Introduction to Scientific Cameras

Dr Louis Keal

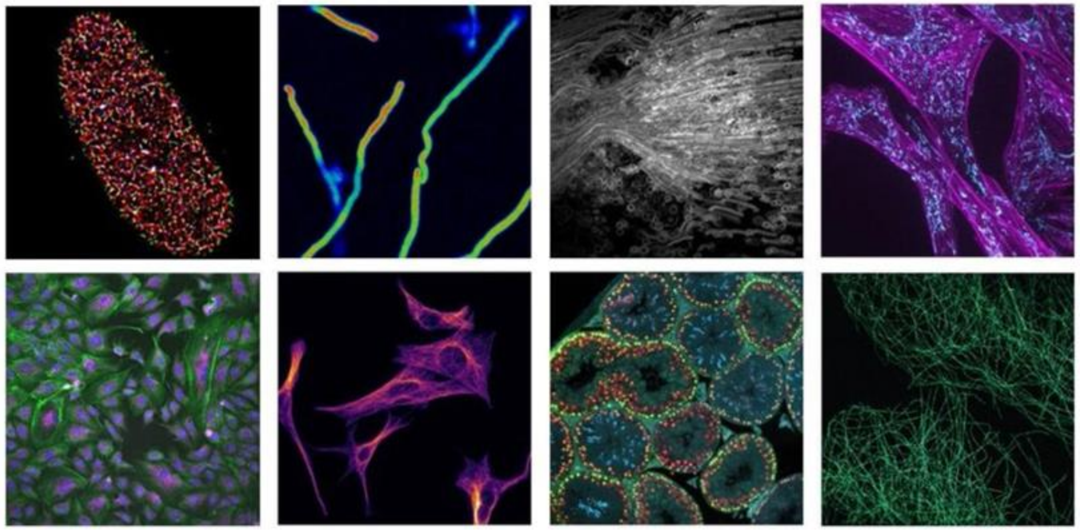
Teledyne Photometrics

About Teledyne Photometrics

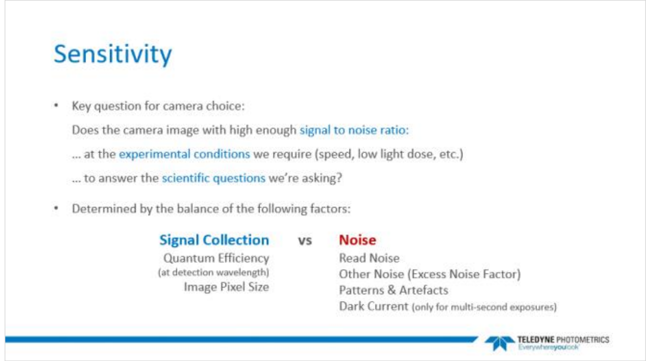
- Design and manufacture **high end** cameras for **low light imaging,** mostly in **biological applications**
- Market Leader for **Scientific CMOS technology**
- Established in 1978 and part of the **Teledyne Imaging Group** since 2019
- Global Headquarters in Tucson, Arizona, **USA**
- Factory in Surrey, British Columbia, **Canada**
- Sales and Marketing HQ in Birmingham **UK**
- Additional offices around the world

TELEDYNE PHOTOMETRICS
Everywhereyoulook™

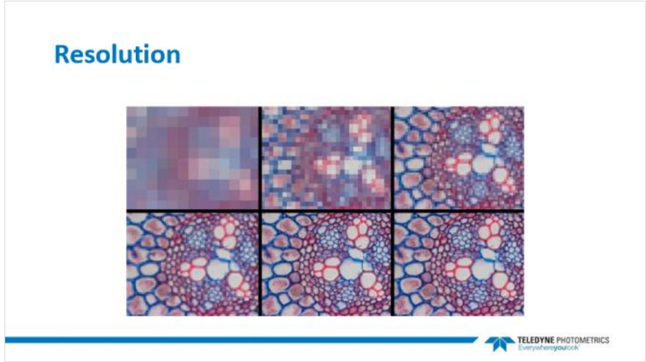
Part of the Teledyne Imaging Group



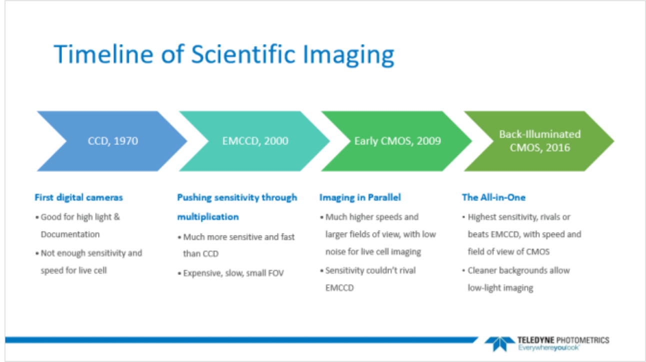
Important Camera Specs & Topics



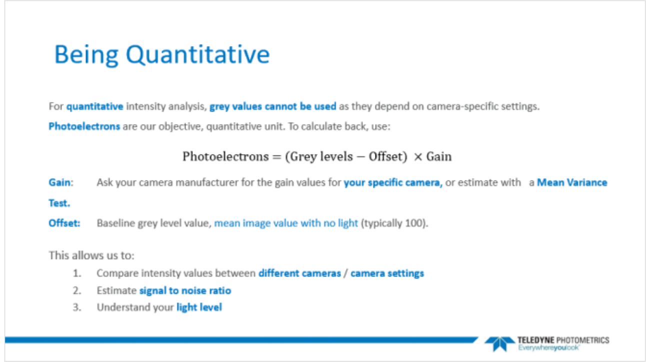
Sensitivity



Resolution



Camera Technologies



Quantitative Imaging

Scientific Cameras

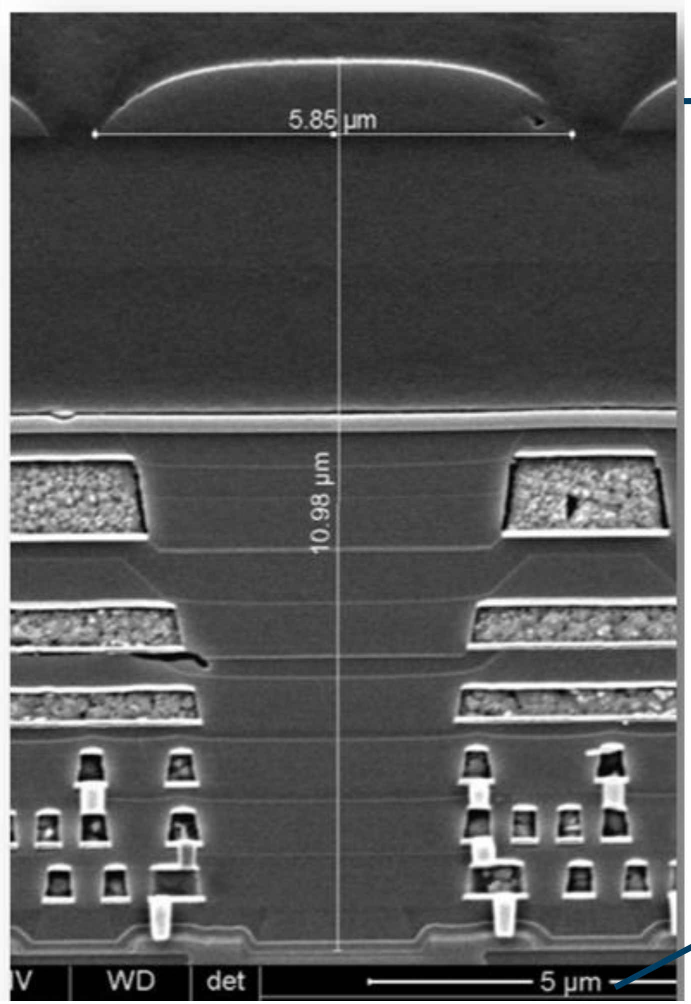
Everyone familiar with standard qualitative ‘consumer’ cameras

Scientific-grade cameras are **quantitative** and **highly sensitive**

The camera measures specific quantities of light

The basic measurable unit of light is the **photon**

This Is A Pixel

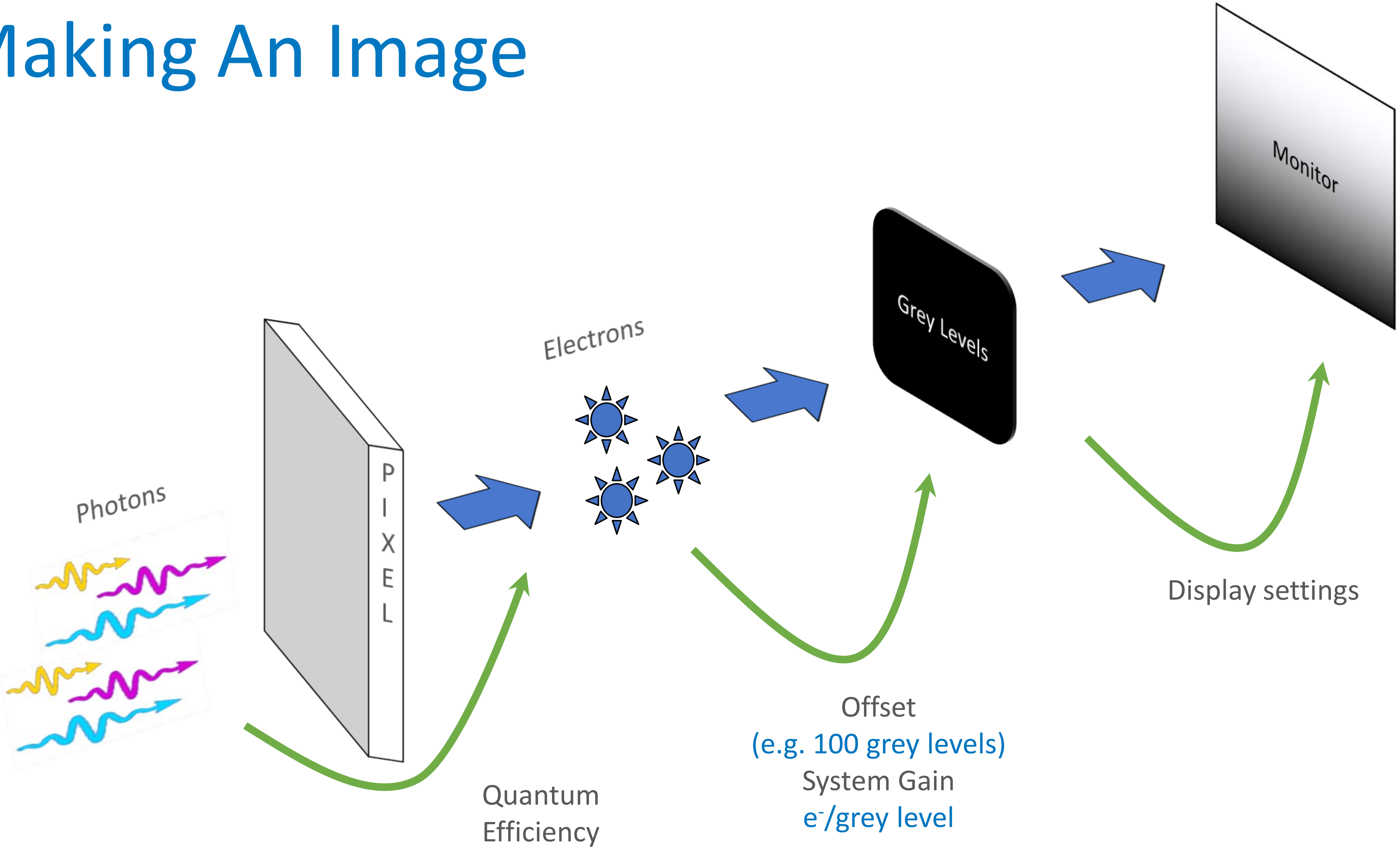


Microlenses

Wiring and electronics

Light-detecting silicon
(below bottom of image)

Making An Image



Sensitivity

- Key question for camera choice:

  Does the camera image with high enough signal to noise ratio:

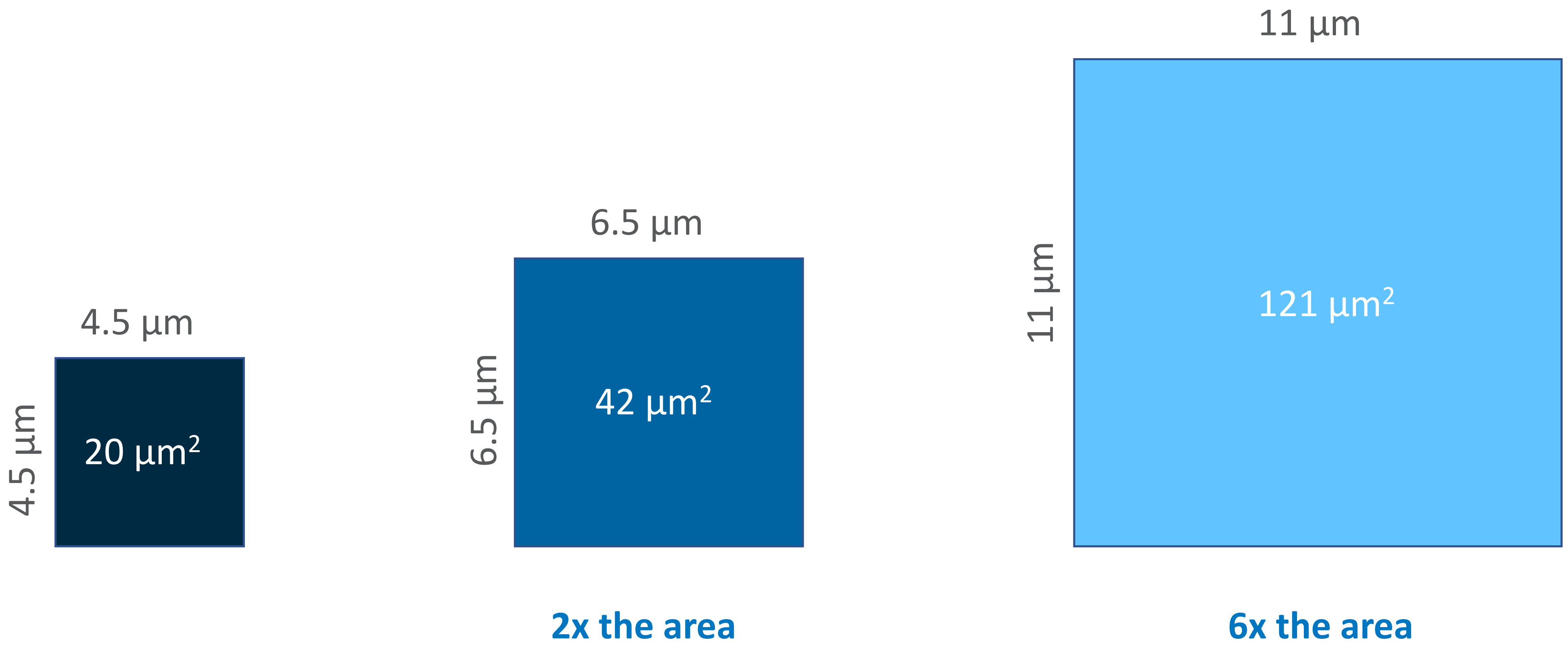
  … at the experimental conditions we require (speed, low light dose, etc.)

