Frequency-Domain Photon Migration in Turbid Media

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Abstract
An analytical model is presented for the propagation of diffuse photon density waves in turbid media. The frequency- and wavelength- dependence of photon density waves are measured using Frequency-domain Photon Migration (FDPM). Media optical properties, including absorption, transport, and fluorescence relaxation times are calculated from experimental results.

Introduction
The spectral and temporal characteristics of endogenous and exogenous molecules in tissues can provide valuable information concerning micro-environment and biochemical disposition. For example, it is well known that hemoglobin absorption spectra can be correlated to oxygen saturation levels. Similarly, fluorescence spectra and lifetime can be used to follow physiological changes such as as those observed during *in vivo* photosensitization [1]. Unfortunately, scattering degrades the information content of diffusely remitted light in turbid, biological media.

In this paper we summarize our efforts to measure absorption, transport, and fluorescence relaxation times in turbid media using Frequency Domain Photon Migration (FDPM) [2,3,4]. In FDPM, an optical fiber probe is used to launch amplitude-modulated light into the sample. Variations in the optical properties of the medium perturb the phase velocity and amplitude of the diffusely propagating photon density waves [4, 5]. Phase delay (ϕ) and demodulation (m) are measured, with respect to the source, by a second fiber placed 1-3 cm away from the launch fiber. Optical properties are calculated by fitting the measured frequency- and distance-dependent behavior to analytical expressions derived from diffusion theory. These properties include the absorption coefficient (β), the effective scattering coefficient (σ_{eff}), and the fluorescence lifetime (τ_f). Since spectral and temporal properties can be used to follow the biochemical disposition of absorbers and scatterers *in situ*, FDPM-spectroscopy may provide a means for non-invasive physiological monitoring.

Theory
(i) Determination of absorption and scattering properties
If a source varies in time with frequencies less than about 1 GHz, the optical fluence rate in most biological tissues satisfies a diffusion equation of the form:

$$\frac{c^2\tau_{tr,s}}{3}\nabla^2\varphi_s - \frac{\varphi_s}{\tau_{l,s}} - \frac{\partial\varphi_s}{\partial t} = -q_s c \qquad (1)$$

where φ_s and q_S are, respectively, the source optical fluence rate and the diffuse photon source density. The parameters c, $\tau_{l,s} = 1/\beta_s c$ and $\tau_{tr,s} = 1/\sigma_{eff,s} c$ are, respectively, the velocity of light, the loss relaxation time and the transport relaxation time in the medium at the same wavelength. Solutions to the form Eq. (1) can be expressed in the form:

$$\varphi \propto e^{-k_s r} \cdot e^{i\omega t} \qquad (2)$$

where r is the distance from the source, ω is the modulation frequency and k_S is the source complex wave number given by,

$$k_s = k_{real} + ik_{imag} = \frac{\sqrt{3}}{c}\sqrt{\frac{1}{\tau_{l,s}\tau_{tr,s}} + \frac{i\omega}{\tau_{tr,s}}} \qquad (3)$$

The real and imaginary part of k_S are:

$$k_{s,imag} = \sqrt{\frac{3}{2}}\frac{1}{c\sqrt{\tau_{l,s}\tau_{tr,s}}}\sqrt{\sqrt{(1+(\tau_{l,s}\omega_s)^2}-1} \qquad (4)$$

$$k_{s,real} = \sqrt{\frac{3}{2}}\frac{1}{c\sqrt{\tau_{l,s}\tau_{tr,s}}}\sqrt{\sqrt{(1+(\tau_{l,s}\omega_s)^2}+1} \qquad (5)$$

OSA Proceedings on Advances in Optical Imaging and Photon Migration, 1994, Vol. 21, R. R. Alfano (ed.)

where $\tau_{l,s}$ and $\tau_{tr,s}$ are, respectively, the loss relaxation time and the transport relaxation time at the wavelength of the external light source.

Multi-frequency measurements of phase (ϕ) and modulation (m) at a given source-detector separation (r) lead to the determination of the optical properties β_S, $\beta_{eff,S}$ and penetration depth, $\delta = 1/\sqrt{3\beta_s(\beta_s + \sigma_{eff,s})}$, since

$$\phi = k_{S,imag} r \text{ and } m = \frac{(AC/DC)_{sample}}{(AC/DC)_{source}} = \exp\left[-\left(k_{s,real} - \frac{1}{\delta}\right)r\right]$$

These expressions assume analytically useful forms under different conditions, however. The precise relationship between ϕ, m, and ω depends upon the absorption relaxation time 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. Because of this variable relationship between modulation frequency and optical properties, we typically evaluate the ϕ and m frequency-dependence in order to reliably calculate β and σ_{eff}.

