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Oxygen Saturation-Dependent Absorption and Scattering of Blood

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We report on the scattering properties of oxygenated and deoxygenated whole blood from 250 to 1000 nm. We determine the complex refractive index of oxygenated and deoxygenated hemoglobin using a Kramers-Kronig analysis and optical coherence tomography measurements. Combining these data with Mie theory, the scattering properties are calculated. The strong oxygen saturation dependent scattering effects should be taken into account in the data analysis of optical oxymetry.

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Hemoglobin (Hb), responsible for the transport of oxygen from the lungs to tissues, is primarily contained inside red blood cells (RBCs) and can reversibly bind up to four oxygen molecules to form oxygenated hemoglobin (HbO2). The oxygen saturation (SO2) is the statistical average of all oxygen bound to hemoglobin, relative to the total amount that can be bound. SO2 dependent absorption of light by blood has been studied extensively [1–4] because optical methods allow for noninvasive and continuous monitoring of saturation. Studies have focused on low-coherence interferometry, e.g., optical coherence tomography measurements. Combining these data with Mie theory, the scattering properties are calculated. The strong oxygen saturation dependent SO2 dependent absorption spectra of hemoglobin, the refractive index of hemoglobin is also expected to be SO2 dependent and, consequently, so is scattering by RBCs. However, SO2 dependent whole blood scattering data are currently not available.

In this Letter, we present a novel method for retrieving the scattering properties of whole blood. From accurately known absorption spectra of Hb and HbO2 we derive their complex refractive index by the Kramers-Kronig (KK) relations in the wavelength range 250–1000 nm similar to the approach taken by Shumilina [7]. The obtained spectra are calibrated using OCT at 800 nm and are subsequently used to calculate the scattering properties of oxygenated and deoxygenated whole blood with Mie theory.

Knowledge of the complex refractive index \( n(\omega) = n(\omega) + i\kappa(\omega) \) of Hb and HbO2 is required to calculate the optical properties of oxygenated and deoxygenated blood. Here, \( \kappa(\omega) \) relates to absorption coefficient \( \mu_a \) of Hb or HbO2 through

\[
\kappa(\omega) = \frac{c\mu_a(\omega)}{2\omega},
\]

where \( c \) is the speed of light in vacuum and \( \omega \) the angular frequency of the light. As for any causal system, \( n(\omega) \) and \( \kappa(\omega) \) are related through KK relations. For convenience we use a subtractive KK analysis [8], defined by

\[
n(\omega) = n(\omega_0) + \frac{2}{\pi} (\omega^2 - \omega_0^2) \int_0^\infty \frac{\omega'\kappa(\omega')}{(\omega^2 - \omega_0^2)(\omega^2 - \omega')^2} d\omega',
\]

where \( n(\omega_0) \) is the refractive index measured at reference frequency \( \omega_0 \) to provide scaling of the calculated curves. In addition, this integral offers greater convergence than the conventional expression when data are available only
vital to keep the hemoglobin samples in physiological

To obtain reliable refractive index measurements it is
to keep the hemoglobin samples in physiological

Thus, knowledge of the absorption

group refractive increment and the extrapolated group

axial resolution is

provide continuous matching of the position of the focus

in the reference arm. In-depth scanning is performed by

125 nm FWHM bandwidth. Dispersion in both interfer-

correlation

can be expressed as the convolution of the source auto-

sii

the group refractive index of the hemoglobin solution and

is obtained from Eq. (2) by differentiation to

Eq. (2). The second right-hand side term of Eq. (4) can be

where

is the refractive index at

as it appears in

Eq. (2). The second right-hand side term of Eq. (4) can be

obtained from Eq. (2) by differentiation to \( \omega \); hence

measurement of \( n_{gr}(\omega) \) suffices to determine \( n(\omega) \). The

accuracy of this setup was tested using glucose-water

10 g/L. Both the determined

index of refraction of water were in excellent agreement

with literature values [11].

