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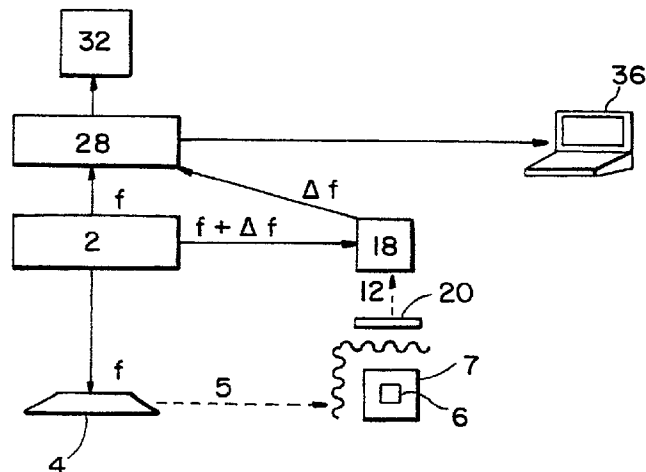(71) LAKOWICZ, Joseph L., US

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(54) **PROCEDE ET APPAREIL PERMETTANT D'EFFECTUER UN CONTROLE TRANSCUTANE D'ANALYSAT**
(54) **A METHOD AND APPARATUS TO PERFORM TRANS-CUTANEOUS ANALYTE MONITORING**

(57) Cette invention concerne un appareil et un procédé permettant d'effectuer des mesures transdermiques avec une invasion minimale afin de mesurer la durée de fluorescence d'un élément implanté (6), et ceci sans faire appel à un réactif et sans avoir recours à un prélèvement de sang douloureux. L'appareil de contrôle (32) affiche la quantité d'une substance choisie présente dans la peau et enregistre les données dans une mémoire (36). Les informations enregistrées peuvent être transmises par modem ou par antenne à une station maître à des fins diagnostiques d'évaluation clinique. Le recours à ce procédé et à cet appareil permet d'améliorer la gestion du contrôle sanguin, et, donc, le suivi à long terme de maladies tout en entraînant moins de complications.

(57) This invention is an apparatus and method to enable minimally invasive transdermal measurements of the fluorescence lifetime of an implanted element (6) without reagent consumption, and not requiring painful blood sampling. The monitoring apparatus (32) displays the quantity of a selected substance present in the skin and stores the data in memory (36). The stored information can be transmitted via modem, or antenna, to a master station for diagnostic purposes of clinical evaluation. The use of this method and apparatus improves control of blood monitoring, and therefore, enhances long-term disease management with fewer complications.
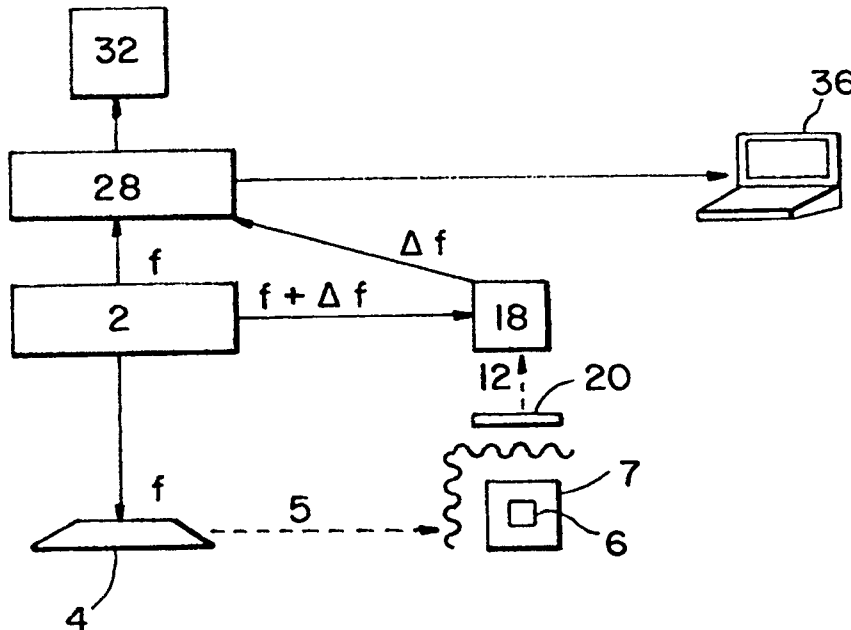
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(54) Title: A METHOD AND APPARATUS TO PERFORM TRANS-CUTANEOUS ANALYTE MONITORING

(57) Abstract

This invention is an apparatus and method to enable minimally invasive transdermal measurements of the fluorescence lifetime of an implanted element (6) without reagent consumption, and not requiring painful blood sampling. The monitoring apparatus (32) displays the quantity of a selected substance present in the skin and stores the data in memory (36). The stored information can be transmitted via modem, or antenna, to a master station for diagnostic purposes of clinical evaluation. The use of this method and apparatus improves control of blood monitoring, and therefore, enhances long-term disease management with fewer complications.

TITLE:

A METHOD AND APPARATUS TO PERFORM

TRANS-CUTANEOUS ANALYTE MONITORING

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The present invention pertains to an apparatus and method to enable minimally invasive trans-cutaneous measurements of the fluorescence of an implanted element without reagent consumption and not requiring invasive sampling. The procedure is completely non-invasive after one implant. The monitoring apparatus displays the quantity of a selected substance present in the skin and stores the data in memory. The stored information can be transmitted via modem, or antenna, to a master station for diagnostic purposes or clinical evaluation. The use of this method and apparatus improves control of blood monitoring, and therefore, enhances long-term disease management with fewer complications.

2. DESCRIPTION OF THE PRIOR ART

Instruments capable of continuously indicating the chemical composition of blood have proved to be useful in regulating operative and postoperative managements of patients, in teaching and research. At first, such instruments were used with sensors mounted directly in the extracorporeal blood circuit that is used for perfusion of open-heart surgery patients. Later,

continuous monitoring of both machine and patients was conducted by means of continuous withdrawal of blood pumped into external cuvettes equipped with appropriate sensors, or by use of implantation of arterial catheters.

5   Other techniques have been employed for measuring biological substances in the blood. For instance, ethanol is currently measured in blood, either directly or by a breath sampling, by classical chemical, gas chromatographic and enzyme methods. One of the alcohol

10   enzyme methods depends upon the polarographic measurement of hydrogen peroxide, while others depend upon the consumption of oxygen.

          The continuous monitoring of blood oxygen by a heated electrode positioned on hyperemic skin has been

15   accomplished. Substances such as halogenated organic compounds, particularly fluorinated compounds, have also been found to diffuse through the skin and have been measured.

          Other methods for cutaneously measuring substances

20   in the blood include contacting the substrate through the skin of a mammal with an enzyme selective for the substrate being analyzed, then reacting the substrate with the enzyme and directly detecting a condition of the skin as a measure of the amount of substrate.

25   The following are representative of the prior art systems available:

          Wider et al., U.S. Patent 5,184,618 discloses a system for invasively measuring blood gas parameters, such as pH, pO_2 or pCO_2, or for measuring other parameters

30   influencing the time constant of the excited state of a fluorescent dye and a correlation between the respective values of the blood gas parameter and the associated values of the time constant of the fluorescent measuring probe.

35   Murray Jr. et al., U.S. Patent 4,752,115, discloses a device for sensing oxygen, particularly for use in

medical applications. The device includes an optical waveguide and an oxygen sensing medium disposed on the waveguide. The sensing medium fluoresces in response to light from a light source such that the intensity of

5   fluorescence is dependent on the partial pressure of oxygen in the environment. The sensing medium includes an oxygen sensitive fluorescent dye in a matrix consisting of a plasticized polymer.

        Lefkowitz et al., U.S. Patent 4,994,396, discloses

10   a sensor and a method for determining the concentration or the partial pressure of oxygen comprising (a) means for transmitting and collecting light to and from a light modifying medium; and (b) a light modifying medium containing a luminescent dye dispersed in or upon a

15   matrix and the dye is accessible to oxygen, wherein said luminescent dye contains a hexanuclear core of molybdenum, tungsten or mixtures thereof having coordination sites, wherein each coordination site is occupied by a ligand.

20   Khalil et al., U.S. Patent 5,043,286 discloses methods of and luminescent substances for measuring oxygen concentration of a test fluid. A test fluid is contacted with a plastic film containing a luminescent substance, the luminescent emission intensity of which is

25   quenched in the presence of oxygen. The film is subjected to irradiation by light that is strongly absorbed by the luminescent substance, and a measure of the time dependence of luminescent emission intensity $I(t)$ is obtained. Three modes of measuring quenching,

30   and thus the oxygen concentration, from $I(t)$ are described.

        Khalil et al., U.S. Patent 5,284,159, discloses a method for converting a value for the partial pressure of oxygen (pO_2) in blood at a measurement temperature to a

35   corresponding value at a reference temperature (37°C). A value for pO_2 is determined by measurements made in a

patient's blood stream using a phosphorescent compound
that is sensitive to the concentration of oxygen. The
phosphorescent compound is illuminated with short pulses
of light, causing it to produce a phosphorescent emission

5   having a rate of decay that varies as the function of the
partial pressure of oxygen in the blood surrounding the
phosphorescent compound. A detector produces an
electrical signal corresponding to the intensity of the
phosphorescent emission, and the electrical signal is

10 converted to a corresponding digital value for input to
a microcomputer. Also supplied to the microcomputer in
digital form is a signal indicative of the temperature at
the measurement site where the phosphorescent compound is
disposed. The microcomputer determines the

15 phosphorescent decay rate and from that value, determines
the pO_2 at the measurement site for the temperature at
which the measurement was made. An initial estimate of
a corresponding value for pO_2 at the reference
temperature is made as a function of the measurement

20 temperature and the pO_2 at that temperature.

       Wolfbeis et al., U.S. Patent 4,580,059, teaches the
simultaneous measurement of the concentrations of several
substances. A number of fluorescence measurements
corresponding to the number of substances to be tested

25 are performed using at least one fluorescent indicator
which is non-specific relative to at least one of the
substances to be tested. Each florescent indicator has
different quenching constants with regard to the
individual substances quenching its intensities. From

30 the known, unquenched fluorescence intensities of the
fluorescent indicators employed, the quenched
fluorescence intensities obtained by measuring, and from
the quenching constants that are known, or rather, have
been determined beforehand by graphical methods or

35 calculation, the concentrations and/or concentration
ratios of the individual substances are obtained.

