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In vivo low-coherence spectroscopic measurements of local hemoglobin absorption spectra in human skin

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Abstract. Localized spectroscopic measurements of optical properties are invaluable for diagnostic applications that involve layered tissue structures, but conventional spectroscopic techniques lack exact control over the size and depth of the probed volume. We show that low-coherence spectroscopy (LCS) overcomes these limitations by measuring local attenuation and absorption coefficient spectra in layered phantoms. In addition, we demonstrate the first in vivo LCS measurements of the human epidermis and dermis only, from the measured absorption in two distinct regions of the dermal microcirculation, we determine total hemoglobin concentration (3.0 ± 0.5 g/l and 7.8 ± 1.2 g/l) and oxygen saturation.

Keywords: spectroscopy; low-coherence; absorption; attenuation; hemoglobin; human skin.

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The derivation of physiological parameters from the spectroscopic determination of tissue optical properties can offer a fast and painless alternative to invasive diagnostic procedures such as tissue biopsies and drawing of blood. For instance, the absorption and scattering coefficients between 480 and 700 nm [bold-faced characters denote wavelength (λ) dependent parameters]. In this study we present, for the first time to our knowledge, quantitative measurements of local μₐ and μₑ spectra within selected volumes of inhomogeneous turbid media. We validate our method by retrieving the dye concentration from the measured μₐ of an Intralipid-dye phantom (μₛ = 4 to 6 mm⁻¹, μₐ = 0 to 5 mm⁻¹), covered by light attenuating layers with varying optical densities (0.39 to 0.89). Subsequently, we demonstrate the first in vivo LCS measurements of μₐ and μₑ of the human epidermis and dermal microcirculation, from which we determine total hemoglobin concentrations and oxygen saturation.

To obtain μₐ and μₑ from a target volume, we measured backscattered power spectra S(λ) at controlled geometrical path lengths ℓ of the light in the medium (path length and depth related parameters are corrected for the refractive index n of the medium). Our LCS system, which is described in detail in Ref. 5, is optimized for the wavelength range of 480 to 700 nm. We controlled ℓ by translating the reference mirror in steps of 27 μm. By translating the sample in the axial direction, focus tracking of the spot size (φ = 5 μm) in the medium is achieved. Around ℓ, the signal is modulated by scanning the piezo-driven reference mirror (23 Hz), resulting in a scanning window of Δℓ ≈ 44 μm in the medium. The optical power at the sample is 6 mW.

A multimode fiber (φ = 62.5 μm) guides the reflected light from both arms to a photodiode. Fourier transformation of the acquired time signal results in spectra S(ℓ) with spectral resolution Δλ = λ³/ln(Δℓ) (4 nm < Δλ < 9 nm). To minimize the influence of speckle noise on S(ℓ), we spatially average 90 to 250 spectra by translating the sample and measuring S(ℓ) every 5 μm. Fitting the single exponential decay model S(ℓ) = A · exp(−μₑ · ℓ) (free running fit parameters A and μₑ) to the background corrected S(ℓ) versus ℓ, results in a μₑ spectrum. Uncertainties in A and μₑ are estimated by their 95% confidence intervals (c.i.).

When S(ℓ) is dominated by single backscattered light, the attenuation coefficient μₑ = μₐ + μₛ. Since the dependence of μₛ on wavelength can be described by a · λ⁻ᵇ, least-squares fitting of μₑ = a · λ⁻ᵇ + ∑ᵢ (cᵢ · μₛᵢ) results in the individual contributions of μₛᵢ and μₛ to the measured μₑ. The free running fit parameters aᵢ, b, and cᵢ are constraint to positive values. The wavelength dependent μₛᵢ are the known absorption spectra (unit: mm⁻¹ per unit concentration) of the contributing chromophores i with contribution cᵢ, which are μₛᵢ of the dye for the phantom measurements and μₛ of deoxygenized hemoglobin (Hb) and oxygenized hemoglobin (HbO₂) for the in vivo skin measurements. The μₛ of the dye was obtained from a...
transmission measurement. \(^5\) Since we are primarily interested in the total hemoglobin concentration ([tHb] = [cHbO2] + cHb) and the oxygen saturation (SO2 = [cHbO2]/[tHb]) with their uncertainty estimates (±95% c.i.) for the in vivo measurements, we directly fit the [tHb] and the SO2 by substituting cHbO2 to the fits (dotted lines) are minimally affected by the covering transmission measurement. \(^5\) Thus, we are primarily interested in both size and position inside the medium, by choosing the region for \(\mu_s\)-contributions to the \(\mu_t\)-fits are shown for all measurements.

