Development of a compact mouse retinal imaging system using spectral domain optical coherence tomography (SD-OCT) with a full field stimulation channel for recording fast optoretinograms (ORGs)

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Keywords: Optical Coherence Tomography, light stimulus, Maxwellian's view illumination, Light evoked optical signal, Optoretinogram

ABSTRACT

While the a-wave of mouse electroretinogram (ERG) occurs within 50 milliseconds after exposure to light, the optoretinogram (ORG) slower than a 20Hz sampling rate could face limitations in observing immediate morphological changes from the retina. In this study, we develop a compact custom-built mouse ORG system based on spectral domain optical coherence tomography (SD-OCT) for 100Hz~1KHz B-scan rates comprised of 100 kHz A-scans. All the optics of the developed ORG system are designed on a 24 x 24 inches optical breadboard to move easily as well as to combine with the ERG system in a dark room. Without using a fundus camera, the OCT system provides en-face images from high-pass filtering and square of the OCT spectral signal for mouse retinal positioning in-vivo before acquiring ORG data. The 490nm LED for light stimulus is generated to make uniform illumination at the mouse retina using the Maxwellian view method. The common path of the OCT scanning light and the visible LED is built with achromatic doublet lens combinations based on optical simulation with Opticstudio®. The developed compact ORG system can not only observe light-evoked responses with 1~10 milliseconds but also be used for the studies of correlations between ORG and ERG in the mouse retina.

Keywords: Optical Coherence Tomography, light stimulus, Maxwellian's view illumination, Light evoked optical signal, Optoretinogram

1. INTRODUCTION

Optoretinogram (ORG) is a noninvasive and optical measurement of neural function in the retina during phototransduction through quantifying visible light-evoked morphological changes in the photoreceptors. The background of these morphological changes is the elongation of the outer segments in photoreceptors during the phototransduction process [1, 2]. Optical coherence tomography (OCT) is an appropriate imaging technique that provides structural information through non-invasive measurements to quantify functional changes in the retina. Over the last decade, ORG studies have been reported using various types of OCT including spectral domain OCT, swept-source full-field OCT with other imaging modalities (scanning laser ophthalmoscopy (SLO), adaptive optics, fundus camera) and processing methods (intensity, phase, and velocity-based ORG processing) in pre-clinical and clinical studies [3-9]. Our previous studies via spectral domain OCT combined with SLO have reported phase and intensity-based ORG results on mice experiments [4, 9]. The ORG results showed light-stimulated outer retinal layer deformations through length differences between two specific layers, an external limiting membrane (ELM) and Bruch's membrane (BrM) containing the outer segment layer.

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We are investigating correlations between these ORG approaches with an electroretinogram which shows an electrical response of the retina from the light. This study is the progress of enhancing the previous OCT system to investigate ORG and ERG data in the same light stimulation and imaging session. Developed OCT system improved sampling time resolution of 1ms (1kHz) and a method of light delivery to mouse eye as well as integrated optics, an animal imaging stage, and ERG instruments on one breadboard. Advanced sampling rates can resolve the time scale difference between ERG signals with 2kHz and ORG signals which was previously acquired with 100Hz. The important signal peaks, the a-wave and b-wave, in mouse ERG occur mostly within hundreds of milliseconds in mouse experiments, and the width of an a-wave is a few milliseconds[10-13]. The purpose of fast ORG with 1kHz time resolution focuses on finding sharp and short peaks like a-wave. In the past study, stimulus light source delivery used a supercontinuum laser as well as shared the beam path including galvoscanner with the imaging beam[4]. The laser source delivered through the scanning pattern for imaging illuminated each region of the scanning area at different times. It means that the previous illumination method was similar to light stimulation for pattern ERG and multi-flash ERG as well as not being able to compare with full-field flash ERG. The developed OCT system provides full-field illumination with a 482-nm LED module through a compact imaging probe. The custom-made imaging probe was based on optical and mechanical design for full-field illumination on the mouse retina during retinal imaging sessions. The developed system for ORG provides quantified mouse outer retinal changes based on ORG of BM-scan data with well-delimiting ELM and BrM.

