Super-resolution bright-field imaging by saturated optical absorption

Kentaro Nishida[1,2], Hikaru Sato[1], Ryosuke Oketani[1], Kentaro Mochizuki[3], Kenta Temma[1,2], Yasuaki Kumamoto[1], Hideo Tanaka[3], and Katsumasa Fujita[1,2]
[1]Osaka University, [2]PhotoBIO-OIL AIST, [3]Kyoto Prefectural University of Medicine
E-mail: fujita@ap.eng.osaka-u.ac.jp

KEY WORDS: Super resolution, bright field microscopy, transmission microscopy, diagnosis

Observing dye-stained tissue sections with a bright-field image is a well-established technique in pathological diagnosis. However, the spatial resolution of bright-field microscopy is limited by the wave nature of the light, and it is difficult to observe the tissue structure finer than about half the wavelength of the incident light. Therefore, some diseases required electron microscopy for definitive diagnosis.

We developed a laser scanning transmission microscope to obtain bright-field images of dye-stained tissue samples with an improved spatial resolution. We utilized the absorption saturation of dyes that occur locally in an illumination spot of the microscope and generates a transmitted signal component showing a nonlinear response to the incident intensity. By selectively detecting the nonlinear transmission signal component, which is generated in a region smaller than the size of the illumination spot, we can reconstruct a bright-field image with a spatial resolution beyond the diffraction limit. We used the harmonic demodulation technique to extract the nonlinear transmission signals, where the sample was irradiated by a temporally modulated illumination light, and the transmitted signal was demodulated at a harmonic frequency of the illumination light [1].

Figure 1 shows a rat kidney tissue section stained with eosin Y observed by the transmission super-resolution microscope we proposed. Figures 1 (A) and 1 (B) show images composed of a linear transmitted signal demodulated at the same frequency as the modulation frequency of the illumination light and a nonlinear transmitted signal demodulated with the second-harmonic frequency, respectively. A CW laser with a wavelength of 532 nm was used as the excitation light, and intensity modulation was applied at a frequency of 10 kHz. An oil-immersed objective lens with an NA of 1.4 was used to illuminate the sample. An oil-immersed condenser lens with an NA of 1.4 was used to collect the transmitted light. The illumination intensities in Fig. 1 (A) and (B) were 0.11 kW/cm^2 and 13 kW/cm^2, respectively. From these experimental results, it was shown that the spatial resolution of the transmission microscope is improved by using our technique. We also confirmed that the images reconstructed by the nonlinear transmitted signals are resolved in 3D, which significantly contribute to improving the image contrast of thick samples.

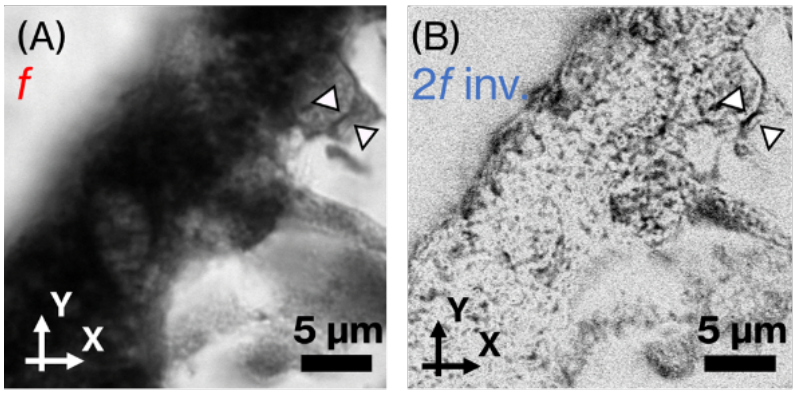

Fig.1 Transmission images of eosinY stained rat kidney tissue reconstructed by the signal demodulated at (A) fundamental frequency (*f*) and (B) second harmonic frequency (2*f*).

[1] Fujita et al., Phys. Rev. Lett., 99 (22), 228105 (2007).