High-Speed Cellular-Resolution Retinal Imaging and Optoretinography Enabled by Spatio-Temporal Optical Coherence Tomography

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We present a fast, adaptive-optics-free approach for *in vivo* structural and functional retinal imaging using spatio-temporal optical coherence tomography (STOC-T). The method enables cellular-resolution imaging of photoreceptors within less than one minute and allows optoretinography (ORG) at the level of individual cones using numerical aberration correction. Preliminary results demonstrate distinguishable stimulus-evoked ORG responses from single cones, highlighting the potential of STOC-T as a rapid and cost-effective tool for combined retinal structure and functional imaging.

Keywords: *In vivo* retinal imaging, OCT, computationl aberration correction, photoreceptors, optoretinography

INTRODUCTION

The human retina contains specialized cells, such as ganglion cells, amacrine cells, and photoreceptors, whose degeneration causes vision disorders and neurodegenerative diseases. Early detection of structural changes is crucial for diagnosis and treatment. Optical Coherence Tomography (OCT) with adaptive optics enables cellular-level imaging but is costly and complex. Fourier Domain OCT (FD-OCT) allows rapid, volumetric retinal imaging, but conventional scanning FD-OCT faces a trade-off between transverse resolution and depth of field. Full-Field FD-OCT (FF-FD-OCT) overcomes this by providing stable spatial phase, uniform axial resolution, and flexible beam shaping without confocal limitations, using ultrafast acquisition. However, spatial coherence induces speckle and cross-talk noise, limiting imaging depth [1, 2]. Spatio-Temporal Optical Tomography (STOC-T) addresses this by reducing spatial coherence with multimode fiber coupling, enabling high-contrast, high-resolution retinal projection imaging [3, 4]. Nevertheless, morphological imaging alone does not provide sufficient information about photoreceptor function. Optoretinography (ORG) is a method that can be described as “measurement of light-evoked changes in the optical properties of retinal neurons” [5]. STOC-T has already proven to be capable of recording ORG signals occurring in response to both single pulse and flickering stimuli [6]. ORG with adaptive optics (AO) enables functional imaging of individual retinal cones and their classification into S, M, and L types [7, 8]. In this study, we present preliminary results demonstrating that meaningful ORG signals from single cones can also be obtained without hardware adaptive optics, using STOC-T combined with numerical aberration correction during post-processing.

METHODS

This study reports recent *in vivo* structural and functional retinal imaging results obtained using an upgraded STOC-T system, extending the configuration previously described in [4]. The principal modification involves replacing the active mode control module, based on a deformable membrane and a 300 m multimode fiber, with a fully passive approach employing a 600 m multimode fiber. This redesign simplifies the optical layout by removing the requirement for dynamic control while

providing effective spatio-temporal phase randomization, which is critical for speckle noise reduction.

For structural imaging, 30 volumetric datasets were sequentially acquired, each consisting of 512 B-scans with 512 × 512 pixels, covering a 980 × 980 μm field of view at a frame rate of 60,000 fps. During optoretinographic measurements, the acquisition rate was increased to 100,000 fps, and 340 volumes were recorded over a 490 × 490 μm field of view. A 3-minute dark adaptation period was applied prior to measurement. Single-cone ORG responses to a 660 nm, 500 ms stimulus were subsequently recorded. Post-processing included numerical aberration correction and multi-stage volume registration, enabling reconstruction of the photoreceptor mosaic and identification of individual cones.

RESULTS

Figure 1 shows B-scan and *en face* images reconstructed from measurement of healthy 20-years volunteer. The study protocol was approved by the ethics committee appointed in accordance with the regulations (approval no. KB 87/ 2021). The following cross-sectional views are shown in Fig. 1: inner plexiform layer (IPL), inner nuclear layer (INL), combined IPL and INL, the layers corresponding to the location of the ends of the photoreceptor outer segments for the S cones, M and L cones. We demonstrate a modified STOC-T experimental system, with an increased lateral resolution of ~3 μm, and optimized illumination to enable *in vivo* cellular-level imaging of the human retina.

