Uniting Structured Illumination and Localization Microscopy (SIMFLUX)

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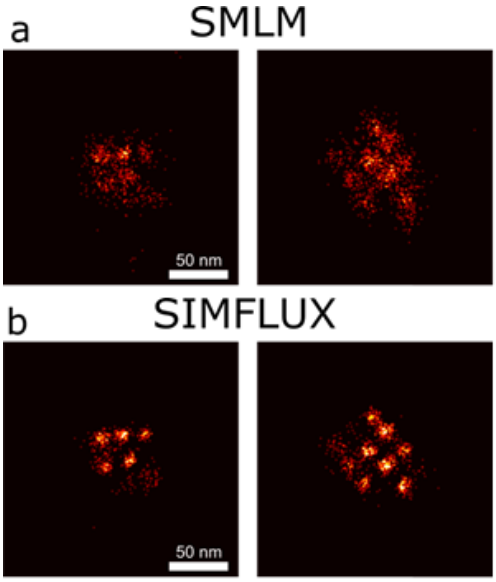
We have developed a novel localization microscopy methodology called SIMFLUX that increases localization precision more than twofold compared to the state-of-the-art localization microscopy. SIMFLUX combines fluorophore intensity measurements as done by MINFLUX [1] with the use of extended illumination patterns similar to those used in Structured Illumination Microscopy (SIM). We have done so by accounting for both the diffraction- and illumination patterns within the localization algorithm. The algorithm that we present is able to estimate the patterns from sparse single-molecule data and sequentially perform the localization using a maximum likelihood approach. We demonstrate our method on GATTAQUANT nano-rulers with 80 nm separations between three neighboring binding sites, as well as on DNA-PAINT [2] labelled biological samples. Our results show a factor of two improvement in localization precision of the binding site point-clouds when compared to Gaussian widefield localization microscopy.



Running SIMFLUX on DNA-PAINT nanorulers show a clear improvement in localization precision.compared to convential localization.

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