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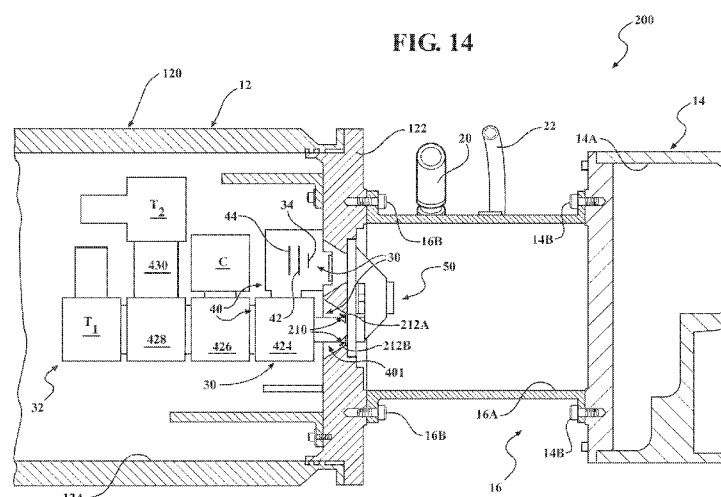
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(54) Title: FLUID SUBMERSIBLE SENSING DEVICE



(57) Abstract: A fluid submersible sensing device is provided comprising: a housing defining an internal chamber and including window structure; sensing structure provided in the internal chamber; light providing apparatus in the internal chamber emitting light capable of passing through the window structure so as to exit the housing; an external structure coupled to the housing and located outside of the housing internal chamber comprising a substantially clear flow cell including an inner surface defining a passage through which a fluid flows and one or more optical elements for causing the light from the housing to pass through the passage and re-enter the housing toward the sensing structure; UV light source apparatus for directing UV light toward the flow cell; and a conduit structure coupled to an inlet of the flow cell so as to provide fluid to be analyzed to the flow cell passage. At least a portion of the conduit structure may be formed from copper. The copper conduit structure portion and the UV light prevent the formation of biofilms on the flow cell inner surface.



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## FLUID SUBMERSIBLE SENSING DEVICE

### TECHNICAL FIELD

The present invention relates to a fluid submersible sensing device and, more particularly, to such a device having sensing structure provided within a fluid-tight housing and an external structure located outside of the fluid-tight housing including a passage through which a fluid sample flows.

### BACKGROUND ART

Flow cytometry is a process for characterization and quantification of microscopic particles suspended in a stream of fluid. A fluid submersible flow cytometer is known comprising a fluid-tight housing having an internal chamber containing a detector, a light source and a flow cell through which a stream of fluid to be analyzed moves. Light from the light source is passed through the stream of fluid and received by the detector for characterization and quantification of the particles within the fluid.

### DISCLOSURE OF INVENTION

In accordance with a first aspect of the invention, a fluid submersible sensing device is provided comprising: a housing defining an internal chamber and including a window structure; sensing structure provided in the internal chamber; light providing apparatus in the internal chamber emitting light capable of passing through the window structure so as to exit the housing; an external structure coupled to the housing and located outside of the housing internal chamber comprising a substantially clear flow cell including an inner surface defining a passage through which a fluid flows and one or more optical elements for causing the light from the housing to pass through the passage and re-enter the housing toward the sensing structure; UV light source apparatus for directing UV light toward the flow cell; and a conduit structure coupled to an inlet of the flow cell so as to provide fluid to be analyzed to the flow cell passage. At least a portion of the conduit structure may be formed from copper. The copper conduit structure portion and the UV light prevent the formation of biofilms on the flow cell inner surface.

The UV light source apparatus may comprise at least one light-emitting diode.

The light providing apparatus may comprise a laser light source and a backlight source.

The sensing structure may comprise an optical analysis and imaging apparatus.

The external structure may further comprise a primary element comprising the one or more optical elements.

5 The primary element may comprise a prism including the one or more optical elements defined by first and second mirrored surfaces on the prism to reflect the light along a desired path such that the light passes through the flow cell including the passage and then re-enters the housing.

The flow cell may comprise a clear body separate from the primary element. The passage may extend through it.

10 The passage has a longitudinal axis substantially parallel with a fluid flow path through the passage and a cross sectional area substantially transverse to the longitudinal axis sized such that generally all fluid flowing through the passage is analyzed by the sensing structure.

15 The device may further comprise a pressure-compensated second housing comprising electronic components and a pressure-compensated third housing containing the external structure. The second housing may be separable from the third housing such that the flow cell in the third housing can be serviced without opening the second housing.

20 The device may further comprise a pump provided in the second housing and coupled to the conduit structure so as to pump the fluid to be analyzed through the conduit structure to the flow cell passage.

The flow cell passage may have a dimension of from about 100 microns to about 3000 microns.

The copper conduit structure portion may have a length of from of about 6 inches to about 24 inches.

25 The UV light source may emit UV light at a nominal UV emission wavelength of about 265 nanometers.

The UV light source apparatus may be located outside of the flow cell passage and UV light from the UV light source apparatus may pass through an outer surface of the flow cell and then into and through the flow cell passage.

30 In accordance with a second aspect of the present invention, a fluid receiving device is provided capable of preventing biofouling comprising: a UV light source apparatus for emitting UV light; a substantially transparent flow cell including an inner surface defining a passage through which a fluid to be analyzed flows, the UV light passing through the

passage; and a conduit structure coupled to an inlet of the flow cell so as to provide fluid to be analyzed to the flow cell passage. At least a portion of the conduit structure may be formed from copper. The copper conduit structure portion and the UV light prevent the formation of biofilms on the flow cell inner surface.

5           The flow cell passage may have a dimension of from about 100 microns to about 3000 microns.

          The copper conduit structure portion may have a length of from about 6 inches to about 24 inches.

          The UV light source apparatus may emit UV light at a nominal UV emission  
10   wavelength of 265 nanometers.

          The UV light source apparatus may be located outside of the flow cell passage and UV light from the UV light source apparatus may pass through an outer surface of the flow cell and then into and through the flow cell passage.

          In accordance with a third aspect of the present invention, a process is provided for  
15   preventing the formation of biofilms on a flow cell inner surface comprising: providing a flow cell having an inner surface defining a passage through which a fluid to be analyzed flows; providing a conduit structure coupled to the flow cell, the conduit structure including at least a portion formed from copper; and passing fluid through the conduit structure such that the fluid and copper<sup>+0</sup> ions or copper<sup>+2</sup> ions leaching from the conduit structure copper  
20   portion flow through the flow cell passage. The UV light causes a photocatalytic conversion of the copper<sup>+0</sup> ions or copper<sup>+2</sup> ions into toxic copper<sup>+1</sup> ions, wherein the copper<sup>+1</sup> ions and UV light prevent the formation of biofilms on the flow cell inner surface.

### **BRIEF DESCRIPTION OF DRAWINGS**

25           Figs. 1 and 2 are side views of a fluid submersible sensing device constructed in accordance with the present invention;

          Fig. 3 is a cross sectional view of the sensing device illustrated in Figs. 1 and 2;

          Fig. 4 is a cross sectional view illustrating an external optics and sample providing structure coupled to a first housing of the sensing device;

30           Figs. 5 and 6 are perspective views of the external optics and sample providing structure coupled to the first housing;

          Fig. 7 is a perspective view of second and third housings of the sensing device illustrated in Figs. 1 and 2;

Fig. 8 is a cross sectional view of an inlet screen assembly;

Fig. 9 is a side view of a portion of a fluid submersible sensing device including a focusing mechanism constructed in accordance with the present invention;

Fig. 10 is a front view of the portion of the fluid submersible sensing device illustrated in Fig. 9;

Fig. 11 is a top view of the portion of the fluid submersible sensing device illustrated in Fig. 9;

Fig. 12 is a cross sectional view of the sensing device including the focusing mechanism illustrated in Fig. 9;

Fig. 13 is a cross sectional view illustrating an external optics and sample providing structure coupled to a first housing of the sensing device illustrated in Fig. 12; and

Fig. 14 is a cross sectional view of a sensing device constructed in accordance with a further embodiment of the present invention.

#### **BEST MODE FOR CARRYING OUT THE INVENTION**

A fluid submersible sensing device 10, constructed in accordance with the present invention, is illustrated in Figs. 1-3. The device 10 is intended to be used underwater, such as in an ocean, to effect a flow cytometry process for characterization and quantification of microscopic particles suspended in a stream of fluid passing through the device 10.

The sensing device 10 comprises a first fluid-tight housing 12 having an internal chamber 12A filled with air, a pressure-compensated second housing 14 having an internal chamber 14A that is preferably filled with a dielectric fluid, e.g., an oil, such as hydraulic or mineral oil, and a pressure-compensated third housing 16 having an internal chamber 16A filled with a liquid having a refractive index (e.g., water having a refractive index = 1.333) that approximately matches the refractive index of glass (borosilicate glass having a refractive index = 1.515), see Fig. 3. The second housing 14 is coupled to the third housing 16 via bolts 14B, see Fig. 3, and the third housing 16 is coupled to the first housing 12 via bolts 16B, see Figs. 1-3. The first housing 12 is sealed relative to the second and third housings 14 and 16.

As noted, the internal chamber 14A of the second housing 14 preferably contains a dielectric fluid since the internal chamber 14A of the second housing 14 contains electronic components, which could be damaged if a fluid other than a dielectric fluid is used, e.g., circuits of the electronic components could be shorted if a conducting fluid were used. Further, the internal chamber 16A of the third housing 16 preferably contains distilled water,

rather than, for example, oil, since oils fluoresce, which could otherwise interfere with measurements taken by a sensing apparatus 30, as will be discussed in detail herein. The chamber 16A preferably contains distilled water because distilled water approximately matches the optical index of glass, it does not fluoresce, and it inhibits

5 biological/microorganism growth. It is noted that regular (undistilled) water meets the first two of these provisions, but may not inhibit biological/microorganism growth. However, inhibiting biological/microorganism growth may be accomplished via other methods, such as, for example, by adding bleach, alcohol or other biocide to the regular water. Other mechanisms, for example, adding a solid substrate containing a biocide to the regular water, 10 autoclaving the regular water, or coating the internal surfaces of the chamber 16A with copper could also inhibit biological/microorganism growth without adding a liquid biocide to the regular water. Yet a further method for inhibiting biological/microorganism growth with regular water would be irradiating the water with UV or other light to disinfect the water.

It is noted that it is preferable to keep the chambers 14A and 16A separate from one 15 another. This is preferable, for example, as the electronic components in the internal chamber 14A of the second housing 14 may shed wear materials, which wear materials could otherwise interfere with measurements taken by the sensing apparatus 30. Alternatively, if the chambers 14A and 16A are not kept separate, some type of filtering device (not shown) would preferably be used to keep the wear materials out of the internal chamber 16A of the 20 third housing 16.

In the illustrated embodiment, the first housing 12 is constructed from materials having sufficient strength and is sealed such that the pressure within the first housing 12 remains at approximately 1 atmosphere when the device 10 is submerged in water and is dropped to a depth, for example, of about 200 meters. A first tube 20 is coupled to and 25 extends between the first and second housings 12 and 14 and contains electrical wiring (not shown) extending between and coupled to the first housing 12 and the second housing 14. The first tube 20 is in fluid communication with the second housing internal chamber 14A and contains oil. The first tube 20 is sealed at the end adjacent the first housing 12 such that oil is not permitted to exit the first tube 20 and enter the first housing internal chamber 12A. 30 However, the wiring extending through the first tube 20 does exit the first tube 20 and enter the first housing internal chamber 12A. A second tube 22 is coupled to and extends from the third housing 16. The second tube 22 is sealed at its end opposite the end coupled to the third housing 16 via a clip 22A or other sealing structure. The first and second tubes 20 and 22 are

exposed to the surrounding water in which the sensing device 10 is submerged. As the sensing device 10 moves deeper into the water, the pressure of the surrounding water increases. The increased pressure of the surrounding water compresses the first and second tubes 20 and 22 so as to vary the pressure of the oil in the second housing internal chamber 14A and the distilled water in the third housing internal chamber 16A such that the pressure of the oil in the second housing internal chamber 14A and the pressure of the water in the third housing internal chamber 16A substantially equals the pressure of the water surrounding the sensing device 10.

