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Abstract. A swept-source dual-wavelength photothermal (DWP) optical coherence tomography (OCT) system is demonstrated for quantitative imaging of microvasculature oxygen saturation. DWP-OCT is capable of recording three-dimensional images of tissue and depth-resolved phase variation in response to photothermal excitation. A 1,064-nm OCT probe and 770-nm and 800-nm photothermal excitation beams are combined in a single-mode optical fiber to measure microvasculature hemoglobin oxygen saturation (SO2) levels in phantom blood vessels with a range of flow speeds (0 to 17 mm/s). A 50-μm-diameter blood vessel phantom is imaged, and SO2 levels are measured using DWP-OCT and compared with values provided by a commercial oximeter at various blood oxygen concentrations. The influences of flow speed and mechanisms of SNR phase degradation on the accuracy of SO2 measurement are identified and investigated. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.18.5.056005]

Keywords: optical coherence tomography; dual-wavelength; imaging; phase-sensitive; microvasculature; hemoglobin oxygen saturation; phantom.

Paper 130033R received Jan. 21, 2013; revised manuscript received Mar. 5, 2013; accepted for publication Mar. 26, 2013; published online May 2, 2013.

1 Introduction

Noninvasive quantitative evaluation of microvascular hemoglobin oxygen saturation (SO2) in tissue is important in early detection and monitoring of the progression of inflammatory and ischemic diseases such as cancer, stroke, and glaucoma.1,2 Various approaches have been used to assess in vivo microvascular oxygen saturation, including oxygen-sensitive microelectrodes,3–7 magnetic resonance imaging (MRI),8–12 reflection spectroscopic oximetry,13–18 and phosphorescence quenching.17,19–24 Measurement by oxygen-sensitive microelectrodes is a point measurement method and is primarily limited to animal studies. MRI has limited spatial (100 to 150 μm) and temporal (tens of seconds to minutes) resolution. The phosphorescence quenching technique has limited spatial resolution (e.g., 50 μm), and no oxygen sensitive dyes that are approved by the United States Food and Drug Administration are available for clinical translation.

A useful three-dimensional imaging technique, optical coherence tomography (OCT), was introduced in 1991 as a time domain approach25 and was later used as a frequency domain method.26,27 OCT implementation in the frequency domain improves signal-to-noise ratio and allows high-speed image acquisition. More recently, Huber et al. showed that, for an equivalent signal-to-noise ratio, swept-source (SS) OCT with balanced detection provides higher acquisition rates compared with spectrometer-based Fourier domain OCT approaches.28

Biomedical investigators are interested in applying OCT approaches to measure microvascular SO2 in tissues. Spectroscopic Fourier domain OCT (SFD-OCT)29,30 has been reported to measure depth-resolved microvascular oxygenation, but an appropriate model has not been given to estimate the attenuation coefficients required to determine blood SO2 levels using OCT light in the near infrared spectral region.31 SFD-OCT has been shown to provide sufficient sensitivity to quantify micro vascular SO2 levels using visible wavelengths (460 to 700 nm) where hemoglobin absorption is relatively large.32,33 However, SFD-OCT using visible wavelength sources is compromised by limited imaging depth, which is restricted by increased scattering.

Photothermal OCT is a functional imaging technique that can measure the optical pathlength variation of OCT light backscattered from tissues in response to an excitation beam. Adler has demonstrated photothermal OCT using a gold nanoparticle contrast agent.34 Skala has developed photothermal OCT for high-resolution molecular imaging.35 and Paranjape has reported using photothermal OCT to detect macrophages in tissue.36 So far, reported applications of photothermal OCT have focused primarily on light absorption by nanoparticles. Previously, we reported using dual-wavelength photothermal OCT (DWP-OCT) to measure microvasculature SO2 in both phantom37 and in vivo38 blood vessels using a common-path phase-sensitive (PhS) OCT system.39 Because we used a common path interferometer, imaging by scanning the beam was difficult, and the results were limited to point microvasculature SO2
measurement. In this study, we report a DWP-OCT system that uses a two-beam interferometer and allows for the imaging and measurement of SO2 levels.

