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(71) Applicant: **CELL MICROSYSTEMS, INC.** [US/US]; 2 Davis Drive, Research Triangle Park, North Carolina 27709 (US).

(72) Inventors: **CORSON, Andrew**; 1101 Rainesview Lane, Apex, North Carolina 27502 (US). **DELGADO, Jessie**; 1809 Pennypacker Lane, Durham, North Carolina 27703 (US). **DOBES, Nicholas C.**; 3702 Chimney Ridge Place, No. 103, Durham, North Carolina 27713 (US). **PACE, Gary M.**; 306 Oregon Circle, Cary, North Carolina 27511 (US). **BLACKBURN, Thomas**; 2609 Ladybank Lane, Fuquay Varina, North Carolina 27526 (US). **GEBHART, Steven C.**; 7114 Kobold Lane, Durham, North Carolina 27707 (US). **HALL, Robert C.**; 215 Lansbrooke Lane, Apex, North Carolina 27502 (US).

(74) Agent: **KNORS, Christopher J.**; P.O. Box 13706, Research Triangle Park, North Carolina 27709 (US).

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(54) **Title**: UNIVERSAL OBJECTIVE MOUNT

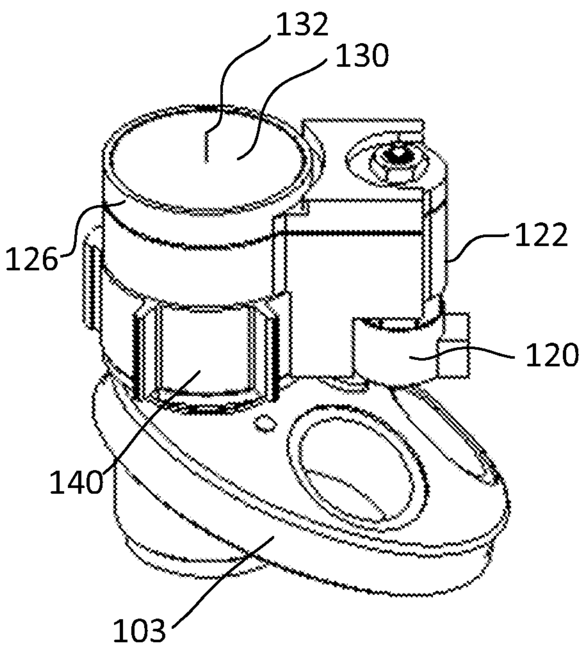

FIG. 3A

(57) Abstract: A mount suitable to be mounted to a microscope objective is described with a first member; a second member engaged with the first member; a first configuration where the second member has a first effective diameter; and a second configuration where the second member has a second effective diameter less than the first effective diameter for securably engaging an outer surface of a microscope objective. Methods of using the mount are also disclosed.

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UNIVERSAL OBJECTIVE MOUNT

CROSS-REFERENCE

This application is related to United States provisional application number 62/194,068, filed July 17, 2015, entitled "UNIVERSAL OBJECTIVE MOUNT", naming Andrew Corson, Jessie Delgado, Nicholas C. Dobes, Gary M. Pace, Thomas Blackburn and Steven C. Gebhart as the inventors. The contents of the provisional application are incorporated herein by reference in their entirety, and the benefit of the filing date of the provisional application is hereby claimed for all purposes that are legally served by such claim for the benefit of the filing date.

BACKGROUND

The selection and isolation of single cells from a mixed population is a common procedure performed throughout biomedical research. For example, during the development of cell lines that are genetically engineered, derived from stem cells, or grown from patient cell lines, single cells must be isolated and then cloned to form a homogeneous population. A variety of strategies exist to selectively identify and collect non-adherent cells from a mixed population, including fluorescence activated cell sorting (FACS), limiting dilution, panning, column chromatography and magnetic sorting; furthermore, new techniques based on microfluidics and dielectrophoresis show promise in this area. To address the need to collect pure or enriched populations of adherent cells, investigators use these procedures by disaggregating or stripping the cells from their growth surface to create cell suspensions. Unfortunately, enzymatic or mechanical release imposes significant drawbacks including loss of cell morphology, removal of cell surface markers, damage to cell membranes, alterations in cellular physiology and loss of viability.

To address this problem a cell isolation and recovery system was developed that uses polydimethylsiloxane (PDMS) microwell arrays comprising releasable, microfabricated elements, termed rafts, formed from biocompatible polystyrene or other materials. One version of such a system is called the CellRaft system. The rafts can be varied in size from tens to hundreds of microns to provide an adequate growth area for single cells or large colonies. The PDMS microwell array comprising the rafts can be visualized using, for example, an inverted microscope. Individual rafts containing the desired cells can released from the array upon mechanical distortion of the microwell array, for example by the application of a gradual energy such as mechanical pushing or continuous vibration. In one example, individual rafts containing the desired cells can be released from the array using mechanical pushing energy that is delivered to the raft using a probe attached to a microscope objective. The probe can be mounted to the objective using a variety of means such as a cylinder with the probe mounted in a window through which the desired raft can be visualized.

Unfortunately, microscope objectives can vary in outside diameter even within the same manufacturer, meaning that a cylindrical mount would have to be custom manufactured for each user. The customization of a cylindrical objective mount for each user is costly and time consuming.

Additionally, probes typically used in cell isolation and recovery systems are not readily positioned with or near an optical axis of a microscope objective when first used with a microscope, resulting in the need to make adjustments of the particular components and the focal plane of the microscope and that of the probe, which can unintentionally change or be lost due to movement of the array during the use of the microscope.

BRIEF SUMMARY OF THE DISCLOSURE

In a first embodiment, an objective mount is provided, comprising: a first member; a second member radially engaged with the first member; a first configuration where the second member has a first effective diameter; and a second configuration where the second member has a second effective diameter less than the first effective diameter for securably engaging an outer surface of an objective. Rotation of the first member relative to the second member about a common longitudinal axis of the first and the second members transitions the objective mount from the first configuration to the second configuration.

In one aspect, the first member is a collar and the second member is a collet. In a second aspect, alone or in combination with any other aspect, the second member comprises a radial wall having one or more axial slots therein forming flexible lobes, and the radial wall having a thickness formed of one or more radial wedges; and the first member comprises a complementary camming inner surface.

In another aspect, alone or in combination with any other aspect, the mount further comprises a cap member engagable with the first member to facilitate transition from the first configuration to the second configuration. In another aspect, alone or in combination with any other aspect, the second member comprises a tapered radial wall having one or more axial slots therein forming flexible lobes and threading on its exterior surface; and the first member comprises a complementary threaded inner surface.

In a second embodiment, the mount alone or in combination with any other aspect of the first embodiment is adapted to an assembly configured for attachment to an inverted microscope objective. In another aspect, the mount is adapted to an assembly configured for microscopic cell sorting.

In a third embodiment, an objective mount is provided, comprising a first member; a second member engaged with the first member; a first configuration where the at least one second member has an effective diameter; and a second configuration where the second member has a second effective diameter less than the first effective diameter for securably engaging an outer surface of an objective. The translation of the first member relative to a longitudinal axis of an objective transitions the objective mount from the first configuration to the second configuration.

In one aspect, the second member is a tapered radial wall having one or more axial slots therein forming flexible lobes; and the first member comprises a complementary unthreaded inner surface.

In another aspect, alone or in combination with any other aspect, the second member comprises one or more cam elements distributed radially along an inner diameter of the second member; and the mount further comprises a ring member positioned between the first member and the second member, the ring member engaged with the first member and configured to translate axially along an outside diameter of the second member and to engage the one or more cam elements, causing at least a portion of the one or more cam elements to deflect inwardly in the second configuration.

In another aspect, alone or in combination with any other aspect, the second member comprises one or more elastomeric elements distributed radially in grooves in an inner surface of the first member. In another aspect, alone or in combination with any other aspect, the second member comprises one or more leaf spring elements distributed about an inner surface of the first member. In another aspect, alone or in combination with any other aspect, the second member comprises one or more spring lobes projecting from an inner surface of the first member.

In another aspect, alone or in combination with any other aspect, the second member comprises one or more whisker elements distributed about an inner surface of the first member. In another aspect, alone or in combination with any other aspect, the second member comprises one or more leaf spring segments that project from an inner surface of the first member.

In a fourth embodiment, the mount alone or in combination with any other aspect of any one of the previous embodiments is adapted to an assembly configured for attachment to an inverted microscope objective. In another aspect, the mount is adapted to an assembly configured for microscopic cell sorting.

In a fifth embodiment, an objective mount is provided, the objective mount comprising a first member; a second member engaged with the first member; a first configuration where the second member has an effective diameter; a second configuration where the second member has a second effective diameter less than the first effective diameter for securely engaging an outer surface of an objective; wherein inflation of the second member with a fluid transitions the objective mount from the first configuration to the second configuration. In another aspect, the second member is an inflatable bladder. In another aspect, alone or in combination with any of the previous aspects, the second member is distributed radially along an inner diameter of the first member. In another aspect, removal of an amount of fluid from the second member transitions the mount from the second configuration to the first configuration. In another aspect, alone or in combination with any of the previous aspects, the mount further comprises a valve for inflating and deflating the second member.

In a sixth embodiment, a base member of an objective mount is provided. The base member comprises a thickness and a first bore extending therethrough, wherein the first bore defines an inner first perimeter, a plurality of second bores defined in the base member, wherein each of the second bores extend from the inner perimeter into the base; a plurality of moveable members, each member located in one of said second bores and comprising a first end adjacent to the inner perimeter and a second end extending into the base member; and bias means associated with each of said movable members, each of said bias means located in one of said second bores adjacent to the second end of a respective moveable member located in the respective second bore, said bias means biasing the respective moveable member inwardly toward the first bore, wherein one or more of the moveable members are configured to move independently and relative to each other in the respective bores and relative to the inner perimeter such that the first ends of the moveable members are spaced apart from each other to form a variable inner second perimeter, where the variable inner second perimeter is smaller than the first inner perimeter for securably engaging an outer surface of an objective.

In one embodiment, alone or in combination with any of the other embodiments, a method is provided comprising providing a mount as defined in any one of above embodiments and aspects, the mount configured to couple with a probe of a release device assembly for cell sorting and self-aligning of a probe with or near an optical axis of a microscope objective.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the present disclosure, reference should now be had to the embodiments shown in the accompanying drawings and described below. In the drawings:

FIG. 1 is a schematic view of an exemplary inverted microscope with imaging objectives.

FIGs. 2A, 2B, 2C and 2D are a schematic view of an exemplary release device with the presently disclosed mount installed on an inverted microscope.

FIGs. 3A, 3B are an assembled and exploded view, respectively, of the release device assembly with the presently disclosed mount, and its interface with a microscope objective.

FIGs. 4A, 4B are a schematic views of the interaction between the needle platform and base parts through their connection to an actuator.

FIGs. 5A, 5B is a schematic view of the interface between the base and mount parts.

FIGs. 6A, 6B, and 6C are a schematic views of a mount embodiment.

FIG. 7 is a schematic view of an alternative mount embodiment.

FIG. 8 is a schematic view of an alternative mount embodiment.

FIGs. 9A, 9B are schematic views of an alternative mount embodiment.

FIG. 10 is a schematic view of an alternative mount embodiment.

FIG. 11 is a schematic partial sectional view of an alternative mount embodiment.

FIGs. 12A, 12B are a schematic views of an alternative mount embodiment.

FIGs. 13A, 13B are schematic views of an alternative mount embodiment.

FIGs. 14A, 14B are schematic views of an alternative mount embodiment.

FIGs. 15A, 15B are schematic views of alternative mount embodiment.

FIGs. 16A, 16B are schematic views of an alternative mount embodiment.

FIGs. 17A, 17B are schematic views of an alternative mount embodiment.

FIGs. 18A, 18B are schematic views of an alternative mount embodiment.

FIGs. 19A, 19B are exploded and top plan views, respectively, of release assembly device platform embodiment.

DETAILED DESCRIPTION

Accordingly, what is provided herein is an adjustable effective diameter mount for attaching and/or releasing from a microscope objective. The mount is suitable for use with raft release mechanisms and systems and can be used with a range of objective diameters.

The present disclosure is now described more fully hereinafter with reference to the accompanying drawings, in which embodiments of the disclosure are shown. This disclosure may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather these embodiments are provided so that this disclosure will be thorough and complete and will fully convey the scope of the disclosure to those skilled in the art.

Like numbers refer to like elements throughout. In the figures, the thickness of certain lines, layers, components, elements or features may be exaggerated for clarity. Where used, broken lines illustrate optional features or operations unless specified otherwise.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the disclosure. As used herein, the singular forms "a," "an" and "the" are intended to include plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, components and/or groups or combinations thereof, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components and/or groups or combinations thereof.

As used herein, the term "and/or" includes any and all possible combinations or one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

As used herein, the term "fluid" is inclusive of gases, liquids, or mixtures thereof.

Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specifications and claims and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. Well-known functions or constructions may not be described in detail for brevity and/or clarity.

It will be understood that when an element is referred to as being "on," "attached" to, "connected" to, "coupled" with, "contacting," etc., another element, it can be directly on, attached to, connected to, coupled with and/or contacting the other element or intervening elements can also be present. In contrast, when an element is referred to as being, for example, "directly on," "directly attached" to, "directly connected" to, "directly coupled" with or "directly contacting" another element, there are no intervening elements present. It will also be appreciated by those of skill in the art that references to a structure or feature that is disposed "adjacent" another feature can have portions that overlap or underlie the adjacent feature.

Spatially relative terms, such as "under," "below," "lower," "over," "upper" and the like, may be used herein for ease of description to describe an element's or feature's relationship to another element(s) or feature(s) as illustrated in the figures. It will be understood that the spatially relative terms are intended to encompass different orientations of the device in use or operation in addition to the orientation depicted in the figures. For example, if the device in the figures is inverted, elements described as "under" or "beneath" other elements or features would then be oriented "over" the other elements or features. Thus the exemplary term "under" can encompass both an orientation of over and under. The device may otherwise be oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein interpreted accordingly. Similarly, the terms "upwardly," "downwardly," "vertical," "horizontal" and the like are used herein for the purpose of explanation only, unless specifically indicated otherwise.

