BRIDGING THE GAP

Impact and Correction of Camera Noise for Computational Microscopy including Precision Localization Nanoscopy

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HAMAMATSU PHOTONICS K.K.
System Division

Cameras are **NOT** perfect!

Why is a
camera manufacturer
proclaiming
that
cameras are not perfect?

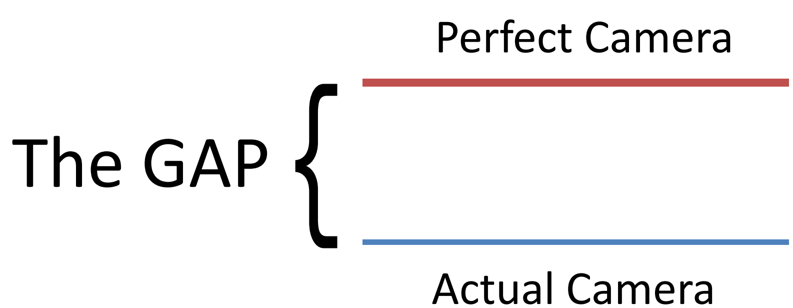
Because NO camera is perfect

&

Because understanding why **matters** to your science

What is the Gap?

The difference between the performance of an actual camera and a theoretically perfect camera



THE AMOUNT OF THE GAP DEPENDS ON:

1. Sensor technology
   - CCD
   - EMCCD
   - sCMOS

2. Camera specifications
   - Quantum Efficiency
   - Camera Noise
     - Read noise
     - Excess Noise
     - Photo-response non-uniformity (PRNU)

3. Input photon level
   - Ultra low light
   - Low Light
   - Intermediate
   - High

UNDERSTANDING WHY THERE IS A GAP ENABLES:

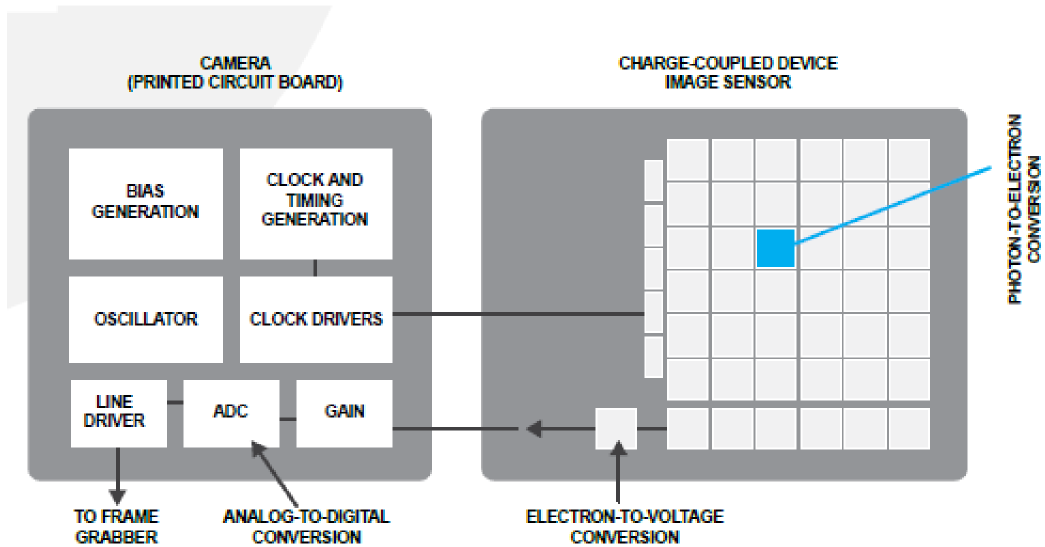
- Appropriate camera selection
- Optimized camera usage
- Optimized experimental design
- More reliable data analysis

} Better Results

The Real Cameras

CCD

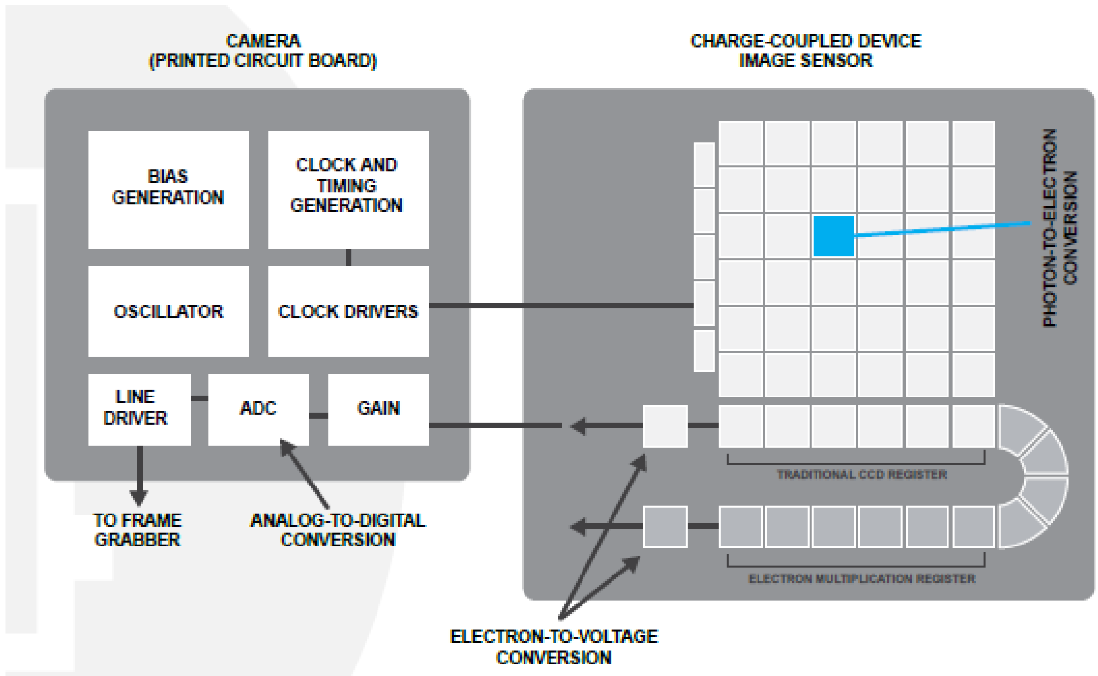
- Well established technology
- All electron to digital conversion done in one chain
- Limited speed
- Moderate read noise
- Very low dark current
- High QE
- Best pixel response uniformity



The Real Cameras

EMCCD

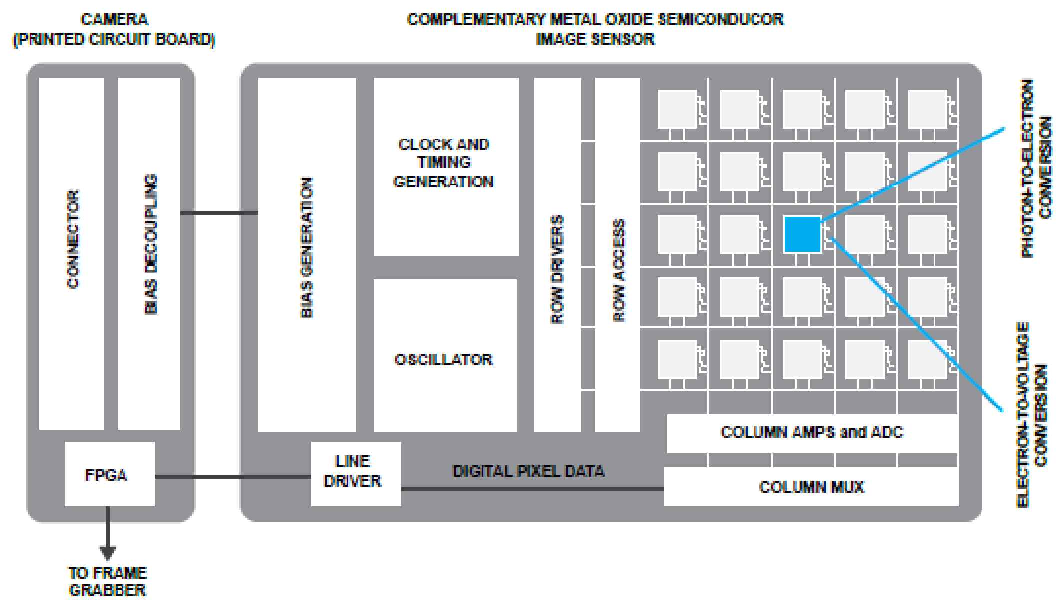
- Back-thinned for increased QE
- High voltage gain register on sensor to achieve on-chip amplification
- All electron to digital conversion through one chain(either for EM or no EM)
- Read noise is low due to gain
- Stochastic EM amplification adds excess noise and long tail