  … to answer the scientific questions we're asking?

- Determined by the balance of the following factors:

Signal Collection	**vs**	**Noise**
Quantum Efficiency (at detection wavelength)		Read Noise
Image Pixel Size		Other Noise (Excess Noise Factor)
		Patterns & Artefacts
		Dark Current (only for multi-second exposures)

Pixel Size Matters



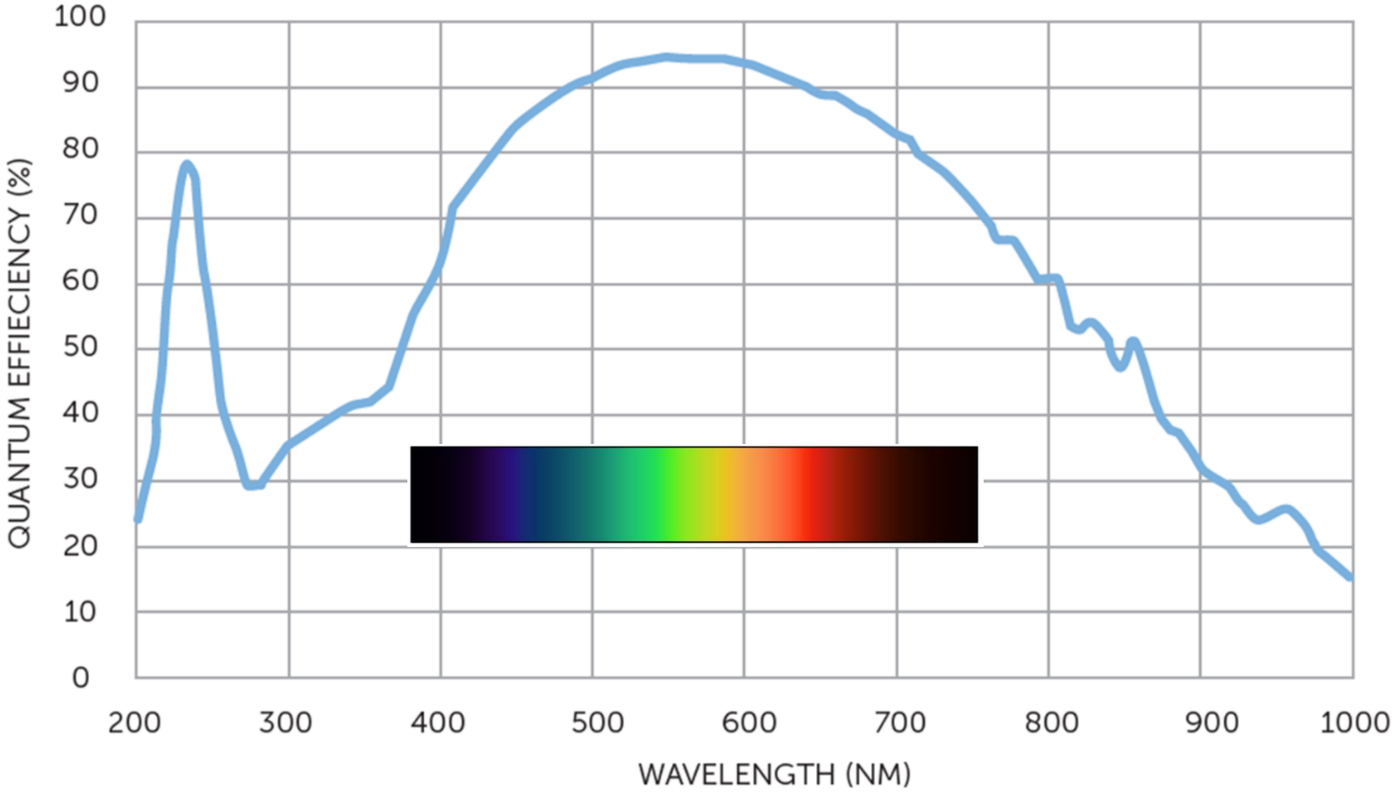
Quantum Efficiency (QE)

QE is the **percentage** of photons that are converted to photoelectrons

If a sensor has 50% QE and is hit with 500 photons, it will result in a signal of 250 electrons

The lower the QE, the more photons are lost

QE **changes with wavelength**



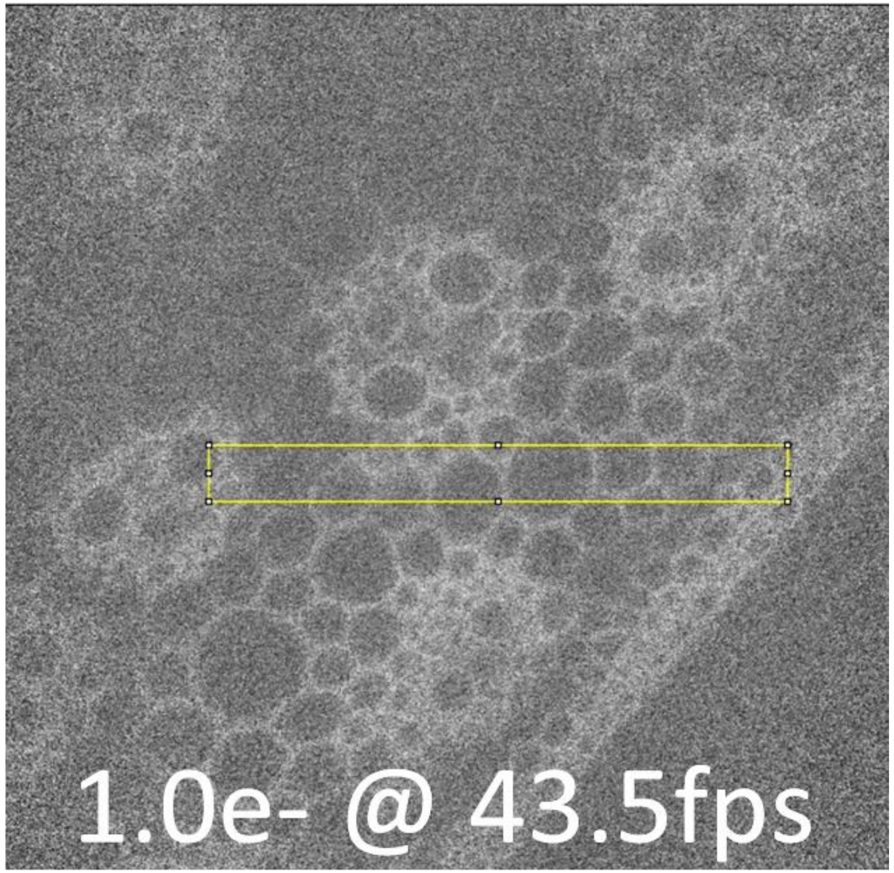
Read Noise

How accurately can we measure how many photoelectrons we collected?

Read noise is primarily determined by quality of electronic design and speed of readout, and has a fixed value for each camera mode.

Prime BSI in CMS mode has a read noise of **±1.0 electrons.**

The lower the light level, the more important it is.



Photon Shot Noise

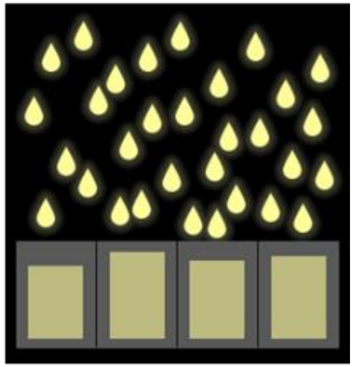
Emission and detection of photons from all sources fluctuates in time.

This is our most commonly visible noise source in imaging.

We may know an average rate of emission, but actual photon events are random (Poisson Distribution).

If we collect N photoelectrons of signal,

Our noise will be $\sqrt{N}$ photoelectrons.



Dark Current Noise

Dark Current Noise is an exposure time and temperature dependent noise source.

Thermal motion of electrons can cause them to enter the pixel well as if they were detected photoelectrons. This is another random Poisson behaviour like Photon Shot Noise.

- **With cooling**, it is typically **negligible for live cell imaging**, and only should be considered for multi-second exposure times.
- **Without cooling**, it must always be considered.

For example, Prime BSI with air cooling achieves D = 0.5 electrons / pixel / second.

Our noise contribution is then $\sqrt{D * t}$, where t is our exposure time.

Even at 1 second exposure, this is still well below our read noise.

