UvA-DARE (Digital Academic Repository)

Toward assessment of blood oxygen saturation by spectroscopic optical coherence tomography

Faber, D.J.; Mik, E.G.; Aalders, M.C.G.; van Leeuwen, T.G.

Link to publication

Download date:20 Jan 2025

Toward assessment of blood oxygen saturation by spectroscopic optical coherence tomography

Dirk J. Faber

Laser Center, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands

Egbert G. Mik

Department of Physiology, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands

Maurice C. G. Aalders

Laser Center, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands

Ton G. van Leeuwen

Laser Center, Academic Medical Center, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, and Biophysical Engineering, Biomedical Technology Institute, University of Twente, PO Box 217, 7500 AE, The Netherlands

The use of spectroscopic optical coherence tomography to assess hemoglobin oxygen saturation of whole blood is investigated. We propose to use the differential attenuation coefficient to determine the degree of saturation. Our data show qualitative agreement between the measured differential attenuation coefficients as a function of saturation and predictions based on the oxygen-saturation-dependent absorption and scattering properties of blood. © 2005 Optical Society of America

OCIS codes: 170.4500, 170.1470.

Spectroscopic optical coherence tomography[1–3] (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[4,5] 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,[4–8] 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 (SO_2) of the blood, where SO_2 is defined as the percentage of HbO_2 concentration of the total Hb concentration; we recently showed that SOCT is capable of retrieving the absorption spectra of oxygenated (HbO_2) and deoxygenated Hb solutions.[8] Optical methods to determine SO_2 rely on differences in absorption between HbO_2 and Hb [Ref. 9 and Fig. 1(A)]. For wavelengths above the isobestic wavelength of 800 nm, absorption cross section σ_a increases with increasing SO_2. 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 ($\sim90~\mu m^3$). The causality principle implies that scattering properties are also SO_2 dependent. We recently determined the full complex refractive-index spectra of HbO_2/Hb by us-

ing a subtractive Kramers–Kronig analysis. We then estimated SO_2-dependent scattering cross section σ_s [Fig. 1(B)] and anisotropy factor g, using Mie theory.[11] SO_2-dependent scattering effects have been suggested[10] but have thus far not been shown experimentally.[12] Because of the large hematocrit in whole blood ($\sim40\%$ for our samples), scattering coefficient μ_s does not scale linearly with hematocrit. This effect is usually addressed by introduction of
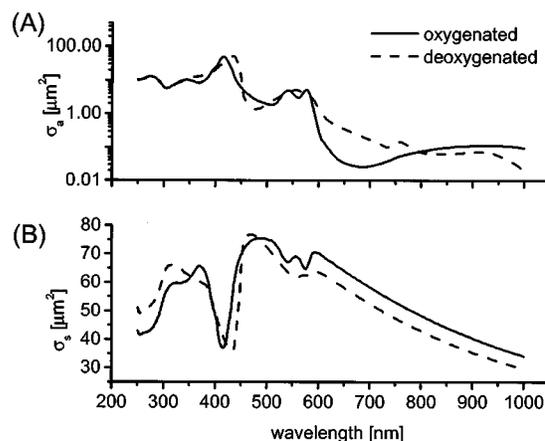

Fig. 1. (A) Absorption cross-section spectra of oxygenated and deoxygenated Hb calculated from their molar extinction coefficients, by use of a concentration value of 150 g/L.[9] (B) Computed scattering cross section of oxygenated and deoxygenated RBCs. The uncertainty in the data is $\sigma_s < 8\%$.[10]
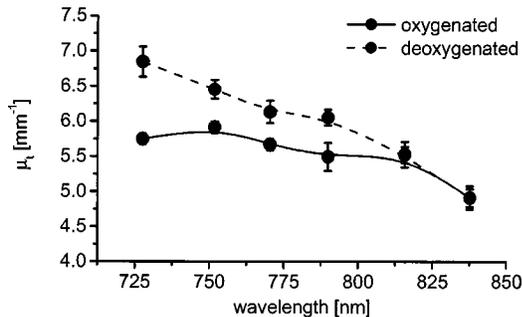
© 2005 Optical Society of America

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

packing factors $W(H)$ such that $\mu_s = W(H)H\sigma_s/V$. 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 spectrocopy (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 (μ_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 (N_2, CO_2) and (N_2, CO_2, O_2) 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 μ_t spectrum was calculated from Beer's law. To decrease noise caused by speckle, 200 μ_t spectra were averaged (Fig. 2). The spectra are consistent with the data shown in Fig. 1: For both oxygenated and deoxygenated blood, μ_t decreases with wavelength, similar to the spectrum of the scattering cross section σ_s. For oxygenated blood, the decrease of μ_t with wavelength as a result of scattering is partly compensated for by the increase in μ_a.

