Visualization of human retinal micro-capillaries with phase contrast high-speed optical coherence tomography

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ABSTRACT

We present high-speed Fourier-domain optical coherence tomography (Fd-OCT) with the phase variance based motion contrast method for visualizing retinal micro-circulation *in vivo*. This technique allows non-invasive visualization of a two-dimensional retinal perfusion map and concurrent volumetric morphology of retinal microvasculature with high sensitivity. The high-speed acquisition rate at 125kHz A-scans enables reduction of motion artifacts with increased scanning area if compared to previously reported results. Several scanning schemes with different sampling densities and scanning areas are evaluated to find optimal parameters for *in vivo* imaging. In order to evaluate this technique, we compare OCT micro-capillary imaging using the phase variance technique with fundus fluorescein angiography (FA). Additionally, volumetric visualization of blood flow for a normal subject is presented.

Keywords: Optical coherence tomography; Phase contrast technique; Imaging system; Medical optics instrumentation

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1 INTRODUCTION

Visualization of the retinal vasculature and microcirculation is essential for clinical diagnostics and treatment monitoring of retinal vascular diseases. Currently, the most widely used technique for examining the circulation of the retina in clinical settings is fundus fluorescein angiography (FA). This is a procedure in which the fundus is photographed through a bandpass filter following excitation of an extrinsic fluorophore, fluoroscein sodium (a compound that fluoresces when absorbing short-wavelength light) as is injected into the blood stream through a vein. Usually the spectral filter is used to measure only light emitted by this compound. In rare instances this injection may cause adverse reactions in patients who are hypertensive or elderly [1]. In contrast to FA, optical coherence tomography (OCT) [2, 3] is a noninvasive imaging technique that doesn't require any contrast agent for ocular imaging and has the benefit of offering depth information about the vasculature. The phase variance based motion contrast technique for Fourier-domain OCT (Fd-OCT) has been implemented successfully in visualizing vasculature of the zebrafish [4], ocular circulation of the mouse [5], and human retina [6]. Recently, several other OCT methods were demonstrated to visualize retinal blood vessel maps [7 - 12].

This manuscript investigates phase variance OCT capabilities for visualization of *in vivo* retinal microvasculature without use of extrinsic contrast agents. High speed image acquisition for Fd-OCT allows a dramatic decrease of motion artifacts and increases the region of interest that can be imaged within a few seconds in a single volume acquisition. To visualize micro-capillaries in two- and three-dimensions, we use the phase variance based motion contrast method described in ref. [6]. Several scanning patterns are used for various sampling densities and scanning areas. Phase variance OCT images are compared with fundus FA images. The processed data show depth-resolved retinal micro-vascular maps for different field of views and total acquisition times. Finally, two-dimensional phase variance data over a selected depth region as well as three-dimensional representation of the vasculature will be presented.

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2 MATERIAL AND METHODS

2.1 Experimental system

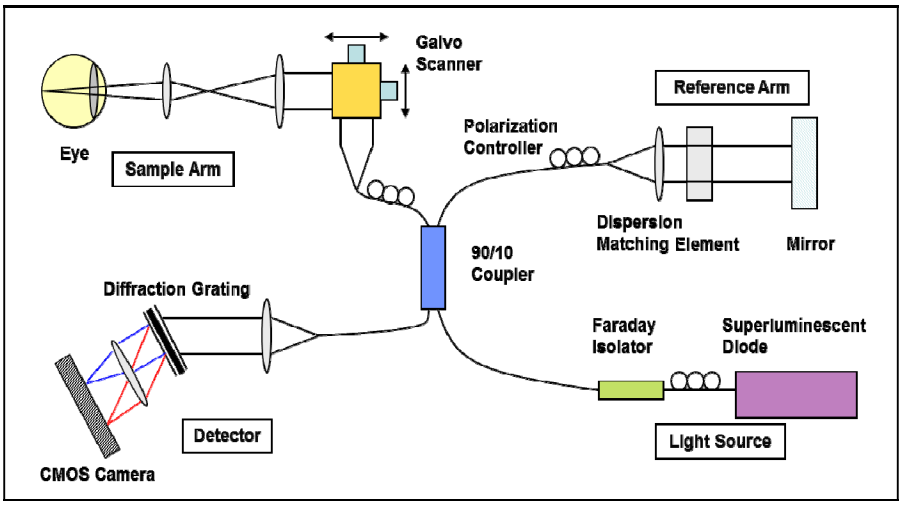

Figure 1. Schematic of the Fourier-domain OCT instrument.

