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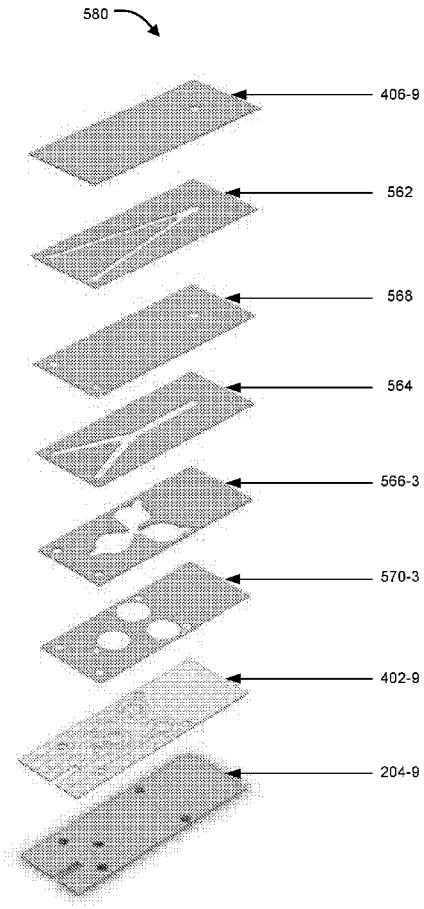


FIG. 5I

(57) Abstract: The present disclosure pertains to a microfluidic device that can simultaneously and independently carry out tests for one or more than one analytes in a given biological sample using biosensor and immune sensor based electrochemical processes. In an aspect the disclosed microfluidic device can be configured to carry out all the tests pertaining to a Clinical Chemistry Panel or a Typical Immunoassay Panel thereby eliminating the need to draw multiple samples from a patient to determine his general health status and evaluate, for example, the body's electrolyte balance and/or the status of several major body organs.

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# MULTIPLEXED MICROFLUIDIC DEVICE

## TECHNICAL FIELD

**[0001]** The present disclosure generally relates to technical field of microfluidic device. In particular the present disclosure relates to a microfluidic device that can simultaneously and independently carry out one or more than one tests on a given biological sample using biosensor and immunosensor based electrochemical processes.

## BACKGROUND

**[0002]** The background description includes information that may be useful in understanding the present disclosure. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

**[0003]** Diagnostic tests play an important role in treatment of patients. Doctors typically wait for diagnostic test results rather than starting treatment based on clinical symptoms. Therefore, in most cases, timely availability of diagnostic test results is crucial, not only for well-being of the patient, but also for achieving an efficient overall cost of treatment.

**[0004]** Many of the diagnostic tests related to blood or other biological fluids are based on biosensor and/or immunosensor that interact (bind or recognize) a macromolecule under study, often referred to as an analyte. Further, a transducer or detector element is also provided to work as means to produce a measurable signal in response to the binding to assess the concentration of the analyte. The transducer or detector element transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transduces) that can be more easily measured and quantified. Typically the detector element works in a physicochemical way such as optical, piezoelectric, electrochemical, etc. however; electrochemical methods are most widely used.

**[0005]** Developments in the fields of microfluidics and microelectronics have provided possibility to miniaturize diagnostic test equipment to be able to provide point of care hand held test facilities. While modern microelectronics allows building microelectrodes that are well

suited for detection of very small volumes of samples (microliters to nanoliters), microfluidics enable fabrication of devices that can handle very small volumes of samples and reagents and facilitate electrochemical reactions between them.

**[0006]** Microfluidics refers to a set of technologies that control the flow of minute amounts of liquids or gases, typically measured in nano and picoliters, in a miniaturized system. Microfluidic devices, first developed in the early 1990s, were initially fabricated in silicon and glass using photolithography and etching techniques adapted from the microelectronics industry, which are precise but expensive and inflexible. The trend recently has moved toward the application of soft lithography-fabrication methods based on printing and molding organic materials.

**[0007]** A microfluidic device can be characterized as having one or more channels with at least one dimension less than 1 mm. These devices are used extensively in the medical field for testing of whole blood samples, bacterial cell suspensions, protein or antibody solutions and various buffers. The microfluidic devices can be used to obtain a variety of interesting measurements including molecular diffusion coefficients, fluid viscosity, pH, chemical binding coefficients and enzyme reaction kinetics. Other applications for microfluidic devices include capillary electrophoresis, isoelectric focusing, immunoassays, flow cytometry, sample injection of proteins for analysis via mass spectrometry, PCR amplification, DNA analysis, cell manipulation, cell separation, cell patterning and chemical gradient formation.

**[0008]** The main advantages of use of microfluidic devices are decreased analyte consumption and thereby limited waste production, rapid analysis and improved automation capacity. The need to limit analyte consumption is highlighted by the increasing number of assays that are performed, Further use of such microfluidic devices has done away with the requirement of collection, storage and transportation of samples as diagnostic and analytic tests can be conducted by introducing the test sample into the microfluidic device since these devices are designed as hand held self-contained test labs.

**[0009]** European Patent Application Publication No. 1391241 discloses a microfluidic device for the detection of target analytes. The microfluidic device employs a solid support, which has a sample inlet port, a storage chamber and a microfluidic channel, connecting the solid

support with the sample inlet port and the storage chambers. The printed circuit boards are used as solid supports on which detection electrodes are provided. The detection electrodes are provided by self-assembled monolayers, which are specific to a particular substrate, for instance thiols.

**[00010]** PCT Application Publication No. WO2010020574 discloses a microfluidic system for assaying a sample, especially a biological sample. The microfluidic system is configured to allow two samples, such as a test sample and a control, to be processed under the same reaction conditions without cross contamination. The invention also relates to a cartridge system comprising the microfluidic system, and to assays performed using the microfluidic system or cartridge system. The microfluidic system comprises two reaction reservoirs, a reagent delivery channel to deliver reagents to the reaction reservoirs, a waste channel, and a means for retaining one or more reagents in each reaction zone, such as magnetic or magnetisable. The reservoirs are connected to waste chambers. The reservoirs have interconnected chambers which store processing components and sample preparation components. Thus, the apparatus is complex with too many interconnections.

**[00011]** US Patent No. 7,419,821 discloses a single use disposable cartridge for the determination of an analyte in biological samples using electrochemical immunosensors or other ligand/ligand receptor based biosensors. The cartridge comprises a cover, a base, and a thin film adhesive gasket, which is disposed between the base and the cover. The analyte measurements are performed in a thin-film of liquid coating an analyte sensor and such thin-film determination are performed amperometrically. The cartridge comprising an immunosensor is micro fabricated from a base sensor of an unreactive metal such as, gold, platinum or iridium.

**[00012]** PCT Application Publication No. WO2004/061418 describes a cartridge for performing a plurality of biochemical assays. The cartridge comprises a flow cell having an inlet, an outlet and a detection chamber. The inlet, outlet and detection chamber define the flow path through the flow cell. The detection chamber comprises plurality of electrodes involving a dedicated working electrode, a dedicated counter electrode and two or more dual-role electrodes, wherein each of the dual-role electrodes is used as a working electrode for measuring an assay dependent signal, and subsequently as a counter electrode for measuring a different assay dependent signal at a different one of said plurality electrode. The fluidic network is formed

within the cartridge employing fabrication method appropriate to the cartridge body material, such as stereo lithography, chemical/laser etching, integral moulding, machining, lamination, etc.

**[00013]** PCT Application Publication No. WO 2013/136115 describes a point of care hand held self-calibrated microfluidic device for rapid screening and diagnosis of various disease markers. It comprises of a polymeric base plate, at least one sensor formed over the polymeric base plate and can be used for detecting at least one target analyte from a sample, the sensor comprising at least one reference electrode and at least one working electrode, wherein a number of nanostructure materials(s) can be deposited over the working electrode for increasing the surface area of the working electrode, and at least one recognition element bound to or deposited over the nanostructures.

**[00014]** The above disclosed microfluidic devices or others available in market are either manufactured by using micromachining and milling techniques and hence, are expensive or their application is limited to conducting one type of analysis/ test on a given sample at a time. However doctors, during any given consultation, typically prescribe groups of tests to determine a person's general health status and evaluate, for example, the body's electrolyte balance and/or the status of several major body organs. These groups of tests, commonly known as Clinical Chemistry Panels, have been standardized with names of the panels and number and type of tests contained in specific panels. Metabolite Panel, Kidney Panel, Electrolytes panel, Coagulation, Liver Panel, Lipid panel are some examples to name a few.

**[00015]** Microfluidic devices that can carry out multiple tests can lead to cost-effective point of care ("POC") clinical diagnostic tools that can be deployed rapidly when needed and are useful in both the developed world and in low resource settings such as semi-urban and rural areas in the developing countries. All areas such as infectious, deficiency or lifestyle diseases could benefit from such tools as much as the detection and treatment of these diseases affecting human beings.

**[00016]** There is therefore a need in the art to design and develop microfluidic devices that can simultaneously analyze and conduct multiple tests simultaneously on a given biological sample.

**[00017]** All publications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

**[00018]** In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

**[00019]** As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

**[00020]** The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification

should be construed as indicating any non-claimed element essential to the practice of the invention.

**[00021]** Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

## OBJECTS OF THE INVENTION

**[00022]** It is an object of the present disclosure to provide a microfluidic device that can be used to simultaneously conduct one or more than one tests on a biological sample.

**[00023]** It is an object of the present disclosure to provide a microfluidic device that independently conducts more than one test on the sample.

**[00024]** It is an object of the present disclosure to provide a microfluidic device that needs only a drop of 100 microliters of blood sample for analysis of multiple analytes.

**[00025]** It is an object of the present disclosure to provide a microfluidic device that eliminates need to draw multiple samples from a patient to determine his general health status and evaluate,

**[00026]** It is an object of the present disclosure to provide a microfluidic device that can carry out all the tests pertaining to a Clinical Chemistry Panel.

**[00027]** It is an object of the present disclosure to provide a microfluidic device that can perform immunoassay tests for multiple analytes simultaneously and independently on a given biological sample.

**[00028]** It is an object of the present disclosure to provide a microfluidic device that can carry out tests for one or more than one analytes in a given biological sample using biosensor and immunosensor based electrochemical processes.

**[00029]** It is an object of the present disclosure to provide a microfluidic device that has microfluidic channels for sample, one or more reagents and waste in different layers.

**[00030]** It is an object of the present disclosure to provide a microfluidic device that is easy to manufacture.

**[00031]** It is an object of the present disclosure to provide a microfluidic device that can be mass produced.

**[00032]** It is an object of the present disclosure to provide a microfluidic device that is economical to produce.

**[00033]** It is an object of the present disclosure to provide a microfluidic device that does not need a separate "desiccant cum oxygen scavenger" in external foil packaging of the cartridge.

## SUMMARY

**[00034]** Aspects of the present disclosure relate to a microfluidic device that can simultaneously and independently carry out tests for one or more than one analytes in a given biological sample using biosensor and immunosensor based electrochemical processes.

**[00035]** In an aspect the disclosed microfluidic device can be configured to carry out all the tests pertaining to a Clinical Chemistry Panel thereby eliminating the need to draw multiple samples from a patient to determine his general health status and evaluate, for example, the body's electrolyte balance and/or the status of several major body organs.

**[00036]** In another aspect, there can be a cartridge for each of the Clinical Chemistry Panels and common Biochemistries such as Metabolite Panel, Kidney Panel, Electrolytes panel, Coagulation, Liver Panel, Lipid panel in whole blood samples or serum or plasma.

