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(54) Title: A FLOW CYTOMETER DEVICE

(57) Abstract: A flow cytometer device to count human blood cells is disclosed. The flow cytometer device may include an optical block and a beam shaped optic assembly configured to be coupled to the optical block. The flow cytometer device may further include a forward scatter device configured to filter light rays other than those emitted by the light source, a side scatter device to split a polarized beam into non-polarized beam, and one or more fluorescence devices with an emission filter of defined wavelength in the visible spectrum. The flow cytometer device may further include a flow cell including a nozzle built into the flow cell, such that the nozzle is configured to transfer the fluid sample via the flow cell.

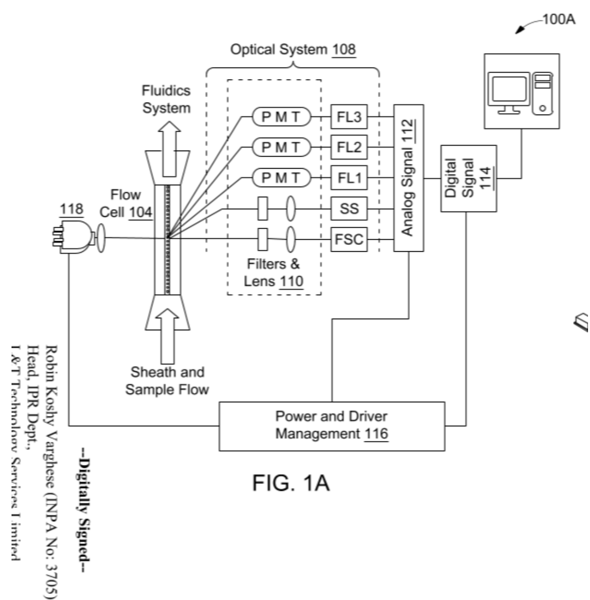

FIG. 1A

FORM 2

THE PATENTS ACT 1970
(39 OF 1970)
&
The Patent Rules, 2003

Complete Specification

(See Section 10 and Rule 13)

1. TITLE OF THE INVENTION

A FLOW CYTOMETER DEVICE

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3. PREAMBLE TO THE DESCRIPTION

COMPLETE

The following specification describes the invention and the manner in which it is to be performed

DESCRIPTION

Technical Field

[001] This disclosure relates generally to a hematology instrument and more specifically to a flow cytometer of a hematology instrument with a modular configuration.

BACKGROUND OF THE INVENTION

[002] Flow cytometry is a technique for counting, examining, and sorting microscopic particles suspended in a stream of fluid, for example blood. Flow cytometry allows simultaneous, multiparametric analysis of the physical and/or biochemical characteristics of single cells flowing through a detection apparatus. Fluidics, optics, and electronics are three main components of a flow cytometer used for hematology applications. The fluidics system of the flow cytometer oversees transporting samples from the sample tube to the flow cell, where they are analyzed. Further, excitation light sources, lenses, and optical filters may be used to collect and move wavelengths of light around the flow cytometer. Electronics component of the flow cytometer converts light signals into electronic signals (voltages) and perform data analysis.

[003] However, the flow cytometers are susceptible to significant performance degradation during operation, even with optical components firmly secured in place, due to ambient vibrations, mechanical shocks. Further, the flow cytometers are susceptible to significant loss of performance, when used to detect extremely small particles, such as platelet cells. Maintaining a high level of performance may necessitate frequent and costly flow cytometer servicing. Furthermore, significant degradation of optical element alignment and damage to fragile equipment may necessitate extensive servicing of the flow cytometer. Most fluidic systems are made up of parts that must be maintained on a regular basis. It is necessary to ensure that air bubbles are not present in the fluidic lines and filters.

SUMMARY OF THE INVENTION

[004] In an embodiment, a flow cytometer device to count human blood cells is disclosed. The flow cytometer device may include an optical block, a beam shaped optic assembly configured to be coupled to the optical block. The flow cytometer device may further include a forward scatter device configured to filter light rays different from the light rays emitted by the light source, for example a Laser, a side scatter device to split a polarized beam to non-polarized beam and one or more fluorescence device with an emission filter of defined wavelength in the visible spectrum.

BRIEF DESCRIPTION OF THE DRAWINGS

[005] The accompanying drawings, which are incorporated in and constitute a part of this disclosure, illustrate exemplary embodiments and, together with the description, serve to
5 explain the disclosed principles.

[006] **FIG. 1A** illustrates a schematic diagram of a flow cytometer device of a hematology instrument, in accordance with an embodiment of the present disclosure.

[007] **FIG. 1B** illustrates a perspective view of the flow cytometer device, in accordance with an embodiment of the present disclosure.

10 **[008]** **FIG. 1C** illustrates an exploded view of the flow cytometer device, in accordance with an embodiment of the present disclosure.

[009] **FIG. 2A** illustrates an exploded view of a beam shaping optics (BSO) device and a flow cell of the flow cytometer device, in accordance with an embodiment

[010] **FIG. 2B** illustrates a light ray diagram of the flow cytometer device, in accordance
15 with an embodiment of the present disclosure.

[011] **FIG. 2C** illustrates a schematic diagram of a forward scatter device (FSC) and a side scatter device (SSC) of the flow cytometer device showing diversion of the light rays, in accordance with an embodiment of the present disclosure.

[012] **FIG. 3A-3B** illustrate different planned views of travel of light via the BSO device of
20 the flow cytometer device, in accordance with an embodiment of the present disclosure.

[013] **FIG. 4A** illustrates a scenario of scattering and diverting of light beam towards the SSC device, in accordance with an embodiment of the present disclosure

[014] **FIG. 4B** illustrates a scenario of scattering and diverting of light beam towards the fluorescence devices (FL), in accordance with an embodiment of the present disclosure

25 **[015]** **FIG. 4C** illustrates a scenario depicting the intensity of light at the SSC device, in accordance with an embodiment of the present disclosure

[016] **FIG. 5A** illustrates a perspective view of the (assembled) flow cytometer device, in accordance with an embodiment.

[017] **FIG. 5B** illustrates a magnified sectional view of the flow cytometer device, in
30 accordance with an embodiment.

[018] **FIG. 5C** illustrates another perspective view of the flow cytometer device along with a housing in which the flow cytometer device is secured, in accordance with an embodiment.

[019] **FIG. 6A** illustrates a perspective view of a conventional flow cell.

[020] **FIG. 6B** illustrates a perspective view of a flow cell, in accordance with an embodiment of the present disclosure.

[021] **FIG. 6C** illustrates a partial view of an (unassembled) integrated silica manifold and flow cell showing the fitting of the flow cell with the silica manifold, in accordance with an embodiment of the present disclosure.

[022] **FIG. 6D** illustrates another view of the (assembled) flow cytometer device showing fitting of the flow cell with other components, in accordance with an embodiment of the present disclosure.

[023] **FIG. 7A** illustrates a perspective view of the (assembled) flow cytometer device, in accordance with an embodiment of the present disclosure.

[024] **FIG. 7B** illustrates a perspective view of the (assembled) flow cytometer device with a housing, in accordance with an embodiment of the present disclosure.

[025] **FIGS. 8A-8C** illustrates various exploded views of detector channels, in accordance with some embodiment of the present disclosure.

