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***In vivo*, three-dimensional imaging of human corneo-scleral and chorio-retinal vasculature with high penetration phase stabilized swept source optical coherence angiography (SSOCA)**

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Abstract: We illustrate a high-speed (100 kHz) high penetration phase stabilized swept source optical coherence angiography (SSOCA) system for *in vivo* volumetric depth-resolved vasculature imaging of anterior and posterior segment of human eye.

OCIS codes: (110.4500) Optical coherence tomography, (110.0110) Imaging systems; (170.3880) Medical and biological imaging; (170.4470) Ophthalmology; (280.2490) Flow diagnostics.

Introduction: OCT provides high-resolution cross-sectional and volumetric imaging non-invasively [1]. It is widely used as a standard procedure in clinical ophthalmology for retinal as well as anterior segment disease diagnosis [2,3]. Most of the current clinical OCT technology, mainly Spectral domain (SD)OCT, is operating in the 800-950 nm spectral window. This technology permits efficient measurements of retinal and corneal structures, including vessels and capillaries, but provides limited ability to examine choroid and its layered vasculature beds. This is mainly because the 800-950 nm bands light is strongly scattered and absorbed by sclera, retinal pigment epithelium (RPE) and the choroid. Swept-source (SS) OCT is now an attractive alternative for 1 μm range OCT over SD-OCT. Its main advantages include robustness to sample motion, a long measurement range in depth due to short instantaneous line-width, linear sampling in wavenumber (k-clock – trigger), compactness, increased detection efficiency (balanced detection scheme) and high imaging speed [4,5]. SSOCA is a non-invasive, non-contact angiographic technique which doesn't need any exogenous dyes like other existing angiographic method fluorescein angiography (FA) and indocyanine green angiography (ICGA). It is based on high speed and high resolution (SSOCT) which utilizes a low coherence interferometry and phase variance contrast (PVC) method. Standard SS-OCT systems often suffer from jitter in synchronization between the wavelength sweep and data acquisition timing; this jitter causes small random spectral shifts among interference spectra and results in phase instability. This phase instability is a critical problem for phase-sensitive OCT modalities including PVC-OCT. We have described recently [6] a simple method for phase stabilization using A-line trigger from a fixed wavenumber Fiber Bragg Grating (FBG) and numerical phase stabilization, without any major additional calibration hardware changes. Here, we describe SSOCA system that allows mapping of blood circulation in different layers of the corneo-scleral and chorioretinal complex of a normal human eye *in-vivo*.

Materials and methods: The scheme of a prototype fiber-based OCT system used in this study is shown in Fig. 1 that allows posterior as well as anterior segment imaging. The light source was an external cavity tunable laser (ECTL), swept-source laser (Axsun Technologies), with a central wavelength of 1060nm, sweep bandwidth of 110 nm, repetition rate of 100 kHz, 46% duty cycle and average output power of ~23 mW.

Fig.1. Schematic diagram of pvSSOCT system. Swept source laser 1060 nm External cavity tunable laser (Axsun Technologies, λ_0 : 1060 nm, $\Delta\lambda$: 110 nm, A-lines/sec 100,000); FBG, Fiber Bragg Grating; Spectrally Balanced Interferometer; FC, Fiber Coupler; GS, galvanometer scanning mirrors; M, mirror; BPD, balanced photo-detector (Thorlabs Inc. PDB 430C-AC, 100Hz-350MHz); PC, polarization controller; LP, linear polarizer; RF Amp, BD, beam dump; UP, unused port; OI, optical isolator; OSA, optical spectrum analyzer; DAQ: A/D converter (Alazartech ATS 9350, 500 MS/s, 12 Bit); RS: retinal scanner; CS: corneal scanner.

Here, a simple method for phase stabilization was implemented. A fixed wavelength reference signal generated by a fiber Bragg grating ($\lambda_0 = 988.9$ nm, reflectivity = 99.91%, $\Delta\lambda = 0.4$ nm) inserted in a transmission mode between the source and the OCT interferometer (Fig. 1) was used to align the start time of each A-line in post-processing. The necessary shifts in the acquired SSOCT signal can be calculated based on the first falling slope of the signal around the FBG signal. The post processing of SSOCT signal and PVC method adopted to generate SSOCA images was described elsewhere [6, 7].

