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(54) **MICROFLUIDIC DEVICE DEFINING AN ARRAY OF SAMPLE CHAMBERS**

(57) A microfluidic device defining an array of sample chambers (211, 212, 221, 222) comprises a bottom control channel layer (1), a sample channel layer (2) and a top control channel layer (3). The bottom control channel layer defines a plurality of bottom control channels (101, 102). The sample channel layer defines at least one microfluidic flow path. The top control channel layer (3) defines a plurality of top control channels (310, 320). Bottom

valves (111, 112, 121, 122) are deflectable into the microfluidic flow path from below; and top valves (311, 312, 321, 322) are deflectable into the microfluidic flow path from above. A particular sample chamber is accessible to a fluid flow in the microfluidic flow path only if both the associated bottom valve and the associated top valve are open.

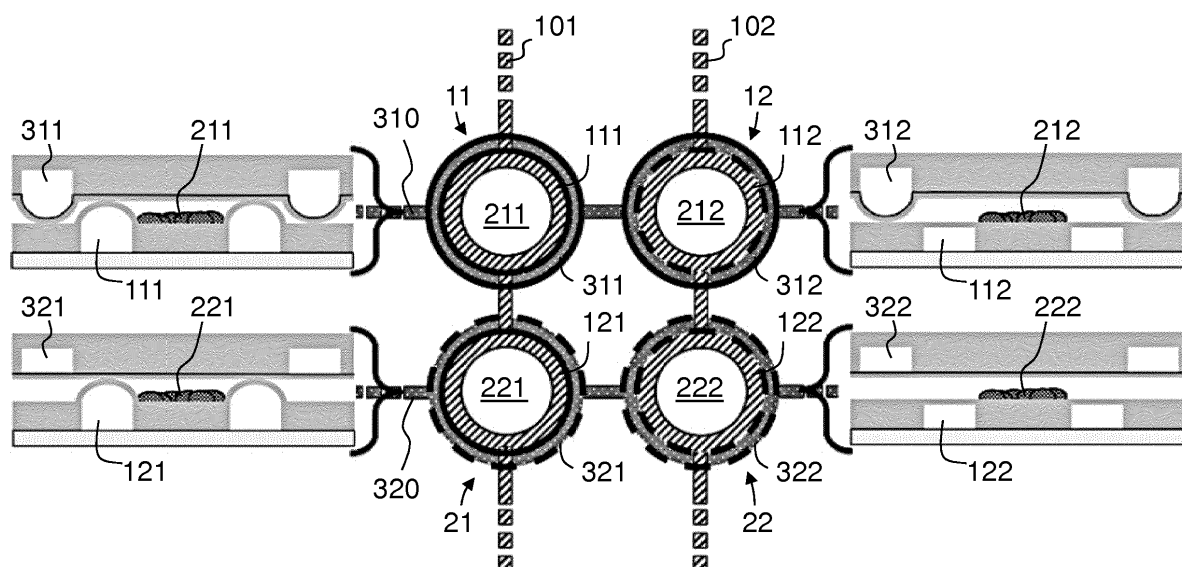


FIG. 7

Description

TECHNICAL FIELD

[0001] The present invention relates to a microfluidic device defining an array of individually addressable sample chambers and to a method of isolating a fluid volume with such a device.

PRIOR ART

[0002] As the need grows to screen larger and larger subsets of conditions for a given biological question, new approaches that increase throughput and decrease costs will be needed to enhance the capabilities and benefits from new biotechnologies. For example, in the post-genomic era, genome sequencing has become cheap enough to become a ubiquitous tool across clinical medicine and basic biological research. These developments make in-depth characterization of genetic factors possible for a wide variety of clinical and research applications, and scientists are increasingly required to provide genome or proteome-scale analysis to explain their conclusions.

[0003] One class of the high-throughput tools to facilitate these new trends is automated fluid handling. This technique has been a ubiquitous tool for researchers across nearly every chemical, biological, and medical discipline for the last few decades. Automated plate-handling systems and fluidic screening platforms have been developed to streamline and accelerate drug screens and basic research in academic and commercial settings. Researchers have pushed towards miniaturization and microtechnology to bring about the next generation of cell culture tools with more throughput and unique capabilities.

[0004] Microfluidics is a prime example of microtechnology applied to high-throughput biology. This automated technology relies on microfabrication techniques from the microcircuit industry to create devices with micron-scale networks of fluid carrying channels to manipulate cells and chemicals with unprecedented precision and reagent consumption savings. A review is provided in Dittrich PS, Manz A, Lab-on-a-chip: microfluidics in drug discovery, Nature Reviews Drug Discovery 5, 2006, 210-218.

[0005] Specifically, multiplexing device logic has been suggested on a chip with two channel layers stacked above each other (Thorsen T, Maerkl SJ, Quake SR, Microfluidic large-scale integration, Science 298, 2002, pp. 580-584). One layer contains elastomeric valves that manipulate biological samples residing on the other layer. Each of these valves is simply a straight channel delimited by a membrane that elastically deforms into the sample channel residing above or below the valve, effectively blocking the fluid flow through this channel. The device logic relies primarily on single-layer multiplexing, which minimizes the number of valves needed to address

any combination of samples on the device. In this approach the valves block off specific subsets of sample channels in a manner that allows one to address specific sample chambers. With a single valve layer, there are a limited number of valves that can occupy a given area on the device, and thus the number of samples one can individually control on these devices is restricted. A review of these techniques is provided in Melin J, Quake SR. Microfluidic large-scale integration: the evolution of design rules for biological automation, Annual Review of Biophysics and Biomolecular Structure 36, 2007, pp. 213-231.

[0006] Eyer K, Kuhn P, Hanke C, Dittrich PS, A micro-chamber array for single cell isolation and analysis of intracellular biomolecules, Lab on a Chip 12, 2012, 765-772 discloses another microfluidic device having a single valve layer.

[0007] New approaches to sample addressing and control are required to overcome the limitations of two-layer devices and to increase sample density and device capabilities of microfluidic automated systems.

SUMMARY OF THE INVENTION

[0008] It is an object of the present invention to provide a microfluidic device that enables simplified sample addressing and control.

[0009] This object is achieved by the microfluidic device of claim 1. Further embodiments of the invention are laid down in the dependent claims.

[0010] The present invention thus provides a microfluidic device defining an array of sample chambers. The microfluidic device comprises:

- a bottom control channel layer, the bottom control channel layer defining a plurality of separate bottom control channels;
- a sample channel layer arranged on top of the bottom control channel layer, the sample channel layer defining at least one microfluidic flow path for a fluid flow,
- a top control channel layer arranged on top of the sample channel layer, the top control channel layer defining a plurality of separate top control channels;
- a plurality of bottom valves, each bottom valve being connected to one of the bottom control channels and being capable of actuation into the microfluidic flow path from below so as to control the fluid flow in the microfluidic flow path;
- and
- a plurality of top valves, each top valve being connected to one of the top control channels and being capable of actuation into the microfluidic flow path from above so as to control the fluid flow in the microfluidic flow path,

wherein the sample chambers are arranged in the sample channel layer, access to each sample chamber being controllable by at least one of the bottom valves and at

least one of the top valves in such a manner that said sample chamber is accessible to the fluid flow in the microfluidic flow path only if both the associated bottom valve and the associated top valve are open. In other words, the sample chamber can be isolated from the fluid flow by closing the associated bottom valve, by closing the associated top valve, or by closing both the associated bottom valve and top valve.

[0011] According to the present invention, the sample channel layer is sandwiched between a bottom control channel layer and a top control channel layer. The sample channel layer defines a microfluidic flow path. In the context of the present invention, a microfluidic flow path is to be understood as a flow path that has, along at least one direction transverse to the direction of flow, a width or height below 1 millimeter, in particular, below 450 micrometers. Access to each sample chamber is commonly controlled by at least two pneumatic or hydraulic valves. One of these valves is a bottom valve or push-up valve controlled through the bottom control channel layer, whereas the other valve is a top valve or push-down valve controlled through the top control channel layer. A selected sample chamber becomes accessible only if both the associated bottom valve and the associated top valve are opened. In any other situation, the sample chamber will remain isolated from the fluid flow.

[0012] The presently proposed sandwich configuration has the potential to increase sample density by orders of magnitude as compared to devices with only a single control channel layer. The method allows one to address, control, and incubate chemical or biological samples (e.g., cells and biochemicals) in micron-scale chambers on a device without cross-contamination or sample loss. In terms of automated screens, the design enables the highest-known density of individually addressable and completely segregated samples achievable on a planar surface of a microfluidic device. The presently proposed unique three-layer sandwich design inherently doubles the density of valves on the device over the current state-of-the-art.

[0013] Since the bottom valves and the top valves are addressed through different control channel layers, a very simple addressing scheme becomes possible. In particular, the bottom valves can be arranged in a plurality of columns, and the bottom valves in each column can be in fluidic communication with a common bottom control channel (column line), so that all these bottom valves can be collectively opened and closed by a pneumatic or hydraulic pressure change in the associated bottom control channel. On the other hand, the top valves can be arranged in a plurality of rows, the rows running across the columns (i.e., at a non-zero angle relative to the columns), and the top valves in each row can be in fluidic communication with a common top control channel (row line), allowing all top valves in a row to be collectively opened and closed by a pressure change in the associated top control channel. A selected sample chamber can be selectively addressed by the column of its asso-

ciated bottom valve and by the row of its associated top valve. For instance, the selected sample chamber can be selectively opened to the microfluidic flow path by depressurizing both the bottom control channel of the associated column and the top control channel of the associated row.

[0014] Preferably, the bottom control channels are parallel to each other and evenly spaced from one another if viewed in the device plane. Likewise, it is preferred that the top control channels are parallel to each other evenly spaced from one another.

[0015] Advantageously, the sample chambers and the associated bottom and top valves can be arranged in a rectangular array, in particular, in a square array. In this case, it is advantageous if the rows run perpendicular (at a 90° angle) to the columns. In this case, the top control channels would advantageously likewise run perpendicular to the bottom control channels. However, any other arrangement of the sample chambers is conceivable, for instance a trigonal arrangement of sample chambers. In this case, it is advantageous if with the rows (or top control channels) run at an angle of 60° to the columns (or bottom control channels).

[0016] A particularly simple configuration of the valves results if the bottom valves are defined by a section of one of the bottom control channels and a bottom elastomeric membrane that is arranged between said section and the microfluidic flow path, said bottom elastomeric membrane being deflectable into the microfluidic flow path so as to control the fluid flow in the microfluidic flow path from below, and if each top valve is defined by a section of one of the top control channels and a top elastomeric membrane that is arranged between said section and the microfluidic flow path, said top elastomeric membrane being deflectable into the microfluidic flow path so as to control the fluid flow in the microfluidic flow path from above. Such a device can be manufactured very cost-efficiently, and no further mechanical components are required for fabricating the valves in the device.

[0017] An important aspect of such a device is that the device comprises membrane portions that form the valves, these membrane portions being elastomeric. The rest of the device does not necessarily need to be elastomeric. The membrane portions of the bottom valves can be formed by the bottom control channel layer, by the sample channel layer, or by a separate bottom membrane layer that is disposed between the bottom control channel layer and the sample channel layer. Likewise, the membrane portions of the top valves can be formed by the top control channel layer, by the sample channel layer, or by a separate top membrane layer that is disposed between the top control channel layer and the sample channel layer.

[0018] In order to improve the sealing action of the valves, the membrane portions that form the valves can be provided with sealing elements, in particular, with sealing ridges that extend from the respective membrane into the microfluidic flow channel. In particular, each bot-

tom valve can comprise a bottom sealing ridge that is provided on the bottom elastomeric membrane, the bottom sealing ridge bearing against a top defining surface of the microfluidic flow path when the bottom valve is closed and being arranged at a distance from the top defining surface when the bottom valve is open. Additionally or in the alternative, each top valve can comprise a top sealing ridge that is provided on the top elastomeric membrane, the top sealing ridge bearing against a bottom defining surface of the microfluidic flow path when the bottom valve is closed and being arranged at a distance from the bottom defining surface when the bottom valve is open. The sealing ridges and the membrane together have a thickness that is greater than the thickness of the membrane alone. Thereby the sealing ridges provide a greater stiffness than the membrane itself. Additionally, the shorter distance between these ridges and the sealing surface, relative to valves without these ridges, potentially allows sealing at a lower pressure applied in either control layer. This capability is due the shorter deformation distance for sealing with these ridged valves.

