Functional mouse retinal imaging using the recently advancing translational eye research STOC-T method

Wiktor Kulesza [a,b,] *, Maciej Wielgo[a,b], Piotr_Węgrzyn[a,b,c], Sławomir Tomczewski[a,b], Katarzyna Kordecka[a,b], Anna Galińska[a,b], Bartłomiej Bałamut[a,b], [b] Egidijus Auksorius[d], Andrzej Foik[a,b], Maciej Wojtkowski[a,b], Dawid Borycki[a,b], Andrea Curatolo[a,b,]

[a]International Centre for Translational Eye Research, Skierniewicka 10a, Warsaw, Poland
[b]Institute of Physical Chemistry PAS, Kasprzaka 44/52, Warsaw, Poland
[c]Faculty of Physics, University of Warsaw, Pasteura 5, Warsaw, Poland
[c]Center for Physical Sciences and Technology (FTMC), Saulėtekio al. 3, Vilnius, Lithuania

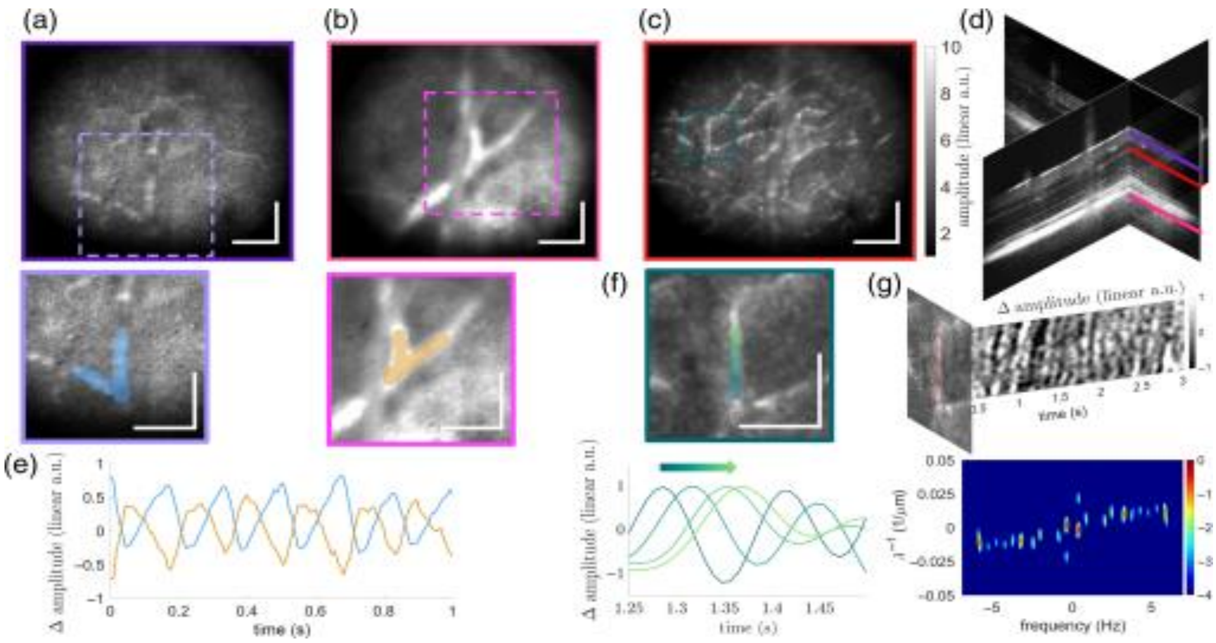
[#]corresponding author email: author@author.com
**Presenting author*

1. Main Text

Advances in laser and detector technology have opened the door to faster, more stable, and powerful biomedical imaging techniques. Beyond structural imaging, researchers now seek functional insights from living tissue, aiming to localize dynamic events for a more accurate understanding of biological mechanisms. Optical methods are ideal for this, offering high-resolution and adaptable imaging. Full-Field Swept-Source Optical Coherence Tomography (FF-SS-OCT) stands out for its ability to rapidly acquire volumetric and phase-sensitive data. Our spatio-temporal optical coherence tomography (STOC-T) method enhances FF-SS-OCT by reducing scattering crosstalk, enabling deeper imaging in the mouse eye [1]. Its 112 Hz acquisition rate captures fast dynamics, supported by digital aberration correction [2]. STOC-T is used in both hemodynamic [3] and optoretinography [4] research.