The volume from which we obtain \(\mu_s\) is controllable in both size and position inside the medium, by choosing the region for lateral averaging and the \(\ell\)-interval for fitting the exponential decay model. When measuring on inhomogeneous media such as skin, spatial information is needed to confine our region of interest to, e.g., the epidermal or dermal layer. Therefore, we support our analysis by reconstructing an OCT image from the individual LCS time signals within every \(\Delta\ell\) in the axial and lateral direction, using depth scaling \(d = \ell/2\). The axial resolution of these “fused” OCT images is given by the coherence length of the light source of \(\sim 1.5\) \(\mu m\) and is therefore higher than the axial resolution of 22 \(\mu m\) for \(\ell(t)\).

Figure 1 shows the measured \(\mu_s\) (dots) of a medium consisting of 1% Intralipid (Intralipid\(^\circledR\) 20%, Fresenius Kabi, Germany) and 10% magenta dye (Ecoline #337, Royal Talens, The Netherlands), uncovered and covered by nonabsorbing silicon-titanium dioxide (TiO\(_2\)) layers. \(^\text{10}\) The three covering layers varied in thickness \(D\) and scattering (layer 1: \(D = 155\) \(\mu m\), \(\mu_s = 2.5\) \(mm^{-1}\)); layer 2: \(D = 170\) \(\mu m\), \(\mu_s = 5\) \(mm^{-1}\); layer 3: \(D = 355\) \(\mu m\), \(\mu_s = 2.5\) \(mm^{-1}\)). Fits on \(\mu_t\) and \(\mu_s\)-contribution to the \(\mu_t\)-fits are shown for all measurements.

Within the dermis, we can distinguish two regions with relatively high (Region 2) and low (Region 3) homogeneity. The measured \(\mu_s\) differ considerably between the two regions [Fig. 2(b)], which can be ascribed to a difference in both scatter power (\(b = 0.5\pm 0.1\) in Region 2, \(b = 2.0\pm 0.1\) in Region 3) and absorption. The \(\mu_s\)-spectra of both regions are shown in Figs. 2(c) and 2(d). The fitted [tHb] of 3.0 ± 0.5 g/l in Region 2 and 7.8 ± 1.2 g/l in Region 3 indicate the presence of blood and can be related to normal dermal blood volume fractions of \(2\%\) and \(5\%\), respectively, when assuming a fixed hemoglobin concentration of 150 g/l for whole blood. \(^4\) The fitted SO2 of \(81\pm 34\%\) in Region 2 and 100± 31% in Region 3 are also within physiological range.

Presumably, Region 3 encloses a flexure line and Region 2 encloses surrounding skin, since relative differences in hemoglobin absorption up to 63% were found between those two palmar skin regions during stretching, \(^7\) which is consistent with our [tHb] results. This also explains the difference in scattering between the two regions, because tissue homogeneity and organization of collagen fiber content, the major contributor to dermal scattering, differ significantly between these skin regions. \(^3\) The value of the \(\mu_s\)-contributions to the measured \(\mu_s\) fall within the physiological range \(^1\) of 1 to 100 \(mm^{-1}\), but the actual dermal \(\mu_s\) may be underestimated due to the contribution of multiple scattering to the LCS signal. \(^6\) Nevertheless, since absorption takes place along the controlled photon path, \(^3\) this contribution does not influence the determination of \(\mu_s\).

