SIMFLUX: INVESTIGATION OF EXPERIMENTAL TOLERANCES FOR TWO-FOLD LOCALIZATION PRECISION IN LOCALIZATION MICROSCOPY WITH STRUCTURED ILLUMINATION

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Recently, a new concept called MINFLUX was introduced in which emitters are localized by probing with a local intensity minimum of excitation light [1]. In MINFLUX a doughnut illumination spot is shifted over an area, and the position of a single molecule in the scan range is determined by triangulation based on the photon count for different spot positions. Here we build upon this insight and introduce SIMFLUX – a combination of structured illumination (SIM) and single molecule localization microscopy (SMLM). Here we illuminate the sample with a series of shifted orthogonal sinusoidal intensity patterns.

To quantify how our proposed measurement scheme increases the photon efficiency regarding the estimation of an emitter position with respect to standard localization, we evaluate its Fisher information and Cramer-Rao Lower Bound (CRLB). Analytical expressions for the localization uncertainty are derived and we can understand it in terms of the width of the Point Spread Function (PSF) and the pattern pitch. For optimal conditions we show an improvement factor over standard SMLM precision [2] of around 2. We investigate this further for a number of experimental variables such as the modulation depth of the pattern, number of exposures, and arrangement of the patterns. For non-zero background and for non-zero-pixel size of the camera we adopt a numerical simulation to study effects on the localization precision.

In this simulation study we use a full vectorial PSF [3] and show that our method achieves the CRLB for a wide range of photon counts and background levels (see Fig. 1). It further reveals that background has the same relative impact as in standard SMLM, implying that SIMFLUX can be used under the same experimental conditions as SMLM. The tolerances for imperfect illumination pattern modulation and the sinusoidal phase and pitch errors are also assessed.

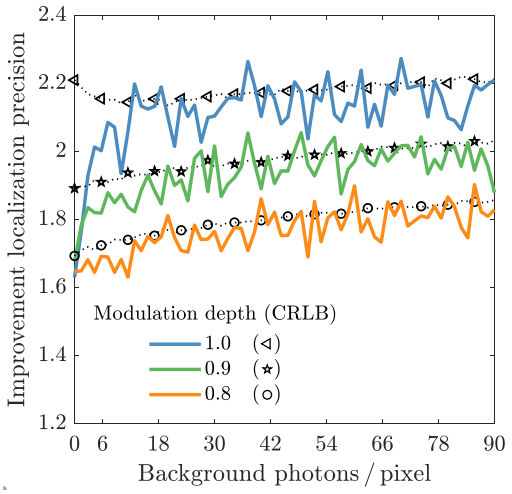

Fig. 1 Improvement in the localization precision of SIMFLUX over SMLM as a function of background level for different pattern modulation. Number of patterns is 6 with a total of 6000 signal photons.

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