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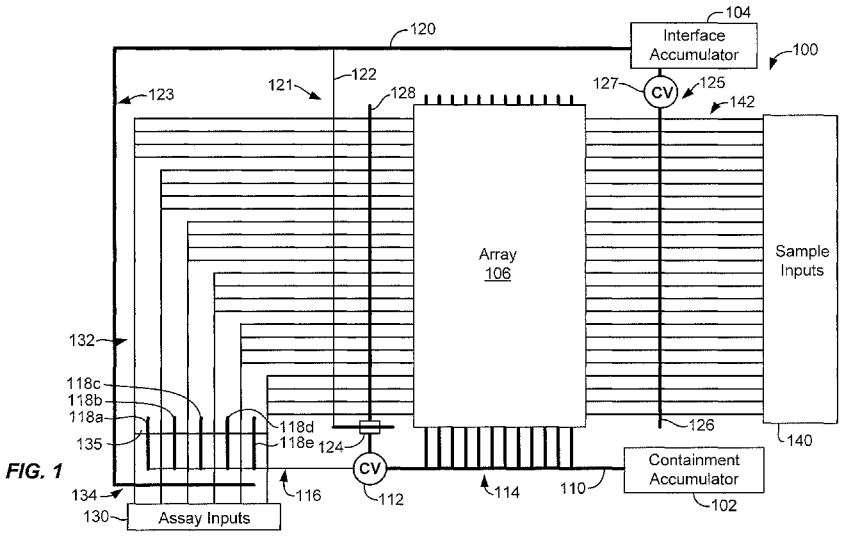

FIG. 1

(57) Abstract: A microfluidic device includes a pressure source and a control line in fluid communication with the pressure source. The microfluidic device also includes a plurality of valves operated via the control line and an independent valve positioned adjacent the control line and between the pressure source and the plurality of valves.

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PROGRAMMABLE MICROFLUIDIC DIGITAL ARRAY

BACKGROUND OF THE INVENTION

[0001] Microfluidic devices can be used for analytical, preparative, metering, and other manipulative functions on a scale not imagined until recently. The advantages of microfluidic devices include conservation of precious reagents and samples, high density and throughput of sample analysis or synthesis, fluidic precision and accuracy at a level scarcely visible to the unaided eye, and a space reduction accompanying the replacement of counterpart equipment operating at the macrofluidic scale. Associated with the reduction in size and the increased density of microfluidic devices is increased complexity and higher engineering and fabrication costs associated with increasingly intricate device architecture.

[0002] Despite these advances in microfluidic design and use, it would be useful to reduce the complexity of microfluidic chips and simplify their operation. Additionally, a need exists for an increased ability to control the flow of fluids and associated reaction processes occurring in microfluidic devices. Thus, there is a need in the art for improved methods and systems related to microfluidic devices.

SUMMARY OF THE INVENTION

[0003] The present invention relates to microfluidic devices. More particularly, the present invention relates to a programmable microfluidic digital array and methods of operating the same. Merely by way of example, the method and apparatus has been applied to a system that provides asynchronous logic functions in a microfluidic chip. Additionally, embodiments of the present invention incorporate unidirectional valves into a digital array, thereby providing for latching control lines. However, it would be recognized that the invention has a much broader range of applicability.

[0004] According to an embodiment of the present invention, a microfluidic device is provided. The microfluidic device includes a pressure source and a control line in fluid communication with the pressure source. The microfluidic device also includes a plurality of valves operated via the control line and an independent valve positioned adjacent the control line and between the pressure source and the plurality of valves.

[0005] According to another embodiment of the present invention, a method of operating a microfluidic device having a valve and a control line having a set of valves associated therewith is provide. The method includes closing the valve and applying a pressure to the control line. The closed valve causes the set of valves associated with the control line to be inoperable.

[0006] According to yet another embodiment of the present invention, a microfluidic device is provided. The microfluidic device includes a first valve, a second valve, and a control line in fluid communication with the first valve and the second valve. The microfluidic device also includes a pressure accumulator in fluid communication with the control line and a unidirectional valve positioned adjacent the control line between the pressure accumulator and the second valve.

[0007] According to an alternative embodiment of the present invention, a microfluidic device is provided. The microfluidic device includes a plurality of reaction chambers disposed in an array configuration. Each of the plurality of reaction chambers has a first valve in fluid communication with a one of the plurality of reaction chambers and a second valve in fluid communication with the one of the plurality of reaction chambers. The microfluidic device also includes a first control line operable to actuate the first valve and the second valve and a set of input lines in fluid communication with the plurality of reaction chambers. The microfluidic device further includes a plurality of sample inlets in fluid communication with the set of input lines and a unidirectional valve disposed in the first control line.

[0008] According to another alternative embodiment of the present invention, a microfluidic device is provided. The microfluidic device includes a plurality of reaction chambers and a plurality of first input ports. Each of the plurality of first input ports are in fluid communication with one or more of the plurality of reaction chambers through one of a first plurality of input lines. The microfluidic device also includes a plurality of second input ports. Each of the plurality of second inputs ports are in fluid communication with the one or more of the plurality of reaction chambers through one of a second plurality of input lines. The microfluidic device further includes a first pressure accumulator in fluid communication with a first control line. The first control line is configured to close the first plurality of input lines. Additionally, the microfluidic device includes a second pressure accumulator in fluid communication with a second control line. The second control line is configured to close the

second plurality of input lines. Moreover, the microfluidic device includes a first unidirectional valve disposed in the first control line between the first pressure accumulator and the first plurality of input lines and a second unidirectional valve disposed in the second control line between the second pressure accumulator and the second plurality of input lines.

[0009] According to yet another alternative embodiment, a method of operating a microfluidic device having a plurality of valves and a unidirectional valve is provided. The method includes applying a first fluid pressure to a control line of the microfluidic device and closing the plurality of valves in response to applying the first pressure. The method also includes closing the unidirectional valve in response to applying the first pressure and applying a second fluid pressure to a second control line of the microfluidic device.

[0010] According to a specific embodiment of the present invention, a method of operating a microfluidic device having a plurality of input ports is provided. The method includes providing an input fluid to one of the plurality of input ports and actuating a set of valves to close a first portion of input lines connected to a subset of the plurality of input ports. The subset does not include the one of the plurality of input ports. The method also includes flowing the input fluid through an input line connected to the one of the plurality of input ports, flowing the input fluid through the input line to a second portion of the input lines and closing a second set of valves to isolate a plurality of reaction chambers.

[0011] According to another specific embodiment of the present invention, a method of operating a programmable microfluidic device having an array of reaction sites in fluid communication with a first set of input lines and a second set of input lines is provided. The method includes actuating a first set of valves operable to obstruct the first set of input lines and actuating a second set of valves operable to obstruct a first portion of a subset of a second set of input lines. The method also includes loading a sample into the reaction sites through a second portion of the second set of input lines and actuating a third set of valves operable to isolate the reaction sites.

[0012] According to yet another specific embodiment of the present invention, a microfluidic device is provided. The microfluidic device includes a predetermined number of input ports, each of the input ports being operable to receive one of a plurality of input fluids and a plurality of input fluid lines, each of the plurality of input fluid lines being in fluid communication with one of the predetermined number of input ports. The microfluidic device also includes a set of valves, each of the set of valves being operable to close one of

the plurality of input fluid lines. A number of the set of valves is less than the predetermined number. The microfluidic device further includes a manifold in fluid communication with each of the plurality of input fluid lines and a second set of valves, each of the second set of valves being operable to close a portion of the manifold.

[0013] According to an embodiment of the present invention, a method of operating a programmable microfluidic device having an array of reaction sites in fluid communication with a first set of input lines, a second set of input lines, and a manifold connecting the second set of input lines is provided. The method includes actuating a first set of valves operable to close the first set of input lines, actuating a second set of valves operable to close a first portion of a subset of a second set of input lines, and actuating a third set of valves operable to deactivate the manifold. The method also includes deactuating the second set of valves, loading a plurality of samples into the reaction sites through a second portion of the second set of input lines, and actuating a fourth set of valves operable to isolate the reaction sites.

[0014] According to another embodiment of the present invention, a method of operating a programmable microfluidic device having an array of reaction sites in fluid communication with a first set of input lines and a second set of input lines is provided. The method includes actuating a first set of valves operable to obstruct the first set of input lines and actuating a second set of valves operable to isolate the reaction sites. The method also includes deactuating the second set of valves, loading a plurality of samples into the reaction sites through a second set of input lines, and actuating the second set of valves.

[0015] According to yet another embodiment of the present invention, a microfluidic device is provided. The microfluidic device includes a plurality of reaction sites and a first set of input lines providing fluid communication between a predetermined number of first input ports and the plurality of reaction sites. A number of the first set is the predetermined number. The microfluidic device also includes a second set of input lines providing fluid communication between a predetermined number of second input ports and the plurality of reaction chambers. Each of the second set of input lines includes a stem portion and a branching portion and a number of the second set is less than the predetermined number. The microfluidic device further includes a programmable input device operable to fill the reaction chambers using the first set of input lines or the second set of input lines.

[0016] According to a specific embodiment of the present invention, a method of configuring a microfluidic device having a plurality of control lines is provided. The method includes actuating a first control line and placing a valve in a first state. The method also includes thereafter, actuating a second control line operable to place a set of valves in a second state. The valve being in the first state prevents the set of valves from being placed the second state.

[0017] According to another specific embodiment of the present invention, a method of configuring a microfluidic device having a plurality of control lines is provided. The method includes establishing a first state of the microfluidic device by actuating a first control line and then actuating a second control line and establishing a second state of the microfluidic device by actuating the second control line and then actuating the first control line.

[0018] According to yet another specific embodiment of the present invention, a microfluidic device is provided. The microfluidic device includes a first valve and a second valve. The microfluidic device also includes a control line in fluid communication with the first valve and the second valve and a pressure source in fluid communication with the control line. The microfluidic device further comprises a unidirectional valve disposed in the control line between the pressure source and the second valve.

[0019] According to another alternative embodiment of the present invention, a microfluidic system is provided. The microfluidic system includes a carrier. The carrier includes a plurality of first input ports and a plurality of first input lines. Each of the plurality of first input lines is in fluid communication with one of the plurality of first input ports. The carrier also includes a plurality of second input ports and a plurality of second input lines. Each of the plurality of second input lines is in fluid communication with one of the plurality of second input ports. The carrier further includes a first pressure source and a second pressure source. The microfluidic system also includes a microfluidic device mounted to the carrier. The microfluidic device includes a plurality of third input lines and a plurality of fourth input lines. Each of the plurality of third input lines is in fluid communication with one of the plurality of first input lines and each of the plurality of fourth input lines is in fluid communication with one of the plurality of second input lines. The microfluidic device also includes a first control line in fluid communication with the first pressure source, a unidirectional valve operable to obstruct at least a portion of the first control line, and a second control line in fluid communication with the second pressure source.

[0020] Many benefits are achieved by way of the present invention over conventional techniques. For example, the present technique allows for customization of a microfluidic device after manufacturing, enabling multiple programmable panel configurations to be provided by the user. These and other embodiments of the invention along with many of its advantages and features are described in more detail in conjunction with the text below and attached figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 is a simplified schematic diagram illustrating a microfluidic device according to an embodiment of the present invention;

[0022] FIG. 2A is a simplified schematic diagram illustrating a unidirectional valve according to an embodiment of the present invention;

[0023] FIG. 2B is a simplified top-view diagram illustrating the unidirectional valve illustrated in FIG. 2A;

[0024] FIG. 3 is a simplified flowchart illustrating a method of operating a microfluidic device according to an embodiment of the present invention;

[0025] FIG. 4 is a simplified flowchart illustrating a method of operating a microfluidic device according to another embodiment of the present invention;

[0026] FIG. 5 is a simplified flowchart illustrating a method of operating a microfluidic device according to yet another embodiment of the present invention;

[0027] FIG. 6A is a simplified illustration of reaction chambers in an array according to an embodiment of the present invention;

[0028] FIG. 6B is a simplified perspective view of control lines, fluid input lines, and reaction chambers in an array according to an embodiment of the present invention;

[0029] FIG. 7 illustrates a simplified method of programming a programmable microfluidic device according to an embodiment of the present invention;

[0030] FIG. 8 illustrates a simplified method of programming a programmable microfluidic device according to another embodiment of the present invention;

[0031] FIG. 9 illustrates a simplified method of programming a programmable microfluidic device according to yet another embodiment of the present invention;

[0032] FIG. 10 is a simplified schematic diagram of a programmable microfluidic device according to an embodiment of the present invention; and

[0033] FIG. 11 is a simplified schematic diagram of a microfluidic system according to an embodiment of the present invention.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

[0034] FIG. 1 is a simplified schematic diagram illustrating a microfluidic device according to an embodiment of the present invention. In a particular embodiment, the microfluidic device illustrated in FIG. 1 includes a programmable high density digital array. The microfluidic device 100 includes a first pressure source 102 and a second pressure source 104. In FIG. 1, the first pressure source 102 is referred to as a containment accumulator since, as described more fully below, the pressure source 102 is in fluid communication with control lines that are operable to close valves associated with reaction chambers (not illustrated) present in array 106. Since actuation using pressure source 102 thus provides for closing of these reaction chamber valves and containment of sample and/or reagents in the reaction chambers, pressure source 102 is referred to as a containment accumulator in some embodiments.

[0035] In operation, a liquid is placed in the pressure accumulator or pressure source, which is then connected to an external positive pressure supply such as a vessel containing pressurized air. The pressurized air or other fluid pushes the liquid in the pressure accumulator into the control lines under pressure, thereby actuating the valves. Thus, the pressure sources do not typically contain pressurized fluids as manufactured, but provide a vessel in which pressurized fluids can be accumulated and stored during operation of the microfluidic device in order to apply pressure to control lines upon activation. As described more fully throughout the present application, the pressure accumulators are operable to maintain pressure in the control lines after activation. Additional description of digital arrays suitable for implementation according to embodiments of the present invention are provided in co-pending and commonly assigned U.S. Provisional Patent Application No. 61/044,417, the entire disclosure of which is hereby incorporated by reference in its entirety for all purposes.

[0036] FIG. 6A is a simplified illustration of reaction chambers in an array according to an embodiment of the present invention. As an example, embodiments of the present invention utilize a unit cell having reaction chambers with a lateral dimension of 100 μm x 60 μm and a height of 125 μm. In this exemplary embodiment, the chamber volume is approximately 0.75 nl. Such small chamber volumes enable the use of smaller sample sizes and reductions in operating costs. Vias 50 μm in diameter connect the reaction chambers to the assay/sample input lines. Reaction chambers are placed with a chamber pitch of 110 μm in a first lateral direction and a chamber pitch of 200 μm in a second lateral direction. 50 μm x 50 μm valves are provided in a separate layer from the layer containing the assay/sample input lines and are operable to prevent flow through the input lines. This particular unit cell geometry is not intended to limit embodiments of the present invention but to merely provide an example of a particular embodiment. In other embodiments, other device geometries are utilized as appropriate to the particular applications.

[0037] According to embodiments of the present invention, input lines with widths ranging from about 5 μm to about 400 μm and depths ranging from about 5 μm to about 75 μm are utilized to provide fluid flow through the microfluidic device. Control lines with widths ranging from about 5 μm to about 400 μm and depths ranging from about 5 μm to about 75 μm are utilized to valve off the fluid flow through the input lines. Reaction chambers with widths ranging from about 10 μm to about 500 μm, lengths ranging from about 10 μm to about 500 μm, and heights ranging from about 5 μm to about 500 μm are utilized according to some embodiments. These device geometries are provided by way of example and are not intended to limit the embodiments described herein.

[0038] As illustrated in FIG. 6A, a plurality of input lines 620 are provided, enabling fluid flow through the input lines in the horizontal direction. Three input lines are illustrated, but embodiments of the present invention utilize more than three input lines, for example, 11 input lines. Utilizing multiple input lines, a single sample can be distributed among the multiple input lines, providing multiple copies of the given sample. The input lines or input channels are at least partially contained in a first layer of the microfluidic device as described more fully in relation to FIG. 6B. Referring to FIG. 1, the input lines are in fluid communication with the assay input lines 132 on the left side of the array 106 and are in fluid communication with the sample input lines 142 on the right side of the array 106. Thus, fluids sourced by either the assay inputs 130 or the sample inputs 140 can be provided to the input lines and the reaction chambers in turn.

[0039] A plurality of control lines 610 are provided running in the vertical direction, enabling control of the fluid flow through the input lines. Two control lines are illustrated, but embodiments of the present invention utilize more than two control lines, for example, 70 control lines. In another embodiment, there are 71 control lines. The multiple control lines form separate reaction chambers along the length of the input line, providing multiple reaction chambers containing the same sample. The control lines or control channels are at least partially contained in a second layer of the microfluidic device as described more fully in relation to FIG. 6B. Referring to FIG. 1, the control lines are in fluid communication with section 114 of latching control line 110, which is in fluid communication with containment accumulator 102. The intersection of the control line with the input line forms a valve 615 that is actuated in response to fluid pressure in the control line and is operative to prevent fluid flow through the input line. Generally, the multilayer microfluidic device includes a number of elastomeric layers and the valves 615 include a deflectable membrane. In the embodiment illustrated in FIGS. 6A and 6B, the deflectable membrane of the valve is deflectable into the fluid channel positioned above the intersection with the control channel. Thus, the illustrated embodiment utilizes "push up" valves in which the deflectable membrane deflects up into the fluid channel to close the fluid channel at the position of the valve. For the valves illustrated in FIGS. 6A and 6B, releasing the fluid pressure present in the control channel will result in the deflectable membrane returning to the undeflected position and thereby opening of the closed valve.

[0040] Fluid flowing through the input lines 620 passes through the vias 625 along a direction normal to the plane of FIG. 6A and flows up into the reaction chambers 630, which are at least partially contained in a third layer of the microfluidic device as described more fully in relation to FIG. 6B. Thus, the vias are at least partially contained in at least the second or the third layer of the microfluidic device. Typically, the vias are formed utilizing a laser ablation process that removes a portion of the second or third layer. Because the microfluidic device is permeable to air, blind fill techniques can be utilized to fill the reaction chambers and perform a wide variety of chemical, biological, or other experiments. As will be evident to one of skill in the art, after fluids are present in the reaction chambers, actuation of the control lines will result in closing of the valves and containment of the fluids in the reaction chambers for a predetermined period of time.

[0041] FIG. 6B is a simplified perspective view of control lines, fluid input lines, and reaction chambers in an array according to an embodiment of the present invention. The

array, for example, array 106 illustrated in FIG. 1 is a portion of a multilayer microfluidic device. Each layer typically includes an elastomeric structure with one or more recesses, channels, chambers, or the like. As depicted here, first layer 601 includes a plurality of control channels 610 disposed as an array of parallel channels and an additional control channel 611. The control channels 610 and the additional control channel 611 are in fluid communication with one or more pressure sources or accumulators. Thus, in an embodiment, the control channels 610 are in fluid communication with pressure accumulator 102 and control channel 611 is in fluid communication with pressure accumulator 104. Although a single control channel 611 is illustrated in FIG. 6B, one of ordinary skill in the art will appreciate that the single illustrated channel is representative of one or more control channels associated with layer 601.

[0042] Additionally, the control channels are not limited to a position associated with layer 601. The control channels may be positioned in other layers as appropriate to the particular application. For example, in one control-on-control implementation, in order for a first control line, for example, control channel 611, to exert control over a second control line, for example, control channel 610, the second control line is positioned in both the first layer 601 and the second layer 602 as a function of the length of the control channel. The second control line switches from first layer 601 to the second layer 602 using a via similar to via 625. By passing the second control line over the first control line, a valve is formed at the intersection of the two control lines. Upon actuation of the first control line, the flexible membrane between the first control line and the second control line at the position of the valve pushes up to obstruct the second control line, which is positioned in layer 602 at the location of the valve. Thus, "push up" valves can be formed between different control lines or between control and fluid input lines as described more fully below. Referring to FIG. 1, the control line associated with valves 128 transitions from the lower layer of the structure into an upper layer using a via and then passes over control line 122 to enable control line 122 to thereby actuate decoupling valve 124. The control line associated with valves 128 then passes back into the first layer through another via in order to pass under and actuate valves for fluid input lines 132. Upon actuation of control line 122, the decoupling valve 124 obstructs flow through the control line associated with valves 128, preventing the typical closure of valves 128 in response to actuation of control line 110.

[0043] Second layer 602 includes a plurality of fluid input channels 620 also disposed as an array of parallel channels. In the embodiment illustrated in FIGS. 6A and 6B, the control

channels 610 and the input channels 620 are arrayed perpendicular to each other. Actuation of valves present in the microfluidic device is accomplished by application of pressure to a liquid (typically a substantially incompressible fluid) present in the control channels 610. Typically, the liquid is placed in the accumulator or pressure source and a supply of pressurized fluid (e.g., air, nitrogen, or the like) is supplied to the accumulator. The increase in pressure in the accumulator forces the liquid into the control lines under pressure. In response to the applied pressure, the deflectable membrane forming the top of layer 601 will deflect up and into the fluid input channels 620. Thus, "push-up" valves are created at the intersections of the control channels and the fluid input channels. Other embodiments can utilized "push-down," "pull-down," or "pull-up" valves by repositioning the various control and fluid layers.

[0044] In embodiments of the present invention that provide for control-on-control, one or more additional control layers may be provided, for example, below layer 601 or by routing a control line into layer 602. The one or more additional control layers may include additional control lines (not illustrated) that, upon actuation, will valve off the control lines present in layer 601. Thus, by applying fluid pressure to a control fluid (e.g., a liquid) present in the one or more additional control lines, a flexible membrane is deflected into the control lines 610, preventing fluid flow through the control lines. Thus, layers of control, also referred to as control-on-control, are provided by embodiments of the present invention. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0045] The second layer also includes a plurality of vias 625, creating a fluid passage that provides for fluid flow from the input channels to the third layer 603. Third layer 603 includes a plurality of reaction chambers 630 that are in fluid communication with the fluid input channels through the vias. In the illustrated embodiment, the reaction chambers 630 are formed to be flush with the bottom of layer 603, that is, the reaction chambers are open from the bottom. Thus, the vias are completely contained in layer 602. In other embodiments, the vias can be contained in both layers 602 and 603 as appropriate to the particular application.

[0046] In some embodiments, a microfluidic device can include one or more layers that have been prepared according to spin or pour fabrication protocols. For example, a spin protocol can involve placing a polymeric material on a patterned disc or mold, and spinning the disc to create a layer of polymer across the disc. Exemplary polymers include polymethylmethacrylate, polystyrene, polypropylene, polyester, fluoropolymers,

polytetrafluoroethylene, polycarbonate, polysilicon, and polydimethylsiloxane (PDMS). A pour protocol can involve pouring a PDMS material, for example, on a patterned template or mold, which can result in a layer of PDMS which can be peeled or pulled off the mold intact. Often, a layer prepared by a pour fabrication technique is thicker than a layer prepared by a spin fabrication technique. Elastomeric blocks can include one or more pour or spin layers, in any desired combination.

[0047] In some embodiments, first layer 603 can be fabricated according to a spin protocol. For example, PDMS can be placed onto a mold that has raised portions corresponding to the various desired control channels 610. The mold can be spun, so as to provide a thin layer of PDMS across the mold. After curing, the first layer 601 can be peeled away from the mold and attached to a suitable rigid substrate such as glass, silicon, or a plastic such as polystyrene. Alternatively, the first layer 601 can remain attached to the mold. First layer 601 can include openings, recesses, or other voids that at least partially form or define the control channels 610.

[0048] To create second layer 602, a spin protocol can be used with PDMS placed onto a second mold that has raised portions corresponding to the various desired fluid input channels. The second mold can also include, for example, raised or contoured portions that form corresponding alignment marks in the second layer 602. These alignment marks can be used during a laser ablation procedure used to form the vias 625, such that the laser ablation is directed toward the alignment marks during the ablation process. The second mold can be spun, so as to provide a thin layer of PDMS across the second mold. Second layer 602 can include openings, recesses, or other voids that at least partially form or define input channels 620. In some cases, second layer 602 can be exposed to one or more laser ablations as described above. An ablative laser beam directed to second layer 602 can form vias 625. After second layer 602 is sufficiently cured, first layer 601 can be peeled away from the second mold, aligned, and contacted with the first layer. The second layer can adhere with the first layer so that both layers are attached to the rigid substrate.

[0049] To create third layer 603 using a pour protocol, PDMS can be poured onto a third mold that has raised portions corresponding to the various desired reaction chambers 630. After curing, the third layer 603 can be peeled off the third mold, aligned, and contacted with the second layer 602. The third layer can adhere with the second layer so that all three layers are adhered to the rigid substrate. Materials from which a microfluidic device can be

fabricated include, without limitation, elastomers, silicon, glass, metal, polymer, ceramic, inorganic materials, and/or combinations of these materials.

[0050] Referring once again to FIG. 1, pressure source 102 is in fluid communication with latching control line 110. The latching control line includes several sections as described more fully below and unidirectional valve 112, also referred to as a check valve. Additional description related to unidirectional valves is provided in co-pending and commonly assigned International Patent Application No. PCT/US07/080489, published as International Publication No. WO 2008/043046 A2, the entire disclosure of which is hereby incorporated by reference in its entirety. A first section 114 receives an actuation pressure from pressure source 102 and is operable to close the containment valves present in array 106. As will be evident to one of skill in the art, array 106 is suitable for performing a wide variety of microfluidic experiments. Samples, reagents, and the like present in reaction chambers in the array can thus be contained in the reaction chambers by closing of the containment valves in response to actuation of the latching control line 110. Section 114 of latching control line 110 is free of a check valve, enabling re-opening of the containment valves upon removal of the actuation pressure at pressure source 102.

[0051] A second section 116 of latching control line 110 is downstream of the unidirectional valve 112. Because the unidirectional valve 112 is operable to prevent fluid flow from section 116 to section 114, actuation of pressure source 102 will result in control fluid, typically a liquid, passing through the unidirectional valve 112 and closing valves 118a - 118e. The thin line associated with latching control line 110 illustrated in FIG. 1 represents a "flyover" section of the control line, wherein the design of the control line prevents closure of the assay input lines 132 (described more fully below) upon actuation of the latching control line 110. The thick lines associated with the valves 118a - 118e represent valves operable to close or deactivate manifold 135 that provides for fluid flow between the various assay input lines 132. Closing of valves 118a - 118e will prevent flow of assays through the manifold 135 from one assay input line to other assay input lines, referred to as deactivation of the manifold.

[0052] After actuation of latching control line 110 by pressure source 102, valves 118a - 118e will close and unidirectional valve 112 will maintain valves 118a - 118e in a closed state after removal of the actuation pressure in the latching control line. In contrast with valves 118a - 118e, the containment valves actuated by section 114 will open after removal of

the actuation pressure. Thus, the spatial position of the check valve in the latching control line provides one or more sections characterized by either latching or non-latching behavior. Use of additional check valves in the control line will provide additional sections with latching behavior as will be evident to one of skill in the art.

[0053] Latching control line 110 further includes a third section 128 with valves operable to close the assay input lines 132 passing from the assay inputs 130 to the array 106. The assay inputs 130 may also be referred to as input ports. Since third section 128 is also downstream of unidirectional valve 112, actuation of latching control line 110 by pressure source 102 may close the valves in third section 128 and prevent flow through the assay input lines 132 to and from the array 106 and the reaction chambers disposed therein. The valves in section 128 will remain closed after removal of the actuation pressure in latching control line 110.

[0054] A second pressure source 104, referred to as an interface accumulator, is provided in fluid communication with a second control line 120. The second control line also includes several sections 121, 123, and 125. Section 121 includes a flyover 122 that enables actuation of independent valve 124 without closing of the assay input lines 132. Additional description related to valve 124, referred to herein as an independent or decoupling valve, is provided throughout the present specification and more particularly below. Although the particular independent or decoupling valve 124 illustrated in FIG. 1 is actuated using second pressure source 104, this is not required by the present invention. In other embodiments, the independent or decoupling valve 124 can be mechanical, electrostatic, fluidic, electromechanical, thermodynamic, piezoelectric, or the like. Thus, although the second pressure source 104 shown in FIG. 1 is utilized to actuate decoupling valve 124, it is not required in some embodiments. Moreover, although multiple sets of valves including the decoupling valve are controlled using the single second pressure source 104, other embodiments could utilize multiple pressure sources, combinations of fluidic actuation and electrostatic actuation, and the like. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0055] Section 123 includes valves 134 operable to close five of the six illustrated assay input lines 132. Since only five valves 134 are provided in the embodiment illustrated in FIG. 1, actuation of valves 134 in section 123 by pressure source 104 will still leave the rightmost assay input line open. Section 125 includes unidirectional valve 127 and valves

126 operable to close sample input lines 142, which are in fluid communication with sample inputs (i.e., input ports) 140 and array 106. It should be noted that although assay and sample inputs are illustrated in FIG. 1, the present invention is not limited to only assays and samples and other inputs are included within the scope of the present invention.

[0056] The placement of unidirectional or check valve 127 in section 125 of the latching control line 120 allows valves 126 to close and remain closed after removal of the applied pressure, preventing fluid flow through the sample input lines to and from the array 106. Since sections 121 and 123 do not include check valves, deactuation of these sections will result in reopening of valves 134 and 124.

[0057] Embodiments of the present invention provide asynchronous logic functions in microfluidic device 100. For example, because the independent or decoupling valve 124 illustrated in FIG. 1 is actuated using a control line 120 that separate and independent from the other illustrated control line 110, embodiments of the present invention provide for control-on-control. Referring to FIG. 1, applying pressure to latching control line 110 prior to actuation of the decoupling valve 124 will latch containment valves 128. On the other hand, actuation of latching control line 120 via interface accumulator 104, thereby closing decoupling valve 124, prior to applying pressure to latching control line 110 via containment accumulator 102, will prevent containment valves 128 from closing. In other words, if the decoupling valve 124 is closed when pressure is initially applied to valves 128, the closed state of the decoupling valve prevents the applied pressure from reaching valves 128. Thus, the order in which the control lines 110 and 120 are actuated results in different valve operation, providing asynchronous logic.

[0058] The control-on-control provided by embodiments of the present invention enables the array 106 to be "programmed." As an example, the array 106 can be used in multiple configurations since it is programmed by the order in which the various valves are closed, latched, or reopened. In embodiments described herein, three different configurations are provided using the two illustrated pressure sources. The unidirectional valves as well as the decoupling valve, which can be considered as one of a set of stacked control valves, are utilized to provide these multiple configurations. The decoupling valve, which can limit the control over valves 128 that is achievable in response to actuation of latching control line 110, is just one example of stacked control valves and other configurations are included within the scope of the present invention.

[0059] The use of check valves in predetermined sections of the control lines provides for partial latching of the control lines. For example, valves 118 and 128 can be latched by initial actuation of control line 110. However, initial actuation of control line 120 will result in closure of the decoupling valve. Subsequent actuation of control line 110 will result in latching of valves 118 but have no effect on valves 128. Subsequent deactuation of control line 120, e.g., by releasing the pressure applied via the interface accumulator, while actuation of control line 110 is maintained, will result in latching of valves 128 upon reopening of the decoupling valve.

[0060] In the microfluidic device 100 illustrated in FIG. 1, six assay input lines 132 split into four input lines apiece, providing a total of 24 assay input lines passing by containment valves 128. Additionally, 24 sample input lines 142 are illustrated. These particular numbers of input lines are only provided by way of example and other particular numbers of input lines are provided by other embodiments. For example, in a specific embodiment, the schematic diagram illustrated in FIG. 1 only represents half of a microfluidic device, e.g., the right side of a device, with a matching set of inputs on the left side of the device. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0061] FIG. 2A is a simplified cross-sectional diagram illustrating a unidirectional valve according to an embodiment of the present invention. FIG. 2B is a simplified top-view diagram illustrating the unidirectional valve illustrated in FIG. 2A. Referring to FIG. 2A, actuation fluid, typically a liquid, flows into the check valve 112/127 through inlet 210 and passes to chamber 220 through via 215. The membrane 230 is either lifted up or maintained in a substantially horizontal position by the flow of the actuation fluid, enabling the actuation fluid to pass from the left to the right through the inlet 210. The actuation fluid flows through the chamber 220, through vias 240a - 240d and via 242, and out through outlet 250. Thus, in the illustrated embodiment, there is one input via and five output vias for a total of six vias per unidirectional valve. As illustrated in the top-view shown in FIG. 2B, additional structural elements and features are provided as appropriate to the particular design.

[0062] When the actuation fluid pressure is removed, the membrane 230 collapses onto layer 260, preventing flow back through the inlet 210. Thus, the unidirectional valve 124/127 provides for flow from the inlet 210 to the outlet 250, but prevents flow in the reverse direction. Additional description related to unidirectional valves is provided in the co-pending and commonly assigned application referenced above.

[0063] Embodiments of the present invention provide microfluidic devices incorporating unidirectional or check valves. In an embodiment, the microfluidic device includes a first valve and a second valve. Typically, the first valve is one of a number of valves making up a first set of valves and the second valve is one of a number of valves making up a second set of valves. A control line in fluid communication with the first valve and the second valve is provided as part of the microfluidic device along with a pressure source in fluid communication with the control line. Referring to FIG. 1, control line 110 is actuated by application of pressure to containment accumulator 102, resulting in actuation of valves 615 in array 106 and valves 118a - 118e to deactivate manifold 135. The microfluidic device also includes a unidirectional valve disposed in the control line between the pressure source and the second valve. As an example, originally open check valve 112 is disposed in control line 110 between the containment accumulator 102 and the valves 118a - 118e. Check valve 112 provides for latching of valves 118a - 118e after pressure in control line 110 is reduced or removed, while the valves present in the array 106, which are not latching valves, are able to reopen, enabling flow of samples into the reaction chambers. Thus, by the use of check valves to latch a predetermined number of valves in the microfluidic device, control of fluid flow and isolation of samples in the microfluidic device are provided that are not available with conventional designs.

[0064] In the embodiment illustrated in FIGS. 1 and 6A, valves 615 are configured to isolate a reaction chamber disposed in the microfluidic device whereas valves 118a - 118e are configured to isolate a first fluid input line of the six lines coupled to the assay input ports 130 from a second fluid input line of the six lines coupled to the assay input ports. As described more fully throughout the present specification, the ability to close and latch the valves 118a - 118e, thereby deactivating the manifold 135, enables the microfluidic device to be programmed in various ways, enabling fluid flow in the input lines 132 to be separated or comingled depending on the particular application.

[0065] In addition to the integration of check valves into microfluidic devices with array configurations, independent valves, also referred to as decoupling valves are provided by some designs. As an example, the decoupling valves, which is controlled independently from other valves in the device, can be operable to prevent actuation of one or more valves that control fluid flow through a fluid input line coupled to a reaction chamber present in the microfluidic device. As an example, decoupling valve 124 can be closed prior to actuation of

control line 110, thereby preventing valves 128 from closing in response to actuation of the control line 110.

[0066] FIG. 10 is a simplified schematic diagram of a programmable microfluidic device according to an embodiment of the present invention. The elements illustrated in FIG. 10 may be provided in a carrier or a microfluidic device attached to the carrier as illustrated in FIG. 11. As illustrated in FIG. 10, the outline of the microfluidic device mounted on the carrier is represented by dashed line 1050. Referring to FIG. 10, two vent sources are provided on opposing sides of the microfluidic device. The vent sources, which are typically provided as part of the carrier, may not be used in all embodiments. 48 sample input lines 1010 are illustrated in an upper portion of FIG. 10, with 24 sample input lines disposed on the left side of the microfluidic device and providing samples to the left array 106 and 24 sample input lines disposed in the right side of the microfluidic device and providing samples to the right array 106. The 48 sample lines are typically provided in the carrier and are in fluid communication with the array 106 through vias 1030 formed in the microfluidic device and aligned with the ends of the sample lines. Sample input lines 142 are provided in the microfluidic device passing from the vias 1030 to the array 106. The 48 sample lines are pressurized by a common pressure source (not shown) that is able to be coupled to a sample portion of a carrier on which the microfluidic device is mounted. Thus, samples in 48 sample ports are able to be loaded into the microfluidic device and pushed through the sample input lines into the array 106. Referring to FIG. 1, the right half of the array 106 is illustrated for purposes of clarity, with the 24 sample input lines from the right portion of FIG. 10 illustrated as sample input lines 142. Thus, embodiments of the present invention provide for 48 sample input lines, with up to 48 different samples. Other implementations provide a different number as appropriate to the particular application.

[0067] A control line 1004, which is coupled to interface accumulator 104 illustrated in FIG. 1, is illustrated in FIG. 10. Additionally, several check valves (CVs) are illustrated in FIG. 10, enabling for one-way flow of fluids through the control lines provided in the microfluidic device. Referring to the lower portion of array 106, a additional CV is provided for control lines coupled to the array at this portion. Hydration lines are provided at outer portions of array 106. These hydration lines provide a source for hydration fluids that serve to reduce or prevent evaporation of fluids from the array.

[0068] The 48 input lines illustrated at the lower portion of FIG. 10 include 12 assay input lines (six for the left array 106 and six for the right array 106). The 12 assay lines are pressurized by a common pressure source (not shown) that is able to be coupled to an assay portion of a carrier on which the microfluidic device is mounted. Thus, assays in 12 assay ports are able to be loaded into the microfluidic device and pushed through the assay input lines into the two arrays 106. Referring to FIG. 1, the right array 106 is illustrated for purposes of clarity, with the 6 sample input lines from the right portion of FIG. 10 illustrated as assay input lines 132. Thus, embodiments of the present invention provide for 12 assay input lines, with up to 12 different assays. The assay input lines pass up through the middle of the microfluidic device between the two arrays 106, with six lines branching to the right array 106 and six lines branching to the left array 106. The use of the illustrated manifold enables less than 12 assays to be provided, for example, as few as a single assay. Other implementations provide a different number as appropriate to the particular application.

[0069] Of the other 36 input lines illustrated in the lower portion of FIG. 10, two lines are used for hydration and 34 are unused in this particular implementation. Assay input line 1020 provides input to a single input line entering array 106 whereas assay input line 1022 is branched into multiple input lines (e.g., four lines) entering array 106.

[0070] An example of three input lines per sample is illustrated in FIG. 6A, but in the embodiment illustrated in FIG. 10, there are 11 input lines. Moreover, in FIG. 6A, two control lines are illustrated in FIG. 6A, but in the embodiment illustrated in FIG. 10, there are 71 control lines operable to form 70 reaction chambers per input line. Thus, for each sample in this embodiment, there are 770 reaction chambers (11 input lines x 70 reaction chambers). With 48 samples partitioned into 770 reaction chambers, a high density integrated fluidic circuit (IFC) is provided. In an embodiment, the microfluidic device is capable of testing up to 48 individual samples at a time. Each of the up to 48 samples is partitioned into separate sets of 770 reaction chambers, thereby delivering, for example, a total of 36,960 simultaneous digital PCR reactions. In another embodiment, some of the programmability of the microfluidic device is removed and the number of reaction chambers per sample is increased for the same device footprint and feature dimensions. For example, one embodiment utilizes up to 48 samples with 814 reaction chambers per sample.