2. METHODS AND MATERIALS

2.1 Custom built optical coherence tomography (OCT) system for ORG with ERG

2.1.1 Spectral domain OCT

We built a custom SD-OCT system with a full field illumination channel for light stimulation on the mouse retina during in-vivo imaging. The LED channel can deliver a maximum of 2.25mW with a 482-nm central wavelength at the end of the imaging probe corresponding to the pupil plane of the mouse eye. The beam path of the LED channel is partially shared with the path of the imaging laser which is a broadband superluminescent diode (SLD) light source (MT-870-HP, Superlum). The imaging light source has an 875-nm central wavelength of 180-nm bandwidth and delivers 825 µW at the mouse pupil. The SLD provides 1.876 µm theoretical axial resolution and is insufficient to bleach or activate rhodopsin during in-vivo ORG data acquisition and imaging sessions. Volume and line scans were operated by a pair of galvoscanner (6215H, Cambridge technology) for X-Y scanning with 100kHz A-scan rates.

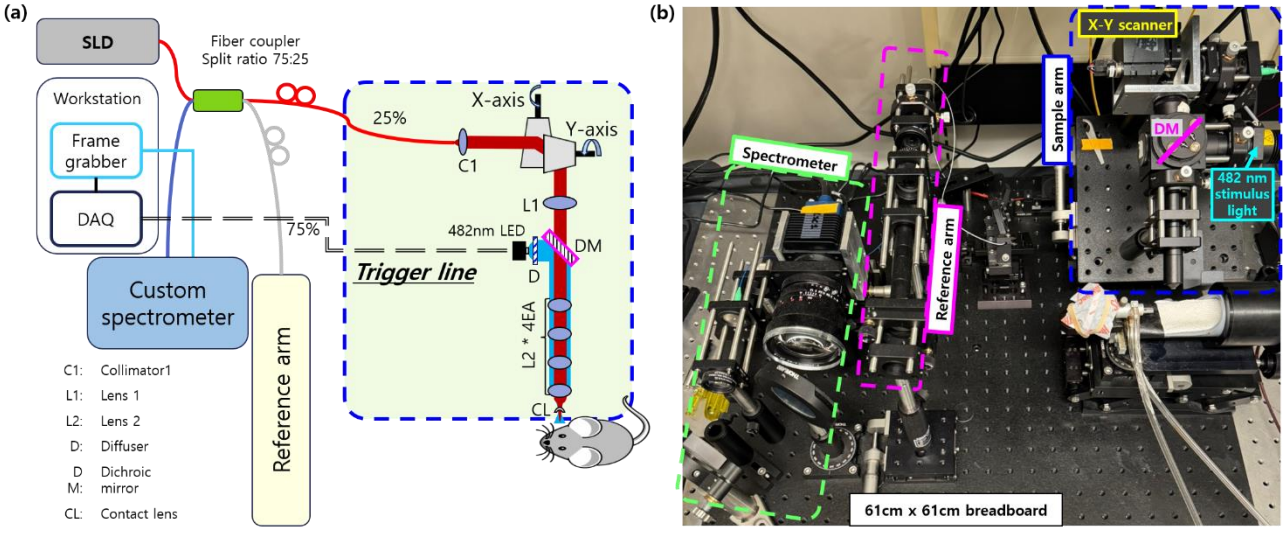

Figure 1. Custom built OCT images. (a) Schematic of OCT system. (b) The system built on the 61cm X 61cm breadboard.

2.1.2 Imaging and light stimulation probe design

Light sources for imaging and light stimulation should share the same beam path in partials of the imaging probe to observe a series of light-evoked changes in the retina. The imaging probe was optically designed to focus imaging light on the mouse retina and to expose the LED light on the pupil plane for widely spread illumination. The estimated LED illuminating angles at the end of the imaging probe was 52.8º based on beam profiling by a CMOS sensor. The engineered diffuser (ED1-C20, Thorlabs) in front of LED light emission enhances the uniformity of 482-nm LED light source (M490L4, Thorlabs). These beams go into the mouse eye through a custom-made imaging adapter, including a contact lens for the mouse cornea.

