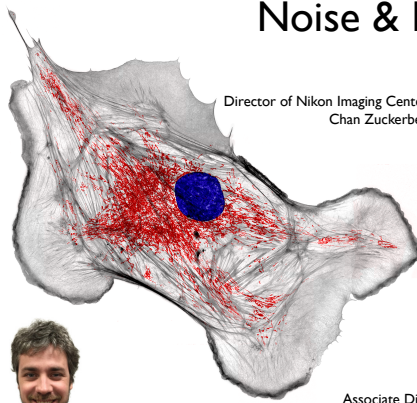
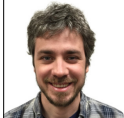


Noise & Detectors

Jennifer Waters, PhD
 Director of Nikon Imaging Center at Harvard Medical School
 Chan Zuckerberg Initiative Imaging Scientist



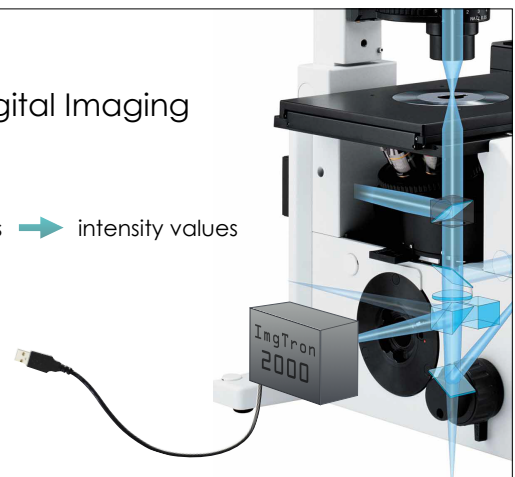
Talley Lambert, PhD
 Associate Director of Imaging Technology



 
 @JenCWaters

Digital Imaging

photons → intensity values



Talley Lambert

Spatial Detectors

- Charge Coupled Device (CCD)
- Complementary Metal-Oxide Semiconductor (CMOS)

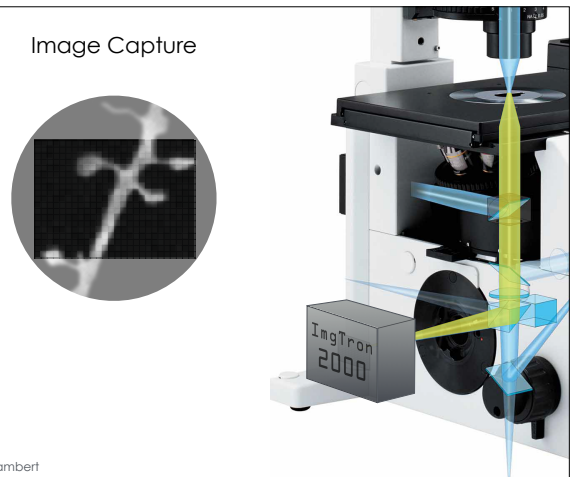
Widefield, Spinning disk, TIRF, light sheet

Non-Spatial Detectors

- Photodiode
- Photomultiplier tube (PMT)

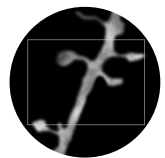
Point scanners

Image Capture

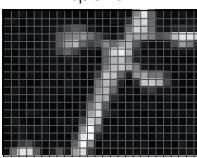


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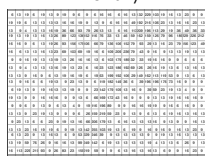
Digital images contain two types of information



spatial



intensity



photons → intensity values

optical image → digital image

intensity values (a.k.a. grayscale values) ≠ photons!!

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Making a Measurement: Accuracy & Precision



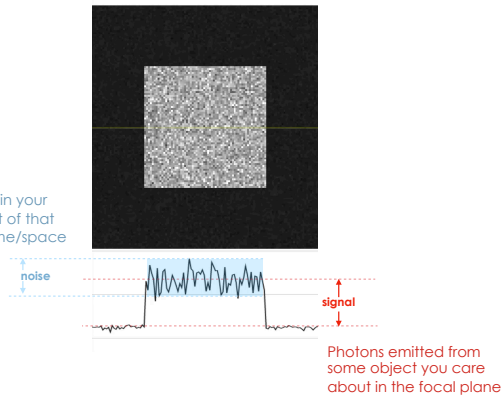
Accuracy: The agreement between a measurement and the ground truth
Precision: The repeatability or uncertainty of a measurement

Example:
 Ground truth = 100.00
 150.00 +/- 0.01 is an inaccurate but precise measurement
 100.00 +/- 50.00 is an imprecise measurement, but the average is accurate

Biology or imprecision?

Signal-to-noise ratio (SNR)

The **variance** in your measurement of that signal over time/space



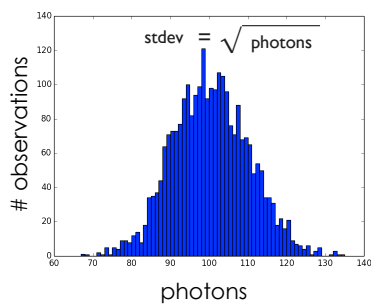
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The Ideal Detector

- Reports exact coordinates of every photon
- Reports exact number of photons

Poisson Noise

If the "true" average intensity is 100 photons, the distribution of your measurements will look something like this...



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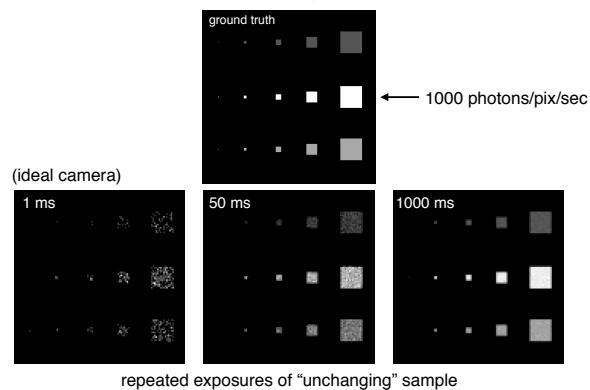
Poisson Noise

As signal increases, so does poisson noise
But the ratio (SNR) also increases

$$\frac{\text{photons}}{\sqrt{\text{photons}}}$$

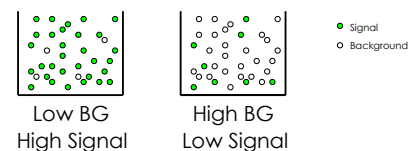
(So it always pays to collect more photons)

Poisson noise on a "perfect" camera



Talley Lambert

The problem with background fluorescence...



$$\text{Poisson noise} = \sqrt{\text{Photons}}$$

Therefore, background reduces signal and increases noise

How do we convert photons to intensity values?

Let's ignore the spatial component for now
starting with a "single pixel camera"....



