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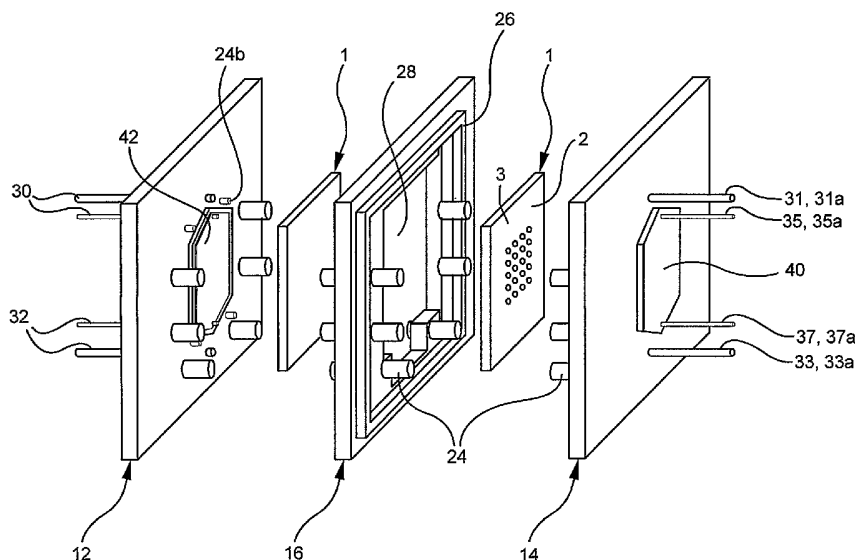
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(54) Title: AUTOMATED REACTION CHAMBER SYSTEM FOR BIOLOGICAL ASSAYS



(57) Abstract: A device for high-capacity, multiplexed processing of a variety of solid-phase-format, biological or biochemical assays, which use fluid media, is described. The device may be part of a larger machine or system, which can be automation friendly. Generally, the device comprises a number of plates, which forms a cassette or chamber module. Each cassette or chamber module has an interior and exterior surface. At least part of an interior wall surface is displaceable. Within each cassette are a number of fluid inlet and outlet passageways and a reaction chamber for receiving at least one microarray substrate. When the cassette is ready to be used, the reaction chamber should be fluid-tight. In the larger machine, a number of cassettes are associated with a rotary drum, from which the chamber modules extend outwardly. A method relating to the use of the present device or system is also provided.



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AUTOMATED REACTION CHAMBER SYSTEM FOR BIOLOGICAL ASSAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

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This application claims the benefit of priority of U.S. Application Serial No. 10/461,280 filed on June 13, 2003.

FIELD OF THE INVENTION

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The present invention relates to the field of biological or chemical assays. More particularly, the invention relates to an apparatus and method for handling small quantities of fluid and reacting biological analytes of interest, to be detected within either a hybridization or other kind of binding-assay chamber.

15

BACKGROUND

Conventional methods for mixing relatively large volumes of fluids utilize mixing devices that mix the fluids by shaking the container, by a rapid mechanical up and down motion, or by a rocking motion that tilts the container back and forth. Conventional mixing methods, however, cannot normally be applied to chemical and biological assays involving small samples in a fluid because the strength of capillary forces of the containment system often exceeds the forces generated by shaking or rocking, thereby preventing or minimizing motion of the fluid. As an illustration, a small amount of fluid sample, when spread across a large reactive, probe-presenting surface, will form a thin film of fluid that may have a thickness of a few microns to a few millimeters. In such situations, the fluid may not adequately contact the entire

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surface or the analyte compounds in the fluid may be very dilute, thereby resulting in a reaction that is limited by the rate of diffusion through the fluid or has an insufficient amount of binding events. Inadequate mixing can adversely affect the reproducibility of results, the sensitivity or specificity of the reaction, the rate of reaction, the extent of reaction, the homogeneity, or the percent yield.

Inadequate mixing is a particular problem in chemical and biological assays performed on microarrays that typically involve very small samples of chemical, biochemical, or biological fluids. In microarrays, numerous molecules of biological interest are immobilized in a spatially addressable manner on a surface of a solid support having a surface area that is in a range from about a few square millimeters to about tens of square centimeters. Each of the respective immobilized molecules is usually confined in a microspot with a typical diameter of several hundred microns. The solid support is typically a glass or fused silica slide that has been treated to facilitate attachment of probe molecules. A sample liquid containing reactants is then brought into contact with the immobilized molecules (e.g., nucleotides) on the solid support. When the microarray is exposed to the liquid, target molecules in the sample selectively and specifically bind to their corresponding binding partners on the microarray. In a typical procedure, the fluid is placed on the support, and the fluid and solid support are covered with another slide or lid, and placed in an environmentally controlled chamber such as an incubator for several hours. Typically, the reactants in the liquid diffuse to the interface where they react with the immobilized probe molecules, and a reaction or binding event occurs, such as a hybridization reaction. For instance, detection of hybridization between an immobilized probe (e.g., nucleotide sequence) and a biological or chemical target in the sample (i.e., complementary nucleotide molecule) offers a convenient and reliable means for isolating, sequencing, and analyzing biological or chemical molecules. But, when diffusion is the only means of bringing the reactants in the liquid in contact with the immobilized probes, problems encountered usually include poor binding or hybridization kinetics, and poor reaction specificity or sensitivity.

Another equally significant and related problem for binding assays on microarrays involves the introduction and movement of fluid media, such as in the washing steps associated with a hybridization protocol, which typically include pre-

hybridization soaks and/or washes, and post-hybridization washes. The efficacy of washing steps is very important, because the cleanliness or extent of how well a microarray is washed can affect the sensitivity of hybridization signal of the microarray (e.g., excess substrate background noise) and the overall outcome quality of the assay.

5 Furthermore, after each pre-hybridization soak and/or wash, or each post-hybridization wash, the microarray substrate may be dried. This drying step is also very important for achieving the best assay results.

To date, washing and drying steps have characteristically been relatively labor-intensive and require much manual manipulation of the microarray substrate. To

10 overcome these labor-intensive procedures, a number of automated systems, which perform automated washes, drying, and incubation for microarray applications, are available commercially (e.g., Discovery™ automated systems, Ventana Medical Systems, Inc., Tucson, AZ; HS 4800 Hybridization Station, Tecan U.S., Inc., Durham, NC; MAUI™ Hybridization System, BioMicro Systems, Inc., Salt Lake City, Utah;

15 GeneTac HybStation™, Genomic Solution, Inc., Ann Arbor, MI; Automated Slide Processor and Lucidea™ SlidePro, Amersham Biosciences Corp., Piscataway, NJ). A common draw back of existing automated systems, however, is that the automated washing and drying steps do not produce results as consistently as those performed by the manual approach (e.g., in a coplin jar). In most cases these different procedures

20 often lead to higher background, poorer hybridization signals, and less reproducibility.

Thus, a need remains for a device or system that not only can address the problems of poor reaction kinetics and poor reaction specificity and sensitivity, but also

1) can perform steps with greater consistency, such as labor-intensive washing and drying, with less effort under automation, using adjustable reaction and washing

25 volumes, in which the fluid components can be actively mixed, 2) can process a multiple number of in parallel, and 3) is easy to use, minimizing user intervention. A system that can promote better fluidic reactions, hybridization or other binding results, as well as wash more substrates at higher throughput and with more consistent quality would be very attractive to persons in academia and industry who have a need for

30 microarray applications.

SUMMARY OF THE INVENTION

The present invention pertains in part to a device for mixing fluids, which can be used to execute assays involving biological reactions or experiments using fluids. In particular, the invention uses a cassette or chamber module that enables one to perform efficiently automated washing, drying, mixing, and other operations and to generate
5 consistent results for binding-assays on array substrates.

Generally, the device comprises a first end plate, a middle plate, and a second end plate, defining parts of a frame assembly. Each of the end plates having an interior and exterior surface, and the middle plate having a side α and a side β . On an interior
10 surface of each end plate or on either side α or side β of the middle plate, are a number of either (a) alignment members, for aligning each of said end plates with said middle plate, or (b) holder members, for holding a microarray substrate in position. A fluid-tight seal is formed between each end plate and the middle plate, respectively, creating within the device a reaction chamber for the microarray. Each end plate or the middle
15 plate has a number of fluid passages running through or incorporated within the plate structure. A moveable or displaceable component, forming part of an interior wall of the reaction chamber, is situated preferably in each end plate or on either side α or side β , or both of sides of the middle plate. A separator may be situated in the middle plate, or on either said side α or side β . The displaceable component or cover permits the
20 cassette to create a reaction chamber of variable volume. Preferably, all three plates, but in particular the displaceable component, are made, at least in part, from a transparent material, which would permit one to observe the interior of the chamber during operations.

At least one microarray substrate, preferably having an industry-standard
25 format, can be placed within each assembled chamber module to perform binding assays. A receptacle holds the microarray substrate and aligns it with the rest of the frame. The receptacle in some embodiments is large enough to accommodate two or more microarray substrates within each cassette or chamber module. This feature of the invention enables researchers to process simultaneously two or more array slides in
30 a single cassette. The probe-presenting surface of a microarray substrate may be placed to face either towards the end plates or towards the middle plate.

Array substrates may be processed in either a single, common chamber, according to devices of a first type of embodiment, or arrays may be placed in separate, fluidically non-communicative chambers, according to a second type of embodiment. In embodiments of the first type, an opening traverses through the middle plate. The opening permits fluid to freely mix throughout the reaction chamber, from one side (α or β) of the middle plate to the other. This kind of arrangement is convenient for faster processing of multiple assays, which have assay steps or use parameters in common. Two or more arrays in a common chamber may be subject, for instance, to common washing or hybridization solutions. Furthermore, researchers may find such an arrangement to enhance empirical control, as well as benefit the reproducibility of assay results. In other words, one may more easily compare multiple assays performed under common or shared assay conditions.

Alternatively, in embodiments of the second type, the middle plate can divide the large single reaction chamber within a cassette into two separate reaction areas. The middle plate may be a non-porous or selectively porous barrier to fluids, incorporating for example a filter, a membrane, or a solid plate. According to an embodiment, a shutter may form part of the middle plate. At desired times during the course of an assay, the shutter would move to either open or close off the opening in the middle plate, possibly by means of either computerized (e.g., hardware and software) or manual control (e.g., a tab or lever) from outside of the reaction chamber or cassette. The two end plates may have configurations that are either identical to each other or different, depending on the dictates of specific assay parameters or functions.

Similarly, depending on the specific configuration, a cassette may be used either as a stand-alone, individual unit or as part of a larger apparatus or system. A plurality of cassettes can be assembled as modules in a machine, such that a series of cassettes or chamber modules, arranged side by side, may perform a number of assays in parallel.

Hence, another aspect of the invention relates to an automation-friendly machine for multiplexed, high-throughput processing of assays. According to certain embodiments, the machine has at least a carousel comprising a rotary drum, from which extends, in a radial fashion, a number of chamber modules. Each chamber module is connected to fluid passages running through the rotary drum. The passages supply each chamber module with the sample or reagent solution fluids that are

consumed during the course of an assay. In addition, other conduits for pneumatic or hydraulic fluids, temperature control and heating elements, as well as electronics used to monitor reactions and operate the machine may be distributed throughout the structure of the machine. In a preferred embodiment, the rotary drum is oriented horizontally.

Conventional automated systems typically orient array substrates in a largely horizontal placement. Horizontal orientation of array substrates, however, often hinders fluids from flowing efficiently across array surfaces. Unlike most existing automated systems, the present invention can work in multiple positions. In other words, array substrates may be rotated or positioned potentially at any angle along a 360° arc. During the course of an assay the orientation of microarrays substrates and cassettes can be either fixed or changed in compliance with the requirements of the assay. According to preferred embodiments, microarrays in the reaction chamber modules are held at least for sometime in a substantially vertical position during the course of executing an assay protocol.

An adjustable reaction chamber volume is another advantage of the present invention. A gap of variable width formed between the probe-presenting surface of a microarray substrate and the displaceable component of the chamber wall may range from a few tens of microns to greater than or equal to 500 μm (e.g., ~ 2-5 millimeters). With a variable gap feature, one can use a large volume for large-volume operations, while still being able to conserve the amount of samples or reagents by executing the binding assay in a small volume.

Furthermore, by placing the reaction chamber in a substantially non-horizontal orientation and increasing the width of the gap between the chamber wall and the substrate during washes or soaks, for example, one may reduce the risk of destabilizing the microspots as a result of hydrodynamic forces generated from the movement of fluids along the surface with excessive pressure. With a large gap, one can guarantee that all surfaces on a substrate are well wetted, since the whole reaction chamber will be filled with solution.

The present invention also describes a method for using the chamber module in an automated machine. The method of performing biological experiments using fluids comprises: a) providing a cassette or chamber-module like that described herein; b)

providing one or more substrates having an array area containing at least one probe molecule (e.g., biomolecule); c) placing or introducing the planar substrate into the chamber-module; d) closing the chamber-module; e) introducing either a fluid medium into the chamber-module; and f) executing or performing an assay according to a predetermined protocol. The performing step may include a variety of further steps, depending on the particular assay protocol. Such additional steps, for example according to nucleic or genomic assays, may include soaking or pre-hybridization washing, target and probe-binding, post-hybridization washing and drying steps, such as by exposure to forced air or other gases. Alternatively, for proteomic applications, additional steps may include pre-blocking (to block non-specific binding of target(s) to surface areas other than microspots), or post binding washing and drying steps. In yet another embodiment, for detecting trace amounts of chemical or biological molecules, such as toxins in gaseous or liquid (e.g., air or water solution) environments, a pre-enrichment step may be added to the pre-blocking or post-binding washing or drying steps. Agitation or thorough mixing of samples reagents and other solutions, can increase binding between targets in the sample and probes-molecules on the microarray surface.

Additional features and advantageous of the present invention will be revealed in the following detailed description. Both the foregoing summary and the following detailed description and examples are merely representative of the invention, and are intended to provide an overview for understanding the invention as claimed.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is a schematic representation, showing an exploded view of individual components of a cassette or chamber module according to an embodiment of the present invention.

FIGURES 2A and 2B are schematic representations of a partially assembled cassette of Figure 1, showing relative alignment and positioning of the respective components. When fully assembled and ready for operation, the module should be closed, encasing a microarray substrate within, and fluid-tight according to the present invention.

FIGURE 3A is a view in three-quarter perspective of an end plate.

FIGURES 3B and 3C each shows a face-on view of the end plate of Figure 3A. Each figure depicts a possible configuration of the inner surface of a displaceable cover or component in the end plate.

FIGURE 4A is a perspective view of an end plate.

5 FIGURES 4B and 4C are cross-sectional views of the end plate of Figure 4A, showing fluidic channels and the movement of the displaceable component.

FIGURE 5A is a diagrammatic view of a fluid flow path through a circular or cylindrical laboratory chamber of the invention.

10 FIGURE 5B is a diagrammatic view of a fluid flow path through a preferred square or rectilinear laboratory chamber of the invention.

FIGURE 5C is a diagrammatic view of a second fluid flow path through an alternate square laboratory chamber of the invention.

15 FIGURE 6A is a schematic representation showing an exploded view of another embodiment of the present invention in which microarray substrates are arranged in an alternate fashion. The microarray substrates are placed within the cassette with their respective array surfaces (i.e., probe specimens) facing towards each other with the middle plate in between. According to variants of the embodiment, the opposing surfaces of the microarrays may be kept apart from each other by the width of a very thin ($\leq 50\text{-}150\text{ }\mu\text{m}$) separator or shutter.

20 FIGURE 6B represents a partially assembled cassette according to the embodiment of Figure 6A, with its individual components aligned and ready to be closed together.

25 FIGURES 6C and 6D are representations of two versions of a shutter or baffle in the middle plate for separating two microarray substrates according to an embodiment of Figure 6A.

30 FIGURE 7 shows an embodiment that uses centrifugal force to drain fluid away from and/or dry microarray substrates (e.g., microscope slides) by spinning on a vertical axis. Remote ends of the fluidic tubing, shown emerging from the chamber modules, can be inserted into the axial cylinder, such as in Figure 8. Two modules are shown in the present illustration, but additional numbers of modules can be attached to or inserted into the central drum or spindle at provided for slots (e.g., friction fit) or other means for ensuring a secure attachment, according to variations of the

embodiment. Although not illustrated, according to a variation of this embodiment, the chamber modules may be attached to the rotary spindle by a hinge, which permits the chamber module to fly upwardly and outwardly as centrifugal forces increase. A waste channel can collect or carry away fluids exiting from the reaction chamber.

5 FIGURE 8 represents an alternate configuration of the device shown in Figure 7. In this embodiment, the rotary spindle or cylinder is oriented horizontally. A number of chamber modules are arranged as extensions from the rotary drum, analogous to a "paddle wheel." A rotary drum and associated chamber modules comprise a carousel unit. Within the core of the rotary drum are a number of passages or channels for
10 delivering or removing fluids, potentially both gaseous and liquid. According to a variation, the surface of the rotary drum may have a number of ports or outlets into which the ends of fluidic tubing can plug to connect the passages in the drum to each chamber module. Each outlet or group of outlets can supply an individual module.

 FIGURE 9 depicts a larger embodiment of the invention, in which a number of
15 carousels are arranged in a line, side by side, for parallel and/or simultaneous processing of a number of assays. In some iterations of this embodiment, each carousel may be removed from a larger machine or system, such as shown in Figures 12-13, and replaced. More commonly, however, individual chamber modules are exchanged by unplugging the fluidic tubing from the drum of the carousel when an assay is finished
20 or the reaction chamber becomes otherwise contaminated. Hence, it is envisioned that chamber modules are preferably designed to be fungible and either recyclable or disposable.

 FIGURE 10 is a schematic cross-sectional view of an embodiment of a rotary drum cylinder. According to the embodiment, passages for reagent and waste fluids are
25 located near the center of the cylinder.

 FIGURE 11A is a schematic cross-sectional representation of a rotary drum-cylinder having a number of slots formed in the drum cylinder. Each slot is configured to receive a cassette or chamber module having internal fluid passages, which are connected to a number of fluid couplers or taps along one end of the cassette. The fluid
30 couplers may be plugged into complementary coupler members associated with fluid passages in the drum cylinder. To prevent leakage of fluids, the fluid couplers can be

either self-sealing or may include a valve associated with the cassette, fluid passages of the drum cylinder, or both. Other passageways are located along the periphery.

Figure 11B is schematic cross-sectional representation of a variation of the embodiment according to Figure 11A. The variation has a number of reservoirs
5 situated in the rotary drum cylinder. Each reservoir is accessible from the outer surface of the drum cylinder. Each reservoir can be filled with a samples or suitable reagent before performing an assay, and an associated regulator or valve (triangle) may be used to control the release of reservoir contents at specified times during an assay protocol.

FIGURES 12A and 12B shows two variations of an apparatus according to the
10 invention, for processing in parallel a plurality of chamber modules arranged according to an embodiment shown in Figures 7-9.

FIGURE 13 shows an embodiment of the machine of Figure 12A in which a computer (including associated hardware and software) controls and monitors the operations of the device and assay reactions. The computer may remotely operate the
15 machine and record data from the chamber modules over the duration of an assay protocol.

FIGURE 14 depicts a front elevation view of an example of a control panel employed with the electronic/control module to set and regulate various parameters of an assay protocol such as in hybridization incubation.

