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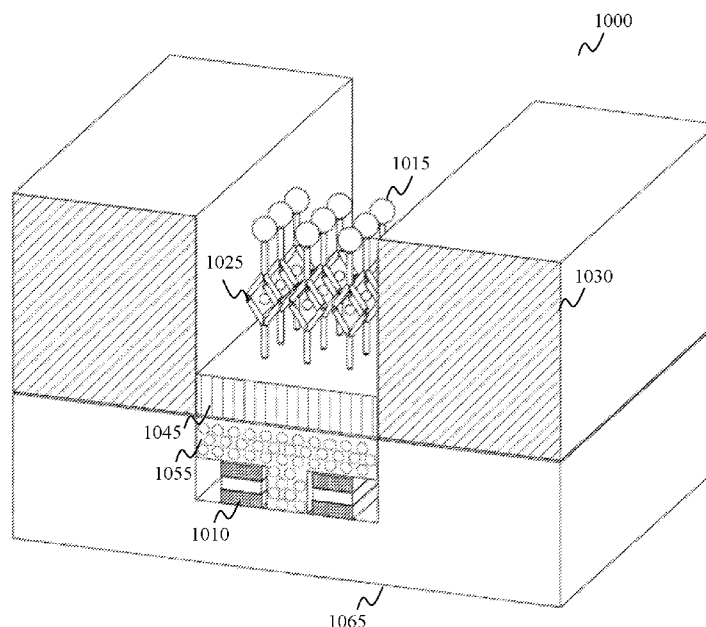


Fig. 10A

(57) Abstract: Methods of detecting the presence of an analyte in a query sample include providing a sensor that includes biomolecules disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor. Modes of operation remove or add magnetic beads from the vicinity of the sensor surface by interactions with the biomolecules. The methods feature detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.



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SYSTEM AND METHOD FOR SENSING ANALYTES IN GMR-BASED DETECTION OF BIOMARKERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/711,396 filed July 27, 2018, which is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] The present disclosure is generally related to systems and methods for sensing analytes in water and biological samples. In particular, the present disclosure relates to analyte sensing using methods of detection based on Giant Magneto-Resistive (GMR) sensors.

[0003] GMR sensors enable development of multiplex assays with high sensitivity and low cost in a compact system, and therefore have the potential to provide a platform suitable for a wide variety of applications. Reliable analyte sensing remains a challenge. The present disclosure provides exemplary solutions.

SUMMARY

[0004] In some aspects, embodiments herein relate to methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a cleavable portion covalently bound to the biomolecule, cleavage being catalyzed by the presence of the analyte in the query sample and a receptor associated with the cleavable portion of the biomolecule, the receptor being capable of binding a magnetic nanoparticle, passing the query sample over the sensor, thereby allowing cleavage and removal of the cleavable portion with the associated receptor from the biomolecule if the analyte is present, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of the analyte in the query sample by measuring resistance

change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

[0005] In other aspects, embodiments herein relate to methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising an antigenic portion that binds an antibody at an antigen binding site, the antibody further comprising a portion separate from the antigen binding site configured to bind a magnetic nanoparticle, passing a mixture of the query sample and the antibody over the sensor, wherein the antigen binding site of the antibody binds the analyte if present in the query sample, thereby preventing binding of the antibody to the antigenic portion of the biomolecule, passing magnetic particles over the sensor after passing the mixture over the sensor, and detecting the presence of the analyte in the query sample by measuring a resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

[0006] In other aspects, embodiments herein relate to method of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding region configured to bind a detection protein, the detection protein also being capable of binding the analyte, wherein when the detection protein binds the analyte, it prevents binding of the detection protein to the binding region of the biomolecule, passing the detection protein over the sensor, passing the query sample over the sensor, passing a reporter protein over the sensor after passing the query sample over the sensor, the reporter protein capable of binding the detection protein and the reporter protein configured to bind to magnetic nanoparticles, passing magnetic particles over the sensor after passing the reporter protein over the sensor, and detecting the presence of the metal ion by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

[0007] In yet other aspects, embodiments herein relate to methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising an associated magnetic particle, passing the query sample over the

sensor, thereby causing removal of the associated magnetic particle from the biomolecule if the analyte is present, and detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing the query sample over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0008] In still other aspects, embodiments herein relate to methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a first biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the first biomolecule comprising a conditional binding site for a second biomolecule comprising a binding site for a magnetic particle, passing the query sample over the sensor, passing the second biomolecule over the sensor, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0009] In yet still further aspects, embodiments herein relate to methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a first biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding site for a magnetic particle when the analyte is present, passing the query sample over the sensor, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0010] In yet still further aspects, embodiments relate to the systems configured to carry out the foregoing methods.

[0011] Other aspects, features, and advantages of the present disclosure will become apparent from the following detailed description, the accompanying drawings, and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Various embodiments of the present disclosure will be described herein below with reference to the Figures wherein:

[0013] FIG. 1 is a perspective view of an exemplary cartridge reader unit used in a system in accordance with an embodiment of the present disclosure.

[0014] FIG. 2A is a perspective view of an exemplary cartridge assembly used in the system, in accordance with an embodiment of the present disclosure.

[0015] FIG. 2B is an exploded view of the cartridge assembly of FIG. 2A, in accordance with an embodiment herein.

[0016] FIG. 2C is a schematic drawing of the cartridge assembly of FIGS. 2A, in accordance with an embodiment herein.

[0017] FIG. 2D shows a cross section of the cartridge assembly of FIG. 2A, illustrating a connection interface between a sample processing card and a sensing and communication substrate thereof.

[0018] FIG. 3 is a schematic diagram of the system in accordance with an embodiment of the present disclosure.

[0019] FIG. 4 shows steps of a method for performing analyte detection in a sample when using features of the herein disclosed system of FIG. 3, in accordance with an embodiment.

[0020] FIG. 5A shows a serpentine channel comprising a plurality of GMR sensors, in accordance with an embodiment.

[0021] FIG. 5B shows an arrangement of a plurality of channels on a substrate for GMR sensing, in accordance with an embodiment.

[0022] FIG. 6A shows a cross-section of a linear length of channel with GMR sensors disposed therein, in accordance with an embodiment.

[0023] FIG. 6B shows a cross-section of a linear length of channel having circular channel expansions where GMR sensors reside, in accordance with an embodiment.

[0024] FIG. 6C shows a cross-section of a linear length of channel having square channel expansions where GMR sensors reside, in accordance with an embodiment.

[0025] FIG. 6D shows a cross-section of a linear length of channel having triangular channel expansions where GMR sensors reside, in accordance with an embodiment.

[0026] FIG. 6E shows a section of a serpentine channel with GMR sensors disposed therein, in accordance with an embodiment.

[0027] FIG. 6F shows a section of a serpentine channel with GMR sensors disposed circular channel expansions, in accordance with an embodiment.

[0028] FIG. 6G shows a section of a channel having a bifurcation and with GMR sensors disposed therein, in accordance with an embodiment.

[0029] FIG. 7 shows a cross-section of a linear length of channel having circular channel expansions where differing GMR sensors reside, in accordance with an embodiment.

[0030] FIG. 8A shows a GMR sensor chip having a plurality of channels with GMR sensors incorporated at circular expansions and the connectivity of the GMR sensors to contact pads via wiring, in accordance with an embodiment.

[0031] FIG. 8B shows an expansion of the area around the GMR sensors in the circular channel expansions showing the wiring network, in accordance with an embodiment.

[0032] FIG. 8C shows the structure of a switch, in accordance with an embodiment.

[0033] FIG. 9 shows a cross-section representation of a circular channel expansion and the GMR residing therein along with attachment to a contact pad via a wire, in accordance with an embodiment.

[0034] FIG. 10A shows a cross-section representation of a channel with no expansion and the GMR residing therein along with a biosurface layer disposed over the GMR sensor, in accordance with an embodiment.

[0035] FIG. 10B shows the basic structure and operating principle of GMR sensors, in accordance with an embodiment.

[0036] FIG. 11A shows a structure state diagram of a subtractive GMR sensing process, in accordance with an embodiment.

[0037] FIG. 11B shows a process flow diagram for the GMR sensing process of FIG. 11A.

[0038] FIG. 12A shows a structure state diagram of an additive GMR sensing process, in accordance with an embodiment.

[0039] FIG. 12B shows a process flow diagram for the GMR sensing process of FIG. 12A.

[0040] FIG. 13A shows another structure state diagram of an additive GMR sensing process, in accordance with an embodiment.

[0041] FIG. 13B shows a process flow diagram for the GMR sensing process of FIG. 13A.

[0042] FIG. 13C shows an alternative flow diagram for the GMR sensing process of FIG. 13A.

[0043] FIG. 14A shows a structure state diagram of an additive GMR sensing process in which an analyte modifies a molecule bound to a biosurface, in accordance with an embodiment.

[0044] FIG. 14B shows a process flow diagram for the GMR sensing process of FIG. 14A.

[0045] FIG. 15A shows an alternative structure state diagram of an additive GMR sensing process in which an analyte modifies a molecule bound to a biosurface, in accordance with an embodiment.

[0046] FIG. 15B shows a process flow diagram for the GMR sensing process of FIG. 15A.

[0047] FIG. 16A shows a structure state diagram of an additive GMR sensing process employing an exemplary “sandwich” antibody process.

[0048] FIG. 16B shows a process flow diagram for the GMR sensing process of FIG. 16A.

[0049] FIG. 17A shows a plot of data generated with a GMR sensor for detecting D-dimer cardiac biomarker: solid line is a positive control; dashed line is a sample run; line indicated with “+” is a negative control.

[0050] FIG. 17B shows a calibration curve for D-dimer using a GMR sensor for detecting D-dimer cardiac biomarker.

[0051] FIG. 17C shows a graph of data generated with a GMR sensor for detecting troponin cardiac biomarker.

[0052] FIG. 18 shows a reaction scheme for the GMR-based detection of lead ion, in accordance with an embodiment.

[0053] FIG. 19 shows a reaction scheme for the GMR-based detection of mercury ion, in accordance with an embodiment.

[0054] FIG. 20 show a reaction scheme for the GMR-based detection of cadmium or arsenic ion, in accordance with an embodiment.

DETAILED DESCRIPTION

[0055] As evident by the drawings and below description, this disclosure relates to a sample handling system (or “system” as noted throughout this disclosure) which may be used for detecting presence of an analyte (or analytes) in a sample. In an embodiment, this system, depicted as system 300 in Fig. 3, may include (1) a sample handling system or “cartridge assembly” that includes sample preparation microfluidic channel(s) and at least one sensing device (or sensor) for sensing biomarkers in a test sample, and (2) a data processing and display device or “cartridge reader unit” that includes a processor or controller for processing any sensed data of the sensing device of the cartridge assembly and a display for displaying a detection event. Together these two components make up the system. In an embodiment, these components may include variable features including, without limitation, one or more reagent cartridges, a cartridge for waste, and a flow control system which may be, for example, a pneumatic flow controller.

[0056] Generally, the process for preparing a sample in the cartridge assembly, in order for detection of analytes, biomarkers, etc. to happen by the assembly and output via the cartridge reader unit, is as-follows: A raw patient sample is loaded onto a card, optionally filtered via a filter membrane, after which a negative pressure generated by off-card pneumatics filters the sample into a separated test sample (e.g., plasma). This separated test sample is quantitated on-card through channel geometry. The sample is prepared on card by interaction with mixing materials (e.g., reagent(s) (which may be dry or wet), buffer and/or wash buffer, beads and/or beads solution, etc.) from a mixing material source (e.g., blister pack, storage chamber, cartridge, well, etc.) prior to flow over the sensor /sensing device. The sample preparation channels may be designed so that any number of channels may be stacked vertically in a card, allowing multiple patient samples to be used. The same goes for sensing microfluidic devices, which may also be

stacked vertically. A sample preparation card, which is part of the cartridge assembly, includes one or more structures providing functionalities selected from filtering, heating, cooling, mixing, diluting, adding reagent, chromatographic separation and combinations thereof; and a means for moving a sample throughout the sample preparation card. Further description regarding these features is provided later below.

[0057] Fig. 1 shows an example of a cartridge reader unit 100, used in system 300 (see Fig. 3) in accordance with an embodiment. The cartridge reader unit 100 may be configured to be compact and/or small enough to be a hand-held, mobile instrument, for example. The cartridge reader unit 100 includes a body or housing 110 that has a display 120 and a cartridge receiver 130 for receiving a cartridge assembly. The housing 110 may have an ergonomic design to allow greater comfort if the reader unit 100 is held in an operator's hand. The shape and design of the housing 110 is not intended to be limited, however.

[0058] The cartridge reader unit 100 may include an interface 140 and a display 120 for prompting a user to input and/or connect the cartridge assembly 200 with the unit and/or sample, for example. In accordance with an embodiment, in combination with the disclosed cartridge assembly 200, the system 300 may process, detect, analyze, and generate a report of the results, e.g., regarding multiple detected biomarkers in a test sample, e.g., five cardiac biomarkers, using sensor (GMR) technology, and further display the biomarker results, as part of one process.

[0059] The display 120 may be configured to display information to an operator or a user, for example. The display 120 may be provided in the form of an integrated display screen or touch screen (e.g., with haptics or tactile feedback), e.g., an LCD screen or LED screen or any other flat panel display, provided on the housing 110, and (optionally) provides an input surface that may be designed for acting as end user interface (UI) 140 that an operator may use to input commands and/or settings to the unit 100, e.g., via touching a finger to the display 120 itself. The size of the display 120 may vary. More specifically, in one embodiment, the display 120 may be configured to display a control panel with keys, buttons, menus, and/or keyboard functions thereon for inputting commands and/or settings for the system 300 as part of the end user interface. In an embodiment, the control panel includes function keys, start and stop buttons, return or enter buttons, and settings buttons. Additionally, and/or alternatively, although not shown in Fig. 1, the cartridge reader 100 may include, in an embodiment, any number of physical input devices, including, but not limited to, buttons and a keyboard. In another

embodiment, the cartridge reader 100 may be configured to receive input via another device, e.g., via a direct or wired connection (e.g., using a plug and cord to connect to a computer (PC or CPU) or a processor) or via wireless connection. In yet another embodiment, display 120 may be to an integrated screen, or may be to an external display system, or may be to both. Via the display control unit 120, the test results (e.g., from a cartridge reader 310, described with reference to Fig. 3, for example) may be displayed on the integrated or external display. In still yet another embodiment, the user interface 140 may be provided separate from the display 120. For example, if a touch screen UI is not used for display 120, other input devices may be utilized as user interface 140 (e.g., remote, keyboard, mouse, buttons, joystick, etc.) and may be associated with the cartridge reader 100 and/or system 300. Accordingly, it should be understood that the devices and/or methods used for input into the cartridge reader 100 are not intended to be limiting. All functions of the cartridge reader 100 and/or system 300 may, in one embodiment, be managed via the display 120 and/or input device(s), including, but not limited to: starting a method of processing (e.g., via a start button), selecting and/or altering settings for an assay and/or cartridge assembly 200, selecting and/or settings related to pneumatics, confirming any prompts for input, viewing steps in a method of processing a test sample, and/or viewing (e.g., via display 120 and/or user interface 140) test results and values calculated by the GMR sensor and control unit / cartridge reader. The display 120 may visually show information related to analyte detection in a sample. The display 120 may be configured to display generated test results from the control unit / cartridge reader. In an embodiment, real-time feedback regarding test results that have been determined / processed by the cartridge reader unit / controller (by receiving measurements from the sensing device, the measurements being determined as a result of the detected analytes or biomarkers), may be displayed on the display 120.

[0060] Optionally, a speaker (not shown) may also be provided as part of the cartridge reader unit 100 for providing an audio output. Any number of sounds may be output, including, but not limited to speech and/or alarms. The cartridge reader unit 100 may also or alternatively optionally include any number of connectors, e.g., a LAN connector and USB connector, and/or other input/output devices associated therewith. The LAN connector and/or USB connector may be used to connect input devices and/or output devices to the cartridge reader unit 100, including removable storage or a drive or another system.

[0061] In accordance with an embodiment, the cartridge receiver 130 may be an opening (such as shown in Fig. 1) within the housing 110 in which a cartridge assembly (e.g., cartridge assembly 200 of Fig. 2) may be inserted. In another embodiment, the cartridge receiver 130 may include a tray that is configured to receive a cartridge assembly therein. Such a tray may move relative to the housing 110, e.g., out of and into an opening therein, and to thereby receive the cartridge assembly 200 and move the cartridge assembly into (and out of) the housing 110. In one embodiment, the tray may be a spring-loaded tray that is configured to releasably lock with respect to the housing 110. Additional details associated with the cartridge reader unit 100 are described later with respect to Fig. 3.

[0062] As previously noted, cartridge assembly 200 may be designed for insertion into the cartridge reader unit 100, such that a sample (e.g., blood, urine) may be prepared, processed, and analyzed. Figs. 2A-2C illustrate an exemplary embodiment of a cartridge assembly 200 in accordance with embodiments herein. Some general features associated with the disclosed cartridge assembly 200 are described with reference to these figures. However, as described in greater detail later, several different types of cartridge cards and thus cartridge assemblies may be utilized with the cartridge reader unit 100 and thus provided as part of system 300. In embodiments, the sampling handling system or cartridge assembly 200 may take the form of disposable assemblies for conducting individual tests. That is, as will be further understood by the description herein, depending on a type of sample and/or analytes being tested, a different cartridge card configuration(s) and/or cartridge assembly(ies) may be utilized. Fig. 2A shows a top, angled view of a cartridge assembly 200, in accordance with an embodiment herein. The cartridge assembly 200 includes a sample processing card 210 and a sensing and communication substrate 202 (see also Fig. 2B). Generally, the sample processing card 210 is configured to receive the sample (e.g., via a sample port such as injection port, also described below) and, once inserted into the cartridge reader unit 100, process the sample and direct flow of the sample to produce a prepared sample. Card 210 may also store waste from a sample and/or fluid used for preparing the test sample in an internal waste chamber(s) (not shown in Fig. 2A, but further described below). Memory chip 275 may be read and/or written to and is used to store information relative to the cartridge application, sensor calibration, and sample processing required, for example. In an embodiment, the memory chip 275 is configured to store a pneumatic system protocol that includes steps and settings for selectively applying pressure to

the card 210 of the cartridge assembly 200, and thus implementing a method for preparation of sample for delivery to a sensor (e.g., GMR sensor chip 280). The memory chip may be used to mistake-proof each cartridge assembly 200 inserted into the unit 100, as it includes the automation recipe for each assay. The memory chip 275 also contain traceability to the manufacturing of each card 210 and/or cartridge assembly 200. The sensing and communication substrate 202 may be configured to establish and maintain communication with the cartridge reader unit 100, as well as receive, process, and sense features of the prepared sample. The substrate 202 establishes communication with a controller in the cartridge reader unit 100 such that analyte(s) may be detected in a prepared sample. The sample processing card 210 and the sensing and communication substrate 202 (see, e.g., Fig. 2B) are assembled or combined together to form the cartridge assembly 200. In an embodiment, adhesive material (see, e.g., Fig. 2D) may optionally be used to adhere the card 210 and substrate 202 to one another. In an embodiment, the substrate 202 may be a laminated layer applied to the sample processing card 210. In one embodiment, the substrate 202 may be designed as a flexible circuit that is laminated to sample processing card 210. In another embodiment, the sample processing card 210 may be fabricated from a ceramic material, with the circuit, sensor (sensor chip 280) and fluid channels integrated thereon. Alternatively, the card 210 and substrate 202 may be mechanically aligned and connected together. In one embodiment, a portion of the substrate 202 may extend from an edge or an end of the card 210, such as shown in Fig. 2A. In another embodiment, such as shown in Fig. 2B, the substrate 202 may be aligned and/or sized such that it has similar or smaller edges than the card 210.

[0063] Fig. 2C schematically illustrates features of the cartridge assembly 200, in accordance with an embodiment. As shown, some of the features may be provided on the sample processing card 210, while other may be associated with the substrate 202. Generally, to receive a test sample (e.g., blood, urine) (within a body of the card), the cartridge assembly 200 includes a sample injection port 215, which may be provided on a top of the card 210. Also optionally provided as part of the card 210 are filter 220 (also referred to herein as a filtration membrane), vent port 225, valve array 230 (or valve array zone 230), and pneumatic control ports 235. Communication channels 233 are provided within the card 210 to fluidly connect such features of the card 210. Pneumatic control ports 235 are part of a pneumatic interface on the cartridge assembly 200 for selectively applying pressurized fluid (air) to the communication channels 233

of the card, for directing flow of fluids (air, liquids, test sample, etc.) therein and/or valve array 230. Optionally, the card 210 may include distinct valve control ports 535 connected to designated communication channels 233 for controlling the valves in the valve array 230. The card 210 may also have one or more metering chambers 240, gas permeable membranes 245, and mixing channels 250 that are fluidly connected via communication channels 233. Metering chamber(s) are designed to receive at least the test sample (either directly or filtered) therein via communication channels 233. Generally, a sample may be injected into the cartridge assembly 200 through port 215 and processed by means of filtering with filter (e.g., filter 220), metering in metering chamber(s) 240, mixing in mixing channel(s) 250, heating and/or cooling (optional), and directing and changing the flow rate via communication channels 233, pneumatic control ports 235, and valve array 230. For example, flow of the fluid may be controlled using internal micro fluidic channels (also generally referred to as communication channels 233 throughout this disclosure) and valves via a connection of a pneumatic system (e.g., system 330 in the cartridge reader unit 100, as shown in Fig. 3) and a pneumatic interface e.g., on the card 210 that has pneumatic control ports 235 or a similar connection section. Optional heating of the test sample and/or mixing materials / fluids within the card 210 may be implemented, in accordance with an embodiment, via a heater 259 which may be in the form of a wire trace provided on a top side of a PCB / substrate 202 with a thermistor. Optional cooling of the test sample and/or mixing materials / fluids within the card 210 may be implemented, in accordance with an embodiment, via a TEC module integrated in the cartridge assembly 200 (e.g., on the substrate 202), or, in another embodiment, via a module integrated inside of the cartridge reader unit 100. For example, if the cooling module is provided in the unit 100, it may be pressed against the cartridge assembly 200 should cooling be required. Processing may also optionally include introduction of reagents via optional reagent sections 260 (and/or blister packs) on the card 210 and/or via reagent cartridges in the housing 110 the cartridge reader unit 100. Reagents may be released or mixed as required by the process for that sample and the cartridge assembly 200 being analyzed. Further, optional blister packs 265 may be provided on the card 210 to introduce materials such as reagents, eluants, wash buffers, magnetic nanoparticles, bead solution, or other buffers to the sample via communication channels 233 during processing. One or more internal waste chambers (also referred to herein as waste tanks for waste reservoirs) 270 may also be optionally provided on the card 210 to store waste from the sample and reagents. An output port

255 – also referred to as a sensor delivery port, or input port to the sensor – is provided to output a prepared sample from the card 210 to a GMR sensor chip 280, as discussed below, for detecting analytes in the test sample. The output port 255 may be fluidly connected to a metering chamber for delivering the test sample and one or more mixing materials to the sensor.

Accordingly, the sensor may be configured to receive the test sample and the one or more mixing materials via the at least one output port 255. In embodiments, an input port 257 – also referred to as a waste delivery port, or output port from the sensor – is provided to output any fluid or sample from the GMR sensor chip 280 to a waste chamber 270. Waste chamber(s) 270 may be fluidly connected to other features of the card 210 (including, for example, metering chamber(s) 240, an input port 257, or both) via communication channels 233.

[0064] The cartridge assembly 200 has the ability to store, read, and/or write data on a memory chip 275, which may be associated with the card 210 or the substrate 202. As noted previously, the memory chip 275 may be used to store information related and/or relative to the cartridge application, sensor calibration, and required sample processing (within the sample processing card), as well as receive additional information based on a prepared and processed sample. The memory chip 275 may be positioned on the sample processing card 210 or on the substrate 200.

[0065] As previously noted, a magnetoresistive sensor may be utilized, in accordance with embodiments herein, to determine analytes (such as biomarkers) within a test sample using the herein disclosed system. While the description and Figures note use of a particular type of magnetoresistance sensor, i.e., a giant magnetoresistance (GMR) sensor, it should be understood that this disclosure is not limited to a GMR sensor platform. In accordance with some embodiments, the sensor may be an anisotropic magnetoresistive (AMR) sensor and/or magnetic tunnel junction (MTJ) sensors, for example. In embodiments, other types of magnetoresistive sensor technologies may be utilized. Nonetheless, for explanatory purposes only, the description and Figures reference use of a GMR sensor as a magnetoresistive sensor.

[0066] The substrate 202 of cartridge assembly 200 may be or include an electronic interface and/or a circuit interface such as a PCB (printed circuit board) that may have a giant magnetoresistance (GMR) sensor chip 280 and electrical contact pads 290 (or electrical contact portions) associated therewith. Other components may also be provided on the substrate 202. The GMR sensor chip 280 is attached at least to the substrate 202, in accordance with an

embodiment. The GMR sensor chip 280 may be placed on and attached to the substrate 202 using adhesive, for example. In an embodiment, a liquid adhesive or a tape adhesive may be used between the GMR sensor 280 and the PCB substrate 202. Such a design may require a bond to the PCB at the bottom and a bond to the processing card at the top, for example. Alternatively, other approaches for attaching the GMR sensor chip 280 to the substrate 202 include, but are not limited to: friction fitting the GMR sensor to the PCB, and connecting a top of the GMR sensor chip 280 directly to the sample processing card 210 (e.g., in particular when the substrate 202 is provided in the form of a flexible circuit that is laminated (to the back) of sample processing card 210. The GMR sensor chip 280 may be designed to receive a prepared sample from the output port 255 of the sample processing card 210. Accordingly, placement of the GMR sensor chip 280 on the substrate may be changed or altered based on a position of the output port 255 on card 210 (thus, the illustration shown in Fig. 2B is not intended to be limiting) – or vice versa. In an embodiment, the GMR sensor chip 280 is positioned on a first side of the substrate 202 (e.g., a top side that faces an underside of the card 210, as shown in Fig. 2B), e.g., so as to receive the prepared sample from an output port that outputs on an underside of the card 210, and the contact pads 290 are positioned on an opposite, second side of the substrate (e.g., on a bottom side or underside of the substrate 202, such that the contact pads 290 are exposed on a bottom side of the cartridge assembly 200 when fully assembled for insertion into the cartridge reader unit 100). The GMR sensor chip 280 may include its own associated contact pads (e.g., metal strips or pins) that are electrically connected via electronic connections on the PCB/substrate 202 to the electrical contact pads 290 provided on the underside thereof. Accordingly, when the cartridge assembly 200 is inserted into the cartridge reader 100, the electrical contact pads 290 are configured to act as an electronic interface and establish an electrical connection and thus electrically connect with electronics (e.g., cartridge reader 310) in the cartridge reader unit 100. Thus, any sensors in the sensor chip 280 are connected to the electronics in the cartridge reader unit 100 through the electrical contact pads 290 and contact pads of the GMR sensor chip 280.

[0067] Figs. 2D shows a view of an exemplary cross section of a mating or connection interface of card 210 and substrate 202. More specifically, Fig. 2D illustrates an interface, in accordance with one embodiment, between an output port 255 on the card 210 and GMR sensor chip 280 of the substrate 202. For example, shown is a PCB substrate 202 positioned below and

adjacent to a card 210 according to any of the herein disclosed embodiments. The substrate 202 may be attached to bottom surface of the card 210. The card 210 has a channel feature, labeled here as microfluidic channel 433 (which is one of many communication channels within the card 210), in at least one layer thereof, designed to direct a test sample that is processed within the card 210 to an output port 255 directed to GMR sensor 280. Optionally, adhesive material may be provided between layers of the card 210, e.g., adhesive 434A may be provided between a layer in the card that has reagent ports 434B and a layer with the channel 433. The substrate 202 includes a GMR sensor chip 280 that is positioned adjacent to the channel 433 and output port 255 of the card 210.

[0068] Magnetic field (from a magnetic coil 365 that is different than magnetic field generator 360, described below with reference to Fig. 3) may be used to excite the nanoparticle magnetic particles located near sensors.

[0069] GMR sensors have sensitivities that exceed those of anisotropic magnetoresistance (AMR) or Hall sensors. This characteristic enables detection of stray fields from magnetic materials at nanometer scales. For example, stray fields from magnetic nanoparticles that bound on sensor surface will alter the magnetization in the magnetic layers, and thus change the resistance of the GMR sensor. Accordingly, changes in the number of magnetic nanoparticles bound to the GMR sensor per unit area can be reflected in changes of the resistance value of the GMR sensor.

[0070] For such reasons, the sensor utilized in cartridge assembly 200, in accordance with the embodiments described herein, is a GMR sensor chip 280.

[0071] Referring now to Fig. 3, an overview of features provided in the system are shown. In particular, some additional features of the cartridge reader unit 100 are schematically shown to further describe how the cartridge reader unit 100 and cartridge assembly 200 are configured to work together to provide the system 300 for detecting analyte(s) in a sample. As depicted, the cartridge assembly 200 may be inserted into the housing 110 of the cartridge reader unit 100. Generally, the housing 110 of the cartridge reader unit 100 may further include or contain a processor or control unit 310, also called a “controller” and/or a “cartridge reader” 310 herethroughout, a power source 320, a pneumatic system 330, a communications unit 340, a (optional) diagnostic unit 350, a magnetic field generator 360, and a memory 370 (or data storage), along with its user interface 140 and/or display 120. Optionally, a reagent opener (e.g.,

puncture system 533 in FIG. 6), e.g., for opening a reagent source on an inserted cartridge assembly or for introducing reagent into the cartridge assembly (e.g., if the reagent is not contained in the assembly in a particular reagent section), may also be provided as part of the cartridge reader unit 100. Once a cartridge assembly 200 is inserted into the housing 110 of the cartridge reader unit 100, and the electrical and pneumatics system(s) are connected, and the cartridge memory chip 275 may be read from the cartridge assembly 200 (e.g., read by cartridge reader 310 / control unit, or PCB assembly, in the unit 100) to determine the pneumatic system protocol that includes steps and settings for selectively applying pressure to the card 210 of the cartridge assembly 200, and thus implementing a method for preparation of sample for delivery to a sensor (e.g., GMR sensor chip 280), and thus the sample placed in the assembly 200 may be prepped, processed, and analyzed. The control unit or cartridge reader 310 may control inputs and outputs required for automation of the process for detecting the analyte(s) in a sample. The cartridge reader 310 may be a real-time controller that is configured to control, among other things, the giant magnetic resistance (GMR) sensor chip 280 and/or memory chip 275 associated with the cartridge assembly 200 and the pneumatic system 330 within the housing 110, as well as the controls from user interface, driving the magnetic field generator 360, and receiving and/or sending signals from/to sensor chip and/or memory associated with the cartridge assembly 200, for example. In an embodiment, the cartridge reader 310 is provided in the form of a PCB (printed circuit board) which may include additional chips, memory, devices, therein. The cartridge reader 310 may be configured to communicate with and/or control an internal memory unit, a system operation initializer, a signal preparing unit, a signal preparing unit, a signal processing unit, and/or data storage (none of which are shown in the Figures), for example. The cartridge reader 310 may also be configured to send and receive signals with respect to the communications unit 340 such that network connectivity and telemetry (e.g., with a cloud server) may be established, and non-volatile recipes may be implemented, for example. Generally, the communications unit 340 allows the cartridge reader unit 100 to transmit and receive data using wireless or wired technology. Power can be supplied to the cartridge reader unit 100 via power source 320 in the form of an internal battery or in the form of a connector that receives power via an external source that is connected thereto (e.g., via a cord and a plug). The pneumatic system 330 is used to process and prepare a sample (e.g., blood, urine) placed into the cartridge assembly 200 by means of moving and directing fluids inside and along the sample processing

card 210 (e.g., via pneumatic connection 235, through its channels and connecting to direct elastomeric valves). The pneumatic system 330 may be a system and/or device for moving fluid, which could use, for example, plungers and/or pistons in contact with fluids (further described later below). The magnetic field generator 360 may be an external magnetic coil or other field generating device that is mounted in the unit 100 or integrated in some fashion with one or more of the chips (e.g., sensor chip 280) provided on the cartridge assembly 200 or provided on the circuit board of the cartridge reader unit 100. The magnetic field generator 360 is used to stimulate magnetic nanoparticles near the GMR sensor chip 280 while reading the signal. In accordance with embodiments, a second magnetic field generator 365, which may be a coil or other field generating device, may be provided as part of the cartridge reader unit 100 and in the housing 110. For example, in accordance with an embodiment, the second magnetic field generator 365 may be separate and distinct from magnetic field generator 360. This second magnetic field generator 365 may be configured to generate a non-uniform magnetic field such that it may apply such a magnetic field to a part (e.g., top, bottom, sides) of the sample processing card 210 during preparation and processing of a sample, e.g., when moving mixing material(s), such as a buffer and/or magnetic beads from a mixing material source, and test sample within the card. In an embodiment, the second magnetic field generator 365 is provided on an opposite end or side of the cartridge reader unit (e.g., located in a top of the housing 110 of unit 100), i.e. away from the magnetic field generator 360, which is used for GMR sensing. In one embodiment, the second magnetic field generator 365 is provided on an opposite end of the cartridge reader unit as compared to the magnetic field generator 360 (e.g., second magnetic field generator is located in a top of the housing 110 of unit 100 and magnetic field generator 360 is provided at a bottom end of the unit 100 (e.g., near cartridge receiver 130)). In an embodiment, the total magnetic field for sensing biomarkers / analytes includes an applied field from magnetic field generator 360 (either external or integrated with the sensor chip) along with any disturbance from magnetic nanoparticles near the GMR sensor chip 280. The reagent opener is optionally used to introduce reagents during the sample processing and reading of the GMR sensor chip 280 (e.g., if the reagent is not contained in the card in a particular reagent section). As described previously, the user interface 140 / display 120 allows an operator to input information, control the process, provide system feedback, and display (via an output display screen, which may be a touch screen) the test results.

