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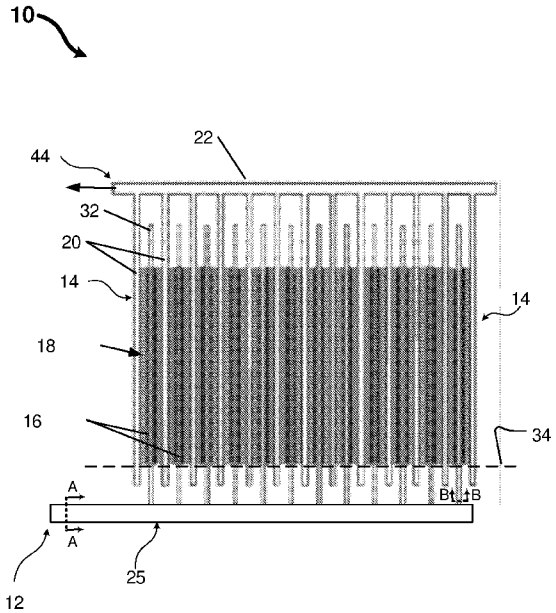


Fig. 1A

(57) **Abstract:** A device comprising: plurality of Stationary Nanoliter Droplet Array (SNDA) components; each SNDA component comprising: at least one primary channel; at least one secondary channel; and a plurality of nano-wells that are each open to the primary channel and are each connected by one or more vents to the secondary channel; the vents are configured to enable passage of air solely from the nano-wells to the secondary channel, such that when a liquid is introduced into the primary channel it fills the nano-wells, and the originally accommodated air is evacuated via the vents and the secondary channel/s; an inlet port and a distribution channel configured to enable a simultaneous introduction of the liquid into all primary channels; and an outlet port and an evacuation channel configured to enable a simultaneous evacuation of the air out of all the secondary channels.

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MULTIPLEXED ARRAY OF NANOLITER DROPLET ARRAY DEVICES

FIELD OF THE INVENTION

[0001] The present invention relates to microfluidic devices. More particularly, the present invention relates to a multiplexed array of nanoliter droplet array devices.

BACKGROUND OF THE INVENTION

[0002] Microfluidic devices that are designed to hold nanoliter-sized droplets of liquids in separate nano-wells, referred to herein as a stationary nanoliter droplet array (SNDA) devices, have been proven to be of use in the execution of various biological and chemical tests and procedures. In a typical procedure, two or more fluids are introduced successively into the device via one or more inlets. The nano-wells are then examined, e.g., visually by: a microscope, an automated image analysis system, or other visualization tools, to determine results of any interactions between the successively introduced liquids, or effects on cells that are suspended in one of the introduced liquids.

[0003] In a typical SNDA device, the introduced fluid may flow from the inlet into a primary channel of the device. The primary channel is lined on both sides by openings to nano-wells, where adjacent nano-wells are being separated one from another by walls. An end of each nano-well that is distal to its opening to the primary channel includes one or more vents that are opened to an air evacuation channel. Thus, as each nano-well is filled with liquid via its opening to the primary channel, air that had previously filled the nano-well escapes through its vent to the air evacuation channel. The openings of the vent are typically small enough so as to prevent the liquid from passing out of the nano-well through the vent. For example, the liquid may be prevented from emerging through the vent by the action of surface tension, viscosity, air pressure, or other forces. Thus, each nano-well may be partially or completely filled by the introduced liquid.

[0004] For example, such SNDA devices have been employed successfully to perform antimicrobial susceptibility testing (AST). When an SNDA device is used for AST, an antibiotic liquid is first introduced into each of the nano-wells. In some cases, the antibiotic may be introduced into the nano-wells in a manner that produces a gradient of concentration of the antibiotic along the length of the primary channel. The antibiotic may be lyophilized

or otherwise treated, e.g., to retain the antibiotic in the nano-wells. A bacterial suspension may then be introduced into the nano-wells. The nano-wells may then be examined to determine the effect of the antibiotic on the bacteria. For example, an image of the SNDA device may be analyzed, either by a human eye or by a processor, to determine the effect of the antibiotic on the bacteria.

SUMMARY OF THE INVENTION

[0005] According to some embodiments of the invention, a new device is provided comprising:

- plurality of Stationary Nanoliter Droplet Array (SNDA) components; each SNDA component comprising: at least one primary channel; at least one secondary channel; and a plurality of nano-wells that are each open to the primary channel and are each connected by one or more vents to the secondary channel; the vents are configured to enable passage of air solely from the nano-wells to the secondary channel, such that when a liquid is introduced into the primary channel it fills the nano-wells, and the originally accommodated air is evacuated via the vents and the secondary channel/s;

  wherein the plurality of the SNDA components are aligned parallel to one another and laterally displaced relative to one another, such that the device comprises a rectangular form;
- an inlet port and a distribution channel configured to enable a simultaneous introduction of the liquid into all primary channels; and
- an outlet port and an evacuation channel configured to enable a simultaneous evacuation of the air out of all the secondary channels.

[0006] According to some embodiments, the diameter D_{DCh}, or the smaller side h_{DCh} of the distribution channel, is selected to be substantially larger than the diameter D_{PCh}, or the smaller side h_{PCh} of the primary channel/s, respectively $D_{DCh} > D_{PCh}$ or $h_{DCh} > h_{PCh}$; such that the distribution channel is configured to be filled via the inlet port with liquid, while withholding the liquid from the primary channels, to about a predetermined threshold of its volume, enabling a liquid pressure, formed there-within, to then simultaneously load all the primary channels.

[0007] According to some embodiments, the device further comprising a plurality of distribution channels, each distribution channel of the plurality of distribution channels connecting the inlet port to the primary channel of a separate SNDA component; and wherein each distribution channel branches off from a single trunk channel that is connected to the inlet.

[0008] According to some embodiments, each distribution channel branches off perpendicularly from the trunk channel.

[0009] According to some embodiments, each of the distribution channels comprises a different cross section, relative to its distance from the common inlet, configured to allow a liquid flow from the common inlet opening, to flow via the common distribution channel, and reach all of the SNDA components concurrently.

[0010] According to some embodiments, the distribution channels are arranged along the trunk channel symmetrically, about a connection of the inlet to the trunk channel.

[0011] According to some embodiments, the connections the plurality of distribution channels with the trunk channel are equally spaced along the trunk channel.

[0012] According to some embodiments, a total length of each of each distribution channel of the plurality of distribution channels, between its connection to the trunk channel and its connection to the primary channel of an SNDA component, is adjusted to enable the substantially equal rates of liquid flow.

[0013] According to some embodiments, the total length of at least one distribution channel of the plurality of distribution channels is lengthened by addition of one or more open loops to said at least one distribution channel.

[0014] According to some embodiments, the lengths of all of the open loops that are added to distribution channels of the plurality of distribution channels are substantially equal.

[0015] According to some embodiments, the length of an open loop of said one or more open loops is equal to a distance between connections of two adjacent distribution channels

of the plurality of distribution channels to the trunk channel, where the connections of the plurality of distribution channels to the trunk channel are equally spaced along the trunk channel.

[0016] According to some embodiments, the number of the open loops that are added to a first distribution channel is smaller than the number of the open loops that are added to a second distribution channel, wherein a connection of the second distribution channel to the trunk channel is more proximal to a connection of the inlet to the trunk channel than to the connection of the first distribution channel to the trunk channel.

[0017] According to some embodiments, a cross section of a distribution channel, of the plurality of distribution channels, is selected to enable the substantially equal rates of liquid flow into each of the primary channels.

[0018] According to some embodiments, a width of a distribution channel having a largest cross-sectional area, is equal to a width of the primary channel of the SNDA component to which that distribution channel is connected.

[0019] According to some embodiments, all of the SNDA components are substantially identical.

[0020] According to some embodiments, the device further comprising a pressure device in communication with the outlet poet, configured to apply simultaneous negative pressure to all the secondary channels via the evacuation channel.

[0021] According to some embodiments of the present invention, there is provided an array of stationary nanoliter droplet array (SNDA) devices. The array may include a plurality of the SNDA devices aligned parallel to one another and laterally displaced relative to one another, each SNDA device of the plurality of SNDA devices comprising a primary channel and a plurality of nano-wells that are each open to the primary channel, each nano-well of said plurality of nano-wells being connected by one or more vents to a secondary channel to enable passage of air from that nano-well to the secondary channel when a liquid that is introduced into the primary channel fills that nano-well.

[0022] The array may also include an inlet for enabling introduction of the liquid into the array; and a plurality of distribution channels, each distribution channel of the plurality of distribution channels connecting the inlet to the primary channel of a separate SNDA device of the plurality of SNDA devices.

[0023] In some embodiments of the present invention, each distribution channel of the plurality of distribution channels branches off from a single trunk channel that is connected to the inlet.

[0024] In some embodiments of the invention, each distribution channel of the plurality of distribution channels branches off perpendicularly from the trunk channel.

[0025] In some embodiments of the invention, the plurality of distribution channels are arranged along the trunk channel symmetrically about a connection of the inlet to the trunk channel.

[0026] In some embodiments of the invention, connections the plurality of distribution channels with the trunk channel are equally spaced along the trunk channel.

[0027] In some embodiments of the invention, a total length of each of each distribution channel of the plurality of distribution channels between its connection to the trunk channel and its connection to the primary channel of an SNDA device of the plurality of SNDA devices is adjusted to enable the substantially equal rates of flow.

[0028] In some embodiments of the invention, the total length of at least one distribution channel of the plurality of distribution channels is lengthened by addition of one or more open loops to said at least one distribution channel.

[0029] In some embodiments of the invention, the lengths of all of the open loops that are added to distribution channels of the plurality of distribution channels are substantially equal.

