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REVIEW ARTICLE

Phase measurement of light absorption and scatter in human tissue

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Analog and digital technologies are presented for precise measurement of propagation delay of photons from source and detector placed on portions of the human body. The goal of the apparatus design is to quantify absorption (μ_a) and scattering (μ_s') induced by biological pigments and biological structures, respectively. Body tissues are highly scattering with a mean distance between scatterers of less than a mm (at 700–850 nm). Significant absorption is mainly due to 5%–10% of the tissue volume occupied by blood. Measurement of μ_a and μ_s' is done by both time and frequency domain equipment. This article focuses upon frequency domain equipment because of its simplicity, reduced noise bandwidth, versatility, and the strong analogy to very high frequency/ultrahigh frequency communication devices, particularly those using phase modulation. Comparisons are made of homodyne and heterodyne systems together with evaluation of single and multiple side band systems, with particular emphasis on methods for multiplexed optical and radio frequencies by frequency encoding or time-sharing technologies. The applications of these phase modulation systems to quantitative brain and muscle blood oximetry, functional activity of the forebrain, and other important problems of medical science, are presented. [S0034-6748(98)01110-1]

I. BACKGROUND OF THE PROBLEM SET TIME: INTRODUCTION TO THE NATURE OF THE MEDICAL DEVICES

The burgeoning interest in medical devices that are safe, economical, and efficacious, combined with recent observations of the feasibility of time domain optical measurements in human and animal brain, has led to significant interest in, and many publications on, the optical characteristics of human tissues,[1] particularly breast tissue,[2] brain tissue,[3,4] and skeletal muscle.[5] These novel techniques not only allow the measurement of the tissue absorption but also allow the independent measurement of tissue scatter which can also be of great clinical significance.[6,7] Most recently, flow, particularly of blood cells through tissue, can be detected using autocorrelation techniques in the time domain.[8] These reduced transport scattering (μ_s') and absorption (μ_a) parameters are measured in what is termed the near infrared (NIR) window lying between the declining absorption of blood and the increasing absorption of tissue water in the wavelength region 700–900 nm (see Fig. 1). In this region, scattering dominates absorption by 2 orders of magnitude and the average distance between isotropic scattering events is approximately 1 mm, while photon propagation pathways in the human head may readily reach 1 m enabling the sampling of large tissue volumes.[9] Thus, measurements at various wavelengths can separate the contributions of the parameters to the total absorption and scattering. For example, for oxy Hb, deoxy Hb, and water three wavelengths will suffice, and since rapidly modulated light is advantageous, laser diodes are most frequently employed as monochromatic light sources at strategic wavelengths in the NIR window, usually 750, 816, and 830 nm for blood studies.

The most important feature of the optical method is the

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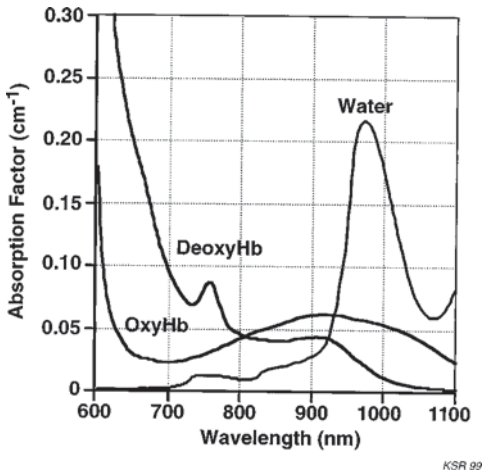

FIG. 1. Near infrared attenuation [$\log_{10}$] for 1 cm depth deoxy hemoglobin (Hb), HbO_2, and water; hemoglobin concentration 210 μM in water.

multiplicity of tissue parameters that can readily be measured. These characteristics are used to identify the presence and the type of a tumor, the presence of brain hemorrhage,[10] aneurysm, etc., all detected by their relative increase of blood content and the deoxygenated state of blood.[11] Scattering itself is an important parameter.[6] One of the principal scatterers is determined to be the numerous mitochondria inside the cells (cardiac muscle is 40% mitochondria), and the variation in the number of mitochondria may afford diagnostic information.[12] Furthermore, tissue refractive index is altered by many chemicals in a nonspecific but useful way which in turn affects the scattering properties of the tissue.[13] Finally and most importantly, highly absorbing compounds can be injected into the blood vessels or, indeed, the tissue, and greatly enhance the contrast between a suspected tumor with increased blood content and the background tissue absorption.[14] Other contrast agents well known to cell biology may be useful in detecting cellular calcium, membrane potentials, etc., providing the absorption of these agents extends to the NIR window.[15]

Radar and Loran technologies exploited pulse time measurements and altimeters frequency domain measurements.[16,17] Furthermore, pulse-code modulation first described in these volumes became one of the most precise methods of communication as taken up by B. M. Oliver (Hewlett–Packard).[18] It was natural, therefore, that both these techniques would be applied to precise measurement of the time delay between light entry and light exit from human tissues. Photon diffusion encodes the tissue characteristics not only upon the timing of the delayed received pulse, but also upon the received intensity time profile. Thus, instead of receiving a ''clean'' replica of the transmitted pulse, the return signals are spread out in time (over 5–10 ns), and are greatly diminished in electrical amplitude (attenuations of 10^{-5}–10^{-10}). Nevertheless, information on the medium traversed is readily obtained.[19] Pulse code or phase modulation assimilates the different times of arrival of photons and gives the mean time delay between source and detector, and then the tissue absorption and scattering can readily be deconvoluted (see below). In contrast, single source/detector continuous wave measurements cannot independently measure optical absorption and scatter.[1]

The analogy between radar and pulse time measurements of the time delay of photons in transmitting the body organs holds well except for the fact that the transmitted powers in human subject studies are severely limited by the intolerance of tissues to heating by light pulses and by photochemical damage to the eye. Thus, microwatts to milliwatts are transmitted and nanowatts are received. Indeed, the concept of the transmit/receive (TR) switch is not necessary and the detector systems may have high gain and fast response: here, rise times of 150 ps are usually adequate. However the frequency domain method suffers from the simultaneous transmission and reception of signals and requires a much more thoughtful design philosophy to avoid unwanted crosstalk between the transmitted and received signals. Phase modulation accepts extraneous signals and gives the mean time delay. Thus, much of this article is dedicated to the differences between phase modulation systems for communication and instrumentation for phase delay measurements in human tissues.

In order to effectively measure these optical pathlengths and their changes, one may use a pulse–time method which follows the relatively standard technology of single photon counting, as in the study of fluorescence lifetimes,[20,21] with the great advantage that in the NIR region pulsed laser diodes afford excellent short pulse light sources for such studies. Frequency domain devices used in phase fluorometry are similarly applicable. Since frequency domain equipment involves amplitude modulation at lower peak powers, slower rise times, and hence smaller bandwidths than the time domain circuits, higher signal-to-noise (S/N) values are obtained and frequency domain circuits appear to be more economic and portable as medical devices. Finally, continuous light may be employed with the caveat that either the optical pathlength [or the differential pathlength factor (DPF)][22] must be determined by frequency or time domain methods. Alternatively, observations with different source–detector distances[23–25] together with calibration standards must be used to obtain quantitative data with continuous light.

This article will focus upon the various types of frequency domain equipment and their performance limitations, particularly in view of the requirements of medical devices for a very high accuracy of phase change (better than 0.03° in a 1 Hz bandwidth) which is beyond specifications generally available for commercial equipment, network analyzers, usual phase detectors, etc. The basic treatment of circuit components here may well be useful for the study of both photon migration in tissues and for lifetimes of fluorescent chemicals.

A. Relation to communication technology

The requirements for multi-frequency, multi-wavelength operation of the phase modulation systems parallels developments in optical fiber communication systems, i.e., time division multiplex (TDM) and wavelength division multiplex (WDM).[26] While we will not use this terminology in our article here, it should be realized that the problems and solutions in the two cases are surprisingly similar, except that

the frequency range in which the multiplexing occurs is much slower than that desirable for cellular communications equipment. While our requirement for medical systems are currently quite modest, i.e., three wavelengths and two frequencies, the advent of imaging technology will increase the need for multiplexing as the first step to 16/32 channels, for imaging of <1 cm^3 objects but eventually to much greater multiplexing for higher resolution imaging systems, which might even use 1000 source–detector combinations for highest resolution imaging. Thus, in presenting the basic developments described here, we have kept in mind the possible input from WDM and TDM systems using digital signal processing (DSP) at lower signal frequencies.

B. Nomenclature of frequency domain devices (FDDs)

We have used the term phase modulation for the process by which biological signals are imprinted upon the phase of a high frequency signal, in common with the terms amplitude, frequency, and time modulation.[16,17] Others, particularly the University College London (UCL) group[27] have used the term amplitude (intensity) modulation which belies the nature of the signal information as a phase change. Therefore, we use phase modulation in this article for frequency domain devices.

The traditional terms ''homodyne'' and ''heterodyne'' are used, respectively, to identify systems that respectively, do not and do, down convert the radio frequency prior to phase detection. Heterodyne systems have been termed ''cross correlation'' by some studying fluorescence spectroscopy,[28] but we shall retain communication terminology and use the term for the device phase delay measurement device (PDMD).

C. Performance criteria phase delay measurement devices (PDMD)

These devices are intended to measure tissue optical properties μ_a and μ_s' to an accuracy to -3% requiring phase and amplitude precisions roughly as follows:

(a) Phase and amplitude noise in a 2 Hz bandwidth should be $<0.03°$ and $<0.1\%$ of total signal at carrier frequency of 50–200 MHz.
(b) Source to detector attenuation may be >100 dB with radio frequency (rf) coupling causing $<0.03°$ phase error.
(c) Amplitude phase crosstalk: Signal attenuation of 10 dB shall not cause more than 0.03° phase error.
(d) Interchannel crosstalk: Multi-frequency operation shall not cause more than 0.03° phase interchannel crosstalk (at 50 dB attenuation). Optical multiplexing employing light sources of different wavelengths shall cause $<0.03°$ interchannel crosstalk.
(e) Bandwidth signal output should be variable from 0.2 to 2 Hz or in special cases of brain study, 40 Hz.
(f) Sufficient information from multiple radio frequency or multi-wavelength operation should be available to permit calculation of absorption coefficient (μ_a cm^{-1}) and transport scattering coefficient (μ_s' cm^{-1}) to 5% and hemoglobin saturation to 3% in the 40%–80% range.

II. PLAN OF TECHNICAL PRESENTATION

This article first discusses the applicable theory, the types of frequency domain instruments used in photon diffusion studies with examples, and second gives details of a few systems which may have optimal characteristics for biomedical studies. These designs of phase modulation systems are intended to operate within the framework of the Federal Drug Administration (FDA) guidelines.

A. Photon diffusion equations

The photon diffusion equation contains separated terms for absorption and scattering (μ_a, μ_s') taking note that D contains μ_s' in Eq. (1). Both time and frequency domain systems afford precise measurement of time delays and amplitude changes due to photon propagation through tissue as a diffusive wave. Appropriate equations for the photon diffusion follow:

(1) The time domain solution for a spatial and temporal impulse in homogeneous infinite medium is:

$$\ln R(\rho,t) = -5/2 \ln t - \mu_a c t - \rho^2/(4Dct) = -5/2 \ln t - \mu_a c t - (3\rho^2\mu_s')/(4ct) \quad \text{for } \mu_s' \gg \mu_a \tag{1}$$

where R is the reflectance signal, a function of input–output separation (ρ) and time (t) and D is the diffusion coefficient and equals $D \approx 1/3(\mu_s')$. μ_a and μ_s' are the absorption and reduced scattering coefficients, respectively, and c is the speed of light in tissue. ρ is the source and detector separation and t is time. In general, μ_s' is much greater than μ_a in biological samples ($>10^2$). At long times, the terminal slope of $\ln R$ vs t is $\mu_a c$.[29]

(2) The equivalent frequency domain solution for amplitude (M) and phase ϕ is:[1]

$$M = \frac{\mathbf{S}_0}{4\pi\mathbf{D}} e^{-\rho A \cos\theta/2}$$

and

$$\phi = -\rho A \sin\frac{\theta}{2} - \rho A \cos\frac{\theta}{2}, \tag{2}$$

where ρ and D are defined as above. $\theta = \tan^{-1}(\omega/\mu_a c)$,

$$\mathbf{A} = \left(\frac{\mu_a^2 c^2 + \omega^2}{c^2 D^2}\right)^{1/4},$$

$-\ln \rho M \propto -\rho A c(\theta/2)$, where ω is the modulation frequency.

Therefore, $\ln(\rho M) \propto -\rho A \cos(\theta/2)$ gives linear dependence of (ρM). $\phi = -\rho A \sin(\theta/2)$ gives linear dependence on ρ. By measuring M and ϕ as functions of ρ and determining the slopes of $\ln(\rho M)$ and ϕ, $\tan(\theta/2)$, θ and $\mathbf{A}$, μ_a and μ_s' are determined.

B. Calculation of tissue parameters from phase information

Equation (2) and Fig. 3 indicate that the phase and amplitude modulation signals increase with frequency and that the sensitivity of phase detection would be higher at higher frequencies. However, the calculation of absorbance from the phase signals is frequency dependent, and is often categorized as a ''low frequency'' or a ''high frequency'' approximation to the diffusion equation that depend upon the tissue optical parameters, i.e., for highly absorbing tissues, such as the skeletal muscle or low absorbing tissues such as breast, $\mu_a = 0.1$ and $\mu_a = 0.05\ \mathrm{cm}^{-1}$. The low $\leqslant$50 MHz and high $\geqslant$500 MHz frequency[1] approximations are, respectively:

$$\theta = \frac{-\sqrt{3\rho}}{2c}\sqrt{\frac{\mu_s'}{\mu_a}}\,f \quad \omega \leqslant \mu_a c, \tag{3}$$

and

$$\theta = a\rho\sqrt{\mu_s' f} \quad \omega \geqslant \mu_a c, \tag{4}$$

where a involves c^{-1} and other constants and the symbols are defined in Eqs. (1) and (2). Furthermore, important calculations, particularly those involving the hemoglobin saturation (Y), depend upon the wavelength used for the determination of optical properties (Fig. 1), as well as the oscillator frequency in the calculation of the absorbance related quantities. Thus, systematic errors may be present in the calculation of Y using low frequency or high frequency, or using a mixture of both. In the region of 50 MHz, the low frequency approximation is valid for highly absorbing tissues such as muscle, while the high frequency approximation is valid at 450 MHz for high and low absorption, and the use of three wavelengths enables determination of the hemoglobin absorption at two wavelengths and the instrument phase delay at the third wavelength (see Fig. 6). A frequency of 450 MHz is near the bandwidth limit of most available photodetectors.[21,30]

C. Phase measurement systems

A number of phase measurement systems are described here with special emphasis on phase rather than amplitude. (1) Amplitudes are relatively simple to measure; (2) amplitude signals can be confounded by stray light under conditions where the amplitude independent phase measurement can still be carried out with acceptable accuracy; and (3) multi-wavelength phase measurements alone are sufficient to afford a calculation of important quantities, such as hemoglobin concentration and hemoglobin oxygenation. Nevertheless, amplitude and phase are traditionally determined by a number of devices designed for measuring the lifetime of various fluorescent chemicals (fluorochrome), particularly the device of Spencer and Weber.[20] Amplitude and phase are also used in the method proposed by Kohl *et al.* to determine absolute μ_a.[31]

III. SPECIFIC SYSTEMS

In order to evaluate the available approaches, the relative qualities of homodyne and heterodyne instruments are outlined as stated. A homodyne system detects the phase shift at the radio frequency, while the heterodyne system shifts the rf to a lower frequency for phase detection. Both may be coupled to analogue and/or digital signal processing circuitry. The combinations of radio and audio frequency devices that yield optimal phase accuracy and stability are emphasized.

The basic difference between homodyne and heterodyne system is, on the one hand, the simplicity of the homodyne systems (replaced in the 1920s by heterodyne communication systems), and the greater complexity and ultimate precision of heterodyne systems, on the other. For purposes of explanation we present the simplest forms of the systems, granting that more complicated forms may have advantages that may not be fully explained in this article.

A. Homodyne systems

Figure 2(A) gives the basic form of a homodyne system with a sine/cosine phase detector in phase-quadrature (IQ) demodulator. The phase difference between the reference oscillator and the signal pathway is detected. The phase shifted path involves a laser diode, an optical detector, an appropriate amplifier, and a narrow band filter. Thus the outputs of the IQ detector are the sine and cosine components of phase (ϕ) and amplitude (M) and thus require trigonometric computation by nonlinear analogue circuitry or by conversion to the digital domain and the use of a look-up table or alike. Quadrature imbalance in the IQ detector is typically 0.3° in ϕ and 0.5 dB in M, but any unwanted signal will be detected and cause variable dc offsets in the output that need to be distinguished from the dc sine/cosine outputs. Nevertheless, accuracies of 0.3° with carrier frequencies of 140 MHz have been observed in this laboratory (Table I) and afford a simple, efficacious system and a convenient PC display. Significant problems of intermodulation exist in the design recently published,[32] as well as problems of coupling large area optical fibers to 0.5 mm avalanche photodiode (APD). These problems have required adoption of the OPTO-8 photomultiplier tube (PMT) detector and time shared wavelengths (see Fig. 8) with performance meeting specifications (Table I). The phase output covers 0°–360° with some increased quadrantal error due to the calculation from $\tan^{-1}\phi$.

