SATURATED TWO-PHOTON EXCITATION MICROSCOPY FOR SUPER RESOLUTION TISSUE IMAGING

Ryosuke Oketani[1], Atsushi Doi[2], Nicholas I. Smith[3], Yasunori Nawa[1], Satoshi Kawata[1], and Katsumasa Fujita[1]

[1] Department of Applied Physics, Osaka University, Japan
[2] Olympus Corporation, Japan
[3] Immunology Frontier Research Center, Osaka University, Japan
E-mail : fujita@ap.eng.osaka-u.ac.jp

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Two-photon excitation microscopy has been utilized for imaging deep inside biological tissues. However, improving the spatial resolution in the depth imaging is still challenging since the scattering and light wave distortion inside the tissue degrade the resolution and even restrict the use of typical super resolution techniques. A simple and robust technique that improves the spatial resolution in 3D is expected for further exploring the interiors of biological tissue.

Recently, we proposed the use of saturated excitation (SAX) of optical effects for improving the spatial resolution in laser scanning microscopy [1-3]. Saturation of optical effects induced predominantly at the center of an excitation focus allows us to probe sample structures smaller than the excitation focal volume. In this research, we demonstrated saturated two-photon excitation microscopy that can be used for super-resolution imaging of tissue samples [4]. We also combined a core-ring illumination with SAX imaging to enhance the high spatial frequency components in the illumination point spread function, which is effective to improve the practical resolution that is limited by the number of fluorescence photons to be detected.

Fig.1 shows the images of fluorescent beads with a diameter of 100 nm embedded at a depth of 100 μm in a mouse brain phantom. Improvement of the spatial resolution in 3D was clearly observed with SAX and the core-ring illumination. The sidelobe effect by the ring illumination was effectively suppressed, which significantly contributed to the improvement of the axial resolution. The proposed technique can be realized by introducing a pupil mask and excitation intensity modulation into a typical two-photon excitation microscope, which allows the implementation of this technique into many practical situations of tissue imaging.

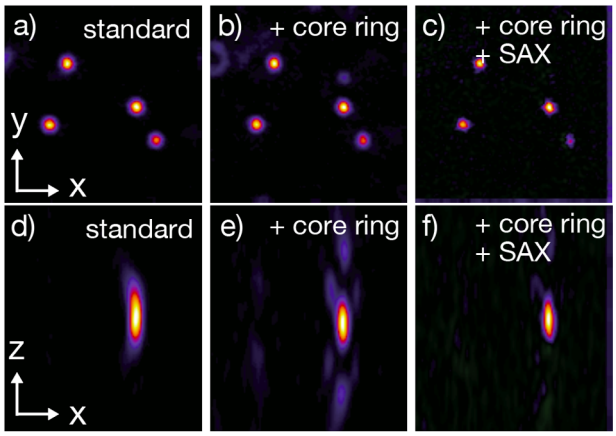

Fig.1 a) - f) Images of fluorescence beads embedded at a depth of 100 μm in a mouse brain phantom. A mode-locked Ti: sapphire laser oscillating at 800 nm was combined with a standard laser scanning microscope (FV1200, Olympus). Fluorescence emission below 680 nm was detected by a non-descanned detector.

References:

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