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Camera Simulation Engine Enables Efficient System Optimization for Super-Resolution Imaging

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ABSTRACT

Quantitative fluorescent imaging requires optimization of the complete optical system, from the sample to the detector. Such considerations are especially true for precision localization microscopy such as PALM and (d)STORM where the precision of the result is limited by the noise in both the optical and detection systems. Here, we present a Camera Simulation Engine (CSE) that allows comparison of imaging results from CCD, CMOS and EM-CCD cameras under various sample conditions and can accurately validate the quality of precision localization algorithms and camera performance. To achieve these results, the CSE incorporates the following parameters: 1) Sample conditions including optical intensity, wavelength, optical signal shot noise, and optical background shot noise; 2) Camera specifications including QE, pixel size, dark current, read noise, EM-CCD excess noise; 3) Camera operating conditions such as exposure, binning and gain. A key feature of the CSE is that, from a single image (either real or simulated "ideal") we generate a stack of statistically realistic images. We have used the CSE to validate experimental data showing that certain current scientific CMOS technology outperforms EM-CCD in most super-resolution scenarios. Our results support using the CSE to efficiently and methodically select cameras for quantitative imaging applications. Furthermore, the CSE can be used to robustly compare and evaluate new algorithms for data analysis and image reconstruction. These uses of the CSE are particularly relevant to super-resolution precision localization microscopy and provide a faster, simpler and more cost effective means of system optimization, especially camera selection.
Keywords: Camera Simulation Engine (CSE), Scientific CMOS, EM-CCD, localization microscopy, super-resolution imaging

1. INTRODUCTION

Quantitative fluorescent imaging requires optimization of the complete optical system, from the sample to the detector. Such considerations are especially true for precision localization microscopy such as PALM and (d)STORM where the precision of the result is limited by the noise in both the optical and detection systems. The noise of the system is ruled by the following parameters: 1) Sample and imaging conditions including optical intensity, wavelength, optical signal shot noise, and optical background shot noise and optical system magnification ; 2) Camera specifications including QE, pixel size, dark current, read noise, EM-CCD excess noise; 3) Camera operating conditions such as exposure time, binning and gain. Optimization of the physical optical imaging systems for specific applications requires a lot of time and is not always practical. On the other hand, accurate simulations of digital image data from cameras for realistic scenarios provides a faster, simpler and more cost effective means of system optimization, including camera selection in precision localization microscopy and other visual and computational imaging applications.

2. CAMERA SIMULATION ENGINE

2.1 Camera Image Generation and Noise Modeling

Our camera simulation engine (CSE) generates digital images that are statistically realistic representations of the digital images which would be obtained from actual cameras. The main inputs of the camera simulation engine are the "ideal" image, which is the image of the object that would be obtain in the absence of *any* noise sources, including photon shot noise and the camera specifications.

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These inputs are then used to generate one or more images that closely represent the actual images acquired with a camera – in other words, the simulation engine includes a realistic statistical model of the noise sources.

Noise in the measurement of optical intensity arises from two distinct sources:

1) Photon physics: photon shot noise, a Poisson distribution
2) Camera characteristics
    a) QE: photons incident on the camera are converted into photoelectrons. As both photons and electrons are quantized, the conversion process is characterized by a binomial distribution
    b) N_r: electronics that convert photoelectrons into digital signals adds noise, usually Gaussian
    c) F_n: EM-CCDs use a many (> 100) stage multiplication process with a small ($g-1 << 1$) gain (g) at each stage to multiply the number of photoelectrons. This process is stochastic, and characterized by a multi-stage binomial distribution, which adds noise, termed "excess noise". In an EM-CCDs operating at typical gains, the excess noise broadens the standard deviation of the output signal[1] by the $\sqrt{2}$, which has the same effect on the pixel signal to noise ratio as reducing QE by 50%. In contrast, CCD and sCMOS detectors have no excess noise ($F_n=1$).

