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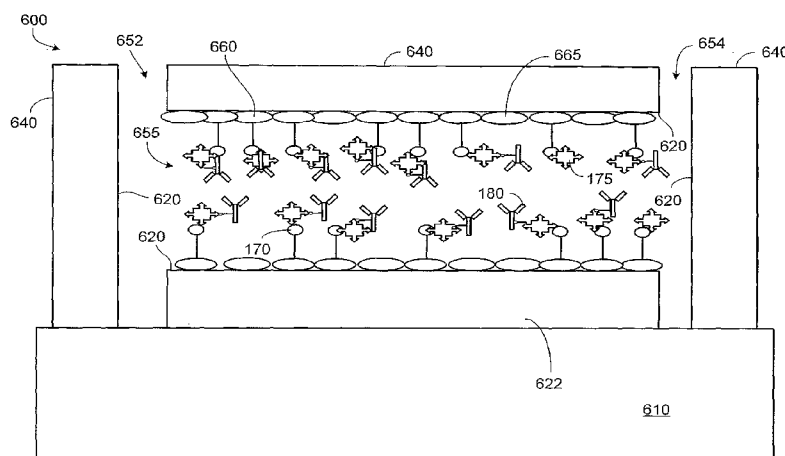


FIG. 1

(57) Abstract: The disclosure relates to the specific capture of an analyte such as a cell from a multi-component sample, such as whole blood. Microfluidic devices contain a channel with a solid phase thermoflowable material with a binding moiety to selectively bind the analyte from the sample. After removing unbound sample components from the channel, the bound analyte can be removed from the channel by converting the thermoflowable material to a liquid phase. The analyte can be detected in the liquid thermoflowable phase, or can be separated from the liquid thermoflowable material outside the channel.



MICROFLUIDIC ANALYTE CAPTURE USING A THERMOFLOWABLE MATERIAL

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Patent Application Serial
5 No. 61/172,943, filed on April 27, 2009, the entire contents of which are hereby
incorporated by reference.

TECHNICAL FIELD

This disclosure relates to the selective capture and release of analytes from
biological samples.

10

BACKGROUND

Isolation of specific cell populations from complex mixtures such as whole blood
has significant utility in both clinical practice and basic medical research. A variety of
approaches may be used to separate cells from a heterogeneous sample. For example,
some techniques can use functionalized materials to capture cells based on cell surface
15 markers that are particular to the target cell population using specific capture moieties
present on in the functionalized materials. Such capture moieties can include antibodies
or other specific binding molecules, such as aptamers or selectins.

A limitation common to many cellular capture techniques is the limited ability to
recover captured cells following isolation. For example, the use of antibody tags on cells
20 or on surfaces is a common way to separate lymphocyte subpopulations. However,
techniques that achieve separation based on size and density are generally unable to
provide adequate resolution between subpopulations of lymphocytes. The ability to
release cells following their specific capture would enable simple and direct nonoptical
detection of the target cell population with much simpler methods and equipment. This
25 capability of releasing specific captured cells may improve the accuracy of target
detection, and can lower associated costs, processing time, and sample manipulation.
Some cell-capture and analysis techniques employ harsh chemical conditions—including
very high or low pH environments—and/or significant variations in temperature or ionic
strength. Such conditions are not compatible with elution of viable cells.

SUMMARY

The disclosure relates to methods and devices for the selective isolation of analytes from a multicomponent sample. The methods and materials described allow the release of specifically captured cells bound to a surface that is functional at a physiologic pH, ionic strength and temperature, and which does not exert undue chemical or mechanical stresses on the cells of interest. The devices can include microfluidic channels configured to selectively capture one or more components from a blood sample. The novel methods and devices are based on the discovery that biological cells such as leukocytes can be selectively isolated from a blood sample by first binding the biological cells to an antibody-functionalized solid surface comprising a thermoflowable material (e.g., gelatin or agar) in a microfluidic channel, then removing unbound blood components from the sample, releasing the captured biological cells by melting the thermoflowable material and removing the captured biological cells from the channel with the liquid thermoflowable material, and finally counting or analyzing the captured biological cells outside the chamber. For example, whole blood can be captured on a solid thermoflowable material containing cell binding moieties (e.g., antibodies) within the microchannel. The thermoflowable material can then be heated to melt the thermoflowable material, and the liquid thermoflowable material can be transferred with the captured cells to a cell counting chamber to release and detect the captured cells.

Methods of isolating a binding analyte from a multi-component sample can include contacting the sample with a solid phase thermoflowable material having a specific binding moiety, retaining a binding analyte to the specific binding moiety on the solid phase thermoflowable, removing unbound components from the sample, converting the solid thermoflowable material with the binding analyte attached thereto into a liquid phase thermoflowable material and then detecting the binding analyte. In one example, the binding analyte is a cell, such as a CD4⁺ leukocyte separated from a whole blood sample. In some embodiments, the thermoflowable material can comprise a protein such as gelatin (e.g. gelatin A or gelatin B), or can be agar. The thermoflowable material can be a solid below a first melting temperature. The multi-component sample can be contacted with the solid thermoflowable material at or below the melting temperature, and the solid thermoflowable material can be converted to the flowable liquid by raising the

temperature of the solid thermoflowable material above the melting temperature after binding the binding analyte to the solid thermoflowable material.

Optionally, the method can further include contacting the solid thermoflowable material with the specific binding moiety under conditions effective to bind the specific binding moiety to the thermoflowable material. The solid thermoflowable material can be contacted with the specific binding moiety before contacting the specific binding moiety and the solid thermoflowable material with the multi-component sample.

For example, a biological cell can be isolated from a multi-component liquid sample such as whole blood. The method can include contacting the sample with a solid thermoflowable material (e.g., comprising gelatin or agar) and an antibody within a chamber under conditions effective to selectively bind the biological cell from the sample to the antibody; removing unbound components from the sample within the chamber; converting the solid thermoflowable material bound to the biological cell to a flowable liquid within the chamber; and detecting the biological cell in the flowable liquid thermoformable material. The thermoflowable material can be a solid below a first melting temperature. The multi-component sample can be contacted with the solid thermoflowable material at or below the melting temperature, and the solid thermoflowable material can be converted to the flowable liquid by raising the temperature of the solid thermoflowable material to second temperature greater than the melting temperature after binding the binding analyte to the solid thermoflowable material. The biological cell can be released from the channel by removing (e.g., flowing) the liquid thermoflowable material from the channel. The biological cell can be viable at the first and second temperatures. Optionally, the flowable liquid can be removed from the chamber prior to detecting the biological cell in the flowable liquid thermoformable material, and the biological cell can be detected outside the chamber.

The methods can be performed in a microfluidic analyte capture device. The device can include a channel having an inner surface defining a fluid flow path of substantially uniform cross-sectional area extending from an inlet to an outlet. The inner surface can include a thermoflowable material in a solid phase comprising a binding moiety specific for an analyte. The microfluidic analyte capture device can include a heating surface configured to melt the thermoflowable material within the channel, for

example by heating the thermoflowable material above a melting point of the thermoflowable material to convert the thermoflowable material from the solid phase to a liquid phase within the channel. The binding moiety can be an antibody and the analyte is an analyte biological cell that specifically binds to the antibody. The analyte biological cells can be CD4+ biological cells

Optionally, the microfluidic analyte capture device can be part of an integrated cell counting chip. The chip can include a first chamber in fluid flow communication with the inlet of the channel, the first chamber configured to retain monocyte cells from the analyte. The chip can also include a second chamber in fluid flow communication with the outlet of the channel, the second chamber configured to count analyte biological cells in the analyte.

The term "thermoflowable material" refers to a material that is converted from a solid to a liquid having a viscosity permitting the liquid material to flow through a microchannel at a melting temperature. Preferably, the thermoflowable material can be reversibly converted from a solid to a liquid phase in a temperature dependent manner. The temperature at which the thermoflowable material is converted from a solid to a liquid preferably permits detection of the binding analyte within the liquid thermoflowable material. In addition, the thermoflowable material is preferably capable of being attached to a binding moiety, does not prevent a binding reaction between the binding moiety and a binding analyte in a microfluidic channel. One example of a thermoflowable material is gelatin. Other examples include agar.

By "binding moiety" is meant a chemical species to which an analyte binds. A binding moiety may be a compound coupled to a surface or the material making up the surface. Exemplary binding moieties include antibodies, antibody fragments (e.g., Fc fragments), oligopolypeptides, nucleic acids, cellular receptors, ligands, aptamers, MHC-peptide monomers or oligomers, biotin, avidin, oligonucleotides, coordination complexes, synthetic polymers, pentamers and carbohydrates.

The term "chamber" is meant to include any designated portion of a microfluidic channel, e.g., where the cross-sectional area is greater, less than, or the same as channels entering and exiting the chamber.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a cross-sectional view along the axis of a microfluidic cell capture chamber.

Figure 2A and 2B are cross-sectional and top views (respectively) of a glass slide as a step in forming a microfluidic cell capture chamber.

Figures 3A and 3B are cross-sectional and top views (respectively) of a gelatin material deposited on a glass slide as a step in forming a microfluidic cell capture chamber.

Figures 4A and 4B are cross-sectional and top views (respectively) showing a micro-transfer molding process step in forming a microfluidic cell capture chamber.

Figures 5A and 5B are cross-sectional and top views (respectively) of a formed gelatin material deposited on a glass slide as a step in forming a microfluidic cell capture chamber.

Figure 6A and 6B are cross-sectional and top views (respectively) of a gelatin material and polymer wall material defining a microfluidic channel, deposited on a glass slide as a step in forming a microfluidic cell capture chamber.

Figures 7A – 7D are a cross-sectional views showing operation of a microfluidic cell capture chamber.

Figure 8 is a fluorescence image showing cells captured on a functionalized gelatin surface.

Figure 9 is a schematic top view of an integrated cell capture and counting system.

Figures 10A-10C are schematic top views showing operation of an integrated cell capture and counting system.

DETAILED DESCRIPTION

Microfluidic analyte capture devices include a solid phase thermoflowable material with a specific binding moiety selected to selectively bind to a binding analyte in a sample (e.g., biological cells in a whole blood sample). As described herein, captured binding analytes can be released by converting the thermoflowable material from a solid phase to a liquid phase. The binding analyte can subsequently be detected in the liquid

thermoflowable material, or can be separated from the thermoflowable material prior to detection. The microfluidic analyte capture devices can be used, for example, to specifically capture and release leukocyte sub-populations from whole blood samples.

Figure 1 is a cross-sectional view of one example of a microfluidic analyte capture device 600. The microfluidic analyte capture device 600 includes a channel 655 with an inner surface 620 defining a fluid flow path of substantially uniform cross-sectional area extending from an inlet 652 to an outlet 654.

The channel 655 can be formed by a suitably configured polymer (e.g., polydimethylsiloxane, "PDMS") bound to the surface of a glass slide 610, around a solid thermoformable material 622 to form wall portions 640 of the inner surface 620 along the length and width of the channel 655. Polymer wall portions 640 can be horizontally spaced apart from the solid thermoformable material 622 to form the inlet 652 and outlet 654, and be vertically spaced apart from the solid thermoformable material 622 to form a suitable channel 655 depth. Alternatively, the channel 655 can be formed by coating a gelatin or other thermoflowable material 622 on other substrate materials (e.g., polymer materials) and forming the wall portions from one or more materials suitable for an intended use. The inner surface 620 includes specific binding moieties such as neutravidin 175, biotin 170 and antibodies 180 (e.g., biotin conjugated to bovine serum albumin, or "BSA"). The inner surface 620 can also include material to prevent or reduce non-specific binding from a sample in the channel 655, such as bovine serum albumin (BSA) 665.

The inner surface 620 includes the thermoflowable material 622 (e.g., gelatin or agar) in a solid phase. The thermoflowable material 622 is preferably thermoreversibly flowable, meaning the material can be melted, reformed and melted by adjusting the temperature above and below the melting temperature. The thermoflowable material 622 is preferably a gelatin having a molecular weight of about 5,000 – 20,000 g/mol, and most preferably about 20,000 g/mol. This allows gelatin to be a gel at room temperature ($T=25^{\circ}\text{C}$) and a liquid at an elevated temperature ($T_{\text{melt}}=37^{\circ}\text{C}$ for 11% wt gelatin). The gelatin layer can be molded into the desired shape and thickness, using for example microcontact printing. Thermoflowable materials 622 can comprise other materials besides gelatin, such as polyethylene glycol (PEG). The thermoflowable material 622

includes a binding moiety specific for an analyte. Figures 2A – 6B show an example of a method for forming a microfluidic analyte capture device 100, described in more detail in Example 1 below. The microfluidic analyte capture device 100 can be subsequently functionalized (e.g., by introducing binding moieties to the inner surfaces) to form the functionalized microfluidic analyte capture device 600 shown in Figure 1.

A microfluidic analyte capture device 100 can be formed on a glass slide 110, shown in a cross-sectional view in Figure 2A along dashed line 180 shown in the top view of the glass slide 110 in Figure 2B. Referring to Figures 3A and 3B, a thermoformable material 122 can be deposited in a liquid phase onto the surface of the glass slide 110 heated above a melting point of the thermoformable material 122. For example, about 20 microliters of liquid gelatin (e.g., 37 degrees C) can be pipetted onto the top of a heated (e.g., 40 degrees C) glass slide. Figure 3A is a cross-sectional view of the glass slide 110 and deposited thermoformable material 122, along dashed line 182 shown in the corresponding top view of Figure 3B.

