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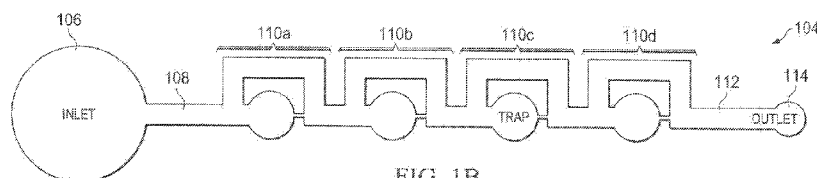


FIG. 1B

(57) Abstract: A microfluidic device includes a substrate and a cover. The substrate has an inlet port, a first microchannel, one or more parking loops, a second microchannel and an outlet port for each microchannel network. The first microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, the parking loops are connected between the first and second microchannels. Each parking loop includes a parking loop inlet, a parking loop output, a fluidic trap connected between the parking loop inlet and the parking loop outlet, and a bypass microchannel connected to the parking loop inlet and the parking loop outlet. The cover is attached to a top of the substrate and has an inlet opening and an outlet opening through the cover for each microchannel network. The inlet and outlet openings of the cover are disposed above the inlet and outlet ports in the substrate.



MICROFLUIDIC DEVICES AND METHODS FOR BIOASSAYS

Field of the Invention

The present invention relates generally to the field of microfluidics, and more particularly to microfluidic devices for bioassays.

5 Background of the Invention

There is a growing interest in conducting drug screens with primary cells derived from human tissues and biofluids to predict patient outcomes. In contrast to immortalized cell lines, primary cells are a scarce resource and yet preclinical studies demand diverse assays probing specific targets, off-targets and cytotoxicity. Therefore there is a need for a technology that can
10 screen drugs with minimal requirements on cell sample and compound volumes. Currently, multiwell plates and pipetting systems are the established methods for drug assays. However, this technology is difficult to scale down to nanoliter volumes due to fluid evaporation and pipetting errors. In recent years, drop-based microfluidics has emerged as a powerful technology to compartmentalize cells in volumes down to picoliters. Despite its great potential,
15 drop-based microfluidics has not been configured to conduct cell based drug assays with the same ease and parallelized fluid handling capability as well plates and pipetting systems.

Summary of the Invention

One embodiment of the present invention provides a microfluidic device having one or more microchannel networks. The microfluidic device includes a substrate and a cover. The
20 substrate has an inlet port, a first microchannel, one or more parking loops, a second microchannel and an outlet port for each microchannel network. The first microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, the one or more parking loops are connected between the first microchannel and the second microchannel. Each parking loop includes a parking loop inlet, a parking loop output, a fluidic trap connected
25 between the parking loop inlet and the parking loop outlet, and a bypass microchannel connected to the parking loop inlet and the parking loop outlet. The cover is attached to a top of the substrate and has an inlet opening and an outlet opening through the cover for each microchannel network. The inlet opening of the cover is disposed above the inlet port in the substrate and the outlet opening is disposed above the outlet port in the substrate.

30 Another embodiment of the present invention provides a method of making a microfluidic device having one or more microchannel networks. An inlet port, a first

microchannel, one or more parking loops, a second microchannel and an outlet port are formed in a substrate for each microchannel network. The first microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, the one or more parking loops are connected between the first microchannel and the second microchannel. Each parking loop includes a parking loop inlet, a parking loop output, a fluidic trap connected between the parking loop inlet and the parking loop output, and a bypass microchannel connected to the parking loop inlet and the parking loop output. An inlet opening and an outlet opening through a cover layer are formed for each microchannel network. The cover is attached to a top of the substrate. The inlet opening of the cover is disposed above the inlet port in the substrate and the outlet opening is disposed above the outlet port in the substrate for each microchannel network.

Yet another embodiment of the present invention provides a method for trapping a sample within a microfluidic device having one or more microchannel networks. A microfluidic device is provided. The microfluidic device includes a substrate having an inlet port, a first microchannel, one or more parking loops, a second microchannel and an outlet port for each microchannel network. The first microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, the one or more parking loops are connected between the first microchannel and the second microchannel. Each parking loop includes a parking loop inlet, a parking loop output, a fluidic trap connected between the parking loop inlet and the parking loop output, and a bypass microchannel connected to the parking loop inlet and the parking loop output. The microfluidic device also includes a cover attached to a top of the substrate that has an inlet opening and an outlet opening through the cover for each microchannel network. The inlet opening of the cover is disposed above the inlet port in the substrate and the outlet opening is disposed above the outlet port in the substrate. A first oil is released into the inlet port of the substrate via the inlet opening using a pipette. The sample is released into the inlet port of the substrate via the inlet opening using the pipette. Each fluidic trap is filled with the substance by creating a suction in the outlet port of the substrate via the outlet opening using the pipette. A second oil is released into the inlet port of the substrate via the inlet opening using the pipette. The sample is removed from the microchannel network, except for each fluidic trap, by creating a suction in the outlet port of the substrate via the outlet opening using the pipette.

The present invention is described in detail below with reference to the accompanying drawings.

Description of the Drawings

The above and further advantages of the invention may be better understood by referring to the following description in conjunction with the accompanying drawings, in which:

FIGURE 1A is an image of a microfluidic device in accordance with one embodiment of the present invention;

FIGURE 1B is a schematic of a single microchannel network within the microfluidic device in accordance with one embodiment of the present invention;

FIGURE 1C is a 3D view of a single parking loop containing a fluidic trap to store a sample in accordance with one embodiment of the present invention;

FIGURE 1D is a cross-sectional view (not to scale) of a portion of the microfluidic device in accordance with one embodiment of the present invention;

FIGURE 2 is a flow chart of a method of making a microfluidic device in accordance with one embodiment of the present invention;

FIGURE 3 illustrates a method using pipette-integrated trapping in a single microchannel network in accordance with one embodiment of the present invention;

FIGURE 4 is a flow chart of a method 400 for trapping a sample within a microfluidic device having one or more microchannel networks in accordance with one embodiment of the present invention;

FIGURES 5A and 5B are images shows the versatility of a pipette-integrated microfluidic well plate device in accordance with the present invention in which different concentration of green dye in distilled water stored in a single microfluidic well plate device (FIGURE 5A) and different dyes in distilled water stored in a single microfluidic well plate device of the SDA (FIGURE 5B);

FIGURE 6 is a graph showing the trapped drop volume conservation data over 2 days in 8-SDAs;

FIGURE 7 is a graph showing the quantification of cell distribution for different stock cell concentrations;

FIGURE 8 is a graph showing the viability of controlled cell assay;

FIGURE 9A is graph showing the effect of the anticancer drug doxorubicin on cell viability, and FIGURE 9B is a graph showing the dose-dependent cytotoxicity of the doxorubicin for the leukemia cell line (CCRF-CEM); and

FIGURE 10 is an image of a high throughput multiwell plate with an industry standard
5 multichannel pipettor in accordance with one embodiment of the present invention.

Description of the Invention

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many
10 applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

Various embodiments of the present invention provide a microfluidic device for storing arrays of nanoliter droplets. Although the microfluidic device 100 described herein is well
15 suited for use with automated multichannel pipettes 102 as shown in FIGURE 1A, the present invention is not limited to use with automated systems. The microfluidic device 100 may include one or more microchannel networks 104 embedded or formed on a substrate. In this example and as shown in FIGURE 1B, the microfluidic device 100 contains a set or array of isolated microchannels networks 104 arranged in a grid format. The non-limiting microfluidic
20 device 100 shown in FIGURE 1A contains 16 microchannel networks 104 arranged in a 2×8 format. This format allows facile interfacing of an 8-channel pipette. This design can be scaled up to a 12×8 format or larger with the total footprint commensurate with a standard multiwell plate.

For example, FIGURE 10 is an image of a high throughput multiwell plate 1000 with an
25 industry standard multichannel pipettor 102. The high throughput multiwell plate 1000 has 192 microchannel networks 104 arranged in a 12×16 format on a footprint equivalent to a standard wellplate that integrates with an 8-channel pipette 102. Other configurations and array sizes can be used.

Each embedded microchannel network 104 contains an inlet port or reservoir 106, a first
30 microchannel 108, one or more parking loops 110 (e.g., four parking loops 110a, 110b, 110c and 110d), a second microchannel 112 and an outlet port 114. The first microchannel 108 is connected to the inlet port 106 and the second microchannel 112 is connected to the outlet port

114. The one or more parking loops 110 are connected between the first microchannel 108 and the second microchannel 112. As shown in FIGURE 1C, each parking loop 110 includes a parking loop inlet 116, a parking loop output 118, a fluidic trap 120 (lower branch) connected between the parking loop inlet 116 and the parking loop outlet 118, and a bypass microchannel 122 (upper branch) connected to the parking loop inlet 116 and the parking loop outlet 118. The fluidic trap 120 includes a trap repository 124 connected to the parking loop inlet 116 and a trap microchannel 126 connecting the trap repository 124 to the parking loop outlet 118. The cross-sectional area of the trap microchannel 126 is smaller than the cross-sectional area of the bypass microchannel 122, which causes the hydrodynamic resistance of the bypass microchannel 122 to be smaller than the hydrodynamic resistance of the fluidic trap 120. The hydrodynamic resistance ratio between the bypass microchannel 122 and fluidic trap 120 is typically from 1.0 to 2.0, and may be between 1.4 to 1.6 in order to trap and store replicates of nanoliter-scale fluid volumes. In the example, each parking loop 110 has the following dimensions: the first microchannel 108, the second microchannel 112 and the bypass microchannel 122 have a width of approximately 200 μm and a height of approximately 200 μm ; the trap repository 124 has a diameter of approximately 450 μm ; the trap microchannel 126 has a width of approximately 40 μm ; the first microchannel 108, the second microchannel 112, the bypass microchannel 122, the trap repository 124 and the trap microchannel 126 have a height of approximately 200 μm ; and the trap microchannel 126 has a length of approximately 100 μm . Each fluidic trap 124 has a volume of only 30 nL in contrast to the 1-10 μL that is currently used in standard multiwell plate. Other dimensions and volumes can be used. For example, volume of the trap repository 124 can be approximately 10, 20, 30, 40, 50, 60, 70, 80, 90 nL or any increment thereof.

