

3D superresolution microscopy on the immunological synapse at isotropic precision below 15 nm

Lukas Velas, Mario O. Brameshuber, Johannes B. Huppa, Elke Kurz, Michael L. Dustin, Philipp Zelger, Alexander Jesacher and Gerhard J. Schütz

T-cells are part of our adaptive immune system and are responsible for recognition of antigens in our bodies. T-cell activation is triggered upon binding of T-cell receptor (TCR) to major histocompatibility complex loaded with antigenic peptide (pMHC) which is presented on the surface of antigen presenting cells (APC). According to the kinetic segregation model of T-cell activation, T-cell topography plays a large role in the antigen recognition process. In this study we have applied a 3D superresolution method to study the spatial organization of the T-cell receptor within the immunological synapse with isotropic localization precision below 15 nm. The method combines stochastic optical reconstruction microscopy (STORM) with defocused imaging that exploits effects of the supercritical angle fluorescence on the shape of the point spread function. Additionally, we correlated the 3D superresolution images with diffraction limited images of the immune synapse obtained by interference reflection microscopy. Experiments were performed on hybrid synapses between primary T-cells and functionalized glass-supported lipid bilayers. We used our method to quantify membrane fluctuations and the cleft size within the synapse by mapping the position of the T-cell receptor (TCR) with respect to the supported lipid bilayer. Our data show average distances of 18 nm up to 31 nm for activating and non-activating bilayers, respectively.