DETAILED DESCRIPTION OF THE DRAWINGS

[026] Exemplary embodiments are described with reference to the accompanying drawings. Wherever convenient, the same reference numbers are used throughout the drawings to refer to the same or like parts. While examples and features of disclosed principles are described herein, modifications, adaptations, and other implementations are possible without departing from the spirit and scope of the disclosed embodiments. It is intended that the following detailed description be considered as exemplary only, with the true scope and spirit being indicated by the following claims. Additional illustrative embodiments are listed below.

[027] **FIG. 1A** illustrates a schematic diagram 100A of a flow cytometer device, in accordance with an embodiment of the present disclosure. **FIG. 1B** illustrates a perspective view 100B of the flow cytometer device, and **FIG. 1C** illustrates an exploded view 100C of the flow cytometer device, in accordance with some embodiments of the present disclosure.

[028] As shown in the FIG. 1A, in one embodiment, the flow cytometer device may include a laser light source 118, a flow cell 104, a fluidic system, and an optical system 108. The optical system 108 may include one or more photomultiplier tubes, and a combination of filters and lenses 110. The flow cytometer device may further include one or more florescence detectors (FL1, FL2, FL3), a side scattering device, a forward scattering device, a signal collector, a power and driver management 116, an analog to digital convertor and a computer system.

[029] It should be noted that a laser diode (light source 118) may direct a light beam into viewing zone of the flow cytometer through which sample fluid carrying particles flows. As shown in the FIG. 1A, the sample fluid may be made to pass through a viewing zone of fluid sheath flow inside the flow cell 104 such that only one cell may pass the laser light at a time.

5 **[030]** The optical system of the flow cytometer may include excitation optics and collection optics. The excitation optics may include the laser and lenses that are used to shape and focus the laser beam. The collection optics may include collection lens to collect the light, emitted after the particle interacts with the laser beam and with the system of optical mirrors that diverts the specified wavelengths of the collected light to the designated optical detectors. One of the 10 detectors may be positioned in front of the laser beam to capture forward scattered light from the cells, and another detector may be positioned to the sides to measure the amount and intensity of light scattered in each direction along with fluorescent labels to detect different cells or components.

[031] Forward scattered light is proportional to the size of the particle, with larger particles 15 producing more forward scattered light. As will be appreciated by those skilled in the art, side-scattered light indicates granularity, with highly granular cells producing more side-scattered light than cells with low granularity. After the cell or particle passes through the laser light, the rays emitted on the side and the fluorescence signals are directed to the photomultiplier tubes (PMTs), and a photodiode collects the signals. Once the light signal strikes one side of the PMT 20 or the photodiode, they are converted into a relative number of electrons that are multiplied to create a more significant electrical current. The greater the fluorescence intensity, the greater the voltage pulse. The electrical current moves to the amplifier and is converted to a voltage pulse. The highest point of the pulse is achieved when the particle strikes the center of the beam (maximum amount of scatter or fluorescence is achieved). An Analog-to-Digital Converter 25 (ADC) then converts the voltage pulse (analog signals 112) to a digital number (digital signals 114) managed by power and driver management 116, which may be further executed by a computer system.

[032] Referring to **FIG. 2A**, another exploded view 200A of a flow cytometer device (corresponding to the flow cytometer device as shown in FIGS. 1A-1C) is illustrated, in 30 accordance with an embodiment of the present disclosure. In an embodiment, the flow cytometer device may include a main optical block 224. The flow cytometer device may further include a beam shaping optics (BSO) device 238, a forward scatter device (FSC) 222, a side scatter device (SSC) 208, and one or more fluorescence devices 204. It should be noted that each of the BSO device 238, the forward scatter device 222, the side scatter device 208, and

the fluorescence devices 204 may have modular configurations, and therefore may be configured to be removably coupled to the optical block 224.

[033] For example, the BSO device 238 (also, referred to as BSO assembly 238) may be configured to be coupled to the optical block 224, for example using one or more M3 shoulder screws. A plug 240 may be used to seal the optical block 224. The BSO device 238 may include a light source for emitting a beam of light. The BSO device 238 may further include two or more optical elements for scattering and narrowing down the beam of light to the required size of a flow cell 228. The BSO device may further include a polarizer and a condenser.

[034] The light beam from the BSO device 238 may be directed towards a fluid sample (for example, blood sample to be inspected). As such, the fluid sample may be drawn into a fluidic system via an inlet tube 232. The inlet tube 232 may be secured to a silicon manifold 230 using one or more M3 button head screws 234. The flow cytometer device may include a flow cell assembly which may include the silicon manifold 230. The fluid sample may be pumped through a flow cell 228 (also known as the flow cell 228), followed by the flow cell locator 226. The fluid sample may be combined with a stream of sheath fluid at a higher differential pressure, and the fluid sample following a laminar flow upon reaching an interrogation point the fluid sample may either flow into a waste line 214 (also, referred to as nozzle 214) or a cell sorter. The fluidic system of the flow cytometer device may be designed to deliver cells in an orderly single file stream to the interrogation point so that only one cell passes through at a time.

[035] The silicon manifold 230 may be coupled to the optical block 224. As such, the optical block 224 acts like a platform that enables the fluid sample running through the flow cell 228 to be subjected to the light beam from the BSO device 238. The light beam is scattered upon passing through the flow cell 228. Further, the optical block 224 allows other inspection devices, such as the forward scatter device (FSC) 222, the side scatter device (SSC) 208, and the fluorescence devices 204 to be individually removably coupled to the optical block 224. The forward scatter device (FSC) 222, the side scatter device (SSC) 208, and the fluorescence devices 204 may receive the scattered light. The FSC 222 may include a filter configured to filter all other wavelengths other than source wavelength, and an obstruction to block 0 degrees rays from the light source. This is further explained in conjunction with FIG. 2B.

[036] Referring now to **FIG. 2B**, another schematic diagram 200B of the flow cytometer device is illustrated. As shown in the FIG. 2B, a light beam from a light source assembly 242 may be directed at a flow cell assembly 244. The light beam may scatter upon passing through

the fluid sample flowing through the flow cell assembly 244. The scattered light may then be received by a scattering detection assembly 246.

[037] The scattering detection assembly 246 may include several detectors carefully placed around a point where the light beam interacts with the fluid sample. One of these detectors, i.e. the forward scatter device (FSC) 222 is in line with the light beam and is used to measure forward scattered light. The forward scatter device 222 may include a ring detector printed circuit board (PCB) 218. The forward scatter device 222 may be configured to be removably coupled to the optical block 224. For example, the forward scatter device may be coupled to the optical block 224 via M3 shoulder screws 220. Another detector, i.e., the side scatter device (SSC) 208 may be placed transverse to the forward scatter device 222 and may be used to measure side scattered light. The side scatter device 208 may be configured to split a polarized beam to non-polarized beam. To this end, the side scatter device 208 may further include two detectors: a polarized side scatter (PSS) 210 and a depolarized side scatter (DSS) 206.

