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# The Absorption Spectra of a Chromophore in Highly Scattering Media

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## ABSTRACT

Frequency domain spectroscopy provides a quantitative measure of the optical properties, namely  $\mu_a$  and  $\mu_s'$  coefficient spectra, of multiply scattering, macroscopically homogeneous media or tissue. The diffusion model for photon transport provides the theoretical framework for the analytical expressions of the optical properties. Experimentally, we intensity modulated (60 MHz) a light emitting diode, which emits between 620-700 nm. From data sets of relative phase shifts and average intensity at different source-detector separations, we calculated on the basis of the analytical expressions a wavelength resolved absorption  $\mu_a(\lambda)$  and scattering  $\mu_s'(\lambda)$  coefficient spectrum. The test material was methylene blue, whose absorption spectrum (maximum 656 nm) closely matches the wavelength profile of the diode source. The multiply scattering, macroscopically homogeneous medium for dissolving the methylene blue was provided by a diluted fat emulsion, Liposyn III. The concentrations of both the absorbing and scattering materials were adjusted to correspond to ranges typical of  $\mu_a$  and  $\mu_s'$  in tissues. We obtained quantitative agreement between the measured  $\mu_a(\lambda)$  in the scattering medium and a control solution measured in a spectrophotometer under non-scattering conditions.

## 1. INTRODUCTION

The quantitative determination of tissue optical properties is becoming important as optical methods are emerging in clinical practice as diagnostics tools and in the monitoring of physiologically relevant processes [1-4]. The appeal of optical methods resides in their non-invasive methodology, non-ionizing interrogating sources, ease and speed of use, good accuracy and relatively modest cost. Knowledge of the quantitative absorption spectrum of a chromophore is important in determining the chromophore's concentration in the highly scattering environment of tissues. The spectral profile also provides characteristic and identifying information about a specific chromophore in mixed systems of absorbing chromophores. In tissue, light transport is attenuated by both absorption and multiple scattering. A variety of approaches have been developed to resolve the relative contributions due to absorption and scattering; these include steady-state

[5] and time-resolved techniques employing time-domain [6] or frequency domain [7 – 9] methods. In our frequency domain photon diffusion experiments [10], the intensity of the light source is sinusoidally amplitude modulated at radio frequencies on the order of 100 MHz. At the given frequency, the detected signals include the average intensity (DC component), the amplitude of the periodic intensity oscillation (AC component) and the phase shift ( $\Phi$ ) of the detected light, relative to the excitation signal. Fishkin and Gratton [11] have developed a frequency domain model based on the diffusion approximation to the Boltzmann transport equation, that provides quantitative determination of the absolute absorption coefficient ( $\mu_a$ ) and the reduced scattering coefficient ( $\mu_s'$ ) from the measured parameters (DC, AC,  $\Phi$ ). These optical parameters are determined for conditions of multiple scattering in a macroscopically homogeneous medium with no contribution from boundary or source effects. Previously, we have shown that analytical solutions to this approach quantitatively track the  $\mu_a$  and  $\mu_s'$  of hemin dissolved in a homogeneous highly scattering medium [12]. In this communication, we describe the wavelength resolution of  $\mu_a(\lambda)$  in a scattering medium by using a multiwavelength source and variable separation distances between source and detector. The influence of wavelength and the sensitivity of the method are examined.

## 2. THEORY

When a sinusoidally intensity modulated point source of visible or near infrared light  $q_0(\mathbf{r},t)$  is immersed in a (quasi-) infinite (negligible boundary effects), macroscopically homogeneous, strongly scattering medium, as in our experiment; then this source is given by the following expression

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

In Equation 1  $\delta(\mathbf{r})$  represents a Dirac-delta function located at the origin,  $S$  is the source strength (in photons per second),  $A$  is the modulation of the source,  $i = \sqrt{-1}$ ,  $\omega$  is the angular modulation frequency of the source, and  $\epsilon$  is an arbitrary phase. Under our experimental conditions, the diffusion approximation to the Boltzmann transport equation holds. The time dependent solution to the equation was articulated by Patterson et al. [6]. For the frequency domain and using the source described by Equation 1, the corresponding solution [11] for the diffusion approximation to the Boltzmann transport equation provides the density of photons  $U(\mathbf{r},t)$ .

