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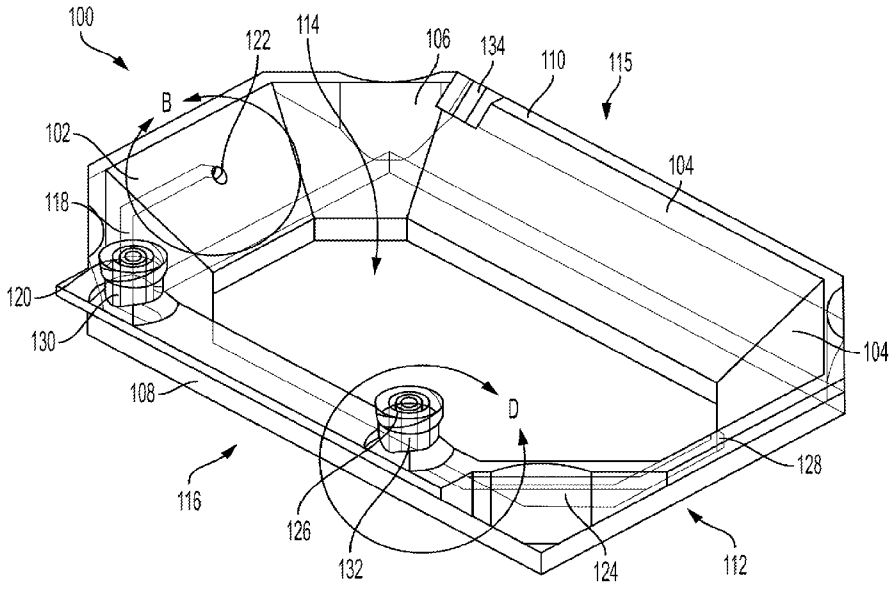

FIG. 1A

(57) Abstract: In some aspects, the present invention relates to a perfusion chamber device having a frame with a plurality of sidewalls extending upwards from the frame, the frame having an inside, outside, top and bottom surface; an internal chamber inside the frame comprising a top and bottom opening; a plurality of channels passing through the sidewalls of the frame, each channel having at least a first opening on a first surface of the frame; and a second opening on a second surface of the frame; and a plurality of locks in fluid communication with the first openings of the plurality of channels configured to connect the plurality of channels to at least one peripheral.

[Continued on next page]

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TITLE

PERFUSION CHAMBER DEVICE FOR ELECTROPHYSIOLOGY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application No. 63/500,127, filed on May 4, 2023, incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under 1944846 awarded by the National Science Foundation. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Perfusion chamber devices are used in the field of electrophysiology to house cells or samples (e.g. brain slices) in a fluid medium to undergo perfusion during experimentation. Current perfusion chamber devices are designed to be mounted onto microscope slides, platforms, heating stages, and other surfaces, in order to have the cells inside undergo perfusion. Current perfusion chambers tend to lack the features and configurability to provide standardized experimentation while allowing other components, such as a transducers, to be used efficiently with the device. Thus, there is a need in the art for a perfusion chamber device that is configured to enable a wide array of configuration and allow transducers and other components to be used efficiently with the device. The present invention satisfies that need.

SUMMARY OF THE INVENTION

[0004] In some aspects, the present invention relates to a perfusion chamber device having a frame with a plurality of sidewalls extending upwards from the frame, the frame having an inside, outside, top and bottom surface; an internal chamber inside the frame comprising a top and bottom opening; a plurality of channels passing through the sidewalls of the frame, each channel having at least a first opening on a first surface of the frame, and a second opening on a second surface of the frame; and a plurality of locks in fluid communication with the first openings of the plurality of channels configured to connect the plurality of channels to at least one peripheral.

[0005] In some embodiments, the device has at least one drain hole passing through the sidewalls of the frame from at least a first surface to a second surface of the frame. In some embodiments, the plurality of sidewalls taper from the bottom surface of the frame to the top surface of the frame. In some embodiments, the frame is a shape selected from the group consisting of: an octagon, a diamond, a polygon, a circle, an oval, a square, a rectangle, a parallelogram. In some embodiments, the device has a width ranging between 10 – 100 mm. In some embodiments, the device has a length ranging between 10 – 100 mm. In some embodiments, the device has a height between 10 – 50 mm. In some embodiments, the sidewalls have a width ranging between 1 – 50 mm.

[0006] In some embodiments, the bottom surface of the frame is flat and configured to mount to a flat surface. In some embodiments, the bottom surface of the frame is a shape selected from the group consisting of: an octagon, a diamond, a polygon, a circle, an oval, a square, a rectangle, a parallelogram. In some embodiments, the bottom surface of the frame comprises at least one mounting feature selected from the group consisting of: a slot, a groove, a pin, a hole. In some embodiments, the bottom surface of the frame comprises an adhesive layer.

In some embodiments, the surfaces of the frame comprise at least one coating. In some embodiments, the coating is a selected from the group consisting of: a biocompatible coating, a biomimetic coating, a hydrophobic coating.

[0007] In some embodiments, the plurality of locks comprise male Luer locks. In some embodiments, the at least one peripheral is selected from the group consisting of: tubing, connectors, Luer locks, perfusion pump, fluid pump, vacuum, fluid medium reservoir, fluid waste reservoir, flow sensor, temperature sensor, pressure sensor, and filter. In some embodiments, the device comprises PLA, PEEK, ABS, PET, PVA, PTFE, PETG, polycarbonate, polypropylene, polyethylene, polyamide, polyvinyl chloride, glass fiber polypropylene (GFPP), borosilicate glass, quartz glass, and UV-curing optical glue.

[0008] In some aspects, the present invention relates to a method of use for a perfusion chamber device, having the steps of, providing any perfusion chamber device of the present invention; mounting a glass slide to the bottom surface; affixing the device to a microscopy platform; placing a sample in the internal chamber; connecting tubing, a fluid reservoir and a fluid pump in fluid communication with the plurality of channels; illuminating the sample with a light source; collecting at least one signal from the sample using at least one transducer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The foregoing purposes and features, as well as other purposes and features, will become apparent with reference to the description and accompanying figures below, which are included to provide an understanding of the invention and constitute a part of the specification, in which like numerals represent like elements, and in which:

[0010] Figure 1A depicts a perspective view of an exemplary perfusion chamber device according to aspects of the present invention.

[0011] Figure 1B depicts an enlarged view of Detail B (as referenced in Fig. 1A) of an exemplary perfusion device according to aspects of the present invention.

[0012] Figure 1C depicts an enlarged view of Detail D (as referenced in Fig. 1A) of an exemplary perfusion device according to aspects of the present invention.

[0013] Figure 1D depicts an overhead view of an exemplary perfusion chamber device according to aspects of the present invention.

[0014] Figure 1E depicts an enlarged view of Detail A (as referenced in Fig. 1D) of an exemplary perfusion device according to aspects of the present invention.

[0015] Figure 1F depicts an enlarged view of Detail C (as referenced in Fig. 1D) of an exemplary perfusion device according to aspects of the present invention.

[0016] Figure 1G depicts a front side view of an exemplary perfusion device according to aspects of the present invention.

[0017] Figure 1H depicts a lateral side view of an exemplary perfusion device according to aspects of the present invention.

[0018] Figures 2A – 2C show an exemplary perfusion chamber device used during electrophysiology experimentation according to aspects of the present invention.

[0019] Figure 3A depicts a perspective view of an exemplary perfusion chamber device according to aspects of the present invention.

[0020] Figure 3B depicts an overhead view of an exemplary perfusion chamber device according to aspects of the present invention.

[0021] Figures 3C and 3D depict side views of an exemplary perfusion chamber device according to aspects of the present invention.

[0022] Figure 3E depicts a perspective view of details of an exemplary perfusion chamber device according to aspects of the present invention.

[0023] Figure 3F depicts an enlarged view of Detail A (as referenced in Fig. 3E) of an exemplary perfusion device according to aspects of the present invention.

[0024] Figure 3G depicts an enlarged view of Detail B (as referenced in Fig. 3E) of an exemplary perfusion device according to aspects of the present invention.

[0025] Figure 3H depicts an enlarged view of Detail C (as referenced in Fig. 3E) of an exemplary perfusion device according to aspects of the present invention.

[0026] Figure 4A depicts an example schematic of an exemplary perfusion chamber device according to aspects of the present invention.

[0027] Figure 4B shows an exemplary perfusion chamber device used during electrophysiology experimentation according to aspects of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0028] It is to be understood that the figures and descriptions of the present invention have been simplified to illustrate elements that are relevant for a clearer comprehension of the present invention, while eliminating, for the purpose of clarity, many other elements found in systems for a perfusion chamber device for electrophysiology. Those of ordinary skill in the art may recognize that other elements and/or steps are desirable and/or required in implementing the present invention. However, because such elements and steps are well known in the art, and because they do not facilitate a better understanding of the present invention, a discussion of

such elements and steps is not provided herein. The disclosure herein is directed to all such variations and modifications to such elements and methods known to those skilled in the art.

[0029] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

[0030] As used herein, each of the following terms has the meaning associated with it in this section.

[0031] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0032] "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to refer to the specified value or encompass variations of ±20%, ±10%, ±5%, ±1%, and ±0.1% from the specified value, as such variations are appropriate.

[0033] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Where appropriate, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2

to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0034] Referring now in detail to the drawings, in which like reference numerals indicate like parts or elements throughout the several views, in various embodiments, presented herein is a perfusion chamber device for electrophysiology.

[0035] Perfusion is utilized in the field of electrophysiology to maintain a tissue sample during whole cell patching or other electrophysiology experiments. These experiments record the electrical activity of neurons or other cells (i.e., cardiovascular cells) that generate an action potential. Electrophysiology provides a high resolution recording of the activity of these cells - often studied to compare healthy and disease states, progressive disease states, to determine cellular activity with varying conditions/perturbations, etc. Electrophysiology encompasses single cell patch clamp electrophysiology (which we are working with in the lab), also higher throughput cell studies using for example the Utah array, implantable microelectrodes, etc. A perfusion chamber device for use in electrophysiology and other experiments is disclosed herein.

[0036] Aspects of the present invention relate to a perfusion chamber device. In some embodiments, the perfusion chamber device is configured to maintain cells, samples, and/or tissue (e.g. brain slices) during any experiment requiring perfusion. In some embodiments, the perfusion chamber device is configured to allow cell perfusion, or the passage of fluid over and/or through the cells, tissue, samples and/or brain slices. In some embodiments, the perfusion chamber device is configured for photoacoustic microscopy, fluorescence microscopy, and other forms of microscopy as would be known by one of ordinary level of skill in the art. In some embodiments, the perfusion chamber device is configured to be placed on a microscope stage or platform. Further, in some embodiments, the perfusion chamber device is configured to hold a

petri dish, coverslip, glass slide, container, and the like. In some embodiments, the perfusion chamber device is configured to allow perfusion of cells, tissue and/or samples while leaving the cells, sample, tissue undisturbed and/or uncontaminated.

