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(54) Title: MICROFLUIDIC DEVICE FOR HYDRODYNAMIC SORTING AND RHEOMETRY MEASUREMENTS OF PARTI-
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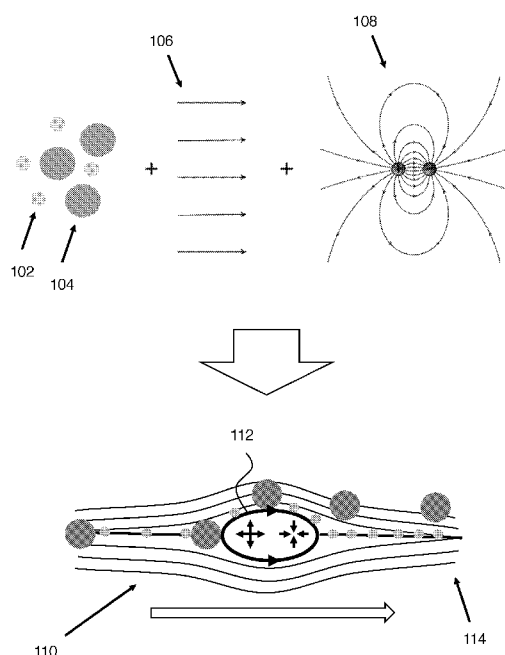


FIG. 1

(57) Abstract: Particle sorting/characterization is provided that has no solid obstacles and is therefore inherently clog-free. Instead of using obstacles/filters, one or more "virtual fluidic obstacles", which can be created by combinations of inlets and outlet flows (sources and sinks) are used to sort/characterize particles. Since particles and cells cannot cross the boundary of these virtual obstacles, they can be used for sorting. However, since there is no physical boundary (only a virtual or hydrodynamic one), the particles have no surface to adhere to and therefore clogging is avoided.



Microfluidic device for hydrodynamic sorting and rheometry measurements of particles

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FIELD OF THE INVENTION

This invention relates to particle sorting and particle rheometry in fluidic devices.

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BACKGROUND

Particles, cells and other biomaterials frequently adhere to obstacles and filter surfaces used for sorting and filtration. As a result, they clog up filters which have to be changed regularly. This clogging problem strongly limits their performance and reliability. Fluidic devices used for particle rheometry -- the study of stress-strain relations in particles -- are also affected by this clogging problem. Accordingly, it would be an advance in the art to provide intrinsically clog-free particle sorting and rheometry.

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SUMMARY

Particle sorting/characterization is provided that has no solid obstacles and is therefore inherently clog-free. Instead of using obstacles/filters, one or more "virtual fluidic obstacles", which can be created by combinations of inlets and outlet flows (sources and sinks), are used to

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sort/characterize particles. Since particles and cells cannot cross the boundary of these virtual obstacles (the electrical analogy is called a "doublet"), they can be used for sorting. However, since there is no physical boundary
5 (only a virtual or hydrodynamic one), the particles have no surface to adhere to and therefore clogging is avoided.

This approach is also tunable, which means that by changing the strength of the source and sink flows, the size of these obstacles is changed. This, in turn, can be
10 used to tune the cut-off size for example.

In addition to sorting, this approach can be used to measure the rheological properties, e.g., stiffness of the cells/particles/capsules etc. We can do this by using combinations of sources and sinks to create, e.g.,
15 extensional flows, pure shear flows, rotating flows and measure the cell deformation in these flows, typically by imaging. The deformations can also be measured while sorting, for example by imaging the deformation of a droplet in a shear flow around a virtual cylinder.

This approach is inherently clog-free. It is tunable (very modular). The flow field around a virtual cylinder (doublets) is known from potential theory, meaning that we can predict the particle trajectory by calculating the imposed hydrodynamic forces. For example, in contrast to
20 flow around physical cylinders which have no-slip boundaries, there are no boundary layers around our virtual cylinders, which eases the prediction of the particle trajectory and/or deformation.

Applications include diagnostics, algal harvesting and
30 other types of concentration/enrichment, rheological measurements (both interfacial and bulk rheology) and teaching/outreach activities.

More detailed consideration of a specific application is helpful to more specifically identify the kinds of problems this new approach can address, and the resulting advantages. Here we consider measuring the mechanical properties of individual corneal epithelial cells (size ~17 μm). The mechanical properties (such as elasticity) can be used to determine the health of a human eye.

Conventional approaches to measure the deformations of corneal cell monolayers tend to be unsuccessful because of the extreme elasticity of these cells.

By imaging the cell deformations as they flow around virtual fluidic obstacles as described above (we can vary the size of the virtual fluidic obstacles by altering the flow rates through the sources and sinks) we should be able to measure mechanical properties of individual cells. Here are some advantages of this approach.