[0030] In some embodiments of the invention, the length of an open loop of said one or more open loops is equal to a distance between connections of two adjacent distribution channels of the plurality of distribution channels to the trunk channel where the connections

of the plurality of distribution channels to the trunk channel are equally spaced along the trunk channel.

[0031] In some embodiments of the invention, the number of the open loops that are added to a first distribution channel is smaller than the number of the open loops that are added to a second distribution channel, wherein a connection of the second distribution channel to the trunk channel is more proximal to a connection of the inlet to the trunk channel than to the connection of the first distribution channel to the trunk channel.

[0032] In some embodiments of the invention, a cross section of a distribution channel of the plurality of distribution channels is adjusted to enable the substantially equal rates of flow.

[0033] In some embodiments of the present invention, a width of a distribution channel of the plurality of distribution channels having a greatest cross-sectional area is equal to a width of the primary channel of the SNDA device of the plurality of SNDA devices to which that distribution channel is connected.

[0034] In some embodiments of the invention, all of the plurality of SNDA devices are substantially identical.

[0035] In some embodiments of the invention, the secondary channels of the plurality of SNDA devices are connected to a single evacuation channel.

[0036] In some embodiments of the invention, the evacuation channel or shared channel includes an opening via which negative pressure is applicable to all of the secondary channels of the plurality of SNDA devices.

[0037] In some embodiments of the invention, plurality of the arrays is each connected to a single input opening via a feeder channel, all of the feeder channels configured to enable concurrent loading of the arrays.

[0038] In some embodiments of the invention, all arrays of the plurality of arrays are oriented parallel to one another.

[0039] In some embodiments of the invention, the feeder channels are branched.

[0040] In some embodiments of the invention, an array of the plurality of arrays is oriented perpendicular to at least one other array of the plurality of arrays.

[0041] In some embodiments of the invention, the secondary channels of the plurality of SNDA devices of all arrays of the plurality of arrays are connected to a single evacuation channel or shared secondary channel.

[0042] In some embodiments of the invention, the evacuation channel or shared channel includes an opening via which negative pressure is applicable to all of the secondary channels of the plurality of SNDA devices of all arrays of the plurality of arrays.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:

[0044] **Fig. 1A** schematically illustrates an example of a plurality of stationary nanoliter droplet array (SNDA) components arranged in an array configuration, forming a rectangular multiplexed SNDA device, according to some embodiments of the invention;

[0045] **Fig. 1B** schematically illustrates another example of a rectangular multiplexed SNDA device, according to some embodiments of the invention;

[0046] **Fig. 1C** schematically illustrates yet another example of a plurality of SNDA components arranged in an array configuration, forming a rectangular multiplexed SNDA device, according to some embodiments of the invention;

[0047] **Fig. 2** schematically illustrates an arrangement of distribution channels of a portion of a multiplexed array of SNDA devices, according to some embodiments of the invention;

[0048] **Fig. 3** schematically illustrates distribution channels a multiplexed array of SNDA devices, the lengths of the channels being adjusted and configured to enable a uniform flow rate, according to some embodiments of the invention;

[0049] **Fig. 4A** schematically illustrates an example of channels of a system of multiple multiplexed SNDA device arrays, where all SNDA devices are oriented parallel to one another, according to some embodiments of the invention; and

[0050] **Fig. 4B** schematically illustrates an example of channels of a system of multiple multiplexed SNDA device arrays, where some SNDA devices are oriented perpendicularly to others, according to some embodiments of the invention.

[0051] It will be appreciated that for simplicity and clarity of illustration, elements shown in the figures have not necessarily been drawn to scale. For example, the dimensions of some of the elements may be exaggerated relative to other elements for clarity. Further, where considered appropriate, reference numerals may be repeated among the figures to indicate corresponding or analogous elements.

DETAILED DESCRIPTION OF THE INVENTION

[0052] In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those of ordinary skill in the art that the invention may be practiced without these specific details. In other instances, well-known methods, procedures, components, modules, units and/or circuits have not been described in detail so as not to obscure the invention.

[0053] Although embodiments of the invention are not limited in this regard, discussions utilizing terms such as, for example, "processing," "computing," "calculating," "determining," "establishing", "analyzing", "checking", or the like, may refer to operation(s) and/or process(es) of a computer, a computing platform, a computing system, or other electronic computing device, that manipulates and/or transforms data represented as physical (e.g., electronic) quantities within the computer's registers and/or memories into other data similarly represented as physical quantities within the computer's registers and/or memories or other information non-transitory storage medium (e.g., a memory) that may store instructions to perform operations and/or processes. Although embodiments of the invention are not limited in this regard, the terms "plurality" and "a plurality" as used herein may include, for example, "multiple" or "two or more". The terms "plurality" or "a plurality" may be used throughout the specification to describe two or more components, devices, elements, units, parameters, or the like. Unless explicitly stated, the method

embodiments described herein are not constrained to a particular order or sequence. Additionally, some of the described method embodiments or elements thereof can occur or be performed simultaneously, at the same point in time, or concurrently. Unless otherwise indicated, the conjunction "or" as used herein is to be understood as inclusive (any or all of the stated options).

[0054] In accordance with some embodiments of the invention, and as demonstrated in **Figs. 1A**, **1B** and **1C**, plurality of stationary nanoliter droplet array (SNDA) components 14 (twelve SNDA components, in these examples) are arranged in an array configuration, forming a rectangular multiplexed SNDA device 10. In the multiplexed SNDA device 10, a liquid may be introduced into the nano-wells 18 of all of the SNDA components 14 of the SNDA device 10 via a common inlet opening 12, also referred to herein as a shared inlet. From the common inlet opening 12, the introduced liquid flows through an arrangement of distribution channels 46 that connects the inlet opening to the primary channel 16 of each SNDA component 14. As the liquid flows along the primary channel of each SNDA component, the liquid fills the nano-wells 18 along that primary channel.

[0055] According to some embodiments, in the multiplexed SNDA device 10, a plurality of SNDA components 14 are arranged substantially parallel to one another and are substantially aligned with one another. In this parallel and aligned configuration, the primary channels 16 of the SNDA components 14 are parallel to one another and are laterally displaced relative to one another. Thus, in this configuration, the connections of all of the primary channels to distribution channel/s lie along a single line 34, e.g., a line that is perpendicular to the orientation of the primary channels.

[0056] According to some embodiments, as the liquid fills the nano-wells 18 of each SNDA component 14, air escapes via vent/s (*not shown/visible*) of each nano-well 18 into a secondary channel/s 20. According to some embodiments, each SNDA component 14 typically includes two secondary channels 20, configured such that air from the nano-wells on either side of the primary channel 16 is enabled to vent out of the nano-well 18. According to some embodiments, in the multiplexed SNDA device 10, all of the secondary channels are arranged to connect to a single evacuation channel 22. According to some embodiments,, concurrently with introduction of liquid, via the common inlet opening 12, negative pressure can be applied to the evacuation channel 22, via an outlet 44, configured

to facilitate removal of the air from the nano-wells, and to facilitate flow of the introduced liquid into the nano-wells.

[0057] According to some embodiments, the distribution channels are configured such that a liquid that is introduced via the common inlet opening 12 flows into each primary channel 16 of the SNDA components 14 of the multiplexed SNDA device 10, at substantially equal flow rates. For example, flow rates may be considered to be substantially equal, when the differences in flow rate between two distribution channels does not exceed 5%, or, in some cases, does not exceed 3%. In this manner, the nano-wells of all of the SNDA components 14, in the multiplexed SNDA device 10, fill concurrently and at a common flow rate.

[0058] According to some embodiments, and as specifically demonstrated in **Fig. 1A**, some SNDA components 14, of the multiplexed SNDA device 10, are closer to the common inlet opening 12 than others. Therefore, according to some embodiments, a wide distribution channel 25 is provided, as a connecting channel between the single inlet 12 and the primary channels 16, feeding the wells 18 of the SNDA components 14. Accordingly, the cross-section of the wide distribution channel 25 is selected to be larger than the cross-section of the primary channels, such that the wide distribution channel 25 is configured to be filled with liquid, to a predetermined level of it's volume, before the liquid pressure that is formed there-within enables the liquid to flow and enter into the primary channel/s 16. According to some embodiments, the cross-section of the distribution channel 25 and/or the primary channel/s comprises a form selected from: a circle, an oval, a rectangle, a square, any polygon and any combination thereof.

[0059] According to some embodiments, the cross-section of the distribution channel 25 and the primary channel/s comprises a circular form. Accordingly the diameter D_{DCh} of the wide distribution channel 25 is selected to be larger than the diameter D_{PCh} of the primary channel/s 16 ($D_{DCh} > D_{PCh}$), such that the wide distribution channel 25 is configured to be filled with liquid to a predetermined threshold (for a non-limiting example about 95%-99%) of its volume, before the liquid pressure that is formed there-within enables to liquid to enter into the primary channel/s 16, in other words, before the liquid pressure that is formed there-within raises high enough, to enable the liquid to flow against the primary channel/s flow resistance.

[0060] According to some related embodiments, where their cross section is circular, an important solution to the Navier-Stokes equations is the Poiseuille (or Hagen-Poiseuille) flow, which applies when a pressure gradient is used to drive a liquid through a capillary or channel. For a capillary with cylindrical cross-section the following expression for the volume flow, Q, exists:

$$Q = \frac{\Delta V}{t} = \frac{\pi R^4 V}{8\eta L}\Delta P \qquad \text{Eq. \{1\}}$$

where R is the radius of the capillary, L is its length and ΔP is the pressure drop across this length (also called hydraulic pressure). The term, $8\eta L/\pi R^4$, of which the reciprocal appears in Eq. {1}, is also called the fluidic resistance. The dependency on $1/R^4$ implies that the fluidic resistance increases drastically as the channel dimensions are reduced. Consequently, higher pressure drops are necessary to move liquid through smaller conduits. For channels with noncylindrical cross sections, expressions similar to those in Eq. {1} can be found, but with different terms for the fluidic resistance.

