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(54) Title: METHOD OF CREATING REMOVABLE BARRIERS IN MICROFABRICATED FLUIDIC DEVICES

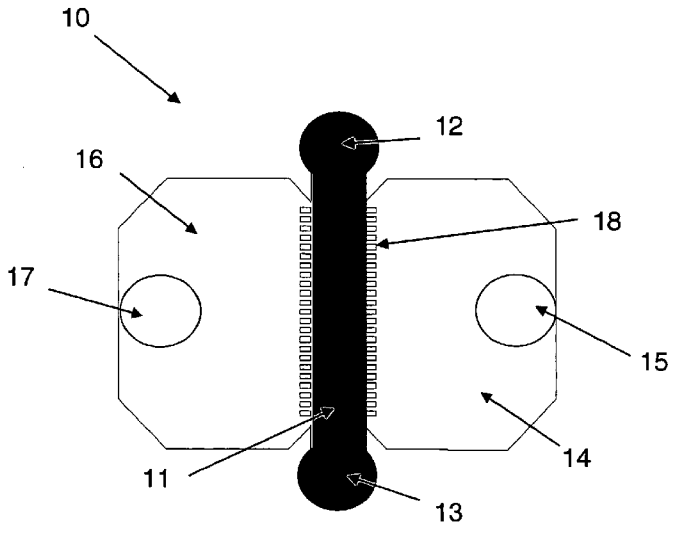

Figure 1

(57) Abstract: A method for creating a removable barrier in a microfluidic apparatus is carried out by: (a) providing a microfluidic apparatus having at least a first chamber and a second chamber, each including at least an inlet, wherein the first and second chamber are separated from one another by a plurality of barrier elements, the plurality of barrier elements defining a barrier zone, (b) introducing a liquid sample into the apparatus through the inlet of the first or second chamber to create a retained continuous liquid phase in one chamber, a continuous gas phase in the other chamber, and a gas/liquid interface between the first and second chamber at the barrier zone, (c) releasing the interface from the barrier elements, and (d) advancing the liquid phase past the barrier zone into the gas chamber displacing the gas phase. In some embodiments the barrier elements are hydrophobic (for example, the barrier elements comprise a hydrophobic polymer. Devices such as chemotaxis devices incorporating such methods are also described.

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METHOD OF CREATING REMOVABLE BARRIERS IN MICROFABRICATED FLUIDIC DEVICES

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Field of the Invention

This invention concerns microfluidic devices and methods of using the same.

Background of the Invention

Microfluidic systems and devices have increasingly been used to acquire chemical, biochemical and biological information. These microfluidic systems are useful for performing chemical, biological, clinical, and environmental analysis of chemical and biological specimens. Additionally, some advantages over conventional chemical or physical laboratory techniques are that the microfluidic devices are able to analyze small sample sizes, the devices themselves may be inexpensive to manufacture, and the devices can provide many specific chemical and biological analytical techniques, such as controlled mixing.

Summary of the Invention

A first aspect of the present invention is a method for creating a removable barrier in a microfluidic apparatus, the method comprising the steps of:

(a) providing a microfluidic apparatus having at least a first chamber and a second chamber, each including at least an inlet, wherein the first and second chamber are separated from one another by a plurality of barrier elements, the plurality of barrier elements defining a barrier zone,

(b) introducing a liquid sample into the apparatus through the inlet of the first or second chamber to create a retained continuous liquid phase in one chamber, a continuous gas phase in the other chamber, and a gas/liquid

interface between the first and second chamber at the barrier zone, (c) releasing the interface from the barrier elements, and (d) advancing the liquid phase past the barrier zone into the gas chamber displacing the gas phase. In some embodiments the barrier elements are hydrophobic (for example, the barrier elements comprise a hydrophobic polymer.

In some embodiments, the microfluidic apparatus comprises a first chamber including an inlet and outlet located at the distal ends of the first chamber, a second chamber including an inlet, and (optionally) a third chamber including an inlet. The first chamber can be located between the second and third chambers when the third chamber is present (while the second and third chambers can in some embodiments be symmetric with one another with respect to the first chamber, in other embodiments they need not). The second and third chambers are each separated from the first chamber by a plurality of barrier elements. In some embodiments, the first, second and third chambers are all equal in height and the second and third chambers are wider than (and/or have a greater volume than) the first chamber. In some embodiments, the second and/or third chambers are open to atmospheric pressure. A device with first, second, and third chambers may be configured for, among other things, use as a chemotaxis device as discussed further below.

The foregoing and other objects and aspects of the present invention are discussed in greater detail in the drawings herein and the specification set forth below.

Brief Description of the Drawings

Figure 1 shows a top view of one embodiment of a microfluidic apparatus of the present invention.

Figure 2 shows a cross-section view of one embodiment of a microfluidic apparatus of the present invention.