1) Various flow configurations are possible, which can be utilized to measure multiple mechanical properties (elasticity, shear rigidity) in a single experiment.

2) Corneal cells live in a biological, sticky fluid that is also elastic. To describe the interactions between cells and the surrounding fluid, it is necessary to characterize the fluid properties as well as the cell properties. Recently, hydrodynamic quadrupoles have become popular to measure fluid properties (rheology). With the present approach, we can create quadrupoles which enables these important measurements.

3) We expect that this approach can be used to sort cells by size, which is also of interest because the mechanical properties can be size dependent. A commonly used technique for cell separation is called Deterministic Lateral Displacement (DLD), where cells are sorted by

laterally displacement using pillars. However, conventional DLD (and all other separation technologies that uses physical obstacles) are prone to clogging. Ultimately, we hope to create a *hydrodynamic* DLD, which is unconditionally clog-free because there are no physical obstacles. Instead, our device uses sources and sinks to create hydrodynamic obstacles which is drastically different from previous methods.

4) We also expect to be able to sort red blood cells (RBCs) by deformability (rigidity), which can be used for on-site detection of various diseases, including malaria. Since an infected RBC is stiffer than a healthy RBC, it has a reduced ability to follow fluid streamlines. The increased rigidity suggests that it can be sorted from healthy cells in a flow field with curved streamlines, such as around hydrodynamic obstacles.

5) No downstream processing required: all relevant parameters are measured *in situ*.

6) Easy to operate: Switching between different operational modes simply by adjusting flowrates, for example using a pneumatic system such as Fluigent, but syringe pumps can also be used to drive the flow.

7) One particularly compelling advantage of this approach is its great flexibility, somewhat analogous to electrical breadboards. Devices according to the present principles can have many ports in communication with the channel, which allows various virtual fluidic obstacle configurations to be set up by providing sources at some ports, providing sinks at other ports, and blocking the remaining ports.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows operating principles of an exemplary embodiment of the invention.

FIGs. 2A-B show exemplary embodiments of the
5 invention.

FIG. 2C shows images of exemplary virtual fluidic obstacles.

FIG. 2D shows another exemplary embodiment of the invention.

10 FIG. 3 is a side view showing one way to reduce source-sink spacing in a virtual fluidic obstacle.

FIGs. 4A-B show further exemplary embodiments of the invention.

15 DETAILED DESCRIPTION

FIG. 1 shows operating principles of an exemplary embodiment of the invention. In this example a mixture of small particles 102 and large particles 104 is placed into a fluid flow 106, which then flows past an arrangement of a
20 fluid source in proximity to a fluid sink that, in isolation, would provide a flow roughly as shown by 108. The combination of these effects will lead to a situation as shown on the bottom half of FIG. 1, where a virtual fluidic obstacle 112 is formed and the flow of particles
25 past virtual fluidic obstacle 112 is similar to what the flow would have been if the obstacle were of a solid material instead of being formed by a fluid flow. In this example, sorting of the particles by size downstream of virtual fluidic obstacle 112 is shown, as referenced by
30 114.

Accordingly, an embodiment of the invention is a method of controlling and/or measuring particles in a fluid flow. The method includes the steps of providing a channel for fluid flow, forming one or more virtual fluidic obstacles in the channel, and flowing a first fluid past the one or more virtual fluidic obstacles in the channel, where the first fluid includes particles. In contrast to some earlier work relating to virtual fluidic structures, here it is the fluid flow *outside* the virtual fluidic structures that is of interest, as opposed to fluid flow or other properties within the virtual fluidic structures.

The example of FIG. 1 relates to a case where the virtual fluidic obstacle is generated by a fluid source having input flow Q_{source} in proximity to a fluid sink having output flow Q_{sink} . All three logical cases are relevant in practice: $Q_{\text{source}} < Q_{\text{sink}}$, $Q_{\text{source}} > Q_{\text{sink}}$, and $Q_{\text{source}} = Q_{\text{sink}}$.

Here a virtual fluidic obstacle is defined as an obstacle to fluid flow provided by: 1) another fluid flow, 2) a gas bubble, or 3) a droplet of a fluid that is distinct from the flowing fluid. Such distinct fluid droplets can be miscible or immiscible with respect to the flowing fluid. In all cases, an obstacle to fluid flow is provided that is much less prone to clogging when particles are included in the fluid flow.

In most cases where a fluid droplet is used as the obstacle, it is preferred to use immiscible fluids. However, if a miscible droplet is very viscous compared to the ambient liquid flowing around it, and if the mutual diffusion coefficient is very small between the two liquids, the droplet will display immiscible behavior for long enough to serve as a virtual obstacle. That is, it will not mix with the ambient liquid on the time scale of a typical experiment and will be distinct from the ambient

liquid. Further details on the use of fluid droplets as virtual obstacles are provided below.