Digital circuitry can effectively be used in the homodyne circuit diagram of Fig. 2(A) where, more conveniently in the 30 MHz region with a 70 MHz clock allowing digitization of the 30 MHz oscillator signal and of the detector output (see Fig. 10 below). The IQ circuit operates in a digital mode followed by an appropriate filter affording phase locked IQ signals which then can be computer processed to give phase and amplitude characteristics of the optical properties of the tissue (Fig. 3).

The analogous homodyne circuit using a zero crossing phase detector has not been tested but would appear to readout phase as a linear function of phase difference without trigonometric calculation. Operation in two quadrants is appropriate to the small phase changes ($\sim$5°) occurring in physiological and pathological alterations of tissue optics [Fig. 2(B)].

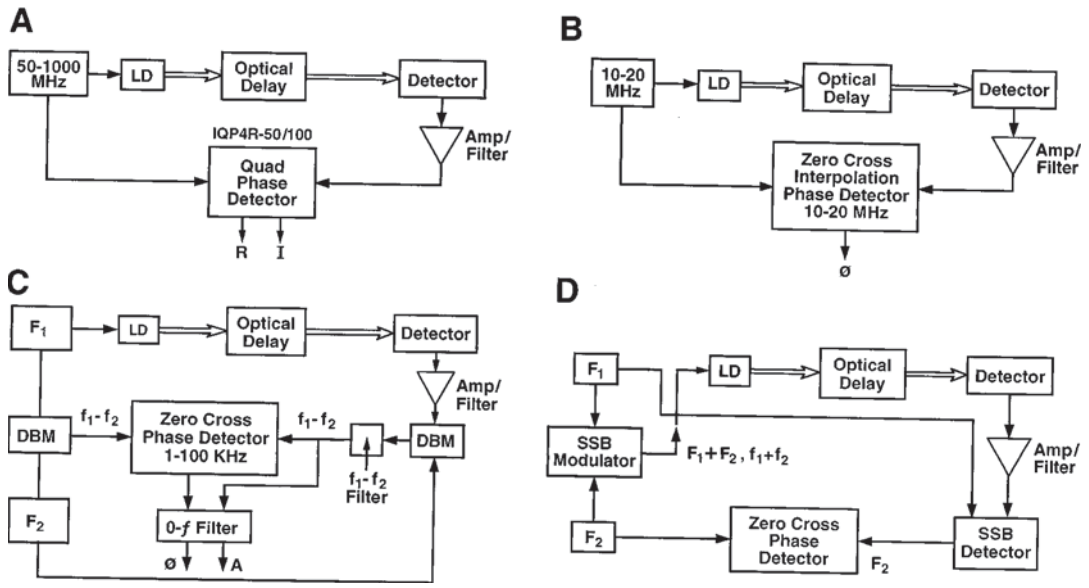

FIG. 2. Illustrating four types of optical propagation delay measurement devices for tissue study: A and B are homodyne devices, A with sine, cosine, IQ circuit, B with zero crossing phase detector. C and D are heterodyne detectors; C is amplitude modulated and D is single sideband with F_1 as the rf oscillator frequency and F_2 is the local oscillator (audio) frequency, the upper sideband F_1+F_2 is used.

B. Heterodyne systems

These are characteristic of nearly all communications equipment instrument [Fig. 2(C)].[33] Since the phase error decreases with decreased oscillator frequency, and decreases with detector bandwidth, instruments designed for the lower frequencies may give higher accuracies. The mixer output gives the sum and difference frequencies of the two oscillators, one of which is selected, amplified, filtered, and coupled to a phase detector as a reference frequency. Propagation of the phase of the transmitter frequency through the optical system, the biological material, the optical detector gives ϕ and M changes. After amplification and filtering, the intermediate frequency is obtained from a second mixer to give the ''measure'' signal for the phase detector. The zero crossing detector now operates at an appropriate low frequency above $1/f$ noise and below frequencies which induce high frequency errors in zero crossing phase detection. Narrow band filtering of the intermediate frequency and the detector output ameliorates the $1/f$ noise problem. The drawback of the heterodyne system is that the oscillators (F_1 and F_2) must have a phase coherence equal to the required system accuracy.

C. Single side band systems

Figure 2(D) indicates an efficient system for light modulation in a single sideband (SSB).[34] The SSB affords both efficient light modulation and efficient signal detection. The SSB system [Fig. 2(D)] differs from the heterodyne system of Fig. 2(C) in two important ways. The carrier modulation and the laser diode modulation are present only when the local oscillator activates the sideband selected. Thus, convenient control of the rf light output is available. The local oscillator frequency can also be in the convenient audio range. Tolerance to alterations in the frequencies of the two oscillators is determined by phase shifts of the narrow band filters employed.

The advantages of SSB over amplitude modulation (AM) include: all of the rf power is in a single narrow band of frequencies set by the low frequency oscillation. Typically, a communications receiver has multiple steps of heterodyne detection with narrow band filters at each frequency to avoid noise overload (Fig. 4). Since the phase and amplitude information is encoded in all these frequencies, narrow band filters will desirably include a flat phase/frequency characteristic.

D. Transmitter/receiver cross coupling

A principle difference of these systems and most communication devices is that transmitter and receiver are operating simultaneously in a PMD. Since the receiver phase is the sum of all signals received, feed-through of the transmitter rf frequencies must be minimized. Careful shielding is required to obtain the desired 120 dB isolation of transmitter and receiver signals.

E. Phase correction algorithms

In spite of the intrinsic accuracy of homodyne and heterodyne systems, it is desirable to have a nearly continuous check of the absolute phase of the device. The instruments described incorporate one of the following methods (see Sec. VII A):

(a) time sharing of single or multiple reference phase;
(b) multiple frequencies or frequency scan (see Sec. VII C);
(c) variable source-detector separation; and
(d) use of model of known μ_a, μ_s' to determine zero phase.

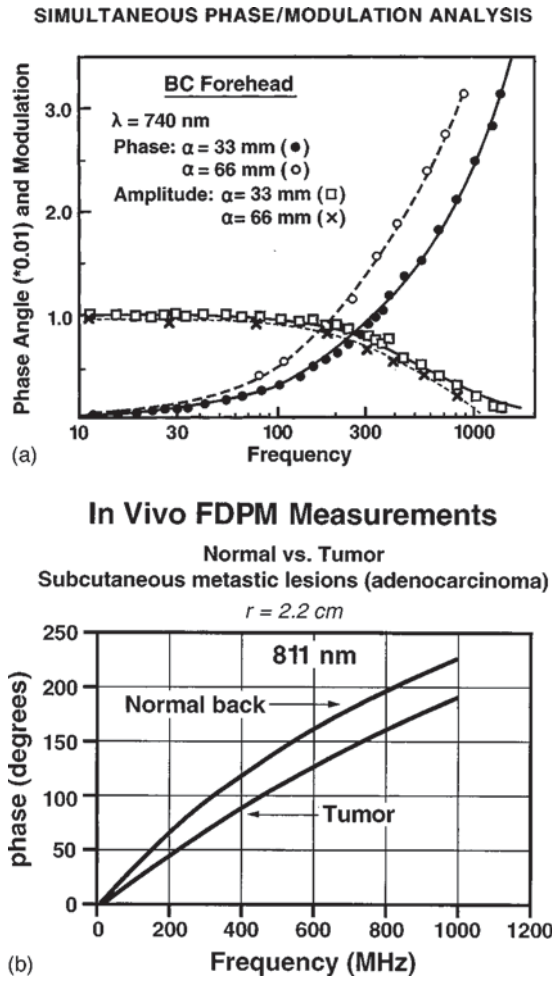

FIG. 3. (A) The phase shift and amplitude attenuation characteristics of adult human brain as a function of light modulation frequency and the separation of sources ρ of 33 and 66 mm, at a wavelength of 740 nm for the forehead of a human subject (see Ref. 33). (B) Illustrating the difference in optical properties in normal and tumor tissue for a subcutaneous metastatic lesion (adenocarcinoma) on the human chest. The upper curve represents the phase shift as a function of frequency for normal tissue, and the lower curve for tumor tissue, indicating the greater absorbance of the tumor tissue, presumably due to its increased blood content (wavelength is 811 nm). These phase shifts are attributed to a shorter pathlength of optical propagation in the tumor tissue which contains more blood absorption at 811 nm (see Fig. 1) (replotted from Tromberg, Ref. 34, with permission).

IV. EFFECT OF PHOTON PROPAGATION UPON OPTICAL SIGNALS

The effect of absorbers/scatterers upon the phase delay of photon propagation in tissue is very distinctive as shown in Fig. 3 for the human brain. The data from the human brain at two source–detector separations (33 and 66 mm) [Fig. 3(A)] indicates suitable frequencies of modulation and Fig. 1, the wavelength of the light source employed:

(a) The frequency range of light modulation desired is generally higher than that conventionally used for fluorescence lifetimes as a consequence of the accuracy required and also as a consequence of the tissue optical characteristics. Figure 3(A)[1–3,5,15,35] shows the desirability of frequencies at 50–500 MHz and little is to be gained below 50 MHz as the phase error is approximately the same over this frequency range. Little increase of accuracy occurs above 500 MHz as the circuitry becomes more complex. The system noise and the circuitry complexity increasing together with decreases of detector sensitivity and laser diode modulation index all contribute to a decreased accuracy in the 500 MHz region. Thus, the increased phase shift indicated by Fig. 3 does not result in an increase of signal to noise ratio in most of the systems described here. Figure 3(B)[36] illustrates the difference between normal and tumor tissue as a function of frequency, showing that over the observed frequency range the tumor has a smaller phase change and hence, higher absorption than the background tissue, in this case than the host tissue. The difference is due to the greater blood content of growing tumors. Thus, wavelengths sensitive to hemoglobin are required (Fig. 1).

(b) The wavelength region of interest is termed the ''NIR window'' (Fig. 1) and is optimal for blood (oxy- and deoxy-hemoglobin), melanin, water, NIR contrast agents, and refractive index determinations, by photon propagation through tissue thickness of up to 15 cm for brain, breast, and limbs.

(c) Multi-wavelength operation is usually desired: complete scanning of absorption/excitation wavelengths and/or emission wavelengths in the high frequency domain involve equipment that is likely to be more complex than that at dedicated wavelengths where the information content is maximal.

(d) The amplitude of the optical signals from tissue may vary widely due to, for example, deoxygenation or due to transfusion of blood or replacement of blood with fluids in certain surgical procedures or in trauma. These signal amplitude changes provide useful information, but the phase changes must be insensitive to amplitude crosstalk.

(e) There is a requirement for the separate calculation of absorption (μ_a) and scattering (μ_s') to an accuracy of $\sim$5%, together with the need to calculate not only concentrations but, of particular interest, the quantity termed hemoglobin saturation (the ratio of oxyhemoglobin to total hemoglobin concentration) to about 3% in the range of 40%–80% saturation. This calculation may require an even better accuracy of μ_a ($<$1%).

(f) The equations for calculating absorption and scattering coefficients from low frequency dual wavelength data require knowledge of the intrinsic phase delay of the system. This quantity termed ϕ can be calculated by extrapolation to zero of the source–detector distance versus the phase reading on a model of properties similar to that of brain, muscle, etc., or by other methods described below. (Dynamic range limitations, i.e., 100–150 dB do not permit both joining of source and detector for phase zero calibration and phase error of optical attenuators of 10^{-5}–10^{-8} in the NIR region is often significant.) Alternatively, measurements of phase shifts as a function of a known concentration of scattering coefficient of an absorber/scatter will allow a calculation of zero phase. Over the wavelength region of Fig. 1, particularly 750–850 nm, blood absorption changes vary rapidly with wavelength, while scattering is relatively independent thereof. A multi-wavelength system allows a qualitative determination of whether scattering or absorption changes are involved. Using this as a qualitative distinction of the two, very useful instruments can be made by time-sharing two

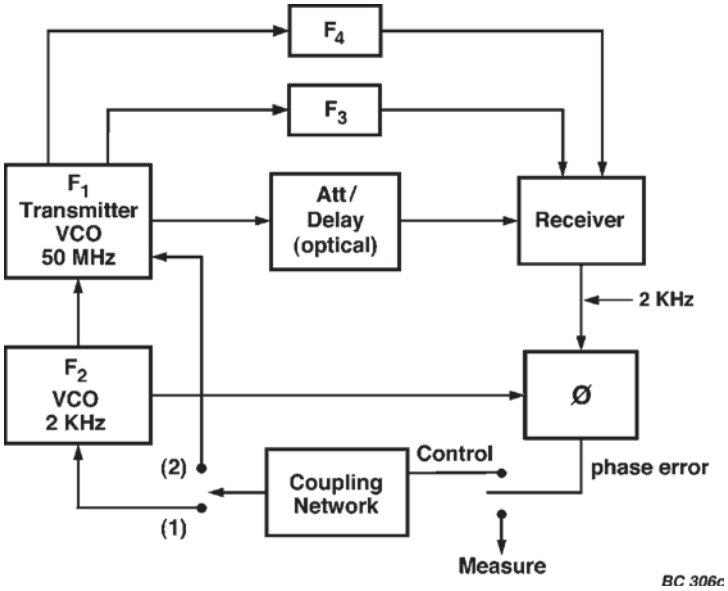

FIG. 4. A typical single sideband radio transmitter provides adequate power output to drive a number of laser diodes which may be optically coupled to the PMT after a tissue optical attenuation of 100–150 dB. The receiver output is between 1 and 2 kHz, dependent upon the VCO input to the transmitter. Transmitter/receiver frequencies may be in the 50–440 MHz region. The pulsed locked loop (PLL) is closed by the varactor control of the rf transmitter phase switch position (2) or low frequency oscillator phase [switch position (1)]. The transmitter and receiver are phase locked through the use of common intermediate frequencies F_3, F_{43}. Sample and hold circuits are useful in the control and measure circuits where the data input is intermitant. F_3, F_4 represent intermediate frequencies of transmitter locked to receiver.

wavelengths according to Fig. 6 in which scattering can be distinguished from absorbance changes (details are given in Fig. 7). Also, continuous light systems using wide range scanning may also use this determination between scattering and absorption, and some use Warren Butler's derivative spectroscopy[37] as used by Cope. However, this article concerns instruments capable of quantification of scattering and absorption with frequency domain technology.

A. The bandwidth of biomedical signals

Given the required signal accuracy of 5% error, the effect of system bandwidth on noise must be considered. Four types of biological signals are under study.

(a) The first and main goal of medical devices is to detect a change from a physiological to a pathological state (oxygen saturation, blood concentration, the presence or absence of brain bleeds, etc.). Here times of change are rarely shorter than 1 min and may extend over several days to detect trends in patient care. Thus, a very low bandwidth system is adequate (time constant $RC > 20$ s).

(b) In the second case, procedures that involve brain hypoxia (O_2 insufficiencies) must be responsive to changes of brain oxygen in a few seconds and examples are: cardiopulmonary bypass which may involve transients of blood oxygen concentration that occur in 5–20 s, with a time of constant $RC \sim 5$ s.

(c) In the third case, great interest has been expressed in brain blood concentration and oxygenation changes related to functional activity of the brain, which have characteristic rise and fall times of approximately 10–30 s when metabolic activation is involved,[38] $RC \sim 0.2$ s.

(d) In the fourth case, light scattering changes occurring during the propagation of optical signals in nerves, cells, and fibers, and occurring in times of milliseconds have been observed in the animal brain[32] for which specialized wide band instruments are available (see Fig. 10) but in which iterative procedures and data averaging are used, $RC \sim 0.05$ s.

For dedicated instruments, which is the usual case with medical devices, the use of a fixed bandwidth is highly desirable for simplicity and for reproducibility of recordings, for example, from patient to patient. Thus, a final bandwidth of 0.2 Hz has been selected to cover the first three cases and is usually obtained with signal processing at a variety of intermediate frequencies as is characteristic of heterodyne communications devices.

B. Signal processing

Phase detection at the single radio frequency is the case with homodyne systems, and at a variety of intermediate frequencies, as is characteristic of heterodyne communication devices, for example, amplitude modulation (AM) and single side band (SSB).[34]

In all of the FD devices, a phase detector is employed which may have many levels of sophistication, ranging from the zero crossing detectors and analog filters to complex lock-in amplifiers with digital signal processing and Fourier filters. Many aspects of the lock-in amplifiers are treated in general[39] or in detail[29] in this article.