Compared to two-beam interferometers, common path interferometry provides higher sensitivity and stability to measure the phase of interference fringes of light backscattered from transparent and scattering media. Despite these advantages, several drawbacks of prior common-path DWP-OCT system design are recognized. Recording en-face images is challenged by incorporating a 2D scanning system into the sample arm. A short working distance associated with common-path DWP-OCT introduces problems for positioning the OCT probe beam at a desired sample measurement location. Also, the short working distance makes intravascular, retinal, and endoscopic applications challenging. We present a DWP-OCT system using a fiber two-beam interferometer to image and measure SO2 levels in a phantom blood vessel.

2 Methods

2.1 DWP-OCT System

In this study, a DWP-OCT system using a fiber Michelson interferometer was constructed for imaging and blood SO2 measurement. Interferometric fringe phase stabilization is a critical feature required for SO2 measurement. In a generic phase-sensitive SS OCT system, two mechanisms contribute to phase noise: inconsistency of the start wavelength between successive A-scans, and nonspecific mechanical movement of optical elements in sample and reference arms. To resolve the first issue, 5% of light in the sample arm is coupled to a high-reflectivity mirror, which is sufficient to form a high SNR interference fringe signal with reference light but too weak to introduce an artifactual autocorrelation and interference signal with light backscattering from the sample. Light reflecting from a high-reflectivity mirror in the sample path introduces a feature-line in recorded B-scans positioned below the imaging media and does not compromise image quality. To minimize the second source of phase noise (due to nonspecific mechanical movement of optical elements), the sample beam scanning system is constructed using a stable mechanical cage system.

Blood SO2 measurement value is dependent on the ratio ($\chi$) of optical pathlength ($op$) signal amplitude at modulation frequencies introduced by 770-nm and 800-nm photothermal excitation light. To balance incident fluence of photothermal excitation beams, the scanning optics is designed to provide nearly equivalent spot sizes for 770-nm and 800-nm light.

The DWP-OCT system (Fig. 1) for imaging and blood SO2 measurement consists of two major systems: a SS PhS OCT system that provides accurate depth-resolved phase measurement with a 300-pm lower bound of detectable $op$ signal amplitude, and two excitation lasers (770 and 800 nm) that are intensity modulated at 400 and 380 Hz, respectively, and introduce a nanometer-scale harmonic $op$ signal amplitude due to blood absorption.

The phase-sensitive OCT system uses a swept source laser (HSL-1000, Santec Corp. Komaki, Aichi, Japan) with a 28-kHz A-line rate, a center wavelength at 1,060 nm, and full-wave-half-maximum spectral width of 58 nm. Single-mode optical fiber (HI1060, Corning Inc., Corning, NY) is utilized to construct the interferometer. Light emitted by the SS laser is split into three subsystems: trigger, sampling clock, and signal interferometer.
The trigger subsystem utilizes a fiber Bragg grating (FBG) to ensure the digitizer starts data acquisition at a consistent and repeatable wavenumber for each A-scan. The sampling clock subsystem consists of a Mach-Zehnder interferometer with a clock rate set by adjusting the interferometric light delay. The sampling clock signal received by a balanced photodetector is input into an external analog circuit, frequency quadrupled, and used as a sampling trigger for the analog-to-digital converter. The third subsystem is the Michelson signal interferometer with sample and reference arms. An optical circulator (1060 PI TGG, Agiltron Inc., Woburn, MA) is used in the sample arm of the Michelson interferometer to increase SNR. The sample arm contains two light paths: a path to the phantom blood vessel with an achromatic scanning system (consisting of two galvanometers and an afocal telescope), and a high-reflectivity mirror used for phase stabilization. The achromatic scanning system is designed and simulated in optical design software (Zemax, Radiant Zemax LLC, Redmond, WA) and provides micrometer-scale lateral resolution for imaging three co-aligned beams; the computed diffraction encircled energy computation gives a 13-μm lateral resolution for 770-nm and 800-nm excitation beams, along with a 14-μm resolution for the 1,060-nm PhS-OCT probe beam.