Fresh porcine blood is anticoagulated before use. Non-

nonscattering hemoglobin solutions are obtained by

cell lysis and filtration as described before [6], resulting

in a reduction of total hemoglobin concentration to 33%,

which is measured to be 93 g/l using a Radiometer OSM3

blood gas analyzer. The solutions are maintained at 37 °C

and oxygen saturated at 0% and 100% using a Minimax

plus hollow fiber clinical oxygenator (Medtronic). Before

applying the subtractive KK analysis, the measured group

indices of refraction are scaled to physiological hemoglo-

concentration using

\[
    n_{gr}(HbX) = n_{gr}(H_2O) + \Delta n_{gr,HbX}[HbX].
\]

where \( n_{gr}(H_2O) \) is the group refractive index of water

[1.3409 at 800 nm, from Ref. [12] and using Eq. (4)],

\( \Delta n_{gr,HbX} \) the refractive increment and

\([HbX]\) the hemoglobin species’ concentration. The measured

indices of refraction of HbO2 and Hb solutions at 800 nm are

1.392 ± 0.001 and 1.388 ± 0.002, respectively, average

±SD of 1000 measurements. These values are in very

close agreement with values reported in the literature,

recalculated at 800 nm when appropriate [13–17]. In

Ref. [7], using a similar KK analysis, but without experi-

tmental scaling, and in Ref. [18] higher values are re-

ported. Deviations are most likely due to differences in

hemoglobin concentration of the used samples and the

fact that these experiments were performed on intact

RBCs rather than hemoglobin solutions. Since these

values are used as reference points for scaling the real

refractive index [obtained by Eq. (2)], we verified that

the means of the two data sets are significantly different

using an unpaired t test \( (p < 0.0001) \) [19]. The imaginary

part of the \( n(\omega) \) is calculated using Eq. (1) with \( \mu_g \)

obtained from Ref. [4]. The KK analysis is performed

by numerical evaluation of Eq. (2) over the frequency

range \( 1.88 \times 10^{15} \) to \( 7.53 \times 10^{15} \) rad/s (250 to 1000 nm

wavelength range). The resulting real part of the \( n(\omega) \) is

shown in Fig. 1.

To calculate the scattering properties of whole blood,

the RBC is assumed to be a homogeneous sphere as in

most theoretical analyses on the interaction of light with

blood. Scattering is then described by Mie theory, which

has been shown to describe single scattering of light by

RBCs well [20]. The basic results are the extinction and

scattering cross sections (\( \sigma_e \) and \( \sigma_s \)) and the scattering

anisotropy factor \( g \) (their formulas can be found, e.g., in

Ref. [21]) which are complex functions of the size

parameter \( x \) and relative refractive index \( m \), given by

\[
    x = \frac{2\pi an_m}{\lambda} \quad \text{and} \quad m = \frac{n_s}{n_m},
\]

where \( n_s \) and \( n_m \) are the complex refractive index of

the sphere and the medium, respectively. \( a \) is the sphere

radius, and \( \lambda \) is the illuminating wavelength in vacuum.
The cross sections \( \sigma_{e,s} \) can be scaled to macroscopic coefficients using [21]

\[
\mu_{e,s} = \frac{H}{V} \sigma_{e,s}.
\]

