporated by reference. The '200 patent describes a sorting device that uses a magnetic deflection module to classify or select particles based on their magnetic properties. The '200 patent further describes processing and separating individual particle streams in parallel.

Summary of the invention

The present invention provides a method and apparatus for sorting particles moving through a closed channel system of capillary size. The particle sorting system of the invention provides a sorting module that can be assembled at low cost while providing an accurate means of sorting large amounts of particles per unit of time. The particle sorting system may include a plurality of closely coupled sorting modules which are combined to further increase the sorting rate. The particle sorting system may comprise a multi-stage sorting device for serially sorting streams of particles, in order to decrease the error rate.

The particle sorting system implements an improved fluidic particle switching method and switching device according to the current invention. The particle sorting system comprises a closed channel system of capillary size for sorting particles. The channel system comprises a first supply duct for introducing a stream of particles and a second supply duct for supplying a carrier liquid. The first supply duct forms a nozzle to introduce a stream of particles into the flow of carrier liquid. The first supply duct and the second supply duct enter a measurement duct, which branches into a first branch and a second branch at a branch point. A measurement region is defined in the measurement duct and is associated with a detector to sense a predetermined characteristic of particles in the measurement region. Two opposed bubble valves are positioned in communication with the measurement duct and are spaced opposite each other. The bubble valves communicate with the measurement duct through a pair of opposed side passages. Liquid is allowed to partly fill these side passages to form a meniscus therein which interfaces the carrier liquid with the reservoir of the bubble valves. An external actuator is also provided for actuating one of the bubble valves. When the external actuator is activated, the pressure in the reservoir of the activated bubble valve increases, deflecting the meniscus and causing a flow disturbance in the measurement duct to deflect the flow therein.

When a sensing means in the measuring region senses a predetermined characteristic in a particle flowing through the measurement region, the sensing means produces a signal in response to the sensed characteristic. The external actuator is responsive to the sensing means to cause a pressure pulse in a compression chamber of a first bubble valve to deflect the particle with the predetermined characteristic, causing the selected particle to flow down the second branch duct.

In one aspect, the invention comprises a method of sorting particles including the steps of providing a measurement duct having an inlet and a branching point at which the duct separates into two branch ducts, conducting a stream of fluid into the duct inlet with a stream of particles suspended therein, such that the particles normally flow through a first one of the branch ducts and providing upstream from the branching point two opposing side passages for momentarily deflecting the stream in the duct. A first one of the side passages is hydraulically connected to a compression chamber of a first bubble valve, which is acted upon by an external actuator for varying the pressure therein. A second of the side passages is hydraulically connected with a buffer chamber of a second bubble valve for absorbing pressure variations. The method further comprises providing a measurement station along the measurement duct upstream of the side passages for sensing a predetermined characteristic of particles in the stream and for producing a signal when the predetermined characteristic is sensed. The method further comprises the step of, in response to sensing the predetermined characteristic, activating the external actuator for creating a flow disturbance in the duct between the side passages, thereby deflecting the particle having the predetermined characteristics and causing the selected particle to flow down the second branch duct.

In further aspects of the invention, the particle sort rate is respectively increased or the type of particles sorted being increased, by respectively connecting a plurality of sorting modules in parallel or serially connecting a plurality of sorting modules in a binary tree like configuration.

According to one aspect of the invention, a particle sorting system is provided. The particles sorting system comprises a first duct for conveying a stream of suspended particles confined in a carrier liquid, comprising an inlet, a first outlet and a second outlet, a sensor for sensing a predetermined characteristic in a particle, a side channel in communication with the

first duct, a sealed chamber positioned adjacent to the side channel, wherein the carrier fluid forms a meniscus in the side channel to separate the sealed chamber from the carrier fluid; and an actuator. The actuator modifies the pressure in the sealed chamber to deflect the meniscus when the sensor senses the predetermined characteristic. The deflection of the meniscus causes the particle having the predetermined characteristic to flow into the second outlet while particles that do not have the predetermined characteristic flow into the first outlet.

According to another aspect of the invention, a particle sorting system is provided. The particle sorting system comprises a first duct for conveying a stream of suspended particles confined in a carrier liquid, a sensor for sensing a predetermined characteristic in a particle and a first side channel in communication with the first duct. The first duct comprises an inlet, a first outlet and a second outlet. The particle sorting system further comprises a sealed actuator chamber positioned adjacent to the first side channel, wherein the carrier fluid forms a meniscus in the first side channel to separate the sealed chamber from the carrier fluid, an actuator for modifying the pressure in the sealed actuator chamber to deflect the meniscus when the sensor senses said predetermined characteristic and a buffer. The deflection of the meniscus creates a transient flow in the first duct which deflects particle having said predetermined characteristic to flow into the second outlet while particles that do not have said predetermined characteristic flow into the first outlet. The buffer absorbs a transient flow in the first duct.

According to another aspect of the invention, a particle sorting system is provided. The particle sorting system comprises a duct for conveying a stream of particles in a carrier fluid, a sensor for sensing a predetermined characteristic in a particle and an actuator. The duct comprises an inlet, a first outlet and a second outlet, wherein the particles normally flow from the inlet into the first outlet. The actuator selectively applies a pressure pulse to the suspension to deflect a particle in the stream of particles into the second outlet when said predetermined characteristic is detected. The particle sorting system further comprises a buffer for absorbing the pressure pulse.

According to yet another aspect of the invention, a particle sorting system is provided. The particle sorting system comprises a duct for conveying a stream of suspended particles

confined in a carrier fluid, comprising an inlet, a first outlet and a second outlet, wherein the particles normally flow from the inlet into the first outlet, a sensor for sensing a predetermined characteristic in a particle, an actuator for selectively applying a pressure pulse to the stream of suspended particles to deflect a particle in the stream of particles when said predetermined characteristic is detected, causing it to flow into the second outlet and a buffer for absorbing the pressure pulse. The buffer comprises a side channel in communication with the duct, a sealed buffer chamber adjacent to the side channel and a meniscus formed by the carrier fluid at an interface between the sealed chamber and the side channel.

According to still another aspect of the invention, a particle sorting system is provided. The particle sorting system comprises a first duct for conveying a stream of suspended particles confined in a carrier liquid, comprising an inlet, a first outlet and a second outlet, a sensor for sensing a predetermined characteristic in a particle, a side channel in communication with the first duct, a sealed chamber positioned adjacent to the side channel, wherein the carrier fluid forms a first meniscus in the side channel to separate the sealed chamber from the side channel an actuator for modifying the pressure in the sealed chamber to deflect the first meniscus when the sensor senses said predetermined characteristic, whereby the deflection of the meniscus causes a particle having said predetermined characteristic to flow into the second outlet while particles that do not have said predetermined characteristic flow into the first outlet and a buffer for absorbing the pressure pulse. The buffer comprises a side channel in communication with the duct, a sealed chamber adjacent to the side channel and a first meniscus formed by the carrier fluid at an interface between the sealed chamber and the side channel.