The Real Cameras

CMOS

- Newest technology
- Every pixel and column has own amplifier
- Very low mean rms read noise
- Pixel dependent read noise
- Fastest speeds and largest field of view
- FPGA processing achieves excellent response uniformity (low PRNU)



SEEING THE PREDICTED GAP

Single Pixel Noise & SNR

Fixed Pattern Noise & Image SNR

(from **specs**)

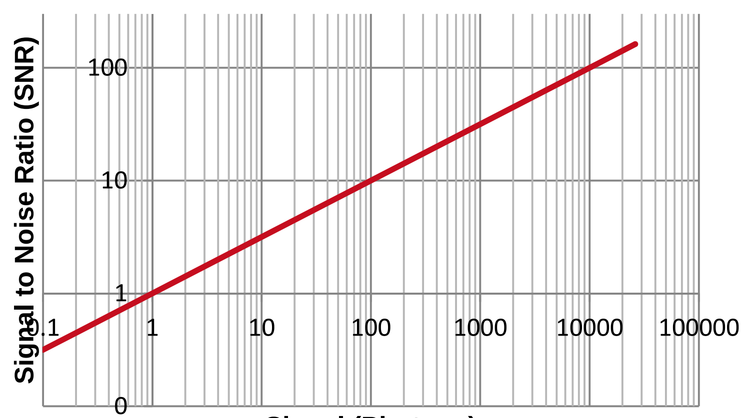
The Perfect Camera

100% QE	Every photon is converted into one electron
0 e- read noise	Every electron is digitized exactly as expected every time
0% fixed pattern noise	Every pixel and amplifier perform identically and predictably

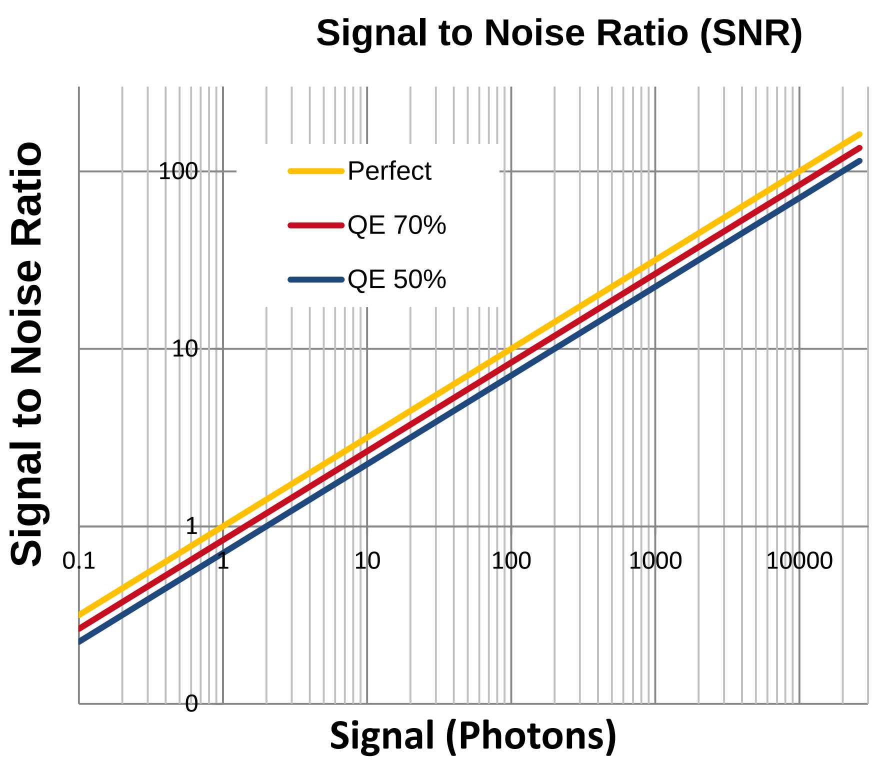
In a perfect camera, the SNR of a single pixel is limited only by the physics of photon statistics… i.e. shot noise.

$$SNR = \sqrt{S}$$

Perfect Camera Signal to Noise Ratio

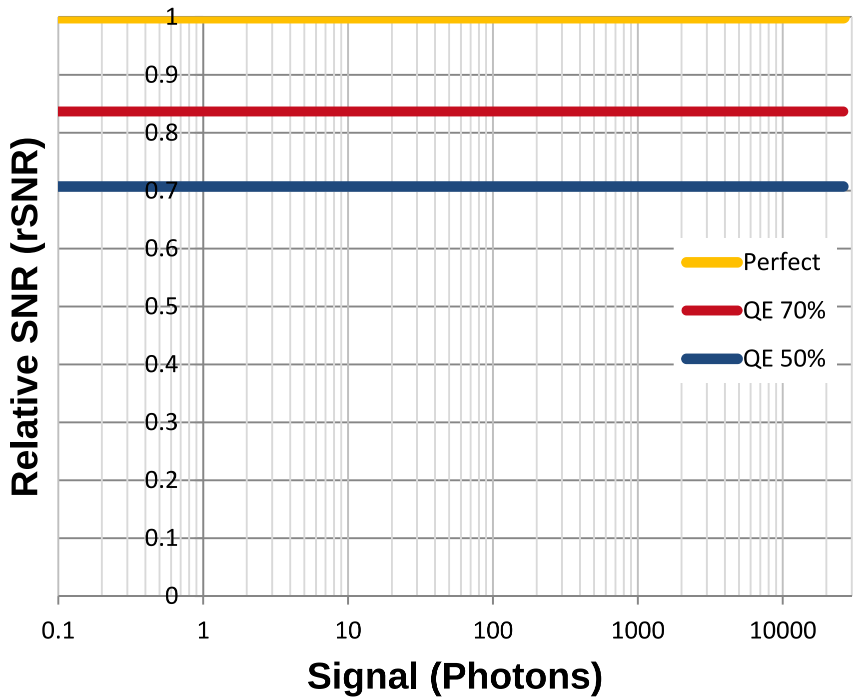


EFFECT OF QE ON THE GAP



A reduction in QE reduces SNR at all light levels

RELATIVE SNR (rSNR) PLOTS CLEARLY SHOW THE GAP



rSNR is the SNR for a camera plotted relative to the perfect camera

rSNR shows differences among cameras over full range of signal level

{All SNR graphs in this talk will be presented as rSNR}

THE SIMPLE SIGNAL TO NOISE RATIO (SNR)

$$SNR = \frac{QE * S}{\sqrt{F_n^2 * QE * (S + I_b) + (N_r / M)^2}}$$

QE: Quantum Efficiency

S: Input Signal Photon Number (photon/pixel)

F: Noise Factor (= 1 for CCD/sCMOS and √2 for EM-CCD)

N_r: Readout Noise

M: EM Gain (=1 for CCD / CMOS)

I_b: Background

"Changing the Game"

EMCCDs: Excess Noise is the Reason for the Gap

SNR for CCD / CMOS

$$SNR = \frac{QE \times P}{\sqrt{QE \times P}} = \sqrt{QE \times P}$$

SNR for EM-CCD

$$SNR = \frac{M \times QE \times P}{F_n \times M \times \sqrt{QE \times P}} = \sqrt{\frac{QE \times P}{F_n^{\ 2}}} = \sqrt{QE_{eff} \times P}$$

$$QE_{eff} = \frac{QE}{F_n^{\ 2}} = \frac{QE}{2}$$

QE: Quantum Efficiency,
P: Input Signal Photon Number,
M: EM Gain
F_n: Noise Factor
(assumes dark current and read noise are negligible)