It will be understood that, although the terms first, second, etc., may be used herein to describe various elements, components, regions, layers and/or sections, these elements, components, regions, layers and/or sections should not be limited by these terms. Rather, these terms are only used to distinguish one element, component, region, layer and/or section, from another element, component, region, layer and/or section. Thus, a first element, component, region, layer or section discussed herein could be termed a second element, component, region, layer or section without departing from the teachings of the present disclosure. The sequence of operations (or steps) is not limited to the order presented in the claims or figures unless specifically indicated otherwise.

"Objective" provides for the collection of light from the sample. The objective is usually a cylinder or other shape configured for containing one or more lenses. The term "objective" and the phrase "objective lens" may be used interchangeably and either is inclusive of an objective with one or more lenses. The lenses of the objective are typically made of glass and/or plastic.

"Cells" for carrying out the present disclosure are, in general, live cells, and can be any type of cell, including animal (e.g., mammal, bird, reptile, amphibian), plant, or other microbial cell (e.g., yeast, gram negative bacteria, gram positive bacteria, fungi, mold, algae, etc.).

"Liquid media" for carrying out the present disclosure, in which cells are carried for depositing on an array as described herein (and specifically within the cavities of the microcups) may be any suitable, typically aqueous, liquid, including saline solution, buffer solutions, Ringer's solution, growth media, and biological samples such as blood, urine, saliva, etc. (which biological samples may optionally be partially purified, and/or have other diluents, media or reagents added thereto).

"Release device" and "release device assembly" are used interchangeably and are inclusive of an apparatus, device, tool, or instrument capable of dislodging a support structure or "rafts" from their underlying well array, causing the rafts to be free and/or unattached from the well in solution. The free and/or unattached raft can be collected and/or removed from the array and solution for transfer to a separate vessel.

The mount members, just as the coupled microwell array, can be of metal and/or plastic construction. The mount members can be produced by any suitable manufacturing technique, such as machining, computer numerical control (CNC), soft lithography, molding, injection molding, embossing, (3D) printing, or microprinting.

Suitable metallic materials for the manufacture of the mount members include, but are not limited to, aluminum, such as 6061-T651, 7075-651 grade, brass, copper, low carbon steel, magnesium, stainless steel such as 17-4,316/316 L and 304/304L, and titanium alloys such as Ti-6Al-4V; Grade 5. Other metals and metal alloys may be used.

Suitable nonmetallic materials for the manufacture of one or more of the mount members include, but are not limited to, thermoplastics, engineering resins, filled or reinforced resins, and epoxies. Examples of nonmetallic materials for the manufacture of one or more of the mount members include styrene butadiene (SB), thermoplastic elastomer/thermoplastic vulcanizates (TPE/TPV), acrylonitrile butadiene styrene (ABS), flame retardant ABS, DELRIN™ 20% glass filled acetal), acetal copolymers, chlorinated polyvinyl chloride (CPVC), high density polyethylene (HDPE), low density polyethylene (LDPE), nylon such as nylon 6, nylon 6/6 and glass filled nylon, polycarbonate (PC), glass filled polycarbonate, polyether ether ketone (PEEK), polyetherimide (PEI), ULTEM™ (30% glass filled PEI), polyethylene terephthalate (PET), polybutylene terephthalate (PBT), polyethylene naphthalate (PEN), polyethylene terephthalate glycol (PETG), polymethyl methyl acrylate (PMMA)/acrylic copolymer, polypropylene (PP) homopolymer, polypropylene copolymers, NORYL™ (polyphenyl ether (PPE) + polystyrene (PS), RADEL ™polyphenylene sulfone (PPSU), high-impact polystyrene (HIPS), UDEL™ (polysulfone), TEFLON™ (polytetrafluoroethylene; PTFE), polyvinyl chloride (PVC), ABS/PC blends, engineered thermoplastic polyurethane (ETPU), liquid crystal polymers (LCPs), cyclic olefinic copolymers (COC's) and ultrahigh molecular weight polyethylene (UHMWPE).

A wide range of epoxies can be used for the manufacture of one or more of the mount members, including the epoxy novolac resins, such as without limitation, EPON 1001F, 1009F, and 1007F. These resins can be used alone or with crosslinkers. Preformulated epoxies, such as Loctite Hysol and other medical device epoxies can also be used. Medical grade polymers of polystyrene (including copolymers thereof, such as poly(styrene-co-acrylic acid) (PS-AA)), poly(methyl methacrylate), polycarbonate, and cyclic olefin copolymer can be used as raft materials and/or mount members. Biodegradable polymers and hydrogels can also be used as mount members. The mounts can be formed of or from a single material or can be a composite of two or more layers of different materials, etc.

The mounts herein disclosed are useful in combination with microwell arrays containing rafts as described above, and can be used to improve methods for collecting or culturing cells or cell colonies, generally involving the depositing a liquid media carrying the cells (including but not limited to non-adherent cells) on the microwell array so that the cells settle on or adhere to the rafts and then after microscopic examination releasing a selected raft having the cells thereon by application of release energy to the selected raft to release the raft from the well in the microwell array in which it is received. Such release energy may be applied as a burst of energy, or may be applied in a gradual manner. The gradual application of release energy can be achieved by, for example, gradual mechanical pushing or vibrating. In some embodiments a probe is used that does not pierce the microwell array; e.g., the probe pierces the microwell array and contacts and dislodges the selected raft. In such releasing arrays, the pushing is aided by or guided by a microscope (e.g., an optical microscope, a fluorescent microscope). In such embodiments, the probe may be connected to or mounted on the microscope objective in a configuration that permits visualization of the selected carrier to be dislodged by the probe through the microscope objective as an aid or guide to carrying out the pushing or dislodgement of the selected carrier. The microscope may be a simple manual optical microscope, with pushing carried out manually, or a partially or fully automated microscope with pushing or dislodgement of the carrier achieved or carried out in an automated manner by movement of the objective (e.g., with a manual or automated XYZ drive stage, and/or with a drive or drive assembly included in the microscope objective assembly).

In one embodiment of a release device mounted on a microscope objective to provide the z-axis manipulation of the release probe to dislodge a raft, a suitable needle is inserted into a cavity in a small transparent polycarbonate plate or other suitable material. Then the polycarbonate plate with fixed needle is attached or mounted to the microscope objective using a cylindrical cap made from a suitable material, such as a thermoplastic or polystyrene, is designed and manufactured by 3D printing, milling, injection molding or other means. In one embodiment, the release device incorporates a motorized device to achieve a greater needle displacement than can be achieved by movement of the microscope objective, or it can be used to move the release needle without changing the focusing of the objective on the microwell array and rafts selected for release.

The presently disclosed mount, couplable to such a release device is designed to fit a range of microscope objective diameters, and is fitted to the microscope objective through either active or passive means. In one aspect, presently disclosed mount, is further configured to permit the self-alignment of the release probe near or with the optical axis of the microscope objective for such release devices. Alignment of the release probe with the optical axis of the microscope objective is important in order to align the probe with the field of view for proper targeting of the raft to be released. It is also preferred that the distance between the focal plane of the objective and the tip of the probe be sized or dimensioned (e.g., about 100 times or more of the depth-of-field of the objective) to ensure that the probe itself is not seen in the focused image when the probe is in the rest position.

The detailed description of the following embodiments provides the information to enable those skilled in the art to practice the disclosure and illustrate the best mode of practicing the disclosure. Upon reading the following description in light of the accompanying drawing figures, those skilled in the art will understand the concepts of the disclosure and will recognize applications of these concepts not particularly addressed herein. It should be understood that these concepts and applications will fall within the scope of the disclosure and the accompanying claims.

With reference to prospective view FIG. 1 and views FIG. 2A, 2B, 2C, and 2D, the present disclosure described herein relates to a mount that is configured to couple with or be integral with a release device assembly 111 so as to attach the release device to the imaging objective of a standard inverted microscope 100. By way of example, the presently disclosed mounts are configured to be used in conjunction with the release device systems described in U.S. Patent Number 9,068,155 and WO 2013/181506, each of the above incorporated by reference herein in its entirety, e.g., as part of the “CellRaft” system. Such a release device assembly, for example, comprises a base 122, a needle probe 132 and window 130 attached to a platform 126, an actuator 120 that is received by female thread pocket 121 and secured via distal end 120a with nut 128. Actuator 120 linearly translates the platform relative to the base 122. Guide rods 124 (only one shown) received by slip fit holes 125 are positioned between the platform and base which constrain lateral movement. FIG. 2B shows a system comprising an exemplary micro well array 105, arranged in a cassette 105a, placed on microscope stage 101 with an objective mount 200 shown mounted to objective 103 of inverted microscope 100.

Mount 200 attaches the base 122 to an outer diameter of the microscope objective 103. Nominal outer diameters for microscope objectives can vary by several millimeters between brands and between magnification powers within a given brand. Actual outer diameter measurements can also vary up to a millimeter or more between units for a given brand and magnification power. In some embodiments, a compressive element of the mount 200 is configured to provide mechanical adjustment around the outer diameter of the microscope objective to compensate for diameter variation and to simultaneously self-align the needle probe of the release device assembly within the field of view of the microscope imaging optics.

Embodiments of the presently disclosed mounts, CellRaft arrays, release device assembly, and methods described above and in this application provides a viable method for isolating single cells, e.g., for downstream molecular analysis. The embodiments of the presently disclosed mounts used in combination with the CellRaft system including the arrays and release device assembly can be used for a number of purposes including, rare cell sorting and the like. Other release device assemblies utilizing a linearly actuated probe can be used with the presently disclosed mounts.

A typical inverted microscope 100 is illustrated in FIG. 1. The specimen to be viewed is mounted on the stage 101 with opening 101a, and a lens objective 103 is attached below the stage for high-magnification imaging of the specimen. The objective transmits an image of the specimen to one or more viewing bodies, which can include one, two, or three oculars 102 for manual viewing or a film-based or digital camera for capturing high-definition images. The microscope often includes coarse and fine focus knobs 104, whose rotation moves the specimen stage or lens objective relative to one another, allowing for imaging focus at the specimen.

FIGs. 2A-2D illustrate a release device assembly 110 with a CellRaft array 105 as specimen and presently disclosed mount 200 installed on an inverted microscope. In some embodiments, mount 200 slides over the lens objective 103 and is oriented with the needle probe 132 positioned below the bottom surface of the array 105 housed in a cassette 105a within a distance less than the maximum vertical travel range of the actuator 120, allowing the tip of the needle probe 132 to puncture the CellRaft array and release individual rafts. Fig. 2D depicts a view of the release device assembly 110 opening 101 a with the CellRaft array 105 removed from the stage 101. The window 130 of the release device that houses the needle probe 132 can be designed flat and transparent to minimize degradation of image transmission through the microscope optics.

An embodiment of the mount 200 coupled to a release device assembly is shown in FIGS. 3A, 3B, 4A, 4B, 5A, 5B, 5C, 6A, 6B and 6C. The release device assembly 110 comprises a base 122, a needle probe 132 and window 130 attached to a platform 126, an actuator 120 that linearly translates the platform relative to the base, guide rods 124 between the platform and base, and a mount 200, having a first member 140 radially engaging a second member 150 that rigidly attaches the base to the microscope objective. In this embodiment, mount 200 provides a compressive force created by collet and collar members as discussed below.

Fig. 3A shows and assembled release device and presently disclosed mount embodiment attached to a microscope objective. FIGs. 3A, 3B, 4A, 4B, depict the base 122 and needle platform 126 are attached via the actuator 120, which is threaded into a female threaded pocket 121 on the base and secured to the platform by a nut 128. Guide rods 124 are press fit into the underside of the platform and ride within slip fit holes 125 in the base, to ensure one-dimensional movement of the two parts relative to one another. The base 122 contains an aperture 127 coincident with the imaging axis of the microscope objective. A flat, transparent window 130 is affixed to the needle platform 126, and the needle probe 132 is secured into a clearance hole in the center of the window (not shown). The collet or second member 150 is designed to circumferentially engage the outer diameter of the microscope objective 103.

As shown in FIGs. 5A-5C, the collet 150 contains female axial slots 152 to accept complementary male tabs 153 on the underside of the base 122 (FIG. 4B), the features 152, 153 are dimensioned to create a friction fit between the two parts. To enable adjustability of the collet inner effective diameter, lobes 156 are created through axial release cuts 154 to allow flexing of the lobes. Radial slots 158 and relief cuts in the outer diameter of the collet 150 further increase the flexibility of the lobes 156.

By inner "effective diameter" or "inner effective perimeter" it is meant the inner void, hole, or spacing of the second member (or collet) in a particular configuration. In some embodiments, the effective diameter or perimeter is determined from a void or space that is not actually circular, for example, where the inner surface of the collet can include lobes, protrusion members or the like, but which otherwise provide physical points extending into the void or space of the collet that can be used to construct an effective diameter. Likewise, in the event that the objective is not actually cylindrical, e.g., hexagonal, etc., the feature of the collet can perform the equivalent compressive force function. The effective diameter or perimeter can be configured to be variable as described below.

As shown in FIGs. 6A-6C, radial wedges 159 on the outer diameter of the collet or second member 150 and radial wedges 149 on the inner diameter of the collar or first member 140 create complementary camming surfaces 148 that translate torque of the collar to inward flexion of the collet lobes 156. The flexion, in turn, creates a compression force onto the outer surface of the microscope objective 103 and generates a securing friction between the two surfaces. The adjustability of the inner diameter of the collet 150 is dictated by the thickness changes in the radial wedges 149, 159 on the collet and collar, respectively.