[0019] In order to improve reliable operation of the bottom and top valves, certain dimensional considerations should advantageously be observed. In particular, it is advantageous if the height of the microfluidic flow path (as measured perpendicular to the layer plane defined by the sample channel layer) and the thickness of the bottom or top elastomeric membrane (as measured perpendicular to said layer plane), in the region of each bottom or top valve, has a ratio between 1 and 7. In absolute numbers, a possible range for the height of the microfluidic flow path, in the region of each bottom or top valve, is 5-35 micrometers. A possible range for the thickness of the bottom or top elastomeric membrane, in the region of each bottom or top valve, is 5-35 micrometers.

[0020] Further dimensional considerations apply to the sections of the bottom control channels that define a bottom valve and to the sections of the top control channels that form a top valve. It is advantageous if these sections have a minimum width and length as compared to the thickness of the bottom and top elastomeric membranes. In particular, both the width and length of each section and the thickness of the bottom or top elastomeric membrane, in the region of each bottom or top valve, may have a ratio of at least 3, preferably of 3-20. In absolute numbers, the width and length of each section may advantageously be in the range of 32-280 micrometers.

[0021] The device can be manufactured in a particularly cost-efficient manner if at least one of the bottom control channel layer, the sample channel layer and the top control channel layer is itself made of an elastomeric material. Specifically, in this case, the bottom elastomeric membranes of the bottom valves and/or the top elastomeric membranes of the top valves can be an integral part of (i.e., integrally formed with) the bottom control channel layer, the sample channel layer, and/or the top control channel layer. Advantageously all three layers are made of elastomeric materials to enable simplified

production.

[0022] In particular, the microfluidic device can comprise a substrate, and the bottom control channel layer can be disposed on top of the substrate. The bottom control channels can then be formed by recesses in the bottom control channel layer and can be commonly delimited by the substrate and by the bottom control channel layer, the bottom control channel layer forming the elastomeric membranes between the bottom control channels and the microfluidic flow path. The microfluidic flow path can be formed by at least one recess in the sample channel layer and can be commonly delimited by the bottom control channel layer and the sample channel layer, the sample channel layer forming the elastomeric membranes between the top control channels and the microfluidic flow path. The top control channels can be formed by recesses in the top control channel layer and can be commonly delimited by the sample channel layer and the top control channel layer.

[0023] The advantages of the novel approach are evident in devices fabricated between millimeter and sub-micron scales. The device can be manufactured using a variation of soft-lithography, in which a photo-activated polymer is coated on a silicon wafer that has been etched with micron-scale features. These features serve as a mold to imprint fluid channels into an elastomeric material (e.g., into a silicone material like polydimethylsiloxane, PDMS) that allows for mechanical deformation or actuation of valve-like structures from above and below the sample channel layer in a manner that allows complete sealing and isolation of the sample from the rest of the device. Possible elastomeric materials include PDMS, other silicones, SU-8, PMMA, Epoxy, thiol-ene (e.g., NOA 61 available from Norland Products), Thermoset Polyester (TPE), Polyurethane Methacrylate (PUMA) or (other) thermoplastic elastomers. For biological applications, the material should be biocompatible.

[0024] In some embodiments, each sample chamber is connected to an individual, separate inlet channel and an individual, separate outlet channel in the sample channel layer. Specifically, the inlet and outlet channels can be formed by recesses in the sample channel layer. Each sample chamber can then be associated with a first bottom valve forming a bottom inlet valve for closing off the inlet channel of said sample chamber from below, and with a first top valve forming a top inlet valve for closing off the inlet channel of said sample chamber from above. The bottom inlet valve and the top inlet valve can be arranged in series along the inlet channel, or they can overlap when viewed in a projection onto the device plane. Each sample chamber can further be associated with a second bottom valve forming a bottom outlet valve for closing off the outlet channel of said sample chamber from below and with a second top valve forming a top outlet valve for closing off the outlet channel of said sample chamber from above. Again, the bottom outlet valve and the top outlet valve can be arranged in series along the outlet channel, or they can overlap completely or par-

tially when viewed in a projection onto the device plane. In this manner, it becomes possible to precisely control to direction of flow through each sample chamber.

[0025] In such embodiments, it may be desirable to open or close the bottom inlet and outlet valves of each sample chamber together, and/or to open or close the top inlet and outlet valves of each sample chamber together. To this end, the bottom inlet valve and the bottom outlet valve of each sample chamber can be interconnected with each other and/or can be in fluidic communication with a common bottom control channel, and/or the top inlet valve and the top outlet valve of each sample chamber can be interconnected with each other and/or can be in fluidic communication with a common top control channel.

[0026] In order to be able to efficiently feed samples to the sample chambers and to efficiently remove samples from the sample chambers, the sample chambers can be arranged in a plurality of rows or columns, wherein the inlet channels of the sample chambers in each row or column are connected to a common sample distribution channel, and wherein the outlet channels of the sample chambers in each row or column are connected to a common sample collection channel. While in some embodiments a separate sample distribution channel and a separate sample collection channel for each row or column can be provided in order to avoid cross-contamination, in other embodiments the sample distribution channel and the sample collection channel of each row or column can coincide, thereby simplifying the layout of the device.

[0027] In some embodiments, each bottom valve has an annular shape, surrounding one of the sample chambers, and each top valve has an annular shape, likewise surrounding one of the sample chambers and being arranged concentrically with the associated bottom valve. In this manner, the sample chambers can laterally be delimited exclusively by the top and bottom valves. No additional structures are needed in the sample channel layer for defining the sample chambers. There is even no requirement of additional structures for defining the microfluidic flow path, i.e., the microfluidic flow path can be essentially a single large cavity. This enables rapid flushing of the sample channel layer. The diameter of the annular top valve can be larger or smaller than the diameter of the annular bottom valve, so that one of these valves surrounds the other when viewed in a projection onto the device plane. In other embodiments, the diameters of the top and bottom valve can be the same, the top and bottom valve overlapping completely or partially when viewed in a projection onto the device plane.

[0028] In order to prevent the microfluidic flow path in the sample channel layer from collapsing, which can in particular be a problem in the absence of additional structures for defining the microfluidic flow path, the sample channel layer can comprise an array of supporting pillars, which are laterally arranged between the sample chambers and/or inside the sample chambers, the pillars ex-

tending all the way from the bottom control channel layer to the top control channel layer, thereby bridging the entire gap between the bottom control channel layer and the top control channel layer to ensure a defined distance between these layers.

[0029] A corresponding method of isolating a fluid volume in a selected sample chamber of a microfluidic device of the type described above comprises:

- opening both the top valves and the bottom valves associated with said selected sample chamber; causing a fluid flow through the selected sample chamber;
- closing both the top valves and the bottom valves associated with the selected sample chamber.

[0030] The method can further comprise flushing the microfluidic flow channel with a flushing fluid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] Preferred embodiments of the invention are described in the following with reference to the drawings, which are for the purpose of illustrating the present preferred embodiments of the invention and not for the purpose of limiting the same. In the drawings,

- Fig. 1 shows a highly schematic plan view of a microfluidic device defining an array of sample chambers, together with bottom and top control channels;
- Fig. 2 shows a schematic plan view of four sample chambers of a microfluidic device according to a first embodiment of the present invention, together with their associated valves;
- Fig. 3 shows a schematic perspective cross-sectional view of one of the sample chambers in Fig. 2, the plane of cross section being plane A-A indicated in Fig. 2, together with its associated valves;
- Fig. 4 shows a schematic cross section of the sample chamber in Fig. 3 with valves open (part (a)) and valves closed (part (b));
- Fig. 5 shows a schematic exploded view of the four sample chambers and associated valves in Fig. 2;
- Fig. 6 shows a schematic perspective view of the four sample chambers and associated valves in Fig. 2;
- Fig. 7 shows an illustration of the four sample chambers and associated valves in Fig. 2 with four different valve states;
- Fig. 8 shows a sequence of steps in a method of isolating a sample volume in a selected sample chamber;

- Fig. 9 shows a photograph of a selected sample chamber of a prototype device with living fibroblast cells in the sample chamber;
- Fig. 10 shows the layout of a microfluidic device comprising a 16 x 16 array of 256 sample chambers;
- Fig. 11 shows a schematic cross-sectional view of one of the sample chambers with its associated valves according to a variant of the first embodiment, part (a) illustrating how the sample chamber comprises a well, and part (b) illustrating how the valves comprise annular sealing ridges;
- Fig. 12 shows a schematic cross section of the sample chamber in Fig. 11 with valves open (part (a)) and valves closed (part (b));
- Fig. 13 and 14 show schematic illustrations of the manufacturing process for the variant of Figs. 11 and 12;
- Fig. 15 shows a schematic plan view of four sample chambers and associated valves of a microfluidic device according to a second embodiment of the present invention;
- Fig. 16 shows a schematic exploded view of the four sample chambers and associated valves in Fig. 15;
- Fig. 17 shows a schematic perspective view of the four sample chambers and associated valves in Fig. 15;
- Fig. 18 shows a schematic plan view of four sample chambers and associated valves of a microfluidic device according to a variant of the second embodiment; and
- Fig. 19 shows a schematic exploded view of the four sample chambers and associated valves in Fig. 18.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0032] Figure 1 illustrates the multiplexing concept used in the present specification.

[0033] Fig. 1(a) shows a highly schematic plan view of a microfluidic device defining a two-dimensional rectangular array of pixels. Each pixel comprises a sample chamber with associated pneumatically or hydraulically actuated bottom valves (push-up valves) and top valves (push-down valves). The setup of the pixels will be explained in more detail below with reference to Figures 2-17. The pixels are arranged in rows and columns. Bottom control channels interconnect the bottom valves of all pixels that belong to the same column (they can also be called "column lines"), and top control channels interconnect the top valves of all pixels that belong to the

same row (they can also be called "row lines").

[0034] Reference numbers are assigned to the various structures according to the following numbering scheme, which will be adhered to throughout this specification:

- 5 Each pixel is assigned a two-digit number, the first digit indicating the row and the second digit indicating the column. For instance, pixel 34 is the pixel in row 3 and column 4. Each bottom valve, sample chamber, top valve and control channel is assigned a three-digit number. For
10 bottom valves, the first digit is always 1; for sample chambers, the first digit is always 2; and for top valves, the first digit is always 3. The second and third digits identify the pixel in which these structures are located. For instance, reference number 221 would refer to a sample chamber (first digit = 2) in row 2 (second digit = 2) and column 1 (third digit = 1). Bottom control channels (column lines) are assigned a three-digit number as follows: first digit = 1, second digit = 0, third digit = column number. Top control channels (row lines) are assigned a three-digit number as follows: first digit = 3, second digit = row number, third digit = 0.

[0035] Fig. 1(b) illustrates how a single selected pixel is addressed. In the present example, pixel 11 is addressed by simultaneously activating bottom control channel (column line) 101 and top control channel (row line) 310.

[0036] Instead of addressing only a single pixel, it is also possible to simultaneously address a plurality of pixels. This is illustrated in Fig. 1(c). In the present example, pixels 33 and 34 are addressed simultaneously by simultaneously activating bottom control channels (column lines) 103 and 104 and top control channel (row line) 330.

[0037] Figures 2-10 illustrate a first embodiment of a microfluidic device according to the present invention.