After the interference fringe signal is acquired uniformly in wavenumber (or optical frequency), computing a fast Fourier transform (FFT) of the signal, we obtain a complex number data array for each A-scan. The complex number amplitude is used to construct an OCT intensity image, and the complex number phase is the sample phase when the interferometer is idle. The phase measurement error due to the delay in data acquisition is corrected for by averaging multiple A-scans. Phase errors at any sample depth (d_s) are eliminated by subtracting the reference phase scaled by depth from the sample phase

\[
\varphi_{sc} = \varphi_s - \frac{d_s}{d} \varphi_r, \tag{1}
\]

where \(\varphi_{sc}\) is the corrected sample phase, \(\varphi_s\) is the sample phase acquired from the raw signal FFT, \(\varphi_r\) is the reference phase obtained from interference between light reflected from the mirror in the sample path and the reference arm, and \(d_s\) and \(d\) are the sample and reference depths, respectively.

The system operates in real-time in either OCT intensity imaging or M-mode phase imaging. Data acquisition and signal processing software are written in Labview (National Instrument Corp., Austin, TX). The system sensitivity is 102 dB (with shot-noise limited sensitivity of 107 dB), and the axial resolution is 13 μm in tissue with the application of a real-time digital dispersion compensation algorithm. The axial resolution is limited by polarization mode dispersion in the circulator. After Fourier transform of M-mode phase data (i.e., 1-s duration), with a calibration process, the phase of light backscattered from a selected sample depth is converted to the optical pathlength (\(\alpha = \lambda * \varphi_{sc}/2\pi\), where \(\lambda\) is the center wavelength and \(\varphi_{sc}\) is the corrected sample phase). The mean noise level in the signal frequency region corresponding to the intensity modulation of photothermal excitation light (360 to 420 Hz) is taken as the \(\alpha\) signal noise floor and measured at 300 pm.

Photothermal excitation beams are emitted from two 100-mW single-mode fiber (HI780, Corning Inc., Corning, NY) pigtailed laser diodes (QFLD-780-100S, QPhotonics LLC, Ann Arbor, MI for 770 nm and QFLD-795-100S for 800 nm). Light from these sources is coupled into the DWP-OCT system’s sample arm through a wavelength division multiplexer (WDM) (PSK-000851, Gould Fiber Optics, Millersville, MD). Both the WDM and the PhS-OCT system are constructed using HI1060 Corning fiber, which is single-mode fiber for 1,060-nm probe light and allows two or three propagation modes at photothermal excitation wavelengths of 770 nm and 800 nm. The temperature of each laser diode is precisely controlled within a fraction of a degree (K) and selected to ensure emission at the desired wavelength as calibrated using a spectrometer. The photothermal excitation power incidents on the sample for 800-nm and 770-nm wavelengths are 2.78 mW and 2.87 mW, respectively; both are within ANSI limits for skin. Intensity modulation frequencies for photothermal excitation light (770 nm [400 Hz] and 800 nm [380 Hz]) are selected in a signal frequency range where the phase noise is low (0.3 nm) and the \(\alpha\) signal amplitude is high. The procedure for determining the optimum photothermal excitation frequency to maximize \(\alpha\) signal-to-noise ratio for blood was reported previously.

OCT probe (1,064-nm) and photothermal excitation (770-nm and 800-nm) beams are co-aligned and coincident on the sample.

2.2 \(\text{SO}_2\) Calculation

We assume that \(\alpha\) signal amplitude due to absorption by blood is linear with the fluence of photothermal excitation light, as derived and reported previously. Neglecting the effect of thermal diffusion, \(\text{SO}_2\) level can be estimated from \(\alpha\) measurement in response to 770-nm (1) and 800-nm (2) photothermal excitation.

\[
\text{SO}_2 = \frac{c_o}{c_o + c_d} = \frac{\alpha_{d1} - \chi_{12} \alpha_{d2}}{\chi_{12} (\alpha_{s2} - \alpha_{d2}) - (\alpha_{s1} - \alpha_{d1})}, \tag{2}
\]

