Two-photon excitation spinning-disk confocal microscopy and its biological applications

Kohei Otomo1,2, Takafumi Kamada2, Ai Goto3, Yumi Yamanaka2, Tomomi Nemoto1,2
(1 Exploratory Research Center on Life and Living Systems; National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan; 2 Research Institute for Electronic Science; Graduate School of Information Science and Technology, Hokkaido University, Sapporo, Japan)
E-mail: otomo_at_nips.ac.jp

KEY WORDS: two-photon microscopy, spinning-disk, second harmonic generation, polarization imaging, linear unmixing, multi-color imaging

Two-photon excitation laser scanning microscopy (TPLSM) is a powerful tool to visualize microstructures in living specimens, because of its superior penetration and less-invasiveness in specimens owing to the near-infrared (NIR) excitation wavelength. Since most of TPLSM systems employ a single-point laser scanning method using moving mirrors, their temporal resolution depends on the speed of the physical movement of these mirrors. On the other hand, multi-point scanning methods have been attempted to achieve high-speed TPLSM imaging. Among them, we have been developed TPLSM systems equipped with a NIR optimized spinning-disk confocal scanning unit (TPLSM-SD) [1]. This presentation will discuss recent technological developments and biological applications of our TPLSM-SD system.

1. TPLSM-SD system requires a high-peak-power laser light source in order to induce two-photon excitation processes at several hundred focal points simultaneously. We introduced high-peak-power 920 nm neodymium laser and 1040 nm ytterbium laser light source for various fluorophores, of which color was from green to red [2; K. Otomo et al., in preparation]. As a result of optimizations, the developed system achieved several tens-times brighter fluorescent intensity than using a conventional mode-locked titanium-sapphire laser light source.

2. Conventional TPLSM system uses one wavelength for excitation, resulting in that the number of excitable fluorophores is limited. Besides, to observe multi-color labeled specimens, overlapping of emission spectra of fluorophores caused a crosstalk of fluorescent signals among detection channels. Such a crosstalk causes a complication to distinguish individual targets. To achieve multi-color imaging, we introduced a fast-switching system to select either of two NIR laser light pulses. We applied the developed system to three fluorescently-labeled organelles in tobacco BY-2 cells and mammalian HeLa cells. As a result, the system visualized 3D movements of individual organelles during cytokinesis without severe phototoxicities. Moreover, we utilized a linear-unmixing method to separate these fluorescent signals more clearly, resulting in finer views of each organelle successfully [T. Kamada et al., in preparation].

3. TPLSM system enables to visualize fluorescent signals and second harmonic generation (SHG) signals, simultaneously. Recently, we introduced a polarization splitting detection system for our TPLSM-SD system and developed high-speed polarization-resolved SHG microscopy for living mouse tissues [3]. The developed system visualized complicated collagen fiber network structures with information of their lateral orientations. Finally, we demonstrated in vivo polarization-resolved SHG imaging of the collagen fibers in the mouse skeletal muscles at video-rate temporal resolution.

Acknowledgements
We thank Mr. Hiroshi Nakayama of Yokogawa Electric Corporation, Prof. Takashi Murata, Prof. Mitsuyasu Hasebe of National Institute for Basic Biology and Prof. Ryota Uehara of Hokkaido University for their kind supports. This work was supported by JSPS KAKENHI 18K06591, JP15H05953 (Resonance Bio) and JP16H06280 (Advanced Bioimaging Support) of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) Japan; the Nano-Macro Materials, Devices, and System Research Alliance (MEXT); the Network Joint Research Center for Materials and Devices (MEXT); and Brain/MINDS, AMED, Japan.

References