[0072] Fig. 4 shows general steps of a method 400 for performing analyte detection in a sample using the herein disclosed system 300. At step 410, the system is initialized. For example, initialization of the system may include: applying power to the system 300 (including cartridge reader unit 100), determining configuration information for the system, reading computations, determining that features (e.g., magnetic coil and carrier signals) are online and ready, etc. At step 415, a whole test sample is added or loaded into the cartridge assembly 200 (e.g., sample is injected into the injection port 215, as shown in Fig. 2C). The order of steps 410 and 415 may be changed; i.e., the addition of the whole test sample to the assembly 200 may be before or after the system is initialized. At step 420, the cartridge assembly 200 is inserted into the cartridge reader unit 100. Optionally, as part of method 400, user instruction may be input to the cartridge reader unit 100 and/or system 300 via the user interface / display 120. Then, at step 425, the processing of sample is initiated via the control unit 310. This initiation may include, for example, receiving input via an operator or user through the user interface/display 120 and/or a system that is connected to the reader unit 100. In another embodiment, processing may be initiated automatically via insertion of the cartridge assembly 200 into the cartridge reader unit 100 and detecting presence of the cartridge assembly 200 therein (e.g., via electrical connection between electrical contact pads 290 on the assembly 200 with the control unit 310, and automatically reading instructions from memory chip 275). The sample is processed at step 425 using pneumatic control instructions (e.g., obtained from memory chip 275) in order to produce a prepared sample. As generally described above (and further later below), the processing of the sample may be dependent upon the type of sample and/or the type of cartridge assembly 200 inserted into the reader unit 100. In some cases, the processing may include a number of steps, including mixing, introduction of buffers or reagents, etc., before the sample is prepared. Once the sample is prepared, the prepared sample is sent (e.g., through channels in the card 210 and to output port 255, via pneumatic control through pneumatic system 330 and control unit 310) to the GMR sensor chip 280. At step 440, analytes in the prepared sample are detected at the GMR sensor chip 280. Then, at step 445, signals from the GMR sensor chip 280 are received and processed, e.g., via cartridge reader 310 (control unit; which may include one or more processors, for example). Once the signals are processed, test results may be displayed at 450, e.g., via the display 120 / user interface. At 455, test results are saved. For example, test results may be saved in a cloud server and/or memory chip 275 on board the cartridge assembly 200. In

embodiments, any fluids or sample may be directed from the GMR sensor chip 280 through an input port 257 to waste chamber 270. Thereafter, once all tests are preformed and read by the sensing device / GMR sensor chip 280, the cartridge assembly 200 may be ejected from the cartridge reader unit 100. In accordance with an embodiment, this may be automatically performed, e.g., mechanics within the housing 110 of the cartridge reader unit 100 may push the assembly 200 out of the housing 110, or performed manually (by way of a button or force) by the operator, for example.

[0073] In an embodiment, the system 300 described herein may utilize a pneumatic control system as disclosed in International Patent Application No. PCT/US2019/____, entitled “SYSTEM AND METHOD FOR GMR-BASED DETECTION OF BIOMARKERS” (Attorney Docket No. 026462-0504846) and filed on the same day, which is hereby incorporated by reference herein in its entirety.

[0074] In an embodiment, the system 300 described herein may utilize a cartridge assembly (e.g., for sample preparation and delivery to the sensor(s)) as disclosed in International Patent Application No. PCT/US2019/____, entitled “SYSTEM AND METHOD FOR SAMPLE PREPARATION IN GMR-BASED DETECTION OF BIOMARKERS” (Attorney Docket No. 026462-0504847) and filed on the same day, which is hereby incorporated by reference herein in its entirety.

[0075] In an embodiment, the system 300 described herein may process signals at the GMR sensor as disclosed in International Patent Application No. PCT/US2019/____, entitled “SYSTEM AND METHOD FOR PROCESSING ANALYTE SIGNALS IN GMR-BASED DETECTION OF BIOMARKERS (Attorney Docket No. 026462-0504850) and filed on the same day, which is hereby incorporated by reference herein in its entirety. For example, as noted above, at step 445, signals from the GMR sensor chip 280 are received and processed, e.g., via cartridge reader 310. In an embodiment, cartridge reader 310 is configured to perform the function of processing results from the GMR sensor chip 280 using a sample preparation control part having a memory reader unit and a sample preparation control unit (e.g., used to receive signals indicating that a cartridge assembly 200 has been inserted into the cartridge reader unit 100, read information stored in the memory chip 275, and generate pneumatic control signals and send them to the pneumatic system 330) and a signal processing part adapted to control electrical elements, prepare and collect signals, and process, display, store, and/or relay detection results to

external systems, including processing measurements signals to obtain test results of the analyte detection, as described in detail in the -0504850 application. Additional features relating to the cartridge reader 310 and signal processor of the unit 100 are provided in greater detail later in this disclosure.

[0076] It should be understood that, with regards to Figs. 1 and 2A-2D, the features shown are representative schematics of a cartridge reader unit 100 and cartridge assembly 200 that are part of the herein disclosed system 300 for detecting the analyte(s) in a sample.

Accordingly, the illustrations are explanatory only and not intended to be limiting.

[0077] Turning back to the features of the sample processing card 210 and cartridge assembly 200 as previously discussed with reference to Fig. 2C, the arrangement, placement, inclusion, and number of features provided on a sample processing card 210 in the cartridge assembly 200 may be based on the test sample being analyzed and/or the test being performed (e.g., detection of biomarkers, detection of metal, etc.), for example. Further, the card 210 may be arranged, in some embodiments, such that there are zones on the card, and/or such that features are provided in different layers (however, such layers do not need to be distinct layers with a body thereof; rather, layered relative to one another at a depth or height (in the Z-direction)). In accordance with embodiments herein, the sample processing card 210 may be formed using parts that are laser cut to form inlets, channels, valve areas, etc. and sandwiched and connected / sealed together. In other embodiments, one or more layers of the sample processing card may be laser cut, laminated, molded, etc. or formed from a combination of processes. The method of forming the sample processing card 210 is not intended to be limiting. For illustrative purposes herein, some of the Figures include a depiction of layers to show positioning of parts of the sample processing card 210 relative to one another (e.g., positioning within the card relative to other features that are placed above and/or below). Such illustrations are provided to show exemplary depths or placement of the features (channels, valves, etc.) within a body of the sample processing card 210, without being limiting.

[0078] Generally, each card 210 has body 214 extending in a longitudinal direction along a longitudinal centerline A—A (provided in the Y-direction) when viewed overhead or from the top. In an embodiment, each card 210 may have dimensions defined by a length extending in the longitudinal direction (i.e., along or relative to centerline A—A), a width extend laterally to the length (e.g., in the X-direction), and a height (or depth or thickness) in the Z-direction, or vertical

direction. In a non-limiting embodiment, the body 214 of the card 210 may be of a substantially rectangular configuration. In one embodiment, the cartridge receiver 130 (and/or any related tray) in the cartridge reader unit 100 is sized to accommodate the dimensions of the sample processing card 210, such that the card 210 may be inserted into the housing of the unit 100.

[0079] The illustrated structural features shown in the Figures of this disclosure are not intended to be limiting. For example, the numbers of sets, valves, metering chambers, membranes, mixing channels, and/or ports are not intended to be limited with regards to those shown. In some embodiments, more channels may be provided. In some embodiments, less channels may be provided. The number of valves is also not intended to be limiting.

[0080] Although the cartridge assembly 200 and sample processing card 210 may be described herein as being used with a reagent and a patient or medical blood sample, it should be noted that the herein disclosed cartridge assembly 200 is not limited to use with blood or solely in medical practices. Other fluids that may be separable and combined with a reagent or reactionary material may be employed in the herein disclosed cartridge for assaying. Other samples may derive from saliva, urine, fecal samples, epithelial swabs, ocular fluids, biopsies (both solid and liquid) such as from the mouth, water samples, such as from municipal drinking water, tap water, sewage waste, ocean water, lake water, and the like.

[0081] A sensing microfluidic device comprises one or more microfluidic channels and a plurality of sensor pads disposed within the one or more microfluidic channels. Referring now to Figure 5A there is shown an exemplary channel 500 in accordance with some embodiments. Channel 500 is shown as serpentine in structure, but it need not be so limited in geometry. Channel 500 comprises a plurality of GMR sensors 510 disposed within the channel body 520. GMR sensors 510 may be all identically configured to detect a single analyte, the redundancy allowing for enhanced detection. GMR sensors 510 may also be all configured differently to detect a myriad of analytes or a combination of differently configured sensors with some redundancies. Channel 500 further comprises a channel entrance 530 where any samples, reagents, bead suspensions, or the like enter channel body 520. Flow through channel body 520 may be mediated under positive pressure at channel entrance 530 or under vacuum applied at channel exit 540.

[0082] Figure 5B shows a plurality of channels 500 disposed within base 550. Each channel 500 features channel expansions 560 which is an expanded area surrounding each GMR

sensor 510 (Fig. 5A; not shown in Fig. 5B for clarity). Without being bound by theory, it is postulated that channel expansions 560 provide a means for better mixing of materials as they pass over the GMR sensors. At the periphery of base 550 are disposed a pair of contact pads 570 which serve as an electrical conduit between the GMR sensors located in channel expansions 560 and the rest of the circuitry. GMR sensors 510 are electronically linked via wiring (not shown) to contact pads 570.

[0083] Figure 6A shows a cross-section of a channel 600 comprising a plurality of GMR sensors 610 in a channel body 620 having a straight configuration. In such embodiments, the flow direction of materials can be from either direction. In other embodiments, as indicated in Figure 6B, channel 600 can comprise a similar plurality of GMR sensors 610 incorporated within channel body 620 at channel expansions 630 that are shaped roughly circular or oval. In still further embodiments, as indicated in Figure 6C, channel 600 can have GMR sensors 610 disposed in channel expansions 630 that are roughly square or rectangular. Although not shown such square or rectangular channel expansions can also be disposed so that the sides, rather than the points of the square or rectangle are part of channel expansion 630 rather than the vertices. Other configurations of channel expansions 1030 are possible, including that shown in Figure 6D where channel 600 has GMR sensors 610 disposed in triangular (or trapezoidal)-shaped. Channel expansions 630 can have any geometry and can be selected for desired flow and mixing properties, as well as residence times over GMR sensors 610.

[0084] As indicated in Figure 6D, channel 600 may have a channel body 620 that is serpentine in shape, with GMR sensors 610 disposed along the length of the serpentine path. In some embodiments, such serpentine structures may allow for more sensors to be packed into a small area compared to a linear channel 600. As shown in Figure 6F, channel 600 can incorporate both a body 620 that is serpentine in structure as well as having channel expansions 630 wherein GMR sensors 610 reside. Further optional structural features of channel 1000 are shown in Figure 6G which shows channel 600 with GMR sensors disposed therein and which has a channel body 620 that incorporates a bifurcation. In some such embodiments, the flow direction can be modulated in either direction, depending on the exact application. For example, when flowing to the left in the drawing, materials can be split into two different pathways. This may represent, for example, the use of different GMR sensors 610 along the two bifurcation arms. The width of channel body 620 can vary before and after the bifurcation and can be selected for specific flow characteristics.

[0085] Referring now to Figure 7, there is shown channel 700 which incorporates within channel body 720, channel expansions 730 in which different GMR sensors 710a and 710b are disposed. Although Figure 7 shows different GMR sensors 710a and 710b alternating, it need not follow this pattern. For example, all of one type of GMR sensors 710a may be clustered together adjacent to each other and likewise all of the other type of GMR sensors 710b may be clustered together. Referring back to Figure 6G, different sensors may also appear along the separated lines of a bifurcation.

[0086] Figs. 8A, 8B and 8C schematically illustrate the structure of a GMR sensor chip 280 which can be mounted on the cartridge assembly 200 according to an embodiment of the present disclosure. As shown in Fig. 8A, the GMR sensor chip 280 includes: at least one channels 810, 820 and 830 arranged approximately in the center of the chip; a plurality of GMR sensors 880 disposed within the channels; electric contact pads 840A, 840B arranged on two opposing ends of the GMR sensor chip; and metal wires 850, 860, 870A, 870B, 870C, 890A, 890B, 890C coupled to the electric contact pads 840A, 840B.

[0087] The channels 810, 820 and 830 each can have a serpentine shape to allow for more sensors to be packed inside. A plurality of channel expansions 885 can be arranged along the channels to receive the plurality of GMR sensors. Fluid to be tested flows into and out of the channels 810, 820, 830 via channel entrances 815A, 825A, 835A and channel exits 815B, 825B, 835B, respectively. Although Fig. 8A shows that the GMR sensors 880 are arranged in an 8×6 sensor array, with 16 sensors received in each of three channels 810, 820, 830, other combinations can be used to satisfy the specific needs of the analyte to be sensed.

[0088] The electric contact pads 840A, 840B comprise a plurality of electric contact pins. The metal wires 850, 860, 870A, 870B, 870C connect the GMR sensors to corresponding electric contact pins 845A, 845B, 875. The electric contact pads 840A, 840B are in turn connected to the electrical contact pads 290 provided on the cartridge assembly 200. When the cartridge assembly 200 is inserted to the cartridge reader 310, electric connection is formed between the GMR sensor chip 280 and the cartridge reader 310 to enable sending of measurement signals from the GMR sensors to the cartridge reader 310.

[0089] Fig. 8B shows more details of the GMR sensors. For example, each GMR sensor can be comprised of five GMR strips which are connected in parallel. At one end, each GMR sensor is connected by one of two main metal wires (i.e., either wire 850 or 860) to one of two

common pins (i.e., either pin 845A or 845B). The other ends of the GMR sensors are connected by separate metal wires 870A, 870B, 870C to distinct pins 875 on the electric contact pads 840A or 840B.

[0090] Fig. 8A also shows fluid detection metal wires 890A, 890B, 890C which are arranged in the proximity of the channel entrances and/or exits, each corresponding to one of the channels. The fluid detection function is carried out by switches 895A, 895B, 895C arranged in the respective fluid detection metal wires. Fig. 8C shows the structure of the switch 895A in detail. In response to recognition that conductive fluid (for example, plasma) flows over it, the switch 895A can couple the wire 896A on one side to the wire 896B on the other side, generating a fluid detection signal.

[0091] The structure and wiring of the GMR sensor chip shown in Figs. 8A-C are only exemplary in nature, it will be apparent to those skilled in the art that other structures and wirings are feasible to achieve the same or similar functions. Referring now to Figure 9, there is shown a cross-sectional view of channel 900 at a channel expansion 930. Disposed within channel expansion 930 is GMR sensor 910 on which is immobilized one or more biomolecules 925. Immobilization of biomolecule 925 to GMR sensor 910 is via conventional surface chemistry (shown in some further detail in Figure 14). Biomolecule 925 may be a peptide or protein, DNA, RNA, oligosaccharide, hormone, antibody, glycoprotein or the like, depending on the nature of the specific assay being conducted. Each GMR sensor 910 is connected by wire 995 to a contact pad 970 located outside of channel 900. In some embodiments, wire 995 is connect to GMR sensor 910 at the bottom of the sensor.

[0092] Referring now to Figure 10A, there is shown a more detailed cross-sectional view of a channel 1000 having a channel body 1030 lacking a channel expansion at the location of a GMR sensor 1010. Biomolecule 1025 is immobilized with respect to the sensor via attachment to a biosurface 1045. Such biosurface immobilization chemistry is known in the art. See, for example, Cha *et al.* "Immobilization of oriented protein molecules on poly(ethylene glycol)-coated Si(111)," *Proteomics* 4:1965-1976, (2004); Zellander *et al.* "Characterization of Pore Structure in Biologically Functional Poly(2-hydroxyethyl methacrylate)-Poly(ethylene glycol) Diacrylate (PHEMA-PEGDA)," *PLOS ONE* 9(5):e96709, (2014). In some embodiments, biosurface 1045 comprises a PEG polymer crosslinked with PHEMA. In some embodiments, the crosslinking group is represented by Formula (I):

PA-LG-PA (I)

wherein each PA is a photo- or metal-activated or activated group, and LG is a linking group. In some embodiments, each PA is the same and in other embodiments each PA is different. In some embodiments PA is photo- or metal-activated to form a nitrene intermediate capable of C-H and/or O-H insertion. See, for example, “Photogenerated reactive intermediates and their properties,” Chapter 2 in *Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Press, 12:8-24 (1983). In some embodiments, PA is metal activated to form a carbene or carbenoid intermediate capable of C-H and/or O-H insertion. See, for example, Doyle *et al.* “Catalytic Carbene Insertion into C-H Bonds,” *Chem. Rev.* 2:704-724 (2010).

[0093] In some embodiments, each PA is an azide ($-N_3$) moiety and photoactivation generates nitrene intermediates capable of C-H and/or O-H insertion thereby mediating crosslinking of PEG and PHEMA polymers. In some embodiments, each PA is a diazo ($-N_2$) and metal catalyzed decomposition reaction forms a carbene or carbenoid intermediate capable of C-H and/or O-H insertion thereby mediating crosslinking of PEG and PHEMA polymers. Both azide and diazo preparations are well known in the art, and in the case of azide are readily prepared by S_N^2 displacement reaction of azide anion, N_3^- with an appropriate organic moiety possessing a leaving group.

[0094] LG in Formula (I) can be any organic fragment that will support the presence of each PA moiety. It can be a simple C_2 - C_{20} hydrocarbon chain that is straight chained or branched. Such hydrocarbons can include fluorinated variants with any degree of fluorine substitution. In some embodiments, LG can include aromatic hydrocarbons including, without limitation, benzene, naphthalene, biphenyl, binaphthyl, or combinations of aromatic structures with C_2 - C_{20} hydrocarbon chains. Thus, in some embodiments, LG can be alkyl, aryl, or aralkyl in structure. In some embodiments, alkyl linking groups may have one or more carbons in their chains substituted with oxygen (O), or an amine (NR), where R is H or C_1 - C_6 alkyl.

[0095] In accordance with the foregoing embodiments, a crosslinked PEG-PHEMA structure may be given by Formula (II):

PEG-A-LG-A-PHEMA

Wherein PEG is the polyethylene glycol moiety, each A is an attachment atom from the catalytic reaction of azide or diazo, i.e., CH₂ or NH, and LG is the linking group as described above.

[0096] In Figure 10A, a magnetic bead-bound entity 1015 is configured to interact with biomolecule 1025 or an analyte of interest, such as in a sandwich complex of antibody-analyte-magnetic bead-bound antibody. Below biosurface 1045 is a further insulating layer 1055. Insulating layer 1055 may be in direct contact with GMR sensors 1010 and may comprise, for example, a metal oxide layer. Biosurface layer 1045 is in direct contact with insulating layer 1055. A base 1065 serves as the scaffold for each component above it, the GMR sensors 1010, insulating layer 1055, and biosurface layer 1045. In some embodiments, base 1065 is made from silicon wafer.

[0097] Fig. 10B schematically illustrates the basic structure and principle of GMR sensors. A typical GMR sensor consists of a metallic multi-layered structure with a non-magnetic conductive interlayer 1090 sandwiched between two magnetic layers 1080A and 1080B. The non-magnetic conductive interlayer 1090 is often a thin copper film. The magnetic layers 1080A and 1080B can be made of ferromagnetic alloy material.

[0098] The electrical resistance of the metallic multi-layered structure changes depending on the relative magnetization direction of the magnetic layers 1080A and 1080B. Parallel magnetization (as shown in the right half of Fig. 10B) results in lower resistance, while anti-parallel magnetization (as shown in the left half of Fig. 10B) results in higher resistance. The magnetization direction can be controlled by a magnetic field applied externally. As a result, the metallic multi-layered structure displays a change in its electrical resistance as a function of the external magnetic field.

[0099] GMR sensors have sensitivities that exceed those of anisotropic magnetoresistance (AMR) or Hall sensors. This characteristic enables detection of stray fields from magnetic materials at nanometer scales. For example, stray fields from magnetic nanoparticles that bound on sensor surface will alter the magnetization in the magnetic layers, and thus change the resistance of the GMR sensor. Accordingly, changes in the number of magnetic nanoparticles bound to the GMR sensor per unit area can be reflected in changes of the resistance value of the GMR sensor.

[0100] Referring now to Figures 11A and 12A, there are shown two exemplary basic modes by which GMR sensors operate in accordance with various assay applications described

herein. In the first mode, exemplified in Figure 11A, magnetic beads 1115 are loaded proximal to a GMR sensor (see Figure 11A, 1010) via biosurface 1165 at the start of the assay. During the assay the presence of a query analyte results in magnetic beads 1115 being displaced from biosurface 1165 (and thus, displaced away from the GMR sensor); this mode is the so-called subtractive mode because magnetic beads are being taken away from the proximity of the sensor surface. The second main mode operation, typified in Figure 12A, is the additive mode. In such assays, there is a net addition of magnetic beads 1215 in the vicinity of the GMR sensor (see Figure 10A, 1010) when a query analyte is present. Either mode, subtractive or additive, relies on the changed state in the number of beads (1115, 1215) proximal to the sensor surface thereby altering the resistance in the GMR sensor system. The change in resistance is measured and query analyte concentrations can be determined quantitatively.

[0101] Referring back to Figure 11A, there is shown a sensor structure diagram illustrating the sensor structures throughout an exemplary subtractive process. At the start of the process the system is in state 1100a in which the GMR sensor has disposed on its biosurface 1165 a plurality of molecules (typically biomolecules) 1125 with associated magnetic beads 1115. The volume above biosurface 1165 may begin dry or with a solvent present. When dry, the detection process may include a solvent priming step with, for example, a buffer solution. After introduction of analyte, the system takes the form of state 1100b in which some of magnetic beads 1115 have been removed from the molecules 1125 in proportion to the concentration of analyte. The change in states 1100a and 1100b provide a measurable change in resistance that allows quantitation of the analyte of interest. In some embodiments, the analyte may simply displace beads directly from molecules 1125. In other embodiments, the analyte may chemically react with molecules 1125 to cleave a portion of the molecule attached to beads 1115, thereby releasing beads 1115 along with the cleaved portion of molecule 1125.

[0102] In embodiments, biosurface 1165 comprises a polymer. The specific polymer may be chosen to facilitate covalent attachment of molecules 1125 to biosurface 1165. In other embodiments, molecules 1125 may be associated with biosurface 1165 via electrostatic interactions. Polymer coatings may be selected for or modified to use conventional linking chemistries for covalently anchoring biomolecules, for example. Linking chemistries include any chemical moieties comprising an organic functional group handle including, without limitation, amines, alcohols, carboxylic acids, and thiol groups. Covalent attachment chemistry includes,

without limitation, the formation of esters, amides, thioesters, and imines (which can be subsequently subjected to reduction, *i.e.*, reductive amination). Biosurface 1165 may include surface modifiers, such as surfactants, including without limitation, anionic surfactants, cationic surfactants, and zwitterionic surfactants.

[0103] Molecules 1125 can include any number of receptor/ligand entities which can be attached to biosurface 1165. In some embodiments molecules 1125 include any of a variety of biomolecules. Biomolecules include DNA, RNA, and proteins that contains free amine groups can be covalently immobilized on GMR sensor surface with functional NHS groups. For the immunoassays, primary antibody (mouse monoclonal IgG) specific to analyte is attached onto GMR surface. All primary antibodies have multiple free amine groups and most proteins have lysine and/or alpha-amino groups. As long as lysine free primary amines are present, antibodies will be covalently immobilized on GMR sensor. To immobilize antibody on sensors surface, 1.2 nL of primary antibody (1 mg/mL in PBS buffer) are injected onto sensors surface using a printer system (sciFLEXARRAYER, Scienion, Germany). All printed surfaces are incubated overnight at 4 °C under a relative humidity of ~85%. The surfaces will be washed three times with blocking buffer (50mM ethanolamine in Tris buffer), and are further blocked with the same buffer for 30 min.

[0104] In embodiments, magnetic beads 1115 may be nanoparticulate, including spheroidal nanoparticles. Such nanoparticles may have effective diameters in a range from about 2 to about 50 nanometers (nm), or about 5 to about 20 nm, or about 5 to about 10 nm. In embodiments, magnetic beads 1115 may be coated to facilitate covalent attachment to molecules 1125. In other embodiments magnetic beads 1115 may be coated to facilitate electrostatic association with molecules 1125. Magnetic beads 1115 may be differentially tagged and/or coated to facilitate multiplex detection schemes. In such embodiments, the differential tagging and/or coating is configured such that the different beads interact with different molecules disposed on different GMR sensors or on a single sensor in which different molecules are spatially organized to create addressable signals.

[0105] Figure 11B shows a process flow 1101 associated with the sensor structure scheme of Figure 11A. The process commences at 1120 by injecting a sample into a cartridge assembly. The sample may then undergo processing at step 1130 through any necessary steps such as filtration, dilution, and/or chemical modification. The sequencing of these pre-process

steps will depend on the nature of the sample and query analyte to be detected. Movement through the system may be controlled pneumatically. Step 1140 involves sending the processed sample to the GMR sensor at a target specified flow rate. Such flow rate may be selected to reflect the kinetics of the chemistry on the GMR sensor surface. Step 1150 provides obtaining readings from the GMR sensors that reflect changes in the concentration of magnetic beads at the surface of the GMR sensor. These readings allow detecting changes in resistance at step 1160. Finally, step 1170 provides computing the detect result based on the changes in resistance.

[0106] Referring now to Figure 12A, there is shown a sensor structure diagram illustrating the sensor structures throughout an exemplary additive process. At the start of the process the system is in state 1200a in which the GMR sensor has disposed on its biosurface 1265 a plurality of molecules (typically biomolecules) 1225. The plurality of molecules 1225 is selected to bind a query analyte 1290, as indicated in second state 1200b. Query analyte 1295 is configured to bind magnetic beads 1215. In some embodiments, query analyte 1295 is associated with the bead prior to passing over biosurface 1265. For example, this may take place during pre-processing of the sample being tested. (In other embodiments, query analyte 1295 may pass over the biosurface first, then query analyte 1295 may be modified with magnetic beads 1215 after the analyte is bound to biosurface 1265, as described below with reference to Figure 13A). In some embodiments, a given query analyte 1295 may require chemical modification prior to binding magnetic particles 1215. In some embodiments, magnetic beads 1215 may be modified to interact with query analyte 1295. The ability to quantitate analyte is provided by changes in measured resistance from state 1200a, where no magnetic beads 1215 are present, to state 1200b, where magnetic beads 1215 are associated with biosurface 1265.

[0107] Figure 12B shows an exemplary process flow 1201 associated with the sensor structure scheme of Figure 12A. The process commences at 1220 by injecting a sample into a cartridge assembly. The sample may then undergo processing at step 1230 through any necessary steps such as filtration, dilution, and/or chemical modification. The sequencing of these pre-process steps will depend on the nature of the sample and query analyte to be detected. Movement through the system may be controlled pneumatically. Step 1240 involves sending the processed sample to a reaction chamber and then in step 1250 beads are introduced into the reaction chamber to modify the query analyte. As described above, such modification may be performed directly on the sensor surface rather than in the reaction chamber. In step 1260, the

modified sample is sent to the GMR sensors at a target flow rate. Such flow rate may be selected to reflect the kinetics of the chemistry on the GMR sensor surface. Step 1270 provides obtaining readings from the GMR sensors that reflect changes in the concentration of magnetic beads at the surface of the GMR sensor. These readings allow detecting changes in resistance at step 1280. Finally, step 1290 provides computing the detect result based on the changes in resistance.

[0108] Referring now to Figure 13A, there is shown a sensor structure diagram illustrating the sensor structures states 1300a-c throughout an exemplary additive process. At the start of the process the system is in state 1300a in which the GMR sensor has disposed on its biosurface 1365 a plurality of molecules (typically biomolecules) 1325. The plurality of molecules 1325 is selected to bind a query analyte 1395, as indicated in second state 1300b. Query analyte 1395 is configured to bind magnetic beads 1315, as indicated in state 1300c. In some embodiments, a given query analyte 1395 may require chemical modification prior to binding magnetic particles 1315. In other embodiments, query analyte 1395 may bind magnetic nanoparticles 1315 without chemical modification. In some embodiments, magnetic beads 1315 are coated or otherwise modified to interact with query analyte 1395. The ability to quantitate query analyte 1395 is provided by changes in measured resistance from state 1300a, where no magnetic beads 1315 are present, to state 1300c, where magnetic beads 1315 are associated with biosurface 1365.

[0109] Figure 13B shows an exemplary process flow 1301a associated with the sensor structure scheme of Figure 13A. The process commences at 1310 by injecting a sample into a cartridge assembly. The sample may then undergo processing at step 1320 through any necessary steps such as filtration, dilution, and/or the like. The sequencing of these pre-process steps will depend on the nature of the sample and query analyte to be detected. At 1330, the process sample is sent to a reaction chamber. Movement through the system may be controlled pneumatically. Step 1340 involves modifying the analyte present in the sample chamber with reagents to allow it to interact with magnetic particles. At step 1350, the modified sample is sent to the GMR sensors at a target flow rate. Such flow rate may be selected to reflect the kinetics of the chemistry on the GMR sensor surface. Next, step 1360 introduces beads into the GMR sensors, which can now interact with the modified analyte. In some embodiments, the beads may be modified as well, such as with a coating or some other linking molecule that will enable interaction with the modified analyte. Step 1370 provides obtaining readings from the GMR

sensors that reflect changes in the concentration of magnetic beads at the surface of the GMR sensor. These readings allow detecting changes in resistance at step 1380. Finally, step 1390 provides computing the detect result based on the changes in resistance.

[0110] Figure 13C shows an alternative exemplary process flow 1301b associated with the sensor structure scheme of Figure 13A. The process commences at 1302 by injecting a sample into a cartridge assembly. The sample may then undergo processing at step 1304 through any necessary steps such as filtration, dilution, and/or the like. The sequencing of these pre-process steps will depend on the nature of the sample and query analyte to be detected. Movement through the system may be controlled pneumatically. At step 1306, the modified sample is sent to the GMR sensors at a target flow rate. Such flow rate may be selected to reflect the kinetics of the chemistry on the GMR sensor surface. Step 1308 involves modifying the analyte present in the sample with reagents to allow it to interact with magnetic particles. Next, step 1312 introduces beads into the GMR sensors, which can now interact with the modified analyte. In some embodiments, the beads may be modified as well, such as with a coating or some other linking molecule that will enable interaction with the modified analyte. Step 1314 provides obtaining readings from the GMR sensors that reflect changes in the concentration of magnetic beads at the surface of the GMR sensor. These readings allow detecting changes in resistance at step 1316. Finally, step 1318 provides computing the detect result based on the changes in resistance.

[0111] Referring now to Figure 14A, there is shown a sensor structure diagram illustrating the sensor structures states 1400a-c throughout an exemplary additive process. At the start of the process the system is in state 1400a in which the GMR sensor has disposed on its biosurface 1465 a plurality of molecules (typically biomolecules) 1425. The plurality of molecules 1425 is selected to interact (chemically react) with a query analyte. Such interaction modifies molecules 1425 (in proportion to analyte concentration) to provide modified molecules 1411, as indicated in second state 1400b. Modified molecules 1411 are configured to bind magnetic beads 1415, as indicated in state 1300c. In some embodiments, modified molecules 1411 may require further chemical modification prior to binding magnetic particles 1415. In other embodiments, modified molecules 1411 may bind magnetic nanoparticles 1415 without chemical modification. In some embodiments, magnetic beads 1415 are coated or otherwise modified to interact with modified molecules 1411. The ability to quantitate query analyte is

provided by changes in measured resistance from state 1400a, where no magnetic beads 1415 are present, to state 1400c, where magnetic beads 1415 are associated with biosurface 1465 via modified molecules 1411. Note, in the overall process, the query analyte is merely serving as a reagent to chemically modify the plurality of molecules 1425 and does not otherwise remain a part of the process once it has performed this function.

