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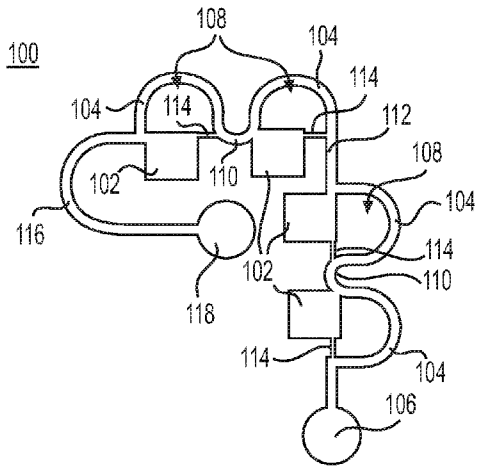

FIG. 1A

(57) Abstract: Described is a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors. The well-plate includes a plurality of fluidic traps, a plurality of hydrophilic capillary constriction channels and a plurality of bypass channels. Each of the plurality of bypass channels is associated with one of the plurality of fluidic traps, each of the plurality of hydrophilic capillary constriction channels is associated with one of the plurality of fluidic traps, and each of the plurality of fluidic traps is associated with one of the plurality of bypass channels and one of the plurality of hydrophilic capillary constriction channels. The well-plate further includes an inlet, an outlet, and a main channel with a plurality of portions that connects the inlet to the plurality of fluidic traps, associated hydrophilic capillary constriction channels and associated bypass channels, and the outlet.

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A MICROFLUIDIC SERIAL DILUTION PLATFORM BASED WELL-PLATE USING AN OIL-FREE IMMISCIBLE PHASE DRIVEN BY MANUAL OR ELECTRONIC PIPETTORS

RELATED APPLICATIONS

This application claims priority of U.S. Provisional Application Serial No. 62/107,132, entitled "A MICROFLUIDIC SERIAL DILUTION PLATFORM BASED WELL-PLATE USING AN OIL-FREE IMMISCIBLE PHASE DRIVEN BY MANUAL OR ELECTRONIC PIPETTORS," and herein incorporated by reference in its entirety.

BACKGROUND

Currently, microfluidic devices that can achieve storage of nano-pico liter volumes of droplets use either upstream mechanisms (such as T-junction or Y-junctions) or external force driven methods (electric, magnetic or acoustic). See, for example, the following published applications and patents US PGPUB 20070195127, WO 2010111231, US Patent No. 723826, US Patent No. 7708949, EP2364774, US Patent No. 8765485 and WO2006096571. Passive methods include the fragmentation of a long slug of fluid into droplets in a hydrophobic microfluidic network by using an immiscible phase (usually oil). In order to use this passive method, the current state of the art requires that the traps be pre-filled with the immiscible oil-phase. This creates a capillary plug in the constriction that immediately follows the trap to ensure that the droplets do not escape from the traps and to accomplish uniform droplet trapping. See, for example, the following published applications and patents: US Patent No. 8592221, CA2521862 and WO2012154688.

Once these droplets are trapped in these pre-defined sites, current methods used to achieve serial dilution of these stored droplets rely on coalescence between the diluting stream and the trapped droplets when these two components meet in the larger entrance channel of the trap. This causes the composition of the diluting plug to be altered which in turn can cause adverse reactions in this plug as it coalesces with droplets further downstream in the network.

There are a number of disadvantages of the above-described current techniques. For example, current techniques require precise fluidic control and additional external manifolds and controls. The immiscible oil phase (a contaminant in some processes) is required in the traps prior to droplet trapping to prevent drops from escaping from the traps. Additionally, the dilution mechanism of current methods can cause contamination of downstream traps in the network. Moreover, the non-uniform trapping of particles (*e.g.,* cells) in traps in methods that use the fragmentation of a long fluidic slug.

SUMMARY

Described herein are embodiments of a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors that overcomes the defects of the prior art. These and other advantages are achieved by a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors. The well-plate includes a plurality of fluidic traps, a plurality of hydrophilic capillary constriction channels and a plurality of bypass channels. Each of the plurality of bypass channels is associated with one of the plurality of fluidic traps, each of the plurality of hydrophilic capillary constriction channels is associated with one of the plurality of fluidic traps, and each of the plurality of fluidic traps is associated with one of the plurality of bypass channels and one of the plurality of hydrophilic capillary constriction channels. The well-plate further includes an inlet, an outlet, and a main channel with a plurality of portions that connects the inlet to the plurality of fluidic traps, associated hydrophilic capillary constriction channels and associated bypass channels, and the outlet.

These and other advantages are also achieved by a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors. The well-plate includes a plurality of fluidic traps, a plurality of hydrophobic capillary constriction channels, and a plurality of bypass channels. Each of the plurality of bypass channels is associated with one of the plurality of fluidic traps, each of the plurality of hydrophobic capillary constriction channels is associated with one of the plurality of fluidic traps, and each of the plurality of fluidic traps is associated with one of the plurality of bypass channels and one of the plurality of hydrophobic capillary constriction channels. The well-plate also includes an inlet, an outlet, and a main channel with a plurality of portions that connects the inlet to the plurality of fluidic traps, associated hydrophobic capillary constriction channels and associated bypass channels, and the outlet.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of a system and method for network data characterization and/or classification are understood and described in conjunction with the following drawings, wherein:

FIGS. 1A-1D are diagrams illustrating a hydrophilic embodiment of a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors.

FIG. 1E is a diagram illustrating an entire well-plate, with cover, that includes an embodiment of a microfluidic serial dilution platform using an oil-free immiscible phase driven by manual or electronic pipettors

FIGS. 2A-2C are diagrams illustrating a hydrophilic operation of a hydrophilic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors, particularly a fluid trapping process of the operation.

FIG. 3 is a series of images illustrating the fluid trapping process of an embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors.

FIGS. 4A-4D are diagrams illustrating a hydrophilic operation of a hydrophilic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors, particularly a serial dilution process of the operation.

FIG. 5 is a series of images illustrating the serial dilution process of an embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors.

FIGS. 6A-6B are diagrams illustrating a hydrophobic operation of a hydrophobic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors, particularly a serial dilution process of the operation.

FIGS. 7A-7C are graphs illustrating data from dilutions using a hydrophilic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors.

FIGS. 8A-8B are graphs illustrating data from dilutions using a hydrophobic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors.

FIG. 9A is a table illustrating results of an experiment using a hydrophobic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors as a hemocytometer.

FIG. 9B is a diagram illustrating a hydrophobic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors used a hemocytometer.

FIG. 9C is a chart illustrating advantages of hydrophobic embodiment of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors versus a standard hemocytometer.

FIGS. 10A and 10B are diagrams illustrating an embodiment of a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors with a cover on top of the well-plate to maintain humidity and control evaporation from the fluidic trap.

FIG. 10C is a graph illustrating average evaporation rates using the embodiment shown in FIGS. 10A and 10B

DETAILED DESCRIPTION

Described herein are embodiments of a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors. Embodiments overcome the problems described above. For example, embodiments provide a passive method based device for storage and serial dilution of fluids in a microfluidic storage network. Embodiments include a microfluidic platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors. Embodiments provide a novel mechanism for storage and serial dilution of droplets in a hydrophilic microfluidic device using conventional pipetting system.

Embodiments overcome the problems of the prior art. For example, by using an air-based immiscible phase for droplet trapping, embodiments eliminate the possibility of a reaction between typically-used oil-based immiscible phases and trapped fluid. Likewise, the novel serial dilution by the formation of micro-droplets in the network provided by embodiments prevents the composition of the diluting slug from being changed. Additionally, the elimination of precise fluidic control allows the storage and dilution of droplets in the network to be accomplished by conventional pipetting systems. The incorporation of these networks into a well-plate based device that integrates with multi-head robotic and manual pipettors eliminates the need for any additional capital equipment. The trapping and dilution is performed in a completely passive manner (without use of electric or magnetic fields), reducing the cost of the device. Embodiments provide a similar or higher throughput compared to currently available robotic high-throughput screening systems. Moreover, embodiments provide the ability to

remove fluids trapped in square hydrodynamic traps with a reversal of the direction of flow in one step without the formation of emulsions. Embodiments also enable uniform trapping of cells and other particles suspended in a fluid over the entire network of traps. This is particularly important in cell-based screening studies.

With reference now to FIGS. 1A-D, shown are four hydrophilic embodiments of a microfluidic serial dilution platform based well-plate **100** using an oil-free immiscible phase driven by manual or electronic pipettors. Each embodiment of well-plate **100** includes a network of one or more fluidic traps **102**. The drawings shown in FIGS. 1A-1C illustrate only a portion a well-plate and the network of fluidic traps **102** on the well-plate. FIG. 1A shows an embodiment of well-plate **100** with a parallel network of four (4) traps **102** with associated bypass channels **104**. In an actual implementation, the well-plate with such network may include multiple repeating networks configured as such. FIG. 1B shows an embodiment of well-plate **100** with a series network of four (4) traps **102** with associated bypass channels **104**. In an actual implementation, the well-plate with such network may include multiple repeating networks configured as such. FIG. 1C shows an embodiment of well-plate **100** with a single trap **102** with associated bypass channels **104**. In an actual implementation, the well-plate may include repeating single trap networks. FIG. 1D shows an embodiment with 176 traps **102** with associated bypass channels **104** connected in a series network. This last embodiment illustrates that the number of traps can be chosen and configured to meet requirements based on the application. The microfluidic serial dilution platform based well-plate designs shown herein may be situated on a chip or other suitable substrate. The well-plates may be made from, for example, poly dimethyl siloxane (PDMS), cyclic olefin copolymer (COC), poly carbonate (PC), or similar materials.

With continuing reference to FIG. 1A, an embodiment includes a fluidic inlet channel **106** connected to traps **102** and associated bypass channels **104** (each trap **102** and bypass channel **104** together forming a well **108**) in parallel arrangement. Fluidic inlet channel **106** includes an interface for pipettor, pipette or other fluid driving mechanism. The embodiment shown further includes main channel **110**, including straight channel portion **112**, connecting individual wells **108** together in parallel circuit as shown, and hydrophilic capillary constriction channels **114** with, *e.g.*, 40 μm width (small constriction inlet) connecting main channel **110** directly to traps **102**. In embodiments, square fluidic traps **102** have, *e.g.*, 1 mm x 1 mm sides and bypass channels **104** have, *e.g.*, 200 μm width. In embodiments, main channel extension **116** connects traps **102** to fluidic outlet **118**. Each trap **102** is an enclosed chamber with openings where main channel **110** and hydrophilic capillary constriction channels **114** connect with trap **102**. Furthermore, while the traps **102** shown in FIGS. 1A-1D are square traps,

embodiments may include any variety of shaped traps, including circular or semi-circular traps. Not shown in FIGS. 1A-1D are covers that cover the entire well-plate and the network of fluidic traps **102** on the well-plate (see FIG. 10 for an example of a cover). The cover encloses each trap **102**, as well as the various channels, creating the enclosed chamber. In a hydrophilic embodiment, such as describe here with reference to FIGS. 1A-1D the cover is also hydrophilic in nature.

With reference again to FIG. 1B, an embodiment includes fluidic inlet channel **106** with an interface for pipettor, pipette or other fluid driving mechanism, straight channel portion **112** of main channel **110** extending from inlet **106** and, with rest of main channel **110**, connecting inlet **106** to fluidic traps **102** and associated bypass channels **104** (together forming a well **108**) in a series circuit with each other, and hydrophilic capillary constriction channels **114** with, *e.g.*, 40 μm width (small constriction inlet), connecting main channel **110** directly to traps **102.** Bypass channels **104** may be fabricated with a, *e.g.*, 200 μm width, and fluidic traps **102** may be fabricated as square, *e.g.*, 1 mm x 1 mm, fluidic traps **102**. Embodiment may include enlarged main channel portions **115** to optimize a reduction of air invading into the fluidic trap **102** and main channel extension **116** connecting traps **102** to fluidic outlet **118**.