Clark, U.S. Patent 4,401,122, discloses cutaneous methods for measuring substrates in mammalian subjects. A condition of the skin is used to measure a number of important substances which diffuse through the skin or are present underneath the skin in the blood or tissue. According to the technique, an enzyme whose activity is specific for a particular substance or substrate is placed on, in or under the skin for reaction. The condition of the skin is then detected by suitable means as a measure of the amount of the substrate in the body. For instance, the enzymatic reaction product or by-product of the reaction is detected directly through the skin as a measure of the amount of substrate. Polarographic electrodes or enzyme electrodes are employed as skin-contact analyzers in the transcutaneous measurement of oxygen or hydrogen peroxide to quantitatively detect substances such as glucose and alcohol. In a preferred quantitative technique, the skin is arterialized, i.e. heated or otherwise treated to arterialize the skin capillaries when the measurements are made. Colorimetric detection methods are also employed.

Among the various clinical measurements, the determination of blood gases (pH, pCO_2, pO_2) is one of the most important. Blood gases are routinely performed on patients in the Critical Care Unit. At present, such measurements are performed mostly by classical methods, such as gas chromatography and pH electrode, which are usually located in a central laboratory.

It is difficult to obtain the blood gas report in less than 30 minutes, by which time the patient's status is often quite different. Additionally, handling of blood by health-care workers is undesirable with regard to the risk of AIDS and other infectious diseases. At present, determination of blood gases is time-consuming and expensive, with a cost of at least $400,000,000 per

year in the United States.

As can be seen by the above, there are a variety of techniques available for the measurement of blood gases and other substances. However a new apparatus and method are desired which can provide non-invasive continuous monitoring, immediate data availability, the ability to automatically save and transmit data for analysis, and can be used as part of a coordinated health monitoring system.

SUMMARY OF THE INVENTION:

A preferred embodiment of the trans-cutaneous analyte monitor comprises, a light emitting means for emitting an excitation light having predetermined wavelength and temporal characteristics; a bio-compatible implant, said bio-compatible implant emitting fluorescence having predetermined wavelength and temporal characteristics upon absorption of said excitation light emitted from said light emitting means; a detector means to detect said fluorescence from said bio-compatible implant, said detector means producing an electrical signal proportional to said fluorescence; a comparison means to compare said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant said comparison means receiving input from said light emitting means and said detector means and having an output; and a computing means to receive said output of said comparison means and relating said comparison of said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible skin implant to the quantity of a biologically

significant substance present.

Another preferred embodiment of this invention comprises, said predetermined wavelength and temporal characteristics of said fluorescence being altered by collisional quenching caused by the presence of a predetermined biologically significant substance.

Another preferred embodiment of this invention comprises, said comparison means to compare said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of intensity based sensing, and wherein said comparison means to compare said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of lifetime-based sensing.

In another preferred embodiment said bio-compatible implant comprises a fluorescent donor and analyte-dependent acceptor which spectrally overlaps said fluorescent donor, said bio-compatible implant emitting fluorescence of a predetermined wavelength and temporal characteristics upon absorption of said excitation light emitted from said light emitter, said fluorescence wavelength and temporal characteristics being altered when a predetermined biologically significant substance is present and said comparison means to compare said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of intensity based sensing.

In another preferred embodiment said bio-compatible implant comprises a fluorescent donor and analyte-dependent acceptor which spectrally overlaps said

fluorescent donor, said bio-compatible implant emitting
fluorescence of a predetermined wavelength and temporal
characteristics upon absorption of said excitation light
emitted from said light emitter, said fluorescence
5   wavelength and temporal characteristics being altered
when a predetermined biologically significant substance
is present and said comparison means to compare said
wavelength and temporal characteristics of said light
emitted from said light emitting means and said
10   wavelength and temporal characteristics of said
fluorescence from said bio-compatible implant is by means
of lifetime-based sensing.

In another preferred embodiment said bio-compatible
implant comprises an analyte-free and analyte-bound form
15   of a fluorescent emitter and wherein the ratio of the
signals at two excitation or emission wavelengths is used
for quantitative measurements of a predetermined
biologically significant substance and said comparison
means to compare said wavelength and temporal
20   characteristics of said light emitted from said light
emitting means and said wavelength and temporal
characteristics of said fluorescence from said bio-
compatible implant is by means of intensity based
sensing.

25   In another preferred embodiment said bio-compatible
implant comprises an analyte-free and analyte-bound form
of a fluorescent emitter and wherein the ratio of the
signals at two excitation or emission wavelengths is used
for quantitative measurements of a predetermined
30   biologically significant substance and said comparison
means to compare said wavelength and temporal
characteristics of said light emitted from said light
emitting means and said wavelength and temporal
characteristics of said fluorescence from said bio-
35   compatible implant is by means of lifetime-based sensing.

Yet another preferred embodiment of the trans-

cutaneous analyte monitor comprises, a light emitting
means for emitting an excitation light having
predetermined wavelength and temporal characteristics; a
bio-compatible implant, said bio-compatible implant

5   emitting fluorescence having predetermined wavelength and
temporal characteristics upon absorption of said
excitation light emitted from said light emitting means;
a detector means to detect said fluorescence from said
bio-compatible implant wherein said detector measures the

10   wavelength and temporal characteristics of said bio-
compatible skin implant fluorescence, and further wherein
said detector emits an electrical signal which is
directly proportional to the fluorescence wavelength and
temporal characteristics received; a comparison means to

15   compare said wavelength and temporal characteristics of
said light emitted from said light emitting means and
said wavelength and temporal characteristics of said
fluorescence from said bio-compatible implant; a
computing means to relate said comparison of said

20   wavelength and temporal characteristics of said intensity
modulated light emitted from said light emitting means
and said wavelength and temporal characteristics of said
fluorescence from said bio-compatible skin implant to the
quantity of a biologically significant substance present;

25   a display means for displaying said quantity of
biologically significant substances; a memory storage
device for receiving said output of said computing means;
and a data transfer means, said data transfer means
receiving input from said memory storage device and

30   whereby said quantity of said biologically significant
substance present stored in said memory storage device.
          Another preferred embodiment of the trans-cutaneous
analyte monitor comprises a frequency synthesizer,
wherein said frequency synthesizer produces a modulated

35   electrical output, wherein said light emitting means
receives said modulated electrical output of said

frequency synthesizer causing said light emitter to emit
an intensity modulated excitation light of predetermined
wavelength and temporal characteristics, and wherein said
detector measures the wavelength and temporal
5   characteristics of said bio-compatible implant
fluorescence, and further wherein said detector emits an
electrical signal which is directly proportional to the
fluorescence wavelength and temporal characteristics
received and wherein said comparison means compares the
10   phase angle difference between said excitation light
predetermined wavelength and temporal characteristics and
said wavelength and temporal characteristics of said bio-
compatible fluorescence, and wherein said computing means
computes the quantity of said biologically significant
15   substance present from said comparison of said light
emitted from said light emitting means and said
wavelength and temporal characteristics of said
fluorescence from said bio-compatible implant and wherein
said light emitting means is a light emitting diode.
20   A preferred method to trans-cutaneously monitor
analytes comprises, emitting a modulated excitation light
from a light emitting means, said excitation light having
predetermined wavelength and temporal characteristics;
implanting a bio-compatible implant, said implant
25   emitting fluorescence having predetermined wavelength and
temporal characteristics upon absorption of said
excitation light emitted from said light emitting means;
detecting said fluorescence wavelength and temporal
characteristics from said bio-compatible implant;
30   comparing said wavelength and temporal characteristics of
said excitation light emitted from said light emitting
means and said wavelength and temporal characteristics of
said fluorescence from said bio-compatible implant; and
computing said wavelength and temporal characteristics of
35   said light emitted from said light emitting means and
said wavelength and temporal characteristics of said

fluorescence from said bio-compatible implant to quantify the amount of a biologically significant substance present under the skin.

Another preferred method comprises displaying said quantity of biologically significant substance, storing in a memory storage device said output of said computing means, transferring said data from said memory storage device to a remote receiving station.

Another preferred method is wherein said bio-compatible implant comprises a fluorophore wherein said bio-compatible implant emits fluorescence of a predetermined wavelength and temporal characteristics, said fluorescence of a predetermined wavelength and temporal characteristics being altered by collisional quenching caused by the presence of a predetermined biologically significant substance.

In another preferred method said bio-compatible implant comprises an analyte-free and analyte-bound form and wherein the ratio of the signals at two excitation or emission wavelengths is used for quantitative measurements.

Another preferred method comprises a fluorescent donor and analyte-dependent acceptor which spectrally overlaps said fluorescent donor, said bio-compatible implant emitting fluorescence of a predetermined wavelength and temporal characteristics upon absorption of said excitation light emitted from said light emitter, said fluorescence wavelength and temporal characteristics being altered when a predetermined biologically significant substance is present.

Yet another preferred method comprises synthesizing a modulated electrical output by means of a frequency synthesizer; inputting said modulated electrical output of said frequency synthesizer means into a light emitting means whereby said light emitting means emits an intensity modulated excitation light, said excitation

light having predetermined wavelength and temporal
characteristics; implanting a bio-compatible implant
wherein said bio-compatible implant emits fluorescence of
a predetermined wavelength and temporal characteristics
5   upon absorption of said excitation light emitted from
said light emitter, said fluorescence wavelength and
temporal characteristics being altered when a
predetermined biologically significant substance is
present; detecting said bio-compatible implant emitting
10   fluorescence of a predetermined wavelength and temporal
characteristics by detection means wherein said detection
means emits an electrical signal which is directly
proportional to the fluorescence wavelength and temporal
characteristics received; comparing said wavelength and
15   temporal characteristics of said excitation light emitted
from said light emitting means and said wavelength and
temporal characteristics of said fluorescence from said
bio-compatible implant; computing the phase angle
difference between said comparison of said light emitted
20   from said light emitting means and said wavelength and
temporal characteristics of said fluorescent light from
said bio-compatible skin implant to the quantity of said
biologically significant substance present and relating
said phase angle difference to the quantity of a
25   biologically significant substance present; and
displaying the quantity of said biologically significant
substance present.

