High-fidelity microscopy investigations of eye diseases

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(a) Background of research

The eye, one of the most complex organs in the body, is an extension of the central nervous system. Abnormalities in the eye have been linked to a range of ocular and systemic conditions, especially neurodegenerative diseases, highlighting the significance of examining eye pathologies. The use of mouse models is widespread in the exploration of eye disease mechanisms and therapies, due to their well-developed genetics and physiological similarities to human eyes. To comprehensively understand eye pathologies, it is crucial to employ methods that allow for early detection of abnormalities and longitudinal tracking of eye structures and functions.

Optical microscopy, owing to its subcellular spatial resolution and non-invasiveness, has become a powerful tool for probing eye pathologies. Non-linear microscopy, due to its intrinsic optical sectioning capability and the retina's minimal responsiveness to near-infrared (NIR) excitation, has unique advantages for studying the structures and functions of the mouse eye. Recent advances in mouse genetics and fluorescent reporters have further enhanced the capabilities of fluorescence microscopy for investigating specific types of neurons and glial cells, as well as vasculature (**Fig. 1A**). Additionally, label-free imaging technologies have been developed and applied to study specific eye structures. Second harmonic generation (SHG) microscopy, for example, can only be produced from non-centrosymmetric structures. In the eye, these structures include the cornea (**Fig. 1A**), sclera, nerve fiber bundles, as well as the deposition of certain proteins, such as senile plaques, which are biomarkers for various eye or systemic disorders and are thus crucial to study. These advancements in optical microscopy have opened new avenues for exploring a variety of eye and systemic disorders. Over recent decades, there has been a surge in the evolution of microscopy technologies, offering unparalleled levels of resolution, depth, speed, and specificity in biological imaging. Despite these advancements, real-world applications of current imaging technologies face significant challenges (**Fig. 1B**) that remain unaddressed, necessitating further refinement for optimal imaging performance.

Over the past six years, the principal investigator (PI) has focused on developing cutting-edge optical microscopy methods for high-resolution brain and eye imaging (**Fig. 2**). Incorporating AO with computational imaging methods based on structured illumination microscopy (SIM), the PI demonstrated the first-time diffraction-limited optical sectioning SIM imaging *in vivo* (**Fig. 2[A1]**; *Science Advances*, 2020) and the first-time synapse-resolving widefield microendoscopic imaging of the mouse brain *in vivo* (**Fig. 2[A2]**; *Optica*, 2020). Applying 2-photon fluorescence microscopy

(2PFM), the PI imaged neuronal activity in the primary visual cortex reported by a newly developed near-infrared (NIR) calcium indicator (**Fig. 2[A3]**; *Nature Biotechnology*, 2021). Recently, we developed a self-supervised deep learning algorithm for computational AO and demonstrated simultaneous hardware-free wavefront estimation and three-dimensional structural information extraction in widefield fluorescence microscopy (**Fig. 2[A4]**; *Nature Machine Intelligence*, in revision). Eye imaging, compared with brain imaging, suffers more from severe and complex optical aberrations from the imperfect eye optics. Recently, we employed AO-assisted 2-photon microscopy to investigate retinal pathology and pharmacology with high fidelity (**Fig. 2[B1]**; *eLife*, 2023). The application of AO-2PFM enabled the identification of small-scale vascular lesions and the detection of abnormal neuronal activities in diseased mouse retinas. Applying 2-photon microscopy, we also probed the structural abnormalities in the crystalline lens of cataract mouse eyes (**Fig. 2[B2]**; *in preparation*).

In this proposed project, the PI aims to address key challenges in *in vivo* imaging of the mouse eye by integrating concepts drawn from astronomy, physics, and machine learning. The primary objective is to develop high-resolution and ultra-sensitive imaging technologies for probing eye pathologies. Specifically, we will develop a machine-learning based adaptive optics (ML-AO) approach for high-resolution eye imaging and a homodyne-detection-based second harmonic generation (SHG) microscopy technique for ultra-sensitive imaging of cornea and sclera. Employing these proposed methods, we will study two specific eye conditions: cataract and myopia.

