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(54) **Title**: DETECTION APPARATUS HAVING A MICROFLUOROMETER, A FLUIDIC SYSTEM, AND A FLOW CELL LATCH CLAMP MODULE

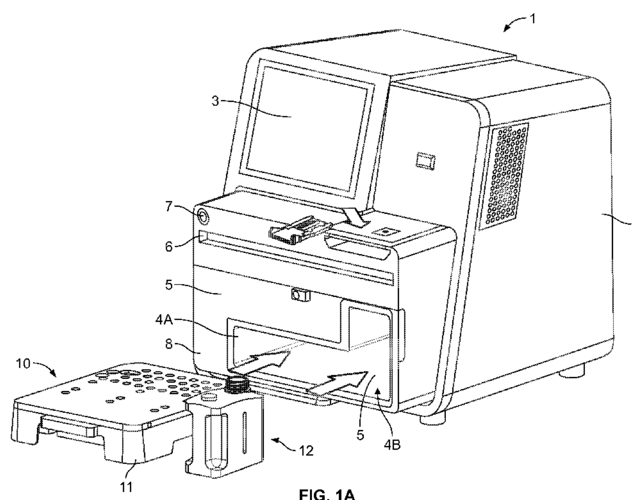

FIG. 1A

(57) **Abstract**: Detection apparatus includes a microfluorometer having an objective, an excitation radiation source, and a detector. The detection apparatus also includes a fluidic system for delivering reagents from a reagent cartridge to a flow cell. The fluidic system includes a manifold body having a plurality of fluidic channels configured for fluid communication between the reagent cartridge and the flow cell. The fluidic system also includes a plurality of reagent sippers. The fluidic system also includes a valve configured to mediate fluid between reagent reservoirs and the flow cell. The detection apparatus also includes a flow cell latch clamp module having a clamp cover for holding the flow cell. The objective is configured to direct excitation radiation from the radiation source to the flow cell and to direct emission from the flow cell to the detector. The microfluorometer is movable to acquire wide-field images of different areas of the flow cell.

WO 2017/123533 A1

DETECTION APPARATUS HAVING A MICROFLUOROMETER, A FLUIDIC SYSTEM, AND A FLOW CELL LATCH CLAMP MODULE

BACKGROUND

5 Embodiments of the present disclosure relate generally to apparatus and methods for fluidic manipulation and optical detection of samples, for example, in nucleic acid sequencing procedures.

Our genome provides a blue print for predicting many of our inherent predispositions such as our preferences, talents, susceptibility to disease and responsiveness to therapeutic 10 drugs. An individual human genome contains a sequence of over 3 billion nucleotides. Differences in just a fraction of those nucleotides impart many of our unique characteristics. The research community is making impressive strides in unraveling the features that make up the blue print and with that a more complete understanding of how the information in each blue print relates to human health. However, our understanding is far from complete and this 15 is hindering movement of the information from research labs to the clinic where the hope is that one day each of us will have a copy of our own personal genome so that we can sit down with our doctor to determine appropriate choices for a healthy lifestyle or a proper course of treatment.

The current bottleneck is a matter of throughput and scale. A fundamental component 20 of unraveling the blue print for any given individual is to determine the exact sequence of the 3 billion nucleotides in their genome. Techniques are available to do this, but those techniques typically take many days and thousands upon thousands of dollars to perform. Furthermore, clinical relevance of any individual's genomic sequence is a matter of comparing unique features of their genomic sequence (i.e. their genotype) to reference 25 genomes that are correlated with known characteristics (i.e. phenotypes). The issue of scale and throughput becomes evident when one considers that the reference genomes are created based on correlations of genotype to phenotype that arise from research studies that typically use thousands of individuals in order to be statistically valid. Thus, billions of nucleotides can eventually be sequenced for thousands of individuals to identify any clinically relevant 30 genotype to phenotype correlation. Multiplied further by the number of diseases, drug responses, and other clinically relevant characteristics, the need for very inexpensive and rapid sequencing technologies becomes ever more apparent.

What is needed is a reduction in the cost of sequencing that drives large genetic correlation studies carried out by research scientists and that makes sequencing accessible in the clinical environment for the treatment of individual patients making life changing decisions. Embodiments of the invention set forth herein satisfy this need and provide other advantages as well.

BRIEF SUMMARY

Provided herein is a detection apparatus including a microfluorometer having an objective, an excitation radiation source, and a detector. The microfluorometer is configured for wide-field image detection. The detection apparatus also includes a fluidic system for delivering reagents from a reagent cartridge to a flow cell. The fluidic system includes a manifold body having a plurality of fluidic channels configured for fluid communication between the reagent cartridge and an inlet of the flow cell. The fluidic system also includes a plurality of reagent sippers extending downward from ports in the manifold body. Each of the reagent sippers is configured to be placed into a reagent reservoir of the reagent cartridge so that liquid reagent can be drawn from the reagent reservoir into the reagent sipper. The fluidic system also includes a valve configured to mediate fluid communication between the reagent reservoirs and the inlet of the flow cell. The fluidic channels fluidly connect the reagent sippers to the valve. The detection apparatus also includes a flow cell latch clamp module having a clamp cover for holding the flow cell. The objective is configured to direct excitation radiation from the radiation source to the flow cell and to direct emission from the flow cell to the detector. The microfluorometer is movable to acquire wide-field images of different areas of an inner surface of the flow cell.

In some aspects, the detection apparatus includes no more than a single microfluorometer. Optionally, the microfluorometer includes a beam splitter that is positioned to direct excitation radiation from the excitation radiation source to the objective and to direct emission radiation from the objective to the detector.

In some aspects, the microfluorometer is an integrated microfluorometer having a compact epifluorescent detection configuration.

In some aspects, the microfluorometer is movable to allow imaging of the flow cell that is larger than a field of view of the microfluorometer.

In some aspects, the detection apparatus also includes a housing and a screen presented on a front face of the housing. The screen functions as a graphical user interface.

In some aspects, the detection apparatus also includes a housing having cartridge receptacle for receiving the reagent cartridge.

In some aspects, the detection apparatus also includes a fluidics automation module including a lift assembly for raising and lowering the reagent cartridge.

5 In some aspects, the detection apparatus also includes a fluidics automation module including a belt assembly that moves the reagent cartridge during loading.

In some aspects, the detection apparatus also includes a fluidics automation module including a lift assembly for raising and lowering the reagent cartridge and a belt assembly that moves the reagent cartridge during loading.

10 In some aspects, the manifold body is formed from multiple layers of solid material bonded together.

In some aspects, the channels are formed in the solid material prior to bonding the multiple layers together.

In some aspects, the fluidic channels are housed entirely within the manifold body.

15 In some aspects, the manifold body has at least sixteen (16) ports that connect to respective reagent sippers.

In some aspects, the valve is configured with inlet ports that correspond to each of the ports of the fluidic channels and configured with a single common outlet port which fluidly connects to the flow cell.

20 In some aspects, the fluidic system has no more than one valve that mediates fluid communication between the reagent reservoirs and the flow cell.

In some aspects, each of the fluidic channels originates from a single port and connects to a corresponding port of the valve.

In some aspects, the manifold body has a single layer of fluidic channels.

25 In some aspects, the reagent sippers have distal ends that are configured to pierce a film or foil layer of the reagent cartridge.

In some aspects, the radiation source produces radiation at different wavelengths.

In some aspects, the detector is a complementary metal–oxide–semiconductor (CMOS) image sensor.

30 In some aspects, the microfluorometer is a component of an imaging module. The imaging module also includes the flow cell latch clamp module. Optionally, the imaging module also includes an XY stage in which an X-motor and a Y-motor provide movement of

the X-stage and the Y-stage, respectively. Optionally, the X-motor moves a camera assembly along an X-axis. The camera assembly has the microfluorometer, wherein the Y-motor moves the flow cell latch clamp module along a Y-axis.

In some aspects, the detection apparatus also includes a fluidic device that includes a flow cell cartridge and the flow cell. The flow cell cartridge is configured to hold the flow cell and facilitate orienting the flow cell for an imaging session.

In some aspects, the fluidic device and the flow cell cartridge are removable such that the flow cell cartridge is removable from the flow cell latch clamp module by an individual or machine without damage to the fluidic device or the flow cell cartridge.

In some aspects, the flow cell cartridge is configured to be repeatedly inserted and removed into the flow cell latch clamp module without damaging the flow cell cartridge or rendering the flow cell cartridge unsuitable for its intended purpose.

In some aspects, the flow cell cartridge includes a housing and a cover member that is coupled to the housing of the flow cell cartridge. The cover member includes a gasket having inlet and outlet passages that are located proximate to one another. The clamp cover includes a cover manifold with inlet and outlet ports that are configured to mate with the inlet passage and the outlet passage of the gasket, respectively.

In some aspects, the flow cell latch clamp module includes a spring-loaded lever that biases the flow cell against dowel pins.

In some aspects, a field diameter for the microfluorometer is at least 0.5 mm. Optionally, a field diameter for the microfluorometer is at least 2 mm. Optionally, a field diameter for the microfluorometer is no larger than 5 mm.

In some aspects, a numerical aperture for the microfluorometer is at least 0.2. Optionally, a numerical aperture for the microfluorometer is no greater than 0.8. Optionally, a numerical aperture for the microfluorometer is no greater than 0.5.

In some aspects, the microfluorometer has a resolution that is sufficient to distinguish features separated by at most 100 μm.

In an embodiments, a sequencing system is provided that includes the detection apparatus set forth above and the reagent cartridge having the reagent reservoirs. The reagent reservoirs include sequencing reagents.

In some aspects, at least one of the reagent reservoirs include a nucleic acid sample.

In some aspects, the reagent cartridge is removable from the detection apparatus.

In some aspects, the sequencing system is configured to perform a sequencing-by-synthesis protocol.

Provided herein is a detection apparatus, comprising (a) a carriage comprising one or more microfluorometers, wherein each of the one or more microfluorometers comprises an objective configured for wide-field image detection, wherein the one or more microfluorometers is positioned to acquire a plurality of the wide-field from a different area of the common plane; (b) a translation stage configured to move the carriage in at least one direction parallel to the common plane; and (c) a fluidic system for delivering reagents from a reagent cartridge to a flow cell comprising: a reagent manifold comprising a plurality of channels configured for fluid communication between a reagent cartridge and an inlet of a flow cell; a plurality of reagent sippers extending downward from ports in the manifold, each of the reagent sippers configured to be placed into a reagent reservoir in a reagent cartridge so that liquid reagent can be drawn from the reagent reservoir into the sipper; at least one valve configured to mediate fluid communication between the reservoirs and the inlet of the flow cell. In certain embodiments, the apparatus comprises no more than a single microfluorometer.

This disclosure further provides a fluidic system for delivering reagents from a reagent cartridge to a flow cell comprising: a reagent manifold comprising a plurality of channels configured for fluid communication between a reagent cartridge and an inlet of a flow cell; a plurality of reagent sippers extending downward from ports in the manifold, each of the reagent sippers configured to be placed into a reagent reservoir in a reagent cartridge so that liquid reagent can be drawn from the reagent reservoir into the sipper; at least one valve configured to mediate fluid communication between the reservoirs and the inlet of the flow cell.

This disclosure further provides a sequencing system comprising: a detection apparatus as described above; and a nucleic acid sample disposed within a reagent cartridge wherein the reagent cartridge is removable from the detection apparatus.

This disclosure further provides a sequencing system comprising: a detection apparatus as described above; and a nucleic acid sample disposed within a flow cell; wherein the flow cell is removable from the detection apparatus.

This disclosure further provides a sequencing method that includes the steps of (a) providing a sequencing system comprising (i) a flow cell comprising an optically transparent

surface, (ii) a nucleic acid sample, (iii) a plurality of reagents for a sequencing reaction, and (iv) a fluidic system for delivering the reagents to the flow cell; (b) providing a detection apparatus comprising (i) a single microfluorometer, wherein the microfluorometer comprises an objective configured for wide-field image detection in an image plane in x and y
5 dimensions, and (ii) a sample stage; and (c) carrying out fluidic operations of a nucleic acid sequencing procedure in the cartridge and detection operations of the nucleic acid sequencing procedure in the detection apparatus, wherein (i) the reagents are delivered to the flow cell by the fluidic system, (ii) wide-field images of the nucleic acid features are detected by the microfluorometer.

10 The details of one or more embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows an integrated optoelectronics and fluidic detection device useful for
15 nucleic acid sequencing.

Figure 1B shows an exploded view of several of the modules and systems that make up the integrated optoelectronics and fluidic detection device shown in Figure 1A.

Figure 2A shows a perspective view of a manifold assembly having reagent sippers, valve and alignment pins. It also shows a reagent cartridge.

20 Figure 2B shows a perspective view of a manifold assembly having reagent sippers, valve and alignment pins.

Figure 2C shows a side view of a manifold assembly having reagent sippers of varying length, valve and alignment pins. It also shows a reagent cartridge.

Figure 2D shows a fluidics map for a fluidic system.

25 Figure 3A shows a top perspective view of a reagent cartridge.

Figure 3B shows a perspective view of a fluidics automation module.

Figure 4A shows an optical layout for an individual microfluorometer having orthogonal excitation and emission beam paths.

Figure 4B shows an optical layout for a microfluorometer.

30 Figure 4C shows a perspective view of an exemplary single microfluorometer.

Figure 5A shows a front perspective view of an imaging module having a single microfluorometer.

Figure 5B shows a side perspective view of an imaging module having a single microfluorometer.

Figure 6 shows a flow cell.

Figure 7A shows a perspective view of a flow cell latch clamp module.

5 Figure 7B shows flow cell latch clamp module without flow cell or cover.

Figure 7C shows flow cell mounted in latch clamp module with cover in unlatched position.

DETAILED DESCRIPTION

10 This disclosure provides methods and apparatus for high-resolution detection of planar areas such as those present on substrate surfaces. A particularly useful application is optically based imaging of a biological sample that is present on a surface. For example, the methods and apparatus set forth herein can be used to obtain images of nucleic acid features that are present in nucleic acid arrays, such as those used in nucleic acid sequencing applications. A 15 variety of nucleic acid sequencing techniques that utilize optically detectable samples and/or reagents can be used. These techniques are particularly well suited to the methods and apparatus of the present disclosure and therefore highlight various advantages for particular embodiments of the invention. Some of those advantages are set forth below for purposes of illustration and, although nucleic acid sequencing applications are exemplified, the 20 advantages can be extended to other applications as well.

In regard to some of the examples set forth herein, salient characteristics of many nucleic acid sequencing techniques are (1) the use of multicolor detection (e.g. often four different fluorophores are used, one for each of the different nucleotide types A, C, G and T (or U) present in nucleic acids), (2) distribution of large numbers of different fragments from 25 a nucleic acid sample (e.g. fragments from a genome sample, RNA sample, or derivative thereof) onto the surface of an array and (3) repeated cycles of fluidic processing and imaging of the arrays. Embodiments of the methods and apparatus disclosed herein are particularly useful for nucleic acid sequencing because they can provide the capability of high resolution imaging of array surfaces in multiple colors and in multiple repetitions. For example, 30 embodiments set forth herein allow an image of a surface to be obtained at a resolution that is in the range of hundreds, tens or even single digit microns. As such, nucleic acid features having nearest neighbor, average center-to-center spacing that is lower than 100 microns, 50

microns, 10 microns, 5 micron or fewer can be resolved. In particular embodiments, wide-field images of surfaces can be acquired, including for example, images that cover an area of 1 mm^2 or more of an array. The images can be acquired in multiple colors simultaneously or sequentially, for example, to identify fluorescent labels uniquely associated with different nucleotide types. Moreover, images can be acquired sequentially for multiple cycles of a sequencing technique. The images from a given area of the array can be reliably compared from each cycle to determine the sequence of color changes detected for each nucleic acid feature on the array. The sequence of color changes can in turn be used to infer the sequences of the nucleic acid fragments in each feature.

In particular embodiments, an apparatus of the present disclosure includes one or more microfluorometers. Each of the microfluorometers can include an excitation radiation source, a detector and an objective to form an integrated subunit of a read head. Other optical components can be present in each microfluorometer. For example a beam splitter can be present to provide for a compact epifluorescent detection configuration, whereby the beam splitter is positioned to direct excitation radiation from the excitation radiation source to the objective and to direct emission radiation from the objective to the detector.

An advantage of using an integrated microfluorometer design is that the microfluorometer can be conveniently moved, for example in a scanning operation, to allow imaging of a substrate that is larger than the field of view of the microfluorometer. In particular embodiments, a single microfluorometer can form a read head. In particular embodiments, several microfluorometers can be combined to form a read head. Various configurations for one or more read heads are set forth below and can be selected to suit a particular format for a substrate that is to be imaged, while maintaining relatively compact size for the overall read head. The relatively small size and low mass of the read head in several embodiments of the present disclosure results in relatively low inertia such that the read head comes to rest quickly after being moved, thereby favoring rapid scanning of a nucleic acid array or other substrate. In some cases, the microfluorometer can be affixed to a carriage such that they are not independently moveable in at least some dimensions during the course of an analytical application such as a nucleic acid sequencing run. The microfluorometer may, however, be independently actuated in the z dimension to provide for independent focus control. Reducing degrees of freedom between several different

microfluorometers of an apparatus of the present disclosure provides for protection against loss of alignment during shipping, handling and use of the apparatus.

