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(54) **DEVICE FOR FACILITATING THE IMAGING OF BIOLOGICAL ENTITIES**

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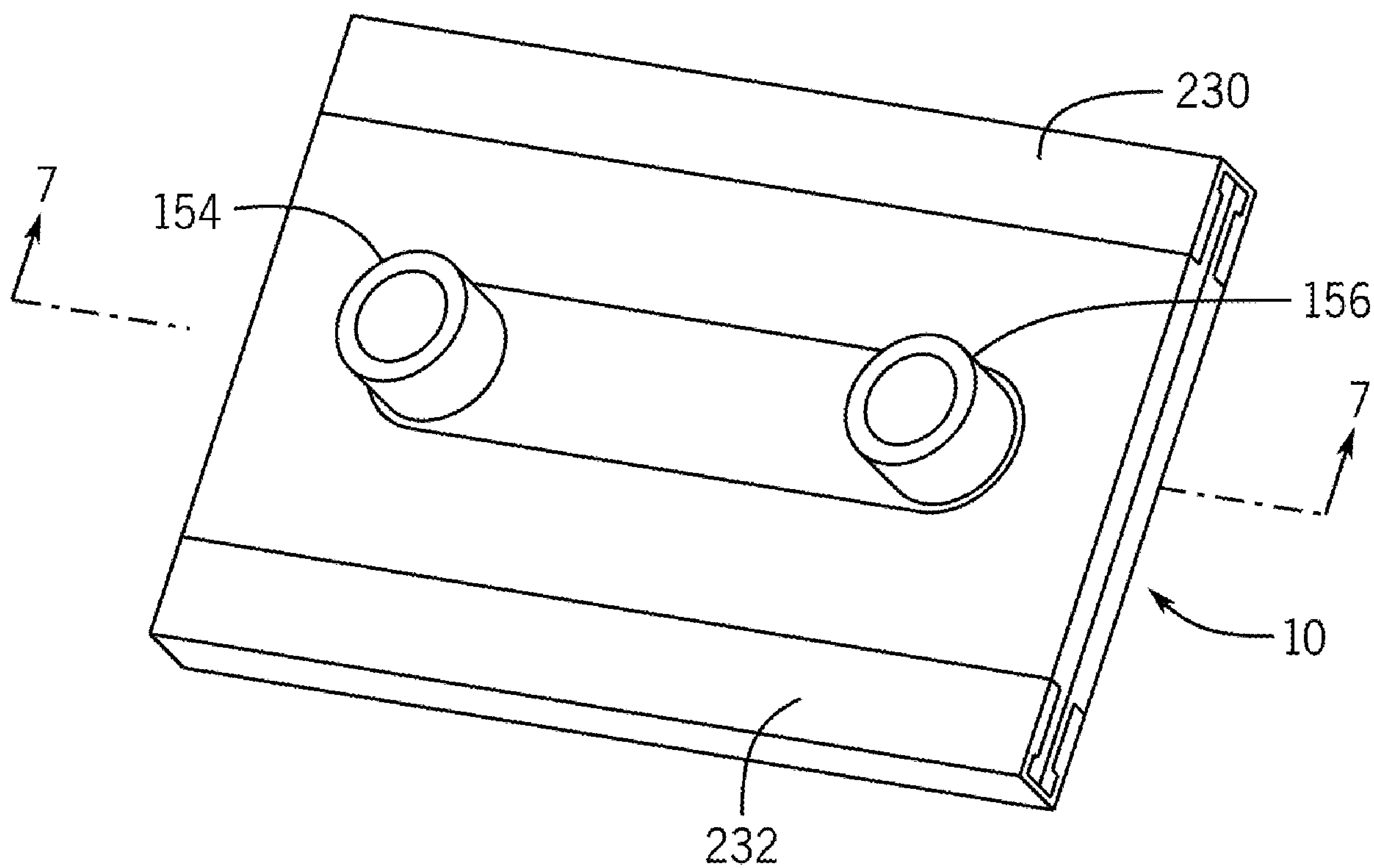
(57) **ABSTRACT**

A device is provided for facilitating the imaging of biological entities. The device includes a body having upper and lower surfaces. The body defines a channel for accommodating fluid flow therethrough and a well communicating with the channel and the lower surface. A microscopy grid is removably received in the body and has a first side communicating with the channel and a second side directed at well. A retainer extends into the channel and is engageable with the microscopy grid to support the microscopy grid.



