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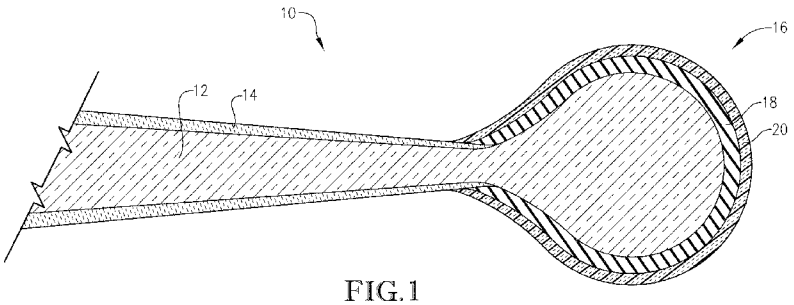

FIG.1

(57) Abstract: A pH micro-probe, a temperature micro-probe, and an immuno-based micro-probe each include a shaft for transmitting an input light signal and a tip for inserting into a cell or other substance for measuring pH, temperature, and/or antigens. The pH micro-probe and the temperature micro-probe each include a luminescent material positioned on the tip of the micro-probe. The light signal excites the luminescent material so that the luminescent material emits a luminescent light signal. The luminescent light signal has a property value dependent on the pH or temperature being measured and reflects back through the shaft for being measured by a light signal measuring device. The immuno-based micro-probe includes a reflective material that has an effective refractive index dependent on the number of antigen-antibody bonds present on the reflective material.

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FIBER-OPTIC MICRO-PROBES FOR MEASURING ACIDITY LEVEL, TEMPERATURE, AND ANTIGENS

GOVERNMENT INTERESTS

[0001] The present invention was developed with support from the U.S. government under a contract with the National Institute of Health, Contract No. 5R21GM104696-03. Accordingly, the U.S. government has certain rights in the present invention.

BACKGROUND

[0002] Probes are often used for measuring acidity level (pH), temperature, and antigens of substances and test samples. Conventional probes typically are too large to make localized measurements, thus making pH, temperature, and antigen measurements in micro-scale such as in a single cell, in a specific local spot, and micro-scale gradient change difficult. This particularly prevents intracellular measurements from being taken. Some conventional probes can make intracellular measurements but not without destroying the cell being probed.

SUMMARY

[0003] The present invention solves the above-described problems and provides a distinct advance in the art of pH, temperature, and immuno-based probes.

[0004] A pH micro-probe constructed in accordance with an embodiment of the present invention comprises a shaft, an ultra-thin mirror coating applied to at least part of the shaft, a tip, and a luminescent dye-doped coating and an ultra-thin mirror coating applied to the tip. The tip of the pH micro-probe may be inserted into a biological cell or similar substance whose pH is being measured. An input light signal is transmitted into the shaft and reflected off of the mirror coating so as to continue traveling into the tip. The input light signal reflects off of the mirror coating applied to the tip so as to excite the luminescent dye-doped coating. The luminescent dye-doped coating emits a pH dependent luminescent light signal. The luminescent light signal travels back through the shaft for being measured by a light signal measuring device.

[0005] A temperature micro-probe constructed in accordance with another embodiment of the present invention comprises a shaft and mirror coating similar to the pH micro-probe and a tip that includes a microcavity extending into the tip and a luminescent material positioned on or in the microcavity. The tip of the temperature micro-probe is inserted into a substance whose temperature is being measured. An input light signal is transmitted through the shaft so as to excite the luminescent material. The luminescent material emits a temperature dependent luminescent light signal. The luminescent light signal travels back through the shaft for being measured by a light signal measuring device.

[0006] An immuno-based micro-probe constructed in accordance with yet another embodiment of the present invention comprises a shaft and mirror coating similar to the pH micro-probe and temperature micro-probe, a tip, and a thin film coated on the tip. The thin film includes a number of nano holes extending therethrough. The tip of the immuno-based micro-probe is inserted into a sample being measured. A number of antibodies will then be immobilized on the thin film. Antigens in the sample will begin to bond to the antibodies. An input light signal is transmitted through the shaft so that some of the input light signal reflects off of the thin film and some of the input light signal passes through the nano holes. The amount of light reflecting off of the thin film is dependent on the number of antigens or ratio of antigen-antibody bonds to total number of antibodies.

[0007] This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the detailed description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other aspects and advantages of the present invention will be apparent from the following detailed description of the embodiments and the accompanying drawing figures.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

[0008] Embodiments of the present invention are described in detail below with reference to the attached drawing figures, wherein:

[0009] FIG. 1 is a partial vertical sectional view of a pH micro-probe constructed in accordance with an embodiment of the present invention;

[0010] FIG. 2 is a partial vertical sectional view of a modified pH micro-probe;

[0011] FIG. 3 is a perspective view of a pH micro-probe having a number of fiber optic fibers;

[0012] FIG. 4 is a partial vertical sectional view of a temperature micro-probe constructed in accordance with another embodiment of the present invention;

[0013] FIG. 5 is a partial perspective view of an immuno-based micro-probe constructed in accordance with another embodiment of the present invention; and

[0014] FIG. 6 is an enlarged partial vertical sectional view of a tip of the immuno-based micro-probe in FIG. 5.

