Automated, robust recognition and extraction of the double-helix point spread function in fluorescence microscope images

EE 368 Project Proposal

Matthew Lew (mlew@stanford.edu) and Scott Hsieh (sshsieh@stanford.edu)

If a microscope sample consists of a single, point-like emitter, then the image of the emitter will be given by point spread function (PSF) of the microscope, which is a small Gaussian-like spot when the emitter is in focus. The double-helix point spread function (DH-PSF) transforms the image of that single point-like emitter into two Gaussian-like spots.[1, 2] These two spots rotate around one another as a function of the axial (*z*) position of the emitter (see **Figure 1**), tracing out a double helix in three-dimensional space. Thus, the angle between the two spots corresponds to the *z* position of the emitter, and the midpoint between the two spots corresponds to the lateral (*xy*) position of the emitter.

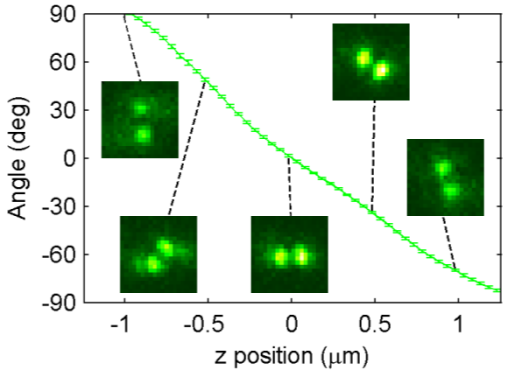

Figure 1. Calibration curve showing how the angle between the two spots of the DH-PSF changes as a function of the axial (*z*) position of a nanoscale fluorescent emitter. Insets show false-color DH-PSF images of a single fluorescent bead at various *z* positions indicated by dashed lines. Figure borrowed from reference [3].

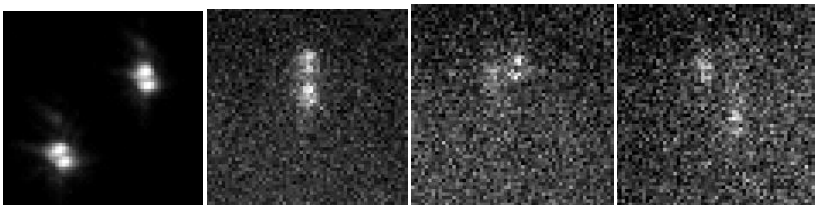

Figure 2. Sample images of the two spots of the DH-PSF at various signal-to-background ratios. The signal-to-background ratio in each image decreases from the leftmost image to the rightmost image. Ideally, at the end of the project, all of these double Gaussians will be successfully extracted by our algorithm.

Photon shot noise and background fluorescence make it difficult to extract the double Gaussians with a simple thresholding algorithm. Thus, the goal of this project will be to build a more sophisticated algorithm to recognize and extract the DH-PSF from experimental data consisting of single molecules within bacterial cells. The algorithm must be robust to 1) rotations of the DH-PSF, 2) a low signal-to-background ratio between the two spots and surrounding pixels, and 3) shot noise in the images of the two spots. Furthermore, the algorithm should 4) run unsupervised, 5) avoid false positives, and 6)

provide data showing the location and frame number of the extracted double Gaussians for later review. Sample images of the two spots to be extracted are shown in **Figure 2**.

Our initial approach could consist of 1) subtraction of the spatially varying (but slowly changing in time) background fluorescence, followed by 2) low-pass filtering and segmentation in order to locate the double Gaussians. This can be refined by making use of the fact that the Gaussians will come in pairs, or by using a more thorough statistical analysis of the data. This project will not involve borrowing a DROID camera phone.

[1] S. R. P. Pavani, M. A. Thompson, J. S. Biteen, S. J. Lord, N. Liu, R. J. Twieg, R. Piestun and W. E. Moerner, "Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function," Proceedings of the National Academy of Sciences **106,** 2995-2999 (2009).

[2] M. A. Thompson, M. D. Lew, M. Badieirostami and W. E. Moerner, "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 (2010).

[3] M. D. Lew, M. A. Thompson, M. Badieirostami and W. E. Moerner, "In vivo three-dimensional superresolution fluorescence tracking using a double-helix point spread function," Proc. SPIE **7571,** 75710Z (2010).