Isotropic Image in Structured Illumination Microscopy Patterned with a Spatial Light Modulator

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Although fluorescence microscopy enables biologists to observe the fine structures of specimens with great contrast and specificity through fluorescent labeling, diffraction limits the spatial resolution over the past century. The extension of spatial resolution beyond the classical diffraction limit is hence of critical importance to reveal the fine structures in increased detail for an improved understanding of their functions and interactions. Structured illumination microscopy (SIM) is a method modified from conventional wide-field illumination to illuminate samples with a structured light pattern [1,2]. Accordingly, undetectable high spatial frequency information is encoded into detectable smaller frequency. Followed with a sequence of calculations, a high resolution image can be reconstructed with a resolution twice better then the conventional fluorescence microscopy.

Three individual images are typically taken with the illumination pattern at three phases to reconstruct an image that has an improved resolution in a direction perpendicular to the line pattern. Rotations of the illumination pattern to at least three orientations – 0°, 60°, and 120°, are required to produce a near isotropic resolution in the lateral direction. However, the phase shifts and rotations of the illumination pattern are time consuming, and require great mechanical precision if a diffraction grating with mechanical motion is used to generate the illumination patterns; additional data processing is also necessary to eliminate the long-time drifts and position offsets. A liquid-crystal spatial light modulator (SLM) can serve as a dynamic grating to generate mutually coherent light beams to form an interference illumination pattern, thus overcoming the shortcomings of mechanical motion required with a grating. However, the former application of SLM is typically limited to pattern orientations 0° and 90° because of the simplicity of pattern design and data analysis, and also because of large variations in periods and complication of data analysis for other pattern orientations. Consequently, a high-resolution image reconstructed merely from 0° and 90° pattern orientations is anisotropic as the overlaps of extended frequencies in other directions, particularly 45° and 135°, is small.

In this work [3], we designed a set of SLM patterns to generate 0°, 45°, 90°, and 135° illumination patterns of high contrast and nearly equivalent periods to achieve a near isotropic enhancement in the lateral resolution on our newly launched SI microscopy system. Images of 100-nm beads reconstructed with SIM were compared with images of conventional fluorescence microscopy to demonstrate the resolution improvement (Fig.1). Images of the dye-labeled actin filaments reconstructed with SIM were also demonstrated to convince the prospective application of SIM on biological samples (Fig.2).

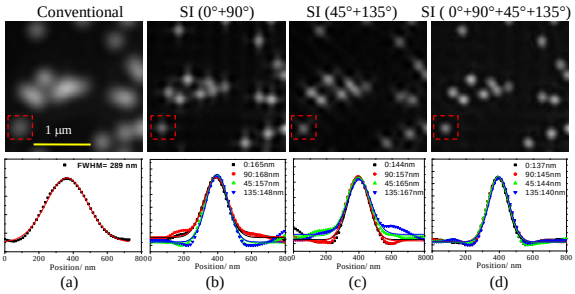

Fig. 1: (a-d) Experimental images and intensity distributions of 100-nm beads for conventional and structured illumination microscopy reconstructed from 0°+90°, 45°+135° and 0°+90°+45°+135° pattern orientations. The solid lines represent Gaussian-fits.

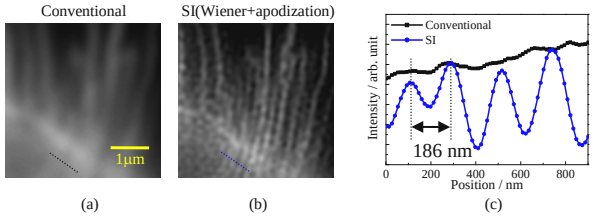

Fig. 2: (a) Conventional image of a dye-labeled actin cytoskeleton, (b) the corresponding SI image after Wiener filter and apodization treatment for comparison, and (c) the intensity distributions along the dotted lines to show the well resolved structures separated by 186 nm in SI image

In conclusion, as shown in Fig. 1, the intensity distribution and the resolution improvement is near isotropic and superior, with an averaged FWHM 144±3 nm of five 100-nm beads. This result shows a resolution improvement of a factor ~2, as compared to the averaged FWHM 290±5 nm in the conventional image. Moreover, the beads that can not be differentiated in the conventional image are well resolved in the structured illumination. As shown in Fig. 2, four fibres of a dye-labeled actin cytoskeleton, entirely unresolved in the conventional image, are well resolved in the SI image with a minimal separation 186 nm. Our results provide true lateral resolution improvement of fluorescence microscopy; the ongoing research is to simultaneously improve axial resolution as a three-dimensional SIM.

[1] M. G. L. Gustafsson, J. Microsc. **198**, 82-87 (2000).
[2] M. G. L. Gustafsson, D. A. Agard, and J. W. Sedat, Proc. SPIE **3919**, 141 (2000).
[3] B.-J. Chang, L.-J. Chou, Y.-C. Chang, and S.-Y. Chiang, Opt. Express **17**, 14710 (2009).