Direct experimental comparison of sCMOS and EMCCD cameras for localization microscopy quantitatively analyzing the interaction of reconstruction algorithms and camera noises

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Scientific CMOS (sCMOS) cameras offer advantages for super resolution microscopy, including a large field of view (4 MP) with high frame and data rates, enabling high speed, high throughput and large area localization nanoscopy.

While papers both theoretically and experimentally demonstrate the expected performance and advantages of sCMOS for localization microscopy [1 – 4], there have not been detailed experimental comparisons of EMCCD and sCMOS cameras for localization microscopy showing the interactions of reconstruction algorithms and camera noises. EMCCD and sCMOS cameras have qualitatively different noise characteristics, specifically signal intensity dependent electron multiplication noise (“excess noise”) for EMCCDs and pixel-dependent noises such as offset, read noise and gain for sCMOS cameras.

To study the interactions of technology-specific camera imperfections and algorithms, we acquired images of beads simultaneously using both a scientific CMOS and EMCCD camera. Identical image sets from both cameras were then reconstructed using several algorithms and the results quantitatively evaluated as a function of brightness of the fluorophores. Four algorithms were analyzed: i) MrSE [5], an algebraic, model-independent utilizing radial symmetry, ii) MaLiang [1], a maximum likelihood estimator using a pure Poisson noise mode, iii) MLE_EMCCD, a maximum likelihood method incorporating a detailed model of the EMCCD electron multiplication noise, and iv) MLE_sCMOS [3], a maximum likelihood estimator including a camera-specific pixel read noise.

Results show that i) at lower light levels, using the appropriate camera-specific algorithm MLE (for EMCCD cameras) or MLE_sCMOS provides the best localization precision ii), surprisingly, while EMCCD electron multiplication noise (“excess noise”) reduces the localization precision over that expected from photoelectron shot noise alone, the localization precision is the same whether reconstructed using a pure Poisson MLE or MLE_EMCCD, and iii) for brighter fluorophores, incorporation of camera-specific noises into the algorithm does not improve localization precision, although MLE algorithms generally provide the best precision.