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(54) Title: TIME AND FREQUENCY DOMAIN SPECTROSCOPY DETERMINING HYPOXIA

(57) Abstract

The present invention discloses methods and apparatus for the quantitation and localization of tissue hypoxia by both time and frequency domain spectroscopy. The present invention provides several alternate embodiments of apparatus by which the saturation of a tissue region may be determined. In the time-resolved embodiment, a simplified system provides data which are directly proportional to the tissue saturation. In the phase modulating embodiments of the present invention, a first embodiment provides phase shift data which may be converted into saturation readings. A second embodiment separtates the real (270) and imaginary portions (272) of the signal and uses these data along with the data gathered from the DC portion of the signal to determine saturation. Methods of determining the hemoglobin concentration/oxygenation of a tissue region are also disclosed.

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TIME AND FREQUENCY DOMAIN SPECTROSCOPY DETERMINING HYPOXIA

Cross-Reference To Related Applications

This is a continuation-in-part of U.S. Patent Application Serial No. 287,847, filed December 21, 1988, entitled "METHODS AND APPARATUS FOR DETERMINING THE CONCENTRATION OF A TISSUE PIGMENT OF KNOWN ABSORBANCE *IN VIVO* USING THE DECAY CHARACTERISTICS OF SCATTERED ELECTROMAGNETIC RADIATION"; and U.S. Patent Application Serial No. 578,063, filed September 5, 1991 entitled "PHASE MODULATED SPECTROSCOPY," which is a continuation of U.S. Patent Application No. 307,066, filed February 6, 1989, now U.S. Patent 4,972,331, issued July 25, 1990, all of which are incorporated by reference as if set forth in their entireties herein.

Background of the Invention

It is clear that a new field of study is emerging where previous limitations to the quantitation of the concentration of absorptive constituents in scattering media by continuous light (CW) approaches are overcome as information on both absorption and scattering parameters become available in homogeneous tissues, and localization possibilities ameliorate problems that arise with inhomogeneous tissues.

The incidence of hypoxia/ischemia and hemorrhage in pre-term neonates is well recognized and the need for

early detection of these syndromes is apparent from the current studies using ultrasound and nuclear magnetic resonance. Other applications to neonatology, particularly brain hypoxia monitoring during cardiopulmonary bypass and other surgical procedures applied to the heart and even monitoring of brain hypoxia of the infant in the birth canal as affected by uterine contractions in prolonged deliveries signify other requirements for a reliable method of quantifying oxy-hemoglobin concentrations *in vivo*, in real time. In adults many similar applications emerge, ranging from brain hypoxia in surgical procedures of cardiopulmonary bypass or AICD testing (ventricular defibrillation), including monitoring of oxygenation of recently transplanted, liver, pancreas, etc., to the detection of altered blood flow or lack of blood flow in chronic brain disease such as Alzheimer's, Parkinson's, and multiple infarct dementia (MID). All of these applications dictate an apparatus which can be readily applied to the exposed tissue and collect sufficient data in times as short as the few seconds that may be required to make a significant reading; time is often of the essence in clinical diagnosis and decision making. Thus calibration procedures, etc., must either be made subsequent to the measurement or the system itself should be rapidly auto-calibrating during the study.

A completely different field of applications which also reflect the need for a reliable quantitative measure of oxy- or deoxy-hemoglobin is to the exercising human body either in a confined exercise test such as rowing ergometry, bicycle ergometry, or in strength testing devices, etc., where the motion of the muscle during contraction requires that the unit be firmly attached to the overlying skin. In this case, setup time prior to the exercise should be minimal and recordings of steady state deoxygenation of the muscle bed during exercise and the transient recovery following the exercise is required. Typical applications are to the testing of national rowers,

to the triathlon (swimming, running and cycling), or equally important, the training and rehabilitation of muscle function following vascular surgery and the study of muscle atrophy due to extended bed rest, geriatric conditions, or space travel.

Thus, it is clear that a device which makes rapid and reproducible readings of hemoglobin deoxygenation and hemoglobin concentration is highly desirable. To be practical, however, such a device requires a high signal-to-noise ratio and a measurement algorithm that is highly robust, with some possibilities for localization.

The brain cortex and larger muscles of the leg (*Vastus Lateralis*, etc.) are relatively homogeneous whilst the inner layers of the brain and the muscles of the forearm are heterogeneous. Furthermore, diseased tissue, infarcted brain, the necrotic portion of tumors represent heterogeneities that are of especial interest in themselves and indeed if included in the measurement of neighboring tissues would give erroneous values of absorption and scattering. Thus, knowledge of photon propagation in tissues and judicious placement of input/output coupling is required for accurate spectroscopy, or acquisition of data sets appropriate for construction of an image.

In principle, time-resolved spectroscopy converts the measurement of concentration or intensities by transmitted or reflected light to the measurement of photon migration time delay or path length. This enables quantitation of concentration changes in highly scattering tissues which was not heretofore possible. The characteristics of such devices and its principles are described, for example, in the co-pending patent applications referenced above.

Summary of the Invention

The present invention utilizes a novel combination of the technology of time and frequency domain spectroscopy to provide a system for imaging hemoglobin

deoxygenation that fulfills and the medical/surgical requirements for patient monitoring and decision making discussed above.

