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### **Single-image molecular analysis for accelerated fluorescence imaging**

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We have developed a new single-molecule fluorescence imaging analysis method, SIMA, to improve the temporal resolution of single-molecule localization and tracking studies to millisecond timescales without compromising the nanometer range spatial resolution [1,2]. In this method, the width of the fluorescence intensity profile of a static or mobile molecule, imaged using submillisecond to milliseconds exposure time, is used for localization and dynamics analysis. We apply this method to three single-molecule studies: (1) subdiffraction molecular separation measurements, (2) axial localization precision measurements, and (3) protein diffusion coefficient measurements in free solution. Applications of SIMA in flagella IFT particle analysis, localizations of UgtP (a cell division regulator protein) in live cells, and diffusion coefficient measurement of LacI in vitro and in vivo will be discussed.

[1] Shawn DeCenzo, Michael C. DeSantis, and Y. M. Wang, "Single-image separation measurements of two unresolved fluorophores," *Optics Express*, 18, 16628-16639, (2010)

[2] M. DeSantis, S. DeCenzo, J. L. Li, and Y.M. Wang, "Precision analysis for standard deviation measurements of single fluorescent molecule images," *Optics Express*, 18, 6563-6576, (2010)