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

In Equation 2,  $v$  is the speed of a photon in the transporting medium (water in our experiments and in most tissues),  $D$  corresponds to the diffusion coefficient

$$D = 1/3 [\mu_a + \mu_s'] \quad (3)$$

with units of distance,  $\mu_a$  is the linear absorption coefficient (namely, the inverse mean free path for photon absorption with units of inverse distance),  $\mu_s'$  is the reduced scattering coefficient

$$\mu_s' = (1-g) \mu_s \quad (4)$$

with  $\mu_s$  the linear scattering coefficient (the inverse mean free path for photon scattering) and  $g$  corresponds to the average of the cosine of the photon scattering angle. Examination of Equation 2 shows that the photon density  $U(r,t)$  produced by the sinusoidally intensity modulated point source propagates through the strongly scattering, (quasi) infinite medium as a spherical wave of constant speed,  $v$ . During propagation, the spherical wave attenuates as a decaying exponential in the distance  $r$ , divided by  $r$  ( $e^{-r}/r$ ). The diffusion approximation of the Boltzmann transport equation remains valid as long as  $\mu_a \ll \mu_s'$  and boundaries are far; at distances from the source which are much greater than the photon mean free path. Consideration of photon transport in homogeneous strongly scattering media as a diffusion process shows that the wave phenomenon framework is appropriate for the treatment of light emitted from a sinusoidally intensity modulated point source and propagating through this medium. Consequently, we designate the study of photon density wave propagation, reflection and refraction as diffusion wave optical spectroscopy.

In a frequency domain experiment, the quantities measured at the detector are the phase lag  $\Phi$  and the modulation, which is the ratio of the average intensity (DC) to the amplitude fluctuation (AC) of the measured signal. From Equations 1 and 2 it is possible to obtain expressions for the experimentally determined parameters

$$\Phi = r \left( \frac{v^2 \mu_a^2 + \omega^2}{v^2 D^2} \right)^{1/4} \sin \left( \frac{1}{2} \tan^{-1} \left( \frac{\omega}{v \mu_a} \right) \right) \quad (5)$$

$$DC = 1/r \exp \left( -r \sqrt{\frac{\mu_a}{D}} \right) \quad (6)$$

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

In Equations 5-7 the unknowns are  $v \mu_a$  and  $vD$ , assuming that the index of refraction is known. Thus, one can calculate  $\mu_a$  and then  $\mu_s'$  (from  $vD$ ). It is possible to obtain the wavelength dependent  $\mu_a(\lambda)$  and  $\mu_s'(\lambda)$  simply by varying the wavelength of the experiment. Further refinements in obtaining the coefficients are attained by varying the modulation frequency  $\omega/2\pi$  or the separation distance  $r$  between source and detector; thereby negating contributions from the source terms  $S$ ,  $A$  and  $\epsilon$  in Equation 2.

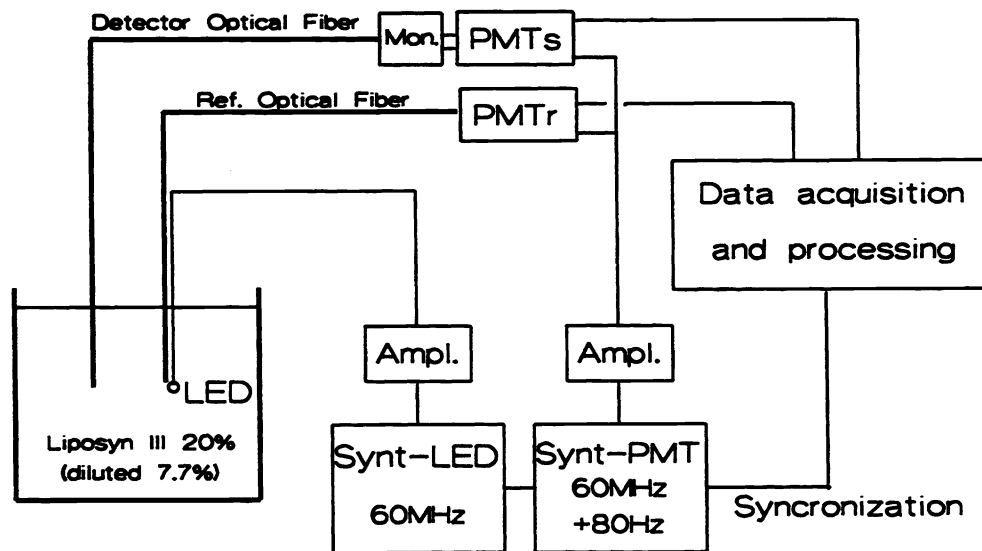


Fig. 1. Experimental arrangement.