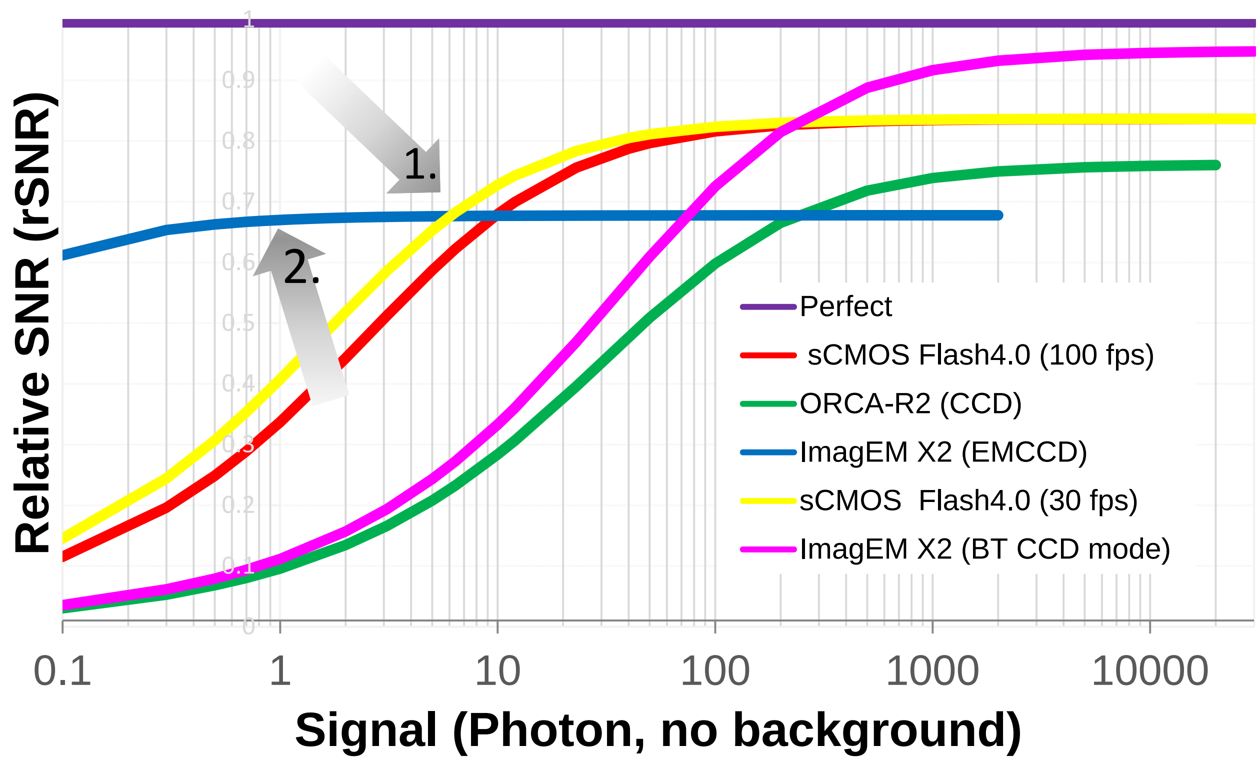
The Real Cameras

	CCD	EMCCD	CMOS
Sensor Type	Charged Coupled Device Interline	Electron Multiplying CCD Back-thinned	Complimentary Metal Oxide Sensor
Camera Name	ORCA-R2	ImagEM x2	ORCA Flash4.0 V2
Pixel Number	1024 x 1344	512 x 512	2048 x 2048
Pixel Size	6.45 µm x 6.45 µm	16 µm x 16 µm	6.5 µm x 6.5 µm
QE (@650 nm)	58 %	90 %	72 %
Frame Rate	18 fps / 8 fps	70 fps	100 fps / 30 fps
Relative read noise (N_r/M), single-frame rms	10 e- / 6 e-	< 0.2 e- (M = 200)	1.9 e- / 1.3 e-
Noise Factor (F_n)	1	$\sqrt{2}$ @ M>10	1

MIND THE GAP: PREDICTED PIXEL rSNR PERFORMANCE FOR THE MOST COMMON CAMERAS

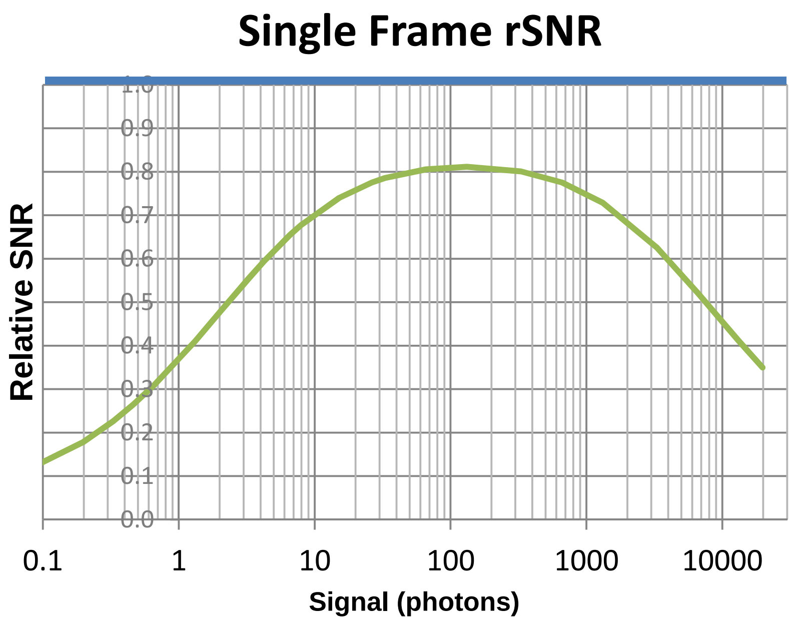
1. A camera with the highest SNR at the lowest light level may not be the best at higher light levels

2. The SNR of an EMCCD above 1 electron/pixel is comparable to a camera with QE_{eff} =QE/2 due to excess noise from EM gain.



λ = 650 nm

BEYOND THE SWEET SPOT: THE GAP EXPANDS AT HIGH LIGHT IF PRNU IS NOT CORRECTED



- PRNU reduces SNR at high light
- Cannot be subtracted from image
- “Raw” PRNU varies by sensor
- Can be corrected in camera to varying degrees

Model:

- QE: 70%
- Noise Factor (F_n): 1
- Read Noise 3 photons rms
- PRNU (σ_s): 1%

Image SNR

$$SNR = \frac{QE * S}{\sqrt{\underbrace{F_n^2 * QE * (S + I_b)}_{\text{Shot Noise}} + \underbrace{\left(\frac{Nr}{M}\right)^2}_{\text{Read Noise}} + \underbrace{[\sigma_s * QE * (S + I_b)]^2}_{\text{Fixed Pattern Noise}}}}$$

All SNR curves will be rSNR @ λ=650 nm

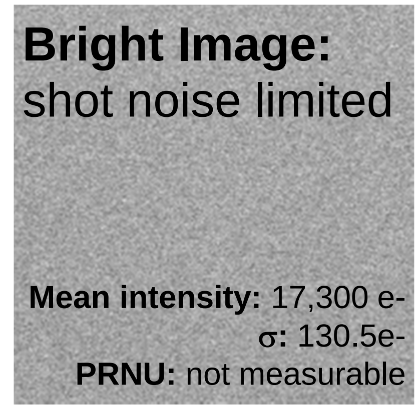
MEASURING THE REAL GAP

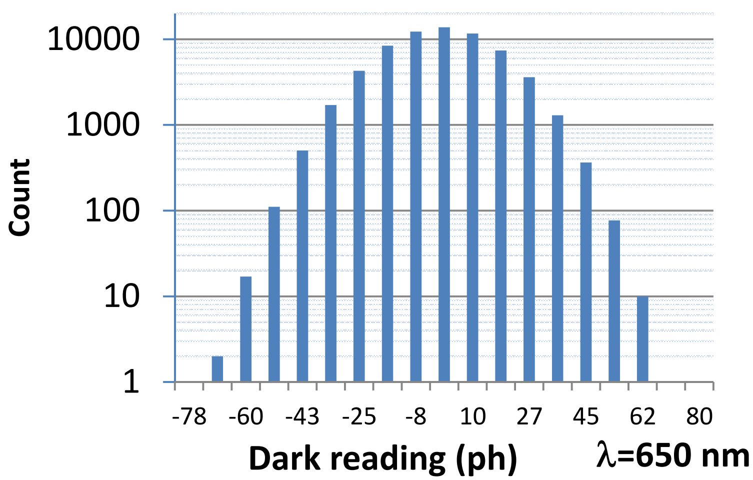
An in-depth look at noise in CCD, EMCCD and CMOS cameras