The top and bottom surfaces of collar 140 are captured between the bottom lip 151 of the collet 150 and the underside of the base 122 (not shown). Male tabs 142 on the outer diameter of the collar 140 facilitate rotation by a user and the third tab 143 serves as a stop to prevent over-rotation and reset of the camming surfaces. When mounted to microscope objective 103 and fully assembled, the mount 200 accepts the objective through its the inner diameter of the collet 150 and allows the objective 103 to be fully inserted until its top surface rests against the underside of the base 122 for viewing of the sample in the CellRaft array, for example.

FIG. 7 shows mount 300 having the same or essentially the same internal components and corresponding function as shown in in FIGS. 3 & 6, which allow the user to rotate the collar 340 relative to the collet 350 for inner diameter adjustability. In mount 300, an adjustment cap 160 is provided that seats in a groove in the upper surface of the needle platform 126 (not shown). Adjustment cap 160 is shown with optional indicia 161 to indicate to user which direction to turn cap 160. A forked feature 162, projecting longitudinally from the outer diameter of the adjustment cap 160, engages a tab 341 on the outer diameter of the collar 340. Rotation of the adjustment cap 160 rotates the collar 340 relative to the collet 350 and transitions the mount to a second configuration from a first configuration where the collet inner effective diameter is reduced relative to that of the effective inner diameter in the first configuration. This mechanism provides for a reversible attachment onto the outer surface of the microscope objective 103. A hood feature 171, on the outer diameter of the base 122, prevents the forked feature 162 from over-rotating the collar and resetting the camming surfaces in the collet 350 and collar 340.

FIG. 8 shows cap 260 (shown in partial cut-away view) and mount 400. In this embodiment, the rotation force is transferred through axial grooves 442 on the outer diameter of the collar 440 designed to accept complementary male tabs 262 along the inner diameter of adjustment cap 260. Mount 400 and cap 260 function similar to the mount shown in FIG. 7 as to attaching and releasing from objective 103 using camming surfaces 451.

In the embodiments of FIGS. 4-8 described above, the exemplary mount embodiments provide for accommodating different diameter objectives while maintaining radial symmetry relative to the objective. FIGs. 9-18 show alternative embodiments of mounts and methods to provide compression force to attach a coupled release device to the microscope objective in a manner that also accommodates variation in diameter while maintaining radial symmetry relative to the objective.

Thus, FIGs. 9A, 9B show an alternative embodiment mount 500 of a compressive element with “active” inner effective diameter adjustment using a tapered thread collet 550 and collar 540 design. As in a previous embodiment, the collet 550 possesses radial release cuts 552 to form multiple lobes 556 that can flex inward during a transition from a first configuration to a second configuration so as to reduce its inner effective diameter from that of the first configuration. As the male collet 550 is threaded with threads 541 of the female collar 540, the tapered thread form produces an inward compressive force on the collet lobes 556, which is transferred to the outer diameter of the microscope objective 103. The adjustability in inner diameter can be adjusted by the taper on the threads of the collet 550.

Mount 600 in FIG. 10 is structurally similar to the tapered collet and collar construction in FIG. 9, but uses unthreaded tapered surfaces 640, 650 for active adjustment of the mount about the objective. In this embodiment, the tapered surface converts axial compression from rod 660 (rather than threading torque) to inward force on the objective. Mount 600 relies on friction between the male part 650 and female part 640 surfaces, respectively, to maintain their relative position and the force on the objective. Male part 650 can be either rigid and slotted as in previously described mounts, or hollow and flexible to allow part deformation. As in FIG. 9A, the diameter adjustability is dictated by the taper angle and is readily determinable from the diameter of the objective.

Mount 700 as shown in FIG. 11 depicts another active adjustment function that uses one or more cams 710 as the second member, distributed radially along the inner diameter of the collar or first member 740. Cams 710 pivot about member 725. A ring 720 is positioned along the outside diameter of the collar to engage the outer cam surfaces by being able to slide axially, causing the cams to deflect toward the center of the collar and decrease the effective diameter (as measured from the points on the cams 710 protruding into the center of the collar 740) that press against the outer surface of the objective. The force generates friction between the cams 710 and objective 103 to hold the release device onto the objective. The diameter adjustability is dictated by the radial deflection and/or length of the cams 710.

In one aspect, the mount is designed to passively engage the objective. Referring to FIGs. 12A, 12B, mount 800 provides a compressive passive adjustment function using elastomeric members 850 as the second member configured radially in the first member 840 to provide compression force on the objective 103. The inner diameter of the collar 840 contains annular grooves 842 designed to receive elastomeric members 850 such as o-rings or hollow tubing. The inner diameter of those members 850 would be smaller than the outer diameter of the microscope objective. Compression of the elastomeric members upon insertion of the objective would create a restoring force that generates friction between the two surfaces. The adjustability in inner diameter can be configured using the compressibility and/or thickness and/or diameter of the elastomeric members.

The passive adjustment mounts 900, 901 in FIGs. 13A, 13B is conceptually similar to FIG. 12B, with the exception that the elastomeric members 950 of mount 900 are distributed radially and extend longitudinally along the inner diameter of the collar 940 . An alternative embodiment in FIG. 13B uses a similar distribution of leaf springs 951 in place of the elastomeric members of collar 940 of mount 901. With the leaf springs 951 attached at both ends to the collar 940, deformation of the springs upon insertion of the microscope objective creates a restoring force and friction on the outer surface of the objective. The diameter adjustability is dictated by the deformation range of the springs.

An alternative mount 1000, depicted in FIGs. 14A, 14B, uses a similar compression mechanism, with leaf springs 1050 as second member mounted radially to create multiple lobes that define an effective diameter 1100. Fig. 14B shows the sectional view of mount 1000 along sectional line 14B-14B. The leaf springs can be distributed in a single annulus (as shown) or with multiple rings distributed axially along the inner length of the collar. The leaf springs 1040 can be independently of different shape or arranged non-symmetrically around the inner diameter of the collar 1040. The leaf springs 1040 can be of other shapes so as to provide lobes of different shape and/or number.

By way of example, mounts 1200, 1201 depicted in FIGs. 15A, 15B, respectively, comprise bristles 1250, 1251, respectively, as members that are distributed radially and longitudinally along the inner diameter of the collar 1240. The inner effective diameter of the extended length of the bristles 1250, 1251 is configured to be smaller than the outer diameter of the objective, such that insertion of the objective leads to deflection of the bristles and a force for attaching the mount 1200. The bristles 1250 are oriented radially (Fig. 15A) or bristles 1251 are oriented radially and tangentially (Fig. 15B) relative to the collar 1240 circumference, creating either axial or axial and radial deflection upon receiving the objective 103. The passive compressive force and friction on the objective is created by the restoring force of the bristles and the diameter adjustability is provided by their deflection range.

Mount 1300 as shown in FIG. 16, is similar in concept to FIG. 15, but uses incomplete leaf springs 1350 distributed radially in place of the bristles to provide an effective diameter 1310 that is less than that of the outer diameter of the objective 103. The leaf springs 1350are oriented radially and tangentially, but only captured at one end to allow significant deflection. Many axial geometries are possible with this embodiment, with the leaf springs comprised of long rectangular elements that span the length of the collar 1340 (as shown) or multiple layers of shorter springs. The diameter adjustability of mount 1300 is dictated by the deformation range of the springs.

An alternate embodiment mount 1700, configured to be coupled or adopted to and used in conjunction with the release device systems, for example, as described in U.S. Patent Number 9,068,155 and WO 2013/181506, is shown in Figs. 17A, 17B. FIG. 17A shows the top plan view along section line 17A-17A and Figs. 17B shows the sectional view along section line 17B-17B of a “collet/collar” mount. A first bore providing an inner first perimeter 1790 through base member 1726 is shown in FIG. 17A. A plurality of second bores1760 extend from the inner first perimeter 1790 into the base member 1726. Each second bore 1760 in base member 1726 is configured to receive bias means 1750. Bias means 1750 can include a spring or the like. The bias means can be secured in the base member 1726 by set screws 1770 or the like, for example, by set screw 1770 threaded into the outside of the inner first perimeter 1790 of the base member 1726. A plurality of moveable members 1780, each moveable member located in one of the second bores 1760 and comprising a first end adjacent to the inner first perimeter 1790 and a second end extending into the base member 1726. Moveable members 1780 can be attached or integral with the bias means 1750. One or more of the moveable members are configured to move independently and relative to each other in the respective bores relative to the inner first perimeter 1790, such that the first ends of the moveable members 1780 are spaced apart from each other to form a variable inner second perimeter for retaining about the outside diameter of the objective 103. Independent and relative symmetric forces between each of the multiple bias means keeps the optical axis of the objective centered with respect to the release device base.

An alternative mount 1800 embodiment is shown in Figs. 18A and 18B. Mount 1800 provides circumferential compression on the outside diameter of the objective through the use of one or more inflatable bladders 1850 mounted along and/or within 1850 the inner circumference of the base component 122a. The bladder(s) 1850 can be filled with a fluid to adjust its volume and fill the gap between the inner diameter 1855 of the base 122a and outer diameter 1895 of the objective 103 and to transmit a force between them via pressure within the bladder 1850, thus modifying the effective diameter of the mount 1800. Bladder 1850 can configured such that upon being filled with a fluid its volume adjust, e.g., expands, in a radial direction. A valve 1890 controls the fluid path allowing the user to control the pressure in the bladder(s) 1850 and thus the effective diameter or effective radial diameter to secure or release the objective 103 from mount 1800. The radial symmetry of the bladder(s) 1850 keeps the optical axis of the objective centered with respect to the release device base 122.

An alternate embodiment for a platform 1400 suitable for combination with a release assembly device is shown in Figures 19A, 19B. platform 1400 is configured for use with any of the above-mentioned mounts of the present disclosure and is also configured to be coupled or adopted to and used in conjunction with the release device systems described in U.S. Patent Number 9,068,155 and WO 2013/181506. Platform 1400 comprises window 130 having an edge 130a, and mounting holes 1450 arranged radially along the circumference of platform 1400 that receive set screws 1452. Inner circumference 130b of platform 1400 provides an effective diameter so as to engage the window circumference edge 130a and hold the window in position via a compression force. This embodiment platform 1400 provides for reduced overall thickness of the platform and shortens the distance between the upper surface of the objective 103 and the tip of the release needle 132 to accommodate objectives with shorter working distances.

Thus the present disclosure provides a mount and a mechanism allowing universal attachment of a releasing device, such as the CellRaft device, to a variety of different diameter microscope objectives and solves the technical problem of providing mounts for microscopes having variation in objective outer diameters. The universal mount and methods can be coupled to or integral with a “release device” assembly, which can further include the motorized window platform, window, probe, etc. The present disclosure therefore facilitates the universality of the attachment of a release device assembly to a range of microscope objective sizes.

The present mount and methods further provides improvement for the general use and applicability of the such release devices that require dislodgement of a raft from a microwell array. For example, the presently disclosed mount embodiments facilitates self-alignment of the probe with or near the optical axis of the objective such that the probe axis is aligned with the target raft. These mount configuration embodiments do not affect existing features of a release device, where the probe is configured to be of a length that is out of the focal plane of the raft, and thus does not require the redesign and manufacture of existing releasing devices.

The present mount and methods further provides an active attachment to the objective a passive attachment to the objective, and a combination of active and passive attachment to the objective.

CLAIMS

1. An objective mount comprising:

a first member;

a second member radially engaged with the first member;

a first configuration where the second member has a first effective diameter; and

a second configuration where the second member has a second effective diameter less than the first effective diameter for securably engaging an outer surface of an objective;

wherein rotation of the first member relative to the second member about a common longitudinal axis of the first and the second members transitions the objective mount from the first configuration to the second configuration.

2. The mount of claim 1, wherein the first member is a collar and the second member is a collet.

3. The mount of claim 1, wherein the second member comprises a radial wall having one or more axial slots therein forming flexible lobes, and the radial wall having a thickness formed of one or more radial wedges; and the first member comprises a camming inner surface with that of the one or more radial wedges.

4. The mount of claim 1, further comprising a cap member engagable with the first member to facilitate transition from the first configuration to the second configuration.

5. The mount of claim 1, wherein the second member comprises a tapered radial wall having one or more axial slots therein forming flexible lobes and threading on its exterior surface; and the first member comprises a complementary threaded inner surface.

6. The mount of any one of claims 1-5, adapted to a release device assembly configured for attachment to an inverted microscope objective.

7. The mount of any one of claims 1-5, adapted to an assembly configured for microscopic cell sorting.

8. An objective mount comprising:

a first member;

a second member engaged with the first member;

a first configuration where the at least one second member has an effective diameter; and

a second configuration where the second member has a second effective diameter less than the first effective diameter for securably engaging an outer surface of an objective;

wherein translation of the first member relative to a longitudinal axis of an objective transitions the objective mount from the first configuration to the second configuration.

9. The mount of claim 8, wherein the second member is a tapered radial wall having one or more axial slots therein forming flexible lobes; and the first member comprises a complementary unthreaded inner surface.

10. The mount of claim 8, wherein the second member comprises one or more cam elements distributed radially along an inner diameter of the second member; and

the mount further comprises a ring member positioned between the first member and the second member, the ring member engaged with the first member and configured to translate axially along an outside diameter of the second member and to engage the one or more cam elements, causing at least a portion of the one or more cam elements to deflect inwardly in the second configuration.

11. The mount of claim 8, wherein the second member comprises one or more elastomeric elements distributed radially in grooves in an inner surface of the first member.

12. The mount of claim 8, wherein the second member comprises one or more leaf spring elements distributed about an inner surface of the first member.

13. The mount of claim 8, wherein the second member comprises one or more spring lobes projecting from an inner surface of the first member.

14. The mount of claim 8, wherein the second member comprises one or more whisker elements distributed about an inner surface of the first member.

15. The mount of claim 8, wherein the second member comprises one or more leaf spring segments that project from an inner surface of the first member.

16. The mount of any one of claims 8-15, adapted to an assembly configured for attachment to an inverted microscope objective.