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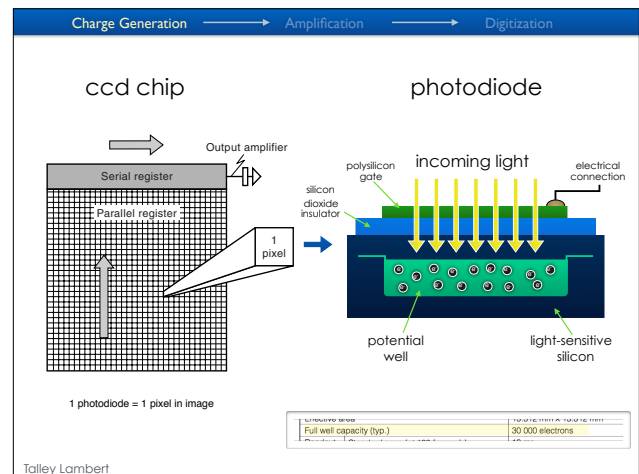
Camera Specifications Sheets



SPECIFICATIONS

Type number	ORCA-R2 (C10600-10B)
Camera head type	Hermetic vacuum-sealed head
Dual cooling	Air cooling / water cooling
Imaging device	ER-150 progressive scan interline CCD
Effective number of pixels	1344 (H) X 1024 (V)
Cell size	6.45 µm (H) X 6.45 µm (V)
Effective area	8.67 mm (H) X 6.60 mm (V)
Dual scan mode	Normal scan / Fast scan
Pixel clock rate	Normal scan: 14.00 MHz/pixel Fast scan: 28.00 MHz/pixel
Readout noise (r.m.s.) typ.	Normal scan: 6 electrons Fast scan: 10 electrons
Full well capacity typ.	High dynamic range mode ①: OFF ON: 18 000 electrons 36 000 electrons
Dynamic range typ. ②	3 000 : 1 (at Normal scan / 1X1)
Cooling method / temperature	Forced-air cooled: -35 °C Water cooled: -40 °C (Water temperature: +20 °C)
Dark current	0.0005 electrons/pixel/s (at -40 °C)
Dual A/D converter	12 bit or 16 bit
Exposure time	10 µs to 4200 s
Binning	2 X 2, 4 X 4, 8 X 8
Sub-array	Yes
Dual light mode	Low light mode / High light mode
High dynamic range mode ①	Yes
Analog gain feature	Yes (10times max.)

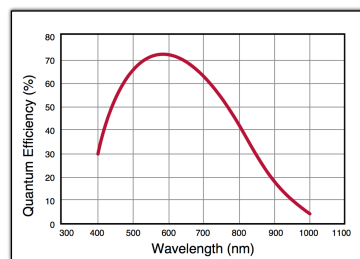
Talley Lambert



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Quantum Efficiency (QE)

wavelength dependent probability that an incident photon will get 'detected' and generate a photoelectron

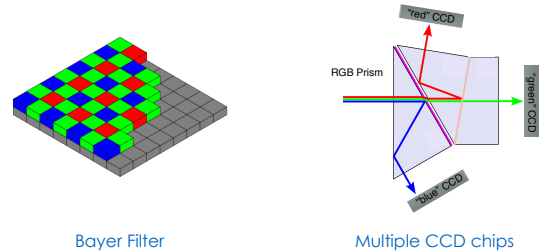


hamamatsucameras.com

Talley Lambert

NOTE: CCDs and sCMOS are monochromatic detectors
(they cannot discriminate between photons of different wavelengths)

2 ways to generate a "color camera"



but ... these filters are not very versatile for quantitative imaging

Charge Generation → Amplification → Digitization

Backed thinned CCDs have the highest QE

front-illuminated back-illuminated

Video Microscopy, 2nd Ed., Inoué and Spring

Charge Generation → Amplification → Digitization

dark current

thermally (spontaneously) generated electrons that DON'T result from photons
(only problematic for multi-second exposures or EMCCD cameras)

Dark Noise versus Temperature

Figure 2

dark current is reduced by cooling the chip

Temperature (°C)	Dark current (electrons/pixel/s)
-40	0.0005

Charge Generation → Amplification → Digitization

We've converted some photons to electrons ... now we need to "count" them

"potential well"

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Charge Generation → Amplification → Digitization

An **amplifier** converts electrons into voltage

The **voltage output** of the amplifier is directly proportional to the number of **input electrons**

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Charge Generation → Amplification → Digitization

read noise

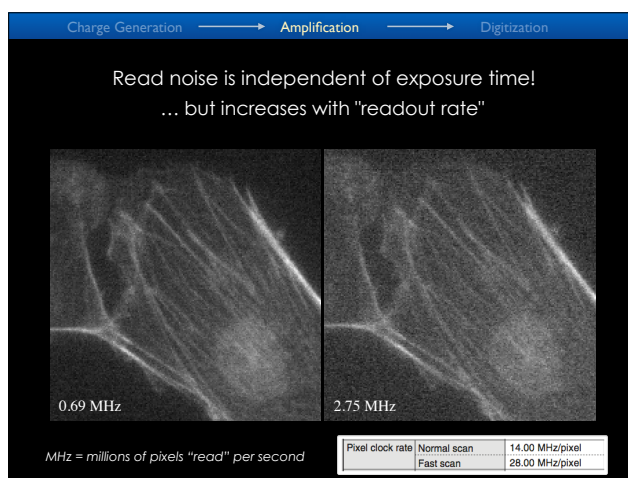
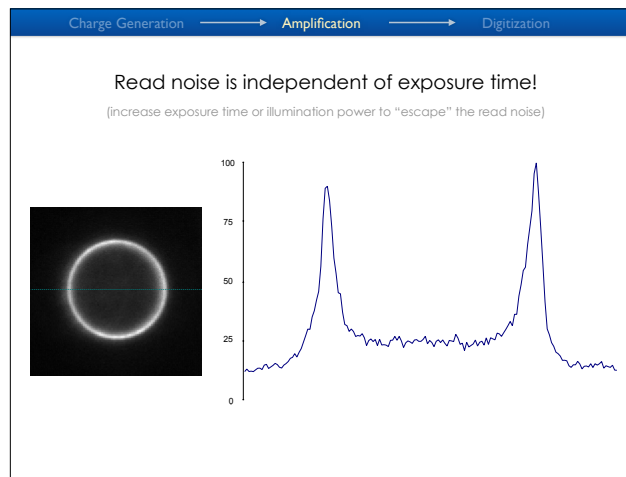
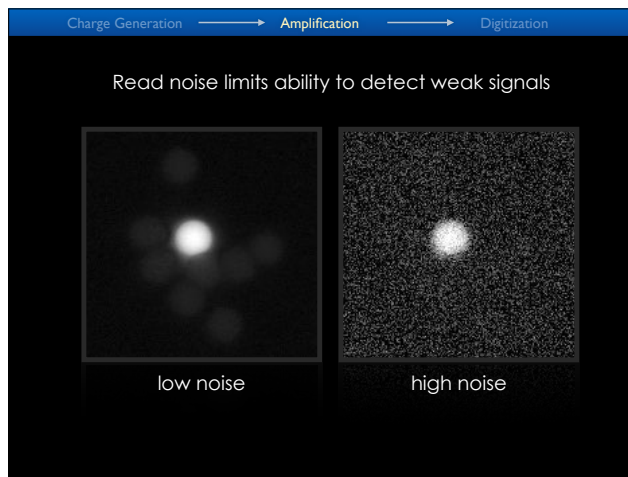
imprecision in the charge-to-voltage conversion
(due to electrical noise in the readout amplifier)

The units are electrons!

Readout noise	Standard scan (at 100 frames/s, typ.)	Slow scan (at 30 frames/s, typ.)
1.9 electrons rms (1.3 electrons median)		
1.5 electrons rms (0.9 electrons median)		

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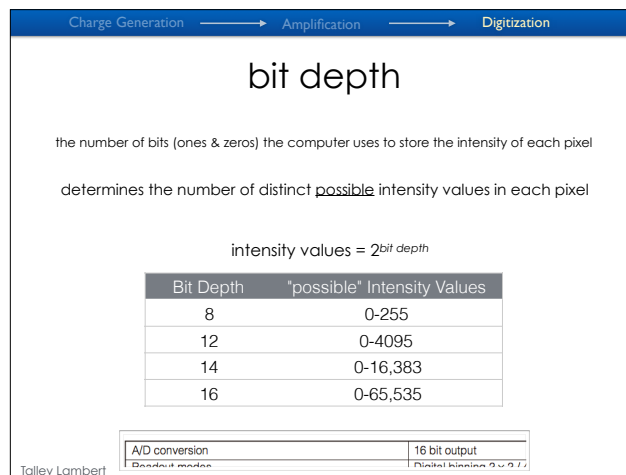
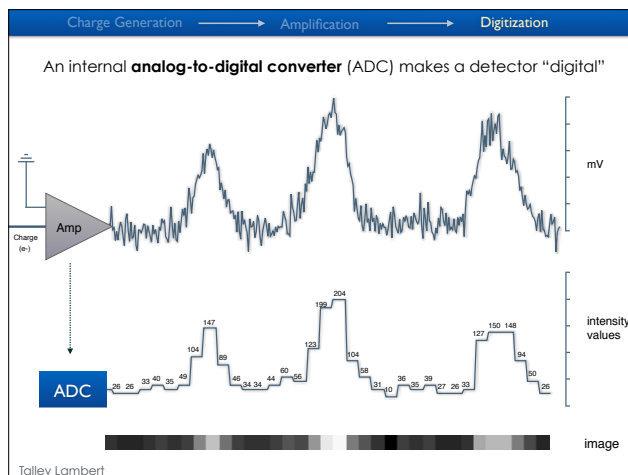