When the rf signal contains a high harmonic or noise content, frequency selection is necessary to avoid overload and intermodulation products in the detector/receiver system. This is especially true in pulse systems where the harmonic content of the pulse and its repetition frequency are used to generate multiple modulation frequencies. Then Fourier transformation and filtering are necessary.[40,41] Otherwise, noise bandwidth must be progressively reduced as the signal is amplified. This presents special problems in homodyne system where the noise bandwidth of photodetectors may be >30 MHz and the overload signal of homodyne detectors is in the millivolt range (see below). In any case, a final 0–F filter is used to obtain a narrow bandwidth mentioned above, for example 0.2 Hz.

Manual control of the bandwidth is used in analog devices and in computer controlled devices, digital control of the bandwidth (R-C values) is possible. When the harmonic

content of the signal is high, digitization and Fourier transformation has been used to select appropriate harmonics of the pulse repetition rate using DSP technology. This is used in certain types of fluorometers applied to chemical studies. In commercial phase fluorometers for chemical applications, both very long and very short lifetimes are measured. This differs significantly from the relatively narrow band phase delays of biological signals. However, many design problems are common to phase meters for both photon migration and fluorescence lifetime measurements.

C. Digital technology: Digital signal processing (DSP)

Digital signal processing can be commonly found in commercial wireless communications, and also in phase modulation technology. In nonmilitary communications, the pressing need for bandwidth conservation at radio frequencies has emphasized frequency modulation (FM) and SSB as contrasted to pulse code modulation (PCM)[16–18,33] as the most efficient in bandwidth conservation and SSB in particular for power consumption. Bandwidth conservation is not a factor in phase modulation, pulse technology, and broadband harmonic generation is employed in multi-frequency phase fluorometry.[40] DSP naturally fits such technology and may be essential to minimize radio frequency interference or crosstalk. However, in SSB phase modulation (PMD) systems, DSP technology follows state-of-the-art communication design, as found in some modern amateur radio receivers where DSP is employed at the lower intermediate and audio frequencies.[42,43] While the principle of DSP is attractive, the two problems of DSP are often overlooked, namely, PMD requires very high frequencies ($>$100 MHz), at which high resolution digitization is not generally available. Thus, DSP is limited to heterodyne systems or to homodyne systems at $\leqslant$30 MHz (see Fig. 10), which is a severe limitation. On the other hand, DSP at IF and audio frequencies are standardized practices in communications equipment and are readily available for a phase modulation system (PMS) as audio DSP filters or DSP lock-in amplifiers. SSB receiver technology also affords DSP at several intermediate frequencies. The principles for application to IF and audio systems are well described[42] and include avoiding alias signals and involving care in matching the signal range to the A-D range. Appropriate filter design algorithms are available.

DSP is flexible and sophisticated, but introduces few new principles. Fourier infrared (FIR) and IIR filters[42,43] mimic well known analog filters, Butterworth, Chebyshev, etc., by sequencing data through various configurations of ''tapped'' filters, without and with feedback.[42,43] Thus, ''better' is a less suitable term to describe DSP technology as compared to analog technology; it is ''different'' and may lead to different systems design as shown in Fig. 10.

D. Laser diode light sources

These afford one of the greatest improvements in phase modulation technology: commercial phase fluorometers usually employ a continuous light source, such as a xenon arc lamp and a Pockels cell modulator and afford relatively low light fluxes and poor modulation depth (10%) that are inadequate for tissue studies of greater than 2 cm input-output separation.[44] Typically, an optical intensity loss of over 10^6 results from transiting significant tissue volumes and requires high power and high modulation depth. Thus, laser diodes with 5–30 mW output power, and whose power at the surface of the body tissue falls within the FDA limits, are discussed below. The great advantage of the laser diodes is that they can be modulated up to the highest frequencies at which reasonably priced photodetectors will effectively operate (approximately 500 MHz). Coupling of laser diodes through lightguide systems or direct coupling to the tissue are employed in tissue spectrometers and fluorometers.

Additional modulation of laser diodes at very low frequencies (10 Hz) for time sharing purposes is adopted by ISS (Champaign, IL) and UCL where the dc current is dropped from $\sim$60 mA to below the lasing threshold of $\sim$10% or 6 mA. Life expectancy does not appear to be deteriorated by this low frequency duty cycle.

The possibility that the frequency of laser diodes can be modulated by thermal perturbation of the cavity, or by external cavity modulation seems very appealing for rapidly sweeping through the region 750–800 nm, thus affording a single light source without the need for time sharing or frequency encoding. Thus the availability of such frequency swept vertical cavity surface emitting lasers (VCSELS) can significantly simplify the multi-wavelength laser diode systems described here.[45]

E. FDA regulations on laser limitations to light intensity

An important factor in signal-to-noise is the input photon intensity. The FDA regulations on the average intensity of coherent laser light used in type I medical devices in the region of 650–900 nm is $\sim$20–100 μW. The type I medical device requires no warning signals, no lock and key, and no comments from the Institutional Initial Review Boards at the hospitals whose duty it is to ensure the safety of medical devices used on human subjects. Type I specification is based upon a happenstance irradiation of the eye with the laser light. This intensity appears adequate for slowly responding systems ($\sim$1 Hz). Higher intensity ($<$1 mW) is required for more rapid changes ($\sim$1 ms). If the laser light is scattered by material covering the end of the fiber optic coupler before it impinges upon the tissues, the highly divergent light may not be identified as ''laser light.'' In the case of multiple light sources that illuminate separate portions of the tissue, their separation should be greater than 5 mm so that there is negligible increase of light due to a single source at any other site of illumination. Designs that employ electromechanical/optical methods of switching from one laser source to another using scattered laser light are most suitable for FDA regulations.[46] rf switching, while convenient, leaves the dc component unchanged. FDA class III allows $>$100 μW but requires fail safe precautions and labeling.

F. Detectors

A wide variety of detectors are available that operate in the time range appropriate to photon migration, especially when the low frequency approximation[32] is employed for calculating absorption and scattering factors from frequency domain systems. The squirrel cage R-928 PMT (Hamamatsu Photonics KK) has been used up to approximately 400 MHz, although it begins to roll off at about 300 MHz, whether or not all dynodes, or the first two dynodes, are used for rf amplification. The extended sensitivity to red light of this PMT makes it most valuable for achieving adequate S/N ratio at 850 nm, as is desirable, but not essential, in hemoglobin spectrophotometers. The large size of the R928 makes it undesirable for mounting directly in a source–detector combination coupled directly to the tissue. The Opto-8 (Hamamatsu Photonics KK) series designed especially for this purpose is highly desirable, first, because of its small size, second, because the S-20 photocathode response extends to 810 mm, third, because the parallel dynode structure gives this photomultiplier good capabilities at 500 MHz for frequency domain systems and for time domain systems. The full width half maximum (FWHM) is ~350 ns. Fourth, with suitable medical grade electrical isolation, it could be mounted directly on the tissue. The current design of the PMT has a gain of approximately 10^5 which is less than optimal for tissue studies, and an S-20 cathode, which does not allow wavelengths longer than 810 nm. A new version of this tube with a gallium–arsenide photocathode is being developed with a flat response over the desired 700–900 nm region for tissue studies, a high quantum efficiency (up to 21%), and a higher gain than the S-20 version.[47] This PMT appears highly desirable for tissue studies, but at the present time has an anode current limitation of 0.2 μA. Special impedance matching networks are used for single frequency operation using a reactive load producing adequate signal voltages well above 50 Ω loads. In broadband 50 Ω operation, care must be exercised to avoid excessive cathode current in developing adequate signal voltage. Numerous other geometries are available and manufacturers should be consulted for the latest developments of detectors appropriate to the ''NIR window.''

Avalanche photodiodes (ADPs) are attractive from the standpoint of high quantum efficiency and bandwidth with built in amplifiers (~40 dB), giving output signals within the range of low gain PMTs. The difficulty with the wideband (500 MHz) APD appears to be the 1 mm diameter of the diode which is less than the >20 mm^2 of exposed tissue surface which is desirable for light collection and available with PMTs. Nevertheless, APDs are desirable for operation above 500 MHz [see Fig. 3(B)], although diameters are reduced further to less than 0.5 mm.

All detectors so far tested have a cross correlation between the amplitude of the input signal and the transit time delay through the detector which may amount to 0.1°/dB for an attenuation of 40 dB.[48]

In summary, the detector characteristics are pivotal for good system performance. All these parameters need to be optimized: quantum efficiency, detector area, internal gain, bandwidth, maximum anode/cathode current and availability of anode wideband rf, and of the cathode for optical coupling to skin. These are all factors which must be considered in selecting an optimal detector. With respect to excessive anode current, the PMT should be shielded from room light by a ''blocking filter.'' Usually, a Wratten 88A or 89B gelatin filter is satisfactory for blocking fluorescent light, but not sunlight or tungsten room light.

G. Time sharing of wavelengths

Low frequency switching among multiple laser diode sources is desirable for multicomponent spectroscopy of oxy and deoxy hemoglobin. The rf relay, or electromechanical switch, has a great number of advantages: (1) no light guides are necessary; (2) the rf isolation can be greater than 60 dB, and with resonant tuning even better. Switching speed can be 10–20 Hz with increased cost and degraded rf performance which can be neutralized. PIN diode rf switches can also be used offering significantly higher switching rates (MHz), although rf isolation is typically only 40 dB and power handling is limited. While manufacturers recommend steady dc (~60 mA) for laser diodes, others have switched both dc and rf drives with success using Sharp laser diodes.

H. Time sharing of fiber optics coupling

One commercially available unit (Dicon Fiberoptics, Inc., Berkeley, CA) is costly, slow, and requires lightguide selection. A rotary light switch is costly, somewhat faster, needs a lightguide, and offers very high attenuation, i.e., greater than 100 dB is available.

I. Duty ratio considerations

The duty ratio of the sinusoidally modulated laser diode shown in Fig. 2 is >50%. In the time sharing devices described subsequently, the duty ratio is decreased by a number of time shares which is 2 in the dual wavelength system and 4 in the 3 wavelength system with a phase locked loop (PLL) channel. In pulsed time systems (not covered here), with 20 ps light pulses and MHz repetition rates, the duty ratios can be as low as one in a million. Of course, the peak power of the light pulses may be quite high. Another system which is disadvantageous with respect to duty ratio is the method of Fedderson, Piston, and Gratton[40] where pulsed light sources of widths of a hundred nanoseconds are used at a frequency of 1 MHz; the duty ratio may be only 10%. Systems employing light pulses are of an intrinsically much greater bandwidth than those employing sinusoids, particularly when the operation of the system depends upon harmonic generation by mixed pulse trains. In a case of the single frequency systems with sinusoidal wave form systems, the bandwidth can be extremely narrow, and crystal filters of Qs of 1000–10 000 can be used with great improvements of S/N ratio as illustrated in Fig. 8. It is for this reason that this article is focused on systems employing sinusoidal wave forms.

J. Interchannel crosstalk

These phase modulation systems differ from communication devices in that they transmit and receive simultaneously. Thus, cross channel interference of optical or rf signals must be down ~100 dB for high precision. Crosstalk between phase and amplitude signals obtained with one excitation wavelength and crosstalk between multiple wavelength signals are key issues in these systems. In addition, crosstalk between blood oxygenation and blood concentration signals creates additional problems; the deconvolution of these two signals is necessary to calculate hemoglobin oxygen saturation. Thus, in the following description frequency encoded and time shared systems are described in which varying degrees of channel isolation are obtained, taking into account that 100 dB channel separation is highly desirable.

V. CIRCUITS COMPONENTS

A. Amplitude selection

The measurement of the moment in time at which a wave form has a certain value is a generic electronic process termed amplitude selection or voltage comparison.[16] It is essential to phase detection, to analog to digital (A/D) conversion, etc., and is common to many basic processes in electronic instrumentation. The process is clear in the flash A/D converter where a wave form is compared against a series of predetermined voltages at specific times and the comparison is carried out by circuit elements which have a sharp voltage dependence of their transfer characteristic. A zero crossing phase detector is similar to a free running 1 bit A/D converter, instead of quantifying the voltage at specific times, it identifies all values in the time at which a wave form reaches a certain level and often taken as the moment in time at which the waveform has zero value. Since phase detection requires a measurement of relative time, the points in time at which two waveforms have zero values are noted. Oldham[49] describes a wide variety of circuit configurations for this purpose. Characteristically, a current integrator (low pass filter) is used to determine the interval between the zero crossings of the reference and tissue signal wave forms. Alternatively, the pulse waveforms may initiate and terminate a sawtooth wave form and its area may be quantified. Also, a counter may be initiated by one zero crossing and terminated by the other, either as a dedicated counter or as the clock of a digital computer. In all cases, the resolution depends upon the rate of rise of the timing wave forms and the inherent instability of the diodes used in amplitude selection.[16]

The reliability, linearity, and noise of transistor (drift) characteristics are important in determining the accuracy of the above mentioned process of amplitude selection. It is often necessary to identify the point in a receiver/phase detector circuit at which crucial amplitude selection occurs and limits the S/N ratio. In the phase detector employed schematically in Fig. 2(A) the measure signal is delayed with respect to the reference signal by the passage of photons through the tissue, a value depending upon the oscillator frequency and the photon path which causes a corresponding delay in the two waveforms. Generally pathlength phase delay is in tens of degrees at 100–200 MHz which must be measured to better than a tenth of a degree.

B. Signal processing

In the case of zero crossing phase detection, narrow band signal processing is done prior to and following the measurement of zero crossing. In the case of A/D conversion of the signal, digitization (quantization) errors are to be avoided and digital signal processing is appropriate to post detection as in Fig. 10. Nevertheless, the limitations of accuracy can be equivalent and are usually set by the properties of the amplitude selector and the stability of its nonlinear characteristic. Nevertheless, DSP has obvious advantages of flexibility and computer computability. Thus, we present in detail a DSP system to which many improvements such as FFT signal processing may be added (see Fig. 10).

In the zero crossing system, two zero crossing detectors are employed, one usually at high S/N ratio with signals directly from a reference oscillator and the other from the measure signal at a low S/N ratio which is processed by a variety of means which may involve phase locked loop tracking, narrow band filtering, level setting, etc., all of which enhance the accuracy of measuring the zero crossing of a sinusoidal wave form.

DSP can be used (as indicated in Fig. 10),[42,43] and can include phase detection by using look-up tables for the calculation of ϕ and M.

The point in the heterodyne circuit at which the amplitude selection noise becomes evident may be in low frequency circuits, whereas in homodyne circuits, amplitude selection occurs at the rf frequency, for example, in the IQ circuits themselves where the signal is mixed with sine and cosines of the reference oscillator which is essentially a phase detection operation with attendant offset and dc drift problems. When these fundamental operations are identified, many of the rhetorical arguments on the ''better performance'' of one system over the other can be reduced to a logical basis. With appropriate attention, it is possible to ensure that electronic instrumental performance is not limiting in the detection of small phase changes and is dependent upon the S/N ratio of the detector itself at photocathode currents less than 10^{-11} A, assuming 10^8 gain and a 50 Ω load.[50]

C. Bandwidth considerations

In heterodyne and homodyne systems described here, low harmonic content sinusoidal wave forms are expected and bandwidth narrowing is employed to avoid noise (shot noise) overload. This may satisfy the needs of zero crossing detectors for signal processing, for harmonic rejection, and for rejection of dc level offsets. For precision phase detection, appropriate filters are desirable pre- and post detection. However, systems that generate harmonic frequencies by pulse mixing require Fourier transform (FT) harmonic rejection circuits. Generally, bandwidth narrowing is necessary in high S/N systems to avoid overload and harmonic intermodulation distortion in the various stages of amplification

at the fundamental and intermediate frequencies as provided in communication equipment by crystal filters. Post detection filtering has advantages of simplicity. A low pass filter is easier to construct than bandpass F_1-F_2 filters.[51,52] At the same time, digital control of the filter bandwidth may be used in cases where wide ranges of bandwidth are required as might be the case in phase fluorometry (not treated in detail here). There, the bandwidth depends upon the nature of the chemical and may vary widely. However, the time constant of biological signals already described in Sec. V A is limited by fundamental properties of the cell/tissue dynamics so that a bandwidth of less than 1 Hz is quite acceptable. Thus, heterodyne receiver technology plus multipole dc to *F* filters afford a reliable and economic solution to signal processing for tissue signals. DSP systems are referred to above and afford digital alternatives to analogue filters. The choice of manual or digital control of signal processing is a topic which has been approached in almost all scientific instrumentation and many solutions are available and have been developed to a particularly fine point in nuclear magnetic resonance and in phase fluorometers where a wide variety of signal bandwidths are encountered in contrast to the low frequency signals of biological tissues.

