A FLEXIBLE AND COMPUTATIONALLY EFFICIENT APPROACH FOR ACCURATE EMITTER LOCALIZATION IN SCMOS CAMERA DATA

Ruisheng Lin[1], Alex Clowsley[1], Isuru Jayasinghe[2], David Baddeley[3], Christian Soeller[1]
[1]Living Systems Institute & Biomedical Physics, University of Exeter, United Kingdom
[2]School of Biomedical Sciences, University of Leeds, United Kingdom
[3]Department of Cell Biology, Yale University, USA
E-mail: C.Soeller@exeter.ac.uk

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Scientific-CMOS (sCMOS) cameras have become increasingly popular for single molecule localization microscopy (SMLM) due to advantages that include high speed, effective quantum efficiency, and large field of view. The main drawback of sCMOS cameras are the non-uniform, camera-specific pixel characteristics which may introduce location-specific bias and degrade the precision in the localization of single molecule fluorescence. Previous work has suggested that applying algorithmic correction which explicitly takes into account the pixel dependent properties can eliminate bias and recover the localization precision [1]. Such algorithms, whilst efficient in 2D localization, require significant algorithmic re-derivation when adapting to different point spread function (PSF) models. Extending the published algorithm beyond 2D localization to tasks such as PSF model based 3D localization [2, 3] or multi-emitter fitting is extremely difficult, partly because an analytical derivative of those types of PSF is often difficult or even impossible to obtain.

We have quantitatively investigated the effect of non-uniform pixel characteristics on the localization of single emitters using a simulation framework. The simulations allowed characterising the conditions where algorithmic correction of sCMOS properties is either critical or essentially superfluous. These conditions include several real-life scenarios where existing ‘uncorrected’ algorithms may be used without compromising localisation properties, despite sCMOS non-uniformities. For scenarios where correction is required we develop an alternative, computationally efficient sCMOS noise model and fitting procedure that restores the localisation properties to that of an equivalent uniform sensor. Our method attains the Cramer-Rao lower bound and can easily be adapted to existing molecule localization schemes, including PSF model based 3D localization and multi-emitter fitting.

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