

TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF) MICROSCOPY: APPLICATIONS TO CELL BIOLOGY

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INTRODUCTION: Total internal reflection fluorescence (TIRF) microscopy (also called "evanescent wave microscopy") provides a means to selectively excite fluorophores in an aqueous or cellular environment very near a solid surface (within ≈ 100 nm) without exciting fluorescence from regions farther from the surface. Fluorescence excitation by this thin zone of electromagnetic energy (called an "evanescent field") results in images with very low background fluorescence, virtually no out-of-focus fluorescence, and minimal exposure of cells to light at any other planes in the sample. The unique features of TIRF have enabled numerous applications in biochemistry and cell biology.

TIRF can be set up in a variety of configurations. All involve only simple add-on modifications of standard upright or inverted fluorescence microscopes and all are easily switchable with other forms of illumination. Some of the configurations involve deployment of an extra prism to direct the excitation light toward the sample plane at an angle exceeding the critical angle for total internal reflection. Other configurations use a very high aperture objective ($NA > 1.4$) now commercially available. Although laser illumination is most convenient, TIRF can also be set up with a conventional arc lamp source.

Three particular applications to quantitative observation of the dynamics of the cell surface are discussed. The first employs the exponential decay of the evanescent field intensity with distance from the surface to characterize the nanometer-scale motions of GFP-marked secretory granules relative to the plasma membrane in both unstimulated and stimulated bovine chromaffin cells. The second takes advantage of the unique polarization properties of the evanescent field to observe dynamic changes in the micromorphology of the diI-labeled plasma membrane of macrophages (e.g., at putative endocytotic sites). The third combines TIRF with fluorescence recovery after photobleaching to measure the spatially-resolved association/dissociation kinetic rates of labelled actin at the submembrane of living smooth muscle cells.