Why Camera Calibration Using Variance Doesn`t Work for Photoresponse Calibration of Scientific CMOS (sCMOS) Cameras (and Other Photodetectors)

Shigeo Watanabe, Teruo Takahashi, Keith Bennett

System Division, Hamamatsu Photonics K.K., Japan, kbennett@hamamatsu.com

Changing the Game

HAMAMATSU
PHOTON IS OUR BUSINESS

INTRODUCTION

A scientific camera should:

- accurately detect the number of **input photons**,
- faithfully represent the **number of input photons in the digital output signal**, and
- provide **predictable noise statistics**

sCMOS camera architecture fundamentally differs from CCD and EMCCD cameras. In scientific digital CCD and EMCCD cameras, conversion from charge to the digital output is generally through a single electronic chain, and the read noise and the conversion factor from photoelectrons to digital outputs is highly uniform for all pixels, although quantum efficiency may spatially vary.

By contrast, in CMOS cameras, the charge to voltage conversion is separate for each pixel, each column has independent amplifiers and analog-to-digital converters, and microlenses introduce pixel-to-pixel variation in quantum efficiency. The “raw” output from the CMOS image sensor includes pixel-to-pixel variability in the read noise, gain, offset and dark current. Therefore, scientific camera manufacturers digitally compensate the raw signal from the CMOS image sensors[1, 2]. Inaccuracies and noise in images can introduce artifacts in computational imaging such as localization microscopy, especially in the case of maximum likelihood estimation methods[3, 4].

The Hamamatsu ORCA-Flash4.0 V3 has well-calibrated pixel response and predictable individual pixel statistics. We show that for this camera (peak QE, 82%):

- individual pixels are accurately factory-calibrated for dark offset and photo-response,
- Output is highly linear to input photons across the entire signal range, and
- individual pixel variance can be accurately modeled as a sum of two pixel-specific terms: read noise and a variance coefficient multiplied by the signal in the pixel (shot noise), however **pixel variance is not an accurate method for calibration of pixel or camera gain.**