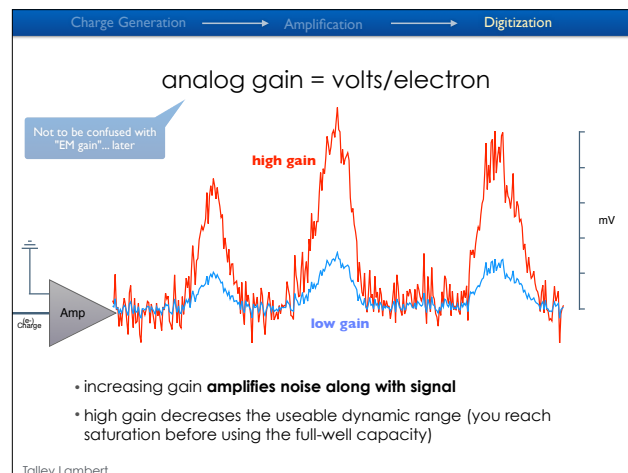
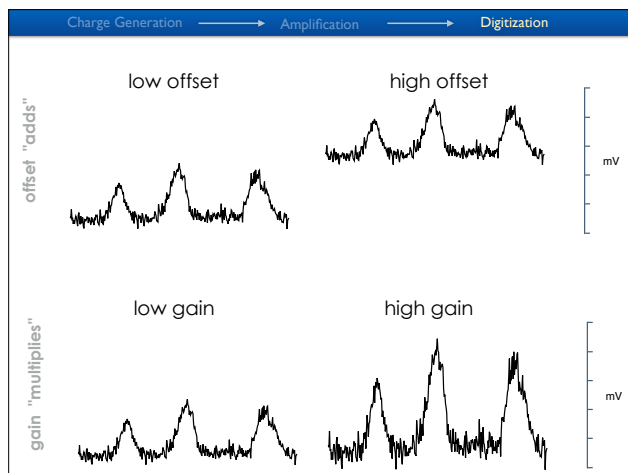
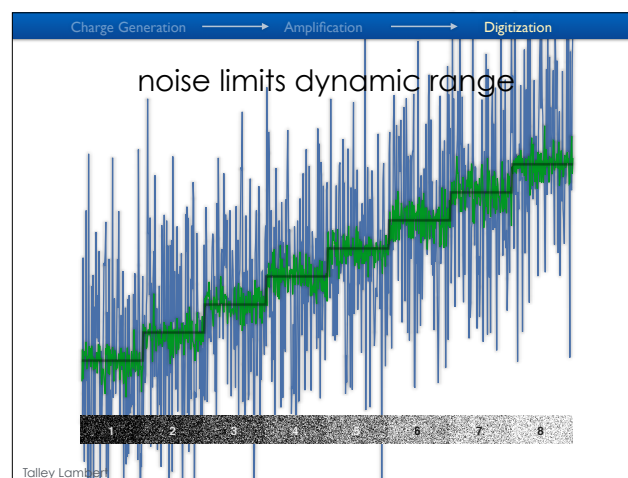
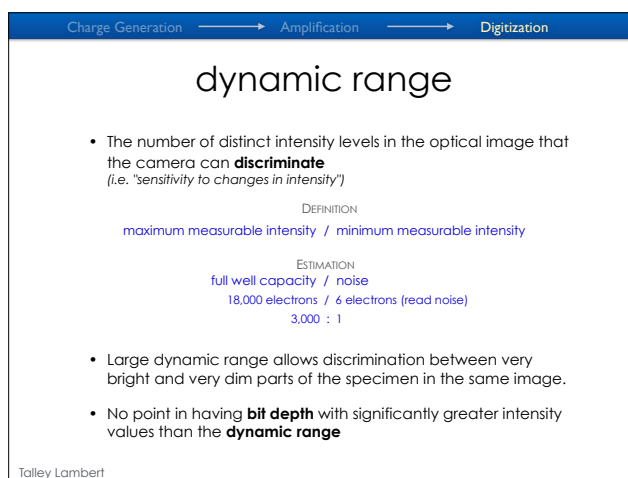
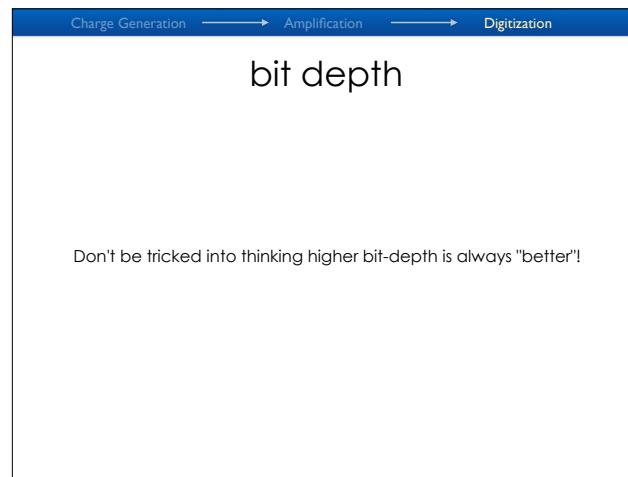
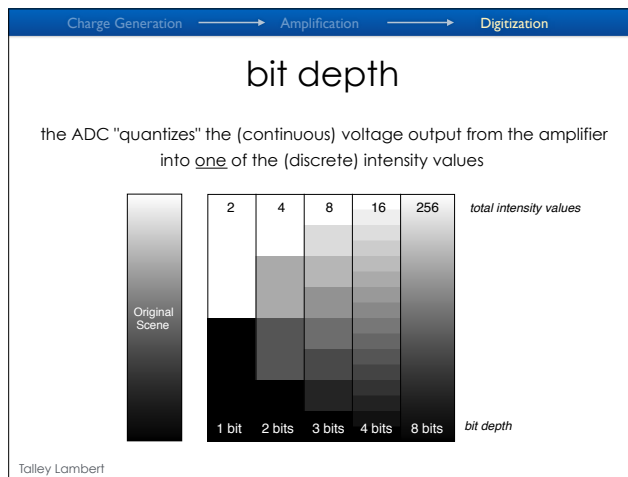