Virtual fluidic obstacles as described above have various applications. One application, shown on FIG. 1, is separating particles by size in fluid flow downstream of at least one of the virtual fluidic obstacles. A second application is measuring particle deformation in flow around at least one of the virtual fluidic obstacles. This can be used to provide information on the stress-strain relations of the particles (i.e., particle rheometry). A third application is measuring particle trajectories in flow around at least one of the virtual fluidic obstacles. This can be used to provide information about the particles (e.g., particle size etc.) and/or the fluid flow (e.g., measuring normal stresses in a viscoelastic fluid by measuring lateral particle propagation velocity, measuring fluid-fluid interface mobility by tracking particles at the boundary streamline etc.).

Practice of the invention does not depend critically on the type of particle being characterized. Suitable particle types include, but are not limited to: gas bubbles, droplets of a second fluid immiscible with the first fluid, droplets of a second fluid miscible with the first fluid, inorganic particles, biological cells, and organic particles.

FIGs. 2A-B show exemplary embodiments of the invention. FIG. 2A is a top view of a planar fluidic device having an inlet 202, channel 204, and outlet 206. As described above, a virtual fluidic obstacle 212 is formed in channel 204 by fluid flow from source 208 to sink 210. FIG. 2B shows an image of an exemplary fabricated device.

FIG. 2C shows images of exemplary virtual fluidic obstacles. These images relate to the device of FIG. 2B, and show the obstacle for various values of the ratio $Q_{\text{source}}/Q_{\text{in}}$, where Q_{source} is the flow rate out of source 208 and Q_{in} is the flow rate into channel 204 from inlet 202. In these examples, the flow rate into sink 210 (Q_{sink}), is less than Q_{source} . The resulting virtual fluidic obstacles tend to have the shape of a circle with a wedge missing.

FIG. 2D shows another exemplary embodiment of the invention. This example is similar to the example of FIG. 2A, except that here virtual fluid obstacle 228 is formed by two sources 224 and 222 and two sinks 220 and 226 arranged in a fluidic quadrupole configuration. Thus one or more of the virtual fluidic obstacles can be generated by two or more fluid sources in proximity to two or more fluid sinks. More generally, any number of sources and sinks can be used to generate the virtual fluidic obstacles.

For applications such as cell sorting by size, it is important to provide sufficiently small virtual fluidic obstacles. To do that, it is usually necessary to reduce the source-sink spacing. FIG. 3 is a side view showing one way to reduce source-sink spacing in a virtual fluidic obstacle. In this example, 302 is the channel, and 304 is the virtual fluidic obstacle formed by source 306 and sink 308. Here a two-layer top plate having layers 310 and 312 can be used to reduce source-sink spacing as shown. Layers 310 and 312 can be fabricated of any convenient material, such as PDMS (polydimethylsiloxane). In experiments, this approach has been used to provide a source-sink spacing of $100\text{ }\mu\text{m}$. This is substantially smaller than the $300\text{ }\mu\text{m}$ spacing shown on FIG. 2B.

FIGs. 4A-B show further exemplary embodiments of the invention. FIG. 4A shows an example where T-junctions formed by secondary inlets 402 and 404 are used to form droplets or bubbles of one liquid or gas in another liquid.

5 However, the droplets can be formed by other configurations including the coflow and the flow focusing techniques as known in the art. Thus practice of these embodiments of the invention does not depend critically on how the bubbles/droplets are formed. The droplet generator is
10 upstream of the virtual fluidic obstacle (separatrix). This functionality adds the capability of producing both single and double emulsions. The droplet or bubble size is a function of the flow rates of each fluid. The flow rates can be controlled by external pumps and can be tuned
15 to get the desired droplet or bubble size. The droplets or bubbles can be sorted downstream when they flow around the hydrodynamic obstacle. Also, by measuring the deformation of droplets around the obstacle, it is possible to extract rheological data of the fluids used.

20 In addition to separating particles, droplets, cells and bubbles by passing them around virtual fluidic obstacles generated by fluid flows, it is also possible to separate them by passing them around stagnant droplets or bubbles. These stagnant bubbles or droplets can easily be
25 produced by injecting a small amount of liquid into the channel from above by using a syringe and needle. The external flow surrounding a stagnant droplet creates an internal flow structure within the droplet. This flow structure generates a mobile interface which cannot be
30 clogged.

Accordingly, virtual fluidic obstacles as described above can be formed in various ways, including but not limited to: gas bubbles, droplets of a second fluid

immiscible with respect to the first fluid, and droplets of a second fluid miscible with respect to the first fluid.