[0015] The drawing figures do not limit the present invention to the specific embodiments disclosed and described herein. The drawings are not necessarily to scale, emphasis instead being placed upon clearly illustrating the principles of the invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0016] The following detailed description of the invention references the accompanying drawings that illustrate specific embodiments in which the invention can be practiced. The embodiments are intended to describe aspects of the invention in sufficient detail to enable those skilled in the art to practice the invention. Other embodiments can be utilized and changes can be made without departing from the scope of the present invention. The following detailed description is, therefore, not to be taken in a limiting sense. The scope of the present invention is defined only by the appended claims, along with the full scope of equivalents to which such claims are entitled.

[0017] In this description, references to “one embodiment”, “an embodiment”, or “embodiments” mean that the feature or features being referred to are included in at least one embodiment of the technology. Separate references to “one embodiment”, “an embodiment”, or “embodiments” in this description do not necessarily refer to the same embodiment and are also not mutually exclusive unless so stated and/or except

as will be readily apparent to those skilled in the art from the description. For example, a feature, structure, act, etc. described in one embodiment may also be included in other embodiments, but is not necessarily included. Thus, the present technology can include a variety of combinations and/or integrations of the embodiments described herein.

[0018] Turning now to the drawing figures, and particularly FIG. 1, a pH micro-probe 10 constructed in accordance with an embodiment of the invention is illustrated. The micro-probe 10 broadly includes a shaft 12, a mirror coating 14 applied to at least part of the shaft 12, a tip 16, and a luminescent dye-doped coating 18 and a mirror coating 20 applied to at least part of the tip 16.

[0019] The shaft 12 allows a light signal to be transmitted therethrough and one embodiment of the shaft 12 is an elongated transparent member formed of glass or other suitable transparent material. The shaft 12 may be tapered so that a distal end of the shaft 12 is narrower or smaller than its proximal end. The shaft 12 also allows an output luminescent light signal to travel from the tip 16 and through the shaft 12, as described below.

[0020] The mirror coating 20 reflects the light signal traveling through the shaft 12 so as to guide the light signal through the shaft 12. The mirror coating 20 may be a thin film at least partially formed of silver, aluminum, gold, or other reflective material and may be applied to a portion or all of an outer surface of the shaft 12.

[0021] The tip 16 allows the luminescent dye-doped coating 18 to interact with the substance being tested and is positioned at the distal end of the shaft 12. The tip 16 may be the distal end of the shaft 12 itself or may be an extension or attachment connected to the shaft 12. The tip 16 may be bulb shaped (elongated, egg shaped, or spherical) and may be wider or larger than the distal end of the shaft 12.

[0022] The luminescent dye doped coating 18 interacts with the light signal to generate a pH dependent luminescent light signal. The luminescent dye-doped coating may be a thin film at least partially formed of 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) or similar material, and may include an aerogel or similar material, organically modified silicate (ORMOSIL) or similar material, or any other suitable material. The luminescent dye-doped coating 18 may be applied to a portion or

all of an outer surface of the tip 16 and may have a thickness on the nano scale or micro scale. In one embodiment the luminescent dye-doped coating 18 may be approximately 100 nm to approximately 2 μm thick.

[0023] The mirror coating 20 at least partially reflects the light signal within the tip 16 and may be a thin film at least partially formed of silver, aluminum, gold, or other reflective material applied over the luminescent dye doped coating 18.

[0024] In one embodiment, a protective film 22 such as a platinum belt may be applied between the luminescent dye doped coating 18 and the mirror coating 20, as shown in FIG. 2. The protective film 22 protects the luminescent dye doped coating 18 and the tip 16 from damage.

[0025] Use of the pH micro-probe 10 will now be described in more detail. The tip 16 of the pH micro-probe 10 may be inserted into a micro-volume solution or the intracellular or intercellular substance (cytoplasm, etc.) of a cell. An input light signal is then transmitted from a light signal generator into the proximal end of the shaft 12. The light signal will reflect off of the mirror coating 20 so as to continue traveling through the shaft 12 to the distal end of the shaft 12. The light signal will then continue into the tip 16 and will reflect off of the mirror coating 20 so as to bounce around inside the tip 16. The light signal will hit and be at least partially absorbed by the luminescent dye doped coating 18. The dye doped coating 18 will emit a luminescent light signal such as a fluorescent light signal. The luminescent light signal will reflect inside of the tip 16 and eventually exit the tip 16 into the shaft 12. The luminescent light signal will reflect off of the mirror coating 14 so as to continue traveling from the distal end of the shaft 12 to the proximal end of the shaft 12. The luminescent light signal will then exit the proximal end of the shaft 12 and continue traveling through fiber optic components to a light signal measuring device.

[0026] The luminescent light signal has a property value indicative of the pH of the measured substance. For example, the intensity of the luminescent light signal may be dependent on the pH of the substance. A fluorescence ratio (the ratio of the fluorescence of the luminescent dye doped coating 18 when excited at a first wavelength compared to a second wavelength (e.g., 560 nm compared to 640 nm) may also be measurable against the pH of the substance.