Figure 1 depicts a schematic of the Fourier-domain OCT system with CMOS (Complementary Metal Oxide Semiconductor) line detector [13]. A superluminescent diode (Superlum, Ireland) with an 855nm central wavelength and a full width half maximum of 75nm is used as a light source. The axial resolution is 4.5µm in the retina (refractive index, n=1.38). The lateral resolution of the system at the retina is estimated at 10µm to 15µm depending on subject's ocular aberrations. A standard fiber-based OCT instrument is operated in Michelson interferometer configuration, connecting the fiber coupler (OZ Optics, Ontario, Canada) with 90/10 splitting ratio. 10% of the input light is sent to the sample arm and the power of the imaging beam at the cornea is 350µW. The remaining 90% of the light is sent toward the reference arm where is back reflected by the mirror and passed to detector arm where it interferes with the back scattered light from the sample arm. The reference arm length matches the position of the imaged sample. The resulting interference in the form of the spectral fringe pattern between the sample and the reference arm is detected in the custom-built spectrometer. The spectrometer consists of a 75mm focal length collimating objective, a 1200 l/mm diffraction grating, and a 150mm objective which focusing the light onto the CMOS detector (spL4096-140 km, Basler). The maximum line rate of the detector is 140kHz at 4096 pixels and 10x10µm (dual line) pixel size. Configuration setups of the CMOS camera are 4tap, 12bit, 80MHz camera link clocks, the line averaging mode, a 125kHz line rate and 2048 active detector pixels.

2.2 Image acquisition and data processing

Fd-OCT data sets were acquired in the Vision Science and Advanced Retinal Imaging laboratory at the University of California Davis Medical Center on a healthy subject with normal ocular media. Written informed consent approved by the Institutional Review Board is obtained prior to testing. Scanning areas of the retina are $3x3mm^2$ or $1.5x1.5mm^2$. Multiple B-scans (3 or 5) are used to gain proper statistics of phase variance. The exposure time during A-line acquisition is 6.3µs. Spectral data are saved in 12-bit tiff format for processing in Labview using standard Fd-OCT procedures including spectral shaping, zero-padding, and dispersion compensation [14-16]. All human retinal images shown in this manuscript have been acquired *in vivo* at 125,000 axial scans (A-scan) per second.

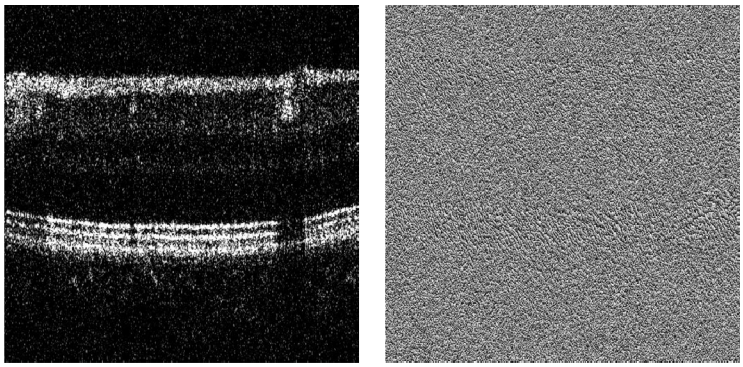

Figure 2. Fourier domain optical coherence tomography: intensity profile ($I(z)$) and phase profile ($\Phi(z)$).

As shown in Figure 2, the Fourier transform of the spectral fringes acquired by the CMOS detector produce a complex OCT signal with intensity $I(z)$ and phase $\Phi(z)$ information. In order to visualize capillaries in the retinal layer, we use the phase variance method based on motion detection [6]. The basic concept of motion detection relies on calculating phase differences between identical structures on consecutive B-scans by

$$\Delta\Phi(z) = \Phi(z, t+T) - \Phi(z, t) \tag{1}$$

which can be interpreted as flow induced phase changes in the sample. Here, $\Phi(z)$ is the phase value in the B-scans at depth position z, and T is the time separation between two consecutive B-scans.