CLAIMS

1. A method of controlling and/or measuring particles in a fluid flow, the method comprising:

5 providing a channel for fluid flow;

 forming one or more virtual fluidic obstacles in the channel; and

 flowing a first fluid past the one or more virtual fluidic obstacles in the channel, wherein the first fluid
10 includes particles.

2. The method of claim 1, wherein at least one of the virtual fluidic obstacles is generated by a fluid source having input flow Q_{source} in proximity to a fluid sink having
15 output flow Q_{sink} , wherein $Q_{\text{source}} < Q_{\text{sink}}$.

3. The method of claim 1, wherein at least one of the virtual fluidic obstacles is generated by a fluid source having input flow Q_{source} in proximity to a fluid sink having
20 output flow Q_{sink} , wherein $Q_{\text{source}} > Q_{\text{sink}}$.

4. The method of claim 1, wherein at least one of the virtual fluidic obstacles is generated by a fluid source having input flow Q_{source} in proximity to a fluid sink having
25 output flow Q_{sink} , wherein $Q_{\text{source}} = Q_{\text{sink}}$.

5. The method of claim 1, wherein at least one of the virtual fluidic obstacles is generated by two or more fluid sources in proximity to two or more fluid sinks.

6. The method of claim 1, wherein at least one of the virtual fluidic obstacles is selected from the group consisting of: gas bubbles, droplets of a second fluid immiscible with respect to the first fluid, droplets of a second fluid miscible with respect to the first fluid, and droplets of a second fluid miscible with respect to the first fluid.

7. The method of claim 1, wherein particles are separated by size in fluid flow downstream of at least one of the virtual fluidic obstacles.

8. The method of claim 1, further comprising measuring particle deformation in flow around at least one of the virtual fluidic obstacles.

9. The method of claim 1, further comprising measuring particle trajectories in flow around at least one of the virtual fluidic obstacles.

10. The method of claim 1, wherein the particles are selected from the group consisting of: gas bubbles, droplets of a second fluid immiscible with the first fluid, inorganic particles, biological cells, and organic particles.

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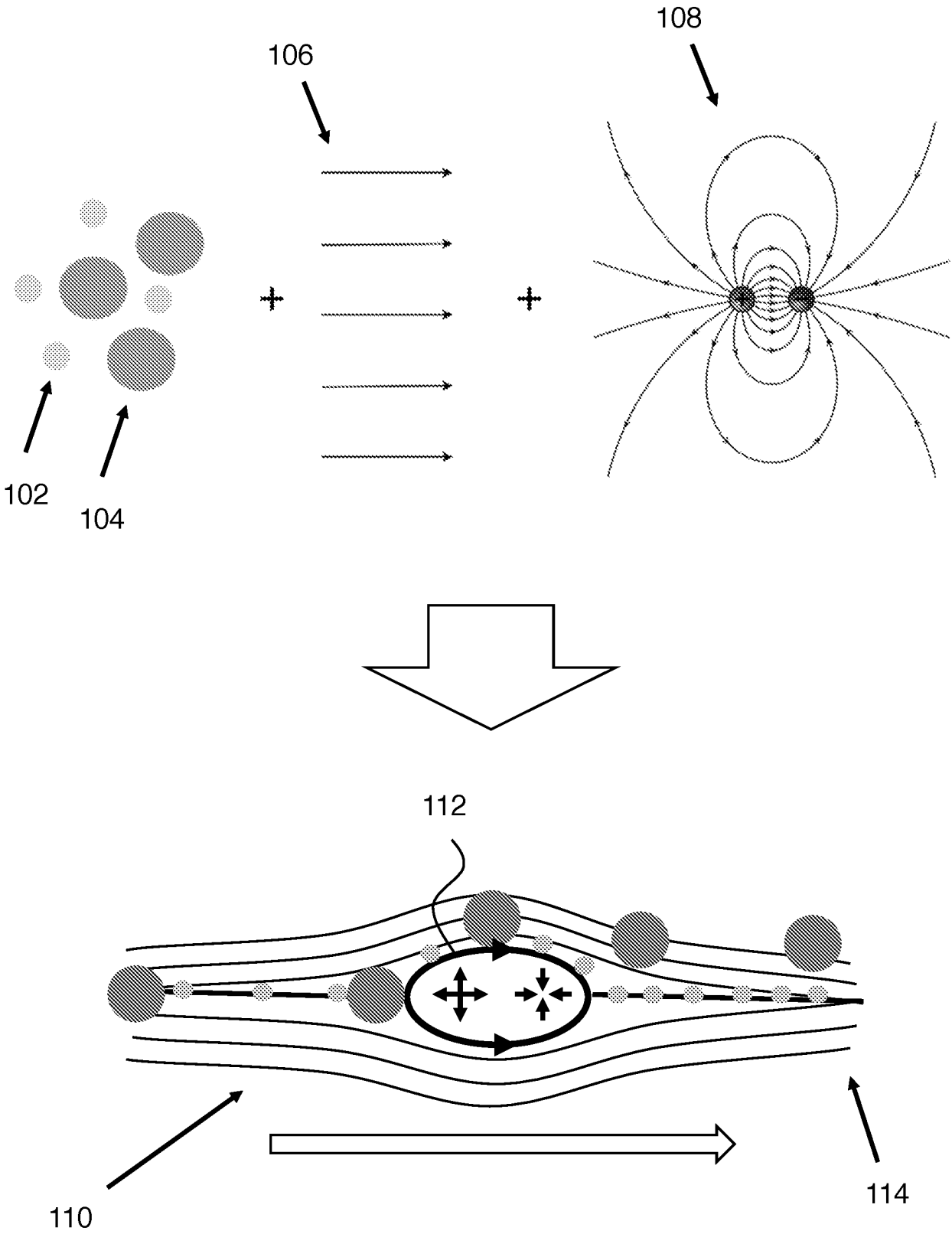