With reference again to FIG. 1C, shown is an embodiment with a fluidic inlet channel **106** with an interface for pipettor, pipette or other fluid driving mechanism, main channel **110** connecting fluidic trap **102** to inlet, hydrophilic capillary constriction channel **114** with, *e.g.*, 40 μm width (small constriction inlet), connecting main channel **110** to trap **102**, enlarged channel **115** to minimize invasion of air into the fluidic trap **102**, and main channel **110** connecting well or trap **102** to fluidic outlet **118**.

With reference again to FIG. 1D, shown is 176 fluidic traps **102** connected in series from fluidic inlet **106** to fluidic outlet **118**. As above, each trap **102** and bypass channel **104** together form well **108** and adjacent trap **102** and bypass channel **104** combinations are connected by main channel **110** and various portions or extensions thereof.

With reference now to FIG. 1E, shown is an entire well-plate **100** with a uniform network of ninety-six fluidic traps. Visible is cover **180**, fluidic inlets **106** and fluidic outlets **118** for each fluidic trap network. Fluidic inlets **106** and fluidic outlets **118** extend through cover **180**.

With reference now to FIGS. 2A-2C shown are schematic diagrams illustrating a fluid trapping process using embodiments of a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors. Shown in FIG. 2A, fluid **200** first enters the bypass channel **204**, through main channel **210** from direction of fluidic inlet (not shown), enhancing or increasing the hydrodynamic resistance in the bypass channel

204, and stops at the larger constriction of the main channel **210** and unfilled fluidic square trap **202**. Also shown in FIG. 2A is unfilled hydrophilic capillary constriction channel **214**.

With reference now to FIG. 2B, fluid **200** is shown now filling the fluidic square trap **202** through the smaller constriction (hydrophilic capillary constriction channel **214**) connected upstream to square trap **202**. Fluid **200** in bypass channel **204** now stops at mouth of the trap **202**.

With reference now to FIG. 2C, air **220** is then passed through the network of fluidic traps **202**. The air **220** passes through bypass channel **204**, removing remaining excess fluid **200** from the bypass channel **204** into main channel **210** and leaving a fragmented droplet of fluid **200** trapped in the hydrodynamic trap **202**. Air **220** will continue to pass through main channel **210** to next well (not shown), removing excess fluid **200** from bypass channel of next well, and so on through network of traps **202** towards fluidic outlet (now shown).

With reference now to FIG. 3, shown are a series of images illustrating the trapping process described above and illustrated in FIGS. 2A-2C. With reference to FIG. 3, shown are a series of micrograph images 1-6 of the series of events that leads to the fragmentation of the long fluid slug and the trapping of fluid in the square trap. Pipettor **330** connected to fluidic inlet channel (*e.g.*, see fluidic inlet channel **106** in FIGS. 1A-D) with an interface for pipettor **330**, pipette or other fluid driving mechanism is shown in these images.

With reference now to FIGS. 4A-D, shown are schematic diagrams illustrating a serial dilution process using an embodiment of microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors. Steps shown in FIGS. 4A-4D take place after the fluid trapping process described above. Together, the fluid trapping process and serial dilution process, shown in FIGS. 2A-2C and 4A-4D, comprise a significant portion of the operation of the microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors.

With reference to FIG. 4A, diluting stream or fluid (white) **425** first displaces air **420** and enters the main channel **410**, then enters the constriction (hydrophilic capillary constriction channel) **414** upstream of the network, invading a part of a first fluid-filled trap **402A**, diluting the fluid **400** in the trap **402**. Fluid **400** is ejected from the trap **402A** into the main channel **410** due to the displacement of fluid **400** from the trap **402A** by the diluting fluid **425**. The portion of fluid **400** that exited the first trap **402A** now enters the second trap **402B** and causes a portion of fluid **400** to leave the second trap **402B**.

With reference to FIG. 4B, shown is trap **402A** filled with diluted fluid **400'**, ejected fluid **400** in main channel **410**, ejected fluid **400** from first trap **402A** that has entered second trap **402B**, connected in series, through hydrophilic capillary constriction channel **414** of second

trap **402B**, and fluid ejected from second trap **402B** into main channel **410**. As an increasing amount of diluting fluid **425** enters the upper section of the first trap **402A** a portion of the diluting stream **425** now enters the second trap **402B** and a series of micro-droplets are formed that enter successive traps causing a serial dilution.

With reference to FIG. 4C, shown is diluted fluid **400'** in first trap **402A**, diluting fluid **425** filling upper section of first trap **402A** and entering second trap **402B**, diluting fluid **425** filing capillary section **414** of the second trap **402B**, and as air is injected into device following the diluting plug, causing fluidic droplets **435** in air phase to form. As air is pumped into the channel **410** following the diluting stream, excess diluting fluid **425** is carried away from the channel into the outlet.

With reference to FIG. 4D, shown is air-filled bypass channel **404** as air **420** is driven into bypass channels **404** after the diluting plug, the diluted fluid **400'** in first trap **402A**, the diluted fluid **400'** in second trap **402B** and main channel **410** with excess fluid **425** flushed out with air phase.

With reference now to FIG. 5 shown are a series of images illustrating the serial dilution process described above with reference FIGS. 4A-4D. Shown in FIG. 5, are five (5) micrograph images of the series of events that leads to serial dilution of the trapped droplets. In image 2, ejected fluid from first trap enters **1** second trap connected in series, bypass channel **2** is filled with air, and fluid is ejected **3** from second trap. In image 3, diluting fluid enters **4** capillary section of first trap, fluid in first trap **5** is now diluted, ejected fluid from second trap forms a fluid drop **6** in air phase. In image 4, diluting fluid fills upper section of first trap 7 and is subsequently ejected, diluting fluid fills upper section of second trap **8** and is ejected from second trap into main channel and ejected micro-droplet **9** from second trap is shown in main channel. In image 5, first trap **10** has the highest amount of diluting fluid, second trap **11** has a comparatively lower amount of diluting fluid compared to first trap and a third trap **12** has the lowest amount of diluting fluid compared to first trap and second trap.

Embodiments enable dilution of different magnitudes carried out in a trapping network. For example, embodiments enable cell cultures to be carried out in the traps, such as breast cancer cells cultures in a matrigel environment in the traps. Embodiments provide a new method for the trapping and dilution of nano to pico liter droplets stored in microfluidic networks. By using a novel design and employing hydrophilic square channels the fluid is pumped in through capillary action and the applied pressure drop of a pipettor. The fluid is driven into the channel in the direction where the smaller constriction of the trap is upstream (as opposed to the conventional system where fluid enters the trap through the larger constriction). The fluidic slug then fills the entire trap by flowing through this constriction (see FIGS. 2 and

3). Consecutive traps in the network are also filled in this manner. Air is then used as the immiscible phase to fragment the long fluidic slug and remove excess fluid contained in the bypass channels.

Consequently, the method utilized by embodiments described herein do not require an immiscible oil phase which can cause adverse results in some applications, although if required an immiscible oil phase can also be used to fragment the fluidic slug in the trap by flowing it through the network at a lower pre-defined flow rate.

Embodiments of the method utilized by embodiments described herein allows for the slug to be trapped and fragmented using larger pressure drops (which are typical for off-the shelf pipetting systems) compared to currently available solutions that require optimization of flow rates or additional external pumping systems (such as syringe pumps).

Embodiments also dilute fluids stored in the traps using a novel dilution method that produces fluidic drops in-air, in-situ in the device. This method with optimization can prevent cross-contamination between compositions of fluids in the various stationary traps. Embodiments of the device described herein can also be used for the three-dimensional culture of cells in the hydrodynamic traps using an appropriate polymer matrix. These cultured cells can then be serially diluted and screened against drugs in an HTS fashion.

In embodiments described herein, a hydrophilic channel microfluidic based network with square (or other shaped) storage traps (see FIGS. 1A-1D) to store and dilute nano-liter droplets. These droplets are produced in-situ in these square traps by flowing a fluidic slug through the fluidic network in a direction where with the smaller capillary junction is upstream of the large entrance channel at the traps. This injection of fluid into the channels is achieved at high flow rates using a commercially available pipetting system. This is followed by fragmenting the long slug of fluid using an immiscible air-phase that removes excess fluid from the channels causing nano to pico liter droplets of fluid to be contained in the square traps (see FIGS. 2 and 3). The need for an immiscible oil-phase is, therefore, completely eliminated.

Embodiments of the method for dilution causes the diluting stream to directly enter into part of the square (or other shaped) trap (amount can be varied based on volume, size of trap and flow rate of the diluting stream) (see FIGS. 4 and 5).

The injection of the diluting stream into the trap causes part of the reagent in the trap to be displaced, which in turn causes the formation of a fluid droplet in the air filled channels (see FIG. 4). This droplet then enters the second trap (where composition is the same as first trap) and causes another fluid-air drop to be produced (see FIG. 5). This mechanism, therefore, can produce serial dilutions without causing contamination of the diluting stream or of the reagents in the traps downstream. Both the trapping and serial dilution of the droplets in the device are

performed using conventional pipetting systems without the need for precise fluid control or lower flow rates.

In another embodiment, only the cover of the microfluidic structure is chosen to be hydrophilic in nature. With reference now to FIGS. 6A-6B, shown are schematic diagrams that illustrate trapping using this such an embodiment. These diagrams again illustrate only a portion of a well-plate and a portion of the network of traps on the well-plate. The fluid is first trapped by injecting it into the channel towards the larger mouth of the hydrodynamic trap that is filled initially with air. The channels are hydrophobic while the base cover used to enclose these channels is hydrophilic in nature. The fluidic slug fills the trap while not entering the hydrophobic capillary section that has a larger hydrodynamic resistance than the fluidic trap and is filled with air. This capillary section filled with air, therefore, acting as an air valve. Once the trap is completely filled, the hydrodynamic resistance of the trap is enhanced and excess fluid from the trap then moves into the bypass channel. This fluid subsequently enters other traps in the network and fills them in the same method as described previously.

The fluids that are filled in these traps can then be diluted by coalescence between the diluting and trapped fluid. See for example US Patent No. WO2012154688 A2. This is depicted in FIG. 6B.

With reference again to FIG. 6A, shown is an outlet for fluid **618**, fluid filled first hydrodynamic trap **602**, bypass channel of fluidic network **604**, capillary section of hydrodynamic trap filled with air **614**, inlet for fluid driven by pipette or other fluid driving mechanism **606**, structure of the fluidic network that is hydrophobic in nature and base **650** of the fluidic network, that is used to cover the hydrophobic structure, which is hydrophilic in nature.

With reference again to FIG. 6B, shown is an outlet for fluid **618**, diluted filled first hydrodynamic trap **602**, diluting fluid injected from the inlet **606**, diluting fluid coalescing with fluid trapped in hydrodynamic trap **602**, inlet for fluid driven by pipette or other fluid driving mechanism **606**, structure of the fluidic network that is hydrophobic in nature and hydrophillic base **650** of the fluidic network that is used to cover the microfluidic hydrophobic structure. Note, the various features described herein with reference to the hydrophilic embodiments (see FIGS. 1A-5, 10A-10B) may be incorporated into the hydrophobic embodiment described in FIGS. 6A-6B.

With reference now to FIGS. 7A-7C, shown are graphs illustrating data from dilutions carried out in the hydrophilic design of FIG. 1C using a Matrix™ sixteen (16) channel electronic pipettor from Thermofisher Scientific. The amount of dilution in the microfluidic chambers can be varied by varying either: (a) the volume of the diluting fluid stream, with the results shown in

FIG. 7A; (b) the number of diluting plugs, with the results shown in FIG. 7B and 7C. As can be seen from the graphs, the co-efficient of variation (CV) of the dilution is typically ≤5%.

With reference now to FIGS. 8A-8B, shown are graphs illustrating data from dilutions carried out in a hydrophobic design of FIG. 1C using a Matrix™ sixteen (16) channel electronic pipettor from Thermofisher Scientific. The graph in FIG. 8A illustrates the varying amount of dilutions that can be accomplished using varying volumes of diluting plugs and varying numbers of such diluting plugs. The graph in FIG. 8B illustrates the varying amounts of dilutions that can be accomplished using varying volumes of diluting plugs.

