Quantitative Diffusive Wave Spectroscopy In Tissues

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ABSTRACT

High frequency, intensity-modulated light waves are attenuated and phase-shifted by the absorption and scattering properties of highly scattering media, such as tissue. The simultaneous measurement of the average light intensity, modulation amplitude, and phase-shift at a fixed distance from a sinusoidally modulated light source, permits a quantitative determination of the absolute values of the absorption and scattering coefficients from a frequency-domain scan. Our studies have established the range of modulation frequencies that give the highest sensitivity to changes of the optical parameters in model systems. We have measured the optical absorption spectra of dyes suspended in highly scattering media. These spectra match those found in non-scattering media. This frequency-domain approach provides a simple method to perform quantitative spectroscopy in highly scattering media.

1. INTRODUCTION

This paper describes a method for obtaining quantitative spectral information in tissues. The determination of the absolute values of the absorption and scattering spectral characteristics of biological tissues has been a subject of a great number of investigations[1-6]. In the transillumination geometry, both scattering and absorption events exponentially decrease the light intensity at the detector as a function of the pathlength. The major problem resides in the separation of the scattering from the absorption contribution. Literature data on the optical properties of tissue is scarce and agreement between different laboratories is "far from ideal, but probably as good as can be expected for a highly multiple scattering system[5]."

Experimentally, ingenious methods have been implemented to separate scatter from absorption, but none of the proposed approaches gives the absolute absorption coefficient. Recently, Patterson, Chance and Wilson[2] have proposed a method that departs from standard approaches. These researchers have circumvented the problem of the quantitation of the effective pathlength by time-resolving a pulse of light passing through the tissue. The onset time of the transmitted pulse provides the pathlength. The absorption coefficient is obtained from the slope of the logarithm of the intensity as a function of time. The validity of this method has been demonstrated both experimentally and using simulations. Shortcomings of this approach include the long time required for acquisition of data at a single wavelength and the necessity of a laser with picosecond pulse duration.

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We present a frequency-domain approach for resolving the time of propagation of photons through strongly scattering media. It has been shown that, in the frequency-domain, the problem of light propagation in strongly scattering media can be treated within the familiar framework of wave phenomena[7]. The method uses an intensity-modulated light source and measures the front of the intensity modulated diffusing light waves. It is well known that time-domain and frequency-domain methods are mathematically equivalent for resolving the time decay of the fluorescence. Of course, this equivalence also holds for the measurement of transmitted light pulses using the Patterson/Chance approach[2]. In this particular situation, the frequency-domain method is advantageous because commercially available frequency-domain fluorometers use a continuous wave light source, such as a high-pressure Xenon arc lamp, which is amplitude-modulated by a Pockels cell modulator. If high frequency and intensity are required, such as for the studies of thick tissue, free electron laser or synchrotron radiation sources can be substituted. These devices provide a source with continuous spectral characteristics. Furthermore, as we report in the experimental section, the measurement at a single modulation frequency is enough to fully characterize the absorption and scattering properties of tissues. In principle, this measurement can be performed in less than a second per wavelength. A frequency-domain approach using multiple modulation frequencies has been used by Tromberg et al.[8] to measure the absorption coefficient in strongly scattering media.

2. ANALYTICAL SOLUTION

A sinusoidally intensity-modulated point source of visible or near-infrared light $q_0(\mathbf{r},t)$ is immersed in a macroscopically homogeneous, strongly scattering medium. A source of this type is given by the following expression

$$q_0(\mathbf{r},t) = \delta(\mathbf{r})S\,(1 + Ae^{-i(\omega t + \varepsilon)}) \qquad (1)$$

where $\delta(\mathbf{r})$ is a Dirac-Delta function located at the origin, S is the fluence of the source (in photons per second), A is the modulation of the source, $i = \sqrt{-1}$, ω is the angular modulation frequency of the source, and ε is an arbitrary phase. It has been shown that when this type of source is inserted into the equations given by the diffusion approximation to the Boltzmann transport equation, the density of photons $U(\mathbf{r},t)$ that is calculated via this approximation for a macroscopically homogeneous, infinite medium is given by [7]

$$U(\mathbf{r},t) = \frac{S}{4\pi vDr}\exp\left(-r\sqrt{\frac{\mu_a}{D}}\right) + \frac{SA}{4\pi vDr}\exp\left(-r\left(\frac{v^2\mu_a^2+\omega^2}{v^2D^2}\right)^{1/4}\cos\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{v\mu_a}\right)\right)\right)\times$$

$$\exp\left(ir\left(\frac{v^2\mu_a^2+\omega^2}{v^2D^2}\right)^{1/4}\sin\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{v\mu_a}\right)\right) - i(\omega t+\varepsilon)\right) \qquad (2)$$

