FandPLimitTool: an application for calculating limits of accuracy for parameter estimation in single molecule microscopy

Anish V. Abraham, Amir Tahmasbi, Jerry Chao, Sripad Ram, Raimund J. Ober
Department of Electrical Engineering, University of Texas at Dallas, Richardson, TX 75080

In single molecule microscopy, the accurate estimation of the location of a single fluorescent particle plays a central role in the analysis of image data, especially in the context of high-accuracy molecule tracking and superresolution image reconstruction. The achievable estimation accuracy, however, depends on a multitude of factors ranging from the wavelength of the photons detected from the fluorescent particle to the pixel size of the detector that is used to capture the image data. It is therefore useful to be able to determine, for a given experimental setup, the best possible accuracy with which a parameter such as the particle location can be estimated from the acquired images. To this end, the FandPLimitTool [1] software program was developed for the calculation of Cramer-Rao lower bound-based limits of accuracy [2] for the estimation of parameters from single molecule microscopy image data. FandPLimitTool is a GUI-based application [3] written in MATLAB, and it currently enables calculation of limits of accuracy for estimating, among other parameters such as the number of photons detected from the molecule(s) of interest, the location of a single molecule and the distance between two single molecules in both two-dimensional and three-dimensional space. For a given estimation problem, options are available in terms of the model for the point spread function, the detector type (e.g., CCD or EMCCD camera), and calculation settings such as the step size for the evaluation of various integrals.

[1] A. V. Abraham, S. Ram, J. Chao, E. S. Ward, and R. J. Ober, “Quantitative study of single molecule location estimation techniques,” *Optics Express*, vol. 17, no. 26, pp. 23352–23373, 2009.
[2] R. J. Ober, S. Ram, E. S. Ward, "Localization accuracy in single-molecule microscopy," *Biophysical Journal*, vol. 86, no. 2, pp. 1185-1200.
[3] Ward Ober Lab, “FandPLimitTool Software Download.” [Online]. Available: http://www.wardoberlab.com/software/fandplimittool/.