Non-contact spectroscopic age determination of bloodstains
Bremmer, R.H.

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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, $\mu' > 10 \cdot \mu_a$. 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, \( \mu_s' \), of tissues vary between 0.5-10 mm\(^{-1}\), whereas the absorption coefficient, \( \mu_a \), of whole blood in this wavelength range can be 0.1-30 mm\(^{-1}\). The use of diffusion theory, requiring \( \mu_s' \gg \mu_a \), 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.

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

In this equation, the following parameters are defined as:

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

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 \( r \), as captured by the collection
fiber, one has to integrate reflectances \( R(\mu^{'}, \mu_{a}) \) and \( R(\mu^{'}, 0) \) over the collection spot size with radius \( r_{c}^{'} \):

\[
\frac{R(\mu^{'}, \mu_{a})}{R(\mu^{'}, 0)} = \frac{\int_{0}^{\rho_{c}} d\rho \int_{0}^{r_{c}'} dr \cdot R(\mu^{'}, \mu_{a}, |\rho - r|) \cdot 2\pi r}{\int_{0}^{\rho_{c}} d\rho \int_{0}^{r_{c}'} dr \cdot R(\mu^{'}, 0, |\rho - r|) \cdot 2\pi r}
\]  \hspace{1cm} (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:

\[
\frac{R(\mu^{'}, \mu_{a})}{R(\mu^{'}, 0)} = \frac{\mu^{'}}{\mu^{'^2} + \mu_{a}} \left\{ e^{-\mu_{a} r_{1}} + e^{-\left(\frac{4A}{3}\right) r_{1}} - z_{0} \cdot e^{-\mu_{a} r_{2}} - \left(1 + \frac{4A}{3}\right) \cdot z_{0} \cdot \frac{e^{-\mu_{a} r_{2}}}{r_{2}} \right\} \to 2 - \frac{1}{\mu^{'}} \cdot \left\{ \frac{1}{r_{1}^{'} + \frac{1 + 4A/3}{r_{2}^{'}}} \right\}
\]  \hspace{1cm} (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 \( \mu_{a} = 0 \). \( r_{c}^{'} \) 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 \( r_{c}^{'} = 3.1 \) mm.

Equations (2) and (3) will be compared with our experimental phantom measurements. Equation 2 from numerical integration of the \( R(\mu^{'}, \mu_{a}) \) and \( R(\mu^{'}, 0) \). 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 \( r_{c}^{'} \), 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-Marquardt fitting algorithm [139] with error margins represented by the 95% confidence intervals. Table 1 summarizes the various parameters for the three situations.
First we have explored the limits of $R(\mu'_a, \mu_s)$ determined from a fit of all phantom experiments. Figures 1 and 2 both show the substantial deviations from the physical parameters $A = 3.1$ and $r_c = 2.7$ mm, as well as for $A = 2.0 \pm 0.4$ and $r_c = 6.7 \pm 0.3$ mm. Also, following Zonios’ approach, from Eq (3) for both physical reflectance, as shown in Eq (2) collected by the collection fiber with radius $r = 2.7$ mm has been determined. Agreement between experimental reflectance measurement and diffusion model, determined from integration, $r^2=0.918$, Zonios’ approach with phantom measurements. Agreement between experimental reflectance measurement $R(\mu'_a, \mu_s)$ and Zonios’ Eq. (3) with fitted parameters shows good correspondence with the experimental results, especially for the low reduced scattering coefficients. However, 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 $R(\mu'_a, \mu_s)$ 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, $r^2=0.994$.

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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Physical value</th>
<th>Fitted Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exact Diffuse (Eq.2)</td>
<td>$\rho$</td>
<td>probe position</td>
<td>from 0 to $r_s$</td>
</tr>
<tr>
<td></td>
<td>$r_s$</td>
<td>Illumination spot radius</td>
<td>3.1 mm</td>
</tr>
<tr>
<td></td>
<td>$r_c$</td>
<td>Collection spot radius</td>
<td>2.7 mm</td>
</tr>
<tr>
<td></td>
<td>$A$</td>
<td>Refractive index parameter</td>
<td>3.1</td>
</tr>
<tr>
<td>Zonios’ Model (Eq. 3)</td>
<td>$r_c$</td>
<td>Effective collection radius</td>
<td>2.7 mm</td>
</tr>
<tr>
<td></td>
<td>$A$</td>
<td>Refractive index parameter</td>
<td>3</td>
</tr>
</tbody>
</table>

**RESULTS**

First we have explored the limits of $R(\mu'_a, \mu_s)/R(\mu'_a,0)$ for four limits of $\mu'_a$ and $\mu_s \rightarrow 0$ and $\infty$.

$$\lim_{\mu_s \rightarrow 0} \frac{R(\mu'_a, \mu_s)}{R(\mu'_a,0)} = 1 \quad \lim_{\mu'_a \rightarrow \infty} \frac{R(\mu'_a, \mu_s)}{R(\mu'_a,0)} = 0 \quad (4)$$

$$\lim_{\mu'_a \rightarrow 0} \frac{R(\mu'_a, \mu_s)}{R(\mu'_a,0)} = \infty \quad \lim_{\mu'_a \rightarrow \infty} \frac{R(\mu'_a, \mu_s)}{R(\mu'_a,0)} = 1 \quad (5)$$

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 $A = 2.0 \pm 0.4$ and $r_c = 6.7 \pm 0.3$ 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 $R(\mu'_a, \mu_s)$ 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, $r^2=0.994$. 
diffusion theory, albeit with additional approximations, as given by Eq. (3) of Zonios et al. We have investigated whether the reflectance spectroscopy model derived from diffusion theory 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 \( \mu_s' \).

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 \( \mu_s' \) varies over two orders of magnitude. We found a remarkable result for the limit of the reduced scattering going to zero; the reflectance ratio goes to infinity.

\[
\lim_{\mu_s' \to 0} R(\mu_s',\mu_a) = \infty
\]

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 \( A = 3.1, r_c = 2.7 \text{ mm} \); the blue lines with fitted parameters \( A = 2, r_c = 6.7 \text{ mm} \). (B) Same figure but the reflectance ratio on a logarithmic scale.

Figure 2. (A) Reflectance Ratio as a function of the reduced scattering coefficient, \( \mu_s' \), for high absorption (\( \mu_a = 10 \text{ mm}^{-1} \)) and low absorption (\( \mu_a = 1 \text{ mm}^{-1} \)). Green line represents Eq (2). The red lines represent Eq. (4) with physical parameters \( A = 3, r_c = 2.7 \text{ mm} \); the blue lines with fitted parameters \( A = 2, r_c = 6.7 \text{ mm} \). (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 \( \mu_s' \).

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 $\mu_a = 0$ and $\mu_s$ 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 $\mu_a$ = 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 $\mu_s$, 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 $\mu'_s,\mu_a$ 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 $\mu'_s$ or $\mu_a$ 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 $\mu'_s$ and $\mu_a$, 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.