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sCMOS noise correction algorithm for microscopy images

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Scientific complementary metal-oxide semiconductor (sCMOS) cameras are rapidly gaining popularity in biological sciences, material sciences and astronomy. The sensor provides significant advances in imaging speed, sensitivity and field of view compared with traditional detectors such as charge-coupled device (CCD) or electron multiplying CCD (EMCCD)[1,2]. However, it introduces pixel-dependent noise — each pixel has its own noise statistics, primarily offset, gain and variance. Left uncorrected, this sCMOS-specific noise generates imaging artifacts and biases in quantification[3]. Previously, specific for single molecule studies, a suite of algorithms was developed to characterize this noise in each pixel and include the noise statistics in the single molecule localization algorithm[3]. However, this correction works exclusively on images with point objects such as in particle tracking or single molecule switching nanoscopy. A more general algorithm that works on conventional microscopy images does not exist to this date. Here we developed an algorithm that dramatically reduces sCMOS noise from microscopy images with arbitrary structures. We

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Data Availability statement

A sub-stack of unprocessed data used in generating Supplementary Fig. 3 and Supplementary Video 1 is included in Supplementary Software. Additional data that support the findings of this study is available from the corresponding author upon request.

Code availability

The developed software package is available at https://github.com/HuanglabPurdue/NCS and in the Supplementary Software.

Contributions

S.L., Z.H. and F.H. conceived the project. M.J.M. built the microscope setup. S.L., Z.H. and F.H. developed the algorithm. M.J.M., Y.R., K.M., D.M.S. and F.H. designed the biological imaging samples. S.L. and M.J.M. performed the COS-7 cell experiments. S.L., M.J.M., Y.R. and K.M. performed the neuronal cell experiments. S.L. wrote the software and performed the simulation and analysis. All authors wrote the manuscript.

Competing financial interests

S.L. and F.H. are co-inventors on a patent application related in part to the material presented here.

show that our new method corrects pixel-dependent noise in fluorescence microscopy using an sCMOS sensor, allowing its performance to approach that of an ideal camera.

The fundamental challenge for sCMOS noise correction is to estimate one of the two variables knowing their sum: each pixel from sCMOS camera gives a digital count representing the sum of two variables, given by photo-electrons and readout noise which we consider to follow Poisson and Gaussian distributions respectively[3]. In the case of detecting point emitters, our extra knowledge is that the photoelectrons form a diffraction-limited spot, modeled, for example, as a Gaussian. Therefore, in spite of the pixel-dependent noise, we demonstrated that the sCMOS-specific maximum-likelihood estimator extracts molecular centers with precision at the theoretical limit[3]. With arbitrary structures, the assumption of single emitters, however, is lost.

Our target is to develop a generalized noise correction algorithm by exploiting the common property of microscopy images: the optical transfer function (OTF). The amplitude of the OTF, defined by the microscope's numerical aperture and the wavelength of detection, dictates the frequency response limit of a microscope system[4,5]. Optical signal from the sample exists only within the frequency limit while outside of this limit lies only the contribution from noise (Fig. 1a and Supplementary Notes 1-5). Assuming of independent readout noise, we focus on minimizing the noise contribution while maximizing the likelihood of our image estimate to recover the underlying signal buried under the readout noise (Fig. 1, Supplementary Fig. 1 and Supplementary Notes 6-8). To this end, we first extract the noise contribution of an image in Fourier space outside or near the theoretical OTF periphery, a conservative estimate of the effective cutoff frequency of a practical system (Supplementary Note 9). Then, based on the sCMOS noise model including the pixel-dependent offset, gain and variance (see Supplementary Note 10 for sensors with multiple readout units per pixel), we calculate the likelihood function for the entire image. By minimizing the sum of the noise contribution in Fourier space and the negative-log-likelihood, we obtain the noise corrected image (Fig. 1b and Supplementary Fig. 1). We find that the pixel-dependent noise is, to a large extent, undetectable in the recovered image (Fig. 1b-e and Supplementary Figs. 2-3 and Supplementary Video 1) and quantification based on the likelihood function shows that the corrected image closely approaches the ideal one (see Supplementary Note 11 on effects of camera sampling rate). Intensity trace comparisons in fluorescence microscopy images of peroxisome membrane protein and end-binding proteins (EB3) both tagged with tdEos, and the time series of F-actin tagged with SiR-actin, show a significant reduction of pixel fluctuation while keeping the original signal level intact (Supplementary Figs. 2-3 and Supplementary Videos 1-2 and Supplementary Methods). Because our algorithm combines the noise and the likelihood for minimization, it minimizes the noise fluctuation while maintaining the underlying expected photon count and resolution of the image (Supplementary Figs. 2 and 4-6 and Supplementary Notes 12-14). To demonstrate the correction over the entire field of view, we calculated the temporal fluctuation of individual pixels from a time series. We noticed that the high readout noise pixels, the hallmark feature of sCMOS images, are absent throughout the entire field of view (Fig. 1c and d and Supplementary Figs. 2, 3 and 6).