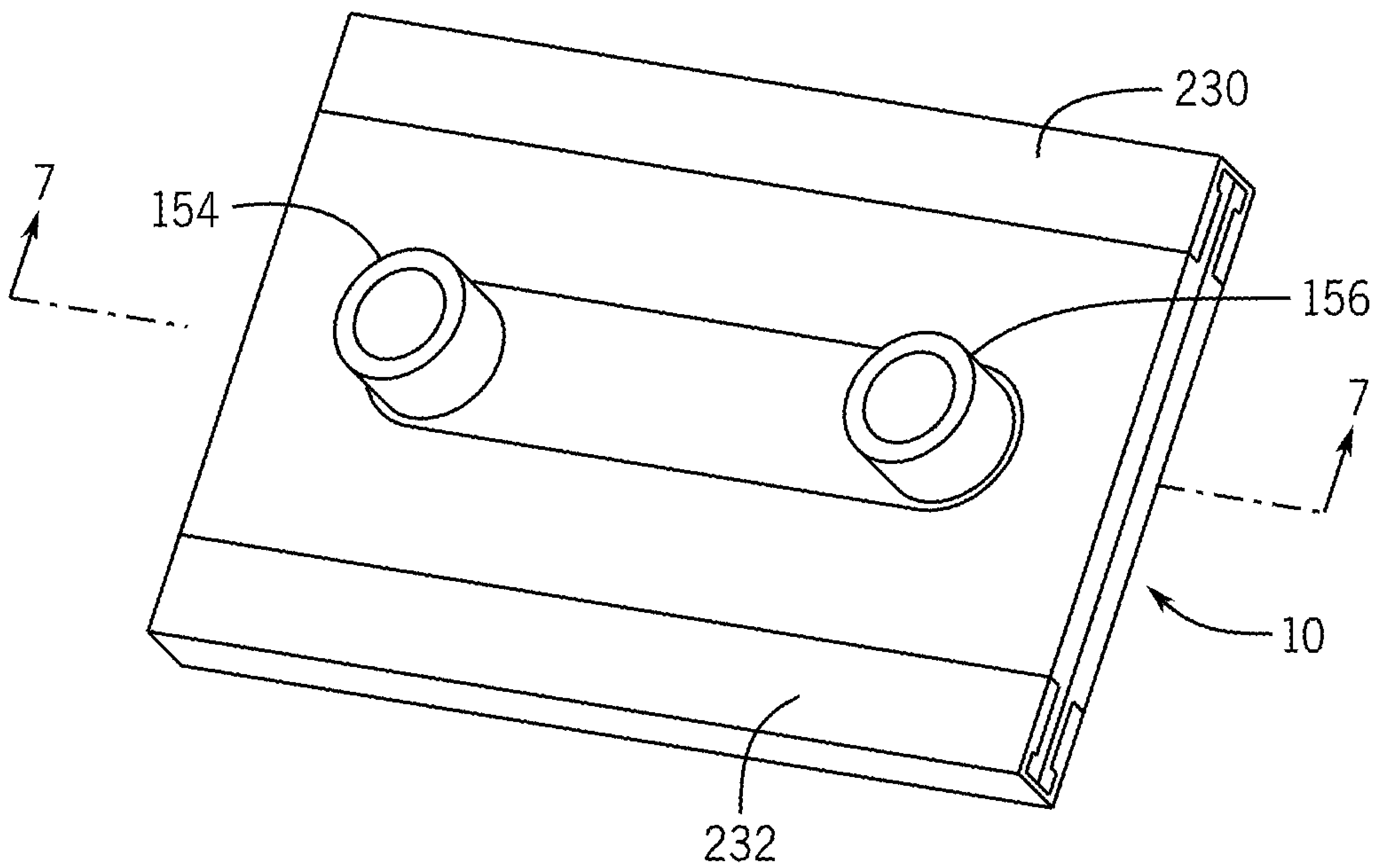


FIG. 1

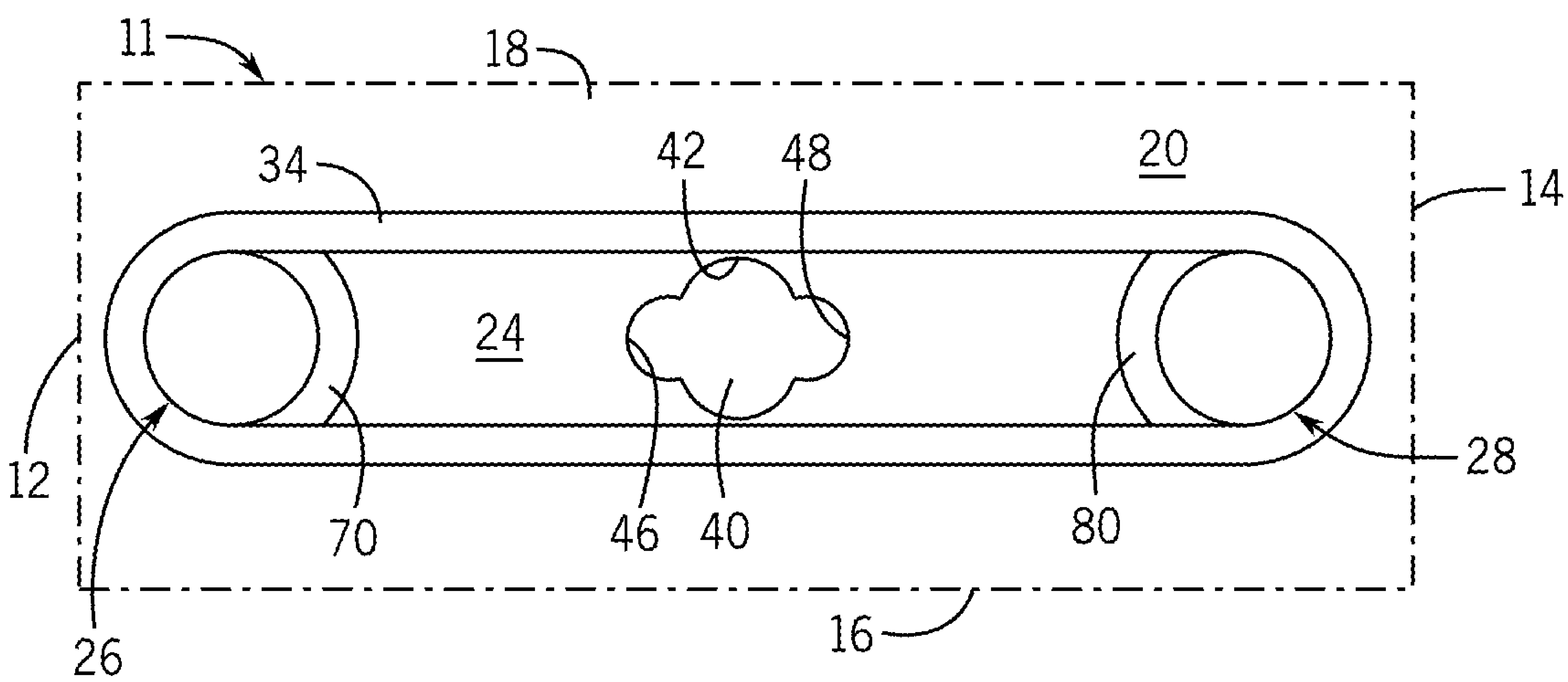


FIG. 2A

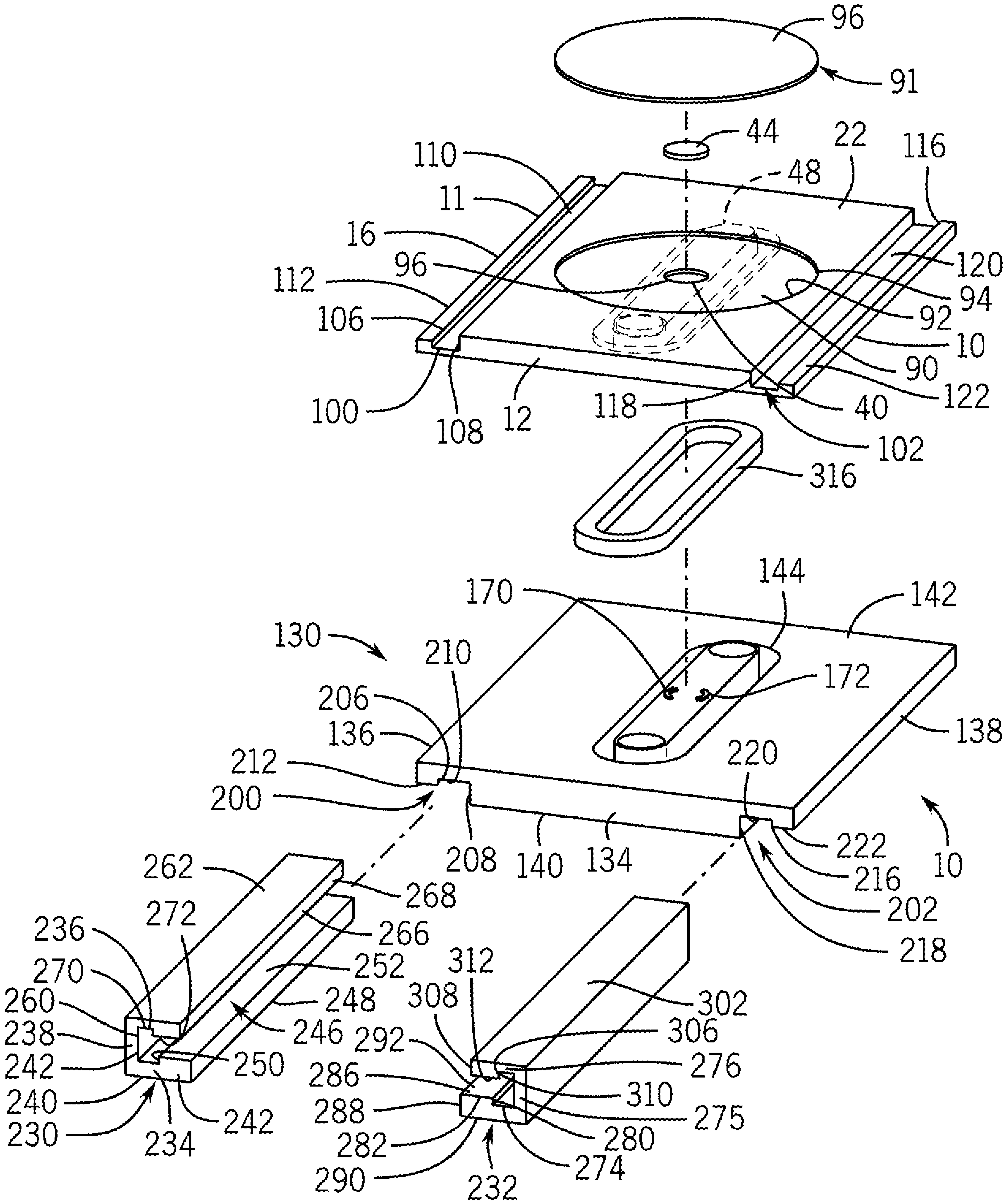

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FIG. 2

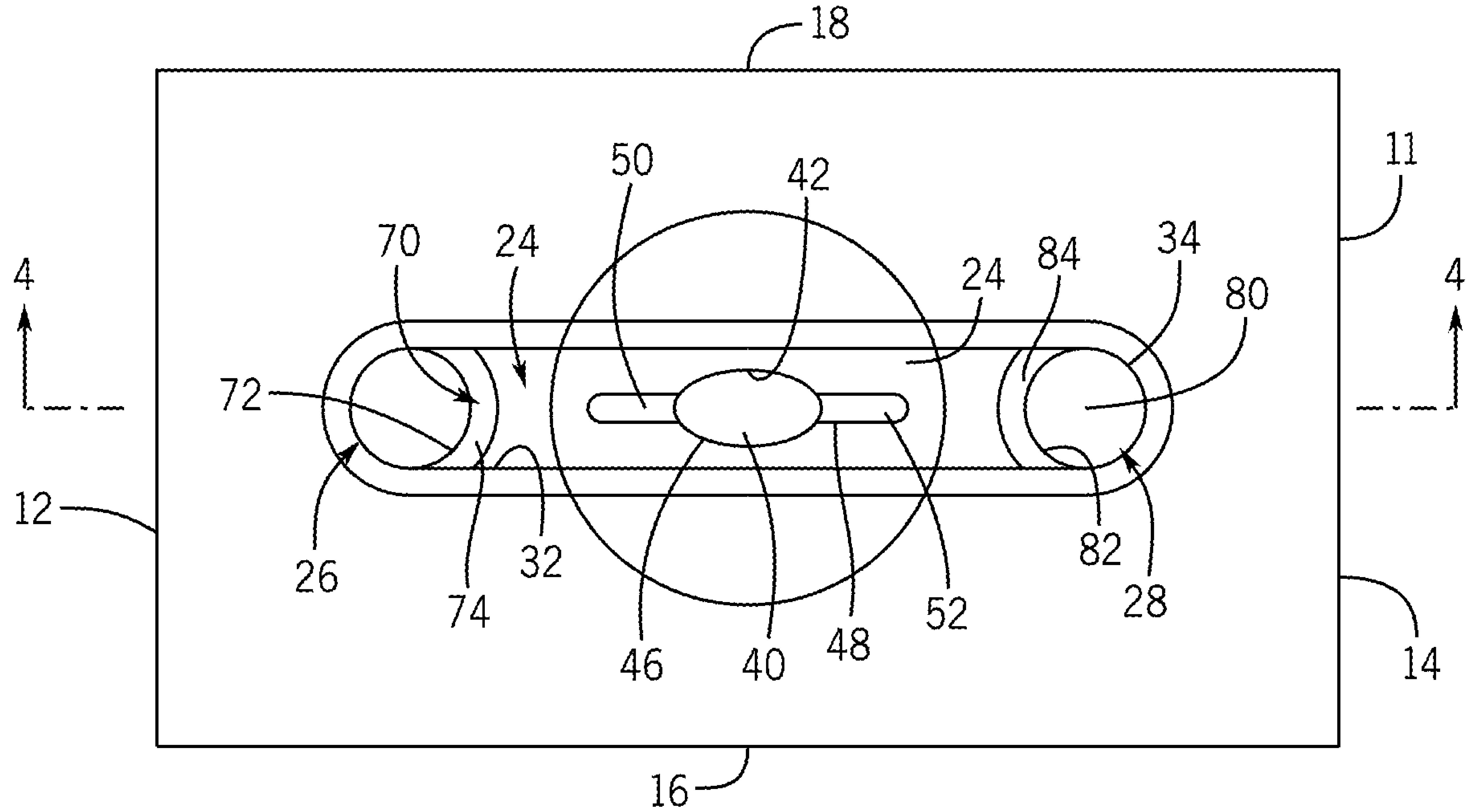
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FIG. 3

