VIDEO-RATE NANOscopy of Stochastically Switching Molecules Using SCMOS-Specific Localization Algorithms

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Nanoscopy techniques that utilize stochastic switching to achieve precise and accurate localization of a large number of single molecules achieve ~25 nm spatial resolution, far beyond the diffraction limit. However, the time resolution of these techniques is limited to typically seconds to minutes due to the necessity of accumulating large numbers of localization estimates [1].

Two of the major limitations are the acquisition speed and the photon budget. Recently introduced sCMOS cameras allow much faster acquisition speeds and significantly higher effective quantum efficiencies than the usually used EMCCD cameras. However, the intrinsic pixel-dependent noise of these cameras causes artifacts in localization and therefore greatly reduces the reliability of the results.

Here, we present video-rate stochastic switching nanoscopy of fixed and living cells at 32 super-resolution images per second. Using a set of novel statistics-based algorithms specifically designed for sCMOS cameras allows us to perform unbiased and precise localization analysis with sCMOS data. We demonstrate that this method achieves Cramer-Rao lower bound-limited single molecule localization. Combining this approach with a recently developed multi-emitter fitting algorithm [2] and optimized imaging condition, we show that this technique shortens the typical acquisition time by up to two orders of magnitude without compromising the field of view.

The here presented method allows to replace EMCCD cameras with SCMOS technology and record faster and more precise super-resolution images without compromises.

Disclaimer: J.B. declares financial interest in Vutara Inc., a start-up company producing a fluorescence microscope utilizing 3D particle localization.