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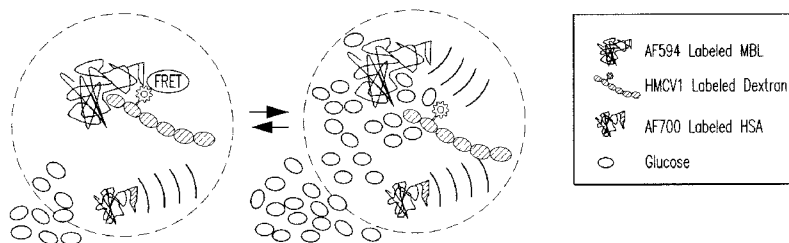


FIG. 3

(57) Abstract: An optical glucose sensor may include an optical fiber and a glucose-permeable membrane having a hollow interior and being coupled to the optical fiber's distal end. The membrane's hollow interior provides a compartment to house a competitive glucose binding affinity assay. The assay may include a glucose analog that may be labeled with a dye, and a glucose receptor that may be labeled with a fluorophore. The optical fiber may include a compound parabolic concentrator tip, and the compartment may additionally house a reflector disposed so as to face the optical fiber's tip. A fluorophore-labeled assay may be interrogated by an optical interrogating system including a light source and a filter substrate having one or more coatings to effect, e.g., an excitation filter and/or an emission filter. The interrogating system may be manufactured as a stacked planar integrated optical system and diced into smaller units.

GLUCOSE SENSOR**RELATED APPLICATION DATA**

This application claims priority from U.S. Provisional Application Serial No. 61/531,449, filed September 6, 2011, and U.S. Provisional Application Serial No. 61/531,451, filed September 6, 2011, and U.S. Provisional Application Serial No. 61/531,456, filed September 6, 2011, and U.S. Provisional Application Serial No. 61/554,057, filed November 1, 2011, and U.S. Provisional Application Serial No. 61/561,146, filed November 17, 2011, and U.S. Provisional Application Serial No. 61/587,819, filed January 18, 2012, and U.S. Provisional Application Serial No. 61/620,563, filed April 5, 2012, and is related to the U.S. Patent Application entitled "Orthogonally Redundant Sensor Systems and Methods", Attorney Docket No. 040088-0405699, which is being filed concurrently herewith, all of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

Embodiments of the present invention relate generally to sensor technology, including sensors used for sensing a variety of physiological parameters, e.g., glucose concentration. More particularly, embodiments of the invention relate to optical sensors, to methods of making and using such sensors, to optical and optoelectronic systems for interrogating optical sensors, and to methods of making and using such optical/optoelectronic systems. More particularly still, embodiments of the invention relate to optical fiber sensors including a fluorophore-labeled assay, to stacked planar optical integrated systems for interrogating such optical fiber sensors, and to methods of making and using such optical fiber sensors and optical integrated systems.

BACKGROUND

Epifluorescence microscopy is a method of fluorescence microscopy that is becoming increasingly used in the biological and medical fields. An epifluorescence microscope is used

primarily to excite a specimen by passing a source light through an objective lens and then onto the specimen. The fluorescence in the specimen generates emitted (fluorescent) light which is focused onto a detector by the same objective lens that is used for the excitation. Since most of the source light is generally transmitted through the specimen, only reflected
5 source light reaches the objective lens together with the fluorescent light. An additional filter between the objective lens and the detector can filter out the remaining source light from fluorescent light.

The underlying principles of epifluorescence microscopy may be used in optical, or optoelectrical, systems for interrogating assay-based glucose sensors. The assay in such
10 sensors may be interrogated using a variety of methods, such as Streak Camera recording, single photon counting, frequency domain lifetime measurement, and steady state fluorescence measurement. In both the frequency domain lifetime and steady state fluorescence interrogation, the function of the optical interrogation system is to excite the assay fluorophore(s) and prevent the excitation light from reaching the detector(s) while, at the same
15 time, transmitting the emitted fluorescence. It is understood that the fluorescence emitted from fluorophore-labeled assays is generally weak. Therefore, it is important to excite the assay as efficiently as possible and to gather as much of the isotropically emitted fluorescence as possible.

In the context of a continuous glucose monitor based on frequency domain lifetime
20 interrogation and steady state fluorescence interrogation, it is important not only to minimize the cost, size, and weight of the (optical system) instrumentation and of the optical sensor, but also to optimize manufacturability of both the instrumentation and the sensor. In this regard, the currently-used optical systems are in general fairly large and expensive, and require precision assembly as they include a number of different optical components. Thus, improved
25 optical systems and optical glucose sensors, including sensors for use with such optical systems, are needed that address the above-mentioned requirements.

SUMMARY

In accordance with one embodiment of the invention, an optical glucose sensor includes an optical fiber with a glucose-permeable membrane joined to its distal end. The membrane may be, e.g., tube-shaped, such that its hollow interior defines a compartment for
5 holding an assay. In one aspect of the invention, the assay is a competitive glucose binding affinity assay that includes a glucose receptor, a glucose analog, a first (donor) fluorophore labeled onto the glucose receptor, and an acceptor dye labeled onto the glucose analog. In a variation of this aspect of the invention, the assay may include a reference fluorophore in addition to the first fluorophore.

10 In accordance with another embodiment of the invention, the optical fiber of the optical glucose sensor includes a compound parabolic concentrator (CPC)-shaped tip that is in direct contact with the assay. In yet another aspect, a reflector may be disposed within the compartment, opposite the CPC-shaped tip, to reflect fluorescence that is emitted from the assay towards the CPC-shaped tip.

15 Embodiments of the invention are also directed to optical systems for lifetime and/or intensity interrogation of the assay. Thus, in one aspect, a fluorophore-labeled assay may be interrogated by an optical interrogating system including a light source and a filter substrate having one or more coatings to effect, e.g., an excitation filter and/or an emission filter. In another aspect, the interrogating system may be manufactured as a wafer-scale stacked planar
20 integrated optical system (SPIOS) and diced into smaller units.

Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings which illustrate, by way of example, various features of embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a glucose binding competitive affinity assay based on Förster Resonance Energy Transfer (FRET) in accordance with an embodiment of the invention;

FIG. 2 shows instrumentation for measuring the lifetime of the excited state for a fluorophore-labeled assay in accordance with an embodiment of the invention;

FIG. 3 shows a glucose binding competitive affinity assay, including a reference fluorophore, for intensity interrogation in accordance with an embodiment of the invention;

FIG. 4A shows instrumentation for interrogating a fluorophore-labeled assay with an internal reference used for an intensity interrogation in accordance with an embodiment of the invention;

FIG. 4B shows instrumentation for interrogating a fluorophore-labeled assay with an internal reference used for an intensity interrogation in accordance with another embodiment of the invention;

FIG. 5A is a perspective view of an optical fiber sensor according to an embodiment of the invention;

FIG. 5B is a side view of the optical fiber sensor shown in FIG. 5A;

FIG. 6A is a perspective view of an optical fiber sensor with a compound parabolic concentrator (CPC)-shaped fiber tip a according to an embodiment of the invention;

FIG. 6B is a side view of the optical fiber sensor shown in FIG. 6A;

FIG. 7A is a perspective view of an optical fiber sensor with a compound parabolic concentrator (CPC)-shaped fiber tip, a reflector, and support structure in accordance with an embodiment of the invention;

FIG. 7B is a side view of the optical fiber sensor shown in FIG. 7A;

FIG. 7C is a side view of an optical fiber with side cut cavities in accordance with an embodiment of the invention;

FIG. 7D is a perspective view of the optical fiber sensor shown in FIG. 7C;

FIG. 8 shows the fluorescence distribution inside the assay compartment of an optical fiber sensor with a straight-cut optical fiber (height = diameter of assay compartment);

FIG. 9 shows the fluorescence distribution inside the assay compartment of an optical fiber sensor with a CPC-shaped optical fiber (height = diameter of assay compartment);

FIG. 10A shows the fluorescence distribution inside the assay compartment of an optical fiber sensor with a CPC-shaped optical fiber and reflector (height = diameter of assay compartment);

FIG. 10B shows the fluorescence distribution inside the assay compartments of an optical fiber sensor with side cut cavities (height = diameter of fiber);

FIG. 11 shows a stacked planar integrated optical system (SPIOS) for lifetime interrogation of a fluorophore-labeled assay in accordance with an embodiment of the invention;

FIG. 12 shows the spectrum of light source, excitation filter, and fluorophore for a lifetime system in accordance with an embodiment of the invention;

FIG. 13 shows a stacked planar integrated optical system (SPIOS) for intensity interrogation of a fluorophore-labeled assay in accordance with an embodiment of the invention;

FIG. 14 shows the spectrum of light source, excitation filter, emission filter, assay fluorophore, and reference fluorophore for a intensity system in accordance with an embodiment of the invention;

FIG. 15 shows a stacked planar integrated optical system (SPIOS) for intensity interrogation of a fluorophore-labeled assay in accordance with another embodiment of the invention;

FIGS. 16A and 16B show examples of a CPC SPIOS-fiber interface in accordance with
5 embodiments of the invention; and

FIG. 17 shows illustrative layers of a wafer-scale stacked planar integrated optical system (SPIOS) in accordance with an embodiment of the invention.

DETAILED DESCRIPTION

In the following description, reference is made to the accompanying drawings which
10 form a part hereof and which illustrate several embodiments of the present invention. It is understood that other embodiments may be utilized and structural and operational changes may be made without departing from the scope of the present invention.

As shown in the drawings for purposes of illustration, embodiments of the invention are directed to optical sensors that may be interrogated by optical, or optoelectronic, systems.
15 Optical sensors may be introduced and/or lodged transdermally, or may be implanted in and/or through subcutaneous, dermal, sub-dermal, inter-peritoneal, or peritoneal tissue. In the discussion herein, preferred embodiments of the devices, systems, and methods of the invention are described with reference to glucose as the analyte whose level/concentration in the blood and/or bodily fluids of the user is to be determined. However, this is by way of
20 illustration and not limitation, as the principles, devices, systems, and methods of the present invention may be used for sensing and/or determining the level of a variety of other physiological parameters, agents, characteristics, and/or compositions.

As will be described in more detail below, an optical glucose sensor having an assay compartment may be formed, e.g., by including a glucose permeable membrane containing the
25 assay at the distal end of an optical fiber. The optical fiber may then be inserted transdermally into the user's body, thereby situating the assay compartment in the user's tissue, while leaving

at least a part of the optical fiber outside the body such that it can be accessed by an interrogating system. Alternatively, the optical sensor may be implantable, e.g., as part of an implantable glucose monitor including an interrogating optoelectronic system and a power source. The assay compartment may be formed between a glucose permeable membrane and an optical interface to the optoelectronic system. The optical sensor may preferably be biodegradable.

As shown in Figure 1, an optical glucose sensor may be based on a competitive glucose binding affinity assay. The assay may include a glucose receptor and a glucose analog (ligand) contained in a compartment where at least a part of the compartment is capable of exchanging small molecules, such as glucose, salts, etc. with the surrounding medium, while retaining macromolecules, such as the assay components.

Several molecules may serve as the glucose receptor in the glucose assay. Examples include, but are not limited to, Concanavalin A, periplasmic glucose/galactose-binding receptor, antibodies raised against glucose-like molecules, Boronic Acids, and Mannan Binding Lectin (MBL). Mannan Binding Lectin is human protein, which is a part of the innate immune system. Thus, the assay may include MBL as the glucose receptor and dextran as the glucose analog.

The binding between MBL and glucose-like molecules (e.g., dextran) is reversible. When no glucose is present, MBL and dextran will predominantly be bound together. When glucose is added to the assay, it will compete off a part of the dextran population, such that the assay enters a new equilibrium state. The equilibrium state at all times corresponds to the glucose concentration. In order to determine this equilibrium state, MBL is labeled with a fluorophore (e.g., Alexa Fluor 594, or AF594), and the dextran is labeled with a dye (e.g., hexamethoxy crystalviolet-1 (HMCV1) – a proprietary crystal violet derivative, Medtronic, Inc.). The donor fluorophore and the acceptor dye together form a Förster Resonance Energy

Transfer (FRET) pair -- i.e., the emission spectrum of the fluorophore and the absorption spectrum of the dye overlap.

The occurrence of FRET affects the lifetime of the excited state and the intensity of the emitted fluorescence and can only occur when the fluorophore and the corresponding dye are in close proximity (i.e., in the range of about 50Å). Thus, the FRET mechanism permits interrogation of the equilibrium state optically by illuminating the assay and measuring either the lifetime of the excited state, and/or the intensity of the emitted fluorescence from the donor fluorophore. It is noted that the donor fluorophore and the acceptor dye are preferably water soluble, as they are to function in an aqueous environment.

Figure 2 shows instrumentation used for frequency domain lifetime interrogation of the above-described assay based on a modified epifluorescence microscope. The instrumentation, or optical interrogation system, is optically coupled to (or aligned with) a sensor 100 carrying the assay. The assay is excited with a periodic signal (e.g., sinusoidal, squarewave, dirac pulse, approximative dirac, sawtooth, etc.), and the modulation frequency is governed by the lifetime of the excited state (τ) for the fluorophore. The optimum modulation frequency may be approximated by:

$$f_{\text{opt}} = 1/(2\pi\tau) \quad \text{Eq. (1)}$$

Thus, for a lifetime of 3ns, e.g., the optimum modulation frequency (f_{opt}) is in the range of about 50 MHz to about 60 MHz.

With reference to Figure 2, an oscillator 105 in combination with a driver circuit 110 modulates a LED 120 with a wavelength range capable of exciting the fluorophore. The LED 120 output is filtered using a multilayer dielectrical filter 130 to select a distinct wavelength region. The filtered LED output is reflected by a dichroic beam splitter 140 and focused onto the sensor 100 (which contains the assay) by a lens 150. The assay emits fluorescence with the same frequency as the excitation (modulated LED output) and phase shifted as a result of the lifetime of the excited state for the fluorophore.

The emitted fluorescence 103 and the reflected excitation light 123 are picked up and collimated by the lens 150. The dichroic beam splitter 140 transmits the fluorescence 103. However, it reflects the majority of the back-reflected excitation light 123. An emission filter 160 with a distinct wavelength region red shifted with respect to, and not overlapping, the pass band of the excitation filter blocks the remaining part of the excitation light 123 and transmits the fluorescence 103. Thus, in effect, only the fluorescence carrying the modulated and phase shifted fluorescence is focused onto a photodetector 180 using a lens 170. The phase lag between the detected fluorescence and the excitation light correlates with the glucose concentration in the assay.

In addition to the lifetime of the excited state, the intensity of the emitted fluorescence also correlates to the glucose concentration. In contrast to a lifetime measurement, the measured intensity of the emitted fluorescence is affected by the intensity of the light source and the coupling between the assay and the optical system. Therefore, the intensity measurement requires an internal reference fluorophore to be incorporated into the assay, as shown in Figure 3.

The reference fluorophore must differ from the assay fluorophore in a way that the emitted fluorescence from the assay and that from the reference may be separated from one another, e.g., by having different absorption spectra or emission spectra. The reference fluorophore may be, e.g., Alexa Fluor 700 (AF700) labeled onto Human Serum Albumin (HSA) or another macro molecule, which largely does not bind to the glucose receptor. See Figure 3. Alexa Fluor 700 may be excited simultaneously with the Alexa Fluor 594 as their absorption spectra spectrally overlap. The emission spectrum from Alexa Fluor 700 is slightly red shifted with respect to Alexa Fluor 594, which makes it possible to detect their respective fluorescence emissions in separate wavelength regions. As they are excited simultaneously by the same light source, any changes in the intensity of the light source will scale fluorescence

from AF594 and AF700 equally. As such, any effect originating from changes in the intensity of the light source may be cancelled out.