Here, \( H \) is the particle volume fraction (for blood called hematocrit), and \( V \) is the sphere volume. The \( \mu_e \) accounts for both scattering and absorption losses. The \( \mu_s \) is obtained by subtracting \( \mu_e \) from \( \mu_s \). For the Mie calculations the code by Zijp et al. [22] was modified to take complex arguments. In Eq. (6) we use \( n_m = 1.340 \) [23] as the refractive index of plasma at 800 nm, and \( a = 2.78 \mu m \), corresponding to a typical RBC volume of \( V = 90 \mu m^3 \). In Eq. (7) \( H = 0.5 \) is taken. Figure 2 shows the calculated scattering coefficient and Fig. 3 the scattering anisotropy vs wavelength of oxygenated and deoxygenated whole blood. In the wavelength range 400–500 nm, corresponding to the location of large absorption peaks in the Hb/HbO\(_2\) absorption spectra, large scattering changes are observed varying from 200 to 425 mm\(^{-1}\). For \( \lambda > 600 \) nm scattering by oxygenated blood is about 10% higher than scattering by deoxygenated blood; in this wavelength range \( \mu_s \) decreases with increasing wavelength. The \( g \) is slightly lower for oxygenated blood for \( \lambda > 600 \) nm. The effect of the uncertainty in the reference measurement on the scattering properties was assessed by repeating the Mie calculations with \( n(\omega) \) now scaled by \( n(\omega_0) \pm \text{SD} \). The deviation at 800 nm and at the clinically relevant wavelengths 405, 580, and 910 nm is depicted by error bars in Figs. 2 and 3. For the whole spectral range, deviations in \( \mu_s \) are less than 4% (8%) for oxygenated (deoxygenated) whole blood; deviations in \( g \) are less than 1%. Consistency of the method was tested by calculating \( \mu_a = \mu_e - \mu_s \). Good agreement with the absorption data of Ref. [4] (Fig. 4) is found. Some care should be taken with regard to the absolute values of the \( \mu_s \). The scaling with \( H \) assumes independent scattering. With \( H = 0.5 \) this assumption is not obvious. Alternative scaling factors have been proposed in literature [24]. These, however, do not change the shape of the presented curves, only their amplitude. For low \( H \), our calculated data show good agreement with the experimental optical data of oxygenated diluted blood by Roggan et al. measured using a double integrating sphere technique [25]. However, contrary to our results, variations of scattering coefficient and anisotropy with SO\(_2\) did not reach significance. Measurements of \( \mu_s \) of oxygenated whole blood using the same technique by
Yaroslavsky et al. [26] showed qualitative agreement with our data in the wavelength range 700–1000 nm. However, an increase in $g$ with wavelength was found. The highly forward scattering of blood, with $g$ reported between 0.985 and 0.997 [23,23–27], makes it difficult to separate scattered from nonscattered light, e.g., required in integrating sphere measurements, implying that the optical properties of whole blood are difficult to determine experimentally. Moreover, aggregation and biochemical responses, such as clotting, make it especially challenging to perform measurements of ex vivo whole blood samples under controlled physiological conditions. Extraction of optical properties from such measurements is complex, and usually requires an inverse Monte Carlo technique [25,26]. Consequently, a wide variety of experimental values have been reported [16,23,25–27]. The approach outlined in this Letter avoids the necessity of measurements on whole blood and using complex reconstruction algorithms. Rather, we determine the refractive index of nonscattering hemoglobin solutions using the Kramers-Kronig relations to obtain the complex refractive index as input for Mie theory. This methodology is particularly advantageous for retrieving scattering properties of oxygenated and deoxygenated whole blood. The choice of Mie theory to perform the latter calculations is not essential. Other, possibly more accurate scattering theories (e.g., Ref. [17,23]) also take the complex refractive index as input, and it is its $\text{SO}_2$ dependence that determines the $\text{SO}_2$ dependent scattering properties. Our method therefore provides a template for accurate evaluation of the blood scattering properties.

To summarize, in this Letter we present experimental values for the refractive index of oxygenated and deoxygenated hemoglobin at 800 nm. The former is significantly larger than the latter, which has not been identified before. The scattering properties of oxygenated and deoxygenated whole blood are determined by a physically straightforward novel method based on accurately known hemoglobin absorption spectra and Kramers-Kronig relations to obtain the complex refractive index as input for Mie theory. This methodology is particularly advantageous for retrieving scattering properties of whole blood since it does not require keeping whole blood under controlled physiological conditions, uses nonscattering hemoglobin solutions instead of highly (forward) scattering blood samples, and does not require complex inverse strategies. It is shown that not only the absorption coefficient but also the scattering properties strongly depend on $\text{SO}_2$. Therefore, to enable quantitative (regional) tissue $\text{SO}_2$ measurements, it is vital to include both in data analysis.

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[11] Measured $n_{gr} = 1.343 \pm 0.001$; $n_{ge} = 1.3409$ [12] and using Eq. (4). Measured $\Delta n_{gr} = (1.8 \pm 0.2) \times 10^4 / \text{g}$; $\Delta n_{ge} = (1.67 \pm 0.01) \times 10^4 / \text{g}$ at 850 nm; J.S. Maier et al., Opt. Lett. 19, 2062 (1994); G.S. Nikolic, M.D. Cakic, and L.A. Ilic, J. Serb Chem. Soc. 66, 397 (2001).