Non-contact spectroscopic age determination of bloodstains

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Diffuse reflectance spectroscopy is a common technique for determining the optical properties of biological samples. Proper data analysis is often based on the diffusion approximation to the radiative transfer equation. This theory is assumed to be only valid for regimes where scattering is dominant over absorption, $\mu_s' > 10 \cdot \mu_a$. However, we observe excellent agreement between reflectance measurements of phantoms and an equation derived by Zonios et al. [100] from diffusion theory. However, two parameters were fitted to all experiments, including strongly absorbing samples, implying that the reflectance equation cannot be considered as being diffusion theory any more. This approach predicts the diffuse reflectance very well (r^2=0.994) for a large range of optical properties and is potentially valid in a larger optical regime than previously assumed.

CHAPTER 7
DIFFUSION APPROXIMATION BEYOND ITS ASSUMED BORDERS OF VALIDITY

In preparation

INTRODUCTION

Interpretation of reflectance spectroscopy measurements requires translating reflectance values into absorption coefficients. In a previous study, we showed that an empirical model connecting path lengths with absorption and reduced scattering coefficients can assist in relating reflectances to absorption, as shown in chapter 4. Defining an empirical path length model for a specific experimental geometry is a recognized method [105-107] for analysis of reflectances and many more (semi-) empirical models have been reported in literature [101-104]. During our search of finding an appropriate empirical path length model we also explored the applicability of an equation proposed by Zonios *et al.* [100], because it allows effortlessly matching theory with our experimental setup. This equation is derived from diffusion theory and hence is not *a priori* applicable to reflectance measurements when tissue absorption dominates over scattering. In the VIS/NIR wavelength range the reduced scattering coefficients, μ_s', of tissues vary between 0.5-10 mm^{-1}, whereas the absorption coefficient, μ_a, of whole blood in this wavelength range can be 0.1-30 mm^{-1}. The use of diffusion theory, requiring $\mu_s' >> \mu_a$, is therefore assumed to be limited to tissue types with small (<1%) blood volume fractions, and excludes studying well perfused organs, bruises [11], let alone whole blood itself.

In this chapter we use phantom measurements to explore the model by Zonios *et al.* [100] including the possible validity of diffusion theory at high absorption coefficients.

METHODS

Diffuse reflectance spectroscopy (DRS)

The DRS measurements presented in this study use the same phantoms and the same measurement setup as presented in chapter 4 and chapter 6, i.e. utilizing the reflectance ratio, $R(\mu_s',\mu_a)/R(\mu_s',0)$, i.e. the diffuse reflectance with absorber, $R(\mu_s',\mu_a)$, versus without absorber, $R(\mu_s',0)$.

Theory

Our starting point is the diffusion theory approach of Farrell *et al.* [138] who calculated the diffuse reflectance as a function of radial distance, *r*, in response to a pencil beam.

$$R(\mu_s',\mu_a,r)=\frac{z_0}{4\pi}\cdot\frac{\mu_s'}{\mu_s'+\mu_a}\cdot\left[\left(\mu+\frac{1}{r_1}\right)\cdot\frac{e^{-\mu r_1}}{r_1^2}+\left(1+\frac{4}{3}A\right)\cdot\left(\mu+\frac{1}{r_2}\right)\cdot\frac{e^{-\mu r_2}}{r_2^2}\right] \qquad (1)$$

In this equation, the following parameters are defined as:

$$\mu=\sqrt{3\mu_a(\mu_a+\mu_{s'})},\ z_0=1/(\mu_a+\mu_s'),\ r_1=\sqrt{(z_0^2+r^2)},\ r_2=\sqrt{((1+4A/3)^2 z_0^2+r^2)}$$

