3D POLARIZED STRUCTURED ILLUMINATION MICROSCOPY

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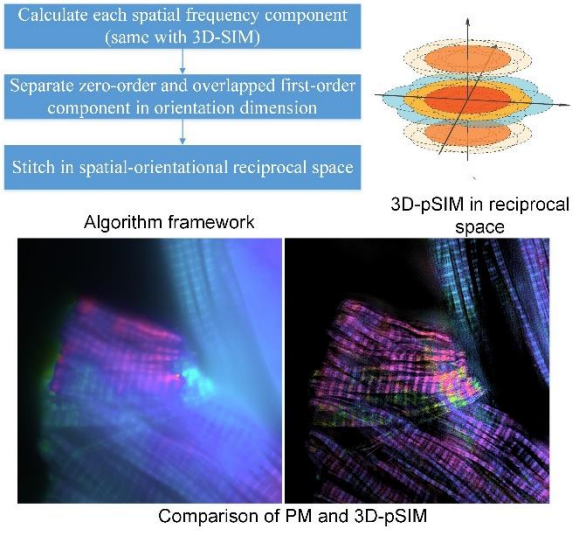
KEYWORDS: Fluorescence polarization microscopy, Structured illumination microscopy

Structured illumination microscopy (SIM) is a fast super-resolution technique with low excitation power, which is suitable for live-cell imaging.[1] Compared to conventional wide-field microscopy, SIM brings the high spatial frequency information of the sample into the observable region with a periodic illumination pattern, resulting in the double resolution in both lateral and axial dimensions.[2] By utilizing the polarization modulation in the SIM setup, Zhanghao et al. recently developed polarized structured illumination microscopy (pSIM) to resolve the dipole orientation further on the super-resolution image.[3] In pSIM, the angular resolution of detected dipole orientation is the same as polarization modulation (PM) in wide-field microscopy, since only the zero-order spatial frequency is under different polarization excitation. It means that in 3D imaging, the out of focus signal will impair the dipole orientation resolving capability on the z-axis. To address this problem, we propose 3D-pSIM in this work, which is illustrated in the Figure.

In 3D-pSIM, both the zero-order spatial frequency (red) and a subset of first-order frequencies (yellow) is in the overlapped region of different polarization excitations, which means that their dipole orientation information can be resolved. [3] Since the first order frequencies brings super-resolution on the z-axis,[2] the axial resolution of dipole orientation detection will be doubled. Especially, the dipole orientation of two intersected filamented overlapped in z-axis can be resolved (Figure). In our framework, we firstly separate each spatial frequency component as 3D-SIM do. Then we apply pSIM to separate the zero-order and overlapped first-order components in orientational dimensional. Moreover, we stitch all of them in the spatial-orientational reciprocal space. Since the relationship between the filament direction and the dipole orientation reveals the insight of biological structure. 3D-pSIM will help to bring more biological structure information.



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[2] Gustafsson, et al. "Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination." Biophysical journal 94.12 (2008): 4957-4970.
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