FIG. 1

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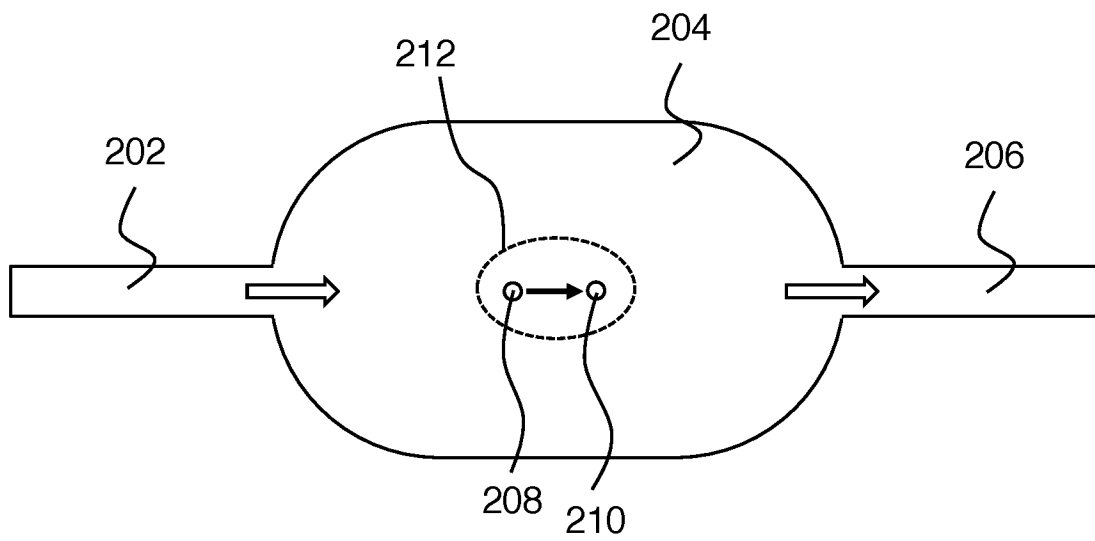