[0112] Figure 14B shows an exemplary process flow 1401 associated with the sensor structure scheme of Figure 14A. The process commences at 1420 by injecting a sample into a cartridge assembly. The sample may then undergo processing at step 1430 through any necessary steps such as filtration, dilution, and/or the like. The sequencing of these pre-process steps will depend on the nature of the sample and query analyte to be detected. Movement through the system may be controlled pneumatically. At 1440, the process sample is sent to GMR sensors at a specified flowrate. Such flow rate may be selected to reflect the kinetics of the chemistry on the GMR sensor surface. Next, step 1450 introduces beads into the GMR sensors, which can now interact with the modified molecules on the biosurface. In some embodiments, the beads may be modified as well, such as with a coating or some other linking molecule that will enable interaction with the modified molecules on the biosurface. Step 1460 provides obtaining readings from the GMR sensors that reflect changes in the concentration of magnetic beads at the surface of the GMR sensor. These readings allow detecting changes in resistance at step 1470. Finally, step 1480 provides computing the detect result based on the changes in resistance.

[0113] Referring now to Figure 15A, there is shown a sensor structure diagram illustrating the sensor structures states 1500a-c throughout an exemplary additive process. At the start of the process the system is in state 1500a in which the GMR sensor has disposed on its biosurface 1565 a plurality of molecules (typically biomolecules) 1525. The plurality of molecules 1525 is selected to interact (chemically react) with a query analyte. Such interaction modifies molecules 1525 (in proportion to analyte concentration) to provide modified molecules 1511, as indicated in second state 1500b. Modified molecules 1511 are configured to prevent binding of magnetic beads 1515, as indicated in state 1500c, in which magnetic beads only bind to molecules 1525 that were not modified by the analyte. In some embodiments, magnetic beads 1515 are coated or otherwise modified to interact with molecules 1525. The ability to quantitate query analyte is provided by changes in measured resistance from state 1500a, where no magnetic beads 1515 are present, to state 1500c, where magnetic beads 1515 are associated with

biosurface 1565 via molecules 1525. Note, in the overall process, the query analyte is merely serving as a reagent to chemically modify the plurality of molecules 1525 and does not otherwise remain a part of the process once it has performed this function.

[0114] Figure 15B shows an exemplary process flow 1501 associated with the sensor structure scheme of Figure 15A. The process commences at 1510 by injecting a sample into a cartridge assembly. The sample may then undergo processing at step 1520 through any necessary steps such as filtration, dilution, and/or the like. The sequencing of these pre-process steps will depend on the nature of the sample and query analyte to be detected. Movement through the system may be controlled pneumatically. At step 1530, the processed sample is sent to GMR sensors at a specified flowrate. Such flow rate may be selected to reflect the kinetics of the chemistry on the GMR sensor surface. Next, step 1540 introduces beads into the GMR sensors, which can now interact with the unmodified molecules on the biosurface. In some embodiments, the beads may be modified, such as with a coating or some other linking molecule that will enable interaction with the unmodified molecules. Step 1550 provides obtaining readings from the GMR sensors that reflect changes in the concentration of magnetic beads at the surface of the GMR sensor. These readings allow detecting changes in resistance at step 1560. Finally, step 1570 provides computing the detect result based on the changes in resistance.

[0115] Referring now to Figure 16A, there is shown a sensor structure diagram illustrating the sensor structure states 1600a-d throughout an exemplary additive process that employs a sandwich antibody strategy for detection of analyte 1695 (state 1600b). At the start of the process the system is in state 1600a in which the GMR sensor has disposed on its biosurface 1665 a plurality of antibodies 1625. Analyte 1695 is then passed over biosurface 1665, allowing binding of analyte 1695 to antibody 1625, as indicated in state 1600b. Analyte 1695 is then modified by binding to a second antibody 1635 to which a covalently linked biotin moiety (B) is provided, as indicated in state 1600c. Magnetic beads 1615 modified with streptavidin (S) are then added, thereby allowing the strong biotin-streptavidin association to provide state 1600d. In some embodiments, streptavidin is provided as a coating on magnetic beads 1615.

[0116] Figure 16B shows an exemplary process flow 1601 associated with the sensor structure scheme of Figure 16A. The process commences at 1610 by injecting a sample into a cartridge assembly. The sample may then undergo processing at step 1620 through any necessary steps such as filtration, dilution, and/or the like. The sequencing of these pre-process steps will

depend on the nature of the sample and query analyte to be detected. Movement through the system may be controlled pneumatically. At step 1630, the processed sample is sent to GMR sensors at a specified flowrate. Such flow rate may be selected to reflect the kinetics of the chemistry on the GMR sensor surface between biosurface-bound antibody and the analyte. Next, step 1640 introduces biotinylated antibody (Ab) to the GMR sensors. This creates the “sandwich” structure of the analyte between two antibodies. At step 1650 streptavidin coated beads are introduced into the GMR sensors, which can now interact with the biotin-bound antibody. Step 1660 provides obtaining readings from the GMR sensors that reflect changes in the concentration of magnetic beads at the surface of the GMR sensor. These readings allow detecting changes in resistance at step 1670. Finally, step 1680 provides computing the detect result based on the changes in resistance.

[0117] The schemes of Figures 16A and 16B were put into practice with cardiac biomarkers and proof of concept results are shown in Figures 17A-C. Figure 17A shows a plot of GMR signal (in ppm) over time (in seconds) in a test run designed to detect cardiac biomarker D-dimer. To generate this data, a biosurface was prepared by printing a D-dimer capture antibody using 2 nL of a 1mg/mL of D-dimer antibody in PBS buffer with 0.05% sodium azide. For testing potential cross reactivity, the biosurface was also functionalized with troponin I capture antibody by printing two combined capture antibodies using 2nL of a solution of 1mg/mL troponin I antibody in PBS buffer with 0.05% sodium azide. Additionally, two other controls were printed on the biosurface. The first is a negative control prepared by printing 2 nL of a solution of 0.5% BSA in PBS buffer with 0.05% sodium azide and the second is a positive control prepared by printing 2 nL of 1mg/mL of biotin conjugated to mouse IgG in PBS buffer with 0.05% sodium azide. The printed sensors were incorporated into a cardiac test cartridge and is configured to use the “sandwich” assay described above in Figures 16A and 16B.

[0118] In the sample test 120 microliters of plasma or whole blood was loaded into a sample well in the cartridge. A membrane filter serves to remove blood cells as the sample is pulled into the flow channel from the sample well. 40 microliters of plasma (or plasma portion of whole blood) is flowed into a metering channel and deposited powder including antibody/biotin conjugates, blockers, and mouse IgG in the channel dissolve into the sample solution. While flowing over the sensor area, the analytes, antibody/biotin conjugates and antibodies immobilized on the sensor surface form a sandwich of antibody-analyte-biotinylated antibody.

Flow rates are modulated depending on the test. For troponin I, the sample is flowed over the sensor for 20 minutes at a flow rate of 1 microliter/minute. For D-dimer, the sample is flowed for 5 minutes at a flow rate of 4 microliters/minute. Following flow of the sample streptavidin-coated magnetic beads were introduced which allow binding to the sensor surface wherever there is a biotinylated antibody bound. The GMR sensor measure bound magnetic beads, which is proportional to the concentration of analytes with the sample. The bead solution is flowed over the sensor for 5 minutes at a flow rate of 4 to 10 microliters/minute. The signals were read from the peak value within 300 seconds after beads started to bind.

[0119] As indicated in the plot of Figure 17A, a negative control with just printed BSA did not bind D-Dimer and thus, the signal remained near baseline as expected. The positive control with biotinylated mouse IgG showed competent bead binding, as expected. A plot of the actual sample of 666.6 ng/mL of human D-dimer appears with a peak detection signal of about 750 ppm indicating successful detection of the D-dimer in an actual sample. There was virtually no cross reactivity with the two bound troponin I capture antibodies (not shown for clarity because these lines were very close to the line with the negative control).

[0120] Figure 17B shows a calibration curve (GMR signal in ppm vs. D-dimer concentration) for D-dimer by running samples with varied, fixed concentrations of D-dimer. The calibration curve allows concentrations to be computed for a future unknown sample containing the D-dimer as the query analyte. A similar plot in Figure 17C is provided for the cardiac biomarker troponin I. Together, these results establish the viability of detecting D-dimer and troponin I in, blood or plasma samples of a subject.

Metal Detection Applications

[0121] Figure 18 shows an application, in accordance with embodiments above, to a lead detection scheme using a GMR sensor platform. Double stranded DNA is printed on the biosurface of the sensor, with one strand being biotinylated (B). If lead is not present, streptavidin-tagged (or coated) magnetic nanoparticles (MNP) can bind to biotin (B), which is part of the DNA substrate strand. When lead is present, a Pb-activated DNase cleaves the biotin-containing substrate strand. When cleaved, the streptavidin-tagged MNP's cannot bind to the via the DNA strand because the biotinylated portion of the strand is no longer present. Thus, MNP's only bind to the GMR surface if lead is not present in the sample. The more lead that is

present, the fewer MNPs bind to the DNA at the biosurface. Such a scheme can be used for the detection of lead in water, blood, or other fluids of interest.

[0122] Figure 19 shows an application, in accordance with embodiments above, to a mercury detection scheme using a GMR sensor platform. A Hg-BSA substrate is immobilized on the a biosurface. In the absence of mercury (Hg) ion (I or II or both), a biotinylated (B) Hg-antibody can bind to the biosurface bound Hg-BSA. In the presence of Hg ion, the ion blocks the biotinylated Hg-antibody's binding site to Hg-BSA, preventing Hg-antibody from binding to Hg-BSA. As described above, streptavidin tagged (or coated) magnetic nanoparticles can bind to biotin. Thus, the more mercury ion present in solution, the fewer magnetic beads will end up bound to the sensor at the biosurface due to the interfering binding of mercury to biotinylated Hg-antibody.

[0123] Figure 20 shows an application, in accordance with embodiments above, to a cadmium or arsenic detection scheme using a GMR sensor platform. A double stranded DNA is printed on the biosurface of the sensor which can bind a detection protein. The detection protein arsR (for arsenate III detection) or Pcad (for cadmium detection) is added in the presence of a sample that may contain the query metal analyte. The detection protein is unable to bind to the DNA double-strand in the presence of their respective heavy metal ion, so DNA-protein binding occurs in proportion to the absence of heavy metal ions in the sample. This is a similar competitive binding event much like the mercury assay described above. A biotinylated (B) reporter protein is then added. This protein can bind to the detection protein. If the detection protein is bound to the DNA double-strand, the biotinylated reporter is immobilized to the DNA-protein complex. Once again, streptavidin tagged magnetic nanoparticles will bind to the biotinylated reporter protein that is bound to the biosurface. Thus, the smaller the concentration of cadmium or arsenic, the more beads will be bound to the biosurface.

[0124] The following is a non-limiting list of applications of analyte sensing that may be accomplished, in accordance with the principles detailed herein.

[0125] (1) Blood samples can include analytes such as proteins or other substance, such as DNA, that can be measured by immunoassay employing the GMR device. Exemplary disease states associated with analytes that may be detected are summarized in Table 1 below.

Table 1

Diseases	Analytes
Cardiac	Apolipoprotein A1, Apolipoprotein B, CK-MB, hsCRP, Cystatin C, D-Dimer, GDF-15, Myoglobin, NT-proBNP, BNP, Troponin I, Troponin T
Cancer	AFP, CA 125, CA 15-3, CA 19-9, CA 72-4, CEA, Cyfra 21-1, hCG plus beta, HE4, NSE, proGRP, PSA free, PSA total, SCC, S-100, Thyroglobulin (TG II), Thyroglobulin confirmatory, b2-Microglobulin
Drugs of Abuse	Acetaminophen/Paracetamol (APAP), Amphetamines (AMP), Methamphetamines (mAMP), Barbiturates (BAR), Benzodiazepines (BZO), Cocaine (COC), Methadone (MTD), Opiates (OPI), Phencyclidine (PCP), THC, and Tricyclic Antidepressants (TCA).
Infectious	Anti-HAV, Anti-HAV IgM, Anti-HBc, Anti-HBc IgM, Anti-Hbe, HBeAg, Anti-HBs, HBsAg, HBsAg confirmatory, HBsAg quantitative, Anti-HCV, Chagas4, CMV IgG, CMV IgG Avidity, CMV IgM, HIV combi PT, HIV-Ag, HIV-Ag confirmatory, HSV-1 IgG, HSV-2 IgG, HTLV-I/II, Rubella IgG, Rubella IgM, Syphilis, Toxo IgG, Toxo IgG Avidity, Toxo IgM, TPLA (Syphilis)
Inflammation	Anti-CCP, ASLO, C3c, Ceruloplasmin, CRP, Haptoglobin, IgA, IgE, IgG, IgM, Immunglobulin A CSF, Immunglobulin M CSF, Interleukin 6, Kappa light chains, Kappa light chains free, Lambda light chains, Lambda light chains free, Prealbumin, Procalcitonin, Rheumatoid factor, a1-Acid Glycoprotein, a1-Antitrypsin, SSA

[0126] (2) GMR systems described herein may be use in urine analyte detection. Any protein, DNA, metal or other substance in urine can be measured and/or detected by the GMR devices described herein. Urine associated protein biomarkers include, without limitation preeclampsia, human chorionic gonadotropin (hCG), kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin (IL)-18, and fatty-acid binding proteins (FABPs), nuclear matrix protein 22 (NMP22), BLCA-4, and epidermal growth factor

receptor (EGFR), etc. Drugs and/or their major urinary metabolites include Acetaminophen/Paracetamol (APAP), Amphetamines (AMP), Methamphetamines (mAMP), Barbiturates (BAR), Benzodiazepines (BZO), Cocaine (COC), Methadone (MTD), Opiates (OPI), Phencyclidine (PCP), THC, and Tricyclic Antidepressants (TCA), etc.

[0127] (3) GMR systems described herein may be use in saliva analyte detection. Any protein, DNA, metal or other substance in saliva or mouth epithelium can be measured and/or detected by the GMR devices described herein. Exemplary biomarkers include, without limitation, matrix metalloproteinases (i.e., MMP1, MMP3, MMP9), cytokines (i.e., interleukin-6, interleukin-8, vascular endothelial growth factor A (VEGF-A), tumor necrosis factor (TNF), transferrins, and fibroblast growth factors, myeloid-related protein 14 (MRP14), profilin, cluster of differentiation 59 (CD59), catalase, and Mac-2-binding protein (M2BP), etc. Drugs include Amphetamines (AMP), Barbiturates (BAR), Benzodiazepines (BZO), Buprenorphine (BUP), Cocaine (COC), Cotinine (COT), Fentanyl (FYL), K2/Spice (K2), Ketamine (KET), Methamphetamine (MET), Methadone (MTD), Opiates (OPI), Oxycodone (OXY), Phencyclidine (PCP), Marijuana (THC), and Tramadol (TML).

[0128] (4) GMR systems described herein may be use in ocular fluid analyte detection. Any protein, DNA, metal or other substance in ocular fluid can be measured and/or detected by the GMR devices described herein. Ocular fluid protein biomarkers include, without limitation α -enolase, α -1 acid glycoprotein 1, S100 A8/calgranulin A, S100 A9/calgranulin B, S100 A4 and S100 A11 (calgizzarin), prolactin-inducible protein (PIP), lipocalin-1 (LCN-1), lactoferrin and lysozyme, b-amyloid 1–40, Neutrophil defensins NP-1 and NP-2, etc, can be measured by sandwich assay in the system.

[0129] (5) Embodiments disclosed herein may employ a liquid biopsy as a sample for query analytes, such as biomarkers. In some such embodiments, there may be provided methods for identifying cancer in patients' blood. Methods described below may be used to detect "rare" mutations in DNA found in the blood. DNA from cancer cells frequently enter the blood stream, however most of the blood borne DNA (>99%) will be from healthy cells. The methods disclosed herein provide for detecting these "rare" mutations and verifying the results. Methods disclosed herein provide for a multistep process to be captured in a single assay using a GMR detection platform.

[0130] Methods disclosed herein comprise extracting DNA from blood, which in accordance with embodiments herein, are automated in cartridge which can perform the requisite extract and purification of DNA from the blood. In some embodiments, a silica membrane is employed as part of the extraction process, but methods herein are not so limited. After extraction and purification, the methods provide for selectively amplifying the query biomarker of interest. In some embodiments, methods for amplifying just the cancer DNA involves the use of locked nucleic acids to act as a blocker to prevent normal DNA from being amplified. Other selective amplification methods are known in the art. The next step in the methods is detecting whether the cancer DNA biomarker of interest is present in the patient sample. In some embodiments, this is achieved using exonuclease to convert double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA). Other ways to convert dsDNA to ssDNA are known in the art. The methods continue with capturing the ssDNA by using a complementary segment of DNA printed on the biosurface. In some embodiments, the ssDNA has a biotin attached to the end, and this biotin captures a streptavidin tagged magnetic bead. In some embodiments, methods include verifying whether the ssDNA (from the patient) is perfectly complementary to the printed probe (synthetic segment of DNA). Verification can be accomplished using heat to denature the binding between two pieces of DNA. Imperfect binding will denature (or separate) at a lower temperature, than the perfect binding. This allows for verification of the signal, determining if the signal is caused by a true-positive or a false-positive. By using this verification step one can achieve a higher level of accuracy in diagnosing patients. There are other methods besides heating to denature DNA are known in the art.

[0131] Provided herein are methods and compositions for analyzing nucleic acids. In some embodiments, nucleic acid fragments in a mixture of nucleic acid fragments are analyzed. Nucleic acid may be isolated from any type of suitable biological specimen or sample (e.g., a test sample). In some embodiments, a sample comprises nucleic acids. A sample or test sample can be any specimen that is isolated or obtained from a subject (e.g., a mammal, a human). Non-limiting examples of specimens include fluid or tissue from a subject, including, without limitation, blood, amniotic fluid, cerebrospinal fluid, spinal fluid, lavage fluid (e.g., bronchoalveolar, gastric, peritoneal, ductal, ear, arthroscopic), a biopsy sample, urine, feces, sputum, saliva, nasal mucous, prostate fluid, lavage, semen, lymphatic fluid, bile, tears, sweat, breast milk, the like or combination thereof. In some embodiments, a biological sample is blood,

or a blood product (e.g., plasma or serum). Nucleic acid may be derived from one or more samples or sources.

[0132] In some embodiments, a sample is contacted with one or more suitable cell lysis reagents. Lysis reagents are often configured to lyse whole cells, and/or separate nucleic acids from contaminants (e.g., proteins, carbohydrates and fatty acids). Non-limiting examples of cell lysis reagents include detergents, hypotonic solutions, high salt solutions, alkaline solutions, organic solvents (e.g., phenol, chloroform), chaotropic salts, enzymes, the like, or combination thereof. Any suitable lysis procedure can be utilized for a method described herein.

[0133] The term “nucleic acid” refers deoxyribonucleic acid (DNA, e.g., complementary DNA (cDNA), genomic DNA (gDNA) and the like) and/or ribonucleic acid (RNA, e.g., mRNA, short inhibitory RNA (siRNA)), DNA or RNA analogs (e.g., containing base analogs, sugar analogs and/or a non-native backbone and the like), RNA/DNA hybrids and polyamide nucleic acids (PNAs), the like and combinations thereof. Nucleic acids can be single- or double-stranded. In some embodiments, a nucleic acid is a primer. In some embodiments, a nucleic acid is a target nucleic acid. A target nucleic acid is often a nucleic acid of interest.

[0134] Nucleic acid may be provided for conducting methods described herein without processing of a sample containing the nucleic acid, in certain embodiments. In some embodiments, nucleic acid is provided for conducting methods described herein after processing of a sample containing the nucleic acid. For example, a nucleic acid can be extracted, isolated, purified, partially purified or amplified from a sample prior to, during or after a method described herein.

[0135] In some embodiments, a nucleic acid is amplified by a process comprising nucleic acid amplification wherein one or both strands of a nucleic acid are enzymatically replicated such that copies or complimentary copies of a nucleic acid strand are generated. Copies of a nucleic acid that are generated by an amplification process are often referred to as amplicons. A nucleic acid amplification process can linearly or exponentially generates amplicons having the same or substantially the same nucleotide sequence as a template or target nucleic acid, or segment thereof. A nucleic acid may be amplified by a suitable nucleic acid amplification process non-limiting examples of which include polymerase chain reaction (PCR), nested (n) PCR, quantitative (q) PCR, real-time PCR, reverse transcription (RT) PCR, isothermal amplification (e.g., loop mediated isothermal amplification (LAMP)), quantitative nucleic acid

sequence-based amplification (QT-NASBA), the like, variations thereof, and combinations thereof. In some embodiments, an amplification process comprises a polymerase chain reaction. In some embodiments, an amplification process comprises an isothermal amplification process.

[0136] In some embodiments, a nucleic acid amplification process comprises the use of one or more primers (e.g., a short oligonucleotide that can hybridize specifically to a nucleic acid template or target). A hybridized primer can often be extended by a polymerase during a nucleic acid amplification process). In some embodiments, a sample comprising nucleic acids is contacted with one or more primers. In some embodiments, a nucleic acid is contacted with one or more primers. A primer can be attached to a solid substrate or may be free in solution.

[0137] In some embodiments a nucleic acid or primer, comprises one or more distinguishable identifiers. Any suitable distinguishable identifier and/or detectable identifier can be used for a composition or method described herein. In certain embodiments a distinguishable identifier can be directly or indirectly associated with (e.g., bound to) a nucleic acid. For example a distinguishable identifier can be covalently or non-covalently bound to a nucleic acid. In some embodiments a distinguishable identifier is attached to a member of binding pair that is covalently or non-covalently bound to a nucleic acid. In some embodiments a distinguishable identifier is reversibly associated with a nucleic acid. In certain embodiments a distinguishable identifier that is reversibly associated with a nucleic acid can be removed from a nucleic acid using a suitable method (e.g., by increasing salt concentration, denaturing, washing, adding a suitable solvent and/or by heating).

[0138] In some embodiments a distinguishable identifier is a label. In some embodiments a nucleic acid comprises a detectable label, non-limiting examples of which include a radiolabel (e.g., an isotope), a metallic label, a fluorescent label, a chromophore, a chemiluminescent label, an electrochemiluminescent label (e.g., Origen™), a phosphorescent label, a quencher (e.g., a fluorophore quencher), a fluorescence resonance energy transfer (FRET) pair (e.g., donor and acceptor), a dye, a protein (e.g., an enzyme (e.g., alkaline phosphatase and horseradish peroxidase), an enzyme substrate, a small molecule, a mass tag, quantum dots, the like or combinations thereof. Any suitable fluorophore can be used as a label. A light emitting label can be detected and/or quantitated by a variety of suitable methods such as, for example, by a photocell, digital camera, flow cytometry, gel electrophoresis, exposure to

film, mass spectrometry, cytofluorimetric analysis, fluorescence microscopy, confocal laser scanning microscopy, laser scanning cytometry, the like and combinations thereof.

[0139] In some embodiments a distinguishable identifier is a barcode. In some embodiments a nucleic acid comprises a nucleic acid barcode (e.g., indexing nucleotides, sequence tags or “barcode” nucleotides). In certain embodiments a nucleic acid barcode comprises a distinguishable sequence of nucleotides usable as an identifier to allow unambiguous identification of one or more nucleic acids (e.g., a subset of nucleic acids) within a sample, method or assay. In certain embodiments a nucleic acid barcode is specific and/or unique to a certain sample, sample source, a particular nucleic acid genus or nucleic acid species, chromosome or gene, for example.

[0140] In some embodiments a nucleic acid or primer comprises one or more binding pairs. In some embodiments a nucleic acid or primer comprises one or more members of a binding pair. In some embodiments a binding pair comprises at least two members (e.g., molecules) that bind non-covalently and specifically to each other. Members of a binding pair often bind reversibly to each other, for example where the association of two members of a binding pair can be dissociated by a suitable method. Any suitable binding pair, or members thereof, can be utilized for a composition or method described herein. Non-limiting examples of a binding pair includes antibody/antigen, antibody/antibody receptor, antibody/protein A or protein G, hapten/anti-hapten, sulfhydryl/maleimide, sulfhydryl/haloacetyl derivative, amine/isotriocyanate, amine/succinimidyl ester, amine/sulfonyl halides, biotin/avidin, biotin/streptavidin, folic acid/folate binding protein, receptor/ligand, vitamin B12/intrinsic factor, analogues thereof, derivatives thereof, binding portions thereof, the like or combinations thereof. Non-limiting examples of a member of a binding pair include an antibody or antibody fragment, antibody receptor, an antigen, hapten, a peptide, protein, a fatty acid, a glyceryl moiety (e.g., a lipid), a phosphoryl moiety, a glycosyl moiety, a ubiquitin moiety, lectin, aptamer, receptor, ligand, metal ion, avidin, neutravidin, biotin, B12, intrinsic factor, analogues thereof, derivatives thereof, binding portions thereof, the like or combinations thereof. In some embodiments, a nucleic acid or primer comprises biotin. In some embodiments, a nucleic acid or primer is covalently attached to biotin.

[0141] In some embodiments a nucleic acid or primer is attached non-covalently or covalently to a suitable solid substrate. In some embodiments, a capture oligonucleotide and/or a

member of a binding pair is attached to a solid substrate. A capture oligonucleotide is often a nucleic acid configured to hybridize specifically to a target nucleic acid. In some embodiments a capture nucleic acid is a primer that is attached to a solid substrate. Non-limiting examples of a solid substrate include surfaces provided by microarrays and particles such as beads (e.g., paramagnetic beads, magnetic beads, microbeads, nanobeads), microparticles, and nanoparticles. Solid substrates also can include, for example, chips, columns, optical fibers, wipes, filters (e.g., flat surface filters), one or more capillaries, glass and modified or functionalized glass (e.g., controlled-pore glass (CPG)), quartz, mica, diazotized membranes (paper or nylon), polyformaldehyde, cellulose, cellulose acetate, paper, ceramics, metals, metalloids, semi-conductive materials, quantum dots, coated beads or particles, other chromatographic materials, magnetic particles; plastics (including acrylics, polystyrene, copolymers of styrene or other materials, polybutylene, polyurethanes, TEFLON™, polyethylene, polypropylene, polyamide, polyester, polyvinylidenedifluoride (PVDF), and the like), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon, silica gel, and modified silicon, Sephadex®, Sepharose®, carbon, metals (e.g., steel, gold, silver, aluminum, silicon and copper), inorganic glasses, conducting polymers (including polymers such as polypyrrole and polyindole); micro or nanostructured surfaces such as nucleic acid tiling arrays, nanotube, nanowire, or nanoparticulate decorated surfaces; or porous surfaces or gels such as methacrylates, acrylamides, sugar polymers, cellulose, silicates, or other fibrous or stranded polymers. In some embodiments, a solid substrate is coated using passive or chemically-derivatized coatings with any number of materials, including polymers, such as dextrans, acrylamides, gelatins or agarose. Beads and/or particles may be free or in connection with one another (e.g., sintered). In some embodiments, a solid substrate refers to a collection of particles. In some embodiments, particles comprise an agent that confers a paramagnetic property to the particles. In some embodiments a first solid substrate (e.g., a plurality of magnetic particles) is non-covalently and/or reversibly attached to a second solid substrate (e.g., a surface). In some embodiments, a second substrate or surface can be magnetized electronically such that magnetic particles are reversibly attached to the second substrate when the surface is magnetized, and the magnetic particles can be released when the second substrate is demagnetized or where the magnetic polarity of the second substrate is changed.

[0142] In some embodiments, a nucleic acid is a capture oligonucleotide. In some embodiments, a capture oligonucleotide is a nucleic acid that is attached covalently or non-covalently to a solid substrate. A capture oligonucleotide typically comprises a nucleotide sequence capable of hybridizing or annealing specifically to a nucleic acid of interest (e.g. target nucleic acid) or a portion thereof. In some embodiments, a capture nucleic acid comprises a nucleic acid sequence that is substantially complementary to a target nucleic acid, or portion thereof. In some embodiments, a capture oligonucleotide is a primer that is attached to a solid substrate. A capture oligonucleotide may be naturally occurring or synthetic and may be DNA or RNA based. Capture oligonucleotides can allow for specific separation of, for example, a target nucleic acid from other nucleic acids or contaminants in a sample.

[0143] In some embodiments, a method described herein comprises contacting a plurality of nucleic acids (e.g., nucleic acids in a sample) with at least one primer comprising a member of a binding pair. In some embodiments, a member of a binding pair comprise biotin. In some embodiments, the plurality of nucleic acids is contacted with a first primer and a second primer, where one of the first or second primers comprise biotin. In some embodiments, a plurality of nucleic acids comprises a target nucleic acid (e.g., a target RNA or DNA molecule). A target nucleic acid is often a nucleic acid of interest (e.g., a gene, a transcript or portion thereof). In some embodiments, a target nucleic acid comprises RNA. In some embodiments a target nucleic acid is amplified by a nucleic acid amplification process. In some embodiments, the nucleic acid amplification process comprises contacting a sample, nucleic acids of a sample and/or a target nucleic acid with a first primer, a second primer that is biotinylated and a polymerase under suitable conditions that promote nucleic acid amplification (e.g., conditions conducive to PCR or isothermal amplification). In some embodiments, a nucleic acid amplification process results in the production of amplicons. In some embodiments, amplicons comprise DNA amplicons, RNA amplicons, or a combination thereof. In some embodiments, amplicons comprise biotinylated DNA amplicons, RNA amplicons, or a combination thereof. In some embodiments, amplicons comprising RNA and biotinylated DNA (e.g., RNA/DNA duplexes) are contacted with a nuclease (e.g., an RNA exonuclease). In some embodiments, DNA amplicons are non-covalently attached to a solid substrate comprising a capture oligonucleotide, where the DNA amplicons, or a portion thereof, hybridize specifically to the capture oligonucleotide. In some

embodiments, biotinylated amplicons are contacted with, and/or are attached to magnetic beads comprising streptavidin, or a variant thereof.

[0144] Accordingly, in some embodiments, there are provided methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a cleavable portion covalently bound to the biomolecule, cleavage being catalyzed by the presence of the analyte in the query sample and a receptor associated with the cleavable portion of the biomolecule, the receptor being capable of binding a magnetic nanoparticle, passing the query sample over the sensor, thereby allowing cleavage and removal of the cleavable portion with the associated receptor from the biomolecule if the analyte is present, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

[0145] In some embodiments, the methods further comprise calculating a concentration of analyte in the query sample based on the resistance change of the GMR sensor.

[0146] In one or more of the preceding embodiments, methods include performing a buffer wash over the sensor prior to passing the query sample over the sensor.

[0147] In one or more of the preceding embodiments, methods include performing a buffer wash over the sensor after passing the query sample over the sensor but before passing the magnetic particles over the sensor.

[0148] In one or more of the preceding embodiments, methods include performing a buffer wash over the sensor after passing the magnetic particles over the sensor.

[0149] In one or more of the preceding embodiments, the analyte is a metal ion.

[0150] In one or more of the preceding embodiments, the query sample is water.

[0151] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0152] In one or more of the preceding embodiments, the biomolecule is double stranded DNA (dsDNA).

[0153] In one or more of the preceding embodiments, the receptor is covalently bound to one of the two strands of the dsDNA.

[0154] In one or more of the preceding embodiments, the dsDNA comprises a DNAzyme, the DNAzyme activated by the metal ion.

[0155] In one or more of the preceding embodiments, methods include determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0156] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0157] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the analyte.

[0158] In one or more of the preceding embodiments, passing the query sample over the detector comprises a flow rate of the query sample over the sensor at a rate of about 1 microL/min to about 20 microL/min.

[0159] In some embodiments, there are provided methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising an antigenic portion that binds an antibody at an antigen binding site, the antibody further comprising a portion separate from the antigen binding site configured to bind a magnetic nanoparticle, passing a mixture of the query sample and the antibody over the sensor, wherein the antigen binding site of the antibody binds the analyte if present in the query sample, thereby preventing binding of the antibody to the antigenic portion of the biomolecule, passing magnetic particles over the sensor after passing the mixture over the sensor, and detecting the presence of the analyte in the query sample by measuring a resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

[0160] In some embodiments, the methods further comprise calculating a concentration of the analyte in the query sample based on the resistance change of the GMR sensor.

[0161] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor prior to passing the mixture over the sensor.

[0162] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor after passing the mixture over the sensor but before passing the magnetic particles over the sensor.

[0163] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor after passing the magnetic particles over the sensor.

[0164] In one or more of the preceding embodiments, the analyte is a metal ion.

[0165] In one or more of the preceding embodiments, the query sample is water.

[0166] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0167] In one or more of the preceding embodiments, the biomolecule is a protein.

[0168] In one or more of the preceding embodiments, the protein is a bovine serum albumin.

[0169] In one or more of the preceding embodiments, determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0170] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0171] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the metal ion.

[0172] In one or more of the preceding embodiments, passing the mixture over the detector comprises a flow rate of the mixture over the sensor at a rate of about 1 uL/min to about 20 uL/min.

[0173] In some embodiments, there are provided methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding region configured to bind a detection protein, the detection protein also being capable of binding the analyte, wherein when the detection protein binds the analyte, it prevents binding of the detection protein to the binding region of the biomolecule, passing the detection protein over the sensor, passing the query sample over the sensor, passing a reporter protein over the sensor after passing the query sample over the sensor, the reporter protein capable of binding the detection protein and the reporter protein configured to bind to magnetic nanoparticles, passing magnetic particles over the sensor after passing the reporter protein over the sensor, and

detecting the presence of the metal ion by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

[0174] In some embodiments, methods may further comprise calculating a concentration of the analyte in the query sample based on the resistance change.

