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(54) **SINGLE-USE TEST DEVICE FOR IMAGING ASSAY BEADS**

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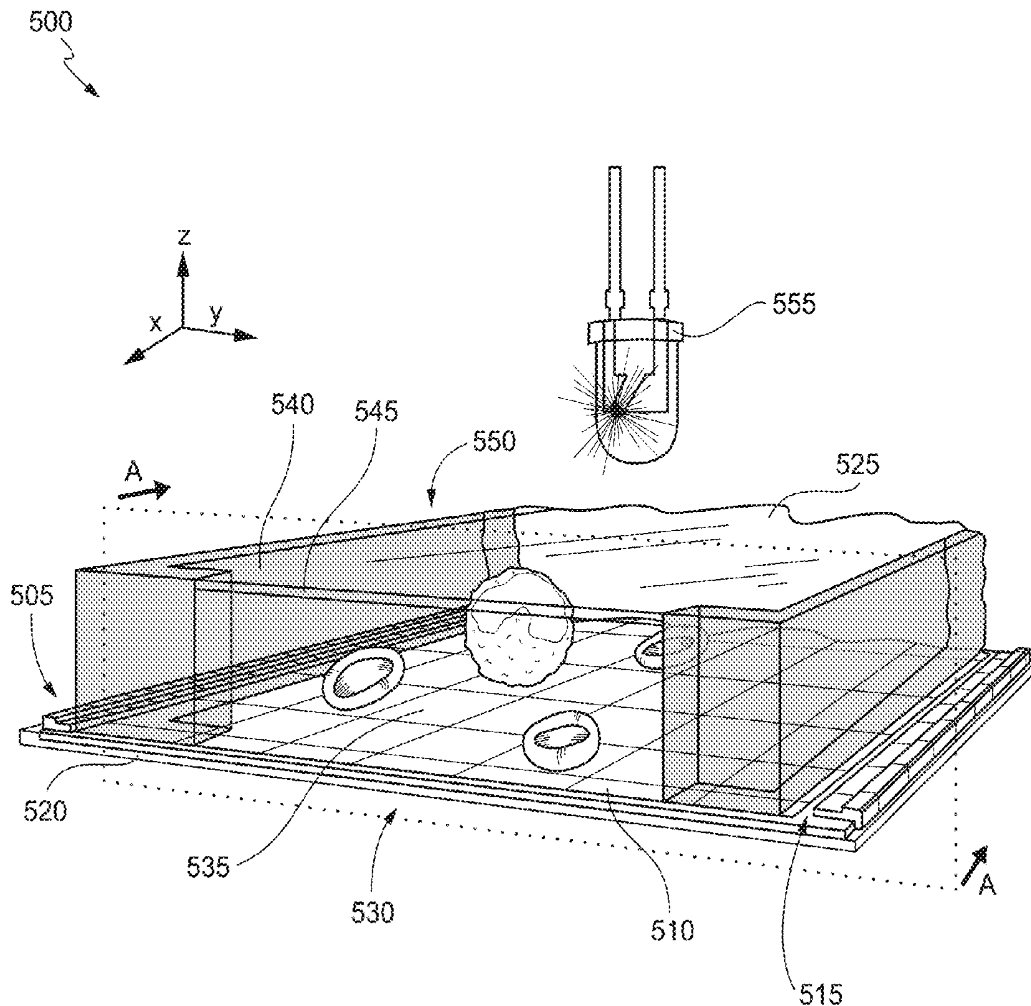
CPC ***G01N 33/5302*** (2013.01); ***G01N 15/1429*** (2013.01); ***G01N 15/1484*** (2013.01)

(57) **ABSTRACT**

This present invention relates generally to devices, systems, and methods for performing bioimaging at the microscopic scale and, more particularly, to devices and systems including a disposable testing device configured to perform bioimaging at the microscopic scale, and methods of performing the bioimaging using the disposable testing device. In some aspects, a testing device is provided for imaging assay beads. The testing device having a sample entry port for receiving the blood sample; a sample testing conduit fluidically connected to the sample entry port, the sample testing conduit including: (i) a planar member, (ii) a transparent planar member, and (iii) a plurality of wells having a predetermined average well height and disposed between the first planar member and the second planar member; and an imager chip forming at least a portion of the planar member.