Referring to the cross-sectional view of Figure 4A, taken along dashed line 184 shown in the top view of Figure 4B, a silanized PDMS template 130 is pressed onto the liquid phase thermoformable material 122 until the PDMS template 130 contacts and adheres to the surface of the glass slide 110. The thermoformable material 122 is subsequently converted to a solid phase, for example by cooling to a temperature (e.g., 4 degrees C) below the melting temperature of the thermoformable material 122.

As shown in the cross-sectional view of Figure 5A taken along dashed line 186 on corresponding top view (Figure 5B), the PDMS template is removed from the glass slide, leaving a solid thermoformable material 122 in a spatially defined area of the surface of the glass slide 110. For example, the PDMS template and excess gelatin (i.e., gelatin outside the mold cavity of the PDMS template) can be manually removed from the surface of the glass slide with a sharp tool (e.g., a razor blade).

Finally, a channel 155 can be formed around the solid thermoformable material 122 as shown in Figures 6A-6B. Figure 6A is a cross-sectional view of the microfluidic device 100 along dashed line 188 shown in a top view of the microfluidic analyte capture device 100 in Figure 6B. The microfluidic device 100 includes the channel 155 with an inner surface 120 defining a fluid flow path 150 of substantially uniform cross-sectional

area extending from an inlet 152 to an outlet 154. The channel 155 can be formed in one or more steps. Formation of the channel 155 can include bonding a suitably configured polymer (e.g., PDMS) to the surface of the glass slide 110, around the solid thermoformable material 122 to form wall portions 140 of the inner surface 120 along the length and width of the channel 155. Polymer wall portions 140 can be horizontally spaced apart from the solid thermoformable material 122 to form the inlet 152 and outlet 154, and be vertically spaced apart from the solid thermoformable material 122 to form a suitable channel 155 depth.

The microfluidic analyte capture device 600 shown in Figure 1 is formed by incorporating a specific binding moiety with the solid thermoflowable material 622. Binding moieties may be attached to channels using methods known in the art. The method employed will depend on the binding moiety and the material used to construct the device. Examples of attachment methods include non-specific adsorption to the surface, either of the binding moiety or a compound to which the binding moiety is attached or chemical binding, e.g., through self assembled monolayers or silane chemistry. For example, a specific binding moiety can be bound to or incorporated into the solid thermoflowable material 122 (Figures 2A-6B). A solution containing one or more specific binding moieties (e.g., one or more antibodies) can be passed through the channel 155. For example, a series of solutions can be passed through the channel 155 to bind a specific binding antibody to the solid thermoflowable material 122. First, a biotinylated bovine serum albumin (BSA) at a suitable pH (e.g., pH 5) can be flowed through a channel 155 containing a solid thermoflowable material 120 comprising gelatin. The volume of the biotinylated BSA passed through the channel 155 can be about four to five times the volume of the microfluidic channel (e.g., about 50 microliters). Second, a neutravidin (e.g., 100 micrograms/mL) in PBS (e.g., pH 5) is passed through the channel 155. Third, a solution of biotinylated antibody (e.g., 20 micrograms/mL) is passed through the channel, preferably in a total amount of about four to five times the volume of the channel 155, thereby coating the channel 155 with a specific binding moiety to form a functionalized solid thermoformable material (e.g., agar or gelatin).

Figures 7A-7D show the operation of a microfluidic analyte capture device 700. As shown in Figure 7A, a multi-component sample (e.g., whole blood) containing one or more binding analytes (e.g., a first biological cell 790) and other components (e.g., a second biological cell 785) can be injected 701 into the inlet of the channel and passed through the channel, exiting 702 from the outlet. The channel contains a solid thermoflowable material 722 comprising a specific binding moieties 775, 780 (e.g., antibodies). The sample is passed through the channel under conditions (e.g., temperature, pressure and/or flow rate) effective to selectively bind the binding analyte 790 from the sample to one or more of the specific binding moiety, without binding the other components (e.g., without binding the second biological cell 785 lacking a surface receptor for the specific binding moieties 775, 780). At least two variables can be manipulated to control the shear stress applied to the channel: the cross sectional area of the chamber and the fluid pressure applied to the chamber. Other factors may be manipulated to control the amount of shear stress necessary to allow binding of desired analytes and to prevent binding of undesired analytes, e.g., the binding moiety employed and the density of the binding moiety in the channel.

Table 1 (below) provides exemplary cell populations, cell surface markers appropriate for the methods and devices described, and the corresponding shear stresses necessary to isolate the indicated cells from a blood sample.

Table 1

Cell-Type	Capture Shear [dynes/cm ²]	Wash [dynes/cm ²]	Capture Molecule	Purity*	Yield
<u>Neutrophil</u>	0.4-0.45	1.5-2.0	Anti- CD66b	> 99	80%
<u>Monocyte</u>	0.7-0.75	2.25-3.0	Anti-CD 14	91 %	
			Anti-CD33		
			Anti-CD36		
Lymphocyte	1.3-1.7	2.5-3	Anti-CD2	95%	80%
			Anti-CD3		
			Anti-CD4		
			Anti-CD8		
Circulating Tumor Cells	8-9	35	Anti-EpCAM	80%#	60%
<u>Neutrophils</u>	1-7	-	<u>E, P Selectins</u>	70 %	80 %
HIV Specific T- cells	0.082	N/A	<u>HLA A2-SL9 pentamer</u>	>99%	N/A
Any-disease specific T-cell	0.07-0.1		<u>Pentamer</u>		

*blood from healthy donor

#blood from patients with cancer stage III-V

After binding analyte 790 to the specific binding moieties 775, 780 within the channel, unbound components from the sample are removed from the sample in the channel. For example, as shown in Figure 7B, non-specifically bound cells (e.g., second biological cell 785) are washed off using BSA in PBS. The flow rate of the washing solution can be faster (e.g., seven times faster) than the flow rate at which the sample was passed through the channel in Figure 7A.

As shown in Figure 7C, the solid thermoflowable material 722 bound to the binding analyte can be converted to a flowable liquid thermoformable material 722 after removing non-specific bound cells. For example, the channel can be heated above a melting point of the thermoformable material 720 to convert the thermoformable material 720 into a flowable liquid. Optionally, the binding analyte 790 and the specific binding moieties 775, 780 can be removed from the channel with the flowable liquid thermoformable material 722. The binding analyte 790 bound to the specific binding moieties 775, 780 can be removed from the channel by converting the thermoflowable material 722 from a solid to a flowable liquid within the channel, and removing the liquid flowable thermoflowable material 722 from the channel. Alternatively, in one example,

shown in Figure 7D, a portion of the specific binding moieties 775, 780 and/or the binding analyte 790 can be retained within the channel after removing the flowable liquid thermoformable material 722 (Figure 7C).

The binding analyte 790 can be detected in the flowable liquid thermoformable material by any suitable method. For example, the binding analyte 790 can be removed from the channel in the liquid flowable thermoformable material 722 and collected in a microfuge tube for further analysis. In one particular example, a population of lymphocyte cells can be captured from whole blood by attachment to antibodies in a thermoflowable material (e.g., gelatin or agar). Figure 8 shows a fluorescence image of cells on an antibody-functionalized gelatin material, stained with CD3-AF647, CD4-AF488 and DAPI. CD3 is a general marker for lymphocytes, and CD4+ T-cells are estimated to comprise about 90% of the captured lymphocyte population.

In one embodiment, the chamber is coated with binding moieties that bind to a cell surface marker of a desired cell population. Through application of an appropriate shear stress, the methods described result in the selective isolation of cells expressing these cell surface markers at a specific concentration. The applied shear stress is preferably great enough to prevent (or substantially reduce) binding of undesired cells that contain the cell surface marker at a concentration lower than the desired population of cells and other non-specific binding interactions. The methods described result, for example, in the isolation of 50%, 60%, 70%, 80%, 90%, 95%, 99%, or 100% of the desired analyte, e.g., cells, in a sample while retaining, for example, less than 20%, 10%, 5%, or 1% of undesired analytes. In addition, while analytes that bind are described as being "desired" and analytes that do not bind are described as being "undesired," either type or both types of analyte may be of actual interest in a particular experiment. For example, the methods described may be used to isolate analytes that either bind to the device or flow through the device channel.

The microfluidic analyte capture device can be part of a cell counting chip system 900, shown in Figure 9, that can include a microfluidic analyte capture channel 920, such as the microfluidic analyte capture channel described above in Figure 1. The inlet to the microfluidic analyte capture channel can receive a modified sample. For example, a whole blood sample 960 can first be passed through a chamber configured to reduce the

concentration of monocytes in the sample, such as the monocyte depletion chamber 910.

The monocyte depletion chamber 910 is described in provisional U.S. patent application 61/143,316, filed January 8, 2009, incorporated by reference herein in its entirety. Briefly, monocytes can be removed from a sample by immunoaffinity isolation.

5 For example, the monocyte depletion chamber 910 can be configured to contact a sample with antibodies such as anti-CD14 MY4 clone to immobilize monocytes from a lysed blood sample or anti-CD14 RMO52 clone. The flow rate of the sample through the monocyte depletion chamber 910 can be selected to maximize monocyte capture. For example, a cell capture of up to 150 cells/square mm can be achieved while applying a
10 shear stress of lower than about 0.5 dyn/square cm. A solution of anti-CD14 antibody at a concentration of about 75 microgram/mL can be passed through the monocyte depletion chamber 910 prior to the sample to coat the sample contact portion of the monocyte depletion chamber 910 with the anti-CD14 antibody.

The cell counting chip system 900 further comprises a means for heating the
15 thermoflowable material within the channel, such as the on-chip heating strips 930. Other examples of means for heating the thermoflowable material can include: flowing pre-heated fluid with the fluid heated outside the device, focused and localized infra-red based heating from outside the device, focused and localized laser based heating from outside the device, focused microwave heating from outside the device, focused magnetic
20 heating from outside the device, or any combination thereof.

Materials used to introduce specific binding antibodies to the solid thermoflowable material within the channel, such as a solution of the specific binding antibodies, can be passed through the chamber and removed 964 from the cell counting chip system 900 using the valve 950. Flowable liquid thermoflowable material
25 containing a binding analyte can be removed from the microfluidic analyte capture channel 920, passed through the valve 950 and injected 962 into a chamber 940 for counting cells in the liquid thermoflowable material.

One example of a chamber 940 for counting biological cells, such as off-chip Coulter counter, off-chip laser based flow cytometer, on-chip microfluidic coupled
30 electrical impedance based cell counter, on-chip microfluidic coupled laser based cell counter, and any combination thereof.

The cell counting chip system can be used in combination with other devices such as fluids, pumps, and/or detectors. Devices may also be combined with reagents, e.g., lysis reagent, labeling reagents, and instructions for use, e.g., for disease diagnosis.

Figures 10A-10C show steps in the operation of a cell counting chip system 1000, which can be identical to the cell counting chip system 900 shown in Figure 9. If required, one or more solution(s) 1062 containing specific binding moieties can be passed through a monocyte depletion chamber 1010 and a microfluidic analyte capture channel 1020, and diverted at a valve 1050 and out of the cell counting chip system 1000. The solution(s) 1062 can include antibodies that are passed through a cell counting chip system 1000 under conditions effective to bind the antibodies to the sample-contact surfaces therein.

Referring to Figure 10B, a whole blood sample 1064 can be introduced to a monocyte depletion chamber 1010, which depletes CD4⁺ monocytes in the blood sample 1064 (*See, e.g.*, 61/143,316, filed January 8, 2009). The monocyte-depleted blood sample 1066 passes through the microfluidic analyte capture channel 1020, where binding analytes (e.g., biological cells) selectively bind to specific binding moieties and are retained attached to the solid thermoflowable material within the channel 1020. The whole blood sample 1064 is flushed through the cell counting chip system 1000, removing other sample components that are not bound to the specific binding moieties and leaving the binding analytes within the channel 1020.

Referring to Figure 10C, the thermoflowable material bound to the binding analytes in the channel 1020 can be converted from a solid to a liquid by heating with heating surfaces 1030. The liquid thermoflowable material and binding analytes can be flushed using a liquid 1066 to transfer the liquid contents of the channel 1020 into the cell counting chamber 1040, where the number of binding analytes (e.g., biological cells) can be counted.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skills in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present methods and devices, suitable methods and materials are described below. All publications, patent

applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

5

EXAMPLES

The methods and devices are further described in the following examples, which do not limit the scope of the claims. Methods of making, analyzing, and characterizing some aspects of the polymer electrolytes are described below.

10 **Example 1: Fabrication of a microfluidic analyte capture device**

The following materials can be used to form a straight channel microfluidic analyte capture device, as described in Cheng et al., "A microfluidic device for practical label-free CD4⁺ T cell counting of HIV-infected subjects," Lab on a Chip, 7, 170-178 (2007) (incorporated by reference herein in its entirety).

