Pulsed-Interleaved MINFLUX as a New Implementation for Super-Resolved Localization of Single Molecules

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Super-resolution microscopy techniques like STED and STORM/PALM have revolutionized the use of optical microscopes to study biological systems at dimensions well below the diffraction limit [1]. The latest development in super-resolution is MINFLUX and combines the stochastic and deterministic nature of the basic techniques. The nanoscale localization resolution to ~1nm is obtained in MINFLUX with a lower number of photons by optimizing the information gained by the detected photons via spatially patterned illumination [2]. Here, we introduce a novel implementation of MINFLUX, combining spatial point spread function engineering with pulsed interleaved excitation to reach a localization precision down to ~1nm for the emitter with an increased temporal resolution. This simple and robust implementation is based on time-correlated single-photon counting, hence it is fully compatible with fluorescence life-time imaging. We will present the basic principle of the setup and first results showing the localization performance for single-molecules as well as how to follow a DNA acrobat [3] walking across a DNA-origami surface.

References

[1] S. J. Sahl et al, *Nat. Rev. Mol. Cell Bio.* (2017) **18**, 685

[2] F. Balzarotti et al., *Science* (2017) **355**, 606

[3] J. Li et al., *Nat. Nanotechnol.* (2018) **13**, 723