(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 December 2005 (08.12.2005)

PCT

(10) International Publication Number
WO 2005/116642 A2

(51) International Patent Classification[7]: G01N 33/48

(21) International Application Number:
PCT/US2005/017503

(22) International Filing Date: 18 May 2005 (18.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/849,715 19 May 2004 (19.05.2004) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 10/849,715 (CIP)
Filed on 19 May 2004 (19.05.2004)

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US (patent), UZ, VC, VN, YU, ZA, ZM, ZW.

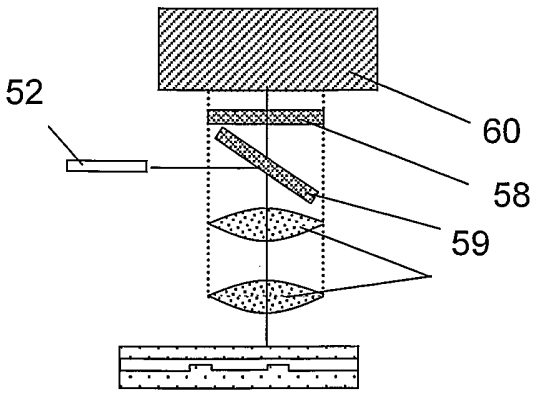
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU,*

[Continued on next page]

(54) Title: PROCESSING OF PARTICLES



(57) Abstract: Particles dispersed in a liquid are assembled in a configuration in which all the particles lie in the same plane, and the assembled particles are processed while in that configuration. The assembled particles can for example be simultaneously exposed to electromagnetic radiation which elicits from the particles a response which provides information about the particles. The particles can for example be cells, cell fragments, or analyte-bearing beads of the type conventionally analyzed in a cytometer.

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ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designation US*

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Title PROCESSING OF PARTICLES

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CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of copending commonly assigned application Serial No. 10/849,715, filed May 19, 2004, by Arnold, Rakestraw, Paul and Leung. This application is related to International Patent Application Number PCT/US 03/36533, which was filed on November 14, 2003, by Eksigent Technologies LLC, claiming priority from U.S. Serial No. 10/295,482. U.S. Serial No. 10/295,482 was published as US 2004-0096977 on May 20, 2004, and PCT/US 03/36533 482 was published as WO 2004/046695 on June 3, 2004 (i.e. both were published after the priority date of the present application). The entire disclosure of each of those applications and publications is incorporated herein by reference for all purposes.

BACKGROUND

The invention relates to the processing of particles, including but not limited to the examination of particles which are conventionally examined by cytometry.

Flow cytometry is widely used in life sciences research and clinical diagnostics for analyzing particles, including cells and beads having analytes attached thereto. A disadvantage of cytometry is that each particle must be examined individually. Other techniques for examining particles are disclosed in, for example, WO 00/12123, WO 01/38865, WO 03/085379, US 6,406,848, and Nature Biotechnology (2000), 18, 630-634, Brenner, S. et al, and Anal. Chem. (2000), 72, 1144-1147, Kitamori, T. et al.

SUMMARY OF THE INVENTION

WO 2004/046695 describes methods, apparatus and systems in which particles dispersed in a liquid are assembled in a configuration in which all the particles lie in the same plane, and the assembled particles are processed while they are in that configuration. For example, substantially all the particles are simultaneously exposed to electromagnetic radiation which elicits from the particles a response which provides information about the particles.

This application discloses a number of novel features which are not disclosed in WO 2004/046695 and which can be used to improve or modify the methods, apparatus

and systems disclosed in WO 2004/040695. These novel features include, but are not limited to, the following.

(1) The processing chamber containing the particles is discarded after the particles have been processed, thus avoiding all danger of residual material interfering with reuse of the processing chamber.

(2) The sample can contain 5 to about 35,000, e.g. 5000 to 25,000, or 10,000 to 25,000, or 15,000 to 25,000, particles per microliter.

(3) The particles in the sample are washed or otherwise modified before the sample is delivered to the processing chamber.

(4) The particles are assembled between a barrier member and an adjacent retention member which is shaped to assist in the location of individual particles in the desired configuration, for example which comprises a plurality of pockets, each of the pockets being capable of receiving one, but only one, of particles.

(5) As an alternative to, or in addition to, the use of a positioning liquid, the flow of the liquid sample itself is used to direct the particles into the desired configuration.

(6) The processing of the particles comprises observing a signal generated by the particles when exposed to a radiation source, and steps are taken to at least partially correct for the intensity profile of the radiation source.

(7) The processing of particles comprises observing a signal generated by the particles when exposed to a radiation source, and the axes of radiation and observation are the same (referred to herein as confocal examination).

(8) Pumps are used to deliver, and if desired to remove, the liquid sample and, if a positioning liquid is used, to control the flow of the positioning liquid; and one or more of the pumps comprises a flexible membrane (also known as a diaphragm) which is moved by working fluid and which is in contact with the sample or positioning liquid.

(9) A plurality of processing chambers are arranged in series, or in series and in parallel; and the particles received in a plurality, and preferably all, of the different processing chambers lie in the same plane and are examined simultaneously or sequentially in substantially the same way.

(10) The processing chamber has a width of about 40 to about 100 μ and a depth of about 10 to about 50 μ and/or each of the width and depth of the processing chamber.

is at least 2.5 times, but less than 15 times, the particle size, e.g. at least 4 times but less than 10 times, the particle size.

Two or more of the features (1)-(10) set out above can be used in combination.

Different countries, and the European Patent Convention, have different laws and practices as to the way in which the methods and apparatus disclosed in WO 2004/046695 and the novel features disclosed in this application should be claimed in one or more national patents, based on one or both of WO 2004/046695 and this application. In this application, the invention is defined and claimed in the broad terms disclosed in WO 2004/046695, with the features (1)-(10) as optional features. In any country where this is not permissible, having regard to the disclosure and/or the claims granted in a national or European application based on WO 2004/046695, Applicant will define and claim the invention of this application in the same way, but, depending upon the law and practice applicable to the national or European application, subject to (1) a disclaimer of the invention now defined and claimed insofar as it is not limited to one or more of the novel features disclosed in this application, and/or (2) a disclaimer of claims granted in a corresponding national or European application based on WO 2004/046695, and/or (3) a disclaimer of subject matter explicitly disclosed in WO 2004/046695, and/or (4) a terminal disclaimer.

