Measurement of position and orientation of single molecules and their assemblies using real-time fluorescence polarization microscope

Shalin B. Mehta¹, Molly McQuilken², Amitabh Verma¹, Amy Gladfelter³, Rudolf Oldenbourg¹,³, Tomomi Tani¹

¹ Cellular Dynamics Program, Marine Biological Laboratory, Woods Hole, MA 02542, USA
² Biological Sciences Department, Dartmouth College, Hanover, NH 03755, USA
³ Physics Department, Brown University, Providence, RI 02912, USA
shalin.mehta@gmail.com, mshalin@mbl.edu

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Understanding the biological function of ordered assemblies, such as lipid membrane and cytoskeletal filaments (e.g., microtubule, actin filament), requires measurement of orientation of constituent molecules in live cells. The molecular and structural specificity required to probe the ordered assemblies can be achieved by imaging the orientation of fluorophores attached to molecules in a rotationally constrained fashion. Orientation of molecules in live cells has been imaged by defocused detection [1] and simultaneous detection along two polarization states [2]. Simultaneous analysis of polarized fluorescence is a promising approach for imaging fast dynamics of live ordered assemblies at high resolution.

A detection architecture that analyzes the fluorescence emission along four polarization channels (termed real-time fluorescence polarization microscope or RTFluorPol) – each with transmission axis 45° provides instantaneous measurement of single fluorophore’s 3D orientation and location. Theoretical studies [3] suggest that such a design provides direct and optimal measurement of the orientation and location of single fluorophores. We have combined, to our knowledge for the first time, total internal reflection fluorescence (TIRF) excitation and RTFluorPol architecture to simultaneously measure position and orientation of single and ensemble of fluorescent molecules. We report algorithms for calibration and analysis algorithms for our microscope such that the experimental performance approaches theoretical capability. Above figure shows the orientation of the ensemble of fluorescent dye, FM 1-43, embedded in E. coli membrane and orientation of single GFP molecules measured with TIRF-RTFluorPol. We present measurements of orientation of cytoskeletal protein septin (labeled with constrained GFP molecules) as they assemble.

References: