Optimal 3D single-molecule super-resolution microscopy with engineered point spread functions

Sean Quirin, Ginni Grover, and Rafael Piestun
Department of Electrical, Computer, and Energy Engineering
University of Colorado at Boulder
Boulder, USA
piestun@colorado.edu

***Abstract*—Three-dimensional single-molecule localization is a fundamental problem in biological and biophysical experiments such as super-resolution microscopy. Point spread function engineering accompanied by corresponding reconstruction procedures provides a unique approach to increase localization precision. Joint optical/digital design and image reconstruction are investigated.**

Superresolution microscopy; Single-molecule localization; Point spread function engineering.

I. INTRODUCTION

Current super-resolution microscopy techniques are creating opportunities to explore sub-cellular structure and function or to visualize materials at the nanoscale [1-8]. Recently, single-molecule photo-activation and photo-switching localization based microscopy has been proposed for far-field super-resolution imaging [1-4]. Techniques such as PALM (Photo-Activated Localization Microscopy) and STORM (Stochastic Optical Reconstruction Microscopy) achieve super-resolution by allowing only a sparse, random set of molecules to emit light at any given time and then localizing each molecule with great precision. The latest extension to three dimensions has opened up unprecedented possibilities to investigate the organization of cells [9-13].

In this presentation we show that proper engineering of the three-dimensional (3D) point spread function (PSF) via specially designed optics improves 3D position estimation and hence resolution. Therefore, we introduce several PSF designs shaped in the form of a double helix and investigate the fundamental tradeoffs. We compare prevalent methods for 3D wide field localization in terms of the Cramer-Rao lower bounds and present an optimal 3D localization estimator in a general framework for noisy, aberrated and/or engineered PSF imaging. A Phase Retrieval (PR) enabled Maximum Likelihood Estimator (MLE) shows to be efficient by reaching the fundamental lower bound for localization precision along x, y and z. Experimental application of the double–helix PSF in conjunction with the PR-MLE reveals low-photon-count 3D wide-field single-molecule localization over an extended depth of field with exceptional precision [14].

II. 3D LOCALIZATION WITH DOUBLE-HELIX POINT SPREAD FUNCTIONS

The Double-helix point spread function (DH-PSF) [15] is an engineered PSF that displays two lobes in the transverse plane that rotate with defocus. The 3D PSF thus generates a double-helix shape (see Fig. 1). The axial position of the imaged point source is encoded in the rotation of the two lobes. The DH-PSF increases the axial and 3D Fisher information transfer of the imaging system [16,17] and can be used for 3D localization of multiple sparse particles. In addition, the DH-PSF provides a long depth-of-field, making it desirable for tracking [15,18,19] and super-resolution imaging [10,13,14].

The localization precision of a system is determined by the number of photons detected, the noise, the optics and the estimator used for localization. Simple estimators are fast but may not reach the limit of precision determined by the number of detected photons, numerical aperture, and noise parameters [13,15,18-20]. Hence, the use of an optimal estimator is required for achieving optimal precision [14,21-24].



Figure 1: A Double Helix PSF for a 1.23NA microscope. Crossections of the PSF show the evolution of the orientation of the two lobes for different axial positions. Scale bar is 1 μm.

III. LOCALIZATION BASED SUPERRESOLUTION IMAGING

Localization based super-resolution methods such as PALM or STORM use engineered molecular proteins or dyes whose emission can be controlled cyclically so that a sparse set of emitters in the field of view can be turned on for single-molecule localization. Repeated activation of different subsets of single molecules over time samples the entire field of view to reconstruct an image of the tagged structure with high resolution.

A DH-PSF based microscope has been shown to be suitable for 3D localization based super-resolution imaging. Figure 2 shows an image of microtubules of PtK1 (Rat Kangaroo Epithelial cells) tagged with an antibody labeled with Alexa-647 and Alexa-488 dyes [2]. The DH-PSF microscope had a 1.45NA 100X Nikon objective and an electron multiplying charged coupled device (EMCCD). A 641 nm diode laser was used for deactivation and the 488 nm line of Argon ion laser was used for activation.

Figure 2(a) shows a normal fluorescence image of microtubules and Fig. 2(b) shows the 3D super-resolution image of the same region where fine details of the microtubule network are easily visible. The depth of the network is shown in color providing details of the imaged volume. Super-resolution imaging with the DH-PSF microscope was achieved over a depth of 1 μm. The single-molecule 3D localization precision achieved for this particular experiment was σ(x, y, z) = (25, 33, 45) nm with 1650 photons per molecule per estimation and 28 photons/pixel background.

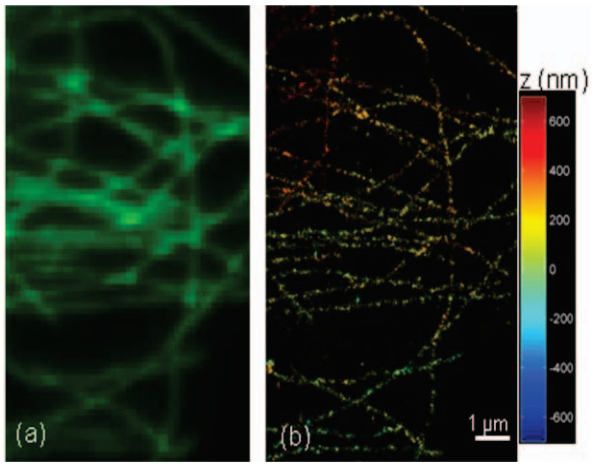


Figure 2: (a) Conventional fluorescence image of microtubule network. (b) 3D super-resolution image of the same region acquired with the DH-PSF localization based microscope. The depth of the microtubules is encoded in color.