**[00037]** In another aspect the disclosure provides a Multiplexed Immunoassay Cartridge (MIC) that can be used to perform immunoassay tests for multiple analytes simultaneously and

independently on a given biological sample wherein only a drop of 100 microliters of blood sample can be applied to the cartridge for analysis of all the analytes.

**[00038]** In another aspect the disclosure provides a Multiplexed microfluidic cartridge that can be configured to carry out more than one tests for multiple analytes involving combination of immunoassay and biochemistry.

**[00039]** In an aspect of the disclosure, the microfluidic cartridge comprises a substrate, a reagent component and a microfluidic laminate. In another aspect the substrate comprises of a polymeric base plate, one or more than one multiplexed sensors formed over the polymeric base plate for detecting one or more than one target analyte contained in a sample.

**[00040]** In another aspect, each one of the one or more than one multiplexed sensors comprises of at least one working electrode and at least one reference electrode and a one or more of nanostructures deposited over the working electrode and a recognition element bound to or deposited over the nanostructures.

**[00041]** In another aspect of the disclosure, the reagent component can have one or more reservoirs for storing reagent(s) and a waste chamber for disposing the used reagent.

**[00042]** In another aspect of the disclosure, the microfluidic laminate is made of plurality of layers comprising a gold sheet, circular adhesives that define area of each of the one or more than one multiplexed sensors and plurality of stacked double sided channel adhesive layers configured with channels for flow of the sample, one or more reagents and waste.

**[00043]** In an aspect, the plurality of stacked double sided channel adhesive layers configured with microfluidic channels can define flow path for the bio sample, the one or more reagents and the waste. Thus the flow path of the bio sample, the one or more reagents and the waste can lie in different layers of the microfluidic laminate.

**[00044]** In an aspect, the microfluidic laminate can further comprise intermediate layers stacked between the plurality of stacked double sided channel adhesive layers. The intermediate layers can incorporate one or more than one holes configured to facilitate transfer of one or more of the sample, the one or more reagents and the waste from one layer to other layer.

**[00045]** In an aspect the microfluidic channels are configured to facilitate simultaneous and independent testing of one or more than one analytes in the biological sample.

**[00046]** In an aspect, the disclosed microfluidic cartridge incorporates a "desiccant cum oxygen scavenger" to enhance the life of the cartridge and this obviates need for a separate "desiccant cum oxygen scavenger" in external foil packaging of the cartridge.

**[00047]** Various objects, features, aspects and advantages of the inventive subject matter will become more apparent from the following detailed description of preferred embodiments, along with the accompanying drawing figures in which like numerals represent like component.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[00048]** The accompanying drawings are included to provide a further understanding of the present disclosure, and are incorporated in and constitute a part of this specification. The drawings illustrate exemplary embodiments of the present disclosure and, together with the description, serve to explain the principles of the present disclosure.

**[00049]** FIG. 1A illustrates an exemplary isometric view of a microfluidic device in accordance with embodiments of the present disclosure.

**[00050]** FIG. 1B illustrates an exemplary orthographic views of the microfluidic device in accordance with embodiments of the present disclosure.

**[00051]** FIG. 2 illustrates an exemplary exploded view of the microfluidic device in accordance with embodiments of the present disclosure.

**[00052]** FIG. 3A, FIG. 3B, FIG. 3C and FIG. 3D illustrate exemplary configurations of substrate of the microfluidic device in accordance with embodiments of the present disclosure.

**[00053]** FIG. 4A and 4B illustrates exemplary exploded views of the microfluidic device with flow direction of sample and reagent in accordance with embodiments of the present disclosure.

**[00054]** FIG. 5A to 5I illustrates exemplary exploded views of various configurations of the microfluidic laminate and substrate in accordance with embodiments of the present disclosure.

**[00055]** FIG. 6 illustrates exemplary images of the microfluidic device in accordance with embodiments of the present disclosure.

## DETAILED DESCRIPTION

**[00056]** The following is a detailed description of embodiments of the disclosure depicted in the accompanying drawings. The embodiments are in such detail as to clearly communicate the disclosure. However, the amount of detail offered is not intended to limit the anticipated variations of embodiments; on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present disclosure as defined by the appended claims.

**[00057]** Each of the appended claims defines a separate invention, which for infringement purposes is recognized as including equivalents to the various elements or limitations specified in the claims. Depending on the context, all references below to the "invention" may in some cases refer to certain specific embodiments only. In other cases it will be recognized that references to the "invention" will refer to subject matter recited in one or more, but not necessarily all, of the claims.

**[00058]** Various terms as used herein are shown below. To the extent a term used in a claim is not defined below, it should be given the broadest definition persons in the pertinent art have given that term as reflected in printed publications and issued patents at the time of filing.

**[00059]** Aspects of the present disclosure relate to a microfluidic device (interchangeably referred to as microfluidic cartridge device or microfluidic cartridge or simply as cartridge hereinafter)that can simultaneously and independently carry out tests for one or more than one analytes in a given biological sample using biosensor and immunosensor based electrochemical processes.

**[00060]** In an embodiment the disclosed microfluidic cartridge comprises a substrate, a reagent component and a microfluidic laminate. In another embodiment the substrate comprises of a polymeric base plate, one or more than one multiplexed sensors formed over the polymeric base plate for detecting one or more than one target analyte contained in the sample.

**[00061]** In another embodiment, each one of the one or more than one multiplexed sensor comprises of at least one working electrode and at least one reference electrode and one or more of nanostructures deposited over the working electrode. In an aspect the working and reference electrodes configured as biosensors can have a corresponding recognition element such as an enzyme deposited over them.

**[00062]** In another embodiment of the disclosure, the reagent component can have one or more than one reservoirs for storing reagent and a waste chamber for disposing the used reagent or waste.

**[00063]** In an embodiment, the microfluidic laminate can be made of plurality of layers comprising a gold sheet, circular adhesives that define area of each of the one or more than one multiplexed sensors and plurality of stacked double sided channel adhesive layers configured with channels for flow of the sample, one or more reagents and waste.

**[00064]** In an embodiment, the gold sheet can be configured to provide the working and reference electrodes and conductive paths. The recognition element and nanostructures can be deposited over the gold sheet as applicable.

**[00065]** In an aspect, the plurality of stacked double sided channel adhesive layers configured with microfluidic channels can define flow path for the bio sample, the one or more reagents and the waste. Thus the flow path of the bio sample, the one or more reagents and the waste can lie in different layers of the microfluidic laminate.

**[00066]** In an aspect, the microfluidic laminate can further comprise intermediate layers stacked between the plurality of stacked double sided channel adhesive layers. The intermediate layers can incorporate one or more than one holes configured to facilitate transfer of one or more of the sample, the one or more reagents and the waste from one layer to other layer.

**[00067]** In an aspect the microfluidic channels are configured to facilitate simultaneous and independent testing of one or more than one analytes in the biological sample. In an aspect the testing of one or more than one analytes can be either biosensor or immunosensor based or a combination of the two.

**[00068]** According to this invention, the term "nanostructures" refers to structures that possess the nanometer size and has partial or complete nanometer effect (e.g. surface effect, size effect). According to this invention, the term "nanoparticles (NPs)" refers to solid particles, which, in three-dimensional space, have at least one-dimensional size less than 500 nm, preferably less than 100 nm, optimally less than 50 nm. According to this invention, the term "nanotubes (NTs)" specifically refers to hollow-core nanostructures with a diameter less than 10 nm.

**[00069]** According to this invention, the term "target analyte" refer to a specific material, the presence, absence, or amount of which is to be detected, and that is capable of interacting with a recognition element. The targets that may be detected include, without limitation, molecules, compounds, complexes, nucleic acids, proteins, such as enzymes and receptors, viruses, bacteria, cells and tissues and components or fragments thereof. Exemplary, samples containing target analyte includes, without limitation, whole blood sample, serum, urine, stool, mucus, sputum and tissues etc.

**[00070]** In an embodiment of the present invention, the recognition element can be selected from the group consisting of antigens, antibodies, enzymes, aptazymes, or aptamers.

**[00071]** In an embodiment of the present invention, the polymer used in the polymeric base plate can be selected from polyester, polystyrenes, polyacrylamides, polyetherurethanes, polysulfones, polycarbonates or fluorinated or chlorinated polymers such as polyvinyl chloride, polyethylenes and polypropylenes. Other polymers include polyolefins such as polybutadiene, polydichlorobutadiene, polyisoprene, polychloroprene, polyvinylidene halides, polyvinylidene carbonate, and polyfluorinatedethylenes. The copolymers, including styrene/butadiene, alpha-methyl styrene/dimethyl siloxane, or other polysiloxanes such as, polydimethylsiloxane, polyphenylmethylsiloxane and polytrifluoropropylmethylsiloxane may also be used. Other alternatives include polyacrylonitriles or acrylonitrile containing polymers such as poly alpha-acrylanitrile copolymers, alkyd or terpenoid resins, and polyalkylenepolysulfonates. However, the material used for forming the polymeric base plate is not limited to those materials listed above and can be any material which has chemical and biological stability and processability.

**[00072]** In another embodiment of the present invention, the sensor can include at least one or more than one working electrode, at least one or more than one reference electrode and optionally a counter electrode. Further these electrodes can be formed over metal coated polymeric base plate by laser technique, such as laser ablation, or sputtering technique, etc. However, it may be apparent to a person skilled in the art to replace sputtering with any other suitable technique known in the art. In accordance with an embodiment of the present invention, the noble metal coated over the polymeric base plate can be selected from gold, platinum or palladium.

**[00073]** In an embodiment of the present disclosure, target analyte containing sample can be selected from whole blood, serum or urine or any other body fluid. In yet another embodiment of the present disclosure, substrate can optionally comprise of at least one fluid detection sensor formed over the polymeric base plate for detecting the presence of the analyte and at least one reagent involving reading reagent or reaction reagent. In yet another embodiment, substrate can compromise at least one or more fluid detection sensor formed over polymeric base plate wherein said sensor can be used for detecting presence of analyte and at least one reagent involving reading reagent or reaction agent.

**[00074]** Embodiments of the present disclosure and their advantages can be best understood by reference to drawings. FIG. 1A illustrates an exemplary isometric view of the microfluidic cartridge device 100 in accordance with embodiments of the present disclosure. Depicted therein is microfluidic cartridge 100 configured with a sample inlet port 102 and connector 104. Sample inlet port 102 can be used to introduce sample to be analysed for target analytes into microfluidic device 100. Connector 104 can be used for electrical coupling of microfluidic device 100 to a test equipment that is configured to receive the microfluidic device 100.

**[00075]** FIG. 1B illustrates exemplary orthographic views of the microfluidic device 100 in accordance with an embodiment of the present disclosure. As illustrated the microfluidic device 100 can have dimensions as small as 64x32x17mm in length, width and thickness.

**[00076]** FIG. 2 illustrates an exemplary exploded view 200 of the microfluidic device 100 in accordance with embodiments of the present disclosure. The exploded view 200 illustrates a top cover 202 that is configured with a sample inlet port 102, a substrate 204, and a reagent

component 206 (interchangeably referred to as bottom cover hereinafter). There can be a microfluidic laminate (shown placed on top face of the substrate) to guide flow of sample from sample port 102 to one or more multiplexed sensors and also flow of reagent from reservoirs (described in subsequent paragraph) to more multiplexed sensors. The microfluidic laminate can comprise a sample transfer port 208 configured to match the sample inlet port 102 of top cover 202 and transfer introduced sample to microfluidic channels configured within the microfluidic laminate. In an aspect the microfluidic channels can be configured to facilitate simultaneous and independent testing of one or more than one analytes in the biological sample.