As will be explained in more detail below and as shown in FIGURE 1D, the microfluidic device 100 also includes a cover or upper layer 130 attached to a top of the substrate or lower layer 132, which reduces evaporation and increases the viability of samples trapped within the microchannel networks 104. Note that the cross-sectional diagram of a portion of the microfluidic device 100 shown in FIGURE 1D is not to scale. The cover 130 can be attached, affixed or integrated to the top of the substrate 132 by any suitable method for the materials in which the substrate 132 and cover 130 are made. For example, the cover 130 can be plasma bonded to the substrate 132 when both the cover 130 and substrate 132 are made of polydimethylsiloxane (PDMS). The cover 130 has an inlet opening 134 and an outlet opening 136 through the cover 130 for each microchannel network 104. Depending on the size of the inlet port 106 and outlet port 114, the cross-sectional area of the inlet opening 134 and outlet

opening 136 can greater than, equal to or less than the cross-sectional area of the inlet port 106 and outlet port 114 in the substrate 132. The inlet opening 134 of the cover 130 is disposed above the inlet port 106 in the substrate 132 and the outlet opening 136 is disposed above the outlet port 114 in the substrate 132. Typically the openings 134 and 136 are aligned with the ports 106 and 114. In one non-limiting example, the inlet opening 134 and the outlet opening 136 of the cover 130 have a diameter of approximately 3 mm, and the cover 130 has a thickness of 1 mm.

More information about other embodiments are described in U.S. Patent Application Serial No. 14/114,304 filed on May 7, 2012 and entitled “Methods and Devices to Control Fluid Volumes, Reagent and Particle Concentration in Arrays of Microfluidic Drops,” which is hereby incorporated by reference in its entirety.

Now referring to FIGURE 2, a flow chart of a method 200 of making a microfluidic device having one or more microchannel networks in accordance with one embodiment of the present invention is shown. An inlet port, a first microchannel, one or more parking loops, a second microchannel and an outlet port are formed in a substrate for each microchannel network in block 202. The first microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, and the one or more parking loops are connected between the first microchannel and the second microchannel. Each parking loop includes a parking loop inlet, a parking loop output, a fluidic trap connected between the parking loop inlet and the parking loop outlet, and a bypass microchannel connected to the parking loop inlet and the parking loop outlet. An inlet opening and an outlet opening are formed through a cover layer for each microchannel network in block 204. The cover is attached to a top of the substrate such that the inlet opening of the cover is disposed above the inlet port in the substrate and the outlet opening is disposed above the outlet port in the substrate for each microchannel network. Note that the forming steps can be repeated to form an array microchannel networks.

Referring now to FIGURE 3, a method 300 using pipette-integrated trapping in a single microchannel network in accordance with one embodiment of the present invention is shown. The method 300 involves 5 times pipetting (3 times dispensing, 2 times suctioning). An empty static droplet array (SDA) (e.g., microchannel network 104) is provided at step 302. In step 304, the channel is primed with oil by releasing 5 μ L oil in the inlet port reservoir of the SDA using a pipette. The oil moves through the channel by a capillary suction. As soon as the oil is reached to the outlet, a 1.5 μ L sample (dye in water or cells in media) is dispensed at the inlet-port using

pipette in step 306. Then suction is created at the outlet port by pressing and releasing the dispense trigger of pipette with an empty tip in step 308. The suction creates a moving plug of the sample from the inlet port, which fills the channel and fluidic traps. Then 10 μ L oil is reloaded at the inlet-port using pipette in step 310. Another suction is created at outlet port using empty pipette tip in step 312. This final suction removes the sample from the channel leaving the trapped samples inside the fluidic traps as shown in step 314. Note that the oil and sample volumes above will vary depending on the size and configuration of the SDA.

Similarly, FIGURE 4 is a flow chart of a method 400 for trapping a sample within a microfluidic device having one or more microchannel networks in accordance with one embodiment of the present invention. A microfluidic device is provided in block 402. The microfluidic device includes a substrate having an inlet port, a first microchannel, one or more parking loops, a second microchannel and an outlet port for each microchannel network. The first microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, the one or more parking loops are connected between the first microchannel and the second microchannel. Each parking loop includes a parking loop inlet, a parking loop output, a fluidic trap connected between the parking loop inlet and the parking loop outlet, and a bypass microchannel connected to the parking loop inlet and the parking loop outlet. The microfluidic device also includes a cover attached to a top of the substrate. The cover has an inlet opening and an outlet opening through the cover for each microchannel network. The inlet opening of the cover is disposed above the inlet port in the substrate and the outlet opening is disposed above the outlet port in the substrate. A first oil is released into the inlet port of the substrate via the inlet opening using a pipette in block 404. The sample is released into the inlet port of the substrate via the inlet opening using the pipette in block 406. Each fluidic trap is filled with the substance by creating a suction in the outlet port of the substrate via the outlet opening using the pipette in block 408. A second oil is released into the inlet port of the substrate via the inlet opening using the pipette in block 410. The sample is removed from the microchannel network, except for each fluidic trap, by creating a suction in the outlet port of the substrate via the outlet opening using the pipette in block 412.

Note that the pipette can be automatically controlled with a processor communicably coupled to the pipette. Moreover, multiple microchannel networks can be filled simultaneously. For example, a first microchannel network can contain the sample having a first concentration, a second microchannel network can contains the sample having a second concentration. Likewise,

a first microchannel network can contain a first sample, and a second microchannel network can contain a second sample. The sample may include one or more drops, cells or compositions.

Various embodiments of the present invention were tested as described below. Solutions of red, green, blue, purple and orange dyes (McCormick) were diluted 10X by distilled water and used as reagent plugs. 0.001% (w/w) biocompatible fluorosurfactant, KrytoxFSH-PEG600-KrytoxFSH (RAN Biotechnologies, Inc., MA) in FC-40 (Sigma- Aldrich) was used as carrier fluid. Human leukemia cancer cell line CCRF-CEM was maintained and grown in RPMI-1640 medium containing 10% fetal bovine serum and 0.1% Insulin-Transferrin-Selenium at 37 °C in 5% CO₂. Cell density of 3.5x10⁶ cells/mL was used in the experiments. LIVE/DEAD® Cell Imaging Kit (Life technologies) was used to stain the cells for the cell viability test. Preliminary cytotoxicity assays performed with the anticancer drug doxorubicin.

The versatility of the pipette-integrated microfluidic well plate device in accordance with the present invention is illustrated in FIGURES 5A and 5B. FIGURE 5A shows different concentrations of green dye in distilled water (10x, 20x, 50x, 100x, 200x and 1000x) stored in a single microfluidic well plate device. FIGURE 5B shows different dyes in distilled water stored in a single microfluidic well plate device of the SDA. The channel and the ports are filled with the residual oil. Different concentrations of the same sample can be trapped in different SDAs (FIGURE 5A) on the single microfluidic well plate device. Likewise, different samples can be trapped in different SDAs (FIGURE 5B) on a single microfluidic well plate device.

To perform cell culture or drug dose response assay in nanoliter-scale, the preservation of drop content over a period of time is very crucial. To avoid the liquid evaporation from the trapped drop over time, oil-filled pipette tips were initially used in both of the ports of the device. However, an ~30% sample evaporation was observed over 48 hours. Therefore, several strategies for reducing the evaporation during the incubation were explored. An effective method involves the plasma bonding of a second layer of PDMS sheet of 1 mm thickness with 3 mm holes aligned with the inlet and outlet ports of each SDA in the bottom layer. Then, the device is soaked in distilled water for 3 days. After 3 days, the device is used and after finishing the droplet trapping process, all of the 3 mm holes in the second layer are filled with oil to create oil reservoirs above the inlet and outlet of the SDAs. Then, the device was put in water filled omni-plate with a lid and stored in the incubator. Potential loss of reagent fluid due to evaporation in this process was less than 10% during 48 hours. The data is shown in FIGURE 6.