According to another aspect of the invention, a particle sorting system for sorting particles suspended in a liquid is provided. The particle sorting system comprises an inlet duct through which flows a liquid containing particles having a predetermined characteristic and particles not having a predetermined characteristic. The inlet duct branches into a plurality of measurement channels which are operated in parallel and simultaneously fed with the liquid, each measurement channel having a sorting module and two outlet channels, and each sorting module having a switch unit for distribution of particles having a predetermined characteristic and particles not having a predetermined characteristic to said two different outlet channel. Each switch unit comprises at least one sensor which detects and classifies

the particles having the predetermined characteristic, a side passage in communication with the inlet duct, a sealed chamber adjacent to and separated from the side passage by a meniscus formed by the liquid and an actuator controlled by each said sensor arranged on each said switch unit for selectively deflecting the meniscus to deflect a particle having the predetermined characteristic into one of said outlet channel. Each of the two outlet channels on each measurement channel is connected to a separate summing channel for the particles having a predetermined characteristic and particles not having a predetermined characteristic selectively distributed to it.

According to still another aspect of the invention, a particle sorting system for sorting particles suspended in a liquid is provided comprising an inlet duct through which flows a liquid containing particles having a predetermined characteristic and particles not having a predetermined characteristic. The inlet duct branches into a plurality of channels which are operated in parallel and simultaneously fed with the liquid, each channel having a sorting module and two outlet channels, and each sorting module having a switch unit for distribution of particles having a first predetermined characteristic and particles not having the first predetermined characteristic between said two different outlet channels. Each sorting module comprises at least one sensor which detects and classifies the particles having the first predetermined characteristic, and an actuator controlled by each of said sensor arranged on each said switch unit for selectively deflecting a particle having the first predetermined characteristic into one of said outlet channels. Each of said two outlet channels on each sorting module is connected to a separate summing channel for the particles having the first predetermined characteristic and particles not having the first predetermined characteristic selectively distributed to it. The system further comprises at least one secondary sorting module connected to the summing channel for the particles having the first predetermined characteristic, each of said secondary sorting modules having a first outlet channel and a second outlet channel, a detector for sensing particles and a switch unit for selectively deflecting a particle having a second predetermined characteristic into one of said outlet channels based on a second predetermined characteristic.

According to another aspect, a particle sorting system is provided, comprising a plurality of parallel primary sorting channels through which flows a stream of suspended particles confined in a carrier liquid. Each primary sorting channel has a detection region for

detecting a predetermined characteristic in a particle and a switching region for separating particles having the predetermined characteristic into a first receiving channel from particles that do not have the predetermined characteristic, which flow into a second receiving channel. The system further comprises an aggregation region for aggregating the particles having the predetermined characteristic from the first receiving channels, and at least one secondary sorting channel in series with the plurality of parallel primary sorting channels for collecting the selected particles from the plurality of first outlet channels and separating particles in the secondary sorting channel having the predetermined characteristic from other particles in the secondary sorting channel.

According to a final aspect, a method of sorting small particles is provided. The method comprises the steps of providing a closed duct having an inlet and a fork at which the duct separates into two branch ducts, conducting a stream of liquid into the duct inlet with a stream of particles suspended therein, the particle stream normally flowing through a first one of the branch ducts surrounding the stream of liquid with a particle free enveloping current of liquid to produce substantially laminar flow and providing a measurement station along the closed duct upstream of the fork for sensing a predetermined property of particles in the stream and for producing a signal when the property is sensed. The method further comprises providing an actuator for applying a pressure on the liquid and providing means for buffering pressure variations in the liquid. The pressure buffering means cooperates with the pressure applying means to result in a momentary deflection of the liquid streaming through the duct substantially perpendicular to the normal direction of flow from the point where the pressure is applied towards the point where the pressure is buffered, causing the specific particle having the predetermined property to flow into the second branch duct without eliminating the laminar flow.

Brief description of the drawings

FIG. 1 is a schematic view of a particle sorting system according to an illustrative embodiment of the invention.

FIGS. 2 through 4 illustrate the operation of the particle sorting system of FIG 1.

FIG. 5 illustrates a particle sorting system showing alternate positions for the actuator chamber and the buffer chamber.

FIG. 6 illustrates the particle sorting system according to another embodiment of the invention.

5 FIG. 7 illustrates a bubble valve suitable for use in the particle sorting system of the invention.

FIG. 8 is a schematic diagram of the particle sorting system of an illustrative embodiment of the invention

FIG. 9 shows a particle sorting system for sorting parallel streams of particles.

10 FIG. 10 shows a particle sorting system binary tree-like configuration of sorting modules.

FIG. 11 illustrates a multi-stage particle sorting system for sorting parallel streams of particles in two stages.

15 FIG. 12 illustrates a parallel particle sorting system according to an alternate embodiment of the invention.

FIG. 13 illustrates a parallel particle sorting system according to another embodiment of the invention.

Detailed Description of the Invention

20 The present invention provides a particle sorting system for sorting particles suspended in a liquid. The particle sorting system provides high-throughput, low error sorting of particles based on a predetermined characteristic. The present invention will be described below relative to illustrative embodiments. Those skilled in the art will appreciate that the present invention may be implemented in a number of different applications and
25 embodiments and is not specifically limited in its application to the particular embodiments depicted herein.

30 Fig. 1 shows a schematic of a particle sorting system according to an illustrative embodiment of the invention. According to one application of the present invention, a particle sorting system 10 comprises a closed channel system of capillary size for sorting particles. The channel system comprises a first supply duct 12 for introducing a stream of particles 18 and a second supply duct 14 for supplying a carrier liquid. The first supply duct 12 forms a nozzle 12a, and a stream of particles is introduced into the flow of carrier liquid. The first supply duct 12 and the second supply duct 14 enter a measurement duct 16 for

conveying the particles suspended in the carrier liquid, which branches into a first branch 22a and a second branch 22b at a branch point 21. A measurement region 20 is defined in the measurement duct 16 and is associated with a detector 19 to sense a predetermined characteristic of particles in the measurement region 20. Two opposed of bubble valves 100a and 100b are positioned in communication with the measurement duct 16 and are spaced opposite each other. The bubble valves 100a, 100b communicate with the measurement duct 16 through a pair of opposed side passages 24a and 24b, respectively. Liquid is allowed to partly fill these side passages 24a and 24b to form a meniscus 25 therein. The meniscus defines an interface between the carrier liquid and a gas in the reservoir of the associated bubble valve 100. An external actuator 26 is also provided for actuating the first bubble valves 100a, which momentarily causes a flow disturbance in the duct to deflect the flow therein when activated by the actuator 26. The second bubble valve 100b serves as a buffer for absorbing the pressure pulse created by the first bubble valve 100a.

