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Nonlinear Deep-UV excitation microscopy for high-resolution multicolor imaging of fluorescent proteins

Masahito Yamanaka[1], Kenta Saito[2], Nicholas I. Smith[3], Satoshi Kawata[1], Takeharu Nagai[2], Katsumasa Fujita[1]

[1] Dept. of Applied Physics, Osaka University, [2] The Institute of Scientific and Industrial Research, Osaka University, [3]Immunology Frontier Research Center, Osaka University
E-mail: yamanaka@ap.eng.osaka-u.ac.jp

Fluorescent proteins (FPs) are essential tools for studies of cellular activities and structures. Multicolor labeling with FPs allows us to visualize the relations between proteins or organelles which evoke a variety of biological functions. Although recent advances in multicolor fluorescence microscopy realized simultaneous imaging of multiple fluorescent probes, there are still issues such as chromatic aberration and difficulty in preparing a sample which provides reasonable fluorescence intensity of each fluorescent probe at a same excitation intensity.

We present a technique to simultaneously image multiple FPs by deep ultraviolet (DUV) two-photon excitation, providing a new spectral window for fluorescence imaging of cellular architectures and behaviors. Although FPs are typically excited by visible wavelengths, it is reported that they can be excited by deep UV wavelength and emit visible fluorescence photons [1]. To enable the concurrent DUV excitation of multiple FPs in cellular imaging, we propose the use of two-photon excitation with visible wavelength laser light, which allows us to utilize optical phenomena in the DUV region with optics for visible light. The nonlinear nature of the excitation enables us to obtain a spatial resolution equivalent to that of a short wavelength of DUV light. Furthermore, because multiple FP targets can be excited by the single laser source, our concept offers a simple method for simultaneous imaging of multiple fluorescent probes with reduced chromatic aberration. The figure shows a fluorescence image of a HeLa cell expressing Sirius, mseCFP, mTFP1, and EGFP, in mitochondria matrix, nucleosome, Golgi apparatus, and nucleoli, respectively, which were excited by 525 nm pulsed laser light with NA 1.4 oil-immersion objective lens [2]. As shown in the figure, we confirmed that the stained organelles are clearly recognized with the high spatial resolution. In this experiment, we also confirmed that the intensity of fluorescence emitted by FPs was about 30 to 450 times higher than that of autofluorescence from endogenous molecules. In addition of the resolution improvement, the nonlinear excitation produced sufficiently high image contrast by eliminating the background signal from the out-of-focus planes.

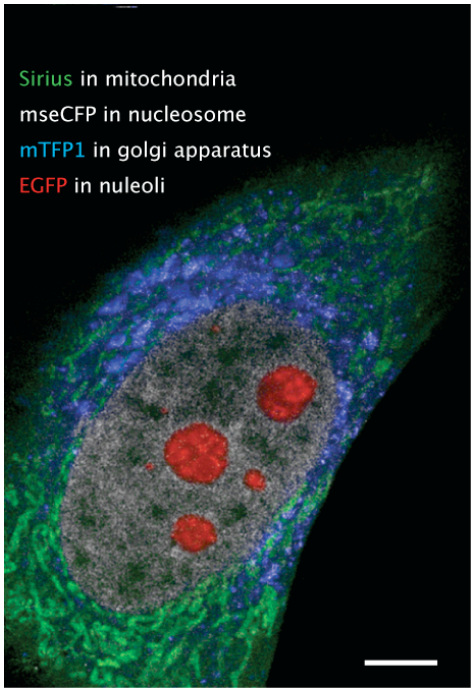

Figure. Fluorescence image of a HeLa cell expressing Sirius in mitochondria , mseCFP in nucleosome, mTFP1 in Golgi apparatus, and EGFP in nucleoli. The scale bar: 5 μm

References: [1] Visser et al., *Biophys. Chem.* **116**, 207-212., [2] Yamanaka et al., submitted.