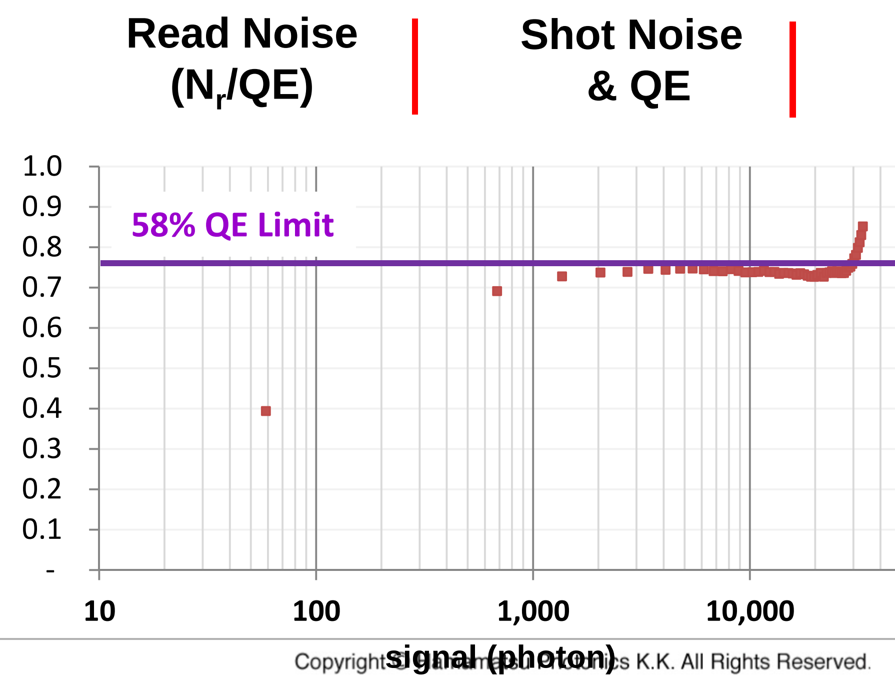
ORCA-R2 Interline CCD: Predictable and Robust

1. PRNU is insignificant

2. Read noise histogram has single Gaussian distribution

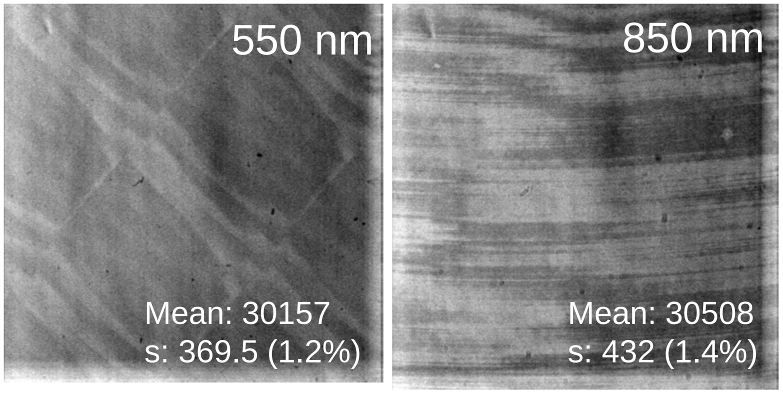




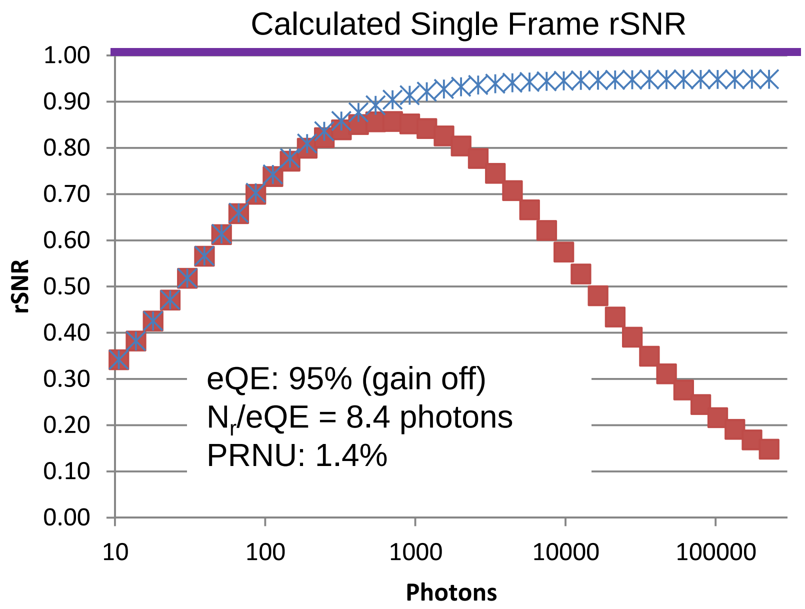


EMCCD: Some Surprising Results

1. { Thickness variations from back-thinning process causes spectrally-dependent PRNU
   - Cannot be removed during manufacturing
   - Must be calibrated by users for *their specific spectrum*.
   - Individual pixel map required for correction



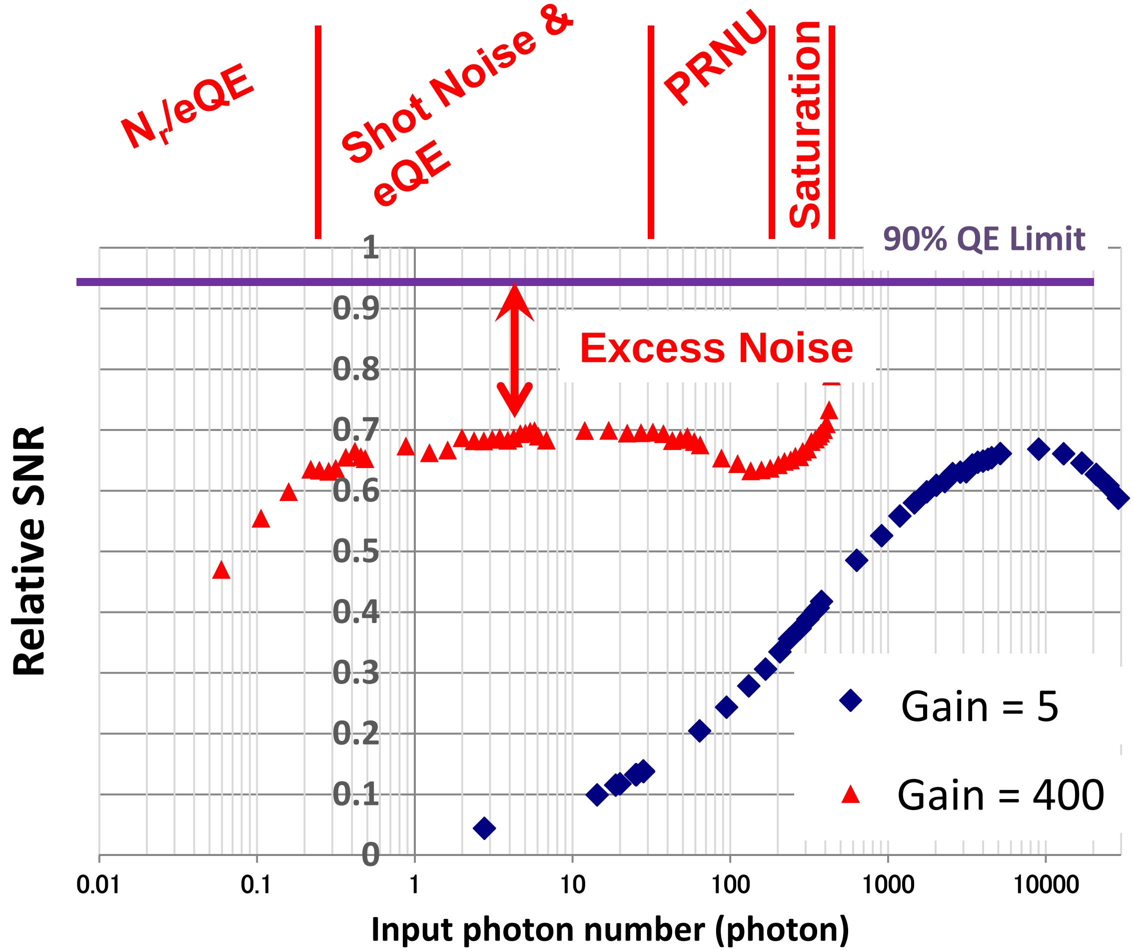
2. { The Gap for EMCCD in CCD mode becomes very wide due to PRNU



