Functional optical coherence tomography: spatially resolved measurements of optical properties
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CHAPTER 7

Towards assessment of blood oxygen saturation by spectroscopic OCT

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 saturation. Our data show qualitative agreement between the measured differential attenuation coefficients as function of saturation, and predictions based on the oxygen saturation dependent absorption and scattering properties of blood.

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7.1 introduction

Spectroscopic Optical Coherence Tomography (SOCT) [1-3] can be used to assess localized absorption spectra of native or foreign chromophores in biological tissue. These spectra can be used for contrast enhancement [4,5], but can also provide valuable 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] which however were all limited to non- or weakly scattering media, thereby underestimating the strong effect of scattering on the SOCT signal. In this chapter we present, for the first time to our knowledge, *in vitro* SOCT experiments on strongly scattering whole blood samples, and correlate the results with the hemoglobin oxygen saturation (SO2) of the blood.

Many areas of medicine (e.g. intensive care management [9]) would benefit from a technique that allows determination of spatially resolved tissue oxygenation. We have recently shown that SOCT is capable of retrieving the absorption spectra of oxygenated and de-oxygenated Hb solutions [8]. SO2 is defined as the percentage of oxygenated Hb (HbO2) concentration of the total Hb concentration. Optical methods to determine SO2 rely on the well known differences in absorption between Hb in its oxygenated and de-oxygenated form [10] (figure 8-1A). In short, for wavelengths above the isobestic wavelength of 800 nm, the absorption cross section $\sigma_a$ increases with increasing SO2 whereas the effect is opposite for wavelengths between 600 nm and 800 nm. The absorption coefficient of blood is then given by $\mu_a = Hct \cdot \sigma_a / V$ where $Hct$ is the volume fraction of RBC’s in blood (called hematocrit) and $V$ is volume of one RBC (approx. 90 $\mu$m$^3$). In whole blood, above 600 nm, scattering is much larger than absorption. We recently determined the complex refractive index spectra of Hb using refractive index measurements and Kramers Kronig analysis on the imaginary part of the complex refractive index to calculate the corresponding real part. We subsequently estimated the SO2-dependent scattering cross section $\sigma_s$ (figure 8-1B) and anisotropy factor $g$ using Mie theory [11].

![Absorption and scattering cross sections](image-url)

*figure 8-1. A: absorption cross-section spectra of oxygenated and de-oxygenated Hb calculated from their molar extinction coefficient, using a concentration value of 150 g/L [10]. B: computed scattering cross section of oxygenated and deoxygennated red blood cells. Uncertainty in data $\sigma_s < 8\%$. [11].*
Due to the large hematocrit in whole blood (approx. 40% for our samples), the individual scatterers' locations are correlated, i.e. the scattering coefficient $\mu_s$ does not scale linearly with hematocrit. This effect is usually addressed by introducing packing factors $W(Hct)$ such that $\mu_s = W(Hct) \mu_t \sigma_i/\nu$. Next to the well known Twersky formula $W_t = 1 - Hct$, packing factors for hard spheres, $W_h = (1 - Hct)^3/(1 + 2 Hct)^2$ and for cylinders, $W_c = (1 - Hct)^3/(1 + Hct)$ are utilized for ultrasound measurements of whole blood. For $Hct = 40\%$ these models lead to estimations of $\mu_s$ of $129 \text{ mm}^{-1}$, $9 \text{ mm}^{-1}$ and $33 \text{ mm}^{-1}$ respectively at $800 \text{ nm}$. Unfortunately, little experimental data are available; because the scattering anisotropy of blood is exceedingly high, it is difficult to separate scattered from non-scattered light. Consequently, even in an OCT measurement multiple forward scattered light will be detected. However, because the scattering properties show the same functional wavelength dependence for oxygenated and de-oxygenated RBC's in the wavelength region $600 - 1000 \text{ nm}$, $SO_2$ dependent attenuation ($\mu_t$-) spectra (including contributions from scattering and absorption) may still be obtained with SOCT. We propose to measure the oxygen 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 [1].

7.2 materials and methods

In this chapter, three in vitro experiments on whole blood samples contained in a cuvette are presented. In the first, we measured the attenuation spectra of oxygenated and de-oxygenated whole blood, using the strong signals from the boundaries of a cuvette, similar to chapter 4 [8]. In the second experiment, we expand on the first, and determine the $\Delta \mu_t$ using the strong signals from the boundaries of a cuvette. In the third experiment we calculate $\Delta \mu_t$ within a blood sample itself.

FIRST EXPERIMENT. – To determine the capability of OCT to assess oxygen saturation of whole blood samples, fresh porcine blood was anti-coagulated with heparin and saturated at 0% and 100% by a clinically used Minimax Plus hollow fiber oxygenator by applying (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 $\mu$m thick flow-through cuvette. At the time of measurement, the flow was stopped to avoid Doppler shifting of the spectrum. After each measurement a blood sample was drawn to determine $SO_2$ in a Radiometer OSM3 (Radiometer) blood gas analyzer. Our OCT setup is described in detail in [8,11]. The light source was a Ti:Sapphire laser (Femtolasers, $\lambda_0 = 800 \text{ nm}$, $\Delta \lambda = 125 \text{ nm}$). To provide accurate dynamic focusing, the scanning mirror and the focusing lens were mounted on independently driven voice coil translators (150 nm measured accuracy; 1 A-scan/s). The photo detector current was band-pass filtered and demodulated by a Lock-In amplifier. Amplitude and phase of the demodulated signal were digitized (8192 points per A-scan) and stored. From each A-scan, data corresponding to the top and bottom cuvette boundary were processed using an STFT algorithm, yielding spectra with 15 nm resolution. From these spectra the $\mu_t$-spectrum is calculated from Beer's law. To increase the SNR of our measurements, 200 attenuation spectra were averaged.

