

PULSED INTERLEAVED MINFLUX

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Super-resolution has revolutionized the power of optical microscopes to study biological systems at resolutions well below the diffraction limit [1]. Among the different techniques, MINFLUX nanoscopy [2] allows to achieve molecular-scale resolution (~ 1 nm) by optimizing the information contained in the detected photons with spatially patterned illumination.

Here, we introduce Pulsed Interleaved MINFLUX, p-MINFLUX [3], a new implementation of the highly photon-efficient single-molecule localization method with a simplified experimental setup

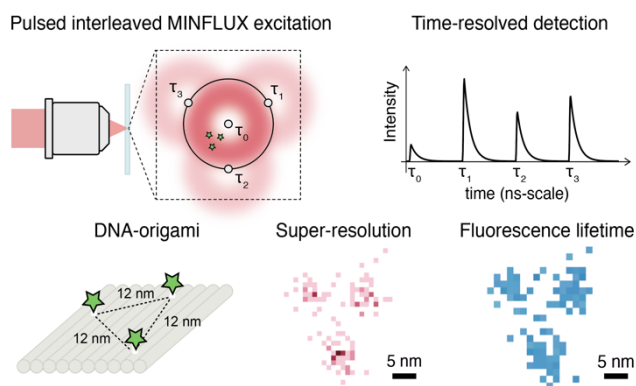


Figure 1. Scheme of p-MINFLUX working principle (top) and fluorescence lifetime nanoscale imaging results (bottom)

and additional fluorescence lifetime information. In contrast to the original MINFLUX implementation, p-MINFLUX uses interleaved laser pulses to deliver the doughnut-shaped excitation foci at a maximum repetition rate. Using both static and dynamic DNA origami model systems, we demonstrate the performance of p-MINFLUX for single-molecule localization nanoscopy and tracking, respectively. p-MINFLUX delivers 1–2 nm localization precision with 2000–1000 photon counts. In addition, p-

MINFLUX gives access to the fluorescence lifetime enabling multiplexing and super-resolved lifetime imaging. p-MINFLUX should help to unlock the full potential of innovative single-molecule localization schemes.

[1] S. J. Sahl et al, *Nature Reviews Molecular Cell Biology*, **18**, 685–701 (2017)

[2] F. Balzarotti et al, *Science*, **355**, 606–612 (2017)

[3] L. A. Masullo et al. “Pulsed Interlaved MINFLUX.” *Nano Letters*, **21**, 1, 840-846 (2021).