Signal To Noise Ratio for CCD & CMOS

$$SNR = \frac{S}{\sqrt{S + D^2 + \sigma_R{}^2}}$$

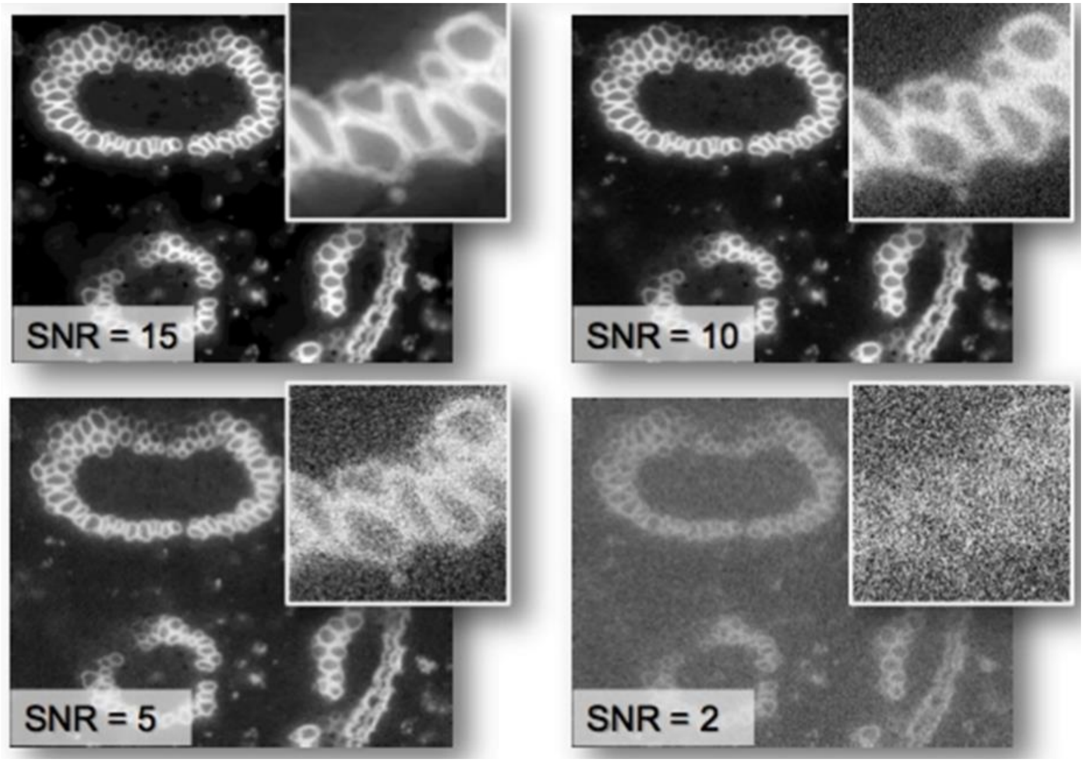
S: Signal

D: Dark current noise

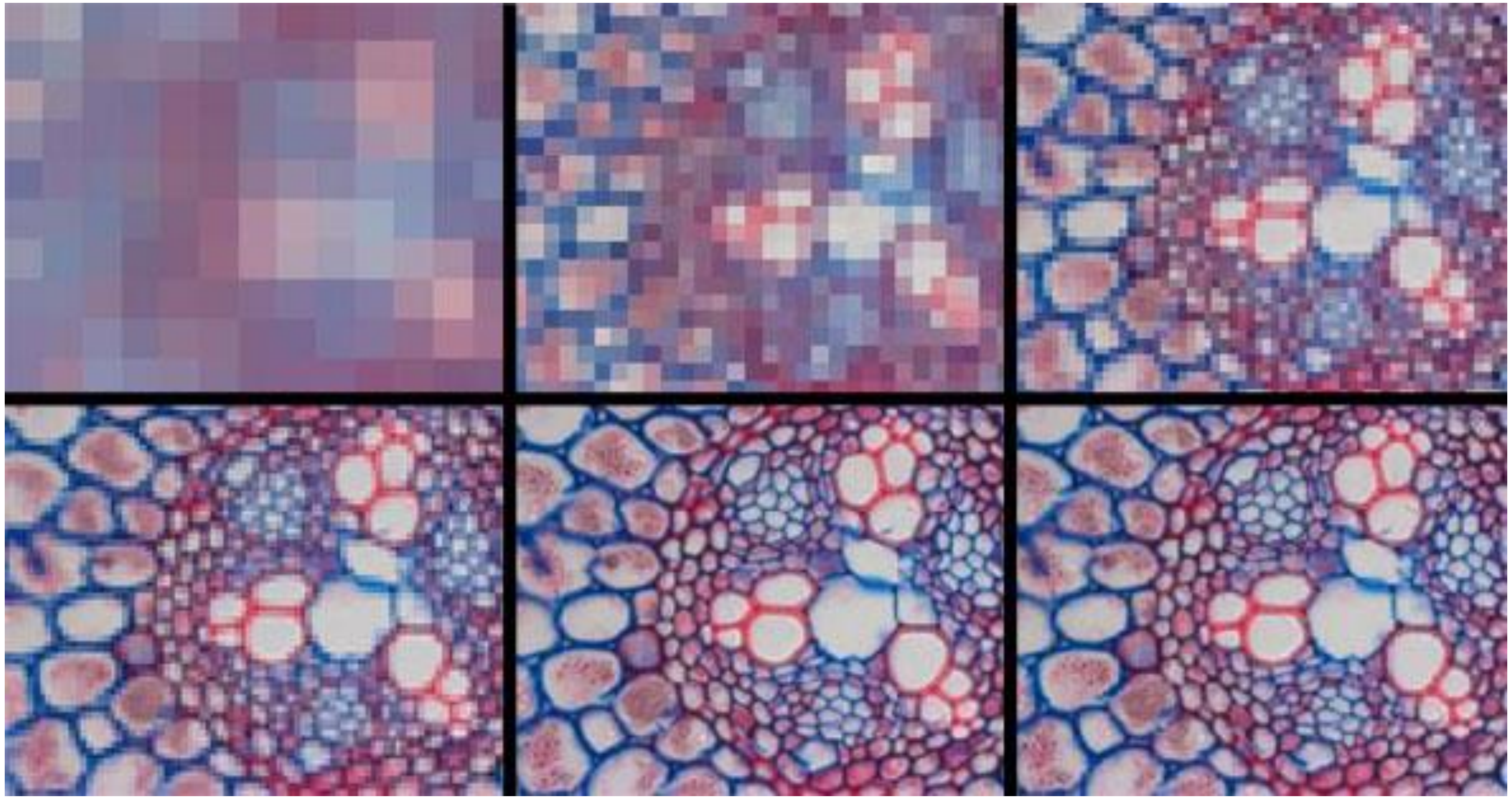
$$D = \sqrt{(Dark\ current\ * exposure\ time)}$$

σ_R: Read noise

√S: Photon shot noise



Resolution

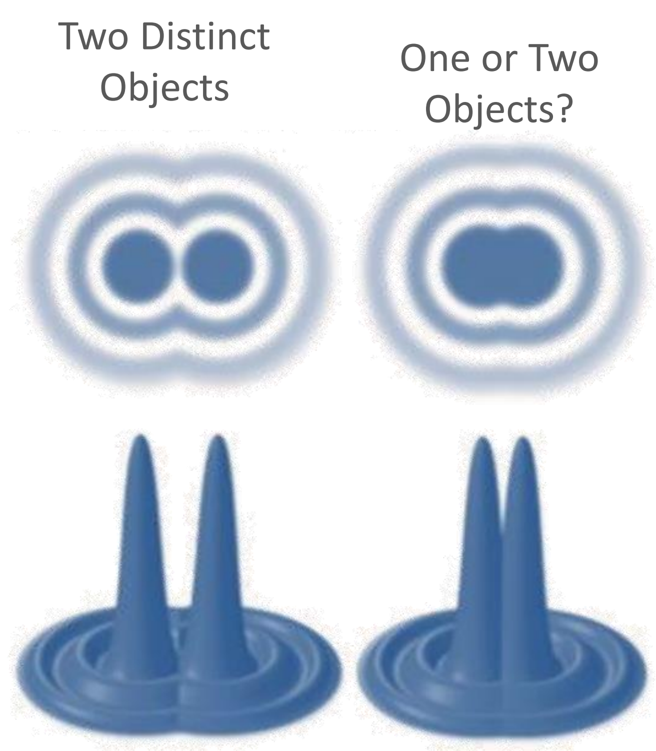


What is Resolution?

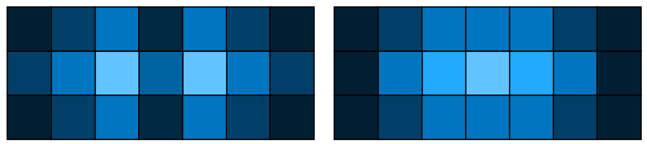
Spatial Resolution in fluorescence microscopy is defined as the shortest distance between two points that can still be distinguished.

Determined by two factors:

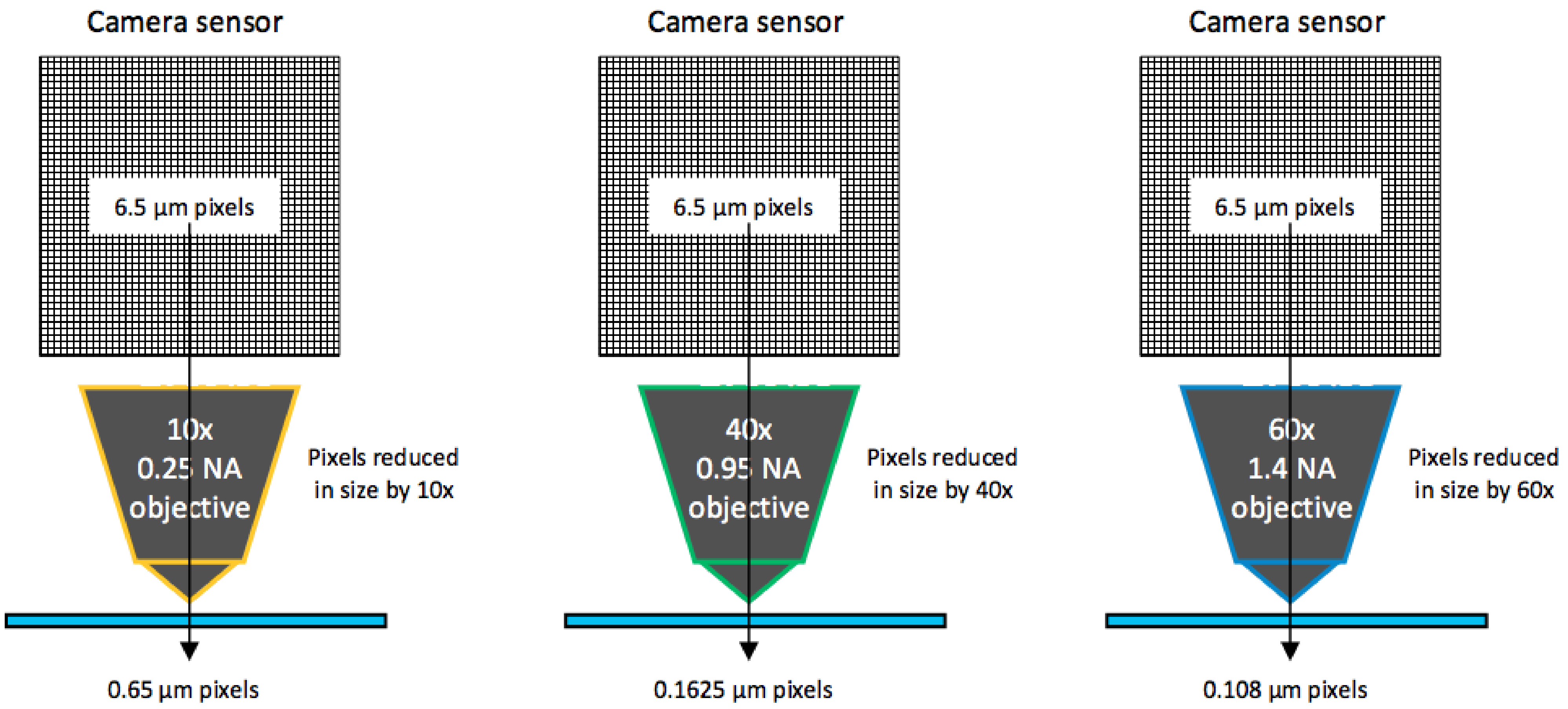
- **Microscope Resolution** – All optics in the light path, numerical aperture of the objective lens, emission wavelength of the sample
- **Camera Resolution** – Pixel size after magnification, plus spatial sampling



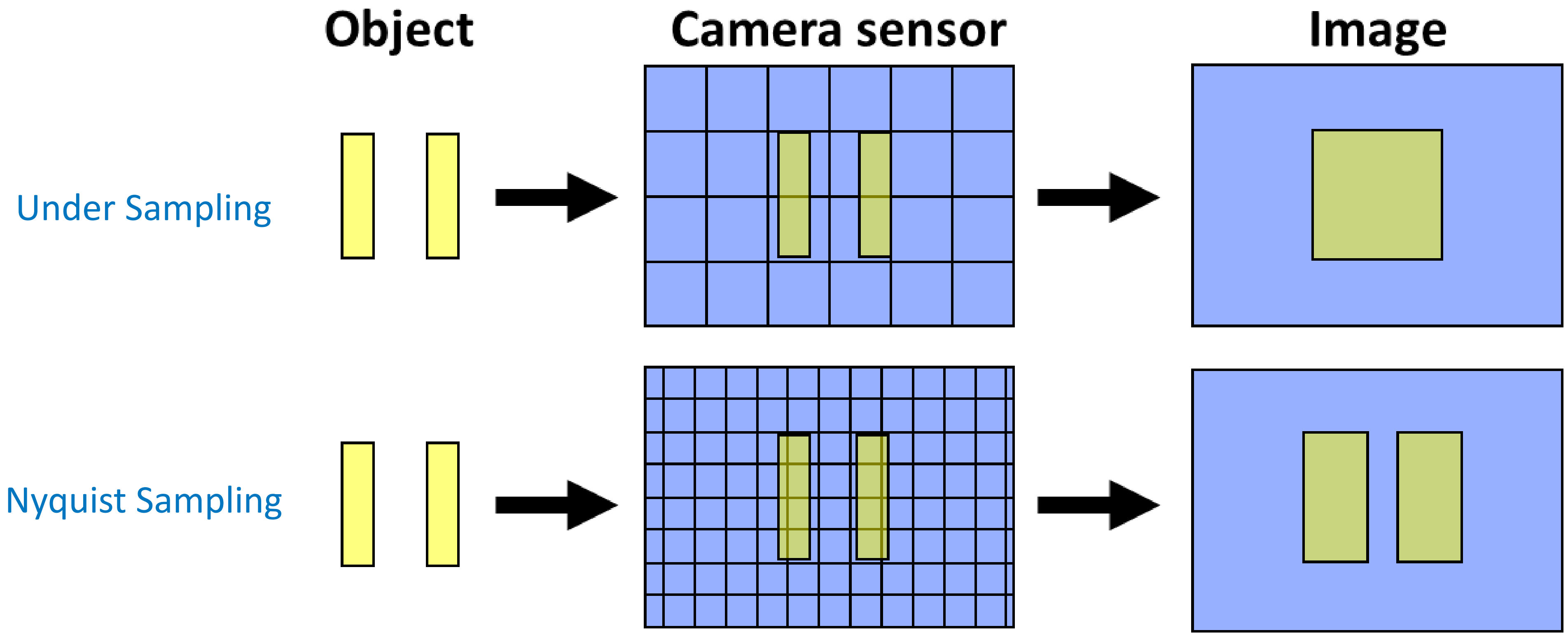
Two Airy disks merge until the two central spots can no longer be differentiated.



Pixel Size After Magnification



Nyquist Sampling



$$\text{Camera Resolution} = \left(\frac{Pixel\ size}{Total\ magnification}\right) \times 2.3$$

When Do I Want To Match Nyquist?

The resolution of your system is limited to the worst component.

When aiming for optimal resolution, match Nyquist.

Camera Resolution

Microscope Resolution

$$\left(\frac{Pixel\ size}{Total\ magnification}\right) \times 2.3 > \frac{1.22 \times Wavelength\ (\mu m)}{2NA_{obj}}$$

If resolution is less important, use **undersampling**: pixels larger than Nyquist requires. This gives greater sensitivity.

If pixels are smaller than Nyquist requires, this is **oversampling**. This is only used for advanced post-processing techniques such as deconvolution.

Field Of View

$$FOV = \frac{Sensor\ size\ (diagonal)}{Total\ magnification}$$

Sensor Sizes:

Blue: 11.6 mm (**EMCCD, 0.25MP**)

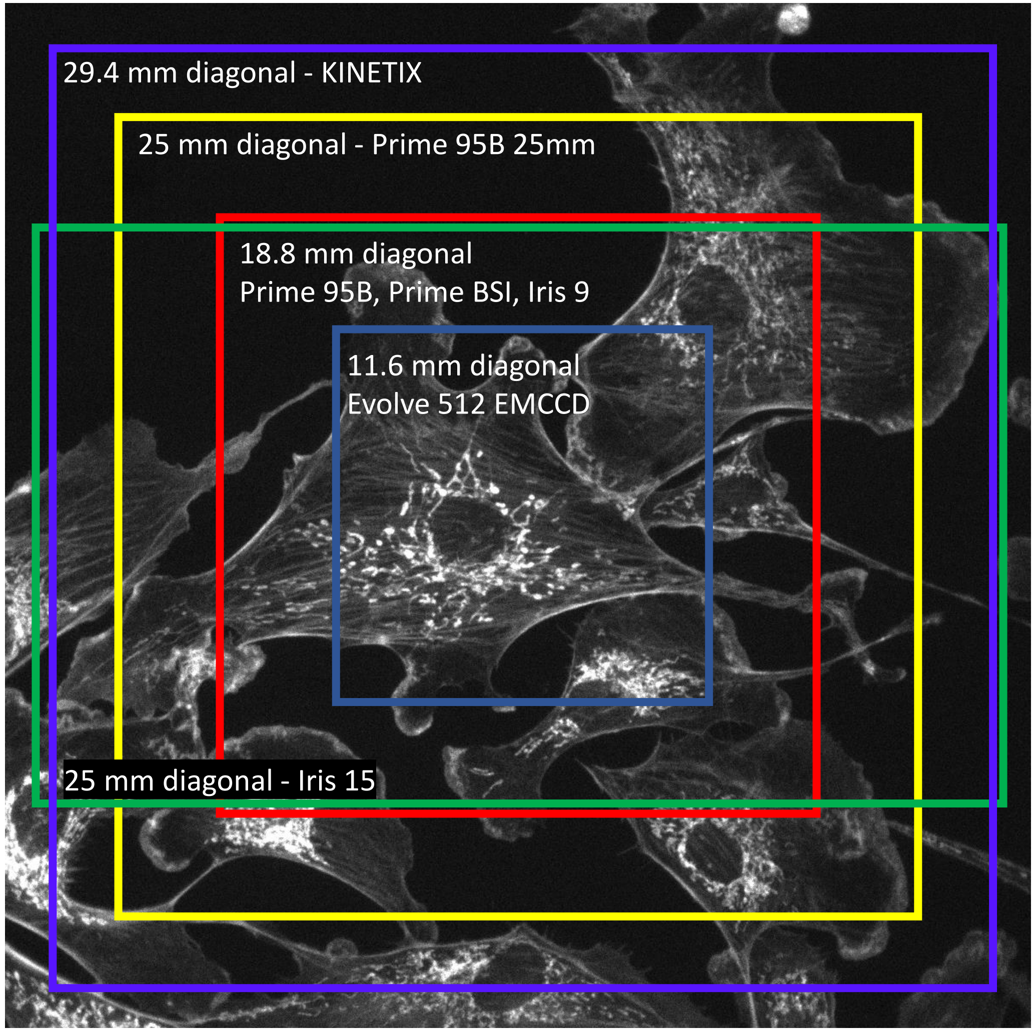
Red: 18.8 mm (**95B 1.4MP, BSI 4.2MP**)

Green: 25 mm (**Iris 15, 15MP**)

Yellow B: 25 mm (**95B 25MM, 2.6MP**)

Purple: 29.4 mm (**Kinetix, 10MP**)

At the same image pixel size, FOV is determined by the number of pixels.



