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Single molecule image deconvolution. I. Standard deviation analysis of immobile fluorescent molecules MICHAEL C. DESANTIS, SHAWN H. DECENZO, Y.M. WANG, Washington University, St. Louis, MO — Single molecule fluorescence imaging has been a powerful technique in studying individual processes not accessible by bulk, ensemble-averaged measurements [1]. Improvements in image analysis are required for high temporal and spatial precision in the localization of single fluorescent molecules. We present the first thorough standard deviation analysis for point spread functions (PSFs) of single immobile fluorescent molecules. Using this new single molecule image deconvolution (SMID) method, we show that 3D localization of individual molecules with sub-nanometer precision can be achieved. We have derived an expression estimating the standard error of the PSF's standard deviation, incorporating experimental effects of the number of collected photons, finite pixel size, and background noise. The localization precision obtained via this expression is approximately 1.5 times better than the current available methods. The use of SMID to extract subexposure dynamics of mobile molecules will also be discussed.
[1]. Wang, Y. M, R. H, Austin, & Cox, E. C. 2006 *Physical Review Letters* **97**, 048302(1-4).

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