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Live Structured-Illumination Microscopy

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Recently, several techniques have been developed which achieve resolutions better than the diffraction limit in fluorescence microscopy including structured illumination microscopy (SIM) [1], stimulated emission depletion microscopy (STED) [2], photo-activated localization microscopy (PALM) [3], stochastic optical reconstruction microscopy (STORM) [4], and fluorescence photo-activation microscopy (FPALM) [5]. The resolution achievable with these techniques ranges from roughly 20nm for PALM and STED to 100nm for linear SIM. All these techniques were originally demonstrated on two-dimensional fixed samples, and currently there is a lot of interest in applying these techniques to live imaging.

We will discuss our recent development of a microscope capable of live SIM using a spatial light modulator (SLM) for pattern creation (Figure 1)[6]. To cleanly image two-dimensional sections we used SIM in concert with total-internal reflection (TIRF) microscopy to image only the first few hundred nanometers below the cover slip. We have demonstrated live TIRF SIM at 100nm resolution at a frame rate of 3.7Hz for a 32μm × 32μm field of view and 11Hz for a 8μm × 8μm field of view. We have imaged EGFP labeled α-tubulin in *Drosophila* S2 cells (Figure 2) where we can clearly observe microtubule movement, polymerization and depolymerization. And we have imaged kinesin-73-EGFP movement along microtubules in S2 cells measuring speeds of roughly 0.8 μm/s.

We would like to extend SIM to live three-dimensional imaging in thick samples. Unfortunately, as the focal plane moves deeper into a live sample, refractive index variations cause optical aberrations which degrade both the structured illumination pattern and the image. We have demonstrated correction of optical aberrations with adaptive optics to correct the microscope point spread function, achieving a 10-fold increase in maximum intensity. We will discuss our current work developing adaptive optics and building a microscope that combines adaptive optics with structured illumination to permit super-resolution imaging deeper into live samples.

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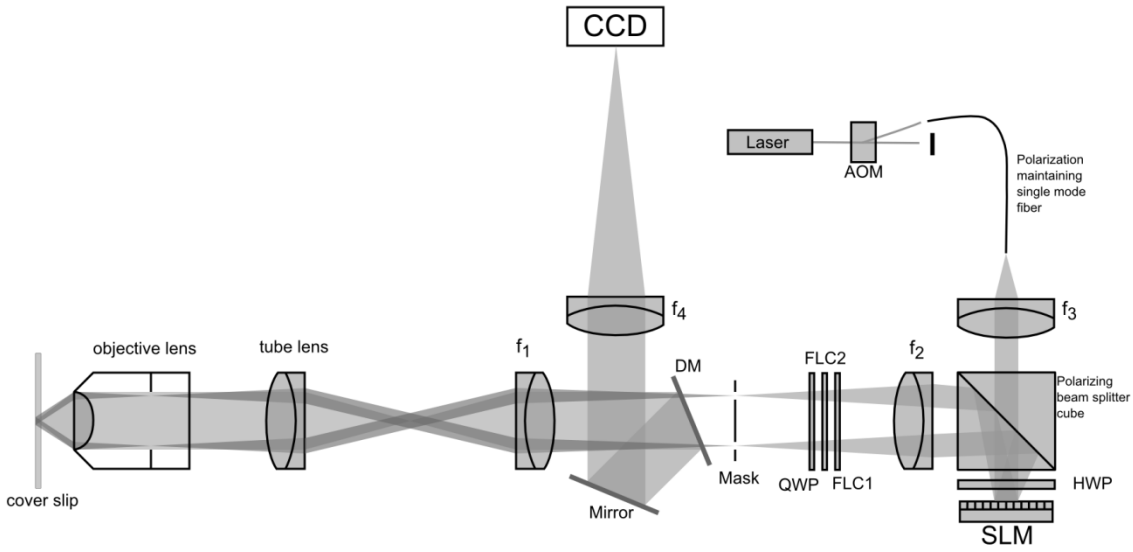

Figure 1: The Live SIM Microscope Layout. DM: dichroic mirror. QWP: quarter-wave plate. FLC: ferro-electric liquid crystal 120º phase retarder. SLM: spatial light modulator. HWP: half-wave plate. AOM: acousto-optic modulator.

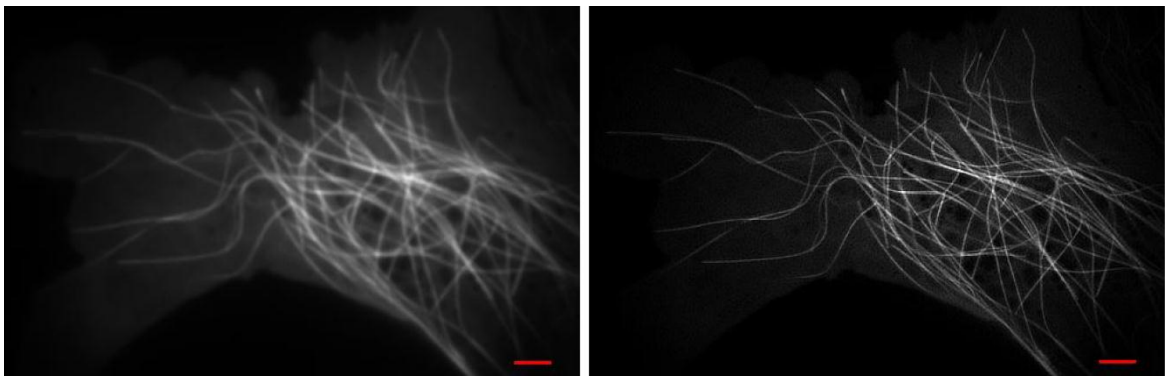

Figure 2: Images from movie of microtubule motion in *Drosophila* S2 cells. Left: conventional image. Right: structured illumination image. The movie is 120 frames taken at 1Hz with a 360ms effective exposure – 9 raw images each with a 40ms exposure are required to construct one super-resolution image. Scale bar is 2 μm.

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