The first housing 12 comprises a generally cylindrical first structure 120 and first and second end caps 122 and 124, respectively, all of which may be made from a metal, such as a 6061-T6 Al alloy, see Fig. 3. The first structure 120, the first end cap 122 and the second end cap 124 are bolted and O-ring sealed or otherwise coupled together to create the sealed inner chamber 12A.

The first end cap 122 is provided with first and second openings 122A and 222A and an adjacent recess 122B surrounding the openings 122A and 222A, see Fig. 4. A window 124A (also referred to herein as “window structure”), formed from clear glass or polymeric material in the illustrated embodiment, is provided in the recess 122B and covers the openings 122A and 222A. The window 124A is coupled to the first end cap 122 via a support plate 126 and bolts 126A, see Fig. 4. It is noted that the window 124A preferably comprises a single piece of glass, plastic, or other suitable material.

The first housing internal chamber 12A contains the sensing structure 30 comprising an optical analysis and imaging apparatus 32, a forward scatter sensor 34, an objective or lens 401, one or more excitation filters 424, a partial mirror 426, one or more fluorescence emission filters 428, and the spacer structure 430, see Fig. 3. The optical analysis and imaging apparatus 32 may comprise a conventional camera C and first and second conventional photo-multiplier tubes  $T_1$  and  $T_2$ , see Fig. 3, although it is noted that more photo-multiplier tubes may be used if desired. The first housing internal chamber 12A further comprises light-providing apparatus 40 comprising an LED backlight 42 and a laser light source 44, see Fig. 3, contained within the optical analysis and imaging apparatus 32. An ultra-violet light source (not shown) may also be provided in the first housing internal chamber 12A. The first housing internal chamber 12A further comprises electronics such as processor apparatus (not shown) for controlling the operation of the sensing structure 30 and the light-providing apparatus 40, see Fig. 3. It is contemplated that the optical analysis and

imaging apparatus 32 may comprise the structure set out in U.S. Patent No. 6,115,119, entitled Device and Method for Studying Particles in a Fluid, by Sieracki et al., the entire disclosure of which is incorporated by reference herein.

An external optics and sample providing structure 50 is coupled to the first housing 12 and located outside of the first housing internal chamber 12A so as not to directly communicate with the first housing internal chamber 12A, see Figs 4-6. In the illustrated embodiment, the structure 50 is contained within the third housing internal chamber 16A. The structure 50 comprises a prism 52 and a flow cell 54, both of which are mounted adjacent the window 124A via a strap 56 bolted to the first end cap 122 via bolts 56A, see Figs 4-6. The prism 52 is formed from glass or a polymeric material and may have first and second mirrored surfaces 52A and 52B (the mirrors are also referred to herein as “optical elements”), see Fig. 4. In the illustrated embodiment, the mirrored surfaces 52A and 52B may be defined by metal layers, which layers are coated with an epoxy to protect the mirror surfaces 52A and 52B from oxidation. The flow cell 54 may be formed from a clear glass or polymeric material and has a passage 54A through which water to be analyzed passes, see Fig. 4. Because the flow cell 54 is located outside of the first housing internal chamber 12A, risk of water leaking from the flow cell 54 and contacting the electronics and the like within the internal chamber 12A is minimized. As noted above, the window 124A preferably comprises a single piece of material. Forming the window 124A from a single piece of material provides for an optimal amount of optical contact between the window 124A and both the flow cell 54 and the prism 52. It is noted that achieving optical contact between the window 124A and both the flow cell 54 and the prism 52 may be difficult if the window 124A is formed from two separate pieces, as each piece would obtain its orientation from the aluminum end cap 122, which cannot be easily machined to optical tolerances.

The flow cell passage 54A comprises an inlet 250 and an outlet 252, see Figs. 5 and 6. A first end 260A of a first conduit 260 is coupled to the passage inlet 250 via a fitting (not shown), friction fit or the like, while the second end (not shown) of the first conduit 260 is coupled within the third housing internal chamber 16A to a first side of an inlet fitting 160 coupled to the third housing 16. A first end 262A of a second conduit 262 is coupled to the passage outlet 252 via a fitting (not shown), friction fit or the like, while a second end (not shown) of the second conduit 262 is coupled within the third housing internal chamber 16A to a first side of an outlet fitting 162 coupled to the third housing 16.

Inlet and outlet screen assemblies 270 and 272 are coupled externally to the second housing 14 via bolts 270A and 272A, see Figs. 7 and 8. The inlet screen assembly 270 comprises an inlet screen 270B and a fitting 270C. An internal cavity 1270 is defined by a recess 270D in a main body 270E and covered by the inlet screen 270B. Passages 1272 and 1274 are also formed in the main body 270E, see Fig. 8. The screen 270B is held in position relative to the main body 270E by a backing plate 270F and first and second side plates 270G and 270H, see Fig. 8. Water flows through the inlet screen 270B, the internal cavity 1270, the first and second passages 1272 and 1274 and into the fitting 270C. The outlet screen assembly 272 comprises an outlet screen 272B and a fitting 272C. An internal cavity (not shown) is defined by a recess (not shown) in an outlet screen assembly main body (not shown) and covered by the outlet screen 272B. Passages (not shown) are also formed in the outlet screen assembly main body. The screen 272B is held in position relative to the main body by a backing plate 272F and first and second side plates 272G and 272H, see Fig. 7. Water flows through the fitting 272C, the second and first passages, the internal cavity, and out the outlet screen 272B. It is noted that the inlet and outlet screens 270B and 272B may be formed from a copper mesh to inhibit microorganism growth.

It is further noted that, while allowing small aquatic species to enter the sensing device 10 through the inlet screen assembly 270 may be desirable, i.e., if it is desired to sense such small aquatic species by the sensing apparatus 30, larger aquatic species are preferably not permitted to enter the sensing device 10 through the inlet screen assembly 270, as these larger aquatic species could clog or become lodged in the components of the sensing device 10. The large inlet screen area, which leads to the narrow passages 1272 and 1274, decreases the chance of the inlet screen 270B becoming completely blocked such that no flow may enter the sensing device 10. The large inlet screen area, which leads to the narrow passages 1272 and 1274, also provides a low flow rate at the inlet screen assembly 270. This may be beneficial as it may decrease the tendency for large particles to get caught on the inlet screen 270 due to the suction generated by a pump 322, discussed below. This may also be beneficial, as some motile aquatic species have an instinct to swim upstream, e.g., so as to keep themselves out of a predator's mouth. Hence, the low flow rate at the inlet screen assembly 270 may reduce the number of motile aquatic species that are startled by the acceleration of being drawn through the inlet screen 270B into the sensing device 10 by remaining below the acceleration threshold that would alert them to swim away from the inlet screen assembly 270. The inlet and outlet screen assemblies 270 and 272 are configured

similarly so that the direction of the flow generated by the pump 322 can be reversed to back flush any particulates that have jammed or become lodged in the sensing device 10.

A third conduit 280, located external to the second housing internal chamber 14A, is coupled to and extends between the inlet screen assembly fitting 270C and a first inlet fitting

284 on the second housing 14. A fourth conduit 290, located internally within the second housing internal chamber 14A, is also coupled to the first inlet fitting 284 and extends to a

valve 300 located within the second housing internal chamber 14A. A fifth conduit 310, located internally within the second housing internal chamber 14A, extends from the valve

300 to a first outlet fitting 312 coupled to the second housing 14. A sixth conduit 314,

located external to the second and third housing internal chambers 14A and 16A, extends from the first outlet fitting 312 to the inlet fitting 160 coupled to the third housing 16. A

seventh conduit 316, located external to the second and third housing internal chambers 14A and 16A, extends from the outlet fitting 162 coupled to the third housing 16 to a second inlet

fitting 318 coupled to the second housing 14. An eighth conduit 320A, located internally

within the second housing internal chamber 14A, extends from the second inlet fitting 318 to a flow meter 321. A ninth conduit 320B extends from the flow meter 321 to the pump 322,

such as a conventional peristaltic pump. A tenth conduit 324, located internally within the second housing internal chamber 14A, extends from the pump 322 to the valve 300. An

eleventh conduit 326, located internally within the second housing internal chamber 14A,

extends from the valve 300 to a second outlet fitting 328 coupled to the second housing 14. A twelfth conduit 330 extends from the second outlet fitting 328 to the outlet screen assembly

fitting 272C.

In the illustrated embodiment, portions of the fifth and tenth conduits 310 and 324 and substantially all of the first, second, third, fourth, sixth, seventh, eighth, ninth, eleventh and

twelfth conduits 260, 262, 280, 290, 314, 316, 320A, 320B, 326 and 330 are formed from a

polymeric material, such as silicone. A portion, e.g., about 12 inches, of each of the fifth and

tenth conduits 310 and 324 may be formed from copper, which copper portions are believed to minimize microorganism growth in the conduits 260, 262, 280, 290, 310, 314, 316, 320A,

320B, 324, 326 and 330.

When the device 10 is operational to analyze water, the valve 300 is opened to allow water to pass through the valve 300 and the fourth and fifth conduits 290 and 310 so as to

move toward the flow cell 54 and allow water moving away from the flow cell 54 to pass

through the valve 300 and the tenth and eleventh conduits 324 and 326. When the device 10

is not operational, the valve 300 is closed to reduce the likelihood that organisms will enter and grow within the flow cell passage 54A. It is also noted that if a ultra-violet (UV) light source is provided in the first housing internal chamber 12A, it is normally activated only when the device 10 is not being used to analyze water passing through the flow cell passage 54A. The UV light source is positioned such that UV light passes through the flow cell passage 54A, whereby the UV light functions to prevent organisms from growing and/or kill organisms contained within the flow cell passage 54A.

Because the external optics and sample providing structure 50 is contained within the third housing internal chamber 16A and the internal chamber 16A may be filled with distilled water, risk that organisms may grow on the structure 50 is minimized.

With the valve 300 in its open state, the pump 322 is actuated to cause water to be pulled through the inlet screen assembly 270 and the third, fourth, fifth, sixth and first conduits 280, 290, 310, 314 and 260 into the flow cell passage 54A. The flow rate through the passage 54A may be from about 0.5 milliliters/minute to about 2.0 milliliters/minute.

While passing through the passage 54A, the water is analyzed in the illustrated embodiment in the following manner.

A laser beam is generated by the laser source 44 forming part of the optical analysis and imaging apparatus 32. The laser beam passes through the one or more excitation filters 424, see Fig. 3, which excitation filters 424 may be used to remove unwanted wavelengths, such as all wavelengths other than, for example, 532 nanometers or 488 nanometers, from the laser beam. The laser beam then passes through the objective 401 and exits the first housing internal chamber 12A through the window 124A and passes into and through the flow cell 54, including the flow cell passage 54A. When a particle in the water flowing through the flow cell passage 54A encounters the laser beam, the particle scatters the laser beam, which scattered laser light continues to generally follow a path P defined by the second and first mirrored surfaces 52B and 52A on the prism 52, back through the window 124A so as to re-enter the first housing internal chamber 12A and moves toward the forward scatter sensor 34. The forward scatter sensor 34 detects the scattered laser light and sends a corresponding signal to the processor apparatus. The processor apparatus then causes the backlight 42 to turn on briefly to provide illumination for the optical analysis and imaging apparatus 32. The light emitted from the backlight 42 generally follows the path P, in a direction opposite to the scattered laser light, such that the light from the backlight 42 travels out of the first housing internal chamber 12A through the window 124A and into the prism 52, where it is reflected

off the first and second mirrored surfaces 52A and 52B and passes through the flow cell 54, including the flow cell passage 54A. A portion of the light from the backlight 42 is blocked by particles in the water passing through the flow cell passage 54A. Light not blocked by particles in the water passing through the flow cell passage 54A re-enters the first housing internal chamber 12A through the window 124A and flows through the objective 401 to the partial mirror 426. The partial mirror 426 directs a portion of the light to the camera C, where the light is imaged by the camera C via a physical light imaging process. A remaining portion of the light passes through the partial mirror 426 to the one or more fluorescence emission filters 428 and on to the first and second photo multiplier tubes  $T_1$  and  $T_2$ , see Fig. 3. This portion of the light may be ignored by the photo multiplier tubes  $T_1$  and  $T_2$ .

