Three-Dimensional Reconstruction of *In Vivo* Blood Vessels in Human Skin Using Phase-Resolved Optical Doppler Tomography

Yonghua Zhao, Kjell Morten Brecke, Hongwu Ren, Zhihua Ding, J. Stuart Nelson, and Zhongping Chen

***Abstract*—Phase-resolved optical Doppler tomography (ODT) has very high sensitivity while maintaining a fast axial scanning rate, making it possible to reconstruct blood flow in three dimensions. Here we demonstrate an ODT system that employed novel signal-processing techniques to remove aliasing effects and artifacts caused by lateral scanning and target movement. The results show that these techniques not only simplified ODT, but also improved lateral scanning speed. Using these signal-processing techniques, three-dimensional images of in vivo blood vessels in human skin were then reconstructed**

***Index Terms*—Biomedical imaging, biomedical optics, optical coherence tomography, optical Doppler tomography.**

PHASE-RESOLVED optical Doppler tomography (ODT) has very high sensitivity while maintaining a fast axial scanning rate, making it possible to reconstruct blood flow in three dimensions. Here, we demonstrate an ODT system that employed novel signal-processing techniques to remove aliasing effects and artifacts caused by lateral scanning and target movement. The results show that these techniques not only simplified ODT, but also improved lateral scanning speed. Using these signal-processing techniques, three-dimensional (3-D) images of *in vivo* blood vessels in human skin were then reconstructed ODT [1]–[3] has emerged as an extension of optical coherence tomography (OCT) [4] for performing high-resolution cross-sectional imaging of tissue structure and blood flow *in vivo* and *in situ*. A recently developed phase-resolved approach to ODT [5] further improved the sensitivity of detectable blood flow velocity (10 μm/s) while maintaining high spatial resolution (10 μm). Phase-resolved ODT [6] has been shown a very good methodology to study the microcirculation of human skin. However, in order to determine capillary structure, 3-D imaging is necessary to obtain vessel topology. In this paper, we describe two techniques to overcome the problems encountered when reconstructing vessels in three dimensions using phase-resolved ODT. We demonstrate the first 3-D images of *in vivo* blood vessels in human skin using phase-resolved ODT.

The principle of OCT is based on low-coherence Michelson interferometry where scanning the optical path length in the reference arm produces interference fringes. The fringe envelope represents the reflectivity and/or scattering amplitude at different depths in the sample. The coherence length of the light source determines axial resolution. Two-dimensional (2-D) tomographic imaging of the sample is then reconstructed from adjacent axial profiles while moving the sample (or optical probe) in the lateral direction. When imaging using conventional ODT, not only is the fringe envelope measured, but also the fringe frequency is calculated using a time-windowed fast Fourier transform (FFT). The Doppler frequency shift caused by blood flow can be detected and used to determine flow velocity in two dimensions. Since the minimum detectable Doppler frequency shift is inversely proportional to the window time of each pixel, it becomes very difficult to locate blood flow when the axial-scanning rate, which is necessary for *in vivo* real-time imaging, is increased. Phase-resolved ODT was developed to overcome this problem by introducing a Hilbert transform to determine phase information for each pixel so that the phase change between sequential axial scans can be used to calculate the Doppler frequency shift [5], [6]. Because this method increases the equivalent window time to that of the axial scanning period, the minimum detectable Doppler frequency is significantly reduced while maintaining high axial scanning speed and high axial resolution. This unique feature makes phase-resolved ODT an excellent methodology for imaging the microvasculature of human skin in three dimensions.

The optical system used in this paper is very similar to the 2-D phase-resolved ODT apparatus previously described [5] except that one more dimension of lateral scanning is added. Briefly, a low-coherence light source with center wavelength of 1310 nm and bandwidth of 80 nm is coupled into a fiber-based Michelson interferometer. The optical probe in the sampling arm has a lateral resolution of 10 μm and is mounted on a 2-D translation stage for lateral scanning. Axial scanning is accomplished in the reference arm by a rapid scanning optical delay-line (RSOD) [7] based on a grating-dispersion system to decouple the group and phase delays. The RSOD is aligned so that the group delay is scanning at a rate of 500 Hz and amplitude of 2 mm (optical path length) while the scanning speed of the phase delay is zero. An electrooptical modulator is used in the reference arm to produce stable interference fringes synchronized for each axial

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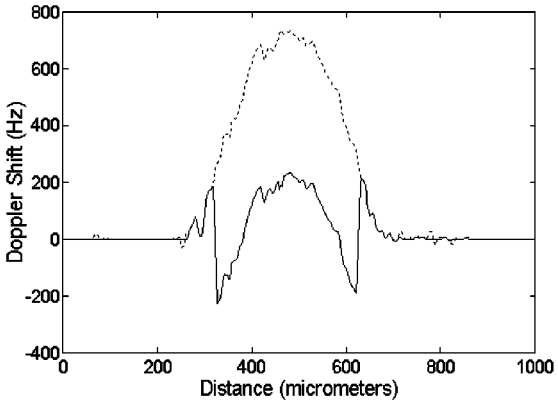

Fig. 1. Doppler shift distribution in the axial direction. Solid line represents values calculated directly from (1) and the dashed line represents corrected values.

