Determination of absorption coefficients in highly scattering media from changes in attenuation and phase

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Received April 4, 1996

The accurate, quantitative analysis of absorption and scattering properties in tissue is a central problem in biochemical optics, in particular for the determination of hemoglobin and oxyhemoglobin concentrations. Because of light scattering, the absolute concentrations of these chromophores (i.e., the absorption coefficient) cannot easily be inferred. A new method for the estimation of the absorption coefficients in scattering media, based on measurements obtained from an intensity-modulated optical spectrometer, is proposed. The ratios of the changes in attenuation and phase that are induced by changes in the absorption coefficient give a good approximation of the absorption coefficient. © 1996 Optical Society of America

Different experimental approaches have been suggested for determining the absorption coefficient in highly scattering media. Most of these methods are based on the measurement of the diffusely reflected light intensity (reflectance R) and the mean arrival time (time of flight) $\langle t \rangle$, or phase Φ, of an intensity-modulated light source. These measurements are then used in a diffusion equation analysis. For a fixed modulation frequency it has been shown that the absorption and transport scattering coefficients (μ_a and $\mu_s{}'$, respectively) can be inferred from measurements of the reflectance and phase data obtained at different source–detector distances.[1–3] Alternatively, measurements can be made with a single distance and multiple modulation frequencies.[4–6] For multiple small source–detector distances the reflectance measurements alone are sufficient for deriving both μ_a and $\mu_s{}'$.[7] Here a different approach for the determination of absorption coefficients is described. Small changes in absorption coefficient induce changes in both R and $\langle t \rangle$ (or Φ). It has been found that the ratio of these changes is largely independent of the scattering properties of the medium and that this ratio gives a good estimate of the (mean) absolute absorption coefficient. The approximation is valid only over a certain range of μ_a and $\mu_s{}'$ values; however, this range encompasses the values typically found in biological tissues for the near-infrared wavelengths. In this Letter we demonstrate in two phantom experiments the feasibility of the suggested method. Changes in μ_a can be induced, first, by changes in the chromophore concentration and, second, by tuning the wavelength over the absorption spectrum of the chromophore.

The transport of light in scattering media has been thoroughly analyzed over recent years, and diffusion theory has become established as a versatile tool for describing R, $\langle t \rangle$, and Φ in terms of the transport scattering coefficient ($\mu_s{}'$), the absorption coefficient (μ_a), and the refractive index (n) of the medium.[8,9] For a pencil beam light source on a semi-infinite half-space, R and $\langle t \rangle$ detected at a distance r from the source can be written as

$$R(r) = z_0(1/\rho + \mu_{\text{eff}})\frac{\exp(-\mu_{\text{eff}}\rho)}{2\pi\rho^2}, \tag{1}$$

$$\langle t \rangle(r) = \frac{\rho^2}{2c(D + \rho\sqrt{\mu_a D})}, \tag{2}$$

respectively, where $\rho = (r^2 + z_0)^{1/2}$ and $z_0 = 1/\mu_s{}'$. The velocity of light in the medium is $c = c_0/n$ (where c_0 is the speed of light in vacuum), the diffusion coefficient is $D = [3(\mu_a + \mu_s{}')]^{-1}$, and $\mu_{\text{eff}} = (\mu_a/D)^{1/2}$ is known as the effective attenuation coefficient. Here the changes of the reflectance and the mean time, with respect to changes in μ_a, are examined, and the scattering properties are assumed to be constant. The derivatives of the attenuation A, defined as $A(r) = \log\{I_0/[R(r)S]\}$, where I_0 is the incident flux and S is the area of the detector, and $\langle t \rangle$ with respect to μ_a are

$$\frac{\partial A}{\partial \mu_a} = \frac{3}{2 \ln 10}\,\frac{\rho}{1/\rho + \mu_{\text{eff}}}(2\mu_a + \mu_s{}'), \tag{3}$$

$$\frac{\partial \langle t \rangle}{\partial \mu_a} = \frac{-3}{2(1/\rho + \mu_{\text{eff}})^2 c}\left(\frac{\rho}{2}\,\frac{\mu_s{}'}{\sqrt{\mu_a D}} - 1\right)\cdot \tag{4}$$

When an intensity-modulated optical spectrometer (IMOS) is used, the phase Φ of a light wave, intensity modulated at the frequency ν_M, rather than the mean time $\langle t \rangle$, is measured. For frequencies considered here, Φ and $\langle t \rangle$ are approximately coupled by the simple linear relationship $\Phi = -2\pi\nu_M\langle t \rangle$.[4,9] The error in this relationship is less than 5%, provided that $\mu_a > 3.2\pi\nu_M/c$. For example, for a modulation frequency of 200 MHz in water, μ_a must be greater than 0.009 mm^{-1}.