- 35 **[0038]** Whereas the microfluidic device can comprise a large two-dimensional array of pixels, only four of these pixels in a 2 x 2 arrangement are shown in Fig. 2. Each pixel comprises a bottom valve 111, 112, 121, 122 and a top valve 311, 312, 321, 322. Each of these valves has an annular shape. For each pixel, the top valve has a larger diameter than the associated bottom valve and is arranged concentrically with the associated bottom valve, surrounding the bottom valve when viewed in the projection of Fig. 2. The bottom and top valves of each pixel together delimit a sample chamber 211, 212, 221, 222. Only when both the bottom and top control valves of a pixel are open, the associated sample chamber is open to a fluid flow 50 that can be established through the device, thereby enabling selective filling and flushing of individual sample chambers.

[0039] All bottom valves in the same column are interconnected by a common bottom control channel (column line) 101, 102 and can be actuated together by pneumatic or hydraulic pressure changes in the respective bottom control channel. All top valves in the same row are interconnected by a common top control channel (row line) 310, 320 and can be actuated together by pressure changes in the respective top control channel. Thereby

the addressing scheme explained above in connection with Fig. 1 is implemented, enabling individual addressing of each sample chamber through its associated column line and row line.

[0040] In Figures 3 and 4, the setup of a single pixel is illustrated in greater detail. The microfluidic device comprises a stack of three elastomeric layers 1, 2, 3 disposed on a solid substrate 4. The first layer is a bottom control channel layer 1. This layer is an essentially flat layer of elastomeric material in which bottom control channels are defined for actuating the bottom (push-up) valves. Each bottom control channel is delimited towards its lower side by the substrate 4, i.e., the substrate 4 forms the channel floor. Each bottom control channel is delimited towards its upper side by a thin membrane 61 of elastomeric material, which is part of the bottom control channel layer 1 and forms the channel ceiling. The subsequent second layer is a sample channel layer 2. Also the sample channel layer 2 is an essentially flat layer of elastomeric material. It defines a shallow cavity that is covered towards its top by a thin elastomeric membrane 62. The cavity forms a microfluidic flow path for the fluid flow 50. The bottom (push-up) valves will extend into this cavity from below, while the top (push-down) valves will extend into this cavity from above to control the fluid flow in the microfluidic flow path. The third layer in the sequence is a top control channel layer 3. Again, this layer is an essentially flat layer of elastomeric material in which top control channels are defined for actuating the top valves. Each top control channel is delimited towards its lower side by the thin elastomeric membrane 62 of the sample channel layer 2, which forms the channel floor. Each top control channel is delimited towards its upper side by a relatively thick bulk of elastomeric material, which forms the channel ceiling.

[0041] Bottom valve 111 is formed by an annular section of a bottom control channel together with the thin, elastomeric membrane 61 of the bottom control channel layer 1 that is arranged above said section, delimiting the section from the microfluidic flow path in the sample channel layer 2. Elastomeric membrane 61 is upwardly deflectable into the cavity that forms the microfluidic flow path all the way up until it touches the top control channel layer 3. The deflection is caused by applying positive pressure to the bottom control channel.

[0042] Similarly, top valve 311 is formed by an annular section of a top control channel together with the thin, elastomeric membrane 62 of the sample channel layer 2 that is arranged below said section, delimiting the section from the microfluidic flow path in the sample channel layer 2. Elastomeric membrane 62 is downwardly deflectable into the cavity that forms the microfluidic flow path all the way down until it touches the bottom control channel layer 3 by applying positive pressure to the top control channel.

[0043] When the control channels of both the bottom valve 111 and the top valve 311 of a particular pixel are at atmospheric pressure, these valves are open and al-

low free fluid exchange between the sample chamber 211 and the microfluidic flow path, as illustrated in Fig. 4(a). As soon as the control channels of at least one of the bottom valve 111 and the top valve 311 are pressurized, they deform the respective membrane 61, 62 into the microfluidic flow path and thereby isolate the sample chamber 211 from the microfluidic flow path, as illustrated in Fig. 4(b). In the present example, the sample chamber contains cells 51.

[0044] In all embodiments that are discussed in the present specification, dimensions will preferably be in the following ranges: The bottom control channel layer and the sample channel layer can each have a thickness in the range of 1-100 micrometers. The top control channel layer can have a thickness exceeding 200 micrometers, preferably exceeding 1 millimeter, e.g., a thickness of approximately 10-25 millimeters. Each of the bottom and top control channels can have a depth of approximately 10-50 micrometers. Each valve membrane 6, 7 can have a thickness of approximately 5-300 micrometers. The control channel section of each valve can have a width of approximately 5-3000 micrometers. In the regions outside the valves, the control channels preferably have a reduced clear width as compared to the sections that form the valves so as to avoid that the membrane which separates the control channel from the sample channel layer can fully deflect into the sample channel layer outside the valves. To this end, structures for limiting the clear width, e.g., longitudinal ribs, can be provided in the control channels, these structures having a height that essentially corresponds to the depth of the respective control channel. Thereby it is avoided that the membrane that covers these portions of the control channels can fully deflect into the microfluidic flow path.

[0045] In all embodiments that are discussed in the present specification, the pressure that is applied to the bottom and top control channels for actuating the respective valves is preferably in the range of 0.5-6 bar, more preferably 0.5-4 bar above atmospheric pressure.

[0046] Figures 5 and 6 illustrate in further views how bottom control channels 101, 102 are arranged below sample channel layer 2, the bottom control channels interconnecting bottom control valves 111, 112, 121, 122 along columns, and how top control channels 310, 320 are arranged above sample channel layer 2, the top control channels interconnecting top control valves 311, 312, 321, 322 along rows.

[0047] Fig. 7 illustrates the states of the valves of pixels 11, 12, 21, 22 when the first bottom control channel (column line) 101 and the first top control channel (row line) 310 are pressurized while second bottom control channel 102 and second top control channel 320 are at atmospheric pressure. For pixel 11, valves 111 and 311 are both closed (indicated by solid lines), isolating a sample in sample chamber 211 from a fluid flow in the sample channel layer. For pixel 12, bottom valve 112 is open (indicated by a broken line), while top valve 312 is closed. As a result, the sample in sample chamber 212 is also

isolated, despite of one of the associated valves being open. For pixel 21, bottom valve 121 is closed, while top valve 321 is open. Again, as a result, the sample in sample chamber 221 is isolated, despite of one of the associated valves being open. Only for pixel 22, both the bottom valve 122 and the top valve 322 are open, rendering sample chamber 222 accessible to the fluid flow.

[0048] Fig. 8 illustrates a method isolating a sample volume in a selected sample chamber. As shown in Fig. 8(a), initially all sample chambers are empty, and the valves are in the states as explained in conjunction with Fig. 7, i.e., only for pixel 22 both the bottom and top valve are open. As shown in Fig. 8(b), a fluid flow 50 is induced in the microfluidic flow channel of the sample channel layer. As shown in Fig. 8(c), only the sample chamber of pixel 22 is filled with a sample 52 of the fluid in the fluid flow 50, while all other sample chambers remain empty. Subsequently, all valves controlling flow into the sample chambers can be closed, and then a buffer wash can enter the flow channel to wash away excess sample in the flow channel outside the sample chambers. Then another sample can be loaded into a different sample chamber by opening another set of top and bottom control valve channels.

[0049] As illustrated in Fig. 2, an array of supporting pillars 63 can be formed in the sample channel layer 2, each pillar 63 extending between the bottom control channel layer 1 and the top control channel layer 3, in order to ensure a defined distance between these layers and to prevent collapse of the cavity.

[0050] Figure 9 shows a photograph of a single pixel in an actual prototype. Fibroblast cells are visible in the sample chamber. The scale bar S indicates a length of 300 micrometers. Figure 10 shows the layout of the complete prototype device. The device defines a 16 x 16 array of 256 sample chambers, which can be individually addressed. Pneumatic valve inputs are indicated by a cross, and fluid sample inputs are indicated by a dot. Bottom control channels (column lines) are indicated by numbers, while top control channels (row lines) are indicated by letters.

[0051] The prototype of Figs. 9 and 10 was fabricated by soft-lithography. First, molds for the two control channel layers and the single sample channel layer were patterned on silicon wafers. The control channel layers were patterned at a height of 25 micrometers using SU-8 3025 negative photoresist (Microchem Inc.), while the sample channel layer was patterned using SU-8 3025 as well as AZ-50XT positive resist (AZ Electronic Materials Co.) at a height of 20 micrometers.

[0052] The control channel layers and the sample channel layer were made from polydimethylsiloxane (PDMS, Momentiv RTV). The PDMS base polymer and curing agent were mixed at a 10:1 ratio for the two control channel layers and mixed at 8:1 ratio for the sample channel layer. Thin layers of PDMS were spin-coated upon the molds for the sample channel layer and for the bottom control layer such that approximately 10-30 micrometers

of the elastomer was coated above the photoresist features. An approximately 1 cm thick layer of the 10:1 PDMS mixture was poured on top of the mold for the top control channel layer. All molds were then placed in an oven set at 80 °C for 1 h to cure the PDMS. The approximately 1 cm thick top control channel layer was then aligned and bonded with the sample channel layer following oxygen plasma treatment (15 seconds, 45 W), and then the bonded pieces were placed in an oven set at 80 °C for at least 2 h. Following this baking step, the resulting two-layer piece was removed from the sample channel mold, and a single 23 gauge (0.6 millimeter) hole was punched to form a first sample fluid inlet to the sample channel layer. Next, the two-layer piece was aligned and bonded with the bottom control channel layer following plasma treatment in the manner just described. If the large sample channel collapsed during bonding, pressurized air was delivered with a syringe through the sample fluid inlet to push the membrane that forms the channel ceiling back to its intended position. The bonded pieces were then placed in the oven set at 80 °C for at least 2 h. Next, the resulting three-layer device was removed from the bottom control channel mold, and 23 gauge (0.6 millimeter) access holes were punched to create the fluid inlets. The bottom side of the device was then cleaned with scotch tape, and finally bonded to a clean microscope glass slide with oxygen plasma treatment (300 s, 45 W), the glass slide forming the substrate. After a 4 h baking step to enhance bonding, experiments could be performed on the device.

[0053] While the prototype of Fig. 10 includes a 16 x 16 array, the presently described design can be readily scaled up to include much larger arrays of sample chambers. Prototypes with almost 10'000 sample chambers have been built, but 10'000 is by no means the upper limit of what is possible.

[0054] In the embodiment illustrated in Figures 1-10, the bottom and top valves are arranged concentrically, one of the valves surrounding the other when viewed in a projection onto the device plane. However, this is not necessary. In particular, it is possible for the top valve and the bottom valve to have the same diameter, meaning that they completely overlap. When one of the valves has a larger diameter than the other, as in the example of Figures 1-10, it is preferred that the difference between the diameters does not exceed 1.5 times the width of the annular top and bottom control channel sections that form the valves so as to keep dead volumes reasonably small.

[0055] A variant of the first embodiment is illustrated in Figs. 11 and 12. In this variant, the device comprises a stack of four elastomeric layers: a bottom control channel layer 1, a lower sample channel layer 2a, an upper sample channel layer 2b, and a top control channel layer 3. In contrast to the first embodiment, the bottom valve membranes 61 are formed by the lower sample channel layer 2a. This is a consequence of a different fabrication process, as described in more detail in conjunction with Figs. 13 and 14 below. Each valve membrane is provided

with an annular sealing ridge to ensure better sealing against the respective opposite surface. In particular, bottom valve (push-up valve) 111 is provided with an annular sealing ridge 64 disposed on top of that portion of membrane 61 that forms bottom valve 111. Likewise, top valve (push-down valve) 311 is provided with an annular sealing ridge 65 disposed on the bottom of that portion of membrane 62 that forms top valve 311. In addition, each sample chamber (only sample chamber 211 being illustrated) comprises a circular well 53 in the center of the associated bottom valve 111, the well 53 extending through bottom sample channel layer 2a and into bottom control channel layer 1 from above. Thereby the volume of the sample chamber is increased and cells can be grown in a lower fluid flow-induced shear stress environment.

