SUPPORTING MATERIAL

Polarization Controlled TIRFM with Focal Drift and Spatial Field Intensity Correction

Daniel S. Johnson[1], Ricardo Toledo-Crow[2], Alexa L. Mattheyses[3], Sanford M. Simon[1*]

1. Laboratory of Cellular Biophysics, The Rockefeller University, New York, New York.
2. Research Engineering Lab, Memorial Sloan-Kettering Cancer Center, New York, NY
3. Department of Cell Biology, Emory University School of Medicine, Atlanta, GA

* Correspondence to: Sanford M. Simon at simon@rockefeller.edu.

Correcting for Beam Ellipticity

A challenge with rotating linearly polarized light is correctly maintaining linear polarization at the sample, because each optical component along the beam path has the potential to alter the polarization state. The transmission or reflection of light at each component can introduce phase delays or even variable attenuation between orthogonal components of the beam. Orientation dependent phase shifts in effect behave as another wave plate in the optical path, turning the desired linearly polarized light into elliptically polarized light. These shifts are particularly common with dielectric mirrors, including broadband mirrors and beam combining dichroic mirrors. For example, a common visible broadband dielectric mirror (BB1-E02, Thorlabs Inc.) had the potential to turn linearly polarized 488 nm laser light into essentially circularly polarized light (Fig. S2 *A*), thus behaving effectively as a quarter-wave plate. We experienced significantly smaller phase shifts with a protected silver coated mirror (PF10-03-P01, Thorlabs Inc.) (Fig. S2 *B*), but the difference in absolute intensity reflected from $\hat{\boldsymbol{s}}$ and $\hat{\boldsymbol{p}}$ incidence was significantly worse than with the silver mirror (2.7% difference - manufacturer spec) compared to a dielectric mirror (0.3% difference). Although phase shifts appear to be more problematic, compensation can be achieved by introducing additional cancelling phase shifts into the beam path. We corrected for the beam ellipticity by adding a variable wave plate, a Berek compensator, following the EOM/quarter-wave plate combo (Fig. 1 *D*). The variable-wave plate was adjusted to maximize the linear polarized state across all rotated states (see Fig. S2 *C* & *D* for analysis before and after compensation).

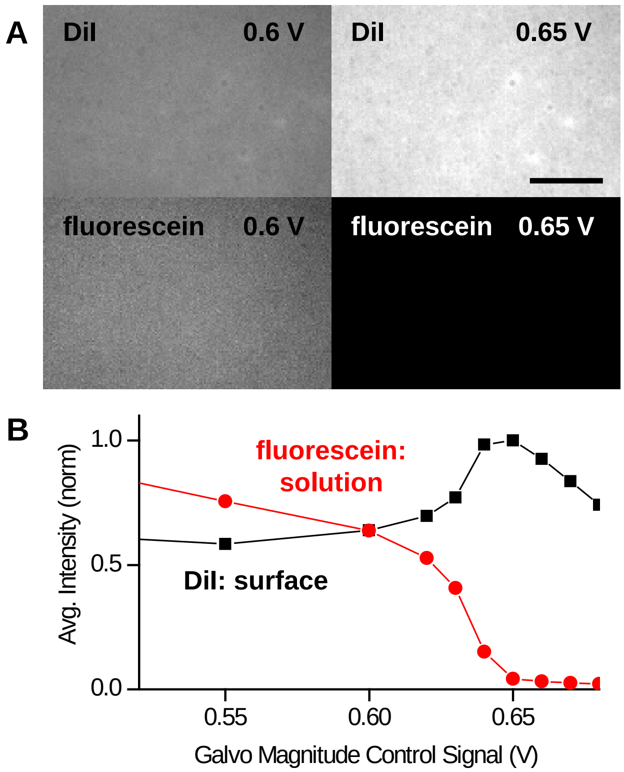

FIGURE S1 – TIR at surface verified by imaging DiI, primarily on the glass surface, and carboxyfluorescein, primarily in the solution, at different laser excitation incident angles. (*A*) Example images both below (left) and above the critical angle (right). Sample prepared by incubating DiI (0.5 mg/ml in EtOH) on glass surface for 10 minutes, followed by washing and replacing the solution with carboxyfluorescein (0.05 mg/ml in H_20). 488 nm laser used for excitation of both dyes with 515/30 nm emission filter used for carboxyfluorescein and 655/40 nm filter used for DiI. (*B*) Normalized fluorescence intensity of DiI (black) and fluorescein (red) at different excitation incident angles (Galvo Magnitude Control Signal). As angle passes beyond critical angle (~0.63V control signal magnitude) excitation of dye in solution decreases while excitation of dye on surface increases. Scale bar 5µm.

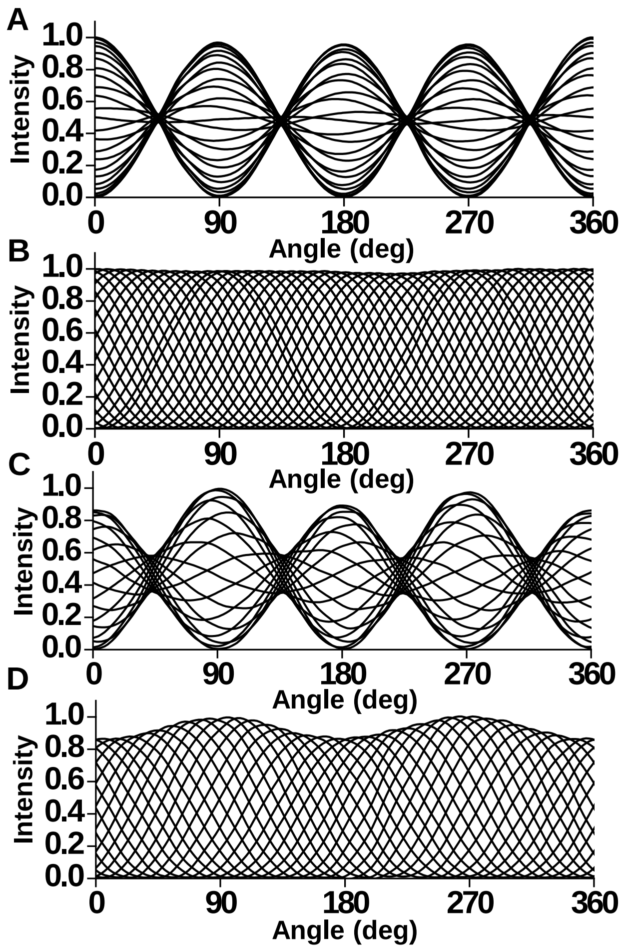

FIGURE S2 – (*A*) Linearly polarized light at different initial polarization angles was reflected off a 45° dielectric mirror (BB1-E02, Thorlabs Inc.) and was analyzed with a linear polarizer at various angles (horizontal axis). Light polarized perpendicular and parallel to the plane of incidence ($\hat{\boldsymbol{s}}$ and $\hat{\boldsymbol{p}}$) remained linearly polarized (full sine wave), but at 45° the reflected light was close to circular (flat analyzed intensity across polarized angle). **(***B***)** Similar to *A*, but analysis of polarization state following reflection from a metal mirror (PF10-03-P01, Thorlabs Inc.). **(***C***)** Analysis of polarization state at objective position prior to correction with Berek compensator. **(***D***)** Analysis of polarization state at objective position following compensation.