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APPLICATION BRIEF

# TIRF Microscopy



Total internal reflection fluorescence (TIRF) microscopy is a highly useful mode of fluorescence-light microscopy that employs a carefully refined illumination scheme to allow direct, time-resolved visualization of various intracellular events, including endocytosis,[1] exocytosis,[2] cytoskeletal dynamics,[3] and dynamic single-molecule imaging.[4]

A relatively recent development in biological fluorescence microscopy, TIRF permits ultrahigh axial resolution — on the order of 100 nm — at the sample/coverslip interface (see **Figure 1**). The benefit of such high optical-sectioning resolution at this interface is that the signal-to-noise ratio for studies involving the direct observation and recording of cell membrane function, vesicle fusion, and membrane proteins will likewise be very high.

TIRF microscopy often finds its greatest utility in the context of low-signal-intensity, high-speed imaging experiments. To best meet the performance challenges posed by these requirements, a growing number of researchers are turning to electron-multiplying CCD (EMCCD) technology.

The Rolera-MGi camera, which incorporates a back-illuminated EMCCD, has been engineered by QImaging® specifically for this type of quantitative, low-light-level, short-exposure-time imaging. The state-of-the-art Rolera-MGi amplifies weak signals above the readout noise floor, delivers 95% peak quantum efficiency in the visible spectrum, and enables precise capture of 16,384 brightness levels. In addition, every Rolera™ camera includes an IEEE 1394 interface for streamlined, standardized connectivity to TIRF workstations along with the ultrafast data transfer needed to reliably record dynamic live-cell events acquired at high frame rates.

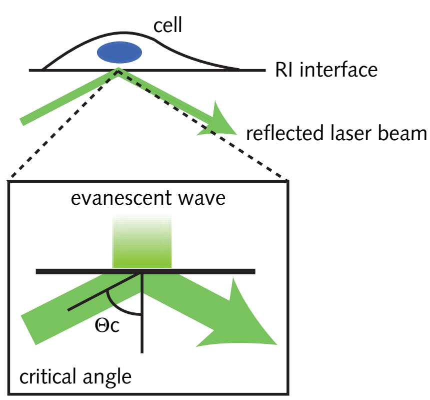


**Figure 1.** Total internal reflection fluorescence. A laser beam incident on an interface between high- and low-refractive-index materials is reflected at 100% efficiency at or above the critical angle, thus setting up an electromagnetic field of the same frequency called an evanescent wave. The evanescent wave excites the specimen only within a couple hundred nanometers of the refractive-index interface.

# Citations

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