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.

- **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.

References