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DETAILED DESCRIPTION OF THE INVENTION

Section I – Definitions

Before describing the present invention in detail, this invention is not necessarily limited to specific compositions, reagents, process steps, or equipment, as
25 such may vary. As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. All technical and scientific terms used herein have the usual meaning conventionally
30 understood by persons skilled in the art to which this invention pertains, unless context defines otherwise.

The term "biological molecule" or "biomolecule" refers to any kind of biological entity, including, such as, oligonucleotides, DNA, RNA, peptide nucleic acid (PNA), peptides, polypeptides, protein domains, proteins, fusion proteins, antibodies, membrane proteins, lipids, lipid membranes, cellular membranes, cell lysates,
5 oligosaccharides, or polysaccharides, or lectins.

The term "biospot" or "microspot" refers to a discrete or defined area, locus, or spot on the surface of a substrate, containing a deposit of biological or chemical material.

The term "complement" or "complementary" refers to the reciprocal or
10 corresponding moiety of a molecule to another. For instance, receptor-ligand pairs, or complementary nucleic acid sequences, in which nucleotides on opposite strands that would normally base pair with each other according to Watson-Crick-base pair (A/T, G/C, C/G, T/A) correspondence.

The term "fluid" or "film of fluid" as used herein refers to a material or medium
15 that can flow such as a gas, a liquid, or a semisolid.

The term "functionalization" as used herein relates to modification of a solid substrate to provide a plurality of functional groups on the substrate surface. The phrase "functionalized surface" as used herein refers to a substrate surface that has been modified to have a plurality of functional groups present thereon.

20 The terms "nucleoside" and "nucleotide" are intended to include those moieties which contain not only the known purine and pyrimidine bases, but also other heterocyclic bases that have been modified. Such modifications include methylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles. In addition, the terms "nucleoside" and "nucleotide" include those moieties that contain
25 not only conventional ribose and deoxyribose sugars, but other sugars as well. Modified nucleosides or nucleotides also include modifications on the sugar moiety, e.g., wherein one or more of the hydroxyl groups are replaced with halogen atoms or aliphatic groups, or are functionalized as ethers, amines, or the like. As used herein, the term "amino acid" is intended to include not only the L-, D- and nonchiral forms of
30 naturally occurring amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), but also

modified amino acids, amino acid analogs, and other chemical compounds which can be incorporated in conventional oligopeptide synthesis, e.g., 4-nitrophenylalanine, isoglutamic acid, isoglutamine, ϵ -nicotinoyl-lysine, isonipecotic acid, tetrahydroisoquinoleic acid, α -aminoisobutyric acid, sarcosine, citrulline, cysteic acid, 5 t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, 4-aminobutyric acid, and the like.

The term "probe" refers to either a natural or synthetic molecule, which according to the nomenclature recommended by B. Phimister (*Nature Genetics* 1999, 21 supplement, pp. 1-60.), is immobilized to a substrate surface. The corresponding 10 microspots are referred to as "probe microspots," and these microspots are arranged in a spatially addressable manner to form a microarray. When the microarray is exposed to a sample of interest, molecules ("targets") in the sample selectively and specifically binds to their binding partners (i.e., probes) in the microarrays. The binding of a "target" to the microspots occurs to an extent determined by the concentration of that 15 "target" molecule and its affinity for a particular probe microspot.

The term "receptor" refers to a molecule that has an affinity for a ligand. Receptors may be naturally-occurring or man-made molecules. They may be employed in their unaltered state or as aggregates with other species. Examples of receptors which may be employed according to this invention may include, but are not limited to, 20 antibodies, monoclonal antibodies and antisera reactive with specific antigenic determinants, pharmaceutical or toxin molecules, oligonucleotides, polynucleotides, DNA, RNA, peptide nucleic acid (PNA), peptides, polypeptides, protein domains, proteins, fusion proteins, cofactors, lectins, oligosaccharides, polysaccharides, viruses, cells, cellular membranes, cell membrane receptors, and organelles. Receptors are 25 sometimes referred to in the art as anti-ligands. A "ligand-receptor pair" is formed when two molecules have combined through molecular recognition to form a complex.

The term "sample" as used herein relates to a material or mixture of materials, typically, although not necessarily, in fluid form, containing one or more components of interest.

30 The term "substrate," "microarray substrate" or "substrate surface" refers to a solid or semi-solid material, which can form a stable support for immobilized probe molecules. The substrate surface can be selected from a variety of materials. For

instance, the materials may be biological (e.g., plant cell walls), non-biological, organic (e.g., silanes, polylucine, hydrogels), inorganic (e.g., glass, ceramics, SiO₂, gold or platinum, or gold- or platinum-coated), polymeric (e.g., polyethylene, polystyrene, polyvinyl, polyester, etc.), or a combination of any of these, in the form of a slide,
5 plate, film, particles, beads or spheres. Preferably, the substrate surface is two dimensional or flat for the printing of an array of biospots, but may take on alternative surface configurations. For example, the substrate may be textured with raised or depressed regions, or it may be porous. Preferably, the substrate surface will have thereon at least one kind of functional or reactive group, which could be amino,
10 carboxyl, hydroxyl, thiol groups, amine-reactive groups, thiol-reactive groups, Ni-chelating groups, anti-His-antibody groups, or the like. More preferably, the surface will be optically transparent.

As used herein, the term "target(s)," "target moieties," "target analyte," "biological target," or "chemical target" refers to a solvated particle, molecule or
15 compound of interest in a sample that is to be detected and identified. Suitable targets include organic and inorganic molecules, biomolecules. In a preferred embodiment, the target may be an environmental pollutant (e.g., such as pesticides, insecticides, toxins, etc); a chemical (e.g., solvents, polymers, organic materials, etc); a therapeutic molecule (e.g., therapeutic and abuse drugs, antibiotics, etc); a biomolecule (e.g.,
20 hormones, cytokines, proteins, peptides, protein domains, fusion proteins, nucleotides, oligonucleotides, DNA, RNA, peptide nucleotide acids (PNA), genomic DNA, lipids, lipid membranes, carbohydrates, cellular membrane antigens, receptors or their ligands, etc); whole cells (e.g., pathogenic bacteria, eukaryotic cells, etc); a virus; or spores, etc.

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Section II – Description

Recent advances in molecular biology have opened opportunities for the biological, pharmaceutical, medical, and other research communities in the areas of drug discovery and development, diagnostic assays, pathogens identification, and other
30 biological research. The explosion in genetic information has produced a need for assays and equipment and methods for performing high-capacity, molecular biological- or chemical-binding assays, such as nucleic acid hybridization assays, faster and more

consistently. Researchers have come to recognize microarrays as useful, high-throughput research tools to measure a variety of biological or biochemical functions, and the microarray format is likely to remain a key research tool into the foreseeable future. Hence, a need exists to miniaturize, automate, standardize and simplify such assays for clinical uses or pharmaceutical drug discovery with high capacity and high throughput, as well as other uses in research laboratories.

The present invention addresses these needs by providing an automated reaction chamber system for solid-phase biological assays, such as microarrays. The present invention will be discussed in detail by reference to one of its functional units, a chamber module, however, the invention is not necessarily so limited. A plurality of such modules may be incorporated into a larger apparatus or machine according to a variety of embodiments, which will be described more fully below.

A. Cassette

In general, a basic operational unit of the invention is a cassette or chamber module **10** configured to accommodate at least one microarray substrate. Each cassette or chamber module has at least three major sections: a first end plate **12**, a second end plate **14** and a middle plate **16**, comprising structural units of a frame assembly, such as shown in Figure 1 and the other figures. Within the cassette **10**, near the center of each plate is a region **18** available to accommodate a microarray substrate **1**. Microarray substrates **1** with active or functionalized surfaces **2** (e.g., microscope slides) for attaching biological molecules **3** can be placed into a receptacle space **18a** between the middle plate **16** and each end plate **12**, **14**. As Figures 1 and 2 illustrate, the three plates define a fluid reaction chamber **11**, in which one can performing biological experiments using fluid media. Preferably, one can simultaneously execute a biological binding-assay, such as hybridization or ligand-binding assays, on two or more microarray substrates.

Each of the end plates has an interior **20** and exterior surface **22**, and the middle plate has a side α and a side β . Located on either an interior surface of each end plate, or either side α or side β of the middle plate are several alignment pins **24a**, which are employed to align the end plates to the middle plate. Also, there are a number of holder-members **24b**, which help to hold a microarray substrate in position within the

chamber. For instance, the particular example of Figure 1 shows six alignment members, two placed to either side of the central region 18 of each plate and two at a lower end of each plate. Also around the end plate are small stopper pins or cushions 24b, numbering four in the example, one at each corner (see detailed Figs. 3B & 3C), which serve as holder members to prevent the probe-immobilized surface of a microarray substrate from bumping or rubbing against the inner surface of the end plate. This same precautionary feature can also be located on the middle plate at corresponding sites. Around the central region of either each end plate or middle plate are a number of gaskets or seals 26 to form a fluid-tight seal around the reaction chamber between the plates when the cassette is fully assembled and ready to use. According to some embodiments, the individual plates of the parts of the cassette may be clamped together. Alternatively, the cassette can be held together using conventional mechanisms (e.g., lap joint for a friction fit, interlocking joint, or hinge), either within or at the interface of the plates, which engage with one another to form a seal.

Figures 2A and 2B illustrate the relative alignment and positioning of each component of the cassette and the microarray substrates encapsulated within. According to the present embodiment, an alignment member 24 should be able to mate with its corresponding counterpart (e.g., male-female), wherein one fits within the other. (Persons of the art will appreciate that alternate engagement mechanisms also may be employed.) The resulting fully-assembled cassette should have a size sufficiently large to accommodate at least one microarray substrate 1, preferably, more simultaneously, and contain microfluidic channels and passages in the respective end or middle plates. A variety of factors may dictate the physical size of each cassette, including the physical dimensions of microarray substrates, or the desirable reaction volume, or availability of reagents or solutions to be used for a particular assay.

The middle plate 16 in the embodiment of Figure 1 has a relatively large aperture 28 traversing a central region of the plate, from side α to side β , and several alignment pins 24 on either side of the opening. A gasket surrounds the opening and is used for sealing and to fix the dimensions of the overall chamber and recess where the microarray sits (>1 mm). The opening permits common fluids, such as soak or wash solutions, to flow and intermix between the reaction areas on either side of the middle

plate. As described more fully with respect to alternative iterations and embodiments in the sections to follow, this feature may be advantageous and convenient for performing comparative assays using at least two microarray slides simultaneously under the same empirical conditions.

5 Figures 1 and 2 depict a number of fluidic inlet **30** and outlet **32** tubing or microchannels located on the outside, leading either towards or away from the chamber module through each end plate. Fluids are delivered to and removed from the reaction chamber **18** through these microchannels. Tubing **31** and **33** are intended to carry fluids or solutions that may be used in common for all parameters of an assay protocol, such as soak and wash solutions. Hence, tubing **31** and **33** feed through each end plate, enabling the fluids to fill and drain the entire reaction chamber. Tubing **35** and **37** serve to carry fluids or reagent solutions aimed at a specific microarray or sub-array of microspots on the microarray substrate. These conduits **35**, **37** terminate at a moveable cover or displaceable component **40**.

15 In the embodiment shown in Figures 1 and 2, a displaceable cover is located on an interior surface of each end plate, preferably in or near a center region. Alternatively, a displaceable cover may be situated on either side α or side β , or both, of the middle plate. Figures 3A, 3B, and 3C are views of an end plate **12** in detail. Figure 3A shows a three-quarter perspective view. Figure 3B and 3C are face-on views of the interior surface **20**, showing two possible designs for the displaceable cover. In the center of the end plate is a displaceable component **40** in an elongated hexagon shape, such as depicted in the examples of Figures 3B and 3C. On the end plate, below and on either side of the displaceable component **40**, are alignment **24a** and holder **24b** members, which help to position and maintain in place a microarray substrate (e.g., a microscope slide), shown in phantom outline. Fluidic inlet **31a** and outlet **33a** are terminal ports in the chamber module of corresponding fluidic tubing **31** and **33**, respectively. Fluidic inlet port **31a** and outlet port **33a** are located at opposing portions – respectively, an upper or top portion and lower or bottom portion – of the end plate **12**. Fluidic inlet **35a** and outlet **37a** are terminal ports in the chamber module of corresponding fluidic tubing **35** and **37**, respectively. Fluidic inlet port **35a** and outlet port **37a** are located, respectively, at an upper or top portion and lower or bottom portion of the movable or displaceable cover **40**. On the inner surface **20a** (the surface

that faces the middle plate) of the displaceable component is a flexible gasket **46** to ensure a good contact and fluid-tight seal with a microarray substrate. The gasket **42** or seal runs along the edge or perimeter of the displaceable cover where it divides from the rest of the end plate. The inlet and the outlet port for specific sample or reagent
5 (e.g., hybridization buffers) are located inside the perimeter defined by this gasket. The gasket, according to an embodiment, can be compressed or expanded depending on the pressure applied to move the displaceable cover.

The displaceable component can move either towards or away from a microarray substrate, depending on the processes in a given binding assay. For the
10 hybridization or binding step of an assay, the displaceable component can be pressed against a microarray substrate to define a micro-volume hybridization or reaction zone. The gasket **42** is used to fix the height (e.g., ≤ 150 or $250\ \mu\text{m}$) of the zone against the substrate **1** and hence the total volume for the reaction assays. The displaceable component simulates a conventional cover slip of a microscope slide. Hence, the
15 displaceable component should be properly aligned, such as to cover the area of the probe-containing surface on the microarray substrate, in a single unit or for each individual sub-array (Fig. 3B and 3C). This feature allows users to minimize the sample volume required and increase the reaction efficiency.

Conversely, in the pre-hybridization or blocking process, or in the post-
20 hybridization washing/drying process, the displaceable component is moved away from the microarray substrate, allowing a larger volume for better efficiency, respectively, for the pre-blocking or post-washing; thereby, increasing the overall assay performance. Some benefits include, for example, a reduced background noise, or reduced risk of destabilizing the microspots due to hydrodynamic forces generated
25 from the movement of fluids along the surface, etc.

Commonly in high throughput assays, a number of sub-arrays, each containing a set of probe molecules in microspots, either of the same or different type from those in a neighboring sub-array, are immobilized on a single microarray substrate. Figure 3B shows a design for a displaceable cover that can accommodate at least one sub-array
30 area of a microarray, and may be used in situations in which the entire surface of the microarray substrate is subjected to a common binding reagents or treatment. Figure 3C shows an example of an alternate design, in which the inner surface **20a** of the

displaceable cover **40** has been sub-divided to provide an individual chamber **45** for each sub-array area of a microarray. Different reagents can be applied to each sub-array area. The dividing partition(s) **47** may align either vertically, as shown, for side by side chambers, or horizontally for top and bottom chambers, or a combination of both for a grid.

Figures 4A, 4B, and 4C are another set of views of an end plate. Figure 4B and 4C are cross-sectional views along a vertical line A-A, which bisects the end plate of Figure 4A. Figure 4B shows the displaceable component at an initial position within the confines of the end plate, and a microarray substrate **1**, in phantom outline, resting on alignment/holder members **24**. Holders or stopper cushions **24b** prevent the microarray surface from contacting the end plate directly. Fluidic inlet **31**, **35** and outlet channels **33**, **37** are also depicted. In Figure 4C, the displaceable component has moved towards the microarray, and the gasket **42** comes either very close to or in contact with the microarray surface. The gasket, preferably, is flexible and responsive to an applied external force. The movable platform **40** has a tight seal all around to prevent liquid from leaking out when it is not pushed forward. A baffle or diaphragm **44** runs around or behind the displaceable component **40** preventing fluids from leaking out from around or behind the component's edges, when the component is deployed against the microarray.

Animation of the displaceable component may be achieved in a number of ways. In rudimentary embodiments, which would require a greater degree of human intervention, the displaceable component can be moved manually. Preferably, however, in automation-friendly embodiments, one may employ a pneumatic cylinder with compress air, or hydraulic pressure. For example, a pneumatic system can fill an air bladder, which pushes the displaceable component against the specimen- or probe-containing surface of the microarray. After completion of a binding assay, the air bladder can deflate restoring the larger gap between the inner surface of the displaceable member and the slide array. In alternate embodiments, the displaceable component may be moved by means of a tension spring, a solenoid, a screw, a motor or other electrical or mechanical agency.

In some embodiments, fluid flows into and out of the reaction chamber through capillary passageways located within the body of an end plate or middle plate, in a

portion of the plate structure, surrounding or peripheral to a hybridization or reaction area, of the chamber. Figures 5A through 5C each depict an iteration of the flow path of a liquid medium, respectively, through capillary passages around the reaction area 11. As the fluid is pushed or drawn through toward inlets, it takes the path of least resistance, and portions of the fluid begins to flow across the open interior of the chamber, as denoted by the fluid paths, assisted in part by gravitational, centrifugal, or centripetal forces. (In particular, if the fluid is introduced from the side opposite the direction of force.)

Figure 5A shows cross-section of an embodiment, a circular chamber 50 in which a microarray substrate is positioned. The chamber has capillary inlet port 52 and capillary outlet port 54 positioned midway along the circumference of opposing sides 55, 56 of the chamber. Fluid flows into the chamber through capillary passages 55, 56 and then into the chamber. Fluid flowing through the inlet port 52, traversing the chamber 50 as indicated by arrows 57-58. The outlet port 54 may be shut to allow fluid to accumulate and fill the chamber. Figure 5B shows another cross-section of a preferred embodiment of the invention having a square or rectilinear configuration. As with the liquid in Figure 5A, the entering liquid 61 takes the path of least resistance flowing at first along the edge of the chamber. However, the liquid begins to separate from the square or rectilinear boundary path 63, 64 and heads across the open chamber toward the outlet port 62, as indicated by flow path arrows 65-66. The liquid path shown distributes the liquid throughout the chamber, wetting over the entire surface of any array placed within the chamber. Figure 5C shows a modification of the preferred embodiment, diagramming an alternate flow path through a square chamber 70 having inlet ports 71 and outlet port 72 positioned at opposing corners of the chamber. As with the previous flow path, fluids flowing through inlet port 71 first attempt to traverse the chamber 70 by travelling along the chamber boundary as denoted by arrows 73 and 74. As the liquid is traversing along that path, however, the fluid is inclined to take the path of least resistance toward outlet tube 72, and portions of the fluid begin to flow from wherever they are along the boundary path in a straight line toward the outlet tube, as denoted by flow path arrows 75-76. In some embodiments it may be preferable for all three plates to have capillary passages for introducing reagents and draining

waste. Variations or combinations of passage designs for different uses and fluids are included within the scope of the invention.

One may fabricate microfluidic channels within the cassette components using a variety of micromachining techniques, among which include, for instance, drilling,
5 laser ablation, photolithography and either isotropic or anisotropic etching, ultrasonic machining with a preformed cutting tool, or computer numerically controlled (CNC) machining.

Likewise, one may create microfeatures on one or more of the inner surfaces of each cassette, preferably on the surface of an end plate and/or a displaceable component
10 of either the end or middle plate. The roughened or textured surface helps to generate turbulence in the fluid flow and aids in mixing. A surface with a fine herringbone or striated pattern is one example, such as described by A.D. Stroock *et al.*, in "Chaotic Mixer for Microchannels," Science, vol. 295, pp. 647- 651, 25 January 2002, incorporated in its entirety herein by reference. Further, agitation of fluids also can
15 increase mixing or binding kinetics. Fluids may be pumped back and forth over a localized reaction space near the surface of a microarray and/or throughout the whole volume of the chamber module.