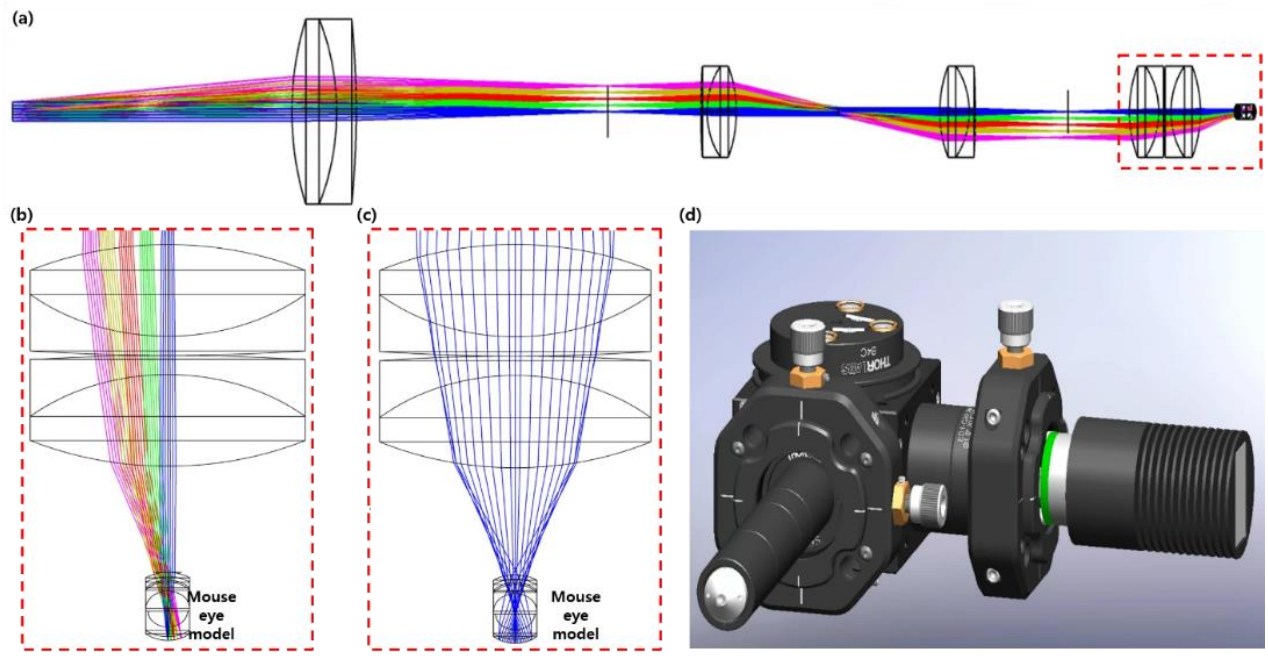

Figure 2. Optical and mechanical probe design. Optical design and scanning simulation were performed using OpticStudio® and mechanical rendering and virtual optics mounting were performed with SolidWorks®. (a) The optical design of beam paths for imaging. (b) Zoomed scanning on the retinal layer of the mouse model eye corresponding to the red dotted box in (a). (c) Zoomed full-field LED illumination on the retinal layer. (d) Rendering optics mounted optomechanics for precise system alignment.

2.2 Experiments protocol

Experiments were based on an animal protocol approved by the IACUC of the University of California, Davis. Albino mice were dark-adapted in a dark box covered with a few layers of blackout curtains for at least 2 hours. Animal anesthesia system (VetEquip Inc) provides insufflation narcosis with 4% isoflurane gas mixed with oxygen in the initial stage for placing mice on the stage and the 2% concentration of mixed gas during imaging sessions. The OCT system acquires three data sets for one mouse experiment: one 1,000 x 1,000 pixels volume scan data and two 100 x 10,000 BM-scans for each 10 seconds as control and experimental data. The volume scan data is processed as simple en-face imaging data for identifying the BM-scan region through high-pass filtering and summation of the volume matrix. For one set of 1kHz BM-scans for 10 seconds, 482-nm LED was operated during designated light exposure sequences. The durations of 482-nm full-field illumination were calculated for each bleaching level considering the estimated exposure area on the retina.