[0175] In one or more of the preceding embodiments, methods may further comprise performing one or more buffer washes.

[0176] In one or more of the preceding embodiments, the detection protein and query sample are mixed prior to passing them over the sensor.

[0177] In one or more of the preceding embodiments, the query sample is passed over the sensor after the detection protein is passed over the sensor.

[0178] In one or more of the preceding embodiments, the analyte is a metal ion.

[0179] In one or more of the preceding embodiments, the query sample is water.

[0180] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0181] In one or more of the preceding embodiments, the biomolecule is double stranded DNA (dsDNA).

[0182] In one or more of the preceding embodiments, the detection protein is an arsenic-binding regulatory protein comprising a tag.

[0183] In one or more of the preceding embodiments, the detection protein is a cadmium-binding regulatory protein comprising a tag.

[0184] In one or more of the preceding embodiments, the tag is glutathione S-transferase.

[0185] In one or more of the preceding embodiments, the tag is a poly-histidine.

[0186] In one or more of the preceding embodiments, the reporter protein is a biotinylated antibody.

[0187] In one or more of the preceding embodiments, the magnetic particles comprise streptavidin-linked nanoparticles.

[0188] In one or more of the preceding embodiments, determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0189] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0190] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the metal ion.

[0191] In one or more of the preceding embodiments, passing the query sample over the detector comprises a flow rate of the query sample over the sensor at a rate of about 1 uL/min to about 20 uL/min.

[0192] In some embodiments, there are provided methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising an associated magnetic particle, passing the query sample over the sensor, thereby causing removal of the associated magnetic particle from the biomolecule if the analyte is present, detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing the query sample over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0193] In some embodiments, there are provided methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a first biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the first biomolecule comprising a conditional binding site for a second biomolecule comprising a binding site for a magnetic particle, passing the query sample over the sensor, passing the second biomolecule over the sensor, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0194] In one or more of the preceding embodiments, the presence of the analyte prevents the binding of the second biomolecule.

[0195] In one or more of the preceding embodiments, the presence of the analyte enables the binding of the second molecule to the first biomolecule.

[0196] In some embodiments, there are provided methods of detecting the presence of an analyte in a query sample comprising providing a sensor comprising a first biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding site for a magnetic particle when the analyte is present, passing the query sample over the sensor, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0197] In one or more of the preceding embodiments, methods may further comprise calculating a concentration of analyte in the query sample based on the resistance change of the GMR sensor.

[0198] In one or more of the preceding embodiments, the biomolecule comprises DNA.

[0199] In one or more of the preceding embodiments, the biomolecule comprises a protein.

[0200] In some embodiments, there are provided systems configured to carry out the methods disclosed herein comprising, the system comprising a sample processing subsystem, a sensor subsystem comprising a microfluidics network comprising a GMR sensor having disposed on a polymer-coated surface of the sensor a biomolecule, a plurality of wires connected to a plurality of contact pads to carry a signal to a processor, a processor, and a pneumatic control subsystem for moving samples, reagents, and solvents throughout the sample processing subsystem and the sensor subsystem.

[0201] In some embodiments, there are provided methods of detecting the presence of a metal ion in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a cleavable portion covalently bound to the biomolecule, cleavage being catalyzed by the presence of the metal ion in the query sample, and a receptor associated with the cleavable portion of the biomolecule, the receptor being capable of binding a magnetic nanoparticle,

passing the query sample over the sensor, thereby allowing cleavage and removal of the cleavable portion with the associated receptor from the biomolecule if the metal ion is present, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of the metal ion in the query sample by measuring a resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the GMR sensor.

[0202] In some embodiments, such methods may further comprise calculating a concentration of metal ion in the query sample based on the resistance change of the GMR sensor.

[0203] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor prior to passing the query sample over the sensor.

[0204] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor after passing the query sample over the sensor but before passing the magnetic particles over the sensor.

[0205] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor after passing the magnetic particles over the sensor.

[0206] In one or more of the preceding embodiments, the metal ion is lead.

[0207] In one or more of the preceding embodiments, the query sample is water.

[0208] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0209] In one or more of the preceding embodiments, the biomolecule is double stranded DNA (dsDNA).

[0210] In one or more of the preceding embodiments, the receptor is covalently bound to one of the two strands of the dsDNA.

[0211] In one or more of the preceding embodiments, the dsDNA comprises a DNAzyme, the DNAzyme activated by the metal ion.

[0212] In one or more of the preceding embodiments, determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0213] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0214] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 10 nanomolar to about 1 micromolar in the metal ion.

[0215] In one or more of the preceding embodiments, passing the query sample over the detector comprises a flow rate of the query sample over the sensor at a rate of about 0.5uL/min to about 5uL/min. The sample is flowed over the sensor in a constant supply of fresh sample. This ensures maximum exposure of the dsDNA to metal ion present in the sample solution. For example, for lead ion, the sample is flowed over the sensor for 30 minutes.

[0216] In some embodiments, there are provided methods of detecting the presence of lead ion in a query sample comprising providing a sensor comprising double stranded DNA (dsDNA) disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the dsDNA comprising a cleavable portion of one strand of the dsDNA, cleavage being catalyzed by the presence of lead ion in the query sample, and a receptor associated with the cleavable portion, the receptor being capable of binding a magnetic nanoparticle, passing the query sample over the sensor, thereby allowing cleavage and removal of the cleavable portion with the associated receptor from the dsDNA if lead ion is present, passing magnetic particles over the sensor after passing the query sample over the sensor, and detecting the presence of lead ion in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the GMR sensor.

[0217] In some such embodiments, methods may further comprise calculating a concentration of lead ion in the query sample based on the resistance change of the GMR sensor.

[0218] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor prior to passing the query sample over the sensor.

[0219] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor after passing the query sample over the sensor but before passing the magnetic particles over the sensor.

[0220] In one or more of the preceding embodiments, may further comprise performing a buffer wash over the sensor after passing the magnetic particles over the sensor.

[0221] In one or more of the preceding embodiments, the query sample is water.

[0222] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0223] In one or more of the preceding embodiments, the receptor is covalently bound.

[0224] In one or more of the preceding embodiments, the dsDNA comprises a DNAzyme, the DNAzyme activated by the lead ion.

[0225] In one or more of the preceding embodiments, determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor

[0226] In one or more of the preceding embodiments, a plurality of dsDNA are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0227] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in lead ion.

[0228] In one or more of the preceding embodiments, passing the query sample over the detector comprises a flow rate of the query sample over the sensor at a rate of about 0.5uL/min to about 5uL/min.

[0229] In some embodiments, there are provided sensors comprising a biomolecule disposed on a polymer-coated surface of the giant magnetoresistance (GMR) sensor, the biomolecule comprising a cleavable portion covalently bound to the biomolecule, cleavage being catalyzed by the presence of a metal ion and a receptor associated with the cleavable portion, the receptor being capable of binding a magnetic nanoparticle; wherein when the cleavable portion is cleaved, the cleavable portion with the receptor is no longer covalently bound to the biomolecule.

[0230] In some such embodiments, the biomolecule is double stranded DNA (dsDNA).

[0231] In one or more of the preceding embodiments, the receptor is covalently bound to one of the two strands of the dsDNA.

[0232] In one or more of the preceding embodiments, the dsDNA comprises a DNAzyme, the DNAzyme activated by the metal ion.

[0233] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0234] In one or more of the preceding embodiments, the surface of the GMR sensor comprises a crosslinked PEG-PHEMA polymer.

[0235] In one or more of the preceding embodiments, the polymer is coated with a surfactant.

[0236] In one or more of the preceding embodiments, the surfactant is cetyl trimethylammonium bromide.

[0237] In one or more of the preceding embodiments, sensors may further comprise a plurality of wires connected to a plurality of contact pads configured to carry an electronic signal from the sensor to a processor.

[0238] In some embodiments, there are provided sensors comprising double stranded DNA (dsDNA) disposed on a polymer-coated surface of the giant magnetoresistance (GMR) sensor, the dsDNA comprising a cleavable portion, cleavage being catalyzed by the presence of a lead ion and a receptor associated with the cleavable portion, the receptor being capable of binding a magnetic nanoparticle, wherein when the cleavable portion is cleaved, the cleavable portion with the receptor is no longer covalently bound to the dsDNA.

[0239] In some such embodiments, the receptor is covalently bound to one of the two strands of the dsDNA.

[0240] In one or more of the preceding embodiments, the dsDNA comprises a DNAzyme, the DNAzyme activated by the lead ion.

[0241] In one or more of the preceding embodiments, a plurality of dsDNA are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0242] In one or more of the preceding embodiments, the surface of the GMR sensor comprises a crosslinked PEG-PHEMA polymer.

[0243] In one or more of the preceding embodiments, the polymer is coated with a surfactant.

[0244] In one or more of the preceding embodiments, the surfactant is cetyl trimethylammonium bromide.

[0245] In one or more of the preceding embodiments, sensor may further comprise a plurality of wires connect to a plurality of contact pads configured to carry an electronic signal from the sensor to a processor.

[0246] In some embodiments, there are provided cartridges for use in detecting metal ions in a query sample, the cartridge comprising (a) a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a cleavable portion covalently bound to the biomolecule, cleavage being catalyzed by the presence of metal ions in the query sample and a receptor associated with the cleavable portion, the receptor being capable of binding a magnetic nanoparticle; wherein when the cleavable portion is cleaved, the cleavable portion with the receptor is no longer covalently bound to the biomolecule; (b) one or more ports to introduce a query sample, magnetic nanoparticles, and optional wash buffers into the cartridge; and (c) a microfluidics system for moving the query sample, magnetic nanoparticles, and optional wash buffers from the one or more ports to the sensor.

[0247] In some such embodiments, such cartridges may further comprise a waste collection area.

[0248] In one or more of the preceding embodiments, the biomolecule is double stranded DNA (dsDNA).

[0249] In one or more of the preceding embodiments, the receptor is covalently bound to one of the two strands of the dsDNA.

[0250] In one or more of the preceding embodiments, the dsDNA comprises a DNAzyme, the DNAzyme activated by the metal ion.

[0251] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0252] In one or more of the preceding embodiments, the surface of the GMR sensor comprises a crosslinked PEG-PHEMA polymer.

[0253] In one or more of the preceding embodiments, the polymer is coated with a surfactant.

[0254] In one or more of the preceding embodiments, the surfactant is cetyl trimethylammonium bromide.

[0255] In one or more of the preceding embodiments, cartridges may further comprise a plurality of wires connect to a plurality of contact pads configured to carry an electronic signal from the sensor to a processor.

[0256] In one or more of the preceding embodiments, cartridges may further comprise one or more filters to filter the query sample.

[0257] In one or more of the preceding embodiments, the metal ions are lead ions.

[0258] In one or more of the preceding embodiments, the microfluidics system is pneumatically controlled.

[0259] In one or more of the preceding embodiments, the cartridge further comprises one or more hardware chips to control flowrate throughout the microfluidics system.

[0260] In some embodiments, there are provided cartridges for use in detecting lead ions in a query sample, the cartridge comprising (a) a sensor comprising double stranded DNA (dsDNA) disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the dsDNA comprising a cleavable portion on one strand of the dsDNA, cleavage being catalyzed by the presence of lead ions in the query sample and a receptor associated with the cleavable portion, the receptor being capable of binding a magnetic nanoparticle; wherein when the cleavable portion is cleaved, the cleavable portion with the receptor is no longer covalently bound to the dsDNA; (b) one or more ports to introduce a query sample, magnetic nanoparticles, and optional wash buffers into the cartridge; and (c) a microfluidics system for moving the query sample, magnetic nanoparticles, and optional wash buffers from the one or more ports to the sensor.

[0261] In some such embodiments, the cartridge may further comprise a waste collection area.

[0262] In one or more of the preceding embodiments, the dsDNA comprises a DNAzyme, the DNAzyme activated by the metal ion.

[0263] In one or more of the preceding embodiments, a plurality of dsDNA are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².

[0264] In one or more of the preceding embodiments, the surface of the GMR sensor comprises a crosslinked PEG-PHEMA polymer.

[0265] In one or more of the preceding embodiments, the polymer is coated with a surfactant.

[0266] In one or more of the preceding embodiments, the surfactant is cetyl trimethylammonium bromide.

[0267] In one or more of the preceding embodiments, cartridges may further comprise a plurality of wires connected to a plurality of contact pads configured to carry an electronic signal from the sensor to a processor.

[0268] In one or more of the preceding embodiments, cartridges may further comprise one or more filters to filter the query sample.

[0269] In one or more of the preceding embodiments, the microfluidics system is pneumatically controlled.

[0270] In one or more of the preceding embodiments, the cartridge further comprises one or more hardware chips to control flowrate throughout the microfluidics system.

[0271] In some embodiments, there are provided methods of making a sensor for the detection of lead ions in a query sample comprising (a) printing double stranded DNA (dsDNA) on a surface of a giant magnetoresistance (GMR) sensor; the dsDNA comprising a cleavable portion on one strand of the dsDNA, cleavage being catalyzed by the presence of lead ions in the query sample; and a receptor associated with the cleavable portion, the receptor being capable of binding a magnetic nanoparticle; wherein when the cleavable portion is cleaved, the cleavable portion with the receptor is no longer covalently bound to the dsDNA; the GMR sensor comprising a polymer coating onto which the dsDNA is printed; and (b) modifying the surface of the polymer coating by adding one or more blocking agents to the polymer coating after the printing step; adding a surfactant to the polymer coating after adding the one or more blocking agents.

[0272] In some such embodiments, the dsDNA comprises a DNAzyme.

[0273] In one or more of the preceding embodiments, the polymer coating comprises a crosslinked PEG-PHEMA polymer.

[0274] In one or more of the preceding embodiments, methods may further comprise one or more washing steps with a buffer wash.

[0275] In one or more of the preceding embodiments, buffer wash is a HEPES buffer.

[0276] In one or more of the preceding embodiments, HEPES buffer has a concentration of 25 mM.

[0277] In one or more of the preceding embodiments, the surfactant is acetyl trimethylammonium bromide (CTAB).

[0278] In one or more of the preceding embodiments, CTAB has a concentration of 1% by weight in 25 mM HEPES.

[0279] In some embodiments, there are provided methods of detecting the presence of a metal ion in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising an antigenic portion that binds an antibody at an antigen binding site, the antibody further comprising a portion separate from the antigen binding site configured to bind a magnetic nanoparticle; passing a mixture of the query sample and the antibody over the sensor, wherein the antigen binding site of the antibody binds the metal ion if present in the query sample, thereby preventing binding of the antibody to the antigenic portion of the biomolecule; passing magnetic particles over the sensor after passing the mixture over the sensor; and detecting the presence of the metal ion in the query sample by measuring a resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the GMR sensor.

[0280] In some such embodiments, methods may further comprise calculating a concentration of metal ion in the query sample based on the resistance change of the GMR sensor.

[0281] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor prior to passing the mixture over the sensor.

[0282] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor after passing the mixture over the sensor but before passing the magnetic particles over the sensor.

[0283] In one or more of the preceding embodiments, methods may further comprise performing a buffer wash over the sensor after passing the magnetic particles over the sensor.

[0284] In one or more of the preceding embodiments, the metal ion is mercury.

[0285] In one or more of the preceding embodiments, the query sample is water.

[0286] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0287] In one or more of the preceding embodiments, the biomolecule is a protein.

[0288] In one or more of the preceding embodiments, the protein is a modified bovine serum albumin. In embodiments, the modified bovine serum albumin is HgBSA and has the

Product Name: Hg²⁺ [BSA] (Cat.No: DAGA-007B) Creative Diagnostics at Ramsey Road Shirley, NY 11967, USA. In embodiments, the antibody paired with HgBSA is HgAb and has Product Name: RHA anti-Hg²⁺ monoclonal antibody, clone Hg2 (Cat.No: HMABPY007) also available from Creative Diagnostics.

[0289] In one or more of the preceding embodiments, determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0290] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the metal ion.

[0291] In one or more of the preceding embodiments, the sample is flowed over the sensor in a loop. In one or more of the preceding embodiments, the sample is flowed over the sensor providing a constant supply of fresh sample. In one or more of the preceding embodiments, B-HgAb (detection antibody) is added to mercury ion containing query sample at a working concentration of about 0.1 µg/mL. Mercury ion (II) in solution competes with HgBSA substrate for the binding site of HgAb. In solutions that have a high concentration of Hg, very little HgAb can bind to HgBSA. This incubation occurs while flowing of the GMR sensor at a flowrate between 1 µl/min and 5 µl/min. A fresh supply of the sample may be continuously being introduced over the sensor to ensure ample binding time of any non-Hg-bound HgAb. In one or more of the preceding embodiments, the query sample may be reacted for about 30 minutes.

[0292] In some embodiments, there are provided methods of detecting the presence of mercury ion in a query sample comprising providing a sensor comprising a protein disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the protein comprising an antigenic portion capable of binding to an antibody at an antigen binding site, the antibody further comprising a portion separate from the antigen binding site configured to bind a magnetic nanoparticle; and passing a mixture of the query sample and the antibody over the sensor, wherein the antigen binding site of the antibody binds mercury ion if present in the query sample, thereby preventing binding of the antibody to the antigenic portion of the protein; passing magnetic particles over the sensor after passing the mixture over the sensor; and detecting the presence of the mercury ion in the query sample by measuring a resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor. In some such embodiments, such methods are used to detect Hg²⁺ ion.

[0293] In some such embodiments, methods may further comprise calculating a concentration of mercury ion in the query sample based on the resistance change of the GMR sensor.

[0294] In one or more of the preceding embodiments, the methods may comprise performing a buffer wash over the sensor prior to passing the mixture over the sensor.

[0295] In one or more of the preceding embodiments, the methods may further comprise performing a buffer wash over the sensor after passing the mixture over the sensor but before passing the magnetic particles over the sensor.

[0296] In one or more of the preceding embodiments, the methods may further comprise performing a buffer wash over the sensor after passing the magnetic particles over the sensor.

[0297] In one or more of the preceding embodiments, the query sample is water.

[0298] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0299] In one or more of the preceding embodiments, the protein is a modified bovine serum albumin. In embodiments, the modified bovine serum albumin is HgBSA and has the Product Name: Hg²⁺ [BSA] (Cat.No: DAGA-007B) Creative Diagnostics at Ramsey Road Shirley, NY 11967, USA. In embodiments, the antibody paired with HgBSA is HgAb and has Product Name: RHA anti-Hg²⁺ monoclonal antibody, clone Hg2 (Cat.No: HMABPY007) also available from Creative Diagnostics.

[0300] In one or more of the preceding embodiments, determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0301] In one or more of the preceding embodiments, the sample is flowed over the sensor in a loop. In one or more of the preceding embodiments, the sample is flowed over the sensor providing a constant supply of fresh sample. In one or more of the preceding embodiments, B-HgAb (detection antibody) is added to mercury ion containing query sample at a working concentration of about 0.1ug/mL. Mercury ion (II) in solution competes with HgBSA substrate for the binding site of HgAb. In solutions that have a high concentration of Hg, very little HgAb can bind to HgBSA. This incubation occurs while flowing of the GMR sensor at a flowrate between 1ul/min and 5ul/min. A fresh supply of the sample may be continuously being

introduced over the sensor to ensure ample binding time of any non-Hg-bound HgAb. In one or more of the preceding embodiments, the query sample may be reacted for about 30 minutes.

[0302] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the mercury ion.

[0303] In some embodiments, there are provided sensors comprising a protein disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the protein comprising an antigenic portion. In some such embodiments, the protein is a modified bovine serum albumin. In embodiments, the modified bovine serum albumin is HgBSA and has the Product Name: Hg²⁺ [BSA] (Cat.No: DAGA-007B) Creative Diagnostics at Ramsey Road Shirley, NY 11967, USA. In embodiments, the antibody paired with HgBSA is HgAb and has Product Name: RHA anti-Hg²⁺ monoclonal antibody, clone Hg2 (Cat.No: HMABPY007) also available from Creative Diagnostics.

[0304] In one or more of the preceding embodiments, a surfactant may be disposed on the polymer-coated surface of the GMR sensor. In some such embodiments, the surfactant is cationic. In some such embodiments, the surfactant is cetyl trimethylammonium bromide.

[0305] In one or more of the preceding embodiments, the protein is spatially organized on the GMR sensor via printing.

[0306] In some embodiments, there are provided sensors comprising a modified bovine serum albumin (BSA) disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the modified bovine serum albumin comprising an antigenic portion that binds an antibody at an antigen binding site. In embodiments, the modified bovine serum albumin is HgBSA and has the Product Name: Hg²⁺ [BSA] (Cat.No: DAGA-007B) Creative Diagnostics at Ramsey Road Shirley, NY 11967, USA. In embodiments, the antibody paired with HgBSA is HgAb and has Product Name: RHA anti-Hg²⁺ monoclonal antibody, clone Hg2 (Cat.No: HMABPY007) also available from Creative Diagnostics.

[0307] In one or more of the preceding embodiments, the sensors may further comprise a surfactant disposed on the polymer-coated surface of the GMR sensor. In some such embodiments, the surfactant is cationic. In some such embodiments, the surfactant is cetyl trimethylammonium bromide.

[0308] In one or more of the preceding embodiments, the modified BSA is spatially organized on the GMR sensor via printing.

[0309] In some embodiments, there are provided cartridges for use in detecting metal ions in a query sample, the cartridge comprising (a) a sensor comprising a protein disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the protein comprising an antigenic portion; (b) a port to introduce a query sample; (c) a storage source of magnetic nanoparticles; (d) a storage source of antibody, the antibody comprising an antigen binding site capable of binding the antigenic portion and a portion separate from the antigen binding site configured to bind the magnetic nanoparticles; and (e) a pneumatically-controlled microfluidics system for moving the query sample, magnetic nanoparticles, and antibody.

[0310] In some such embodiments, the cartridge further comprises a waste collection area.

[0311] In one or more of the preceding embodiments, the protein is a modified bovine serum albumin. In embodiments, the modified bovine serum albumin is HgBSA and has the Product Name: Hg²⁺ [BSA] (Cat.No: DAGA-007B) Creative Diagnostics at Ramsey Road Shirley, NY 11967, USA. In embodiments, the antibody paired with HgBSA is HgAb and has Product Name: RHA anti-Hg²⁺ monoclonal antibody, clone Hg2 (Cat.No: HMABPY007) also available from Creative Diagnostics.

[0312] In one or more of the preceding embodiments, a surfactant is disposed on the polymer-coated GMR sensor. In some such embodiments, the surfactant is cetyl trimethylammonium bromide.

[0313] In one or more of the preceding embodiments, the sensor is configured to be in electronic communication with a plurality of contact pins to carry an electronic signal from the sensor to a processor.

[0314] In one or more of the preceding embodiments, the cartridge may comprise one or more filters to filter the query sample.

[0315] In one or more of the preceding embodiments, the cartridge may further comprise one or more hardware chips to control the pneumatically-controlled microfluidics system.

[0316] In some embodiments, there are provided cartridges for use in detecting mercury ions in a query sample, the cartridges comprising (a) a sensor comprising a modified bovine serum albumin (BSA) disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the modified BSA comprising an antigenic portion; (b) a port to introduce a query sample; (c) a storage source of magnetic nanoparticles; (d) a storage source of antibody,

the antibody comprising an antigen binding site capable of binding the antigenic portion and a portion separate from the antigen binding site configured to bind the magnetic nanoparticles; and (e) a pneumatically-controlled microfluidics system for moving the query sample, magnetic nanoparticles, and antibody. In embodiments, the modified bovine serum albumin is HgBSA and has the Product Name: Hg²⁺ [BSA] (Cat.No: DAGA-007B) Creative Diagnostics at Ramsey Road Shirley, NY 11967, USA. In embodiments, the antibody paired with HgBSA is HgAb and has Product Name: RHA anti-Hg²⁺ monoclonal antibody, clone Hg2 (Cat.No: HMABPY007) also available from Creative Diagnostics.

[0317] In some such embodiments, the cartridge may further comprise a waste collection area.

[0318] In one or more of the preceding embodiments, a surfactant may be disposed on the polymer-coated GMR sensor. In some such embodiments, the surfactant may be cetyl trimethylammonium bromide.

[0319] In one or more of the preceding embodiments, the sensor is configured to be in electronic communication with a plurality of contact pins to carry an electronic signal from the sensor to a processor.

[0320] In one or more of the preceding embodiments, the cartridge may comprise one or more filters to filter the query sample.

[0321] In one or more of the preceding embodiments, the cartridge further comprises one or more hardware chips to control the pneumatically-controlled microfluidics system.

[0322] In some embodiments, there are provided methods of making a sensor for the detection of mercury ions in a query sample comprising printing a protein comprising an antigenic portion on a polymer-coated GMR sensor. In embodiments, the protein is a modified bovine serum albumin. In embodiments, the modified bovine serum albumin is HgBSA and has the Product Name: Hg²⁺ [BSA] (Cat.No: DAGA-007B) Creative Diagnostics at Ramsey Road Shirley, NY 11967, USA. In embodiments, the antibody paired with HgBSA is HgAb and has Product Name: RHA anti-Hg²⁺ monoclonal antibody, clone Hg2 (Cat.No: HMABPY007) also available from Creative Diagnostics.

[0323] In one or more of the preceding embodiments, the polymer coating is a crosslinked PEG-PHEMA polymer.

[0324] In some embodiments, there are provided methods of detecting the presence of a metal ion in a query sample comprising providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding region configured to bind a detection protein, the detection protein also being capable of binding the metal ion; wherein when the detection protein binds the metal ion, it prevents binding of the detection protein to the binding region of the biomolecule; passing the detection protein over the sensor; passing the query sample over the sensor; passing a reporter protein over the sensor after passing the query sample over the sensor, the reporter protein being capable of binding the detection protein and the reporter protein configured to bind to magnetic nanoparticles; passing magnetic particles over the sensor after passing the reporter protein over the sensor; and detecting the presence of the metal ion by measuring a resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the GMR sensor.

[0325] In some such embodiments, the methods may further comprise calculating a concentration of metal ion in the query sample based on the resistance change of the GMR sensor.

[0326] In one or more of the preceding embodiments, the methods may further comprise performing one or more buffer washes.

[0327] In one or more of the preceding embodiments, the detection protein and query sample are mixed prior to passing them over the sensor.

[0328] In one or more of the preceding embodiments, the query sample is passed over the sensor after the detection protein is passed over the sensor.

[0329] In one or more of the preceding embodiments, the metal ion is arsenic.

[0330] In one or more of the preceding embodiments, the metal ion is cadmium.

[0331] In one or more of the preceding embodiments, the query sample is water.

[0332] In one or more of the preceding embodiments, the query sample is derived from the blood of a subject.

[0333] In one or more of the preceding embodiments, the detection protein is an arsenic-binding regulatory protein comprising a tag.

[0334] In one or more of the preceding embodiments, the detection protein is a cadmium-binding regulatory protein comprising a tag.

[0335] In one or more of the preceding embodiments, wherein the tag is glutathione S-transferase.

[0336] In one or more of the preceding embodiments, the tag is a poly-histidine.

[0337] In one or more of the preceding embodiments, the biomolecule is double stranded DNA (dsDNA).

[0338] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5'-CTT ACA CAT TCG TTA AGT CAT ATA TGT TTTATGA CTT ATC CGC TTC GAA GA/3AmMC6T/-3' SEQ ID NO. 1.

[0339] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5'-TCT TCG AAG CGG ATA AGT CAA AAA CAT ATA TG ACTT AAC GAA TGT GTA AG-3' SEQ ID NO. 2.

[0340] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5' – TGA GTC GAA AAT GGT TAT AAT ACA CTC AAA TAA ATA TTT GAA TGA AGA TG/3AmMC6T/ - 3' SEQ ID NO. 3.

[0341] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5' – CAT CTT CAT TCA AAT ATT TAT TTG AGT GTA TTA TAA CCA TTT TCG ACT CA – 3' SEQ ID NO. 4.

[0342] In one or more of the preceding embodiments, the reporter protein is a biotinylated antibody.

[0343] In one or more of the preceding embodiments, the magnetic particles comprise streptavidin-linked nanoparticles.

[0344] In one or more of the preceding embodiments, determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

[0345] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm² on the biosensor.

[0346] In one or more of the preceding embodiments, Pcad-Ocad-F-Amine strand may be printed on the surface in a concentration of between 10uM and 25uM.

[0347] In one or more of the preceding embodiments, a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the metal ion.

[0348] In one or more of the preceding embodiments, passing the query sample over the detector comprises a flow rate of the query sample over the sensor at a rate of about 1ul/min and 5ul/min. In some such embodiments, reaction duration may be about 30 minutes. In some embodiments, this reaction time was determined to be sufficient by testing flow of biotinylated Pcad-Ocad-R over printed Pcad-Ocad-F. Signal was obtained when streptavidin-labeled magnetic nanoparticles were introduced, which confirmed that hybridization of the two Pcad-Ocad strands was occurring. In some embodiments, R-strand hybridization is always done in concentrations at least equal to the highest available F-strand concentration.

[0349] In some embodiments, there are provided sensors for detecting a metal ion comprising a biomolecule disposed on a polymer-coated surface of the giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding region configured to bind a detection protein, the detection protein also being capable of binding the metal ion; wherein when the detection protein binds the metal ion, it prevents binding of the detection protein to the binding region of the biomolecule.

[0350] In some such embodiments, the biomolecule is double stranded DNA (dsDNA).

[0351] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5'-CTT ACA CAT TCG TTA AGT CAT ATA TGT TTTATGA CTT ATC CGC TTC GAA GA/3AmMC6T/-3' SEQ ID NO. 1.

[0352] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5'-TCT TCG AAG CGG ATA AGT CAA AAA CAT ATA TG ACTT AAC GAA TGT GTA AG-3' SEQ ID NO. 2.

[0353] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5' – TGA GTC GAA AAT GGT TAT AAT ACA CTC AAA TAA ATA TTT GAA TGA AGA TG/3AmMC6T/ - 3' SEQ ID NO. 3.

[0354] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5' – CAT CTT CAT TCA AAT ATT TAT TTG AGT GTA TTA TAA CCA TTT TCG ACT CA – 3' SEQ ID NO. 4.

[0355] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm² on the biosensor.

[0356] In one or more of the preceding embodiments, the surface of the GMR sensor comprises a polymer comprising a crosslinked PEG-PHEMA.

[0357] In one or more of the preceding embodiments, the polymer of the polymer-coated surface is overcoated with a surfactant. the surfactant is cetyl trimethylammonium bromide.

[0358] In one or more embodiments, sensors may further comprise a plurality of wires connected to a plurality of contact pads configured to carry an electronic signal from the sensor to a processor.

[0359] In one or more of the preceding embodiments, the metal ion comprises arsenic or cadmium.

[0360] In some embodiments, there are provided cartridges for use in detecting metal ions in a query sample, the cartridge comprising (a) a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding region configured to bind a detection protein, the detection protein also being capable of binding the metal ion; wherein when the detection protein binds the metal ion, it prevents binding of the detection protein to the binding region of the biomolecule; (b) one or more ports to introduce a query sample, magnetic nanoparticles, and optional wash buffers into the cartridge; and (c) a microfluidics system for moving the query sample, magnetic nanoparticles, and optional wash buffers from the one or more ports to the sensor.

[0361] In some such embodiments, the cartridges may further comprise a waste collection area.

[0362] In one or more of the preceding embodiments, the biomolecule is double stranded DNA (dsDNA).

[0363] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5'-CTT ACA CAT TCG TTA AGT CAT ATA TGT TTTATGA CTT ATC CGC TTC GAA GA/3AmMC6T/-3' SEQ ID NO. 1.

[0364] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5'-TCT TCG AAG CGG ATA AGT CAA AAA CAT ATA TG ACTT AAC GAA TGT GTA AG-3' SEQ ID NO. 2.

[0365] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5' – TGA GTC GAA AAT GGT TAT AAT ACA CTC AAA TAA ATA TTT GAA TGA AGA TG/3AmMC6T/ - 3' SEQ ID NO. 3.

[0366] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5' – CAT CTT CAT TCA AAT ATT TAT TTG AGT GTA TTA TAA CCA TTT TCG ACT CA – 3' SEQ ID NO. 4.

[0367] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm² on the biosensor.

[0368] In one or more of the preceding embodiments, the surface of the GMR sensor comprises a crosslinked PEG-PHEMA polymer.

[0369] In one or more of the preceding embodiments, the polymer is coated with a surfactant. In some such embodiments, the surfactant may be cetyl trimethylammonium bromide.

[0370] In one or more of the preceding embodiments, sensors in a cartridge may further comprise a plurality of wires connected to a plurality of contact pads configured to carry an electronic signal from the sensor to a processor.

[0371] In one or more of the preceding embodiments, cartridges may comprise one or more filters to filter the query sample.

[0372] In one or more of the preceding embodiments, the metal ion comprises arsenic or cadmium.

[0373] In one or more of the preceding embodiments, the microfluidics system is pneumatically controlled.

[0374] In one or more of the preceding embodiments, the cartridge further comprises one or more hardware chips to control flowrate throughout the microfluidics system.