Microarray substrates can be placed within the cassette with their respective biospot-presenting surfaces oriented to face either toward the end plates, as depicted in
20 Figure 1, or toward each other with the middle plate in between, as in Figures 6A and 6B. In certain other embodiments, it is envisioned that as many as four microarray slides can be processed simultaneously in a single cassette. That is on each side of the middle plate, two slides can be placed back-to-back, with the probe-free surfaces facing each other and array surfaces, each respectively, oriented towards the end and middle
25 plates.

Figure 6A depicts an exploded perspective of a second embodiment of a cassette. In this second embodiment, when the chamber module is assembled, as illustrated in Figure 6B, the two probe-bearing surfaces *2* of the microarray substrates *1* will be in very close proximity to each other. The middle plate *16* and end plates *12, 14*
30 are modified to effectuate an adjustable volume in between the two array surfaces, as well as prevent the opposing surfaces from directly touching each other.

At the center of the middle plate **16**, a separator **80**, such as a shutter, baffle, or gasket, is sandwiched in between the two array surfaces. Preferably, a separator is very thin, on the order of a few microns (≤ 250) microns thickness (e.g., ~ 1 or $2\text{-}250\text{ }\mu\text{m}$, preferably about $5\text{-}175\text{ }\mu\text{m}$, $25\text{-}100\text{ }\mu\text{m}$, or $10\text{-}50\text{ }\mu\text{m}$). The thickness of the separator delimits the size of gap or reaction zone **81** between the substrates. The separator according to certain embodiments functions as a displaceable component, since the thickness of separator may be adjustable. Figure 6C shows a face-on view of a separator **80** with a hexagon-shaped opening **83**. The aperture, preferably, is large enough to surround or frame an array of probe molecules on the surface **2** of a microarray substrate **1**, analogous to the displaceable cover on an end plate. Figure 6D is a second iteration for a separator, with a rectilinear opening **83a**. The volume of the reaction zone is adjustable. When used as a hybridization chamber the zone is a capillary space. Referring to Figure 6A, fluidic conduits **84a**, **84b**, to which tubing may be attached, extends from upper **85a** and lower **85b** ends of the middle plate **16**, though the structure of the middle plate to the top *x* and bottom *y* parts of the separator, respectively. The fluidic conduits permit one to introduce fluids directly into the reaction area **81** towards which the probe-bearing surfaces are directed. Often, for hybridization or other biological applications, the amount of sample or reagent needed is quite small, with volumes on the order of microliters or less. Although designed to conveniently handle small volumes, the present device is, however, not necessarily so limited, since large volumes can also be handled with ease.

With regard to the end plates, in the second embodiment, instead of a displaceable component **40**, as described above, each end plate can have a vacuum or pneumatic agency **86**, such as shown in Figures 6A and 6B. The microarray substrate **1** could be drawn back away from the middle plate **16** toward the outer end plate **12**, **14** using a vacuum to create suction against the back of the microarray substrate. Once a substrate is pulled back away from the middle plate, the volume between the two substrates increases. This feature makes both washing and mixing operations easier, since fluids that pass through do not need to compete with fluidic tension and capillary forces, associated with spaces of small volumes. Also, once fluid surface tension is broken, one can more easily remove the microarray substrates from the chamber module after an assay.

One may fashion the structural components of a chamber module from a variety of materials, which may include, for example: inorganic glass, glass-ceramic, or ceramic materials (e.g., borosilicate (Pyrex[®]), boroaluminosilicate, aluminosilicate glasses, fused silica, or metal oxides); metal materials (e.g., stainless steel, titanium, or aluminum); inorganic or polymer (e.g., Plexiglas, or poly(ethylene-co-norbornene), known commercially as Topas from Celanese AG) or composite materials; or combinations of such materials. Preferably, the material is chemically inert and can withstand higher temperatures of up to about 100-105°C. Parts made from metals may have a coating of Teflon[®] (e.g., FEP (fluorinated ethylene-propylene) and PFA (perfluoroalkoxy alkane)) or like materials to modify or protect the surfaces exposed to assay or reaction environments. Some polymer components, fittings, tubing, and associated accessories have proven superior to stainless steel in many analytical applications. Examples of other possible polymer materials, selected according to their desired properties, may include the following: polypropylene, polyphenylene sulfide (PPS), polyphenylsulphone, acetal resin (e.g., Delrin[®]), ethylene-chlorotrifluoroethylene (ECTFE), polychloro-trifluoroethylene (PCTFE), ethylene-tetrafluoroethylene (ETFE), polyetheretherketone (PEEK[™]), or ultra-high molecular weight polyethylene (UHMWPE). (See, *Technical Resources*, Upchurch Scientific, <http://www.upchurch.com/PDF/Lit/Technical.pdf>.) The gasket that seals against the microarray substrate preferably is made from an elastomeric material, such as silicone rubber, poly(dimethylsiloxane), or combinations thereof, which can be self-adhering to the substrate.

Over the course of an assay protocol the chamber module may be subjected to various chemical and physical conditions in the biological reactions. For example, for hybridization applications using DNA microarrays the incubation temperature typically may vary from around 40°C to about 65°C, and acidic or alkaline reagents. Hence, the preferred materials from which a chamber module is fabricated should be tolerant of temperatures up to about 100-105°C, and be chemically inert or resistant to solvent solutions. Other potential assay protocols, however, may require lower temperatures. Thus, depending on the choice of material, chamber modules should be able also to withstand hotter or cooler temperatures in certain situations or particular applications.

In some embodiments, the end and middle plates are preferably transparent, allowing for optical observation.

The gaskets or sealing agents can be fabricated out of a nature or synthetic rubber, such as neoprene, cork, paper, silicone, polysiloxane, waxes, paraffin or Parafilm® (American National Can, Chicago, IL), or other polymer or elastomeric materials used for sealing. Preferably, the sealing material, like the rest of the chamber module, should be able to tolerate temperatures of up to about 100-105°C without deteriorating sealing functionality or losing structural integrity.

B. Carousel

Having described the basic cassette or chamber module in detail, the invention also so envisions incorporating a number of such chamber modules with a shaft to create a larger system or apparatus, such as depicted in any one of Figures 7-9. At least one chamber module **10** extends outward from a central axis of a rotary drum body **100**. The rotary drum may take any shape, but preferably has a circular or rectilinear cross sectional profile. A rotary drum having a combination or grouping of at least one chamber module is referred to as a “carousel” or “carousel unit” **102**. A carousel unit, at a minimum, includes a single chamber module and associated rotary drum, and can be as large or as wide in physical dimensions as one would desire to satisfactorily accommodate a number of microarray substrates. Actual physical dimensions (i.e., height, length, width, diameter, or circumference) of each carousel may vary depending on the number and sizes of chamber modules associated with the rotary drum. Preferably, the carousel **102** has at least two chamber modules **10** on the rotary drum **100**, like in Figures 7, 8, 11A or 11B, for balance. A carousel **102** may be relatively long along the length of the drum **100** and is able to carry, length-wise, two or more chamber modules **10** in a line, like in Figure 9. A carousel may be employed as a part of a larger device or machine, as described in detail below.

The rotary drum **100** may be oriented in a number of potential configurations. In one version the unit is oriented vertically, such as in Figure 7, wherein the apparatus is part of a centrifuge – looking like a spindle with wings, or a washing-machine agitator. According to a second variation, the rotary drum can be oriented horizontally, such as in Figures 8 and 9, wherein the chamber modules **10** and cylinder **100** assembly may be arranged like a “paddle wheel.” A series of chamber-module and cylinder units

could be either stacked one atop another according to the first version, or arranged side by side or end to end according to the second version of Figures 8 and 9. Different designs or modifications with multiple carousels (chamber-module and rotary drum) units can help researchers process a large number (e.g., dozens, hundreds, or thousands) of assays in parallel. As persons of the art will appreciate, a large number of samples and analytes may be processed simultaneously using multiple chamber modules. This feature may be useful for mass commercial applications. (Actual numbers of assays may depend on the number of units operating.)

Rotation of the drum or cylinder can aid fluid mixing and drainage, depending on the speed or rate of revolution (rpm) at which the cylinder is spun. The revolution speed can be tailored or controlled so that it meets the parameters of specific bio-binding assay processes. A low or slower speed is more helpful in mixing, since movement of the unit would tend to agitate fluids within. A high or faster speed, on the other hand, creates greater centrifugal force, which pushes fluids toward peripheral extremes of the chamber modules and out drain conduits.

The rotary drum can have either a hollow or dense construction. That is, in the first instance, a drum can be fabricated with an interior composed of mostly open space, defined by an exterior surface **101** or curved shell, and having structural or support elements within to hold a number of passageways running through the body of the drum stable and open space. According to this type of construction, a user with access to the interior space can make alterations and adjustments to suit his uses fairly easily, just by adding, eliminating, removing or rearranging the internal passages.

Alternatively, in the second instance, the drum may be fabricated out of a solid material or a combination of materials, as long as the rotary drum contains within its structure a number of passages to accommodate passageways **104** for a variety of uses. Figures 10, 11A and 11B are cross-sectional views of rotary drums according to the second type of construction, with a dense drum body **103**. For both types of construction, channels or tubes **105** of various sizes can carry fluids, either for ingress **105a**, egress **105b**, or waste uses. Other conduits **107** for electrical wiring, mechanical elements, or gas passages are also integrated within the drum cylinder. The drum preferably is made from materials (e.g., glass, ceramic, stainless steel, aluminum, other metals, inorganic,

polymer or composite materials) that are durable and can tolerate physical stresses associated with operation.

Cassettes **10** can be attached or detached fairly easily from the rotary drum cylinder **100**, such as illustrated in Figures 7-9, and in particular Figures 11A and 11B.

5 A chamber module may be secured in its place on the drum cylinder by means of several attachment mechanisms, such as practiced in the mechanical arts. For instance, a tab or flange on a peripheral portion of the module could be plugged into a mating slot **106** in the drum. Alternatively, friction-fit clamps or a variety of other systems could hold the chamber in place. Such a feature would allow the module to be snapped
10 on or off fairly quickly. In Figures 11A and 11B a slot is provided in the drum to receive a cassette.

Once secure, tubes and other conduits can be plugged into either the cylinder drum or the chamber module. A variety of fluid transfer elements (e.g., couplers, fittings, connectors, lines, valves, adapters, flanged and flangeless ferrules and/or pump
15 accessories) such as used for gas or liquid chromatography, can be incorporated in the chamber modules to facilitate its joining to the drum cylinder. According to one embodiment, such as in Figures 7-9, the chamber modules may have fluidic conduits either pre-fabricated or connected on each end plate or middle plate, ready to be plugged into corresponding outlets on the cylinder drum. According this embodiment,
20 inlet and outlet conduits are located either in a major surface of an end plate **12**, **14**, or along an edge or end of the middle plate **16**.

In a second, more preferred, embodiment, shown in Figures 11A and 11B, the chamber modules can be attached or detached from the rotary drum cylinder by way of a "snap-in" or "plug-in" approach. According to this method of assembly, a cassette is
25 first prepared with a microarray substrate, closed, and aligned with the slot on the drum cylinder. In a section of the slot are arranged a group of ports for fluids. On an edge or side of the cassette **10** are located a number of fluid coupler members **110** or taps, which serve as inlets and outlets to the reaction chamber **11** and fluid passageways **111**, **113** in each module. Preferably, the inlets and outlets of the chamber module are
30 located on the same end of the middle plate. The inlet channel **112** introduces fluids or samples into the reaction chamber **11**. Starting at one end or along a side of the reaction chamber, the fluid passes over the whole surface of the microarray enclosed

within the reaction chamber. An outlet channel *114*, beginning at an end of the reaction chamber away from the inlet, runs in a side passage back towards the drum cylinder *100* and allows sample solution to exit from the chamber. Arrows *115*, *117* indicate the general direction of fluid circulation. In addition to using centrifugal force to remove liquids from the reaction chamber, such as in a drying step, in some embodiments one may also employ either a vacuum to draw the liquids out, or inject a gas to push liquids out of the reaction chamber concurrently with the spinning of the centrifuge.

As the cassette *10* is aligned with the slot *106*, each fluid coupler *110* also lines up with a corresponding port *116* in the drum *100*. The fluid couplers *110* or taps, associated with a peripheral edge of the cassette, are arranged to insert themselves easily into the ports. Each tap and port forms a junction between the two. A chamber module, once connected, can use these ports as inlet and outlet conduits through which to access the fluid passages *104* that carry sample solutions or common washing/soaking/pre-blocking solutions in the drum cylinder. According to this embodiment, each port in the drum leads to a channel *118*, which preferably, branches off of a main passageway *104*.

When the module is disengaged from the drum, the cassette and port both should not leak fluid. Any suitable type of coupler may be used. For instance, a two-part coupler may seal both the cassette and the ports in the drum cylinder. The port or cassette may have a valve (e.g., unidirectional check valve) or regulator to control fluid flow through the junction of the two. Alternatively, when disconnected, the outlets can automatically re-seal (e.g., elastomeric gaskets) the conduit to prevent leakage. Also a part of the branch or the coupler may be relatively long and narrow, forming a capillary that self-seals by surface tension if a liquid.

In certain embodiments, one may have one or more reservoirs, located in a section of either the end plate or drum cylinder. For example, the reservoir may be situated within the end plates of a chamber module, either as part of an enlarged section of a capillary passage (Figure 5), or separate from the capillary passage. In other embodiments, the reservoir, connecting fluid channel, and/or other passages may be incorporated within the rotary drum (Figures 10, 11A and 11B). The reservoir *120* can hold reagents or samples, until they are ready to be use during an assay. Connecting conduits *122* link the chamber module *10* to the reservoir *120* or other fluid

passageways **104**. Typically, a reservoir will service at last one corresponding chamber module. A reservoir **120** proximal – preferably immediately adjacent – to a chamber module **10** can supply reagent or samples when a regulator **124** or some other mechanism activates and releases the content **121** (e.g., either a liquid or gas) of the reservoir into a waiting reaction chamber **11** through the conduit **122**, such as depicted in Figure 11B. Each reservoir may sit in a cavity in the drum and may be accessed from either inside or outside the drum, either by automation or manually, such as by a syringe or pipette, to introduce or re-supply reagents. In automated embodiments, a computer or other electro-mechanical agents can control the release of the contents of these reservoirs, as well as its rotation and other processes or mechanics of the device. At an appropriate time, for example, reagents from the reservoir would be injected into the reaction chamber. A reservoir could have a replaceable container **126** that allows different reagent or samples to be exchanged for different assays. Such a design may also avoid potential inappropriate mixing of fluids or contamination from reusing a permanent container.

In washing operations, for example, volumes of wash media larger than that which the reservoir can hold typically will be used. Hence, outboard or remote containers may be connected to the drum. The washing media can be piped through the channels or tubing in the cylinder body. Waste stream can be carried away in other passages to be either collected or discharged. According to an embodiment, the device may include passages for delivering air or other gas media, which may be pump into each reaction chamber to dry the substrate, according to the needs and parameters of specific assays. Specific configurations of the fluidic plumbing system will depend on the particular design of a carousel unit, and the type of assay to be performed.

If the chamber module have already been connected to the rotary drum of the carousel, then it is preferred that the chamber module be set at an upright position when loading the chamber, such as show in Figures 1-6. The microarray substrates may be slipped into the appropriate receptacle spaces, one either side of the middle plate. Users of the device, however, may also pre-assemble a chamber module, with microarray substrate enclosed, before plugging the chamber module into the carousel as described above. To pre-assemble the chamber module, according to one possible process, one would provide a first end plate. Then, place a microarray substrate in the

provided position between alignment and holder members. Attach the middle plate. As an option, one may place a second microarray substrate against the middle plate, and close with the second end plate. The three plates with microarray substrates in between can be snapped shut and secured fluid-tight.

5 A carousel unit may function either individually or in common with other units. Located at each end of a carousel unit is a stationary housing having a pilot hole to receive the carousel, and for the fluid-handling and/or drive mechanisms (e.g., a spindle *127*, a drive shaft, a cam, belts, gears or flywheel, bushing, etc.), which powers the rotation of the carousel. The mechanical details relating to tubing or channels
10 connecting fluid containers with the fluid passageways inside of drum cylinder are discussed below. Preferably, the stationary housing, with its associated fluid and rotary mechanisms, is located at a final terminus of an extended series of interconnected carousel units, like that of Figure 9. The mass of the rotary drum in each carousel should be physically supported without interfering with its rotary motion. Drum
15 cylinders can be mounted on supports at each end of the cylinder, in a manner allowing free rotation of the drum or carousel. For embodiments of extended length, such as of Figure 9, bushings or rotating bearings *128* can be placed around the drum cylinder to help support the drum.

20 C. System Device

As mentioned in the foregoing discussion, it is envisioned that a number of carousel units can be used in a larger machine or system device. Thus, the invention also provides an automated, multiplexed machine *150* for high-throughput processing of microarray-based assays. The machine comprises a housing or cabinet *152*, defining
25 within a space or compartment *204* for holding at least one, preferably plurality of carousels *102* as described herein, each having at least one, preferable two or more, chamber modules. Figures 12A and 12B depict two examples according to an embodiment of such a machine *150*, containing a number of carousels *102* within the housing *152*. In Figure 12A the carousels are oriented horizontally, while in Figure
30 12B, the carousels are set vertically. The housing may be made of a double-walled construction for thermal insulation and to accommodate a plethora of fluid tubing. The carousel-holding compartment *154* in the housing *152* can be accessed through a door,

removable panel, hatch, or cover **156**. According to certain embodiments, each carousel or a number of carousels may be mounted to a stationary housing, as described in the previous sub-section, above. One end of the rotary drum cylinder may be first inserted into a pilot hole having a bearing structure, and the second end slipped into a
5 separate slot.

According to the depicted embodiment, a number of containers or reservoirs **158**, such as tanks, vessels, or flasks storing gases, reagents, or solutions are located in an adjacent compartment **160**. Each container is hooked-up to a fluid handling system, which supplies the chamber modules. One may use a fitting or coupler to connect the
10 rotary drum with a fluid conduit linked to the stationary container. The coupler should have a configuration in which it can accommodate the relative rotation of the drum cylinder on one side, and the stationary fluid conduit on the other side of the coupler, without the stationary conduit becoming twisted or loose when the drum rotates. The drum cylinder at a first end can have a stepped design, with a number, preferably a
15 plurality, of inter-nesting lands or grooves to transfer fluid from stationary containers, connected through lines, to passages in the rotary drum. Preferably each kind of fluid will have its own coupler unit, so as to prevent contamination. Bearings at each terminus of the drum cylinder can help support the carousel once mounted in the machine. Other features may include a sliding mount or housing to hold the drum
20 cylinder, a pilot hole and rotary drive mechanism, and associated lip or radial seals. At the other or second end, in some designs the arrangement at the first end may be repeated, or there may be either a common or separate waste reservoirs to collect spent fluids, or an end plate with a return connection to the first end. Specific details for building such a machine and its variations are familiar to persons in the mechanical
25 arts.

A worker can easily exchange or substitute one container for another by unplugging the container from its linkage to either the fluid conduit or the coupler, when different reagents or fluids are to be used or when the content of the container is consumed. Preferably, the fluid system is pressurized to help fluid flow, and may be
30 driven by a pump mechanism. It is envisioned that the present machine may entertain a variety of inter-linkages between fluid passages, using valves or regulators within the machine to control the timing and kind of fluid passing therethrough.

