ADVANCES IN MULTI-FOCUS MICROSCOPY:
ACQUIRING AND VISUALIZING 3D SUPER-RESOLUTION IMAGES

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Super-resolution microscopy beyond the conventional diffraction limit is an invaluable tool for understanding biological processes, making it possible to infer the morphology of molecular assemblies, examine the composition of intracellular complexes, and understand the dynamic behavior of single-molecules. An important challenge for studying biological specimen is the development of robust methods for accessing super-resolution information in 3D. Our recently demonstrated multi-focus microscopy (MFM) technique enables the acquisition of 3D stacks in a parallel and ultrasensitive manner, with a measurement depth of ~4 µm (exceeding astigmatism, double-helix, and biplane techniques) [1].

Here we present the application of MFM to super-resolution imaging. Using PALM/STORM approaches, we can record 3D images with a lateral resolution down to 20 nm along the x/y axes, and 50 nm along the z axis. Importantly, MFM allows probing of volumes with an axial extension comparable to that of cultured cells. We next discuss the challenges of properly representing 3D super-resolution images consisting of millions of individual localization events. To do so, we have developed a single-molecule visualization tool capable of treating dense super-resolution data. Important features of our software (soon to be public) include 3D volume rendering and cluster segmentation, multi-channel capabilities, and experimentally relevant filtering and quantification tools.

The performance of our MFM technique and our visualization tool is demonstrated in an illustration of tracking in yeast cells (Figure 1A), and a surface reconstruction of mitochondria in a mammalian cell (Figure 1B).

Figure 1. A) Multi-focus microscopy illustration of tracking between nine planes. B) Surface reconstruction of mitochondria in a mammalian cell (units in um).