15 The 3-Mercaptopropyl trimethoxysilane was purchased from Gelest (Morrisville, PA). Ethanol (200 proof), glass coverslips (35 6 60 mm, no. 1), hemacytometer and microslide field finder were obtained from Fisher Scientific (Fair Lawn, NJ). For chamber fabrication, SU-8 photoresist and developer were obtained from MicroChem (Newton, MA); silicone elastomer and curing agent were obtained from Dow Corning
20 (Midland, MI). Phosphate buffered saline (PBS) was obtained from Mediatech (Herndon, VA). Lyophilized bovine serum albumin (BSA) was obtained from Aldrich Chemical Co. (Milwaukee, WI). The coupling agent GMBS (N-y-maleimidobutyryloxy succinimide ester) and NeutrAvidin were obtained from Pierce Biotechnology (Rockford, IL). Biotinylated mouse anti-human anti-CD4 (clone 13b8.2) was purchased from Beckman
25 Coulter (Somerset, NJ). Biotinylated mouse antihuman anti-CD36 (clone SMO) was obtained from Ancell (Bayport, MN). Alexa Fluor1 488-conjugated mouse antibody to human CD4 (AF488-anti-CD4, clone 289-14120), Alexa Fluor1 647-conjugated mouse antibody to human CD3 (AF647-anti-CD3, clone 289-13801) and 49-6-diamidino-2-phenylindole (DAPI) were obtained from Molecular Probes (Eugene, OR). Phycoerythrin
30 (PE)-conjugated mouse antihuman CD14 monoclonal antibody (PE-anti-CD14, clone

M5E2) was purchased from BD Bioscience (San Diego, CA). Paraformaldehyde was obtained from Electron Microscopy Sciences (Hatfield, PA).

A straight flow channel was formed as described above with respect to Figures 2A-2D. A straight channel device for efficient isolation of CD4⁺ T lymphocytes under fixed shear stress within the shear stress range optimized for pure CD4⁺ T cell capture without contaminating monocytes (Figure 1). This simple device had an internal volume of 10 mL, which serves as a sample volume metering mechanism. The 10 mL volume allows for convenient delivery of a small-volume sample obtained from a study subject, and sufficient sample size for statistically valid cell counts. The elongated chamber design increases the interaction time of blood with the functional surface. The channel provides a constant shear stress along the channel length and has a footprint of 2 square cm. The width, length and height of the channel were 4 mm, 50 mm and 50 micrometers respectively. The straight channel device was used for actual cell capture and counting experiments.

The channel was functionalized with a specific antibody for affinity selection of target cells. Accordingly, the chambers were pretreated with 4% (v/v) solution of 3-mercaptopropyl trimethoxysilane in ethanol for 30 min at room temperature, followed with incubating the chambers with 0.01 mmol mL⁻¹ GMBS in ethanol for 15 min at room temperature. Afterwards, NeutrAvidin was immobilized to GMBS by incubating the chamber surfaces with 10 mg mL⁻¹ NeutrAvidin solution in PBS for at least 1 h at 4 °C. Finally, 10 mg mL⁻¹ biotinylated anti-CD4 solution in PBS containing 1% (w/v) BSA and 0.09% (w/v) sodium azide was injected to react at room temperature for 15 min. After each step, the surfaces were rinsed with either ethanol or PBS, depending on the solvent used in the previous step, to flush away unreacted molecules.

Controlled shear stress applied in the microfluidic channel enabled specific and efficient selection of CD4⁺ T cells versus monocyte and other white blood cells from a small volume sample compatible with fingerprick collection. We injected 10 mL of whole blood at shear stresses ranging from 0.2–7 dyn /square cm (i.e., cm²) into the linear device, collected samples before and after flow through the chamber, and analysed them by flow cytometry to study the capture efficiency within this device. For example, within the optimal lymphocyte-binding window (1–3 dyn /cm²), the shear force exerted on a cell

10 mm in diameter is 8–25 pN. This is of the same order as the binding force of a single antibody–antigen pair. Whole blood (10 microliter samples) from donors was flowed into linear chambers at the desired flow rates (1–20 mL/min). The flow rate of the sample was optimized to capture a desired analyte by providing a desired shear force to analytes bound to the specific binding moieties along the channel. For example, at a shear force of 1.7 dyn/cm², which yielded 95% target cells, a narrow cell density peak of around 200 adherent cells/mm² was seen within 10 mm from the device inlet; this density quickly dropped below 20 cells/mm² at greater distances from the inlet. In contrast, at a less efficient shear of 7 dyn/cm², surface-captured cells remain at a relatively constant, low density throughout the length of the chamber.

Immediately after sample delivery, PBS containing 1% BSA (w/v) and 1 mM EDTA was flowed through the chamber at 40 mL/min for 5 min to rinse off the unbound cells. The cells were then fixed on the surfaces by incubating with 1% paraformaldehyde, followed with incubating with an antibody mixture containing AF647-anti-CD3/AF488-anti-CD4/PE-anti-CD14 for 15 min.

After rinsing off the unbound antibody with PBS containing 1% BSA (w/v) and 1 mM EDTA, the number of adhered cells were counted by placing a field finder under the chambers and counting cells at select points along the device axis using an inverted microscope (Nikon Eclipse TE2000, Nikon, Japan). Monocytes can be identified by staining with antibody to CD14, CD4⁺ T cells can be recognized by CD3⁺/CD4⁺/CD14² staining, and the total number of adherent nucleated cells were determined by staining with DAPI or direct observation under the phase contrast microscope. For each point, three measurements were made, corresponding to three 1 mm squares in that vicinity, and averaged. Images were obtained at 10⁶ magnification using fluorescein, rhodamine and Cy5 excitation/emission filters. DAPI staining was performed afterwards by incubating the surface-attached cells with 300 nM DAPI in PBS at room temperature for 5 min and rinsing with PBS. The cells were counted either manually or using Image J software (<http://rsb.info.nih.gov/ij/>). To avoid competitive binding between the capture antibody and the labelling antibody, CD4 antibodies were selected to bind to different epitopes.

Example 2: Fabrication of a Cell Counting System With a Monocyte Depletion Chamber

Experiment 1 was repeated, except that the whole blood sample was first passed through a monocyte depletion chamber. The monocyte depletion chamber was formed in as described with respect to the channel in Example 1, except that the chamber was functionalized by contacting the sample contact surfaces with anti-human CD14 (MY4 clone). The anti-human CD14 (MY4 clone) was first purified using the Melon 5 Gel IgG purification kit and biotinylated using the Sulfo-NHS-Biotinylation kit following the recommended kit protocols. Afterwards, the antibody concentration was measured using a UV-Vis spectrometer and diluted to the desired final concentration using PBS containing 1% BSA.

The monocyte depletion chamber can be a single straight flow channel, with dimensions of 50 mm × 4 mm × 50 μm (Figure 1) for CD4+ T cell capture. The chamber can be fabricated in PDMS and bonded permanently to clean glass cover slips using standard clean room techniques.

A monocyted depletion microfluidic chamber coated with antibodies specific to leukocytes can be employed for depletion of white blood cells prior to isolation of an analyte such as circulating tumor cells (CTCs) in a channel. By pre-depleting the contaminant leukocytes prior to capturing circulating tumor cells, the purity of capture increased by orders of magnitude. Higher purity allows better genotyping and phenotyping of CTCs, which can provide detailed insight into the metastatic process and permit direct exploration of targeted treatment strategies. Depletion efficiency can be increased by using a red blood cell lysis step prior to depletion of leukocyte cells. Alternatively, a "cocktail" of multiple binding moieties for white bloods cells, e.g., anti-CD66, anti-CD3, and anti-CD45, improved capture efficiency.

Example 3: Gelatin performance characterization in terms of CD4+ T-cells and CD66b+ cells

In another experiment, the gelatin device was characterized with respect to two different cell types: neutrophils (CD66b) and lymphocytes (CD4+). Table 2 presents the

results in terms of three parameters: cell capture purity, release efficiency from the gelatin layer itself, and the released cell viability.

Following the functionalization with biotin BSA and then NeutrAvidin, the gelatin devices used to test the capture of the CD4⁺ T-cells were treated with 20 µg/mL of biotinylated anti-CD4 solution (Beckman Coulter, Somerset, NJ) and the devices used to test the capture of CD66b⁺ neutrophils, were treated with 30 µg/mL of biotinylated anti-CD66b solution (Abd Serotec). The devices were allowed to react with the antibody solutions overnight at 4°C. Before running whole blood to perform the capture experiments, the devices were washed with ice-cold 1% BSA.

Institutional Review Board (IRB) approval of the protocol used in this work was obtained prior to initiation of the study and all subjects provided written informed consent. Blood was drawn by a trained phlebotomist and collected through venipuncture into EDTA Vacutainer collection tubes (BD Biosciences, San Jose, CA, USA). All samples were run on the microfluidic devices on the same day as the blood draw. All experiments were conducted at room temperature conditions. A commercially available syringe pump (PHD 2000 from Harvard Apparatus, Holliston, MA, USA) is used to propel all the fluids into the device. Unprocessed whole blood from the healthy volunteers was introduced into the microfluidic device using the syringe pump at the appropriate flow rate (6.7 µL/min for the CD4⁺T-cell capture and 1.8 µL/min for the CD66b⁺ granulocyte capture). PBS (phosphate buffer saline, pH 7.8) was used as both the wash and release buffer, and was pumped in at 20 µL/min. Blood and the buffer were filled using disposable syringes (BD Biosciences), with needle tips and tygon tubing (both from Small Parts) attached to the ends of the needle tips and inserted into the appropriately sized inlets/outlets of the gelatin device.

Cell count and purity

In order to quantify the cell purity of the captured cells in the gelatin device, the non-specific cells were first washed off, and then the remaining captured cells were fixed by flowing 1% (v/v) formaldehyde [prepared from paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) in 1X PBS and stored at 4°C for future use], and incubating the solution for 20 minutes at room temperature or longer at 4°C. The

fixed cells were then rinsed with PBS, incubated with an antibody mixture containing fluorescein isothiocyanate (FITC) anti-CD66b and Alexa Fluor 647 anti-CD3 (all from BD Biosciences, San Jose, CA, USA), followed by Hoechst 33342 stain (Invitrogen, Carlsbad, CA, USA), with all the dye solutions diluted in 1% BSA solution in 1X PBS, followed by being imaged on an inverted microscope (Nikon Eclipse TE2000, Nikon, Japan). All the gelatin devices were treated with the same cocktail mixture of dyes and were imaged using the same UV, FITC and Cy5 excitation/emission filters. The cells were counted manually using tools from ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2009.). It should be pointed out that in order to avoid competitive binding between the capture and the labeling antibodies, they were all selected to be of different clones. The cell purity was measured as the ratio of the total number of nucleated (Hoechst positive) target cells (FITC anti-CD66b positive for neutrophils and AlexaFluor 647 anti-CD3 positive for CD4+ T-cells) to the total number of nucleated cells (Hoechst positive).

Released cell efficiency characterization

In order to quantify the release efficiency of the gelatin layer, bright field microscopy imaging was performed in order to count the captured cells that were present before the release process. Following the release of the cells from the gelatin device, another count was performed in order to get the number of cells that remained. It should be noted that only the cells that were captured on the gelatin surface were counted, and not the cells that were captured on the non-gelatin parts of the device (for example on the top of the channel made of PDMS or on the side walls or edges of the channels)

Cell viability characterization

In order to quantify for the viability of the released cells, a live/dead cell assay (Invitrogen) in conjunction with a microplate spectrofluorometer reader (Spectra Max Gemini XS, Molecular Devices, Sunnyvale, CA) was used. This method involves staining the cells with a mixture of calcein AM (fluoresces green for live cells) and ethidium homodimer-1 (fluoresces red for dead cells) and interrogating the cell and dye mixture at two different wavelengths (465 nm and 525 nm) using the microplate reader.

Controls of live and dead cells were used in order to calibrate the signal from the released cells. The live and dead cells were obtained from whole blood in which the red blood cells were lysed. The dead cells control sample was obtained by treating the leukocytes obtained after lysing the red blood cells with 4% paraformaldehyde followed by 0.2 %
 5 Triton-X-100. The live cells were obtained by using the leukocytes from the RBC-lysed blood as is in PBS. This method was used in order to get a more objective analysis of the cell viability.

Table 2:

Cell Type	Cell Capture Purity	Release Efficiency*	Cell Viability
CD4+ T-cells	97.0 $\pm$ 2.0	99.7 $\pm$ 0.5	80.3 $\pm$ 5.2
CD66b+ Neutrophils	99.0 $\pm$ 0.8	99.5 $\pm$ 0.8	84.2 $\pm$ 7.3

10 *Release efficiency was measured in terms of the cells that were captured and released from the gelatin layer itself, and not from the cells that were captured in the non-gelatin parts (for example the PDMS parts) of the microfluidic channel

It is to be understood that while the methods and devices have been described in conjunction with the detailed description thereof, the foregoing description is intended to
 15 illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. For example, these methods and devices can be used in conjunction with other devices. In one application of the gelatin release device, neutrophils can be enriched from a whole blood sample. The released neutrophils can then be used a point of care chemotaxis assay. Other aspects, advantages, and modifications are within the
 20 scope of the following claims. For example, where the present description refers to gelatin, other thermoflowable materials can also be used, e.g., agar or carrageenan.

CLAIMS:

We Claim:

1. A method of isolating a binding analyte from a multi-component sample,
5 the method comprising
 contacting the multi-component sample with a solid thermoflowable material
 comprising a specific binding moiety under conditions effective to selectively bind a
 binding analyte from the sample to the specific binding moiety;
 removing unbound components from the sample;
10 converting the solid thermoflowable material bound to the binding analyte to a
 flowable liquid thermoformable material; and
 detecting the binding analyte in the flowable liquid thermoformable material.
2. The method of claim 1, wherein the binding analyte is a biological cell,
 particularly wherein the binding analyte is a CD4+ biological cell or wherein the binding
15 analyte is a leukocyte.
3. The method of claim 1, wherein the multi-component sample comprises
 blood.
4. The method of claim 1, further comprising contacting the solid
 thermoflowable material with the specific binding moiety under conditions effective to
20 bind the specific binding moiety to the thermoflowable material, particularly herein the
 solid thermoflowable material is contacted with the specific binding moiety before
 contacting the specific binding moiety and the solid thermoflowable material with the
 multi-component sample.
5. The method of claim 1, wherein
25 the thermoflowable material is a solid below a first melting temperature;
 the multi-component sample is contacted with the solid thermoflowable material
 at or below the melting temperature; and

the solid thermoflowable material is converted to the flowable liquid by raising the temperature of the solid thermoflowable material above the melting temperature after binding the binding analyte to the solid thermoflowable material.

6. The method of claim 5, wherein the thermoflowable material

5 7. The method of any of the preceding claims, wherein the thermoflowable material is a protein or wherein the thermoflowable material comprises a gelatin.

8. The method of any of the preceding claims, wherein the specific binding moiety is an antibody.

9. A method of isolating a biological cell from a multi-component liquid
10 sample, the method comprising

contacting the sample with a solid thermoflowable material comprising an antibody within a chamber under conditions effective to selectively bind the biological cell from the sample to the antibody;

removing unbound components from the sample within the chamber;

15 converting the solid thermoflowable material bound to the biological cell to a flowable liquid within the chamber; and

detecting the biological cell in the flowable liquid thermoformable material.

10. The method of claim 9, further comprising removing the flowable liquid from the chamber prior to detecting the biological cell in the flowable liquid
20 thermoformable material.

11. The method of claim 9, wherein the biological cell is detected outside the chamber.

12. The method of any of claims 9-11, wherein

the thermoflowable material is a solid below a first melting temperature;

25 the multi-component sample is contacted with the solid thermoflowable material at or below the melting temperature;

the solid thermoflowable material is converted to the flowable liquid by raising the temperature of the solid thermoflowable material to second temperature greater than the melting temperature after binding the binding analyte to the solid thermoflowable material; and

5 the biological cell is viable at the first and second temperatures.

13. The method of any of claims 9-12, wherein
the thermoflowable material comprises a gelatin;
the specific binding moiety is an anti-CD4 antibody; and
the biological cell is a CD4+ biological cell.

10 14. A microfluidic analyte capture device comprising:
a channel having an inner surface defining a fluid flow path of substantially
uniform cross-sectional area extending from an inlet to an outlet, the inner surface
including a thermoflowable material in a solid phase comprising a binding moiety
specific for an analyte; and

15 a means for heating the thermoflowable material within the channel above a
melting point of the thermoflowable material to convert the thermoflowable material
from the solid phase to a liquid phase within the channel.

15. The microfluidic analyte capture device of claim 14, wherein the binding
moiety is an antibody and the analyte is an analyte biological cell that specifically binds
20 to the antibody.

16. The microfluidic analyte capture device of claim 15, wherein device
further comprises a first chamber in fluid flow communication with the inlet of the
channel, the first chamber configured to retain monocyte cells from the analyte,
particularly wherein device further comprises a second chamber in fluid flow
25 communication with the outlet of the channel, the second chamber configured to count
analyte biological cells in the analyte.

17. A method of isolating a cell from a blood sample, the method comprising

contacting the blood sample with a solid thermoflowable material within a channel, the solid thermoflowable material comprising gelatin retaining an antibody specific for the cell under conditions effective to selectively bind the cell from the blood sample to the antibody retained on the gelatin;

5 removing unbound components from the channel while retaining the cell bound to the solid thermoflowable material within the channel; and

 converting the solid thermoflowable material bound to the cell to a flowable liquid thermoformable material within the channel to release the cell bound to the antibody from the channel.

10 18. The method of claim 17, further comprising detecting the cell in the flowable liquid thermoformable material.

 19. The method of claim 17, wherein the cell is a first cell of a first cell type, and the blood sample further comprises a second cell of a second cell type, the antibody capable of binding both the first cell type and the second cell type.

15 20. The method of claim 19, wherein the method further comprises applying a shear force to the cell bound to the antibody to remove a second cell from the channel while retaining a first cell in the channel.

 21. The method of claim 20, wherein the second cell type is a monocyte.

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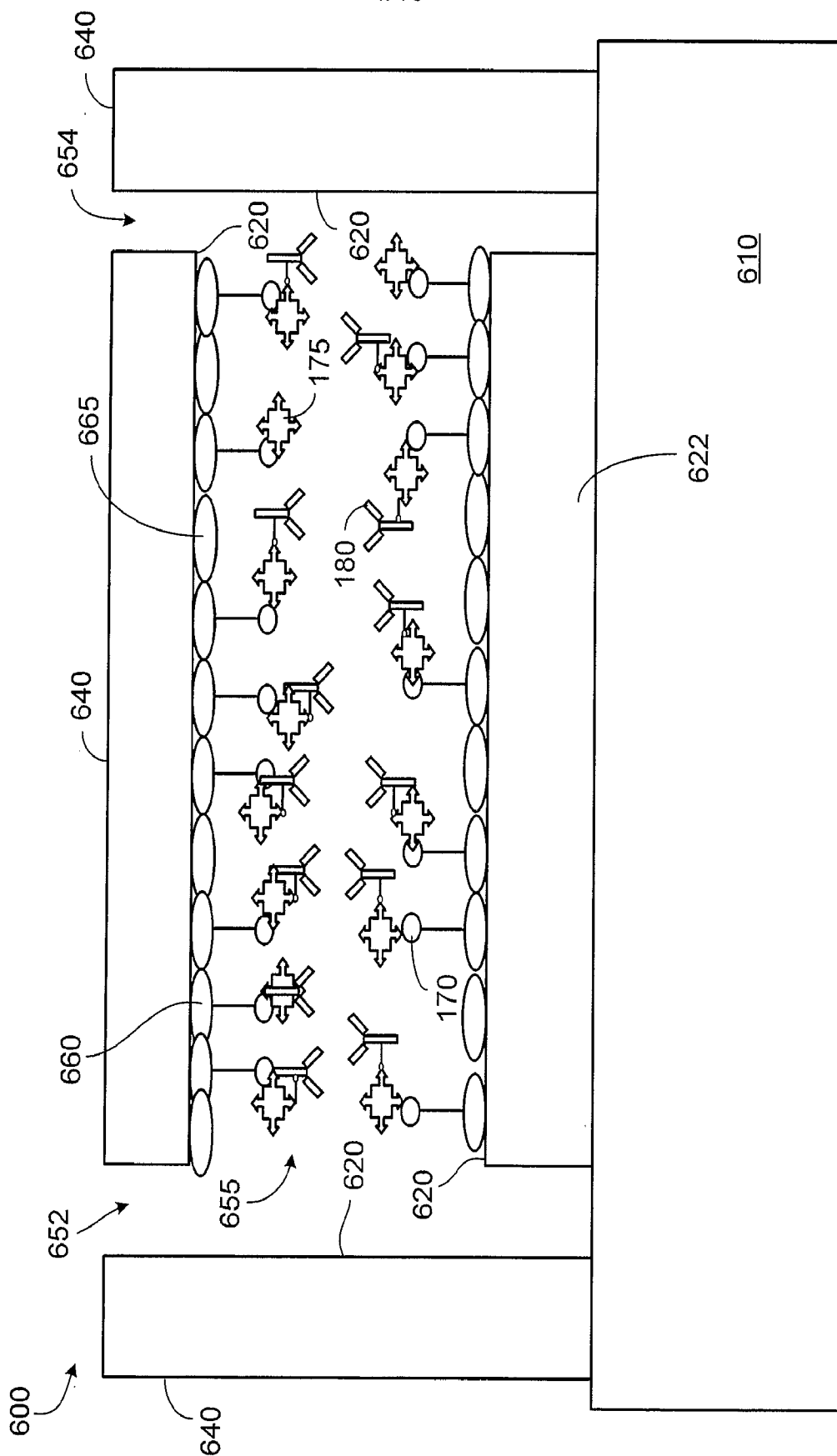


FIG. 1

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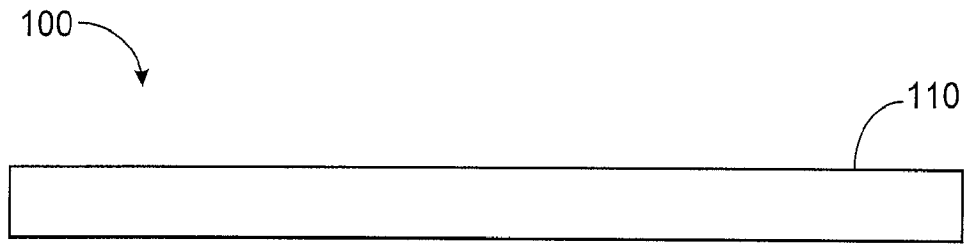