The laser beam may also simultaneously cause the particles to fluoresce. Some of the light emitted by a particle fluorescing passes out from the flow cell passage 54A, re-enters through the window 124 and flows through the objective 401 to the partial mirror 426. The partial mirror 426 allows a portion of the light to pass therethrough to the one or more fluorescence emission filters 428, which fluorescence emission filters 428 may permit only certain wavelengths of light, e.g., 660 nanometers for chlorophyll analysis and  $575 \pm 20$  nanometers for phycoerythrin analysis, therethrough to pass to the first and second photo multiplier tubes  $T_1$  and  $T_2$ . It is noted that a spacer structure 430, shown in Fig. 3, spaces the second photo multiplier tube  $T_2$  a desired distance from the fluorescence emission filters 428. A second portion of the light emitted by the particle fluorescing is directed by the partial mirror 426 to the camera C. The camera C may ignore this portion of the light.

The processor apparatus, based on the imaging effected by the camera C and the first and second photo multiplier tubes  $T_1$  and  $T_2$ , uses conventional techniques to characterize and quantify the microscopic particles suspended in the stream of water passing through the flow cell passage 54A.

It is noted that the flow cell passage 54A has a longitudinal axis  $A_L$ , see Fig. 6, substantially parallel with a fluid flow path through the passage 54A and a cross sectional area substantially transverse to the longitudinal axis  $A_L$  sized such that generally all fluid flowing through the passage 54A is analyzed by the sensing structure. That is, the flow cell passage cross sectional area is preferably sized to substantially match a field of view and depth of focus of the optical analysis and imaging apparatus 32.

After passing through the flow cell passage 54A, the water leaves the flow cell 54 via the second, seventh, eighth, ninth, tenth, eleventh and twelfth conduits 262, 316, 320A, 320B 324, 326 and 330 and then exits the device 10 via the outlet screen 272B.

The position of the flow cell 54 may be adjusted relative to the imaging apparatus 32 via three screws in the illustrated embodiment (only one screw 400 is illustrated in Fig. 5), which screws pass through threaded bores in projections 402, forming part of the support plate 126, see Fig. 5.

As noted above, the internal chamber 16A of the third housing 16 is preferably filled with a liquid, e.g., distilled water, which approximately matches the optical index of glass, i.e., the index of refraction of water is 1.333 and the index of refraction of the window 124A, if formed from borosilicate glass, is 1.515. The use of distilled water is preferable, since the liquid in the internal chamber 16A surrounds the window 124A, which, as noted above, is preferably formed from glass or polymeric material, and which is imaged by the sensing structure 30. By using a liquid that approximately matches the optical index of the window 124A, reflections encountered by the sensing structure 30 are believed to be reduced. Hence, the light throughput for imaging is improved and laser reflections are reduced, which are believed to improve the instrument fluorescence and scatter sensitivity. Specifically, Snell's law states that when light travels from a medium of index of refraction  $n1$  to a medium of index of refraction of  $n2$ , the angle of transmission,  $\theta t$ , is related to the angle of incidence,  $\theta i$ , by the equation:

$$n1 \cdot \sin \theta i = n2 \cdot \sin \theta t$$

Further, Fresnel's formula states that when optical radiation travels from a medium of index of refraction  $n1$  to a medium of index of refraction of  $n2$ , the light component perpendicular to the surface has a portion reflected in an amount given by the equation:

$$R = \left( \frac{(n1 \cdot \cos \theta i - n2 \cdot \cos \theta t)}{n1 \cdot \cos \theta i + n2 \cdot \cos \theta t} \right)^2$$

Moreover, the transmitted portion of this same perpendicular component of light is given by the equation:

$$T = \left( \frac{(2 \cdot n_1 \cdot \cos \theta_i)}{n_1 \cdot \cos \theta_i + n_2 \cdot \cos \theta_t} \right)$$

For the light component parallel to the plane of incidence, the light has a portion reflected in an amount given by the equation:

5

$$R = \frac{\left( \left( \frac{1}{n_2} \right) \cdot \cos \theta_t - \left( \frac{1}{n_1} \right) \cdot \cos \theta_i \right)}{\left( \frac{1}{n_2} \right) \cdot \cos \theta_t + \left( \frac{1}{n_1} \right) \cdot \cos \theta_i}$$

For this same parallel component of light, the portion of transmitted from medium of index of refraction  $n_1$  to medium of index of refraction  $n_2$  is given by the equation:

10

$$T = \frac{\left( \left( \frac{2}{n_2} \right) \cdot \cos \theta_i \right)}{\left( \frac{1}{n_2} \right) \cdot \cos \theta_t + \left( \frac{1}{n_1} \right) \cdot \cos \theta_i}$$

As the indices of refraction in these equations approach each other, the reflection goes down and the transmission coefficients increase. When  $n_1$  and  $n_2$  are equal, Snell's law states that the angles  $\theta_i$  and  $\theta_t$  are equal. In that case, the reflections can be seen to go to zero and the transmissions go to unity for perfect transmission.

15

In other words, for imaging and measuring optics in the sensing structure 30, as the index of refraction of the fluid between the window 124A and the flow cell prism 52 gets closer to the index of refraction of the glass from which the window 124A is formed and the glass from which the prism 52 is formed, the laser light used to excite fluorescence in the sample, the scatter light from the sample, and the imaging light used to illuminate the sample for imaging do not reflect off of the glass interfaces. Rather, these lights travel in the direction they are intended to, resulting in more laser light getting to the sample, more scatter and fluorescence light getting to the appropriate detectors, and more imaging light getting to the sensing structure 30 for better images. It also means that there is less laser light back-reflected to the camera C and to the fluorescence measuring photo-multiplier tubes  $T_1$  and  $T_2$ .

20

25

Referring now to Figs. 9-13, a sensing structure 130 according to another aspect of the invention is shown, where elements similar to those described above with respect to Figs. 1-8 include the same reference numbers. The sensing structure 130 comprises an optical analysis

and imaging apparatus 32, a forward scatter sensor 34, an objective or lens 401, one or more excitation filters 424, a partial mirror 426, one or more fluorescence emission filters 428, and a spacer structure 430. The sensing structure 130 may also include a computer controlled electro-mechanical focus mechanism 400, such as, for example, an Extended Motorized  
5 MicroMini Stage model number MM-3M-EX-1.0, which is commercially available from National Aperture, Inc. Electronics such as a processor apparatus (not shown) is provided for controlling the operation of the sensing structure 130 and light-providing apparatus 40.

The focus mechanism 400 may be used to move the objective or lens 401 relative to the optical analysis and imaging apparatus 32 (which comprises a camera C and first and  
10 second photo-multiplier tubes  $T_1$  and  $T_2$ ), the forward scatter sensor 34, the excitation filters 424, the partial mirror 426, the fluorescence emission filters 428, and the spacer structure 430, to an optimal position relative to the flow cell passage 54A during operation of the sensing structure 130. The focus mechanism 400 can move the objective 401 toward or away from the flow cell 54 and the flow cell passage 54A to adjust the imaging quality of the  
15 camera C and/or to adjust the fluorescence measuring of the photo-multiplier tubes  $T_1$  and  $T_2$ . Such movement of the objective 401 may be useful to compensate for temperature or mechanical variations in instrument dimensions, which variations may cause the camera C to go out of focus. The focus mechanism 400 can be controlled automatically by conventional focusing algorithms, such as, for example, by the processor apparatus sensing a decrease in  
20 focus of the camera C by monitoring data retrieved from the camera C. This data can be used by the processor apparatus to control actuation of the focus mechanism 400 to move the objective 401. Similar focusing algorithms are used in point and shoot cameras and video cameras and are commonly referred to as “contrast-detect auto focus.” It is also noted that the focus mechanism 400 could be controlled manually, e.g., by an operator located remotely  
25 from the sensing device 10.

As shown in Figs. 9-11, the focus mechanism 400 comprises a slider apparatus 402 and a motor apparatus 404. The motor apparatus 404 is coupled to a baseplate 406 via a bracket 408 and a plurality of mounting screws 410. The baseplate 406, in turn, is coupled to a floor of the first housing 12 (not shown in Figs. 9-11). The motor apparatus 404 can be  
30 controlled automatically by focusing algorithms or manually by an operator as discussed above. The slider apparatus 402 is coupled to the objective 401 via a bracket 412 and an objective plate 414, which objective plate 414 is coupled to the bracket 412 and to the objective 401. The motor apparatus 404 comprises an encoder (not shown), an electric motor

(not shown) and a lead screw (not shown) coupled to the motor and the slider apparatus 402.

The motor apparatus 404, when actuated, turns the lead screw to effect movement of the slider apparatus 402 and, hence, the bracket 412 and the objective plate 414, to effect movement of the objective 401. Movement of the objective plate 414 is guided by rods 416  
5 coupled to a support cube 418, which is located underneath the excitation filters 424 (see Fig. 11), and extending through corresponding openings 420 in the bracket 412. It is noted that the focus mechanism 400 may derive all power from a USB bus connection to the processor apparatus that controls the operation of the sensing structure 30. It is also noted that the focus mechanism 400 may communicate with the processor apparatus via the USB bus connection.

10 During operation of the sensing structure 130, a laser source 44 emits a laser beam as discussed above with reference to Figs. 1-8. The laser beam may pass through the one or more excitation filters 424, see Figs. 9-12, which excitation filters 424 are used to remove unwanted wavelengths, such as all wavelengths other than, for example, 532 nanometers or 488 nanometers, from the laser beam.

15 Referring to Figs. 12 and 13, the laser beam then passes through the objective 401 and exits the first housing internal chamber 12A through the window 124A and passes into and through the flow cell 54. When a particle in the water flowing through the flow cell passage 54A encounters the laser beam, the particle scatters the laser beam and the light from the laser beam re-enters the first housing internal chamber 12A through the window 124A. The light  
20 is detected by the scatter sensor 34, which sends a corresponding signal to the processor apparatus, wherein the processor apparatus causes a backlight 42 to turn on briefly to provide illumination for the optical analysis and imaging apparatus 32.

A portion of the light from the backlight 42 is blocked by particles in the water passing through the flow cell passage 54A, and light not blocked by particles in the water  
25 passing through the flow cell passage 54A re-enters the first housing internal chamber 12A through the window 124A and flows through the objective 401 to the partial mirror 426. The partial mirror 426 allows a portion of the light to pass to the camera C, where the light is imaged by the camera C via a physical light imaging process.

The laser beam may also simultaneously cause the particles to fluoresce, wherein  
30 some of the light emitted by a particle fluorescing, re-enters through the window 124 and flows through the objective 401 to the partial mirror 426. The partial mirror 426 allows a portion of the light to pass therethrough, e.g., 660 nanometers for chlorophyll analysis and 575 +/-20 nanometers for phycoerythrin analysis, to the one or more fluorescence emission

filters 428, which fluorescence emission filters 428 may permit only certain desirable wavelengths of light therethrough to pass to the first and second photo multiplier tubes  $T_1$  and  $T_2$ . As discussed above, a spacer structure 430, shown in Fig. 11, spaces the second photo multiplier tube  $T_2$  a desired distance from the fluorescence emission filters 428.