[0071] Utilizing products available from the present assignee, it is possible to complete the entire digital PCR process in less than four hours. Moreover, the microfluidic device

described herein is a component of a complete system for genetic analysis. Such systems can include the microfluidic devices, a controller for the microfluidic device, a BioMark™ System or Stand-Alone Thermal Cycler, an EP1 Reader, and associated software. The microfluidic devices described herein are compatible with off-the-shelf reagents and standard micro well format dispensing layouts. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0072] FIG. 11 is a simplified schematic diagram of a microfluidic system according to an embodiment of the present invention. The microfluidic system includes a carrier 1100 and a microfluidic device 1108 mounted on the carrier. The microfluidic device 1108 incorporates elements as described in relation to FIG. 1. The carrier includes a plurality of first input ports or wells 1105 arranged in bank 1106a. The carrier also has a plurality of first input lines 1115 (e.g., 48 input lines) in fluid communication with the plurality of first input ports. A peripheral lip surrounds the plurality of first input ports, enabling the first input ports to be pressurized using a common pressure source. In an example use, 48 samples are loaded into the first input ports and application of pressure to the ports from the top side of the carrier results in the 48 samples being pushed through the plurality of first input lines and eventually into fluid lines in the microfluidic device.

[0073] The carrier also includes a plurality of second input ports 1105 arranged in bank 1106b and a plurality of corresponding second input lines 1115. Like the plurality of first input ports, the plurality of second input ports are surrounded by a peripheral lip, enabling fluids dispensed into the second input ports to be pushed through the second input lines and into fluid lines in the microfluidic device.

[0074] In order to provide for fluid communication between the carrier and the microfluidic device mounted on the carrier, the microfluidic device has vias 1114 formed in the lower portion of the microfluidic device that are aligned with an end portion of the first input lines and the second input lines. Fluid flowing through the input lines passes through the vias and up into fluid lines provided in the microfluidic device. As illustrated in FIG. 10, fluid lines connected to the sample input ports pass from the vias 1030 arrayed vertically along the left and right sides of the microfluidic device in the figure, into the opposing sides of the microfluidic device, and towards the center of the microfluidic device. Referring to FIG. 6A, these sample input lines can correspond to input lines 620 flowing horizontally through FIG.

6A. Using these 48 sample input lines, the reaction chambers can be filled with different samples in each row of the microfluidic device.

[0075] Vias 1040 are also provided in the lower portion of the microfluidic device and aligned with the plurality of second input lines 1020. As illustrated in FIG. 10, fluids such as assays loaded in the second input ports flow through the second input lines 1020, through the vias 1040, and into manifold 1060. The microfluidic device includes valves operable in conjunction with the manifold to result in either 1 fluid (sample or assay) or 12 different fluids being provided to the reaction chambers in the two arrays. If the application calls for a single input fluid, the manifold is opened and the input fluid flows to all the fluid lines passing vertically in FIG. 10 through the center of the array. The fluid lines branch out as they pass through the center of the array, eventually passing by the reaction chambers as they pass towards vias 1030 at the sides of the array. Referring to FIG. 6A, these assay input lines can correspond to input lines 620 passing under the reaction chambers. In this example, flow in the left array is from the center to the left and flow in the right array is from the center to the right.

[0076] Alternatively, the manifold 135 can be blocked, allowing for introduction of 12 different fluids (e.g., assays) to be introduced into the reaction chambers (i.e., 6 different assays in each array. As illustrated in FIG. 10, the fluids flow vertically between the arrays, then branch out, with each of the 12 fluids flowing into multiple rows of the array, passing horizontally from the center towards the edges adjacent vias 1030.

[0077] Pressure accumulators 1106c and 1106d are provided on the carrier to enable actuation of the control lines and check valves present in the microfluidic device. In a manner similar to the fluid lines, control lines 1002 and 1004 in the carrier are in fluid communication with control lines 110 and 120 in the microfluidic device through vias formed in the microfluidic device. Thus, although FIG. 1 illustrates the interface and containment accumulators of the carrier as well as the control lines in the microfluidic device, it will be appreciated that this schematic diagram is simplified for purposes of clarity and ease of illustration. Additional details related to carriers on which microfluidic devices can be mounted is provided in U.S. Patent Application Publication No. 2005/0214173, the disclosure of which is hereby incorporated by reference in its entirety for all purposes.

[0078] The carrier 1100 has integrated pressure accumulator wells 1101 and 1102, each having therein a drywell 1103, 1104 for receiving a valve, preferably a check valve attached

to a cover. Carrier 1100 further includes one or more well banks 1106a, b, c, and d, each having one or more wells 1105 (also referred to as input ports) located therein. Each of the wells 1105 of carrier 1100 have channels leading from well 1105 to the microfluidic device 1108 mounted on the carrier in position 1107. The well banks 1106c and 1106d are typically used to provide pressure used to actuate control lines present in the microfluidic device 1108. Fluid lines for the control fluid are provided to connect the wells in the well banks to the valves or other control mechanisms present in the microfluidic device. The microfluidic device is preferably an elastomeric block formed from two or more layers of elastomeric material having microfabricated recesses or channels formed therein.

[0079] Within the microfluidic device are one or more channels in fluid communication with one or more vias 1114, which in turn provide fluid communication between the channels within the microfluidic device and channels within the carrier which then lead to wells 1105 within well rows 1106a-d to provide for fluid communication between wells 1105 of the carrier 1100 and the channels within microfluidic device 1108. Accumulator well tops 1109 and 1110 are attached to accumulator wells 1101 and 1102 to form accumulator chambers 1115 and 1116. Accumulator well tops 1109 and 1110 include valves 1112 and 1111, respectively, which are preferably check valves for introducing and holding gas under pressure into accumulator chambers 1115 and 1116. Valves 1111 and 1112 are situated inside of drywells 1102 and 1104 to keep liquid, when present in accumulator chambers 1115 and 1116, from contacting valves 111 and 1112. Valves 1111 and 1112 preferably may be mechanically opened by pressing a shave, pin or the like, within a preferred check valve to overcome the self closing force of the check valve to permit release of pressure from the accumulator chamber to reduce the pressure of the fluid contained within the accumulator chamber.

[0080] Carrier 1100 and its associated components may be fabricated from polymers, such as polypropylene, polyethylene, polycarbonate, high-density polyethylene, polytetrafluoroethylene PTFE or Teflon (R), glass, quartz, or a metal (for example, aluminum), transparent materials, polysilicon, or the like. Accumulator well tops 1109 and 1110 further may comprise access screws that can be removed to introduce or remove gas or liquid from accumulator chambers 1115 and 1116. Preferably, valves 1112 and 1111 can be actuated to release fluid pressure otherwise held inside of accumulator chambers 1115 and 1116. Notch 1117 is used to assist correct placement of the microfluidic device into other

instrumentation, for example, instrumentation used to operate or analyze the microfluidic device or reactions carried out therein.

[0081] FIG. 3 is a simplified flowchart illustrating a method of operating a microfluidic device according to an embodiment of the present invention. In the embodiment illustrated in FIG. 3, a 48 panel configuration for the digital array is provided. A first pressure is applied to pressure source 102 (containment accumulator), resulting in actuation of latching control line 110 (310). In a specific embodiment, the first pressure is 30 psi. In other embodiments, other pressures are utilized as appropriate to the particular application. Referring to FIG. 1, all the valves (not illustrated) in array 106 will close due to pressure in section 114. Pressure will be applied through unidirectional valve 112 to section 116, which will result in valves 118a - 118e closing and latching. The flyover portion of section 116 will prevent closing of the assay input lines as a result of actuation of section 116. The closing of valves 118 will shut off the flow of input fluids through manifold 135, thereby separating each of the six assay input lines connected to the assay inputs 130.

[0082] It should be noted that in the control flow illustrated in FIG. 3, pressure source 104 is not utilized. Thus, there is no actuation of control line 120 or closing of decoupling valve 124. Thus, in response to actuation of latching control line 110, containment valves 128 close and latch. Thus, after applying the first pressure via the containment accumulator (e.g., 30 psi), the array valves are closed and valves 118 and 128 are closed and latched.

[0083] The first pressure from pressure source 102 is reduced (312), enabling the array valves (not illustrated) to reopen. In some embodiments, the pressure is removed at process (312) so that the applied pressure is equal to zero. As stated above, valves 118 and 128 will remain latched in the closed position. Samples are loaded (314) into the reaction chambers in the array through the sample input lines 142, fed from the sample inputs or ports 140. The chip design provides for loading through the sample input lines 142, the assay input lines 132, or both. Because, in this embodiment, the assay input lines are shut by latched valves 128, loading is via the sample input lines. After sample loading in completed, a second pressure is reapplied to pressure source 102 (316), thereby shutting the array valves and isolating the samples in the reaction chambers. In some embodiments, the second pressure is equal to the first pressure (e.g., 30 psi), although this is not required by the present invention. Other pressures that are suitable for closing of the array valves are included within the scope of the present invention. Moreover, although the first pressure is removed in some

embodiments, this is not required by the present invention, since some embodiments may reduce the pressure to a non-zero value that still provides for sufficient fluid flow to load the samples.

[0084] Thus, the embodiment illustrated by FIG. 3 provides for loading of 48 samples (i.e., 24 illustrated sample input lines x 2 sides of the device). As described below, other sample configurations are also provided by the programmable digital array described herein.

[0085] It should be appreciated that the specific steps illustrated in FIG. 3 provide a particular method of operating a microfluidic device according to an embodiment of the present invention. Other sequences of steps may also be performed according to alternative embodiments. For example, alternative embodiments of the present invention may perform the steps outlined above in a different order. Moreover, the individual steps illustrated in FIG. 3 may include multiple sub-steps that may be performed in various sequences as appropriate to the individual step. Furthermore, additional steps may be added or removed depending on the particular applications. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0086] FIG. 4 is a simplified flowchart illustrating a method of operating a microfluidic device according to another embodiment of the present invention. In the embodiment illustrated in FIG. 4, a single panel configuration is provided in contrast to the 48 panel configuration described in relation to FIG. 3. A first pressure is applied to pressure source 104 (410), resulting in actuation of latching control line 120. Pressure will be applied through unidirectional valve 127 to close and latch valves 126 in section 125. Thus, flow through the sample input lines 142 will be prevented in this configuration. Flyover section 122 will transmit the control pressure to close the decoupling valve 124.

[0087] Section 123 will transmit the control pressure in latching control line 120 to valves 134 associated with five of the six assay input lines 132. Thus, valves 134 will be closed, enabling flow through only the rightmost assay input line.

[0088] A single sample is loaded (412) through the rightmost assay input line. Because section 116 of latching control line 110 has not been actuated yet in this configuration, the manifold 135 is open, allowing the single sample contained in the rightmost sample input to be provided to all the assay input lines 132. Thus, the array is loaded though the assay input lines using a single sample from the rightmost sample assay input. A second pressure is applied to pressure accumulator 102, actuating the latching control line 110 and closing the

array valves associated with section 114. Thus, utilizing different control flows, multiple configurations are possible using a single programmable digital array, for example, a single panel configuration.

[0089] It should be appreciated that the specific steps illustrated in FIG. 4 provide a particular method of operating a microfluidic device according to another embodiment of the present invention. Other sequences of steps may also be performed according to alternative embodiments. For example, alternative embodiments of the present invention may perform the steps outlined above in a different order. Moreover, the individual steps illustrated in FIG. 4 may include multiple sub-steps that may be performed in various sequences as appropriate to the individual step. Furthermore, additional steps may be added or removed depending on the particular applications. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0090] FIG. 5 is a simplified flowchart illustrating a method of operating a microfluidic device according to yet another embodiment of the present invention. In the embodiment illustrated in FIG. 5, a 12 panel configuration for the digital array is provided. A first pressure is applied to pressure source 104 (510). In a particular embodiment, the first pressure is 45 psi although this is not required by the present invention and other suitable pressures can be utilized. As discussed in relation to process (410) of FIG. 4, latching control line 120 is actuated. Pressure is applied through unidirectional valve 127 to close and latch valves 126 in section 125. Thus, flow through the sample input lines 142 will be prevented in this configuration. Flyover section 122 will transmit the control pressure to close the decoupling valve 124. As will be described below, temporary closure of the decoupling valve will prevent containment valves 128 from closing upon subsequent actuation of control line 110. Section 123 will transmit the control pressure in latching control line 120 to valves 134 associated with five of the six assay input lines 132. Thus, valves 134 will be temporarily closed.

[0091] A second pressure is applied to pressure source 102 (512), actuating latching control line 110. In a specific embodiment, the second pressure is 30 psi, which is less than the first pressure (e.g., 45 psi). As described below, the second pressure is sufficient to close predetermined valves without opening the decoupling valve 124, which was closed during process (510). Other pressures may also be utilized as appropriate to the particular application. Actuation of section 114 will close the valves in the array 106. Pressure will be

applied through unidirectional valve 112 to section 116, which will result in valves 118a - 118e closing and latching. The flyover portion of section 116 will prevent closing of the assay input lines as a result of actuation of section 116. The closing of valves 118 will shut off the flow of input fluids through manifold 135, thereby separating each of the six assay input lines connected to the assay inputs 130.

[0092] In contrast with initial actuation of control line 102, initial actuation of control line 104 closes the decoupling valve, thereby providing a programming sequence in which actuation of control line 102 does not result in latching of valves 128 associated with the assay input lines 132. This control-on-control feature provides for asynchronous logic functions utilizing embodiments of the present invention that are not available using conventional microfluidic devices.

[0093] The pressure applied to pressure source 102 is reduced, for example, to zero (514). Because section 116 is downstream of unidirectional valve 112, valves 118 remain latched in the closed position after deactuation of section 116, preventing fluid flow between the six assay input lines. The deactuation of section 114 opens the valves in the array, enabling loading of samples in a subsequent loading process. As discussed above, valves 128 were not latched at process (512).

[0094] The pressure applied to pressure source 104 is reduced, for example, to zero (516). Valves 134 are reopened by reduction of pressure in section 123. As described below, the assay inputs 130 will be utilized to provide inputs for the reaction chambers in the array. The decoupling valve 124 is reopened by reduction of pressure in flyover 122 in section 121. Since no pressure is applied to control line 110 at this stage of the programming process, valves 128 remain in their open state. Although pressure reductions to zero are illustrated, these particular pressures are not required by the present invention and other suitable pressures may be utilized.

[0095] Samples are loaded from the six assay inputs (518). Separation between the assay inputs is maintained via the non-functionality of the manifold 135 resulting from the previous latching of valves 118. Thus, in this configuration, a 12 panel configuration (six assay inputs x two sides of the device) is provided. A third pressure is applied to pressure source 102 (520) to close the array valves associated with section 114. Additionally, since the decoupling valve 124 is now reopened, valves 128 are latched closed.

[0096] It should be appreciated that the specific steps illustrated in FIG. 5 provide a particular method of operating a microfluidic device according to yet another embodiment of the present invention. Other sequences of steps may also be performed according to alternative embodiments. For example, alternative embodiments of the present invention may perform the steps outlined above in a different order. Moreover, the individual steps illustrated in FIG. 5 may include multiple sub-steps that may be performed in various sequences as appropriate to the individual step. Furthermore, additional steps may be added or removed depending on the particular applications. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0097] Table 1 provides actuation pressures and loading processes to provide one of multiple possible panel configurations from the programmable digital array microfluidic device described herein. The pressures applied to the pressure sources 102 and 104 are illustrated with exemplary pressures, although other suitable pressures are included within the scope of embodiments of the present invention.

Programming Step	48 Panel Configuration	1 Panel Configuration	12 Panel Configuration
1	102 = 30 psi	104 = 30 psi	104 = 45 psi
2	102 = 0 psi	Load Sample	102 = 30 psi
3	Load Samples	102 = 30 psi	102 = 0 psi
4	102 = 30 psi		104 = 0 psi
5			Load Samples
6			102 = 30 psi

Table 1

[0098] The integration of the unidirectional valves and the decoupling valve with the digital array provides for multiple user-programmed configurations using a single high density digital array chip. Thus, cost savings and improvements in experiment throughput are provided by embodiments of the present invention. The serial or asynchronous logic described herein provides for increased device functionality since the state of a particular valve is dependent not only on the actuation of the control line in fluid communication with the particular valve, but also on the state of the decoupling valve, which is actuated by a separate control line. Thus, the order of pressurization drives the logical outcome of the panel configuration. As a result, embodiments of the present invention provide for device configurations and functionality not available using conventional microfluidic devices.

[0099] According to an embodiment of the present invention, a method of configuring a microfluidic device having a plurality of control lines is provided. Serial or asynchronous logic is performed according to this method. The method includes actuating a first control line and placing a valve in a first state. The valve, which may be one of a number of valves, may be placed in a closed state in response to the actuation of the first control line. As an example, decoupling valve 124 can be closed in response to application of pressure to interface accumulator 104 and the pressurization of control line 120. As described more fully throughout the present specification and more particularly below, because the decoupling valve is disposed between valves 128 and the containment accumulator 102, the decoupling valve can provide a control-on-control function which provides for serial logic operations.

[0100] The method also includes, after placing the valve in the first state, actuating a second control line operable to place a set of valves in a second state. The fact that the valve is in the first state (i.e., closed) prevents the set of valves from being placed the second state, for example, in a closed state. As illustrated in FIG. 1, closing of the decoupling valve 124 prior to actuation of control line 110 prevents valves 128 from being closed (and latched) in response actuation of control line 110. Thus, embodiments of the present invention provide for serial logic functions in which the order of valve actuation produces a different outcome selected from several final states. In the present example, initial actuation of control line 110 results in closing and latching of valves 128. On the other hand, initial actuation of control line 120 results in closing of decoupling valve 124, which while maintained in the closed state, prevents the closing and latching of valves 128. Although FIG. 1 illustrates control lines 110 and 120 actuated by application of pressure to independent pressure sources 102 and 104, other methods of actuation are included within the scope of the present invention.

[0101] According to another embodiment of the present invention, another method of performing serial logic using a microfluidic device is provided. The method of configuring the microfluidic device, which has a plurality of control lines, includes establishing a first state of the microfluidic device by actuating a first control line and then actuating a second control line. The first state of the microfluidic device may include setting valves in an open or closed state in response to actuation of the various control lines. For example, as discussed above, actuating control line 120 prior to actuating control line 110 results in closing of the decoupling valve 124. As a result, valves 128 do not latch closed in response to actuation of control line 110.

[0102] The method also includes establishing a second state of the microfluidic device by actuating the second control line and then actuating the first control line. The programmable nature of the microfluidic devices described herein provides for different final states of the microfluidic device depending on the order of valve actuation. Thus, in this example, actuating control line 110 prior to actuating control line 120 results in closing and latching of valves 128 and then closing of the decoupling valve 124. The closing of the decoupling valves after latching of valves 128 is inconsequential since check valve 112 maintains a constant pressure in the control line downstream of the check valve, preventing the closure of the decoupling valve from impacting the state of the microfluidic device.

[0103] As examples of microfluidic devices implementing serial logic, the first state of the microfluidic device may include a first set of input lines in a closed or obstructed state (e.g., input lines 142 obstructed by valves 126) and a second set of input lines (e.g., input lines 132) in fluid communication with a plurality of reaction chambers disposed in the microfluidic device. The second state may include the first set of input lines (e.g., input lines 142) being in fluid communication with the plurality of reaction chambers disposed in the microfluidic device and the second set of input lines in a closed or obstructed state (e.g., input lines 132 obstructed by valves 128). The second state may further include a set of valves in a closed state (e.g., valves 118a - 188e), thereby preventing fluid flow through a manifold connecting the second set of input lines.

