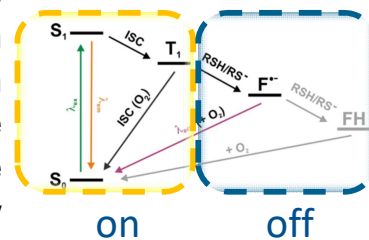


Establishment of different dSTORM fluorophores

Motivation

Different single-molecule localization microscopy methods exist to circumvent the diffraction limit. One of these methods is the direct stochastic optical reconstruction microscopy (dSTORM). This approach uses photoswitchable fluorescent dyes and can visualize cellular structures with near-molecular resolutions. However it comes with some drawbacks: The photophysical behaviour of the fluorophores reacts very sensitively to the measurement environment. Imaging buffers and excitation lasers have to be adjusted very precisely. And if several structures are to be examined at the same time, a new fluorophore with different spectral characteristics must be used and calibrated for each of them.



Task Description

The goal is to improve dSTORM measurements and enable multi-color pictures with different fluorophores. Therefore microtubules will serve as a model system. We will immunostain this structure in U2OS cells with different fluorophores known from the literature and measure dSTORM on a TIRF setup. Afterwards, we will analyse the measurements with the Picasso software and evaluate them with regard to resolution and photophysics. By varying different imaging buffers and excitation lasers, they will be established in the working group.

Key References

1. Dempsey, G. T., Vaughan, J. C., Chen, K. H., Bates, M., & Zhuang, X. (2011). "Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging" *Nature methods*, 8(12), 1027–1036. <https://doi.org/10.1038/nmeth.1768>
2. M. S. Dietz and M. Heilemann, "Analysis of sparse molecular distributions in fibrous arrangements based on the distance to the first neighbor in single molecule localization microscopy" *Nanoscale*, 2019, 11, 17981—17991 <https://doi.org/10.1039/C9NR06364A>

Work Area

Laboratory	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input 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 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
April-May 2022

Duration
4 weeks

Contact

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Language