[0061] According to some related embodiments, where their cross section is circular, the ratio between D_{DCh} : D_{PCh} is respectively selected from: *10:1, 9:1, 8:1, 7:1, 6:1, 5:1* and any combination thereof. According to some embodiments, the ratio between D_{DCh} : D_{PCh} is respectively *4 or more : 1*. According to some embodiments, the ratio between D_{DCh} : D_{PCh} is respectively selected *X:1* where *X* is selected between: $10>X>4$.

[0062] According to some embodiments, the cross-section of the distribution channel 25 and the primary channel/s comprises a rectangular form. For this example, shown in **Fig. 1A**, AA is the cross section of the wide distribution channel, where h_{DCh} is the smaller side and w_{DCh} is the other side of the AA rectangular cross section and BB is the cross section of the primary channel , where h_{PCh} is the smaller side and w_{PCh} is the other side of the BB rectangular cross section. According to such embodiments, the wall dimension h_{DCh} of the wide distribution channel 25 is selected to be larger than the wall dimension h_{PCh} of the primary channel/s 16 ($h_{DCh} > h_{PCh}$), such that the wide distribution channel 25 is configured to be filled with liquid to a predetermined threshold (for a non-limiting example about 95%-99%) of its volume, before the liquid pressure that is formed there-within enables to liquid to enter into the primary channel/s 16, in other words, before the liquid pressure that is formed there-within raises high enough, to enable the liquid to flow against the primary

channel/s resistance. According to a non-limiting example: $AA = w_{DCh} \times h_{DCh} = 0.3mm \times 0.3mm$ and $BB = w_{PCh} \times h_{PCh} = 0.15mm \times 0.1mm$.

[0063] According to some related embodiments, where their cross section is rectangular, an important solution to the Navier-Stokes equations is the Poiseuille (or Hagen-Poiseuille) flow, which applies when a pressure gradient is used to drive a liquid through a capillary or channel. For a capillary with rectangular cross-section the following expression approximation for the volume flow, Q, exists:

$$Q = \frac{\Delta V}{t} = \frac{h^4}{12\eta La}\Delta P(1 - 0.63a) \qquad \text{Eq. } \{2\}$$

where h is the smaller wall, and w is the other wall of the capillary, L is its length, $a=h/w$ is the aspect ratio of capillary walls, and ΔP is the pressure drop across this length (also called hydraulic pressure). The term, $12\eta La/h^4$, of which the reciprocal appears in Eq. {2}, is also called the fluidic resistance. The dependency on $1/h^4$ implies that the fluidic resistance increases drastically as the channel dimensions are reduced. Consequently, higher pressure drops are necessary to move liquid through smaller conduits.

[0064] According to some related embodiments, where their cross section is rectangular, the ratio between h_{DCh} : h_{PCh} is respectively selected from: *10:1, 9:1, 8:1, 7:1, 6:1, 5:1* and any combination thereof. According to some embodiments, the ratio between h_{DCh} : h_{PCh} is respectively *4 or more : 1*. According to some embodiments, the ratio between h_{DCh} : h_{PCh} is respectively selected *X:1* where *X* is selected between: $10>X>4$.

[0065] According to some embodiments, and as demonstrated in **Fig. 1B**, **1C**, **2**, **3**, **4A** and **4B**, some SNDA components 14 of the multiplexed SNDA device 10 are nearer/closer to the common inlet opening 12 than others. Therefore, a distribution channel 24f,27f that connects the common inlet opening 12 to a nearer SNDA component is configured to resist, or introduce a delay, into the flow through that distribution channel, relative to a distribution channel 24a,27a that connect a more distant SNDA component to the common inlet opening.

[0066] According to some embodiments, the distribution channel/s 24 that connect the common inlet opening 12 with the SNDA components that are close/r to the inlet opening are configured to be lengthened by an addition of bends or open loops 24b, 24c, 24d, 24e, 24f. In this manner, the lengths of all distribution channels 24a, 24b, 24c, 24d, 24e, 24f that connect each SNDA component to the common inlet opening are equal. For example, where

the flow through the distribution channels is assumed to be laminar, and where all of the distribution channels have substantially identical cross sections, the resistance to flow is assumed to be simply proportional to the length of the channel. In this case, where the flow rate is assumed to be equal to the pressure difference divided by the resistance to flow (analogous to Ohm's law for electrical current, potential difference, and electrical resistance, respectively), a calculation of the required additional length that is to be added to each distribution channel to ensure identical flow rates may be similar to analogous calculations for simple electrical circuits based on Kirchhoff's rules for electrical circuits.

[0067] According to some embodiments, alternatively or in addition, the cross-sectional area of a shorter distribution channel, e.g., that connects the common inlet opening to a nearer SNDA device is configured with a narrower diameter than a longer distribution channels that connects the common inlet opening to a more distant SNDA component.

[0068] According to some embodiments, and as specifically demonstrated in **Fig. 1C**, a flow resistance to the liquid entering from common inlet 12 via a common distribution channel 28 is configured to be made significantly low at a distal distribution channels of a distal SNDA component 14a (for example 27a is distal from inlet 12), compared with a proximal distribution channels of a proximal SNDA component 14f (for example 27f is proximal to inlet 12), such that flow rate entering to each of the primary channels is about equal. According to some embodiments, a reduced cross-sectional area is configured to reduce a flow rate, through a proximal distribution channel, relative to the flow rate through a distal distribution channel. In this way, the liquid that flows through the distribution channels 27 from the common inlet opening 12, via the common distribution channel 28, reaches all of the SNDA components 14 concurrently.

[0069] In some related embodiments, the connecting channels are designed differently one from another (by length as in 24 Fig. 1B, or by width as in 27 Fig. 1C) and/or that the resistance at the common distribution channel (as in 25 Fig. 1A) is configured to be reduced, such that fluid can first fill the common distribution channel 25,28 and then flow through the SNDAs' main channels to enable simultaneous loading of the SNDA components.

[0070] According to some embodiments, in addition to the introduction of a liquid into all of the SNDA components 14 of the multiplexed SNDA device 10, via the common inlet opening 12, the primary channel of each SNDA device can include an individual opening

32, configured to enable selective introduction of liquid into selected individual SNDA components 14. Typically (but not necessarily), the individual opening of each primary channel is located at an end of the primary channel that is opposite the opening of the primary channel to the distribution channels. For example, different experiments can be conducted concurrently, by introducing different antibiotic solutions, or that reagent solutions can be introduced into different SNDA component. According to some embodiments, no antibiotic or reagent solutions should be introduced into an SNDA component that is to function as a control measure.

[0071] According to some embodiments, the multiplexed SNDA device 10 comprises a flat rectangular form, such that all SNDA components 14 are arranged in an array configuration and are oriented parallel to one another and linearly displaced relative to one another along a single pair of orthogonal axes. This rectangular arrangement within the multiplexed SNDA device 10 is advantageous over other arrangements of SNDA devices (e.g., a circular arrangement, where SNDA devices extend radially from an inlet opening). For example, the rectangular arrangement is configured to enable more efficient use of space/volume, e.g., more compact filling, than an arrangement where adjacent SNDA components are rotated relative to one another. The rectangular arrangement is configured to enable efficient and easy control of the SNDA components, for example when positioning (whether manually or by an automatically controlled stage) a successive SNDA component within a field of view of a viewing or imaging device.

[0072] According to some embodiments, a plurality of rectangular multiplexed SNDA devices 10 are configured to be connected to a common inlet, as demonstrated in **Figs. 4A** and **4B**. For example, the plurality of rectangular multiplexed SNDA devices 10 can be connected to the common inlet in a symmetric manner such that the lengths of channels that connect the common inlet to the inlet opening of each of the multiplexed SNDA device 10 are equal to one another. In some cases, one or more of the multiplexed SNDA device arrays can be rotated 90° relative to other of the multiplexed SNDA device. When one multiplexed SNDA device is rotated by 90° relative to another, the aforementioned advantages of efficient use of space and ease of control may still be present.

[0073] Reference is made again to **Fig. 1B**, which schematically illustrates an example of a rectangular multiplexed array 10 of stationary nanoliter droplet array (SNDA) components 14, according to some embodiments of the invention.

[0074] In some embodiments, the multiplexed SNDA device 10 is provided a plurality of SNDA components 14, which are arranged parallel to one another. A liquid may be introduced concurrently into all SNDA components 14 via common inlet 12, also referred to herein as shared inlet 12. For example, common inlet 12 may connect to an opening in a cover (not shown) that covers multiplexed SNDA device 10.

[0075] According to some embodiments, the common inlet 12 is connected to each of the SNDA components 14 via a distribution channel 24. In the example shown, distribution channels 24 branch off of a single distribution trunk channel 28. According to some embodiments, and as in the shown example, distribution channels 24 branch off perpendicularly from distribution trunk channel 28. In other examples/embodiments, distribution channels 24 can otherwise connect to common inlet 12. For example, a distribution channel 24 can connect to common inlet 12 via a diagonal or curved segment of that distribution channel 24, can branch off of distribution trunk channel 28 at an oblique angle, or may otherwise connect to common inlet 12.

[0076] According to some embodiments, and as in the shown example, common inlet 12 is located at symmetry axis 30, and distribution channels 24 are arranged symmetrically about symmetry axis 30. In other examples/embodiments, common inlet 12 can be located closer to one lateral side of multiplexed SNDA device array 10, e.g., such that a distance between common inlet 12 and an SNDA component 14 at one end of multiplexed SNDA device 10 is less than the distance between common inlet 12 and an SNDA component 14 at the other end of multiplexed SNDA device 10.