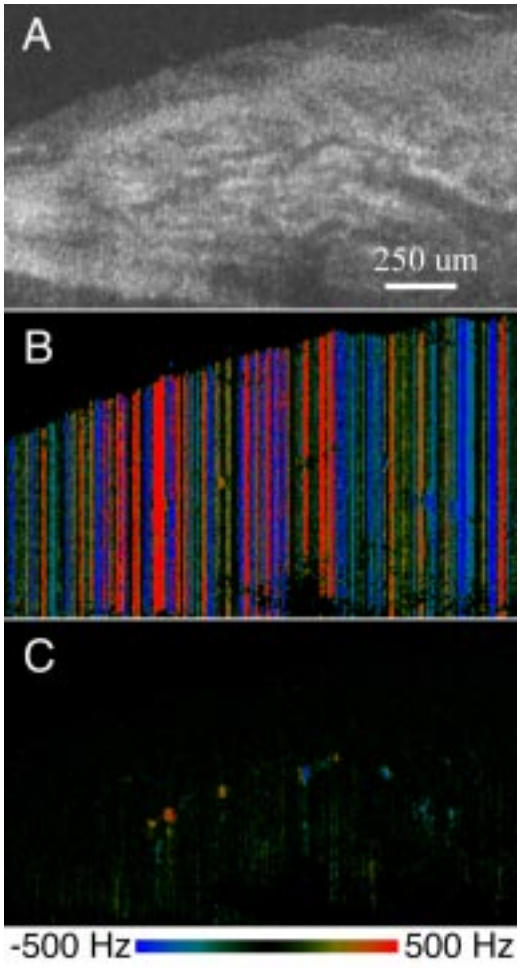

Fig. 2. (A) OCT image and (B) and (C) ODT images from *in vivo* normal human skin. The background movement shown in (B) was removed in (C) and now the blood vessels are clearly apparent.

scan. The fringe phase difference between each sequential axial scan should be zero if the scattering particle in the sample does not move during the scan. Any nonzero phase difference indicates a moving object. We determine the phase at any point in the interference fringe by a Hilbert transform and then calculate the Doppler shift (Δf) by the following equation [5]:

$$\Delta f = \frac{1}{2\pi T}\tan^{-1}\left(\frac{\mathrm{Im}\left(\sum_{j=1}^{n}\Gamma_{i,j}\cdot\Gamma_{i,j+1}^{*}\right)}{\mathrm{Re}\left(\sum_{j=1}^{n}\Gamma_{i,j}\cdot\Gamma_{i,j+1}^{*}\right)}\right). \quad (1)$$

Here, T is the axial scanning period; $\Gamma_{i,j}$ is the digitized fringe signal in a complex format after the Hilbert transform; i represents different data points along each axial scan and j represents different axial scans; and n is the number of sequential scans that are averaged. In practice, sensitivity and the signal-to-noise ratio in the ODT images are significantly improved when $n \geq 4$.

There is a compromise between velocity sensitivity and dynamic range. High sensitivity usually comes with a small measurable range. As noted in (1), a limitation is that the range of phase differences determined by the inverse tangent function is only π, from $-\pi/2$ to $\pi/2$. This range can be extended to 2π if the sign of the denominator is checked before calculating the inverse tangent function, which means that the measurable Doppler shifts will be from $-f_s/2$ to $f_s/2$, where f_s is axial scanning frequency. Any Doppler shift outside this range will cause an aliasing effect, which is the same phenomenon observed in pulsed Doppler ultrasound imaging. By increasing the axial scanning frequency, the aliasing effect can be avoided. However, the galvanometer used in the RSOD has a maximum scanning frequency of approximately 1 kHz. Consequently, Doppler shifts determined by the phase-resolved method range from -500 to 500 Hz, which is approximately 1 mm/s assuming a probe angle of 45°. Such a value is relatively small when compared with the 0.5 mm/s mean blood flow velocity in human capillaries. Changing the probe angle may increase the upper limit if the direction of blood flow is known. However, in actual practice, when imaging the complex microvascular network in human skin, this is very difficult.