COMPLEX BEHAVIOR: A CLOSER LOOK AT EMCCD SNR WITH HIGH AND LOW GAIN

Complex Behavior

- Excess noise (eQE)
- PRNU
- Saturation
- High read noise (34 e- @ M=5, 70 fps)
- Gain hard to measure

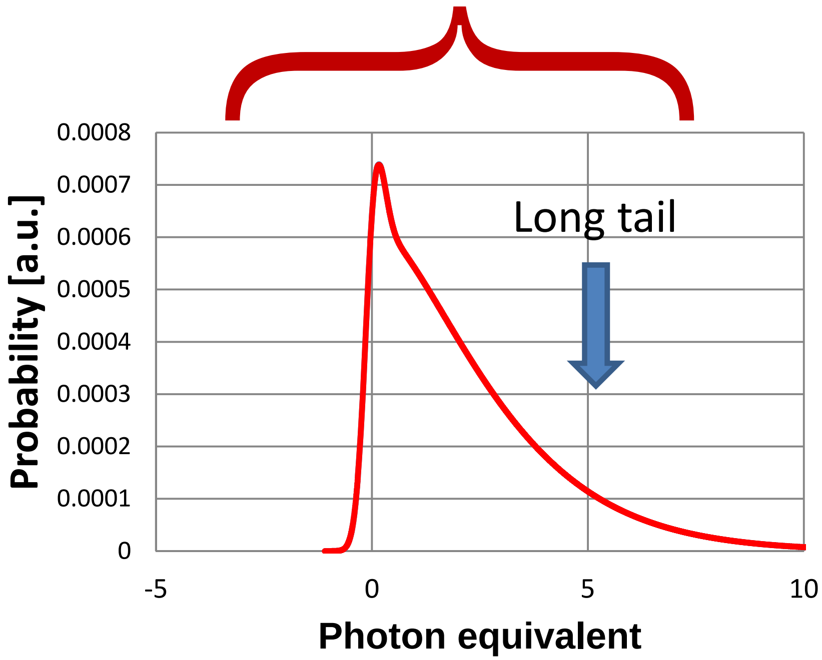


Changing the Game

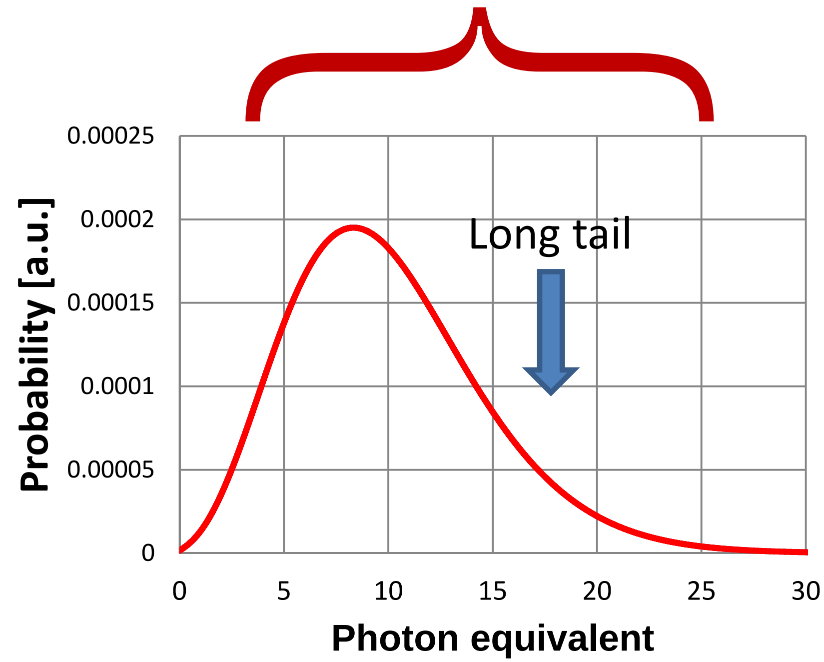
EMCCD Gain Causes Uneven Probability Distributions

In simulated probability distribution functions for EMCCD, the output at high gain is not Poisson due to the electron multiplication process!