Figure 3 shows a microscope image of the barrier elements and gas/liquid interface at the barrier zone.

Figure 4 shows cell chemotaxis data displaying the tracks of cells during an experiment. Experimental details are provided in Example 3.

Figure 5 shows removal of the gas/liquid interface at the barrier zone using a pulsed laser. Experimental details are provided in Example 4.

Figure 6 shows reformation of the removable barrier at one set of the barrier elements using a pulsed laser. Experimental details are provided in Example 4.

Detailed Description of the Invention

The present invention is now described more fully hereinafter with reference to the accompanying drawings, in which embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather these embodiments are provided so that this disclosure will be thorough and complete and will fully convey the scope of the invention to those skilled in the art.

Like numbers refer to like elements throughout. In the figures, the thickness of certain lines, layers, components, elements or features may be exaggerated for clarity. Where used, broken lines illustrate optional features or operations unless specified otherwise.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a," "an" and "the" are intended to include plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements components and/or groups or combinations thereof, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components and/or groups or combinations thereof.

As used herein, the term "and/or" includes any and all possible combinations or one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specification and claims and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. Well-known functions or constructions may not be described in detail for brevity and/or clarity.

It will be understood that when an element is referred to as being "on," "attached" to, "connected" to, "coupled" with, "contacting," etc., another element, it can be directly on, attached to, connected to, coupled with and/or contacting the other element or intervening elements can also be present. In contrast, when an element is referred to as being, for example, "directly on," "directly attached" to, "directly connected" to, "directly coupled" with or "directly contacting" another element, there are no intervening elements present. It will also be appreciated by those of skill in the art that references to a structure or feature that is disposed "adjacent" another feature can have portions that overlap or underlie the adjacent feature.

Spatially relative terms, such as "under," "below," "lower," "over," "upper" and the like, may be used herein for ease of description to describe an element's or feature's relationship to another element(s) or feature(s) as illustrated in the figures. It will be understood that the spatially relative terms are intended to encompass different orientations of the device in use or operation in addition to the orientation depicted in the figures. For example, if the device in the figures is inverted, elements described as "under" or "beneath" other elements or features would then be oriented "over" the other elements or features. Thus the exemplary term "under" can encompass both an orientation of over and under. The device may otherwise be oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein interpreted accordingly. Similarly, the terms "upwardly," "downwardly," "vertical," "horizontal" and the like are used herein for the purpose of explanation only, unless specifically indicated otherwise.

It will be understood that, although the terms first, second, etc., may be used herein to describe various elements, components, regions, layers and/or sections, these elements, components, regions, layers and/or sections should not be limited by these terms. Rather, these terms are only used to

distinguish one element, component, region, layer and/or section, from another element, component, region, layer and/or section. Thus, a first element, component, region, layer or section discussed herein could be termed a second element, component, region, layer or section without departing from the teachings of the present invention. The sequence of operations (or steps) is not limited to the order presented in the claims or figures unless specifically indicated otherwise.

The term "microfluidic apparatus" as used herein refers to a device or system having one or more fluid passages, chambers, channels or conduits that are able to handle microvolumes of liquids, typically wherein one or more of the dimensions is less than 500 μm.

The term "liquid" as used herein refers to liquids or aqueous solutions, including suspensions of particulates in liquids, such as cells or beads.

The term "chamber" as used herein refers to a continuous or uninterrupted liquid pathway or channel through the microfluidic apparatus with an opening at either end or top or bottom of the microfluidic apparatus, i.e. an inlet and an outlet, to allow the passage of a liquid therethrough. A "chamber" typically can hold or accommodate microscale amounts (e.g., microliters or less, such as for example, in nanoliters to microliters) of liquid. For example, in some embodiments, the "first" chamber as described herein can have a size or volume of 1, 5 or 10 μl, up to 100 or 500 μl. The size or volume of "second" and "third" chambers as described herein can likewise be 1, 5 or 10 μl, up to 100 or 500 μl, but in some embodiments the size of the second and third chambers can be 10, 100 or 1,000 times that of the first chamber (*e.g.*, a size or volume up to 1, 5 0r 10 ml. The size of second and third chambers can be the same as one another or different from one another.

"Barrier zone" as used herein refers to a region defined by barrier elements at which a physical (*e.g.*, gas) barrier is formed in the microfluidic apparatus (that is, the barrier elements are not themselves the barrier, but enable the creation of the barrier). The physical barrier created at the barrier zone may be overcome by chemical or physical mechanisms.

"Barrier element" as used herein may be any geometric structure, in any pattern, that define or are arranged in a barrier zone at which a physical

barrier can be formed. Examples include, but are not limited to: posts, columns, walls, columns, bumps, protrusions or the like, and including combinations thereof. The barrier elements may be: continuous or discontinuous; arranged in uniform, random, or partially random patterns with respect to one another, be of high or low aspect ratio, etc.

