

SUPERRESOLUTION STED NANOSPECTROSCOPY WITH ENVIRONMENT-SENSITIVE PROBES

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Polarity-sensitive membrane probes, which shift their emission spectrum according to the local lipid order (such as Laurdan), have played a pivotal role in the research of the (domain) structure and function of biological membranes. But as the heterogeneities in cellular membranes are smaller than the resolution of confocal microscopes, an integration with a super-resolved imaging approach is needed. Stimulated emission depletion (STED) microscopy would in principle be a good candidate due to its speed and live cell compatibility compared to the localisation-based techniques, but the traditional polarity-sensitive dyes do not meet its special demands, especially on photostability.

We have recently identified and characterised several STED-compatible polarity-sensitive membrane dyes (Di-4-ANEPPDHQ, Di-4-AN(F)EPPTEA, and NR12S), achieving approx. 3-fold improvement in spatial resolution compared to confocal, which allowed us to detect even smaller variations in local lipid order in vesicles and live cell membranes [1]. However, the two-channel detection and representation in terms of the so-called generalised polarisation (GP), applied previously, does not offer as high a spectral sensitivity as spectrally resolved data analysed by spectral fitting or phasors. In addition, the broad spectra of these polarity-sensitive dyes hinder a simultaneous use of another probe to stain other structures of interest (e.g. proteins in green or far-red channel).

To mitigate both of the above limitations, we integrated a 16-channel spectral detector into a STED microscope, which allows us to record spectrally resolved super-resolution microscopy images, yielding better environmental sensitivity as well as the capability to decompose the signal from spectrally overlapping dyes. We use it to study the nanoscale architecture of the membranes of activating T cells and thereby better understand the molecular mechanisms of the immune response.

[1] E. Sezgin, F. Schneider, V. Zilles, I. Urbančič, E. Garcia, D. Waithe, A. Klymchenko, and C. Eggeling, "Polarity-Sensitive Probes for Superresolution Stimulated Emission Depletion Microscopy," *Biophysical Journal*, **113**, 1321–1330 (2017).