Parameter *A* depends on the refractive index mismatch of the air-tissue boundary [138]. For a phantom reflective index of 1.35, parameter *A* is equal to 3. To calculate the reflectance ratio in response to an irradiation with radius r_i as captured by the collection fiber, one has to integrate reflectances R(μ_s',μ_a) and R(μ_s',0) over the collection spot size with radius r_c:

$$\frac{R(\mu_s',\mu_a)}{R(\mu_s',0)}=\frac{\int_0^{r_i} d\rho \int_0^{r_c} dr\cdot R(\mu_s',\mu_a,|\rho-r|)\cdot 2\pi r}{\int_0^{r_i} d\rho \int_0^{r_c} dr\cdot R(\mu_s',0,|\rho-r|)\cdot 2\pi r} \quad (2)$$

Because Eq. (2) cannot be evaluated analytically, a simple analytical expression for R was derived by Zonios assuming point delivery of light and collection over a circular spot with an effective radius of r_c of the detecting fiber. This resulted in the following equation:

$$\frac{R(\mu_s',\mu_a)}{R(\mu_s',0)}=\frac{\frac{\mu_s'}{\mu_s'+\mu_a}\cdot\left\{e^{-\mu z_0}+e^{-\left(1+\frac{4}{3}A\right)\mu z_0}-z_0\cdot\frac{e^{-\mu r_1}}{r_1}-\left(1+\frac{4}{3}A\right)\cdot z_0\cdot\frac{e^{-\mu r_2}}{r_2}\right\}}{2-\frac{1}{\mu_s'}\cdot\left\{\frac{1}{r_1'}+\frac{1+4A/3}{r_2'}\right\}} \quad (3)$$

Here, r_1 and r_2 have become a function of r_c instead of r, replacing r by r_c. Further, r_1' and r_2' are the values for r_1 and r_2 for μ_a=0. r_c is defined by Zonios *et al.* as the effective spot radius [100]. The geometry setup used in [100] is similar to reflectance measurements in previous chapters, only difference is interchanging of collection and illumination fibers. Therefore, to describe the reflectance measurements r_c has become the effective collection radius. The numerical aperture of our fibers is 0.22; accordingly the acceptance angle is 13°. The fiber radius is 0.20 mm. At a height of 17 mm above the phantom, the collection spot size radius was determined to be, r_c = 2.7 mm. The six, equally sized, illumination fibers around the collection fiber will produce an illumination spot size with radius of r_i 3.1 mm.

Equations (2) and (3) will be compared with our experimental phantom measurements. Equation 2 from numerical integration of the R(μ_s',μ_a) and R(μ_s',0). Equation (3), first, with the physical parameters A = 3 and collection area radius r_c = 2.7 mm when placing the probe at a height of 17 mm. Second, because the irradiation in our experimental geometry is not by a pencil beam but covers an area with radius r_i, while the detection fiber only covers the diffuse reflectance coming from a smaller area with radius r_c, we also followed Zonios *et al.* [100], and used the parameters A, r_c that best described the phantom measurements using a Levenberg-Marquandt fitting algorithm [139] with error margins represented by the 95% confidence intervals. Table 1 summarizes the various parameters for the three situations.

Table 1. Overview of additional parameters in Eq (2) and Eq (3).

	Symbol	Definition	Physical value	Fitted Value
Exact Diffuse (Eq.2)	ρ	probe position	from 0 to r_i	-
	r_i	Illumination spot radius	3.1 mm	-
	r_c	Collection spot radius	2.7 mm	-
	A	Refractive index parameter	3.1	-
Zonios' Model (Eq. 3)	r_c	Effective collection radius	2.7 mm	6.7 mm
	A	Refractive index parameter	3	2.0

RESULTS

First we have explored the limits of $R(\mu_s', \mu_a)/R(\mu_s', 0)$ for four limits of μ_s' and $\mu_a \rightarrow 0$ and ∞

$$\lim_{\mu_a \to 0} \frac{R(\mu_s', \mu_a)}{R(\mu_s', 0)} = 1 \qquad \lim_{\mu_a \to \infty} \frac{R(\mu_s', \mu_a)}{R(\mu_s', 0)} = 0 \tag{4}$$

$$\lim_{\mu_s' \to 0} \frac{R(\mu_s', \mu_a)}{R(\mu_s', 0)} = \infty \qquad \lim_{\mu_s' \to \infty} \frac{R(\mu_s', \mu_a)}{R(\mu_s', 0)} = 1 \tag{5}$$

We found a remarkable result for the limit of the reduced scattering going to zero; the reflectance ratio goes to infinity.