With reference to FIGS. 9A-9C, tables and diagrams are shown illustrating the embodiments of a microfluidic serial dilution platform based well-plate that may also be used as a hemocytometer. The embodiment shown is a hydrophobic design (*e.g.*, see FIGS. 6A-6B). With reference to FIG. 9A, a table is shown illustrating cell counts made with a standard hemocytometer as well as an embodiment of a microfluidic serial dilution platform based well-plate. The table illustrates two cell counting experiments: (1) an experiment performed with a standard hemocytometer diluted with trypan blue, a dye used to determine between live/dead cells, and a microfluidic serial dilution platform based well-plate without dilution by trypan blue and (2) an experiment performed with a standard hemocytometer diluted with trypan blue and a microfluidic serial dilution platform based well-plate with dilution by trypan blue. The cell counting formula used is a common protocol associated when using a hemocytometer, where the cell count is multiplied by both the dilution factor and the conversion factor. With reference to FIG. 9B, a hydrophobic implementation of a microfluidic serial dilution platform based well-plate **900** with traps gridded into quadrants each for cell counting. A depiction of a standard gridded hemocytometer 990 is also shown.

A cell suspension may be loaded using either a manual or electronic pipette into a gridded microfluidic trap. The cells within the traps were counted and used to calculate the total population. In the first experiment, the cell count using a standard hemocytometer with trypan blue took six minutes and twenty-two seconds (6:22) and produced counts of 180, 182, 225, and 83 totaling to 670. Applying the conversion factor, the count became $670e^4$ which is the same as $6.7x10^6$ cells/ml. Including the entire suspension volume, the total count from the standard gridded hemocytometer resulted in $20.1x10^6$ total cells. The count using an implementation of microfluidic serial dilution platform based well-plate **900** with traps gridded into quadrants took only thirty seconds (0:30). Within six traps, the quadrants totaled to 681, 659, 667, 610, 641, and 734, producing an average of 665 cells/0.1 µl, which converts to 6653 cells/µl, equaling $6.65x10^6$ cells/ml. With the same suspension volume, this method calculated to a total of

$19.9x10^6$ cells. The results for the second experiment were obtained using the same methods and produced values that were equally close.

A comparison of results obtained from the implementation of a microfluidic serial dilution platform based well-plate and those obtained using a conventional hemocytometer shows reasonably similar cell counts. The microfluidic serial dilution platform based well-plate shows excellent reproducibility and presents further advantages over conventionally used hemocytometers one such being the time required, as illustrated in FIG. 9C.

Experiments with embodiments described herein demonstrate that microfluidic serial dilution platform based well-plate may perform various assays completely on-chip (*i.e.*, on a chip or other suitable substrate containing (how many traps within a given chip. For example, experiments showed Clenbuterol ELISA assays may be performed completely on-chip using an electronic pipette. In an experiment, the varying degrees of dilution of the sample using the buffer and substrate solutions were accomplished using the graphs for dilution presented in FIGS. 7A-7C and FIGS. 8A-8B. Microscopic images of the experiments demonstrated that the samples with most amount of drug had no coloration where samples with the least amount of drug below the threshold value have higher values of coloration as expected. Advantages of using ELISA-based assays with embodiments of the present invention include: (a) reduced time to conduct tests (nearly one-third the time compared to conventional methods; (b) higher sensitivity due to an increased surface to volume ratio; and (c) increased accuracy due to a conserved volume that does not change.

With reference now to FIGS. 10A-C, shown is an embodiment of microfluidic serial dilution platform based well-plate that controls the evaporation of fluid from the fluidic traps. If evaporation of fluid from the traps needs to be controlled as is the case when performing cell based assays the device is configured as shown. With reference to FIG. 10A, shown is a top view of this configuration which includes a hole **1100** drilled into each trap **1002** of the microfluidic network (specifically, hole **1100** is drilled through cover enclosing trap **1002**). In this manner, trap **1002** is not fully enclosed by cover. However, this hole **1100** is covered at all times while microfluidic serial dilution platform based well-plate is in use by a material different than the cover. As discussed above, each trap in the microfluidic trap network is covered by cover (*e.g.*, cover **1200**) while hole 1100, which extends through cover, is covered by cellophane or similar material that is airtight. The covered hole maintains humidity in the trap to prevent evaporation of fluid in the trap. Note: the fluidic trap **1002** shown in FIG. 10A is not square; rather trap **1002** has a rounded or semi-circular bottom. With reference to FIG. 10B, shown is a perspective view of this embodiment of microfluidic serial dilution platform based well-plate **1000**. A reservoir **1150**, situated on well-plate 1000 and filled with fluid, such as water, at a

fixed pressure, surrounds the microfluidic network. After the trap **1002** is filled with fluid, *e.g.*, a cell solution, a cover **1200** is placed over the microfluidic serial dilution platform based well-plate covering the inlet **1006**, outlet **1018**, the hole **1100** and the reservoir **1150** (note cover **1200** is shown in suspended above microfluidic serial dilution platform based well-plate; in use, it would be directly in contact and actually cover microfluidic network). With reference to FIG. 10C, shown is a graph depicting the amount of evaporation in the well after various time points using the embodiment illustrated in FIGS. 10A-B. As can be seen the maximum evaporation is less than fifteen percent (15%) after a time period of seventy-two (72) hrs.

The terms and descriptions used herein are set forth by way of illustration only and are not meant as limitations. Those skilled in the art will recognize that many variations are possible within the spirit and scope of the invention as defined in the following claims, and their equivalents, in which all terms are to be understood in their broadest possible sense unless otherwise indicated.

What is claimed is:

1. A microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors comprising:

a plurality of fluidic traps;

a plurality of hydrophilic capillary constriction channels;

a plurality of bypass channels, wherein each of the plurality of bypass channels is associated with one of the plurality of fluidic traps, each of the plurality of hydrophilic capillary constriction channels is associated with one of the plurality of fluidic traps, and each of the plurality of fluidic traps is associated with one of the plurality of bypass channels and one of the plurality of hydrophilic capillary constriction channels;

an inlet;

an outlet; and

a main channel with a plurality of portions that connects the inlet to the plurality of fluidic traps, associated hydrophilic capillary constriction channels and associated bypass channels, and the outlet.

2. The microfluidic serial dilution platform based well-plate of claim 1 wherein the inlet includes an interface for a pipettor, pipette or other fluid driving mechanism.

3. The microfluidic serial dilution platform based well-plate of claim 1 wherein the well-plate is located on a chip.

4. The microfluidic serial dilution platform based well-plate of claim 1 wherein the plurality of fluidic traps are arranged in a serial circuit with each other.

5. The microfluidic serial dilution platform based well-plate of claim 1 wherein the plurality of fluidic traps are arranged in a parallel circuit with each other.

6. The microfluidic serial dilution platform based well-plate of claim 1 wherein the plurality of fluidic traps comprises a plurality of networks of fluidic traps each including at least four fluidic traps.

7. The microfluidic serial dilution platform based well-plate of claim 1 wherein the plurality of fluidic traps comprises more than one hundred fluidic traps.

8. The microfluidic serial dilution platform based well-plate of claim 1 wherein the plurality of fluidic traps comprises a number of fluidic traps configured to meet requirements based on a desired application of the microfluidic serial dilution platform based well-plate.

9. The microfluidic serial dilution platform based well-plate of claim 1 further comprising a plurality of enlarged main channel portions configured to optimize a reduction of air invading into the plurality of fluidic traps, wherein one of the plurality of enlarged main channel portions is located on an outlet side of each of the plurality of fluidic traps.

10. The microfluidic serial dilution platform based well-plate of claim 1 wherein each of the plurality of fluidic traps is a square-shaped trap.

12. The microfluidic serial dilution platform based well-plate of claim 1 wherein the hydrophilic capillary constriction channels are located on an inlet side of the associated fluidic trap.

13. The microfluidic serial dilution platform based well-plate of claim 1 wherein the inlet, the outlet, the main channel, and the plurality of fluidic traps with the associated hydrophilic capillary constriction channels and associated bypass channels form a network, and the well-plate further comprises:

a reservoir, filled with water substantially at a constant pressure, that surrounds the network of inlet, outlet, main channel, fluidic traps, hydrophilic capillary constriction channels and bypass channels; and

a cover that covers plurality of fluidic traps, the reservoir and the network of inlet, outlet, main channel, fluidic traps, hydrophilic capillary constriction channels and bypass channels, wherein the cover defines a plurality of holes, each hole extending into one of the plurality of fluidic traps and in which the plurality of holes are configured to control the humidity in the fluidic traps.

14. A microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors comprising:

a plurality of fluidic traps;

a plurality of hydrophobic capillary constriction channels;

a plurality of bypass channels, wherein each of the plurality of bypass channels is associated with one of the plurality of fluidic traps, each of the plurality of hydrophobic capillary constriction channels is associated with one of the plurality of fluidic traps, and each of the plurality of fluidic traps is associated with one of the plurality of bypass channels and one of the plurality of hydrophobic capillary constriction channels;

an inlet;

an outlet; and

a main channel with a plurality of portions that connects the inlet to the plurality of fluidic traps, associated hydrophobic capillary constriction channels and associated bypass channels, and the outlet.

15. The microfluidic serial dilution platform based well-plate of claim 14 wherein the hydrophobic capillary constriction channels are located on an outlet side of the associated fluidic trap.

16. The microfluidic serial dilution platform based well-plate of claim 14 wherein each of the plurality of fluidic traps is a square-shaped trap.

17. The microfluidic serial dilution platform based well-plate of claim 14 wherein the inlet, the outlet, the main channel, and the plurality of fluidic traps with the associated hydrophobic capillary constriction channels and associated bypass channels form a network, the outlet each of the plurality of fluidic traps defines a hole in a top surface of the fluidic trap, and the well-plate further comprises:

a reservoir, filled with water substantially at a constant pressure, that surrounds the network of inlet, outlet, main channel, fluidic traps, hydrophobic capillary constriction channels and bypass channels; and

a cover that covers the holes in the plurality of fluidic traps, the reservoir and the network of inlet, outlet, main channel, fluidic traps, hydrophobic capillary constriction channels and bypass channels.

18. A method of operating a microfluidic serial dilution platform based well-plate using an oil-free immiscible phase driven by manual or electronic pipettors comprising:

inserting fluid into a main channel of the well-plate through an inlet;

causing the fluid to enter into a bypass channel associated with a fluidic trap;

causing the fluid to enter into the fluidic trap through a hydrophilic capillary constriction channel associated with the fluidic trap, wherein the fluidic trap is filled with the fluid;

inserting air into the main channel of the well-plate through the inlet; and

causing the air to push the fluid from the bypass channel past the fluidic trap, wherein the fluid in the fluidic trap remains in the fluidic trap.

19. The method of claim 18 further comprising:

inserting diluting fluid into the main channel of the well-plate through the inlet; and

causing the diluting fluid to enter into the fluidic trap through the hydrophilic capillary constriction channel associated with the fluidic trap.

