Phase-resolved functional optical coherence tomography: simultaneous imaging of in situ tissue structure, blood flow velocity, standard deviation, birefringence, and Stokes vectors in human skin

Hongwu Ren, Zhihua Ding, Yonghua Zhao, Jianjun Miao, J. Stuart Nelson, and Zhongping Chen

Received March 15, 2002

We describe a phase-resolved functional optical coherence tomography system that can simultaneously yield in situ images of tissue structure, blood flow velocity, standard deviation, birefringence, and the Stokes vectors in human skin. Multifunctional images were obtained by processing of analytical interference fringe signals derived from two perpendicular polarization-detection channels. The blood flow velocity and standard deviation images were obtained by comparison of the phases from pairs of analytical signals in neighboring A-lines in the same polarization state. The analytical signals from two polarization-diversity detection channels were used to determine the four Stokes vectors for four reference polarization states. From the four Stokes vectors, the birefringence image, which is not sensitive to the orientation of the optical axis in the sample, was obtained. Multifunctional in situ images of a port wine stain birthmark in human skin are presented.

Optical coherence tomography (OCT) is a noninvasive, noncontact imaging modality that uses coherence gating to obtain high-resolution cross-sectional images of tissue microstructure. OCT is analogous to ultrasound imaging, except that infrared light waves rather than acoustic waves are used. Consequently, the spatial resolution of OCT is more than an order of magnitude higher than that of ultrasound. Several extensions of OCT have been developed for functional imaging of tissue physiology. Optical Doppler tomography combines the Doppler principle with coherence gating for tomographic imaging of tissue microstructure and blood flow simultaneously. Polarization-sensitive OCT combines polarization-sensitive detection with OCT to determine tissue birefringence. Both techniques use phase information from the interference fringes to yield physiologically important information. Although several potential clinical applications have been demonstrated, to date, optical Doppler tomography and polarization-sensitive OCT imaging have been performed with separate systems. However, there are many clinical indications where determination of both blood perfusion and tissue birefringence is important. For example, the loss of cutaneous blood perfusion and changes in tissue birefringence are two critical factors used to determine burn depth. Simultaneous imaging of the changes in blood perfusion and collagen birefringence by functional optical coherence tomography (F-OCT) may permit better clinical management of burn injuries.

In this Letter we describe a phase-resolved F-OCT system that can simultaneously obtain in situ images of tissue structure, blood flow velocity, standard deviation, and birefringence, and Stokes vectors in human skin. The system is based on a phase-resolved signal processing method whereby the multifunctional images are obtained from the analytical interference fringe signals derived from two perpendicular polarization-detection channels. A fiber-based high-speed F-OCT system for multifunctional imaging is shown in Fig. 1. A 1.3-μm partially coherent light source with a bandwidth of 80 nm is used. Light entering a 2 × 2 fiber splitter is divided equally into the sample and reference arms of a Michelson interferometer. In the reference arm, a rapid-scanning optical delay line is aligned such that only group-delay scanning at 500 Hz is...
generated without phase modulation. A stable phase modulation at 500 kHz is generated with an electro-optic modulator for heterodyne detection.\(^6\) The fringe signals from two polarization channels are detected by two photodetectors, high-pass filtered, amplified, and digitized by a dual-channel, 5-MHz, 12-bit analog-to-digital conversion board. A polarization modulator is used to control the polarization state of light in the reference arm, which varies rapidly between states that are orthogonal in the Poincaré sphere at 125 Hz. The choice of orthogonal polarization states in the Poincaré sphere is important because it ensures that the birefringence measurements will be independent of the orientation of the optical axis in the sample. The Stokes vectors, are accurately measured by generation of four states of light polarization for each lateral location. For each polarization state, four A-line scans are performed sequentially. Therefore, a total of 16 A-line scans are used to obtain images of tissue structure, formed sequentially. Therefore, a total of 16 A-line scans are per-

\[
\mathbf{S}_{0,n} = \sum_{m=-(n-1)M}^{nM} \frac{1}{4} \sum_{j=1}^{4} \left[ \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) + \tilde{\mathbf{F}}^*_j(t_m) \tilde{\mathbf{F}}_j(t_m) \right],
\]

\[
\mathbf{S}_{1,n} = \sum_{m=-(n-1)M}^{nM} \frac{1}{4} \sum_{j=1}^{4} \left[ \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) - \tilde{\mathbf{F}}^*_j(t_m) \tilde{\mathbf{F}}_j(t_m) \right],
\]

\[
\mathbf{S}_{2,n} = \sum_{m=-(n-1)M}^{nM} \frac{1}{4} \sum_{j=1}^{4} \left[ 2 \text{Re} \left[ \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) \right] \right],
\]

\[
\mathbf{S}_{3,n} = \sum_{m=-(n-1)M}^{nM} \frac{1}{4} \sum_{j=1}^{4} \left[ 2 \text{Im} \left[ \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) \right] \right],
\]

where \( S_0, S_1, S_2, \) and \( S_3 \) are the four components of the Stokes vector. \( S_1, S_2, \) and \( S_3 \) are the normalized coordinates of the Stokes vector in the Poincaré sphere that characterize the polarization state of the backscattered light, \( S_0 \) is the module of the Stokes vector characterizing light intensity, \( \tilde{\mathbf{F}}_j(t_m) \) and \( \tilde{\mathbf{F}}^*_j(t_m) \) are the complex signals detected from the two orthogonal polarization channels at axial time \( t_m \), for the \( j \text{th} \) A-scan, and \( \tilde{\mathbf{F}}_j(t_m) \) and \( \tilde{\mathbf{F}}^*_j(t_m) \) are their conjugates. From the Stokes vectors for the four states of light polarization, the structural image is obtained by averaging of the four \( S_0 \). The phase-retardation image, which characterizes the birefringence distribution in the sample, is calculated by rotation of the Stokes vectors in the Poincaré sphere.\(^7\)

