Nanoscale T-cell membrane protein imaging across complex topography using accelerated large depth-of-field localisation microscopy

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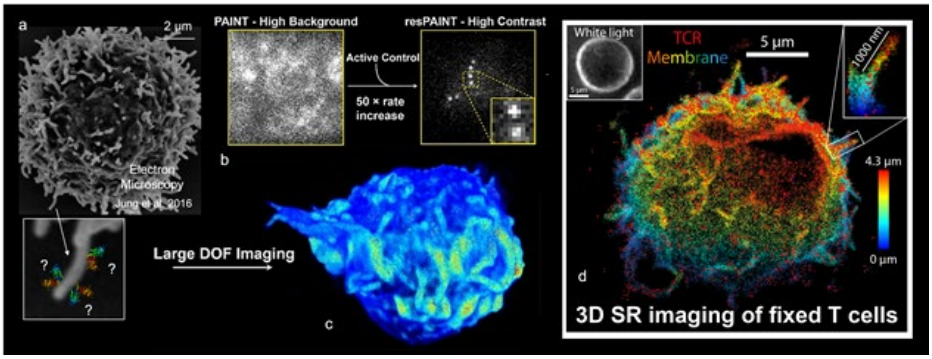
Background incl. aims
The nanoscale topography of cells plays a key role in a variety of biological processes, particularly for T cells, where finger-like structures scan the environment to sense pathogens. Conventional imaging often considers individual protein distributions, which can result in perceived clustering due to the membrane curvature. Without considering topography, it becomes impossible to decipher protein distributions and therefore its role in biological processes. Imaging cell topography (Fig. 1a) with localisation microscopy in 3D is thus important, but challenging, due to the relatively large scales involved and the requirement to achieve sufficient localisation densities. Point accumulation for imaging in nanoscale topography (PAINT) can be used overcome these challenges, but is typically hampered by high background, particularly for large depth-of-field 3D imaging.

Methods
Here we use actively controlled probes to greatly increase the effective concentration in PAINT, without influencing the background. By building up a concentrated and reversible reservoir of photoactivatable or spontaneously blinking probes the localisation rate of PAINT can be greatly accelerated. We combine this method with double-helix point spread function engineering that enables large depth-of-field localisation microscopy, which we apply to image the cell membrane of fixed T cells. Finally, the membrane imaging is combined with conventional membrane protein localisation microscopy to characterise nanoscale T-cell protein distributions across the complex topography of the cell membrane.

Results
We demonstrate that our approach (resPAINT) can increase the localisation rate of PAINT up to 50-fold without increasing background (Fig. 1b), which is essential for achieving suitable localisation densities to study membrane topography. We then show that our approach is suitable for imaging topography as we apply it to volumetric super-resolution imaging of entire T-cell membranes (Fig. 1c). Finally, we achieve correlative membrane and protein imaging (Fig 1d) to resolve the distribution of key T-cell membrane proteins: the T-cell receptor, the phosphatase CD45 and the microvilli marker CD62. By considering the local distribution of neighbouring proteins and membrane topography, we demonstrate how eigenvector analysis can be used to decipher preferential organisation and clustering of membrane proteins.

Conclusion
We have developed a technique for performing correlative membrane topography and membrane protein imaging, which we show is key for understanding how proteins distribute at the nanoscale. This represents a new approach to understanding how proteins organise and redistribute to achieve important biological functions, such as the recognition of pathogens by the adaptive immune response.

Graphic:



Keywords:

Super-resolution, Single-molecule, Membrane proteins, Biophysics