In a first preferred aspect, this invention provides a method of processing particles, the method comprising the steps of

(A) placing a sample containing the particles dispersed in a liquid in a processing chamber containing a particle receptor;

(B) causing at least some of the particles in the placed sample to be received by the particle receptor so that the received particles are all in the same plane; and

(C) processing at least some of the particles received by the particle receptor.

The method can include the step of (D) removing the particles from the processing chamber so that the processing chamber can be reused. Alternatively, the processing chamber containing the particles can be discarded after step (C). The term "removing the particles" in step (D) is used to include removing the particles in the original or in a changed state.

In a second preferred aspect, this invention provides a novel processing chamber comprising

(i) a sample inlet;

(ii) an elongate particle retention passage (EPRP) which is in fluid communication with the inlet and which has a first end and a second end; and

(ii) between the first and second ends of the EPRP, an elongate particle receptor comprising an elongate barrier member which permits passage of liquid but not the passage of the particles to be processed, e.g. particles having a size greater than a selected size in the range of 0.1 to 50 micron, preferably a size greater than a selected size in the range of 1 to 20 micron.

In such a processing chamber, the particle-bearing liquid can be directed towards the barrier member, so that the particles are received by the particle receptor and the liquid passes beyond the barrier member.

In a third preferred aspect, this invention provides apparatus for carrying out the method of the first aspect of the invention, the apparatus comprising a processing chamber according to the second aspect of the invention and means for processing particles received by the receptor, preferably a source of electromagnetic radiation which elicits from the particles a response which provides information about the particles. The term "apparatus" is used in a wide sense to include any system comprising a plurality of cooperating parts.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated in the accompanying drawings, which, with the exception of Figures 11-12, are diagrammatic and not to scale, and in which

Figures 1 and 2 are cross-sections showing successive stages in the operation of a processing chamber,

Figure 3 illustrates a system comprising a plurality of processing chambers,

Figures 4-7 are cross-sections through a processing chamber in which the flow of the liquid sample positions the particles,

Figures 8 shows confocal examination of retained particles,

Figures 9 and 10 show images of particles processed using different types of illumination,

Figures 11 and 12 show (with normalized intensity on the vertical axis and location on the horizontal axis) the signals provided by particles when the signal depends upon the intensity of the illumination when no correction has been made for the intensity profile of the light source (Figure 11) and when such correction has been made (Figure 12),

Figures 13 and 14 show pumping systems for delivering samples and positioning fluids to a processing chamber, and

Figure 15 shows a system of the invention.

Figures 1-16, and the discussion thereof, in WO 2004/046695 further illustrate the invention.

DETAILED DESCRIPTION OF THE INVENTION

In the Summary of the Invention above, in the Detailed Description of the Invention and the claims below, and in the accompanying drawings, reference is made to particular features of the invention, including for example components, ingredients, devices, apparatus, systems, steps and embodiments. It is to be understood that the disclosure of the invention in this specification includes all possible combinations of such particular features. For example, where a particular feature is disclosed in the context of a particular aspect or embodiment of the invention, or a particular Figure, or a particular claim, that feature can also be used, to the extent possible, in combination with and/or in the context of, other particular aspects and embodiments of the invention, and in the invention generally. The invention claimed herein includes the use of features which are not specifically described herein, but which provide functions which are the same as, equivalent to or similar to, features specifically described herein.

The term "comprises", and grammatical equivalents thereof, are used herein to mean that other components, ingredients, steps etc. are optionally present in addition to the component(s), ingredient(s), step(s) specifically listed after the term "comprises". For example, a system "comprising" (or "which comprises") components A, B and C can contain only components A, B and C, or can contain not only components A, B and C, but also one or more other components. Where reference is made herein to a

method comprising two or more defined steps, then, unless the context requires otherwise, the defined steps can be carried out in any order or simultaneously, and the method can include one or more other steps which are carried out before any of the defined steps, between two of the defined steps, or after all the defined steps. The term "at least" followed by a number is used herein to denote the start of a range beginning with that number (which may be a range having an upper limit or no upper limit, depending on the variable being defined). For example "at least 1" means 1 or more than 1, and "at least 80%" means 80% or more than 80%. The term "at most" followed by a number is used herein to denote the end of a range ending with that number (which may be a range having 1 or 0 as its lower limit, or a range having no lower limit, depending upon the variable being defined). For example, "at most 4" means 4 or less than 4, and "at most 40%" means 40% or less than 40 %. When, in this specification, a range is given as "(a first number) to (a second number)" or "(a first number) - (a second number)", this means a range whose lower limit is the first number and whose upper limit is the second number. The numbers given herein should be construed with the latitude appropriate to their context and expression. The terms "plural" and "plurality" are used herein to mean two or more. The term "elongate" is used herein to refer to an article or passageway having one dimension (its length) which is substantially greater than, preferably at least 4 times, e.g. at least 6 times, each of its other dimensions. The abbreviation "EPRP" is used herein to refer to an elongate particle retention passage.

Where reference is made herein to “a”, "an", "one" or "the" feature, it is to be understood that, unless the context requires otherwise, there can be a single such feature or more than one such feature. For example, when reference is made herein to a feature selected from a list of features, it is to be understood that, unless the context requires otherwise, the feature can be a single one of the listed features or two or more of the listed features.

When reference is made herein to a first feature and/or a second feature, it is to be understood that, unless the context requires otherwise, such terminology is used for convenience in identifying such features, and means that either or both features can be present, and that when both features are present, they can be the same or different.

Where reference is made herein to two or more components (or parts or portions etc.), it is to be understood that the components can be, unless the context requires

otherwise, separate from each other or integral parts of a single structure or a single component acting as the two or more specified components.

Particles

Page 3, line 24, to Page 4, line 14, of WO 2004/046695 provides a detailed disclosure of particles which can be used in this invention.

Preparation, Pretreatment, and Dilution or Concentration of Samples

The sample can be prepared from the sample source in any way. For example, a multi-port rotor valve can be connected to the sample source, a pump, a sample loop, a waste reservoir, and a delivery conduit. In a first position of the valve, particle-containing liquid is pumped from the sample source into the sample loop (the amount of liquid preferably being greater than that required to fill the sample loop, in order to ensure that the loop is completely filled); the excess liquid flows to the waste reservoir. In a second position of the valve, the sample is pumped down the delivery conduit towards the processing chamber.