Our phase-resolved F-OCT system was used to perform \textit{in situ} imaging of a port wine stain birthmark in human skin. Simultaneous imaging of tissue structure, blood flow velocity, standard deviation, birefringence, and the Stokes vectors is shown in Fig. 3. The image area is \( 2 \text{mm} \times 1.5 \text{mm} \), with \( 200 \times 150 \) pixels and 3200 axial scans. With a 500-Hz axial scanner in the delay line, the image acquisition time is approximately 7 s. Image acquisition times of less

\[
\frac{d}{dt} = \frac{2}{2\pi T} \tan^{-1} \left[ \text{Im} \left( \sum_{m=-(n-1)M}^{nM} \sum_{j=1}^{4} \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) \right) \right] \left/ \text{Re} \left( \sum_{m=-(n-1)M}^{nM} \sum_{j=1}^{4} \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) \right) \right],
\]

\[
\sigma_n^2 = \frac{1}{2n^2T^2} \left[ 1 - \frac{\sum_{m=-(n-1)M}^{nM} \sum_{j=1}^{4} \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m)}{\sum_{m=-(n-1)M}^{nM} \sum_{j=1}^{4} \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m)} \right],
\]

\[
\langle \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) \rangle = \frac{1}{2} \left[ \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) + \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) + \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) + \tilde{\mathbf{F}}_j(t_m) \tilde{\mathbf{F}}^*_j(t_m) \right],
\]

where \( \tilde{\mathbf{F}}_j(t_m) \) and \( \tilde{\mathbf{F}}^*_j(t_m) \) are the complex signals at axial time \( t_m \), corresponding to the \( j \text{th} \) A-scan and its conjugate, respectively, \( T \) is the time duration between A-scans, and \( M \) is an even number that denotes the window size in the axial direction for each pixel. Note that Eq. (5) is slightly different from our previously published work.\(^9\) We replaced term \( \Gamma_j(t_m) \Gamma_j^*(t_m) \) with \( 1/2[\Gamma_j(t_m) \Gamma_j^*(t_m) + \Gamma_j(t_m) \Gamma_j^*(t_m)] \) to ensure nonnegativity in the variance values. The modified algorithm presented above also effectively reduces speckle noise. The calculated Doppler frequency shifts and standard deviation values for four polarization states are then averaged to yield the Doppler shift and variance images. The velocity sensitivity of phase-resolved F-OCT is \( \sim 10 \mu \text{m/s} \). The Doppler flow image in phase-resolved F-OCT is very sensitive to any environmental disturbances such as sample stability. However, because we are interested in relative motion of blood flow with respect to tissue, we correct motion artifacts by choosing tissue as a stable reference point for phase measurement in each axial scan.\(^12\)
Fig. 2. Synchronizing time-clock diagram for the F-OCT system. Channel (Ch) 1 is the signal generated from the synchronized trigger output of function generator 1 and arms the analog-to-digital conversion. Channel 2 is the signal generated by function generator 1 to control the polarization modulator. Channel 3 is the triangle signal from the function generator 3 to control the galvanometer scanning. Channel 4 is the signal generated by synchronized output of function generator 3 through a digital delay. This signal is used to trigger the phase modulation and data acquisition.

Fig. 3. Simultaneous imaging of the Stokes vectors, structure, blood flow velocity, standard deviation, and birefringence from *in vivo* human skin. The top four panels are the Stokes vector image corresponding to the four reference polarization states. The bottom four panel images are A, structural image; B, blood flow velocity image; C, standard deviation image; D, phase-retardation image.

than 1 s are possible if an 8-kHz resonator scanner is used. The computation time to derive all the images with a Pentium IV PC is less than 10 s. The top four panels are the Stokes vectors corresponding to the four different polarization states shown in Fig. 2. From left to right, they are $S_0$, $S_1$, $S_2$, and $S_3$, obtained by averaging of four A-lines in every polarization state. The bottom panel images are tissue structure, Doppler frequency shift (blood flow velocity), standard deviation, and birefringence. Implementation of the polarization-diversity detection in the intensity, Doppler and standard deviation images significantly reduces the artifact due to tissue birefringence. In addition, speckle noise is also greatly reduced. The greater detail noted in the structural image compared with the $S_0$ image indicates that the speckle noise is greatly reduced when polarization-diversity detection is used and multiple scans are averaged. Small vessels can be clearly identified in the Doppler frequency shift and standard deviation images. The arrows in the Doppler frequency shift and standard deviation images indicate that the blood vessels are located approximately 500 μm below the skin surface. The nonuniform birefringence of human skin can also be identified in the phase-retardation image.

In summary, we have developed a phase-resolved F-OCT system capable of simultaneous imaging of *in situ* tissue structure, blood flow velocity, standard deviation, birefringence, and the Stokes vectors in human skin.

This work was supported by research grants awarded from the National Institutes of Health (EB-00293, RR-01192, and EB-00255) and the National Science Foundation (BES-86924). Institutional support from the U.S. Air Force Office of Scientific Research (F49620-00-1-0371) and the Beckman Laser Institute Endowment is also gratefully acknowledged. Please address all correspondence to Z. Chen at zchen@bli.uci.edu.

**References**