

SATURATED-EXCITATION FLUORESCENCE MICROSCOPY FOR BIOLOGICAL IMAGING BEYOND THE DIFFRACTION LIMIT

Katsumasa Fujita, Masahito Yanamaka, Shogo Kawano,
Nicholas I. Smith, and Satoshi Kawata

Department of Applied Physics, Osaka University
2-1 Yamadaoka, Suita, Osaka 565-0871 Japan
E-mail: fujita@ap.eng.osaka-u.ac.jp

KEY WORDS: high-resolution microscopy, saturated excitation, confocal, fluorescence

In the recent developments in high resolution laser microscopy, saturation effects in optical phenomena, such as stimulated emission depletion, fluorescence excitation, and photoswitching of fluorescence molecules have been effectively used. Since the saturation effects induce nonlinear responses between excitation and fluorescence intensity, it can be used to improve the spatial resolution. Unlike multiphoton excitation, these techniques do not require a longer wavelength of light, such as near-infrared light, to excite visible-fluorescence dyes, and therefore, the spatial resolution can be effectively improved.

In this research, we have used the saturation effect in single-photon excitation to improve the spatial resolution of confocal fluorescence microscopy [1]. We saturate the population of fluorescence molecules at the excitation state with high excitation intensity to induce the strong nonlinear fluorescence response. Since the nonlinear fluorescence response can be prominently observed at the center of the laser focus, extraction of the nonlinear response from the fluorescence signal allows us to image structures smaller than the focal spot. To extract the nonlinear fluorescence signals, we modulate the excitation intensity at a frequency (ω) and demodulate the fluorescence signals at higher-order harmonic frequencies (2ω , 3ω , ...). This microscope system can be simply realized by implementing laser modulation and lock-in detection of fluorescence signal into a typical confocal fluorescence microscope.

We observed microtubules in a HeLa cell stained with ATTO488 to confirm the improvement of the spatial resolution by saturated excitation [2]. The sample was excited with CW laser light at a wavelength of 488 nm and an objective lens with an NA of 1.4. The laser was modulated at 10 kHz, and the fluorescence signal was demodulated at 20 kHz for harmonic detection (saturated excitation, Fig. 1 a) and at 10 kHz for conventional confocal detection (Fig. 1 b)). The sample was excited with an intensity of 66 kW/cm² and 2 kW/cm² for Fig. 1 a) and b), respectively. From the comparison of the intensity profiles on the dotted lines in the image, we confirmed the improvement of spatial resolution by saturated excitation.

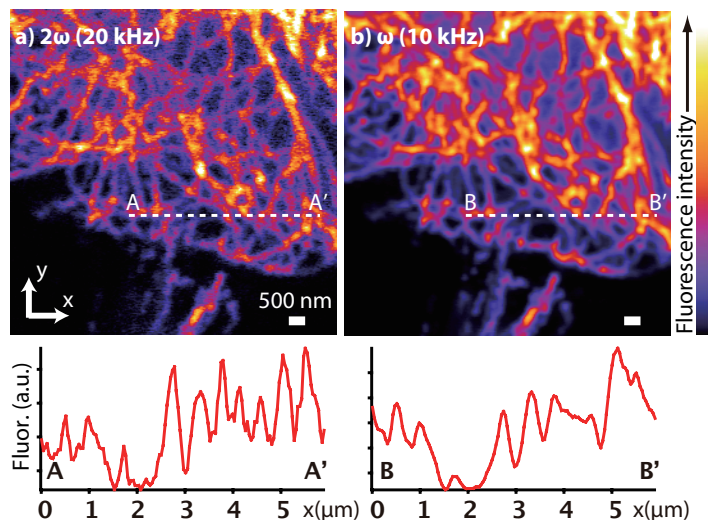


Fig. 1 The fluorescence image of microtubules in a HeLa cell with a) saturated excitation and b) conventional confocal microscopy.

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- [2] Yamanaka, Kawano, Fujita, et al., J. Biomed. Opt., 13, 050507 (2008).