Devices of the invention can be formed from any suitable material including but are not limited to silica based substrates, such as glass, quartz, silicon or polysilicon, as well as other substrate materials, such as gallium arsenide and the like. Other suitable materials include but are not limited to polymeric materials, e.g., plastics, such as polymethylmethacrylate (PMMA), polycarbonate, polytetrafluoroethylene (TEFLON.TM.), polyvinylchloride (PVC), polydimethylsiloxane (PDMS), polysulfone, polystyrene, polymethylpentene, polypropylene, polyethylene, polyvinylidine fluoride, ABS (acrylonitrile-butadiene-styrene copolymer), and the like, as well as polymerized photoresists, e.g., SU-8, 1002F and the like.

Substrate materials are often selected based upon their compatibility with known techniques, such as microfabrication. Suitable substrate materials are also generally selected for their compatibility with the full range of conditions to which the microfluidic devices may be exposed, including extremes of pH, temperature, salt concentration, and application of electric fields. Accordingly, the substrate material may include materials normally associated with the semiconductor industry. 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 some embodiments, the substrates used to fabricate the microfluidic apparatus are silica-based, more preferably glass or quartz, due to their inertness to the conditions described above, as well as the ease with which they are microfabricated. In other embodiments 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. These polymeric materials may include treated surfaces, e.g., derivatized or coated surfaces, to enhance their utility in the microfluidic system, e.g., provided enhanced fluid direction, e.g., as described in U.S. Pat.

No. 5,885,470, and which is incorporated herein by reference in its entirety for all purposes. In other embodiments the microfluidic apparatus is fabricated using a combination of materials, such as silica-based and polymeric materials.

The material of the microfluidic device may be opaque, translucent or transparent. The device can be formed of a single layer substrate of a single material or a laminated or multi-layer configuration of the same or different material substrates. The device may be a single layer monolithic substrate or (more typically) a multiple layer device (*e.g.*, having two or three layers or more) and having a thickness that is between about 0.2 mm to about 15mm. The thickness of the device is not critical, as the thickness of top and bottom parts of the device are not critical, so long as the proper inner chamber dimensions are provided for the intended use. The device can comprise a bioactive agent that is formed in a matrix of the substrate and/or applied or coated on a primary surface thereof to define one or more analytical sites on the device for analysis and/or to define a barrier zone.

The term "bioactive" includes the term "bioreactive" and means an agent or material or composition that alone or when combined with another agent and exposed to a test sample will form a chemical reaction and/or be altered in appearance or in another optical or electronically readable or detectable manner when a target analyte, *e.g.*, constituent, antigen, antibody, bacterium, virus, ligand, protein contaminant, toxin and/or other material is present in the test sample. *See, e.g.*, U.S. Patent No. 6,294,107, the contents of which are hereby incorporated by reference as if recited in full herein.

Devices of the invention can be made by any suitable technique including but not limited to microfabrication techniques, such as photolithography, wet chemical etching, laser ablation, reactive ion etching (RIE), air abrasion techniques, injection molding, LIGA methods, metal electroforming, embossing, and other techniques. Suitable techniques may also be those employed in the semiconductor industry. Other suitable techniques include but are not limited to molding techniques, such as injection molding, embossing or stamping, or by polymerizing the polymeric precursor material within the mold (See U.S. Pat. No. 5,512,131).

In operation, the gas/liquid interface can be released from the barrier elements by any suitable chemical or physical technique, or combination thereof. For example, the interface can be released by: (a) producing a laser generated shock wave, (b) increasing the hydraulic pressure of the liquid phase, (c) decreasing the atmospheric pressure of the gas phase, (d) generating an electric field, (e) heating the liquid phase, (f) adding a surfactant (*e.g.,* to the liquid in one or both chambers), (g) producing a magnetic field, (h) generating an ultrasonic signal, (i) disrupting a chemical interaction by cleaving a chemical bond (*e.g.,* to disrupt a coating on the barrier elements), (j) altering the ionic conditions of the liquid phase, (k) changing the pH of the liquid phase, (l) adding excess of a first member of a specific binding pair (*e.g.,* to disrupt a coating on the barrier elements) , or any combination thereof.

Advantageously, once released, the interface or removable barrier can be reformed by any suitable technique, such as by focusing a pulsed laser beam between a set of the barrier elements and firing the pulsed laser until a bubble of adequate size reforms the gas/liquid interface. Or, the barrier can be reformed by removing the liquid from the apparatus and refilling one chamber of the apparatus.

When devices of the invention are used to create a concentration gradient (*e.g.,* as in a chemotaxis device), the gradient can be created by any suitable technique, such as by: (a) introducing a first liquid sample into the apparatus through the inlet of the first chamber, (b) releasing the interface from the barrier elements, (c) advancing the liquid phase past the barrier zone and into the second chamber, (d) introducing a second liquid sample into the apparatus through the inlet of the second chamber, and (e) diffusing the second liquid sample across the apparatus.