Here v is the speed of a photon in the transporting medium (i.e. water in our experiments), D is the diffusion coefficient,

$$D = \{3[\mu_a + \mu_s (1 - g)]\}^{-1} \quad (3)$$

μ_a is the linear absorption coefficient (i.e. the inverse of the mean free path for photon absorption, with units of 1/distance), μ_s is the linear scattering coefficient (i.e. the inverse of the mean free path for photon scattering), and g is the average of the cosine of the photon scattering angle. Examination of equation 2 shows that the photon density $U(\mathbf{r},t)$ generated by a sinusoidally intensity-modulated point source immersed in a strongly scattering, infinite medium constitutes a scalar field that is propagating at a constant speed in a spherical wave and attenuates as $e^{-\alpha r}/r$ as it propagates. The approach of regarding photon transport in strongly scattering media as a diffusional process shows that light emitted from a sinusoidally intensity-modulated point source in such a medium can be treated within the framework of wave phenomena; we therefore refer to $U(\mathbf{r},t)$ as a "photon-density wave." We refer to the study of the propagation, reflection, and refraction of these waves as "diffusive wave optical spectroscopy."

Equation 2 is the Fourier transform equivalent of

$$\rho(\mathbf{r},t) = \frac{1}{(4\pi vDt)^{3/2}} \exp\left(-\frac{r^2}{4vDt} - \mu_a vt\right) \quad (4)$$

which is the time dependent solution of the diffusion equation as reported by Patterson et al [2]. Here, $\rho(\mathbf{r},t)$ is the photon density that satisfies the diffusion approximation to the Boltzmann transport equation when the source term is a narrow pulse given by $q_0(\mathbf{r},t) = \delta(\mathbf{r})\delta(t)$. Equations 2 and 4 show the practical difference between describing photon diffusion in the frequency-domain with respect to its Fourier transform equivalent in the time-domain: the photon density generated by a sinusoidally intensity-modulated source at any given modulation frequency propagates with a single phase velocity, while pulses undergo dispersion due to the different phase velocity of each frequency component of the pulse.

The quantities that are measured in a frequency-domain experiment are the phase lag Φ of the signal at the detector relative to the source and the demodulation M, which is defined as

$$M \equiv \frac{AC_{source}/DC_{source}}{AC_{detector}/DC_{detector}} \quad (5)$$

where the DC and AC are, respectively, the average intensity and amplitude of the measured signal. Equations 1 and 2 yield expressions for these experimentally determined quantities in a uniform, infinite medium:

$$\Phi = r\left(\frac{v^2\mu_a^2 + \omega^2}{v^2D^2}\right)^{1/4} \sin\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{v\mu_a}\right)\right) \quad (6)$$

$$\ln(M) = r\sqrt{\frac{\mu_a}{D}} - r\left(\frac{v^2\mu_a{}^2 + \omega^2}{v^2D^2}\right)^{1/4} \cos\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{v\mu_a}\right)\right) \quad (7)$$

From equations 6 and 7 it is calculated that

$$\frac{\ln(M)}{\Phi} = \frac{1 - \left(1 + \frac{\omega^2}{v^2\mu_a{}^2}\right)^{1/4} \cos\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{v\mu_a}\right)\right)}{\left(1 + \frac{\omega^2}{v^2\mu_a{}^2}\right)^{1/4} \sin\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{v\mu_a}\right)\right)} \quad (8)$$

is only dependent on the parameter $\omega/v\mu_a$, and that μ_a is the only unknown quantity in equation 8 for a given measurement of $\ln(M)/\Phi$.