The excitation, as well as the detection, of the emitted fluorescence for the assay and the reference follow the same optical path from the optical system to the assay. As such, the
5 detected signal from the reference serves as a measure for the optical coupling between the optical interrogating system and the assay. Any effect originating from changes in the optical coupling such as alignment may be cancelled out.

Figure 4A shows one embodiment of instrumentation used for fluorescence interrogation of the above-described assay based on another modification of an epifluorescence
10 microscope. A driver circuit 310 modulates a LED 320 at a low frequency--solely with the purpose of eliminating the 1/f noise and canceling out ambient light--with a wavelength range capable of simultaneously exciting the assay and reference fluorophores. The LED output is filtered using a multilayer dielectrical filter 330 to select a distinct wavelength region. The filtered LED output is reflected by a first dichroic beam splitter 340 and focused onto the
15 sensor 300, which includes the assay and the reference, by a lens 350.

The assay and the reference emit fluorescence. The emitted fluorescence 301 and the reflected excitation light 323 are picked up and collimated by the lens 350. The first dichroic beam splitter 340 transmits the fluorescence 301. However, it reflects the majority of the back reflected excitation light 323. A second beam splitter 344 reflects the reference fluorescence at
20 a 90° angle 307, but it transmits the assay fluorescence 309. An assay emission filter 360 with a distinct wavelength region red shifted with respect to, and not overlapping, the pass band of the excitation filter and matching the desired part of the assay fluorescence spectrum then blocks the remaining part of the excitation light and transmits the assay fluorescence.

Similarly, a reference emission filter 364 with a distinct wavelength region red shifted
25 with respect to, and not overlapping, the pass band of the excitation filter and matching the desired part of the reference fluorescence blocks the remaining part of the excitation light and

transmits the reference fluorescence 307. Thus, in effect, only the fluorescence from the assay and the fluorescence from the reference are focused onto their respective photo detectors 380, 384 using respective lenses 370, 374. The ratio between the detected assay fluorescence and the detected reference fluorescence correlates with the glucose concentration in the assay. As mentioned previously, any changes in light-source intensity or optical coupling will be cancelled out as they scale the assay and reference fluorescence equally.

Figure 4B shows another embodiment of the instrumentation used for fluorescence interrogation. Here, as in Figure 4A, driver circuit 310 modulates a LED 320 at a low frequency--solely with the purpose of eliminating the 1/f noise and canceling out ambient light--with a wavelength range capable of simultaneously exciting the assay and reference fluorophores. The LED output is filtered using a multilayer dielectrical filter 330 to select a distinct wavelength region. The filtered LED output is reflected by a first dichroic beam splitter 340 and focused onto the sensor 400, which includes the assay and the reference, by a lens 350. The sensor 400 is a fiber optical sensor, as described more fully hereinbelow.

As described in connection with Figure 4A, the assay and the reference emit fluorescence. The emitted fluorescence 301 and the reflected excitation light 323 are picked up and collimated by the lens 350. The first dichroic beam splitter 340 transmits the fluorescence 301. However, it reflects the majority of the back reflected excitation light 323. A second beam splitter 344 reflects the assay fluorescence at a 90° angle 309, but it transmits the reference fluorescence 307. A reference emission filter 364 with a distinct wavelength region red shifted with respect to, and not overlapping, the pass band of the excitation filter and matching the desired part of the reference fluorescence spectrum then blocks the remaining part of the excitation light and transmits the reference fluorescence.

Similarly, an assay emission filter 360 with a distinct wavelength region red shifted with respect to, and not overlapping, the pass band of the excitation filter and matching the desired part of the assay fluorescence blocks the remaining part of the excitation light and

transmits the assay fluorescence 309. Thus, in effect, only the fluorescence from the assay and the fluorescence from the reference are focused onto their respective photo detectors 380, 384 using respective lenses 370, 374. The ratio between the detected assay fluorescence and the detected reference fluorescence correlates with the glucose concentration in the assay. Again, as mentioned previously, any changes in light-source intensity or optical coupling will be cancelled out as they scale the assay and reference fluorescence equally.

Figures 5A and 5B show an embodiment of the invention, wherein a fiber optical sensor 400 is made by placing the assay in a compartment 420 that is distal to the distal end 412 of an optical fiber 410. In this embodiment, a test tube-shaped glucose permeable membrane 430 containing the assay is slid over the end of the optical fiber 410 and sealed (e.g., heat sealed). The distal end 412 of the fiber 410 is straight cut and polished, and is in direct contact with the assay. In embodiments of the invention, the glucose permeable membrane 430 may be made of a biocompatible, biodegradable polymer such as, e.g., PolyActive™ (Integra Orthobiologics, Irvine, CA), Poly-lactide-glycolic-acid, polycaprolactone and non-biodegradable polymers exhibiting molecular weight cut-off properties like Cellulose (Spectrum Laboratories, Rancho Dominguez, CA) or Polysulfone (Spectrum Laboratories, Rancho Dominguez, CA).

With the above configuration, the assay may now be excited through the optical fiber 410, and the resulting fluorescence collected by the optical fiber. As the fluorescence from the assay radiates isotropically, the amount of the emitted fluorescence, which can be picked up by the optical fiber 410, is set by the numerical aperture of the fiber.

The numerical aperture (NA) of the optical fiber is a function of the refractive index of the fiber core (n_1) and the refractive index of the cladding (n_2):

$$NA = \sqrt{n_1^2 - n_2^2} \quad \text{Eq. (2)}$$

Generally, light entering the optical fiber at an angle less than a critical angle will be transmitted through the optical fiber due to total internal reflection in the core/cladding boundary, whereas light entering at an angle larger than the critical angle will simply exit the fiber through the cladding. Commercially available optical fibers have a high refractive index core and a low refractive index cladding. Typical refractive indices for the core and the cladding for a plastic optical fiber are about 1.49 and about 1.40, respectively, which, based on Eq. (2), results in a numerical aperture of about 0.51. Per Eq. (3) below, this corresponds to a critical angle (θ) of about 30.6°, or a solid angle of about 0.88sr:

$$NA = n \sin \theta_{\max} \quad \text{Eq. (3)}$$

For the ideal case, this translates into about a 7% pickup of the total emitted fluorescence--where isotropic radiation is $4\pi\text{sr}$, and $0.88\text{sr}/4\pi\text{sr} \approx 7\%$. The maximum fluorescence pickup is thus set by the optical fiber.

Furthermore, some of the excitation light will spill out of the assay compartment through the glucose permeable membrane as the excitation light is coupled into the assay at angles corresponding to the fiber numerical aperture.

The Optical Invariant theorem states that the product of the source area (A) multiplied by the solid angle (Ω) is constant:

$$A_1\Omega_1 = A_2\Omega_2 \quad \text{Eq. (4)}$$

In Eq. (4), A_1 is equal to the cross sectional area of the optical fiber, Ω_1 equals the solid angle corresponding to the numerical aperture of the optical fiber, and A_2 and Ω_2 are set according to a trade-off between the maximum fiber tip length, saturation intensity for the assay, and manufacturable tip geometry.

Applied to the fiber sensor 400, this means that, with the right optical component and fiber tip design, the excitation light transmitted through the optical fiber 410 may be focused

down to a smaller area moving the excitation away from the glucose permeable membrane 430 and thereby reduce the spillage of excitation light out of the sensor. In addition, the numerical aperture of the fiber tip may be increased resulting in an increased fluorescence pickup from the assay.

5 The Compound Parabolic Concentrator (CPC) is a non-imaging component, which has an entrance aperture, a parabolic shaped reflective surface, and an exit aperture. The CPC may be formed as an air filled void or an optical material with a parabolic mirror surface, or it may be formed by an optical material with a refractive index which is higher than that of the surrounding material. The parabolic shaped part of the CPC is formed as a parabola which
10 ensures total internal reflection due to the high-low refractive index transition.

The radial coordinate of points on the CPC as a function of the z coordinate along the axis is given by the positive real root of the following quadratic equation:

$$C^2r^2 + 2(CSz + aP^2)r + (z^2S^2 - 2aCQz - a^2PT) = 0 \quad \text{Eq. (5)}$$

Where $C=\cos\theta$, $S=\sin\theta$, $P=1+S$, $Q=1+P$, and $T=1+Q$

15 Shaping the tip of the optical fiber 410 as a CPC with the right dimensions will lead to the desired properties. As an example, a CPC shape applied to the tip of a 250 μm optical fiber reduces tip diameter to 125 μm . Area is thus reduced four times, leading to a theoretical four-fold increase in numerical aperture, which corresponds to a four-fold increase in fluorescence pickup.

20 An embodiment of the present invention employing a CPC tip geometry is shown in Figures 6A and 6B. As shown, the CPC shaped fiber tip 414 is in direct contact with the assay, which has a refractive index similar to water (1.33). As this is significantly lower than the refractive index of the optical fiber cladding, a CPC designed to have a cladding on the parabolic part will work with, as well as without, cladding.

25 It is noted that the theoretical four-fold increase in fluorescence pickup is based on the assumption that the fluorophore(s) of the assay are excited at the tip-assay transition, i.e., the

CPC-assay optical interface 415. However, since the fluorescence occurs in a volume in front of the fiber tip 414, the increase in fluorescence pickup may be significantly less than the four-fold increase predicted by crude theoretical calculations.

Thus, even though, theoretically, the numerical aperture of the CPC fiber tip 414 will be increased dramatically compared to the conventional straight cut fiber tip 412, in operation, the CPC design generally cannot pick up more than about 50% of the emitted fluorescence. Nevertheless, in an embodiment of the invention, the fluorescence pick-up percentage may be increased by placing a concave mirror 417 in front of the fiber tip 414 to reflect fluorescence emitted in a direction that is opposite to the fiber tip and focus it into the fiber.

As shown in Figures 7A and 7B, there must still be a gap between the fiber tip 414 and the mirror 417 to leave room for the assay, i.e., to constitute the assay compartment 420. The mirror 417 obviously needs to be kept in place in front of the fiber tip 414. This may be accomplished, e.g., by the support structure 419, which allows glucose to diffuse into the space in front of the fiber tip 414. At the same time, the support structure 419 serves the purpose of supporting the glucose permeable membrane 430.

The straight cut fiber tip 412, the CPC shaped fiber tip 414, and the CPC shaped fiber tip in combination with a reflector 414, 417 have been modeled using Zemax optical design software. For each of the three designs, the model included the following: (1) an excitation light source at the proximal (free) end of the fiber sensor, coupling light into the optical fiber and exciting the assay at the (distal, in-situ) tip of the optical fiber; (2) an optical fiber with the selected fiber tip geometry; (3) an assay-filled compartment including assay absorption and assay fluorescence processes; and (4) a detector with a fluorescence filter only selecting the fluorescence picked up and transmitted back through the optical fiber.

For all three designs, the ratio between the excitation and the detected fluorescence was calculated. As can be seen from the results shown in Table 1 below, the Zemax simulations shows significantly lower fluorescence pickup from the CPC designs than the Optical Invariant

theorem predicts. As stated, this is due to the fact that the excitation and resulting fluorescence emission occur in a volume in front of the fiber tip rather than directly at the fiber tip/assay boundary.

TABLE 1		
	Without Reflector	With Reflector
Straight Cut	100%	N/A
CPC	166%	277%

5 In embodiments of the invention employing the CPC tip geometry described above, the assay, including the reference dye, can be carried or contained by a hydrogel in order to ease production and stabilize the assay. Specifically, the glucose assay is first dissolved in a hydrogel. Next, the CPC-shaped fiber tip may be dipped into the hydrogel containing the assay, and a droplet may be left in front of the CPC-shaped fiber tip. Finally, the hydrogel may
 10 be cross-linked so as to provide a glucose sensor. Suitable cross-linking hydrogels may include, e.g., Poly acryl based hydrogels (such as poly-Hydroxy-Ethyl-Methacrylate (pHEMA), PMMA-pHEMA co-polymers, etc.), Polyurethanes, Polyesters, Polyethers, etc.

Where the hydrogel containing the assay is not cross-linked, the entire embodiment could be coated by any of the polymers suitable for glucose sensors, i.e., polymers that allow
 15 glucose diffusion through the polymer. Suitable non-crosslinking hydrogels may include, e.g., poly-vinyl alcohol (PVA), Poly-ethylene glycol (PEG), poly-propylene glycol (PPG), poly-Hydroxy-Ethyl-Methacrylate (pHEMA), etc., and co-polymers thereof.

Figures 7C and 7D show another embodiment of the invention, wherein a fiber optical sensor 1400 is made by placing the assay in the compartments 1420a, 1420b proximate the
 20 distal end 1412 of an optical fiber 1410. The compartments are formed as side cuts in the fiber cutting through the fiber cladding as well as core. In this embodiment, a tube-shaped glucose

permeable membrane 1430 is slid over the side cuts and sealed to the cladding of the fiber leaving glucose permeability in the regions of the assay compartments.

Two cuts are made on opposite sides of the fiber and displaced from each other sufficiently to maintain the structural strength of the fiber. For the first side cut cavity 1420a, the surface 1421a parallel to the fiber axis is preferably optical quality. As the refractive index of the assay is significantly lower than the fiber core, this will provide total internal reflection for excitation light 1423 traveling from the proximal end of the fiber sensor to the second assay compartment 1420b and furthermore provide total internal reflection for fluorescence emitted from the second assay compartment 1420b and back to the proximal end of the fiber. The above-described configuration provides structural strength to the fiber sensor, which is advantageous, especially for fiber sensors where a soft glucose permeable membrane in itself does not provide sufficient structural strength to ensure the stability of the assay compartment.

It is noted that, in embodiments of the invention, fewer or more assay compartments may be included. For example, with reference to Figures 7C and 7D, the second assay compartment 1420b may be omitted when, e.g., a single assay compartment provides sufficient fluorescence. Also, in additional embodiments, as an alternative to the combination of a liquid assay and a glucose permeable membrane, a hydrogel with embedded assay as described above may be cast into the one or more side cavities to form the assay compartment(s).

Figures 8-10A show the fluorescence intensity distribution inside the assay compartment 420 for each of the aforementioned designs. Clearly, while, with the straight cut fiber, the fluorescence emission zone extends out to the membrane, the CPC concentrates the excitation and the fluorescence emission in the center of the assay compartment. Figure 10B shows the fluorescence intensity distribution inside the assay compartments for the side cut cavities shown in Figures 7C and 7D.

It is noted that the CPC configuration applied to the fiber sensor in the above description is illustrative, and other geometries, e.g., a CPC with a rectangular cross sectional

area, as well as other imaging or non-imaging geometries that also change the numerical aperture of the fiber tip may also be applied.

Embodiments of the invention are also directed to improved optical systems for interrogating fluorophore-labeled assays contained within optical sensors and, in particular,
5 fiber optical sensors of the types described above.

As noted previously, in typical interrogation systems (e.g., epifluorescence microscopy), fluorescence applications commonly operate with fairly intense excitation of the fluorophore in the absorption band of the fluorophore and detection of the weak fluorescence emitted by the fluorophore. In such applications, the dichroic beam splitter serves as a crude
10 filtering of the light, whereas the heavy filtering occurs in the excitation filter and the emission filter, which are used in transmission mode and depend on 10^6 times attenuation of wavelengths outside the pass band in a single pass. Such filters are normally based on dielectric multilayer filters consisting of a substrate with optical coatings on both sides consisting of up to 100 layers with alternating refractive indices. In the pass band, the filters
15 have up to 99% transmittance, which is most probably caused by reflection losses in the air-coating transition on both sides of the filter in spite of the fact that filter stacks on both sides of the substrate generally include an anti-reflective coating.

