High speed imaging for green fluorescent proteins by utilizing multi-point scanning two-photon microscopy

Kohei Otomo[1, 2], Ai Goto[2], Yumi Yamanaka[2], Hiroshi Nakayama[3], Takashi Hori[4], Tomomi Nemoto[1, 2]

([1]Research Institute for Electronic Science, Hokkaido University, Kita 20 Nishi 10, Kita-ku, Sapporo 001-0020, Japan, [2]Graduate School of Information Science and Technology, Hokkaido University, Kita 14 Nishi 9, Kita, Sapporo 060–0814, Japan, Japan, [3]Yokogawa Electric Corporation, 2-3 Hokuyodai, Kanazawa 920–0177, Japan, [4]IMRA America, Inc., 1044 Woodridge Avenue, Ann Arbor, Michigan 48105, USA)

E-mail: otomo@es.hokudai.ac.jp

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Two-photon excitation laser scanning fluorescence microscopy (TPLSM) has been widely used as an analytical method for direct visualization of dynamical molecular and cellular phenomena in live specimens. This is because of its superior penetration depth and less-invasiveness in specimens owing to its near-infrared excitation laser wavelength compared with the wavelength of single-photon excitation-based systems. Although the TPLSM system is equipped with a spinning-disk confocal scanning unit to improve the temporal resolution, the insufficient energy of the conventional Titanium-Sapphire (Ti-Sa) laser source restricts the field of view (FOV) to a narrow region [1]. Therefore, we introduced a high-peak-power Ytterbium (Yb)-based 1040-nm laser to extend the FOV [2]. This system provided three and four-dimensional yellow or red fluorophore imaging of a sufficiently deep and wide region of a live specimen. Furthermore, to visualize the general green fluorophores, we developed a Neodymium (Nd)-based 920-nm laser light source and introduced it in our system. This laser source generates high-peak-power pulses instead of lower repetition rates (Table). In general, such a low-repetition-rate laser cannot be used for high-speed scanning in a single-point TPLSM because of the laser's short pixel dwell time. In contrast, the spinning-disk unit scanned a specimen making use of approximately 200 excitation beams, and the entire FOV could be scanned once for every per half rotation (5 ms at 6,000 rpm) of the disks, i. e., approximately two million pulses were used for two-photon excitation within one scan run, resulting in sufficient conditions to acquire a video-rate image. By utilizing the Nd-based laser, we achieved high-temporal-resolution biological green fluorophore imaging for calcium oscillation and exocytosis in live pancreatic acinar cells of mouse.

Table. Properties of excitation lasers

*[1]MaiTai eHP deepsee (Spectra Physics), *[2]Developed laser (IMRA America), *[3]FemtoTrain (Spectra Physics)

	Ti-Sa (680-1040 nm)*[1]	Nd (920 nm) *[2]	Yb (1040 nm)*[3]
Average power	1.5 W@920 nm, 0.5 W@1040 nm	1.2 W	3.8 W
Pulse width	100 fsec	200 fsec	300 fsec
Repetition rate	80 MHz	2 MHz	10 MHz

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