20. The method of claim 18 further comprising repeating the steps for additional fluidic traps on the well-plate.

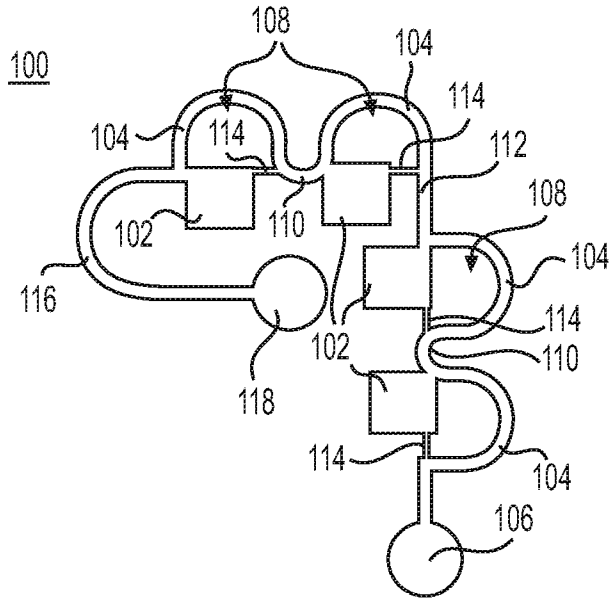

FIG. 1A

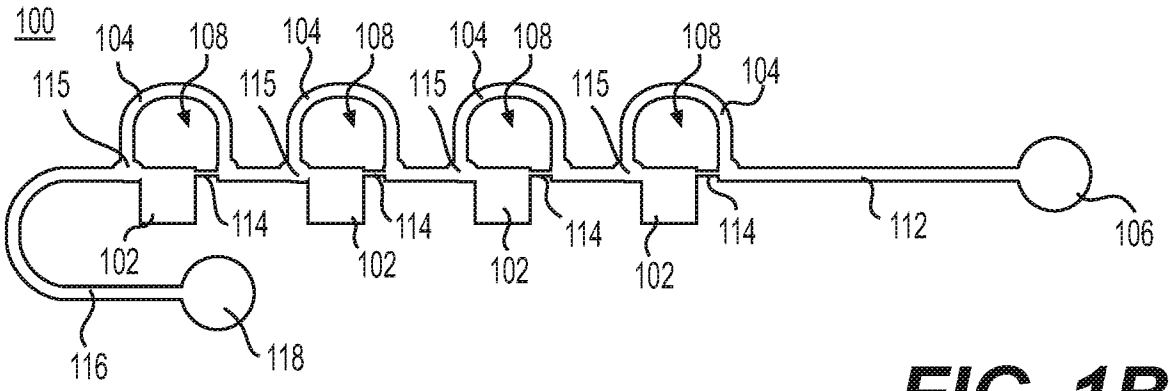

FIG. 1B

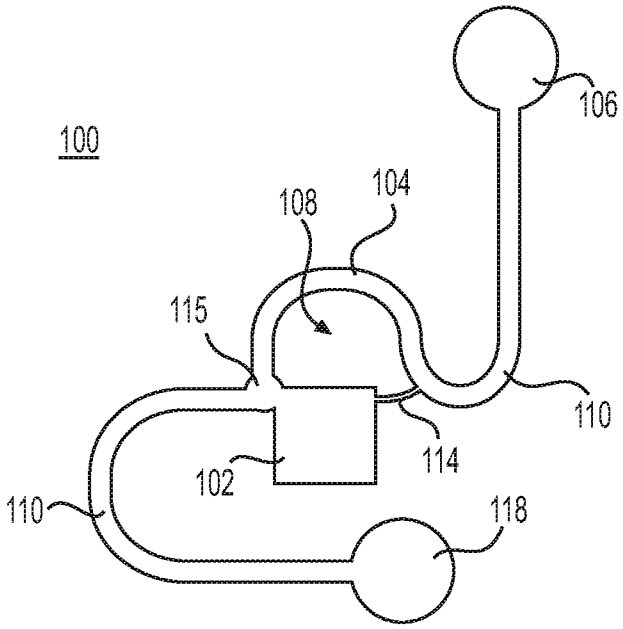

FIG. 1C

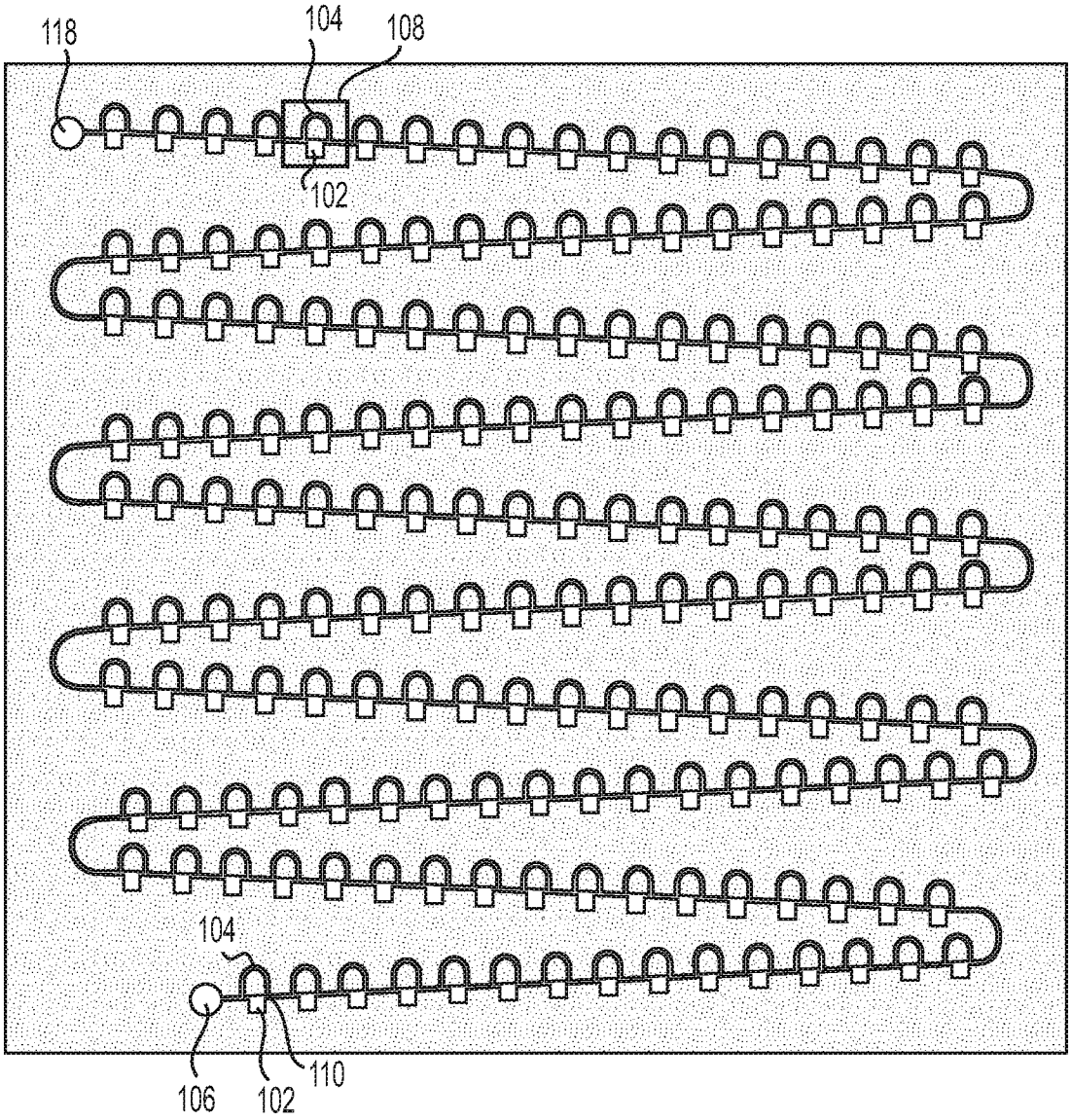

FIG. 1D

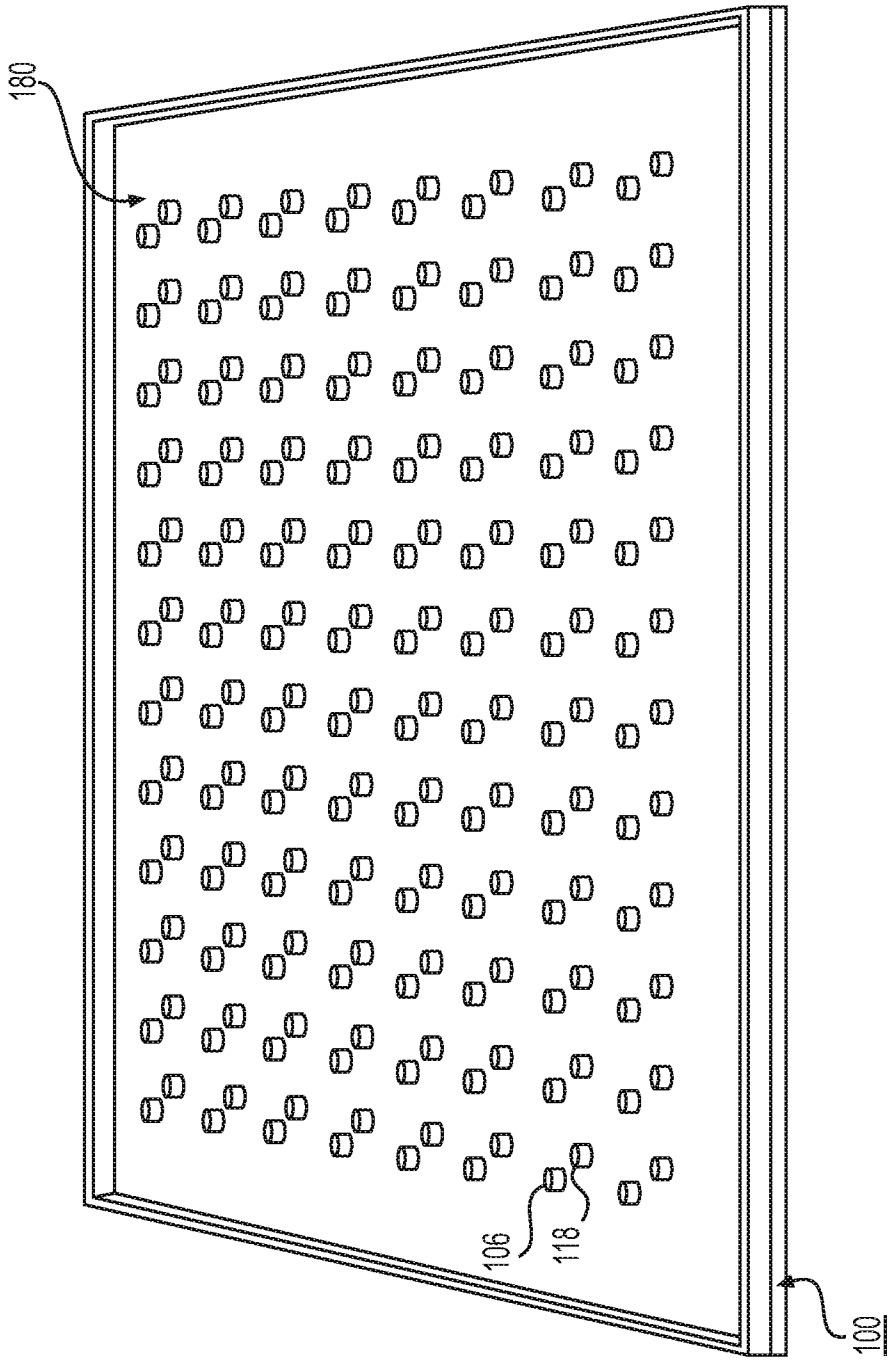

FIG. 1E

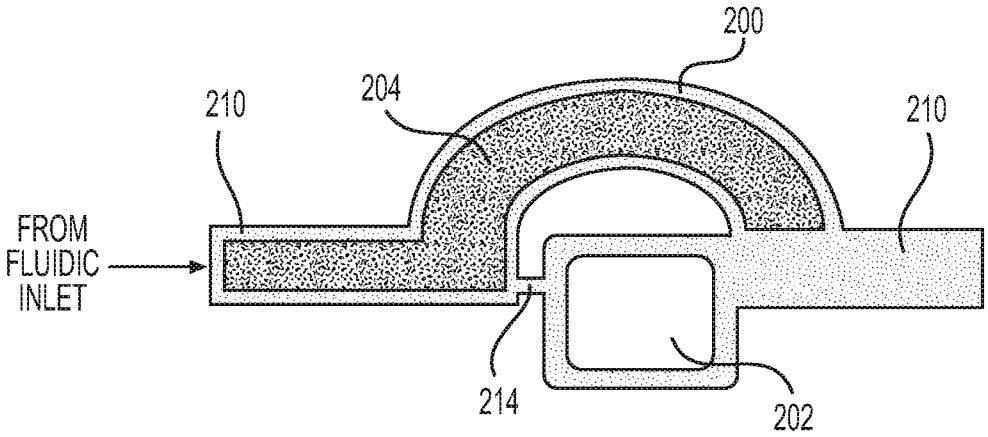

FIG. 2A

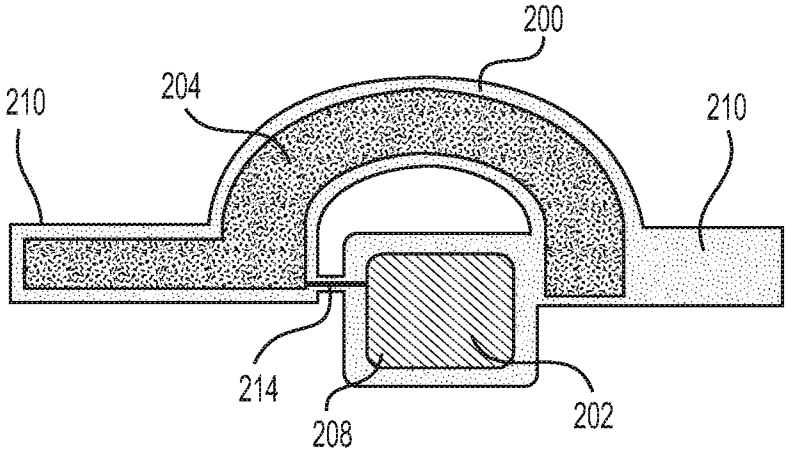

FIG. 2B