(ii) Determination of fluorescence lifetimes

If the source is used to excite fluorescence in turbid media, the corresponding expressions for the real and imaginary part of k for the fluorescent light can be expressed by:

$$k_{f,imag} = \sqrt{\frac{3}{2}} \frac{1}{c\sqrt{\tau_{a,f}\tau_{tr,f}}} \sqrt{\sqrt{(1+(\tau_{a,f}\omega)^2} - 1} \qquad (6)$$

$$k_{f,real} = \sqrt{\frac{3}{2}} \frac{1}{c\sqrt{\tau_{a,f}\tau_{tr,f}}} \sqrt{\sqrt{(1+(\tau_{a,f}\omega)^2} + 1} \qquad (7)$$

where $\tau_{a,f}$ and $\tau_{tr,f}$ are, respectively, the loss relaxation time and the transport relaxation time at the wavelength of the fluorescent light.

The excitation of a fluorophore can be expressed,

$$\frac{\partial N}{\partial t} + \frac{N}{\tau_{nr}} + \frac{N}{\tau_r} = \frac{\beta_f \varepsilon_f \varphi_e}{\hbar\omega_e} \qquad (8)$$

where N is the number of excited fluorophores per unit volume and $1/\tau_{nr}$ and $1/\tau_r$ are, respectively, the non-radiative and the radiative contributions to the measured fluorescence decay rate. The absorption coefficient of the fluorophore at the source wavelength is β_f, the efficiency of the excitation is ε_f, the fluence rate of the exciting light is φ_s and ω_s is the angular frequency of the exciting light. The source density for the fluorescence emission is, therefore:

$$q_f = \hbar\omega_f \frac{N}{\tau_r} = \frac{\tau_f}{1+i\omega\tau_f} \frac{\varepsilon_f \beta_f}{\tau_r} \frac{\omega_s}{\omega_f} \varphi_s \qquad (9)$$

where q_f is the source density and ω_f is the angular frequency of the fluorescent light. The total lifetime of the excited state, τ_t, is given by:

$$\tau_f = \frac{\tau_{nr}\tau_r}{\tau_{nr} + \tau_r} \qquad (10)$$

and the efficiency of the excitation, $\varepsilon_f = \tau_f/\tau_r$.

In a planar model where the exciting light is launched at position x=0, the detected fluorescent light at position x=a can be expressed as:

$$\begin{aligned}\varphi_f(x=a) &= \int_0^{\xi=a} I \frac{\tau_f}{1+i\omega\tau_f} \frac{\varepsilon_f\beta_f}{\tau_r} \frac{\omega_s}{\omega_f} \frac{3(1+i\omega\tau_{tr,s})}{2c\tau_{tr,s}k_s} x \\ &\frac{3(1+i\omega\tau_{tr,f})}{2c\tau_{tr,f}k_f} e^{-k_s\xi} e^{-k_f(a-\xi)} d\xi \\ &= I \frac{\tau_f}{1+i\omega\tau_f} \frac{\varepsilon_f\beta_f}{\tau_r} \frac{\omega_e}{\omega_f} \frac{3(1+i\omega\tau_{tr,s})}{2c\tau_{tr,s}k_s} x \\ &\frac{3(1+i\omega\tau_{f,tr})}{2c\tau_{tr,f}k_f} \frac{e^{-k_s a} - e^{-k_f a}}{k_f - k_s}\end{aligned} \qquad (11)$$

The explicit expressions for the modulus and phase of the fluorescent signal in a scattering medium can be determined from the real and imaginary components of Eq. (11).

Materials and Methods

(i) Instrumentation

The frequency-domain photon migration (FDPM) instrument is a modified multiharmonic Fourier Transform phase and modulation fluorometer (SLM, 48000-MHF, Champaign, IL). A brief description of the instrument is presented in an accompanying paper; details have been reported elsewhere [4].

(ii) Materials

Measurements were performed in a large cylindrical vessel filled with an emulsified fat solution, Intralipid™ (KabiVitrum, Inc., Clayton, NC). Fibers (flat-cut faces, 600 μm core diameter) were positioned in the center of the liquid, facing each other. This central location was selected in order to simulate an infinite medium. The distance between source and collection fibers was systematically varied between 5 and 30 mm. Reference (i.e. source) measurements were recorded in air with input and collection fibers facing each other.