Figure 1 shows the reflectance ratio for varying absorption coefficients and two reduced scattering coefficients (1 and 11.5 mm^{-1}). Figure 2 shows the reflectance ratio for varying reduced scattering coefficients and two absorptions (1 and 10 mm^{-1}). The total reflectance, as shown in Eq (2) collected by the collection fiber with radius r = 2.7 mm has been determined. Also, following Zonios' approach, from Eq (3) for both physical parameters A = 3.1 and r_c = 2.7 mm, as well as for A= 2.0 ± 0.4 and r_c = 6.7 ± 0.3 mm determined from a fit of all phantom experiments. Figures 1 and 2 both show the substantial deviations between these three approaches. Exact diffusion theory, Eq. (2), and Zonios' Eq. (3) with physical A, r_c values show substantial deviations from the experimental results, especially for the low reduced scattering coefficients. However, Zonios' model Eq. (3) with fitted parameters shows good correspondence with the phantom measurements. Agreement between experimental reflectance measurement and diffusion model, determined from integration, r^2=0.918, Zonios' approach with physical parameters, r^2=0.922, and with fitted parameters can be expressed as, r^2=0.994.

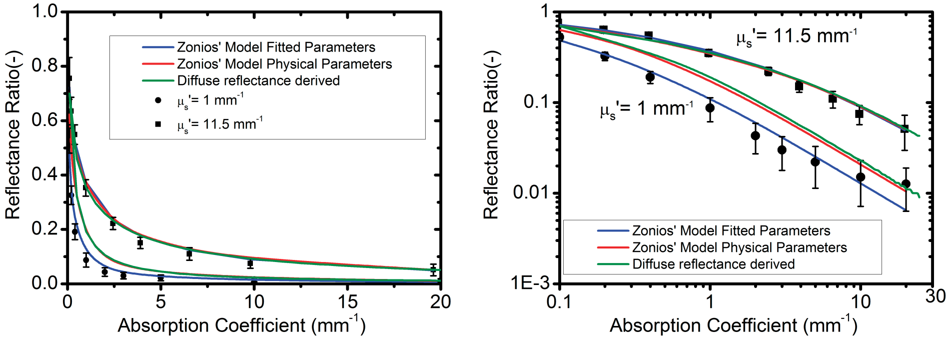

Figure 1. (A) Reflectance Ratio as a function of the absorption coefficient, for high (squares) and low (dots) scattering. Green line represents Eq (2). The red lines represent Eq. (4) with physical parameters A = 3.1, r_c = 2.7 mm; the blue lines with fitted parameters A = 2, r_c = 6.7 mm. (B) Same figure but the reflectance ratio on a logarithmic scale.