17. The mount of any one of claims 8-15, adapted to an assembly configured for microscopic cell sorting.

18. An objective mount comprising:

a first member;

a second member engaged with the first member;

a first configuration where the at least one second member has an effective diameter; and

a second configuration where the second member has a second effective diameter less than the first effective diameter for securably engaging an outer surface of an objective;

wherein inflation of the second member with a fluid transitions the objective mount from the first configuration to the second configuration.

19. The mount of claim 18, wherein the second member is an inflatable bladder.

20. The mount of claim 18, wherein the second member is distributed radially along an inner diameter of the first member.

21. The mount of claim 18, wherein the second member comprises one or more elastomeric elements distributed radially in grooves in an inner surface of the first member.

22. The mount of claim 18, further comprising a valve for inflating and deflating the second member.

23. A method comprising

providing a mount as defined in any one of claims 1-22, the mount configured to couple with a probe of a release device assembly for cell sorting and an objective of a microscope; and

self-aligning the probe with or near an optical axis of a microscope objective.

24. A base member of an objective mount comprising:

a thickness and a first bore extending therethrough, wherein the first bore defines an inner first perimeter;

a plurality of second bores defined in the base member, wherein each of the second bores extend from the inner perimeter into the base;

a plurality of moveable members, each member located in one of said second bores and comprising a first end adjacent to the inner perimeter and a second end extending into the base member; and

bias means associated with each of said movable members, each of said bias means located in one of said second bores adjacent to the second end of a respective moveable member located in the respective second bore, said bias means biasing the respective moveable member inwardly toward the first bore,

wherein one or more of the moveable members are configured to move independently and relative to each other in the respective bores relative to the inner perimeter, such that the first ends of the moveable members are spaced apart from each other to form a variable inner second perimeter, where the inner second perimeter is smaller than the first inner perimeter for securably engaging an outer surface of an objective.

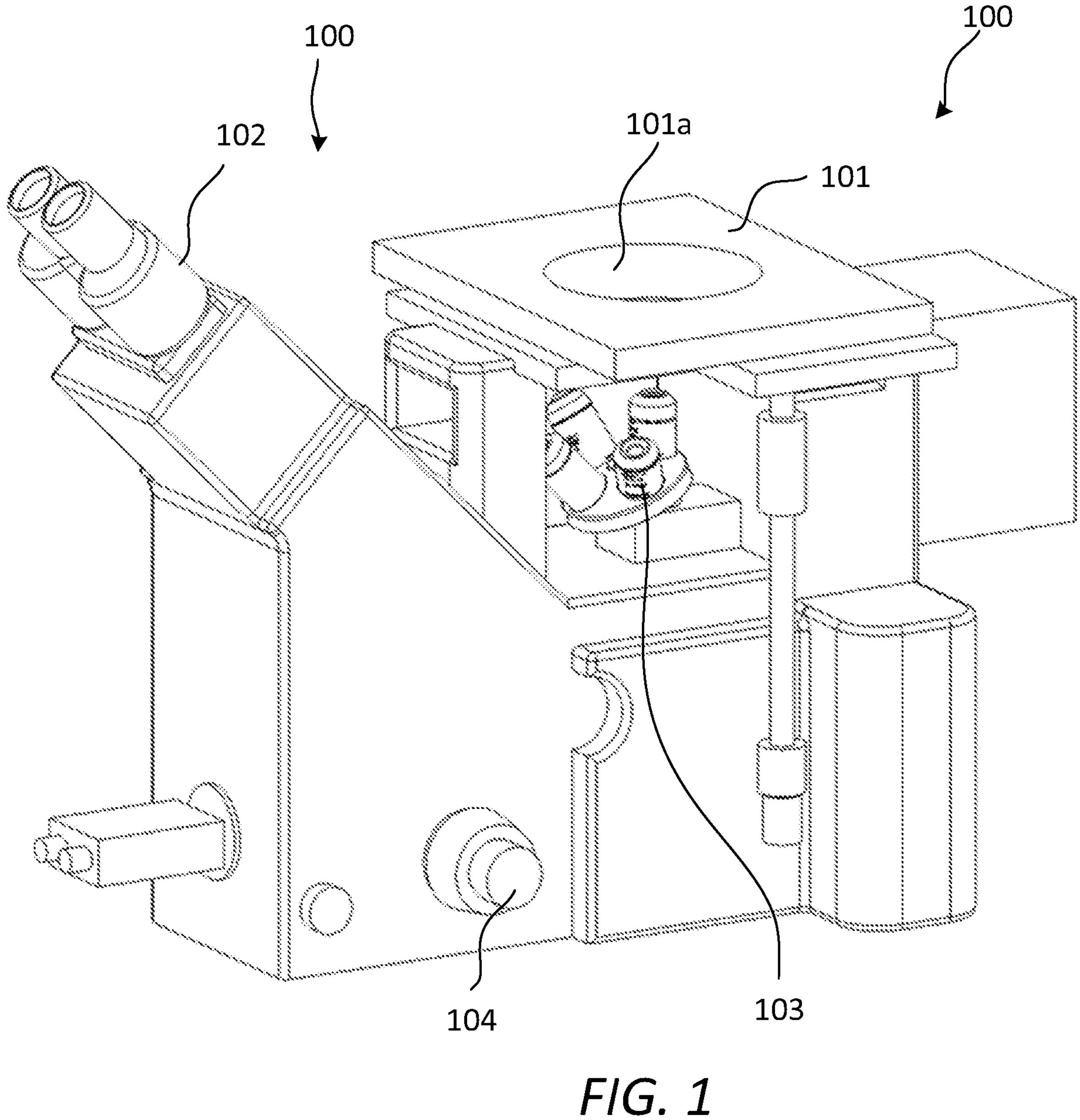

FIG. 1

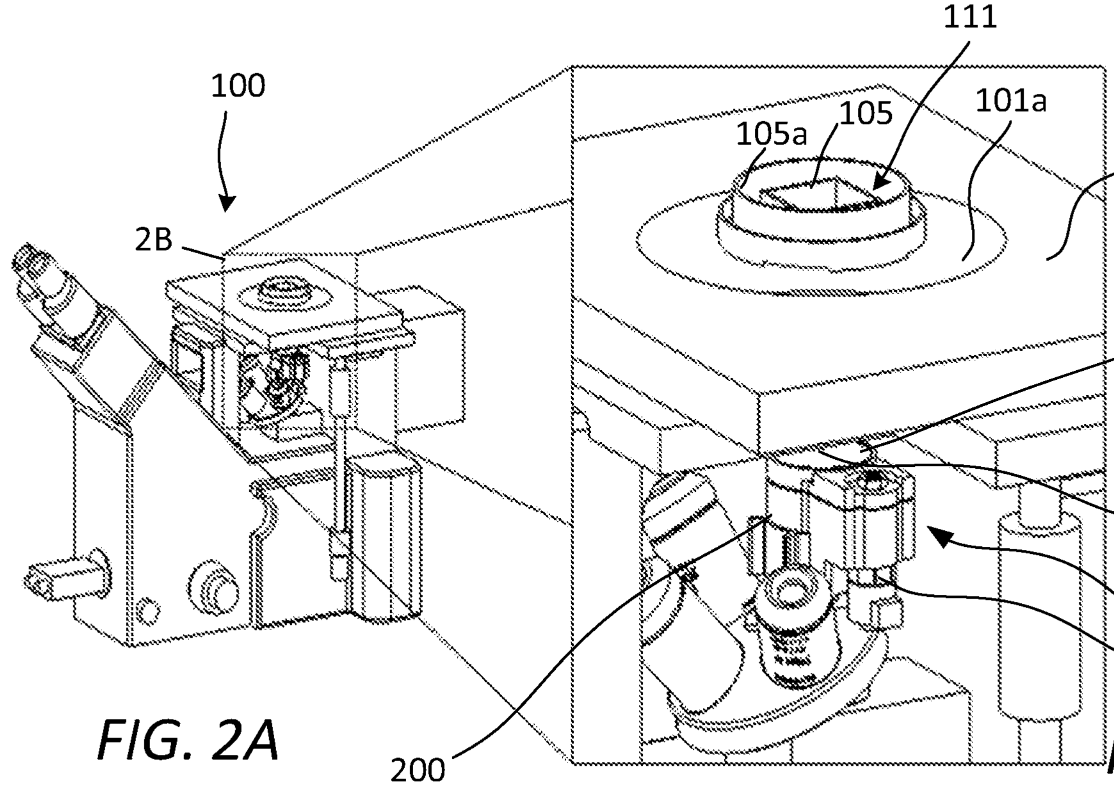

FIG. 2A

FIG. 2B

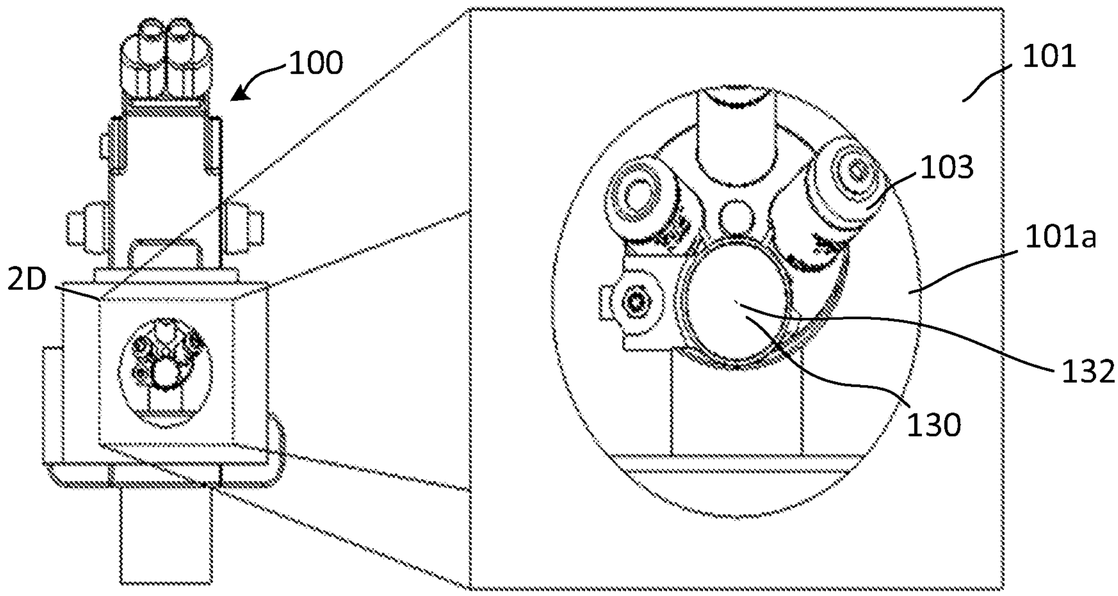

FIG. 2C

FIG. 2D

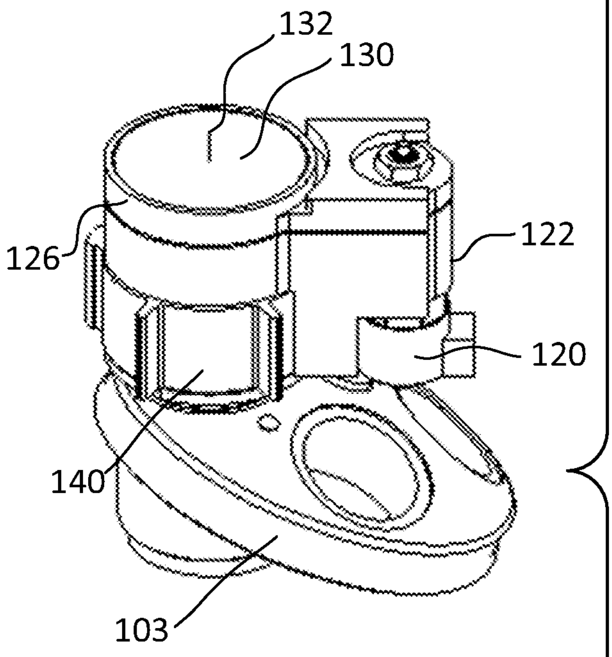
132
130
126
122
120
140
103

FIG. 3A

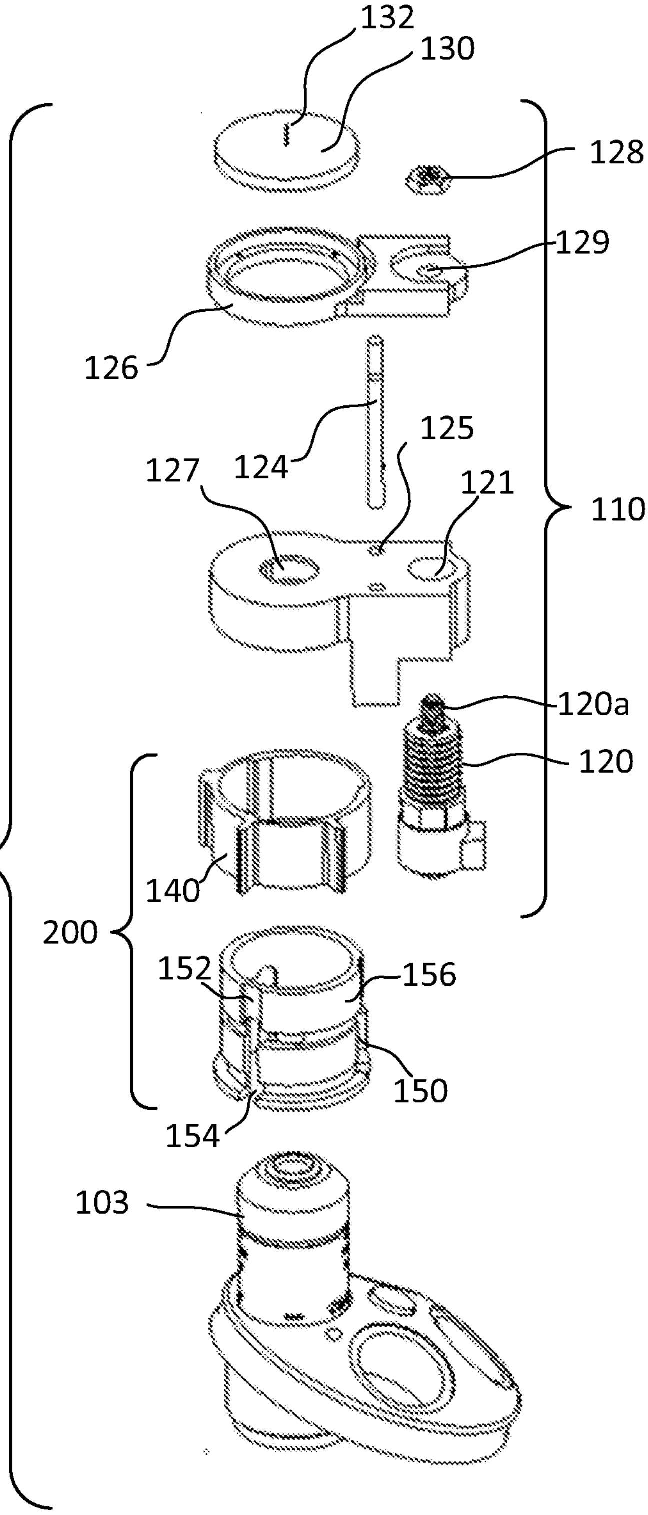
132
130
128
129
126
124
125
127
121
110
120a
120
140
200
152
156
150
154
103

