A SIMPLE MODIFICATION OF IMAGE SCANNING MICROSCOPY ENABLES THREE-DIMENSIONAL IMAGING AT ONE SINGLE FOCUS WITH SPATIAL RESOLUTIONS BEYOND THE DIFFRACTION LIMIT

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We present a novel fluorescence imaging technique that acquires three-dimensional images of the specimen without any change of focus. We modify a conventional image scanning microscopy system [1] by removing the confocal pinhole and inserting a phase plate, such that the generated point spread functions (PSFs) can provide accurate spatial information for all three dimensions. In this work, we use ring-like PSFs where the centers of the rings indicate X and Y positions and the ring diameters indicate Z positions. We then process the acquired images with least-square estimation algorithms to achieve the final three-dimensional reconstruction of the specimen.

To our surprise, the preliminary result of our implementations shows spatial resolutions superior to conventional confocal microscopy (Fig. 1), which represents the gold standard of diffraction-limited, three-dimensional imaging. To validate our results, we further developed a numerical tool that estimates the spatial resolutions of our approach, and found that the observed resolutions are consistent with the numerical estimations. In particular, our numerical analysis suggests that the spatial resolution of our technique depends not only on the point spread function, but also the signal-to-noise ratio of the acquired image. Requiring no change of focus during acquisition, we believe that our discovery opens a new approach to high-speed and high-resolution three-dimensional imaging, with the added benefit that it can be implemented via a simple modification to a standard widefield microscope.

Fig. 1: Optical sectioning comparison of (a) a confocal microscope and (b) our microscopy system. The fluorescence emission wavelength of the specimen (F36924, Thermo Fisher Scientific) is ~630 nm. The images are single sections from the two systems. The line plots show the lateral (solid blue lines) and axial (dashed yellow lines) intensity profiles.