FIG. 2A

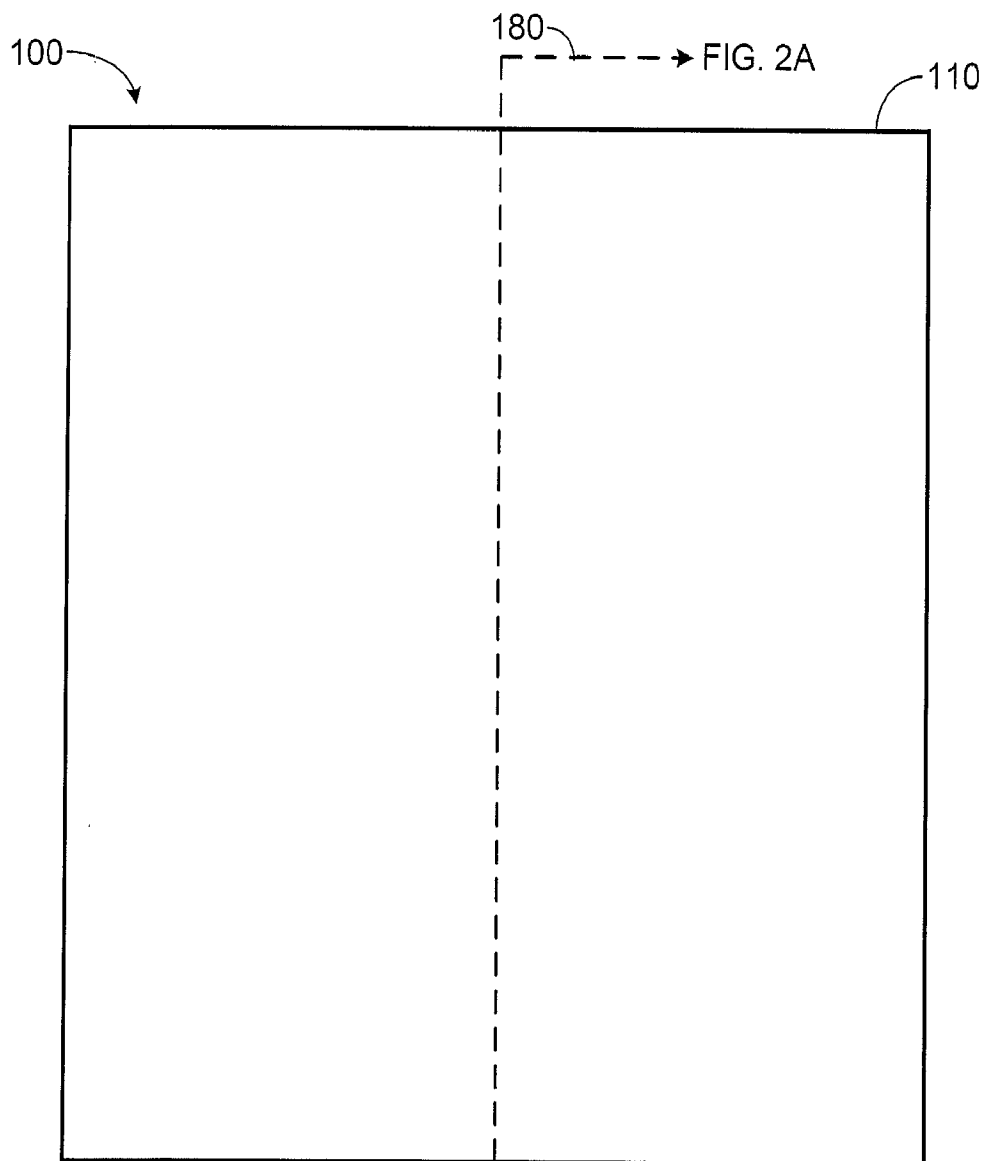


FIG. 2B

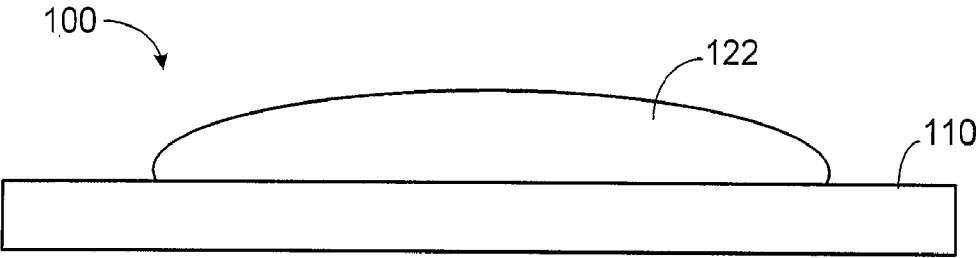


FIG. 3A

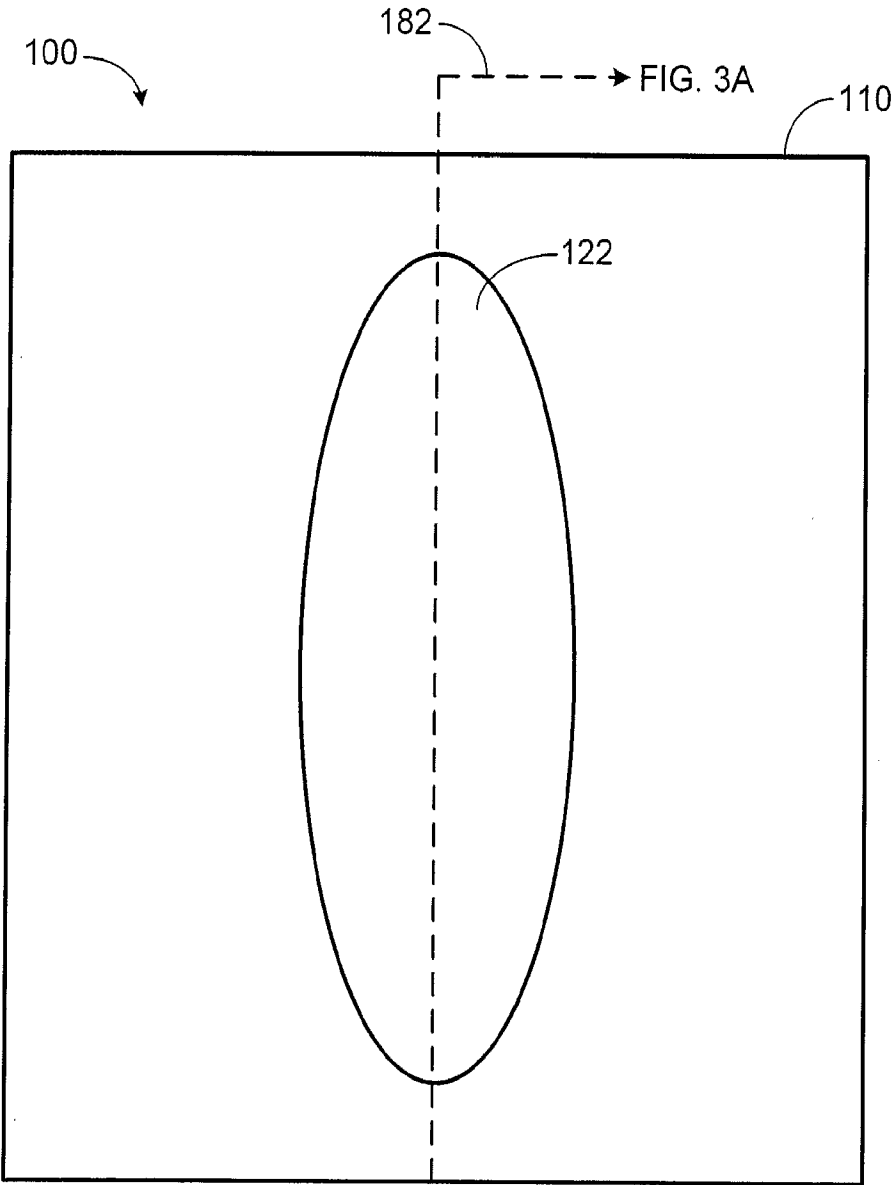


FIG. 3B

FIG. 3A

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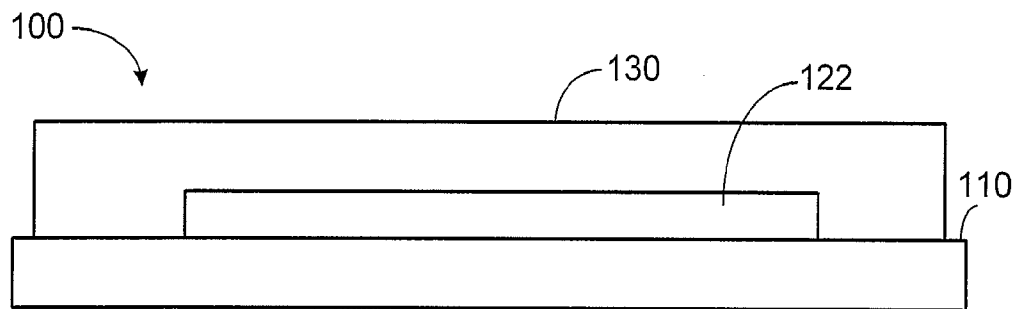
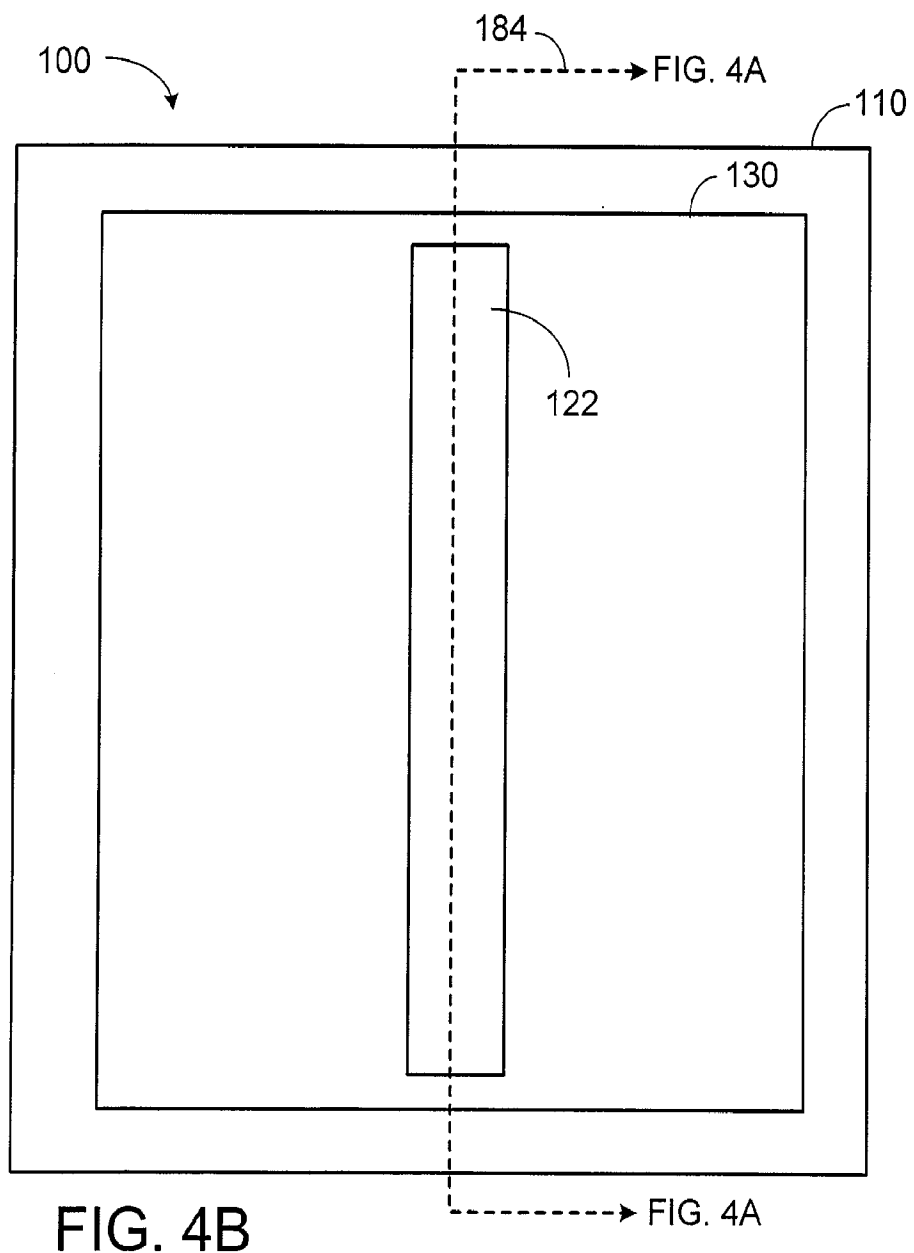


FIG. 4A



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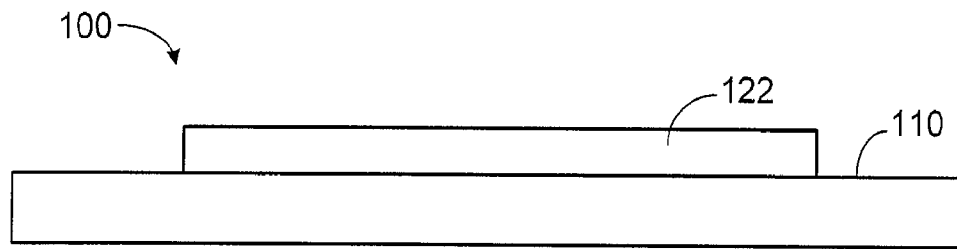