FIG. 3B

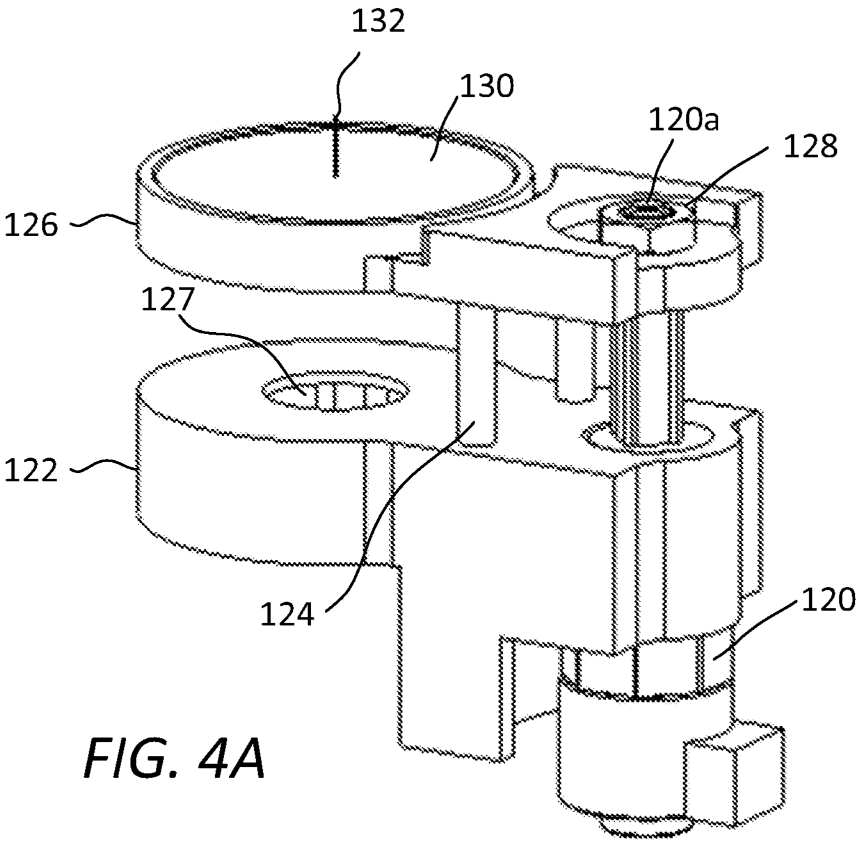

FIG. 4A

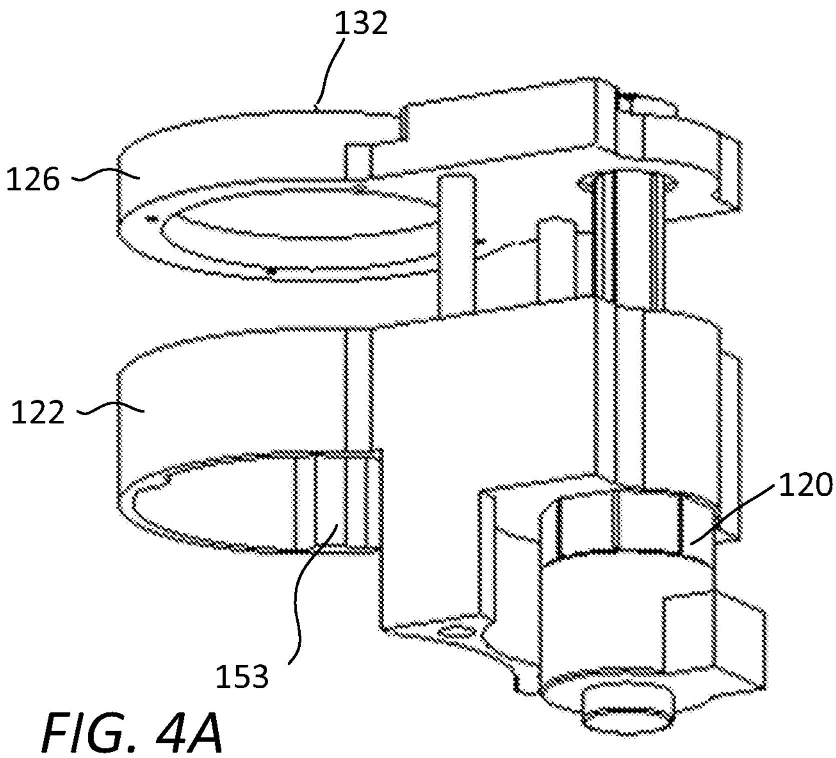

FIG. 4A

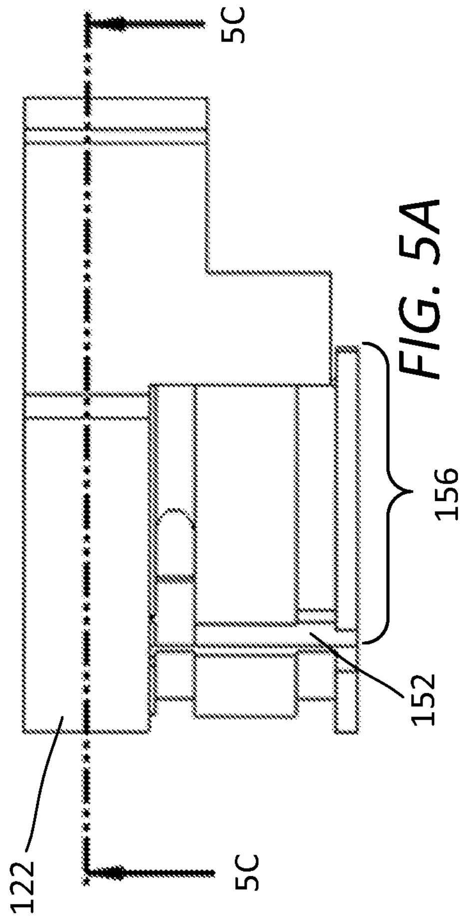

FIG. 5A

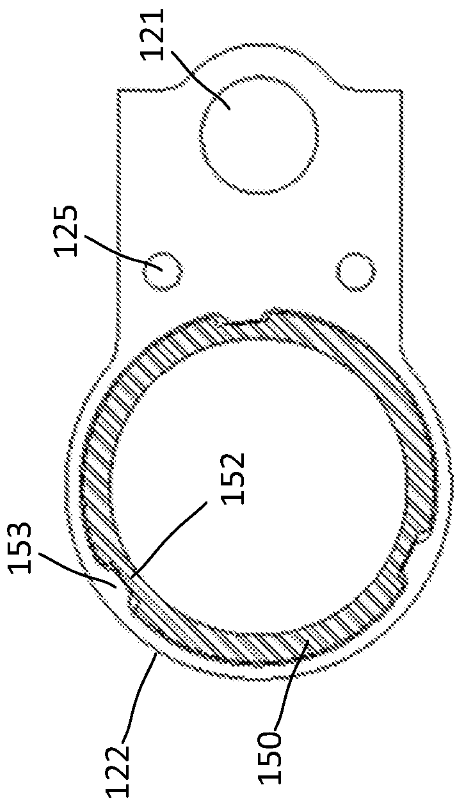

FIG. 5C

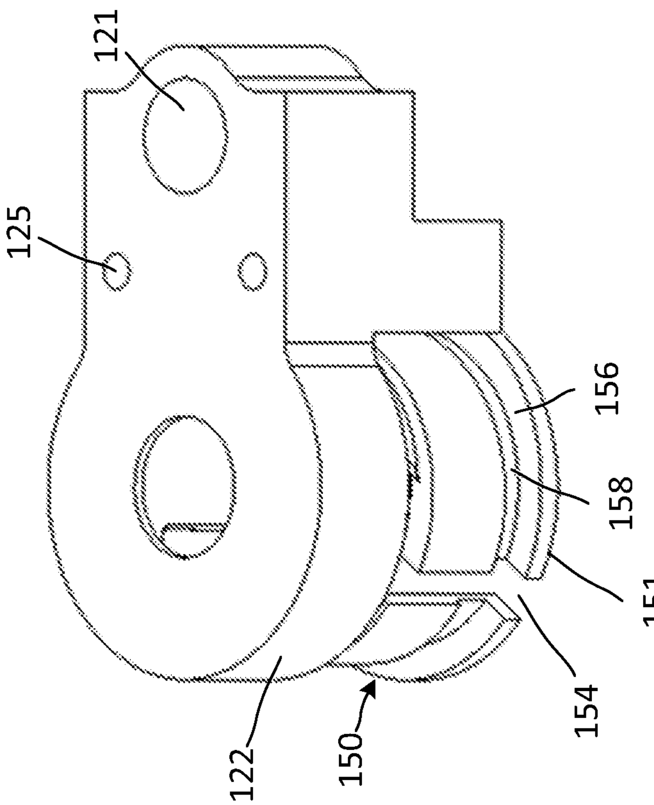

FIG. 5B

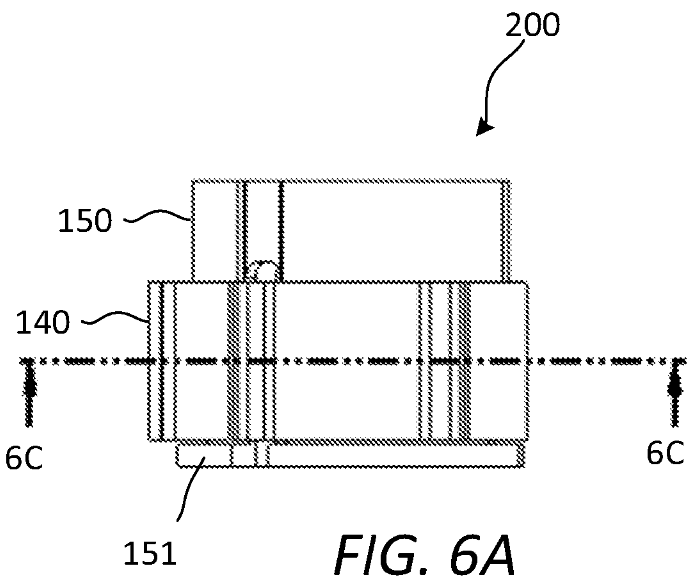

FIG. 6A

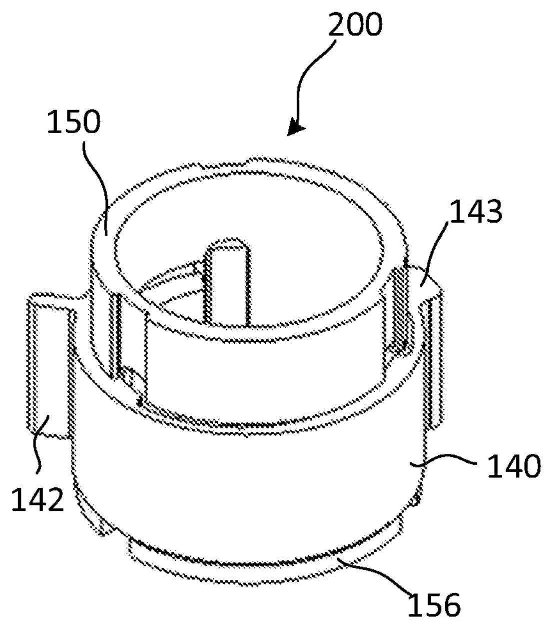

FIG. 6B

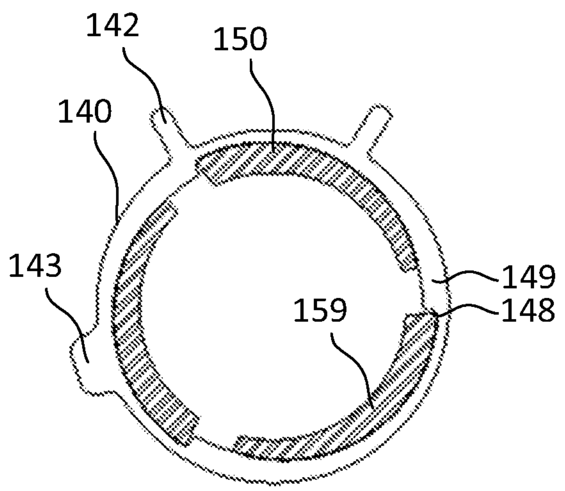

FIG. 6C

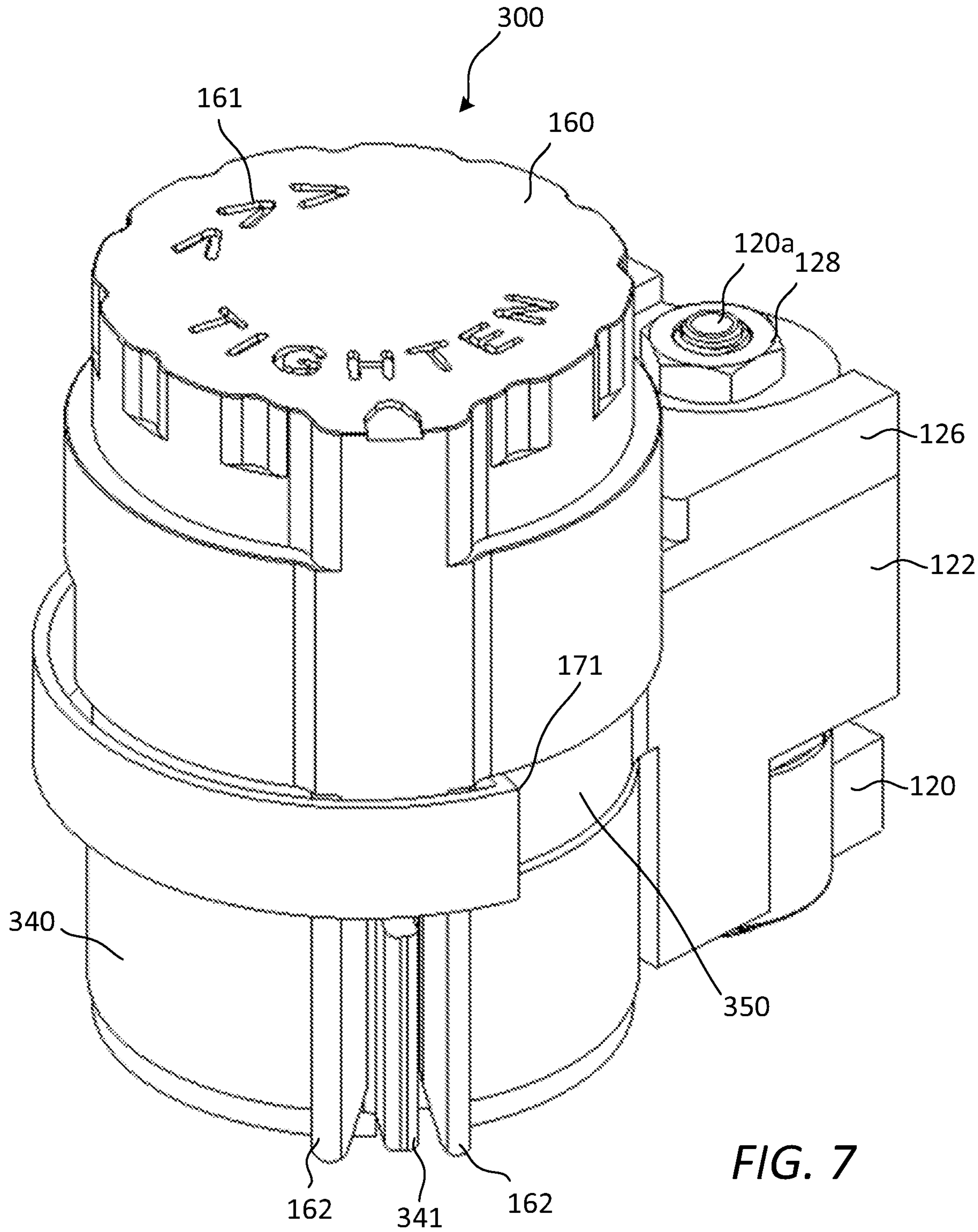
300
161
160
TIGHTEN
120a
128
126
122
171
120
340
350
162
341
162

