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Investigation of the effect of directional (off-axis) illumination on the reflectivity of retina layers in mice using swept-source optical coherence tomography

Ratheesh K. Meleppat[a*], Myeong Jin Ju[b], Pengfei Zhang[a], Yifan Jian[b], Suman Manna[a], Daniel J. Wahl[b], Marinko V. Sarunic[b], Edward N. Pugh Jr.[a], and Robert J. Zawadzki[a,c*]
[a]UC Davis Eye-pod, Department of Cell Biology and Human Anatomy, University of California Davis, Davis, CA 95616
[b]School of Engineering Science, Simon Fraser University, Burnaby BC, V5A 1S6, Canada
[c]UC Davis Eye Center, Dept. of Ophthalmology & Vision Science, University of California Davis, 4860 Y Street, Suite 2400, Sacramento, California 95817
*rjzawadzki@ucdavis.edu ; *rkmeleppat@ucdavis.edu

ABSTRACT

Changes in visibility of the Henle fiber layer and photoreceptor bands of the human retina with illumination directionality have been reported in OCT clinical imaging. These are a direct consequence of the changes in back scattering due to fibrous tissue orientation and to waveguiding properties of the photoreceptors respectively. Here we report the preliminary results of a study on the effects of retinal images acquired with OCT of illumination directionality in the mouse retina. The quantitative assessment of the reflectivity of retinal layers of a BALB/c and WT pigmented mice was performed *in-vivo* using a swept-source optical coherence tomography system. The intensities of backscattered signals from different outer retinal layers were measured and compared.

Keywords: Directional illumination, Optical coherence tomography, Retinal Imaging

1. INTRODUCTION

In the retinal imaging systems, the illumination angle on the retina is in part determined by the pupil entry position. Because the reflectivity of retinal structures might depend on illumination angle, the visibility of these structures will vary as a function of entrance pupil position. Various approaches based on objective reflectometers, scanning laser ophthalmoscopy (SLO), snapshot imaging using CCD array, and imaging spectrographs have been used for the assessment of directionality of illumination in both human subjects and in animals [1-5]. Over the past two decades optical coherence tomography (OCT) has emerged as a powerful tool for retinal imaging because of its capability to provide high resolution cross-sectional and volumetric images of retina in vivo[6]. OCT is based on low coherence interferometry that measures the depth encoded back-scattered signals from the sample. Recently, Fourier domain OCT systems has been demonstrated as a powerful tool for studying the directional reflectance properties of human retina[7-9]. Among Fourier domain OCT systems, swept source OCT systems (SSOCT) are gaining interest as an alternative to spectrometer based Spectral domain OCT systems for retinal imaging due to their ability to perform high-speed imaging with higher sensitivity. In order to characterize the cellular microstructure and to understand the biophysical mechanisms of the living retina, high-resolution retinal imaging of rodent eyes is desirable. This makes the mice an important model organism in ophthalmic research. In this paper, we investigate the angular-dependent reflectivity of retinal layers of BALB/c (Albino) and WT (pigmented) mice using a SS-OCT system.

2. METHODS

2.1 Swept-source optical coherence tomography

A schematic of our SS-OCT system is shown in Figure 1. The swept laser source has a center wavelength of 1060 nm and tuning range of 100 nm. The axial resolution (in air) and sensitivity of the SS-OCT are 7.15 μm and 101 dB, respectively. The incident power of the eye is measures to be as ~1mW. The optical configuration comprises of three 50/50 couplers.[10,11]

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The optical power from source is directed to the interferometer coupler (FC2) via first coupler (FC1). The two output ports of the coupler FC2 constitute the two arms of an Michelson interferometer. In the reference arm, the light is collimated and projected onto a mirror through a dispersion compensator and achromatic lenses, whereas, in the sample arm, the probe beam is scanned over the retina using a galvo mirror-based beam steering setup. A scanning lens (L3) and a tube lens (L4) delivers collimated beam of diameter of 500 μm on the mouse cornea. The reflected light from the two interferometric arms is combined at the coupler and the interferogram is detected by a dual-balanced photodetector. Both the calibration signal (k-clock) and the detected signal are simultaneously acquired by the two channels of the digitizer (ATS9350, AlazarTech). The galvo mirror scanning unit synchronized with the data acquisition system is controlled by a DAQ Card (PCI6202, National Instruments). The imaging probe of our OCT system was mounted on a X-Y-Z translation stage as shown in Figure 1(b). This allows controlled positioning of the entrance pupil with respect to the dilated mouse pupil by translating the micrometer screw.