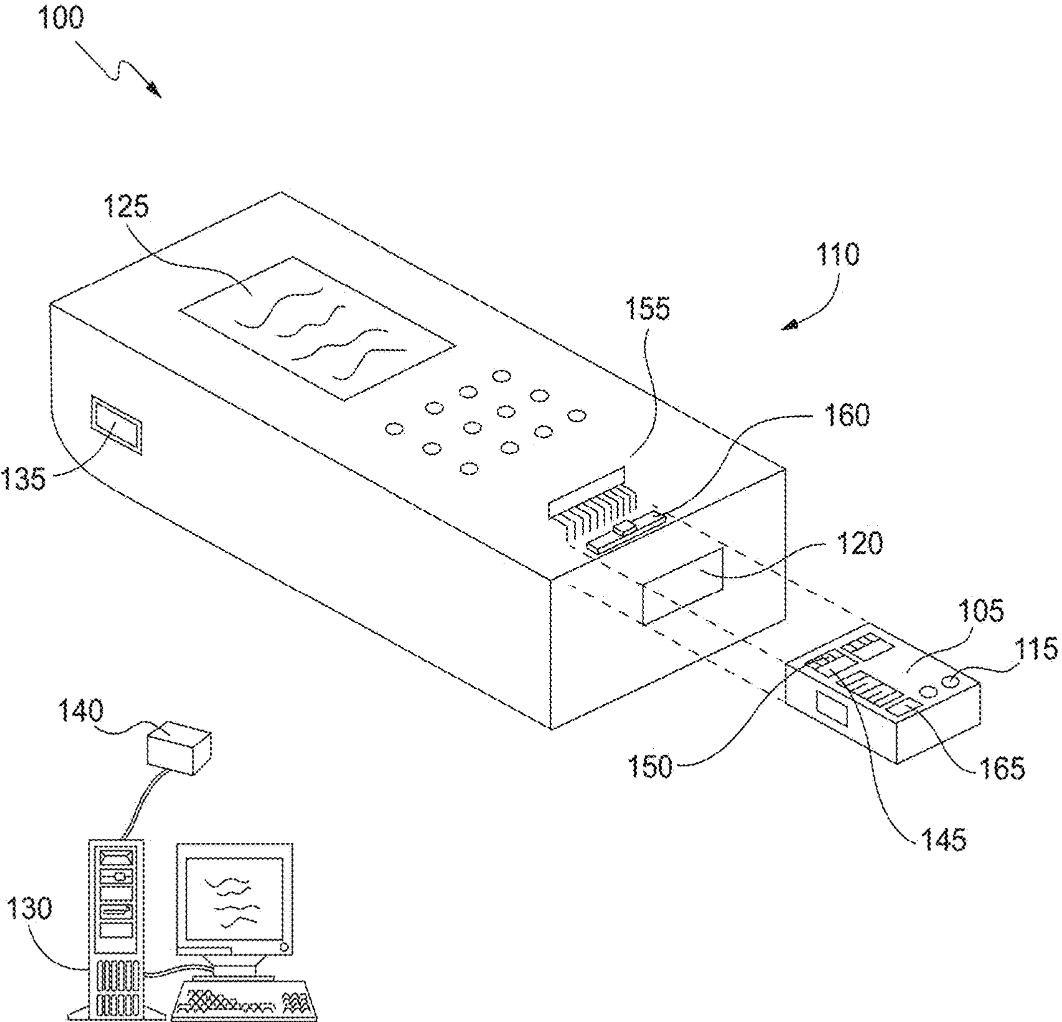


FIG. 1

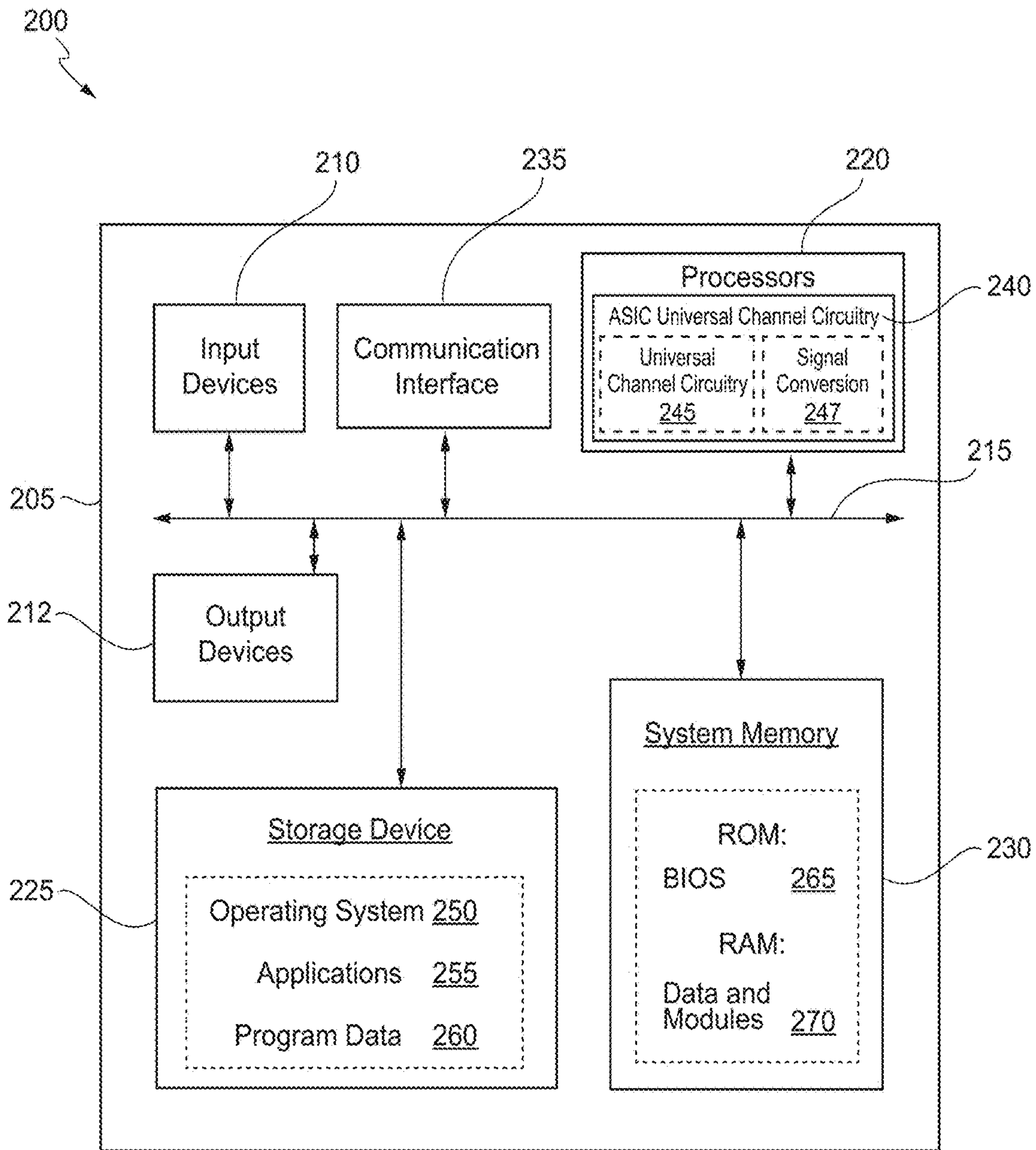


FIG. 2

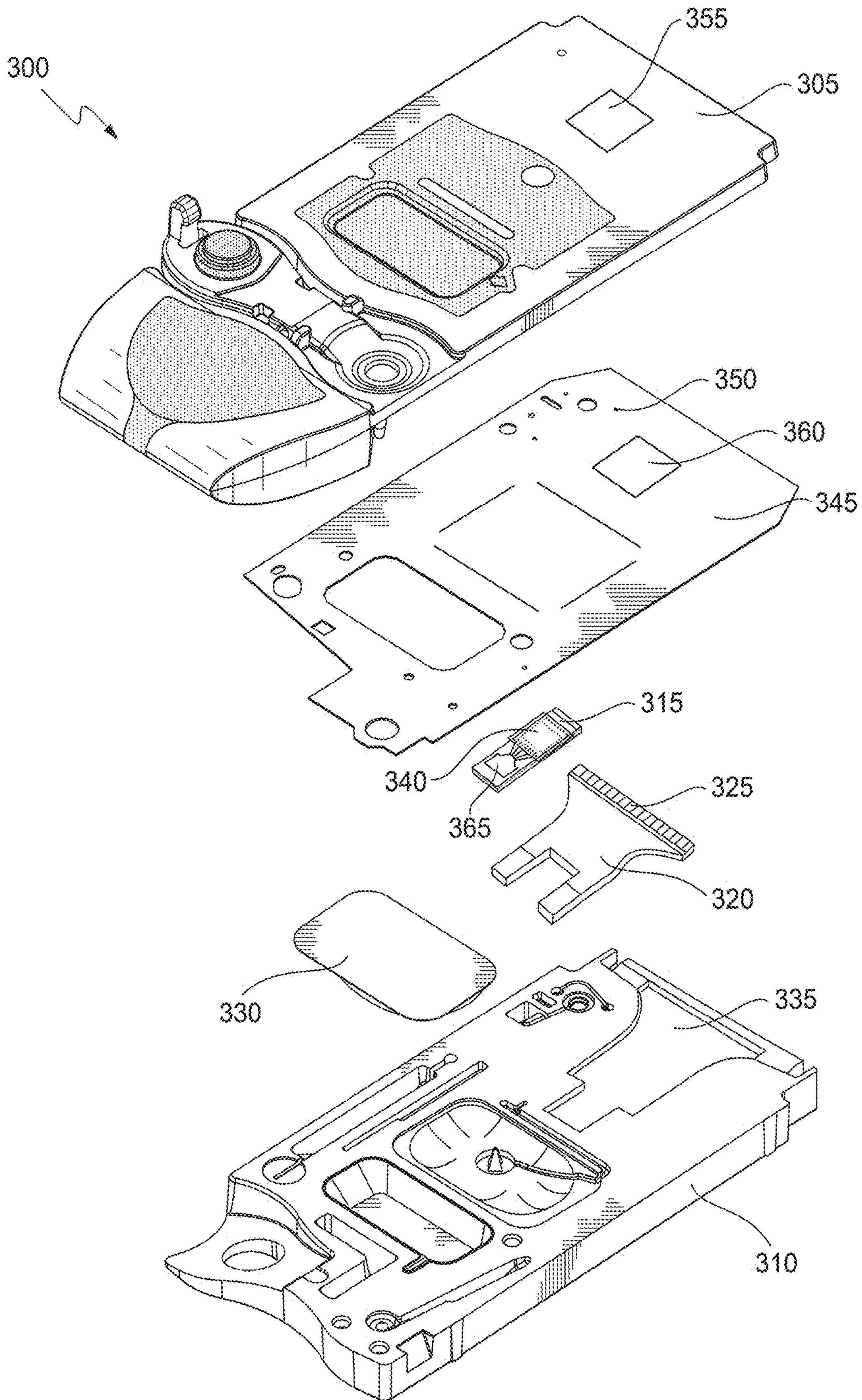


FIG. 3

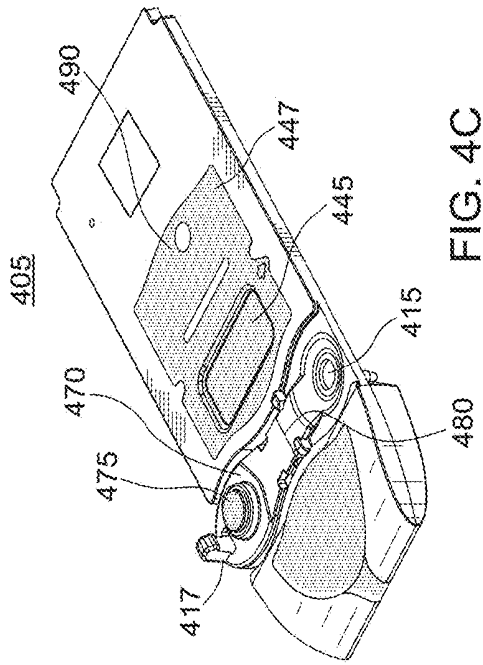


FIG. 4C

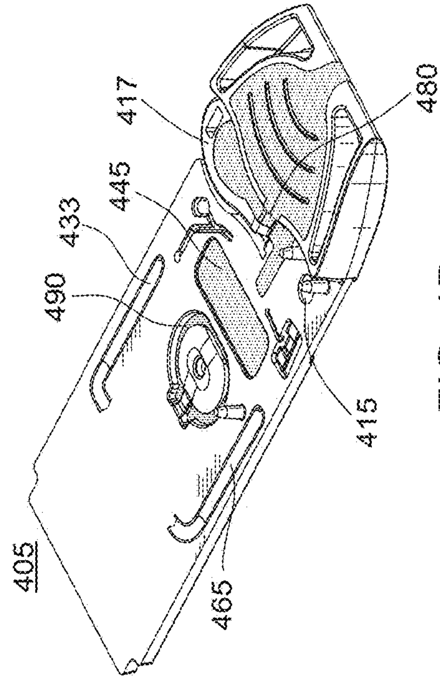


FIG. 4D

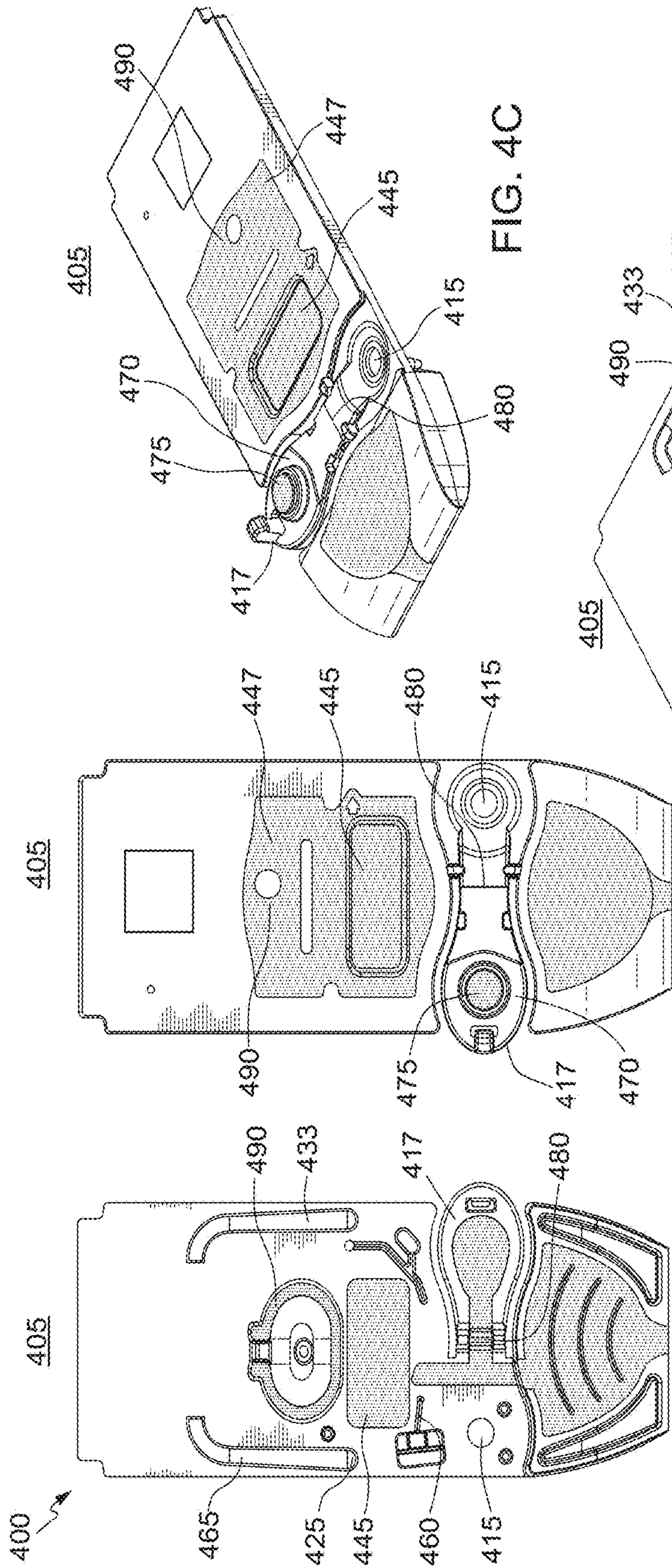


FIG. 4A

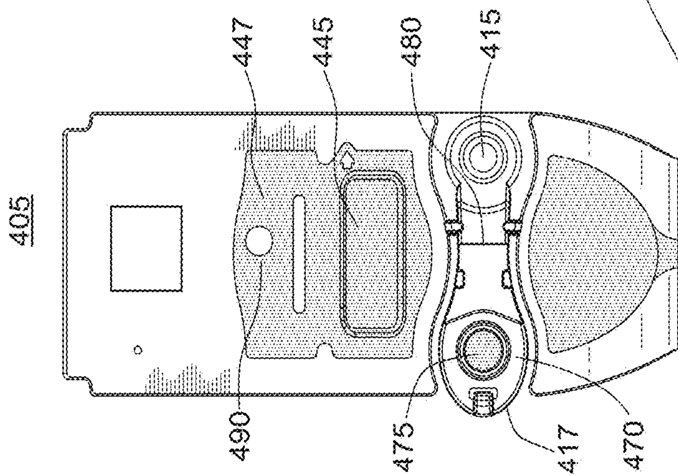


FIG. 4B

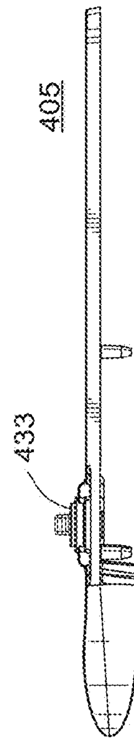


FIG. 4E

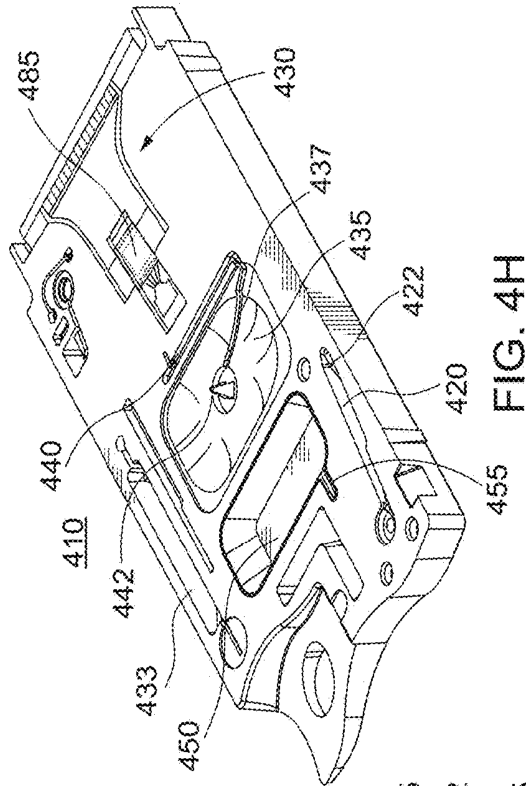


FIG. 4H

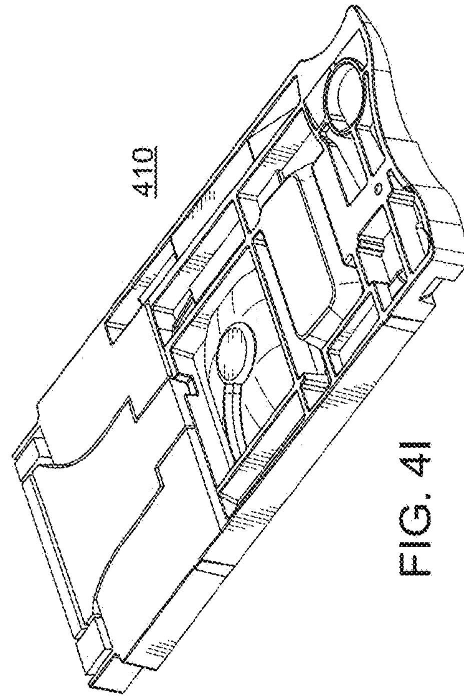


FIG. 4I

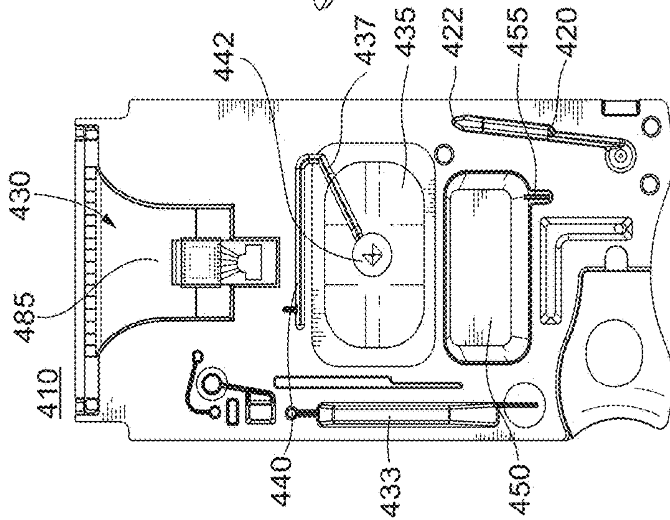


FIG. 4G

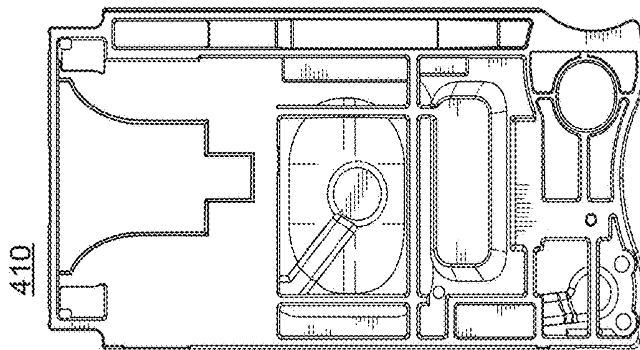


FIG. 4F

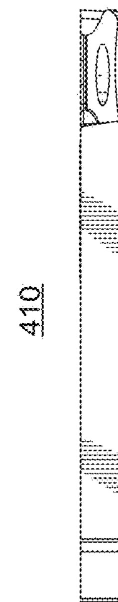


FIG. 4J

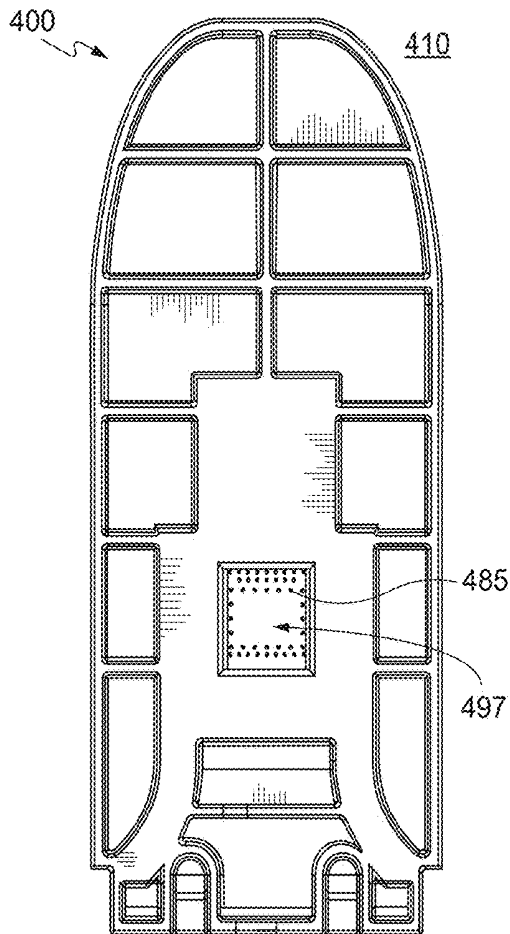


FIG. 4L

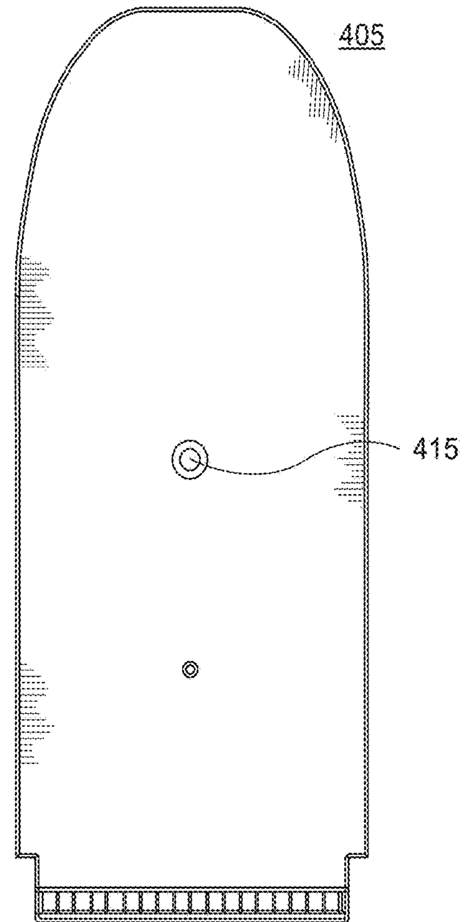


FIG. 4K

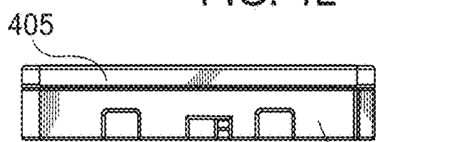


FIG. 4M

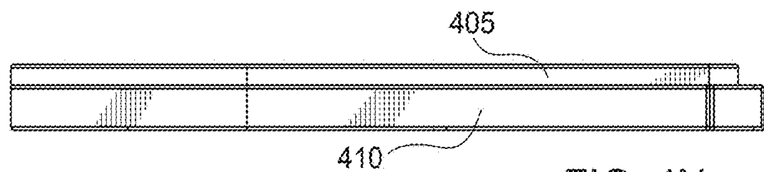


FIG. 4N

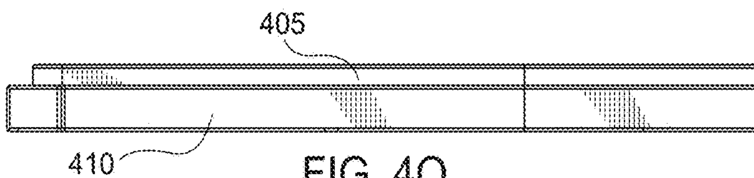


FIG. 4O

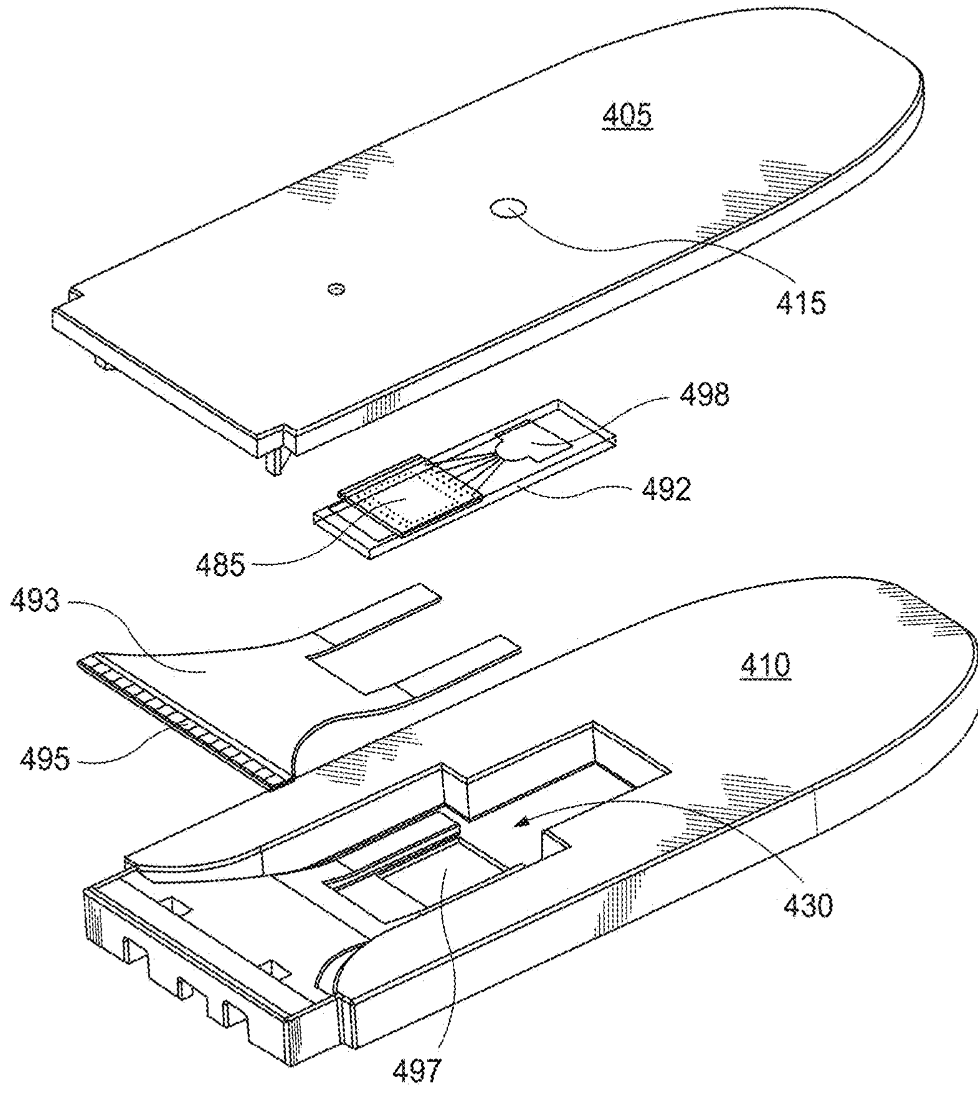


FIG. 4P

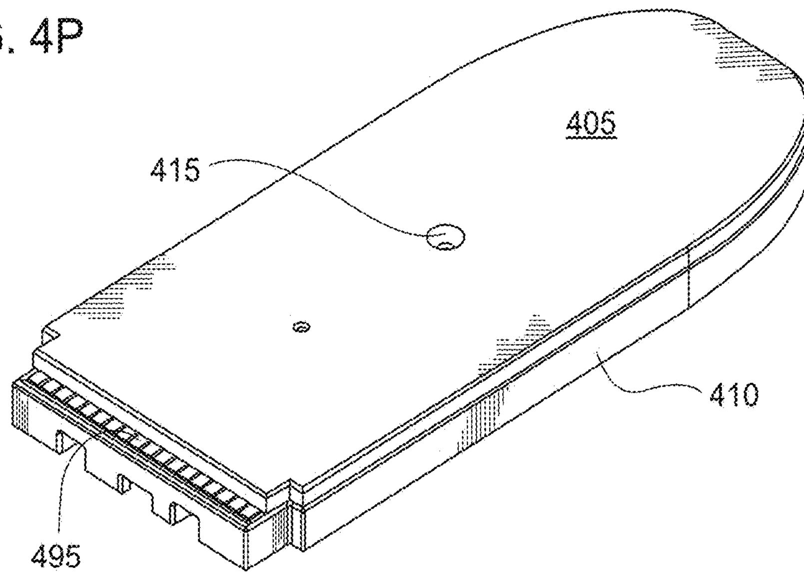


FIG. 4Q

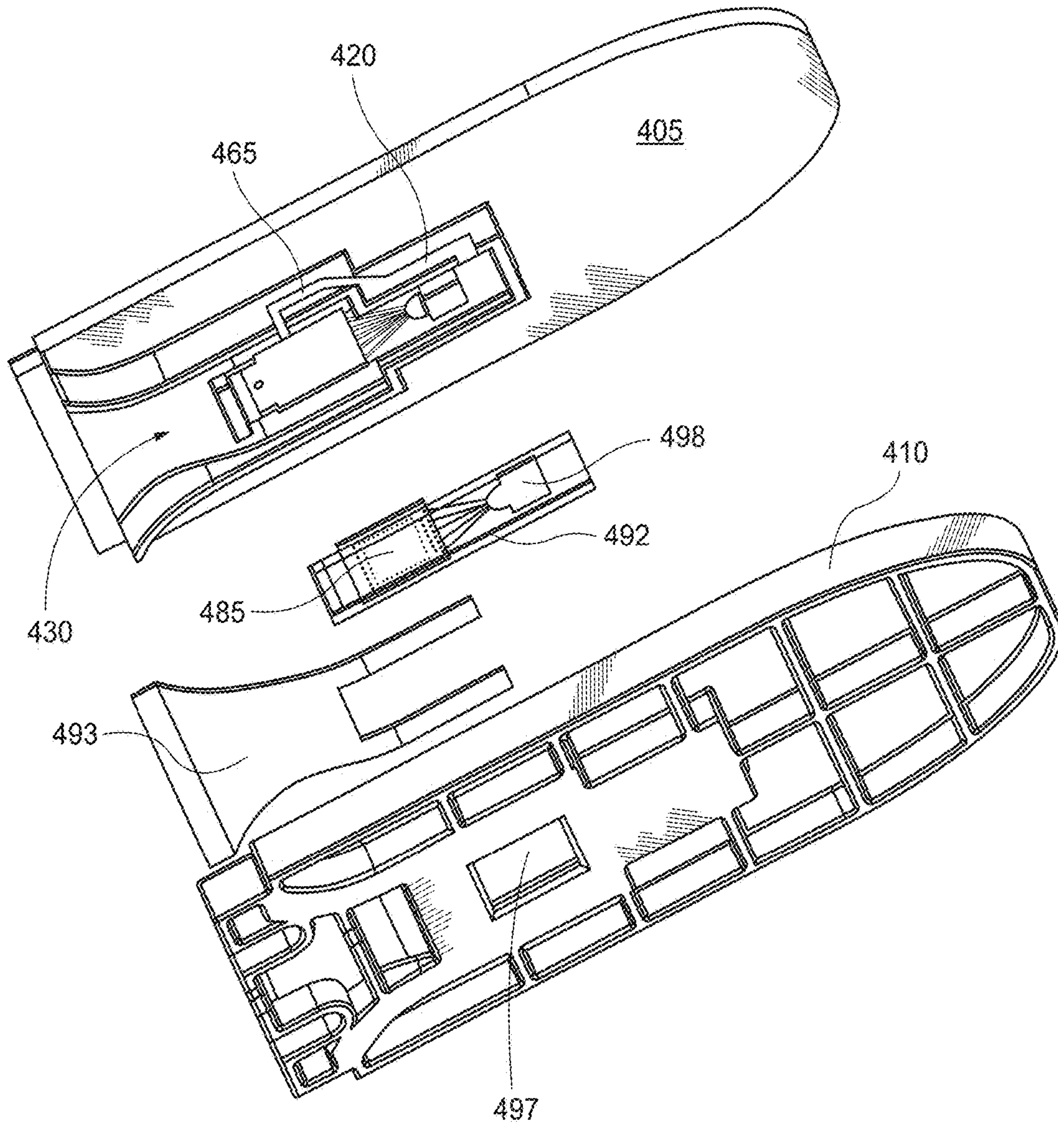


FIG. 4R

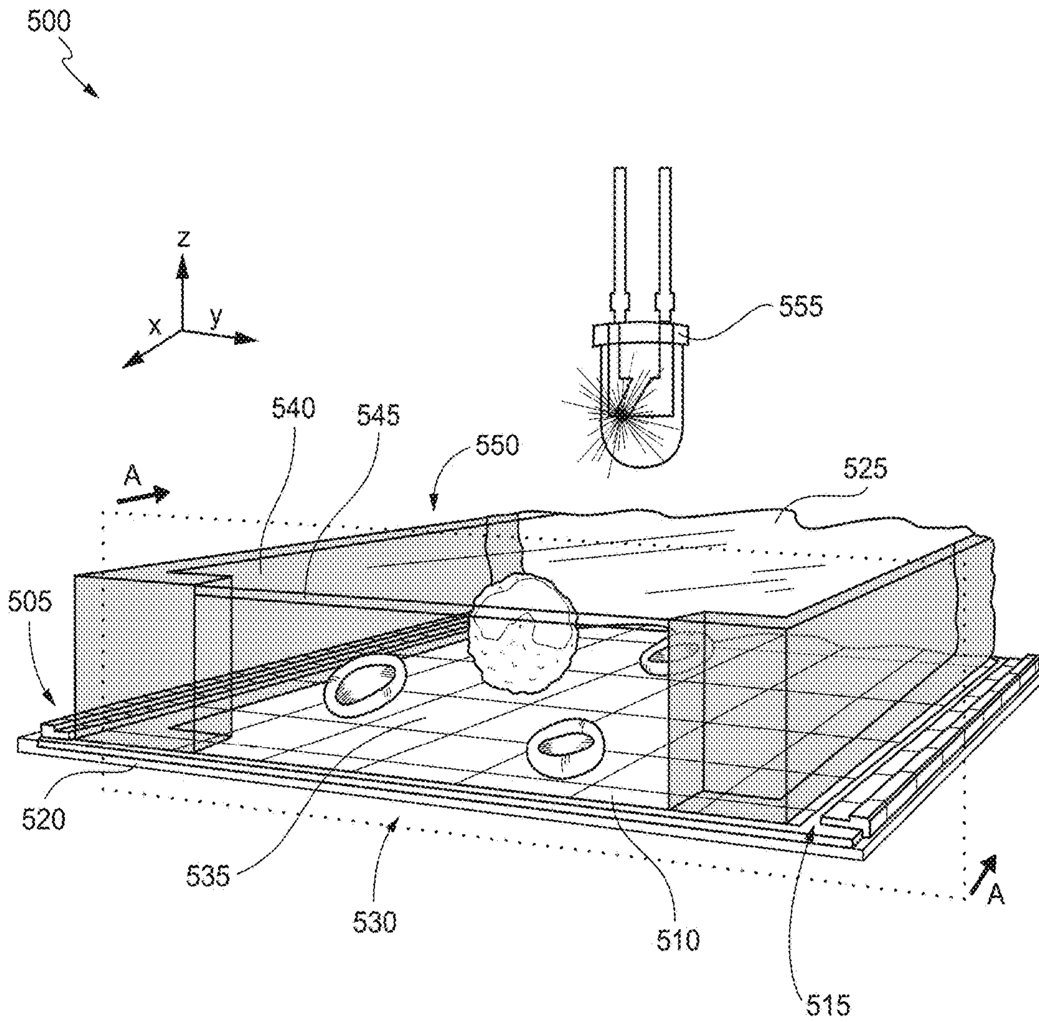


FIG. 5

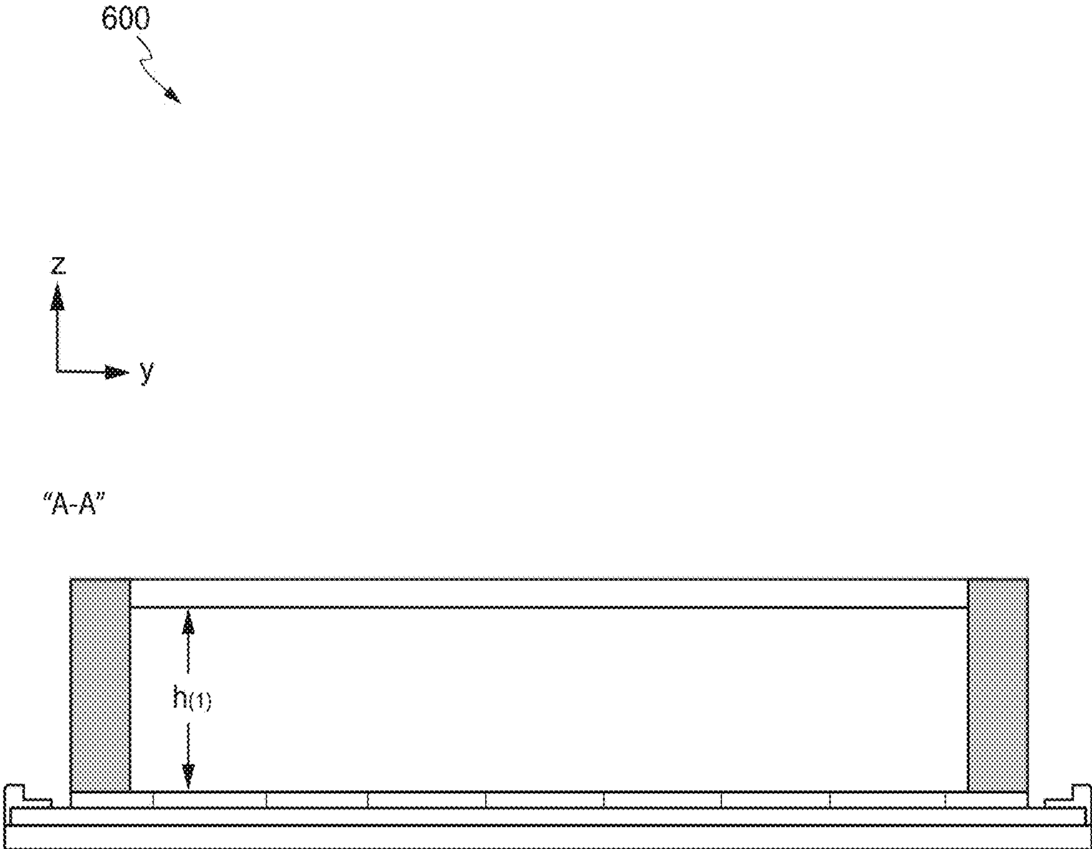


FIG. 6

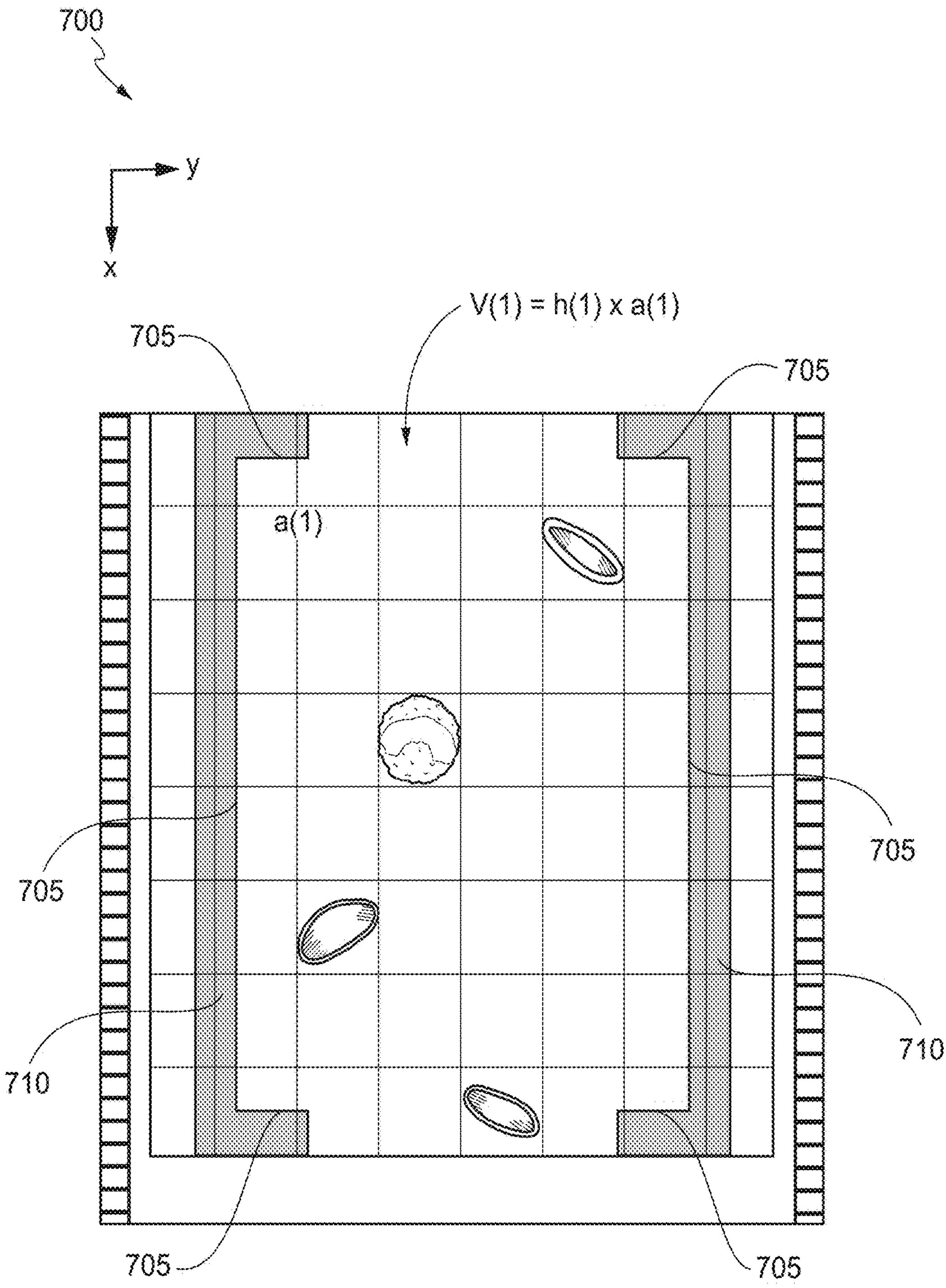


FIG. 7

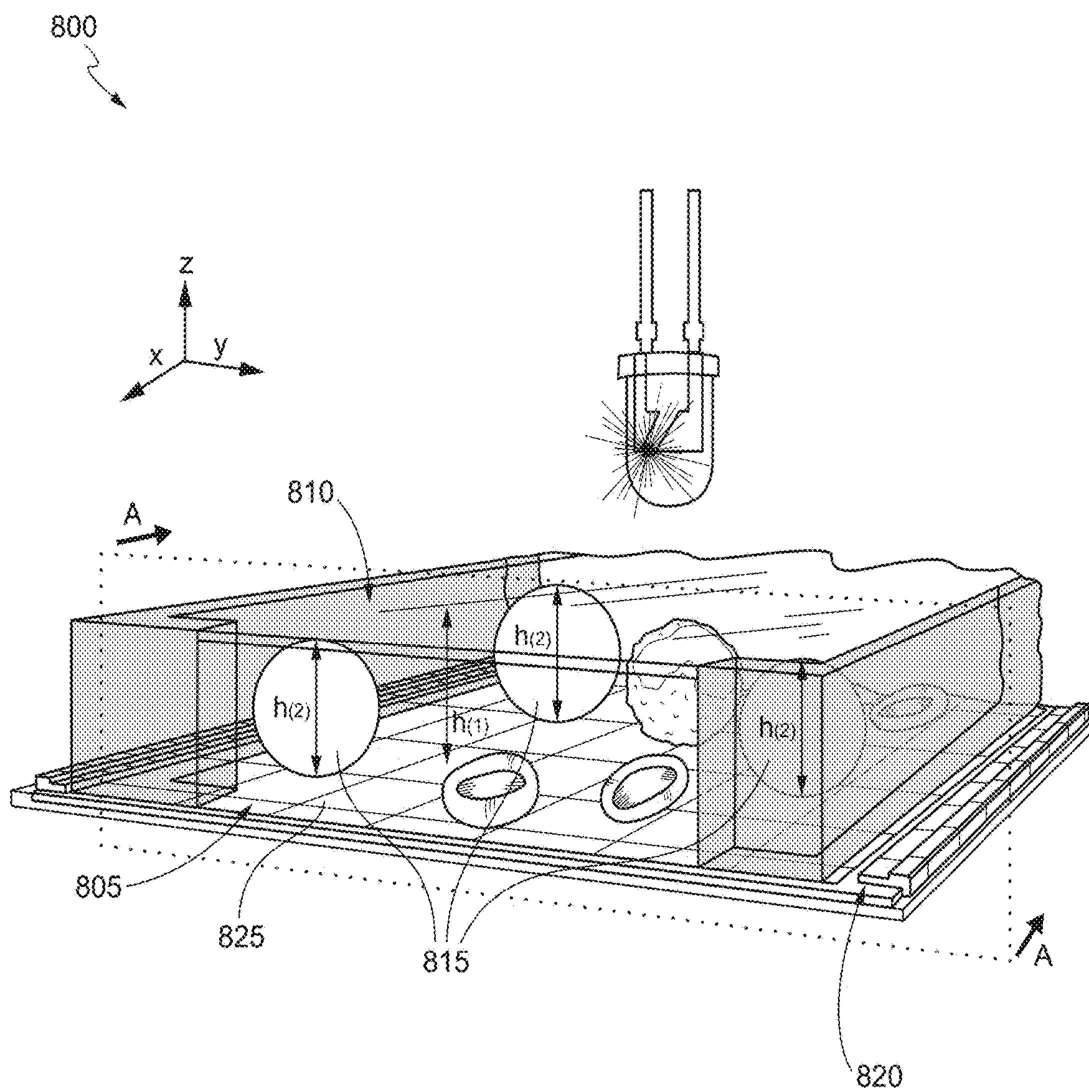


FIG. 8

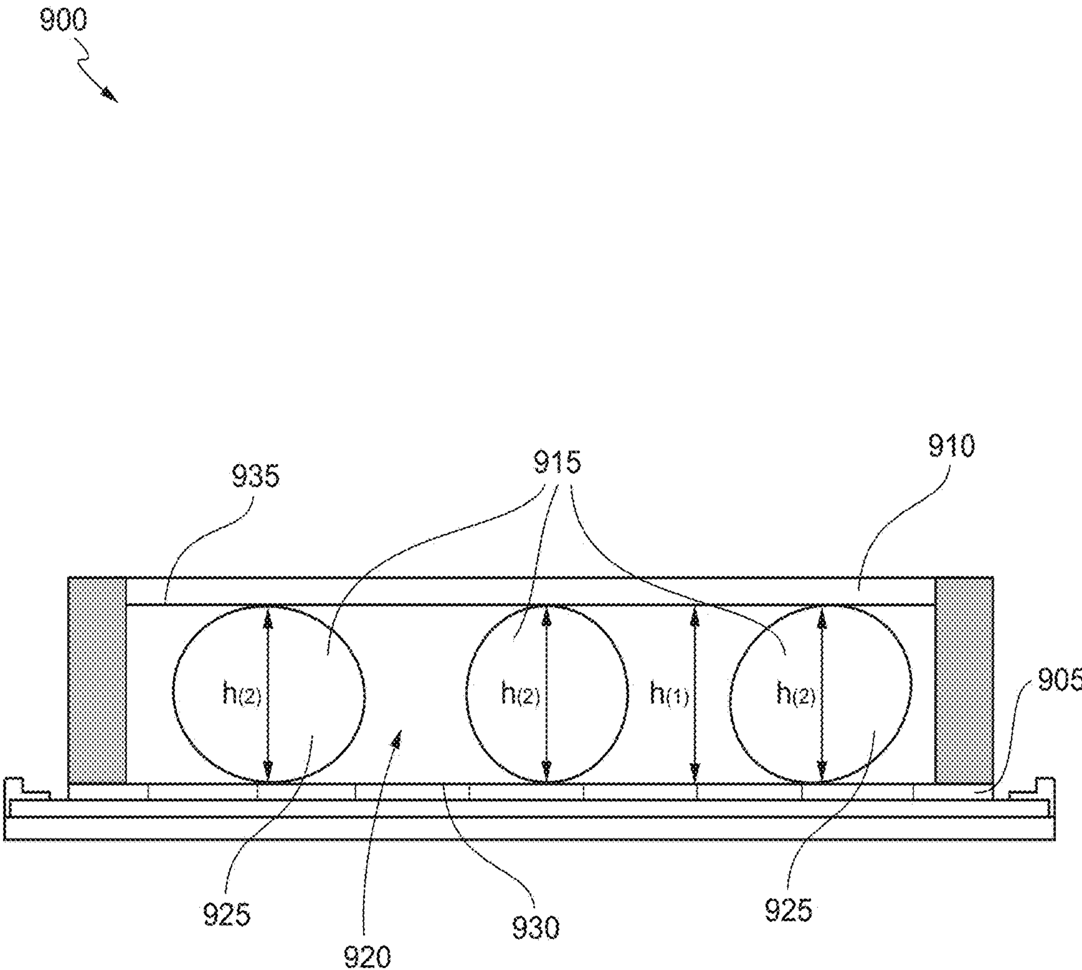


FIG. 9

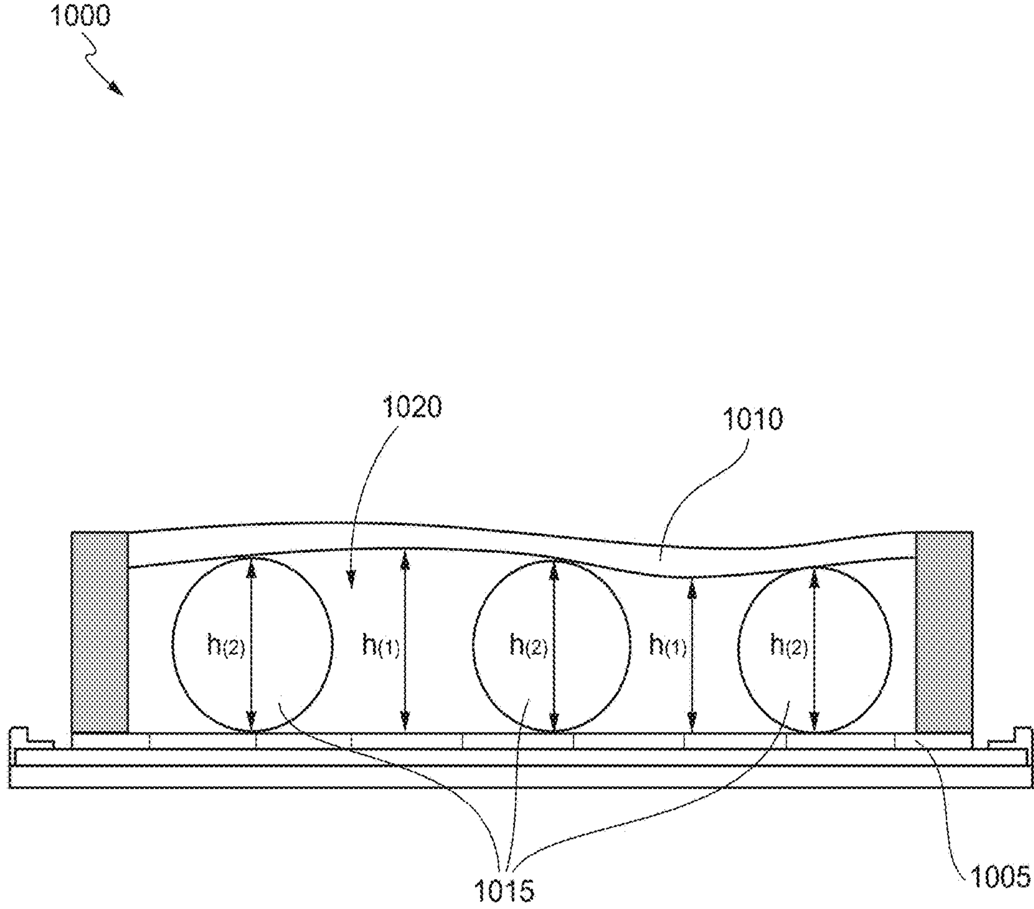


FIG. 10

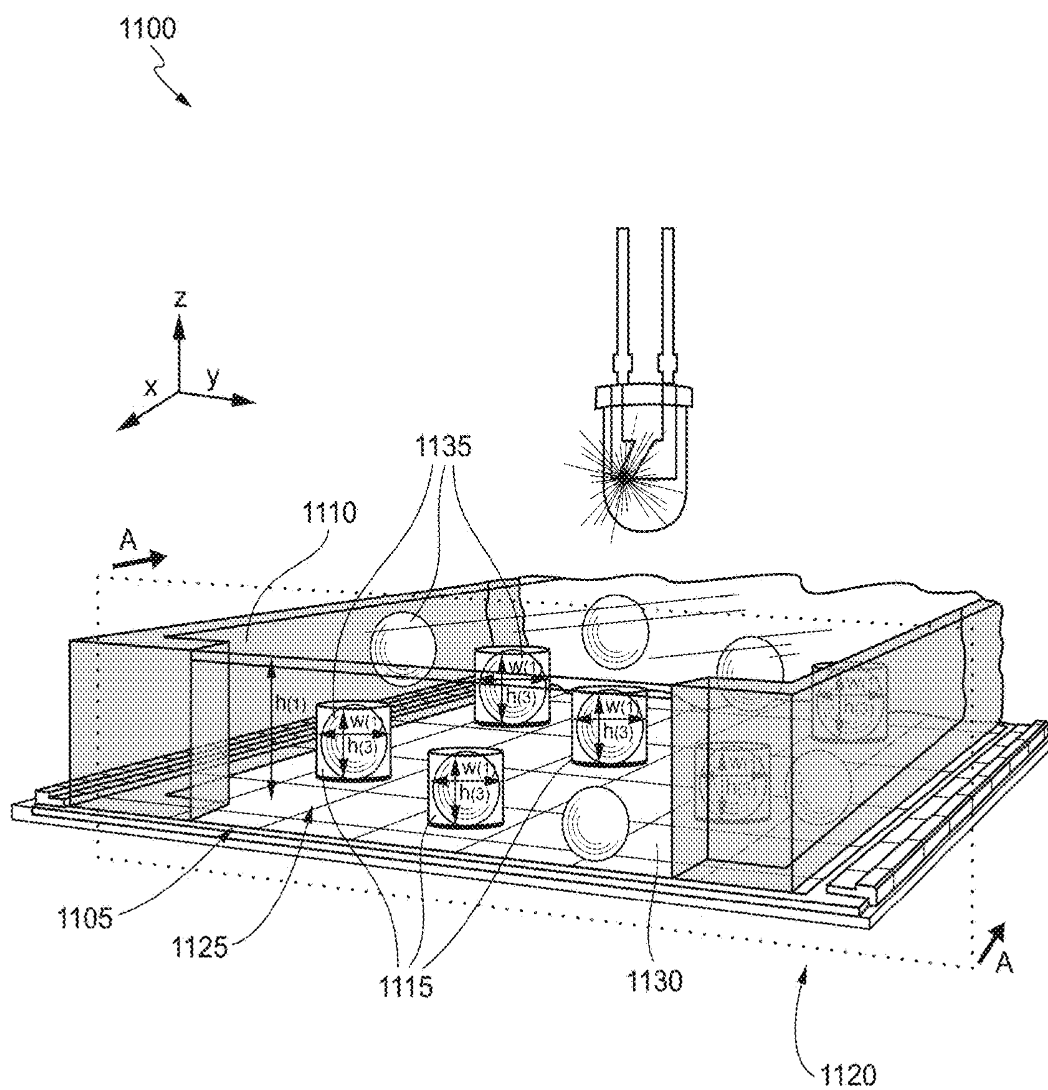


FIG. 11A

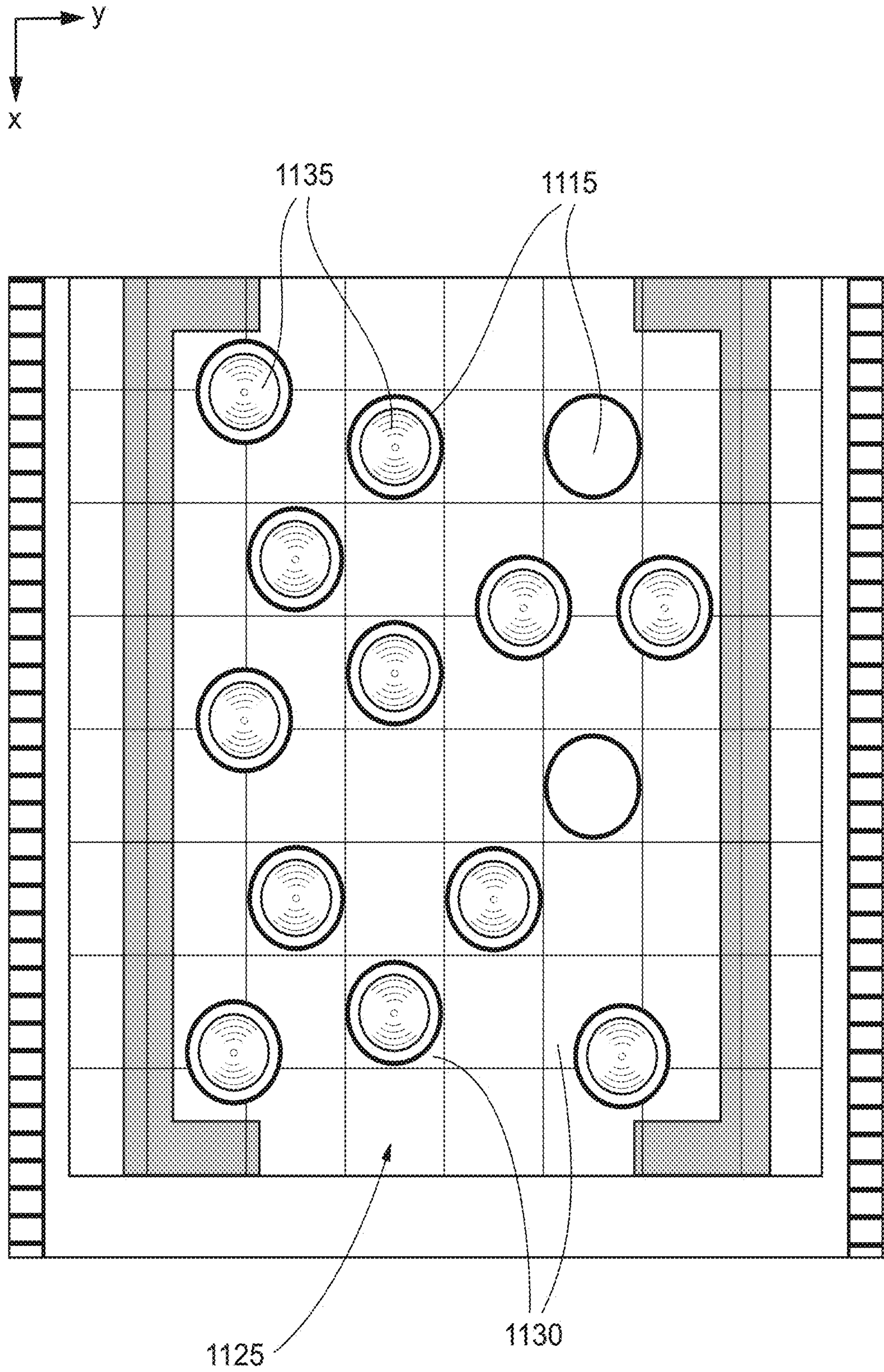


FIG. 11B

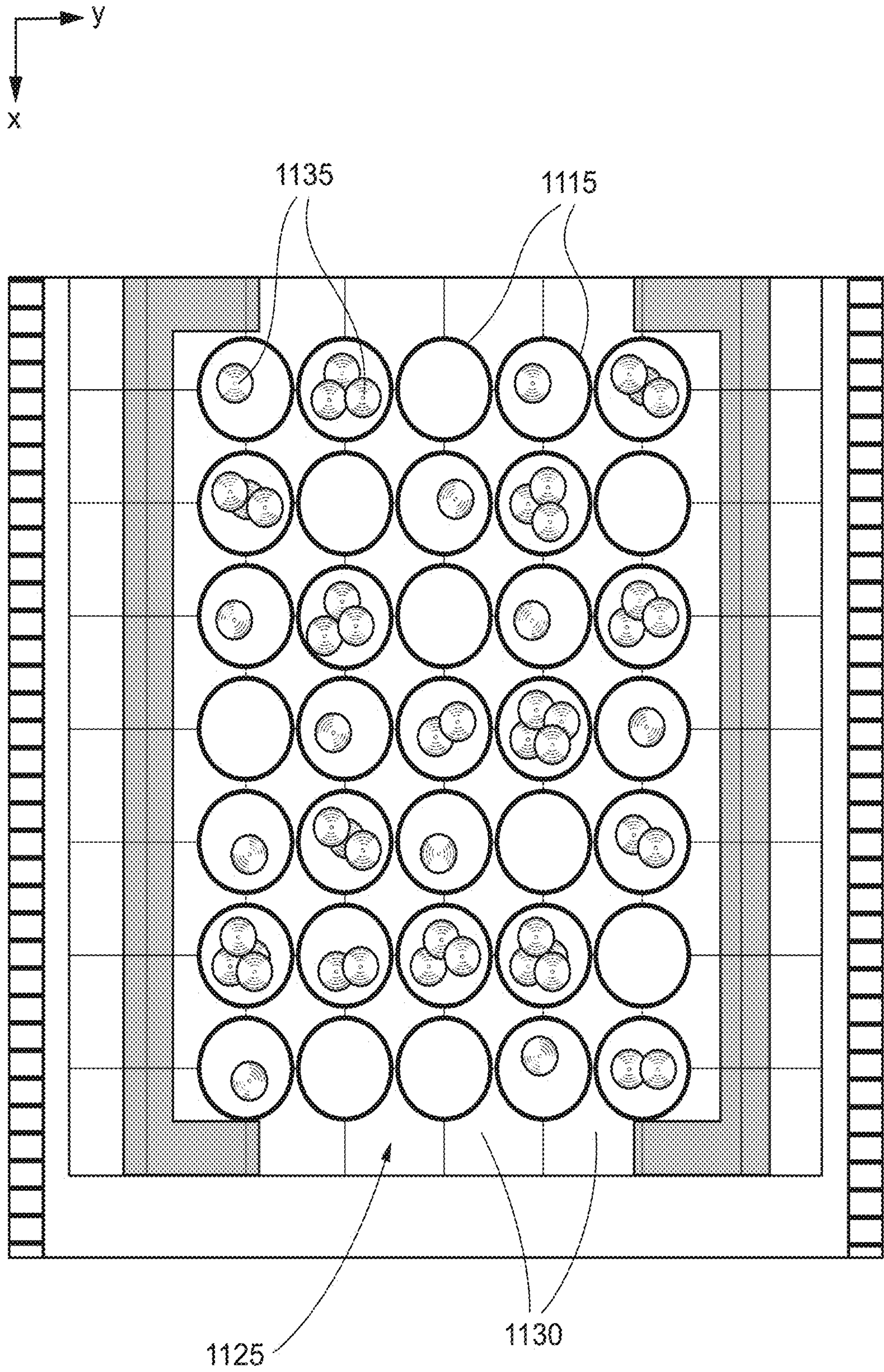


FIG. 11C

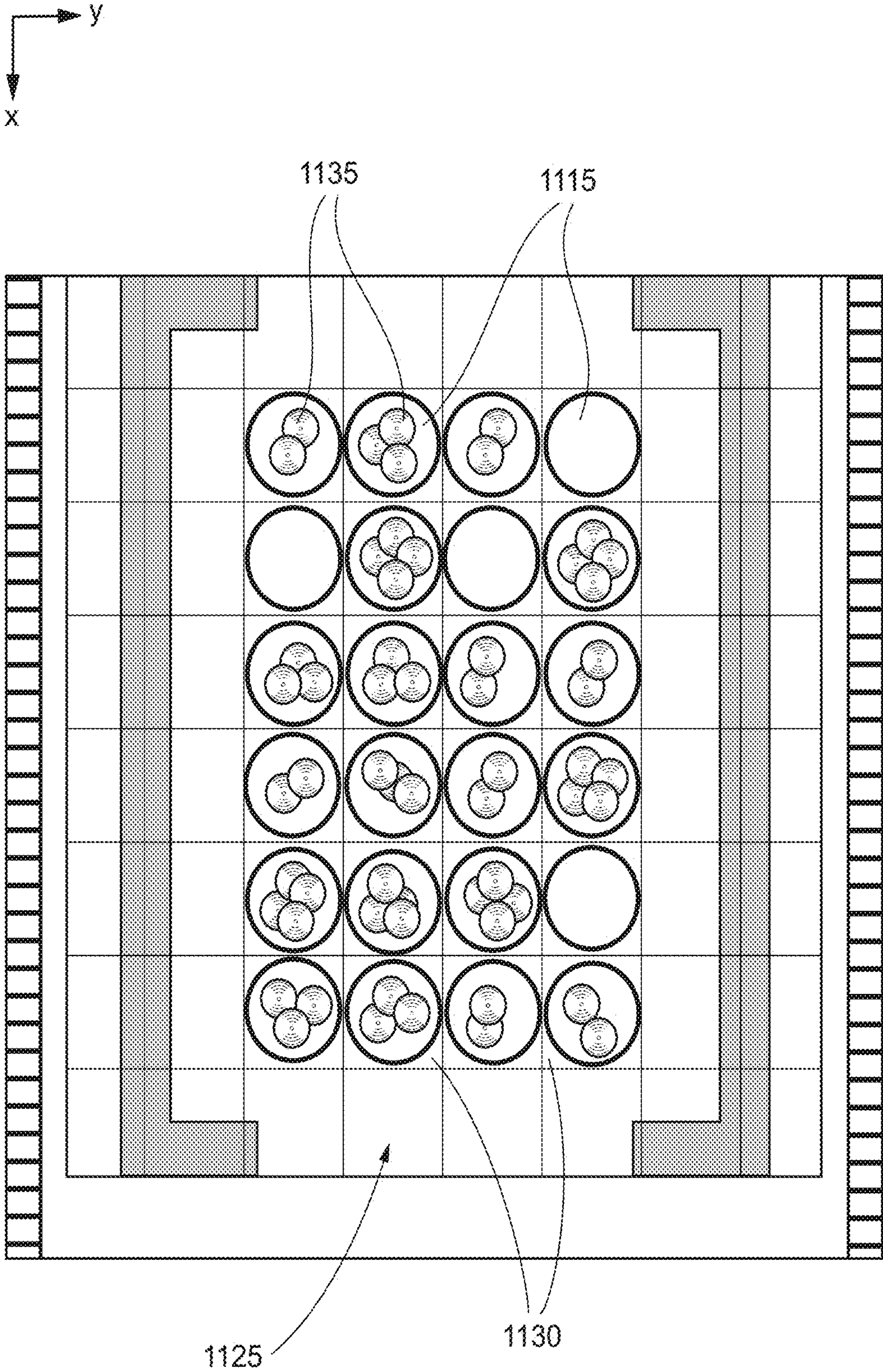


FIG. 11D

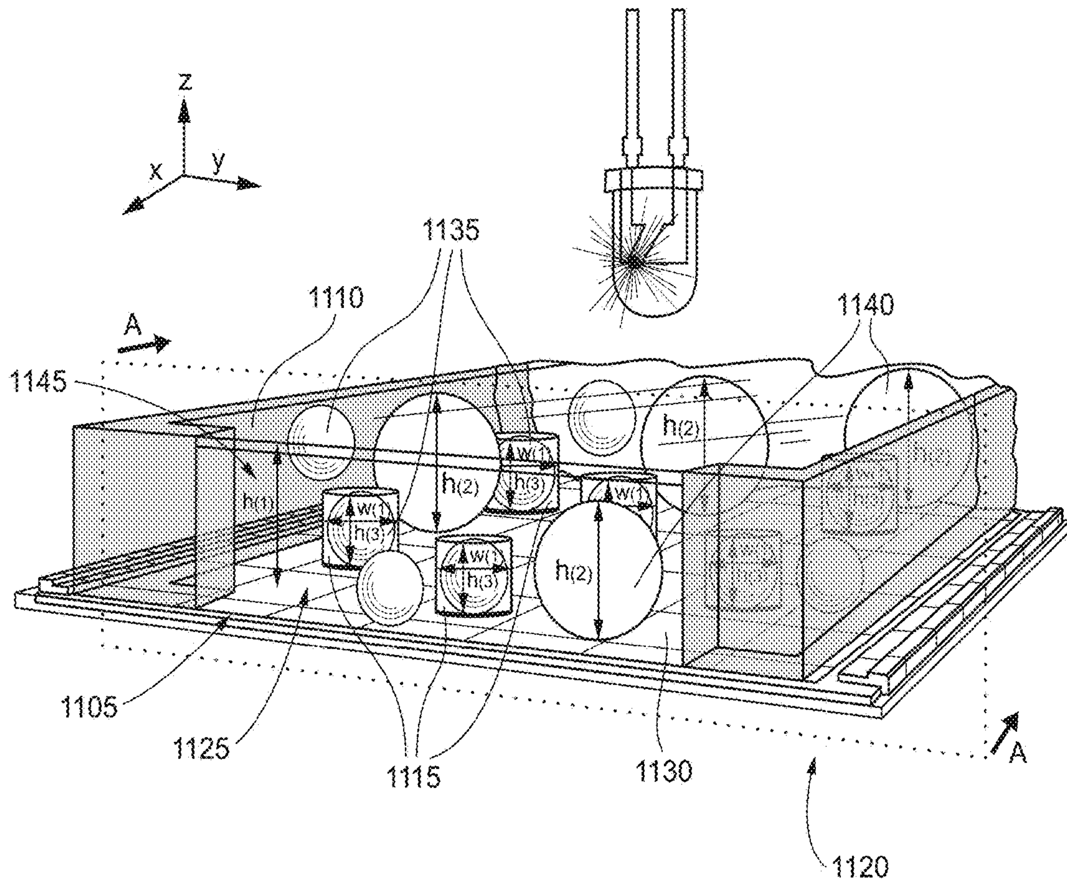


FIG. 11E

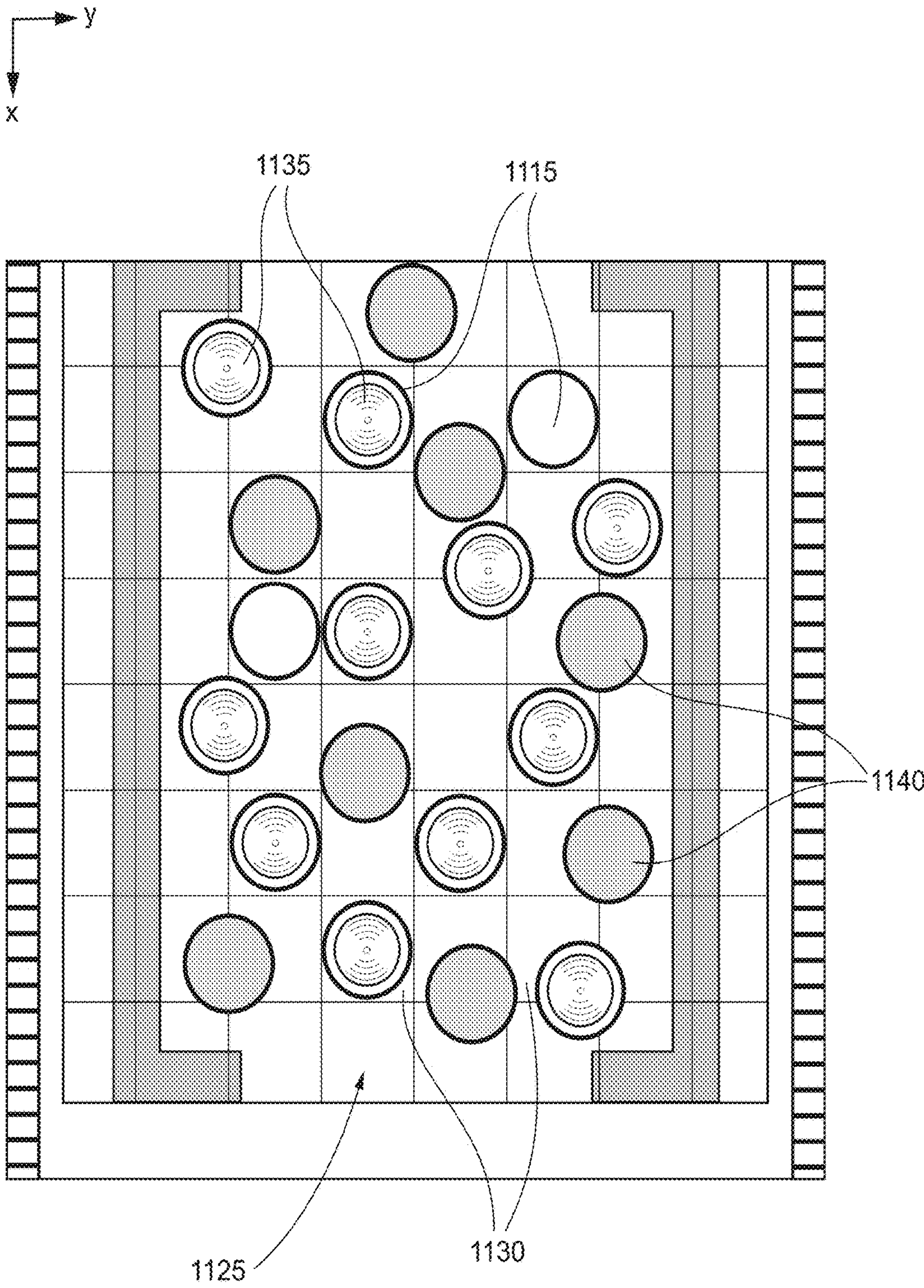


FIG. 11F

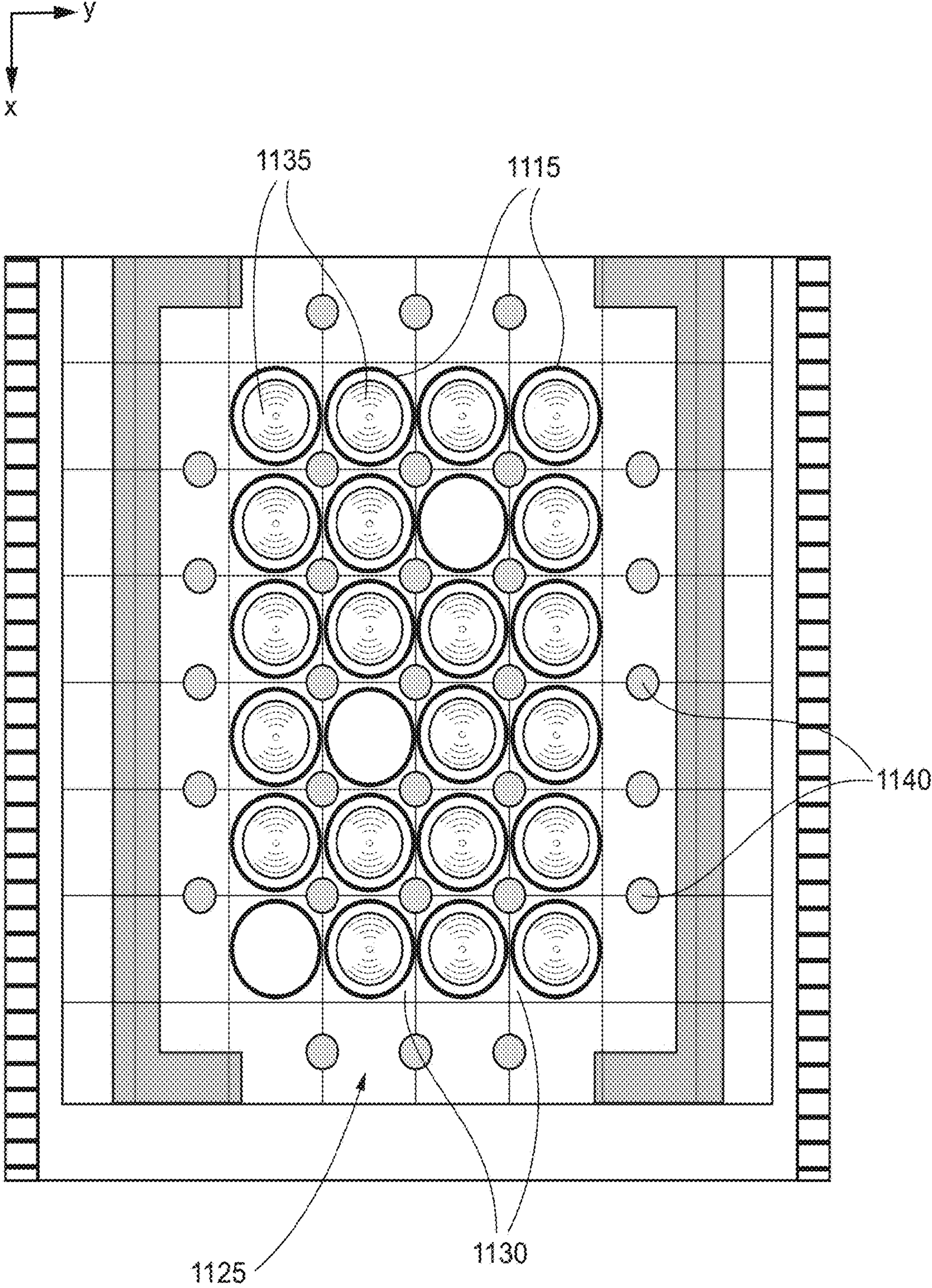


FIG. 11G

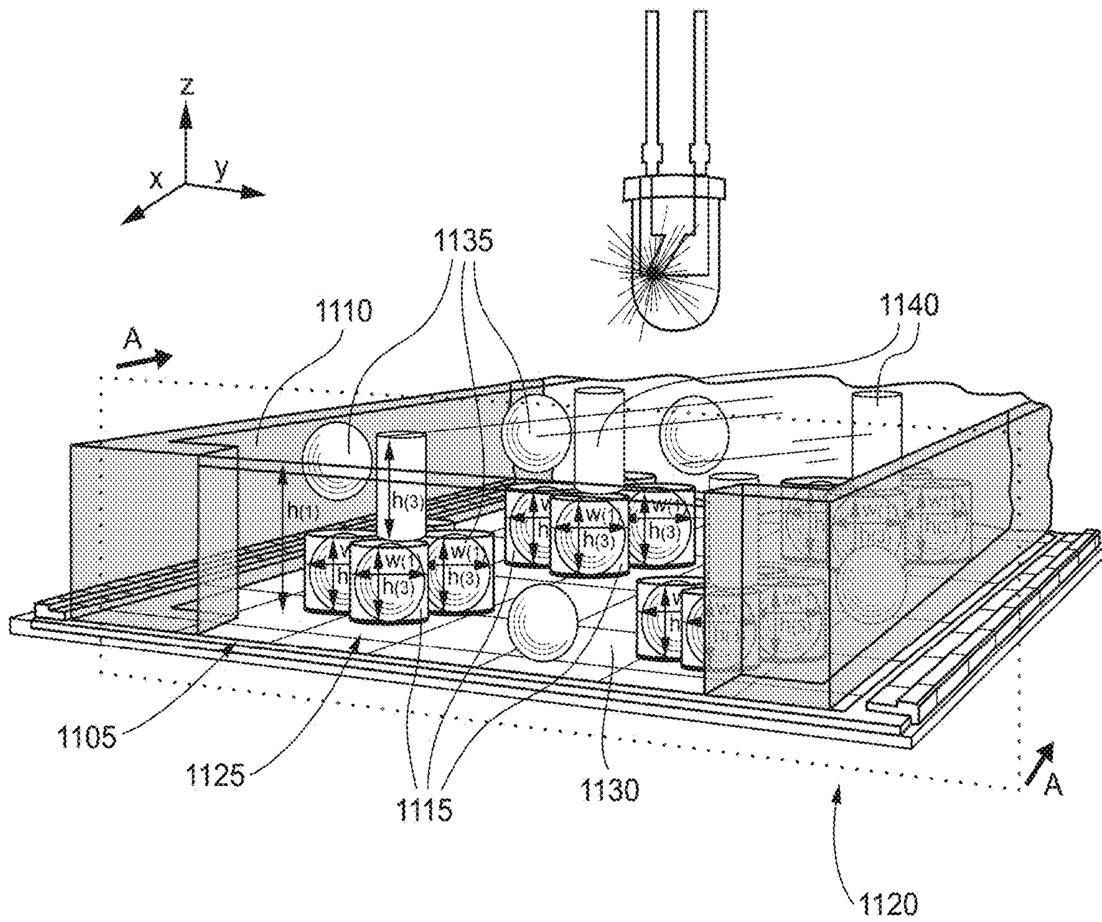


FIG. 11H

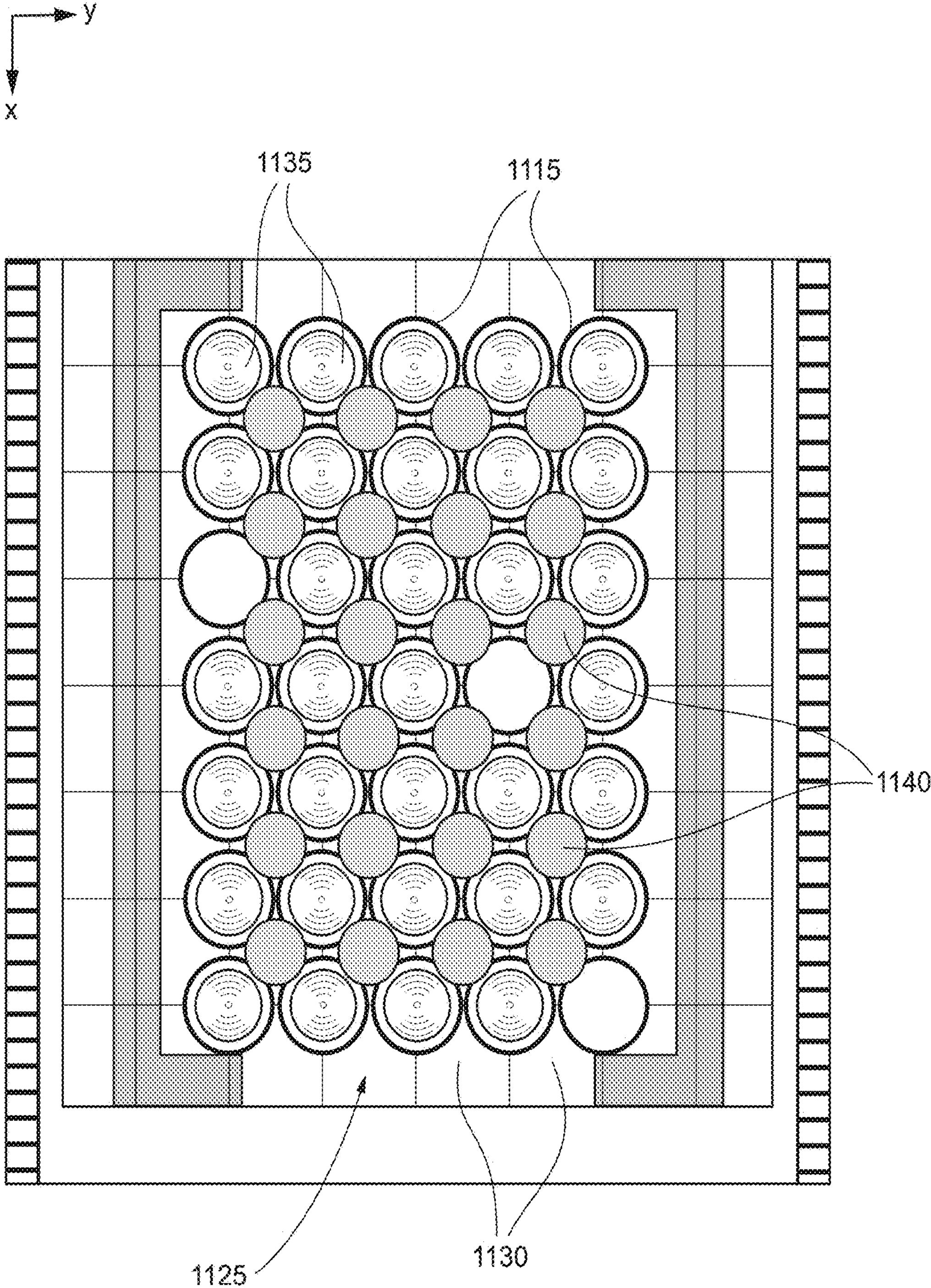


FIG. 11I

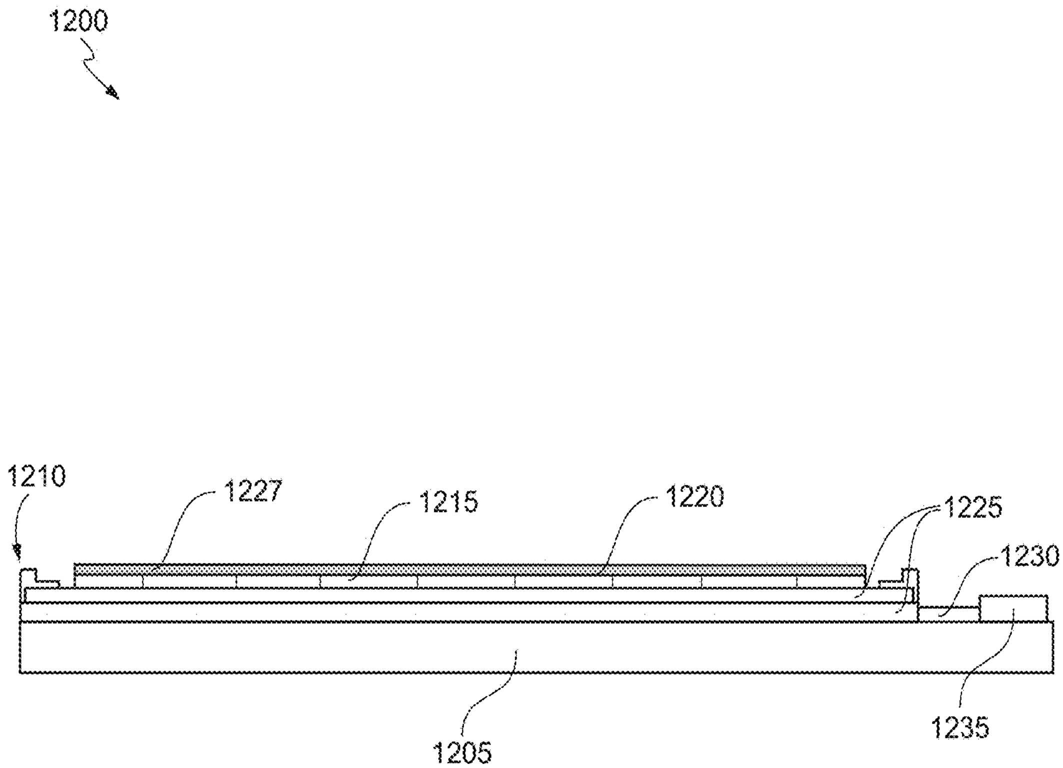


FIG. 12

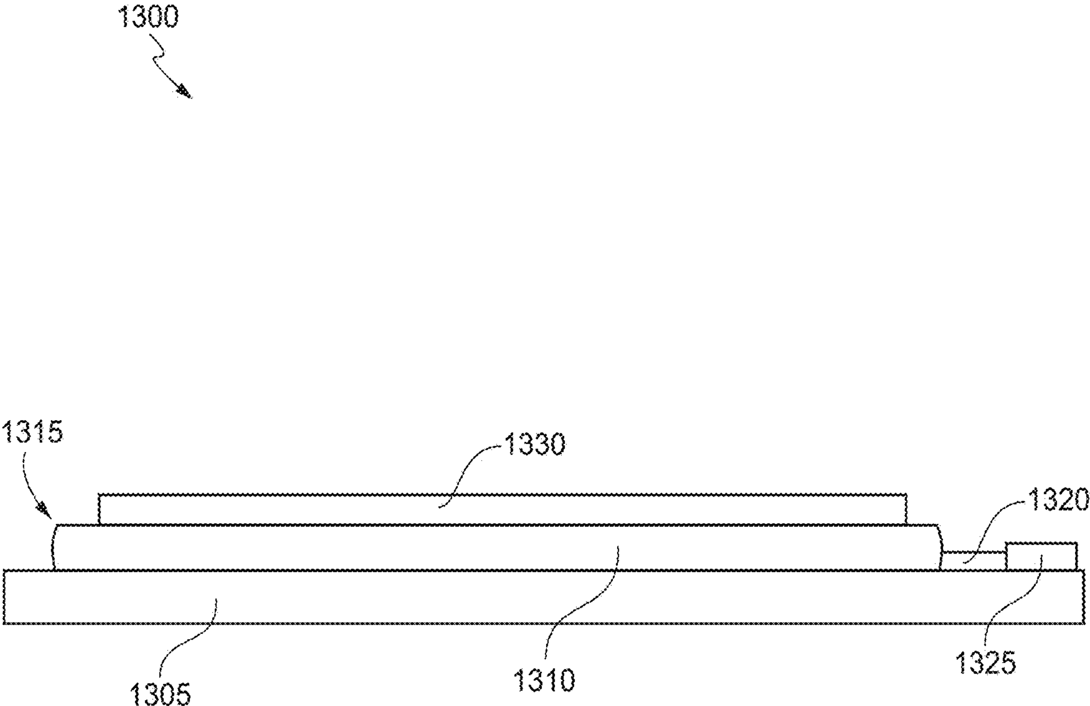


FIG. 13

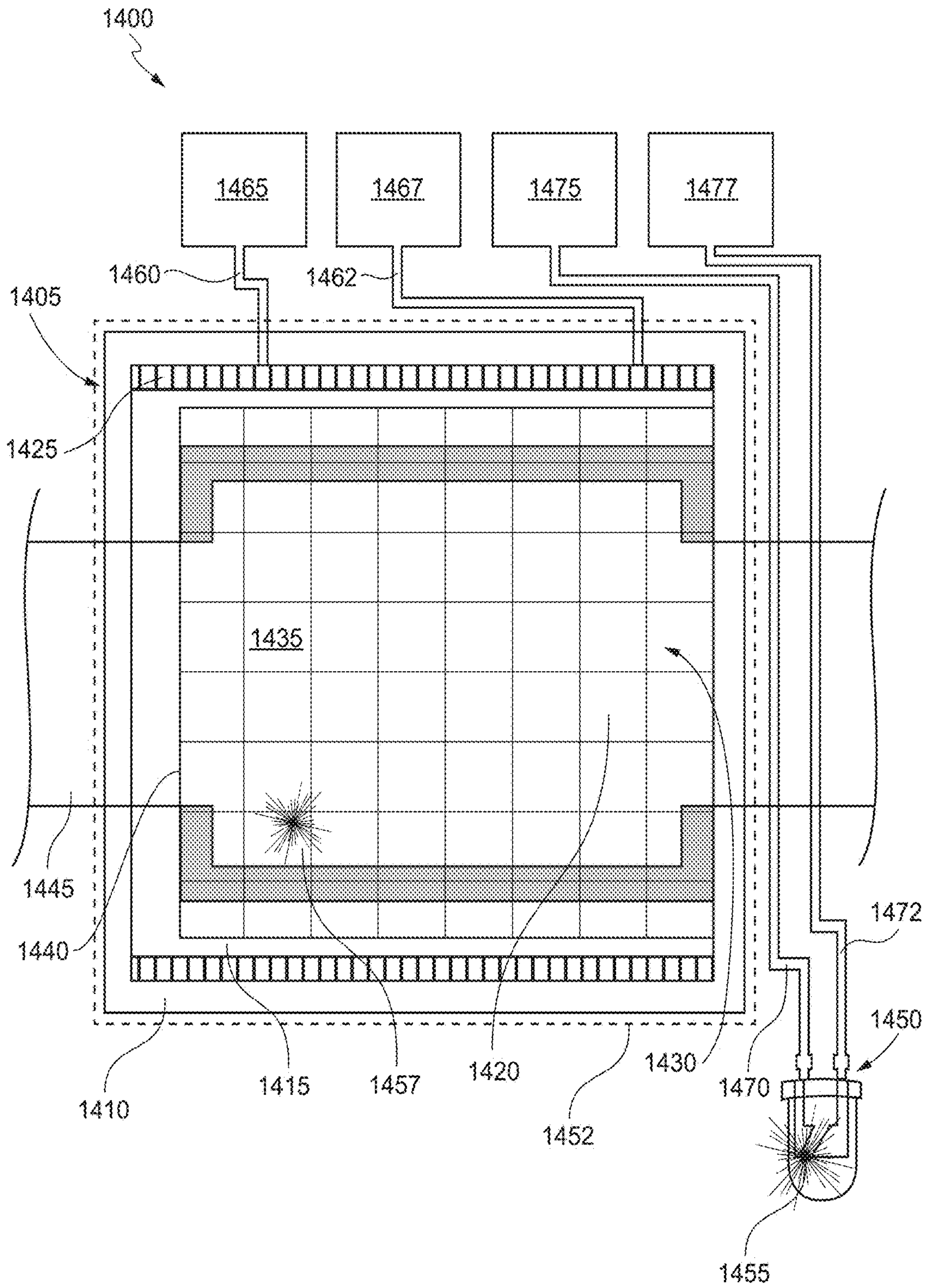


FIG. 14

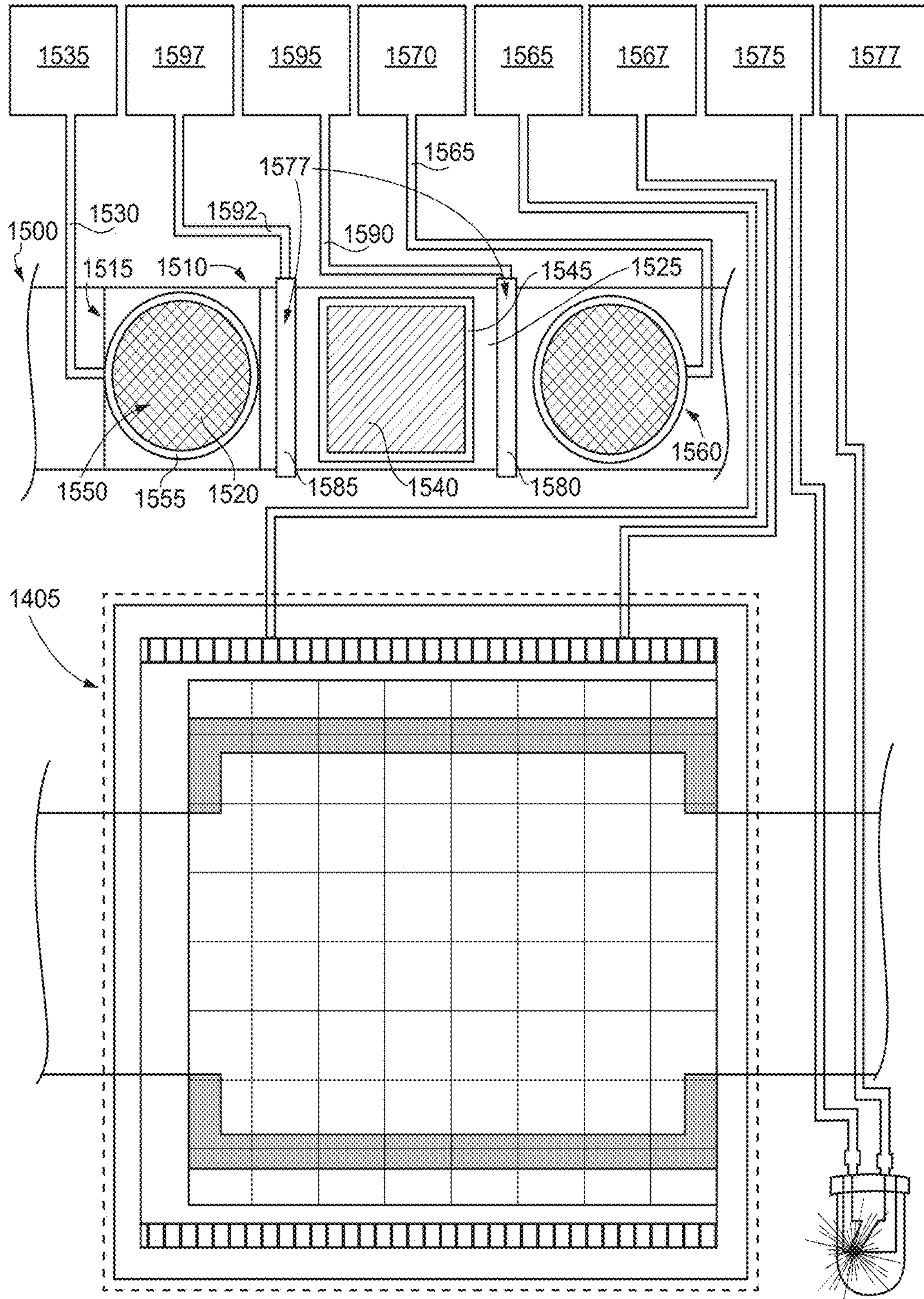


FIG. 15

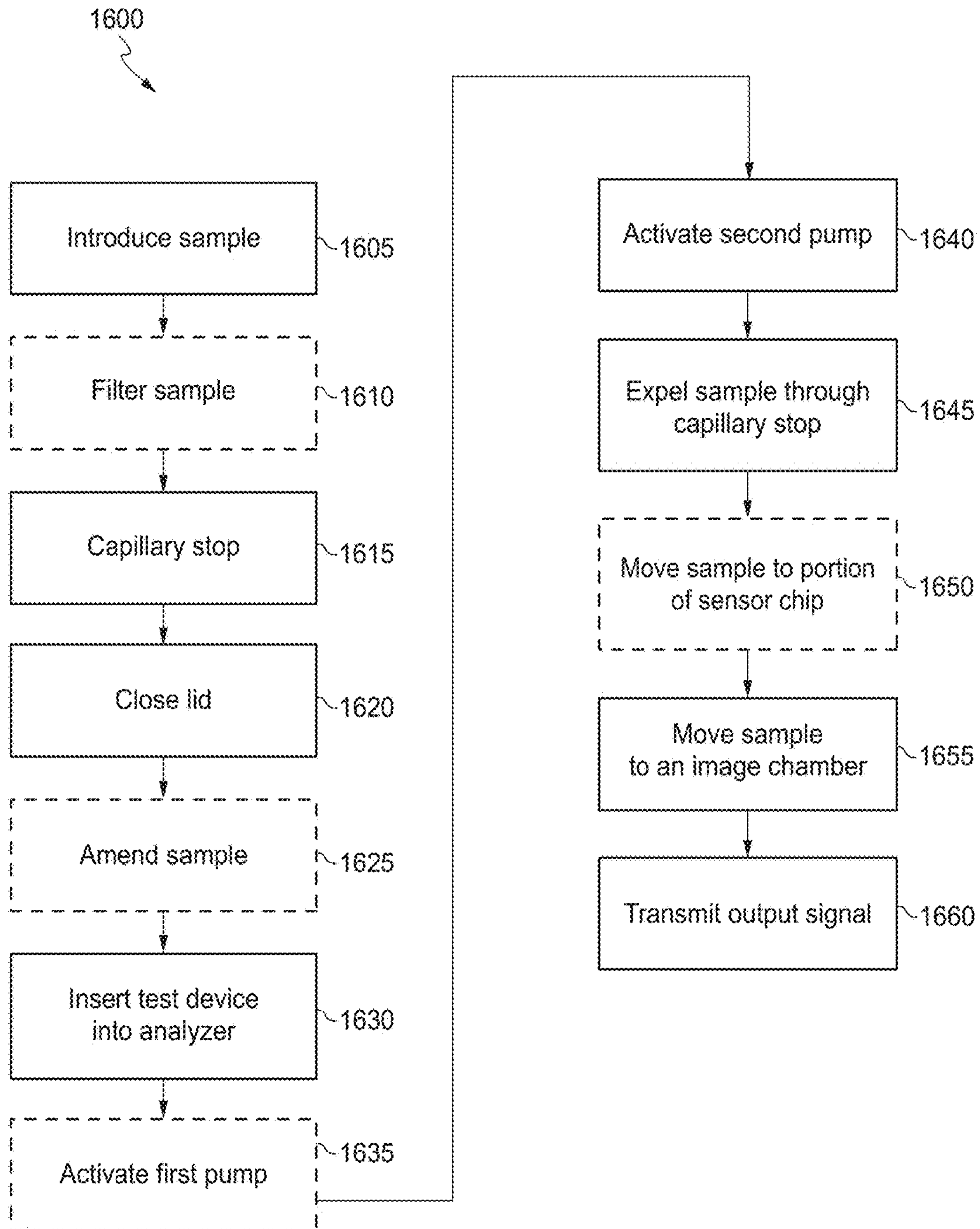


FIG. 16

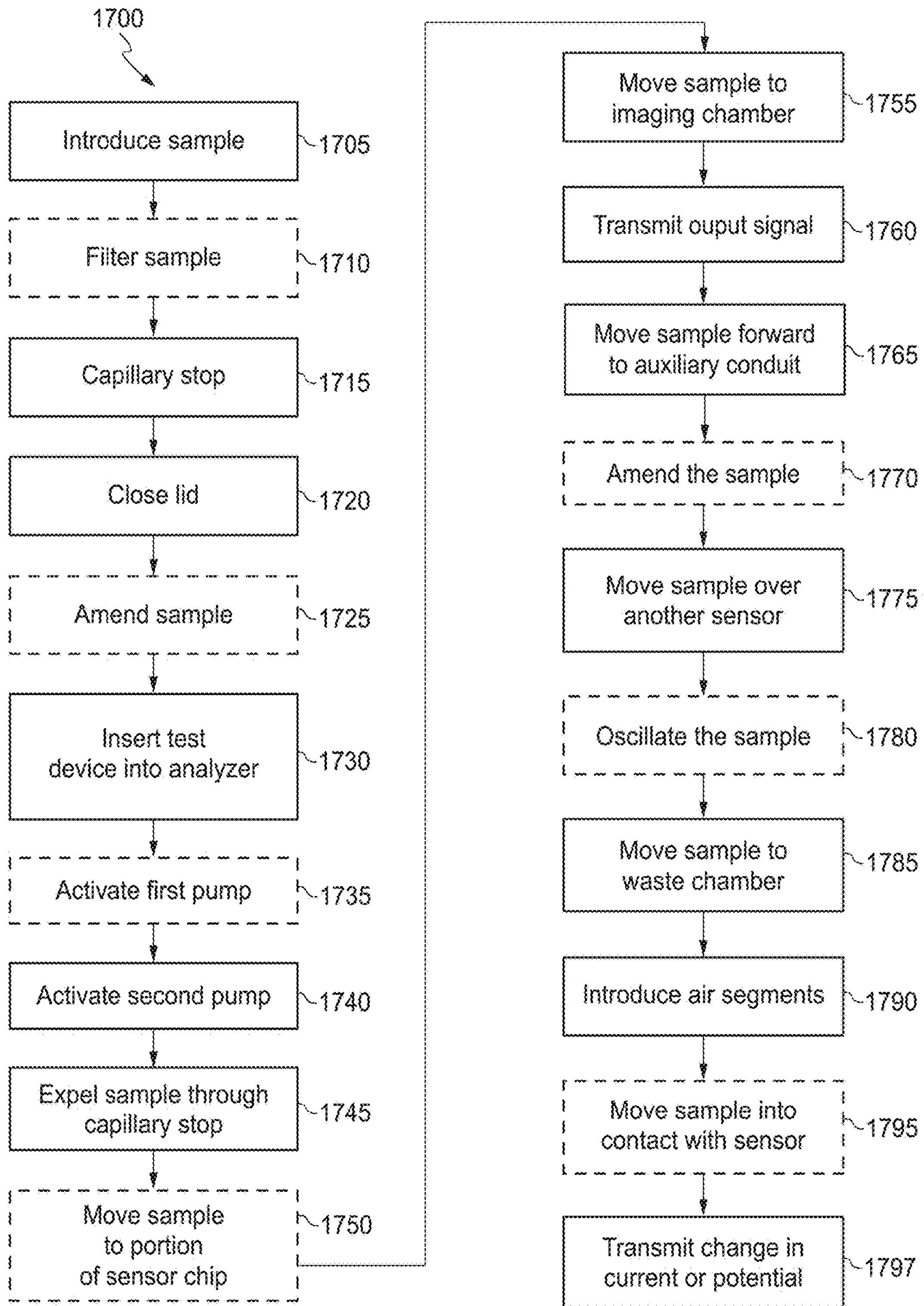


FIG. 17

1800

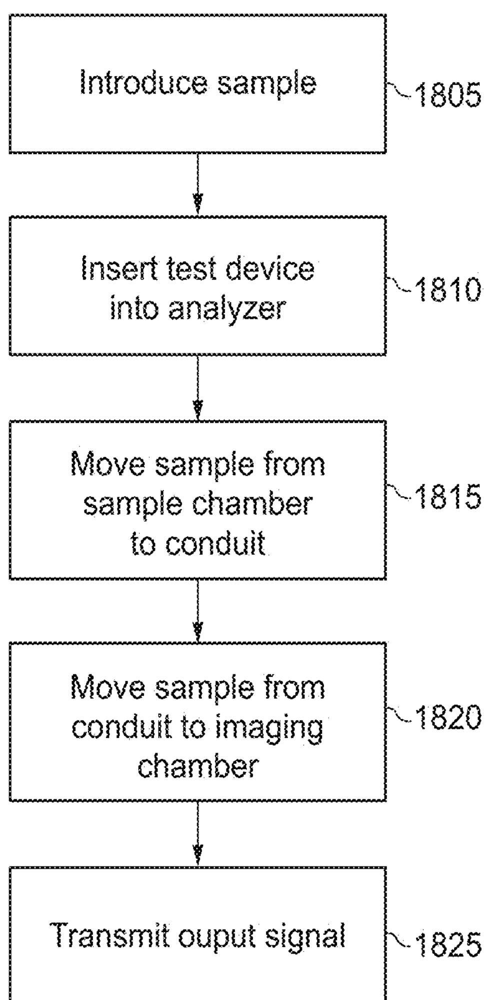


FIG. 18

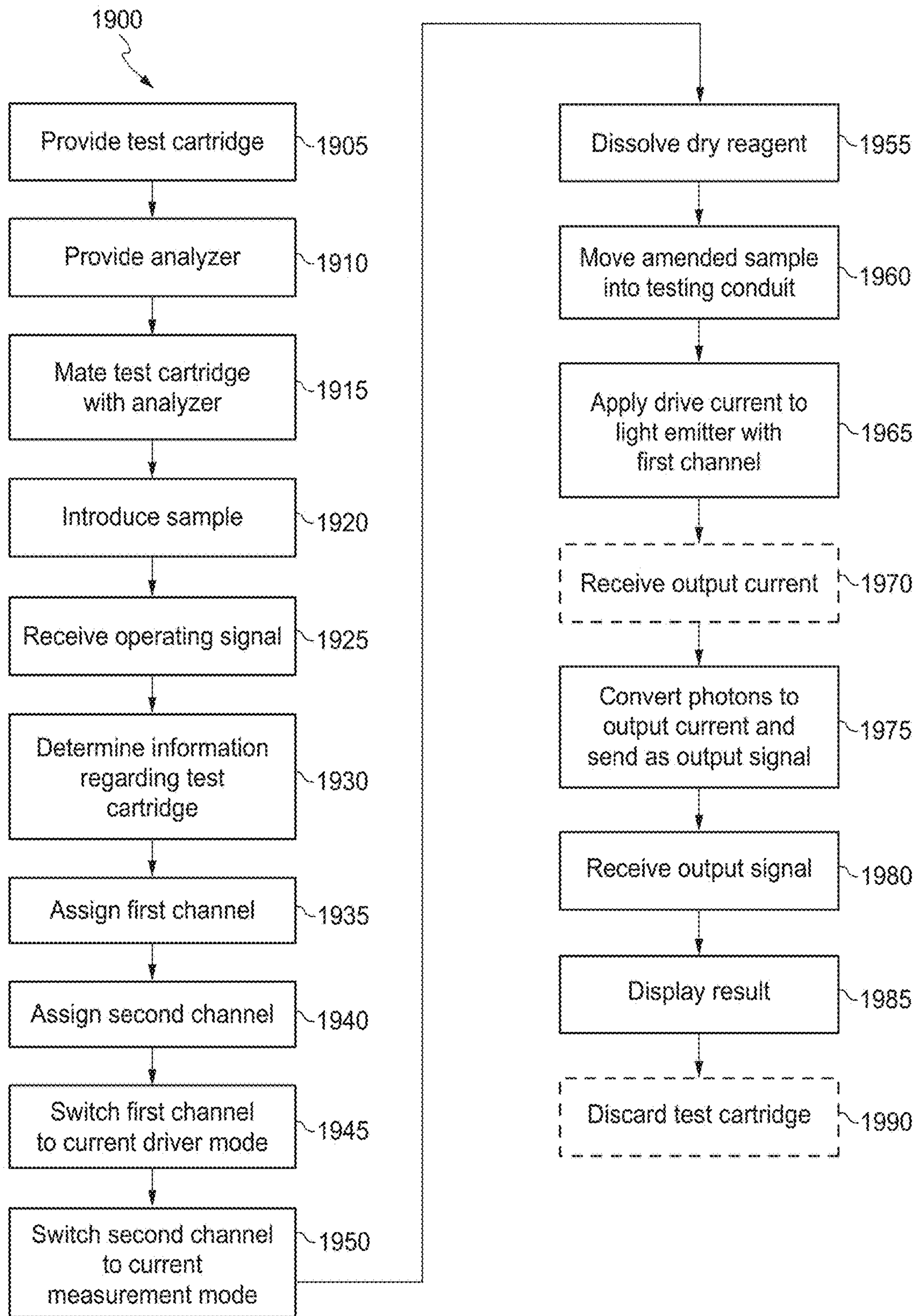


FIG. 19

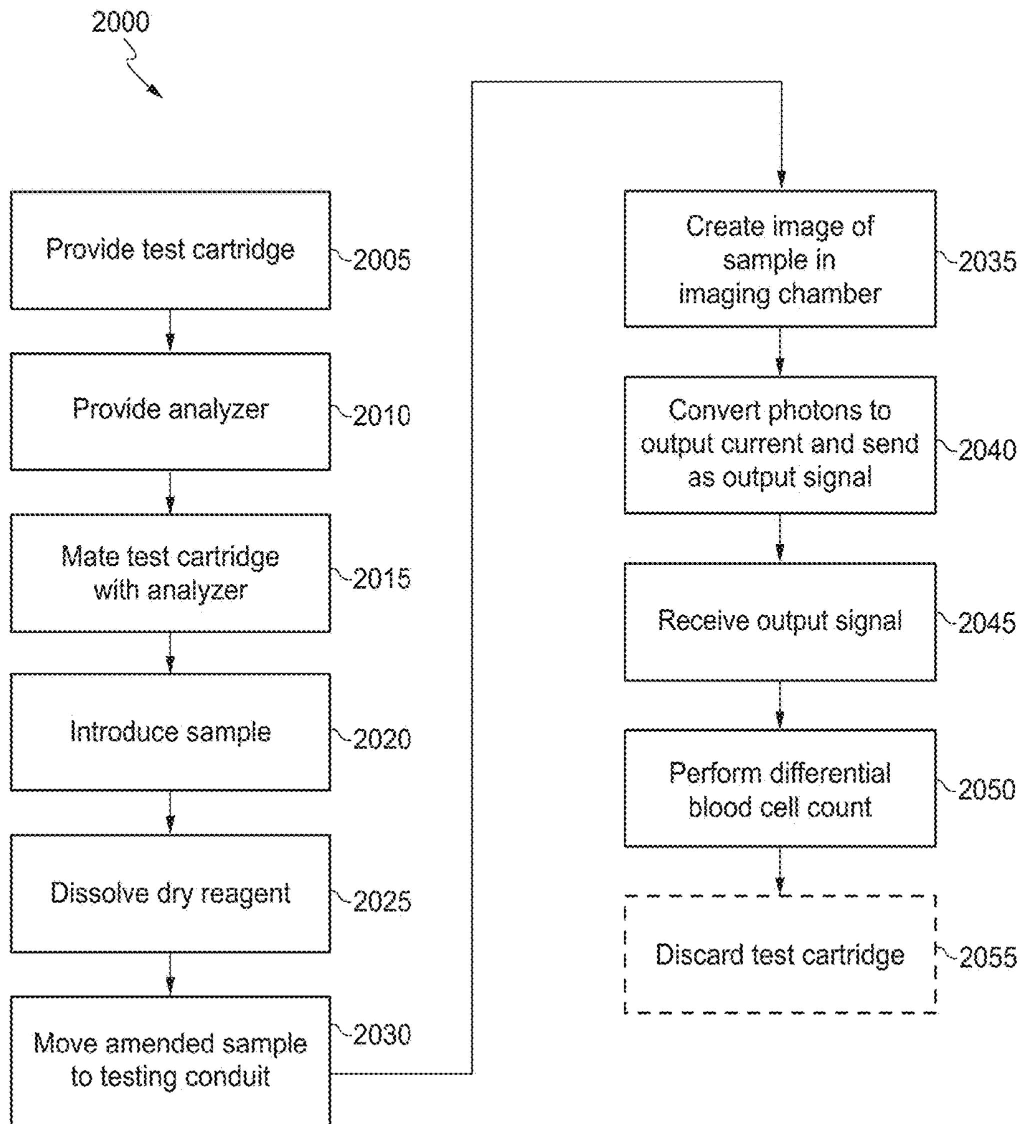


FIG. 20

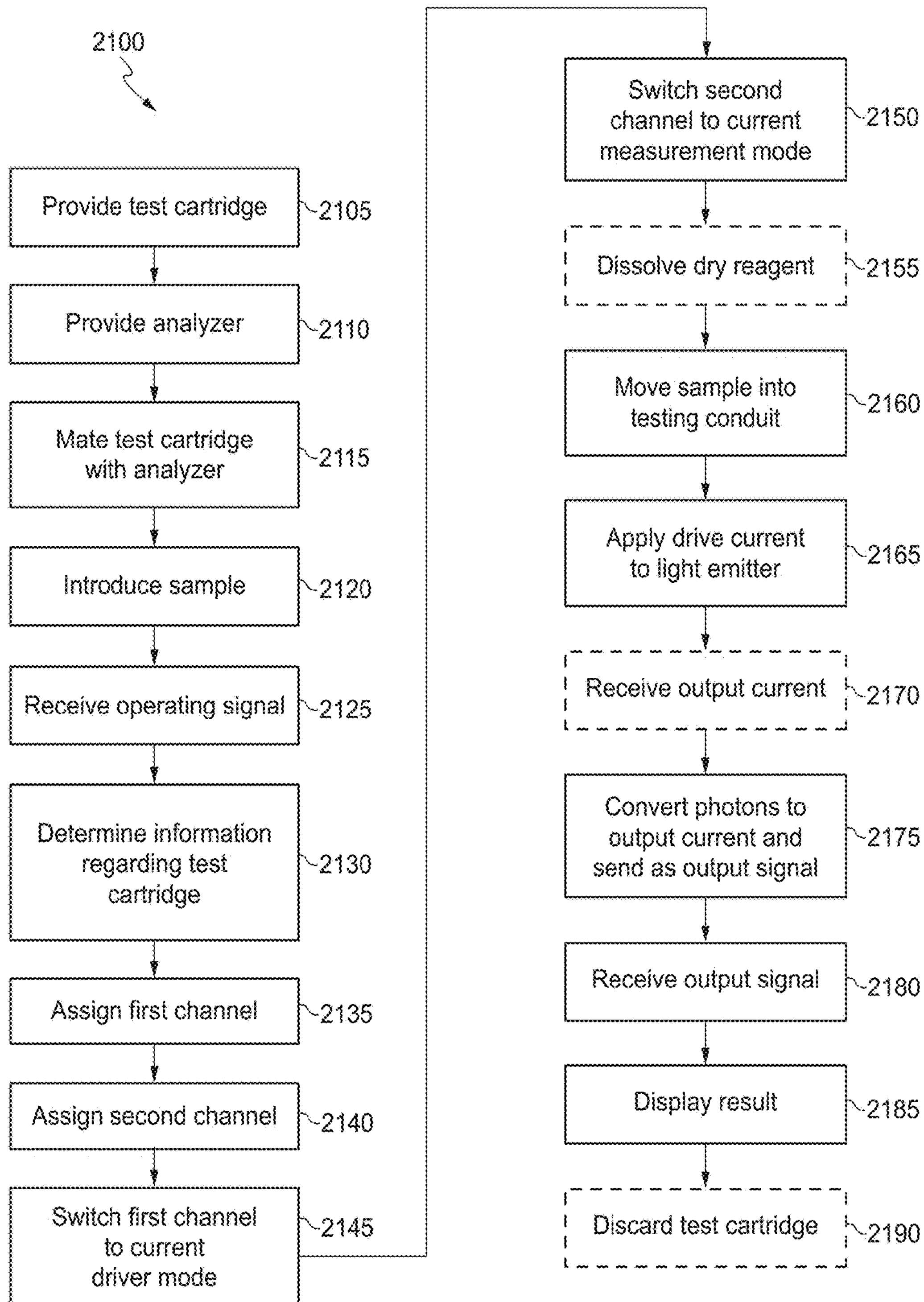


FIG. 21

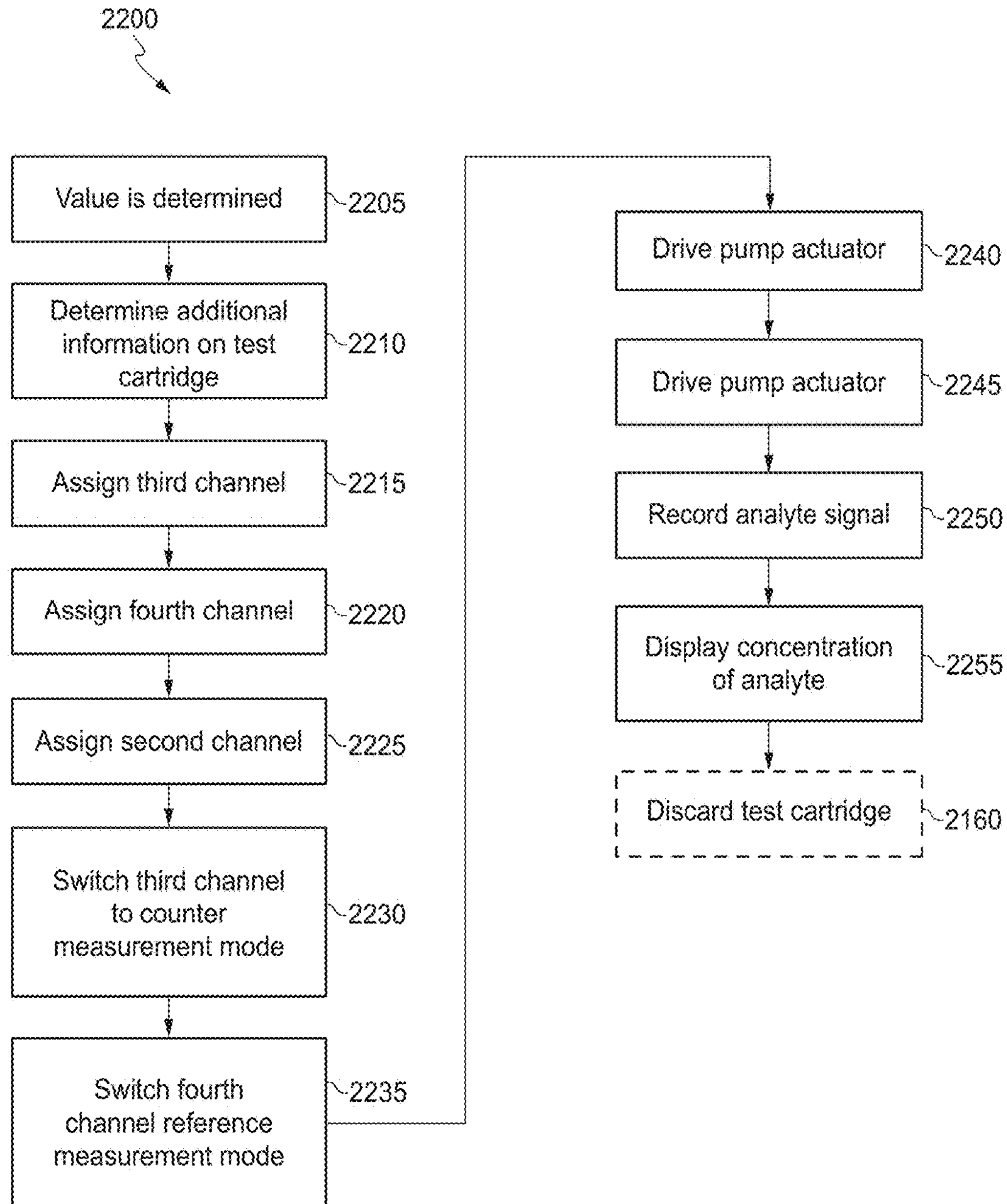


FIG. 22

SINGLE-USE TEST DEVICE FOR IMAGING ASSAY BEADS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/546,713 filed on Aug. 17, 2017 and U.S. Provisional Application No. 62/647,423 filed on Mar. 23, 2018, the entireties of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This present invention relates generally to devices, systems, and methods for performing bioimaging at the microscopic scale and, more particularly, to devices and systems including a disposable testing device configured to perform bioimaging at the microscopic scale, and methods of performing the bioimaging using the disposable testing device.

BACKGROUND OF THE INVENTION

[0003] Point-of-care (POC) sample analysis systems are typically based on one or more re-usable hand-held analyzers (i.e., instruments or reading apparatus) that perform sample tests using a single-use disposable testing device, e.g., a cartridge or strip that contains analytical elements, e.g., electrodes or optics for sensing analytes such as pH, oxygen and glucose, as well as various types of proteins, enzymes and blood cells. The disposable testing device may include fluidic elements (e.g., conduits for receiving and delivering the sample to sensing electrodes or optics), calibrant elements (e.g., aqueous fluids for standardizing the electrodes and optics with a known concentration of analyte), and dyes with known extinction coefficients for standardizing optics. The instrument or reading apparatus may contain electrical circuitry and other components for operating the electrodes or optics, making measurements, and performing computations. The instrument or reading apparatus may also have the ability to display results and communicate those results to laboratory and hospital information systems (LIS and HIS, respectively), for example, via a computer workstation or other data management system. Communication between the instrument or reading apparatus and a workstation, and between the workstation and a LIS or HIS, may be via, for example, an infrared link, a wired connection, wireless communication, or any other form of data communication that is capable of transmitting and receiving electrical information, or any combination thereof. A notable point-of-care system (The i-STAT® System, Abbott Point of Care Inc., Princeton, N.J.) is disclosed in U.S. Pat. No. 5,096,669, which is incorporated herein by reference in its entirety. The i-STAT® System comprises one or more disposable testing devices, operating in conjunction with a hand-held analyzer, for performing a variety of measurements on biological specimens such as blood.

[0004] One benefit of point-of-care sample testing systems is the elimination of the time-consuming need to send a sample to a central laboratory for testing. Point-of-care sample testing systems allow a nurse or doctor (user or operator), at the bedside of a patient, to obtain a reliable quantitative analytical result, comparable in quality to that which would be obtained in a laboratory. In operation, the nurse selects a testing device with the required panel of tests, draws a biological sample from the patient, dispenses the biological sample into the testing device, optionally seals the testing device, and inserts the testing device into the instrument or reading apparatus. While the particular order in which the steps occur may vary between different point-of-care systems and providers, the intent of providing rapid sample test results close to the location of the patient remains the same. The instrument or reading apparatus then performs a test cycle, i.e., all the other analytical steps required to perform the tests. Such simplicity gives the doctor quicker insight into a patient's physiological status and, by reducing the turnaround time for diagnosis or monitoring, enables a quicker decision by the doctor on the appropriate treatment, thus enhancing the likelihood of a successful patient outcome.

[0005] As discussed herein, point-of-care sample testing systems typically include an instrument or analyzer configured to perform sample tests using single-use disposable testing device for the determination of analytes in biological samples. The type of sample tests performed may vary and can be implemented using one or more disposable testing devices including, for example, a qualitative or semi-quantitative testing device (e.g., lateral flow or microarray assays), a quantitative testing device (e.g., an electrochemical assay), or a combined qualitative or semi-quantitative testing device and a quantitative testing device (e.g., a testing device with both lateral flow or microarray assays and an electrochemical assay). In order to perform the sample tests, the instrument or analyzer includes an optical sensor configured to process a signal from the qualitative or semi-quantitative testing device and/or an electrical connector configured to process a signal from the quantitative testing device (see, e.g., U.S. Pat. No. 9,194,859, which is incorporated herein by reference in its entirety). In particular, the optical sensor includes an optical imager configured to image an assay of an optical test cartridge. The assay is a qualitative or semi-quantitative lateral flow test or microarray test (e.g., a one or more lateral flow test strips or microarrays disposed in a conduit of the optical test cartridge). The optical sensor further includes a processor configured to process a signal generated by the optical imager to display a qualitative or semi-quantitative test result.

[0006] However, a problem associated with these conventional instruments or analyzer is that they are incapable of performing bioimaging at the microscopic scale, which is important for hematology assays (e.g., cell counting). Moreover, the optical imager is a set type hardwired to a non-disposable instrument or analyzer, and thus there is limited flexibility in performing multiple types of assays (e.g., hematology and immunoassays) within the testing devices without hardware changes. The limitations imposed by having the optical sensor within the non-disposable instrument or analyzer combined with the hardwired design adversely affects the capability of the instruments or analyzer to perform bioimaging at the microscopic scale and multiple tests or measurements without hardware changes.

[0007] Conventionally, bioimaging at the microscopic scale (e.g., performing hematological assays such as cell counting and differentials) has been performed using lenses, such as with optical microscopy which utilizes objective lenses for magnification of blood cells. Recently, however, imaging without lenses has matured as a modality competitive with conventional lens-based microscopy. In lensless

microscopy, a diffraction pattern resulting from an object (based on, e.g., scattering or fluorescence) is recorded directly on a digital image sensor array without being optically imaged or magnified by any lens elements. The recorded diffraction pattern is then computationally reconstructed to form an "image" of the object(s). The recent maturation of lensless microscopy was made possible largely by the mass production of inexpensive digital image sensors with small pixel size and high pixel counts, along with improvements in computing power and reconstruction algorithms used to process the captured diffraction patterns. Compared with conventional lens-based microscopy, lensless approaches impart several key advantages including: a large space-bandwidth product (large field of view and high resolution simultaneously), high resolution, cost-effectiveness, and portability.

[0008] In order to take advantage of lensless microscopy, an alternative type of assay system has been developed using digital image sensors and specially designed chambers that permit the enumeration of particulate matter (e.g., blood cells) within the sample. For example, U.S. Pat. No. 7,850,916, which is incorporated herein by reference in its entirety, describes a chamber for the enumeration of particulate matter (e.g., blood cells) that includes a first planar member that is flexible, a second planar member, and at least three separators. The characterization of the blood cells (e.g., a white blood cell differential count) within the chamber may be performed by classifying each individual blood cell as it is encountered using either traditional image-processing methods or by the techniques described in U.S. Pat. Nos. 5,321,975, 6,235,536, 6,350,613, 8,797,527, and 9,041,790, US Patent Publication Nos. 20130169948 and 20120034647, and Aydogan Ozcan and Euan McLeod, *Lensless Imaging and Sensing,* Annu. Rev. Biomed. Eng. 2016. 18:77-102, doi:10.1146/annurev-bioeng-092515-010849, all of which are incorporated herein by reference in their entirety.

[0009] However, a problem associated with these lensless microscopy assay systems is that typically one or more components of the system such as the digital image sensor array, the light source, the imaging chamber, and the image processing software/hardware are not designed appropriately to lend themselves to point-of-care utilization. Specifically, these conventional lensless microscopy testing systems lack portability and disposability which are aspects normally associate with point-of-care testing devices. Therefore, there exists within the field of bioimaging at the microscopic scale, and in particular for applications in which cells and analytes are to be determined within biological samples such as blood, a need for devices that can rapidly and simply determine the presence, count, identity, and/or concentration of cells and analytes at a patient's point-of-care, and can be performed by less highly trained staff than is possible for conventional laboratory-based testing. It would, for example, be of benefit in the diagnosis and treatment of critical medical conditions for the attending physician or nurse to be able to obtain optical assay results such as cell counts and differentials at the patient's bedside without delay.

