Sensor-less adaptive optics in aperture correlation confocal microscopy

Osaka Univ. [1], Univ. of Oxford [2], Univ. of Oxford, Micron [3], ○Toshiki Kubo[1,3], Syed Hussain[2], Nicholas Hall[3], Karen Hampson[2], Michael Phillips[3], Dalia Gala[3], Ian Dobbie[3], Jacopo Antonello[2], Matthew Wincott[2], Katsumasa Fujita[1], Ilan Davis[3], Martin Booth[2]

E-mail: kubo@ap.eng.osaka-u.ac.jp

Laser-free spinning disk confocal microscopy is a powerful technique for observation of biological specimens in 3D with high temporal resolution. However, the observation depth for thick specimens is limited due to the spatial variations of refractive index in the specimen [1].

Here we implemented a sensorless adaptive optics (AO) system into a spinning disk laser-free confocal microscope [2]. In the optical setup, a deformable mirror (DM) was introduced at an intermediate pupil plane located between a microscope body and a spinning disk confocal module (Clarity, Aurox Ltd., UK) to compensate the aberration induced for both excitation and detection light. To compensate the aberration, phase distributions that provide different degrees of spherical aberration and coma were applied, and fluorescence images under each condition was recorded and used to calculate an optimal phase condition that provides a high image contrast [3]. Control of devices for phase control, image acquisition and phase optimization were performed by using the open-source software, Python cockpit, microscope, and microscope-aotools [4].

We performed aberration correction for fluorescence imaging of *Drosophila melanogaster* larval neuro muscular junction (NMJ) with their nuclei and synapse labelled respectively by DAPI and Alexa Fluor™ 488. Fig.1 shows the obtained images with and without aberration correction. The images with aberration correction provided a higher contrast, revealed the finer structures in the sample to make the synapses, which were barely observed without correction, visible.

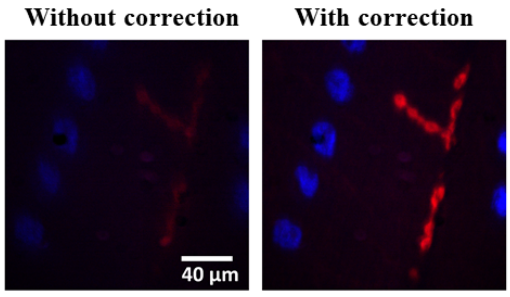

Fig.1 Fluorescence image of NMJ, with nuclei (blue) and the synapse (red). An oil-immersion objective lens (Olympus, 1.4NA, 60x) was used for observation.

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