[0056] As apparent from Fig. 12 (a), the sealing ridges have a height that is smaller than the height of the microfluidic flow path in sample channel layer 2 so as to ensure that a fluid flow is possible through the microfluidic flow path past the respective valve when the respective valve is open. As apparent from Fig. 12 (b), when the bottom valve 111 is closed, the sealing ridge 64 of the bottom valve 111 bears against the surface 66 that delimits the microfluidic flow path to the top, and when the top valve 311 is closed, the sealing ridge 65 of the top valve 311 bears against the surface 67 that delimits the microfluidic flow path to the bottom.

[0057] Fabrication of the device according to the variant of Figs. 11 and 12 is illustrated in Figs. 13 and 14.

[0058] With reference to Fig. 13, initially photoresist structures 72 are patterned upon a substrate 71, thereby creating a first mold (step a). This mold is spin-coated with an elastomer (here: degassed PDMS) and left uncured to create bottom control channel layer 1 (step b). On a separate substrate 73, photoresist structures 74 are deposited, thereby creating a second mold (step c). The second mold is spin-coated with elastomer in such a manner that at least some of the photoresist structures 74 are covered by the elastomer thereby creating the lower sample channel layer 2a (step d). The second mold with the elastomer is then flipped by 180° and aligned with the first mold, and the elastomer of the second mold comes in contact with the elastomer of the first mold. The placement of a weight on the backside of the second mold applies pressure on the structures to ensure maximum contact between the two surfaces. These pieces are then placed in an oven with the weight to cure the elastomer (step e). Substrate 73 and photoresist 74 are then removed to leave the top surface of layer 2a exposed (step f).

[0059] With reference to Fig. 14, photoresist structures 76 are patterned upon a further substrate 75 to create a third mold (step a). The third mold is spin-coated with elastomer to form upper sample channel layer 2b (step b). A fourth mold is created by patterning a photoresist structure 78 on yet another substrate 77 (step c). Elastomer is poured upon the fourth mold to create top control

channel layer 3 (step d). After curing, top control channel layer 3 is removed from the fourth mold (step e) and bonded to the top of upper sample channel layer 2b (step f). The resulting layer structure of layers 2b and 3 is removed from the third mold and bonded to the top of lower sample channel layer 2a. Subsequently substrate 71 and photoresist 72 are also removed from bottom control channel layer 1, and the exposed bottom surface of layer 1 is bonded to a glass slide that forms substrate 4 (step g).

[0060] Figures 15-17 illustrate a second embodiment of a microfluidic device according to the present invention. Elements that perform similar functions as in the first embodiment carry the same reference signs as in the first embodiment. As in the first embodiment, the device is a three-layer device comprising a bottom control channel layer, a sample channel layer and a top control channel layer. The sample chamber layer defines an array of sample chambers arranged in parallel rows and columns, only four of these sample chambers 211, 212, 221, 222 being shown in Figs. 15-17. Each sample chamber is in fluidic communication with an associated inlet channel and an associated outlet channel, only inlet channel 211a and outlet channel 211b of sample chamber 211 being specifically indicated in Fig. 16. The inlet channels and the outlet channels of the sample chambers of each column are connected to a common sample distribution and collection channel 201, 202.

[0061] An associated bottom inlet valve 111a is arranged below the inlet channel 211a of each sample chamber to close off the inlet channel from below, and an associated top inlet valve 311a is arranged above the inlet channel, slightly downstream of the bottom inlet valve 111a along the inlet channel, to close off the inlet channel from above. In this manner, access to the sample chamber through the inlet channel can be controlled both from below and from above, whereby access is provided only if both the associated bottom inlet valve 111a and the associated top inlet valve 311a are open. Likewise, an associated bottom outlet valve 111b is arranged below the outlet channel 211b to close off the outlet channel from below, and an associated top outlet valve 311b is arranged above the outlet channel, slightly upstream of the bottom outlet valve 111b along the outlet channel, to close off the outlet channel from above. In this manner, access to the sample chamber through the outlet channel can be controlled both from below and from above, whereby access is provided only if both the associated bottom outlet valve 111b and the associated top outlet valve 311b are open.

[0062] In the present example, the bottom inlet valve 111a and the bottom outlet valve 111b are interconnected by a bottom control channel segment 111c. In fact, the bottom inlet valves and the bottom outlet valves of all sample chambers in the same column are connected to a common bottom control channel 101. In this manner, all bottom valves of all sample chambers in the same column are opened and closed together by pressure changes in the common bottom control channel 101.

[0063] Likewise, the top inlet valve 311a and the top outlet valve 311b of each sample chamber are interconnected by a top control channel segment 311c, and are connected to the same top control channel 310. In fact, the top inlet valves and the top outlet valves of all sample chambers in the same row are connected to the same top control channel 310. In this manner, all top valves of all sample chambers in the same row are opened and closed together by pressure changes in the common top control channel 310.

[0064] Access to a particular sample chamber for a fluid flow in the associated sample distribution and collection channel 201, 202 is only possible if both the bottom control channel of the column in which the sample chamber resides and the top control channel of the row in which the sample chamber resides are depressurized. In this manner, each sample chamber can be addressed individually by selecting its row and column.

[0065] A variant of the second embodiment is illustrated in Figs. 18 and 19. Elements that perform similar functions as in the second embodiment carry the same reference signs as in the second embodiment. In this embodiment, the bottom inlet valve 111a and the top inlet valve 311a of each sample chamber 111 completely overlap when viewed in a projection onto the device plane. Likewise, the bottom outlet valve 111b and the top outlet valve 311b of each sample chamber completely overlap in a projection onto the device plane. This results in a more compact arrangement.

[0066] From the foregoing description it is apparent that a large number of modifications are possible. For instance, in the first embodiment, the sample channel layer can have additional structures in addition to or instead of the supporting pillars 63. In the second embodiment, instead of a common sample distribution and collection channel per row, a separate sample distribution channel and a separate sample collection channel can be provided for each row, the inlet channels of the sample chambers in each column being connected to the common sample distribution channel, and the outlet channels of the sample chambers in each column being connected to the common sample collection channel. For instance, the sample distribution channel could be provided in the same location as channel 201 in Figs. 15-17, whereas the sample collection channel could be provided adjacent to the next sample distribution channel 202, between the sample chambers 211, 221 and sample distribution channel 202. Of course, the order of the top and bottom valves along each inlet and outlet channel can be reversed. It is conceivable to separately actuate inlet and outlet valves, e.g., in order to enable diffusion only through either the inlet or the outlet. Many different arrangements of the inlet and outlet channels, of the inlet and outlet valves on these channels, and of their connection to distribution and control channels are conceivable.

Claims

1. A microfluidic device defining an array of sample chambers (211), the microfluidic device comprising:

a bottom control channel layer (1), the bottom control channel layer (1) defining a plurality of bottom control channels (101);
 a sample channel layer (2) arranged on top of the bottom control channel layer (1), the sample channel layer (2) defining at least one microfluidic flow path for a fluid flow (50),
 a top control channel layer (3) arranged on top of the sample channel layer (2), the top control channel layer (3) defining a plurality of top control channels (310);
 a plurality of bottom valves (111), each bottom valve (111) being connected to at least one of the bottom control channels (101) and being configured to be actuated into the microfluidic flow path from below; and
 a plurality of top valves (311), each top valve (311) being connected to at least one of the top control channels (310) and being configured to be actuated into the microfluidic flow path from above,

wherein the sample chambers (211) are arranged in the sample channel layer (2), access to each sample chamber (211) being controllable by at least one of the bottom valves (111) and at least one of the top valves (311) in such a manner that said sample chamber (211) is accessible to the fluid flow in the microfluidic flow path only if both the associated bottom valve (111) and the associated top valve (311) are open.

2. The microfluidic device of claim 1,

wherein the bottom valves (111) are arranged in a plurality of columns, the bottom valves (111) in each column being in fluidic communication with a common bottom control channel (101), and

wherein the top valves (311) are arranged in a plurality of rows, the rows running across the columns, the top valves (311) in each row being in fluidic communication with a common top control channel (310).

3. The microfluidic device of claim 1 or 2,

wherein each bottom valve (111) is defined by a section of one of the bottom control channels (101) and a bottom elastomeric membrane (61) that is arranged between said section and the microfluidic flow path, said bottom elastomeric membrane (61) being deflectable into the micro-

fluidic flow path; and

wherein each top valve (311) is defined by a section of one of the top control channels (310) and a top elastomeric membrane (62) that is arranged between said section and the microfluidic flow path, said top elastomeric membrane (62) being deflectable into the microfluidic flow path.

4. The microfluidic device of claim 3,

wherein each bottom valve (111) comprises a bottom sealing ridge (64) that is provided on the bottom elastomeric membrane (61), the bottom sealing ridge (64) bearing against a top defining surface (66) of the microfluidic flow path when the bottom valve is closed, and/or

wherein each top valve (311) comprises a top sealing ridge (65) that is provided on the top elastomeric membrane (62), the top sealing ridge (65) bearing against a bottom defining surface (67) of the microfluidic flow path when the bottom valve is closed.

5. The microfluidic device of claim 3 or 4,

wherein the microfluidic flow path has a height, wherein each bottom elastomeric membrane (61) and each top elastomeric membrane (62) has a thickness, and wherein the height of the microfluidic flow path and the thickness of the bottom or top elastomeric membrane (61, 62), in the region of each bottom or top valve (111, 311), has a ratio between 1 and 7.

6. The microfluidic device of any one of claims 3-5,

wherein each section of the bottom control channels (101) that defines a bottom valve (111) and each section of the top control channels (310) that forms a top valve (311) has a width; wherein each bottom elastomeric membrane (61) and each top elastomeric membrane (62) has a thickness, and wherein the width of each section and the thickness of the bottom or top elastomeric membrane (61, 62), in the region of each bottom or top valve (111, 311), has a ratio of at least 3, preferably of 3-20.

7. The microfluidic device of any one of the preceding claims, wherein at least one of the bottom control channel layer (1), the sample channel layer (2) and the top control channel layer (3) are made of an elastomeric material.

8. The microfluidic device of any one of the preceding

claims,

further comprising a substrate (4), wherein the bottom control channel layer (1) is disposed on top of the substrate (4), wherein the bottom control channels (101) are formed by recesses in the bottom control channel layer (1) and are commonly delimited by the substrate (4) and the bottom control channel layer (1), the bottom control channel layer (1) forming the elastomeric membranes (61) between the bottom control channels (101) and the microfluidic flow path;

wherein the microfluidic flow path is formed by at least one recess in the sample channel layer (2) and is commonly delimited by the bottom control channel layer (1) and the sample channel layer (2), the sample channel layer (2) forming the elastomeric membranes (62) between the microfluidic flow path and the top control channels (310); and

wherein the top control channels (310) are formed by recesses in the top control channel layer (3) and are commonly delimited by the sample channel layer (2) and the top control channel layer (3).

9. The microfluidic device of any one of the preceding claims,

wherein each sample chamber (211) is connected to an inlet channel (211a) and an outlet channel (211b) in the sample channel layer (2), wherein each sample chamber (211) is associated with a first bottom valve forming a bottom inlet valve (111a) for closing off the inlet channel (211a) of said sample chamber (211) from below, and with a first top valve forming a top inlet valve (311a) for closing off the inlet channel (211a) of said sample chamber (211) from above, and

wherein each sample chamber (211) is further associated with a second bottom valve forming a bottom outlet valve (111b) for closing off the outlet channel (212b) of said sample chamber (211) from below and with a second top valve forming a top outlet valve (311b) for closing off the outlet channel of said sample chamber (211) from above.

10. The microfluidic device of claim 9,

wherein the bottom inlet valve (111a) and the bottom outlet valve (111b) of each sample chamber (211) are interconnected with each other and/or are in fluidic communication with a common bottom control channel (101), and/or wherein the top inlet valve (311a) and the top

outlet valve (311b) of each sample chamber (211) are interconnected with each other and/or are in fluidic communication with a common top control channel (310).

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11. The microfluidic device of claim 9 or 10,

wherein the sample chambers (211) are arranged in a plurality of columns,
wherein the inlet channels (211a) of the sample chambers (211) in each column are connected to a common sample distribution channel (201),
and

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wherein the outlet channels (211b) of the sample chambers (211) in each column are connected to a common sample collection channel,
wherein optionally the sample distribution channel (201) and the sample collection channel of each column coincide.

