Imaging and tracking of single molecules with exceptional spatial and temporal resolution by MINFLUX microscopy

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Imaging single molecules at localization precisions of below 2 nm and being able to follow their positions with a temporal resolution of >10 kHz has always been on the wish list of many scientists among various fields of research. MINFLUX microscopy enables the localization of a single molecule by reading out the fluorescence signal at pre-defined points in its vicinity. Using a central minimum in the excitation beam the emitted fluorescence is kept minimal allowing MINFLUX to achieve its localization with a very small number of photons and consequently within a spatio-temporal regime exceeding other alternative techniques [1].

We report on imaging results of various biological samples with localization precisions down to a few nm performed on a novel optical MINFLUX setup based on a commercial microscope stand.

In single particle tracking by means of fluorescence the limited photon budget and emission frequencies put constraints on the achievable tracking performance [2]. Here, MINFLUX plays out its strength because it uses photons very efficiently.

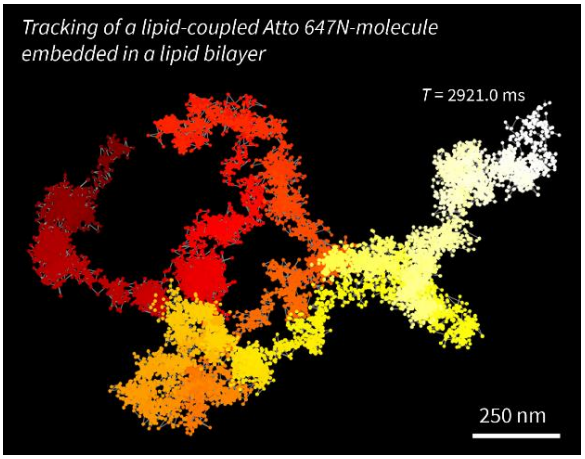

Figure 1: Tracking of a single Atto 647N molecule coupled to a lipid in a supported lipid bilayer.

We show single fluorophore tracks on supported lipid bilayers recorded with thousands of localizations acquired over several seconds with an extraordinary temporal and spatial resolution.

The results point towards MINFLUX's great potential for future spt experiments, e. g. tracking of proteins on cell membranes using fluorescent markers or tracking of fluorescent proteins in living cells, keeping the disturbance of the endogenous cellular environment at a minimum.

[1] Balzarotti ET AL. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science **355**, 606–612 (2017).
[2] Carlo Manzo and Maria F Garcia-Parajo. A review of progress in single particle tracking: from methods to biophysical insights. Rep. Prog. Phys. **78** 124601 (2015).