SUMMARY OF THE INVENTION

[0010] In various embodiments, a test device is provided for imaging assay beads, including: a sample entry port for receiving a biological sample. The test device also includes a sample receiving chamber fluidically connected to the sample entry port; and a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit including: (i) a first planar member, (ii) a second planar member, and (iii) a plurality of wells having a predetermined average well height and disposed between the first planar member and the second planar member. The test device also includes where the second planar member includes an imager chip including an array of pixels. The test device also includes where each of the plurality of wells is aligned vertically with one or more of the pixels in the array of pixels.

[0011] Implementations of the test device may include one or more of the following features. The test device where each of the plurality of wells has a width from about 2 μm to about 20 μm. The test device further including a plurality of assay beads. The test device where at least one assay bead from the plurality of assay beads is disposed in each of the plurality of wells. The test device where the plurality of assay beads include a reagent, which includes an antibody, antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, or combinations thereof. The test device where the plurality of assay beads include a reagent, which includes an optical marker dye identifying a type of assay bead. The test device where the plurality of assay beads have a diameter from about 0.1 μm to about 20 μm. The test device where the plurality of assay beads are immobilized in a portion of each of the plurality of wells. The test device where the sample testing conduit further includes a plurality of spacer elements having a predetermined average spacer height and disposed between the first planar member and the second planar member to form a chamber having a predetermined average chamber height extending between the first planar member and the second planar member. The test device where the predetermined average well height plus the predetermined average spacer height is substantially equal to the predetermined average chamber height. The test device where the predetermined average spacer height is substantially equal to the predetermined average chamber height. The test device where: the imager chip includes a substrate and a photosensitive surface. The test device may also include the photosensitive surface includes the array of pixels. The test device may also include the plurality of wells are in direct contact with the photosensitive surface. The test device where the array of pixels include at least 5 mega pixel resolution with at least a 150 ppi pixel density. The test device where each pixel of the array has an area of less than about two μm. The test device further including a light emitter positioned over the sample testing conduit, where the light emitter is configured to transmit light through the chamber to the imager chip at one or more wavelengths from about 300 nm to about 1000 μm. The test device further including a housing, where the imager chip, the sample testing conduit, and the light emitter are housed within the housing.

[0012] In various embodiments, a test device is provided for imaging assay beads, including: a housing including a sample entry port for receiving a biological sample. The test device also includes an imager chip formed in the housing and including an array of pixels, where each pixel of the array has an area of less than about two μm. The test device also includes a sample testing conduit fluidically connected to the sample entry port and including a first wall and a

second wall, where at least a portion of the imager chip forms the first wall. The test device also includes a plurality of wells abutting the portion of the imager chip, where each of the plurality of wells has a width from about 2 μm to about 20 μm. The test device also includes a conformable transparent material layer contacting the plurality of wells and forming the second wall of the sample testing conduit. The test device also includes where each of the plurality of wells is aligned vertically with one or more pixels in the array of pixels and each of the plurality of wells include at least one assay bead.

[0013] Implementations of the test device may include one or more of the following features. The test device where the at least one assay bead includes a reagent, which includes an antibody, antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, or combinations thereof. The test device where the at least one assay bead includes a reagent, which includes an optical marker dye identifying a type of assay bead. The test device where each pixel of the array has an area from about 0.5 μm to about 1.5 μm. The test device where each pixel of the array has an area of about 1.1 μm. The test device where the array of pixels includes at least 5 mega pixel resolution with at least a 150 ppi pixel density. The test device where the imager chip has a width from about 1 mm to about 20 mm and a length from about 1 mm to about 20 mm. The test device where the imager chip has a width of about 5 mm and a length of about 6 mm. The test device where the housing further includes a window adjacent to the transparent material layer for illuminating the sample testing conduit. The test device where at least a portion of the transparent material layer includes one or more fiduciary features for calibration of the imager chip. The test device where at least a portion of the transparent material layer includes one or more fiduciary features for calibration of the biological sample. The test device where the transparent material layer includes a Mylar™ film a polycarbonate film, polyethylene terephthalate (pet), or cyclo-olefin polymer (cop).

[0014] Implementations of the test device may also include one or more of the following features. The test device where at least a portion of the sample testing conduit has a width from about 0.5 mm to about 2 cm. The test device where at least a portion of the sample testing conduit has a length from about 0.5 mm to about 2 cm. The test device further including a light emitter positioned over the sample testing conduit, where the light emitter is configured to transmit light through the chamber to the imager chip at one or more wavelengths from about 300 nm to about 1000 μm. The test device where the light emitter is located adjacent to the transparent material layer. The test device where the light emitter is one or more light-emitting diodes. The test device where the imager chip is configured to measure absorbance. The test device where the imager chip includes a filter layer and is configured to measure fluorescence. The test device further including a pump configured to move the biological sample from the sample entry port into the sample testing conduit. The test device further including an auxiliary conduit fluidically connected to the sample entry port and including an electrochemical sensor for detecting an analyte in the biological sample. The test device further including an auxiliary conduit fluidically connected to the sample entry port and including a conductivity sensor for detecting a position of the biological sample in the auxiliary conduit. The test device where the sample testing conduit further includes a plurality of spacer elements having a predetermined average spacer height and disposed between the first wall and the second wall to form a chamber having a predetermined average chamber height extending between the first wall and the second wall. The test device where the plurality of wells have a predetermined average well height, and the predetermined average well height plus the predetermined average spacer height is substantially equal to the predetermined average chamber height. The test device where the predetermined average spacer height is substantially equal to the predetermined average chamber height.

[0015] In various embodiments, a system is provided for imaging assay beads, including: an analyzer including: a port, a multi-terminal connector, a processor connected to the multi-terminal connector, and memory coupled to the processor; and a test cartridge including:. The system also includes a plurality of discrete connector contacts. The system also includes a sample receiving chamber configured to receive a biological sample, a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit including: (i) a first wall, (ii) a second wall, and (iii) a plurality of wells having an average well height and disposed between the first wall and the second wall, and an analyte assay region including: a portion of the sample testing conduit and an imager chip, where the imager chip is electrically connected to at least one of the plurality of discrete connector contacts, and at least a portion of the imager chip forms a portion of the first wall of the sample receiving chamber. The system also includes where each of the plurality of wells is aligned vertically with one or more pixels of the imager chip and each of the plurality of wells include at least one assay bead. The system also includes where the test cartridge is insertable into the port such that the multi-terminal connector is in electrical contact with the plurality of discrete connector contacts. The system also includes where the memory is encoded with a set of instructions configured to perform an analytical test on the biological sample. The system also includes where to perform the analytical test, (i) the processor is electrically connected to a light emitter, (ii) the processor is electrically connected to the imager chip via at least one of the plurality of discrete connector contacts and the multi-terminal connector, (iii) the processor is configured to drive the light emitter to generate light projected into the portion of the sample testing conduit, (iv) the imager chip is configured to convert light received from the portion of the sample testing conduit to an output signal, and (v) the processor is configured to convert the output signal of the imager chip to a value indicative of a reaction of the biological sample with the at least one assay bead in each of the plurality of wells.

[0016] Implementations of the system may include one or more of the following features. The system where each of the plurality of wells has a width from about 2 μm to about 20 μm. The system where the at least one assay bead includes a reagent, which includes an antibody, antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, or combinations thereof. The system where the at least one assay bead includes a reagent, which includes an optical marker dye identifying a type of assay bead. The system where the at least one assay bead has a diameter from about 0.1 μm to about 20 μm. The system where the at least one assay bead is immobilized in a portion of each of the plurality of wells.

The system where the test cartridge includes the light emitter, and the light emitter is electrically connected to at least one of the plurality of discrete connector contacts. The system where the test cartridge further includes a housing, where the imager chip, the sample testing conduit, and the light emitter are housed within the housing. The system where the analyzer includes the light emitter, the test cartridge further includes a housing including a window adjacent to the sample testing conduit for illuminating the portion of the sample testing conduit, and the test cartridge is insertable into the port such that the light emitter is aligned over the window and the portion of the sample testing conduit. The system where the analyzer further includes a pump actuator, the test cartridge further includes a pump, and the test cartridge is insertable into the port such that the pump actuator is aligned with the pump.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0017] The present invention will be better understood in view of the following non-limiting figures.

