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Fluorescence lifetime measurements with high optical resolution using time-resolved nonlinear fluorescence microscopy constructed of a pump-probe setup

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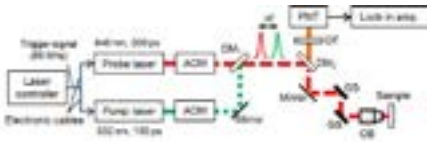
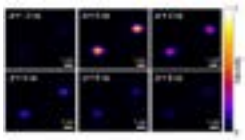
In fluorescence microscopy, fluorescence lifetime information enables fluorophores with the same emission spectrums and different lifetimes to be distinguished, which can improve molecular discrimination ability. Fluorescence lifetime generally depends on molecular conformation and the manner in which the molecule interacts with its environment, so that fluorescence lifetime imaging can reveal molecular interactions and dynamics at the molecular scale. We propose a time-domain approach for fluorescence lifetime measurements using nonlinear fluorescence microscopy constructed of two-color laser pulses. Our method is based on the pump-probe setup, where wavelengths of the pump and probe beams overlap the absorption spectrum and the fluorescence emission spectrum of the fluorescent dye, respectively and fluorescence wavelength to be detected is different from both wavelengths of incident beams. Nonlinear fluorescence signals generated by fluorescence reduction due to stimulated emission were detectable through a lock-in technique. The signal is produced by the multiplicative combination of incident beams, resulting in an improvement of the three-dimensional optical resolution. In the experiment, we modulated intensities of the pump and probe beams with frequencies of $f1$ and $f2$, respectively and demodulated the signal with $f1–f2$ to extract nonlinear fluorescence signal. Changing the time delay between the two-color pulses enables acquisition of a time-resolved nonlinear fluorescence signal, which directly reflects the fluorescence lifetime of the sample and is thus applicable to fluorescence lifetime imaging. We also quantitatively demonstrate that nonlinear fluorescence microscopy possesses better optical resolution than conventional laser-scanning fluorescence microscopy. Experimental trials indicated that straightforward fluorescence lifetime imaging with high optical resolution is readily available.





Biography

Fumihiro Dake, Senior researcher, Research & Development Division, Nikon Corporation, entered Nikon Corporation in 2009. He worked as an optical designer from 2009 to 2013. Then, he got to work on research of optical microscopy. He has researched and developed nonlinear optical microscopy.

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