BRIEF DESCRIPTION OF THE DRAWINGS:

30
      Fig. 1   is a graph depicting lifetime-based (frequency
                domain) phase-modulation.
      Fig. 2   Experimental geometry for lifetime-based
                sensing in turbid media.
35   Fig. 3   Effect of multiple scattering events on time-
                domain   (3A)   or   phase-modulation   (3B)

measurements of a fluorescence lifetime. The figure shows the pulsed or modulated excitation (·····), the time-delayed scattered light (———), and the fluorescence (— — —) which is further delayed by the lifetime τ.

Fig. 4   Absorption and emission spectra of the pH sensor Carboxy SNARF-6 (CF6) and the reference fluorophore Texas Red Hydrazide (TRH). Also shown (·····) is the transmission profile of the 620 nm interference filter used to isolate the emission. Intralipid was not present.

Fig. 5   Frequency-response of TRH in the microcuvette at various depths below the intralipid surface.

Fig. 6   Frequency-response of CS6, in the microcuvette 4 mm below the intralipid surface, when measured relative to the scattered light (A), relative to the TRH standard (B), and without intralipid (C).

Fig. 7   pH sensing at a 4 mm depth in 0.25% intralipid using CS6. Excitation was the mode-locked output of an Argon-ion laser at 514 nm. The emission was detected at 640 nm. Phase and modulation data were obtained without intralipid (O), in intralipid versus the scattered light from the surface (●) and in intralipid versus the TRH standard (■).

Fig. 8   shows the change in phase angle differences of fluorescent emissions from various excitation intensities measured through layers of chicken skin.

Fig. 9   is a block diagram of a representative system
            for trans-cutaneous analyte monitor.

Fig. 10   is representative of the interaction between
5   the biologically significant substance, an
            analyte donor and a absorbing acceptor for the
            detection of glucose.

Fig. 11   is a graph displaying the phase and life time
10   shifts that occur when glucose displaces the
            acceptor from the donor.

Fig. 12 is a view of a portable trans-cutaneous analyte
            monitor with a display and keyboard.
15

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

There are two common types of illumination detection
sensor systems: absorption and luminescent. The present
20 invention is used in conjunction with a luminescent
system, of which there are two types: phosphorescent and
fluorescent.   Such systems operate on the concept of
measuring the radiation emitted by the excitation of a
substance.
25   Optical sensors, particularly fluorescence sensors,
offer advantages over electrochemical sensors.   These
include the absence of electrical interferences (magnetic
or electrical fields), no analyte consumption, the
possibility of multiple measurements using optical fibers
30 and the possibility of miniaturization. Fiber-optics can
be readily adapted for chemical sensing and be fabricated
as miniature devices suitable for remote sensing and safe
operation in chemical environments. Perhaps more
importantly, optical detection of analytes can be
35 accomplished without the use of radioactive tracers, and
can   minimize   the   need   for   sample   handling   and

manipulation. Fluorescence sensing provides the additional advantage of good sensitivity, with the specificity being obtained from either the properties of the fluorophore or its fabrication within the sensing
5   element.

    At present, most fluorescence sensors are based on intensity measurements, that is, intensity-based sensing, in which the change of intensity in response to the analyte of interest is measured. These intensity changes
10   can be due to changes in extinction coefficient due to probe ionization, changes in quantum yield of the probe upon analyte binding, or due to inner filtering resulting from the optical density changes of indicators.

    The fluorescence intensity measurement depends on
15   the intensity of exciting light, the extinction coefficient and concentration of the probe, the optical density at the excitation and the emission wavelengths, the optical path length, the fluorescence quantum yield of the probe, and the detector sensitivity. The
20   fluorescence intensity can also vary due to light scattering and/or absorption characteristics of the sample.

    An alternate fluorescent sensor measurement is fluorescent lifetime. The fluorescence lifetime of a
25   sample is the mean duration of time the fluorophore remains in the excited state. Following pulsed excitation, the intensity decays of many fluorophores are single exponential

$$I(t) = I_o \exp(-t/\tau), \quad (1)$$

where I_o is the intensity at t = 0, and τ is the
30   lifetime. A variety of molecular interactions can influence the decay time. The excited fluorophore can return to the ground state by the radiative (emission) pathway with a rate k_r. The inverse of this rate constant ($\tau_r = k_r^{-1}$) is usually called the intrinsic or

radiative lifetime. The radiative decay rate k_r is generally of intramolecular origin, with only a modest dependence on the local environment. There are several formulas to calculate radiative decay rate using the absorption and emission spectra. The simplest of them requires only integration of the first absorption band

$$k_r = 2900 \, n^2 \, \bar{v}_0^{-2} \int \varepsilon(\bar{v}) \, d\bar{v}, \quad (2)$$

where n is the refractive index of the solvent, $\bar{v}_0$ the wavenumber of the maximum of the absorption band, and $\varepsilon(\bar{v})$ in the wavenumber-dependent extinction coefficient. The approximate value of k_r can be estimated from a simplified form of Eq. (2), $k_r = 10^4 \epsilon_{max}$. Upon binding the analyte the absorption spectrum of the probe in many cases changes (spectral shift and/or change in extinction coefficient), which can result in changes the radiative decay rate and thus affect the fluorescence decay time.

The measured fluorescence lifetime τ is usually shorter than the radiative lifetime τ_r because of presence of other decay rates which can be dependent on intramolecular processes and intermolecular interactions. The measured fluorescence lifetime (τ) is given by the inverse of the total rate of dynamic processes that cause deactivation from the excited (mostly singlet S_1) state

$$\tau = \frac{1}{k_r + k_{nr} + k_Q[Q] + k_T}. \quad (3)$$

Non-radiative processes (k_{nr}) can occur with a wide range of rate constants. Molecules with high k_{nr} values display low quantum yields due to rapid depopulation of the excited state by this route. The measured lifetime in the absence of collisional or energy transfer quenching is usually referred to as τ_0, and is given by

$\tau_0 = (k_r + k_{nr})^{-1}$.

There are two widely used methods to measure the

fluorescence decay time.   These are the pulse or time-
domain method and the phase-modulation or frequency-
domain method.   In the pulse method, one excites the
sample with a brief pulse of light, typically shorter

5   than the fluorescence decay time, followed by measurement
of · the time-dependent decay of the emission.   The
lifetime can be calculated from the rate of decay.   An
alternative method is to excite the sample with an
intensity modulated light source.   The lifetime is then

10   determined by the phase shift of the emission relative to
the phase of the incident light.   The lifetime can also
be determined from the relative modulation of the
emission compared to the modulation of the incident
light.

15   Due to the absorption of the fluorescence by the
acceptor the donor emission displays a phase angle shift
in relation to the excitation, which is dependent on the
analyte concentration around the sensor.   This is
illustrated in Figure 1. The excitation and emission are

20   modulated at the same circular frequency ($\omega=2\pi$ x
frequency in Hz).   The emission is delayed by the phase
angle (Φ).   The relative amplitude of the variable
portion of the emission (B/A) is smaller than that of the
excitation (b/a).   The phase angle (Φ) can be used to

25   calculate the lifetime of the fluorophore.   The
demodulation factor [m=(Ba/bA)] can also be used to
independently calculate the lifetime (τ) using the
equations, which is valid for a single exponential decay:

30       $$\tan \Phi = \omega\tau \text{ and } m = [1 + \omega^2\tau^2]^{-1/2} \qquad (4)$$

These phase values are correlated to acceptor
concentrations. Values or data at several frequencies
can be taken, increasing the precision and/or reliability

35   of the measurements.
Another characteristic of lifetime-based sensing is

the absolute nature of the measurement and after a one-time calibration, the sensor never needs recalibration. This feature is ideal for an implanted sensor.

The electronics for phase-modulation sensing can be based on relatively low-cost components, and phase and modulation at a single frequency are easily measured in a second or less of data acquisition.

The following is a discussion of various means of detecting fluorescent intensity and lifetime-based changes based on the physical mechanisms causing the changes. One mechanism for creating·· changes is collisional quenching. A variety of substances act as quenchers, including oxygen, nitrous oxide, heavy atoms, Cl^-, and amines, to name a few. By consideration of the lifetime in the absence (τ_0) and presence (τ) of collisional quenchers (no resonance energy transfer), one can easily see that such quenching decreases the lifetime of the excited state

$$\frac{\tau_0}{\tau} = 1 + \tau_0 k_q [Q] \, , \quad (5)$$

where k_q is the biomolecular quenching constant. In fluid solution with efficient quenchers k_q is typically in the range of 10^9 to 10^{10} $M^{-1}sec^{-1}$. Collisional quenching by oxygen can be used for intensity-based and lifetime-based sensing of oxygen using the long-lifetime transition metal complexes resulting with large amount of quenching by oxygen. Collisional quenching also results in the transfer of electrons which are induced by excitation. Binding of cations prevents electron transfer from the electron-donating group, thus preventing intramolecular quenching, resulting in an enhancement of quantum yield upon binding metal cations without observable spectral shifts.

Another mechanism which decreases the fluorescence intensity and decay time is fluorescence resonance energy transfer (FRET). An energy-transfer-based sensor consists of two kinds molecules. Donors can be selected for excitability with inexpensive light sources. Acceptors are selected with an analyte-dependent absorbance that spectrally overlaps the donor fluorescence when added to the sensor. Fluorescent resonance energy transfer (FRET) from the donor to the acceptor will quench the fluorescence and alter both the fluorescence intensity and lifetime. By using the phenomena of fluorescent resonance energy transfer (FRET), the donor need not be sensitive to a particular analyte, and the acceptor does not need to be fluorescent. This energy transfer takes place without the appearance of a photon, and the transfer rate depends primarily on the extent of overlap of the emission spectrum of the donor with that of the absorption spectrum of the acceptor, and the distance between the two.