The first side passage 24a is hydraulically connected to a compression chamber 70a in the first bubble valve 100a, so that if the pressure in this chamber is increased, the flow in the measurement duct near the side passage is displaced inwards, substantially perpendicular to the normal flow in the duct. The second side passage 24b, positioned opposite of the first side passage 24a is hydraulically connected to a buffer chamber 70b in the second bubble valve 100b for absorbing pressure transients. This second side passage 24b co-operates with the first side passage 24a to direct the before mentioned liquid displacement caused by pressurizing the compression chamber 70a, so that the displacement has a component perpendicular to the normal flow of the particles through the measurement duct.

Upon pressurizing the compression chamber 70a an amount of liquid is transiently discharged from the first side passage 24a. The resiliency of the second side passage 24b results upon a pressurized discharge, in a transient flow of the liquid in the duct into the second side passage 24a. The co-operation of the two side passages and the fluidic structures they interconnect causes the flow through the measurement duct 16 to be transiently moved sideways back and forth upon pressurizing and depressurising of the compression chamber 70a induced by the external actuator 26 in response to the signal raised by the detection means 19. This transient liquid displacement, having a component perpendicular to the normal flow in the duct, can be applied in deflecting particles having predetermined characteristics to separate them from the remaining particles in the mixture.

As shown, the measurement duct 16 branches at the branch point 21 into two branches 22a, 22b and the flow rates in these branches are adjusted so that the particles normally stream through the second of the two branches 22b. The angle between the branches 22a, 22b is between 0 and 180 degrees, preferably between 10 and 45 degrees. However, the angle can even be 0 degrees, which corresponds to two parallel ducts with a straight separation wall between them.

The particles to be sorted are preferably supplied to a measurement position in a central fluid current, which is surrounded by a particle free liquid sheath. The process of confining a particle stream is known, and often referred to as a 'sheath flow' configuration. Normally confinement is achieved by injecting a stream of suspended particles through a narrow outlet nozzle into a particle free carrier liquid flowing in the duct 16. By adjusting the ratio of flow rates of the suspension and carrier liquid, the radial confinement in the duct as well as the inter particle distance can be adjusted. A relative large flow rate of the carrier liquid results in a more confined particle stream having a large distance between particles.

In a suspension introduced by the first supply duct 12, two types of particles can be distinguished, normal particles 18a and particles of interest 18b. Upon sensing the predetermined characteristic in a particle 18b in the measurement region 20, the detector 19 raises a signal. The external actuator 26 activates the first actuator bubble valve 100a, when signaled by the detector 19 in response to sensing the predetermined characteristic, to create a flow disturbance in the measurement duct 16 between the side passages 24a, 24b. The flow disturbance deflects the particle 18b having the predetermined characteristic so that it flows down the first branch duct 22a rather than the second branch duct 22b. The detector communicates with the actuator 26, so that when the detector 19 senses a predetermined characteristic in a particle, the actuator activates the first bubble valve 100a to cause pressure variations in the reservoir 70a of the first bubble valve. The activation of the first bubble valves deflects the meniscus 25a in the first bubble valve 100a and causes a transient pressure variation in the first side passage 24a. The second side passage 24b and the second bubble valve 100b absorb the transient pressure variations in the measurement duct 16 induced via the actuator 26. Basically, the reservoir 70b of the second bubble valve 100b is a buffer chamber having a resilient wall or containing a compressible fluid, such as a gas. The resilient properties allow the flow of liquid from the measurement duct into the second side

passage 24b, allowing the pressure pulse to be absorbed and preventing disturbance to the flow of the non-selected particles in the stream of particles.

At the measurement region 20, individual particles are inspected, using a suitable sensor means 19, for a particular characteristic, such as size, form, fluorescent intensity etc. Examples of applicable sensing means, known in the art, are various types of optical detection systems such as microscopes, machine vision systems and electronic means for measuring electronic properties of the particles. Particularly well known systems in the field are systems for measuring the fluorescent intensity of particles. These systems comprise a light source having a suitable wavelength for inducing fluorescence and a detection system for measuring the intensity of the induced fluorescent light. This approach is often used in combination with particles that are labelled with a fluorescent marker, i.e. an attached molecule that upon illuminating with light of a particular first wavelength produces light at another particular second wavelength (fluorescence). If this second wavelength light is detected, the characteristic is sensed and a signal is raised.

Other examples include the measurement of light scattered by particles flowing through the measurement region. Interpreting the scattering yield information on the size and form of particles, which can be adopted to raise a signal when a predetermined characteristic is detected.

The actuator 26 for pressurizing the compression chamber of the first bubble valve may comprise an external actuator that responds to a signal from the sensor that a particle has a selected predetermined characteristic. There are two classes of external actuators that are suitable for increasing the pressure. The first class directly provides a gas pressure to the liquid in the first side passage 24a. For example, the actuator may comprise a source of pressurized gas connected with a switching valve to the liquid column in the side passage 24a. Activation of the switch connects the passage to the source of pressurized gas, which deflects the meniscus in the liquid. Upon deactivation, the switch connects the passage 24a back to the normal operating pressure.

Alternatively, a displacement actuator may be used in combination with a closed compression chamber having a movable wall. When the displacement actuator displaces the wall of the compression chamber inward, the pressure inside increases. If the movable wall is

displaced back to the original position, the pressure is reduced back to the normal operating pressure. An example of a suitable displacement actuator is an electromagnetic actuator, which causes displacement of a plunger upon energizing a coil. Another example is the use of piezoelectric material, for example in the form of a cylinder or a stack of disks, which upon the application of a voltage produces a linear displacement. Both types of actuators engage the movable wall of the compression chamber 70 to cause pressure variations therein.

Figures 2-4 illustrate the switching operation of switch 40 in the particle sorting system 10 of Figure 1. In Figure 2, the detector 19 senses the predetermined characteristic in a particle and raises a signal to activate the actuator 26. Upon activation of the actuator, the pressure within the reservoir 70a of the first bubble valve 100a is increased, deflecting the meniscus 25a and causing a transient discharge of liquid from the first side passage 24a, 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 24b, because of the resilient properties of the reservoir of the second bubble valve 100b. This movement of liquid into the second side passage 24b is indicated with an arrow. As a result, as can be seen in the figure, the flow through the measurement duct 16 is deflected, causing the selected particle of interest 18b located between the first side passage 24a and the second side passage 24b to be shifted perpendicular to its flow direction in the normal state. The flow resistances to the measurement duct 16, the first branch 22a and the second branch 22b is chosen so that the preferred direction of the flow to and from the first side passage 24a and the second side passage 24b has an appreciable component perpendicular to the normal flow through the measurement duct 16. This goal can for instance be reached by the first branch 22a and the second branch 22b so that their resistances to flow is large in comparison with the flow resistances of the first side passage 24a and the second side passage 24b.