where \(c_o\) is the concentration of oxygenated hemoglobin (mM); \(c_d\) is the concentration of deoxygenated hemoglobin (mM); \(\chi_{12} = (\alpha_{p1}/\Phi_1)/(\alpha_{p2}/\Phi_2)\); \(\Phi\) is the measured optical pathlength signal amplitude; \(\tau\) represents the fluence over one period; \(\tau\) is the period of photothermal excitation; \(I\) is the average intensity of excitation light on the blood vessel; \(\alpha_{s}\) and \(\alpha_{d}\) are the tabulated molar extinction coefficients of oxygenated and deoxygenated hemoglobin (\(\text{cm}^{-1}\text{mM}^{-1}\)), respectively; and subscripts 1 (770 nm) and 2 (800 nm) correspond to the wavelengths of excitation light (\(\alpha_{s1} = 0.65 \text{ cm}^{-1}\text{mM}^{-1}\), \(\alpha_{d1} = 0.79 \text{ cm}^{-1}\text{mM}^{-1}\), \(\alpha_{s2} = 1.31 \text{ cm}^{-1}\text{mM}^{-1}\), \(\alpha_{d2} = 0.793 \text{ cm}^{-1}\text{mM}^{-1}\)). The ratio of the two excitation beams’ fluence (\(\Phi_2/\Phi_1\)) at the sample is calibrated before measurement. The \(\alpha\) signal amplitude at each photothermal excitation wavelength (\(\alpha_{p1}\) and \(\alpha_{p2}\)) is determined by computing the magnitude of the signal phase oscillation at the respective modulation frequencies of excitation light (Fig. 2).

2.3 Blood Vessel Phantom and Blood Flow

A 50-μm inner diameter polytetrafluoroethylene conduit (SUBL 060, Braintree Scientific Inc., Braintree, MA) containing porcine blood is used as a blood vessel phantom. A desired blood \(\text{SO}_2\) level is achieved by adding sodium dithionite to the blood sample to deoxygenate. Six blood samples are prepared at different \(\text{SO}_2\) levels (99.6%, 89.2%, 84.1%, 69.0%, 57.3%, and 3.0%). To provide a scattering background for imaging, the phantom blood vessel is placed on a sheet of...
white-colored copy paper. After imaging, blood \( \text{SO}_2 \) measurements are recorded in an M-mode acquisition at a selected position in the lumen of the phantom vessel (Fig. 3).

To investigate the effect of blood flow on \( \text{SO}_2 \) measured by DWP-OCT, a digital syringe pump (AL-1000, World Precision Instruments, Sarasota, FL) is used to introduce blood flow in the phantom vessel at a fixed \( \text{SO}_2 \) level (98.2%) corresponding to an arteriole. At the fixed \( \text{SO}_2 \) level, DWP-OCT \( \text{SO}_2 \) measurements are recorded at blood flow speeds from 0 to 17 mm/s. For each blood flow speed, \( \text{SO}_2 \) levels are also measured at the same position in the lumen of the phantom vessel.

3 Results

We observed \( \text{op} \) signal amplitude in the phantom vessel containing blood resulting from photothermal excitation with 770-nm and 800-nm light. In a control experiment, with the phantom vessel containing water, no \( \text{op} \) signal was detected in response to photothermal excitation. Three experiments were completed to investigate the functionality of the DWP-OCT system: en-face imaging of the blood vessel phantom, blood \( \text{SO}_2 \) measurement without flow, and influence of blood flow speed on \( \text{SO}_2 \) measurement.

3.1 Phantom Image

A two-vessel phantom was constructed to demonstrate DWP-OCT imaging of an arterial-venous vessel pair. Two 50-\( \mu \)m inner-diameter phantom vessels were attached to a sheet of white-colored copy paper to provide a scattering background for imaging. The two phantom vessels were filled with porcine blood, and digital syringe pumps were used to introduce flow (2.8 mm/s) in opposite directions in each phantom vessel (Fig. 4). Average flow speed was calculated by dividing the syringe pump infusion flow rate (0, 20, 40, 60, 80, 100, and 120 \( \mu \)L/h) by the phantom vessel’s lumen cross-sectional area (1.96 \( \times 10^3 \) \( \mu \)m\(^2\)).

3.2 Blood \( \text{SO}_2 \) Measurement in Phantom Vessel without Flow

DWP-OCT phase data was recorded over a time period of 1-s at the bottom of the lumen in one of the phantom vessels (Fig. 3). The \( \text{op} \) signal amplitude was determined for each 0.5-s data acquisition period by computing the fast Fourier transform (FFT) of the phase (\( \varphi_{\text{sc}} \)) data. For each 1-s of acquired DWP-OCT data, 15 sub-segments were analyzed with a 1/28-s offset between successive 0.5-s data segments. For each 0.5-s data segment, the \( \text{op} \) signal amplitudes at 380 and 400 Hz were calculated, and the \( \text{SO}_2 \) level was estimated according to Eq. (2). In the experiment, DWP-OCT data segments longer than 1-s were not recorded due to phase drift. Estimates of \( \text{op} \) were obtained using a moving average approach, which is preferred for short signal durations to reduce high-frequency noise. Phase noise in the \( \text{op} \) signal amplitude increases variance in the computed \( \text{SO}_2 \) levels (see error propagation model in Sec. 4.1). The mean of \( \text{SO}_2 \) values derived from 15 sub-segments gives a better estimate, and a moving window will smooth the time variation