[0027] The above-described pH micro-probe 10 provides several advantages over conventional pH measuring methods. For example, the pH micro-probe 10 allows the pH of the cellular substance of a single cell or a very small sample to be measured. The pH micro-probe 10 confines the pH measurement to the cellular or other substance in contact with the tip 16 so that the pH of adjacent cells or adjacent material does not affect the measurement. This is particularly useful in heterogeneous environments such as biological cells. The pH micro-probe 10 also allows for the measurement of pH via fluorescence ratios, which eliminates the need to account for certain factors such as how much dye doped coating 20 is used.

[0028] In another embodiment of the invention, a pH micro-probe 100 similar to the above-described micro-probe 10 includes a shaft 102 having a central portion 104 and one or more surrounding fibers 106, as shown in FIG. 3.

[0029] The central fiber 104 allows an input light source to travel therethrough and includes a core 108 similar to the shaft 12 and a mirror coating 110 similar to the mirror coating 20 described above.

[0030] The surrounding fibers 106 allow the luminescent light signal to travel therethrough and each include a core and mirror coating similar to the core 108 and mirror coating 110 of the central fiber 104. The surrounding fibers 106 may be twisted around the central fiber 104 or may maintain an initial orientation in relation to the central fiber 104. In one embodiment there are six surrounding fibers 106 forming a hexagonal pattern around the central fiber 104.

[0031] The pH micro-probe 100 is used in substantially the same manner as the pH micro-probe 10 described above except that the light signal is transmitted through the central fiber 104 and the luminescent light signal is reflected back through the surrounding fibers 106 and optionally the central fiber 104.

[0032] Turning now to FIG. 4, a temperature micro-probe 200 constructed in accordance with another embodiment of the invention is illustrated. The temperature micro-probe 200 broadly includes a shaft 202, a tip 204, at least one microcavity 206 extending into the tip 204, and a luminescent material 208 positioned in the microcavity 206.

[0033] The shaft 202 allows a light signal to be transmitted therethrough and may be substantially similar to the shaft 12 described above.

[0034] The tip 204 allows the light signal to interact with the luminescent material 208 and may be substantially similar to the tip 16 described above. In one embodiment, the tip 204 is the distal end of the shaft 202.

[0035] The microcavity 206 allows the luminescent material 208 to adhere to the tip 204 and interact with the light signal and may be formed in the tip 204 near the distal end of the shaft 202. The microcavity 206 may be formed by chemical etching via hydrofluoric acid (such as a 20% hydrofluoric acid solution) and may extend less than 30 μm along the tip 204. In one embodiment, the microcavity 206 has a volume of 5 μm by 5 μm by 5 μm or less.

[0036] The luminescent material 208 interacts with the light signal to generate a temperature dependent luminescent light signal and may be a thin film or coating of fluorescent material or a number of quantum dots. In one embodiment, the luminescent material 208 is a Rhodamine dye such as R6G. In another embodiment, the luminescent material 208 is a number of quantum dots in a liquid phase or solid phase coating on the surface. In yet another embodiment, the luminescent material 208 is a phosphor or phosphorescent material

[0037] Use of the temperature micro-probe 200 will now be described in more detail. The tip 204 of the temperature micro-probe 200 is inserted into the substance whose temperature is to be measured. An input light signal is then transmitted into the proximal end of the shaft 202 from a light signal generator. The light signal travels to the distal end of the shaft 202 until it reaches the microcavity 206. The light signal will then be at least partially absorbed by the luminescent material 208. The luminescent material 208 will emit a luminescent light signal such as a fluorescent light signal. The luminescent light signal will travel to the proximal end of the shaft 202, exit the proximal end of the shaft 202, and continue traveling through fiber optic components to a light signal measuring device.

[0038] The luminescent light signal has a property value indicative of the temperature of the substance. For example, the wavelength of the luminescent light signal may be dependent on the temperature of the substance. In one embodiment, the

full width of half of the maximum value of the wavelength (FWHM) may be measured. As another example, the intensity or peak intensity of the luminescent light signal at a specified wavelength may be dependent on the temperature of the substance. In another embodiment, the luminescent decay time may be measured to determine the temperature of the substance. As an example, the luminescent decay time of phosphor may be dependent on temperature.

[0039] The above-described temperature micro-probe 200 provides several advantages over conventional temperature measuring instruments. For example, the temperature micro-probe 200 allows the temperature of very small amounts of substances (including intracellular and intercellular substances) to be measured. The temperature micro-probe 200 allows for localized measurements to be made without influence of nearby temperatures. This is particularly useful in heterogeneous environments such as biological cells.

[0040] Turning now to FIGS. 5 and 6, an immuno-based micro-probe 300 constructed in accordance with another embodiment of the invention is illustrated. The immuno-based micro-probe 300 broadly includes a shaft 302, a tip 304, a thin film 306, and a number of nano holes 308.

[0041] The shaft 302 allows a light signal to be transmitted therethrough and may be substantially similar to the shafts 12, 202 described above.