[0018] FIG. **1** shows a disposable testing device and instrument in accordance with various embodiments;

[0019] FIG. **2** shows an illustrative architecture of a computing system implemented in accordance with various embodiments;

[0020] FIGS. **3** and **4A-4**R show a testing device or cartridge in accordance with various embodiments;

[0021] FIG. **5** shows an imaging device in accordance with various embodiments;

[0022] FIG. **6** shows a cross-section of an imaging chamber in accordance with various embodiments;

[0023] FIG. **7** shows a top planar view of an imaging chamber in accordance with various embodiments;

[0024] FIG. **8** shows an imaging chamber comprising a first planar member, a second planar member, and a plurality of spacer elements disposed between the first planar member and the second planar member in accordance with various embodiments;

[0025] FIG. **9** illustrates the plurality of spacer elements of the imaging chamber may be formed from a material that has greater flexibility than the first planar member and the second planar member in accordance with various embodiments;

[0026] FIG. **10** illustrates the second planar member of the imaging chamber may be formed from a material that has greater flexibility than the plurality of spacer elements and the first planar member in accordance with various embodiments;

[0027] FIGS. **11A-11**I show an imaging chamber comprising a first planar member, a second planar member, and a plurality of wells disposed between the first planar member and the second planar member in accordance with various embodiments;

[0028] FIG. **12** shows wafer-level micro-fabrication of an imager chip in accordance with various embodiments;

[0029] FIG. **13** shows wafer-level micro-fabrication of an electrochemical chip in accordance with various embodiments;

[0030] FIG. **14** shows a sensor chip configuration in accordance with various embodiments;

[0031] FIG. **15** shows an alternative sensor chip configuration in accordance with various embodiments; and

[0032] FIGS. **16-22** show exemplary flowcharts for performing process steps in accordance with various embodiments.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0033] Various embodiments of the present invention are directed to devices, systems, and methods for performing optical and optionally electrochemical assays. For example, FIG. **1** shows an exemplary system **100** that may comprise a self-contained disposable testing device or cartridge **105** and an instrument or reading apparatus **110** (e.g., an analyzer) that is portable or stationary and battery powered or line powered. In some embodiments, the testing device **105** is a single-use device configured to be disposable after the single-use. A fluid sample (e.g., whole blood) to be measured is drawn into a sample receiving chamber via a sample entry orifice **115** in the testing device **105**, and the testing device **105** may be inserted into the analyzer **110** through a port **120**. The analyzer **110** may comprise a processor configured to perform processes including but not limited to: driving a light emitter, optical sensor, a pump, and/or electrochemical sensor; obtaining an output signal of at least one of absorbance and fluorescence (optical), current (amperometric), potential or charge accumulation (potentiometric), a conductive property of a medium between electrodes (conductometric), and impedance (both resistance and reactance); and converting the output signal to: (i) a number count or percentage for each type of cell in a blood sample, or (ii) a value indicative of a reaction of the biological sample with at least one assay bead. Measurements and determinations performed by the analyzer **110** (for example, (i) the number count or percentage for each type of cell in the blood sample, or (ii) the value indicative of the reaction) may be output to a display **125** or other output device, such as a printer or data management system **130** via a port **135** on the analyzer **110** to a computer port **140**. Transmission can be via wired or wireless communication such as a telephone network, Internet connection, Wi-Fi, Bluetooth link, infrared and the like. The sensor(s) **145** (e.g., an optical sensor) in the testing device **105** include a plurality of discrete connector contacts **150** that make electrical contact with the analyzer **110** via a multi-terminal connector **155** when the testing device **105** is inserted into the port **140**. For example, the multi-terminal connector **155** may be of the design disclosed in U.S. Pat. No. 4,954,087, which is incorporated herein by reference in its entirety. The analyzer **110** may further comprises a pump actuator **160** and the testing device **105** may further comprise a pump **165**. In some embodiments, the inserting the testing device **105** into the port **120** of the analyzer **105** places the pump actuator **160** aligned with the pump **165**, and the fluid sample may be moved into a sample testing conduit of the testing device **105** by driving the pump actuator **160** to actuate the pump **165** and displace the fluid sample into the sample testing conduit. In certain embodiments, the analyzer **110** is further configured to perform a method for automatic fluid flow compensation in the testing device **105**, as disclosed in U.S. Pat. No. 5,821,399, which is also incorporated herein by reference in its entirety.

[0034] To specifically address problems associated with conventional instruments or reading apparatus, some

embodiments described herein are directed to devices and systems including a disposable testing device configured to perform bioimaging at the microscopic scale, and methods of performing the bioimaging using the disposable testing device. In one embodiment, a test device for imaging blood cells in a blood sample is provided that includes: a sample entry port for receiving the blood sample; a sample receiving chamber fluidically connected to the sample entry port; a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit comprising: (i) a planar member, (ii) a transparent planar member, and (iii) a plurality of spacer elements having an average spacer height and disposed between the planar member and the transparent planar member to form a chamber having an average chamber height extending between the planar member and the transparent planar member; and an imager chip forming at least a portion of the planar member.

[0035] In another embodiments, a system for imaging blood cells in a blood sample is provided for that comprises: an analyzer comprising: a port, a multi-terminal connector, a processor connected to the multi-terminal connector, and memory coupled to the processor; and a test cartridge comprising: a plurality of connector contacts, a sample receiving chamber configured to receive the blood sample, a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit comprising: (i) a first wall, (ii) a second wall, and (iii) a plurality of spacer elements having a predetermined average spacer height and disposed between the first wall and the second wall to form a chamber having a predetermined average chamber height extending between the first wall and the second wall, and an analyte assay region comprising: a portion of the chamber and an imager chip, wherein the imager chip is electrically connected to at least one of the plurality of connector contacts, and at least a portion of the imager chip forms a portion of the first wall of the sample receiving chamber. The test cartridge is insertable into the port such that the multi-terminal connector is in electrical contact with the plurality of connector contacts.

[0036] In another embodiment, a method is provided for performing a differential blood cell count comprising: providing a test cartridge comprising a sample entry port, a sample testing conduit fluidically connected to the sample entry port, and an imager chip comprising an array of pixels; providing an analyzer comprising a processor and display; mating the test cartridge with the analyzer; introducing a blood sample into the sample entry port before or after the mating the test cartridge with the analyzer; dissolving a dry reagent into the blood sample to generate an amended blood sample; moving the amended blood sample into the sample testing conduit, wherein the sample testing conduit comprises a first wall formed from at least a portion of an imager chip, a second wall formed from a transparent material layer, and a plurality of spacer elements having an average spacer height and disposed between the first wall and the second wall, and wherein the average spacer height defines an average chamber height of a chamber between the portion of the imager chip and the transparent material layer; driving a light emitter to project light through the chamber and the amended blood sample; recording an output signal of at least one of absorbance and fluorescence at the array of pixels based on the light received from the chamber and the amended blood sample; converting the output signal using the processor to a number count or percentage for each type of cell in the blood sample; and displaying the number count or percentage for each type of cell in the blood sample on the display.

[0037] In another embodiment, a test device is provided for imaging assay beads, comprising: a sample entry port for receiving a biological sample; a sample receiving chamber fluidically connected to the sample entry port; and a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit comprising: (i) a first planar member, (ii) a second planar member, and (iii) a plurality of wells having a predetermined average well height and disposed between the first planar member and the second planar member. The second planar member comprises an imager chip comprising an array of pixels; and each of the plurality of wells is aligned vertically with one or more of the pixels in the array of pixels.

[0038] In another embodiment, a system is provided for imaging assay beads, comprising: an analyzer comprising: a port, a multi-terminal connector, a processor connected to the multi-terminal connector, and memory coupled to the processor; and a test cartridge comprising: a plurality of discrete connector contacts, a sample receiving chamber configured to receive a biological sample, a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit comprising: (i) a first wall, (ii) a second wall, and (iii) a plurality of wells having an average well height and disposed between the first wall and the second wall, and an analyte assay region comprising: a portion of the sample testing conduit and an imager chip, wherein the imager chip is electrically connected to at least one of the plurality of discrete connector contacts, and at least a portion of the imager chip forms a portion of the first wall of the sample receiving chamber. Each of the plurality of wells is aligned vertically with one or more pixels of the imager chip and each of the plurality of wells comprise at least one assay bead. The test cartridge is insertable into the port such that the multi-terminal connector is in electrical contact with the plurality of discrete connector contacts. The memory is encoded with a set of instructions configured to perform an analytical test on the biological sample, and to perform the analytical test, (i) the processor is electrically connected to a light emitter, (ii) the processor is electrically connected to the imager chip via at least one of the plurality of discrete connector contacts and the multi-terminal connector, (iii) the processor is configured to drive the light emitter to generate light projected into the portion of the sample testing conduit, (iv) the imager chip is configured to convert light received from the portion of the sample testing conduit to an output signal, and (v) the processor is configured to convert the output signal of the imager chip to a value indicative of a reaction of the biological sample with the at least one assay bead in each of the plurality of wells.