[0104] It should be noted that although decoupling valve is actuated by control line 120, other embodiments can utilize another decoupling valve actuated by control line 110 in addition to or in place of the illustrated decoupling valve. Thus, for example, a flyover line could run from containment accumulator 102 to an additional decoupling valve positioned between interface accumulator 104 and check valve 127. Closing of this additional decoupling valve prior to application of pressure to section 125 of the control line coupled to the interface accumulator 104 will prevent closing and latching of valves 126 in response to actuation of control line 120. Thus, although a particular implementation is illustrated in FIG. 1, the present invention is not limited to this particular implementation and other microfluidic device designs are included within the scope of the present invention.

[0105] Moreover, although the embodiment illustrated in FIG. 1 and the alternative geometry discussed above utilize decoupling valves that are actuated by application of pressure to one of two control lines, thereby providing two levels of control-on-control, additional levels of control-on-control are provided by embodiments of the present invention. For example, an additional independent valve (e.g., actuated electrostatically) could be integrated into the design of the microfluidic device, providing for a third level of control. Initial actuation of this additional independent valve could then drive the logical outcome of the device state in addition to the actuation of the two illustrated control lines.

[0106] FIG. 7 illustrates a simplified method of programming a programmable microfluidic device according to an embodiment of the present invention. In the embodiment illustrated in FIG. 7, the microfluidic device is programmed to partition the reaction chambers of the microfluidic device into 48 separate panels. The particular number of panels will depend on

the actual design of the microfluidic device and may be a different number of panels in alternative embodiments, for example, 12 or 192 panels. The reaction chambers in each of the separate panels can be filled with a different sample, providing concurrent testing of up to 48 different samples in this embodiment.

[0107] As illustrated in FIG. 1, the microfluidic device has an array of reaction sites or reaction chambers disposed in the array 106. The reaction sites are in fluid communication with a first set of input lines 132 and a second set of input lines 142. The method includes actuating a first set of valves (710) operable to obstruct the first set of input lines (712). As an example, the first set of valves are valves 128, also referred to as containment valves that are operable to block or close input fluid lines 132. Closing of valves 128, for example, by application of pressure to containment accumulator 102, prevents the flow of fluid through the input lines 132. Since the presence of unidirectional or check valve 112 makes control line 110 a latching control line, after the containment valves 128 are closed, they remain closed.

[0108] The method also includes actuating a second set of valves (714) operable to isolate the reaction sites (716). As illustrated in FIG. 6B, the reaction chambers 630 are defined in elastomeric layer 603 of the microfluidic device. The second set of valves are disposed in the array and are illustrated by valves 615 in FIG. 6A. In addition to the first set of valves 128 and the second set of valves 615, a third set of valves 118a - 118e are actuated concurrently with the first set of valves. The presence of the check valve 112 also causes valves 118a - 118e to latch once they are closed. In the embodiment illustrated in FIG. 1, the first set of valves and the second set of valves are actuated or closed concurrently in response to the application of pressure to control line 110.

[0109] After the first, second, and third sets of valves are closed, the second set of valves is deactuated (718), for example, by reducing or eliminating the pressure applied to control line 110 by pressure source 102. Deactuation of the valves results in opening of the valves, which allows for fluid flow through the input lines associated with the valves. A plurality of samples are loaded into the reaction sites through a second set of input lines 142 (720). In FIG. 1, the samples are contained in sample inputs or ports 140. The two sides of the microfluidic device, each containing 24 different input ports, provide a total of 48 input ports, although this particular number can vary depending on the particular design of the microfluidic device. The sample ports may contain 48 different samples or some fewer

number of total samples if a single sample is provided in multiple input ports. Thus, although Table 1 refers to a 48 panel configuration, the programmable microfluidic device actually provides for up to 48 separate panels depending on the samples provided in the 48 sample ports. After the samples are loaded into the reaction sites, the second set of valves are actuated a second time to isolate the reaction chambers from each other (722).

[0110] It should be appreciated that the specific steps illustrated in FIG. 7 provide a particular method of programming a microfluidic device according to an embodiment of the present invention. Other sequences of steps may also be performed according to alternative embodiments. For example, alternative embodiments of the present invention may perform the steps outlined above in a different order. Moreover, the individual steps illustrated in FIG. 7 may include multiple sub-steps that may be performed in various sequences as appropriate to the individual step. Furthermore, additional steps may be added or removed depending on the particular applications. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0111] FIG. 8 illustrates a simplified method of programming a programmable microfluidic device according to another embodiment of the present invention. The method illustrated in FIG. 8 provides a method for operating the programmable microfluidic device as a single panel, with all reaction chambers containing the same sample. As illustrated in FIG. 1, the programmable microfluidic device has an array of reaction sites that are in fluid communication with a first set of input lines and a second set of input lines. In the exemplary embodiment illustrated in FIG. 8, the first set of input lines are input fluid lines 142 coupled to sample inputs 140 and the second set of input lines are input fluid lines 132 coupled to the assay inputs 130. The first set of valves are actuated into a closed position (810), obstructing flow through (i.e., shutting) the first set of input lines (812). Thus, in an example, pressurizing the interface accumulator 104 results in valves 126 closing, preventing fluid flow through the input lines 142. The presence of check valve 127 results in the first set of valve latching in the closed position.

[0112] The method also includes actuating a second set of valves (814) operable to obstruct a first portion of a subset of a second set of input lines (816). Referring to FIG. 1, the first portion of the second set of input lines is the portion of input lines 132 below the valves 134 and the second set of valves are valves 134, which are operable to obstruct or shut five of the six input lines 132 coupled to the six assay inputs 130. Thus, in this embodiment, the subset

of the input lines is five of the six input lines and the sample to be loaded into the reaction chambers or sites will be provided using the rightmost assay input port illustrated in FIG. 1, which is connected to the rightmost input line. The input line not included in the subset (i.e., the rightmost input line) is open and used to load the sample into the reaction chambers as described more fully below. The other assay input ports will not be utilized in this particular implementation. In the embodiment illustrated in FIG. 1, valves 126 and valves 134 are actuated concurrently by application of pressure from interface accumulator 104.

[0113] Referring to FIG. 1, no actuation of valves through the use of control line 110 has been performed. As a result, loading of the sample from the rightmost assay input port will result in the flow of the sample past section 116 and through manifold 135 to a second portion of the second set of input lines (i.e., the portion of input lines 132 above the valves 134. The sample is loaded through the second set of input lines into the reaction chambers or sites present in the array 106 (818). It should be noted that the manifold is deactivated (i.e., flow through the manifold is prevented) by actuation via section 116 of control line 110, which has not yet been pressured in this embodiment. As a result, the manifold is open and connects all six input fluid lines at the initial section of the second portion of the input lines 132. Passing through the manifold 135, the sample will be distributed to the branching portions of input lines 132, eventually flowing through the 24 input lines passing over valves 128, which are open. Thus, a single sample is provided to all the reaction chambers in the array 106. In order to isolate the sample in the reaction chambers (822), a third set of valves is actuated (820), for example, containment valves 615 illustrated in FIG. 6A. Application of pressure to control line 110 by the use of containment accumulator 102 will provide pressure to close valves 615 in the array 106.

[0114] It should be appreciated that the specific steps illustrated in FIG. 8 provide a particular method of programming a microfluidic device according to another embodiment of the present invention. Other sequences of steps may also be performed according to alternative embodiments. For example, alternative embodiments of the present invention may perform the steps outlined above in a different order. Moreover, the individual steps illustrated in FIG. 8 may include multiple sub-steps that may be performed in various sequences as appropriate to the individual step. Furthermore, additional steps may be added or removed depending on the particular applications. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0115] FIG. 9 illustrates a simplified method of programming a programmable microfluidic device according to yet another embodiment of the present invention. The programmable microfluidic device has an array of reaction sites in fluid communication with a first set of input lines, a second set of input lines, and a manifold connecting the second set of input lines. The method includes actuating a first set of valves (910) operable to obstruct the first set of input lines (912). Referring to FIG. 1, the first set of valves can be valves 126 downstream of check valve 127. The presence of check valve 127 causes valves 126 to latch in the closed position after actuation. A second set of valves is actuated (914) in order to obstruct a first portion of a subset of a second set of input lines (916). Application of pressure to control line 120 from interface accumulator 104 will result in actuation of valves 126 and valves 134.

[0116] Referring to FIG. 1, the first portion of the second set of input lines is the portion of input lines 132 below the valves 134 and the second set of valves are valves 134, which are operable to obstruct or shut five of the six input lines 132 coupled to the six assay inputs 130. Thus, in this embodiment, the subset of the input lines is five of the six input lines. Because the valves 134 are not latched by a unidirectional valve, when these valves are reopened, fluid is able to flow through the first portion of all of the six input lines 132 toward the manifold 135. In the embodiment illustrated in FIG. 1, the valves 126 and the valves 132 are actuated concurrently by application of pressure from interface accumulator 104.

[0117] The method also includes actuating a third set of valves (918) operable to deactivate the manifold (920). In an exemplary embodiment, actuating the third set of valves includes applying a predetermined pressure to a pressure source in fluid communication with the second set of valves, for example, containment accumulator 102. Referring to FIG. 1, actuation of valves 118a - 118e by application of pressure to section 116 of control line 110 will obstruct flow through the manifold, providing for separation of fluid flowing through the input lines 132. The presence of check valve 112 will result in valves 118a - 118e latching in the closed position. As described more fully below, the deactivation of manifold 135 enables 12 samples (6 samples per side of the microfluidic device) to be loaded into the reaction chambers from the six assay input ports 130. As with the embodiment discussed in relation to FIG. 7, less than 12 samples can be loaded by providing the same sample to more than one of the assay inputs. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0118] Referring once again to FIG. 1, it should be noted that initial actuation of control line 120 prior to actuation of control line 110 not only results in the closing and latching of valves 126 and valves 134, but closing of decoupling valve 124. Because the decoupling valve is disposed between the containment accumulator 102 and valves 128, the closing of the decoupling valve is able to prevent closing of valves 128 in response to actuation of control line 110. In the embodiment illustrated in FIG. 9, the pressure utilized to actuate control line 120 is a predetermined pressure sufficient to prevent actuation of control line 110 resulting in the closure of valves 128. As shown in Table 1, a pressure of 45 psi is sufficient to maintain valves 128 in an open position despite the application of a pressure of 30 psi to control line 110. The particular values of 45 psi and 30 psi are not required by the present invention, but other pressures can be utilized to perform this control-on-control function.

[0119] The method also includes deactuating the second set of valves (922). Typically, the second set of valves are deactuated to assume an open position by reducing the first pressure applied to the first pressure source, for example, to zero psi. The reduction in the pressure applied to the control line enables the flexible membrane that was deflected into the input fluid line to return to a substantially undeflected position, enabling fluid flow through the input fluid line to resume. After the valves 134 are reopened, samples provided in the assay inputs 130 are loaded into the reaction sites (924). As shown in FIG. 1, the first portion of each of the second set of input lines (e.g., the portion of the input lines 134 below valves 134) is in fluid communication with an assay input port, which are configured to receive one of the plurality of samples. The samples flow through the first and second portion of the second set of input lines to the reaction sites. As illustrated in FIGS. 6A and 6B, the reaction sites may be formed as a plurality of reaction chambers, with each of the reaction chambers in fluid communication with the second portion of input fluid lines 132 through a via passing from the input fluid line to the reaction chamber, which may be disposed in a layer of the elastomeric microfluidic device above the layer containing the fluid line.

[0120] As illustrated in FIG. 1, the portion of input lines 132 positioned above valves 134 branch into sets of four input lines that are in fluid communication with the reaction sites or chambers present in the array 106. By providing six different samples in each of the assay input ports, a total of 12 panels can be defined in the array since FIG. 1 only illustrates a first side of the microfluidic device. Use of one sample in more than one input port will result in less than 12 panels as will be evident to one of skill in the art. In order to isolate the reaction sites, a fourth set of valves are actuated (926), for example, the valves 615.

[0121] In some implementations, the method also includes actuating a valve to prevent closure of a fifth set of valves, wherein the fifth set of valves are operable to close the second portion of the second set of input lines. As shown in FIG. 1, closure of the decoupling valve 124 prevents valves 128 from closing in response to actuation of control line 110. In the embodiment illustrated in FIG. 9, the pressure of 45 psi initially applied to control line 120 results in closure of the decoupling valve, enabling the second portion of input lines 132 to remain open while the manifold is deactivated by closing valves 118a - 118e. The decoupling valve may be actuated by application of pressure to a control line or may be actuated in other ways including, but not limited to mechanical, electrostatic, or the like. In applications utilizing pressure-based control lines, multiple valves and/or sets of valves can be actuated concurrently using a single control line. Thus, decoupling valve 124 as well as valves 126 and valves 134 are actuated concurrently in response to actuation of control line 120 when interface accumulator 104 is pressurized.

[0122] The programmable nature of the microfluidic devices provided herein is demonstrated by the fact that exchanging the order of the steps of valve actuation will result in the valves in the array, and therefore, the state of the array, being in alternative states. For example, in the embodiment illustrated in FIG. 9, 12 samples can be loaded into the array by closing the decoupling valve 124, then applying pressure to control line 110, which deactivates the manifold 135, then reducing the pressure on accumulator 102, and then reducing the pressure on accumulator 104. In this example, maintaining the pressure on accumulator 104 while pressure is applied through accumulator 102 prevents the valves 128 from closing in response to actuation of control line 110 by application of pressure using accumulator 102. On the other hand, releasing the pressure applied to accumulator 104 while pressure is applied through accumulator 102 will result in the valves 128 closing. Thus, two different states of the microfluidic device, the capability of loading samples through input lines 132 or blocking input lines 132 are achieved by varying the order in which the valves are actuated. This programmable characteristic provides benefits not available using conventional designs.

[0123] It should be appreciated that the specific steps illustrated in FIG. 9 provide a particular method of programming a microfluidic device according to yet another embodiment of the present invention. Other sequences of steps may also be performed according to alternative embodiments. For example, alternative embodiments of the present invention may perform the steps outlined above in a different order. Moreover, the individual

steps illustrated in FIG. 9 may include multiple sub-steps that may be performed in various sequences as appropriate to the individual step. Furthermore, additional steps may be added or removed depending on the particular applications. One of ordinary skill in the art would recognize many variations, modifications, and alternatives.

[0124] It is understood that the invention is not limited to the particular methodology, protocols, and reagents, etc., described herein, as these may vary as the skilled artisan will recognize. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only, and is not intended to limit the scope of the invention. It is also to be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art.

[0125] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which the invention pertains. The embodiments of the invention and the various features and advantageous details thereof are explained more fully with reference to the non-limiting embodiments and examples that are described and/or illustrated in the accompanying drawings and detailed in the following description. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale, and features of one embodiment may be employed with other embodiments as the skilled artisan would recognize, even if not explicitly stated herein. Descriptions of well-known components and processing techniques may be omitted so as to not unnecessarily obscure the embodiments of the invention. The examples used herein are intended merely to facilitate an understanding of ways in which the invention may be practiced and to further enable those of skill in the art to practice the embodiments of the invention. Accordingly, the examples and embodiments herein should not be construed as limiting the scope of the invention, which is defined solely by the appended claims and applicable law. Moreover, it is noted that like reference numerals reference similar parts throughout the several views of the drawings.

[0126] Accordingly, provided immediately below is a "Definition" section, where certain terms related to the invention are defined specifically for clarity, but all of the definitions are consistent with how a skilled artisan would understand these terms. Particular methods, devices, and materials are described, although any methods and materials similar or

equivalent to those described herein can be used in the practice or testing of the invention. All references referred to herein are incorporated by reference herein in their entirety.

[0127] Definitions

[0128] PNA is peptide nucleic acid

[0129] LNA is locked nucleic acid

[0130] DA is dynamic array

[0131] PCR is polymerase chain reaction

[0132] BSA is bovine serum albumin

[0133] FRET is fluorescence resonance energy transfer

[0134] GT is genotyping

[0135] PEG is polyethylene glycol

[0136] PLP is padlock probe

[0137] The term "adjacent" as used herein, generally refers to the positioning of the primer with respect to the probe on its complementary strand of the target nucleic acid analyte. The primer and probe may be separated in a range of about 1 to about 20 nucleotides, more specifically, in a range of about 1 to about 10 nucleotides, or may directly abut one another.

[0138] The term "analyte" as used herein, generally refers to a nucleic acid molecule or mixture of nucleic acid molecules, defined *infra*, that is to be detected or quantified using the methods of the invention. The terms "target nucleic acid analyte" and "nucleic acid analyte" are used interchangeably with the term "analyte" for the purposes of this invention.

[0139] The terms "complementary" or "complementarity" as used herein, may include the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands and in the design and use of molecules.

[0140] The term "covalently attached" as used herein, generally refers to an attachment of one molecular moiety to another molecular moiety through covalent chemical bonds.

[0141] The term "dye" as used herein, generally refers to any organic or inorganic molecule that absorbs electromagnetic radiation at a wavelength greater than or equal 340 nm.

[0142] The term "fluorescent dye" as used herein, generally refers to any dye that emits electromagnetic radiation of longer wavelength by a fluorescent mechanism upon irradiation by a source of electromagnetic radiation, such as a lamp, a photodiode, or a laser.

[0143] The term "GT sample buffer," as used herein generally refers to a buffer that is capable of blocking binding sites on the surface of the reaction channels and chambers in a DA chip. The buffer protects the reaction components from depletion during the chip loading process or reaction. It may also reduce the usage of additional Taq-Gold Polymerase by less than about 80% for reagent costs. A 20x GT buffer may include a combination of betaine (FW 117.15), BSA, Superblock® T20 (in PBS) (Thermo Scientific, Rockford, IL), Superblock® (in PBS) (Thermo Scientific, Rockford, IL), Superblock® (in TBS) (Thermo Scientific, Rockford, IL), Superblock® T20 (in TBS) (Thermo Scientific, Rockford, IL), glycerol, PEG 20,000, PEG MME550, PEG MME5000, and Tween 20.

[0144] The term "homogenous assay" as used herein, generally refers to a method to detect or quantify a nucleic acid analyte that requires no post-assay processing to record the result of the assay. The homogenous assays may be carried out in closed tubes or microfluidic arrays where no further addition of reagents or supplementary chemicals are necessary to record the result once the assay is started. Homogenous assays allow recordation of the result of the assay in real time, meaning that the result of the assay can be continuously recorded as the assay progresses in time.

[0145] The term "hydrolysis probes" as used herein are generally described in U.S. Patent No. 5,210,015 incorporated herein by reference in its entirety. Hydrolysis probes take advantage of the 5'-nuclease activity present in the thermostable Taq polymerase enzyme used in the PCR reaction (TaqMan® probe technology, Applied Biosystems, Foster City CA). The hydrolysis probe is labeled with a fluorescent detector dye such as fluorescin, and an acceptor dye or quencher. In general, the fluorescent dye is covalently attached to the 5' end of the probe and the quencher is attached to the 3' end of the probe, and when the probe is intact, the fluorescence of the detector dye is quenched by fluorescence resonance energy transfer (FRET). The probe may anneal downstream of one of the primers that defines one

end of the amplification target site on the nucleic acid target analyte in the PCR reaction. Using the polymerase activity of the Taq enzyme, amplification of the target nucleic acid analyte is directed by one primer that is upstream of the probe and a second primer that is downstream of the probe but anneals to the opposite strand of the target nucleic acid. As the upstream primer is extended, the Taq polymerase reaches the region where the labeled probe is annealed, recognizes the probe-template hybrid as a substrate, and hydrolyzes phosphodiester bonds of the probe. The hydrolysis reaction irrevocably releases the quenching effect of the quencher dye on the reporter dye, thus resulting in increasing detector fluorescence with each successive PCR cycle. In particular, the hydrolysis probes of the invention may capable of detecting 8-mer or 9-mer motifs that are common in the human and other transcriptomes and may have a high T_m of about 70°C enabled by LNA analogs.