5           The processor apparatus, based on the imaging effected by the camera C and the first and second photo multiplier tubes  $T_1$  and  $T_2$ , uses conventional techniques to characterize and quantify the microscopic particles suspended in the stream of water passing through the flow cell passage 54A.

Referring now to Fig. 14, a fluid submersible sensing device 200 is provided  
10           according to a further embodiment of the present invention, where elements similar to those described above with respect to Figs. 1-8 include the same reference numbers. Further provided in the fluid tight housing internal chamber 12A is UV light source apparatus 210 comprising one or more light-emitting diodes (LED), which emit UV light in the illustrated embodiment at a nominal UV emission wavelength of about 265 nanometers. In the  
15           illustrated embodiment, first, second and third light-emitting diodes are equally spaced apart about the lens 401. Only the first and second light-emitting diodes 212A and 212B are illustrated in Fig. 14. In the illustrated embodiment, the first, second and third light-emitting diodes are only activated when testing of fluid flowing through the flow cell passage 54A is not taking place, i.e., when test data is not being collected.

20           As noted above, the flow cell 54 may be formed from a clear glass or polymeric material and has a passage 54A through which water to be analyzed passes, see Fig. 4. The flow cell has an inner surface, which may be rectangular in shape, defining the passage 54A. As also noted above, the passage 54A has a cross sectional area substantially transverse to the longitudinal axis  $A_L$ , which may be rectangular in shape. A width of the flow cell passage  
25           54A may fall within the range of from about 1000 microns to about 3000 microns and a depth of the flow cell passage 54A may fall within the range of from about 100 microns to about 300 microns. The flow cell passage 54A extends completely through a length of the flow cell 54.

Hence, in the illustrated embodiment, the first, second and third UV light-emitting  
30           diodes are located outside of the flow cell passage 54A and UV light from the UV light-emitting diodes passes through a first outer surface of the flow cell 54 and then into and through the flow cell passage 54A and out through a second outer surface of the flow cell 54, which second outer surface is opposite to the first outer surface of the flow cell 54.

The first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh and twelfth conduits 260, 262, 280, 290, 310, 314, 316, 320A, 320B, 324, 326 and 330 define a conduit structure. All or a portion of each of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh and twelfth conduits 260, 262, 280, 290, 310, 314, 316, 320A, 320B, 324, 326 and 330 may be formed from copper. In a preferred embodiment, at least about 12 inches of each of the fifth and tenth conduits 310 and 324 is formed from copper.

When fluid is passed through the copper portions of the conduit structure, copper<sup>+0</sup> ions or copper<sup>+2</sup> ions leach from the conduit structure copper portion(s) through natural corrosion processes and flow through the flow cell passage 54A. The UV light passing through the flow cell passage 54A causes a photocatalytic conversion of the copper<sup>+0</sup> ions and/or copper<sup>+2</sup> ions into toxic copper<sup>+1</sup> ions. The toxic copper<sup>+1</sup> ions and the UV light are believed to prevent the formation of biofilms due to the adhesion of dissolved organic matter to the flow cell inner surface and growth of organisms on the flow cell inner surface when the sensing device 200 in which the flow cell 54 is incorporated is submerged in natural waters, e.g., a lake or ocean, for extended periods of time.

Testing was conducted using UV grade synthetic fused silica flow cells having an internal cavity with a width of about 1000 microns and a depth of about 100 microns. A first group of flow cells were coupled to copper tubing so as to provide nominal copper concentrations > 300 µg/L and were not exposed to UV light; a second group of flow cells were exposed to UV light (estimated at about 430 µW at 265 nm wavelength) and were not coupled to copper tubing; a third group of flow cells were coupled to copper tubing so as to provide nominal copper concentrations > 300 µg/L and exposed to UV light (estimated at about 430 µW at 265 nm wavelength); and a fourth group of flow cells (control cells) were not coupled to copper tubing and were not exposed to UV light. Natural seawater filtered to remove particles larger than 5 microns was pumped through each flow cell during a 63 day testing period and at an initial flow rate of about 1 mL/minute. Visual observations and flow measurements showed that the flow cells exposed to UV treatment alone resisted biofilm formation better than those coupled to copper tubing and not exposed to UV light, and had flow rates of about 0.75 ml/minute at the end of the testing period. All control cells were heavily fouled with biofilm formation and had flow rates of 0.0-0.03 ml/minute at the end of the testing period. The flow cells coupled to copper tubing and not exposed to UV light sustained the initial 1 ml/minute flow rate until the last 15 days of the testing period, at which

point the flow rates slowed to about 0.15 ml/minute. The flow cells coupled to copper tubing and exposed to UV light had flow rates ranging from 0.95 to 1.02 ml/minute at the end of the 63 day testing period. The flow cells exposed to UV light and coupled to copper tubing performed better than the other three groups of flow cells because less organic matter and bacterial cells (biofilm) accumulated on the inner walls of the flow cell as shown by an epifluorescent microscope and fluorescent dyes.

A second test, using seawater and flow cells as above, was conducted for 36 days with copper only exposure and copper plus UV light exposure. The flow cells were exposed to flowing water at about 1 ml/minute with copper concentrations of about 800 µg/L with subsets exposed to different doses of UV light (~265nm wavelength) by varying LED time-on intervals, i.e., duty cycles, (100%, 66%, 33%, 0% time-on). Biofilm formation as measured by a confocal microscope using fluorescent dyes was observed on the copper only exposed flow cells surfaces but not on the copper plus UV light exposed flow cells.

While the flow cell 54 is disclosed herein as being incorporated into the sensing device 200, it is contemplated that the flow cell 54 in combination with LEDs emitting UV light and conduit structure coupled to the flow cell comprising portions formed from copper may be incorporated into any other sensing device. Such sensing devices may include instruments using conductivity cells to measure conductance of water, optical probes and sensors that depend on glass or other optically transparent windows to emit light into and from water, or flow cells used to deliver water to sensing devices contained inside of pressure housings.

While a particular embodiment of the present invention has been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention.

## CLAIMS

What is claimed is:

1. A fluid submersible sensing device comprising:  
a housing defining an internal chamber and including window structure;  
5 sensing structure provided in said internal chamber;  
light providing apparatus in said internal chamber emitting light capable of passing  
through said window structure so as to exit said housing;  
an external structure coupled to said housing and located outside of said housing  
internal chamber comprising a substantially clear flow cell including an inner surface  
10 defining a passage through which a fluid flows and one or more optical elements for causing  
the light from the housing to pass through said passage and re-enter said housing toward said  
sensing structure;  
UV light source apparatus for directing UV light toward said flow cell; and  
a conduit structure coupled to an inlet of said flow cell so as to provide fluid to be  
15 analyzed to said flow cell passage, at least a portion of said conduit structure being formed  
from copper, said copper conduit structure portion and said UV light preventing the formation  
of biofilms on said flow cell inner surface.
2. The device as set out in claim 1, wherein said UV light source apparatus comprises at  
20 least one light-emitting diode.
3. The device as set out in claim 1, wherein said light providing apparatus comprises a  
laser light source and a backlight source.
- 25 4. The device as set out in claim 1, wherein said sensing structure comprises an optical  
analysis and imaging apparatus.
5. The device as set out in claim 1, wherein said external structure further comprises  
a primary element comprising said one or more optical elements.  
30
6. The device as set out in claim 5, wherein said primary element comprises a prism  
including said one or more optical elements defined by first and second mirrored surfaces on

said prism to reflect the light along a desired path such that the light passes through said flow cell including said passage and then re-enters said housing.

7. The device as set out in claim 5, wherein said flow cell comprises a clear body  
5 separate from said primary element and has said passage extending through it.

8. The device as set out in claim 7, wherein said passage has a longitudinal axis  
substantially parallel with a fluid flow path through said passage and a cross sectional area  
substantially transverse to said longitudinal axis sized such that generally all fluid flowing  
10 through said passage is analyzed by said sensing structure.

9. The device as set out in claim 5, further comprising a pressure-compensated second  
housing comprising electronic components and a pressure-compensated third housing  
containing said external structure, said second housing separable from said third housing such  
15 that said flow cell in said third housing can be serviced without opening said second housing.

10. The device as set out in claim 9, further comprising a pump provided in said second  
housing and coupled to said conduit structure so as to pump the fluid to be analyzed through  
said conduit structure to said flow cell passage.

11. The device as set out in claim 1, wherein said flow cell passage has a dimension of  
from about 100 microns to about 3000 microns.

12. The device as set out in claim 1, wherein said copper conduit structure portion has a  
25 length of from of about 6 inches to about 24 inches.

13. The device as set out in claim 1, wherein said UV light source emits UV light at a  
nominal UV emission wavelength of about 265 nanometers.

14. The device as set out in claim 1, wherein said UV light source apparatus is located  
30 outside of said flow cell passage and UV light from said UV light source apparatus passes  
through an outer surface of said flow cell and then into and through said flow cell passage.

15. A fluid receiving device capable of preventing biofouling comprising:  
a UV light source apparatus for emitting UV light;  
a substantially transparent flow cell including an inner surface defining a passage  
through which a fluid to be analyzed flows, the UV light passing through said passage; and

5 a conduit structure coupled to an inlet of said flow cell so as to provide fluid to be  
analyzed to said flow cell passage, at least a portion of said conduit structure being formed  
from copper, said copper conduit structure portion and said UV light preventing the formation  
of biofilms on said flow cell inner surface.

10 16. The device as set out in claim 15, wherein said flow cell passage has a dimension of  
from about 100 microns to about 3000 microns.

17. The device as set out in claim 15, wherein said copper conduit structure portion has a  
length of from about 6 inches to about 24 inches.

15 18. The device as set out in claim 15, wherein said UV light source apparatus emits UV  
light at a nominal UV emission wavelength of 265 nanometers.

19. The device as set out in claim 15, wherein said UV light source apparatus is located  
20 outside of said flow cell passage and UV light from said UV light source apparatus passes  
through an outer surface of said flow cell and then into and through said flow cell passage.

20. A process for preventing the formation of biofilms on a flow cell inner surface  
comprising:

25 providing a flow cell having an inner surface defining a passage through which a fluid  
to be analyzed flows;

providing a conduit structure coupled to said flow cell, the conduit structure including  
at least a portion formed from copper; and

passing fluid through the conduit structure such that the fluid and copper<sup>+0</sup> ions or copper<sup>+2</sup>

30 ions leaching from the conduit structure copper portion flow through said flow cell passage,  
said UV light causing a photocatalytic conversion of the copper<sup>+0</sup> ions or copper<sup>+2</sup> ions into  
toxic copper<sup>+1</sup> ions, wherein the copper<sup>+1</sup> ions and UV light prevent the formation of biofilms  
on said flow cell inner surface.