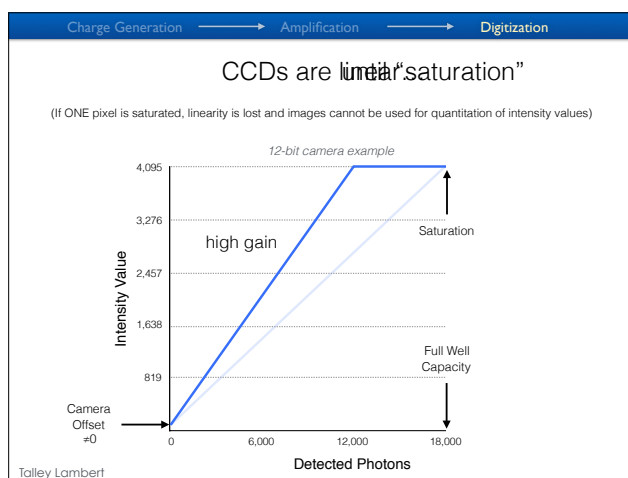
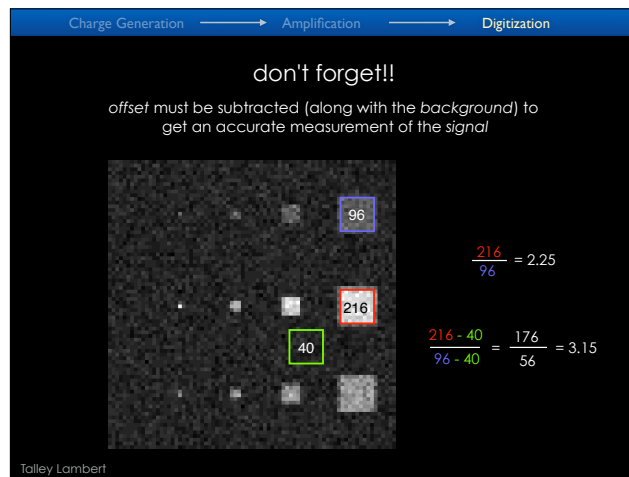
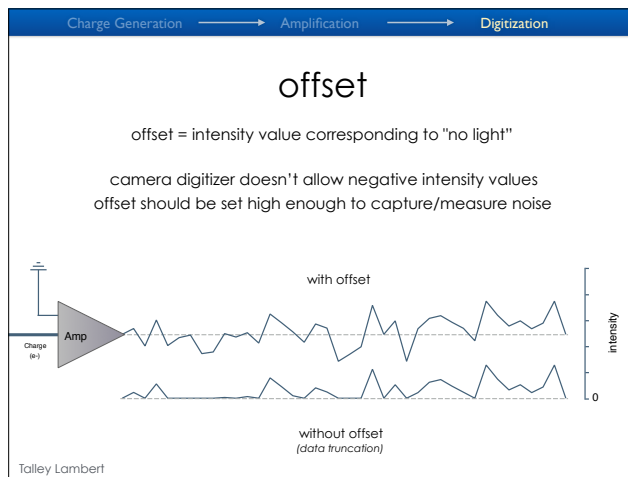
Charge Generation → Amplification → Digitization

Up until this point, we've just been dealing with (analog) electrons and voltages...

there's been nothing "digital" at all!

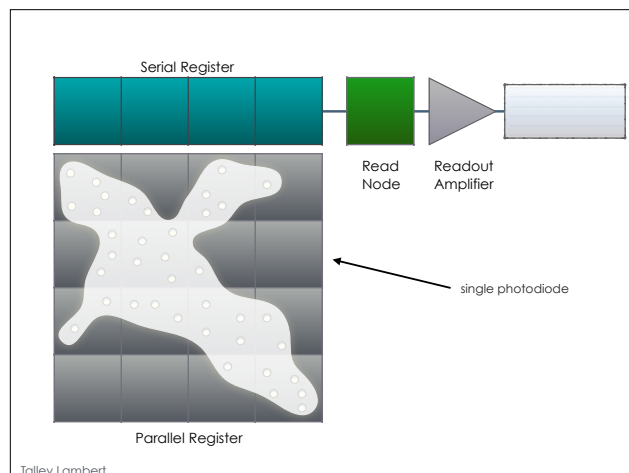
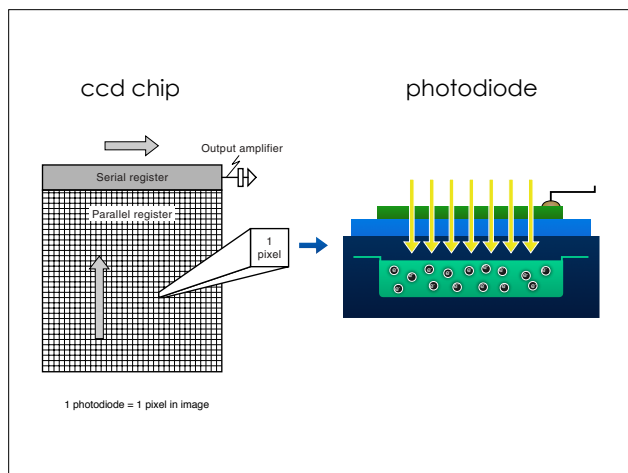


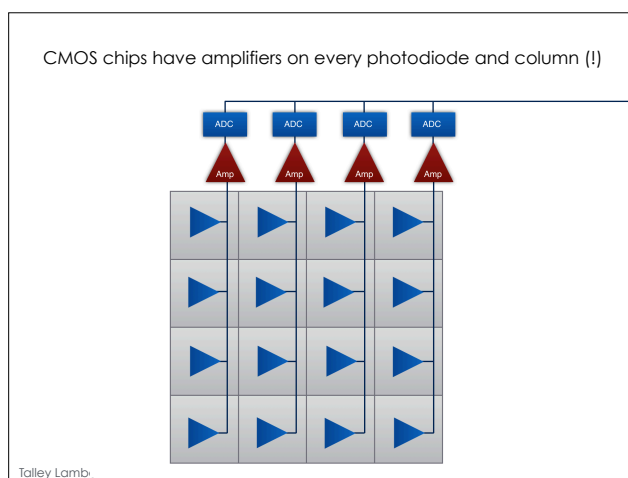
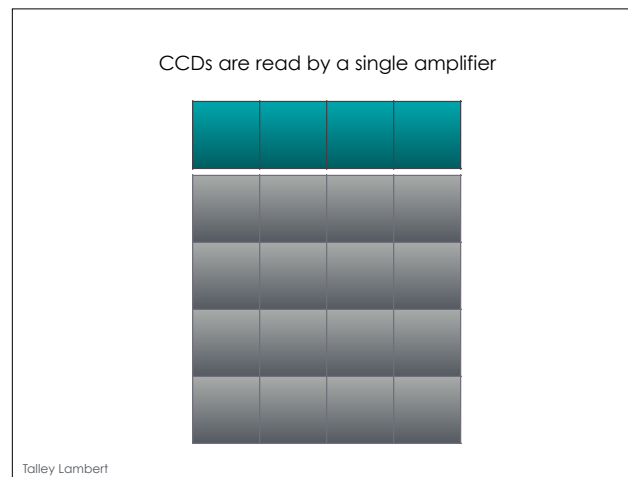
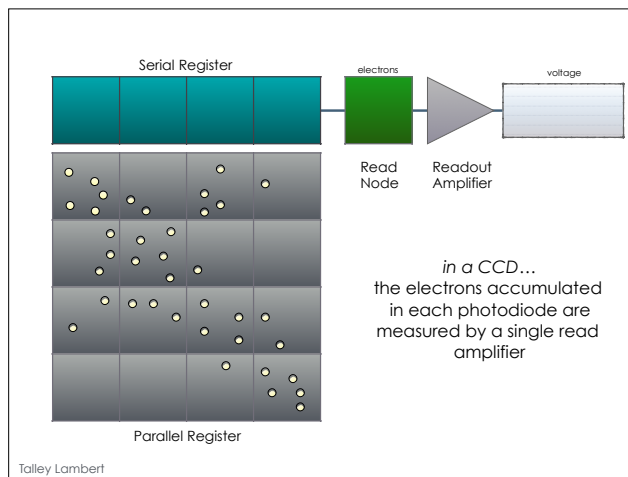