To obtain μ_a from the ratio $\ln(M)/\Phi$, one refers to the graph shown in figure 1, which represents equation 8. To use this graph, the measured value of $\ln(M)/\Phi$ must be identified on the y-axis and the corresponding value of the parameter $\omega/v\mu_a$ is obtained on the x-axis. Because the angular modulation frequency of the source ω and the speed of the photon v in the medium containing the scattering particles are known, the absorption of the system μ_a may be calculated from the value determined for $\omega/v\mu_a$. Once μ_a has been determined, a value for the diffusion coefficient D may be calculated from equations 6 or 7, and this value for D may then be used in equation 3 to calculate a value for $(1 - g)\mu_s$.

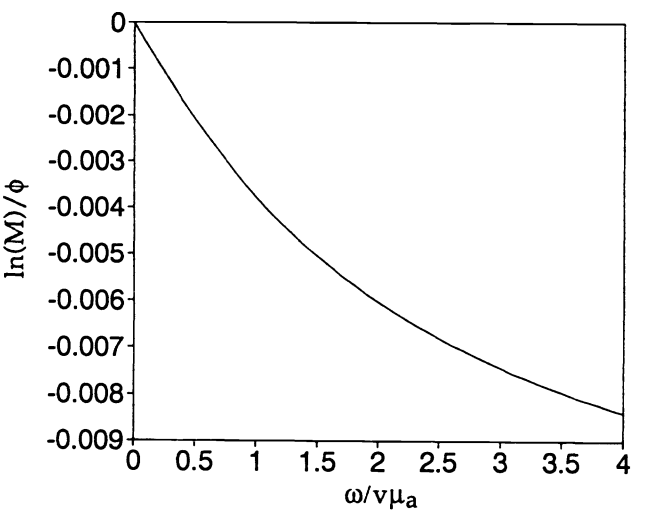

Fig. 1. Plot of equation 8 (i.e. $\ln(M)/\Phi$ vs. $\omega/v\mu_a$). In this plot the phase is in units of degrees.

As mentioned above, time-domain and frequency-domain methods are fully equivalent in regard to the information they provide about a scattering and absorbing medium. However, as demonstrated by equations 6, 7, and 8, frequency-domain methods are advantageous because scattering properties of a medium can be fully

characterized by a measurement of Φ and M at a single modulation frequency, which allows for a more rapid determination of the spectral properties of a medium.

3. APPARATUS AND METHOD

We modified the laser-based frequency-domain fluorometer used in our laboratory by inserting a fiber optic bundle system in the light path as shown in figure 2. The light source is a HeCd laser with emission at 442 nm and 7 mW output power. Amplitude