**[00077]** In an embodiment, internal space in the top cover 202 can be used for storing a desiccantor a "desiccant cum oxygen scavenger". In an aspect the desiccant or a "desiccant cum oxygen scavenger" stored in the cover 202 can ensure a long shelf-life of the cartridge and also does away with requirement of a separate desiccant or a "desiccant cum oxygen scavenger" in external foil packaging of the cartridge. This can reduce the cost of packaging the cartridge.

**[00078]** In another embodiment, the reagent component/bottom cover 206 can have one or more reservoirs for storing reagent(s) and a waste chamber for disposing the used reagent. In an embodiment the one or more reservoirs of the reagent component 206 can be configured with silicon valves such as 214 and 216 to work as one way valves to allow flow of gas /reagent in one direction but prevent their return back.

**[00079]** The exploded view 200 further illustrates an end cover 210 that can be configured with one or more means such as septa 212 to provide fluidic connectivity to the one or more reservoirs configured in the reagent component 206. Thus when the microfluidic device 100 is coupled with suitable test equipment, one or more needle(s) can puncture the one or more septa212 and provide supply of a gas such as air to the reservoirs to help move the reagents to the test area in the microfluidic channels.

**[00080]** In an embodiment the reagent component 206 can be configured with silicon valves such as 214 and 216 to work as one way valves to allow flow of gas /reagent in one direction but prevent their return back.

**[00081]** In an embodiment the top cover 202 and the reagent component 206 can be configured with means to hold them together after the substrate 204 and microfluidic laminate

have been placed between them. These means could be but not limited to clips or locks that can be molded along with either one or both these components at manufacturing stage.

**[00082]** FIG. 3A, FIG. 3B, FIG. 3C and FIG. 3D illustrate various exemplary configurations of sensor(s) on substrate 204 of the microfluidic device 100 in accordance with embodiments of the present disclosure. The substrate of the disclosed microfluidic device 100 incorporates sensors that are configured to transduce results of electrochemical reaction between analyte and a reagent. In accordance with an embodiment, the sensor includes at least one working electrode, at least one reference electrode and optionally a counter electrode. These electrodes can be formed over metal coated polymeric base plate by using a laser technique, such as laser ablation.

**[00083]** According to an embodiment, the metals are coated over the polymeric base plate by sputtering technique. Alternatively, the sensor may also be formed over polymeric base plate using a screen printing technique. However, it may be apparent to a person skilled in the art to replace these techniques with any other suitable technique known in the art. In accordance with an embodiment of the present invention, the noble metal coated over the polymeric base plate is selected from gold, platinum or palladium. According to an embodiment of the present invention, the polymeric base plate is sputtered with gold and the sensors are ablated with laser technique over the base plate. Alternatively, the sensors are printed on polymeric base plate using screen printing. In an alternate embodiment a gold sheet can be configured to provide the working electrode and reference electrodes along with corresponding conducting paths. The recognition element and nano structures can be deposited over the gold sheet as required.

**[00084]** FIG. 3A illustrates a configuration 320 of the substrate wherein substrate 320 can be configured with one sensor 322. The sensor 322 can comprise of a working electrode 324 and a reference electrode 326. The working electrode 324 and reference electrode 326 can be connected to a conductive paths 328 and 330 respectively so that when the microfluidic device 100 is coupled with suitable test equipment, the electrical signal from these electrodes can be sensed by the equipment. The substrate 320 can be configured with a port 332 to transfer sample after test to waste chamber in the reagent component 206.A substrate such as 320 can be used for clinical chemistry or enzyme based chemistry or an immunoassay test of a single target analyte.

**[00085]** FIG. 3B illustrates another exemplary configuration 340 of the substrate in an embodiment of the present disclosure wherein substrate 340 can be configured with two sensor 342-1 and 342-2 with their respective working electrodes 344-1 and 344-2 and corresponding reference electrodes 346-1 and 346-2 respectively. Each of these electrodes can be connected to a conductive path 348-1, 350-1, 348-2 and 350-2 respectively. The substrate 340 can be configured with ports such as 352-1 and 352-2 and these ports can be used for transfer of sample after test to waste chamber in the reagent component 206. Such a substrate configuration can be used for clinical chemistry or enzyme based chemistry of two target analytes such as for diabetic profile (Hb& HbA1C), kidney profile (urea, creatinine), metabolite profile (glucose, lactate), lipid profile (cholesterol, triglyceride), liver profile (ALT,AST).

**[00086]** FIG. 3C illustrates an exemplary configuration 360 of a substrate as yet another embodiment of the present disclosure wherein substrate 360 can be configured with three sensors 362-1, 362-2 and 362-3, each having a working electrode and a reference electrodes and corresponding conductive path. The substrate 340 can be configured with a ports such as 364-1, 364-2 and 364-3 and these ports can be used for transfer of sample after test to waste chamber in the reagent component 206. There can be additional ports such as 366-1 and 366-2 that can be used to transfer reagents stored in reservoirs in the reagent component 206 to the test area through the micro fluidic channels configured on the microfluidic laminate. Such a substrate can be used for testing and analyses of three target analytes, one of them can be immunoassay based test for example a cardiac markers test (CnTni, CK-mb, Myoglobin).

**[00087]** FIG. 3D illustrates an exemplary configuration 380 of a substrate as yet another embodiment of the present disclosure wherein substrate 380 can be configured with four sensors elements 382-1, sensor 382-2, sensor 382-3 and sensor 382-4. Such substrate configuration can be used for testing and analyses of four target analytes for example in the measurement of electrolyte panel of Na,K,Cl along with reference sensor or a coagulation sensor.

**[00088]** In yet another embodiment of the present invention, the working electrodes can be deposited with a nanostructures for increasing the surface area of the working electrodes wherein increase in surface area of the working electrode can result in increased sensitivity and accuracy of the assay to be performed even with very low quantity of the target analyte. The non-limiting exemplary nanostructures according to the present invention can be selected from carbon

nanotubes (CNTs) or gold nanoparticles. In accordance with an embodiment of the present invention, the nanostructures are gold nanoparticles deposited over the working electrode using the electro deposition technique. In an embodiment, the nanostructures are carboxylated carbon nanotubes and the percentage of carboxylation of carbon nanotubes is 3% to 5%. In accordance with an embodiment of the present invention, the working and /or the reference electrode of the sensor can be ablated in the form of concentric arcs, circle, spiral, helix or any polygonal shape to increase the deposition of nanostructures. The "polygonal" shape is a multi-sided, closed planar shape. The Polygons may include trigons (or triangles), tetragons (or quadrilaterals), pentagons, hexagons, heptagons, octagons, and the like. Tetragons may include squares and rectangles, which have four sides connected at four right angles. Tetragons also may include rhombi (e.g. diamond- shaped polygons or parallelograms), which do not include four right angles. According to an embodiment of the present invention, the working electrode is ablated in the form of concentric arcs. The concentric arcs of the working and /or the reference electrode can be in a hill-valley type arrangement providing better deposition of the nanostructure. The working electrode can have a diameter in the range of 2mm to 8mm.

**[00089]** FIG. 4A and 4B illustrates exemplary exploded views 400 and 450 of the microfluidic device with flow direction of sample and reagent in accordance with embodiments of the present disclosure. As shown the microfluidic laminate is made of plurality of layers comprising a gold sheet 402, circular adhesives that define area of each of the multiplexed sensors and the double sided channel adhesive layer 404 for the multiplexed sensors. There can be an additional layer such as an upper layer 406 to cover the side of the double sided channel adhesive layer 404 that is opposite to the side facing gold sheet 402.

**[00090]** In an embodiment of the disclosure, the microfluidic laminate can comprise plurality of stacked double sided channel adhesive layers configured with channels for flow of the sample, one or more reagents and waste.

**[00091]** In an aspect, the plurality of stacked double sided channel adhesive layers configured with microfluidic channels can define flow path for the bio sample, the one or more reagents and the waste. Thus the flow path of the bio sample, the one or more reagents and the waste can lie in different layers of the microfluidic laminate.

**[00092]** In an aspect, the microfluidic laminate can further comprise intermediate layers stacked between the plurality of stacked double sided channel adhesive layers. The intermediate layers can incorporate one or more than one holes configured to facilitate transfer of one or more of the sample, the one or more reagents and the waste from one layer to other layer.

**[00093]** In an aspect the microfluidic channels can be configured to facilitate simultaneous and independent testing of one or more than one analytes in the biological sample. In an aspect the testing of one or more than one analytes can be either biosensor or immunosensor based or a combination of the two.

**[00094]** FIG. 4A shows movement/flow of sample fluid in the device 100 configured with plurality of receptor based biosensors for all the tests pertaining to a Clinical Chemistry Panel. As shown sample bio sample can enter through the sample port 104 in the top cover 202 and flow through the microfluidic channels and spread to the plurality of sensors. After the analytes have been sensed the sample can flow to waste chambers as shown in the exploded view 400.

**[00095]** FIG. 4B shows movement/flow of regents in a device 100 configured with at least one immunosensor for at least one immunoassay test among the multiple analytes being tested simultaneously and independently on a given biological sample. The sample bio fluid can enter through the sample port 104 in the top cover 202 and flow through the microfluidic channels and spread to the sensors (flow path not shown). Reagents stored in respective chambers in the reagent component 206 can move under influence of a compressed gas such as air supplied to the reservoirs. The reagent can move to the sensor chambers through the one or more silicon valves such as 214.After the analyte has been sensed mixture of the sample and the regent can flow to waste chambers in the reagent component 206 as shown in the exploded view 450.

**[00096]** FIG. 5A to 5F illustrate exemplary exploded views 500 to 550 respectively of various configurations of the microfluidic laminate and substrate in accordance with embodiments of the present disclosure. These microfluidic laminates can be configured to carry out tests on varying numbers of analytes in a given biological sample using biosensor based electrochemical processes. Some of them can incorporate an additional layer such as 502 configured to direct bio sample to the sensors and to the waste chamber. The exemplary figures show the microfluidic laminate that can be configured to test up to 9 analytes. The device 100 in these cases can have a reagent component 206 that does not have any reagent

chambers/reservoirs. The reagent component 206 only needs to have a chamber/reservoir for storing waste.

**[00097]** FIG. 5G to 5I illustrate further exemplary exploded views 560 to 580 respectively of various embodiments of the microfluidic laminate and substrate in accordance with embodiments of the present disclosure. Views 560, 570 and 580 show exploded views of stacked layers of plurality of double sided channel adhesive layers and intermediate layers configured to carry out tests for one two and three analytes respectively wherein at least one analyte is detected using immunoassay based electrochemical process requiring movement of one or more reagents stored in the reservoirs of the reagent component.

**[00098]** In an embodiment, the plurality of stacked double sided channel adhesive layers such as 562, 564 and 566, configured with microfluidic channels can define flow path for the bio sample, the one or more reagents and the waste. Thus the flow path of the bio sample, the one or more reagents and the waste can lie in different layers of the microfluidic laminate.

**[00099]** In an embodiment, the microfluidic laminate can further comprise intermediate layers such as 568 and 566 stacked between the plurality of stacked double sided channel adhesive layers and other layers of the microfluidic laminate. The intermediate layers 568 and 566 can incorporate one or more than one holes configured to facilitate transfer of one or more of the sample, the one or more reagents and the waste from one layer to other layer. The layer 570 can define the sensor area/chamber and also incorporate holes to facilitate transfer of fluids from upper layers to the reagent chamber or vice versa.

**[000100]** In an aspect the microfluidic channels on the plurality of stacked double sided channel adhesive layers and the holes in the intermediate layers are configured to facilitate simultaneous and independent testing of one or more than one analytes in the biological sample wherein at least one of the analytes is detected using immunoassay electrochemical process.