Thus, in a preferred embodiment of the preferred invention a time sharing, time resolved spectroscopic apparatus for the quantitation of hemoglobin is disclosed which comprises a laser that transmits pulses of light alternately between two wavelengths via a fiber coupler into the subject. A detector receives migrating light and creates a signal which is amplified and then transmitted to a discriminator to provide TTL pulses. The apparatus uses a time to amplitude convertor to create amplitude signals which are then synchronized with the alternations of the frequency and transmitted to two dedicated multichannel analyzers which create an output signal. Finally, a signal processor converts the output signals into a ratio of the terminal slopes of the intensity of the pulses over time and this ratio is directly proportional to the hemoglobin saturation in the tissue of the subject.

In another preferred embodiment of the present invention frequency domain spectroscopic apparatus are provided which can provide quantitation data for the hemoglobin concentration in the tissue of the subject. In this embodiment, a laser and detector are again used to alternately pulse two wavelengths of electromagnetic radiation into a subject where the received signals are amplified. A phase detector is used to determine the phase shift between the transmitted pulse and the output signal detected. An electronic switch means which is synchronized

with the alternations of the pulses separates the alternate wavelengths and transmits them into a signal processor which create output signals indicative of the sum, the difference and a ratio of the phase signals. These data can be converted into a value of hemoglobin saturation.

In still another embodiment of the present invention, frequency domain spectroscopic apparatus for the quantitation of hemoglobin concentration and in tissue region are disclosed which again use an alternately pulsed laser and detector to transmit light through the tissue of a subject. The received signal is again amplified and is then transmitted to two double balanced mixers as well as being transmitted to a phase detector. The double balanced mixers also receive a reference phase shift signal which has been set to zero and fed through a 90° splitter. The output of each of the double balanced mixers is transmitted to sychronizing circuits which respectively separate the real and imaginary portions of the signal for each of the two wavelengths. The phase detector signal is also transmitted to a synchronizing circuit which separates the signal into DC portions corresponding to each of the respective wavelengths. Finally, signal processing means are provided which obtain the process signals indicative of the phase shift and amplitude of the detected signal. These processed signals can then be converted into signals indicative of the modulation index of the tissue at each of the wavelengths.

Methods of determining the concentration of hemoglobin within a tissue region are also disclosed.

Brief Description of the Drawings

FIG. 1 is a schematic representation of a time-resolved spectrophotometer.

FIG. 2 is a schematic representation of a frequency domain spectrophotometer.

FIG. 3 is a schematic representation of a time-shared/phase modulated spectrophotometer.

Detailed Description of the Preferred Embodiments

The availability of pulsed laser diode light sources and the knowledge that large tissue volumes such as the adult head exhibit photon migration lasting 5-10 nsec open the possibility that "slow" detection systems such as the squirrel cage photomultipliers, become extremely attractive especially since the latter can be obtained with extended red response which is most suitable for tissue measurements. Silicon diodes, especially of the avalanche type, are of adequate speed but have such a small sensitive area that the need for multiple detectors or appropriate light gathering systems has currently limited their application to large tissue volumes such as adult brain, etc. These components, together with simplified photon counting systems, make time-resolved systems practical for portable use. In fact, currently available tissue spectrophotometers can readily be converted to time domain

spectroscopy by the use of pulsed laser diodes and improved photon counting technology.

The configuration of a simplified and portable time domain system for tissue studies is shown in the block diagram of FIG. 1, where a "squirrel cage" photomultiplier detector 10 and a laser diode light source 20 are employed. This system serves the need for a more sophisticated and compact time correlated single photon counting (TCSPC) system. As shown in FIG. 1, in a preferred embodiment of this system, Hamamatsu PLP-10 pulsed laser diodes 22, 24 are operated at 10 MHz repetition frequency and at wavelengths of 754 nm and 810 nm. The laser diodes 22, 24 are driven by a 100 MHz pulse generator 18 connected to a 5 mW pulser 19 that drives both diodes. In this embodiment, a fiber coupler 28 conducts pulses of light into the subject 50. The light from the two laser diodes 22, 24 is time shared electromechanically by a 60 Hz vibrating mirror 26 so that they alternately illuminate the fiber coupler 28. The transmitted photons migrate through the subject 50 to the detector 10. Using this configuration the extended red sensitive squirrel cage photomultiplier 10 can be employed for studies of human brain at input/output fiber separations of greater than 5 cm as shown. The instrument function shows a 1 nanosecond FWHM response for this detector. The R928 photomultiplier tube 10 for non-imaging spectroscopy can be coupled directly to the forehead to provide a detector area of 200 square millimeters. In an alternate embodiment, a fiber optics coupling (not shown)

with an area of 20 square millimeters can be employed -- with an obvious decrease in the signal to noise ratio, but providing increased spatial resolution.