Figure 3 shows the particle sorting system 10 during the relief of the first bubble valve reservoir when the particle of interest 18b has left the volume between the first side passage 24a and the second side passage 24b. The actuator 26 is deactivated, causing the pressure inside the reservoirs 70a, 70b to return to the normal pressure. During this relief phase there is a negative pressure difference between the two reservoirs 70a, 70b of the bubble valves, causing a liquid flow through the first side passage 24a and the second side passage 24b opposite to the liquid flow shown in the previous figure and as indicated by the arrows.

Figure 4 illustrates the particle sorting system 10 after completion of the switching sequence. The pressures inside the reservoirs of the bubble valves are equalized, allowing the flow through the measurement duct 16 to normalize. As the particle of interest 18b has been displaced radially, it will flow into the first branch 22a, while the other particle continue to flow into the second branch 22b, thereby separating the particles based on the predetermined characteristic.

This process of detecting and selective deflecting of particles may be repeated many times per second for sorting particles at a high rate. Adopting the fluid switching as described, switching operations may be executed up to around several thousand switching operations per second, yielding sorting rates in the order of million sorted particles per hour.

According to another embodiment of the invention, the actuator bubble valve 100a and the buffer bubble valve 100b may be placed in different positions. For example, as shown in Figure 5, the actuator bubble valve 100a and the first side passage 24a and/or the buffer bubble valve 100b and the second side passage 24b may be place upstream from the branch point 21. The components may be placed in any suitable location, such that the flow resistance between the actuator chamber 70a and the buffer chamber 70b is less than the flow resistance between any of these latter components and other pressure sources. More particularly, the actuator chamber 70a and the buffer chamber 70b may be placed such that the flow resistance between them is less than the flow resistance between a selected particle and a subsequent particle in the stream of particles. The positioning of the components in this manner thus prevents a pressure wave generated by the above described method of deflecting a single selected particle, from travelling upstream or downstream and affecting the flow of the remaining particles in the stream of particles. The larger the difference in flow resistances, the larger the level of isolation of the fluidic switching operation with associated pressure transients from the flow characteristics in the rest of the system. Moreover, the in-situ dampening of generated pressure pulses applied for sorting allows the implementation of sorting networks comprising a plurality of switches 40, each of which is hydraulically and pneumatically isolated from the others.

According to another embodiment, shown in Figure 6, the particle sorting system of the invention may use any suitable pressure wave generator (in place of a bubble valve) in

combination with the buffer bubble valve 100b. For example, the pressure wave generator 260 may comprise an actuator such as a piezoelectric column or a stepper motor, provided with a plunger that can act upon the flowing liquid, either directly or via deflection of the channel system, to selectively deflect particles when the actuator is activated by a signal.

5 Other suitable pressure wave generators include electromagnetic actuators, thermopneumatic actuators and a heat pulse generator for generating vapor bubbles in the flowing liquid by applying heat pulses. The buffer bubble valve 100b is positioned to absorb the pressure wave created by the pressure wave generator 260 to prevent flow disturbance in the other particles of the particle stream. The spring constant of the buffer 100b may be varied according to the
10 particular requirements by varying the volume of the buffer chamber 70b, the cross-sectional area of the side passage 24b and/or the stiffness or the thickness of a flexible membrane (reference 72 in Figure 7) forming the buffer chamber 70b.

Fig. 7 illustrates an embodiment of a bubble valve 100 suitable for creating a pressure
15 pulse to separate particles of interest from other particles in a stream of particles and/or acting as a buffer for absorbing a pressure pulse according to the teachings of the present invention. As shown, the bubble valve 100 is formed adjacent to a side passage 24a or 24b formed in a substrate which leads to the measurement duct 16. The side passage 24a includes a fluid interface port 17 formed by an aperture in the side wall of the passage. A sealed compression
20 chamber 70 is positioned adjacent to the side passage 24a and communicates with the side passage through the fluid interface port. The illustrative chamber 70 is formed by a seal 71 and a flexible membrane 72. The carrier fluid in the side passage 24a forms a meniscus 25 at the interface between the side passage and the chamber. The actuator 26 depresses the flexible membrane to increase the pressure in the chamber, which deflects the meniscus and
25 causes a pressure pulse in the carrier fluid.

Fig. 8 shows a sorting module 50 having an appropriate supply duct 52 for providing a stream of particles to be sorted as well as an outlet duct 54 and a second outlet duct 56 carrying the particles sorted in the sorting module 50. The sorting module 50 comprises
30 detector system 19 for sensing particles entering the sorting module 50 via the supply duct 52 operationally connected to a switch 40 for providing the required switching capabilities to sort particles. The first branch 22b and second branch 22a are in fluidic connection with the outlet duct 54 and second outlet duct 56.

Fig. 9 shows a particle sorting system 500 according to an alternate embodiment of the invention, comprising a plurality of sorting module 50 operating in parallel. The individual outlet duct 54 of the sorting module 50 are forwarded to a first combined outlet 58, the individual second outlet duct 56 are forwarded to a second combined outlet 60. The parallel arrangement of sorting modules yields a system of combined sorting module 50 having an overall sorting rate of N times the sorting rate of an individual sorting module 50, where N is the number of parallel connected sorting module 50.

Fig. 10 shows a particle sorting system 550 according to another embodiment, comprising a first sorting module 50a and a second sorting module 50b in series with the first sorting module 50a. The second sorting module 50b may be equipped for sorting out particles having a predetermined characteristic different than the predetermined characteristic of the particles sorted out by the first sorting module 50a. The particle stream enters the first sorting module 50a through the supply duct 52 and may contain at least two types of particles. A first type of particles is sorted out in the first sorting module 50a and leaves through the first outlet duct 54a. The remaining particles leave the first sorting module 50a through second outlet duct 56a and are fed into the second sorting module 50b via the second supply duct 52b. From this stream of particles, particles having the other predetermined characteristic are sorted out and leave through the second outlet duct 54b. Particles that possess neither of the two predetermined characteristics leave the second sorting module 50b via the second outlet duct 56b.

Figure 11 shows a hierarchical architecture for high throughput-low error sorting according to another embodiment of the invention. The embodiment shown is a two-stage particle sorting system 800 for sorting a plurality of parallel particles streams in a first stage, aggregating the outputs of the first stage and then performing a secondary sorting process on the output of the first stage. An input stream of particles in suspension 80 from a particle input chamber 88 is split among N single sorting channels 81a-81n, each channel being capable of sorting a selected number of particles per second. Each channel 81 includes a detection region 84 for examining the particles and identifying particles that have a predetermined characteristic and a switching region 82 for separating the particles having the predetermined characteristic from the other particles in the stream, as described above. The switching region 82 produces two output streams of particles: a "selected" stream and a "rejected" stream in its switching region 82 based on the measured particle characteristics at

the detection region 84. The “selected” streams from each channel are aggregated in an aggregation region 86 into one stream to be sorted again in a secondary sorting channel 810. As shown, the secondary sorting channel 810 repeats the sorting process of detecting and sorting based on a predetermined characteristic.