Second experiment. We propose to assess SO_2 by measuring the saturation-dependent differential attenuation coefficient $\Delta\mu_t(\lambda_1, \lambda_2, SO_2) = \mu_t(\lambda_1, SO_2) - \mu_t(\lambda_2, SO_2)$ 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 (N_2, CO_2, 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, μ_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 $W(H)$.
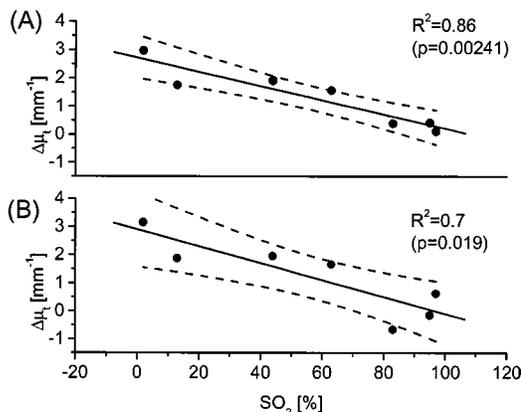

Fig. 3. $\Delta\mu_t$ at 780 and 820 nm determined from the cuvette boundaries [second experiment (A)] and from the blood layer itself [third experiment (B)]. Errors in $\Delta\mu_t$ are <0.25 mm^{-1}. Solid lines, fits of the measured data with their correlation coefficients and statistical significance. Dashed curves, 95% confidence bounds of the linear fit.

Third experiment. 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 $I(z,\lambda) = S_{ii}(\lambda)R(z,\lambda)$, where $S_{ii}(\lambda)$ is the source spectrum and $R(z,\lambda)$ is the spectral sample reflectivity.[8] The magnitude of $R(z,\lambda)$ represents the attenuation of the sample at λ as a function of depth, and for a homogeneous medium it can be expressed as $|R(z,\lambda)| = r(\lambda,z)\exp[-\mu_t(\lambda)z]/G$, where $r(\lambda,z)$ is the sample reflectivity at depth z and G is a geometry function including confocal parameters. We then calculate μ_t at a certain wavelength λ_x by fitting this model to measurements of $|R(z,\lambda_x)|$, using a nonlinear least-squares fitting algorithm. Because the fitted section of the A scans is much smaller than the depth of focus (≈ 200 compared with $\approx 750 \ \mu$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.[15] 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 ($p = 0.019$) is due to a decreased signal-to-noise ratio compared with that of data from the cuvette boundaries. The exponential decay that we used is likely unsuitable for describing multiple scattering in a dense medium, especially given the highly forward-directed scattering by blood[11]; however, from a practical point of view it could well be fitted to the data. We attempted to fit more-comprehensive, multiple scattering models (e.g., from Ref. 16) to our data. These fits did not converge, most likely owing to their dependence on $1-g$: a very small change in g heavily influences the fit. A robust description of the SOCT signal is required for quantitative SO_2 measurements, with multiple dependent scattering (of blood) and the confocal properties of the OCT system taken into account. For *in vivo* measurements in large vessels, flow-induced Doppler shifts need to be dealt with, although this may not be necessary when spectra can be obtained from, e.g., vessel walls. In the microcirculation it is likely that conditions that correspond to more-diluted blood samples will occur, and analyses such as those in Refs. 6, 7, and 17 may be applied. The minimum thickness of the probe volume needed for accurate results has 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.

References

1. J. M. Schmitt, S. H. Xiang, and K. M. Yung, J. Opt. Soc. Am. A **15**, 2288 (1998).
2. U. Morgner, W. Drexler, F. X. Kärtner, X. D. Li, C. Pitris, E. P. Ippen, and J. G. Fujimoto, Opt. Lett. **25**, 111 (2000).
3. R. Leitgeb, M. Wojtkowski, A. Kowalczyk, C. K. Hitzenberger, M. Sticker, and A. F. Fercher, Opt. Lett. **25**, 820 (2000).
4. C. Xu, J. Ye, D. L. Marks, and S. A. Boppart, Opt. Lett. **29**, 1647 (2004).
5. C. Yang, M. A. Choma, L. E. Lamb, J. D. Simon, and J. A. Izatt, Opt. Lett. **29**, 1396 (2004)
6. C. Xu, D. L. Marks, M. N. Do, and S. A. Boppart, Opt. Express **12**, 4790 (2004), http://www.opticsexpress.org.
7. B. Hermann, K. Bizheva, A. Unterhuber, B. Považay, H. Sattmann, L. Schmetterer, A. F. Fercher, and W. Drexler, Opt. Express **12**, 1677 (2004), http://www.opticsexpress.org.
8. D. J. Faber, E. G. Mik, M. C. G. Aalders, T. G. van Leeuwen, Opt. Lett. **28**, 1436 (2003).
9. Data tabulated from various sources compiled by S. Prahl, http://omlc.ogi.edu/spectra.
10. A. G. Borovoi, E. I. Naats, and U. G. Oppel, J. Biomed. Opt. **3**, 364 (1998).
11. D. J. Faber, M. C. G. Aalders, E. G. Mik, B. A. Hooper, M. J. C. van Gemert, and T. G. van Leeuwen, Phys. Rev. Lett. **93**, 028102 (2004).
12. A. Roggan, M. Friebel, K. Dörschel, A. Hahn, and G. Müller, J. Biomed. Opt. **4**, 36 (1999).
13. V. Twerksy, J. Opt. Soc. Am. **60**, 1084 (1970).
14. V. V. Tuchin, ed., *Handbook of Biomedical Optics* (SPIE Press, Bellingham, Wash., 2002).
15. R. O. Esenaliev, K. V. Larin, I. V. Larina, and M. Motamedi, Opt. Lett. **26**, 992 (2001).
16. J. M. Schmitt, and A. Knüttel, J. Opt. Soc. Am. A **14**, 1231 (1997).
17. D. J. Faber, F. J. van der Meer, M. C. G. Aalders, and T. G. van Leeuwen, Opt. Express **12**, 4353 (2004), http://www.opticsexpress.org.