These in vivo measurements show that LCS can be used to measure hemoglobin concentration and oxygenation in the microcirculation. Although no gold standard exists to confirm our in vivo [tHb] and SO2 determinations, their values are convincing biologically and the optical properties from which they were derived are within the range of optical properties that were validated in our phantom study. The accuracies at which we determined [tHb] (\(\sim 15\%\)) and SO2 (\(\sim 30\%\)) are influenced by the homogeneity of their distribution within the investigated region, and by the accuracy of the determination of \(\mu_t\). The latter is affected by the size of the investigated region (i.e., the number of spatial averages and the length of the \(\ell\)-interval for fitting the exponential decay model) and the OD of the medium covering this region, which limits the maximum probing depth of LCS to \(\sim 0.5\) to 1 mm \(in vivo\). The limiting accuracy of the in vivo determination of [tHb] can be expected to be \(8\%\), as found for the dye concentration in the phantoms. Since the epidermal OD (\(\sim 0.8\)) is comparable to the OD’s of the layers in our phantom study, the extra inaccuracy of the [tHb] and SO2 determination can be ascribed to the skin’s heterogeneity. \(^7\) Although the size of the investigated region improves accuracy, it negatively affects measurement speed. Faster acquisition can be achieved by optimizing this trade-off, and by investigating the possibility for Fourier domain acquisition. In contrast to time domain

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**Fig. 1** Measured \(\mu_s\) (dots) of a 1% Intralipid-10% dye phantom behind no layer and three TiO\(_2\)-silicon layers: 1: \(D = 155\) \(\mu m\), \(\mu_s = 2.5\) \(mm^{-1}\); 2: \(D = 170\) \(\mu m\), \(\mu_s = 5\) \(mm^{-1}\); 3: \(D = 355\) \(\mu m\), \(\mu_s = 2.5\) \(mm^{-1}\). Fits on \(\mu_t\) and \(\mu_s\)-contributions to the \(\mu_t\)-fits are shown for all measurements.
acquisition, the latter will require correction for unwanted signal attenuation due to out-of-focus detection and sensitivity roll-off in depth.\textsuperscript{8,12}

Potentially, the dermal [\(\text{Hb}\)] can be related to the hemoglobin concentration in whole blood, if the blood volume within the investigated region can be assessed. A possible method that deserves further investigation for this purpose is obtaining the blood volume from the OCT image by assessing the vessel density using advanced signal analysis, for instance as described in Ref. 11. When measuring other tissue types, the contribution of additional chromophores (e.g., bilirubin, melanin) to the measured \(\mu_t\) may need to be incorporated in our analysis. Also, a correction for Doppler broadening or shifting of the measured \(\mu_t\) spectra may be needed for tissues that exhibit blood flow. We did not observe any of those influences on the measured \(\mu_t\) spectra in Fig. 2(b), which can be explained by a temporary decrease of blood flow due to applied pressure on the skin.

In conclusion, we have shown that we can use LCS to locally obtain absorption coefficient spectra within confined volumes of optically inhomogeneous media. This enabled us to perform the first \textit{in vivo} LCS measurements of hemoglobin concentration and oxygen saturation inside the dermal microcirculation. By confining the measurement volume to specific tissue structures, LCS overcomes the limitations of conventional spectroscopic techniques. LCS therefore offers a potential alternative to invasive drawing of blood for the determination of whole blood hemoglobin concentration and oxygen saturation.

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\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{\textit{In vivo} measurement on the skin of the palmar side of a finger joint. The measured \(\mu_t\) fits on \(\mu_t\) and \(\mu_r\)-contributions to the \(\mu_t\)-fits are shown in (a) for the epidermis (Region 1) and (b) for the dermis (Regions 2 and 3). The \(\mu_r\)-contributions to the \(\mu_t\)-fits are shown in (c) for Region 3 and (d) Region 2 (note the difference in vertical axis scaling). The selected regions are shown in the OCT image in the upper right corner.}
\end{figure}

\begin{thebibliography}{99}
9. Data tabulated from various sources compiled by S. Prahl, \texttt{http://omlc.ogi.edu/spectra}.
\end{thebibliography}