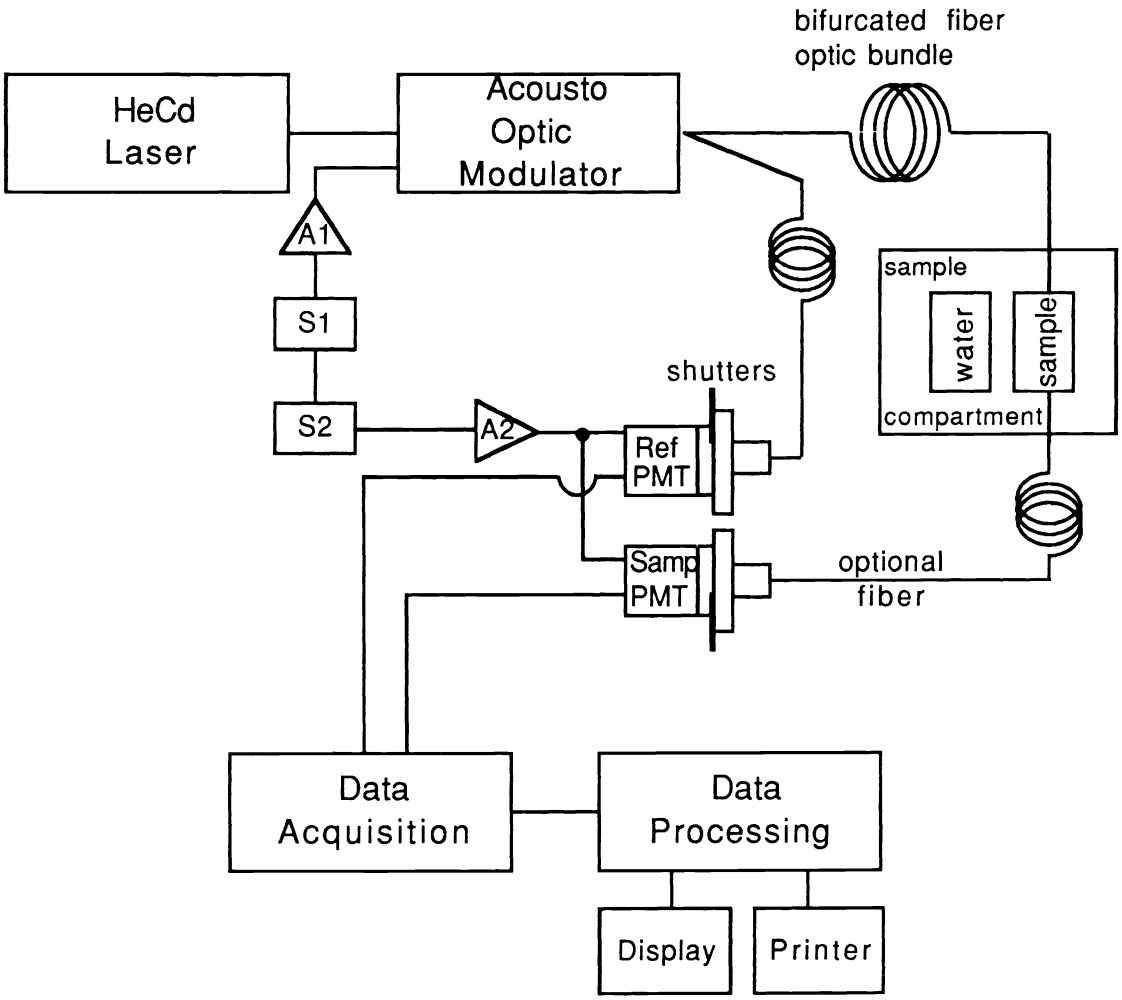

Fig. 2. A1, 2 = radio frequency amplifier; S1, 2 = frequency synthesizer; PMT = photomultiplier tube.

modulation was obtained using a double acousto-optic modulator designed by Piston et al.[9]. The central diffraction dot after the modulator was focused on a bifurcated fiber optic bundle and brought to the sample compartment and reference photomultiplier tube (PMT), respectively. The transmitted light from the sample was collected by the standard collection optics of the fluorometer and thereby transferred to the sample photomultiplier tube of the fluorometer. The photomultiplier signals were processed by

a cross-correlation electronics system using the digital acquisition system described by Feddersen et al.[10].

The validity of the proposed frequency-domain determination of absorption coefficients has been tested on a sample composed of a highly scattering solution (20% Intralipid™ suspension, diluted 13:1 with water) with an equivalent optical density due to the turbidity of the scatterer greater than 200, measured with a spectrophotometer using a cuvette with a 0.1 mm pathlength. Increasing amounts of hemin were added to the suspension (final concentration: 0.1 μM to 2.0 μM) and the phase and amplitude of the diffusive wave after transversing the sample was measured at 51 MHz. The hemin concentrations we have employed are typical of metabolite concentrations in tissues.

4. RESULTS

In figure 3, we report the value of the absolute phase delay (in degrees) with respect to a water sample versus the hemin concentration. The phase decreases as the hemin concentration increases, as predicted by equation 6. This delay depends on the phase velocity of the diffusive wave and defines the effective optical pathlength at any given frequency. As the concentration of the hemin increases, the phase delay decreases due to the higher absorption probability for the longer paths in the solution. In the limit of very high concentration, equation 2 is no longer valid. The modulation of the diffusive wave increases only slightly as the hemin concentration is increased (data not shown) as predicted by equation 7. The logarithm of the absolute modulation divided by the absolute phase (in degrees) is plotted versus the inverse of the hemin concentration and is figure 4. Values for the absorption coefficient μ_a at each hemin concentration in the scattering medium are calculated from these values of $\ln(M)/\Phi$ and shown in Table I under the heading *Calculated Absorption Coefficient (using eq. 8)*. We have independently determined the value of the absorption coefficient μ_a in a solution of identical hemin concentration, but no scatter, using standard spectrophotometric methods (these results are shown in

