Deep-tissue super-resolution imaging of Drosophila brain

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ABSTRACT

Since the days of Cajal, optical microscopy has been a vital tool for physiology, and neuroscientists have accumulated significant amount of information on structures and functions of isolated neurons. However, to understand the emergent properties of a brain, functional observation of complicated neuronal networks is necessary, leading to the request of deep-tissue imaging with dendritic level resolution. In this talk, I report our recent progresses on drosophila brain imaging, including world-record 20-nm resolution across a whole brain, and three-photon imaging penetrating through a living drosophila brain.

In the first part [1], the spatial resolution is provided by molecular localization of a photoconvertible fluorescent protein Kaede, whose blinking state is reported for the first time. The photoconvertibility allows replenishing of blinking fluorophores, potentially solving structure discontinuity issue in super-resolution imaging. The deep-tissue observation is enabled by optical sectioning capability of spinning disk microscopy, as well as reduced scattering of optical clearing. Together these techniques are readily available for many biologists without the need of upgrading hardware, and provide unprecedented depth/resolution performance to three-dimensionally resolve densely entangled dendritic fibers from top to bottom in a complete Drosophila brain.

In the second part [2], a surprising fact is that two-photon microscopy cannot penetrate through the 200- μm-thick brain, due to the extraordinarily strong aberration/scattering from tracheae. Here we achieve whole-Drosophila-brain observation by degassing the brain or by using three-photon microscopy at 1300-nm, while only the latter provides *in vivo* feasibility, reduced aberration/scattering and exceptional optical sectioning capability. Furthermore, by comparing one-photon (488-nm), two-photon (920-nm), and three-photon (1300-nm) excitations in the brain, we not only demonstrate first quantitative reduction of both scattering and aberration in trachea-filled tissues, but unravel that the contribution of aberration exceeds scattering at long wavelengths. Our work paves the way toward constructing functional connectome in a living Drosophila.

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