Functional optical coherence tomography: spatially resolved measurements of optical properties
Faber, D.J.

Citation for published version (APA):
Faber, D. J. (2005). Functional optical coherence tomography: spatially resolved measurements of optical properties

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CHAPTER 5

Absorption spectra of (oxy-)hemoglobin assessed by spectroscopic optical coherence tomography

The combination of optical coherence tomography and spectroscopy may allow for highly localized, quantitative measurements of tissue spectral properties. In this chapter, quantitative measurements of the absorption coefficient of phantoms and of hemoglobin and oxygenated hemoglobin with spectroscopic optical coherence tomography (SOCT) are presented. The results suggest that SOCT will be able to provide localized, quantitative oxygenation measurements.

Part of this work has been published: Dirk J. Faber, Egbert G. Mik, Maurice C.G. Aalders, Ton G. van Leeuwen, Light absorption of (oxy-)hemoglobin assessed by optical coherence tomography, Optics Letters 28(16), 1436-1438 (2003).
5.1 introduction

Optical Coherence Tomography (OCT) has developed from a novel imaging technique into a versatile diagnostic modality. Currently, OCT is used for high-resolution, non- or minimally invasive morphological imaging of tissue. Functional information of the imaged tissue can also be deduced from the OCT signal, e.g. flow information from Color Doppler OCT [1] and birefringence from Polarization Sensitive OCT [2]. Since a broadband light source is required for obtaining a high axial resolution, spectroscopic OCT seems to be a natural extension [3-6].

SOCT may overcome two drawbacks of spectroscopic techniques currently used in the clinic (such as pulse oxymetry), both originating from lack of exact measurement or control of the optical path length: poor localization of measured spectra and, consequently, the need for calibration due to the underlying theoretical models. The spectra obtained with SOCT originate from a highly localized tissue volume because of the combination of coherence and confocal gating. Attenuation of light can therefore be assessed quantitatively inside the tissue using Beer’s law, because the optical path length of the detected light is exactly known. Moreover, the spectroscopic data are obtained in combination with morphological images and, if needed, other functional information which may help to discriminate between different tissues or tissue pathologies. However, future applications of SOCT will relate to the combination of shallow penetration depth (1 to 2 mm) and the confinement of the available spectral bandwidth to the light source spectrum. Lasers operating around 800 nm have melanin, hemoglobin (Hb) and oxygenated hemoglobin (HbO2) as the main absorbers in their spectral window. The latter two are of specific interest since SOCT may allow for spatially confined, quantitative oxygenation measurements which can be valuable as a research tool and in the clinic, for example intensive care medicine [7]. We examine the feasibility of using OCT with a commercially available Ti:Sapphire laser to quantitatively measure absorption coefficients of hemoglobin and oxygenated hemoglobin in solution. Figure 5-1 shows the absorption spectrum of Hb and HbO2, with known isobestic point around 800 nm at which the Hb and HbO2 absorption coefficient are equal [8].

![Absorption spectra of Hb and HbO2](image)

*figure 5-1: absorption spectra of hemoglobin [Hb] and oxygenated hemoglobin [HbO2] using tabulated values of the molar extinction coefficient (cm²/M) of hemoglobin and oxygenated hemoglobin [8] and a concentration value of 150 g/L and 66500 g/mole. The inset shows the absorption coefficient on a linear scale, along with the power spectrum of the Ti:Sapphire laser used in the experiments.*
5.2 theory

In OCT, the interference signal $i(\lambda)$ measured by a photodetector is proportional to the cross correlation of the fields returning from sample and reference arms of the interferometer. Here $z = \Delta l/2$ is the position in the sample corresponding to the total path length difference $\Delta l$ between both arms of the interferometer. The reference field is a shifted version of the source field; the sample field is a convolution of the source field with the backscatter profile $h(z)$ of the sample. Thus the interferometric cross correlation can be written as the convolution of the source autocorrelation $R_s(\lambda)$ and the backscatter profile $h(z)$. The convolution in the $\Delta l$-domain can be expressed as a multiplication in the $k$-domain:

$$I(k) = S_s(k)H(k)$$ (5-1)

where $S_s(k)$ is the source spectrum, $H(k)$ the spectral sample reflectivity and $k = 2\pi/\lambda$. The magnitude of $H(k)$ represents the attenuation of the sample at wavenumber $k$, the phase its dispersion at $k$. Localized spectroscopic information at $z_0$ is obtained from time-frequency analysis i.e. through a wavelet transform [5] or windowed short time Fourier transform (STFT), on a segment of $i(\lambda)$ centered around $z_0$. The magnitude of $H(k)$ can then be written as $\exp(-\mu(k,z)dz)$ which reduces to $\exp(-\mu(k)dz)$ for a homogeneous medium. When the amplitude spectra $I_1$ and $I_2$, taken at two positions in the sample separated by a distance $d$ are calculated, the attenuation coefficient follows from Beer’s law and equation 5-1, now written in terms of wavelength, as:

$$\mu(\lambda) = \frac{1}{d} \ln \left( \frac{I_2(\lambda)}{I_1(\lambda)} \right)$$ (5-2)

Since the experiments presented here were carried out on non-scattering samples, the attenuation coefficient equals the absorption coefficient. The segmentation causes a trade-off between spectral and temporal (spatial) resolution. Defining the desired spectral resolution $\Delta \lambda = \Delta \lambda / N$ where $\Delta \lambda$ is the FWHM bandwidth of the source spectrum, the length of the STFT window (and the resulting spatial resolution) can be computed from [9]:

$$l_{\text{STFT}} = \frac{\pi}{4 \ln 2} l_c N$$ (5-3)

where $l_c$ is the coherence length of the light source.