The output of the photomultiplier tube 10 is directly connected to a wide band amplifier 12 with appropriate roll-off to give good pulse shape and optimal signal to noise ratio. A high/low level discriminator 13 receives an output signal from the amplifier 12 and gives TTL pulses to a time to amplitude convertor (TAC) 14. Following the time to amplitude conversion, the counts corresponding to the two wavelengths are separately summed in two multichannel analyzers (MCA) 30, 32. The outputs of the multichannel analyzers 30, 32 can be used to calculate signals 40, 41 indicative of the terminal slopes: $\mu_a\lambda_1$ and $\mu_a\lambda_2$. These are divided in a division step 44, since their ratio is simply converted into the saturation of hemoglobin. The pulses are then preferably accumulated in a 1,000 bin multichannel analyzer 46 over a sufficient interval so that approximately 10^5 counts are collected at the maximum in order that the logarithmic slope be followed down for three or four decades of intensity. The stored information on the slopes of the two wavelengths is then processed by creating a set of ratios and a logarithm is employed in order to conveniently calculate saturation using the formula:

$$38-17\frac{\mu_a^{754}}{\mu_a^{816}}$$

$$Y\ (x\ 100\%) = \frac{}{25+9\ \frac{\mu_a^{754}}{\mu_a^{816}}} \qquad (1)$$

This embodiment of the present invention also permits the scattering factor to be calculated, particularly when fits to the data have been obtained by a diffusion equation. If input/output distances smaller than 5 centimeters are desired, then the input function can be convoluted with the solution to the diffusion equation, which then is fitted to the experimental curve. An instrument made in accordance with this embodiment of the present invention has attractive possibilities of portability, and the readout algorithm for saturation is readily computed from the extinction coefficients according to the equation set forth above. This embodiment, with the aid of an extended red sensitive photomultiplier, can accumulate satisfactory data in several minutes, but does not approach the speed of a phase modulated system.

As understood by those of ordinary skill, the data collected and signals obtained by the embodiment described above can be further analyzed. The Fourier transform of the domain kinetics give phase and amplitude compounds that contain all the time domain information, and adequate information for concentration determinations in the dual wavelength mode, which is available for both.

The present invention also provides novel frequency domain systems which determine hemoglobin concentrations. Phase modulation spectroscopy has been shown to be well adapted to the measurement of photon migration parameters in the human brain and in model systems. Multi-frequency systems are available and have proved invaluable in this research for studies of the frequency/phase diagrams and for phase modulation imaging of tissues. It has been shown to be desirable to design simple phase modulation systems for clinical applications.

Such designs however, require significant precision, since, as discussed above, it is convenient to employ frequencies within the range of the squirrel cage photomultiplier in systems where the limitations of the photodetector frequencies to 200 MHz, phase shifts of the order a few degrees, and path length changes of a few centimeters are characteristic. Thus, oscillator precision, leakage of excitation signal into the receiving channel, drift of the phase detector, and cross-talk between the channels representing the two wavelengths employed have all been problems which can be readily solved by the present invention, as described below.

A simplified system for frequency domain studies is illustrated in FIG. 2. Rather than using a pulse generator, this embodiment utilizes a 200 MHz precision oscillator 118 which drives two laser diodes 22, 24, again at 760 and 816 nm, the outputs of which are time shared into a fiber optic coupling 28 to the head 50 as illustrated. At this frequency, the input/output separations can be varied from 10-5 cm as desired and either the total output from the sensitive area of photocathode (200 square millimeters) can be used or that of a fiber optics coupling from a smaller area, as in the case of the time domain system described above with reference to FIG. 1. Whilst usually an oscillator displaced some 20 KHz from the 200 MHz oscillator is used to provide a low frequency heterodyne signals, as described in my co-pending patent application referenced above, the availability of wide band phase detectors makes it attractive to couple the 200 MHz signal directly into such a wide band phase detector chip 160 as indicated in FIG. 2, with time shared outputs corresponding to the two light intensities. In order to demodulate these outputs, an electronic switch 162 synchronized with the vibrating mirror 26 is employed so that the phase delay at the two wavelengths is available as the ratio the difference, or

the sum for appropriate calculations of the saturation according to the equality:

$$\frac{\theta^{\lambda_1} - \theta_0^{\lambda_1}}{\theta^{\lambda_2} - \theta_0^{\lambda_2}} = \frac{\mu_a^{\lambda_1}}{\mu_a^{\lambda_2}}$$

Research has shown that θ_0 can be determined from the hemoglobin free scattering properties of the brain tissue. Thus, the three outputs of the electronic switch 162 may be fed to processing circuits 164, 166 which create a sum and difference respectively. The phase outputs are also combined in a circuit 168 to create a ratio. The selection and construction of the logic devices, e.g., the integrated circuits disclosed herein and the like, is well known to those of ordinary skill.

The term θ_0 limits the applications of single-frequency modulated spectroscopy to tissues where $(1-g)\mu_s$ can be estimated *a priori* and is not expected to change such as might happen during disease, treatments such as radiotherapy, or even transfer from animal models to tissue. There are, however, two approaches to utilizing the approximation which circumvent this problem: (i) to employ an additional third wavelength; and (ii) to employ dual-wavelength, dual-frequency phase-modulation techniques. In the first approach, the ratio of absorption coefficients for two sets of wavelengths are used to solve for θ_0 such that both ratios of absorption coefficients predict identical hemoglobin saturations, Y. In the second approach, measurement of phase shifts at dual-wavelength and dual-frequencies, where $2\pi f_1$, $2\pi f_2 >> \mu_a^{\lambda 1} c$, $\mu_a^{\lambda 2} c$, can give information of hemoglobin saturation from transmittance geometries.