FIG. 2A

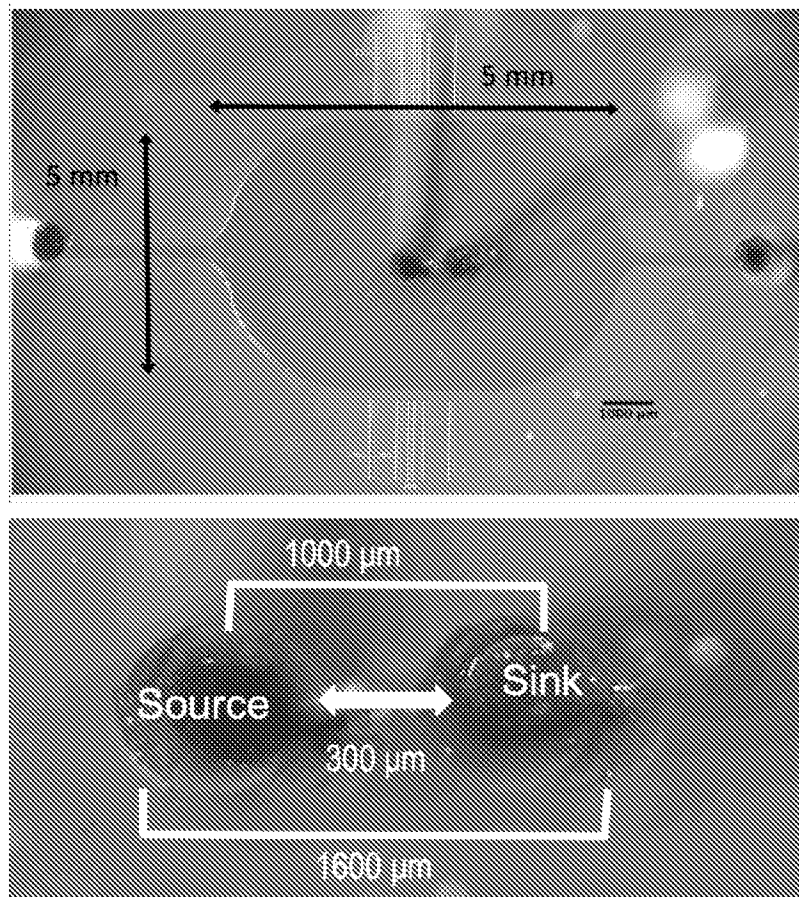


FIG. 2B

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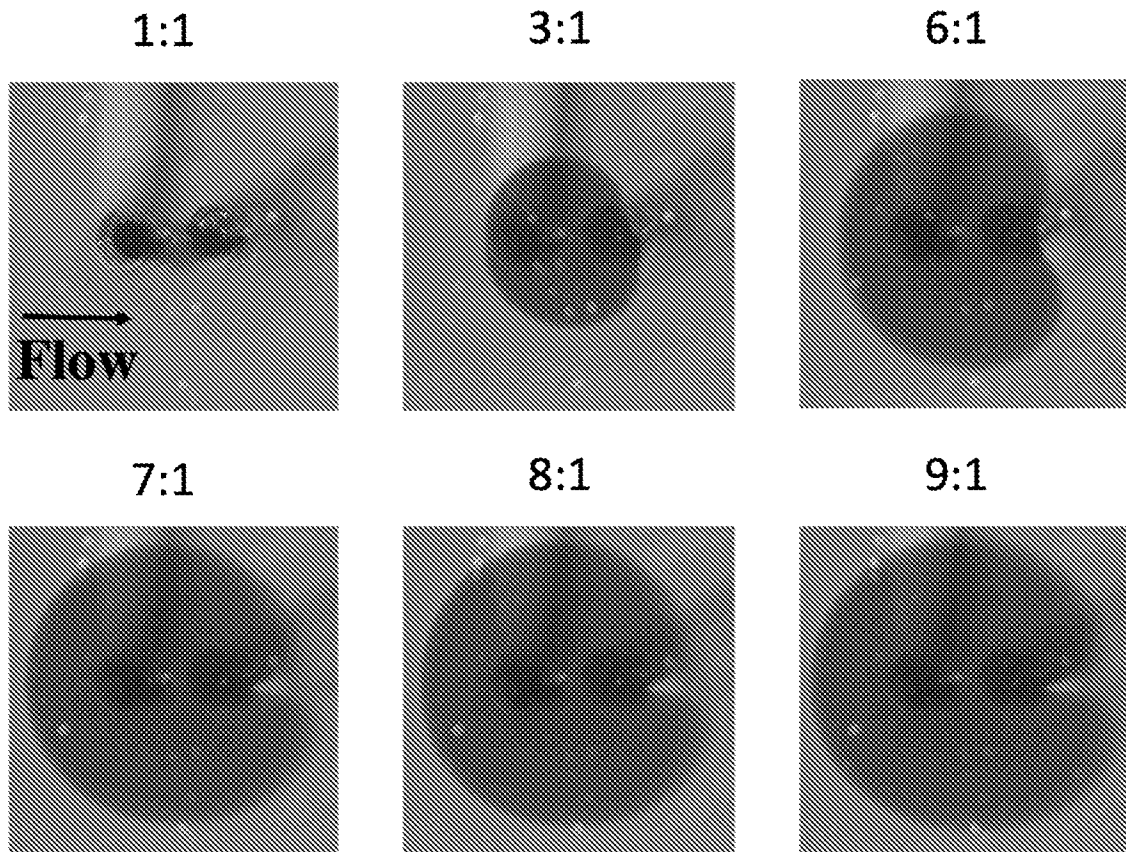