Speed And Interfaces

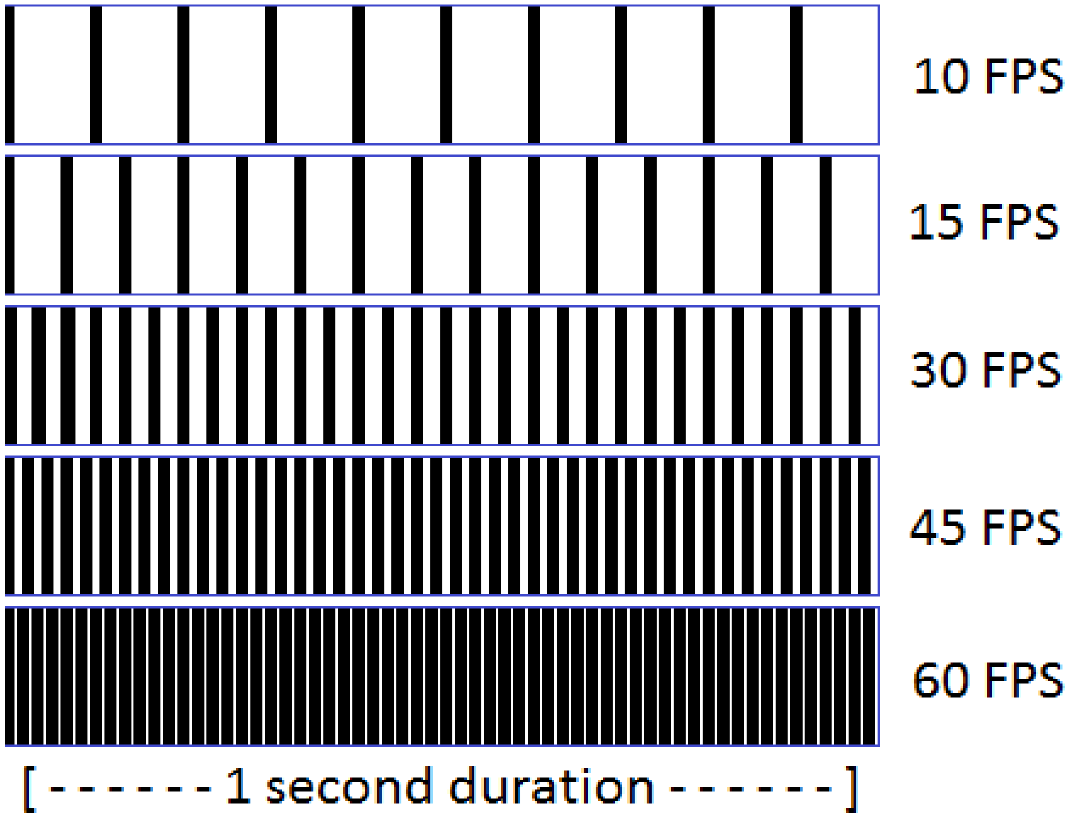
Required exposure time is the first determining factor for speed → **sensitivity** is a major factor!

Imaging at **50 ms** exposure results in **20 fps**

Other speed factors:

- Sensor size – row time & number of rows
- Digital interface – USB3 vs 3.1g2 vs PCIe
- Readout Method – CMS vs High Speed

New CMOS technology is allowing speeds up to **500fps for 10 Megapixels**, maintaining low noise.



Timeline of Scientific Imaging

CCD, 1970 → EMCCD, 2000 → Early CMOS, 2009 → Back-Illuminated CMOS, 2016

First digital cameras

- Good for high light & Documentation
- Not enough sensitivity and speed for live cell

Pushing sensitivity through multiplication

- Much more sensitive and fast than CCD
- Expensive, slow, small FOV

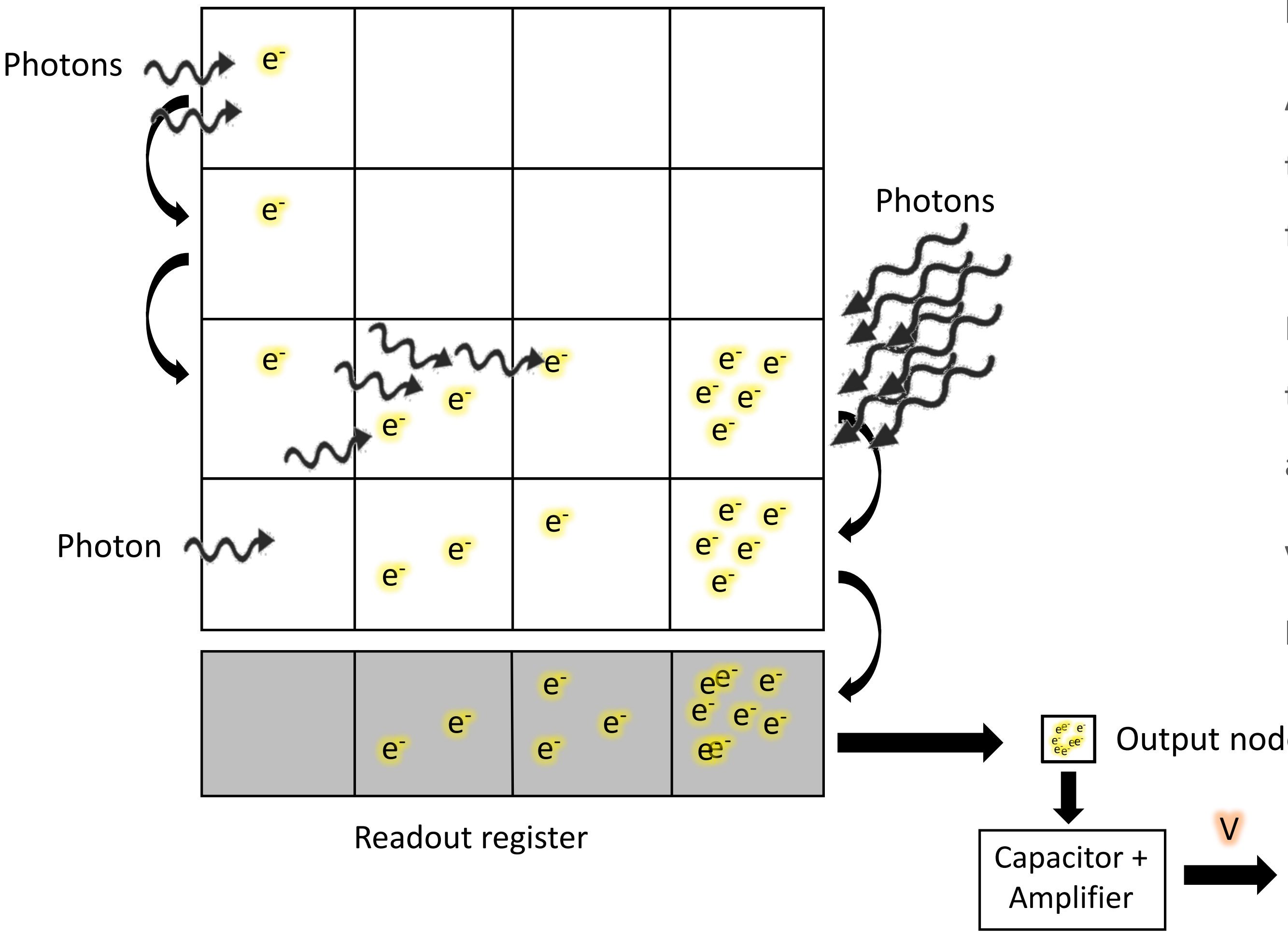
Imaging in Parallel

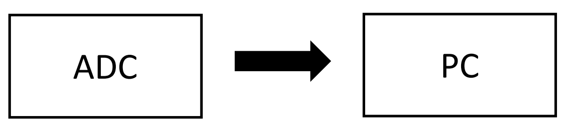
- Much higher speeds and larger fields of view, with low noise for live cell imaging
- Sensitivity couldn't rival EMCCD

The All-in-One

- Highest sensitivity, rivals or beats EMCCD, with speed and field of view of CMOS
- Cleaner backgrounds allow low-light imaging

CCD Fundamentals





The number of electrons created should be linearly proportional to the number of photons hitting the pixel

After exposure, electrons are moved down, row by row, until they reach the readout register - a row that doesn’t prevented from seeing light

Readout registers shuttle the bunches of electrons one at a time into the output node which is connected to a capacitor and amplifier

Voltage is sent to an analogue to digital convertor which provides the digital signal on a computer

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EMCCD: Faster and More Sensitive

Used for faster, more sensitive imaging than CCD

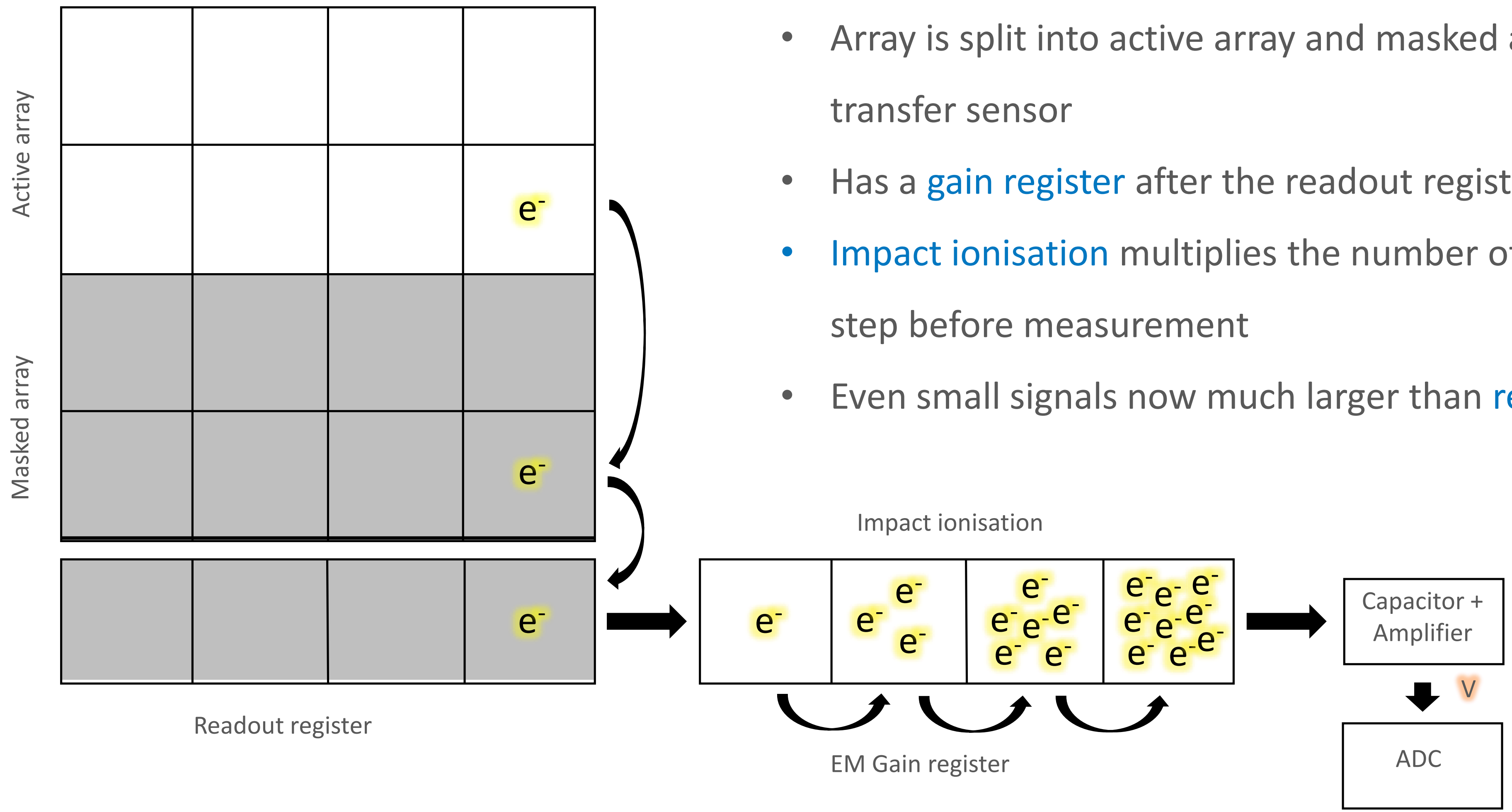
Photometrics introduced the first scientific grade camera (Cascade 650) in 2000