The quantification of cell distribution in drops was also tested. Different stock cell densities were used to get an idea of cell distribution in each microchannel using pipette-integrated droplet trapping. Two stock solutions of cells (density of 3×10^6 and 8×10^6 cells/mL) with a target of 50-250 cells/drop were prepared to get about 200-1000 cells per SDA, which gave a better statistic for future assay analysis. The actual cell distribution in 6- SDAs is shown in FIGURE 7, which is less than 50 % of the expected number of cells/SDA.

Previously, cell viability in 16nL drop of a density of 30 cells/drop was tested. The previous data showed greater than 90% cells are viable up to 4.5 hours without any media replacement. A similar cell viability test was performed using the microfluidic device in accordance with the present invention with a 30 nL drop volume. A cell density of 3×10^6 cells/mL was used to get about 100 cells per SDA to assess the viability of cells during the culture. Cells diluted with only media and without any drugs are used as controls. The cells are staining with live and dead fluorescent dyes prior to the trapping process. Standard epifluorescence microscopy automated stage was used with to image viable cells. A greater than 90% cell viability over 20 hours in 8-SDAs without any media replacement was observed. The results are shown in FIGURE 8.

In addition, preliminary cytotoxicity assays were performed using the anticancer drug doxorubicin in the microfluidic device in accordance with the present invention. A concentration 0.75×10^6 cells/mL strained with live/dead cell imaging kit was used. In one SDA controlled cell trapping (without drug) was performed and in another SDA the cells were exposed to 1 μ M doxorubicin before trapping and the cell viability over time was observed in both of the SDAs. Preliminary cytotoxicity assays with the anticancer drug doxorubicin showed greater than 99% cell death over 6 hour incubation period (FIGURE 9A).

Further, to determine dose-dependent cytotoxicity of the doxorubicin, leukemia cells were exposed to different concentrations of drug and each concentration treated cells were trapped in a separate SDA of the microfluidic device, and then the device was incubated for 1 hour, after which the cell viability was determined by standard image based epifluorescence microscopy. The IC₅₀ value of the doxorubicin in the leukemia cell lines was found 430 nM (FIGURE 9B).

The foregoing description of the apparatus and methods of the invention in preferred and alternative embodiments and variations, and the foregoing examples of processes for which the invention may be beneficially used, are intended to be illustrative and not for purpose of

limitation. The invention is susceptible to still further variations and alternative embodiments within the full scope of the invention, recited in the following claims.

Claims

1. A microfluidic device having one or more microchannel networks comprising:
a substrate having an inlet port, a first microchannel, one or more parking loops, a second
5 microchannel and an outlet port for each microchannel network, wherein the first microchannel
is connected to the inlet port, the second microchannel is connected to the outlet port, the one or
more parking loops are connected between the first microchannel and the second microchannel,
and each parking loop comprises a parking loop inlet, a parking loop output, a fluidic trap
connected between the parking loop inlet and the parking loop outlet, and a bypass microchannel
10 connected to the parking loop inlet and the parking loop outlet; and
a cover attached to a top of the substrate, the cover having an inlet opening and an outlet
opening through the cover for each microchannel network, wherein the inlet opening of the
cover is disposed above the inlet port in the substrate and the outlet opening is disposed above
the outlet port in the substrate.
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2. The microfluidic device as recited in claim 1, wherein the fluidic trap comprises:
a trap repository connected to the parking loop inlet; and
a trap microchannel connecting the trap repository to the parking loop outlet, wherein a
cross-sectional area of the trap microchannel is smaller than a cross-sectional area of the bypass
20 microchannel.
3. The microfluidic device as recited in claim 2, wherein the trap repository has a volume of
approximately 10, 20, 30, 40, 50, 60, 70, 80 or 90 nL.
- 25 4. The microfluidic device as recited in claim 2, wherein:
the first microchannel, the second microchannel and the bypass microchannel have a
width of approximately 200 μm and a height of approximately 200 μm ;
the trap repository has a diameter of approximately 450 μm ;
the trap microchannel has a width of approximately 40 μm ; and
30 the first microchannel, the second microchannel, the bypass microchannel, the trap
repository and the trap microchannel have a height of approximately 200 μm .
5. The microfluidic device as recited in claim 4, wherein the trap microchannel has a length

of approximately 100 μm .

6. The microfluidic device as recited in claim 1, wherein a hydrodynamic resistance ratio between the bypass microchannel and fluidic trap is from 1.0 to 2.0, and a hydrodynamic
5 resistance of the bypass microchannel is smaller than a hydrodynamic resistance of the fluidic trap.

7. The microfluidic device as recited in claim 1, wherein a hydrodynamic resistance ratio between the bypass microchannel and fluidic trap is from 1.4 to 1.6, and a hydrodynamic
10 resistance of the bypass microchannel is smaller than a hydrodynamic resistance of the fluidic trap.

8. The microfluidic device as recited in claim 1, wherein the one or more comprise at least four parking loops.

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9. The microfluidic device as recited claim 1, wherein the one or more microchannel networks comprise two or more microchannel networks forming an array microchannel networks.

20 10. The microfluidic device as recited in claim 1, wherein a diameter of the inlet opening and the outlet opening of the cover have a diameter of approximately 3 mm.

11. The microfluidic device as recited in claim 1, wherein:
the inlet port in the substrate comprises a reservoir; and
25 the inlet opening of the cover is aligned with the reservoir in the substrate.

12. A method of making a microfluidic device having one or more microchannel networks comprising:

forming an inlet port, a first microchannel, one or more parking loops, a second microchannel and an outlet port in a substrate for each microchannel network, wherein the first
30 microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, the one or more parking loops are connected between the first microchannel and the second microchannel, and each parking loop comprises a parking loop inlet, a parking loop output, a fluidic trap connected between the parking loop inlet and the parking loop output, and a bypass

microchannel connected to the parking loop inlet and the parking loop outlet;

forming an inlet opening and an outlet opening through a cover layer for each microchannel network; and

attaching the cover to a top of the substrate, wherein the inlet opening of the cover is disposed above the inlet port in the substrate and the outlet opening is disposed above the outlet port in the substrate for each microchannel network.

13. The method as recited in claim 12, wherein the fluidic trap comprises:

a trap repository connected to the parking loop inlet; and

a trap microchannel connecting the trap repository to the parking loop outlet, wherein a cross-sectional area of the trap microchannel is smaller than a cross-sectional area of the bypass microchannel.

14. The method as recited in claim 13, wherein the trap repository has a volume of 10, 20,

30, 40, 50, 60, 70, 80 or 90 nL.

15. The method as recited in claim 13, wherein:

the first microchannel, the second microchannel and the bypass microchannel have a width of approximately 200 μm and a height of approximately 200 μm ;

the trap repository has a diameter of approximately 450 μm ;

the trap microchannel has a width of approximately 40 μm ; and

the first microchannel, the second microchannel, the bypass microchannel, the trap repository and the trap microchannel have a height of approximately 200 μm .

16. The method as recited in claim 15, wherein the trap microchannel has a length of approximately 100 μm .

17. The method as recited in claim 12, further comprising repeating both forming steps to form an array microchannel networks.

18. The method as recited in claim 12, wherein a hydrodynamic resistance ratio between the bypass microchannel and fluidic trap is from 1.0 to 2.0, and a hydrodynamic resistance of the bypass microchannel is smaller than a hydrodynamic resistance of the fluidic trap.

19. The method as recited in claim 12, wherein a hydrodynamic resistance ratio between the bypass microchannel and fluidic trap is from 1.4 to 1.6, and a hydrodynamic resistance of the bypass microchannel is smaller than a hydrodynamic resistance of the fluidic trap.

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20. The method as recited in claim 12, wherein the one or more comprise at least four parking loops.

21. The method as recited in claim 12, wherein a diameter of the inlet opening and the outlet opening of the cover have a diameter of approximately 3 mm.

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22. The method as recited in claim 12, wherein:
the inlet port in the substrate comprises a reservoir; and
the inlet opening of the cover is aligned with the reservoir in the substrate.

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23. A method for trapping a sample within a microfluidic device having one or more microchannel networks comprising:

providing the microfluidic device comprising:

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a substrate having an inlet port, a first microchannel, one or more parking loops, a second microchannel and an outlet port for each microchannel network, wherein the first microchannel is connected to the inlet port, the second microchannel is connected to the outlet port, the one or more parking loops are connected between the first microchannel and the second microchannel, and each parking loop comprises a parking loop inlet, a parking loop output, a fluidic trap connected between the parking loop inlet and the parking loop output, and a bypass microchannel connected to the parking loop inlet and the parking loop output, and

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a cover attached to a top of the substrate, the cover having an inlet opening and an outlet opening through the cover for each microchannel network, wherein the inlet opening of the cover is disposed above the inlet port in the substrate and the outlet opening is disposed above the outlet port in the substrate;

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releasing a first oil into the inlet port of the substrate via the inlet opening using a pipette;
releasing the sample into the inlet port of the substrate via the inlet opening using the pipette;

filling each fluidic trap with the substance by creating a suction in the outlet port of the substrate via the outlet opening using the pipette;

releasing a second oil into the inlet port of the substrate via the inlet opening using the pipette; and

5 removing the sample from the microchannel network, except for each fluidic trap, by creating a suction in the outlet port of the substrate via the outlet opening using the pipette.