In some cases, it may be desirable to wash and/or to modify the particles in the sample before the sample is delivered to the processing chamber. In such cases, the sample can for example be passed into a pretreatment chamber through which liquid can pass, but which retains the particles, e.g. an elongate pretreatment chamber which includes a barrier member of the kind used in the processing chamber. The particles can be exposed to a single liquid or sequentially to two or more liquids. The liquid(s) can merely wash the particles to remove unwanted components of the original sample, for example excess components of an assay mixture, and/or can produce some desired change, e.g. a chemical reaction, in the particles, in which case the wash fluid flows across the particles for the length of time necessary to complete the reaction. A single reaction or a series of reactions can be carried out. The progress of the reaction(s) can in some cases be monitored by observing signals from the particles (for example if appropriate fluors are added as part of the reaction sequence). The treated particles can be removed from the pretreatment chamber by a reverse flow of a suitable liquid.

In some cases, it may be desirable to increase or decrease the concentration of particles in the sample before the sample is delivered to the processing chamber. In such cases, the sample can be delivered to a dilution chamber in which additional liquid is added to it. If the particles have been washed or otherwise pretreated, they can be

flushed out of the pretreatment chamber into a dilution chamber having a volume corresponding to the desired concentration.

For example, if the EPRP contains 100 to 500 particle parking sites and has a volume between 4 and 20 nL, a preferred sample contains about 25,000 particles per microliter. To obtain such a concentration from an original sample containing about 300 particles in a volume of 25 microliters, the particles in the original sample are separated, e.g. by causing the liquid in the original sample to pass over a weir which retains the particles, and are then flushed into a dilution chamber having a suitable volume (e.g. with a volume in the tens of nL range, depending upon the design of the EPRP).

Elongate Particle Retention Passages (EPRP's)

The EPRP can be straight; or can include straight and curved portions, thus forming for example a serpentine shape; or can be continuously curved. The cross-section of the EPRP, which is preferably constant, can be of any shape, e.g. part-round or rectangular (including square). The passage may have, for example, transverse dimensions in the range of about 10 to about 100 micron, e.g. a width of about 40 to about 100 micron and a depth of 10 to 50 micron, such that the particle-bearing liquid can freely flow therethrough, while minimizing the volume of the sample. Preferably each of the transverse dimensions of the EPRP is at least about 2.5 times, particularly at least about 4 times, the particle size.

The processing chamber can comprise a single EPRP or a plurality of EPRP's which lie in the same plane and which are preferably parallel to each other. When the processing chamber comprises a plurality of EPRP's, adjacent EPRP's can share an elongate wall which provides an elongate particle receptor to one or both of the EPRP's. When the processing chamber comprises a plurality of EPRP's, all of them can be in liquid communication with a single inlet, or there can be a plurality of different inlets, each communicating with one or more of the EPRP's.

Further information about EPRP's is provided by page 4, line 16 -- page 6, line 21, of WO 2004/046695.

Auxiliary Chambers

The processing chamber can include at least one elongate auxiliary chamber which runs alongside the EPRP and (i) receives liquid which has passed over or through the barrier member or (ii) serves as a reservoir for liquid to be delivered into the EPRP. If there are plural parallel EPRP's sharing adjacent elongate walls, the auxiliary chambers run alongside the outermost EPRP's. The auxiliary chambers improve the uniformity of the processing along the length of the EPRP's.

Barrier Members

In preferred embodiments of the invention, the barrier member is a weir, the clearance gap above the weir being less than the size of particles which are to be retained. The particles, when directed against the weir, are received in a line at the top of the weir. Further information about barrier members is provided by page 4, line 30 -- page 5, line 24, of WO 2004/046695.

Pockets for Receiving Particles

The barrier member and/or the retention member can optionally be shaped to assist in the location of individual particles in the desired configuration, for example can comprise a plurality of pockets, each of the pockets being capable of receiving one, but only one, of the particles. Further information about pockets is provided by page 5, lines 10-20, of WO 2004/046695.

Manufacture of Processing Chambers

The processing chamber can conveniently be made as described on page 6, lines 12-21 of WO 2004/046695.

In some embodiments, the top and bottom member of the processing chamber, although sealed together, are separable. A single bottom member can then be used with a selected one of a number of different top members, or vice versa. In this way, if only one of the top and bottom members includes pockets for locating particles, it can be replaced by a different member having differently-sized pockets, when there is a change in the size of particles to be processed. In addition, if either member becomes contaminated or damaged, it can be replaced.

Causing Particles to be Received by Receptors

In some embodiments, as described for example on page 6, lines 23-30, of WO 2004/046695, a positioning liquid is used to direct the particles towards the receptors. After the particles have been processed, the flow direction of the positioning liquid can be reversed to assist in the removal of the particles from the EPRP.

As an alternative to, or in addition to, the use of a positioning liquid, the flow of the liquid sample itself into and through the EPRP can be used to direct the particles into the particle receptors. This can be done for example by appropriate arrangement of the EPRP or EPRP's relative to the direction of flow of the sample from the inlet to an outlet of the processing chamber and/or by appropriately controlled pumping of the liquids within the processing chamber.

In one embodiment in which the flow of the liquid sample itself is used to direct the particles towards the particle receptor, the processing chamber comprises an inlet and an outlet, the inlet being connected to an entry conduit having a plurality of parallel coplanar EPRP's connected thereto. Each of the EPRP's

(i) has a cross-section throughout its length such that one or more particles can enter and pass longitudinally down the EPRP, and

(ii) has its axis at an angle to the axis of the entry conduit, so that particles can move from the conduit into the EPRP; the angle can vary widely, e.g. from 30 to 150°, but angles of 30 to 60°, e.g. 45°, are often convenient, so that particles follow an obtuse-angled path as they pass from the entry conduit into the EPRP.

Each of the transverse dimensions of the EPRP's (in this and also in other embodiments) is preferably at least about 2.5 times, preferably at least about 4 times, e.g. 2.5 to 15 times or 4 to 10 times, the particle size. Liquid entering each of the EPRP's passes over the barrier member into an auxiliary chamber, and all the auxiliary chambers are connected to an exit conduit connected to the outlet. The outlet is optionally connected to a pump which helps to maintain a desired rate of flow and helps to draw particles into the EPRP's. There can be two or more coplanar sets of parallel, coplanar EPRP's. For example, that can be two sets in which the EPRP's are mirror images of each other and which share an exit conduit which runs between them. If each of the auxiliary chambers is substantially identical to, but a mirror image of, its

associated EPRP, then the system can be operated with flow in the opposite direction, the exit conduit becoming the entry conduit, the entry conduit becoming the exit conduit, the auxiliary chambers becoming the EPRP's, and the EPRP's becoming the auxiliary chambers. Such an arrangement is shown in Figures 4-7.