FIG. 7

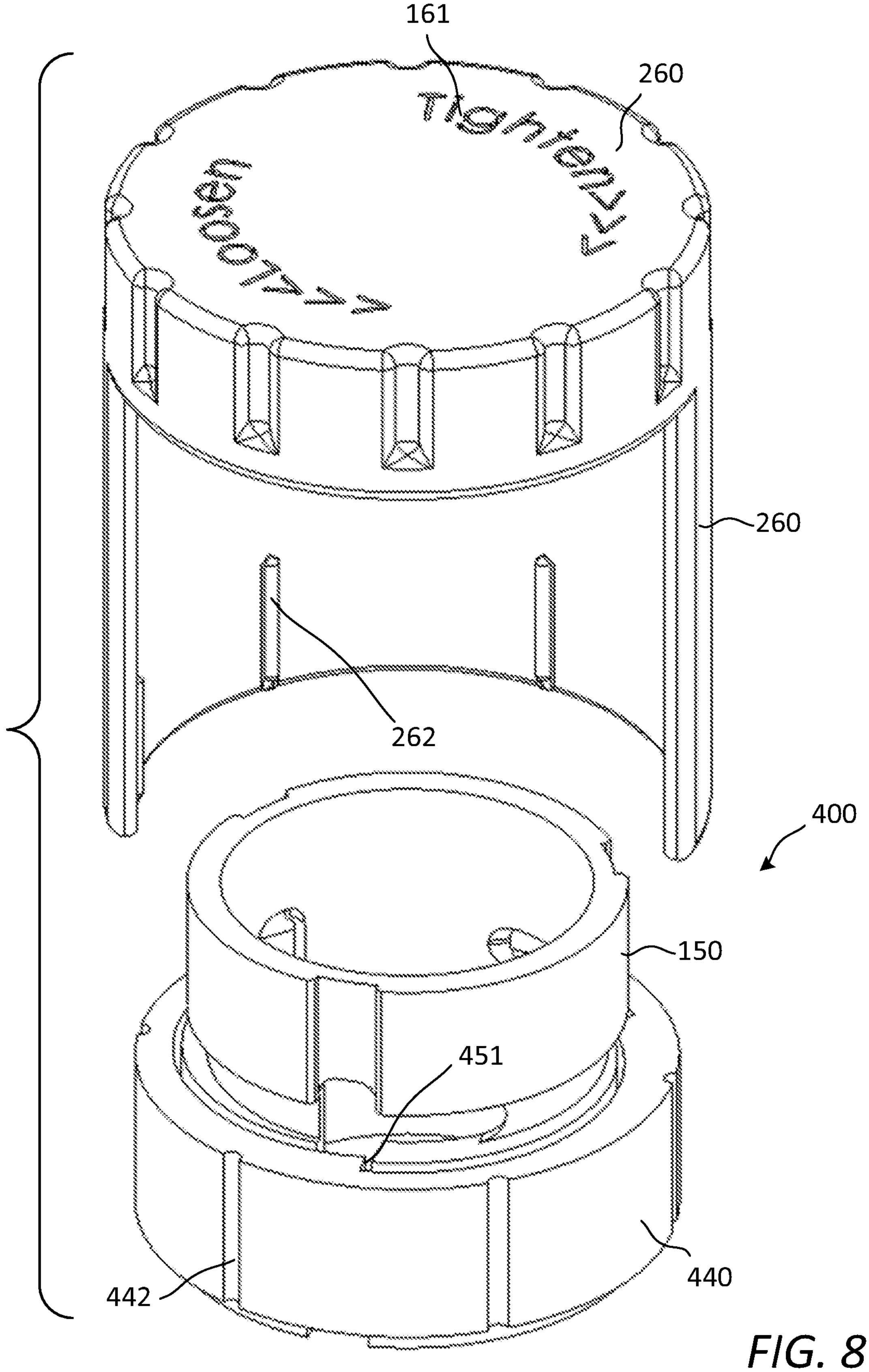

FIG. 8

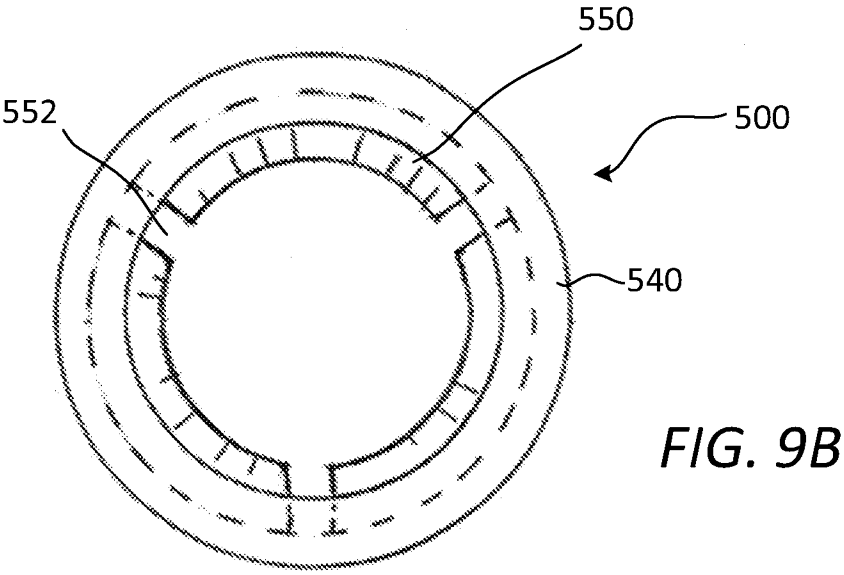

FIG. 9B

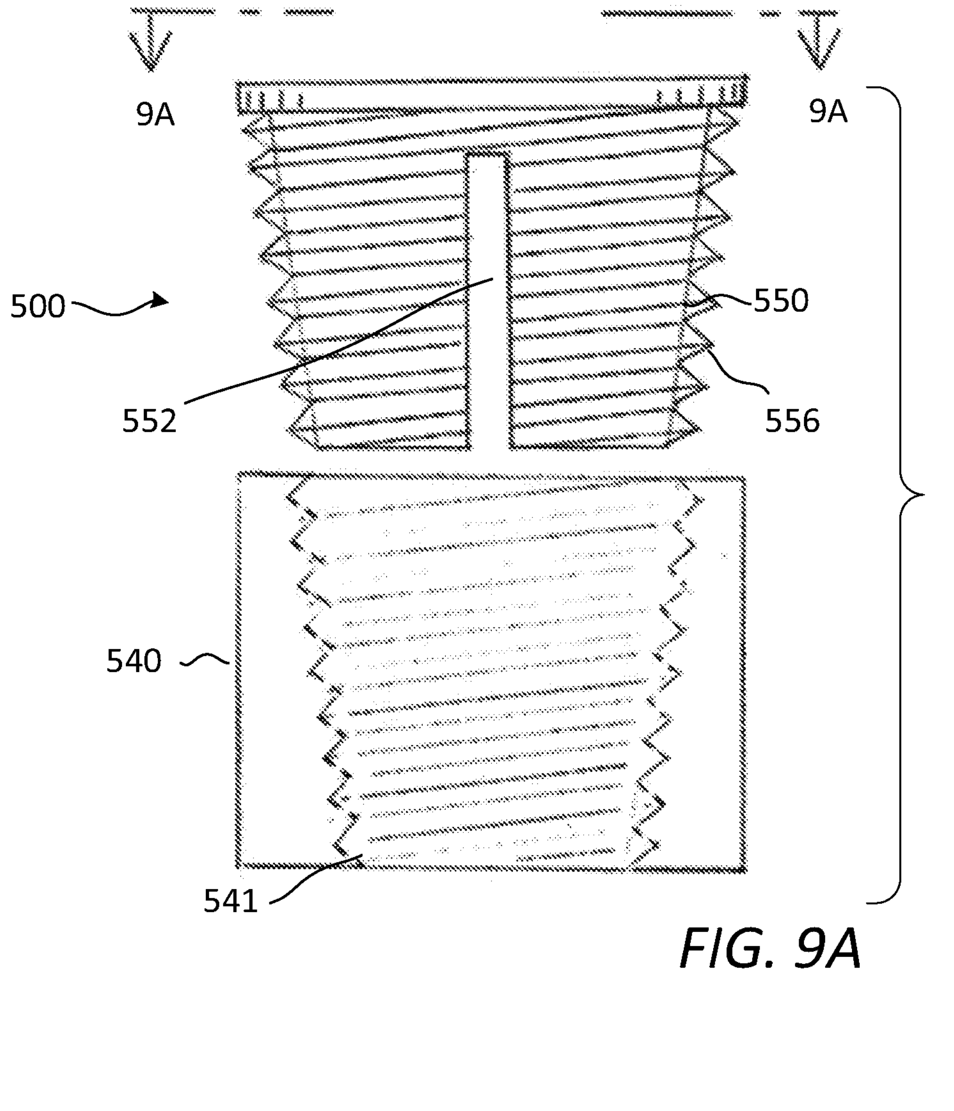

FIG. 9A

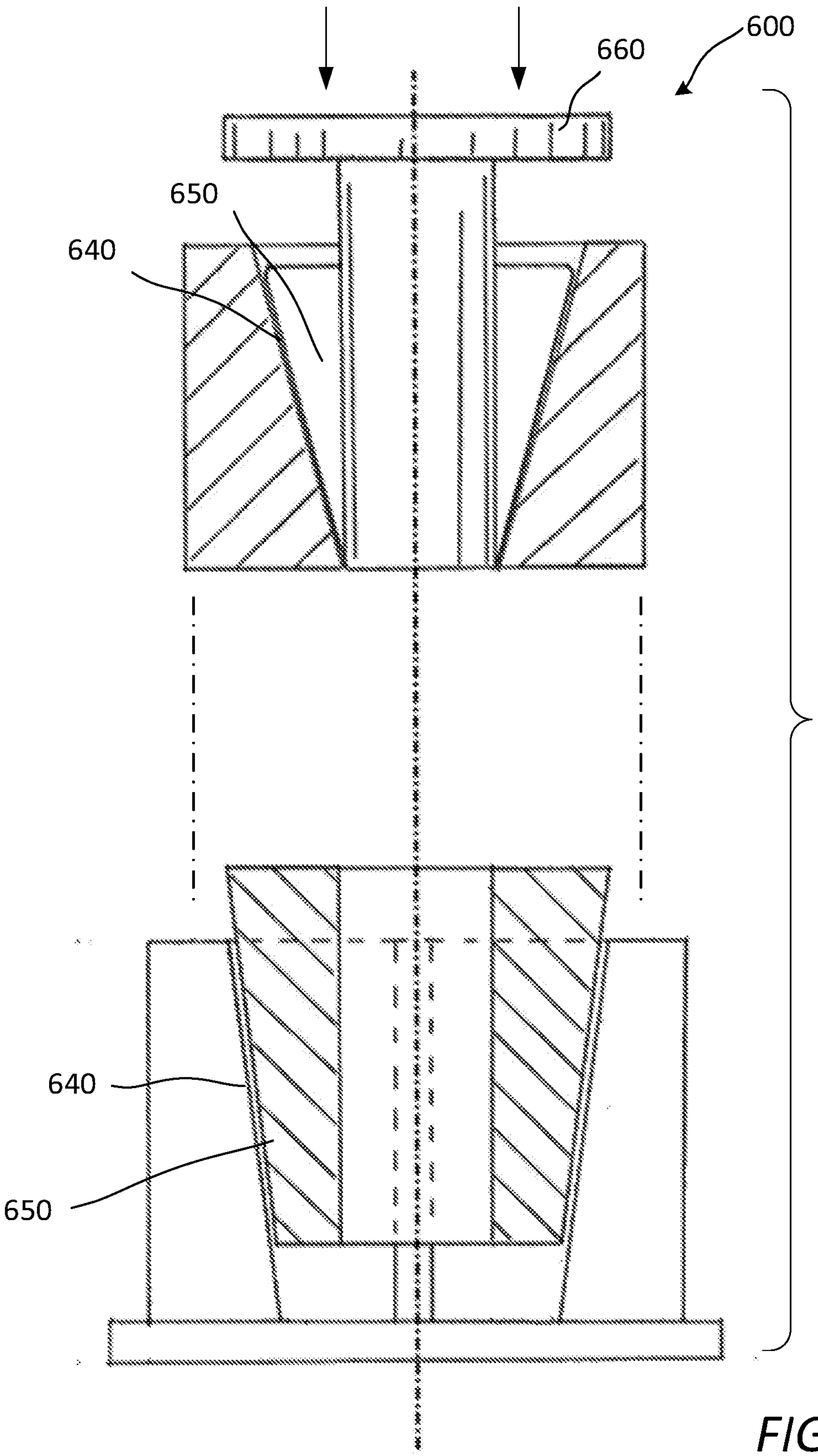

FIG. 10

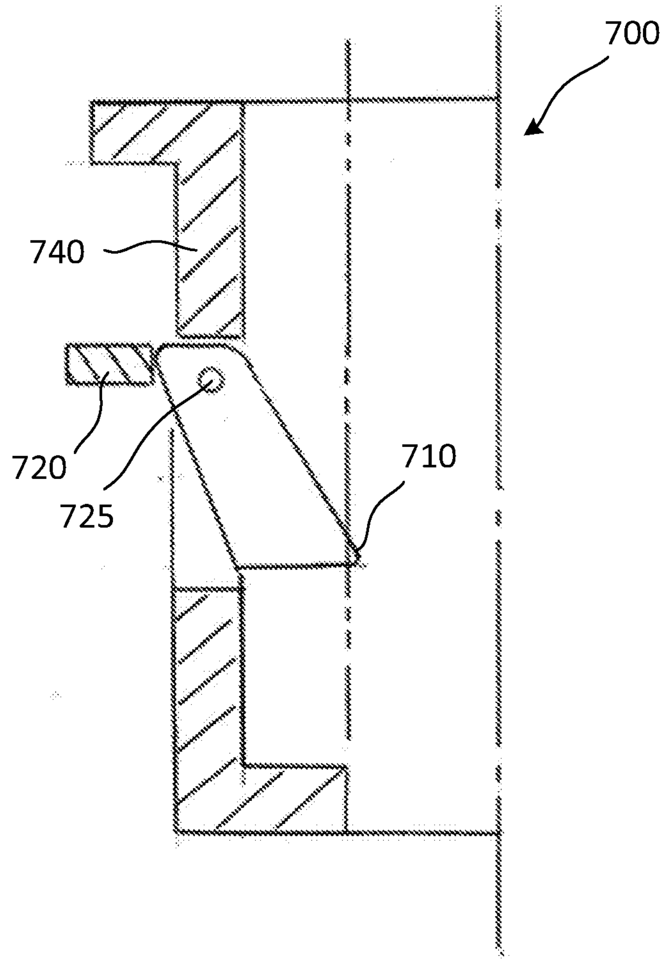

FIG. 11

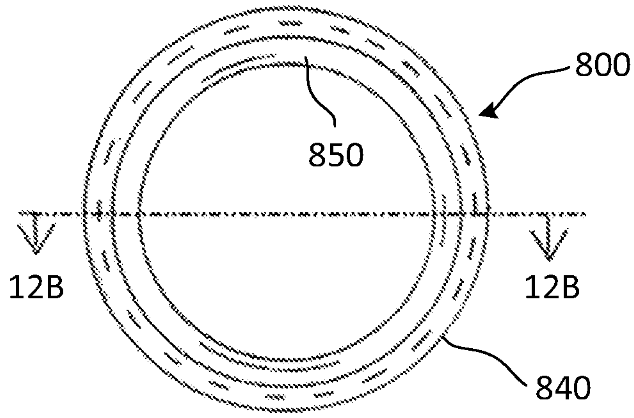

FIG. 12A

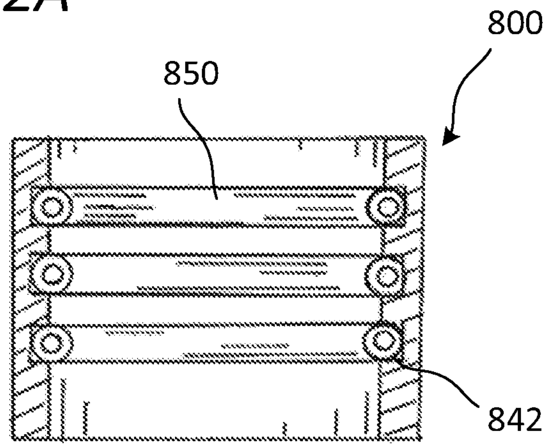

FIG. 12B

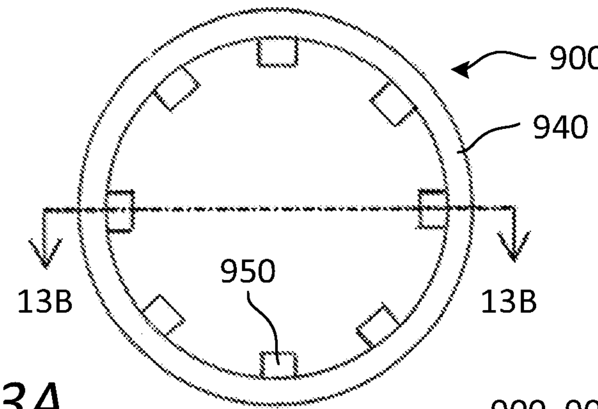

FIG. 13A

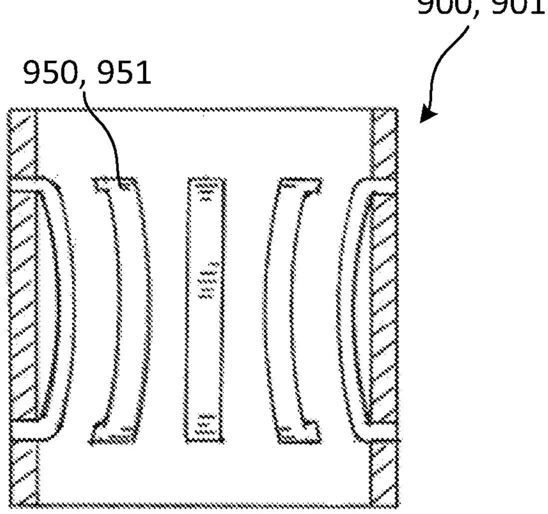

FIG. 13B

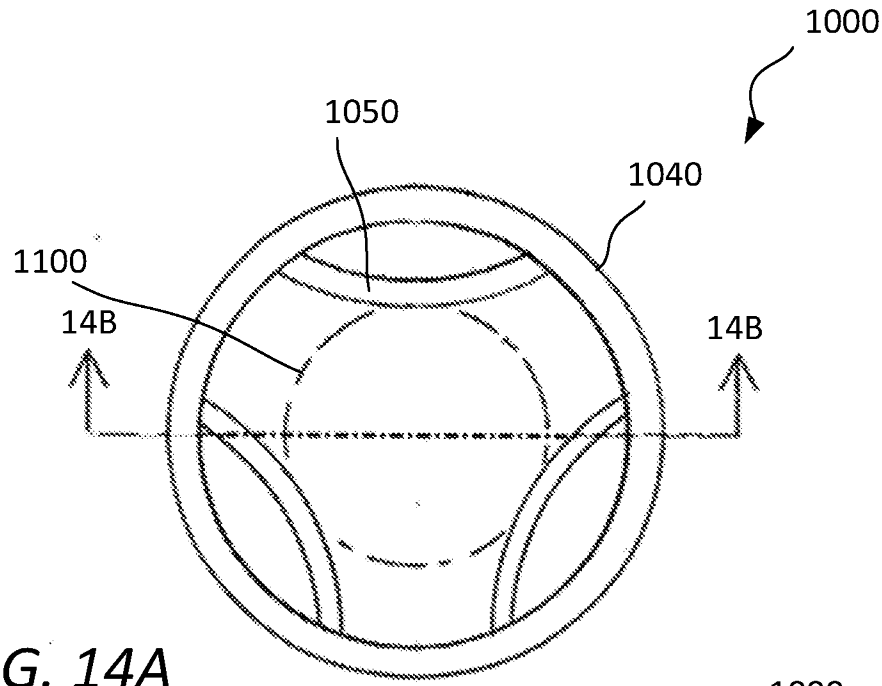

FIG. 14A

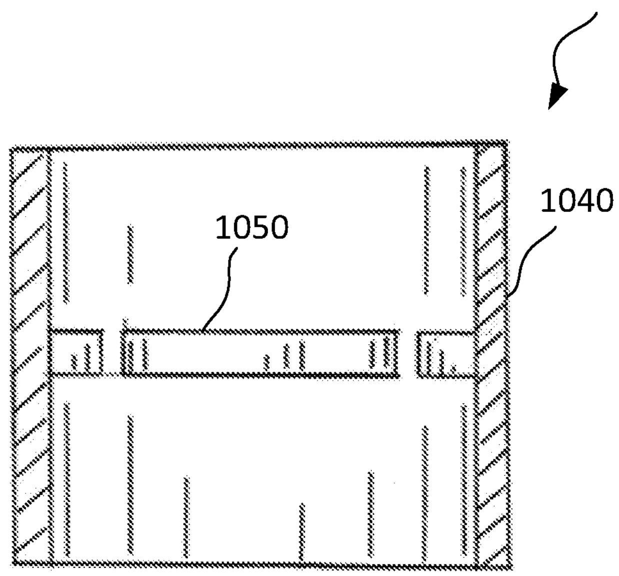

FIG. 14B

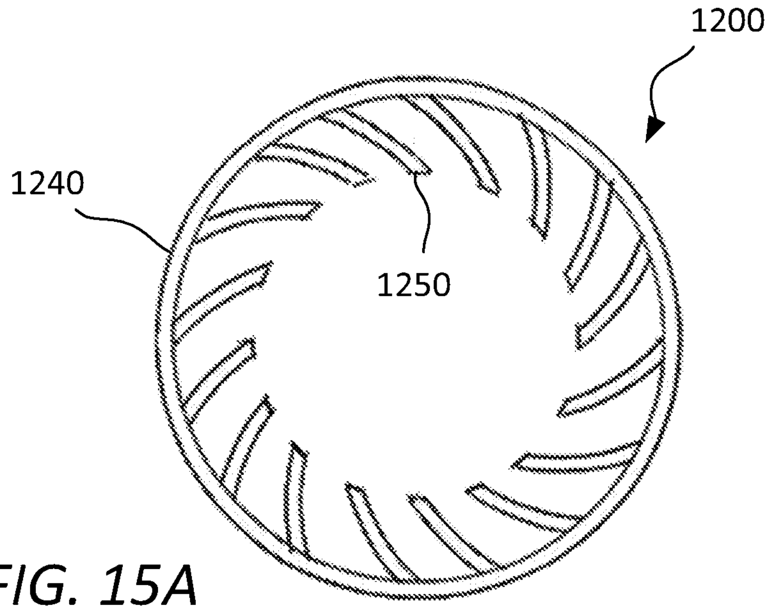

FIG. 15A

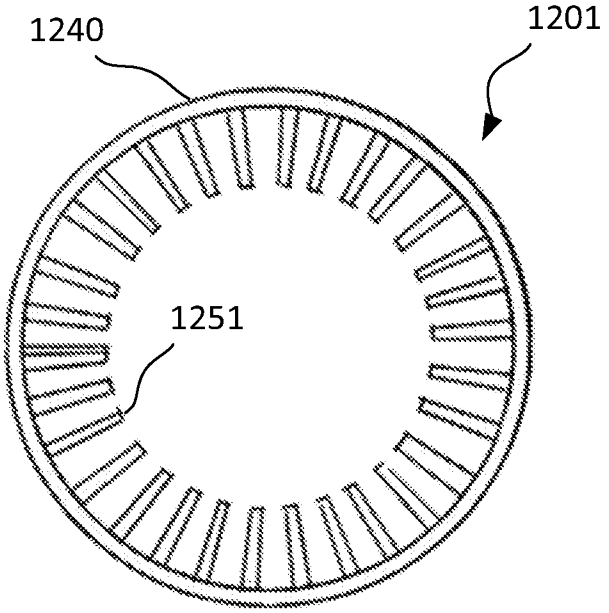

FIG. 15B

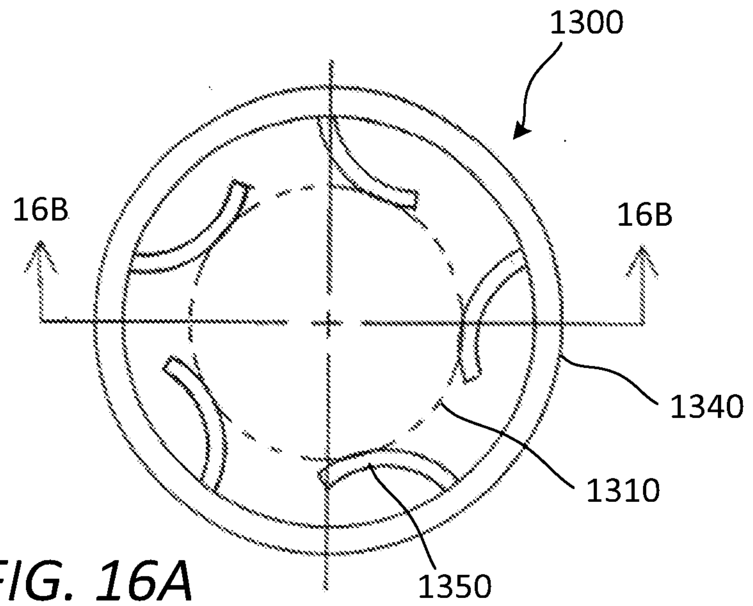

FIG. 16A

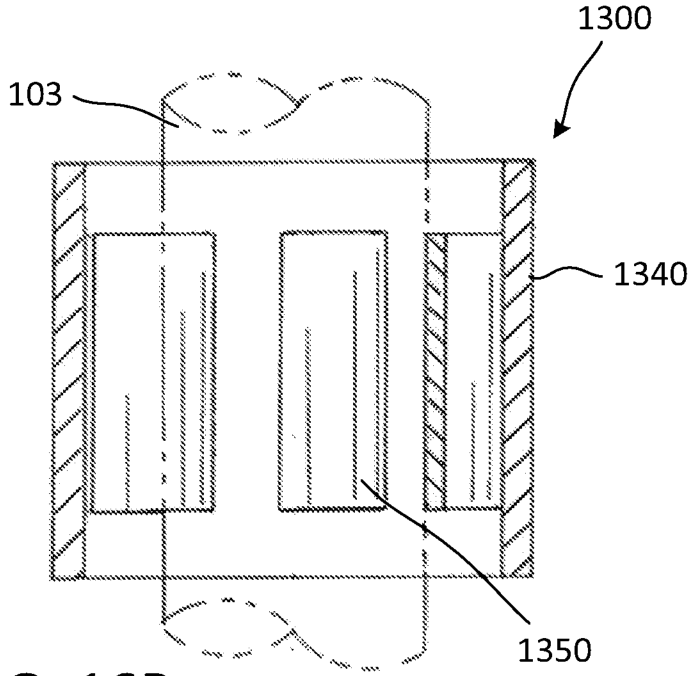

FIG. 16B

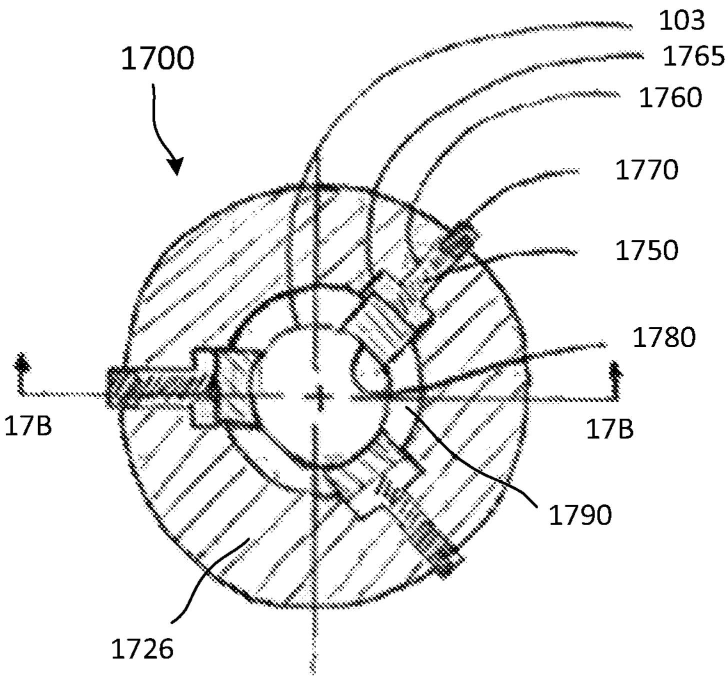

FIG. 17A

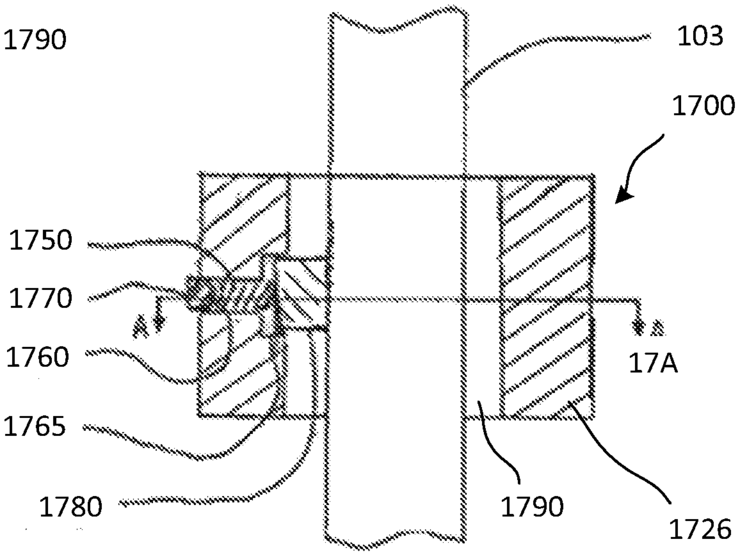

FIG. 17B

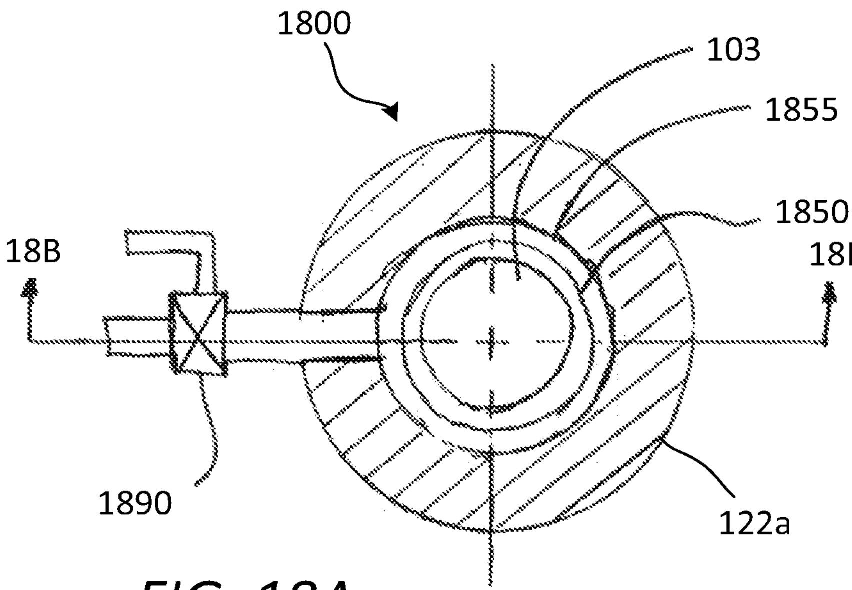

FIG. 18A

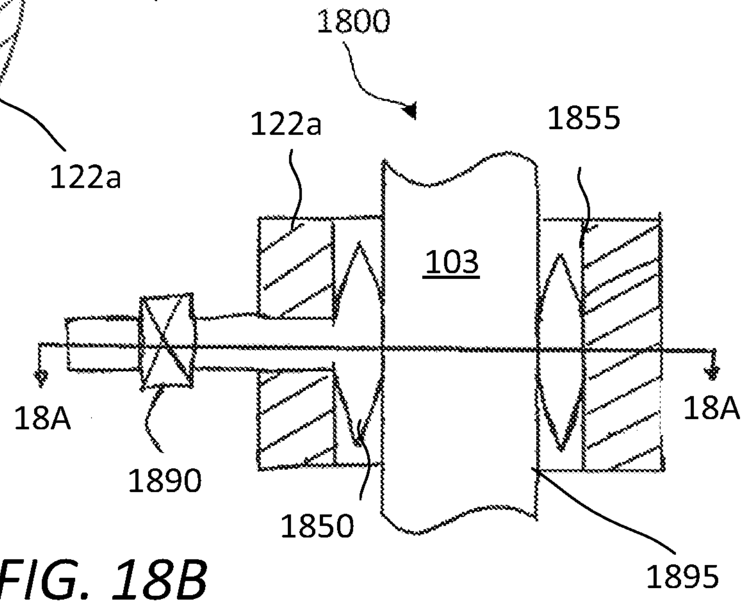

FIG. 18B

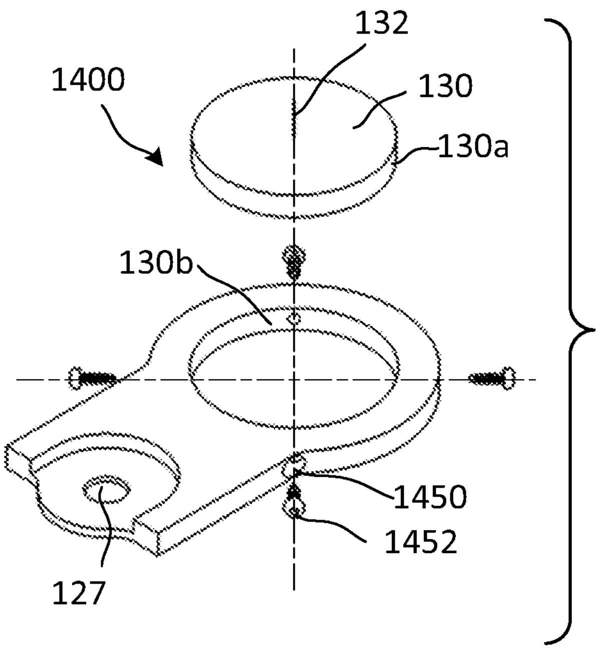

FIG. 19A