**[000101]** FIG. 6 illustrates exemplary images 600 of the microfluidic device 100 in accordance with embodiments of the present disclosure. Shown therein are top and bottom images. the top image clearly shows the sample inlet port 102 while the back side is shown with a bar code that can help in identifying each of the device 100 and linking it with a patient/sample. For traceability, record keeping, and report preparation.

**[000102]** While the foregoing describes various embodiments of the invention, other and further embodiments of the invention may be devised without departing from the basic scope thereof. The scope of the invention is determined by the claims that follow. The invention is not limited to the described embodiments, versions or examples, which are included to enable a person having ordinary skill in the art to make and use the invention when combined with information and knowledge available to the person having ordinary skill in the art.

## ADVANTAGES OF THE INVENTION

**[000103]** The present disclosure provides a microfluidic device that can be used to simultaneously conduct one or more than one tests on a biological sample.

**[000104]** The present disclosure provides a microfluidic device that independently conducts more than one test on the sample.

**[000105]** The present disclosure provides a microfluidic device that needs only a drop of 100 microliters of blood sample for analysis of multiple analytes.

**[000106]** The present disclosure provides a microfluidic device that eliminates need to draw multiple samples from a patient to determine his general health status and evaluate,

**[000107]** The present disclosure provides a microfluidic device that can carry out all the tests pertaining to a Clinical Chemistry Panel.

**[000108]** The present disclosure provides a microfluidic device that can perform immunoassay tests for multiple analytes simultaneously and independently on a given biological sample.

**[000109]** The present disclosure provides a microfluidic device that can carry out tests for one or more than one analytes in a given biological sample using biosensor and immunosensor based electrochemical processes.

**[000110]** The present disclosure provides a microfluidic device that has microfluidic channels for sample, one or more reagents and waste in different layers.

**[000111]** The present disclosure provides a microfluidic device that is easy to manufacture.

**[000112]** The present disclosure provides a microfluidic device that can be mass produced.

**[000113]** The present disclosure provides a microfluidic device that is economical to produce.

**[000114]** The present disclosure provides a microfluidic device that does not need a separate "desiccant cum oxygen scavenger" in external foil packaging of the cartridge.

**We Claim:**

1. A microfluidic device configured to carry out tests for one or more than one analytes in a sample, the device comprising:

   a base plate configured with one or more than one multiplexed sensors formed over the polymeric base plate for detecting one or more than one target analytes contained in the sample;

   a microfluidic laminate comprising circular adhesives that define area of each of the one or more than one multiplexed sensors and plurality of stacked double sided channel adhesive layers configured with channels for flow of the sample, one or more reagents and waste; wherein flow path of the bio sample, the one or more reagents and the waste lies in different layers.

2. The device of claim 1, wherein the device further comprises a reagent component configured with one or more reservoirs for storing reagent(s) and a waste chamber for disposing the used reagent.

3. The device of claim 1, wherein the microfluidic laminate further comprises intermediate layers with one or more than one holes configured to facilitate transfer of one or more of the sample, the one or more reagents and the waste from one layer to other layer.

4. The device of claim 1, wherein the device further comprises a top cover configured with a sample inlet port.

5. The device of claim 1, wherein at least one of the one or more than one multiplexed sensors is configured for testing at least one of the one or more than one analytes in the sample using immunosensor based electrochemical processes.

6. The device of claim 1, wherein the one or more than one multiplexed sensors comprise at least one working electrode and at least one reference electrode.

7. The device of claim 6, wherein the at least one working electrode has one or more of nanostructures deposited over it.

8. The device of claim 1, wherein the device is configured to carry out all the tests pertaining to a Clinical Chemistry Panel and common Biochemistries.

9. The device of claim 8, wherein the Clinical Chemistry Panel and common Biochemistries are selected from the group containing Metabolite Panel, Kidney Panel, Electrolytes panel, Liver Panel, Lipid panel in whole blood samples or serum or plasma.

10. The device of claim 8, wherein the panel is Coagulation panel.

11. A microfluidic device configured to carry out tests for one or more than one analytes in a sample, the device comprising:

    a base plate configured with one or more than one multiplexed sensors formed over the polymeric base plate for detecting one or more than one target analytes contained in the sample, wherein each of the one or more than one multiplexed sensors is a bio sensor;

    a microfluidic laminate comprising circular adhesives that define area of each of the one or more than one multiplexed sensors and at least one double sided channel adhesive layer configured with channels for flow of the sample to the one or more than one multiplexed sensors.

12. The device of claim 11, wherein the one or more than one multiplexed sensors comprise at least one working electrode and at least one reference electrode.

13. The device of claim 12, wherein the at least one working electrode has one or more of nanostructures deposited over it and a recognition element bound to or deposited over the nanostructures

14. The device of claim 13, wherein the recognition element is selected from the group consisting of antigens, antibodies, enzymes, aptazymes, and aptamers.

15. The device of claim 11, wherein the device is configured to carry out all the tests pertaining to a Clinical Chemistry Pane.

16. A microfluidic device configured to carry out tests for one or more than one analytes in a sample, the device comprising:

a base plate configured with one or more than one sensors formed over the polymeric base plate for detecting one or more than one target analytes contained in the sample;

a microfluidic laminate configured with channels for flow of the sample to the one or more than one sensors; and

a top cover wherein the top cover is configured to store a desiccant or a desiccant cum oxygen scavenger.

