Toward assessment of blood oxygen saturation by spectroscopic optical coherence tomography
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Spectroscopic optical coherence tomography (SOCT) can be used to assess localized absorption spectra of native or exogenous chromophores in biological tissue. These spectra can be used for contrast enhancement and can provide information on the composition and function of normal or pathological tissue. The use of SOCT for this purpose has been the subject of a number of recent studies, all of which were limited to nonscattering or weakly scattering media, thereby underestimating the strong effect of scattering on the SOCT signal. In this Letter we present in vitro SOCT experiments with strongly scattering whole-blood samples and correlate the results with the hemoglobin (Hb) oxygen saturation (SO₂) of the blood, where SO₂ is defined as the percentage of HbO₂ concentration of the total Hb concentration; we recently showed that SOCT is capable of retrieving the absorption spectra of oxygenated (HbO₂) and deoxygenated Hb solutions. Optical methods to determine SO₂ rely on differences in absorption between HbO₂ and Hb [Ref. 9 and Fig. 1(A)]. For wavelengths above the isobestic wavelength of 800 nm, absorption cross section increases with increasing SO₂. The effect is opposite for wavelengths of 600–800 nm. The absorption coefficient of blood is then given by \( \mu_a = H \sigma_a / V \), where \( H \) is the volume fraction of red-blood cells (RBCs) in blood (hematocrit) and \( V \) is the RBC volume (\( \sim 90 \) \( \mu m^3 \)). The causality principle implies that scattering properties are also SO₂ dependent. We recently determined the full complex refractive-index spectra of HbO₂/Hb by using a subtractive Kramers–Kronig analysis. We then estimated SO₂-dependent scattering cross section \( \sigma_s \) [Fig. 1(B)] and anisotropy factor \( g \), using Mie theory. SO₂-dependent scattering effects have been suggested but have thus far not been shown experimentally. Because of the large hematocrit in whole blood (\( \sim 40\% \) for our samples), scattering coefficient \( \mu_s \) does not scale linearly with hematocrit. This effect is usually addressed by introduction of

![Fig. 1. (A) Absorption cross-section spectra of oxygenated and deoxygenated Hb calculated from their molar extinction coefficients, by use of a concentration value of 150 g/L. (B) Computed scattering cross section of oxygenated and deoxygenated RBCs. The uncertainty in the data is \( \sigma < 8\% \).](image-url)
packing factors \( W(H) \) such that 
\[
\mu_s = W(H) \mu_H/V.
\]

The expression derived by Twersky,\(^{13}\) \( W_T = 1 - H \), is often used (Ref. 14, and references therein); packing factors for hard spheres, \( W_S = (1 - H)^4/(1 + 2H)^2 \), and for cylinders, \( W_C = (1 - H)^3/(1 + H) \), are utilized for ultrasound measurements of whole blood. For \( H = 40\% \) these models lead to \( \mu_s = 129, 9, 33 \) mm\(^{-1} \), respectively, at 800 nm. Unfortunately, few experimental data are available. Because the \( g \) of blood is high, it is difficult to separate scattered from nonscattered light. Consequently, even in an optical coherence spectroscopy (OCT) measurement, multiple forward-scattered light will be detected. Because the scattering properties show the same functional wavelength dependence for oxygenated and deoxygenated RBCs in the wavelength region 600–1000 nm, SO\(_2\)-dependent attenuation \( (\mu_t) \) spectra (including contributions from scattering and absorption) may still be obtained with SOCT.

First experiment. To determine the capability of OCT to assess oxygen saturation of whole-blood samples we measured the attenuation spectra of oxygenated and deoxygenated whole blood. First, fresh porcine blood was anticoagulated with heparin and saturated at 0% and 100% by a clinically used MiniMax Plus hollow fiber oxygenator by application of gas mixtures to the blood. OCT data were taken from the blood in a 250-μm-thick flow-through cuvette. At the time of measurement, the flow was stopped to prevent Doppler shifting of the spectrum, which would lead to incorrect mapping of the spectrum to the wavelength axis. After each measurement we drew a blood sample to determine SO\(_2\) in a Radiometer OSM3 blood gas analyzer. Our OCT setup is described in detail in Refs. 8 and 11. The light source was a Ti:sapphire laser (Femtolasers; \( \lambda_0 = 800 \) nm, \( \Delta \lambda = 125 \) nm). To provide accurate dynamic focusing, we mounted the scanning mirror and the focusing lens upon independently driven voice coil translators (150-nm accuracy measured with a He–Ne-based interferometer; 1 A scan/s). The photodetector current was bandpass filtered and demodulated by a lock-in amplifier; amplitude and phase (8192 points/A scan) were stored. From each A scan, data corresponding to the top and bottom cuvette boundaries were processed with a short-time Fourier-transform algorithm, yielding spectra with a 15-nm spectrogram bin size. From these spectra the \( \mu_t \) spectrum was calculated from Beer’s law. To decrease noise caused by speckle, 200 \( \mu_t \) spectra were averaged (Fig. 2). The spectra are consistent with the data shown in Fig. 1: For both oxygenated and deoxygenated blood, \( \mu_t \) decreases with wavelength, similar to the spectrum of the scattering cross section \( \sigma_s \). For oxygenated blood, the decrease of \( \mu_t \) with wavelength as a result of scattering is partly compensated for by the increase in \( \mu_t \).