In accordance with embodiments of the present invention, an optical system may be used for interrogating a fluorophore-labeled assay either with, or without, an internal reference.
20 The inventive optical system is based on a filter substrate with one or multiple optical coatings separated in location on the surfaces of the substrate. The coatings may be dielectrical multilayer coatings forming short wave pass, longwave pass, band pass filters, and anti-reflection coatings. Furthermore, coatings may be metallic reflective coatings.

More specifically, embodiments of the present invention utilize the fact that a
25 dielectrical multilayer filter reflects what is not transmitted. Therefore, a filter in accordance with embodiments of the present invention may include a first coating on the filter substrate

which transmits a certain wavelength range of the excitation, but reflects wavelengths outside the pass band. This enables the emitted fluorescence to be reflected once or multiple times by the coating, while allowing reflected excitation light to be transmitted out of the optical system. The filtered fluorescence then exits the filter substrate when it reaches a part of the substrate
5 that is not coated with the first coating, and is picked up by one or more detectors.

Subsequent filtering may also be achieved by applying a second coating which transmits a desired wavelength range originating from a first fluorophore, but reflects wavelengths outside the transmission band – in particular, remains of the excitation light. In this way, the desired wavelength range will be transmitted out of the substrate where it may be
10 picked up by one or more suitable detectors. In addition, anti-reflective coatings may be applied to areas of the substrate where light is coupled into, or out of, the substrate to reduce reflection losses.

In an intensity interrogation configuration, where both a first fluorophore and a second fluorophore are used, the above-mentioned second coating may transmit the desired
15 wavelength range associated with the first fluorophore and reflect wavelengths outside the transmission band – in particular remains of the excitation light and fluorescence related to the second fluorophore. The filtered fluorescence originating from the second fluorophore then exits the filter substrate when it reaches a part of the substrate that is not coated with the first or second coating, such that it can be picked up by a suitable detector(s). Subsequent filtering of
20 fluorescence originating from the second fluorophore may be achieved by applying a second coating which transmits the desired wavelength range associated with the second fluorophore and reflects wavelengths outside the transmission band – in particular, remains of the excitation light. In this way, the desired wavelength range may be transmitted out of the substrate where it may be picked up by a suitable detector(s). In other embodiments, the
25 system may be expanded to include multiple light sources and/or multiple wavelength ranges to be detected.

Implementation of the above-described filter configurations requires imaging optical elements such as lenses, mirrors or diffractive optical elements to focus, pick up, and collimate light. Furthermore, apertures and light traps may be required to control light path and to absorb undesired wavelengths that are transmitted out of the filter substrate, such as, e.g., the excitation light to be blocked. In this embodiment, the above-mentioned optical elements and light traps are shown with an air gap between the elements and the (glass) substrate. However, the optical elements and light traps may also be formed in an optically transparent material with mirror coating and absorbing coating on the surface facing away from the filter substrate. Such a configuration is generally more advantageous, as the optical elements and light traps are better index matched to the filter substrate/filter coating than is the case when an air gap is present.

The above-mentioned elements may be individually aligned and fitted onto both sides of the coated filter substrate as discrete units. Similarly, one or more light sources and one or more detectors may be aligned and fitted onto the filter substrate as packaged units, as raw dies laminated directly onto the coated filter substrate, or mounted as raw dies onto a printed circuit board and mounted as a unit onto the coated filter substrate.

Suitable light sources may include, e.g., light emitting diodes (LEDs) and laser diodes, and suitable detectors may include, e.g., photodiodes, avalanche photodiodes, silicon photomultipliers, photomultipliers, and phototransistors. In addition, the assembled optical system may be coated or placed in an enclosure to block out ambient light.

A stacked planar integrated optical system (SPIOS) for interrogating a single fluorophore in accordance with an embodiment of the invention is shown in Figure 11. With reference to Figures 11 and 12, a LED 510 emits light into a filter substrate 500 with a wavelength range overlapping the absorption spectrum of the fluorophore to be interrogated. The LED output is limited to a certain wavelength range by an excitation filter 520 before entering the filter substrate 500. The filtered excitation light exits the filter substrate 500

through an identical excitation filter on the opposite side of the filter substrate and is collimated by a first mirror 530. The collimated excitation light passes through the filter substrate to reach a second mirror 540 and is thereby focused onto a sensor 590 through an optical window 550. It is noted that, in this embodiment, the sensor 590 is a fiber optical sensor--including an assay in an assay compartment 595--of the types described above in connection with Figures 5-10.

Traveling through the sensor 590, the excitation light 591 reaches the assay compartment 595, where it excites the fluorophore in the assay such that the fluorophore emits fluorescence 593. Furthermore, excitation light is reflected and back scattered from the optical window and sensor. The fluorescence 593 and reflected/back scattered excitation light are picked up and collimated by the second mirror 540 and enter the filter substrate 500 through an uncoated or anti-reflection coated area 503 on the filter substrate. The emitted fluorescence is reflected between the two coated surfaces (i.e., the excitation filters 520) while the excitation light is transmitted through the coatings 520 and absorbed by the light traps 560. The filtered fluorescence exits the filter substrate 500 where the coated area ends 507 and is focused by a third mirror 570 onto a detector 580 through an uncoated, or anti-reflection coated, region of the filter substrate.

In an alternative embodiment, a stacked planar integrated optical system (SPIOS) for interrogating a sensor with an assay fluorophore and a reference fluorophore is shown in Figure 13. With reference to Figures 13 and 14, a LED 510 emits light into a filter substrate 500 with a wavelength range overlapping the absorption spectrum of the two fluorophores to be interrogated. The LED output is limited to a certain wavelength range by an excitation filter 520 before entering the filter substrate 500. The filtered excitation light exits the filter substrate 500 through an identical excitation filter on the opposite side of the filter substrate and is collimated by a first mirror 530. The collimated excitation light passes through the filter substrate to reach a second mirror 540 and is thereby focused onto a sensor 590 through an

optical window 550. It is noted that, in this embodiment, the sensor 590 is a fiber optical sensor--including an assay in an assay compartment 595--of the types described above in connection with Figures 5-10.

Traveling through the sensor 590, the excitation light 591 reaches the assay
5 compartment 595, where it excites the fluorophore in the assay such that the fluorophore emits fluorescence 593. Furthermore, excitation light is reflected and back scattered from the optical window and sensor. The fluorescence 593 and reflected/back scattered excitation light are picked up and collimated by the second mirror 540 to enter the filter substrate 500 through an uncoated or anti-reflection coated area 503 on the filter substrate. The emitted fluorescence is
10 reflected between the two excitation filter coatings 520 while the excitation light is transmitted through the coatings and absorbed by the light traps 560.

The filtered fluorescence exits the filter substrate 500 where the excitation filter coatings end 507, and an emission filter 525 transmits a wavelength range relating to a first fluorophore (the assay fluorophore). The filtered fluorescence from the first fluorophore is
15 focused by a third mirror 570 onto a first detector 580, while the fluorescence associated with the second fluorophore (the reference fluorophore) is reflected between the emission filter 525 on the two sides of the filter substrate 500. The filtered fluorescence from the second fluorophore exits the filter substrate 500 where the coated area ends 509 or is replaced with an anti-reflection coating, and is focused by a fourth mirror 575 onto a second detector 585
20 through an uncoated, or anti-reflection coated, region of the filter substrate.

As noted previously, the optical elements, apertures, and light traps of the SPIOS for interrogating a sensor with an assay fluorophore and a reference fluorophore may be disposed with an air gap between the elements and the (glass) substrate. See Figure 13. However, the optical elements and light traps may also be formed in an optically transparent material with
25 mirror coating and absorbing coating on the surface facing away from the filter substrate. The latter configuration is generally more advantageous, as the optical elements and light traps are

better index matched to the filter substrate/filter coating than is the case when an air gap is present. An example of such a configuration is shown in Figure 15, where connecting material 1501 is provided between the optical components.

It is noted that the light emitting area of a LED chip has an area, which is comparable to a 500 μm multimode fiber. Furthermore, the LED chip emits in a large angular space. For a SPIOS equipped with a LED interrogating a fiber sensor, the fluorescence output gradually becomes limited by the ability to focus light from the LED onto the fiber when the fiber diameter is reduced. Also the positioning of the proximal end of the fiber relative to the optical system becomes more critical.

As shown in Figures 16A and 16B, the ability to couple light from the LED into the fiber sensor may be enhanced by placing a compound parabolic concentrator (CPC) 1505 in the interface between the SPIOS and the fiber sensor 1590 if the numerical aperture of the SPIOS in relation to the fiber is smaller than the numerical aperture of the fiber itself. The CPC may then be used to match the numerical aperture of the fiber and thereby reduce the spot size of the focused excitation light originating from the LED. This, in turn, enables more light to be coupled from the LED into the fiber.

The CPC may be an integral part of the SPIOS, as shown, e.g., in Figure 16A. However, the CPC may also be formed on the proximal end of the fiber, whereby the area to be targeted by the SPIOS is increased, thus reducing the requirements on the positioning of the fiber (with CPC at the proximal end) relative to the SPIOS.

In yet another embodiment shown in Figure 17, the inventive optical interrogating system may be designed to be manufactured as a wafer-scale stacked planar integrated optical system, or wafer-scale SPIOS (also referred to as a "Wafer Scale Optical System" or a "Wafer Level Optical System"). As shown in Figure 17, the SPIOS includes various layers that are stacked and aligned. In the wafer layer 610, one or more light sources (e.g., LEDs and photodiodes) and detectors may be laid out on a wafer. Alternatively, they may be naked chips

(e.g., sold by Avago Technologies or Hamamatsu), which are individually aligned and laminated onto the SPIOS units.

One or more optical layers 620 may include mirrors and absorbers laid out on a wafer-sized injection molded disk. Mold inserts defining optical surfaces are made by a diamond turning/milling company (e.g., Kaleido Technology in Denmark). Gold or protected silver is applied to mirror surfaces, e.g., by sputtering, while any absorbers are masked off during the process.

The optical filter layer 630 includes a wafer-sized glass substrate with optional coatings. Specifically, multilayer optical coatings may be applied on both sides of the glass substrate using ion-assisted sputtering to form durable coatings. The technique is similar to that used in manufacturing fluorescence filters by, e.g., Semrock in the United States and Delta in Denmark.

As shown in Figure 17, in one embodiment, a wafer layer 610 may be followed by an optical layer 620, an optical filter layer 630, and another optical layer 620. The entire stack is then thoroughly aligned and laminated, e.g., by gluing, and the connections are bonded onto the chips. The stack is then diced 640 using, e.g., a diamond saw to form multiple assembled SPIOS units 670.

The above-described system may be made small and is suitable for large-scale production. The system may be used for interrogating a sensor in a light scattering environment, such as a sensor implanted into the skin, as well as a fiber sensor. Coating or packaging may be used to block out ambient light.

While the description above refers to particular embodiments of the present invention, it will be understood that many modifications may be made without departing from the spirit thereof. The accompanying claims are intended to cover such modifications as would fall within the true scope and spirit of the present invention.

The presently disclosed embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

WHAT IS CLAIMED IS:

1. An optical glucose sensor comprising:
 - an optical fiber having a proximal end and an opposing distal end;
 - a glucose-permeable membrane having a hollow interior, an open proximal end, and a
 - 5 closed distal end, wherein the membrane's proximal end is coupled to the optical fiber's distal end so as to define a compartment in said hollow interior between the optical fiber's distal end and the membrane's distal end; and
 - a competitive glucose binding affinity assay disposed in said compartment, the assay including a glucose receptor and a glucose analog.
- 10 2. The optical glucose sensor of claim 1, wherein the compartment is placed within a user's tissue.
3. The optical glucose sensor of claim 2, wherein said proximal end of the optical fiber is external to the user's body.
4. The optical glucose sensor of claim 3, wherein the proximal end of the optical fiber is
- 15 optically coupled to an assay interrogating system.
5. The optical glucose sensor of claim 4, wherein the interrogating system is a stacked planar integrated optical system.
6. The optical glucose sensor of claim 2, wherein the proximal end of the optical fiber is optically coupled to an assay interrogating system.
- 20 7. The optical glucose sensor of claim 6, wherein the interrogating system is an optical interrogating system.
8. The optical glucose sensor of claim 1, wherein said assay is a fluorophore labeled assay.
9. The optical glucose sensor of claim 1, wherein said glucose receptor is labeled with a
- 25 first fluorophore.

10. The optical glucose sensor of claim 9, wherein the assay further includes a reference fluorophore different from said first fluorophore.

11. The optical glucose sensor of claim 10, wherein said first fluorophore and said reference fluorophore have either different absorption spectra, or different emission spectra, or
5 both.

12. The optical glucose sensor of claim 10, wherein said first fluorophore is Alexa Fluor 594 (AF594).

13. The optical glucose sensor of claim 10, wherein said reference fluorophore is Alexa Fluor 700 (AF700).

10 14. The optical glucose sensor of claim 10, wherein said reference fluorophore is labeled onto a macro molecule.

15. The optical glucose sensor of claim 1, wherein said glucose receptor is selected from the group consisting of Concanavalin A, glucose galactose binding protein, an antibody, Boronic Acid, and Mannan Binding Lectin (MBL).

15 16. The optical glucose sensor of claim 1, wherein the glucose analog is dextran.

17. The optical glucose sensor of claim 1, wherein the glucose analog is labeled with a dye.

18. The optical glucose sensor of claim 1, wherein the glucose receptor is labeled with a fluorophore, the glucose analog is labeled with a dye, and said fluorophore and dye form a Förster Resonance Energy Transfer pair.

20 19. The optical glucose sensor of claim 1, wherein the glucose receptor is Mannan Binding Lectin (MBL), and the glucose analog is dextran.

20. The optical glucose sensor of claim 19, wherein the Mannan Binding Lectin (MBL) is labeled with an Alexa Fluor fluorophore.

21. The optical glucose sensor of claim 20, wherein the fluorophore is water soluble.

22. The optical glucose sensor of claim 20, wherein the fluorophore is Alexa Fluor 594 (AF594).
23. The optical glucose sensor of claim 19, wherein the dextran is labeled with a dye.
24. The optical glucose sensor of claim 23, wherein the dye is water soluble.
- 5 25. The optical glucose sensor of claim 23, wherein the dye is hexamethoxy crystalviolet-1 (HMCV1).
26. The optical glucose sensor of claim 1, wherein the glucose receptor is Mannan Binding Lectin (MBL) labeled with Alexa Fluor 594 (AF594), and the glucose analog is dextran labeled with hexamethoxy crystalviolet-1 (HMCV1).
- 10 27. The optical glucose sensor of claim 26, wherein the assay further includes a macro molecule labeled with Alexa Fluor 700 (AF700) as a reference fluorophore.
28. The optical glucose sensor of claim 1, wherein said membrane is tube-shaped.
29. The optical glucose sensor of claim 1, wherein said membrane comprises a biocompatible polymer.
- 15 30. The optical glucose sensor of claim 29, wherein said polymer is biodegradable.
31. The optical glucose sensor of claim 1, wherein the membrane's open proximal end is sealably fitted over the optical fiber's distal end.
32. The optical glucose sensor of claim 1, wherein the distal end of the optical fiber is straight cut and polished.
- 20 33. The optical glucose sensor of claim 1, wherein the distal end of the optical fiber is in direct contact with the assay.
34. The optical glucose sensor of claim 1, wherein the distal end of the optical fiber is in the shape of a compound parabolic concentrator.