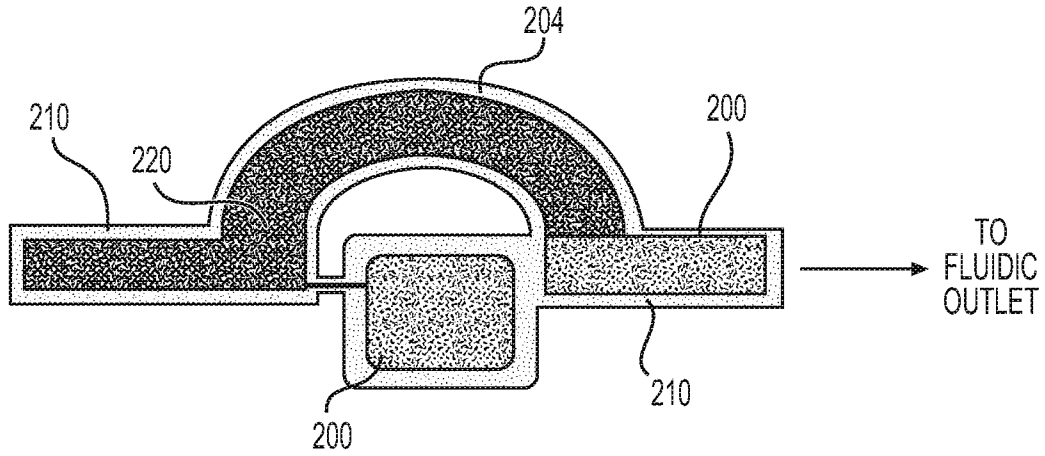

FIG. 2C

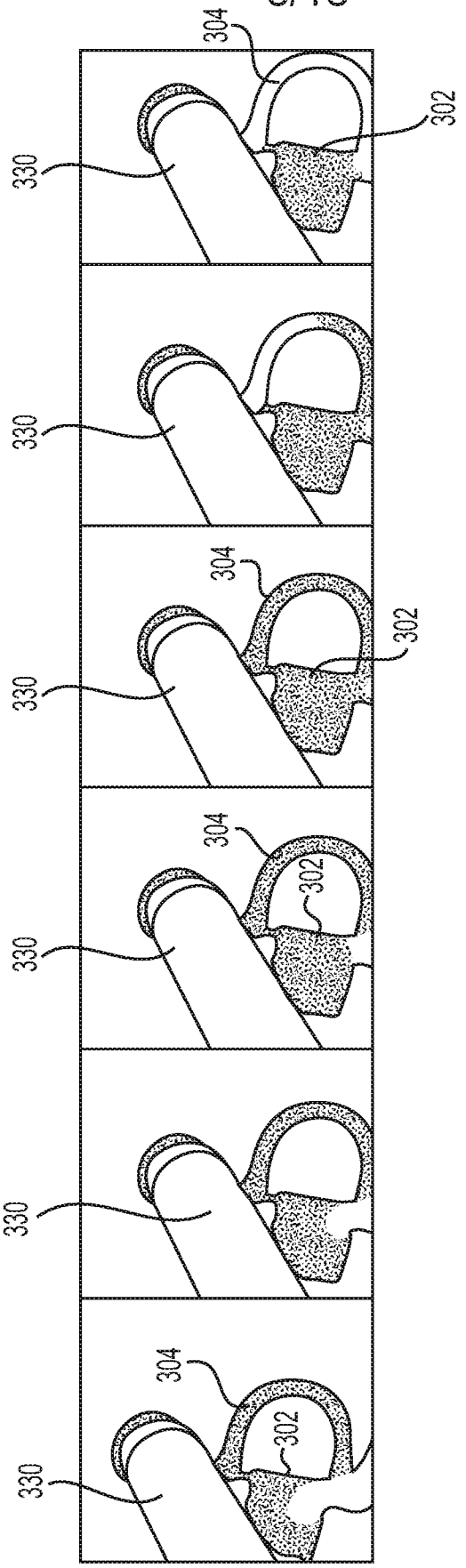

FIG. 3

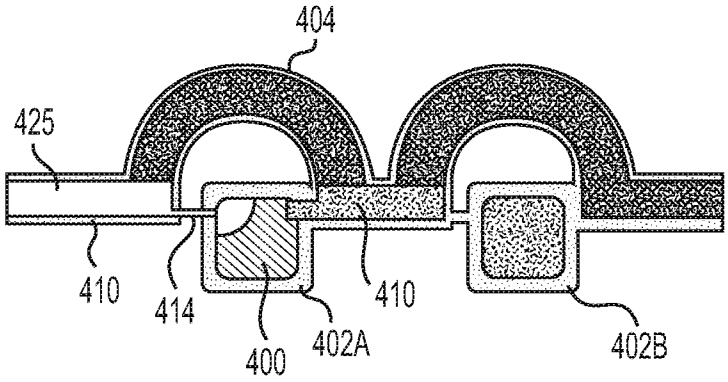

FIG. 4A

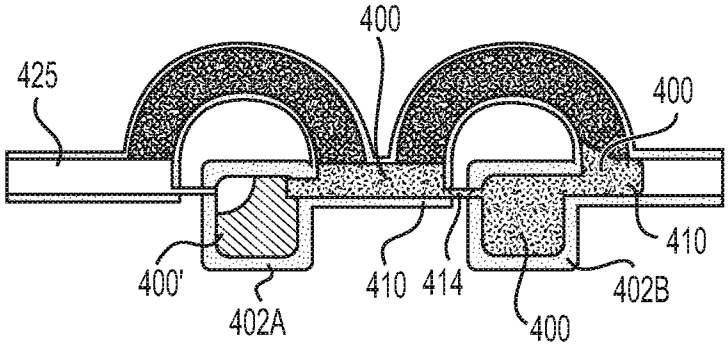

FIG. 4B

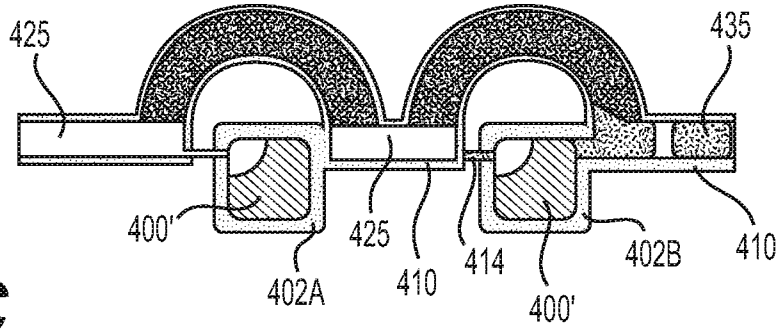

FIG. 4C

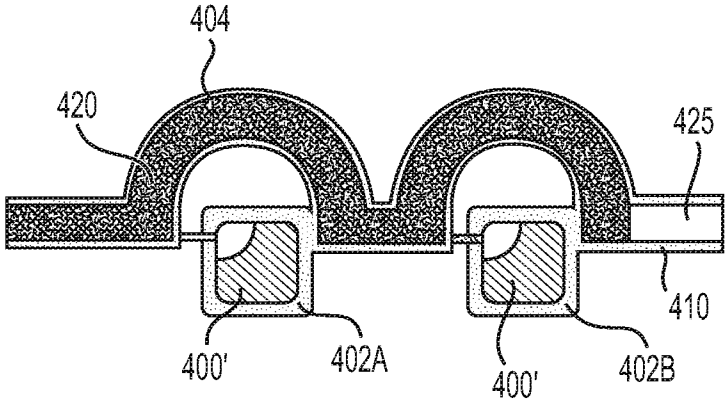

FIG. 4D

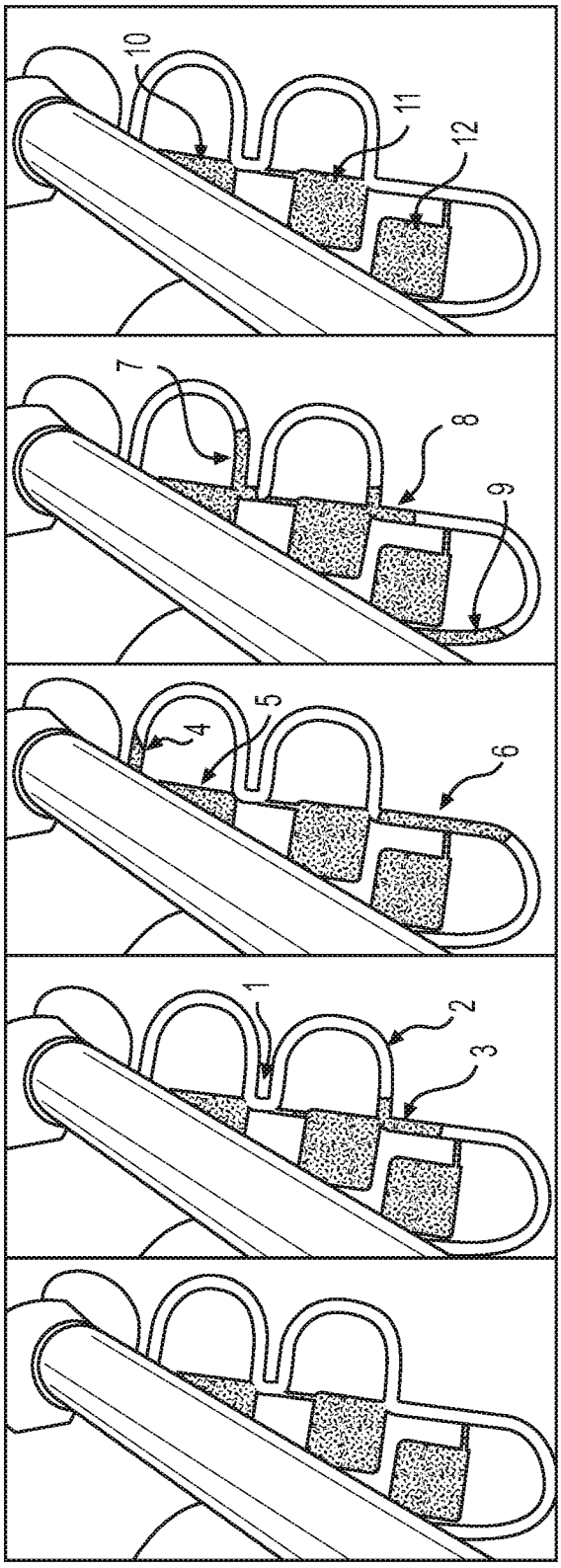

FIG. 5

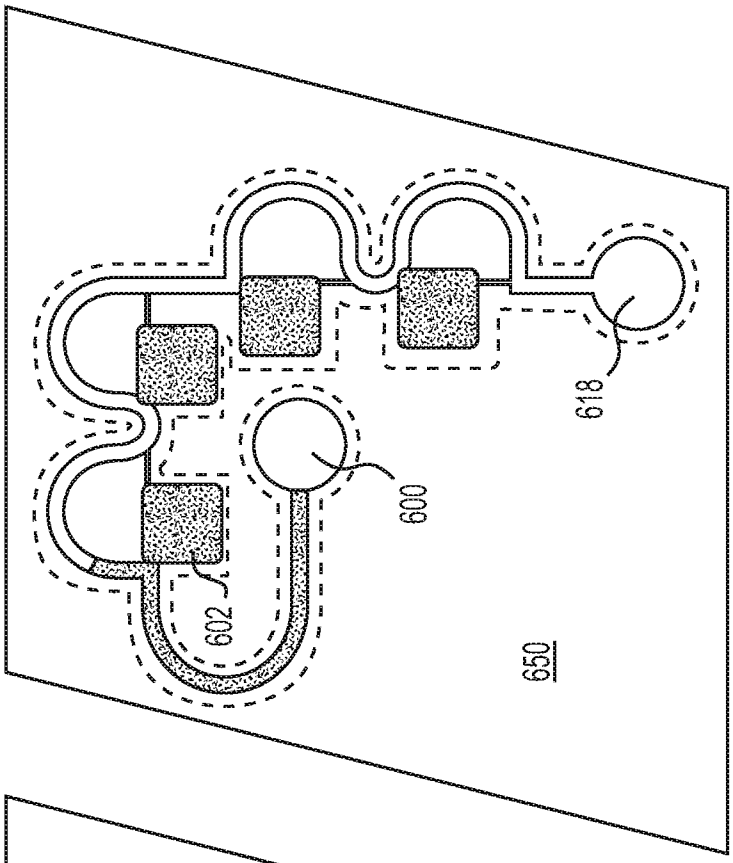

FIG. 6B

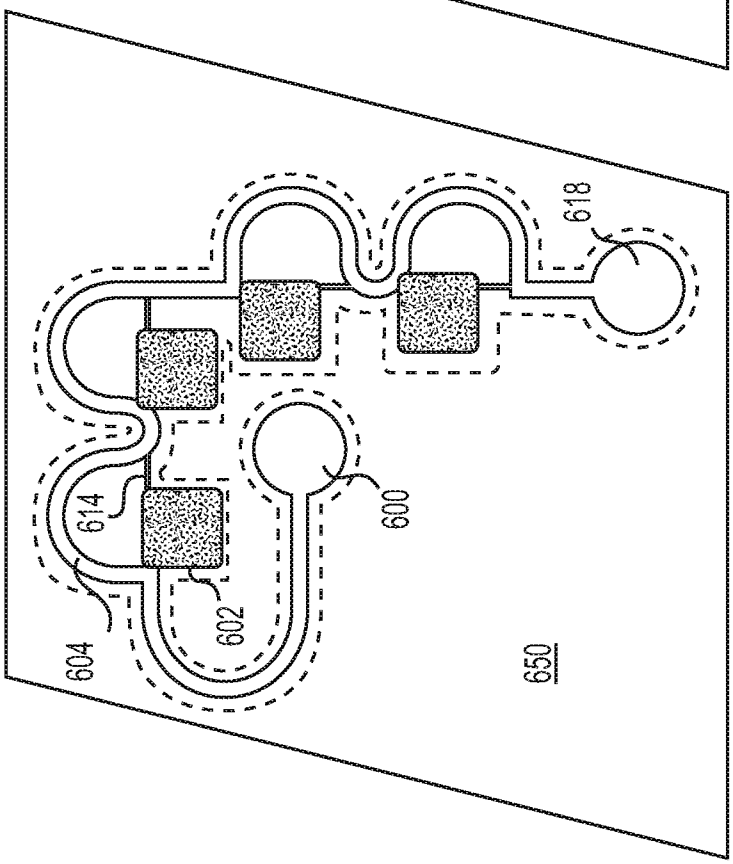

FIG. 6A