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12. The microfluidic device of any one of claims 1-8,

wherein each bottom valve (111) has an annular shape, surrounding one of the sample chambers (211), and

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wherein each top valve (311) has an annular shape, surrounding one of the sample chambers (211), and being arranged concentrically with the associated bottom valve (111).

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13. The microfluidic device of claim 12, wherein each sample chamber (211) comprises a well in the bottom control channel layer (1).

14. The microfluidic device of claim 12 or 13, wherein the sample channel layer (1) comprises an array of supporting pillars (63) that extend from the bottom control channel layer (1) to the top control channel layer (3) to ensure a defined distance between the bottom control channel layer (1) and the top control channel layer (3).

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15. A method of isolating a fluid volume in a selected sample chamber (211) of the microfluidic device of any one of the preceding claims, the method comprising:

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opening both at least one of the top valves (311) and at least one of the bottom valves (111) associated with said selected sample chamber (211);

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causing a fluid flow through the selected sample chamber (211);

closing the top valves (311) and/or the bottom valves (111) associated with the selected sample chamber (211).

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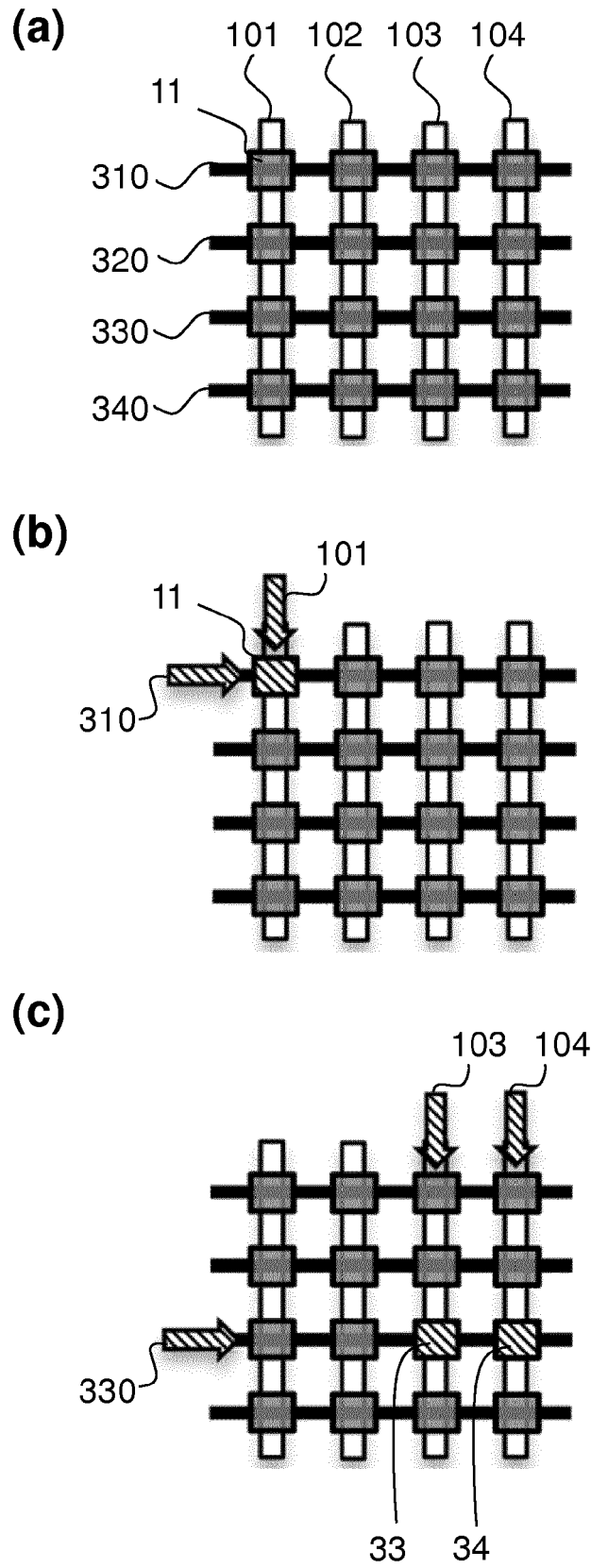