35. The optical glucose sensor of claim 34, wherein said compound parabolic concentrator extends into said compartment and is in direct contact with the assay in the compartment.
36. The optical glucose sensor of claim 34, further including a reflector disposed longitudinally spaced apart from a distal end of the compound parabolic concentrator.
- 5 37. The optical glucose sensor of claim 36, wherein the reflector reflects fluorescence emitted from the assay towards the distal end of the optical fiber.
38. The optical glucose sensor of claim 36, wherein the reflector is a concave mirror.
39. The optical glucose sensor of claim 36, wherein the assay is disposed in a space between the reflector and the distal end of the compound parabolic concentrator.
- 10 40. The optical glucose sensor of claim 34, wherein the compound parabolic concentrator has a rectangular cross-section.
41. The optical glucose sensor of claim 34, wherein the optical fiber and the compound parabolic concentrator have circular cross-sections.
42. The optical glucose sensor of claim 1, wherein the sensor is implanted within a user's
15 body.
43. The optical glucose sensor of claim 42, wherein the proximal end of the optical fiber is optically coupled to an assay interrogating system.
44. The optical glucose sensor of claim 43, wherein the interrogating system is an optical interrogating system.
- 20 45. The optical glucose sensor of claim 44, wherein the interrogating system is a stacked planar integrated optical system.
46. The optical glucose sensor of claim 1, wherein the sensor is biodegradable.

