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Point Spread Diffraction Patterns and Super-Resolution Particle Localization STEPHEN ANTHONY, SRIPAD RAM, ANISH ABRAHAM, JERRY CHAO, E. SALLY WARD, Dept. of Immunology, UT Southwestern, RAIMUND OBER, Dept. of Electrical Eng., UT Dallas — Sub-diffraction limit localization of fluorophores depends upon fitting the observed photon distribution to the point spread function (PSF). As such, accurate knowledge of the PSF is important to super-resolution microscopy and critical to determining the trajectories and dynamics of molecules within cells. While simple geometrical optics serves well to represent light propagation on the macroscopic level, more elaborate wave representations are necessary to describe light propagation within a few wavelengths of focal points, such as single fluorophores imaged by microscope objectives. As a result, numerous theoretical approximations to experimental PSFs exist as the exact theoretical PSF is unknown. Further consideration must be given that for realistic experiments, frequently events of interest will not entirely match the design conditions of the microscope; most events of interest will not be perfectly in focus, nor can the index of refraction of a living cell be controlled. Additionally, a number of imaging modes explicitly rely upon out-of-focus images. Through serial sectioning microscopy, we explore the experimental PSF in comparison with various models, determining which models are robust and provide accurate sub-diffraction limit localization for realistic data.

Stephen Anthony
Dept. of Immunology, UT Southwestern

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