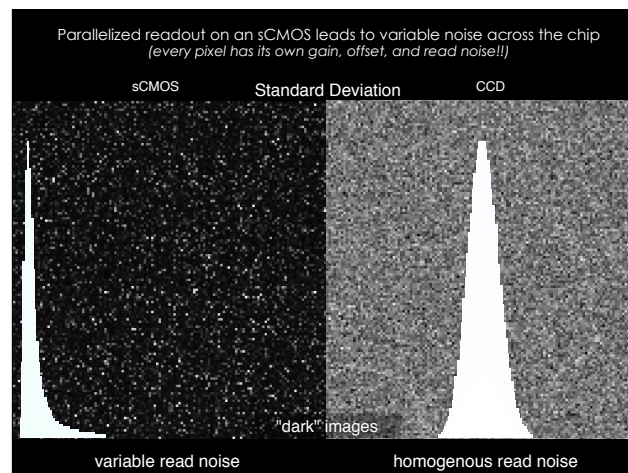
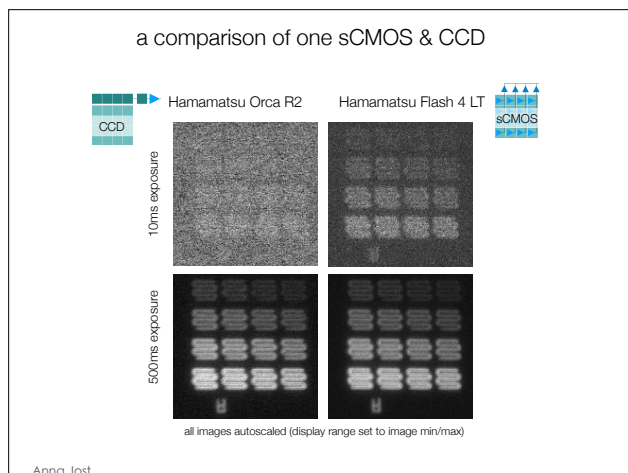
Ok... back to the spatial spatial component

(creating a 2D image from a grid of photodiodes....)



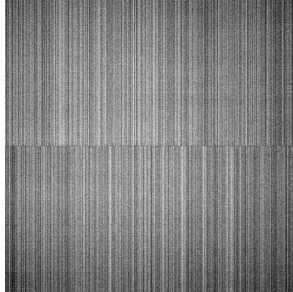


- Parallelized readout on an sCMOS enables:
- lower read noise
 - very fast frame rates
 - large field of view (more pixels)



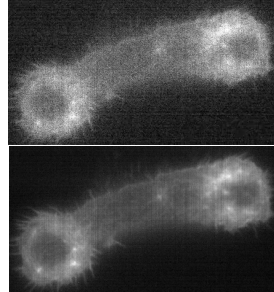
pixel-dependent gain and offset cause "fixed pattern noise" on an sCMOS

example time lapse



"Flat Field Image" revealing variable gain
(don't over-interpret the magnitude of variability without numbers)

Talley Lambert



average of the time-lapse

Rolling Shutter Readout

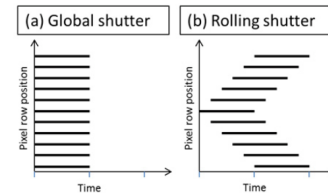
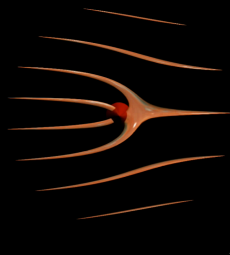
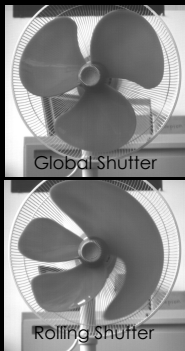


Figure 2: Exposure timing by pixel row position
hamamatsu.com

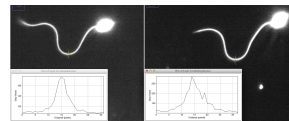
Rolling shutter artifact in a CMOS



Anton River - YouTube

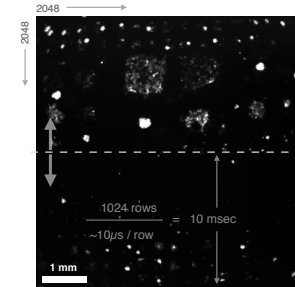
When might you need to worry about rolling shutter?

Very rapidly moving objects



<http://blog.mshalin.com/2012/08/global-exposure-with-scientific-cmos.html>

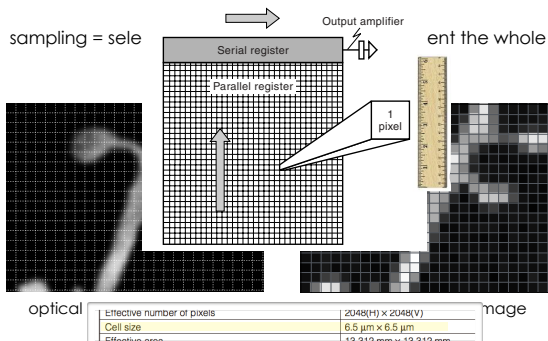
Temporal correlation across FOV



50 fps, realtime playback
Worley et al (2017) doi: 10.1364/BOE.8.005794

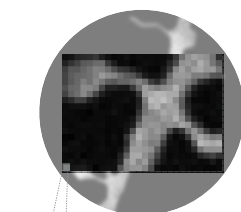
Talley Lambert

Photodiode **size** affects spatial sampling ...



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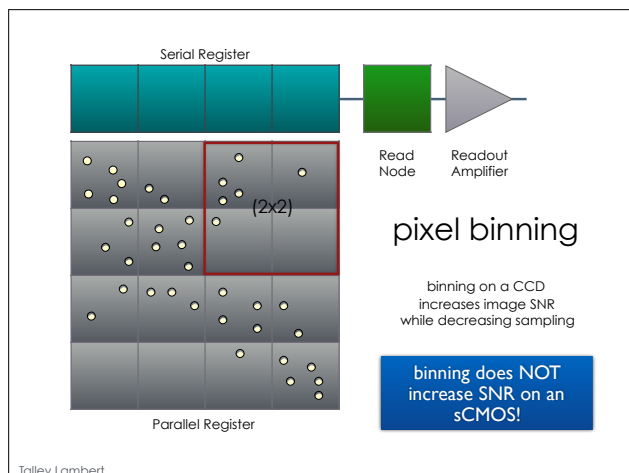
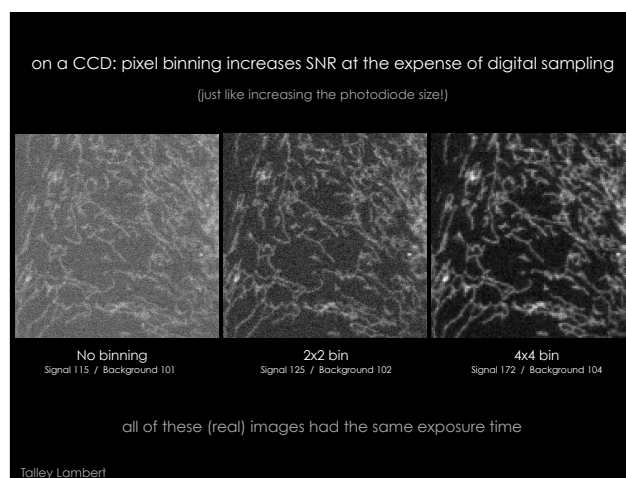
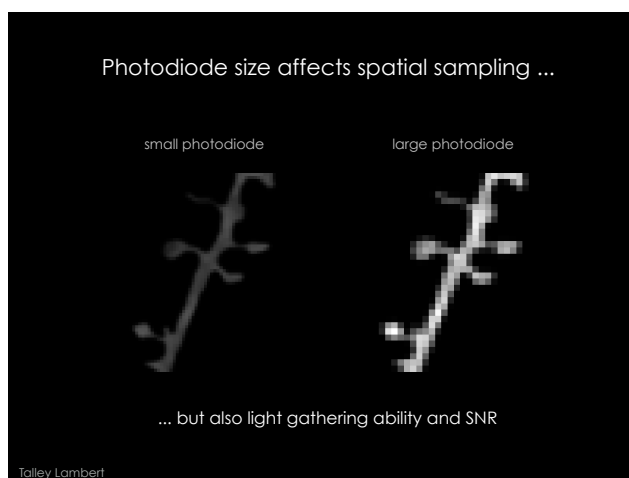
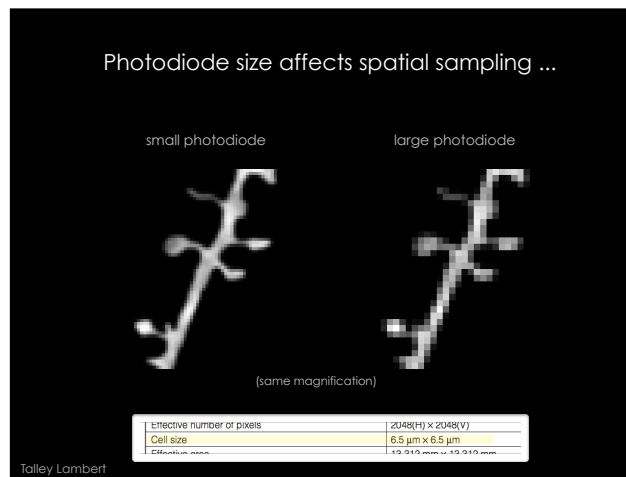
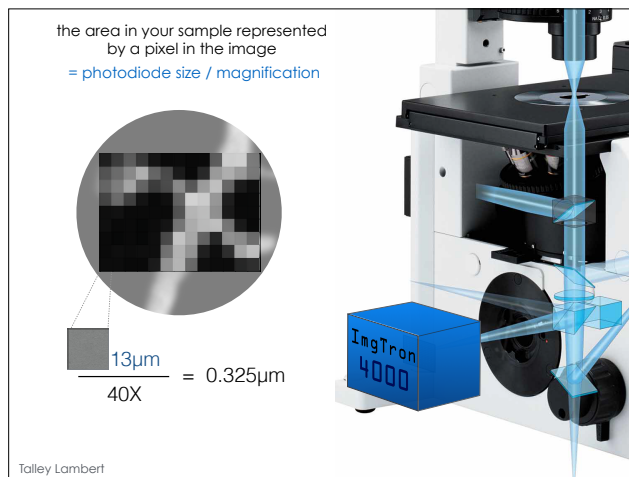
the area in your sample represented by a pixel in the image
= photodiode size / magnification