In some embodiments, multiple microfluorometers that are present in a read head or carriage can each have a dedicated autofocus module. Accordingly, each microfluorometer can be independently focused. In some embodiments, a particular autofocus modules in a read head, although dedicated to actuation of a particular microfluorometer, can nevertheless receive information from at least one other autofocus module in the read head and the information from that particular autofocus module and from the at least one other autofocus module can be used to determine an appropriate actuation to achieve desired focus for the particular microfluorometer. In this way focus for any given microfluorometer can be determined by consensus between two or more microfluorometers present in the same read head or carriage.

In particular embodiments, a sample that is to be detected can be provided to a detection chamber using a fluidic system as provided herein. Taking the more specific example of a nucleic acid sequencing application, the fluidic system can include a manifold assembly that can be placed into fluidic communication with one or more of reservoirs for holding sequencing reagents, reservoirs for holding sample preparation reagents, reservoirs for holding waste products generated during sequencing, and/or pumps, valves and other components capable of moving fluids through a flow cell.

In particular embodiments a fluidic system can be configured to allow re-use of one or more reagents. For example, the fluidic system can be configured to deliver a reagent to a flow cell, then remove the reagent from the flow cell, and then re-introduce the reagent to the flow cell. An advantage of re-using reagents is to reduce waste volume and reduce the cost of processes that utilize expensive reagents and/or reagents that are delivered at high concentrations (or in high amounts). Reagent re-use takes advantage of the understanding that depletion of reagent occurs only or primarily at the flow cell surface, and therefore a majority of the reagent goes unused and may be subject to re-use.

Fig. 1A shows an exemplary detection device **1** that exploits advantages of integrated optoelectronics and fluidic systems that are provided by several embodiments set forth herein. The exemplary detection device **1** includes a housing **2** that contains various fixed components including, for example, optical components, computational components, power source, fan and the like. A screen **3** present, for example, on the front face of the housing **2**

functions as a graphical user interface that can provide various types of information such as operational status, status of an analytical procedure (e.g. a sequencing run) being carried out, status of data transfer to or from the detection device **1**, instructions for use, warnings or the like. A cartridge receptacle **4A** is also present on the front face of the housing **2**. A waste reservoir receptacle **4B** is also present on the front face of the housing **2**. As shown, the cartridge receptacle **4A** and waste reservoir receptacle **4B** can be configured as a slot having a protective door **5**. A status indicator **6,** in the form of an indicator light on the frame of the cartridge receptacle in this example, is present and can be configured to indicate the presence or absence of a reagent cartridge in the detection device **1**. For example the indicator light **6** can change from on to off or from one color to another to indicate presence or absence of a cartridge. A power control button **7** is present on the front face of the housing **2** in this example as is identifying indicia **8** such as the name of the manufacturer or instrument.

Also shown in **Fig. 1A** is an exemplary reagent cartridge **10** that can be used to provide a sample and reagents to the detection device **1.** The reagent cartridge **10** includes a cartridge housing **11** that protects various fluidic components such as reservoirs, fluidic connections, and the like. A bar code or other machine readable indicia can optionally be present on the cartridge housing **11**, for example, to provide sample tracking and management. Other indicia can also be present on the housing for convenient identification by a human user, for example, to identify the manufacturer, analytical analysis supported by the fluidic cartridge, lot number, expiration date, safety warnings and the like.

Also shown in **Fig. 1A** is a waste reservoir **12** that can be inserted into the waste reservoir receptacle **4B**. The waste reservoir 12 is configured to receive waste fluid through an opening on the top of the reservoir.

The device shown in **Fig. 1A** is exemplary. Further exemplary embodiments of the methods and apparatus of the present disclosure that can be used alternatively or additionally to the example of **Fig. 1A** are set forth in further detail below.

Fig. 1B shows components of the exemplary detection device **1**, including a fluidic pump **1001** that is in fluid communication with a fluidics automation module (FAM) **1002**. FAM **1002** is configured to be placed in fluid communication with reagent cartridge **1003**, flow cell **1004** and waste reservoir **1005** so as to move liquid reagents from reagent cartridge **1003** onto flow cell **1004** and to waste reservoir **1005**. Also shown in **Fig. 1B** is XY stage

1006, including flow cell clamp module **1007**. Also shown in **Fig. 1B** is power supply unit **1008** and main printed circuit board (PCB) **1009**.

Fig. 2A shows an exemplary fluidic system **100** having reagent sippers **103** and **104** and valves **102** that exploits advantages of fluidic systems that are provided by several embodiments set forth herein. The fluidic system **100** includes a manifold assembly **101** that contains various fixed components including, for example, reagent sippers, valves, channels, reservoirs and the like. A reagent cartridge **400** is present having reagent reservoirs **401** and **402** (hereinafter referred to as "wells") configured to simultaneously engage a set of reagent sippers **103** and **104** along a dimension *z* such that liquid reagent can be drawn from the reagent reservoirs into the sippers.

Shown in **Fig. 2B** is an exemplary manifold assembly **101** that can be used to provide liquid reagents from reagent reservoirs to a flow cell. The manifold assembly **101** includes reagent sippers **103** and **104** extending downward in a dimension *z* from ports **106** in the manifold. The reagent sippers **103** and **104** can be placed into one or more reagent reservoirs (not shown) in a reagent cartridge. The manifold body **108** also includes fluidic channels **107** fluidly connecting the reagent sipper **103** to a valve **102**. The reagent sippers **103** and **104,** the fluidic channels **107** and the valve **102** mediate fluid communication between the reagent reservoirs and a flow cell (not shown). Valves **102** and **109** may individually, or in conjunction, select reagent sippers **103** or **104,** and through fluidic channels such as **107**, mediate fluid communication between the reagent reservoirs and a flow cell (not shown).

Fig. 2C shows a side view of an exemplary fluidic system **100** having reagent sippers and valves. The manifold has alignment pins **105** protruding downward from the manifold in an axis parallel to the reagent sippers. The alignment pins **105** are longer along the *z* dimension compared to the reagent sippers, although in alternative embodiments they can also be of equal length or shorter. The alignment pins **105** are configured to engage with one or more corresponding interface slots on a reagent cartridge (not shown). The reagent sippers **103** and **104** are coupled to the manifold via ports **106** that are housed in a manifold body **108** of the manifold assembly **101**. Reagent sippers **104** are longer in comparison to reagent sippers **103**, in order to draw liquid from reagent reservoirs of varying depth that corresponds to the depth of the reagent sipper **103** or **104**. In alternative embodiments, reagent sippers **103** and **104** can be of equal lengths, or may switch dominant lengths.

Fig. 2B shows a view of a manifold assembly **101** displaying one possible layout of fluidic channels **107** within the manifold body **108**. Each of the fluidic channels **107** originates from a single port **106** and connects a corresponding port **106** to the valve **102**. In some embodiments, certain channels can include a cache reservoir which has sufficient volume to allow a quantity of liquid reagent to flow from a flow cell (not shown) to the cache reservoir such that liquid reagent from the flow cell is not directed back to the reagent reservoir (not shown) after contacting the flow cell. Also shown in **Fig. 2B** are exemplary positions of one or more alignment pin **105**. The manifold assembly shown in **Fig. 2B** also includes inlet ports **111** for shared buffers. Valve **102** is configured with inlet ports corresponding to each port **106**, and with a common out port **110** which fluidly connect to a flow cell and a waste port which fluidly connect to a waste receptacle.

As demonstrated by the exemplary embodiments above, a fluidic system for delivering reagents from a reagent cartridge to a flow cell can include a reagent manifold comprising a manifold body having a plurality of channels configured for fluid communication between a reagent cartridge and an inlet of a flow cell. Use of a manifold body in fluidic systems provides several advantages over the use of tubing alone. For example, a manifold body with fixed fluidic channels reduces the likelihood of error during assembly, such as misplacement of tubing attachments, as well as over- or under- tightening of connections. In addition, a manifold body provides ease of maintenance, allowing, for example, quick replacement of an entire unit rather than time-intensive testing and replacement of individual lines.

The one or more of the channels of the manifold can include a fluidic track through a solid material. The track can be of any diameter to allow desired level of fluid transfer through the track. The track can have an inner diameter of, for example, less than 0.1 mm, 0.2 mm, 0.3 mm 0.4 mm, 0.5 mm, 0.6 mm, 0.7 mm, 0.8 mm, 0.9 mm, 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm or less than 10 mm in diameter. The track configuration can be, for example, straight or curved. Alternatively or additionally, the track can have a combination of curved portions and straight portions. The cross section of the track can be, for example, square, round, “D”-shaped, or any other shape that enables a desired level of fluid transfer through the track.

The channel between the sipper and the valve can be housed entirely within the manifold body **108**. Alternatively or additionally, the channel can include one or more portions that are external to the manifold. For example, tubing such as, for example, flexible

tubing can connect a portion of the fluidic track to another portion of the track on the manifold. Alternatively or additionally, flexible tubing can connect a flow cell to fixed fluidic components of the system, including, for example, pumps, valves, sensors and gauges. As an example, flexible tubing can be sued to connect a flow cell or a channel of the present system
5 to a pump such as a syringe pump or a peristaltic pump.

The manifold body 108 can be, for example, made of any suitable solid material that is capable of supporting one or more channels therein. Thus, the manifold body 108 can be a resin such as polycarbonate, polyvinyl chloride, DELRIN® (Polyoxymethylene); HALAR®; PCTFE (PolyChloroTriFluoroEthylene); PEEK™ (Polyetheretherketone); PK (Polyketone);
10 PERLAST®; Polyethylene; PPS (Polyphenylene Sulfide); Polypropylene; Polysulfone; FEP; PFA; High Purity PFA; RADEL® R; 316 Stainless Steel; TEFZEL® ETFE (Ethylene Tetrafluoroethylene); TPX® (Polymethylpentene); Titanium; UHMWPE (Ultra High Molecular Weight Polyethylene); ULTEM® (polyetherimide); VESPEL® or any other suitable solid material that is compatible with the solvents and fluids transported through the channels
15 of the manifold in the embodiments presented herein. The manifold body can be formed from a single piece of material. Alternatively or additionally, the manifold body can be formed from multiple layers that are bonded together. Methods of bonding include, for example, the use of adhesives, gaskets, and diffusion bonding. The channels can be formed in the solid material by any suitable method. For example, channels can be drilled, etched or milled into
20 the solid material. Channels can be formed in the solid material prior to bonding multiple layers together. Alternatively or additionally, channels can be formed after bonding layers together.

The manifold assemblies presented here are configured for delivery of liquid reagents from a reagent cartridge to a flow cell. Thus, the manifold can have any number of ports
25 coupled to reagent sippers. More specifically, the number of ports can correspond to the number and configuration of reagent reservoirs in a reagent cartridge. In some embodiments, the manifold comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or at least 30 ports, each port configured to couple a reagent sipper to a channel in fluid communication with the at least one valve.

30 The fluidic systems presented herein can also include an array of sipper tubes extending downward along the z dimension from ports in the manifold body, each of the reagent sippers configured to be inserted into a reagent reservoir in a reagent cartridge so that

liquid reagent can be drawn from the reagent reservoir into the sipper. The reagent sippers can comprise, for example, a tubular body with a proximal end and a distal end. The distal end can taper to a sharp tip that is configured to pierce a film or foil layer used as a seal over a reagent reservoir in a reagent cartridge. The reagent sippers can be provided with, for
5 example, a single lumen running through the tubular body from the distal to the proximal end. The lumen can be configured to provide fluid communication between the reagent cartridge on one end of the sipper and the reagent manifold on the other end of the sipper. As shown in exemplary **Fig. 2B**, reagent sippers **103** and **104** are coupled to the manifold body **108** via ports **106** that are housed in the manifold body **108**.

10 In some embodiments, as exemplified in **Fig. 2C**, a subset of the reagent sippers is of a length that is shorter than other reagent sippers. For example, the length of the subset can be at least 1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or at least 2.0 mm shorter than the other reagent sippers. The manifold and reagent sippers can be used in a device having an elevator mechanism configured to move a reagent cartridge bi-directionally along the *z* dimension
15 such that the reagent sippers are inserted into corresponding wells or reservoirs in the reagent cartridge. In certain embodiments, the reagent wells may be covered with protective foils. Thus, an advantage of providing sippers of varying length is a reduction in the force required by the elevator mechanism to accommodate a foil-piercing force when a reagent cartridge is brought into contact with the piercing sippers. The difference in sipper length can
20 advantageously correspond to the depth of reagent wells in a reagent cartridge, so that each sipper reaches a desired depth in its corresponding reagent well when the sippers and the cartridge are in a fully engaged position.

The sippers can be formed of any suitable material that allows fluid transfer through a lumen and which is compatible with the solvents and fluids transported through the channels
25 of the manifold in the embodiments presented herein. The sippers can be formed from a single tube. Alternatively or additionally, one or more sippers can be made of multiple segments that together form a sipper of a desired length and diameter.

In some embodiments, at least one of the reagent sippers includes a compliant tip configured to flex when the tip impinges upon the bottom of a reagent well in a reagent
30 cartridge. By flexing or deforming, a compliant tip allows the lumen of the sipper to more fully approach or even contact the bottom of the reagent well, thereby reducing or even eliminating the evacuation volume in the reagent well. A compliant tip can be especially

advantageous for uptake of sample or reagents where small volumes are used, or in situations where it is desirable for uptake of most or all of the liquid in a reagent reservoir. The body of the sipper having a compliant tip can be made entirely of the same flexible material as the tip. Alternatively or additionally, the body of the sipper can be made of a distinct material than the tip. The compliant tip can be made of any suitable material such that the compliant tip may deform or yield when urged into contact with the bottom of a reagent reservoir. Some suitable materials include polymeric and/or synthetic foams, rubber, silicone and/or elastomers, including thermoplastic polymers such as polyurethane.

The fluidic systems presented herein may also include, for example, pumps and valves that are selectively operable for controlling fluid communication between the reservoirs and the inlet of the flow cell. As exemplified by the manifold assembly **101** shown in **Fig. 2D,** channel outlets on the manifold body **108** can be configured to connect with corresponding inlet ports on the valve **102** such that each fluidic channel **107** is in fluid communication with an inlet port on the valve **102**. Thus, via the fluidic channels **107** of the manifold body **108**, one or more or each of the inlet ports can be in fluid communication with a reagent sipper. The valve **102** can be configured with a common out port **110** which fluidly connects to an inlet of one or more lanes on a flow cell. Alternatively or additionally, the valve **102** can be configured with a waste port **112** fluidly connected to one or more waste reservoirs **1005**.

The apparatuses shown in **Figs. 2A, 2B, 2C and 2D** are exemplary. Further exemplary embodiments of the methods and apparatus of the present disclosure that can be used alternatively or additionally to the example of **Figs. 2A, 2B, 2C and 2D** are set forth in further detail below.

An exemplary reagent cartridge is shown in **Fig. 3A.** The reagent cartridge **400** shown in **Fig. 3A** can include wells **401** of varying depths along the *z* dimension compared to those of wells **402**. More specifically, the reagent cartridge exemplified in **Fig. 3A** has wells designed to accommodate the length of a corresponding reagent sipper (not shown) such that each sipper reaches a desired depth in its corresponding reagent well when the sippers and the cartridge are in a fully engaged position. In the reagent cartridge exemplified in **Fig. 3A**, the wells are arranged in row or column along the *y* dimension, where those wells **401** on the outside of the row or column extend downward further along the *z* dimension than those wells **402** on the inside of the row or column. Some or all of the wells can be of varying depths. Alternatively or additionally, some or all of the wells can be of the same depth. When the

sippers and the cartridge are in a fully engaged position, the penetration depth of any sipper tip (i.e., the distance from the bottom surface of the well to the end of the sipper tip) can be equivalent to the penetration depth of any other sipper tip in any other given well in the reagent cartridge. The penetration depth of any sipper tip need not be the same as the penetration depth of any other given well in the reagent cartridge. Where at least some reagent wells have a different well depth, the well depth can be, for example, at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or at least 2.0 mm shorter than the other reagent sippers. Similarly, when the sippers and the cartridge are in a fully engaged position, the penetration depth of any sipper tip can be at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or at least 2.0 mm different than the penetration depth of any other sipper tip in the reagent cartridge.

As shown in the exemplary reagent cartridge **400** in **Fig. 3A**, the cartridge includes a plurality of reagent reservoirs **401A**, **401B**, **402A** and **402B**. The reagent reservoirs in **Fig. 3A** are arranged in x and y dimensions into rows. Also shown in **Fig. 3A**, the cartridge includes alignment slots **403** and **404** configured to engage with corresponding alignment pins of a manifold assembly (not shown). The cartridge may also include protective foil covering any number of the reagent wells or reservoirs, which can be pierced by piercing sippers when the cartridge is brought into contact with the piercing sippers.