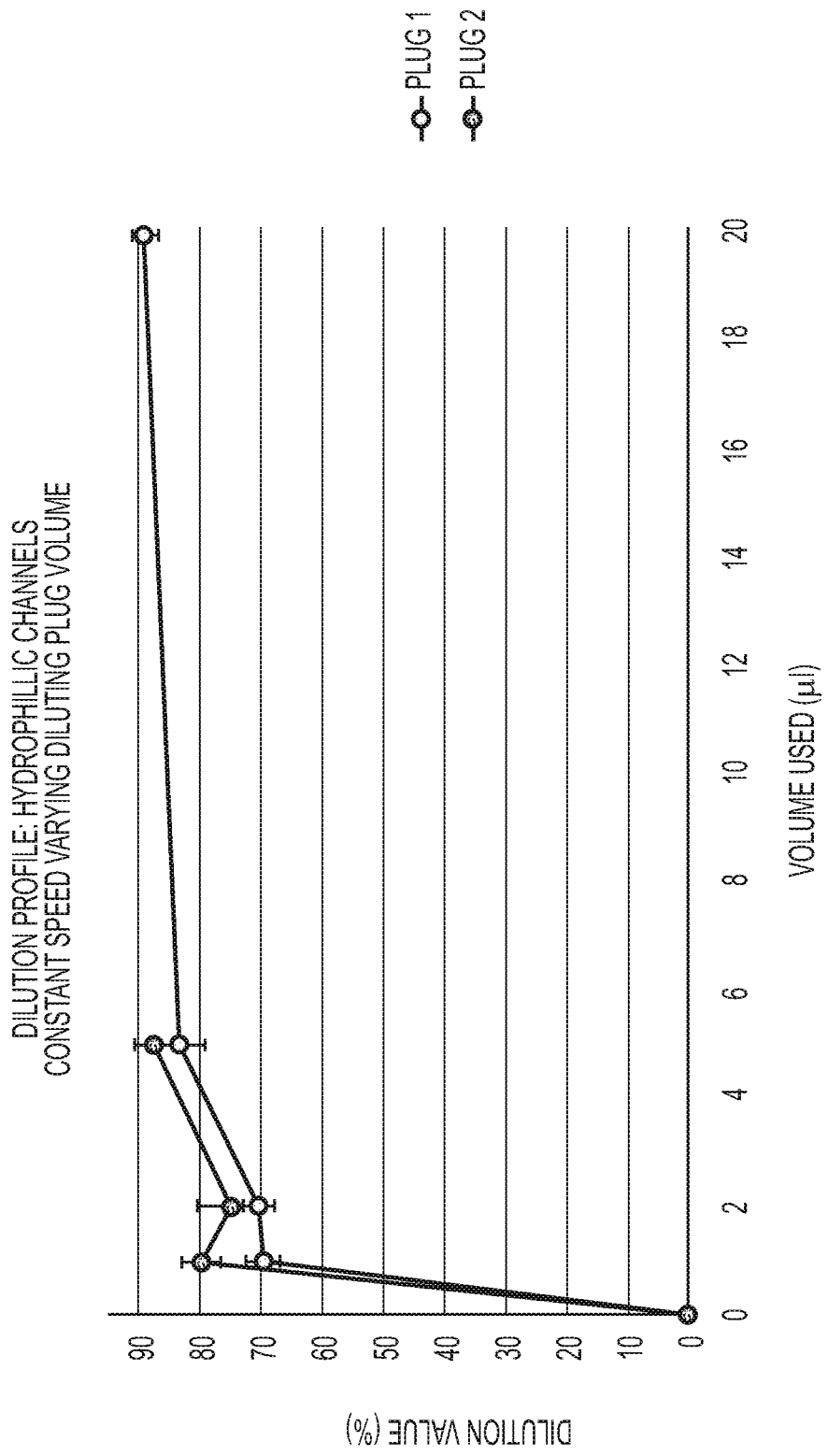
PLUG 1
PLUG 2
DILUTION PROFILE: HYDROPHILLIC CHANNELS
CONSTANT SPEED VARYING DILUTING PLUG VOLUME
DILUTION VALUE (%)
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
VOLUME USED (µl)

FIG. 7A

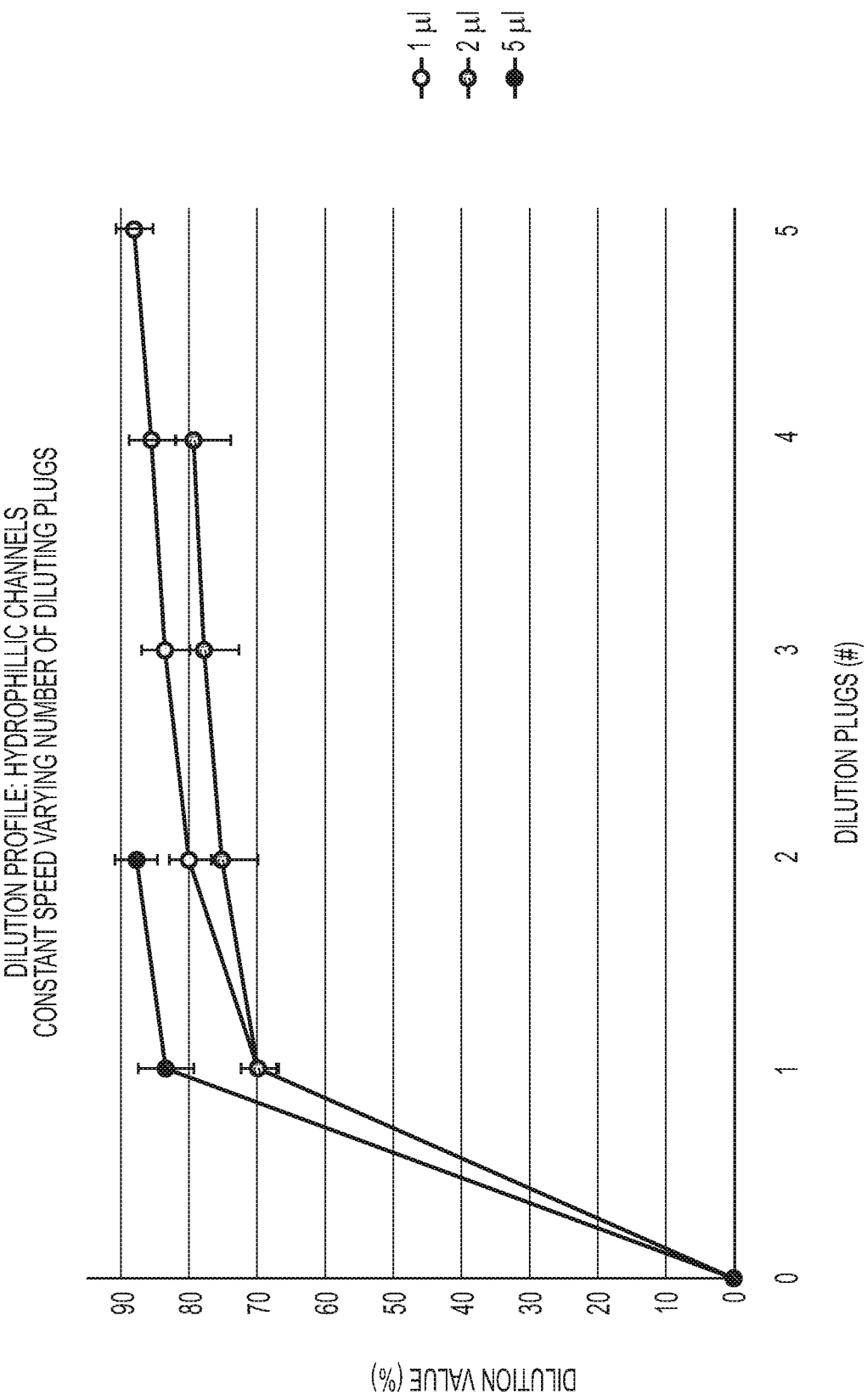
1 µl
2 µl
5 µl
DILUTION PROFILE: HYDROPHILLIC CHANNELS
CONSTANT SPEED VARYING NUMBER OF DILUTING PLUGS
DILUTION VALUE (%)
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
DILUTION PLUGS (#)