EMCCD sensors are also back-illuminated (high QE) and have large pixels (up to 24x24 µm)

Electron Multiplication of detected photoelectrons to overcome read noise, increasing speed and sensitivity above CCDs

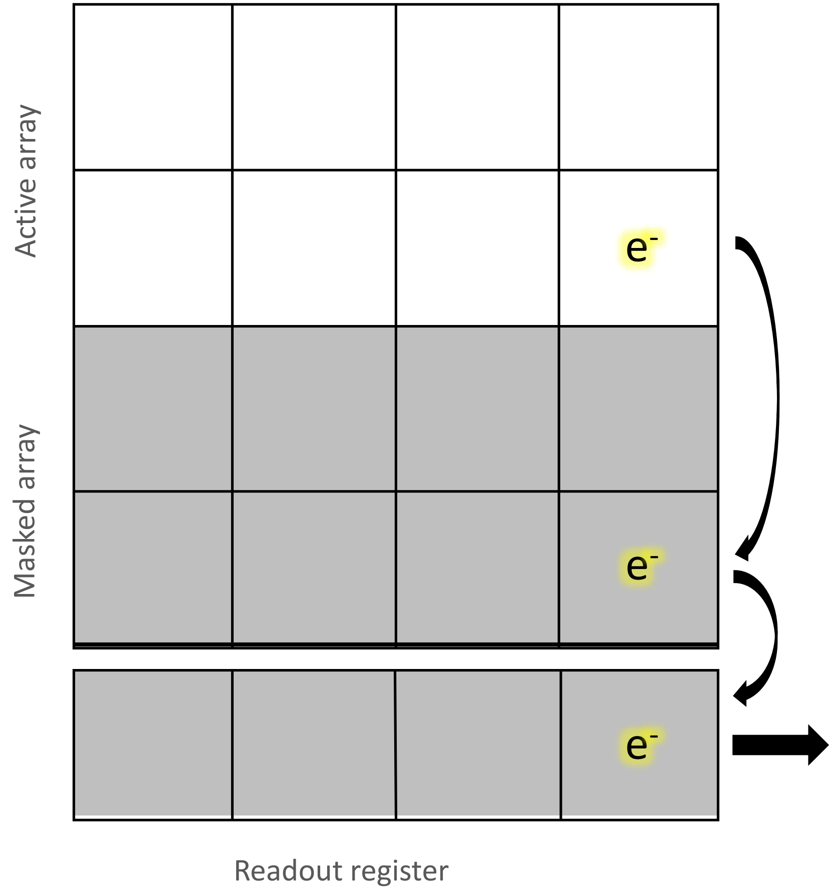
photometrics®
evolve™
DELTA

EMCCD Fundamentals

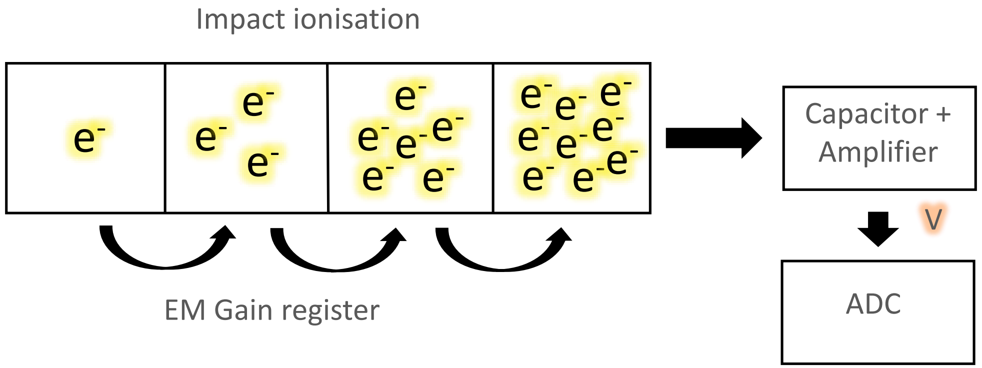


- Array is split into active array and masked array on a frame transfer sensor
- Has a gain register after the readout register
- Impact ionisation multiplies the number of electrons step-by-step before measurement
- Even small signals now much larger than read noise floor.

Issues With EMCCD Cameras



- **EM Gain decay** – cameras have **limited lifespan** and require regular calibration for any **quantitative** measurement or **reproducibility**.
- EM Gain Stability – temperature and other fluctuations affect values
- **Excess Noise Factor** – due to randomness of EM multiplication, photon shot noise (our largest noise source) is multiplied by **√2 (1.4x)**



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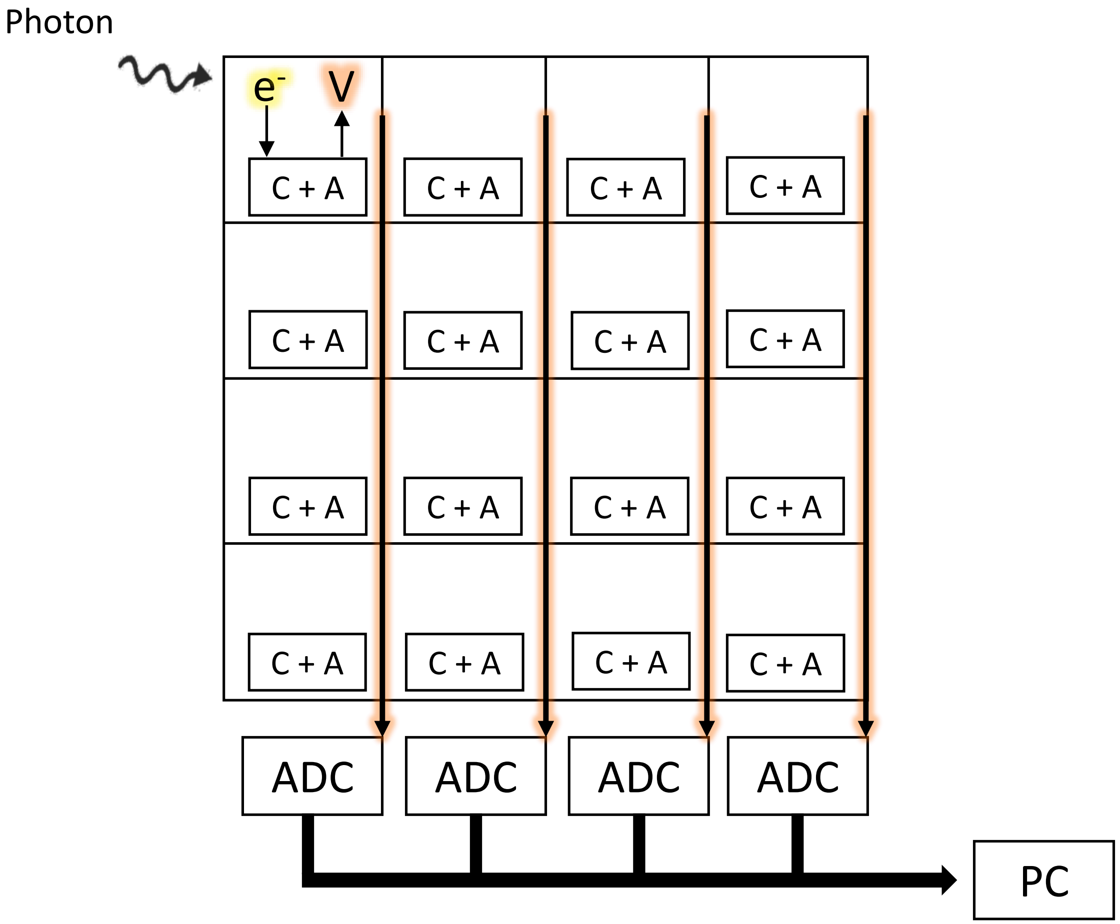
CMOS Fundamentals



- Capacitor and amplifier on **every pixel**
- An ADC for **every column**
- sCMOS cameras deliver **bigger fields of view** at much **faster speeds**
- Low read noise to detect weak fluorescence and work with live cells
- However early sCMOS sensitivity & image quality couldn't rival EMCCD.

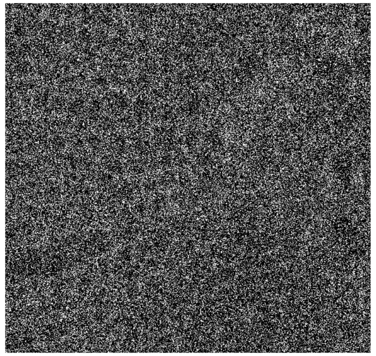
Competing With EMCCD Sensitivity

sCMOS brought many advantages over EMCCD, not least being significantly cheaper!

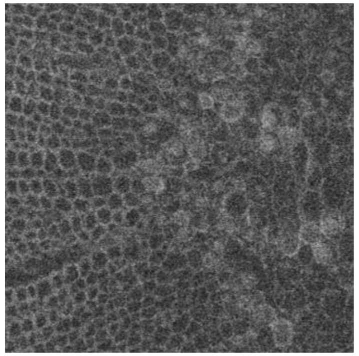
However, sCMOS couldn't perform the low-light imaging tasks of the more sensitive EMCCD,

due to:

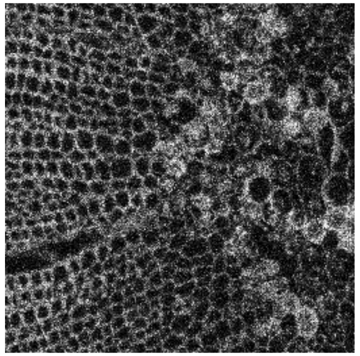
- Front-illumination, Lower QE
- Higher noise
- Problems with patterns & artefacts
- Much smaller pixels



Interline CCD (5e$^-$ read noise)

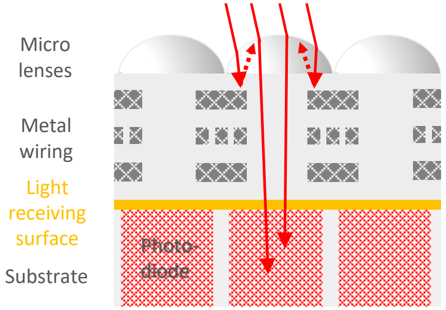


sCMOS (1.5e$^-$ read noise)

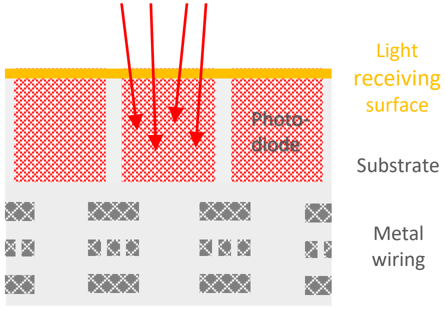


EMCCD (<1e$^-$ read noise)

Front-illuminated



Back-illuminated



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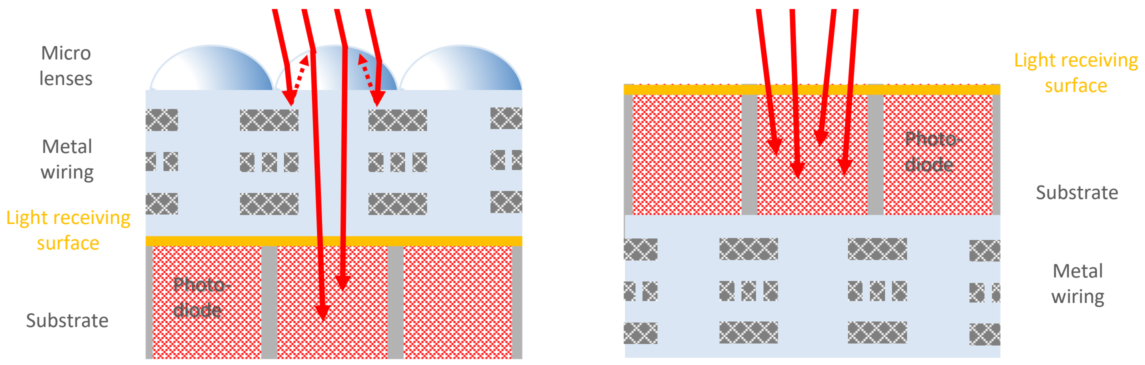
Back-Illuminated CMOS

Avoid the reflecting layers completely by bringing the light in from the back of the sensor. Requires thinning of silicon to around 1.1um.

