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Sub-diffraction limit differentiation of single fluorophores using Single Molecule Image Deconvolution (SMID) SHAWN H. DECENZO, MICHAEL C. DESANTIS, Y. M. WANG, Department of Physics Washington University, St. Louis Missouri, 63130 USA — In order to better understand biological systems, researchers demand new techniques and improvements in single molecule differentiation. We present a unique approach utilizing an analysis of the standard deviation of the Gaussian point spread function of single immobile fluorescent molecules. This technique, Single Molecule Image Deconvolution (SMID), is applicable to standard TIRF instrumentation and standard fluorophores. We demonstrate the method by measuring the separation of two Cy3 molecules attached to the ends of short double-stranded DNA immobilized on a surface without photobleaching. Preliminary results and further applications will be presented.

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