In Fig. 1, $Q_a = (\partial A/\partial \mu_a)/(\partial \Phi/\partial \mu_a)$ is shown as a function of μ_a, calculated for the transport scattering coefficients $\mu_s{}' = 0.75$, 1.0, 1.25, 1.50 mm^{-1}. A source–detector distance $r = 30$ mm, a modulation frequency $\nu_M = 200$ MHz, and a refractive index of $n = 1.33$ were assumed. To a good first-order approximation, Q_a varies linearly with μ_a. The influence of $\mu_s{}'$

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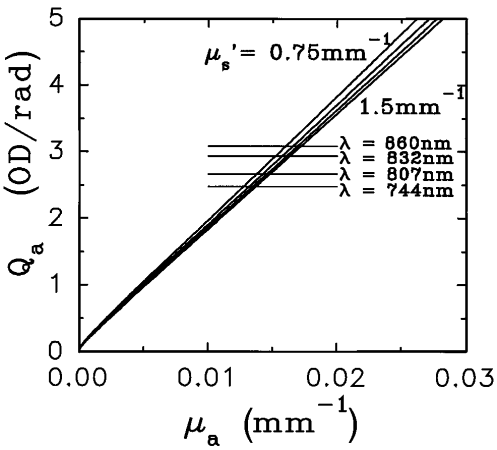

Fig. 1. $Q_a = (\partial A/\partial \mu_a)/(\partial \Phi/\partial \mu_a)$ as a function of μ_a calculated for values of $\mu_s' = 0.75, 1.0, 1.25, 1.5\ \text{mm}^{-1}$ ($\nu_M = 200$ MHz, $n = 1.33$, $r = 30$ mm). The horizontal lines indicate Q_a, obtained for the different laser wavelengths of the IMOS in the phantom experiment described below (compare with Figs. 2 and 3).

on Q_a is small. Therefore, under the conditions of a fixed geometry and pure absorption changes, Q_a is dependent primarily on μ_a. In this Letter we argue that this finding can be exploited for the estimation of μ_a.

Equations (1)–(4) assume a simplified boundary condition that sets the fluence rate equal to zero at the boundary. In the experimental measurements the fluence rate was not zero and was determined by the refractive-index mismatch of the scattering medium and its surrounding medium. When the physically more correct mismatched boundary condition is included,[3] R, $\langle t \rangle$, and Φ and their differentials with respect to μ_a can be compared with values from Eqs. (1)–(4). For typical optical properties ($\mu_s' = \text{mm}^{-1}$, $\mu_a = 0.0135\ \text{mm}^{-1}$, $n = 1.33$, $r = 30$ mm) and a refractive index of 1 for the outside medium, R is greater by more than a factor of 2 and $\langle t \rangle$ is larger by ~1% with the more physically correct boundary condition. However, the slopes $\partial A/\partial \mu_a$ and $\partial \Phi/\partial \mu_a$ differ by less than 1%, and hence Q_a differs by only 1% compared with the values given in Fig. 1. The use of the simplified matched boundary condition is therefore justified.