The photosensitizing porphyrin dye, tetraphenyl porphine tetrasulfonate ($TPPS_4$) (Porphyrin Products, Logan, UT) was added to the scattering system so the effect of absorber and fluorophore could be quantitatively determined. All measurements were performed using 514-nm light from an Argon-ion laser. Bandpass filters (Corion Corp, Holliston, MA) were placed at the entrance to the PMT housing in order to isolate the green absorption (514 nm) and red fluorescence (650 nm).

Results and Discussion

Fig. 1 shows the frequency-dependent phase response in 2% Intralipid, with and without 2 μg/ml $TPPS_4$, under a variety of measurement conditions.

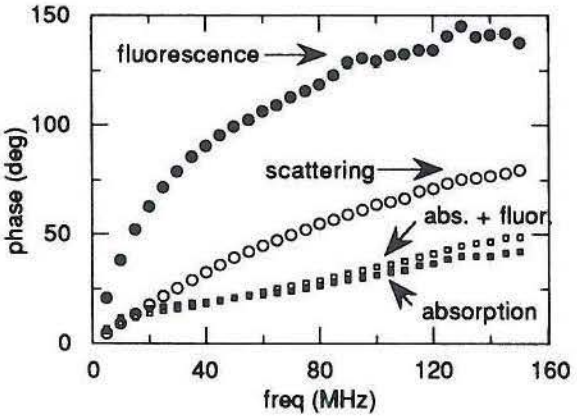

Figure 1. Phase vs. frequency response for TPPS in Intralipid.

The upper-most *fluorescence* curve was obtained by isolating the $TPPS_4$ emission with a 650 nm bandpass filter. The *scattering* response was acquired in pure 2% Intralipid (no $TPPS_4$ absorber). The *absorption* and *abs. + fluor.* data were acquired in $TPPS_4$/Intralipid with and without a source-isolating 514 nm bandpass filter. These data were combined with the modulation response in order to calculate the absorption and scattering parameters at 514 nm. Table 1 summarizes the measured parameters used to estimate fluorescence lifetime.

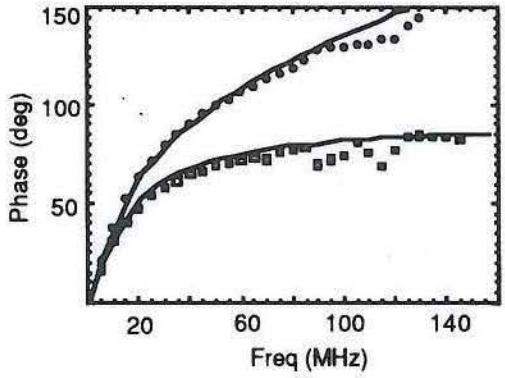

Figure 2. Phase vs. frequency data for TPPS fluorescence and Intralipid scattering medium (circles) and TPPS in water (squares). Solid lines are simulated response (see text for parameters).

$TPPS_4$ fluorescence was measured using the same fiber format (1cm separation) without Intralipid scatterer. These data are displayed in Figure 2 (open squares), along with the fluorescence obtained in the scattering medium (open circles). The difference can be qualitatively understood in terms of the additional path, and hence phase delay, experienced by fluorescent photons. The solid lines through the data represent the simulated response using Table 1 parameters and a 10 ns fluorescence lifetime; a value which agrees well with conventionally-derived $TPPS_4$ fluorescence lifetimes.

Table 1. Calculated relaxation times for $TPPS_4$ in Intralipid

source loss relaxation time: $\tau_{l,s}$	source transport relaxation time: $\tau_{tr,s}$	fluorescence loss relaxation time: $\tau_{l,f}$	fluorescence transport relaxation time: $\tau_{tr,f}$
200 ps	2.2 ps	1000 ps	2.4 ps

Acknowledgements

This work was supported by the Whitaker Foundation (WF16493), the National Institutes of Health (R29GM50958) and Beckman Instruments Inc.. In addition, we acknowledged program support from the Beckman Laser Institute and Medical Clinic including grants from the Office of Naval Research (N00014-91-0134), the Department of Energy (DE-FG-3-91-ER61227), and the National Institutes of Health (5P41RR01192-15). The authors are grateful to the following for their assistance: Alexander Kam, Eric Cho, David Pham, Cuong Ly, and Tuan Ho.

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