DARK OFFSET & UNIFORMITY

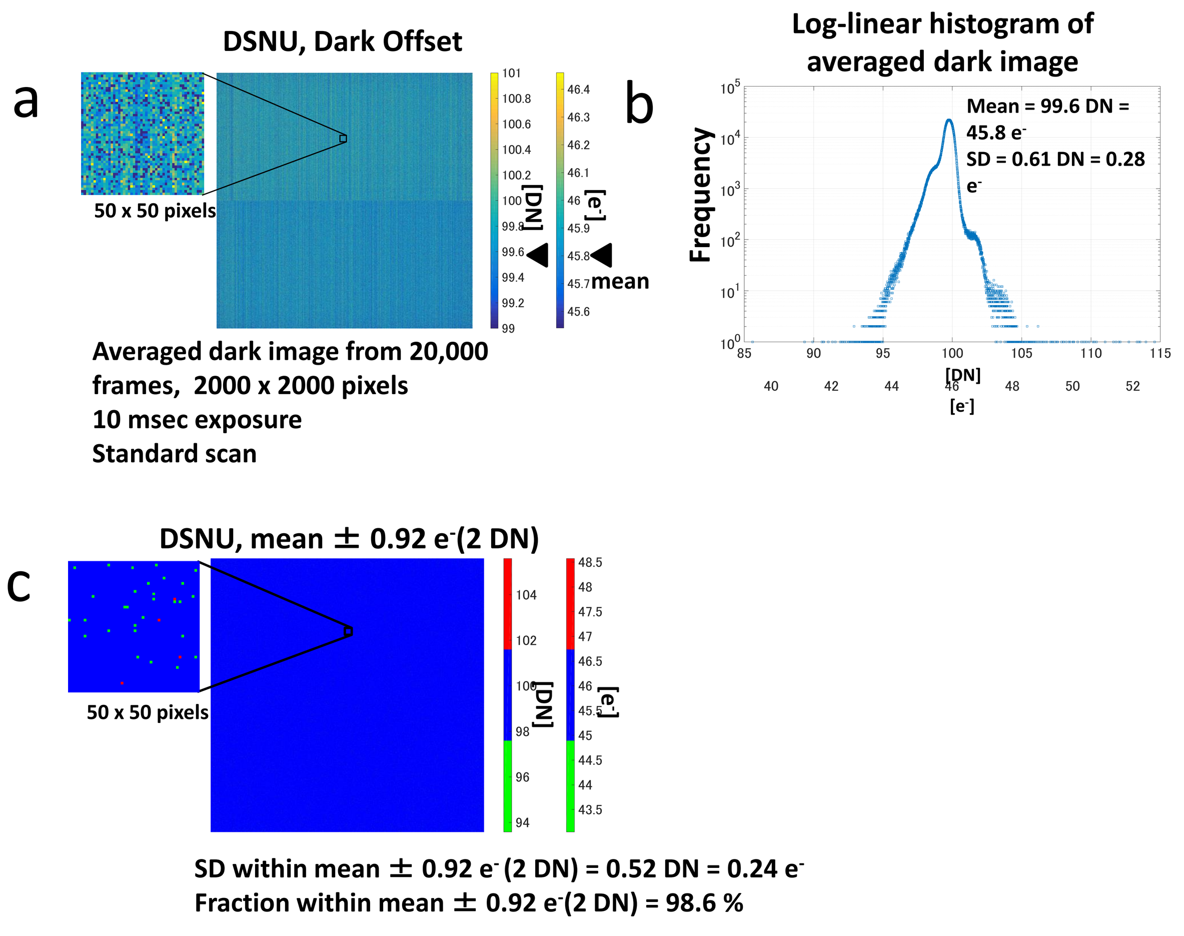


Figure 1. DSNU a) map, b) offset histogram and c) threshold map of pixels > 2 DN (0.92 e-) from the mean.