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Given that each single channel sorting process produces some error (y) rate (y is a probability less than one of a particle being “selected” by mistake) of mistaken selections, the hierarchical architecture produces an lower error rate of y^2 for a 2-stage hierarchy as drawn or y^n for an n -stage hierarchy. For example, if the single channel error rate is 1% the 2-stage error rate is 0.01% or one part in 10^4 .

10

Alternatively, the architecture could have M primary sets of N sorting channels per secondary channel. Given that the application wants to capture particles that have a presence in the input at rate z and single channel sorters have a maximum sorting rate x particles per second. The system throughput is $M*N*x$ in particles per second. The number of particles aggregated in N channels per second is $N*x*z$ and so $N*z$ must be less than 1 so that all particles aggregated from N channels can be sorted by a single secondary channel. To increase throughput above $N=1/z$ one must add parallel groups of N primary + 1 secondary channels. Overall throughput then comes from $M*N*x$ with M secondary channels.

15

20

Figure 12 show a parallel-serial particle sorting system 160 according to another embodiment of the invention. The parallel-serial particle sorting system 160 includes a first parallel sorting module 161 and a second parallel sorting module 162. The first sorting module 161 is applied in multiple marked particles and particles having both markers are sorted out and conveyed through the exit channel 165.

25

Figure 13 shows another parallel-serial particle sorting system 170. The first parallel sorting module 171 separates particles having a first marker, collects the particles from the different channels and conveys the particles having the first marker through the first exit channel 175. All other particles are then fed into a second parallel sorter 172 for sorting particles having a second marker. The particles having the second marker are collected and conveyed through a second exit channel 176. Particles having neither the first marker nor the second marker are conveyed through a third exit channel 177.

30

The present invention has been described relative to an illustrative embodiment. Since 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.

5

It is also to be understood that the following claims are to cover all generic and 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.

10

Having described the invention, what is claimed as new and protected by Letters Patent is:

Claims

1. A method of sorting small particles comprising the steps of:

providing a closed duct having an inlet and a fork at which the duct separates into two branch ducts;

conducting a stream of liquid into the duct inlet with a stream of particles suspended therein, the particle stream normally flowing through a first one of the branch ducts;

surrounding the stream of liquid with a particle free enveloping current of liquid to produce substantially laminar flow;

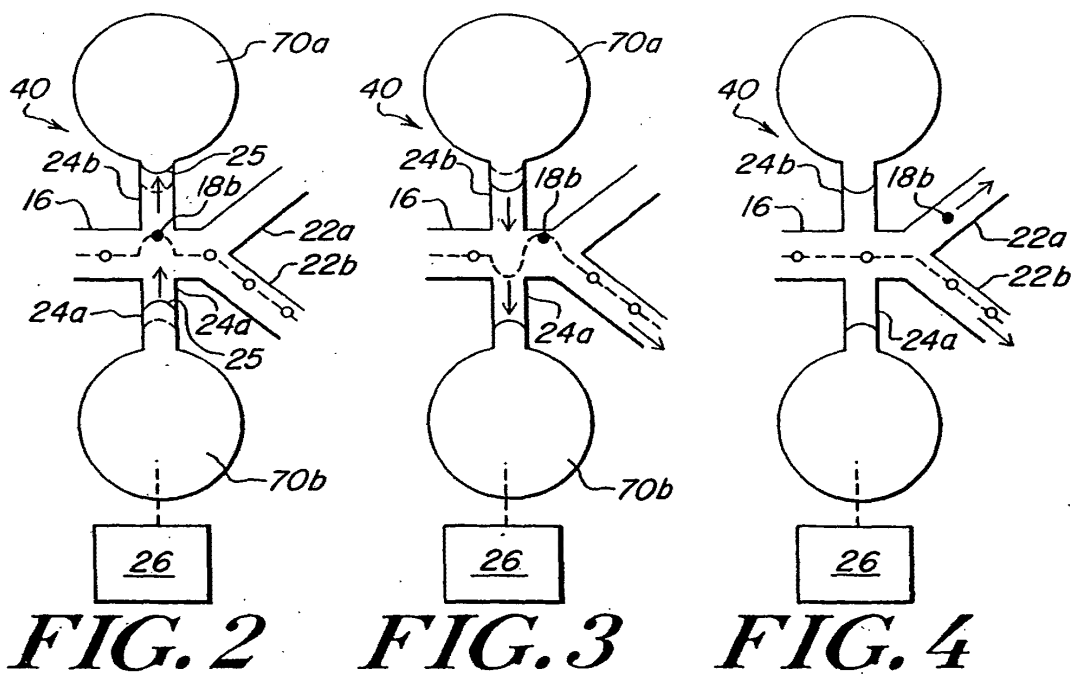
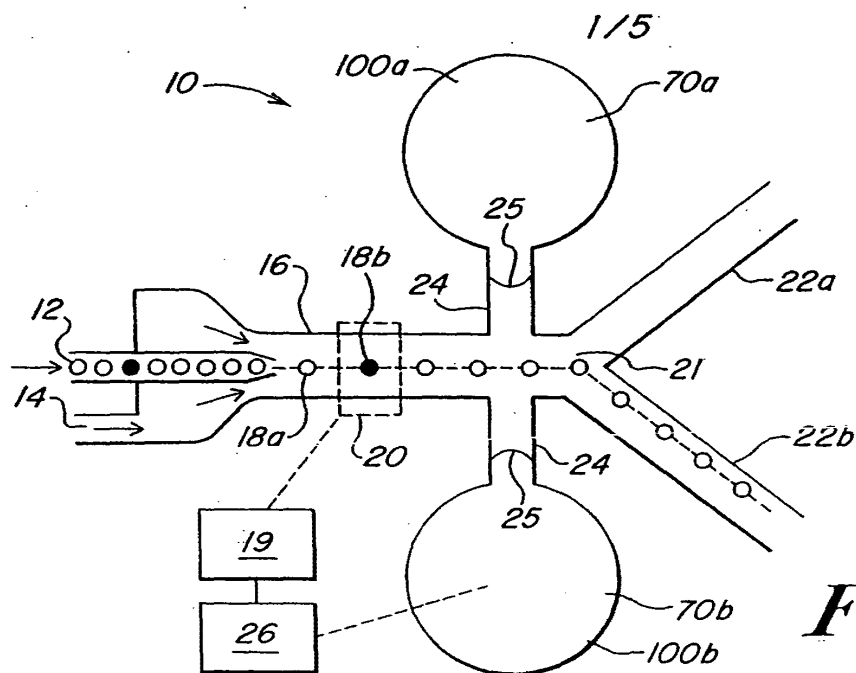
providing a measurement station along the closed duct upstream of the fork for sensing a predetermined property of particles in the stream and for producing a signal when the property is sensed;

providing an actuator for applying a pressure on the liquid; and

providing means for buffering pressure variations in the liquid, the pressure buffering means co-operating with the pressure applying means to result in a momentary deflection of the liquid streaming through the duct substantially perpendicular to the normal direction of flow from the point where the pressure is applied towards the point where the pressure is buffered, causing the specific particle having the predetermined property to flow into the second branch duct without eliminating the laminar flow.