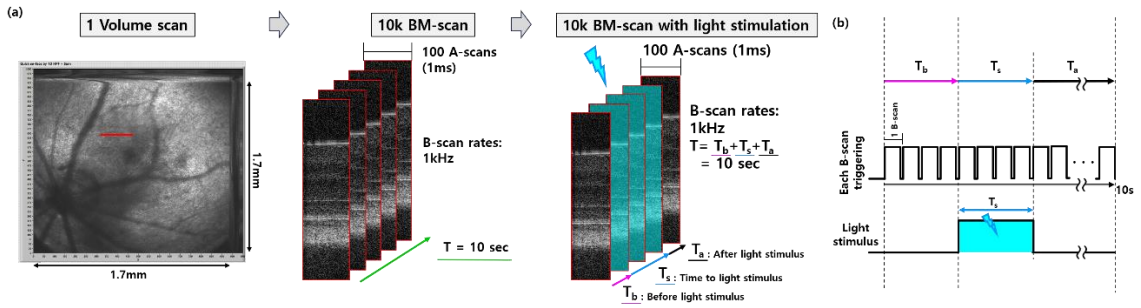

Figure 3. Data acquisition protocols. (a) imaging steps in order 1 volume scan for acquiring simple en-face imaging to select a B-scan region, 10,000 BM-scan during 10 sec with the 1kHz acquisition speed, and 10,000 BM-scan with light stimulation in the same period. (b) Timeline of 10,000 BM-scans with light stimulation in the 3rd step of (a) including the timing of each B-scan acquisition and light stimulation.