(d) Figures

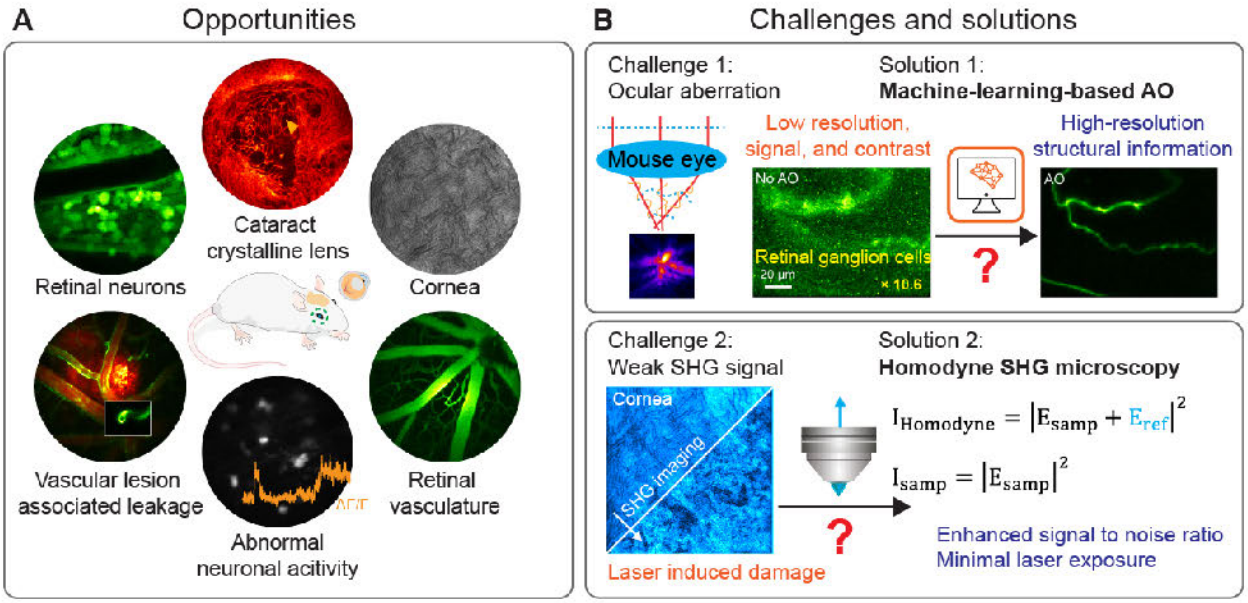

Figure 1. Project overview. **(A)** Opportunities in applying 2PFM and SHG microscopy for eye imaging. **(B)** Challenges and solutions in non-linear eye imaging. Proposed technologies: ML-AO (top) and ultra-sensitive homodyne SHG microscopy (bottom). Images: *eLife* (2023) and *unpublished*.