According to certain adaptations, not only are the fluid containers, which supply the system, designed to be interchangeable, but the individual cassettes as a whole or parts of chamber modules, as well as carousel units are also fungible. For example, once a chamber-module and cylinder unit has been used or becomes contaminated and unfit for further assay processing, a carousel unit may be removed from the machine for cleaning or other servicing. The unit is disconnected from its corresponding stationary housing, taken out of the larger machine, and replaced with a fresh carousel unit.

According to yet another embodiment, each chamber module is design to be temperature controllable. Heating or cooling elements, or other temperature control devices may be integrated into the chamber-module. For example, heating coils or plates can be incorporated within the end plates of each chamber module to provide individualized incubation, in certain assays, for each microarray substrate.

Alternatively, the entire cabinet or housing of the machine in Figures 12-13, may be constructed and heated in a manner such as described in U.S. Patent No. 5,360,741, to J.E. Hunnell, the disclosure of which is incorporated herein by reference. A heat pump such as a blower may be mounted in a plenum space of the housing and this blower is joined in gas outflow relationship to a gas discharge unit for introducing heated gas into the interior volume of the carousel-holding compartment. Alternatively, a pump for heated water also may be employed to provide radiant heat through the ceiling, floor, or wall surfaces of the carousel-holding compartment. Hence, the carousel-holding compartment should be thermally insulated in particular embodiments. As the embodiments of Figures 12A, 12B, and 13 show, the present machine **150** has a number of displays **146** for monitoring the temperature of the individual chamber modules **10** in the carousel-holding compartment **204**. Temperature control and heating or cooling features afford users of the device the ability to regulate thermal conditions within the chamber, and provide an environment conducive for performing certain assays, such as DNA hybridization.

According to a multiplexed embodiment, the automated machine is envisioned to be able to process simultaneously within a number of sets of carousels a corresponding number of sets of microarray substrates. A single carousel may have at least two, preferably four or more, chamber modules. Actual numbers of chamber modules per carousel, however, are not necessarily limited. For practical

considerations, depending on the carousel's radius and arc size of the rotary drum cylinder, the number of chamber modules which may extend outward from a single carousel can be, for example, up to 8, 10, 12, 16, 20, 24 or more. Each module may contain within it at least one, preferably two or more, microarray substrates. For
5 multiplexed operations, individual sets of carousels may be programmed to operate independently or in concert with adjacent carousels with respect to the particular reagents, buffer solutions, washing or other common fluids used. Each set of microarray substrates may undergo a distinct binding assay protocol and/or use several different reagents. For instance, two or more carousels are arranged within a machine
10 as generally described herein, and depicted schematically in Figures 12-13. One carousel may be used to perform a first type of binding assay with a first specific reagent species, while a second carousel, its neighbor, may be processing a second type of binding assay with a second different reagent.

To operate the larger machine **150**, a computer **215** or other electronic or
15 electromechanical means can be integrated, such as in Figure 13. In addition, one may oversee the various parameters of an assay using methods and equipment familiar to persons skilled in the electrical or mechanical arts and laboratory testing instruments. For instance, one may employ a thermocouple to monitor temperature, or use a linear transducer to measure the fluid flow rate of the reagents, gases and solutions. These
20 instruments preferably are integrated within the machine **150**. Assay parameters may be set, or data may be controlled or displayed with a panel such as depicted in Figure 14.

Figure 14 is an example of a display panel, typically used for hybridization purposes. As shown in Figure 14, the monitoring/control display **148** on panel **190**
25 features an on-off pressure switch **192**, which may be a membrane switch or other suitable switch device which is manually actuatable. At the upper portion of the on-off switch **192** is a display light **291** which is illuminated when the electronic/control module is turned on. At the left-hand portion of the display is a digital temperature display **194** and to the right of such digital temperature display are temperature set
30 point switches **196** and **198**, switch **196** upon application of manual pressure thereto upwardly or increasingly incrementing the set point temperature by a predetermined

increment, e.g., 1°C., and the switch **198** corresponding decreasing or decrementing the set point temperature value, as display on display **194**.

On the right-hand portion of display **148** is provided a temperature/time readout display **200**, and to the left of such readout display are time-switch **214** and temperature
5 **212**, which may be selectively depressed to choose the display modality of display **200**, as showing the time or the temperature, as desired.

Below temperature/time display **200** are sets switches **216** and **218**. Switch **216** is a time set switch which is adapted for fast incrementing of the time, to select a rough time setting value, and switch **218** is a corresponding slow set switch for more closely
10 selecting the desired time, so that switches **216** and **218** are in effect "rough" and "fine" time set switches.

In use, the display **148** may be actuated by the on-off switch **192**, and the set point temperature for the incubation chamber may be set by means of set point switches **196** and **198** to display the desired set point temperature on display **194**.

Subsequent to establishment of a desired set point temperature for the hybridization chamber, the time parameter of the hybridization operation and any appropriate time-temperature schedule (in the event the hybridization is carried out under more than one temperature value) is set by means of the time set switches **216** and **218**, and the temperature is correspondingly reset for different phases of the multi-
20 temperature hybridization sequence, by means of temperature set switches **196** and **198**.

During the subsequent operation of the hybridization apparatus, the time switch **214** and temperature switch **212** may be alternatively actuated to display the actual temperature and elapsed time. The display **148** shown in Figure 14 may be associated with suitable microprocessor and microcircuitry elements of a type well known and
25 within the skill of the art and the field of biomedical instrumentation. The temperature settings and time selections may thus be stored in the microprocessor and employed during the hybridization to selectively adjust the intensity of heating in the heating element associated with gas delivery means, as for example a resistance heating element disposed in the inlet or outlet passage of the blower.

D. Method of Use

In another aspect, the present invention details a method for researchers to perform consistently and efficiently wash, mixing, and other steps of any microarray-based applications. The method essentially comprises: a) providing a device defined by a frame having a first end plate, a middle plate, and a second end plate as described
5 here; b) placing at least one microarray substrate into a holder in the chamber module, with a specimen or probe-presenting array surface oriented either towards or away from the middle plate; c) closing and securing the chamber module; and d) performing an assay protocol involving fluid media. The method may further comprise repeating the various steps of the assay protocol(s).
10

One may illustrate the function of a chamber module by way of an example drawn from genomic or nucleic acid hybridization assays. Nucleic acid hybridization assays are advantageously performed using a probe array technology, which target nucleic samples bind onto immobilized nucleic (e.g., oligonucleotide) probes. The
15 detection limit of a nucleic acid hybridization assay is influenced by the sensitivity of the detection device and the amount of target nucleic acid available to be bound. Hybridization using oligonucleotides or cDNA arrays need to be performed in a volume in which a small amount of target DNA or other nucleic acid can efficiently anneal to the immobilized probes. For diagnostic assays, DNA molecules are used in minute ($\leq$
20 500 picomole) quantities. In conventional practice, it can take several (e.g., ten of) hours for hybridization to be substantially completed at low levels of target nucleic acid concentration. The present invention, it is envisioned can reduce the amount of time required and increase the assay efficiency.

A microscope slide prepared with microarrays is placed in the recess on each
25 side of the middle plate of a chamber module. According to the embodiments described herein, slides may rest on the two lower alignment pins near the bottom of the reaction chamber. In preferred embodiments, a vertical position is favored for either loading substrates or draining fluids from the chamber module. The slides are positioned with their respective probe-containing surfaces oriented either toward the middle plate or toward the end plates. As Figures 1 and 2 illustrate, the middle
30 platform is sandwiched between the first and second (left and right) end plates, which are fastened together to form a fluid-tight seal. A battery of solutions and reagents may

be introduced into the chamber. For instance, a soak/wash solution is injected into the whole chamber through a soak/wash solution inlet, at top, and the solution is drained from an outlet at bottom. The inlet and the outlet also may serve as the inlet and the outlet for air, nitrogen or other inert gas to dry the slides. In another embodiment, the slides can be dried by spinning using centrifugal force, such as in Figure 7, which depicts two chamber-modules attached to an upright column or drum that can rotate.

When the chamber module includes a displaceable component or cover set in an end plate, one can adjusted the volume of the chamber over the course of an assay protocol. Initially, for instance, the displaceable component is kept within the end plate so that the distance (gap) between the surface of the displaceable component and a substrate surface printed with an array can be a relatively large. A relatively large gap is suitable for soaking, washing or mixing operations which use relatively large volumes of fluid. For washing, this gap distance can be increase to permit the soak or wash solution to fill and drain properly from the chamber in an efficient manner. A larger gap-size (e.g., $> 250 \mu\text{m}$, preferably $\sim 1\text{-}2 \text{ mm}$) within the device can reduce fluid pressure associated with capillaries, allowing the chamber to be filled quickly, and guaranteeing that the whole chamber will be filled with solution so that every surface on the substrate will be wetted, i.e., comes in contact with the solutions. Surfaces that are not wetted tended to produce an uneven wash and leave higher background signals.

During hybridization or target-probe binding, the displaceable cover can move to a predetermined distance from the surface of the microarray substrate to create a low or micro-volume reaction chamber against the surface of the micrarray substrate. The displaceable component of the first and second end plates is moved or displaced such that the hybridization chamber gasket comes in contact with the surface of a slide and forms a liquid-tight hybridization chamber. For hybridization, the width of the gap can be reduced to capillary dimensions, $\leq 500 \mu\text{m}$ (preferably ~ 5 or $10\text{-}250 \mu\text{m}$, or more preferably about $25\text{-}150 \mu\text{m}$). Subsequently, hybridization buffer is introduced into the hybridization chamber and subject to a constant temperature for incubation. At the completion of hybridization, the two displaceable components retreated to their original positions, thus enlarging the distance between array surface and the inner surface of the displaceable component. Next, a wash solution is introduced for a post-hybridization

wash, and air or nitrogen gas can be used to dry the slides. Finally, the slides are taken out of the module for data analysis.

Likewise for embodiments where array surfaces are oriented to face each other across the middle plate, such as illustrated in Figure 6A, fluid can be pumped, injected
5 or otherwise introduced through passages in the middle plate into the reaction chamber to perform the preparatory work, pre- and post-reaction washes, and other reaction/assay steps. Located on the outer surface of the middle plate may be one or more access ports, which serve as either inlet or outlet passages for fluids, either liquid or gas, and to which conduits may be attached to connect the reaction chamber. These
10 passages permit the introduction and removal of fluids into a reaction zone between the two adjacent microarray substrates.

E. Examples of Array Species & Their Associated Binding-Assays

The devices of the invention help the performance of binding assays detect
15 targets in samples. The target analytes preferably bind with probe molecule(s) immobilized on a surface of a substrate. Even though the inventive device and method has been described in the context of nucleic acid reactions for illustrative purposes, the present invention is not necessarily limited only to nucleic acid hybridization assays. Alternate applications that may benefit from the present invention may include other
20 biological binding-assay formats, such as protein-membrane arrays, which may be useful for the emerging field of proteomics. A description of several examples of different kinds of microarrays and their various uses follow.

When a set of probe nucleic acid molecules with known sequences are tethered or immobilized onto a surface in confined locations in a DNA microarray, the target
25 analyte can be a nucleic acid. The target sequence may be a portion of a gene, a regulatory sequence, genomic DNA, cDNA, RNA including mRNA and rRNA, or others. The target sequence is preferred used to be in a single-stranded format; however, the target sequence in a double stranded conformation (e.g., genomic DNA) may be used after denaturation. The target sequence is preferably labeled with a
30 detectable moiety or moieties, such as fluorescence dye molecule(s) to allow detection of the binding of the target sequence to the probe microspots directly using fluorescence imaging techniques, or with biotin moieties in which a sequential

detection step using labeled anti-biotin or anti-biotin coated gold nanoparticle is required for detection the binding of the target sequence to the probe microspots (Bao *et al.* Anal. Chem. 2002, 74, 1792-1797). A "probe nucleic acid" or "probe sequence" refers to a nucleic acid sequence with known sequence or defined sequence. Preferably, the probe nucleic acid is a cDNA, a oligonucleotide with defined sequence, or a modified oligonucleotide with defined sequence. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramidate (Beaucage *et al.*, Tetrahedron 1993, 49,1925), peptide nucleic acid backbones and linkages (Egholm, J. Am. Chem. Soc. 1992, 114,1895); Nielsen, Nature, 1993,365,566). As will be appreciated by those in the art, all of these nucleic acid analogs may find use in the present invention.

According to an embodiment, a pharmacological compound or ligand may be the target compound or ligand when using a probe protein microarray. A "target compound" or "target ligand" in this context refers to a chemical or biochemical or biological compound whose identity, abundance, or binding affinity and specificity is to be detected. The target compound can be synthetic, naturally occurring, or biological produced. The target compound may be an addictive or abused drug, a pharmaceutical drug candidate, a chemical (an organic or inorganic compound, including ionic salt), a biochemical (e.g., synthetic lipids, oligosaccharides, peptides, amino acids, nucleotides, nucleosides, etc), or a biological (e.g., a naturally occurring lipids, a protein, an antigen, an antibody, a growth factor, etc.). The target compound may be an activator, an inhibitor, an effector, a binding partner, or an enzyme substrate of the probe protein(s). The target compound can be part of a selected or random compound library. A "probe protein" or "probe polypeptide" refers to a polypeptide with a known sequence. The probe proteins may be obtained from natural sources or, optionally, be overexpressed using recombinant DNA methods. The probe proteins may be either purified using conventional approaches or un-purified (e.g., cell lysates). The probe protein includes, but not limited to, intracellular proteins, cell surface proteins, soluble proteins, toxin proteins, synthetic peptides, bioactive peptides, and protein domains. Examples of intracellular proteins include, but are not limited to: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, kinases,

phosphoproteins, and mutator transposons, DNA or RNA associated proteins (for example, homeobox, HMG, PAX, histones, DNA repair, p53, RecA, ribosomal proteins, etc.), electron transport proteins (e.g., flavodoxins); adaptor proteins; initiator caspases, effector caspases, inflammatory caspases, cyclins, cyclin-dependent kinases, cytoskeletal proteins, G-protein regulators, small G proteins, mitochondria-associated proteins, PDZ adaptor proteins, PI-4-kinases, etc.. Recombinant proteins of unknown functions may also be used. Applicable cell surface proteins include, but are not limited to: G-protein coupled receptors (e.g., adrenergic receptor, angiotensin receptor, cholecystikinin receptor, muscarinic acetylcholine receptor, neurotensin receptor, galanin receptor, dopamine receptor, opioid receptor, serotonin receptor, somatostatin receptor, etc), G proteins, ion-channels (e.g., nicotinic acetylcholine receptor, sodium and potassium channels, etc), receptor tyrosine kinases (e.g., epidermal growth factor (EGF) receptor), immune receptors, integrins, and other membrane-bound proteins. Mutants or modifications of such proteins or protein functional domains or any recombinant forms of such proteins may also be used. Toxin proteins include, but are not limited to, cholera toxin, tetanus toxin, shiga toxin, heat-labile toxin, botulinum toxin A & E, delta toxin, pertussis toxin, etc. Toxin domains or subunits may also be used. In this embodiment, the probe protein microarrays may be used to identify small molecule-binding proteins (Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, S., Houfek, T., *et al.* "Global analysis of protein activities using proteome chips" *Science* 2001, 293, 1201-2105), or used to measure protein kinase activities (Houseman, B.T., Huh, J.H., Kron, S.J., Mrksich, M. "Peptide chips for the quantitative evaluation of protein kinase activity", *Nature Biotechnology* 2002, 20, 270- 274), or used to profile compounds for pharmacological uses (binding affinity, selectivity, and specificity) and screen compounds (Fang, Y., *et al.* "Membrane protein microarrays". *J.Am. Chem.Soc.* 2002, 124, 2394-2395; and Fang, Y. *et al.* "Membrane biochips" *BioTechniques*, 2002, 33, S62-S65).

In a further embodiment, the target analyte may be an antigen, a hormone, a cytokine, an immune antibody, a protein, a lipid, or a mixture of un-purified cell lysate, when a probe antibody microarray is used. By "target biologicals" herein means a biological from a biofluid or an organelle or a living cell whose identity/abundance is to be detected. The probe antibody includes, but not limited to, an immunoglobulin (e.g,

IgEs, IgGs and IgMs), a therapeutically or diagnostically relevant antibodies (e.g.,
 antibodies to human albumin, apolipoproteins including apolipoprotein E, human
 chorionic gonadotropin, cortisol, a-fetoprotein, thyroxin, thyroid stimulating hormone,
 antithrombin; antibodies to antiepileptic drugs (phenytoin, primidone, carbamazepine,
 ethosuximide, valproic acid, and phenobarbital), cardioactive drugs (digoxin, lidocaine,
 5 procainamide, and disopyramide), bronchodilators (theophylline), antibiotics
 (chloramphenicol, sulfonamides), antidepressants, immunosuppressants, abused drugs
 (amphetamine, methamphetamine, cannabinoids, cocaine and opiates)), an antibody to
 any viruses (e.g., antibodies to orthomyxoviruses such as influenza virus,
 10 paramyxoviruses (e.g., respiratory syncytial virus, mumps virus, measles virus),
 adenoviruses, rhinoviruses, coronaviruses, reoviruses, togaviruses (e.g., rubella virus),
 parvoviruses, poxviruses (e.g., variola virus, vaccinia virus), enteroviruses (e.g.,
 poliovirus, coxsackievirus), hepatitis viruses (including A, B and C), herpesviruses
 (e.g., Herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr
 15 virus), rotaviruses, Norwalk viruses, hantavirus, arenavirus, rhabdovirus (e.g., rabies
 virus), retroviruses (including HIV, HTLV-I and -II), papovaviruses (e.g.,
 papillomavirus), polyomaviruses, and picornaviruses, and the like), and anthrax, etc.),
 an antibody to bacteria (e.g., antibodies to a wide variety of pathogenic and non-
 pathogenic prokaryotes of interest including *Bacillus*; *Vibrio*, e.g., *V. cholerae*;
 20 *Escherichia*, e.g., Enterotoxigenic *E. coli*, *Shigella*, e.g., *S. dysenteriae*; *Salmonella*,
 e.g., *S. typhi*; *Mycobacterium* e.g., *M. tuberculosis*, *M. leprae*; *Clostridium*, e.g., *C.*
botulinum, *C. tetani*, *C. difficile*, *C. perfringens*; *Corynebacterium*, e.g., *C. diphtheriae*;
Streptococcus, *S. pyogenes*, *S. pneumoniae*; *Staphylococcus*, e.g., *S. aureus*;
Haemophilus, e.g., *H. influenzae*; *Neisseria*, e.g., *N. meningitidis*, *N. gonorrhoeae*;
 25 *Yersinia*, e.g., *Y. lambia*, *Y. pestis*; *Pseudomonas*, e.g., *P. aeruginosa*, *P. putida*;
Chlamydia, e.g., *C. trachomatis*; *Bordetella*, e.g., *B. pertussis*; *Treponema*, e.g., *T.*
palladium; and the like)), an antibody to bacteria toxin (e.g., antibodies to diphtheria
 toxin, anthrax toxin, tetrodotoxin, saxitoxin, batrachotoxin, grayanotoxin, veratridine,
 actonitine, scorpion, sea anemone venom, scorpion charybdotxins, dendrotoxins,
 30 hanatoxins, sea anemone toxins, hololena, calcicludine, bungarotoxin, cholera toxin,
 conantokin, etc).