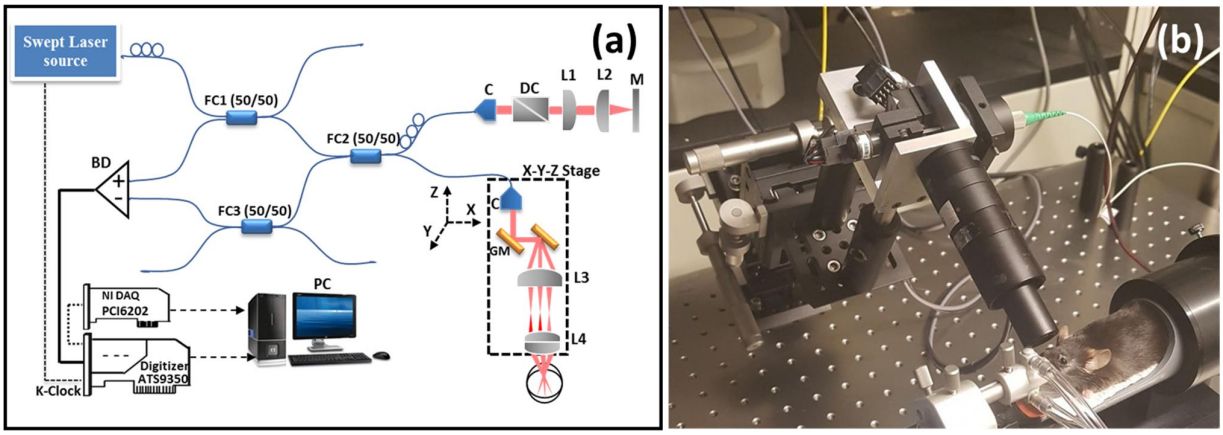

Figure 1. (a) Schematic of the SSOCT system used for directional OCT imaging. DC: Dispersion compensator, BD: Balanced detector, GM: Galvo mirror, C: Collimator, L1-L4: Achromat. (b). Picture of OCT probe with translation stage during mouse imaging session

2.2 Mouse Handling

All mouse husbandry and handling including imaging were in accordance with an animal study protocol approved by the University of California Animal Care and Use Committee, which is accredited by Association for Assessment and Accreditation of Laboratory Animal Care International and strictly adhere to all the National Institutes of Health (NIH) guidelines. Both mice (C57BL/6J and BALB/c) used in the study were obtained from The Jacksons Laboratory (Sacramento, Ca). During image acquisition, mice were anesthetized with the inhalational anesthetic isoflurane (2% in O_2). Pupils were dilated with tropicamide and phenylephrine. In order to keep the cornea hydrated, lubricating eyedrops (Z-drop Vet PLUS) were supplied over regular interval throughout the duration of imaging. No mouse contact lens was used during imaging session.

2.3 Image acquisition and Averaging

The directional imaging is performed by laterally shifting the entrance pupil position along horizontal (X) direction. This alters the directionality of the imaging light at the retina. Cross-sectional images (B-scans) of the same retinal region corresponding to various beam entry position were acquired. Fifty B-scans are acquired from each position and performed inter-frame motion correction using a custom written MATLAB Script. This is followed by the spatial averaging of the B-scans. The tilt in averaged B-scans are corrected using Image J. Thirty A-scans are selected from each B-scan and overall

1500 A-scans are used for averaging. The averaged A-scan profiles were used to extract changes in the reflectivity of the retinal layers. Figure 2. shows the averaged OCT axial scan profiles of a retina of pigmented and Albino mouse. All highlighted peaks in the A-scan profile represent the reflections from the major retinal layers. Reflections from the outer retinal layers, including the External Limiting Membrane (ELM), the Inner Segment-Outer Segment junction (IS/OS), and Bruch's Membrane (BrM)/Retinal Pigment Epithelium (RPE) with respect to the illumination angle were measured.