[0375] In some embodiments, there are provided methods of making a sensor for the detection of arsenic or cadmium ions in a query sample comprising (a) printing double stranded DNA (dsDNA) on a surface of a giant magnetoresistance (GMR) sensor; the dsDNA comprising a binding region configured to bind a detection protein, the detection protein also being capable of binding the arsenic or cadmium ions; wherein when the detection protein binds the metal ion, it prevents binding of the detection protein to the binding region of the biomolecule; the GMR sensor comprising a polymer coating onto which the dsDNA is printed; and (b) modifying the surface of the polymer coating by: adding one or more blocking agents to the polymer coating after the printing step; and optionally adding a surfactant to the polymer coating after adding the one or more blocking agents.

[0376] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5'-CTT ACA CAT TCG TTA AGT CAT ATA TGT TTTATGA CTT ATC CGC TTC GAA GA/3AmMC6T/-3' SEQ ID NO. 1.

[0377] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5'-TCT TCG AAG CGG ATA AGT CAA AAA CAT ATA TG ACTT AAC GAA TGT GTA AG-3' SEQ ID NO. 2.

[0378] In one or more of the preceding embodiments, a forward strand of the dsDNA has a sequence 5' – TGA GTC GAA AAT GGT TAT AAT ACA CTC AAA TAA ATA TTT GAA TGA AGA TG/3AmMC6T/ - 3' SEQ ID NO. 3.

[0379] In one or more of the preceding embodiments, a reverse strand of the dsDNA has a sequence 5' – CAT CTT CAT TCA AAT ATT TAT TTG AGT GTA TTA TAA CCA TTT TCG ACT CA – 3' SEQ ID NO. 4.

[0380] In one or more of the preceding embodiments, a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm² on the biosensor.

[0381] In one or more of the preceding embodiments, Pcad-Ocad-F-Amine strand is printed on the surface in a concentration of between 10uM and 25uM.

[0382] In one or more of the preceding embodiments, the polymer coating comprises a crosslinked PEG-PHEMA polymer.

[0383] It will be understood that all embodiments disclosed herein may be combined in any manner to carry out a method of detecting an analyte and that such methods may be carried out using any combination of embodiments disclosed herein describing the various system components.

[0384] While the principles of the disclosure have been made clear in the illustrative embodiments set forth above, it will be apparent to those skilled in the art that various modifications may be made to the structure, arrangement, proportion, elements, materials, and components used in the practice of the disclosure.

[0385] It will thus be seen that the features of this disclosure have been fully and effectively accomplished. It will be realized, however, that the foregoing preferred specific embodiments have been shown and described for the purpose of illustrating the functional and structural principles of this disclosure and are subject to change without departure from such

principles. Therefore, this disclosure includes all modifications encompassed within the spirit and scope of the following claims.

What is claimed is:

1. A method of detecting the presence of an analyte in a query sample comprising:
providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising:
a cleavable portion covalently bound to the biomolecule, cleavage being catalyzed by the presence of the analyte in the query sample; and
a receptor associated with the cleavable portion of the biomolecule, the receptor being capable of binding a magnetic nanoparticle;
passing the query sample over the sensor, thereby allowing cleavage and removal of the cleavable portion with the associated receptor from the biomolecule if the analyte is present;
passing magnetic particles over the sensor after passing the query sample over the sensor; and
detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.
2. The method of claim 1, further comprising calculating a concentration of analyte in the query sample based on the resistance change of the GMR sensor.
3. The method of any one of claims 1 or 2, further comprising performing a buffer wash over the sensor prior to passing the query sample over the sensor.
4. The method of any one of claims 1 to 3, further comprising performing a buffer wash over the sensor after passing the query sample over the sensor but before passing the magnetic particles over the sensor.
5. The method of any one of claims 1 to 4, further comprising performing a buffer wash over the sensor after passing the magnetic particles over the sensor.
6. The method of any one of claims 1 to 5, wherein the analyte is a metal ion.
7. The method of any one of claims 1 to 6, wherein the query sample is water.

8. The method of any one of claims 1 to 6, wherein the query sample is derived from the blood of a subject.
9. The method of any one of claims 1 to 7, wherein the biomolecule is double stranded DNA (dsDNA).
10. The method of claim 9, wherein the receptor is covalently bound to one of the two strands of the dsDNA.
11. The method of claim 9 or 10, wherein the dsDNA comprises a DNAzyme, the DNAzyme activated by the metal ion.
12. The method of any one of claims 1 to 11, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.
13. The method of any one of claim 1 to 12, wherein a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².
14. The method of any one of claims 1 to 13, wherein a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the analyte.
15. The method of any one of claims 1 to 14, wherein passing the query sample over the detector comprises a flow rate of the query sample over the sensor at a rate of about 1 uL/min to about 20 uL/min.
16. A method of detecting the presence of an analyte in a query sample comprising:
providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising:

an antigenic portion that binds an antibody at an antigen binding site, the antibody further comprising a portion separate from the antigen binding site configured to bind a magnetic nanoparticle;

passing a mixture of the query sample and the antibody over the sensor, wherein the antigen binding site of the antibody binds the analyte if present in the query sample, thereby preventing binding of the antibody to the antigenic portion of the biomolecule;

passing magnetic particles over the sensor after passing the mixture over the sensor; and

detecting the presence of the analyte in the query sample by measuring a resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

17. The method of claim 16, further comprising calculating a concentration of the analyte in the query sample based on the resistance change of the GMR sensor.

18. The method of claim 16 or 17, further comprising performing a buffer wash over the sensor prior to passing the mixture over the sensor.

19. The method of any one of claims 16 to 18, further comprising performing a buffer wash over the sensor after passing the mixture over the sensor but before passing the magnetic particles over the sensor.

20. The method of any one of claims 16 to 19, further comprising performing a buffer wash over the sensor after passing the magnetic particles over the sensor.

21. The method of any one of claims 16 to 20, wherein the analyte is a metal ion.

22. The method of any one of claims 16 to 21, wherein the query sample is water.

23. The method of any one of claims 16 to 21, wherein the query sample is derived from the blood of a subject.

24. The method of any one of claims 16 to 23, wherein the biomolecule is a protein.
25. The method of claim 24, wherein the protein is a bovine serum albumin.
26. The method of any one of claims 16 to 25, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.
27. The method of any one of claim 16 to 26, wherein a plurality of biomolecules are attached on the surface of the sensor in a density of about 1×10^9 to about 5×10^{10} biomolecules per/mm².
28. The method of any one of claims 16 to 27, wherein a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the metal ion.
29. The method of any one of claims 16 to 28, wherein passing the mixture over the detector comprises a flow rate of the mixture over the sensor at a rate of about 1 uL/min to about 20 uL/min.
30. A method of detecting the presence of an analyte in a query sample comprising:
providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising:
a binding region configured to bind a detection protein, the detection protein also being capable of binding the analyte;
wherein when the detection protein binds the analyte, it prevents binding of the detection protein to the binding region of the biomolecule;
passing the detection protein over the sensor;
passing the query sample over the sensor;
passing a reporter protein over the sensor after passing the query sample over the sensor, the reporter protein capable of binding the detection protein and the reporter protein configured to bind to magnetic nanoparticles;

passing magnetic particles over the sensor after passing the reporter protein over the sensor; and

detecting the presence of the metal ion by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor.

31. The method of claim 30, further comprising calculating a concentration of the analyte in the query sample based on the resistance change.

32. The method of claim 30 or 31, further comprising performing one or more buffer washes.

33. The method of any one of claims 30 to 32, wherein the detection protein and query sample are mixed prior to passing them over the sensor.

34. The method of any one of claims 30 to 33, wherein the query sample is passed over the sensor after the detection protein is passed over the sensor.

35. The method of any one of claims 30 to 34, wherein the analyte is a metal ion.

36. The method of any one of claims 30 to 35, wherein the query sample is water.

37. The method of any one of claims 30 to 35, wherein the query sample is derived from the blood of a subject.

38. The method of any one of claims 30 to 37, wherein the biomolecule is double stranded DNA (dsDNA).

39. The method of any one of claim 30 to 38, wherein the detection protein is an arsenic-binding regulatory protein comprising a tag.

40. The method of any one of claim 30 to 38, wherein the detection protein is a cadmium-binding regulatory protein comprising a tag.
41. The method of claim 39 or 40, wherein the tag is glutathione S-transferase.
42. The method of claim 39 or 40, wherein the tag is a poly-histidine.
43. The method of any one of claims 30 to 42, wherein the reporter protein is a biotinylated antibody.
44. The method of any one of claims 30 to 43, wherein the magnetic particles comprise streptavidin-linked nanoparticles.
45. The method of any one of claims 30 to 44, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.
46. The method of any one of claims 30 to 45, wherein a plurality of biomolecules are attached on the surface of the sensor in a density 1×10^9 to about 5×10^{10} biomolecules per/mm².
47. The method of any one of claims 30 to 46, wherein a sensitivity limit of detection is in a range from about 1 nanomolar to about 10 nanomolar in the metal ion.
48. The method of any one of claims 30 to 47, wherein passing the query sample over the detector comprises a flow rate of the query sample over the sensor at a rate of about 1 uL/min to about 20 uL/min.

49. A method of detecting the presence of an analyte in a query sample comprising:
providing a sensor comprising a biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising an associated magnetic particle;
passing the query sample over the sensor, thereby causing removal of the associated magnetic particle from the biomolecule if the analyte is present;
detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing the query sample over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

50. A method of detecting the presence of an analyte in a query sample comprising:
providing a sensor comprising a first biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the first biomolecule comprising a conditional binding site for a second biomolecule comprising a binding site for a magnetic particle;
passing the query sample over the sensor;
passing the second biomolecule over the sensor;
passing magnetic particles over the sensor after passing the query sample over the sensor; and
detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

51. The method of claim 50, wherein the presence of the analyte prevents the binding of the second biomolecule.

52. The method of claim 50, wherein the presence of the analyte enables the binding of the second molecule to the first biomolecule.

53. A method of detecting the presence of an analyte in a query sample comprising:
providing a sensor comprising a first biomolecule disposed on a polymer-coated surface of a giant magnetoresistance (GMR) sensor, the biomolecule comprising a binding site for a magnetic particle when the analyte is present;
passing the query sample over the sensor;
passing magnetic particles over the sensor after passing the query sample over the sensor; and
detecting the presence of the analyte in the query sample by measuring resistance change of the GMR sensor based on determining resistance before and after passing magnetic particles over the sensor, wherein determining resistance change of the GMR sensor comprises using at least one reference resistor to perform phase-sensitive solution of resistance change of the GMR sensor.

54. The method of any one of claims 49-53, further comprising calculating a concentration of analyte in the query sample based on the resistance change of the GMR sensor.

55. The method of any one of claims 49-54, wherein the biomolecule comprises DNA.

56. The method of any one of claims 49-54, wherein the biomolecule comprises a protein.

57. A system configured to carry out the methods of any one of claims 1 to 56 comprising:
a sample processing subsystem;
a sensor subsystem comprising a microfluidics network comprising a GMR sensor having disposed on a polymer-coated surface of the sensor a biomolecule;
a plurality of wires connected to a plurality of contact pads to carry a signal to a processor;
a processor; and
a pneumatic control subsystem for moving samples, reagents, and solvents throughout the sample processing system and the sensor subsystem.