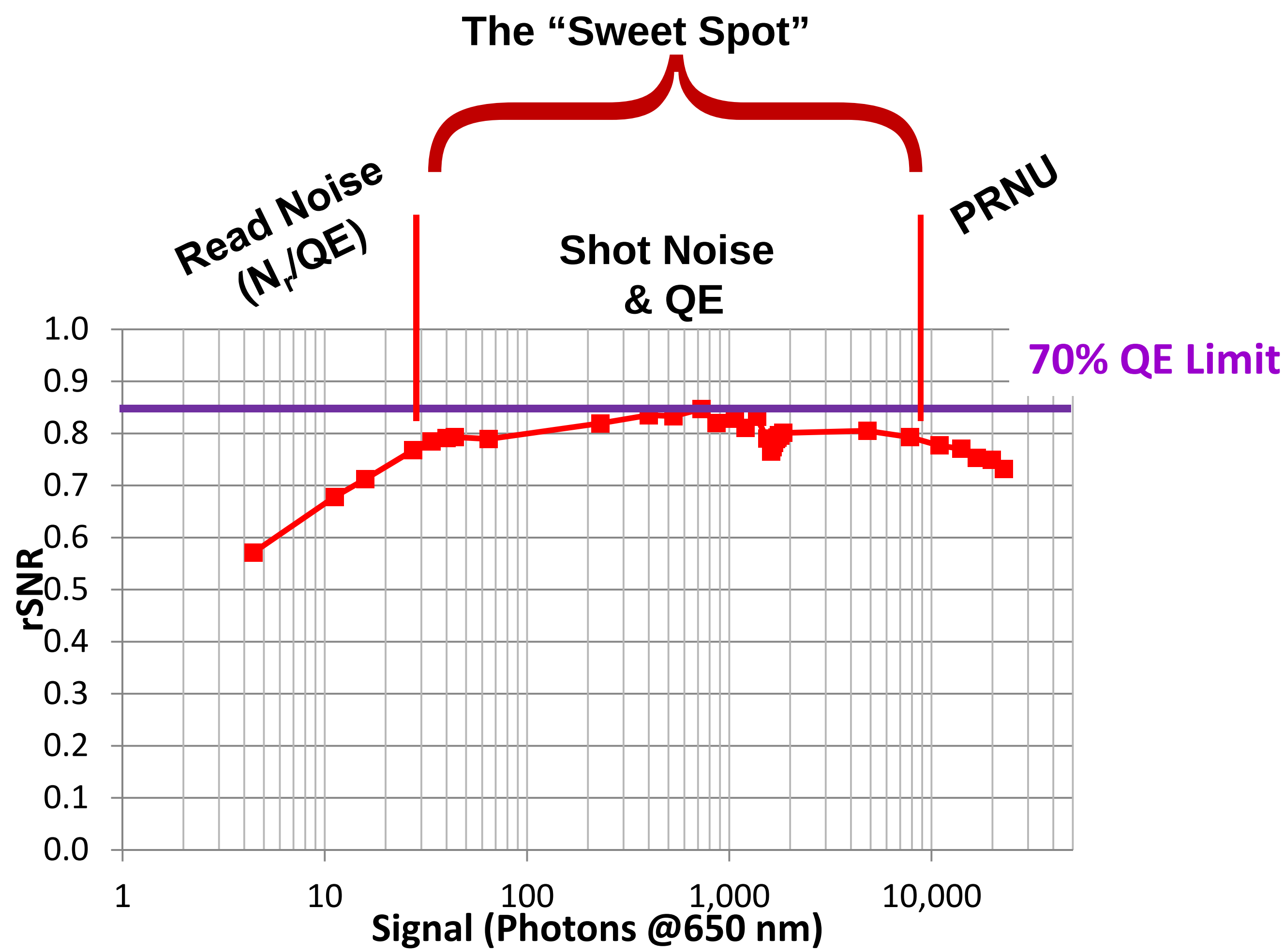
2 Photon Average Input
Gain = 200



10 Photon Average Input
Gain = 200

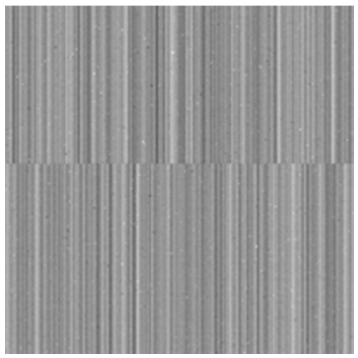


ORCA-FLASH4.0 V2 (sCMOS): A VERY COMFORTABLE SWEET SPOT



NOT ALL CAMERAS ARE CREATED EQUAL: FLASH4.0 SWEET SPOT BROUGHT TO YOU BY HAMAMATSU CAMERA ENGINEERS

Raw Data
Bright Image



PRNU: ~2%

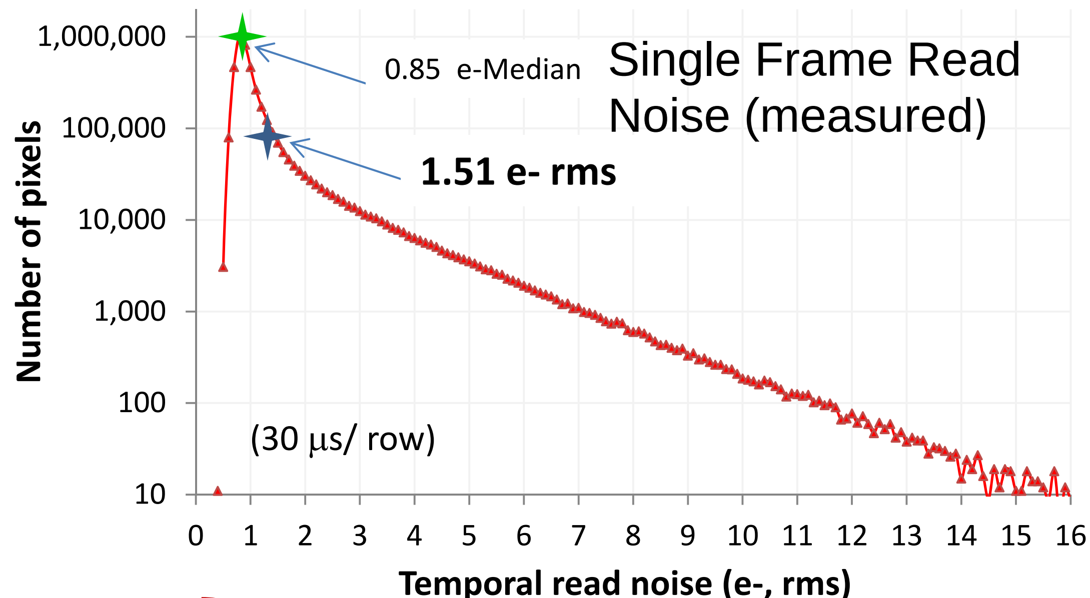
Corrected Data
Bright Image

PRNU: ~0.5%

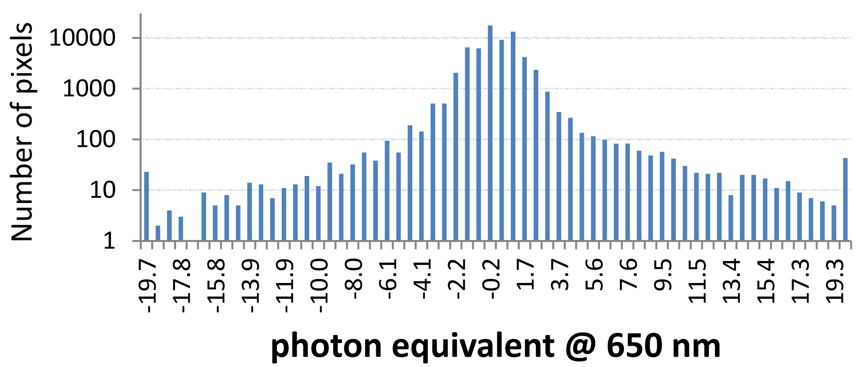
Signal amplified and digitized in column-parallel ADC. FPGA provides offset and gain correction to the raw digitized signal.

sCMOS: Pixel-Dependent Read Noise

Rms read noise matches single frame rSNR.



Single Frame Dark Histogram



Does not fit a Gaussian distribution, i.e. is not completely modeled by a single "read noise."

BRIDGING THE GAP

Using knowledge of camera noise to get highest camera performance provides improved Precision Localization Results

What is **MOST** important?

throughput

field of view

sample contrast

frame *rate*

RESOLUTION

ACCURACY

SAMPLE **BRIGHTNESS**

distance measurement

background

minimum bleaching rate

Two Phases of Precision Localization Microscopy

1. Collect Image Data
   - Prepare Sample
   - Minimize Background
   - Optimize Optical System
   - Consider Camera Induced Noise

2. Reconstruct Superresolution Image
   - Calibrate Camera
   - Implement Noise Corrections
   - Apply Statistical Algorithms

STANDARD PRACTICE IS NOT THE BEST PRACTICE: USING EMCCD WITH GAIN YIELDS LEAST ACCURATE RESULTS

Mean photon count	Ultimate accuracy limit (nm)	Conventional EMCCD accuracy limit (nm)	UAIM at 900× accuracy limit (nm)	UAIM at 4500× accuracy limit (nm)	CCD accuracy limit[a] (nm)
200	4.84	9.71 [100.6%]	5.41 [11.8%]	5.13 [6.0%]	7.94 [64.0%]
400	3.42	6.94 [102.9%]	3.92 [14.6%]	3.63 [6.1%]	5.26 [53.8%]
800	2.42	4.93 [103.7%]	2.85 [17.8%]	2.57 [6.2%]	3.45 [42.6%]
1600	1.71	3.49 [104.1%]	2.08 [21.6%]	1.82 [6.4%]	2.32 [35.7%]
3200	1.21	2.48 [105.0%]	1.52 [25.6%]	1.30 [7.4%]	1.57 [29.8%]

[a]Computed at near-optimal magnification (i.e., magnification that yields approximately the best localization accuracy limit) of 128.6×, 185.7×, 185.7×, 185.7×, and 242.9× for mean photon count of 200, 400, 800, 1600, and 3200, respectively.

CCD QE: 100%, read noise = 1.8 ph, no background; No fixed pattern noise.