[0037] Referring now to Fig. 1A, a perfusion chamber device 100 is now described. In some embodiments, perfusion chamber device 100 comprises a frame 102 having a plurality of walls or sidewalls 104 extending upwards from the frame. In some embodiments, plurality of walls 104 comprises an inside surface 106, an outside surface 108, a top surface 110 and a bottom surface 112. In some embodiments, device 100 comprises at least one internal chamber 114 disposed within frame 102 and having a top opening 115, and a bottom opening 116, and configured to hold a fluid when device 100 is placed on a surface. In some embodiments, the input and output of a fluid to and from internal chamber 114 is supported by a plurality of channels. In some embodiments, the plurality of channels comprises a first channel 118 and a second channel 124.

[0038] Aspects of the present invention relate to an internal chamber for a perfusion chamber device. Still referring to Fig. 1A, in some embodiments, internal chamber 114 is configured to hold and/or retain a fluid (e.g. artificial cerebral spinal fluid (aCSF)) and cells, tissue and/or samples (e.g. brain slices) while undergoing perfusion experimentation. In some embodiments, internal chamber 114 is sized and/or configured to allow at least a portion of a light source, microscope objective and/or transducer to pass through top opening 115. In some embodiments, at least a portion of the light source, objective and/or transducer is then submerged in the fluid medium. In some embodiments, device 100 is configured for photoacoustic detection of a sample during perfusion. In some embodiments, device 100 is configured to allow continuous perfusion throughout the experiment using a fluid pump. In some embodiments, internal chamber 114 may

be an open-top perfusion chamber. In some embodiments, internal chamber 114 may be a closed-top perfusion chamber. In some embodiments, device 100 retains the fluid medium under sterile conditions. In some embodiments, internal chamber 114 may be sealed to avoid evaporation of the fluid medium, avoid contamination of the fluid medium and/or sample, and provide control over environmental variables, such as, but not limited to, pH, temperature, carbon dioxide concentration, O_2 concentration, and the like. For example, in some embodiments internal chamber 114 has a top opening 115 and/or a bottom opening 116 sized to accept a transparent cover (e.g. microscope glass slide, microscope cover slip) to protect the cells and the fluid medium from external factors. In some embodiments, device 100 comprises an internal chamber 114 with bottom opening 116 sized and shaped to accept a microscope glass cover slip. In some embodiments, bottom opening 116 is sized and shaped to accept microscope glass slide and/or plate. In some embodiments, bottom opening 116 is sized and shaped to fit over and/or envelope a microscope glass slide and/or plate. Internal chamber 114 and bottom opening 116 may accept insertion of the glass cover slip and/or slide wherein the slip or slide fits in place with a compression fit, thereby closing the bottom opening and 116 and allowing internal chamber 114 to retain a fluid. In some embodiments, the glass slide is fixedly attached to bottom opening 116 with an adhesive. In some embodiments, the adhesive is a UV curing optical glue.

[0039] Aspects of the present invention relate to a plurality of channels for a perfusion chamber device. In some embodiments, the plurality of channels are configured to provide fresh fluid medium, and collect spent fluid medium during experiments. In some embodiments, the plurality of channels are disposed on opposite ends of internal chamber 114, and configured to provide a flow of fluid across internal chamber 114. In some embodiments, first channel 118 comprises at least a first opening 120 and a second opening 122 in fluid communication with

channel 118. In some embodiments, second channel 124 comprises at least a first opening 126 and a second opening 128 in fluid connection with channel 124. In some embodiments first opening 120 and first opening 126 are outside internal chamber 114, and second opening 122 and second opening 128 are inside internal chamber 114. In some embodiments, second opening 122 and second opening 128 are holes on the inside surface 106 of the plurality of sidewalls 104.

[0040] In some embodiments, the plurality of channels may be fluidly connected to a plurality of connectors, allowing connection to components external to device 100, such as perfusion pumps, vacuums, filters, and the like. In some embodiments, first opening 120 is disposed within a hollow post 130 extending out from frame 102. In some embodiments, hollow post 130 extends upwards from top surface 110 of frame 102. In some embodiments, first opening 126 is disposed within a hollow post 132 extending out from frame 102. In some embodiments, hollow post 132 extends upwards from top surface 110 of frame 102. In some embodiments, first opening 120 and hollow post 130 are configured as a connector (e.g. luer lock). In some embodiments, first opening 126 and hollow post 132 are configured as a connector (e.g. luer lock). In some embodiments, the individual channels of plurality of channels have any shape or size as would be used and known by one of ordinary level of skill in the art. In some embodiments, plurality of channels are round channels. In some embodiments, plurality of channels have a diameter ranging between 0.1 mm and 10 mm. In some embodiments, the channels are different sizes and shapes.

[0041] Further, in some embodiments, internal chamber 114 is configured as a well or container to hold cells, sample, tissue (e.g. brain slices) while submerged in a fluid medium. In some embodiments, internal chamber 114 may comprise a plurality of wells or chambers. In some embodiments, the wells or chambers are configured to hold fluid medium along with cells,

tissue and/or brain slices and are separated in order to limit interaction of samples or cross-contamination. In some embodiments, a secondary chamber is used as an overflow chamber, to account for variations in water level or flow. This overflow chamber would enable steady flow to the main chamber. In some embodiments, the fluid medium may be any sort of liquid suitable for perfusion of cells as would be known by one of ordinary level of skill in the art. In some embodiments, the fluid medium comprises any reagent, solution, saline, blood, and the like, that allow for perfusion of cells during the experimentation. In some embodiments, the fluid medium comprises Artificial cerebral spinal fluid (aCSF) and/or Dulbecco's Modified Eagle Medium (DMEM). Additionally, in some embodiments, device 100 is arranged such that heating or cooling may be applied to internal chamber 114. In some embodiments, heating may be applied to internal chamber 114 by the use of an inline heater attached to device 100.

[0042] Still referring to Fig. 1A, in some embodiments, device 100 comprises a plurality of walls 104 extending upwards from frame 102 and configured to retain a fluid medium. The shape of plurality of walls 104 may be any shape as would be known by one of ordinary level of skill in the art. For example, in some embodiments, plurality of walls 104 may be tapered such that the bottom surface 112 of the plurality of walls is wider than the top surface 110 of the plurality of walls. Similarly, in some embodiments, plurality of walls 104 may be slanted, angled and/or curved, such that the internal chamber 114 is configured in the shape of a bowl or dish. Now referring to Fig. 1D, in some embodiments, plurality of walls have a width 146 ranging from 0.5 mm to 10 mm.

[0043] Aspects of the present invention relate to a frame for a perfusion chamber device. Frame 102 may be any size or shape as would be known by one of ordinary level of skill in the art. For example, in some embodiments, frame 102 may have an octagonal shape. In some

embodiments, frame 102 may have an oval, circle, square, diamond, rectangle, parallelogram, polygonal, or irregular shape. In some embodiments, frame 102 may be any shape that allows for a sample and fluid medium to be retained within device 100.

[0044] Aspects of the present invention relate to mounting a perfusion chamber device on a surface. Device 100 may be configured to mount to any flat surface. In some embodiments, device 100 comprises a flat bottom surface 112 configured to stabilize device 100 a surface of microscopy stage and/or platform. In some embodiments, bottom surface 112 of device 100 may be mounted to an upright microscope, an inverted microscope, a perfusion platform, a heating stage, a cooling stage, a lab bench, and/or any suitable lab and/or microscopy surface as would be known to one of ordinary level of skill in the art. In some embodiments, device 100 may be mounted to a surface intended for electrophysiology experiments. The mounting of device 100 may include any method known in the art that ensures device 100 will not move during experimentation. For example, an adhesive may be used to mount device 100 to a surface. In other examples, bottom surface 112 comprises tabs, slots, grooves, and the like, for fixedly attaching device 100 to a surface or reciprocal feature. In another example, bottom surface 112 comprises tabs, slots, indents, reliefs, and the like, configured to retain a glass microscopy slide.

[0045] Aspects of the present invention relate to a plurality of channels for a perfusion chamber device. Continuing to refer to Fig. 1A, in some embodiments, device 100 may comprise a plurality of channels originating from at least a first surface, proceeding through the plurality of walls 104, and terminating at inside surface 106. In some embodiments, the channels support input and/or output of a fluid medium from internal chamber 114. In some embodiments, plurality of channels terminate on opposing ends of internal chamber 114. In some embodiments, plurality of channels are configured to provide a laminar flow across internal chamber 114. In

some embodiments, plurality of channels allow a fluid medium to be quickly added and removed. In some embodiments, plurality of channels may fluidly connect to a perfusion pump, a fluid medium reservoir, a vacuum, and/or similar components as would be known by one of ordinary level of skill in the art. In some embodiments, plurality of channels may originate at a first opening within a hollow posting extending upwards from top surface 110. In some embodiments, plurality of channels may comprise flow-controlling features including, but not limited to, valves, gates, connectors, shut-offs, filters, screens, stoppers, and the like, to control the flow of the fluid in and out of internal chamber 114.

[0046] Aspects of the present invention relate to materials for a perfusion chamber device. In some embodiments, device 100 may comprise any suitable material for use in laboratory experimentation as would be known and used by one of ordinary level of skill in the art. For example, but without limitation, device 100 may comprise any glass, plastic, resin, silicon, metal, and/or metal alloys. In some embodiments, device 100 comprises a 3D printed material. In some embodiments, device 100 comprises PLA, PEEK, ABS, PET, PVA, PTFE, PETG, polycarbonate, polypropylene, polyethylene, polyamide, and polyvinyl chloride, glass fiber polypropylene (GFPP), and the like. In some embodiments, device 100 may comprise borosilicate glass and/or quarts glass. In some embodiments, device 100 may comprise UV-curing optical glue. In some embodiments, device 100 comprises a material that is biocompatible, hydrophobic and/or thermoresistant. In some embodiments, device 100 comprises at least one coating. In some embodiments, inside surface 106, outside surface 108, top surface 110 and bottom surface 112 may comprise at least one coating. In some embodiments, bottom surface 112 comprises an adhesive layer.

[0047] Aspects of the present invention relate to size dimensions for a perfusion chamber device. Referring now to Fig. 1D, 1G and 1H, an exemplary perfusion chamber device with exemplary dimensions are shown. In some embodiments. device 100 may have a width 140 ranging between 10 – 200 mm, a length 142 ranging between 10 – 200 mm, and a height 144 ranging between 10 – 200 mm. In some embodiments, internal chamber 114 has a volume ranging between 10 mL to 250 mL. Now referring to Fig. 1F, dimensions for an exemplary hollow post with opening are shown. In some embodiments, the hollow post has an outside diameter ranging between 1 mm and 20 mm. In some embodiments, the opening in the hollow post has a diameter ranging between 1 mm and 10 mm. In some embodiments, hollow post has a height ranging between 1 mm and 10 mm.

[0048] Now referring back to Fig. 1A, in some embodiments, device 100 may comprise at least one drainage feature extending outward from at least one surface of device 100. In some embodiments, feature 134 is a hollow tube positioned at least partially on inside surface 106 and top surface 110. In some embodiments, feature 134 is a configured as a drain to prevent fluid from overflowing out of device 100. In some embodiments, feature 134 fluidly connects internal chamber 114 with at least one drain tube. In some embodiments, feature 134 is an alignment feature for aligning at least one sensor and/or tool to position the sensor and/or tool to reside within the plurality of walls and/or internal chamber 114. In some embodiments, feature 134 aligns and positions a reference electrode (e.g. a ground electrode) in contact with the fluid medium.