The camera simulation engine (CSE) using MATLAB that we developed generates images with noise distributions in accordance with these models.

2.2 Validation of the Camera Simulation Engine

To validate our CSE, we compared real captured images and simulated images for an EM-CCD and a Gen II sCMOS camera. We imaged 200 nm diameter fluorescent beads with a bandpass filter near 510 nm wavelength. Fig. 1 shows the "ideal optical distribution" used for the simulations, and was generated by averaging 100 captured images with long exposure time using a Gen II sCMOS camera (ORCA-Flash4.0, Hamamatsu Photonics). The combination of the low read noise of the ORCA-Flash4.0, long integration time and averaging provides an input image for the simulation engine with negligible noise. To compare the performance of different cameras, images of 200 nm diameter fluorescent beads were simulated for the optical system and camera specifications shown in Table 1. Individual images obtained with the cameras for various exposure times and the corresponding simulated images are shown in Fig. 2, which shows a 100 x 100 pixel region of image so that the noise properties can be visualized more clearly. The photon intensity in the beads are approximately 4, 14, 42 photons / pixel for exposure times of 3.3, 11, and 33 ms respectively. At very low photon intensities, the differences in the noise properties of the two cameras are clearly shown in the figures. For the ORCA-Flash4.0, black and white pixels are visible in a gray background (2A, 2B). For the EM-CCD (ImagEM, Hamamatsu Photonics), white pixels are visible in a black background (2G, 2H). Increasing the number of photons through longer exposure times, results in relatively smaller noise (2C, 2I). Simulated images calculated using the "ideal" image shown in Fig. 1 are shown in Fig. 2D-2F for the ORCA-Flash4.0, and 2J-2L, for the EM-CCD. Visually, each simulated image is very similar to the corresponding real images demonstrating the validity of the CSE.

Table 1. Specifications and measurement conditions for each camera

Camera	Gen II sCMOS (ORCA-Flash4.0) (Hamamatsu Photonics)	EM-CCD (ImagEM) (Hamamatsu Photonics)
Pixel size [μm x μm]	6.5 x 6.5	16 x 16
QE @ 510nm	65 %	91 %
Read noise [e^-]	1.3	100
Optical Magnification	100	100 x 2.5 (with relay lens)
EM Gain		1000
Exposure time [ms]	3.3, 11, 33	

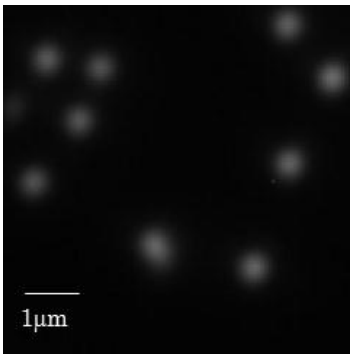

Figure 1. "Ideal" input image for simulation. This image is made by averaging 100 images captured with long exposure time by the ORCA-Flash4.0, and has negligible noise.

3. CAMERA OPTIMIZATION FOR LOCALIZATION MICROSCOPY

Noise in the measurement of the optical intensity is a significant factor determining the localization precision of localization microscopy. Although EM-CCD cameras have traditionally been used for localization microscopy due to their very low input-referred read noise (<1 e-) and high apparent quantum efficiency (QE), excess noise arising from the electron multiplication process broadens the statistical distribution of the camera digital output[1] significantly reducing localization precision[2,3]. Excess noise in EM-CCDs has roughly the same impact on reconstructed images as reducing the quantum efficiency by a factor of 2. On the other hand, CMOS camera achieves both low read noise and high frame rate simultaneously without the excess noise of EM-CCDs. Huang *et al.*[4] demonstrated the feasibility of using Gen I sCMOS cameras for localization microscopy. In this paper, we use the CSE to compare the expected performance of Gen II sCMOS cameras with EM-CCDs for localization microscopy.