[038] A series of dichroic mirrors (DM) and emission filters 212 may route the light signals to the detectors. Fluorescence signals, which are frequently weak, may be detected by photomultiplier tubes (PMTs). A filter may be placed in front of the PMT, so that only a narrow range of wavelengths may reach the detector. The specificity of a detector for a specific fluorescent dye can be optimized. The fluorescently labelled cell components may get excited by the laser and emit light at a longer wavelength than the light source and then further the light so emitted may be detected by fluorescence detectors.

[039] When the light beam passes through the fluid sample, the side scattered beam maybe directed towards and collected by side scatter device 208. Similarly, fluorescence signals may get diverted to the one or more fluorescence devices (FL1, FL2, FL3) 204, as shown in **FIG. 2C** which shows another schematic diagram 200C of the flow cytometer device showing diversion of the light rays. As such, the fluorescence signals may get collected by a photodiode. The one or more fluorescence devices 204 may be configured to be removably coupled to the optical block 224, for example, using M3 shoulder screws 202.

[040] The photodiode may convert the light signal into a current, which may be recorded by the electronics system. The light entering the photodetectors (i.e. the PMT or the photodiode) may get converted into electrons and received by a computing device. The electrons may further get multiplied creating amplified current which may further transform into voltage pulses that may be processed by the computer system.

[041] Referring now to **FIG. 3A-3B**, different schematic views 300A, 300B of travel of light via the various modules of the flow cytometer device, the beam shaping optics (BSO) device

238 (polarized light source), the forward scatter device (FSC) 222, the side scatter device (SSC) 208, and the one or more fluorescence devices 204 are illustrated, in accordance with some embodiments of the present disclosure. As mentioned above, the beam shaping optic (BSO) device 238 may include a light source, an optical element for scattering the beam of light, and
5 an optical element for narrowing down the beam of light. Further, the BSO device 238 may be configured to be coupled to the optical block 224. The BSO device 238 may ensure that each individual beam is delivered to the flow cell in the correct dimensions and focus.

[042] The light source may be used to emit a beam of light. The scattered light may be collected with the help of a detectors placed in line with the laser path (on the opposite side of
10 the stream), i.e. the FSC device 222. The FSC device 222 may be equipped with a filter that filters all wavelengths other than the source wavelength, as well as obscuration to block 0 degree rays from the laser source. Many photons may pass through the cytoplasm because cells are translucent. When a photon collides with a cell organelle, it may be reflected at a greater angle than those generated by forward scatter. A second detector, perpendicular to the laser
15 path, i.e. the SSC device 208 may be used to collect light scattered in this way. The SSC device 208 may be equipped with an emission filter and a beam splitter to convert a polarized beam to a non-polarized beam. Fluorescence measurements at various wavelengths can provide quantitative and qualitative information about fluorophore-labeled cell surface receptors or intracellular molecules like DNA and cytokines. The fluorescence has a low intensity and is
20 uniformly radiated across the entire solid angle. As a result, fluorescence may be condensed by a condenser lens with a large numerical aperture and detected by an ultrasensitive photodetector known as the photomultiplier tube (PMT) detector or the fluorescence devices (FL1, FL2, FL3) 204, placed just behind the flow cell.

[043] Referring now to **FIG. 4A**, various views of the scattering of the light through the
25 flow cytometer are illustrated. FIG. 4A shows scenario 400A of scattering and diverting of light beam towards the SSC device 208. **FIG. 4B** shows a scenario 400B of scattering and diverting of light beam towards one of the fluorescence channel (FL1) 204. **FIG. 4C** shows a scenario 400C depicting the intensity of light at the SSC device 208.

[044] Simulations may be performed for evaluating the performance of the scattered light
30 from forward scatter to side scatter. The conditions of the simulation are described below:

1. Beam shaping optics till flow cell from laser source
2. Gaussian scatter was assumed, and the distribution is fixed at normal to axis for study purposes.

3. Solid spheres (bubbles) are used as blood cells for simulation purposes with modified surface properties.

4. Tracepro is used for fluorescence detection optic design

5. The intensity radiance loss in transmission is negligible from source to detector when no flow happens (ideal condition)

6. The focal length of the source is positioned just behind the flow cell

7. The beam width in the interrogation zone is maintained at 16μm

[045] Referring now to **FIG. 5A**, a perspective view 500A of the (assembled) flow cytometer device is illustrated, in accordance with an embodiment. Further, **FIG. 5B** illustrates a magnified sectional view 500B of the flow cytometer. **FIG. 5C** illustrates another perspective view 500C of the flow cytometer along with a housing in which the flow cytometer may be secured. As shown in the FIG. 5A-5B, one or more lenses may be assembled at the Fluorescence block (i.e. FL1, FL2, Fl3) which may then be mounted in the main optical block 224 using a fit tolerance. The fit design may ensure concentric and angular alignment. The housing tolerance may be used to manage the concentric alignment. One or more shoulder screws may keep angular tolerances. A single integrated optical block 224 may ensure the alignment of forward, side scatter, and fluorescence detectors. Tolerances may be maintained and controlled using this arrangement, results in an increased availability.

[046] Referring now to **FIG. 6A** a perspective view 600A of a conventional flow cell is illustrated. Referring to **FIG. 6B** a perspective view 600B of the flow cell 228 is illustrated. The circular profile of the flow cell 228 may simplify manufacturing and facilitate assembly and disassembly with the housing block. As will be understood, the flow cell 228 may be configured to receive a fluid sample pumped into the flow cell 228 and expose the fluid sample to the light beam emitted by the light source. The flow cell 228 may include a nozzle 602 (corresponding to the nozzle 214) build into the flow cell 228. The nozzle 602 may be configured to drain out fluid sample fluid after interrogation in the flow cell 228.

[047] Due to absence of edges (as compared to the conventional flow cell), the circular profile of the flow cell 228 also ensures uniform dispersion when compared to a cuboid structure. With a 30-degree angle, the nozzle design may be aligned with the assistance of top and bottom alignment bush to the silica manifold for easy assembly with the flow cell. The flow cell module and nozzle may be combined into a single block by direct welding with a local melting of glass interface.

[048] **FIG. 6C** illustrates a partial view 600C of the (unassembled) flow cytometer device showing the fitting of the flow cell 228 with the silica manifold 230. **FIG. 6D** illustrates another view 600D of the (assembled) flow cytometer device showing the fitting of the flow cell 228 with the other components. As shown in FIG. 6D, the nozzle 602 may be aligned with the
5 assistance of top and bottom alignment bush to the silica manifold for easy assembly with the flow cell. The flow cell 228 and the nozzle 602 may be combined into a single block by direct welding with a local melting of glass interface.

[049] As will be appreciated by those skilled in the art, conventionally, the nozzle is attached to the flow cell assembly during assembly. The nozzle is manufactured as a machined part with
10 high machining tolerance. The nozzle is then aligned to the flow cell and then glued to attain the alignment. However, in the current embodiment of the present disclosure, the nozzle is welded to the flow cell via the silica manifold to provide the alignment.

[050] Referring now to **FIG. 7A**, a perspective view 700A of the (assembled) flow cytometer is illustrated. Referring to **FIG. 7B**, a perspective view 700B of the (assembled) flow
15 cytometer with a housing is illustrated. It should be noted that calibration at a sub-assembly level may also be possible. The modular design may reduce reliance on optical base alignment. While integrating subsystems, there may be a reduction in complexity and assembly time. Service may be cut in half during the downtime.