Second experiment. We propose to assess SO\(_2\) by measuring the saturation-dependent differential attenuation coefficient \( \Delta \mu_t (\lambda_1, \lambda_2, \text{SO}_2) = \mu_t (\lambda_1, \text{SO}_2) - \mu_t (\lambda_2, \text{SO}_2) \) at a wavelength pair within the source bandwidth, similar to the analysis presented in Ref. 1. In a new set of experiments we saturated porcine whole-blood samples from 0% to 100% by varying the composition of \((\text{N}_2, \text{CO}_2, \text{O}_2)\) gasses applied to the blood. Because it is vital to maintain the blood samples in specific physiological conditions during the measurements it is desirable to reduce measurement time. Therefore depth scanning was performed with a rapid-scanning optical delay line operating at 25 Hz. The scan speed was limited, because a constant scan velocity is required. Using a rapid-scanning optical delay reduced the available bandwidth to 95 nm at the expense of lower wavelengths in the source spectrum (owing to alignment) and did not permit dynamic focusing. However, it is expected that clinical OCT systems will also not facilitate dynamic focusing. The focus in the sample arm was fixed at the lower cuvette boundary. As above, \( \mu_t \) spectra were calculated from the upper and lower cuvette boundaries. Figure 3(A) shows \( \Delta \mu_t \) for (780–820 nm versus SO\(_2\). A clear correlation between \( \Delta \mu_t \) and SO\(_2\) can be seen (\( p = 0.024 \)). The values of \( \Delta \mu_t \) found in this second set of experiments are slightly larger than those found in the first set (Fig. 2), possibly because of differences in hematocrit between the two blood volumes. Note that the actual influence of a change in \( H \) on \( \Delta \mu_t \) depends on packing factor \( W(H) \).

![Fig. 2. Attenuation spectra of oxygenated and deoxygenated whole blood (first experiment). The lines were drawn as a guide to the eye.](image)

![Fig. 3. \( \Delta \mu_t \) at 780 and 820 nm determined from the cuvette boundaries [second experiment (A)] and from the blood layer itself [third experiment (B)]. Errors in \( \Delta \mu_t \) are \(<0.25 \) mm\(^{-1} \). Solid lines, fits of the measured data with their correlation coefficients and statistical significance. Dashed curves, 95% confidence bounds of the linear fit.](image)
In tissues, clear boundaries (and the corresponding strong reflections from which the spectra can be determined) may not be available. Therefore we processed the data by using a different approach. The result of time–frequency-domain analysis of the OCT signal can be written as $I(z, \lambda) = S_d(\lambda)R(z, \lambda)$, where $S_d(\lambda)$ is the source spectrum and $R(z, \lambda)$ is the spectral sample reflectivity. The magnitude of $R(z, \lambda)$ represents the attenuation of the sample at $\lambda$ as a function of depth, and for a homogeneous medium it can be expressed as $|R(z, \lambda)| = r(\lambda, z) \exp[-\mu_s(\lambda)z]/G$, where $r(\lambda, z)$ is the sample reflectivity at depth $z$ and $G$ is a geometry function including confocal parameters. We then calculate $\mu_s$ at a certain wavelength $\lambda$, by fitting this model to measurements of $|R(z, \lambda)|$, using a nonlinear least-squares fitting algorithm. Because the fitted section of the $A$ scans is much smaller than the depth of focus ($\approx 200$ compared with $=750 \mu m$), geometry function $G$ is set constant in the fitting. This method of analysis is comparable to that described in recent publications in which the OCT signal slope was correlated to glucose concentration. The result of this procedure for the wavelength pair $780–820$ nm is shown in Fig. 3(B). We performed the same analysis for other wavelength pairs; these showed comparable results. Good correspondence with the data from Fig. 3(A) was found. The lower significance results have yet to be established.

In conclusion, we have determined differential attenuation coefficients of whole-blood samples as a function of $SO_2$. Our results show a decrease of differential attenuation with saturation, which corresponds quantitatively to predictions based on the known differential absorption coefficient and recent determinations of the $SO_2$-dependent scattering properties of blood. We consider these results to be an important step toward obtaining spatially resolved quantitative oxygenation measurements in tissue. The need for exact knowledge of the scattering properties of blood to facilitate quantitative SOCT is evident.

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