[0077] According to some embodiments, each SNDA component 14 comprises a primary channel 16 that connects to one of distribution channels 24. Thus, a liquid that is introduced into common inlet 12 can flow from common inlet 12 and into primary channels 16 of all SNDA components 14 of multiplexed SNDA device 10 via distribution channels 24 that connect common inlet 12 to all primary channels 16.

[0078] According to some embodiments, a separate inlet 32 (located at an opening in a cover of multiplexed SNDA device 10) to each primary channel 16 can be located at an end of primary channel 16 that is opposite to an end that is connected via distribution channel 24 to common inlet 12. Accordingly, liquid can be introduced into primary channel 16 of a selected SNDA components 14 of the multiplexed SNDA device 10 via separate inlets 32 of the selected SNDA components 14, without being introduced into other SNDA components 14 of the multiplexed SNDA device array 10.

[0079] According to some embodiments, a liquid that flows into a primary channel 16 of an SNDA component 14 can flow into nano-wells 18 that are open to that primary channel 16. As each nano-well 18 is filled, any air or gas that had previously filled that nano-well 18 is enabled to flow outward via one or more vents of that nano-well 18 (not visible at the scale of Fig. 1B) to a secondary channel 20 that is adjacent to that nano-well 18. For example, a typical SNDA component 14 includes two secondary channels 20, on opposite sides of its primary channel 16.

[0080] In some embodiments, each nano-well 18 typically has a volume that is less than 100 nanoliters. In some embodiments, each vent has a length of a few (less than or about 10) μm. In some embodiments, each nano-well 18 has a length about 400 μm, a width of about 200 μm, and a height of about 100 μm, each vent has a width of about 7 μm and a height of about 100 μm, each primary channel 16 (and, possibly, each distribution channel 24) has a width of about 150 μm, and each secondary channel 20 has a width of about 1 mm. In other examples, structure of a multiplexed SNDA device 10 can have different dimensions.

[0081] In the example shown, all secondary channels 20 of multiplexed SNDA device 10 connect to a single evacuation channel 22. In this manner, air from all nano-wells 18 can be evacuated via a single opening 44. According to some embodiments, negative pressure that is applied to evacuation channel 22 is, therefore, applied to all secondary channels 20 and to all nano-wells 18. Thus, application of negative pressure to evacuation channel 22 facilitates flow of liquid into nano-wells 18.

[0082] According to some embodiments, the structure of multiplexed SNDA device 10, including channels (e.g., common inlet 12, distribution trunk channel 28, distribution channels 24, primary channels 16, separate inlets 32, secondary channels 20, evacuation

channel 22, and other channels) and nano-wells 18, can be formed together with a base that forms the bottom of each of the structures. For example, the base and structure can be formed using any applicable method, for example, by a molding, spin coating, stamping process, hot embossing, three-dimensional (3D) printing, etc., or can be formed by applying an etching, micromachining, or photolithography process to a block of material. According to some embodiments a cover can then be attached to the base and structure to cover the structure. Typically, the cover is transparent to enable optical or visual examination of the contents. Typically, the cover includes openings to enable introduction of liquids into the structure. For example, one or more openings can be positioned so as to enable introduction of liquids into common inlet 12, and, at least in some cases, into one or more separate inlets 32. One or more openings 44 can be positioned to enable evacuation of air there-through, or application of negative pressure to evacuation channel 22.

[0083] According to some embodiments, the length (or, in some cases, the cross-sectional area, or both) of each distribution channel 24 is selected such that the rate of the flow of a liquid that is introduced into that distribution channel 24, via common inlet 12, is substantially equal to the rate of flow in all of the other distribution channels 24. In the example shown, in order to achieve the equal flow rates, the lengths of each of distribution channels 24b to 24f is increased by the addition of one or more extensions, such as open loops 26. In the example shown, all open loops 26 are of substantially equal, having predetermined length, and are approximately U-shaped (e.g., with a curved or flat bottom). In the schematic example shown, the length of each open loop 26 is equal to separation distance *d* between two adjacent connection nodes 40, where adjacent distribution channels 24 connect to distribution trunk channel 28. The number of open loops 26 added to each distribution channel 24 is selected to retard the rate of flow in a distribution channel 24 (e.g., in distribution channel 24f) that connects common inlet 12 to a more proximal (e.g., to common inlet 12 or to inlet connection 36) SNDA component 14 to equal the rate of flow in a distribution channel 24 (e.g., distribution channel 24a) that connects common inlet 12 to a more distal SNDA component 14.

[0084] It may be noted that, in the schematic example shown, the number of open loops 26 that are added to each distribution channel 24 is based on a simple calculation, in which the number of open loops 26 of length *d* that are added to each distribution channel 24b to 24f that branches off of distribution trunk channel 28, at a connection node 40, is equal to

the distance between that connection node 40 and the most distal node (e.g., the connection node 40, where distribution channel 24a connects to distribution trunk channel 28). A more accurate calculation that takes into account different flow rates through different sections of distribution trunk channel 28 is described below.

[0085] In other examples, the lengths of different distribution channels 24 can be otherwise adjusted, cross sectional areas of different distribution channels 24 can be adjusted, surface properties of different distribution channels 24, or other adjustments to distribution channels 24 can be made to achieve equal rates of flow through all distribution channels 24.

[0086] According to some embodiments, when a pressure difference between common inlet 12 and evacuation channel 22 is constant (e.g., due to negative pressure that is applied to evacuation channel 22), the rate of flow in each distribution channel 24 of a liquid that is introduced into multiplexed SNDA device 10, via common inlet 12, can be inversely proportional to the resistance of each distribution channel 24 to flow (e.g., analogous to Ohm's law that states that current is equal to potential difference divided by electrical resistance). In the case of laminar flow, resistance to flow can be a function of at least the viscosity of the liquid, cross sectional area of a conduit, and length of the conduit.

[0087] In the example shown, the cross-sectional areas of all distribution channels 24, as well as of distribution trunk channel 28, are substantially identical. Therefore, in the event of laminar flow of a single incompressible liquid through all distribution channels 24, the rate of flow through a distribution channel 24 can be adjusted by adjusting the length of that distribution channel 24. Furthermore, it may be assumed that the resistances to flow through all SNDA component 14 of multiplexed SNDA device 10 are substantially identical. Therefore, it may be assumed that, when substantially equal flow rates are achieved, the difference in pressure between inlet connection 36 between common inlet 12 and distribution trunk channel 28, and the connection (along SNDA device connection line 34) of each distribution channel 24 to its connected SNDA components 14 is the same for all distribution channels 24.

[0088] Accordingly, a calculation of a length of each distribution channel 24, or, equivalently, of a number of open loops 26 (of predetermined length) that are to be included

in each distribution channel 24, can be based on an analogy to Kirchhoff's rules for electrical circuits.

[0089] According to some embodiments, in such an analogous calculation, the pressure difference between two points that are connected by one or more conduits is analogous to a difference in electrical potential, or voltage. As in the electrical analog, the pressure difference is the same for all parallel conduits that connect the two points. The flow rate is analogous to electrical current. As in the electrical analog, at a node where a single conduit branches into two or more branch conduits, the total flow rate into the node (e.g., through the single node) is equal to the total flow rate out of the node (e.g., through all the branch conduits). Resistance to flow in each conduit is analogous to electrical resistance. Thus, as in Ohm's law of the electrical analog, the rate of flow in a conduit is equal to the pressure difference between the ends of the conduit divided by the resistance to flow in that conduit.

[0090] Therefore, as in the electrical analog, when conduits are connected in series, the total resistance to flow R_s is the sum of the resistances to flow of the connected conduits:

[0091] $R_s = R_1 + R_2 + \ldots + R_n$,

[0092] where $R_1, R_2, \ldots R_n$ are the resistances to flow of each of the connected conduits. Similarly, when n conduits are connected in parallel, the total resistance to flow R_p may be calculated from the formula:

[0093] $1/R_p = 1/R_1 + 1/R_2 + \ldots + 1/R_n$.

[0094] In an example where laminar flow may be assumed (e.g., slow flow rates and low Reynold's number), and where all of the conduits have similar walls and cross sections, the resistance to flow is substantially proportional to the length of the conduit. Therefore, in such a case, lengths of conduit sections may be substituted for the resistances in the above formulae.

[0095] Multiplexed SNDA device 10 is configured to enable substantially equal flow rates through all of distribution channels 24. In particular, calculations based on the analogy to electrical current can be applied to distribution trunk channel 28 and distribution channels 24 between inlet connection 36 and SNDA device connection line 34. The purpose of the

calculation is to determine any additional resistance to flow that is to be added to distribution channels 24, in order to enable substantially equal flow rates in all distribution channels 24.

[0096] According to some embodiments, by making the flow rates equal in all distribution channels 24, all SNDA components 14 can be filled concurrently and the terms applied on SNDAs are identical. In the absence of a configuration that enables equal flow rates, an SNDA component 14 that is nearest to common inlet 12 (e.g., an SNDA component 14 that is connected to distribution channel 24f) would be likely to completely fill before an SNDA component 14 that is further from common inlet 12 (e.g., an SNDA component 14 that is connected to any of distribution channels 24a to 24e) has completed filling, or perhaps has not even begun to fill. Such uneven filling could adversely affect results of testing that entails comparison of results in different SNDA components 14 of multiplexed SNDA device 10.

[0097] **Fig. 2** schematically illustrates an arrangement of distribution channels of a portion of a multiplexed array of SNDA components, according to some embodiments of the invention.

[0098] As shown in **Fig. 2**, all unlengthened distribution channels 42a to 42f are shown without any loops. As shown, unlengthened distribution channels 42a to 42f are shown with their minimum lengths for connecting inlet connection 36 with SNDA components 14, prior to adjustment in order to provide a uniform flow rate in all of unlengthened distribution channels 42a to 42f. The length of each of unlengthened distribution channels 42a to 42f, e.g., from its connection to distribution trunk channel 28 at one of connection nodes 40a to 40f, to its connection to an SNDA component 14, at SNDA device connection line 34, is channel minimum length *D*. The lateral center-to-center distance between adjacent connection nodes 40a to 40f is separation distance *d*.