PHOTORESPONSE NON-UNIFORMITY

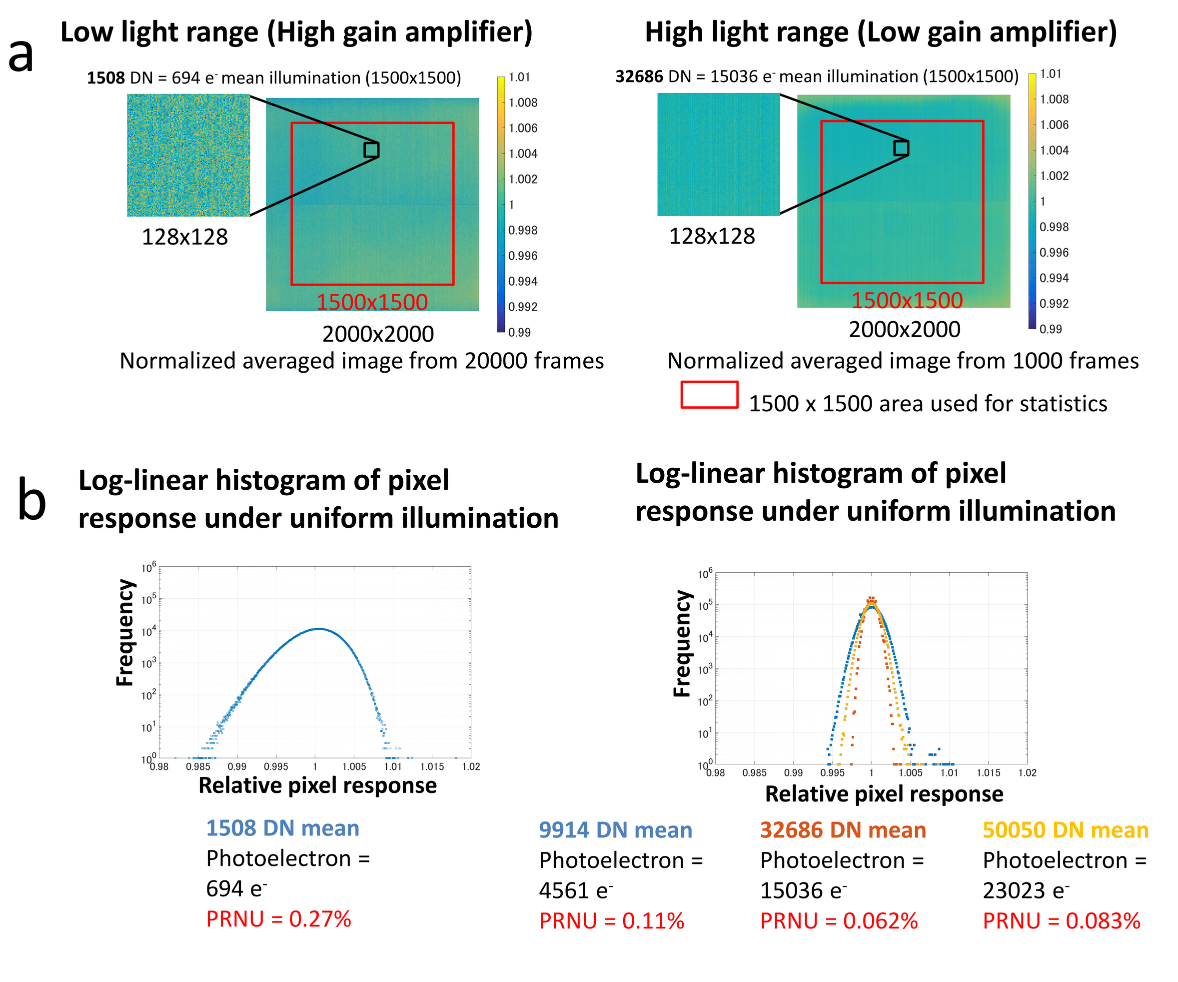


Figure 2. a) PRNU and b) log-linear histograms of pixel response uniformity in both low and high light ranges.