Thus, a preferred embodiment of the invention disclosed herein employs phase modulated spectrophotometers capable of time-shared, dual-wavelength, dual-frequency measurements.

Referring still to FIG. 2, a block diagram of a time shared phase modulation system in which two wavelengths are available is shown. This system provides appropriate sums and differences and ratios of the signal utilizing a vibrating mirror 26 for time sharing of the two laser wavelengths. This simplifies the oscillator system, as only two are required and only one phase detector. The difference or ratio circuits afford cancellation of common mode errors to a great extent affording high performance with a simple system.

An alternate way of handling the data output from the system of FIG. 2 is illustrated in FIG. 3 where the detector output is put through two wide band double balance mixers (DBM) 270, 272 which are fed through a 90° phase splitter 274 so that real (R) and imaginary (I) portions of the signal are obtained. The double balance mixers 270, 272 preferably operate at the modulation frequency. The phase ϕ is the angle whose tangent is the imaginary over real part, whilst the amplitude is the square root of the sum of the squares of these values, providing the phase shift has been taken out as the residual phase shift ϕ set to zero as indicated. Thus this embodiment of the present invention provides the modulation index, which is the quotient of the amplitude over the amplitude plus the DC component obtained from a narrow band detector 276 as shown in FIG. 3. A synchronous detector de-codes the phase shifts for the phase and amplitude values for the two wavelengths so that the ratio of the phase shifts may be obtained as indicated in the previous diagrams. In order to obtain the proper functions, the ratio of the θ's and the correct value of amplitude, a ratio circuit divides the I and R terms and the angle is computed; in this case, the small angle approximation is valid and the ratio circuit

computes $\theta\lambda_1/\theta\lambda_2$ as required for the equation set forth with reference to Fig. 2 above. In the case of the amplitude function, the square root of the sum of the squares are computed to obtain the amplitude and the summing and dividing circuits calculate the modulation index at the two wavelengths.

Some of the components of the embodiments of the present invention described above with reference to Figs. 1-3 are of especial interest, e.g., the requirements for the laser diodes 22, 24 are that the low power, 5 mW laser diodes be modulated near 100% to give signals appropriate for tissue studies. The phase modulated information is obtained from a significant volume of tissue surface. A second and important feature is the stability of crystal oscillators requiring appropriate electronic construction and tuning parameters. The performance values are drift of 0.2mm/hr and phase noise < 0.9mm in a 0.1 Hz band width. Signal changes are usually 1-2 cm in a 23 cm path length. Wavelengths of the laser diodes 22, 24 are preferably on opposite sides of the cross over or isosbestic point between the oxy- and deoxy- hemoglobin absorption spectrum (about 800 nm) are used so that the difference of the signals represents a change on deoxygenation whilst the sum represents the total amount of hemoglobin present of the desirable quantities mentioned above.

Data display is of considerable importance to the functioning of the apparatus of the present invention, as is computer coupling. A running time LCD display is preferably used for monitoring in order to ensure that signals are within the linear range of the phase detector, together with a LED indicator, indicating that the signal amplitude does not reach predetermined limits. In addition, the computer coupling to obtain manipulated data from wave saturation may be obtained as necessary.

Various formulations by which the path length changes measured in time and frequency domain studies can be simply converted into concentration changes using

appropriate wavelengths and appropriate extinction coefficients have been presented. Whilst the biochemist requires the determination of a tissue concentration, the physiologist is content with the saturation percentage change of one form with respect to another, usually the oxy with respect to the total (oxy + deoxy) in the case of hemoglobin. These algorithms generally involve the ratio of the path lengths determined at a pair of wavelengths as indicated in the discussion above. However, those of ordinary skill will appreciate that other absorbers can also be studied. For example, injected indocyanine-green (a flow indicator), or naturally occurring absorbers such as fat, protein, water may be studied by photon migration techniques such as those utilized by the present invention.

The coupling of the system to the subject 50 is of importance and whilst this can be done satisfactorily with plastic light guides 28, usually of an area for receiving of 2 square centimeters, direct contact of a large area detector with the subject's skin is desirable if the input/output separation exceeds 5-10 cm, as is the case in an adult. Similarly, whilst spectroscopy requires signal acquisition from large surface areas, imaging upon this area sets limitations to about 1 cm signals from a large number of points around the circumference of the human head are desirable for planar imaging of brain bleeding.

The performance of the phase modulation systems disclosed above have been experimentally determined, together with quantification of the drift, noise and other parameters. For example, the performance of the laser diode light source and squirrel cage detector as applied to a model system and a human head have been noted. While animal models provide excellent systems for quantifying the performance of both time resolved and phase modulated systems the exercising human muscle is optimal for the validation of the functionality of the system, particularly

in terms of the signal-to-noise ratio in an actual *in vivo* system.

This application has presented three systems for time and frequency domain spectroscopy, each one capable of measurement of hemoglobin saturation and blood volume. The most comprehensive measurement is given by that of Figure 1 since the entire time profile of photon decay is measured and if measured at longer times, is independent of μ_s. Perhaps the most important feature of the instrumentation is that the variation of time delay with photomultiplier voltage is not as a primary factor, as long as the logarithmic slopes, μ_a values, are read out at longer times, i.e., 5-10 ns. Thus, this system is optimal for the adult human head where long path lengths and large separations of input/output are available.