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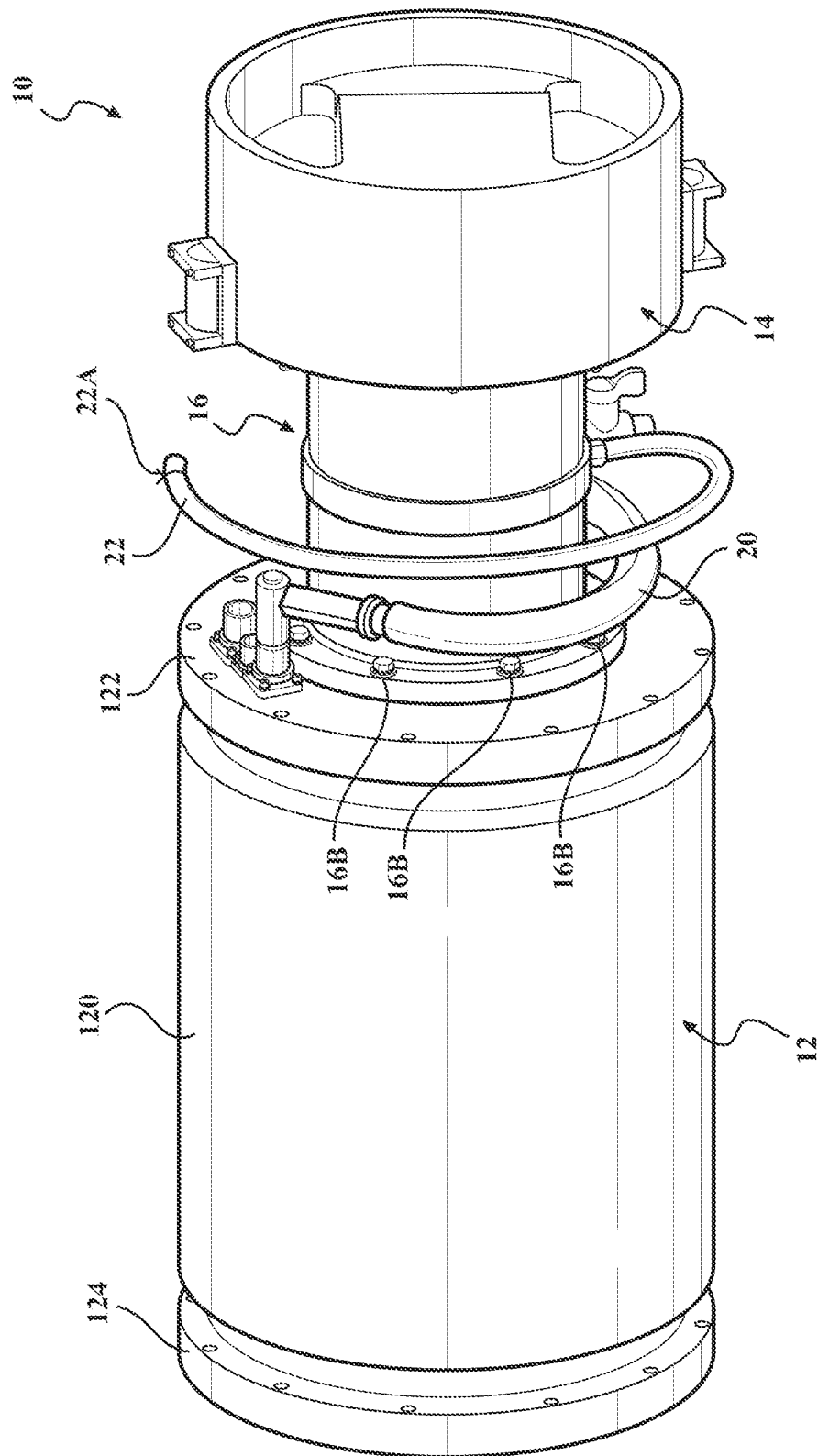


FIG. 1

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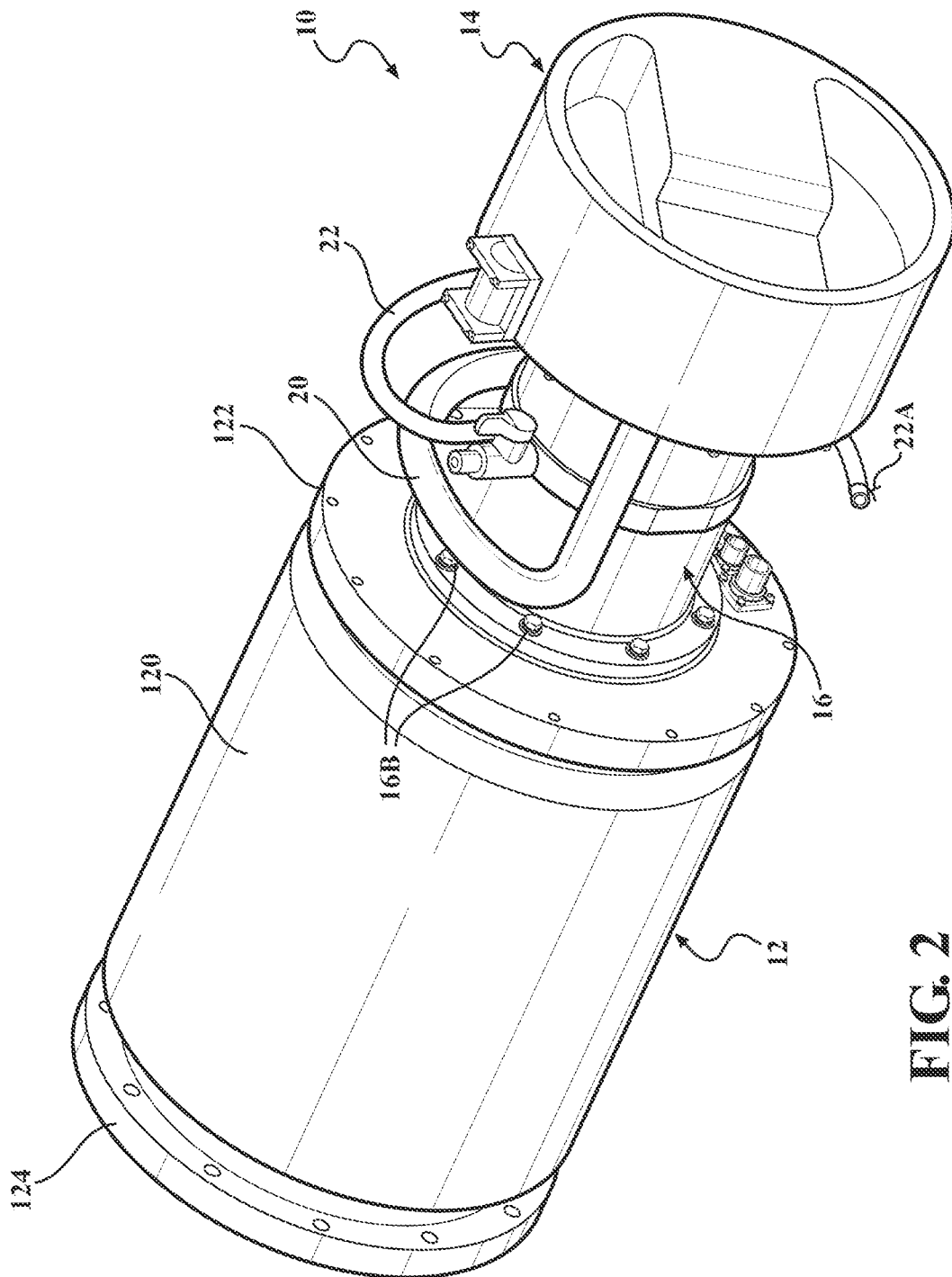
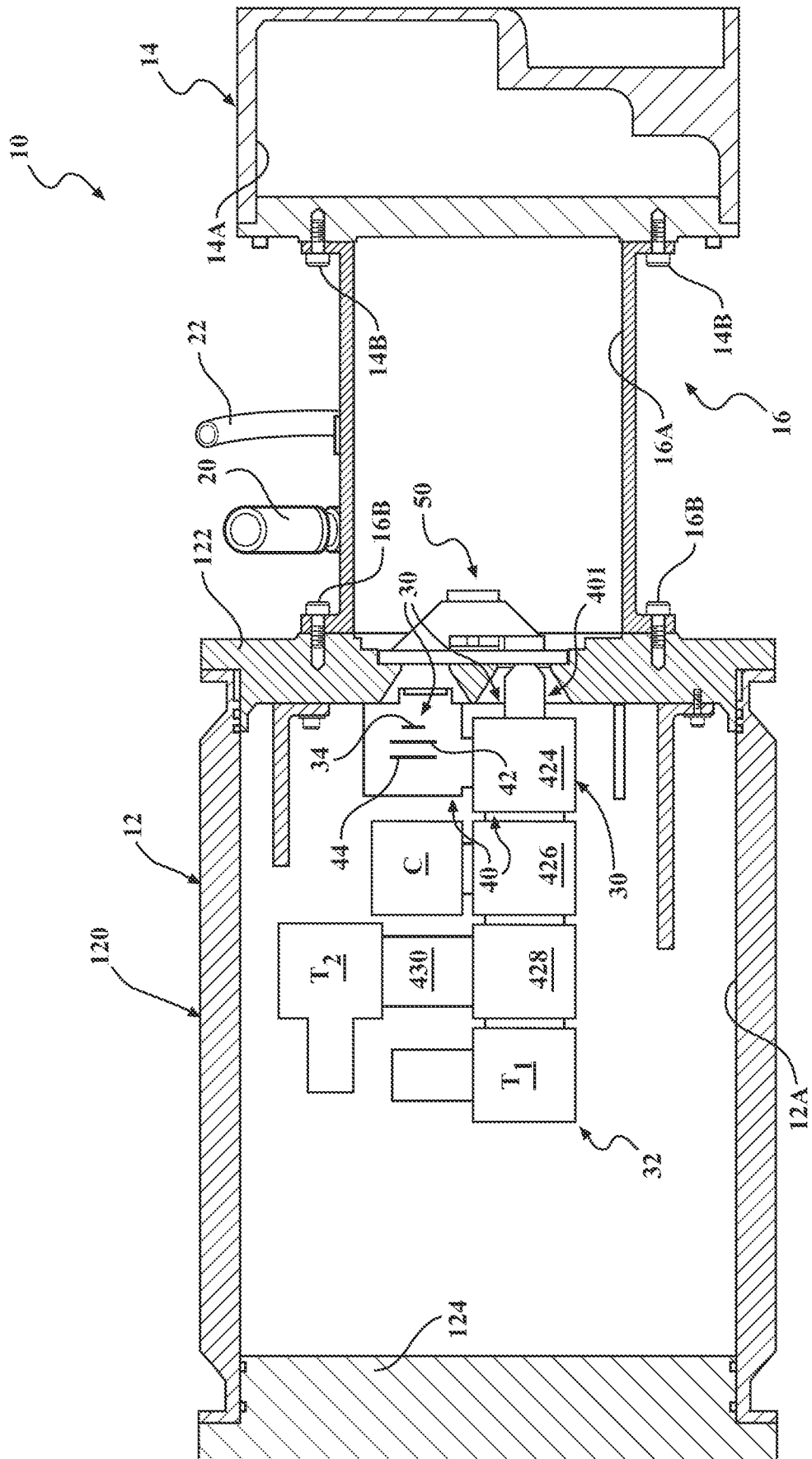