[051] Referring now to **FIGS. 8A-8C.** FIG. 8A shows an exploded view 800A of the
20 forward scatter device (FSC), FIG. 8B shows an exploded view 800B of the side scatter device (SSC), and FIG. 8C shows an exploded view 800C of the fluorescence device (FL). As will be appreciated, the existing optic assemblies of the flow cytometer are glued to the base plate. However, the optic assemblies, for example of the fluorescence device (FL) 204 are modular in nature. The design for assembly (DFA) consideration may result in a significant reduction
25 in assembly time. The PMT, detector, and machined part may all be aligned with the optic housing.

[052] According to an embodiment, the modular nature of the optical assembly design allows for the seamless replacement of subassemblies/components while maintaining the required positional accuracy. The optical block assembly may be connected to all sub-
30 assemblies. The flow cell assembly including silica manifold/nozzle alignment design saves alignment time and may allow the entire sub assembly to be dismantled and easily replaced with another during a clog, in the flow. Furthermore, the modular design aids in the configuration of the fluorescence detection system, which may include the detector and the

beam splitter. For safety and to avoid interference from ambient light, the entire ray scatter may be concealed.

[053] According to an embodiment, a flow cytometer device may offer faster assembly and servicing, accurate self-alignment, rugged construction by design avoiding gluing. Another
5 significant benefit of flow cytometer device is that it causes lower cost of goods sold and lower levels of inventory to support the same field population.

[054] It is intended that the disclosure and examples be considered as exemplary only, with a true scope and spirit of disclosed embodiments being indicated by the following claims.

WE CLAIM:

1. A flow cytometer device comprising:

an optical block (224);

a beam shaping optics (BSO) device (238), wherein the BSO device (238) comprises;

a light source to emit a light beam;

at least one optical element to scatter the light beam; and

at least one optical element to narrow the light beam to a predetermined size;

a forward scatter device (222) configured to filter light rays different from the light rays emitted by the light source;

a side scatter device (208) to split a polarized beam to non-polarized beam; and

one or more fluorescence devices (204), each of the one or more fluorescence devices (204) comprising an emission filter of defined wavelength in the visible spectrum,

wherein each of the BSO device (238), the forward scatter device (222), the side scatter device (208), and each of the one or more fluorescence devices (204) is configured to be removably coupled to the optical block (224).

2. The flow cytometer device as claimed in claim 1, wherein the BSO device (238) comprises:

a polarizer; and

a condenser.

3. The flow cytometer device as claimed in claim 1, wherein the forward scatter device (222) comprises:

a filter configured to filter all other wavelengths other than source wavelength;

an obstruction to block 0 degrees rays from the light source.

4. The flow cytometer device as claimed in claim 1, wherein the side scatter (208) device comprises:

an emission filter; and

a beam splitter to split the polarized to non-polarized beam.

5. The flow cytometer device as claimed in claim 1, wherein each of the one or more fluorescence devices (204) comprises:

an emission filter of defined wavelength in the visible spectrum.

6. The flow cytometer device as claimed in claim 1, further comprising:

a flow cell (228) configured to:

receive a fluid sample pumped into the flow cell 228; and

expose the fluid sample to the light beam emitted by the light source.

7. The flow cytometer device as claimed in claim 7, wherein the flow cell (228) comprises:

a nozzle (602) build into the flow cell (228), wherein the nozzle (602) is configured to drain out fluid sample after interrogation in the flow cell (228).

Dated this 15th Day of July 2022

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ABSTRACT

A FLOW CYTOMETER DEVICE

A flow cytometer device to count human blood cells is disclosed. The flow cytometer device may include an optical block and a beam shaped optic assembly configured to be coupled to the optical block. The flow cytometer device may further include a forward scatter device configured to filter light rays other than those emitted by the light source, a side scatter device to split a polarized beam into non-polarized beam, and one or more fluorescence devices with an emission filter of defined wavelength in the visible spectrum. The flow cytometer device may further include a flow cell including a nozzle built into the flow cell, such that the nozzle is configured to transfer the fluid sample via the flow cell.