24. The method as recited in claim 23, wherein the fluidic trap comprises:

a trap repository connected to the parking loop inlet; and

10 a trap microchannel connecting the trap repository to the parking loop outlet, wherein a cross-sectional area of the trap microchannel is smaller than a cross-sectional area of the bypass microchannel.

25. The method as recited in claim 24, wherein the trap repository has a volume of
15 approximately 10, 20, 30, 40, 50, 60, 70, 80 or 90 nL.

26. The method as recited in claim 24, wherein:

the first microchannel, the second microchannel and the bypass microchannel have a width of approximately 200 μm and a height of approximately 200 μm ;

20 the trap repository has a diameter of approximately 450 μm ;

the trap microchannel has a width of approximately 40 μm ; and

the first microchannel, the second microchannel, the bypass microchannel, the trap repository and the trap microchannel have a height of approximately 200 μm .

25 27. The method as recited in claim 26, wherein the trap microchannel has a length of approximately 100 μm .

28. The method as recited in claim 23, further comprising the step of automatically controlling the pipette with a processor communicably coupled to the pipette.

30

29. The method as recited claim 28, wherein:

the one or more microchannel networks comprise two or more microchannel networks forming an array microchannel networks;

the pipette comprises one pipette for each microchannel network; and
the method is performed simultaneously for the microchannel networks.

30. The method as recited claim 23, wherein:

the one or more microchannel networks comprise at least a first microchannel network
and a second microchannel network;

the first microchannel network contains the sample having a first concentration; and
the second microchannel network contains the sample having a second concentration.

31. The method as recited claim 23, wherein:

the one or more microchannel networks comprise at least a first microchannel network
and a second microchannel network;

the sample comprises a first sample and a second sample;
the first microchannel network contains the first sample; and
the second microchannel network contains the second sample.

32. The method as recited in claim 23, wherein the sample comprises one or more drops,
cells or compositions.

33. The method as recited in claim 23, wherein a hydrodynamic resistance ratio between the
bypass microchannel and fluidic trap is from 1.0 to 2.0, and a hydrodynamic resistance of the
bypass microchannel is smaller than a hydrodynamic resistance of the fluidic trap.

34. The method as recited in claim 23, wherein a hydrodynamic resistance ratio between the
bypass microchannel and fluidic trap is from 1.4 to 1.6, and a hydrodynamic resistance of the
bypass microchannel is smaller than a hydrodynamic resistance of the fluidic trap.

35. The method as recited in claim 23, wherein the one or more comprise at least four
parking loops.

36. The method as recited in claim 23, wherein the one or more microchannel networks
comprise two or more microchannel networks forming an array microchannel networks.

37. The method as recited in claim 23, wherein a diameter of the inlet opening and the outlet opening of the cover have a diameter of approximately 3 mm.
38. The method as recited in claim 23, wherein:
- 5 the inlet port in the substrate comprises a reservoir; and
the inlet opening of the cover is aligned with the reservoir in the substrate.

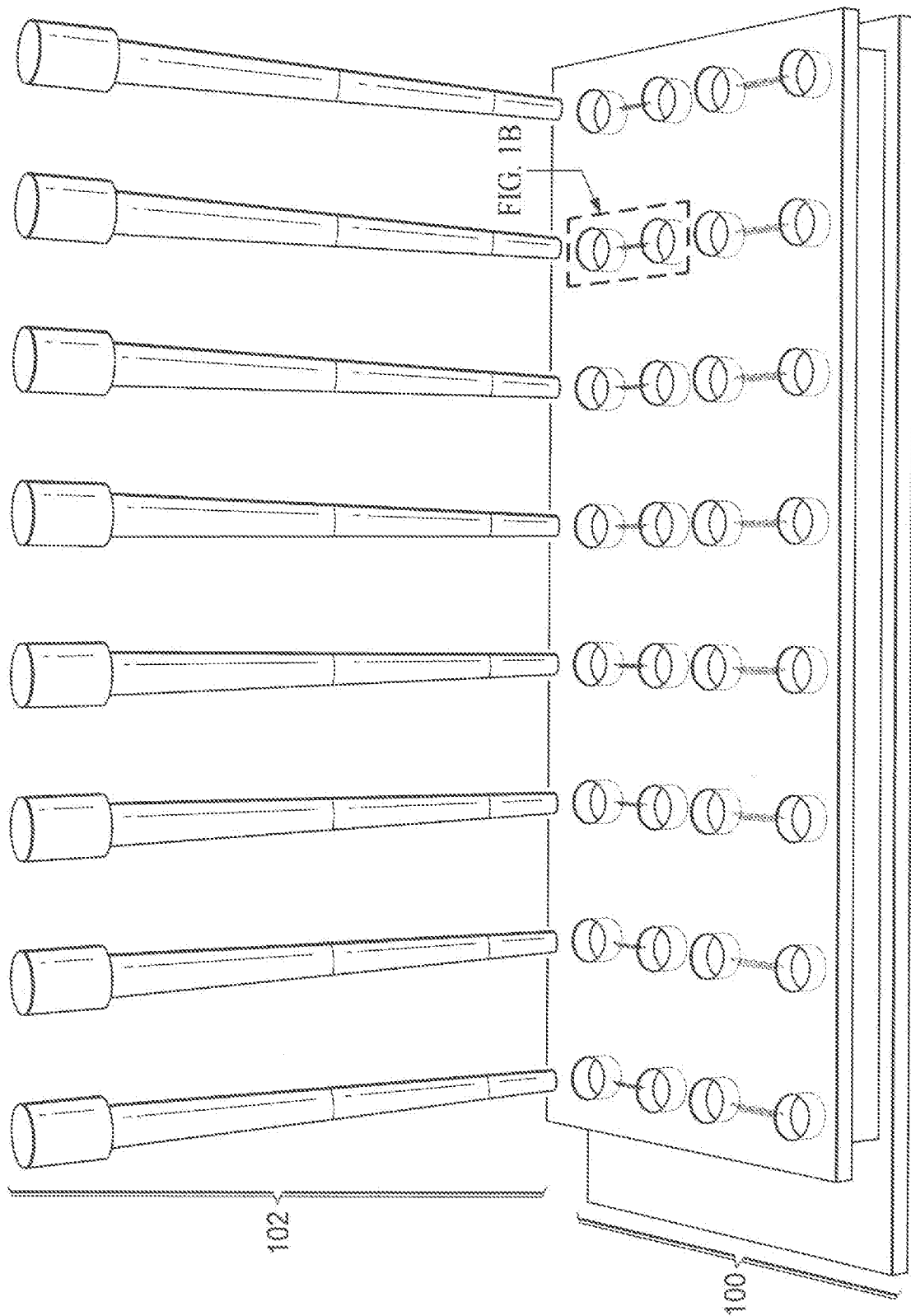


FIG. 1A

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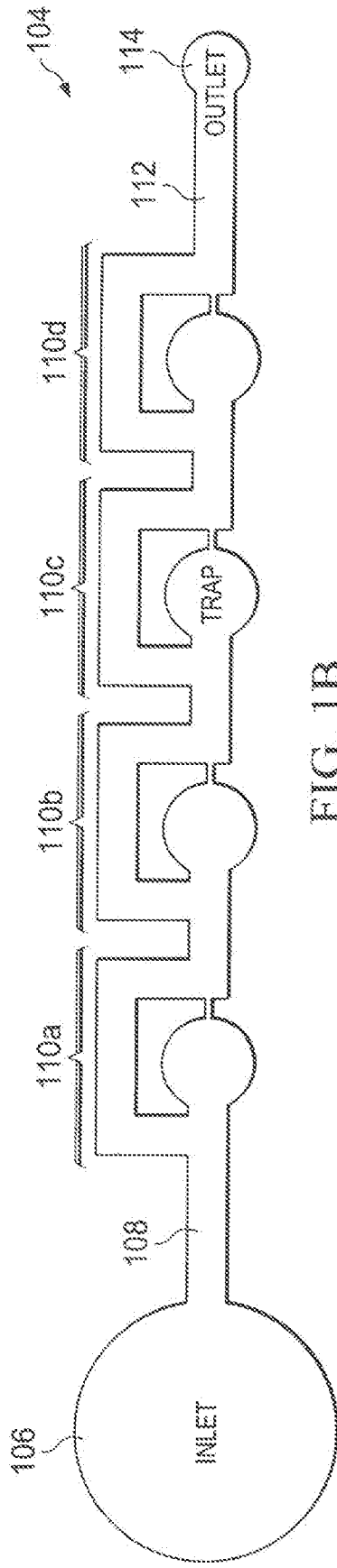


