# SCANNING EPIC MICROSCOPY: TOWARD SIMULTANEOUS SUPER-RESOLUTION OF CONTINUOUS 3D FLUORESCENT STRUCTURES AT SPEED

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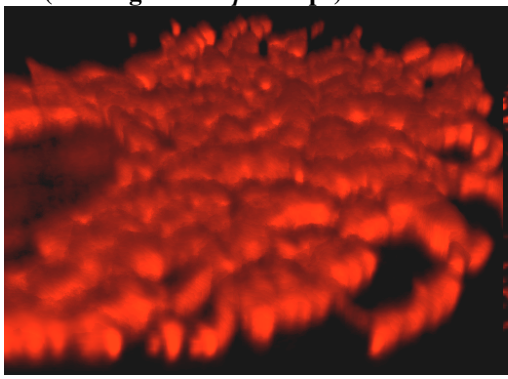
Microscope developers and biologists have long sought an instrument that can *simultaneously* produce nano-scale resolution images of continuous three-dimensional structures in living cells at high speed. Our ongoing work in PSF engineering and optimization image processing, called *expanded point information content (EPIC*) microscopy, continues to make progress toward this ambitious goal. In this presentation we demonstrate how a recent breakthrough has allowed us to achieve super-resolution of two-dimensional and three-dimensional *continuous* biological structures via a few simple modifications to a standard widefield fluorescence microscope. These include adding a focused spot scanning illumination mechanism, that is step scanned once across the 3D sample (from just one position of focus), and recording a small sub-image on an sCMOS camera at each scanning step. These sub-images are then processed using well-known optimization techniques to achieve resolutions on the order of 50-100nm in X, Y *and Z*, even though the recorded sub-image PSFs are spaced much closer than the Rayleigh criterion for resolution.

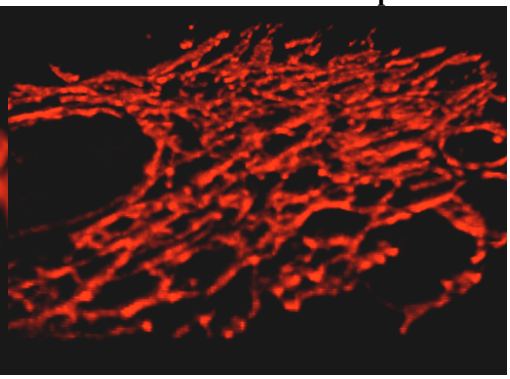
For imaging 3D thick structures, an additional modification to the microscope involves inserting a specially designed phase plate near the back focal plane of the objective. This phase plate alters the normal PSFs, converting them into ring shaped structures that encode precise depth information about the photo-emitters in the sample. Using these specially designed ring PSFs allows us to dispense with the need to change the microscope focus or build up an image stack. The result is the new microscope is able to record 3D images of dynamic cell structures at speeds an order of magnitude faster than existing microscopes and *simultaneously* super-resolve continuous features over an extended depth *without the need to change the original focus!* Results include comparisons to 3D confocal renderings (see below) and demonstrate that our new *Scanning EPIC* microscope shows great promise toward achieving our ultimate goal of simultaneous 3D super-resolution imaging at speed.

**Confocal: 3D rendering from z-stack (20 images at 0.2$\mu$m steps)**



X-Y resolution ~ 250nm and Z ~700nm,

**Scanning EPIC: 3D image from one X-Y scan at one focus position**



X-Y resolution ~150nm and Z ~200nm
Objective: 63x, NA 1.4