FIG. 2

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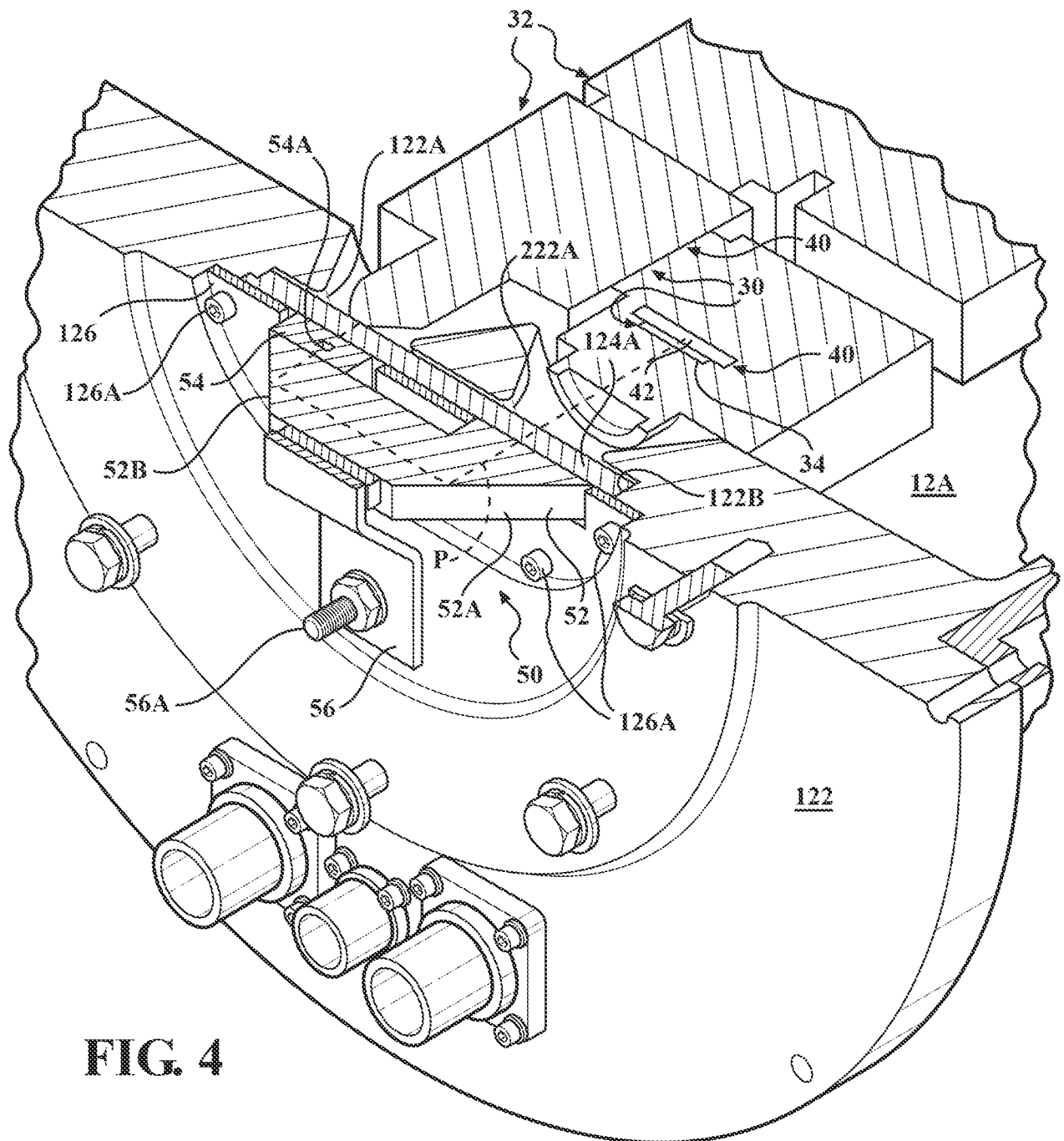


FIG. 4

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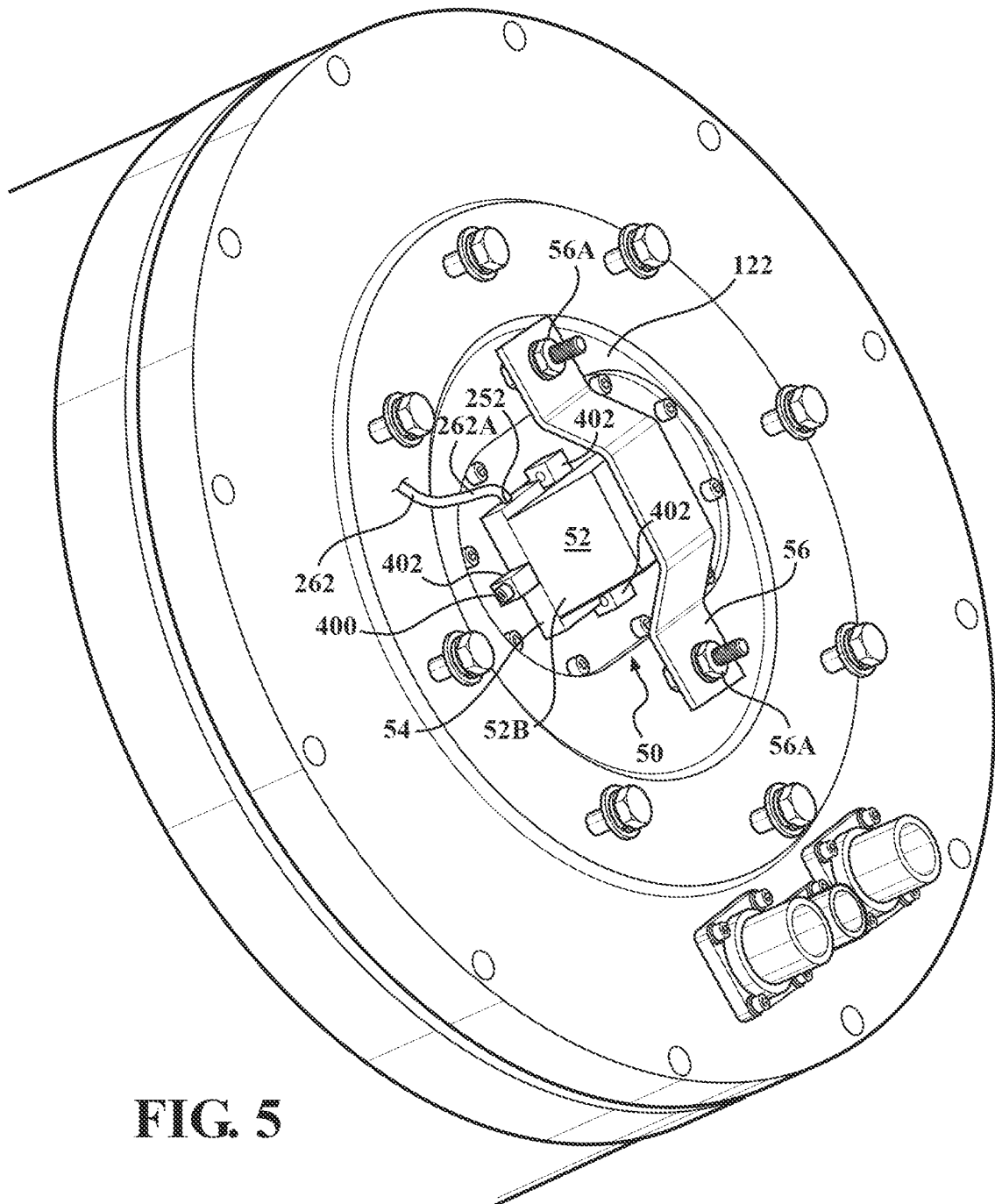


FIG. 5

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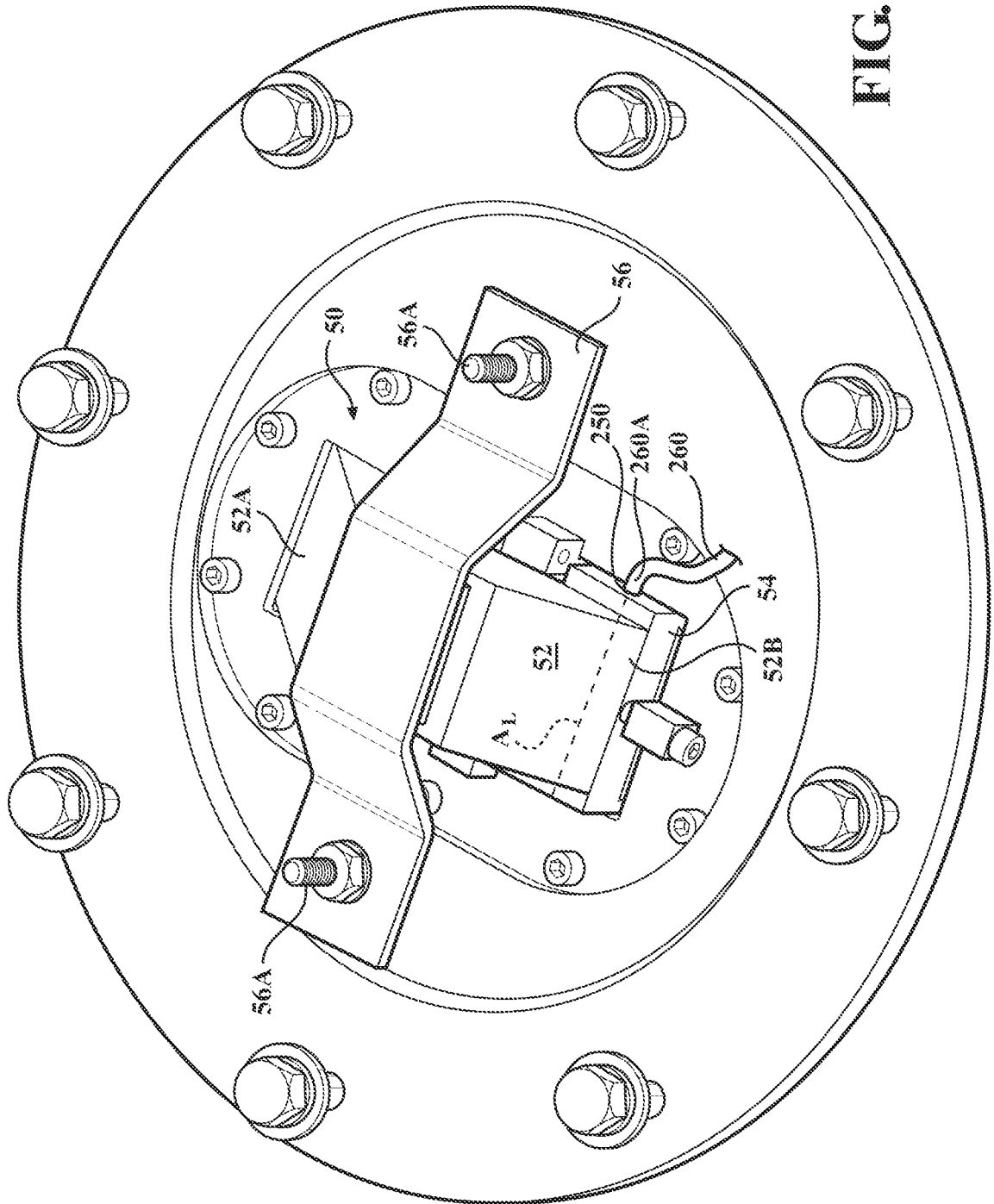
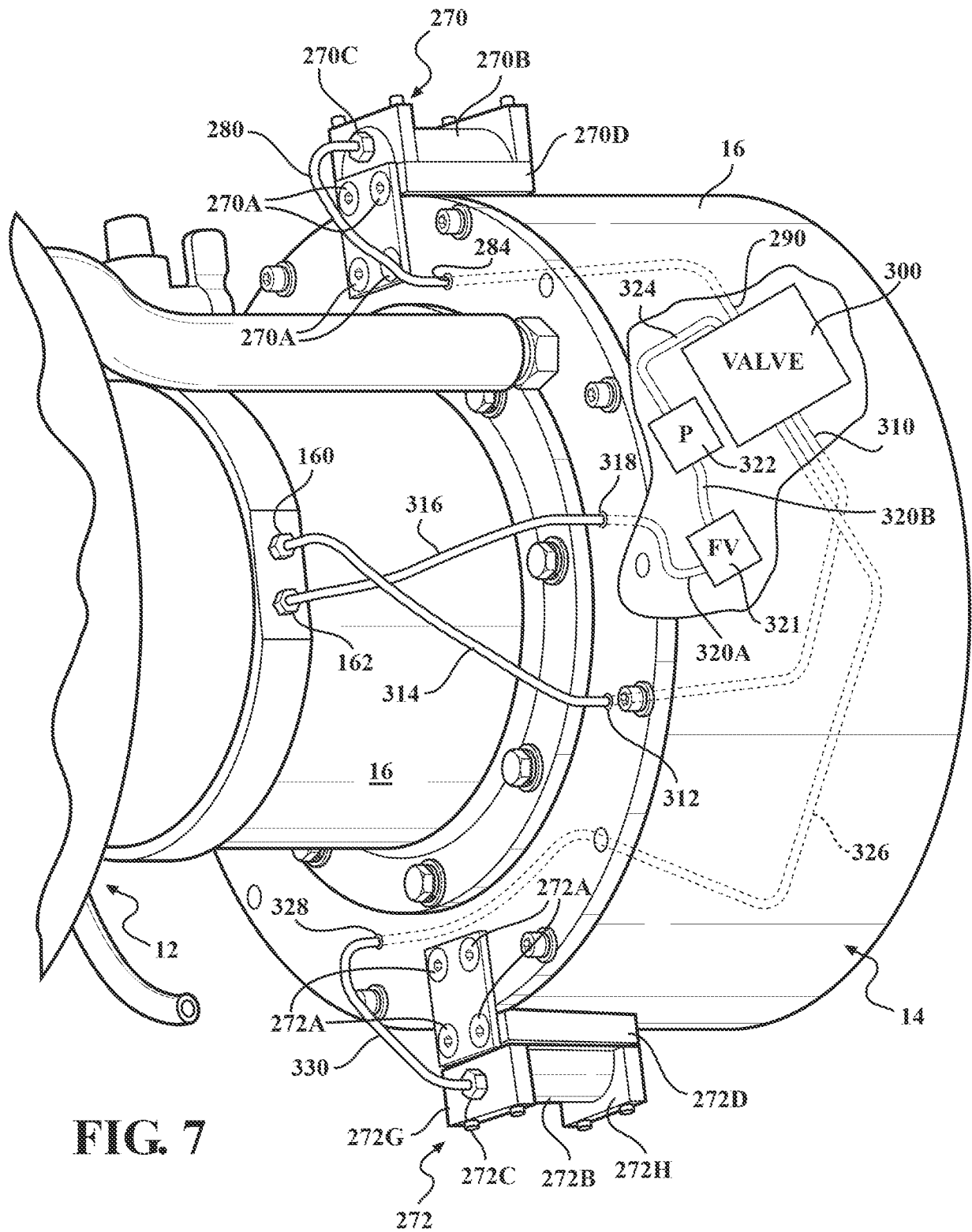


