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Computational Recovery of Engineered Point Spread Functions in Single Molecule Localization Microscopy using the Double Helix 3DTRAX Software

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Single molecule localization microscopy (SMLM) techniques like STORM, PALM, DNA-PAINT, etc. have overcome the traditional optical diffraction limit of 200 nm [1]. By trading temporal resolution for spatial resolution, these techniques make use of photoconvertible molecules such that only a sparse subset of molecules are visible in any given image frame. The center position of the molecules is then determined by fitting it to the expected point spread function (PSF) model of the imaging system. The final image is reconstructed by combining the localized positions of the molecules over typically 1000's of image frames.

Although SMLM enables high precision imaging in the range of 10-20 nm in the lateral dimension, it lacks axial (z) precision, especially near focus. This limitation is due to the fact that in the absence of aberrations, the Airy Disc PSF is symmetric about focus leading to ambiguity in the sign of the axial position. Furthermore, within the depth of field of the microscope, the Airy Disc PSF does not change shape leading to further ambiguity in axial localization.

The Double-Helix Point Spread Function (DH-PSF) offers a solution to this problem by enabling large-depth and high-precision 3D imaging [2]. The DH-PSF refers to an optical response that modifies the point spread function on the microscope such that, instead of an Airy Disc, the image of each point source is in the form of two well separated lobes. Unlike the blurring of the Airy Disc PSF, the two lobes rotate around their midpoint as the emitter moves along the axial dimension. The axial position of the emitter is therefore encoded in the orientation angle of the two lobes while the centroid between them indicates the lateral position [3]. The DH-PSF technique has been shown to be fundamentally more precise than other 3D methods, providing the best combination of precision and the greatest depth of field [4]. This unique combination of precision and depth can be used for 3D-SMLM and 3D-particle tracking.

A core component of implementing engineered PSFs is the efficient recovery of the axial and lateral position of the emitters based on their PSF shape. This can be either done by fitting an analytical function to the PSF or by matching the resulting PSF shape to previously captured calibration data. However, most previous localization algorithms required sparse, well-separated images of the PSF. This leads to fewer localizations per frame increasing the image capture time, data size and post processing times. Furthermore, for densely labeled samples, it is not always possible to reduce the emission density to avoid overlap between the PSFs.

A possible solution to this was introduced by Barsic, et. al. using an algorithm based on matching pursuit to resolve overlapping emitters as described in [5]. The approach involves creating a dictionary of subpixel sampled PSFs at various x-y-z positions, and matching the dictionary elements to the acquired images of the PSF. The accuracy of localization thus depends on the accuracy of the PSF model which can be affected by inherent aberrations of the microscope. To address this, Quirin, et. al. used a phase-retrieval [6] approach to more accurately model the pupil plane and hence the PSF based on captured calibration data.

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We have combined, and built upon, these two approaches to create a Fiji (ImageJ) compatible software, 3DTRAX® that enables high precision extended depth 3D localization of overlapping emitters for a wide variety of experimental setups and engineered PSF designs including the double helix and tetrapod. The software also provides for simultaneous tracking of multiple particles across an extended depth to allow for continuous study of particles across time [7].

We will present details of our approach for achieving high precision 3D localization using 3DTRAX for overlapping emitter imaging, simultaneous two-color imaging and particle tracking. The software also contains modules for two color alignment and drift correction and has been implemented to enable multithreading and parallel processing with GPU. The graphic user interface for localization analysis and the various modules is shown in Figure 1.

The addition of engineered PSFs and computational recovery using 3DTRAX to microscopy techniques such as nanometer-scale single-molecule imaging[8], [9], light sheet[10], and 3D particle tracking [11], [12] are already playing a pivotal role in the studies of cancer [12], immunology, and neuroscience[13]. As an example, Figure 2, shows a comparison between a 2D and a 3D (using DHPSF and 3DTRAX) single molecule image of microtubules in a Cos7 cell.