[0146] The term "label" as used herein refers to any atom or molecule which can be used to provide a detectable and/or quantifiable signal. In particular, the label can be attached to a nucleic acid or protein. Labels may provide signals detectable by fluorescence, radioactivity, colorimetric, X-ray diffraction or absorption, magnetism, enzymatic activity, and the like.

[0147] The term "nucleic acid" as used herein generally refers to cDNA, DNA, RNA, single-stranded or double-stranded and any chemical modification thereof, such as PNA and LNA. LNAs are described in U.S. Patent Nos. 6,794,499, 6,670,461, 6,262,490, and 6,770,748 herein incorporated by reference in their entirety. Nucleic acids may be of any size. Nucleic acid modifications may include addition of chemical groups that incorporate additional charge, polarizability, hydrogen bonding, electrostatic interaction, and functionality to the individual nucleic acid bases or to the nucleic acid as a whole. Such modifications may include modified bases such as 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at cytosine exocylcic amines, substitutions of 5-bromo-uracil, backbone modifications, methylations, unusual base pairing combinations such as the isobases isocytidine and isoguanidine and the like. The nucleic acid can be derived from a completely chemical synthesis process, such as a solid phase mediated chemical synthesis, or from a biological origin, such as through isolation from almost any species that can provide nucleic acid, or from processes that involve the manipulation of nucleic acids by molecular biology tools, such as DNA replication, PCR amplification, reverse transcription, or from a combination of those processes.

[0148] The term "nucleic acid probe" as used herein is a nucleic acid that carriers at least one covalently attached dye, such as a fluorescent dye. In particular, the probe does not contain a sequence complementary to sequences used to prime the PCR reaction.

[0149] The term "padlock probe" or "PLP" as used herein, generally refers to linear oligonucleotides having a length of about 100 base pairs. The sequences at the 3' and 5' ends of the PLP are complementary to adjacent sequences in the target nucleic acid analyte. In the central, noncomplementary region of the PLP there is a "tag sequence" that may be used to identify the specific PLP. The tag sequence may be flanked by universal primer sites or unique and/or specific primer sites, which allow PCR amplification of the tag sequence. Upon hybridization to the target, the 5' and 3' ends of the PLP are brought into close proximity and may be subsequently ligated. The resulting product is a circular probe molecule catenated to the target nucleic acid analyte. The tag regions of circularized PLPs may be amplified and quantified and/or detected using TAQMAN® Real Time PCR, for example. The presence and amount of amplicon may be correlated with the presence and quantity of target sequence in the sample. For descriptions of PLPs *see, e.g.*, Landegren et al., 2003, Padlock and proximity probes for in situ and array-based analyses: tools for the post-genomic era, *Comparative and Functional Genomics* 4:525-30; Nilsson et al., 2006, Analyzing genes using closing and replicating circles *Trends Biotechnol.* 24:83-8; Nilsson et al., 1994, Padlock probes: circularizing oligonucleotides for localized DNA detection, *Science* 265:2085-8. The above references are incorporated by reference herein in their entirety.

[0150] The term "PCR," as used herein, generally refers to a method for amplifying, detecting, or quantifying a specific region of an analyte. One skilled in the art appreciates that there are several variations on the basic PCR technique such as allele-specific PCR, assembly PCR or polymerase cycling assembly (PCA), colony PCR, helicase-dependent amplification, hot start PCR, intersequence-specific (ISSR) PCR, inverse PCR, ligation-mediated PCR, methylation-specific PCR, multiplex ligation dependent probe amplification, multiplex PCR, nested PCR, overlap-extension PCR, quantitative PCR, quantitative real-time PCR, RT-PCR, thermal asymmetric interlaces (TAIL) PCR, touchdown PCR, and PAN-AC. Additionally, one skilled in the art would understand how to practice these variations on the basic PCR technique.

[0151] The term "purification," as used herein, generally refers to any process by which proteins, polypeptides, or nucleic acids are separated from other elements or compounds on the basis of charge, molecular size, or binding affinity.

[0152] The term "quencher" as used herein, generally refers to dye that reduces the emission of fluorescence of another dye.

[0153] The term "querying" as used herein, generally refers to determining whether a target-specific probe is associated with (e.g., bound to or catenated with) the nucleic acid analyte, and optionally quantifying the amount of target-specific probe in the sample.

[0154] A "sample" as used herein, generally refers to a sample of tissue or fluid from a human or animal including, but not limited to plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal and genitourinary tracts, tears, saliva, blood cells, tumors, organs, tissue and sample of in vitro cell culture constituents. In particular, the sample may be single cells, paraffin embedded tissue samples, and needle biopsies. Moreover, a sample may include environmental samples such as lake water, and food samples.

[0155] The phrase "substantially purified," or "substantially isolated," as used herein generally includes nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least about 60% free, specifically at least about 75% free, and most specifically at least about 90% free from other components with which they may be associated with, and includes recombinant or cloned nucleic acid isolates and chemically synthesized analogs or analogs biologically synthesized by systems.

[0156] Given the tremendous diversity of polymer chemistries, precursors, synthetic methods, reaction conditions, and potential additives, there are a huge number of possible elastomer systems that could be used to make elastomeric blocks, layers, membranes, microvalves, pumps, and the like. Variations in the materials used may in some cases be driven by the need for particular material properties, i.e. solvent resistance, stiffness, gas permeability, or temperature stability. There are many, many types of elastomeric polymers. A brief description of the most common classes of elastomers is presented here, with the intent of showing that even with relatively "standard" polymers, many possibilities for bonding exist. Common elastomeric polymers include polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicones or polysiloxanes.

[0157] Polyisoprene, polybutadiene, and polychloroprene are all polymerized from diene monomers, and therefore have one double bond per monomer when polymerized. This double bond allows the polymers to be converted to elastomers by vulcanization (generally, sulfur is used to form crosslinks between the double bonds by heating). This would easily allow homogeneous multilayer soft lithography by incomplete vulcanization of the layers to be bonded; photoresist encapsulation would be possible by a similar mechanism.

[0158] Pure polyisobutylene has no double bonds, but is crosslinked to use as an elastomer by including a small amount (.about.1%) of isoprene in the polymerization. The isoprene monomers give pendant double bonds on the polyisobutylene backbone, which may then be vulcanized as above.

[0159] Poly(styrene-butadiene-styrene) is produced by living anionic polymerization (that is, there is no natural chain-terminating step in the reaction), so "live" polymer ends can exist in the cured polymer. This makes it a natural candidate for a photoresist encapsulation system (where there will be plenty of unreacted monomer in the liquid layer poured on top of the cured layer). Incomplete curing would allow homogeneous multilayer soft lithography (A to A bonding). The chemistry also facilitates making one layer with extra butadiene ("A") and coupling agent and the other layer ("B") with a butadiene deficit (for heterogeneous multilayer soft lithography). SBS is a "thermoset elastomer", meaning that above a certain temperature it melts and becomes plastic (as opposed to elastic); reducing the temperature yields the elastomer again. Thus, layers can be bonded together by heating.

[0160] Polyurethanes are produced from di-isocyanates (A--A) and di-alcohols or di-amines (B--B); since there are a large variety of di-isocyanates and di-alcohols/amines, the number of different types of polyurethanes is huge. The A vs. B nature of the polymers, however, make them useful for heterogeneous multilayer soft lithography just as RTV 615 is: by using excess A--A in one layer and excess B--B in the other layer.

[0161] Silicone polymers have great structural variety, and provide a great number of commercially available formulations. The vinyl-to-(Si--H) crosslinking of RTV 615 (which allows both heterogeneous multilayer soft lithography and photoresist encapsulation) has already been discussed, but this is only one of several crosslinking methods used in silicone polymer chemistry.

[0162] In addition to the use of the simple "pure" polymers discussed above, crosslinking agents may be added. Some agents (like the monomers bearing pendant double bonds for

vulcanization) are suitable for allowing homogeneous (A to A) multilayer soft lithography or photoresist encapsulation; in such an approach the same agent is incorporated into both elastomer layers. Complementary agents (i.e. one monomer bearing a pendant double bond, and another bearing a pendant Si--H group) are suitable for heterogeneous (A to B) multilayer soft lithography. In this approach complementary agents are added to adjacent layers.

[0163] The following is a non-exclusive list of elastomeric materials which may be utilized in connection with the present invention: polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicone polymers; or poly(bis(fluoroaLkoxy)phosphazene) (PNF, Eypel-F), poly(carborane-siloxanes) (Dexsil), poly(acrylonitrile-butadiene) (nitrile rubber), poly(1-butene), poly(chlorotrifluoroethylene-vinylidene fluoride) copolymers (Kel-F), poly(ethyl vinyl ether), poly(vinylidene fluoride), poly(vinylidene fluoride-hexafluoropropylene) copolymer (Viton), elastomeric compositions of polyvinylchloride (PVC), polysulfone, polycarbonate, polymethylmethacrylate (PMMA), and polytertrafluoroethylene (Teflon).

[0164] Allcock et al, Contemporary Polymer Chemistry, 2nd Ed. describes elastomers in general as polymers existing at a temperature between their glass transition temperature and liquefaction temperature. Elastomeric materials exhibit elastic properties because the polymer chains readily undergo torsional motion to permit uncoiling of the backbone chains in response to a force, with the backbone chains recoiling to assume the prior shape in the absence of the force. In general, elastomers deform when force is applied, but then return to their original shape when the force is removed. The elasticity exhibited by elastomeric materials may be characterized by a Young's modulus. Materials having a Young's modulus of between about 1 Pa to about 1 TPa, or between about 10 Pa to about 100 GPa, or between about 20 Pa to about 1 GPa, or between about 50 Pa to about 10 MPa, or between about 100 Pa to about 1 MPa are useful in accordance with embodiments of the present invention, although materials having a Young's modulus outside of these ranges could also be utilized depending upon the needs of a particular application. In some cases, materials can have a Young's modulus of about 100 MPA (megapascals) or less. In other embodiments, the Young's modulus of the material is about 75 MPA or less, about 50 MPa or less, about 25 MPa or less, about 10 MPa or less, about 8 MPa or less, about 5 MPa or less, or about 2 MPa or less.

[0165] Embodiments of the present invention provide a microfluidic device that includes features such as channels, valves, and chambers, that are at least partially contained, embedded, or formed by or within one or more layers or levels of an elastomeric block. An exemplary microfluidic device has a reagent flow channel, or reagent line, formed in a first layer of an elastomer. The reagent flow channel includes a containment valve and a chamber conduit. The microfluidic device may also have a control channel, or containment line, formed in a second layer of the elastomer adjacent to the first layer. Further, the microfluidic device may contain a sample flow channel, or sample line, formed in a third layer of the elastomer adjacent to the second layer. The sample flow channel may include a containment valve and a chamber conduit. The control channel can be in operative association with both the reagent flow channel containment valve and the sample flow channel containment valve. The microfluidic device can include a reagent chamber in fluid communication with the reagent line, and a sample chamber in fluid communication with the sample line. The reagent chamber and the sample chamber may be in fluid communication with each other by way of a reaction flow channel or reaction line, formed in the third layer of the elastomer. The reaction line may include an interface valve. The microfluidic device may also include an interface channel formed in a fourth layer of the elastomer adjacent to the third layer. The interface channel can be in operative association with the reaction flow channel interface valve.

[0166] Embodiments of the present invention also encompass methods of making and using the microfluidic devices disclosed herein. For example, operation of a microfluidic device can involve opening one or more isolation valves, closing one or more interface valves, and flowing material past the isolation valves and into one or more chambers, optionally under pressure. Techniques may also include changing the pressure in a containment line to close the isolation valves, so as to seal off the individual chambers, and changing the pressure in an interface line, so as to open an interface valve. A first material in a first chamber can flow past an open interface valve and into a second chamber, where the first material mixes or reacts with a second material contained therein.

[0167] It is also understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A microfluidic device comprising:

a pressure source;

a control line in fluid communication with the pressure source;

a plurality of valves operated via the control line; and

an independent valve positioned adjacent the control line and between the pressure source and the plurality of valves.

2. The microfluidic device of claim 1 wherein the independent valve is constructed and arranged to obstruct fluid flow through the control line.

3. The microfluidic device of claim 1 wherein the independent valve is in fluid communication with a second pressure source.

4. The microfluidic device of claim 1 wherein the control line comprises a latching control line including a unidirectional valve.

5. The microfluidic device of claim 4 wherein the unidirectional valve is configured to prevent fluid flow from the valves to the pressure source.

6. The microfluidic device of claim 5 further comprising a plurality of chamber valves.

7. The microfluidic device of claim 6 wherein the control line provides an actuation pressure to the plurality of valves and the plurality of chamber valves.

8. The microfluidic device of claim 1 wherein the independent valve is operable to prevent the plurality of valves from closing.

9. The microfluidic device of claim 1 further comprising a second plurality of valves operated via a second control line.

10. The microfluidic device of claim 9 wherein the second control line comprises a second latching control line including a second unidirectional valve.

11. A method of operating a microfluidic device having a valve and a control line having a set of valves associated therewith, the method comprising:

closing the valve; and

applying a pressure to the control line; wherein the closed valve causes the set of valves associated with the control line to be inoperable.

12. The method of claim 11 wherein closing the valve comprises:

applying a second pressure to a second control line; and

closing the valve in response to the second pressure.

13. The method of claim 11 wherein a first set of valves closes in response to applying the first pressure to the first control line.

14. The method of claim 13 wherein the first set of valves latch closed.

15. The method of claim 11 further comprising:

maintaining the second pressure applied to the second control line; and

applying a second pressure less than the first pressure to the first control line, wherein the set of valves associated with the second control line close in response to applying the second pressure.

16. The method of claim 15 wherein the set of valves latch closed.

17. A microfluidic device comprising:

a first valve;

a second valve;

a control line in fluid communication with the first valve and the second valve;

a pressure accumulator in fluid communication with the control line; and

a unidirectional valve positioned adjacent the control line between the pressure accumulator and the second valve.

18. The microfluidic device of claim 17 wherein the control line between the pressure accumulator and the first valve is free of a unidirectional valve.

19. The microfluidic device of claim 17 wherein the first valve is configured to isolate a reaction chamber disposed in the microfluidic device.

20. The microfluidic device of claim 17 wherein the second valve is configured to isolate a first fluid input line from a second fluid input line.

21. The microfluidic device of claim 17 further comprising a third valve in fluid communication with the control line.

22. The microfluidic device of claim 21 wherein the third valve is operable to prevent fluid flow through a fluid input line coupled to a reaction chamber.

23. The microfluidic device of claim 21 further comprising a decoupling valve constructed and arranged to obstruct fluid flow through the control line, wherein the decoupling valve is positioned along the control line between the pressure accumulator and the third valve.

24. The microfluidic device of claim 23 wherein the decoupling valve is configured to receive an actuation pressure from a second control line in fluid communication with a second pressure accumulator.

25. The microfluidic device of claim 24 wherein the second control line is independent from the control line.

26. The microfluidic device of claim 23 wherein the decoupling valve is operable to prevent the third valve from closing in response to actuation of the control line.

27. A microfluidic device comprising:

a plurality of reaction chambers disposed in an array configuration, each of the plurality of reaction chambers having a first valve in fluid communication with a one of the plurality of reaction chambers and a second valve in fluid communication with the one of the plurality of reaction chambers;

a first control line operable to actuate the first valve and the second valve;

a set of input lines in fluid communication with the plurality of reaction chambers;

a plurality of sample inlets in fluid communication with the set of input lines; and

a unidirectional valve disposed in the first control line.

28. The microfluidic device of claim 27 further comprising a first set of valves in fluid communication with a first pressure accumulator and operable to obstruct a portion of the set of input lines.

29. The microfluidic device of claim 27 wherein the first set of valves are downstream of the unidirectional valve.

30. The microfluidic device of claim 27 further comprising:

a second set of input lines in fluid communication with the plurality of reaction chambers; and

a second plurality of sample inlets in fluid communication with the second set of input lines.

31. The microfluidic device of claim 30 further comprising a second set of valves operable via a second control line.

32. The microfluidic device of claim 31 wherein the second control line is in fluid communication with a second pressure accumulator and operable to obstruct a portion of the second set of input lines.

33. The microfluidic device of claim 31 further comprising a second unidirectional valve disposed in the second control line.

34. The microfluidic device of claim 31 further comprising a decoupling valve disposed in the second control line and operable to obstruct fluid flow through the first control line.

35. A microfluidic device comprising:

a plurality of reaction chambers;

a plurality of first input ports, wherein each of the plurality of first input ports are in fluid communication with one or more of the plurality of reaction chambers through one of a first plurality of input lines;

a plurality of second input ports, wherein each of the plurality of second inputs ports are in fluid communication with the one or more of the plurality of reaction chambers through one of a second plurality of input lines;

a first pressure accumulator in fluid communication with a first control line, wherein the first control line is configured to close the first plurality of input lines;

a second pressure accumulator in fluid communication with a second control line, wherein the second control line is configured to close the second plurality of input lines;

a first unidirectional valve disposed in the first control line between the first pressure accumulator and the first plurality of input lines; and

a second unidirectional valve disposed in the second control line between the second pressure accumulator and the second plurality of input lines.

36. The microfluidic device of claim 35 further comprising a set of valves operable to isolate each of the plurality of reaction chambers.

37. The microfluidic device of claim 35 further comprising a valve operable to obstruct the second control line and disposed between the second pressure accumulator and the second control line.

38. The microfluidic device of claim 37 wherein the first control line is operable to actuate the valve.

39. The microfluidic device of claim 35 further comprising a set of valves operable to obstruct a subset of the second plurality of input lines.

40. The microfluidic device of claim 35 further comprising a set of valves operable to obstruct a manifold providing fluid communication between the second plurality of input lines.

41. A method of operating a microfluidic device having a plurality of valves and a unidirectional valve, the method comprising:

applying a first fluid pressure to a control line of the microfluidic device;

closing the plurality of valves in response to applying the first pressure;

closing the unidirectional valve in response to applying the first pressure; and

applying a second fluid pressure to a second control line of the microfluidic device.

42. The method of claim 41 further comprising reducing the first fluid pressure applied to the control line.

43. The method of claim 42 wherein the plurality of valves remain in a closed state after reducing the first fluid pressure applied to the control line.

44. The method of claim 42 wherein reducing the first fluid pressure comprises applying no fluid pressure to the control line.

45. The method of claim 41 wherein applying the second fluid pressure to the second control line comprises closing a decoupling valve.

46. The method of claim 45 wherein the decoupling valve is operable to prevent fluid communication between the plurality of valves and the control line.

47. The method of claim 41 wherein the microfluidic device has a plurality of reaction chambers and a set of valves associated with each of the plurality of reaction chambers.

48. A method of operating a microfluidic device having a plurality of input ports, the method comprising:

providing an input fluid to one of the plurality of input ports;

actuating a set of valves to close a first portion of input lines connected to a subset of the plurality of input ports, wherein the subset does not include the one of the plurality of input ports;

flowing the input fluid through an input line connected to the one of the plurality of input ports;

flowing the input fluid through the input line to a second portion of the input lines; and

closing a second set of valves to isolate a plurality of reaction chambers.

49. The method of claim 48 wherein closing the second set of valves comprises applying a fluid pressure to a first pressure source.

50. The method of claim 49 wherein closing the second set of valves comprises closing a manifold providing fluid communication between the input lines.

51. The method of claim 49 wherein actuating the set of valves comprises applying a fluid pressure to a second pressure source.

52. The method of claim 48 wherein flowing the input fluid to the second portion of the input lines comprises flowing the input fluid through a manifold providing fluid communication between the input lines.

53. The method of claim 48 wherein actuating the set of valves is performed prior to closing the second set of valves.

54. The method of claim 48 further comprising flowing the input fluid through a second set of input lines branching off of the second portion of each of the input lines.