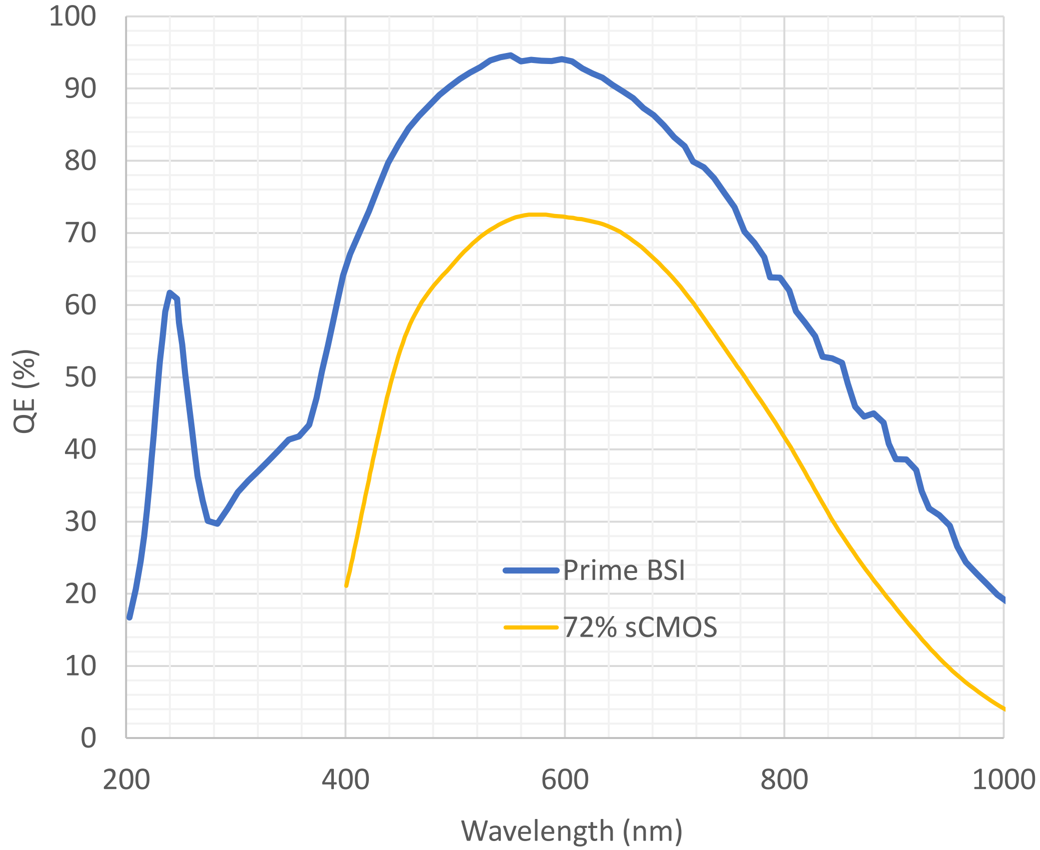
Front-illuminated Back-illuminated



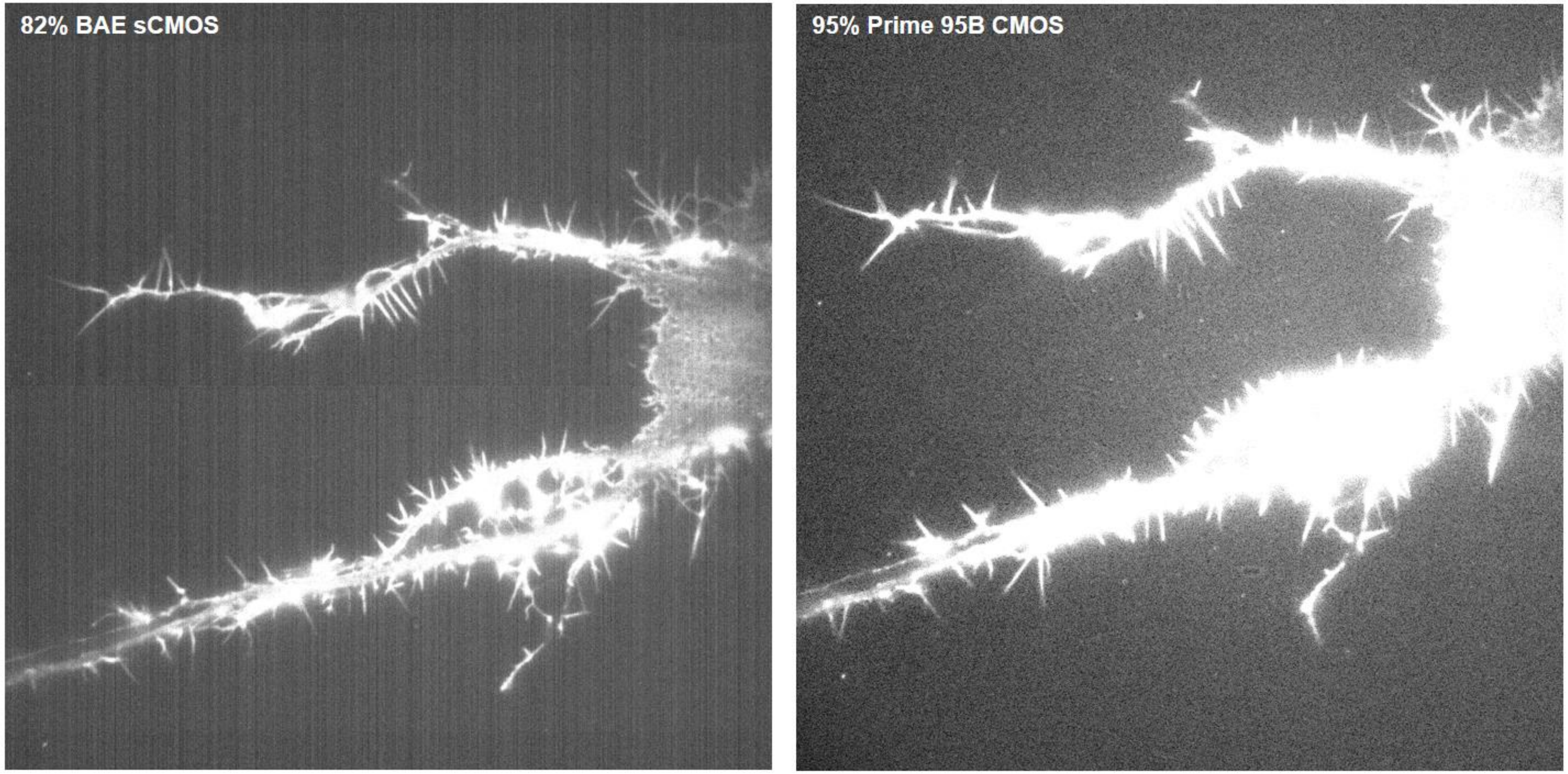
Achieve **95% peak QE**

Maintain 1.0 - 1.6e^- Read Noise

Outperforms EMCCD in **sensitivity**, speed, FOV and more

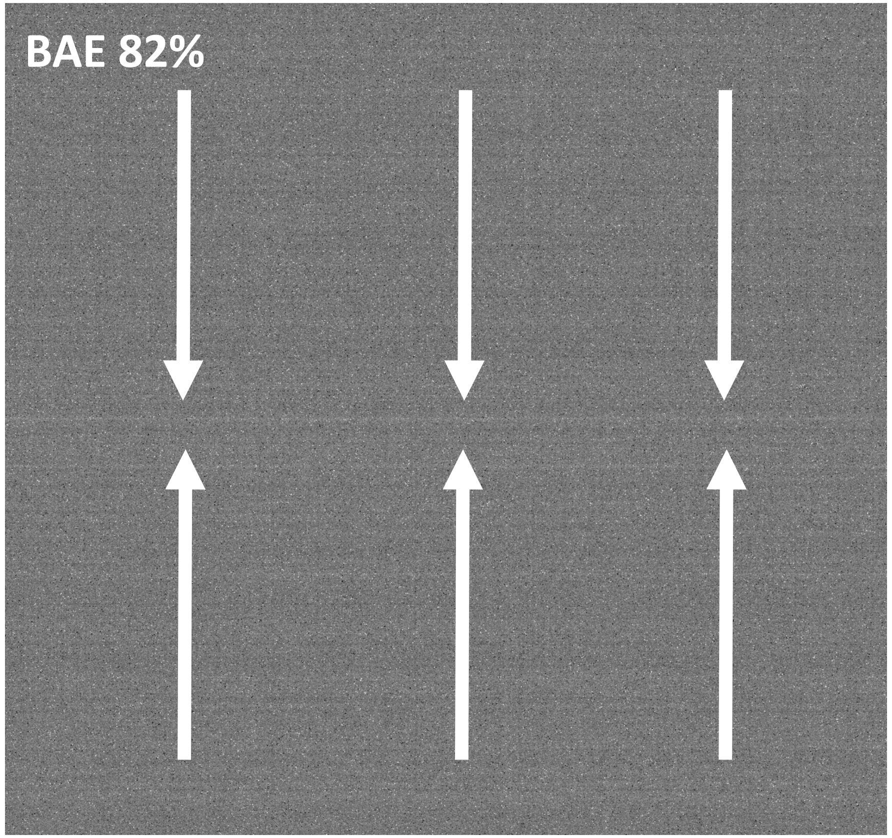


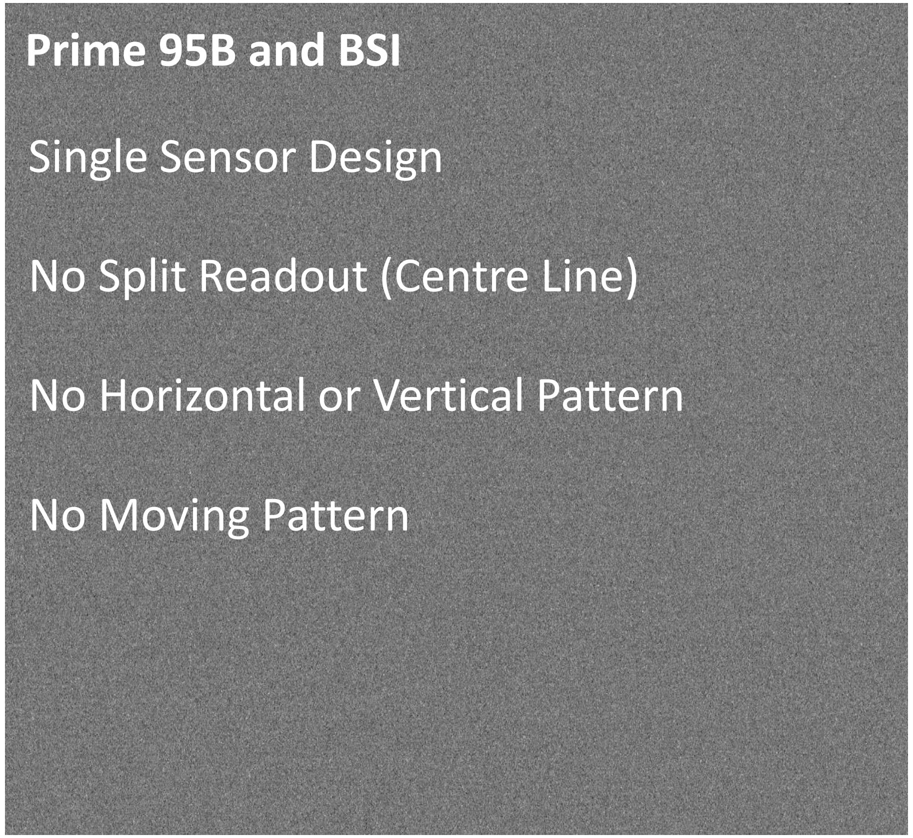
Fixed pattern column noise



Average of 100 frames, 30 ms exposure time

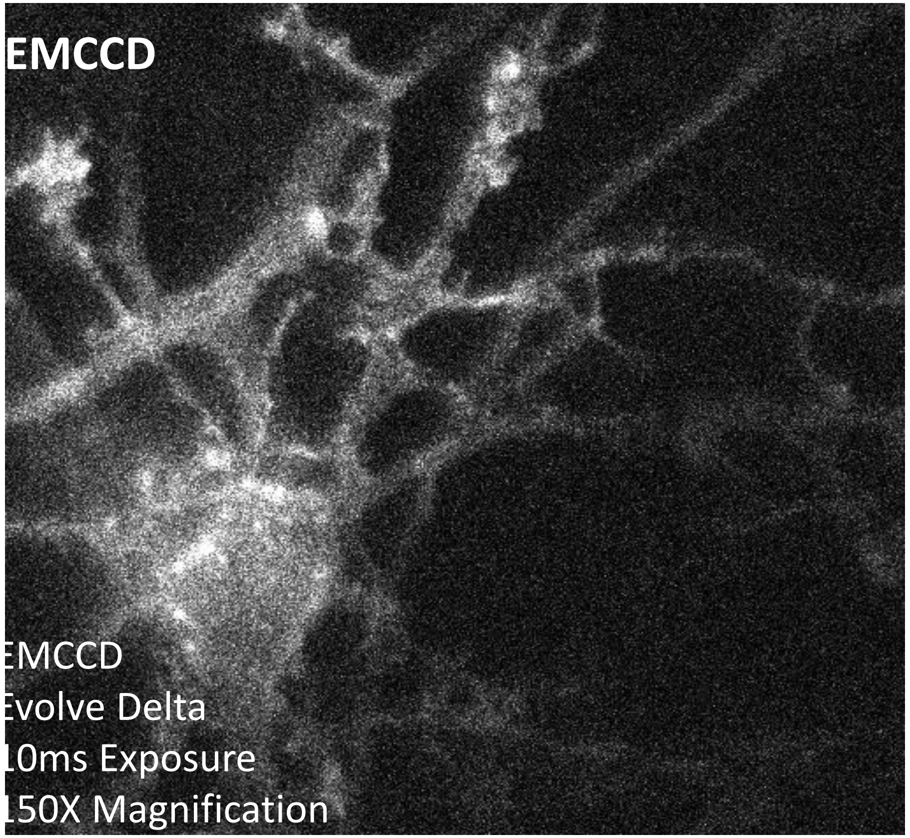
Excellent Background Quality

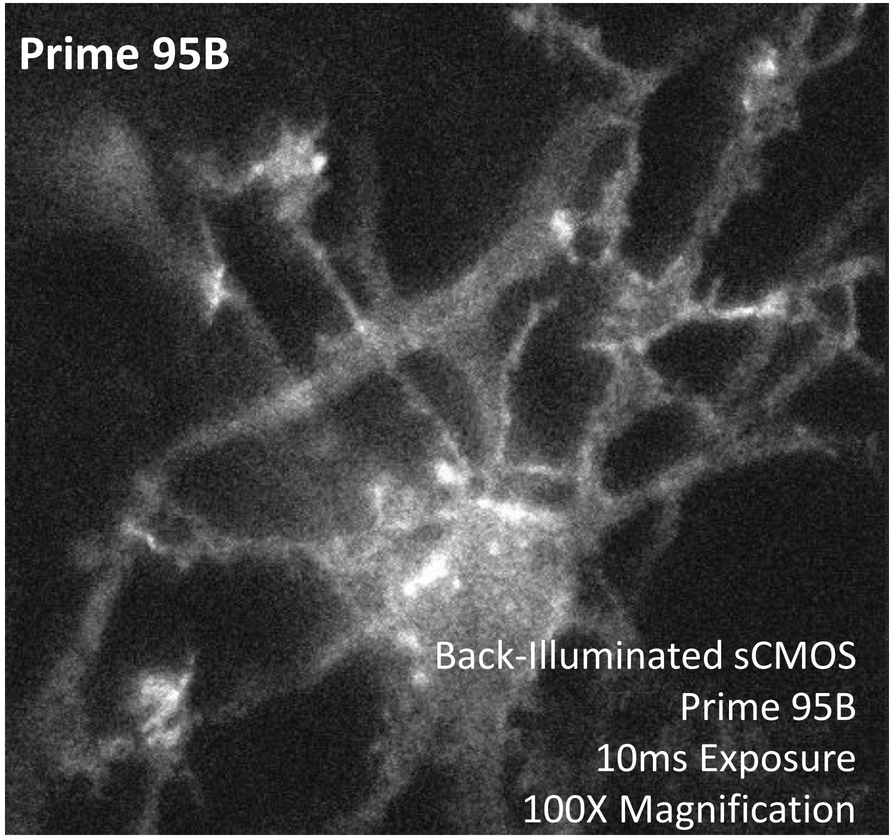




Background quality with the Prime 95B and Prime BSI is excellent, offering the very best low light imaging performance