The reagent cartridges presented herein can include any number of reagent reservoirs or wells. The reagent reservoirs or wells can be arranged in any format along the x and y dimensions to facilitate transport and storage of reagents in the cartridge. Alternatively or additionally, reagent reservoirs or wells can be arranged in any format along the x and y dimensions suitable for interaction with an array of sipper tubes extending downward along the *z* dimension from ports in the manifold. More specifically, the reagent reservoirs or wells can be arranged in any format suitable for simultaneously engaging a matrix of reagent sippers such that liquid reagent can be drawn from the reagent reservoir into the sippers.

Not all reagent wells need interact simultaneously with all sipper tubes of a manifold assembly. For example, the reagent cartridge can include a subset of one or more reagent reservoirs or wells that are configured to remain in a non-interacting state while other reservoirs or wells are engaged by an array of sipper tubes. As one example, a cartridge presented herein can comprises a plurality of wash reservoirs arranged in a configuration corresponding to the plurality of reagent reservoirs, whereby wash reservoirs are configured

to simultaneously engage the reagent sippers when the reagent sippers are not engaged with the reagent reservoirs so that wash buffer can be drawn from the wash reservoirs into the sippers. An exemplary embodiment is presented in **Fig. 3A**, which shows a row of reagent wells **401A**. The cartridge also includes a row of corresponding wells **401B** which retains the
5 same orientation in the *x* dimension with respect to each other, but which are offset in the *y* dimension from wells **401A**. The offset wells **401B** can include a wash buffer, for example, provided for rinsing sipper tubes and fluidic lines after using one cartridge and before using another cartridge.

Alternatively or additionally, other reservoirs that are empty, or which hold buffer,
10 sample or other reagents can be present on the cartridge. The additional reservoirs can, but need not interact with a sipper tube. For example, a reservoir can be configured to be filled with waste or overflow reagent or buffer over the course of cartridge use. Such a reservoir may be accessed, for example via a port that does not interface with a sipper tube.

To facilitate correct alignment of cartridge reservoirs with corresponding sipper tubes,
15 alignment slots can be positioned in the cartridge. For example, in particular embodiments where an array of sipper tubes is removed from one set of reservoirs and translocated to another set of reagent or wash reservoirs, alignment slots can be positioned in the cartridge to ensure correct alignment of the array of reagent sippers with one or both sets of reservoirs. As shown in **Fig. 3A**, the exemplary cartridge includes alignment slots **404** which retain the
20 same orientation in the *x* dimension, but which are offset in the *y* dimension with respect to corresponding alignment slot **403**. A cartridge of the embodiments presented herein can have any number of alignment slots which provide suitable alignment with the features of a fluidic assembly. For example, a cartridge can include 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more alignment slots configured to engage with corresponding alignment pins of the fluidic system
25 so that reagent sippers of the fluidic system are positioned in alignment with the reagent and/or wash reservoirs.

An exemplary fluidics automation module (FAM) is shown in **Fig. 3B**. FAM **470** can comprise lift assembly **471** for raising and lowering the reagent cartridge for piercing and bubble creation. Belt assembly **473** moves the reagent cartridge receptacle **474** during
30 cartridge loading, and moves the reagent cartridge outside the device during unloading. A waste reservoir receptacle **475** is also present on the front face of the FAM **470** and is

configured to accept waste reservoir **476**. FAM **470** further comprises a manifold assembly **472**, which is set forth in greater detail in **Fig. 2B**.

Provided herein is a detection apparatus, having (a) a carriage including a one or more microfluorometers, wherein each of the microfluorometers includes an objective configured for wide-field image detection, wherein the one or more microfluorometers is positioned to acquire one or more wide-field images in a common plane, and wherein each of the wide-field images is from a different area of the common plane; (b) a translation stage configured to move the carriage in at least one direction parallel to the common plane; and (c) a sample stage configured to hold a substrate in the common plane.

A detection apparatus (or an individual microfluorometer) of the present disclosure can be used to obtain one or more images at a resolution that is sufficient to distinguish features on a micron scale. For example, a microfluorometer that is used in a detection apparatus can have a resolution that is sufficient to distinguish features that are separated by at most 500 μm, 100 μm, 50 μm, 10 μm, 5 μm, 4 μm, 3 μm, 2 μm or 1 μm. Lower resolution is also possible, for example, a resolution that distinguishes features that are separated by more than 500 μm.

A detection apparatus (or an individual microfluorometer) of the present disclosure is well suited for high-resolution detection of surfaces. Accordingly, arrays having features with average spacing in the micron range are especially useful substrates. In particular embodiments, a detection apparatus or microfluorometer can be used to obtain one or more images of an array having features with center-to-center spacing for nearest neighbors that is on average at or below 500 μm, 100 μm, 50 μm, 10 μm, 5 μm, 4 μm, 3 μm, 2 μm or 1 μm. In many embodiments the features of an array are non-contiguous being separated, for example, by less than 100 μm, 50 μm, 10 μm, 5 μm, 1 μm, or 0.5 μm. However, the features need not be separated. Instead some or all of the features of an array can be contiguous with each other.

Any of a variety of arrays (also referred to as “microarrays”) known in the art can be used. A typical array contains features, each having an individual probe or a population of probes. In the latter case, the population of probes at each site is typically homogenous having a single species of probe. For example, in the case of a nucleic acid array, each feature can have multiple nucleic acid species each having a common sequence. However, in some embodiments the populations at each feature of an array can be heterogeneous. Similarly,

protein arrays can have features with a single protein or a population of proteins typically, but not always, having the same amino acid sequence. The probes can be attached to the surface of an array for example, via covalent linkage of the probes to the surface or via non-covalent interaction(s) of the probes with the surface. In some embodiments, probes, such as nucleic acid molecules, can be attached to a surface via a gel layer as described, for example, in US 2011/0059865 A1, which is incorporated herein by reference.

Exemplary arrays include, without limitation, a BeadChip Array available from Illumina®, Inc. (San Diego, CA) or others such as those where probes are attached to beads that are present on a surface (e.g. beads in wells on a surface) such as those described in U.S. Patent Nos. 6,266,459; 6,355,431; 6,770,441; 6,859,570; or 7,622,294; or PCT Publication No. WO 00/63437, each of which is incorporated herein by reference. Further examples of commercially available microarrays that can be used include, for example, an Affymetrix® GeneChip® microarray or other microarray synthesized in accordance with techniques sometimes referred to as VLSIPS™ (Very Large Scale Immobilized Polymer Synthesis) technologies. A spotted microarray can also be used in an apparatus or system according to some embodiments of the invention. An exemplary spotted microarray is a CodeLink™ Array available from Amersham Biosciences. Another microarray that is useful is one that is manufactured using inkjet printing methods such as SurePrint™ Technology available from Agilent Technologies.

Other useful arrays include those that are used in nucleic acid sequencing applications. For example, arrays having amplicons of genomic fragments (often referred to as clusters) are particularly useful such as those described in Bentley et al., *Nature* 456:53-59 (2008), WO 04/018497; US 7,057,026; WO 91/06678; WO 07/123744; US 7,329,492; US 7,211,414; US 7,315,019; US 7,405,281, or US 2008/0108082, each of which is incorporated herein by reference. Another type of array that is useful for nucleic acid sequencing is an array of particles produced from an emulsion PCR technique. Examples are described in Dressman et al., *Proc. Natl. Acad. Sci. USA* 100:8817-8822 (2003), WO 05/010145, US 2005/0130173 or US 2005/0064460, each of which is incorporated herein by reference in its entirety. Although the above arrays have been described in the context of sequencing applications, it will be understood that the arrays can be used in other embodiments including, for example, those that do not include a sequencing technique.

Whether configured for detection of an array or other sample, one or more microfluorometers that are present in a detection apparatus can be configured for wide-field detection. The field diameter for an individual microfluorometer can be, for example, at least 0.5 mm, 1 mm, 2 mm, 3 mm, 4 mm, 5 mm or larger. By choice of appropriate optical components the field diameter can be limited to a maximum area as well and, as such the field diameter can be, for example, no larger than 5 mm, 4 mm, 3 mm, 2 mm or 1 mm. Accordingly, in some embodiments an image obtained by an individual microfluorometer can have an area that is in a range of 0.25 mm^2 to 25 mm^2.

In addition to being configured for wide-field detection, a microfluorometer can be configured to have a numerical aperture (NA) that is greater than 0.2. For example, the NA of an objective used in a microfluorometer of the present disclosure can be at least 0.2, 0.3, 0.4, or 0.5. Alternatively or additionally, it may be desirable to restrict the NA of the objective to be no greater than 0.8, 0.7, 0.6 or 0.5. The methods and apparatus set forth herein are particularly useful when detection occurs through an objective having a NA between 0.2 and 0.5.

In array detection embodiments, a detection apparatus (or individual microfluorometer) can be configured to obtain a digital image of the array. Typically, each pixel of the digital detection apparatus (or individual microfluorometer) will collect signal from no more than a single feature in any given image acquisition. This configuration minimizes unwanted 'cross talk' between features in the image. The number of pixels that detect signal from each feature can be adjusted based on the size and shape of the features imaged and based on the configuration of the digital detection apparatus (or individual microfluorometer). For example, each feature can be detected in a given image by no more than about 16 pixels, 9 pixels, 4 pixels, or 1 pixel. In particular embodiments, each image can utilize on average 6.5 pixels per feature, 4.7 pixels per feature or 1 pixel per feature. The number of pixels used per feature can be reduced, for example, by reducing variability in the position of features in the pattern of the array and tightening the tolerance for alignment of the detection apparatus to the array. Taking as an example a digital detector that is configured to use fewer than 4 pixels per feature, image quality can be improved by using an array of ordered nucleic acid features in place of an array of randomly distributed nucleic acid clusters.

It will be understood that a detection apparatus having one or more microfluorometers can detect an area of a common plane that is roughly equivalent to the number of

microfluorometers multiplied by the wide-field area detected by each microfluorometer. The areas need not be contiguous. For example, 2 or more microfluorometers can be positioned to detect discrete regions of a common plane that are separated by an undetected area. However, if desired, multiple microfluorometers can be positioned to detect areas that are contiguous, but not overlapping. In alternative embodiments a detection apparatus having one or more microfluorometers can detect an area of a common plane that is substantially less than the number of microfluorometers multiplied by the wide-field area detected by each microfluorometer. This can result, for example, when multiple microfluorometers are positioned to detect areas that have at least a partial overlap. As set forth in further detail elsewhere herein, multiple images need not be acquired in a format that is used for or that even supports reconstruction of a complete image of an array or other common plane that has been detected.

The detection apparatus can make use of any of the detection apparatus configurations and sequencing methods set forth in US Patent Application Serial Number 13/766,413 filed on February 13, 2013 and entitled "INTEGRATED OPTOELECTRONIC READ HEAD AND FLUIDIC CARTRIDGE USEFUL FOR NUCLEIC ACID SEQUENCING," the content of which is incorporated by reference in its entirety.

The exemplary detection apparatus presented herein is shown having a single microfluorometer. In other embodiments, the detection apparatus can have two or more microfluorometer, in any one of a variety of configurations. It will be appreciated that a single microfluorometer can be advantageous from a variety of standpoints. First, the single microfluorometer provides advantages from the standpoint of production and maintenance costs, especially when other configurations require calibration of each individual microfluorometer. In the single microfluorometer configuration presented herein, the optical readhead can be presented in an intact unit that can be incorporated into the detection apparatus in a modular approach, simplifying manufacturing and reducing costs further. These costs savings can be passed along to the end user, allowing users access to a large number of nucleic acid array detection applications. Another advantage of the single microfluorometer configuration presented herein comes from the relative simplicity of the interactions with the fluidic components of the system. For example, a flow cell used in the system can be configured with a single flow channel, thus reducing the requirements for multiple valves and pumps found in prior systems. Whereas flow cells having 2 or more flow

channels may require switching fluid flow between different flow channels, a single flow channel can have a dedicated supply line in fluid communication with a single valve. Additionally, fluidic manifold bodies can be used having a single layer of channels, again simplifying the design and manufacture of the fluidic system and reducing costs of fabrication
5 and maintenance of the system.

An exemplary optical layout for a microfluorometer **500** is shown in **Fig. 4A**. The microfluorometer **500** is directed to a flow cell **600** having an upper layer **671** and a lower layer **673** that are separated by a fluid filled channel **675**. In the configuration shown, the upper layer **671** is optically transparent and the microfluorometer **500** is focused to an area
10 **676** on the inner surface **672** of the upper layer **671**. In an alternative configuration the microfluorometer **500** can be focused on the inner surface **674** of the lower layer **673**. One or both of the surfaces can include array features that are to be detected by the microfluorometer **500**.

The microfluorometer **500** includes an objective **501** that is configured to direct
15 excitation radiation from a radiation source **502** to the flow cell **600** and to direct emission from the flow cell **600** to a detector **508**. In the exemplary layout, excitation radiation from the radiation source **502** passes through a lens **505** then though a beam splitter **506** and then through the objective on its way to the flow cell **600**. In the embodiment shown the radiation source includes two light emitting diodes (LEDs) **503** and **504**, which produce radiation at
20 different wavelengths from each other. The emission radiation from the flow cell **600** is captured by the objective **501** and is reflected by the beam splitter through conditioning optics **507** and to the detector **508** (e.g. a CMOS sensor). The beam splitter **506** functions to direct the emission radiation in a direction that is orthogonal to the path of the excitation radiation. The position of the objective can be moved in the z dimension to alter focus of the
25 microfluorometer. The microfluorometer **500** can be moved back and forth in the y direction to capture images of several areas of the inner surface **672** of the upper layer **671** of the flow cell **600**.

Fig. 4B shows an exploded view of an exemplary microfluorometer for purposes of demonstrating functional arrangement for various optical components. Two excitation sources
30 are shown, including a green LED (LEDG) and a red LED (LEDR). Excitation light from each passes through a green LED collector lens (L6) and red LED collector lens (L7), respectively. The green excitation radiation passes from the green LED collector lens (L6) to

the combiner dichroic (F5) which reflects the green excitation radiation through an excitation filter (F2), then through a an LED field lens (L8) and laser diode beam splitter (F3), then through an excitation field stop (FS), then through an excitation projection lens group L2 to an excitation/emission dichroic (F4) which reflects the green excitation radiation through an objective lens group (L4) to the surface of a flow cell (FC). An LED fold mirror (M1) reflects the red excitation radiation to a combiner dichroic (F5) after which the red excitation radiation follows the same path as the green excitation radiation to the surface of the flow cell (FC). As shown in the figure, focusing is actuated by moving the translating objective lens group (L4) up and down (i.e. along the z dimension). Emission from the flow cell (FC) surface passes back through the translating objective lens group (L4), to the excitation/emission dichroic (F4) which passes the emission radiation to the emission projection lens group (L1) through to the emission filter and then to the CMOS image sensor (S1). A laser diode (LD) is also directed via a laser diode coupling lens group (L5) to the laser diode beam splitter (F3) which reflects the laser diode radiation through the excitation field stop (FS), the excitation projection lens group (L2), the excitation/emission dichroic (F4), objective lens group (L4) to the flow cell (FC). An LED heat sink (HS) is disposed above the green LED (LEDG) and red LED (LEDR).

Fig. 4C shows an exemplary embodiment of a single camera module, including laser diode (LD) for autofocus system, CMOS image sensor (S1) and Z stage voice coil motor for adjusting objective lens (L4). LEDG and LEDR are not shown in this view.

As demonstrated by the exemplary embodiments of **Fig. 4A, 4B** and **4C**, each of the microfluorometers can include a beam splitter and a detector, wherein the beam splitter is positioned to direct excitation radiation from an excitation radiation source to the objective and to direct emission radiation from the objective to the detector. As shown in the figures, each microfluorometer can optionally include an excitation radiation source such as an LED. In this case, each microfluorometer can include a dedicated radiation source, such that the read head includes several radiation sources each separated into individual microfluorometers. In some embodiments, two or more microfluorometers can receive excitation radiation from a common radiation source. As such the two or more microfluorometers can share a radiation source. In an exemplary configuration, a single radiation source can direct radiation to a beam splitter that is positioned to separate the excitation radiation into two or more beams and directs the beams to two or more respective microfluorometers. Additionally or alternatively,

excitation radiation can be directed from a radiation source to one, two or more microfluorometers via one or more optical fibers.

It will be understood that the particular components shown in the figures are exemplary and can be replaced with components of similar function. For example, any of a variety of radiation sources can be used instead of an LED. Particularly useful radiation sources are arc lamps, lasers, semiconductor light sources (SLSs), or laser diodes. LEDs can be purchased, for example, from Luminus (Billerica, Mass). Similarly, a variety of detectors are useful including, but not limited to a charge-coupled device (CCD) sensor; photomultiplier tubes (PMT's); or complementary metal–oxide–semiconductor (CMOS) sensor. A particularly useful detector is a 5-megapixel CMOS sensor (MT9P031) available from Aptina Imaging (San Jose, CA).

Figs. 4A, 4B and **4C** provide exemplary embodiments of a microfluorometer that includes two excitation sources. This configuration is useful for detecting at least two fluorophores that are excited at different wavelengths, respectively. If desired, a microfluorometer can be configured to include more than two excitation sources. For example, a microfluorometer can include at least 2, 3, 4 or more different excitation sources (i.e. sources producing different wavelengths from each other). Alternatively or additionally, beam splitters and optical filters can be used to expand the range of excitation wavelengths available from an individual radiation source. Similar use of multiple radiation sources and/or optical filtering of split excitation beams can be used for embodiments where several microfluorometers share excitation from one or more radiation sources. As set forth in further detail elsewhere herein, the availability of multiple excitation wavelengths is particularly useful for sequencing applications that utilize several different fluorophore labels.