FIG. 1B

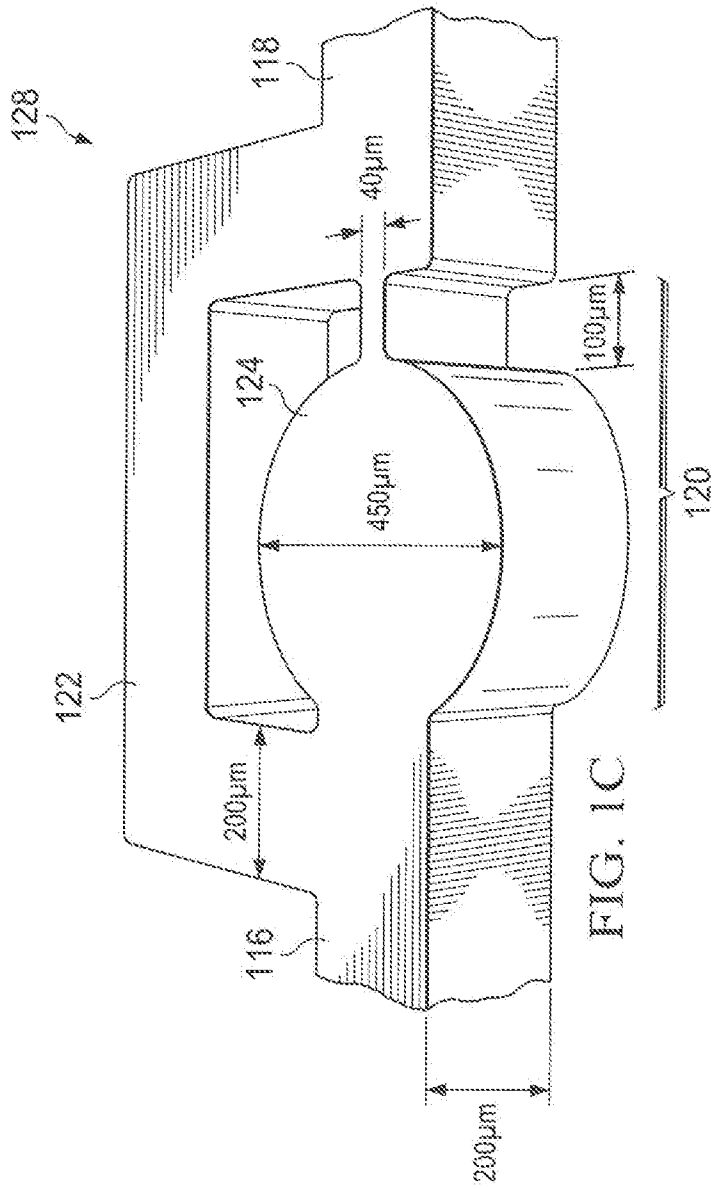


FIG. 1C

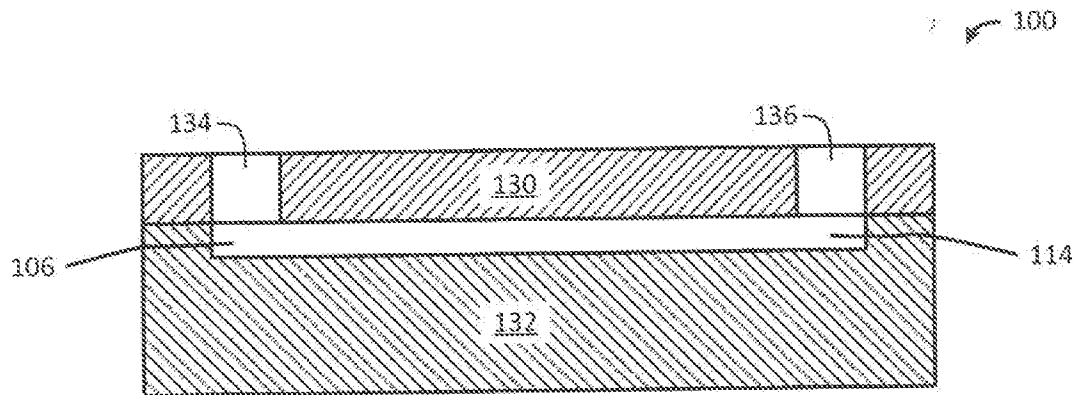


FIG. 1D

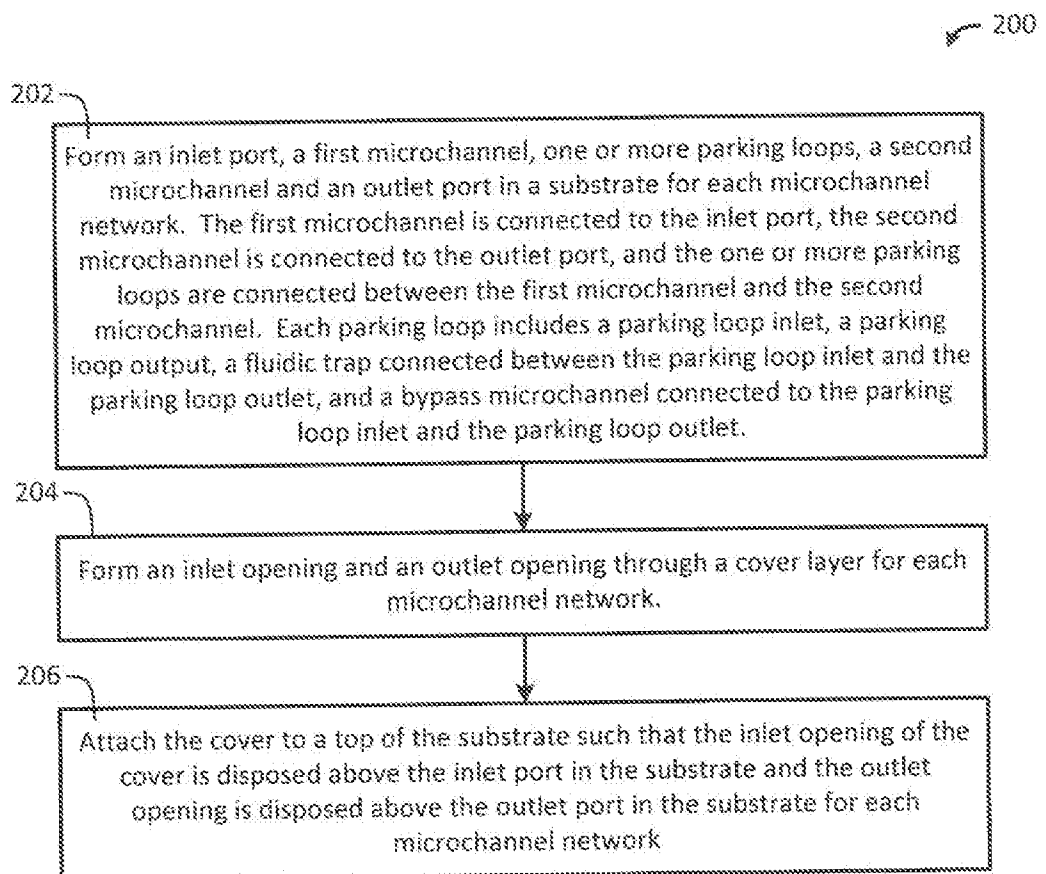


FIG. 2

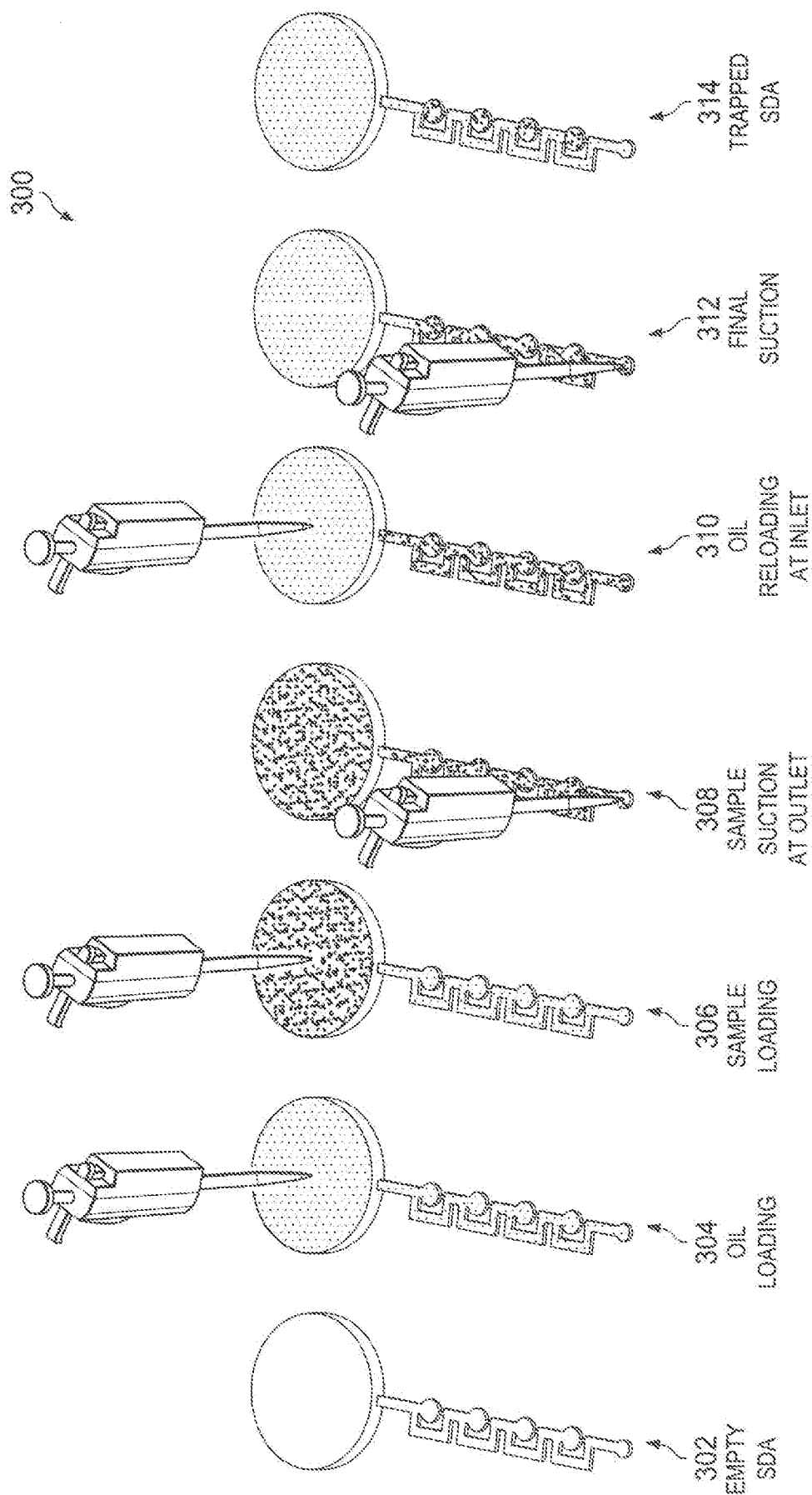


