

2 光子顕微鏡で神経活動を計測・制御する新提案

An alternative method to control and monitor neural activity in 2 photon imaging

○全 香玉¹, 加藤 大輔², 和氣弘明², 栗辻 安浩³, 的場 修¹

Kobe Univ.¹, Nagoya Univ.², Kyoto Inst. of Tech.³, °Xiangyu Quan¹, Daisuke Kato², Hiroaki Wake²,

Yasuhiro Awatsuji³, Osamu Matoba¹

E-mail: zen@rabbit.kobe-u.ac.jp

Two-photon(2P) imaging method is outstanding in its ability to form a clear image, and penetrate deep inside of tissue. Modern neuroscience benefits from 2P imaging, however is also suffer from the trade-off of recording speed. 2P imaging requires scanning and sectioning, which prevent fast-recordings of a whole field activity. In this presentation, we will review some alternative methods to perform fast functional imaging using 2P method.

Because the neural activity happens in a fraction of time, electrophysiology is often used to monitor action potentials in the nerve cells. However, this method causes unnecessary damage to the subjects. In order to improve imaging speed using optical method, region-of-interest (ROI) is often used with the price of limited number of target cells. Some studies prove that using spatial light modulator (SLM) to selectively illuminate multiple cells, and record resultant fluorescence reactions using CCD image sensor is valid [1]. Computational method to demix spatio-temporal activity from each illumination spots are also actively studied [2]. Our lab has been developing a holographic stimulation system in 2P microscopes [3]. Here, we propose an alternative method to stimulate selected spots in the whole field of view, and also use holographically illuminated multi-spots to probe fluorescence functional signals using a high-sensitivity image sensor. This method also has a huge potential in simultaneous 3D stimulation and imaging because the SLM produces multi-spots in 3D space. Care should be taken to the intensities of multi-spots, because the diffraction efficiency is depending on to the diffraction angle. An adaptive method to equalize spot intensities in the FOV is also discussed (Fig. 1).

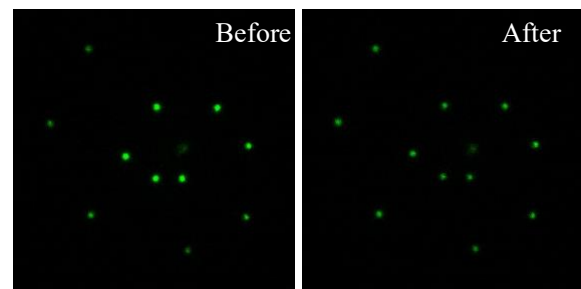


Fig. 1. Intensity correction by adaptive method.

This research is supported by JSPS KAKENHI (20K15193, 18H03888).

[1]. C. Moretti and S. Gigan. "Readout of fluorescence functional signals through highly scattering tissue." *Nature Photonics* 14.6 (2020): 361-364.

[2]. A. Giovannucci, et al. "CalmAn an open source tool for scalable calcium imaging data analysis." *Elife* 8 (2019): e38173.

[3]. X. Quan et al. "Three-dimensional stimulation and imaging-based functional optical microscopy of biological cells." *Optics letters* 43.21 (2018): 5447-5450.