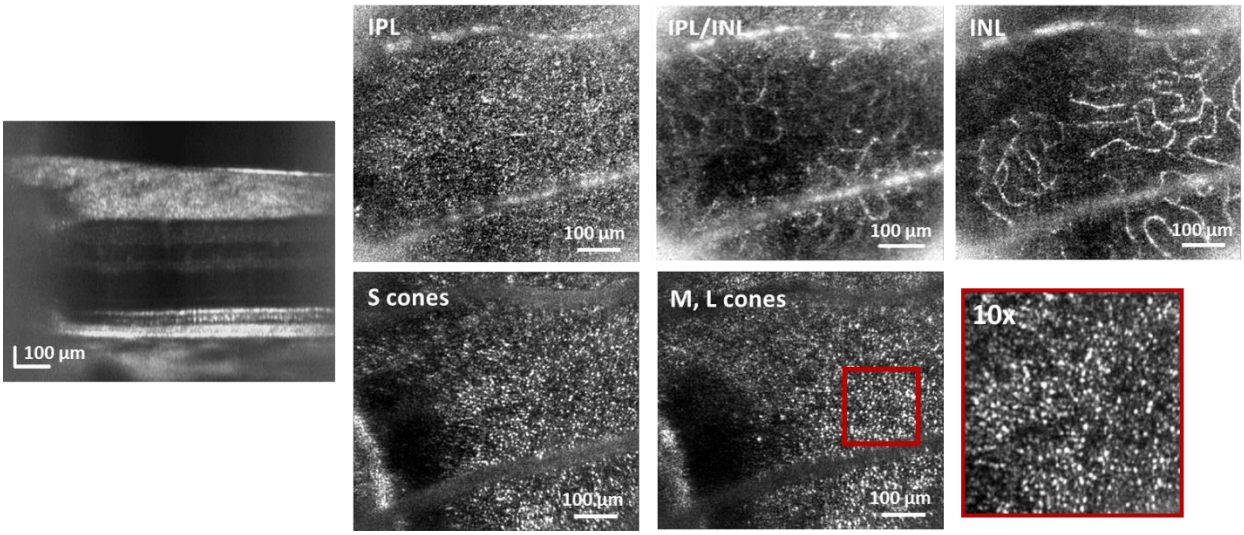

Figure 1. STOC-T imaging of retinal microstructure: (a) cross-sectional image of the internal microstructure (B-scan), (b) inner plexiform layer (IPL), (c) combined IPL and inner nuclear layer (INL), (d) inner nuclear layer (INL), the layers corresponding to the location of the ends of the photoreceptor outer segments for the (e) S cones, (f-g) M and L cones.

Figure 2 shows results for functional measurement: (a) reconstructed photoreceptors mosaic with marked cones from measurement of healthy 29-years volunteer; (b) representative single-cone ORG responses to a 660 nm stimulus with stimulus onset at 0.25 s, showing distinct baseline and stimulus-evoked signals; (c) histogram of mean ORG responses computed over the 0.25–1 s interval after stimulus onset, revealing two response populations attributed to L- and M-cones. Reduced separation compared to AO-based studies is observed. Figure 2(d) shows cone-type classification overlaid on the reconstructed cone mosaic.

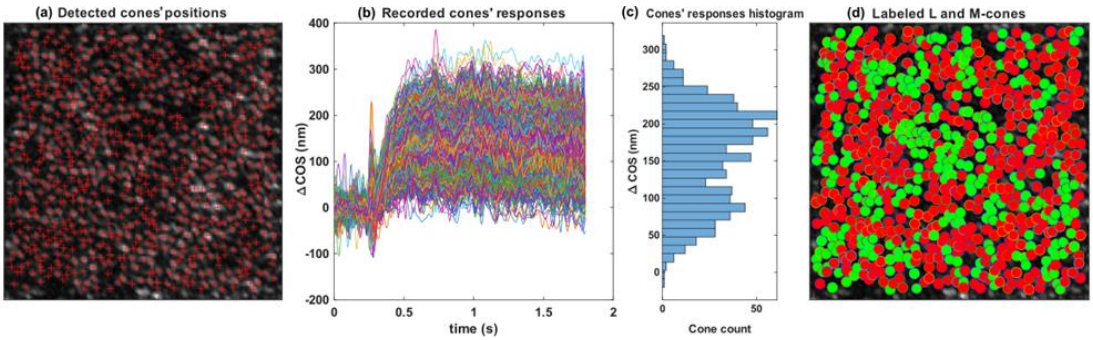

Figure 2. STOC-T functional measurements of the reconstructed photoreceptor mosaic.

CONCLUSIONS

STOC-T enables fast, high-resolution in vivo imaging of retinal structure and function without hardware adaptive optics. Preliminary findings demonstrate the feasibility of functional cone assessment using numerical aberration correction alone, with both baseline and stimulus-evoked ORG responses clearly resolved at the single-cone level. Cone-type separation is observed for 660 nm stimulation. Ongoing studies focus on evaluating phase stability, measurement reproducibility, and the feasibility of complete classification of S-, M-, and L-cones. Overall, STOC-T represents a promising and cost-effective platform for combined structural and functional retinal imaging, with potential applications in early diagnosis and monitoring of retinal and neurodegenerative diseases.

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