55. A method of operating a programmable microfluidic device having an array of reaction sites in fluid communication with a first set of input lines and a second set of input lines, the method comprising:

actuating a first set of valves operable to obstruct the first set of input lines;

actuating a second set of valves operable to obstruct a first portion of a subset of a second set of input lines;

loading a sample into the reaction sites through a second portion of the second set of input lines; and

actuating a third set of valves operable to isolate the reaction sites.

56. The method of claim 55 wherein actuating the first set of valves comprises latching the first set of valves.

57. The method of claim 55 wherein actuating the first set of valves comprises applying a first pressure to a first pressure source in fluid communication with the first set of valves.

58. The method of claim 55 wherein actuating the first set of valves and actuating the second set of valves is performed concurrently.

59. The method of claim 55 wherein actuating the third set of valves comprises applying a second pressure to a second pressure source in fluid communication with the third set of valves.

60. The method of claim 59 wherein actuating the third set of valves is performed after loading the plurality of samples into the reaction sites.

61. The method of claim 55 wherein the sample flows from the first portion of an input line not included in the subset of the second set of input lines to the second portion of the second set of input lines through a manifold connecting the second portions of the second set of input lines.

62. The method of claim 55 wherein an input line not included in the subset of the second set of input lines is in fluid communication with an input port configured to receive the sample.

63. A microfluidic device comprising:

a predetermined number of input ports, each of the input ports being operable to receive one of a plurality of input fluids;

a plurality of input fluid lines, each of the plurality of input fluid lines being in fluid communication with one of the predetermined number of input ports;

a set of valves, each of the set of valves being operable to close one of the plurality of input fluid lines, wherein a number of the set of valves is less than the predetermined number;

a manifold in fluid communication with each of the plurality of input fluid lines; and

a second set of valves, each of the second set of valves being operable to close a portion of the manifold.

64. The microfluidic device of claim 63 wherein the predetermined number is 12.

65. The microfluidic device of claim 63 further comprising a first pressure source in fluid communication with the set of valves.

66. The microfluidic device of claim 65 further comprising a second pressure source in fluid communication with the set of valves.

67. The microfluidic device of claim 63 wherein the portion of the manifold comprises fluid lines connecting one of the plurality of input fluid lines to another of the plurality of input fluid lines.

68. The microfluidic device of claim 63 wherein the number of the set of valves is one less than the predetermined number.

69. The microfluidic device of claim 63 wherein each of the plurality of input fluid lines branches into multiple input fluid lines.

70. A method of operating a programmable microfluidic device having an array of reaction sites in fluid communication with a first set of input lines, a second set of input lines, and a manifold connecting the second set of input lines, the method comprising:

actuating a first set of valves operable to close the first set of input lines;

actuating a second set of valves operable to close a first portion of a subset of a second set of input lines;

actuating a third set of valves operable to deactivate the manifold;

deactuating the second set of valves;

loading a plurality of samples into the reaction sites through a second portion of the second set of input lines; and

actuating a fourth set of valves operable to isolate the reaction sites.

71. The method of claim 70 wherein actuating the first set of valves comprises latching the first set of valves.

72. The method of claim 70 wherein actuating the first set of valves and actuating the second set of valves comprises applying a first pressure to a first pressure source in fluid communication with the first set of valves and the second set of valves.

73. The method of claim 72 actuating the third set of valves comprises applying a second pressure to a second pressure source in fluid communication with the second set of valves.

74. The method of claim 73 wherein the second pressure is less than the first pressure.

75. The method of claim 70 wherein deactuating the second set of valves comprises reducing the first pressure applied to the first pressure source.

76. The method of claim 70 wherein actuating the third set of valves comprises latching the third set of valves.

77. The method of claim 70 further comprising actuating a valve to prevent closure of a fifth set of valves operable to close the second portion of the second set of input lines.

78. The method of claim 77 wherein the valve is in fluid communication with a first pressure source in fluid communication with the first set of valves.

79. The method of claim 70 wherein the first portion of each of the second set of input lines is in fluid communication with an input port configured to receive one of the plurality of samples.

80. The method of claim 70 wherein the reaction sites comprise a plurality of reaction chambers, each of the plurality of reaction chambers being in fluid communication with the second portion of one of the second set of input fluid lines through a via.

81. The method of claim 70 wherein the first portion of each of the second set of input lines is in fluid communication with a different input port.

82. A method of operating a programmable microfluidic device having an array of reaction sites in fluid communication with a first set of input lines and a second set of input lines, the method comprising:

actuating a first set of valves operable to obstruct the first set of input lines;

actuating a second set of valves operable to isolate the reaction sites;

deactuating the second set of valves;

loading a plurality of samples into the reaction sites through a second set of input lines; and

actuating the second set of valves.

83. The method of claim 82 wherein actuating the first set of valves comprises latching the first set of valves.

84. The method of claim 82 wherein actuating the first set of valves comprises applying a first pressure to a first pressure source in fluid communication with the first set of valves.

85. The method of claim 82 further comprising actuating a third set of valves concurrently with actuating the first set of valves.

86. The method of claim 85 wherein actuating the third set of valves comprises latching the third set of valves.

87. The method of claim 82 wherein deactuating the first set of valves comprises reducing the first pressure applied to the first pressure source.

88. The method of claim 82 wherein each of the second set of input lines is in fluid communication with a different input port.

89. The method of claim 82 wherein actuating the first set of valves and actuating the second set of valves is performed concurrently.

90. The method of claim 82 wherein the reaction sites comprise reaction chambers defined in an elastomeric layer of the microfluidic device.

91. A microfluidic device comprising:

a plurality of reaction sites;

a first set of input lines providing fluid communication between a predetermined number of first input ports and the plurality of reaction sites, wherein a number of the first set is the predetermined number;

a second set of input lines providing fluid communication between a predetermined number of second input ports and the plurality of reaction chambers, wherein each of the second set of input lines includes a stem portion and a branching portion and a number of the second set is less than the predetermined number; and

a programmable input device operable to fill the reaction chambers using the first set of input lines or the second set of input lines.

92. The microfluidic device of claim 91 wherein the plurality of reaction sites are disposed in an array configuration.

93. The microfluidic device of claim 91 wherein each of the plurality of reaction sites comprises a reaction chamber in fluid communication with one of the first set of input fluid lines through a via.

94. The microfluidic device of claim 91 wherein each of the plurality of reaction sites comprises a reaction chamber in fluid communication with one of the branching portions of the second set of input fluid lines through a via.

95. The microfluidic device of claim 91 wherein the branching portion of each of the set of input lines form four fluid channels from the stem portion.

96. The microfluidic device of claim 91 wherein the programmable input device comprises a set of valves operable to close the first set of input fluid lines.

97. The microfluidic device of claim 96 wherein the set of valves are in fluid communication with a unidirectional valve operable to latch the set of valves in a closed state.

98. The microfluidic device of claim 91 wherein the programmable input device comprises a set of valves operable to close the branched portion of the second set of input fluid lines.

99. The microfluidic device of claim 98 wherein the set of valves are in fluid communication with a unidirectional valve operable to latch the set of valves in a closed state.

100. The microfluidic device of claim 98 further comprising an independent valve disposed adjacent the set of valves and operable to prevent the closure of the set of valves.

101. A method of configuring a microfluidic device having a plurality of control lines, the method comprising:

actuating a first control line;

placing a valve in a first state;

thereafter, actuating a second control line operable to place a set of valves in a second state, wherein the valve being in the first state prevents the set of valves from being placed the second state.

102. The method of claim 101 wherein the first state is closed.

103. The method of claim 101 wherein the second state is closed.

104. The method of claim 101 wherein actuating the first control line comprises applying a first pressure to a first pressure source.

105. The method of claim 104 wherein actuating the second control line comprises applying a second pressure to a second pressure source.

106. The method of claim 105 wherein the second pressure source is independent from the first pressure source.

107. A method of configuring a microfluidic device having a plurality of control lines, the method comprising:

establishing a first state of the microfluidic device by actuating a first control line and then actuating a second control line; and

establishing a second state of the microfluidic device by actuating the second control line and then actuating the first control line.

108. The method of claim 107 wherein the first state comprises a first set of input lines in a closed state and a second set of input lines in fluid communication with a plurality of reaction chambers disposed in the microfluidic device.

109. The method of claim 108 wherein the second state comprises the first set of input lines in fluid communication with the plurality of reaction chambers disposed in the microfluidic device and the second set of input lines in a closed state.

110. The method of claim 108 wherein the second state further comprises a set of valves in a closed state preventing fluid flow through a manifold connecting the second set of input lines.

111. The method of claim 107 wherein the first control line is in fluid communication with a first pressure source.

112. The method of claim 111 wherein the second control line is in fluid communication with a second pressure source.

113. The method of claim 107 wherein the microfluidic device comprises an elastomeric material.

114. A microfluidic system comprising:

a carrier comprising:

a plurality of first input ports;

a plurality of first input lines, each of the plurality of first input lines being in fluid communication with one of the plurality of first input ports;

a plurality of second input ports;

a plurality of second input lines, each of the plurality of second input lines being in fluid communication with one of the plurality of second input ports;

a first pressure source; and

a second pressure source

a microfluidic device mounted to the carrier, the microfluidic device comprising:

a plurality of third input lines, each of the plurality of third input lines being in fluid communication with one of the plurality of first input lines;

a plurality of fourth input lines, each of the plurality of fourth input lines being in fluid communication with one of the plurality of second input lines;

a first control line in fluid communication with the first pressure source;

a unidirectional valve operable to obstruct at least a portion of the first control line; and

a second control line in fluid communication with the second pressure source.

115. The microfluidic system of claim 114 wherein the plurality of first input ports are operable to receive a common pressure source.

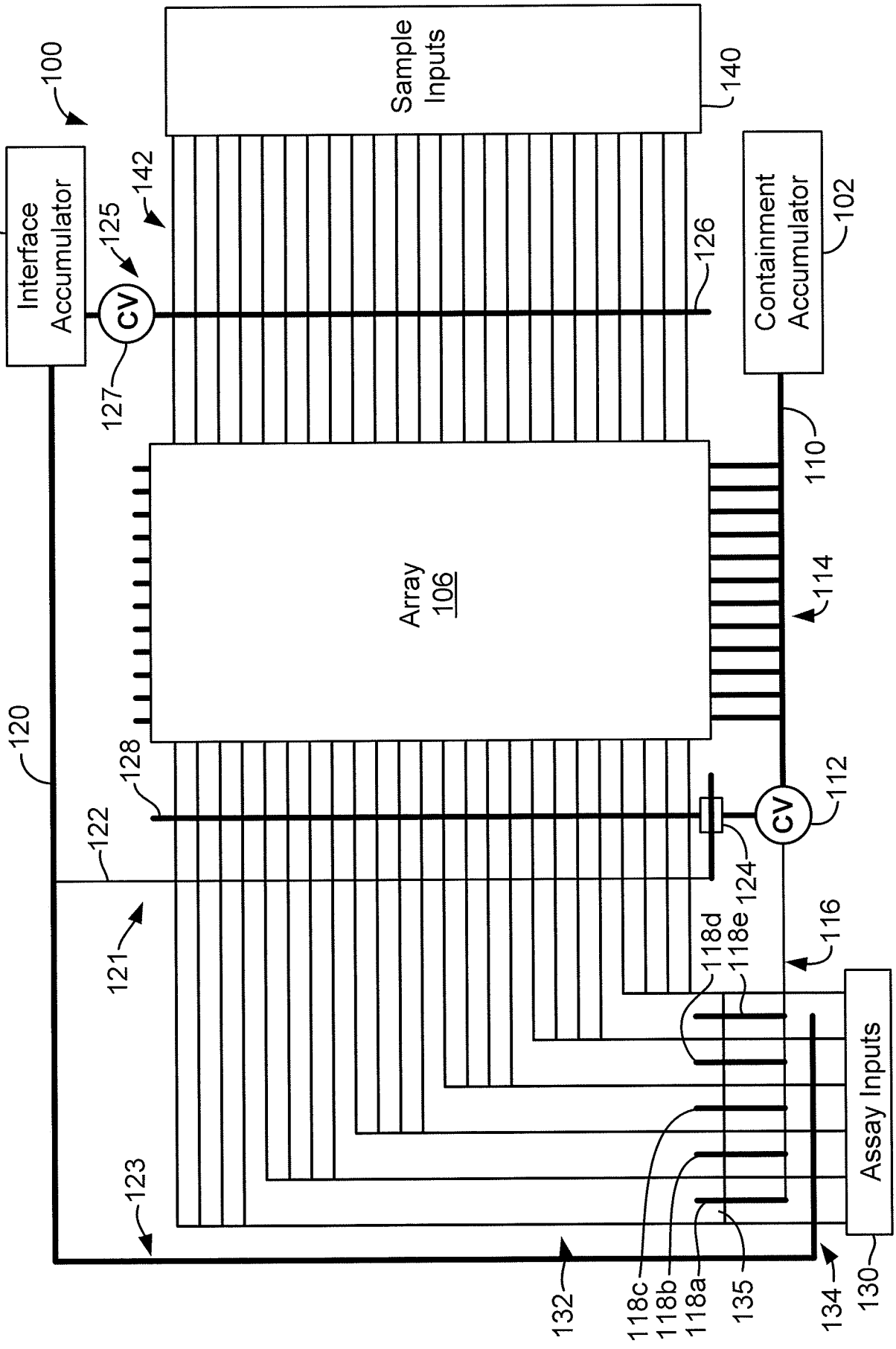
116. The microfluidic system of claim 114 wherein the plurality of second input ports are operable to receive a common pressure source.

117. The microfluidic system of claim 114 further comprising a second unidirectional valve operable to obstruct at least a portion of the second control line.

118. The microfluidic system of claim 114 wherein the microfluidic device further comprises a manifold coupling the plurality of second input lines in the carrier to the plurality of fourth input lines in the microfluidic device.

119. The microfluidic system of claim 118 further comprising a set of valves operable to obstruct portions of the manifold.

120. The microfluidic system of claim 114 wherein the microfluidic device further comprises a plurality of sets of reaction chambers, each reaction chamber of each set of reaction chambers being in fluid communication with one of the plurality of third input lines and one of the plurality of fourth input lines.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Sample Inputs
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104
Interface Accumulator
142
125
CV
127
126
Containment Accumulator
102
110
Array
106
114
120
128
122
112
124
121
116
118d
118e
123
Assay Inputs
132
118c
118b
118a
135
134
130