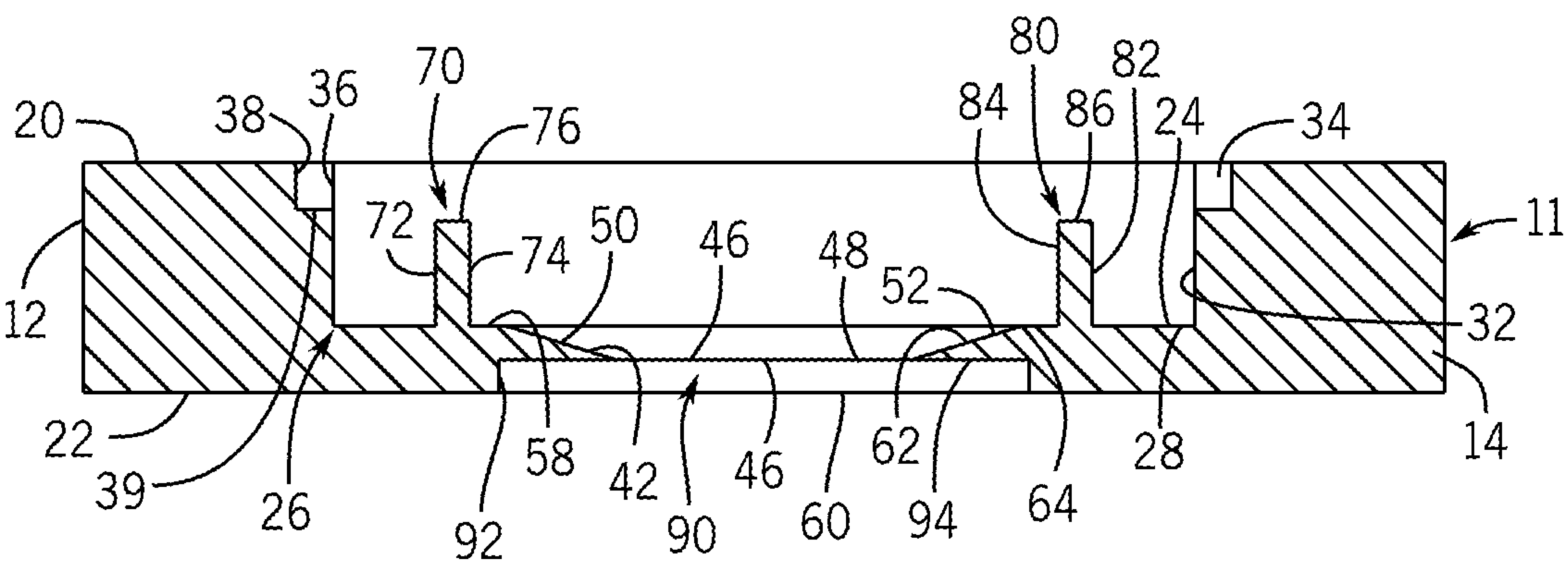
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FIG. 4

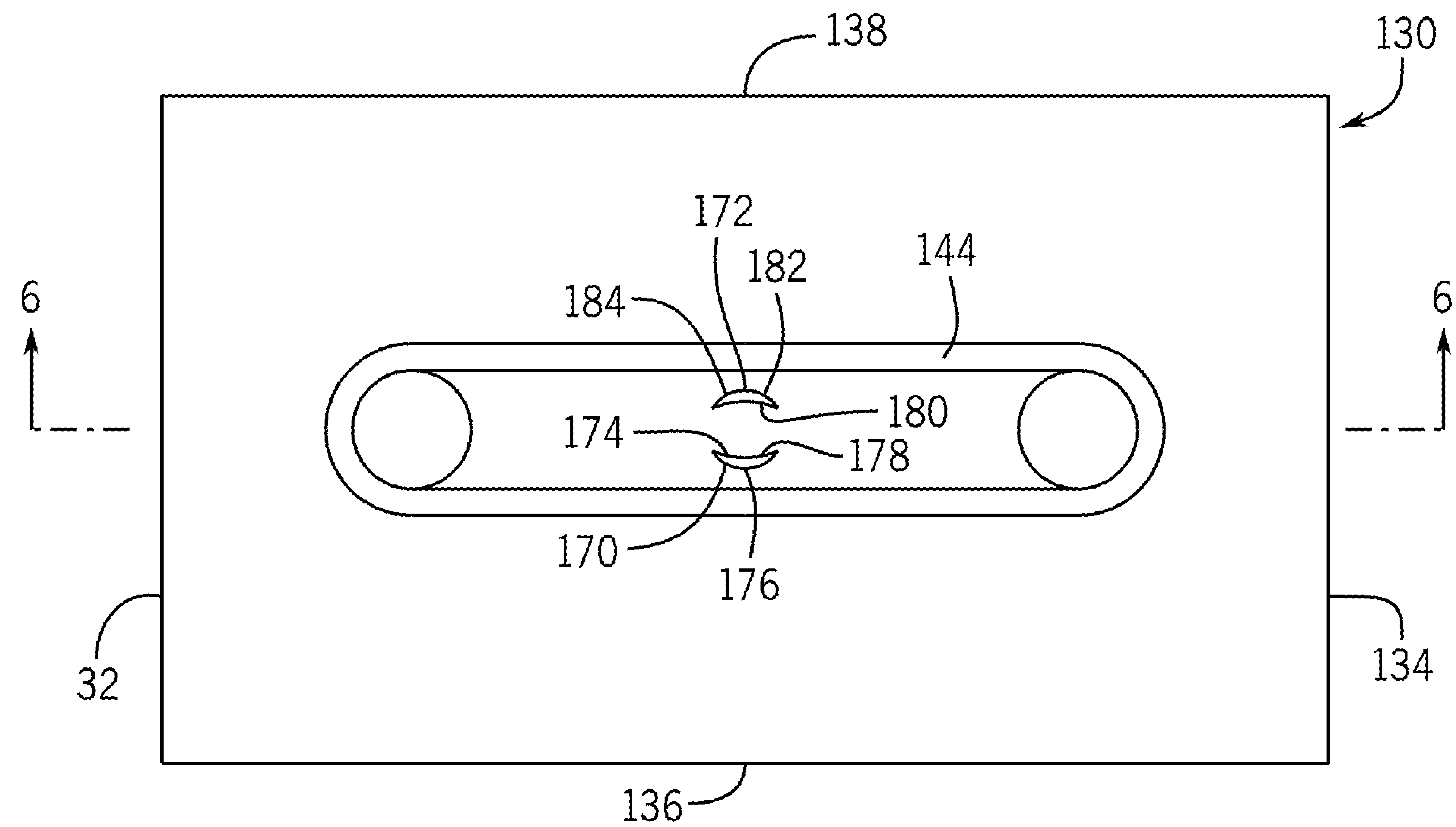
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FIG. 5

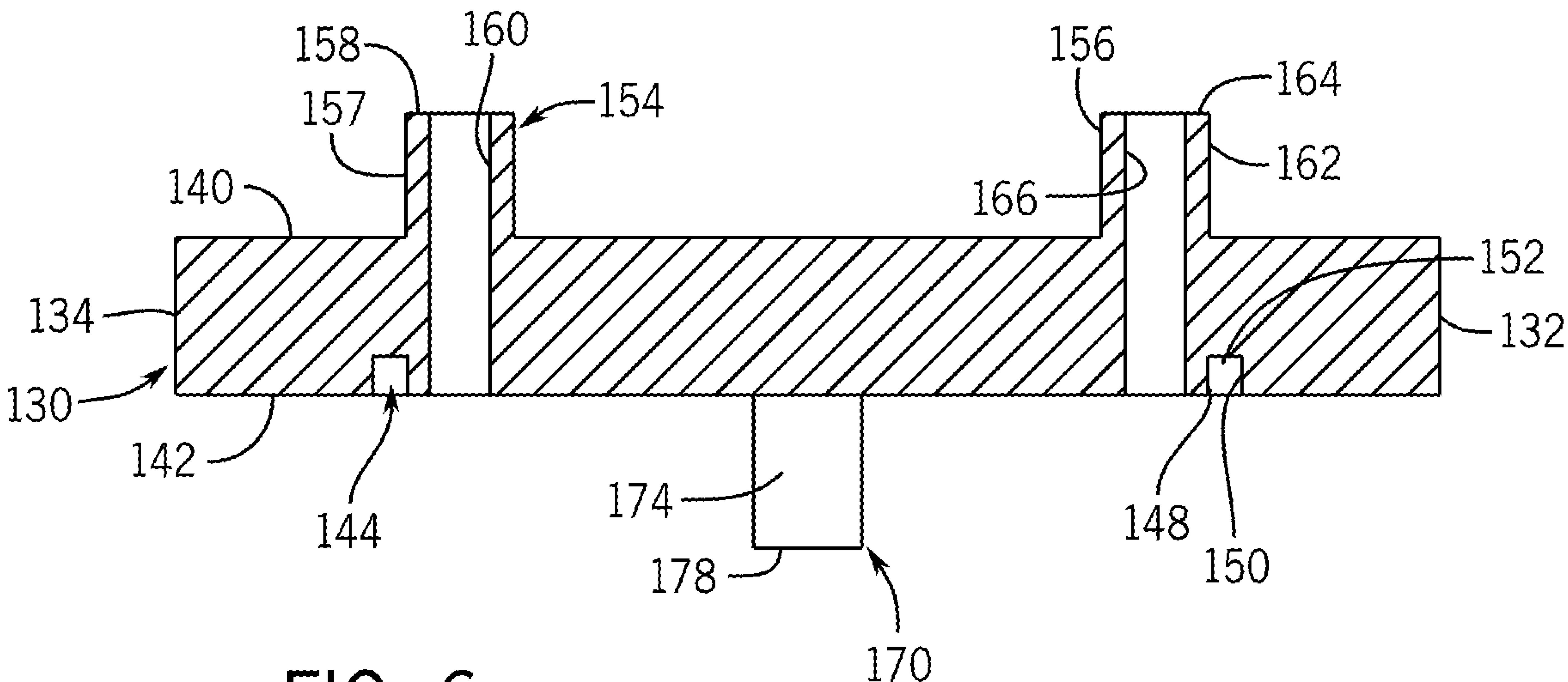
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FIG. 6

