

Abstract Submitted  
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**“Calibration-on-the-spot”: How to calibrate an EMCCD camera from its images**<sup>1</sup> KIM I. MORTENSEN, HENRIK FLYVBJERG, Department of Micro- and Nanotechnology, Technical University of Denmark — In localization-based microscopy, super-resolution is obtained by analyzing isolated diffraction-limited spots imaged, typically, with EMCCD cameras. To compare experiments and calculate localization precision, the photon-to-signal amplification factor is needed but unknown without a calibration of the camera. Here we show how this can be done *post festum* from just a recorded image. We demonstrate this (i) theoretically, mathematically, (ii) by analyzing images recorded with an EMCCD camera, and (iii) by analyzing simulated EMCCD images for which we know the true values of parameters. In summary, our method of *calibration-on-the-spot* allows calibration of a camera with unknown settings from old images on file, with no other info needed. Consequently, *calibration-on-the-spot* also makes future camera calibrations before and after measurements unnecessary, because the calibration is encoded in recorded images during the measurement itself, and can at any later time be decoded with *calibration-on-the-spot*.

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