An advantage of using fluorescent resonance energy transfer (FRET) as the transduction mechanism is that this interaction can be reliably predicted to occur for any Donor-Acceptor pair displaying suitable spectral overlap. Consequently, Donor-Acceptor pairs may be selected which are suitable for sensing in tissues, which are excited with simple light sources, and which are advantageous for simplicity in design and robustness. While the signal levels will be attenuated by tissue absorption and/or scatter the intensity decays are not significantly perturbed. The time scale of photon migration in tissues is near 200 picoseconds. For such a sensor the donor need not be sensitive to the analyte, and the acceptor typically displays a change in absorption spectrum due to the analyte.

Yet another approach is the use of wavelength-

ratiometric probes, where the ratio of signals at two
excitation or emission wavelengths is used for
quantitative measurements, however the ratio is
independent of the probe concentration. The change in
5   lifetime (from τ_F to τ_B), characteristic of the analyte-
free (F) and analyte-bound (B) forms of the probes, is
due to binding of the analyte resulting in changes of the
radiative and/or nonradiative decay rates. Such a probe
must display different absorption and/or emission spectra
10   when free in solution and when bound to the analyte.
The absorption spectra overlap and are excited at the
same wavelength. The fluorescence intensity decay of
such probe is generally not a single exponential, but is
given as a sum of the exponentials with decay times τ_F
15   and τ_B.

        Multi-frequency phase and modulation instruments
.which operate at preset frequencies for a specific
application are easily designed, and are well known to
those skilled in the art. In detecting transcutaneous
20   emissions, the most difficult obstacles to overcome are
the absorbance and scatter of light caused by skin and
the lack of fluorescent sensing probes which can be
excited at wavelengths over 600 nm. Red light readily
penetrates skin as diffusely scattered light. Light at
25   580 nm is stopped by skin absorbance, while light at 600
nm and 620 nm penetrates skin. Therefor a subcutaneous
system must emit light at a wavelength greater than 600nm
and even longer wavelengths are preferred. Based on
current integrated circuit designs, well known in the
30   art, and using a diode light source, the phase
fluorometer could be constructed on a single printed
circuit board.

                            EXPERIMENT I

        We now describe how time-resolved or lifetime-based
35   sensing is used in real-world situations, such as quasi
non-invasive measurements performed through skin. The

use of lifetime-based sensing in scattering media is
supported by a computational study using the known
scattering and absorption properties of tissues. These
simulations suggested that properly referenced phase and

5   modulation measurements can be used to recover the
lifetime of fluorophores uniformly dispersed in
scattering media. In figure 2 a sensing fluorophore is
localized below the surface of the turbid media. The
sensing fluorophore is contained within a capillary tube

10   below the surface of an intralipid suspension. The
microcuvette contains a fluorophore whose lifetime is
sensitive to the analyte, which is pH in this instance.
The measurement the pH of the "sensor" embedded in the
intralipid is performed by using the time-dependent

15   fluorescence which re-emerges from the surface of the
sample. However, because there is no wavelength change
with light scatter, it is not possible to selectively
observe light scattered from the same region as the
embedded sensor. This difficulty is circumvented by using

20   a reference fluorophore of known decay time.

       Fluorescence intensity decays were determined using
the frequency-domain method. Excitation was provided by
the 3.795 MHz cavity-dumped output of a Rhodamine 6G dye
laser at 570 nm, or using the 75.9 MHz mode-locked output

25   of an Argon-Ion laser at 514 nm, which provides pulse
widths near 5 and 100 ps, respectively. The emission was
isolated with an interference filter at 620 or 640 nm.

       The sample consisted of a suspension of 20%
intralipid, diluted 20-fold to 1%. The incident light

30   was directed onto the surface of the intralipid using a
1 mm diameter fiber bundle. The emission or scattered
light was collected from the surface with a similar fiber
optic bundle and directed to the Photo Multiplier Tube
(PMT).

35   The fluorescent samples were contained in about 1 mm
(inner) diameter capillary tubes of the type used for

melting point determinations, which allowed control of the probe's environment and knowledge of its lifetime(s). Texas Red Hydrazide (TRH) was used as a pH-insensitive reference fluorophore, and Carboxy SNARF-6 (CS6) was used
5   as the pH lifetime sensor.   Phase and modulation measurements were performed relative to scattered excitation from the surface of the intralipid at the excitation wavelength, or from the reference fluorophore at the same depth under the intralipid surface using an
10   interference filter, to isolate the emission.

The frequency-domain method is used to measure the lifetimes of fluorophores in scattering media. The basis of these measurements can be understood by considering the effect of the scattering media on the time-domain
15   data. When a sample is exposed to a δ-function pulse of light, the light pulse is time-delayed and broadened due to multiple scattering events prior to arriving at the fluorescent sensor (Figure 3A). The broadened and time-delayed pulse excites the fluorophores, which may then
20   decay by a single or multi-exponential decay law. The emitted light is again time-delayed and broadened as it migrates to the detector located at the surface of the sample. At the surface of the scattering media the re-emerged light (————) is time-delayed and broadened
25   relative to the incident light (·····). The fluorescence emission (— — —) is further delayed relative to the scattered light because of the lifetime τ of the excited state.

A similar effect is seen in the phase-modulation
30   measurements (Figure 3B). In this case, the re-emergent scattered (————) light is delayed in phase and demodulated relative to the intensity-modulated incident light (·····). The re-emergent fluorescent light is further phase shifted and demodulated due to the
35   fluorescent lifetime (— — —). Our goal is to measure the phase (ϕ) and modulation (m) of the emission, independent

of the phase shift and demodulated due to multiple
scattering events.

When a fluorescent-sample is illuminated with
intensity-modulated light, the emission is delayed in
phase by an angle (ϕ_ω) due to the lifetime (τ) of the
excited state. The emission is also demodulated by a
factor m_ω relative to the modulation of the incident
light. For a single exponential decay the phase and
modulation are related to the decay time τ by

$$m_\omega = (1 + \omega^2 \tau^2)^{-1/2} \quad (6)$$

here ω is the light modulation frequency in radians/sec.
If the fluorescence decay is a single exponential then
the same lifetime is recovered independently of light
modulation frequency; where $\omega = 2\pi \times$ frequency (in Hz),
and

$$N_\omega J = \sum_i \frac{\alpha_i \omega \tau_i^2}{(1 + \omega^2 \tau_i^2)}$$

and

$$D_\omega J = \sum_i \frac{\alpha_i \tau_i}{(1 + \omega^2 \tau_i^2)}, \quad (7)$$

and

$$J = \sum_i \alpha_i \tau_i \quad (8)$$

When the light pulse enters the scattering media it
is time delayed (t_L) due to multiple scattering events.
The magnitude of the time-delay t_L is expected to
increase with increasing depth in the sample.
Additionally, the pulse is broadened due to multiple path
lengths, which is most evident on the rising side of the

pulse. This effect is analogous to a transit time spread in a photomultiplier tube (PMT), and hence we refer to this time as Δt. And finally the light decays due to sample absorption and/or migration away from the

5 detector. We refer to the decay time as t_D, not to be confused with a fluorescence decay time (τ or τ_i).

For the phenomenological model the phase angle of the re-emergent light, without interacting with any

10 fluorophore, is given by

$$\varphi_{\omega} = \arctan(\omega\, t_D) + \omega\, t_L. \quad (9)$$

The phase angle due to the time delay (t_L) is given by $\phi_L = \omega t_L$, and is therefore strongly dependent on the light

15 modulation frequency ω (radians/sec). The decay time t_D behaves analogously to the fluorescence lifetime (Eq. 9). The modulation of the re-emergent scattered light is given by

$$m_{\omega} = m_{\Delta t} m_D, \quad (10)$$

where

$$m_{\Delta t} = (1 + \omega^2 \Delta t^2)^{-1/2}, \quad (11)$$

$$m_D = (1 + \omega^2 t_D^2)^{-1/2}, \quad (12)$$

20 Equation (10) is analogous to the usual relationship between a decay time and demodulation. We note that the modulation values are not sensitive to the phase of the scattered light, and hence not sensitive to t_L, since t_L does not appear in Equations (11)-(12). This model was

25 found to account for the frequency-domain data, so the parameters t_D, t_L and Δt are adequate to describe the re-emerged scattered light.

The fluorophore is excited by the migrating light

pulse but the "excitation pulse" is not the original
δ-function, but rather the time-delayed and broadened
pulse. Measurements of the emission, relative to the
pulse which arrives at the sensor, will provide the
5   correct decay time of the sensor. However, because there
is no wavelength change with scattering, it is not
experimentally possible to select the light which has
been scattered from the same position as the sensor. The
detected scattered light will be dominantly due to
10   scattering from the surface of the solution or skin. In
contrast, the fluorescence will be excited with the
delayed and broadened pulse which has migrated down to
the sensor, and the emission will be delayed and
broadened as it migrates to the surface. Consequently,
15   the phase and modulation values (ϕ_ω^s and m_ω^s), measured
relative to light scattered from the surface(s), will
contain contributions from both time-dependent photon
migration and the lifetime of the fluorophore. For a
single exponential lifetime (τ) these values will be

$$\phi_\omega^s = \arctan(\omega\,\tau) + \arctan(\omega\,t_D) + \omega\,t_L \quad (13)$$

20

$$m_\omega^s = (1 + \omega^2\,\tau^2)^{-1/2}\,(1 + \omega^2 t_D^2)^{-1/2}\,m_{\Delta t}. \quad (14)$$

Equations (13) and (14) will strictly correct for
measurement of the emission relative to the excitation
pulse, as could be observed with a mirror in front of the
25   fibers in place of the scattering solution.
       Examination of Equations (13) and (14) reveal that
the effects of τ or t_D are mathematically similar, so
that it is not possible to recover both values (τ and t_D)
from measurement of the emission relative to the light
30   scattered from the surface. The time delay t_L appears in
a linear manner, and does not contribute to the
modulation, so this value can be found from the

frequency-domain data. Fortunately, the values of the
decay time t_D are modest for all but the shortest
lifetimes. For instance, the value of t_D for a position
4 mm deep in the 1% intralipid in only 140 ps. Hence,
5   for lifetimes 1 ns and longer use of the values of ϕ_ω^s and
m_ω^s provide a good approximation (τ_{app}) to the true
lifetime (τ)

$$\phi_\omega^s = \arctan(\omega\,\tau_{app}) + \omega\,t_L \quad \textbf{(15)}$$

$$m_\omega^s = (1 + \omega^2\,\tau_{app}^2)^{-1/2}\,m_{\Delta t}. \quad \textbf{(16)}$$

In practice the values of t_L and Δt are determined from
the frequency-domain data (ϕ_ω^s and m_ω^s) because of their
10 distinct contribution in Equations (15) and (16). In
particular, we found that the use of τ_{app} from Equations
(15) and (16) was adequate to recover a single
exponential decay.