FIG. 5A

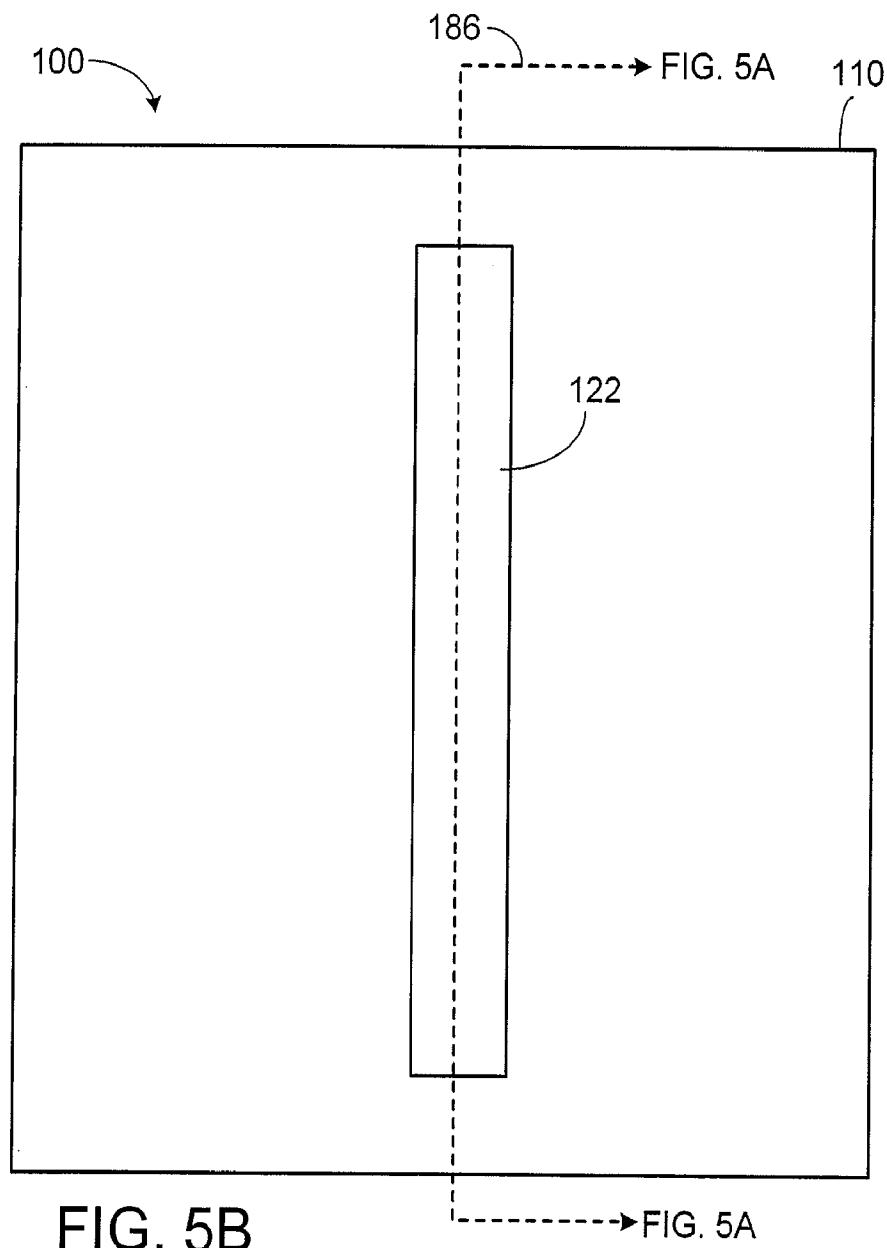


FIG. 5B

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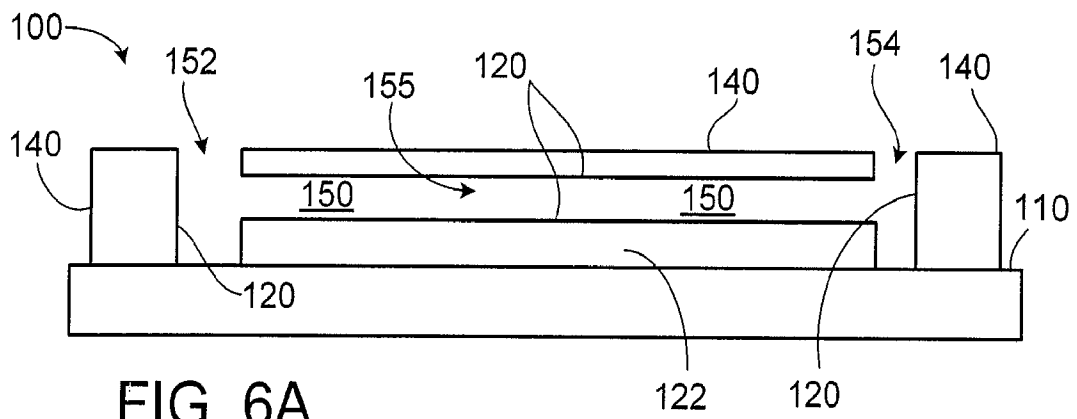


FIG. 6A

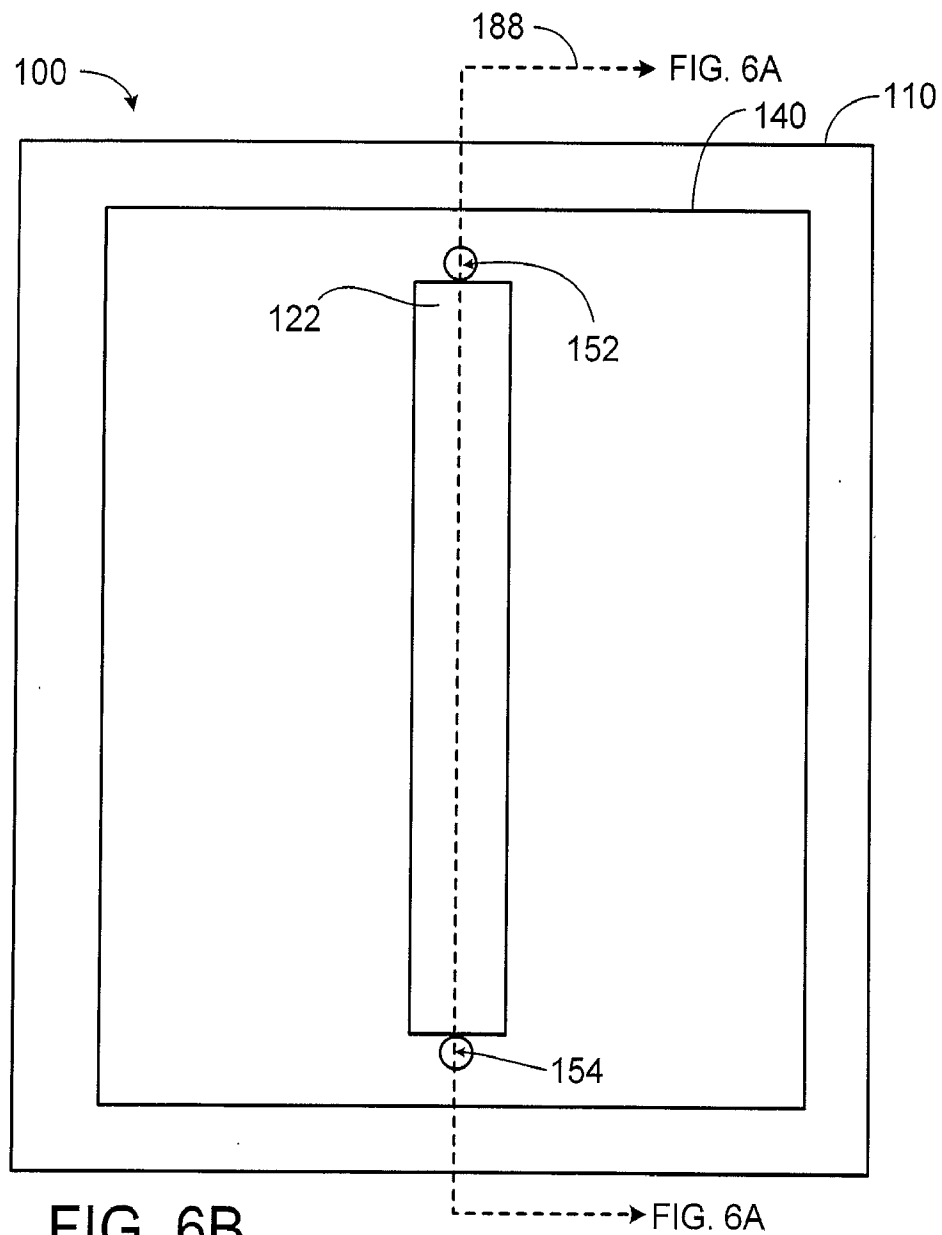


FIG. 6B

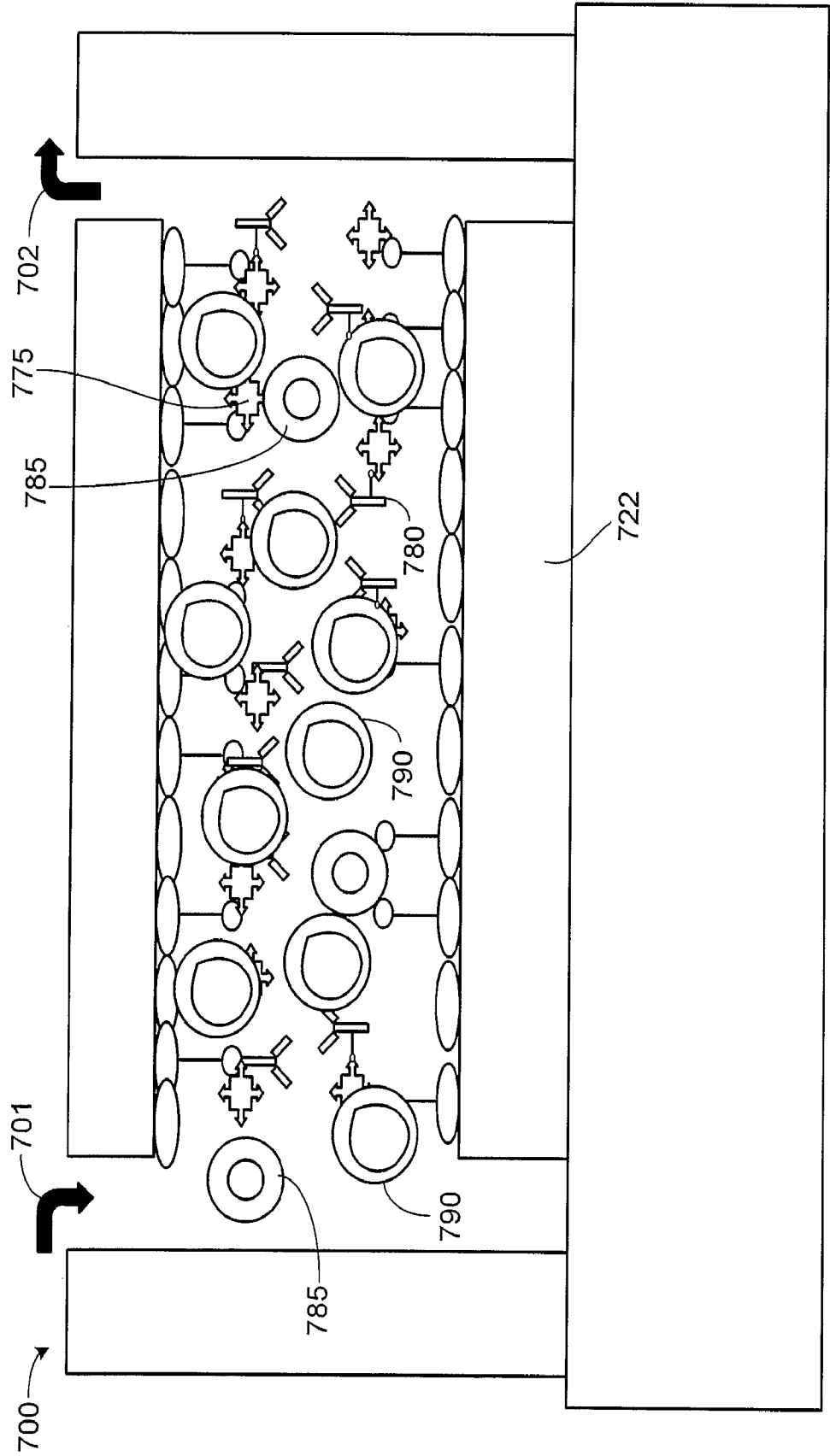


FIG. 7A

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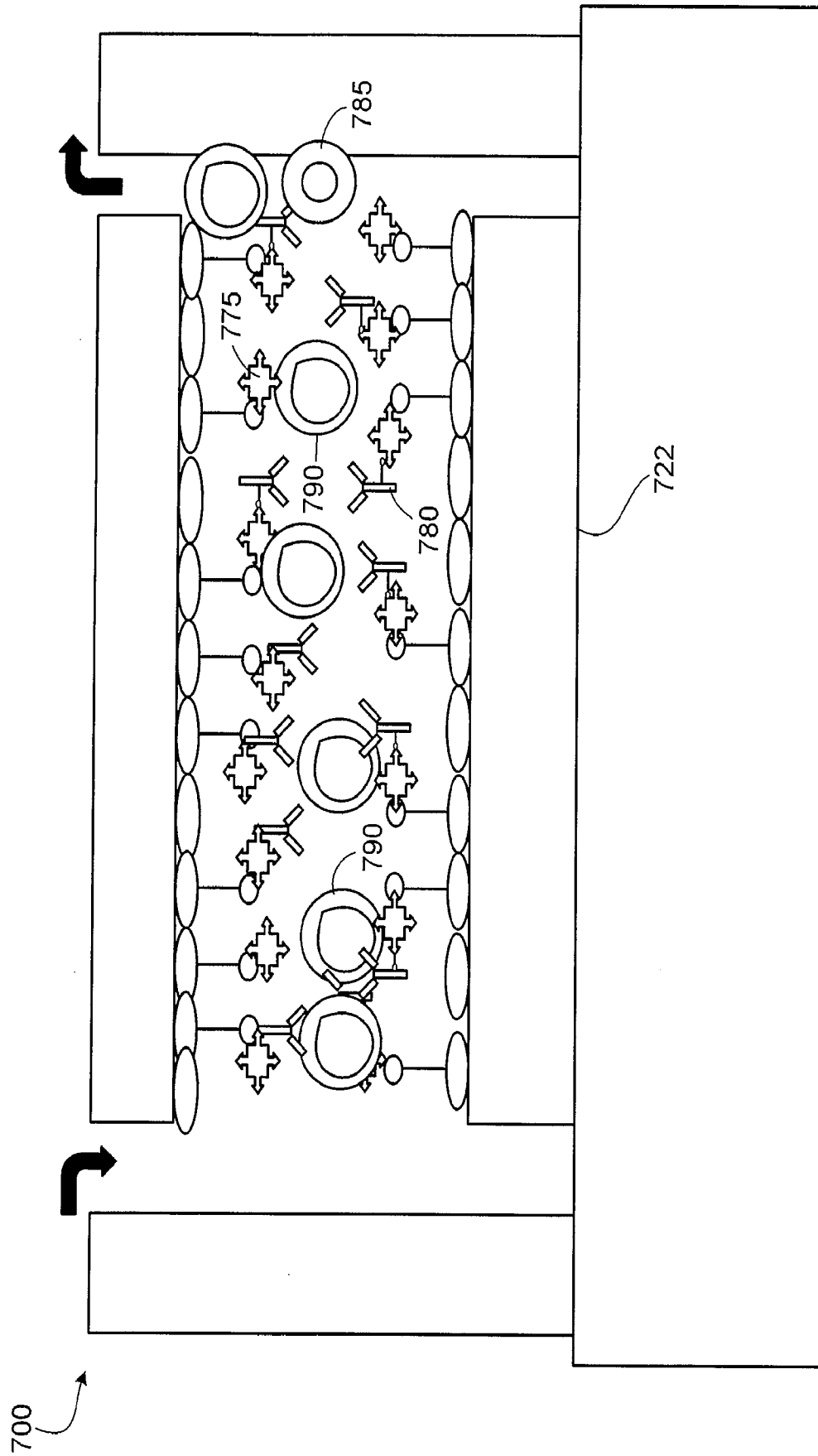