3.1 Simulation Model and Conditions

Fig. 3A shows the simulation sample structure consisting of line pairs whose intervals are 20, 25, 30, 35, 40, 45, 50 and 55 nm. This sample structure enables easy comparison of simulated super resolution images at a glance. To avoid images with overlapping fluorescence patterns, each frame is simulated with only a single molecule (Fig. 3B). The intensity of each fluorophore was set to a total mean of 500 collected photons, which corresponds to a maximum intensity on the camera of 87 photons in a single pixel (Fig. 3C). The model also includes optical background, typically caused by non-specific fluorescence or stray light. The CSE allows both fluorophore strength and optical background to be easily changed.

3.2 Simulated Results

The single molecule fluorescence images created by our CSE for the sCMOS camera and EM-CCD cameras with specifications given in Table 2 are shown in Fig. 4A, 4B, These images include a uniform optical background of 43.5 mean photons / pixel frame, which corresponds to a value of ½ of the peak intensity from a single molecule. A subset of the corresponding reconstructed super resolution images (30, 35, 40, 45nm spacing line pairs) is shown (Fig. 4C and 4D). MaLiang[5], a maximum likelihood reconstruction algorithm provided courtesy of Prof. Zhen-li Huang was used for the reconstruction. Visually, the line pairs in the images of ORCA-Flash4.0 (Fig. 4D) are more clearly separated than those of the EM-CCD (Fig. 4C). For a more quantitative comparison, we obtained the integrated line profiles by summing image intensity along long axis of the line pair (Fig. 5). The profiles shows that the line pairs of ORCA-Flash4.0 are more clearly separated than that of EM-CCD. Since we know the true position of each emitting molecule, we can readily calculate localization precision (Fig. 6) for a several values of the temporal mean of the optical background. It is clear that the ORCA-Flash4.0 shows better precision than the EM-CCD.