![Figure 2](https://neurophotonics.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)  
Fig. 2 Spectra of \( \text{op} \) signal amplitude induced by 770-nm (5-nm, 400-Hz) and 800-nm (6-nm, 380-Hz) excitation light.

![Figure 3](https://neurophotonics.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)  
Fig. 3 B-scan image of a phantom vessel with a 50-\( \mu \)m inner diameter containing blood positioned on a sheet of white-colored copy paper.

![Figure 4](https://neurophotonics.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)  
Fig. 4 (a) En-face image of an arterial-venous phantom vessel pair. (b) B-scan image at the indicated site. Arrows in (a) indicate blood flow direction.
of oxygen saturation. Averaging SO\textsubscript{2} values over the sub-segments suppresses the phase noise in the \( op \) signal amplitude.

To demonstrate DWP-OCT for blood SO\textsubscript{2} measurement, the six blood samples prepared at different SO\textsubscript{2} levels were measured (99.6%, 89.2%, 84.1%, 69.0%, 57.3%, and 3.0%) with a commercial blood oximeter (AVOximeter 1000E, International Technidyne Corp., Edison, NJ). Each blood sample was separated into two volumes to ensure DWP-OCT and oximeter measurements could be carried out simultaneously, thus reducing measurement variation due to differences in reoxygenation. The DWP-OCT measurement time of a single blood sample was shorter than 30 min to minimize the effect of drift in the blood SO\textsubscript{2} levels (blood samples were deoxygenated by sodium dithionite).\textsuperscript{46} The DWP-OCT SO\textsubscript{2} measurement results of the blood samples are shown in Fig. 5. Each plot indicates the SO\textsubscript{2} level deduced from Eq. (2) and derived from the 15 segments lasting 0.5-s each. The solid line (green) and dashed lines (red and blue) represent the mean and standard deviation, respectively, of the 15 segments’ DWP-OCT SO\textsubscript{2} values. The SO\textsubscript{2} levels measured by a commercial oximeter are indicated in the right portion of each plot.

The six blood samples’ SO\textsubscript{2} levels cover a substantially wider range than physiological variation [from 70% (veins) to 97 to 99% (arteries)]. For each measured level, the oximeter SO\textsubscript{2} measurement results are within the experimental error of DWP-OCT measurement values (Fig. 6). The AVOximeter 1000E features a specified accuracy of ±1% and a precision of ±0.5% for blood SO\textsubscript{2} measurements.

### 3.3 Influence of Blood Flow on DWP-OCT SO\textsubscript{2} Measurement

To determine the impact of blood flow on DWP-OCT SO\textsubscript{2} measurement, we recorded the \( op \) signal amplitude in a 50-μm inner-diameter phantom blood vessel at different average blood flow speeds introduced by the syringe pump. For each DWP-OCT measurement, the SO\textsubscript{2} level was fixed at 98.2%. At increasing blood flow speeds, the \( op \) signal amplitude induced...
by blood absorption of each photothermal excitation beam was reduced, as shown in Fig. 7(a). A substantial reduction (80%) in the op signal amplitude was observed at the greatest average blood flow speed (17 mm/s).

The DWP-OCT measurements were recorded at average blood flow speeds from stationary to 17 mm/s, and the op signal amplitudes for 770-nm (400-Hz) and 800-nm (380-Hz) light were normalized by respective amplitudes at the stationary condition, as shown in Fig. 7(b).

