# High-resolution multimodal optical coherence tomography and scanning laser ophthalmoscopy for *in vivo* mouse retinal imaging

Siyu Song,[1,2] Guangru Ben Liang,[1,2] Tristan Hormel,[1] Yukun Guo,[1,2] Min Gao,[1,2] Benjamin Sivyer,[1] Siyu Chen,[1,2] Yifan Jian,[1,2] J. Peter Campbell,[2] Yali Jia[1,2,*]
[1]Casey Eye Institute, Oregon Health & Science University, Portland, Oregon 97239, USA
[2]Department of Biomedical Engineering, Oregon Health & Science University, Portland, Oregon 97239, USA
*jiaya@ohsu.edu

## ABSTRACT

We developed a compact lens-based multimodal optical coherence tomography (OCT) and scanning laser ophthalmoscopy (SLO) high-resolution system for *in vivo* mouse retinal imaging that enables the visualization of retinal laminar microstructures, microvasculature, and labeled neuron cells.

## 1. INTRODUCTION

Mouse models of retinal and optic nerve diseases are important for understanding the mechanisms of disease development and evaluating the efficacy of therapies. In these models, *in vivo* retinal imaging allows for the study of physiological processes such as microglia activation, one of the events before retinal vasculopathy, ganglion cell death, or nerve fiber loss [1]. To better visualize specific types of neuron cells, and their roles in the development of retinal diseases and optic neuropathy, high-resolution, non-invasive imaging modalities are highly desirable. Among various ophthalmic imaging modalities, OCT can image both microstructures and microvasculature three-dimensionally, and SLO enables endogenous and exogenous fluorescence signal imaging at single-cell resolution. In our prior work, we have also demonstrated visible light band offers higher axial resolution for OCT. Here, we describe a lab-built multimodal visible-light OCT and SLO system for *in vivo* mouse retinal imaging. By registering OCT and SLO images, we can localize microglia within specific retinal layers with respect to nearby capillaries, veins, or arteries.

## 2. METHODS

A schematic for our system is presented in Fig. 1. A supercontinuum laser and a tunable filter provide a central wavelength of 580 nm with a bandwidth of ~100 nm illumination for the OCT system. A 488 nm laser was used for the SLO system and fluorescence excitation. Both light sources were connected to a 90/10 single-mode optical fiber coupler for co-alignment, and the output light was divided into a sample and reference arm by another 75/25 fiber coupler. In the sample arm, an electronically focus tunable lens was located before a 2D galvanometer-scanner to alter the focal plane in the sample. And the eyepiece consisted of three achromatic lenses and a slit lamp lens. The OCT back-scattered signal and SLO emission signal were separated by a customized dichroic mirror mounted at 45 degrees. Then, the OCT signal was directed to a commercial spectrometer with a line rate of 50 kHz, and OCT volumes were sampled at 2048×300×300 points. The theoretical axial and transverse OCT resolutions were 2.12 and 8.78 μm, respectively. The SLO signal was collected by a collimator connected to a multimode fiber and then directed to a photo-multiplier tube. The SLO subsystem could operate alone at 1 MHz pixel rate with a sampling density of 500×500 points, and the theoretical transverse resolution was 7.1 μm. During acquisition, OCT and SLO images were displayed in real-time for fine adjustment using our custom GPU-accelerated software written in C/C++ [2,3]. Then OCT images were generated by standard post-processing steps in Matlab, and OCT B-scans were generated by averaging 5 consecutive B-scans, while *en face* images were obtained using mean intensity projection (MIP) over the whole depth. SLO images were generated by averaging 10 frames acquired successively within 5 seconds.

Transgenic mice (B6.129P2-Cx3cr1tm1Litt/J) with Enhanced Green Fluorescent Protein (EGFP)-labeled retinal microglia were used in this study. Prior to imaging, the mice were anesthetized with inhaled 2.5% isoflurane and the eyes were dilated with 1% tropicamide. During imaging, mice were kept in a warm blanket circulated with 37 °C water to maintain body temperature and comfort, and artificial tears were used to prevent dehydration of the cornea. The incident power of OCT and SLO were 0.8 mW and 0.2 mW, respectively.