Systems of Figures 2 and 3 are suitable for shorter path lengths as are observed in skeletal muscle or neonates. The system of Figure 3 gives comprehensive information which if available at a number of frequencies and appropriately Fourier transformed would have the same information content as the pulse time method. When restricted to particular carrier frequencies, the method nevertheless retains its quantitation of hemoglobin saturation through the ratio of the θ values and affords in addition the modulation index. Thus, this unit would have unique properties for imaging, brain bleeding, or other localized depots of hemoglobin. The system is in effect to be significantly faster than that of Figure 1 by a factor of 10 or perhaps more.

The system of Figure 2 is the simplest system requiring only a small number of chips to afford the ratio of phase shifts necessary for the calculation of saturation. This system is also quite fast, time constants of 5 sec probably being appropriate for brain recording.

The system of Figure 1 is not expected to need calibration except for its own instrument function which is expected to be constant for a given dyode voltage on the

photomultiplier and to vary insignificantly with small changes thereof. The system of Figures 2 and 3 will require calibration for the values of θ although it should be noted that the ratio of these values is acceptable and thus the calibration errors will only be of secondary importance. Obviously, the rapid development of available electronic circuitry, particularly in the region of 100-500 MHz will result in further simplifications of the apparatus displayed here. At the present time, the use of time shared laser diode wavelengths and the calculation from the ratio of phase shifts of the saturation value allows much greater freedom from drift and background signals than for a single wavelength system. Obviously, as many wavelengths as desired can be obtained by time sharing.

The goal of the certain preferred embodiments of the present invention therefore, is to indicate what is needed in order to acquire appropriate data for the study of brain tissue hypoxia. The first requirement is obviously of a system of adequate accuracy and reproducibility; the requirements being approximately 1 mm in distance or 0.001 in logarithmic slope (i.e., a corresponding value in mm). Absolute stability is less stringent, but nevertheless, the measurement requires the difference or ratio of slopes for the determination of saturation and for the absolute concentration. The second major requirement is to select wavelengths at which an adequate signal is generated, i.e., one in which the change of hemoglobin saturation or concentration gives a significant change of path length. Also, ready calibration is necessary. For animal models, 100% change can readily be obtained by ischemia and hypoxia, however, for the study of human subjects, the range from 40% to 80% saturation is the maximum that could be expected under conditions of patient stability. The "normal" variations may be 1/5th of this for 8 to 10%. Thus, the "oxygen saturation" of the brain study requires a very high level of stability and reproducibility and stable calibration.

Certain embodiments of the present invention can determine hemoglobin saturation from measurements of phase-shift and modulation at varying modulation frequency. It has been found that μ_a^{λ} may be identified from reflectance and transmittance measurements of phase-shift, θ, and demodulation of the detected signal, M, as a function of modulation frequency, f. Theoretical considerations show that the critical phase and modulation frequencies, f_o^{θ} and f_o^{M}, at which the magnitude of the θ and M versus f slopes reach minima, are functions μ_a alone, regardless of $(1-g)\mu_s$ or of the source and detector configuration. From the ratio of critical frequencies identified from phase-shift and modulation spectra, the ratio of absorption coefficients can be found:

$$\frac{f_o^{M,\lambda_1}}{f_o^{M,\lambda_2}} = \frac{f_o^{\theta,\lambda_1}}{f_o^{\theta,\lambda_2}} = \frac{\mu_a^{\lambda_1}}{\mu_a^{\lambda_2}}$$

Sensitivity of this technique increases with enhanced tissue scattering and minimal absorption as is the characteristic of the brain. Thus, f_o^{θ} and f_o^{M}, might be identified from experimental spectra of media with physiological scattering properties to determine whether differential frequency-resolved spectroscopy may be used to accurately quantitate tissue oxygenation.

Although certain embodiments of the present invention have been set forth above with particularity, the invention is by no means limited to these embodiments. Upon review of the instant specification those of ordinary skill will realize numerous variations or adaptations to the methods and apparatus disclosed. For example, certain modifications can be made to the circuits disclosed or their application which still lie within the spirit of the invention disclosed. Accordingly, reference should be made to the appended claims in order to determine the scope of the present invention.

What is claimed is:

1. Time-sharing, time resolved spectroscopic apparatus for the quantitation of hemoglobin concentration in a tissue region of a subject comprising:

laser means for transmitting pulses of electromagnetic energy alternately at two predetermined wavelengths which alternate at an alternating frequency fiber coupler means for transmitting the pulses to the subject;

detector means for receiving the pulses after migration through a tissue region, the detector means including a photomultiplier tub and creating an output signal;

amplifier means for amplifying the output signal and transmitting an amplified signal to a discriminator means for providing TTL pulses;

a time to amplitude convertor for creating amplitude signals corresponding to each of the alternately transmitted wavelengths;

a synchronizing circuit synchronized with the alternating frequency for transmitting the amplitude signals to dedicated multichannel analyzers corresponding to each wavelength and each creating an output signal; and

signal processor means for converting the output signals into a ratio of the terminal slopes of the intensity of the pulses over time;

whereby the ratio is directly proportional to the hemoglobin saturation in the tissue of the subject.