FIG. 1

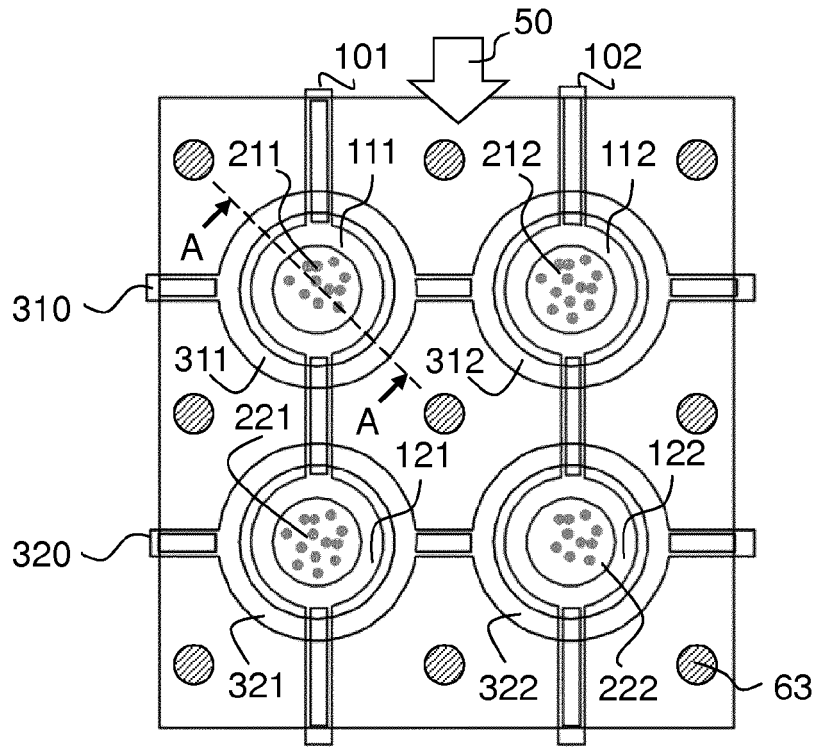


FIG. 2

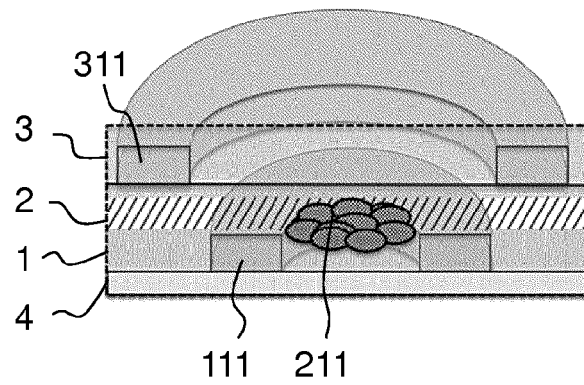


FIG. 3

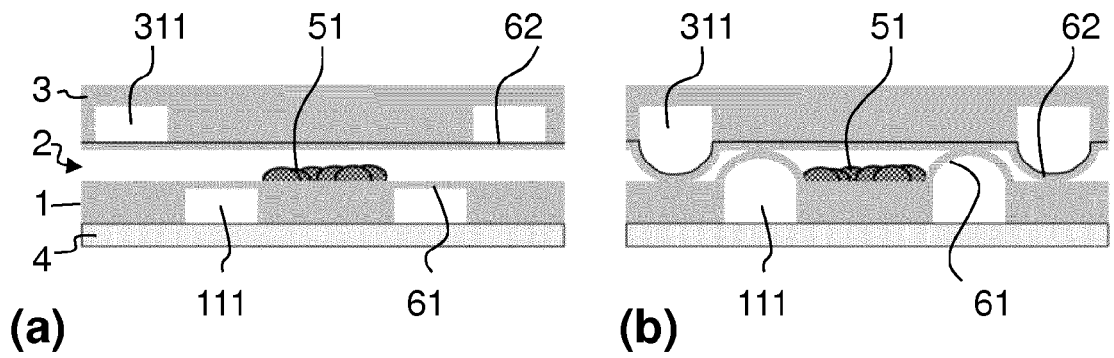


FIG. 4

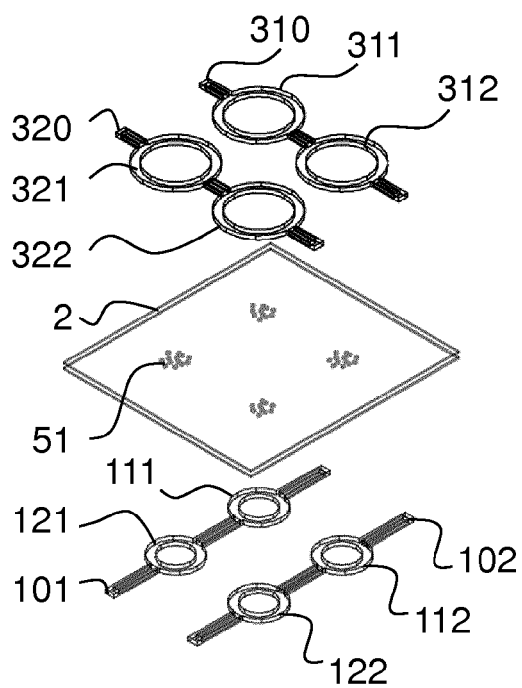


FIG. 5

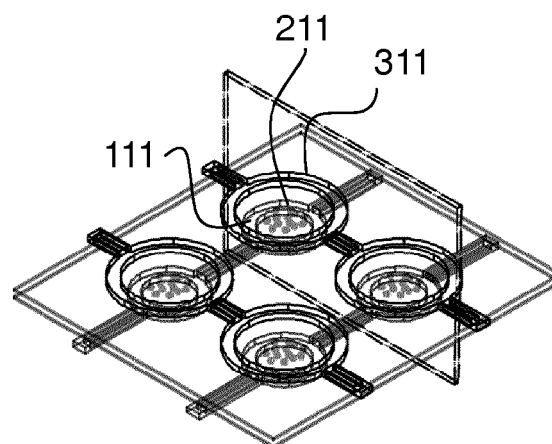


FIG. 6

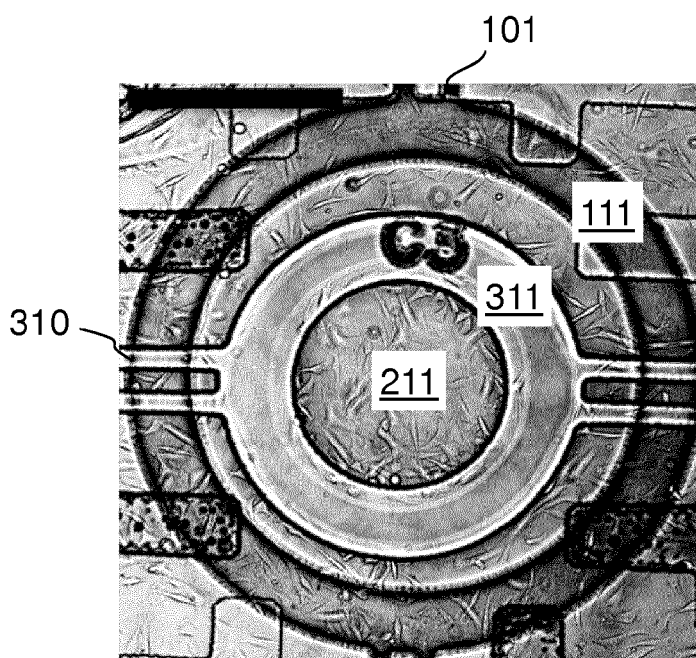


FIG. 9

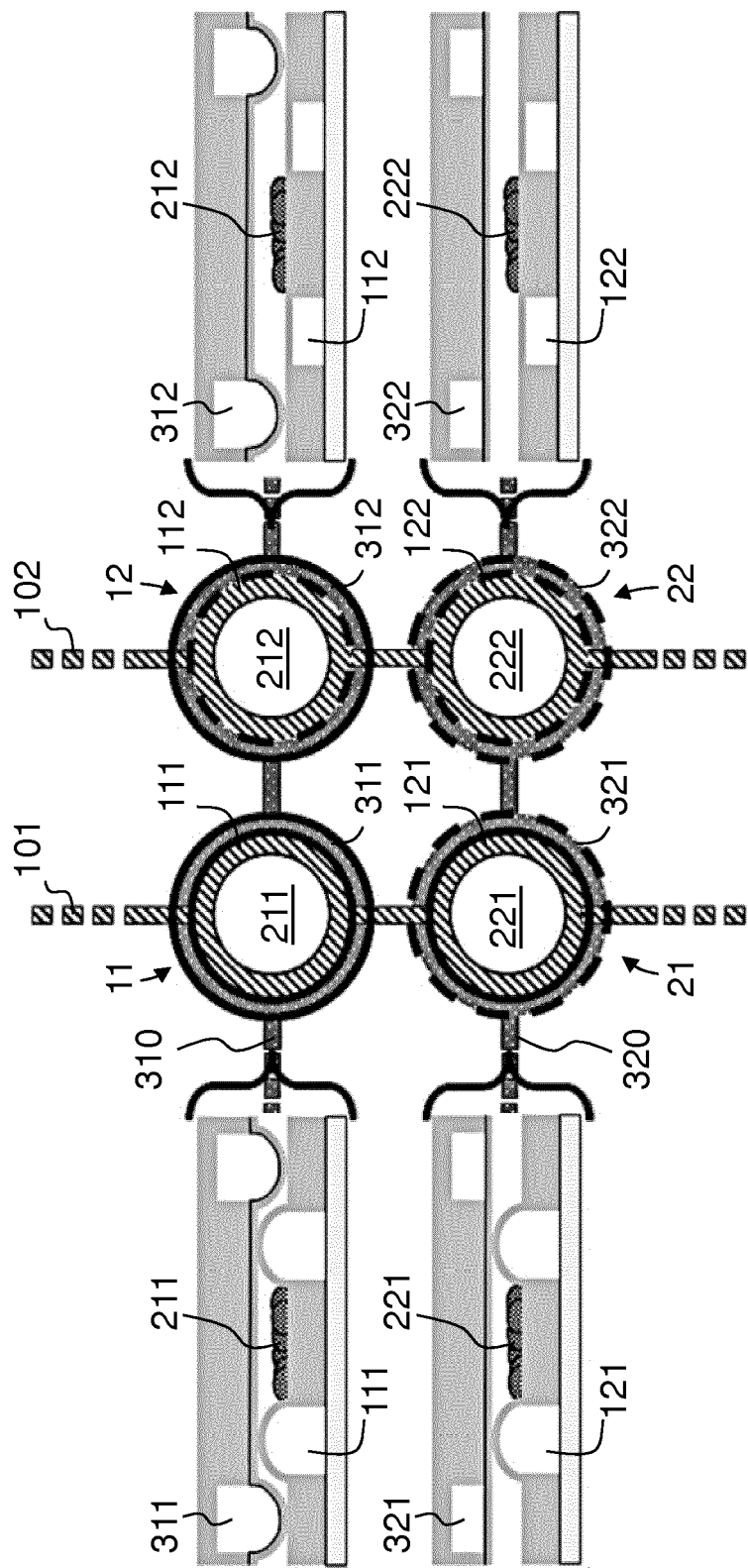


FIG. 7

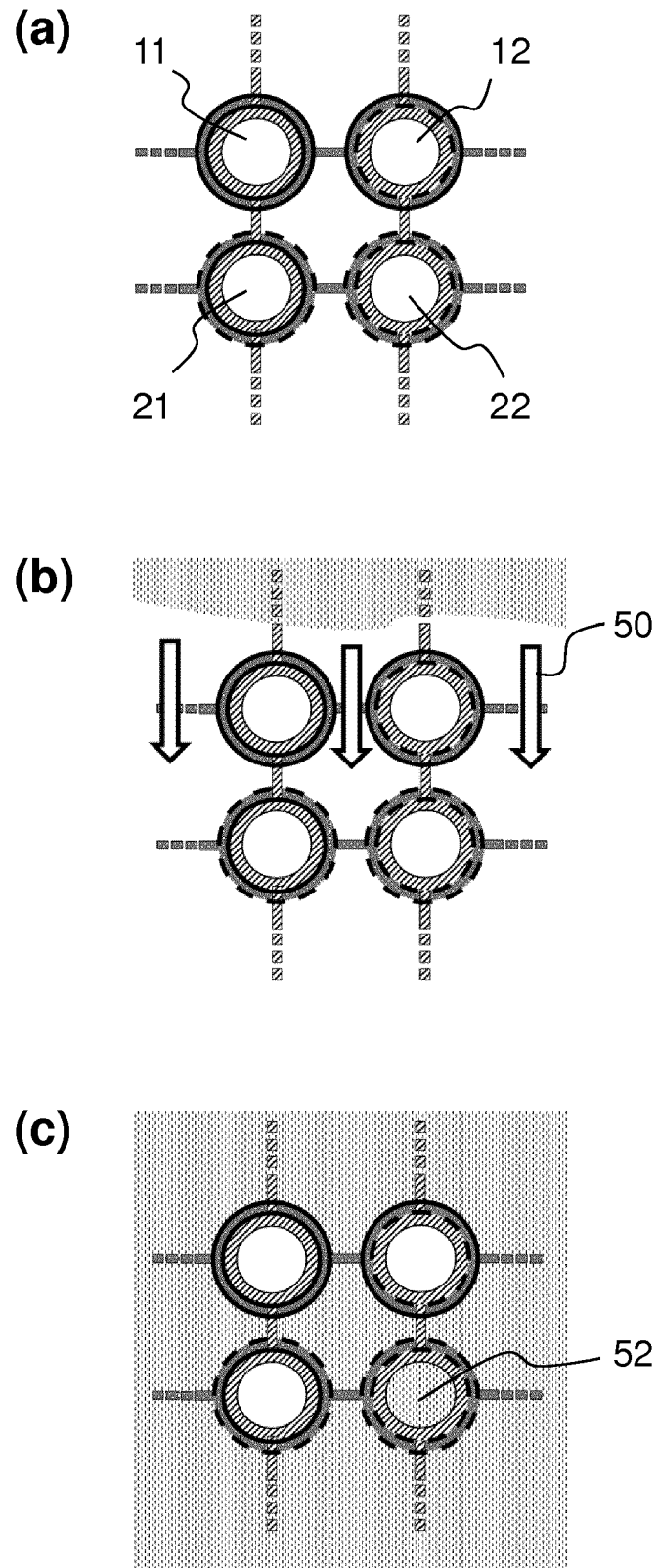
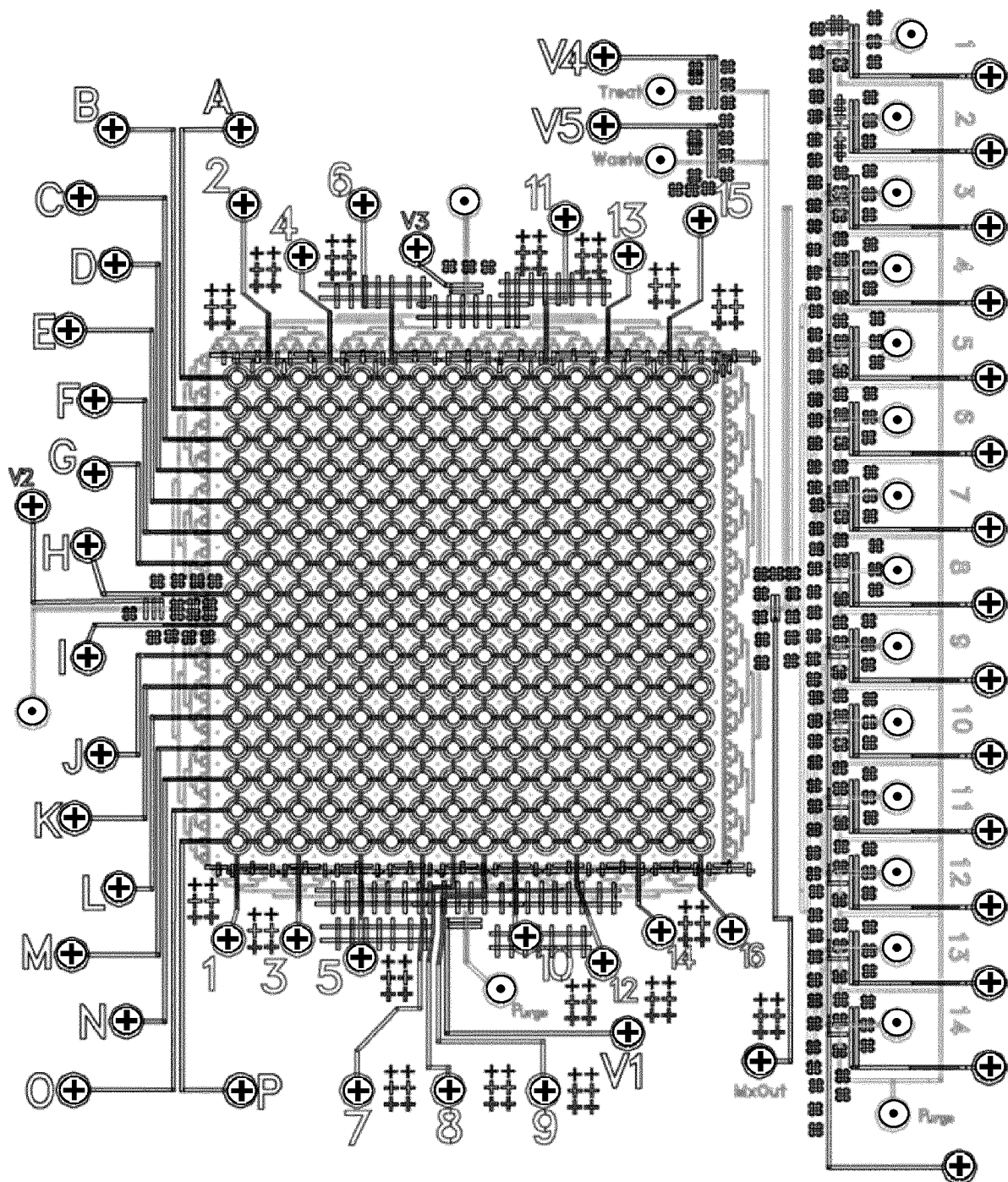


