

SIMFLUX: LOCALIZATION MICROSCOPY AT DOUBLED PRECISION

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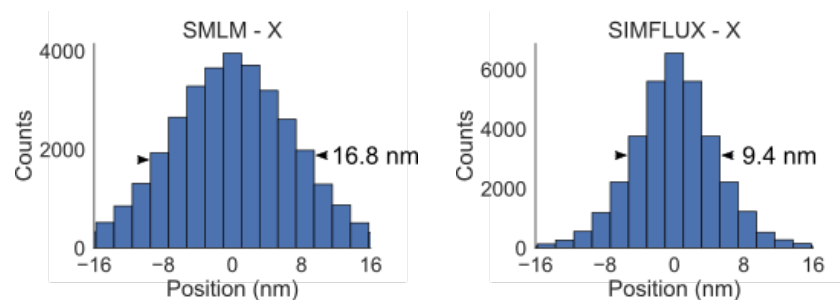
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Centroid estimation of single, isolated molecule fluorescence is one of the essential steps in localization microscopy to obtain resolutions below the diffraction limit [1]. Recently, MINFLUX introduced doughnut shaped illumination and triangulation as an alternative channel of information on the molecule's positions [2]. Breakthrough precisions have been realized compared to widefield illumination, but the technique suffers from a limited, nanometer sized, field-of-view and long acquisition times. Here, we introduce SIMFLUX, in which we combine centroid estimation and illumination pattern induced photon count variations in conventional widefield localization microscopy to extract position information of all molecules in parallel in a typical micron sized field-of-view. We introduce a sinusoidal illumination pattern that can be shifted and rotated on the millisecond scale, enabled by a novel polarization optics based architecture for the illumination branch. In this way three modulated intensities of a single molecule per dimension during a typical on-time of the stochastically switching fluorophores are recorded. The localization precision of SIMFLUX is :

$$\Delta x = \frac{\sigma}{\sqrt{N} \sqrt{1 + 2\pi^2 \sigma^2 / p^2}}$$

where N is the number of detected photons, σ the PSF width, and p the illumination pattern pitch. This results in on average twice better localization precision compared to standard Single-Molecule Localization Microscopy (SMLM). In the presentation we will highlight the underlying principles, present a performance benchmark with simulations, and show experimental results on DNA-origami samples.



Localization precision (FWHM) of SIMFLUX compared to standard SMLM on DNA-origami nano-rulers, indicating a twofold improvement factor.

[1] T. Klein, S. Proppert, and M. Sauer, *Histochem Cell Biology*, 141, 561-575, 2014.

[2] F. Balzarotti et al, *Science*, 355, 606-612, 2016.