In another embodiment, the processing chamber is connected to a plurality of pumps, each which can exert positive and/or negative pressure on liquids in the processing chamber so as to urge particles towards or away from the particle receptor. Preferably the processing chamber is connected to (1) a delivery pump which provides primary control of the rate at which the sample is delivered to the inlet of the processing chamber, and (2) first and second positioning pumps which provides primary control over the rate and direction at which positioning fluid flows across the EPRP. The delivery pump can also provide primary control over the rate at which the particles are removed after they have been processed. Preferably, the processing chamber is also connected to an extraction pump which provides primary control over the rate at which liquid is removed from the EPRP. The term "primary control" is used above because the way in which the other pumps operated can also influence the various rates and directions of liquid flow.

The different pumps can be controlled by electropneumatic actuators to provide a desired sequence of liquid flows within the processing chamber. With a correct configuration, sample can be fed and then positioned at the same time to the analysis chamber. For example, when loading a sample into the EPRP, there should be a positive pressure drop down the sample inlet line and a negative pressure drop (i.e. suction) down the outlet line. Particles in the sample can then be pushed towards the particle receptors. During this process, the sample outlet line can be pressurized to reduce or prevent liquid from leaving the EPRP. The selection of suitable pressure settings at different lines can assist in the removal of contaminants from the EPRP while minimizing the loss of sample particulates. We have found that it is useful, in particular in reducing contamination of the EPRP, to remove a minor proportion of the liquid sample from the EPRP during at least part of one or more of steps (A)-(C). The rate of removal can for example be 0.05 to 0.2 times the rate at which the sample is supplied during step (A).

In suitable pressure-controlled configurations, multiple electro pneumatic actuators can be used to provide pressure heads over a wide operating pressure range,

typically 0 to 100psi (0 to 7 kg/cm^2), making it possible to use high flow rates for processing large volume of sample and/or cleaning. For example, the pressure can be set at 0 to 60 psi (0 to 4.2 kg/cm^2) at different lines to get a pressure drop up to 60psi (4.2 kg/cm^2) for sample loading, and up to 100psi (7 kg/cm^2) at inlet and positioning outlet lines to provide maximum flush for releasing a sample. A relatively large amount (e.g. 10 to 20μL.) of sample fluid can be processed in a short period of time (e.g. less than 1 minute). The sample-loading and sample-releasing processes can be repeated in a cyclic manner to process small portions in multiple cycles. Alternatively, all the particles can be captured in one sample-loading process. In the one-time capture mode, processing is simple and only a single detection step is needed after the sample-loading step is stopped. However, long sample loading times tend to capture small substance in the sample which sticks to the particles and particle receptors, and which can make it difficult to remove the particles in the releasing step. In the cyclic loading mode, less small sticky substance is captured, and the particles are relatively easy to remove. Multiple detection steps are needed for multiple cycles of loading, and the control of the cycle steps can be complicated if there is significant transient delay in the fluidic system.

In some embodiments, the particles have electrical properties which can be used to direct the particles towards the receptors, as disclosed on page 6, line 31 -- page 7, line 2, of WO 2004/046695.

Techniques for Processing Received Particles

Particles received by the particle receptors can be processed by any appropriate technique, as disclosed, for example, on page 7, line 4 -- page 8, line 9, of WO 2004/046695.

When the processing of the particles involves measuring the intensity of a signal generated by the particles, it is desirable to take steps which will at least partially correct for the intensity profile of the radiation source. This can be done, for example, in a separate normalization procedure in which (a) the light source to be used (or already used) to interrogate the particles illuminates an area which is similar to, preferably somewhat larger than, e.g. 25% larger than, the area containing the received particles, and which is composed of uniformly fluorescent material, e.g. a liquid or gel, and (b) the resulting signal is observed by the detection system to be used (or already

used) when the particles are interrogated. The fluorescent area can for example be provided by a separate chamber which is physically attached to the processing chamber, or by a chamber which is used during the processing of particles, e.g. a dilution chamber, which is, however, disconnected and filled with the fluorescent material in order to carry out the normalization procedure. The results of this normalization procedure are used to normalize the signals obtained from the received particles of the sample. Figures 11 and 12 illustrate the improvement that can be obtained in this way.

In some types of examination, fluorescence from the particles is observed along an axis which is different from the axis of the illumination, as shown for example in Figures 14 and 15 of WO 2004/046695. Such examination is referred to herein as off-axis examination. In other types of examination, the axes of observation and illumination are the same, as shown for example in Figure 8. Such examination is referred to herein as confocal examination.

Off-axis examination results in an image which suffers from shadowing of one side of the particle. Confocal examination, on the other hand, provides a shadowless image. The different images are illustrated in Figures 9 and 10. These facts make it possible to use confocal examination to distinguish between (a) a particle whose response to the radiation is substantially uniform throughout the particle (e.g. a dye-infused particle), and which therefore produces an image whose intensity has a Gaussian profile brightest at the center, and (b) a particle whose response to radiation is only at the surface of the particle (e.g. a hybridized particle), and which therefore produces an image which is brighter at the periphery than at the center. This makes it possible to use a particle which is infused with an encoding dye and which, if it has reacted with an analyte, has been hybridized with a signal dye, the encoding and signal dyes responding differently to the radiation, e.g. producing different fluorescent signals when exposed to a laser. Such particles can be examined through a first filter to identify the encoding dye and then through a second filter to identify the concentration of the signal dye.

It is also possible for the received particles to be processed so that part or all of the particles (e.g. analyte fragments forming part of the particles) undergoes physical and/or chemical change, as disclosed for example on page 27, line 27 --page 8, line 5, of WO 2004/046695.

It is also possible for the processing chamber to be used for sample preparation, pre-concentration, extraction and clean up. Discrimination for low concentrations of target analytes and high background analyte concentrations can be achieved by passing a sample over capture particles positioned in the processing chamber.