SECOND EXPERIMENT. – Porcine whole blood samples were saturated 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 keep the blood samples under physiological conditions during the measurements it is desirable to reduce measurement time. To accomplish this, depth scanning was performed using a rapid
scanning optical delay line operating at 25 Hz, which however reduced the available optical bandwidth to 95 nm at the expense of lower wavelengths in the source spectrum and did not allow dynamic focusing. For convenience, the focus in the sample arm was fixed at the lower cuvette boundary. Attenuation spectra were calculated as described above from the upper and lower cuvette boundaries. From the attenuation spectra, differential attenuation coefficients are determined at two different wavelengths.

THIRD EXPERIMENT. – In tissues, clear boundaries (and the corresponding strong reflections from which the spectra can be determined) may not be available. Therefore, we also processed the data using a different approach. The result of time-frequency domain analysis of the OCT signal can be written as $I(z,\lambda) = S_0(\lambda)H(z,\lambda)$ where $S_0(\lambda)$ is the source spectrum and $H(z,\lambda)$ the spectral sample reflectivity [8]. The magnitude of $H(z,\lambda)$ represents the attenuation of the sample at $\lambda$ as a function of depth, and for a homogeneous medium can be expressed as:

$$|H(z,\lambda)| = r(\lambda, z) \cdot \exp(-\mu_\lambda z)/G$$

(7-1)

where $r(\lambda, z)$ is the sample reflectivity at depth $z$ and $G$ is a geometry function including confocal parameters. The attenuation coefficient at a certain wavelength $\lambda_\alpha$ is then calculated by fitting equation 1 to measurements of $|H(z, \lambda_\alpha)|$. Because the fitted section of the A-scans is much smaller then the depth of focus ($\approx 200 \mu m$ vs. $\approx 750 \mu m$) the geometry function $G$ is set constant in the fitting. This method of analysis is comparable to recent publications in which the OCT signal slope was correlated to glucose concentration [12].

7.3 results and discussion

The result of the first experiment is shown in figure 8-2. The spectra are consistent with the data shown in figure 1: for both oxygenated and de-oxygenated 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 due to scattering is partly compensated by the increase in $\mu_a$.

![figure 8-2. Attenuation spectra of oxygenated and de-oxygenated whole blood (first experiment). The lines are drawn as guide to the eye.](image)

Based on these results, it seems feasible to correlate the differential attenuation coefficient to oxygen saturation. Figure 8-3A shows the results of the second experiment, the differential attenuation coefficient for (780–820) nm vs. $SO_2$. A clear correlation between $\Delta\mu_t$ and $SO_2$ is
found \((p=0.024)\). The values of \(\Delta \mu_t\) found in this second set of experiments are slightly larger than those found in the first set (figure 8-2), possibly due to differences in hematocrit between the two blood volumes. Note that the actual influence of a hematocrit change on \(\Delta \mu_t\) depends on the packing factor \(W(Hct)\).

\[\text{(A)} \quad \text{and } R^2=0.86 \quad (p=0.00241)\]

\[\text{(B)} \quad \text{and } R^2=0.7 \quad (p=0.019)\]

\(\Delta \mu_t \text{[mm}^{-1}]\)

\(\text{SO}_2 \text{[\%]}\)

**Figure 8-3.** Differential attenuation coefficients at wavelengths 780 nm and 820 nm determined from the cuvette boundaries (second experiment, A) and by direct exponential fitting of spectral magnitude vs depth according to eq. 1, i.e. from the blood layer itself (third experiment, B). Experimental errors in \(\Delta \mu_t < 0.25 \text{mm}^{-1}\). Solid lines: fit of the measured data with their correlation coefficient and statistical significance. Dashed lines: 95% confidence bounds of the fit.

The result of calculating the differential attenuation coefficient for the wavelength pair (780–820) nm using equation 1 (third experiment) is shown in figure 8-3B. We performed the same analysis for other wavelength pairs; these showed comparable results. Good correspondence with the data from figure 8-3A is found. The lower significance \((p=0.019)\) is due to decreased signal to noise ratio, compared to data from the cuvette boundaries. The exponential decay in equation 8-1 is likely unsuitable for multiple scattering in a dense medium. However, from a practical point of view, equation 1 could well be fitted to the data. To facilitate quantitative \(\text{SO}_2\) measurements by OCT, a robust theoretical description of the OCT signal is required, taking into account multiple, dependent scattering, and the confocal gating properties of the OCT system under these conditions. Also, better understanding of the scattering properties of very dense, highly forward scattering media such as whole blood is needed. For measurements in the microcirculation it is likely that conditions corresponding to more diluted blood samples occur, and analysis such as in [6,7,14] may be applied.

### 7.4 Conclusions

In conclusion, we have determined differential attenuation coefficients of whole blood samples as a function of hemoglobin oxygen saturation. 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 oxygen saturation dependent scattering properties of blood. We consider these results to be an important step toward spatially resolved quantitative oxygenation measurements in tissue. The presented results stress the need for exact knowledge of the scattering properties of the investigated tissue to enable quantitative SOCT.
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REFERENCES AND LINKS
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