[0049] Now referring to Figs. 2A, 2B and 2C, an exemplary device 100 is shown installed on a microscope stage 150 and configured for electrophysiology experimentation with an objective 155 and transducer 160. As shown in Fig. 2B, in some embodiments, device 100 comprises a

plurality of channels originating with two male Luer locks and internal chamber 114 contains a red fluid medium. As shown in this example, tubing with female Luer locks connect to the first post 130 and second post 132. Also shown is feature 134 configured as a drain. In this example, feature 134 enables fluid communication between internal chamber 114 and a fluid medium waste reservoir. In some embodiments, a glass cover is positioned to enclose internal chamber 114 and close top opening 115. In some embodiments, at least a portion of objective 155 and/or transducer 160 is submerged in the fluid medium contained in internal chamber 114.

[0050] Some aspects of the present invention may be made using an additive manufacturing (AM) process. Among the most common forms of additive manufacturing are the various techniques that fall under the umbrella of "3D Printing", including but not limited to stereolithography (SLA), digital light processing (DLP), fused deposition modelling (FDM), selective laser sintering (SLS), selective laser melting (SLM), electronic beam melting (EBM), and laminated object manufacturing (LOM). These methods variously "build" a three-dimensional physical model of a part, one layer at a time, providing significant efficiencies in rapid prototyping and small-batch manufacturing. AM also makes possible the manufacture of parts with features that conventional subtractive manufacturing techniques (for example CNC milling) are unable to create. In some embodiments, an AM process may comprise building a three dimensional physical model from a single material, while in other embodiments, a single AM process may be configured to build the three dimensional physical model from more than one material at the same time.

[0051] Aspects of the present invention relate to a method of use for a perfusion chamber device. In some embodiments, a method of use for a perfusion chamber device comprises the steps of: providing any device 100 of the present invention, mounting a glass slide to bottom

surface 112 of device 100, affixing device 100 to a microscopy platform, placing a sample in internal chamber 114, connecting tubing, a fluid reservoir and a fluid pump (e.g. a peristaltic pump) in fluid communication with the plurality of channels, heating the fluid with an inline heater (e.g. a ThermoClamp heater), carbogenating the fluid, oxygenating the fluid, illuminating the sample with a light source, collecting at least one signal from the sample using at least one transducer. In some embodiments, the signal is any of a photoacoustic signal, an ultrasound signal, an optical signal, an electrophysiological signal, and other signals as would be known by one of ordinary level of skill in the art. In some embodiments, the method does not include a transducer. In some embodiments, the method includes traditional blind or image-guide (e.g. fluorescence-guided) electrophysiology.

[0052] The present invention may be used for, in some examples, perfusion of a tissue sample during electrophysiology experiments to maintain a tissue sample during whole cell patching or other experiments known in the art. The device is configured to keep neurons in a tissue sample alive and firing for as long as possible. The fluid supplies warmed, oxygenated (or carbogenated) medium. The fluid also serves as a ground for a reference electrode. In some embodiments, the fluid medium is configured to optimized for a specific neuron subtype or levels of firing activity (i.e. excitatory or inhibitory). In some embodiments, the electrophysiology experiments record the electrical activity of neurons or other cells (i.e., cardiovascular cells) that generate an action potential. Electrophysiology provides a high resolution recording of the activity of these cells—often studied to compare healthy and disease states, progressive disease states, to determine cellular activity with varying conditions/perturbations, etc. Electrophysiology encompasses single cell patch clamp electrophysiology, and also higher throughput cell studies using for example the Utah array,

implantable microelectrodes, and other methods, using materials and instruments known in the art.

[0053] Figs. 3A-3H depict various views of a perfusion chamber device 300. It is noted that device 300 includes the same or similar features as device 100. The like reference numerals of Figs. 1A-1H and 3A-3H represent like parts and assemblies, and as such, a description of these parts and assemblies is not repeated. Device 300 is distinguishable from device 100 in that device 300 includes a reservoir 302, an opening 308 disposed within a hollow post 316, an opening 312 disposed within a hollow post 314, and a port 318.

[0054] The opening 312 and hollow post 314 may be configured as a connector (e.g., a luer lock 319). The luer lock 319 may be an output luer lock 319 fluidly connected to the internal chamber 114. The opening 308 and hollow post 316 may be configured as a connector (e.g., luer lock 317). The luer lock 317 may be fluidly connected to the reservoir 302 and provide input media to the internal chamber 114. In some embodiments, the device 300, and in particular, the reservoir 302, luer lock 317, luer lock 319, and their respective channels 318, 322, and 324 that fluidly connect one or more portions of the device 300 are configured to modify one or more of a flow rate, flow direction, and turbulence within the internal chamber 114. For example, the flow rate may be modified by increasing or decreasing the diameter of the channels and/or by increasing or decreasing the number of channels. In another example, the flow direction may be controlled based on the position of the openings of the one or more channels into, for example, the internal chamber 114. In some embodiments, the plurality of channels, such as channels 318, 322, and 324, are configured to provide fresh fluid medium, and collect spent fluid medium during experiments. In some embodiments, the plurality of channels may be fluidly connected to

a plurality of connectors, allowing connection to components external to device 300, such as perfusion pumps, vacuums, filters, and the like.

[0055] The reservoir 302 may be a rigid body formed from one or more walls 304 that define a receptacle 310. The body of the reservoir 302 may be formed in a variety of shapes, for example, but not limited to, a rectangular shape, a square shape, an oval shape, and the like. In some embodiments, the reservoir 302 is positioned on one side of the device 300. For example, the reservoir 302 may be positioned along a side that defines a width W1 of the device 300. In another example, the reservoir 302 may be positioned along a side that defines a length L of the device 300. In some embodiments, the reservoir 302 is positioned on two adjacent sides of the device 300. For example, a portion of the reservoir 302 may be positioned along a side that defines the width W1 of the device 300, and another portion of the reservoir 302 may be positioned along a side that defines the length L of the device 300.

[0056] The receptacle 310 of the reservoir 302 may be configured to store a fluid medium, such as input media for the internal chamber 114. The receptacle 310 of the reservoir 302 may be fluidly connected to the internal chamber 114 via one or more channels, such as channel 318. For example, referring to Fig. 3G, device 300 may include a channel 318 that has openings 326 and 328 disposed on both ends of the channel 318 and are in fluid connection with the channel 318. Opening 328 may terminate on an inside surface 106 of the wall 104. Opening 326 may terminate on an inside surface of wall 304 that interfaces with the receptacle 310. As such, the channel 318 may provide a laminar flow between the receptacle 310 of the reservoir 302 and the internal chamber 114. In some embodiments, a channel fluidly connecting the receptacle 310 and the internal chamber 114, such as channel 318, may have any shape or size as would be used and known by one of ordinary level of skill in the art. In some embodiments, the channel may be a

round channel. In some embodiments, the channel may have a diameter ranging between 0.1 mm and 10 mm. In some embodiments, the channels may have different sizes and shapes.

[0057] In some embodiments, referring to Fig. 3G, the luer lock 319 is disposed on the top surface 110 of the frame 102. The hollow post 314 of the luer lock 319 may protrude from the top surface 110 at a variety of angles. For example, the hollow post 314 protrudes vertically from the top surface 110 of the frame 102. In some embodiments, the luer lock 319 may be disposed on another surface, such as the outside surface 108, and protrude from such surfaces at a variety of angles, such as laterally from the outside surface 108. In some embodiments, the channel 324 has openings 312 and 314 disposed on both ends of the channel 324 and are in fluid connection with the channel 324. Opening 314 is positioned inside the internal chamber 114 and may terminate on an inside surface 106 of the wall 104. Opening 312 is positioned outside the internal chamber 114 and disposed within the hollow post 314. Opening 312 may terminate on a distal surface of the post 314. The channel 324 may provide a laminar flow between the internal chamber 114 and an external connector coupled to the luer lock 319 allowing connection to components external to device 300, such as perfusion pumps, vacuums, filters, and the like. In some embodiments, channel 324 may have any shape or size as would be used and known by one of ordinary level of skill in the art. In some embodiments, the channel 324 may be a round channel. In some embodiments, the channel 324 may have a diameter ranging between 0.1 mm and 10 mm. Although device 300 is described as having one luer lock 319 and one channel 324, it should be understood that multiple luer locks may be disposed around the frame 102 in a same or similar fashion as luer lock 319 and channel 324. The additional luer locks may provide laminar flow between the internal chamber 114 and external connectors coupled to a respective luer lock.

[0058] In some embodiments, referring to Figs. 3A and 3F, reservoir 302 includes a body 306 that provides a pathway for channel 322 to extend between the opening 308 of the luer lock 317 and the opening 320 that interfaces with the receptacle 310. In some embodiments, the body 306 protrudes laterally from a sidewall 307 of the walls 304 that define the reservoir 302. The luer lock 317 may be disposed on a surface (e.g., a top surface 321 or a side surface) of the body 306. The body 306 may be formed in a variety of shapes. For example, the body 306 may taper from the top surface 321 to the bottom surface 312 of the device 300. In another example, the body 306 may be rectangularly shaped. As an alternative to the body 306 supporting the luer lock 317, the sidewall 307 of the reservoir 302 may be thick enough such that the luer lock 317 may be disposed on a surface (e.g., a top surface or a side surface) of the sidewall 307.

[0059] The hollow post 316 of the luer lock 317 may protrude from a surface (e.g., the top surface 321) of the body 306 at a variety of angles. For example, the hollow post 316 protrudes vertically from the top surface 321 of the body 306. In another example, the luer lock 317 may be disposed on another surface, such as a side surface of the body 306, and protrude from such surfaces at a variety of angles, such as laterally from the side surface. In some embodiments, the channel 322 has openings 308 and 320 disposed on both ends of the channel 322 and are in fluid connection with the channel 322. Opening 320 is positioned inside the reservoir 302 and may terminate on an inside surface of the reservoir 302. The opening 320 may interface with the receptacle 310. Opening 308 is positioned outside the internal chamber 114 and the receptacle 310. Opening 308 is disposed within the hollow post 314. Opening 308 may terminate on a distal surface of the post 316. The channel 322 may provide a laminar flow between an external connector coupled to the luer lock 317 and the receptacle 310 of the reservoir 302 allowing connection to components external to device 300. In some embodiments, channel 322 may have

any shape or size as would be used and known by one of ordinary level of skill in the art. In some embodiments, the channel 322 may be a round channel. In some embodiments, the channel 322 may have a diameter ranging between 0.1 mm and 10 mm. Although device 300 is described as having one luer lock 317 and one channel 322, it should be understood that multiple luer locks may be disposed around the reservoir 302 in a same or similar fashion as luer lock 317 and channel 322. The additional luer locks may provide laminar flow between external connectors coupled to a respective luer lock and the receptacle 310 of the reservoir 302.

