Camera sensor technologies for biological microscopy

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Teledyne Photometrics

About Teledyne Photometrics

- Design and manufacture **high end cameras for *low light biological imaging***
- Market leader for **Scientific CMOS technology**
- Established in **1978** as division of Roper Technologies, now **Teledyne Technologies**
- Global Headquarters in **Tucson, Arizona**, USA
- Sales & Marketing Headquarters in **Birmingham**, UK
- Factory in **Surrey**, British Columbia, Canada
- Also in our portfolio are cameras for
  *high light applications (**Teledyne Qimaging**)*

TELEDYNE PHOTOMETRICS
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Part of the Teledyne Imaging Group

Contents

1. How does a camera work? CCD Sensors
2. Sensitivity & Noise
3. Measuring signals: understanding camera gain
4. EMCCDs
5. CMOS & Back-Illuminated CMOS (the future)

What is a camera image?

What do we want a camera sensor to do?

Take an image (an array of measurements of light in photoelectrons):

- Quickly / As fast as we can
- Limited noise → Good signal to noise ratio
- Low light levels → Avoiding photobleaching / phototoxicity
- Signals of differing emission wavelengths
- Good dynamic range
- Enough resolution to see detail

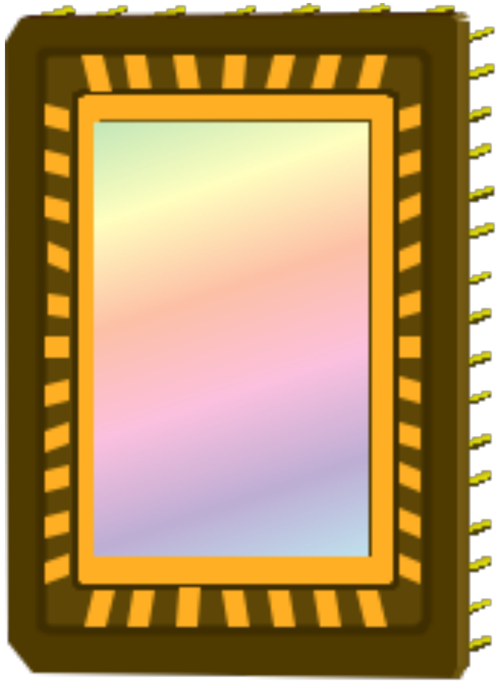
CCD – Charge Coupled Device

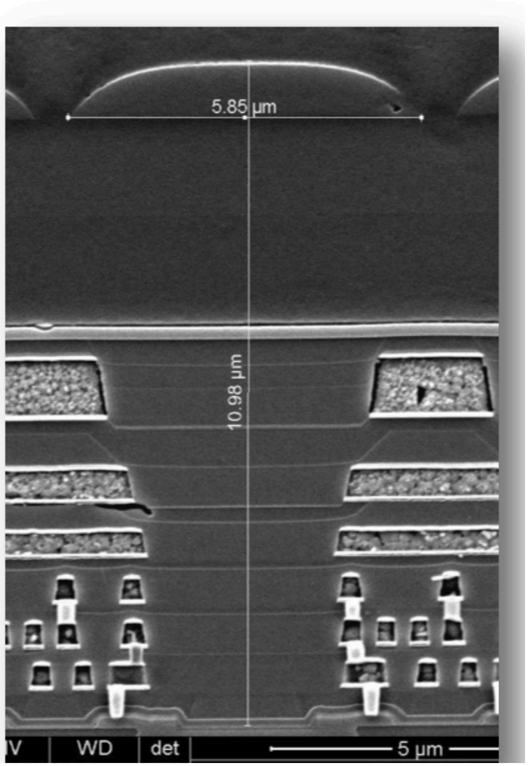
Invented in 1970 at Bell Labs

A silicon chip that converts an image into an electrical signal

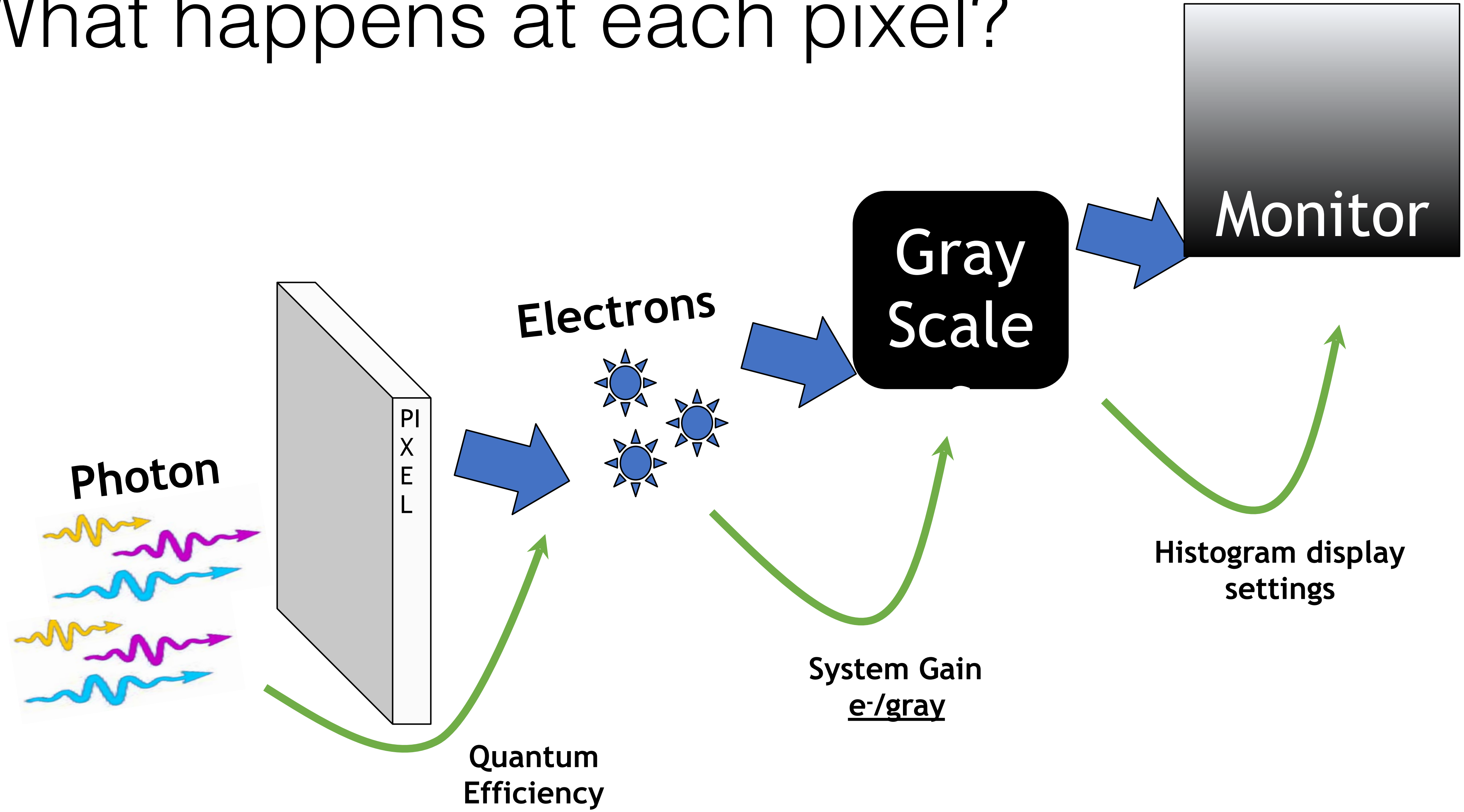
Made up of multiple pixels, with a characteristic:

- Physical size (xy)
- Efficiency (ability to capture light)
- Well depth / full well (maximum signal)