FIG. 7B

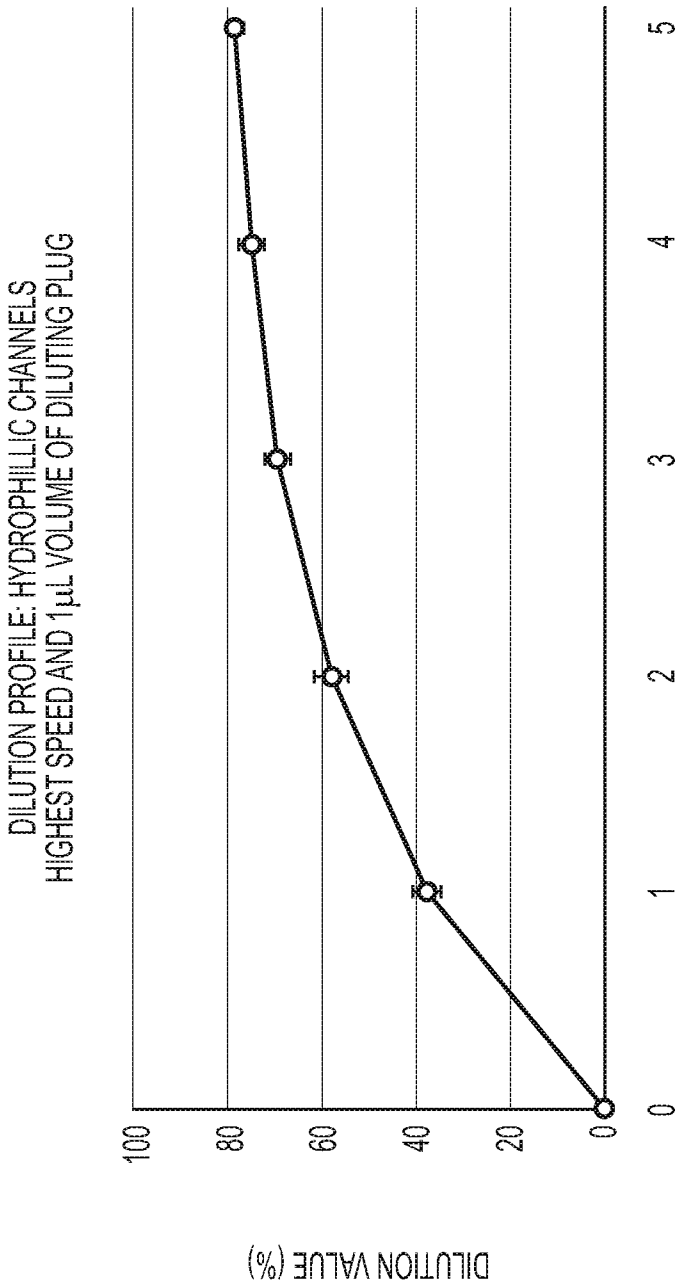
DILUTION PROFILE: HYDROPHILLIC CHANNELS
HIGHEST SPEED AND 1µL VOLUME OF DILUTING PLUG
DILUTION VALUE (%)
100
80
60
40
20
0
0
1
2
3
4
5
DILUTION PLUGS (#)

FIG. 7C

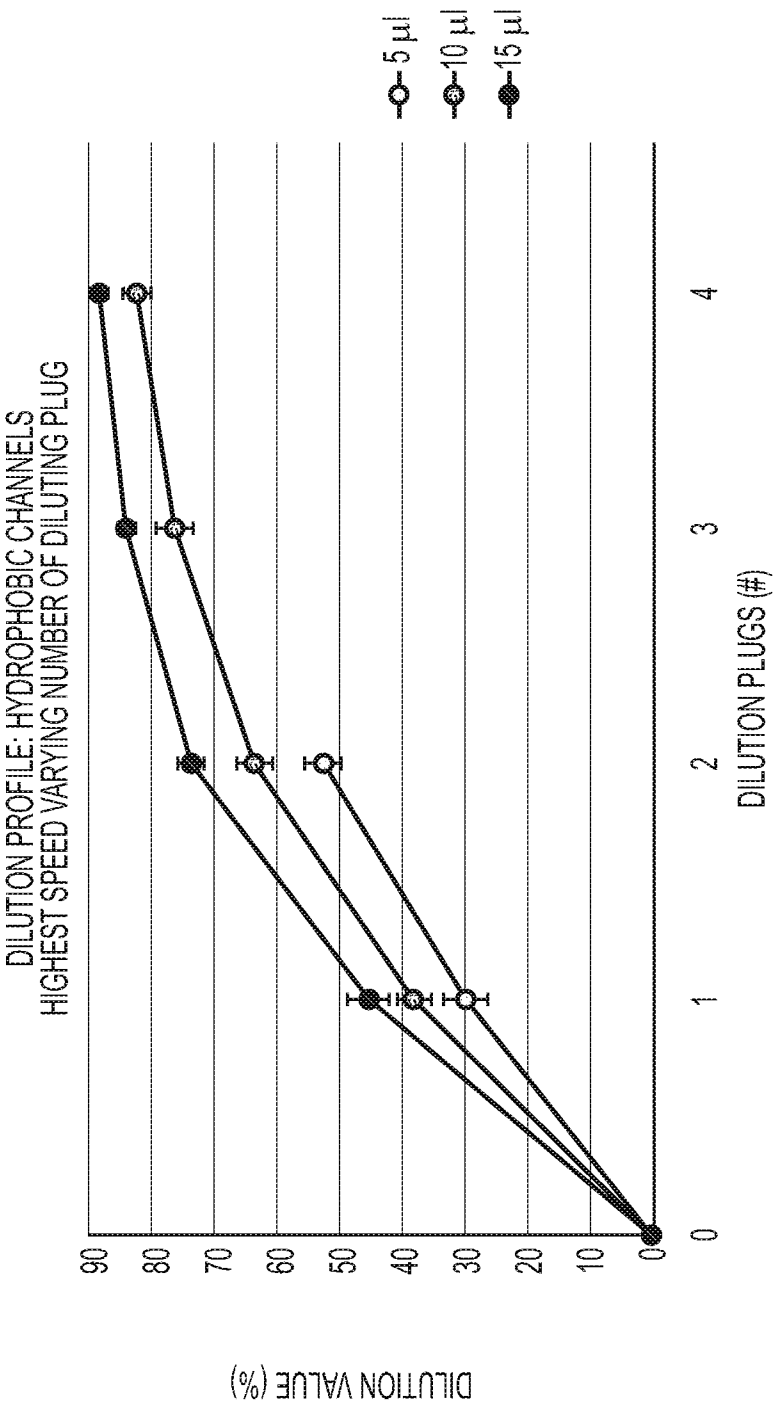
DILUTION PROFILE: HYDROPHOBIC CHANNELS
HIGHEST SPEED VARYING NUMBER OF DILUTING PLUG
5 µl
10 µl
15 µl
DILUTION VALUE (%)
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
DILUTION PLUGS (#)

FIG. 8A

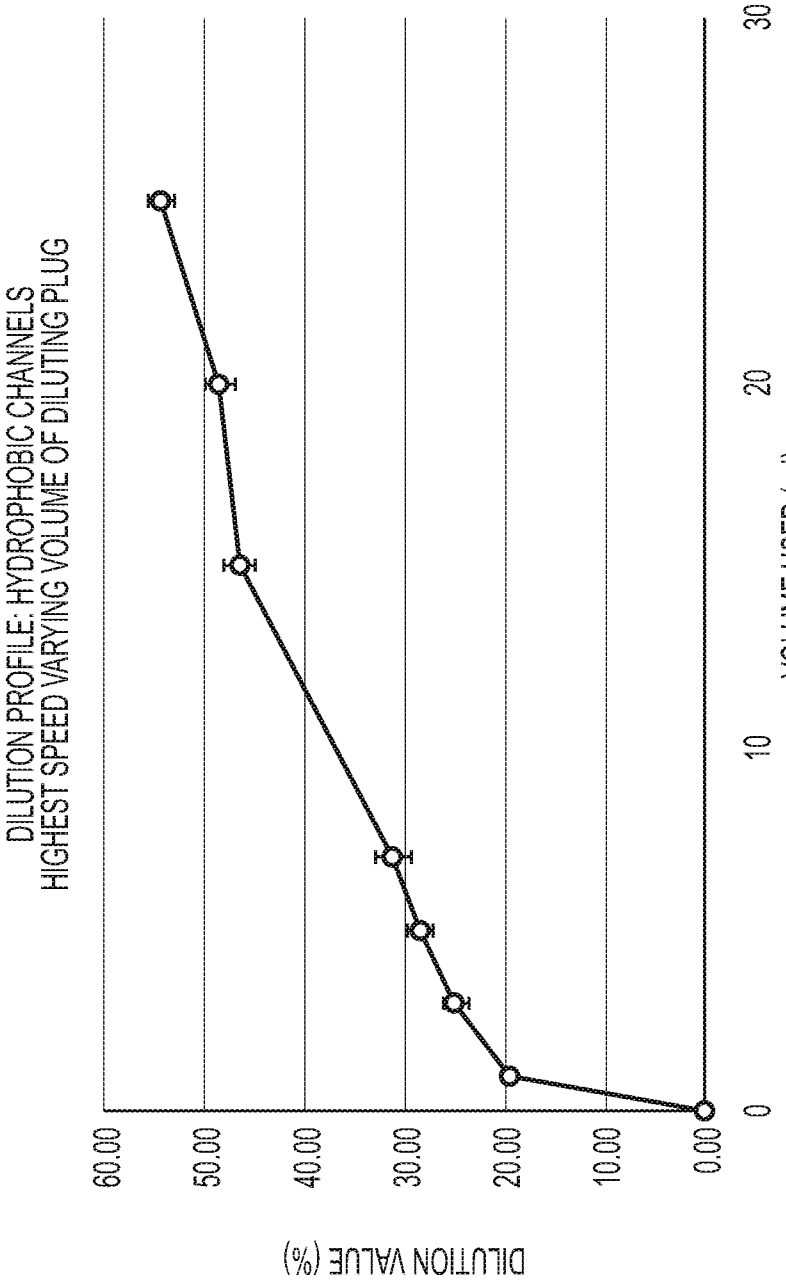
DILUTION PROFILE: HYDROPHOBIC CHANNELS
HIGHEST SPEED VARYING VOLUME OF DILUTING PLUG
DILUTION VALUE (%)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
10
20
30
VOLUME USED (µl)

FIG. 8B

CELL COUNTING EXPERIMENTS	HEMOCYTOMETER	CHIP W/O TRYPAN BLUE	CHIP WITH TRYPAN BLUE
TEST 1	$6.7X10^6$ CELLS/ml	$6.65X10^6$ CELLS/ml	X
TEST 2	$4.7X10^6$ CELLS/ml	X	$5.01X10^6$ CELLS/ml

