

Press Release

March 11th, 2020

Visualization of *Drosophila*'s mind

- Developing novel optical microscopies to unravel the mystery of brain function

Brain, which governs our mind and behaviors, is arguably the most important organ in our body, but is also the functionally least understood one. Although the function of a single neuron or interaction of a few neurons have been well studied, the number of neurons ranges from hundred thousands in *Drosophila* to billions in human brains, and the emerging properties from the massive connection among these neurons are unknown yet. The main obstacle is lacking a suitable tool that allows us to observe the physiological dynamics of brain with enough spatiotemporal resolution.

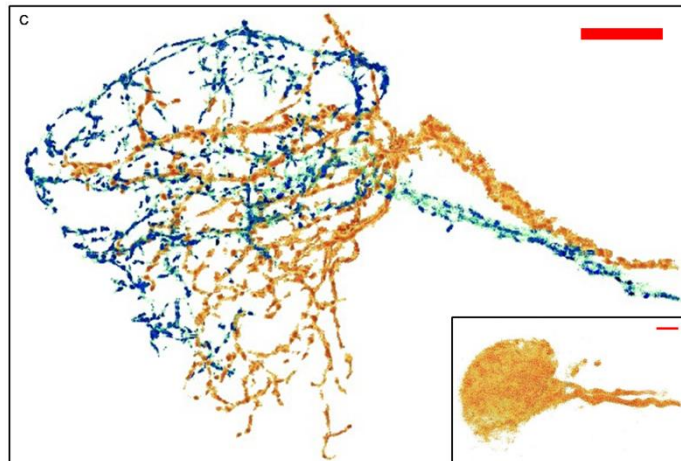
Under the support of the “Disease-oriented Brain and Mind Research Program” (2016 – 2019) and “Taiwan Brain Technology Development and International Raising Program” (2019 – 2021) (MOST, R.O.C.), Prof. Shi-Wei Chu (Physics, NTU) established an interdisciplinary team, including Prof. Meng-Lin Li (EE, NTHU), Prof. Shang-Da Yang (EE, NTHU), Prof. Shun-Chi Wu (Engineering and System Science, NTHU), Prof. Ming-Che Chan (Photonics, NCTU), and Dr. Yen-Yin Lin (JelloX Co.), to develop novel optical micro-imaging platform to study *Drosophila* brain, whose neural network connection is similar to human brain. The platform features high temporal and spatial resolutions that are capable to capture physiological dynamics of neurons in an intact living *Drosophila* brain. Our technical innovations are:

1. First high-speed volumetric imaging system that provides millisecond temporal resolution to observe 3D neuronal firing dynamics in a

living *Drosophila* brain. The result was published in a leading optics journal from Optical Society of America, *Optics Letters*, in 2019 , and was selected as “Editor’s pick”. (reference 1)

2. Combining the high-speed volumetric imaging system with a home-built optical neuronal stimulation system, we have achieved volumetric all-optical physiology observation, that allows us to resolve the 3D neural connection and coding of visual circuits in a *Drosophila* brain. The result was published in a new interdisciplinary journal of Cell Press, *iScience*, in 2019. (reference 2)
3. We have developed deep-tissue super-resolution imaging (COOL, Confocal lOcalization deep-imaging with Optical cLearning), which combines advanced techniques of fluorescence protein labeling, confocal scanning microscopy, optical clearing, and localization microscopy to achieve 20-nm spatial resolution across a whole brain of *Drosophila*. 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. The method not only paves the way toward whole-brain neural network studies, but also will be applicable to other high-resolution imaging in biological tissues. This was published in *iScience* 2019 (reference 3). Due to the contribution in the field of super-resolution microscopy, Prof. SW Chu was invited to write a News and Views article in a top optics journal, *Light: Science and Applications* (IF > 14), promoting the visibility of Taiwan academic society. (reference 4)

(Figure : The deep-tissue super-resolution imaging can distinguish two tightly entangled neural fibers in an intact brain tissue. The inset shows conventional confocal imaging of the same neural fibers, whose structures are not differentiated at all)



4. We also explained a mystery that a deep-tissue imaging technique, two-photon microscopy, which typically provides 1 mm penetration depth in mouse brain, cannot penetrate more than 0.1 mm in *Drosophila's* brain. The reason is optical aberration due to air in trachea of insect's brain (mouse has blood in vessel, whose refractive index is similar to that of brain tissues). Surprisingly, no study has reported optical properties of trachea-filled tissues. We are not only the first group that quantify the optical decay in trachea-filled *Drosophila's* brain, but we also demonstrate that using long-wavelength three-photon microscopy enables whole-brain imaging in a living *Drosophila*. This was published in another leading optics journal from Optical Society of America, *Biomedical Optics Express*, in 2019. (reference 5)

Currently the whole research team is devoted to develop novel optical techniques under the support of the "Taiwan Brain Technology Development and International Raising Program" (2019 – 2021) (MOST, R.O.C.). In addition, we work closely with MOST- and MoE-funded NTHU "Brain Research Center" (2018-2023), which was led by Academician Ann-Shyn Chiang (Systems

Neuroscience, NTHU). Our future goal is to realize observation of “functional whole-brain connectome” in *Drosophila*, i.e. the connections among every single neuron during learning and memory formation, with high temporal resolution (millisecond), high spatial resolution (sub-micrometer to nanometer), and high penetration depth (millimeter), to unravel the mysteries of brain function.

Reference:

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<https://doi.org/10.1364/OL.44.003190>
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<https://doi.org/10.1016/j.isci.2019.11.011>
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4. **S.-W. Chu** “Optical microscopy approaches angstrom precision, in 3D!”, *Light Sci. Appl.* 8, 117 (2019) **Invited News and Views article**
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5. K.-J. Hsu, Y.-Y. Lin, A.-S. Chiang, **S.-W. Chu***, “Optical properties of adult *Drosophila* brains in one-, two-, and three-photon microscopy”, *Biomed. Opt. Exp.* 10, 1627-1637 (2019). <https://doi.org/10.1364/BOE.10.001627>

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