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Are quantitative attenuation measurements of blood by optical coherence tomography feasible?

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We present optical coherence tomography (OCT) measurements on fully physiologically oxygenated blood samples of varying hematocrit. We show that attenuation coefficients cannot be extracted quantitatively using the currently accepted models for the OCT signal from scattering media, because the confidence intervals obtained in the fitting procedure cannot be used as reliable uncertainty estimates of the attenuation coefficients. Better modeling of the hematocrit-dependent OCT signal is needed.© 2009 Optical Society of America

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Optical techniques are clinically used to estimate hemoglobin oxygen saturation (the percentage of oxygenated hemoglobin over total hemoglobin, \( \text{SO}_2 = \text{HbO}_2 / (\text{HbO}_2 + \text{Hb}) \)). Methods such as pulse oximetry are based on differences in light absorption between \( \text{HbO}_2 \) and \( \text{Hb} \) along the path of the detected light. Quantitative measurement of \( \text{SO}_2 \), e.g., a measurement with sufficient sensitivity including a reliable uncertainty estimate, is difficult because this path length is unknown. This drawback may be overcome by spectroscopic optical coherence tomography, \((S)OCT\), where the path length can be accurately controlled. After our [1–3] and others’ [4–6] work using time-domain OCT, Lu et al. [7] and Kagemann et al. [8] have published changes in the Fourier-domain OCT signal related to oxygenation state. They report important technological steps toward clinical application of \( \text{SO}_2 \) measurements using SOCT. Nevertheless, before quantitative \( \text{SO}_2 \) measurements are feasible, there are a number of issues that need to be overcome and that are insufficiently addressed in the available literature. In general, \( \text{SO}_2 \) is determined from a spectrally resolved measurement of the attenuation coefficient \( \mu_s(\lambda) \) by fitting the OCT signal \( I_{OCT}(\lambda) \) versus depth to an analytical model. The \( \mu_s(\lambda) \) has absorption \[ \mu_a(\lambda) \] and scattering \[ \mu_s(\lambda) \] contributions, both of which are \( \text{SO}_2 \) dependent [2]. Appropriate fitting of \( \mu_s \) is a prerequisite for quantitative \( \text{SO}_2 \) measurements.

In this Letter, we discuss modeling and fitting challenges associated with determining \( \mu_s \) in blood and illustrate those using measurements on fully oxygenated blood of varying red blood cell concentration (hematocrit, \( H \)). The fit model needs to account for a significant contribution of multiple scattered light because of the high hematocrit found \textit{in vivo} (\( \sim 50\% \)), and the highly forward directed scattering of red blood cells (anisotropy factor \( g \) reported between 0.985 and 0.997) [10]. The scattering coefficient of undiluted blood is difficult to measure but can be estimated theoretically [2] and experimentally [9,10] from measurements on diluted blood to be of the order of 300 mm\(^{-1}\) at 800 nm. Multiple scattering leads to decreased signal attenuation with depth. Moreover, at higher \( H \), the individual scatterer’s locations are correlated so that \( \mu_s \) may not scale linearly with \( H \). Packing factors \( W(H) \) are introduced such that \( \mu_s = H \cdot W(H) \). Twersky’s expression, \( W = 1 − H \), is often used. For low \( H \), \( \mu_s \) is expected to scale linearly with hematocrit but, with increasing \( H \), both multiple and spatially correlated scattering effects cause an increasing deviation from this linear trend.