[0042] The tip 304 allows the light signal to interact with the thin film 306 and may be substantially similar to the tips 16, 204 described above. In one embodiment, the tip 304 is the distal end of the shaft 302.

[0043] The thin film 306 allows half antibodies to be immobilized thereon and may be applied to or coated on at least a portion of the tip 304. The thin film 306 may be formed of gold or any other suitable material.

[0044] The nano holes 308 allow at least a portion of the light signal to pass through the thin film 306 and extend through the thin film 306 so that the thin film 306 exhibits a porous texture.

[0045] Use of the immuno-based micro-probe 300 will now be described in more detail. The tip 304 of the immuno-based micro-probe 300 is inserted into a sample to be measured. An input light signal is then transmitted into the proximal end of the shaft

302 from a light signal generator. The light signal will travel to the distal end of the shaft 302 and will continue into the tip 304. Some of the input light signal will reflect off of the thin film 306 and some of the input light signal will pass through the nano holes 308. Antigens in the sample will bind to a layer of half antibodies immobilized on the thin film 306. As more antigens bind to the antibodies, the reflection intensity at spectral wavelength will increase. That is, more of the input light signal will reflect back into the shaft 302 for being detected by a light signal measuring device. As such, the number of antigen-antibody bonds or the ratio of antigen-antibody bonds to non-bonds can be measured as a function of the measured reflection intensity of the reflected light signal.

[0046] The above-described immuno-based micro-probe 300 provides several advantages over conventional immuno-based detection methods. For example, the immuno-based micro-probe 300 allows the antigens of a very small sample, such as a single cell, to be measured. The immuno-based micro-probe 300 allows for localized measurements to be made. This is particularly useful in heterogeneous environments.

[0047] Although the invention has been described with reference to the embodiments illustrated in the attached drawing figures, it is noted that equivalents may be employed and substitutions made herein without departing from the scope of the invention as recited in the claims.

[0048] Having thus described various embodiments of the invention, what is claimed as new and desired to be protected by Letters Patent includes the following:

CLAIMS:

1. A micro-probe for measuring a non-cellular substance or an intracellular or intercellular pH of a cellular substance, the micro-probe comprising:

an elongated transparent shaft for transmitting a light signal therethrough;

a mirror coating applied to at least part of the shaft;

a tip extending from a distal end of the shaft for contacting or being inserted into the substance;

a luminescent dye doped coating applied to at least part of the tip; and

a mirror coating applied to at least part of the tip.

2. The micro-probe of claim 1, wherein the micro-probe is configured such that the light signal transmitted into a proximal end of the shaft will reflect off of the mirror coating of the shaft as the light signal travels through the shaft so as to continue travelling through the shaft, enter into the tip, reflect off of the mirror coating of the tip, and be at least partially absorbed by the dye doped coating so that the dye doped coating emits a luminescent light signal having a property value indicative of the pH of the substance.

3. The micro-probe of claim 2, wherein the micro-probe is further configured such that the luminescent light signal will enter into the shaft from the tip, reflect off of the mirror coating of the shaft as the luminescent light signal travels through the shaft so as to continue travelling through the shaft, and exit the proximal end of the shaft for being received by a light signal measuring device so that the property value can be measured for determining the pH of the substance.

4. The micro-probe of claim 3, wherein the shaft includes a central fiber for transmitting the light signal to the tip and one or more fibers surrounding the central fiber for transmitting the luminescent light signal from the tip to the measuring device for measuring the property value.

5. The micro-probe of claim 1, wherein the shaft is tapered so that the distal end is smaller than the proximal end.

6. The micro-probe of claim 1, wherein the tip is bulb shaped.

7. The micro-probe of claim 1, wherein the dye doped coating is formed of 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF).

8. The micro-probe of claim 1, wherein the dye doped coating comprises an aerogel.

9. The micro-probe of claim 1, wherein the dye doped coating comprises an organically modified silicate.

10. The micro-probe of claim 1, wherein the dye doped coating is approximately 100 nm to approximately 2 μm thick.

11. A micro-probe for measuring a temperature of a substance, the micro-probe comprising:

an elongated transparent shaft for transmitting a light signal therethrough;

a tip extending from a distal end of the shaft for contacting or being inserted into the substance;

at least one microcavity extending into the tip; and

a luminescent material positioned in the microcavity.

12. The micro-probe of claim 11, wherein the micro-probe is configured such that the light signal transmitted into a proximal end of the shaft will travel through the shaft, enter into the tip, and be at least partially absorbed by the luminescent material so that the luminescent material emits a luminescent light signal having a property value indicative of the temperature of the substance.

13. The micro-probe of claim 12, wherein the micro-probe is further configured such that the luminescent light signal will enter into the shaft from the tip, travel through the shaft to the proximal end of the shaft, and exit the proximal end of the shaft for being received by a light signal measuring device so that the property value can be measured for determining the temperature of the substance.

14. The micro-probe of claim 11, wherein the microcavity is formed via chemical etching.

15. The micro-probe of claim 12, wherein the microcavity is etched by hydrofluoric acid.

16. The micro-probe of claim 11, wherein the microcavity has a volume of 5 micrometers by 5 micrometers by 5 micrometers or less.

