FIX YOUR MEMBRANE RECEPTOR IMAGING: ACTIN CYTOSKELETON AND CD4 MEMBRANE ORGANIZATION DISRUPTION BY CHEMICAL FIXATION

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Single molecule localization microscopy (SMLM) techniques allow near molecular scale resolution (~20 nm) as well as precise and robust analysis of protein organization at different scales. Protocol reliability and artefact identification emerged as an increasingly important aspect of super-resolution microscopy. Here, we explore how different fixation approaches affect the actin cytoskeleton, CD4 organization, membrane protein mobility, and general sample preservation. Live-fixation was automated by using microfluidics on microscopes [1]. Distortions of the cytoskeleton were quantified by our recently developed quality metric tool SQUIRREL [2]. We show that fixation-mediated disruption of the actin cytoskeleton may introduce artefacts such as altering CD4 membrane organization. Single-particle tracking of transmembrane proteins and GPI-anchored protein showed that size and anchoring mode in the plasma membrane supersede fixation conditions. We highlight how careful sample preparation is essential for extracting meaningful results from super-resolution microscopy images. To this end we present a reproducible imaging approach to effectively determine preservation of the overall cellular structure during chemical fixation for super-resolution microscopy [3].

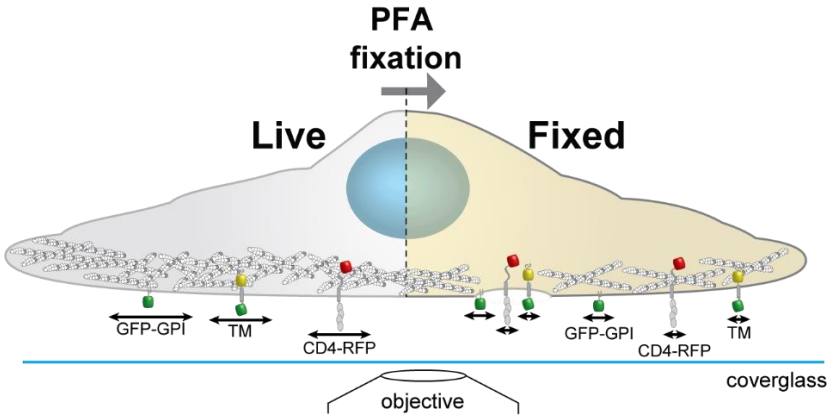

Figure 1: During imaging of live cells and subsequent chemical fixation changes in actin cytoskeleton architecture and membrane associated proteins mobility were observed in a protocol dependant manner.

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