FIG. 8



⊕ = Valve input

⊙ = Fluid sample input

FIG. 10

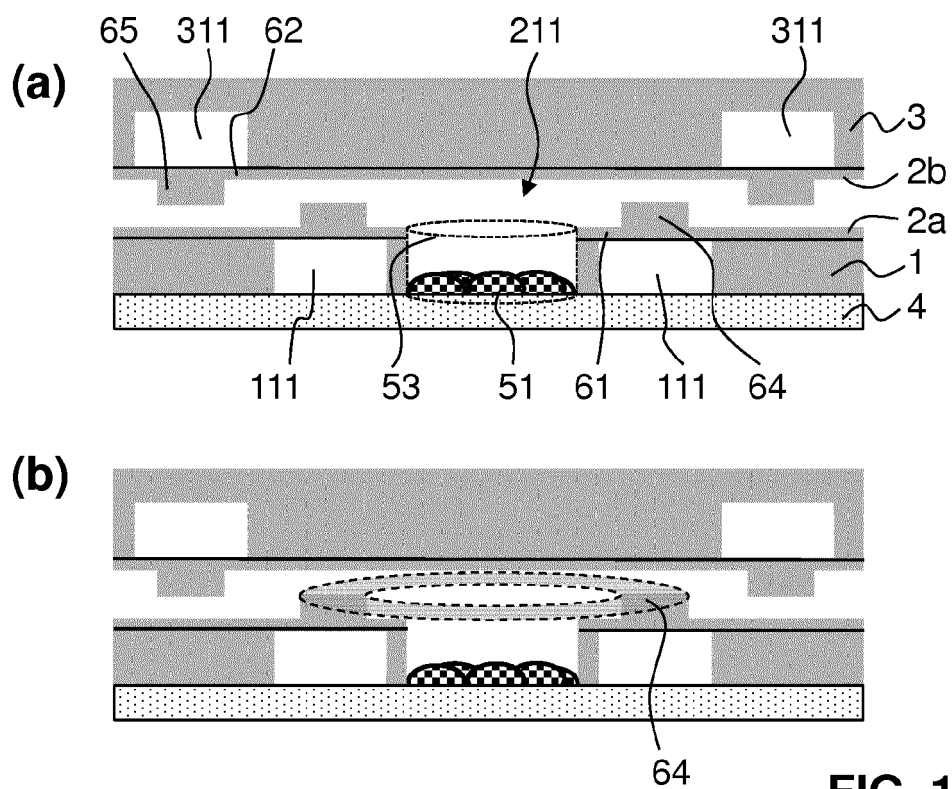


FIG. 11

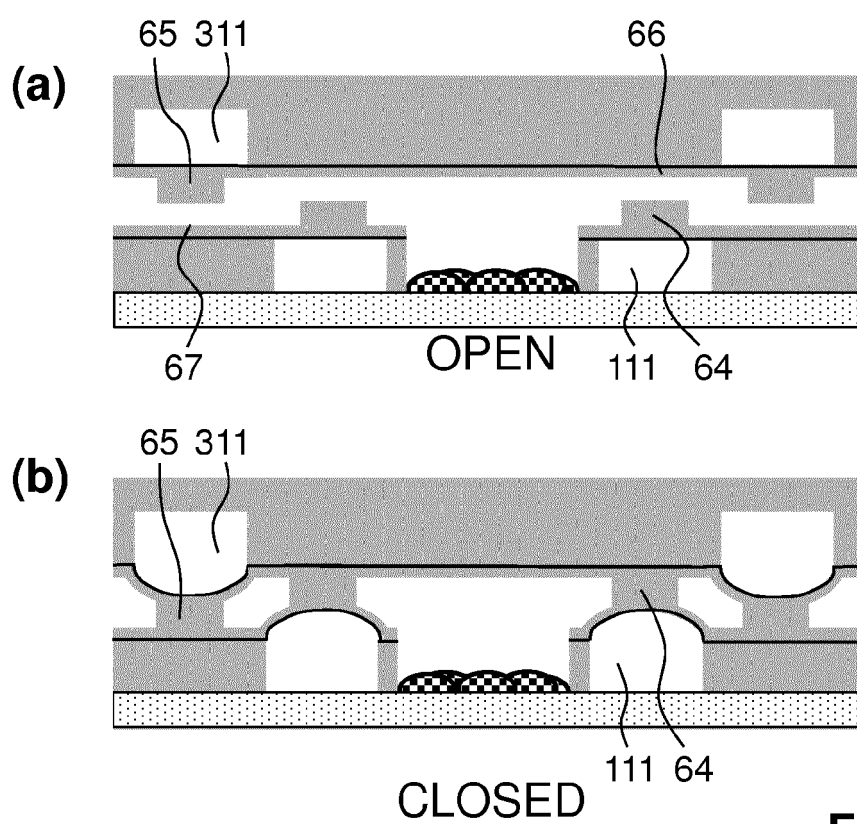


FIG. 12

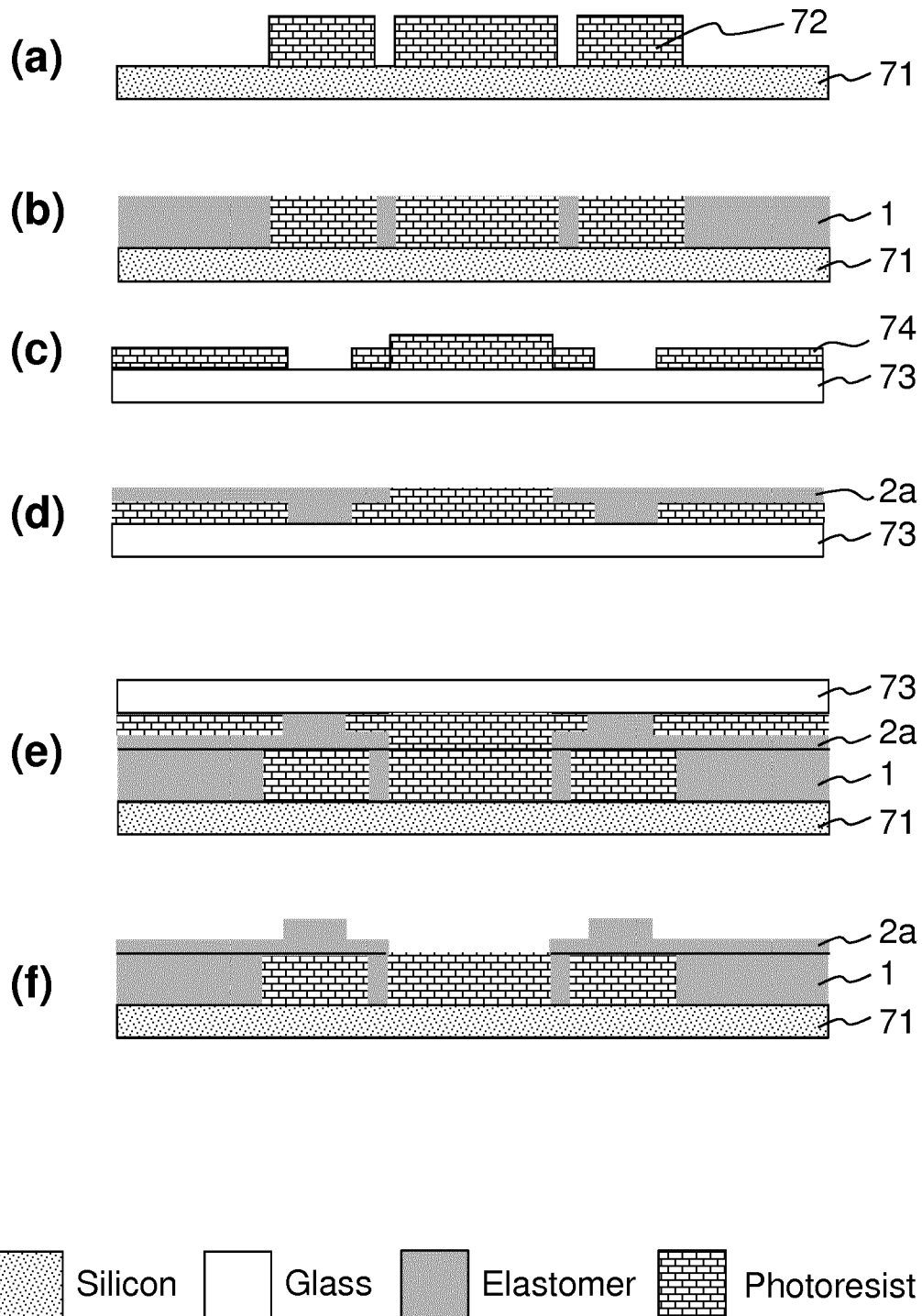


FIG. 13

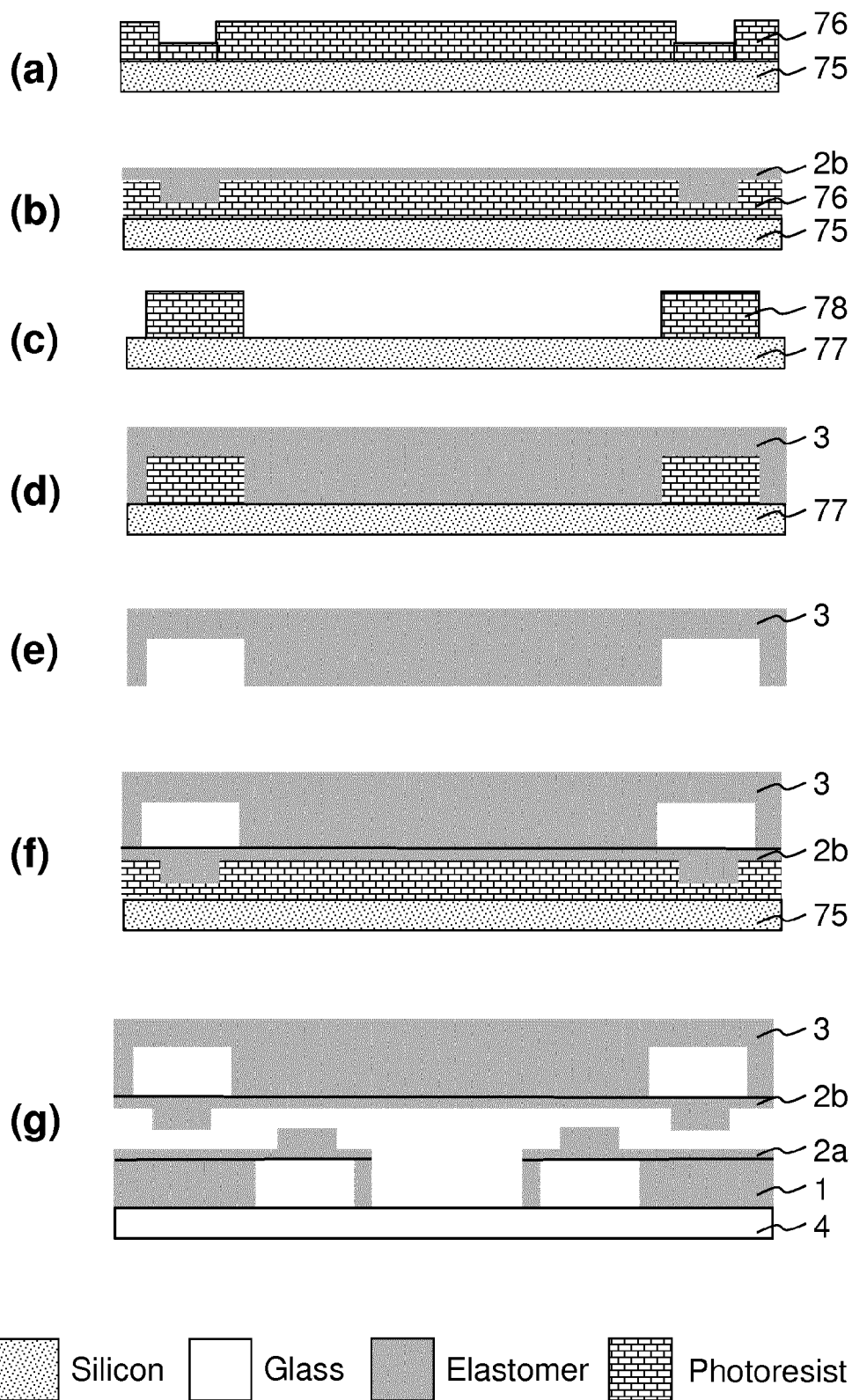


FIG. 14

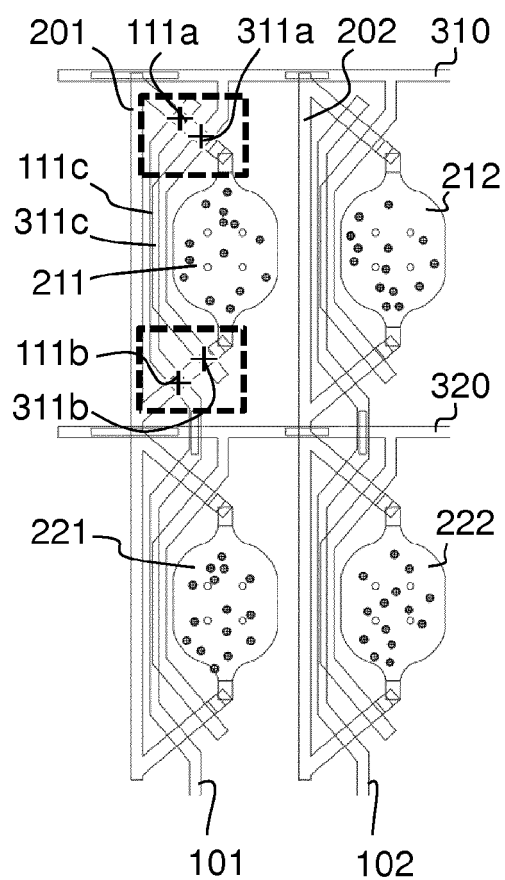


FIG. 15

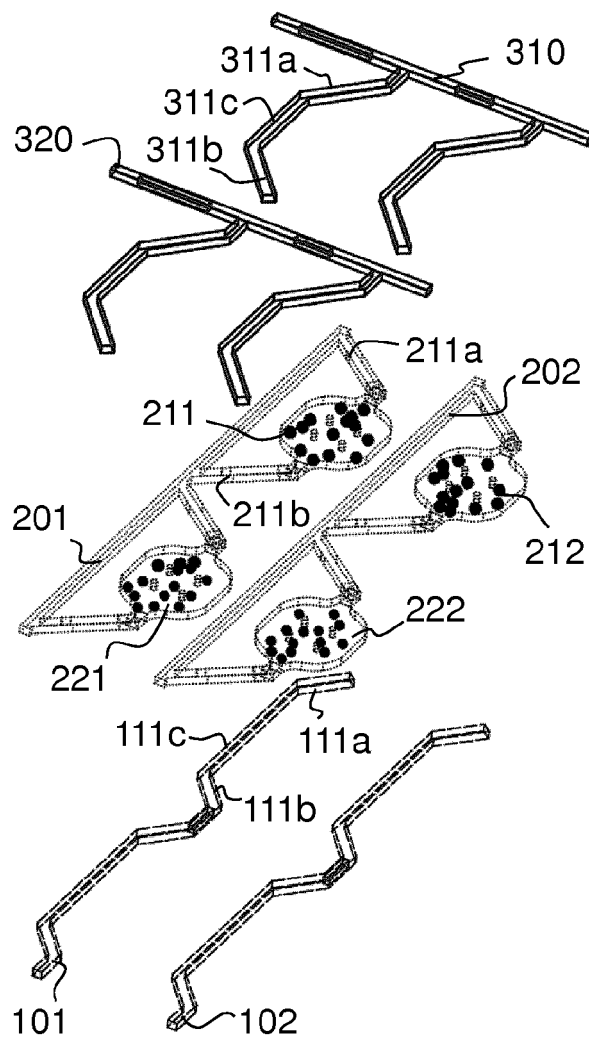


FIG. 16

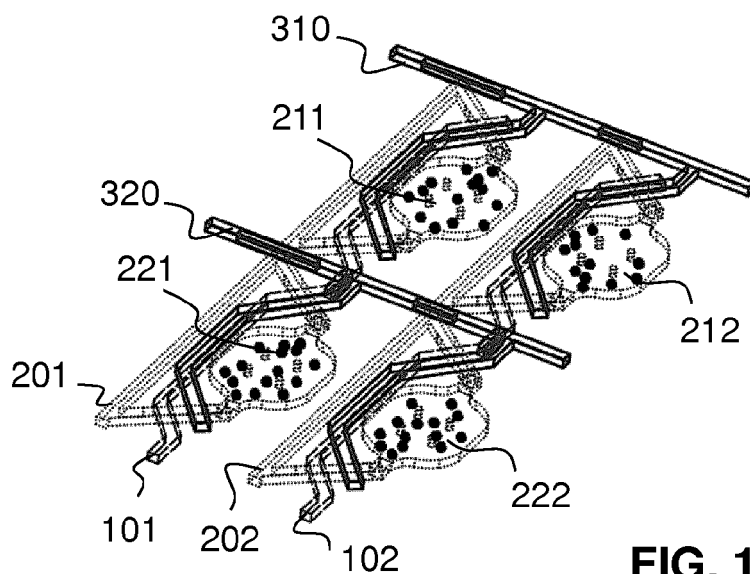


FIG. 17

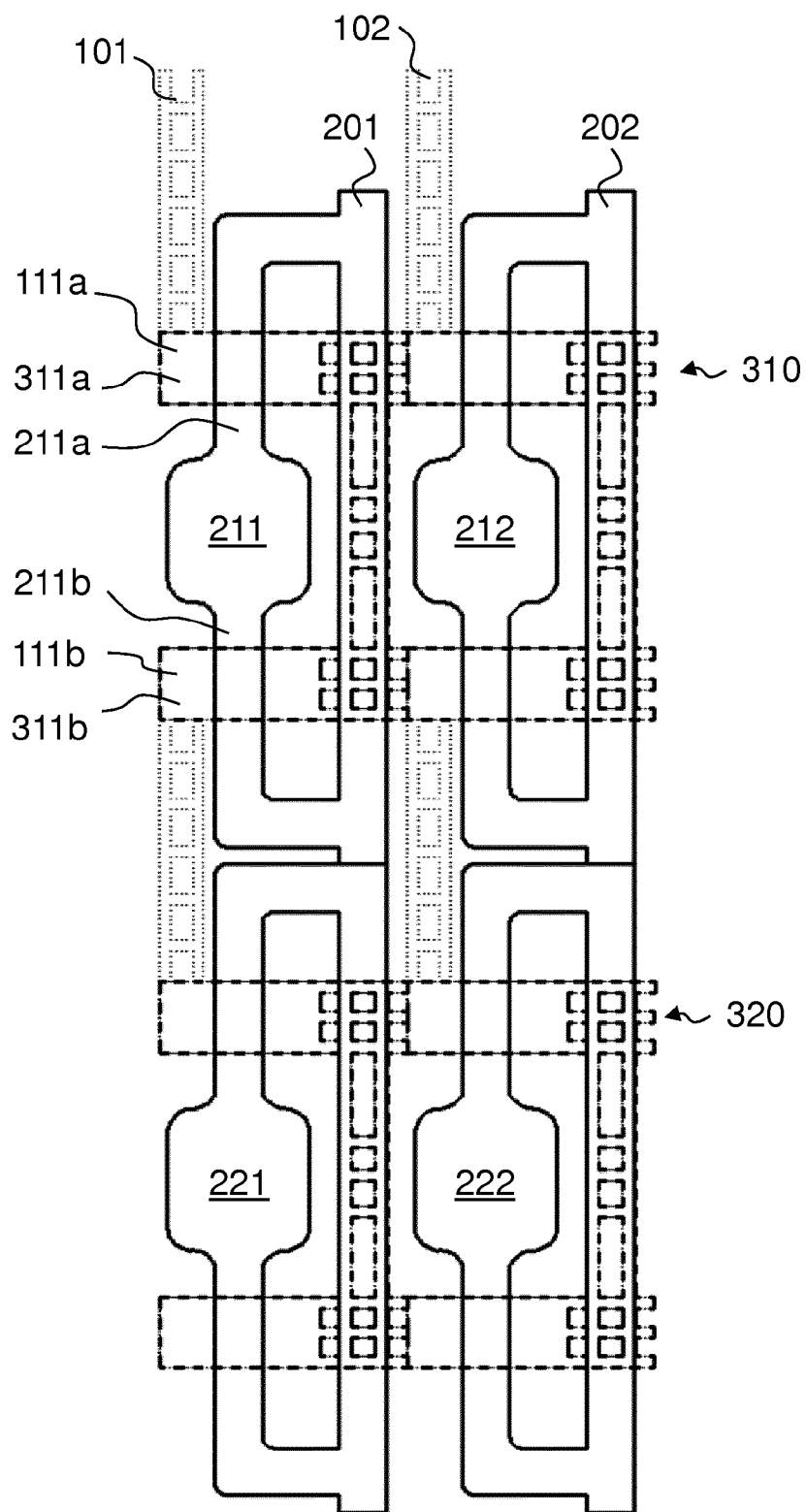


FIG. 18