17. The micro-probe of claim 11, wherein the luminescent material comprises a plurality of quantum dots.

18. The micro-probe of claim 17, wherein the quantum dots are in a liquid phase.

19. The micro-probe of claim 17, wherein the quantum dots are in a solid phase.

20. The micro-probe of claim 11, wherein the luminescent material comprises a phosphor or phosphorescent material.

21. The micro-probe of claim 11, wherein the luminescent material has a luminescent decay time corresponding to the temperature of the substance.

22. A micro-probe for measuring antigens, the micro-probe comprising:

an elongated transparent shaft;

a tip extending from a distal end of the shaft;

a thin film applied to at least part of the tip; and

a number of nano holes extending through the thin film, the thin film being configured to immobilize a number of antibodies thereon.

23. The micro-probe of claim 22, wherein the micro-probe is configured such that a light signal transmitted into a proximal end of the shaft will:

travel through the shaft;

enter into the tip;

at least partially pass through the nano holes;

at least partially reflect off of the thin film depending on the number of antigens bound to the antibodies so that a remaining portion of the light signal has a property value indicative of the number of antigens bound to the antibodies;

enter into the shaft from the tip after being at least partially absorbed by the thin film;

travel through the shaft to the proximal end of the shaft; and

exit the proximal end of the shaft so that the property value can be measured for determining the number of antigens bound to the antibodies or determining the ratio of antigen-antibody bonds to total antibodies.

24. A measurement system for measuring an intracellular pH of a cellular substance, the measurement system comprising:

a light signal generator for generating a light signal;

a micro-probe comprising:

an elongated transparent shaft for transmitting the light signal therethrough;

a mirror coating applied to at least part of the shaft;

a tip extending from a distal end of the shaft for contacting or being inserted into the substance;

a luminescent dye doped coating applied to at least part of the tip; and

a mirror coating applied to at least part of the tip; and

a light signal measuring device connected to the micro-probe.

25. The measurement system of claim 24, wherein the micro-probe is configured such that:

the light signal generated by the light signal generator and transmitted into a proximal end of the shaft will:

reflect off of the mirror coating of the shaft as the light signal travels through the shaft so as to continue travelling through the shaft;

enter into the tip;

reflect off of the mirror coating of the tip; and

be at least partially absorbed by the dye doped coating so that the dye doped coating emits a luminescent light signal having a property value indicative of the pH of the substance; and

the luminescent light signal will:

enter into the shaft from the tip;

reflect off of the mirror coating of the shaft as the luminescent light signal travels through the shaft so as to continue travelling through the shaft; and

exit the proximal end of the shaft,

the light signal measuring device being configured to receive the luminescent light from the proximal end of the shaft so that the property value can be measured for determining the pH of the substance.