        As described above, the scattered light is dominated
15 by light scattered from near the surface, whereas the
fluorescence originates from the embedded sensor deeper
in the sample. Consequently, the value of ϕ_ω^s and m_ω^s
contains contributions from t_D, t_L and Δt (Eqs. 15 and
16). This difficulty can be avoided using a reference
20 fluorophore of known lifetime at the same location as the
sensor. In this way, we can use an emission filter to
separate the emission of the reference from the scattered
light.

        In the present experiments we replaced the sensor
25 with a reference fluorophore emitting at the same
wavelength as the sensor. Alternatively, one could use
adjacent sensors and reference fluorophores which emit at
different wavelengths, and isolate the desired emission
with optical filters. The use of a reference fluorophore
30 corrects for the effects of light scatter while the
excitation is traveling to the sensor, and the emission
is migrating to the surface. In both cases the effects

of scattering are the same, and the only difference in the re-emergent light is due to the different lifetime of the sample and reference fluorophore. In the present analysis we are ignoring the effects of wavelength, which

5   are expected to be minor for the small wavelength difference between the scattering and emission wavelengths.

The use of a reference fluorophore requires correction for its decay time. Suppose the reference

10   displays a single exponential decay τ_R. The measured phase angle of the sensor, relative to the reference fluorophore, is smaller than the true value due to the phase of the reference fluorophore (ϕ_R). Similarly, the modulation of the sensor, measured relative to the

15   reference, is larger than the true value due to the decreased modulation of the emission of the reference compared to the modulation of the excitation. The actual phase (ϕ_ω) and modulation (m_ω) values can be calculated from the observed values relative to the reference

20   fluorophore using

$$\varphi_\omega = \varphi_\omega^{obs} + \varphi_R \quad (17)$$

$$m_\omega = m_\omega^{obs} (1 + \omega^2 \tau_R^2)^{-1/2} \quad (18)$$

where tan $\phi_R = \omega\tau_R$. In this case, the measured phase and modulation values are corrected for the effects of time-dependent photon migration, and if desired, can be used

25   to recover a multi-exponential decay.

We examined the effects of a scattering media on the frequency-domain measurements, using fluorophores contained in microcuvettes located below the surface of the intralipid (Figure 2). By placing the fluorophores

30   within the microcuvette, we avoided changes in the lifetime due to possible molecular interactions of the fluorophore with the intralipid. Two fluorophores were

selected for these experiments: Texas Red Hydrazide
(TRH) and Carboxy SNARF-6 (CS6). Absorption and emission
spectra of these fluorophores are shown in Figure 4.
Both fluorophores can be excited with the 570 nm cavity-
5   dumped output of our R6G dye laser, or by the mode-locked
output of the Argon-ion laser at 514 nm.   CS6 was
selected as a pH indicator whose mean lifetime depends on
pH.   TRH was chosen because its emission could be
observed at the same wavelengths as used for CS6. The
10   intensity decay TRH in the absence of intralipid is
essentially a single exponential, and .there are no
significant effects of pH on its lifetime.

Frequency-domain intensity decay data of TRH are
shown in Figure 5.   These data were obtained for TRH in
15   the microcuvette, located at various depths below the
intralipid surface. The phase and modulation values were
measured in the usual manner, by comparing the phase and
modulation of the emission at 620 nm with the phase and
modulation of the scattered light at the excitation
20   wavelength of 570 nm.   In the absence of intralipid the
phase values show the usual dependence on light
modulation frequency;   at high light modulation
frequencies the phase angle approaches 90°, and the
modulation decreases toward zero.   In the presence of
25   intralipid, the phase angles exceed 90°, which cannot be
explained by decay of directly excited species. Phase
angles in excess of 90° can result only from excited
state reactions or time delays in the detected signal due
to some process such as time-dependent photon migration,
30   different path lengths for scattered and fluorescent
light, or color effects in the detector.   In the present
case the rapidly increasing phase angles are due to the
effects of the delay time t_L, which results in increasing
large phase angles at higher light modulations
35   frequencies (Eqs.13 and 17).   It is apparent that the
effects of t_L on the phase angles prevents direct use of

the phase angle for determining the lifetime. In contrast, t_L does not affect the modulation, and the effects of Δt are modest. Consequently, the modulation values, measured relative to the surface scatter, can provide a reasonable estimate of the lifetime of the embedded probe.

The data in Figure 5 shows the apparent decay time was taken as representative of the decay time of TRH (Table I). We also examined the frequency-domain data for CS6 at pH 9.2 when the intensity decay is dominated by a component with τ = 1.15 ns. As the depth of the microcuvette is increased, the delay time increases, as does the transit time spread. The values of the delay time increase approximately linearly with depth, and are consistent with a mean path length about 10- to 15-fold larger than the time-of-flight for the geometric distance between the surface of the intralipid (excitation source), the position of the microcuvette, and surface of the intralipid back (emission collection). The transit time spread also increases with depth, which suggests that the emission is affected by broadening of the pulse prior to its arrival at the sample, and again during migration of the fluorescence to the surface.

The decay times (τ) recovered for TRH and CS6 are close to the expected values of 4.1 and 1.15 ns, respectively for all depths of the microcuvette in the intralipid (Table I).

Table I. Intensity decays of Texas Red Hydrazide (TRH)
          and Carboxy SNARF-6 (CS6) in capillary tubes
          at   various   depths   in   the   intralipid
          suspension[a].

Depth (mm)	Dye	τ_{app} (ns)	t_L (ps)	Δt (ps)	χ^2_R
No intralipid	CS6	1.09	-18	<0>	1.3
	TRH	4.10	-34 ± 2	67 ± 83	1.5
0.5	TRH	3.84	59	157 ± 36	1.9
1.0	TRH	3.94	132	156 ± 35	1.7
2.0	CS6	1.22	208	190 ± 10	1.0
	TRH	3.99	226	205 ± 66	· 8.2
4.0	CS6	1.33	473	350 ± 32	15.0
	TRH	4.20	480	376 ± 86	18.0
6.0	TRH	3.89	847	957 ± 170	65.9

[a]1.0% intralipid suspension, 20°C, excitation at
570 nm, emission at 620 nm. The Carboxy SNARF-6 was in
80 mM tris, pH 9.2.

Also, the FD data appears to be adequately fit by a
single exponential decay, as can be seen from the
reasonable values of χ^2_R from 1 to 2. The elevated values

of χ_R^2 for sensors 4 and 6 mm deep in the intralipid appears to be the result of a decreased signal-to-noise ratio for the weaker signal. The values of χ_R^2 were not improved by using a multi-exponential decay time. These

5   results suggest that for lifetimes longer than 1 ns, the effects of time-dependent photon migration did not significantly alter the apparent decay times (τ_{app}) relative to the time values.

Lifetime measurements can be obtained by using a

10   reference fluorophore at the same location as the sensor. These measurements are shown in Figure 6 where the sensing fluorophore is CS6 and the reference fluorophore is TRH. For these measurements, the CS6 and the TRH reference were contained in the microcuvettes. When

15   measured relative to the surface scattered light, the phase angles and modulations show the usual effects of the time delay and transit time spread (Figure 6). When measured relative to TRH, the phase angles (B) and modulations are essentially identical to that found in

20   the absence of intralipid (C). These results illustrate how the use of a reference fluorophore corrects for the effects of time-dependent photon migration.

Table II. Intensity decays of Carboxy SNARF-6 (pH 9.2)
          measured relative to the excitation source and
          to the fluorescent reference TRH[a].

Scattering Condition	τ or τ_{app} (ns)	t_L (ps)	Δt (ps)	χ_R^2
	Relative to the excitation source			
	τ_{app} (ns)			
No intralipid	1.09	-18	~0	1.3
In intralipid,[b] 2 mm	1.22	208	190	1.0
In intralipid, 4 mm	1.33	473	351	15.0
	Relative to the fluorescent reference TRH			
	τ (ns)			
No intralipid	1.17	-33	~0	1.2
In intralipid, 2 mm	1.14	-46	~0	2.7
In intralipid, 4 mm	1.18	-22	~0	7.0

[a]Excitation at 570 mm, emission at 620 nm, 20°C.
1.0% intralipid suspension. The Carboxy SNARF-6 was in
80 mM tris, pH 9.2.
    [b]Depth under surface of intralipid suspension.
    Additional data and analyses using the reference
fluorophore are shown in Table II. When measured
relative to surface scatter, the analysis using Equations

(20) and (21) shows that significant values of t_L and Δt are required to account for the data. When measured relative to TRH (Eqs. 22 and 23), the values of t_L and Δt are near zero, indicating that it is no longer necessary to consider these effects when using a reference fluorophore.

To further illustrate the usefulness of the reference fluorophore a multi-exponential analysis of the intensity decay of CS6 using Equations (5)-(9)was performed. In the absence of intralipid, the intensity decay is a double exponential (Table III), as can be seen from the 10-fold decrease in χ_R^2 from 6.7 to 0.6 for the two exponential fit.

Table III.   Multiexponential intensity decay analysis
                of   Carboxy   SNARF-6   (pH   9.2)   measured
                relative to its excitation source and to
                the fluorescent reference TRH.

Reference/Sample	τ_i (ns)	α_i	$f_i{}^a$	χ_R^2
Surface scatter/	1.06	1	1	6.7
CS6 without intralipid	0.46	0.188	0.084	
	1.15	0.812	0.916	0.6
TRH/	1.10	1	1	20.9
CS6 without intralipid	0.16	0.243	0.040	
	1.19	0.757	0.960	0.9
TRH/	1.03	1	1	38.7
CS6 under 2 mm of	0.17	0.310	0.062	
intralipid	1.16	0.690	0.938	1.6
TRH/	1.12	1	1	10.4
CS6 under 4 mm of	0.30	0.128	0.036	
intralipid	1.18	0.872	0.964	5.0

[a]The fractional contribution of each component to the
steady-state intensity can be calculated using f_i =
$\alpha_i \tau_i / \Sigma_j \alpha_j \tau_j$.