D. Detection systems

Communications technology has developed single and double balanced mixers of extraordinarily good carrier rejection, linearity, and noise level. Prior to the availability of such mixers, Spencer and Weber[20] proposed to modulate the second dynode of a nine stage electron photomultiplier phototube as a heterodyne detector; the idea being that the time spread of the photon packet through two elements would be less than that through all of the elements. Variable frequency operation requires ''flat'' or frequency independent drive, i.e., 50 Ω @ 90 V=162 W. Thus, decreases of modulation are required to avoid rf heating and undesired coupling. Nonetheless, the circuit is simple and minimizes delays due to dynode voltage changes. Since the development of photomultiplier tubes of vastly improved frequency response, the PMT can be followed by a double balanced mixer. The FWHM of the pulse response of the Opto-8 series of Hamamatsu has been measured to be approximately 220 ps, a factor of 3 or 4 better than the early designs. Thus, the Opto-8 PMT can used at 450 MHz and possibly higher frequencies. In addition, the newly developed APDs, particularly those of small window diameter, are available up to 1 GHz but with approximately 100-fold less gain than the photomultiplier tubes. However, the Gratton group has held to second dynode modulation.[24] The dynode modulation appears to be advantageous in cases where the limitations in some systems on the cathode or anode current are severe and a narrow band output circuit from the PMT (0–100 kHz in some cases, down to zero to 1 Hz in others) may be advantageous. Additionally, dynode modulation may be advantageous when trying to obtain the maximum frequency response possible from a given PMT.

E. Dynode feedback

Control of the PMT accelerating voltage by the signal amplitude, i.e., dynode feedback control, is used in many commercial spectrophotometers[53] and provides the appropriate dynode voltages to give a predetermined constant signal output level. It has the following advantages:

(a) The photomultiplier is protected from excessive anode currents and with additional sophistication, excessive cathode currents.
(b) The photomultiplier is an element in a feedback loop, and its output is converted almost exactly logarithmically (although each PMT will be to a different logarithmic base).
(c) The amplifier and phase detector are supplied with a constant amplitude and thus have minimal contribution to drift or phase delay.
(d) A disadvantage is that there is a device dependent change of transit time delay which is ~0.2°/V of total dynode voltage for the Hamamatsu R928 PMT.[54]

Since the amplitude phase/crosstalk of the PMT is a reproducible quantity and depends upon the physical characteristic of electron multiplication, the compensation for the amplitude phase crosstalk can be readily entered by computer algorithm or a simple algebraic subtraction system depending upon the appropriate signal from the amplitude detector.[55]

In multisource systems such as Fig. 8, in which dynode feedback regulation is to be employed, it is necessary to adjust the dynode voltage for each signal level depending upon source intensity and tissue transmission and for variations of signal level which may occur in the course of an experimental study where, for example, blood concentration and oxygenation changes are involved. The receiver output signal as obtained from the amplitude detectors, usually automatic gain control (AGC) systems, gives an appropriate amplitude signal which will shift the dynode voltage of the PMT to track amplitude changes and normalize them in a straightforward manner. The frequency response of the dynode feedback control system needs to be approximately 10 Hz in order to accurately follow the 1 Hz time sharing system. At the same time, algorithms to correct for the change of transit time of the PMT with dynode voltage are obtainable from a ''look up'' in the associated computer. Thus, corrected phase data are available for calculation of the hemoglobin saturation and blood concentration values as are needed from the system. In short, the dynode feedback maintains a very nearly constant signal into the receiver so that its amplitude/phase delay characteristics are minimized. The automatic gain control circuit of the receiver detects an error signal that regulates the high voltage and maintains its input nearly constant. This error signal is, however, the amplitude signal and affords useful data for amplitude/phase calculation and for the amplitude, phase crosstalk. In short, every value of PMT dynode voltage affords a computer ''look-up table'' correction factor for the particular time-shared wavelength.

Preliminary tests with the multichannel circuit of the

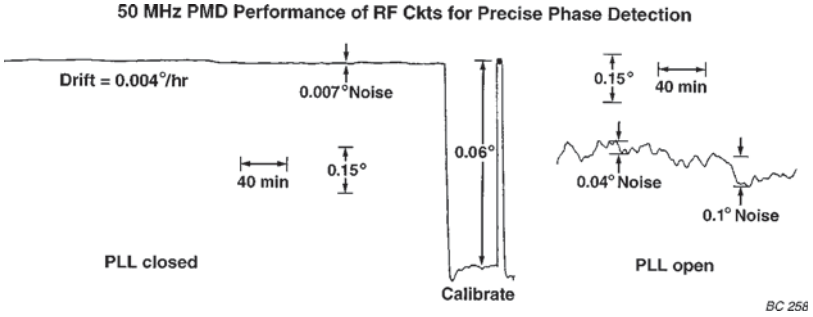

FIG. 5. The electrical noise performance achievable at 50 MHz with transmitter and receiver coupled through 100 dB attenuation according to the diagram of Fig. 4 of the SSB system (no optical coupling).

type of Fig. 8 suggests that dynode feedback affords a reliable and stable correction factor for amplitude phase crosstalk anywhere in the system.

F. Accuracy of phase and amplitude values

The key to the operation of phase detectors is the generation of very sharp pulses from the zero crossing of the sine wave inputs and the minimization of diode contact potential variation. The noise levels of phase detectors due to contact potential variation in the diode detector gives errors of $<0.10°$ at a bandwidth of about 0.2 Hz at 50 and 200 MHz (Table I). This corresponds to <0.7 ps or a <0.2 mm pathlength error at 200 MHz. Brain oximetry requires a ~ 1 mm pathlength error in order to achieve a $\sim 5\%$ hemoglobin saturation error at 70% saturation using 754 and 816 nm. Amplitude–phase crosstalk for sinusoidal wave forms input to a commercial zero crossing phase detector may be as low as 0.004°/dB (Log_{10}) (Krohn Hite 6200).

G. Phase lock loop tracking circuit

A phase locked loop[56] can also be used to correct phase error between F_1 and F_2 as shown in Fig. 4. In that case, it is desirable to time share the optical signals through ''reference'' and ''measure'' materials. The former controls the local oscillator frequency and phase and the latter measures the pathlength changes in the tissue (attenuation and delay). Thus, this system combines the advantages of low frequency phase detection with high frequency phase delay measurement at any frequency that the light source and optical detector are capable of. Time-shared phase locked loops require phase memory for control of the phase locked loop due to intermittent phase error signals. Thus, phase corrections are only feasible at a fraction of the frequency at which the time sharing occurs (usually 4–10 Hz).

H. Phase memory

The handling of intermittent data by sample hold circuits[12] was first used in radar technology for processing signal data during prolonged scans of the radar antenna.[16,17] The technique is generally used with intermittent data as indeed is the case with the time shared PLL where phase memory is required, i.e., the phase output is time shared among the several wavelengths but a reference phase must be retained in a memory over the interval of the time sharing at a low duty ratio, (25% for a four-wavelength system). Thus, the sample and hold circuits are used for the phase memory with storage time constants of $\gg 20$ s. Integration times of a few seconds are, for example, acceptable for biological signals. Such a system is indicated in Fig. 6 below. If the phase detector output is digitized and stored, the sample and hold technology is useful in reducing quantization error in A/D conversion.

I. Phase detection (SSB system)

By introducing a low frequency SSB modulation into the oscillator system as generically indicated by Fig. 4 above, it is possible to modulate the radio frequency at an appropriate value for optimal accuracy of phase detection (i.e., 1 kHz). In these systems, the transmitter and receiver share identical local oscillators F_2, F_3. The phase shift of the radio frequency caused by the phase delay in the biological material will directly alter the phase of the SSB signal and provide precise phase detection of the difference between the low frequency signal modulating the transmitter and the phase shift at low frequency as caused by light propagation through tissue.

VI. CIRCUITS FROM COMMUNICATION EQUIPMENT

Phase locked loops are used extensively in communication systems[56] to stabilize the local oscillators in heterodyne technology and can be used to obtain optimal phase accuracy of the instruments used in optical phase delay measurements. An example of a PLL is based upon communications equipment is shown in Fig. 4. This consists of a 50 MHz SSB type FT 690 transmitter and FT 690 receiver (Yaesu), together with a model 6200 phase detector (Krohn Hite) with an RC integrate/differentiate coupling network and a voltage controlled oscillator (VCO) (Hewlett Packard 3010). Alternatively, the phase of the rf oscillator is controlled by a varactor VCO (Fig. 4). The performance of the radio frequency system only (no optical coupling) is shown in Fig. 5. An attenuation of 100 dB between transmitter and receiver is used with a final time constant of approximately 10 s for the drift test. The calibration is obtained by altering the electrical pathlength with calibrated cables to give the 0.6° phase shift shown. On the right hand side, the phase noise without PLL and with only rf coupling between the transmitter and receiver is shown to be 0.04° for average peak-to-peak noise with singular peaks of 0.1°.[57]

The PLL loop controlling the VCO is then closed and the noise is less than 0.01° with a drift over several hours is less than 0.01°/h. When an optical link is placed between transmitter and receiver and the attenuation is ~ 100 dB, the phase noise is increased to 0.1° (Fig. 5). Similar results have

been obtained at higher frequencies. The timing error is approximately the same at 140, 220, and 450 MHz and as the timing precision increases with frequency so does the phase noise (unpublished observation).

VII. CHARACTERISTICS OF OXIMETRY SYSTEMS

A. Multiwavelength, multifrequency instruments

A number of system requirements involve some combination of multiple wavelength, multiple frequencies, multiple sources, and multiple detectors. Multiwavelength systems are used for quantification of chemical species which may be abetted by using multiple frequencies [see Figs. 3(A) and 3(B)]. The monotonicity of the relationships suggests that spot frequencies will do as well as swept frequencies depending upon the technical considerations involved in broadband and narrow band systems, and the latter is to be preferred wherever possible because of the design difficulties in broadband such as network analyzers as opposed to narrow band single frequency systems. Multi-source, multi-detector systems are appropriate to imaging problems, and can be multi-wavelength and multi-frequency, resulting in relatively complex systems. The general principles that are involved here, i.e., for multichannel systems, involve the usual considerations of communication systems, namely, time or frequency encoding. The basic arguments for time and frequency encoding encountered in communication systems are likely to apply here. However, the intrinsic narrow bandwidth nature of biomedical signals allows the use of electromechanical multiplex systems which would not be possible for wider bandwidth systems. Nevertheless, frequency multiplexing has a great deal to offer, at these lower frequencies. In the following, we shall first describe briefly frequency multiplexing and finally give an example of source–detector multiplexing suitable for use in an imaging application.

In order to simplify the calculation of optical parameters and to afford some redundancy in the calculation, systems using three wavelengths and one or more frequencies are described. In these systems not only can the parameters of the three absorbers be obtained, but also a segregation of absorption and scattering due to the predominance of scattering with high frequency measurements as contrasted to low frequency measurements where the effect of absorption and scattering are more equally weighted. One of the caveats of the multi-frequency method ranging from 0.3 to 1000 MHz is that the penetration depth of the high frequency modulation is less than that of the low frequency modulation.

B. Time sharing multiplexing systems

The simplest form of multiplexing is simply to time (Fig. 6) or frequency (Fig. 8) encode the laser diodes. In the former case, one transmitter/receiver suffices, and in the latter case, replicate transmitters/receivers or frequency shift encoding of a single transmitter/receiver is required. Some instrumentation is common to all channels and thus it is expected that slow systematic fluctuations as in Fig. 6 would be similar and would be largely canceled by the phase lock loop except for individual circuits related to the laser diodes. Optical detectors generate broadband shot noise, some of which

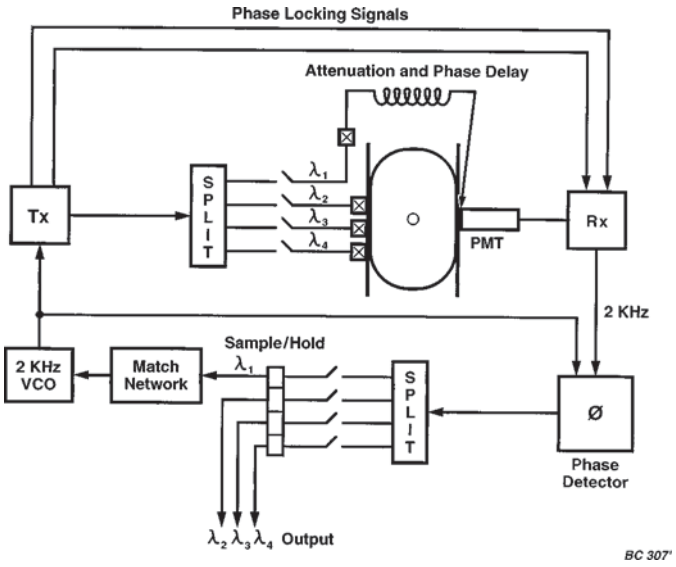

FIG. 6. Illustrating the time shared coupling of transmitter and receiver in order to provide λ_1 with phase lock loop control, and $\lambda_{2,3,4}$ with selective responses to spectroscopic changes particularly those of the deoxyhemoglobin spectrum show in Fig. 1, i.e., 750, 816, and 830 nm in a frequency in the range of 50–440 MHz. Source/detector separation is typically 4 cm.

falls within the frequency band of interest. Time sharing of a variety of signals through the SSB system involves decreased duty ratio and some degradation of the S/N ratio. Time sharing is done at a frequency low compared to the 10 kHz local oscillator frequency, for example at 100 Hz or even 1 Hz. Lower frequencies may be necessary when PMT output stabilization is used with feedback.

Time sharing of the oscillator frequency is illustrated in Fig. 6 with a series of laser diodes operating in sequence and sequential switching of the output of the phase detector to provide multichannel operation. These switching operations are normally computer controlled.

The system in Fig. 6 uses the same radio frequency components as in Fig. 4. The rf output of the transmitter is coupled to a four-way power splitter and a sequence of four electromechanical rf switches to selectively activate laser diodes at $\lambda_1-\lambda_4$. λ_1 serves as a reference signal that is delayed by a 10 ns optical delay. The optical link feeds back over the total system. The received tissue signals (for example, through the human breast) are detected by the PMT, coupled to the receiver, down converted, and sequentially filtered to a 1 kHz bandwidth at 2 kHz and coupled to the phase detector. The output of the phase detector is also split four ways, and is coupled to four electromechanical switches synchronized with the rf switches. Sample and hold output circuits are used, one of which is used to couple into the matching network for phase locked loop control of a varactor tuned radio frequency source or of the 2 kHz VCO.

Time sharing of the information through different channels with electromechanical switches affords excellent channel separation, i.e., 60 dB. The power loss due to the duty ratio of time sharing can be compensated by using higher peak power.

C. Dual frequency, dual wavelength systems

In the above description, transmitters and receivers were nominally in the same frequency band with sufficient chan-

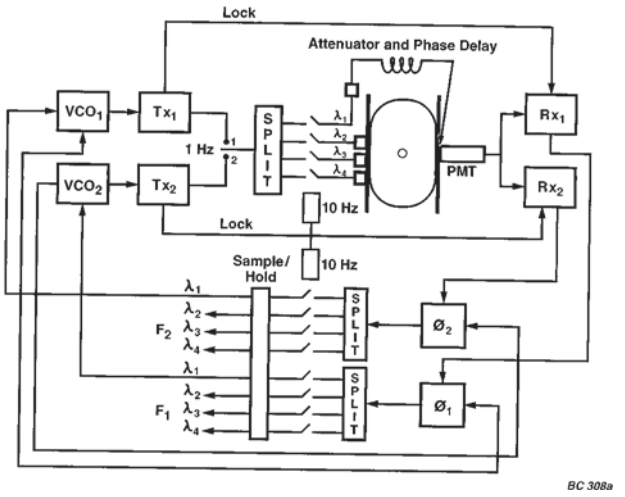

FIG. 7. A 200 MHz three-channel frequency encoded PMD for wide band output at three wavelengths.

nel separation to avoid crosstalk between the two frequencies. In other applications, it is desirable to acquire data in widely different frequency regions, for example, the high and low frequency approximations of the solution of the diffusion equation for the frequency domain instruments gives different formulas for calculation of μ_a and μ_s'. The photon diffusion pathway through the sample will be deep or shallow at low and high frequencies, respectively. Here we propose transmitter/receiver combinations which are widely separated in frequency, for example, 50 and 440 MHz may be possible. Thus, the diagram of Fig. 4 can be configured to use these widely different frequencies as in Fig. 7. In order to employ the same laser diodes and operate at two frequencies, we time share the laser diodes through an electromechanical switch (10 Hz) energizing them at Tx_1 and Tx_2 frequencies, for example, 50 and 440 MHz. The drivers for the laser diodes must be sufficiently wideband to accommodate this frequency range. The PLL attenuation bypasses the tissue as usual. The photodetector is wideband enough to detect the two frequencies and the outputs are connected in parallel to two receivers at 50 and 440 MHz. The two receiver outputs are coupled into the phase detectors and the phase detector output is time shared into the four sample and hold detectors, two of which carry PLL control. The output of two sample and hold circuits can then be directly connected to the appropriate VCOs so that they hold their frequency and phase in the time-sharing interval. The phase detectors accept 1 kHz sine waves from the two receivers and from the two VCOs and give out phase voltages for the eight signals which are then decoded as $\lambda_1-\lambda_4$ at F_1 and $\lambda_1-\lambda_4$ at F_2.