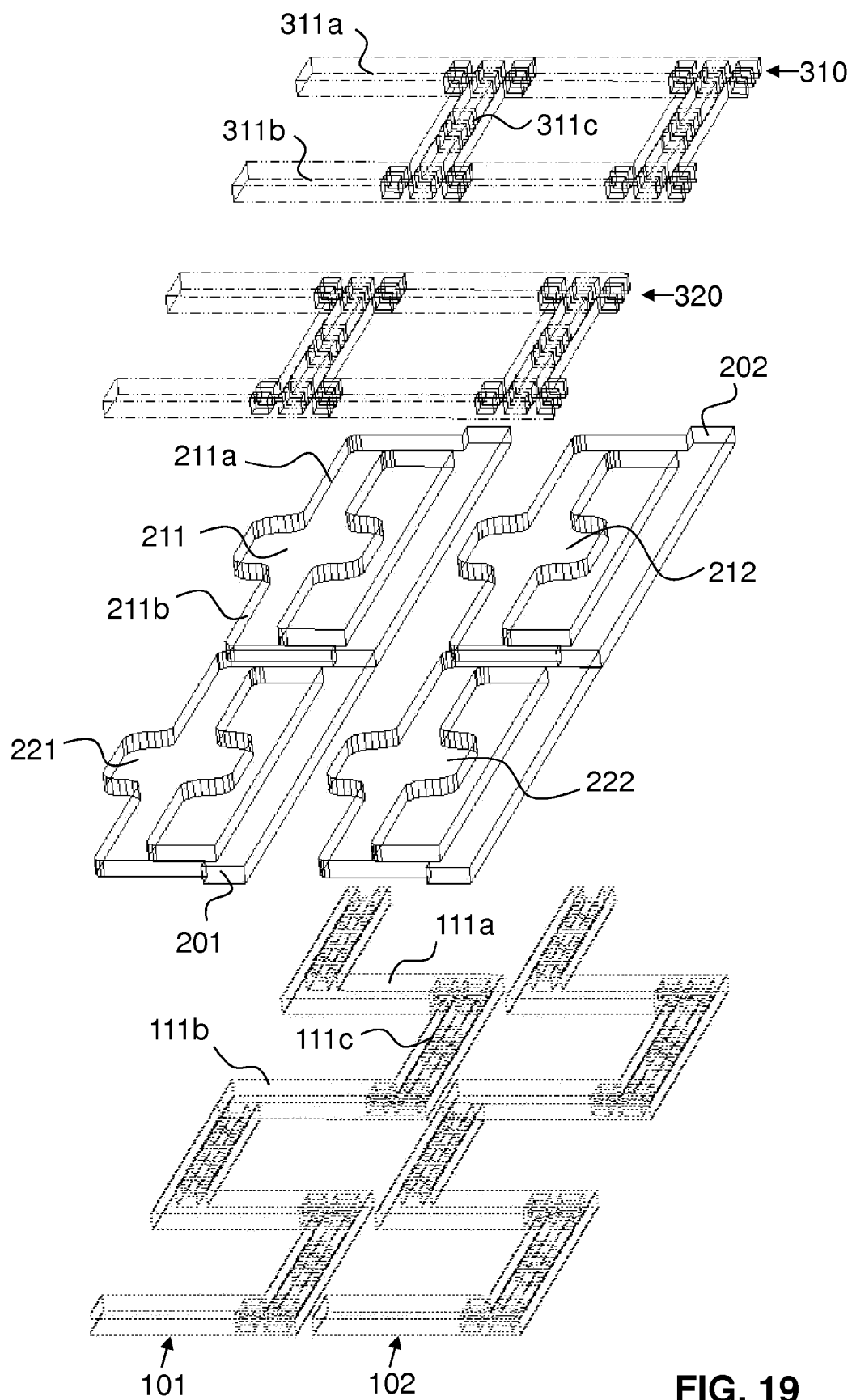


FIG. 19



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 Application Number
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The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 1 December 2016	Examiner Bischoff, Laura
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Place of search The Hague		Date of completion of the search 1 December 2016	Examiner Bischoff, Laura
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