$$\text{e.g. } \frac{6.5\mu\text{m}}{20X} = 0.325\mu\text{m}$$



Talley Lambert



$$\text{Image pixel size} = \frac{\text{Photodiode size}}{\text{Total Magnification}}$$

example calculation with high NA lens

6.5 μm camera photodiode
60X objective

$$\text{image pixel} = \frac{6.5 \mu\text{m}}{60\text{X}} = 108\text{nm}$$

GFP (525nm emission)
1.42 NA Objective

$$\text{airy disk diameter} = 2 \times \frac{0.61 \times 525\text{nm}}{1.42} = 451\text{nm}$$

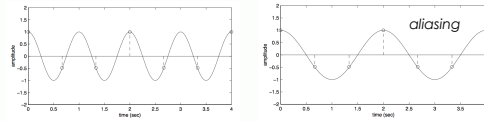
(abbe equation)

$$\text{sampling rate} = \frac{\text{pixels}}{\text{airy disk}} = \frac{451}{108} = 4.2 \text{ pixels per airy disk}$$

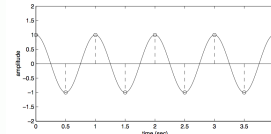
Nyquist Sampling Theorem

Sampling rate should be at least twice the highest frequency contained in the signal

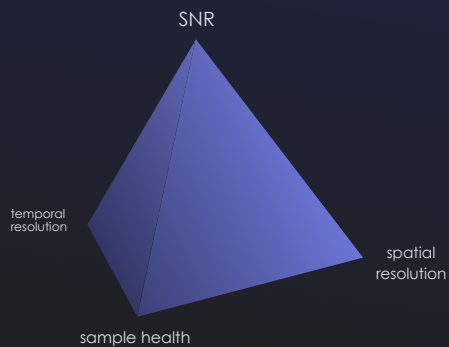
Under-sampling:



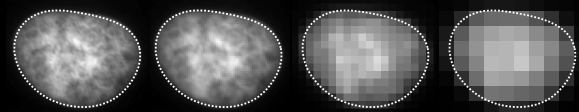
Nyquist sampling:



Here's where you're on your own...



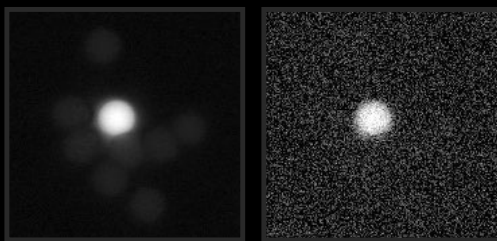
think about what you are trying to "resolve"



you don't always need the highest spatial sampling
(... and you will "pay" for it with SNR)

Talley Lambert

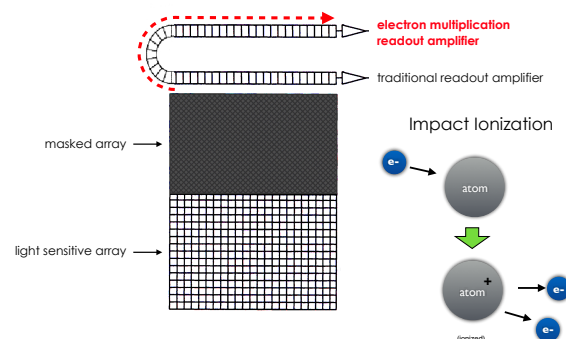
What if your signal is below the noise?



(this can happen with extremely low light or high speed imaging)

Charge Generation → Amplification → Digitization

Electron Multiplying CCDs (EMCCDs):
when signal is below the read noise



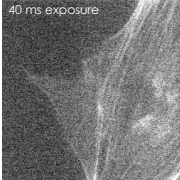
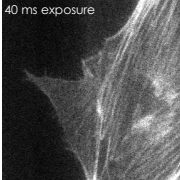
Talley Lambert

Charge Generation → Amplification → Digitization

Electron Multiplying CCDs (EMCCDs):

Major Benefit:

- Can amplify very low signals until they are detectable above the read noise

no EM gain EM gain

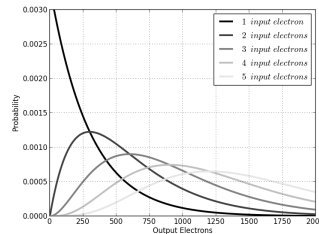
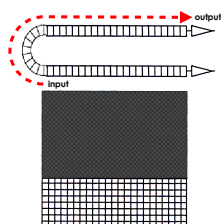
Talley Lambert

Charge Generation → Amplification → Digitization

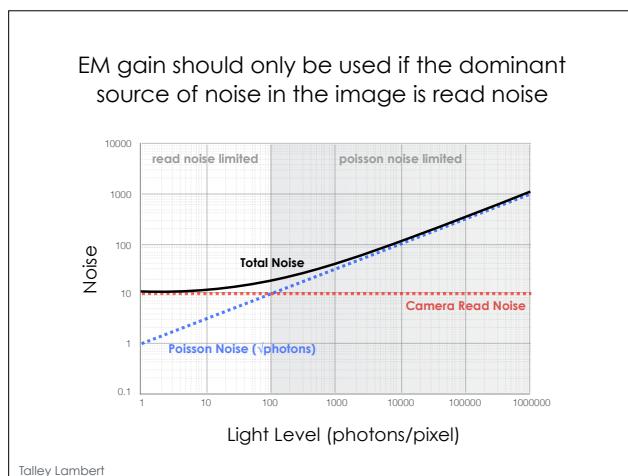
Electron Multiplying CCDs (EMCCDs):

Major Disadvantage:

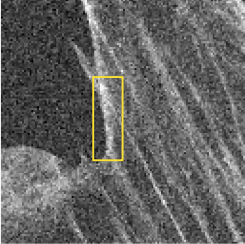
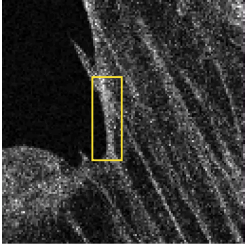
Electron multiplication adds a new source of noise!
"Multiplicative noise"

Lambert, T. J., & Walters, J. C. (2014). Methods in Cell Biology, 123



Which image has higher SNR?