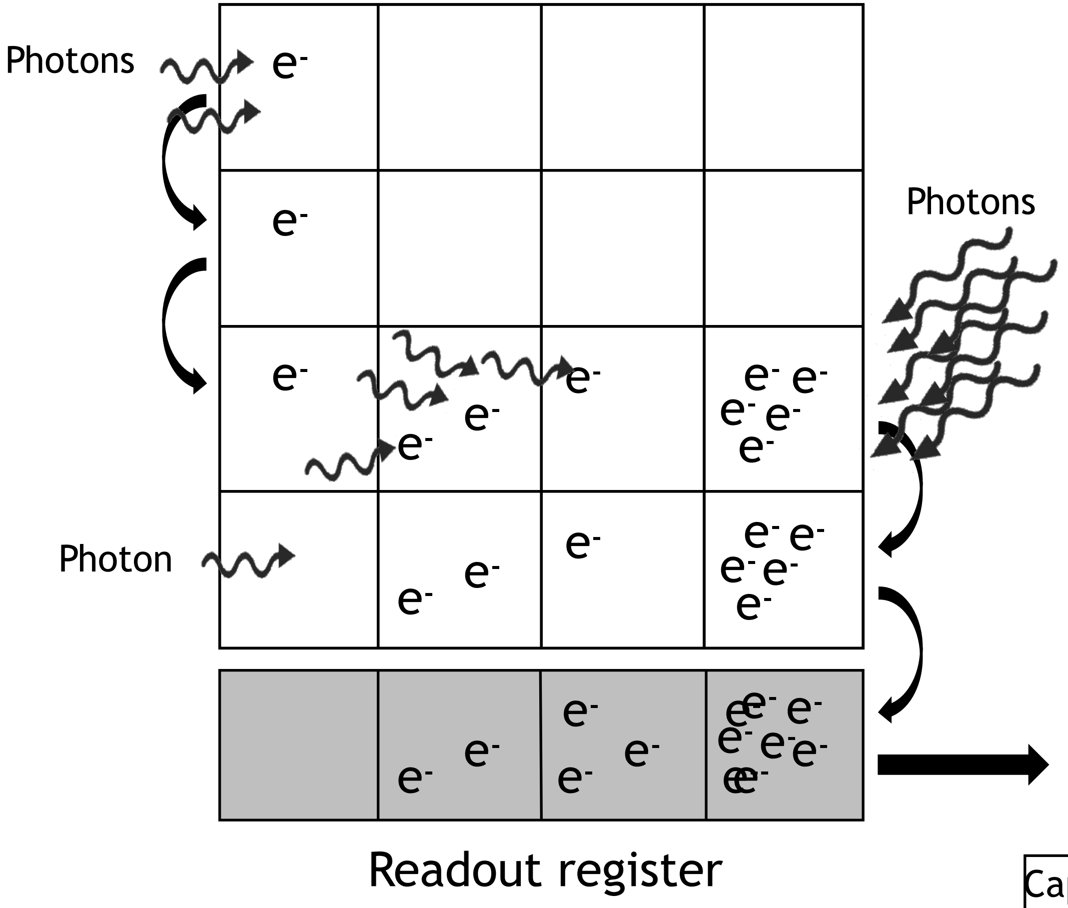


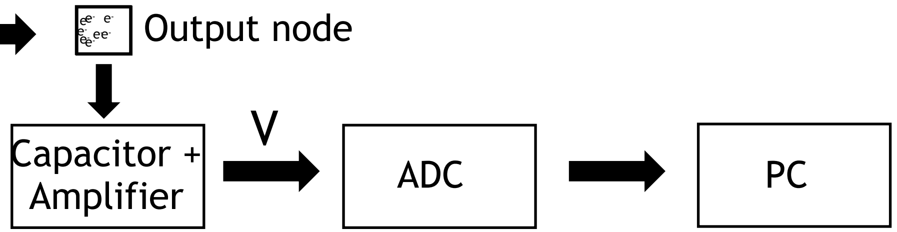


What happens at each pixel?