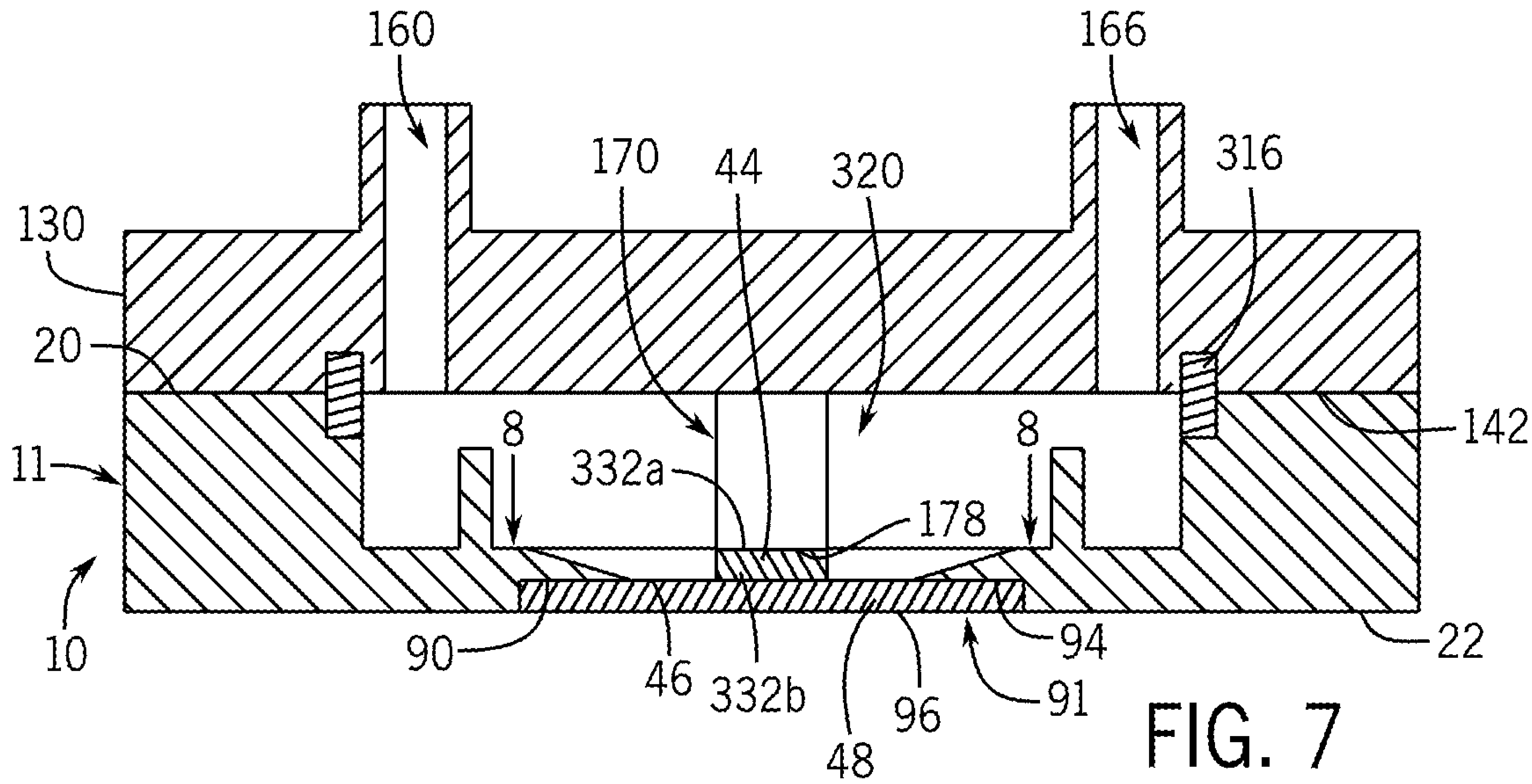
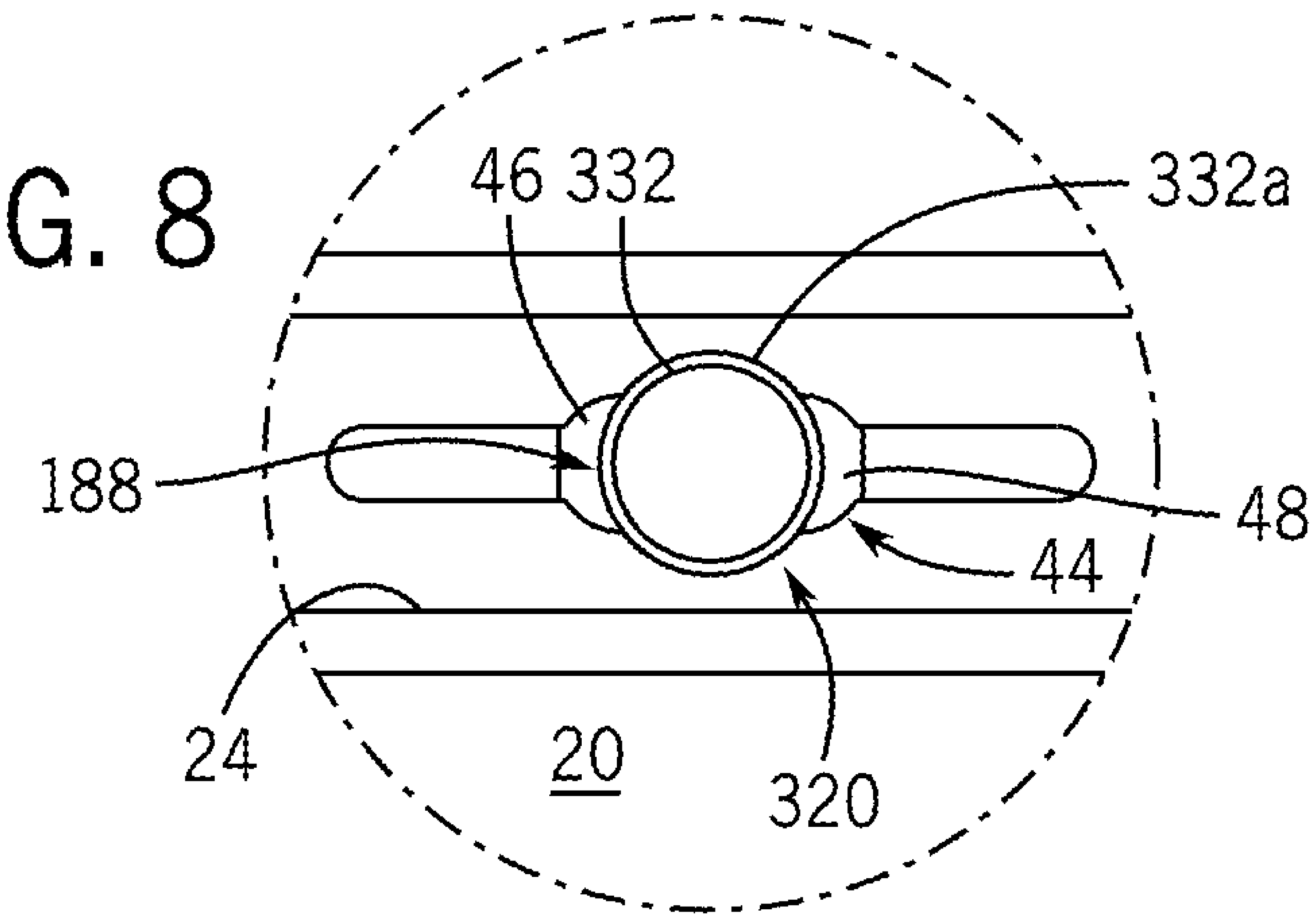


