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Progress on Adaptive Optics for Multimodal OCT and Confocal Microscopy

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Abstract: We present our progress on image-guided (sensor-less) adaptive optics for multi-modal imaging of retinal tissue using high numerical aperture OCT and confocal microscopy using a custom developed instrument. Images of *ex vivo* tissues are compared to data acquired *in vivo*.

1. Introduction

Imaging the retina non-invasively is a valuable tool for vision research. Optical Coherence Tomography (OCT) is highly valued for the ability to provide structural images of the retinal layers, but lacks molecular contrast. Complementary imaging modalities are required to identify specific cells and tissues of interest. Fluorescence is a convenient source of contrast in the retina due to the presence of endogenous fluorophores and the ease of introducing extrinsic fluorophores. As an example, fluorescein is commonly used clinically for labeling the retinal vasculature. In preclinical imaging, the relevant cells can be labelled with a fluorophore such as Green Fluorescent Protein (GFP), complementing autofluorescence from layers such as the Retinal Pigment Epithelium (RPE). The RPE is of particular interest due to its connection to diseases such as Age Related Macular Degeneration (AMD). AMD is the leading cause of vision loss for adults in the UK, affecting more than 500,000 people with a total annual economic cost of £2.6 billion [1]. Progress in the imaging of this layer would make great strides in the development of novel therapies for such diseases.

We are performing a comparative study of imaging with OCT and fluorescently labeled targets with high resolution. When imaging the retina through an ophthalmoscope, and given that the focal length of the eye is fixed, the numerical aperture (NA) is maximized by filling the pupil, which introduces monochromatic aberrations. This severely distorts the focal spot, and therefore dims the image significantly.

To improve the images in the presence of aberrations, we use adaptive optics (AO) to obtain a near-diffraction limited focused spot at the retina. We employ a sensor-less adaptive optics (SAO) approach via an image-based hill-climbing algorithm to determine which aberration corrections result in the sharpest image. We have previously reported on small animal retinal imaging systems that can perform image-guided aberration corrections using SAO (see for example [2] and [3]. In this report, we present on our progress on evaluating the performance of our multimodal imaging system combining AO, OCT and scanning laser confocal reflectance and fluorescence on *ex vivo* retina samples.

2. Previous Work

The details of our system are described in detail in [3] and are outlined here in brief. Using the concepts outlined above, we designed a multimodal system capable of OCT and confocal scanning laser reflectance and fluorescence microscopy. The SAO proved to be very beneficial to improve image quality, and allowed for monitoring of cellular dynamics such as microglia motility. The structural imaging with OCT may be highly complementary to related confocal imaging techniques [4].

3. Methods and Results

The multimodal imaging system was reconfigured with a fold mirror to direct the imaging beam in a downward direction, which is more suitable for imaging samples on microscope slides and well plates. The final optical relay and objective lens are readily interchangeable to investigate imaging performance versus numerical aperture (NA). The imaging system is shown schematically in Figure 1. The OCT light source was a superluminescent diode (SLD) with a centre wavelength of 850nm (Superlum, Ireland). The light source for reflectance and fluorescence excitation

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was a multi-line laser (Toptica Photonics, Germany). A variable focus lens (VFL, A-39N1, Corning, USA) was used to change the axial location of the focal spot to image different depths in the retina. Following this is the deformable mirror (DM, DM-69, ALPAO, France) for aberration correction, and an XY mounted pair of galvanometer-scanning mirrors (GM, 6215H, Cambridge Technology Inc., USA) to scan the focal spot across the retina. These three elements were optically conjugated to the mouse eye with lens-based relays. The back-scattered light from the excitation was recombined with the light from the reference arm and directed into a spectrometer.

