

Structured illumination in TIRF microscopy
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TIRF microscopy [1] has become an indispensable tool in the live sciences, as it allows monitoring of very faint processes or very rapid dynamics at the plasma membrane owing to its high sensitivity and the shallow penetration depth of the evanescent field used for illumination. Here I present that structured illumination can significantly improve the lateral resolving power as well as the optical sectioning power in TIRF microscopy.

To improve the lateral resolution, TIRF-SIM illuminates the sample with a series of evanescent standing waves where both the phase and orientation of the pattern is varied. The fine interference patterns encode high resolution information, which after numerical processing can improve the spatial resolution up to 2.5 fold [2]. Examples of live cell TIRF-SIM imaging are shown and challenges are discussed.

Both conventional TIRF and TIRF-SIM can be plagued by imperfections of the evanescent field [3], which can generate out-of-focus blur that compromises image analysis and can lead to severe image artifacts in TIRF-SIM. Here I show that by illuminating the sample with evanescent standing waves with coarse line spacing, out-of-focus blur can be robustly rejected (see also Fig 1). This drastically improves the contrast in TIRF images of densely labeled or highly scattering samples, which is demonstrated by imaging a cytosolic translocation biosensor in cancer cells and endocytosis in a dense monolayers of cells.

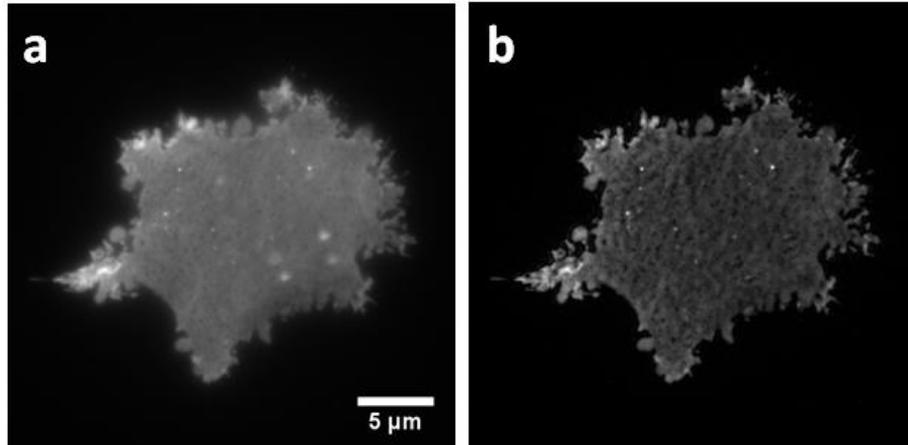


Figure 1. Comparison of normal TIRF (a) and TIRF with structured illumination (b) by imaging an MV3 cell labeled with a PI3 Kinase biosensor. The line spacing of the illumination pattern in (b) was chosen to best fill the missing cone of the optical transfer function.

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