FIG. 7

FIG. 8



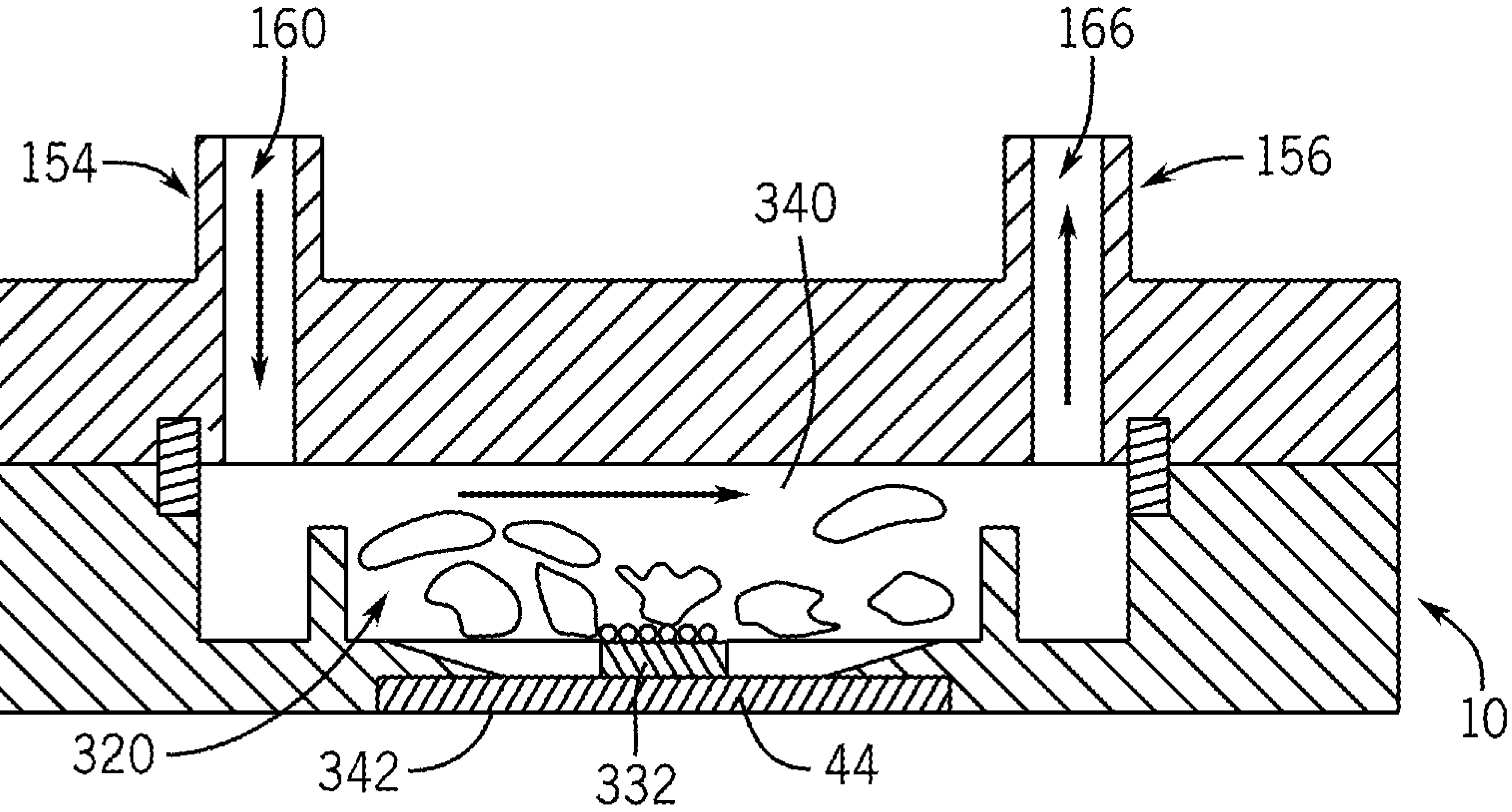


FIG. 9

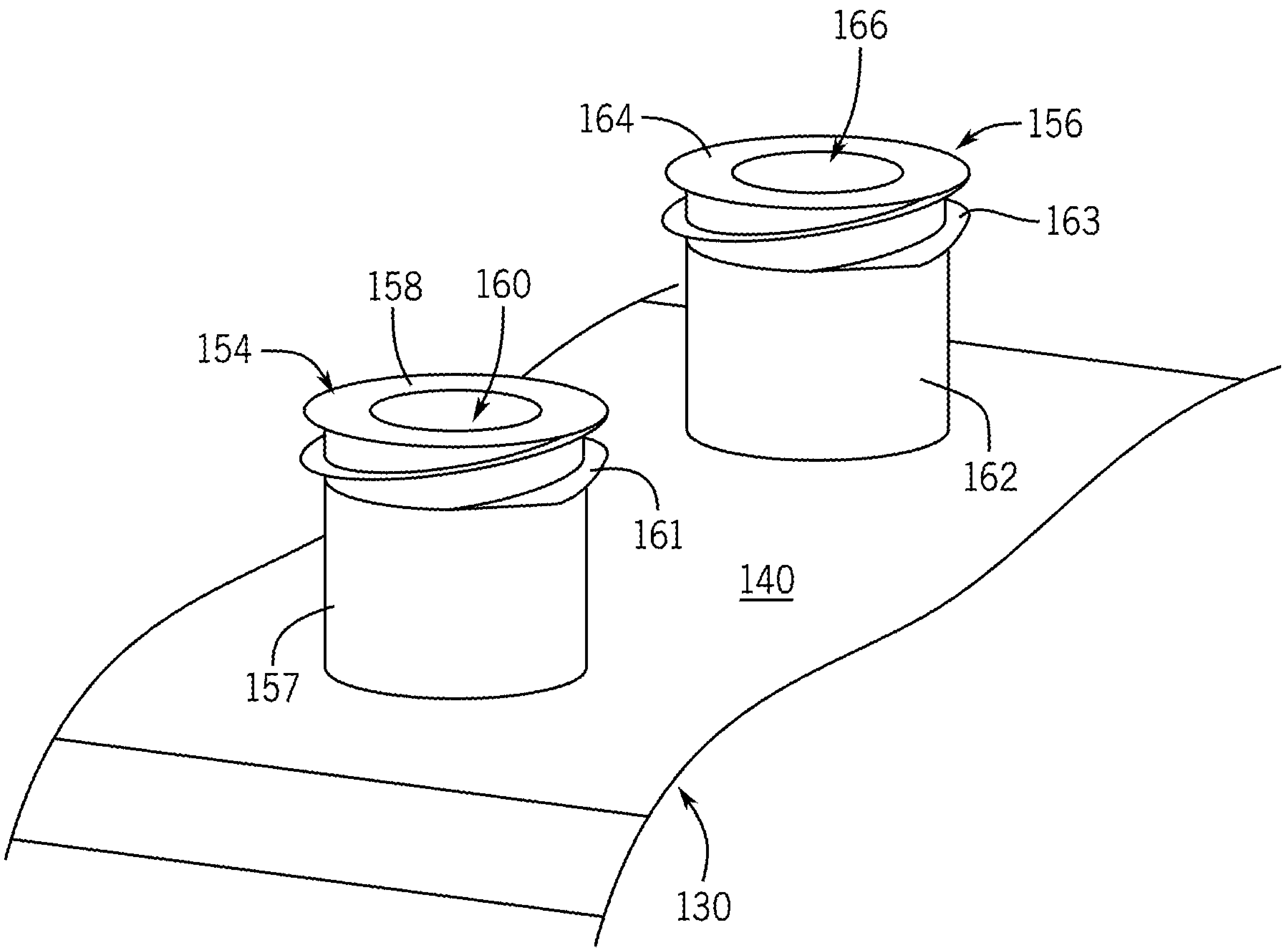


FIG. 10

DEVICE FOR FACILITATING THE IMAGING OF BIOLOGICAL ENTITIES

REFERENCE TO GOVERNMENT GRANT

[0001] This invention was made with government support under GM104540 and GM139168 awarded by the National Institutes of Health and under DE-SC0018409 awarded by the US Department of Energy. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the imaging of biological entities, and in particular, to a device that allows for the live-cell imaging of biological entities in a liquid environment that mimics their native environment and allows for the biological entities to be preserved in place for cryogenic electron microscopy imaging and downstream data processing and analysis.

BACKGROUND AND SUMMARY OF THE INVENTION

[0003] The most common approach for studying dynamic cellular events is light microscopy. Typically, in light microscopy, biological entities (e.g., cells, organoids, tissue) are affixed to a slide or coverslip. A bright light source illuminates the biological entities and a resulting image is formed by the contrast between the biological entities and their surroundings. It can be appreciated that beyond the mere observation of biological entities, light microscopy may also be utilized to capture live-cell images in a liquid environment which mimics their native environment. While functional for its intended purpose, the resolution of the images utilizing light microscopy is somewhat limited.

[0004] Cryogenic electron microscopy (cryo-EM) and cryogenic electron tomography (cryo-ET) are imaging techniques wherein the three-dimensional (3D) structure of biological molecules, complexes, and intact cells are illustrated at high- to atomic resolutions. In this technique, an aqueous solution of the biological sample is applied to a grid/mesh and is rapidly cooled in liquid ethane or a mixture of liquid ethane and propane to cryogenic temperatures, e.g., below −150° Celsius (C), so as to preserve the biological sample in an environment of vitreous ice. An electron microscope is used to image the biological sample while it is held at cryogenic temperatures. In single particle cryo-EM, a set of thousands of two-dimensional (2D) images of nearly identical objects are captured by a camera. For cryo-ET, uniquely shaped biological entities are examined by the collection of individual tilt-series that are composed of 2D images acquired over a range of angles. In both approaches, advanced computational algorithms are then used to reconstruct 3D maps and models of the biological sample from the collected 2D images.

[0005] Cryogenic electron microscopy (cryo-EM) continues to grow in popularity, largely due to the near-atomic resolution of samples, including biological specimens. Improvements in sample preparation and reconstruction software are driving an uptake in usage of cryo-EM in many applications and industries, including pharma. However, cryo-EM is still limited in certain applications, particularly those where dynamic native conditions have an immense impact on behavior and sample development (e.g., bacterial biofilm formation, generation of organoids, and cellular expansion). As a result, researchers are pursuing complimentary technology for cryo-EM sample preparation that could marry improvements in cell culture (e.g., flow cells) with reliable/repeatable electron microscopy (EM) sample grid preparation.

[0006] In view of the foregoing, it can be appreciated that it would highly desirable to provide a device that utilizes an electron microscopy (EM) substrate which is held in place during the seeding of biological entities (cells, organoids, tissue), allows for the growth and expansion of the biological entities to be followed by live-cell imaging (in a liquid environment that mimics their native environment), and allows for the biological entities to be preserved in place for cryogenic electron microscopy imaging and downstream data processing and analysis.

[0007] Therefore, it is primary object and feature of the present invention to provide a device that allows for the live-cell imaging of biological entities in a liquid environment that mimics their native environment and allows for the biological entities to be preserved in place for cryogenic electron microscopy imaging and downstream data processing and analysis.

[0008] It is a further object and feature of the present invention to provide a device for facilitating the imaging of biological entities that utilizes an electron microscopy (EM) substrate which is held in place during the seeding of biological entities (cells, organoids, tissue).

[0009] It is a still further object and feature of the present invention to provide a device for facilitating the imaging of biological entities that is simple to utilize and inexpensive to manufacture.

[0010] In accordance with the present invention, a device is provided for facilitating the imaging of biological entities. The device includes a body having upper and lower surfaces. The body defines a channel for accommodating fluid flow therethrough and a well communicating with the channel and the lower surface. A microscopy grid is removably received in the body and has a first side communicating with a channel and a second side directed at well. A retainer extends into the channel and is engageable with the microscopy grid to support the microscopy grid. The channel includes an inlet and an outlet at opposite ends thereof.

[0011] A transparent coverslip is attached to the lower surface of the body and overlaps the well. In addition, the body includes a grid aperture extending between the channel and the well and being axially aligned with the channel. The grid aperture has a diameter and the well has a diameter. The diameter of the grid aperture is less than the diameter of the well. The grid aperture is generally circular and has outer periphery. The grid aperture includes first and second enlarged portions projecting radially from opposite sides thereof. The first enlarged portion of the grid aperture extends toward the inlet of the channel and the second enlarged portion is directed at the outlet.