The microfluorometer of the exemplary embodiment is a component of an imaging module. **Fig. 5A** sets forth a front view of one exemplary embodiment. Shown is imaging module **700**, made up of camera assembly **701**, XY stage **702**, and flow cell latch clamp module **900**, mounted to fixed base **704**. Camera assembly comprises heat sink **711** above camera housing **705**, which in turn is mounted to fixed base **704** and provides an enclosure for the lower half of the microfluorometer set forth in **Fig. 4A, 4B** and **4C**. Two motors provide movement of X-stage and Y-stage, individually. X-motor **706** drives X lead screw **707** which moves camera assembly **701** along X-axis as indicated. Y-motor (**712**, not shown) drives movement of Y lead screw **708** which moves flow cell latch clamp module **900** along Y-axis

on guide rail **709** as indicated. The flow cell latch clamp module **900** can also comprise an RFID module **710** for detecting RFID encoded flow cells.

Fig. 5B shows a side view of the imaging module **700**. Shown is Y-motor **712**, Y lead screw **708**, guide rail **709** and X-lead screw **707**.

5 In accordance with one embodiment, a fluidic device for analyzing samples is provided. The fluidic device includes a flow cell having inlet and outlet ports and a flow channel extending therebetween. The flow cell is configured to hold a sample-of-interest. The fluidic device also includes a housing having a reception space that is configured to receive the flow cell. The reception space is sized and shaped to permit the flow cell to float 10 relative to the housing. The fluidic device also includes a gasket that is coupled to the housing. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively.

15 In another embodiment, a removable cartridge configured to hold and facilitate positioning a flow cell for imaging is provided. The cartridge includes a removable housing that has a reception space configured to hold the flow cell substantially within an object plane. The housing includes a pair of housing sides that face in opposite directions. The reception space extends along at least one of the housing sides so that the flow cell is exposed to an 20 exterior of the housing through said at least one of the housing sides. The cartridge also includes a cover member that is coupled to the housing and includes a gasket. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is configured to be mounted over an exposed portion of the flow cell when the flow cell is held by the housing.

25 Exemplary fluidic devices, including flow cells suitable for use with the devices described herein are set forth greater detail in US Patent Application Serial Number 13/766,413 filed on February 13, 2013 and entitled "INTEGRATED OPTOELECTRONIC READ HEAD AND FLUIDIC CARTRIDGE USEFUL FOR NUCLEIC ACID SEQUENCING," the content of which is incorporated by reference in its entirety.

30 **Fig. 6** illustrates a fluidic device **800** formed in accordance with one embodiment. As shown in **Fig. 6**, the fluidic device **800** includes a cartridge (or flow cell carrier) **802** and the

flow cell **850**. The cartridge **802** is configured to hold the flow cell **850** and facilitate orienting the flow cell **850** for an imaging session.

In some embodiments, the fluidic device **800** and the cartridge **802** may be removable such that the cartridge **802** may be removed from an imaging system (not shown) by an
5 individual or machine without damage to the fluidic device 800 or cartridge **802**. For example, the cartridge **802** may be configured to be repeatedly inserted and removed into the imaging system without damaging the cartridge 802 or rendering the cartridge **802** unsuitable for its intended purpose. In some embodiments, the fluidic device **800** and the cartridge **802** may be sized and shaped to be handheld by an individual. Furthermore, the fluidic device **800**
10 and the cartridge **802** may be sized and shaped to be carried by an automated system.

As shown in **Fig. 6**, the cartridge **802** may include a housing or carrier frame **804** and a cover member **806** that is coupled to the housing **804**. Also shown in **Fig. 6**, the fluidic device **800** may have a device window that passes entirely through the cartridge **802** along the Z-axis. With respect to **Fig. 6**, the cover member **806** may include a cover body **840** and a
15 gasket **842** that are coupled to each other. The gasket **842** includes inlet and outlet passages **846** and **844** that are located proximate to one another. In the illustrated embodiment, the cover body **840** and the gasket **842** are co-molded into a unitary structure. When formed, the cover body **840** and the gasket **842** may have different compressible properties. For example, in particular embodiments, the gasket **842** may comprise a material that is more compressible
20 than material of the cover body **840**. However, in alternative embodiments, the cover body **840** and the gasket **842** may be separate parts that are coupled together (e.g., mechanically or using an adhesive). In other embodiments, the cover body **840** and the gasket **842** may be different portions or regions of a single continuous structure.

The cover member **806** may be movably coupled to the housing **804**. For example, the
25 cover member **806** may be rotatably coupled to the base member **826** of the housing **804**. The housing **804** may define a cartridge cavity that is accessible when the cover member **806** is in the disengaged position. In some embodiments, an identification transmitter may be positioned within the cartridge cavity. The identification transmitter is configured to communicate information about the flow cell **850** to a reader. For example, the identification
30 transmitter may be an RFID tag. The information provided by the identification transmitter may, for example, identify the sample in the flow cell **850**, a lot number of the flow cell or sample, a date of manufacture, and/or the assay protocol to be performed when the flow cell

850 is inserted into the imaging system. The identification transmitter may communicate other information as well.

Shown in **Fig. 7A** is a flow cell latch clamp module (FCLM) **900** (hereinafter referred to as latch clamp module), is mounted directly on the Y-plate of the XY stage **702**. Flow cell latch clamp cover **901** holds the flow cell 800 and aligns it to the camera in the microfluorometer. Within clamp cover **901** is manifold **902** with inlet and outlet ports **944, 946** configured to mate with inlet passage **846** and outlet passage **844** positions in the gasket of flow cell housing. A heat block **903** is mounted to Y-plate and provides heating to the flow cell to enable chemistry. Latch button **904** can be depressed to open clamp cover **901**.

Fig. 7B shows a cutaway view of latch clamp module **900**, showing heat block **903** mounted directly to imaging module Y-plate **702**. **Fig. 7C** shows latch clamp module **900** with flow cell **850** mounted in position. Flow cell datum edges **807** and **808** are biased against another set of dowel pins **907** and **908** mounted to heater block. Spring-loaded lever **909** biases the flow cell **850** against the heater block dowel pins **907** and **908**. As shown, flow cell **850** is registered in X and Y positions against reference pins and in Z position against heater block surface. The flow cell clamp module **900** provides sealing for fluidics, supporting negative and positive pressure in the fluidic connection between the FAM and the flow cell gasket.

The embodiments shown in **Fig. 7A, 7B and 7C** are exemplary. Further exemplary embodiments of the methods and apparatus of the present disclosure that can be used alternatively or additionally to the example of **Fig. 7A, 7B and 7C** are set forth in further detail in US Patent Application Serial Number 13/766,413 filed on February 13, 2013 and entitled "INTEGRATED OPTOELECTRONIC READ HEAD AND FLUIDIC CARTRIDGE USEFUL FOR NUCLEIC ACID SEQUENCING," the content of which is incorporated by reference in its entirety.

In particular embodiments a fluidic system can be configured to allow re-use of one or more reagents. For example, the fluidic system can be configured to deliver a reagent to a flow cell, then remove the reagent from the flow cell, and then re-introduce the reagent to the flow cell. One configuration is exemplified in the apparatus and methods set forth in US Patent Application Serial Number 14/453,868 filed on August 7, 2014 and entitled "FLUIDIC SYSTEM FOR REAGENT DELIVERY TO A FLOW CELL," the content of which is incorporated by reference in its entirety.

Embodiments of the present fluidic systems and methods find particular use for nucleic acid sequencing techniques. For example, sequencing-by-synthesis (SBS) protocols are particularly applicable. In SBS, extension of a nucleic acid primer along a nucleic acid template is monitored to determine the sequence of nucleotides in the template. The underlying chemical process can be polymerization (e.g. as catalyzed by a polymerase enzyme) or ligation (e.g. catalyzed by a ligase enzyme). In a particular polymerase-based SBS embodiment, fluorescently labeled nucleotides are added to a primer (thereby extending the primer) in a template dependent fashion such that detection of the order and type of nucleotides added to the primer can be used to determine the sequence of the template. A plurality of different templates can be subjected to an SBS technique on a surface under conditions where events occurring for different templates can be distinguished. For example, the templates can be present on the surface of an array such that the different templates are spatially distinguishable from each other. Typically the templates occur at features each having multiple copies of the same template (sometimes called "clusters" or "colonies"). However, it is also possible to perform SBS on arrays where each feature has a single template molecule present, such that single template molecules are resolvable one from the other (sometimes called "single molecule arrays").

Flow cells provide a convenient substrate for housing an array of nucleic acids. Flow cells are convenient for sequencing techniques because the techniques typically involve repeated delivery of reagents in cycles. For example, to initiate a first SBS cycle, one or more labeled nucleotides, DNA polymerase, etc., can be flowed into/through a flow cell that houses an array of nucleic acid templates. Those features where primer extension causes a labeled nucleotide to be incorporated can be detected, for example, using methods or apparatus set forth herein. Optionally, the nucleotides can further include a reversible termination property that terminates further primer extension once a nucleotide has been added to a primer. For example, a nucleotide analog having a reversible terminator moiety can be added to a primer such that subsequent extension cannot occur until a deblocking agent is delivered to remove the moiety. Thus, for embodiments that use reversible termination a deblocking reagent can be delivered to the flow cell (before or after detection occurs). Washes can be carried out between the various delivery steps. The cycle can then be repeated n times to extend the primer by n nucleotides, thereby detecting a sequence of length n. Exemplary sequencing techniques are described, for example, in Bentley et al., *Nature*

456:53-59 (2008), WO 04/018497; US 7,057,026; WO 91/06678; WO 07/123744; US 7,329,492; US 7,211,414; US 7,315,019; US 7,405,281, and US 2008/0108082, each of which is incorporated herein by reference.

For the nucleotide delivery step of an SBS cycle, either a single type of nucleotide can be delivered at a time, or multiple different nucleotide types (e.g. A, C, T and G together) can be delivered. For a nucleotide delivery configuration where only a single type of nucleotide is present at a time, the different nucleotides need not have distinct labels since they can be distinguished based on temporal separation inherent in the individualized delivery. Accordingly, a sequencing method or apparatus can use single color detection. For example, a microfluorometer or read head need only provide excitation at a single wavelength or in a single range of wavelengths. Thus, a microfluorometer or read head need only have a single excitation source and multiband filtration of excitation need not be necessary. For a nucleotide delivery configuration where delivery results in multiple different nucleotides being present in the flow cell at one time, features that incorporate different nucleotide types can be distinguished based on different fluorescent labels that are attached to respective nucleotide types in the mixture. For example, four different nucleotides can be used, each having one of four different fluorophores. In one embodiment the four different fluorophores can be distinguished using excitation in four different regions of the spectrum. For example, a microfluorometer or read head can include four different excitation radiation sources. Alternatively a read head can include fewer than four different excitation radiation sources but can utilize optical filtration of the excitation radiation from a single source to produce different ranges of excitation radiation at the flow cell.

In some embodiments, four different nucleotides can be detected in a sample (e.g. array of nucleic acid features) using fewer than four different colors. As a first example, a pair of nucleotide types can be detected at the same wavelength, but distinguished based on a difference in intensity for one member of the pair compared to the other, or based on a change to one member of the pair (e.g. via chemical modification, photochemical modification or physical modification) that causes apparent signal to appear or disappear compared to the signal detected for the other member of the pair. As a second example, three of four different nucleotide types can be detectable under particular conditions while a fourth nucleotides type lacks a label that is detectable under those conditions. In an SBS embodiment of the second example, incorporation of the first three nucleotide types into a nucleic acid can be

determined based on the presence of their respective signals, and incorporation of the fourth nucleotide type into the nucleic acid can be determined based on absence of any signal. As a third example, one nucleotide type can be detected in two different images or in two different channels (e.g. a mix of two species having the same base but different labels can be used, or a single species having two labels can be used or a single species having a label that is detected in both channels can be used), whereas other nucleotide types are detected in no more than one of the images or channels. In this third example, comparison of the two images or two channels serves to distinguish the different nucleotide types.

The three exemplary configurations in the above paragraph are not mutually exclusive and can be used in various combinations. An exemplary embodiment is an SBS method that uses reversibly blocked nucleotides (rbNTPs) having fluorescent labels. In this format, four different nucleotide types can be delivered to an array of nucleic acid features that are to be sequenced and due to the reversible blocking groups one and only one incorporation event will occur at each feature. The nucleotides delivered to the array in this example can include a first nucleotide type that is detected in a first channel (e.g. rbATP having a label that is detected in the first channel when excited by a first excitation wavelength), a second nucleotide type that is detected in a second channel (e.g. rbCTP having a label that is detected in the second channel when excited by a second excitation wavelength), a third nucleotide type that is detected in both the first and the second channel (e.g. rbTTP having at least one label that is detected in both channels when excited by the first and/or second excitation wavelength) and a fourth nucleotide type that lacks a label that is detected in either channel (e.g. rbGTP having no extrinsic label).

Once the four nucleotide types have been contacted with the array in the above example, a detection procedure can be carried out, for example, to capture two images of the array. The images can be obtained in separate channels and can be obtained either simultaneously or sequentially. A first image obtained using the first excitation wavelength and emission in the first channel will show features that incorporated the first and/or third nucleotide type (e.g. A and/or T). A second image obtained using the second excitation wavelength and emission in the second channel will show features that incorporated the second and/or third nucleotide type (e.g. C and/or T). Unambiguous identification of the nucleotide type incorporated at each feature can be determined by comparing the two images to arrive at the following: features that show up only in the first channel incorporated the first

nucleotide type (e.g. A), features that show up only in the second channel incorporated the second nucleotide type (e.g. C), features that show up in both channel incorporated the third nucleotide type (e.g. T) and features that don't show up in either channel incorporated the fourth nucleotide type (e.g. G). Note that the location of the features that incorporated G in this example can be determined from other cycles (where at least one of the other three nucleotide types is incorporated). Exemplary apparatus and methods for distinguishing four different nucleotides using detection of fewer than four colors are described for example in US Pat. App. Ser. No. 61/538,294, which is incorporated herein by reference.

In some embodiments, nucleic acids can be attached to a surface and amplified prior to or during sequencing. For example, amplification can be carried out using bridge amplification to form nucleic acid clusters on a surface. Useful bridge amplification methods are described, for example, in US 5,641,658; US 2002/0055100; US 7,115,400; US 2004/0096853; US 2004/0002090; US 2007/0128624; or US 2008/0009420, each of which is incorporated herein by reference. Another useful method for amplifying nucleic acids on a surface is rolling circle amplification (RCA), for example, as described in Lizardi et al., *Nat. Genet.* 19:225-232 (1998) and US 2007/0099208 A1, each of which is incorporated herein by reference. Emulsion PCR on beads can also be used, for example as described in Dressman et al., *Proc. Natl. Acad. Sci. USA* 100:8817-8822 (2003), WO 05/010145, US 2005/0130173 or US 2005/0064460, each of which is incorporated herein by reference.

As set forth above, sequencing embodiments are an example of a repetitive process. The methods of the present disclosure are well suited to repetitive processes. Some embodiments are set forth below and elsewhere herein.

Accordingly, provided herein are sequencing methods that include (a) providing a fluidic system comprising (i) a flow cell comprising an optically transparent surface, (ii) a nucleic acid sample, (iii) a plurality of reagents for a sequencing reaction, and (iv) a fluidic system for delivering the reagents to the flow cell; (b) providing a detection apparatus comprising (i) a plurality of microfluorometers, wherein each of the microfluorometers comprises an objective configured for wide-field image detection in an image plane in x and y dimensions, and (ii) a sample stage; and (c) carrying out fluidic operations of a nucleic acid sequencing procedure in the cartridge and detection operations of the nucleic acid sequencing procedure in the detection apparatus, wherein (i) the reagents are delivered to the flow cell by the fluidic system, (ii) wide-field images of the nucleic acid features are detected by the

plurality of microfluorometers, and (iii) at least some reagents are removed from the flow cell to a cache reservoir.

Throughout this application various publications, patents and/or patent applications have been referenced. The disclosure of these publications in their entireties is hereby
5 incorporated by reference in this application.

The term comprising is intended herein to be open-ended, including not only the recited elements, but further encompassing any additional elements.

A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made. Accordingly, other embodiments are within the
10 scope of the following claims.

What is claimed is:

1. A detection apparatus, comprising:

a microfluorometer comprising an objective, an excitation radiation source, and a detector, the microfluorometer configured for wide-field image detection;

5 a fluidic system for delivering reagents from a reagent cartridge to a flow cell, the fluidic system comprising:

a manifold body having a plurality of fluidic channels configured for fluid communication between the reagent cartridge and an inlet of the flow cell;

a plurality of reagent sippers extending downward from ports in the manifold 10 body, each of the reagent sippers configured to be placed into a reagent reservoir of the reagent cartridge so that liquid reagent can be drawn from the reagent reservoir into the reagent sipper; and

a valve configured to mediate fluid communication between the reagent reservoirs and the inlet of the flow cell, the fluidic channels fluidly connecting the 15 reagent sippers to the valve; and

a flow cell latch clamp module having a clamp cover for holding the flow cell, the objective configured to direct excitation radiation from the radiation source to the flow cell and to direct emission from the flow cell to the detector, the microfluorometer being movable to acquire wide-field images of different areas of an inner surface of the flow cell.