EMBODIMENTS

[0060] The invention includes at least the following numbered embodiments:

1. A perfusion chamber device comprising: a frame with a plurality of sidewalls extending upwards from the frame, the frame having an inside, outside, top and bottom surface; an internal chamber inside the frame comprising a top and bottom opening; a plurality of channels passing through the sidewalls of the frame, each channel having at least a first opening on a first surface of the frame, and a second opening on a second surface of the frame; and a plurality of locks in fluid communication with the first openings of the plurality of channels configured to connect the plurality of channels to at least one peripheral.

2. The device of embodiment 1, wherein the device comprises at least one drain hole passing through the sidewalls of the frame from at least a first surface to a second surface of the frame.

3. The device of embodiment 1 or embodiment 2, wherein the plurality of sidewalls taper from the bottom surface of the frame to the top surface of the frame.

4. The device of any of embodiments 1-3, wherein the frame is a shape selected from the group consisting of: an octagon, a diamond, a polygon, a circle, an oval, a square, a rectangle, a parallelogram.

5. The device of any of embodiments 1-4, wherein the device has a width ranging between 10 – 100 mm.

6. The device of any of embodiments 1-5, wherein the device has a length ranging between 10 – 100 mm.

7. The device of any of embodiments 1-6, wherein the device has a height between 10 – 50 mm.

8. The device of any of embodiments 1-7, wherein the sidewalls have a width ranging between 1 – 50 mm.

9. The device of any of embodiments 1-8, wherein the bottom surface of the frame is flat and configured to mount to a flat surface.

10. The device of any of embodiments 1-9, wherein the bottom surface of the frame is a shape selected from the group consisting of: an octagon, a diamond, a polygon, a circle, an oval, a square, a rectangle, a parallelogram.

11. The device of any of embodiments 1-10, wherein the bottom surface of the frame comprises at least one mounting feature selected from the group consisting of: a slot, a groove, a pin, a hole.

12. The device of any of embodiments 1-11, wherein the bottom surface of the frame comprises an adhesive layer.

13. The device of any of embodiments 1-12, wherein the surfaces of the frame comprise at least one coating.

14. The device of any of embodiments 1-13, wherein the coating is a selected from the group consisting of: a biocompatible coating, a biomimetic coating, a hydrophobic coating.

15. The device of any of embodiments 1-14, wherein the plurality of locks comprise male Luer locks.

16. The device of any of embodiments 1-15, wherein the at least one peripheral is selected from the group consisting of: tubing, connectors, luer locks, perfusion pump, fluid pump, vacuum, fluid medium reservoir, fluid waste reservoir, flow sensor, temperature sensor, pressure sensor, and filter.

17. The device of any of embodiments 1-16, wherein the device comprises any of PLA, PEEK, ABS, PET, PVA, PTFE, PETG, polycarbonate, polypropylene, polyethylene, polyamide, polyvinyl chloride, glass fiber polypropylene (GFPP), borosilicate glass, quartz glass, and UV-curing optical glue.

18. A method for a perfusion chamber device, comprising the steps of: providing the device of any of embodiments 1-17; mounting a glass slide to the bottom surface; affixing the device to a microscopy platform; placing a sample in the internal chamber; connecting tubing, a fluid reservoir and a fluid pump in fluid communication with the plurality of channels; illuminating the sample with a light source; collecting at least one signal from the sample using at least one transducer.

EXPERIMENTAL EXAMPLE

[0061] Figs. 4A and 4B illustrate the device 300 used during electrophysiology experimentation. The number of tests able to be conducted per brain slice sample is currently limited, as cells lose adhesion and morphology as soon as 30-minutes after experiments begin. With proper conditions, acute brain slices post-euthanasia have been shown to last between 4 and 6 hours. These conditions include: (1) maintaining a constant room temperature, (2) carbogenation (95% O2 and 5% CO2) of artificial cerebrospinal fluid, and (3) consistent fluid flow profiles. The devices 100 and 300 described herein operate under these conditions while providing an all-in-one perfusion chamber. The devices are tested on B35 neuron cells.

[0062] Maintaining stability in neurons before patch clamping involves several challenges. These challenges include the need for laminar flow and precise temperature control. Furthermore, the setup should accommodate spatial constraints imposed by the DIC microscope, such as the requirement for the microscope's objective lens to be positioned directly above the B35 cells. Additionally, the concentrically aligned micropipette and fiber optic must approach the neurons from an angle off to the side of the experimental chamber. Given these parameters, the devices described herein provide a perfusion chamber that ensures the viability of neurons during experiments. The devices described herein improve the viability of the neurons, which enables additional experiments to be performed per glass slide. As such, time in between experiments is reduced, the health of the neurons is improved, and the number of neurons required per experiment is reduced. Upon successful perfusion of neurons in the chamber, further work can be done on whole brain tissue slices during real time automated patch clamping.

[0063] The specifications for the device 300 during the example electrophysiology experimentation are laminar flow, constant temperature at 37 degrees Celsius, and

carbogenation. The luer locks 317 and 319 serve as the inlet and outlet, respectively, for the device 300. A temperature probe is provided in the overflow chamber at the back of the device 300. The carbogenation line is connected to a 50 mL tube that is heated on a hot plate off site of the device 300. At approximately 9 mL per minute, the media will flow into the internal chamber 114 from the openings in the overflow chamber and exit the internal chamber 114 to the 50 mL tube through the luer lock 319.

[0064] To conduct the experiment, the device 300 is placed under the DIC microscope to image the B35 neurons. $1/16^{th}$ inch silicone tubes are connected to the inlet and outlet ports (e.g., luer locks 317 and 319) to allow media flow into and out of the chamber. A thermocouple is attached before the inlet port to heat up the media prior to entering the perfusion chamber overflow tank. This heating element regulates the temperature of the media. The thermocouple has an inbuilt temperature probe and a proportional integral derivative (PID) controller to modulate the temperature. A peristaltic pump moves the fluid. The peristaltic pump is able to push the media through the lines at about 9 mL per minute. The media is initially stored offsite in a 50 mL tube. By moving the offsite, the media is able to be carbogenated and heated on a hot plate.

[0065] The hot plate is set to a temperature of 75 degrees to keep the beaker with the 50 mL tube warmed. Tin foil is wrapped around the beaker to preserve its heat. The 50 mL tube had the carbogenation stone placed inside the vial along with an inlet and outlet silicone tube line. The silicone tube lines were connected to each other with Leur locks. The perfusion pump imaged above the hot plate and before the green LED box was connected to the 50 mL tube. From there, the silicone tube lines were heated with the thermocouple and entered the perfusion chamber underneath the microscope.

[0066] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A perfusion chamber device comprising:

a frame with a plurality of sidewalls extending upwards from the frame, the frame having an inside, outside, top and bottom surface;

an internal chamber inside the frame comprising a top and bottom opening;

a plurality of channels passing through the sidewalls of the frame, each channel having at least a first opening on a first surface of the frame, and a second opening on a second surface of the frame; and

a plurality of locks in fluid communication with the first openings of the plurality of channels configured to connect the plurality of channels to at least one peripheral.

2. The device of claim 1, wherein the device comprises at least one drain hole passing through the sidewalls of the frame from at least a first surface to a second surface of the frame.

3. The device of claim 1, wherein the plurality of sidewalls taper from the bottom surface of the frame to the top surface of the frame.

4. The device of claim 1, wherein the frame is a shape selected from the group consisting of: an octagon, a diamond, a polygon, a circle, an oval, a square, a rectangle, a parallelogram.

5. The device of claim 1, wherein the device has a width ranging between 10 – 100 mm.

6. The device of claim 1, wherein the device has a length ranging between 10 – 100 mm.

7. The device of claim 1, wherein the device has a height between 10 – 50 mm.

8. The device of claim 1, wherein the sidewalls have a width ranging between 1 – 50 mm.

9. The device of claim 1, wherein the bottom surface of the frame is flat and configured to mount to a flat surface.

10. The device of claim 1, wherein the bottom surface of the frame is a shape selected from the group consisting of: an octagon, a diamond, a polygon, a circle, an oval, a square, a rectangle, a parallelogram.

11. The device of claim 1, wherein the bottom surface of the frame comprises at least one mounting feature selected from the group consisting of: a slot, a groove, a pin, a hole.

12. The device of claim 1, wherein the bottom surface of the frame comprises an adhesive layer.

13. The device of claim 1, wherein the surfaces of the frame comprise at least one coating.

14. The device of claim 13, wherein the coating is a selected from the group consisting of: a biocompatible coating, a biomimetic coating, a hydrophobic coating.

15. The device of claim 1, wherein the plurality of locks comprise male Luer locks.

16. The device of claim 1, wherein the at least one peripheral is selected from the group consisting of: tubing, connectors, luer locks, perfusion pump, fluid pump, vacuum, fluid medium reservoir, fluid waste reservoir, flow sensor, temperature sensor, pressure sensor, and filter.

17. The device of claim 1, wherein the device comprises any of PLA, PEEK, ABS, PET, PVA, PTFE, PETG, polycarbonate, polypropylene, polyethylene, polyamide, polyvinyl chloride, glass fiber polypropylene (GFPP), borosilicate glass, quartz glass, and UV-curing optical glue.

18. A method for a perfusion chamber device, comprising the steps of:

providing the device of claim 1;

mounting a glass slide to the bottom surface;