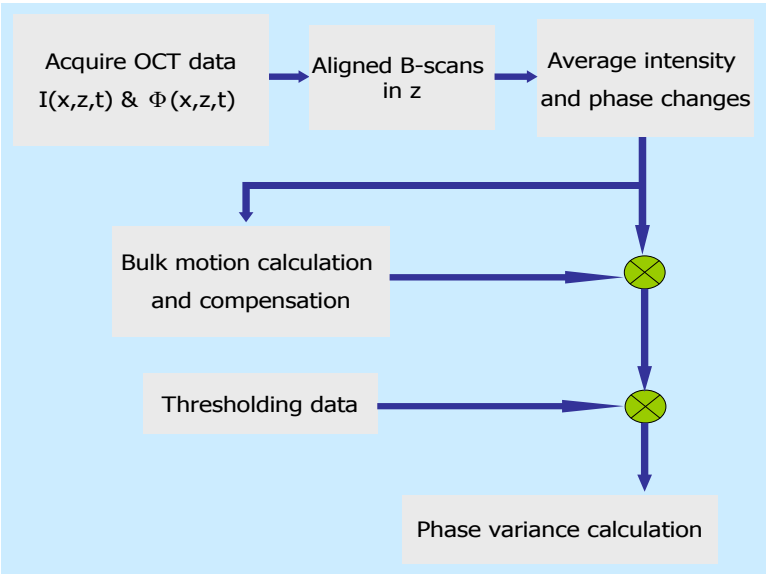

Figure 3. Diagram of data processing with the phase variance method [6].

The flow chart of the data processing method is illustrated by Figure 3. The Biological Imaging Center at the California Institute of Technology post-processes phase variance values using data sets of the OCT intensity and phase information. Average intensity images and phase differences are calculated from sets of B-scans acquired at the same location. The phase unwrapping method is applied to obtain the phase difference values. Additionally, bulk motion removal and histogram-based thresholding processing are implemented to remove phase shifts caused by eye motion. The phase variance based motion contrast procedure generates B-scans of retinal flow. We use the *en face* projection to view the results in 2D vascular maps similar to the ones obtained with FA.

3 RESULTS AND DISCUSSION

The retinal vascular flow image shown in Figure 4 (a), is reconstructed using a summation of the retinal contributions of the phase variance Fd-OCT data. These data were obtained from the Fd-OCT volume acquired over a 3x3mm^2 retinal area captured in approximately 3.6 seconds. Figure 4 (b) shows the FA image over the same region for comparison. The phase variance OCT image is similar to the FA image blood vessel networks of the retina. Increased imaging speed of the system allows shorter acquisition time, resulting in reduction of imaging artifacts caused by involuntary eye motion.

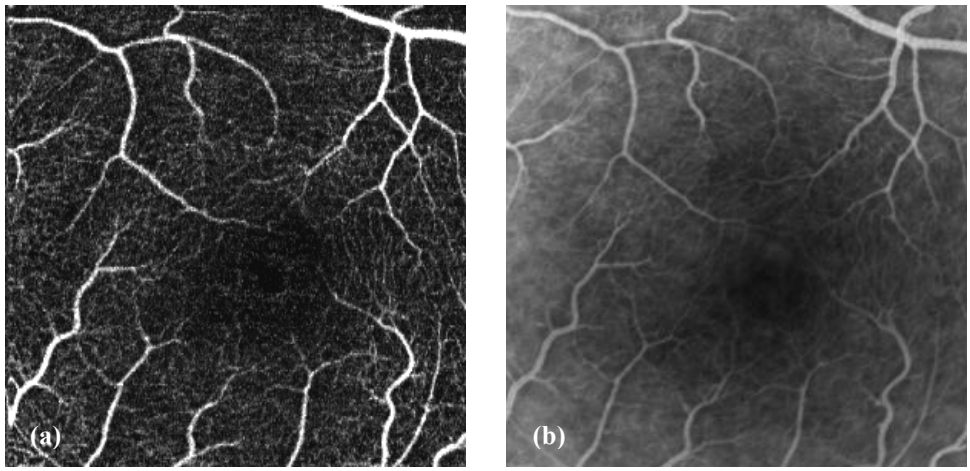

Figure 4. Human retinal vasculature images. Image size is 3x3mm^2. (a) Phase variance OCT retinal summation image. The imaging acquisition time is 3.6 seconds. (b) Fluorescein angiography (FA).

Next the pvOCT images acquired over of the human central retina are illustrated in Figure 5. We compare them to fundus FA acquired at the same location. Since our method includes depth information of micro-capillary locations we included this in a color depth-coded projection view as shown in Figure 5 (c). The RGB color map codes axial location of the vessels; red color vessels as a top layer, green color capillaries as an intermediate vascular bed, and finally blue color micro-capillaries as a deeper vascular plexus layer.