5.3 materials and methods

In the experiments, we used an OCT setup illuminated by a commercially available Ti:Sapphire laser source (FemtoSource Pro, Femtolasers, Vienna), operating at 800 nm with a 125 nm FWHM bandwidth. In the reference arm of the interferometer a folded grating-lens pair [10] was used as a dispersion compensator. In depth scanning was performed by moving the compensator’s double-pass mirror which was mounted on a voice coil translator (QuickScan V-102.2L, Physik Instrumente), driven with a 1 Hz round-off triangular waveform. In the sample arm, the focusing lens ($f=25$ mm) was mounted on a similar but independently driven voice-coil translator to provide dynamic focusing. The axial resolution of this system was 3 $\mu$m with a dynamic range of 110 dB. The lateral resolution, defined as the waist of the field in the focus of the lens was calculated to be 3.6 $\mu$m. The interferometric
signal was detected by a photodiode, 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 in a computer. To extract the spectrum $I(k)$ at a depth $z_0$ the interferometric data were processed using a STFT algorithm with a Hanning window function. A 512-point data segment (length: 312 μm in air) centered at $z_0$ was used, which resulted in a spectral resolution of 1 nm. To increase the SNR of our measurements, absorption spectra calculated from equation 5-3 were averaged over 1000 measurements. Hemoglobin solutions were obtained from fresh porcine blood using a standard clinical protocol. Porcine blood has an average hemoglobin concentration of 115-155 g/l; due to the addition of water to achieve cell lysis the total hemoglobin concentration in our solutions was reduced by 33%. The hemoglobin solutions, maintained at 37 °C, were saturated at 0% and 100% by a clinically used Minimax Plus hollow fiber oxygenator (Medtronic, #22046313), by varying the composition of (N₂, CO₂, O₂) gasses applied to the solutions. To facilitate OCT imaging, the hemoglobin solutions were perfused through a cuvette with a rectangular lumen of 3 mm thickness. The cuvette was incorporated in a circulation containing the oxygenator and a pump. Dispersion of the cuvette wall was compensated in the reference arm. The index of refraction of the hemoglobin solution was estimated at 1.33. OCT images (0.46 μm per data point) were taken from the cuvette, and the spectra at the glass-liquid boundaries were obtained and used to determine the absorption coefficients. After each measurement a sample was drawn to determine the saturation and to monitor electrolyte values and pH by a Radiometer OSM3 blood gas analyzer. Electrolyte values and pH did not change during the course of the experiment and the total hemoglobin concentration was 93 g/l for all samples, which was in good agreement with the expected values around 100 g/l.

5.4 results and discussion

To determine the ability of our setup to do spectral measurements, we determined the absorption spectrum of a glass filter with an absorption edge in the wavelength range of the laser (Maico πc13, $n=1.58$).

![figure 5-2: Measured absorption coefficient of a glass filter (Maico πc13) measured by spectroscopic optical coherence tomography. The grey line represents the absorption spectrum from spectrometer measurements. Spectral resolution was 1 nm. Data is the average of 1000 absorption spectra, the error bars correspond to the standard deviation. (not all error bars are plotted for clarity.)](image)

From its specifications we expected the absorption coefficients to be of the same order of magnitude as the absorption coefficients of the hemoglobin solutions. OCT images (0.38 μm...
per data point) of the glass filter were taken, the spectra at the air-glass (front) interface and glass-air (back) interface were obtained and used to determine the absorption coefficients. The absorption spectrum was also measured by an Ocean Optics SD 2000 spectrometer. The result is shown in figure 5-2. The absorption spectrum measured by SOCT corresponds well to that measured by the spectrometer in the wavelength range 780-825 nm. The small offset of the values with respect to the spectrometer data may be due to slight defocusing in our dynamic focusing setup.

We proceeded to determine the absorption spectra of Hb and HbO₂ using SOCT. The calculated spectra are shown in figure 5-3.

![Figure 5-3: Absorption spectra of hemoglobin (dashed line) and oxygenated (solid line) hemoglobin measured by spectroscopic optical coherence tomography. Spectral resolution was 1 nm. The plotted lines are the average of 1000 absorption spectra; the error bars correspond to the standard deviation. (not all error bars are plotted for clarity.)](image)

Again the measured values are in good agreement with literature values. The isobestic point around 800 nm can be clearly seen. For both figure 5-2 and 5-3 the accuracy of the absorption coefficient was largest for the wavelengths with highest spectral power densities. Consequently, for oxygenation measurements a trade-off exists between signal to noise ratio and spectral contrast in a certain wavelength band. The ability to determine blood oxygenation from SOCT measurements will therefore depend on the choice of analysis wavelengths.

### 5.5 conclusions

In conclusion, we quantitatively determined absorption coefficients of hemoglobin and oxygenated hemoglobin solutions with spectroscopic optical coherence tomography. The ability of SOCT to distinguish both oxygenation states implies that localized, quantitative oxygenation measurements of whole blood based on differences in attenuation will be possible. In clinical practice, however, dynamic focussing makes the OCT imaging slow. Fortunately, if the confocal properties of the sample arm are known, focus tracking is not a prerequisite for quantitative measurements on the attenuation of light by OCT [11]. Furthermore, for clinical application the effect of oxygenation state on scattering properties of blood, albeit often ignored, and the effect of flow have to be investigated.
REFERENCES AND LINKS