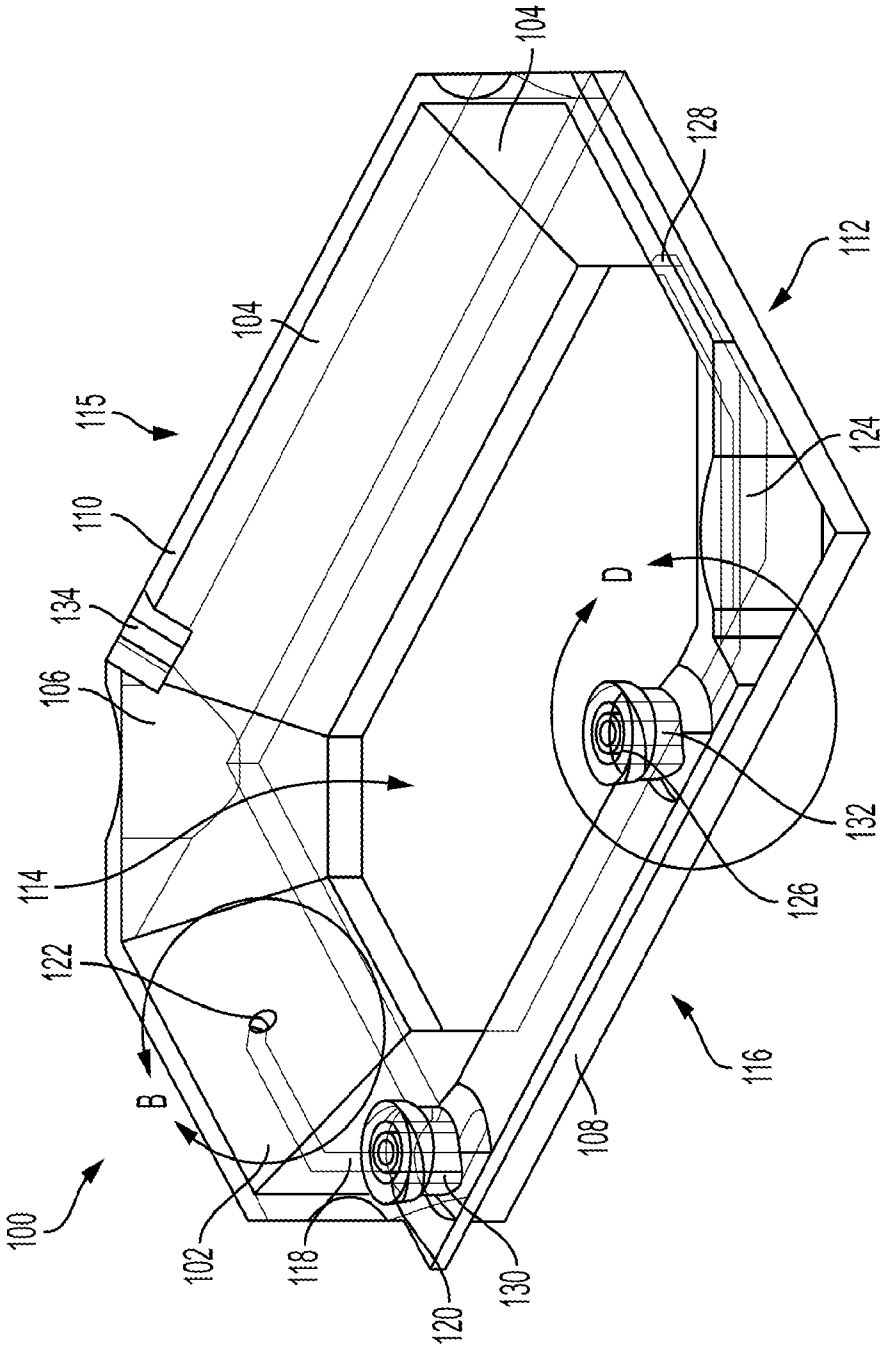
affixing the device to a microscopy platform;

placing a sample in the internal chamber;

connecting tubing, a fluid reservoir and a fluid pump in fluid communication with the plurality of channels;

illuminating the sample with a light source;

collecting at least one signal from the sample using at least one transducer.


104
128
112
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D
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114
126
122
B
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120

FIG. 1A

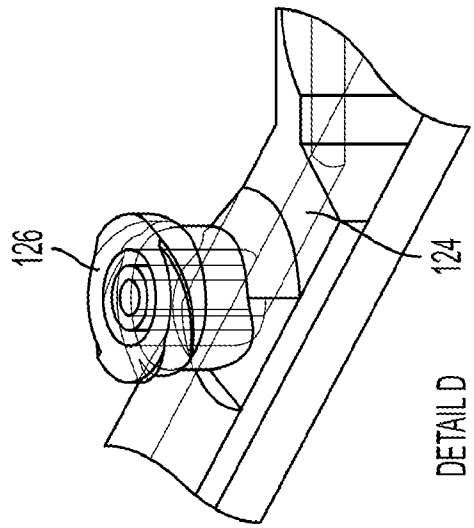


FIG. 1C

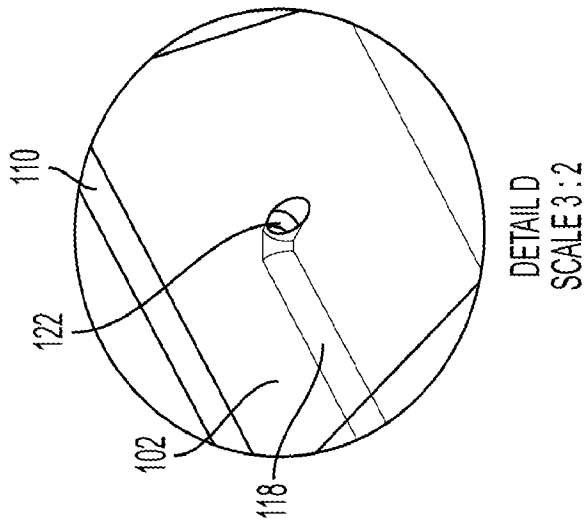


FIG. 1B

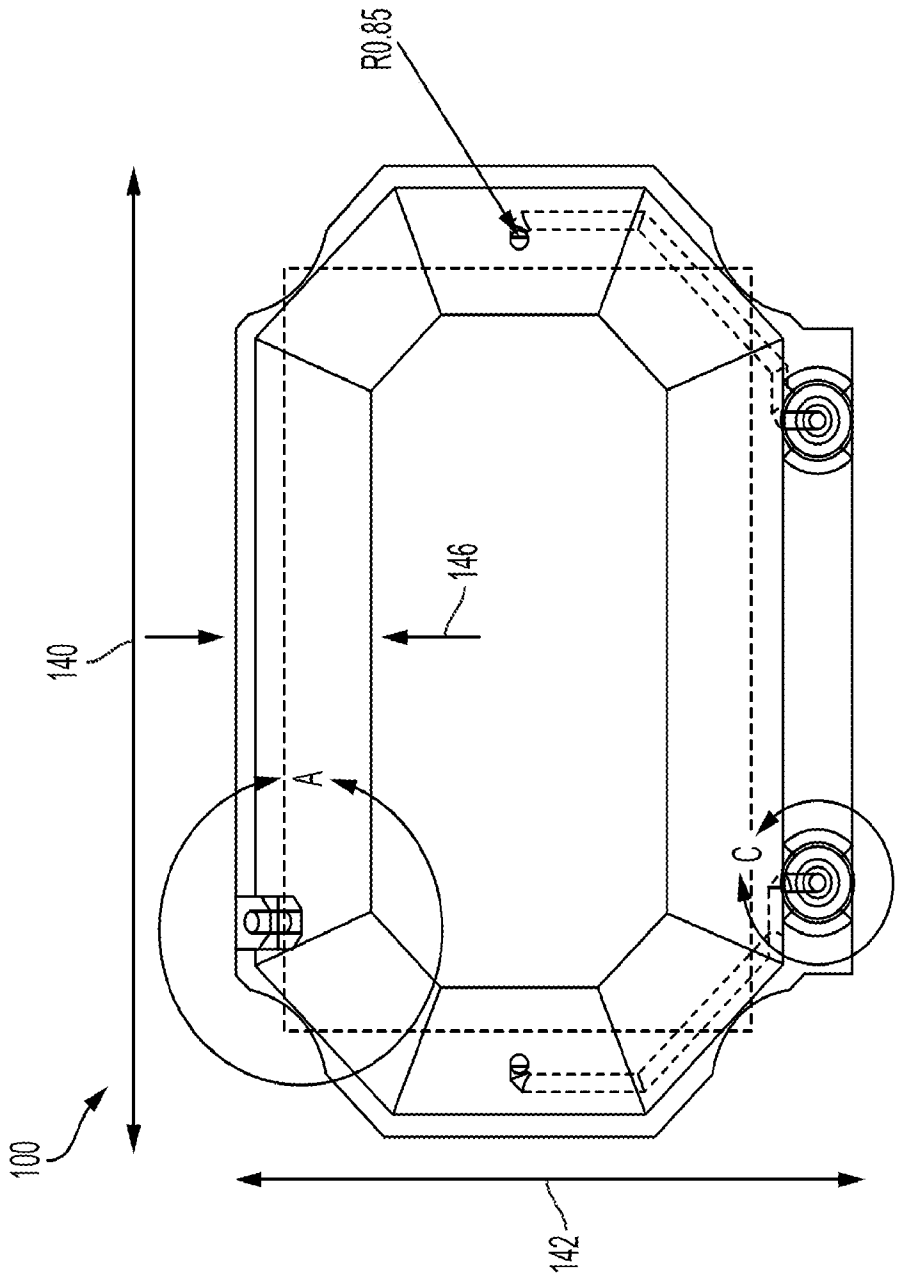


FIG. 1D

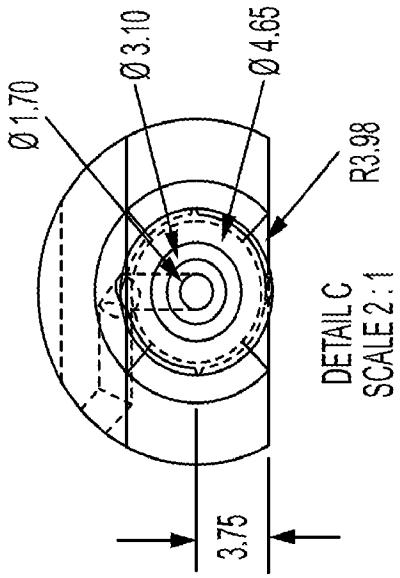
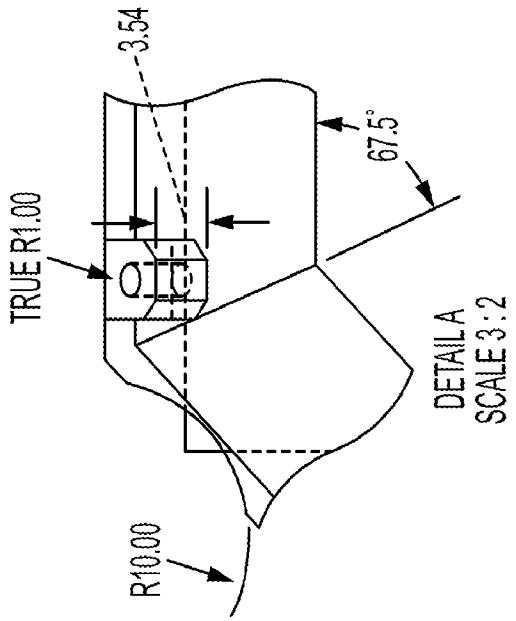

Ø 1.70
Ø 3.10
Ø 4.65
R3.98
DETAIL C
SCALE 2 : 1
3.75

FIG. 1F


3.54
67.5°
TRUE R1.00
DETAIL A
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R10.00