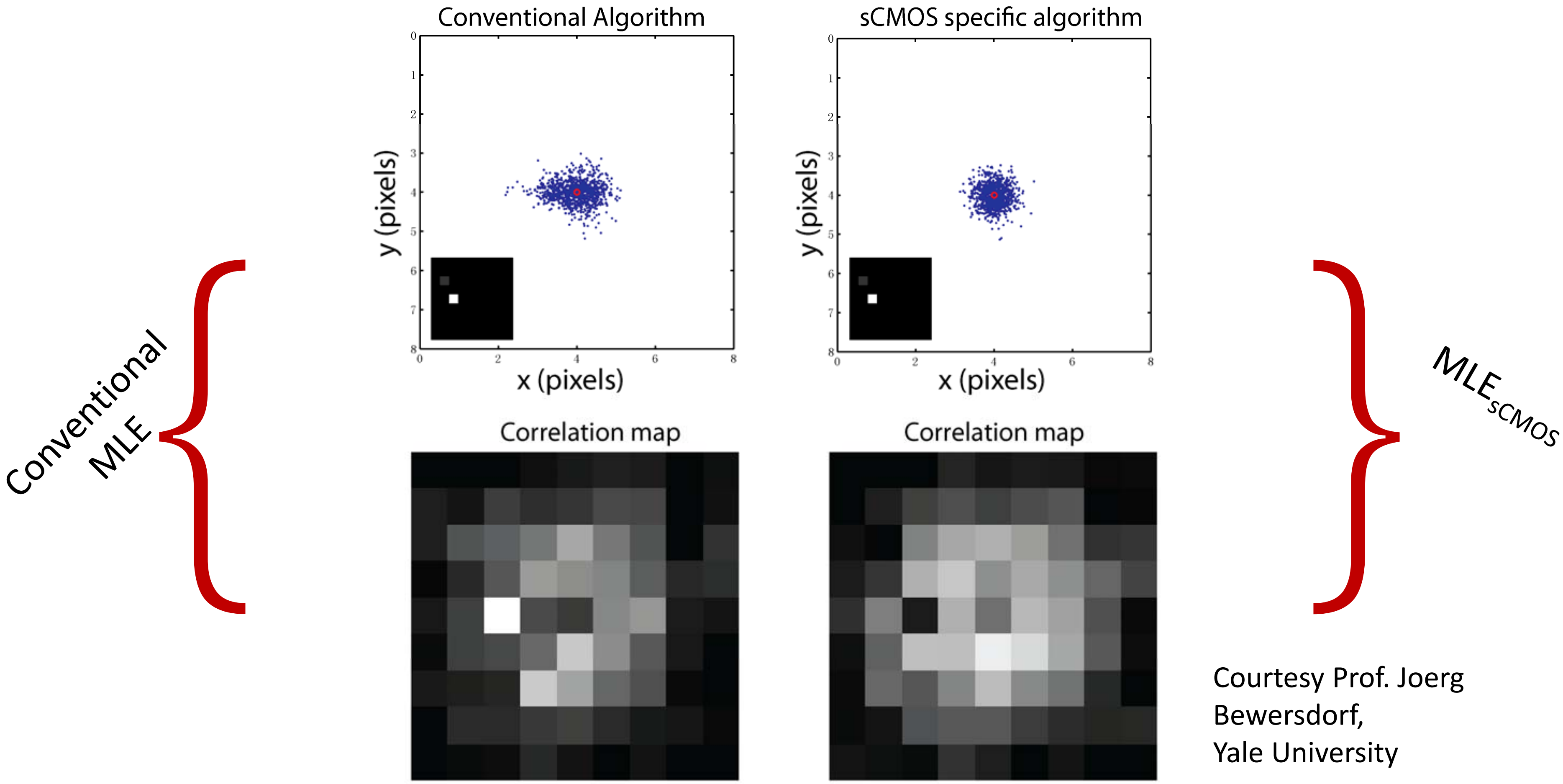
… "the fact that the noise coefficient approaches 1 with increasing photon count demonstrates the suitability of the CCD (CMOS) detector when enough light is available…."

… "even with a low readout noise of σ=2 electrons, the CCD detector is unsuitable for extreme low light imaging and implementing UAIM."

Adapted from: J. Chao et al (Ober Lab), Nat. Meth10, 2013) doi:10.1038/nmeth.2396

http://www.wardoberlab.com/

COMPENSATING READ NOISE VARIATION



Courtesy Prof. Joerg Bewersdorf, Yale University

Incorporating pixel-specific read noise into the Maximum Likelihood Probability Model eliminates and narrows the asymmetric distribution of localized molecules caused by higher read noise pixels.

Courtesy F. Huang, Bewersdorf Lab

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Selecting and Using Cameras: Case Studies

CCD

Subnanometre single-molecule localization, registration and distance measurements

Alexandros Pertsinidis[1,2], Yunxiang Zhang[1,2] & Steven Chu[1,2,3,4]†

EMCCD

Ultrahigh accuracy imaging modality for super-localization microscopy

Jerry Chao[1–3], Sripad Ram[1–3], E Sally Ward[2] & Raimund J Ober[1,2]

sCMOS

Video-rate nanoscopy using sCMOS camera–specific single-molecule localization algorithms

Fang Huang[1], Tobias M P Hartwich[1–3,9], Felix E Rivera-Molina[1,9], Yu Lin[4,5], Whitney C Duim[1], Jane J Long[6], Pradeep D Uchil[7], Jordan R Myers[1], Michelle A Baird[8], Walther Mothes[7], Michael W Davidson[8], Derek Toomre[1] & Joerg Bewersdorf[1,4,5]

Subnanometre single-molecule localization, registration and distance measurements

Alexandros Pertsinidis[1,2], Yunxiang Zhang[1,2] & Steven Chu[1,2,3,4]†

Results

Accurate measurement of the *distance* between two fluorophores of different colors. $\sigma_{distance}$ ~0.77 nm using a dichroic beamsplitter to direct each color of light to separate halves of the CCD camera.

Camera Correction

Measured PRNU maps for each color. Improved localization relative accuracy by ~2– 4 nm.

Details

Speed: 5 – 50 s / measurement
Light: ~4,000 – 10,000 ph/ mol/frame
~10^5 ph / mol before bleaching

Imaging: Simultaneous 2 color
Camera: Back-thinned EM-CCD, gain off

Nature (2010)| doi:10.1038/nature09163

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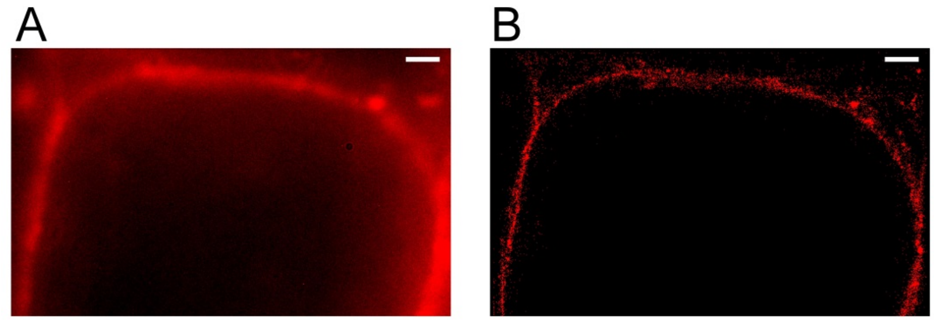
Ultrahigh accuracy imaging modality for super-localization microscopy

Jerry Chao[1–3], Sripad Ram[1–3], E Sally Ward[2] & Raimund J Ober[1,2]



Cholera toxin B subunit

scale bar: 1 μm

Results — **Localization Microscopy with Minimal Bleaching.** Plasma membrane dynamics for > 60 s (594 frames). 40% better localization precision than "conventional" EMCCD localization

Camera Correction — Implemented detailed statistical EM noise model into maximum likelihood reconstruction probability model.

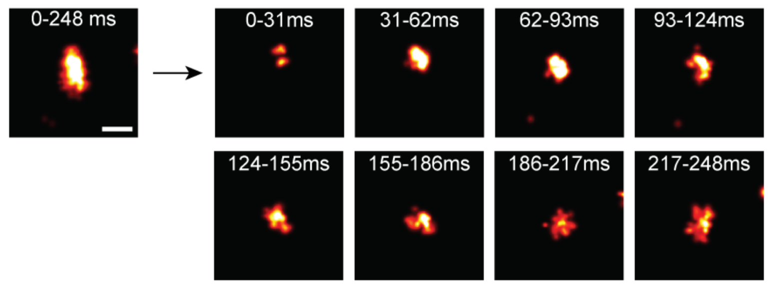
Details —
Speed: ~60s / reconstructed image
Light: ~100 photons /molecule frame
Mag: 630X
Camera: EM-CCD, Gain ~1000

Courtesy of J. Chao et al (Ober Lab)
Adapted from Nat Meth (2013) doi:10.1038/nmeth.2396

Video-rate nanoscopy using sCMOS camera–specific single-molecule localization algorithms

Fang Huang[1], Tobias M P Hartwich[1–3,9], Felix E Rivera-Molina[1,9], Yu Lin[4,5], Whitney C Duim[1], Jane J Long[6], Pradeep D Uchil[7], Jordan R Myers[1], Michelle A Baird[8], Walther Mothes[7], Michael W Davidson[8], Derek Toomre[1] & Joerg Bewersdorf[1,4,5]

Results: 32 reconstructed frames / sec (6.6 x 6.6 μm²) field of view; fixed and living cells showed cellular dynamics not visible in reconstructions using longer data collection times.