For quantitative \( \mu_s \) measurements, reliable uncertainty estimates must be computed for \( \mu_s \) and ultimately \( \mu_s(\lambda) \) for quantitative \( \text{SO}_2 \) measurements. It is common to use the 95% confidence intervals (c.i.) of the fitted \( \mu_s \) as uncertainty estimates. They are derived from the covariance matrix reported by the fitting algorithm and quantify the range in which each fit parameter can be varied without influencing the overall fit statistics. They should be interpreted with caution [11]. A fit using an inappropriate model can yield very small c.i. of the fit parameters, because a slight change would worsen the fit (case A). Likewise, an overparameterized model can lead to very large c.i., because the fit is not significantly influenced by that parameter or the change in one parameter can be compensated by a change in the others (case B). Only two analytical models have been reported to quantitatively extract \( \mu_s \) from OCT data, and the corresponding fitting procedures to the OCT data have been well established. For weakly scattering media, the single-scattering model is valid up to about seven scattering mean-free paths and yields an estimation for \( \mu_s \) with an accuracy of \( \sim 1 \) mm\(^{-1}\) [12]. This method does not account for multiple forward scattering. The extended Huygens Fresnel (EHF) model [13] determines the scattering coefficient \( \mu_s \), accounting for multiple scattering, so there is no range of validity in terms of depth. It is applicable to small-angle for-
ward scattering. This model features an additional parameter, the rms scattering angle \( \theta_{\text{rms}} \), which is related to the more commonly used scattering anisotropy \( g \). Within the range of validity, \( \mu_t \) is determined with an accuracy of \( \sim 1 \text{ mm}^{-1} \) [14]. For convenience we use \( \mu_t \) instead of \( \mu_s \) in the discussion of our results.

To illustrate the effect of these modeling and fitting challenges, we evaluated OCT data of blood samples of varying \( H \) and \( \text{SO}_2 \) fixed at 100% using the two analytical models. The dynamic focusing, time domain OCT setup (\( \lambda_0 = 800 \text{ nm} \); \( \Delta_\lambda = 125 \text{ nm} \)) used in the experiments is described in [1–3]. Fresh human blood was anticoagulated before use. Samples with \( H < 50\% \) were obtained by diluting the blood with plasma. OCT images were taken with the blood inside a closed cuvette. Oxygen saturation was fixed at 100% under physiological conditions, using the methods described in [1–3]. At the time of imaging, the blood was not stirred or flowing. By visual inspection, only parts of the OCT images where no cell grouping or sedimentation of the blood took place were used in the analysis. From the appropriate depth range, average A-scans±standard deviation are calculated. The latter is used for weighting in the fitting procedure, which minimizes the sum of the squared difference between the data and fit \( \chi^2 \). An offset (fixed at the average noise level) and amplitude (free-running parameter) were added to the models to allow for scaling. In all fits the correlation coefficient between the data and fit \( R^2 \) is 0.995. For higher assumed \( \theta_{\text{rms}} \) values versus \( H \) are shown in Fig. 1, open circles. The upper inset shows the averaged signal profile and fit using the single-scattering model for the measurement at \( H = 28\% \) (not all error bars are plotted). For all \( H \), the depth fitting range was set to \( \sim 60 \text{ \mu m} \). Longer depth intervals are outside the range of validity of the single-scattering model; shorter depth intervals could not be fitted accurately.

The fitted \( \mu_t \) values do not scale linearly with \( H \), likely due to the increasing contribution of multiply and spatially correlated scattering to the OCT signal, in which both lead to an underestimation of \( \mu_t \). The 95% c.i. values, however, are very small because a small change in \( \mu_t \) would lead to a (much) worse fit and are therefore unreliable uncertainty estimates of the actual \( \mu_t \) value (case A). These measurements are obtained directly from the blood layer and are larger than those previously measured from cuvette boundaries [3]. The EHF fitted \( \mu_t \pm 95\% \) c.i. values versus \( H \) with \( \theta_{\text{rms}} \) as a free-running parameter are shown in Fig. 1, closed squares. The lower inset shows the averaged signal profile and fit using the EHF model for the measurement at \( H = 9\% \). The \( \theta_{\text{rms}} \) did not converge to a realistic value, \( \theta_{\text{rms}} \in (0.25, 0) \) or \( g \in (0.75, 1) \). Large confidence intervals are obtained in the range from 40% to 60% of the fitted value of \( g \). These indicate that the fit statistics do not rely heavily on this parameter (case B). Therefore in subsequent fitting \( \theta_{\text{rms}} \) was fixed to a value of 0.35, corresponding to \( g = 0.8 \). Even though this value is much lower than expected for blood, it is within the range of validity of the model and is found to optimize fit statistics (low \( \chi^2 \) and high \( R^2 \)). The fitted \( \mu_t \) (Fig. 2, open circles) are much higher compared to Fig. 1 and do scale linearly with \( H \). The same observations are made when \( \theta_{\text{rms}} \) is fixed at higher values. Figure 1, closed squares, shows the fit corresponding to \( g = 0.995 \). For higher assumed \( g \), the fitted \( \mu_t \) also increases because the assumed contribution of multiple scattering to the OCT signal is increased (at the expense of worse fit statistics as \( \chi^2 \) and c.i. on the fit parameters). Since both multiple scattering and spatially correlated scattering have similar effects on the dependence of \( \mu_t \) on \( H \), the EHF model accounts for both simultaneously through the assumed contribution of multiply scattered light. However, because the packing factors could not be successfully incorporated in the models, it was not possible to separate effects arising from spatially correlated scattering or...
multiple forward scattering in our measured data. Both models discussed in this Letter therefore yield unreliable uncertainty estimates for the actual $\mu_t$ values and also values for the fitted optical properties that do not agree with literature. They therefore do not appear to be appropriate for quantitative extraction of optical properties of blood as a function of hematocrit. Unfortunately, as outlined above, it is not clear how the effects of spatially correlated scattering should be included in quantitative data analysis. Note that this does not disqualify the use of these models to extract optical properties from much less scattering tissues [14,15].