Pumps

Pumps are generally used to deliver and remove the liquid samples and, if positioning liquids are used, to control the flow of the positioning liquids. Preferably the pumps are pneumatic or electroosmotic (also known as electrokinetic) pumps. Such pumps can be precisely controlled and can accurately introduce very small quantities of liquid, e.g. about 100 picoliters to about 100 nanoliters of liquid per cycle. One or more of the pumps can act directly on the sample or positioning liquid. Alternatively or additionally, one or more of the pumps can comprise a flexible membrane (also known as a diaphragm) which is moved by working fluid and which is in contact with the sample or positioning liquid. This makes it possible to use a pump having a working fluid which would not be satisfactory to use as a positioning fluid or a pump which would not be satisfactory for the sample or positioning fluid. It also permits orthogonal control of the flow in the EPRP without cross-talk between the pumps. Suitable electroosmotic pumps are described for example in U.S. Patent Nos. 6,277,257, 6,013,164 and 3,923,426, US Patent Publication Nos. 2004/0074768and 2004/0074784, and WO2004/03604, the entire disclosures of which are incorporated herein by reference.

Methods

Further information about method steps of the invention is provided by page 8, lines 21-28, of WO 2004/046695.

Processing Chambers connected in Parallel and/or in Series

A plurality of processing chambers can be arranged in parallel and/or in series, preferably in a way such that several, and preferably all, of the particles received in a plurality, and preferably all, of the different processing chambers lie in the same plane and can, therefore, be examined simultaneously or can be examined sequentially in substantially the same way. Processing chambers connected in series are connected to a single inlet, so that they receive substantially identical samples; when a positioning liquid is used, it can be supplied from the same source but separately to each processing chamber through a manifold or can be supplied to run sequentially through

two or more processing chambers. Further information about the use of a plurality of processing chambers is provided by page 8, line 30 -- page 9, line 9 of WO 2004/046695.

Drawings

The invention is illustrated in the accompanying drawings, in which the same or similar components are denoted by the same reference numerals.

Figures 1 and 2 are partial cross-sections of a processing chamber in which there are pockets 151 in the top member 2 adjacent the weir 15.

Figure 3 is similar to Figure 15 of WO 2004/046695, but has a much larger number of turns, so that a larger number of particles can be processed per cycle. The sample enters through inlet 11 and leaves through outlet 12. The positioning liquid enters through inlet 16 and leaves through outlet 17.

Figures 4-7 show a processing chamber in which the flow of the liquid sample positions the particles. EPRP's 12a1/a2, 12b1/b2 ... are arranged in pairs with parallel weirs 14a, 14b ... between them. There are also parallel weirs 14a/a', 14b/b' ... between the EPRP's 12a2 and 12b1, 12b2 and 12c1 ... The first EPRP in each pair is connected at an angle to manifold 111 connected to inlet 11 for the particle-bearing sample. The second EPRP in each pair is connected at an angle to exit conduit 131, which is connected to outlet 13 for particle-free liquid which passes over the weirs into the second EPRP, which functions as an auxiliary chamber. The particles enter and travel down the EPRP's 12a1, 12b1 ... and are retained on the weirs. After the received particles have been processed, they can be removed by reversing the liquid flow. The reverse liquid flow can be provided by a second particle-bearing sample, so that the second EPRP's retain the second particles, and the first EPRP functions as an auxiliary chamber

Figure 8 shows confocal examination of particles retained in an EPRP. A laser 52 directs a diffuse beam having a selected wavelength at a dichroic reflector 59 and onto the particles via optics 56. Light emitted by the particles (at a different, typically longer, wavelength) is collected and analyzed by detector 60.

Figures 9 and 10 show typical results from the off-axis illumination (Figure 9) and confocal illumination (Figure 10) of particles retained by an EPRP.

Figure 11 shows a typical result, with more than 50% variation, when the signal from a particle depends upon the intensity of the incident light, for the intensity of the signal from a dye-infused particle when there has been no flat-field correction of the signal. Figure 12 shows the same data, with about 16% variation, when flat-field correction has been carried out.

Figure 13 shows the use of diaphragm-containing pumps to control the delivery and removal of samples and positioning fluids to a processing chamber. Sample pump 2 includes pump fluid 210 confined by diaphragms 211 and 212, movement of which pumps particle-bearing liquid 215 through a processing chamber containing EPRP 12. Sample pump 4 includes pump fluid 412 confined by diaphragms 411 and 412, movement of which pumps positioning fluid 415 through the processing chamber.

Figure 14 shows the use of pumps 2A and 2B to control delivery and removal of the sample to an EPRP 12 and the use of pumps 4A and 4B to control delivery and removal of positioning liquid to the EPRP. The pumps can be under electropneumatic control, so that different pressures can be applied to different inlets and outlets simultaneously, according to a program which produces desired flow rates in different parts of the system at different times.

Figure 15 shows a system of the invention in which the particles are pretreated before being processed. The sample is introduced through a sample loop assembly 32 that is in the line from the sample introduction pump to the processing chamber. The loop assembly comprises sample inlet port 302, a multiport rotor valve 304 with a sample loop 305, and a waste conduit to a waste reservoir 303. With the valve in the first (sample introduction) position the loop is in line with the sample port and waste lines. An excess of sample is introduced through 302 into the sample loop 305 with the overflow going to waste 303. Once the sample is introduced, the valve is switched to a second position (run position) in which the sample loop is in the line between the sample introduction pump and the processing chamber, thereby allowing the pump to push the fluid from the loop 305 towards the processing chamber.

The sample passes through conduit 32 into chamber 36 and the excess fluid is allowed to flow to the waste reservoir over weir 37 (which does not allow particles to pass) through conduit 35. An optional shutoff valve 39 is shown. Once the particles have been collected along the weir 37, wash fluid is introduced through conduit 34 to

wash off excess components of the assay mixture. The wash fluid can for example contain reactants that are necessary for an assay, in which case the wash fluid flows across the particles for the length of time necessary to complete the reaction. The wash line can be connected to a manifold (not shown) which is connected to a plurality of reactant mixtures, so that a series of chemical reactions can be carried out. The progress of the reaction(s) can in some cases be monitored by observing signals from the particles (for example if appropriate fluors are added as part of the reaction sequence). The wash line can be placed at other positions in the chamber 37. Again, the fluid from the wash reservoir passes out of the chamber over the first weir through the conduit 35 to the waste reservoir.