In certain embodiments, the probe antibody arrays may be used for protein profiling, measurement of protein abundance in blood, measurement of cytokine abundances, detection of bacteria toxins in samples (such as environmental water, or food resources), as well as capture of leukocytes/phenotyping leukemias. These target species may be present in any number of different sample types, including, but not limited to, bodily fluids including blood, lymph, saliva, vaginal and anal secretions, urine, feces, perspiration and tears, and solid tissues, including liver, spleen, bone marrow, lung, muscle, brain, etc. Conversely, the "probes" can also be antigens, in which the antigen arrays may be used for reverse immunoassay to measure immuno-antibodies and allergens.

In another embodiment, a carbohydrate microarray having oligosaccharides, polysaccharides, or oligosaccharides-derived lipids (e.g., gangliosides) immobilized on to a surface at defined locations may be used to detect carbohydrate-binding protein target(s) in a sample (Fukui, S., Feizi, T., Galustian, C., Lawson, A.M., and Chai, W. "Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate-protein interactions" *Nature Biotechnology*, 2002, 20, 1011-1017), or for identifying cross-reactive molecular markers of microbes and host cells (Wang, D., Liu, S., Trummer, B.J., Deng, C., and Wang, A., "Carbohydrate microarrays for recognition of cross-reactive molecular markers of microbes and host cells" *Nature Biotechnology*, 2002, 20, 275-281), or for identifying specific viruses or bacteria or spores.

The present invention has been described both in general and in detail by way of examples. Persons skilled in the art will understand that the invention is not limited necessarily to the specific embodiments disclosed. Modifications and variations may be made without departing from the scope of the invention as defined by the following claims or their equivalents, including equivalent components presently known, or to be developed, which may be used within the scope of the present invention. Hence, unless changes otherwise depart from the scope of the invention, the changes should be construed as being included herein.

CLAIMS

We Claim:

1. A device for performing biological experiments using fluids comprising:
a first end plate, a middle plate, and a second end plate, defining parts of a frame assembly;
each of said end plates having an interior and exterior surface, and said middle plate having a side α and a side β ;
a fluid-tight seal formed between each end plate and said middle plate;
a reaction chamber for receiving at least a microarray substrate, serviced by a number of fluid passages in said plates; and
having either a displaceable component or a separator.
2. The device according to claim 1, wherein said fluid passages have inlet and outlet ports.
3. The device according to claim 1, wherein said reaction chamber has an inlet and an outlet situated opposite relative to each one other within said chamber.
4. The device according to claim 1, wherein said fluid passages extend from said end plate.
5. The device according to claim 1, wherein each of said end plates on its interior surface, or said middle plate on either said side α or said side β , has a number of either
(a) alignment members, for aligning each of said end plates with said middle plate, or
(b) holder members, for holding said microarray substrate in position;
6. The device according to claim 1, wherein said reaction chamber can contain a plurality of microarray substrates simultaneously.
7. The device according to claim 6, wherein said reaction chamber can process as many as four microarray substrates simultaneously.

8. The device according to claim 1, wherein said displaceable component is situated in at least one of said end plates, or situated on either said side α or said side β or both of said middle plate.
9. The device according to claim 1, wherein said displaceable component is in or near a central region of said end plate.
10. The device according to claim 1, wherein said reaction chamber has microfeatures on least one wall surface.
11. The device according to claim 10, wherein said microfeatures are on the surface of either said end plate, said middle plate, or said displaceable component.
12. The device according to claim 11, wherein said microfeatures assists mixing of fluids.
13. The device according to claim 10, wherein said microfeature is a herringbone or striated pattern.
14. The device according to claim 1, wherein said microarray substrate has at least a surface with a biospot-presenting surface oriented toward said displaceable component.
15. The device according to claim 1, wherein a gap having an adjustable span is between said displaceable component and a biospot-presenting surface of said microarray substrate.
16. The device according to either claim 15, wherein the span of said gap is adjustable according to parameters for executing an assay protocol.
17. The device according to claim 1, wherein said middle plate has an aperture traversing from said side α to said side β .

18. The device according to claim 1, wherein said middle plate is either a non-porous or selectively porous barrier to fluids.
19. The device according to claim 18, wherein said middle plate incorporates a filter, a membrane, or a solid plate.
20. The device according to claim 1, wherein said separator is situated in said middle plate or one either said side α or said side β , or both.
21. The device according to claim 20, wherein said separator includes at least an opening sized to encompass each array surface.
22. The device according to claim 20, wherein said separator is a shutter.
23. The device according to claim 1, wherein said middle plate has a number of fluid passages integrated therein.
24. The device according to claim 1, wherein said microarray substrate has a biospot-presenting surface oriented toward said middle plate.
25. The device according to claim 1, wherein said device has a plurality of fluid passages, each to receive a different kind of fluid.
26. The device according to claim 1, further comprising a rotary drum having a surface with a number of outlets into which ends of said fluid passages of said device connects with passageways in said rotary drum.
27. The device according to claim 1, wherein fluidic movement across said biospot-presenting surface of said microarray substrate within said device can be directed either forward or backward using either pneumatic, hydraulic, or mechanical means.

28. The device according to claim 1, wherein said fluidic movement across said biospot-presenting surface of said microarray substrate within said device is controlled using either gravitational, centrifugal or centripetal forces.

29. The device according to claim 1, wherein a gasket forms a fluid tight seal with said microarray substrate.

30. The device according to claim 1, wherein said frame assembly has at least two couplers adapted to connect a fluid conduit with another.

31. The device according to claim 30, wherein at least one of said couplers connects to a conduit supplying a sample or a reagent into said reaction chamber.

32. The device according to claim 30, wherein said coupler connects to a waste channel.

33. A device for performing biological experiments using fluids comprising:
a first end plate, a middle plate, and a second end plate, defining parts of an assembly;
each of said end plates having an interior and an exterior surface and said middle plate having a side α and a side β ;
a fluid-tight seal formed between each end plate and said middle plate;
a reaction chamber situated within said assembly of plates;
a number of fluid passages within said assembly of plates;
a displaceable component forming a part of said reaction chamber wall, with a flexible, fluid-tight sealing element situated along a perimeter of said displaceable component; and
a number of fluid inlet and outlet channels situated in or around said displaceable component.

34. The device according to claim 33, wherein said reaction chamber has an inlet and an outlet situated opposite relative to each one other within said chamber.

35. The device according to claim 33, wherein said reaction chamber has microfeatures present on at least one wall surface to assist mixing of fluids.
36. The device according to claim 35, wherein said microfeature is a herringbone or striated pattern.
37. The device according to claim 33, wherein fluidic movement across said biospot-presenting surface of said microarray substrate within said device is directed forwards or backwards using either pneumatic, hydraulic, or mechanical pump mechanism.
39. The device according to claim 33, wherein said fluidic movement across said biospot-presenting surface of said microarray substrate within said device is controlled using either gravitational, centrifugal or centripetal forces.
40. The device according to claim 33, wherein said device is associated with rotary drum body having a number of passageways therein.
42. The device according to claim 33, The device according to claim 33, wherein said microarray substrate has at least a surface with a biospot-presenting surface oriented toward said displaceable component.
43. The device according to claim 33, wherein between said displaceable component and a biospot-presenting surface of said microarray substrate is an adjustable gap.
44. The device according to either claim 43, wherein said gap has a width adjustable according to parameters for executing an assay.
45. The device according to claim 33, wherein said gap has a width that ranges from about 5 microns up to about 5000 microns.

45. The device according to claim 33, wherein a gasket forms a fluid tight seal with said microarray substrate.

46. The device according to claim 33, wherein said assembly has at least two couplers adapted to connect with a fluid conduit.

47. The device according to claim 46, wherein at least one of said couplers is connected to a conduit supplying a sample or a reagent into said reaction chamber.

48. The device according to claim 46, wherein said coupler is connected to a waste channel.

49. The device according to claim 33, wherein said fluid passages extend from said end plates.

50. The device according to claim 33, wherein said displaceable component is in or near a central region of said end plate.

51. The device according to claim 33, wherein said middle plate has an aperture traversing from said side α to said side β .

52. The device according to claim 33, wherein said middle plate is either a non-porous or selectively porous barrier to fluids.

53. The device according to claim 52, wherein said middle plate incorporates a filter, a membrane, or a solid plate.

54. The device according to claim 33, wherein said separator is situated in said middle plate or one either said side α or said side β , or both.

55. The device according to claim 55, wherein said separator is a shutter.

56. The device according to claim 33, wherein said middle plate has a number of fluid passages integrated therein.

57. The device according to claim 33, wherein said microarray substrate has a biospot-presenting surface oriented toward said middle plate.

58. The device according to claim 57, wherein adjacent microarray substrates on either side α or side β of said middle plate have at least a surface with an array area oriented towards each other and said separator is in between.

59. The device according to claim 33, wherein said device has a plurality of fluid passages, each to receive a different kind of fluid.

60. The device according to claim 33, wherein a plurality of microarray substrates is processed simultaneously within said reaction chamber.

61. An apparatus for performing biological reactions, the device comprising:
a number of modules, each defined by a first plate, a second plate, and a third plate, said third plate being situated in between said first and second plates, a number of fluid passages situated in or extend from said first, second, or third plates, a reaction chamber within each module, and a fluid-tight seal formed between said third plate and said first and second plates, respectively; and
a rotary drum body having an axis of rotation and a drum surface, from which said modules extend outward.

62. The apparatus according to claim 61, wherein said rotary drum body has a number of fluid passageways in said drum body.

63. The apparatus according to claim 61, wherein said rotary drum body has a number of ports.

64. The apparatus according to claim 63, wherein each of said ports leads to a channel connected to at least one of said number of fluid passageways in said rotary drum body.
65. The apparatus according to claim 61, wherein said rotary drum body has an engagement mechanism for receiving said module.
66. The apparatus according to claim 61, wherein said rotary drum has a slot on said drum surface for said module to engage with said rotary drum body.
67. The apparatus according to claim 63, wherein said ports are situated in said slot on said drum surface.
68. The apparatus according to claim 61, wherein said modules extend outward in a radial fashion.
69. The apparatus according to claim 61, wherein each module has a set of fluid couplers for engaging with said fluid passageways in said drum body.
70. The apparatus according to claim 69, wherein at least one of said couplers is connected to a channel supplying a sample or a reagent into said reaction chamber.
71. The apparatus according to claim 69, wherein at least one of said fluid couplers is connected to a waste channel.
72. The apparatus according to claim 61, wherein said rotary drum is part of a centrifuge.
73. The apparatus according to claim 61, wherein said rotary drum body has a number of reservoirs situated within said drum body, and having a conduit connectable

to either said reaction chamber in said module, or said fluid passageways in said drum body.

74. The apparatus according to claim 61, wherein a number of either (a) alignment members, for aligning each of said end plates with said middle plate, or (b) holder members, for holding said microarray substrate in position are associated with a surface of either said first, second, or third plate, respectively.

75. The apparatus according to claim 61, wherein each module includes a temperature control device.

76. The apparatus according to claim 77, wherein said temperature control device is a heating or cooling element.

77. The apparatus according to claim 61, wherein said reaction chamber has an inlet and an outlet.

78. The apparatus according to claim 77, wherein said inlet and outlet are situated in opposing parts of said chamber relative to each other.

79. The apparatus according to claim 61, wherein said module has a displaceable component forming at least part a wall surface of said reaction chamber.

80. The apparatus according to claim 74, wherein said microarray substrate has at least a surface with a biospot-presenting surface oriented toward said displaceable component.

81. The apparatus according to claim 61, wherein a gap having an adjustable span is between said displaceable component and a biospot-presenting surface of said microarray substrate.

82. The apparatus according to either claim 81, wherein the span of said gap is adjustable according to parameters for executing an assay protocol.

83. The apparatus according to claim 61, wherein a plurality of microarray substrates are processed simultaneously within said reaction chamber.

84. The apparatus according to claim 61, wherein said reaction chamber has microfeatures on least one wall surface for mixing of fluids.

85. A system for high throughput, parallel processing of a number of binding-assays, the system comprising:

a machine having:

a) at least one carousel, said carousel further comprising:

(1) a number of cassettes,

each defined by a first plate, a second plate, and a third plate, said third plate being situated between said first and second plates; having

a fluid-tight seal between each of said plates;

a reaction chamber; connected to

a number of fluid passages in either said first, second, or third plates; and

(2) a rotary drum body having an axis of rotation and a drum surface, from which said cassettes extend outward;

b) a housing to contain said carousel; and

c) an electro-mechanical element for operating said machine or for monitoring said cassettes.

86. The system according to claim 85, wherein said rotary drum body has a number of fluid passageways in said drum body.

87. The system according to claim 85, wherein said rotary drum body has a number of ports.

88. The system according to claim 87, wherein each of said ports leads to a channel connected to at least one of said number of fluid passageways in said rotary drum body.

89. The system according to claim 85, wherein said rotary drum body has an engagement mechanism for receiving said cassette.

90. The system according to claim 89, wherein said rotary drum has a slot on said drum surface for said cassette to engage with said rotary drum body.

91. The system according to claim 85, wherein said ports are situated in said slot on said drum surface.

92. The system according to claim 85, wherein said cassettes extend outward in a radial fashion.

93. The system according to claim 85, wherein each cassette has a set of fluid couplers for engaging with said fluid passageways in said rotary drum body.

94. The system according to claim 93, wherein at least one of said couplers is connected to a channel supplying a sample or a reagent into said reaction chamber.

95. The system according to claim 93, wherein at least one of said fluid couplers is connected to a waste channel.

96. The system according to claim 85, wherein said rotary drum is part of a centrifuge.

97. The system according to claim 85, wherein said rotary drum body has a number of reservoirs situated within said drum body, and each reservoir having a conduit connectable either to said reaction chamber in said cassette, or to said fluid passageways in said drum body.

98. The system according to claim 85, wherein a number of either (a) alignment members, for aligning each of said end plates with said middle plate, or (b) holder members, for holding said microarray substrate in position are associated with a surface of either said first plate, second plate, or third plate, respectively.

99. The system according to claim 85, wherein each cassette includes a temperature control device.

100. The system according to claim 99, wherein said temperature control device is a heating or cooling element.

101. The system according to claim 85, wherein said reaction chamber has an inlet and an outlet.

102. The system according to claim 101, wherein said inlet and outlet are situated in opposing parts of said chamber relative to each other.

103. The system according to claim 85, wherein said cassette has a displaceable component forming at least part a wall surface of said reaction chamber.

104. The system according to claim 98, wherein said microarray substrate has at least a surface with a biospot-presenting surface oriented toward said displaceable component.

105. The system according to claim 85, wherein a gap having an adjustable span is between said displaceable component and a biospot-presenting surface of said microarray substrate.

106. The system according to either claim 85, wherein the span of said gap is adjustable according to parameters for executing an assay protocol.

107. The system according to claim 85, wherein a plurality of microarray substrates are processed simultaneously within said reaction chamber.

108. The system according to claim 85, wherein said reaction chamber has microfeatures on least one wall surface for mixing of fluids.

109. The system according to claim 85, wherein said axis of rotation is oriented either horizontally or vertically.

110. The system according to claim 85, wherein said electro-mechanical element includes a fluid-handling mechanism for storing or carrying fluid media, a drive mechanism for rotating said rotary drum body, or a computer for coordinating operations or monitoring assay conditions.

111. The system according to claim 85, wherein said machine further comprises a compartment to contain reagents and samples.

112. A method of performing biological experiments using fluids comprising:
- a) providing a chamber-module defined by a frame comprising: a first end plate, a middle plate, and a second end plate, having a fluid-tight seal in between said middle plate and each of said end plates, a reaction chamber, and a number of fluid passages within either said first or second end plates, or said middle plate;
 - b) providing one or more microarray substrates having probes immobilized on at least a section of a substrate surface;
 - c) introducing one or more of said substrates into said chamber-module;
 - d) closing said chamber-module;
 - e) introducing a fluid medium into said reaction chamber;
 - f) performing an assay according to an assay protocol.

113. The method according to claim 112, further comprises agitating said fluid medium by moving the fluid back and forth in said reaction chamber.

114. The method according to claim 112, further comprises draining said fluid medium from said reaction chamber.
115. The method according to claim 112, further comprises processing simultaneously a plurality of microarray substrates within a single chamber-module.
116. The method according to claim 112, further comprises orienting said microarray substrates such that a probe-presenting surface is facing either towards a displaceable component, which forms at least part of a wall surface of said reaction chamber, or said middle plate having a separator, or both.
117. The method according to claim 112, further comprises moving said displaceable component a distance towards or away from a probe-presenting surface of said microarray substrate.
118. The method according to claim 112, wherein a gap of variable width is formed between said displaceable component and said probe-presenting surface of said microarray substrate.
119. The method according to claim 118, wherein said gap has a width that ranges from about 5 μm up to about 5000 μm .
120. The method according to claim 120, wherein said gap has a width that ranges from about 20 μm up to about 2000 μm .
121. The method according to claim 112, wherein fluid movement within said chamber-module is assisted by either: pneumatic, hydraulic, mechanical, gravitational, centrifugal or centripetal forces.
122. The method according to claim 112, further comprises repeating step e of claim 112, or any one of the further steps in claims 113-117.

123. The method according to claim 112, further comprises controlling the temperature of said reaction chamber.
124. The method according to claim 112, further comprises engaging said chamber-module with a rotary drum body.
125. The method according to claim 124, wherein said chamber-module is fluidly integrated with fluid passageways situated within said rotary drum body.
126. The method according to claim 125, wherein said chamber-module and rotary drum body are part of a system for high throughput, parallel processing of a number of binding-assays, according to claim 86.

