Structured illumination microscopy for simultaneous targeted improvement of spatiotemporal resolution in different ROIs

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Structured illumination microscopy (SIM) is one of the fastest superresolution (SR) microscopy techniques, which can be reconstructed by using only 9 or 15 widefield (WF) images (2D or 3D) while illuminating diffraction-limited fringe patterns[1]. Because of its high temporal resolution and relatively simple experimental setup compared with other SR microscopy systems, SIM has been developed popularly to observe biological processes smaller than the diffraction limit. Although SIM has higher temporal resolution than other SR microcopies, multiple SIM raw images are still required for the reconstruction of a single SR-SIM image, which degrades the imaging speed by 1/9 or 1/15 times. In conventional SIM microscopy, fringe patterns are illuminated to the entire field of view, and the imaging speed is decreased evenly across the entire image. Recently, we presented a new SIM method that enables custom targeted wide-field and SR imaging regions in a single frame[2].

Here, the microscopic system enables the enhancement of spatiotemporal resolution based on tunable SIM (customized varying illumination patterns across the field of view). By adopting a digital micro-mirror device (DMD) based system, the field of view can be arbitrarily divided to generate fringe patterns to SIM regions and flat field plane wave to WF regions. The temporal resolution in WF region is higher than SIM region by 9 or 15 (3 or 5 in rolling reconstruction) times, but is limited by the maximum frame rate of cameras. We successfully demonstrated the simultaneous observation of both the U87 cell's subcellular structure changes and the fast viscous flow surrounding the cells by introducing different temporal and spatial resolutions to arbitrary regions in a single image. We also show that specifically tuned stroboscopic illumination can be applied to WF region by utilizing the fast frame rate of DMD to resolve fast-moving objects[3]. As adopting strobe illumination against WF regions, afterimages shown as long tails could be resolved with the accurate location and the original object's correct shape. In this system, the spatial resolution could be doubled as conventional SIM for specific regions as required, and the temporal resolution also could be improved by introducing strobe illumination to the corresponding regions with WF regions in a tunable SIM system.

Reference

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