Two phase detectors can be used in order to avoid switching inputs and outputs. Also, transmitter switching can be done by modulation of 1 kHz local oscillator VCO_1, VCO_2 (Fig. 7). In other words, when Tx_1 is on, there will be signals from Rx_1 into ϕ_1 with phase locked control of the appropriate VCO and give λ_1, λ_2 signals at F_1. There is a similar sequence for F_2.

D. Time-shared IQ systems

A novel two wavelength time shared IQ system is shown in Fig. 8. The circuit consists of a 140 MHz oscillator which operates two time-shared laser diodes at 750 and 786 nm which are coupled to the tissue and a 9 mm diam cathode (Opto-8) PMT. A 20 dB gain amplifier thereafter is required for obtaining sine/cosine output from the IQ circuitry. These IQ outputs are connected to low pass filters to give the IQ outputs appropriately from 754 to 786 nm. These are coupled to either analog or digital circuitry to separate and to calculate the appropriate phase and amplitude signals at the two wavelengths, from which the oxygen saturation of the hemoglobin and the blood concentration are obtained.

Calibration by insertion of three known phase delays into the reference or measure pathways prior to, during, or after an experimental study is feasible via sequence phase delays of 2, 4, and 6 cm by using cut wire delays and computer controlled relays.

E. Frequency encoding multiplex

Frequency encoding has been the classical mode of multichannel communication but involves different transmitter/local oscillator/receiver frequency systems for each wavelength of light. In heterodyne systems, it requires channel separation at least 60 dB to compare favorably with the time

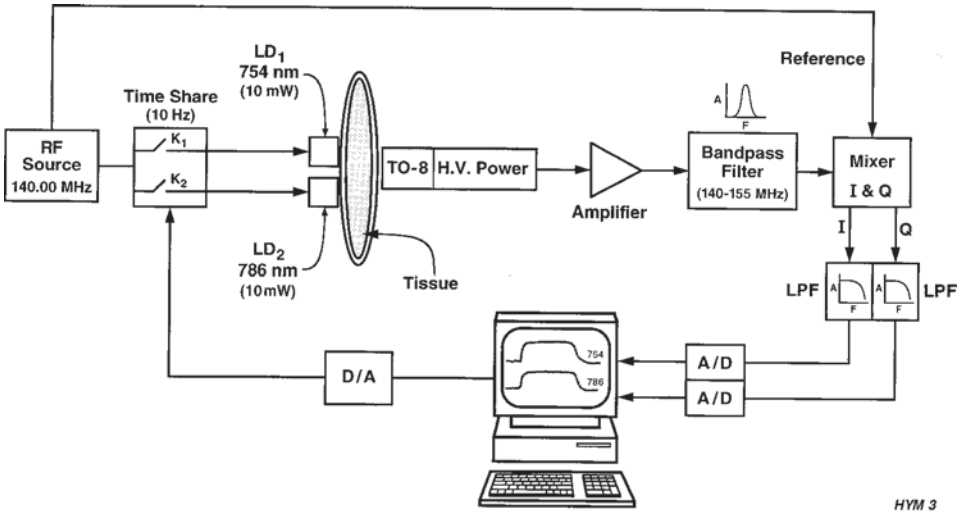

FIG. 8. A dual wavelength homodyne phase measurement system using IQ detection; time sharing is used to separate the two channels of laser diode wavelengths. The IQ detectors operate at 140 MHz. The phase and amplitude values are calculated from the IQ values by analogue or digital methods.

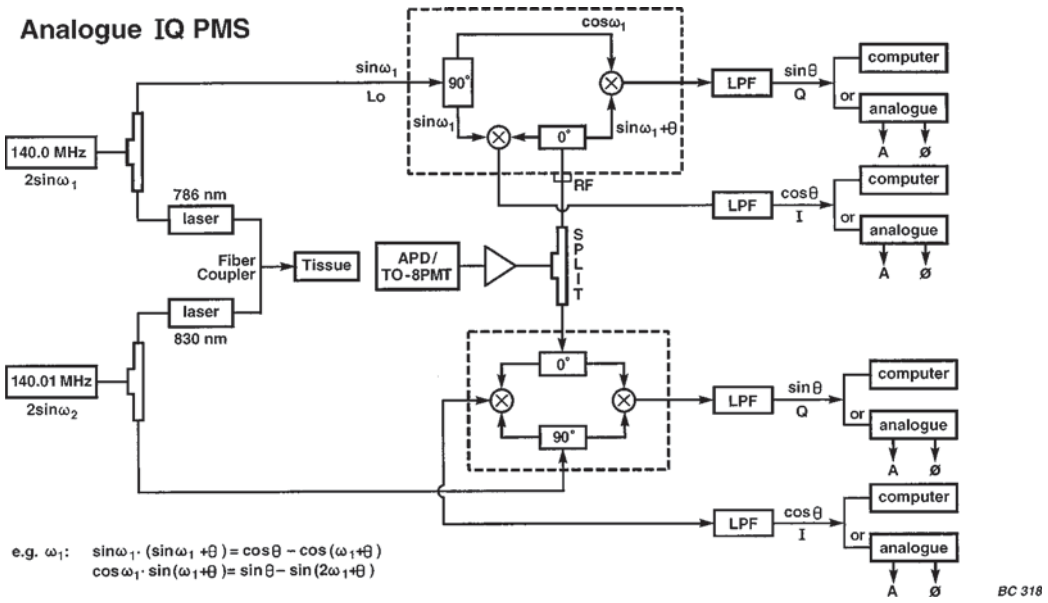

FIG. 9. A two wavelength frequency encoded PMS operating at 140 MHz. This system is especially adapted for wideband recordings of pathlength changes at the two wavelengths.

shared system and when executed with technology similar to the scheme of Figs. 6 and 7, involves replication of transmitter and receiver for each channel. It is indeed simple to replicate the communication system illustrated in Fig. 4, employing single sideband technology, consisting of a coupled transmitter–receiver and phase locked loop. A large number of combinations of radio frequency technology are possible in the frequency encoded system (see below).

When multiple oscillator frequencies are used in the circuits of Figs. 2(A)–2(D) in order to afford multichannel operation, the phase detector and/or mixer will receive multiple frequencies and intermodulation distortion crosstalk will appear in the mixer output unless appropriate filtering is used and single frequencies are coupled to the mixer. Appropriate techniques are available, i.e., analog and digital filters including FT systems, phase lock loop tracking systems, etc. to minimize intermodulation distortions. This usually means that the frequencies for the different wavelengths of the laser diode may be significantly different, requiring manipulation of the data in order to obtain correct phase shifts on the one hand, and the possibility of error due to intermodulation distortion (IMD) on the other. It is for this reason that most of the practical multi-wavelength systems employ time sharing of the laser diodes. In heterodyne systems multi-section filters can be constructed which would give adequate channel separation for a 2.5-fold difference ratio of frequencies of Fig. 9; in homodyne systems, the intermodulation signals due to the impingement of the two radio frequency carriers simultaneously upon the detector system can cause significant and complex intermodulation products especially when the transfer characteristic of the second dynode of the PMT is relied upon (see elsewhere for this description).[40] Such a system can be based on time-sharing technology (see Fig. 8).

F. A wide band dual wavelength system using frequency encoding

In cases where the narrow bandwidth of the time-sharing systems limits the response time, frequency encoding may be used in a heterodyne system where three oscillators are employed giving two intermediate frequencies in the kilohertz region according to the general diagram of Figs. 2(C) or 2(D) (see Fig. 10). Dual wavelength operation is satisfactory for quantitative measurements of scattering and absorption provided that a calculated μ_s', μ_a model is used to determine zero phase 200 MHz is used (2.4° phase shift per centimeter pathlength). Thus, F_1, [Fig. 2(C)] 200.000 MHz and F_2 is 200.025 MHz give one channel with a 25 kHz F_2-F_1 carrier and zero crossing phase detector (I). A third oscillator F_3 is employed, which is offset by 55 kHz to give a second difference frequency.

The two frequencies are separated to 50 dB by multipole filters and connected to separate phase detectors and hence zero to F filters of 0.01–5 s time constants. The two laser diode sources are merged into a Y connection fiber so that a single 3 mm fiber source and an 8 mm detection fiber are conveniently applied to human or animal models. In this case, the system employs dynode voltage modulation of the PMT. A single frequency, F_1, is used for modulating the second dynode of the PMT. The 50 Ω drive for the second dynode is transformed by a resonant circuit in voltage and impedance to approximately 1 kΩ, providing a 50 V drive for the second dynode and giving approximately 50% modulation at $\frac{1}{20}$ the wattage that would be needed with a 50 Ω drive.

The successful operation of this system depends critically upon the suppression of unwanted signals due to intermodulation in the dynode modulated PMT, and crosstalk between the 25 and 55 kHz signals in the bandpass filters. An analysis of the circuit shows that shielding of the output of the PMT from its rf input is sufficient to avoid overload in the low frequency amplifier (25–55 kHz). The 25 kHz filter is also designed with extreme care to ensure that the attenuation at 55 kHz is 50 dB or more. These design problems are evaluated in detail in a separate article.[54] Time sharing may afford a more economical solution.

The unit has been found to be robust with phase, noise,

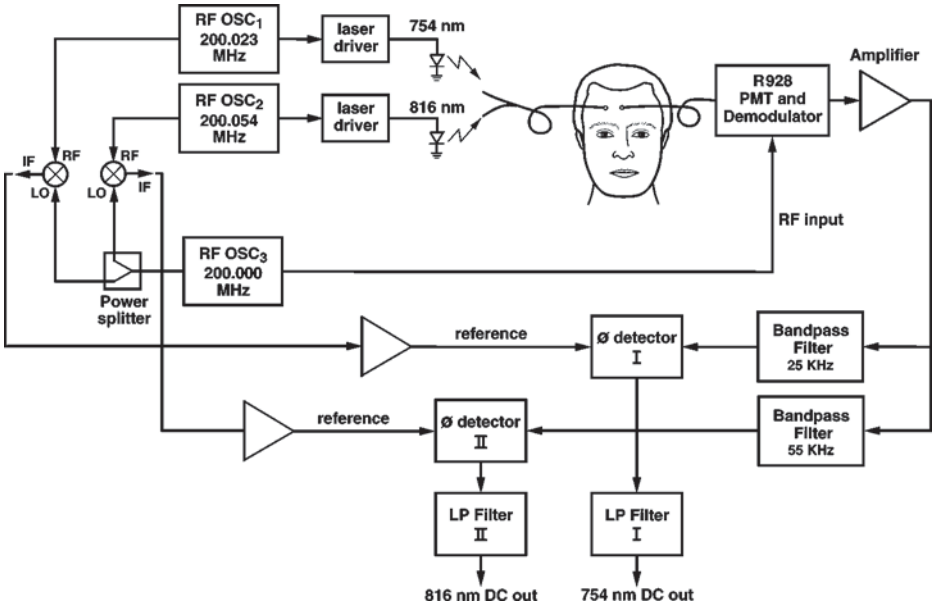

FIG. 10. 200 MHz phase modulatron device.

and drift of $<0.3°$ for a 2 h period. Amplitude signals can readily be provided by detecting the filter outputs of the 25 and 55 kHz signals. Frequency encoding acquires data continuously at both frequencies.

G. Frequency/phase crosstalk

In narrow band frequency encoded systems, drift of the oscillator frequency and/or shift of a filter phase band pass will cause phase errors. Thus, stable frequency sources and flat-topped filters prior to the phase detector with post phase detector narrow band filters are preferred, for example, DSP filters. In other systems, a filter in the dc to F region may be easier to construct and may be more efficient in terms of rise time per unit bandwidth than that between F_1 and F_2 at an intermediate frequency.[50]

H. Digital IQ circuitry using frequency encoding

If digital operation is desired, economic and available components require that the frequency be dropped from 140 to 30 MHz to allow the use of a 70 MHz digitizing frequency as in an untested system proposal (see Refs. 42 and 43). Thus, each oscillator frequency is digitized prior to coupling to the IQ circuit as a reference. Second, the two outputs of the PMT are similarly digitized prior to coupling to the digital IQ circuit. Thereafter, DSP of the low frequency components of the IQ output (LPF) is possible and affords versatility and precision in the calculation of the phase and amplitude values for the two wavelengths.

Such a digital system for phase measurement is shown in Fig. 11. The signals received from the PMT detector, suitably amplified to a level of 0 dB or greater, are coupled to the 70 MHz A/D converter (16 bit) and then to the IQ detector and low pass filter. The synthesized local oscillatory frequency gives 30 MHz digitized sine waves, as obtained from the 70 MHz clock frequency and an A/D converter without aliasing problems. In this way, the system is securely phased locked, and the IQ signals from the low pass filter are accurately related to the phase and amplitude changes of the PMT signal. Processing of the IQ signals can be with an appropriate computer program or give the phase and amplitude of the signals. A digital filter prior to each mixer at 30.00 and 30.040 MHz is essential to minimize intermodulation distortion.

VIII. CHARACTERISTICS OF IMAGING SYSTEMS

A. Comparison of systems: Nonimaging and imaging

The simple continuous wave devices have great utility where trends in the parameter are of importance, for example the change of hemoglobin saturation caused by cardiopulmonary bypass, stroke, etc., or indeed, muscular exercise. On the other hand, some applications require other quantified measures as a basis for medical decision making. In this case, the frequency and time domain instruments are required, particularly the former with accuracies of a few percent, for example, in hemoglobin saturation. While many pathologies can be discovered in lateralized organs such as brain, skeletal muscle, breast, etc., decision making in clinical medicine is best based upon quantified data where frequency and time domain methods appear necessary. Furthermore, the S/N ratio of a particular measurement of a pathology may be vastly increased by imaging, particularly where small pathologies are involved, i.e., in early tumor detection, small strokes, aneurysms, and thus quantification and localization appear necessary and some examples are already described.[3,58,59] Indeed it may be that S/N ratio maximization, particularly in a tissue volume exhibiting maximum deviation from the norm, may be more important than the average value in clinical decision making in the evaluation of the tissue at risk, particularly in brain, or indeed, in early tumors, small aneurysms, etc. Finally, functional imaging, i.e., identifying portions of the brain in which functional activity, evokes blood concentration and blood oxygenation changes require imaging in order to ensure that their stimu-

lation has evoked the response in the appropriate anatomical location.

B. Imaging algorithms

It is not the purpose of this article to describe in any detail algorithms by which the photon migration data can be converted into images. However, the general categories are those imagers which, first, select photons which are said to be ''ballistic'' and are not deviated by scattering in their source to detector transit.[60] These images are accumulated using gating pulses, and hence, time resolution of approximately 20 ps. Second, there are those that use snake-like photons[60] and are accumulated in times of near 300 ps duration. Third, a variety of imaging algorithms accept more and more of the migrating photons, until the whole gamut of time delays of photon diffusion is accepted.[61] In the frequency domain (the Fourier transform of the time domain), the analogous transformation gives very high frequency to low frequency modulated data.

Generally, for short optical pathlengths between source and detector, the assumption of rectilinear propagation appears justified and simple back projection algorithms are usable. These short photon pathlengths can be used in the remission mode and afford images of reasonable resolution particularly useful for studies of the brain cortex.[3,60,62,63]

For longer migration pathlengths, where rectilinear propagation can no longer be assumed, more complicated algorithms are necessary. Nevertheless, a fast two-dimensional 2D scan involves a Fourier spatial filter (*K* space) while other algorithms,[64,65] which create 2D or 3D images and involve solution of the inverse problem, are generally much slower. It is not clear what type of image algorithm will be optimal, but it is already obvious that different problems will be solved by different approaches, for example prefrontal imaging. This is readily solved by simple back-projection algorithms which co-register well with the nuclear magnetic resonance imaging (MRI) data.[3,59] Breast imaging, however, while currently satisfied with 2D images, requires penetration to distances of 5–7 cm, and a sensitivity/specificity criteria sufficient for diagnostic purposes. Imaging of structures within the brain have similar requirements for sensitivity of specificity, but have to deal with a greater degree of heterogeneity of background. While the final form of instrument design in these cases is unknown and subject to considerable evolution, the fact is that none of the devices is expensive when compared with PET, MRI, x-ray, or computed tomography (CT). In terms of diagnostic information, optical instrumentation may be significantly more economical than ultrasound. Again, the fact that the NIR technology at low light intensities is innocuous even to the eye is much to its advantage, and may be a deciding factor in its use with many patient populations, and in many medical and industrial applications.

C. Multiplex, multisource, multidetector systems for imaging

Special considerations in multi-source, multi-detector systems are used for imaging. Generally, imagers of the most rudimentary design require between 16 and 32 source positions which can be obtained by light guide coupling of the light sources to a fiber-optics multiplex system (Dicon Fiberoptics, Inc., Berkeley, CA) so that each source is illuminated sequentially, at approximately 1 per second. A similar arrangement can be used if a single detector serves a mechanically multiplexed fiber-optic coupler to all detector positions. However, this results in an acquisition time of over 200 s which is unacceptable for a human subject study and thus parallel detection is usually employed in which each detector position has a separate photomultiplier, or APD, generally necessitating duplication of the phase and amplitude detection systems.