[0012] The body may be defined by an upper portion and a lower portion. The channel extends between the upper and lower portions of the body. A sealing gasket is positioned between the upper and lower portions of the body. The sealing gasket extends about the channel. The lower portion of the body includes an upper surface. The upper surface includes a portion partially defining the channel. The portion of the lower surface includes a first ramp having a first end spaced from the inlet of the channel and a second end adjacent the microscopy grid and a second ramp having a first end spaced from the outlet of the channel and a second end adjacent the microscopy grid. A wall projects into the channel adjacent the inlet. The wall is configured to generate turbulence in fluid flowing through the channel.

[0013] In accordance with a further aspect of the present invention, a device is provided for facilitating the imaging of biological entities. The device includes a body defined by upper and lower portions. The upper portion has a lower surface that includes a portion partially defining a channel. The lower portion has an upper surface that includes a portion partially defining the channel. A well extends through the lower portion and communicates with the channel. A grid has a first side communicating with the channel and a second side directed at well. A retainer extends from the lower surface into the channel and is engageable with the grid to support the grid. A coverslip is attached to a lower surface of a lower portion and overlaps the well.

[0014] The channel includes an inlet and an outlet and the lower portion of includes a grid aperture extending between the channel and the well. The grid aperture has a diameter and the well has a diameter. The diameter of the grid aperture is less than the diameter of the well. The grid aperture is generally circular and has outer periphery. In addition, the grid aperture includes first and second enlarged portions projecting radially from opposite sides thereof. The first enlarged portion of the grid aperture extends toward the inlet of the channel and the second enlarged portion is directed at the outlet.

[0015] The portion of the upper surface of the lower portion includes a first ramp having a first end spaced from the inlet of the channel and a second end intersecting the first enlarged portion of the grid aperture and a second ramp having a first end spaced from the outlet of the channel and a second end intersecting the second enlarged portion of the grid aperture. A sealing gasket is positioned between the upper and lower portions of the body and extends about the channel. A wall projects into the channel. The wall is configured to generate turbulence in fluid flowing through the channel. A clamp is removably engageable with the upper and lower portions of the body. The clamp maintains the upper surface of the lower portion against the lower surface of the upper portion.

[0016] In accordance with a still further aspect of the present invention, a device is provided for facilitating the imaging of biological entities. The device includes a body defined by upper and lower portions. The upper portion has a lower surface including a portion partially defining a channel having an input end and an output end. The lower portion has an upper surface including a portion partially defining the channel. A well extends through the lower portion and a grid aperture extends between the channel and the well. The gird aperture has a diameter less than the diameter of the well. A grid is removably received in the grid aperture. The grid has a first side communicating with the channel and a second side directed at well. A coverslip is attached to a lower surface of the lower portion and overlaps the well. A retainer extends from the lower surface of the upper portion into the channel and is engageable with the grid, the retainer retaining the grid against the coverslip.

[0017] The grid aperture is generally circular and has outer periphery. The grid aperture includes first and second enlarged portions projecting radially from opposite sides thereof. The first enlarged portion of the grid aperture extends toward the inlet of the channel and the second enlarged portion is directed at the outlet. The portion of the upper surface of the lower portion includes a first ramp having a first end spaced from the inlet of the channel and a second end intersecting the first enlarged portion of the grid aperture and a second ramp having a first end spaced from the outlet of the channel and a second end intersecting the second enlarged portion of the grid aperture. A sealing gasket is positioned between the upper and lower portions of the body. The sealing gasket extends about the channel. A wall projects into the channel. The wall is configured to generate turbulence in fluid flowing through the channel. The retainer is engageable with an outer periphery of the grid. The retainer may be first retainer and may also include a second retainer extending from the lower surface of the upper portion into the channel and being engageable with the grid. The first and second retainers have a generally crescent-shaped configuration and are circumferentially spaced from each other.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The drawings furnished herewith illustrate a preferred methodology of the present invention in which the above advantages and features are clearly disclosed as well as others which will be readily understood from the following description of the illustrated embodiment.

[0019] In the drawings:

[0020] FIG. **1** is an isometric view of a microfluidic device in accordance with the present invention;

[0021] FIG. **2** is an exploded view of the microfluidic device of FIG. **1**;

[0022] FIG. **2**A is a top plan view of a lower portion of the microfluidic device of FIG. **1**;

[0023] FIG. **3** is a top plan view of an alternate configuration of the lower portion of the microfluidic device shown in FIG. **2**A;

[0024] FIG. **4** is a cross-sectional view of the lower portion of the microfluidic device of the present invention taken along line **4-4** of FIG. **3**;

[0025] FIG. **5** is a bottom plan view of an upper portion of the microfluidic device of FIG. **1**;

[0026] FIG. **6** is a cross-sectional view of the upper portion of the microfluidic device of the present invention taken along line **6-6** of FIG. **5**;

[0027] FIG. **7** is a cross-sectional view of the microfluidic device of the present invention taken along line **7-7** of FIG. **1**;

[0028] FIG. **8** is a cross-sectional view of the microfluidic device of the present invention taken along line **8-8** of FIG. **7**;

[0029] FIG. **9** is a cross-sectional view, similar to FIG. **7**, showing the microfluidic device of the present invention in operation; and

[0030] FIG. **10** is an isometric view showing a part of the upper portion of the microfluidic device of the present invention.

DETAILED DESCRIPTION OF THE DRAWINGS

[0031] Referring to FIGS. **1-2**, a microfluidic device in accordance with the present invention is generally designated by the reference numeral **10**. Microfluidic device **10** may be formed from a photopolymer resin, however, other materials are contemplated as being within the scope of the present invention. As best seen in FIGS. **2-4**, in the depicted embodiment, microfluidic device **10** includes lower portion **11** having first and second ends **12** and **14**, respectively; first and second sides **16** and **18**, respectively; and upper and lower surfaces **20** and **22**, respectively. It is contemplated for microfluidic device **10** to have dimensions generally equal to the dimensions of a standard-sized light microscopy slide. Recessed surface **24** is provided in upper surface **20** of lower portion **11** of microfluidic device **10** and extends along axis. Recessed surface **24** includes a first input end **26** and a second output end **28** and has an outer edge **32** extending about the outer periphery thereof.

[0032] Referring to FIG. 2A, grid receipt aperture **40** extends through recessed surface **24** and is defined by generally cylindrical sidewall **42**. Grid receipt aperture **40** has diameter less than the width of recessed surface **24** and has a sufficient dimension to accommodate grid **44**, as hereinafter described. Grid receipt aperture **40** further includes first and second enlarged portions **46** and **48**, respectively, extending toward corresponding input end **26** and output end **28**, respectively. First and second enlarged portions **46** and **48**, respectively, of grid receipt aperture **40** have generally crescent-shapes to allow grid **44** to be grasped by a pair of tweezers or the like.

[0033] As best seen in FIGS. **3-4** and **7-8**, in an alternate configuration, ramps **50** and **52** may be provided in recessed surface **24** to further facilitate the grasping of grid **44** with a pair of tweezers or the like. Ramp **50** includes angled surface **56** has a first end **58** intersecting recessed surface **24** at a location spaced from first input end **26** of recessed surface **24** and a second end **60** communicating with first enlarged portion **46** of grid receipt aperture **40**. Ramp **52** includes angled surface **62** has a first end **64** intersecting recessed surface **24** at a location spaced from second output end **28** of recessed surface **24** and a second end **66** communicating with second enlarged portion **48** of grid receipt aperture **40**.

[0034] Referring to FIGS. **2A-4** and **7-8**. seal receiving channel **34** extends about outer edge **32** of recessed surface **24** and is defined by a first inner wall **36** depending from outer edge **32** and an outer wall **38** spaced from inner wall **36** by lower surface **39**. Lower surface **39** is generally parallel to upper surface **20** of lower portion **11** of microfluidic device **10** and perpendicular to inner and outer walls **36** and **38**, respectively.

[0035] First arcuate wall **70** extends from recessed surface **24** at between first input end **26** of recessed surface **24** and first end **58** of angled surface **56** of ramp **50**, if present. First arcuate wall **70** is defined by a generally arcuate first side **72** directed towards first input end **26** of recessed surface **24** and a generally arcuate second side **74** directed away from first input end **26** of recessed surface **24**. First and second sides **72** and **74**, respectively, are interconnected by upper surface **76** lying in a plane vertically spaced from upper surface **20** of lower portion **11** of microfluidic device **10**, for reasons hereinafter described.

[0036] Second arcuate wall **80** extends from recessed surface **24** at a location between second output end **28** of recessed surface **24** and first end **64** of angled surface **62** of ramp **52**, if present. Second arcuate wall **80** is defined by a generally arcuate first side **82** directed towards second output end **28** of recessed surface **24** and a generally arcuate second side **84** directed away from second output end **28** of recessed surface **24**. First and second sides **82** and **84**, respectively, are interconnected by upper surface **86** lying in a plane vertically spaced from upper surface **20** of lower portion **11** of microfluidic device **10**, for reasons hereinafter described.

[0037] As best seen in FIGS. **2** and **4**, lower surface **22** of lower portion **11** of microfluidic device **10** includes a coverslip well **90** formed therein. Coverslip well **90** is defined by generally cylindrical surface **92** projecting from the outer periphery of a generally ring-shaped ledge **94**. Ledge **94** has an outer diameter generally equal to the outer diameter of a generally circular, transparent coverslip **91** and an inner diameter greater than the diameter of grid **44**. As described, ledge **94** is adapted for receiving coverslip **91** thereon, as hereinafter described. Ledge **94** is spaced from lower surface **22** of lower portion **11** of microfluidic device **10** by a distance generally equal to the thickness of coverslip **91**, such that outer face **96** of coverslip **91** is substantially flush with lower surface **22** of lower portion **11** of microfluidic device **10** with coverslip **91** received on ledge **94**.

