

Visible-wavelength two-photon excitation for multicolor fluorescent protein imaging with improved image contrast

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Simultaneous multicolor fluorescence imaging provides us the insight of the biological functions of cells through the visualization of the intracellular molecules and their interactions. Recently, we proposed the use of visible-wavelength two-photon excitation (V2PE) in confocal microscopy for simultaneous excitation of fluorescent proteins with different emission wavelengths [1] via light absorption at the DUV region which is commonly seen in most of the fluorescent proteins. V2PE also provides the improvement in spatial resolution and image contrast through the nonlinear light-matter interaction by two-photon excitation at a relatively shorter wavelength (500 nm – 600 nm).

In this research, we introduced slit-scanning to V2PE microscopy in order to improve the image acquisition speed with the capability of spectral detection, which allows us to extract images of different fluorescent proteins. The line illumination enables us to detect the fluorescence spectrum in parallel at each position, allowing spectrum analysis for extracting the distributions of target molecules. We demonstrated multicolor imaging of a living HeLa cell expressing Sirius and mseCFP on mitochondria and histone H2B, respectively. Pulsed laser light at 521 nm was used for excitation, and the detection wavelength range was set to 420 – 496 nm. The fluorescence spectra indicating Sirius and mseCFP were found in the cell at the positions corresponding to the distribution of mitochondria and histone H2B, respectively.

We also implemented V2PE into a spinning disk confocal microscope for further improvement of the image acquisition speed. We demonstrated time-lapse 2D and 3D imaging of a living HeLa cell. We also calculated the effective point spread functions and confirmed the improvement of axial resolution by two-photon excitation which effectively contributes to the improvement of the image contrast in 3D images.

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References:

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