FIG. 1

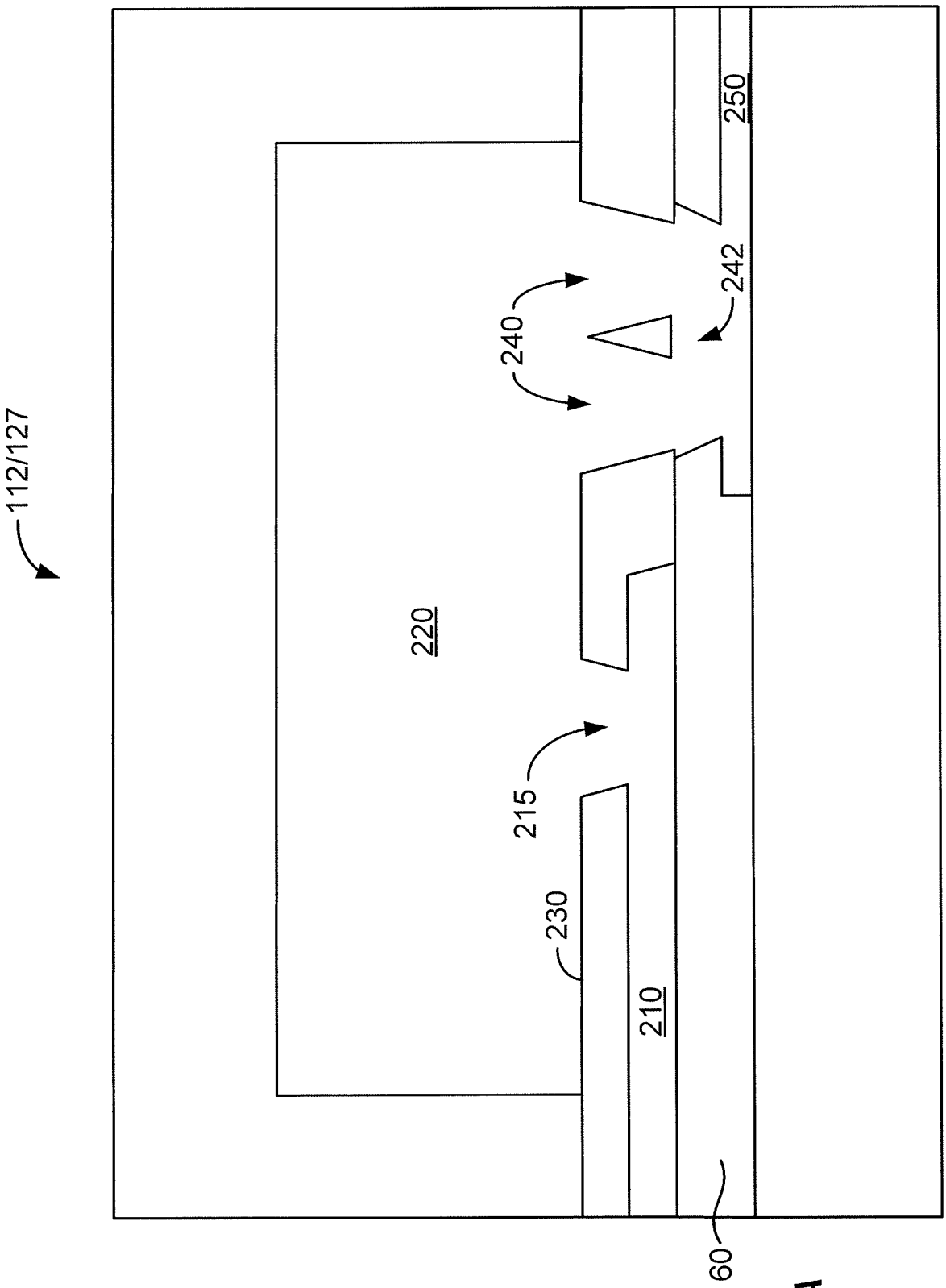

FIG. 2A

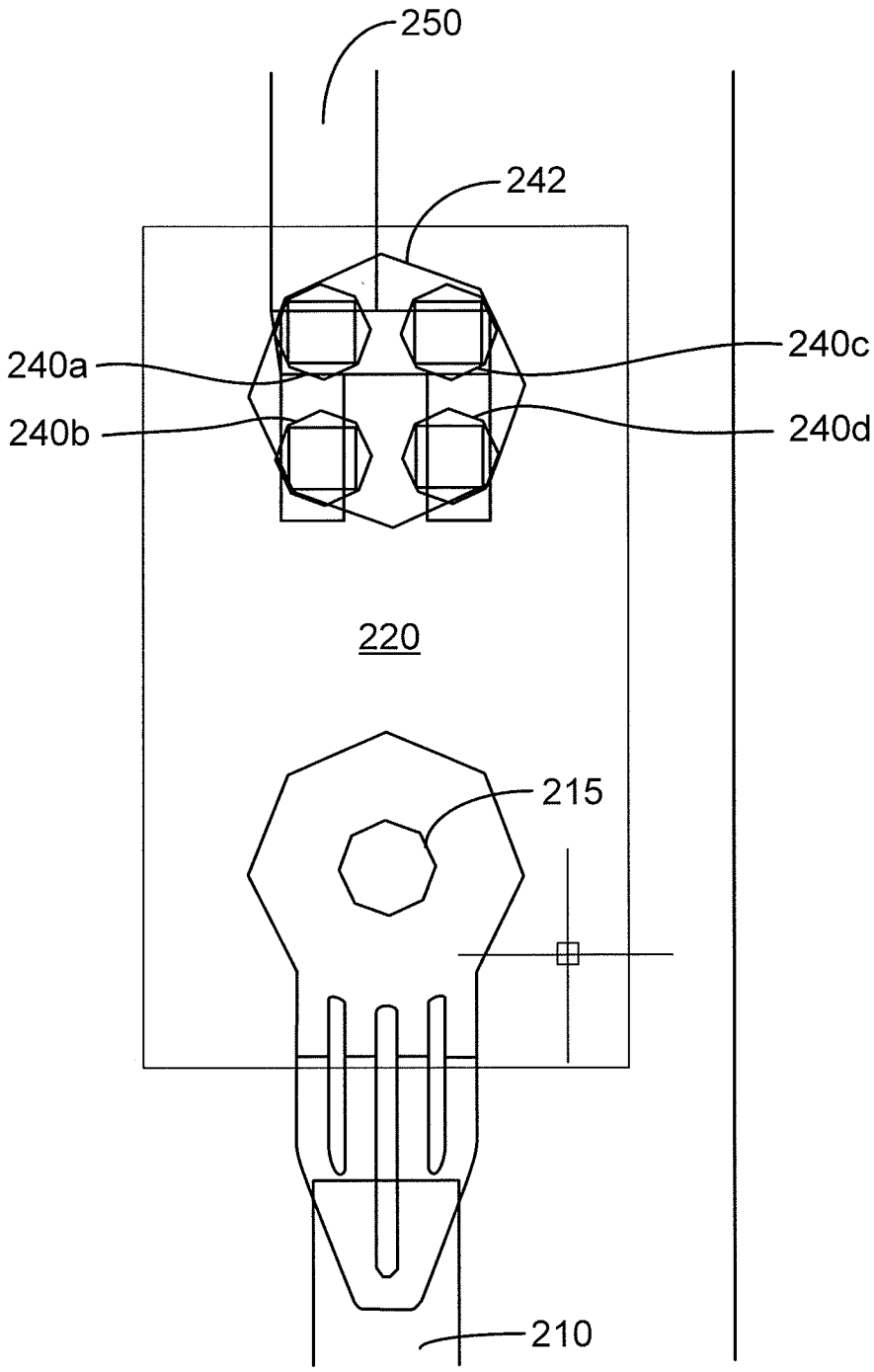

FIG. 2B

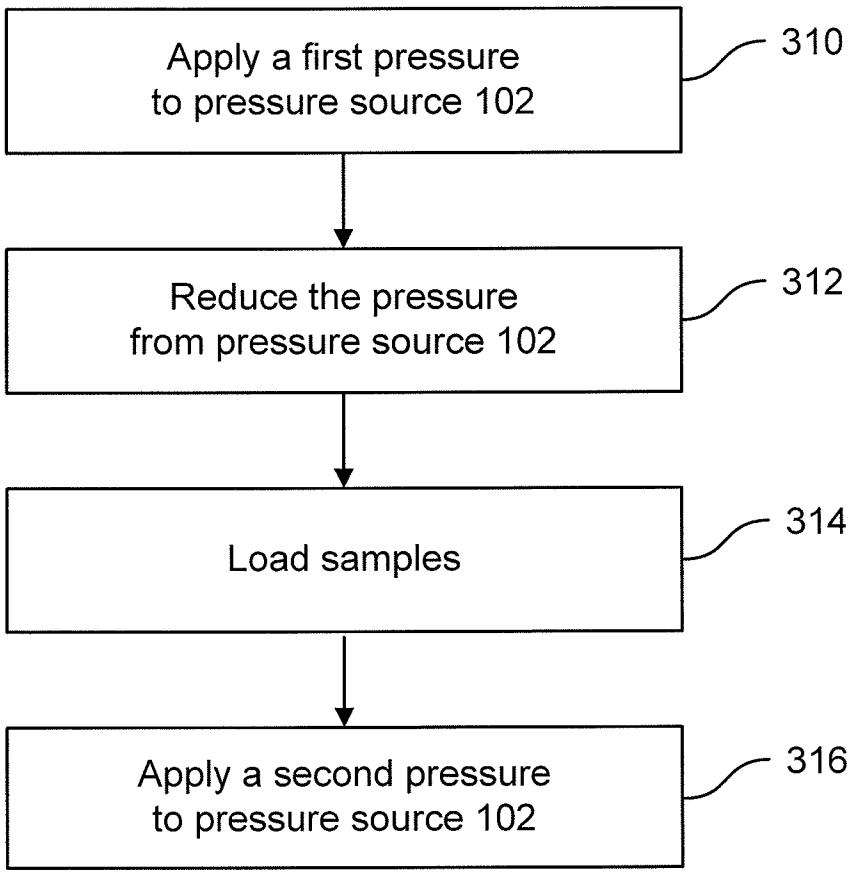
Apply a first pressure to pressure source 102
310
Reduce the pressure from pressure source 102
312
Load samples
314
Apply a second pressure to pressure source 102
316

FIG. 3

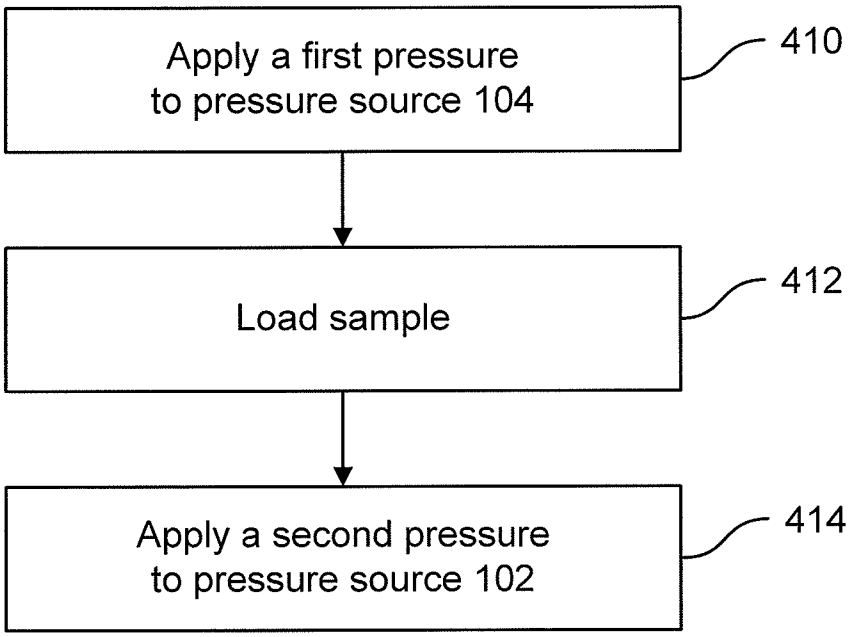
Apply a first pressure to pressure source 104
410
Load sample
412
Apply a second pressure to pressure source 102
414

FIG. 4

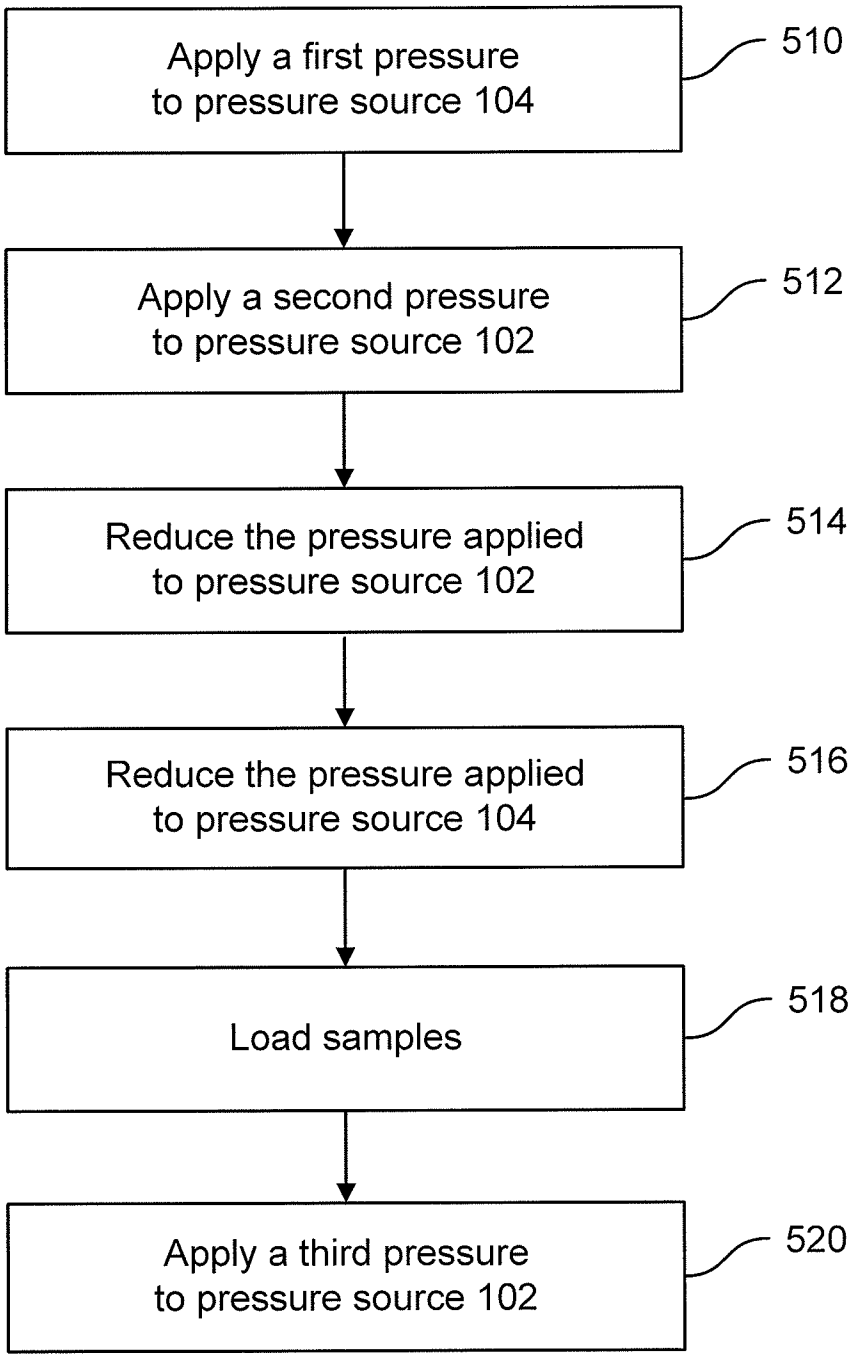
Apply a first pressure to pressure source 104
510
Apply a second pressure to pressure source 102
512
Reduce the pressure applied to pressure source 102
514
Reduce the pressure applied to pressure source 104
516
Load samples
518
Apply a third pressure to pressure source 102
520

FIG. 5

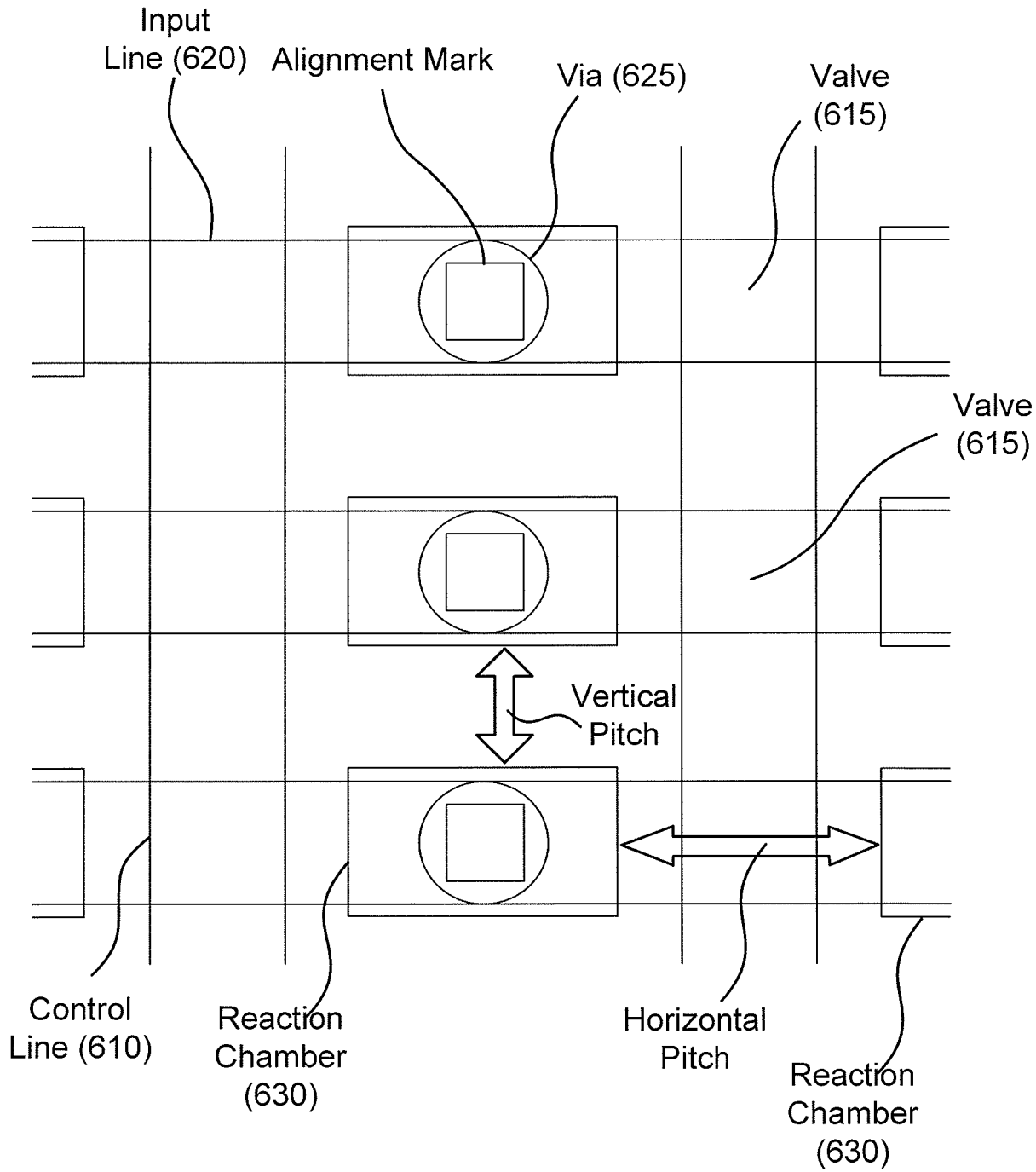
Input
Line (620)
Alignment Mark
Via (625)
Valve
(615)
Valve
(615)
Vertical
Pitch
Control
Line (610)
Reaction
Chamber
(630)
Horizontal
Pitch
Reaction
Chamber
(630)

