ULTRAFAST 3D CONFOCAL FLUORESCENCE MICROSCOPY AT 100 VOLUMES/SECOND ENABLED BY TELECOMMUNICATION TECHNOLOGY

Hideharu Mikami, Yasuyuki Ozeki, and Keisuke Goda
The University of Tokyo
Hongo 7-3-1, Bunkyo-ku, Tokyo, Japan
E-mail: mikami@chem.s.u-tokyo.ac.jp

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3D fluorescence microscopy is a powerful method in biological and medical research as it allows investigating the whole structure of biological tissues and cells, but its low data acquisition speed critically limits its applications, especially in real-time observation of dynamic samples. Here, we propose and demonstrate ultrafast confocal fluorescence microscopy that reaches volume rates over 100 volumes/sec by harnessing telecommunication techniques such as frequency-division multiplexing (FDM) and quadrature amplitude modulation (QAM) [1,2]. In our method, as schematically shown in Figure 1, multiple excitation beam spots having different modulation frequencies ($f_1, f_2, \ldots, f_n$) and phases [0°: in-phase (I), 90°: quadrature (Q)] are scanned over a sample in two directions. A fluorescence signal is detected by a single-pixel photodetector (e.g., avalanche photodetector) through a slit aperture and is decomposed into the signal components that correspond to the multiple excitation beam spots by digital signal processing, allowing the 3D image reconstruction by a 2D scan. As a proof-of-concept demonstration, we observed flow motion of microalgal cells (*Euglena gracilis*) with 3D two-color fluorescence [green fluorescence of SYTO9 (nucleus stain) and red autofluorescence of intracellular chlorophyll] images at a record high volume rate of 104 volume/sec (Figure 2).

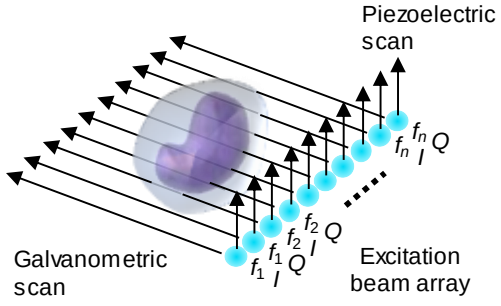

Figure. 1: Confocal fluorescence microscopy with FDM and QAM.

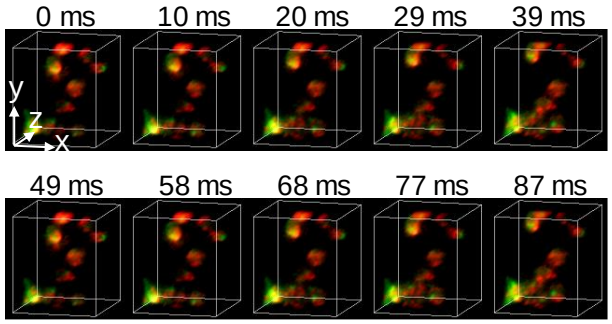

Figure. 2: 3D images of *Euglena gracilis* cells at a volume rate of 104 volumes/sec. Box size: 70 μm (x) × 80 μm (y) × 90 μm (z).

[1] E. Diebold *et al*., Nature Photon. **7**(10), 806 (2013).
[2] H. Mikami *et al*., Optica (2018), in press.