Exploiting Fluorescence Lifetime in Pulsed Interleaved MINFLUX for Colocalization and Energy Tansfer Experiments

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Super-resolution microscopy techniques like STED and STORM/PALM have revolutionized the use of optical microscopes to study biological systems [1] as well as polymers and anorganic nanoparticles [2] revealing structures well below the diffraction limit.
One of the latest developments in super-resolution microscopy that increased the optical precision to the limit of the label size is MINFLUX [3] which we recently extended with pulsed interleaved excitation (p-MINFLUX) [4] to make the fluorescence lifetime information accessible. Both, the nanoscale position localization resolution of ~1nm as well as the nanosecond fluorescence lifetime is obtained in p-MINFLUX [4].
Here, we show applications of p-MINFLUX in different DNA origami based model systems where the fluorescence lifetime can be used as a parameter for super-resolution multiplexing at distances without direct dye-dye interaction. Below these distances nanometer-resolution fluorescence lifetime imaging can be applied to track energy transfer pathways between a donor-acceptor dye pair (smFRET) as well as between a fluorescent dye and an energy accepting surface, e.g. graphene. This enables super-resolution in the third dimension [5] and in combination with p-MINFLUX it enables an isotropic optical resolution of ~1-2 nm.

References

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