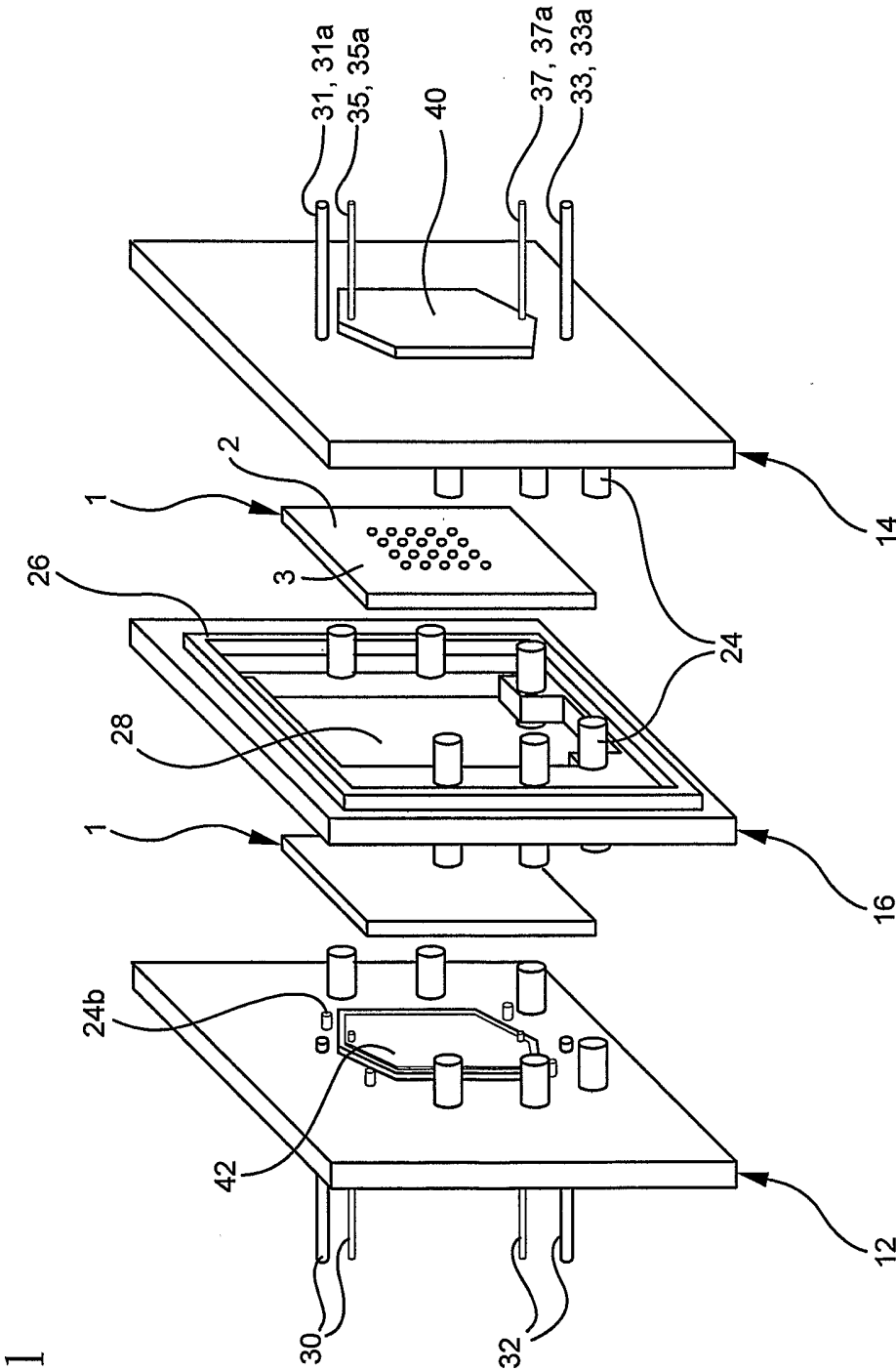


FIG. 1

FIG. 2B

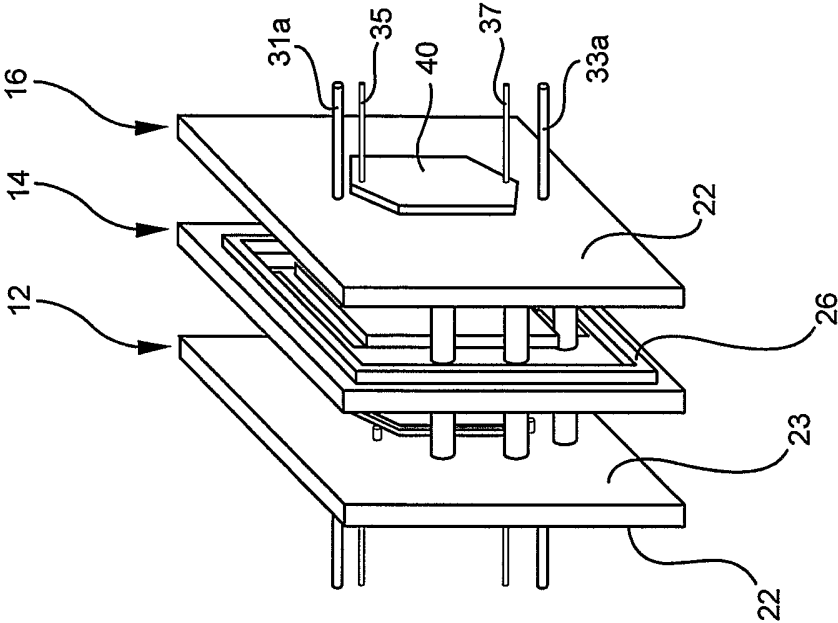


FIG. 2A

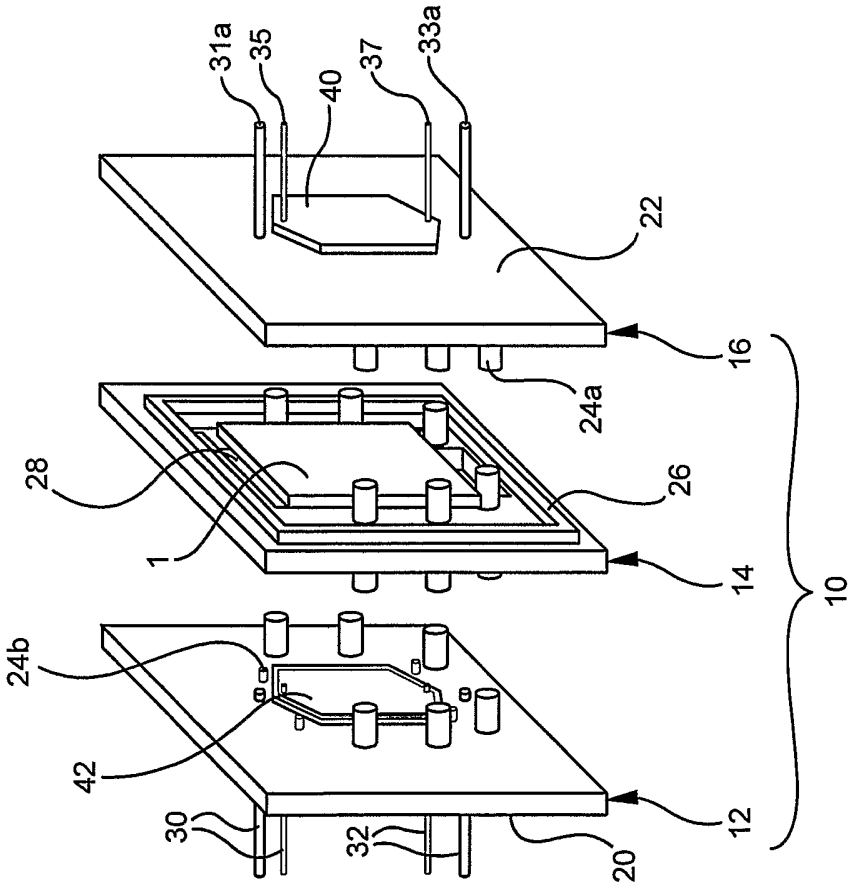


FIG. 3A

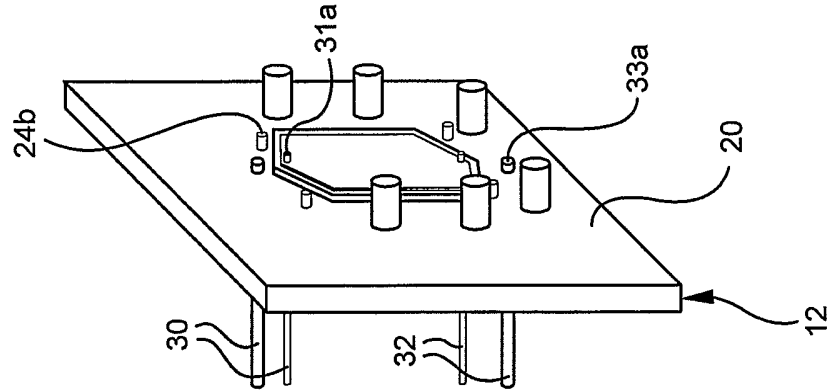


FIG. 3B

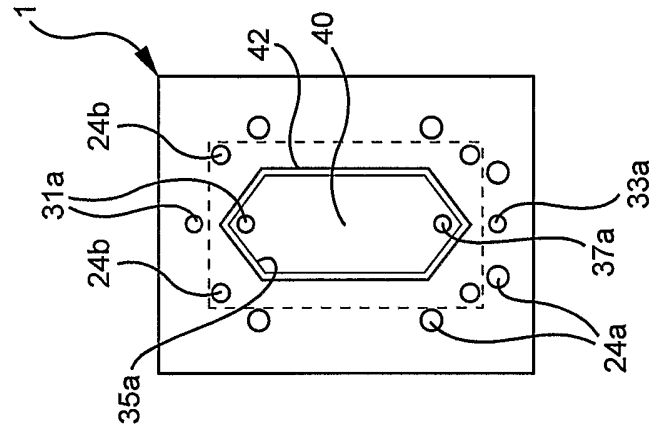


FIG. 3C

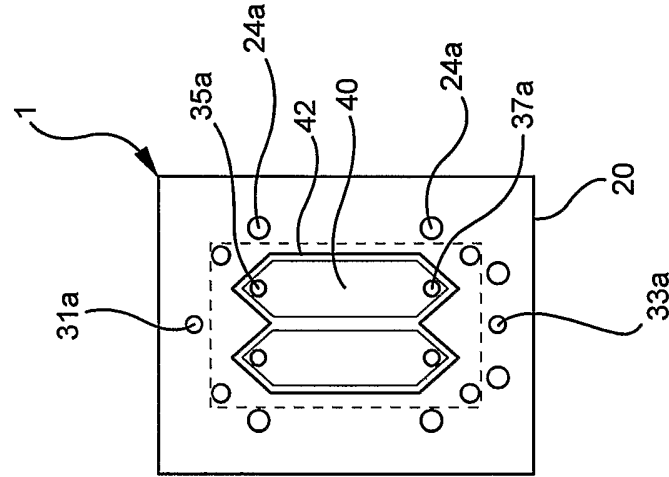


FIG. 4C

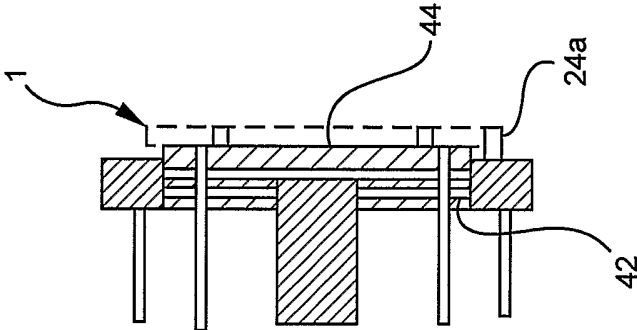


FIG. 4B

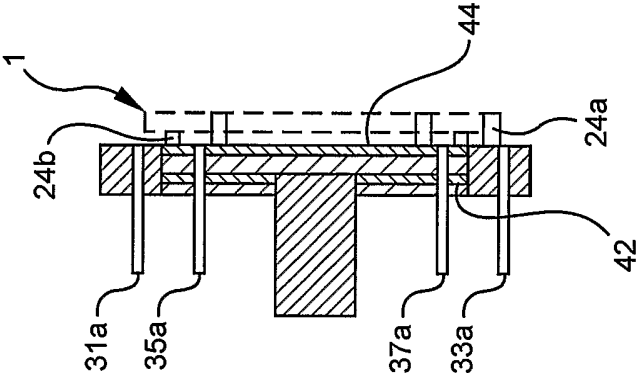
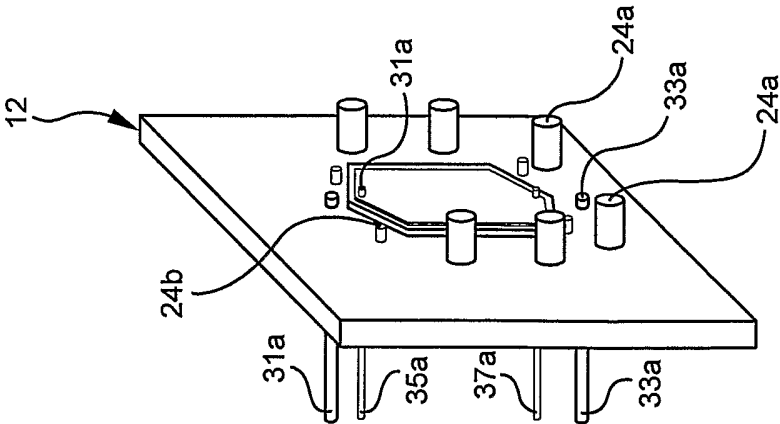


FIG. 4A



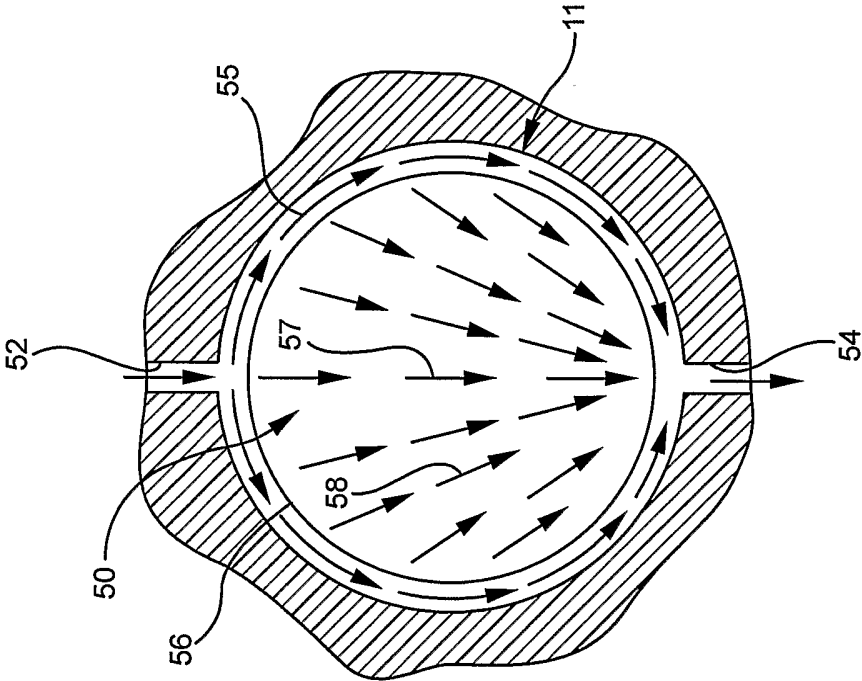
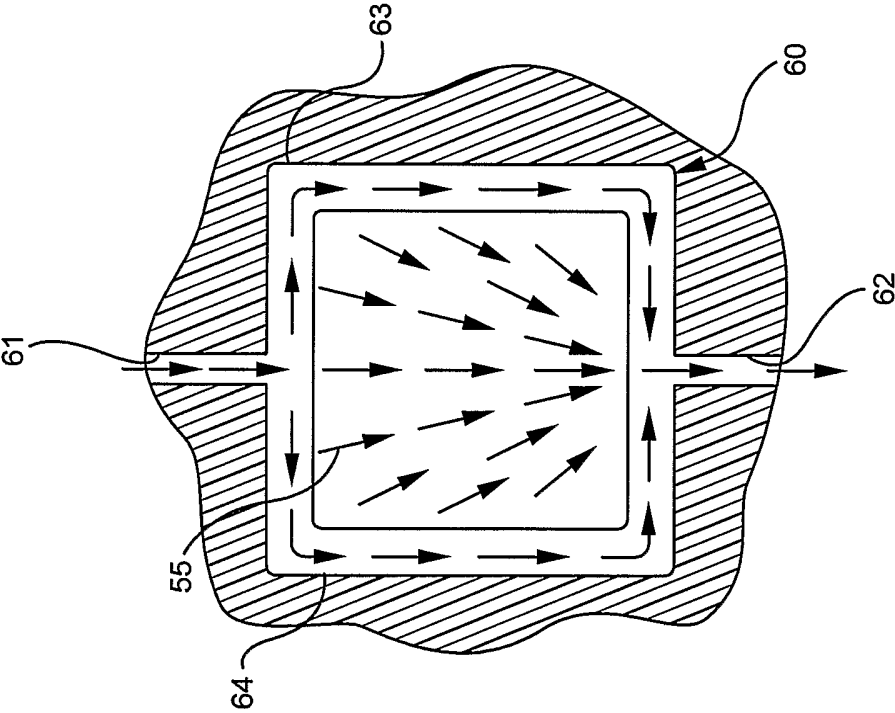


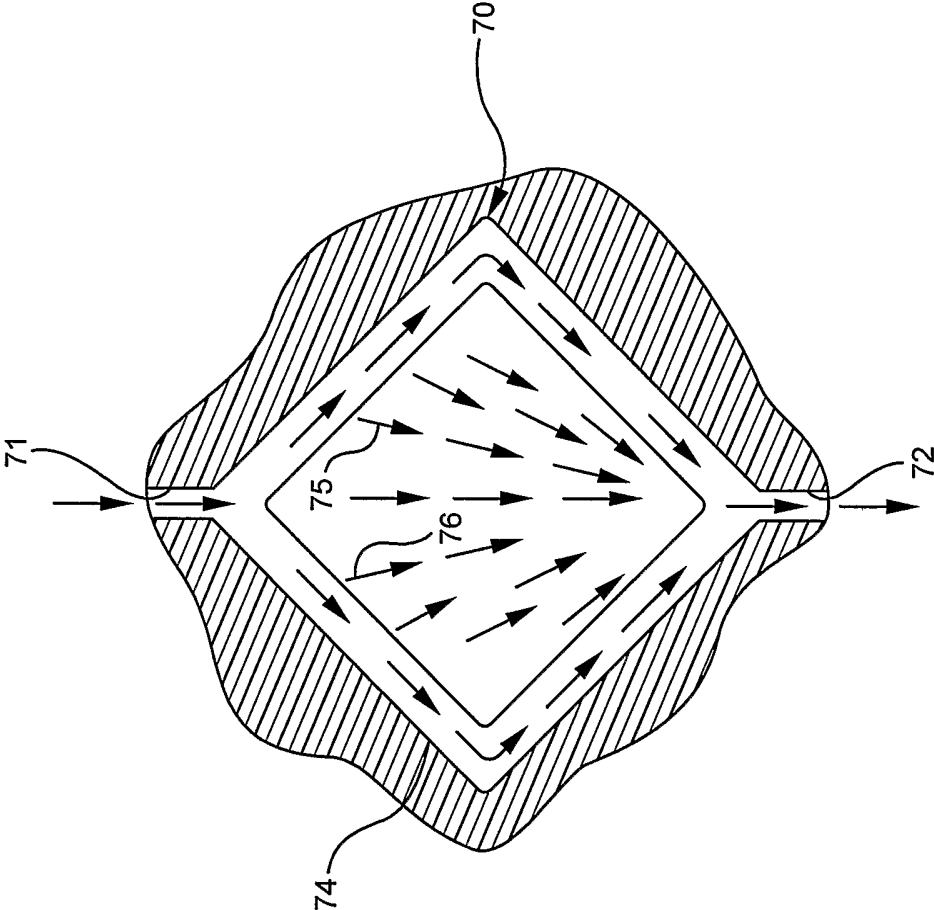
FIG. 5A

FIG. 5B



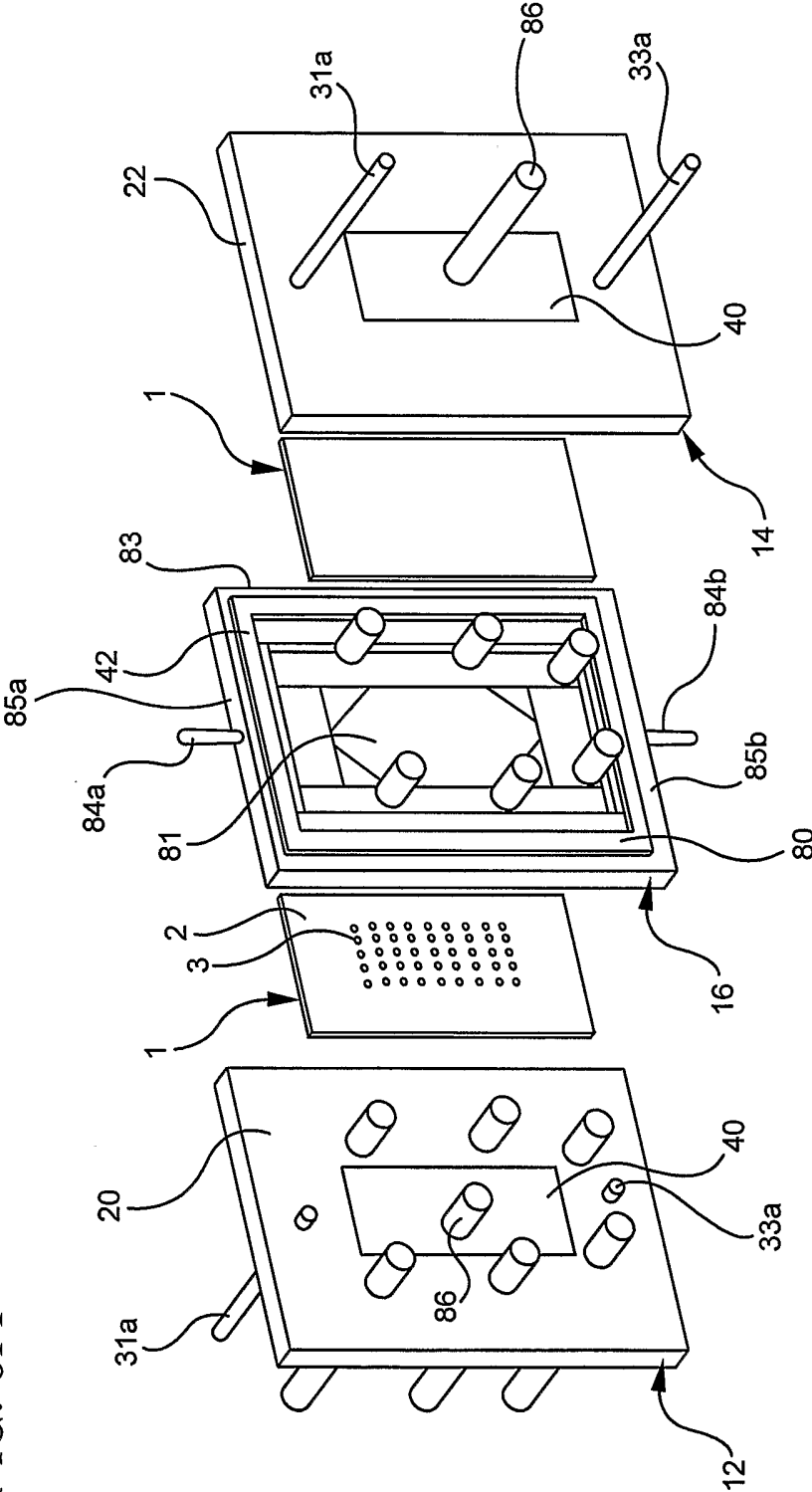
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FIG. 5C