LINEARITY

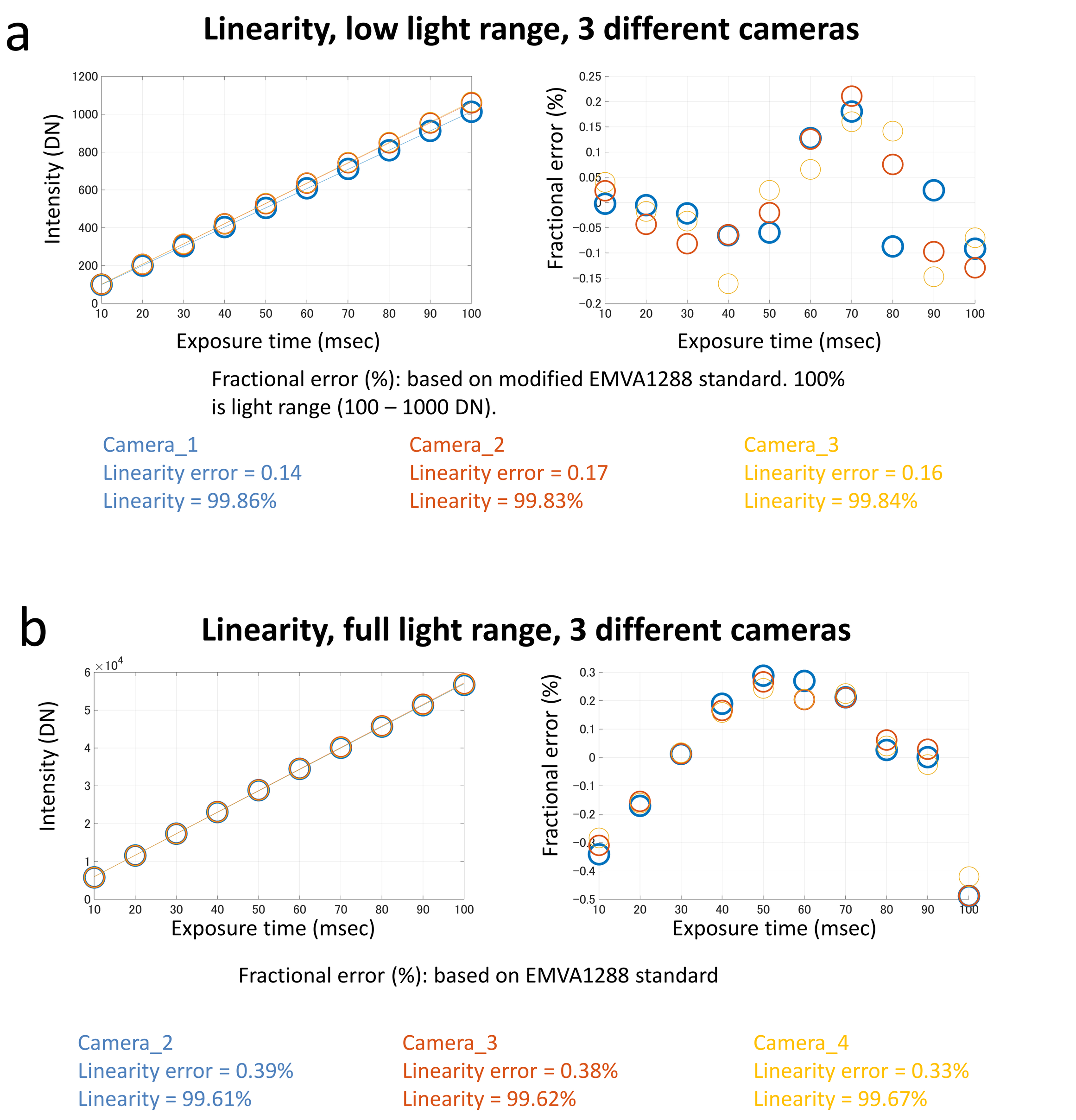


Figure 3. Linearity of 3 different cameras in both a) low and b) high light ranges.

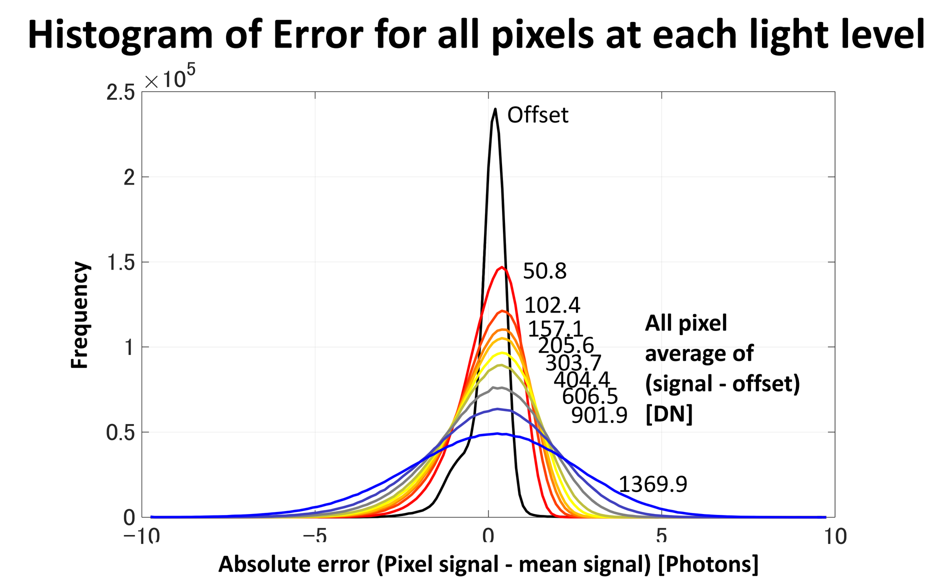
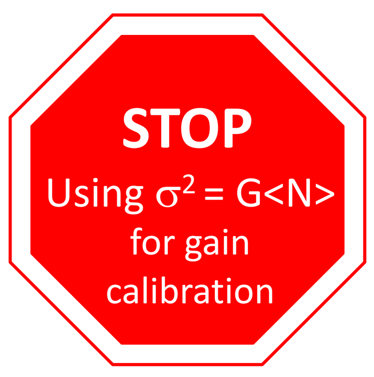


Figure 4. Distribution of absolute response error at various light levels

CAN VARIANCE BE USED FOR ACCURATE GAIN CALIBRATION?

While the photon transfer curve[5] technique is a good method to validate camera (and pixel) performance, it is not a reliable method for camera photo-response or gain calibration.



- The relationship between the variance and the mean for a photoelectron signal, $\sigma^2 = G\langle N\rangle$, where, G= camera gain and <N> = mean number of photoelectrons / measurement assumes i) perfect counting statistics and ii) all measurements are statistically independent of any other measurements. In practice, (analog) coupling between individual pixel measurements may be >10%.
- Many other noises contribute to the variance, including i) instability in the illumination, ii) excess noise (from electron multiplication processes in EMCCD and iCCD cameras), iii) read noise, and iv) electrical pickup, such as clock-induced charge.
- The conversion from photons to electrons (QE), and hence photoresponse, may vary from pixel to pixel (or camera to camera).
- Careful validation of the statistical model (including all noises) is required to extrapolate quantitative parameters from noise.