The developed algorithm (Supplementary Software and Supplementary Note 15) can generally be applied to sCMOS based detection and quantitative analysis in a broad spectrum of microscopy techniques, for example, light sheet microscopy, total internal reflection fluorescence microscopy, fluorescence resonance energy transfer microscopy, speckle microscopy, wide-field calcium imaging, as well as conventional fluorescence imaging. The fundamental principle can be applied to other fields where a maximum cutoff frequency exists such as astronomy and photonics. We hope that these fields can now benefit from the increased quantum efficiency, field of view and imaging speed of sCMOS cameras without compromising its quantitative nature of detection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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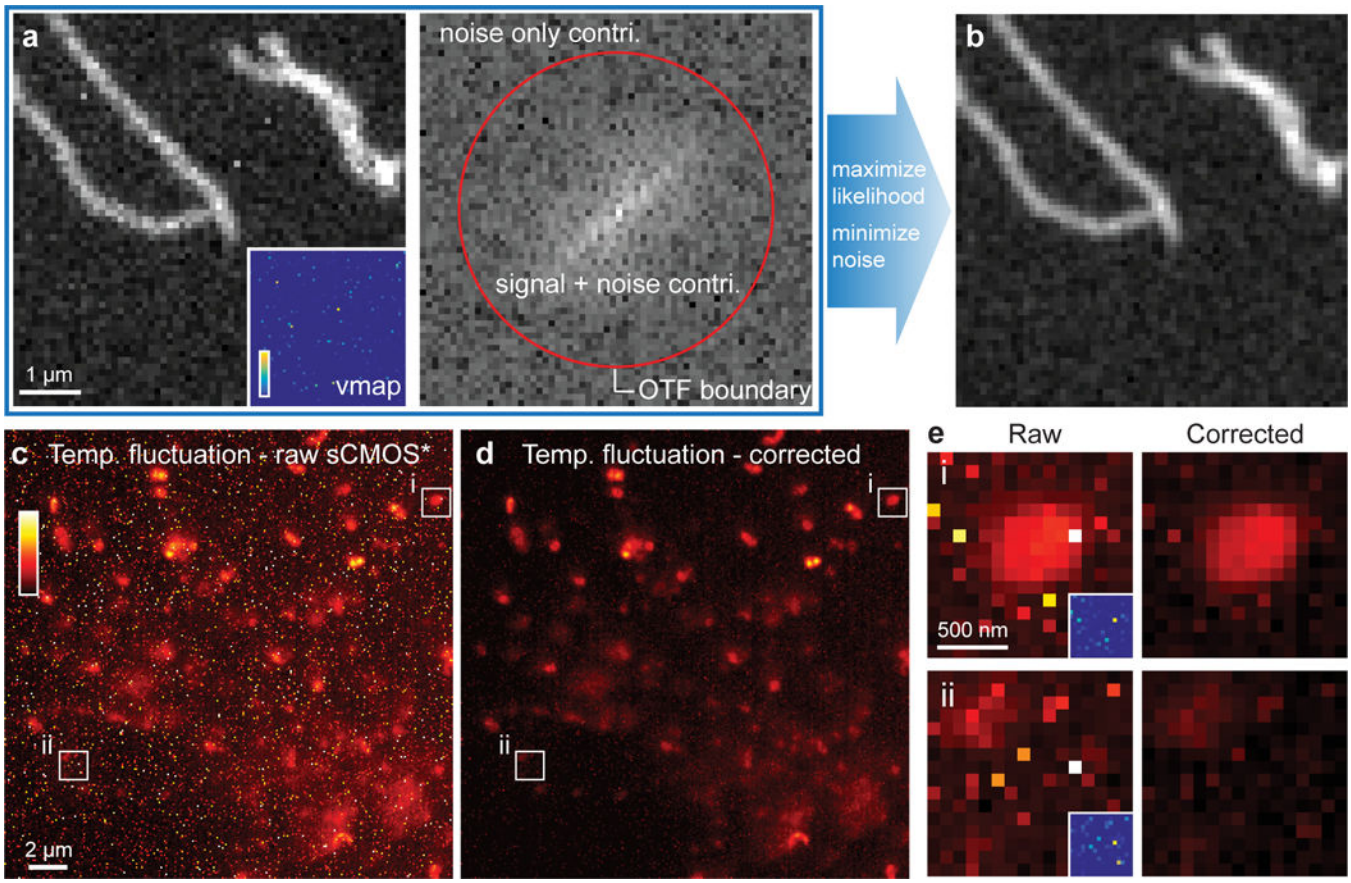

Figure 1. Concept and results of noise correction algorithm for sCMOS camera

(**a**) Simulated raw sCMOS image and its components in Fourier space. The inlet image shows the variance map of the readout noise. The image in Fourier space consists of contributions from both noise and signal. Colormap of variance map linearly scales from 2.8 to 2000 ADU^2 (camera count squared). (**b**) The noise corrected image of the sCMOS image in **a**. (**c**) Temporal pixel fluctuation map (standard deviation in each pixel over time) over 400 sCMOS frames from experimental data. Color map indicates low (standard deviation: 1.5 ADU) to high (standard deviation: 8 ADU) temporal fluctuation per pixel. (**d**) Temporal pixel fluctuation map over 400 noise corrected images of the sCMOS frames in **c**. (**e**) Zoom-in images of selected sub-regions **i** and **ii** in **c** and **d**. The inlet images are the variance maps of the corresponding sub-regions showing the correlation with pixels with high temporal fluctuation in raw sCMOS frames. *****Raw sCMOS frames are corrected by sCMOS gain and offset maps to facilitate visual comparison.