When devices of the invention are used to control mixing of reagents, that mixing can be controlled or regulated by techniques such as: (a) introducing a first liquid sample into the apparatus through the inlet of the second chamber, (b) introducing a second liquid sample into the apparatus through the inlet of the third chamber, and (c) retaining the first liquid sample in the second chamber and the second liquid sample in the third

chamber until time to mix reagents, (d) releasing the interface from the barrier elements, and (e) mixing the first and second liquid sample.

In some applications, the devices of the invention can be used to separates two compartments from each other by: (a) introducing a first liquid sample into the apparatus through the inlet of the second chamber, (b) introducing a second liquid sample into the apparatus through the inlet of the third chamber, and then (c) retaining the first liquid sample in the second chamber and the second liquid sample in the third chamber.

The present invention is explained in greater detail in the following non-limiting Examples.

EXAMPLE 1

The following example demonstrates a chemotaxis experiment. The microfluidic apparatus was made by creating a microfabricated apparatus from poly(dimethyl siloxane) (PDMS). As shown in Figure 1, the microfluidic apparatus **10** possessed a first chamber **11** with an inlet **12** and an outlet **13**, a second chamber **14** with an inlet **15**, a third chamber **16** with an inlet **17**, and a plurality of barrier elements **18** defining a barrier zone separating the first and second chamber as well as the first and third chamber. As shown in Figure 2 the inlets of the second **15** and third chambers **17** were larger than the width of the first chamber **11** in order to create a concentration gradient across the first chamber (it is the different concentrations of the reagents in the second and third chambers that sets up a linear gradient in the first chamber). The apparatus **10** was reversibly bonded to the glass part of a "Delta T" (Biotechs Inc., Bulter, PA, USA) culture dish that provided temperature control during the chemotaxis experiment. A cover for the apparatus was made from PDMS to seal the inlet and outlet of the first chamber and both the inlet of the second or third chamber and to prevent any gravity flow after the addition of liquid into the apparatus. The cover contained two holes of different diameter positioned over either the inlet of the second or third chamber. The larger hole was for loading additional liquid and the smaller hole allowed for air to escape during the loading process.

The experiment was carried out as follows. A coating reagent (fibronectin, collagen, etc.) for increasing cell adherence within the chamber

was loaded into the first chamber from either the inlet or outlet of the first chamber by flowing the solution into the chamber. As shown in Figure 3, the barrier elements **18** confined the liquid **21** in the first chamber due to surface tension. A continuous gas phase **22** remained in the second and third chambers. Thus, a gas/liquid interface **20** was created between the first and second chambers and between the first and third chambers at the barrier zone. Once the coating process was complete, a cell suspension was loaded through either the inlet or outlet of the first chamber and gravity flow loaded the cells into the first chamber. The barrier elements again confined the cell suspension in the first chamber. The cells were allowed to attach to the surface of the first chamber. Manual pressure was then applied at either the inlet or outlet of the first chamber to overcome the surface tension between the barrier elements and release the removable barrier from the barrier elements. The liquid then advanced into both the second and third chambers. The cover was reversibly bonded on the top of the microfluidic apparatus and chemotaxis reagent was loaded into the apparatus through the larger hole of the cover. Cell migration was monitored by microscopy and time-lapse photography.

EXAMPLE 2

A concentration gradient in the microfluidic apparatus was created by diffusion. The microfluidic apparatus and cover were made as described above in Example 1. RPMI cell culture media was loaded into the first chamber of the microfluidic apparatus by manual pressure releasing the removable barrier from the barrier elements and allowing the liquid to fill the second and third chambers. The cover was reversibly bonded on the top of the apparatus and the pair of holes in the cover were positioned over the inlet of the second chamber. Through the larger hole of the cover, 0.8 micron beads in suspension were loaded into the apparatus. Movement of the beads was monitored by microscopy and recorded by a CCD camera. The beads were observed to slowly diffuse from the input region over the second chamber across the first chamber to the low density region, the third chamber. This behavior occurred only after removal of the removable barrier. The beads displayed movement only by Brownian motion. There was no evidence

of bead displacement by fluid flow within the apparatus, indicating the absence of gravity or other pressure driven flow.