FIG. 1E

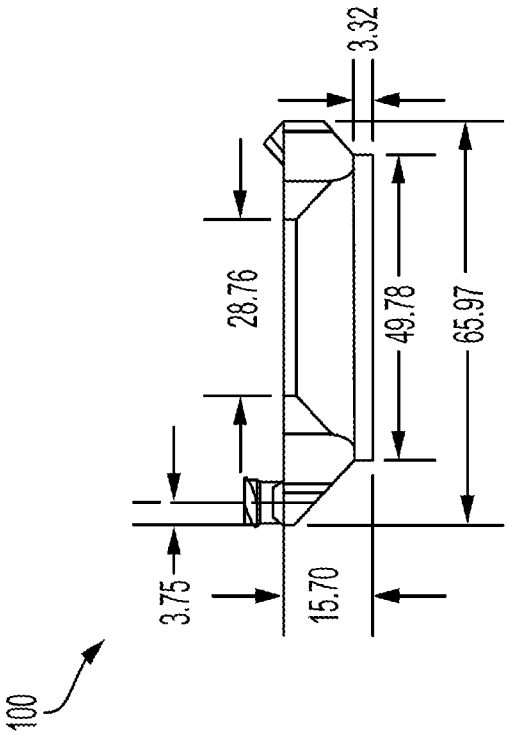

FIG. 1H

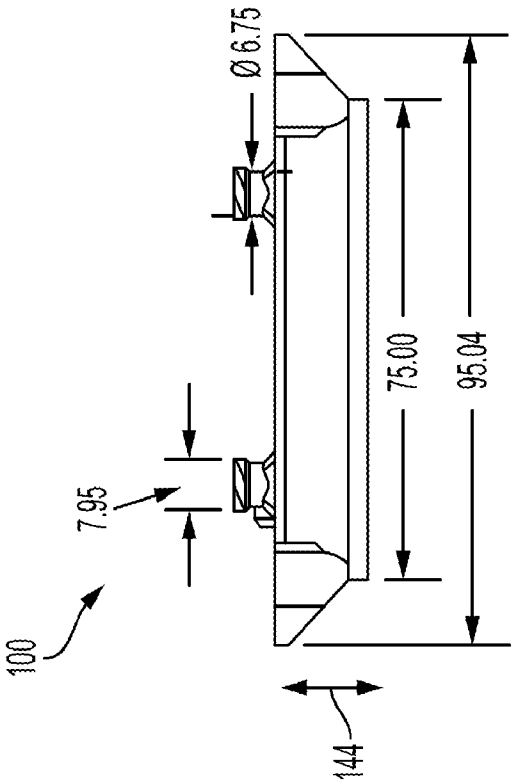

FIG. 1G

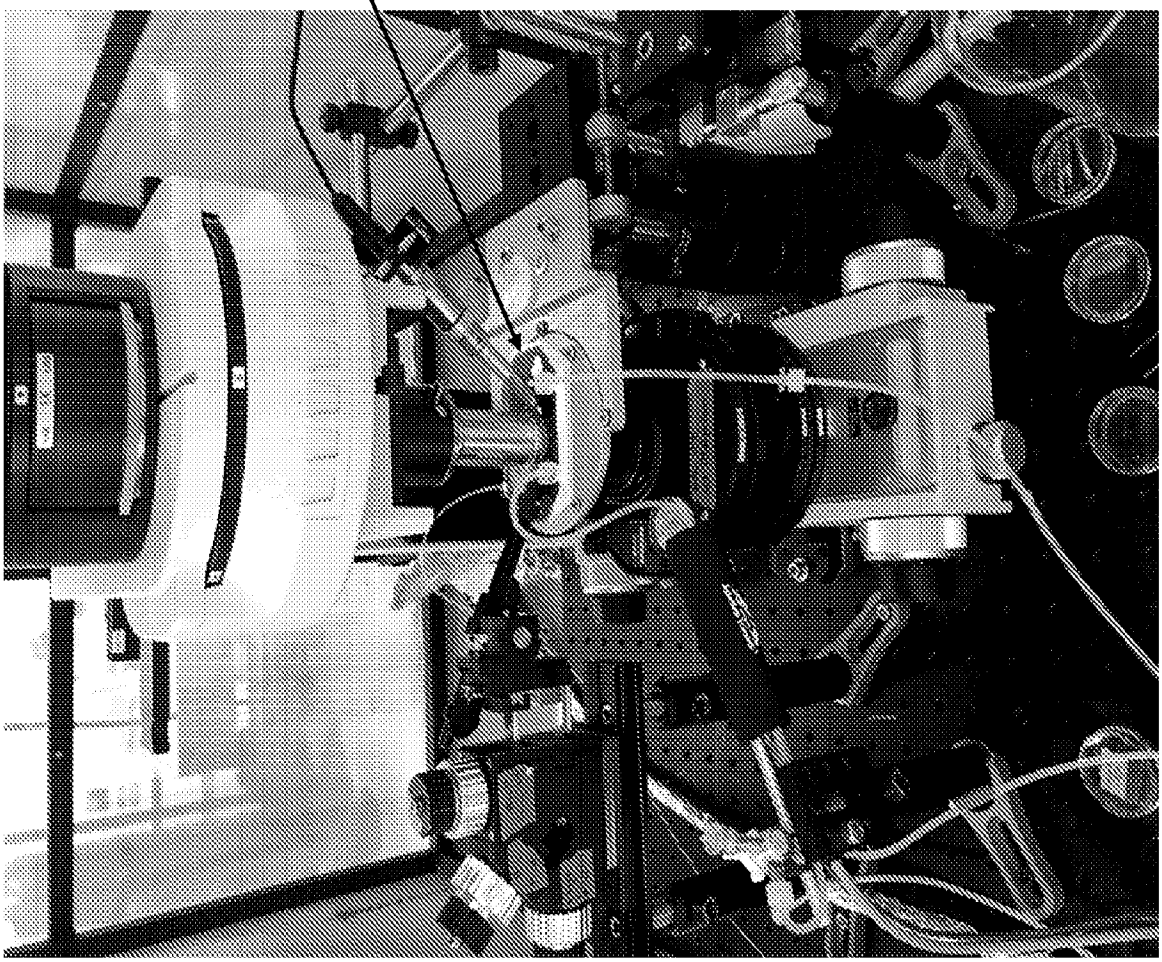

FIG. 2A

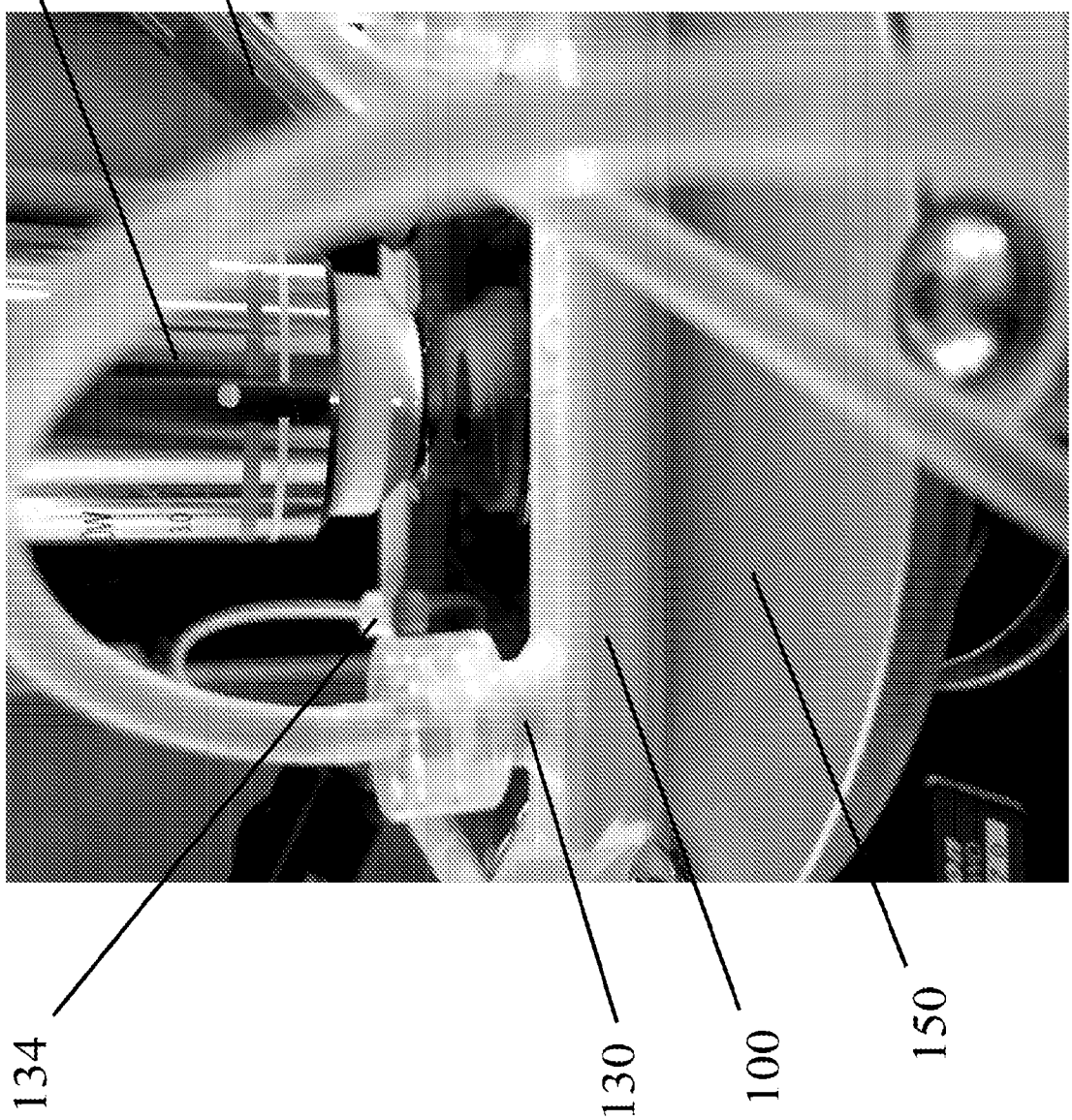

