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 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.