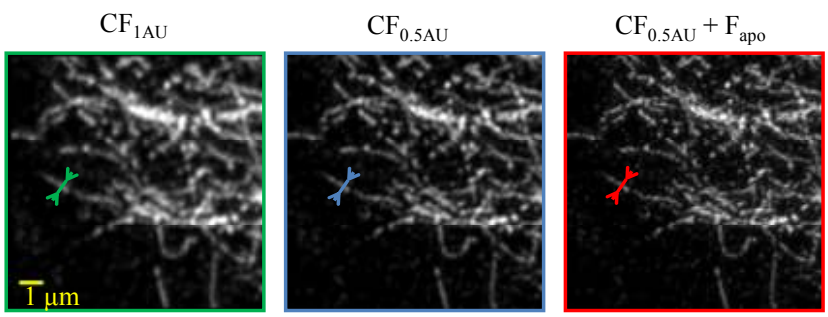
Superresolution Confocal Microscopy via Analogy with Structured Illumination Microscopy

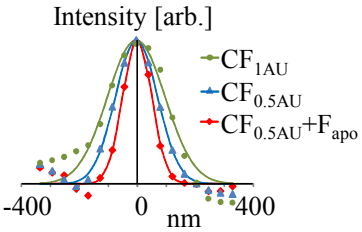
Olympus Corp., °Shinichi Hayashi

E-mail: sh_hayashi@ot.olympus.co.jp

Structured illumination microscopy (SIM) is a wide-spread superresolution (SR) fluorescence microscopy technique with a two-fold higher lateral resolution than conventional wide-field fluorescence (WF) microscopy. SIM is considered to be the most compatible with biological applications because of its higher acquisition speed and wider selection of fluorescence probes than other superresolution microscopy techniques [1]. The super-resolution of SIM is attributed to the two-fold higher optical cut-off frequency than that of WF. By contrast, confocal fluorescence (CF) microscopy has approximately the same optical cut-off frequency as SIM, but the maximum theoretical increase in resolution over that of WF is 1.4-fold with an infinitesimal pinhole diameter.

To compensate for this discrepancy, analytical formulae have been derived to compare SIM imaging with CF imaging. Numerical calculations based on the analytical formulae reveal that SIM reconstructed images before post-processing are nearly identical to CF images recorded with an infinitesimal pinhole diameter. As post-processing of the SIM reconstructed images is used to obtain the two-fold increase in lateral resolution over that of WF images, an application of similar post-processing to CF images would also double the resolution. However, using an infinitesimal pinhole diameter blocks the entire signal from the object and is impractical. Here, CF microscopy with a finite pinhole diameter and post-processing has been investigated and an apodised Fourier inverse filter has been proposed for a practical solution [2]. This approach can easily be applied to conventional laser scanning CF, spinning disk CF, and multi-photon microscopes with minor modifications [3].





	FWHM
CF_{1AU}	233 nm
$CF_{0.5AU}$	171 nm
$CF_{0.5AU}$+F_{apo}	117 nm

Fig. Experimental results with microtubule stained BPAE cells.
$CF_{\beta AU}$: a CF image with the pinhole diameter β Airy unit.
+F_{apo} : additional application of an apodized Fourier inverse filter.

[1] C. Coltharp and J. Xiao, Cell. Microbiol., **14**, 1808 (2012).

[2] S. Hayashi, to be published in Jpn. J. Appl. Phys.

[3] S. Hayashi and Y. Okada, Mol. Biol. Cell, **26**, 1743 (2015).

© 2016年 応用物理学会