FIG. 9A

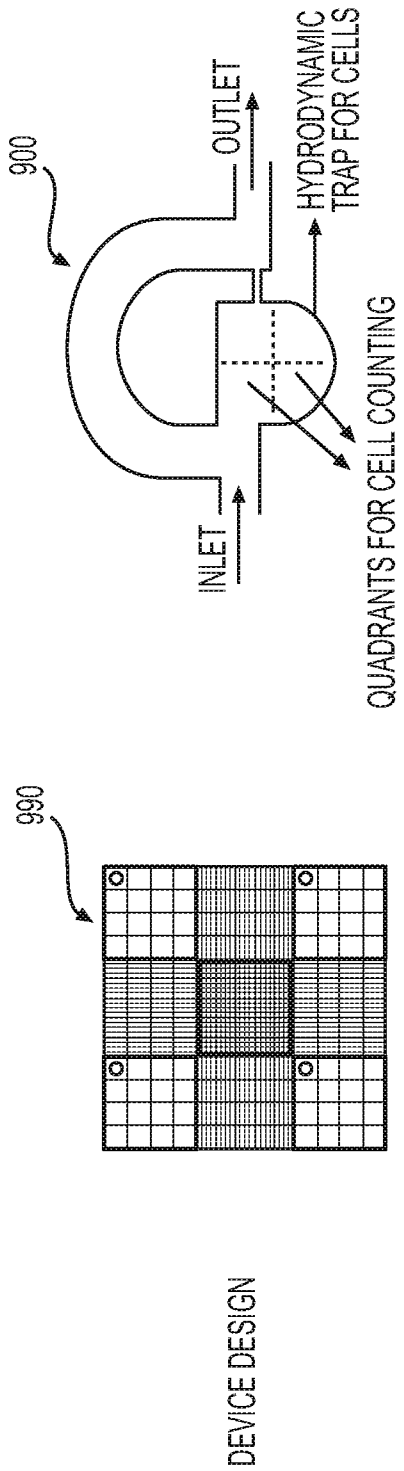
900
OUTLET
HYDRODYNAMIC TRAP FOR CELLS
INLET
QUADRANTS FOR CELL COUNTING
990
DEVICE DESIGN

FIG. 9B

PARAMETERS	HEMOCYTOMETER	NEOPLATE
TIME TO LOAD CELLS	45-60 SEC	5-6 SEC
TIME TO COUNT CELLS	5-6 MIN	1.5-2 MIN
TOTAL TIME TO GET CELL COUNT	7-8 MIN	2-2.5 MIN (70-75% REDUCTION IN TIME)
DISTRIBUTION OF CELLS IN 4 QUADRANTS	UNEVEN	EVEN
EASE OF HANDLING	NOT EASY	VERY EASY
HOW DELICATE?	VERY DELICATE	ROBUST
REUSABILITY	REUSABLE	REUSABLE
OTHER FUNCTIONS	NO	CAN BE USED FOR CELL BASED EXPERIMENTS SUCH AS ASSAY, STAINING ETC.

FIG. 9C

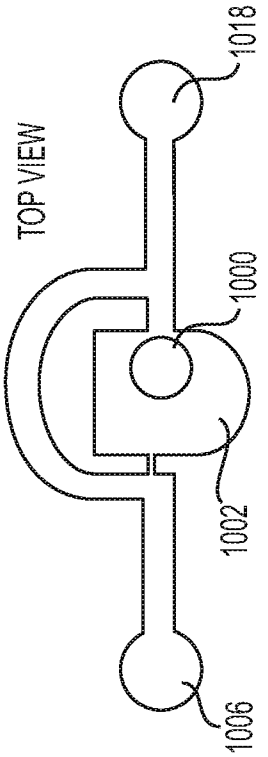

FIG. 10A

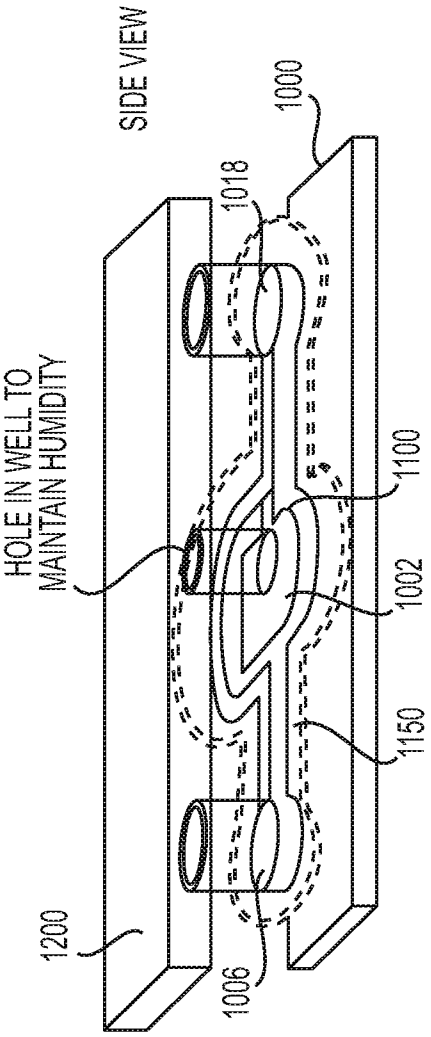

FIG. 10B

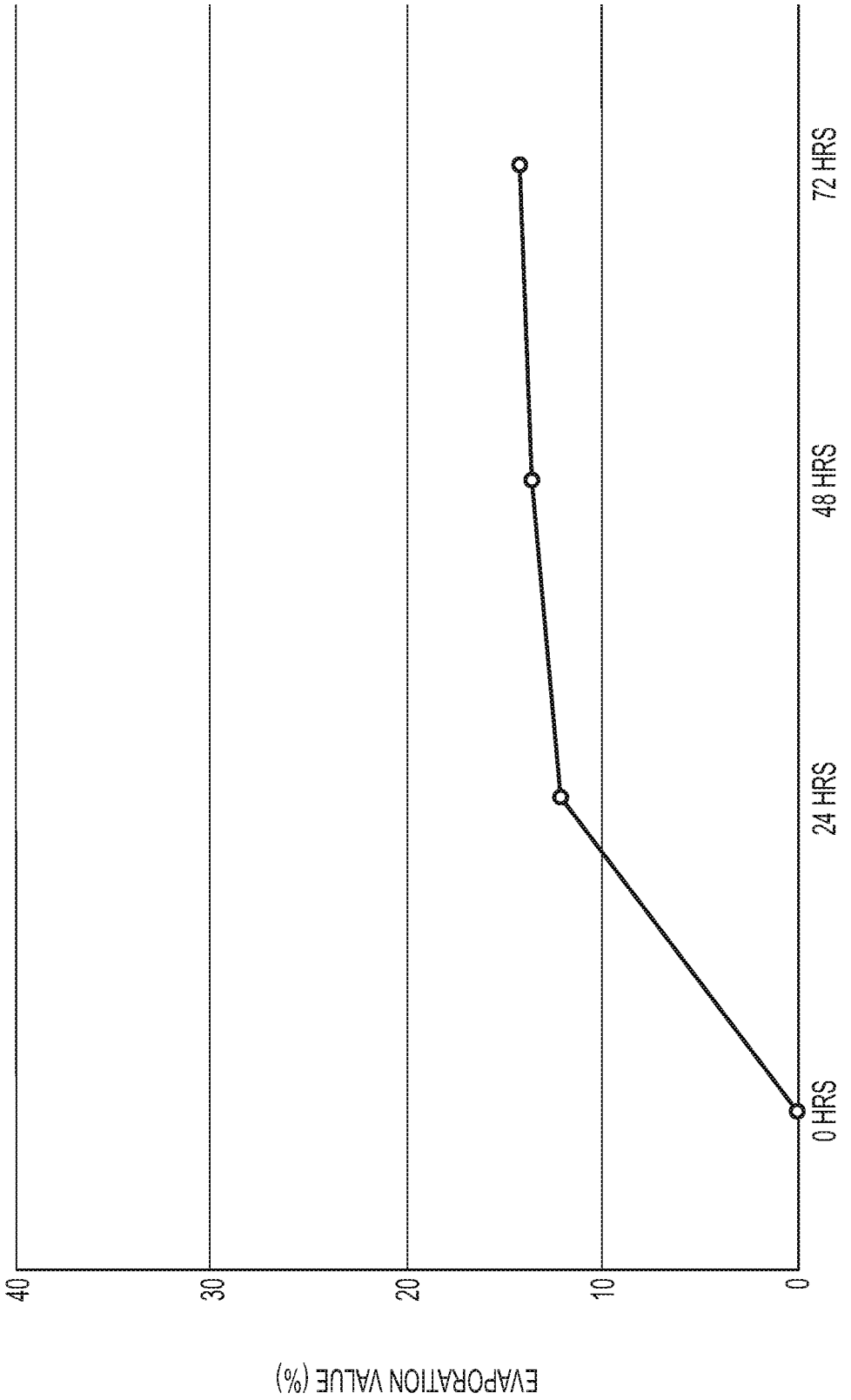
AVERAGE EVAPORATION OVER TIME
EVAPORATION VALUE (%)
40
30
20
10
0
0 HRS
24 HRS
48 HRS
72 HRS

FIG. 10C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/14704

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - B81B 7/04; G01F 11/28; G01N 1/18 (2016.01)
CPC - B81B 7/04; G01F 11/28; G01N 1/18
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)
-***-Continued Within the Next Supplemental Box-***-

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; EBSCO; IP.com; traps, capillaries, restricting, bypass, channel, microfluidic, serial, dilution, platform, seal, water, pressure, humidity, parallel, hydrophobic, hydrophilic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2004/0206408 A1 (PETERS, RP et al.) 21 October 2004; figures 1A-4B, 11; paragraphs [0005]-[0006], [0014]-[0016], [0047]-[0048], [0055]	1-2, 4-5, 8, 14-15 --- 3, 6-7, 9-10, 12-13, 16-17
Y	US 2003/0138829 A1 (UNGER, M et al.) 24 July 2003; figures 1A; 7A-7B; paragraphs [0006], [0008], [0059], [0072], [0083], [0204], [0219]-[0220]	3, 6-7, 10, 13, 16-17
Y	US 2002/0075363 A1 (MCNEELY, MR et al.) 20 June 2002; figures 4-5; paragraphs [0025]-[0028]	9
Y	US 2013/0337578 A1 (DELAMARCHE, E et al.) 19 December 2013; figure 2; paragraph [0048]	12
A	US 2014/0246098 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE, et al.) 04 September 2014; entire document	1-10, 12-17

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2016 (14.03.2016)	02 JUN 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/14704

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group I: Claims 1-17
Group II: Claims 18-20
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-17 are directed toward a plurality of capillary constriction channels and bypass channels associated with one of the plurality of fluidic traps and a main channel with a plurality of portions that connects the inlet to the plurality of fluidic traps, associated hydrophilic capillary constriction channels and associated bypass channels, and the outlet.

Group II: Claims 18-20 are directed toward a method of operating a bypass channel and a constriction capillary associated with a fluidic trap by sequentially filling the system with fluid and using air to purge part of the system.

-***-Continued Within the Next Supplemental Box-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10, 12-17

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

-***-Continued from Box B. FIELDS SEARCHED -***-

IPC(8): B01L 3/00; B81B 7/00, 7/04; G01F 11/00, 11/28, 13/00; F15C 4/00, 5/00; G01N 1/00, 1/10, 1/18, 33/00, 35/00, 35/08, 35/10 (2016.01)

CPC: B01L 3/00, 3/50, 3/5027, 3/502784, 2200/061; B81B 7/00, 7/04; G01F 11/00, 11/28, 13/00; F15C 4/00, 5/00; G01N 1/00, 1/10, 1/18, 33/00, 35/00, 35/08, 35/10

-***-Continued from Box No. III Observations where unity of invention is lacking -***-

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a plurality of capillary constriction channels and bypass channels associated with one of the plurality of fluidic traps and a main channel with a plurality of portion, which are not present in Group II; the special technical features of Group II include sequentially filling the system with fluid and using air to purge part of the system, which are not present in Group I

The common technical features of Groups I-II are a bypass channel and a hydrophilic constriction capillary associated with a fluidic trap.

These common technical features are disclosed by US 2004/0206408 A1 to Peters, et al. (hereinafter 'Peters'). Peters discloses a bypass channel (bypass channels 4; figures 1A-1D; paragraphs [0023]-[0024]) and a hydrophilic constriction capillary associated (a stopping means, such as a capillary stop 20, is provided by having a junction between a hydrophilic portion and a hydrophobic portion; figure 11; paragraphs [0007], [0038]) with a fluidic trap (a microfluidic switch has channels have chambers or cavities, specifically numbered as 3, bypass channels 4, and capillary stops 20; figures 1A-1D; paragraphs [0006], [0014], [0023]-[0024]).

Since the common technical features are previously disclosed by Peters, these common features are not special and so Groups I-II lack unity.