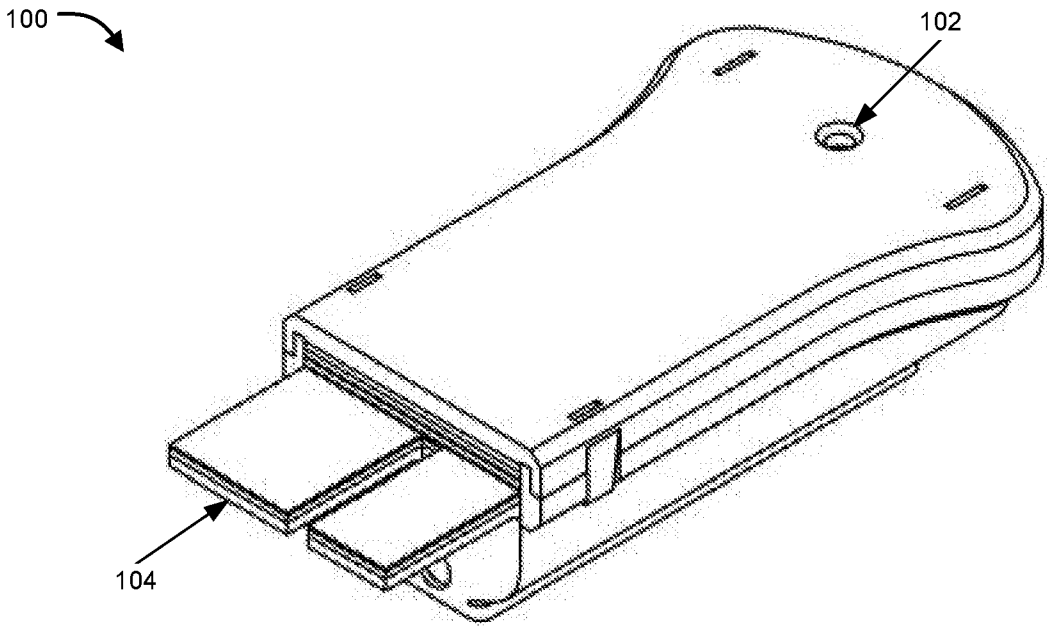


FIG. 1A

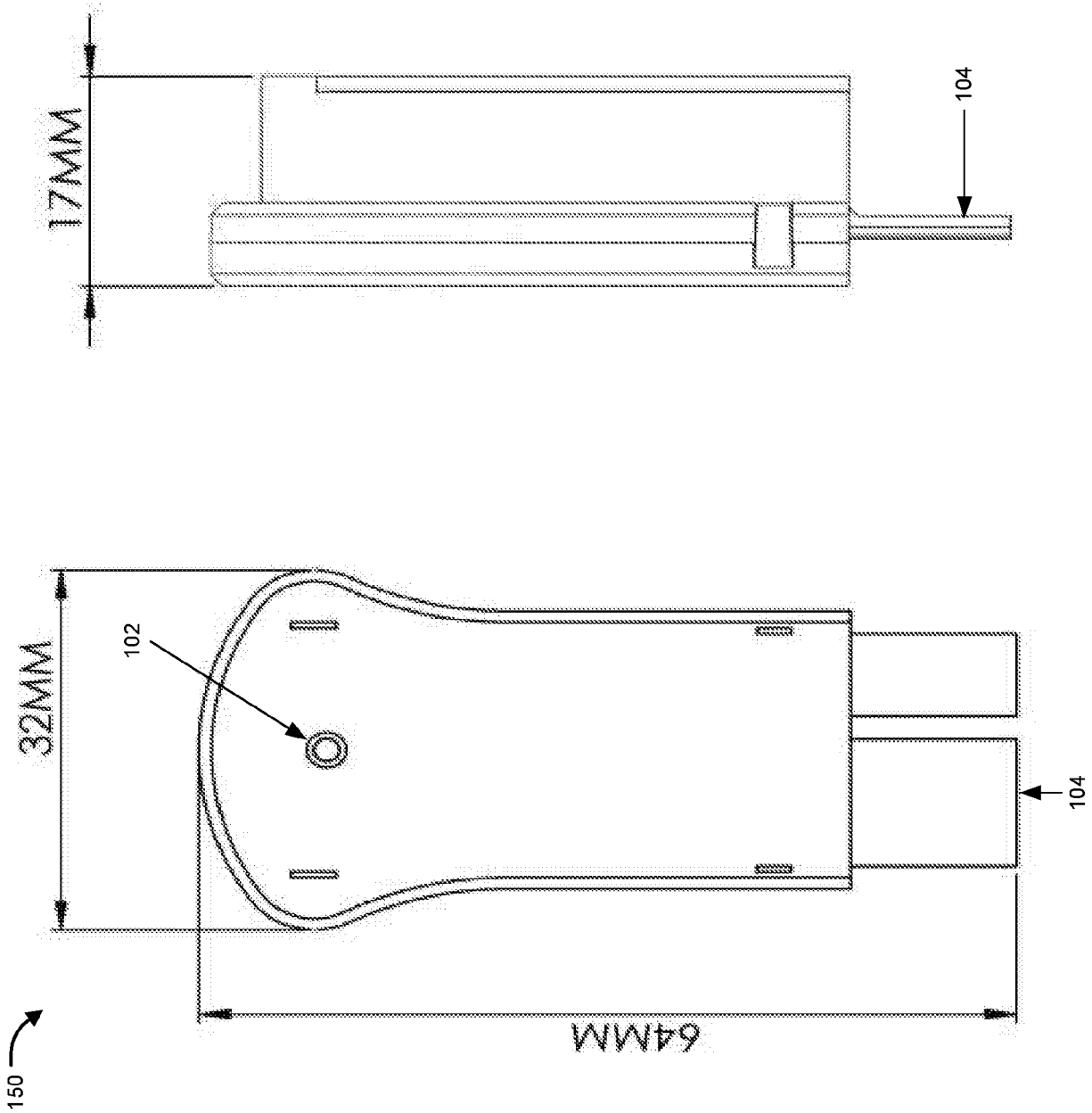


FIG. 1B

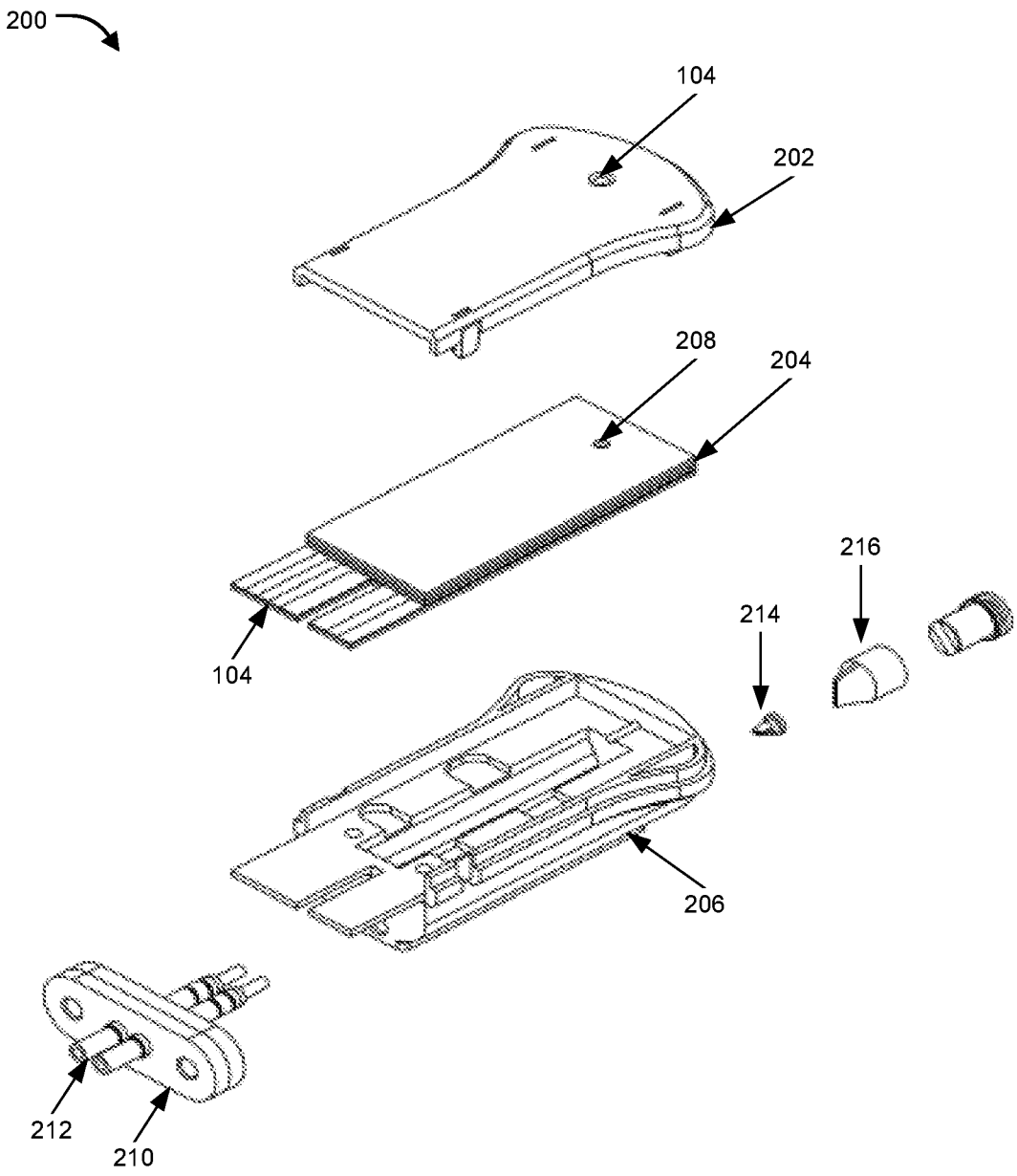


FIG. 2

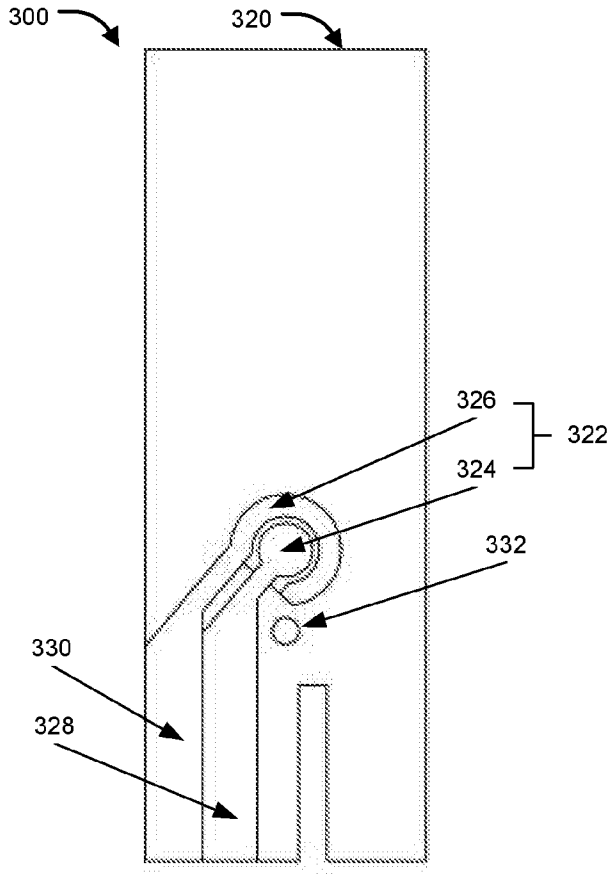


FIG. 3A

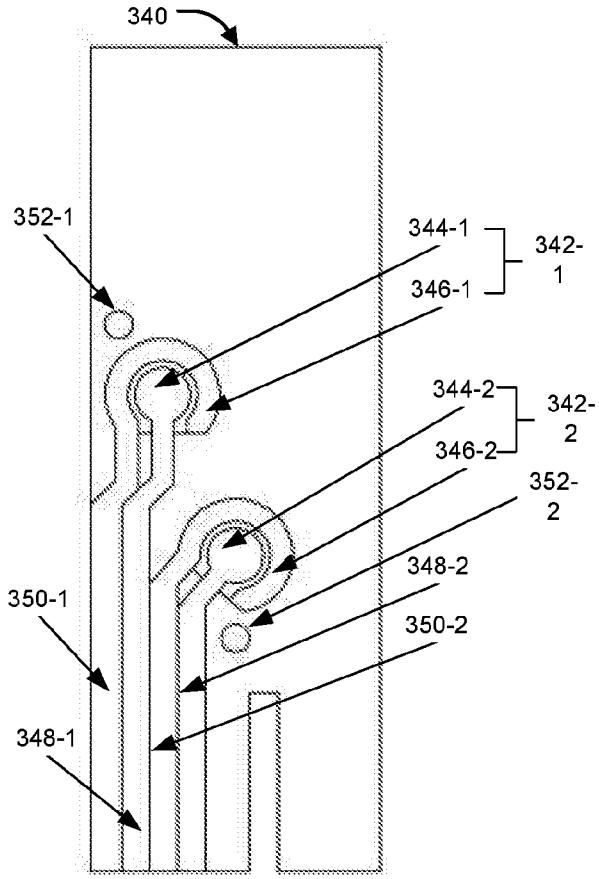


FIG. 3B

