Mouse retina hemodynamics analysis using advanced optical imaging, estimating pulse wave frequency, phase and velocity.

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1. Main Text

Non-invasive *in vivo* retinal hemodynamics analysis is particularly valuable for diabetic retinopathy, and glaucoma diagnostics [1]. Fourier Domain Full-Field Optical Coherence Tomography (FD-FF-OCT) overcomes the volumetric acquisition speed issue typical for Doppler OCT [2], and it has been employed for hemodynamic analysis, including pulse wave propagation in human retinal vessels [3]. This work describes a system based on Spatio-Temporal Optical Coherence Tomography (STOC-T), a variant of FD-FF-OCT reducing the influence of scattering crosstalk, opening access to deeper layers in the eye [4], designed for mouse retinal imaging [5]. The system can be controlled to focus on a specific retinal or choroidal vascular bed in the mouse eye. The phase enabled OCT system across the *en-face* layer in conjunction with ultrafast acquisition speeds facilitates combined intensity and phase processing. Either of the methods allow for the extraction of hemodynamic parameters, including pulse trace, blood pulse wave propagation, across the different layers within the mice eye.

2. Methods and results

By connecting the swept-source laser to the optical system with a long multimode fiber, we can implement effective suppression of the coherent crosstalk noise [4]. The system benefits from a white light fundus imaging system for guiding the mouse eye into focus for the STOC-T system [5]. The optical system volumetric acquisition rate of 112 Hz provides high temporal resolution data, which underwent a digital aberration correction processing pipeline [6]. For hemodynamic purposes, the layers of interest focused on the choroid, retinal nerve fiber layer (NFL) and outer plexiform layer (OPL), as these layers have a significant density of blood vessels compared to the other layers, so the layers indicated by colour in the OCT volume in Figure 1 a) were analysed in greater detail. By analysing the time- change of the amplitude for NFL and choroid, we can derive the deviation from mean amplitude over time Figure 1 f) within the chosen region of interest (ROI) shown in Figure 1 b-c). Furthermore, by tracking the change of amplitude time-series in small sections along the vessel indicated in Figure 1 d), we can see the spatial amplitude wave propagation. The blood wave propagation within a vessel can be tracked in time as seen in Figure 1 e). Figure 1 h) presents the 2D Fourier Transform of the space-time representation (from Figure 1 e). Different frequency components group along a constant spatial frequency – temporal frequency ratio, which corresponds with the propagation velocity within the blood vessel. We estimated the propagation velocity (v) to lie in a range between 350-600μm/s by means of $v = \lambda \cdot f$, however it needs further investigation with a controlled metrological test sample, to confirm the estimation accuracy of our method.

The animal studies were approved by the I Local Ethical Commission for animal experiments in Warsaw (resolution no 1401P3/2022).

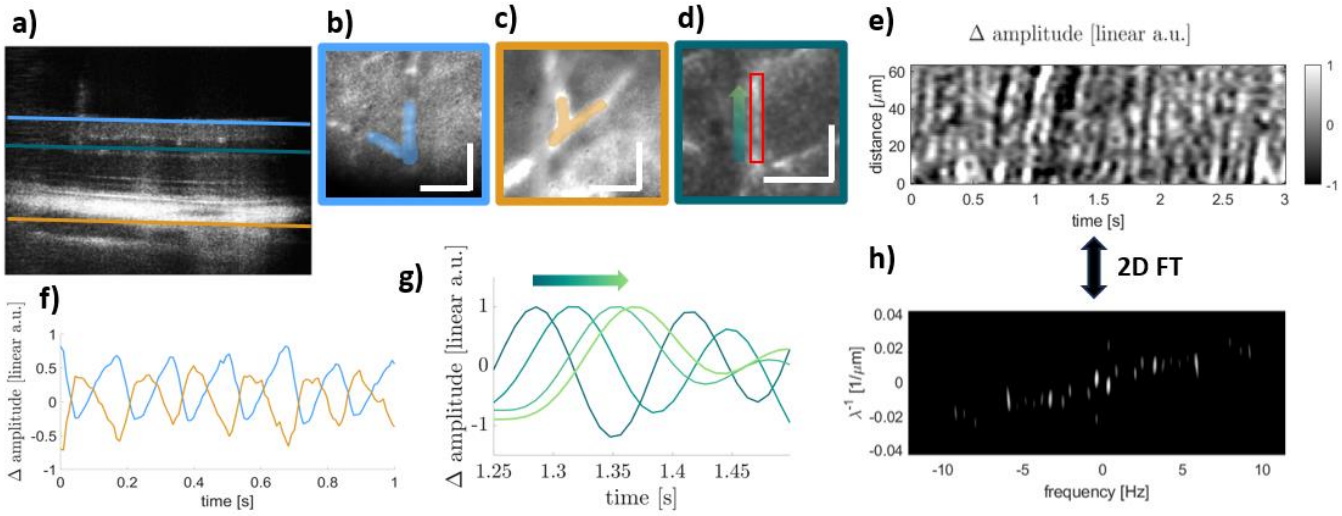

Fig. 1. **a**) Retinal B-scan of B6 albino wild mouse with color-coded layers that underwent further analysis. **b** – **d**) Respectively the enface ROI from the images of the NFL, choroid layer and OPL. **f**) Time-change of the amplitude in the STOC-T signal analyzed on the examples of NFL **b**) and choroid **c**). **g**) Time-change of amplitude in the STOC-T signal analyzed in four sections (ROI) of the vessel from Figure 1 **d**). **e**) Space-time representation of the vessel section from Figure 1 **d**). **h**) Spatial frequency – temporal frequency representation of space-time signal (from Figure 1 **e**))

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4. References

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