EXAMPLE 3

The following experiment demonstrates that a gradient generated using the microfluidic apparatus can be used to induce chemotaxis. Rat-2 cells were adapted to CO_2-independent DMEM microscopy media (Sigma Aldrich) containing 5% fetal bovine serum (HyClone) for three days prior to the experiment. The apparatus and cover were made as described above in Example 1. Through either the inlet or outlet of the first chamber, 10 μg/ml fibronectin (BD Biosciences) was loaded into the first chamber and allowed to coat the glass within the chamber for one hour. The first chamber was then washed with PBS and a suspension of Rat-2 cells in microscopy media containing 5% serum was loaded into the first chamber. The cells were allowed to adhere to the surface for two hours in the presence of serum. After two hours the removable barrier was released from the barrier elements by a pressure differential caused by the addition of serum-free microscopy media into the first chamber. This caused the liquid to advance into both the second and third chambers. The cover was reversibly bonded on the top of the apparatus and the cells were serum-starved for five hours. After five hours, serum was added through the larger hole of the cover to a final concentration of 10%. The cells were imaged under the microscope and recorded with a CCD camera every five minutes for five hours. As shown in Figure 4, cells were imaged chemotaxing up a gradient of serum ranging from 0-10%.

EXAMPLE 4

Removal or formation of the removable barrier can be accomplished using a pulsed laser. The microfluidic apparatus was made as described above in Example 1. The first chamber was loaded with a liquid and the liquid was retained within the first chamber due to surface tension. This created a gas/liquid interface at the barrier zone between the first and second chamber and between the first and third chamber. A Nd:YAG pulsed laser (5 nanosecond pulse duration, 532 nm, Minilase II-20, New Wave Research, Sunnyvale, CA, USA) was focused by the microscope optics in the liquid

between a set of barrier elements. Pulsing of the laser formed a plasma in the liquid, which produced a cavitation bubble and shock wave of sufficient magnitude that the resultant local increase in pressure was able to overcome the surface tension resulting in the local removal of the barrier. As shown in Figure 5, the gas/liquid interface **20** can be seen. With the gas/liquid interface removed between one set of barrier elements, the liquid **21** advanced into the adjacent chamber displacing the gas phase **22** due to the hydrophilic nature of the glass slide on which the apparatus is attached. The advancing liquid merged with the liquid in the first chamber and removed the removable barrier along its length.

To reform the removable barrier, as shown in Figure 6, the pulsed laser was focused between a set of barrier elements **18** and continuously fired as above. A plasma was formed, which generated cavitation bubbles **30**. These bubbles merged into a bubble of adequate size to locally reform the removable barrier **31** between the set of barrier elements. This process was continued until the removable barrier was reformed between all barrier elements.

`The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A method for creating a removable barrier in a microfluidic apparatus comprising the steps of:

(a) providing a microfluidic apparatus having at least a first chamber and a second chamber, each including at least an inlet, wherein said first and second chamber are separated from one another by a plurality of barrier elements, said plurality of barrier elements defining a barrier zone,

(b) introducing a liquid sample into said apparatus through said inlet of said first or second chamber to create a retained continuous liquid phase in one chamber, a continuous gas phase in the other chamber, and a gas/liquid interface between said first and second chamber at said barrier zone,

(c) releasing said interface from said barrier elements, and

(d) advancing said liquid phase past said barrier zone into said gas chamber displacing said gas phase.

2. The method of claim 1, wherein said barrier elements are hydrophobic.

3. The method of claim 2, wherein said barrier elements comprise a hydrophobic polymer.

4. The method of claim 1, wherein said microfluidic apparatus comprises a first chamber including an inlet and outlet located at the distal ends of the first chamber, a second chamber including an inlet, and a third chamber including an inlet, wherein said first chamber is located in between said second and third chamber and said chambers are separated by a plurality of barrier elements.

5. The method of claim 1, wherein said gas/liquid interface is chemically or physically released from said barrier elements.

6. The method of claim 5, wherein said interface is released by:

(a) producing a laser generated shock wave,

(b) increasing the hydraulic pressure of the liquid phase,

(c) decreasing the atmospheric pressure of the gas phase,

(d) generating an electric field,

(e) heating the liquid phase,

(f) adding a surfactant,

(g) producing a magnetic field,

(h) generating an ultrasonic signal,

(i) disrupting a chemical interaction by cleaving a chemical bond,

(j) altering the ionic conditions of the liquid phase,

(k) changing the pH of the liquid phase,

(l) adding excess of a first member of a specific binding pair, or any combination thereof.

7. The method of claim 1 further comprising reforming said removable barrier.

8. The method of claim 7, wherein said barrier is reformed by focusing a pulsed laser beam between a set of said barrier elements and continuously firing said pulsed laser until a bubble of adequate size reforms said gas/liquid interface.

9. The method of claim 7, wherein said barrier is reformed by removing said liquid from said apparatus and refilling one chamber of said apparatus.

10. The method of claim 1, wherein said second chamber is open to atmospheric pressure.

11. The method of claim 1, wherein said method generates a concentration gradient by:

(a) introducing a first liquid sample into said apparatus through said inlet of said first chamber,

(b) releasing said interface from said barrier elements,

(c) advancing said liquid phase past said barrier zone and into said second chamber,

(d) introducing a second liquid sample into said apparatus through said inlet of said second chamber, and

(e) diffusing said second liquid sample across said apparatus.

