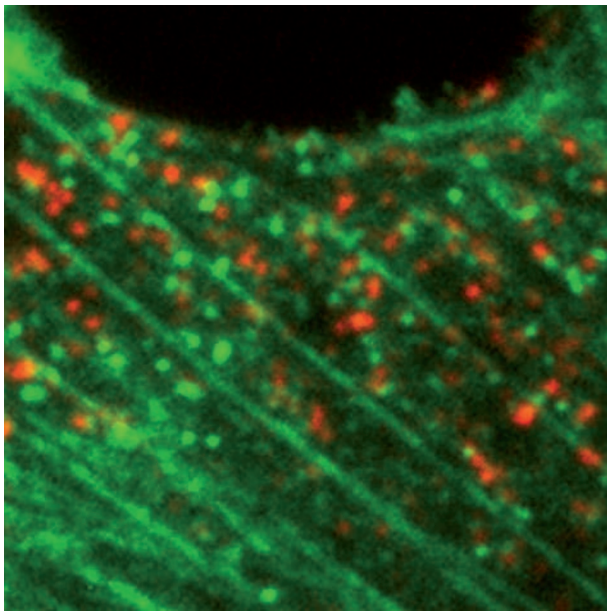
MILESTONE 13

TIRF: imaging at the cellular edge



Clathrin-coated pits (red), actin stress fibres (parallel green lines) and local foci of actin polymerization (green dots) near the plasma membrane of a fibroblast. Image courtesy of W. Almers, Vollum Institute, Portland, Oregon, USA.

If you want to visualize what happens at a biological interface or on the surface of a cell, it is vital to eliminate the background that comprises all the stuff that sits beyond that first layer. This is exactly the approach used in total internal reflection fluorescence microscopy (TIRF). Building on studies from the late 1800s on the detection of scattered photons from TIR excitation, Daniel Axelrod built a microscope in which a laser beam hits, at an angle, a glass slide — on the other side of which the sample is placed. The incident angle is greater than the critical angle, and therefore the beam is completely reflected back into the slide. At the same time, a tiny part of the beam called an evanescent wave manages to penetrate a few hundred nanometres into the sample and can stimulate fluorescence in a biological medium that is tagged with chromophores.

> TIRF in live cells concerns the plasma membrane and the adjacent 100 nm of cytosol.
> *Wolfhard Almers*

The evanescent wave penetrates the sample only for some 200 nm and its intensity decreases exponentially, thereby producing exquisite z-axis sensitivity. Restricting illumination to a thin section at the interface of the sample and the glass slide eliminates background fluorescence from deeper in the sample and allows longer imaging periods by limiting bleaching of chromophores that can move into or out of the illuminated layer during imaging. In 1981, Axelrod demonstrated the imaging capabilities of the technique by visualizing cellular focal adhesions on a glass slide.

In the mid-1990s, Yanagida and colleagues took the thin illumination layer provided by TIRF to its logical consequence and performed single-molecule detection of biological events in aqueous solutions and in real time. They reported individual ATP turnover events by a single myosin molecule in 1995. This setup predated confocal microscopy by several years, and to a large extent it is much simpler and less expensive because it can be easily fitted on to conventional inverted fluorescence microscopes.

In 2000, Almers and colleagues used the high z-sensitivity of TIRF to image the process of exocytosis of synaptic vesicles, and visualized the vesicles approaching the plasma membrane and unloading their contents. In the same year, Axelrod and colleagues similarly imaged the fusion of post-Golgi carriers with the plasma membrane. These studies are also early examples of through-the-objective TIRF, which, through the use of high numerical aperture objectives, further simplifies the setup and sample handling. Given the number of important events that take place at the cellular membrane, including endocytosis, exocytosis, receptor activation and ion transport, having a technique that can access this layer has set the stage for what can be done with TIRF at present.

Stefano Tonzani, Associate Editor, Nature

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