

## **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 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. Our results show a factor of two improvement in localization precision of the binding site point-clouds when compared to Gaussian widefield localization microscopy.