[0039] In another embodiment, a method is provided for imaging assay beads comprising: mating a test cartridge with an analyzer, wherein the test cartridge comprises a sample entry port, a sample testing conduit fluidically connected to the sample entry port, and an imager chip, and the analyzer comprises a processor and display; introducing a biological sample into the sample entry port before or after the mating the test cartridge with the analyzer; moving the biological sample into the sample testing conduit, wherein the sample testing conduit comprises a first wall formed from at least a portion of an imager chip, a second wall formed from a transparent material layer, and a plurality of

wells having an average well height and disposed between the first wall and the second wall, and wherein each of the plurality of wells is aligned vertically with one or more pixels of the imager chip and at least a portion of the plurality of wells comprise at least one assay bead; driving a light emitter to project light through the plurality of wells; recording an output signal of at least one of absorbance and fluorescence at the pixels of the imager chip based on the light received from the plurality of wells; converting the output signal using the processor to a value indicative of a reaction of the biological sample with the at least one assay bead in each of the plurality of wells; and displaying the value on the display.

[0040] Advantageously, these approaches provide devices, systems, and methods with greater flexibility in testing device design including: (i) the combination of tests in any given testing device, (ii) the combination of tests on any given sensor chip, (iii) the position of sensors within the testing device, (iv) extending utility of the analyzers to perform various types of assays without hardware changes, and (v) increasing the point-of-care testing opportunities. In addition, these approaches may also reduce the number of different testing device bases (which accommodate the sensor chips) used to manufacture all the different testing devices for the various test. In addition, these approaches allow for bioimaging at the microscopic scale, and in particular for applications in which the count and/or identification of cells and optionally the concentration of analytes at patient point-of-care can be performed by less highly trained staff than is possible for conventional laboratory-based testing.

System Environment

[0041] FIG. **2** is an illustrative architecture of a computing system **200** implemented in various embodiments. The computing system **200** is only one example of a suitable computing system and is not intended to suggest any limitation as to the scope of use or functionality of the various embodiments. Also, computing system **200** should not be interpreted as having any dependency or requirement relating to any one or combination of components illustrated in computing system **200**.

[0042] As shown in FIG. **2**, computing system **200** includes a computing device **205**. The computing device **205** can be resident on a network infrastructure such as within a cloud environment, or may be a separate independent computing device (e.g., a computing device implemented within the environment of an analyzer such as analyzer **110** as described with respect to FIG. **1**)). The computing device **205** may include one or more input devices **210**, one or more output devices **212**, a bus **215**, processor **220**, a storage device **225**, a system memory (hardware device) **230**, and a communication interface **235**.

[0043] The one or more input devices **210** may include one or more mechanisms that permit an operator to input information to computing device **205**, such as, but not limited to, a touch pad, dial, click wheel, scroll wheel, touch screen, one or more buttons (e.g., a keyboard), mouse, game controller, track ball, microphone, camera, proximity sensor, light detector, motion sensors, biometric sensor, and combinations thereof. The one or more output devices **212** may include one or more mechanisms that output information to an operator, such as, but not limited to, audio speakers, headphones, audio line-outs, visual displays, antennas, infrared ports, tactile feedback, printers, or combinations thereof. The bus **215** permits communication among the components of computing device **205**. For example, bus **215** may be any of several types of bus structures including a memory bus or memory controller, a peripheral bus, and a local bus using any of a variety of bus architectures to provide one or more wired or wireless communication links or paths for transferring data and/or power to, from, or between various other components of computing device **205**.

[0044] The processor **220** may be one or more integrated circuits, printed circuits, controllers, microprocessors, or specialized dedicated processors that include processing circuitry operative to interpret and execute computer readable program instructions, such as program instructions for controlling the operation and performance of one or more of the various other components of computing device **205** for implementing the functionality, steps, and/or performance of the embodiments discussed herein. In certain embodiments, processor **220** interprets and executes the processes, steps, functions, and/or operations, which may be operatively implemented by the computer readable program instructions. For example, processor **220** can receive an operating state signal from a test cartridge indicative of a type of cartridge inserted into an analyzer; determine that the type of cartridge is the test cartridge having a contact connected to a sensor chip configured to image blood cells in a blood sample; drive a pump actuator to actuate a pump on the test cartridge and move the blood sample from a sample receiving chamber into a sample testing conduit, drive a light emitter to project light through the sample testing conduit and the blood sample, record an output signal of at least one of absorbance and fluorescence at an array of pixels of a sensor chip based on the light received from the sample testing conduit and the blood sample, and convert the output signal to a number count or percentage for each type of cell in the blood sample. In some embodiments, the information obtained or generated by the processor **220**, e.g., type of test cartridge, number count or percentage for each type of cell, circuit configurations for channels, a tally for various operations, output current, look-up tables, potential to be applied, etc., can be stored in the storage device **225**. In certain embodiments, the processor **220** comprises a thermal controller for controlling a temperature of the biological sample or specimen in a portion of a conduit.

[0045] In various embodiments, the processor **220** comprises an application-specific integrated circuit **240** that includes universal channel circuitry **245**, and analog to digital signal converter **247**. In other embodiments, the processor **220** is in communication with the application-specific integrated circuit **240** that includes the universal channel circuitry **245**. The application-specific integrated circuit **240** is an integrated circuit (IC) customized for performing a number of functions including an analog to digital signal interface, current to voltage conversion, multiplexing, resistor selection, signal amplification, potential and conductance generation and/or measurement, and the performance of multiple types of assays. The universal channel circuitry **245** includes circuitry that can be implemented in conjunction with computer readable program instructions, data structures, program modules and other data to switch between various modes or configurations (e.g., a potentiometric mode, an amperometric mode, a

conductance mode, an optical mode, etc.) and contribute to the performance of multiple types of assays.

[0046] The storage device **225** may include removable/non-removable, volatile/non-volatile computer readable media, such as, but not limited to, non-transitory machine readable storage medium such as magnetic and/or optical recording media and their corresponding drives. The drives and their associated computer readable media provide for storage of the computer readable program instructions, data structures, program modules and other data for operation of computing device **205**. In various embodiments, storage device **225** stores operating system **250**, application programs **255**, and/or program data **260**. In some embodiments, the application programs **255**, and/or program data **260** may include a database, index, or table, and algorithms, for example, firmware or software for image analysis, data storage, illumination control, data display, sample analysis, and sample movement. In some embodiments, the computing system **200** implements algorithms, which provide the instructions for execution of processor **220**, to enhance, detect, analyze, characterize, and measure images of cells and other specimens of interest and to display or transmit the result of these algorithms to a human operator and/or a second computer-based system, such as a personal computing device (e.g., a smartphone) or storage system including hospital record storage systems. In certain embodiments, the computing system **200** implements qualitative, semi-quantitative, or quantitative value algorithms that include components for determining a presence and/or amount of target analyte in a biological specimen or sample, a position determining algorithm for determining the location of a biological sample within a test device based on detected conductance, a cell count and differential algorithm, and a hematocrit determination algorithm for determining a hematocrit of a biological sample based on detected conductance across a biological sample, which provide the instructions for execution of processor **220**.

[0047] The system memory **230** may include one or more storage mediums, including for example, non-transitory machine readable storage medium such as flash memory, permanent memory such as read-only memory ("ROM"), semi-permanent memory such as random access memory ("RAM"), any other suitable type of non-transitory storage component, or any combination thereof. In some embodiments, an input/output system **265** (BIOS) including the basic routines that help to transfer information between the various other components of computing device **205**, such as during start-up, may be stored in the ROM. Additionally, data and/or program modules **270**, such as at least a portion of operating system **250**, application programs **255**, and/or program data **260**, that are accessible to and/or presently being operated on by processor **220**, may be contained in the system memory **230**.

[0048] The communication interface **235** may include any transceiver-like mechanism (e.g., a network interface, a network adapter, a modem, or combinations thereof) that enables computing device **205** to communicate with remote devices or systems, such as other analyzers, a hospital information system, a mobile device or other computing devices such as, for example, a server in a networked environment, e.g., cloud environment. For example, computing device **205** may be connected to remote devices or systems via one or more local area networks (LAN) and/or one or more wide area networks (WAN) using communication interface **235**.

[0049] As discussed herein, computing system **200** may be configured to perform one or more analytical tests (e.g., a hematology assay). In particular, computing device **205** may perform tasks (e.g., process, steps, methods and/or functionality) in response to processor **220** executing program instructions contained in non-transitory machine readable storage medium, such as system memory **230**. The program instructions may be read into system memory **230** from another computer readable medium (e.g., non-transitory machine readable storage medium), such as data storage device **225**, or from another device via the communication interface **235** or server within or outside of a cloud environment. In some embodiments, hardwired circuitry of computing system **200** is used in place of or in combination with the program instructions to implement the tasks, e.g., steps, methods and/or functionality, consistent with the different aspects discussed herein. Thus, the steps, methods and/or functionality disclosed herein can be implemented in any combination of hardware circuitry and software.

Testing Device or Cartridge

[0050] In one embodiment, as shown in FIG. **3**, a testing device or cartridge **300** (e.g., testing device **105** as described with respect to FIG. **1**) comprises a top portion **305** (e.g., a cover) and a bottom portion **310** (e.g., a base) in which are mounted at least one microfabricated sensor chip **315** disposed on a imager chip carrier **320** (e.g., a substrate) with electrical contacts **325** and optionally a pouch **330** containing a fluid, e.g., a calibrant fluid, a diluent fluid, a reagent, and/or a wash fluid. In some embodiments, the composition of the fluid in the pouch **330** is selected from the group consisting of water, calibrant fluid, reagent fluid, control fluid, wash fluid and combinations thereof. The sensor chip **315** and imager chip carrier **320** may be positioned in recessed region **335** and configured to generate electric signals based on, for example, light transmitted through an imaging chamber **340** of the sensor chip **315** and a biological specimen, e.g., a blood sample from a patient. A gasket **345** may be situated between the top portion **305** and the bottom portion **310** to bond them together, and to define and seal several cavities and conduits within the cartridge **300**. The gasket **345** may cover substantially the entire area between the top portion **305** and the bottom portion **310** of the cartridge **300**, as shown in FIG. **3**, or may be localized over and between only predetermined structural features, e.g., the sensor chip **315** of the cartridge **300** (not shown). The gasket **345** may include apertures **350** to enable physical, fluidic and/or gaseous communication between structural features of the top portion **305** and the bottom portion **310**. The gasket **345** may or may not have an adhesive surface, and may have an adhesive surface on both sides thereof, i.e., forming a double-sided adhesive layer. In some embodiments, in which a light emitter is provided by an external element such as the analyzer (e.g., the reading apparatus **110** as discussed with respect to FIG. **1**), the top portion **305** and the gasket **345** (or optionally the bottom portion **310**) comprise a transparent window or cut-out **355**, **360**, respectively (shown in top portion **305** and the gasket **345** for illustrative purposes). In other embodiments, in which a light emitter **365** is provided within the testing device or cartridge **300**, the light emitter **365** is provided on the sensor

chip **315** or imager chip carrier **320**, and the transparent window or cut-out **355**, **360** are not included in the top portion **305** and the gasket **345** (or optionally the bottom portion **310**).

[0051] As shown in FIGS. **4**A-**4**J, in some embodiments, the testing device or cartridge **400** (e.g., cartridge **300** as described with respect to FIG. **3**) has a housing that comprises a top portion **405** (e.g., a cover) and a bottom portion **410** (e.g., a base) formed of rigid and flexible zones of material. As shown in FIGS. **4**A-**4**J, the rigid zones (non-shaded portions) of the cover **405** and the base **410** respectively are preferably each a single contiguous zone; however, the molding process can provide a plurality of non-contiguous substantially rigid zones. The flexible zones (shaded portions) of the cover **405** and the base **410** respectively are preferably a set of several non-contiguous zones. For example, the flexible zone around a displaceable membrane may be separate and distinct from the flexible zone at a closeable sealing member. Alternatively, the flexible zones may comprise a single contiguous zone.

[0052] The testing device or cartridge **400** further comprises a sealable sample entry port **415** and a closable sealing member **417** for closing the sample entry port **415**, a sample receiving chamber **420** located downstream of the sample entry port **415**, an optional capillary stop **422**, an optional filter **425** between the sample receiving chamber **420** and a sensor region **430** (i.e., assay region), and a waste chamber **433** located downstream of the sensor region **430**. In certain embodiments, the filter **425** is configured to retain blood cells from a biological sample and permit passage of plasma into the sensor region **430**. Preferably, the cross-sectional area of a portion of the sample receiving chamber **420** decreases distally with respect to the sample entry port **415**. In some embodiments, a pouch (e.g., the pouch **320** described with respect to FIG. **3**) is disposed in a recessed region **435** and in fluid communication with a conduit **437** leading to the sensor region **430**, optionally via conduit **440**. The pouch may be of the design described in U.S. Pat. No. 5,096,669 or, more preferably, in U.S. Pat. No. 8,216,529, both of which are incorporated herein by reference in their entireties. Recessed region **435** preferably includes a spike **442** configured to rupture the pouch, upon application of a force upon the pouch, for example, by reader or analyzer (e.g., analyzer **110** as described with respect to FIG. **1**). Once the pouch is ruptured, the system is configured to deliver the fluid contents from the pouch into conduit **437**. Movement of the fluid into the conduit **437** and to the sensor region **430** and/or within the conduit **440** may be effected by a pump, e.g., a pneumatic pump connected to the conduit(s) **437** or **440**. Preferably, the pneumatic pump comprises a displaceable membrane **445** formed by a portion of a flexible zone **447** of the housing formed over a recessed region or air bladder **450**. In the embodiment shown in FIGS. **4**A-**4**J, upon repeatedly depressing the displaceable membrane **445**, the device pumps via conduits **455** and **460** causing fluid from the ruptured pouch to flow through the conduit **437**, optionally into the conduit **440**, and over the sensor region **430** via conduit **465**.

[0053] The closable sealing member **417**, in some embodiments, includes a portion of the rigid zone that forms a sealing member **470**, and a portion of the flexible zone that forms a seal **475**. The sealing member **417** can rotate about hinge **480** and engage the seal **475** with the sample entry port **415** when in a closed position, thus providing an air-tight seal. Alternatively, an air-tight seal may be formed by contact of two flexible materials, e.g., a thermoplastic elastomer (TPE) on TPE. Optionally, the sealable sample entry port **415** also includes a vent hole (not shown). In an alternative embodiment, a portion of the rigid zone forms a sealing member, and a portion of the flexible zone forms a perimeter seal around the sample entry port, whereby the sealing member can rotate about a hinge and engage the perimeter seal when in a closed position, thus providing an air-tight seal. Alternatively, the perimeter seal may be formed by contact of two flexible materials. In yet another embodiment, the sealing member may include a slidable closure element as described in pending U.S. Pat. No. 7,682,833, the entirety of which is incorporated herein by reference.

[0054] The sensor region **430**, in some embodiments, contains a sensor array comprising one or more sensors for analysis such as cell counting or determination one or more target analytes. For example, the sensor array may include an optical sensor for cell counting and optionally an electrochemical sensor for determining one or more target analytes. The optical sensor may include one or more light detectors positioned near conduit **465** for receiving light through a biological specimen, e.g., a blood sample in the conduit **465**. In certain embodiments, the one or more light detectors are constructed based on similar technology (e.g., a photosensitive surface comprising an array of pixels) found in complementary metal-oxide semiconductor (CMOS) or charge-coupled device (CCD) image sensors. In some embodiments, the electrochemical sensor includes a base sensor or sensing electrode on a substantially planar chip where the sensing electrode is positioned in an auxiliary conduit (not shown) for receiving a sample mixed with a reagent.

[0055] In some embodiments, a portion of the conduit **465** forms an imaging chamber **485**. For example, a portion of the conduit **465** may include (i) a planar member, (ii) a transparent planar member, and (iii) a plurality of spacer elements having an average spacer height and disposed between the planar member and the transparent planar member to form the imaging chamber **485** having an average chamber height extending between the planar member and the transparent planar member. In certain embodiments, the one or more light detectors form at least a portion of the planar member. Preferably, the portion of the conduit **465** includes a uniform width dimension in the range of about 0.5 mm to about 2 cm, a uniform length dimension in the range of about 0.5 mm to about 2 cm, and a uniform height dimension in the range of about 1.5 μm to about 35 μm, for example, about 2 μm to about 20 μm. As used herein, the terms "substantially," "approximately" and "about" are defined as being largely but not necessarily wholly what is specified (and include wholly what is specified) as understood by one of ordinary skill in the art. In any disclosed embodiment, the term "substantially," "approximately," or "about" may be substituted with "within [a percentage] of what is specified, where the percentage includes 0.1, 1, 5, and 10 percent.

[0056] The analytes/properties to which the sensors respond may be selected from among particles (e.g., blood cells or microparticles), human chorionic gonadotropin, pH, partial pressure CO_2, partial pressure O_2, glucose, lactate, creatinine, urea, sodium, potassium, chloride, calcium, magnesium, phosphate, hematocrit, prothrombin time (PT), acti-

vated partial thromboblastin time (APTT), activated clotting time (ACT), D-dimer, prostate-specific antigen (PSA), creatine kinase-MB (CKMB), brain natriuretic peptide (BNP), troponin I (TnI), cardiac troponin (cTnl), human chorionic gonadotrophin, troponin T, troponin C, myoglobin, neutrophil gelatinase-associated lipocalin (NGAL), galectin-3, prostate-specific antigen (PSA), parathyroid hormone (PTH), galectin-3, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, bilirubin, alkaline phosphatase (ALP), and the like, and combinations thereof. In various embodiments, an optical sensor is configured to convert light received from cells within a portion of the imaging chamber to an output signal, and a processor connected to the optical sensor is configured to convert the output signal to a number count or percentage for each type of cell in the blood sample. In some embodiments, a differential blood cell count is a measurement of a number or percentage of each type of cell (e.g., white blood cells (WBCs)) that is in a whole blood sample. Cells types include erythrocytes and leukocytes and platelets. Imaging can distinguish various types of leukocytes including neutrophils, lymphocytes, granulocytes, eosinophils, basophils and monocytes. The differential blood cell count may also reveal if there are any abnormal or immature cells. Preferably, the analytes/properties are tested in a liquid sample that is whole blood, however other samples can be used including blood, serum, plasma, urine, cerebrospinal fluid, saliva and amended forms thereof. Amendments can include dilution, concentration, addition of regents such as anticoagulants and the like. Whatever the sample type, it can be accommodated by the sample entry port **415** of the cartridge **400**.

[0057] In some embodiments, the cartridge **400** further comprises a portion **490** of the flexible zone **447** positioned over the recessed region **435** that is configured for being actuated upon like a pump to apply pressure within the recessed region **435**. In certain embodiments, the flexible zone **447** includes a generic symbol description to indicate to the user that pressure should not be applied to the flexible zone **447** by the user. For example, the symbol may comprise an embossed circle with a crossbar. The portion **490** of the flexible zone **447** provides a surface that can accommodate an actuator feature of the analyzer (e.g., analyzer **110** as described with respect to FIG. **1**) to apply a force and burst the underlying pouch in the recessed region **435**. The thickness of the plastic in the portion **490** of the flexible zone **447** may be preferably from about 200 to about 800 μm, for example about 400 μm. Essentially, the portion **490** of the flexible zone **436** should be sufficiently thin to flex easily, but sufficiently thick to maintain physical integrity and not tear.

[0058] In various embodiments, a portion of the sensor region **430** (e.g., a top surface of the substrate of a sensor), a wall of the conduit **465**, and/or a wall of the sample receiving chamber **420** is coated with one or more dry reagents to amend the biological sample. The one or more dry reagents may comprise a one or more non-fluorescent or fluorescent dyes such as Eosin, Methylene Blue , Acridine Orange (also referred to as “Basic Orange **15**” or “ACO”), or Astrazon Orange (also referred to as “AO” or Basic Orange **21**), a component to bind to nucleic DNA in cells (e.g., blood cells such as WBCs), an anticoagulant, an antibody, an antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, a substrate, an optical marker dye identifying a type of assay bead, and/or combinations thereof. In some embodiments, the one or more dry reagent are in the sample receiving chamber **420** and dissolve into the sample before the sample arrives at the sensor region **430**. In other embodiments, a portion of the sensor region **430** (e.g., a top surface of the substrate of a sensor) includes a reagent region coated with a reactant and/or substrate for cells or an analyte of interest. The reagent region may be defined by a containment ring structure. In some embodiments, the containment ring structure is a hydrophobic ring of polyimide or another photolithographically produced layer. A microdroplet or several microdroplets (approximately 5-40 nL in size) or a series of about a 100 nanodroplets (approximately 50 to 1000 pL in size) containing the one or more dry reagents in some form may be dispensed or printed on the surface of the sensor or adjacent to the sensor. The photodefined ring structure contains this aqueous droplet allowing the reagent region to be localized to a precision of a few microns. The reagent region can be made from 0.03 to approximately 2 mm^2 in size. The upper end of this size is limited by the size of the conduit and sensor chip **400** in present embodiments, and is not a limitation of the invention.

[0059] The biological sample or a fluid may be passed at least once over the dry reagent, e.g., the reagent region to dissolve the reagent within the biological sample or fluid. Within a segment of the biological sample or fluid, the reagent can be preferentially dissolved and concentrated within a predetermined region of the segment. This is achieved through control of the position and movement of the segment. Thus, for example, if only a portion of a segment, such as the leading edge, is reciprocated over the reagent, then a high local concentration of the reagent can be achieved close to the leading edge. Alternatively, if a homogenous distribution of the reagent is desired, for example if a known concentration of a reagent is required for a quantitative analysis, then further reciprocation of the sample or fluid will result in mixing and an even distribution.

[0060] As shown in FIGS. **4K-4R**, in alternative embodiments, the testing device or cartridge **400** (e.g., cartridge **300** as described with respect to FIG. **3**) has a housing that comprises a top portion **405** (e.g., a cover) and a bottom portion **410** (e.g., a base) formed of rigid material. As shown in FIGS. **4A-4J**, the rigid material of the cover **405** and the base **410** respectively are preferably each a single contiguous zone; however, the molding process can provide a plurality of non-contiguous substantially rigid zones. The testing device or cartridge **400** further comprises a sample entry port **415**, a sample receiving chamber **420** located downstream of the sample entry port **415**, and a conduit **465** fluidically connecting the sample receiving chamber **420** to a sensor region **430** (i.e., assay region). Sample motion in the sample receiving chamber **420**, conduit **465**, and sensor region **430** may be controlled by capillary action where fluidic paths and conduits are dimensioned to promote capillary action. The surfaces of the fluidic paths and/or conduit may also be treated to make them more or less hydrophilic and hydrophobic to further promote capillary action using established techniques known in the art.

[0061] In some embodiments, the sensor region **430** comprises at least one microfabricated sensor chip **492** disposed on a imager chip carrier **493** (e.g., a substrate) with electrical contacts **495**. The sensor chip **492** and imager chip carrier **493** may be positioned in the sensor region **430** and con-

figured to generate electric signals based on, for example, light transmitted through an imaging chamber **485** of the sensor chip **492** and a biological specimen, e.g., a blood sample from a patient. In some embodiments, in which a light emitter is provided by an external element such as the analyzer (e.g., the reading apparatus **110** as discussed with respect to FIG. **1**), the top portion **405** or bottom portion **410** comprise a transparent window or cut-out **497** (shown in bottom portion **410** for illustrative purposes). In other embodiments, in which a light emitter **498** is provided within the testing device or cartridge **400**, the light emitter **498** is provided on the sensor chip **492** or imager chip carrier **493**, and the transparent window or cut-out **497** is not included in the top portion **405** or bottom portion **410**. In some embodiments, a portion of the conduit **465** forms the imaging chamber **485**. For example, a portion of the conduit **465** may include (i) a planar member, (ii) a transparent planar member, and (iii) a plurality of spacer elements having an average spacer height and disposed between the planar member and the transparent planar member to form the imaging chamber **485** having an average chamber height extending between the planar member and the transparent planar member. In certain embodiments, the one or more light detectors form at least a portion of the planar member. Preferably, the portion of the conduit **465** includes a uniform width dimension in the range of about 0.5 mm to about 2 cm, a uniform length dimension in the range of about 0.5 mm to about 2 cm, and a uniform height dimension in the range of about 1.5 μm to about 35 μm, for example, about 2 μm to about 20 μm.

[0062] The analytes/properties to which the sensors respond may be selected from among particles (e.g., blood cells or microparticles), human chorionic gonadotropin, pH, partial pressure CO_2, partial pressure O_2, glucose, lactate, creatinine, urea, sodium, potassium, chloride, calcium, magnesium, phosphate, hematocrit, prothrombin time (PT), activated partial thromboblastin time (APTT), activated clotting time (ACT), D-dimer, prostate-specific antigen (PSA), creatine kinase-MB (CKMB), brain natriuretic peptide (BNP), troponin I (TnI), cardiac troponin (cTnl), human chorionic gonadotrophin, troponin T, troponin C, myoglobin, neutrophil gelatinase-associated lipocalin (NGAL), galectin-3, prostate-specific antigen (PSA), parathyroid hormone (PTH), galectin-3, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, bilirubin, alkaline phosphatase (ALP), and the like, and combinations thereof. In various embodiments, an optical sensor is configured to convert light received from cells within a portion of the imaging chamber to an output signal, and a processor connected to the optical sensor is configured to convert the output signal to a number count or percentage for each type of cell in the blood sample. In some embodiments, a differential blood cell count is a measurement of a number or percentage of each type of cell (e.g., white blood cells (WBCs)) that is in a whole blood sample. Cells types include erythrocytes and leukocytes and platelets. Imaging can distinguish various types of leukocytes including neutrophils, lymphocytes, granulocytes, eosinophils, basophils and monocytes. The differential blood cell count may also reveal if there are any abnormal or immature cells. Preferably, the analytes/properties are tested in a liquid sample that is whole blood, however other samples can be used including blood, serum, plasma, urine, cerebrospinal fluid, saliva and amended forms thereof. Amendments can include dilution, concentration, addition of regents such as anticoagulants and the like. Whatever the sample type, it can be accommodated by the sample entry port **415** of the cartridge **400**.

[0063] In various embodiments, a portion of the sensor region **430** (e.g., a top surface of the substrate of a sensor), a wall of the conduit **465**, and/or a wall of the sample receiving chamber **420** is coated with one or more dry reagents to amend the biological sample. The one or more dry reagents may comprise Acridine Orange (also referred to as "Basic Orange 15" or "ACO"), Astrazon Orange (also referred to as "AO" or Basic Orange 21), a component to bind to nucleic DNA in cells (e.g., blood cells such as WBCs), an anticoagulant, an antibody, an antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, a substrate, an optical marker dye identifying a type of assay bead, and/or combinations thereof. In some embodiments, the one or more dry reagent are in the sample receiving chamber **420** and dissolve into the sample before the sample arrives at the sensor region **430**. In other embodiments, a portion of the sensor region **430** (e.g., a top surface of the substrate of a sensor) includes a reagent region coated with a reactant and/or substrate for cells or an analyte of interest. The reagent region may be defined by a containment ring structure. In some embodiments, the containment ring structure is a hydrophobic ring of polyimide or another photolithographically produced layer. A microdroplet or several microdroplets (approximately 5-40 nL in size) or a series of about a 100 nanodroplets (approximately 50 to 1000 pL in size) containing the one or more dry reagents in some form may be dispensed or printed on the surface of the sensor or adjacent to the sensor. The photodefined ring structure contains this aqueous droplet allowing the reagent region to be localized to a precision of a few microns. The reagent region can be made from 0.03 to approximately 2 mm^2 in size. The upper end of this size is limited by the size of the conduit and sensor chip **400** in present embodiments, and is not a limitation of the invention. The biological sample or a fluid may be passed over the dry reagent by capillary action, e.g., the reagent region, to dissolve the reagent within the biological sample or fluid.

Imaging Device

[0064] In order to implement lensless microscopy in point-of-care applications (e.g., the analyzer and cartridge systems described with respect to FIGS. **1**, **2**, **3**, and **4**A-**4**R), several aspects of the present invention are directed to imaging devices comprising: (i) one or more light detectors, (ii) an imaging chamber, and (iii) one or more light emitters. Various embodiments of imaging devices are illustrated by the examples shown in FIGS. **5**-**10** and **11**A-**11**I. In some embodiments, an imaging device **500** comprises a light detector such as an imager chip **505** having an inner surface or photosensitive surface **510** presented by an imaging integrated circuit **515** formed on a substrate. The imaging integrated circuit **515** (e.g., a very-large-scale integrated (VLSI) circuit) may have a high-resolution photosensitive array including a multi-dimensional array of pixels presented at its surface **510** and non-photosensitive supporting circuitry for processing and readout. The imaging integrated circuit **515** may be electrically and mechanically attached to a sensor chip **520**, which is a printed circuit board whose components connect to one or more electrical connections (e.g., an electrical connection comprising a plurality of

discrete contacts) that are capable of connecting the photosensitive surface **510** to one or more conductive pins such as a temporary electrical connector of an analyzer (e.g., analyzer **110** as described with respect to FIG. **1**).

[0065] The multi-dimensional array of pixels may be light sensors or photodetectors formed of semiconductor materials used in very-large-scale or larger integrated circuits. The defining property of a semiconductor material is that it can be doped with impurities that alter its electronic properties in a controllable way; in some embodiments, the array is formed substantially of a crystalline inorganic solid such as silicon; and in other embodiments the array is formed substantially of a compound semiconductor comprised of elements of at least two different species. The compound semiconductor may be comprised of elements in groups 13-15 (old groups III-V), for example of elements from group 13 (old group III, boron, aluminum, gallium, indium) and from group 15 (old group V, nitrogen, phosphorus, arsenic, antimony, bismuth). The range of possible formulae for the compound semiconductor may include binary (two elements, e.g., gallium (III) arsenide (GaAs)), ternary (three elements, e.g., indium gallium arsenide (InGaAs)), and quaternary (four elements, e.g., aluminum gallium indium phosphide (AlInGaP)) alloys. In some embodiments, the array of pixels are light sensors or photodetectors such as PD(s), e.g., a silicon photo PIN diode(s) having an undoped intrinsic semiconductor region sandwiched between a p-type semiconductor region and an n-type semiconductor region. Alternatively, other light sensors or detectors with or without filters to control wave lengths may be used without departing from the spirit and scope of the present invention. The spectral response of the multi-dimensional array of pixels may be in the range of 300 nm to 1000 nm. This provides the capability to cover a wide spectrum of LED wavelengths. The size of the photosensitive surface **510** may be selected to fit with other components (e.g., the conduits or sensor region) of the testing device, e.g., the photosensitive surface **510** available as surface mount diode (SMD) and chip scale packaging (CSP) may be used to fit a variety of testing devices. In some embodiments, the sensor chip **520** may have a width from about 1 mm to about 20 mm and a length from about 1 mm to about 20 mm (e.g., a width of about 5 mm and a length of about 6 mm), in order to accommodate a low profile photosensitive surface **510** that has the industry standard 2.0 mm×1.25 mm footprint, which provides high efficiency light detection and low power consumption. In accordance with various aspects , the sensitivity of the photosensitive surface **510** is within the range of 0.5 uA/cm^2-4 uA/cm^2, for example substantially 1 uA/cm^2.

[0066] As described herein, the multi-dimensional array of pixels form part of a high-resolution photosensitive array. The term "high-resolution" as used herein refers to a resolution that equals or exceeds the resolution of standard lens-based optical microscopes. The resolution of a standard lens-based optical microscopes is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities. For example, depending on the context of the application, high-resolution means less than 5 μm, less than 2 μm, less than 1 μm, less than about 0.5 μm, or even less. Resolution in an optical sensor is primarily determined by the pixel size of the photosensitive array. Some photosensitive arrays have many million square pixels each slightly more than 1 μm on a side, resulting in a resolution of about 1 μm; the resolution achievable will improve with decreasing pixel sizes, theoretically exceeding, for example, 1 billion pixels, each as small as 200 nm or less on a side, as the design and fabrication techniques of integrated circuits or other devices improve. Pixels per inch (PPI) or pixels per centimeter (PPCM) are measurements of the pixel density of the optical sensor. The resolution of the optical sensor is the count of pixels that contribute to the final image and is typically measured in megapixels (meaning millions of pixels). For example, a photosensitive array comprising 1280×720 pixels has 921,600 pixels or less than 1 mega pixel resolution, and a photosensitive array comprising 1920×1080 pixels has 2,073,600 pixels or about 2.1 mega pixel resolution. In some embodiments, the photosensitive surface **510** is comprised of an array of pixels having at least 5 mega pixel resolution with at least a 150 ppi pixel density. In some embodiments, each pixel of the array has a length and width of equal to or less than 10 μm, equal to or less than 5 μm. equal to or less than 1 μm, equal to or less than 500 nm, or equal to or less than 250 nm. In certain embodiments, each pixel of the array has an area of about 0.9 μm^2, 1.1 μm^2, 1.4 μm^2, or 1.8 μm^2. In terms of ranges each pixel of the array may have an area of less than about 10.0 μm^2, less than about 5.0 μm^2, less than about 2.0 μm^2, for example, from about 0.5 μm^2 to about 1.5 μm^2.

[0067] Micro-fabrication techniques (e.g., photolithography and plasma deposition) may be utilized for construction of multilayered sensor structures in confined spaces. In some embodiments, the imaging integrated circuit **515** is fabricated to include a CCD. In other embodiments, the imaging integrated circuit **515** is fabricated using CMOS technology. CCDs have advantages for contact optical microscopy applications, including the ability to detect light over the full exposed surface of the chip (100% fill factor), though they have slower readout speeds relative to CMOS due to requirement for sequential transfer of charge from light-sensing (parallel register) to readout (serial register) elements. Various configurations of CCD can be used: full-frame architecture may be used to maximize the proportion of the chip available for imaging, but requires an external shutter to prevent image smearing during readout; whereas frame-transfer architecture avoids image smearing, but in the process requires a masked, non-photosensitive area of the parallel register of about the same size as the photosensitive area of the parallel register, with the result that the imaging integrated circuit has about half the photosensitive area of a full-frame architecture. Because of the small area of the individual pixels in the arrays used in accordance with various aspects discussed herein, the charge collected in each pixel will be small under many imaging conditions; however, as the specimen is in contact, or nearly in contact, with the pixel, the pixel's effective acceptance angle for photons emanating from the specimen is larger than that achieved by lenses in conventional microscopy. In some CCD embodiments, to increase the sensitivity further, CCDs of any architecture additionally employ electron multiplying gain, in which high clock voltages applied to an extended region of the serial register(s) amplify the charge of each pixel as it is shifted to the output node(s).

[0068] CMOS devices have alternative advantages for these applications, including less expensive fabrication, signal processing by electronic elements embedded in individual pixels, and the ability to read out independently-addressed pixel values individually without sequential

transfer. In some CMOS embodiments, thinned back-side illuminated arrays are used; though previously requiring expensive and complex fabrication methods, these may be fabricated cheaply using bonded wafer processes such as those that use silicon-on-insulator substrates with a buried oxide layer as an etch-stop to yield a uniformly optimally thinned light-absorbing back layer (see as an example, U.S. Pat. No. 7,425,460, which is incorporated herein by reference). Light entering ordinary (front-side illuminated) imaging integrated circuits typically passes through overlying layers that scatter light and whose metal circuit elements block the underlying photosensitive layer; in back-side illuminated imaging integrated circuits the photosensitive layer is close to the surface, above the metal circuit-bearing layers, typically resulting in less light blocking (larger "fill factors") and consequently higher effective quantum efficiency.

[0069] In various embodiments, the imaging device **500** further comprises a sample testing conduit **525** fluidically connected to a sample receiving chamber (e.g., the sample receiving chamber **420** of FIGS. **4**A-**4**R). A portion of the sample testing conduit **525** may include: (i) a planar member **530** having an inner surface **535** (e.g., the imager chip **505** having the photosensitive surface **510**) and (ii) a transparent planar member **540** having an inner surface **545** to form an imager chamber **550** having an average chamber height extending between the planar member and the transparent planar member. The imaging chamber **550** structured to obtain images of at least a portion of a sample residing in the imaging chamber **550**. In some embodiments, the imaging chamber is structured to obtain images of cells within the sample (e.g., a monolayer of red blood cells and/or white blood cells). In other embodiments, the imaging chamber is structured to obtain images of one or more assay beads used in the performance of an analytical test (e.g., a qualitative or semi-quantitative analytical test for a target analyte within the sample). FIG. **6** shows a cross-section of an imaging chamber **600** having a predetermined height (hl) measured on the Z-axis. In some embodiments, the chamber height (h**1**) is selected to accommodate the analysis of cells (e.g., the formation of a monolayer of cells) and will be from about 2 μm to about 20 μm, from about 2 μm to about 6 μm, or from about 3 μm to about 5 μm, for example about 4 μm, about 2 μm, or about 6 μm. In other embodiments, the chamber height (h**1**) is selected to accommodate the analysis of assay beads and will be from about 0.5 μm to about 40 μm, from about 0.5 μm to about 20 μm, or from about 2 μm to about 10 μm, for example about 4 μm, about 8 μm, or about 10 μm. FIG. **7** shows a top planar view of an imaging chamber **700** having an area (a**1**) measured on the X-Y plane and a volume ((v**1**)=(h**1**)×(a**1**)) measured on the x, y, and z axis. The lateral boundaries **705** of the imaging chamber **700** may be defined, for example, by structural features **710** (e.g., sides of the conduit, glue lines, or hydroscopic material disposed on a planar member surface that inhibit lateral travel) extending between the inner surfaces of the planar member and the transparent planar member, respectively.