### 3. MATERIALS AND METHODS

The instrumental configuration used in determining the wavelength ( $\lambda$ ) dependent absorption  $\mu_a(\lambda)$  and reduced scattering  $\mu_s'(\lambda)$  coefficients is schematically shown in Figure 1. To obtain wavelength tunability, we used a Hewlett-Packard (HLMP-4101) light emitting diode. The diode intensity was modulated by a sinusoidally applied voltage delivered by a frequency synthesizer (Marconi Instruments, Model 2022A), which was radio frequency amplified (ENI, model # 403). Striving for a relatively high modulation frequency of the diode, we selected 60 MHz which provided a good AC signal (modulation ratio 60%) at the diode output. Under the experimental conditions, the output of the light emitting diode was centered at 665 nm with a 30 nm width at half maximum. The optical power was 0.2 mW. The spectral span of the diode, 620-700 nm, encompassed the absorption peak of our chromophore methylene blue (656 nm maximum). The light from the amplitude modulated diode was collected at a separation distance  $r$  by a detector (glass) optical fiber bundle (3mm diameter). The light was wavelength resolved (to 4 nm) by a 10 cm monochromator (Instruments SA), stepped through in 1nm increments and detected at a sample photomultiplier tube (PMTs; Hamamatsu R928). Cross correlation techniques [13] and digital acquisition methods [14], which are common in frequency domain instrumentation, provided the DC, AC and phase delay heterodyned down to 80 Hz. These values from the PMTs were referenced against the signal at a reference photomultiplier (PMTr), which collected its signal from a fiber optic bundle in proximity to the diode source. This rationing procedure is standard in frequency domain systems, since it corrects for any variations in the source's output characteristics during the measurement.

The absorbing chromophore was dissolved in a highly scattering, macroscopically homogeneous fat emulsion of Liposyn III 20% (Abbott Laboratories). The light emitting diode and the two fiber optic bundles were immersed in this mixture in a 2.3 l cylindrical container 18 cm in diameter. The Liposyn scattering solution was diluted by water to 7.7% by volume to yield an average scattering coefficient on the order of  $20 \text{ cm}^{-1}$ . This value approximates the scattering of soft tissues [15]. We used the extinction coefficient of methylene blue ( $182 \text{ cm}^{-1} \text{ mM}^{-1}$  at 664 nm) to measure its absorption spectrum (maximum 656 nm) and concentration in a spectrophotometer (Perkin Elmer Lambda V) in a non-scattering solution. In the Liposyn suspension, the methylene blue concentration was set at  $0.225 \text{ }\mu\text{M}$  to approximate the  $\mu_a$  typical of tissues ( $0.01$  to  $0.1 \text{ cm}^{-1}$ ) [15].

### 4. RESULTS

To obtain a wavelength resolved, quantitative, absorption coefficient  $\mu_a(\lambda)$  spectrum of methylene blue dispersed in the multiply scattering Liposyn suspension, we performed a control experiment to correct for the water and Liposyn

absorption. Between 620-700 nm the  $\mu_a(\lambda)$  spectrum (data not shown) of the aqueous Liposyn dispersion was about  $0.006 \text{ cm}^{-1}$  and contained contributions from both water and Liposyn, and any boundary effects arising from the finite size of our measurement container. The measured scattering coefficient spectrum  $\mu_s'(\lambda)$  of about  $20 \text{ cm}^{-1}$  agreed closely with the spectrum predicted on the basis of Mie theory calculations for a similar fatty emulsion [16]. The results for the methylene blue wavelength resolved, absorption coefficient spectrum  $\mu_a(\lambda)$  are presented in Figure 2.