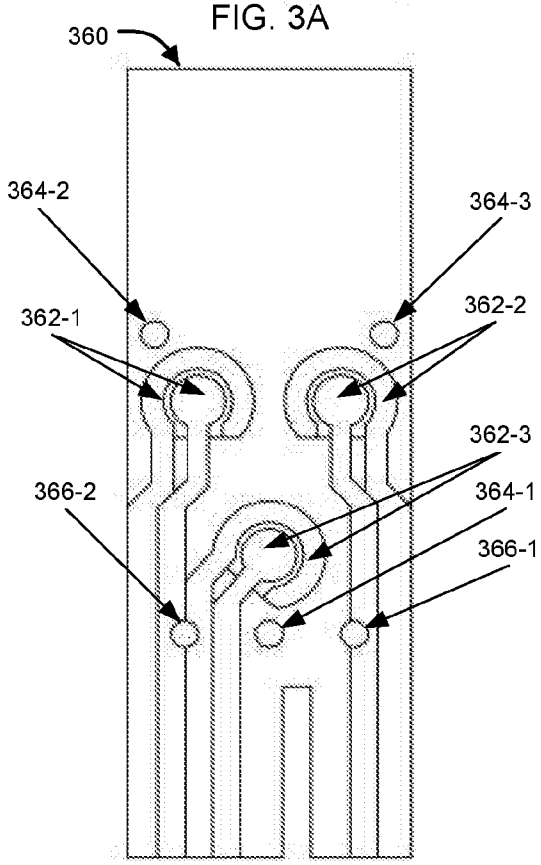


FIG. 3C

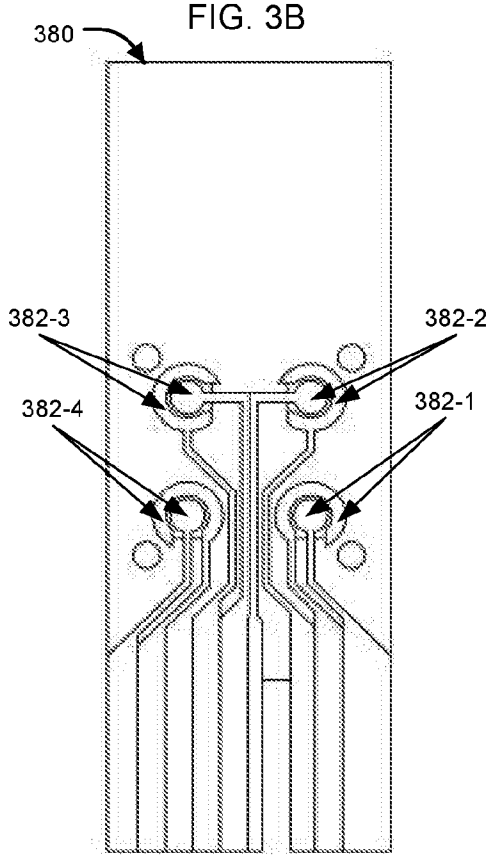


FIG. 3D

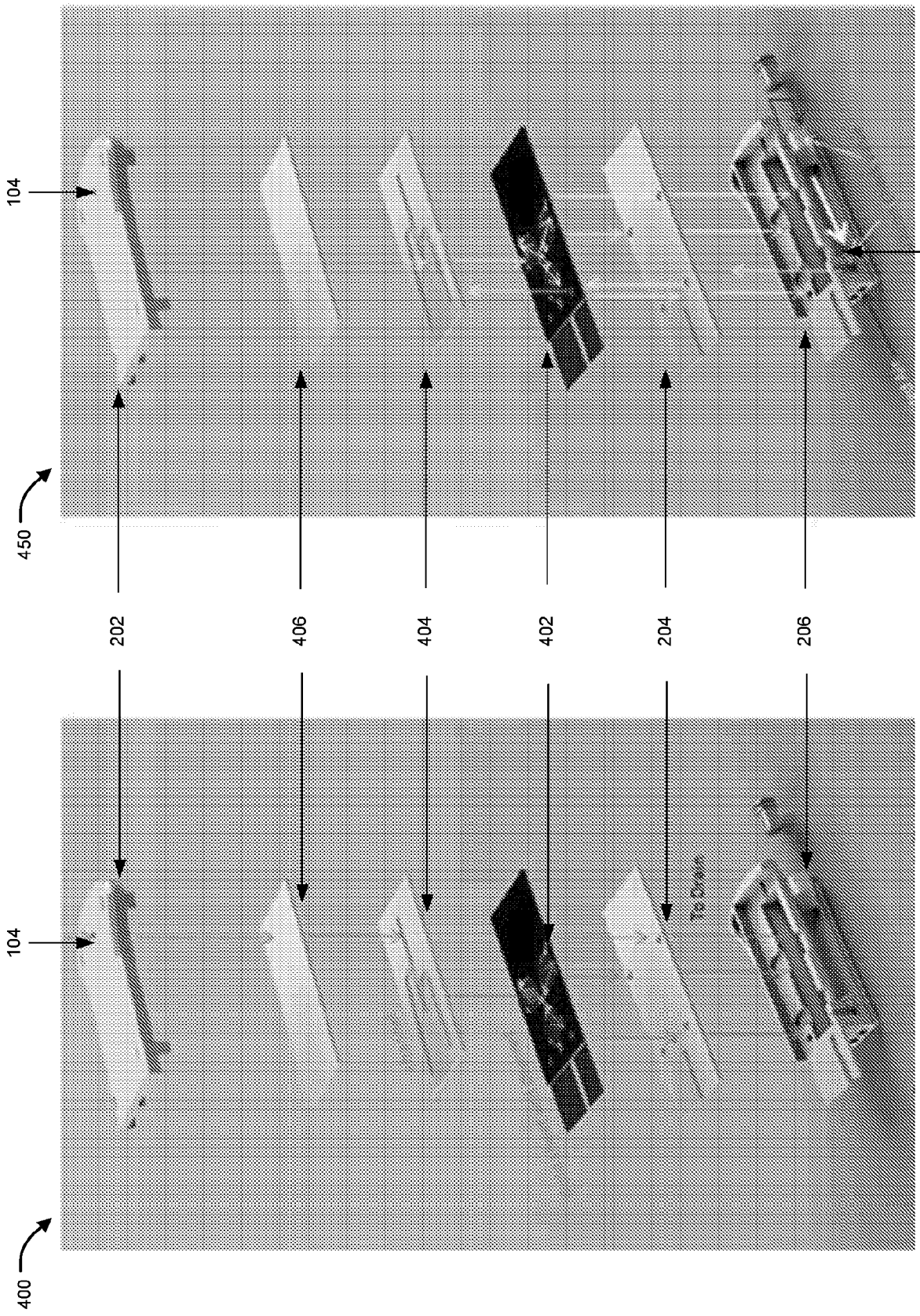


FIG. 4B

FIG. 4A

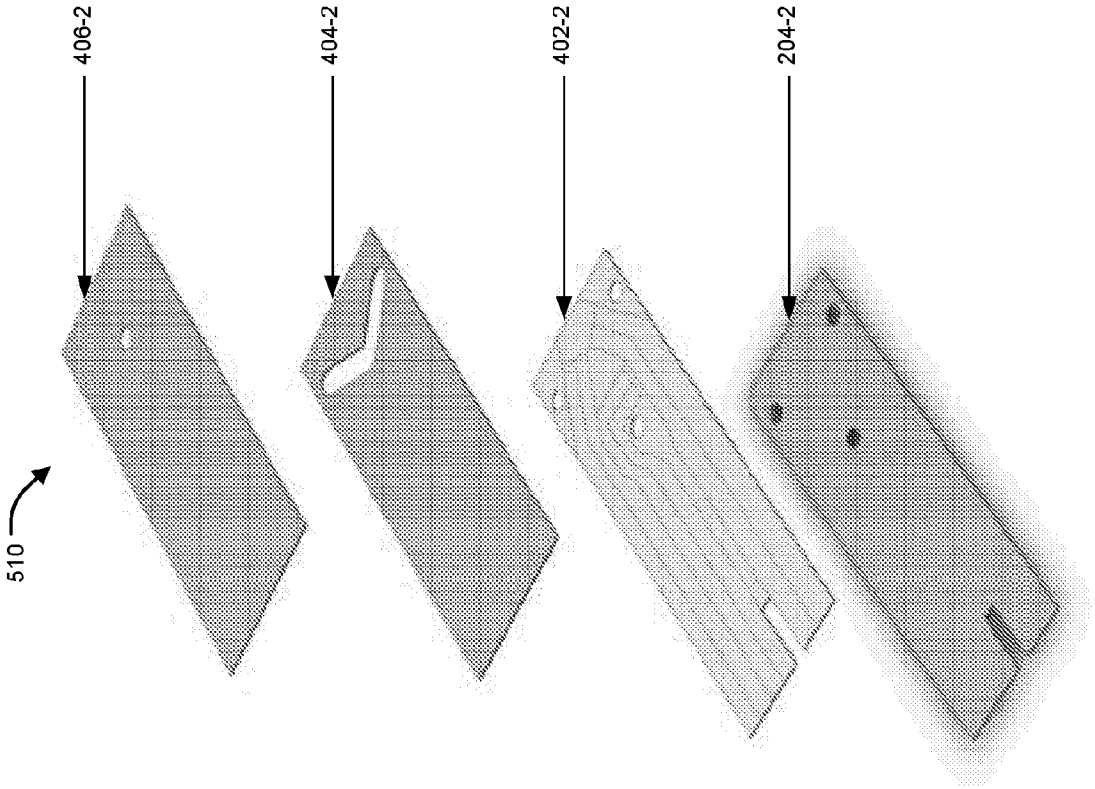


FIG. 5B

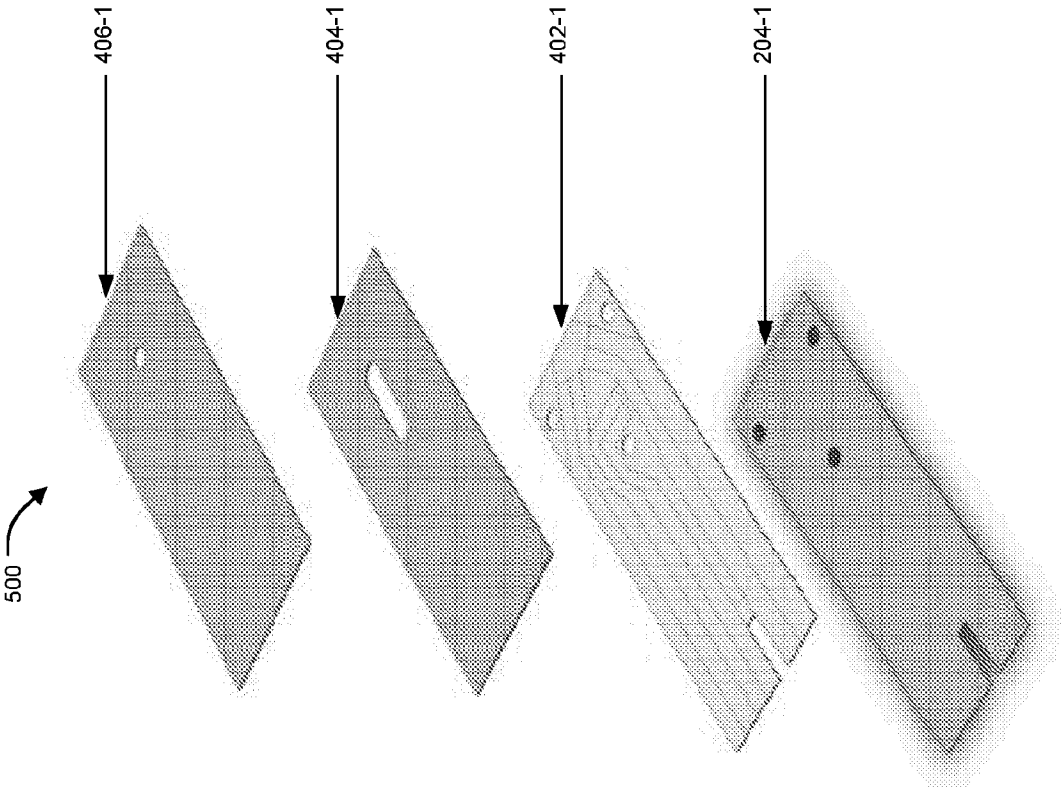


FIG. 5A