FIG. 3

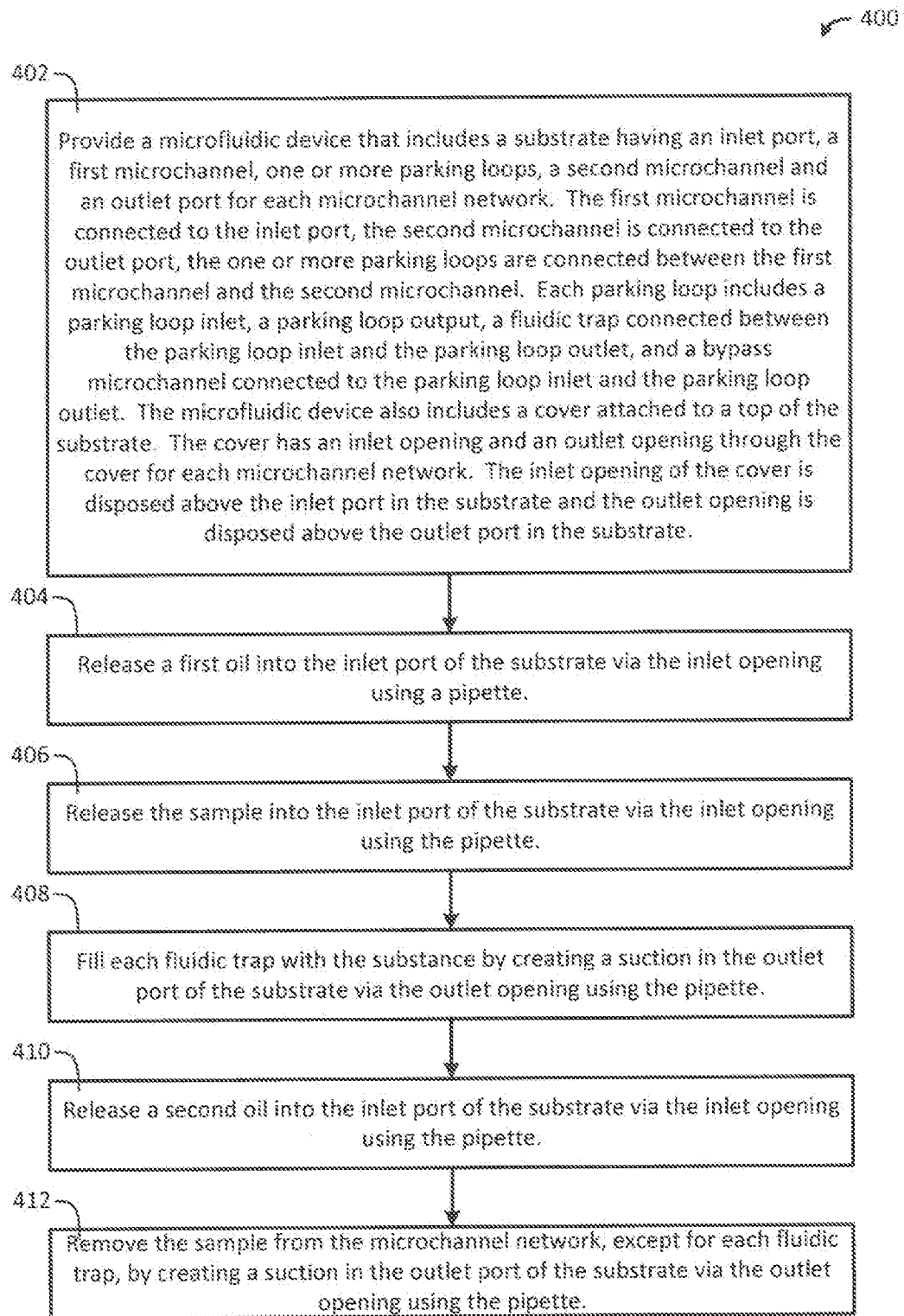


FIG. 4

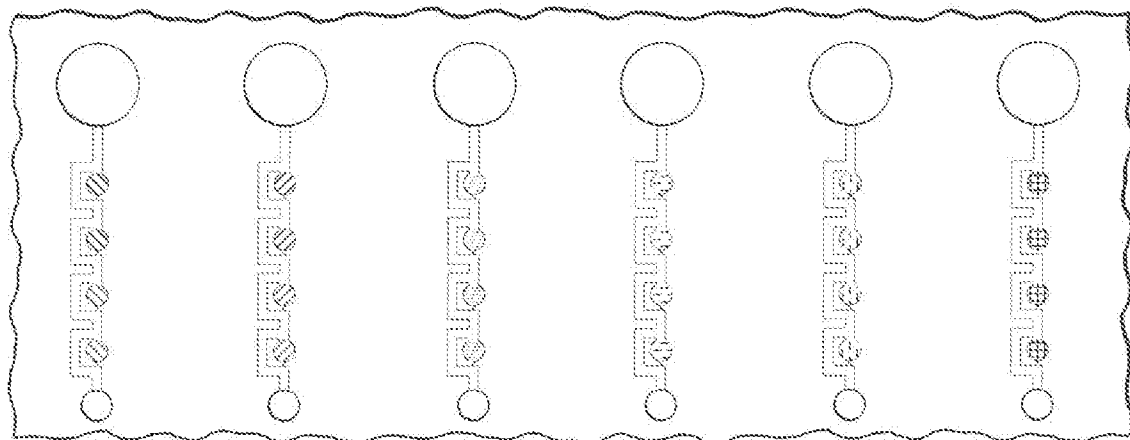


FIG. 5B

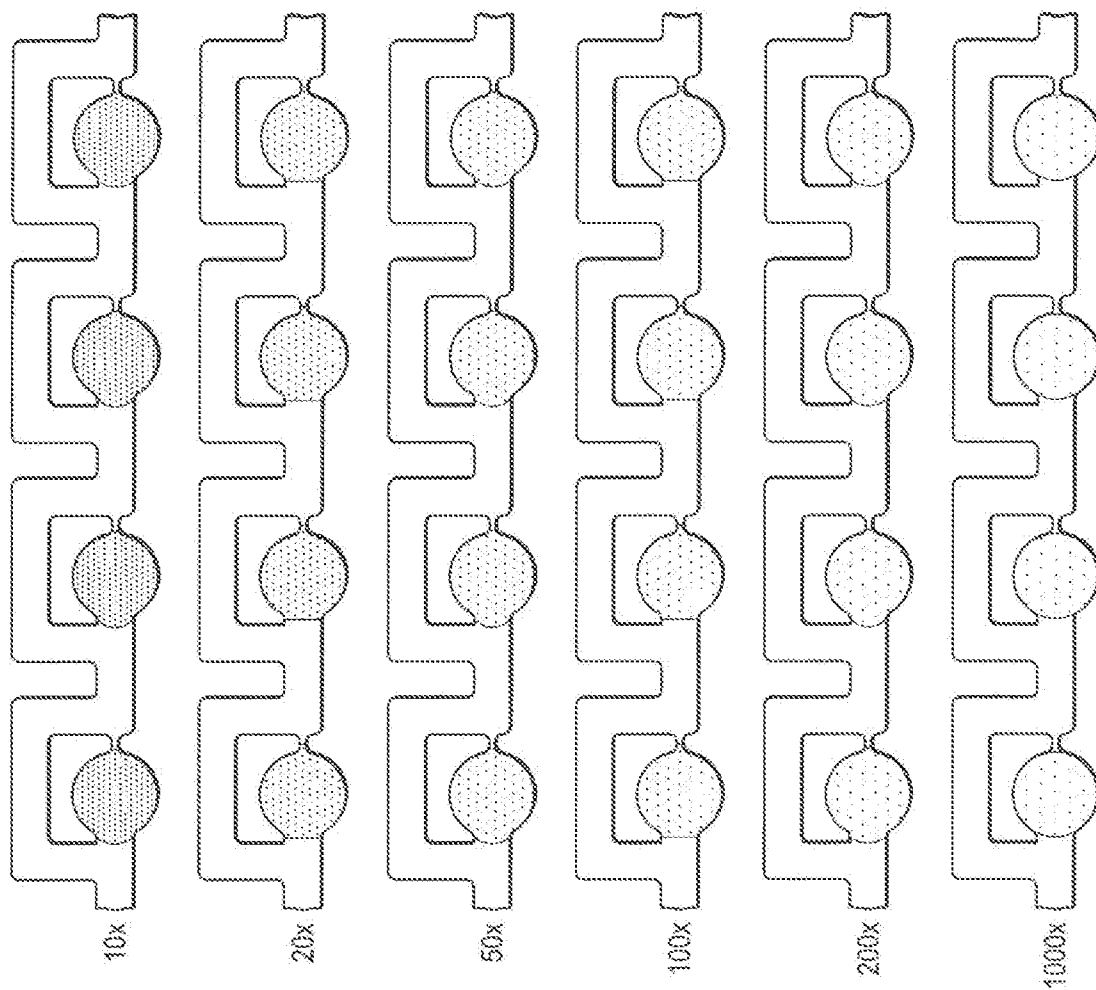


FIG. 5A

FIG. 6