12. The method of claim 5, wherein said method controls mixing of reagents by:

(a) introducing a first liquid sample into said apparatus through said inlet of said second chamber,

(b) introducing a second liquid sample into said apparatus through said inlet of said third chamber, and

(c) retaining said first liquid sample in said second chamber and said second liquid sample in said third chamber until time to mix reagents,

(d) releasing said interface from said barrier elements, and

(e) mixing said first and second liquid sample.

13. The method of claim 5, wherein said method separates two compartments from each other by:

(a) introducing a first liquid sample into said apparatus through said inlet of said second chamber,

(b) introducing a second liquid sample into said apparatus through said inlet of said third chamber, and

(c) retaining said first liquid sample in said second chamber and said second liquid sample in said third chamber.

14. A microfluidic apparatus comprising,

(a) a first chamber including an inlet and an outlet located at the distal ends of the chamber,

(b) a second chamber including an inlet,

(c) a third chamber including an inlet, and

(d) a plurality of barrier elements defining a barrier zone located between said first and second chamber and between said first and third chamber, wherein said chambers are equal in height and said second and third chambers are wider than said first chamber.

15. The microfluidic apparatus of claim 14, wherein either said second or third chamber is open to atmospheric pressure.

16. The device of claim 14, wherein said device is a chemotaxis device.

17. In a chemotaxis device for establishing a chemical gradient across a chamber to which cells are attracted or repelled, the improvement comprising:

providing means for establishing a removable gas barrier associated with said chamber through which said gradient can form.

18. The chemotaxis device of claim 17, wherein said means for establishing a removable gas barrier comprise a plurality of barrier elements operatively associated with said chamber.