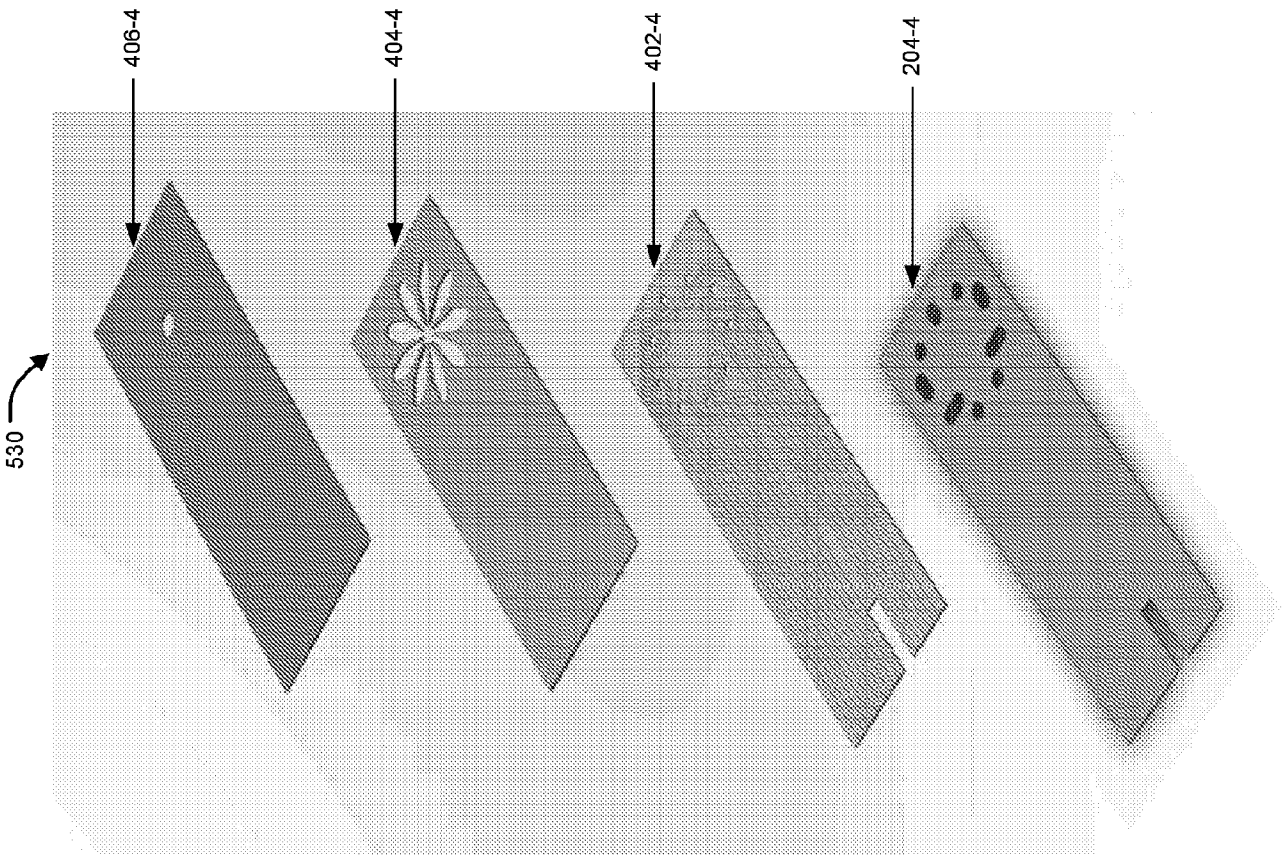


FIG. 5D

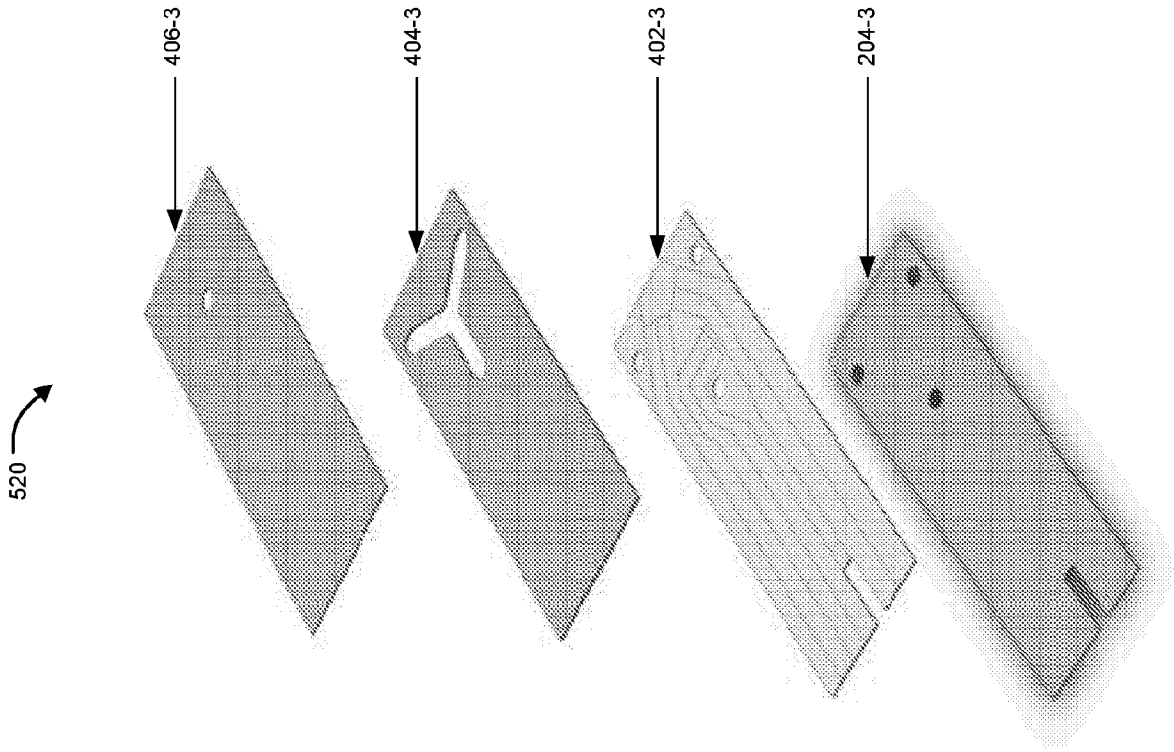


FIG. 5C

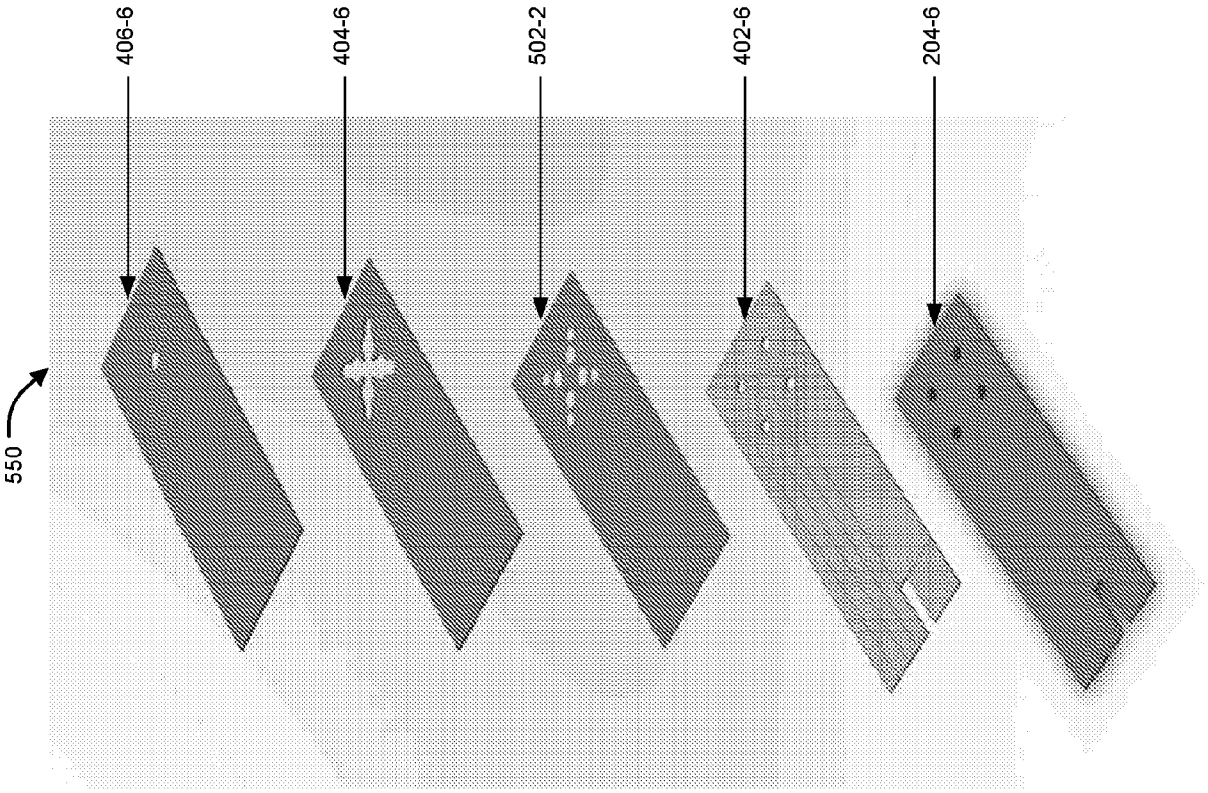


FIG. 5F

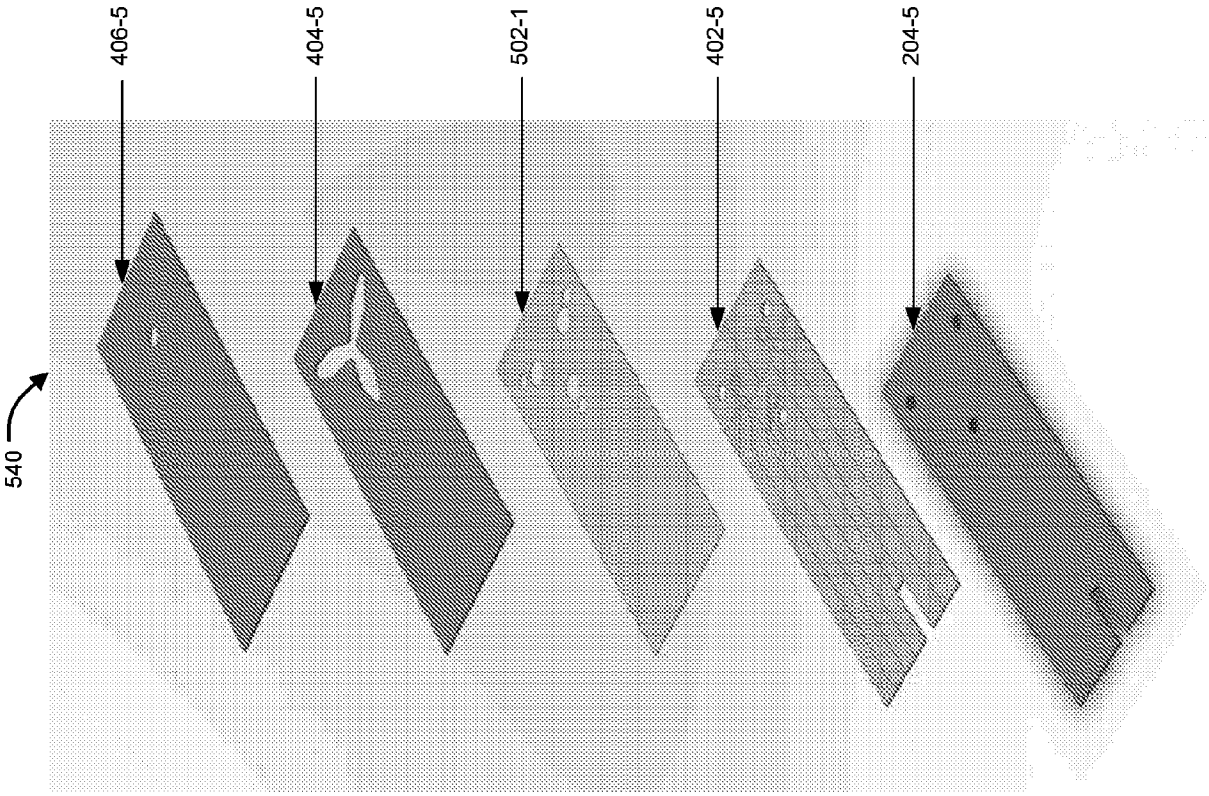


FIG. 5E

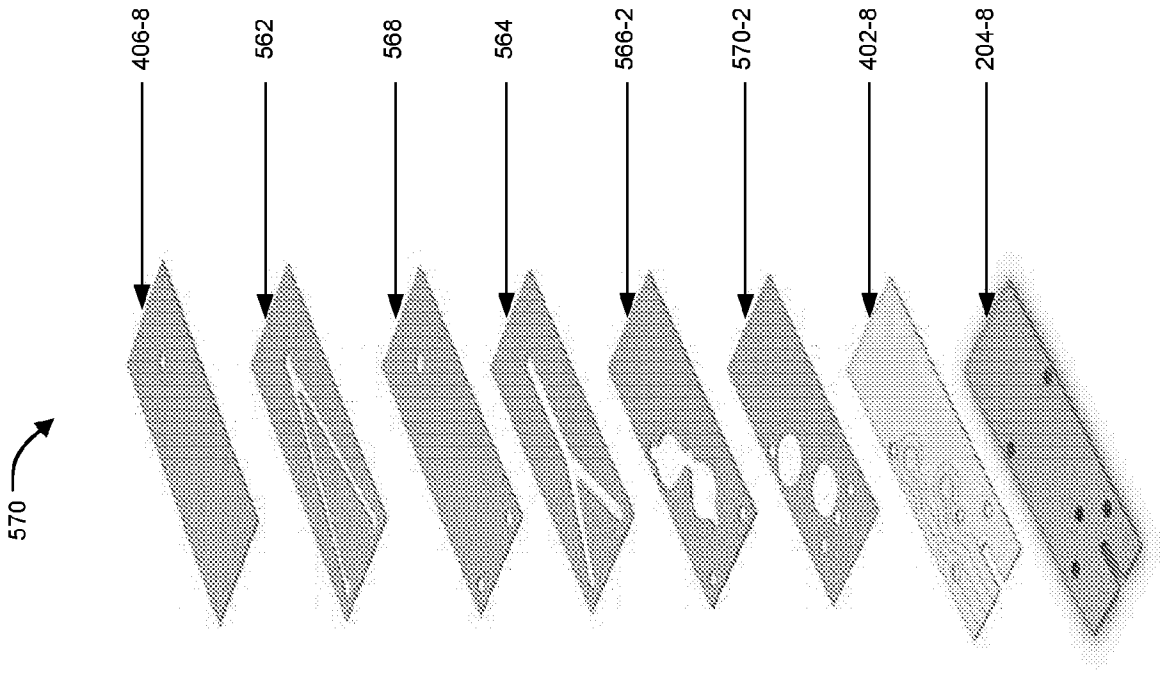
570
406-8
562
568
564
566-2
570-2
402-8
204-8