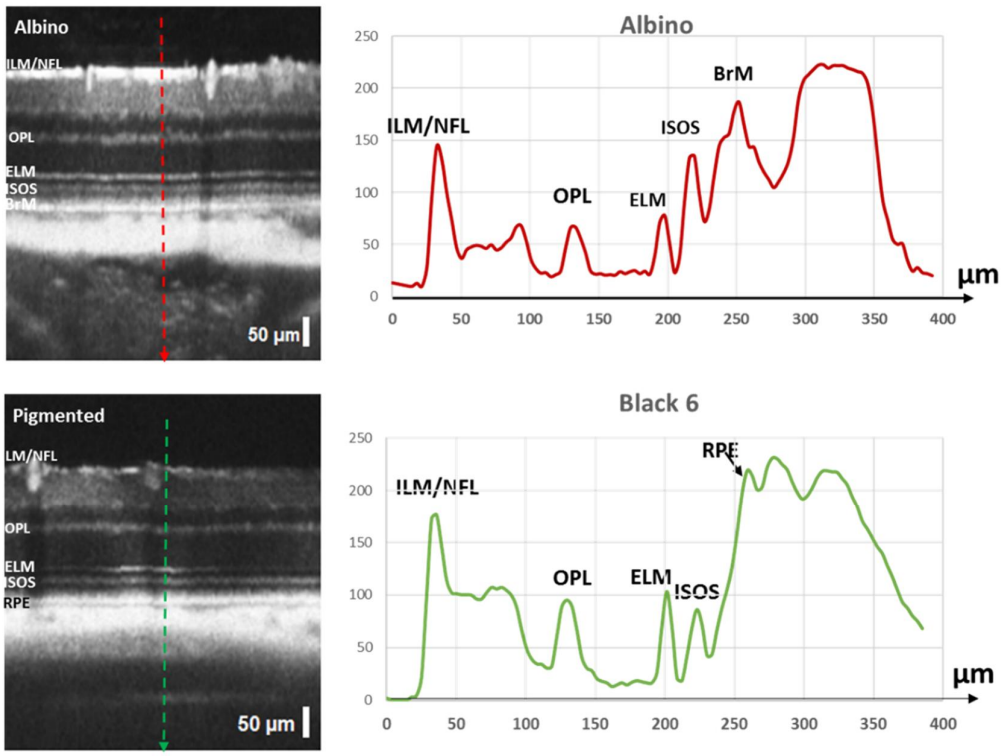

Figure 2. Representative OCT B-scans and corresponding A-Scan profiles from retinal layers for (a) Albino (BALB/c) (b) WT Pigmented.

3. RESULTS AND DISCUSSION

Figure 3 shows the directional OCT B-scans acquired along horizontal directions with corresponding directional reflectivity profiles for an Albino mouse. The red dotted lines in the Figure 3(a) and 4(a) shows the direction of the movement of OCT probe. The entrance pupil position is shifted to maximum of 1 mm on either side of the pupil center with a step movement of 100 µm. Illustrated figures shows that shifting the pupil position of the beam, apparently tilt the B-scan images. Furthermore, the angle of tilt increases as the pupil position moves away from the center. The angle of tilt is measured from the B-scan images. The angular reflectivity of the outer retinal layers (ELM, IS/OS and BrM) measured from each beam entry position is shown in Figure 3(e).

