Saturated excitation microscopy with image subtraction

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Saturated excitation (SAX) induces a nonlinear relation between excitation and emission of fluorescent probes. Since the nonlinear response is confined in a region smaller than the illumination laser spot, extraction the nonlinear fluorescence signals realizes the spatial resolution beyond the diffraction limit in confocal microscopy [1]. In SAX microscopy, the increase of signal-to-noise ratio (SNR) realizes the further improvement of the spatial resolution by extracting higher-order nonlinear relations [2].

In this research, we developed an image processing technique to extract high-order nonlinear fluorescence responses. The technique extracts the nonlinear fluorescence signals from two or three fluorescence images obtained with different excitation intensities. Compared to the original SAX microscopy technique, the image subtraction technique allows the increase of SNR in detecting second and third order nonlinear signals by a factor of √8 and √32, respectively. Using the developed technique, we confirmed similar increase of the SNR with the theoretical expectation and visualized the distribution of actin-filaments in the HeLa cells with the sub-diffraction-limit resolution and high SNR (Fig. 1).

Fig. 1 Fluorescence images of actin-filaments in HeLa cells stained with ATTO Rho6G phallolidin, with a) confocal and b) image-subtraction-based saturated excitation (S-SAX) microscopy.

Reference