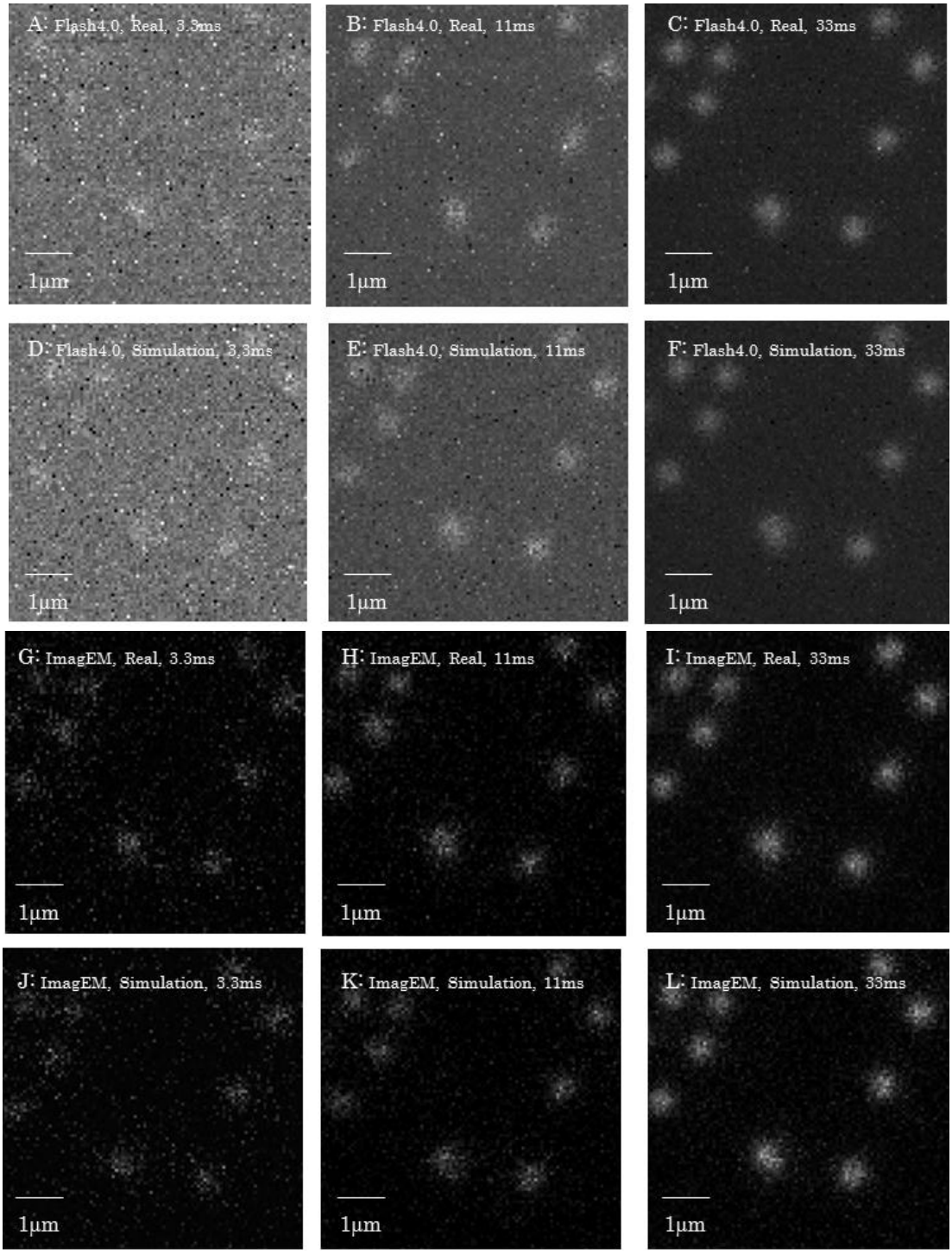

Figure 2. Comparison between real images and simulated images of 200 nm diameter fluorescent beads (510 nm bandpass filter). Each image is 100 x 100 pixels, and each pixel corresponds to 65 nm (ORCA-Flash4.0) or 64 nm (EM-CCD) at the sample. Real images (A, B, C) captured by the ORCA-Flash4.0; Corresponding simulated images are shown in (D, E, F); Real images captured by ImagEM under the same conditions are shown as (G,H,I) along with the corresponding simulations (J,K,L). Exposure times are shown on each image (3.3, 11 and 33 msec). To improve visualization, the look-up table for grey values is adjusted for each image.

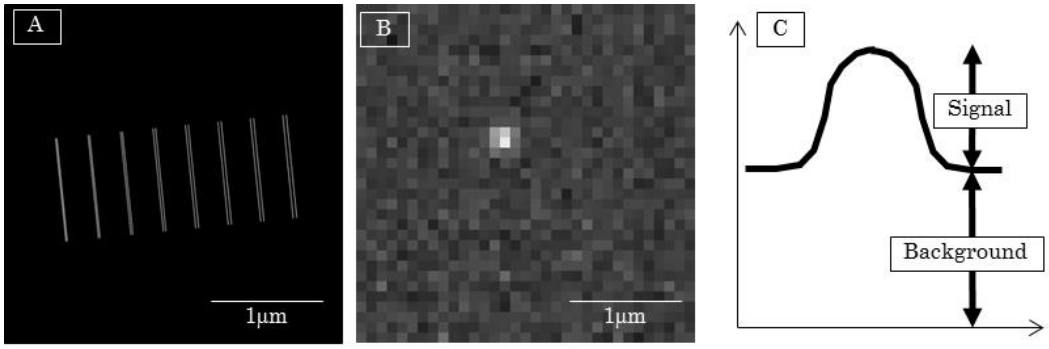

Figure 3. Simulation model. (A) Sample structure: line pairs spacing of 20, 25, 30, 35, 40, 45, 50, and 55 nm. (B) Example of a simulated camera image. Each frame includes emission from a single fluorophore. (C) Transverse profile model of the intensity from the fluorophore (Gaussian) on an optical background.