FIG. 7B

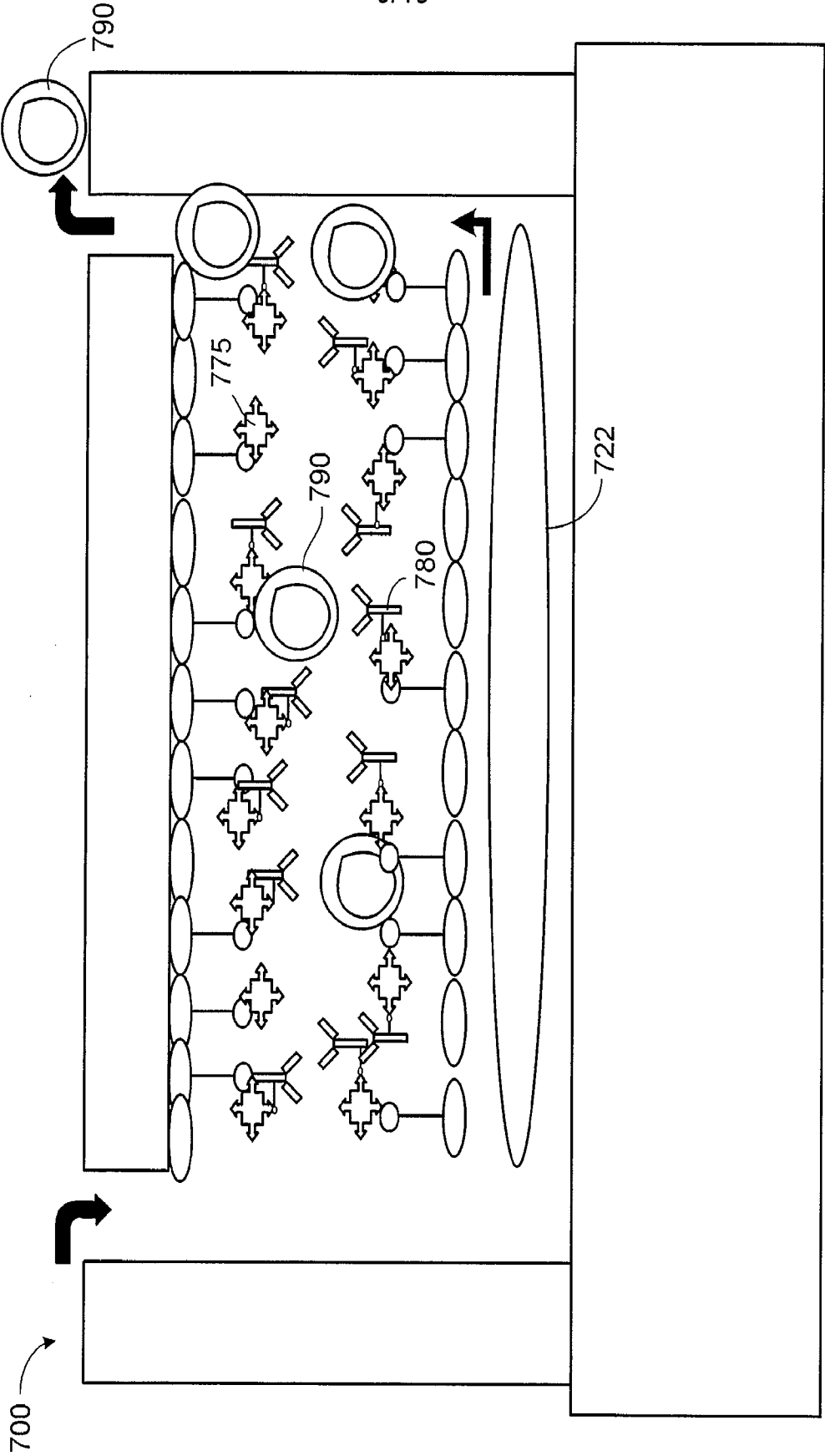


FIG. 7C

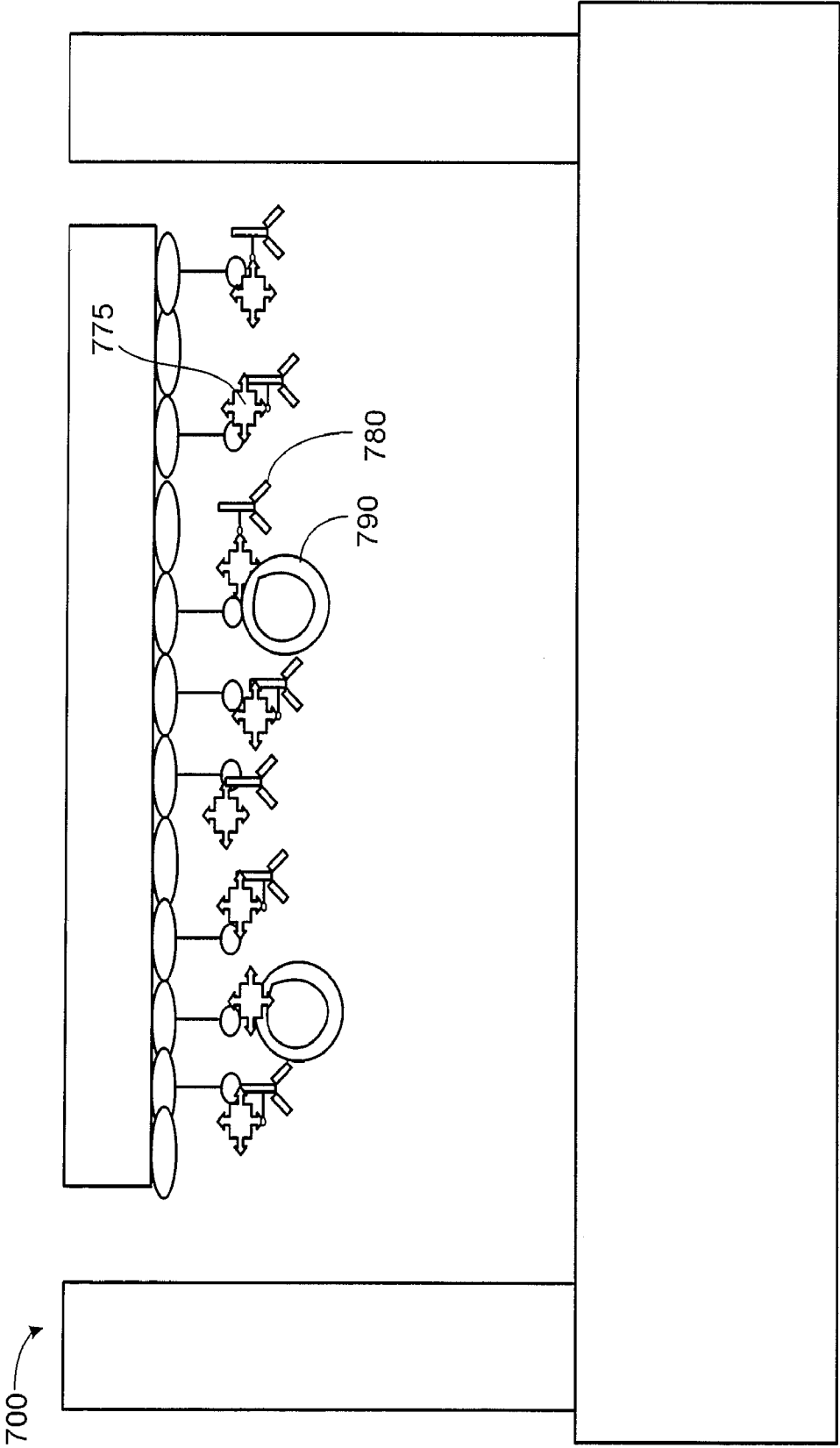


FIG. 7D

11/15

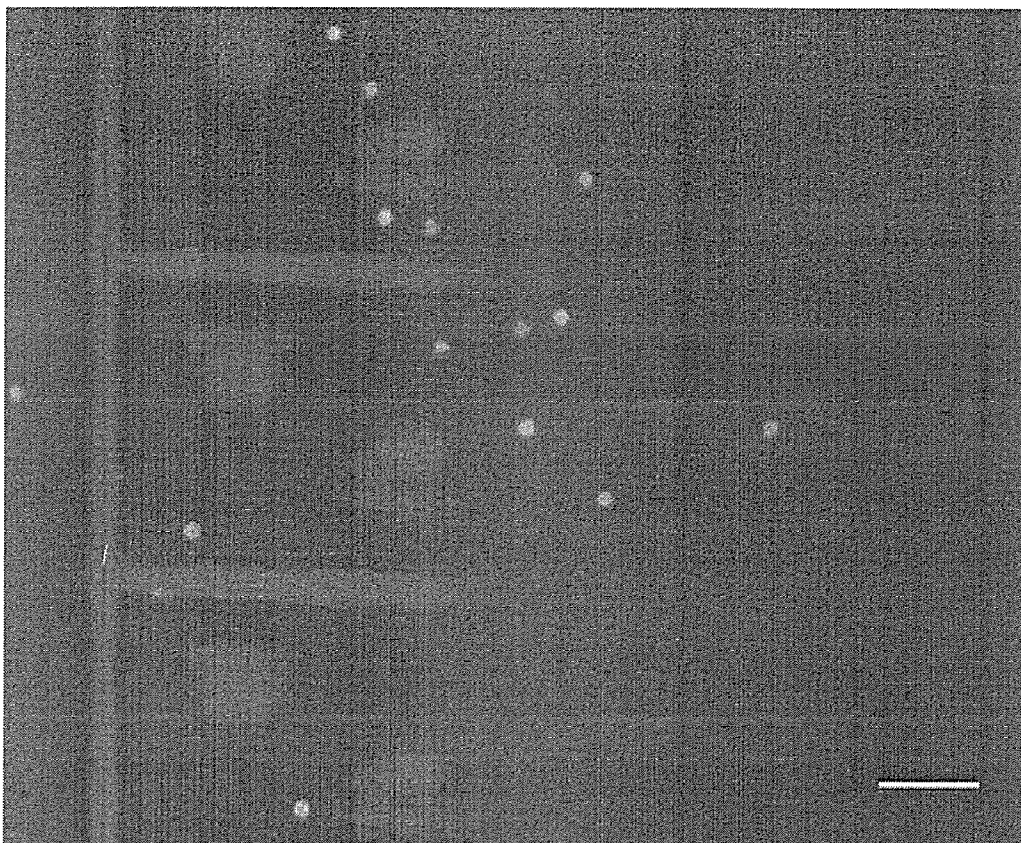


FIG. 8