2. The apparatus of Claim 1, wherein the laser means comprises a pulse generator and a pulser for producing pulses of electromagnetic radiation.

3. The apparatus of Claim 2, wherein the pulse generator operator at about 100 MHz and the pulser produces about 5 mW.

4. The apparatus of Claim 1, wherein the predetermined wavelengths are about 754 mm and about 810 nm.

5. The apparatus of claim 1 wherein the fiber coupler means and the detector means are separated by more than about 5 cm.

6. The apparatus of Claim 1, wherein the predetermined wavelengths are alternated by mechanical means comprising a vibrating mirror.

7. The apparatus of Claim 1, wherein the ratios of the terminal slopes are stored over time to create a data set and the signal processor calculates saturation, Y, according to the formula:

$$Y\ (\times 100\%) = \frac{38-17\frac{\mu_a^{754}}{\mu_a^{816}}}{25+9\frac{\mu_a^{754}}{\mu_a^{816}}} \qquad (1)$$

8. A method of quantifying the hemoglobin concentration in the tissue of a subject comprising the steps of:

creating alternate pulses of electromagnetic energy at two predetermined wavelengths which alternate at an alternating frequency;

transmitting the pulses to the subject;

detecting the pulses after migration through the tissue of the subject and creating an output signal;

amplifying and processing the output signal to create TTL pulses;

converting the pulses to amplitude signals corresponding to each of the alternately transmitted wavelengths;

synchronizing the transmission of the amplitude signals to multichannel analyzers which create and output signal; and

processing said output signal into a ratio directly proportional to the hemoglobin saturation in the tissue of the subject.

9. The method of Claim 8, further comprising the steps of the storing the processed signals as a data set over time and calculating saturation, Y, according to the formula:

$$Y\ (\times 100\%) = \frac{38-17\dfrac{\mu_a^{754}}{\mu_a^{816}}}{25+9\dfrac{\mu_a^{754}}{\mu_a^{816}}}$$

10. Frequency domain spectroscopic apparatus for the quantitation of hemoglobin concentration in a tissue region of a subject comprising:

laser means for transmitting pulses of electromagnetic energy alternately at two predetermined wavelengths which alternate at an alternating frequency fiber coupler means for transmitting the pulses to the subject;

detector means for receiving the pulses after migration through a tissue region, the detector means including a photomultiplier tub and creating an output signal;

amplifier means for amplifying the output signal and transmitting an amplified signal;

phase detector means for determining the phase shift between the transmitted pulse and the output signal of the detector;

electronic switch means, synchronized the alternating frequency, for creating phase signals corresponding to each of the alternating wavelengths; and

signal processing means for the processing the phase signals to create output signals indicative of the sum, the difference and a ratio of the phase signals, whereby the output signals are converted into a value for the saturation of hemoglobin.

11. The apparatus of Claim 10 wherein the laser means comprises an oscillator and a driver for producing pulses of electromagnetic radiation.

12. The apparatus of Claim 11, wherein the oscillator operates at 200 MHz and the driver produces about 5 mW.

13. The apparatus of claim 10 wherein the predetermined wavelengths are about 754 mm and about 810 nm.

14. The apparatus of claim 10 wherein the fiber coupler means and the detector means are separated by more than about 5 cm.

15. The apparatus of claim 10 wherein the predetermined wavelengths are alternated by mechanical means comprising a vibrating mirror.

16. The apparatus of Claim 10, wherein the output signals are converted to a saturation value according to the formula:

$$\frac{\theta^{\lambda_1} - \theta_0^{\lambda_1}}{\theta^{\lambda_2} - \theta_0^{\lambda_2}} = \frac{\mu_a^{\lambda_1}}{\mu_a^{\lambda_2}}$$

17. A method of quantifying the hemoglobin concentration in the tissue of a subject comprising the steps of:

creating alternate pulses of electromagnetic energy at two predetermined wavelengths which alternate at an alternating frequency;

transmitting the pulses to the subject;

detecting the pulses after migration through the tissue of the subject and creating an output signal;

amplifying and processing the output signal to create TTL pulses;

amplifying and processing the output signal to determine the phase shift between the transmitted pulse and the output signal of the detector to create a phase signal;

synchronously switching the phase signal at the frequency the wavelengths are alternated to produce phase signals corresponding to each of the wavelengths;

processing the phase signals to create output signals indicative of the sum, difference and a ratio of the phase signals; and

converting the output signals into a value for the saturation of hemoglobin.

18. The method of Claim 17, further comprising the steps of calculating a saturation value according to the formula:

$$\frac{\theta^{\lambda_1} - \theta_0^{\lambda_1}}{\theta^{\lambda_2} - \theta_0^{\lambda_2}} = \frac{\mu_a^{\lambda_1}}{\mu_a^{\lambda_2}}$$

19. Frequency domain spectroscopic apparatus for the quantitation of hemoglobin concentration in a tissue region of a subject comprising:

laser means for transmitting pulses of electromagnetic energy alternately at two predetermined wavelengths which alternate at an alternating frequency

fiber coupler means for transmitting the pulses to the subject;

detector means for receiving the pulses after migration through a tissue region, the detector means including a photomultiplier tube and creating an output signal;

amplifier means for amplifying the output signal and transmitting an amplified signal;

quadrature reference signal means for providing a reference phase signal which may be set to a predefined value and create a reference phase signal;

first and second double balanced mixers for receiving input signals from the amplifier and for receiving the reference phase signal, wherein the mixers to create a real output signal and an imaginary output signal;

synchronous detector means for decoding the phase shifts for the phase and amplitude values for the two wavelengths;

signal processing means for providing a signal indicating the DC component of the amplified signal corresponding to each wavelength;

signal processing means for synchronously separating the real output signal and the imaginary output signal for each wavelength; and

signal processing means for obtaining processed signals indicative of the phase shift and amplitude, whereby the processed signals are converted into signals indicative of the modulation index of the tissue at each of the wavelengths.