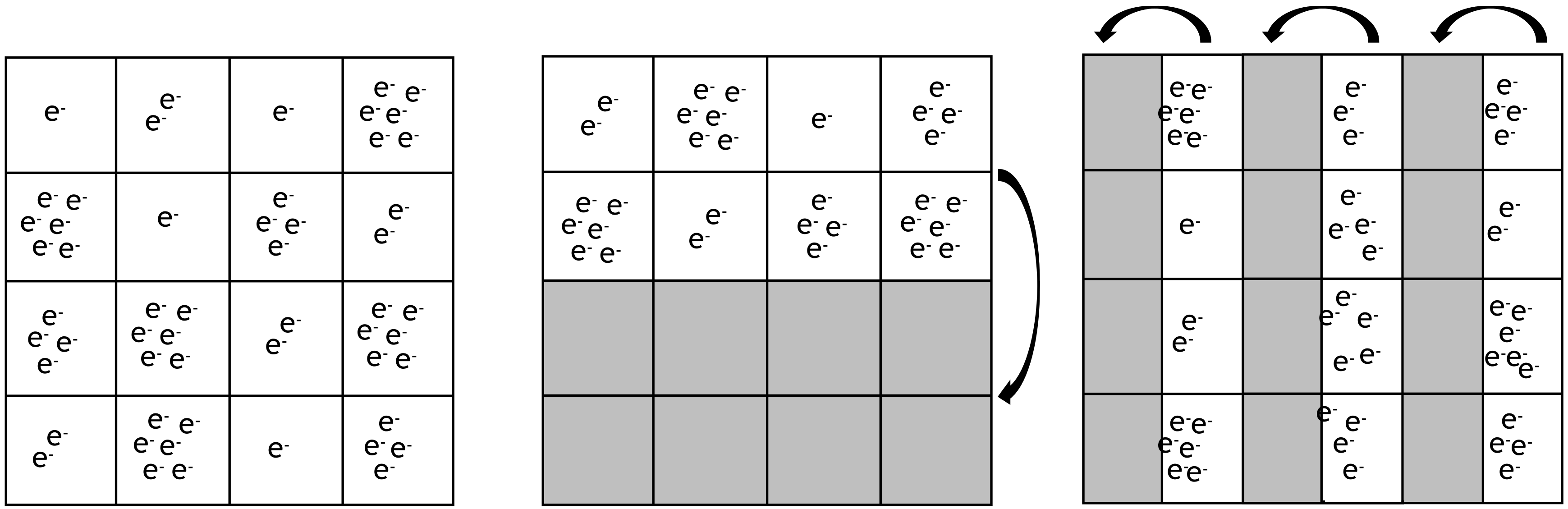
CCD fundamentals





- The amount of electrons created is directly proportional to the amount 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 receive light
- Readout register shuttles the row 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 and we can then interpret the digital signal on a computer

Sensor formats and electronic shutters



Full Frame
(needs physical shutter)

Frame transfer
(EMCCD)

Interline transfer

Light ***sensitive*** pixel

Light ***insensitive*** pixel

Sensitivity

- Sensitivity is commonly confused with quantum efficiency – in fact, there are many contributing factors:

Explaining sensitivity:

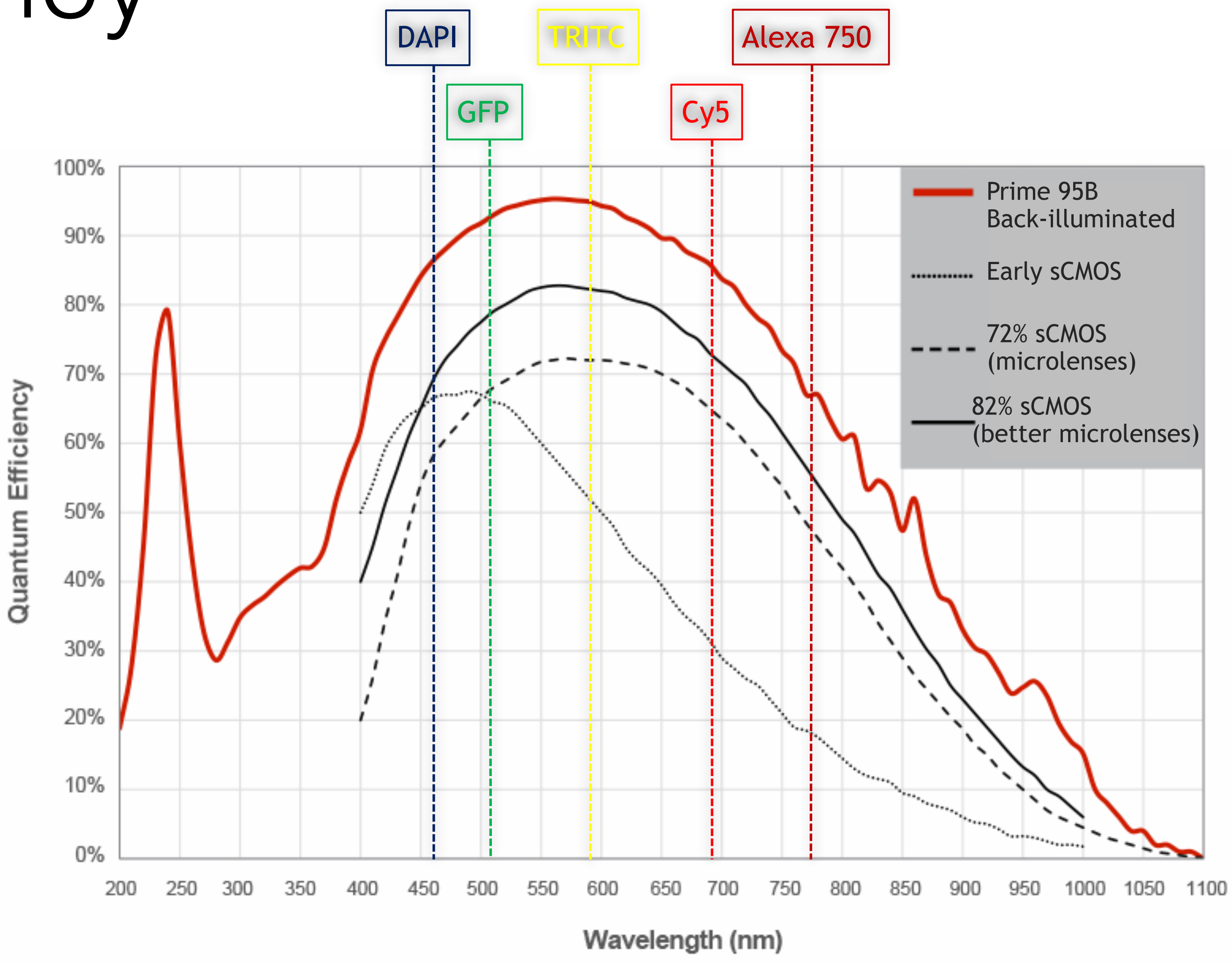
1. The percentage conversion of photons to electrons in the sensor is defined as **quantum efficiency (QE)**
2. Sensors convert photons of some wavelengths better than others
3. The number of photons that interact with the pixel will depend on the **physical size** of the pixel
4. We can have a sensor that collects a lot of light but if our **noise** is significant at our acheieved signal level, our image quality & contrast will be poor
5. **Patterns** from the sensor will contribute to 'messy' images and challenging analysis

→ The ultimate indication of sensitivity is 'Do my required imaging conditions give high enough **signal to noise** for my experimental needs?'

Quantum Efficiency

- QE curves are present on most scientific-grade camera data sheets

- The QE curve plots the % conversion of photons to electrons at any specific wavelength