     However, the amplitude of the shorter 0.16 to 0.46
ns component is weak and contributes only 4 to 8% of the
total intensity. In spite of this weak contribution, two

decay times and amplitudes of CS6 were recovered from 2 and 4 mm of intralipid when measured using the reference fluorophore. This result suggests that even complex intensity decays can be quantitatively recovered from
5   sensors embedded in highly scattering media.

To avoid confusion, we note that double-exponential intensity decays of CS6 were not recovered when using Equations (16) and (17) (Table II).

In this case there is a weak contribution of the
10 short-lived component, and it appears that the value of t_L can vary and to some extent account for the short-lived component in the decay of CS6. For instance, in the absence of intralipid (Table II), the value of t_L = 18 ps may be a consequence of the multi-exponential decay
15 of CS6. An advantage of using the reference fluorophore is that the data can be analyzed using Equations (5)-(9), without the use of t_L and Δt as additional parameters.

Experiment II
20

To illustrate the possibility of non-invasive pH measurements, we examined a series of CS6 solutions of various pH. The experimental conditions were changed to 514 nm excitation and 640 nm emission, which selects for
25 excitation of the acid form and detection of the base form of CS6, and results in an apparent pK_a value near 7.5 as seen from the phase angle and 8 for the modulation. The calibration curve for these experimental conditions is shown in Figure 7. When measured relative
30 to surface scatter, the phase angles (●) are larger than the true values and do not reveal the correct pH. When measured relative to the TRH standard, the correct phase angles are observed (■) demonstrating the possibility of non-invasive pH sensing in scattering media. It is
35 interesting to notice that for CS6 under these experimental conditions, essentially the same modulation

values are measured versus surface scatter (●) as measured without intralipid (O).

Experiment III

- To demonstrate transcutaneous sensing, experiments were conducted using layers of chicken skin to block both excitation and emission light paths during spectra acquisition. Chicken skin is optically similar to human skin. Figure 8 shows real time fluorescence intensity and phase angle measurements (at 750KHz) as oxygen and nitrogen were alternately bubbled through the fluorophore solution. The measurements were made first with no skin, one layer of skin and finally with two layers of skin in both the excitation and emission paths. While a 50 fold decrease in the intensity response occurred, the phase angle measurements were unaffected by the presence of the skin. This demonstrates the independence of phase angle measurements to changes in skin thickness and attenuation in excitation light intensity. Provided a minimum measurable emission is available (the quantity of light required for measurement at a tolerable signal to noise level, is governed entirely by the photodector) the phase angles are independent of the attenuating effects that limit fluorescence intensity based measurements.

The results described above demonstrate that lifetime-based sensing is possible for sensors imbedded in turbid media. The measurements were performed using a mode-locked Argon-ion laser and a ps dye laser light source. Based on these results it would be apparent for one skilled in the art to adapt simple instrumentation for use in the doctor's office, at home, or even as portable devices.

Medical phase-modulation fluorometers can be constructed using currently available probe and electro-optics technology. Such a device is shown in Figure 1,

which illustrates one possible configuration for non-invasive measurements. The sensing patch may be implanted under the skin, and of course, would be permeable to the analyte of interest. The lifetime of the sensing patch could be measured using the methods described in this report. There has been rapid development of long-wavelength fluorophores, many of which can be excited with currently available laser diodes. It is well known that the optical output of laser diodes can be intensity modulated to GHz frequencies, and that wavelengths above 620 nm readily penetrate the skin, so that laser diodes can be the light source for imbedded phase-modulated sensors. Laser diodes have already been used for frequency-domain (FD) lifetime measurements, for FD lifetime measurements through fibers, and for a fiber-optic pH sensor. New classes of fluorescence sensors are emerging, which display long decay times over 100 ns, and that such long-lived fluorophores can be excited with simple modulated light sources such as an electroluminescent device. It has even been possible to develop a sterilizable sensor using these long-lived fluorophores. For these longer lifetimes, the effect of time-dependent photon migration will be less significant, and can possibly be neglected. Lifetime-based sensing in tissues is clinically practical with currently available technology.

Advances in the chemistry of molecular recognition allow tailored recognition of analytes, thus providing a whole new class of biochemically significant substances that can be instantaneously and non-invasively monitored. Among the representative blood components that can be measured include blood gas levels, metal content, anion and cation levels, hormones, fats, and carbohydrates. Elements suitable for monitoring in this manner are pO_2, pH, pCO_2, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO^{2-}, Estrogen, Progesterone, Thyroxine, IL-1, high and low density cholesterol,

glucose levels and mixtures thereof. These emerging
applications of fluorescence can be seen by the growth
and introduction of improved methods for immunoassays,
enzyme-linked immunoassays (ELISA), protein and DNA
5   staining, and protein and DNA sequencing.

Other objects, features and advantages of the
present invention will become apparent to those skilled
in the art from the following detailed description and
attached drawings. For simplicity and clarity, a limited
10   number of preferred embodiments of the present invention
are described, but it is understood that it is not to be
construed as limiting the invention thereto, but rather
it should be viewed as representative embodiments of the
broader inventive concept.

15   One preferred embodiment of the invention pertains
to glucose detection as a means for controlling diabetes
which is discussed in detail below.

While the acute problems of diabetes can be
controlled, diabetics suffer a number of long-term
20   adverse health effects which result from inadequate
control of blood glucose. The health problems include
blindness and cardiovascular diseases, which appear to be
a consequence of the accumulation of glycosylated
proteins and their metabolic products. Amelioration of
25   these long term effects seems possible if blood glucose
can be controlled within normal physiological limits.
Unfortunately, such control is not available, in part due
to our present inability to continuously monitor blood
glucose. Present methods to measure blood glucose
30   require a sample of fresh blood, which is painful and
inconvenient, so that patients perform the least frequent
testing possible and thus suffer extreme fluctuations in
glucose levels.

Non-invasive measurement of blood or tissue glucose
35   would be a significant advance for diabetics. Tissue
glucose closely follows blood glucose, with about a 10

minute time lag, so that a sensor for subdermal tissue glucose can be expected to provide adequate information for controlling glucose levels. Many attempts have been made to develop a method for non-invasive glucose

5   sensing, including transdermal NIR spectroscopy the measurement of glucose in the eye by optical rotation, or transducing the glucose concentration to changes in pH, or oxygen concentration. However, the lack of a commercially available alternative to (invasive) blood

10   sample based enzymatic glucose analyzers suggests that all these approaches are yet to materialize as practical, low-cost devices.

An advantage of phase-modulation fluorometry for sensing is the use of cross-correlation detection, which

15   results in an increased signal-to-noise ratio and rejection of undesired harmonics. The gain of the photo-multiplier tube (PMT) detector is modulated by a small voltage applied to one of its dynodes. The gain modulation frequency is offset from the light modulation

20   frequency by a small amount, typically 25 or 40 Hz. The phase and modulation information are preserved in the low frequency signal, and θ_ω and m_ω then easily measured with simple electronics. The AC component of the signal is typically passed through a 1 Hz wide filter, which

25   rejects undesired harmonics. Cross-correlation electronics or numerical filtering results in phase and modulation measurements which are correct irrespective of the form of the light and gain modulation. The measurements may be made with either sinusoidal or pulse

30   source modulation. The frequency synthesizers for a sensing application require that the two frequencies (f and f+Δf) be phase-locked.

Figure 9 depicts an example of a flow scheme for using the apparatus of this invention. Frequency

35   synthesizer 2 transmits an excitation frequency to light emitting source 4. The light emitting source 4, such as

a laser diode (i.e. Toshiba model TOLD9520) emits excitation light 5 with a -3db bandwidth of .07 to 1.7 MHz in concert with the frequency of frequency synthesizer 2. Subcutaneous sensor patch 6 is implanted
5   in a patient's arm, or other readily accessible portion of the body. Multiple patches may be implanted if required for diagnostic reasons, or a single sensor patch 6 capable of detecting multiple biologically significant substances can be used.
10   Sensor patch 6 is a permeable subcutaneous bio-compatible implantable element containing a fluorescent donor labelled glucose binding protein and a fluorescent acceptor labelled sugar. The sensor may be of a planar or cylindrical shape with a membrane 7 permeable to the
15   relevant substance. In this embodiment, cellulosic dialysis membranes are used. The process of fluorescent emission is discussed in greater detail below with reference to Figures 9 and 10. The acceptor fluorescent emission is detected by detector 18. High pass filter 20
20   may be incorporated before detector 18 to reduce noise. Detector 18 is comprised of one or more photomultiplier tubes 22 for collection of emitted light 12 from sensor patch 6. Detector 18 may be enclosed in a portable housing 30 (as shown in figure 12) which allows light
25   entry through an aperture (not shown).
         The received emission light 12 wavelength and temporal characteristics is compared to the signal generated by frequency synthesizer 2 in comparison unit 28, wherein an algorithm correlates the wavelength and
30   temporal characteristics of the change in emission light 12 from excitation light 5 to quantify the amount of biologically significant substances present. The algorithm is computed from empirical data for the various biologically significant substances, and the process of
35   developing a mathematical model of natural phenomena is well known to those skilled in the art. The comparison

can be performed by any integrated circuits of this type
well known in the art, including the Analog Devices Model
630 IC. This preferred embodiment uses the phase shift
between emitted light 12 and excitation light 5 to solve

5   an algorithm to determine the amount of relevant
substances present at sensor patch 6. Display 32
communicates the quantitative result of the comparison
algorithm to the operator, and the quantity is
electrically stored in memory storage device 36.