2. Methods and results

For hemodynamic studies, high-quality in vivo volumetric images of the mouse retina were digitally corrected to reveal detailed retinal and choroidal structures, including microvasculature across key layers like the choroid, nerve fiber layer (NFL), and outer plexiform layer (OPL) [3]. These layers were chosen for their dense vascular networks. Numerical methods were used to estimate wave propagation by analyzing frequency components at constant spatial frequencies. In the OPL, capillary pulse wave velocity averaged 0.35 mm/s, and both arterial and venous pulsation frequencies and phase delays were identified. Tissue displacement near major vessels—up to 150 nm—was measured, shedding light on retinal biomechanics. Delays in displacement due to pulse wave propagation were also characterized.



Mouse chorioretinal hemodynamics were analyzed in the NFL, OPL, and choroid using STOC-T amplitude signal variations over time in an albino mouse. STOC-T en-face images illustrate the NFL (a) and choroid (b), with zoomed-in regions highlighting blood vessels. En-face imaging of the OPL (c) includes an indicated region of interest (ROI). Scale bars are provided. Cross-sectional views of the STOC-T volume (d) depict the relative depths of the analyzed layers (NFL, OPL, and choroid). The deviation from mean pixel amplitude over time was averaged within the shaded areas in (a) (cyan) and (b) (orange) for their respective ROIs (e). A green gradient arrow overlays a capillary in the OPL ROI (f), indicating blood flow direction. Below, a graph shows STOC-T signal amplitude variations over time at different points along the vessel, demonstrating pulse wave propagation. The time-space representation (top) of amplitude variation in (g) along the vessel corresponds to a 2D Fourier transform amplitude (bottom), depicting different frequency components propagating at a group velocity.

In optoretinography studies, a PDE6 inhibitor (sildenafil) was used to explore how conformational changes in PDE6 affect light-induced length changes in rodent photoreceptor outer segments. Under scotopic conditions, nanometer-scale responses to a single light pulse were detected—with and without the inhibitor. Findings suggest that angstrom-level structural shifts in PDE6 are amplified to the nanometer scale through the architecture of the outer segment and sequential activation. This highlights the potential of photopic flicker optoretinography to link molecular phototransduction events to measurable structural changes in rods and cones, positioning it as a promising diagnostic tool.