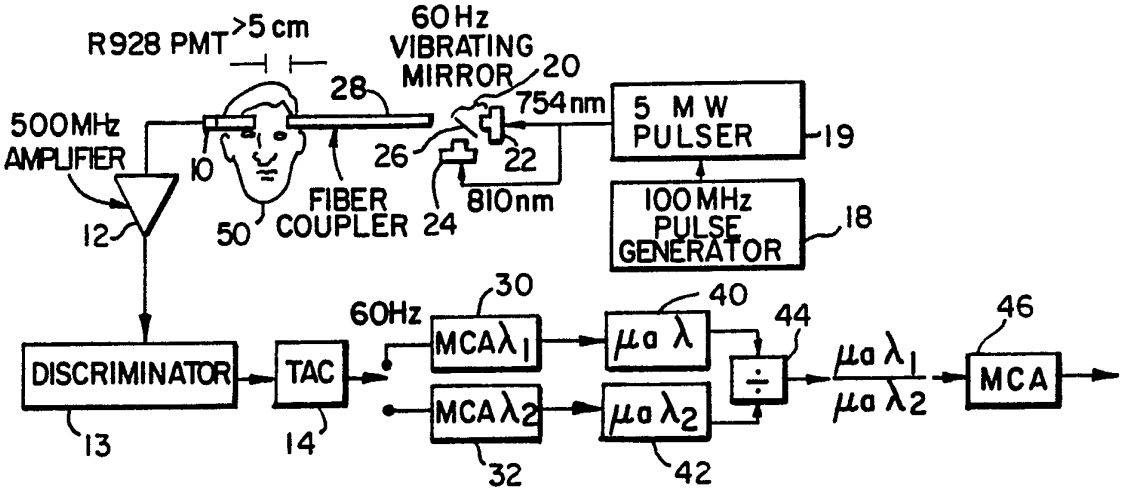

R928 PMT
>5 cm
60 Hz
VIBRATING
MIRROR
20
28
754nm
5 MW
PULSER
19
500MHz
AMPLIFIER
10
26
22
12
50
FIBER
COUPLER
24
810nm
100MHz
PULSE
GENERATOR
18
30
40
44
46
60Hz
MCAλ1
μa λ
DISCRIMINATOR
TAC
MCAλ2
μa λ2
μa λ1
μa λ2
MCA
13
14
32
42

