AN OPTICAL FORCE-FLUORESCENCE MICROSCOPE FOR MOLECULAR BIOMECHANICAL STUDIES

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Optical tweezers and single molecule fluorescence methods provide the ability to mechanically probe a substrate with piconewton forces and simultaneously report on the structure with fluorescence detected from a single molecule. Our goal was to develop a method to combine mechanical measurements with fluorescence detection for simultaneous and coincident application for both single molecule biophysics measurements and cell level studies. In addition to the technical hurdles of interfacing the required instrumentation, efforts to combine these two powerful single molecule biophysics methods have been complicated due to enhanced photobleaching and other destructive photophysical properties that fluorophores experience when simultaneously irradiated with the excitation and trapping lasers. Because of this, the method having only been demonstrated in a coincident geometry with rhodamine [1], a particular stable dye, has remained elusive for favored single molecule probes such as the Cyanine and Alexa dyes. Our instrument design achieves a combination of these techniques for coincident measurements so that photobleaching is minimized. We report on the performance of our method for a range of favored fluorophores through bulk, ensemble level assays using labeled beads and surface bound probes. We present single molecule level studies of Cy3 photobleaching histograms, a dye that is particularly susceptible to destruction in the presence of the trap. Our methods shows over an order of magnitude decrease in photobleaching rate for Cy3. In addition we present the first demonstration of combined coincident optical trapping mechanical measurement and single molecule fluorescence detection using Cy3. Here force from the optical trap is used to unzip a 15 basepair strand of DNA with simultaneous detection of the strand separation through single molecule fluorescence detection of Cy3. In addition to showing that our instrument maintains fluorescence detection capability, we demonstrate that the integrity of the optical trap is not compromised.

Reference:

[1] M.J. Lang, P.M. Fordyce, A.M. Engh, K.C.Neuman and S.M. Block, "Simultaneous, coincident optical trapping and single-molecule fluorescence," Nature Methods, vol. 1, p1-7, (2004)

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