FIG. 2C

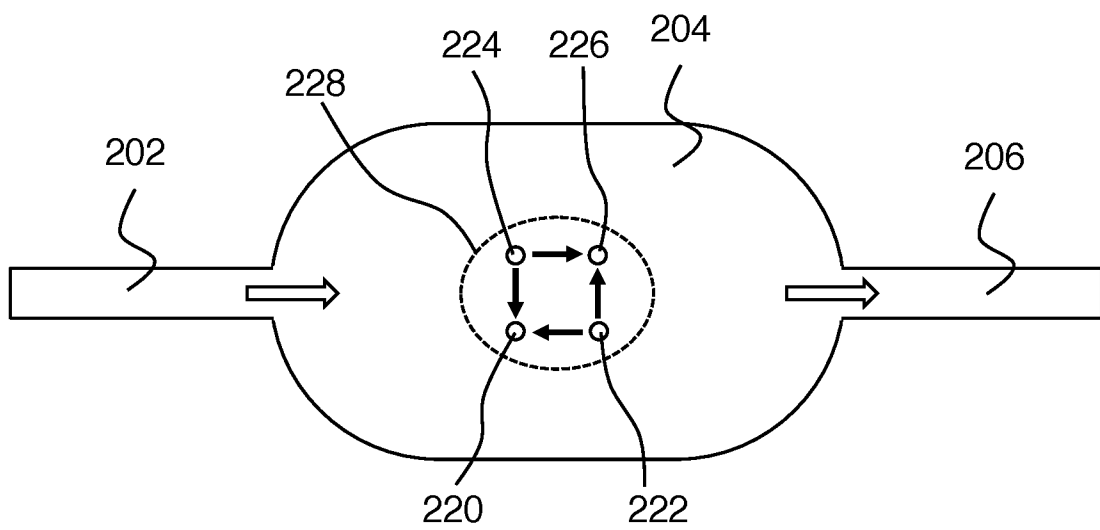


FIG. 2D

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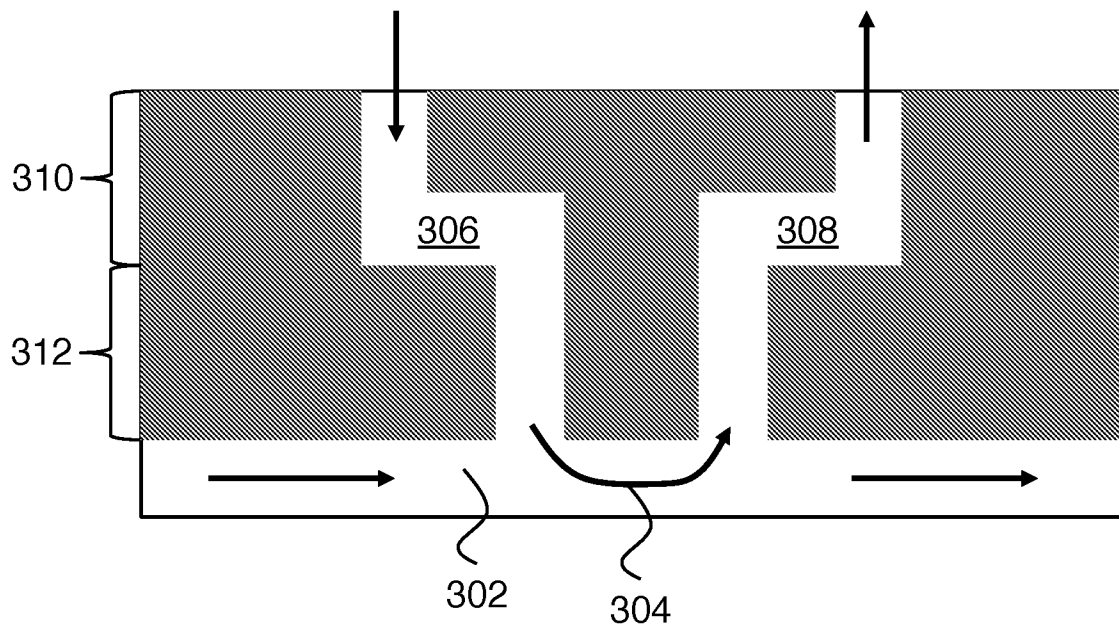