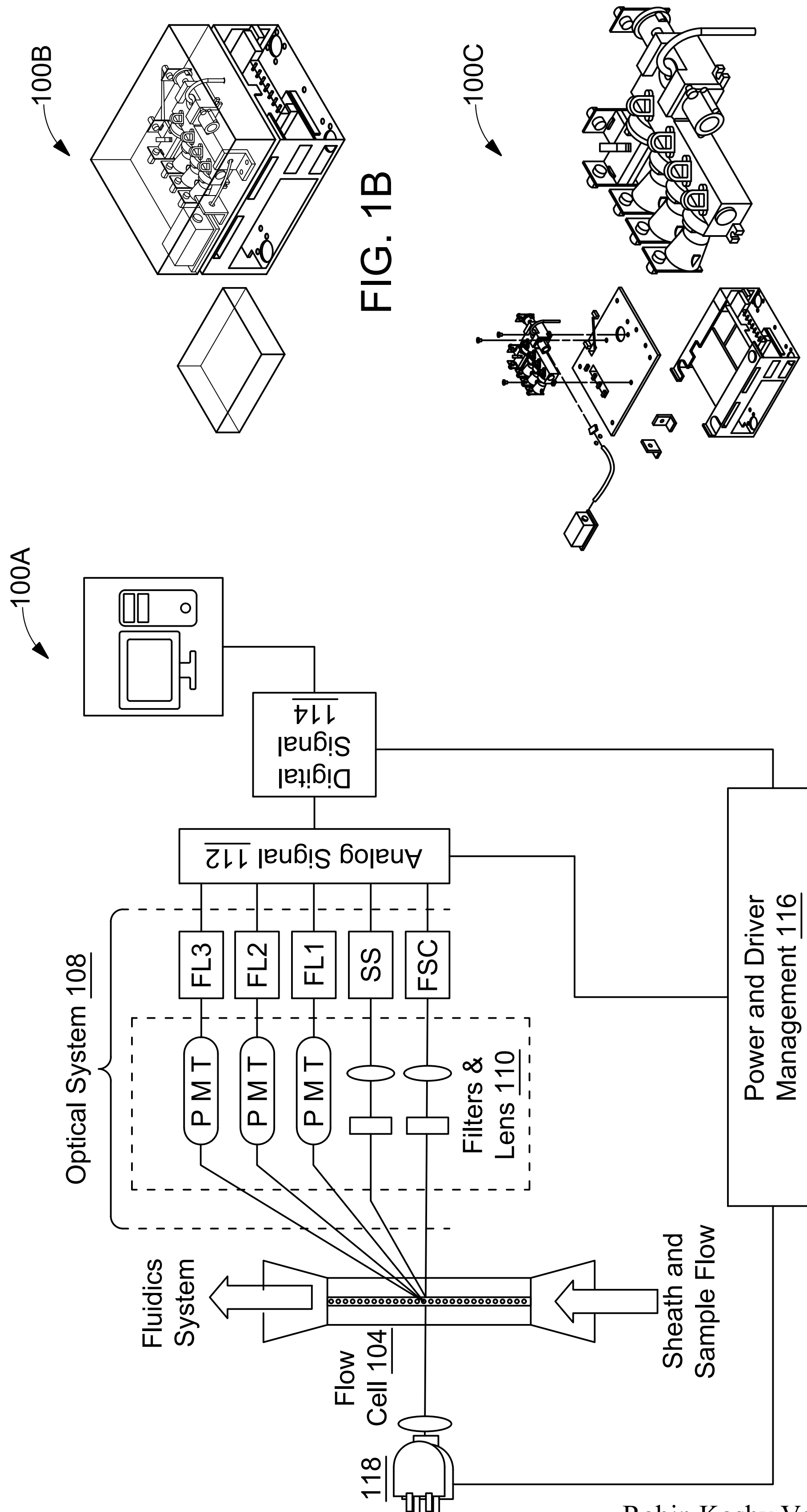

FIG. 1A

FIG. 1B

FIG. 1C

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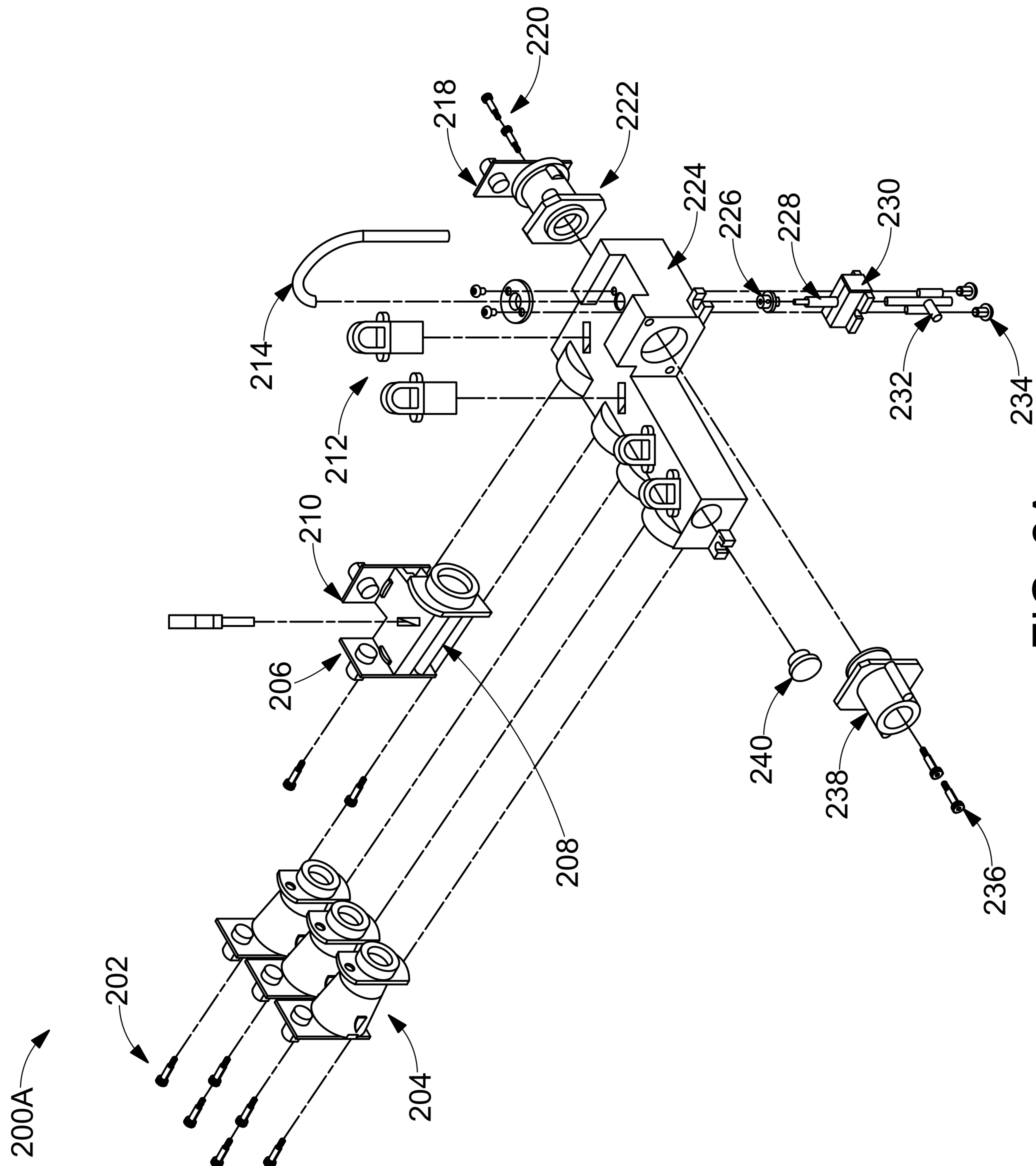

FIG. 2A

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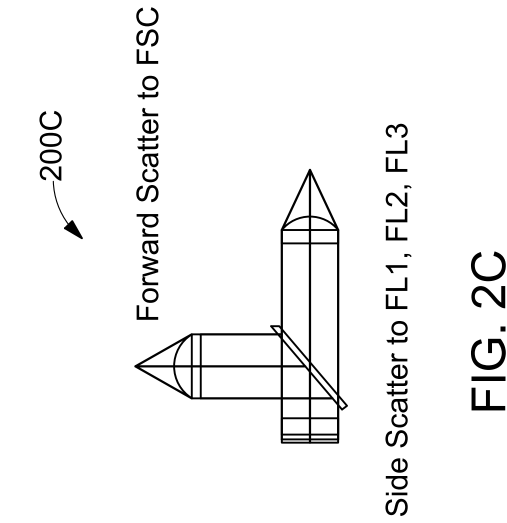