[0099] In this example, since the path between inlet connection 36 and SNDA device connection line 34, via unlengthened distribution channel 42a, is longer than the path via other unlengthened distribution channels 42b - 42f, any adjustments to the lengths of distribution channels 24a to 24f may require lengthening of unlengthened distribution channels 42b to 42f, rather than shortening unlengthened distribution channel 42a. In other examples/embodiments, e.g., where diagonal or other variants of distribution channels are allowed, adjustment can include shortening distribution channels.

[00100] According to some embodiments, the calculation yields a total channel length *L*, for each of distribution channels 24a to 24f, that enables a uniform flow rate through all of the distribution channels 24a - 24f. As stated above, in the current example, total length L_a of distribution channel 24a between connection node 40a and SNDA device connection line 34 is equal to minimum length *D*.

[00101] According to some embodiments, at connection node 40b, in order that the flow rate via distribution channel 24b between connection node 40b and SNDA device connection line 34 equal that via distribution channel 24a, the resistances to flow via distribution channels 24a and 24b, and thus total lengths L_a and L_b, respectively, are to be made equal. The length of a path between connection node 40b and SNDA device connection line 34 via unlengthened distribution channel 42a is the sum of *D*, the length of unlengthened distribution channel 42a, and *d*, the distance between connection node 40b and connection node 40a. Therefore, total channel length L_b for distribution channel 24b (corresponding to unlengthened distribution channel 42b, with an added open loop 26) can be calculated as:

[00102] $L_b = D + d$.

[00103] Accordingly, distribution channel 24b includes an open loop 26 of length *d* (or a plurality of loops whose total length is *d*).

[00104] According to some embodiments, at connection node 40c, a calculated total length L_c of distribution channel 24c is to result in equal flow rates between connection node 40c and SNDA device connection line 34 via each of distribution channels 24a to 24c. Using the aforementioned formula for series and parallel resistances, the equivalent resistance to flow between connection node 40c and SNDA device connection line 34 via parallel flow through distribution channels 24a and 24b is proportional to $(D + 3d) / 2$. Further noting that the flow rate via the section of distribution trunk channel 28 between connection node 40c and 40b (and thus through the combination of distribution channels 24a and 24b) is double the flow rate through distribution channel 24c, the total length L_c of distribution channel 24c that enables a uniform flow rate can be calculated to be:

[00105] $L_c = D + 3d$.

[00106] Accordingly, distribution channel 24c includes one or more open loops 26 of total length *3d*. It may be noted that the length of open loops 26 that are added to distribution channel 24c in this calculation for L_c of distribution channel 24c, as well as the calculations below for distribution channels 24d to 24f, differs from the number of open loops 26 shown in the general layout illustration in **Fig. 1B**, and which are based on a different calculation.

[00107] Similarly, according to some embodiments, at connection node 40d, a calculated total length L_d of distribution channel 24d is to result in equal flow rates between connection node 40d and SNDA device connection line 34 via each of distribution channels 24a to 24d. Using the aforementioned formula for series and parallel resistances, the equivalent resistance to flow between connection node 40d and SNDA device connection line 34 via parallel flow through distribution channels 24a through 24c is proportional to $(D + 3d) / 3$. Further noting that the flow rate via the section of distribution trunk channel 28 between connection node 40d and 40c (and thus through the combination of distribution channels 24a to 24c is triple the flow rate through distribution channel 24d, the total length L_d of distribution channel 24d that enables a uniform flow rate can be calculated to be:

[00108] $L_d = D + 6d$.

[00109] Accordingly, distribution channel 24d includes one or more open loops 26 of total length *6d*.

[00110] Similarly, according to some embodiments, at connection node 40e, a calculated total length L_e of distribution channel 24e is to result in equal flow rates between connection node 40e and SNDA device connection line 34 via each of distribution channels 24a to 24e. Using the aforementioned formula for series and parallel resistances, the equivalent resistance to flow between connection node 40e and SNDA device connection line 34 via parallel flow through distribution channels 24a through 24d is proportional to $(D + 6d) / 4$. Further noting that the flow rate via the section of distribution trunk channel 28 between connection node 40e and 40d (and thus through the combination of distribution channels 24a to 24d is quadruple the flow rate through distribution channel 24e, the total length L_e of distribution channel 24e that enables a uniform flow rate can be calculated to be:

[00111] $L_e = D + 10d$.

[00112] Accordingly, distribution channel 24e includes one or more open loops 26 of total length 10d.

[00113] Finally (in the example shown), according to some embodiments, at connection node 40f, a calculated total length L_f of distribution channel 24f is to result in equal flow rates between connection node 40f and SNDA device connection line 34 via each of distribution channels 24a to 24f. Using the aforementioned formula for series and parallel resistances, the equivalent resistance to flow between connection node 40f and SNDA device connection line 34 via parallel flow through distribution channels 24a through 24e is proportional to $(D + 10d) / 5$. Further noting that the flow rate via the section of distribution trunk channel 28 between connection node 40f and 40e (and thus through the combination of distribution channels 24a to 24e is five times the flow rate through distribution channel 24f, the total length L_f of distribution channel 24f that enables a uniform flow rate can be calculated to be:

[00114] $L_f = D + 15d.$

[00115] Accordingly, distribution channel 24f includes one or more open loops 26 of total length 15d.

[00116] According to some embodiments, this calculation can be continued in a similar manner for numbers of distribution channels 24 greater than six. When the number of distribution channels 24 is fewer than six, the calculation can proceed as described above until the lengths L of all distribution channels 24 have been calculated.

[00117] It may be noted that, when distribution channels 24 are arranged symmetrically about symmetry axis 30, calculations need be performed only on one side of symmetry axis 30. When symmetrically arranged, the calculated total lengths L of each pair of symmetrically arranged distribution channels 24 that are equidistant from symmetry axis 30 are identical to one another. In the event of an asymmetric arrangement of distribution channels 24, or where the distance between adjacent connection nodes 40 is not the same for all pairs of adjacent distribution channels 24, calculation may be modified in accordance with the asymmetric positions of distribution channels 24.

[00118] **Fig. 3** schematically illustrates distribution channels of the right side of the symmetry plane of a multiplexed array of SNDA components, according to some embodiments of the invention, where the lengths of the channels being adjusted to enable a uniform flow rate.

[00119] According to some embodiments, in channel arrangement 46, a total length of each of distribution channels 24a to 24d is as calculated in the examples above. The length of each of distribution channels 24b to 24d includes one or more open loops 26. In the example shown, the length of each open loop 26 is equal to separation distance *d*. Therefore, the number of open loops 26 in each of distribution channels 24a to 24d is equal to the multiple of *d* that is added to channel minimum length *D* to yield total length *L* for each of distribution channels 24a to 24d.

[00120] For example, in accordance with the calculation above, distribution channel 24a includes no (zero) open loops 26, distribution channel 24b includes one open loop 26, distribution channel 24c includes three open loops 26, and distribution channel 24d includes six open loops 26. Identical numbers of open loops 26 can be included in distribution channels 24 that extend from distribution trunk channel 28 at positions that are symmetrical about symmetry axis 30 to those of distribution channels 24a to 24d.

[00121] It may be noted that a maximum distance between distribution trunk channel 28 and SNDA device connection line 34 can be limited by various considerations. Accordingly, there can be various reasons for limiting the number of open loops 26 that can be added to a distribution channel 24. Other considerations can limit a minimum size of *d*. Thus, the number of distribution channels 24 that extend from distribution trunk channel 28 may be limited. In the examples shown in Figs. 1B and 3, the maximum number of open loops 26 that can be included in a single distribution channel 24 is limited to about six. In this case, if the added length is calculated as described above, no more than four distribution channels 24 can extend from distribution trunk channel 28 on either side of symmetry axis 30.

[00122] Alternatively, or in addition to adjusting a total length of each distribution channel 24, a cross section of each distribution channel 24 can be designed to enable substantially identical flow rates through all distribution channels 24. For example, channel arrangement in such a case can be similar to the arrangement of **Fig. 2**, where each unlengthened distribution channel 42 has a different cross section.

[00123] For example, results of a flow simulation may yield a width of each unlengthened distribution channel 42 required to provide identical flow rates through all of unlengthened distribution channels 42.

[00124] In one example simulation, the widths of unlengthened distribution channel 42a and of distribution trunk channel 28 were set to 150 μm (e.g., to match the width of primary channels 16), d was set to 2.35 mm, and D was set to 11 mm. In this simulation, the calculated widths ranged from 14 μm for unlengthened distribution channel 42b to about 10 μm for unlengthened distribution channel 42f. It may be noted that, in this example, the differences in width among unlengthened distribution channels 42b to 42f are small relative to the width of unlengthened distribution channel 42a. Different results can be obtained from simulations based on other dimensions.

[00125] According to some embodiments, the rectangular shape of multiplexed SNDA device 10 can enable connecting a plurality of component multiplexed SNDA devices 10 into a multi-array system. The multi-array system can include a single inlet port into which a liquid is to be introduced to flow to all the component multiplexed SNDA devices 10 via an arrangement of feeder channels. Similarly, all secondary channels 20 can be connected to a single evacuation channel (e.g., having a rectangular form) to which negative pressure can be applied.

[00126] **Fig. 4A** schematically illustrates an example of channels of a system 51 of multiple multiplexed SNDA devices, according to some embodiments of the invention, where all SNDA devices 10a – 10h are oriented parallel to one another.