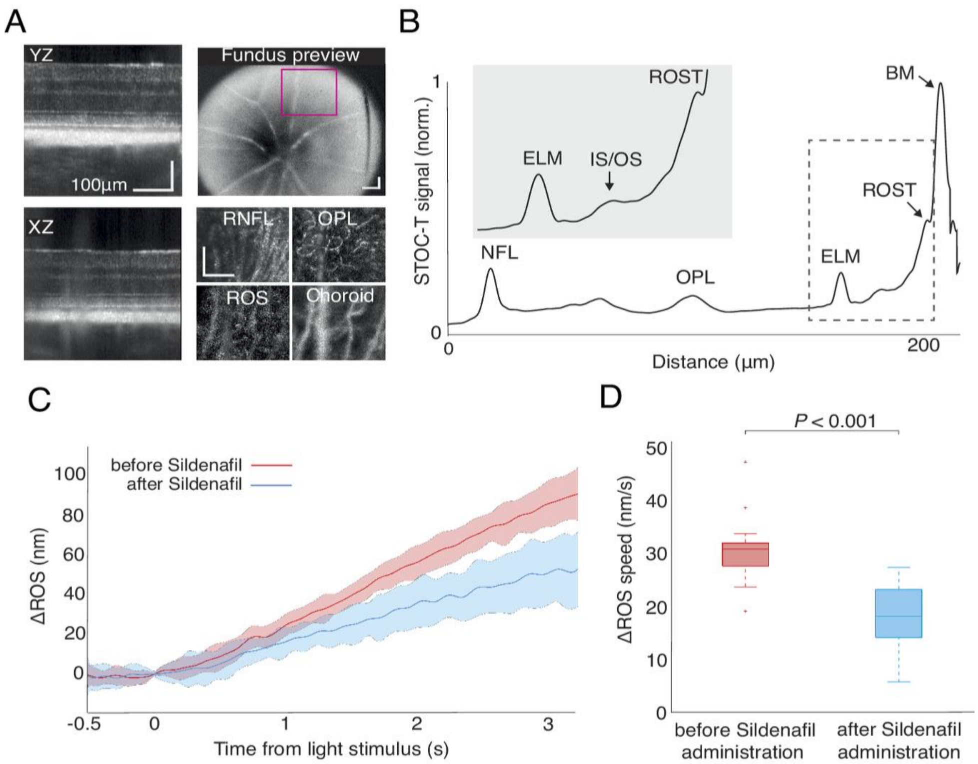


Figure 2 Scotopic single-pulse optoretinography (ORG) was conducted in vivo in the mouse eye to assess sildenafil's effects on rod length changes in response to light stimulation. (A) STOC-T images of a BALB/c albino mouse retina are shown alongside a fundus camera preview. (B) An STOC-T averaged A-scan acquired before sildenafil administration under scotopic conditions is presented, with an inset highlighting the ELM, IS/OS, and ROST regions. (C) Single-pulse, rod-mediated scotopic ORG recordings before (red) and after (blue) sildenafil administration are displayed, with the centerline indicating the mean and the shaded area representing ±one standard deviation. (D) A boxplot illustrates the differences in the linear component of rod elongation velocity before (red) and after (blue) sildenafil administration. The central mark indicates the median, with the box edges representing the 25th and 75th percentiles. Whiskers extend to the most extreme non-outlier data points, with individual outliers plotted separately. A Welch's t-test confirmed a statistically significant difference in the scotopic ORG response pre- and post-sildenafil administration (P < 0.001).

The development of such system provides insights into the hemodynamics of the mouse retina and choroid that could be beneficial in the study of neurovascular coupling and vasculature and flow speed anomalies in neurological and neuro-ophthalmic conditions.

3. Acknowledgement

Funding: ICTER: The International Centre for Translational Eye Research (FENG.02.01-IP.05-T005/23) project is carried out within the International Research Agendas programme of the Foundation for Polish Science co-financed by the European Union under the European Funds for Smart Economy 2021-2027 (FENG)

4. References

1. E. Auksorius, D. Borycki, P. Wegrzyn, B. L. Sikorski, K. Lizewski, I. Zickiene, M. Rapolu, K. Adomavicius, S. Tomczewski, and M. Wojtkowski, "Spatio-temporal optical coherence tomography provides full thickness imaging of the chorioretinal complex," iScience **25**, 105513 (2022).
2. D. Borycki, E. Auksorius, P. Węgrzyn, and M. Wojtkowski, "Computational aberration correction in spatiotemporal optical coherence (STOC) imaging," Opt. Lett. **45**, 1293-1296 (2020)
3. Piotr Węgrzyn, Wiktor Kulesza, Maciej Wielgo, Sławomir Tomczewski, Anna Galińska, Bartłomiej Bałamut, Katarzyna Kordecka, Onur Cetinkaya, Andrzej Foik, Robert J. Zawadzki, Dawid Borycki, Maciej Wojtkowski, Andrea Curatolo, "*In vivo* volumetric analysis of retinal vascular hemodynamics in mice with spatio-temporal optical coherence tomography," Neurophoton. 11(4) 045003 (8 October 2024) https://doi.org/10.1117/1.NPh.11.4.045003
4. S. Tomczewski, A. Curatolo, A. Foik, P. Węgrzyn, B. Bałamut, M. Wielgo, W. Kulesza, A. Galińska, K. Kordecka, S. Gulati, H. Fernandes, K. Palczewski, & M. Wojtkowski, Photopic flicker optoretinography captures the light-driven length modulation of photoreceptors during phototransduction, Proc. Natl. Acad. Sci. U.S.A. 122 (7) e2421722122, https://doi.org/10.1073/pnas.2421722122 (2025).