FIG. 6



**FIG. 7**

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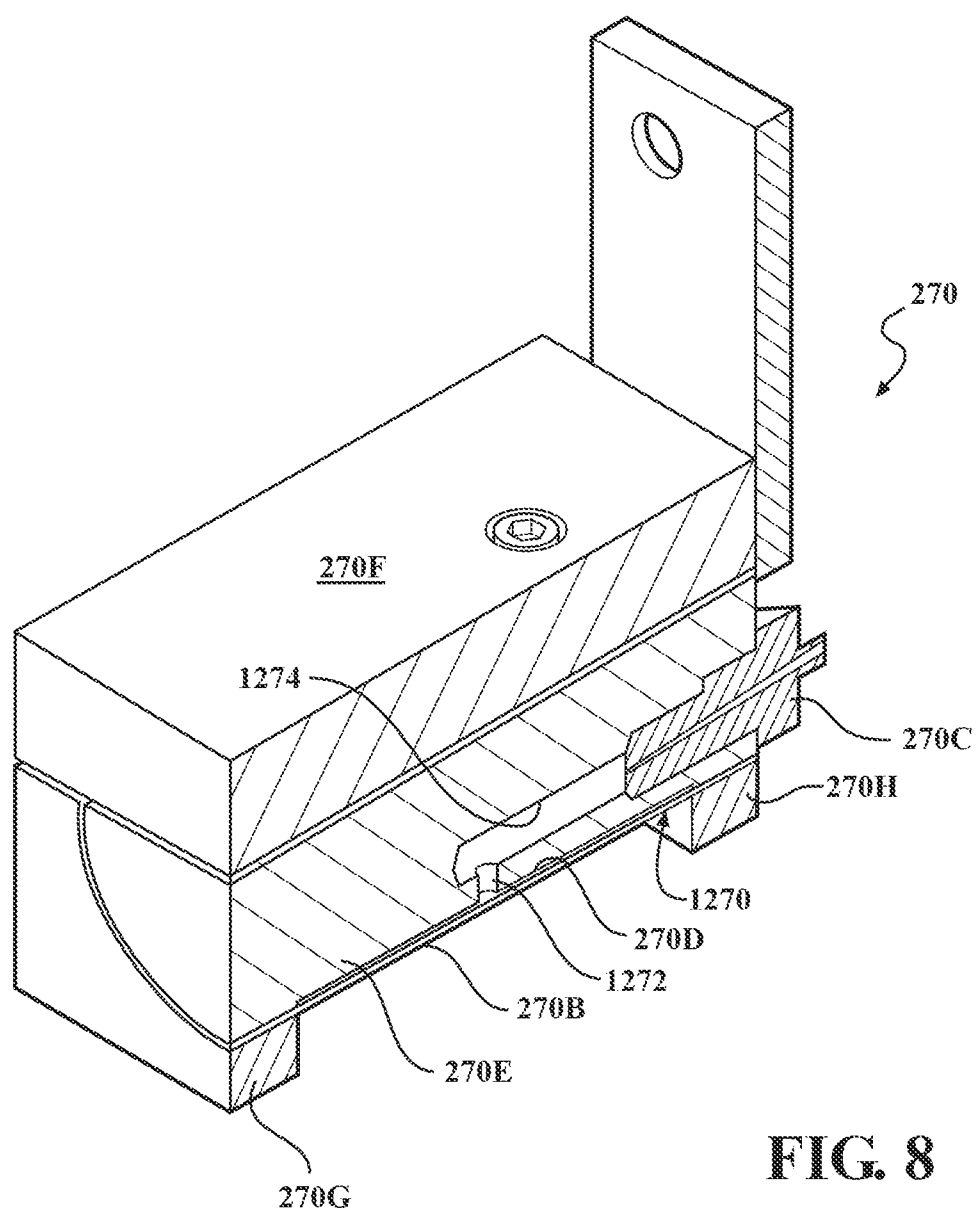
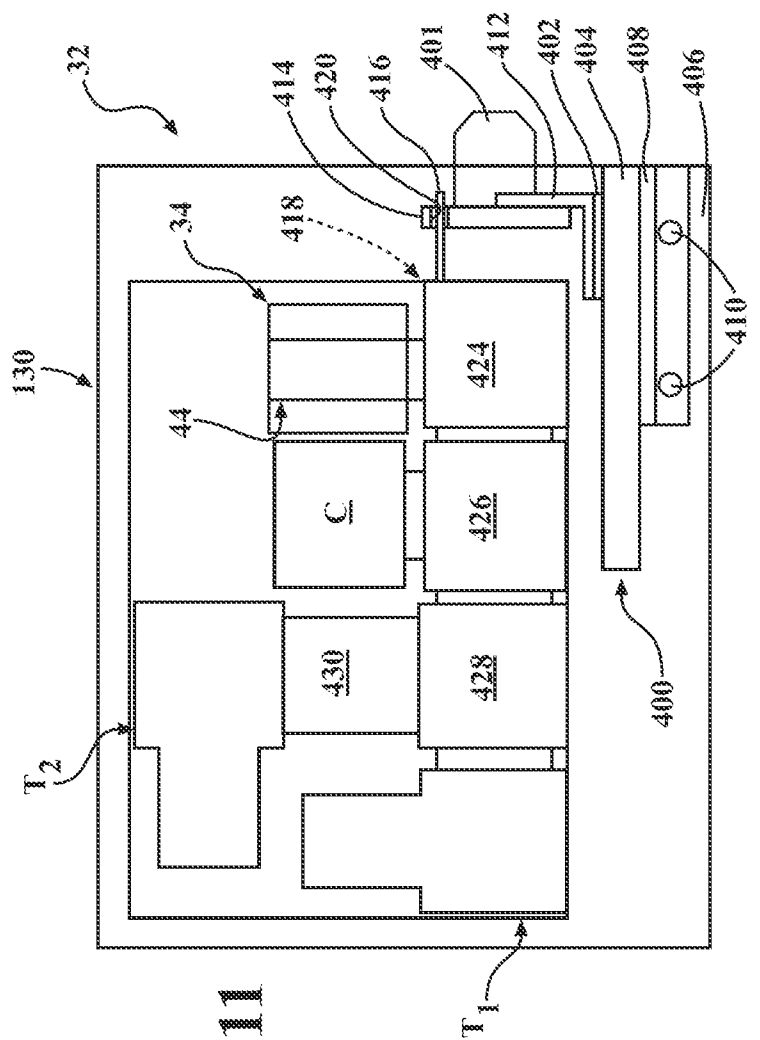
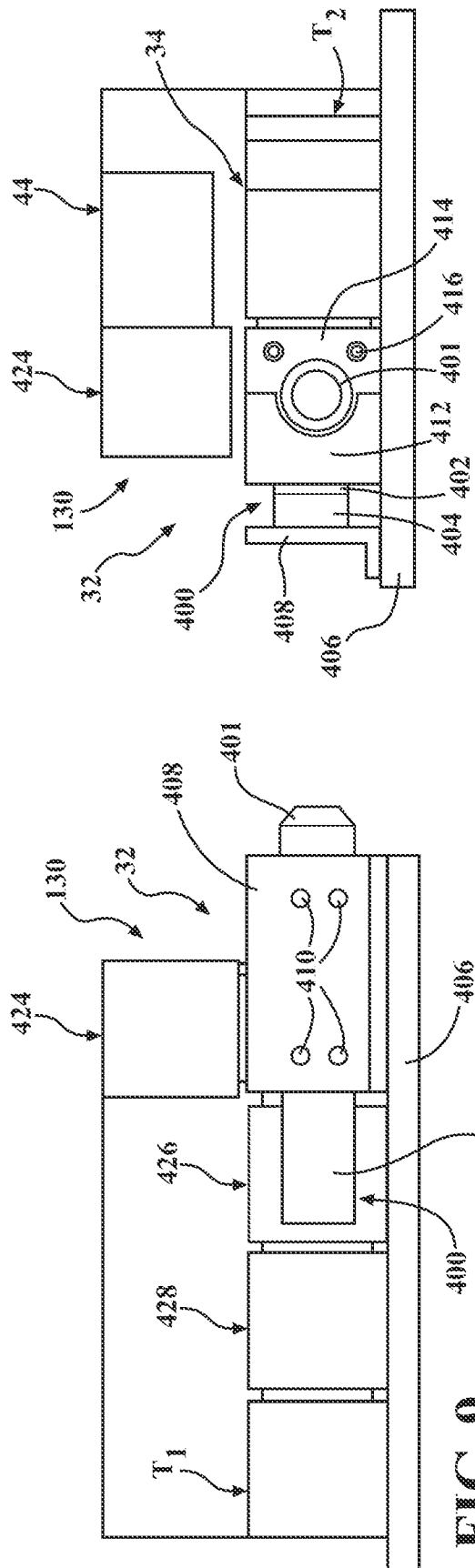