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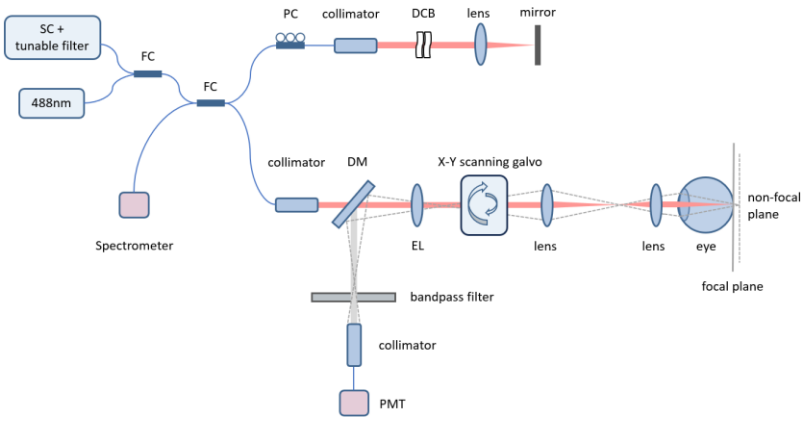


Fig. 1. Schematic diagram of multimodal OCT and SLO system. SC: supercontinuum laser; FC: fiber coupler; PC: polarization controller; DCB: dispersion compensation block; DM: dichroic mirror; EL: electronically focus tunable lens; PMT: photo-multiplier tube.

## 3. RESULTS

Two male transgenic mice were imaged. In the SLO channel, we were able to clearly visualize microglia structures including the microglia soma, and the dendrites (Fig. 2a). In the OCT channel, *en face* OCT demonstrated the branches of the central retinal artery and vein at the optic nerve head region and the inter-plexus capillaries (Fig. 2b). Due to the mouse's respiration during data acquisition, the discontinuity of vessels and line artifacts were also observed (Fig. 2b). On cross-sectional OCT (Fig. 2c and 2d), vascular boundaries and retina layers can be distinguished.

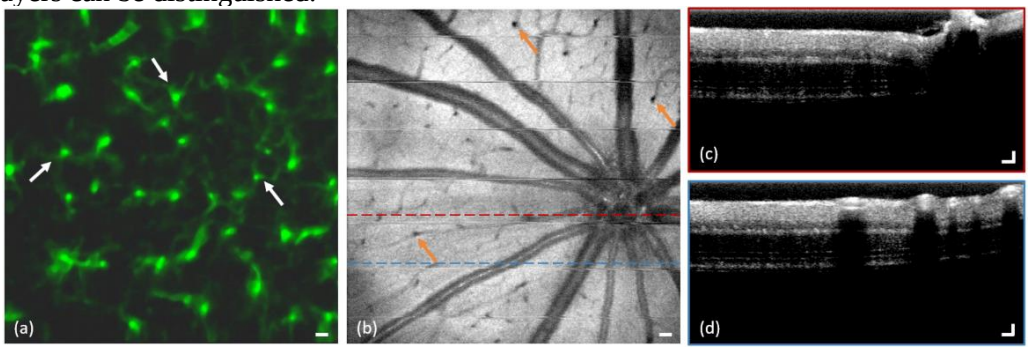


Fig. 2. (a) SLO image of a mouse retina with EGFP-labeled microglia. Microglia soma (green bright dots), and dendrites (white arrows) were demonstrated. (b) *en face* OCT of the same FOV, showing large vessels from the optic nerve head and interplexus capillaries as dark spots (orange arrows) due to greater light absorption than neighboring capillaries. (c, d) representative cross-sectional OCT located at two positions shown in (b). The vascular boundaries and retinal layers are distinctly resolved. The scale bar is 50 $\mu m$.

## 4. DISCUSSION AND CONCLUSIONS

We have successfully demonstrated a multimodal system for mouse retinal imaging *in vivo*. The *en face* and cross-sectional OCT illustrate retinal structure with great clarity, while SLO fluorescence imaging visualizes individual microglia cells with distinguishable dendrites. The system holds potential for longitudinal studies of microglia behavior in retinal vascular diseases or optic nerve disorders. For the next step, we will improve the system's robustness to enable retinal oximetry for detailed analysis of microvascular dysfunctions.

## 5. REFERENCES

1. A. Bosco, C. O. Romero, K. T. Breen, A. A. Chagovetz, M. R. Steele, B. K. Ambati, and M. L. Vetter, "Neurodegeneration severity can be predicted from early microglia alterations monitored in vivo in a mouse model of chronic glaucoma," Dis. Model. Mech. **8**(5), 443–455 (2015).
2. Y. Jian, K. Wong, and M. V. Sarunic, "Graphics processing unit accelerated optical coherence tomography processing at megahertz axial scan rate and high resolution video rate volumetric rendering," J. Biomed. Opt. **18**(2), 026002 (2013).
3. X. Wei, A. Camino, S. Pi, T. T. Hormel, W. Cepurna, D. Huang, J. C. Morrison, and Y. Jia, "Real-time cross-sectional and en face OCT angiography guiding high-quality scan acquisition," Opt. Lett. **44**(6), 1431–1434 (2019).