SNR = 8 SNR = 4

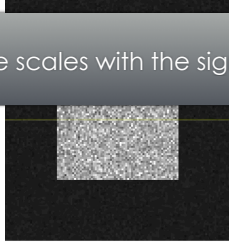
Talley Lambert

CCD



SNR = 8

EMCCD



SNR = 4

EM (multiplicative) noise scales with the signal

sign

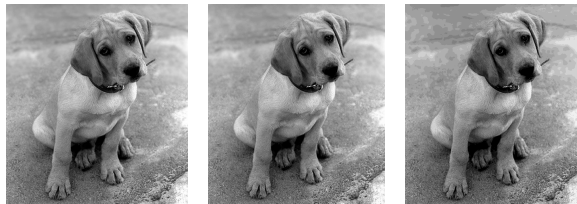
Talley Lambert

Data ≠ Image

Data = Numbers

Judge image quality by looking at the
image intensity values,
not the image on the monitor!

Do not judge image quality based on how the image looks on the monitor...



256 Gray levels

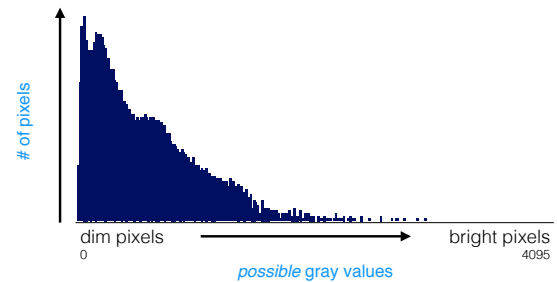
64 Gray levels

16 Gray levels

Images from Butch Moomaw, Hamamatsu

Use software to monitor intensity values

(The image histogram is your best friend)



Talley Lambert

Image processing for display

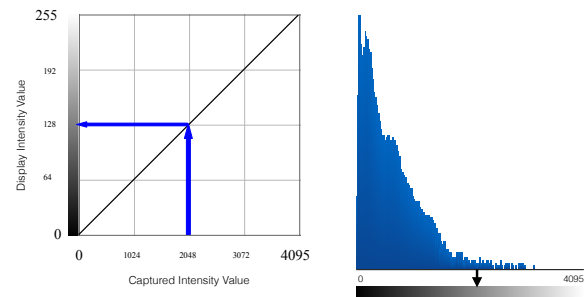
mapping image intensity to display intensity

photons
optical image



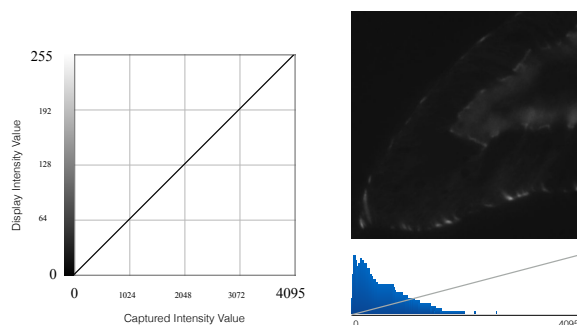
intensity values
digital image

mapping image intensity to monitor intensity
("look up tables")



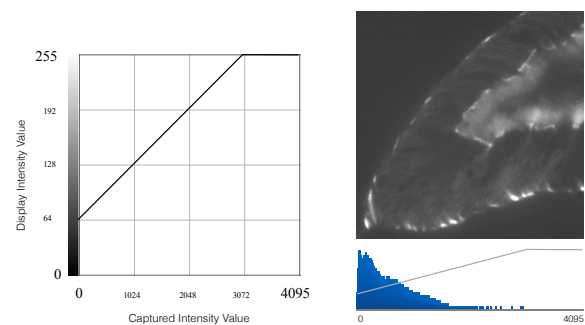
Talley Lambert

Full Scale

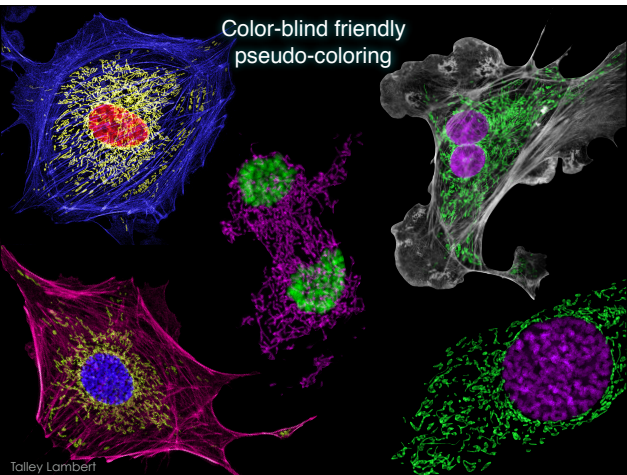
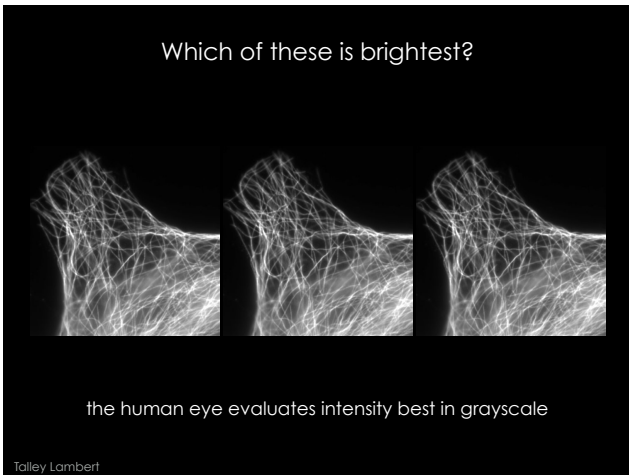
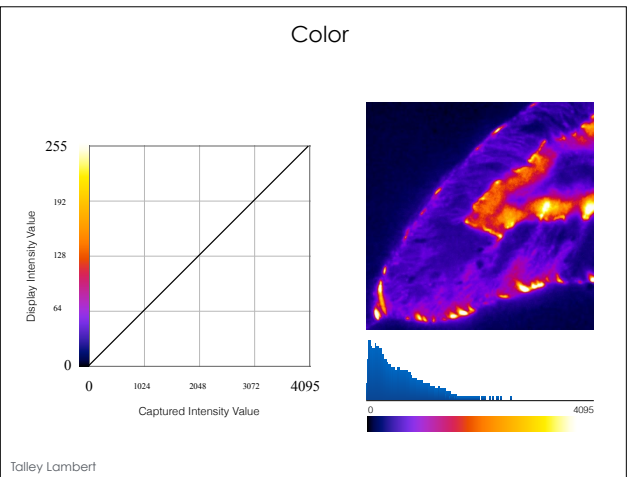
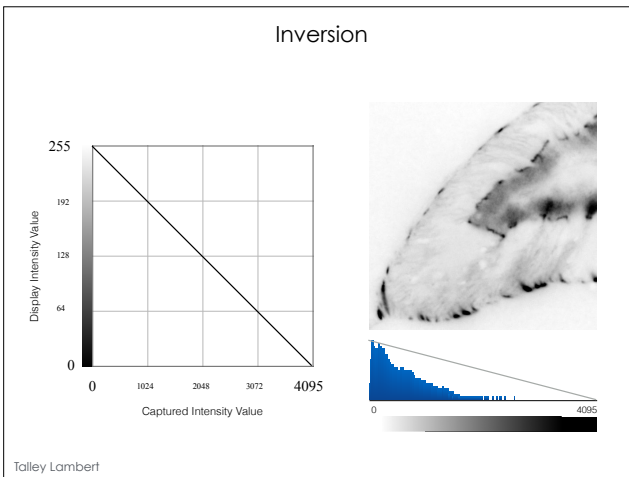
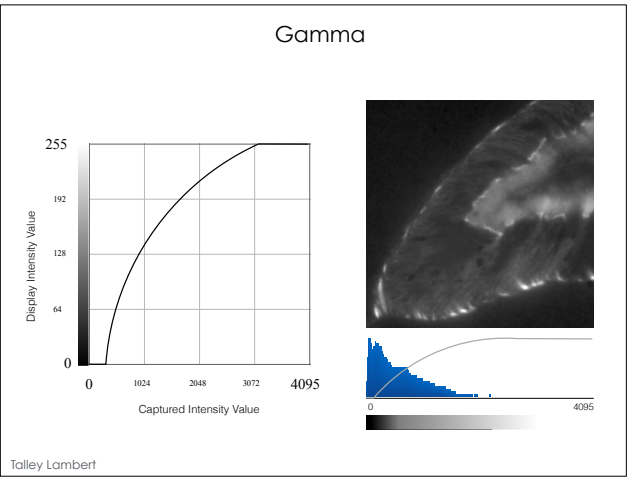
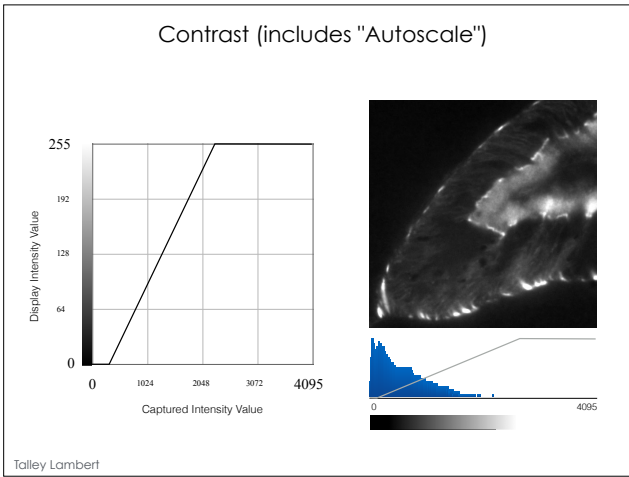


