THREE-DIMENSIONAL LIVE-CELL SUPER-RESOLUTION IMAGING WITH MULTI-ANGLE TOTAL INTERNAL REFLECTION FLUORESCENCE-STRUCTURED ILLUMINATION MICROSCOPY

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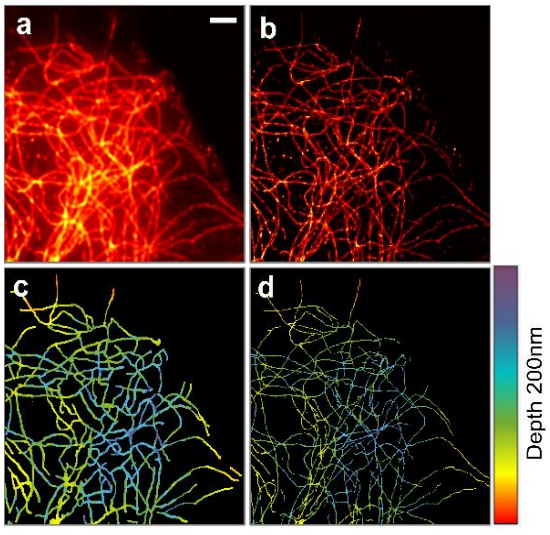
1. PRINCIPLE

Evanescent wave illumination is widely used in optical microscopy to illuminate the characteristics of cells and materials on axial scale down to hundreds of nanometers, which makes it possible to dissect nanostructures with axial super resolution [1].

We introduce a new technique for noninvasive three-dimensional (3D) super-resolution fluorescence microscopy that combines structured illumination microscopy (SIM) with multi-angle total internal reflection fluorescence microscopy (MA-TIRFM). Firstly, lateral super-resolution imaging is performed by detecting the high-frequency information of the sample with SIM under evanescent wave illumination. On the latter, we select Region Of Interest (ROI) with lateral super-resolution image for all raw images obtained by MA-TIRFM after local-background-subtracting and thresholding. Finally, 3D super-resolution image is reconstructed either by curve fitting or inverse problem solving in which the depth information is represented with different colors.

2.EXPERIMENT RESULTS



Using this approach, we obtain the wide-field image [Fig. 1(a)], axial super-resolution image [Fig. 1(b)], lateral super-resolution image [Fig. 1(c)] and 3D super-resolution image [Fig. 1(d)] of microtubules. The axial and 3D super-resolution images have the same depth distribution, which also all coincide with the information distribution in raw images.

Figure 1: Imaging results of microtubules. (a) Wide-field image. (b) Lateral super-resolution image. (c) Axial super-resolution image. (d) 3D super-resolution image.

REFERENCE：

1. Boulanger J, Gueudry C, Münch D, et al. “Fast high-resolution 3D total internal reflection fluorescence microscopy by incidence angle scanning and azimuthal averaging,” Proceedings of the National Academy of Sciences of the United States of America, 111(48):17164-9 (2014).