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FIG. 6A



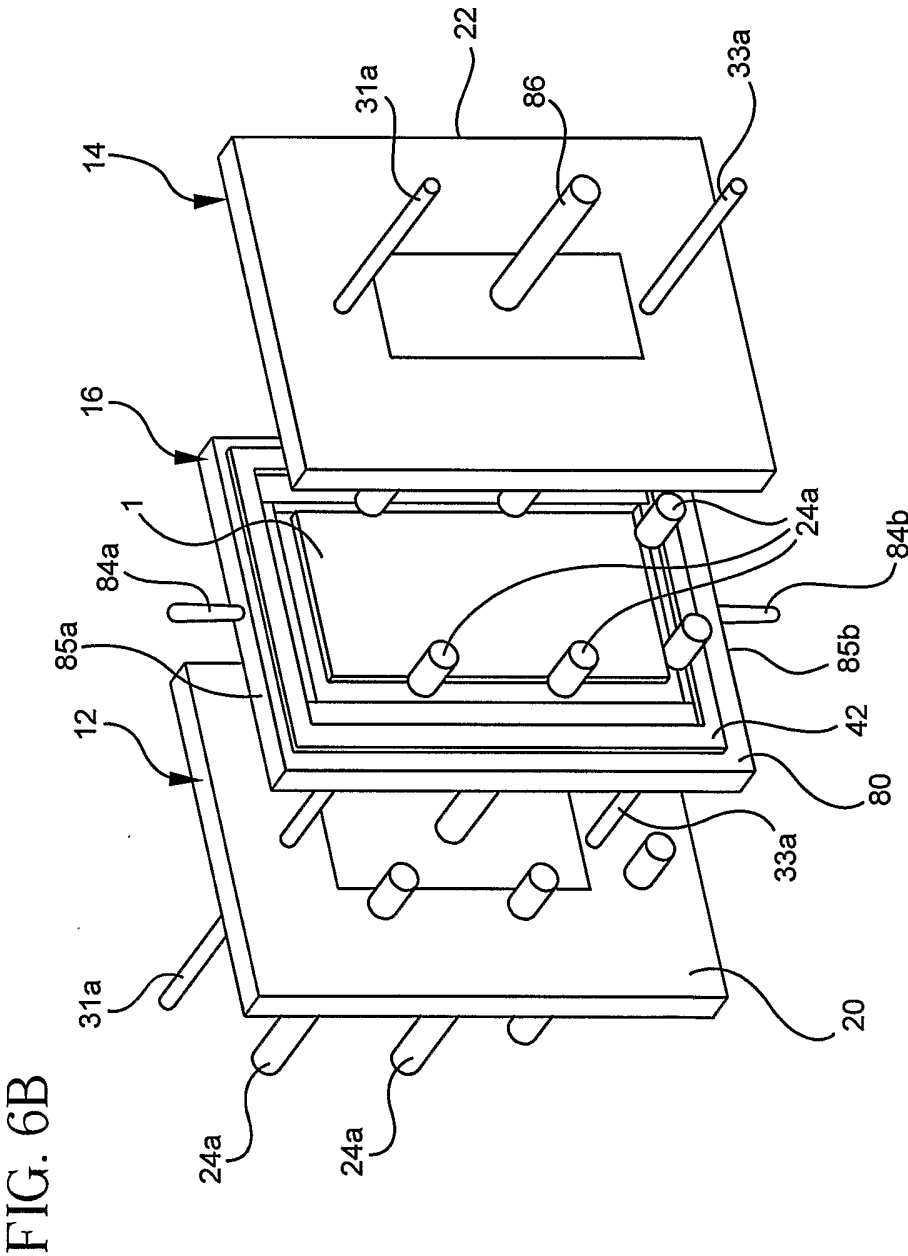


FIG. 6D

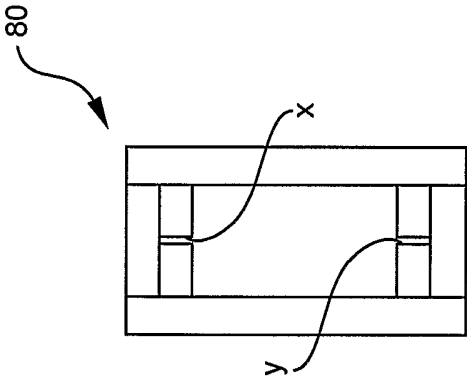
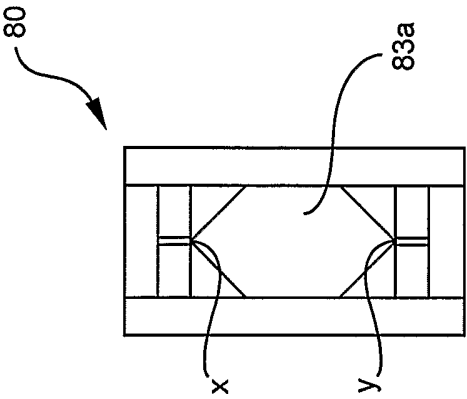


