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ABSTRACT

In frequency domain photon migration (FDPM), amplitude-modulated light is launched into a turbid medium, e.g. tissue, which results in the propagation of density waves of diffuse photons. Variations in the optical properties of the medium perturb the phase velocity and amplitude of the diffusing waves. These parameters can be determined by measuring the phase delay and demodulation amplitude of the waves with respect to the source. More specifically, the damped spherical wave solutions to the homogeneous form of the diffusion equation yield expressions for phase (ϕ) and demodulation (m) as a function of source distance, modulation frequency, absorption coefficient (β), and effective scattering coefficient (σ_{eff}).

In this work, we present analytical expressions for the variable dependence of ϕ and m on modulation frequency. A simple method for extracting absorption coefficients from ϕ and m vs. frequency plots is applied to the measurement of tissue phantoms. Using modulation frequencies between 5 MHz and 250 MHz, absorption coefficients as low as 0.024 cm^{-1} are measured in the presence of effective scattering coefficients as high as 144 cm^{-1}. Our results underscore the importance of employing multiple modulation frequencies for the quantitative determination of optical properties.

1. INTRODUCTION

In order to *non-invasively* measure chromophore concentration in opaque tissues, light scattering effects must be clearly separated from absorption phenomena.[1,2] Patterson et al.[3] demonstrated that this scattering limitation can be removed by the use of *time-resolved* photon migration, the time-domain analog of frequency-domain photon migration (FDPM).

Sevick and Chance[4] have reported measurements of hemoglobin saturation using FDPM phase measurements at a single modulation frequency. Frequency-domain techniques place less stringent demands on the bandwidth of light source and detector, and the efforts of Sevick and Chance were aimed at reducing instrumentation costs by employing laser diodes and inexpensive photomultiplier tubes.

Multifrequency photon migration measurements have been demonstrated by Lakowicz, *et al.* who explored frequencies to 3 GHz.[5] Fishkin and Gratton,[6] have described intensity-modulated light diffusion in turbid media based on the diffusion approximation to one-speed linear transport theory. We employ diffusion theory in a similar manner, however the frequency dependence of phase and modulation is strongly emphasized. Analyses are conducted on the commonly-used tissue phantom, Intralipid, using modulation frequencies between 5 and 250 MHz. A porphyrin absorber (tetraphenylporphine tetrasulfonate, $TPPS_4$) is systematically added and a general model for the influence of absorption and scattering on the properties of optical waves is presented.

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2. FDPM THEORY

When light propagates through a highly scattering medium, e.g. tissue, the flux of diffuse photons from a region of high fluence rate, φ, to a region of low fluence rate can be described by the diffusion equation:[3, 6, 7]

$$\frac{1}{c}\frac{\partial\varphi}{\partial t} - \zeta\nabla^2\varphi + \beta\varphi = S(\vec{r},t) \qquad (1)$$

where c is the speed of light in tissue, ζ is the photon diffusion constant in tissue, β is the absorption coefficient, S is the light source, r is distance, and t is time. In an infinite homogeneous medium, the damped spherical wave solutions to the homogeneous form of this equation yield the following expressions for the phase (ϕ) and demodulation (m):

$$\phi = r\sqrt{\frac{3}{2}}\sqrt{\sqrt{1+\left(\frac{\omega}{\beta c}\right)^2}-1}\sqrt{\beta[\beta+(1-g)\sigma]} \qquad (2)$$

$$-\ln(m) = r\sqrt{\frac{3}{2}}\left[\sqrt{\sqrt{1+\left(\frac{\omega}{\beta c}\right)^2}+1}-\sqrt{2}\right]\sqrt{\beta[\beta+(1-g)\sigma]} \qquad (3)$$

where r is the distance from the source, ω is the angular modulation frequency, and σ and g are the tissue optics parameters: scattering coefficient, and mean cosine of the scattering angle, respectively. The values of ϕ and m are defined in the usual manner where ϕ = the measured phase lag between source and sample response and $m = (AC_{sample}/DC_{sample})/(AC_{source}/DC_{source})$.