100

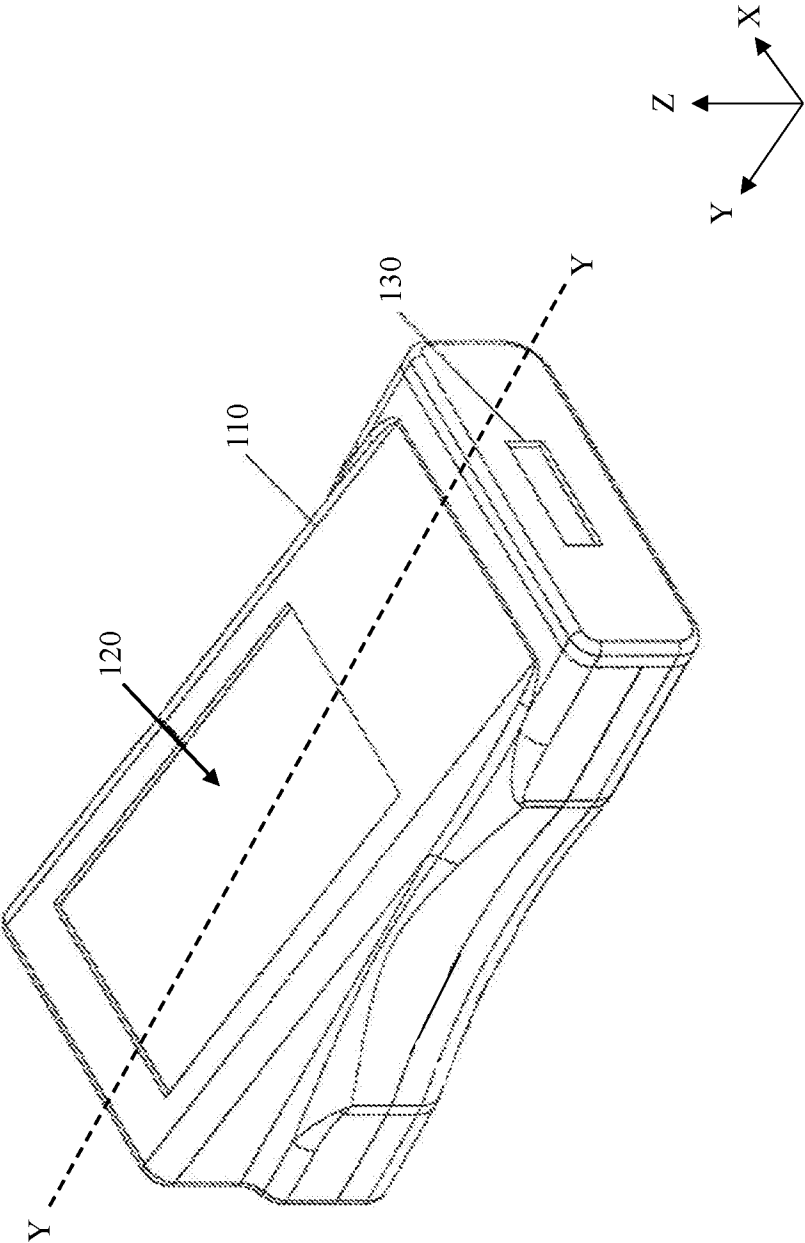


Fig. 1

200

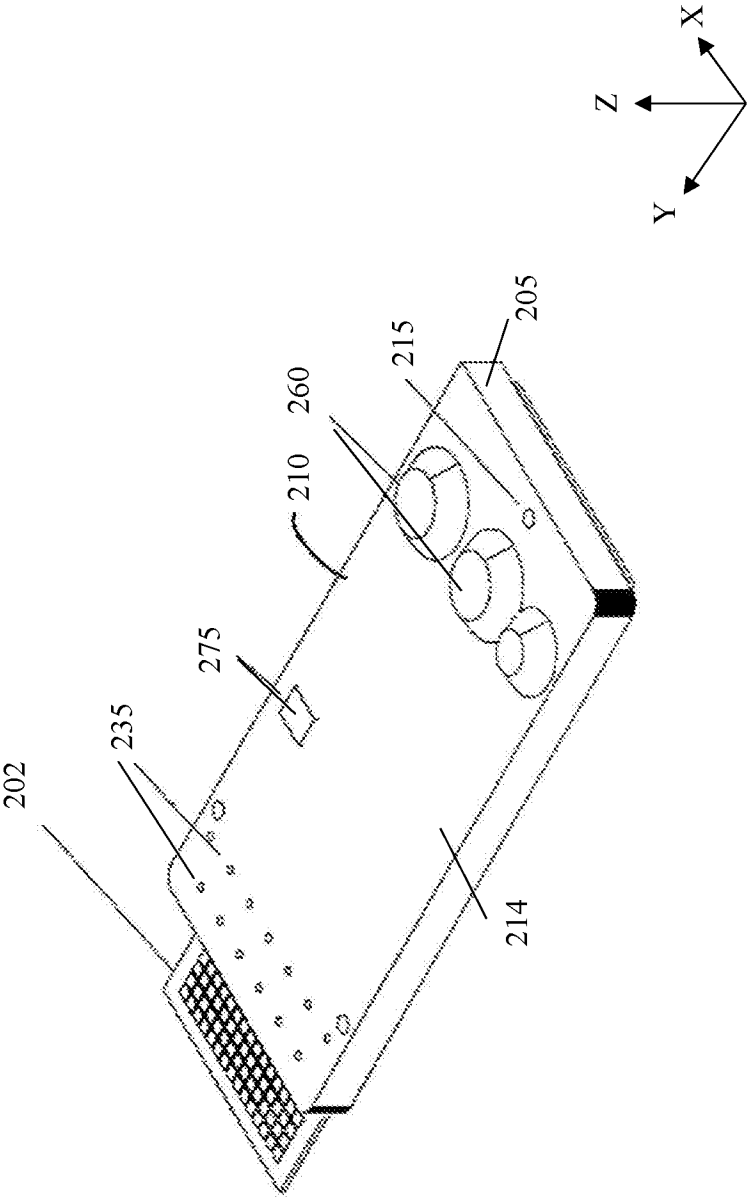


Fig. 2A

200

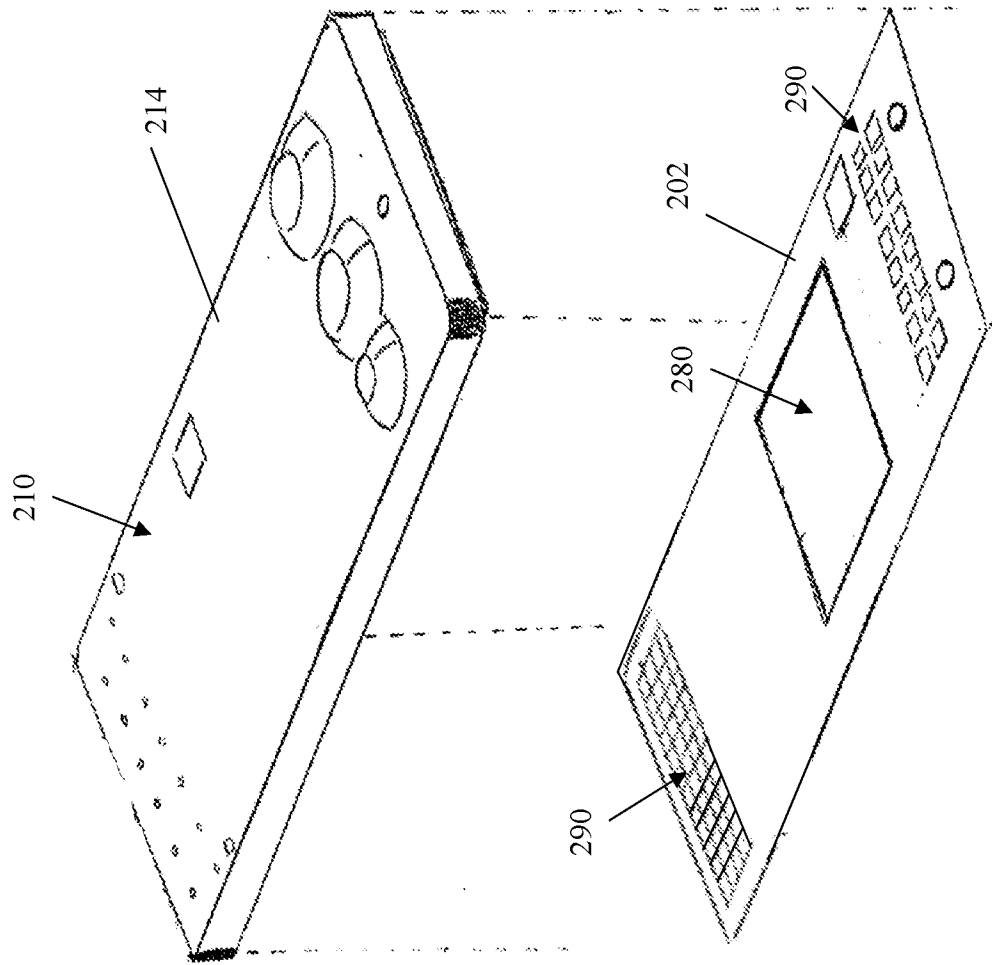


Fig. 2B

200

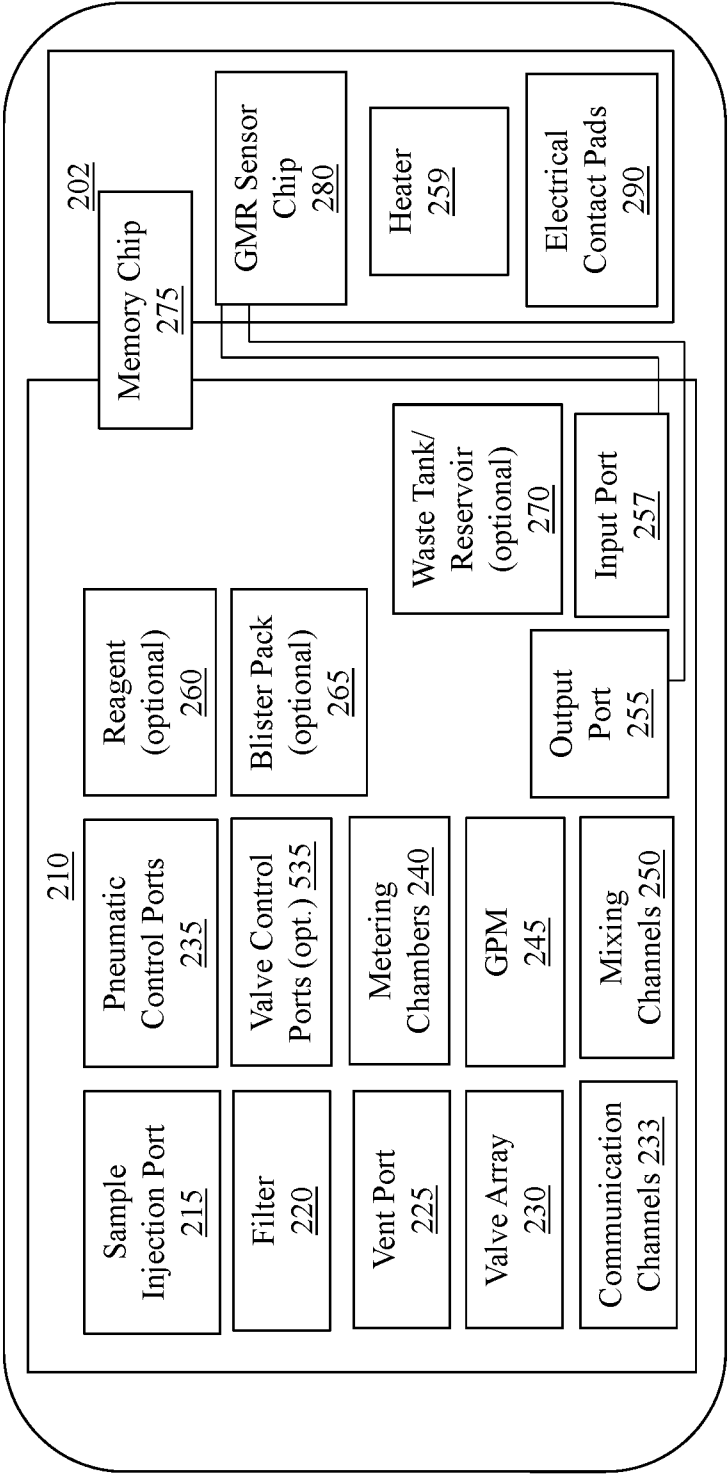


Fig. 2C

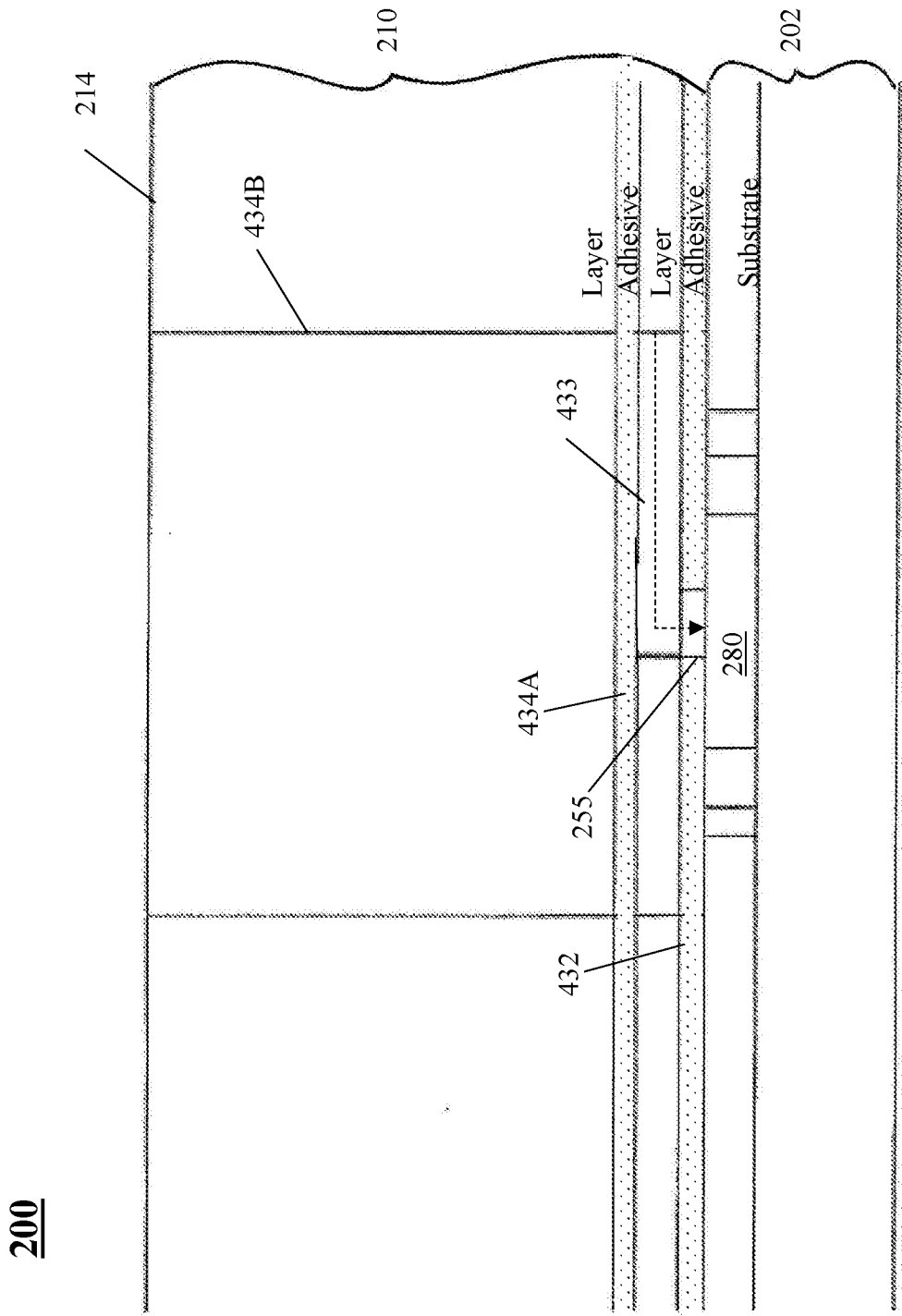


Fig. 2D

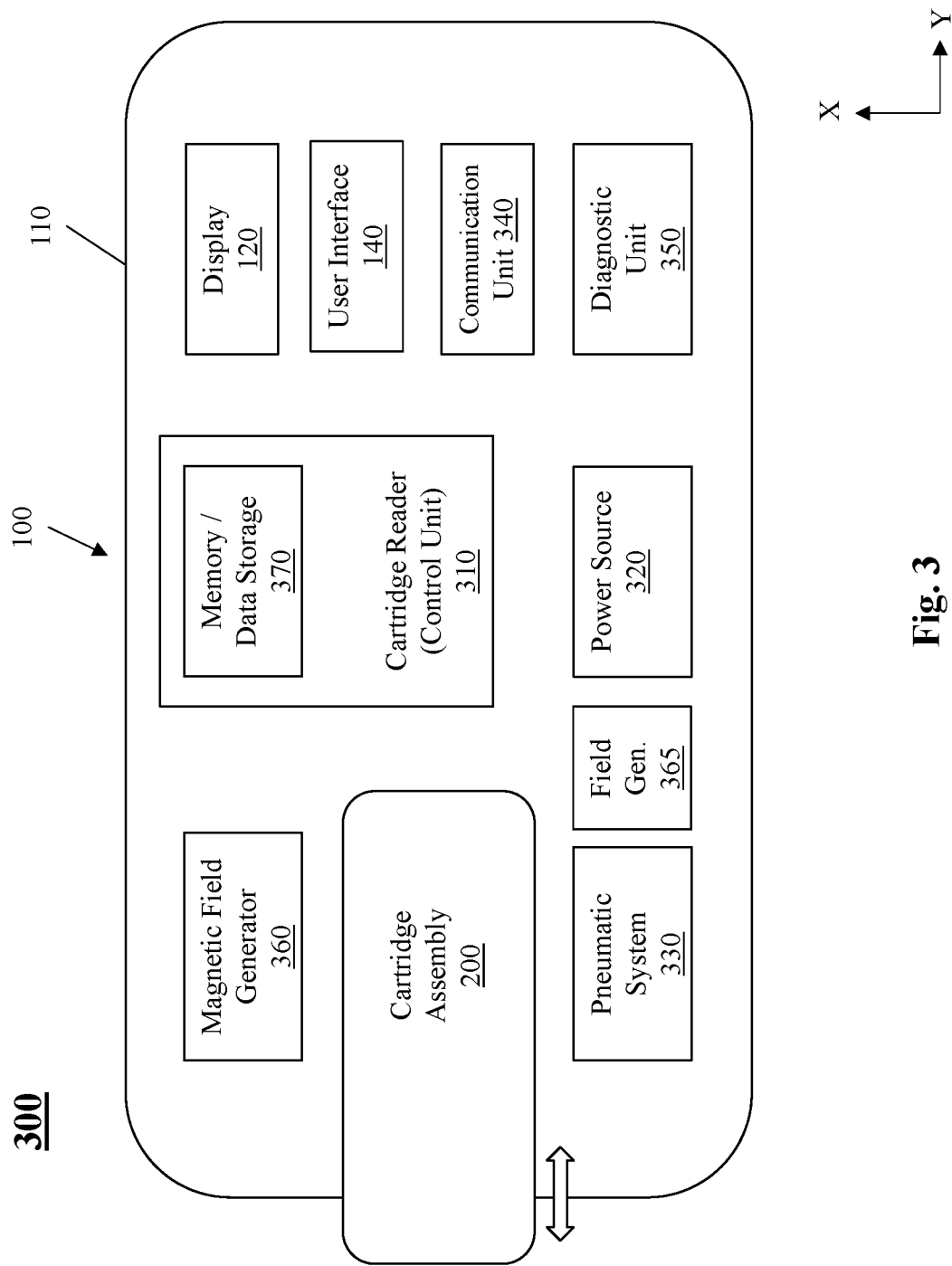


Fig. 3

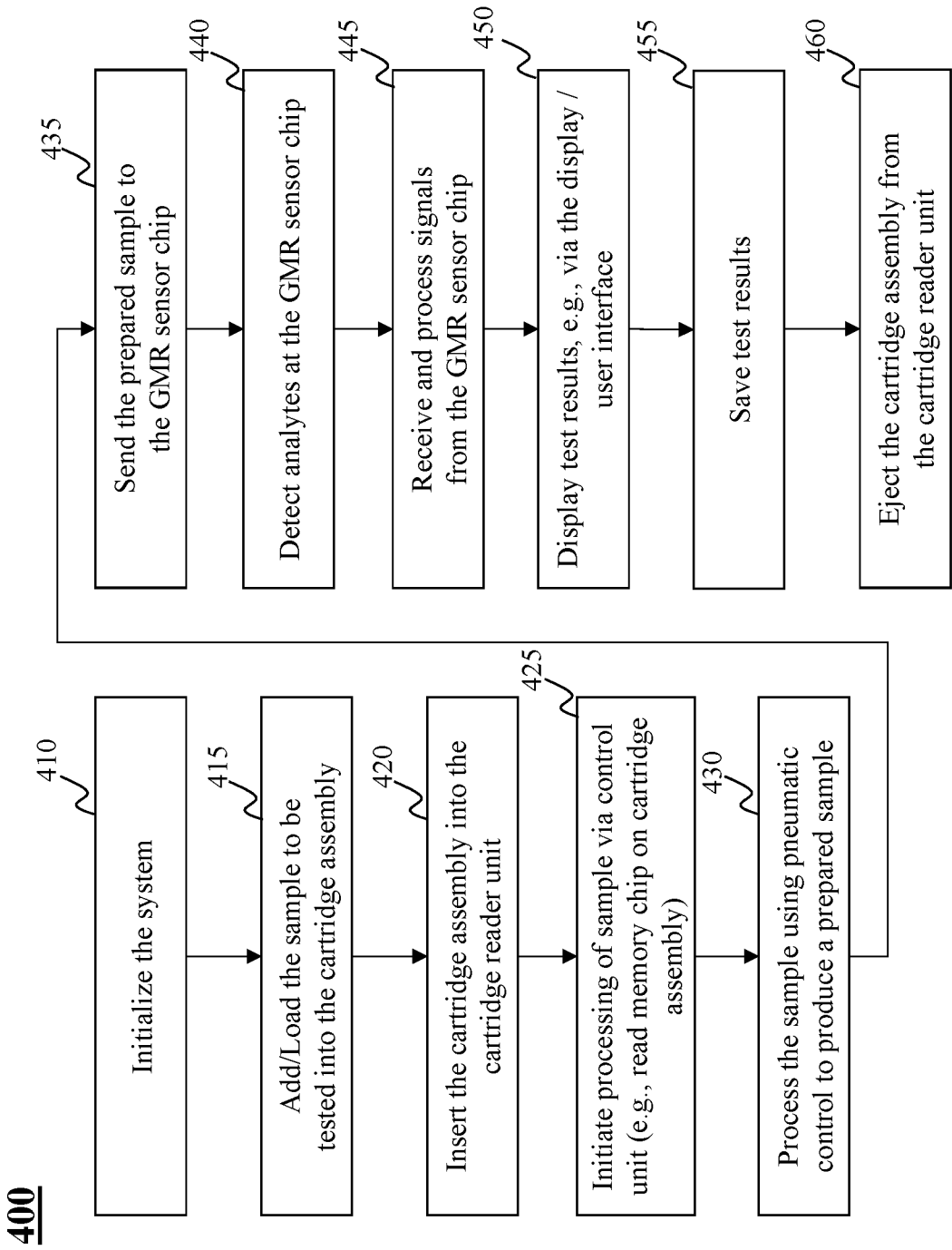


Fig. 4

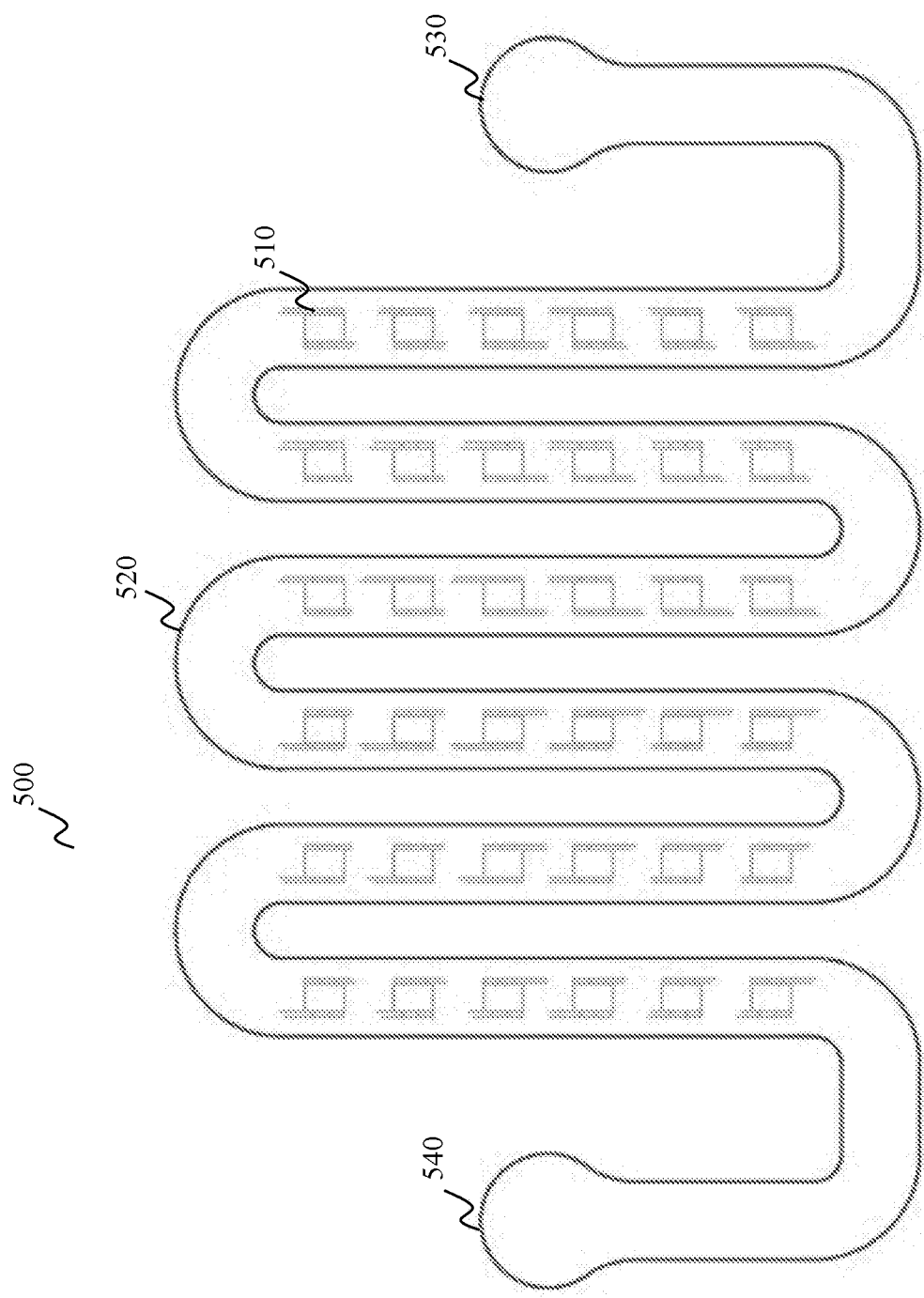


Fig. 5A

(Multiple channels)

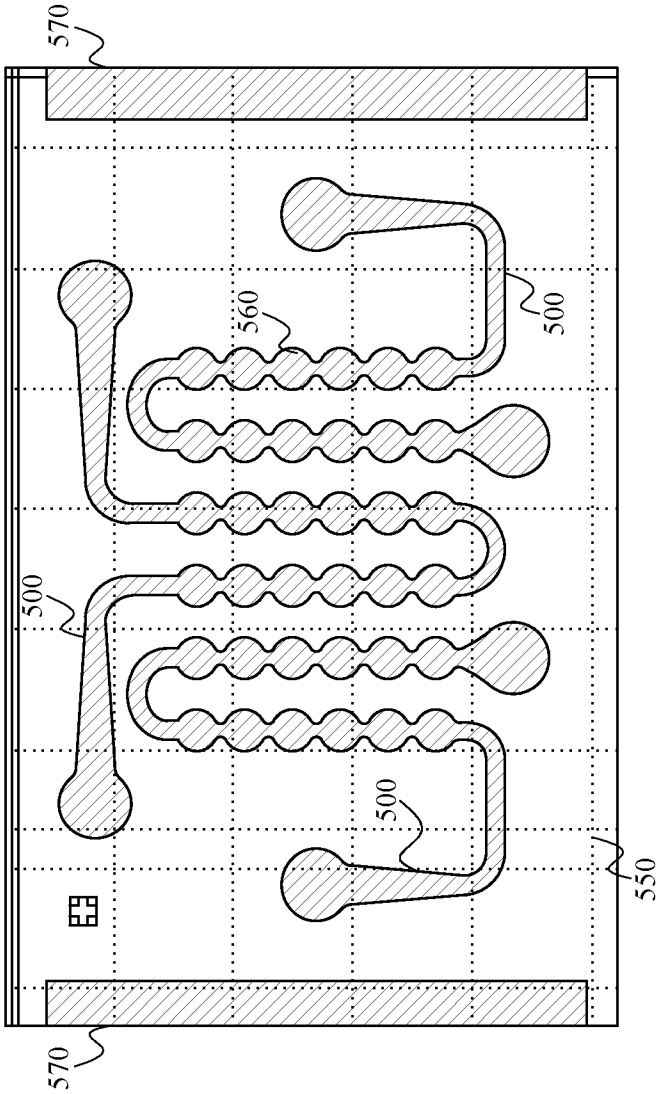


Fig. 5B

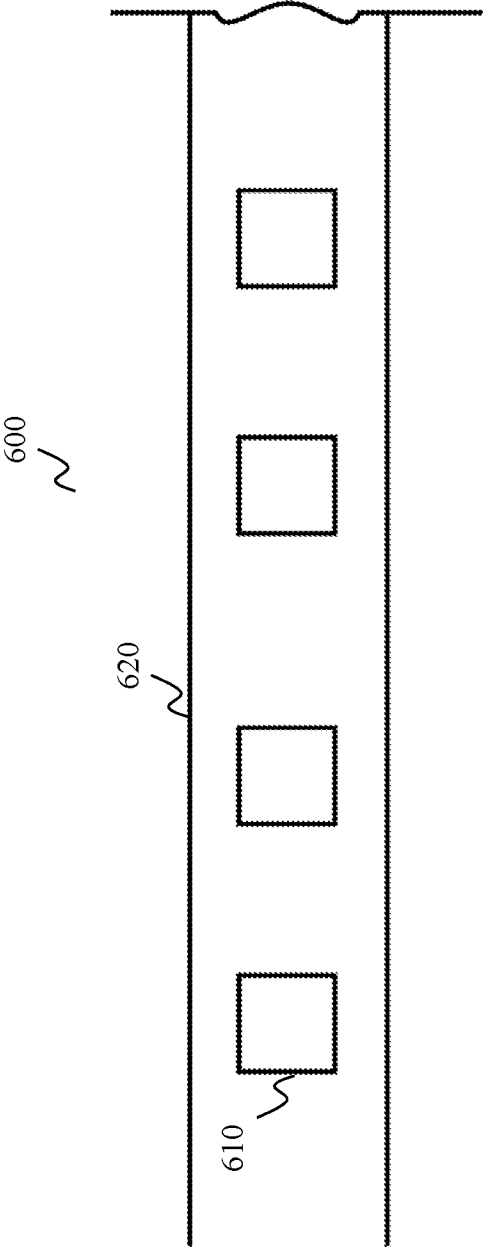


Fig. 6A

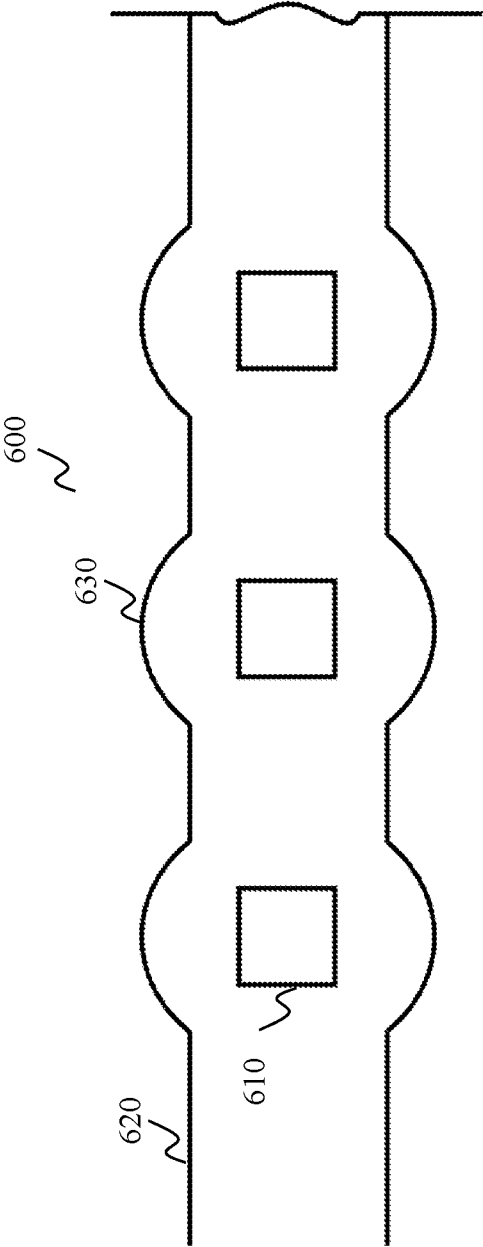


Fig. 6B

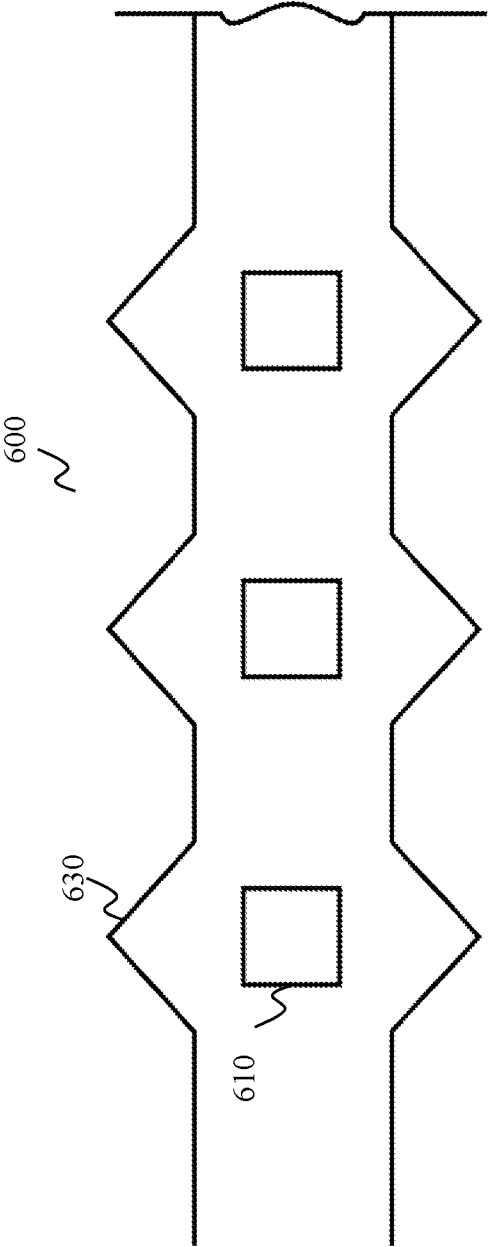


Fig. 6C

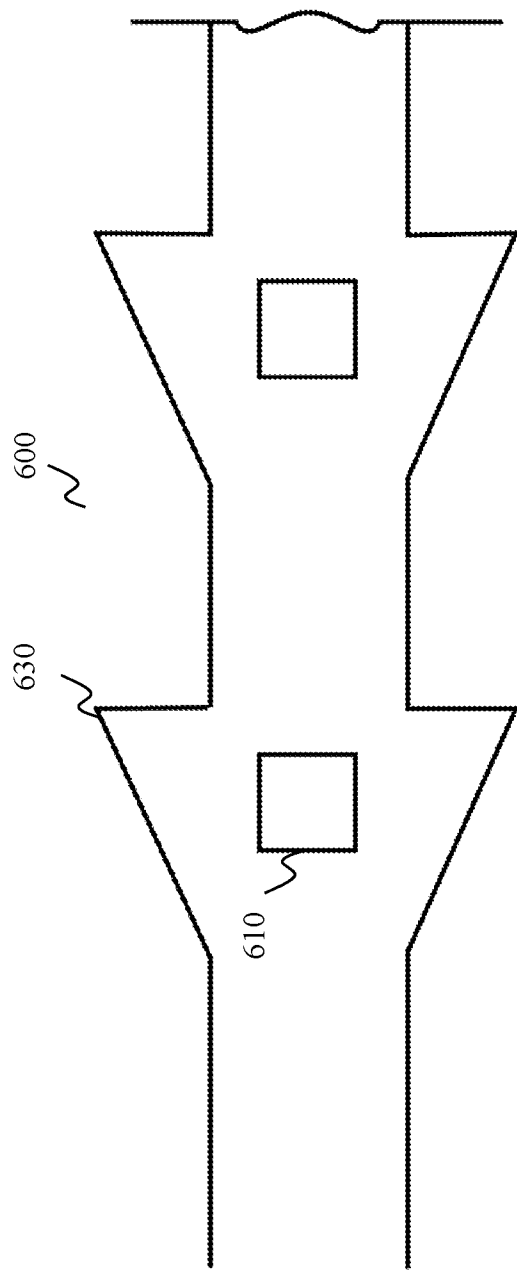


Fig. 6D

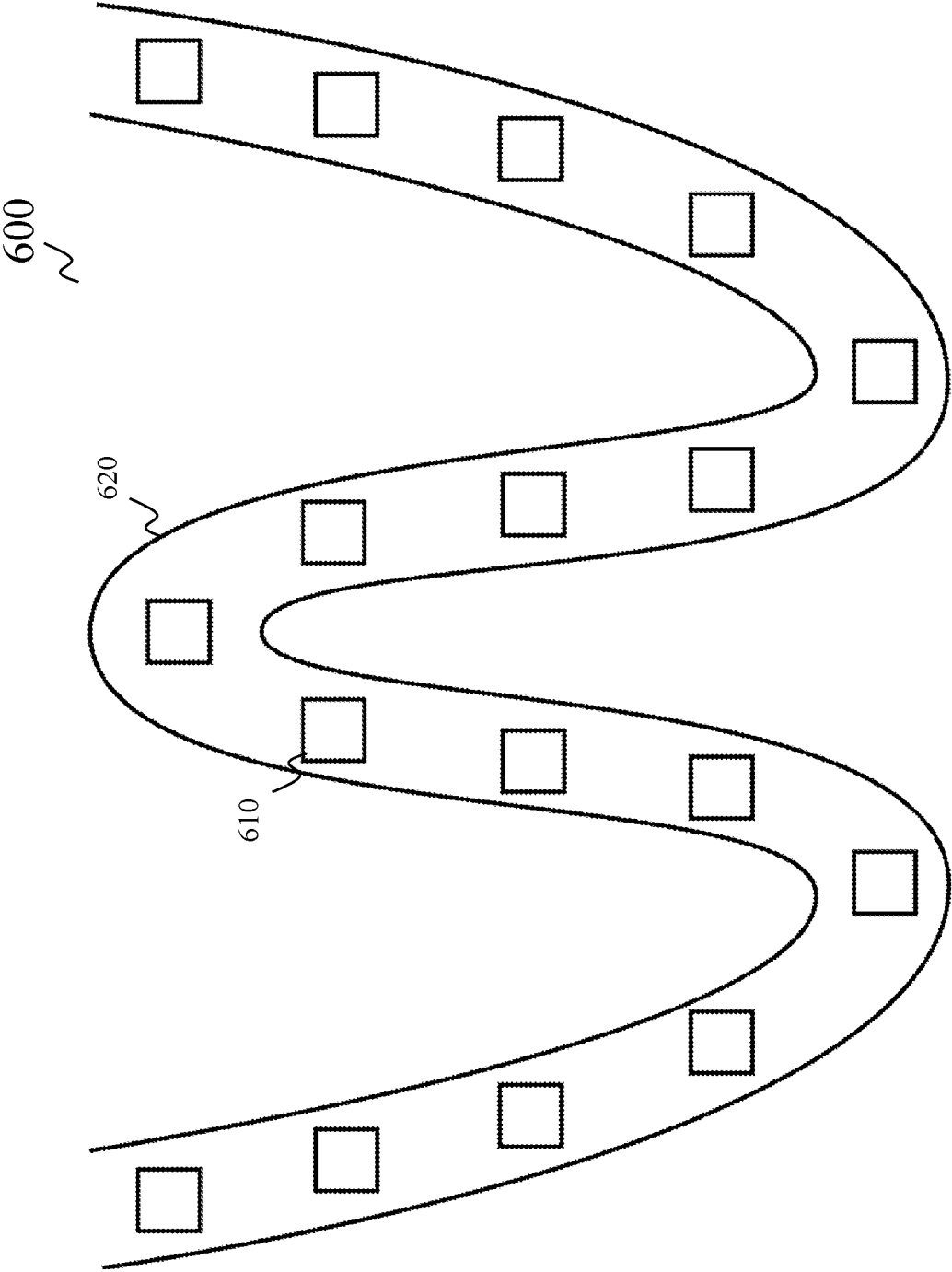


Fig. 6E

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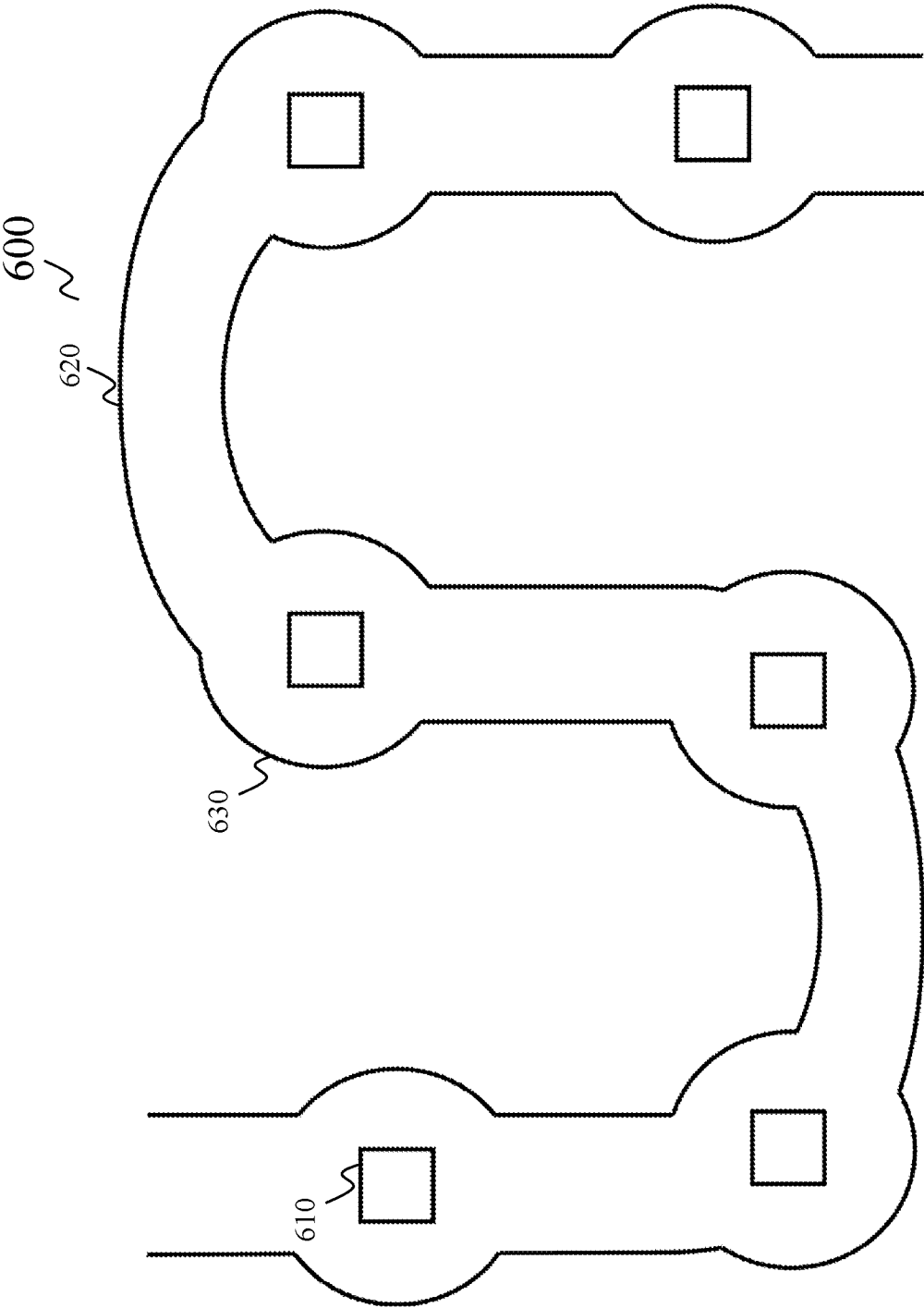


Fig. 6F

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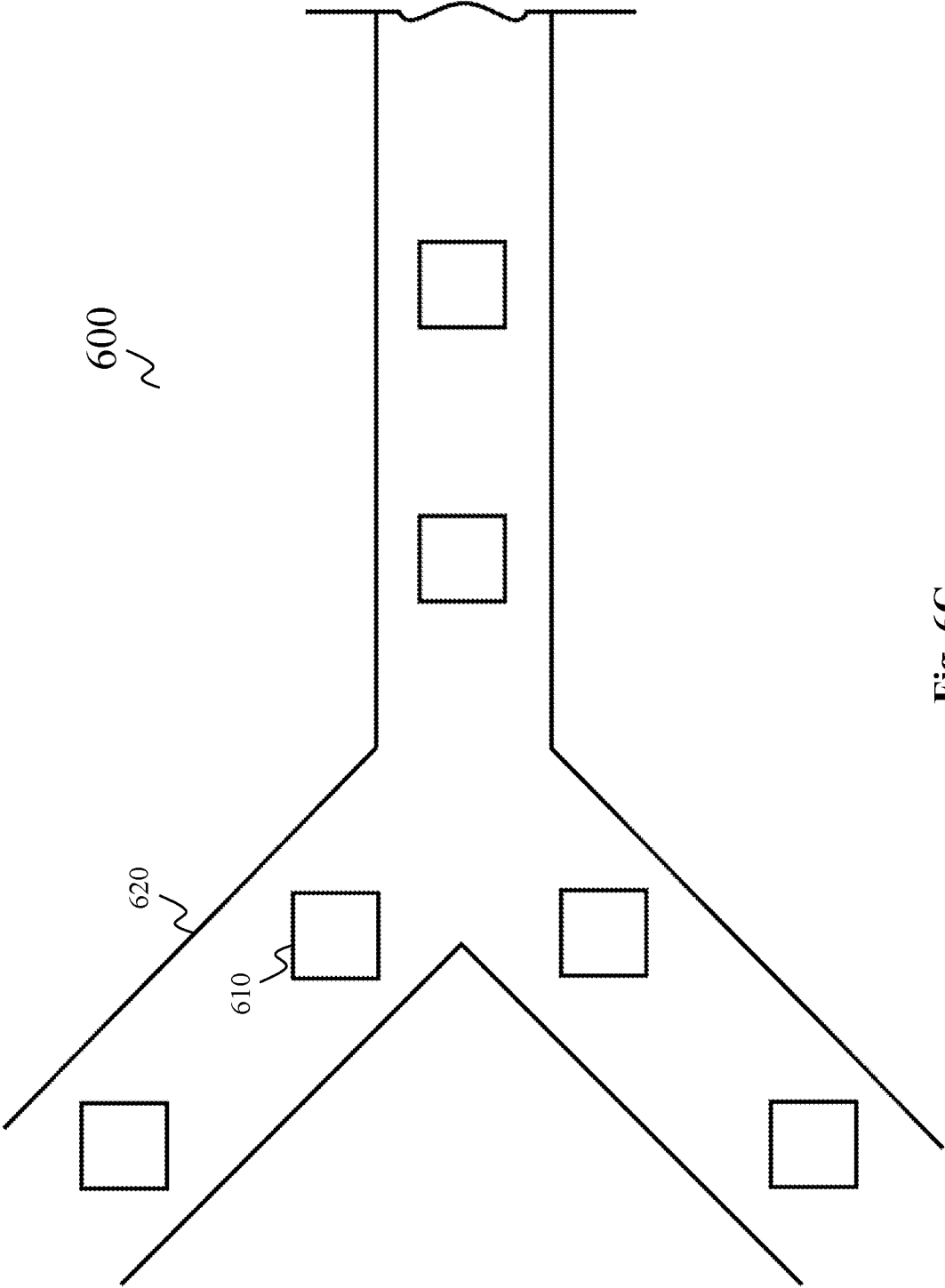


Fig. 6G

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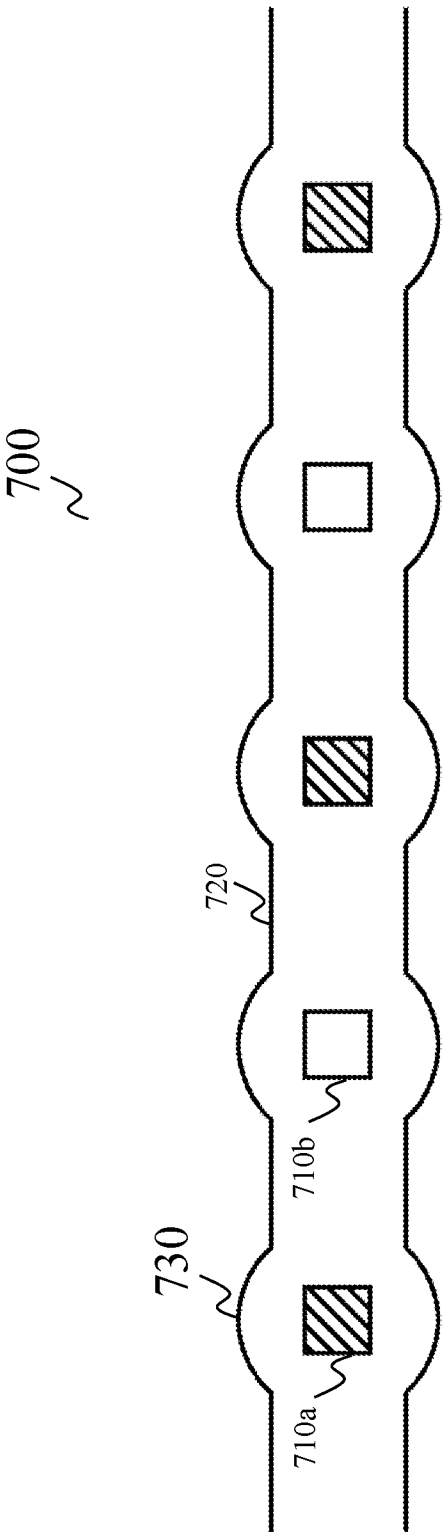


