# SUPER-RESOLVED ENERGY TRANSFER IMAGING BY INTENSITY-BASED STED-FRET

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Förster Resonance Energy Transfer (FRET) imaging methods provide unique insight into the spatial distribution of energy transfer and (bio-)molecular interaction events, though they deliver average information for an ensemble of events included in a diffraction-limited volume. Coupling super-resolution fluorescence microscopy and FRET has shown to be a challenging and elusive task.

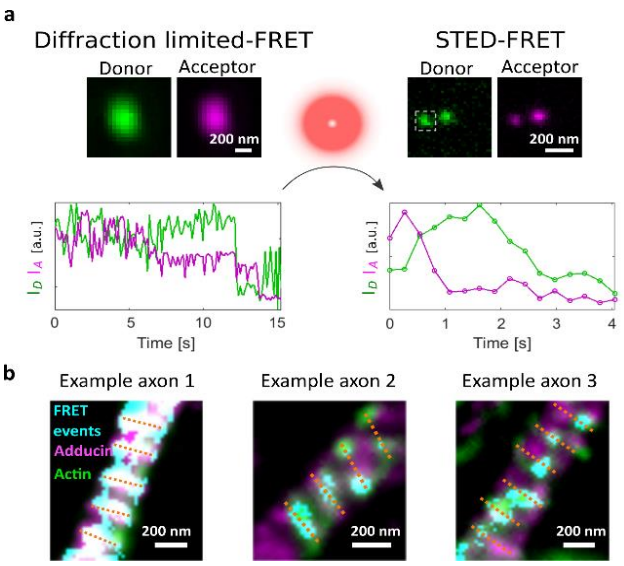


Figure 1. Example images of STED-FRET in (a) two DNA-origamis and (b) studying actin/adducin interactions in neurons.

Here, we present STED-FRET, a method of general applicability to obtain super-resolved energy transfer images through intensity-based calculations of FRET from STED images. In addition to higher spatial resolution, STED-FRET provides a more accurate quantification of interaction and has the capacity of suppressing contributions of non-interacting molecular partners, which are otherwise masked by averaging in conventional imaging.

STED-FRET maintains the resolution and acquisition speed of STED, and delivers high sensitivity down to single-molecule level. We demonstrate the performance of STED-FRET in DNA-origami model systems and in biological samples (Figure 1). In the latter, STED-FRET reveals biomolecular contacts between actin and adducin within the sub-diffracition structure of the membrane-associated periodic skeleton (MPS) of neurons.