FIG. 2B

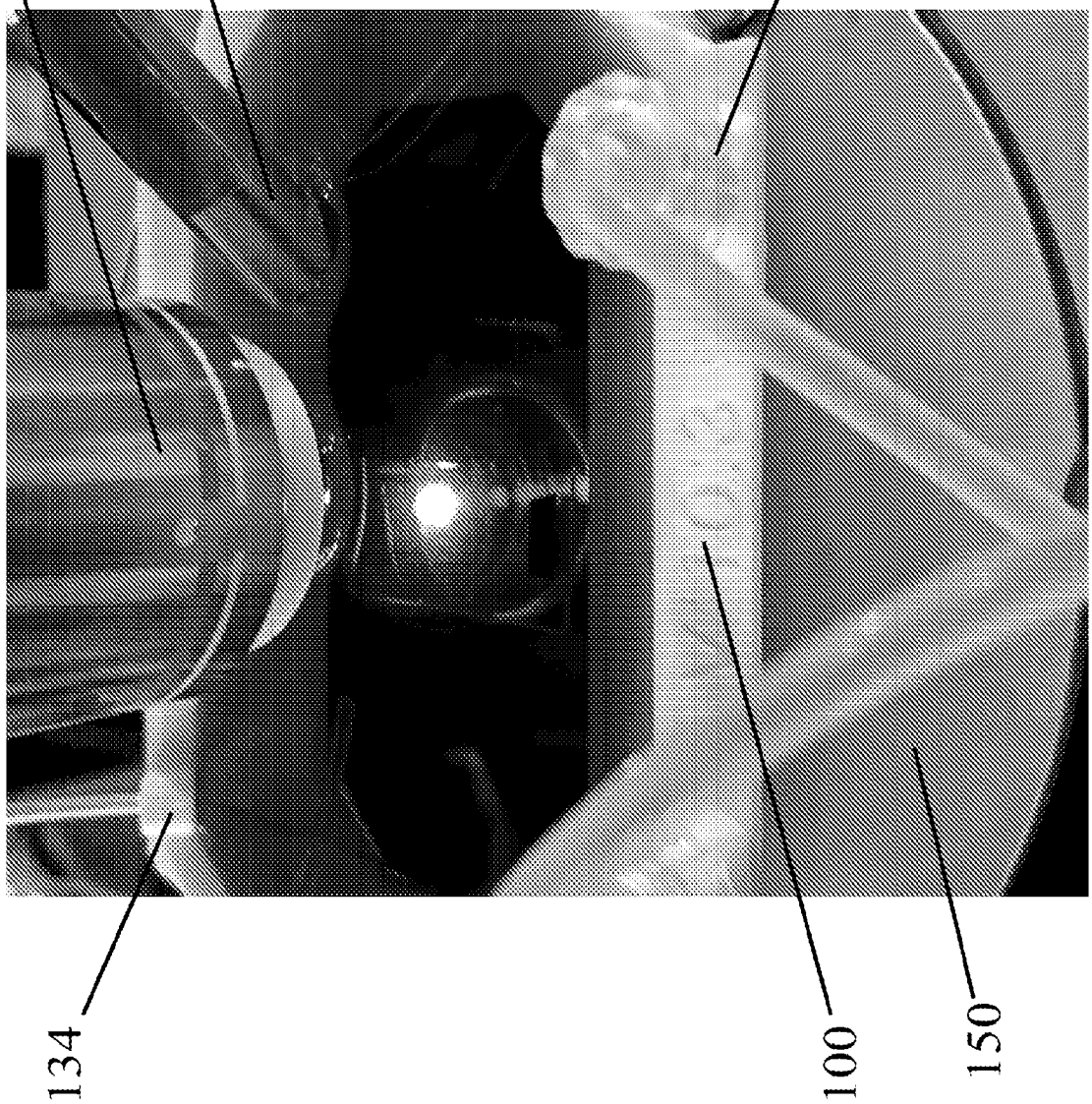

FIG. 2C

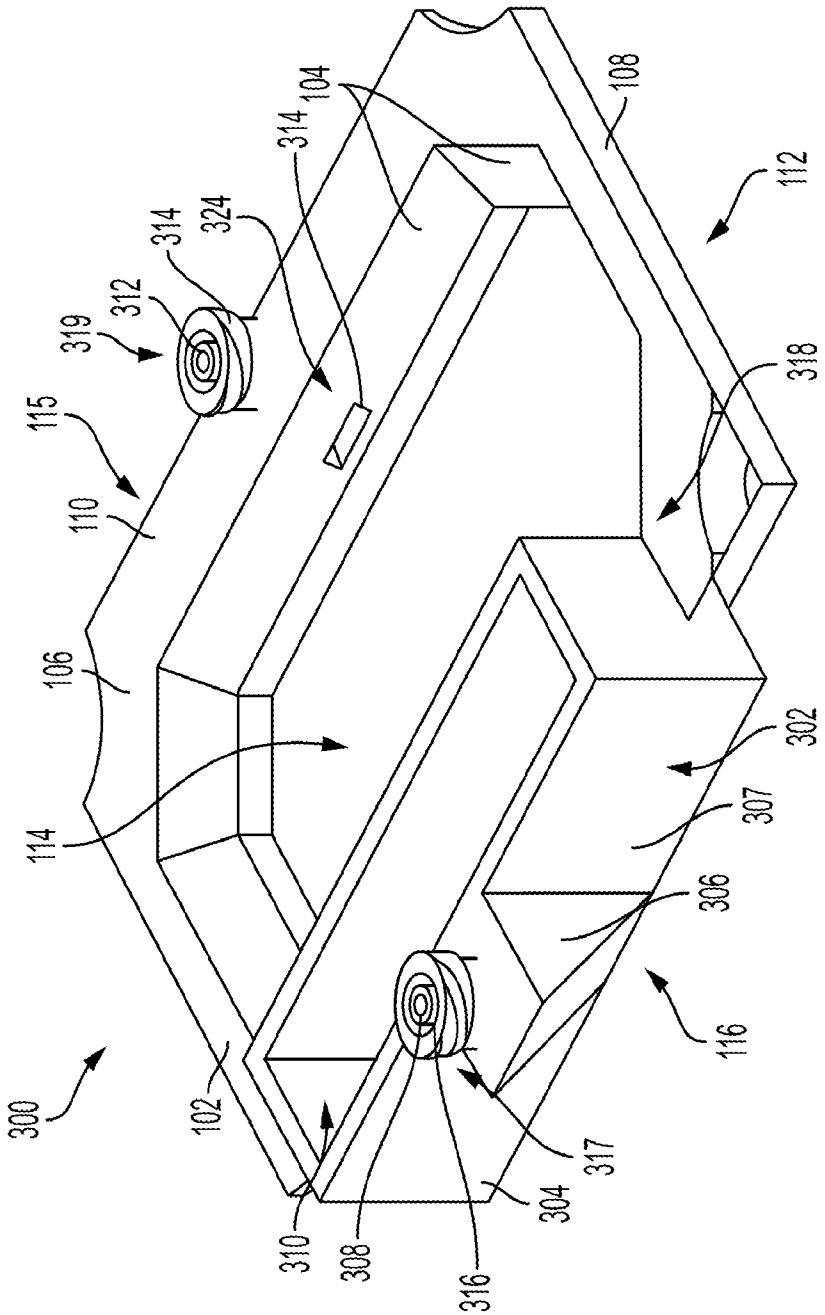
300
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FIG. 3A