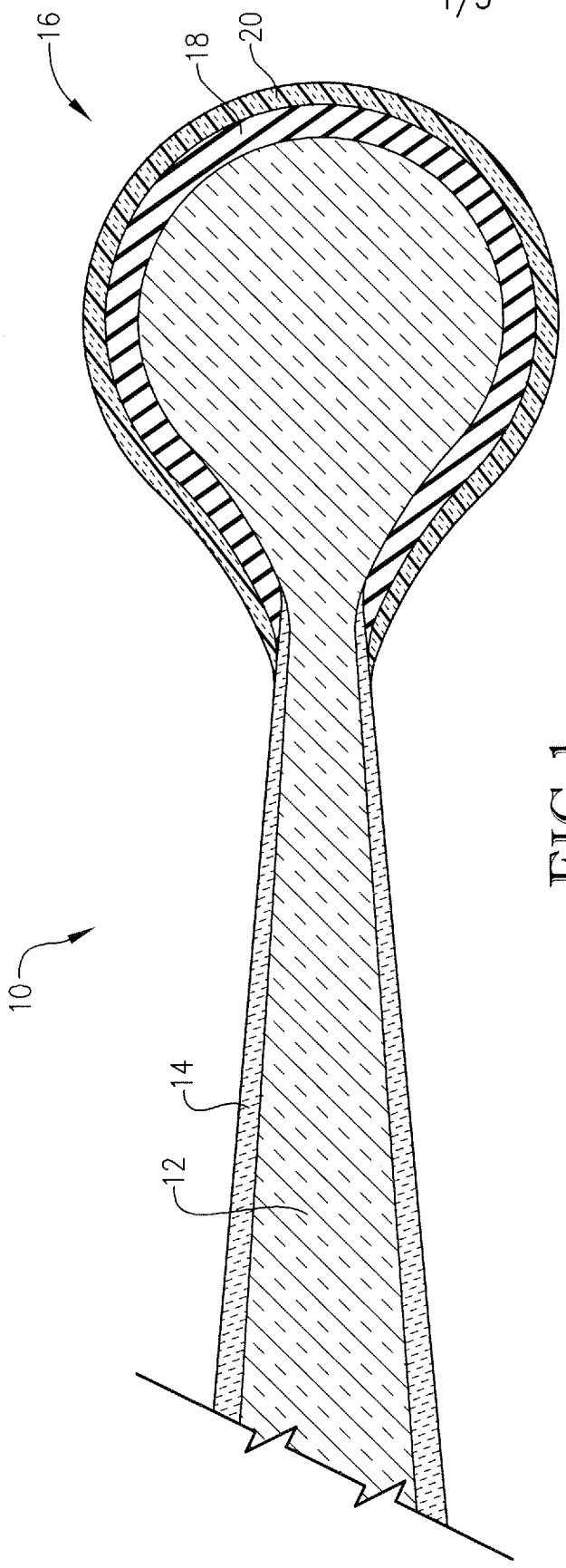

FIG. 1

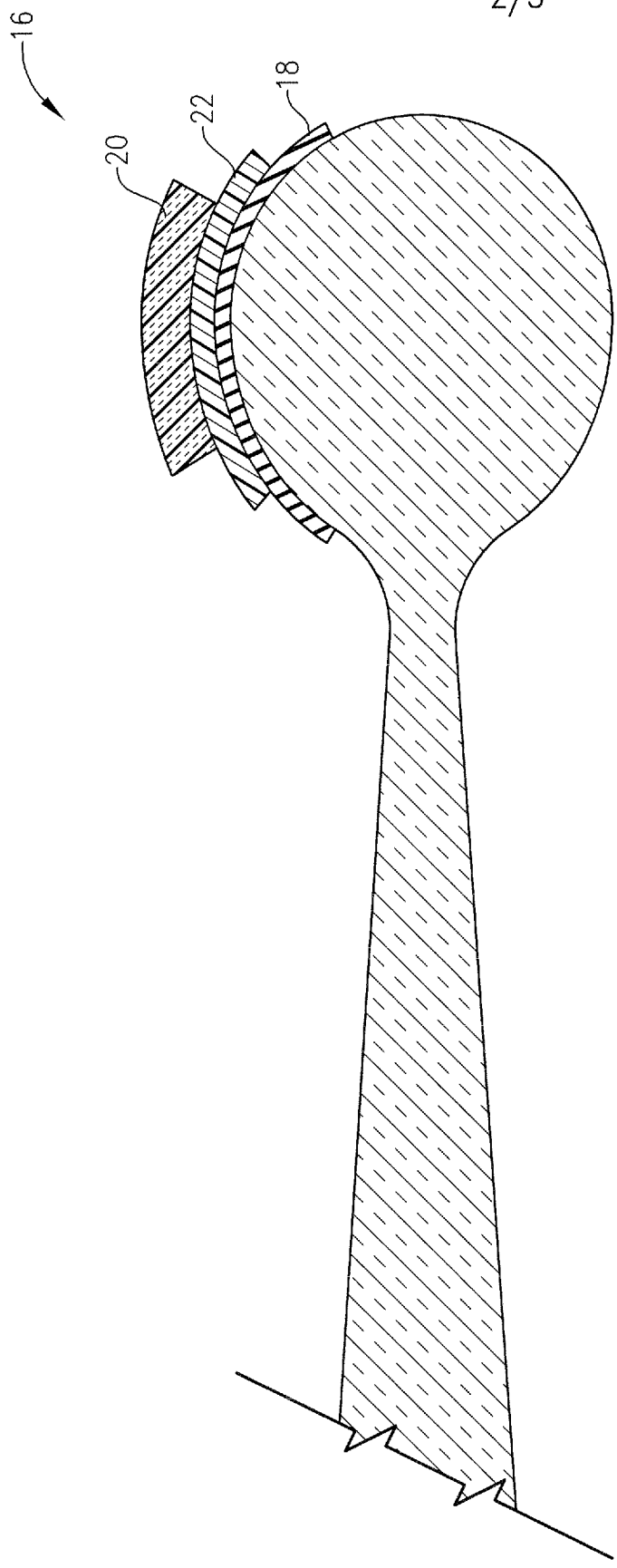

FIG. 2

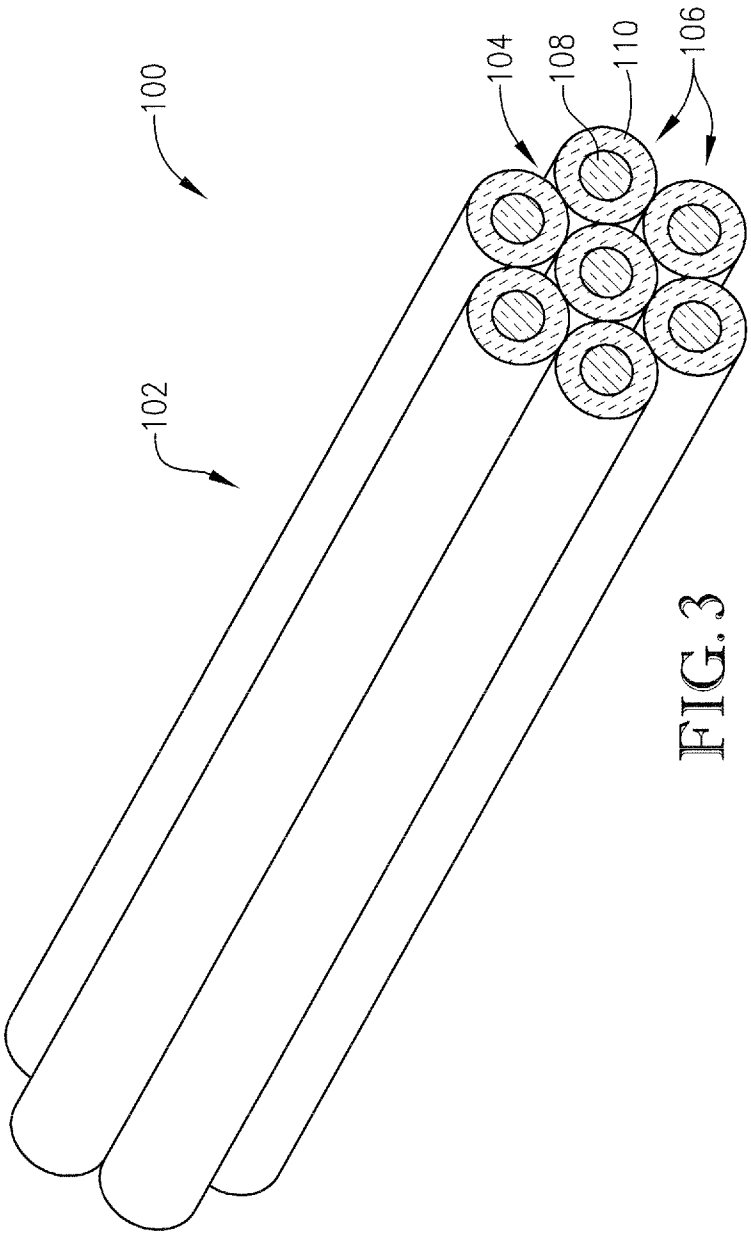

FIG. 3

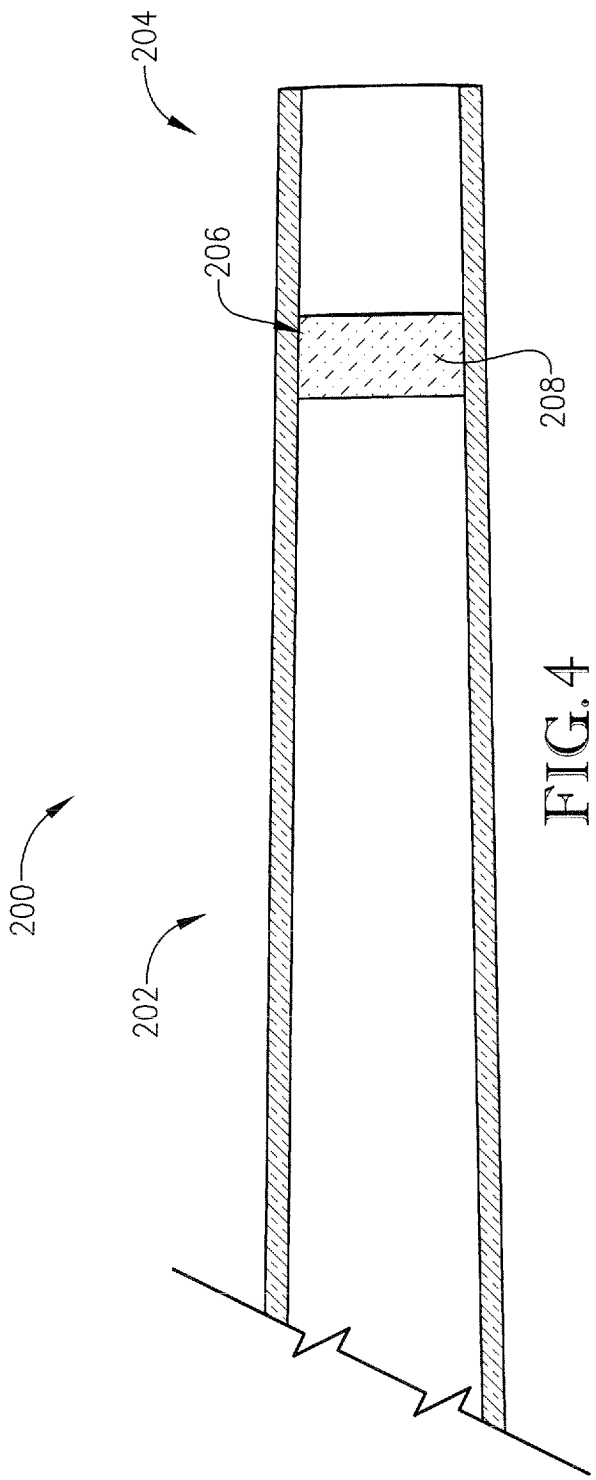

FIG. 4

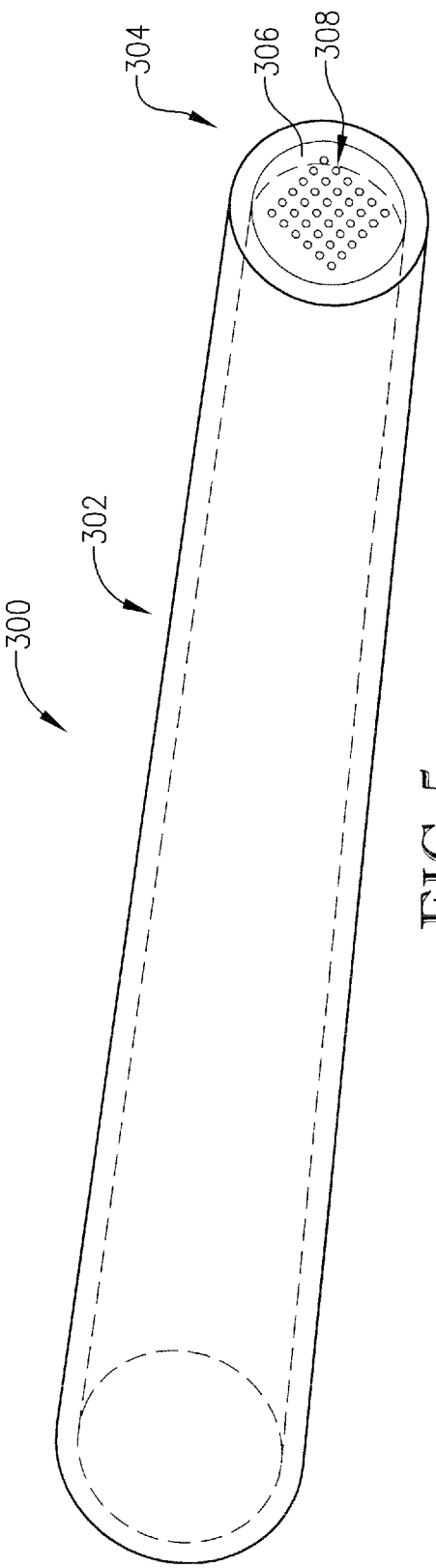

FIG. 5

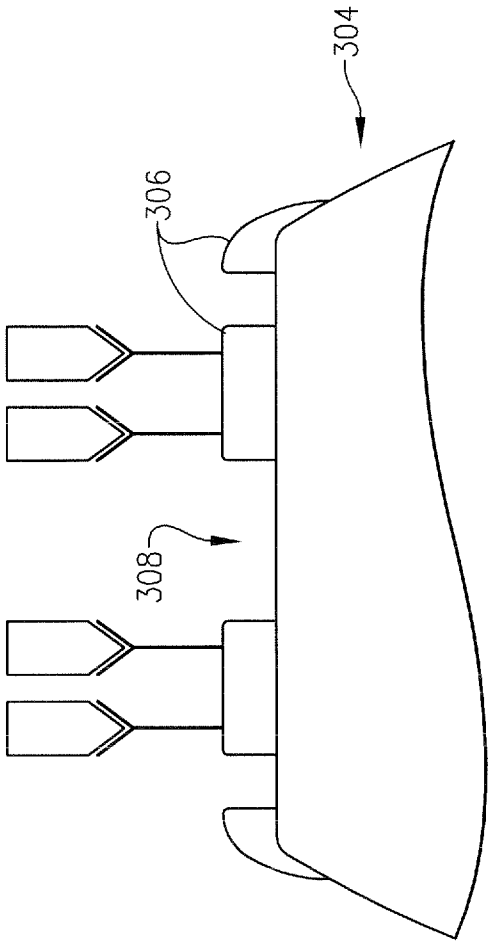

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060139

A. CLASSIFICATION OF SUBJECT MATTER

G01N 21/64(2006.01)i, G01N 33/53(2006.01)i, G01K 13/00(2006.01)i

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 21/64; G02B 6/04; G01N 21/00; G01N 33/53; G01K 13/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: probe, luminescent, mirror, shaft, acid, optic and fiber

