Volumetric Super-Resolution Imaging Using Multifocus Microscopy

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Keywords: 3D imaging, super-resolution microscopy, whole-cell

Abstract:
Single molecule localization-based microscopy techniques that overcome the optical diffraction limit have become a prominent tool for researchers. Increased interest in single molecule imaging in biological studies, however, has led to new technical challenges. Among the most pressing issues is the ability to rapidly image and localize molecules in thick 3D volumes. Our recently developed multifocus microscopy technique (MFM) enables instantaneous acquisition of volumetric images by tiling fluorescence images of nine axial sections on the same detector [1].

Here, we show that MFM can be combined with super-resolution imaging techniques such as PALM or STORM [2]. When imaged using the MFM, single molecules can be localized axially within ~4 \(\mu\)m, a depth comparable to the dimensions of organelles. Moreover, this axial probing depth surpasses all other 3D super-resolution localization microscopy techniques [3]. We demonstrate the volumetric imaging capability of the MFM through super-resolution imaging of mitochondria network in HeLa cells, and by two-color imaging in yeast (Figure 1). Additionally, we discuss the different parameters dictating image quality, and accordingly characterize performance of the system over the imaging depth. Recent efforts to further extend the range of axial detection and improve precision are also presented.

![Figure 1](image)

Figure 1: Two colors volumetric super-resolution imaging of whole yeast cell during cell division using the MFM. In red-orange the cell wall labeled with Alexa 647, and in blue-white tubulin fibers expressing photo-convertible fluorescent protein tdEos. Grid: 1 micrometer.

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