FIG. 5H

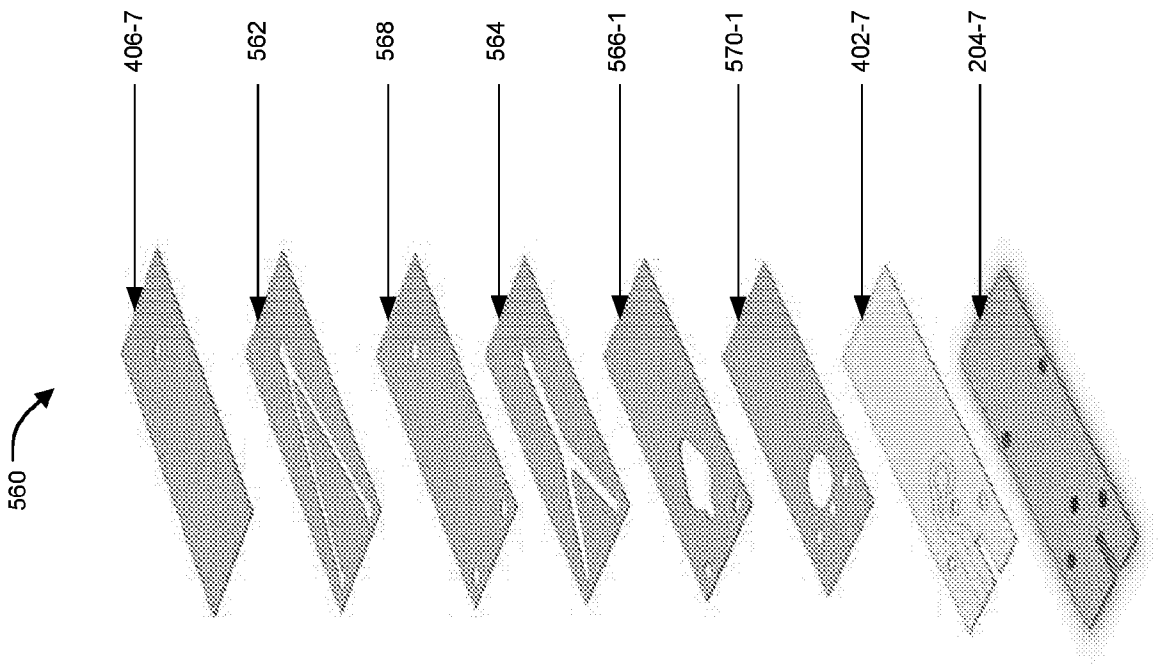
560
406-7
562
568
564
566-1
570-1
402-7
204-7

FIG. 5G

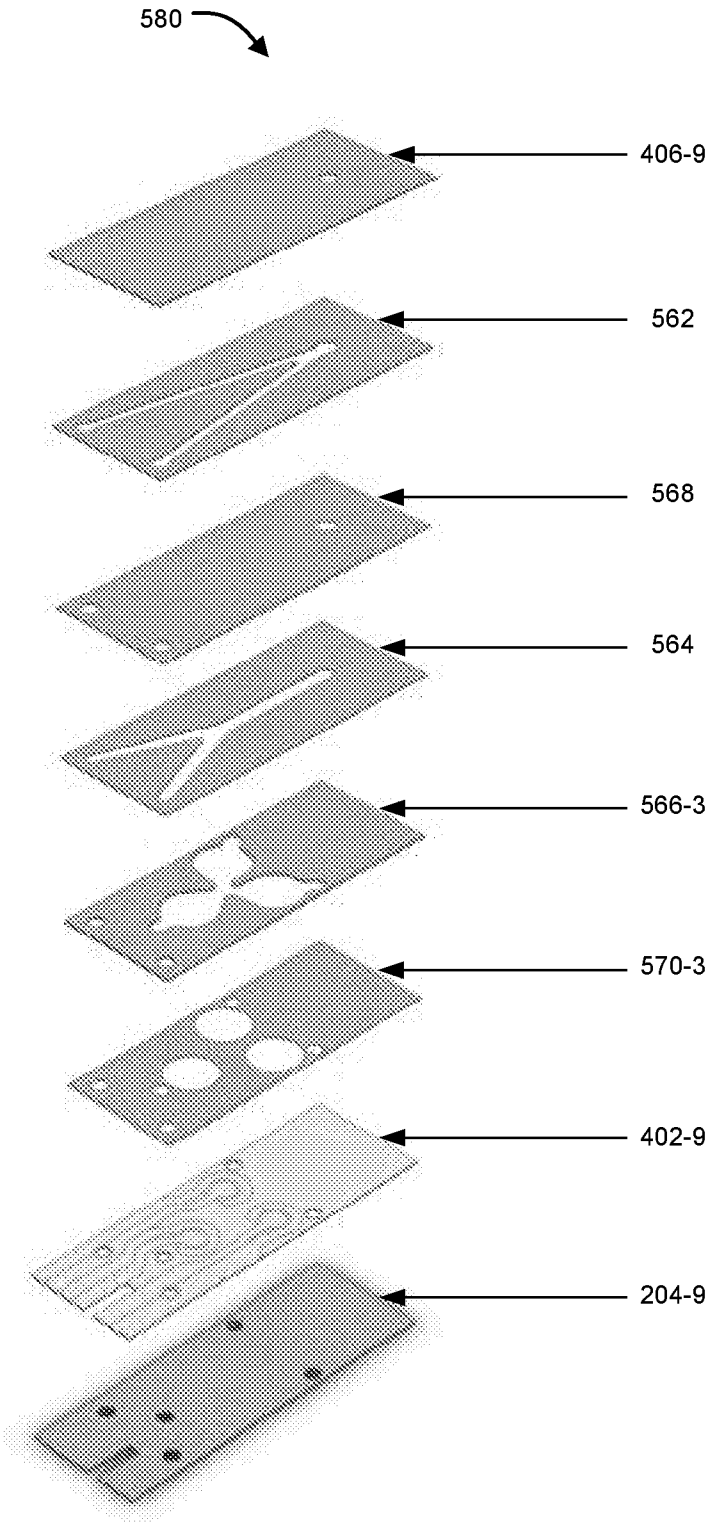


FIG. 5I

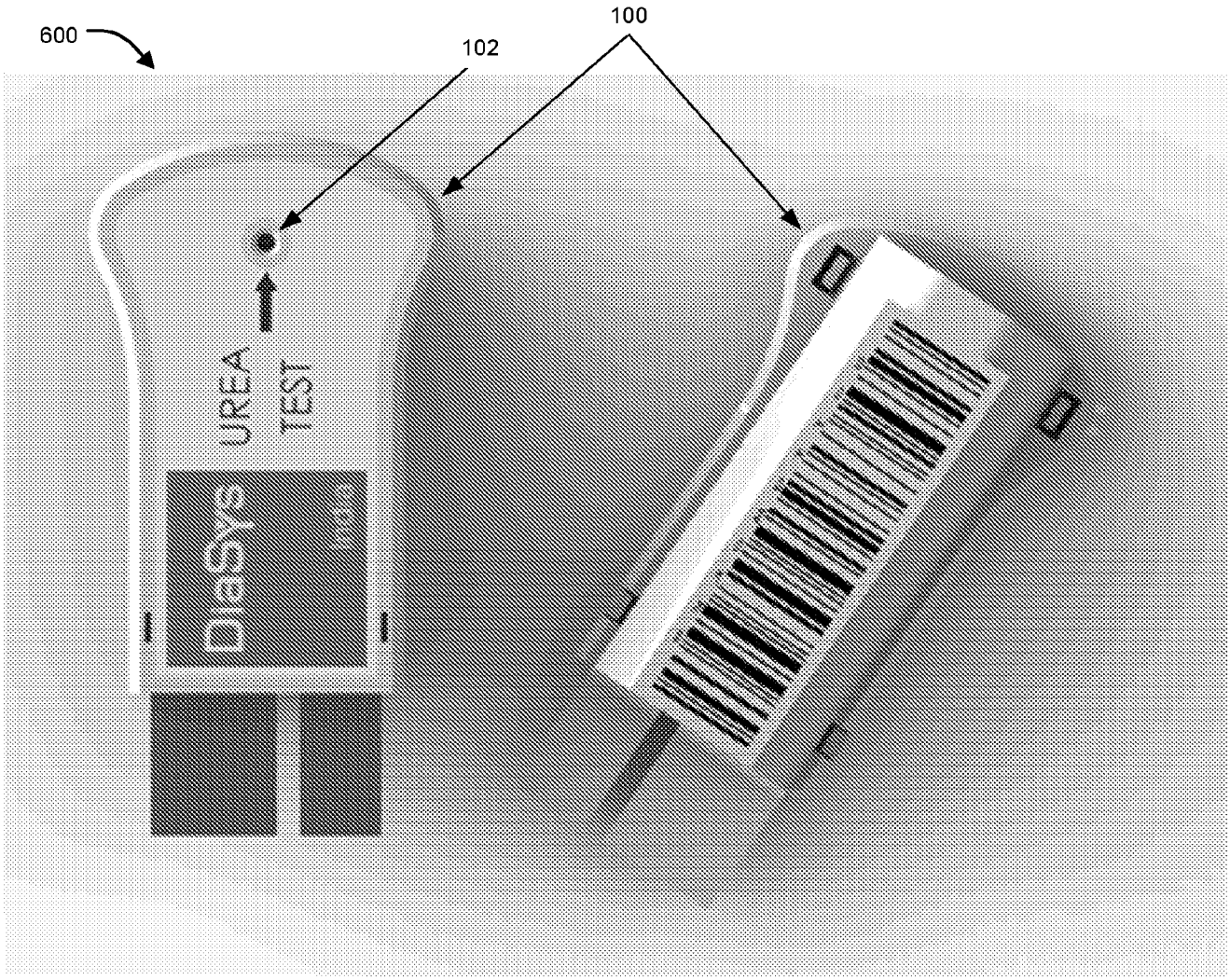


FIG. 6

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 15/56918

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - B01L 3/00; G01N 33/53, 35/00 (2016.01)
CPC - B01L 3/5027, 3/502707
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - B01L 3/00; G01N 33/53, 35/00 (2016.01)
CPC B01L 3/5027, 3/502707

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - B01L 3/502715, 2200/10, 2300/0819, 2300/0887; G01N 27/44791, 33/53, 33/5302, 33/5304, 2035/00158

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase; Google Patents; Google Scholar; Google Web; Espacenet; Search Terms: adhe*, analyte*, cap, circle*, circular*, cover*, desiccant*, different, double*, exit*, laminate*, layer*, microfluid*, multiplex*, outlet*, reagent*, sample*, scaveng*, sensor*, separate, side*, unique*, waste*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>A | US 2002/0042125 A1 (Petersen et al.) 11 April 2002 (11.04.2002), Figs. 16-17, para [0198]-[0202] | 16<br>---<br>1-15 |
| A | US 2008/0050830 A1 (Floriano et al.) 28 February 2008 (28.02.2008), para [0133]-[0134], [0208] | 1-15 |
| A | US 2011/0189719 A1 (Kuo et al.) 04 August 2011 (04.08.2011), para [0112] | 1-15 |
| A | US 2006/0264782 A1 (Holmes et al.) 23 November 2006 (23.11.2006), para [0189] | 1-16 |
| A | US 2011/0318728 A1 (Phan et al.) 29 December 2011 (29.12.2011), para [0013] | 1-16 |

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

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

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 19 January 2016 (19.01.2016) | 12 FEB 2016 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (January 2015)