

## Testing imaging conditions for STED microscopy with exchangeable protein labels

### Motivation

Stimulated emission depletion (STED) microscopy is an optical super-resolution microscopy technique that provides sub-diffraction spatial resolution and high temporal resolution. There are many factors that affect the spatial resolution in STED microscopy, such as pinhole size, excitation laser intensity, STED laser intensity, pixel size, accumulation and dwell time. Using exchangeable dyes as labels for STED microscopy significantly reduces photobleaching and are based on the principles of point accumulation for imaging in nanoscale topography (PAINT; e.g. membrane stains such as Nile Red) and DNA-PAINT (using DNA-barcoded antibodies and weak-affinity duplex formation).

### Task Description

The stable U2OS cell line containing Halo Tag labeled vimentin will be used in this project. Your first task will be to label proteins such as TOM20 (mitochondria), alpha-tubulin (cytoskeleton) and KDEL (endoplasmic reticulum) with DNA docking strands-labeled antibodies. You will apply the principles of exchange-PAINT and DNA-PAINT for STED imaging. As different labels require different imaging conditions, your main task will be to find the best imaging conditions for different cellular structures to obtain high-resolution STED images. The image quality will be evaluated by analyzing the spatial resolution.

### Key References

- Spahn, C.K., Grimm, J. B., Lavis, L. D., Lampe, M., & Heilemann, M. (2019). Whole-Cell, 3D, and Multicolor STED Imaging with Exchangeable Fluorophores. *Nano Letters* 19(1), 500-505.
- Spahn C, Hurter F, Glaesmann M, Karathanasis C, Lampe M & Heilemann M (2019) Protein-specific, multi-color and 3D STED imaging in cells with DNA-labeled antibodies. *Angew Chemie*, DOI 10.1002/anie.201910115.

### Work Area

Laboratory	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Microscopy	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Data Analysis	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Programming	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

### Time

#### Possible Start

Summer 2022

#### Duration

4 Weeks

### Contact

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Language English