To overcome this limitation, we have developed a new approach to Doppler image processing. Using this approach, the Doppler shift is not only determined by (1), but also dependent on the values of neighboring points. If the difference between neighboring points exceeds half the axial scanning frequency, the Doppler shift will be added (or subtracted) by a value equal to that of the axial scanning frequency, f_s. Fig. 1 shows an example of this approach. The sample used in this experiment is a 5% concentration of polystyrene beads (diameter: 0.356 μm) flowing in water through a glass tube. The average flow velocity calculated from the pump speed is 0.264 mm/s. The axial scanning frequency is 500 Hz and the angle between the probe and flow direction is 60°. The axial profile of Doppler shifts along the center of the tube is shown in Fig. 1. The solid line is derived directly from (1) and the frequency variation caused by aliasing can be seen clearly. The dashed line is the corrected values, which are very similar to a parabolic distribution predicted by theory. Further experiments have shown that this method can extend the measurable Doppler frequency shift up to three times that of axial scanning frequency.

The high sensitivity of phase-resolved ODT allows capillary blood flow to be imaged. However, phase-resolved ODT is also very sensitive to any environmental disturbances such as optical

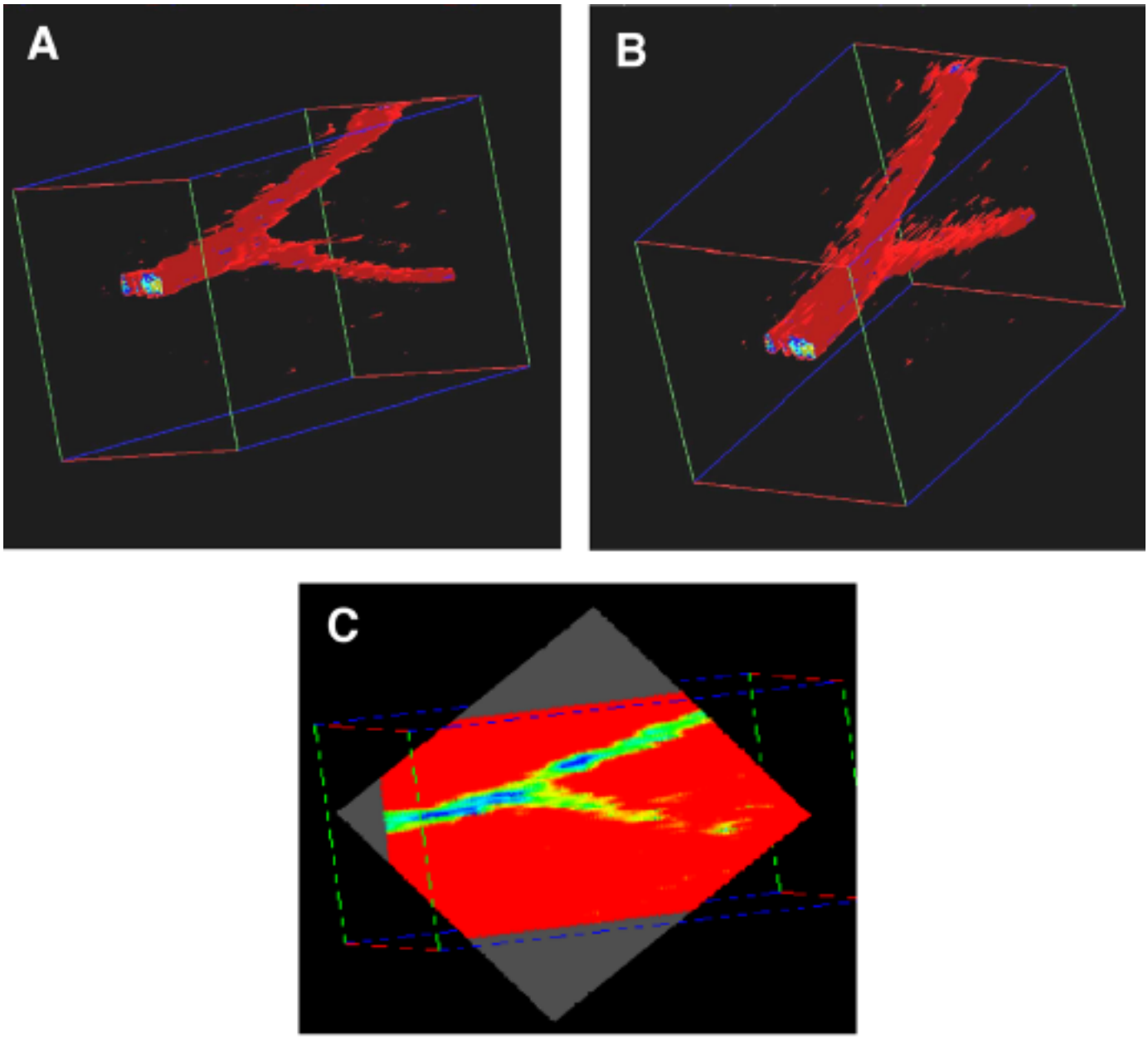

Fig. 3. (A) and (B) Three-dimensional ODT images of a rodent blood vessel viewed from two different angles. (C) Cross-sectional image through an arbitrary plane.