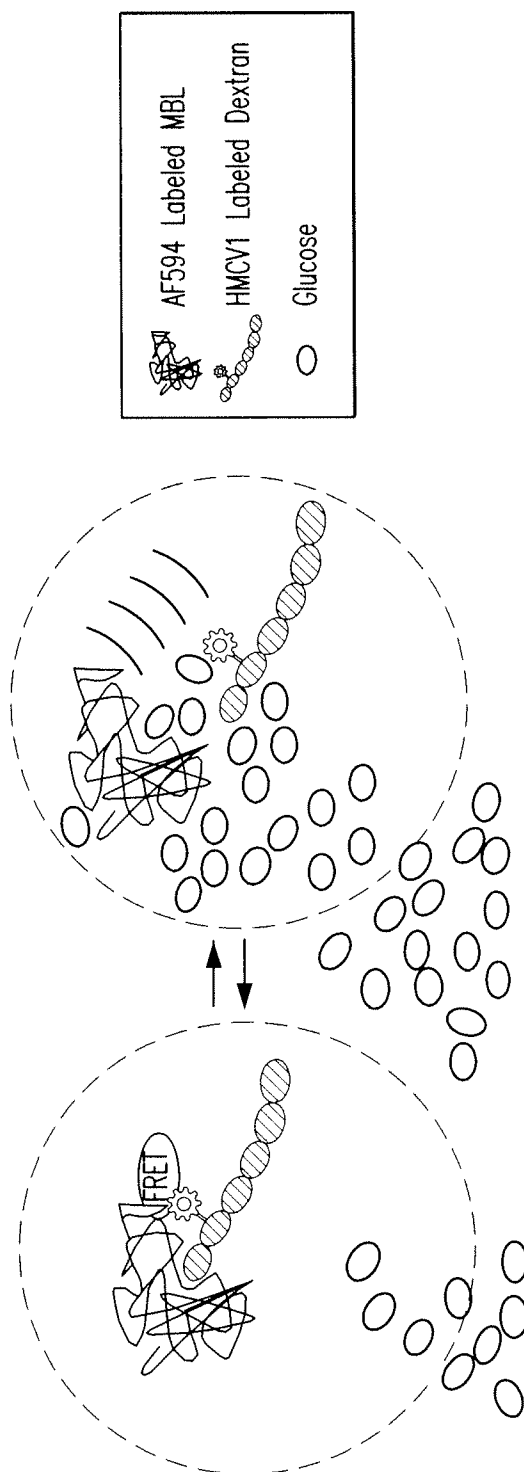


FIG. 1

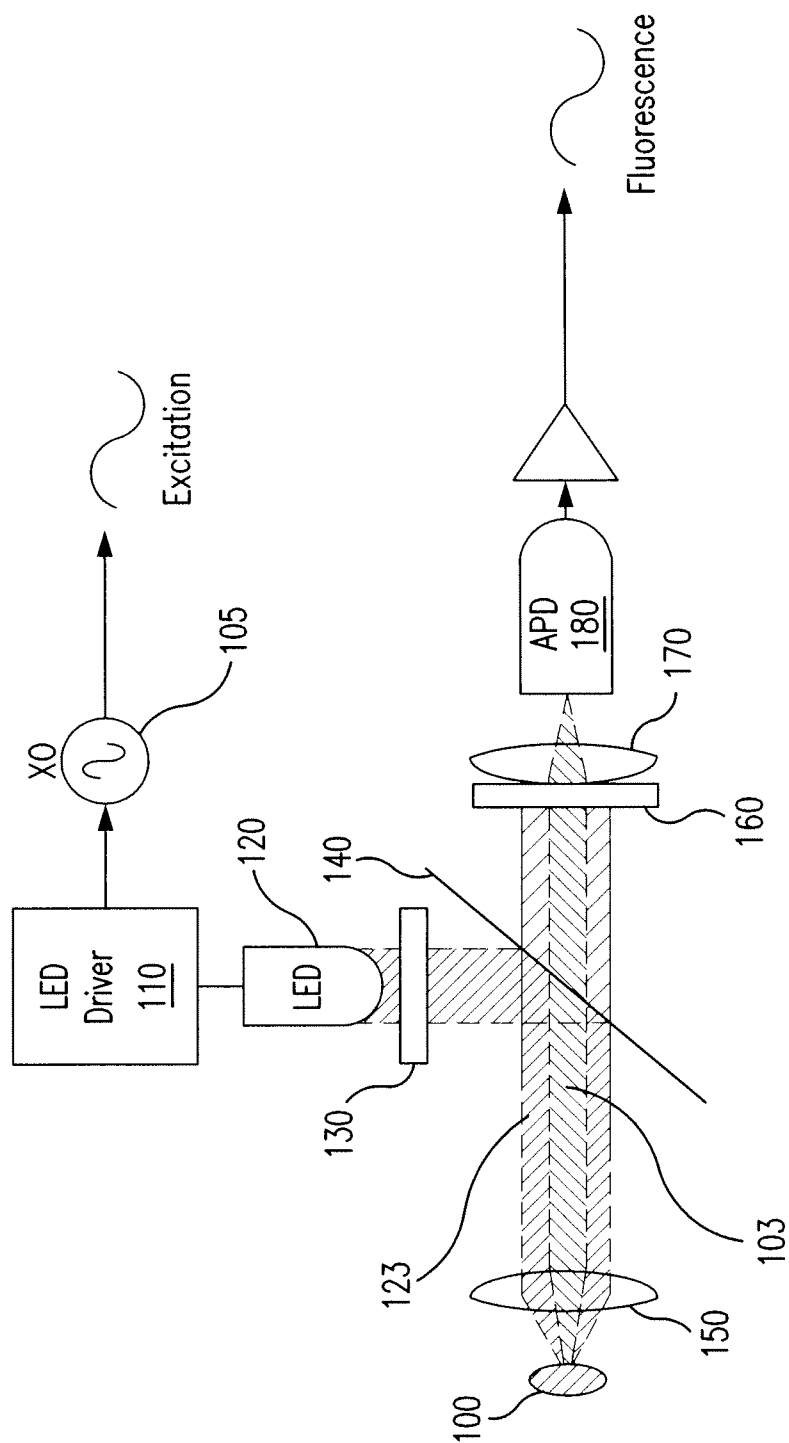


FIG. 2

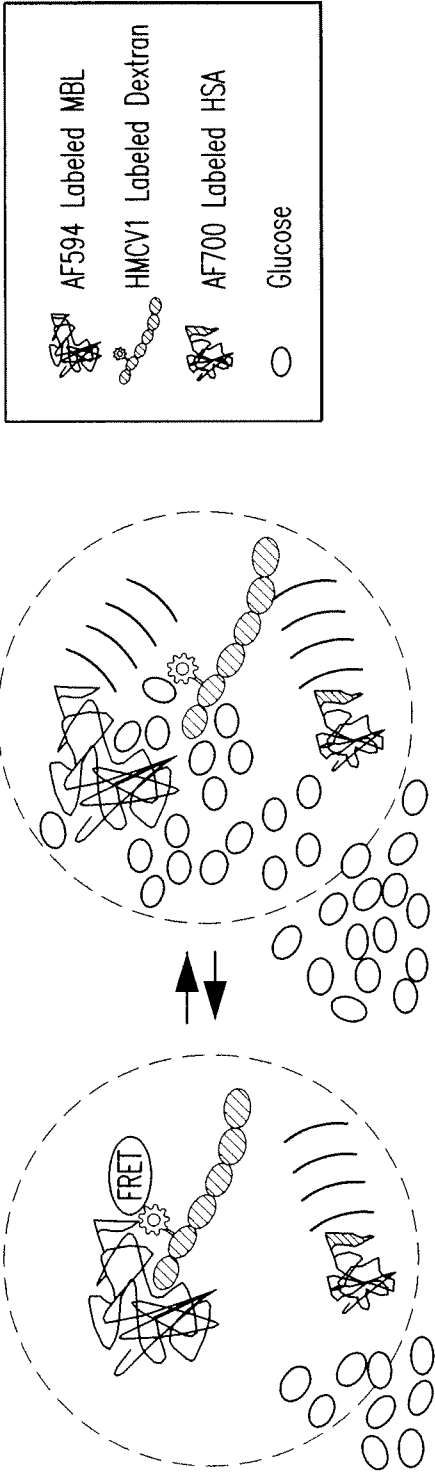


FIG. 3

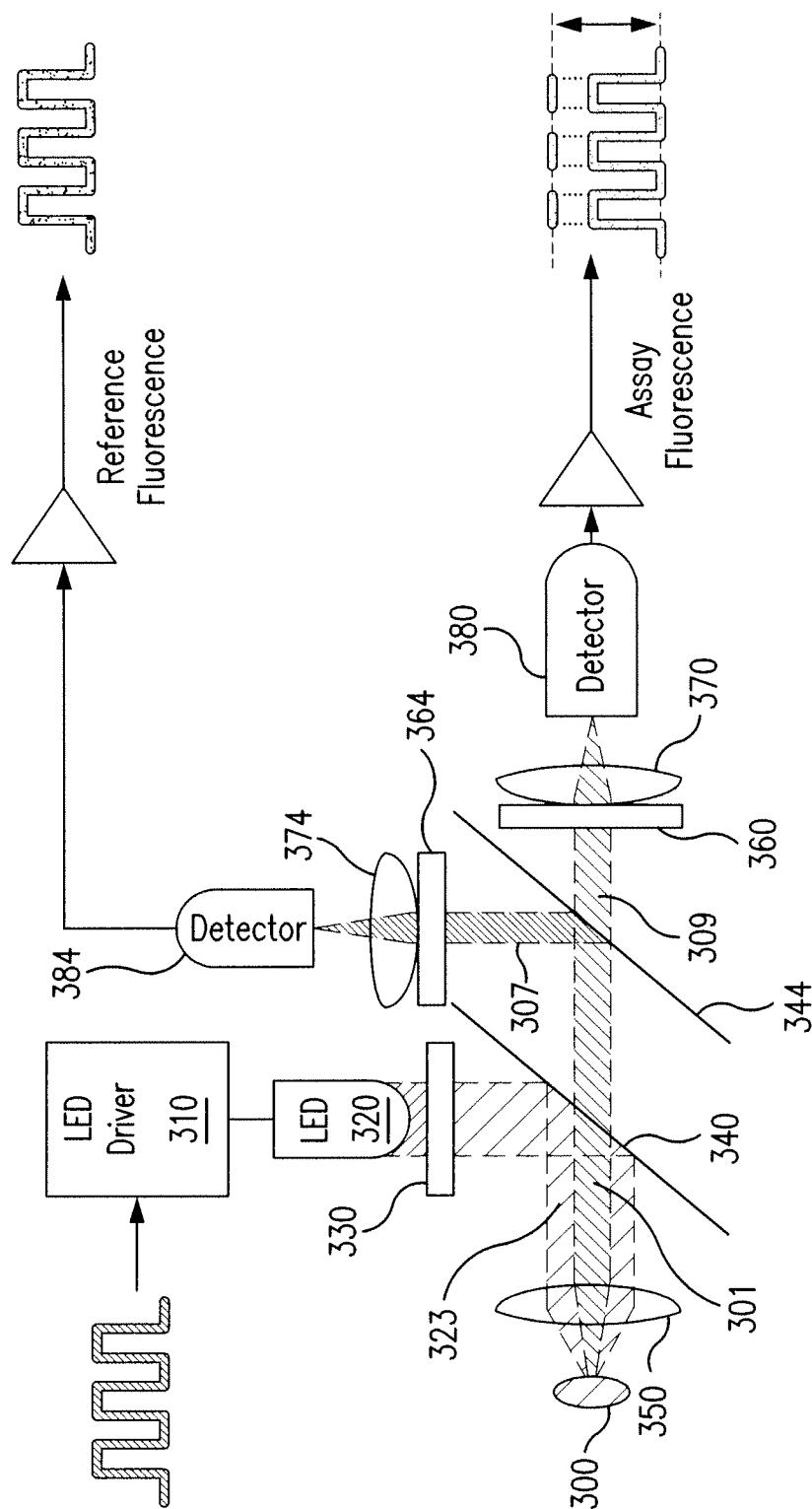


FIG. 4A

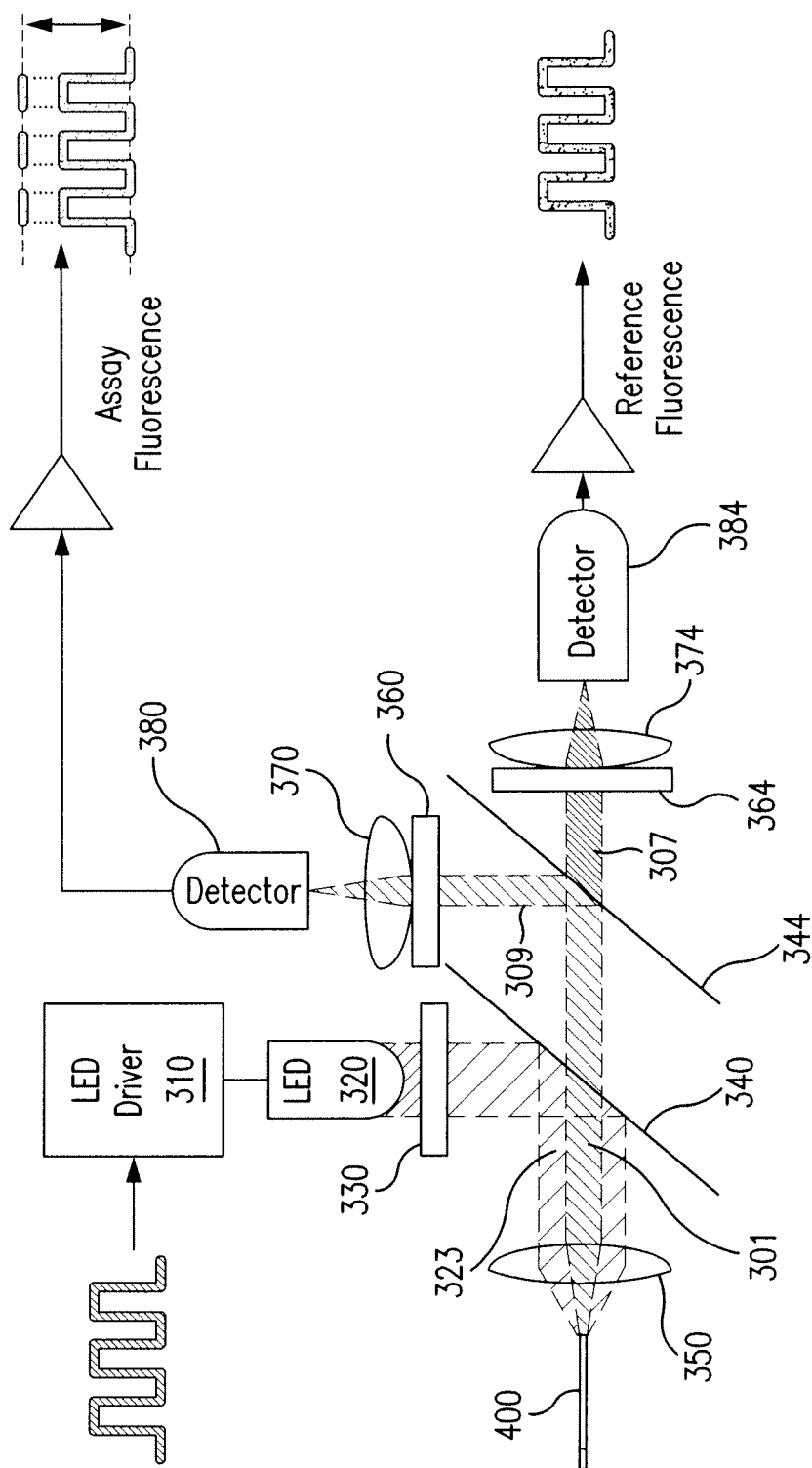


FIG. 4B

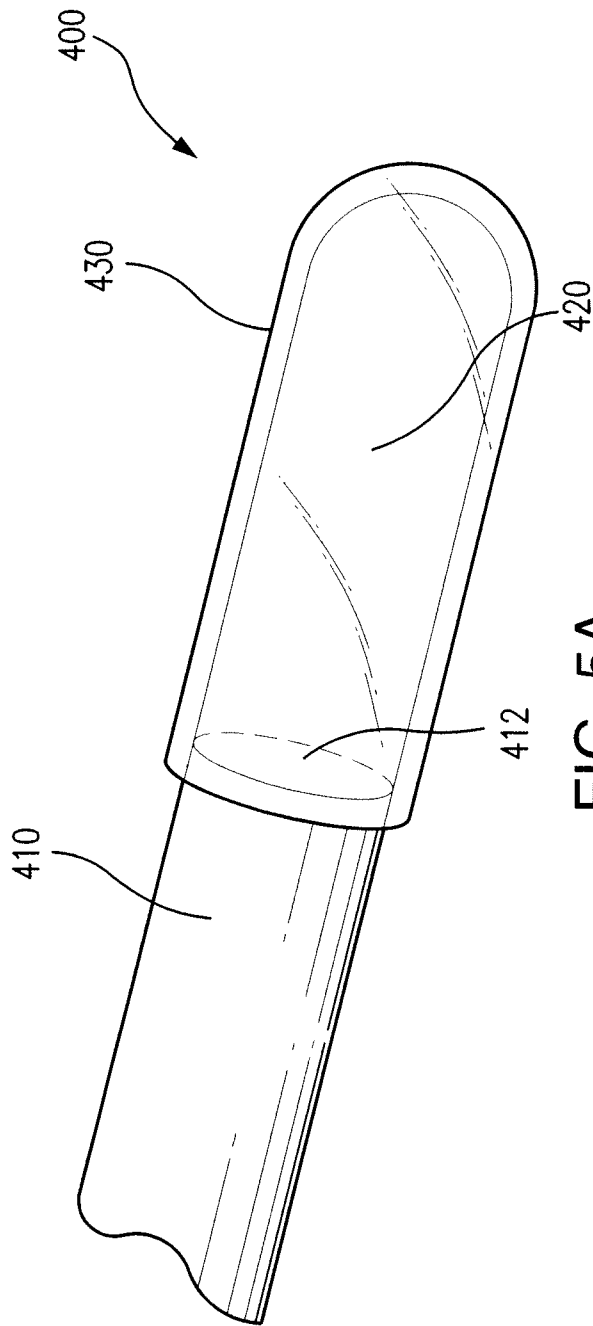


FIG. 5A

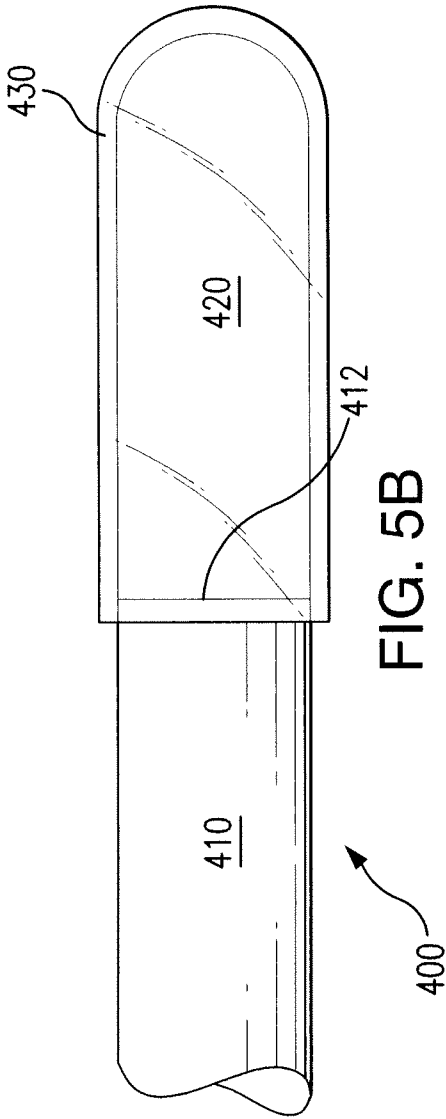
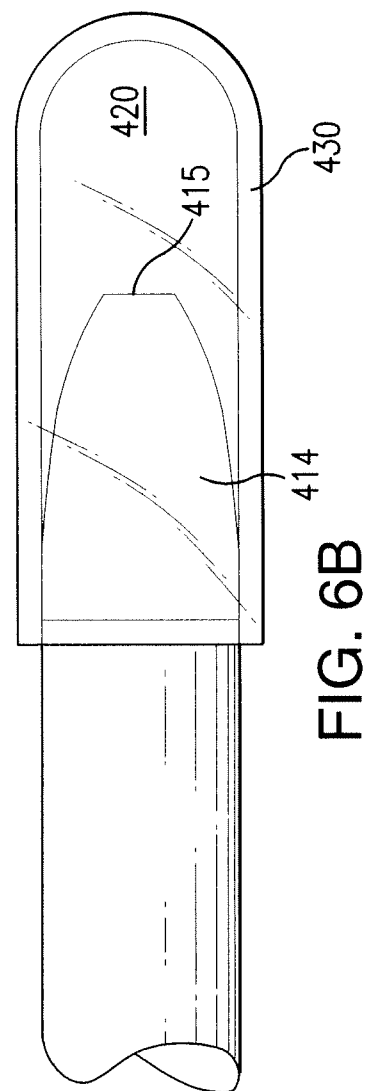
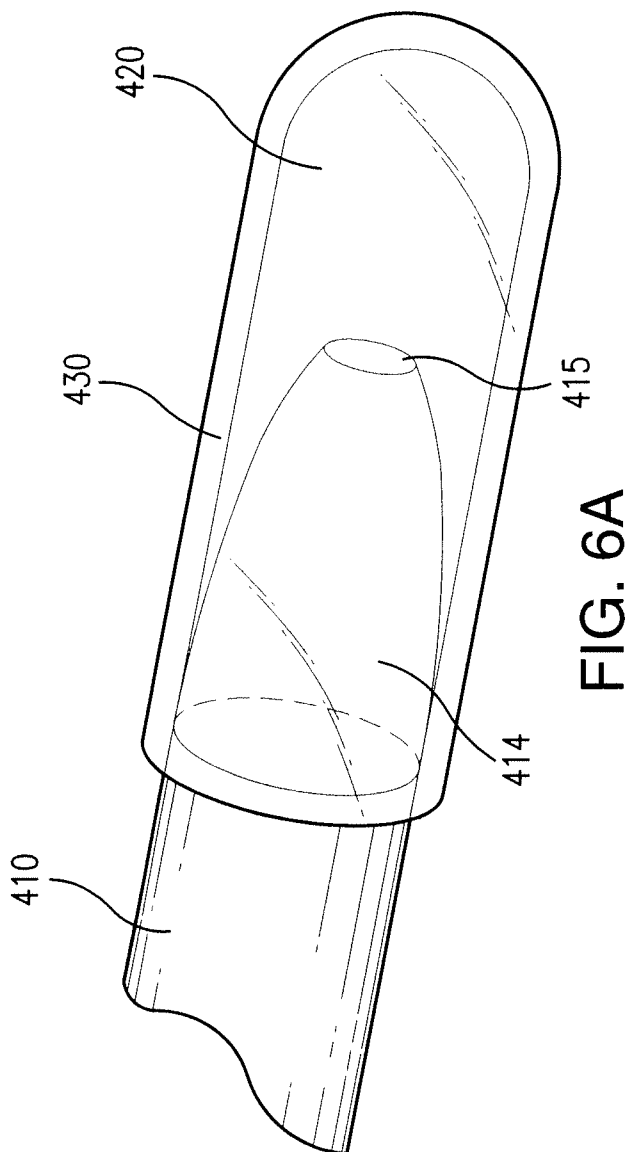
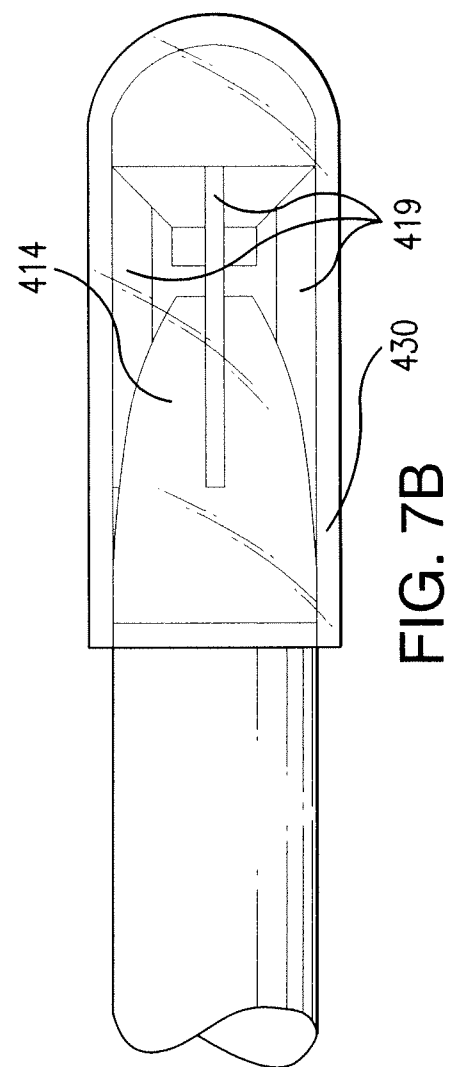
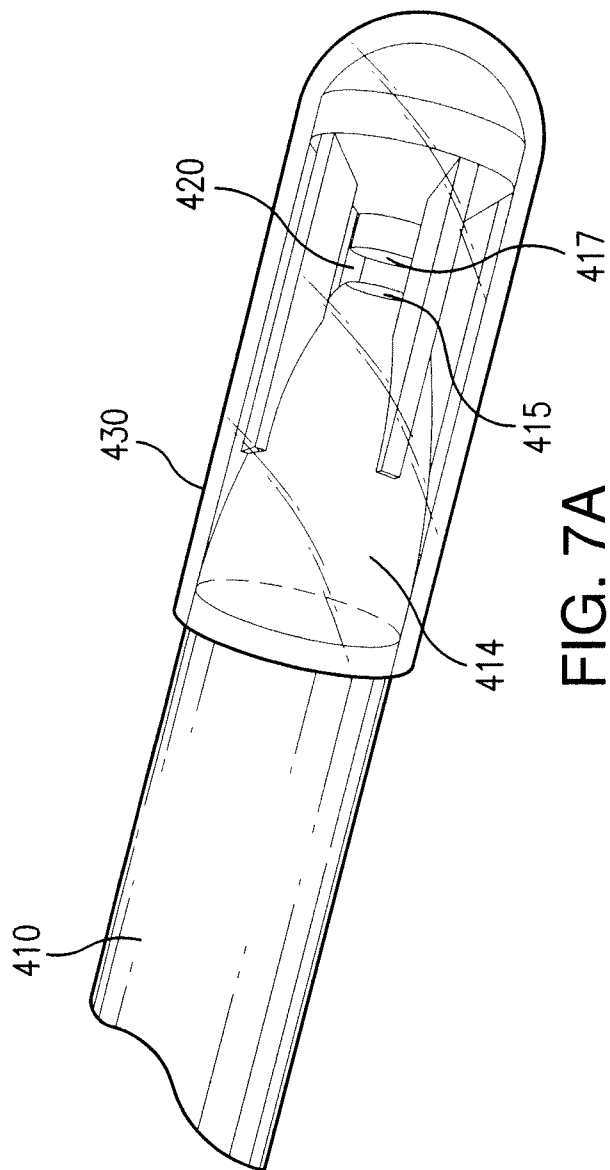
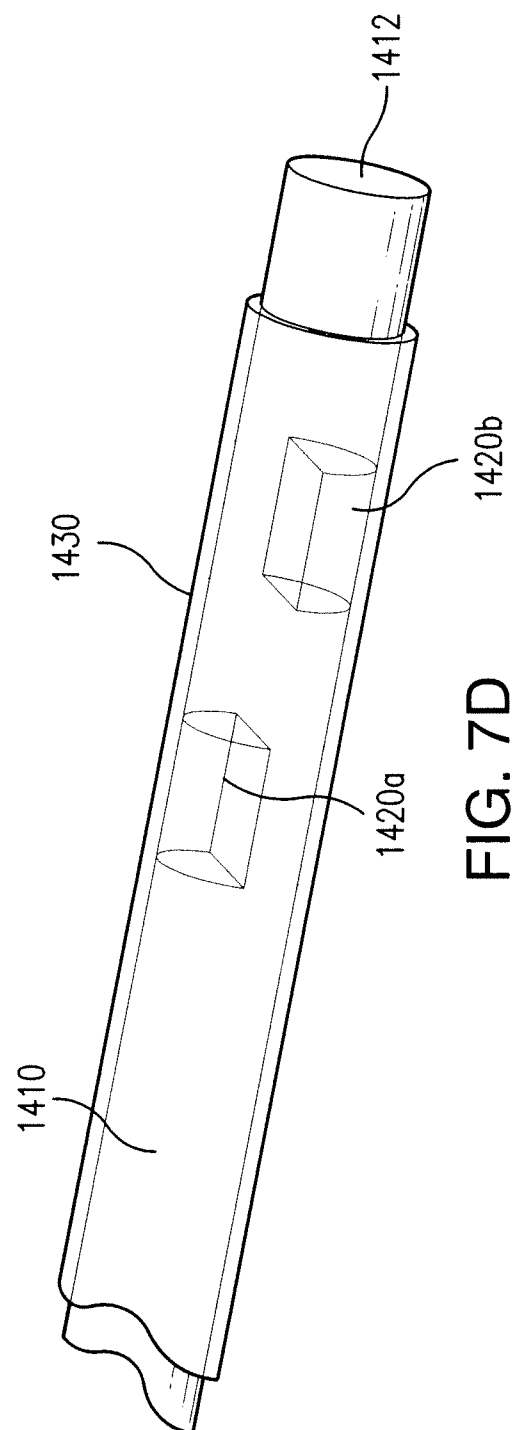
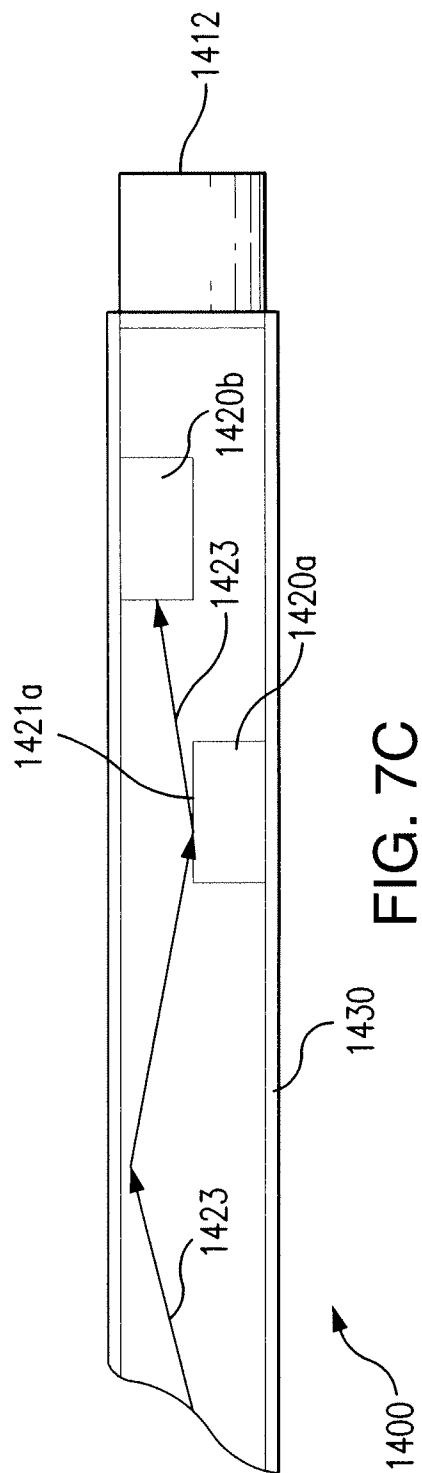