[00127] In the example shown of channeling system 50, eight multiplexed SNDA devices 10a-10h, and their associated channel arrangements 46, are connected to a single input port 52. A liquid that is introduced into channeling system 50 via input port 52 can flow from input port 52 to multiple channel arrangements 46 via feeder channels 54. Feeder channels 54 are configured such that the lengths of all paths from input port 54 to each of channel arrangements 46 are substantially identical. In the example shown, feeder channels 54 are arranged in a branched pattern in which all branches are of equal length.

[00128] According to some embodiments, a single evacuation channel (not shown), for example having a rectangular shape or a U-shape, can surround all of the multiplexed

SNDA devices 10a-10h that are connected to input port 52, via feeder channels 54 and channel arrangements 46. According to some embodiments, the evacuation channel can include a single port via which negative pressure can be applied to all component multiplexed SNDA devices 10a-10h.

[00129] **Fig. 4B** schematically illustrates an example of channels of a system 61 of multiple multiplexed SNDA devices 10i-10l, according to some embodiments of the invention, where some SNDA devices are oriented perpendicularly to others.

[00130] In the example shown of channeling system 60, four multiplexed SNDA devices 10i-10l, and their associated channel arrangements 46a and 46b, are connected to a single input port 52. A liquid that is introduced into channeling system 60 via input port 52 can flow from input port 52 to multiple channel arrangements 46a and 46b via feeder channels 62. In the example shown, feeder channels 62 are in the form of segments with resistance that can be substantially lower than the resistance at 46a and 46b entry port, ensuring that all feeding channels are filled prior to reaching the 46a,b complexes.

[00131] In the example shown, channel arrangements 46a are arranged opposite one another across input port 52. Similarly, channel arrangements 46b, each rotated 90° to channel arrangements 46a, are arranged opposite one another across input port 52.

[00132] According to some embodiments, a single evacuation channel (not shown), e.g., that is rectangular, can surround all of the multiplexed SNDA devices 10i-10l that are connected to input port 52 via feeder channels 54 and channel arrangements 46a and 46b. The evacuation channel can include a single port via which negative pressure can be applied to all component multiplexed SNDA devices 10i-10l.

[00133] Different embodiments are disclosed herein. Features of certain embodiments may be combined with features of other embodiments; thus, certain embodiments may be combinations of features of multiple embodiments. The foregoing description of the embodiments of the invention has been presented for the purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed. It should be appreciated by persons skilled in the art that many modifications, variations, substitutions, changes, and equivalents are possible in light of the above teaching.

It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

[00134] While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

1. A device comprising:

   - plurality of Stationary Nanoliter Droplet Array (SNDA) components; each SNDA component comprising: at least one primary channel; at least one secondary channel; and a plurality of nano-wells that are each open to the primary channel and are each connected by one or more vents to the secondary channel; the vents are configured to enable passage of air solely from the nano-wells to the secondary channel, such that when a liquid is introduced into the primary channel it fills the nano-wells, and the originally accommodated air is evacuated via the vents and the secondary channel/s;

     wherein the plurality of the SNDA components are aligned parallel to one another and laterally displaced relative to one another, such that the device comprises a rectangular form;

   - an inlet port and a distribution channel configured to enable a simultaneous introduction of the liquid into all primary channels; and

   - an outlet port and an evacuation channel configured to enable a simultaneous evacuation of the air out of all the secondary channels.

2. The device of claim 1, wherein the diameter D_{DCh} or the smaller side h_{DCh} of the distribution channel is selected to be substantially larger than the diameter D_{PCh} or the smaller side h_{PCh} of the primary channel/s, respectively $D_{DCh} > D_{PCh}$ or $h_{DCh} > h_{PCh}$; the distribution channel is configured to be filled via the inlet port with liquid, while withholding the liquid from the primary channels, to about a predetermined threshold of its volume, enabling a liquid pressure formed there-within, to then simultaneously load all the primary channels.

3. The device of claim 1, further comprising a plurality of distribution channels, each distribution channel of the plurality of distribution channels connecting the inlet port to the primary channel of a separate SNDA component; and wherein each distribution channel branches off from a single trunk channel that is connected to the inlet.

4. The device of claim 3, wherein each distribution channel branches off perpendicularly from the trunk channel.

5. The device of claim 4, wherein each of the distribution channels comprises a different cross section, relative to its distance from the common inlet, configured to allow a liquid flow from the common inlet opening, to flow via the common distribution channel, and reach all of the SNDA components concurrently.

6. The device of claim 4, wherein the distribution channels are arranged along the trunk channel symmetrically, about a connection of the inlet to the trunk channel.

7. The device of claim 4, wherein the connections the plurality of distribution channels with the trunk channel are equally spaced along the trunk channel.

8. The device of claim 4, wherein a total length of each of each distribution channel of the plurality of distribution channels, between its connection to the trunk channel and its connection to the primary channel of an SNDA component, is adjusted to enable the substantially equal rates of liquid flow.

9. The device of claim 8, wherein the total length of at least one distribution channel of the plurality of distribution channels is lengthened by addition of one or more open loops to said at least one distribution channel.

10. The device of claim 9, wherein the lengths of all of the open loops that are added to distribution channels of the plurality of distribution channels are substantially equal.

11. The device of claim 10, wherein the length of an open loop of said one or more open loops is equal to a distance between connections of two adjacent distribution channels of the plurality of distribution channels to the trunk channel, where the connections of the plurality of distribution channels to the trunk channel are equally spaced along the trunk channel.

12. The device of claim 10, wherein the number of the open loops that are added to a first distribution channel is smaller than the number of the open loops that are added to a second distribution channel, wherein a connection of the second distribution channel

to the trunk channel is more proximal to a connection of the inlet to the trunk channel than to the connection of the first distribution channel to the trunk channel.

13. The device of claim 3, wherein a cross section of a distribution channel, of the plurality of distribution channels, is selected to enable the substantially equal rates of liquid flow into each of the primary channels.

14. The device of claim 13, wherein a width of a distribution channel having a largest cross-sectional area, is equal to a width of the primary channel of the SNDA component to which that distribution channel is connected.

15. The device of claim 1, wherein all of the SNDA components are substantially identical.

16. The device of claim 1, further comprising a pressure device in communication with the outlet poet, configured to apply simultaneous negative pressure to all the secondary channels via the evacuation channel.

