Novel Optical Microscopies to Unravel Brain Function

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ABSTRACT

Brain function stems from emergent properties of interconnected neuron networks, which requires *in vivo* volumetric imaging with high spatial/temporal resolution. Here, we introduce our recent technical developments for *Drosophila* brain imaging.

INTRODUCTION

The brain is one of the most important organs in our body, but it is functionally the least understood one. It is composed of millions of neurons, whose interconnection, i.e. connectome, determines its function. Although the interaction of neurons *in vitro* has been well studied in the past century, no existing tool can capture whole-brain emergent properties at single neuron or even synapse resolution. To understand functional connectome, an imaging system that can cover a whole brain *in vivo* with spatial resolution of micrometers (neuron) to nanometers (synapse) as well as temporal resolution in sub-seconds (calcium) to milliseconds (action potential) is highly desirable. In this focus article, we introduce our recent efforts to improve optical microscopy in terms of speed, depth, and spatial resolution, toward the goal of understanding the brain of *Drosophila*, which offers a small brain with sophisticated functions and genetic control capabilities.

High speed volumetric imaging with millisecond temporal resolution

Neurons are distributed in three dimensions in the brain, and their firing dynamic is in the millisecond scale. To observe their functional connections *in vivo*, we developed a high-speed volumetric imaging system by combining a conventional two-photon microscope with a tunable acoustic gradient-index (TAG) lens [1]. The TAG lens is based on acoustic resonance, which in turn creates periodic gradient index modulation up to MHz speed, thus enabling high-speed axial scanning when combined with a microscope objective [2, 3]. Fig. 1 (a) shows that we can convert a plane scan in the xy plane into volumetric imaging with sub-second imaging speed. Fig. 1(b) is a line scan in the xy plane converted into a "ribbon" scan in xyz volume, with temporal resolution approaching the millisecond scale.

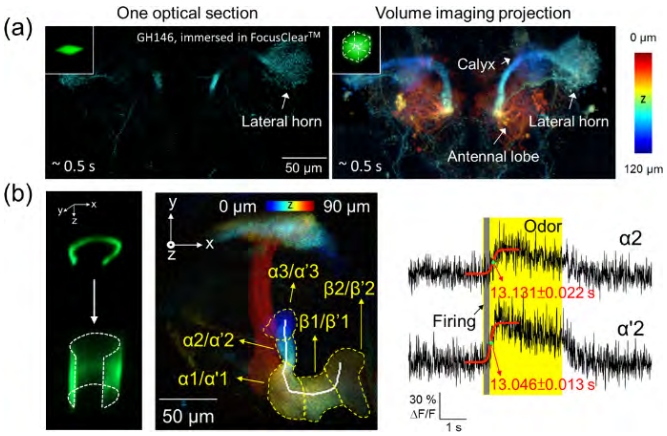

Fig. 1: Fast three-dimensional imaging in *Drosophila* brains. (a) left: conventional two-photon imaging, taking 0.5 s for one 2D optical section; right: our new system observes 3D brain volume with the same acquisition time. (b) Ribbon imaging with 3.8 millisecond temporal resolution, see [1] for more details.

Volumetric all-optical physiology

To further unravel the *in vivo* functional connections, it is necessary to incorporate high-precision stimulation capabilities into a volumetric imaging system, i.e., a volumetric all-optical physiology that uses photons to manipulate and report neuron activities [4]. Fig. 2 presents our results in *Drosophila*'s visual pathway, where we were able to stimulate upstream neurons and record the

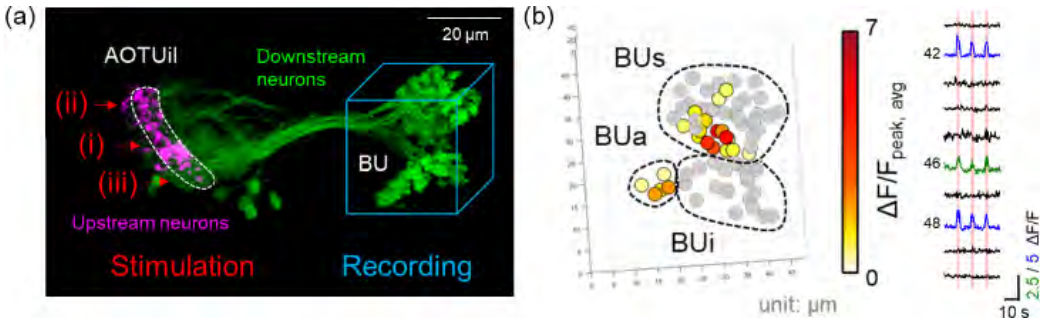

Fig. 2: In-vivo volumetric all-optical physiology in *Drosophila*'s visual pathway. (a) Structures of upstream (AOTU) and downstream (BU) neurons, where the neural signal coding is hidden in >70 tightly assembled 2-μm BU microglomeruli. (b) Volumetric response of downstream BU microglomeruli, some of whose temporal responses are shown in the right-hand side [4].