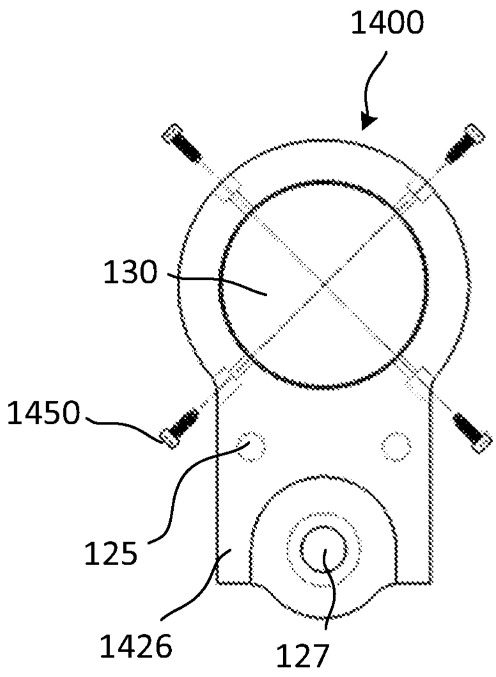

FIG. 19B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/042406

A. CLASSIFICATION OF SUBJECT MATTER
INV. G02B7/00 G02B21/24 G02B21/34
ADD. G02B21/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G02B

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 296 16 666 U1 (KAPPA MESSTECHNIK GMBH [DE]) 23 January 1997 (1997-01-23)	1-5
Y	figures 1-11 page 9 - page 13	6,7,23
X	DE 33 41 876 A1 (LEMKE GMBH [DE]) 30 May 1985 (1985-05-30)	8-15
Y	figures 1-4 page 9 - page 14	16,17,23
X	US 2010/135647 A1 (LAI YUK SHING [HK]) 3 June 2010 (2010-06-03)	1-4,24
Y	figures 18-23 paragraph [0002] paragraph [0044] - paragraph [0047] paragraph [0055] - paragraph [0057]	6,7,23

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

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2016	31/10/2016

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/042406

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/065795 A1 (ALLBRITTON NANCY [US] ET AL) 14 March 2013 (2013-03-14) figures 15-17 paragraph [0174] - paragraph [0179] -----	6,7,16, 17,23