10   Figure 10 depicts bio-compatible sensor patch 6. In
this embodiment, donor-labeled protein Concanavalin A
(ConA) is used in contact with acceptor-labeled dextran.
Since both the donors and acceptors are high molecular
weight species, they can be trapped behind glucose-

15   permeable membrane 7. Endogenous glucose will bind to
ConA and displace it from the dextran. Consequently, the
donor lifetime will increase due to decreased proximity
to the acceptor, which will be measured as an increase in
phase angle, as depicted in Figure 11.

20   Normally, sensor patch 6 will be subcutaneously
implanted for hygiene and cosmetic reasons. However,
there may be a donor-acceptor combination needed to
detect a particular biochemically relevant substance for
which the excitation light frequency of the donor is

25   incapable, or only marginally capable, of skin
penetration.

          The inventive detection system is compatible with
either digital or analog circuit design, and with fiber
optic or wire electrical connections. The preferred

30   embodiment for reliability, low cost and speed is a
digital circuit using fiber optic transmission lines.

          Graphical displays 32, consisting of liquid
crystals, or paper printouts, are used to communicate the
quantitative result of the comparison algorithm, and the

35   data is stored in memory storage device 36, such as a
semiconductor, computer or other electronic data storage

device.

Keyboard 40, as shown in Figure 12, can be added to the housing for inputting patient identification numbers, time information, or telephone numbers to identify,

5   amplify, and transmit the data collected from the sensor patch. The data can be internally stored, or transmitted immediately via telephone, radio, microwave, infrared or equivalent means to a remote location.

The amount of storage capacity incorporated in the

10   apparatus is design dependent. A unit used in a hospital which will be in continuous or near continuous contact with the main data storage system will require little data storage capacity. A unit designed to be used at remote sites or in field hospitals will require more

15   internal storage capacity if there are longer periods and/or more individual patient's data inserted before each data transmission. As the costs for memory storage have declined, the addition of enough memory to retain daily readings for later transmission to a physician will

20   not be cost prohibitive or bulky.

Another embodiment of this invention can be used to detect levels of oxygen. The clinical assessment of oxygenation in arteries and other tissues is of significant importance in monitoring critically ill

25   patients. Arterial oxygenation problems are a common indication of loss of hemoglobin or of deteriorating lung function and indicates tissue hypoxia with resulting loss of function. In general, disturbances in oxygenation result in decreased performance from organs highly

30   dependent on oxygen, such as the hear and brain.

An Osmium based metal-ligand complex is chosen as a donor, for example $[Os(bpy)_3]$ I_2 and Malachite green as the acceptor with a light emitting source 4 chosen which emits a red excitation light. An alternate embodiment

35   contains a Ruthenium ligand complex $[Ru(bpy)_3]Cl_2$ as a donor with an Acceptor of reactive Blue and a doubled

frequency blue excitation light. In both examples there is adequate spectral overlap between the donor emission and the acceptor absorption to expect fluorescent resonance energy transfer (FRET) to occur.

5   The excitation source is an inexpensive blue LED that is modulated simply by varying its supply current. The phase fluorimetric oxygen sensor measures the quenching by oxygen of the transition metal complex, tris [4,7-diphenyl-1,10-phenanthroline] ruthenium (II)$^{2+}$. This

10   transduction scheme generally follows a first-order-type response to oxygen tension resulting in higher oxygen sensitivities at low oxygen concentrations. The fluorophore is immobilized in a membrane which has high oxygen solubility and diffusivity. The sensor is equally

15   sensitive to gaseous and dissolved oxygen tensions. The matrix selected for gaseous analytes because of its hydrophobic nature, adding to sensor specificity. In addition, the hydrophobic matrix and the water insolubility of the ruthenium complex add to long-term

20   sensor stability by preventing washing out and leaching of the probe.

       Another preferred embodiment of this invention is a lifetime-based pH sensor. Two dyes, a high-quantum-yield fluorescence donor and a pH-sensitive acceptor whose

25   absorbance overlaps significantly with the donor's emission are place in a hydrophilic matrix and sub-cutaneously implanted. Optimal energy transfer is obtained when the donor-acceptor pair are within 40-70 Å of each other. Red laser diodes (> 635 nm) that permit

30   MHz modulation are used as the light emitter source. Texas Red Hydrazide is used as the donor and Bromothymol blue as the acceptor.

       Yet anther embodiment of this invention is a life-time-based CO_2 sensor.

35   In another embodiment the sensor patch may only be needed for short periods, and does not require a long

indwelling device such as in post surgery recovery. Furthermore, the full implantation procedure in such situations is not cost effective or beneficial. In these cases the sensor 6 may be transcutaneously implanted

5   microspheres or particles implanted beneath the skin by injection. The apparatus may be used to monitor patients in a hospital where each patient's information is preceded by an identifying number. The stored information is entered into a central computer file for all patients

10   at one time, while a local record is printed for the attending physician and the data is transmitted via modem to another location for consultation.

In another embodiment a patient in a remote location can self monitor blood chemistry, or transmit the data to

15   a physician for evaluation. This obviates the need for costly and time consuming visits to laboratories for testing and delays in waiting for the results.

In another embodiment, Emergency Medical Technicians, or military field hospital personnel, can

20   implant the sensor patch and have quick access to various readings to aid in the diagnosis of conditions while in areas remote from analysis facilities or situations where time is critical.

In yet another preferred embodiment, multiple

25   analyte measurement methods could be combined in one instrument, such as the combination of O_2, CO_2, and pH sensors for blood-gas monitoring.

The invention being thus described, it will be obvious that the same may be varied in many ways. Further

30   advances will undoubtedly increase the number of biomedically significant analytes that can be measured by phase fluorometry. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be

35   included within the scope of the following claims.

What is claimed is:

1. A trans-cutaneous analyte monitor, comprising:

5   a light emitting means for emitting an excitation
light having predetermined wavelength and temporal
characteristics;

           a bio-compatible implant, said bio-compatible implant
10   emitting fluorescence having predetermined wavelength and
temporal characteristics upon absorption of said
excitation light emitted from said light emitting means;

           a detector means to detect said fluorescence from
15   said bio-compatible implant, said detector means producing
an electrical signal proportional to said fluorescence
wherein the fluorescence detected by the detector means
varies dependent on a quantity of a biologically
significant substance present;
20

           a comparison means to compare said wavelength and
temporal characteristics of said light emitted form said
light emitting means and said wavelength and temporal
characteristics of said fluorescence from said bio-
25   compatible implant said comparison means receiving input
from said light emitting means and said detector means and
having an output; and

           a computing means to receive said output of said
30   comparison means and relating said comparison of said
wavelength and temporal characteristics of said light
emitted from said light emitting means and said wavelength
and temporal characteristics of said fluorescence form
said bio-compatible skin implant to the quantity of a
35   biologically significant substance present.

2.   The trans-cutaneous analyte monitor of claim 1,
wherein:

said   predetermined   wavelength   and   temporal
5 characteristics of said fluorescence is altered by
collisional quenching caused by the presence of a
predetermined biologically significant substance.

3. The trans-cutaneous analyte monitor of claim 2,
10 further comprising:

said comparison means to compare said wavelength and
temporal characteristics of said light emitted from said
light emitting means and said wavelength and temporal
15 characteristics of said fluorescence from said bio-
compatible implant is by means of intensity based
sensing.

4.   The trans-cutaneous analyte monitor of claim 2,
20 wherein:

said comparison means to compare said wavelength and
temporal characteristics of said light emitted from said
light emitting means and said wavelength and temporal
25 characteristics of said fluorescence from said bio-
compatible implant is by means of time domain lifetime-
based sensing.

5. The trans-cutaneous analyte monitor of claim 2,
30 wherein:

said comparison means to compare said wavelength and
temporal characteristics of said light emitted from said
light emitting means and said wavelength and temporal
35 characteristics of said fluorescence from said bio-
compatible implant is by means of frequency domain

lifetime-based sensing.

6. The trans-cutaneous analyte monitor of claim 1, wherein:

. said bio-compatible implant comprises a fluorescent donor and analyte-dependent acceptor which spectrally overlaps said fluorescent donor, said bio-compatible implant emitting fluorescence of a predetermined wavelength and temporal characteristics upon absorption of said excitation light emitted from said light emitter, said fluorescence wavelength and temporal characteristics being altered when a predetermined biologically significant substance is present.

7. The trans-cutaneous analyte monitor of claim 6, wherein:

said comparison means to compare said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of intensity based sensing.

8. The trans-cutaneous analyte monitor of claim 6, wherein:

said comparison means to compare said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of time domain lifetime-based sensing.

9. The trans-cutaneous analyte monitor of claim 6,

wherein:

said comparison means to compare said wavelength and temporal characteristics of said light emitted from said
5   light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of frequency domain lifetime-based sensing.

10   10.   The trans-cutaneous analyte monitor of claim 1, wherein:

said bio-compatible implant comprises an analyte-free and analyte-bound form of a fluorescent emitter and
15   wherein the ratio of the signals at two excitation or emission wavelengths is used for quantitative measurements of a predetermined biologically significant substance.

20   11. The trans-cutaneous analyte monitor of claim 10, wherein:

said comparison means to compare said wavelength and temporal characteristics of said light emitted from said
25   light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of intensity based sensing.

30   12. The trans-cutaneous analyte monitor of claim 10, wherein:

said comparison means to compare said wavelength and temporal characteristics of said light emitted from said
35   light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-

compatible implant is by means of time domain lifetime-based sensing.

13. The trans-cutaneous analyte monitor of claim 10, wherein:

said comparison means to compare said wavelength and temporal characteristics of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant is by means of frequency domain lifetime-based sensing.

14. The trans-cutaneous analyte monitor of claim 1, wherein:

a display means for displaying said quantity of biologically significant substances.

15. The trans-cutaneous analyte monitor of claim 1, further comprising:

a memory storage device for receiving said output of said computing means.

16. The trans-cutaneous analyte monitor of claim 1, further comprising:

a data transfer means, said data transfer means receiving input from said memory storage device and whereby said quantity of said biologically significant substance present stored in said memory storage device is transferred to a remote receiving station.

17. The trans-cutaneous analyte monitor of claim 16, wherein said data transfer means is a modem for data

transfer to a computer.

18. The trans-cutaneous analyte monitor of claim 16, wherein said data transfer means is an antenna and
5 transmitter to transmit said data to a receiver.