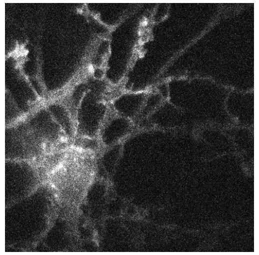
EMCCD vs Back sCMOS: Sensitivity



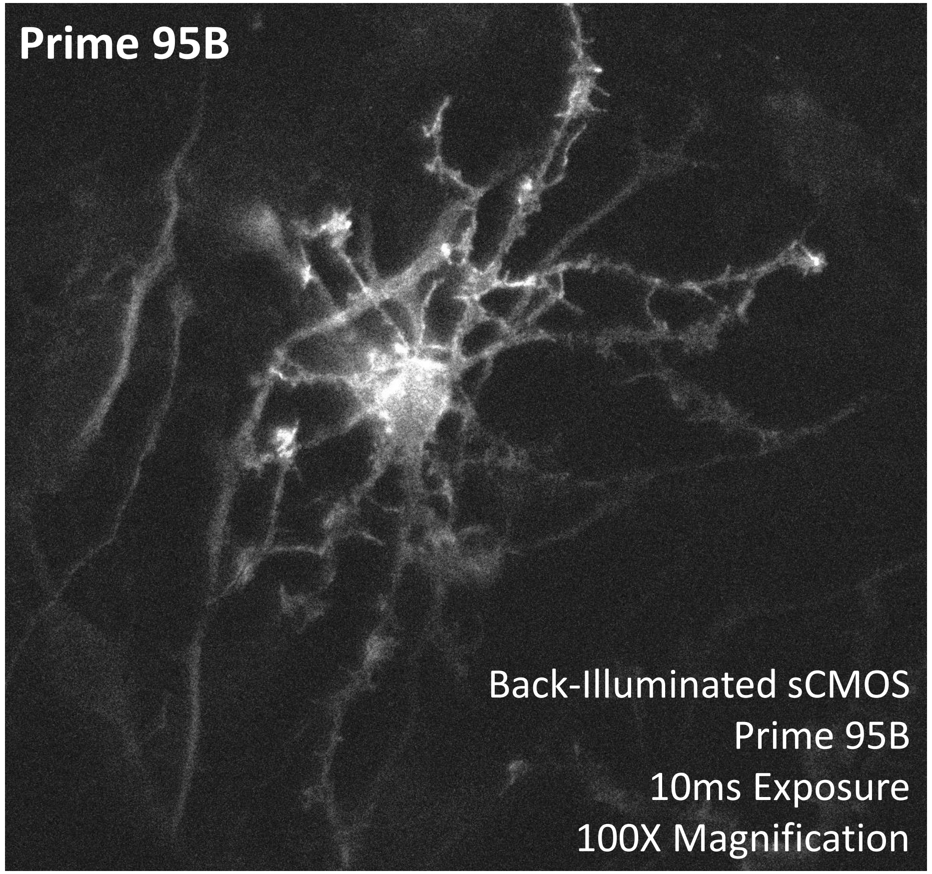


Better SNR than the EMCCD due to absence of Excess Noise Factor

EMCCD vs Back sCMOS: Sensitivity



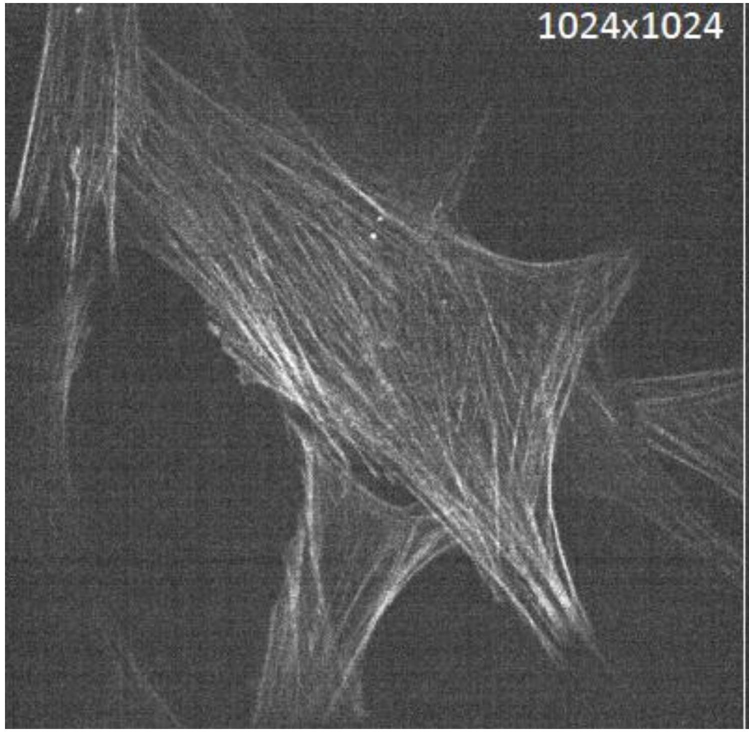
EMCCD
Evolve Delta
10ms Exposure
150X Magnification

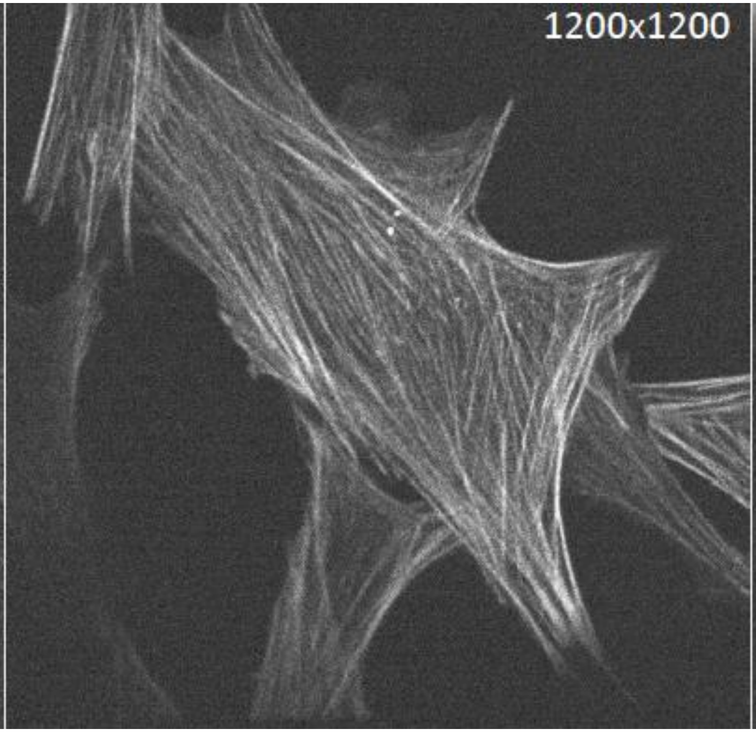


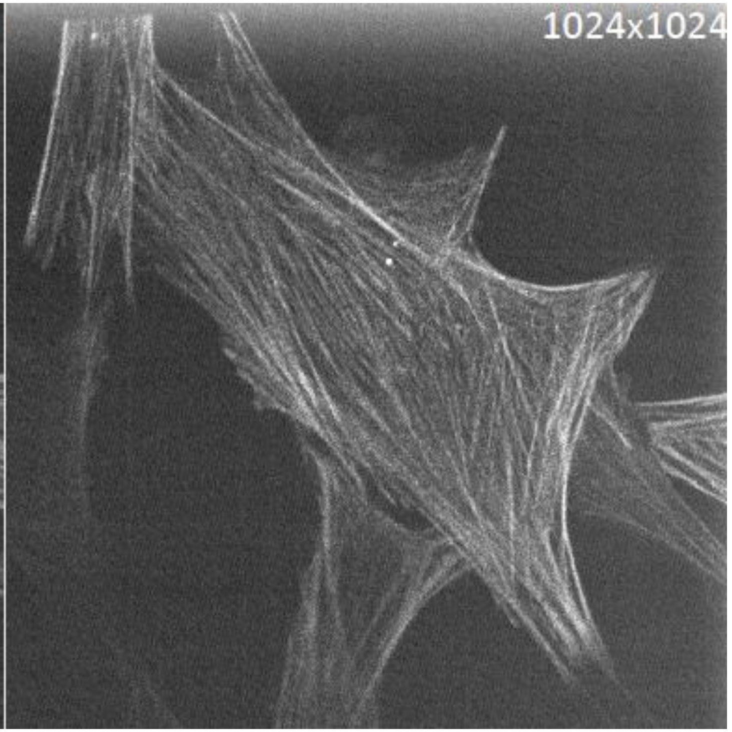
Better SNR than EMCCD, with faster frame rate and drastic FOV improvement

2x2 Binned CMOS vs 95B vs 1k EMCCD

Fixed sample, Spinning disk confocal, same exposure time & focal plane







sCMOS 2x2Bin - 13 micron
Pixel Area 169µm^2

Prime 95B - 11 micron
Pixel Area 121 µm^2

1024 EMCCD- 13 micron
Pixel Area 169µm^2

Being Quantitative

For **quantitative** intensity analysis, **grey values cannot be used** as they depend on camera-specific settings.

Photoelectrons are our objective, quantitative unit. To calculate back, use:

$$\text{Photoelectrons} = (\text{Grey levels} - \text{Offset}) \times \text{Gain}$$

Gain: Ask your camera manufacturer for the gain values for **your specific camera,** or estimate with a **Mean Variance Test.**

Offset: Baseline grey level value, mean image value with no light (typically 100).

This allows us to:

1. Compare intensity values between **different cameras** / **camera settings**
2. Estimate **signal to noise ratio**
3. Understand your **light level**

How is Gain set?

Gain represents the number of **detected photoelectrons** that each **grey level** (**ADU**) represents. Choosing the value is a **balance** of **precision** and **dynamic range:**

Dynamic Range:	**Bit depth x Gain = Full Well Capacity** (in e-)
Precision & Contrast:	**Gain (in e-/grey) = smallest measurement step**

'**Higher**' gain means a **smaller number** of **e-/grey** (e.g. 0.25e-/grey), resulting in each detected photoelectron providing more grey levels and **better contrast for low light imaging**.

At high light levels, low gain (e.g. 2e-/grey) is necessary to detect strong signals.

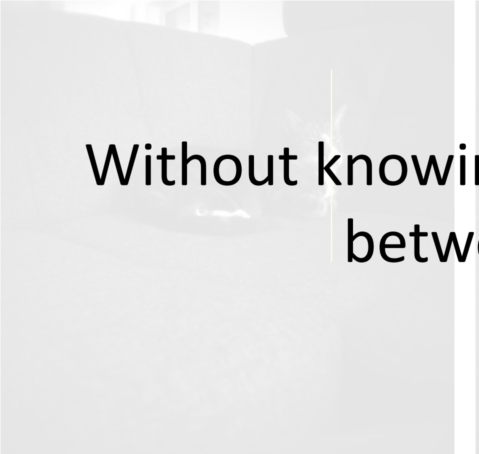
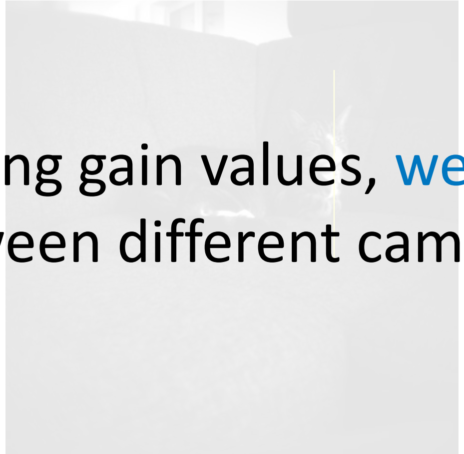
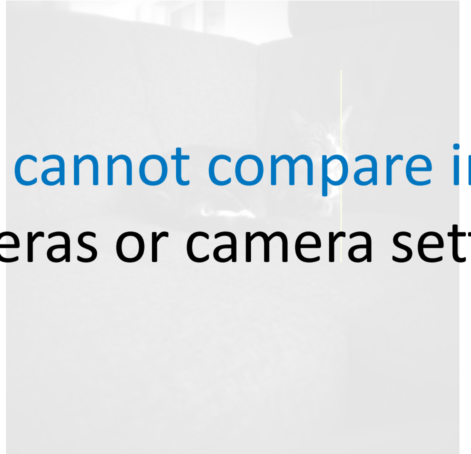
Gain States With Cimba

12-bit sensitivity
= 0.6 e^-/grey level

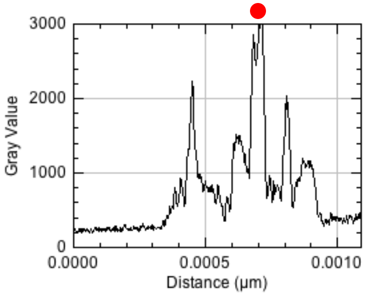
12-bit balanced
= 1.2 e^-/grey level

12-bit full well
= 2.4 e^-/grey level

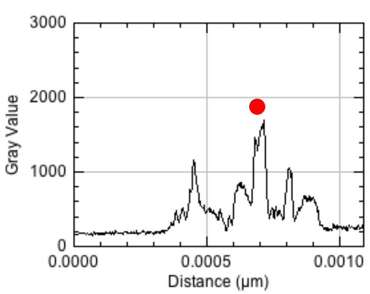
16-bit HDR
= 0.9 e^-/grey level

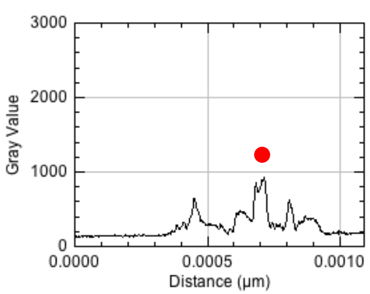

Without knowing gain values, we cannot compare intensity values between different cameras or camera settings



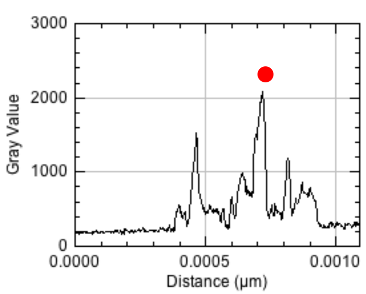
Max grey 2900 = 1700 e^-



Max grey 1500 = 1700 e^-



Max grey 800 = 1700 e^-



Max grey 2100 = 1700 e^-

Thank you for attending! Summary:

Sensitivity: **Quantum Efficiency** most important factor,
Read Noise also important at low light levels.