FIG. 6A

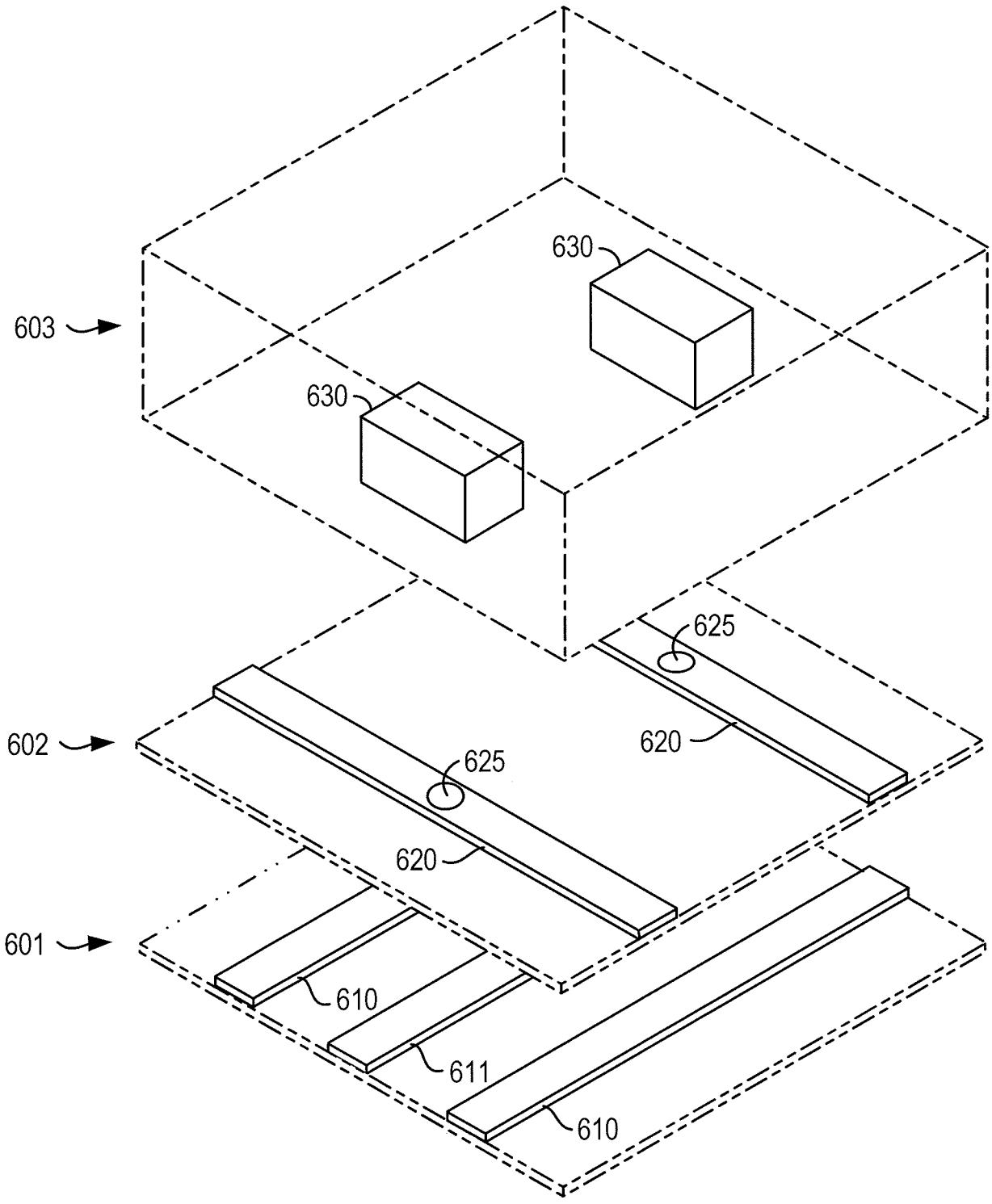

FIG. 6B

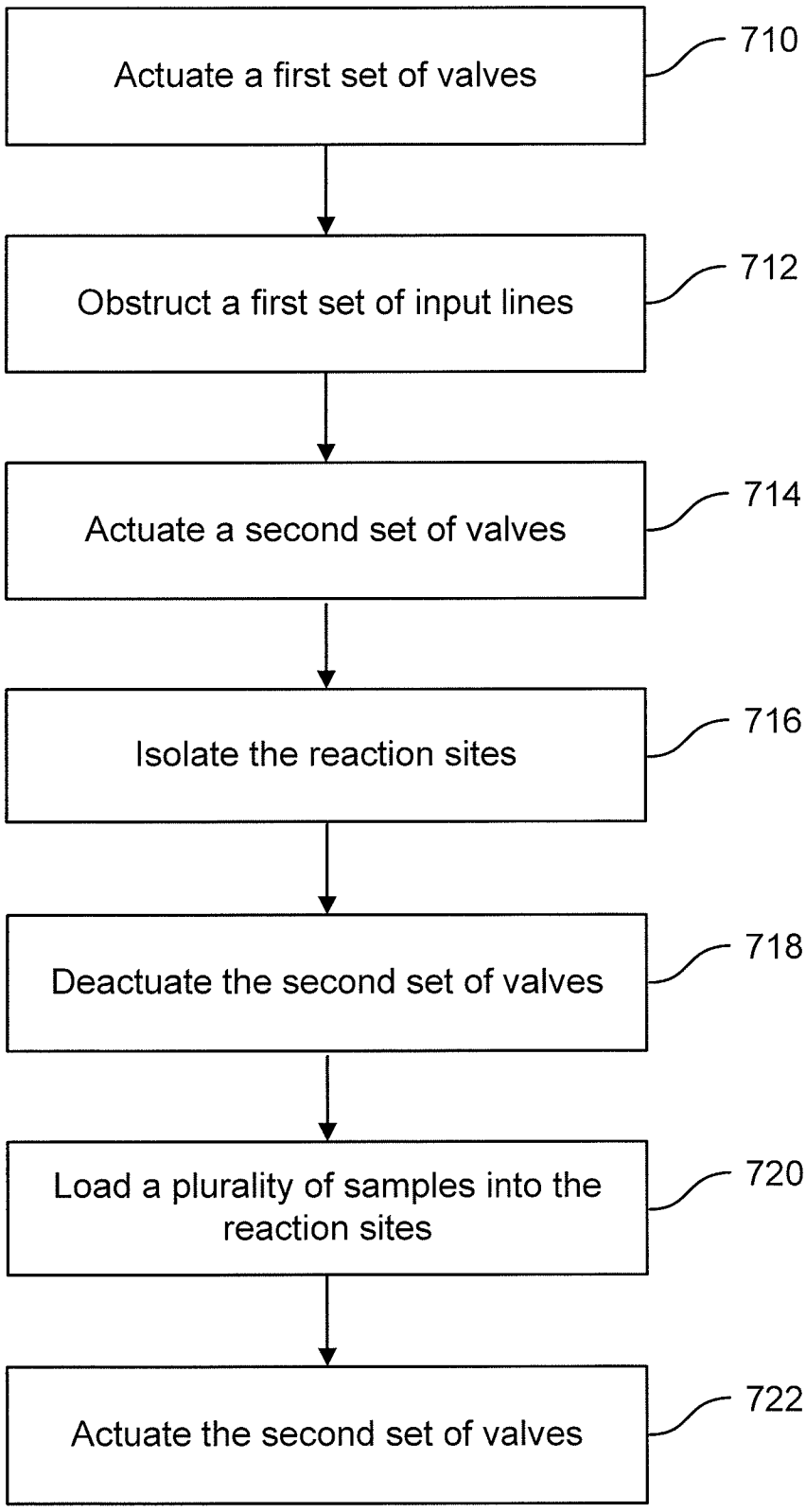
Actuate a first set of valves
710
Obstruct a first set of input lines
712
Actuate a second set of valves
714
Isolate the reaction sites
716
Deactuate the second set of valves
718
Load a plurality of samples into the reaction sites
720
Actuate the second set of valves
722

FIG. 7

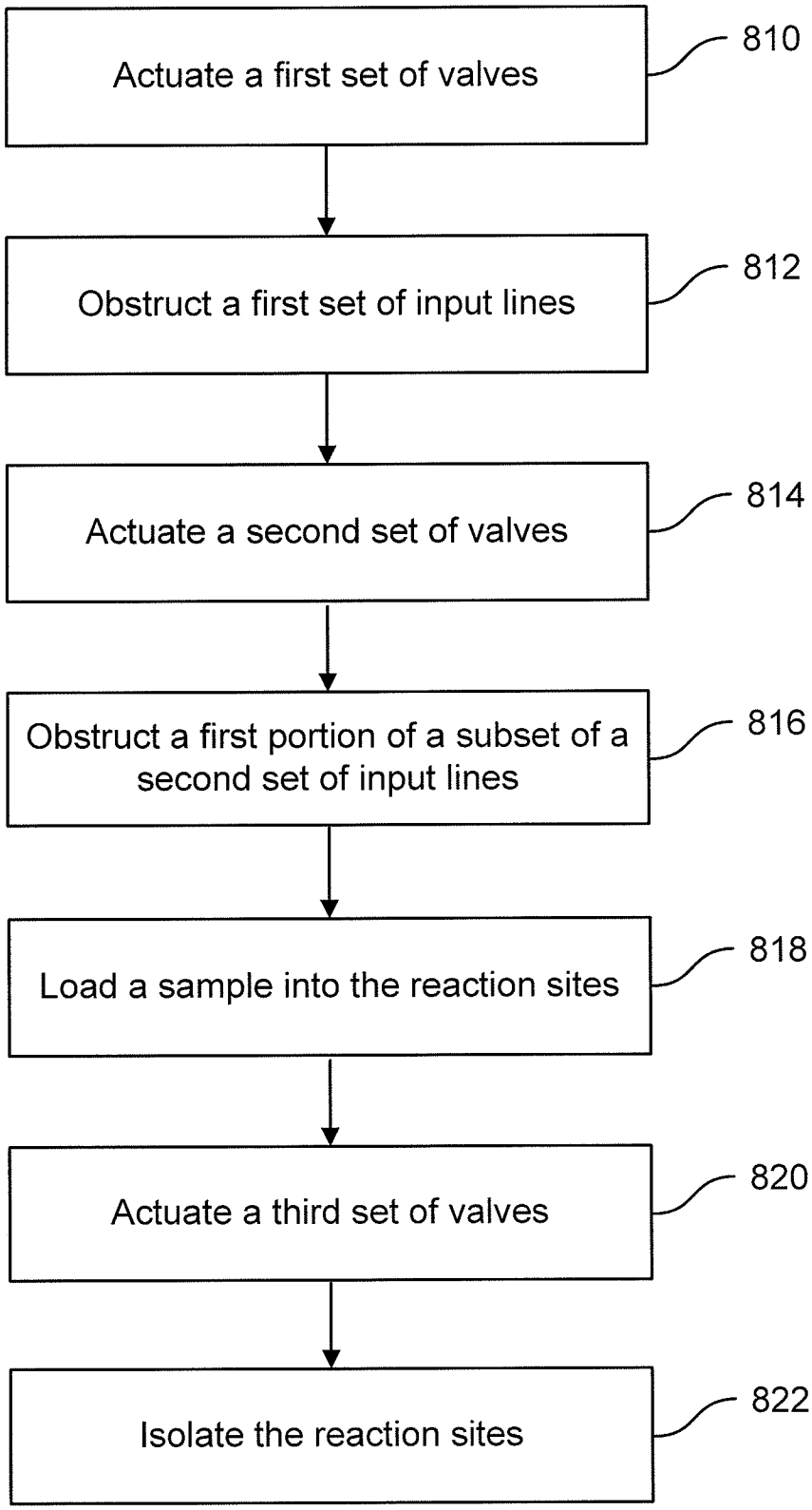
Actuate a first set of valves
810
Obstruct a first set of input lines
812
Actuate a second set of valves
814
Obstruct a first portion of a subset of a second set of input lines
816
Load a sample into the reaction sites
818
Actuate a third set of valves
820
Isolate the reaction sites
822

FIG. 8

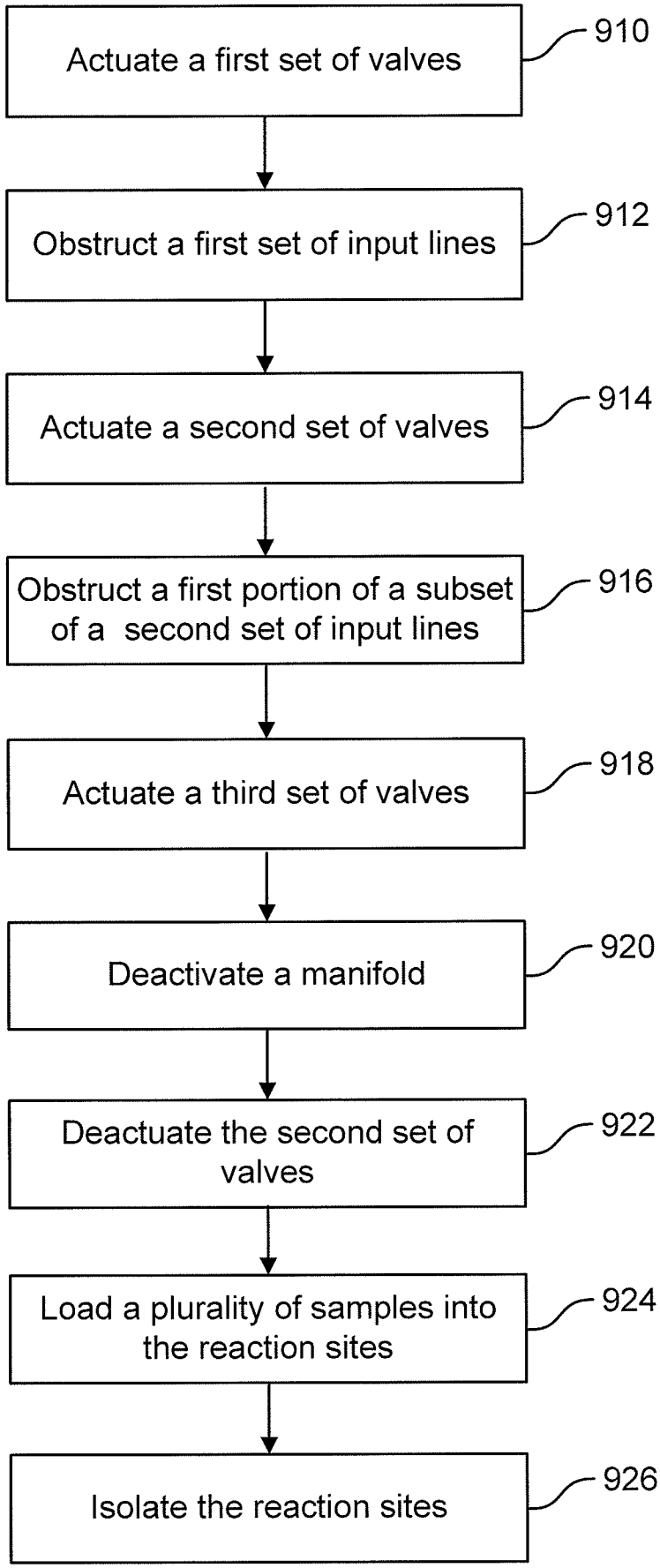
Actuate a first set of valves
910
Obstruct a first set of input lines
912
Actuate a second set of valves
914
Obstruct a first portion of a subset of a second set of input lines
916
Actuate a third set of valves
918
Deactivate a manifold
920
Deactuate the second set of valves
922
Load a plurality of samples into the reaction sites
924
Isolate the reaction sites
926

FIG. 9

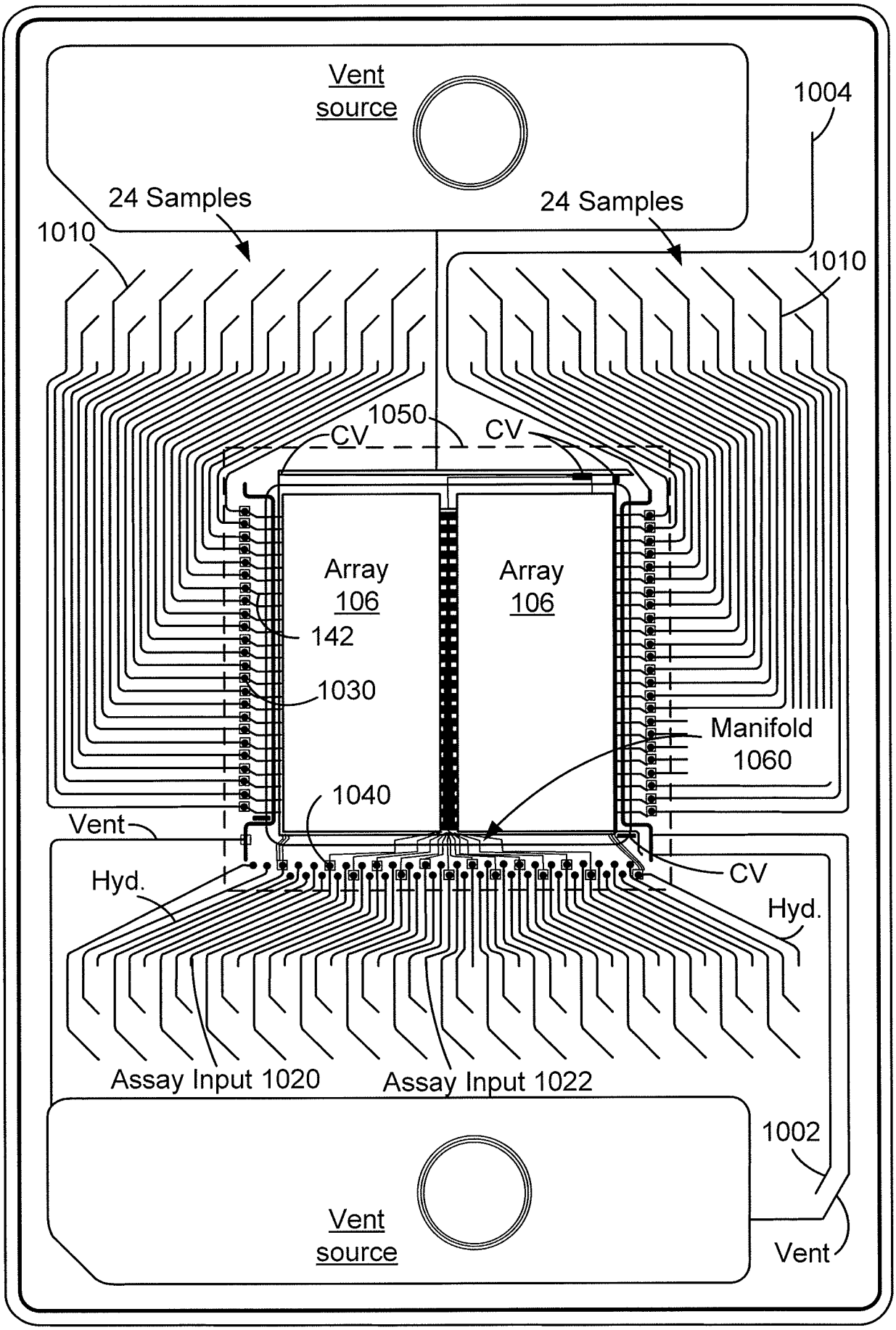
Vent source
1004
24 Samples
24 Samples
1010
1010
1050
CV
CV
Array 106
Array 106
142
1030
Manifold 1060
1040
Vent
Hyd.
CV
Hyd.
Assay Input 1020
Assay Input 1022
1002
Vent source
Vent

FIG. 10

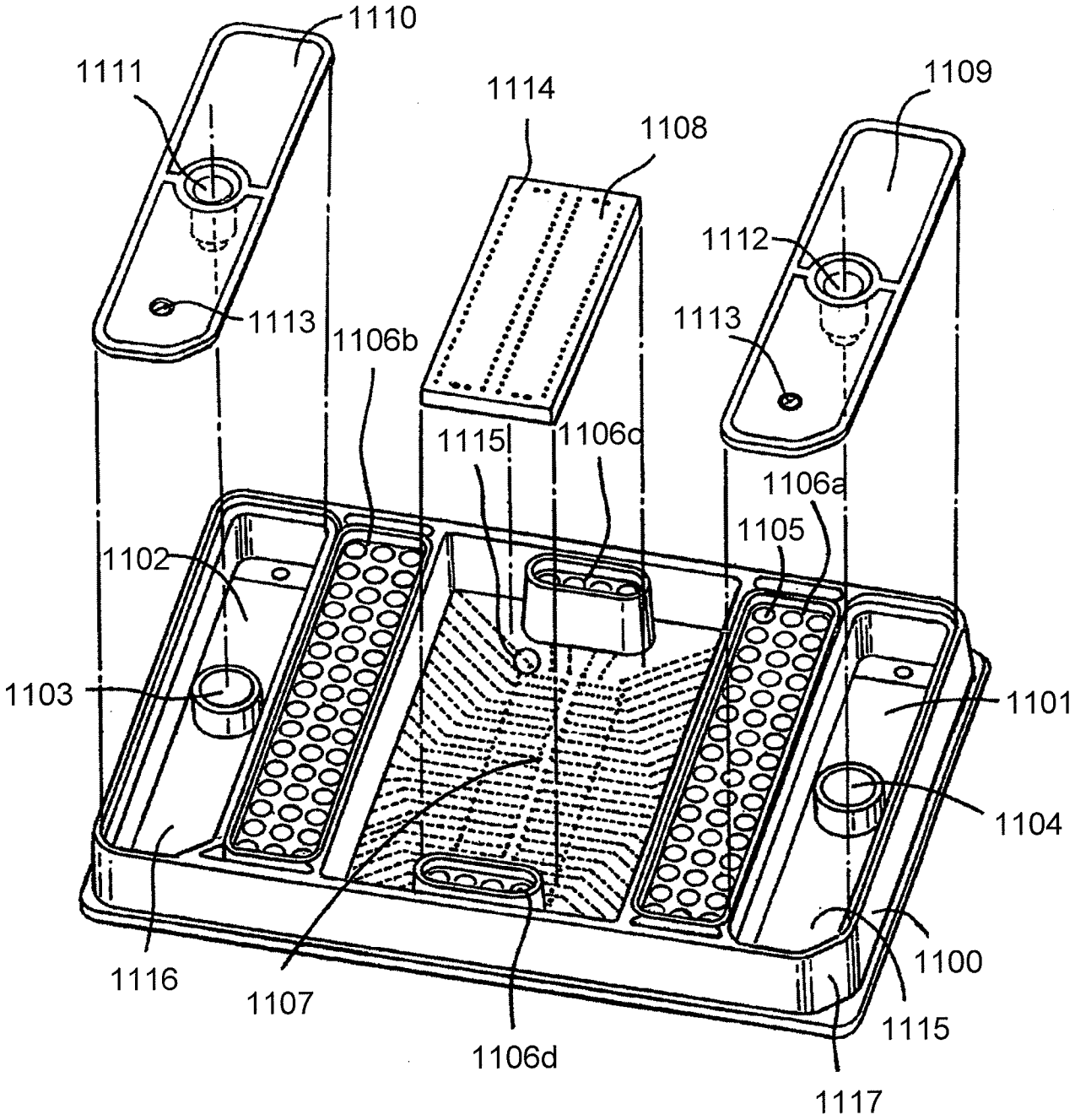
1110
1111
1114
1108
1109
1112
1113
1113
1106b
1115
1106c
1106a
1105
1102
1103
1101
1104
1100
1116
1107
1106d
1115
1117

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/067037

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - B01L 11/00 (2010.01)
USPC - 204/451
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)
IPC(8) - B01L 11/00 (2010.01)
USPC - 204/451

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent, Freepatentsonline, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/0112442 A1 (MAERKL et al) 17 June 2004 (17.06.2004) entire document	107, 111, 113
---		---
Y		1-26, 41-47, 101-106, 108-110, 112
Y	US 2008/0289710 A1 (UNGER et al) 27 November 2008 (27.11.2008) entire document	1-26
Y	US 2007/0237686 A1 (MATHIES et al) 11 October 2007 (11.10.2007) entire document	4-7, 10, 14-16
Y	US 2007/0074972 A1 (NASSEF et al) 05 April 2007 (05.04.2007) entire document	17-26, 41-47, 101-106, 108-110, 112

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
31 March 2010	12 APR 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/067037

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

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

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

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

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

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

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

See supplemental page

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-26, 41-47 and 101-113

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.