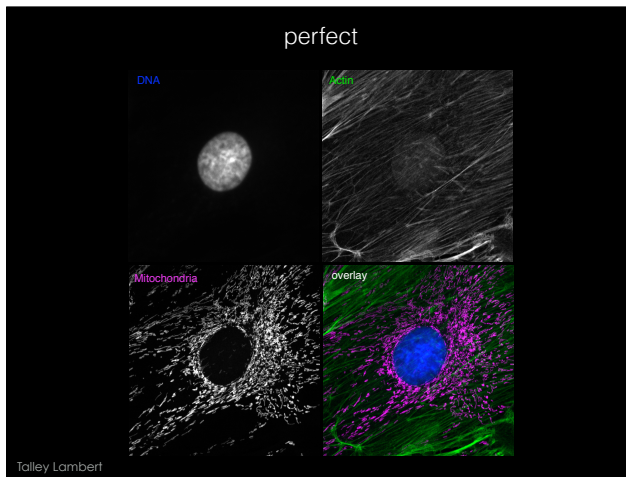
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Scaling / Brightness

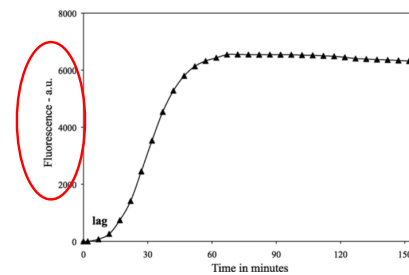


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What units are used to report fluorescence intensity?



What units are used to report fluorescence intensity?

A.U.s

The correct units are *photons*

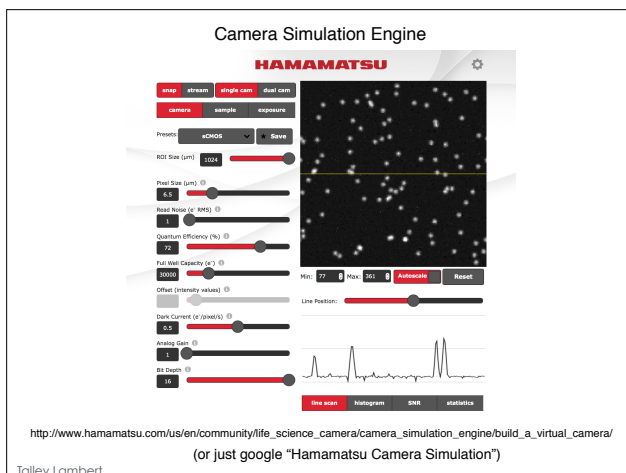
Reporting in photons allows the reader to understand the contribution of Poisson noise and allows for *direct* comparisons between studies

Estimating photons per intensity value...

Number of detected photons per intensity value

$\approx \text{full well capacity} / (\text{max intensity} - \text{offset})$

$$\text{Poisson noise} = \sqrt{\text{Photons}}$$



FPbase Find FP properties, spectra, sequences, & references...

- Community editable
- Moderated
- Create & share collections
- Spectra viewer
- FRET calculator

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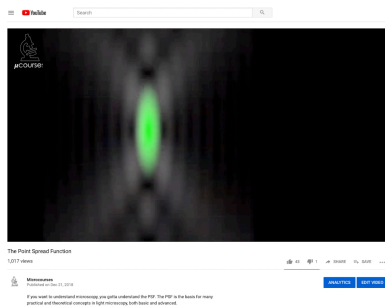
Build a custom spectra viewer for your microscope configuration & evaluate photon efficiency

explore relationships between FP properties



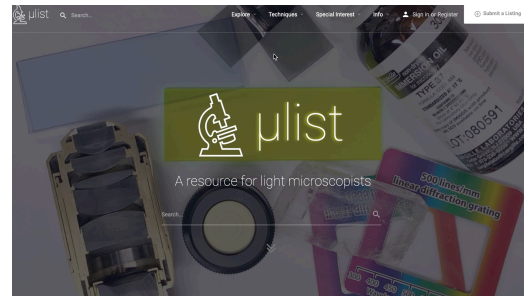
Microcourses YouTube channel

Short educational videos



Microlist.org

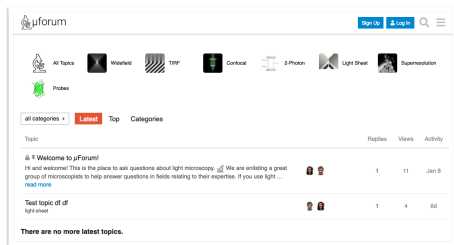
Resources for microscopists



Microforum

Direct interaction with academic
microscopy developers & experts

Launching on Tuesday!



Josh Vaughan
Joachim Goedhart
Hesper Rego
Abhishek Kumar
Brian Beliveau
Reto Fiolka
Patrick Oakes
Ulrike Boehm
Michael Weber
Seamus Holden
Cathy Galbraith
Nicola Maghelli
Nico Stuurman
Andrea Stout
Susan Cox
Melike Lakadamyali
Lin Shao
David Hoffman
Wes Legant
Mark Bates
Sara Abrahamsson
Christoph Lafertre