

**TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY (TIRFM):
ADVANCED TECHNIQUES FOR TOPOLOGY AND MEMBRANE DYNAMICS OF
LIVING CELLS**

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KEY WORDS: Living cells, TIRFM techniques, cell-substrate topology, membrane dynamics, cholesterol

Total internal reflection fluorescence microscopy (TIRFM) has gained considerable importance in membrane studies of living cells. For selective excitation of membrane associated fluorophores by an evanescent electromagnetic field two different innovative setups have been developed. The first one consists of a specific microscope condenser with variable-angle excitation using a hemispherical prism and additional optics for transillumination (prism type TIRFM), whereas the second one includes a high aperture objective lens with a laser beam being focused close to the edge of its aperture (objective type TIRFM). Both setups are described, and their properties are compared with one another as well as with a commercially available TIRFM system. Prism type TIRFM provides an excellent depth resolution, variable amplification and numerous advantages for physiological measurements, whereas objective type TIRFM provides some advantages of optical alignment.

Measurements were combined with single cell spectroscopy and fluorescence lifetime imaging (FLIM) and concentrated on U373-MG glioblastoma cells incubated either with the cytoplasm marker calcein or the membrane marker laurdan, as well as T47-D human breast cancer cells transfected with a gene coding for a yellow fluorescent membrane protein (EYFP-Mem). In addition to cell-substrate topology, we studied membrane dynamics as a function of temperature and cholesterol content. The latter was modified using defined protocols of depletion or enrichment. A comparison of measurements with total internal reflection (TIR) illumination and transillumination proved that membrane stiffness decreased with temperature and was always larger for the plasma membrane than for intracellular membranes. In addition, intracellular membranes were more affected by cholesterol depletion than the plasma membrane.

A correlation between membrane parameters and cholesterol content might be useful to detect some specific diseases (with alteration of membrane properties) and to measure the influence of pharmaceutical agents on membrane properties. For this purpose a TIR reader system has been developed which might be suitable for high throughput screening (HTS).

(submitted for oral presentation)