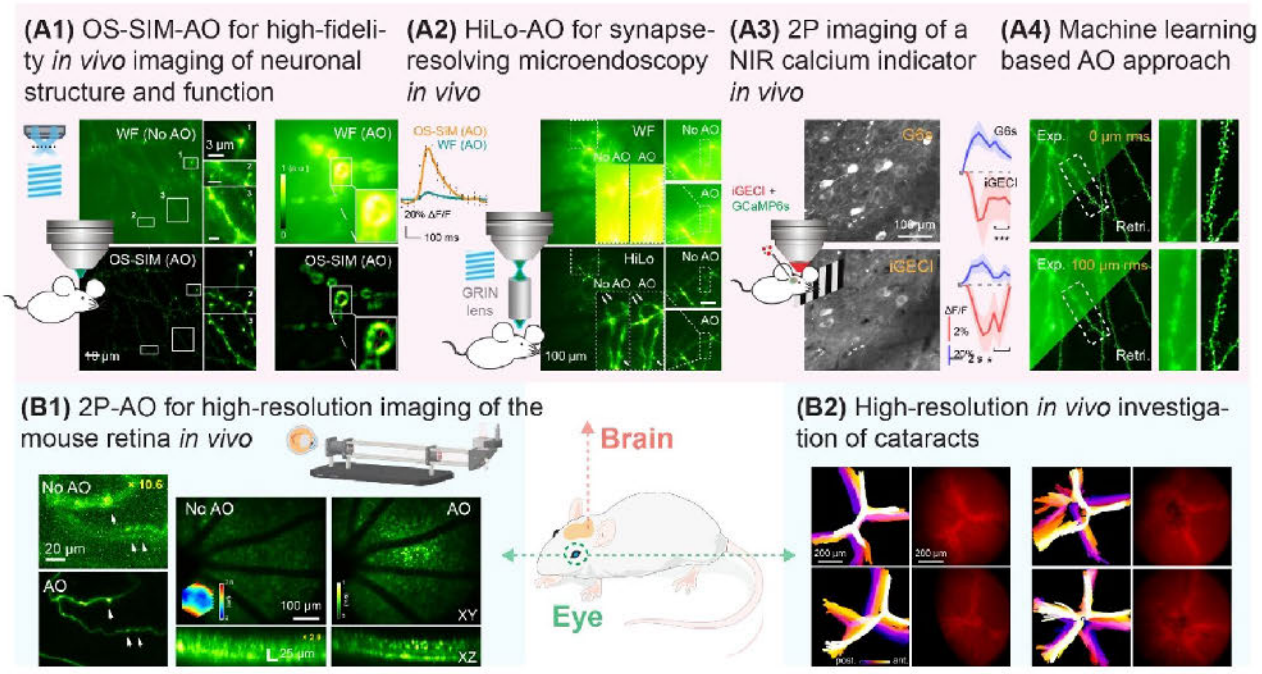

Figure 2. Summary of the PI's past research. Development of high-resolution microscopy technologies for *in vivo* imaging of the mouse brain **(A1-A4)**, the mouse retina **(B1)**, and the mouse crystalline lens **(B2)**. Images from: *Sci. Adv.* (2020), *Optica* (2020), *Nat. Biotechnol.* (2020), *Nat. Mach. Intell.* (*in revision*), *eLife* (2023), and (*manuscript in prep*).

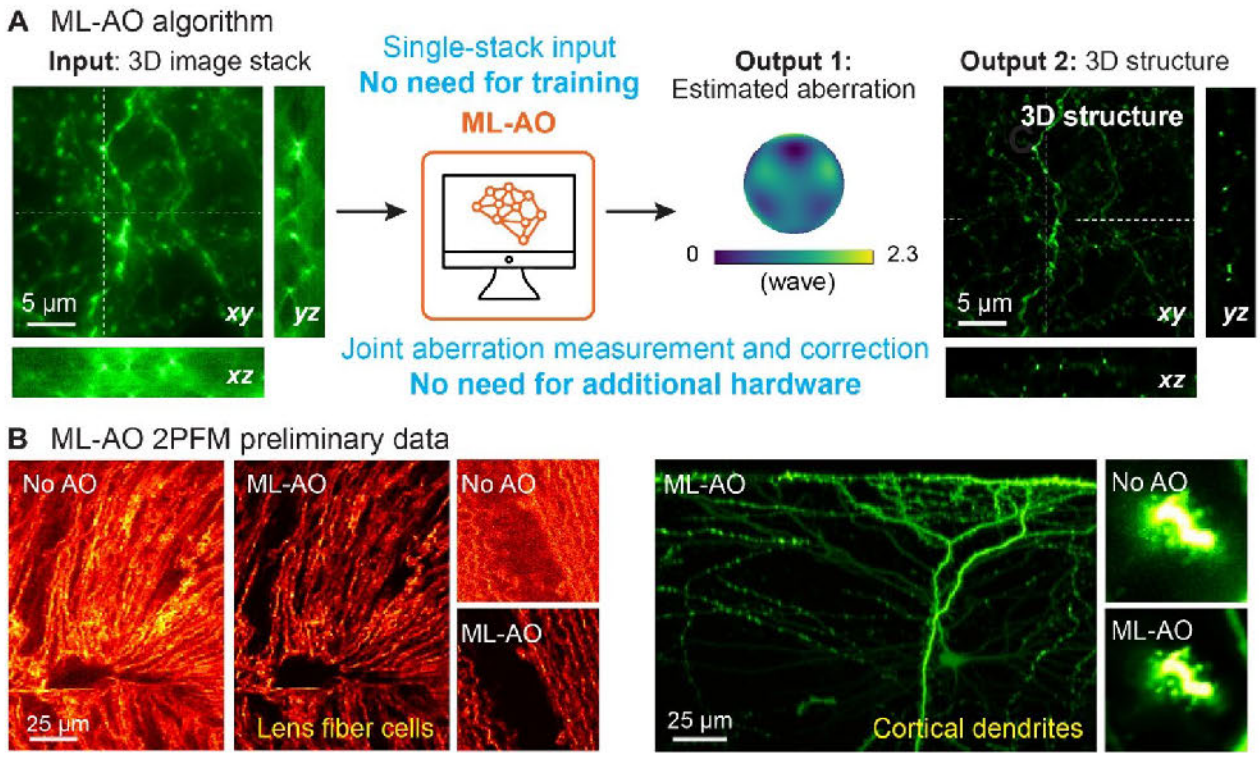

Figure 3. Proposed technology: Machine learning based adaptive optics approach (ML-AO). **(A)** ML-AO for widefield microscopy. *Nat. Mach. Intell.* (*in revision*). **(B)** Preliminary results of ML-AO applied with 2PFM.

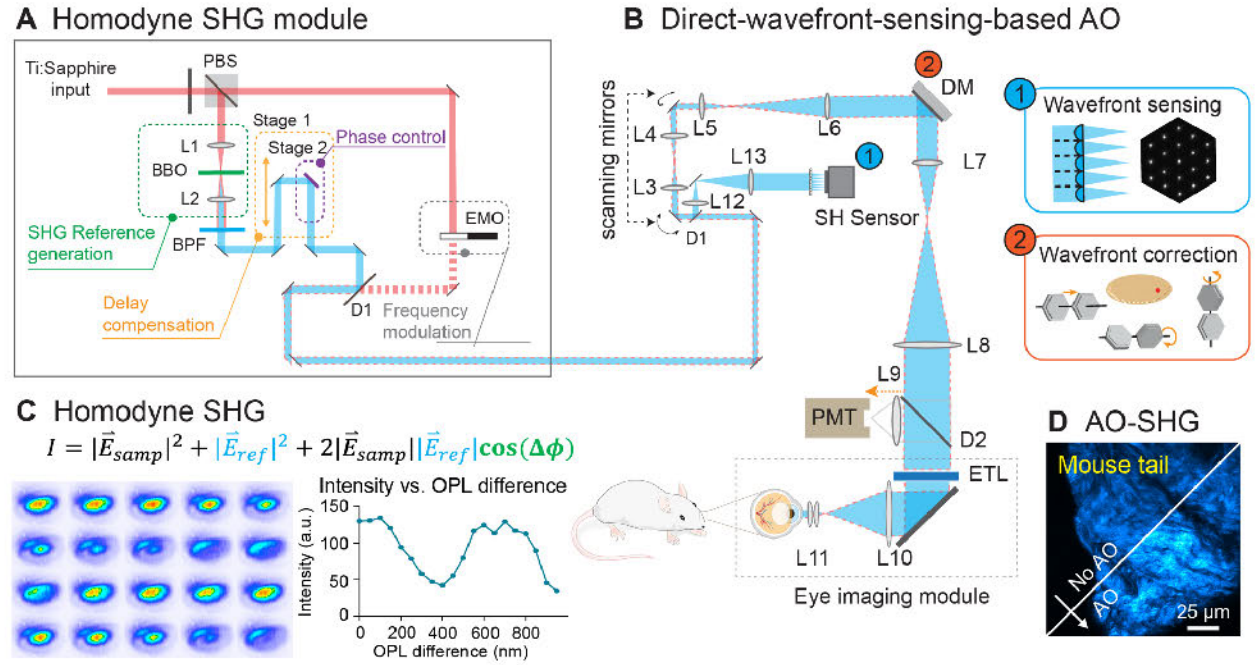

Figure 4. Proposed technology: ultra-sensitive homodyne SHG microscopy. **(A)** Schematics of the homodyne SHG module. **(B)** Direct-wavefront-sensing-based AO. Inset 1: direct wavefront sensing using a Shack-Hartmann (SH) sensor, inset 2: wavefront correction using a deformable mirror (DM). **(C)** Preliminary results of homodyne wave mixing at a single focal point. OPL: optical path length. (D) Preliminary results of AO applied with SHG microscopy for imaging the mouse tail tissue.