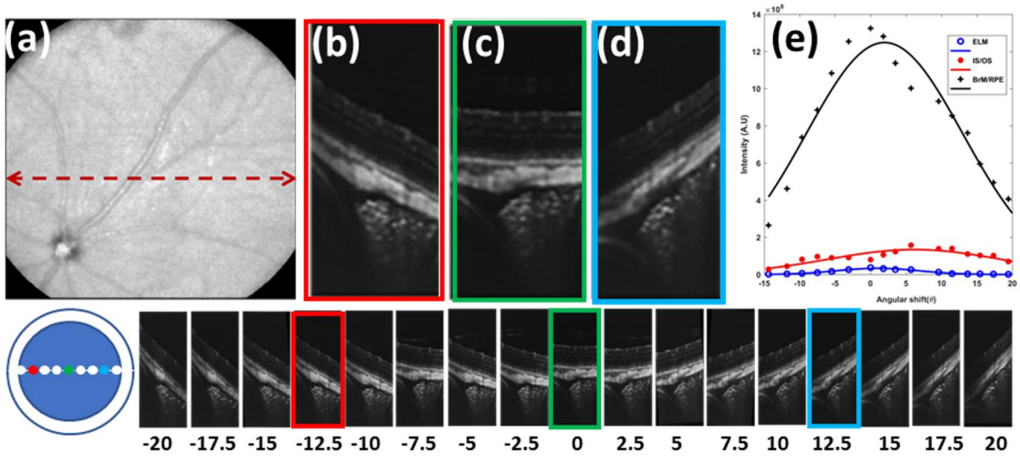

Figure.3 (a) OCT fundus image of Albino (b), (c) and (d): Directional OCT B-scans acquired at -12.5, 0 and +12.5 Degrees along X-direction. (e): Angular reflectivity Profiles. The dark blue shaded circle shows the mouse pupil. The white and colored dots represent different beam entry positions.

Figure 4 shows the directional OCT B-scans acquired along horizontal directions with corresponding directional reflectivity profiles for a WT pigmented mouse. The angular reflectivity of the outer retinal layers (ELM, IS/OS and RPE) measured from each beam entry position is shown in Figure 4(e).

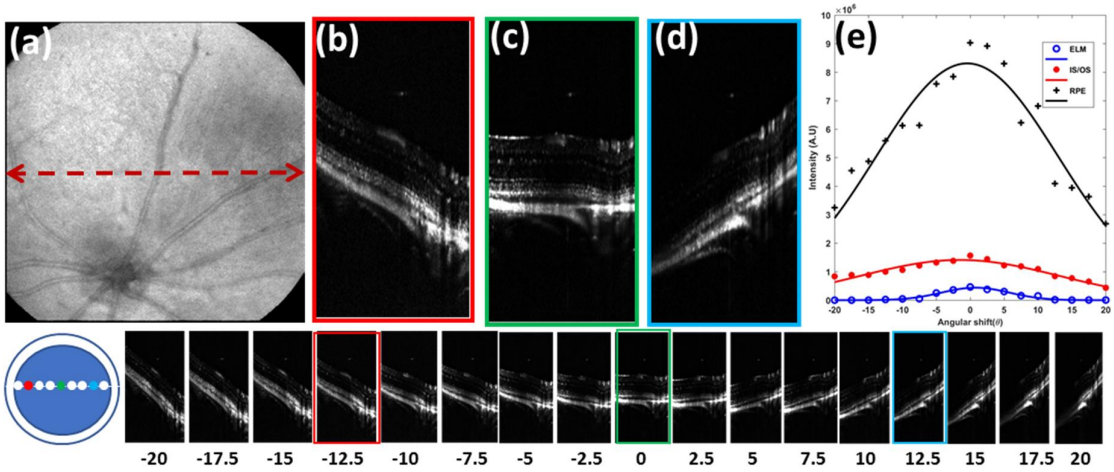

Fig.4 (a) OCT fundus image of WT pigmented mouse (b),(c) and (d): Directional OCT B-scans acquired at -12.5, 0 and +12.5 Degrees along X-direction. (e): Angular reflectivity Profiles. The dark blue shaded circle shows the mouse pupil. The white and colored dots represent different beam entry positions.

From Figure 3 and 4, it is evident that the reflections from the major outer retinal layers are highly sensitive to directional illumination and their angular reflectivity have approximately Gaussian profiles. However, limited axial resolution of the SSOCT system restricts the study of the angular dependent reflectivity of the Photoreceptor Outer segment tips.

4. CONCLUSION

The results of the study on directional reflectivity of retinal layers in BALB/c and WT pigmented using a SSOCT system has been presented. We found that the reflections from the major outer retinal layers are highly sensitive to directional illumination and that their angular reflectivity have approximately Gaussian profiles. OCT imaging at higher axial resolution would enable us to study the reflectivity from the photoreceptors outer segment tips (mostly rods outer segment tips (ROST) in mice). Potential future work includes an automated pupil offset positioning and high resolution directional OCT imaging that allows the reflectivity studies from all retinal layers in multi directional beam entry positions.
The changes in the angular reflectivity of retina layers hold promise of sensing microscopic changes in cellular and sub-cellular morphology that might precede and/or follow disease progression in the retina. We intend to apply this technique to study mice with different retina degenerations to allow testing the feasibility of this technique.

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