X	US 5 540 393 A (STAFFORD ROBERT G [US] ET AL) 30 July 1996 (1996-07-30) figures 2,2A -----	18-22
X	FR 2 793 567 A1 (JEULIN SA [FR]) 17 November 2000 (2000-11-17) figures 1-6 -----	1,2,24
A	US 4 066 330 A (JONES ERIC MYNARD) 3 January 1978 (1978-01-03) figure 4 -----	24
A	US 1 477 440 A (GRIER JR FREDERICK A) 11 December 1923 (1923-12-11) the whole document -----	24

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/042406

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 29616666 U1	23-01-1997	NONE	
DE 3341876 A1	30-05-1985	NONE	
US 2010135647 A1	03-06-2010	US 2010135647 A1 WO 2010063990 A1	03-06-2010 10-06-2010
US 2013065795 A1	14-03-2013	CA 2789761 A1 EP 2536819 A1 US 2013065795 A1 US 2015251151 A1 US 2015259637 A1 WO 2011103143 A1	25-08-2011 26-12-2012 14-03-2013 10-09-2015 17-09-2015 25-08-2011
US 5540393 A	30-07-1996	NONE	
FR 2793567 A1	17-11-2000	NONE	
US 4066330 A	03-01-1978	DE 2723652 A1 DE 7716597 U1 US 4066330 A	22-12-1977 23-11-1978 03-01-1978
US 1477440 A	11-12-1923	NONE	