FIG. 8



# FIGURE

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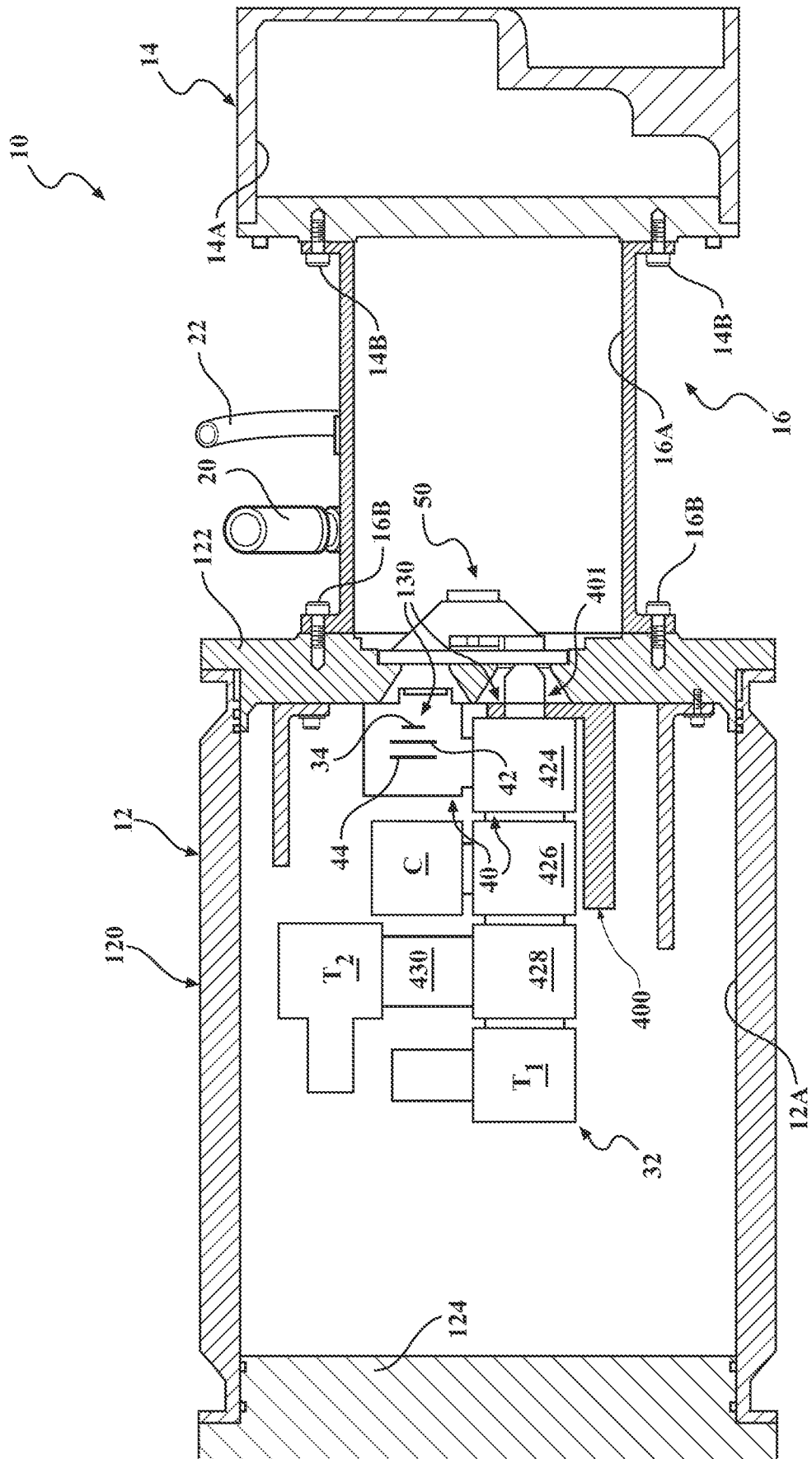
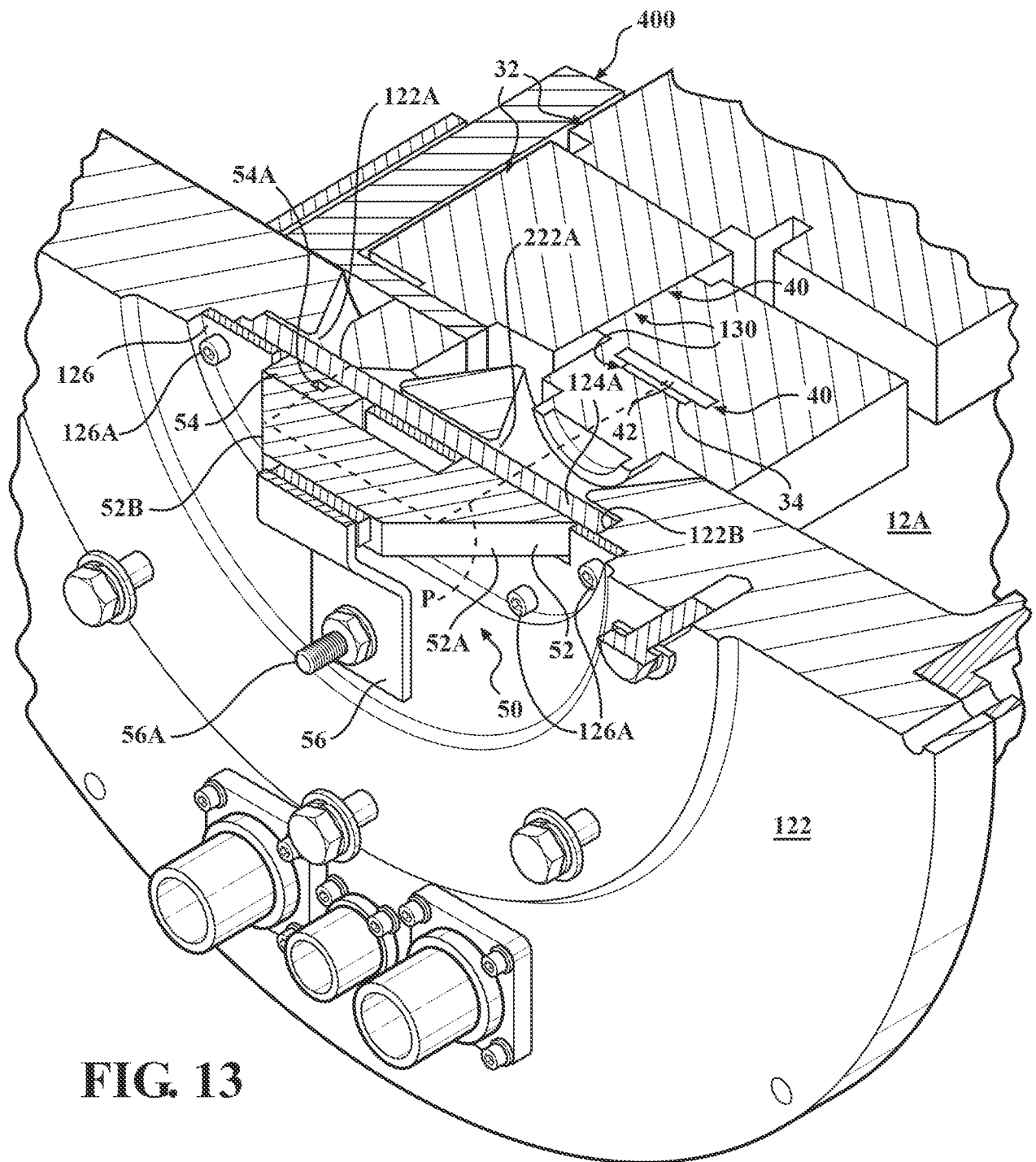
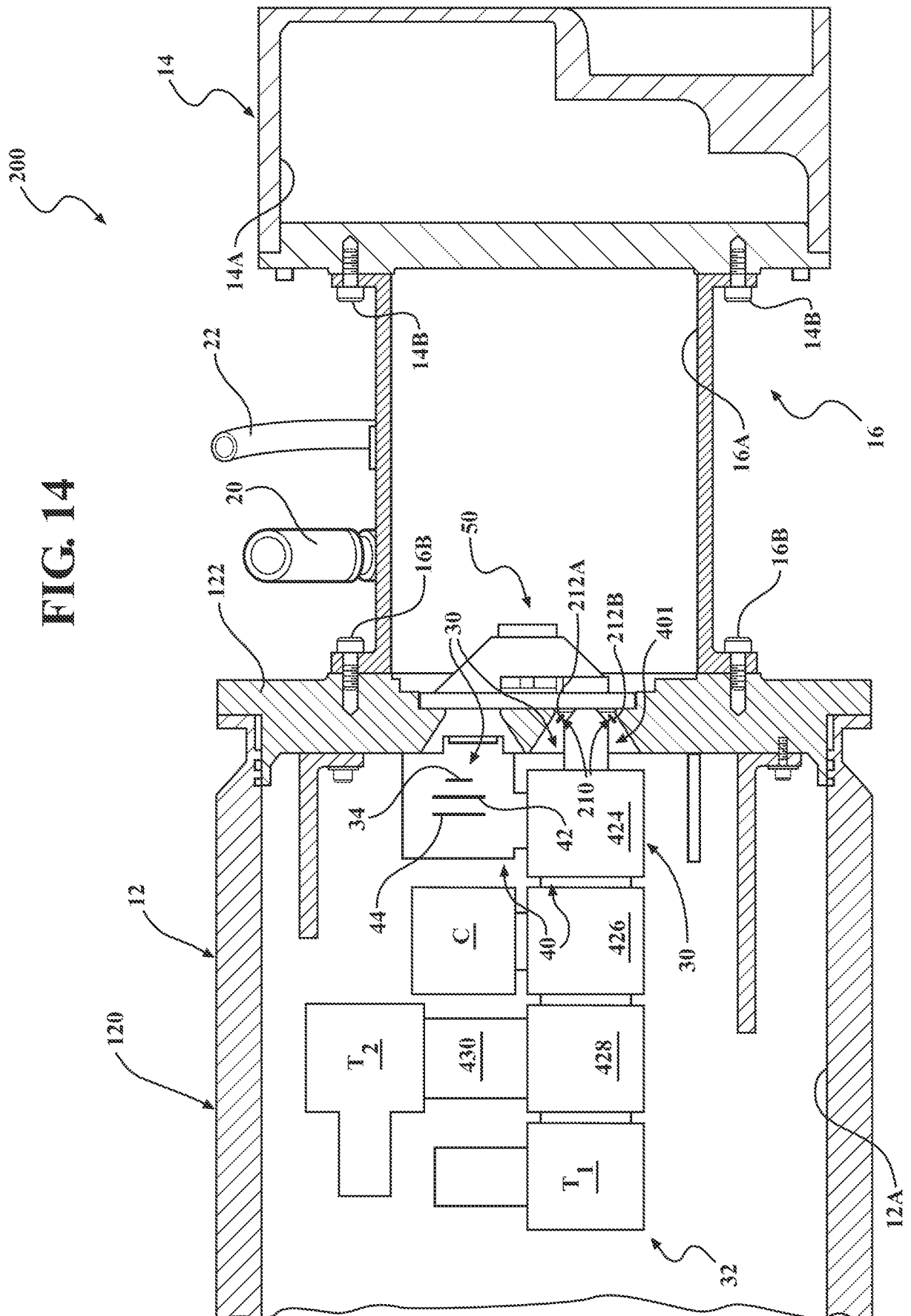


FIG. 12

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# FILE



## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2011/056842

## A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N1/14 G01N33/18  
 ADD. G01N15/14

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

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 practical, search terms used)

EPO-Internal, WPI Data, INSPEC, COMPENDEX

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                 | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
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| Y,P       | 29 December 2010 (2010-12-29)<br>page 4, lines 22-25<br>page 9, lines 15-20<br>the whole document                                                                                                                                                                                                                                                  | 20                    |
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Further documents are listed in the continuation of Box C.



See patent family annex.

## \* Special categories of cited documents :

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Date of the actual completion of the international search

23 March 2012

Date of mailing of the international search report

02/04/2012

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 Fax: (+31-70) 340-3016

Authorized officer

van Lith, Joris

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2011/056842

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                       | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A         | XUZHU WANG ET AL.: "Underwater cytometer for in situ measurement of marine phytoplankton by a technique combining laser-induced fluorescence and laser Doppler velocimetry", OPTICS LETTERS, vol. 30, no. 10, 15 May 2005 (2005-05-15), pages 1087-1089, XP002600619, abstract; figure 1<br>column 1, line 1 - column 3, line 10<br>-----                                                | 1                     |
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2011/056842

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date         |
|-------------------------------------------|---------------------|----------------------------|-----------------------------|
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| -----                                     |                     |                            |                             |
| WO 2004051367                             | A2                  | 17-06-2004                 | AU 2003298902 A1 23-06-2004 |
|                                           |                     | WO 2004051367 A2           | 17-06-2004                  |
| -----                                     |                     |                            |                             |
| US 2009109432                             | A1                  | 30-04-2009                 | NONE                        |
| -----                                     |                     |                            |                             |