20 2. The detection apparatus of claim 1, wherein the detection apparatus comprises no more than a single microfluorometer.

3. The detection apparatus of claim 1 or claim 2, wherein the microfluorometer includes a beam splitter that is positioned to direct excitation radiation from the excitation radiation source to the objective and to direct emission radiation from the objective to the 25 detector.

4. The detection apparatus of any one of claims 1-3, wherein the microfluorometer is an integrated microfluorometer having a compact epifluorescent detection configuration.

5. The detection apparatus of any one of claims 1-4, wherein the 30 microfluorometer is movable to allow imaging of the flow cell that is larger than a field of view of the microfluorometer.

6. The detection apparatus of any one of claims 1-5, further comprising a housing and a screen presented on a front face of the housing, the screen functioning as a graphical user interface.

7. The detection apparatus of any one of claims 1-5, further comprising a housing
5 having cartridge receptacle for receiving the reagent cartridge.

8. The detection apparatus of any one of claims 1-7, further comprising a fluidics automation module including a lift assembly for raising and lowering the reagent cartridge.

9. The detection apparatus of any one of claims 1-7, further comprising a fluidics automation module including a belt assembly that moves the reagent cartridge during loading.

10 10. The detection apparatus of any one of claims 1-7, further comprising a fluidics automation module including a lift assembly for raising and lowering the reagent cartridge and a belt assembly that moves the reagent cartridge during loading.

11. The detection apparatus of any one of claims 1-10, wherein the manifold body is formed from multiple layers of solid material bonded together.

15 12. The detection apparatus of claim 11, wherein the channels are formed in the solid material prior to bonding the multiple layers together.

13. The detection apparatus of any one of claims 1-12, wherein the fluidic channels are housed entirely within the manifold body.

14. The detection apparatus of any one of claims 1-13, wherein the manifold body
20 has at least sixteen (16) ports that connect to respective reagent sippers.

15. The detection apparatus of any one of claims 1-14, wherein the valve is configured with inlet ports that correspond to each of the ports of the fluidic channels and configured with a single common outlet port which fluidly connects to the flow cell.

16. The detection apparatus of any one of claims 1-15, wherein the fluidic system
25 has no more than one valve that mediates fluid communication between the reagent reservoirs and the flow cell.

17. The detection apparatus of claim 16, wherein each of the fluidic channels originates from a single port and connects to a corresponding port of the valve.

18. The detection apparatus of any one of claims 1-17, wherein the manifold body
30 has a single layer of fluidic channels.

19. The detection apparatus of any one of claims 1-18, wherein the reagent sippers have distal ends that are configured to pierce a film or foil layer of the reagent cartridge.

20. The detection apparatus of any one of claims 1-19, wherein the radiation source produces radiation at different wavelengths.

21. The detection apparatus of any one of claims 1-20, wherein the detector is a complementary metal–oxide–semiconductor (CMOS) image sensor.

5 22. The detection apparatus of any one of claims 1-21, wherein the microfluorometer is a component of an imaging module, the imaging module also including the flow cell latch clamp module.

23. The detection apparatus of claim 22, wherein the imaging module also includes an XY stage in which an X-motor and a Y-motor provide movement of the X-stage and the Y-

10 stage, respectively.

24. The detection apparatus of claim 23, wherein the X-motor moves a camera assembly along an X-axis, the camera assembly having the microfluorometer, wherein the Y-motor moves the flow cell latch clamp module along a Y-axis.

25. The detection apparatus of any one of claims 1-24, further comprising a fluidic

15 device that includes a flow cell cartridge and the flow cell, the flow cell cartridge configured to hold the flow cell and facilitate orienting the flow cell for an imaging session.

26. The detection apparatus of claim 25, wherein the fluidic device and the flow cell cartridge are removable such that the flow cell cartridge is removable from the flow cell latch clamp module by an individual or machine without damage to the fluidic device or the

20 flow cell cartridge.

27. The detection apparatus of 25, wherein the flow cell cartridge is configured to be repeatedly inserted and removed into the flow cell latch clamp module without damaging the flow cell cartridge or rendering the flow cell cartridge unsuitable for its intended purpose.

28. The detection apparatus of any one of claims 1-27, wherein the flow cell

25 cartridge includes a housing and a cover member that is coupled to the housing of the flow cell cartridge, the cover member including a gasket having inlet and outlet passages that are located proximate to one another, wherein the clamp cover includes a cover manifold with inlet and outlet ports that are configured to mate with the inlet passage and the outlet passage of the gasket, respectively.

30 29. The detection apparatus of any one of claims 1-28, wherein the flow cell latch clamp module includes a spring-loaded lever that biases the flow cell against dowel pins.

30. The detection apparatus of any one of claims 1-29, wherein a field diameter for the microfluorometer is at least 0.5 mm.

31. The detection apparatus of any one of claims 1-30, wherein a field diameter for the microfluorometer is at least 2 mm.

5 32. The detection apparatus of any one of claims 1-31, wherein a field diameter for the microfluorometer is no larger than 5 mm.

33. The detection apparatus of any one of claims 1-32, wherein a numerical aperture for the microfluorometer is at least 0.2.

34. The detection apparatus of any one of claims 1-33, wherein a numerical 10 aperture for the microfluorometer is no greater than 0.8.

35. The detection apparatus of any one of claims 1-34, wherein a numerical aperture for the microfluorometer is no greater than 0.5.

36. The detection apparatus of any one of claims 1-35, wherein the microfluorometer has a resolution that is sufficient to distinguish features separated by at most 15 100 μm.

37. A sequencing system that includes the detection apparatus of any one of claims 1-36 and the reagent cartridge having the reagent reservoirs, wherein the reagent reservoirs include sequencing reagents.

38. The sequencing system of claim 37, wherein at least one of the reagent 20 reservoirs include a nucleic acid sample.

39. The sequencing system of claim 37 or claim 38, wherein the reagent cartridge is removable from the detection apparatus.

40. The sequencing system of claim 37 or claim 38 or claim 39, wherein the sequencing system is configured to perform a sequencing-by-synthesis protocol.