4 Discussion

In this study, we constructed a DWP-OCT system for the imaging and measurement of static and flowing blood SO2 level in a phantom vessel. From Eq. (2), we find that the relative uncertainty in DWP-OCT blood SO2 values can be written as

$$\frac{\delta SO_2}{SO_2} = \frac{(a_{s2} - a_{i2}) - (a_{s1} - a_{i1})}{\chi_{12} - \chi_{12}}.$$

(3)

The variation in $\chi_{12}$ [$\delta \chi_{12}$/$\chi_{12}$; see Eq. (4)] can originate from phase variation in the optical pathlength ($op_1$ or $op_2$) or the fluence ($\Phi_1$ or $\Phi_2$) of photothermal excitation beams.

$$\left(\frac{\delta \chi_{12}}{\chi_{12}}\right)^2 \approx \left(\frac{\delta op_1}{op_1}\right)^2 + \left(\frac{\delta op_2}{op_2}\right)^2 + \left(\frac{\delta \Phi_1}{\Phi_1}\right)^2 + \left(\frac{\delta \Phi_2}{\Phi_2}\right)^2.$$

(4)

We define the op signal-to-noise ratio [SNR; see Eq. (5)], where op is the optical pathlength signal amplitude in response to photothermal excitation (380 Hz or 400 Hz), and $\delta$ op corresponds to the optical pathlength variation due to either the DWP-OCT system or relative motion between the DWP-OCT source beams (PhS-OCT probe beam and photothermal excitation beams) and sample constituents.

$$SNR(dB) = 10 \log \left(\frac{op}{\delta op}\right).$$

(5)

4.1 DWP-OCT Static Blood SO2 Measurement Error

In phantom vessel static blood SO2 measurement, low-power (~2.8 mW) photothermal excitation light gives op amplitudes of 2 to 5 nm, and a 0.3-nm uncertainty in $op$ amplitude gives a relative uncertainty $\delta op/\rho p = 6$ to 15% ($op$ SNR 8.2 to 12.2 dB). Laser power fluctuation can introduce a 2% uncertainty in $\Phi/\Phi$. Based on Eqs. (3) and (4), the effect of the op SNR on the relative blood SO2 measurement error ($\delta SO_2/\rho p$) was estimated (Fig. 8). Relative uncertainty in $\chi_{12}$ decreases with an increasing op SNR, as shown in Fig. 8(a). Relative uncertainty in DWP-OCT blood SO2 increases with decreased SO2 values, as shown in Fig. 8(b). At any blood SO2 level, $\delta SO_2/\rho p$ increases with increasing relative uncertainty in $\chi_{12}$. Each of the six measured blood samples’ relative SO2 measurement errors in a single (0.5-s) segment was deduced and plotted, as shown in Fig. 8(b), and they have values close to curves corresponding to 20% and 30% relative uncertainty in $\chi_{12}$.

To reduce DWP-OCT’s relative blood SO2 measurement error to within 5% (SO2 above 60%), relative uncertainty in $\chi_{12}$ must be less than 5%, requiring an op SNR above 15 dB ($\delta op/\rho p$ below 3%). A substantial increase in DWP-OCT SO2 measurement errors observed in 57.3% and 3% SO2 blood levels are consistent with computed values, as shown in Fig. 8(b). To increase DWP-OCT blood SO2 measurement accuracy and reliability, system phase stabilization is critical.

4.2 Effect of Blood Flow on SNR of the Optical Pathlength Signal

The accuracy of DWP-OCT SO2 measurement at various blood flow speeds can be determined by analysis of the SNR of the op signal in response to laser excitation [Eq. (5)]. SNR degradation with respect to increasing blood flow speed, illustrated in Fig. 9(a), suggests that the most reliable DWP-OCT SO2 measurements can be obtained at blood flow speeds up to 13 mm/s.

The SO2 level is calculated for average blood flow speeds up to 17 mm/s, as shown in Fig. 9(b). The SO2 measured by DWP-OCT is within the experimental error of values measured by a commercial oximeter for average blood flow speeds less than 13 mm/s. A 13-mm/s average blood flow speed is found in retinal arterioles30 to 40 μm in diameter. Relative blood SO2 measurement error increases with increasing blood flow speed, as shown in Fig. 9(c). The op SNR is a critical factor that determines accuracy of measured SO2 levels, as shown in Fig. 9(b). The results suggest that when the op SNR degradation exceeds 10 dB, SO2 levels measured by DWP-OCT are...
Fig. 8 (a) Relative $\chi_{12}$ error ($\delta\chi_{12}/\chi_{12}$) versus op SNR. (b) Relative blood $SO_2$ measurement error ($\delta SO_2/SO_2$) versus $SO_2$ for various levels of relative $\chi_{12}$ error. Horizontal axis: blood $SO_2$ level, vertical axis: relative error of $SO_2$. Solid curves represent conditions when the relative variations of $\chi_{12}$ are 5%, 10%, 20%, and 30%. Dashed lines: $SO_2$ of veins (70%) and arteries (97%), circles: relative blood $SO_2$ measurement error in six blood samples.