Abstract

A method and apparatus for sorting particles moving through a closed channel system of capillary size comprises a bubble valve for selectively generating a pressure pulse to separate a particle having a predetermined characteristic from a stream of particles. The particle sorting system may further include a buffer for absorbing the pressure pulse. The particle sorting system may include a plurality of closely coupled sorting modules which are combined to further increase the sorting rate. The particle sorting system may comprise a multi-stage sorting device for serially sorting streams of particles, in order to decrease the error rate.



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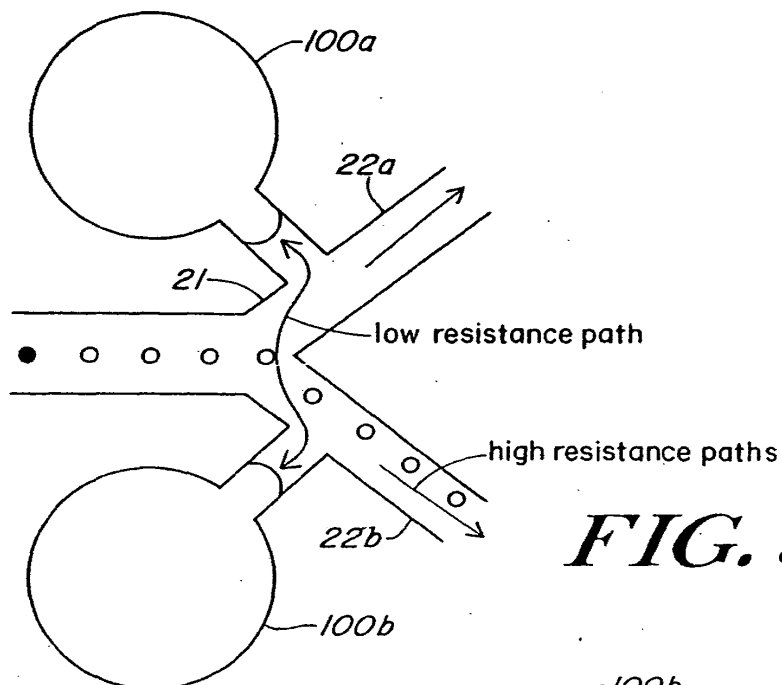


FIG. 5

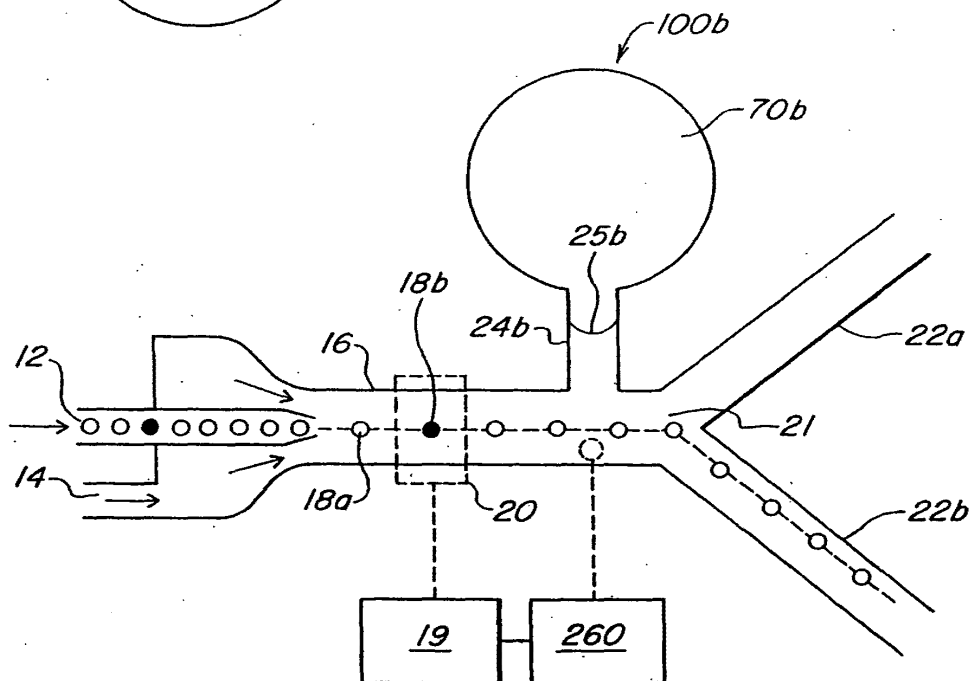


FIG. 6

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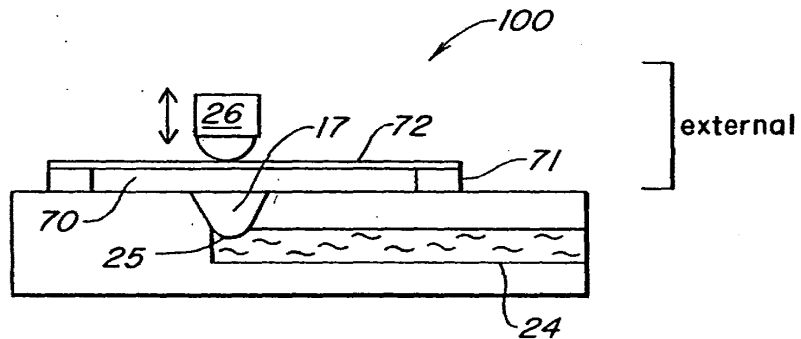