2.4 Phase based ORG processing

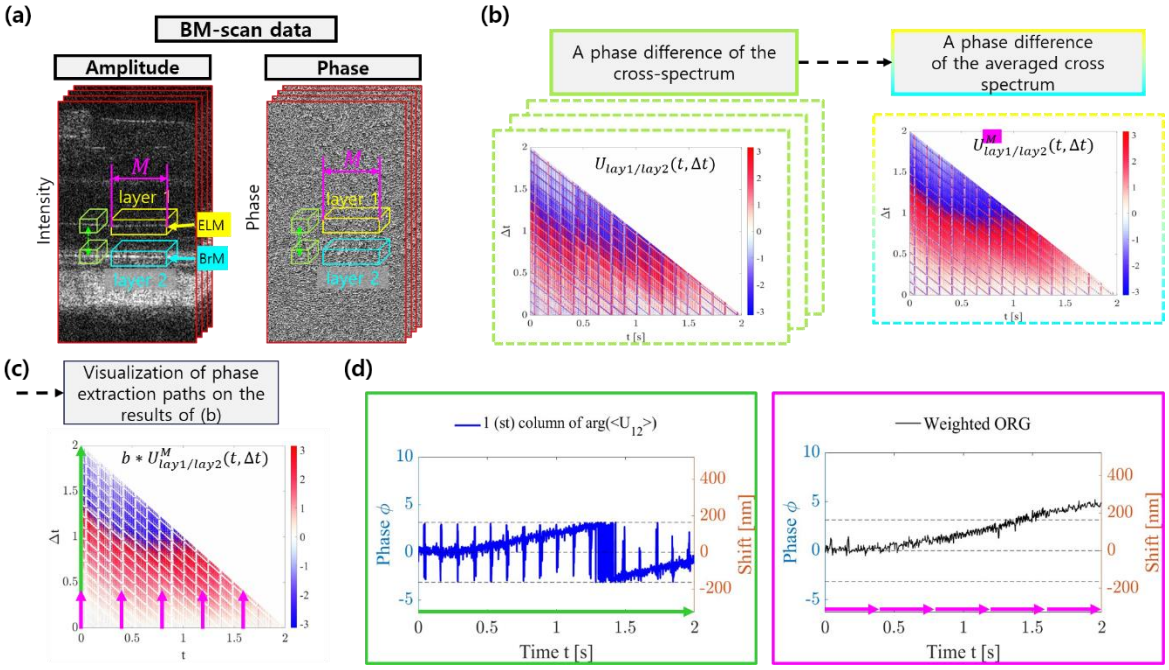

Figure 4. Summary of phase-based ORG signal extracting process with processing results. (a) OCT BM-scans data. (b) Phase difference graph of cross-spectrum between layer pixels corresponding to External layer membrane (ELM) and Bruch's membrane (BrM). (c) Visualized extracting phase differences on the processed graph (b). Applying weight b to reduce signals by breathing and heartbeats on the result of (b). (d) Processed ORG data showing deformation between ELM and BrM by the light stimulation.

The optoretinogram (ORG) processing software reported by Ewelina et al. (2021) in this study estimated the distance between the external limit membrane (ELM) and the Bruch's membrane (BrM) in retinal cross-section images of B6 albino mice[9]. These layers encompass the outer segment (OS) of photoreceptors which are elongated as one of the effects induced by absorbing photons in photoreceptors. Quantifying the distance between the two layers can indirectly show light-evoked morphological changes in the OS. The phase-based ORG processing has been reported as a more sensitive method than our previous intensity-based ORG processing. The main idea of phase-based ORG processing is to calculate the difference of the cross-spectrum in the two layers over the time series. A summary of the phase-based ORG processing is shown in Fig.4, and the detailed whole progress was described in Ewelina's report.

3. RESULTS

Full-field LED illumination channel provided light energy with 47.92μJ (1.843 mW for 26 ms) to bleach 20% for rhodopsin. The light was distributed as about 52.8º on the 4.061mm^2 of mouse retina at 1 sec after the imaging session was started. Following ORG signals showed the effects of this light stimulation.

3.1 Mouse optoretinogram(ORG) by phase-based ORG processing

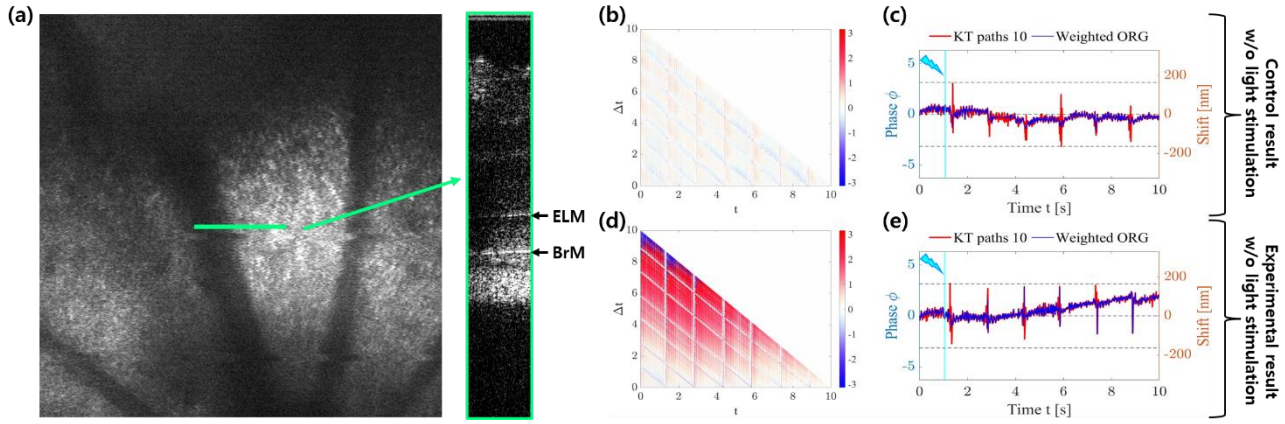

Figure 5. ORG dataset and ORG processing results. (a) Acquired image data. An en-face view (left), One of the BM-scans corresponding to green line of the en-face image(right). (b) and (d) are he averaged phase difference graph of cross-spectrum, (c) and (e) Comparison of Knox-Thompson signal without thresholding (red line) and with weights (blue line)

After the mouse retina was exposed to full-field illumination, the phase difference distance between the Bruch's membrane (BrM) and External limiting membrane (ELM) was gradually enlarged. The extended distance is about 105 nm which is shown at the 10 sec of Fig. 5(e).