In spectroscopy, as described previously, the source–detector distance is optimized to reach a particular depth at which data are to be accumulated. In imaging, a wide range of source–detector combinations are required; for example in a matrix of sources and detectors, the most remote coupling signals involving the largest separation of source and detector are often desirable in order to fill the data matrix. For this reason, the dwell time for any particular source–detector combination is a minimum of 1 s, and may in some cases be considerably longer. However, the duty ratio may be 1/16, thus, higher intensities could be considered (ftmt). It should be noted (see Sec. IV E) that the FDA limit is 50 μW in the NIR for class I laser light sources.

Electromechanical or radio frequency switching of multiple sources is necessary since few imaging algorithms are appropriate for data inversion in any system in which simultaneous illumination of the object from many positions is involved. Multipixel FD imaging using gain modulated image intensifiers was first used in fluorometry by Gratton[66] and extended to photon migration by Sevick *et al.*[1] and Knuttel *et al.*[67]

A number for forms of imagers simply have been adapted for the phase modulation systems described here with multiple sources and multiple detectors, for example, the three wavelength 50 MHz of Fig. 6 has been found to be convenient and useful for the study. Alternatively, time multiplex systems used for example in Figs. 6 and 7 have been found convenient in systems using 10 sources and 20 detectors. One feature not described here is the use of amplitude or phase cancellation to enhance the object location, for example, if the radio frequency phase for the laser diodes is split 0 and 180 deg, and they are equidistant from a detector, cancellation of phase will occur so that the residual value is 90 deg. The system, normalized on a model system, or on normal tissue, and transferred to an abnormal tissue, will sensitively detect the presence of a small object that unbalances the phase cancellation. Similarly, two detectors equidistant from a single source will give the same effect, except here it is convenient to cancel the amplitudes in a dc system. Numerous configurations of sources and detectors can be used in this type of imaging study. Such amplitude and cancellation technologies appear to greatly enhance the quality of the images. One feature of such imagers not usually required of oximeters described here is automatic balancing of phase and amplitude on model systems prior to the perturbations which are to be imaged. This is conveniently done by

computer controlled chips, which are suitable for dc signals or by using pin diode system designed by K. Simons for balancing intensities at radio frequencies. The latter may introduce phase shifts as a function of attenuation. Furthermore, software packages are necessary to program the activation of source/detector combinations in the scanning or data acquisition modes. Images are generally processed at present by simple back projection algorithms using available (MATlab) imaging programs. Extensive studies have been made of brain activation through sensorimotor, visual and especially cognitive activations, and related studies involving muscle activation have been found to be very useful in basic medical research and in clinical applications. Most prominent, however, is the detection of breast tumors based upon the congruent images of enhanced blood volume through anglogenesis, and enhanced deoxygenation through tumor hypermetabolism.

IX. COMPARISONS OF SPECIFIC PMS SYSTEMS

In order to afford insight and evaluation of the current technology of the several laboratories involved in the development of phase modulation devices for optical spectroscopy and imaging, Table I gives a summary of the state-of-the-art for phase modulation tissue spectroscopy. The equipment is classified generally by the multiplicity of wavelengths and the type of detection system, either heterodyne or homodyne (for example see Fig. 2). A summary of general characteristics follows.

A. Frequency range

The frequency is either fixed or variable and the range is indicated. The lowest frequency system that has been constructed is 50 MHz. However the analytic equations for the performance of these devices suggests that even frequencies lower than 50 MHz are feasible, especially since the phase accuracy, as indicated by the noise figures, can be very high at the low frequencies. Heterodyne systems involve an intermediate frequency, the choice of which may indeed govern the ultimate accuracy of phase detection and is seen to vary from 10 kHz *down* to that of the homodyne systems. The radio frequencies can be increased above 500 MHz, although the phase errors and rf couplings may increase.

B. Light sources

Laser diodes appear to be favored by almost all contributors to this technology, and thus the wavelengths recited are simply those of available laser diodes. It is noteworthy that the wavelengths favored by finger-pulse oximetry do not appear in this list (often 670 and 940 nm) mainly because maximal tissue penetration is desired for the human subject studies, particularly brain and breast.

Considerable variation in the laser source output is observed, as is the power delivered to the tissue. The 20 μW values at 760 nm indicate that the system is designed to comply with the FDA class I laser delivery, i.e., to be ''retina safe''. The ''International'' laser safety standard, EN60825, accepts nearer 200 μW as being class I for a ''collimated beam.'' The other systems are apparently used in human studies under special waiver from the local medical institution boards, and presumably proper labeling and proper safety devices are employed by those exceeding the appropriate safety authority power levels of light delivery to tissues.

C. Detectors

The Hamamatsu R928 PMT appears to be the detector of choice that is currently available because of its extended red response. This response is available in other PMT types which may be more suitable for special purposes where space is at a premium or where higher frequencies are required. The high voltages are typical of those used for the test of the device on the human forehead at 3 cm separation, and gives an indication of the amplifier gain following the detector. The consequent S/N ratio is measured in a 1 Hz bandwidth and varies significantly among the devices.

D. Test data

We are fortunate to have had the communication and cooperation of those working on phase modulation devices. The key parameters and performance are first the calibration as a device for measuring the velocity of light in air, listed as ϕ/L (°/cm). The error of this value with respect to the velocity of light in water—whichever test is made—varies from 20% to 0.5%. Any calibration error needs to be accounted for before converting the output data to μ_a/μ_s using the algorithm of choice.[31]

The phase change per absorbance unit in the absence of a pathlength change is an important criterion (ϕ/M) in determining whether the unit will change its calibration under conditions where light absorption and optical pathlength vary simultaneously, as in the case of the deoxygenation of hemoglobin, or blood volume changes. Any significant crosstalk requires that the unit afford some compensation for this error, in order to correctly determine the phase shift. The product $\mu_a\ \varphi/M$ varies from 0.04°/dB in heterodyne systems to 1°/dB in the homodyne system shown here.[54]

Instrument noise is determined for both phase and amplitude, and is seen to vary significantly with laser power. This is indeed the criterion of performance and values less than a tenth of a degree in phase and less than a tenth of a percent in amplitude seem to be achievable and are required to ensure that instrument accuracy does not determine the accuracy of hemoglobin saturation in human subjects. If values given for amplitude and phase are taken after an initial warm-up time, a tenth of a degree in phase and a tenth of a percent over a 1 h operating interval is expected.

Another important test of performance is interchannel interference, listed here for two pairs of wavelengths, as represented over the performance of multi-wavelength devices. Such an effect can occur, for example, when the signal or corresponding harmonic at one wavelength (channel) leaks into the other wavelength channel. Such conditions (as given here for a 20 dB [1 ΔOD change] can cause significant error and should be maintained at less than a tenth of a degree change for a 20 dB signal change. The following presents specific details of the various devices.

X. PERFORMANCE OF SPECIFIC PMS SYSTEMS

A. Homodyne system (IBBR)

This is a two wavelength time shared system at 140 MHz with wavelengths of 754 and 780 nm.[31] The laser output is 10 mW and the intensity at tissue is on the order of 20 μW. A Hamamatsu T0-8 S20 surface is acceptable for these two wavelengths and is operated at 600–900 V to give a S/N ratio of 550 at 3 cm separation for the brain test. The attenuation is over 50 dB under these conditions. The calibration of phase per cm separation in air is given as 1.2°/cm which is 20% from the theoretical value.

The amplitude/phase crosstalk is 1.4°/dB. The phase noise in a 1 Hz bandwidth is 0.1° and the amplitude noise is 0.2%. The absolute drift of amplitude and phase are 0.05°/h and 0.2°/h. In this case, intermodulation has been tested in detail and for a 20 dB change of one wavelength at 754 nm, 0.1° crosstalk appears in the other channel and vice versa. The laser diode modulation is over 90%. The real (χ) and imaginary (Y) components of phase and amplitude are processed directly in a PC to give φ and M. This unit is characterized by great simplicity of components having three active elements and four passive elements plus a PC.

B. Heterodyne systems

The HP8505 network analyzer affords a frequency sweep phase meter of $\pm 3°$ accuracy from 0.3 to 1000 MHz and an amplitude/phase crosstalk of $\sim$1 dB/60 dB.[68,69] The unit ''Tromberg'' is built around an HP network analyzer.[70] It is a time shared four wavelength system, currently, with a projected seven wavelengths. The current wavelengths available are 647, 811, 849, and 956 nm with add-ons at 780, 940, and 980 nm in an 8×8 multiplex system wherein the sample can be sequentially illuminated by any one of the above wavelengths with a mechanical sequencer (Dicon Fiberoptics, Inc., Berkeley, CA). The laser diode output is typically 100 mW with approximately 25 mW delivered to the tissue. This is a relatively high power; 1000 times the ''retina safe'' power and one would except $1000^{1/2}$ improved S/N ratio over system operation at <100 μ. The Hamamatsu avalanche photodiode detection is used to cover this frequency range. Connection from the 2 mm fiber to the 0.5 mm aperture of the APD (AC 5658 0.5 mm diameter) with an appropriate optical coupler. A voltage of approximately -300 V is applied to the APD and the noise level is -48 dB m or 16 nW with a linear range to 3 mW giving typical S/N for the human forehead of <5, at $\rho = 3$ cm at 200 MHz. The HP 8505 permits an attenuation of 100 dB at the receiver input.

Since the phase error increases with frequency, the error is specified as a length $1<1$ mm. The crosstalk between amplitude and phase is 0.05°/dB and might be expected to be significant over the wide frequency range covered by the network analyzer. This is the subject of a recent paper on the APD.[48]

The noise, which also depends upon modulation frequency is cited to be 0.3° and the corresponding value in amplitude is 0.6% or 0.002 OD. The drift of phase is variable between 0.007 and 7.5°/h and (0.7°/h at 1 GHz) while the corresponding drift in amplitude is from 0.6 to 20%/h (9%/h for 200 MHz). These values are reduced to a 1 Hz bandwidth by dividing by 3. These values are said to vary with detector, and a new instrument gives values at the low end of the above ranges. The drift is such that recalibration every 15 min is desirable to reduce systematic errors of optical properties to 1%. Since multiple wavelengths are used, the crosstalk between wavelength channels is of great interest (not available). The modulation of the laser diodes varies from 80% at 1 MHz to 20% at 1 GHz.

Dr. H. Siebold of Seimens AG[71] has tested two PMS systems, the most recent in a homodyne ''lock in'' at four frequencies (69–75 MHz) and four optical wavelengths: 690, 755, 790, and 856 nm with 90% modulation of the laser diodes, at respective powers of 30, 10, 40, and 50 mW (70% is delivered to the tissue). Hamamatsu R943-02 responds adequately at 856 nm. In view of these intensities and high detector quantum efficiency, the performance is good, 0.01°/Hz noise (increasing to 0.08°/Hz while scanning). The amplitude noise of 1.5×10^{-3}% also increases to 3.1×10^{-2}% while scanning. The amplitude phase crosstalk is 0.05°/dB over 40 dB; other parameters are not available.

Five rather similar heterodyne systems have been tested: one by Cope, one by Wallace, two from IBBR, and one from NIM (Thayer). These systems (Cope, Wallace and Thayer) are heterodyne systems with intermediate frequencies in the kHz region and all rely upon basic oscillator stability to avoid drift and phase noise. Cope's system is variable in frequency range from 1 to 500 MHz. Tromberg also has assembled a variable frequency heterodyne system based upon the H-P network analyzer.

The early prototype of this class is a 200 MHz frequency encoded heterodyne system with intermediate frequencies for the two light sources of 25 and 55 kHz.[72] The wavelengths are 754 and 816 nm and the laser output is 5 mW delivering 20 μW to the tissue through lightguides.[58] The R928 detector is modulated at the second dynode to give the intermediate frequency and typical high voltages of 800 V for a S/N ratio of 25 with a 3 cm spacing on the forehead, corresponding to 100 dB attenuation. The phase/pathlength calibration is 2.32° cm within 5% of the theoretical for air. The amplitude phase crosstalk is 0.1°/dB over 40 dB. The phase noise in a 1 Hz bandwidth is 0.3° while the amplitude noise is 1.1%, the drift being 0.16°/h in phase and 1%/h in amplitude. The interchannel crosstalk from 25 to 55 kHz channels is 0.9° over a 10 dB range due to the difficulties of making a sharp roll off of the 25 kHz filter over 1 octave. The crosstalk from 55 to 25 kHz is negligible (0.5°) over a 10 dB range. The modulation of the source is 90% and in this case, the modulation of the detector at the second dynode is approximately 50%, taking into account the available power and its effect upon the sensitive parts of the circuit, and the leakage into undesirable sensitive portions of the radio frequency circuit.

This device has a two companion systems in which the light from the two laser diodes is time shared by a 60 Hz vibrating reed[73] and in the other by a 100 Hz rotating disk. Calibration of these units can be obtained with multiple switched phase delays. In this case, the inter channel crosstalk is less.

1. SRI system

The Faris and Wu system[74] is a heterodyne operating at 107 MHz at 671 nm with a source power of 3.2 mW and 800 μW delivered to the tissue. The detector is R928. The noise (per Hz) is 0.3 in phase and 0.36% per Hz in amplitude. The drift is 0.25°/h in phase, and 0.4%/h in amplitude. The modulation is 90%. The calibration of phase shift per cm separation in air is 1.2°/cm SRS (not associated with SRI) manufacturers a variable frequency ''lock in.'' We have observed frequency dependent offset.

2. 50 MHz SSB system

An innovative IBBR heterodyne system,[73] takes advantage of single sideband modulation and demodulation to afford increased S/N ratio. It operates at 50 MHz and the SSB frequency is 1 kHz. Three wavelengths are presently used: 754, 790, and 830 nm and a third channel employing 790 nm is put through a fixed optical delay line in order to provide continuous monitoring and control of the phase delay through the electro-optical system using a phase locked loop which is coupled to a varator diode for control of the transmitter phase. This system has a nominal laser output of 5 mW and a delivered power of 20 μW. The detector is either an R928 or the S20 Opto 8 from Hamamatsu. At a bias voltage of 750 V, the S/N ratio on the forehead at a 3 cm separation is 46 for the 754 channel and for the 830 channel, corresponding to an attenuation of $\overline{25}$ dB.

The amplitude/phase shift crosstalk is 0.04°/dB. It should be noted that this system employs dynode voltage regulation controlled by the automatic gain control signal of the SSB receiver. This minimizes amplitude/phase shift in the electronic system because the signal amplitude is held accurately constant. Adjustment of the PMT voltage in order to achieve constant input to the receiver, itself causes a phase error. This error is linear with high voltage and thus readily corrected with a simple computer algorithm resulting in a residual error of 0.04°/dB.

The phase noise is very low: 0.05°/Hz as is the amplitude noise (0.5%) because of the PLL. The drift is minimal, 0.01°/h. Intermodulation is minimal due to the fact that the light sources are time shared.

The source modulation is 90% and since an SSB receiver and detector are used, the modulation fraction is very high. The demodulation is very efficient (see Figs. 12 and 13).

3. UCL system

Another heterodyne system is that due to Cope, which uses commercially available components of very high quality where available.[75] This employs a pair of phase locked oscillators for the heterodyne system, and allows a frequency variation between 1 and 500 MHz (see Fig. 12). The intermediate frequency is chosen to be 10 kHz and four wavelengths are time shared. The laser power being delivered to the tissue is 3 mW. The R5841 detector (Hamamatsu Photo-

TABLE I. Summary of instrument performance.

