Three-Dimensional Super-Resolution Microscopy using Selective Plane Excitation

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We have developed a large-scale, three dimensional super-resolution microscope based on the selective plane illumination (SPIM). The implementation of SPIM in the localization microscopy offers several advantages over conventional approaches, such as thinner optical sectioning, minimal optical damage, a larger field of view and faster data acquisition [1]. The signal-to-noise ratio can be further improved using spontaneously blinking dyes, HMSiR, which are not fluorescent in their native states and can be activated with relative low power density threshold [2]. The combination of SPIM and HMSiR dye enables one to image densely labeled samples, where 1.7 fluorophores can be registered per um^2 per frame on average. The field of view can be extended by sample scanning. To increase the volume-scanning speed, we optimized the labeling density, excitation power and exposure time. Our system can image a volume of 75 μm x 70 μm x 9 μm within 3 seconds with a localization uncertainty of 20 nm. Using Fourier ring correlation (FRC) analysis, a reconstructed image resolution of better than 40 nm can be achieved by accumulating 300 volumes.

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