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YANG et al. `Reflection-mode micro-spherical fiber-optic probes for in vitro real-time and single-cell level pH sensing`, SENSORS AND ACTUATORS B: CHEMICAL, 01 November 2014, Vol. 207, pp. 571-580 See pages 572-574, 578 and figures 1-3.	1-25
Y	YUAN et al. `All-in-fiber optofluidic sensor fabricated by femtosecond laser assisted chemical etching`, OPTICS LETTERS, 09 April 2014, Vol. 39, No. 8, pp. 2358-2361 See pages 2358-2360 and figures 1, 3.	1-21,24-25
Y	LAN et al. `Reflection based extraordinary optical transmission fiber optic probe for refractive index sensing`, SENSORS AND ACTUATORS B: CHEMICAL, 23 November 2013, Vol. 193, pp. 95-99 See pages 96, 98 and figure 2.	22-23
A	US 2008-0089635 A1 (HECHT et al.) 17 April 2008 See paragraphs [0029]-[0034], claims 1, 13 and figure 2.	1-25
A	US 2011-0236988 A1 (REED et al.) 29 September 2011 See paragraphs [0131]-[0132], claim 10 and figures 2-3.	1-25

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
12 February 2016 (12.02.2016)	**12 February 2016 (12.02.2016)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2015/060139

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