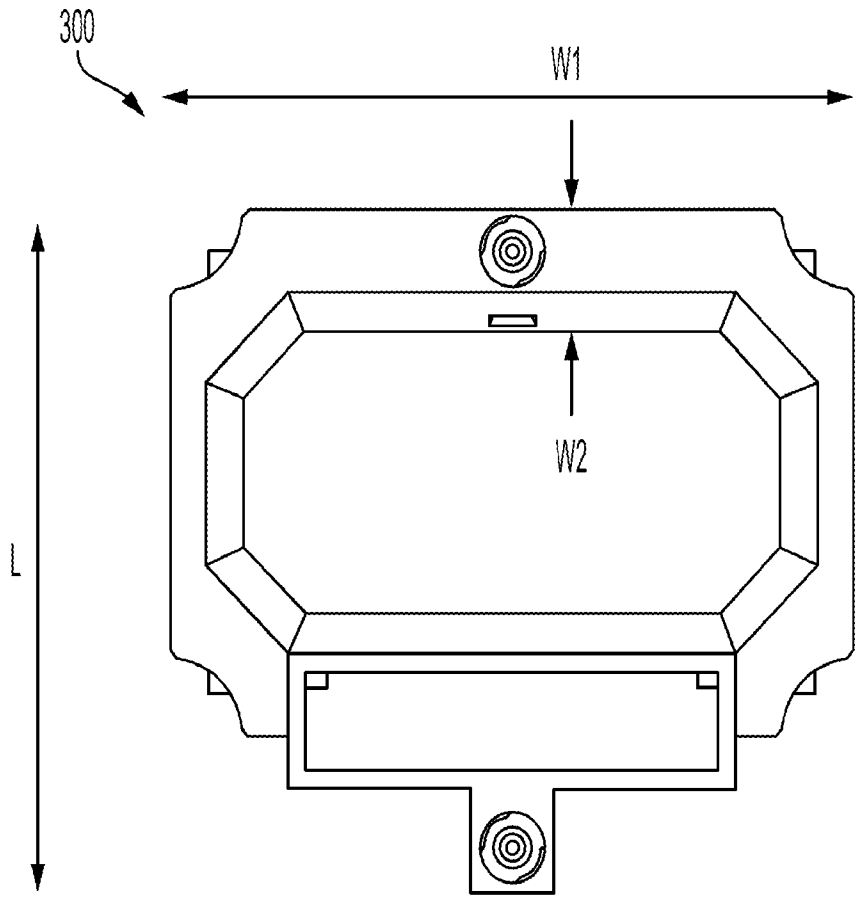

FIG. 3B

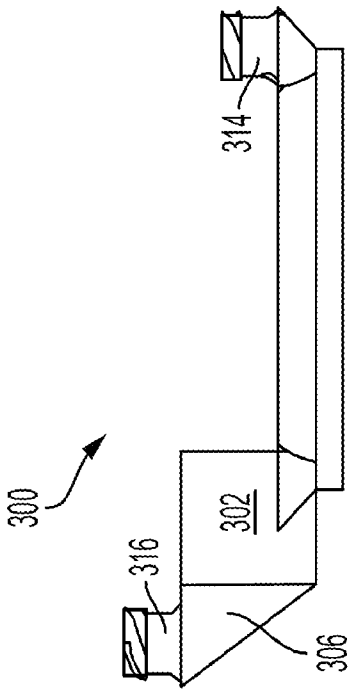

FIG. 3D

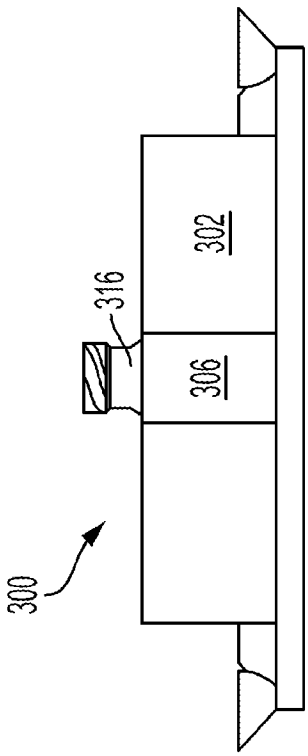

FIG. 3C

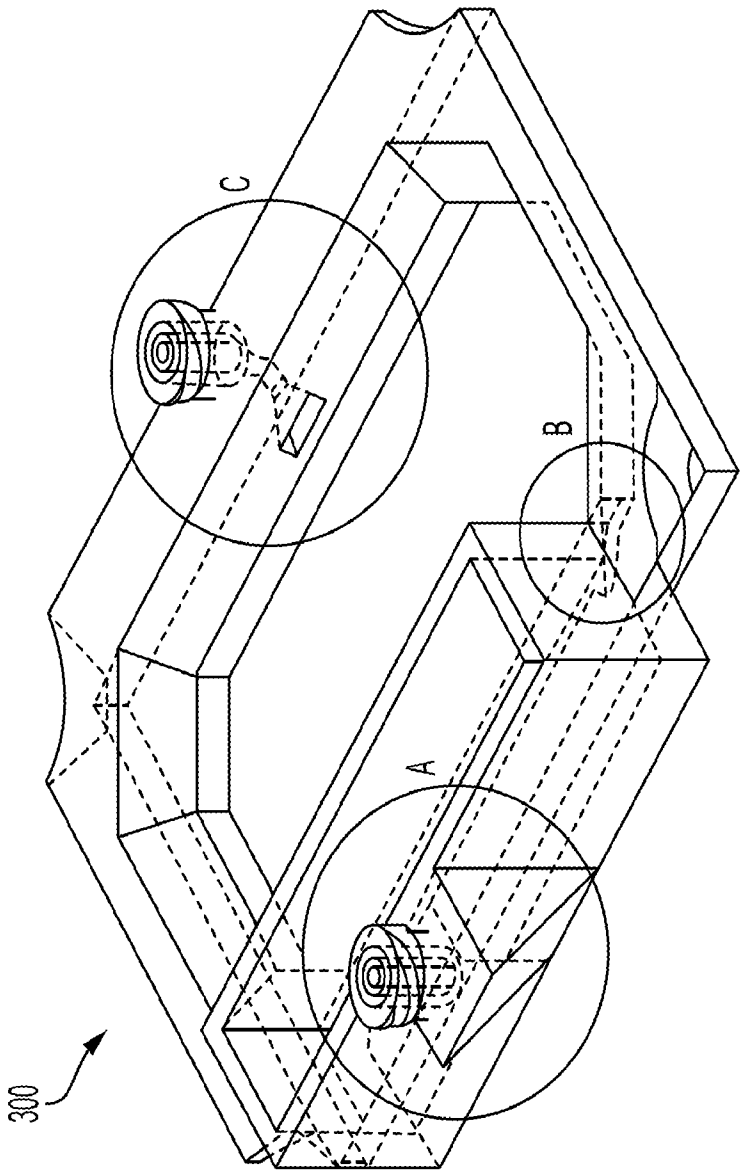

FIG. 3E

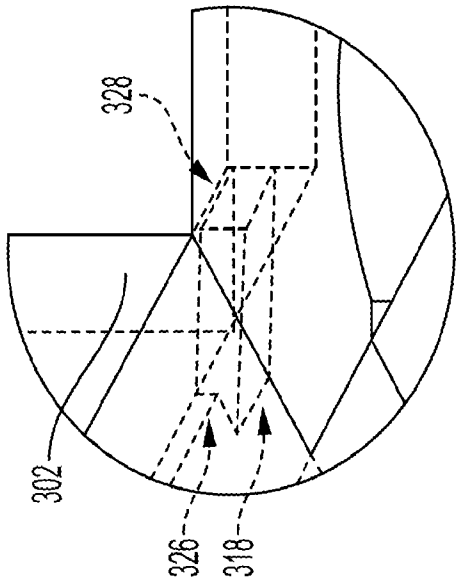

FIG. 3G

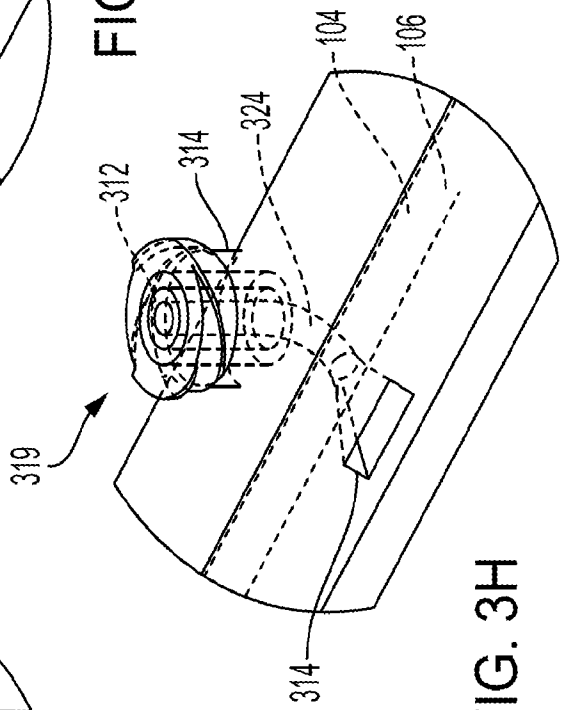

FIG. 3H

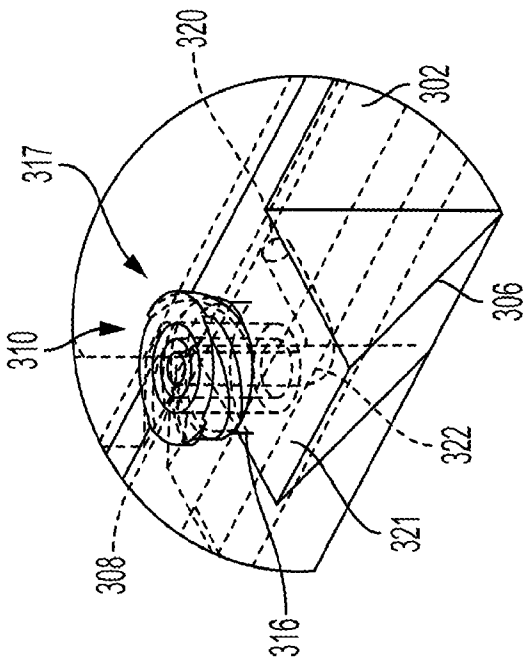

FIG. 3F

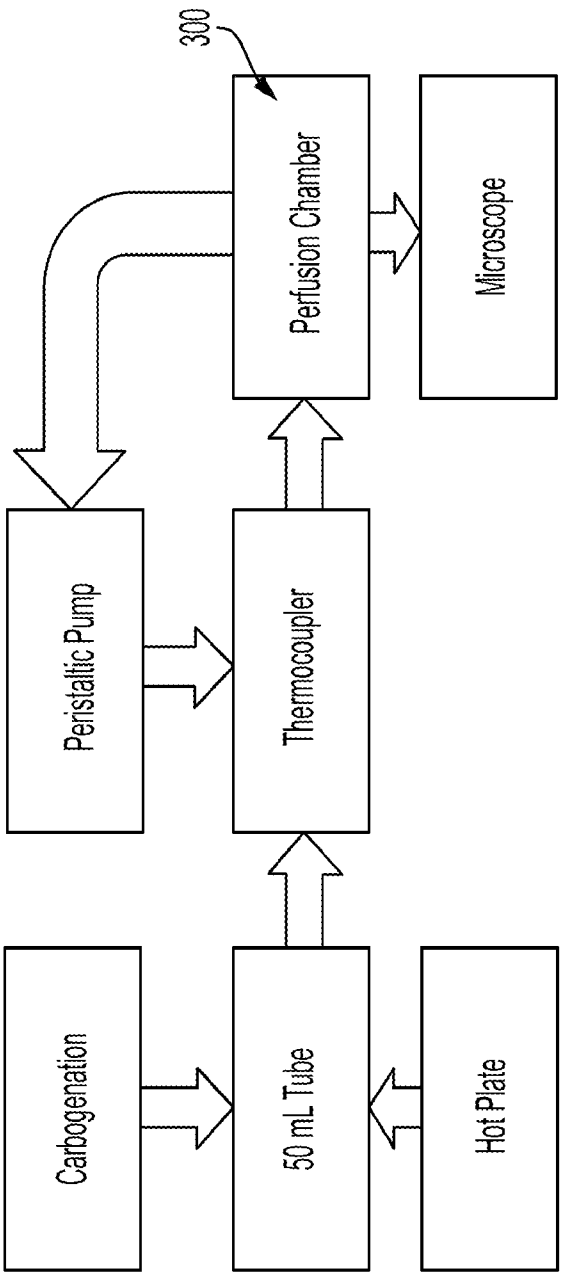
300
Perfusion Chamber
Microscope
Peristaltic Pump
Thermocoupler
Carbogenation
50 mL Tube
Hot Plate

FIG. 4A

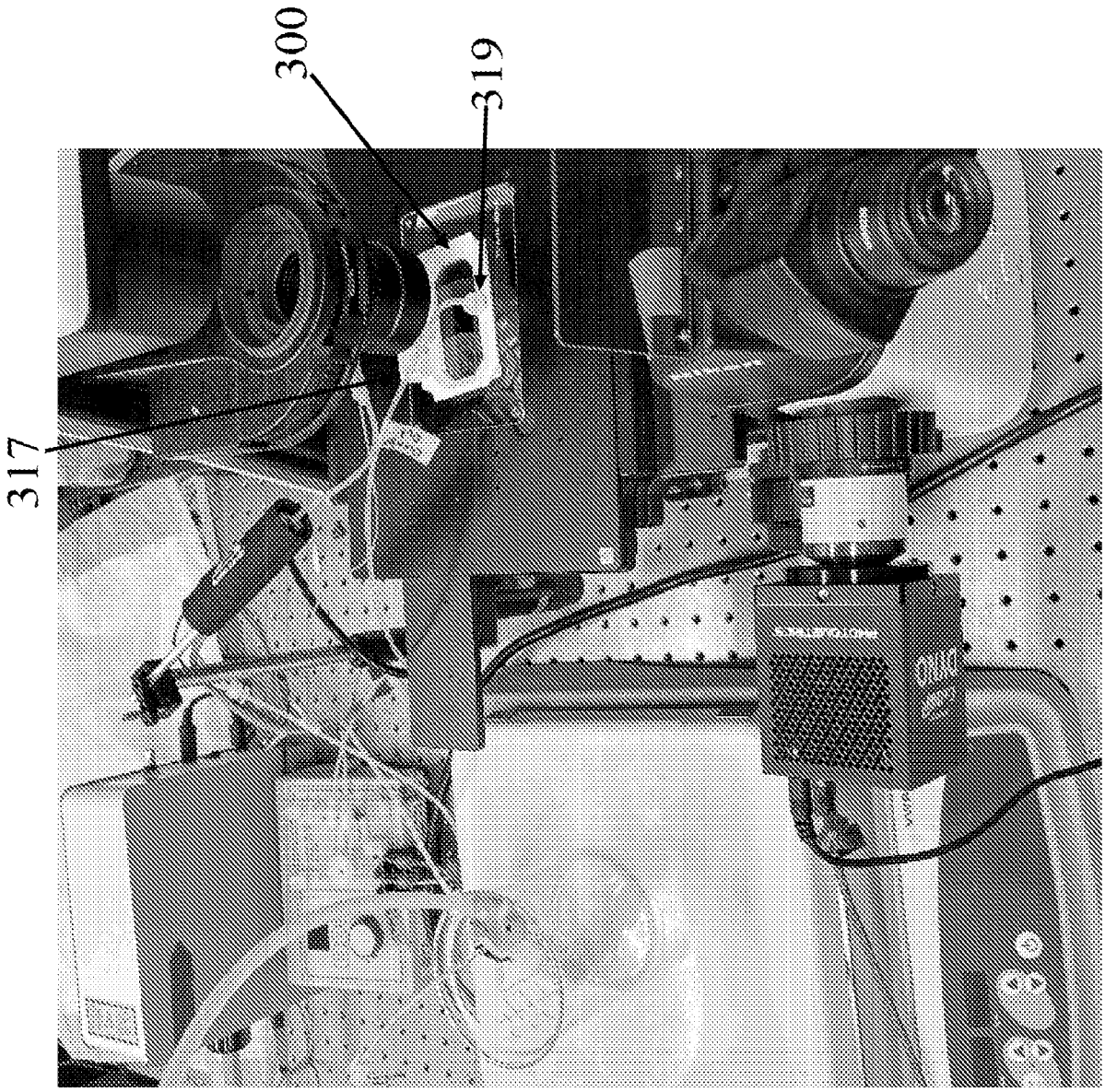

FIG. 4B