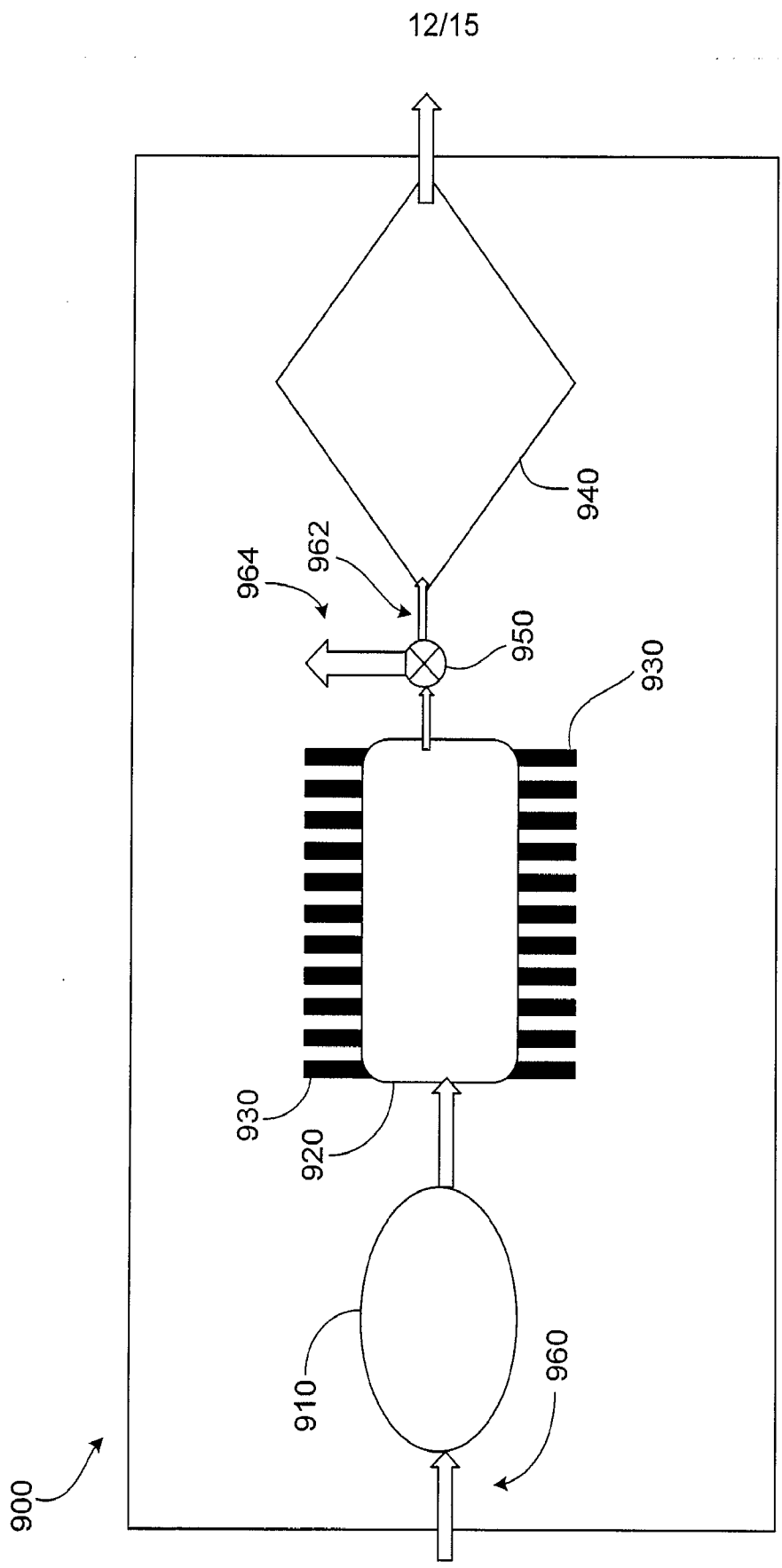


FIG. 9

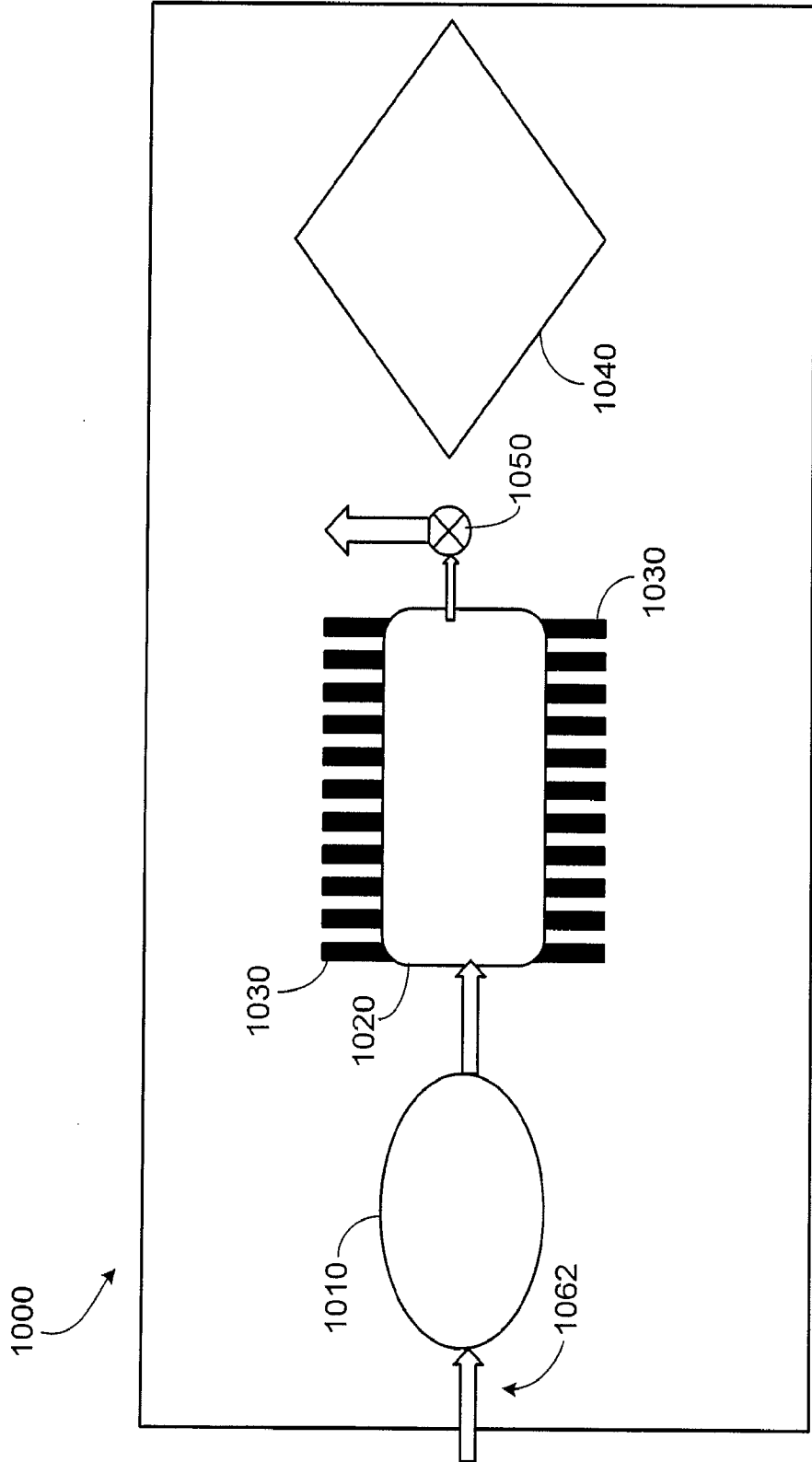


FIG. 10A

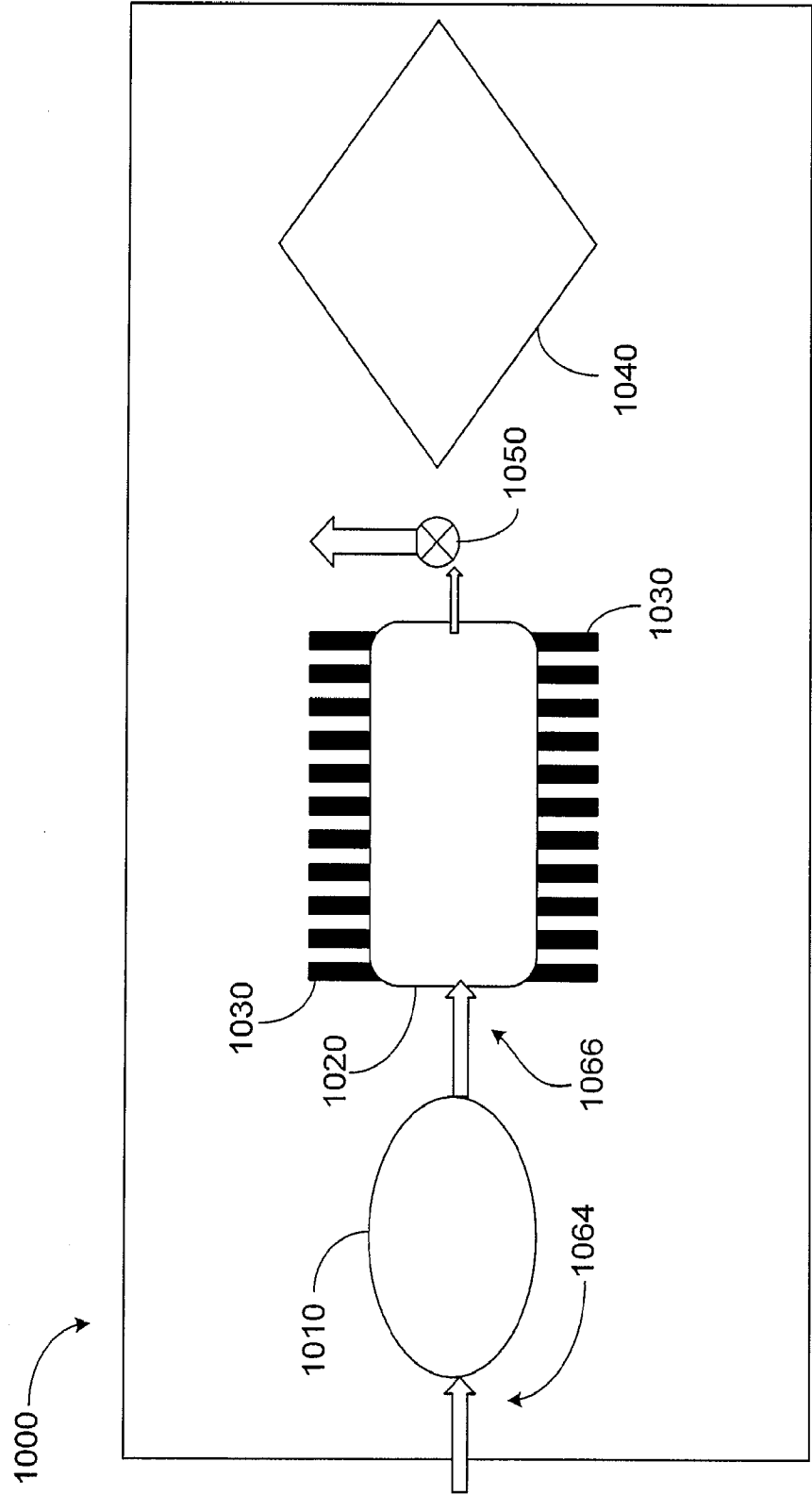


FIG. 10B

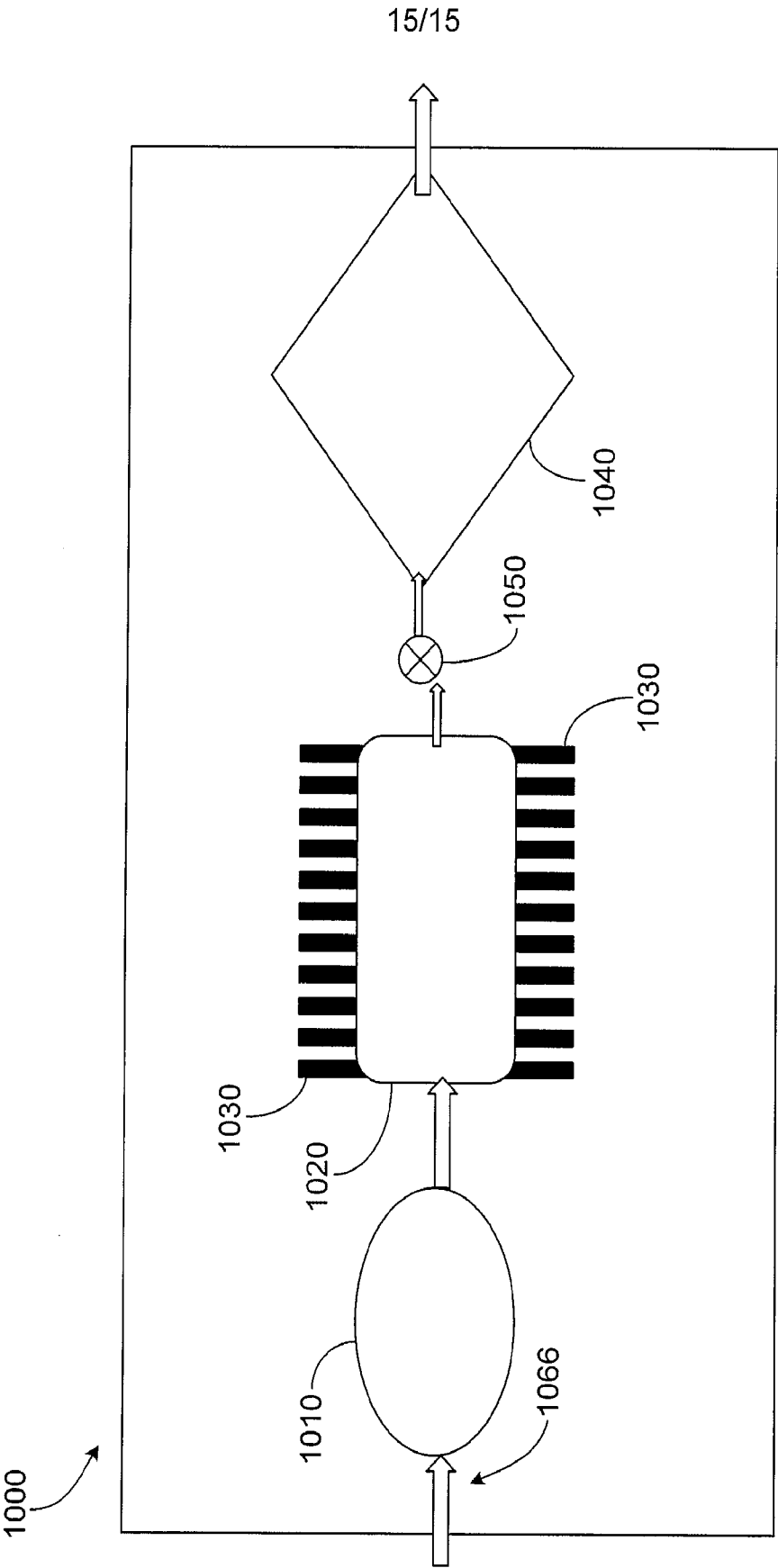


FIG. 10C