Photon-free characterization of CMOS cameras removes bias from SMLM measurements

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We propose an approach for photon-free characterization of CMOS cameras for gain, offset, noise and dark current in each pixel. We show that this approach can be effectively applied to experimental single-molecule localization microscopy (SMLM) data and specifically DNA-PAINT data where it removes localization bias.

Complementary metal-oxide semiconductor (CMOS) cameras have evolved into a preferred camera architecture. Such detectors typically feature pixel-to-pixel variations in terms of noise, offset and gain. When being applied to single molecule localization microscopy (SMLM), this effect can for instance impair the localization precision as well as bias the localization coordinates [1, 2]. An appropriate characterization of the camera allows the reconstruction software to circumvent this effect and restore the theoretically achievable uncertainties. So far, the pixel-to-pixel variations in terms of dark current and thermal noise are often neglected. However, the localizations can be considerably biased in the vicinity of pixels featuring relatively high dark current. For measurements at long exposure times such as DNA-PAINT, this bias can well exceed the localization precision.

We demonstrate an approach that uses thermally generated electrons at different exposure times to characterize each camera pixel. The dark current and the associated thermal noise can be used for a complete camera characterization including offset, noise and gain. To this end, we propose a photon-free calibration scheme. This approach allows for similar data processing as in the traditional approach of using photoelectrons, but without the need of external stimuli. The thermally generated signal particularly plays a role for uncooled industry-grade CMOS cameras that are recently gaining popularity in the SMLM community [3, 4, 5]. Comparison to scientific-grade cameras has proven that only little compromise has to be made in terms of image quality for SMLM [6, 7]. At the same time, industry-grade devices offer easily applicable solutions at significantly reduced cost as compared to scientific-grade detectors.

We discuss the entire pipeline of an automated industry-grade CMOS camera characterization and how it is effectively used to remove the localization bias in experimental DNA-PAINT data. We furthermore demonstrate that our approach can be similarly applied dSTORM and PALM.

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[2] R. Lin et al. Optics Express 25, 11701-11716 (2017)
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[4] H. Babcock. Scientific Reports 8, 1726 (2018)
[5] R. Diekmann et al. Scientific Reports 7, 14425 (2017)