[0038] Lower surface **22** of lower portion **11** of microfluidic device **10** further includes first and second, generally parallel grooves **100** and **102**, respectively, therein. First groove **100** extends between first and second ends **12** and **14**, respectively, of lower portion **11** adjacent first side **16** thereof. First groove **100** is defined by first and second generally parallel sidewalls **106** and **108**, respectively, interconnected by flat surface **110**. Sidewall **106** is interconnected to first side **16** of lower portion **11** by planar surface **112**, which lies in a plane spaced from and parallel to lower surface **22** of lower portion **11**. Sidewall **108** intersects lower surface **22** of lower portion **11**. Surface **110** lies in a plane spaced from and parallel to lower surface **22** of lower portion **11**.

[0039] First groove **100** extends between first and second ends **12** and **14**, respectively, of lower portion **11** adjacent first side **16** thereof. First groove **100** is defined by first and second generally parallel sidewalls **106** and **108**, respectively, interconnected by flat surface **110**. First sidewall **106** is interconnected to first side **16** of lower portion **11** by planar surface **112**, which lies in a plane spaced from and parallel to lower surface **22** of lower portion **11**. First sidewall **108** intersects lower surface **22** of lower portion **11**. Surface **110** lies in a plane spaced from and parallel to lower surface **22** of lower portion **11**.

[0040] Second groove **102** extends between first and second ends **12** and **14**, respectively, of lower portion **11** adjacent second side **18** thereof. Second groove **102** is defined by first and second generally parallel sidewalls **116** and **118**, respectively, interconnected by flat surface **120**. First sidewall **116** is interconnected to second side **18** of lower portion **11** by planar surface **122**, which lies in a plane spaced from and parallel to lower surface **22** of lower portion **11**. Second sidewall **118** intersects lower surface **22** of lower portion **11**. Surface **120** lies in a plane spaced from and parallel to lower surface **22** of lower portion **11**.

[0041] Referring to FIGS. **2** and **5-6**, microfluidic device **10** further includes an upper portion **130** having first and second ends **132** and **134**, respectively; first and second sides **136** and **138**, respectively; and upper and lower surfaces **140** and **142**, respectively. Seal receiving channel **144** is formed in lower surface **142** and is defined by a first inner wall **148** and an outer wall **150** spaced from inner wall **148** by upper surface **152**. Upper surface **152** is generally parallel to lower surface **142** of upper portion **130** of microfluidic device **10** and perpendicular to inner and outer walls **148** and **150**, respectively. As described, it is intended for seal receiving channel **144** to corresponding in size, shape and dimension to seal receiving channel **34** in lower portion **11** of microfluidic device **10**.

[0042] Upper portion **130** of microfluidic device **10** further includes an input port **154** and an output port **156** projecting from upper surface **140** thereof. Input port **154** is defined by a cylindrical outer surface **157** and a terminal surface **158** spaced from and parallel to upper surface **140**. Passageway **160** extends axially from terminal surface **158** through input port **154** and upper portion **130** to lower surface **142** of upper portion **130** such that passageway **160** communicates with lower surface **142** of upper portion **130** at a location within seal receiving channel **144**. As best seen in FIG. **10**, it is contemplated to provide threading **161** on outer surface **157** of input port **154** to facilitate the connection of input port **154** to a source of cell culture media utilizing a standard leur lock connector.

[0043] Referring back to FIGS. **2** and **5-6**, output port **156** is defined by a cylindrical outer surface **162** and a terminal surface **164** spaced from and parallel to upper surface **140**. Passageway **166** extends axially from terminal surface **164** through output port **156** and upper portion **130** to lower surface **142** of upper portion **130** such that passageway **166** communicates with lower surface **142** of upper portion **130** at a location within seal receiving channel **144**. It is contemplated to provide threading **163** on outer surface **162** of output port **156** to facilitate the connection of output port **156** to an output for cell culture media utilizing a standard leur lock connector, FIG. **10**.

[0044] First and second crescent-shaped retainers **170** and **172**, respectively, depending from lower surface **142** of upper portion **130**. First retainer **170** is defined generally arcuate first side **174** directed towards second retainer **172** and a generally arcuate second side **176** directed away from second retainer **172**. First and second sides **174** and **176**, respectively, are interconnected by engagement surface **178** lying in a plane vertically spaced from lower surface **142** of upper portion **130**. Second retainer **172** is defined generally arcuate first side **180** directed towards first retainer **170** and a generally arcuate second side **182** directed away from first retainer **170**. First and second sides **180** and **182**, respectively, are interconnected by engagement surface **184** lying in a common plane with engagement surface **178** of first retainer **170**. As described, the distance between second sides **176** and **182** of first and second retainers **170** and **172**, respectively, is generally equal to the outer diameter of grid **44** such that engagement surfaces **178** and **184** of first and second retainers **170** and **172**, respectively, are engageable with outer rim **188** of grid **44**, as hereinafter described.

[0045] Upper surface **140** of upper portion **130** of microfluidic device **10** further includes first and second, generally parallel grooves **200** and **202**, respectively, therein. First groove **200** extends between first and second ends **132** and **134**, respectively, of upper portion **130** adjacent first side **136** thereof. First groove **200** is defined by first and second generally parallel sidewalls **206** and **208**, respectively, interconnected by flat surface **210**. Sidewall **206** is interconnected to first side **136** of upper portion **130** by planar surface **212**, which lies in a plane spaced from and parallel to upper surface **140** of upper portion **130**. Sidewall **208** intersects upper surface **140** of upper portion **130**. Surface **210** lies in a plane spaced from and parallel to upper surface **140** of upper portion **130**.

[0046] Second groove **202** extends between first and second ends **132** and **134**, respectively, of upper portion **130** adjacent second side **138** thereof. Second groove **202** is defined by first and second generally parallel sidewalls **216** and **218**, respectively, interconnected by flat surface **220**. First sidewall **216** is interconnected to second side **138** of upper portion **130** by planar surface **222**, which lies in a plane spaced from and parallel to upper surface **140** of upper portion **130**. Second sidewall **218** intersects upper surface **140** of upper portion **130**. Surface **222** lies in a plane spaced from and parallel to upper surface **140** of upper portion **130**.

[0047] Microfluidic device **10** further includes first and second generally C-shaped clamps **230** and **232**, respectively, are provided. First clamp **230** extends along an axis and is defined by generally parallel, upper and lower spaced walls **234** and **236**, respectively, interconnected and spaced by sidewall **238**. Upper wall **234** includes a planar upper surface **240** and a lower surface **242** spaced from and parallel to upper surface **240**. First key **246**, having a generally rectangular configuration, depends from lower surface **242** and is defined by generally parallel first and second sides **248** and **250**, respectively, interconnected by generally planar, terminal surface **252**. For reasons hereinafter described, it is intended for first key **246** to form a mating relationship with first groove **200** in upper surface **140** of upper portion **130** of microfluidic device **10**.

[0048] Lower wall **236** of first clamp **230** includes an upper surface **260** and a generally planar, lower surface **262** spaced from and parallel to upper surface **260**. Second key **266**, having a generally rectangular configuration, extends from upper surface **260** and is defined by generally parallel first and second sides **268** and **270**, respectively, interconnected by generally planar, terminal surface **272**. For reasons hereinafter described, it is intended for second key **266** to form a mating relationship with first groove **100** in upper surface **20** of lower portion **11** of microfluidic device **10**.

[0049] Second clamp **232** extends along an axis and is defined by generally parallel, upper and lower spaced walls **274** and **276**, respectively, interconnected and spaced by sidewall **278**. Upper wall **274** includes a planar upper surface **280** and a lower surface **282** spaced from and parallel to upper surface **280**. First key **286**, having a generally rectangular configuration, depends from lower surface **282** and is defined by generally parallel first and second sides **288** and **290**, respectively, interconnected by generally planar, terminal surface **292**. For reasons hereinafter described, it is intended for first key **286** to form a mating relationship with second groove **202** in upper surface **140** of upper portion **130** of microfluidic device **10**.

[0050] Lower wall **276** of second clamp **232** includes an upper surface **300** and a generally planar, lower surface **302** spaced from and parallel to upper surface **300**. Second key **306**, having a generally rectangular configuration, extends from upper surface **300** and is defined by generally parallel first and second sides **308** and **310**, respectively, interconnected by generally planar, terminal surface **312**. For reasons hereinafter described, it is intended for second key **306** to form a mating relationship with first groove **102** in lower surface **22** of lower portion **11** of microfluidic device **10**.

[0051] Referring to FIGS. **2** and **7-8**, in order to assemble microfluidic device **10**, seal or gasket **316** is positioned in seal receiving channel **144** is formed in lower surface **142** of upper portion **130** of microfluidic device **10**. Thereafter, upper surface **20** of lower portion **11** of microfluidic device

10 is positioned on lower surface **146** of upper portion **130** of microfluidic device **10** such that first and second ends **12** and **14**, respectively, of lower portion **13** are aligned with and lie in common planes with corresponding first and second ends **132** and **134**, respectively, of upper portion **130** and first and second sides **16** and **18**, respectively, lower portion **11** are aligned with and lie in common planes with corresponding first and second sides **136** and **138**, respectively, of upper portion **130**. With lower portion **11** positioned on upper portion **130**, seal **316** is received in seal receiving channel **34** in upper surface **20** of lower portion **11** and first and second retainers **170** and **172**, respectively, are received in grid receipt aperture **40**.

[0052] After positioning lower portion **11** on upper portion **130**, as heretofore described, first and second clamps **230** and **232**, respectively, are interconnected to lower portion **11** and upper portion **130** to retain lower portion **11** and upper portion **130** together. More specifically, first clamp **230** is positioned adjacent to either first end **12** of lower portion **11** and first end **132** of upper portion **130** or second end **14** of lower portion **11** and second send **134** of upper portion **130** such that first key **246** is axially aligned with first groove **200** in upper surface **140** of upper portion **130** and second key **266** is axially aligned with first groove **100** in lower surface **22** of lower portion **11**. Thereafter, first clamp **230** is slid axially such the first key **246** is received within first groove **200** in upper surface **140** of upper portion **130** and second key **266** is received within first groove **100** in lower surface **22** of lower portion **11**.