Fig. 7

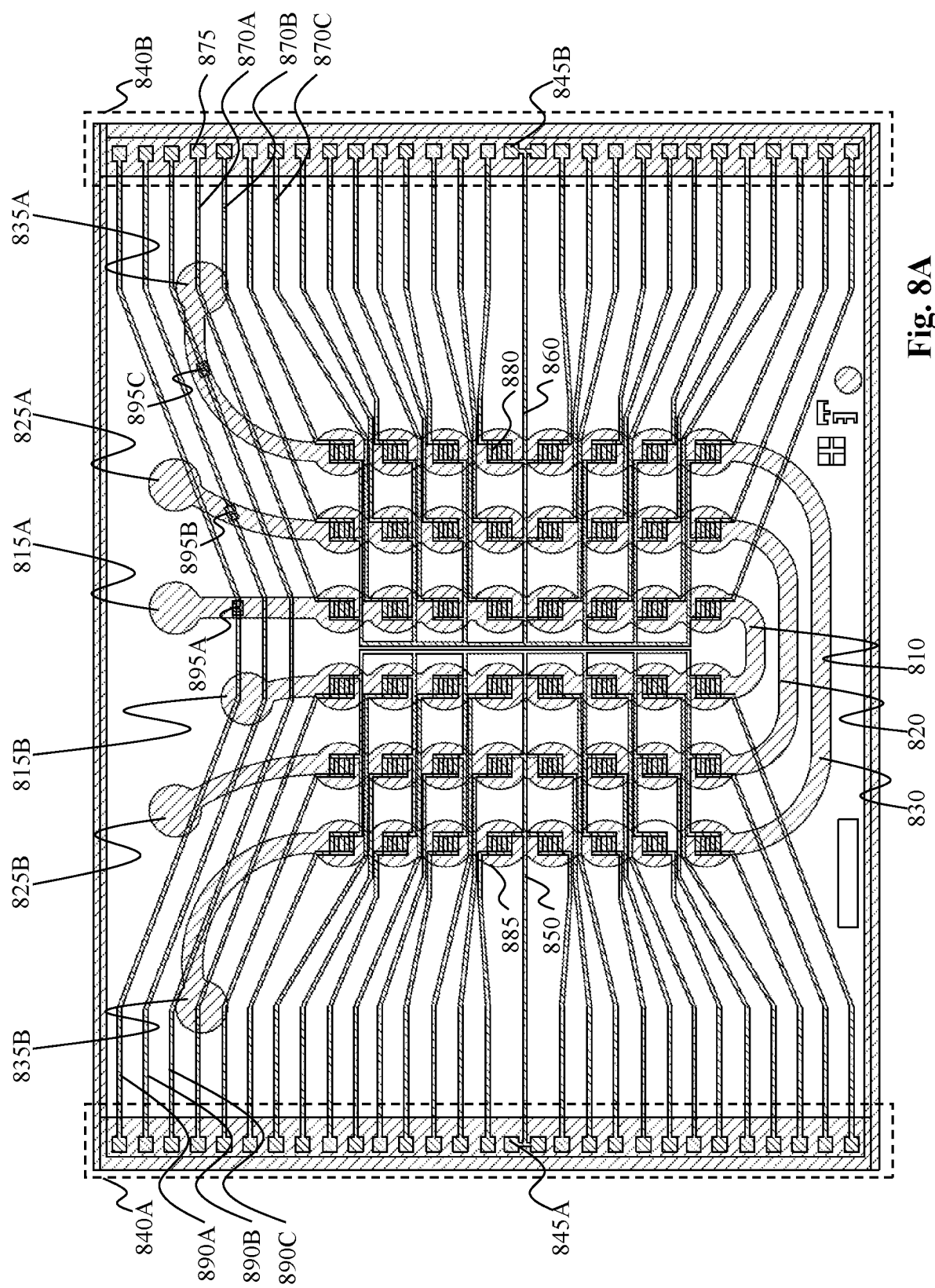


Fig. 8A

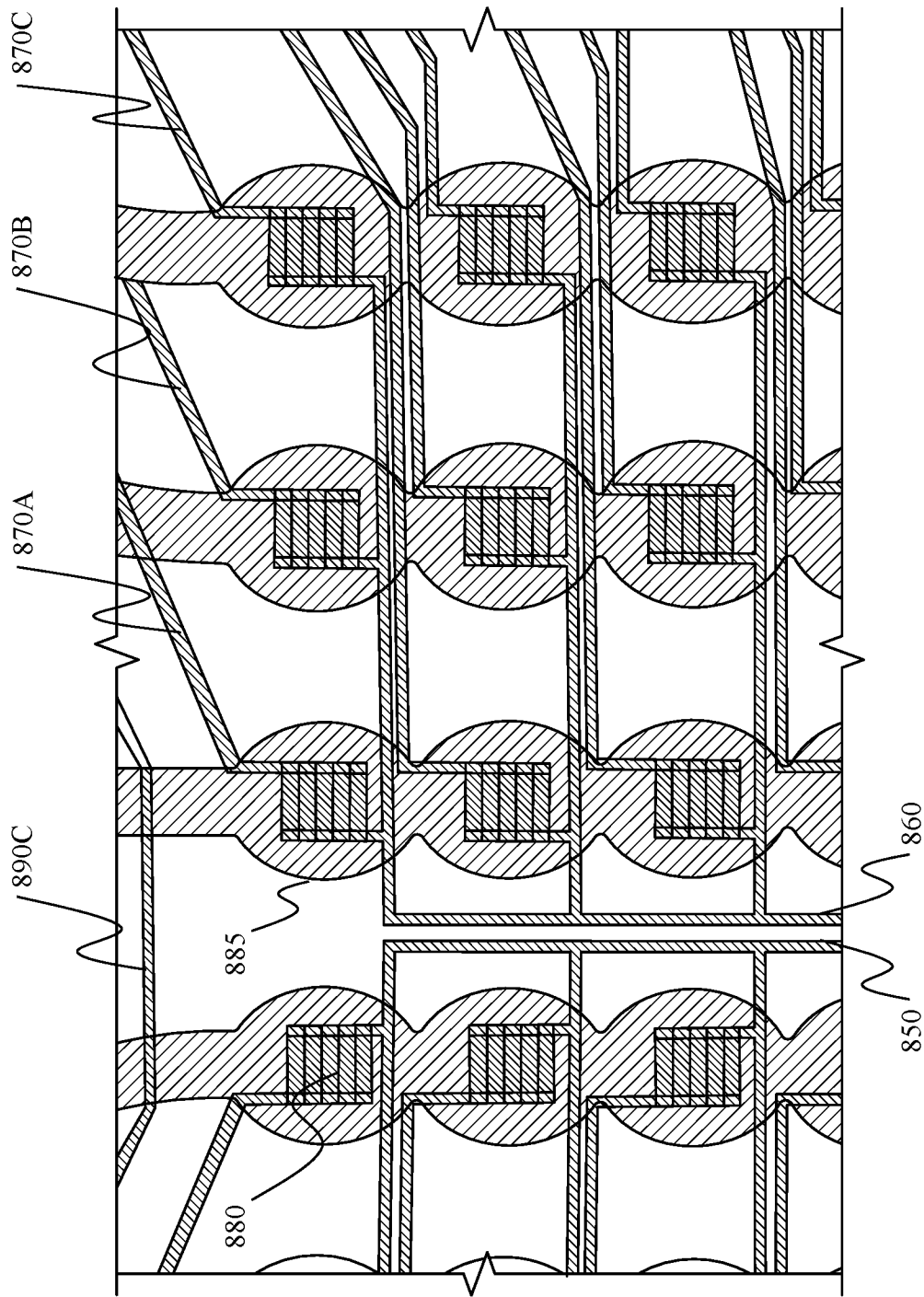


Fig. 8B

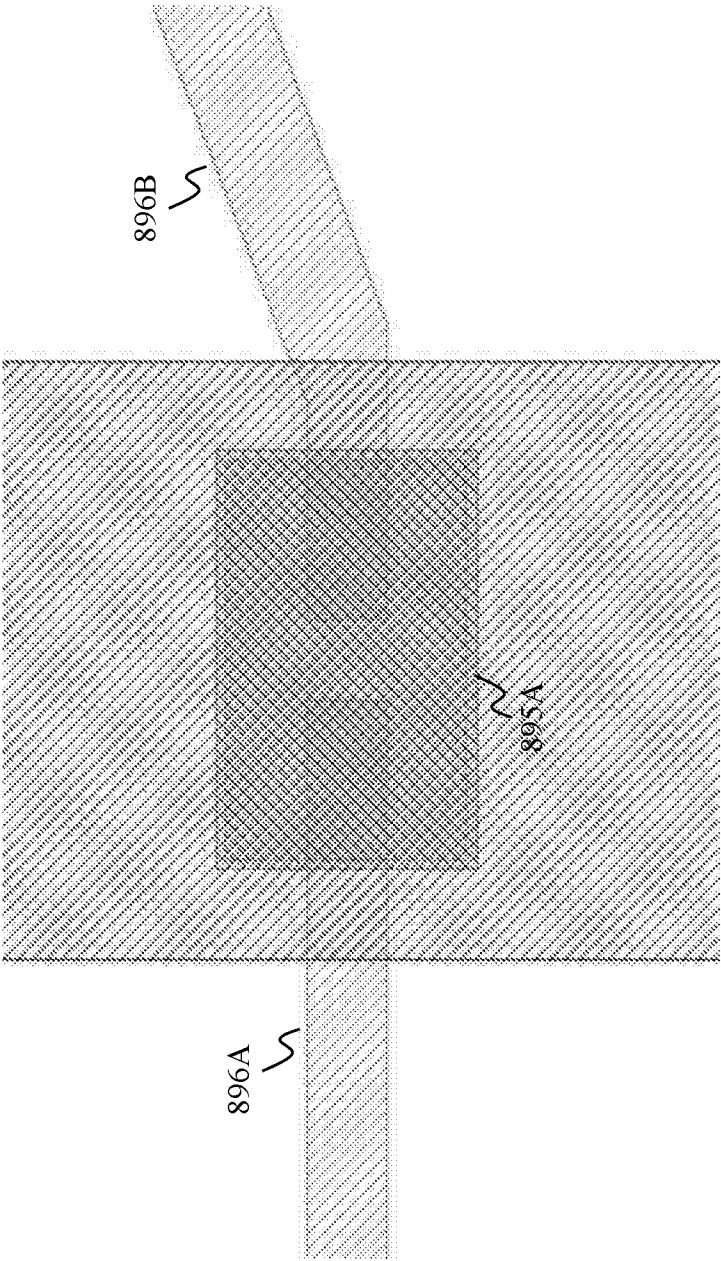


Fig. 8C

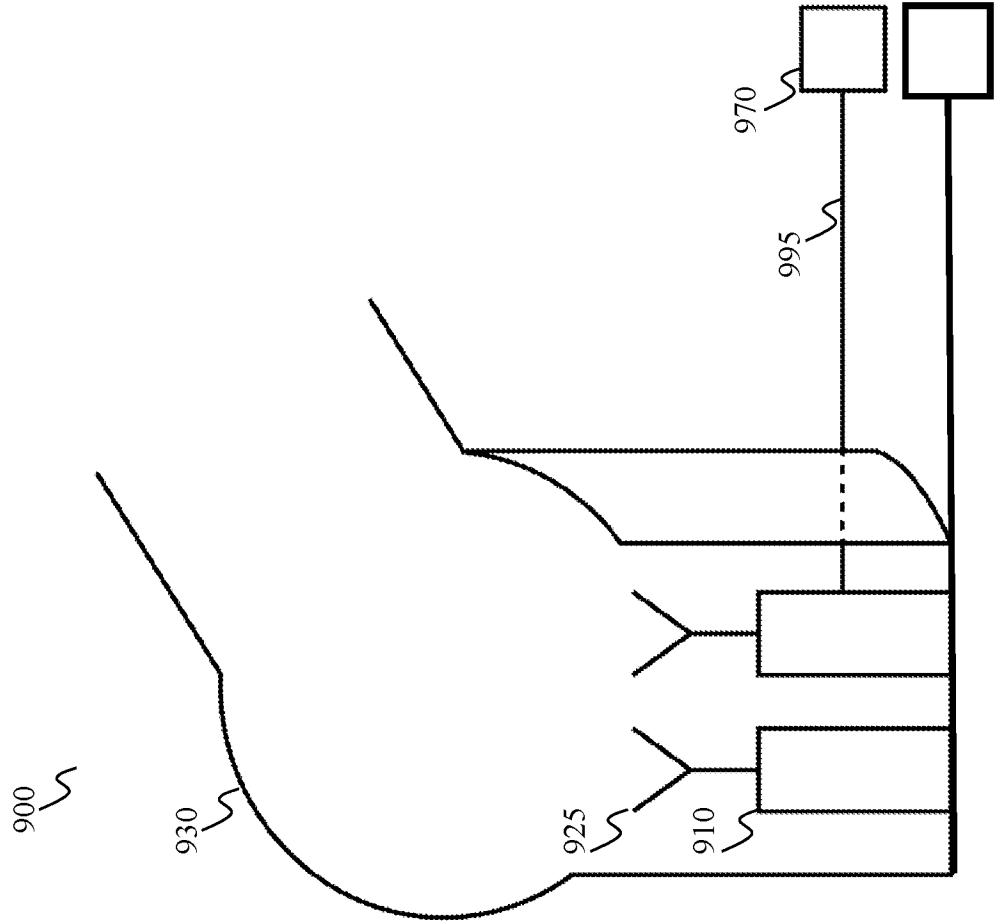


Fig. 9

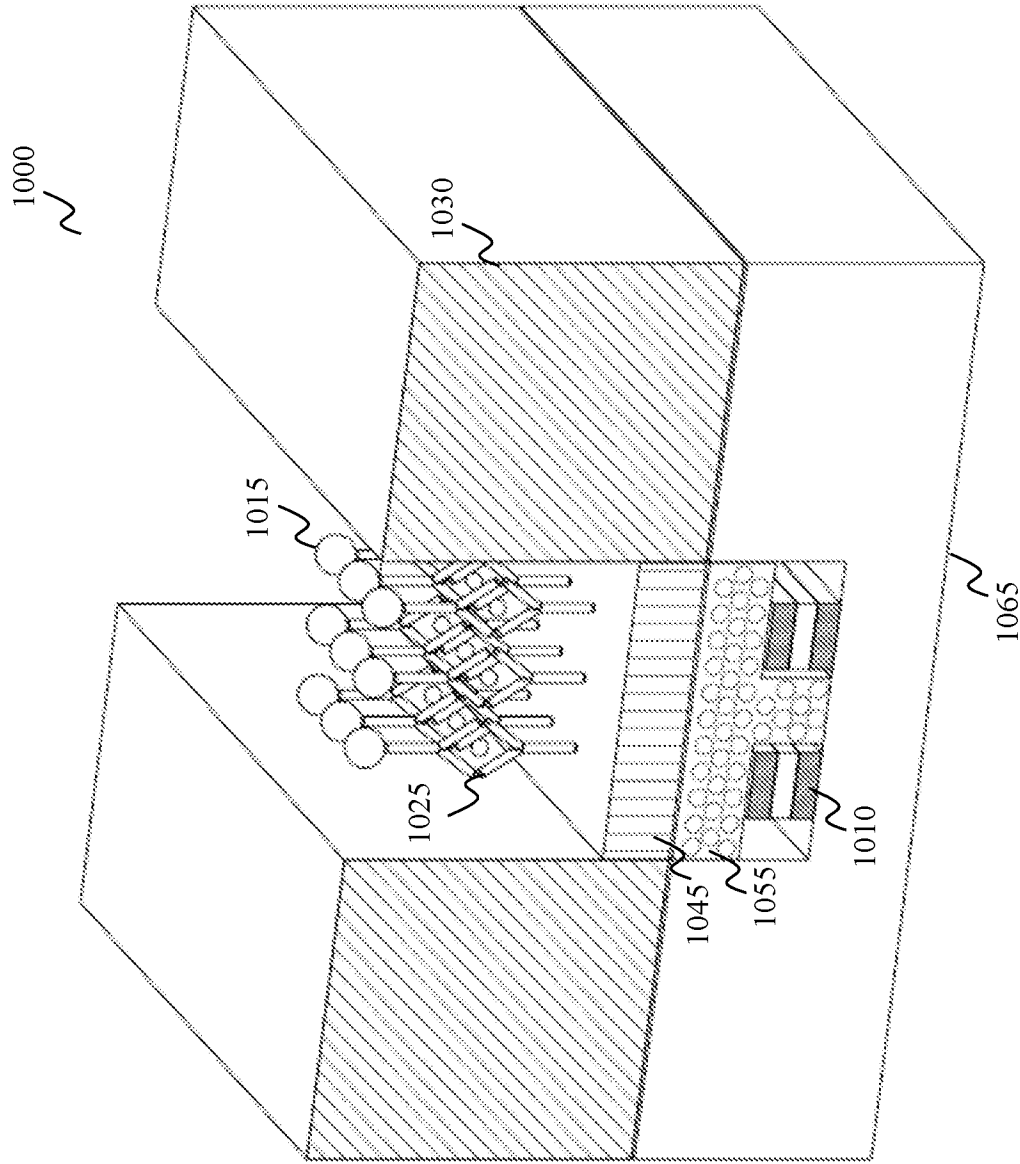


Fig. 10A

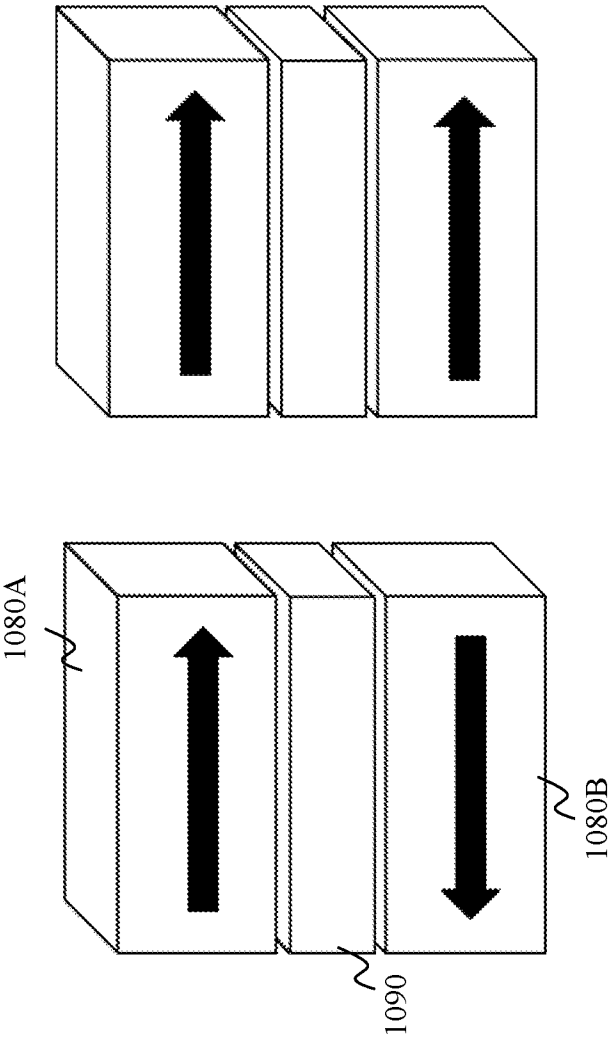


Fig. 10B

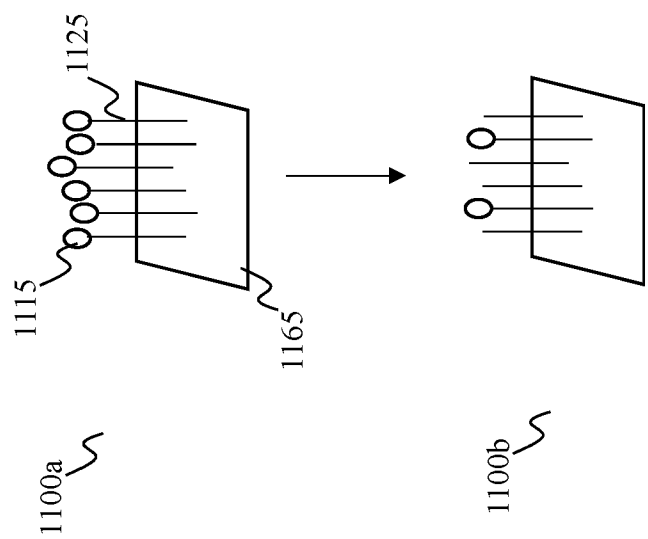


Fig. 11A

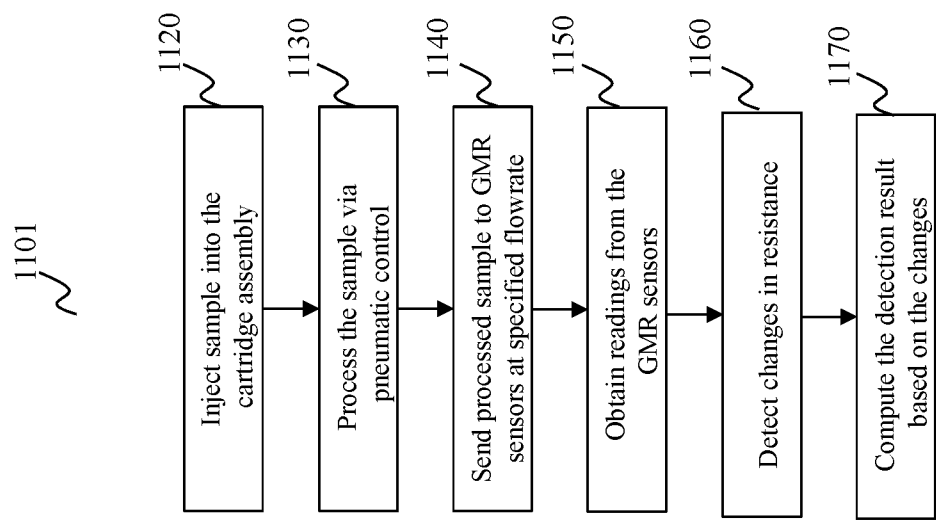


Fig. 11B

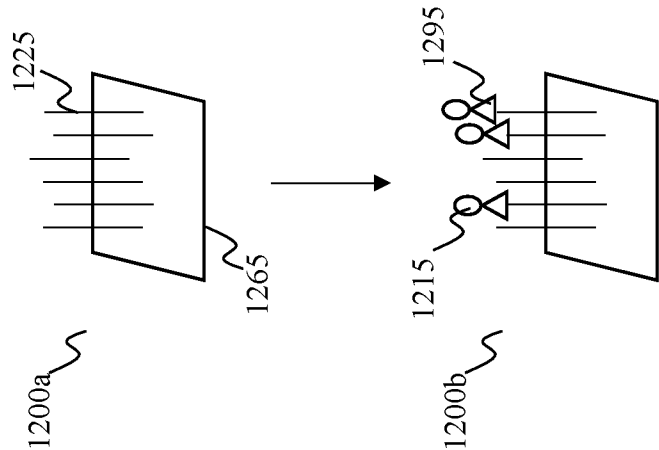


Fig. 12A

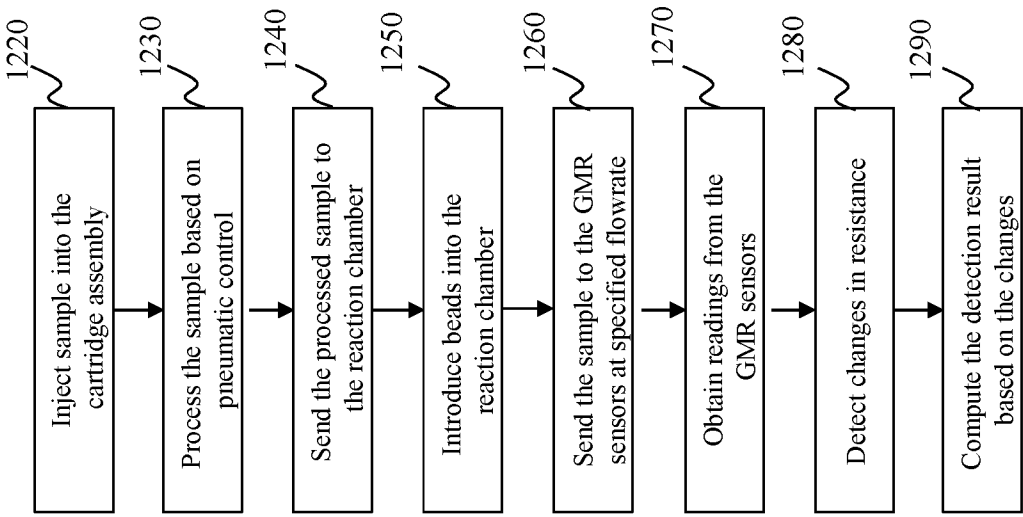


Fig. 12B

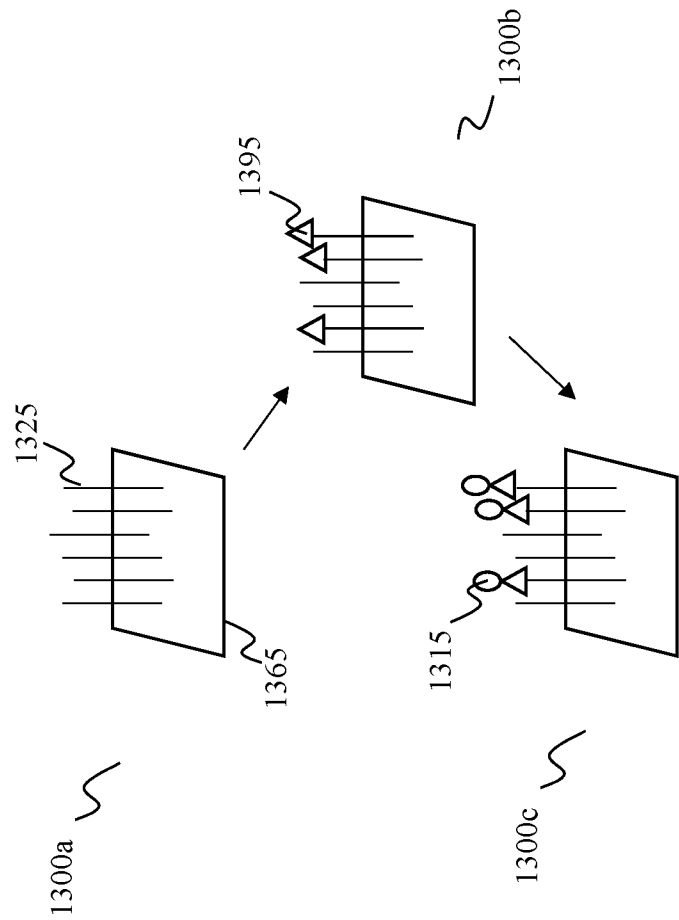


Fig. 13A

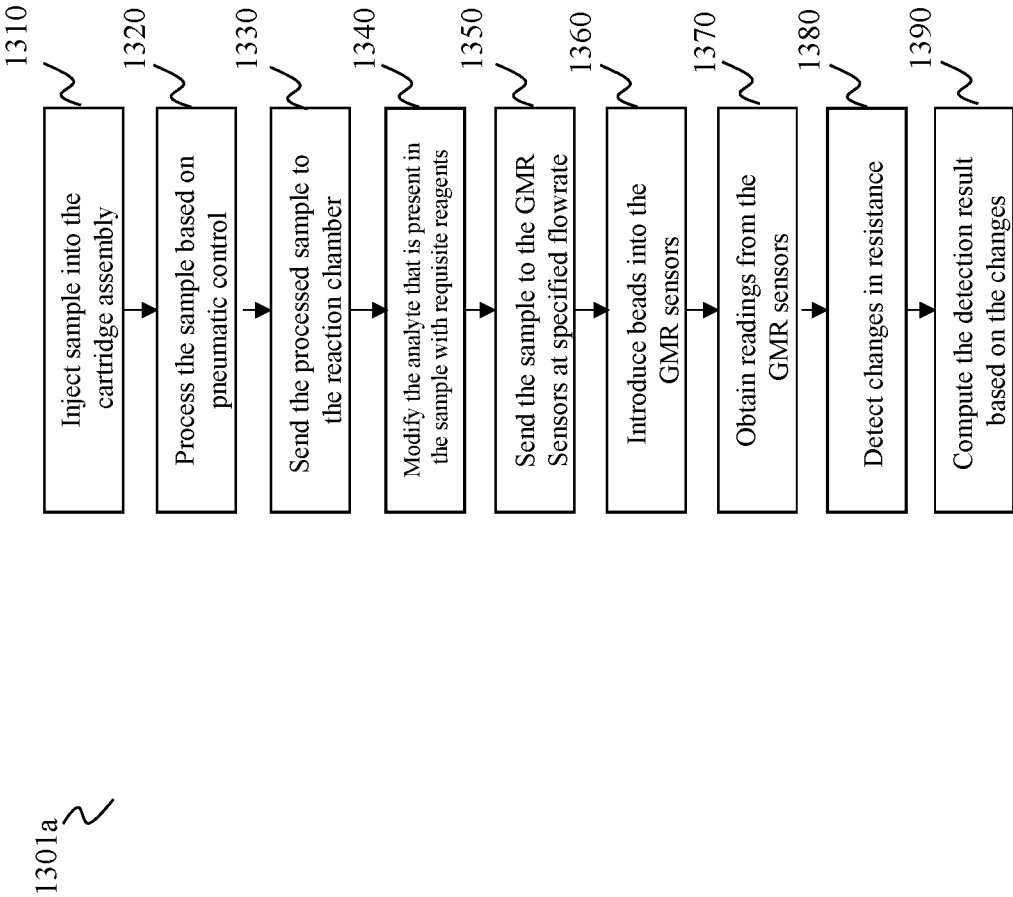


Fig. 13B

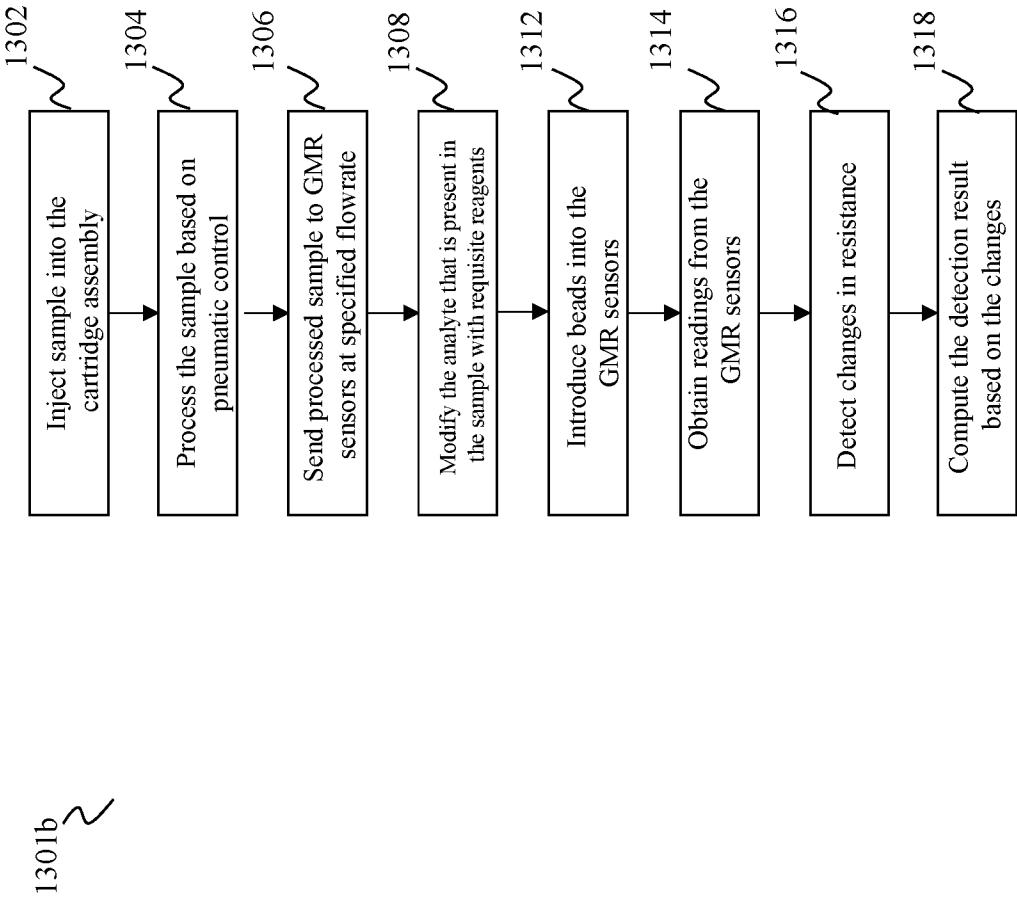


Fig. 13C

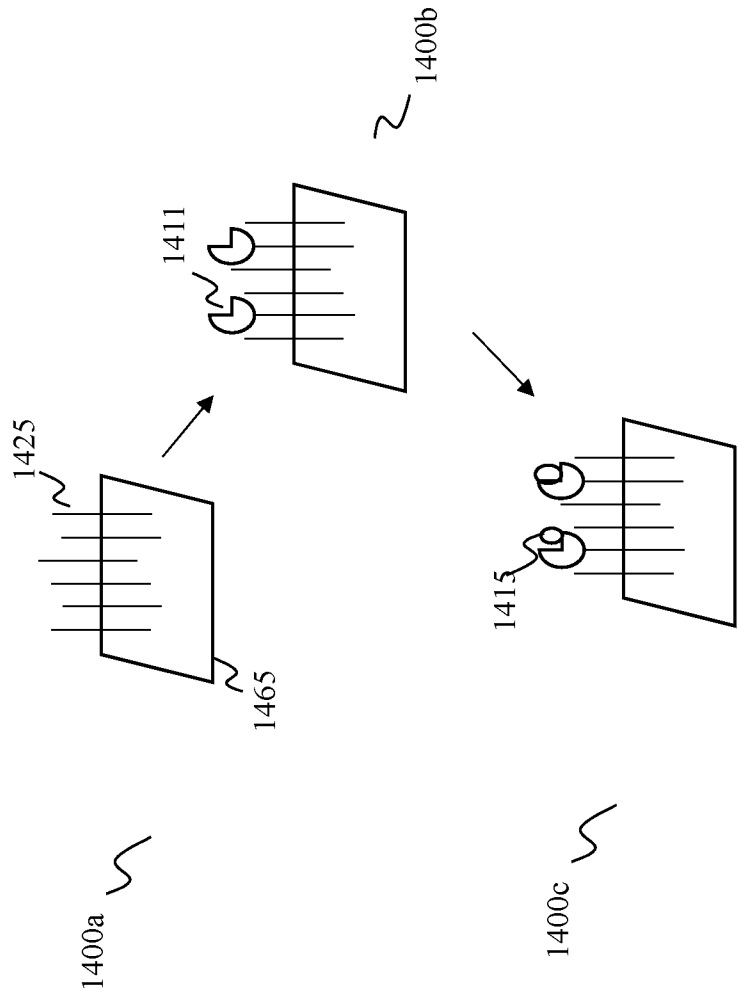


Fig. 14A

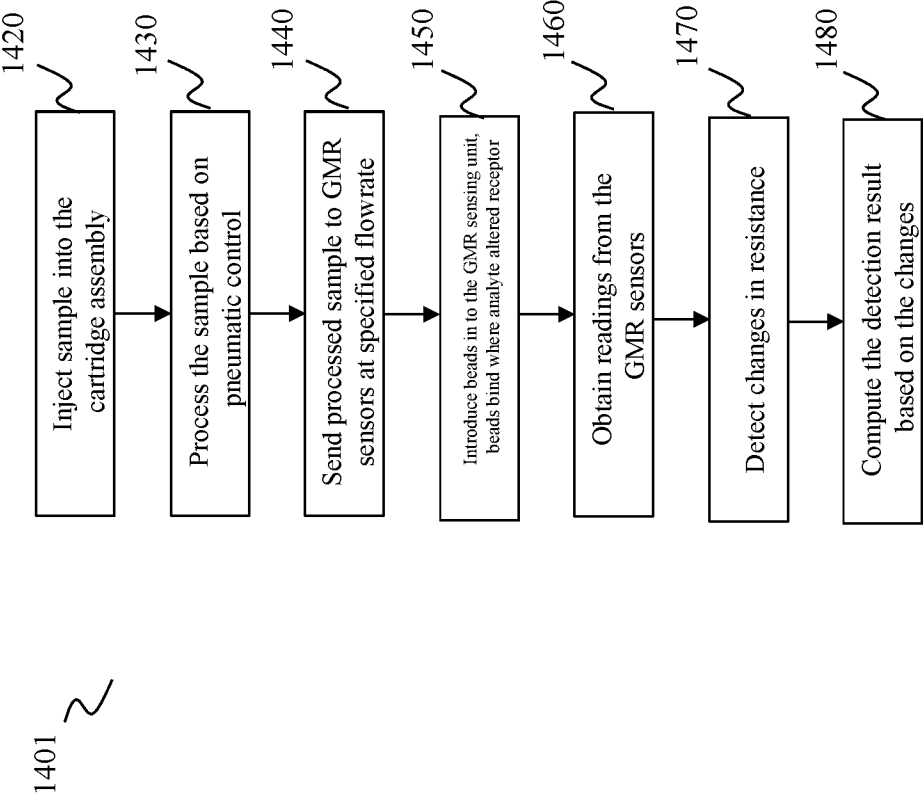


Fig. 14B

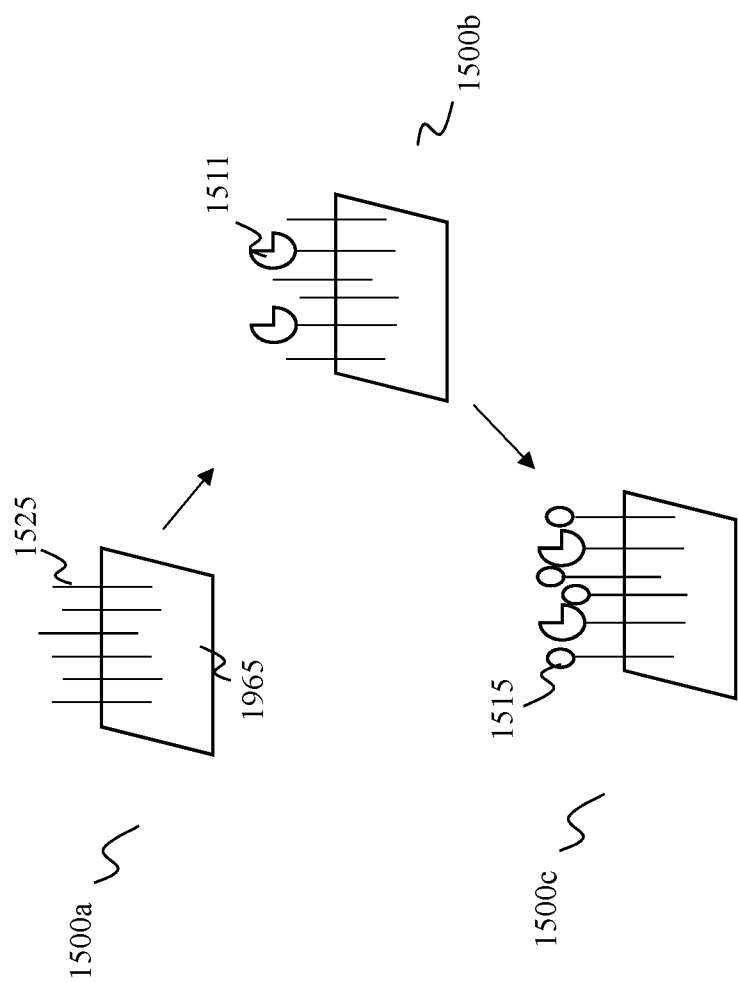


Fig. 15A

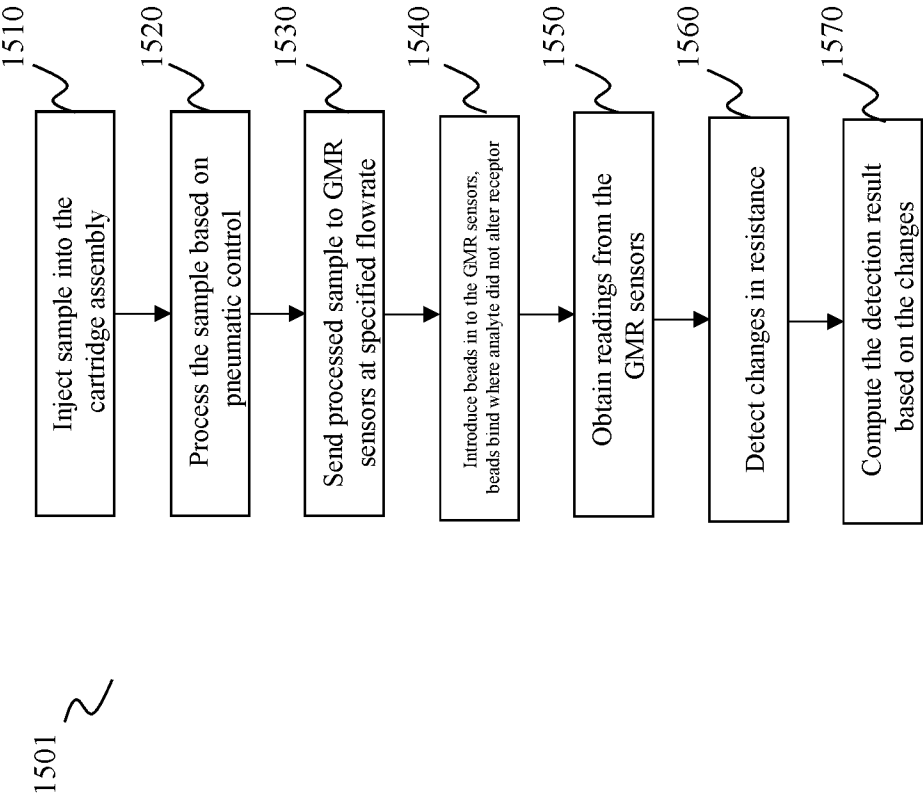


Fig. 15B

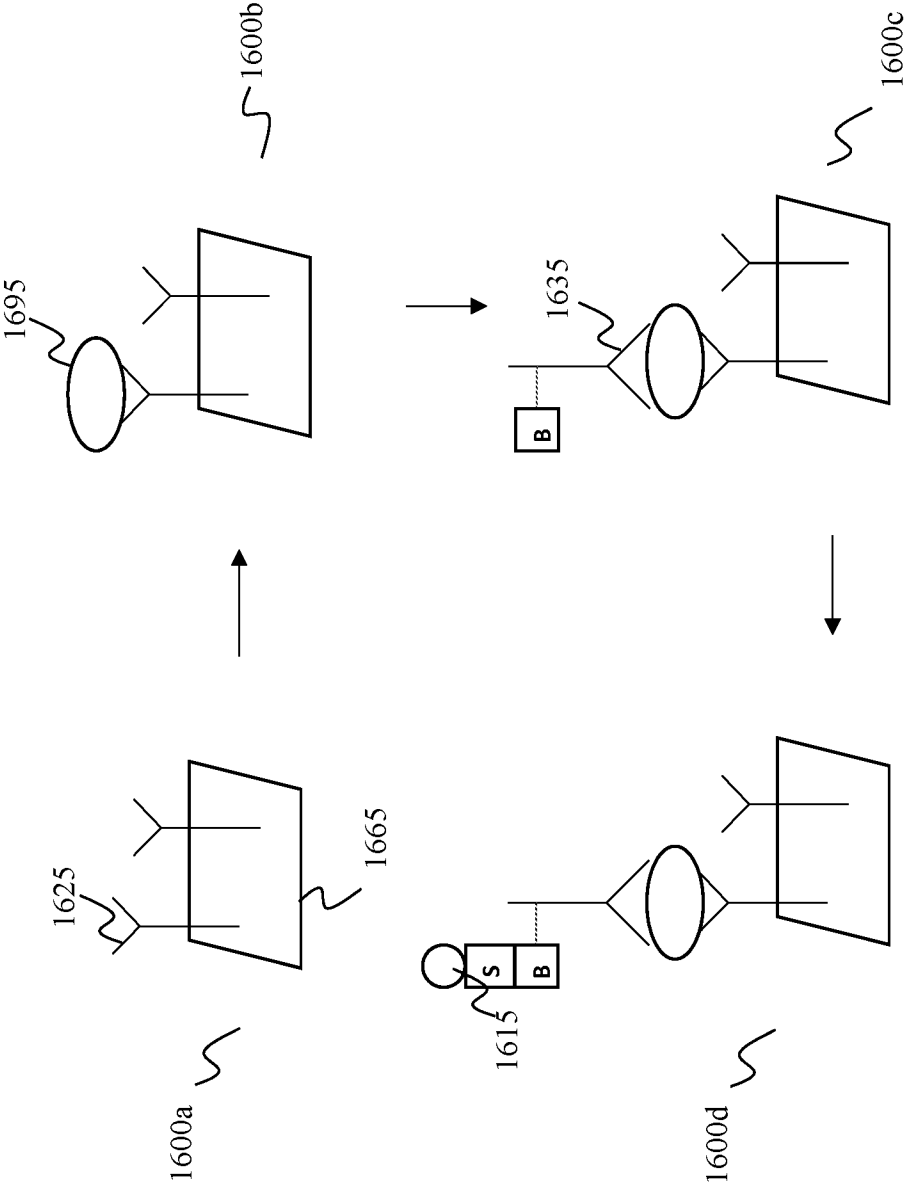


Fig. 16A

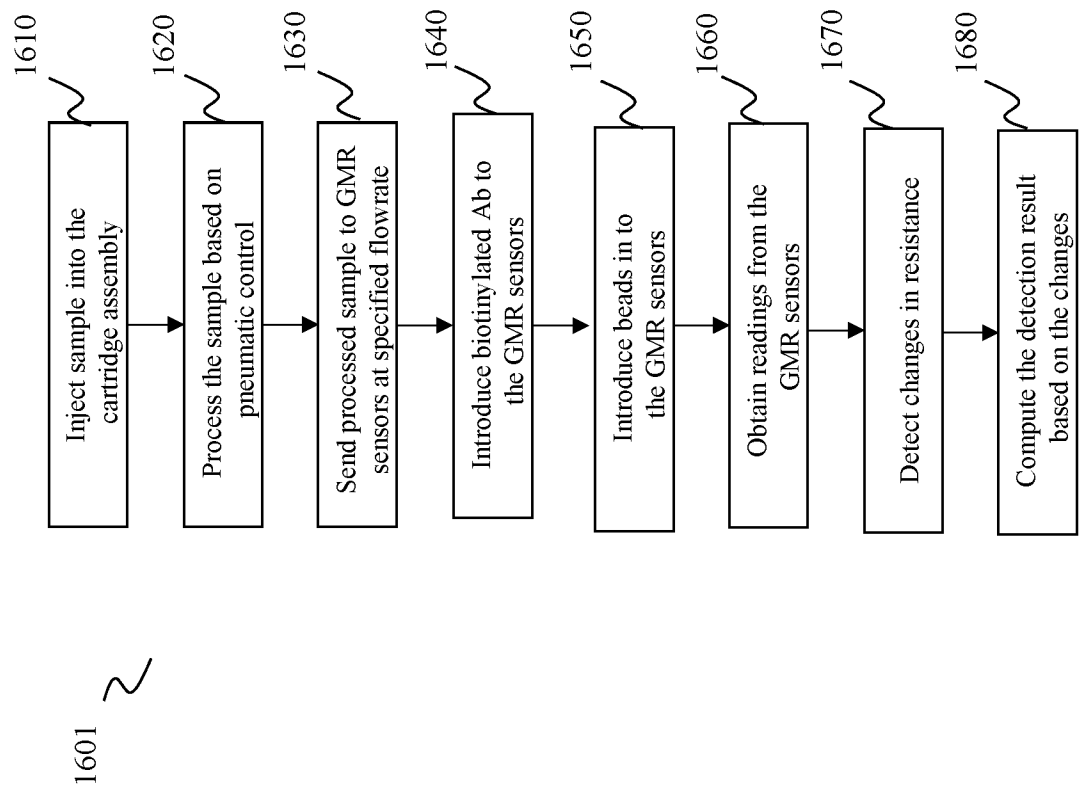


Fig. 16B

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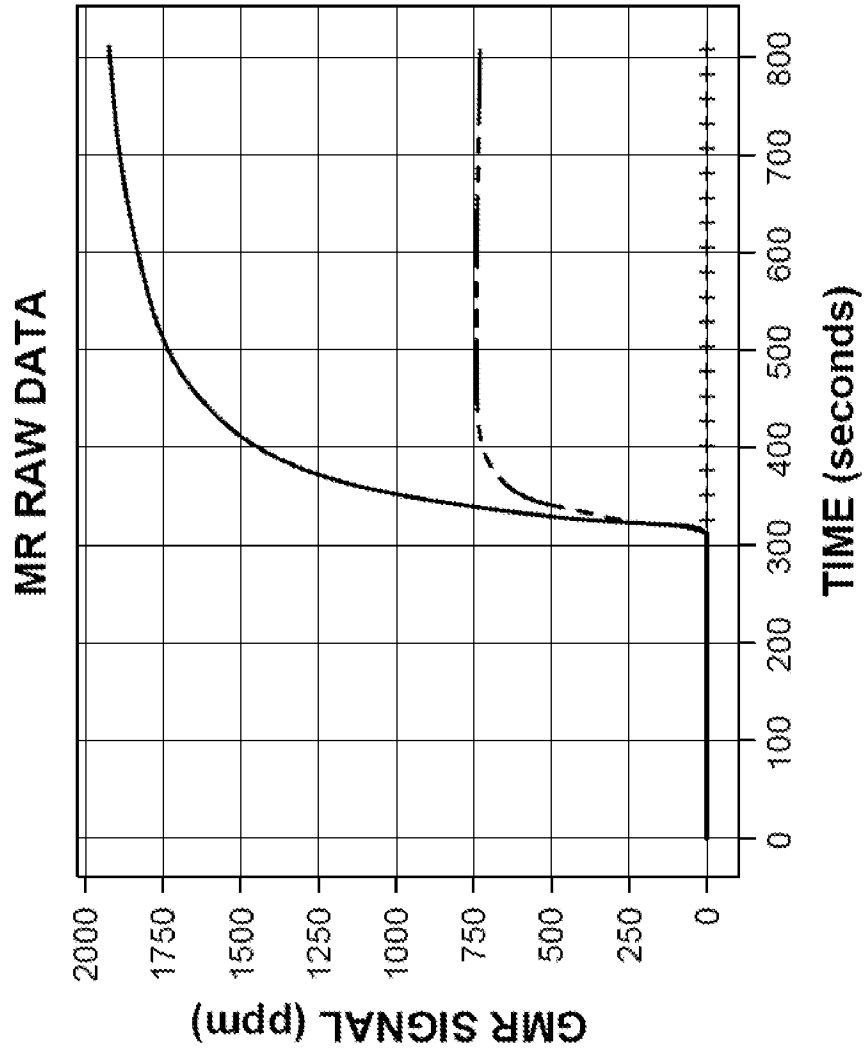


Fig. 17A

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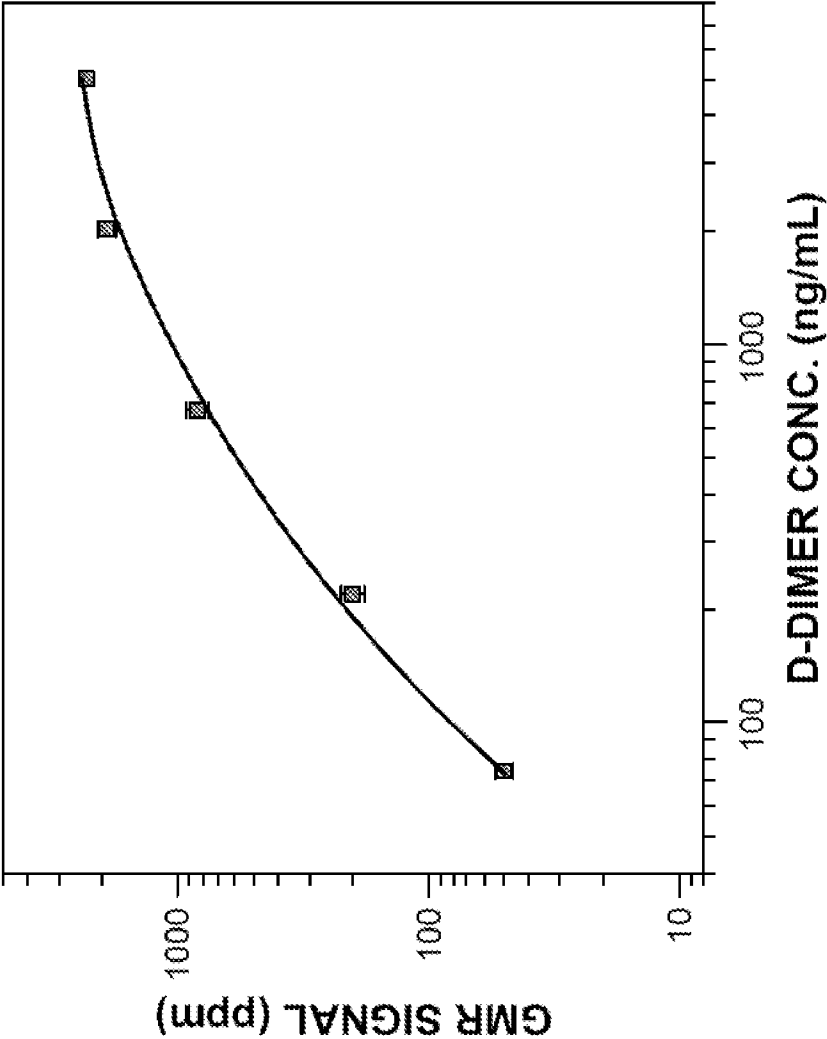


Fig. 17B

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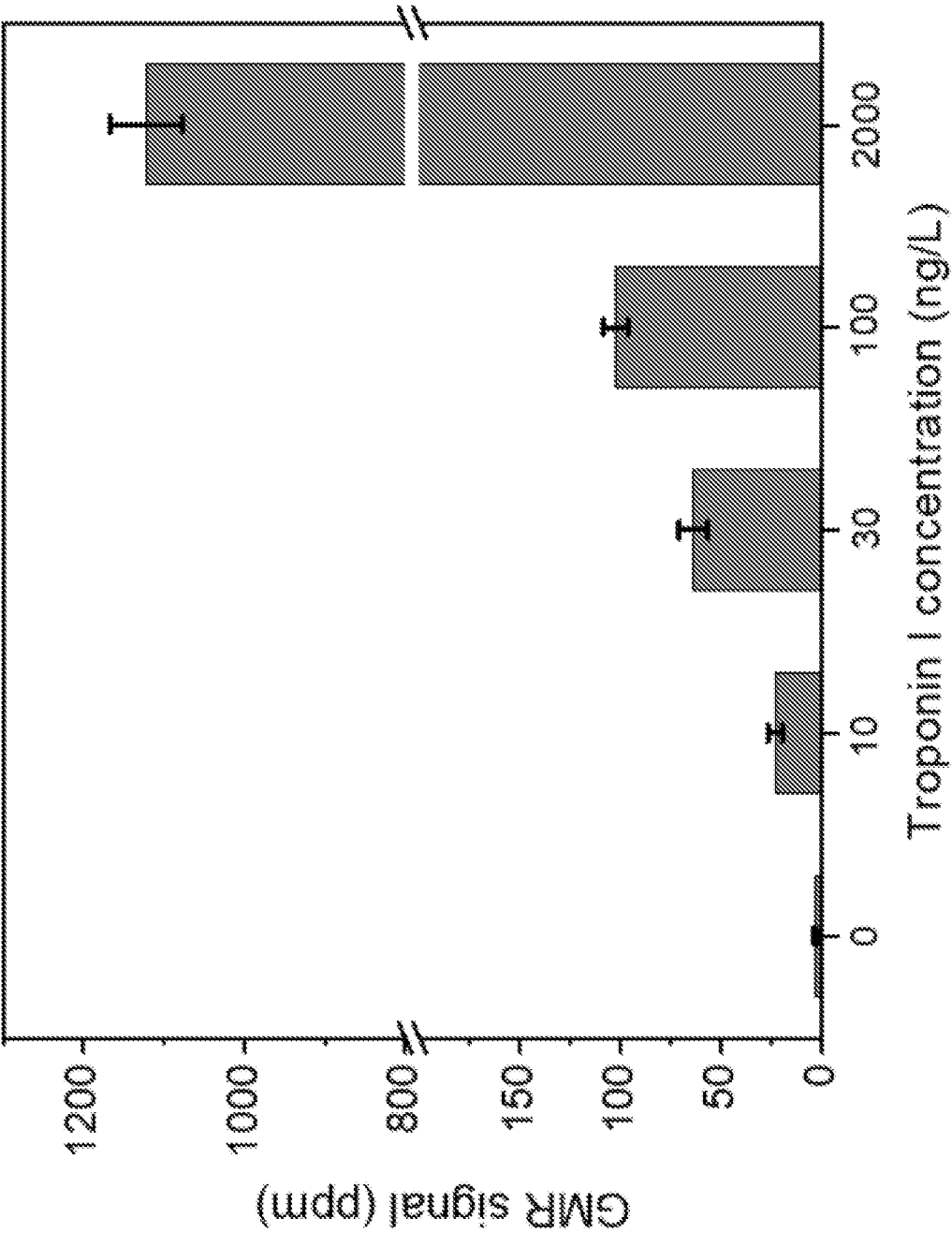


Fig. 17C

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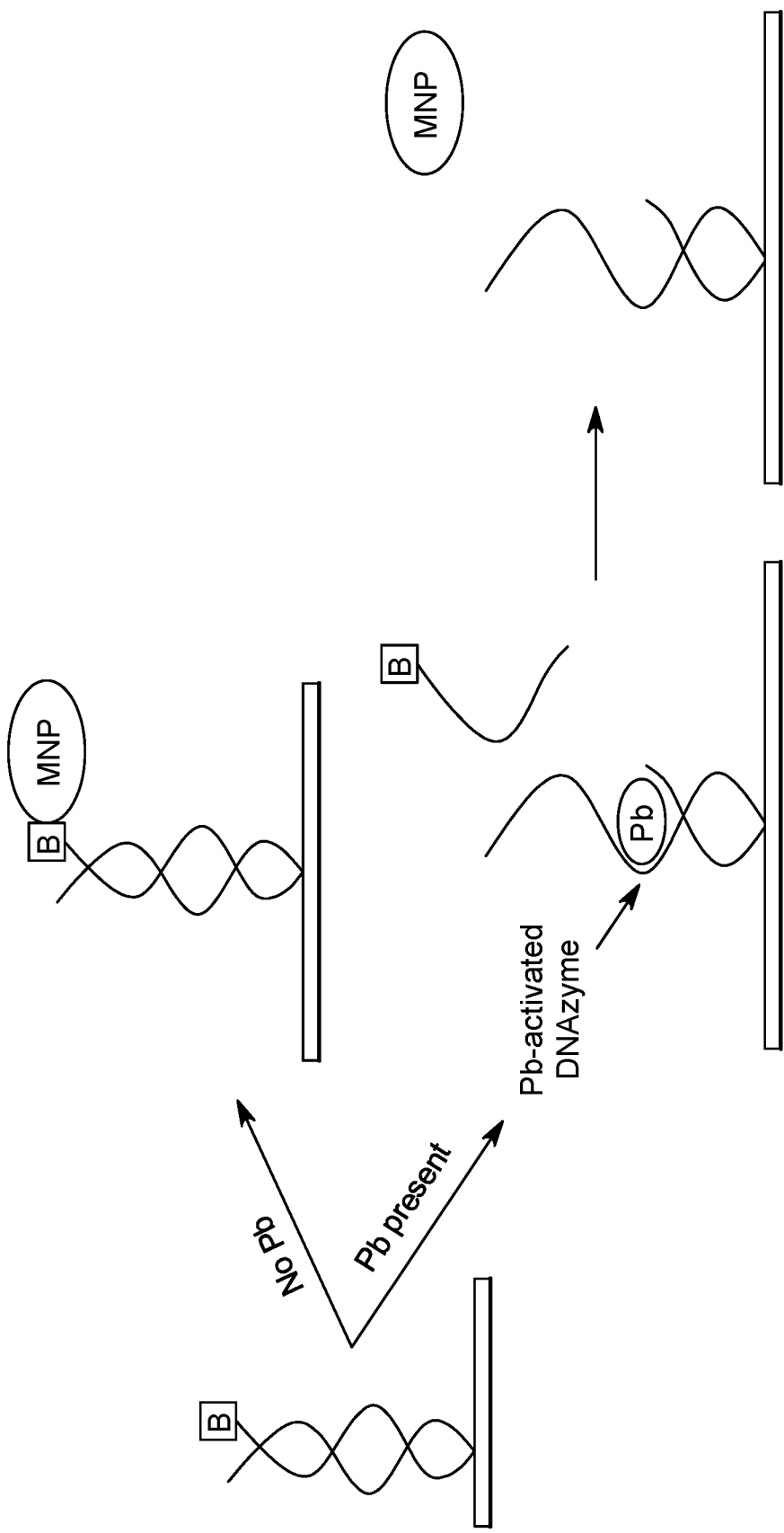


Fig. 18

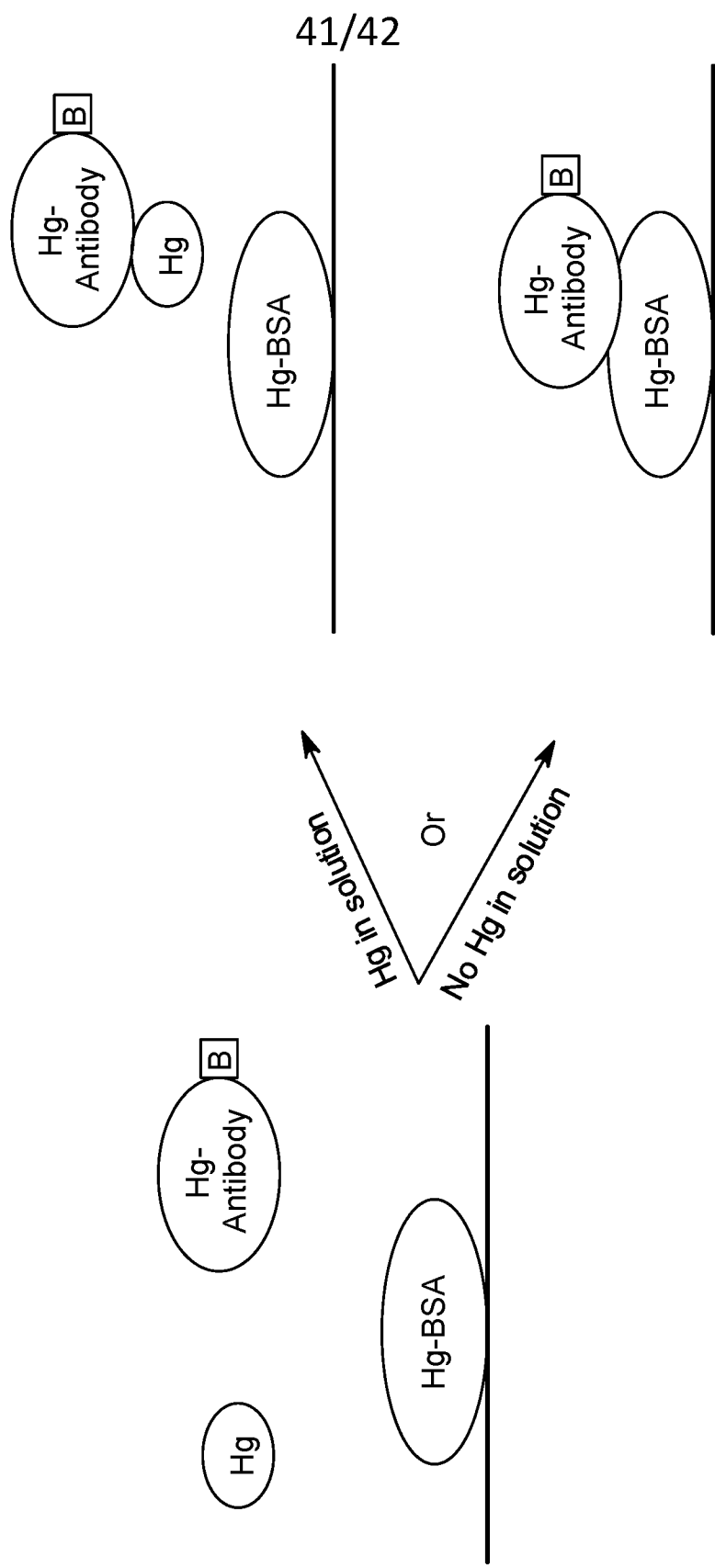


Fig. 19

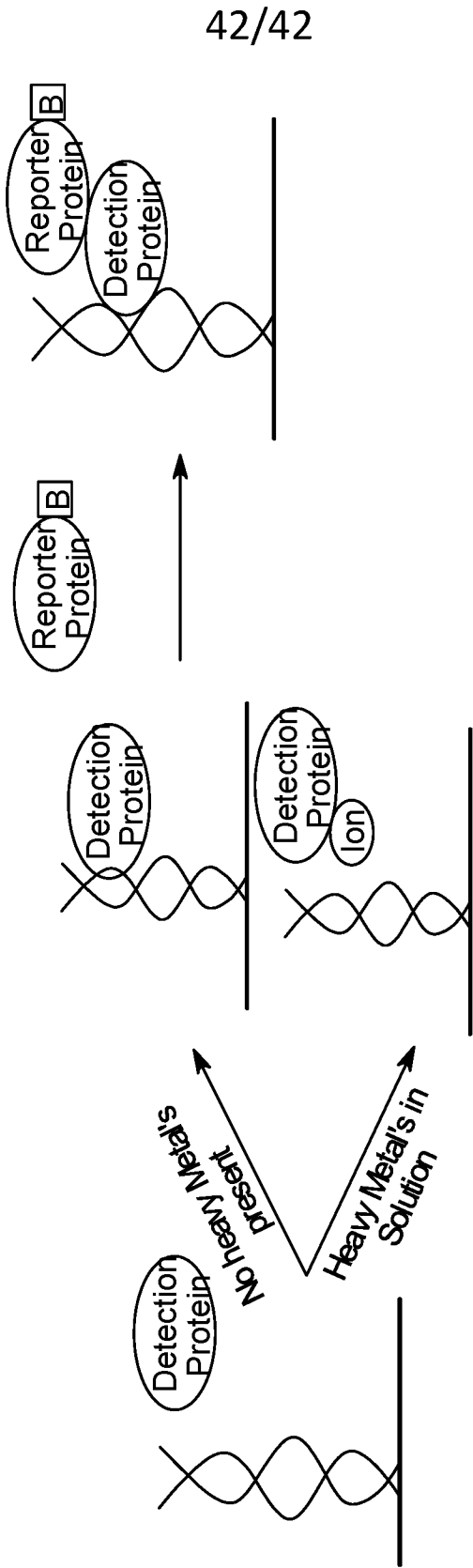


Fig. 20

A. CLASSIFICATION OF SUBJECT MATTER**G01N 33/543(2006.01)i, G01R 33/09(2006.01)i, G01N 33/18(2006.01)i, G01N 33/49(2006.01)i, G01N 35/00(2006.01)i**

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)