FIG. 5B







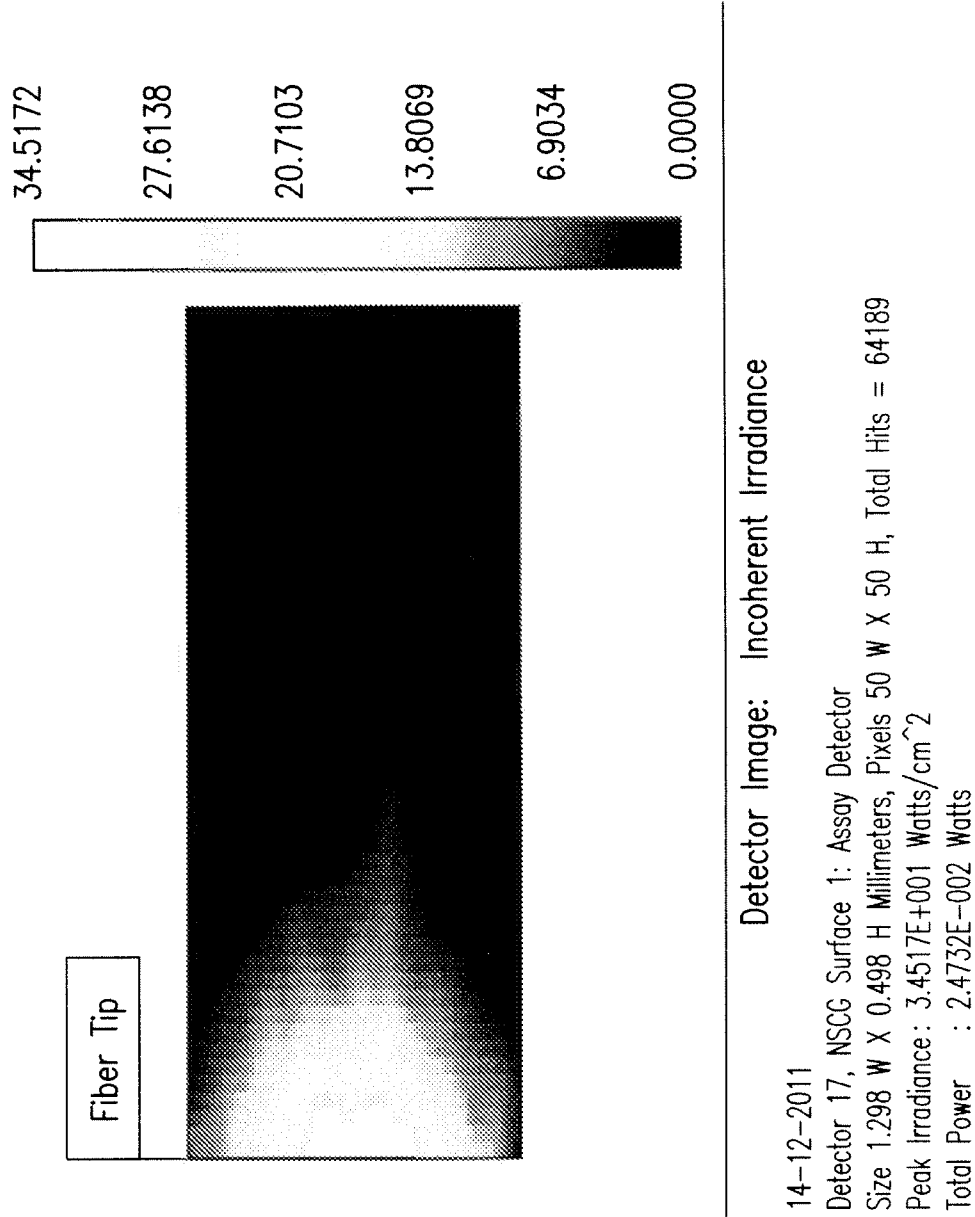
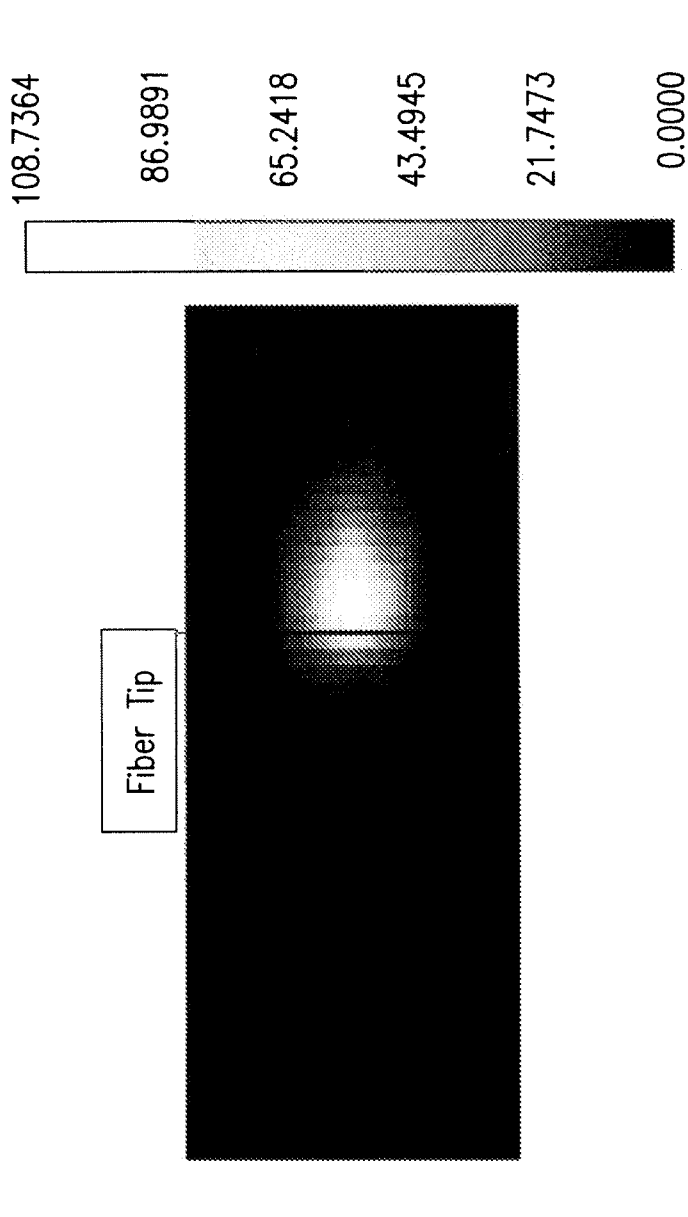


FIG. 8



Detector Image: Incoherent Irradiance

14-12-2011
Detector 17, NSCG Surface 1: Assay Detector
Size 1.298 W X 0.498 H Millimeters, Pixels 50 W X 50 H, Total Hits = 100530
Peak Irradiance: 1.0874E+002 Watts/cm²
Total Power : 2.9066E-002 Watts

FIG. 9

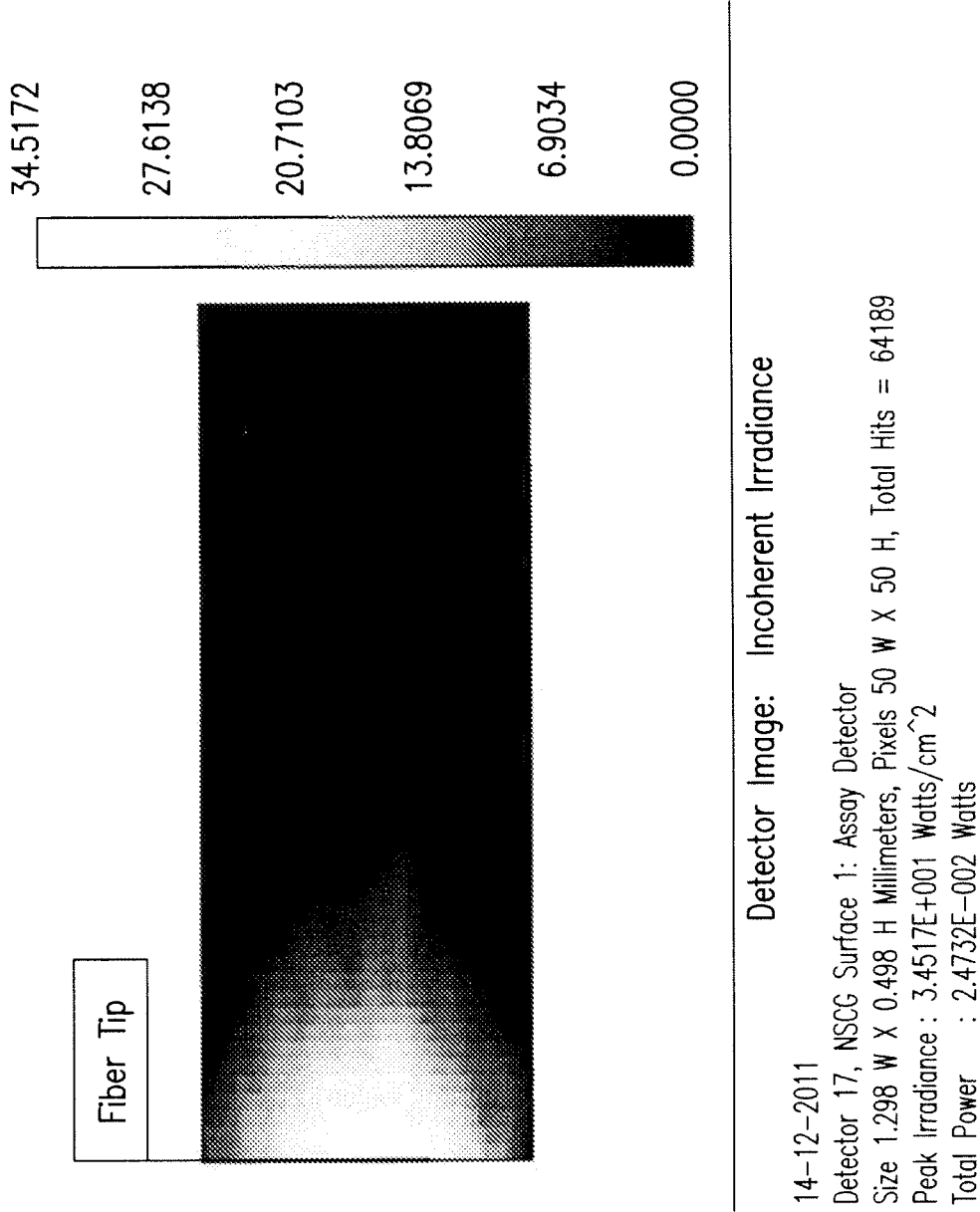
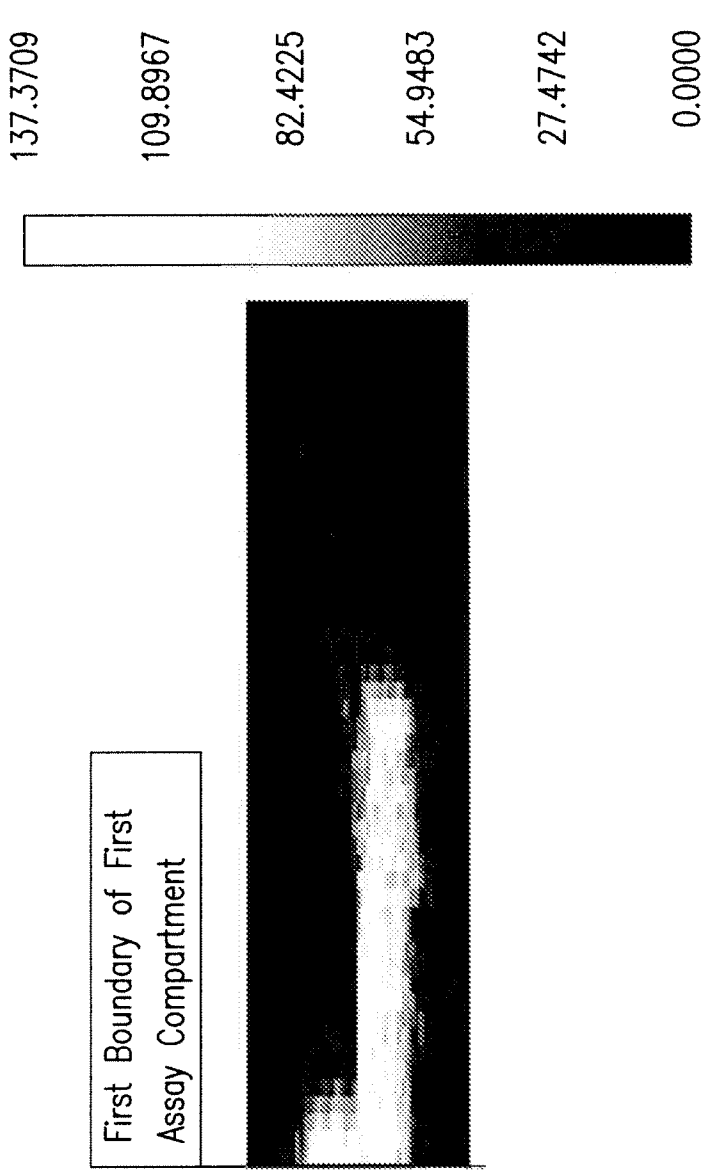


