# TIRF TECHNOLOGIES FOR QUANTITATION OF GLUCOSE TRANSPORTER 4 (GLUT4) TRANSLOCATION

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Stimulation of glucose transporters is a key factor for treatment of type 2 diabetes mellitus. In particular, glucose absorption from the blood by adipocytes or muscle cells occurs after translocation of the glucose transporter 4 (GLUT4) from intracellular compartments to the plasma membrane upon application of insulin or insulin-mimetic compounds. Total Internal reflection (TIRF) microscopy is a valuable technique to visualize and quantitate this translocation in living cells and thus to probe the efficiency of these compounds. We therefore, established various TIRF techniques:
- *Prism-based TIRF microscopy* with variable-angle illumination in combination with spectral imaging and fluorescence lifetime imaging (FLIM) in order to get detailed information on a cellular as well as on a molecular level.
- *Super-resolution TIRF* upon Structured Illumination (SIM) for visualizing intracellular translocation at 100 nm resolution [1]. Using objective-based TIRF microscopy two interfering laser beams, e.g. the first diffraction orders of a spatial light modulator (SLM), are focused close to the edge of the microscope aperture and interfere in the plane of the sample, as shown in Figure 1.

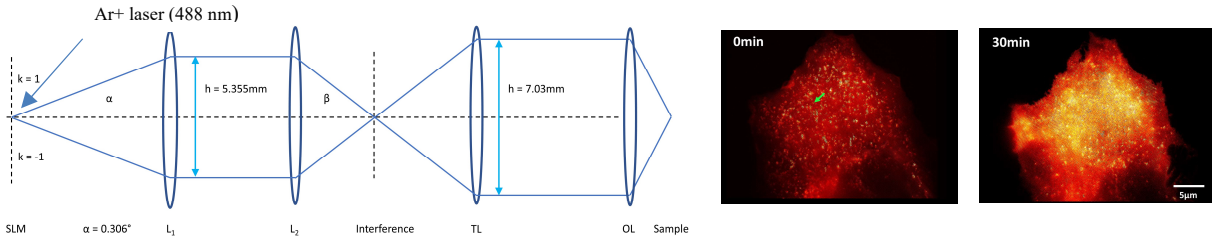


Fig. 1. Principle of super-resolution TIRF-SIM; translocation of GLUT4-myc-GFP fusion proteins from intracellular vesicles to the plasma membrane in CHO-K1 cells prior to (0 min) and subsequent to stimulation (30 min) with *Bellis perennis* extract (common daisy; 10 mg/l).

- *TIRF reader* technology for the simultaneous and quantitative analysis of larger cell populations [2]. Up to 96 wells of a microtiter plate are illuminated simultaneously under TIRF conditions, and a continuous increase of GFP fluorescence in the plasma membrane is measured over a range of $10^{-12}$ to $10^{-6}$ mol/l insulin up to 30 min.

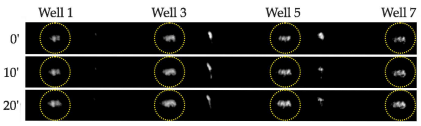


Fig. 2. Fluorescence of GLUT4-myc-GFP in selected wells at 0, 10 and 20 min. after stimulation with insulin.

## References

[1] V. Richter, P. Lanzerstorfer, J. Weghuber, H. Schneckenburger: Super-resolution live cell microscopy of membrane-proximal fluorophores, Int. J. Mol. Sci. 21(19) (2020), 7099.
[2] V. Stadlbauer et al.: Fluorescence microscopy-based quantitation of GLUT4 translocation: high-throughput or high-content? Int. J. Mol. Sci. 21 (2020) 7964.