table oscillation or electromechanical fluctuations in the motorized translation stage, both of which will cause Doppler shifts in the fringe signal. In structural OCT images, these shifts in fringe frequency will cause a negligible effect on the demodulated amplitude signal because they are much smaller than the detection bandwidth. However, these disturbances will cause a large phase shift, resulting in a background signal that is comparable with the original signal. The large background signal forms stripes, as shown in Fig. 2. These images were taken from *in vivo* normal human skin. Fig. 2(A) and (B) shows the structural and Doppler images, respectively. Background noise in the Doppler image can be identified easily by its characteristic vertical striations. The consistent color in each stripe indicates that variations in the Doppler shift caused by the environment are very low within the time window of each A-line scan and, therefore, negligible. These results suggest that the background Doppler shift can be removed if we can designate an object as a reference for the initial phase in each A-line scan. In most biological tissues, we are interested in determining blood flow velocity relative to static tissue constituents. Consequently, the phase shift from static tissue can be used as a reference for the initial phase and zero background. Fig. 2(C) shows the result after applying this method. The background noise is removed efficiently and blood vessels are now clearly apparent. This method also makes synchronization of phase modulation between sequential axial scans less critical because it can compensate for the initial phase difference at the beginning of each axial scan.

Three-dimensional vessel reconstruction based on phase-resolved ODT is demonstrated in Fig. 3. Axial scanning is in the Z direction at a speed of 500 Hz. The optical probe in the sampling arm performs the X scan first at a velocity of 0.5 mm/s. After the

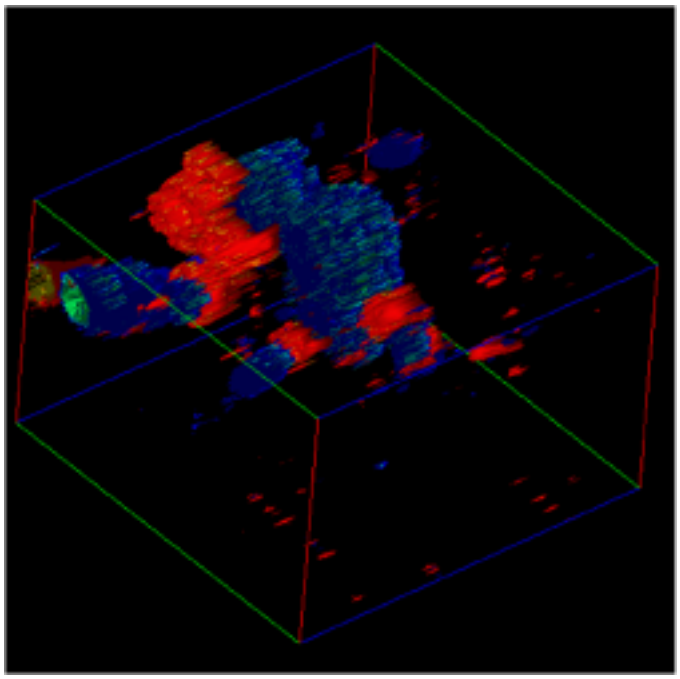

Fig. 4. Three-dimensional ODT images that shows multiple blood vessels in human skin from a patient with a PWS birthmark.

X scan is finished, the optical probe will return to the zero-X position quickly and then move to the next Y position to start another X scan. A dual-processor computer performs the data acquisition and signal processing simultaneously. The scanning range $(X \times Y \times Z)$ is $2 \times 2 \times 1$ mm and the distance between neighboring X scans is 100 μm. Fig. 3(A) and (B) shows a clear blood vessel shaped as a Y structure in rodent ear viewed from two different angles. A cross-sectional image can be obtained in an arbitrary plane, as shown in Fig. 3(C). Fig. 4 shows multiple blood vessels imaged in human skin from a patient with port wine stain (PWS) birthmark. Different colors represent different signs of the Doppler shift, which depends on the angle between the direction of flow and probing beam. The convoluted nature of the blood vessels is consistent with the typical vasculature observed in PWS patients.

In conclusion, we have presented the first 3-D images of *in vivo* blood vessels in human skin using phase-resolved ODT. Novel signal-processing techniques were described to eliminate aliasing effects and remove background noise in the images.

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