FIG. 2C

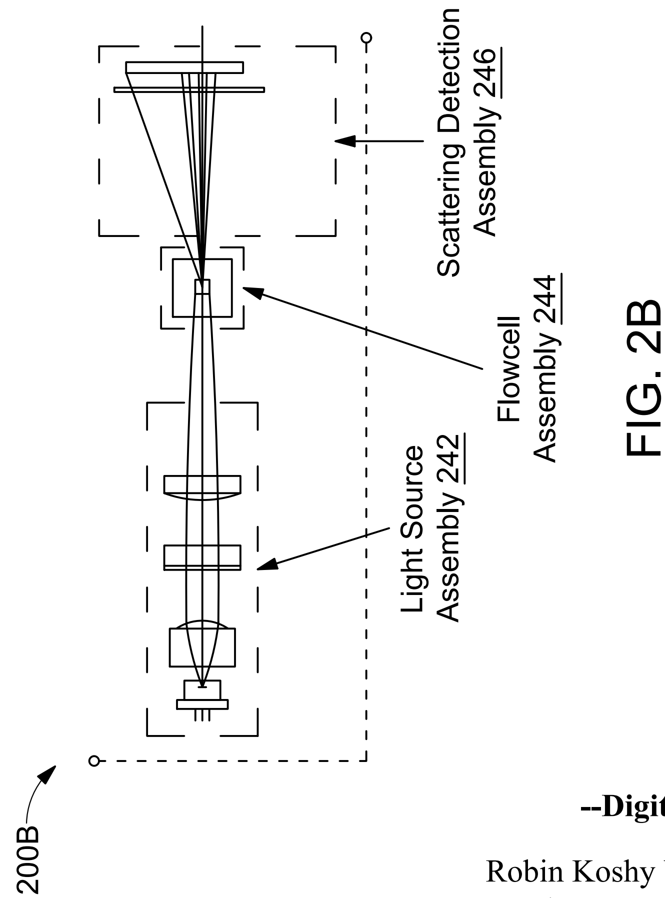

FIG. 2B

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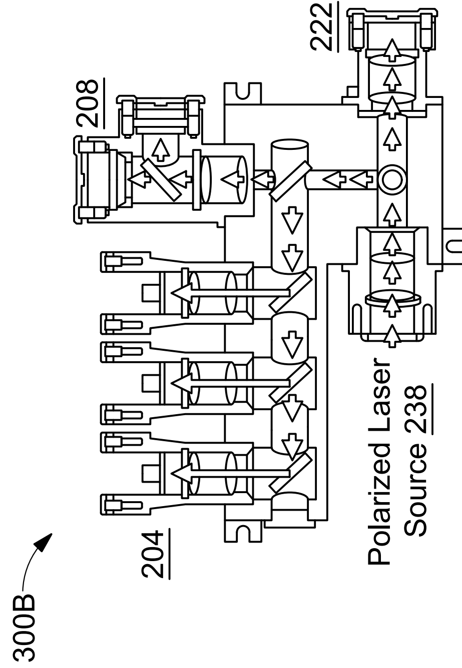

FIG. 3B

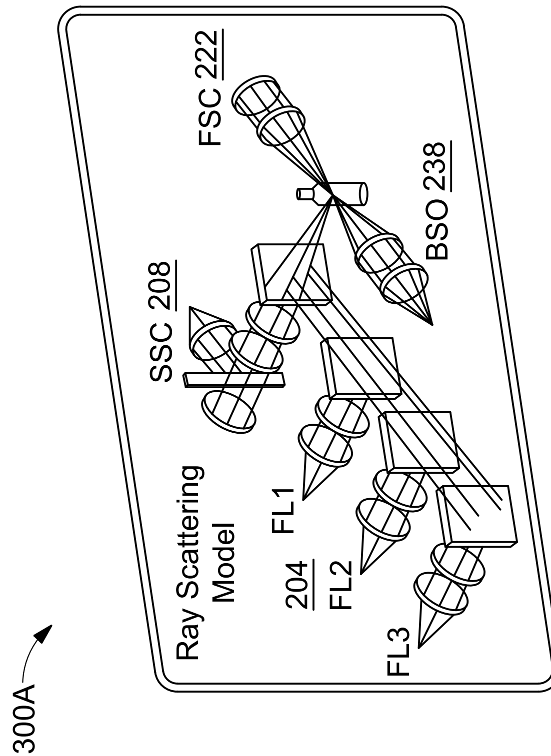

FIG. 3A

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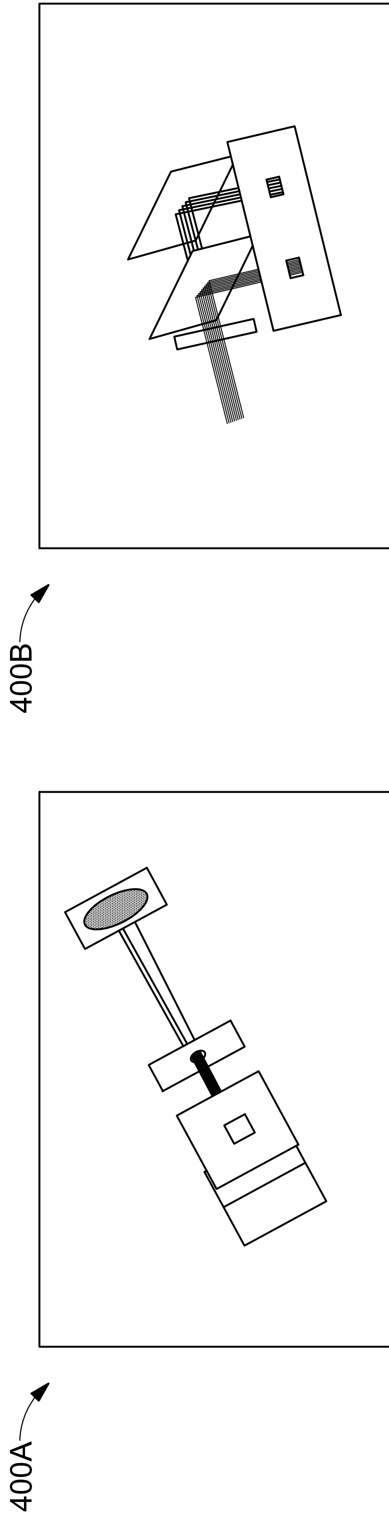


400A

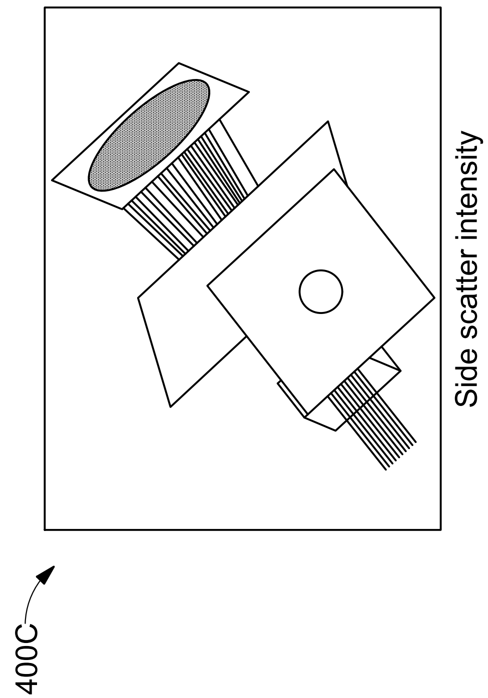
Forward to Slide scatter

FIG. 4A

400B

Side scatter to FL1 channel

FIG. 4B



400C

Side scatter intensity

FIG. 4C

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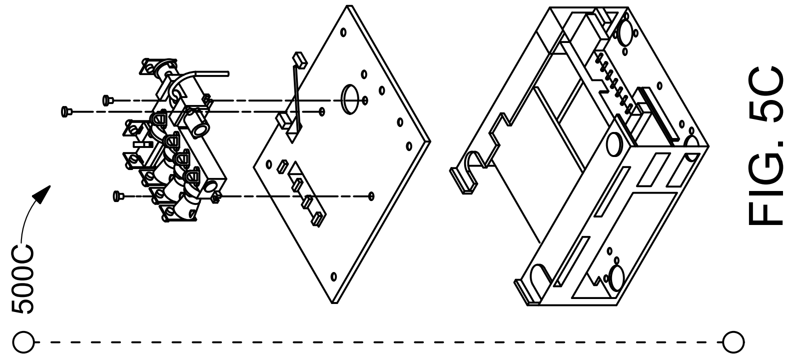