Fig. 9 (a) SNR degradation versus blood flow speed. Circle: SNR in response to 770-nm excitation, dashed line: linear fit, diamond: SNR in response to 800-nm excitation, solid line: linear fit, dotted line: 10-dB SNR op degradation. (b) $SO_2$ measurement in blood vessel phantom at various blood flow speeds. Diamond: $SO_2$ measured by DWP-OCT, solid line: $SO_2$ measured by oximeter (98.2%), dashed line: threshold speed above which SNR degradation exceeds 10 dB. (c) Relative blood $SO_2$ measurement error ($\delta SO_2/SO_2$) for a single segment (0.5 s) versus blood flow speed. Dashed line is linear fit.
no longer reliable. Experimental results suggest that a DWP-OCT system utilizing low-power (∼2.8 mW) photothermal excitation has sufficient stability and sensitivity to measure SO₂ levels in a 50-μm-diameter stationary blood vessel phantom with average blood flow speeds from stationary up to 13 mm/s.

Brownian motion and blood flow can also contribute to an increased op signal noise floor; in the blood flow experiments reported here, the difference in refractive indices between red blood cells (RBC) and blood plasma is one source of increased op signal noise. The time dependent optical pathlength \( [op(t)] \) of the probe beam traveling through the phantom vessel lumen can be expressed as

\[
op(t) = n_{RBC}l_{RBC}(t) + n_{plasma}l_{plasma}(t), \tag{6}
\]

where \( n_{RBC} \) and \( n_{plasma} \) are the group refractive indices of red blood cells and plasma, respectively; and \( l_{RBC} \) and \( l_{plasma} \) are the physical pathlengths that probe beam travels through RBC and blood plasma, respectively. The values of \( l_{RBC} \) and \( l_{plasma} \) vary randomly due to blood flow; a higher blood flow speed will cause \( op \) signal amplitude to change more rapidly, as indicated in Eq. (6), which results in an increased \( op \) signal noise floor between successive A-scans. In the case of stationary blood, Brownian RBC motion contributes to the \( op \) signal noise. For the phantom blood vessel tested here (with a 50 μm inner-diameter), the effect of Brownian RBC motion on \( op \) signal noise is approximately equivalent to the increase associated with a 6-mm/s blood flow speed relative to the stationary state.\(^{48}\) An increased \( op \) signal noise floor is observed in a larger vessel (300 μm inner-diameter) due to a longer physical pathlength. The SO₂ measurement has also been recorded in a 300-μm-diameter phantom blood vessel. At an equivalent average blood flow speed (11.8 mm/s), the \( op \) signal noise floor (1.82 nm) in the larger phantom vessel (330 μm inner-diameter) increases by 1.3 nm over the signal noise floor (0.52 nm) in the phantom vessel with a 50-μm inner-diameter.

For in vivo measurements, the relative motion between the DWP-OCT source beams and the bulk tissue is an additional noise source that degrades the \( op \) SNR. Tissue motion artifacts can be suppressed by increasing either the modulation frequency or the DWP-OCT A-scan rate. SS laser sweep rates of up to 5 MHz have been demonstrated.\(^{49}\) A higher modulation frequency will require photothermal excitation lasers with greater instantaneous power (corresponding to a shorter excitation period) to maintain fluence at the same level as the system presented here. In studies reported here, the incident radiant power (∼2.8 mW) is within the ANSI limits for skin. For retinal applications, the photothermal excitation power must be less than 0.75 mW.

Acknowledgments

This study was partially supported by NIH KL2 training grants (Parent Grant Nos. UL1RR025767 and KL2RR025766); by the San Antonio Area Foundation (Grant No. 130977); and by research support from Carl Zeiss Meditec to RVK and TEM, from the Department of Veterans Affairs (VA MERIT Award) to TQD, and by the NIH (R01 EY018855 and R01 EY014211) to TQD. The authors also gratefully acknowledge support from the National Institutes of Health (NIH R01EY016462).

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