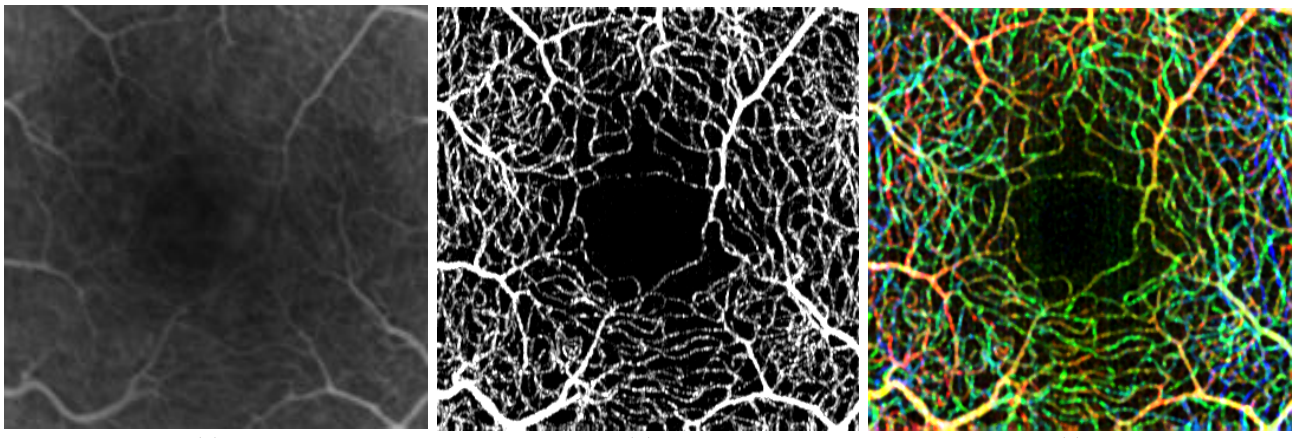

Figure 5. Human retinal micro-capillary network. Image size is 1.5x1.5mm^2. (a) Fluorescein angiography (FA). (b) Projection view of pvOCT. (c) Color depth-coded projection view of pvOCT. The imaging acquisition time of pvOCT is 3.5 seconds.

Figure 6 shows three-dimensional visualization of color depth-coded image of pvOCT from Figure 5 (c). This image shows volumetric information of the vessel network at the parafoveal region in the human retina. Note that the red-colored, larger diameter, capillaries have green colored shadows underneath. This is because the pvOCT method creates a shadow artifact (non-zero phase variance values) below relatively large vessels.

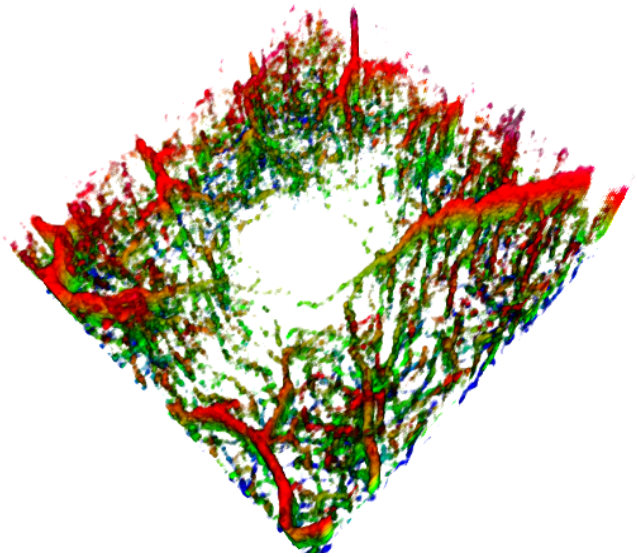

Figure 6. Three-dimensional color depth-coded image of pvOCT, using the same data presented in Figure 5 (c).

4 CONCLUSIONS

We demonstrate an imaging system and processing algorithm enabling two-dimensional visualization of the capillary network over human foveal and parafoveal regions as well as three-dimensional representation of the vasculature in the human retina. Vasculature imaging with phase variance OCT produces results comparable to fundus FA imaging without using any contrast agents. Phase variance OCT with rapid image acquisition at an A-scan speed of 125kHz reduces acquisition time, hence decreasing eye motion artifacts. pvOCT could play a pivotal role in early diagnosis of retinal vascular diseases including diabetic retinopathy and vascular-related macular degeneration.

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