G01N 33/543; G01N 27/12; G01N 33/20; G01N 33/53; G01R 3/00; G01R 33/12; G01R 33/09; G01N 33/18; G01N 33/49; G01N 35/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: GMR, giant magnetoresistance, sensor, metal, DNA, DNzyme, protein, albumin, BSA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014-0178900 A1 (ELECTRONICS AND TELECOMMUNICATIONS RESEARCH INSTITUTE) 26 June 2014 See paragraph [0042]; claim 13; and figures 1A-3B.	1-3
A		16-18, 30-32, 49-54
Y	US 2018-0067094 A1 (ZEPTO LIFE TECHNOLOGY, LLC) 08 March 2018 See abstract; and paragraphs [0052]-[0055].	1-3
A	US 2016-0011182 A1 (ZEPTO LIFE TECHNOLOGY, LLC) 14 January 2016 See the whole document.	1-3, 16-18, 30-32, 49-54
A	US 2014-0292318 A1 (REGENTS OF THE UNIVERSITY OF MINNESOTA) 02 October 2014 See the whole document.	1-3, 16-18, 30-32, 49-54
A	US 2016-0209405 A1 (REGENTS OF THE UNIVERSITY OF MINNESOTA) 21 July 2016 See the whole document.	1-3, 16-18, 30-32, 49-54



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"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

15 November 2019 (15.11.2019)

Date of mailing of the international search report

15 November 2019 (15.11.2019)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

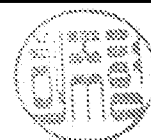
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Authorized officer

HEO, Joo Hyung

Telephone No. +82-42-481-8150



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/043766

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

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

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

2. ☒ Claims Nos.: 10,25
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 10 and 25 are unclear since they are referring to the multiple dependent claims which do not comply with PCT Rule 6.4(a).

3. ☒ Claims Nos.: 4-9,11-15,19-24,26-29,33-48,55-57
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2019/043766

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014-0178900 A1	26/06/2014	KR 10-1979256 B1 KR 10-2014-0083536 A US 9535035 B2	28/08/2019 04/07/2014 03/01/2017
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