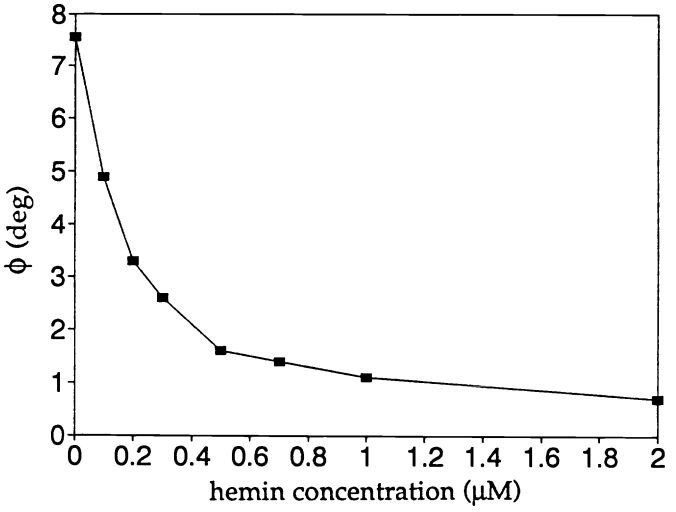

Fig. 3. Phase (in degrees) vs. the hemin concentration in the 20 % Intralipid™ solution (diluted 13:1 with water).

Table I under the heading *Absorbance 442 nm (cm^{-1})*. The values obtained in the highly scattering medium are in close agreement with the values measured by absorption in the absence of scattering (Table I).

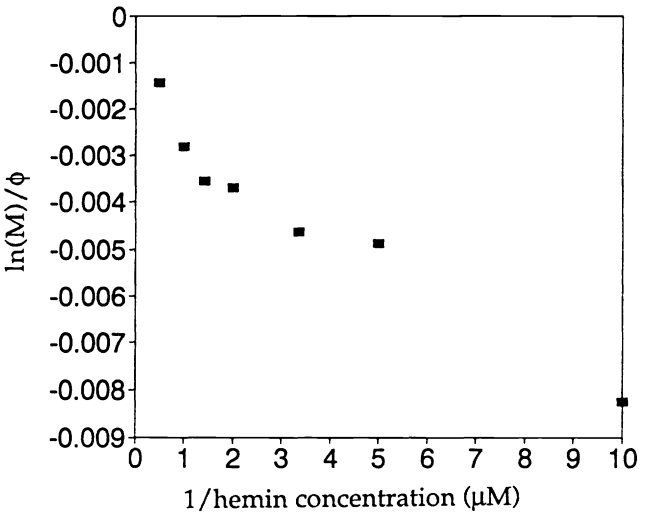

Fig. 4. Natural logarithm of the absolute modulation divided by the absolute phase delay (in degrees) is plotted vs. the inverse of the hemin concentration in 20% Intralipid™ solution (diluted 13:1 with water).

Table I. Hemin absorbance in Intralipid.

[Hemin] Micromolar	*Absorbance 442 nm (cm^{-1})*	*Calculated Absorption Coefficient (using Eq. 8)*
0.0	-0-	-0-
0.1	0.003	0.004
0.2	0.006	0.010
0.3	0.007	0.011
0.5	0.012	0.014
0.7	0.017	0.016
1.0	0.025	0.024
2.0	0.049	0.048

5. CONCLUSIONS

The readily accessible range of the quantity $\omega/v\mu_a$ is from 0 to about 4 (see figure 1). Representative absorption coefficients in tissues range from 0.1 to 1.0 cm^{-1}. Figure 2 can be used to determine the appropriate modulation frequency range for measurement of absorption coefficients in tissues, which is in the 100 MHz region. This frequency range is typical for frequency-domain fluorometers. Such order of magnitude calculations underscore the high sensitivity of the proposed method, since low absorption coefficients can be easily measured at low frequencies where the signal-to-noise ratio is better.

Of special importance in our test experiment is the fact that due to turbidity the scattering suspension has a very high optical density (>200), which totally prevents the use of normal spectroscopic methods. This is the situation normally encountered in the analysis of spectroscopic properties of tissues. The hemin concentrations we have employed are typical of metabolite concentrations in tissues. These preliminary results demonstrate that our technique can detect submicromolar concentrations of metabolites in highly scattering suspensions.

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