Equations 2 and 3 indicate that measurements of ϕ and m at multiple modulation frequencies can lead to the determination of β and $\sigma_{eff} = (1\text{-}g)\sigma$. These equations assume analytically useful forms under different conditions, however. The precise relationship between ϕ, m, and ω depends upon the absorption relaxation time ($\tau = 1/\beta c$) of the medium. At low frequencies the phase increases linearly, but at high frequencies the phase increases as the square root of frequency. The transition occurs at an angular frequency (ω) equal to the absorption coefficient times the speed of light in the tissue ($\omega = \beta c$). Similar arguments can be made for the frequency dependence of m.[7] Because of this variable relationship between modulation frequency and optical properties, the phase and demodulation frequency dependence should be determined in order to correctly calculate β and σ_{eff}.

3. MATERIALS AND METHODS

3.1 Instrument

The photon migration instrument is a modified multiharmonic Fourier Transform phase and modulation fluorometer (SLM, 48000-MHF, Champaign, IL). Light is provided by a water-cooled Argon-ion laser

(Coherent Innova 90-5). A Pockel's cell, driven either directly by a frequency synthesizer or indirectly by the amplified output of a harmonic comb generator is used to modulate light at single frequencies or produce pulses with high harmonic content. The harmonic comb generator output, in the frequency domain, is a fundamental frequency (typically 5 MHz) and its integer harmonics at 5 MHz, 10 MHz, 15 MHz, etc. to 250 MHz. Modulated light is coupled to a 600 μm-diameter fused-silica fiber optic probe and directed on to the sample.

A second fused-silica fiber (same dimensions) collects the scattered light and transmits it to the measurement photomultiplier tube (PMT, Hammatsu R928). The gain of the photomultiplier tube is modulated either by a second harmonic comb generator (in the case of multiharmonic measurements) or directly by the frequency generator (for single frequency measurements). The PMT is driven at the frequency of the Pockel's cell plus a small difference frequency (the cross correlation frequency). The sample's phase and amplitude response at each modulation frequency is contained within the corresponding cross correlation frequency. For example, in the case of multiharmonic operation, phase and modulation information from 5 Mhz to 250 MHz is contained within a 3 Hz to 150 Hz spectrum. Using the multi-harmonic technique, we can acquire phase shifts and demodulations for 50 frequencies (5-250 MHz) in a few seconds.[8]

3.2 Materials

In order to simulate the optical properties of tissue, a one liter vessel was filled with 10% Intralipid. Fibers were positioned in the center of the vessel either facing or adjacent to one another. This central location was carefully selected in order to minimize boundary losses and approximate an infinite medium. Reference (i.e. source) measurements were recorded with input and collection fibers touching and a small amount of index-matching gel placed between the fiber faces. The distance between source and collection fibers was systematically varied, typically between 2.5 and 20 mm, for each measurement series.

A porphyrin compound, tetraphenyl porphine tetrasulfonate, $TPPS_4$ (Porphyrin Products, Logan, UT) was added to the Intralipid so the effect of absorber could be recorded. A 514 nm laser line filter (Corion Corp, Holliston, MA) was placed at the entrance to the PMT housing in order to block $TPPS_4$ fluorescence and isolate the scattered light.

4. RESULTS AND DISCUSSION

Figure 1 illustrates the linear distance distance dependence for phase (Fig. 1a) and -ln(demodulation) (Fig. 1b) predicted by equations 2 and 3. Data is presented for 25, 50 and 100 MHz modulation frequencies. The measured phase lag increases and "m" decreases (-ln(demod) increases) with increasing distance. This behavior can be understood by considering the effect the medium has on the propagating waves. As the waves (with modulation wavelength, $\lambda_m = 2\pi/k_i$, angular frequency, $\omega = 2\pi f$, and a phase velocity $V_p = \omega/k_i$ where k_i is the imaginary part of the complex angular wavenumber[7]) travel, they experience a distance-dependent attenuation and retardation due to media optical properties. At greater distances light has travelled further and encountered more scatterers, hence the attenuation and phase lag are larger.