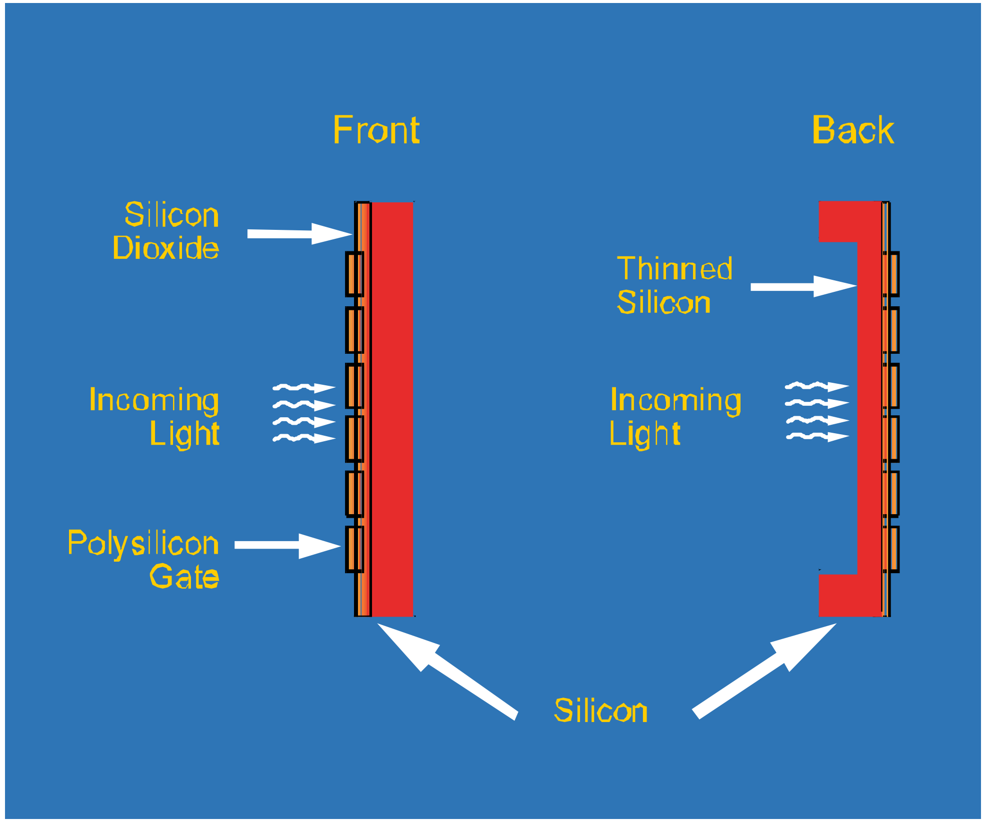


Front and Back illumination

- Typical front illuminated QE 40-75% at best wavelength

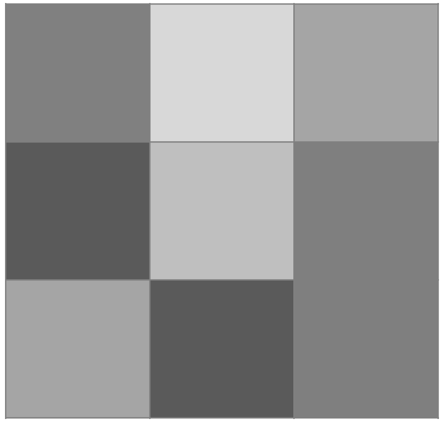
- Some cameras are back thinned and back illuminated to be as efficient as possible with incoming light

- Typical back illuminated QE >90% at best wavelength



What is noise?

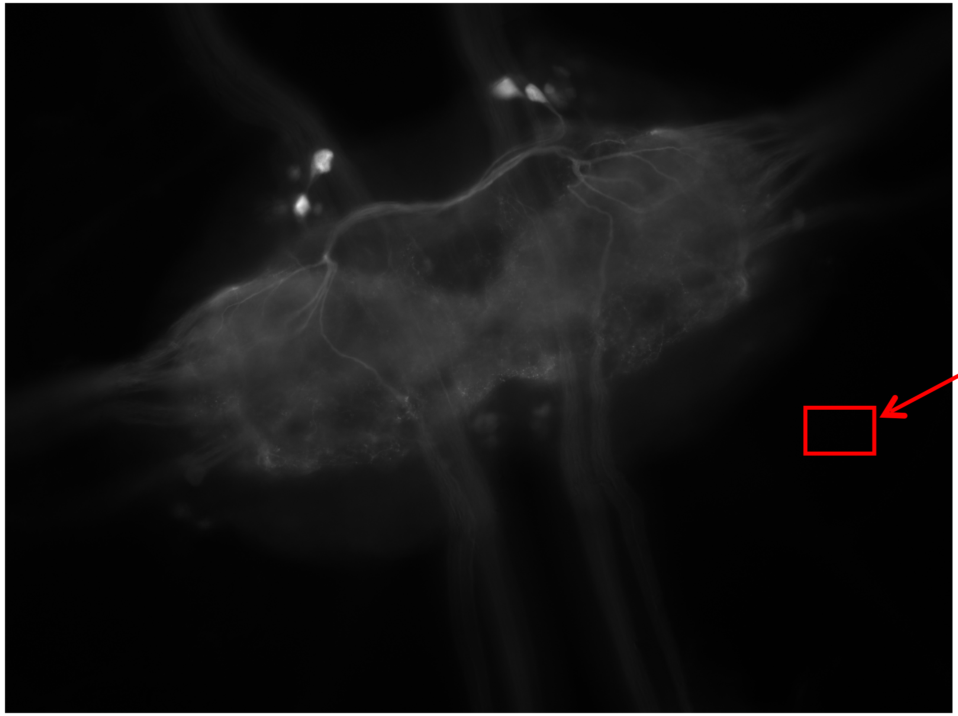
- Noise is NOT background
- Noise is uncertainty
- Noise is plus or minus
- Noise is driven by statistics
- Noise can be calculated