[0053] Similarly, second clamp **232** is positioned adjacent to either first end **12** of lower portion **11** and first end **132** of upper portion **130** or second end **14** of lower portion **11** and second end **134** of upper portion **130** such that first key **286** is axially aligned with second groove **202** in upper surface **140** of upper portion **130** and second key **306** is axially aligned with second groove **102** in lower surface **22** of lower portion **11**.

[0054] Thereafter, second clamp **232** is slid axially such the first key **286** is received within second groove **202** in upper surface **140** of upper portion **130** and second key **306** is received within second groove **102** in lower surface **22** of lower portion **11**.

[0055] With first and second clamps **230** and **232**, respectively, securing upper portions **130** and lower portion **11** together, channel **320** is formed between upper portions **130** and lower portion **11**. Channel **320** is defined by lower surface **142** in upper portion **130** and recessed surface **24** in lower portion **11** and includes a first end **322** in communication with passageway **160** through input port **154** and a second end **324** in communication with passageway **166** through output port **156**. First and second clamps **230** and **232**, respectively, act to compress seal **316** with seal receiving channels **34** and **144** such that seal **316** in seal receiving channels **34** and **144** retains fluid flowing in channel **320** and prevents leakage therefrom.

[0056] First and second arcuate walls **70** and **80**, respectively, project into channel **320** such that upper surfaces **76** and **86** of first and second arcuate walls **70** and **80**, respectively, are vertically spaced from lower surface **142** of upper portion **130**. It is intended for first and second arcuate walls **70** and **80**, respectively, to generate turbulence in fluid flowing through channel **320**.

[0057] Tweezers or the like (not shown) may be used to deposit grid **44** on first and second retainers **170** and **172**, respectively, such that outer rim **188** of grid **44** is supported on engagement surfaces **178** and **184** of first and second retainers **170** and **172**, respectively, first side **332*a*** of central grid portion **332** of grid **44** is communication with channel **320**, and second side **332*b*** of central grid portion **332** of grid **44** is directed towards and in communication with coverslip well **90**. Once grid **44** is positioned on first and second retainers **170** and **172**, respectively, coverslip **91** is positioned within coverslip well **90** such that the radially outer portion of upper surface **314** of coverslip **91** is positioned on ledge **94**. The radially outer portion of upper surface **314** of coverslip **91** is bonded to ledge **94** is a conventional manner, e.g., by utilizing an ultraviolet bonding resin. Once coverslip **91** is bonded to ledge **94**, outer surface **96** of coverslip **91** is substantially flush with lower surface **22** of lower portion **11** of microfluidic device **10**. Thereafter, microfluidic device **10** may be flipped such that upper surface **140** of upper portion **130** of microfluidic device **10** is directed upwardly.

[0058] With microfluidic device assembled, it is contemplated to load channel **320** with a biological sample, e.g. a cell culture media **340**. More specifically, cell culture media **340** is introduced into passageway **160** of input port **154** and flows into channel **320** wherein cells **342** in cell culture media **340** are allowed to incubate and attach to grid portion **332** of grid **44**. As noted above, grid portion **332** of grid **44** is communication with channel **320**. Cell culture media **340** may be replenished during incubation to maintain cell culture hydration during. Thereafter, minimal media flow may be continued to provide a liquid environment that mimics the cells' native environment, while preserving cells **340** for subsequent analysis.

[0059] It can be appreciated that during the incubation and growth of cells **340** on grid portion **188** of grid **44**, microfluidic device **10** may be positioned on a stage of a light microscope, thereby allowing for long term live imaging of cells **340** in channel **320** through coverslip **91**. As previously noted, microfluidic device **10** has dimensions generally equal to the dimensions of a standard-sized light microscopy slide, thereby enhancing the compatibility of microfluidic device **10** with the stage of the light microscope. Further, it can be understood grid **44** may be removed from microfluidic device **10** for facilitating imaging of cells **340** by an electron microscope. More specifically, in order to remove grid **44** from microfluidic device **10**, first and second clamps **230** and **232**, respectively, may be removed from upper and lower portions **140** and **11**, respectively, thereby allowing lower portion **11** to be separated from upper portion **140**. With lower portion **11** separated from upper portion **140**, a user may use tweezers or the like (not shown) to remove grid **44** from first and second retainers **170** and **172**, respectively. Thereafter, grid **44** may be frozen via plunge or high pressure freezing and imaged in a cryo-transmission electron microscope in a conventional manner.

[0060] Various modes of carrying out the invention are contemplated as being within the scope of the following claims particularly pointing out and distinctly claiming the subject matter, which is regarded as the invention.

We claim:

1. A device for facilitating the imaging of biological entities, comprising:

a body including upper and lower surfaces, the body defining a channel for accommodating fluid flow therethrough and a well communicating with the channel and the lower surface;

a microscopy grid removably received in the body and having a first side communicating with the channel and a second side directed away from the channel; and

a retainer extending into the channel and being engageable with the microscopy grid to support the microscopy grid;

wherein:

the channel includes an inlet and an outlet at opposite ends thereof.

2. The device of claim **1** further comprising a transparent coverslip attached to the lower surface of the body and overlapping the well.

3. The device of claim **2** wherein the body includes a grid aperture extending between the channel and the well and being axially aligned with the channel.

4. The device of claim **3** wherein the grid aperture has a diameter and the well has a diameter, the diameter of the grid aperture is less than the diameter of the well.

5. The device of claim **3** wherein the grid aperture is generally circular and has outer periphery, the grid aperture includes first and second enlarged portions projecting radially from opposite sides thereof.

6. The device of claim **5** wherein first enlarged portion of the grid aperture extends toward the inlet of the channel and the second enlarged portion is directed at the outlet.

7. The device of claim **1** wherein the body is defined by an upper portion and a lower portion, the channel extending between the upper and lower portions of the body.

8. The device of claim **7** further comprising a sealing gasket positioned between the upper and lower portions of the body, the sealing gasket extending about the channel.

9. The device of claim **7** wherein:

the lower portion of the body includes an upper surface, the upper surface including a portion partially defining the channel;

the portion of the lower surface including a first ramp having a first end spaced from the inlet of the channel and a second end adjacent the microscopy grid and a second ramp having a first end spaced from the outlet of the channel and a second end adjacent the microscopy grid.

10. The device of claim **1** further comprising a wall projecting into the channel adjacent the inlet, the wall configured to generate turbulence in fluid flowing through the channel.

11. A device for facilitating the imaging of biological entities, comprising:

a body defined by:

an upper portion having a lower surface, the lower surface including a portion partially defining a channel;

a lower portion having an upper surface, the upper surface including a portion partially defining the channel; and

a well extending through the lower portion and communicating with the channel;

a grid having a first side communicating with the channel and a second side directed at well;

a retainer extending from the lower surface into the channel and being engageable with the grid to support the grid; and

a coverslip attached to a lower surface of a lower portion and overlapping the well.

12. The device of claim **11** wherein the channel includes an inlet and an outlet and wherein the lower portion includes a grid aperture extending between the channel and the well.

13. The device of claim **12** wherein the grid aperture has a diameter and the well has a diameter, the diameter of the grid aperture is less than the diameter of the well.

14. The device of claim **12** wherein:

the grid aperture is generally circular and has outer periphery;

the grid aperture includes first and second enlarged portions projecting radially from opposite sides thereof; and

the first enlarged portion of the grid aperture extends toward the inlet of the channel and the second enlarged portion is directed at the outlet.

15. The device of claim **14** wherein the portion of the upper surface of the lower portion includes a first ramp having a first end spaced from the inlet of the channel and a second end intersecting the first enlarged portion of the grid aperture and a second ramp having a first end spaced from the outlet of the channel and a second end intersecting the second enlarged portion of the grid aperture.

16. The device of claim **11** further comprising a sealing gasket positioned between the upper and lower portions of the body, the sealing gasket extending about the channel.

17. The device of claim **11** further comprising a wall projecting into the channel, the wall configured to generate turbulence in fluid flowing through the channel.

18. The device of claim **11** further comprising a clamp removably engageable with the upper and lower portions of the body, the clamp maintaining the upper surface of the lower portion against the lower surface of the upper portion.

19. The device for facilitating the imaging of biological entities, comprising:

a body defined by:

an upper portion having a lower surface, the lower surface including a portion partially defining a channel having an input end and an output end;

a lower portion having an upper surface, the upper surface including a portion partially defining the channel;

a well extending through the lower portion;

a grid aperture extending between the channel and the well, the gird aperture having a diameter less than the diameter of the well;

a grid removably received in the grid aperture, the grid having a first side communicating with the channel and a second side directed at well;

a coverslip attached to a lower surface of the lower portion and overlapping the well;

a retainer extending from the lower surface of the upper portion into the channel and being engageable with the grid, the retainer retaining the grid against the coverslip.

20. The device of claim **18** wherein the grid aperture is generally circular and has outer periphery;

the grid aperture includes first and second enlarged portions projecting radially from opposite sides thereof; and

the first enlarged portion of the grid aperture extends toward the inlet of the channel and the second enlarged portion is directed at the outlet.

21. The device of claim **20** wherein the portion of the upper surface of the lower portion includes a first ramp having a first end spaced from the inlet of the channel and a second end intersecting the first enlarged portion of the grid aperture and a second ramp having a first end spaced from the outlet of the channel and a second end intersecting the second enlarged portion of the grid aperture.

22. The device of claim **19** further comprising a sealing gasket positioned between the upper and lower portions of the body, the sealing gasket extending about the channel.

23. The device of claim **19** further comprising a wall projecting into the channel, the wall configured to generate turbulence in fluid flowing through the channel.

24. The device of claim **19** wherein the retainer is engageable with an outer periphery of the grid.

25. The device of claim **19** wherein the retainer is first retainer and wherein the device further comprises:

a second retainer extending from the lower surface of the upper portion into the channel and being engageable with the grid, the first and second retainers have a generally crescent-shaped configuration and are circumferentially spaced from each other.

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