The IMOS used here was described in detail previously.[4] It incorporates four intensity-modulated laser diodes ($\lambda = 744, 807, 832, 860$ nm; $\nu_M = 200$ MHz) and phase-sensitive detection. Optical fibers were used to transport the light between the light source, the scattering medium, and the detector. For the first experiment a phantom consisting of an aqueous suspension of spherical polystyrene particles serving as light-scattering centers was employed. Mie theory[10] was used to derive μ_s' for these spheres (diameters of 0.6–2.5 μm). For a scattering sphere concentration $c_s = 1\%$ volume per volume (vol/vol), $\mu_s' = 1.2\ \text{mm}^{-1}$ was calculated, almost independently of wavelength between 740 and 860 nm. The absorption coefficient of the phantom was the sum of that of water ($\mu_a{}^w$) and of a dye ($\mu_a{}^d$) (S109564, ICI, Manchester, UK) added in known quantities: $\mu_a = \mu_a{}^w + \mu_a{}^d$. The spectra of $\mu_a{}^w$ and $\mu_a{}^d$ for a dye concentration $c_d = 1.40 \times 10^{-5}$ vol/vol are shown in Fig. 2. The ends of both the light delivery and detection fibers were submerged approximately 2 mm into the phantom (volume 100 mm × 80 mm × 60 mm) 30 mm apart. A change in μ_a of the phantom was induced by variation of the concentration of the dye in 14 steps each of 1.44% (corresponding to $\Delta\mu_a = 1.56 \times 10^{-4}\ \text{mm}^{-1}$ at $\lambda = 744$ nm; mean $\mu_a = 0.0133\ \text{mm}^{-1}$). The concentration of the scattering microspheres was unchanged. The attenuation changes and the phase shift were recorded with the IMOS as a function of the absorption changes [see Fig. 3(a) for $\lambda = 744$ nm].

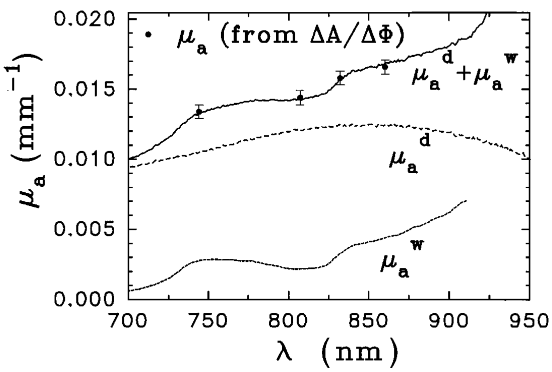

Fig. 2. Absorption coefficient $\mu_a = \mu_a{}^w + \mu_a{}^d$ of the liquid phantom, where $\mu_a{}^w$ is the water absorption and $\mu_a{}^d$ is the dye absorption for a concentration of $c_d = 1.40 \times 10^{-5}$ vol/vol. The dots are the experimental values derived from measurements of Q_a and the true μ_s' values (see Figs. 1 and 3). The error bars indicate the influence of variations by ±50% in the assumed μ_s' value.

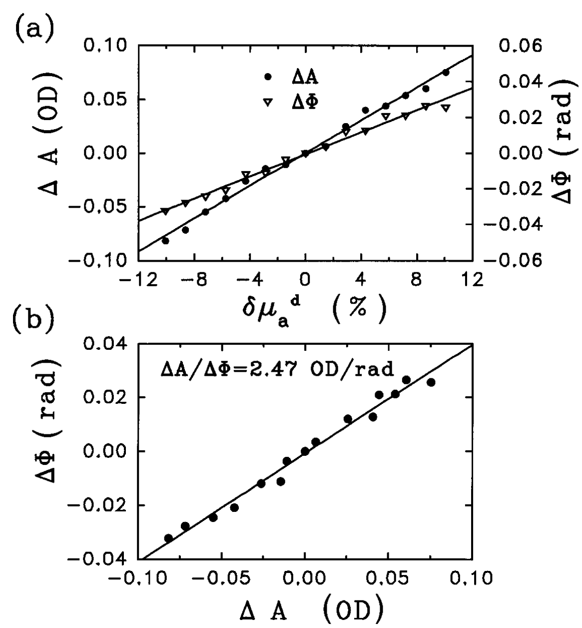

Fig. 3. (a) Measured changes in attenuation (ΔA) and phase ($\Delta\Phi$) as a function of fractional changes in dye absorption $\delta\mu_a{}^d$ ($\lambda = 744$ nm, mean dye concentration $c_d = 1.40 \times 10^{-5}$ vol/vol). The lines give a first-order regression fit of the experimental data. (b) Correlation plot of ΔA and $\Delta\Phi$ for the data shown in (a). The regression line has a slope of $Q_a = 2.47$ OD/rad.