Once the particles have been washed, they can be moved from the weir 37 to a second weir 36 by flowing wash fluid through conduit 34 through the chamber over the second weir and through conduit 20 for a short time. Once the particles have been placed on weir 36, they can be moved towards the processing chamber by sending fluid through conduit 20. As the particles move towards the processing chamber, they pass through a dilution chamber 301, in which the particles distribute themselves as the fluid expands into the dilution chamber. The volume of the dilution chamber serves as a liaison between the particle density received into the particle analysis system from the assay and the particle density that is optimum for the number of particle parking sites in the EPRP.

CLAIMS

1. A method of processing a sample comprising particles dispersed in a liquid, the method comprising

(A) placing the sample in a processing chamber containing a particle receptor;

(B) causing at least some of the particles to be received by the particle receptor so that the received particles are all in the same plane; and

(C) processing at least some of the particles received by the particle receptor.

2. A method according to claim 1 which has at least one of the following features: --

(1) the processing chamber containing the particles is discarded after the particles have been processed;

(2) the sample contains 5000 to 25,000, or 10,000 to 25,000, or 15,000 to 25,000, particles per microliter;

(3) the particles in the sample are washed or otherwise modified before the sample is delivered to the processing chamber;

(4) the particles are assembled between a barrier member and an adjacent retention member which comprises a plurality of pockets, each of the pockets being capable of receiving one, but only one, of particles;

(5) the flow of the liquid sample itself is used to direct the particles into the desired configuration;

(6) the processing of the particles comprises observing a signal generated by the particles when exposed to a radiation source, and the signal is processed so as to at least partially correct for the intensity profile of the radiation source;

(7) the processing of particles comprises observing a signal generated by the particles when exposed to a radiation source, and the axes of radiation and observation are the same;

(8) pumps are used to deliver, and if desired to remove, the liquid sample and, if a positioning liquid is used, to control the flow of the positioning liquid; and one or more of pumps comprises a flexible membrane which is moved by working fluid which is in contact with the sample or positioning liquid;

(9) a plurality of processing chambers are arranged in series, or in series and in parallel; and the particles received in a plurality of processing chambers which lie in the same plane are examined simultaneously or sequentially in substantially the same way;

(10) The processing chamber has a width of about 40 to about 100 μ and a depth of about 10 to about 50 μ and/or each of the width and depth of the processing chamber is at least 2.5 times, but less than 15 times, the particle size.

3. A method according to claim 2 wherein the barrier member is a weir; the only way in which liquid can pass the barrier member is over the weir; the particle receptor includes an elongate retention member which is adjacent to the weir, forms an elongate corner with the weir, and includes a plurality of pockets, each of the pockets being capable of receiving one, but only one, of the particles.

4. A method according to claim 2 or 3 wherein in step (B) the flow of the liquid sample directs the particles towards the particle receptor.

5. A method according to claim 4 wherein the EPRP

(i) has a cross-section throughout its length such that one or more particles can enter and pass longitudinally down the EPRP, and

(ii) has its axis at an angle of 30 to 60° to the axis of the entry conduit, so that particles can move from the conduit into the EPRP.

6. A method according to any one of claims 2 to 5 which comprises observing a signal generated by the particles when exposed to a radiation source, and wherein the axes of radiation and observation are the same.

7. A method according to any one of claims 2 to 6 which comprises observing a signal generated by the particles when exposed to a radiation source; the information depends on the intensity of the signal; and a flat-field correction is used to reduce the dependence of the information on the intensity profile of the radiation source.

8. A method according to any one of claims 2 to 7 wherein the sample placed in the processing chamber has been prepared by separating the particles from an original sample, and dispersing the separated particles in a liquid at a concentration of 5 to 25,000 particles per microliter.

9. A processing chamber for processing particle-containing liquid samples by a method as claimed in any one of the preceding claims, the processing chamber comprising

(i) a sample inlet;

(ii) an elongate particle retention passage (EPRP) which is in liquid communication with the inlet and which has a first end and a second end; and

(ii) between the first and second ends of the EPRP, an elongate particle receptor comprising an elongate barrier member which permits passage of liquid but not passage of the particles to be processed.

10. A processing chamber according to claim 9 wherein the particle receptor includes an elongate retention member which is adjacent to the weir, forms an elongate corner with the weir, and includes a plurality of pockets, each pocket having a maximum depth of about 2 to about 20 micron and a width of about 3 to about 50 micron.