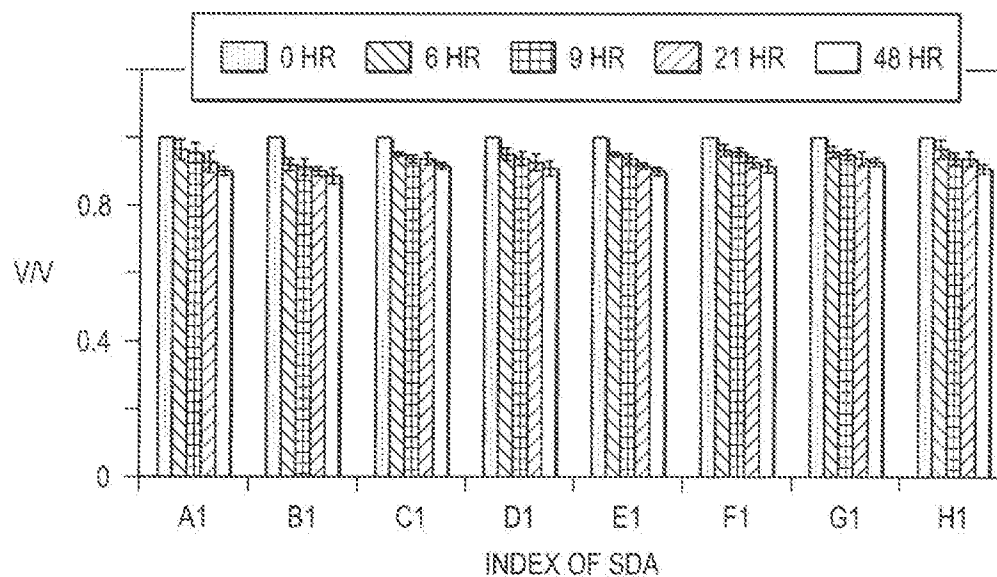
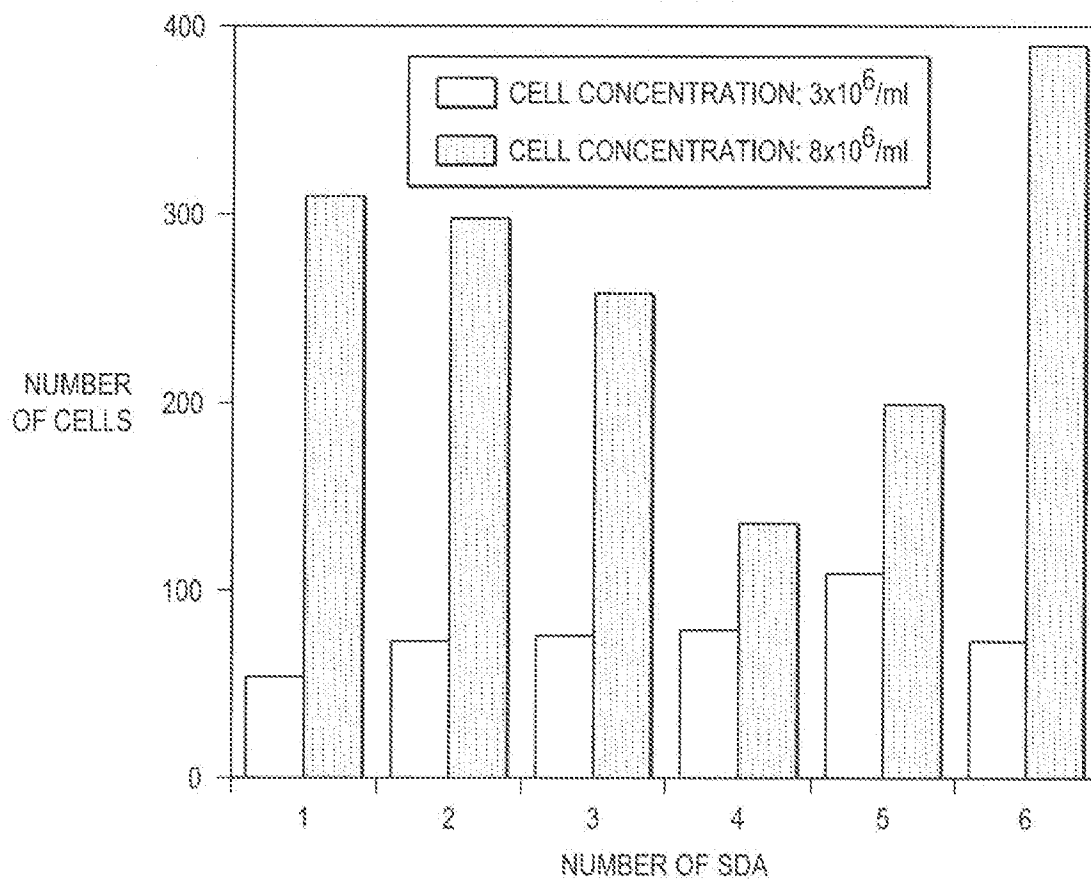
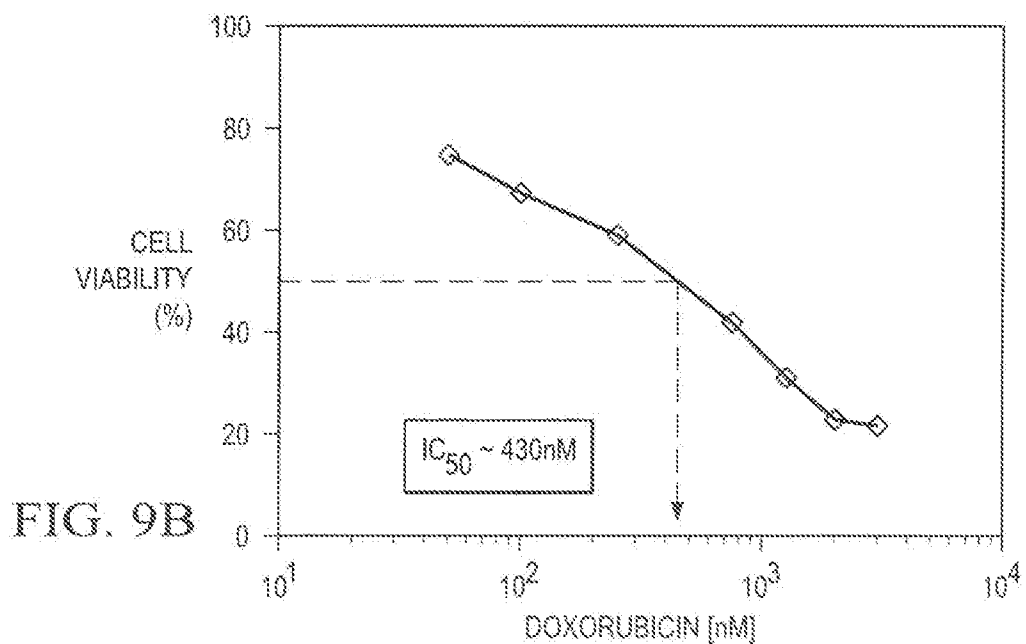
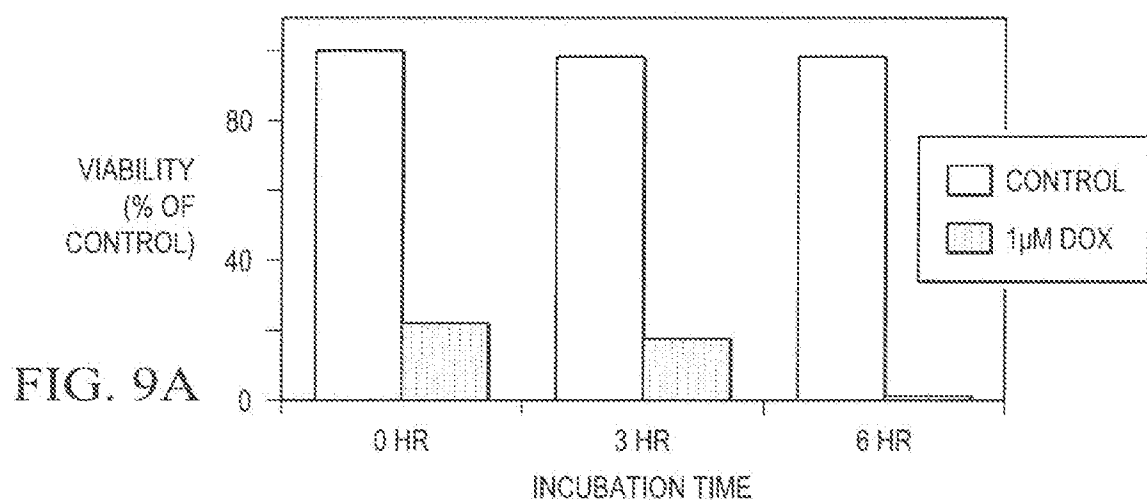
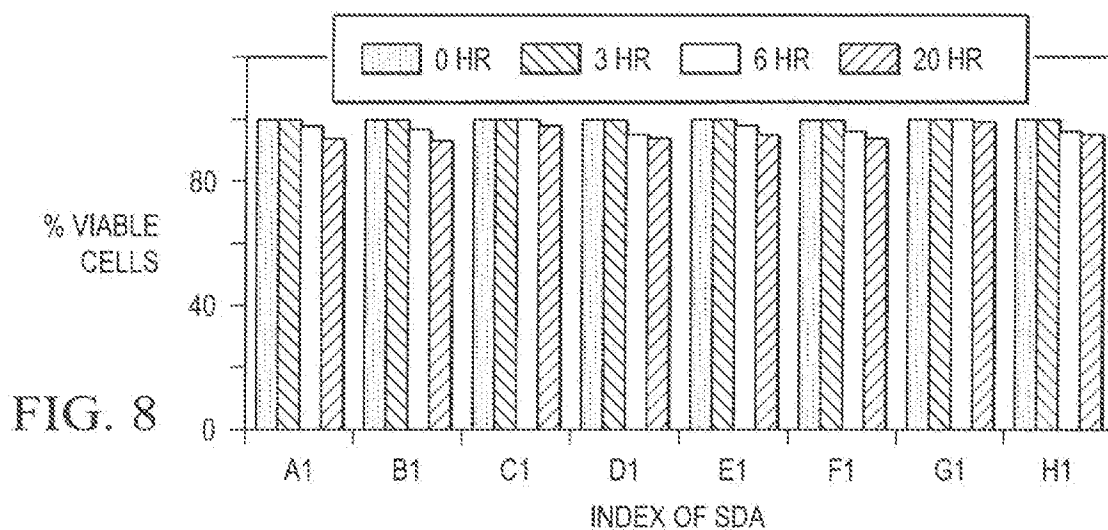