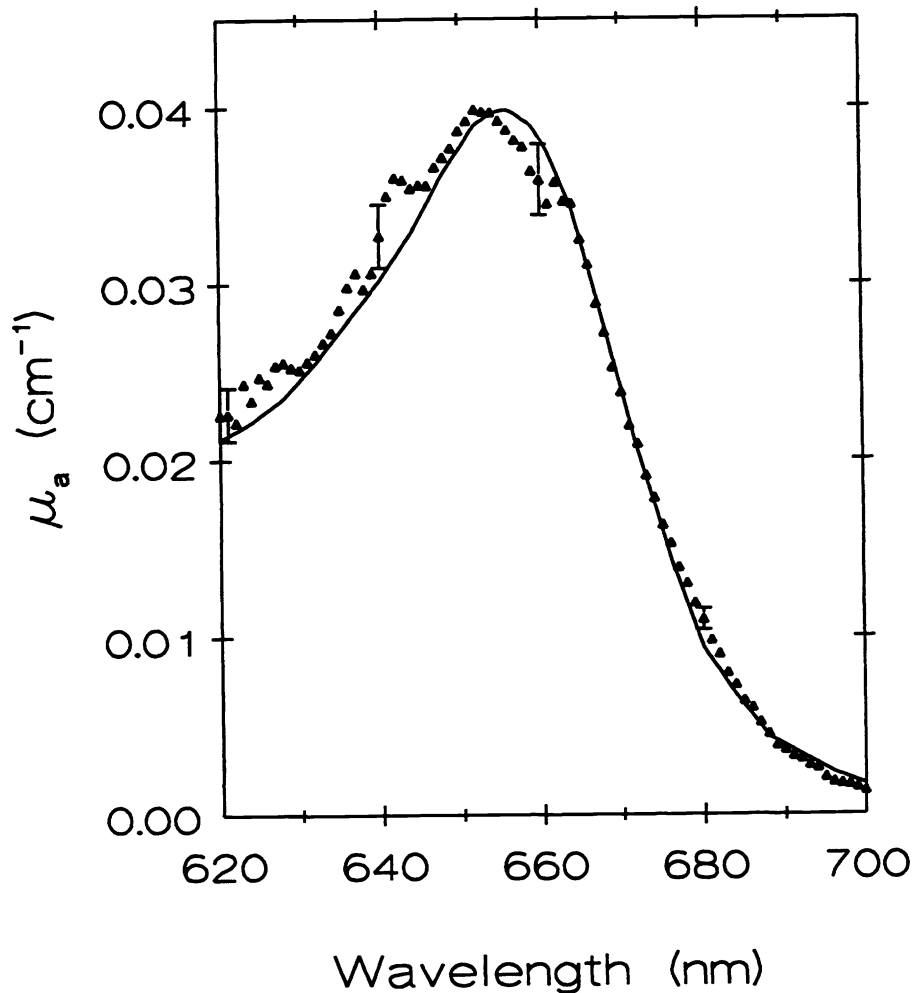


Fig. 2. Quantitative comparison between methylene blue absorption spectra measured in the multiply scattering case by the light emitting diode method (symbols) and in the non-scattering regime by a standard spectrophotometer (solid line). The spectra were acquired for the same methylene blue concentration of  $0.225 \mu\text{M}$ . Error bars for the data relative to the multiply scattering case are shown every 20 nm.

The data were collected in nanometer steps and the contribution from the blank absorption of aqueous Liposyn was subtracted. The same concentration of methylene blue (0.225  $\mu\text{M}$ ) was measured in a non-scattering solution (in a spectrophotometer) and its spectrum is drawn as the solid line in Figure 2. The agreement between  $\mu_a(\lambda)$  for the two regimes – multiply scattering and non-scattering – is essentially quantitative. We also verified the dependence of  $\mu_a$  on methylene blue concentration (data not shown) and found it to be linear in accordance with our previous work [12]. The wavelength resolved reduced scattering coefficient  $\mu_s'(\lambda)$  was independent of methylene blue concentration.