Results and discussion: The effect of fixed-pattern noise removal is shown in an intensity image of the cornea and macula of a healthy volunteer (Fig. 2). The image has a width of 5 mm consisting of 1500 A-lines; a displayed depth of 2.8 mm. Eye from one normal subject was imaged with SSOCA system as per approved protocol.

Fig. 2. SSOCT intensity images of cornea (A), retina (B) before and after (C, D) phase stabilization. 2000 A-scans over 6 mm. Arrows shows artifact due to phase un-stability. Scale bar: 1 mm.

Fig. 3. *In vivo* images of human sclera using SSOCA. Top and bottom panels show images before and after phase stabilization, respectively, (A) Average intensity image of three B-scans (B) Phase-variance processed image using phase data from the same three B-scans, (C) composite image of (A) and (B), (D) Total intensity projection image of (A), (E) Total phase variance projection of (B). The scanning size of the lateral direction is 2 mm and the sampling density of the lateral direction is 5.6 μm . Scale bar: 1 mm.

Phase variance data were calculated from sets of 3 B-scans acquired at each location (BM-scans) in the volume. These data highlight motion at each cross-section, primarily the flow of blood cells, yielding the depth and size of vessels within the retina. $2 \times 2 \text{ mm}^2$ areas around the diseased region were scanned with densities of 360 BM-scans consisting of 440 A-lines per scan. To In subject's eyes, distinguishable scleral vasculatures were visible (Fig. 4 C1, D1). It was observed that, most of the blood vessels are located at conjunctiva and episcleral layer. Perfusions were observed at scleral and deep scleral layer. A comparison between SSOCA projections and corresponding FA images from chorio-retinal complex are presented in Fig. 5. Flow signal from different retinal and choroidal layers has been overlaid over the intensity image shown in a composite B-scan image (Fig 5E). A volumetric manual segmentation (Fig 5E) was performed to extract each vascular layer based on existing literature. The retinal vascular network

Fig.4. (A) Three-dimensional reconstruction of sclera after flattening. Virtual C-scans (projections) from intensity (B, C, D, E) and corresponding phase variance (B1, C1, D1, E1) data set showing vascular networks in conjunctiva (1), episclera (2, 3) and (4) sclera respectively. (F) En face projection ($2 \times 2 \text{ mm}^2$) of structural images (A), (F1) Projection image of phase-variance OCT scleral layers (G) 3D volume rendering of vasculature layers in false color scale. Scale bar: 1 mm.

(within the nerve fiber layer to outer nuclear layer) is presented (Fig. 5C). It was observed that SSOCA has higher resolution and contrast to than FA (micro vessels region). Choriocapillaris (5D), Sattler's layer (5F) and Haller's layer (5G) were also presented. The size of the smallest resolvable capillary is $10\sim 12 \mu\text{m}$. The pseudo-color (RGB) depth-coded projection is also presented in Fig. 5H. Deep penetration pv-SSOCT clearly permits *in vivo* visualization of the chorioretinal complex.

Fig.5. SSOCA volumetric scan over $1.5 \times 1.5 \text{ mm}^2$ at 6° nasal retina. (A) FA image of retina, (B) zoomed FA image within white rectangle, (C) corresponding segmented depths for en-face projection images for retinal layers (RE), (D) $6 \mu\text{m}$ below Bruch's membrane for choriocapillaris (CC), (E) B-scan image showing flow signal in different retinal and choroidallayers overlaid over intensity image (position mentioned by yellow dotted lines from B), (F) $26 \mu\text{m}$ to $34 \mu\text{m}$ below Bruch's membrane for Sattler's layer (SL); (G) $61 \mu\text{m}$ to $90 \mu\text{m}$ below Bruch's membrane for Haller's layer (HL); and, (H) depth color-coded enface projection. Yellow arrows in (D) show the shadow artifact from big retinal vessels projected on choriocapillaris layer. Scale bars, $300 \mu\text{m}$. Morphological features here resemble histological images by corrosion vascular casts and scanning electron microscopy.

Conclusion: We present *in-vivo*, noninvasive, volumetric imaging system for 2D and 3D visualization of vasculature in corneo-scleral and chorio-retinal tissue. Our data from normal eye suggest that SSOCA has potential in the early diagnosis of ocular vascular diseases including age-related macular degeneration, better understanding of pathogenesis, and studies of treatment response and efficiency of pharmaceutical agents

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