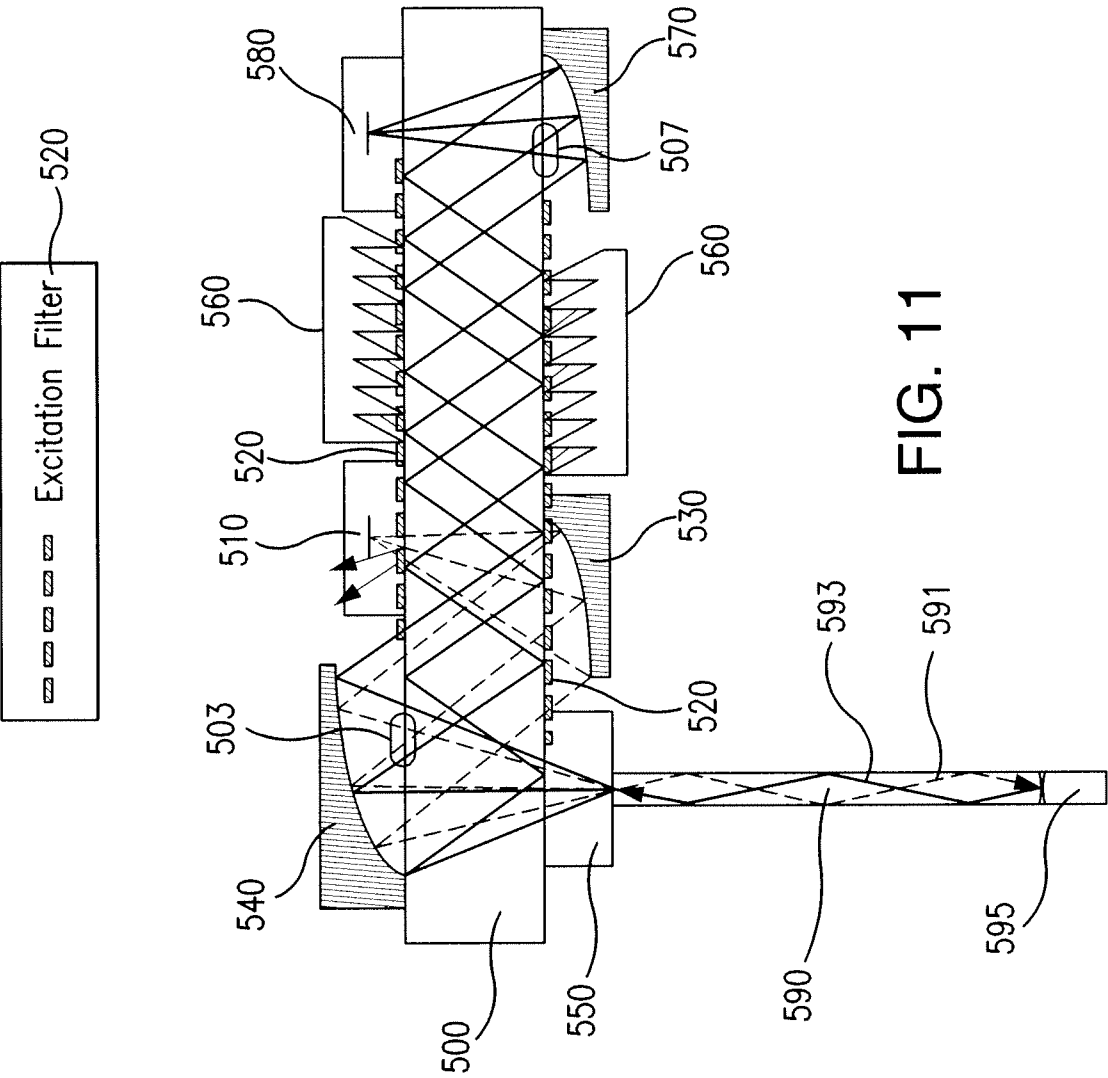
FIG. 10A

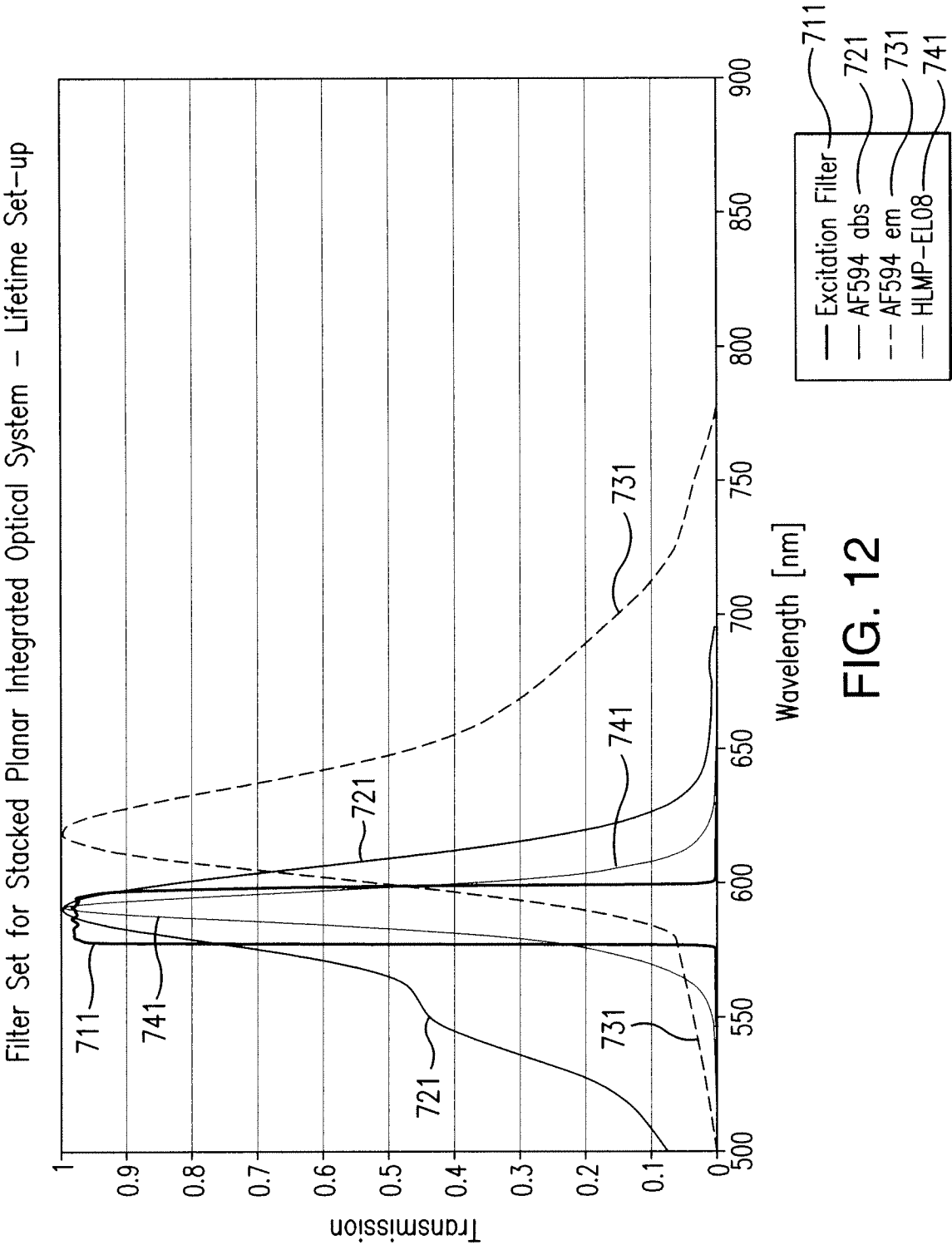


Detector Image: Incoherent Irradiance

11-05-2012
Detector 26, NSCG Surface 1: Assay Detector
Size 2.000 W X 0.500 H Millimeters, Pixels 50 W X 50 H, Total Hits = 2837379
Peak Irradiance : 1.3737E+002 Watts/cm²
Total Power : 2.7147E-001 Watts

FIG. 10B





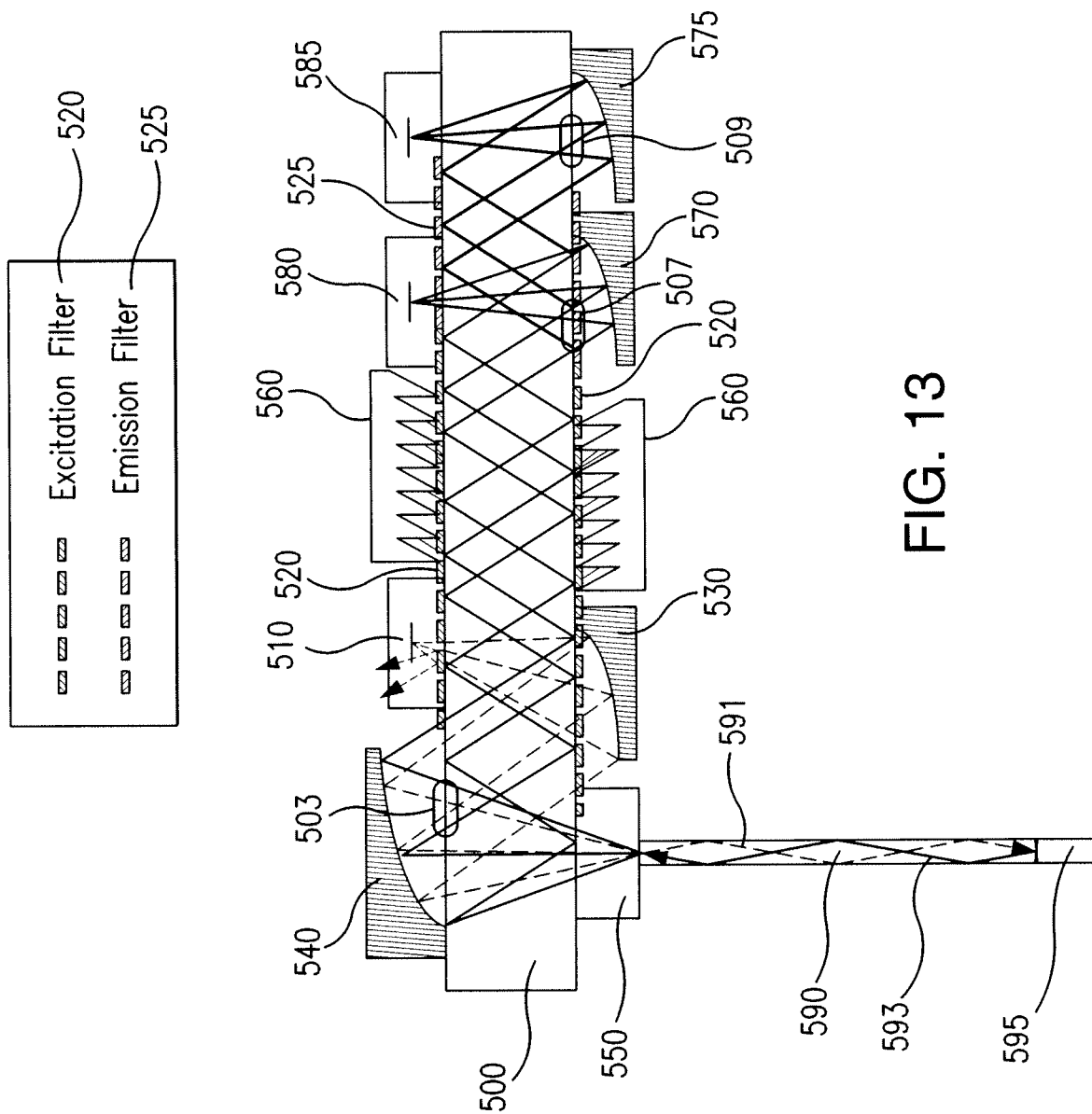


FIG. 13

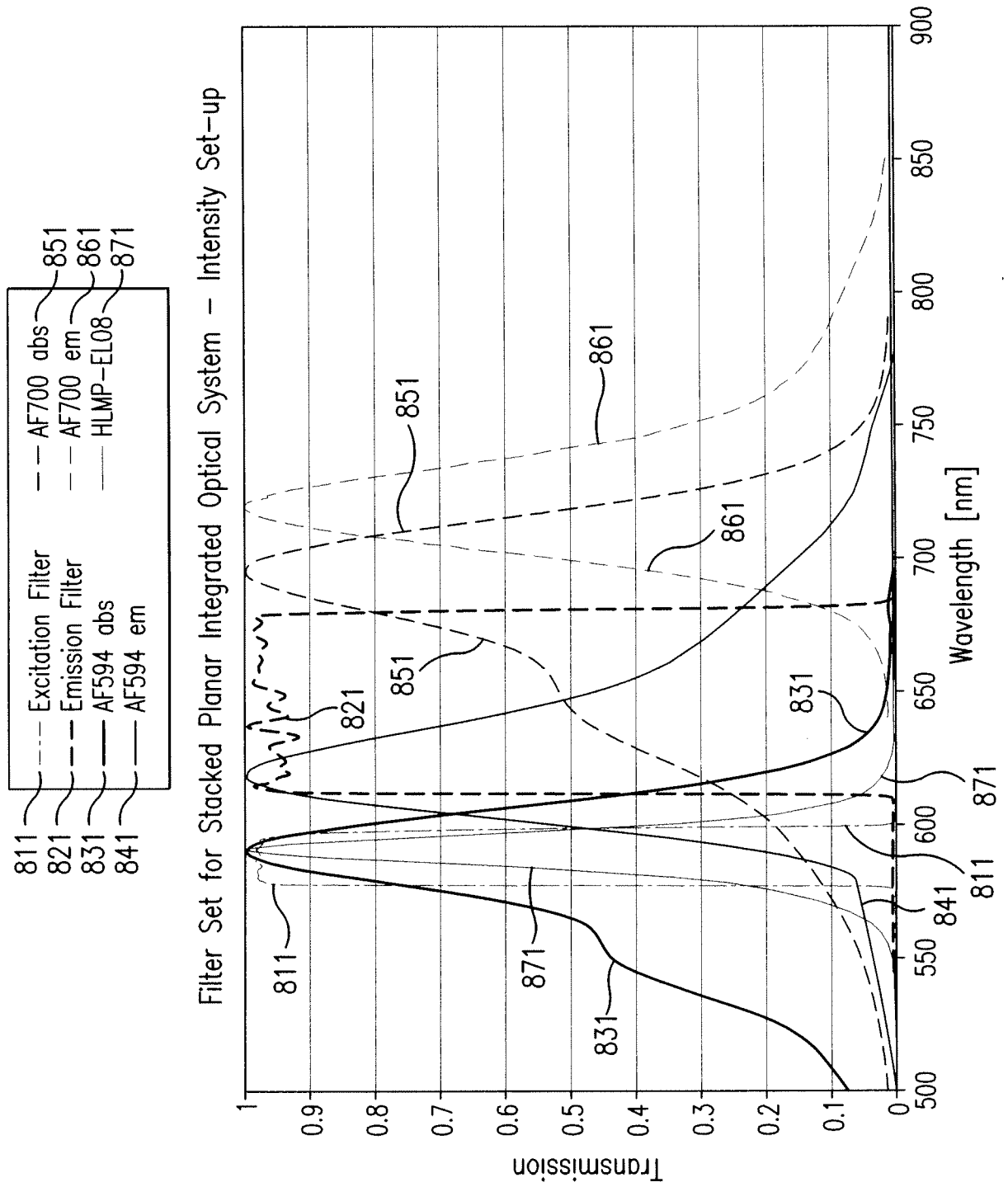
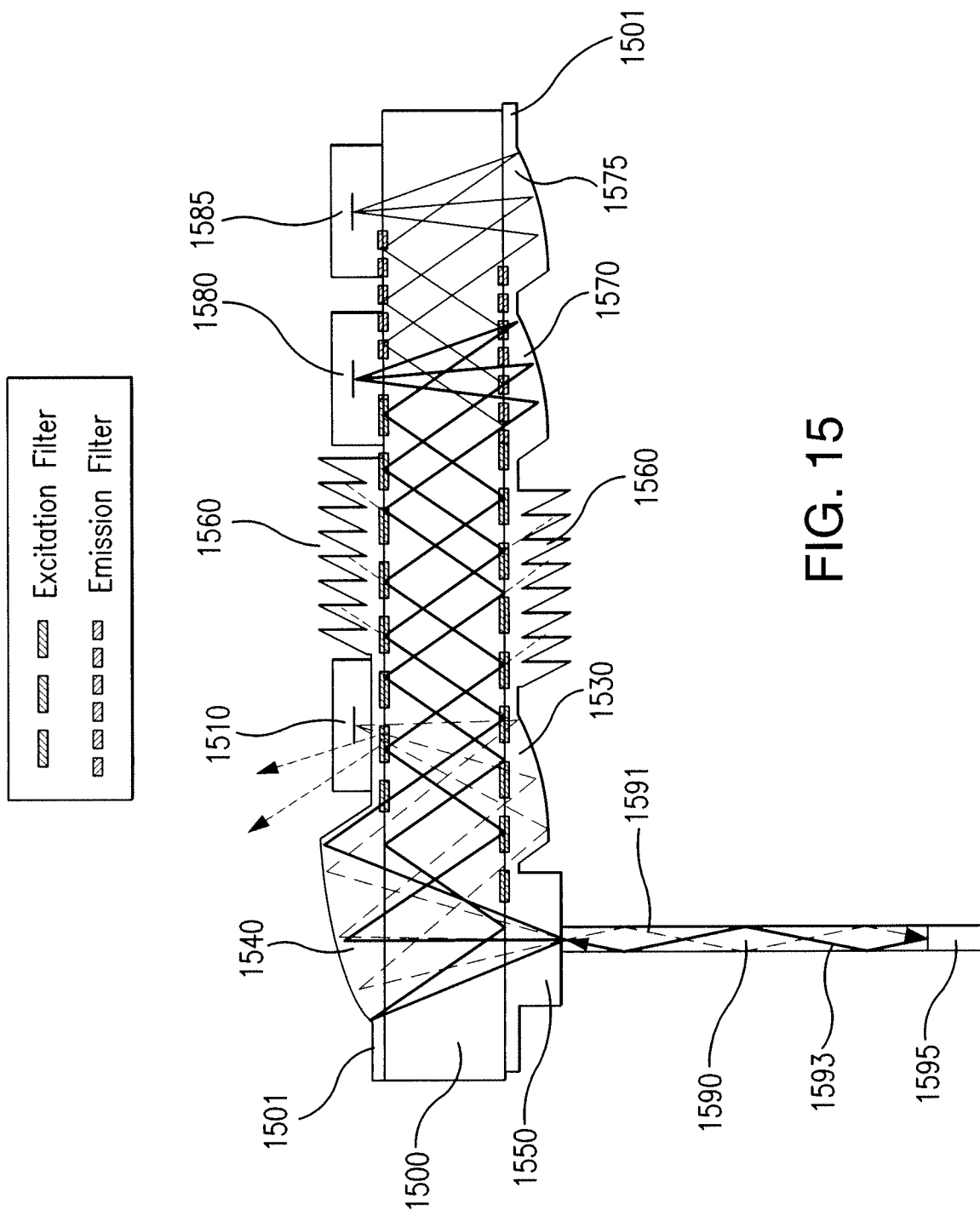


