In vivo whole-brain observation in *Drosophila* by three-photon microscopy

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ABSTRACT

One long-standing dream in brain functional study is to visualize every spike from every neuron in the whole brain. *Drosophila*, whose brain is small and its functional behavior is similar to human, should be the best model animal [1]. However, current two-photon microscope based on 900-1000 nm excitation, cannot image through the whole *Drosophila* brain *in vivo*, even though the whole brain is only about 200 μm in depth, due to strong aberration/scattering from trachea structures and high-density neuron distribution inside the brain [2]. To improve imaging depth, here we demonstrate three-photon fluorescence microscopy [3, 4], in living *Drosophila*, based on a high-energy 1300-nm femtosecond laser. The long wavelength excitation significantly reduces both scattering and aberration, while three-photon fluorescence offers better excitation confinement to suppress background from out-of-focus layers. As a result, *in vivo* whole-brain observation in *Drosophila* is achieved for the first time, with sub-cellular resolution maintained throughout the imaging volume. Combined with functional genetic probes, this method is promising for studying connectomics in *Drosophila* brain.

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