Our results question the feasibility of quantitative SO$_2$ measurements by spectroscopic OCT using this approach. Translating the single-scattering, EHF, or a newly developed model of the OCT signal to a model for extracting SO$_2$ is not trivial, because next to hematocrit dependence, it requires extensive knowledge of the SO$_2$-dependent absorption and scattering properties of blood (which complicates the presented experiments, where SO$_2$ was fixed at 100%). The obtained confidence intervals will ultimately determine the uncertainty estimate of the clinical SO$_2$ measurement. Nevertheless, an engineering approach can still be possible where measured signal attenuations are related to careful calibration measurements. Such calibrations must be performed under physiological conditions. In normal arterial blood, pO$_2$ is approximately 102 mmHg, corresponding to 100% oxygen saturation; normal venous blood has pO$_2$ of $\sim$40 mmHg, corresponding to SO$_2$ $\sim$70%, at pH=7.4. The conversion between pO$_2$ and SO$_2$ depends on, e.g., temperature and pH. In the experiment described in [7], a volume of blood is placed in a chamber filled with pure oxygen in which pO$_2$ was varied between $\sim$250 mmHg and $\sim$650 mmHg, far above the physiologically found maximum and leading to samples that remain 100% saturated. Their measured signals can therefore not be quantitatively correlated with clinically relevant SO$_2$ changes. To determine the accuracy and sensitivity of the SOCT measurement, multiple measurements must be made along the disassociation curve under laboratory conditions that need to be as close to physiological conditions as possible.

In conclusion, inherent control of the path length of the detected light can in principle provide quantitative measurements of attenuation coefficients, from which SO$_2$ can be inferred. Quantitative SO$_2$ measurements using SOCT comprise the following steps. From measurements (in vivo or under physiological conditions in the laboratory), $\mu_s$(SO$_2$, $\lambda$)$=\mu_s$(SO$_2$, $\lambda$) + $\mu_s$(SO$_2$, $\lambda$) values are extracted using an appropriate fit model that reports reliable uncertainty estimates. The second step translates these measured quantities to a SO$_2$ value and corresponding uncertainty estimate.

We present measurements on fully physiologically oxygenated blood samples of varying hematocrit and analyze the OCT data using analytical models to describe the OCT signal. Using the single-scattering model and the EHF model with $\theta_{ms}$ as a free-running parameter, we obtained values for $\mu_t$ that are much lower than expected based on literature (Fig. 1). The resulting confidence intervals are consequently not suitable as uncertainty estimates for the actual $\mu_t$. For the EHF model with fixed $\theta_{ms}$ the fitted $\mu_t$ values are in closer agreement with literature but depend heavily on the chosen $\theta_{ms}$. Reliable uncertainty estimates for $\mu_t$ could not be obtained from the resulting confidence intervals (Fig. 2).

Before the question of whether or not OCT can quantitatively measure the attenuation coefficient of blood can be answered affirmatively, better understanding and modeling of the hematocrit dependent OCT signal from blood is needed.

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