25

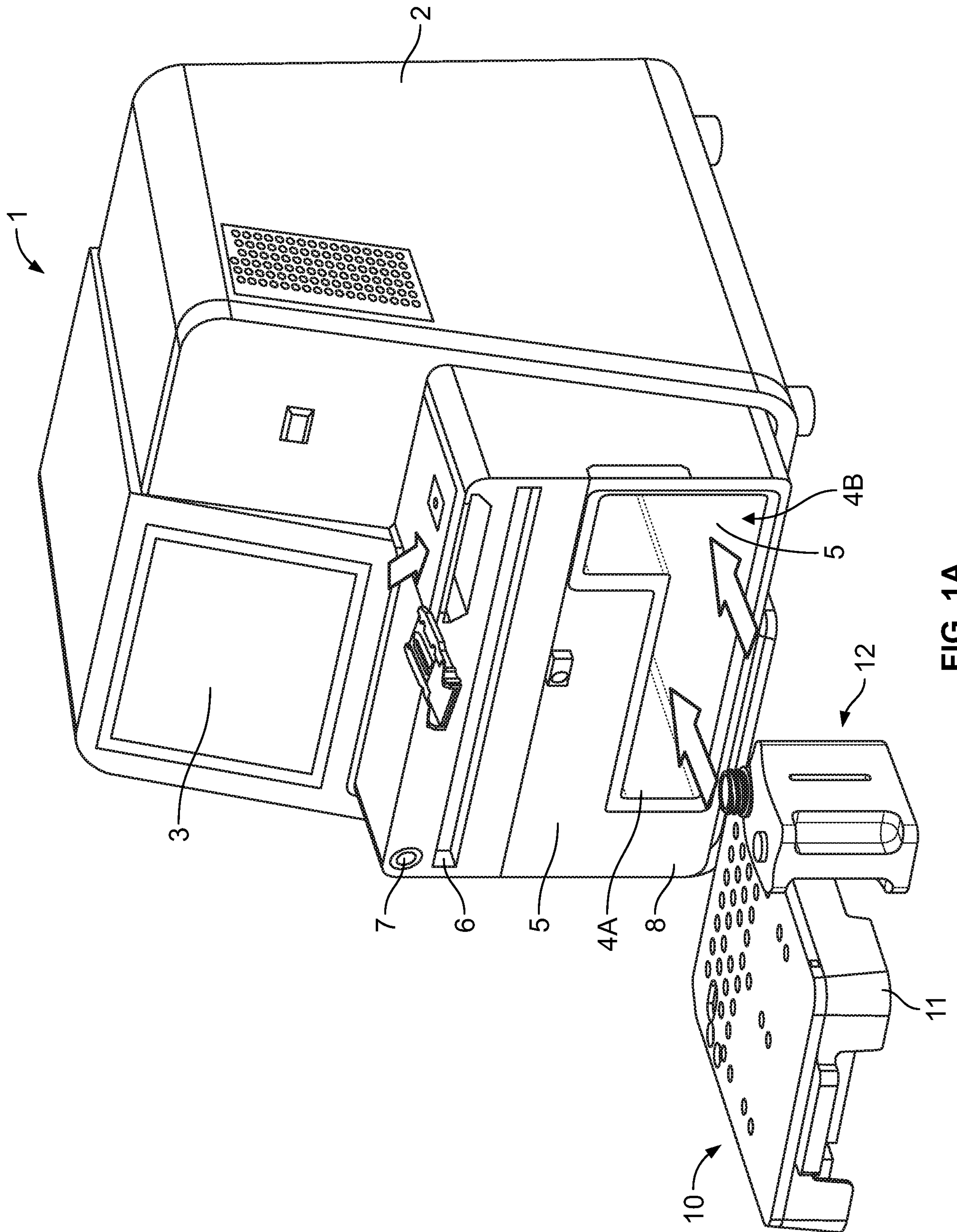

FIG. 1A

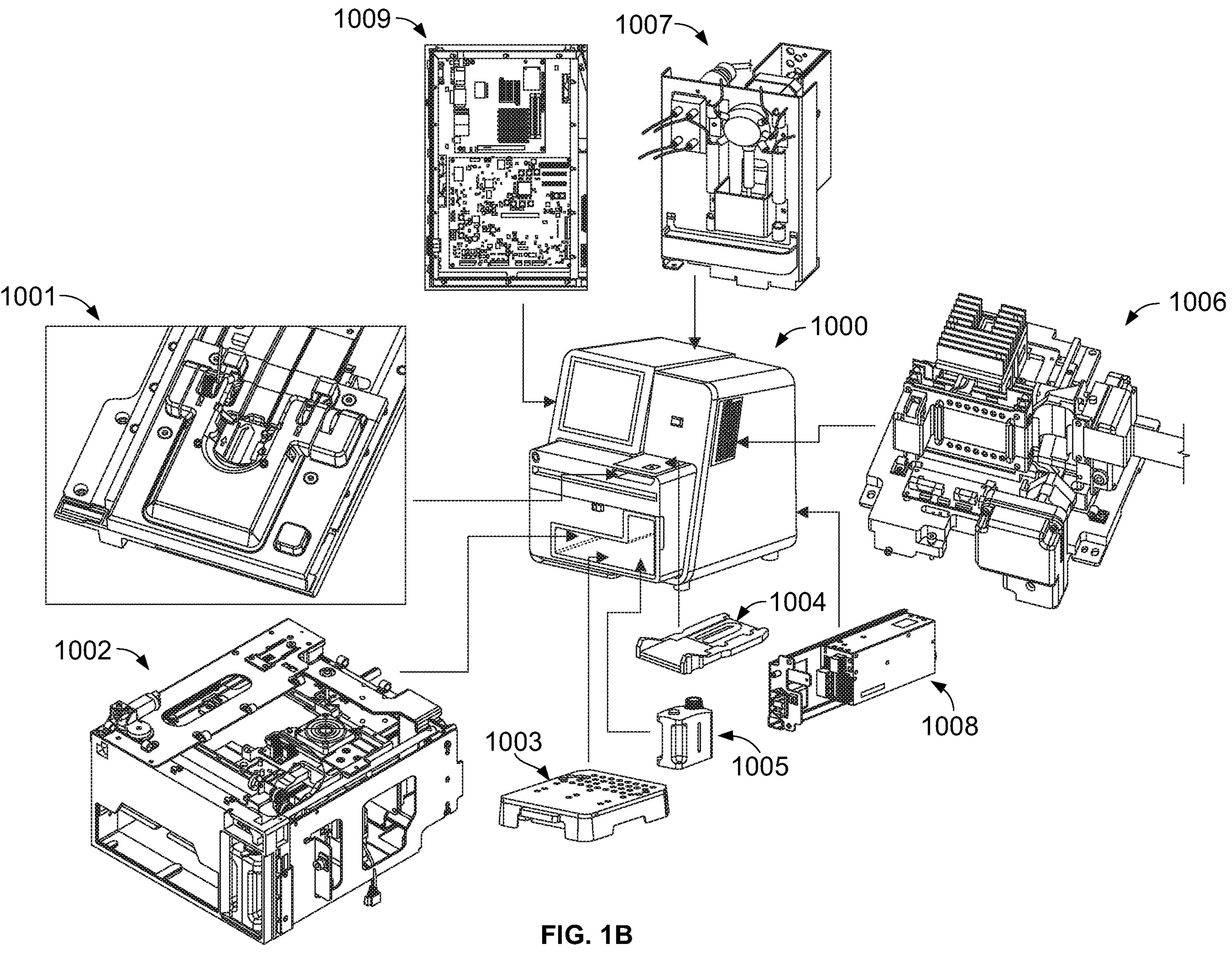

FIG. 1B

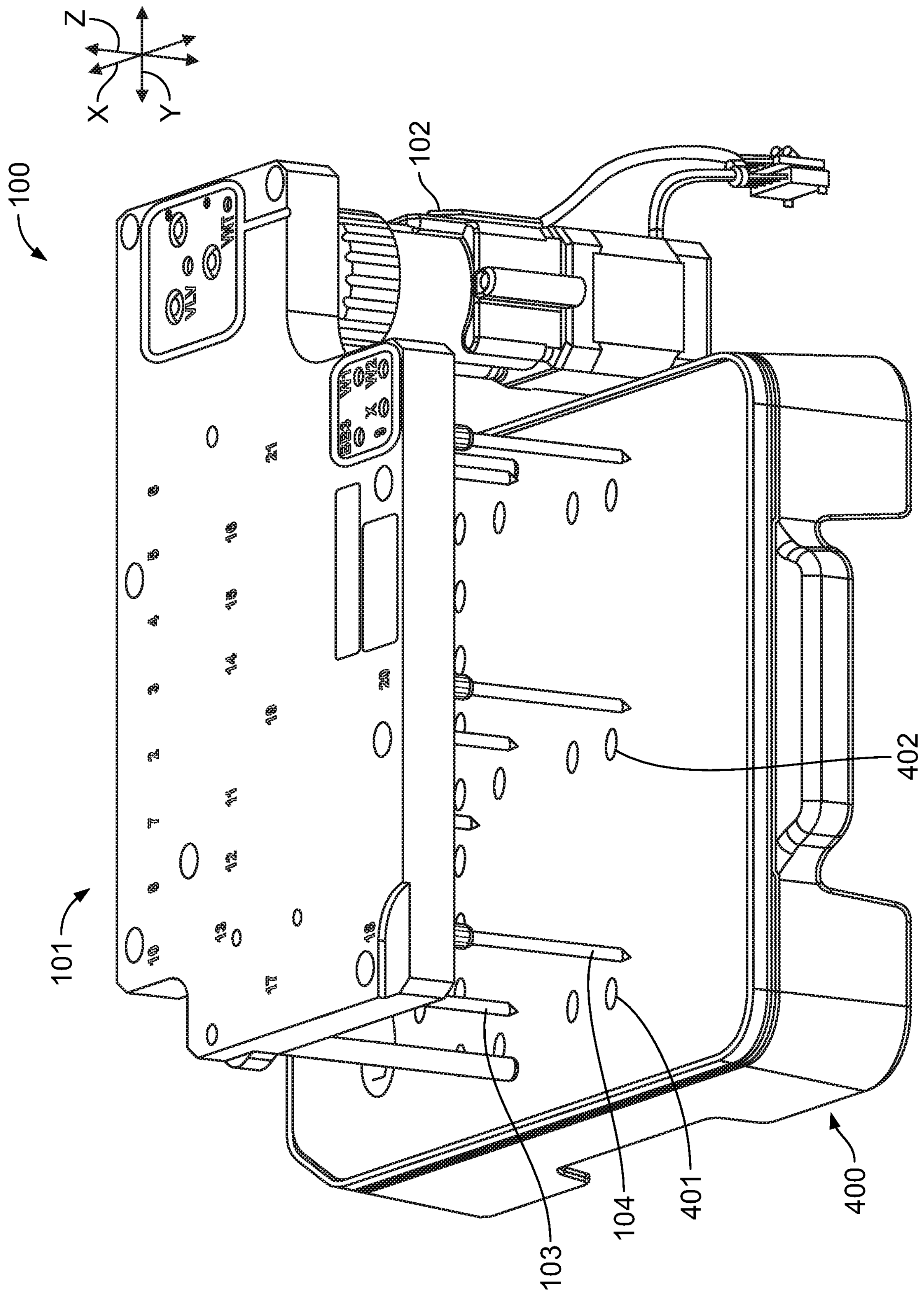

FIG. 2A

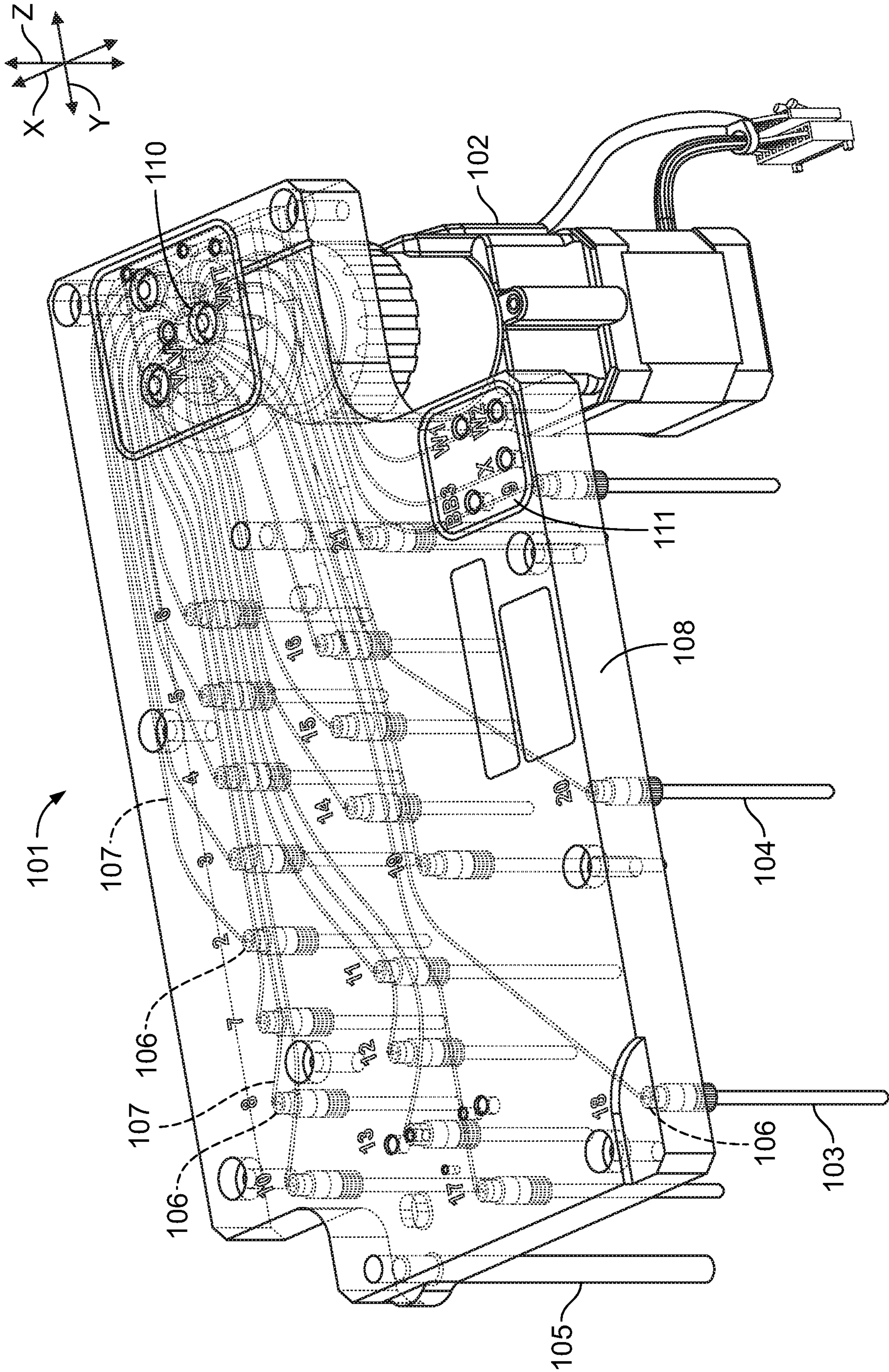

FIG. 2B

102

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FIG. 2C

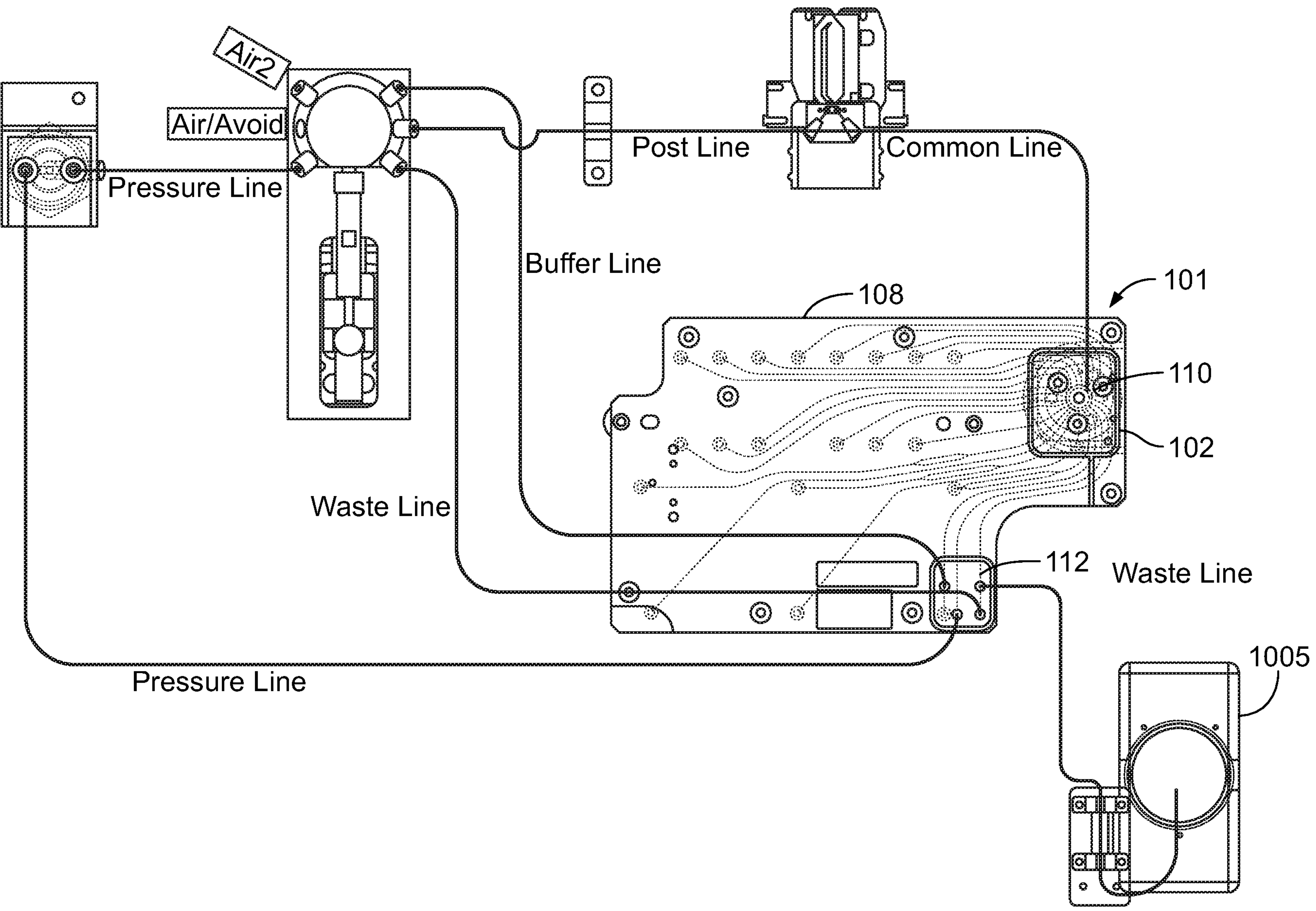


FIG. 2D

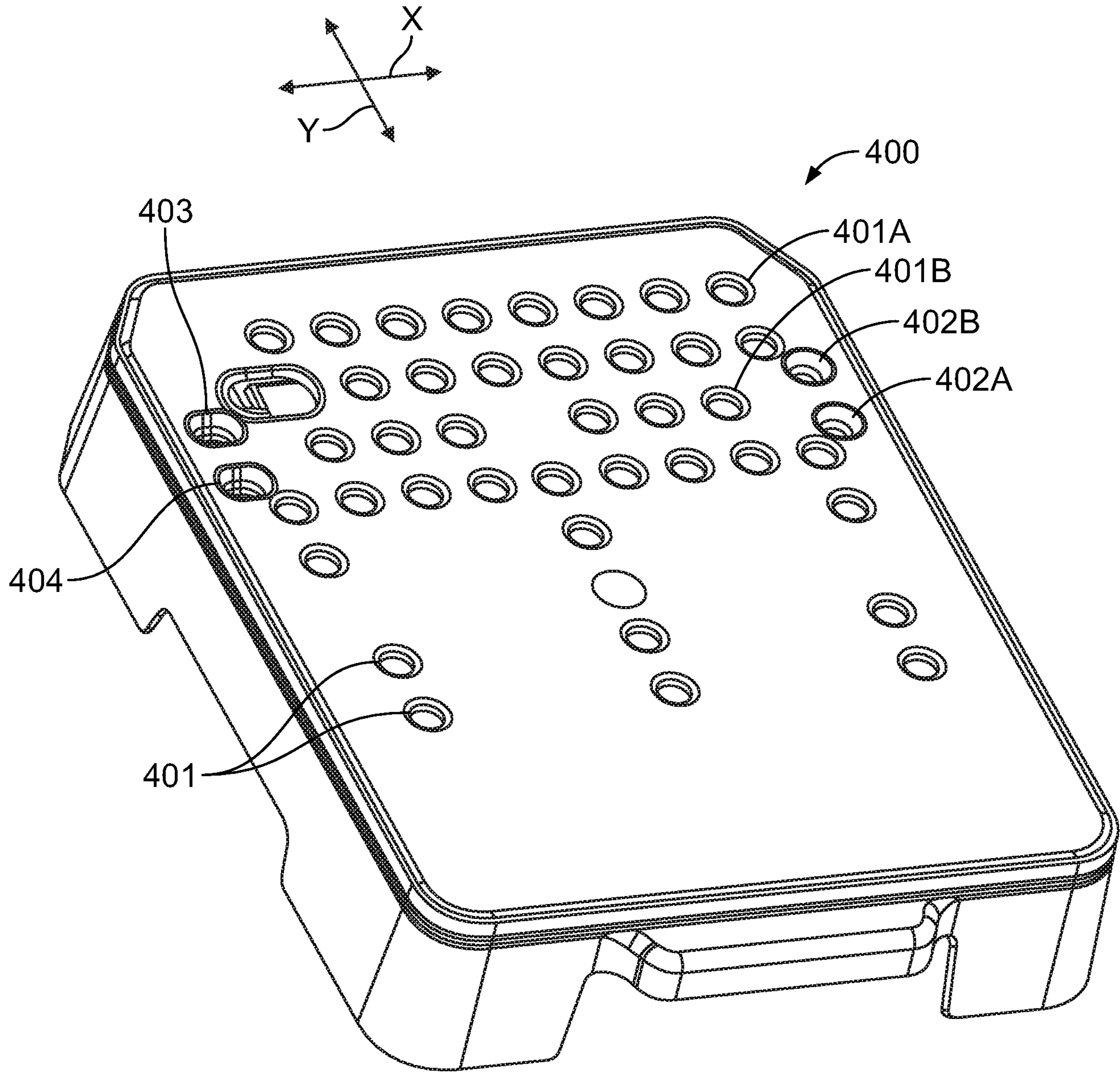


FIG. 3A

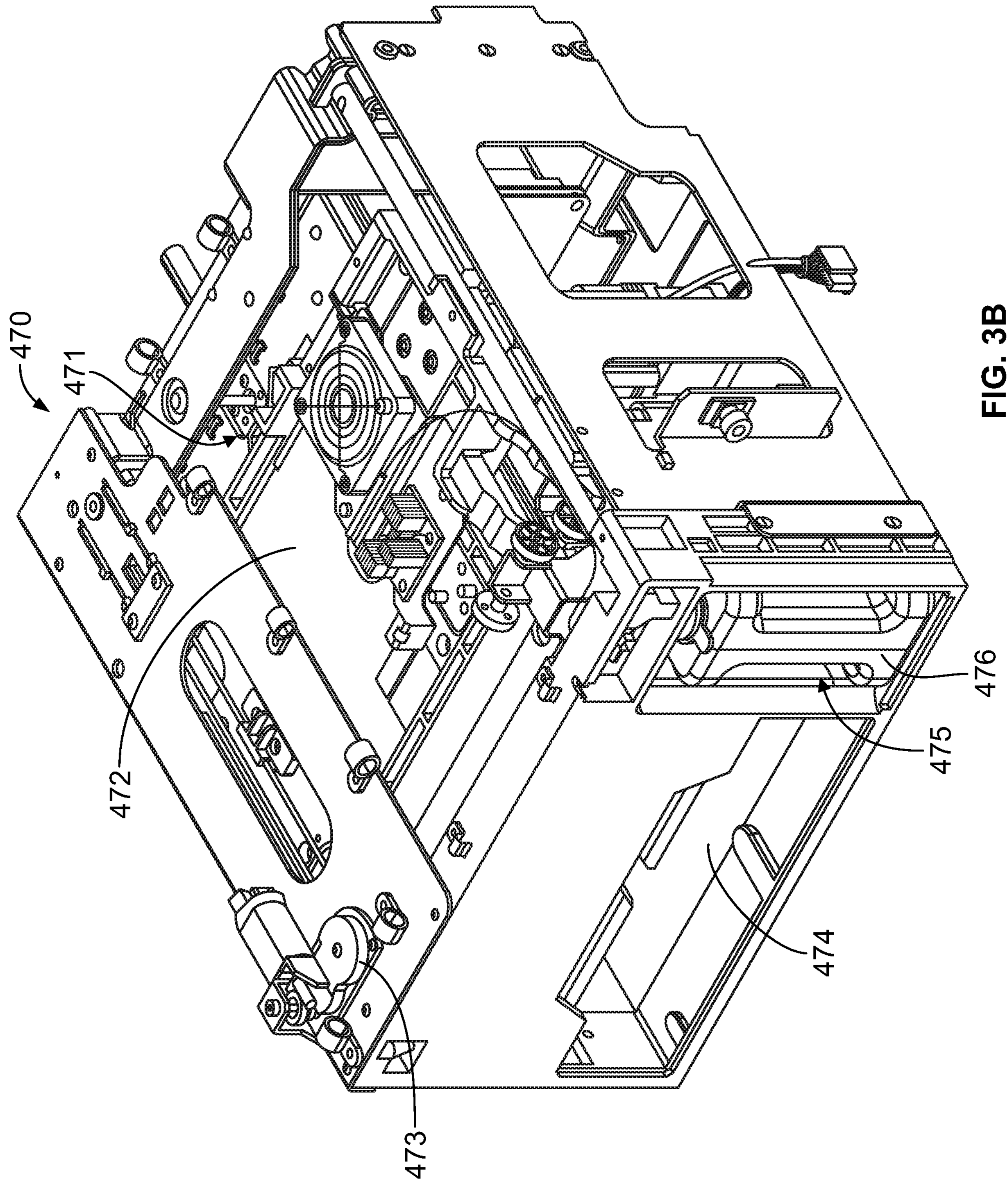


FIG. 3B

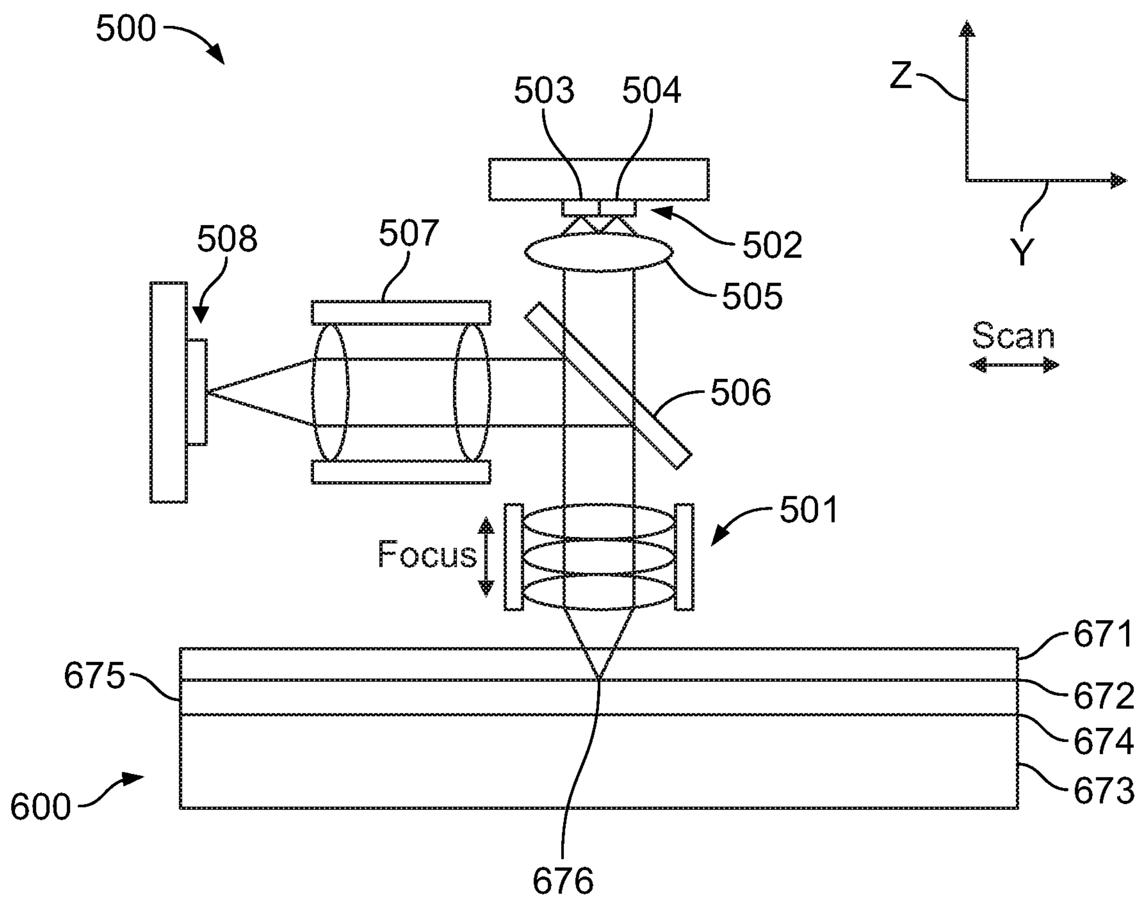
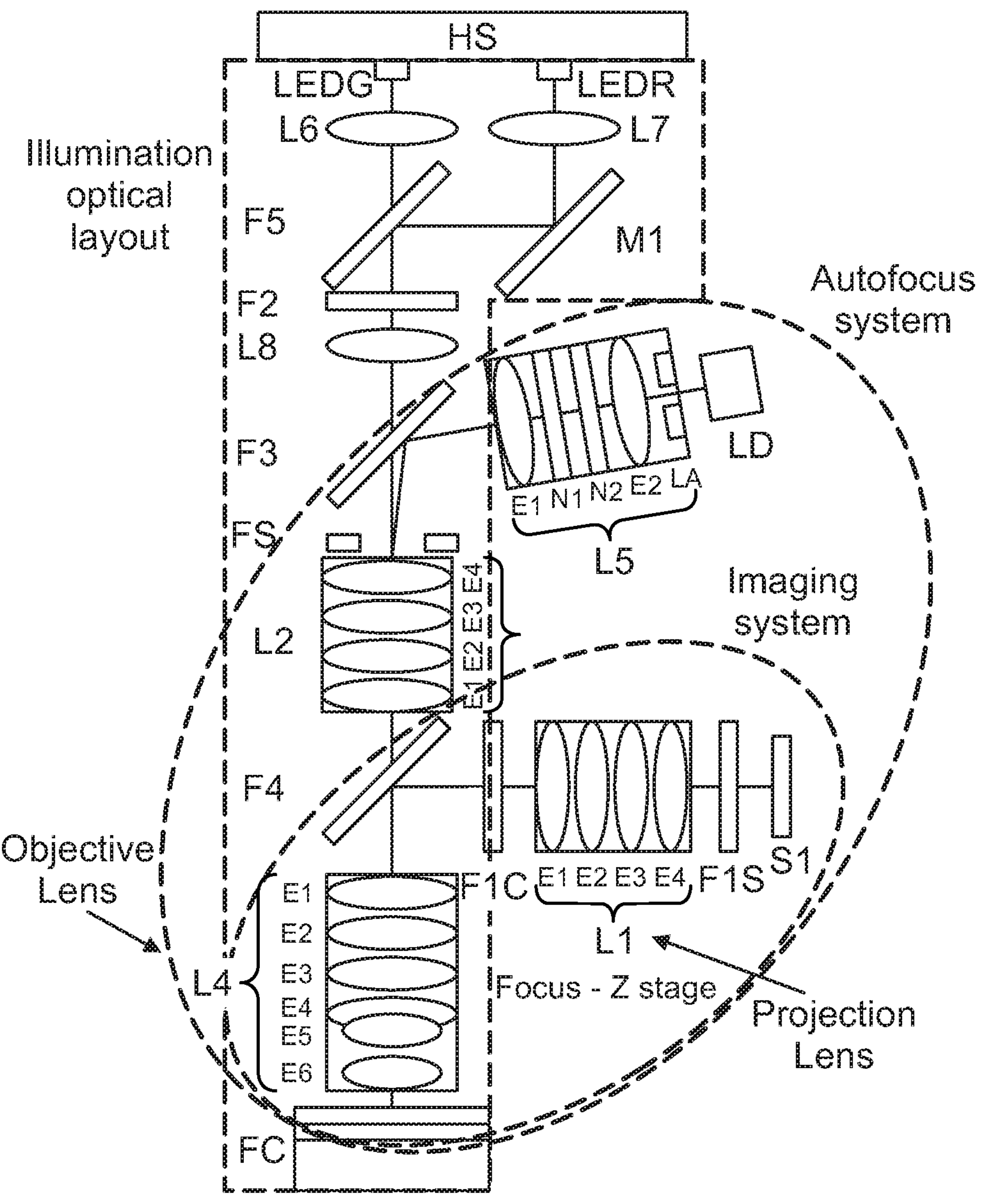


FIG. 4A



Identifier	Description
F1S	Emission filter (square)
F1C	Emission filter (circular)
F2	Excitation filter
F3	Laser diode beamsplitter
F4	Excitation/emission dichroic
F5	Combine dichroic
FC	Flowcell
FS	Excitation field stop
HS	LED heat sink
L1	Emission projection lens group
L2	Excitation projection lens group
L4	Objective lens group
L5	Laser diode coupling lens group
L6	Green LED collector lens
L7	Red LED collector lens
L8	LED field lens
LA	Laser diode aperture
LD	Laser diode
LEDG	Green LED die
LEDR	Red LED die
M1	LED fold mirror
N1	Neutral density filter 1
N2	Neutral density filter 2
S1	CMOS image sensor

