

**Session Title:** Molecular Mechanics & Force Spectroscopy  
**Presentation Number:** 2833-Pos  
**Abstract Title:** **Reducing Enhanced Photobleaching for Combined, Coincident Optical Tweezers and Single Molecule Fluorescence**  
**Location:** Halls A/B/C/D  
**Topic:** 9H Molecular Mechanics and Force Spectroscopy  
**Author Block:** **Ricardo R. Brau**, Jorge M. Ferrer, Peter B. Tarsa, Peter Lee, David C. Appleyard, Matthew J. Lang.  
MIT, Cambridge, MA, USA.

Optical tweezers and single molecule fluorescence techniques provide different, but complementary, insights about the biophysical properties of single proteins and molecules. However, efforts at combining these two technologies into a single instrument have been complicated by the enhanced photobleaching that occurs when fluorescent dyes are simultaneously irradiated by trapping and excitation lasers. We provide a simple solution to this problem by employing common laser manipulation techniques for an integrated combination of force and fluorescence spectroscopy. Beads coated with Cy3 chromophores were examined to test this new arrangement. As expected, the photobleaching rate in the unmodified system was increased by over an order of magnitude in the presence of the trapping laser. Conversely, beads inspected with the modified apparatus exhibited no enhanced photobleaching effects, allowing the fluorescence decay rate to recover to nearly the rate observed in non-trapping conditions. Similar behavior was observed for surface-immobilized Cy3 at the single molecule level and for other commonly used single molecule dyes, such as TMR and Alexa 555, at the ensemble level. In addition to this fluorescence photobleaching rate improvement, our results show that this technique does not compromise the integrity of the trap. These results are being applied to unzip a 15 base pair portion of dye-labeled dsDNA using combined, coincident optical tweezers and single molecule fluorescence.

**Commercial Relationship:** **R.R. Brau**, None; **J.M. Ferrer**, None; **P.B. Tarsa**, None; **P. Lee**, None; **D.C. Appleyard**, None; **M.J. Lang**, None.