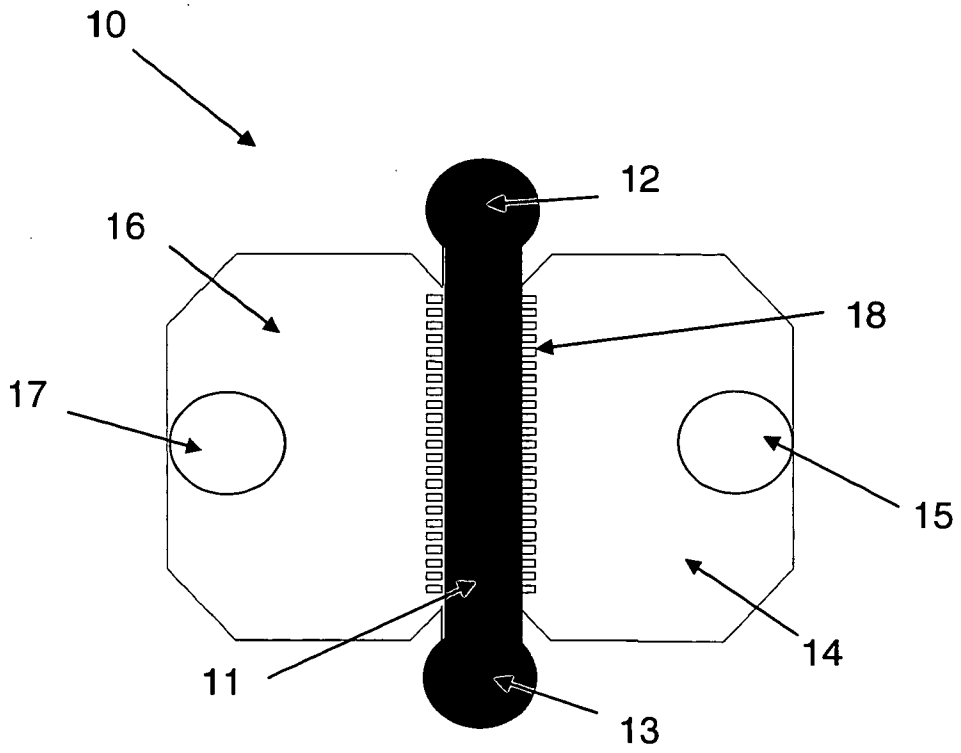

Figure 1

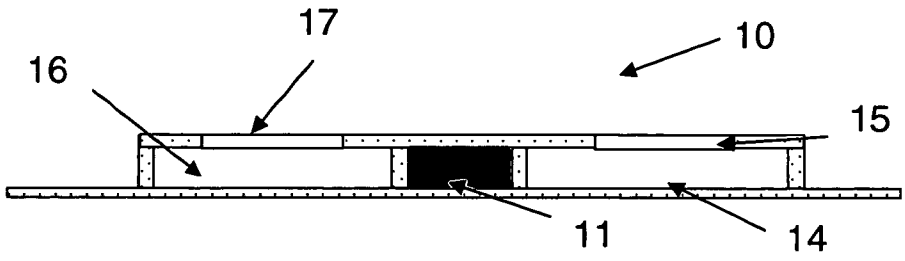

Figure 2

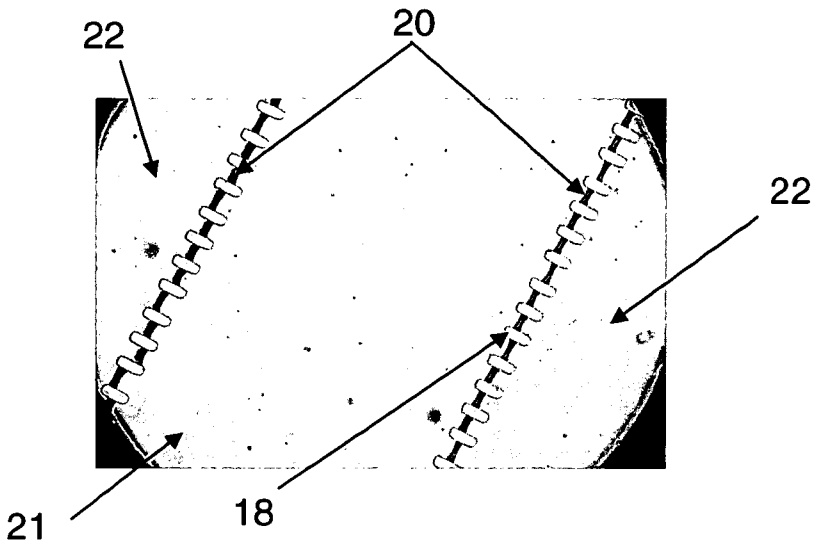

Figure 3

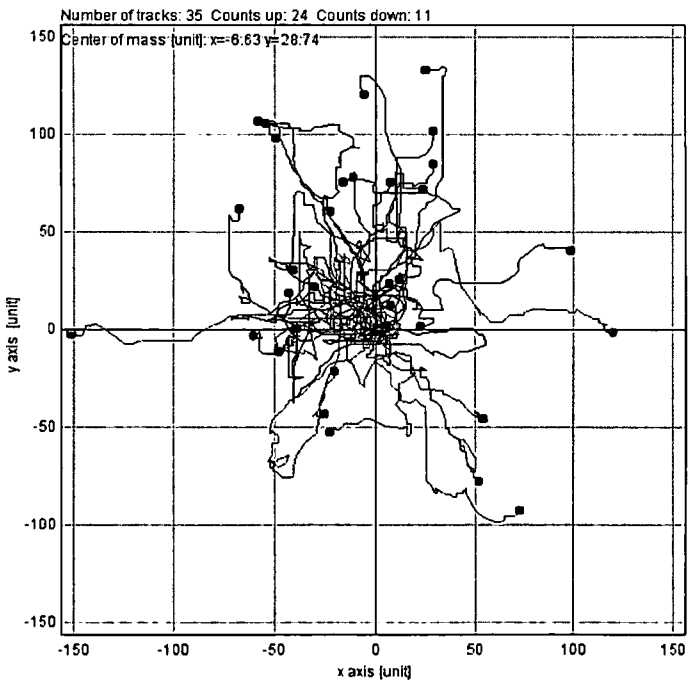

Figure 4

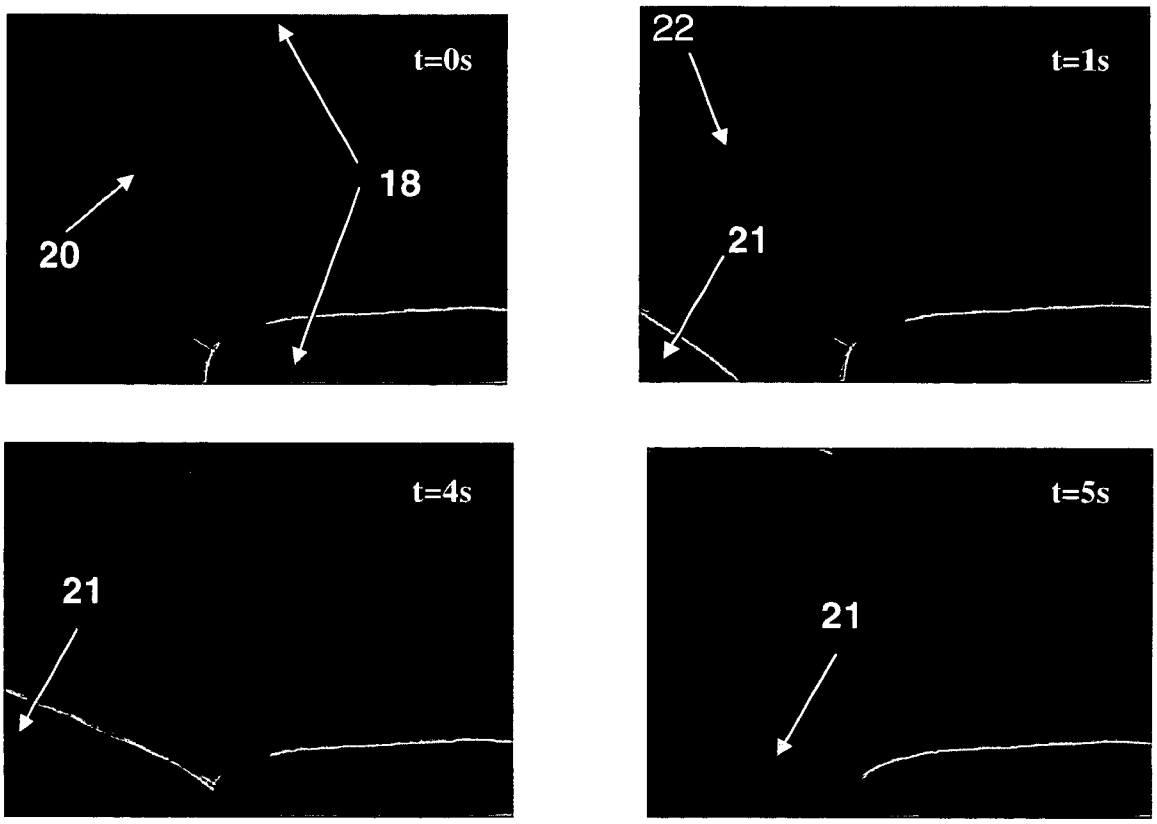

Figure 5

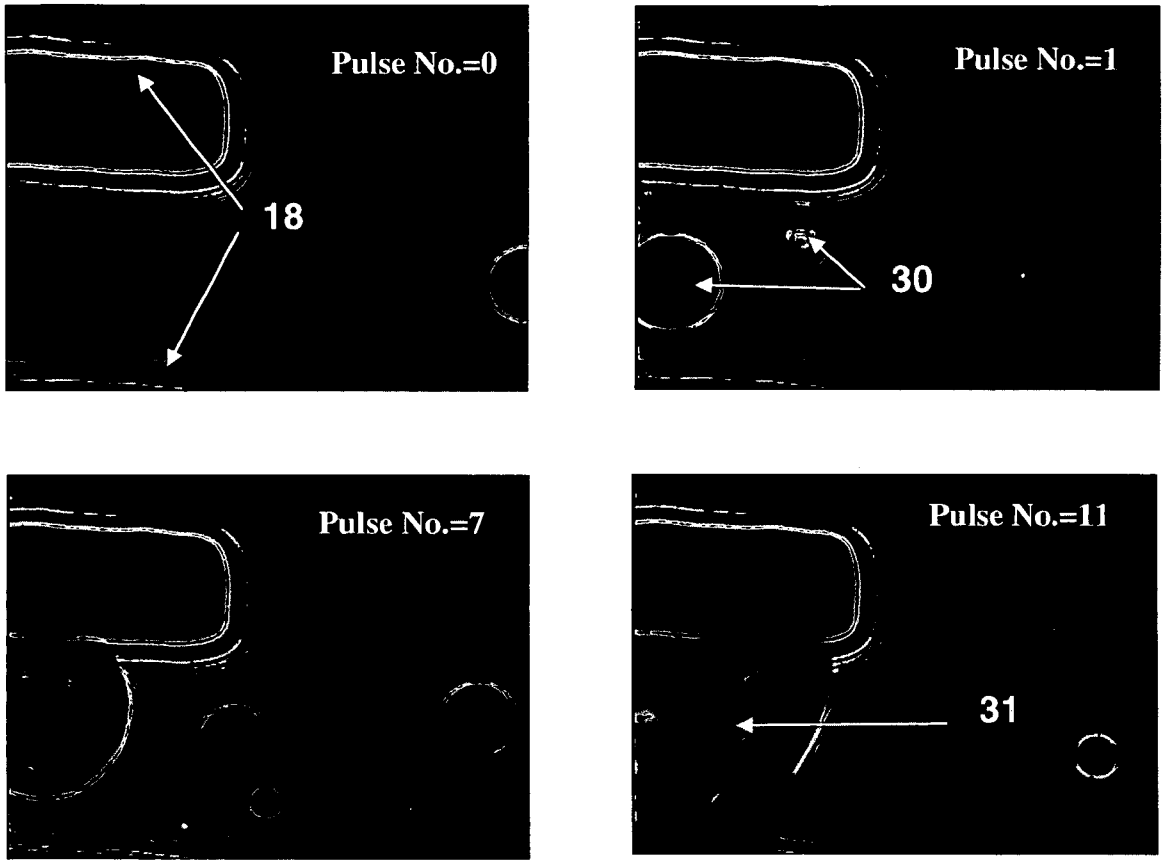

Figure 6