

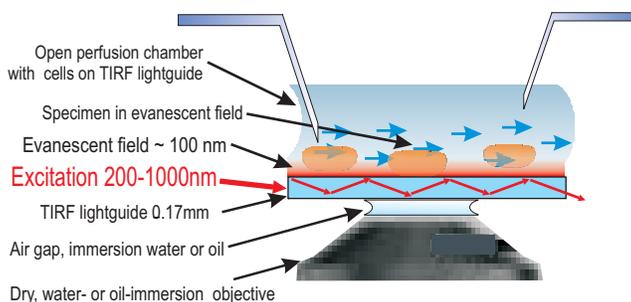
Axon Growth and Pathfinding Studies of Intracellular Pathways that Govern Axon Behavior

During neuronal development proteins are produced in the cell body as well as in axons and growth cones. Axons and growth cones contain mRNAs and ribosomes and are capable of translating endogenous and heterologous mRNAs into proteins. Axon growth involves dynamic changes of the cytoskeleton, such as beta-actin (de)polymerization at the plasma membrane. Many of the proteins are ubiquitously expressed inside the axon and growth cone. However, only a small fraction of them actively regulates the cytoskeleton at the membrane. Conventional epi-fluorescence microscopy visualizes all proteins and does not distinguish between membrane and bulk proteins.

Unlike conventional epi-fluorescence microscopy, TIRF microscopy provides exceptional spatial selectivity, approximately one order of magnitude better than that in confocal microscopy. In TIRF only a thin layer of the specimen (~100 nm) is excited and fluoresces, while the bulk of the specimen does not fluoresce. This feature allows for selective identification of proteins at the membrane. TIRF visualizes only proteins that are at the membrane and, therefore, is well suited for monitoring of the response of membrane proteins to a stimulus, as opposed to the bulk of all protein in the axon.

Figures 1 and 2 compare TIRF and epi-fluorescence images of 293FT cells and the network of axons of dorsal root ganglia explants cultured for 3 days in open perfusion chambers attached to the surface of polylysine coated TIRF lightguides. 24 hours before the experiments, the cells were infected with a Sindbis virus driving expression of a membrane-anchored version of EGFP throughout the cells (SR-IRES-myrEGFP). Unlike epi-fluorescence, TIRF excites and images only ~100 nm of the specimen adjacent to the surface of TIRF lightguide. Thus, the membrane-anchored EGFP is visualized by TIRF. Epi-fluorescence reveals the presence of EGFP in the bulk of the specimen.

Schematics of lightguide-based TIRF microscopy experiment



Features and Compatibility of Ig-TIRFM

Ig-TIRFM is multicolor, factory-aligned, flexible system

Objectives: dry, water- and oil-immersion lenses

Light sources: LED, lasers, Xe and Hg arc lamps, etc.

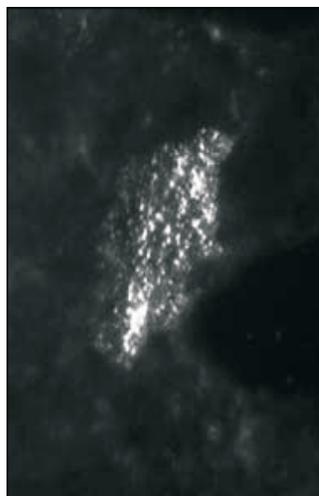
Open perfusion and microliter-volume closed flow cells

Microscopes: Nikon, Olympus, Zeiss, Leica, other

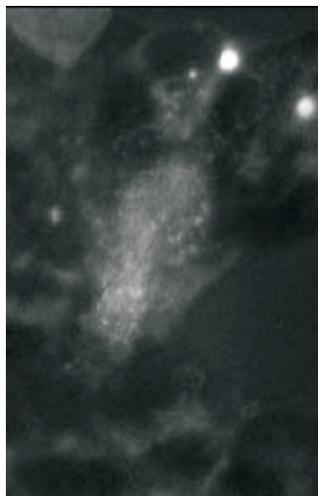
Superior signal/background ratio

No dependency on specialized objectives and laser light sources. Can be used virtually with any objective and light source.

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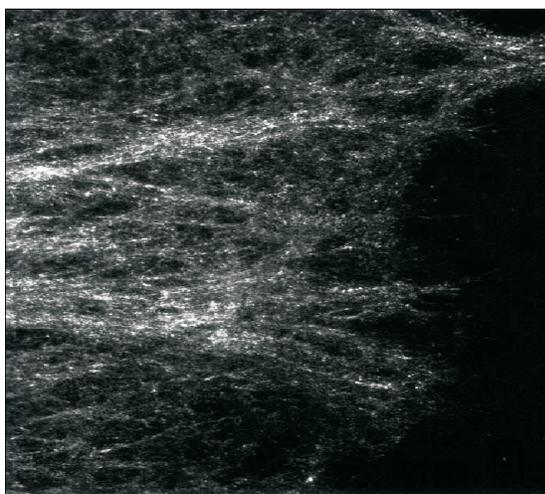


TIRFM

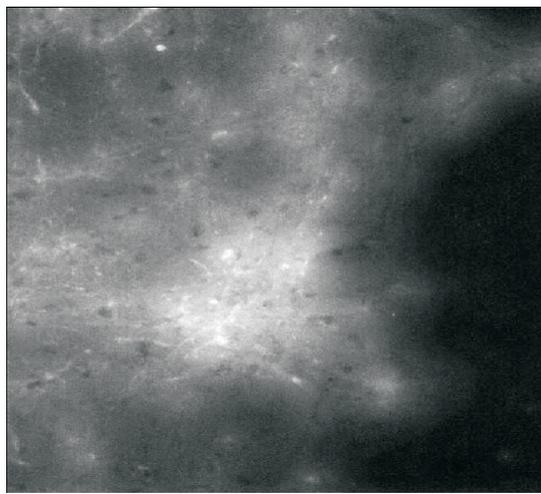


Epifluorescence

Figure 1. Cells 293FT were cultured for 3 days with 0.5 ml growth medium inside the perfusion chamber attached to TIRFM lightguide surface. 24 hours before the experiments, the cells were infected with a Sindbis virus driving expression of a membrane-anchored version of EGFP throughout the cells. Left image – TIRFM; right image - epifluorescence. TIRF excitation - blue LED 460-490 nm, emission range -510-560 nm. Objective Nikon CFI Plan Achromat DL 40X, NA 0.65, WD 0.57. Images - courtesy of Dr. Ulrich Hengst, and Dr. Samie Jaffrey, Department of Pharmacology, Weill Medical College, Cornell University, New York.



TIRFM



Epifluorescence

Figure 2. Dorsal root ganglia explants (DRG) were cultured for 3 days with growth medium inside the perfusion chamber attached to TIRFM lightguide. 24 hours before the experiments, the DRG cells were infected with a Sindbis virus driving expression of a membrane-anchored version of EGFP throughout the cells. Left – TIRFM image of DRG axons; right- the same specimen imaged in epifluorescence mode. TIRF excitation - blue LED 460-490 nm, emission — 510-560 nm. Objective Nikon CFI Plan Achromat DL 40X, NA 0.65, WD 0.57 mm. Images - courtesy of Dr. Ulrich Hengst, and Dr. Samie Jaffrey, Department of Pharmacology, Weill Medical College, Cornell University, New York.