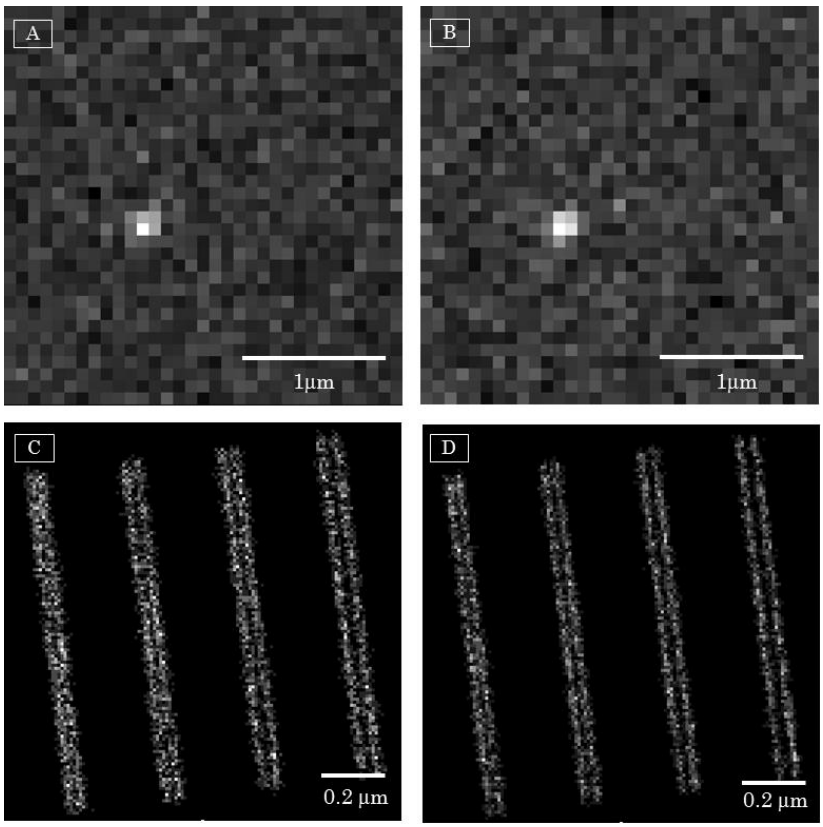

Figure 4. (A) and (B) Representative frames of the simulated camera image with a single molecule. The simulated single molecular fluorescence images are for the camera specifications of Table 2 and 500 photons collected per molecule per frame. A uniform optical background of 43.5 photons / pixel / frame is modeled. (A) EM-CCD, (B) ORCA-Flash4.0; (C) and (D) Super resolution images reconstructed from simulated single molecule fluorescence image stacks (21,000 frames). (C) EM-CCD, (D) ORCA-Flash4.0.

Table 2. Specifications and simulation conditions for each camera

Camera	**Gen II sCMOS (ORCA-Flash4.0) (Hamamatsu Photonics)**	**EM-CCD (ImagEM) (Hamamatsu Photonics)**
Pixel size [μm x μm]	6.5 x 6.5	16 x 16
QE @ 550nm	72 %	92 %
Read noise [e^-]	1.3	100
Optical Magnification	60 x 1.20 (with relay lens)	60 x 2.96 (with relay lens)
EM Gain	-	500
Field of view in a pixel [nm]	90	