FIG. 3

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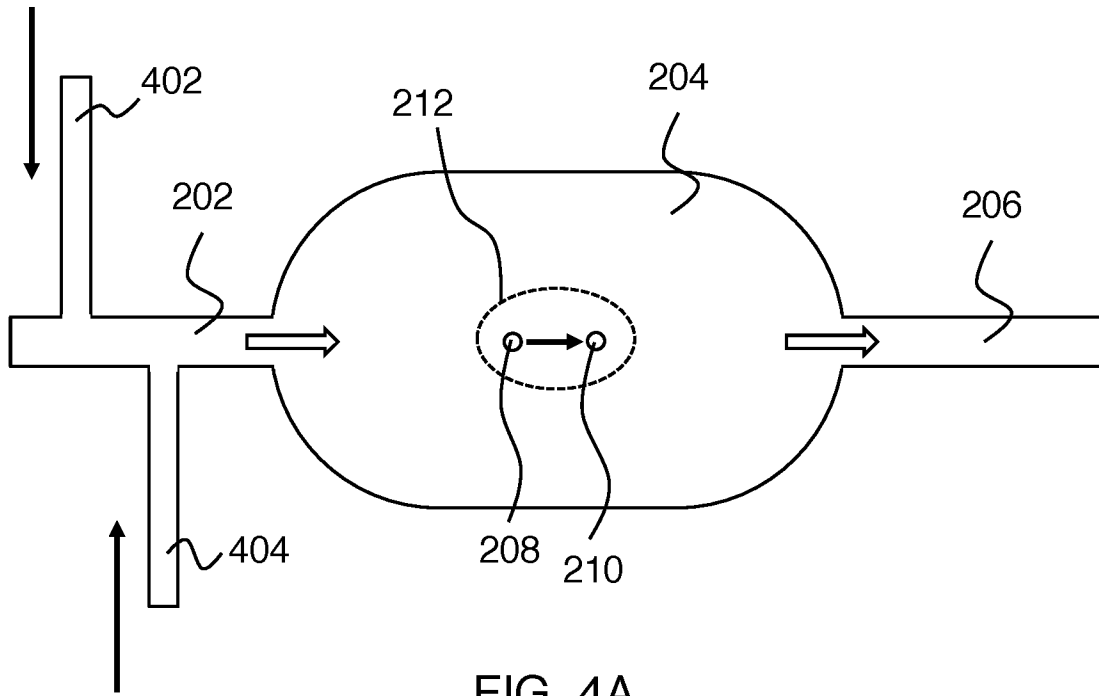


FIG. 4A

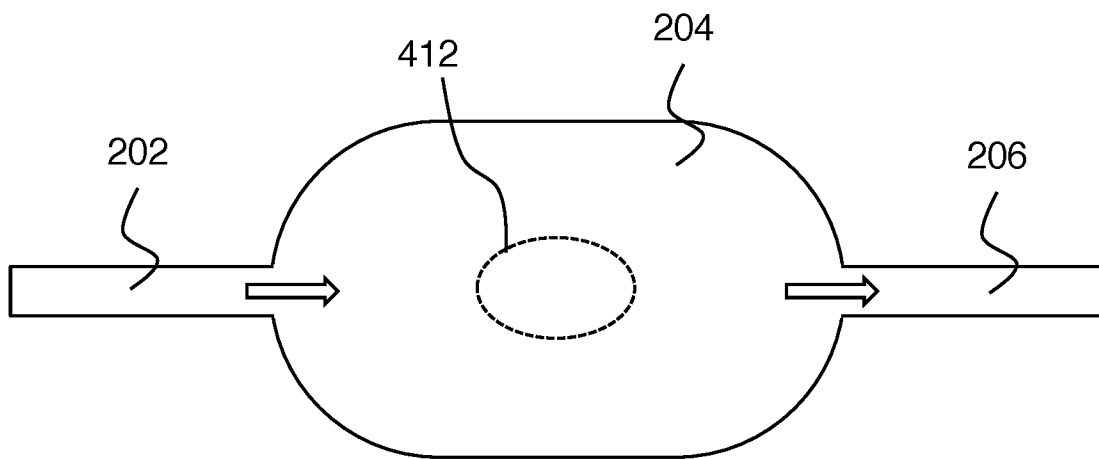


FIG. 4B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/21216

A. CLASSIFICATION OF SUBJECT MATTER

IPC - B01L 3/00; C12M 1/34 (2020.01)

CPC - B01L 3/5027; B01L 2200/0652; C12M 23/16

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- A	Pamme. "Continuous flow separations in microfluidic devices" Lap Chip, Vol 7 Issue 12 (02 Nov 2007); pages 1644-1659; entire document, but especially: abstract, page 1654 col 2 para 2, table 1, fig. 10	1, 7, 9-10 ----- 2-6, 8
A	US 2010/0234674 A1 (Wheeler et al.) 16 September 2010 (16.09.2010); para [0011], para [0047], para [0049], fig. 3	2-5
A	WO 2013/020965 A1 (SAW Instruments GmbH) 14 February 2013 (14.02.2013); para [11], para [17]	6
X	WO 2017/141048 A1 (Cambridge Enterprise Limited et al.) 24 August 2017 (24.08.2017); entire document	1, 10
X	US 2018/0264482 A1 (New Mexico Tech Research Foundation) 20 September 2018 (20.09.2018); entire document	1, 10
A	US 2016/0363523 A1 (Reichenach) 15 December 2016 (15.12.2016); entire document	1-10

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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06 May 2020

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