The effect of increasing modulation frequency can be explained in a similar manner. At a given distance, an increased modulation frequency results in a shorter modulation wavelength. Under these conditions, there are more photon density fluctuations per unit distance. This results in an increase in the imaginary wavenumber, k_i, and enhanced attenuation and phase lag are subsequently observed.

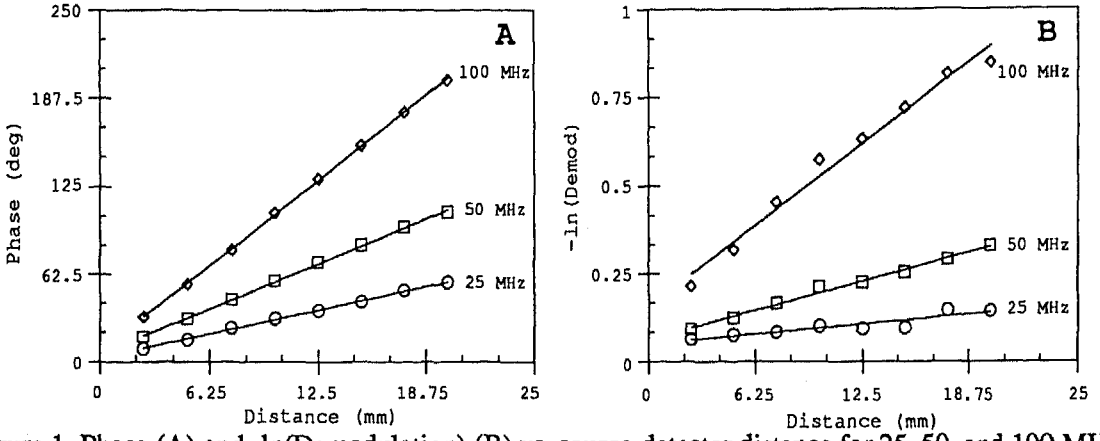

Figure 1. Phase (A) and -ln(Demodulation) (B) vs. source-detector distance for 25, 50, and 100 MHz modulation frequencies; 10% Intralipid solution.

In Figure 2, phase (Fig. 2a) and demodulation (Fig. 2b) are displayed as a function of several modulation frequencies (5-250 MHz) for fibers placed 7.5 mm apart in 10% Intralipid. The smooth lines through the data are the non-linear least squares fits to equations 2 and 3. The corresponding fitted optical properties, determined from a series of measurements over 8 distances (to 20 mm), are: Phase fits: $\beta = 0.0229 \pm 0.0024$ cm^{-1} and Demodulation fits: $\beta = 0.0234 \pm 0.0043$ cm^{-1}. The σ_{eff} was calculated to be 144 cm^{-1} using either phase or demodulation data.