ACKNOWLEDGMENT

We thank Jennifer DeLuca and Keith DeLuca for providing the biological samples and assisting in the experiments. This work was supported by NSF awards DBI-0852885 and DGE-0801680.

REFERENCES

[1] Betzig E, et al. (2006) Imaging intracellular fluorescent proteins at nanometer resolution. *Science* 313:1642-1645

[2] Rust MJ, Bates M, Zhuang X (2006) Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature Methods* 3:793-796

[3] Hess ST, Girirajan TPK, Mason MD (2006) Ultra-high resolution imaging by fluorescence Photoactivation Localization Microscopy. *Biophysical Journal* 91:4258-4272

[4] Egner A, et al. (2007) Fluorescence nanoscopy in whole cells by asynchronous localization of photoswitching emitters. *Biophysical Journal* 93:3285-3290

[5] Hell SW, Wichmann J (1994) Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt. Letters* 19:780-782

[6] Gustafsson MGL (2005) Nonlinear structured-illumination microscopy: Wide-field fluorescence imaging with theoretically unlimited resolution. *Proc Natl Acad Sci USA* 102:13081-13086

[7] Tang J, Akerboom J, Vaziri A, Looger LL, Shank CV (2010) Near isotropic 3D optical nanoscopy with photon limited chromophores. Proc Natl Acad Sci USA 107:10068-10073

[8] Pertsinidis A, Zhang Y, Chu S (2010) Subnanometre single-molecule localization, registration and distance measurements. Nature 466:647-651

[9] Shtengel G, et al. (2009) Interferometric fluorescent super-resolution microscopy resolves 3D cellular ultrastructure. *Proc Natl Acad Sci USA* 106:3125-3130

[10] Pavani SRP, DeLuca J, Piestun R (2009) Polarization sensitive, three-dimensional, single-molecule imaging of cells with a double-helix system. *Opt. Express* 17:19644-19655

[11] Juette MF, et al. (2008) Three-dimensional sub-100nm resolution fluorescence microscopy of thick samples. *Nature Methods* 5:527-529

[12] Huang B, Wang W, Bates M, Zhuang X (2008) Three-dimensional super-resolution imaging by Stochastic Optical Reconstruction Microscopy. *Science* 319:810-813

[13] Pavani SRP, et al. (2009) Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function. *Proc Natl Acad Sci USA* 106:2995-2999

[14] Quirin S, Pavani SRP, Piestun R, "Optimal 3D single-molecule localization for superresolution microscopy with aberrations and engineered point spread functions," *Proc. Natl. Acad. Sci.* Published online before print December 30, 2011

[15] Pavani SRP, Piestun R (2008) Three dimensional tracking of fluorescent microparticles using a photon-limited double-helix response system. *Opt. Express* 16:22048-22057

[16] Ober R, Ram S, Ward ES (2004) Localization accuracy in single-molecule microscopy. *Biophysical Journal* 86:1185-1200

[17] Grover G, Pavani SRP, Piestun R (2010) Performance limits on three-dimensional particle localization in photon-limited microscopy. *Opt. Letters* 35:3306-3308

[18] Thompson MA, Lew MD, Badieirostami M, Moerner WE (2010) Localizing and tracking single nanoscale emitters in three dimensions with high spatiotemporal resolution using a double-helix point spread function. *Nano Letters* 10:211-218

[19] Thompson MA, Casolari JM, Badieirostami M, Brown PO, Moerner WE (2010) Three-dimensional tracking of single mRNS particles in Saccharomyces cerevisiae using a double-helix point spread function. *Proc Natl Acad Sci USA* 107:17864-17871

[20] Thompson RE, Larson DR, Webb WW (2002) Precise nanometer localization analysis for individual fluorescent probes. *Biophysical Journal* 82:2775-2783

[21] Quirin S, Pavani SRP, Piestun R, "Pattern Matching Estimator for Precise 3-D Particle Localization with Engineered Point Spread Functions," in *Digital Holography and Three-Dimensional Imaging*, OSA Technical Digest (CD) (Optical Society of America, 2010), paper DMC3

[22] Aguet F, Van De Ville D, Unser M (2005) A maximum-likelihood formalism for sub-resolution axial localization of fluorescent nanoparticles. *Opt. Express* 13:10503-10522

[23] Mortensen KI, Churchman LS, Spudich JA, Flyvbjerg H (2010) Optimized localization analysis for single-molecule tracking and super-resolution microscopy. *Nature Methods* 7:377-381

[24] Smith CS, Joseph N, Rieger B, Lidke KA (2010) Fast, single-molecule localization that achieves theoretically minimum uncertainty. *Nature Methods* 7:373-375