FIG. 1

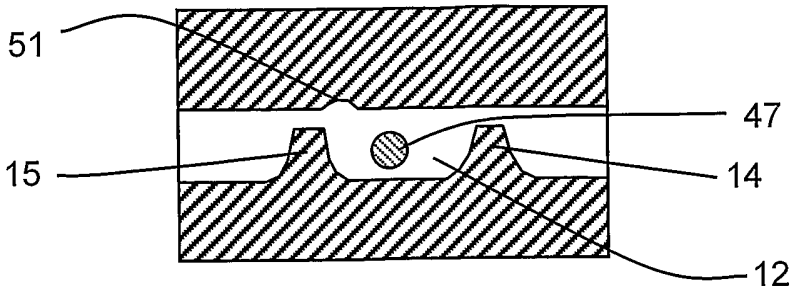

FIG. 2

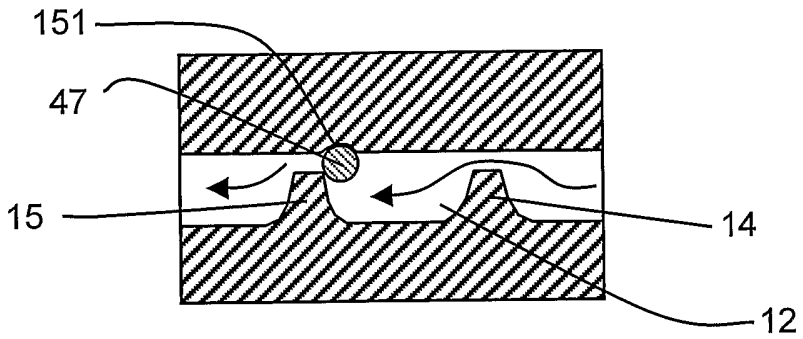

FIG. 3

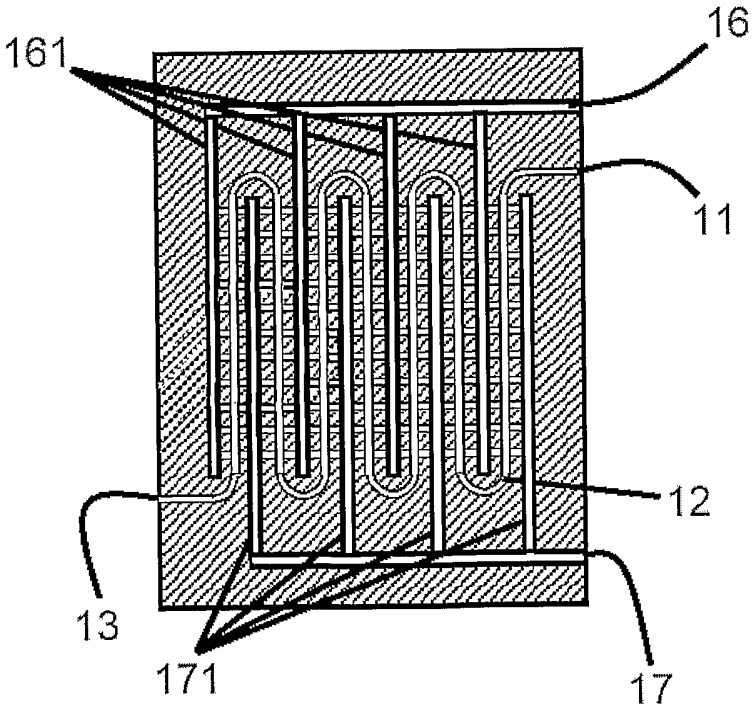

FIG. 4

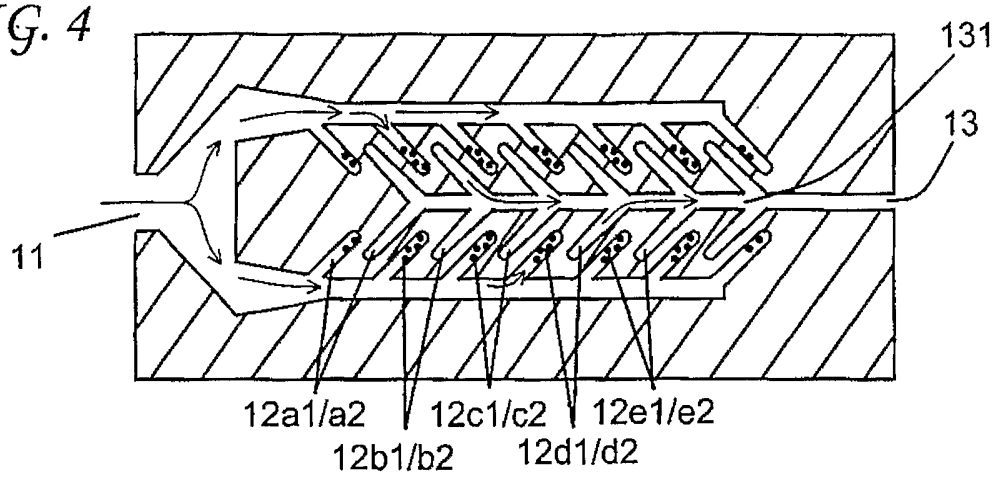

FIG. 5

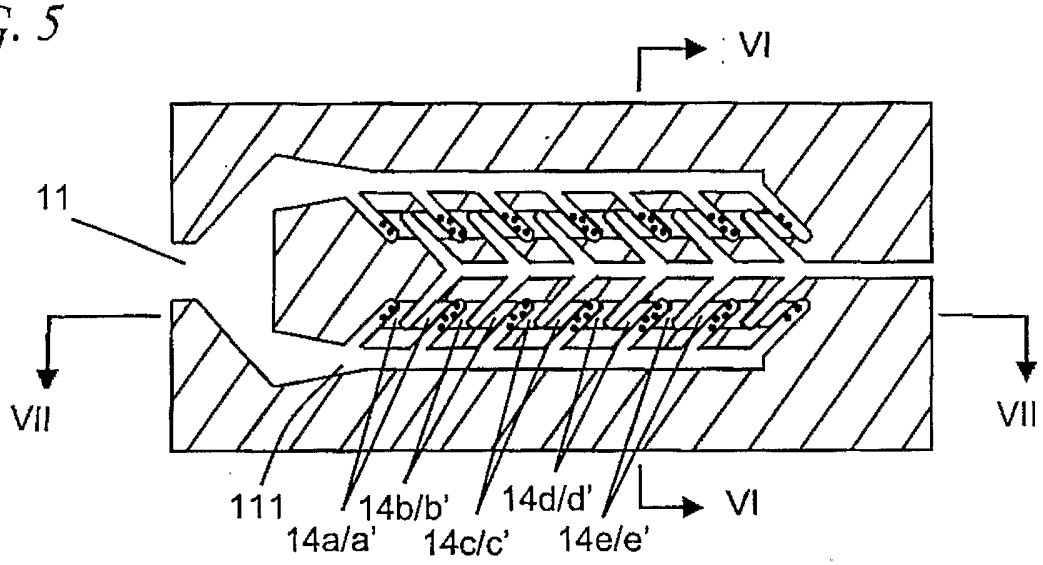

FIG. 6

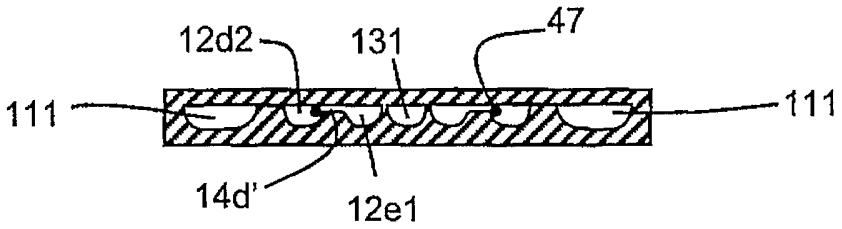

FIG. 7

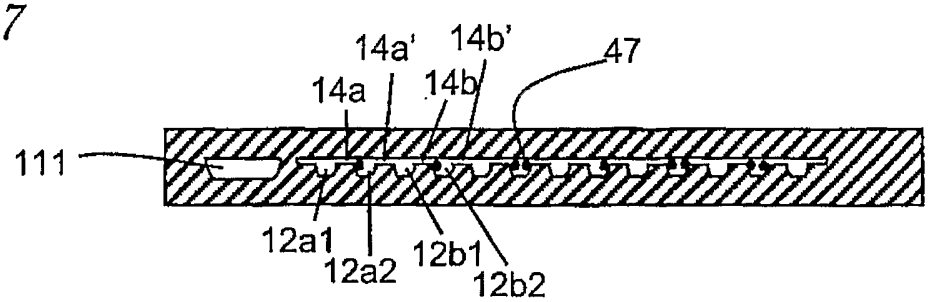

FIG. 8