FIG. 7

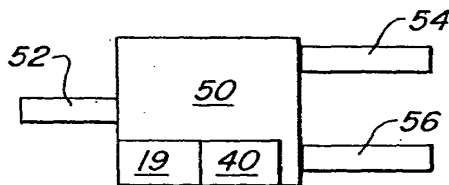


FIG. 8

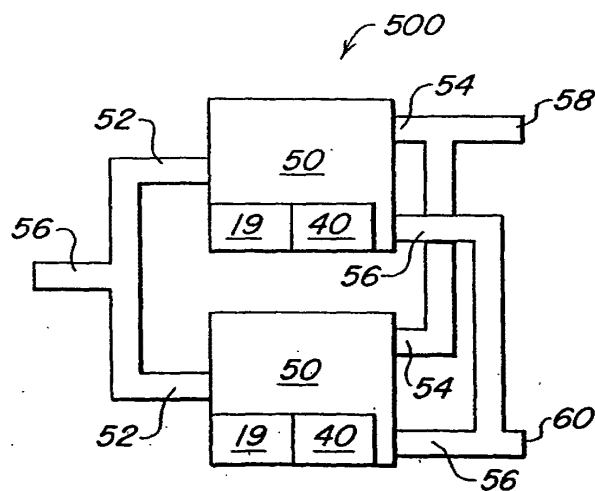


FIG. 9

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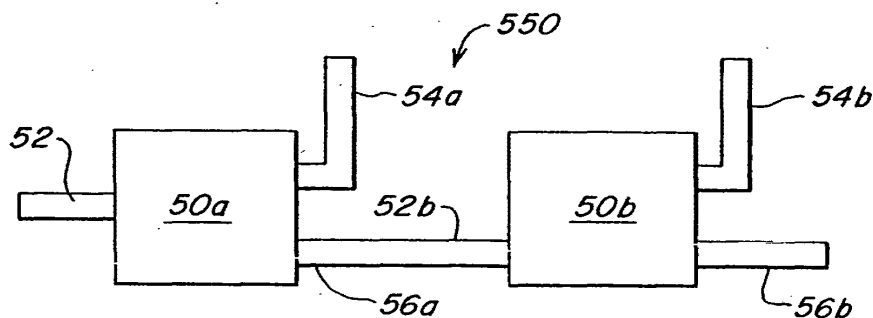


FIG. 10

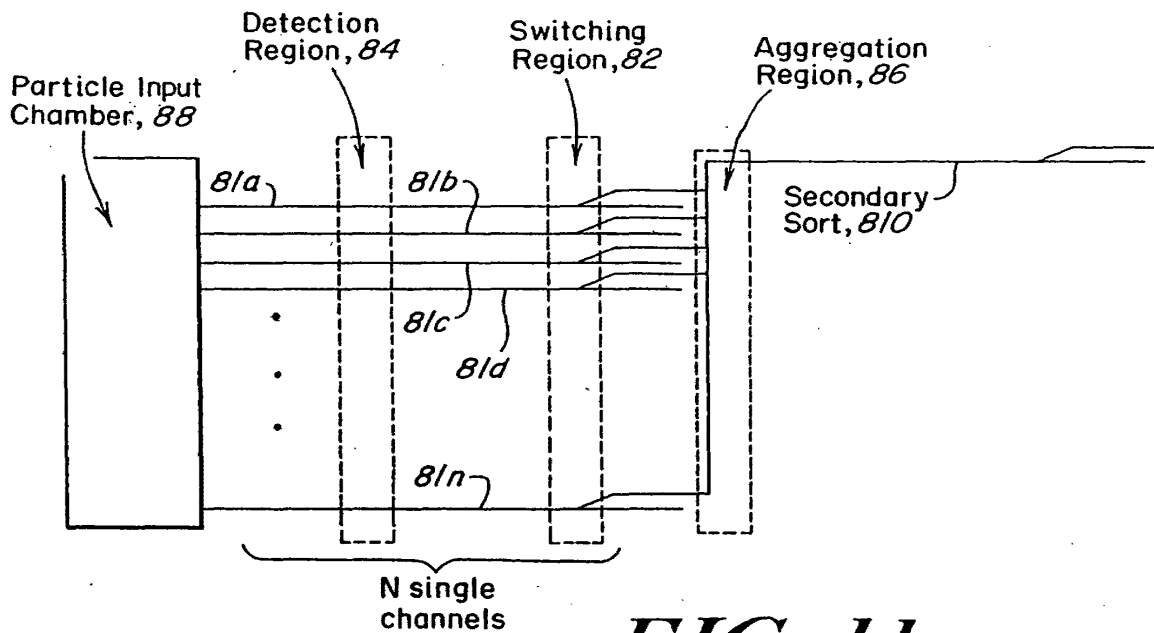


FIG. 11

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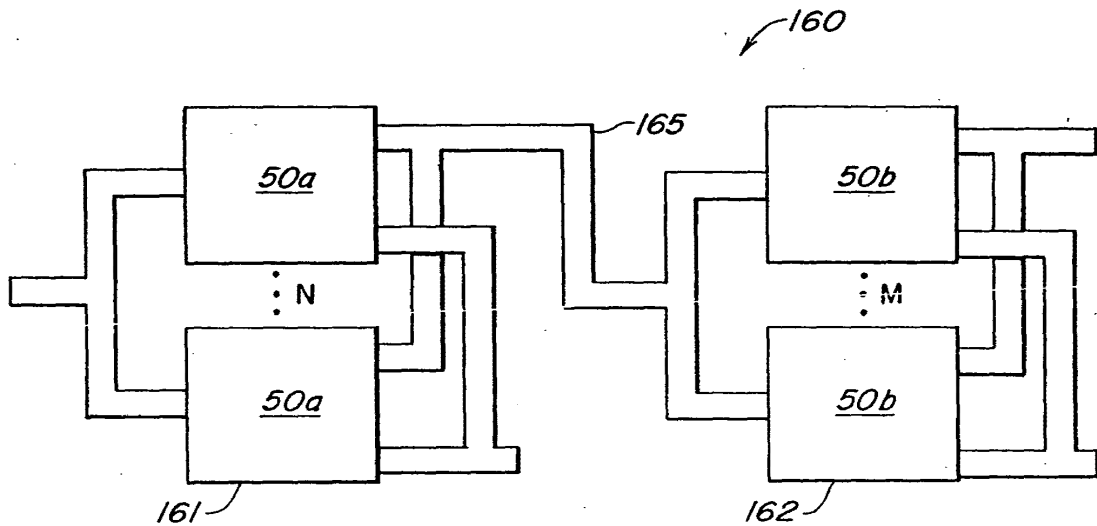


FIG. 12

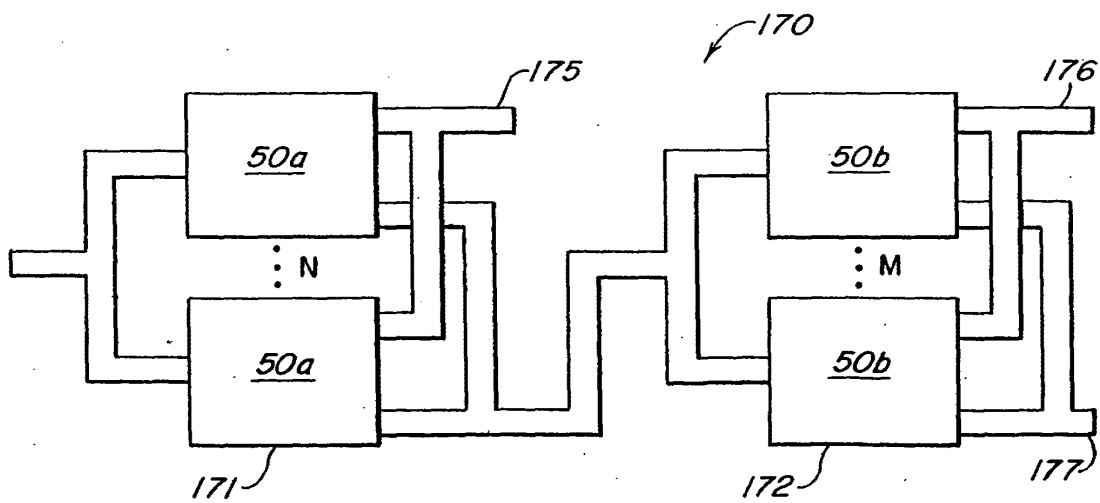


FIG. 13

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Number CVZ-021

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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:

METHOD AND APPARATUS FOR SORTING PARTICLES

the specification of which:

is attached hereto.