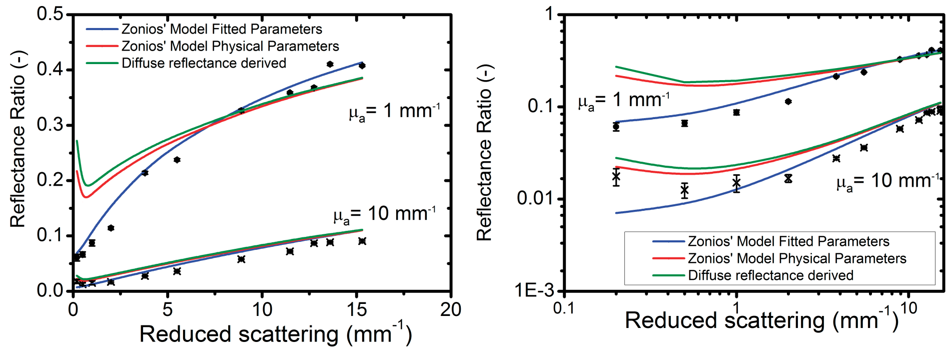

Figure 2. (A) Reflectance Ratio as a function of the reduced scattering coefficient, μ_s', for high absorption (μ_a = 10 mm^{-1}) and low absorption (μ_a = 1 mm^{-1}). Green line represents Eq (2). The red lines represent Eq. (4) with physical parameters A = 3, r_c = 2.7 mm; the blue lines with fitted parameters A = 2, r_c = 6.7 mm. (B) Same figure but on a logarithmic scale.

DISCUSSION

We have investigated whether the reflectance spectroscopy model derived from diffusion theory, albeit with additional approximations, as given by Eq. (3) of Zonios *et al.* can be used to model the reflectance ratio measurements on phantoms with controlled optical properties.

First, the limit of measuring the reflectance ratio in the situation of the reduced scattering going to zero is purely hypothetical, yet the ratio of $R(\mu_s',\mu_a)/R(\mu_s',0)$ should not be able to be larger than one, let alone to go infinity. Therefore, the left hand part of Eq. (5) at least implies that Eq. (3) incorrectly describes reflectance ratios for small μ_s'.

Secondly, we showed that Eq. (3) describes the diffuse reflectance ratio of phantom experiments with good agreement, provided that parameters A, r_c are fitted to experimental results, including cases where $\mu_a >> \mu_s'$. For large scattering coefficients, Eq. (2) and Eq. (3) with physical A, r_c parameters (table 1) reasonably model the reflectance ratio too. This raises the interesting question why diffuse reflectance spectroscopic experiments under certain conditions allow analysis by equations derived from diffusion theory far beyond the conditions of validity of this theory, in contrast to e.g. fluence rate distributions (see e.g. Figure 6.2 of Star [140]).

When using physical parameters in Eq (3) it only describes the qualitative shape of $R(\mu_s',\mu_a)/R(\mu_s',0)$ versus absorption, since it correctly describes the limits for μ_a = 0 and μ_a is large, i.e. $R(\mu_s',\mu_a)/R(\mu_s',0) = 1$ for $\mu_a = 0$ while it tends to zero for for μ_a = large, i.e. Eq. (4) and Eq. (5). Yet, when the two parameters r_c and A are fitted to all experimental results including those with large μ_a, it is appears that Eq. (3) with numerically fitted parameters describes the experiments also quantitatively. However, Eq. (3) with fitted parameters has become a semi-empirical model, with no physical meaning of parameters A and r_c.

Additionally, the structure of Eq. (3) is such that the reflectance ratio for larger values of μ_s',μ_a depends less and less on the actual values of A, r_c. This is because then, the third and fourth terms inside the brackets of the nominator, which include A, r_c, become small relative to the first and second terms, while the second term, which includes A, also becomes small relative to the first. Hence, Eq. (3), either with exact or with fitted parameters approaches the same outcome when μ_s' or μ_a become large. Here, it helps that also r_c is relatively large. Thus, diffusion theory described by Eq. (2) and approximated by Eq. (3) with physical parameters A and r_c indeed predicts reflectance ratios accurately for larger values of μ_s' and μ_a, including those far beyond the validity limits of diffusion theory.

In conclusion, by comparison with experimental reflectance measurements, remarkable agreement is observed with the diffusion approximation at high scattering and high absorption properties. When using fitted parameters (A and r_c), the model also showed good agreement at low scattering and high absorption properties. Thus, Zonios' model, expressed by Eq. (3) is able to describe reflectance ratios accurately for a large range of optical properties and is potentially applicable beyond the generally assumed limits of validity of diffusion theory.