Group	Type	RF (MHz)	IF (kHz)	Wavelength (nm)	Modulation	Laser power (mW)	Laser power at tissue (μW)	Detector
Cope	four wavelength HET	1–500	10	744,807,832,859	80	10–50	3000	R5841
Chance, Guan	three wavelength SSB	50	10	754,790,830	90	3	20	R928
Chance, Zhou	two wavelength HET	200	25,55	754,816	90	5	20	R928
Chance, Ma	two wavelength HOM	140	140 000	754,780	90	10	20	T08
ISS	two wavelength HET	110	5	750,830 (4×4)	⋯	⋯	800–2300	R928
Tromberg	network analyzer	0.3–1000[a]	4	674,811,849,956	20–80	—	25 000	APD
NIM	three wavelength HET	200	30	754,785,816	90	10 to 40	180	R928

Group	Type	High voltage	S/N (Calc)	S/N[a] (Exp)	ϕ/L (°/cm)	Error (°/cm)	ϕ/M (°/dB)	Noise ϕ(°/Hz)	Noise M (%/Hz)	Drift (0.2 Hz) ϕ (°/h)	Drift (0.2 Hz) M (%h)
Cope	four wavelength HET	2500	⋯	1000	2.4	0.01	0.06	0.34	1.9	0.06	0.25
Chance, Guan	three wavelength SSB	750	⋯	2300	⋯	⋯	0.04	0.05	0.6	0.01	⋯
Chance, Zhou	two wavelength HET	640	7000	1250	2.32	0.08	0.5	0.3	1.1	0.16	1
Chance, Ma	two wavelength HOM	740	6000	550	1.23	0.36	0.1	0.12	0.2	0.05	0.2
ISS	two wavelength HET	750	⋯	⋯	⋯	⋯	⋯	⋯	⋯	⋯	⋯
Tromberg	network analyzer	300	⋯	⋯	±1°	±1°	0.05° dB	0.3	0.6	0.007–7.5	0.6–20
NIM	three wavelength HET	800	⋯	2000	2.5,2.7,2.6	5,11,10	0.5,0.6,0.9	0.1	0.5	0.2	0.4
Siebold	four λ homodyne	500–900	⋯	10^3	⋯	⋯	0.05° dB	0.08	0.03	⋯	⋯

Group	Type	$\Delta\phi(\lambda_1-\lambda_2)$ (°)	$\Delta\phi(\lambda_2-\lambda_1)$ (°)	$\Delta M(\lambda_1-\lambda_2)$ (%)	$\Delta M(\lambda_2-\lambda_1)$ (%)
Cope	four wavelength (HET)	0.1	⋯	⋯	⋯
Chance, Guan	three wavelength SSB	⋯	⋯	⋯	⋯
Chance, Zhou	two wavelength HET	1.9	⋯	0.05∼	∼
Chance, Ma	two wavelength HOM	0.3	0.3	0.30	0.03
Barbieri	eight wavelength HET	⋯	⋯	⋯	⋯
Tromberg	network analyzer	⋯	⋯	⋯	⋯
NIM	three wavelength HET	0.4	0	0	0

[a]The phase error is +3° over this frequency range.

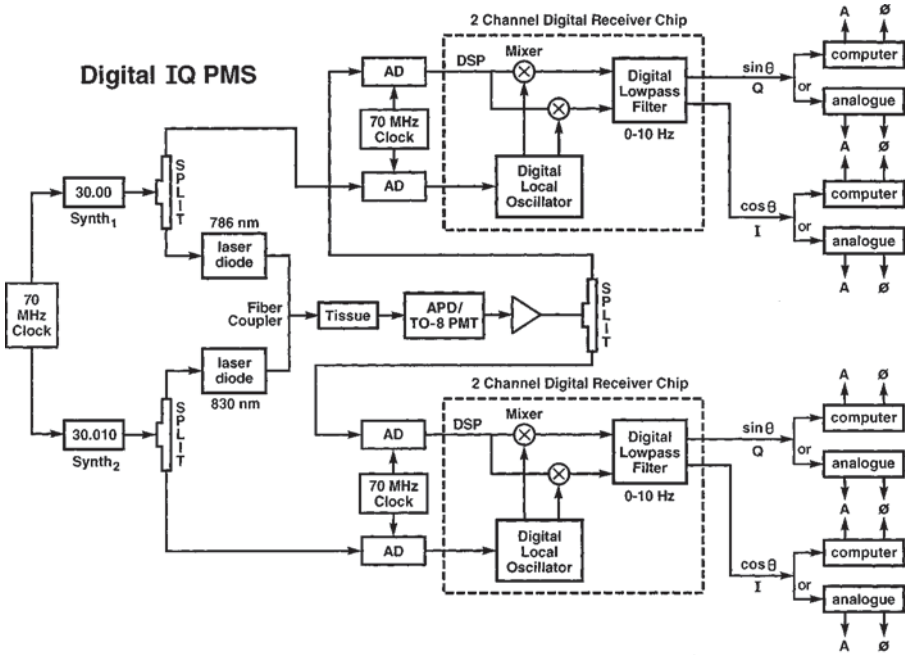

FIG. 11. A digital phase modulation system with digital signal processing operating at 30 MHz with a 70 MHz clock frequency (see Refs. 42 and 43).

nics) operates at a variable voltage but is normally near its maximum value of 2500 V. The S/N ratio for average intensity at 3 cm optode separation on the human forehead is 60 dB (3 OD) at a 1 Hz bandwidth. The maximum attenuation is 6 OD or 120 dB (corresponding to 4 cm optode separation on an adult head). The calibration of phase shift per cm 2.40°/cm at 60 dB attenuation, 200 MHz modulation, and $\rho = 3.0$ cm as expected. The crosstalk of intensity variations into phase shift is 0.06°/dB. The phase and modulation noise is 0.34° and 0.09%, respectively, at 3 cm optode separation on the human forehead in a 1 Hz bandwidth, while phase and modulation depth drift is 0.06°/h and 0.25%/h, respectively. Laser sources are modulated at a depth of 80% and the detector attenuates the modulation depth to a further 4 dB at 200 MHz. This attenuation increases to approximately 10 dB at 500 MHz. rf power to the laser diodes responds to control signals from a PC, which in turn digitizes intensity, phase, and modulation depth data from two detectors appropriate to the active laser diode. A silicon diode detector was used to improve drift performance, but this has not proved to be the case. A second PMT detector can be added to allow dual site measurements.

4. ISS oximeter

The ISS oximeter is in its second phase of development at ISS.[40] This ISS oximeter has many of the features of the earlier unit, but has improved performance, particularly with respect to thermal drift. The unit uses dual wavelengths 830 and 750 nm from laser diodes instead of light-emitting diodes (LEDs) but retains the use of several source–detector distances to determine the optical properties of tissue with reference to a silicone rubber block of known μ_a/μ_s'. The source–detector distances are usually 1.5–2.5 and 3 cm, as compared to single source–detector separations of 3–4 cm for other oximeters. The light intensity delivered to the tissue varies from 0.08 to 2.2 mW. The system operates at 110 MHz in a heterodyne fashion with an offset frequency of 5 kHz. The system retains second dynode modulation of the R928 PMT. A sequence of the laser diodes takes 0.32 s to give 2.56 s for a data acquisition diodes. The phase noise on the standard is 0.06°/Hz, which is a noise level maintained under practical conditions of brain observation (with source detector separations varying from 1.5 to 3.0 cm). This noise level approximately doubles for the increased absorption of skeletal muscle. The calculated noise in hemoglobin saturation determinations is 0.3% for a 3 s acquisition time on the brain phantom. The thermal drift is quoted as ±1.5% with a 3.5 s time constant and gives ~1% fluctuation in oxygen saturation when HbO_2 is 95%. A number of test data values are absent, as indicated by the blanks in Table II, particularly the amplitude/phase and interchannel crosstalk values, as is the absolute calibration in the instrument as a time measuring device (phase shift per centimeter change in pathlength).

5. A three wavelength 50 MHz time shared phase modulation system with dynode feedback

As an example of current technology, the performance of the system of Fig. 6 is illustrated by the following tests, and dynode feedback is included. The phase shift due to changes in absorption caused by the oxygenation and deoxygenation due to yeast in 0.6% hemoglobin in 0.6% intralipid are illustrated in Fig. 13. In addition, the response to scattering changes are afforded by a single addition with 4 ml/ℓ of 20%L intralipid at a source–detector separation of 4 cm. The response to a scattering change is nearly identical in the three channels while the response to hemoglobin oxygenation is in accordance with the absorption difference spectrum of hemoglobin shown in Fig. 1. Thus, 750 nm gives a large response, and 780 and 830 nm give small and approximately equal and opposite responses.

Tests of the linearity of the system are afforded by intralipid titration where increasing amounts of intralipid give

TABLE II. Detection sensitivity for optical spectroscopy and imaging.

Quantity Substance	μ_a						μ_s'	
	$Hb+HbO_2$	$HbO_2/Hb+HbO_2$	Cyto. Oxidase	ICG	ICG Fluor.	H_2O	Cell proliferation	Solutes
Potential variation in breast tissue concentration (ΔmM)	0.2	0.05/0.2	0.005	0.002	0.002	10,010	20%	100
Wavelength (nm)	816	754,780	850	780	830	950	780	850
Extinction coeff. ($cm^{-1}\,mM^{-1}$) (log_{10})	0.4	0.2/0.4	0.4	100	---	3×10^{-6}	---	---
μ_s' concentration	---	---	---	---	---	---	0.8 (cm^{-1}/% Vol)	-0.001 (cm^{-1}/mM)
Value of $\Delta\mu_a$ or $\Delta\mu_s'$ (cm^{-1})	0.08	---	0.005	0.400	---	0.10	0.1	0.1
Photon fluence ($cm^{-2}\,s^{-1}$)	10^{10}	10^{10}	10^{10}	10^{10}	10^{8}	10^{9}	10^{10}	10^{10}
PMT(GaAs) quantum yield(%)	10	10	10	10	10	10	10	10
Photoelectrons at photocathode (s^{-1})	2×10^{6}	2×10^{6}	2×10^{6}	2×10^{6}	10^{4}	2×10^{3}	2×10^{6}	2×10^{6}
S/N at photocathode	10^{3}	10^{3}	10^{3}	10^{3}	10^{2}	50	10^{3}	10^{3}
Detection sensitivity (S/N)	80	20	5	400	5	5	100	100

$\mu_a = 0.022\ cm^{-1}$ and a μ_s' change of approximately 6 cm^{-1} that is very close to standard values. The accuracy with which the phase is shifted as a function of intralipid concentration is high, the error of μ_s' at the three wavelengths is, respectively, 0.5%, 1.3%, and 1.3% at 780, 750, and 830 nm. Furthermore, the phase noise of the recordings as shown by the calibration mark of 1° is less than 0.1° at a bandwidth of 0.2 Hz. The concept of single sideband modulation, phase lock loop frequency/phase stabilization, and the time sharing of the optical signals through electromagnetic switches at a low frequency, is shown to be feasible. However, rf switching of laser diodes gives dc shot noise in photodetectors. In addition to these features (in order to regularize the amplitude/phase crosstalk), the amplitude of the PMT anode current controls the dynode voltage of the PMT, and this affords a reproducible voltage dependent phase offset which can be compensated by an appropriate computer look-up table or by a simple analog correction over the range of 400–900 bias voltage.

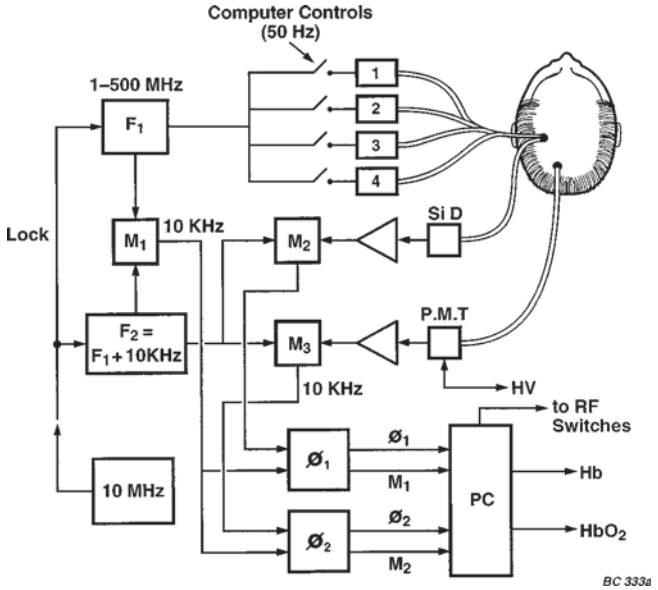

FIG. 12. The University College London system designed by Dr. M. Cope. A multi-frequency multi-wavelength heterodyne system with time shared laser diodes and a compensatory signal pathway is shown.

C. Example of an application to human subject

In order to illustrate typical problems and solutions in studying human subjects in Fig. 14 at an optode spacing of 4 cm, we demonstrate the response of the adult human arm to

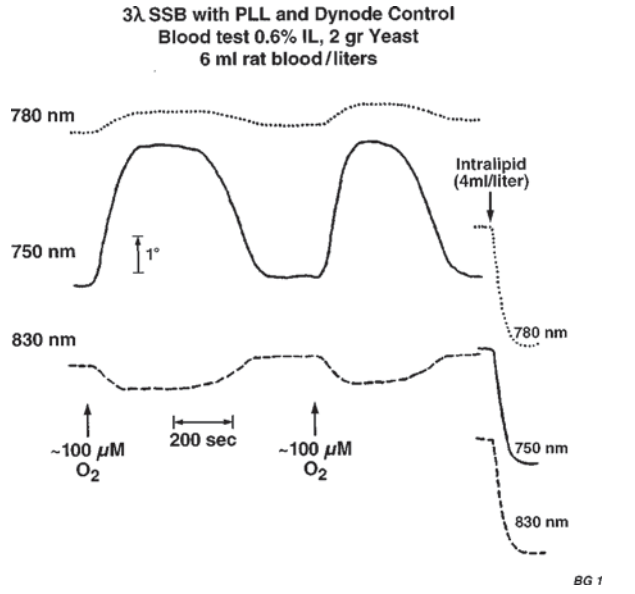

FIG. 13. Illustrating sensitivity of the phase delay to absorbance changes due to the oxygenation of blood and to scattering changes due to the addition of more scatterer (Intralipid). The response of the three wavelength system 780, 750, and 830 nm using a 50 MHz SSB system with dynode control is illustrated. Source–detector separation=4 cm. Blood test, 0.6% IL, 2 g yeast; 6 ml rat blood.

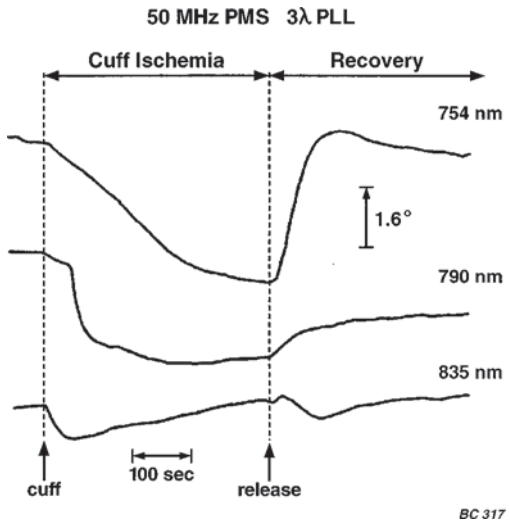

FIG. 14. Illustrating raw unprocessed data on phase changes due to blood concentration and deoxygenation/reoxygenation changes in arm cuff ischemia using the 3λ phase modulation instrument of Fig. 6. Source–detector separation=4 cm.

the blockade of circulation with subsequent absorption spectrum changes according to Fig. 1, where the absorption spectrum changes from that of oxyhemoglobin to largely deoxyhemoglobin because of the occlusion of circulation in the arm.

The initial state of the arm at rest is relatively highly oxygenated, and baselines are established for the three wavelengths of the apparatus of Fig. 14. Rapid inflation of the cuff to a pressure of 240 Torr is sufficient to cut off the arterial and venous circulation simultaneously for a typical arterial pressure of 120 Torr. Initially, no change is observed while the cuff is being inflated manually to 250 Torr. Thereafter, the cuff pressure redistributes the oxyhemoglobin in the arm so that an increased absorption is observed at 780 and 830 nm. The trace at 750 nm is responsive mainly to the slow conversion from oxy- to deoxyhemoglobin causing decreased optical pathlengths and transmission at 750 nm and at 780 nm, but in the opposite direction at 830 nm (see corresponding changes in the model of Fig. 13 and Fig. 1). The changes continue over an interval of approximately 8 min and reach a plateau when most of the oxyhemoglobin has been deoxygenated by tissue metabolism (there being no blood flow and no new oxygen supply). On releasing the cuff, oxygenated hemoglobin flows into the arm and the direction of the traces is rapidly reversed. There is a slight overshoot seen at 780 nm because the inrush of blood exceeds the normal supply level. It should be noted that the traces obtained with this instrument are quite noiseless, that the response is adequate to measure the transients of blood concentration on inflating the cuff and upon releasing the cuff. This test is routinely used in the study of the human blood circulation on the one hand, and on evaluating optical instruments on the other (see Fig. 13).

System performance considerations. Overall, the phase modulation oximeters are expected to track the deoxygenation, reoxygenation of blood, theoretically from 0% to 100%, practically from 20% to 80% (because the tissue survival limits are encompassed by the latter range). The requisite accuracy is not known since we have no statistical data on the significance of a change of saturation upon long term human brain survival. Presumably, the range from 80% to 60% is relatively benign and a 5% error would not be of importance in clinical decision making. However, from 60% to 30% may be quite important and life threatening situations may rapidly evolve particularly in the case of a sudden stroke, rupture of an aneurysm, a head injury causing herneation of brain circulation, etc., where a few percent of saturation might represent the border between brain damage and brain survival. As instrumentation developers, it is therefore necessary to cite conservative limits and to this point, propagation of errors from phase into saturation are important and somewhat algorithm dependent. Generally, however, the phase error of half the saturation error would be a conservative approach and thus if we cite that $\pm 5\%$ from 60 to 80 and $\pm 2\%$ from 30 to 60 would be a requirement, then we come to a phase of approximately $\pm 1\%$ in the middle lower region. Typically, phase shift from oxy to deoxy signals involve pathlength changes of 5 cm in the region of interest, 50–200 MHz, which when measured to 1% would be 0.05 mm comes to a small fraction of a degree, i.e., 0.05°. Thus, we have named a value of 0.03° as a target in this article taking into account that as the frequency increases, the tolerance on phase error increases, proportionately. However, we have not been able to get increased accuracy at high frequencies as compared with low frequencies for technical reasons that are discussed elsewhere in this article.