4. CONCLUSION

In this study, we have developed a compact mouse retinal imaging system (OCT). The OCT has a full-field visible light illumination channel with an about 52.8º output angle at the end of the imaging probe. This imaging system acquired BM-scan data with 1kHz sampling rates to calculate the time evolution phase differences of the cross-spectrum. Light-evoked morphological changes in retinal layers are quantified through the ORG processing step using phase-based ORG processing software. This quantification of the enlarged phase difference between ELM and BrM validated the availability of a developed OCT system to capture the nanometer scale changes. This system will be combined with an electroretinogram system in future experiments to understand the physiological origins of ORG signals. Additionally, future works will apply several types of mouse models, animal models, ORG processing algorithms, and ERG signal filtering.

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References

[1] M. Mazzolini, G. Facchetti, L. Andolfi *et al.*, “The phototransduction machinery in the rod outer segment has a strong efficacy gradient,” Proceedings of the National Academy of Sciences, 112(20), E2715-E2724 (2015).

[2] R. S. Jonnal, “Toward a clinical optoretinogram: a review of noninvasive, optical tests of retinal neural function,” Annals of translational medicine, 9(15), (2021).

[3] V. Srinivasan, M. Wojtkowski, J. Fujimoto *et al.*, “In vivo measurement of retinal physiology with high-speed ultrahigh-resolution optical coherence tomography,” Optics letters, 31(15), 2308-2310 (2006).

[4] P. Zhang, R. J. Zawadzki, M. Goswami *et al.*, “In vivo optophysiology reveals that G-protein activation triggers osmotic swelling and increased light scattering of rod photoreceptors,” Proceedings of the National Academy of Sciences, 114(14), E2937-E2946 (2017).

[5] D. Hillmann, H. Spahr, C. Pfäffle *et al.*, “In vivo optical imaging of physiological responses to photostimulation in human photoreceptors,” Proceedings of the National Academy of Sciences, 113(46), 13138-13143 (2016).

[6] M. Azimipour, D. Valente, K. V. Vienola *et al.*, “Optoretinogram: optical measurement of human cone and rod photoreceptor responses to light,” Optics letters, 45(17), 4658-4661 (2020).

[7] R. J. Zawadzki, D. Valente, R. K. Meleppat *et al.*, "Full-field swept source optical coherence tomography (FF-SS-OCT) system for in vivo evaluation of light evoked activation of photoreceptors (optoretinography) in mice." PC119410G.

[8] K. V. Vienola, D. Valente, R. J. Zawadzki *et al.*, “Velocity-based optoretinography for clinical applications,” Optica, 9(10), 1100-1108 (2022).

[9] E. Pijewska, P. Zhang, M. Meina *et al.*, “Extraction of phase-based optoretinograms (ORG) from serial B-scans acquired over tens of seconds by mouse retinal raster scanning OCT system,” Biomedical Optics Express, 12(12), 7849-7871 (2021).

[10] S. Liu, F. Yuan, and M. Xiang, “Comparative analysis of electroretinogram with subdermal and invasive recording methods in mice,” Biochemical and Biophysical Research Communications, 655, 118-126 (2023).

[11] J. Robson, H. Maeda, S. Saszik *et al.*, “In vivo studies of signaling in rod pathways of the mouse using the electroretinogram,” Vision research, 44(28), 3253-3268 (2004).

[12] N. Tanimoto, R. L. Muehlfriedel, M. D. Fischer *et al.*, “Vision tests in the mouse: Functional phenotyping with electroretinography,” Frontiers in Bioscience-Landmark, 14(7), 2730-2737 (2009).

[13] V. Chrysostomou, and J. G. Crowston, “The photopic negative response of the mouse electroretinogram: reduction by acute elevation of intraocular pressure,” Investigative ophthalmology & visual science, 54(7), 4691-4697 (2013).