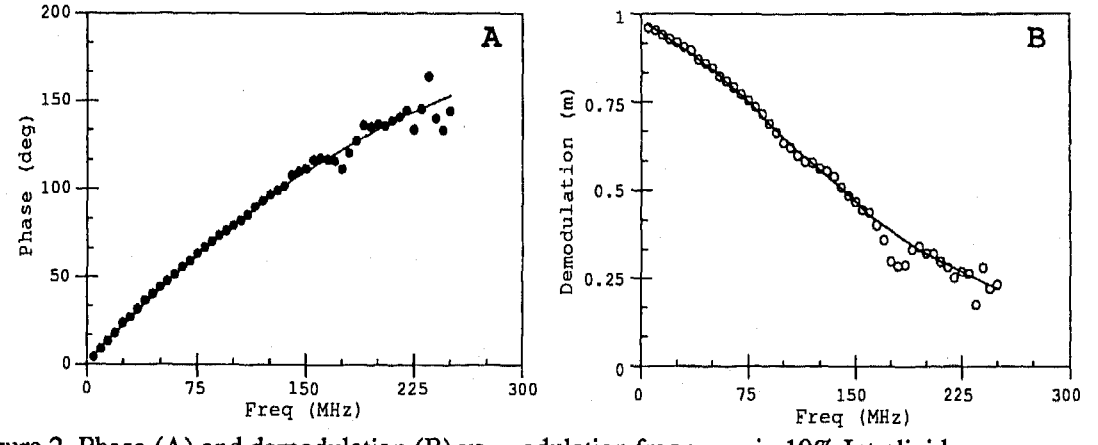

Figure 2. Phase (A) and demodulation (B) vs. modulation frequency in 10% Intralipid.

The phase and demodulation vs. frequency response changes in a number of ways when absorber is added to the scattering medium. Figure 3 displays phase (Fig. 3a) and demodulation (Fig. 3b) measurements for 10% Intralipid and $TPPS_4$ absorber. Modulation frequencies to 165 MHz are shown at a source-detector separation of 7.5 mm.

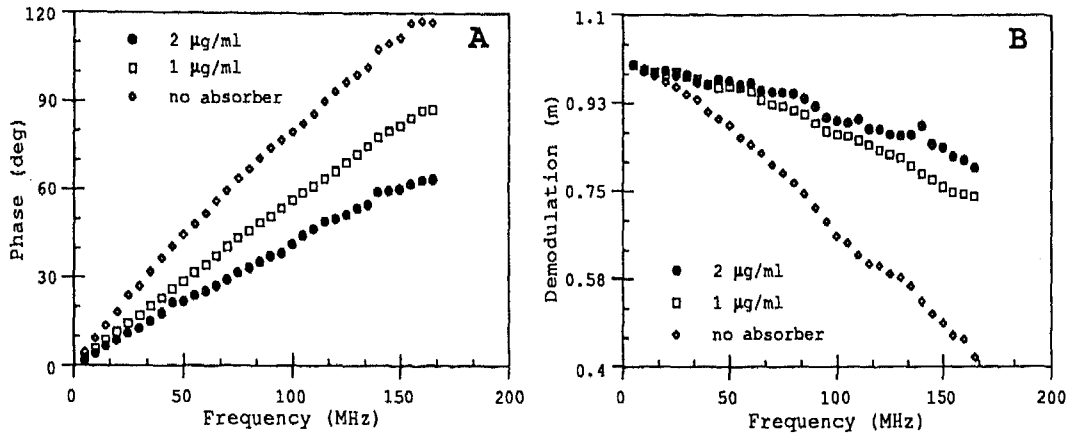

Figure 3. Phase (A) and Demodulation (B) vs. Frequency for 10% Intralipid with and without added absorber. Absorber solutions were 1 and 2 µg/ml $TPPS_4$. Demodulation values are normalized to 1.

From these results, it is clear that phase decreases and "m" increases (i.e. larger Demodulation *values*) with increasing absorber concentration. The additonal absorbers, in effect, "capture" light and the measured photons follow shorter paths to the detector. Fewer scattering events are recorded and a decrease in phase delay and demodulation is observed.

When the absorption relaxation time, $\tau = 1/\beta c$ (where c = the speed of light in the medium) is short compared to $1/\omega$ (i.e. high absorption conditions), ϕ assumes a linear dependence on modulation frequency and -ln(m) is proportional to ω^2. Conversely, an $\omega^{1/2}$ dependence is observed at low absorber concentrations.[9] This variable dependence on modulation frequency is qualitatively illustrated in Figures 2 and 3. There is distinct non-linearity to the ϕ and m vs frequency curves of Figure 2, however as absorber is added (Figure 3) the ϕ vs. frequency response begins to straighten out. The high absorption linear and squared frequency dependence for ϕ and -ln(m), respectively, is illustrated in Figure 4.