Pixel Size: **Larger pixels** are more sensitive
Smaller pixels can offer better resolution unless limited by microscope

Being Quantitative:

Calculating back to electrons gives true comparison
then can **Calculate Signal to Noise Ratio**

Camera Technologies:

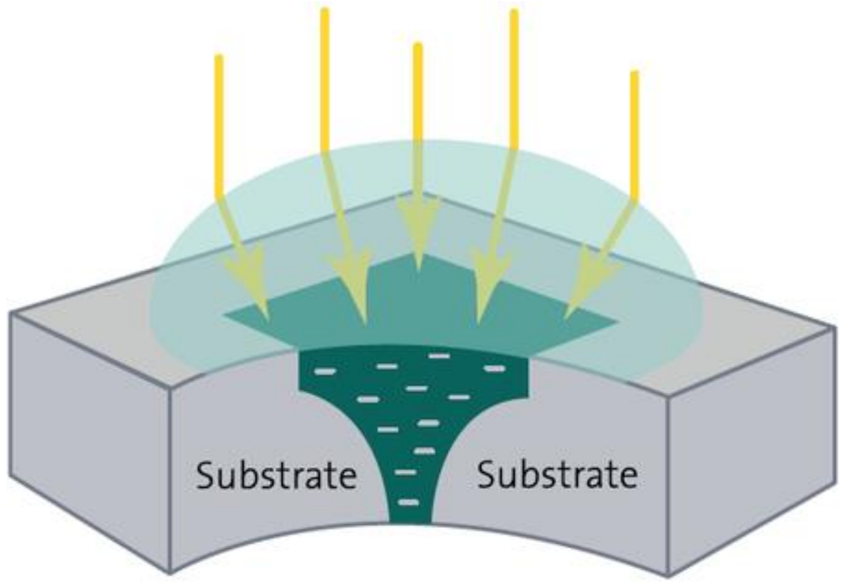
Front-Illuminated CMOS: Fast but not as sensitive as EMCCD
EMCCD: Sensitive but slow & small FOV
CCD: Good for documentation, but not for live cell
…Back-Illuminated CMOS: Fastest, biggest FOV, most sensitive

Well Depth / Full Well Capacity

How many photoelectrons of signal can a pixel store during an exposure?

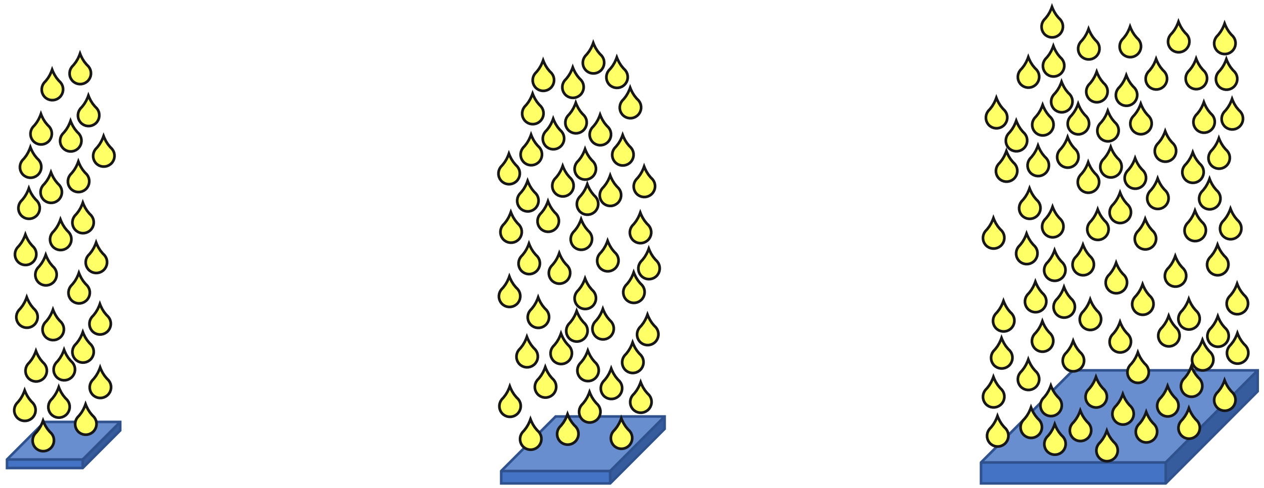
After photons are converted to photoelectrons in a pixel, they are stored in the **well** of a pixel.

Too many photoelectrons in a well and it will **saturate** – no additional light can be collected in that pixel.



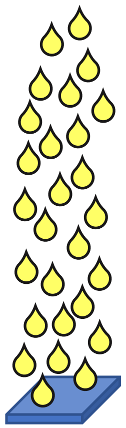
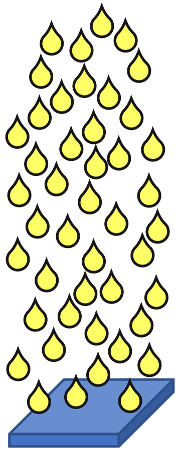
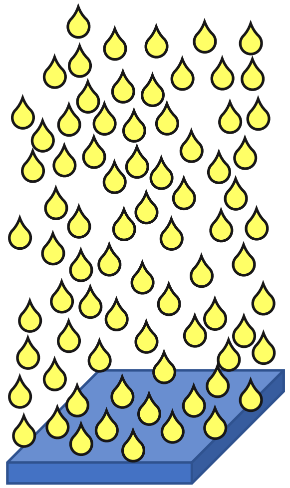
Full-well Capacity and Dynamic Range

Pixel Size	4.25µm	6.5µm	11µm



Full-Well Capacity	12,000e-	45,000e- (~3.8X more e-)	80,000e- (~6.3X more e-)

Full-well Capacity and Dynamic Range

Pixel Size	4.25µm	6.5µm	11µm
			
Full-Well Capacity	**12,000e-**	**45,000e-** (~3.8X more e-)	**80,000e-** (~6.3X more e-)
Dynamic Range:	**8000: 1**	**30,000 : 1**	**53,000 : 1**

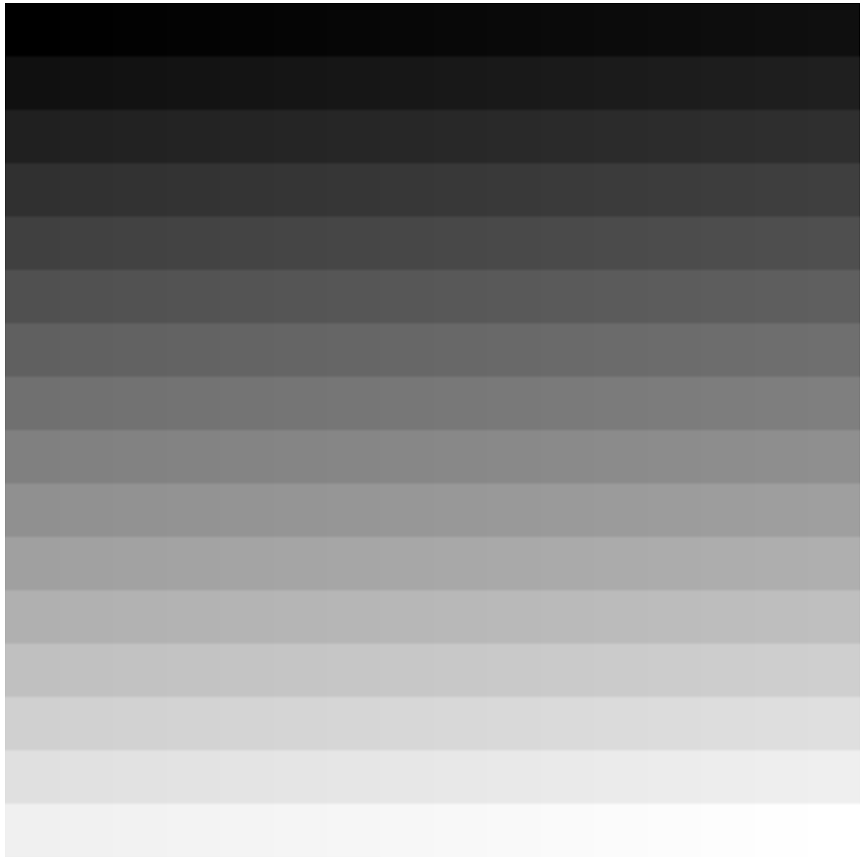
Full-well Capacity / Read Noise (1.5e-)

Grey Levels

The voltage from photoelectrons is converted into an **arbitrary** intensity unit using an **analogue to digital converter (ADC)**

As our cameras are monochrome, these colors are **gray levels**

The maximum number of displayable grey levels depends on the **bit depth**



Bit Depth

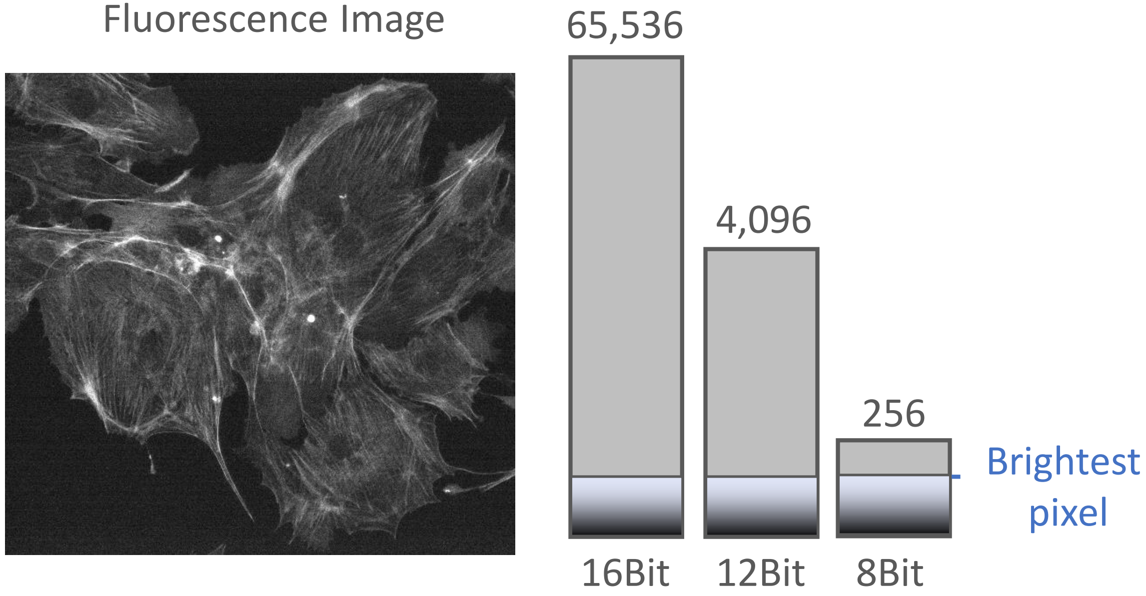
8 bit (**256** grey levels)

5 bit (**32** grey levels)

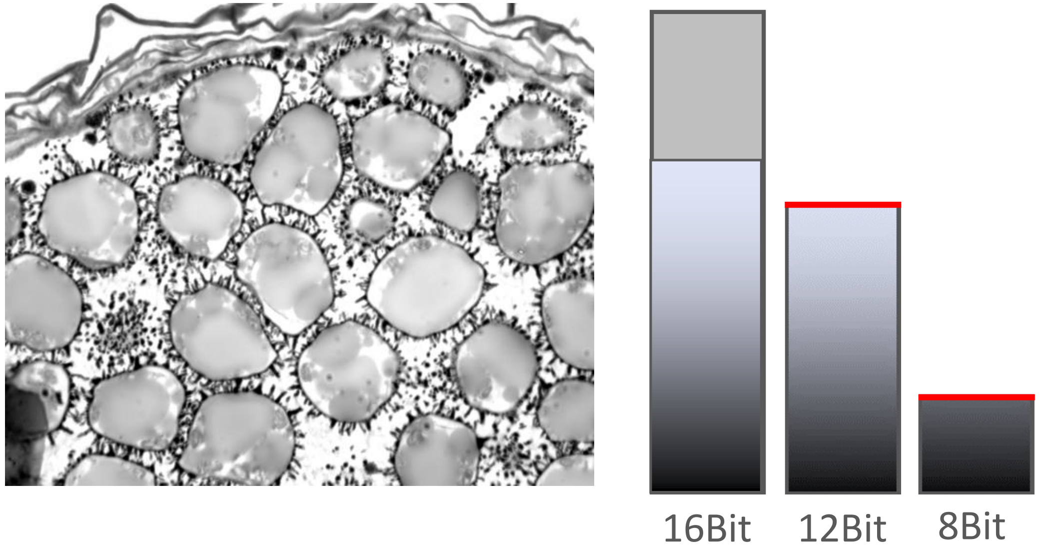
2 bit (**4** grey levels)

Bit Depth

Fluorescence Image



Brightfield Image



- High bit depth allows precise capture of high contrast samples, such as bright fixed cell fluorescence and brightfield.
- Bit depth is typically not relevant for low light imaging