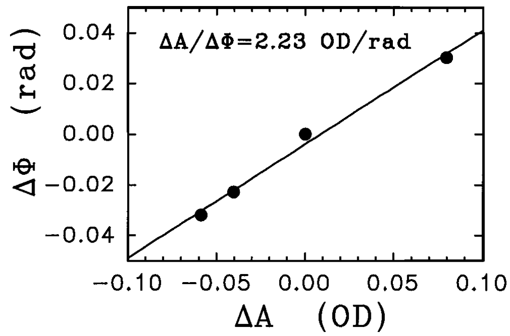

Fig. 4. Correlation of changes in attenuation and phase measured on a solid phantom for wavelengths between 753 and 761 nm. The solid line represents the first-order regression line of the experimental values (dots) with a shape of 2.23 OD/rad.

ΔA and $\Delta\Phi$ are strongly correlated [Fig. 3(b)] with a first-order regression slope of $\Delta A/\Delta\Phi = 2.47$ optical density (OD)/rad. By using this measured value of $\Delta A/\Delta\Phi$ as an approximation of Q_a together with the known $\mu_s{}'$ of the phantom, we calculated a (mean) $\mu_a = 0.0135\ \text{mm}^{-1}$ from Fig. 1. The experimental μ_a values were found to be in excellent agreement with the true μ_a value (see Fig. 2) for all four laser wavelengths. Variations in the assumed $\mu_s{}'$ values of $\pm 50\%$ result in errors in the estimate of μ_a of only $\pm 4\%$.

The second approach to inducing small changes in absorption coefficient is to scan over the absorption spectrum of the medium by tuning the wavelength λ. This requires either that the scattering coefficient stay unchanged over the wavelength shifts used or that changes in $\mu_s{}'$ be accounted for. A solid, light-scattering phantom[11] with well-characterized optical properties at $\lambda = 758$ nm ($\mu_a = 0.0160\ \text{mm}^{-1}$, $\mu_s{}' = 0.934\ \text{mm}^{-1}$, $n = 1.56$, $\delta\mu_a = +1.5\%/\text{nm}$, $\delta\mu_s{}' = -0.05\%/\text{nm}$) was used. A temperature-tunable diode laser was employed to change the wavelength between $\lambda = 753$ nm and $\lambda = 761$ nm (FWHM = 2 nm). The light reflected from the phantom was detected at a distance $r = 30$ mm from the light delivery fiber. The attenuation and phase changes were corrected for wavelength-dependent variations in the diode laser output by reference measurements of intensity and phase at a distance $r = 7$ mm. Figure 4 shows the correlation of the measured attenuation and phase changes for this wavelength range. The first-order regression of the correlation gives a slope of $Q_a = 2.23$ OD/rad. By analyzing this according to Eqs. (3) and (4) we calculated an absorption coefficient of $\mu_a = 0.016\ (\pm 0.0006)\ \text{mm}^{-1}$ for $0.75\ \text{mm}^{-1} < \mu_s{}' < 1.5\ \text{mm}^{-1}$. This is in excellent agreement with the true value.

In this Letter the change in A and Φ with respect to absorption changes was discussed and exploited to yield μ_a. Similarly, one can use changes with respect to distance to derive an estimate of μ_a from $(\partial A/\partial r)/(\partial\Phi/\partial r)$. Furthermore, similar considerations are valid for the changes in phase and modulation depth (M) of an intensity-modulated light wave, where μ_a can be derived from $(\partial\Phi/\partial\mu_a)/(\partial M/\partial\mu_a)$.

In conclusion, a method is described that allows the absorption coefficient μ_a of a turbid medium to be estimated when its scattering properties are only approximately known. For tissue, $\mu_s{}'$ typically has values[12] of 0.5–1.5 mm^{-1}, resulting in uncertainties in μ_a of only ± 4–6%. The main advantage of this method over others is its simplicity: measurements are made at only one detector position, and changes in attenuation and phase rather than absolute values are measured. μ_a can be simply estimated from the ratio of attenuation and phase changes. Preliminary experiments in tissue *in vivo* have shown that only small changes in blood volume, oxygenation, or probe distance are sufficient to generate the attenuation and phase changes.

We acknowledge the support of The Wellcome Trust.

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