Field:

Chemistry/Biochemistry

Session Topic:

Super-resolution Imaging

Speaker:

Katsumasa FUJITA, Osaka University

1. Introduction

The optical microscope has been used as an inevitable tool for observation of biological specimens since it allows us to observe the specimen in physiological conditions. However, the spatial resolution of the optical microscope is limited to about 200 nm, which is incomparably lower than other microscopic techniques such as electron microscopy or atomic force microscopy.

The limitation in the spatial resolution of optical microscopy is caused by the wave nature of light. Since the light intensity distribution is produced by the interference effect of light waves, light cannot be focused into a size smaller than half of the wavelength. This causes the limitation in the spatial resolution of optical microscopy, which is known as "the diffraction limit", described by Abbe in 1887. Tremendous efforts have been made to overcome this limit.

2. Super resolution microscopy

The recent development of super resolution microscopy has successfully broken the barrier set by the diffraction limit [1]. These techniques utilized saturation or switching effect of the fluorescence excitation of molecules that are used to label a specimen. These "nonlinear" interactions between light and fluorescent probe allow us to derive information about a specimen from a region smaller than the diffraction-limited light focus. Stimulated emission depletion (STED) microscopy and saturated excitation (SAX) microscopy utilize the saturation effect observed in stimulated emission and fluorescence excitation, respectively, to restrict the volume of fluorescence detection beyond the diffraction limit. Photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) use the photoswitching effect of fluorescent molecules to measure the positions of individual molecules existing in a specimen accurately to construct a high resolution image.

3. SAX microscopy

SAX microscopy is one of the super resolution techniques that we recently introduced [2, 3]. In SAX microscopy, fluorescence molecules are excited with an excitation intensity high enough to cause saturation in the fluorescence signal. Since the saturation effect is prominently seen at the center of the excitation focus, extracting the nonlinear fluorescence signals induced by saturation gives information about molecular concentration in a smaller region of the laser focus.

To extract the nonlinear fluorescence signal, the excitation intensity is modulated temporally at a single frequency, and the fluorescence signal is demodulated at harmonic frequencies, by which nonlinear fluorescence responses are detected separately from the linear response.

Figure 1 shows fluorescence images of actin in a HeLa cell stained with the fluorescence probe ATTO Rho6G obtained by a conventional confocal microscope and a SAX microscope. The fluorescence molecules were excited by light with a wavelength of 488 nm. The SAX image was obtained by demodulating the fluorescence signal at the 2nd harmonic frequency. The improvement of the spatial resolution for both lateral and axial directions was confirmed from the comparison of the images.

The advantage of SAX microscopy compared to other super resolution techniques is the simple configuration of the optical system. Since the improvement of spatial resolution can be given simply with the harmonic demodulation of fluorescence signal, a SAX microscope can be realized by addition of a laser modulator and a lock-in amplifier into a typical confocal microscope. SAX microscopy is therefore useful for applications that require higher spatial resolution in three dimensions and particularly suited for improving the spatial resolution by a simple and minimal modification of existing microscope setup and laser sources.

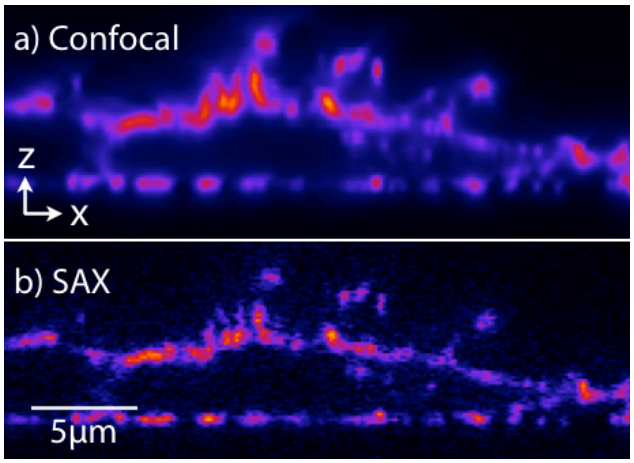

Fig. 1 Fluorescence images (x-z cross section) of actin in a HeLa cell obtained by a) a conventional confocal microscope and b) by a SAX microscope.

4. Conclusion

We demonstrated the improvement of the spatial resolution of laser scanning confocal microscopy by using saturated excitation of fluorescence. The simple principle and configuration are the main advantages of this technique. The limitation of the technique is photobleaching of the fluorescence molecules. Since saturated excitation requires high excitation intensity, fluorescence probes can be bleached more easily. However, since saturation phenomena are essential to any kind of fluorescence material, the technique can be applied to various forms of samples. In addition, the saturation effect is not limited only to fluorescence, but also to other optical phenomena. Therefore, SAX technique can be applied to improve the spatial resolution in other types of optical microscopy.

5. References

[1] S. Hell, Science 316, 1153-1158 (2007).
[2] K. Fujita, M. Kobayashi, et al., Phys. Rev. Lett., 99, 228105 (2007).
[3] M. Yamanaka, S. Kawano, K. Fujita, et al., J. Biomed. Opt. 13, 050507 (2008).