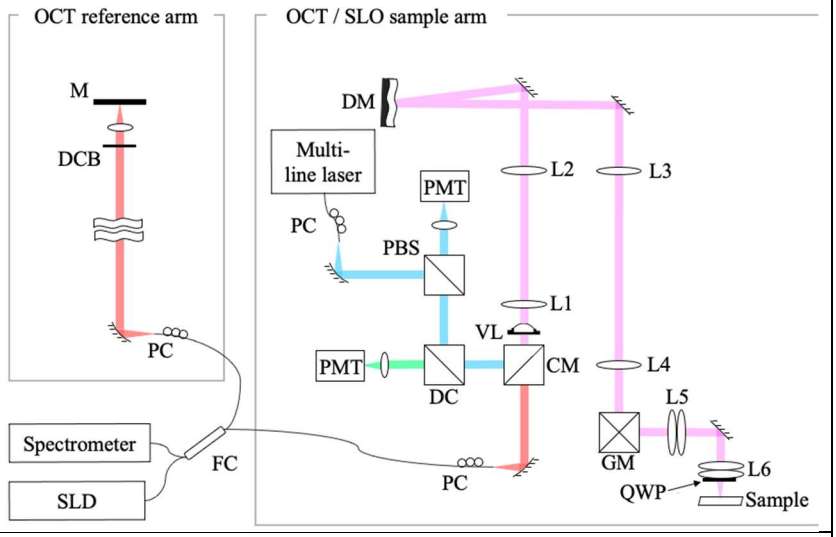

Figure 1. Schematic of the system used for Sensorless Adaptive Optics Optical Coherence Tomography and confocal imaging in ex vivo samples. PMT: photo multiplier tube; DC: dispersion compensation; PBS: pellicle beam splitter; VL: variable lens; DC: dichroic mirror; GM: galvanometer-scanning mirror; DM: deformable mirror; QWP: quarter wave plate; PC polarization controller; CM: cold mirror.

The A-scan rate of the OCT was 100 kHz, with a sampling density of 1024 x 400 x 200. The digitization of the PMT signal was synchronized with the OCT A-scans to ensure that it and the confocal reflectance and fluorescence could be operated simultaneously and were co-registered. This gives the confocal images complimentary information on 3D structure and direct visualization of the position of the focal plane in the retinal cross-section. The OCT detection can be turned off to achieve higher confocal acquisition rates to allow for better averaging in post-processing.

Representative images of an *ex vivo* human retina are presented in Figure 2. The retina was obtained at 22 hours post-mortem and fixed with paraformaldehyde. Prior to imaging, the retina was stained using tomato lectin with Dylight488 conjugated. The 'eye-cup' preparation was placed in fluoromount on a glass slide with coverslip for imaging. The light source imaging intensity was maintained at similar levels as used for *in vivo* imaging. The retinal layers in the cross-sectional OCT B-scan show indications of slight denegation in the post mortem tissue.

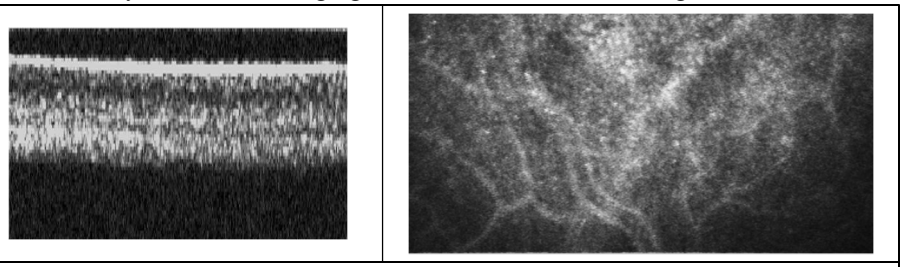

Figure 2. Representative images of *ex vivo* human retina acquired with the multimodal imaging system. (Left) OCT (Right) Fluorescence.

4. References

1] "Time-to-Focus," Fight-for-Sight, 2020. [Online]. Available: www.fightforsight.org.uk/our-research/timetofocus

[2] M. J. Ju, C. Huang, D. J. Wahl, Y. Jian, and M. V. Sarunic, "Visible light sensorless adaptive optics for retinal structure and fluorescence imaging," *Optics Letters*, vol. 43, no. 20, pp. 5162–5165, 2018.

[3] D. J. Wahl, R. Ng, M. J. Ju, Y. Jian, and M. V. Sarunic, "Sensorless adaptive optics multimodal en-face small animal retinal imaging," *Biomed Opt Express*, vol. 10, no. 1, pp. 252–267, Jan. 2019.

[4] A. J. Radtke *et al.*, "IBEX: A versatile multiplex optical imaging approach for deep phenotyping and spatial analysis of cells in complex tissues," *Proc Natl Acad Sci U S A*, vol. 117, no. 52, pp. 33455–33465, Dec. 2020.