FIG. 1

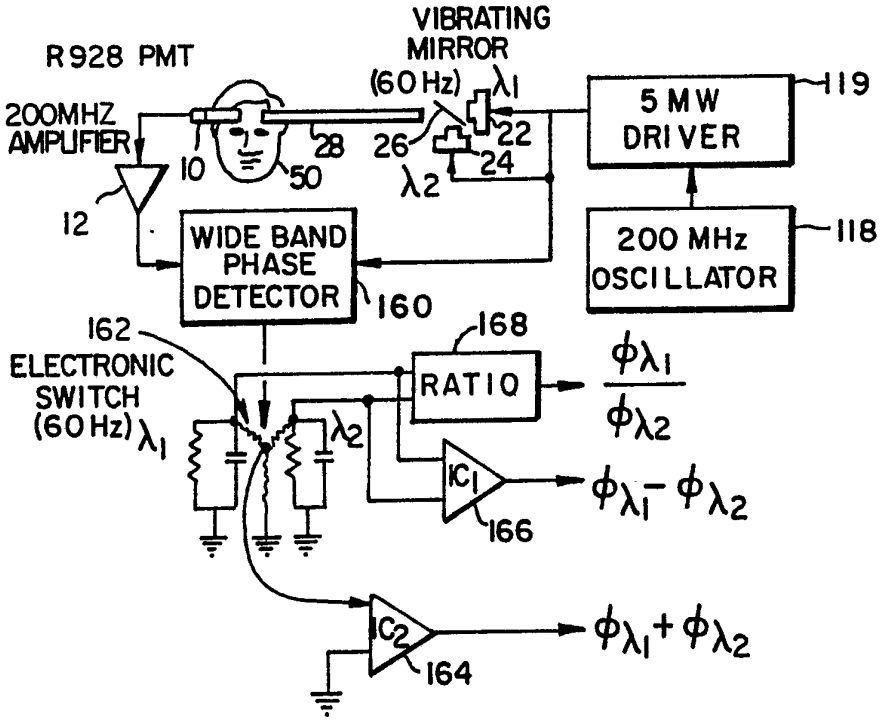
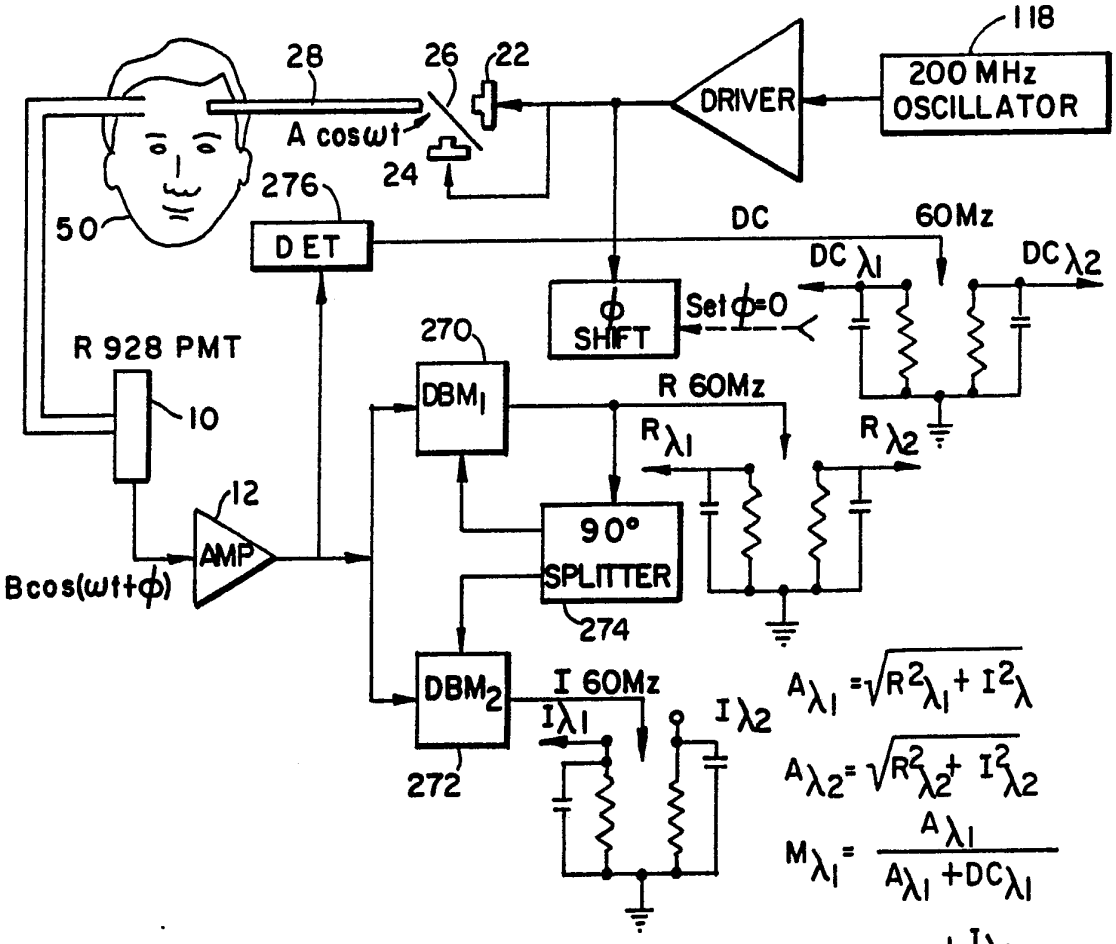

VIBRATING
MIRROR
R928 PMT
(60 Hz)
λ1
5 MW
DRIVER
119
200MHZ
AMPLIFIER
10
28
26
22
50
24
λ2
12
WIDE BAND
PHASE
DETECTOR
160
200 MHz
OSCILLATOR
118
162
168
ELECTRONIC
SWITCH
(60 Hz) λ1
RATIO
φλ1
φλ2
λ2
IC1
φλ1 - φλ2
166
IC2
φλ1 + φλ2
164

FIG. 2



$$A_{\lambda 1} = \sqrt{R^2_{\lambda 1} + I^2_{\lambda}}$$

$$A_{\lambda 2} = \sqrt{R^2_{\lambda 2} + I^2_{\lambda 2}}$$

$$M_{\lambda 1} = \frac{A_{\lambda 1}}{A_{\lambda 1} + DC_{\lambda 1}}$$

$$\frac{\theta_{\lambda 1}}{\theta_{\lambda 2}} = \frac{\tan^{-1} \frac{I_{\lambda 1}}{R_{\lambda 1}}}{\tan^{-1} \frac{I_{\lambda 2}}{R_{\lambda 2}}}$$

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/00463

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5) A61B 5/00 US:128/633

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US.	128/633,664-666;364/413.09,447,525,550,554,575;356/39,40 40,318,319,346,333;250/338.5,339-341,393-395,206,564,565, 208.5

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	J.OPT. Soc. Am, A, Vol. 4, No.3, March 1987, R.F Bonner et al., "Model for photon migration in turbid biological media" pp.423-432	1-19
Y	Photon Migration in Tissues, Academic Press, N.Y. 1989, J.R. Lackowicz "Gigaherty Frequency Domain Flourimitry: Resolution of Complex intensity Decays, Processes and future Developments" pp 169-186	1-19

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 June 1992	27 JUL 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	David Shay