FIG. 7





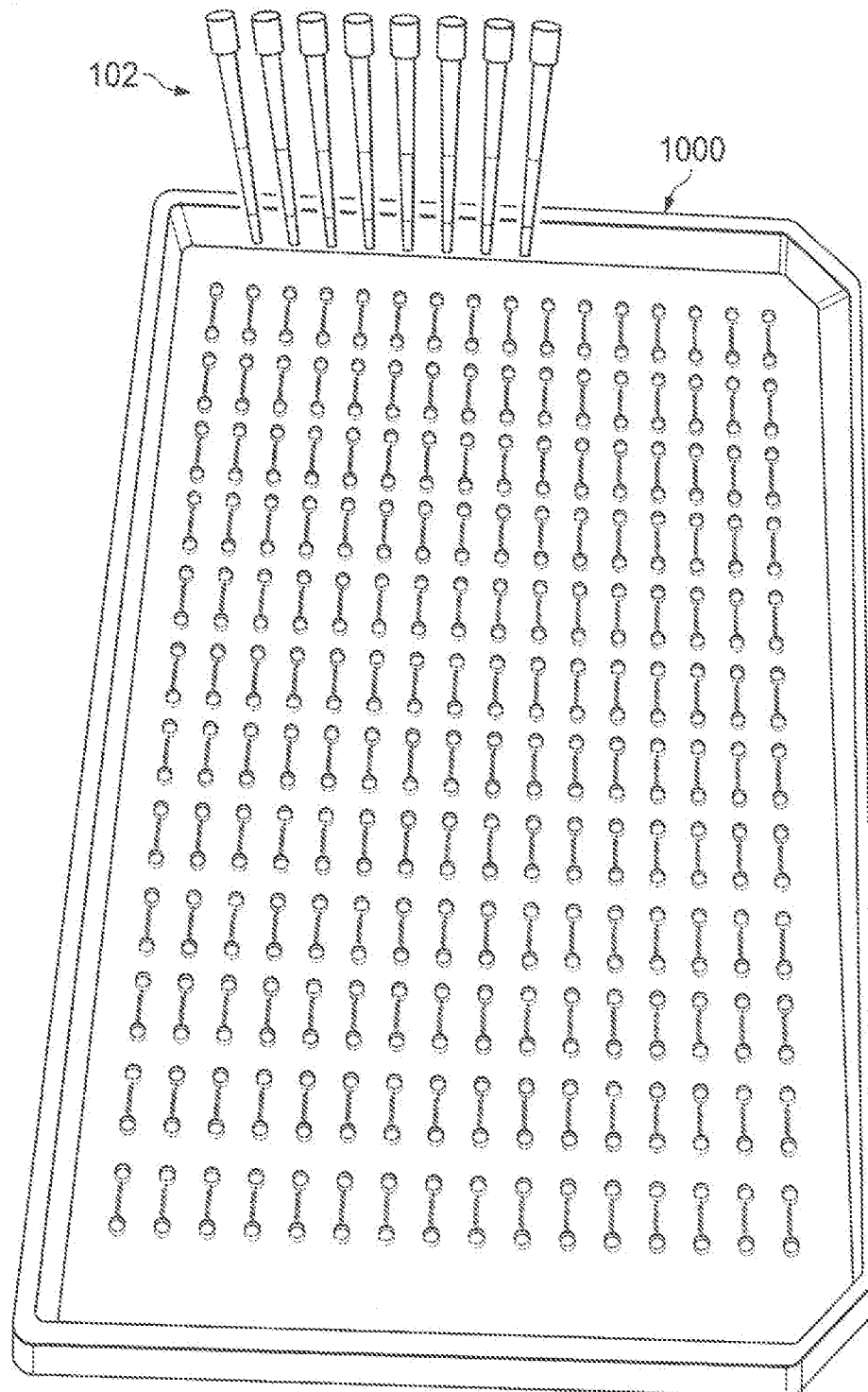


FIG. 10

A. CLASSIFICATION OF SUBJECT MATTER**B01L 3/00(2006.01)I**

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)

B01L 3/00; C12M 1/00; F15C 1/02; F17D 1/00; G01N 27/447

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & keywords: microfluidic device, microchannel, substrate, parking loop, fluidic trap, bypass

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014-0051062 A1 (VANAPALLI, S. A. et al.) 20 February 2014 See paragraphs [0010]–[0064]; claims 1–28; figures 1–12.	1–38
X	US 2010-0163109 A1 (FRADEN, S. et al.) 1 July 2010 See paragraphs [0060]–[0155]; claims 1–25; figures 1A–9.	1–38
X	US 2010-0252118 A1 (FRADEN, S. et al.) 7 October 2010 See paragraphs [0044]–[0155]; claims 1–6, 10–11, 15 and 24–32; figures 1A–11.	1–38
A	US 2009-0266421 A1 (LINDER, V. et al.) 29 October 2009 See the whole document.	1–38
A	US 2003-0138359 A1 (CHOW, A. W. et al.) 24 July 2003 See the whole document.	1–38



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

13 September 2016 (13.09.2016)

Date of mailing of the international search report

26 September 2016 (26.09.2016)

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/036787

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014-0051062 A1	20/02/2014	WO 2012-154688 A2 WO 2012-154688 A3	15/11/2012 10/01/2013
US 2010-0163109 A1	01/07/2010	US 2014-0295572 A1 US 2015-0118761 A1 US 8772046 B2 US 9017623 B2 WO 2008-097559 A2 WO 2008-097559 A3	02/10/2014 30/04/2015 08/07/2014 28/04/2015 14/08/2008 09/10/2008
US 2010-0252118 A1	07/10/2010	US 2014-0246098 A1 US 2015-0276562 A1 US 8592221 B2 US 9068699 B2 WO 2008-130623 A1	04/09/2014 01/10/2015 26/11/2013 30/06/2015 30/10/2008
US 2009-0266421 A1	29/10/2009	EP 2285491 A1 US 2012-0241013 A1 US 8222049 B2 WO 2009-131677 A1	23/02/2011 27/09/2012 17/07/2012 29/10/2009
US 2003-0138359 A1	24/07/2003	AU 1999-45529 A1 AU 1999-45529 B2 CA 2332919 A1 EP 1084391 A1 US 6274089 B1 US 6551836 B1 WO 99-64836 A1	30/12/1999 16/05/2002 16/12/1999 21/03/2001 14/08/2001 22/04/2003 16/12/1999