It should be pointed out that the anesthesiologists who are most likely to use the instruments expect a quantum jump of precision, reproducibility, and usability over the continuous light devices which have the intrinsic fault of failing to measure optical pathlengths. Finally, the finger-pulse oximeter has conditioned anesthesiologists to expect to read saturation to three significant figures. Thus, this article which approaches the problem from the instrumental standpoint tries to set a realizable but difficult goal for instrumentation specialists.

XI. CALCULATION OF EXPECTED SENSITIVITIES IN BIOLOGICAL SYSTEMS

Hemoglobin in blood is the principal absorber, and it is determined as a total amount (blood volume $Hb+HbO_2$) or as is oxygen carrying capacity or saturation $((HbO_2/Hb+HbO_2))$. This can be used to determine the ''biological'' accuracy of the phase modulation systems discussed. The magnitude of the six more principal optical signals obtainable from the human body are listed together with their optical properties and nominal concentrations in Table II. The appropriate parameters are given together with nominal concentrations, as seen by the optical method. This is significantly less than the total concentration of blood, because of the improbability of photon migration through large blood vessels.

Cytochromes (cell colors) are located in the ''powerhouse'' of the cell, the mitochondria, which use oxygen delivered by hemoglobin to form adenosine–triphosphate (ATP) which drives nearly all the biological reactions. Cyto-

chrome oxidase has a copper component with an NIR absorption band peaking at approximately 830 nm. This is similar to the absorption band of oxy-hemoglobin (see Fig. 1) in the region 800–900 nm, and indeed is easily mistaken for it. Thus, since cytochrome oxidase has a low concentration (see Table II), it is not only difficult to detect, but difficult to deconvolute from the oxy-hemoglobin signal (Matcher *et al.*[76]).

The mitochondria themselves are organelles of approximately the same size of the wavelength of the light used, and again, having a slightly higher refractive index, are effective scatterers and in fact comprise a major component of the scattering of the cell,[9,68] thus, the biological system provides signals of both absorption and scattering changes. Scattering changes can be induced by altering the refractive index of the water surrounding the cell, or of the cell itself. Substances, termed solutes, are routinely used in trauma therapy. Mannitol, for example, is administered intravenously to patients who have had head injury in order to diminish the volume of the brain. These solutes give scattering changes because they alter the relative refractive index of the cell and its surrounding extracellular water. They may also cause changes of cell volume. Water has a very small absorption coefficient, however, its ~50 M concentration in biological systems makes it important not only to optical methods but also to magnetic resonance methods. Thus it is a further chromophore which must be reckoned with. Melanin and bilirubin are two other pigments, one physiological and the other pathological, which are to be reckoned with, although melanin's absorption is decreasing rapidly as the NIR window is approached (Fig. 1).

The most important class of absorbing/fluorescence substances are contrast agents, substances which are delivered intravenously and occupy the blood vessels or may be transferred into the extracellular space or the intracellular space of tissues. While only one contrast agent is currently approved for human use, indocyanine green (ICG), many others are expected to be developed in the near future to allow probing of many important functions of the body with optical means. The characteristics for ICG given in the table, identify its very high extinction coefficient and significant fluorescence (in spite of the low quantum yield).

XII. SUMMARY

This article contains a review of principles and practices that are well known over many years, but have been recently specialized to the needs of phase modulation spectroscopy, where small changes of photon propagation at a variety of wavelengths and modulation frequencies are required for precise biomedical computations of blood oxygen concentrations. The performance of both homodyne and heterodyne systems operating in the region of 30–450 MHz has been examined as having both advantages and disadvantages; the homodyne system has advantages of simplicity of construction and execution, while the heterodyne system, particularly single sideband system, enjoys a higher precision and the possibility of low frequency phase detection not enjoyed by the homodyne system. Both systems can be constructed by readily available components.

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NOMENCLATURE

ICG	Indocyanine Green, an optical contrast agent
ISS	A Fluorescence Instrumentation NIR Imaging Company, Champaign, IL
UCL	University College London
SRI	Stranford Research Institute
Hb	Deoxyhemoglobin
HbO_2	Oxyhemoglobin
NIM, Inc.	A small Instrumentation company at the University City Science Center, 3624 Market Street, Philadelphia, PA
UCSC	University City Science Center, 3624 Market Street, Philadelphia, PA
IBBR	Institute for Biochemical and Biophysical Research, a part of the UCSC
APD	Avalanche photodiode
PMS	phase modulation system
PDMD	phase delay measurement device
FDD	frequency domain devices
TMD	time domain multiplex device
FDA	Federal Drug Administration
RC	resistance capacity
TC	time constants
NIR	near infrared
Wratten	trade name for Kodac *k* filters
IQ	inplane/quadature
DSB	digital signal processing
OPTO-8	A size category for Hamamatsu Photomultiplier Tube, approximately 1 cm^2
PLL	phase lock loop
VCO	voltage controlled oscillator
SRS	Stanford Research System
SRI	Stanford Research Institute
AM	amplitude modulation
FM	frequency modulation
PMT	photomultiplier tube
IMD	intermodulation distortion
SSB	single sideband
MRI	magnetic resonance imaging
DPF	Factor used to correct for pathlength error, formally the ratio of the actual optical pathlength to the input–output separation

[1] E. M. Sevick and B. Chance, Anal. Chem. **195**, 330 (1991).
[2] K. A. Kang, B. Chance, S. Zhao, S. Srinivasan, E. Patterson, and R. Trouping, Proc. SPIE **1888**, 487 (1993).
[3] B. Chance, J. S. Leigh, H. Miyake, D. S. Smith, S. Nioka, R. Greenfeld, M. Finander, K. Kaufmann, W. Levy, M. Young, P. Cohen, H. Yoshioka, and R. Boretsky, Proc. Natl. Acad. Sci. USA **85**, 4971 (1988).
[4] B. Chance, Q. Luo, S. Nioka, D. C. Alsop, and J. A. Detre, Philos. Trans. R. Soc. London, Ser. B **352**, 707 (1997).
[5] T. Hamaoka, H. Iwane, T. Shimomitsu, T. Katsumura, N. Murase, S. Nishio, T. Osada, Y. Kurosawa, and B. Chance, J. Appl. Physiol. **81**, 1410 (1996).
[6] B. Chance, T. Kitai, H. Liu, and Y. Zhang, Anal. Biochem. **227**, 351 (1995).
[7] B. Chance, A. Mayevsky, B. Guan, and Y. Zhang, ''Hypoxia triggers a light scattering event in rat brain,'' *Oxygen Transport to Tissue XIX (ISOTT)*, edited by D. Harrison and D. Delpy (Plenum, New York, in press).
[8] D. A. Boas, I. V. Meglinsky, L. Zemany, L. E. Campbell, B. Chance, and A. G. Yodh, ''Flow properties of heterogeneous turbid media probed by diffusing temporal correlation,'' *OSA Trends in Optics and Photonics on Advances in Optical Imaging and Photon Migration*, edited by R. R. Alfano and J. G. Fujimoto (Optical Society of America, Washington, DC, 1966), Vol. 2, pp. 175–177.
[9] M. A. O'Leary, D. A. Boas, B. Chance, and A. G. Yodh, Phys. Rev. Lett. **69**, 2658 (1992); see also D. A. Boas, M. A. O'Leary, B. Chance, and A. G. Yodh, *ibid.* **47**, R2999 (1993).
[10] C. S. Robertson, S. P. Gopinath, and B. Chance, J. Biomed. Opt. **2**, 31 (1997).
[11] P. Vaupel, ''Pathophysiological mechanisms of hyperthermia in cancer therapy,'' in *Biological Basis of Oncologic Thermotherapy*, edited by M. Gautherie (Springer, New York, 1990), pp. 73–134.
[12] B. Beauvoit, T. Kitai, and B. Chance, Biophys. J. **67**, 2501 (1994).
[13] B. Chance, J. Biol. Chem. **240**, 2729 (1965).
[14] B. Chance, Ann. (N.Y.) Acad. Sci. **838**, 29 (1997).
[15] B. M. Salzberg, A. M. Okaid, and H. Gainer, J. Gen. Physiol. **86**, 395 (1985).
[16] B. Chance, B. Hughes, E. F. MacNichol, D. Sayre, and F. C. Williams, *Waveforms* MIT Radiation Laboratories Series (Boston Technical Lexington, MA, 1949), Vol. 19.
[17] B. Chance, R. I. Hulsizer, E. F. MacNichol, Jr., and F. C. Williams, *Electronic Time Measurements*, MIT Radiation Laboratories Series (Boston Technical, Lexington, MA, 1949), Vol. 20.
[18] R. Finnerty, J. Pollett, J. Phillips and B. Youfdren, Hewett Packard Laboratories, Tribute to Barney Oliver, United Methodist Church, Palo Alto, CA (1995).
[19] D. A. Boas, M. A. O'Leary, B. Chance, and A. G. Yodh, Proc. Natl. Acad. Sci. USA **91**, 4887 (1994).
[20] R. P. Spencer and G. Weber, Ann. (N.Y.) Acad. Sci. **158**, 3631 (1969).
[21] J. Sipior, G. M. Carter, J. R. Lakowicz, and G. Rao, Rev. Sci. Instrum. **68**, 2666 (1997).
[22] D. T. Delpy, M. Cope, P. van der Zee, S. Arridge, S. Wray, and J. Wyatt, Phys. Med. Biol. **33**, 1433 (1988).
[23] T. J. Farrell, B. C. Wilson, and M. S. Patterson, Phys. Med. Biol. **37**, 2281 (1992).
[24] J. S. Maier, S. A. Walker, S. Fantini, M. A. Franceschini, and E. Gratton, Opt. Lett. **19**, 2062 (1994).
[25] H. Liu, B. Beauvoit, M. Kimura, and B. Chance, J. Biomed. Opt. **1**, 200 (1996).
[26] Ref. to radio Communication paper.
[27] M. Cope and D. T. Delpy, Med. Biol. Eng. Comput. **26**, 289 (1988).
[28] Berks and Little, Proc. R. Soc. London, Ser. A **66**, 921 (1953).
[29] A. Mandelis, Rev. Sci. Instrum. **65**, 3309 (1994).
[30] K. Simons (personal communication).
[31] M. Kohl, R. Watson, and M. Cope, Opt. Lett. **21**, 1519 (1996); Appl. Opt. (in press).
[32] H. Y. Ma, C. W. Du, and B. Chance, Proc. SPIE **2979**, 826 (1977).
[33] *The ARRL Handbook* (The American Radio Relay League, Newington, CT, 1998), p. 12.8.
[34] *The ARRL Handbook* (The American Radio Relay League, Newington, CT, 1998), pp. 12.2 and 12.3
[35] B. Chance, M. Maris, J. Sorge, and M. Z. Zhang, Proc. SPIE **1204**, 481 (1990).
[36] J. B. Fishkin, O. Coquoz, E. R. Anderson, M. Brenner, and B. J. Tromberg, Appl. Opt. **36**, 10 (1997).
[37] W. L. Butler and K. H. Norris, Arch. Biochem. Biophys. **87**, 31 (1960); see also *The Science of Photobiology*, edited by K. C. Smith (Plenum, New York, 1977), p. 400.
[38] A. Villringer and B. Chance, Trends Neurosci. **20**, 435 (1997).
[39] S. Cova and A. Longoni, Chem. Anal. **50**, Chap 7 (1979).
[40] B. A. Fedderson, D. W. Piston, and E. Gratton, Rev. Sci. Instrum. **60**, 2929 (1989).
[41] *The ARRL Handbook* (The American Radio Relay League, Newington, CT, 1998), p. 18.1
[42] *The ARRL Handbook* (The American Radio Relay League, Newington, CT, 1998), pp. 18.1–8.
[43] Y. E. Rgidem, OEX Winter, p. 22 (1997); IEEE Commun. Mag..
[44] B. W. Pogue and M. S. Patterson, J. Biomed. Opt. **1**, 311 (1996).
[45] S. A. Walker, A. Cerussi, and E. Gratton, in *Proceedings of Optical Tomography, Photon Migration, and Spectroscopy of Tissue and Model Media: Theory, Human Studies, and Instrumentation*, edited by B. Chance and R. R. Alfano (SPIE, Bellingham, WA, 1995), Vol. 2389, pp. 350–357.
[46] *Compliance Guide for Laser Products* (U.S. Department of Health and Human Services, FDA, MD, 1985), HHS Publication FDA86-8260.
[47] M. Miwa, Hamamatsu Photonics, KK (personal communication).
[48] APD delay (Drexel and or Nimmu Student has).
[49] N. M. Oldham, J. A. Kramar, P. S. Hetrick, and E. C. Teague, Precis. Eng. **15**, 173 (1993).
[50] J. B. Johnson and Llewellyn, Bell Syst. Tech. J. **14**, 85 (1935).
[51] G. E. Valley and H. Wallman, (1948) *Rad Lab Series* (McGraw Hill, New York, 1948), Vol. 18; (personal communication).
[52] B. Chance, Rev. Sci. Instrum. **18**, 601 (1947).
[53] B. Chance, D. S. Greenstein, J. Higgins, and C. C. Yang, Arch. Biochem. Biophys. **37**, 322 (1952).
[54] N. Ramanujam, C. Du, and H. Ma, Rev. Sci. Instrum. (in press).
[55] B. Chance, J. Franklin Inst. **229**, 455 (1940); **229**, 737 (1940).
[56] *The ARRL Handbook* (The American Radio Relay League, Newington, CT, 1998), p. 14.33
[57] A. T. Williams, W. Tarpley, and C. Clark, Trans. Am. Inst. Electr. Eng. **67**, 1 (1948).
[58] B. Chance, C. Hirth, C. Hyman, O. Luo, and S. Nioka, ''INIR Functional Imaging with Near Infrared,'' Brain '97 XVIIIth International Symposium on Cerebral Blood Flow and Metabolism, Baltimore, MD, June 15–19, 1997 (in press).
[59] B. Chance, Spatial and Temporal Resolution of Frontal Cognitive Response using NIR Tomography, Proceedings of the International Symposium on Brain Mapping OISO '96 (in press).
[60] R. Alfano, P.-P. Ho, and K.-M. Yoo, Phys. World, p. 37 (1992).
[61] M. Ishii, J. S. Leigh, and J. C. Schotland, *Proceedings of Optical Tomography, Photon Migration, and Spectroscopy of Tissue and Model Media: Theory, Human Studies, and Instrumentation* edited by (B. Chance and R. R. Alfano (SPIE, Bellingham, WA, 1995), Vol. 2389, pp. 312–319.
[62] D. Boas, Ph.D. thesis, University of Pennsylvania, Philadelphia, PA, 1996.
[63] M. O'Leary, Ph.D. thesis, University of Pennsylvania, Philadelphia, PA, 1996.
[64] D. A. Boas, M. A. O'Leary, B. Chance, and Y. Yodh, Appl. Opt. **36**, 75 (1997).
[65] X. D. Li, T. Durduran, B. Chance, and A. G. Yodh, ''Detectability and characterization limits of fluorescent objects in turbid media,'' CLEO '97 (in press).
[66] E. Gratton and M. Limkeman, Biophys. J. **44**, 315 (1983).
[67] A. Knuttel, J. M. Schmitt, and J. R. Knutson, Appl. Opt. **32**, 381 (1992).
[68] S. R. Arridge, Appl. Opt. **34**, 78395 (1995); **34**, 8026 (1995).
[69] *HP 8505 Instruction Manual on Network Analyzer* (Hewlett-Packard, Santa Rosa, CA, 95404).
[70] S. J. Madsen, E. R. Anderson, R. C. Haskell, and B. Tromberg, Opt. Lett. **19**, 1934 (1994).
[71] H. Siebold, Seimens AG (personal communication).
[72] Report on 3000 PMF 3000 NIM, Inc., Philadelphia, PA.
[73] B. Guan, Y. Zhang, and B. Chance, Proc. SPIE **2979**, 838 (1997).
[74] X. Wu, L. Stinger, and W. Faris, Proc. SPIE **2979**, 300 (1997).
[75] M. Kohl, R. Watson, and M. Cope, Proc. SPIE (in press).
[76] S. J. Matcher, C. E. Elwell, C. E. Cooper, M. Cope, and D. T. Delpy, Anal. Biochem. **227**, 54 (1995).