FIG. 6C



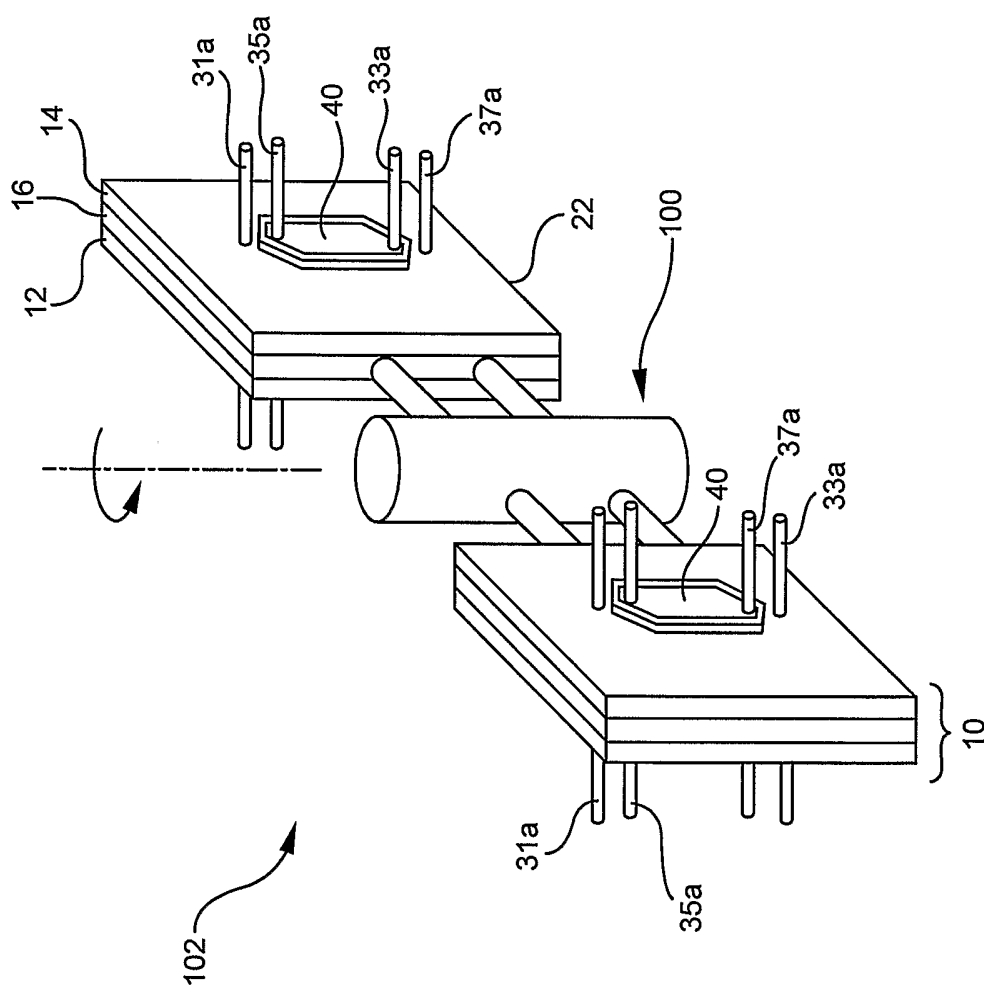


FIG. 7

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FIG. 8

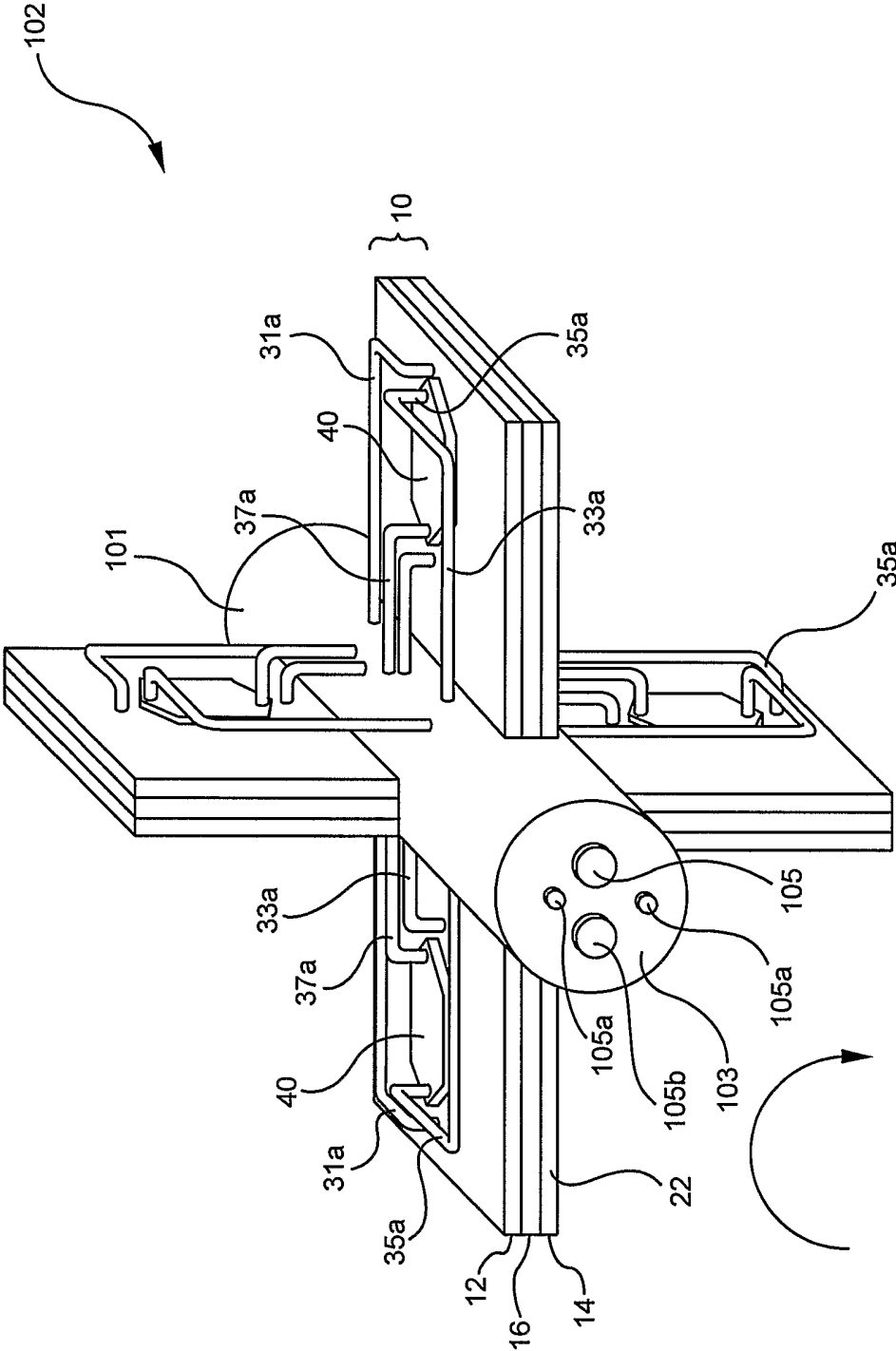


FIG. 9

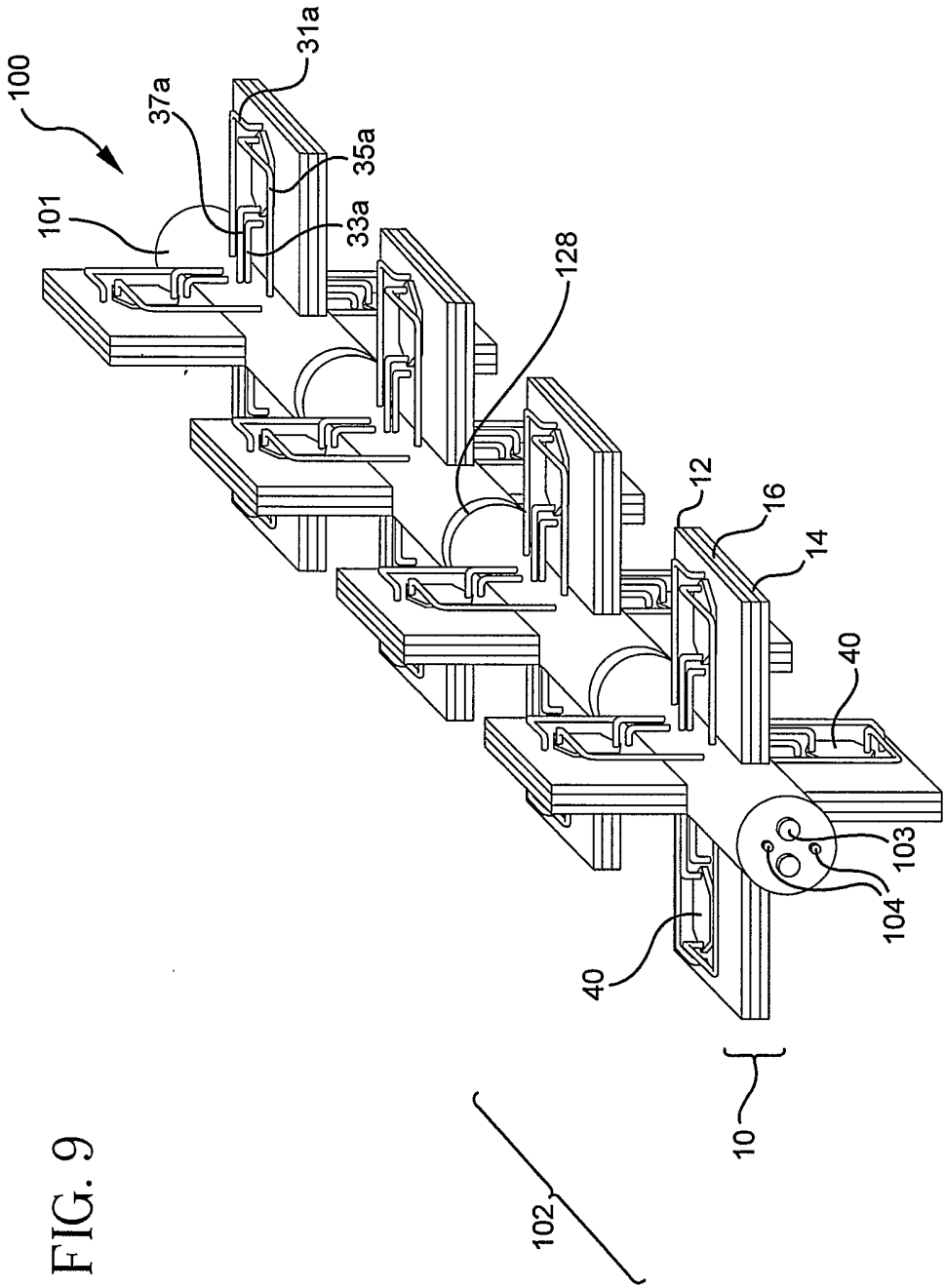


FIG. 10

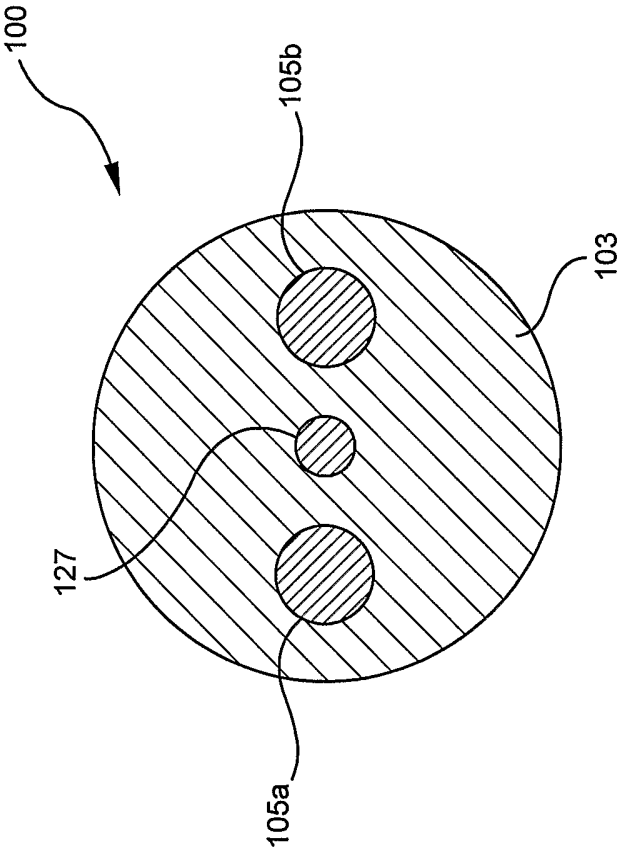
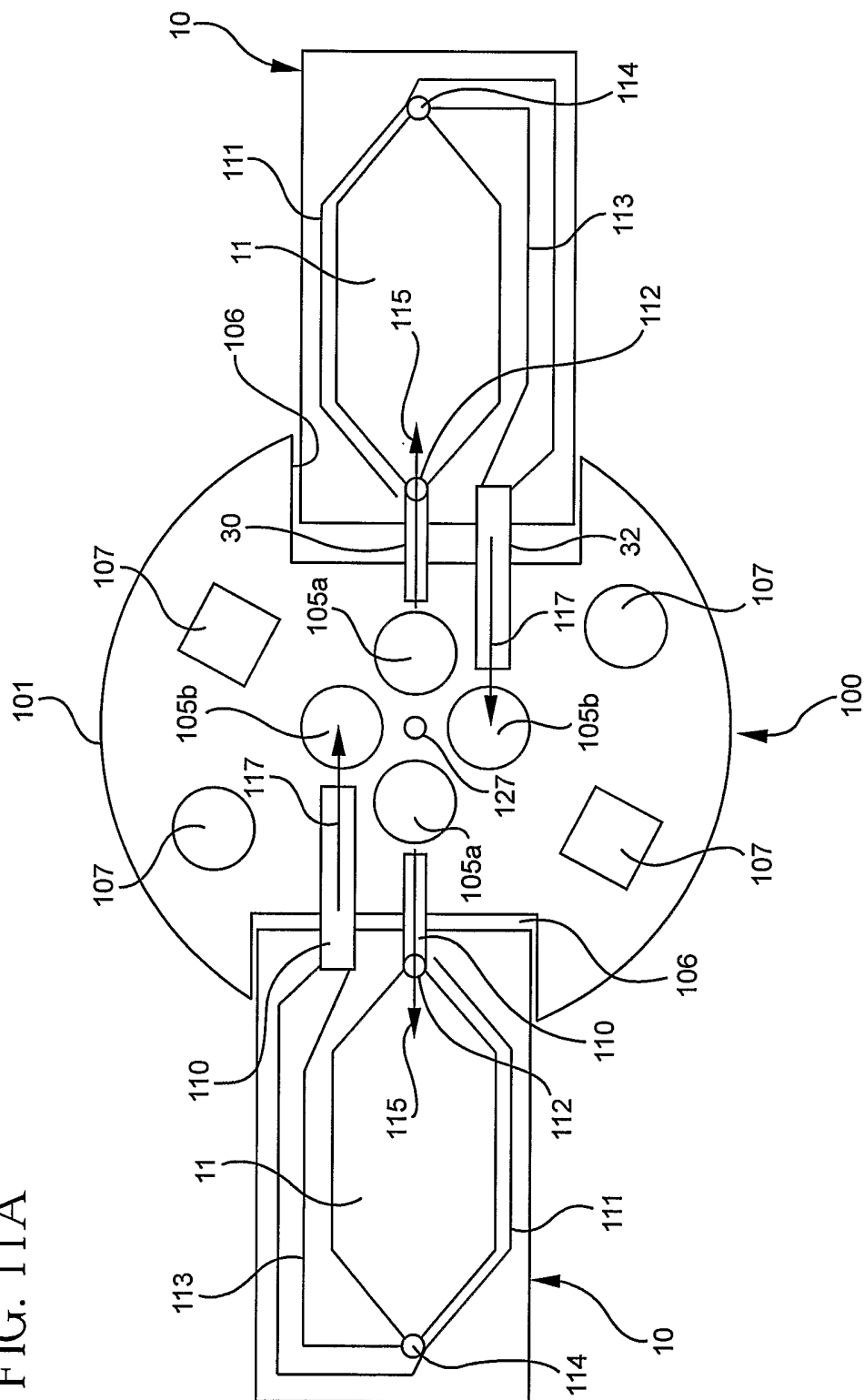


FIG. 11A



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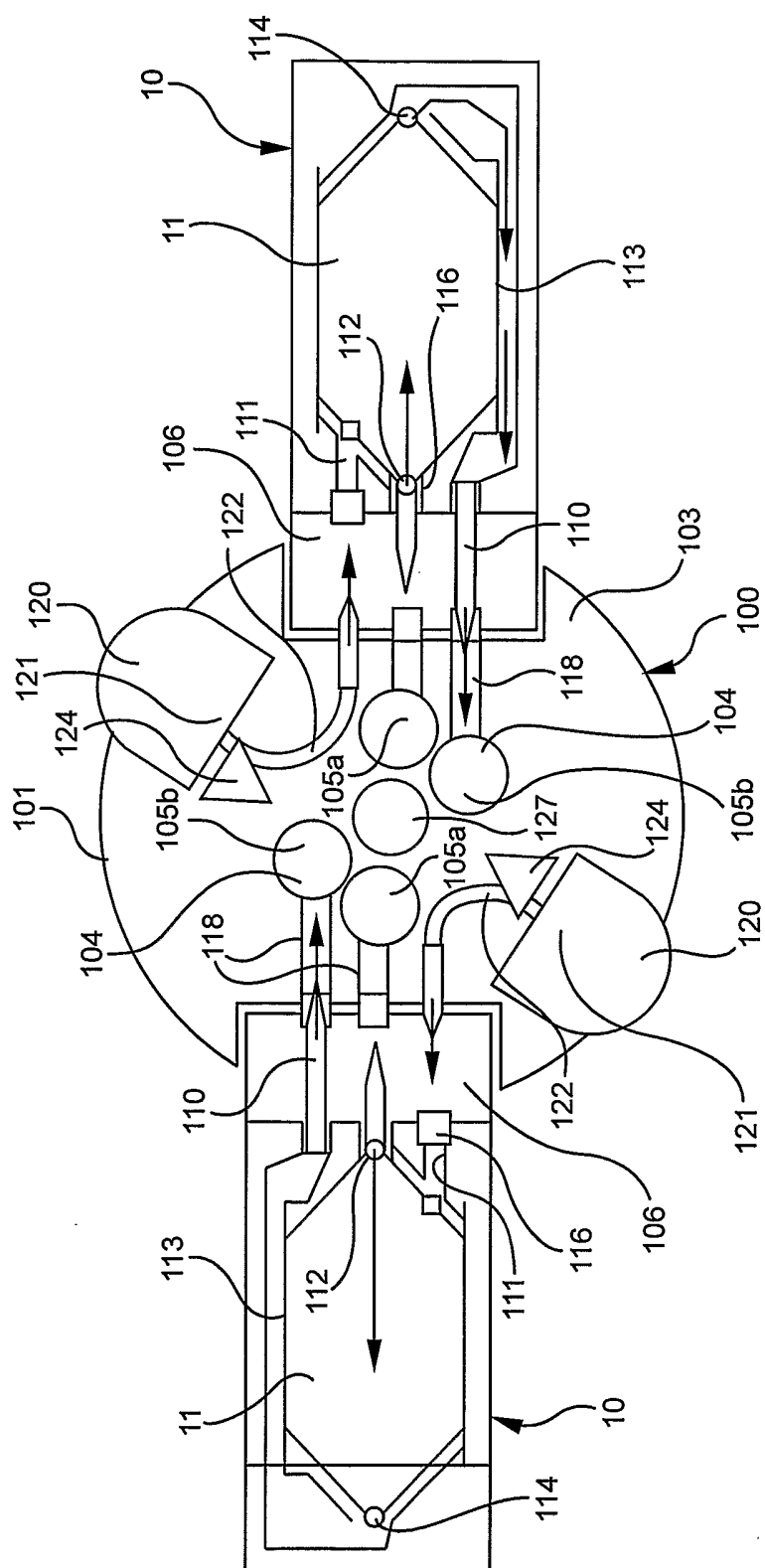


FIG. 11B

FIG. 12A

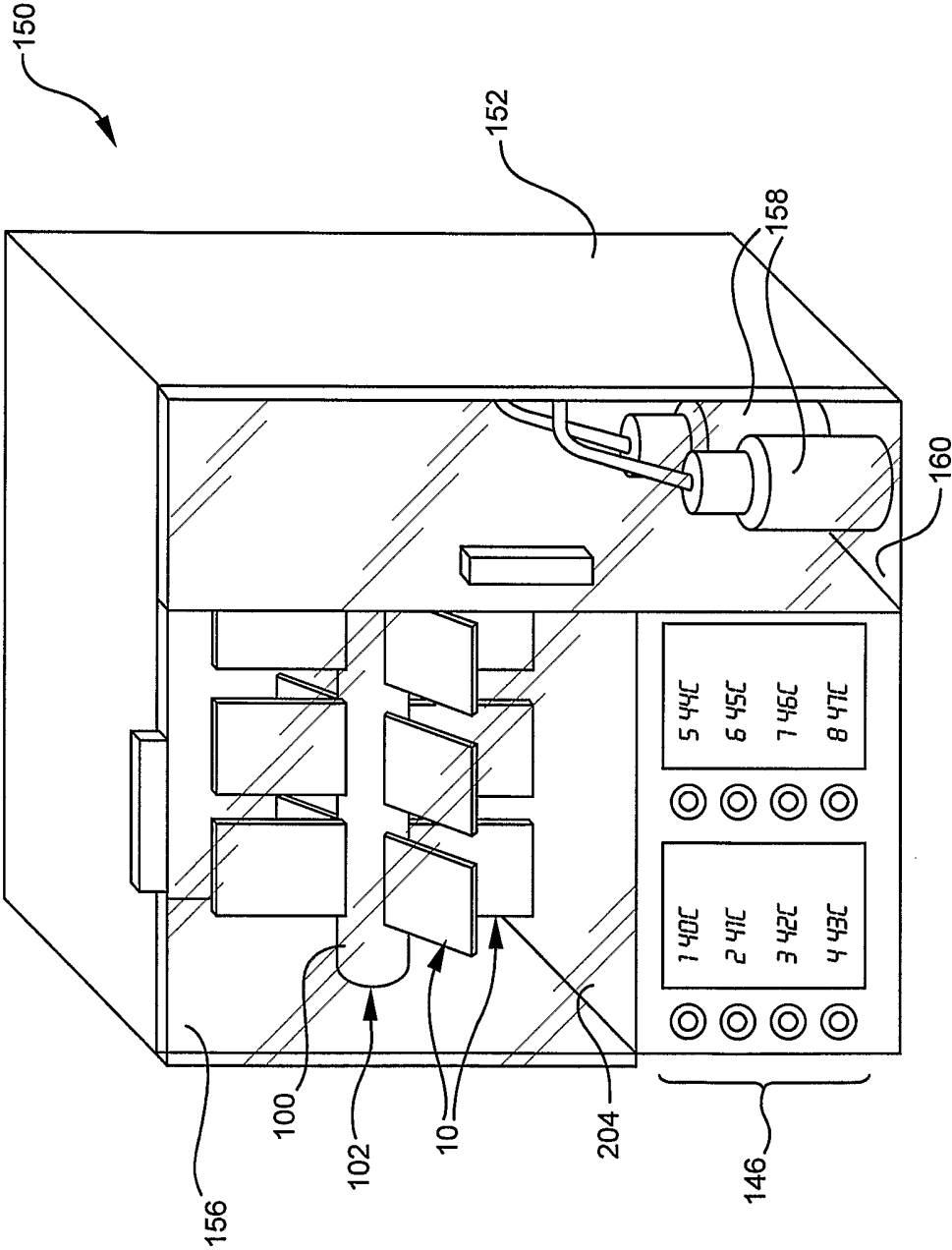
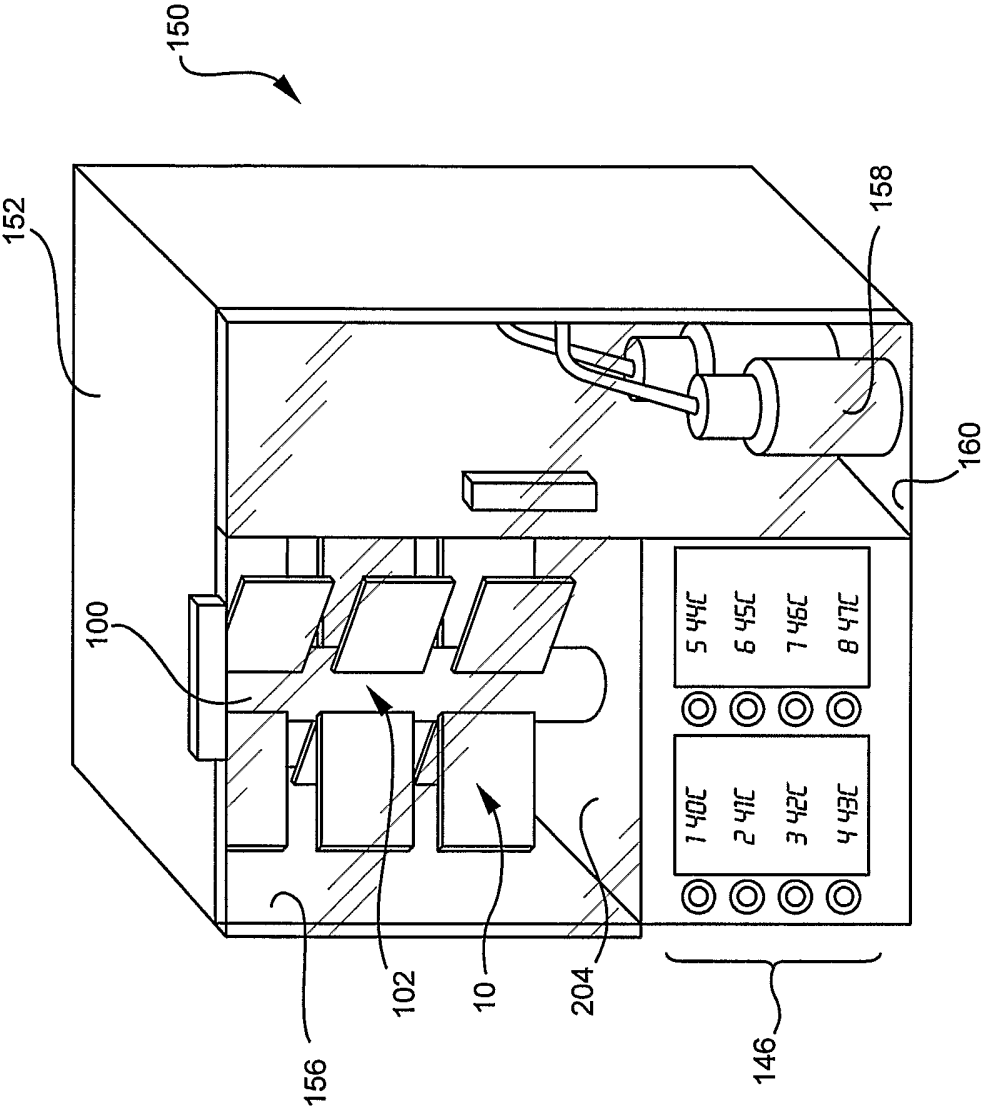


FIG. 12B



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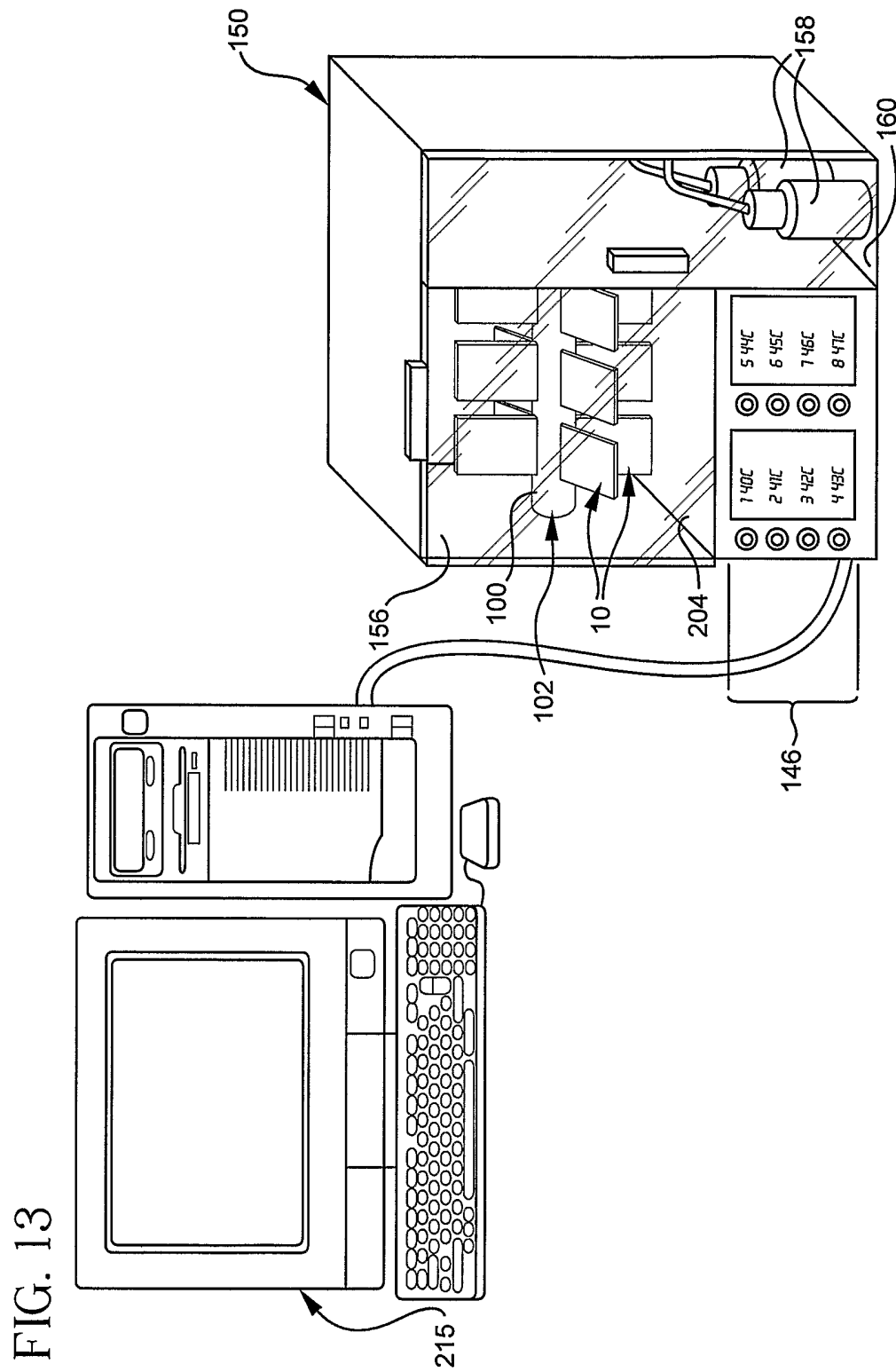


FIG. 14

