Saturated-Excitation Image Scanning Microscopy for super-resolution imaging

Kenta Temma[1,2], Ryosuke Oketani[1], René Richter[3], Toshiki Kubo[1,2], Nicholas I. Smith[4], Rainer Heintzmann[3], Katsumasa Fujita[1,2,5]

[1]Department of Applied Physics, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan, [2]AIST-Osaka Univ. PhotoBIO-OIL, 2-1 Yamadaoka, Suita, Osaka, 565-0871, japan, [3]Friedrich-Schiller-Universtät Jena Institut für Physikalische Chemie, Helmholtzweg 4, Jena, 07743, Germany, [4]IFReC, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan, [5]OTRI, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: fujita@ap.eng.osaka-u.ac.jp

Confocal microscopy can achieve a spatial resolution beyond the diffraction limit by using a detection pinhole with a diameter smaller than an Airy unit (A.U.), resulting in a trade-off between spatial resolution and signal amount. Image Scanning Microscopy (ISM) overcomes this trade-off by using a detector array to detect and analyze the spatial distribution of signal light [1,2]. We introduced ISM into saturated excitation (SAX) microscopy [3] in order to improve the signal to noise ratio (SNR), which benefits extracting nonlinear fluorescence signals that carry high spatial frequency information in SAX imaging. Fig.1 shows fluorescence images of HeLa cell detected by a detector array with a pixel size equivalent to 0.2 A.U. ISM, SAX and SAX-ISM images are constructed from signal detected by a part of the array equivalent to areas of 0.6 A.U. and 1.6 A.U. respectively. The ISM images were reconstructed by the pixel reassignment method described in ref. [4]. We extracted the nonlinear signal by the differential excitation technique used in dSAX microscopy [5]. We can confirm that SAX-ISM achieves higher SNR and spatial resolution compared to SAX microscopy and ISM respectively.

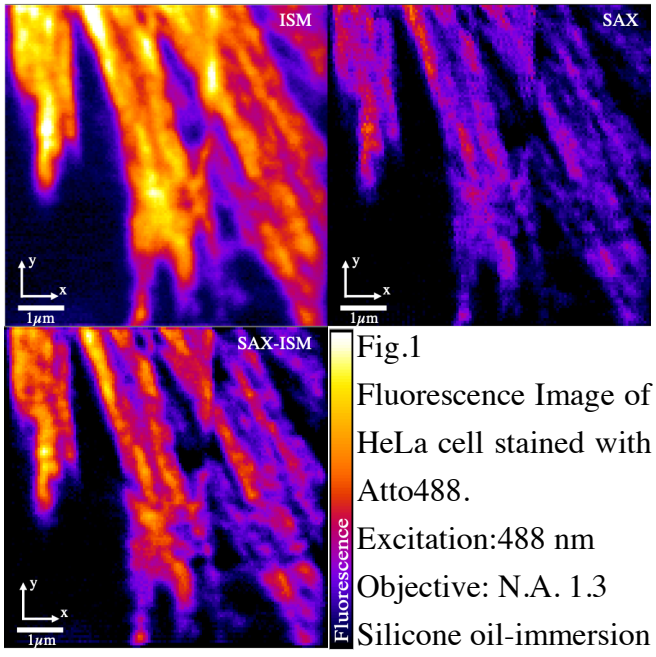

Fig.1
Fluorescence Image of HeLa cell stained with Atto488.
Excitation:488 nm
Objective: N.A. 1.3
Silicone oil-immersion

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[3] K. Fujita et al., “High-Resolution Confocal Microscopy by Saturated Excitation of Fluorescence” *Phys. Rev. Lett.*, **99**, 228105(2007)

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[5] Y.Nawa et al., "Saturated Excitation microscopy using differential excitation for efficient detection of nonlinear fluorescence signal", *APL Photonics*, **3**, 080805 (2018).