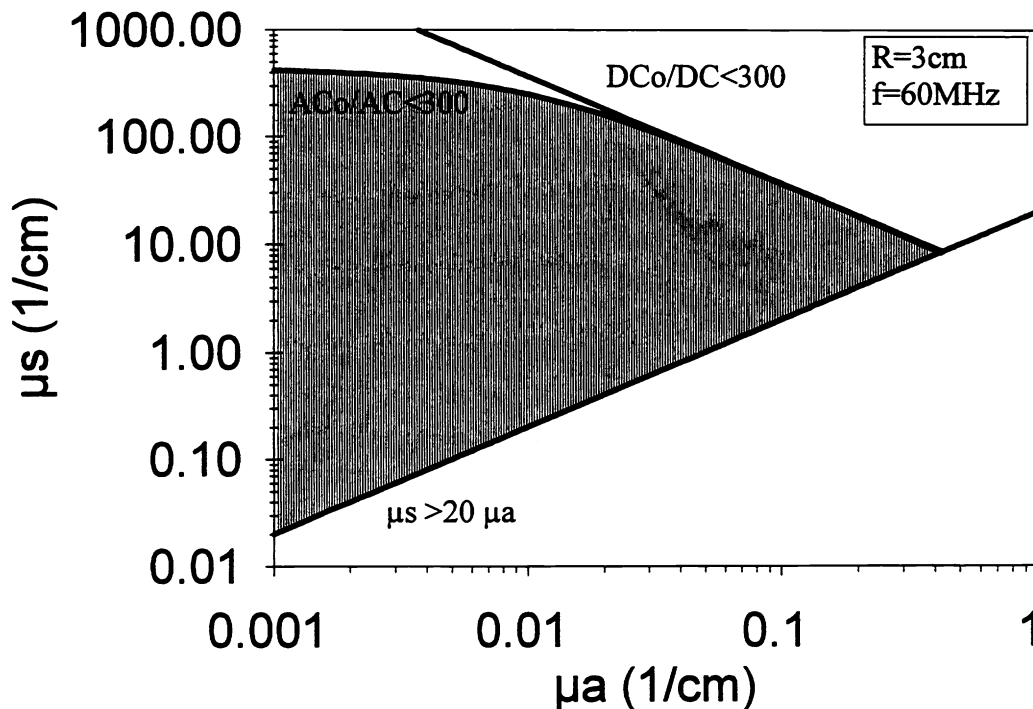


Fig. 3. Values of  $\mu_a$  and  $\mu_s'$  that can be measured by frequency-domain methods. The conditions discussed in the text define the measurable region, shown shaded, in the  $\mu_s'$  -  $\mu_a$  plane. Note that the typical values of  $\mu_a$  and  $\mu_s'$  in tissues ( $\mu_a \sim 0.01\text{-}0.1 \text{ cm}^{-1}$ ,  $\mu_s' \sim 1\text{-}100 \text{ cm}^{-1}$ ) fall inside the measurable region.



## 5. DISCUSSION

When one applies the diffusion model to obtain  $\mu_a(\lambda)$  and  $\mu_s'(\lambda)$  from frequency domain experiments in homogeneous, multiply scattering media certain conditions must be fulfilled:

- i)  $\mu_s'$  must be much larger than  $\mu_a$  (e.g.  $\mu_s' > 20 \mu_a$ ).
- ii) the dynamic range of the detectors can be photon limiting if the source-detector separation is too large, resulting in unrealistically weak DC and AC terms.

These conditions are graphically represented in Figure 3, which shows the ranges for accurately measurable  $\mu_a(\lambda)$  and  $\mu_s'(\lambda)$  values in a log-log plot. The figure is plotted for a modulation frequency of 60 MHz and a distance  $r = 3$  cm, corresponding to our typical experimental conditions. The shaded area designates the experimentally accessible range of optical parameters. These values include typical ranges of soft tissue optical properties [15]. The sensitivity of our approach to monitoring changes in  $\mu_a(\lambda)$  and  $\mu_s'(\lambda)$ , follows our ability to resolve these parameters to within a few percent. Application of this methodological approach to *in situ* measurement of the optical properties of tissues encounters the problems of tissue heterogeneity (in both scattering and absorption processes) and the finite geometry of most tissues. To a first approximation, the macroscopically homogeneous, multiply scattering medium assumption has yielded reasonable results for *in vivo* applications [6,16]. A number of groups are considering different boundary conditions, with surface orientations of sources and detectors, to deal with the second concern of non-invasive aspects of optical spectroscopy. These approaches will invariably add additional constraints on the diffusion approximation equations.

In conclusion, this communication demonstrates the power of the frequency domain approach, within the constraints of the photon diffusion approximation, to obtain absolute and quantitative, wavelength resolved absorption  $\mu_a(\lambda)$  and reduced scattering  $\mu_s'(\lambda)$  coefficient spectra. These coefficients are important in establishing chromophore concentrations, as well as the spectral identification of the chromophore(s) of interest. Finally, from an experimental perspective, we have shown the utility of wavelength resolved, modulated light emitting diodes as sources for optical spectroscopy experiments in the frequency domain. Tunable laser sources or other wide band point sources would also fulfill these requirements.

## 6. ACKNOWLEDGMENTS

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