REFERENCES

[1] Fullerton, S., et al., “Camera simulation engine enables efficient system optimization for super-resolution imaging,” Proc. SPIE 8228, Single Molecule Spectroscopy and Superresolution Imaging V, 822811 (2012)

[2] Fullerton, S., et al., “Optimization of Precision Localization Microscopy using CMOS Camera Technology,” Proc. SPIE 8228, Single Molecule Spectroscopy and Superresolution Imaging V, 82280T (2012)

[3] Huang, Z. L et al., “Localization-based super-resolution microscopy with an sCMOS camera,” Optics Express 19(20), 19156-19168 (2011).

[4] Huang, F., et al., “Video-rate nanoscopy using sCMOS camera-specific single-molecule localization algorithms,” Nat Methods 10(7), 653-658 (2013).

[5] Janesick, J. R., Photon Transfer, SPIE Press Book, (2007).

[6] Pertsinidis, et al., “Subnanometre single-molecule localization, registration and distance measurements,” Nature 466(7306), 647-651 (2010).

TEMPORAL READOUT NOISE & VARIANCE MODEL

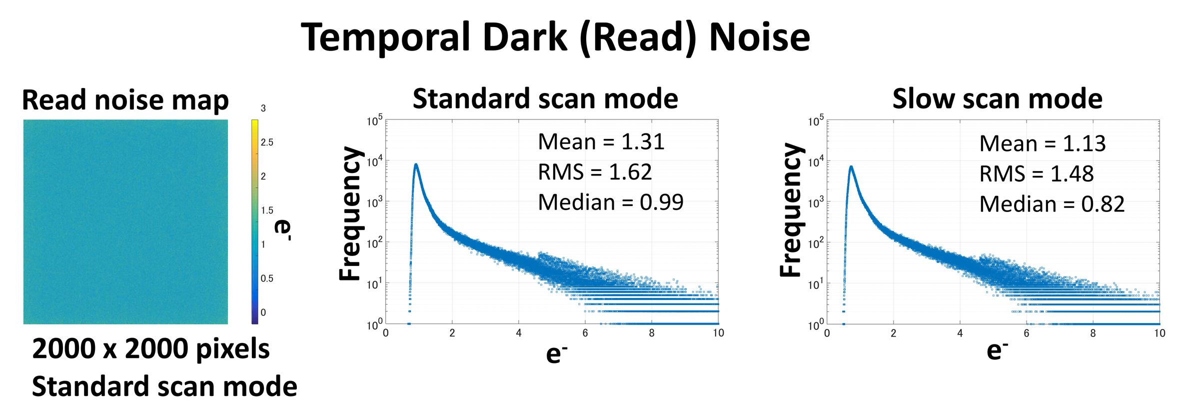


Figure 5. Pixel-specific temporal dark read noise of ORCA-Flash4.0 V3 sCMOS camera.