[0070] The imaging chamber **500**, **600**, **700** is typically sized to hold about 0.2 to about 2.0 μL of sample, but the imaging chamber **500**, **600**, **700** is not limited to any particular volume capacity, and the capacity can vary to suit the analysis application. For example, sized to create a monolayer of red blood cells or white blood cells for cell identification and counting. In some embodiments, the imaging chamber **500**, **600**, **700** is operable to quiescently hold a liquid sample. The term "quiescent" is used herein to describe that the sample is deposited within imaging chamber **500**, **600**, **700** for analysis, and is not purposefully moved during the analysis. To the extent that motion is present within the blood sample, it will predominantly be due to Brownian motion of formed constituents within the blood sample, which motion is not disabling of the use of this invention. However, in other embodiments, the imaging chamber **500**, **600**, **700** is operable to actively hold a liquid sample. The term "actively" is used herein to describe that the sample is deposited within imaging chamber **500**, **600**, **700** for analysis, and is purposefully moved during the analysis (e.g., via a pump such as a displaceable membrane **426** formed by a portion of a flexible zone **427** as described with respect to FIGS. **4**A-**4**R).

[0071] As shown in FIG. **5**, in various embodiments, the imaging device **500** further comprises a light emitter **555** positioned near the sample testing conduit **525** (e.g., on a side of the conduit or above the conduit). In some embodiments, the light emitter **555** is provided by an external element such as the analyzer (e.g., the reading apparatus **110** as discussed with respect to FIG. **1**). In other embodiments, the light emitter **555** is provided on the imager chip or imager chip carrier within the testing device or cartridge (e.g., the testing device or cartridge **110** as discussed with respect to FIG. **1**). In some embodiments, the light emitter **555** is positioned so that a path of light from the light emitter **555** to the photosensitive surface **510** of the sensor chip **520** is at an angle of 45 degrees or more to the photosensitive surface **510**. In some embodiments, the light emitter **555** is positioned so that a path of light from the light emitter **555** to the photosensitive surface **510** is at an angle of at most 45 degrees to the photosensitive surface **510**. In some embodiments, the light emitter **555** is positioned so that a path of light from the light emitter **555** to the photosensitive surface **510** is approximately perpendicular or parallel to the photosensitive surface **510**.

[0072] Images of the specimen can be obtained in the presence of the light emitter **555**. The light emitter **555** may produce light of at least one wavelength for which the imaging integrated circuit **515** is responsive. In some embodiments, the light emitter **555** includes a laser and the predetermined wavelength is the substantially monochromatic wavelength of the laser. In some embodiments, the light emitter **555** includes a blackbody and the predetermined wavelength band is a segment of the electromagnetic spectrum which the blackbody is suitably efficient at producing, with or without use of a bandpass spectral filter interposed between the light emitter **555** and the specimen. In some embodiments, the light emitter **555** comprises one or more light-emitting diodes, for example, an organic light-emitting diode array, oriented so as to produce light in the predetermined wavelength band or bands. In some embodiments, the light emitter **555** is continuous. In some embodiments, the light emitter **555** is pulsed. In some embodiments, the light emitter **555** is polarized. In some embodiments, the light emitter **555** includes any ambient, incandescent, or fluorescent light source. In certain embodiments in which a fluorescent dye is used as a reagent, the light emitter **555** includes a fluorescence illuminator. In some embodiments, the light emitter **555** is structured, such as a periodic grating of bright bars. In conjunction with appropriate oblique, pulsed, polarized, structured, or other

forms of illumination, some embodiments can generate additional useful information corresponding to methods known in the art of microscopy, including but by no means limited to dark field, fluorescence, fluorescence lifetime, optical tomography, and polarization microscopy. In some embodiments, the specimen is itself the light emitter **555**; for example through chemi-luminescence, or in an instance where the photosensitive array is treated to render the pixels sensitive to radiation emitted by a radioactive specimen. In some embodiments, there may be additional light emitters capable of operating as any of the aforementioned light emitters.

[0073] The spectra of the light source(s) may lie in any predetermined region of the electromagnetic spectrum detectable using photosensitive arrays, with or without specialized treatments to extend the effective ranges of wavelengths detectable by such arrays. In some embodiments, the predetermined wavelength or wavelength band is in the infrared spectrum. In some embodiments, the predetermined wavelength or wavelength band is in the ultraviolet spectrum. In some embodiments, the predetermined wavelength or wavelength band is in the visible spectrum. In certain embodiments, the light emitter **555** is configured to transmit light through the imaging chamber **550** to the sensor chip **520** at a wavelength from about 300 nm to about 1000 μm. In other embodiments, the light emitter **555** is configured to transmit light through the imaging chamber **550** to the sensor chip **520** at a plurality of wavelengths from about 300 nm to about 1000 μm.

[0074] In various embodiments, the light emitter **555** is located adjacent to the imaging chamber **530** (i.e., near the transparent planar member, for example within about 1 mm, about 2 mm, or about 3 mm). In certain embodiments, the testing cartridge or test device comprises a housing, and the sensor chip **520**, the sample testing conduit **525**, and the light emitter **555** are housed within the housing. For example, the test cartridge may comprise the light emitter **555**, and the light emitter **555** may be electrically connected to at least one of a plurality of connector contacts, and the light emitter **555** may be configured to transmit light through the portion of the imaging chamber **550** to the sensor chip **520** at one or more wavelengths from about 300 nm to about 1000 μm. In other embodiments, the analyzer comprises the light emitter **555**, and the test cartridge further comprises a housing comprising a window adjacent to the sample testing conduit **525** for illuminating the portion of the imaging chamber **550**, and the test cartridge is insertable into the port of the analyzer such that the light emitter **555** is aligned over the window and the portion of the imaging chamber **550** to transmit light through the portion of the imaging chamber **550** to the sensor chip **520** at one or more wavelengths from about 300 nm to about 1000 μm.

[0075] In some embodiments, the light emitter **555** includes individually controlled light-emitting diodes (LEDs) selected for their spectral emission characteristics and their uniformity of emitted light, and positioned so as to facilitate the analyses contemplated. In some embodiments, the light emitter **555** is positioned so as to uniformly illuminate the imaging chamber **550**. The LEDs may be controlled, for example, by an embedded controller incorporated within the cartridge or the analyzer. The LEDs may be controlled, for example, either singly or in groups so as to facilitate the analyses to be contemplated, including but not limited to conventional microscopy wherein the illuminator, the specimen and the imaging system are substantially aligned, and dark-field microscopy wherein the specimen is illuminated from an angle outside the acceptance angle of the pixel. In addition, through appropriate selection of the LEDs in the light emitter **555**, the contemplated imaging devices can be used for, but not be limited to, e.g., color imaging, fluorescence microscopy, polarization microscopy, infra-red and ultra-violet microscopy. Some embodiments will incorporate multiple light emitters **555**, each of which may have different characteristics so as to facilitate the conduct of a wider range of analyses. In some embodiments the light emitter **555** will be easily interchangeable. In some embodiments the light emitter **555** includes organic LED (OLED) or active matrix organic LED (AMOLED) panel with selective addressing. Some embodiments facilitate both uniform specimen illumination and rapid illumination variation so as to facilitate analyses yet to be contemplated with both stationary and moving specimens. In some embodiments, an AMOLED panel is used to illuminate the specimen through appropriate control of the panel photoemitters. In some examples, the light emitter **555** can include LEDs, organic LED panels, fluorescent panels, ultraviolet sources, ambient illumination such as sunlight or room light, incandescent sources, or any other light source, including none, e.g., for chemiluminescent specimens, and combinations of these examples. Configurations of the light emitter **555** include, but are not limited to, flat panels, rectangular or other grid layouts of sources, movable sources, multi-color sources, and sources affixed to the inside or a hemispherical shell mounted over the imaging chamber **550** with the center of the chamber as the center of the shell, or combinations of them. Control of the light emitter **555** may include, but not be limited to, steady illumination, selectively exciting one or a plurality of light emitters **555** simultaneously or in sequence, controlling the intensity of any one or a plurality of light emitters **555**, controlling each or a plurality of light emitters **555** so as to have a specific temporal illumination pattern, or using any one or any combination of them and others (including future technologies). The controller for the light emitter **555** may include, but not be limited to, a manual controller such as a switch or knob, an automated embedded computing system, an external computing system such as onboard the analyzer, an external computing system such as a desktop or laptop computer, or a combination of the foregoing. Imaging Cells

[0076] As shown in FIG. **8**, an imaging chamber **800** comprises a first planar member **805**, a second planar member **810**, and a plurality of spacer elements **815** disposed between the first planar member **805** and the second planar member **810**. A height (h**1**) of the imaging chamber **800** is predetermined such that the sample residing within the imaging chamber **800** will travel laterally within the imaging chamber **800** via capillary forces. In various embodiments, at least one of the first planar member **805** and the second planar member **810** is transparent (e.g., a transparent planar member). Transparent plastic films comprising acrylic or polystyrene are examples of acceptable materials for the first planar member **805** and the second planar member **810**. In some embodiments, at least a portion of the first planar member **805** or the second planar member **810** (e.g., a planar member) is formed from the imager chip **820**. For example, typically optical sensors have a protective window over the photosensitive surface **825**. However, in certain embodiments, the optical sensors do not comprise a

protective window and instead the imager chip **820** is windowless in order for the sample to come close enough to the photosensitive surface **825** to achieve high resolution, as defined herein, without computational image processing. For portions of the sample within half a pixel width of the photosensitive surface **825**, the resolution of the image is limited by the size of the pixels making up the photosensitive surface **825**. For example, when a point on the sample (e.g., a cell) is less than half a pixel width from the center of the closest pixel, nearly all the light emitted or scattered from that point toward the array will predominantly be incident on, and therefore excite, only the closest pixel; under these conditions, the resolution may be determined by the pixel size or, more precisely, by the size of a circle of equivalent area (e.g., about a 450 nm resolution for a 400 nm×400 nm pixel), although resolution may be further enhanced by computation, sample flow, or other means. A near-field criterion may be considered to be reached, for example, when the distance between the photosensitive surface and the sample is less than the wavelength of interest. No lenses or any other optical components are required to achieve these conditions, and thus to achieve such pixel-limited resolution.

[0077] In other embodiments, the photosensitive surface **825** is treated with one or more thin layers. The layers may be considered thin when the aggregate thickness of such layers as applied to the photosensitive surface **825** still allows for the near-field criterion described herein to be satisfied or substantially satisfied. In several embodiments, the layers are thin enough for the sample to come within half a pixel width of the photosensitive surface **825**. In some embodiments, the layers are thin enough in the direction of the optical path so that the total distance that the optical path takes through the layers is no more than about the wavelength of interest. In some embodiments, a thin layer of transparent chemically resistant material coats the photosensitive surface **825**. Such a thin-film substrate may be any sufficiently transparent and insulating material, including but not limited to silicon oxide, titanium oxide, aluminum oxide, tantalum oxide, magnesium fluoride, lanthanum fluoride, aluminum fluoride, silicon nitride, and silicon oxynitride; and it may be deposited by a variety of means including but not limited to magnetron sputtering, chemical vapor deposition, thermal or vacuum arc plasma evaporation. In some embodiments, the substrate is a dielectric thin film acting as an interference filter, thereby restricting the spectral sensitivity of the underlying pixels as appropriate to a given application. In some embodiments, the substrate is used to effect certain forms of color imaging. In certain embodiments, the substrate is substantially transmissive to a portion of a predetermined wavelength band, such as a band-pass filter. In other embodiments such as for fluorescence or emission microscopy, the substrate is substantially transmissive to an alternative predetermined wavelength band which corresponds to the wavelength band produced by fluorescence, emission, or in other ways, of the sample. In some embodiments, the substrate includes a dielectric thin film acting as an anti-reflection coating. In some embodiments, there are multiple substrates situated in close contact to each other. In some embodiments, the photosensitive surface **825** is silanized so as to decrease adhesion between the surface and the sample. In some embodiments, the chemically resistant material includes diamond, deposited in a suitably thin layer as, for example, by chemical vapor deposition. In some embodiments, the chemically resistant material includes Al_2O_3 or Si_3N_4, deposited in a suitably thin layer as, for example, by chemical vapor deposition. Such materials can impart more robust characteristics to the photosensitive surface **825**, allowing for ease of cleaning as well as protection of the surface from abrasive samples. In some embodiments, a passivation layer, typically of Si_3N_4, coats the imaging integrated circuit, resulting in reduced conductivity when used with metallic or other conductive samples such as salt solutions. In some embodiments, a thin layer of polarizing material coats the photosensitive surface **825**. In some embodiments, a thin layer of absorptive material coats the photosensitive surface **825**. In some embodiments, a thin layer of interference material coats the photosensitive surface **825**. In some embodiments, a thin layer of surface plasmon generating material coats the photosensitive surface **825**. Technology is available to deposit such layers as a thin film and in arbitrary pixel-by-pixel patterns.

[0078] In various embodiments, the plurality of spacer elements **815** are any structure that is disposable between the first planar member **805** and the second planar member **810**, and operable to space the first planar member **805** and the second planar member **810** apart from one another and maintain the chamber height (h**1**). The height of each of the plurality of spacer elements **815** typically do not equal one another exactly, but are substantially equal and within commercially acceptable tolerance for spacing means used in similar analysis apparatus. As such the height of the plurality of spacer elements **815** is characterized as the average spacer height (h**2**). In some embodiments, the average spacer height (h**2**) is selected to accommodate the analysis of cells (e.g., the formation of a monolayer of cells) and will be from about 2 μm to about 20 μm, from about 2 μm to about 6 μm, or from about 3 μm to about 5 μm, for example about 4 μm, about 2 μm, or about 6 μm. In other embodiments, the average spacer height (h**2**) is selected to accommodate the analysis of assay beads and will be from about 0.5 μm to about 40 μm, from about 0.5 μm to about 20 μm, or from about 2 μm to about 10 μm, for example about 4 μm, about 8 μm, or about 10 μm. The height of the spacer elements can be determined by any known analytical technique typically used to measure the height or size of an object, such as flow cytometry, laser device, SEM imaging, particle size analyzer, etc. As used herein, the "average spacer height" means the average height of at least 90% of the spacer elements used to construct the chamber. Average is understood as a calculated "central" value of a set of numbers (the sum of the set of numbers divided by the count), where the set of numbers is the set of height values for at least 90% of the spacer elements used to construct the chamber.

[0079] In some embodiments, the plurality of spacer elements **815** are spherical beads (e.g., uniform polymer, silica or magnetic microsphere products for diagnostic, research and flow cytometry applications commercially available from, for example, Bangs Laboratories, Inc. In some embodiments, the plurality of spacer elements **815** are pillar structures fabricated on: (i) at least a portion of a surface of the first planar member **805** (e.g., the photosensitive surface **825** of the sensor chip **820**), and/or (ii) at least a portion of a surface of the second planar member **810**. In some embodiments, the plurality of spacer elements **815** are structures embossed on: (i) at least a portion of a surface of the first planar member **805** (e.g., the photosensitive surface

825 of the sensor chip **820**), and/or (ii) at least a portion of a surface of the second planar member **810**. In some embodiments, the plurality of spacer elements **815** are formed directly (physical contact between the objects) on: (i) at least a portion of a surface of the first planar member **805** (e.g., the photosensitive surface **825** of the sensor chip **820**), and/or (ii) at least a portion of a surface of the second planar member **810**. In some embodiments, the plurality of spacer elements **815** are formed indirectly (no physical contact between the objects) on: (i) at least a portion of a surface of the first planar member **805** (e.g., the photosensitive surface **825** of the sensor chip **820**), and/or (ii) at least a portion of a surface of the second planar member **810**.

[0080] In several embodiments, the plurality of spacer elements **815** comprise a material that has greater flexibility than one or both of the first planar member **805** and the second planar member **810**; i.e., relatively speaking, one or both of the first planar member **805** and the second planar member **810** may be considered to be rigid relative to the plurality of spacer elements **815** and the plurality of spacer elements **815** may be considered to be flexible relative to one or both of the first planar member **805** and the second planar member **810**. In other embodiments, the plurality of spacer elements **815** comprise a material that has less flexibility than one or both of the first planar member **805** and the second planar member **810**; i.e., relatively speaking, one or both of the first planar member **805** and the second planar member **810** may be considered to be flexible relative to the plurality of spacer elements **815** and the plurality of spacer elements **815** may be considered to be rigid relative to one or both of the first planar member **805** and the second planar member **810**.

[0081] In particular, it has been discovered that if the imaging chamber **800** is formed using spacer elements disposed between planar members, and if at least one the first planar member **805** and the second planar member **810**, and/or the plurality of spacer elements **815** is flexible, the imaging chamber **800** behaves differently than the conventional hemocytometer chambers, and the difference is highly advantageous. When the imaging chamber **800** is filled with a liquid (e.g., a blood sample), the capillary forces tend to pull the first planar member **805** and the second planar member **810** together, thus exerting a slight pressure on the retained plurality of spacer elements **815**. This pressure will cause the flexible element to deform in such a manner as to cause the chamber height (h**1**) to approximate, on average, the mean dimension of the plurality of spacer elements **815** (the average spacer height (h**2**)) disposed between the first planar member **805** and the second planar member **810**. For example, if both the first planar member **805** and the second planar member **810** are rigid and the plurality of spacer elements **815** are flexible, separators larger than the mean diameter will be compressed, and the first planar member **805** and the second planar member **810** will approximate until more and more separators come into contact with the first planar member **805** and the second planar member **810**, preventing further approximation. At that point, the height of the chamber (h**1**) will have an average height which substantially approximates the average height (h**2**) of the plurality of spacer elements **815** and is readily ascertainable, provided the standard deviation of the heights of the plurality of spacer elements **815** is acceptable and the plurality of spacer elements **815** are sufficiently flexible. As used herein, the term "flexibility" is the ability of a material to deform elastically and return to its original shape when the applied force or stress is removed (defined as the displacement caused by a unit force). As used herein, the terms "flexible" and "sufficiently flexible" are defined as an object having a Taber stiffness of less than 0.4 mN/m. As used herein, the terms "rigidity" and "stiffness" are the inverse of flexibility and is the extent to which a material capable resisting deformation upon the application of a force or stress (defined as the force required to produce a unit displacement). As used herein, the term "rigid", "stiff", "sufficiently rigid", and "sufficiently stiff" are defined as an object having a Taber stiffness of greater than 0.4 mN/m. In another example, if the plurality of spacer elements **815** are rigid and the first planar member **805** and/or the second planar member **810** is flexible, the first planar member **805** and/or the second planar member **810** will deform and be "tented-up" in a small area around each of the larger separators and be lower over smaller separators. The height of the chamber (h**1**) will have an average height which substantially approximates the average height (h**2**) of the plurality of spacer elements **815**, provided the first planar member **805** and/or the second planar member **810** is sufficiently flexible.

[0082] The height of the spacer elements can be determined by any known analytical technique typically used to measure the height or size of a structure, such as laser device, SEM imaging, internal standard means, etc. An example of an internal standard includes a flexible or flowable material which is not miscible with the sample and which contains a known, stable and uniform concentration of a sensible optical dye. The material can be dyed flexible beads, dyed oil or the like, and may be present in one or more areas of the chamber. Since the optical density is in direct proportion to the thickness of the calibrator material, measurement of the optical density of the part of the calibrator material, which completely fills the chamber height, will allow the calculation of the exact chamber height at a set location to within the precision capabilities of the optical system. As used herein, the "average chamber height" means the average height of at least 90% of the chamber. Average is understood as a calculated "central" value of a set of numbers (the sum of the set of numbers divided by the count), where the set of numbers is the set of height values for at least 90% of the chamber.

[0083] As shown in FIG. **9**, in some embodiments, an imaging device **900** comprises a first planar member **905** and a second planar member **910** separated by a plurality of spacer elements **915** that define an imaging chamber **920** between the first planar member **905** and the second planar member **910**. The plurality of spacer elements **915** are formed from a material that has greater flexibility than the first planar member **905** and the second planar member **910**; i.e., the first planar member **905** and the second planar member **910** may be considered to be rigid relative to the plurality of spacer elements **915** and the plurality of spacer elements **915** may be considered to be flexible relative to the first planar member **905** and the second planar member **910**. In this example, larger spacer elements **925** may be compressed to the point where the first planar member **905** and the second planar member **910** have approximated to the point where most of the plurality of spacer elements **915** are touching the interior surfaces **930**, **935** of the first planar member **905** and the second planar member **910**, respectively, thereby making the average spacer height (h**2**) sub-

stantially equal to the chamber height (h1) when the blood sample is received in the sample testing conduit. Testing indicates that that the desired chamber height (h1) can be controlled to 1% or better at chamber heights of less than four microns.

[0084] As shown in FIG. **10**, in other embodiments, an imaging device **1000** comprises a first planar member **1005** and a second planar member **1010** separated by a plurality of spacer elements **1015** that define an imaging chamber **1020** between the first planar member **1005** and the second planar member **1010**. The second planar member **1010** is formed from a material more flexible than the plurality of spacer elements **1015** and the first planar member **1005**, and will overlay the plurality of spacer elements **1015** in a tent-like fashion i.e., relatively speaking, the second planar member **1010** may be considered to be flexible relative to the plurality of spacer elements **1015**, and the plurality of spacer elements **1015** may be considered to be rigid relative to the second planar member **1010**. In this example, it should be apparent although small local areas of the imaging chamber **1020** will deviate from the desired chamber height (h**1**), the average height of all the tented areas will approximate the average spacer height (h**2**), thereby making the average spacer height (h**2**) substantially equal to the chamber height (h**1**) when the blood sample is received in the sample testing conduit. Testing indicates that that the desired chamber height (h**1**) can be controlled to 1% or better at chamber heights of less than four microns.

Imaging Assay Beads

[0085] As shown in FIG. **11**A, an imaging chamber **1100** may comprise a first planar member **1105**, a second planar member **1110**, and a plurality of wells **1115** disposed between the first planar member **1105** and the second planar member **1110**. A height (h1) of the imaging chamber **1100** may be predetermined such that the sample residing within the imaging chamber **1100** will travel laterally within the imaging chamber **1100** via capillary forces. In various embodiments, at least one of the first planar member **1105** and the second planar member **1110** is transparent (e.g., a transparent planar member). Transparent plastic films comprising acrylic or polystyrene are examples of acceptable materials for the first planar member **1105** and the second planar member **1110**. In some embodiments, at least a portion of the first planar member **1105** or the second planar member **1110** (e.g., a planar member) is formed from the imager chip **1120**, as similarly described with respect to imaging chamber **800** illustrated in FIG. **8**.

[0086] As shown in FIGS. **11**B, **11**C, and **11**D, the plurality of wells **1115** may be arranged on the photosensitive surface **1125** in a pattern such that the wells are aligned vertically with one or more of the pixels **1130** in the array of pixels. In some embodiments, the plurality of wells **1115** are arranged on the photosensitive surface **1125** such that at least 50% of the wells are aligned vertically with one or more of the pixels **1130** in the array of pixels (see, e.g., FIG. **11**B). In several embodiments, the plurality of wells **1115** are arranged on the photosensitive surface **1125** such that each of the wells is aligned vertically with one or more of the pixels **1130** in the array of pixels (see, e.g., FIG. **11**C). In certain embodiments, the plurality of wells **1115** are arranged on the photosensitive surface **1125** such that each of the wells is aligned vertically with exactly one of the pixels in the array of pixels (see, e.g., FIG. **11**D). Consequently, the alignment between the wells and the pixels facilitates satisfaction of the near-field criterion and resolution of the assay beads to be captured within the wells.

[0087] In various embodiments, the plurality of wells **1115** are any structure that is disposable between the first planar member **1105** and the second planar member **1110**, and operable to hold one or more assay beads **1135**. In certain embodiments, each well **1115** is sized and each assay bead **1135** is sized such that each well **1115** is structured to hold exactly one assay bead **1135** (see, e.g., FIG. **11**B). In other embodiments, each well **1115** is sized and each assay bead **1135** is sized such that each well **1115** is structured to hold at least one assay bead **1135** (see, e.g., FIG. **11**C). In other embodiments, each well **1115** is sized and each assay bead **1135** is sized such that each well **1115** is structured to hold a plurality of assay beads **1135** (see, e.g., FIG. **11**D). The height of each of the plurality of wells **1115** typically do not equal one another exactly, but may be substantially equal. As such the height of the plurality of wells **1115** may be characterized as the average well height (h**3**). In some embodiments, the average well height (h**3**) is selected to accommodate the analysis of the assay beads **1135** and will be from about 0.5 μm to about 40 μm, from about 0.5 μm to about 20 μm, or from about 2 μm to about 10 μm, for example about 4 μm, about 8 μm, or about 10 μm. The width of each of the plurality of wells **1115** typically do not equal one another exactly, but may be substantially equal. As such the width of the plurality of wells **1115** may be characterized as the average well width (w**1**). In some embodiments, the average well width (w**1**) is selected to accommodate the analysis of assay beads **1135** and will be from about 0.5 μm to about 40 μm, from about 2.0 μm to about 20 μm, or from about 2 μm to about 10 μm, for example about 4 μm, about 10 μm, or about 15 μm.

[0088] In some embodiments, the plurality of wells **1115** are columns (e.g., round or square uniform polymer, silica or polystyrene columns). In some embodiments, the plurality of wells **1115** are hollow pillar structures fabricated on: (i) at least a portion of a surface of the first planar member **1105** (e.g., the photosensitive surface **1125** of the sensor chip **1120**), and/or (ii) at least a portion of a surface of the second planar member **1110**. In some embodiments, the plurality of wells **1115** are hollow structures embossed on: (i) at least a portion of a surface of the first planar member **1105** (e.g., the photosensitive surface **1125** of the sensor chip **1120**), and/or (ii) at least a portion of a surface of the second planar member **1110**. In some embodiments, the plurality of wells **1115** are formed directly (physical contact between the objects) on: (i) at least a portion of a surface of the first planar member **1105** (e.g., the photosensitive surface **1125** of the sensor chip **1120**), and/or (ii) at least a portion of a surface of the second planar member **1110**. In some embodiments, the plurality of spacer elements **1115** are formed indirectly (no physical contact between the objects) on: (i) at least a portion of a surface of the first planar member **1105** (e.g., the photosensitive surface **1125** of the sensor chip **1120**), and/or (ii) at least a portion of a surface of the second planar member **1110**.

[0089] In various embodiments, the assay beads **1135** are microparticles having a general shape such as spherical, cylinder, cube, dodecahedron, elliptical, or other regular or irregular shapes. In some embodiments, the assay beads **1135** are formed of a polymer such as a latex, glass, silica, or polystyrene. In other embodiments, the assay beads **1135**

are formed of a magnetic material such that they exhibit magnetic properties when placed in a magnetic field with no residual magnetism once removed from the magnetic field. The assay beads **1135** may have a diameter, width and/or length from about 0.1 μm to about 35 μm, from about 0.1 μm to about 20 μm, or from about 0.1 μm to about 10 μm. The assay beads **1135** may be coated with a reagent capable of binding a target antigen in a sample. The reagent may comprise an antibody, an antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, an optical marker dye identifying a type of assay bead, and/or combinations thereof

[0090] In some embodiments, the assay beads **1135** are mobile within the imagining chamber **1100**, which can accelerate binding reactions, making the capture step of the assay faster. For example, the imaging chamber **1100** can contain mobile assay beads **1135** capable of interacting with an analyte and being localized over the photosensitive surface **1125**, whereby motion of specimen via capillary forces, active forces (e.g., via a pump) and/or gravity in the imaging chamber **1100** is used to capture the mobile assay beads **1135** in the plurality of wells **1115**. Alternatively, the imaging chamber **1100** can contain mobile magnetic assay beads **1135** capable of interacting with an analyte and being localized over the photosensitive surface **1125**, whereby magnetic forces are used to capture the mobile magnetic assay beads **1135** in the plurality of wells **1115**. In some embodiments, exactly one assay bead **1135** is capable of being captured (by capillary action, active action, gravity or magnetic force) in each of the plurality of wells **1115**; however, not all wells **1115** must capture a bead **1135** (see, e.g., FIG. **11**B). For example, the plurality of wells **1115** are arranged such that at least 50% of the wells **1115** capture an assay bead **1135**, at least 65% of the wells **1115** capture an assay bead **1135**, or at least 85% of the wells **1115** capture an assay bead **1135**. In other embodiments, at least one assay bead **1135** is capable of being captured (by capillary action, active action, gravity or magnetic force) in each of the plurality of wells **1115**; however, not all wells **1115** must capture at least one assay bead **1135** (see, e.g., FIG. **11**C). For example, the plurality of wells **1115** are arranged such that at least 60% of the wells **1115** capture at least one assay bead **1135**, at least 75% of the wells **1115** capture at least one assay bead **1135**, or at least 85% of the wells **1115** capture at least one assay bead **1135**. In other embodiments, a plurality of assay beads **1135** are captured (by capillary action, active action, gravity or magnetic force) in each of the plurality of wells **1115**; however, not all wells **1115** must capture a plurality of beads **1135** (see, e.g., FIG. **11**D). For example, the plurality of wells **1115** are arranged such that at least 65% of the wells **1115** capture a plurality of assay beads **1135**, at least 75% of the wells **1115** capture a plurality of assay beads **1135**, or at least 85% of the wells **1115** capture a plurality of assay beads **1135**.

[0091] In other embodiments, the assay beads **1135** are immobilized within the imagining chamber **1100**. For example, the assay beads **1135** may be dispensed into the plurality of wells **1115**, forming an adhered, porous bioactive layer. The bioactive layer has the property of binding specifically to the analyte of interest, or of manifesting a detectable change when the analyte is present, and is most preferably an immobilized antibody directed against a target analyte. In some embodiments, exactly one assay bead **1135** from the plurality of assay beads **1135** is immobilized in each of the plurality of wells **1115**. In some embodiments, exactly one assay bead **1135** from the plurality of assay beads **1135** is immobilized in at least 50% of the plurality of wells **1115** (see, e.g., FIG. **11**B). In other embodiments, at least one assay bead **1135** from the plurality of assay beads **1135** is immobilized in each of the plurality of wells **1115**. In other embodiments, at least one assay bead **1135** from the plurality of assay beads **1135** is immobilized in at least 50% of the plurality of wells **1115** (see, e.g., FIG. **11**C). In other embodiments, a plurality of assay beads **1135** from the plurality of assay beads **1135** are immobilized in a portion of each of the plurality of wells. In other embodiments, a plurality of assay beads **1135** from the plurality of assay beads **1135** are immobilized in a portion of at least 50% of the plurality of wells (see, e.g., FIG. **11**D).

[0092] As shown in FIGS. **11**E, **11**F, **11**G, and **11**H, in various embodiments, the imaging chamber **1100** further comprises plurality of spacer elements **1140**. The plurality of spacer elements **1140** may be any structure that is disposable between the first planar member **1105** and the second planar member **1110**, and operable to space the first planar member **1105** and the second planar member **1110** apart from one another and maintain the chamber height (h**1**), as described with respect to the plurality of spacer elements **815** illustrated in FIG. **8**. In some embodiments, the plurality of spacer elements **1140** have a predetermined average spacer height (h**2**) and are disposed between the first planar member **1105** and the second planar member **1115** to form a chamber **1145** having a predetermined average chamber height (h**1**) extending between the first planar member **1105** and the second planar member **1115**. FIGS. **11**E and **11**F show that the plurality of spacer elements **1140** maybe spherical beads. FIG. **11**G shows the plurality of spacer elements **1140** may be pillar structures fabricated on: (i) at least a portion of a surface of the first planar member **1105** (e.g., the photosensitive surface **1125**), and/or (ii) at least a portion of a surface of the second planar member **1110**. In several embodiments, the plurality of spacer elements **1140** comprise a material that has greater flexibility than one or both of the first planar member **1105** and the second planar member **1110**. In other embodiments, the plurality of spacer elements **1140** comprise a material that has less flexibility than one or both of the first planar member **1105** and the second planar member **1110**. For example, at least one of: (i) the second planar member **1110** and (ii) the plurality of spacer elements **1135**, may be deformable such that the predetermined average spacer height (h**2**) is substantially equal to the predetermined average chamber height (h**1**) (e.g., (h**2**)=(h**1**)). Alternatively, at least one of: (i) the second planar member **1110** and (ii) the plurality of spacer elements **1135**, may be rigid such that the predetermined average spacer height (h**2**) is substantially equal to the predetermined average chamber height (h**1**) (e.g., (h**2**)=(h**1**)).

[0093] In other embodiments, the plurality of spacer elements **1140** are disposable between the first planar member **1105** and the second planar member **1110** and disposable above or below the wells **1115** such that the spacer elements **1140** and the wells **1115** are operable together to space the first planar member **1105** and the second planar member **1110** apart from one another and maintain the chamber height (h**1**). FIGS. **11**H and **11**I show the plurality of spacer elements **1140** may be pillar structures fabricated on: (i) at least a portion of a surface of one or more wells **1115**, and/or (ii) at least a portion of a surface of the second planar

member **1110**. For example at least one of: (i) the second planar member **1110** and (ii) the plurality of spacer elements **1135**, is deformable such that the predetermined average well height (h**3**) in addition to the predetermined average spacer height (h**2**) is substantially equal to the predetermined average chamber height (h**1**) (e.g., (h**3**)+(h**2**)=(h**1**)) when the blood sample is received in the sample testing conduit. Alternatively, at least one of: (i) the second planar member **1110** and (ii) the plurality of spacer elements **1135**, is rigid such that the predetermined average well height (h**3**) in addition to the predetermined average spacer height (h**2**) is substantially equal to the predetermined average chamber height (h**1**) (e.g., (h**3**)+(h**2**)=(h**1**)) when the blood sample is received in the sample testing conduit.

Sensor and Chip Designs

[0094] In one embodiment, a microfabricated sensor chip (e.g., the at least one sensor chip **315** described with respect to FIG. **3**) comprises a sensor or transducer (e.g., an optical sensor). In other embodiments, a microfabricated sensor chip comprises a sensor array including at least a first sensor (e.g., an electrochemical sensor) and a second sensor (e.g., an optical sensor). In some embodiments, the sensors are fabricated singularly, as adjacent structures within a sensor array of a single chip. In other embodiments, the sensors are fabricated singularly, separated from another within a sensor array of a plurality of chips, or any combination thereof. In certain embodiments, the microfabricated sensor chip(s) comprises a plastic, polyester, polyimide, or silicon planar substrate, a plastic, polyester, polyimide, or silicon non-planar substrate, a transparent plastic, polyester, polyimide, or silicon substrate, a printed circuit board (PCB), and the like.

[0095] In various embodiments, the microfabricated sensor chip is an imager chip comprising one or more optical sensors such as a light detector. For example, wafer-level micro-fabrication of a preferred embodiment of an imager chip **1200** may be achieved as shown in FIG. **12**. A non-conducting substrate **1205** having a planar top and bottom surface may be used as a base for the imager chip **1200**. An imaging integrated circuit **1210** may be provided or formed on the substrate **1205** by conventional means, e.g., a micro-fabrication technique known to those of skill in the art to form a multi-dimensional array of pixels **1215** presented at its surface **1220** (i.e., a photosensitive surface) and non-photosensitive supporting circuitry **1225** for processing and readout. In certain embodiments in which a fluorescent dye such as acridine orange is used as a reagent, the imager chip **1200** further comprises a filter layer **1227** (e.g., an absorptive or dichroic filter layer) between the sample and the multi-dimensional array of pixels **1215**. For example, the filter layer **1227** may be disposed on the top surface of the multi-dimensional array of pixels **1215**. The filter layer **1227** is an optical filter layer which absorbs at a specific wavelength, or one or more bands of wavelengths, e.g. red, blue, green, etc. The filter layer **1227** may be manufactured on the imager chip **1200** as a coating, e.g., dispensed and dried to form the layer. Alternatively, the filter layer **1227** may be manufactured on the imager chip **1200** as patterned photoresist material as described for example in U.S. Pat. No. 5,200,051, which is incorporated herein by reference in its entirety. Alternatively, the filter layer **1227** may be manufactured on the imager chip **1200** as a separate film layer fixed to a surface of the imager chip **1200**, e.g., the top surface of the multi-dimensional array of pixels **1215**.

[0096] The microfabricated imager chip **1200** may further comprise an electrical connection **1230** (e.g., an electrical connection comprising a plurality of discrete contacts) that connects the imaging integrated circuit **1210** to one or more conductive pins **1235** such as a temporary electrical connector. The size of the multi-dimensional array of pixels **1215** may be selected to fit with other components (e.g., the conduits or sensor region) of the testing device, e.g., a multi-dimensional array of pixels **1215** may be provided as a surface mount diode (SMD) and chip scale packaging (CSP) may be used to fit a variety of testing devices. In some embodiments, the imager chip **1200** may have a width from about 1 mm to about 20 mm and a length from about 1 mm to about 20 mm (e.g., a width of about 5 mm and a length of about 6 mm), in order to accommodate a low profile multi-dimensional array of pixels **1215** that has the industry standard 2.0 mm×1.25 mm footprint, which provides high efficiency light detection and low power consumption. In accordance with various aspects of the present invention, the sensitivity of the multi-dimensional array of pixels **1215** may be within the range of 0.5 uA/cm^2-4 uA/cm^2, for example substantially 1 uA/cm^2.

[0097] In various embodiments, the microfabricated sensor chip is an electrochemical sensor chip comprising one or more electrochemical sensors such as an amperometric electrode. For example, wafer-level micro-fabrication of a preferred embodiment of the electrochemical sensor chip **1300** may be achieved as shown in FIG. **13**. A non-conducting substrate **1305** having a planar top and bottom surface may be used as a base for the electrochemical sensor chip **1300**. A conducting layer **1310** may be deposited on the substrate **1305** by conventional means, e.g., screen printing, or micro-fabrication technique known to those of skill in the art to form at least one component **1315** (e.g., an amperometric electrode). The conducting layer **1310** may comprise a noble metal such as gold, platinum, silver, palladium, iridium, or alloys thereof, although other unreactive metals such as titanium and tungsten or alloys thereof may also be used, as many non-metallic electrodes of graphite, conductive polymer, or other materials may also be used. In certain embodiments, the one or more of the electrochemical sensors may be formed as electrodes with gold surfaces coated with a photo defined polyimide layer **1330** that includes openings to define a grid of small gold electrodes (e.g., a gold microarray electrode) at which an electroactive species may be oxidized. The electrochemical sensor chip **1300** may also comprise an electrical connection **1320** that connects each component of the conducting layer **1310** to one or more conductive pins **1325** such as a temporary (make and break) electrical connector.

