

LIVE-CELL THREE-DIMENSIONAL SUPER-RESOLUTION IMAGING WITH SIMULTANEOUS TWO-ANGLE AXIAL RATIOOMETRY

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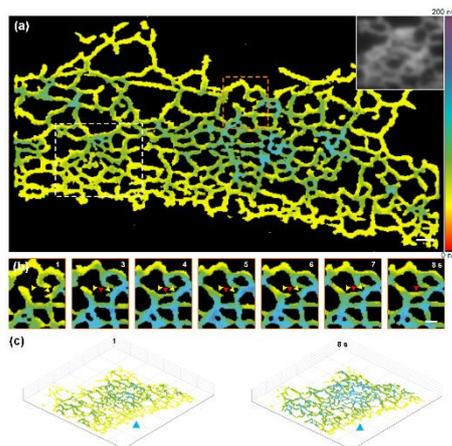
KEY WORDS: Live-cell imaging, three-dimensional super-resolution imaging, total internal reflection fluorescence microscopy.

1. PRINCIPLE

Visualizing 4D (spatial and temporal) super-resolution cellular structures and functions is essential in biological research [1, 2]. Recently, significant developments have increased the three-dimensional (3D) spatial resolving capacity of optical microscopy but at the expense of a long acquisition time, a high illumination intensity, or the use of specific dyes. Such limitations make these techniques incompatible with 4D super-resolution cell imaging in their native states.

In light of this, we present a new technique for 4D super-resolution imaging, called simultaneous two-angle axial ratioetry (STARII). By acquiring only two raw total internal reflection fluorescence microscopy images at two incident angles with very low illumination intensity, STARII can simultaneously provide <20 nm axial resolution, the absolute depth of an emitter relative to an interface, and real-time and long-term axial super-resolution live-cell imaging. Furthermore, by combining STARII with lateral super-resolution techniques, 3D live-cell super-resolution imaging can be achieved in millisecond scope.

2. EXPERIMENT RESULTS



A time-lapse 3D super-resolution image stack is presented in Fig. 1, showing the 3D morphology evolution of the local endoplasmic reticulum (ER) tubules. The tubules continuously formed, extended, retracted, and disappeared with high remodeling speed. When focusing on the axial dimension, we could observe the bending and fluctuation of the ER tubules. A clearer 3D view of the fluctuation, migration, and remodeling of the whole ER meshwork is displayed in Fig. 1(c).
Figure 1: Live-cell 3D super-resolution imaging of ER meshwork remodeling using STARII-SIM.

REFERENCE :

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