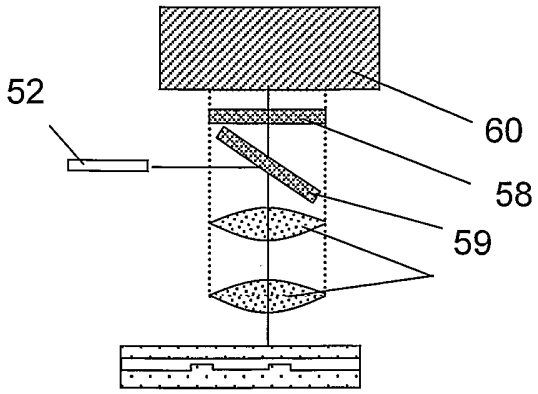

FIG. 9

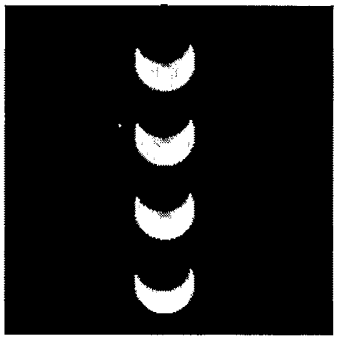

FIG. 10

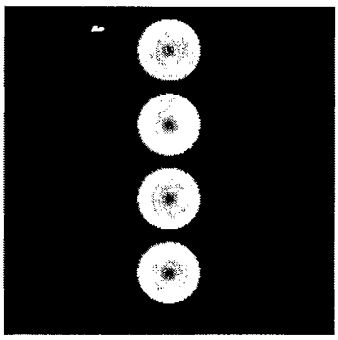

FIG. 11

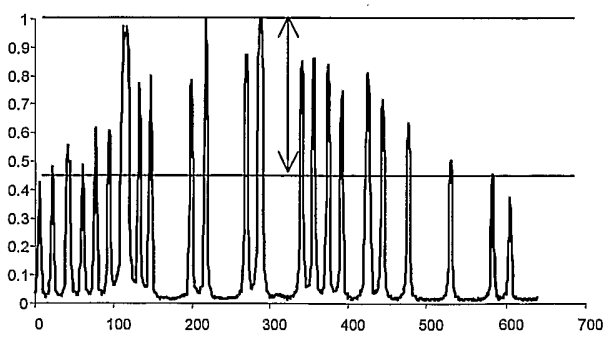

FIG. 12

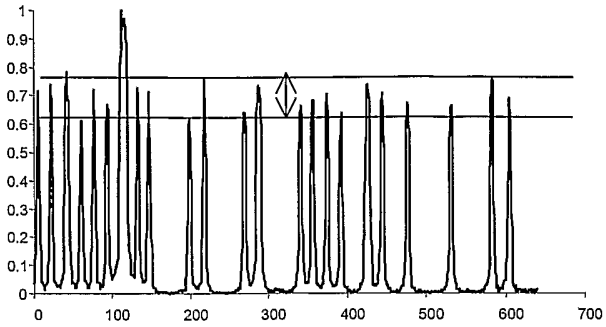

FIG. 13

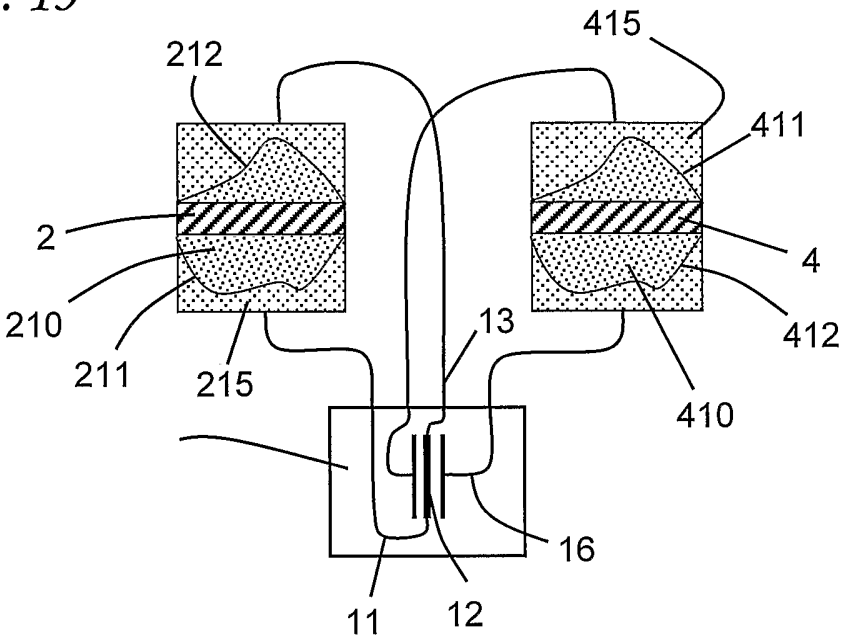

FIG. 14

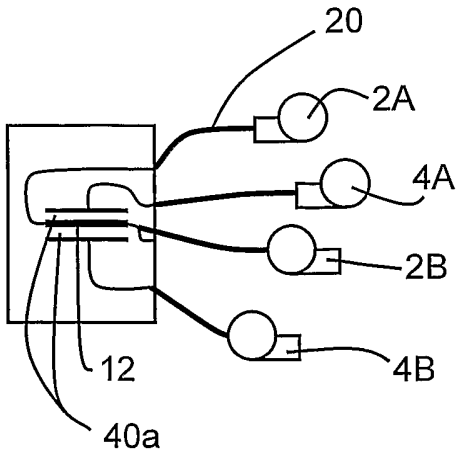

FIG. 15