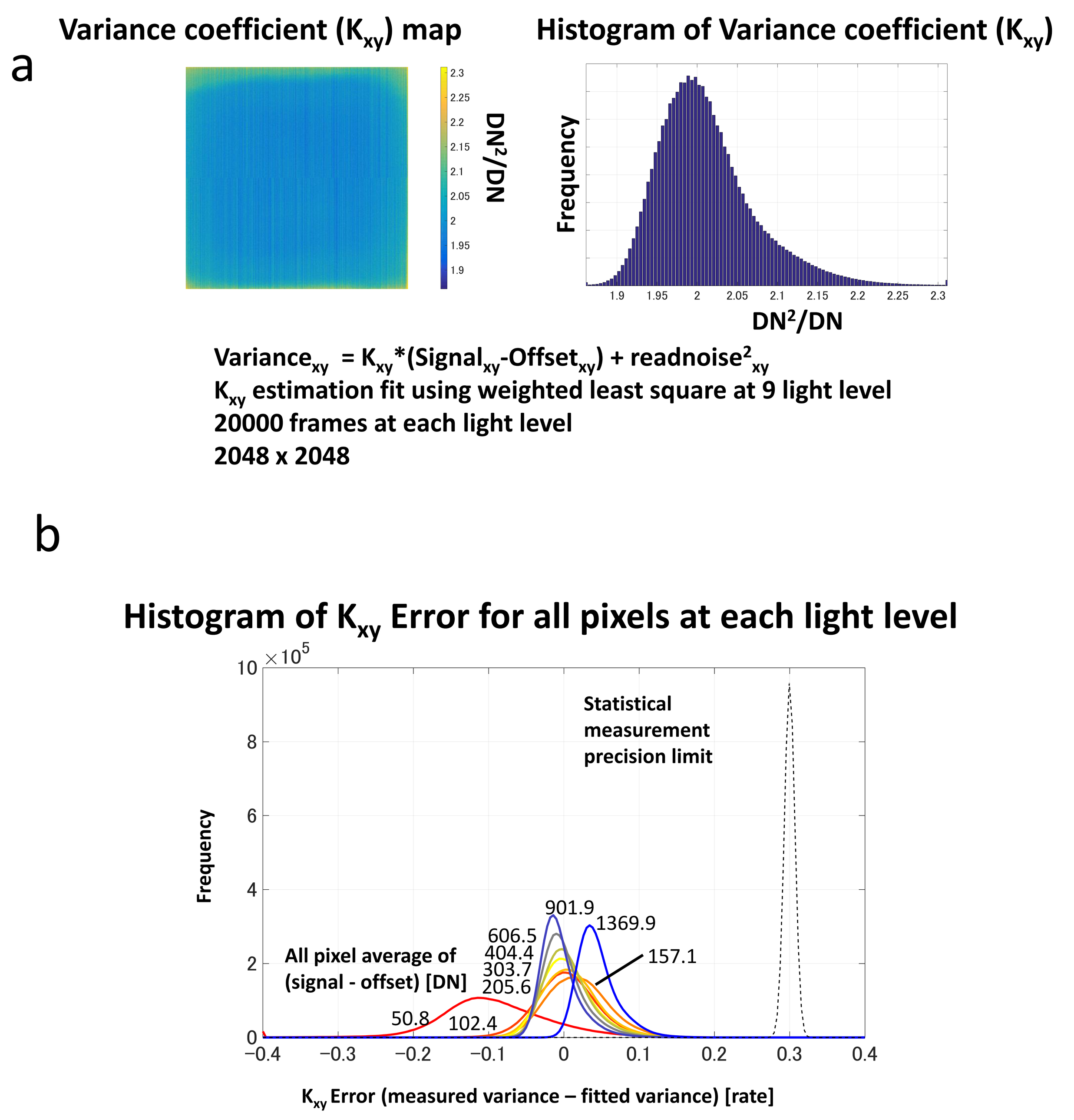


Figure 5. a) Variance coefficient map, b) histogram of variance coefficient K_{xy} and residual K_{xy} error as a function of light level. The curve labeled “Statistical measurement precision limit” shows the statistical uncertainty in measurement of the variance of a single pixel using 20,000 measurements arising solely from statistical considerations.

CONCLUSIONS

- **Production ORCA-Flash4.0 V3 sCMOS cameras with on-board correction have low dark offset non-uniformity.**
- **Photoresponse uniformity is comparable to front-illuminated CCDs (0.25%) and better than back-illuminated image sensors (e.g. EMCCDs, ~1%) for narrowband illumination.**
- **The digital output accurately represents the input intensity (photons) for each pixel.**
- **Uniform response (DN / photon) requires compensation for both QE and electronic gain variation.**
- **Using variance to estimate the gain does not correct for PRNU because of QE variations and other factors**
- **Variance is not a reliable method for accurate photodetector gain calibration as measurements without verification of the photodetectors statistics. Although the PTC provides an estimate of the electronic “gain,” the “photon transfer curve” method theory is based upon the statistical independence of each measurement, absence of excess noise, and no other noise sources., which is not generally correct.**

ORCA-Flash4.0 V3 specification	
Dark Signal Non-Uniformity (DSNU) [1]	0.3 e- rms
Photoresponse Non-Uniformity (PRNU) at half level of full light range (15,000 e-) [1]	0.06 % rms.
Photoresponse Non-Uniformity (PRNU) at low light level (700 e-) [1]	0.3 % rms.
Linearity error, full light range (EMVA 1288 standard) [1]	0.5 %
Linearity error, low light range (< 500 e- signal) [1]	0.2 % / Less than approx. 1 e- absolute error
Dark Current (electrons/pixel/s) – Air Cooled to -10° C	0.06
Readout Noise (N_r) median in electrons at slow scan [1]	0.8
Readout Noise (N_r) rms in electrons at slow scan [1]	1.4
Readout Noise (N_r) median in electrons at standard scan [1]	1.0
Readout Noise (N_r) rms in electrons at standard scan [1]	1.6

[1]Typical value