FIG. 14



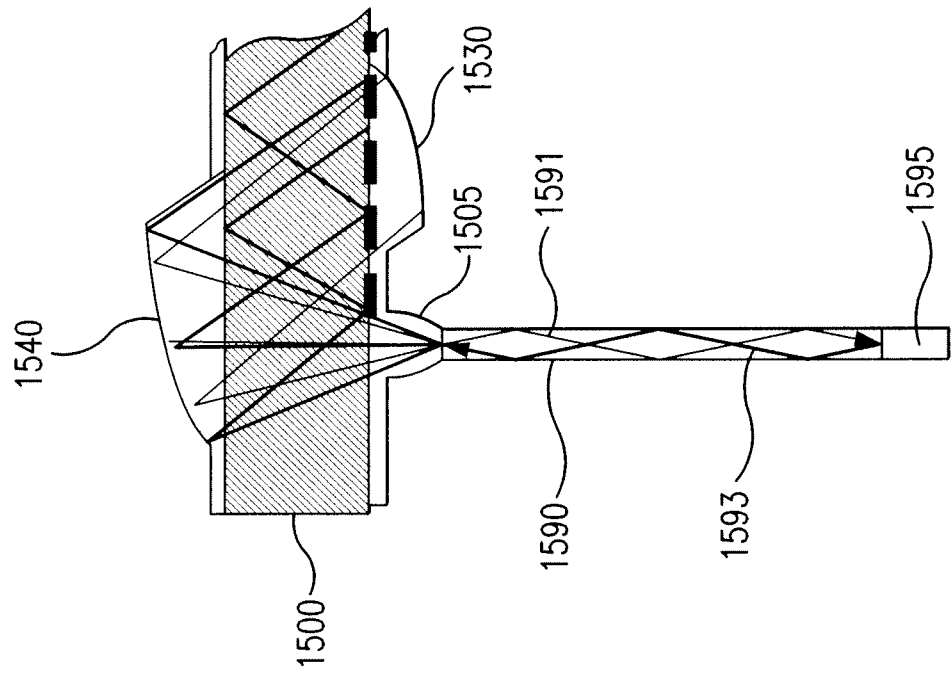


FIG. 16A

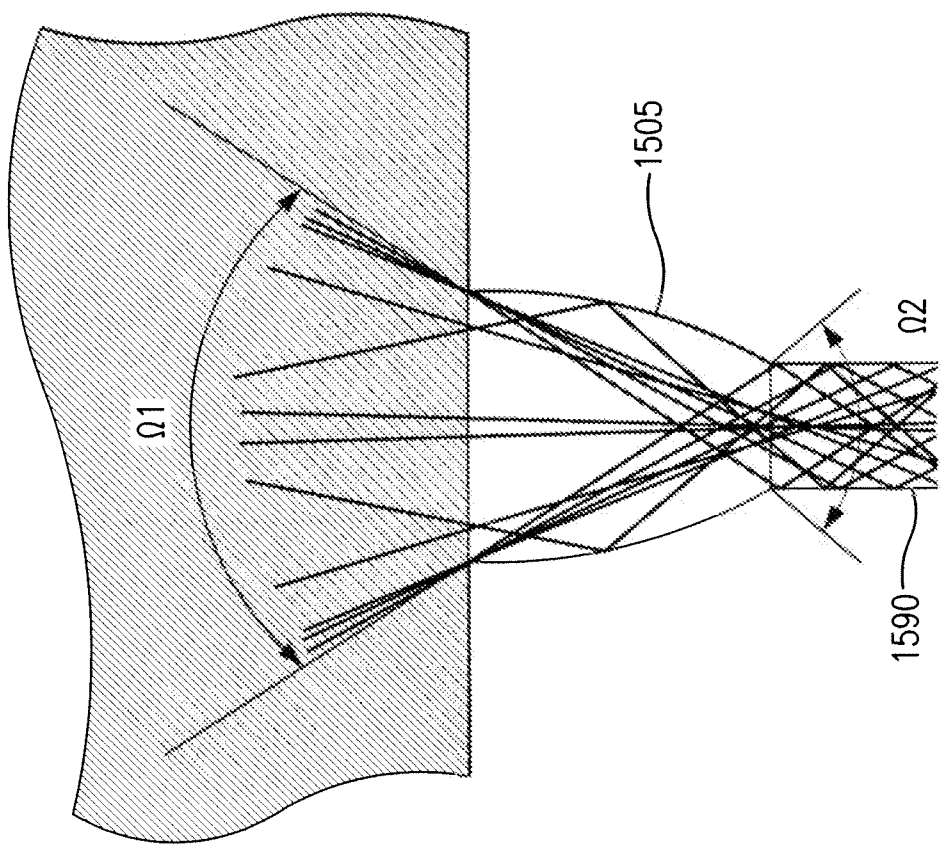


FIG. 16B

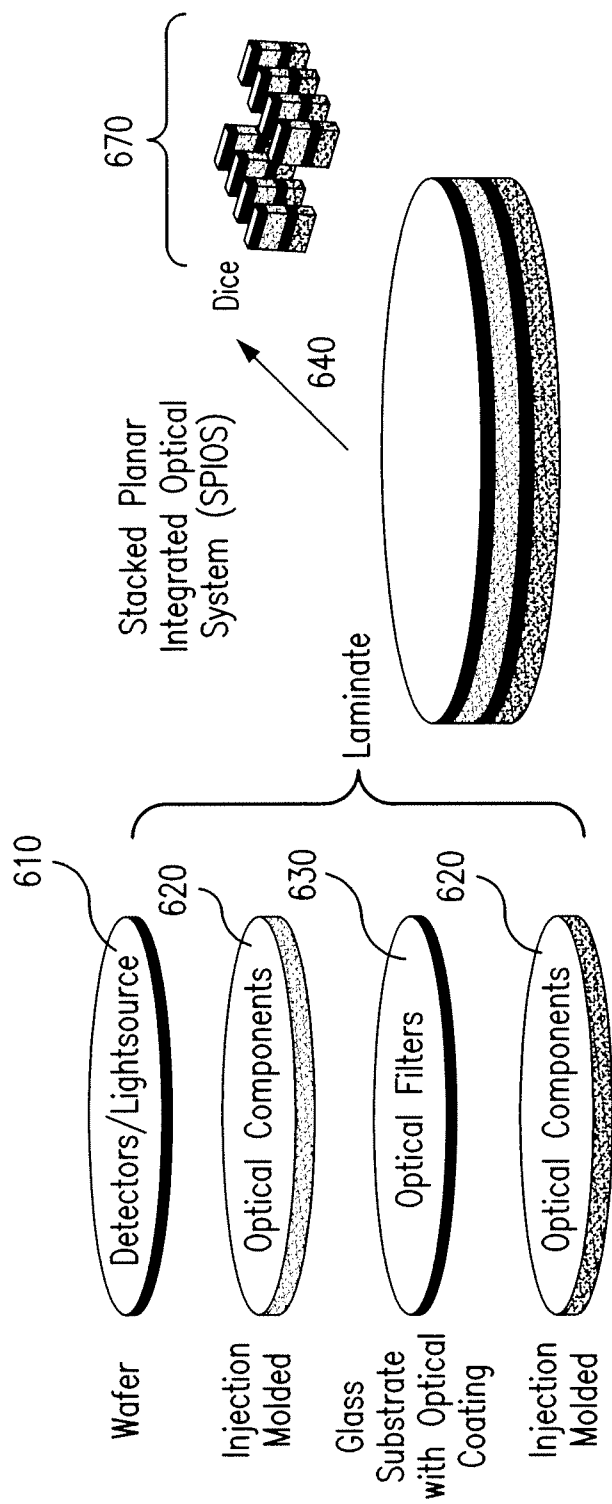


FIG. 17

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/053704

A. CLASSIFICATION OF SUBJECT MATTER INV. G01N21/64 G01N21/77 A61B5/145 A61B5/1459 ADD.		
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 A61B		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ralph Ballerstadt ET AL: "Fiber-Coupled Fluorescence Affinity Sensor for 3-Day in Vivo Glucose Sensing", J Diabetes Sci Technol, vol. 1, no. 3 May 2007 (2007-05), pages 384-393, XP055044981, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2769588/pdf/dst-01-0384.pdf [retrieved on 2012-11-20]	1-4,6-9, 15,16, 28-33, 42-44
Y	pages 385-387 ----- -/--	10-14, 17-27, 29,30
<div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex. </div>		
<div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search	Date of mailing of the international search report	
30 January 2013	06/02/2013	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Hoogen, Ricarda	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/053704

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/171666 A1 (LOEB GERALD E [US] ET AL) 11 September 2003 (2003-09-11) paragraphs [0026] - [0036], [0062]; figures 1-3 -----	1-4,6-9, 15,16, 31-33, 42-44,46
Y	WO 2011/075575 A1 (BAYER HEALTHCARE LLC [US]; CHINNAYELKA SWETHA [US]; RIPLEY PAUL [US]) 23 June 2011 (2011-06-23) paragraphs [0083] - [0085] -----	10-14,27
Y	JANNIK K NIELSEN ET AL: "Clinical Evaluation of a Transcutaneous Interrogated Fluorescence Lifetime-Based Microsensor for Continuous Glucose Reading", JOURNAL OF DIABETES SCIENCE AND TECHNOLOGY, vol. 3, no. 1, 1 January 2009 (2009-01-01), pages 98-109, XP055042790, page 100 -----	17-27
Y	WO 2006/061207 A1 (PRECISENSE AS [DK]; KRISTENSEN JESPER SVENNING [DK]; GREGORIUS KLAUS []) 15 June 2006 (2006-06-15) page 29, line 30 - page 30, line 15 page 30, lines 12-13 page 34, lines 7-10 page 34, lines 27-32 page 48, lines 4-10 page 56, lines 14-27 -----	17-27, 29,30
Y	TANAKA K ET AL: "COMPOUND PARABOLIC CONCENTRATOR PROBE FOR EFFICIENT LIGHT COLLECTION IN SPECTROSCOPY OF BIOLOGICAL TISSUE", APPLIED OPTICS, OPTICAL SOCIETY OF AMERICA, WASHINGTON, DC; US, vol. 35, no. 4, 1 February 1996 (1996-02-01), pages 758-763, XP000577616, ISSN: 0003-6935, DOI: 10.1364/AO.35.000758 page 758 - page 759 -----	34-41
Y	NIKOLAY A. DENISOV: "<title>Comparison of competing fiber optic probes for tissue fluorescence analysis</title>", PROCEEDINGS OF SPIE, vol. 4161, 16 November 2000 (2000-11-16), pages 234-243, XP055051756, ISSN: 0277-786X, DOI: 10.1117/12.409334 page 234 - page 238 ----- -/--	34-41

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/053704

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005/113658 A1 (JACOBSON ROSS W [US] ET AL ALARCON JAVIER [US] ET AL) 26 May 2005 (2005-05-26) paragraphs [0046] - [0050], [0054] figures 3B, 3E -----	36-39

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/053704

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-4, 6-44, 46
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4, 6-9, 15, 16, 28, 31-33, 42-44

A fiber-optic glucose sensor based on a competitive glucose binding affinity assay, the sensor comprising: an optical fiber having a proximal end and an opposing distal end; a glucose-permeable membrane having a hollow interior, an open proximal end, and a closed distal end, wherein the membrane's proximal end is coupled to the optical fiber's distal end so as to define a compartment in said hollow interior between the optical fiber's distal end and the membrane's distal end; and a competitive glucose binding affinity assay disposed in said compartment, the assay including a glucose receptor and a glucose analog. Not novel claims 2-4, 6-8, 15, 16, 31-33, and 42-44 were grouped with invention 1.

2. claims: 5, 45

The fiber-optic glucose sensor based on a competitive glucose binding affinity assay of claim 1, wherein the optical fiber is optically coupled to a stacked planar integrated optical system.

3. claims: 10-14

The fiber-optic glucose sensor based on a competitive glucose binding affinity assay of claim 1, further comprising a reference fluorophore different from a first fluorophore labeling the glucose receptor.

4. claims: 17-27

The fiber-optic glucose sensor based on a competitive glucose binding affinity assay of claim 1, wherein the glucose receptor is Mannan Binding Lectin (MBL) and the glucose analog is dextran, and wherein the MBL is labeled with the fluorophore Alexa Fluor 594 and dextran is labeled with a dye, in particular hexamethoxy crystalviolet-1.

5. claims: 29, 30, 46

The fiber-optic glucose sensor based on a competitive glucose binding affinity assay of claim 1, wherein the membrane comprises a biocompatible polymer, in particular a biodegradable polymer, or wherein the sensor is biodegradable.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. claims: 34-41

The fiber-optic glucose sensor based on a competitive glucose binding affinity assay of claim 1, wherein the distal end of the optical fiber is in the shape of a compound parabolic mirror.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/053704

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