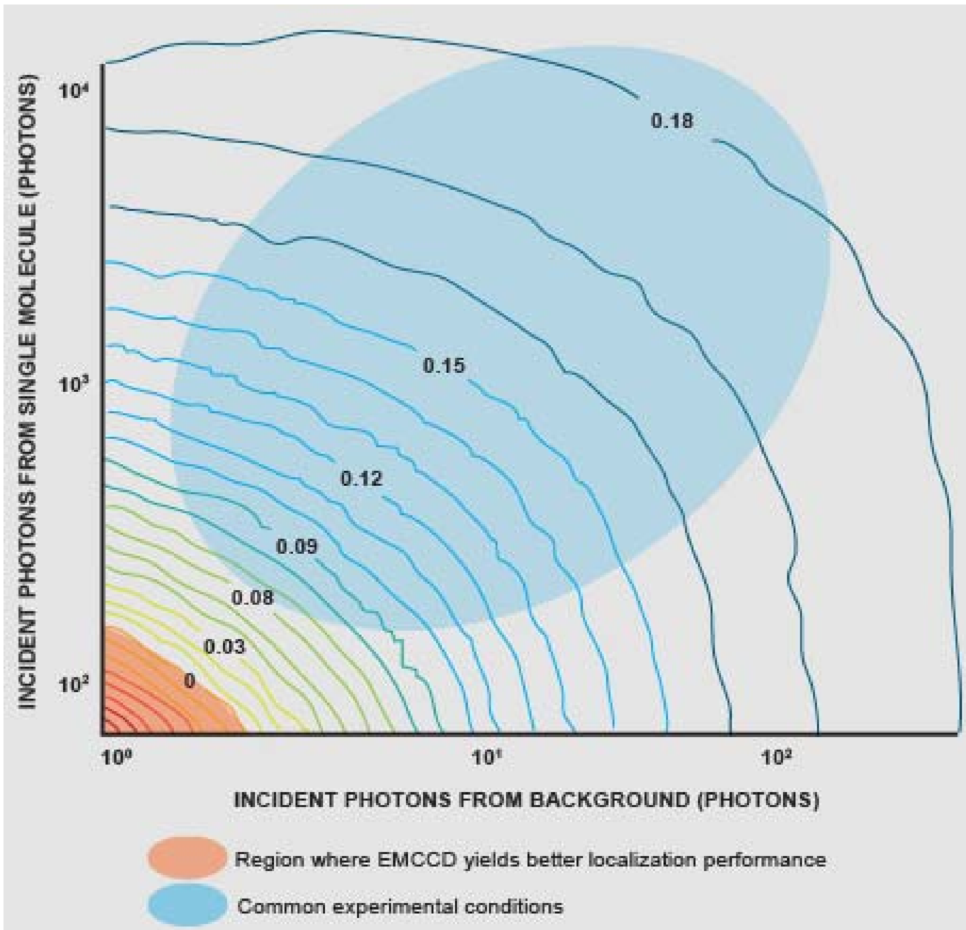
Camera Correction: Implemented pixel-specific read noise into probability model for MLEM.

Details:

Speed: 0.03 s/ reconstructed image
Light: ~3,000 photons /mol/ frame
Mag: 60X,
Camera: sCMOS, 3200 fps

Courtesy of F. Huang,(Bewersdorf Lab)

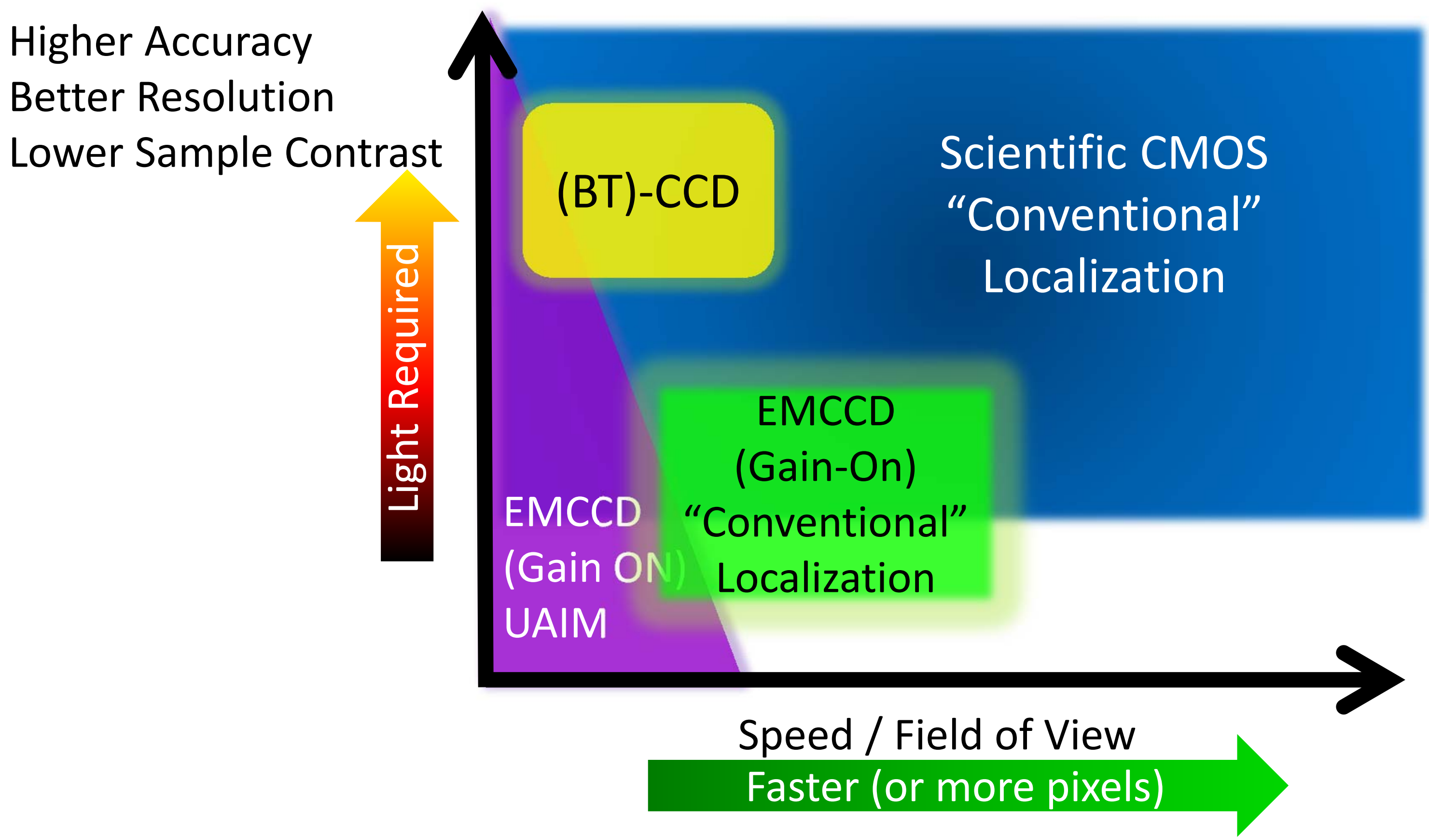
Localization Precision "conventional" EMCCD vs. sCMOS



Courtesy of F. Huang. Bewersdorf Lab, Yale

Adapted from F. Huang *et al.*, Nature Methods 10(7): 653-658 (2013)

MINIMIZING THE GAP: MATCHING THE CAMERA TO YOUR NEEDS



HAVE YOU DONE A GAP ANALYSIS?

1. How much light do I have?

   The relative performance of CCD, back-thinned CCD, EMCCD and sCMOS cameras is light-level dependent.

2. Do I know my camera's strengths and weaknesses?

   No camera is perfect; proper use is required for the best results and to avoid errors

3. What is the goal of my experiment?

   The most appropriate choice of camera depends upon the specific super resolution / localization experiment

Acknowledgements

Prof. Zhen-li Huang, Huanzhong University of Science and Technology
F. Long et al, OPTICS EXPRESS 17741 (2012)

Prof. Joerg Bewersdorf, Yale University
F. Huang *et al.*, Nature Methods 10(7): 653-658 (2013)

Prof. Raimund Ober, Texas Southwestern University
J. Chao et al, Nat Meth (2013) doi:10.1038/nmeth.2396

See a movie of 32 fps dynamics in the supplementary materials of the article by Huang et al.

<u>Hamamatsu</u>
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