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Strategies for Fluorescence Correlation with Photosensitive/Low-Yield Probes to Measure Receptor Dimerization[1] ALEXANDRIA DOERFLER, JAMES THOMAS, Department of Physics and Astronomy, University of New Mexico, UNM PHYSICS JAMES THOMAS LAB TEAM — Fluorescence Correlation Spectroscopy (FCS) is a well-established technique used to measure diffusion coefficients and concentrations of fluorescently labeled particles. If two distinct fluorescent labels are used with two detection channels, FCS can also measure particle-particle association, such as protein dimerization. Photobleaching adversely affects the measurement of associations: both the fluorescence signal and the fraction of dual-labeled receptor dimers are not stationary during the course of the measurement, but decay over time. We have adopted three strategies to deal with these difficulties. First, total internal reflection illumination is used to reduce background, allowing for dimmer illumination and lower count rates. Second, we make a direct measurement of photon counts in both channels (rather than using real-time correlation.) Third, a dynamic estimate of dimer concentration is made using very short measurement intervals (properly accounting for the large uncertainties in measurement) followed by fitting to a model that includes photobleaching effects. The design and calibration of the microscope will be described, along with proof-of-principle model fitting.

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