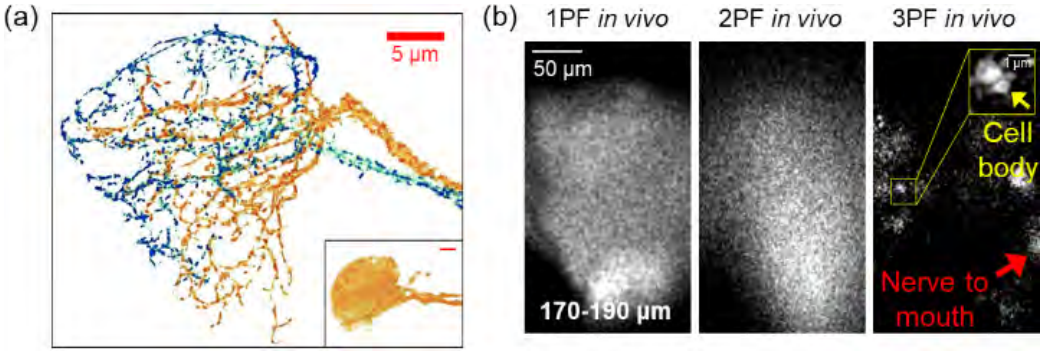

Fig. 3: Deep-tissue imaging in *Drosophila*. (a) Intertwined neural fibrils of two neurons imaged by COOL and a confocal microscope (inset), respectively. Only COOL can distinguish fibrils with nanometer resolution [5].
(b) Comparison of single-, two-, three-photon *in-vivo* imaging at the bottom of a *Drosophila* brain (170-190 μm), with excitation wavelengths at 488-, 920-, and 1300-nm, respectively. Only three-photon imaging provides whole-brain observation [6].

downstream neuronal responses in 3D, thus resolving the neural coding scheme of vision.

Whole-brain super-resolution imaging

In the above results, optical microscopy provides sub-μm spatial resolution, limited by diffraction. The neural fibers and synapses inside a *Drosophila* brain can be much smaller, so super-resolution techniques that breaks the diffraction barrier are required. However, conventional super-resolution modalities are mostly not applicable at the tissue level due to their susceptibility for aberration and scattering. We recently developed COOL (Confocal lOcalization deep-imaging with Optical cLearing) [5], which combines advanced techniques using blinking fluorescence proteins, confocal microscopy, optical clearing, and localization microscopy to achieve 20-nm spatial resolution across a whole brain of *Drosophila*. Fig. 3(a) shows resolving densely entangled dendritic fibers in an intact *Drosophila* brain with unprecedented depth/resolution performance in 3D (inset: in the same structure mapped by confocal microscopy, no fibrils can be observed).

Whole-brain imaging in a living *Drosophila* brain

It is well known that two-photon microscopy provides ~1-mm penetration depth in a mouse brain, but when imaging the *Drosophila* brain, it is mysterious that the imaging depth cannot exceed 0.1 mm! We recently unraveled the underlying mechanism as an strong optical aberration from the trachea, which delivers oxygen in insects. As shown in Fig. 3(b), we used long-wavelength three-photon microscopy to reduce the aberration and therefore achieved whole-brain observation with single neuron resolution in a living *Drosophila* brain [6].

CONCLUSION

For developing a bio-imaging system, the most important factors are contrast, resolution, speed, and depth. By combining interesting physics concepts (such as acoustic resonance for volumetric imaging, high-precision focus-

ing for precise stimulation, localization calculations for super-resolution microscopy, and three-photon excitation for deep-tissue imaging) with innovative biological concepts, (including calcium/voltage sensitive fluorescent protein labeling, optogenetic stimulation, and optical clearing), we push the limits of optical microscopy in all four areas. These novel techniques will benefit not only brain science research, but also studies in other bio-tissues.

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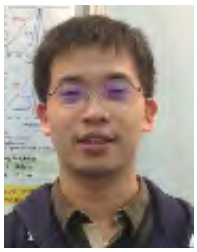

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