[0098] In some embodiments, the electrochemical sensor chip **1300** comprises an array of 5-10 μm noble metal disks, e.g., 7 μm noble metal disks, on 15 μm centers. The array of noble metal disks or electrodes may cover a region, e.g., a circular region, approximately 300 to 900 μm in diameter, optionally 400-800 μm or about 600 μm in diameter, and may be formed by photo-patterning a thin layer of polyimide or photoresist of thickness up to 1.5 μm over a substrate made from a series of layers comprising Si,SiO2,TiW, and/or Au, or combinations thereof. In some embodiments, the electrodes have a working area of about 130,000 to 300,000 sq μm (i.e., a microelectrode), the volume of sample

directly over the electrodes may be about 0.1-0.3 and the volume of the sample over the sensor chip may be 1-3 μL. In accordance with these aspects of the present invention, the conduit (e.g., the conduit **465** described with respect to FIG. **4**A and **4**D) in a region of the electrodes (e.g., the one or more sensor recesses **430** described with respect to FIGS. **4**G- and **4**H) has a volume to sensor area ratio of less than about 64, to about 1 square mm.

[0099] As shown in FIG. **14**, in some embodiments, a microfabricated sensor chip **1400** includes an optical sensor **1405**. The optical sensor **1405** may be formed of a non-conducting substrate **1410** and an imaging integrated circuit **1415** may be provided or formed on the substrate **1410**. The imaging integrated circuit **1415** may include a multi-dimensional array of pixels **1420** for light detection and non-photosensitive supporting circuitry **1425** for processing and readout. The multi-dimensional array of pixels **1420** create a photosensitive surface **1430** of the optical sensor **1405**. In certain embodiments, each pixel of the array of pixels **1420** comprises a photodetector (e.g., one or more light detectors) and optionally an amplifier in order to measure properties of light such as absorbance and florescence from an imaging chamber **1435** disposed adjacent to the optical sensor **1405**. As described with respect to FIGS. **5-10** and **11**A-**11**I, at least a portion of the photosensitive surface **1430** forms a portion of the wall or planar member **1440** of conduit **1445** (e.g., the conduit **465** described with respect to FIGS. **4**A and **4**D).

[0100] In various embodiments, one or more light emitters **1450** are positioned near the conduit **1445** (e.g., on a side of the conduit or above the conduit) and configured to transmit light through a portion of another wall or transparent planar member **1452** and the imaging chamber **1435** to the sensor chip **1400**. In some embodiments, the one or more light emitters **1450** are located adjacent to the transparent planar member **1452** of the conduit **1445**, and configured to transmit light through the transparent planar member **1452** and the imaging chamber **1435** to the sensor chip **1400**. As such, the sensor chip **1400**, the conduit **1445**, and the one or more light emitters **1450** are contained within a same housing of the testing device (as described with respect to FIGS. **4**A-**4**R). In other embodiments, the one or more light emitters **1450** are positioned near the transparent planar member **1452** of the conduit **1445** but not within same housing of the testing device, and instead are provided within the instrument or analyzer. As such, the sensor chip **1400** and the conduit **1445** are housed within the same housing (as described with respect to FIGS. **4**A-**4**R), and the housing further comprises a window adjacent to the transparent planar member **1452** for illuminating the imaging chamber **1435** from the outside environment. In certain embodiments, the one or more light emitters **1450** are configured to transmit light through the window, the transparent planar member **1452**, and the imaging chamber **1435** to the sensor chip **1400**.

[0101] In several embodiments, the optical sensor **1405** is configured to measure the absorption of radiation (i.e., light), as a function of frequency or wavelength, due to the interaction of the radiation with a biological sample in the conduit **1445**. In accordance with these aspects, the one or more light emitters **1450** are arranged to transmit incident light **1455** of one or more wavelengths through the conduit **1445** having the biological sample. Upon the incident light **1455** striking the sample, photons that match an energy gap of cells, a target analyte, or a chromatic substance related to a presence of the cells or the target analyte present in the biological specimen are absorbed. Other photons of light **1457** transmit through the conduit **1445** and biological specimen unaffected. The optical sensor **1405** is arranged to collect the photons of light **1457** transmitted through the conduit **1445** and the biological sample, and convert the transmitted photons of light **1457** into current. By comparing the attenuation of the photons of light **1457** with the incident light **1455**, an absorption spectrum can be obtained to identify the presence, identity, count, and/or concentration of cells or the target analyte in the biological specimen.

[0102] In various embodiments, the optical sensor **1405** is connected via wirings **1460**, **1462** to a conductive contact **1465**, **1467**, respectively (e.g., temporary electrical connector). In embodiments in which the one or more light emitters **1450** are provided within the same housing as the optical sensor **1405**, the one or more light emitters **1450** are connected via wirings **1470**, **1472** to another conductive contact **1475**, **1477**, respectively (e.g., temporary electrical connector). The wirings **1460**, **1462**, **1470**, **1472** may be formed with gold surfaces that are optionally coated with a photo defined polyimide or photoresist layer such that the wirings **1460**, **1462**, **1470**, **1472** are insulated from exposure to the environment of the sensor region (e.g., the biological sample disposed within the conduit **1445**). The wirings **1460**, **1462**, **1470**, **1472** terminate at the conductive contacts **1465**, **1467**, **1475**, **1477**, respectively (e.g., the discrete connector contacts **150** as described with respect to FIG. **1**), which are used to make electrical contact with a connector (e.g., the multi-terminal connector **155** as described with respect to FIG. **1**) in the analyzer (e.g., an i-STAT® cartridge reader as described in U.S. Pat. No. 4,954,087, the entirety of which is incorporated herein by reference). The design and arrangement of sensor chip **1400**, optical sensor **1405**, one or more light emitters **1450**, wirings **1460**, **1462**, **1470**, **1472**, and/or conductive contacts **1465**, **1467**, **1475**, **1477** is preferably selected based on printing and performance characteristics (e.g., minimize interference between multiple sensors, maximize transmission of light through the conduit and biological specimen, avoidance of interfering light, size constraints, etc.). However, it should be understood to those of ordinary skill in the art that any design or arrangement for the components is contemplated without departing from the spirit and scope of the present invention.

[0103] In some embodiments, the universal channel circuitry of the analyzer applies a drive current (e.g., a voltage greater than 2V and a current less than 1 mA), optionally via the conductive contact **1475**, to the one or more light emitters **1450**, and measures output current from the one or more light emitters **1450** via an optional conductive contact **1477**. The output current is channeled from the conductive contact **1477** into the universal channel circuitry, and feedback resistor(s) of the universal channel circuitry may us the output current to set a nominal range of 0.5 mA to 4 mA, for example substantially 2 mA, which can provide over 1 mA at up to 4 V. The feedback resistor(s) are able to establish a constant current to continually drive the one or more light emitters **1450** for a predetermined period of time. The universal channel circuitry of the analyzer may control the optical sensor **1405** via the conductive contact **1467**. The optical sensor **1405** channels output current (i.e., the current converted from the photons of light **1457** received from the one or more light emitters **1450**) to the conductive contact

1465. The output current is channeled from the conductive contact **1465** into the universal channel circuitry and converted to a measurable voltage proportional to the amount of light detected by the optical sensor **1405**. The processor (e.g., the processor **220** described with respect to FIG. **2**) converts the measurable voltage to a qualitative, semi-quantitative, or quantitative value proportional to: (i) a number count or percentage for each type of cell in the blood sample, (ii) or an amount of target analyte in the biological specimen.

[0104] As shown in FIG. **15**, in alternative embodiments, a microfabricated sensor chip **1500** includes a first sensor **1505** (e.g., an optical sensor) and optionally a different sensor chip **1510** or the same sensor chip **1500** includes a second sensor **1515** (e.g., an electrochemical sensor). The first sensor **1505** may be an optical sensor constructed as similarly described with respect to FIG. **14**. The second sensor **1515** may be an electrochemical sensor constructed with an array of metal disks or electrodes that cover a region of the sensor chip **1500/1510**. In some embodiments, the electrochemical sensor may be formed as electrodes **1520** with gold surfaces that are exposed (e.g., no polyimide or photoresist covering) to the inside environment of an auxiliary conduit **1525** and configured to directly contact the biological sample disposed within the conduit **1525**. The second sensor **1515** may be connected via wiring **1530** to a conductive contact **1535** (e.g., temporary electrical connector). The wiring **1535** may be formed with a gold surface that is optionally coated with a photo defined polyimide or photoresist layer such that the wiring **1530** is insulated from exposure to the environment of the sensor region (e.g., the biological sample disposed within the conduit **1445**). The wiring **1530** terminates at the conductive contact **1535** (e.g., the discrete connector contacts **150** as described with respect to FIG. **1**), which are used to make electrical contact with a connector (e.g., the multi-terminal connector **155** as described with respect to FIG. **1**) in the analyzer (e.g., an i-STAT® cartridge reader as described in U.S. Pat. No. 4,954,087, the entirety of which is incorporated herein by reference). The design and arrangement of sensor chips **1500/1510**, first sensor **1505**, second sensor **1515**, wiring **1530**, and/or conductive contact **1535** is preferably selected based on printing and performance characteristics (e.g., minimize interference between multiple sensors, maximize transmission of light through the conduit and biological specimen, avoidance of interfering light, size constraints, etc.). However, it should be understood to those of ordinary skill in the art that any design or arrangement for the components is contemplated without departing from the spirit and scope of the present invention.

[0105] In some embodiments, a portion of the sensor chip **1500/1510** (e.g., a top surface of the substrate), a wall of the auxiliary conduit **1525**, and/or a wall of the sample receiving chamber (e.g., the sample receiving chamber **420** described with respect to FIGS. **4A-4R**) can be coated with one or more dry reagents to amend the biological sample for an electrochemical assay. For example, the sensor chip **1500/1510** may include a reagent region **1540** coated with an antibody-enzyme conjugate for an analyte of interest. The reagent region **1540** may be defined by a containment ring structure **1545**. In some embodiments, the containment ring structure **1545** is a hydrophobic ring of polyimide or another photolithographically produced layer. A microdroplet or several microdroplets (approximately 5-40 nL in size) or a series of about a 100 nanodroplets (approximately 50 to 1000 pL in size) containing the antibody-enzyme conjugate in some form may be dispensed or printed on the surface of the sensor chip **1500/1510**. The photodefined ring structure **1545** contains this aqueous droplet allowing the reagent region **1540** to be localized to a precision of a few microns. The reagent region **1545** can be made from 0.03 to approximately 2 mm^2 in size. The upper end of this size is limited by the size of the conduit and sensor chip the sensor chip **1500/1510** in present embodiments, and is not a limitation of the invention.

[0106] The biological sample or a fluid may be passed at least once over the dry reagent, e.g., the reagent region **1540** to dissolve the reagent within the biological sample or fluid. Reagents used to amend biological samples or fluid within the cartridge may include the antibody-enzyme conjugate, magnetic beads coated with capture antibodies, or blocking agents that prevent either specific or non-specific binding reactions among assay compounds. Within a segment of the biological sample or fluid, the reagent can be preferentially dissolved and concentrated within a predetermined region of the segment. This is achieved through control of the position and movement of the segment. Thus, for example, if only a portion of a segment, such as the leading edge, is reciprocated over the reagent, then a high local concentration of the reagent can be achieved close to the leading edge. Alternatively, if a homogenous distribution of the reagent is desired, for example if a known concentration of a reagent is required for a quantitative analysis, then further reciprocation of the sample or fluid will result in mixing and an even distribution.

[0107] In certain embodiments, the second sensor **1515** is an immunosensor positioned in the auxiliary conduit **1525** for receiving a biological sample mixed with the antibody-enzyme conjugate that is configured to bind to a target analyte within the biological sample. For example, the second sensor **1515** may be configured to detect an enzymatically produced electroactive species (e.g., 4-aminophenol) from the reaction of a substrate (e.g., 4-aminophenylphosphate) with an antibody-enzyme conjugate (e.g., one or more antibodies bound to alkaline phosphatase (ALP)). In accordance with these aspects, the second sensor **1515** contains a capture region or regions **1550** coated with capture antibodies that are configured to bind to a target analyte bound to the antibody-enzyme conjugate. The capture region **1550** may be defined by a containment ring structure **1555**. In some embodiments, the containment ring structure **1535** is a hydrophobic ring of polyimide or another photolithographically produced layer. A microdroplet or several microdroplets (approximately 5-40 nL in size) containing capture antibodies in some form, for example bound to beads or microspheres, may be dispensed on the surface of the second sensor **1515**. The photodefined ring structure **1555** contains this aqueous droplet allowing the capture region **1550** to be localized to a precision of a few microns. The capture region **1550** can be made from 0.03 to approximately 2 mm^2 in size. The upper end of this size is limited by the size of the auxiliary conduit **1525** and sensor chip **1500/1510** in present embodiments, and is not a limitation of the invention.

[0108] In various embodiments, the microfabricated sensor array (e.g., a sensor array on the microfabricated sensor chip **1500**, the different sensor chip **1510**, or a completely different sensor chip such as a ground chip) further com-

prises a reference sensor or electrode **1560**. In accordance with certain aspects, in which the second sensor **1515** is an amperometric sensors, the reference electrode **1560** is configured as a counter electrode to complete the circuitry. In a preferred embodiment, the reference electrode **1560** may comprise silver metal (Ag) and its silver salt (AgCl) deposited on a solid substrate (i.e., an Ag/AgCl reference electrode). The reference electrode **1560** may be connected via wiring **1565** to a reference contact **1570** (e.g., temporary electrical connector). The microfabricated sensor array may be designed such that the ground chip is positioned upstream of the semiconductor chip **1500/1510**. However, it should be understood that other arrangements for sensor and ground chips are possible without departing from the spirit and scope of the present invention. For example, the sensor array may further comprise one or more additional sensor chips (not shown) configured to detect various analytes of potential interest, such as troponin I, troponin T, CKMB, procalcitonin, bHCG, HCG, NTproBNP, proBNP, BNP, myoglobin, parathyroid hormone, d-dimer, NGAL, galectin-3, and/or PSA, among other analytes.

[0109] In certain embodiments, the universal channel circuitry of the analyzer applies a potential via the conductive contact **1535** to the second sensor **1515** and the reference electrode **1560**, and measures current changes generated by oxidation current from the substrate as an electrochemical signal. The electrochemical signal being proportional to the concentration of the analyte in the biological sample. The second sensor **1515** may have an applied potential of approximately +0 mV to 90 mV, e.g., 60 mV versus the reference electrode **1560** and, in another embodiment, the second sensor **1515** has an applied potential of approximately +40 mV versus the reference electrode **1560**. The signal generated by the enzyme reaction product at approximately +10 mV is distinguishable from the signal generated by the unreacted substrate at approximately +200 mV. It should be noted that the exact voltages used to amperometrically detect the substrate and the analyte will vary depending on the chemical structure of the substrate. It is important that the difference in the voltages used to detect the substrate be great enough to prevent interference between the readings.

[0110] In various embodiments, the sensor chip **1500/1510** further includes one or more conductometric sensors **1577** (e.g., hematocrit sensors). The one or more conductometric sensors **1577** are configured to determine biological sample arrival and/or departure at the reagent region **1540** and biological sample arrival and/or departure at the first and second sensors **1505** and **1515**. More specifically, the one or more conductometric sensors **1577** lie perpendicular to a length of the conduit **1525** or sensor conduit, and an electrical resistance between pairs of electrodes for the sensor **1577** may be used to monitor a relative position of a fluid front of the biological sample. For example, at the extremes, an open circuit reading may indicate that the biological sample has been pushed off the reagent region **1540** and a closed circuit reading may indicate the reagent region **1540** is covered with the biological sample.

[0111] In some embodiments, the one or more conductometric sensors **1577** comprise at least two electrodes **1580** and **1585** (i.e., electrode pair). The electrode **1580** may be positioned upstream from the reagent region **1540**, and the electrode **1585** may be position downstream of the reagent region **1540** and upstream of the second sensor **1515**. As shown in FIG. **15**, the electrodes **1580** and **1585** may be connected via wirings **1590** and **1592** to a conductive contact **1595**, which functions as a conductometric low pin, and a conductive contact **1597**, which functions an alternating current source or conductometric high pin, respectively. The wirings **1590** and **1592** may be formed with a gold surface that is coated with a photo defined polyimide or photoresist layer such that the wirings **1590** and **1592** are insulated from exposure to the biological sample disposed within the conduits. As such, in some embodiments, the biological sample or fluid reaches the reagent region **1540** after departing the sample receiving chamber and passing over the electrode **1580**, then the biological sample subsequently arrives at the second sensor **1515** after departing the reagent region **1540** and passing over the electrode **1585**.

[0112] While some embodiments are disclosed herein with respect to certain types of sensors (e.g., optical, electrochemical, and conductometric sensors) being electrically connected to certain pins, this is not intended to be restrictive. Instead, it should be understood to those of ordinary skill in the art that any design or arrangement for the sensors and pins is contemplated without departing from the spirit and scope of the present invention. For example, the universal channel circuitry is configured in such a manner that any pin and connector connection can be used as a channel for optical, amperometric, conductometric, and/or potentiometric measurements.

Assay Methods

[0113] FIGS. **16-22** show exemplary flowcharts for performing the process steps of the present invention. The steps of FIGS. **16-22** may be implemented using the computing devices and systems described above with respect to FIGS. **1-15**. Specifically, the flowcharts in FIGS. **16-22** illustrate the architecture, functionality, and operation of possible implementations of the systems, methods and computer program products according to several embodiments of the present invention. In this regard, each block in the flowcharts may represent a module, segment, or portion of code, which comprises one or more executable instructions stored on non-transitory machine readable storage medium that when executed by one or more processors (e.g., a processor of the analyzer) cause the one or more processors to perform the specified logical function(s) within the one or more executable instructions. It should also be noted that, in some alternative implementations, the functions noted in the blocks may occur out of the order noted in the figure. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the flowchart illustrations, and combinations of blocks in the flowchart illustrations, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

[0114] FIG. **16** illustrates a method **1600** (with reference to the testing device **400** as illustrated in FIGS. **4A-4J**) of using a testing device to perform an optical assay in accordance with one embodiment of the invention. At step **1605**, an unmetered biological sample may be introduced into a sample receiving chamber (e.g., the sample receiving chamber **420** described with respect to FIGS. **4G** and **4H**) of a testing device, through a sample entry port (e.g., sealable sample entry port **415** described with respect to FIGS. **4B**

and **4**C). Optionally at step **1610**, the biological sample may be filtered to remove cells ( ) such that only a plasma fraction of the sample reaches the sensors (e.g., in some embodiments (e.g., assays performed using the assay beads), if the cells are not substantially removed they may scatter the light from the LED and affect assay performance). In some embodiments, the sample receiving chamber comprises the filter material such that only the plasma fraction reaches the sample metering portion of the device. In other embodiments, a first conduit (e.g., conduit **465** described with respect to FIG. **4**A) comprises the filter material such that the metered portion of the sample is filtered to remove the cells. At step **1615**, a capillary stop (e.g., capillary stop **422** described with respect to FIGS. **4**G and **4**H) may prevent passage of the sample into the first conduit (e.g., conduit **465** described with respect to FIG. **4**A) at this stage, and the sample chamber is filled with the sample. The capillary stop at the end of the sample chamber delimits a metered portion of the biological sample. At step **1620**, a lid (e.g., closable sealing member **417** described with respect to FIGS. **4**A and **4**B) maybe closed to prevent leakage of the biological sample from the sensing device. While the biological sample is within sample chamber or the first conduit, the biological sample may be optionally amended at step **1625** with a compound or compounds (e.g., reagents such as dyes, enzymes, enzyme substrate, activators, stabilizers, binders, anticoagulants, buffers, enzyme-labeled antibody conjugate and the like) present initially as a dry coating on the inner surface of the sample chamber or first conduit.

[0115] At step **1630**, the testing device may be inserted into an analyzer (e.g., analyzer **105** described with respect to FIG. **1**) in accordance with some aspects of the present invention. Optionally at step **1635**, insertion of the sensing device into the analyzer may activate a first pump (e.g., the portion of the flexible zone **490** as described with respect to FIGS. **4**A and **4**B) or mechanism that punctures a fluid-containing package when the package is pressed against a spike (e.g., spike **442** as described with respect to FIGS. **4**G and **4**H). Fluid (e.g., a substrate) may thereby be expelled into a second conduit (e.g., conduit **437** as described with respect to FIGS. **4**G and **4**H) that is in fluidic communication with the first conduit. A constriction in the second conduit prevents further movement of the fluid. At step **1640**, operation of a second pump (e.g., displaceable membrane **445** as described with respect to FIGS. **4**A, **4**B, **4**G, and **4**H) by the analyzer applies pressure to an air-bladder of the sensing device, forcing air through a third conduit (e.g., conduit **455** as described with respect to FIGS. **4**G and **4**H) and into the sample chamber at a predetermined location.

[0116] At step **1645**, the metered portion of the biological sample is expelled through the capillary stop by air pressure produced within the air-bladder at step **1640** into the first conduit. Optionally at step **1650**, the biological sample is moved forward within the first conduit to a portion of the first conduit (e.g., conduit **465** as described with respect to FIGS. **4**A) that is exposed to a sensor chip (e.g., sensor chip **1400** as described with respect to FIG. **14**) by air pressure produced within the air-bladder such that the biological specimen can be amended with a compound or compounds (e.g., reagents such as enzymes, enzyme substrate, activators, stabilizers, buffers, enzyme-labeled antibody conjugate and the like) present initially as a dry coating on a portion of the sensor chip (i.e., one or more reagent regions). Additionally or alternatively, the fluid in the second conduit may be moved past the constriction into the first conduit and into contact with the biological specimen by air pressure produced by the first pump. The fluid may include a substrate that may be acted upon by the biological specimen and/or amended compounds to produce a chromatic substance. To facilitate the dissolution of the substrate, compound or compounds in the biological sample and/or promote efficient reaction, the biological sample may be oscillated by air pressure produced within the air-bladder. In one embodiment, an oscillation frequency of between about 0.2 Hz and about 5 Hz is used, most preferably about 0.7 Hz.

[0117] At step **1655**, the biological sample is move forward within the first conduit to a portion of the first conduit forming an imaging chamber (e.g., imaging chamber **530** as described with respect to FIG. **5**) that is exposed to the sensor chip (e.g., sensor chip **505** or **1400** as described with respect to FIGS. **5** and **14**) by air pressure produced within the air-bladder such that analysis (e.g., optical analysis) of the biological specimen can be performed. Once the biological sample is move forward to the imaging chamber, the biological specimen is dispersed through-out the imaging chamber by capillary action. In various embodiments, the biological sample dispersed though-out the imaging chamber over the photosensitive surface such that one or more light emitters can transmit incident light of one or more wavelengths into the portion of the imaging chamber and the biological specimen. Upon the incident light striking the biological sample, photons that match an energy gap of cells, a target analyte, or a chromatic substance related to a presence of cells or the target analyte present in the biological specimen are absorbed. Other photons transmit through the imaging chamber and biological specimen unaffected. The photosensitive surface collect the photons of light transmitted through the imaging chamber and the biological sample, and convert the transmitted photons of light into current. At step **1660**, the current is transmitted to the analyzer as an output signal via a conductive contact, and the analyzer compares the attenuation of the transmitted light with the incident light to obtain an absorption spectrum and converts the output signal to: (i) to a number count or percentage for each type of cell in the blood sample, or (ii) an analyte signal proportional to the light received from the imaging chamber and collected by the photosensitive surface.

[0118] FIG. **17** illustrates a method **1700** (with reference to the testing device **400** as illustrated in FIGS. **4**A-**4**J) of using a testing device to perform an optical assay and an electrochemical assay in accordance with one embodiment of the invention. At step **1705**, an unmetered biological sample may be introduced into a sample chamber (e.g., the sample holding chamber **420** described with respect to FIGS. **4**G and **4**H) of a testing device, through a sample entry port (e.g., sealable sample entry port **415** described with respect to FIGS. **4**B and **4**C). Optionally at step **1710**, the biological sample may be filtered to remove cells such that only a plasma fraction of the sample reaches the sensors (e.g., in some embodiments, if the cells are not substantially removed they may scatter the light from the LED and affect assay performance). In some embodiments, the sample receiving chamber comprises the filter material such that only the plasma fraction reaches the sample metering portion of the device. In other embodiments, a first conduit (e.g., conduit **465** described with respect to FIG. **4**A) comprises the filter material such that the metered portion of the

sample is filtered to remove the cells. At step **1715**, a capillary stop (e.g., capillary stop **422** described with respect to FIGS. **4**G and **4**H) may prevent passage of the sample into the first conduit (e.g., conduit **465** described with respect to FIG. **4**A) at this stage, and the sample chamber is filled with the sample. The capillary stop at the end of the sample chamber delimits a metered portion of the biological sample. At step **1720**, a lid (e.g., closable sealing member **417** described with respect to FIGS. **4**A and **4**B) maybe closed to prevent leakage of the biological sample from the sensing device. While the biological sample is within sample chamber or the first conduit, the biological sample may be optionally amended at step **1725** with a compound or compounds (e.g., reagents such as dyes, enzymes, enzyme substrate, activators, stabilizers, binders, anticoagulants, buffers, enzyme-labeled antibody conjugate and the like) present initially as a dry coating on the inner surface of the sample chamber or first conduit.

[0119] At step **1730**, the testing device may be inserted into an analyzer (e.g., analyzer **105** described with respect to FIG. **1**) in accordance with some aspects of the present invention. Optionally at step **1735**, insertion of the sensing device into the analyzer may activate a first pump (e.g., the portion of the flexible zone **490** as described with respect to FIGS. **4**A and **4**B) or mechanism that punctures a fluid-containing package when the package is pressed against a spike (e.g., spike **442** as described with respect to FIGS. **4**G and **4**H). Fluid (e.g., a substrate) may thereby be expelled into a second conduit (e.g., conduit **437** as described with respect to FIGS. **4**G and **4**H) that is in fluidic communication with the first conduit and/or an auxiliary conduit. A constriction in the second conduit prevents further movement of the fluid. At step **1740**, operation of a second pump (e.g., displaceable membrane **445** as described with respect to FIGS. **4**A, **4**B, **4**G, and **4**H) by the analyzer applies pressure to an air-bladder of the sensing device, forcing air through a third conduit (e.g., conduit **455** as described with respect to FIGS. **4**G and **4**H) and into the sample chamber at a predetermined location.

[0120] At step **1745**, the metered portion of the biological sample is expelled through the capillary stop by air pressure produced within the air-bladder at step **1740** into the first conduit. Optionally at step **1750**, the biological sample is moved forward within the first conduit to a portion of the first conduit (e.g., conduit **465** as described with respect to FIGS. **4**A) that is exposed to a sensor chip (e.g., sensor chip **1400** as described with respect to FIG. **14**) by air pressure produced within the air-bladder such that the biological specimen can be amended with a compound or compounds (e.g., reagents such as enzymes, enzyme substrate, activators, stabilizers, buffers, enzyme-labeled antibody conjugate and the like) present initially as a dry coating on a portion of the sensor chip (i.e., one or more reagent regions). Additionally or alternatively, the fluid in the second conduit may be moved past the constriction into the first conduit and into contact with the biological specimen by air pressure produced by the first pump. The fluid may include a substrate that may be acted upon by the biological specimen and/or amended compounds to produce a chromatic substance. To facilitate the dissolution of the substrate, compound or compounds in the biological sample and/or promote efficient reaction, the biological sample may be oscillated by air pressure produced within the air-bladder. In one embodiment, an oscillation frequency of between about 0.2 Hz and about 5 Hz is used, most preferably about 0.7 Hz.

[0121] At step **1755**, the biological sample is move forward within the first conduit to a portion of the first conduit forming an imaging chamber (e.g., imaging chamber **530** as described with respect to FIG. **5**) that is exposed to the sensor chip (e.g., sensor chip **505** or **1400** as described with respect to FIGS. **5** and **14**) by air pressure produced within the air-bladder such that analysis (e.g., optical analysis) of the biological specimen can be performed. Once the biological sample is move forward to the imaging chamber, the biological specimen is dispersed through-out the imaging chamber by capillary action. In various embodiments, the biological sample dispersed though-out the imaging chamber over the photosensitive surface such that one or more light emitters can transmit incident light of one or more wavelengths into the portion of the imaging chamber and the biological specimen. Upon the incident light striking the biological sample, photons that match an energy gap of cells, a target analyte, or a chromatic substance related to a presence of cells or the target analyte present in the biological specimen are absorbed. Other photons transmit through the imaging chamber and biological specimen unaffected. The photosensitive surface collect the photons of light transmitted through the imaging chamber and the biological sample, and convert the transmitted photons of light into current. At step **1760**, the current is transmitted to the analyzer as an output signal via a conductive contact, and the analyzer compares the attenuation of the transmitted light with the incident light to obtain an absorption spectrum and converts the output signal to: (i) to a number count or percentage for each type of cell in the blood sample, or (ii) an analyte signal proportional to the light received from the imaging chamber and collected by the photosensitive surface.

[0122] At step **1765**, the biological sample is move forward within the first conduit to an auxiliary conduit (e.g., auxiliary conduit **1525** as described with respect to FIG. **15**) that is exposed to the sensor chip (e.g., sensor chip **1500**/**1510** as described with respect to FIG. **15**) by air pressure produced within the air-bladder such that analysis (e.g., electrochemical analysis) of the biological specimen can be performed. Optionally at step **1770**, the biological sample is moved forward such that the biological specimen can be amended with a compound or compounds (e.g., reagents such as an enzyme and enzyme substrate-labeled antibody conjugate) present initially as a dry coating on a portion of the sensor chip (i.e., one or more reagent regions). To facilitate the dissolution of the compound or compounds in the biological sample and/or promote efficient reaction, the biological sample may be oscillated over the one or more reagent regions by air pressure produced within the air-bladder. In one embodiment, an oscillation frequency of between about 0.2 Hz and about 5 Hz is used, most preferably about 0.7 Hz. At step **1775**, the biological sample is moved forward within the auxiliary conduit to a position over a second sensor (e.g., an amperometric sensor) by air pressure produced within the air-bladder. Optionally at step **1780**, to promote efficient reaction product formation sandwich formation on or near the surface of the second sensor comprising a biolayer, the biological sample may be oscillated over the second sensors by air pressure produced within the air-bladder. In one embodiment, an oscillation

frequency of between about 0.2 Hz and about 5 Hz is used, most preferably about 0.7 Hz

[0123] At step **1785**, the biological sample is displaced from the auxiliary conduit by further pressure applied to air-bladder, and the biological sample passes to a waste chamber (e.g., waste chamber **433** as described with respect to FIGS. **4**A and 4G.). At optional step **1790**, one or more air segments (meniscus) may be produced within the first conduit by any suitable means, including a passive means, an embodiment of which is described in detail in U.S. Pat. No. 7,682,833, which is incorporated herein by reference in its entirety, or an active means including a transient lowering of the pressure within the first conduit using the second pump whereby air is drawn into the first conduit through a flap or valve. The one or more air segments are extremely effective at clearing or rinsing the biological sample-contaminated fluid from the first conduit. For example, a leading and/or trailing edge of the one or more air segments may be passed a number of times over the first and second sensors to rinse and resuspend extraneous material that may have been deposited from the biological sample. Extraneous material includes any material other than specifically bound analyte or analyte/antibody-enzyme conjugate complex. However, in accordance with various embodiments, the clearing or rinsing step **1790** using the one or more air segments is not sufficiently protracted or vigorous so as to promote substantial dissociation of specifically bound analyte or analyte/antibody-enzyme conjugate complex from a biolayer.

[0124] Optionally at step **1795**, the fluid in the second conduit is moved past the constriction into the auxiliary conduit and into contact with the second sensor by air pressure produced by the first pump. The fluid may include a substrate or signal agent and the enzyme remaining within the auxiliary conduit and immobilized on or near the second sensor either produces an electroactive species from an electro-inactive substrate or destroys an electroactive substrate. In some embodiments, the fluid may be applied to the second sensor to wash the biological sample from the second sensor. At step **1797**, a change in current or potential generated by the production or destruction of the electroactive species at the second sensor and the change is transmitted as a function of time to the analyzer via a conductive contact, and the analyzer performs analysis of the change in current or potential to identify the presence and/or concentration of the target analyte in the biological specimen.

[0125] As should be understood, the previous steps could be split up into two or more processes for using two or more testing devices to perform an optical assay and an electrochemical assay in accordance with alternative embodiments of the invention. For example, the steps pertaining to the optical assay could be performed via an optical testing device and subsequently the steps pertaining to the electrochemical assay could be performed via an electrochemical testing device, or vice versa.

[0126] FIG. **18** illustrates a method **1800** (with reference to the testing device **400** as illustrated in FIGS. **4**K-**4**R) of using a testing device to perform an optical assay in accordance with one embodiment of the invention. At step **1805**, an unmetered biological sample may be introduced into a sample receiving chamber (e.g., the sample receiving chamber **420** described with respect to FIG. **4**R) of a testing device, through a sample entry port (e.g., sample entry port **415** described with respect to FIGS. **4**K and **4**P). While the biological sample is within the sample chamber, the biological sample may be optionally passively amended (e.g., dissolution) with a compound or compounds (e.g., reagents such as dyes, enzymes, enzyme substrate, activators, stabilizers, binders, anticoagulants, buffers, enzyme-labeled antibody conjugate and the like) present initially as a dry coating on the inner surface of the sample chamber.

[0127] At step **1810**, the testing device may be inserted into an analyzer (e.g., analyzer **105** described with respect to FIG. **1**) in accordance with some aspects of the present invention. At step **1815**, the biological sample moves passively from the sample chamber into a conduit (e.g., conduit **465** described with respect to FIG. **4**R). For example, capillary action may facilitate the passive movement of the biological sample from the sample chamber into the conduit. In some embodiments, the surfaces of the fluidic paths and/or conduit may be treated to make them more or less hydrophilic and hydrophobic to further promote capillary action using established techniques known in the art. While the biological sample is within the conduit, the biological sample may be optionally amended passively (e.g., dissolution) with a compound or compounds (e.g., reagents such as dyes, enzymes, enzyme substrate, activators, stabilizers, binders, anticoagulants, buffers, enzyme-labeled antibody conjugate and the like) present initially as a dry coating on the inner surface of the conduit.

[0128] At step **1820**, the biological sample moves passively through the conduit to a portion of the conduit forming an imaging chamber (e.g., imaging chamber **485**, **530** as described with respect to FIGS. **4**P and **5**) that is exposed to the sensor chip (e.g., sensor chip **505** or **1400** as described with respect to FIGS. **5** and **14**). For example, capillary action may facilitate the passive movement of the biological sample through the conduit and into the imaging chamber. While the biological sample is within the imaging chamber, the biological sample may be optionally amended passively (e.g., dissolution) with a compound or compounds (e.g., reagents such as dyes, enzymes, enzyme substrate, activators, stabilizers, binders, anticoagulants, buffers, enzyme-labeled antibody conjugate and the like) present initially as a dry coating on the inner surface of the imaging chamber.

[0129] Once the biological sample is move forward to the imaging chamber, the biological specimen is dispersed through-out the imaging chamber by capillary action. In various embodiments, the biological sample dispersed though-out the imaging chamber over the photosensitive surface such that one or more light emitters can transmit incident light of one or more wavelengths into the portion of the imaging chamber and the biological specimen. Upon the incident light striking the biological sample, photons that match an energy gap of cells, a target analyte, or a chromatic substance related to a presence of cells or the target analyte present in the biological specimen are absorbed. Other photons transmit through the imaging chamber and biological specimen unaffected. The photosensitive surface collect the photons of light transmitted through the imaging chamber and the biological sample, and convert the transmitted photons of light into current. At step **1825**, the current is transmitted to the analyzer as an output signal via a conductive contact, and the analyzer compares the attenuation of the transmitted light with the incident light to obtain an absorption spectrum and converts the output signal to: (i) to a number count or percentage for each type of cell in the

blood sample, or (ii) an analyte signal proportional to the light received from the imaging chamber and collected by the photosensitive surface.

[0130] FIG. **19** illustrates a method **1900** of performing a differential blood cell count in a biological sample (e.g., whole blood) in accordance with various embodiments of the invention. At step **1905**, a test cartridge is provided that comprises a sample entry port, a sample testing conduit fluidically connected to the sample entry port, optionally a pump, and an imager chip comprising an array of pixels. In some embodiments, the test cartridge further comprises a plurality of discrete connector contacts, and the imager chip is electrically connected to at least one of the plurality of discrete connector contacts. At step **1910**, an analyzer is provided comprising a processor and display. In some embodiments, the analyzer further comprises a multi-terminal connector and optionally a pump actuator. At step **1915**, the test cartridge is mated with the analyzer. The mating may comprise inserting the testing device into a port of the analyzer. Mating or inserting the test cartridge into the port of the analyzer places the multi-terminal connector in electrical contact with the plurality of discrete connector contacts. Mating or inserting the test cartridge into the port of the analyzer places may also place the pump actuator aligned with the pump in the test cartridge. At step **1920**, a blood sample (e.g., whole blood) is introduced into the sample entry port before or after the mating the test cartridge with the analyzer.

[0131] At step **1925**, an operating state signal is received that is indicative of a type of test cartridge inserted into the analyzer. In some embodiments, the operating state signal comprises a value of a measured resistance between contacts of the test cartridge and a shorting bar. For example, in order to impart cartridge identification functionality into a test cartridge, an additional mechanism or means may be included in the sensor chip arrangement for cartridge identification. In certain embodiments, a resistor can be implemented between contacts. The resistance of the resistor may be measured by a detector (e.g., processor) by applying a small voltage, e.g., 1 mV, between the contacts, subsequent to (e.g., immediately after) the cartridge being inserted into the analyzer. The value of the measured resistance can then be used for cartridge identification. For example, each cartridge type (e.g., i-STAT® cartridges EC8+, CG8+, EG7+, CHEM8+, etc.) may be associated with a certain resistance or resistance range such that a measured resistance of the cartridge may be used to identify the type of cartridge using a look-up table.

[0132] In alternative embodiments, the operating state signal comprises a value obtained from a barcode located on the test cartridge or a package of the test cartridge. For example, an imaging area of the test cartridge may be used to scan a barcode to obtain a value using the barcode reader **135** of the instrument **110**, as described with respect to FIG. **1**. The value of barcode can then be used for cartridge identification. For example, each cartridge type (e.g., i-STAT® cartridges EC8+, CG8+, EG7+, CHEM8+, etc.) may be associated with a certain value such that a scanned value of the cartridge may be used to identify the type of cartridge using a look-up table retained in the instrument.

[0133] At step **1930**, information regarding sensors of the test cartridge are determined based on the identified type of cartridge. In certain embodiments, determining the information comprises: identifying, based on a value of the operating state signal, the type of test cartridge using a look-up table, and obtaining, based on the type of test cartridge, the information regarding the sensors from a database, where the database has information for each type of test cartridge. In various embodiments, the information indicates the type of sensors of the test cartridge (e.g., one or more optical sensors, one or more reference electrode, one or more electrochemical sensors, etc) and the position of conductive contacts connected to the sensors of the test cartridge In addition or alternative to obtaining information regarding the type of sensors and the position of conductive contacts from the database via the identified type of testing cartridge, the type of sensors and position of the conductive contacts may be identified using information obtained regarding the connector pins in contact with the various conductive contacts of the testing cartridge. For example, the analyzer connector may be a linear array of connector pins, e.g., pins one to twenty. The type of sensors and position of the conductive contacts may be identified via the position of each pin relative to the contacts. For example, a light emitter may be connected via a contact to a pin "x" (e.g., pin **11**) and a light detector of the optical sensor may be connected via another contact to a pin "y" (e.g., **12**), and thus since both pins **11** and **12** are being used, the type of sensor (optical) and components (e.g., light emitter and light detector) connected to the contacts can be identified via the database. Consequently, as described herein, the analyzer may then assign channels of the universal circuitry to the appropriate pins for the types of sensors determined to be in the identified testing cartridge. As should be understood, once a test cycle is run and the testing cartridge is removed from the instrument or analyzer, the channels of the universal circuitry can be reassigned to the same or different connector pins when a new testing cartridge is inserted into the analyzer.

[0134] At step **1935**, a first channel is assigned to the light emitter via: (i) the first contact and a corresponding first pin, and optionally, (ii) the second contact and a corresponding second pin. At step **1940**, a second channel is assigned to the light detector via the third contact and a corresponding third pin. At step **1945**, the circuitry of the first channel is switched to a current driver mode. In some embodiments, the switching the circuitry of the first channel comprises modifying switching elements of the circuitry such that the first channel is configured to apply the drive current via the first contact and the corresponding first pin to the light emitter. At step **1950**, the circuitry of the second channel is switched to a current measurement mode. In some embodiments, the switching the circuitry of the second channel comprises modifying switching elements of the circuitry such that the second channel is configured to convert output current received from the light detector to a measurable voltage proportional to an amount light detected by the light detector.

[0135] At step **1955**, a dry reagent is dissolved into the blood sample to generate an amended blood sample. In some embodiments, dissolving the dry reagent may include driving the pump actuator to actuate the pump on the test cartridge and move the blood sample into contact with the dry reagent (e.g., cause the blood sample to oscillate over the dry reagent), which ultimately dissolves the dry reagent in the blood sample. In other embodiments, dissolving the dry reagent may include the blood sample moving passively into contact with the dry reagent, which ultimately dissolves the

dry reagent in the blood sample. At step **1960**, the amended blood sample is moved into a sample testing conduit. The sample testing conduit may comprise a first wall formed from at least a portion of an imager chip, a second wall formed from a transparent material layer, and a plurality of spacer elements having an average spacer height and disposed between the first wall and the second wall. In certain embodiments, the average spacer height defines an average chamber height of a chamber between the portion of the imager chip and the transparent material layer. In some embodiments, moving the blood sample into the sample testing conduit includes driving the pump actuator to actuate the pump on the test cartridge and move the blood sample from a sample receiving chamber into the sample testing conduit. In other embodiments, moving the blood sample into the sample testing conduit includes the blood sample moving passively from a sample receiving chamber into the sample testing conduit.

[0136] At step **1965**, a drive current is applied to the light emitter using the first channel. The applying the drive current to the light emitter causes the light emitter to generate output current and light comprising a predetermined wavelength that is projected through the chamber and the amended blood sample. Optionally at step **1970**, the output current generated by the light emitter is received at the first channel from the second contact and the corresponding second pin, and the output current is applied to a feedback resistor to establish a constant current for the drive current.

[0137] At step **1975**, the light detector converts the photons of light received from the light emitter to an output current and sends the output current to the third contact as an output signal. In some embodiments, the output signal is at least one of absorbance and fluorescence and is recorded at the array of pixels based on the light received from the light emitter. At step **1980**, the output signal from the light detector is received at the second channel via the third contact and the corresponding third pin. The output signal may be converted, using the second channel, to a number count or percentage for each type of cell in the blood sample. At step **1885**, the number count or percentage for each type of cell in the blood sample may be displayed on the display. Optionally at step **1990**, the test cartridge is unmated from the analyzer and the test cartridge is discarded in the trash.

[0138] FIG. **20** illustrates a method **2000** of performing a differential blood cell count in a biological sample (e.g., whole blood) in accordance with various embodiments of the invention. Medical diagnostics often include analyses of a whole blood sample from a patient. One of the more popular diagnostics is a complete blood count (referred to as a "CBC"), which is a suite of tests that may include, in addition to the enumeration of the cellular components, red blood cell metrics, reticulocyte counts, and a leukocyte differential count ("LDC"; sometimes referred to as a "white blood cell differential"), which is the identification and enumeration of the types of white blood cells (WBCs) present in the blood sample. At step **2005**, a test cartridge is provided that comprises a sample entry port, a sample testing conduit fluidically connected to the sample entry port, optionally a pump, and an imager chip comprising an array of pixels. In some embodiments, the test cartridge further comprises a plurality of discrete connector contacts, and the imager chip is electrically connected to at least one of the plurality of discrete connector contacts. At step **2010**, an analyzer is provided comprising a processor and display. In some embodiments, the analyzer further comprises a multi-terminal connector and optionally a pump actuator. At step **2015**, the test cartridge is mated with the analyzer. The mating may comprise inserting the testing device into a port of the analyzer. Mating or inserting the test cartridge into the port of the analyzer places the multi-terminal connector in electrical contact with the plurality of discrete connector contacts. Mating or inserting the test cartridge into the port of the analyzer places may also place the pump actuator aligned with the pump in the test cartridge. At step **2020**, a blood sample (e.g., whole blood) is introduced into the sample entry port before or after the mating the test cartridge with the analyzer.

[0139] At step **2025**, a dry reagent is dissolved into the blood sample to generate an amended blood sample. In some embodiments, dissolving the dry reagent may include driving the pump actuator to actuate the pump on the test cartridge and move the blood sample into contact with the dry reagent (e.g., cause the blood sample to oscillate over the dry reagent), which ultimately dissolves the dry reagent in the blood sample. In other embodiments, dissolving the dry reagent may include the blood sample moving passively into contact with the dry reagent, which ultimately dissolves the dry reagent in the blood sample. The reagent may be added to the sample to facilitate distinguishing one constituent from another within the sample. For example, the reagent may be a dye or colorant such as Acridine Orange (also referred to as "Basic Orange 15" or "ACO") and Astrazon Orange (also referred to as "AO" or Basic Orange 21), which emit light at particular wavelengths when mixed with whole blood and subjected to an excitation wavelength from the light emitter. The light emitter may be operable to produce light at wavelengths associated with one or more of red, green, and blue light. The red light is typically produced in the range of about 600-700 nm, with red light at about 660 nm preferred. The green light is typically produced in the range of about 515-570 nm, with green light at about 540 nm preferred. The blue light is typically in the range of about 405-425 nm, with blue light at about 413 nm preferred. Light transmitted through the sample, or fluoresced from the sample, is captured using the imager chip, and a signal representative of the captured light is sent to the analyzer, where it is processed into an image. The image is produced in a manner that permits the light transmittance or fluorescence intensity captured within the image to be determined on a per unit basis; e.g., "per unit basis" being an incremental unit of which the image of the sample can be dissected, such as a pixel.

[0140] At step **2030**, the amended blood sample is moved into a sample testing conduit. In certain embodiments, the sample is quiescently residing within the chamber. The sample testing conduit may comprise a first wall formed from at least a portion of an imager chip, a second wall formed from a transparent material layer, and a plurality of spacer elements having an average spacer height and disposed between the first wall and the second wall. In certain embodiments, the average spacer height defines an average chamber height of a chamber between the portion of the imager chip and the transparent material layer. In some embodiments, moving the blood sample into the sample testing conduit includes driving the pump actuator to actuate the pump on the test cartridge and move the blood sample from a sample receiving chamber into the sample testing

conduit. In other embodiments, moving the blood sample into the sample testing conduit includes the blood sample moving passively from a sample receiving chamber into the sample testing conduit.

[0141] At step **2035**, at least one image of the sample residing within the chamber is created using the imager chip. In some embodiments, the imager chip comprises a CCD type image sensor that converts light passing through (or from) the sample into an electronic data format image. In other embodiments, the imager chip comprises a CMOS type image sensor that converts light passing through (or from) the sample into an electronic data format image. The signals from the imager chip provide information for each pixel of the image, which information includes, or can be derived to include, intensity, wavelength, and optical density. Intensity values may be assigned an arbitrary scale of, for example, 0 units to 4095 units ("IVUs"). Optical density ("OD") is a measure of the amount of light absorbed relative to the amount of light transmitted through a medium; e.g., the higher the "OD" value, the greater the amount of light absorbed during transmission. OD may be quantitatively described in optical density units ("OD") or fractions thereof; e.g., a MilliOD is a $1/1000^{th}$ of an OD. One "OD" unit decreases light intensity by 90%. "OD" or "MilliOD" as a quantitative value can be used for images acquired or derived by transmission light, for example, the transmission blue light.

[0142] In some embodiments, the information from the imager chip is separated into multiple channels, for example, three channels, which provides particular utility for determining a four part LDC. However, the present invention is not limited to a three channel embodiment. A first of the three channels may be directed toward information relating to light emitted from the sample at a first wavelength (e.g., 540 nm, which appears green). A second channel may be directed toward information relating to light emitted from the sample at a second wavelength (e.g., 660 nm, which appears red). A third channel may be directed toward information relating to light passing through the sample at a third wavelength (e.g., 413 nm, which is used to determine blue optical density—"OD"). These wavelength values and the number of channels have particular utility when an LDC is being performed on a whole blood sample. However, the present invention is not limited to these particular wavelengths or number of channels. Additional channels can be implemented to gather information at different wavelengths and/or transmission values. That information, in turn, can be used to evaluate additional constituents within the sample and/or to increase the accuracy of the analysis. For example, in applications where it is desirable to further differentiate basophils within the sample, a fourth and a fifth channel can be added. The fourth channel can be directed toward information relating to light passing through the sample at a fourth wavelength (e.g., 540 nm), which is used to determine green OD, and the fifth channel can be directed toward information relating to light passing through the sample at a fifth wavelength (e.g., 660 nm), which is used to determine red OD. These OD values, in turn, can be used to identify basophils.

[0143] At step **2040**, the imager chip converts the photons of light received from the light emitter to an output current and sends the output current to the analyzer. At step **2045**, the output signal from the imager chip is received at the analyzer. For example, the analyzer is in communication with the test cartridge, light emitter, and imager chip, and may be adapted (e.g., programmed) to send and receive signals from one or more of the cartridge, light emitter, and imager chip. For example, the analyzer is adapted to: (i) send signals to the light emitter to produce light at defined wavelengths (or alternatively at multiple wavelengths); and (ii) send and receive signals from the imager chip to capture light for defined periods of time. The analyzer is further adapted to process the signals (e.g., the output signal) received from the imager chip according to one or more predetermined algorithms. The specifics of a particular algorithm will depend upon the analysis at hand. As indicated above, the present invention has particular utility when applied to perform an LDC on a whole blood sample, and to illustrate that utility the invention is described herein as performing an LDC. However, the present invention is not limited to this particular analysis.

[0144] At step **2050**, a differential blood cell count is performed using the output signal received from the imager chip. In some embodiments, a differential blood cell count includes: (i) identifying the cells, for example white blood cells, within the sample residing within the chamber; (ii) quantitatively analyzing at least some of the identified cells within the image relative to one or more predetermined quantitatively determinable features; and (iii) identifying at least one type of cell from the identified cells using the quantitatively determinable features. For example, to perform the differential blood cell count such as a LDC, the algorithm utilizes a set of identifying features, each of which features is distinguishable from the other features and each of which is quantitatively determinable from an image of the sample. Each WBC can be characterized by the presence or absence of certain identifying features, and/or by quantitative information associated with certain features. For purposes of providing an enabling disclosure, the present invention is described herein in terms of an exemplary set of identifying features that can be used to selectively identify and distinguish WBCs. This set is not inclusive of all possible features, and therefore the present invention is not limited to this particular set.

[0145] For a WBC analysis, if for example acridine orange is used, an exemplary set of identifying features includes those entitled: Cell, Nucleus, number of Lobes, Cell Area, Nucleus Area Ratio of Large Granules, Ratio of Nucleus, Red-Green Ratio, Nucleus Shape, Cell Shape, Nucleus Brightness, Cytoplasm Brightness, Average Cell Absorption at a Given Wavelength, Nucleus Texture, Cytoplasm Texture, Cell Absorption Texture at a Given Wavelength, Nucleus Hollowness, and Cytoplasm Hollowness; each of which is described in US. Patent Publication No. 20120034647, which is incorporated herein by reference. In some instances, certain features directly provide information about a particular cell (e.g., Nucleus Shape). In other instances, a feature (e.g., Cell Area) can be used to indirectly provide information about a particular cell (e.g., ratio of Nucleus Area to Cell Area—referred to above as "Ratio of Nucleus", etc.). The identifying features are based on quantifiable characteristics such as light intensity, light color. OD, area, and relative position (e.g., shape). As indicated above, the colors may be created by one or more fluorescent colorants admixed with the sample, which upon excitation, produce fluorescent light emission at particular wavelengths associated with particular colors. As should be understood,

this principal also applies to non-fluorescent dye detection based on absorbance of a particular wavelength associated with particular colors.

[0146] An example of an acceptable colorant that can be used when performing an LDC on a whole blood sample is Acridine Orange ("ACO"). ACO is a fluorescent dye that, when mixed with a whole blood sample, selectively stains constituents within the sample; e.g., white blood cells, platelets, reticulocytes, and nucleated red blood cells. With respect to WBCs, the ACO permeates through the respective WBC and stains its DNA and RNA. The color(s) emitted by the dye within the WBC arc a function of a number of factors, including: the quantity of RNA and DNA within the dye, the concentration of the dye in the constituent, and the pH of the constituent. The present invention is not limited to using ACO, and other dyes (e.g., Astrazon Orange) may be used in place of ACO or in combination with ACO. Using ACO and white blood cells as an example, if the sample is subjected to an excitation light at or about a wavelength of 470 nm, the ACO bound to materials (e.g., DNA) within the nucleus of a white blood cell will emit light at about 540 nm (which appears green), and the ACO bound to materials (e.g., RNA) within the cytoplasm of a white blood cell will emit light at about 660 nm (which appears red).

[0147] As indicated above, OD values within the sample are a function of absorptivity of light at predetermined wavelengths by materials that naturally occur within the cell (e.g., hemoglobin), and/or may be a function of colorant absorbed (or not absorbed) by constituents within the sample. The identification of particular groups of pixels at one or more defined wavelengths can be performed using a variety of different techniques. For example, segmentation techniques can be used to produce a masked image depicting only those pixels within the image that meet the criteria (e.g., intensity and color). For those analyses that derive information only from the green light portions (e.g., the nuclei) or the red light portions (e.g., cytoplasm) of the image, or both, the sample image can be masked to produce a partial image depicting only those pixels showing green, or red, or both, and may also be distinguished by a predetermined intensity threshold. The present invention is not limited to any particular segmentation technique, and a specific technique can be chosen in view of the application at hand. For example, a hard segmentation technique can be used wherein a pixel is assigned as either belonging to an object or not. "Hard" segmentation techniques can be implemented using thresholding, region grow, or watershed type routines. Alternatively, soft segmentation techniques can be utilized; e.g., a "fuzzy" segmentation, where each pixel is assigned a value in the range of 0 to 1, which value describes the likelihood that the particular pixel belongs to the object. The description of each of the identifying features below will provide clear examples of how quantitative data such as that associated with wavelength and intensity can provide a basis for distinguishing one WBC from another. The present invention is also not limited to using a segmentation technique, and can use other techniques that select (i.e., "pick") pixels or otherwise distinguish pixels having particular attributes.

[0148] As indicated above, an LDC is an analysis wherein the different types of WBCs are identified and enumerated. The results can be expressed in terms of the relative percentages of the identified WBC types. Consequently, at step **2050**, the output signal output signal may be converted, using the analyzer, to a number count or percentage for each type of cell in the blood sample. At step **2050**, the number count or percentage for each type of cell in the blood sample may be displayed on the display. Optionally at step **2055**, the test cartridge is unmated from the analyzer and the test cartridge is discarded in the trash.

[0149] FIG. **21** illustrates a method **2100** of imaging assay beads in a biological sample (e.g., plasma) in accordance with various embodiments of the invention. At step **2105**, a test cartridge is provided that comprises a sample entry port, a sample testing conduit fluidically connected to the sample entry port, optionally a pump, and an imager chip comprising an array of pixels. In some embodiments, the test cartridge further comprises a plurality of discrete connector contacts, and the imager chip is electrically connected to at least one of the plurality of discrete connector contacts. At step **2110**, an analyzer is provided comprising a processor and display. In some embodiments, the analyzer further comprises a multi-terminal connector and optionally a pump actuator. At step **2115**, the test cartridge is mated with the analyzer. The mating may comprise inserting the testing device into a port of the analyzer. Mating or inserting the test cartridge into the port of the analyzer places the multi-terminal connector in electrical contact with the plurality of discrete connector contacts. Mating or inserting the test cartridge into the port of the analyzer places may also place the pump actuator aligned with the pump in the test cartridge. At step **2020**, a blood sample is introduced into the sample entry port before or after the mating the test cartridge with the analyzer.

[0150] At step **2125**, an operating state signal is received that is indicative of a type of test cartridge inserted into the analyzer. In some embodiments, the operating state signal comprises a value of a measured resistance between contacts of the test cartridge and a shorting bar. For example, in order to impart cartridge identification functionality into a test cartridge, an additional mechanism or means may be included in the sensor chip arrangement for cartridge identification. In certain embodiments, a resistor can be implemented between contacts. The resistance of the resistor may be measured by a detector (e.g., processor) by applying a small voltage, e.g., 1 mV, between the contacts, subsequent to (e.g., immediately after) the cartridge being inserted into the analyzer. The value of the measured resistance can then be used for cartridge identification. For example, each cartridge type (e.g., i-STAT® cartridges EC8+, CG8+, EG7+, CHEM8+, etc.) may be associated with a certain resistance or resistance range such that a measured resistance of the cartridge may be used to identify the type of cartridge using a look-up table.

[0151] In alternative embodiments, the operating state signal comprises a value obtained from a barcode located on the test cartridge or a package of the test cartridge. For example, an imaging area of the test cartridge may be used to scan a barcode to obtain a value using the barcode reader **135** of the instrument **110**, as described with respect to FIG. **1**. The value of barcode can then be used for cartridge identification. For example, each cartridge type (e.g., i-STAT® cartridges EC8+, CG8+, EG7+, CHEM8+, etc.) may be associated with a certain value such that a scanned value of the cartridge may be used to identify the type of cartridge using a look-up table retained in the instrument.

[0152] At step **2130**, information regarding sensors of the test cartridge are determined based on the identified type of

cartridge. In certain embodiments, determining the information comprises: identifying, based on a value of the operating state signal, the type of test cartridge using a look-up table, and obtaining, based on the type of test cartridge, the information regarding the sensors from a database, where the database has information for each type of test cartridge. In various embodiments, the information indicates the type of sensors of the test cartridge (e.g., one or more optical sensors, one or more reference electrode, one or more electrochemical sensors, etc.) and the position of conductive contacts connected to the sensors of the test cartridge In addition or alternative to obtaining information regarding the type of sensors and the position of conductive contacts from the database via the identified type of testing cartridge, the type of sensors and position of the conductive contacts may be identified using information obtained regarding the connector pins in contact with the various conductive contacts of the testing cartridge. For example, the analyzer connector may be a linear array of connector pins, e.g., pins one to twenty. The type of sensors and position of the conductive contacts may be identified via the position of each pin relative to the contacts. For example, a light emitter may be connected via a contact to a pin "x" (e.g., pin **11**) and a light detector of the optical sensor may be connected via another contact to a pin "y" (e.g., **12**), and thus since both pins **11** and **12** are being used, the type of sensor (optical) and components (e.g., light emitter and light detector) connected to the contacts can be identified via the database. Consequently, as described herein, the analyzer may then assign channels of the universal circuitry to the appropriate pins for the types of sensors determined to be in the identified testing cartridge. As should be understood, once a test cycle is run and the testing cartridge is removed from the instrument or analyzer, the channels of the universal circuitry can be reassigned to the same or different connector pins when a new testing cartridge is inserted into the analyzer.

[0153] At step **2135**, a first channel is assigned to the light emitter via: (i) the first contact and a corresponding first pin, and optionally, (ii) the second contact and a corresponding second pin. At step **2140**, a second channel is assigned to the light detector via the third contact and a corresponding third pin. At step **2145**, the circuitry of the first channel is switched to a current driver mode. In some embodiments, the switching the circuitry of the first channel comprises modifying switching elements of the circuitry such that the first channel is configured to apply the drive current via the first contact and the corresponding first pin to the light emitter. At step **2150**, the circuitry of the second channel is switched to a current measurement mode. In some embodiments, the switching the circuitry of the second channel comprises modifying switching elements of the circuitry such that the second channel is configured to convert output current received from the light detector to a measurable voltage proportional to an amount light detected by the light detector.

[0154] Optionally, at step **2155**, a dry reagent is dissolved into the blood sample to generate an amended blood sample. In some embodiments, dissolving the dry reagent may include driving the pump actuator to actuate the pump on the test cartridge and move the blood sample into contact with the dry reagent (e.g., cause the blood sample to oscillate over the dry reagent), which ultimately dissolves the dry reagent in the blood sample. In other embodiments, dissolving the dry reagent may include the blood sample moving passively into contact with the dry reagent, which ultimately dissolves the dry reagent in the blood sample. At step **2160**, the amended blood sample is moved into a sample testing conduit. The sample testing conduit may comprise a first wall formed from at least a portion of an imager chip, a second wall formed from a transparent material layer, and a plurality of wells having an average well height and disposed between the first wall and the second wall. In some embodiments, each of the plurality of wells is aligned vertically with one or more pixels of the imager chip, and at least a portion of the plurality of wells comprise at least one assay bead. In some embodiments, moving the blood sample into the sample testing conduit includes driving the pump actuator to actuate the pump on the test cartridge and move the blood sample from a sample receiving chamber into the sample testing conduit. In other embodiments, moving the blood sample into the sample testing conduit includes the blood sample moving passively from a sample receiving chamber into the sample testing conduit.

[0155] At step **2165**, a drive current is applied to the light emitter using the first channel. The applying the drive current to the light emitter causes the light emitter to generate output current and light comprising a predetermined wavelength that is projected through the sample testing conduit and the amended blood sample. Optionally, at step **2170**, the output current generated by the light emitter is received at the first channel from the second contact and the corresponding second pin, and the output current is applied to a feedback resistor to establish a constant current for the drive current.

[0156] At step **2175**, the light detector converts the photons of light received from the light emitter to an output current and sends the output current to the third contact as an output signal. In some embodiments, the output signal is at least one of absorbance and fluorescence and is recorded at the array of pixels based on the light received from the light emitter. At step **2180**, the output signal from the light detector is received at the second channel via the third contact and the corresponding third pin. The output signal may be converted, using the second channel, to a value indicative of a reaction of the biological sample with the at least one assay bead in each of the plurality of wells. At step **2185**, value indicative of a reaction of the biological sample may be displayed on the display. Optionally at step **2190**, the test cartridge is unmated from the analyzer and the test cartridge is discarded in the trash.

[0157] FIG. **22** illustrates a method **2200** of performing an optical assay and electrochemical assay using a same testing device. At step **2205**, a qualitative, semi-quantitative, or quantitative value is determined based on a measurable voltage that is proportional to cell types or an amount of target analyte in the biological specimen in accordance with steps **1905-1985** of method **1900** or **2105-2185** of method **2100**. At step **2210**, additional/alternative information regarding sensors of the test cartridge is determined based on the type of the test cartridge and/or the pins being used. In various embodiments, the information indicates that a fourth contact is connected to a counter electrode, a fifth contact is connected to a reference electrode, and the third contact or a sixth contact is connected to a working electrode (e.g., an amperometric electrode).

[0158] At step **2215**, a third channel is assigned to the counter electrode via the fourth contact and a corresponding

fourth pin. At step **2220**, a fourth channel is assigned to the reference electrode via the fifth contact and a corresponding fifth pin. At step **2225**, the second channel is assigned to the working electrode via the third contact and the corresponding third pin or the sixth contact and a corresponding sixth pin. At step **2230**, the circuitry of the third channel is switched to a counter measurement mode. In some embodiments, the switching the circuitry of the third channel comprises modifying switching elements of the circuitry such that the third channel is configured to apply a potential that is optionally not measured and is adjusted so as to balance the reaction occurring at the working electrode. This configuration allows the potential of the working electrode to be measured against a known electrode (i.e., the counter electrode) without compromising the stability of the reference electrode by passing current over the reference electrode. At step **2235**, the circuitry of the fourth channel is switched to a reference measurement mode. In some embodiments, the switching the circuitry of the fourth channel comprises modifying switching elements of the circuitry such that the fourth channel is configured to apply a stable potential to the reference electrode, which may be used as a reference for measurements made by the working electrode.

[0159] At step **2240**, the pump actuator is driven to actuate the pump on the test cartridge to split the blood sample into a first portion and a second portion. In some embodiments, the first portion of the blood sample is moved into the sample testing conduit. At step **2245**, the pump actuator is driven to actuate the pump on the test cartridge to move the second portion of the blood sample into an auxiliary conduit comprising an electrochemical sensor for detecting an analyte in the blood sample. At step **2250**, an analyte signal from the electrochemical sensor is recorded based on performance of an electrochemical analytical test in the auxiliary conduit, and a qualitative, semi-quantitative, or quantitative value is determined proportional to an amount of the analyte in the blood sample based on the analyte signal. In various embodiments, the performing the electrochemical analytical test comprises: (i) applying a potential to the counter electrode using the third channel; (ii) applying a potential to the reference electrode using the fourth channel; (iii) applying a potential to the working electrode using the second channel; (iv) measuring a current change across the biological specimen, using the second channel, that is proportional to a concentration of target analyte within the biological specimen; and (v) determining the concentration of target analyte within the biological specimen based on the current change across the biological specimen. In various embodiments, the counter electrode and the reference electrode are used in conjunction with the working electrode to measure the current change across the biological specimen. At step **2255**, the concentration of target analyte within the biological specimen may be displayed on the display. Optionally at step **2260**, the test cartridge is unmated from the analyzer and the test cartridge is discarded in the trash.

[0160] While the invention has been described in detail, modifications within the spirit and scope of the invention will be readily apparent to the skilled artisan. It should be understood that aspects of the invention and portions of various embodiments and various features recited above and/or in the appended claims may be combined or interchanged either in whole or in part. In the foregoing descriptions of the various embodiments, those embodiments which refer to another embodiment may be appropriately combined with other embodiments as will be appreciated by the skilled artisan. Furthermore, the skilled artisan will appreciate that the foregoing description is by way of example only, and is not intended to limit the invention.

We claim:

1. A test device for imaging assay beads, comprising:

a sample entry port for receiving a biological sample;

a sample receiving chamber fluidically connected to the sample entry port; and

a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit comprising: (i) a first planar member, (ii) a second planar member, and (iii) a plurality of wells having a predetermined average well height and disposed between the first planar member and the second planar member,

wherein the second planar member comprises an imager chip comprising an array of pixels; and

wherein each of the plurality of wells is aligned vertically with one or more of the pixels in the array of pixels.

2. The test device of claim **1**, wherein each of the plurality of wells has a width from about 2 μm to about 20 μm.

3. The test device of claim **1**, further comprising a plurality of assay beads.

4. The test device of claim **3**, wherein at least one assay bead from the plurality of assay beads is disposed in each of the plurality of wells.

5. The test device of claim **3**, wherein the plurality of assay beads comprise a reagent, which comprises an antibody, antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, or combinations thereof.

6. The test device of claim **3**, wherein the plurality of assay beads comprise a reagent, which comprises an optical marker dye identifying a type of assay bead.

7. The test device of claim **3**, wherein the plurality of assay beads have a diameter from about 0.1 μm to about 20 μm.

8. The test device of claim **3**, wherein the plurality of assay beads are immobilized in a portion of each of the plurality of wells.

9. The test device of claim **1**, wherein the sample testing conduit further comprises a plurality of spacer elements having a predetermined average spacer height and disposed between the first planar member and the second planar member to form a chamber having a predetermined average chamber height extending between the first planar member and the second planar member.

10. The test device of claim **9**, wherein the predetermined average well height plus the predetermined average spacer height is substantially equal to the predetermined average chamber height.

11. The test device of claim **9**, wherein the predetermined average spacer height is substantially equal to the predetermined average chamber height.

12. The test device of claim **1**, wherein:

the imager chip comprises a substrate and a photosensitive surface;

the photosensitive surface comprises the array of pixels; and

the plurality of wells are in direct contact with the photosensitive surface.

13. The test device of claim **12**, wherein the array of pixels comprise at least 5 mega pixel resolution with at least a 150 ppi pixel density.

14. The test device of claim **13**, wherein each pixel of the array has an area of less than about two μm^2.

15. The test device of claim **1**, further comprising a light emitter positioned over the sample testing conduit, wherein the light emitter is configured to transmit light through the chamber to the imager chip at one or more wavelengths from about 300 nm to about 1000 μm.

16. The test device of claim **15**, further comprising a housing, wherein the imager chip, the sample testing conduit, and the light emitter are housed within the housing.

17. A test device for imaging assay beads, comprising:

a housing comprising a sample entry port for receiving a biological sample;

an imager chip formed in the housing and comprising an array of pixels, wherein each pixel of the array has an area of less than about two μm^2;

a sample testing conduit fluidically connected to the sample entry port and comprising a first wall and a second wall, wherein at least a portion of the imager chip forms the first wall;

a plurality of wells abutting the portion of the imager chip, wherein each of the plurality of wells has a width from about 2 μm to about 20 μm; and

a conformable transparent material layer contacting the plurality of wells and forming the second wall of the sample testing conduit,

wherein each of the plurality of wells is aligned vertically with one or more pixels in the array of pixels and each of the plurality of wells comprise at least one assay bead.

18. The test device of claim **17**, wherein the at least one assay bead comprises a reagent, which comprises an antibody, antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, or combinations thereof.

19. The test device of claim **17**, wherein the at least one assay bead comprises a reagent, which comprises an optical marker dye identifying a type of assay bead.

20. The test device of claim **17**, wherein each pixel of the array has an area from about 0.5 μm^2 to about 1.5 μm^2.

21. The test device of claim **17**, wherein each pixel of the array has an area of about 1.1 μm^2.

22. The test device of claim **17**, wherein the array of pixels comprises at least 5 mega pixel resolution with at least a 150 ppi pixel density.

23. The test device of claim **17**, wherein the imager chip has a width from about 1 mm to about 20 mm and a length from about 1 mm to about 20 mm.

24. The test device of claim **17**, wherein the imager chip has a width of about 5 mm and a length of about 6 mm.

25. The test device of claim **17**, wherein the housing further comprises a window adjacent to the transparent material layer for illuminating the sample testing conduit.

26. The test device of claim **17**, wherein at least a portion of the transparent material layer comprises one or more fiduciary features for calibration of the imager chip.

27. The test device of claim **17**, wherein at least a portion of the transparent material layer comprises one or more fiduciary features for calibration of the biological sample.

28. The test device of claim **17**, wherein the transparent material layer comprises a Mylar™ film , a polycarbonate film, polyethylene terephthalate (PET), or cyclo-olefin polymer (COP).

29. The test device of claim **17**, wherein at least a portion of the sample testing conduit has a width from about 0.5 mm to about 2 cm.

30. The test device of claim **17**, wherein at least a portion of the sample testing conduit has a length from about 0.5 mm to about 2 cm.

31. The test device of claim **17**, further comprising a light emitter positioned over the sample testing conduit, wherein the light emitter is configured to transmit light through the chamber to the imager chip at one or more wavelengths from about 300 nm to about 1000 μm.

32. The test device of claim **31**, wherein the light emitter is located adjacent to the transparent material layer.

33. The test device of claim **31**, wherein the light emitter is one or more light-emitting diodes.

34. The test device of claim **17**, wherein the imager chip is configured to measure absorbance.

35. The test device of claim **17**, wherein the imager chip comprises a filter layer and is configured to measure fluorescence.

36. The test device of claim **17**, further comprising a pump configured to move the biological sample from the sample entry port into the sample testing conduit.

37. The test device of claim **17**, further comprising an auxiliary conduit fluidically connected to the sample entry port and comprising an electrochemical sensor for detecting an analyte in the biological sample.

38. The test device of claim **17**, further comprising an auxiliary conduit fluidically connected to the sample entry port and comprising a conductivity sensor for detecting a position of the biological sample in the auxiliary conduit.

39. The test device of claim **17**, wherein the sample testing conduit further comprises a plurality of spacer elements having a predetermined average spacer height and disposed between the first wall and the second wall to form a chamber having a predetermined average chamber height extending between the first wall and the second wall.

40. The test device of claim **39**, wherein the plurality of wells have a predetermined average well height, and the predetermined average well height plus the predetermined average spacer height is substantially equal to the predetermined average chamber height.

41. The test device of claim **39**, wherein the predetermined average spacer height is substantially equal to the predetermined average chamber height.

42. A system for imaging assay beads, comprising:

an analyzer comprising: a port, a multi-terminal connector, a processor connected to the multi-terminal connector, and memory coupled to the processor; and

a test cartridge comprising:

a plurality of discrete connector contacts,

a sample receiving chamber configured to receive a biological sample,

a sample testing conduit fluidically connected to the sample receiving chamber, the sample testing conduit comprising: (i) a first wall, (ii) a second wall, and (iii) a plurality of wells having an average well height and disposed between the first wall and the second wall, and

an analyte assay region comprising: a portion of the sample testing conduit and an imager chip, wherein the imager chip is electrically connected to at least one of the plurality of discrete connector contacts, and at least a portion of the imager chip forms a portion of the first wall of the sample receiving chamber,

wherein each of the plurality of wells is aligned vertically with one or more pixels of the imager chip and each of the plurality of wells comprise at least one assay bead,

wherein the test cartridge is insertable into the port such that the multi-terminal connector is in electrical contact with the plurality of discrete connector contacts,

wherein the memory is encoded with a set of instructions configured to perform an analytical test on the biological sample, and

wherein to perform the analytical test, (i) the processor is electrically connected to a light emitter, (ii) the processor is electrically connected to the imager chip via at least one of the plurality of discrete connector contacts and the multi-terminal connector, (iii) the processor is configured to drive the light emitter to generate light projected into the portion of the sample testing conduit, (iv) the imager chip is configured to convert light received from the portion of the sample testing conduit to an output signal, and (v) the processor is configured to convert the output signal of the imager chip to a value indicative of a reaction of the biological sample with the at least one assay bead in each of the plurality of wells.

43. The system of claim **42**, wherein each of the plurality of wells has a width from about 2 μm to about 20 μm.

44. The system of claim **42**, wherein the at least one assay bead comprises a reagent, which comprises an antibody, antibody fragment, an ionophore, an enzyme, a set of enzymes, a peptide with a cleavable detectable moiety, or combinations thereof.

45. The system of claim **42**, wherein the at least one assay bead comprises a reagent, which comprises an optical marker dye identifying a type of assay bead.

46. The system of claim **42**, wherein the at least one assay bead has a diameter from about 0.1 μm to about 20 μm.

47. The system of claim **42**, wherein the at least one assay bead is immobilized in a portion of each of the plurality of wells.

48. The system of claim **42**, wherein the test cartridge comprises the light emitter, and the light emitter is electrically connected to at least one of the plurality of discrete connector contacts.

49. The system of claim **48** , wherein the test cartridge further comprises a housing, wherein the imager chip, the sample testing conduit, and the light emitter are housed within the housing.

50. The system of claim **42**, wherein the analyzer comprises the light emitter, the test cartridge further comprises a housing comprising a window adjacent to the sample testing conduit for illuminating the portion of the sample testing conduit, and the test cartridge is insertable into the port such that the light emitter is aligned over the window and the portion of the sample testing conduit.

51. The system of claim **42**, wherein the analyzer further comprises a pump actuator, the test cartridge further comprises a pump, and the test cartridge is insertable into the port such that the pump actuator is aligned with the pump.

* * * * *

patsnap

专利名称(译)	用于成像测定珠的一次性测试装置		
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当前申请(专利权)人(译)	雅培医疗点Inc.的.		
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摘要(译)

本发明一般涉及用于在微观尺度上进行生物成像的装置，系统和方法，更具体地说，涉及包括配置成在微观尺度上进行生物成像的一次性测试装置的装置和系统，以及使用该装置和系统进行生物成像的方法。一次性测试设备。在一些方面，提供了用于成像测定珠的测试装置。该测试装置具有用于接收血液样本的样本入口;样品测试导管流体连接到样品入口端口，样品测试导管包括：（i）平面构件，（ii）透明平面构件，和（iii）具有预定平均孔高度的多个孔并设置在它们之间第一平面构件和第二平面构件;以及形成平面构件的至少一部分的成像器芯片。