FIG. 5C

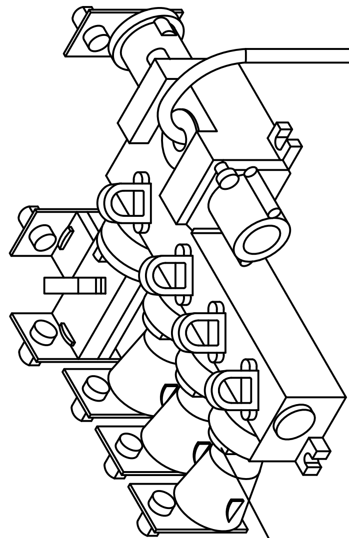

FIG. 5A

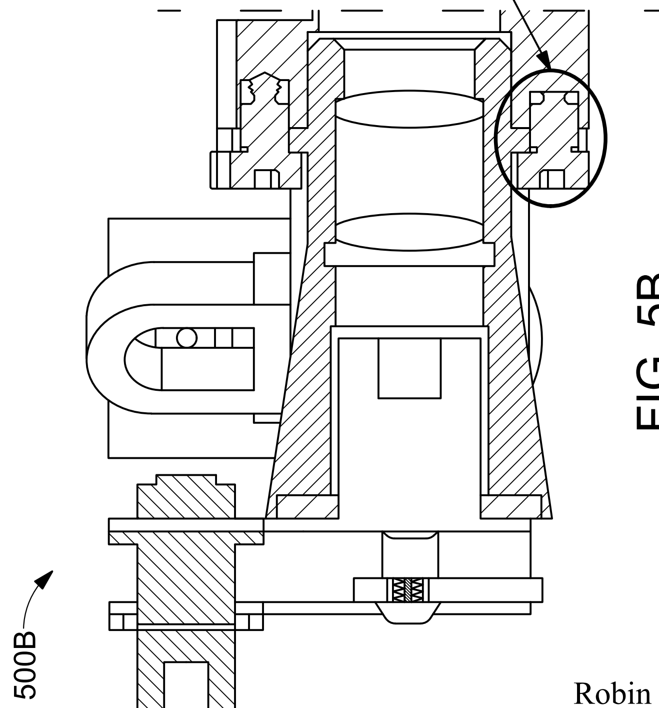

FIG. 5B

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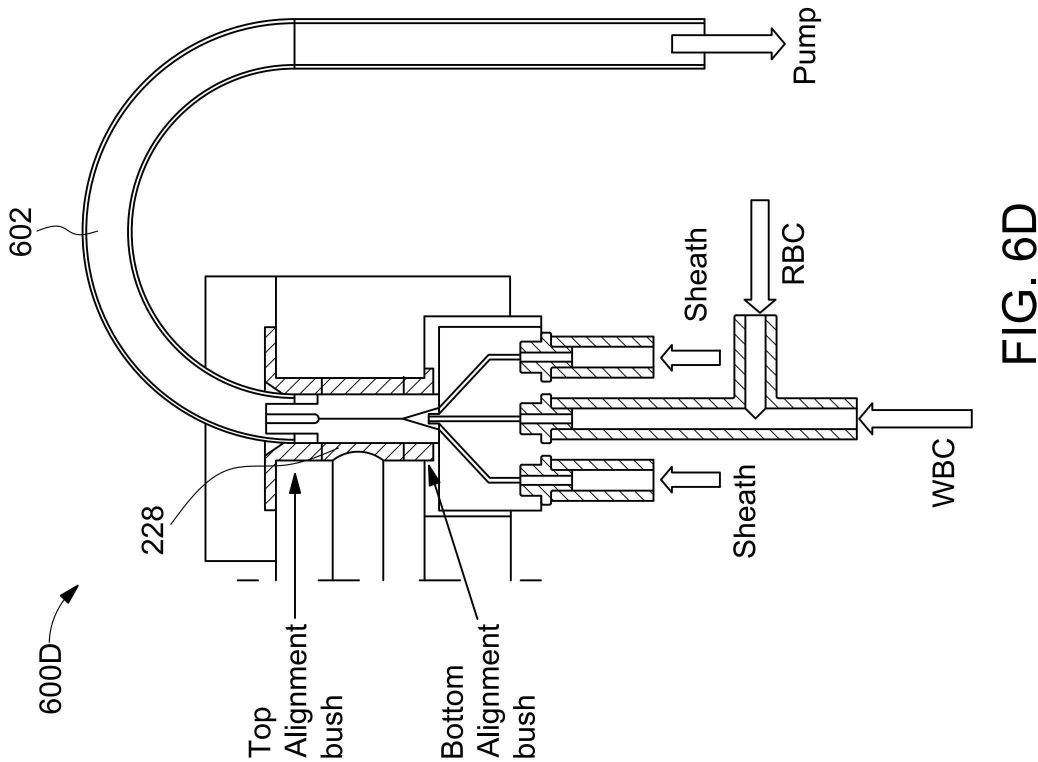

FIG. 6D

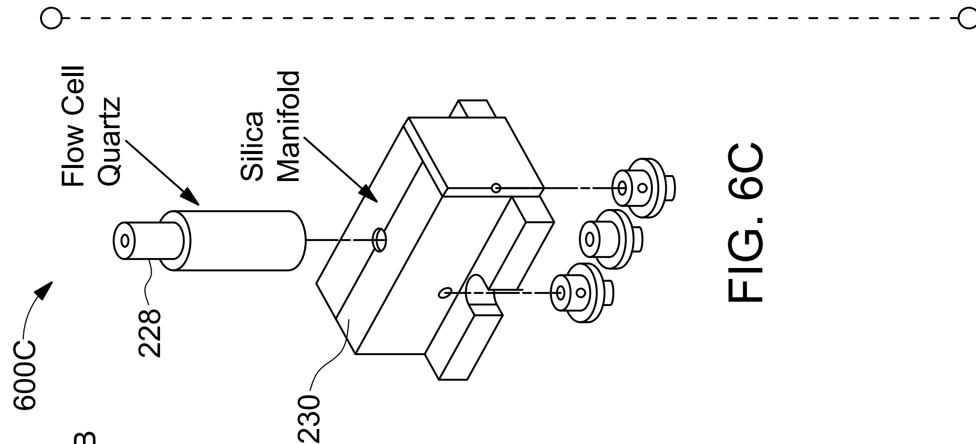

FIG. 6C

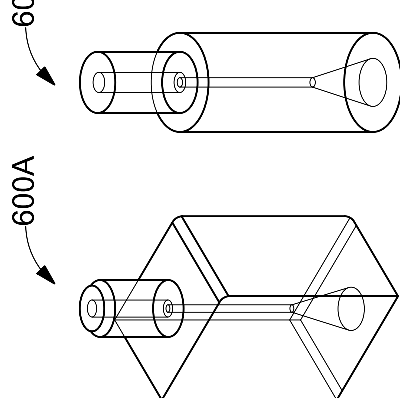

FIG. 6A (Prior Art)

