# INDUCING SPARSITY WITH POLARISED STED TOWARDS CRYOGENIC SUPER-RESOLUTION

**C.N. Hulleman, R.J. Moerland, S. Stallinga and B. Rieger**
**Department of Imaging Physics**
**Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands**
**Email: c.n.hulleman@tudelft.nl**

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In single-molecule localisation microscopy (SMLM) the localisation precision is limited by the number of captured photons per individual emitter [1]. At cryogenic temperatures (photobleaching) reaction rates are reduced leading to an increased photostability of fluorophores and potentially more photons per emitter for localisation. However, the reduced reaction rates also hamper sparsity inducing techniques as applied at room temperature e.g. PALM, STORM. We proposed to utilise the fixed molecular dipole at cryogenic temperatures to our advantage [2] and now use orthogonal linearly polarised excitation and depletion beams to selectively image an angular subset of emitters, illustrated in Figure 1 a).

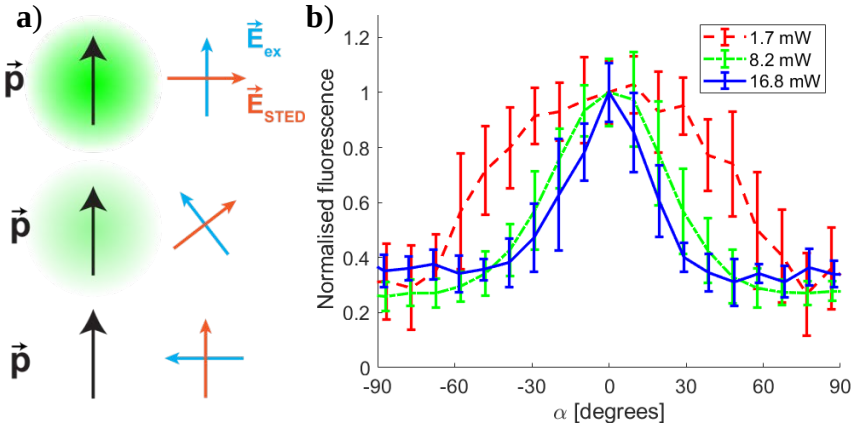


Figure 1: **a**) Illustration of (de)excitation scheme; **p** fluorophore dipole moment, $\mathbf{E}_{ex}$ excitation polarisation and $\mathbf{E}_{STED}$ depletion polarisation. **b**) Reduction of angular distribution of fluorescence of a single fluorophore as a function of STED power.

We applied this excitation/depletion scheme with a liquid nitrogen cryostat on a single molecule concentration spin coated ATTO 647N sample. Figure 1 b) shows the modulation of single molecule emitters averaged over multiple periods. At zero degree the molecular dipole orientation is aligned with the excitation polarisation and orthogonal to the STED polarisation. With increasing STED power the set of angles effectively fluorescing decreases leading to a narrow set of molecular dipole orientations that are imaged simultaneously. Currently we have achieved a FWHM of ~36 degrees at 16.8 mW of STED power. We are working towards improving the modulation depth and sharpness of the angular subset through understanding the electronic state transition pathways of fluorophores at cryogenic temperatures under intense STED de-excitation.

[1] C.N. Hulleman, W. Li, I. Gregor, B. Rieger, J. Enderlein, ChemPhysChem, 19:1774-1780, 2018
[2] C.N. Hulleman, M. Huisman, R.J. Moerland, D. Grünwald, S. Stallinga B. Rieger, Small Methods, 1700323, 2018