4D IMAGING FROM A SINGLE PLANE OF FOCUS USING EXPANDED POINT INFORMATION CONTENT (EPIC) MICROSCOPY

Carol Cogswell, Jiun-Yann Yu, Simeng Chen, Jian Xing, Robert Cormack, Ramzi Zahreddine, Bruce Wallin, Ute Herzfeld, Jolien Tyler and Mark Winey
University of Colorado at Boulder
Boulder, CO 80309, USA

KEY WORDS: PSF engineering, high-speed 3D imaging, extended depth super-localization.

In this presentation we demonstrate how the mainstream microscopes found in nearly every biology lab can be easily adapted to perform high-resolution 3D imaging that exceeds the acquisition speed of confocal and other 3D fluorescence microscopes while achieving similar or better 3D localization accuracy. By carefully engineering the microscope’s PSF using a custom-designed, add-on optical component, we have produced a new prototype system that overcomes a high NA objective’s limited depth of field (~600nm for 1.4 NA). It does so by collecting all fluorescence photons from an extended sample volume (that is at least 20 times thicker than the depth of field) and redirecting these photons into a single image plane, doing away with the need to change the microscope focus (or build up an image stack).

The new system is based on our novel approach to optical design, called expanded point information content (EPIC), and is built on the premise that it is possible to encode more information about the original three-dimensional sample into a standard microscope image and then easily retrieve this information using a custom signal processing algorithm. Unlike confocal systems where much of the light emitted by the sample is rejected by the imaging pinhole, our new EPIC configuration collects all of the photons emitted from the extended sample volume and forms a series of ring-like PSFs whose diameters correspond to the precise depths of each of the fluorescent features in the sample (see figure). The result is the new microscope is able to record high-resolution 3D images of dynamic cell structures at speeds an order of magnitude faster than existing microscopes and simultaneously locate features over an extended depth without the need to change the original focus! Results to be shown include 3D videos of yeast centrosome division and mitotic spindle tracking.

In addition to imaging point-like fluorescent structures, the EPIC microscope can be adapted to acquire volumetric information from extended objects and display it in several forms such as 3D renderings or as a series of optical sections, all from a single recorded plane of focus. Results from imaging extended objects such as mitochondria clusters show that (surprisingly) the new EPIC system can produce optical sections with higher lateral and axial resolution (i.e. ~150nm/300nm for lateral/axial resolution) when compared to a confocal microscope.