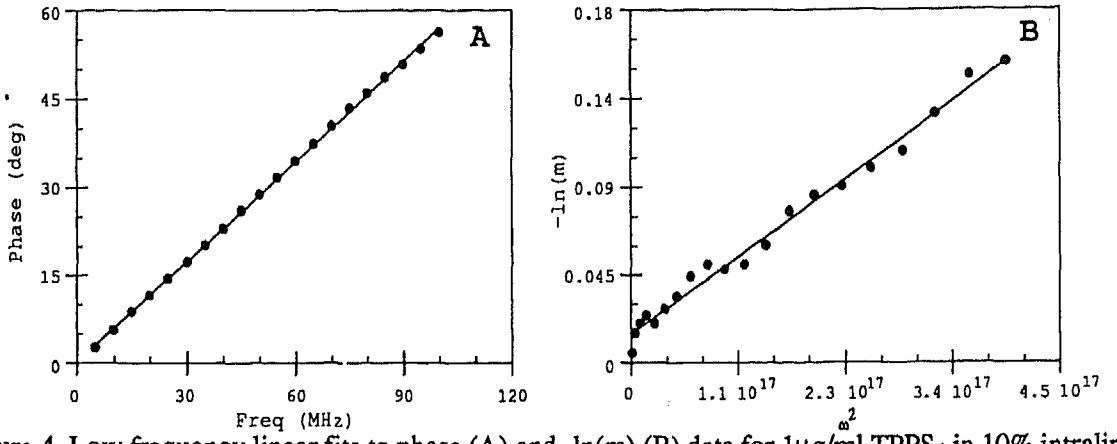

Figure 4. Low frequency linear fits to phase (A) and -ln(m) (B) data for 1μg/ml $TPPS_4$ in 10% intralipid. Source-detector separation = 10 mm.

Under high absorption conditions (i.e. when $\omega\tau \ll 1$), the linear ϕ and m frequency dependence limits the accuracy of non-linear least squares fits to equations 2 and 3. Furthermore, under these conditions "m" may be close to (or equal to) 1 since the AC and DC components of the signal will be damped at approximately equal rates. Two general options are available for the determination of optical properties under these conditions. If insufficient demodulation is observed, the distance between source and detector fibers must be accurately known. The distance-dependent DC signal (or AC signal since, in the absence of demodulation, AC = DC) can be used to calculate the 1/e penetration depth, $\delta = 1/\sqrt{3\beta(\beta + \sigma_{eff})}$. This information can be combined with either the phase vs. frequency response or the phase velocity ($V_p = \omega/k_i$) in order to calculate optical properties.

If a small amount of demodulation is observed, as in Fig. 4b, phase and modulation data can be combined to determine β. Under these conditons, it is not necessary to know the source-detector distance. Using this technique, the absorption coefficient for 0, 1, and 2 μg/ml $TPPS_4$ solutions was determined. The molar concentration of $TPPS_4$ in Intralipid was calculated from the relationship between the linear absorption coefficient, β, and the molar extinction coefficient, ε, i.e. $\beta = 2.3\varepsilon C$, where $\varepsilon(TPPS_4) = 2.5 \times 10^4\ M^{-1}\ cm^{-1}$ at 514 nm. These results are summarized in Table 1.

Table 1. Summary of measured optical properties in 10% Intralipid.

$TPPS_4$ (μg/ml)	$[TPPS_4]$ (moles/L)	β measured (cm^{-1})	$[TPPS_4]$ measured (moles/L)	σ_{eff} measured (cm^{-1})
0	0	0.0235	0	133
1	8.0×10^{-7}	0.0532	5.2×10^{-7}	143
2	1.6×10^{-6}	0.0622	6.7×10^{-7}	111
4	3.2×10^{-6}	0.109	1.9×10^{-6}	120

The analytical power of FDPM is particularly evident when one considers that extremely low absorber concentrations are detectable in a solution which, at 514 nm, has an effective scattering coefficient more than 5000 times greater than β. When expressed in the more familiar terms of Beer's Law (which, of course is not applicable under these multiple-scattering conditions), as little as 0.02 Absorbance Units are detectable despite the opacity of the medium.

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