FIG. 6B

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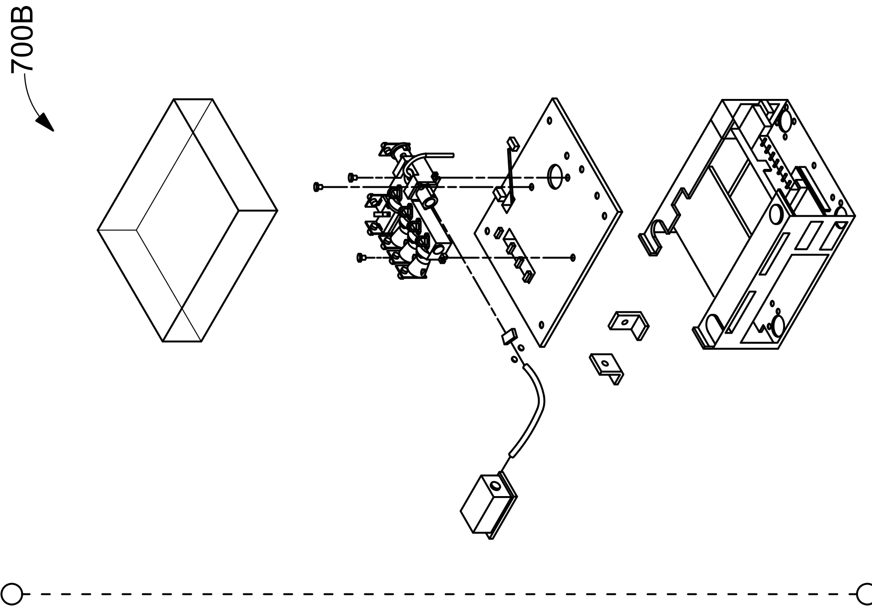

FIG. 7B

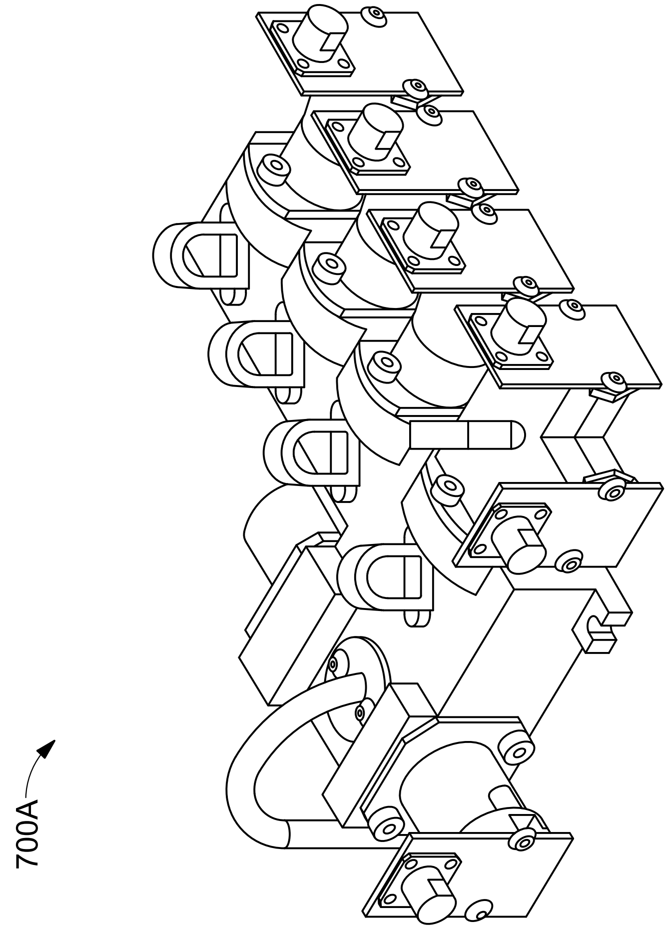

FIG. 7A

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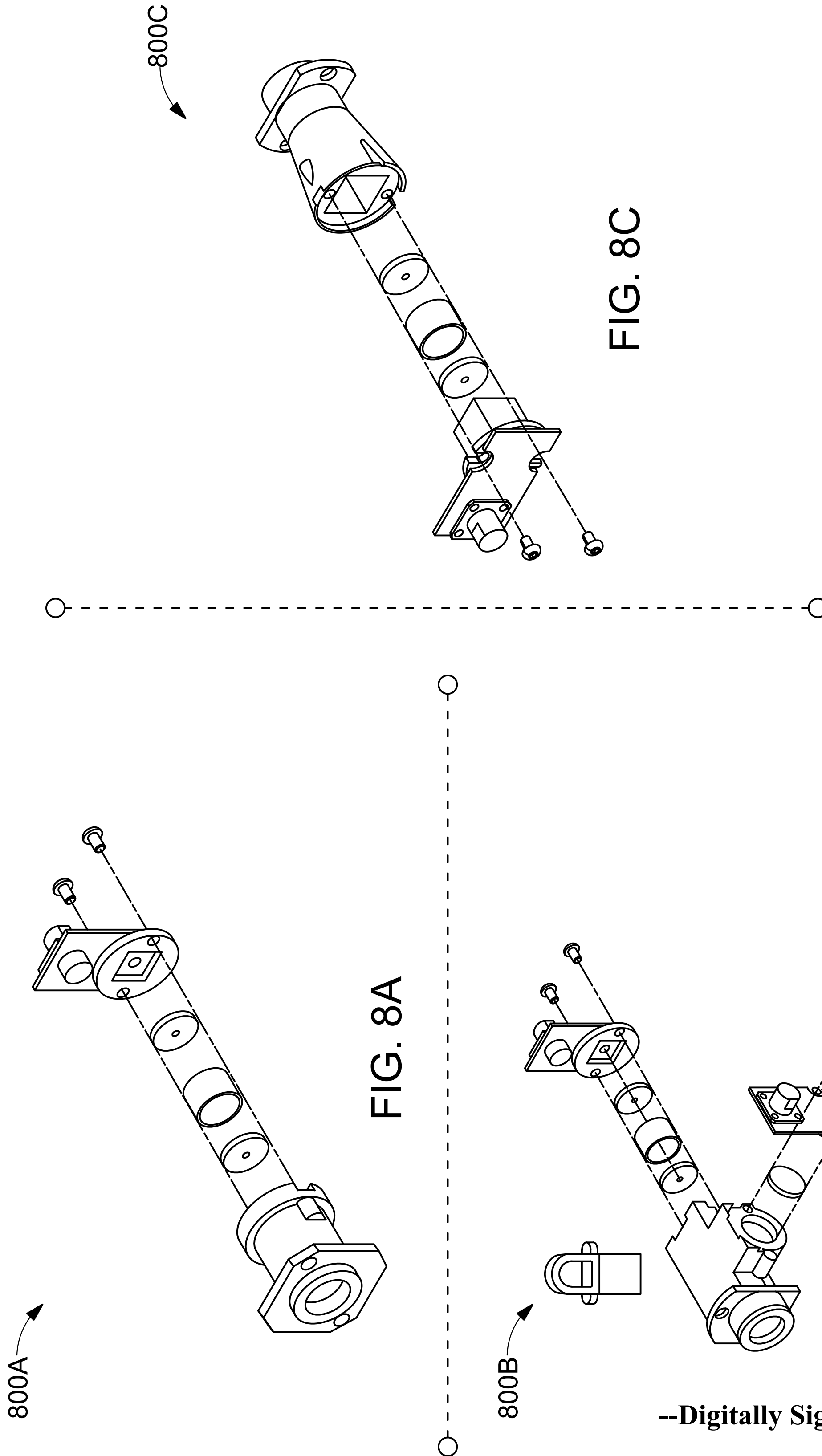

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