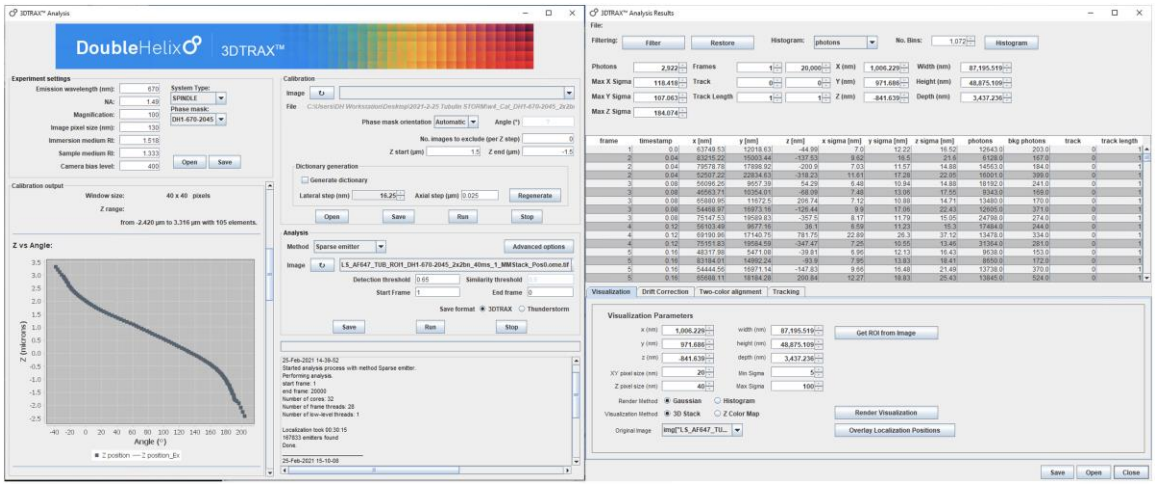

Figure 1. Left: 3DTRAX analysis panel for calibrating and localizing the PSF for a given system and phase mask setup. Also shown is the z vs angle calibration curve. Right: 3DTRAX Analysis results window showing a table of the 3D localization data and corresponding precision values for each localized emitter. Also shown are the visualization, drift correction, two-color alignment and particle tracking modules.

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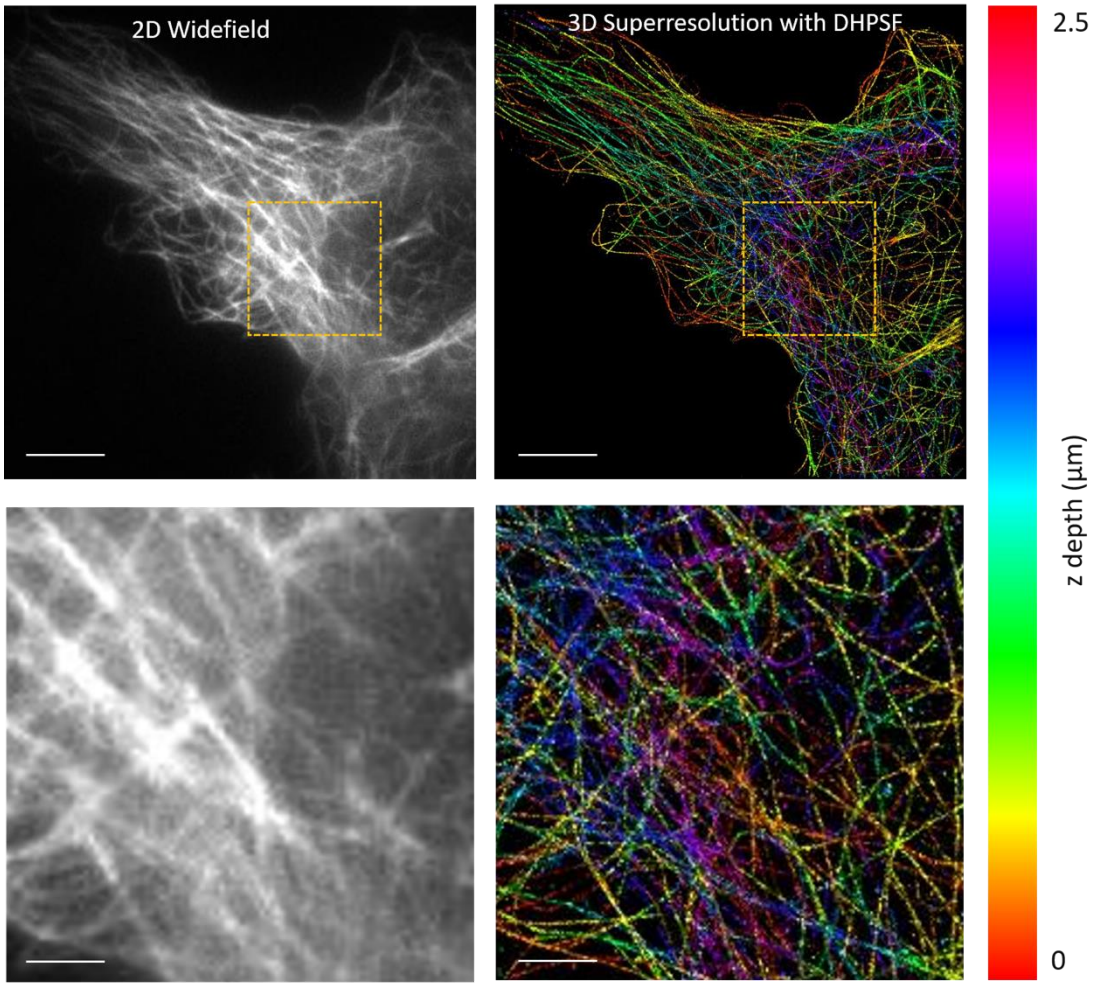

Figure 2. Super-resolution image of microtubules in a Cos7 cell. Left: Conventional 2D widefield image Right: Double Helix 3D reconstruction. Depth encoded in color, scale at bottom. Top panel scale bars are 10 μm, bottom panel scale bars are 2 μm.

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