19. The trans-cutaneous analyte monitor of claim 16, wherein said data transfer means is a satellite relay.

10 20. The trans-cutaneous analyte monitor of claim 1, wherein said light emitting means is a light emitting diode.

21. The trans-cutaneous analyte monitor of claim 1,
15 wherein said light emitting means is a laser emitting diode.

22. The trans-cutaneous analyte monitor of claim 20, wherein said light emitting diode emits an excitation
20 light in the infrared wavelength.

23. The trans-cutaneous analyte monitor of claim 22, wherein said light emitting diode emits an excitation light of a wavelength greater than about 600nm.
25
24. A trans-cutaneous analyte monitor, comprising:

a light emitting means for emitting an excitation light having predetermined wavelength and temporal
30 characteristics;

a bio-compatible implant, said bio-compatible implant emitting fluorescence having predetermined wavelength and temporal characteristics upon absorption of said excitation light emitted from said light emitting means;

a detector means to detect said fluorescence from said bio-compatible implant wherein said detector measures the wavelength and temporal characteristics of said bio-compatible skin implant fluorescence, and further wherein said detector emits an electrical signal which is directly proportional to the fluorescence wavelength and temporal characteristics received wherein the fluorescence detected by the detector means varies dependent on a quantity of a biologically significant substance present;

a comparison means to compare said wavelength and temporal characteristics of said light emitted form said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant;

a computing means to relate said comparison of said wavelength and temporal characteristics of said intensity modulated light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence form said bio-compatible skin implant to the quantity of a biologically significant substance present;

a display means for displaying said quantity of biologically significant substances;

a memory storage device for receiving said output of said computing means; and

a data transfer means, said data transfer means receiving input from said memory storage device and

whereby said quantity of said biologically significant substance present stored in said memory storage device.

25. The trans-cutaneous analyte monitor of claim 24, further comprising:

a frequency synthesizer, wherein said frequency synthesizer produces a modulated electrical output:

wherein said light emitting means receives said modulated electrical output of said frequency synthesizer causing said light emitter to emit an intensity modulated excitation light of predetermined wavelength and temporal characteristics;

wherein said detector measures the wavelength and temporal characteristics of said bio-compatible implant fluorescence, and further wherein said detector emits an electrical signal which is directly proportional to the fluorescence wavelength and temporal characteristics received;

wherein said comparison means compares the phase angle difference between said excitation light predetermined wavelength and temporal characteristics and said wavelength and temporal characteristics of said bio-compatible fluorescence; and

wherein said computing means computes the quantity of said biologically significant substance present from said comparison of said light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said bio-compatible implant.

26. The trans-cutaneous analyte monitor of claim 25,

wherein:
        said light emitting means is a light emitting diode.

27.   The trans-cutaneous analyte monitor of claim 25,
wherein:
        said light emitting means is a laser emitting diode.

28.   A method to trans-cutaneously monitor analytes,
comprising:

        emitting a modulated excitation light from a light
emitting means, said excitation light having predetermined
wavelength and temporal characteristics;

        implanting a cio-compatible implant, said implant
emitting fluorescence having predetermined wavelength and
temporal characteristics upon absorption of said
excitation light emitted form said light emitting means;

        detecting said fluorescence wavelength and temporal
characteristics from said bio-compatible implant wherein
the fluorescence detected varies dependent on a quantity
of biologically significant substance present;

        comparing said wavelength and temporal
characteristics of said excitation light emitted form said
light emitting means and said wavelength and temporal
characteristics of said fluorescence form said bio-
compatible implant; and

        computing said wavelength and temporal
characteristics of said light emitted form said light
emitting means and said wavelength and temporal
characteristics of said fluorescence form said bio-
compatible implant to quantify the amount of a
biologically significant substance present in the skin.

28. The method to trans-cutaneously monitor analytes of claim 28, comprising:

    displaying said quantity of biologically significant
5   substance.

30. The method to trans-cutaneously monitor analytes of claim 29, further comprising:

10   storing in a memory storage device said output of said computing means.

31. The method to trans-cutaneously monitor analytes of claim 28, further comprising:
15
    transferring said data from said memory storage device to a remote receiving station.

32. The method to trans-cutaneously monitor analytes of
20 claim 28 wherein:

    said bio-compatible implant comprises a fluorophore wherein said bio-compatible implant emits fluorescence of a predetermined wavelength and temporal characteristics,
25 said fluorescence of a predetermined wavelength and temporal characteristics being altered by collisional quenching caused by the presence of a predetermined biologically significant substance.

30 33. The method to trans-cutaneously monitor analytes of claim 28 wherein:

    said bio-compatible implant comprises an analyte-free and analyte-bound form and wherein the ratio of the
35 signals at two excitation or emission wavelengths is used for quantitative measurements.

34. The method to trans-cutaneously monitor analytes of claim 28 wherein:

said bio-compatible implant comprises a fluorescent
5   donor and analyte-dependent acceptor which spectrally overlaps said fluorescent donor, said bio-compatible implant emitting fluorescence of a predetermined wavelength and temporal characteristics upon absorption of said excitation light emitted from said light emitter,
10   said fluorescence wavelength and temporal characteristics being altered when a predetermined ·biologically significant substance is present.

35. A method to trans-cutaneously monitor analytes,
15   comprising:

synthesizing a modulated electrical output by means of a frequency synthesizer;

20   inputting said modulated electrical output of said frequency synthesizer means into a light emitting means whereby said light emitting means emits an intensity modulated excitation light, said excitation light having predetermined wavelength and temporal characteristics;
25

implanting a bio-compatible implant wherein said bio-compatible implant emits fluorescence of a predetermined wavelength and temporal characteristics upon absorption of said excitation light emitted from
30   said light emitter, said fluorescence wavelength and temporal characteristics being altered when a predetermined biologically significant substance is present;

35   detecting said bio-compatible implant emitting fluorescence of a predetermined wavelength and temporal

characteristics by detection means wherein said detection means emits an electrical signal which is directly proportional to the fluorescence wavelength and temporal characteristics received;

5

comparing said wavelength and temporal characteristics of said excitation light emitted from said light emitting means and said wavelength and temporal characteristics of said fluorescence from said

10   bio-compatible implant;

computing the phase angle difference between said comparison of said light emitted from said light emitting means and said wavelength and temporal characteristics of

15   said fluorescent light from said bio-compatible skin implant to the quantity of said biologically significant substance present and relating said phase angle difference to the quantity of a biologically significant substance present; and

20

displaying the quantity of said biologically significant substance present.

FIG. 1

$$m = \frac{B/A}{b/a}$$

TIME (nanoseconds)

FIG. 2

Excitation   Emission

dye

microcuvette

Intralipid

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FIG.3A

Excitation pulse, Observed pulses in scattered medium. INTENSITY vs TIME. τ

FIG.3B

Exc. modulation. Observed frequency domain responses in scattered medium. Exc. phase angle. PHASE ANGLE (degrees) vs log FREQUENCY. τ
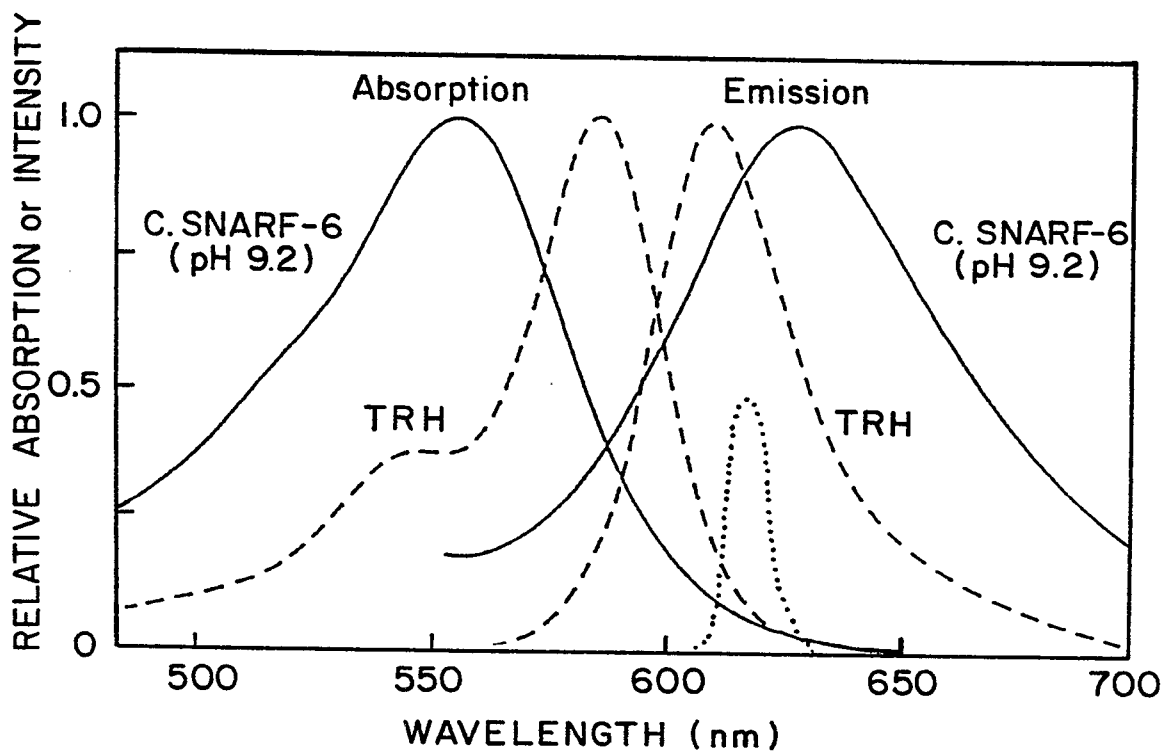
FIG.4

FIG.5

5 / 9

FIG.6

FIG.7

o No intralipid

● In intralipid vs. excitation source

■ In intralipid vs. fluorescent dye

FIG.8

FIG.9

FIG.10

Excitation   Emission

Skin

▼ Glucose

Σ Osmium probe-
Concanavalin A
(Fluorescent Donor)

◐ Malachite
green-Dextran
(Absorbing Acceptor)

SENSOR PATCH

Barrier membrane

FIG.11

FIG.12