X was filed on June 24, 2002. as

X Application Serial No. 10/179,488

_____ 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.

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Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119	
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
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60/373,256
(Application Serial No.)

April 17, 2002
(Filing Date)

(Application Serial No.)

(Filing Date)

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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.

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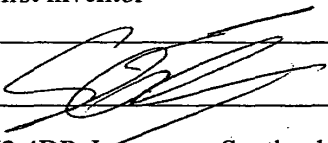
LAHIVE & COCKFIELD, LLP, 28 State Street, Boston, MA 02109

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.

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Docket
Number CVZ-021

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the specification of which:

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			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

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(Filing Date)

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any earlier United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the earlier application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date(s) of the earlier application(s) and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier application(s), if any, described below, I do not know and do not believe that the same was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date(s) of said earlier application(s), or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date(s) of said earlier application(s) on an application filed more than twelve months (six months if this application is for a design) before the filing of said earlier application(s); and I acknowledge that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

(Application Serial No.)	(Filing Date)	(Status) (patented,pending,aband.)

(Application Serial No.)	(Filing Date)	(Status) (patented,pending,aband.)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

James E. Cockfield	Reg. No. 19,162	Maria C. Laccotripe Zacharakis	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. 51,619
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. Ridders	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

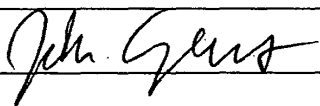
Direct Telephone Calls to:

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 Sebastian Böhm	
Inventor's signature	Date
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Inventor's signature 	Date <u>12/28/02</u>
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Citizenship US	
Post Office Address (if different)	

Full name of third inventor, if any Manish Deshpande	
Inventor's signature 	Date 10/28/12
Residence 34 Apple Blossom Way, Canton, MA 02021	
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Post Office Address (if different)	

Docket No.: TGZ-021DVCN
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sebastian Böhm *et al.*

Application No.: Not Yet Assigned

Confirmation No.: N/A

Filed: Concurrently Herewith

Art Unit: N/A

For: METHOD AND APPARATUS FOR SORTING
PARTICLES

Examiner: Not Yet Assigned

DESIGNATION OF RECORD ATTORNEYS

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

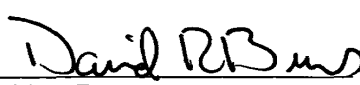
Dear Sir:

Pursuant to 37 C.F.R. 1.32(b) the following patent practitioners named in the Power of Attorney filed in this case are to be recognized by the Office as being of record in the above referenced application and in any patent issued thereon.

Thomas V. Smurzynski	Reg. No. 24,798
Giulio A. DeConti	Reg. No. 31,503
Elizabeth A. Hanley	Reg. No. 33,505
Amy E. Mandragouras	Reg. No. 36,207
Anthony A. Laurentano	Reg. No. 38,220
Kevin J. Canning	Reg. No. 35,470
Jane E. Remillard	Reg. No. 38,872
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Dated: August 7, 2006

Respectfully submitted,

By 
David R. Burns
Registration No.: 46,590
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PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

08/09/2006 HMARZ11 00000015 120080 11499953

01 FC:2011	150.00 DA
02 FC:2111	250.00 DA
03 FC:2311	100.00 DA

PTO-1556
(5/87)

U.S. Government Printing Office: 2002 — 499-267/80033

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875 Effective December 8, 2004

Application or Docket Number

11499953

APPLICATION AS FILED - PART I

(Column 1)

(Column 2)

SMALL ENTITY

OR

OTHER THAN
SMALL ENTITY

FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A
EXAMINATION FEE (37 CFR 1.16(d), (e), or (f))	N/A	N/A
TOTAL CLAIMS (37 CFR 1.16(i))	1 minus 20 =	-
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	-
APPLICATION SIZE FEE (37 CFR 1.16(e))	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).	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))		

RATE (\$)	FEE (\$)
N/A	150.00
N/A	\$250
N/A	\$100
X\$ 25 =	
X100 =	
+180=	
TOTAL	500

RATE (\$)	FEE (\$)
N/A	300.00
N/A	\$500
N/A	\$200
X\$50 =	
X200 =	
+360=	
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
Total (37 CFR 1.16(g))	Minus	**	=
Independent (37 CFR 1.16(h))	Minus	***	=
Application Size Fee (37 CFR 1.16(s))			
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))			

RATE (\$)	ADDITIONAL FEE (\$)
X\$ 25 =	
X100 =	
+180=	
TOTAL ADD'L FEE	

RATE (\$)	ADDITIONAL FEE (\$)
X\$50 =	
X200 =	
+360=	
TOTAL ADD'L FEE	

(Column 1)

(Column 2)

(Column 3)

AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total (37 CFR 1.16(g))	Minus	**	=
Independent (37 CFR 1.16(h))	Minus	***	=
Application Size Fee (37 CFR 1.16(s))			
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))			

RATE (\$)	ADDITIONAL FEE (\$)
X\$ 25 =	
X100 =	
+180=	
TOTAL ADD'L FEE	

RATE (\$)	ADDITIONAL FEE (\$)
X\$50 =	
X200 =	
+360=	
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.

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 1460, Alexandria, VA 22313-1460.

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

Application Data Sheet

Application Information

Application Type::	Regular
Subject Matter::	Utility
Suggested Group Art Unit::	3651
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	METHOD AND APPARATUS FOR SORTING PARTICLES
Attorney Docket Number::	TGZ-021DVCN
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	5
Small Entity?::	Yes
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

Applicant Information

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State or Province of mailing address:: MA
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Correspondence Information

Correspondence Customer Number:: 00959

Representative Information

Representative Customer Number:: 00959

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Continuation of	10/940143	09/13/04
10/940143	Division of	10/179488	06/24/02
10/179488	An application claiming the benefit under 35 USC 119(e)	60/373256	04/17/02

Foreign Priority Information**Assignee Information**

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Postal or Zip Code of mailing address:: 02210