

INVITED LECTURE T10

Total internal reflection with fluorescence correlation spectroscopy

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The combination of total internal reflection illumination with fluorescence correlation spectroscopy (TIR-FCS) allows one to examine in quantitative detail a variety of biophysical properties related to the motions and interactions of fluorescent molecules near the interface of a transparent planar surface and an adjacent solution. Several experimental and theoretical aspects of this combination will be discussed. TIR-FCS has allowed characterization of local diffusion coefficients and concentrations of fluorescently labeled antibodies in solution but very close to substrate-supported phospholipid bilayers. TIR-FCS has also been used to examine the interaction kinetics of fluorescently labeled mouse IgG specifically and reversibly associating with the mouse receptor Fc γ RII, which was purified and reconstituted into substrate-supported planar membranes. This method also has the potential of providing information about the kinetics of nonfluorescent molecules which compete with fluorescent reporters for surface-immobilized receptors.