FIG. 4B

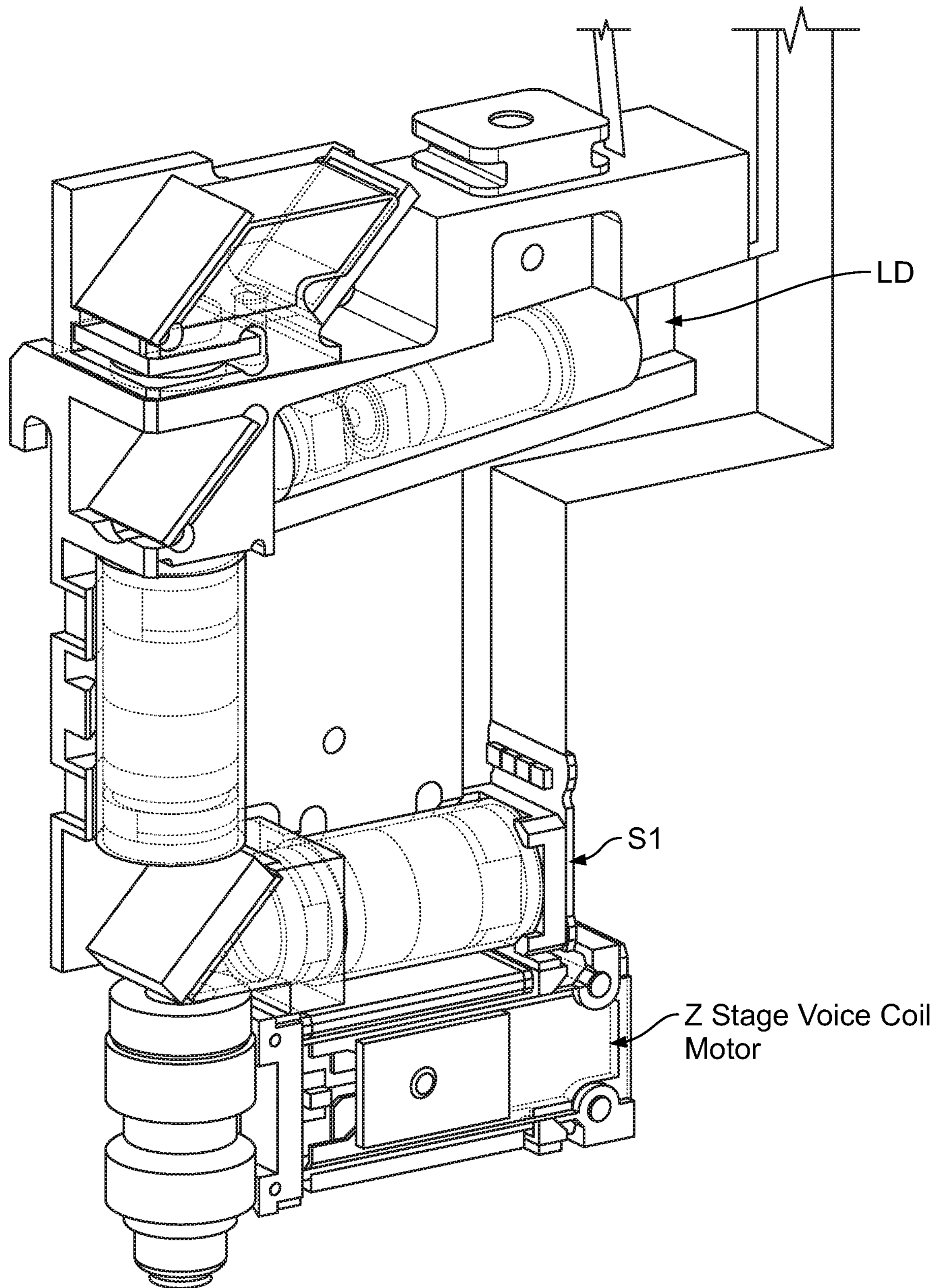


FIG. 4C

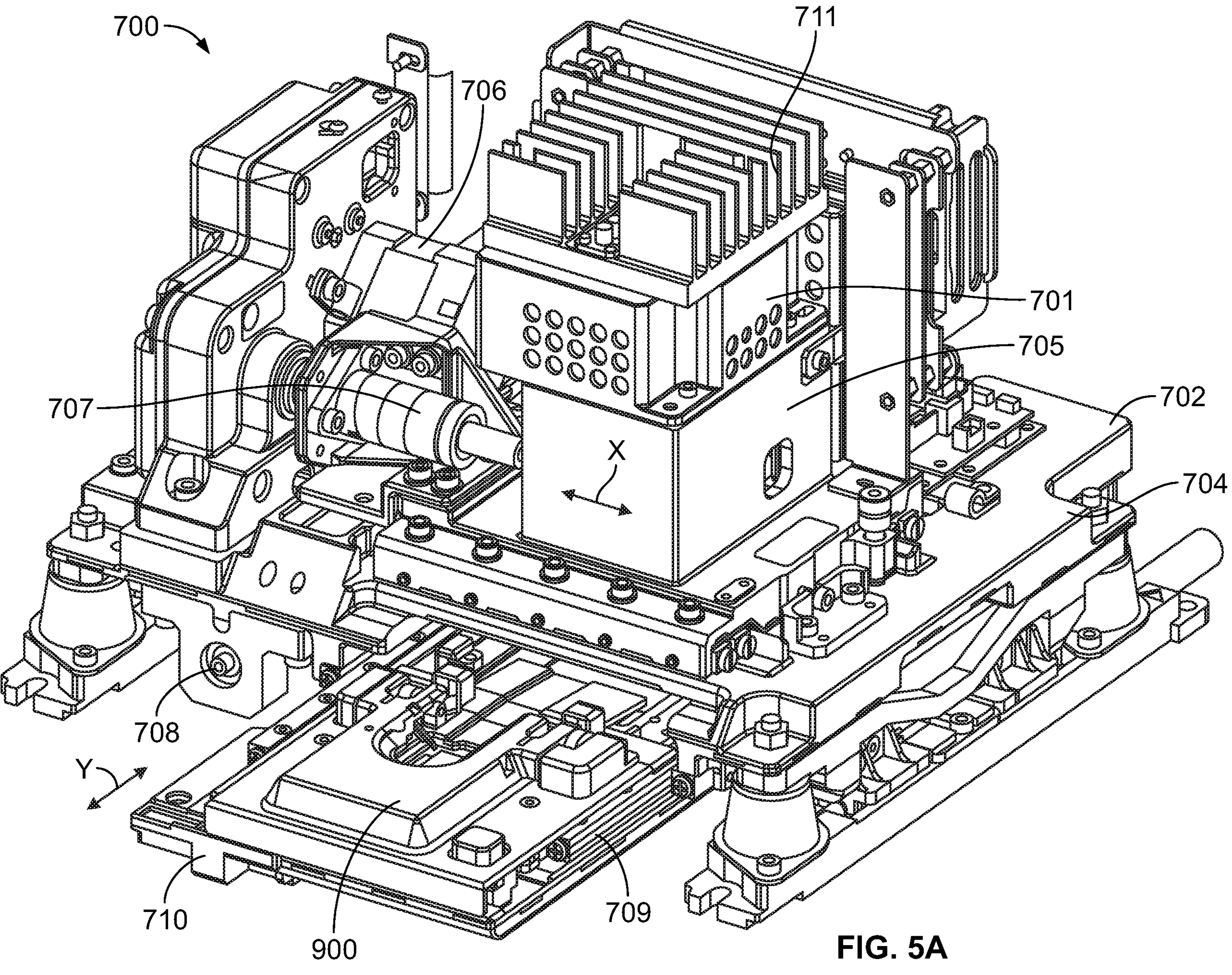


FIG. 5A

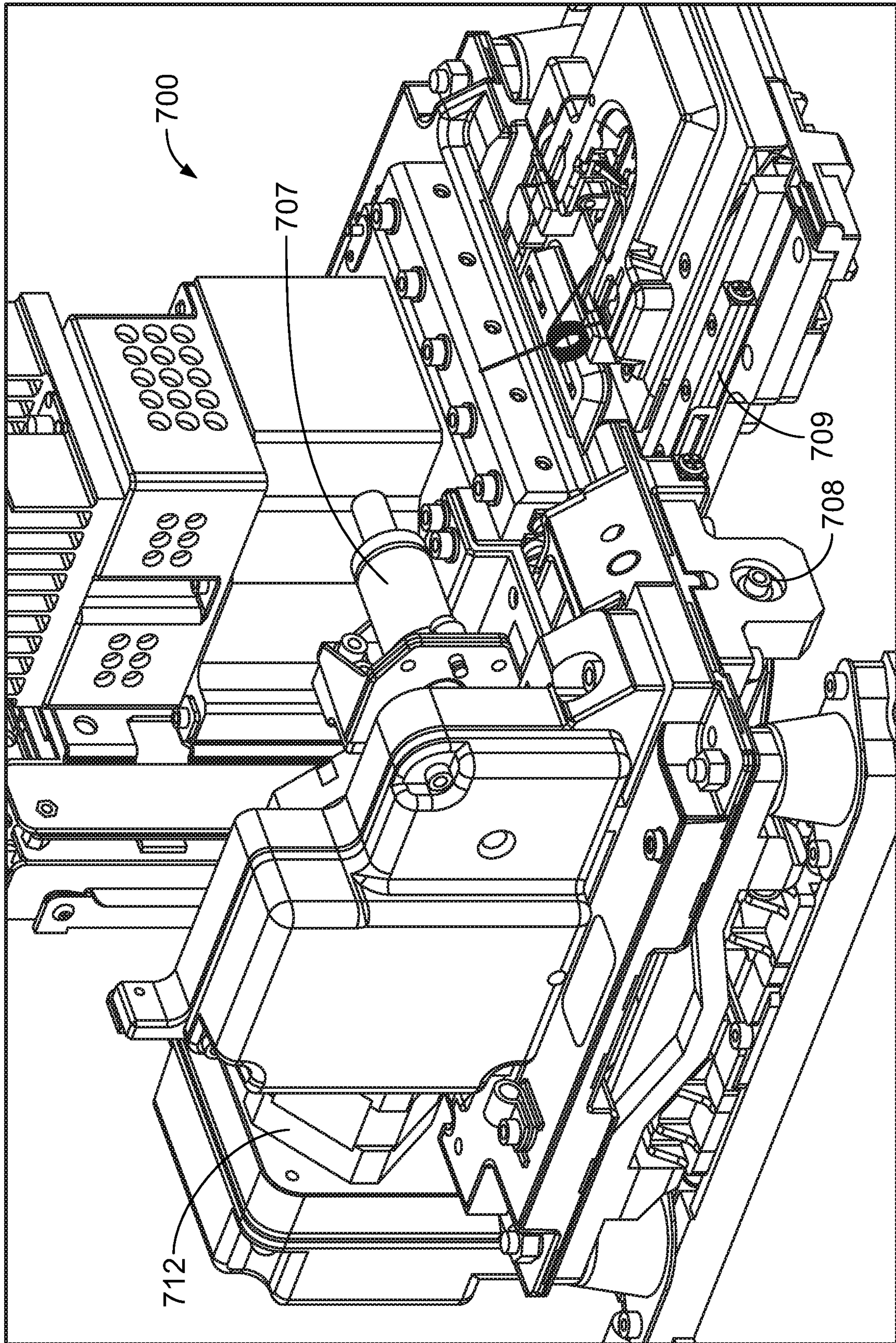


FIG. 5B

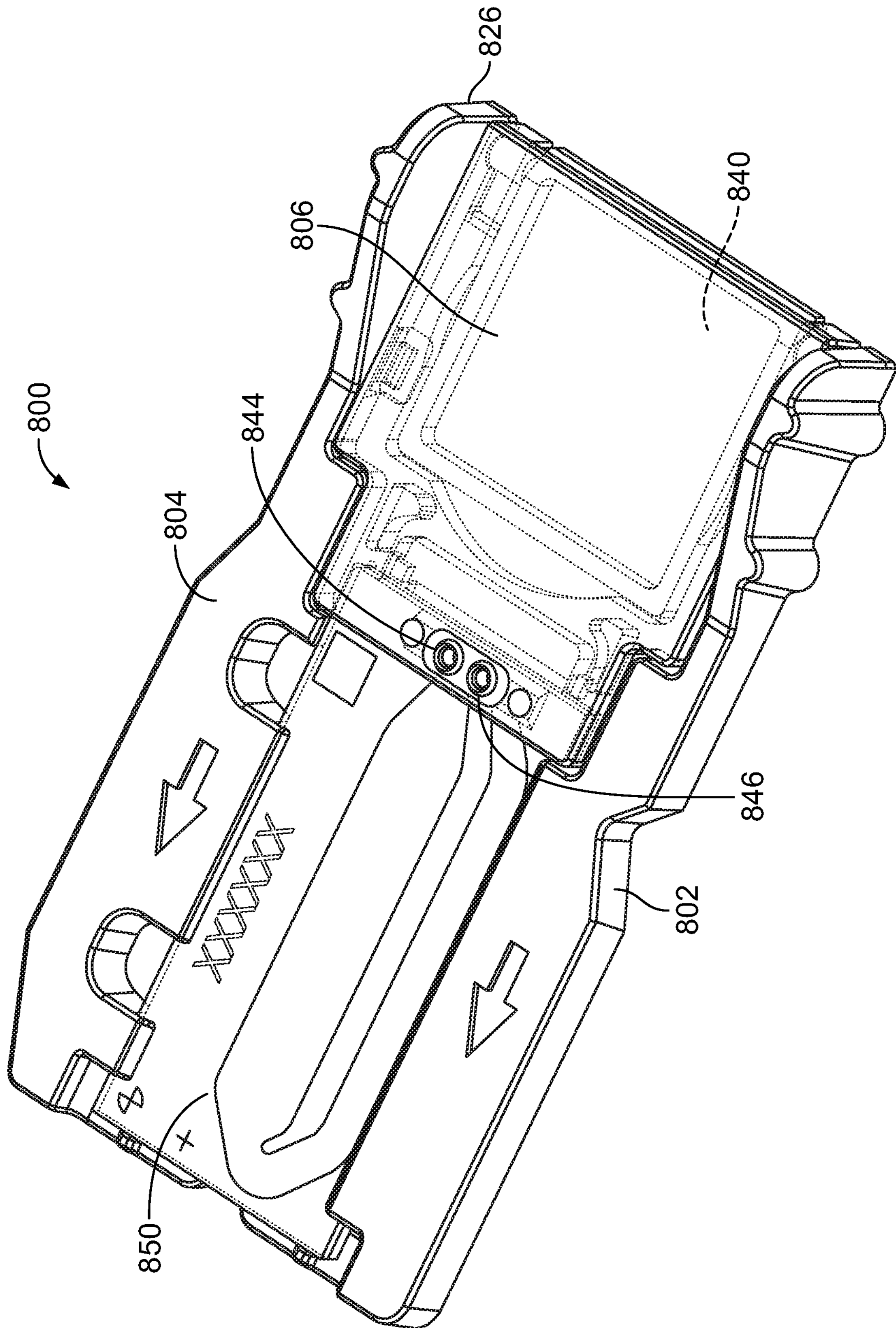


FIG. 6

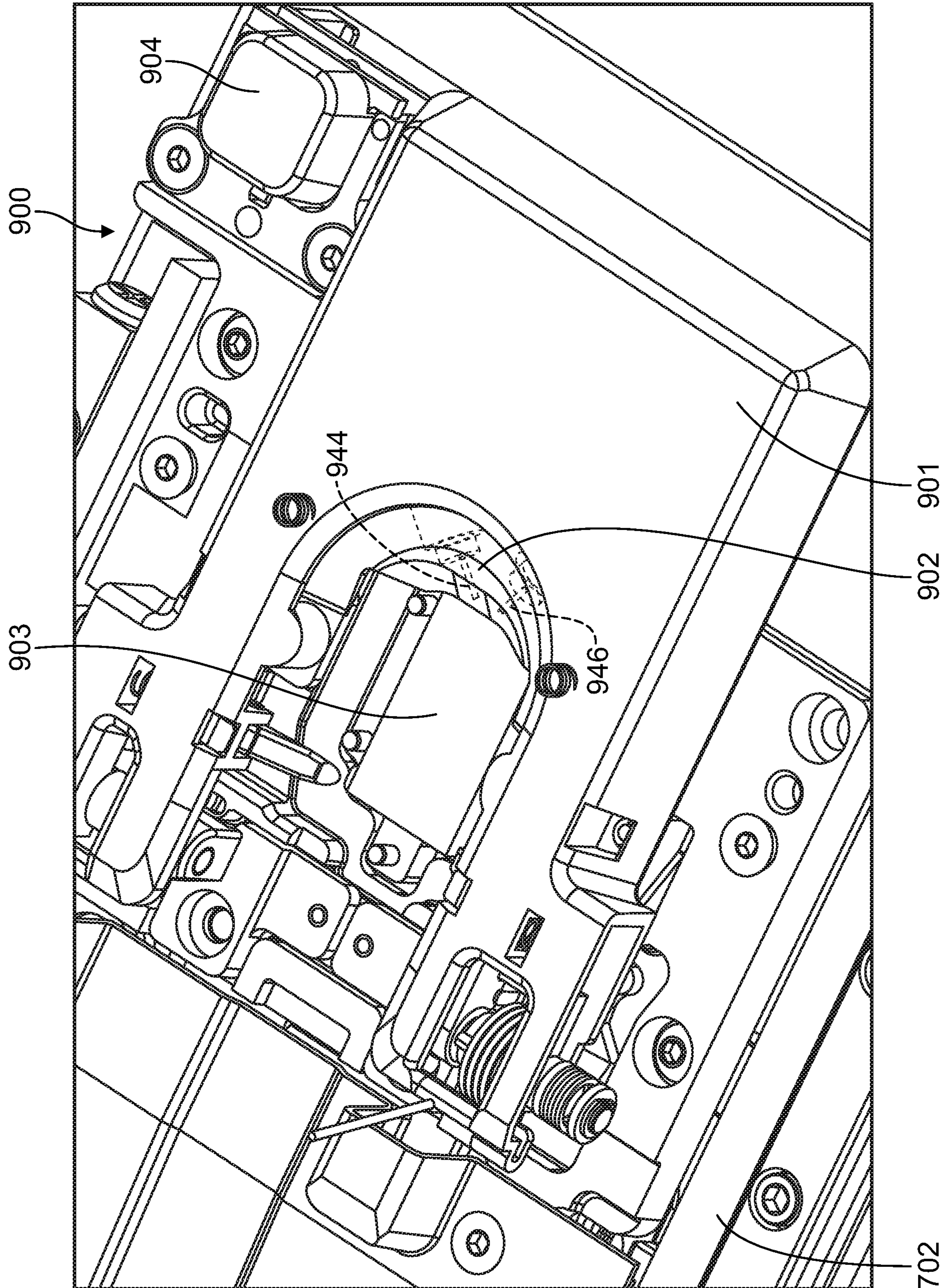


FIG. 7A

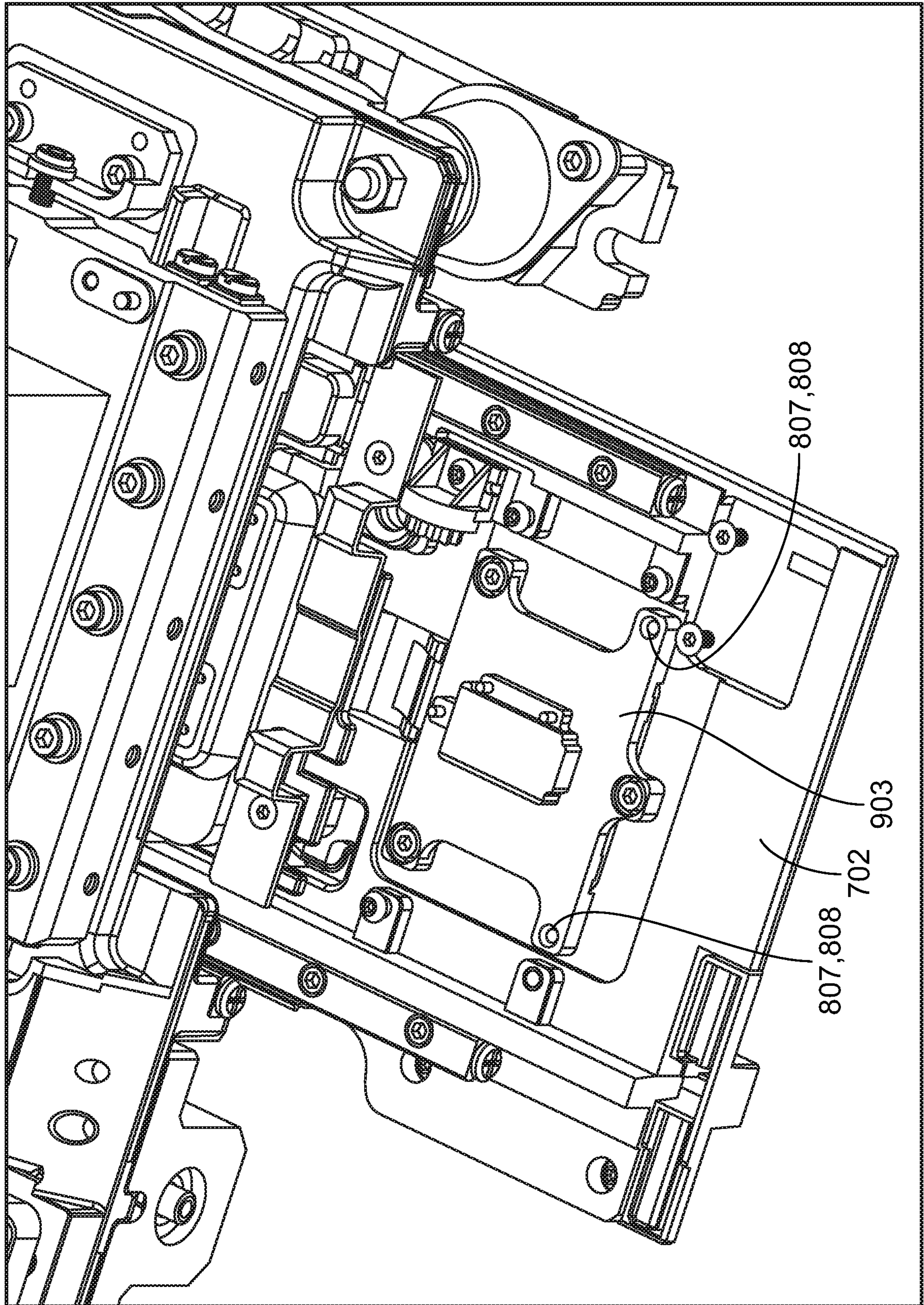


FIG. 7B

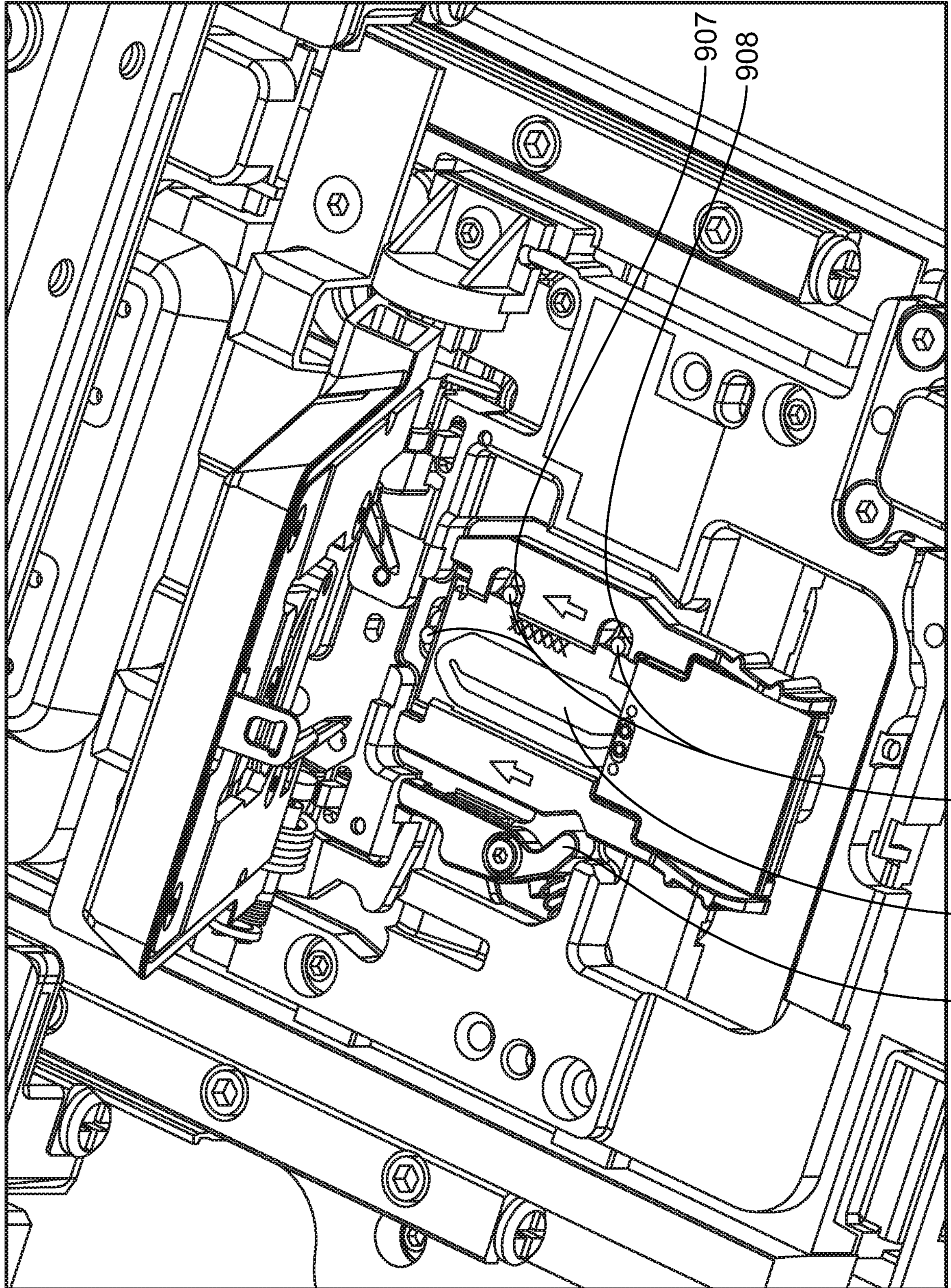


FIG. 7C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/012822

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/64
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N B01L C12Q

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/045234 A1 (STONE MICHAEL [US] ET AL) 12 February 2015 (2015-02-12) paragraphs [0028] - [0033], [0046]; figures 1-3,9,10	1-40
Y	US 2013/260372 A1 (BUERMANN DALE [US] ET AL) 3 October 2013 (2013-10-03) paragraphs [0031], [0046] - [0051], [0055], [0057], [0065], [0068]; figures 2,3,6,7	1-40
Y	AU 2014 259 551 A1 (ILLUMINA INC) 27 November 2014 (2014-11-27) paragraphs [0112], [0142], [0158] - [0161], [0178] - [0180]; figures 9,10, 12, 13, 16	1-40

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2017	13/04/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Meacher, David

1

International application No
PCT/US2017/012822

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/031596 A1 (ILLUMINA INC [US]) 5 March 2015 (2015-03-05) page 13, line 25 - page 14, line 31 -----	24

1

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/012822

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CN 204198724 U	11-03-2015
		DE 202014006405 U1	08-12-2014
		EP 3030645 A1	15-06-2016
		JP 3193848 U	23-10-2014
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		AU 2016222420 A1	22-09-2016
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