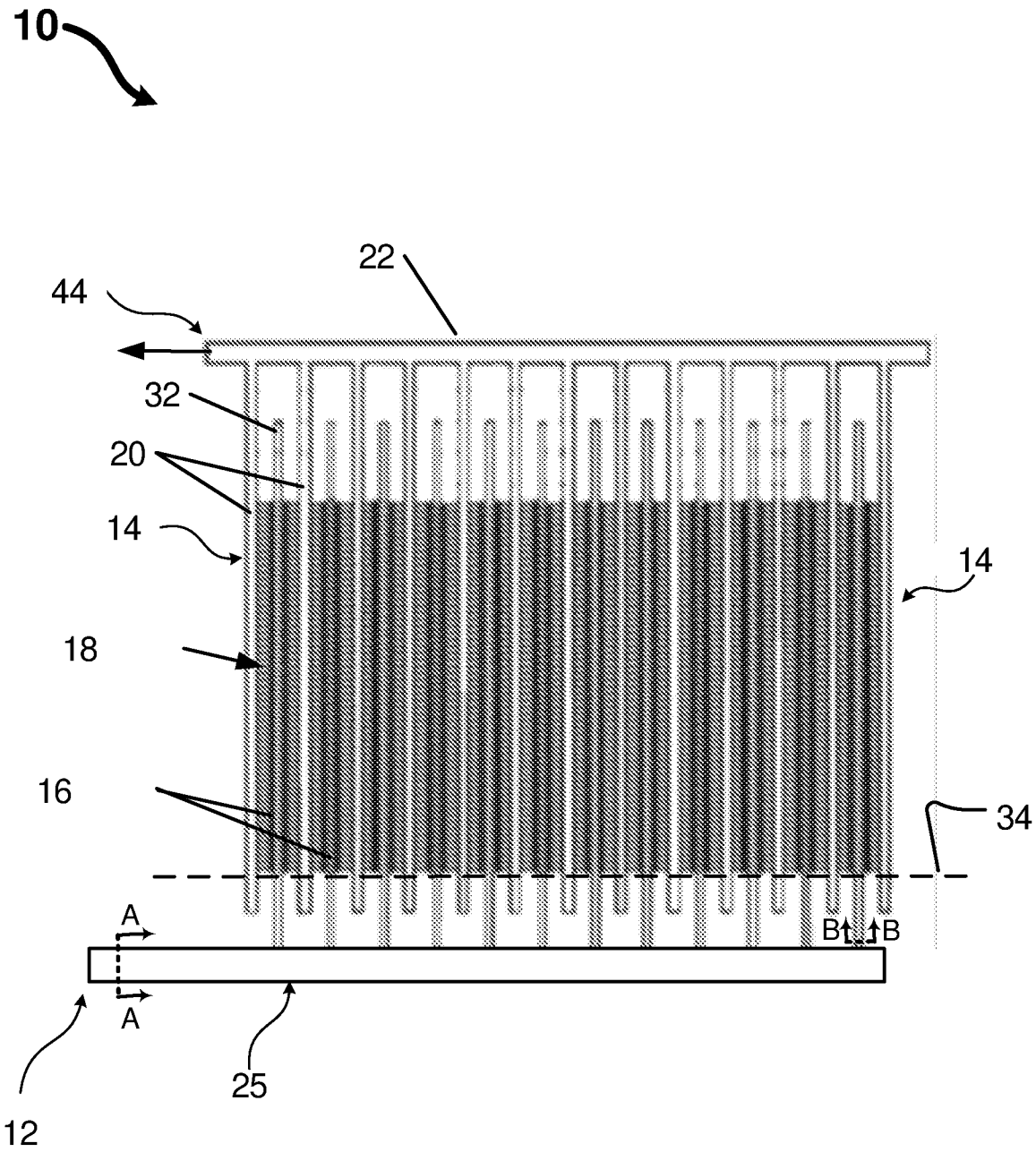


Fig. 1A

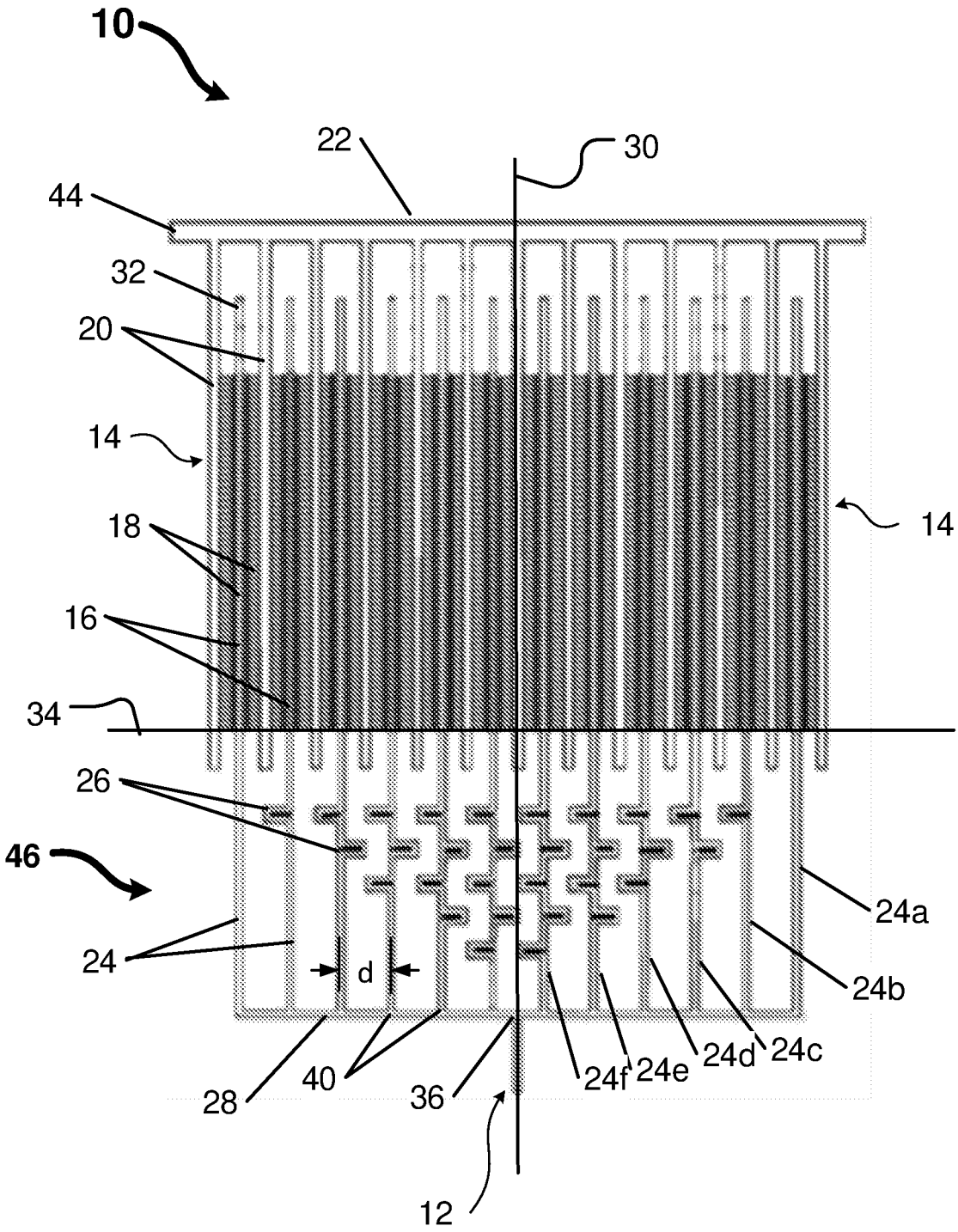

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12

Fig. 1B

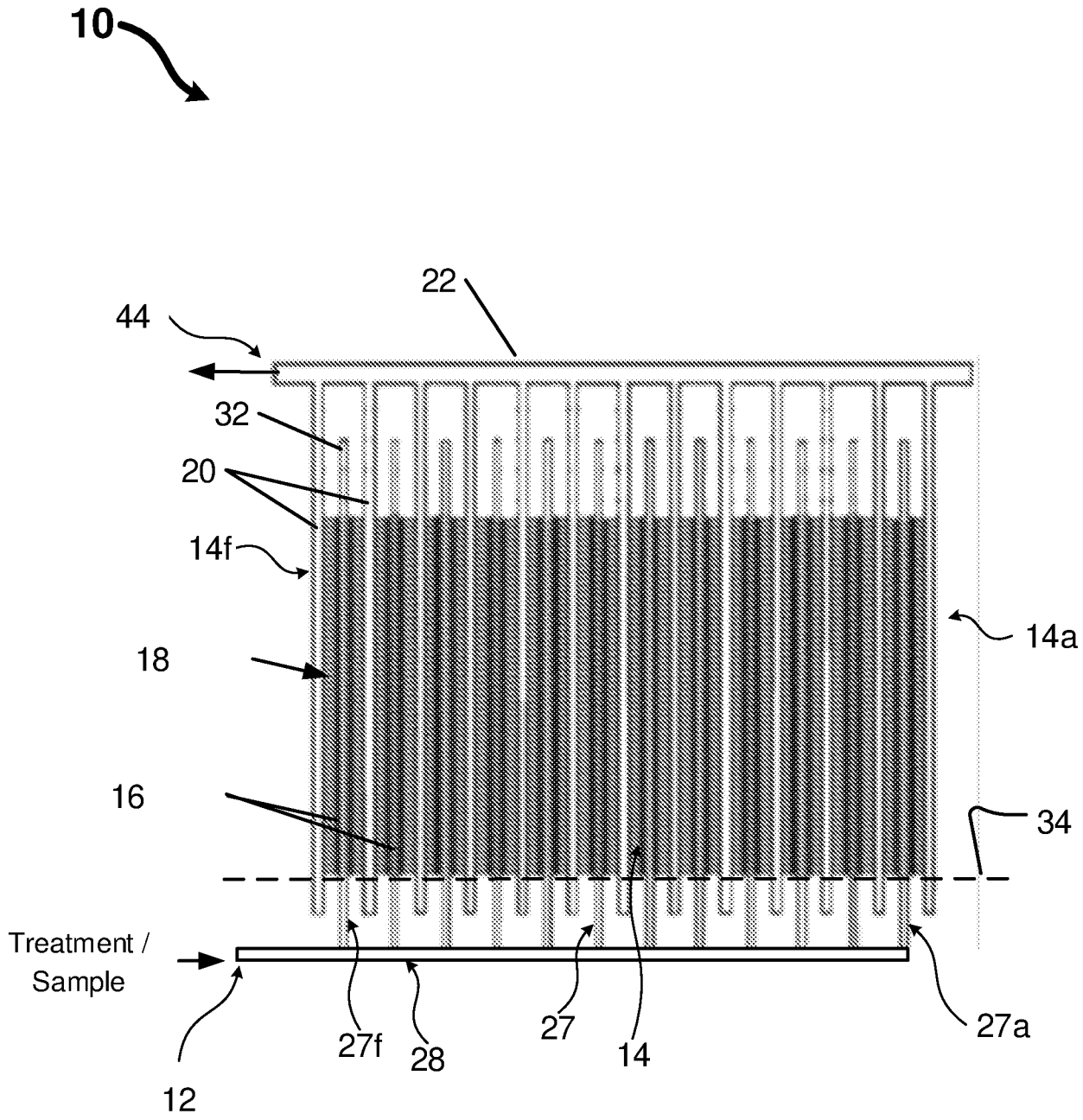

10
22
44
32
20
14f
18
14a
16
34
Treatment /
Sample
12
27f
28
27
14
27a

Fig. 1C

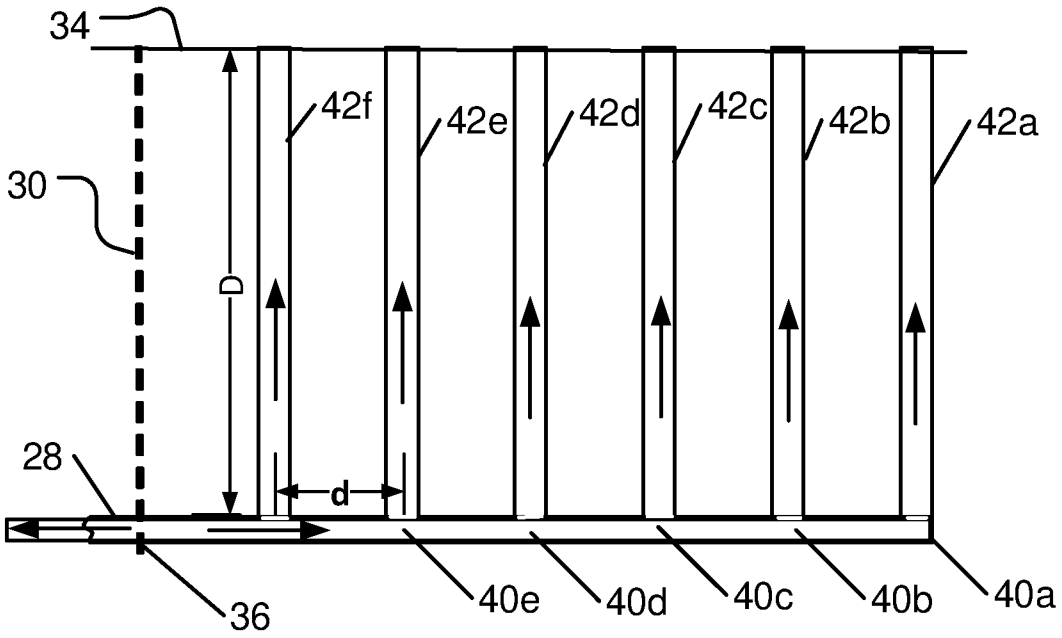

34
30
28
D
d
42f
42e
42d
42c
42b
42a
36
40e
40d
40c
40b
40a

Fig. 2

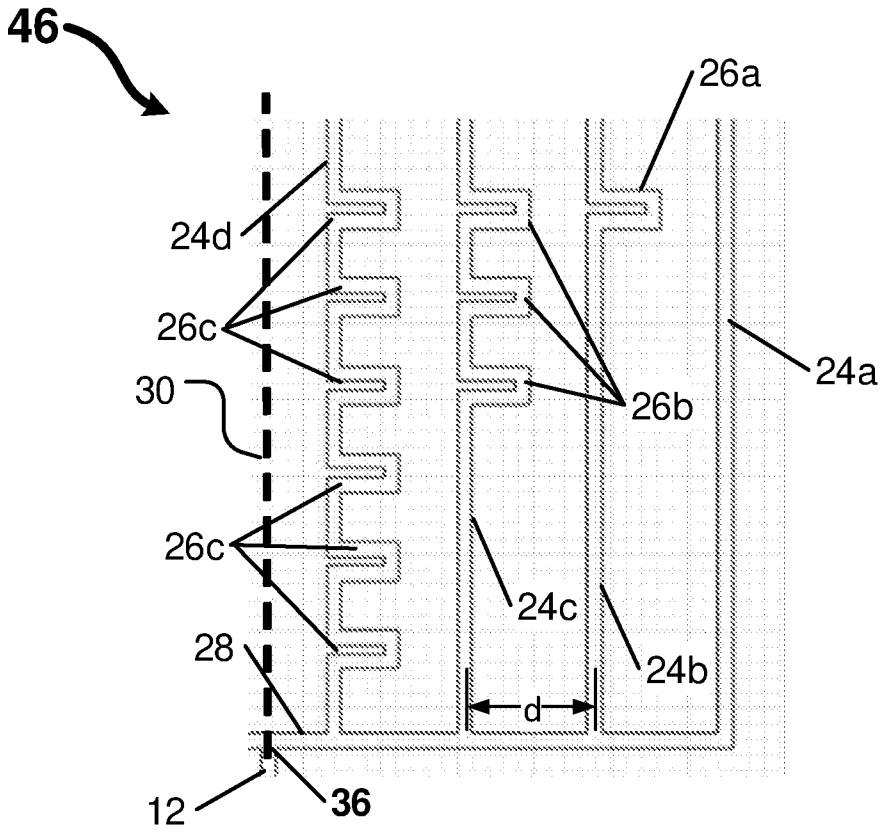

46
26a
24d
26c
30
26b
24a
26c
24c
28
24b
d
12
36

Fig. 3

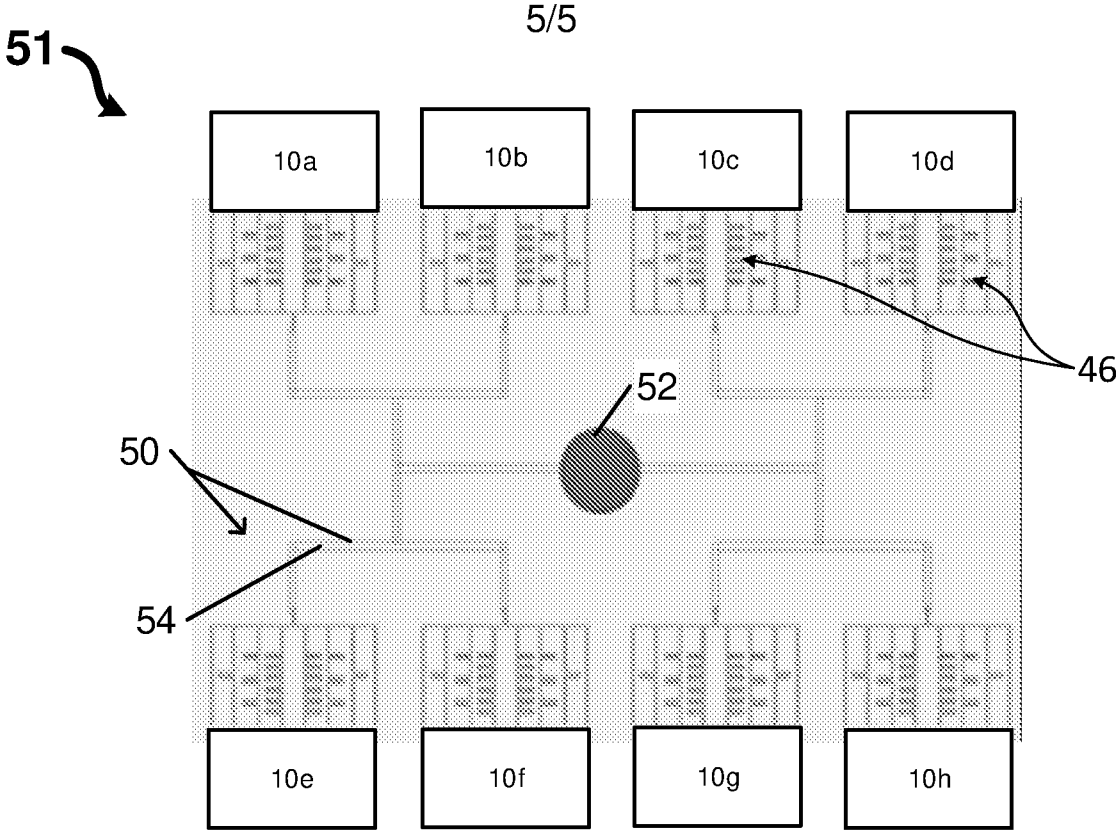

51
10a
10b
10c
10d
46
52
50
54
10e
10f
10g
10h

Fig. 4A

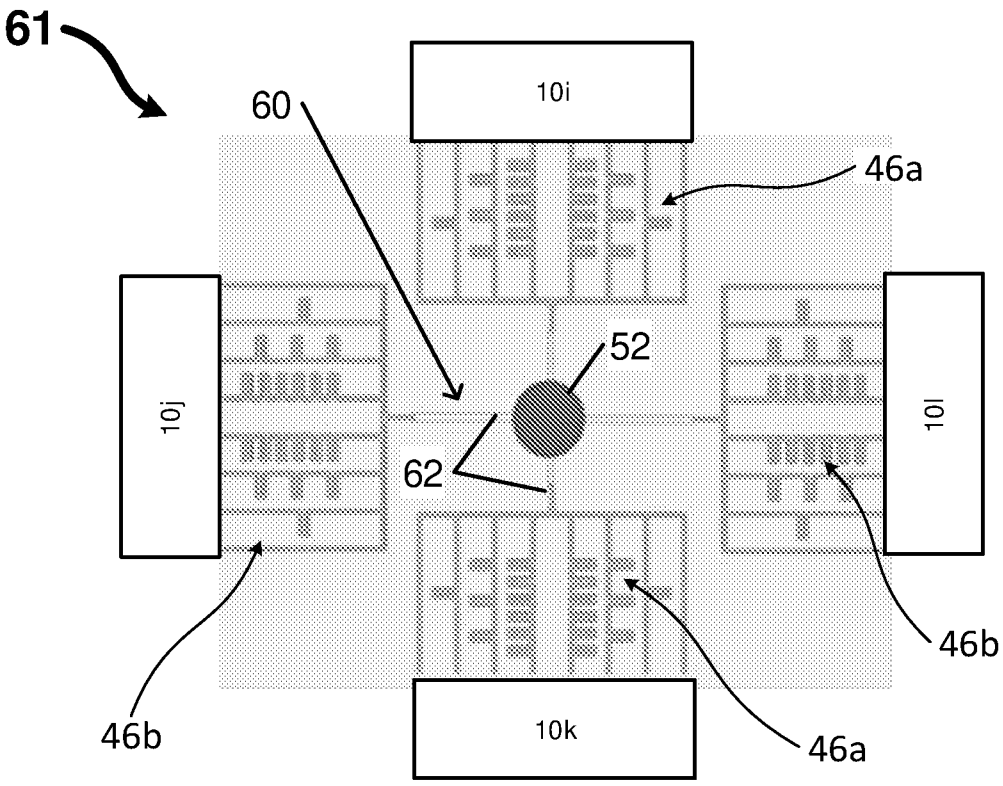

61
60
10i
46a
52
10j
10l
62
46b
46b
10k
46a

Fig. 4B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2020/050414

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B01L 3/00; G01N 1/28; G01N 1/38; G01N 31/20 (2020.01)

CPC - B01L 3/5027; B01L 3/502; B01L 3/5025; B01L 3/502723; B01L 3/502746; B01L 2300/0861; B01L 2300/0864 (2020.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

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

see Search History document

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

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0155128 A1 (PETERS et al) 18 June 2009 (18.06.2009) entire document	1, 3-7, 13, 15 --- 16
Y	US 2008/0213755 A1 (GEISER et al) 04 September 2008 (04.09.2008) entire document	16
A	US 2009/0042256 A1 (HANAFUSA et al) 12 February 2009 (12.02.2009) entire document	1-16
A	US 2003/0138819 A1 (GONG et al) 24 July 2003 (24.07.2003) entire document	1-16
A	US 2011/0143964 A1 (ZHOU et al) 16 June 2011 (16.06.2011) entire document	1-16

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

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 July 2020	22 JUL 2020
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