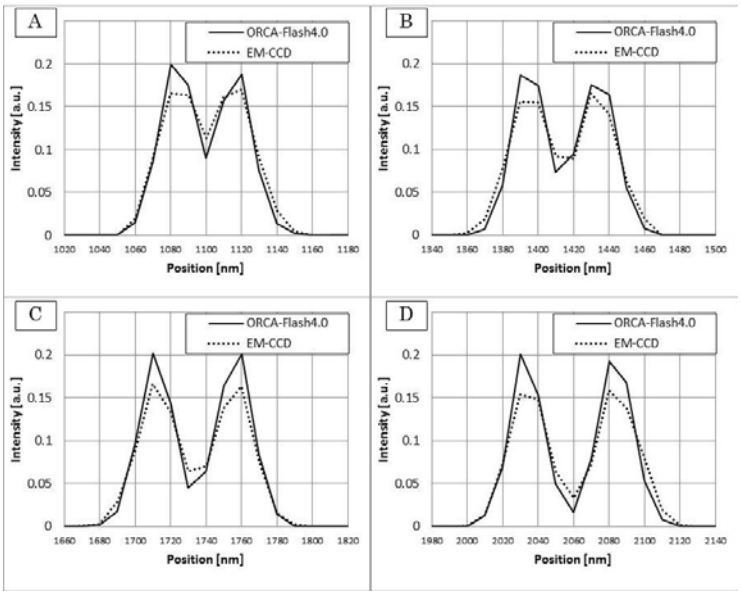

Figure 5. Profile comparison. Each profile is calculated by summing image intensities along long axis of the line pair. Spacing of the line pairs are (A) 30nm, (B) 35nm, (C) 40nm, (D) 45nm

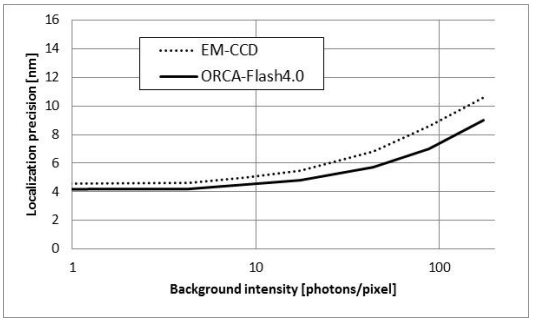

Figure 6. Localization precision as a function of optical background, under the same conditions as Figure 4, with the exception that the optical background is varied. The ORCA-Flash4.0 shows better precision than the EM-CCD for all levels of optical background, and the relative performance of the sCMOS camera improves with higher optical background.

4. DISCUSSION

With advances in detector technology, selecting a camera for a certain application is more complicated than simply finding a camera with a specification of low read noise or high quantum efficiency. Yet, combined with the development of sophisticated imaging techniques, the process of testing a camera that offers the performance required for a given system can be very time-consuming and tedious. Accurate simulation of camera images streamlines this process by emulating the conditions encountered in a laboratory imaging setting and provides realistic images that can be used to evaluate cameras in the context of the entire system, including image processing or analysis algorithms

We have carefully modeled the dominant noise sources in commercial scientific cameras and in Fig. 2 show that simulated images from two high performance scientific cameras, an EM-CCD and Gen II sCMOS are visually indistinguishable from actual acquired data.

To demonstrate the usefulness of the CSE for application specific camera selection, we chose precision localization or super-resolution microscopy. Precision localization microscopy is regarded as one of the most challenging quantitative imaging techniques and camera choice for this application is both critical and difficult to test *in situ*. We generated a large image stack of simulated data using image system and optical intensity parameters typically found in localization microscopy of a weak fluorescent molecule (500 collected photons/molecule/frame) for two cameras of differing technologies. These simulated image data sets were then processed as real images would be, using the MaLiang precision localization reconstruction method. The simulations show that Gen II sCMOS is expected to provide better precision localization results than an EM-CCD. Although it might be predicted that EM-CCDs would offer the best performance for localization microscopy because of their high quantum efficiency and low noise, in fact their performance is significantly degraded because of the excess noise due to electron multiplication.

The CSE is not meant to substitute for camera evaluations but it can be a first step to choosing the most appropriate camera since it provides a realistic estimate of results that would be obtained in specific applications or usage scenarios. The CSE bridges the gap between camera specifications on a data sheet and complicated *in situ* demonstrations.

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