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Simultaneous Single and Two-photon Excitation of Fluorescent Proteins For Multicolor Imaging of Cellular Structures

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Fluorescent proteins (FPs) have been widely used to observe the distribution, structures and activities of cellular organelles in living cells. Since FPs can be selectively expressed in different organelles, we can simultaneously map different organelles in a single cell by using a combination of different FPs. However, because different FPs have different absorption spectra at visible light region, we have to use different excitation lights for each FPs, which means that different FPs have to be imaged at different times and the co-localization study of labeled organelles become less convincing. To know the complex structures of different organelles in living cells, multiple cell organelles should be observed at the same time.

Here we proposed a way to simultaneously excite multiple FPs by ultrashort pulsed laser of visible light. FPs have a common absorption peak at the deep ultraviolet (DUV) region in addition to the one at the visible light region[1]. Therefore, multiple FPs can be simultaneously excited by a single laser light. In a previous work, we reported the multi-color imaging of FPs by the two-photon DUV excitation with visible light to improve the spatial resolution and optical sectioning ability while imaging[2]. In this study, we excited FPs by absorption in both DUV and visible lights at the same time. This enables us to easily separate each FP and expand the number of FPs for imaging.

As shown in Fig.1, we observed four organelles in the same cell transfected with four different FPs: mTFP1, EGFP, Venus and DsRed expressed at Golgi apparatus, Nucleoli, Actin filament and Mitochondria respectively. We achieved the simultaneous four-color imaging of living cells by exciting the mTFP1 and EGFP with two-photon excitation and Venus and DsRed with single photon excitation by using 525nm ultrashort pulsed laser.

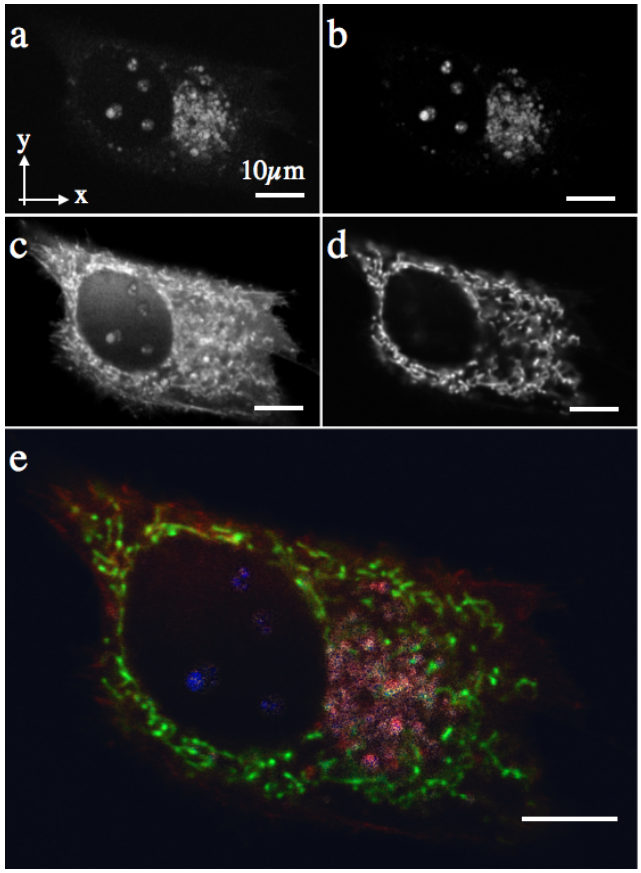

Fig. 1 Fluorescence images of a living HeLa cell expressing mTFP1, EGFP, Venus and DsRed at Golgi apparatus, nucleoli, actin filaments and mitochondria respectively. Fluorescence was detected by four photomultiplier tubes of wavelength reneges of (a) 410-490 nm, (b) 490-500 nm, (c) 540-560nm, (d) 560-580nm. (e) Multicolor images reconstructed by overlaying the unmixed fluorescence distribution

References

[1] N. V. Visser, et al., *Biophys. Chem.* **116**, 207-212 (2005)
[2] Yamanaka, et al. (Submitted)

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