THREE-DIMENSIONAL SUPER-RESOLUTION IMAGING OF LIVE WHOLE CELLS USING STRUCTURED ILLUMINATION MICROSCOPY

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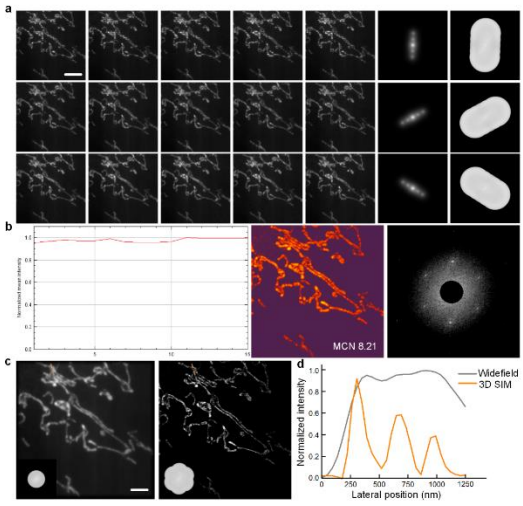
1. PRINCIPLE

Structured illumination microscopy (SIM) improves the spatial resolution via shifting the high-frequency information of a sample into the acceptable passband of the imaging system. In conventional SIM systems, a diffraction grating is used to diffract the incident light interfering at the sample plane to produce the structured illumination pattern, which lengthens the imaging speed. Faster SIM can be achieved by replacing the grating with a spatial light modulator (SLM). In recent years, the SIM theory have been greatly developed to enhance the spatial and temporal resolution. However, these developments were mainly focused on two dimensions [1]. Only a few studies have been conducted with three-dimensional (3D) SIM, especially for live-cell imaging.

With routine fast biological applications in mind, we present here a more flexible system for 3D SIM based on two galvanometer sets conveniently controlling the incident angle (i.e. pattern period) and azimuth angle (i.e. pattern orientation) of the illumination light. The phase of the 3D structured illumination pattern is shifted by two piezoelectric stages. As the galvanometer and piezoelectric stage allow the pattern to be rotated and phase-shifted at the order of kHz, the imaging speed of our system is high enough to observe the sub-cellular dynamics.

2. EXPERIMENT RESULTS



A 3D SIM image stack is presented in Fig. 1. Contrast to the widefield image, we can observe the hollow globular mitochondrial morphology, the intermediate mitochondrial structures, and the interconnected tubular network morphology with more details. The frequency distribution, modulation contrast, and channel intensity profile are also shown, illustrating the performance of our system.

Figure 1: Experimental results of mitochondria.

REFERENCE :

1. Y. Wu, and H. Shroff. “Faster, sharper, and deeper: structured illumination microscopy for biological imaging,” *Nature Methods*. **15**, 1011–1019 (2018).