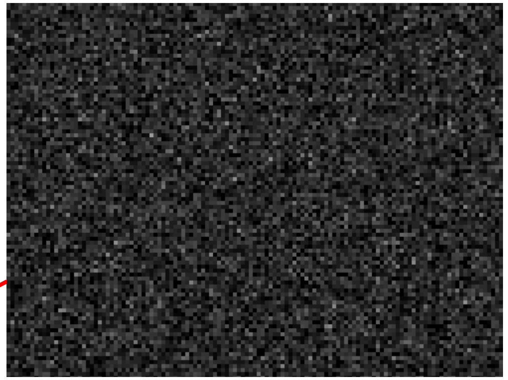


8	12	10
4	11	8
10	4	8

Living with noise

- Noise exists on every camera and in every measurement
- Depending on the image scale used, you may or may not see it.





Noise Sources

All cameras suffer from at least 3 types of noise:

- **Read Noise** – noise of reading the signal - fixed
- **Dark Current** – noise from heat - exposure time and temperature dependent
- **Photon Shot Noise** – square route of signal - signal dependent

Other noise sources:

- **Excess Noise Factor** – EMCCD
- **Clock Induced Charge** – All but mainly observed in EMCCD
- **Random Telegraph Noise** - CMOS

Read Noise

- Generated by electronics as electrons on the pixel are converted to a voltage which is then passed to the Analog to Digital Converter (ADC) to convert that voltage into the digital value (Grey Level)

- Called read noise because it's noise created when the image is read

- E.g. the calculated read noise of the Photometrics Prime 95B has a median value of 1.6e^- so the electron signal on each pixel can fluctuate by ±~1.6e^-

- Minimized by careful electronic design and slower readout

- Read noise is not as significant in high light applications

Photon shot noise

- Photon fluctuation
- Photons are emitted at random times, we can't guarantee the same amount land every time
  → Poisson behaviour
- Grows with signal, but more significant at low signal
- Square root relationship between signal and noise: For a signal of N photons,

$$SNR = \frac{N}{\sqrt{N}} = \sqrt{N}$$

- When photon noise exceeds system noise, data is photon (shot) noise limited

Dark Current

- Thermally generated electrons which build up on the pixels even when not exposed to light
- Can be reduced by cooling
- Dark current reduction is sensor dependent e.g. many sensors halve dark current for every 7 degrees of cooling; some require more cooling
- Other technologies can be applied which reduce the cooling required

  Retiga SRV (cooled to -30) Dark Current 0.15 e^-/p/s

  Exi Blue (cooled to zero) Dark Current 0.005 e^-/p/s

- We know the average but not when each electron will occur → Poisson noise source
- Dark Current Noise = $\sqrt{\text{Dark Current} \times \text{Exposure Time}}$

Signal To Noise (For CCD, CMOS)

Noise **cannot be estimated from the image,** it must be calculated based on camera performance parameters:

Standard SNR Equation:

$$SNR = \frac{S}{\sqrt{S + D2 + \sigma R2}}$$

S = Signal in Photoelectrons

D = Dark Current Noise = $\sqrt{\text{(Dark Current * Exposure)}}$

σR = Read Noise

All values must be compared in units of **electrons,** not grey levels!

How to measure signals? Know your gain!

- To calculate Signal to Noise, or know detected photoelectrons, we have **to convert back from grey levels.**
- Camera grey values are **entirely arbitrary** and not a quantitative measurement of intensity

  …but it's easy to convert to **photoelectrons:**

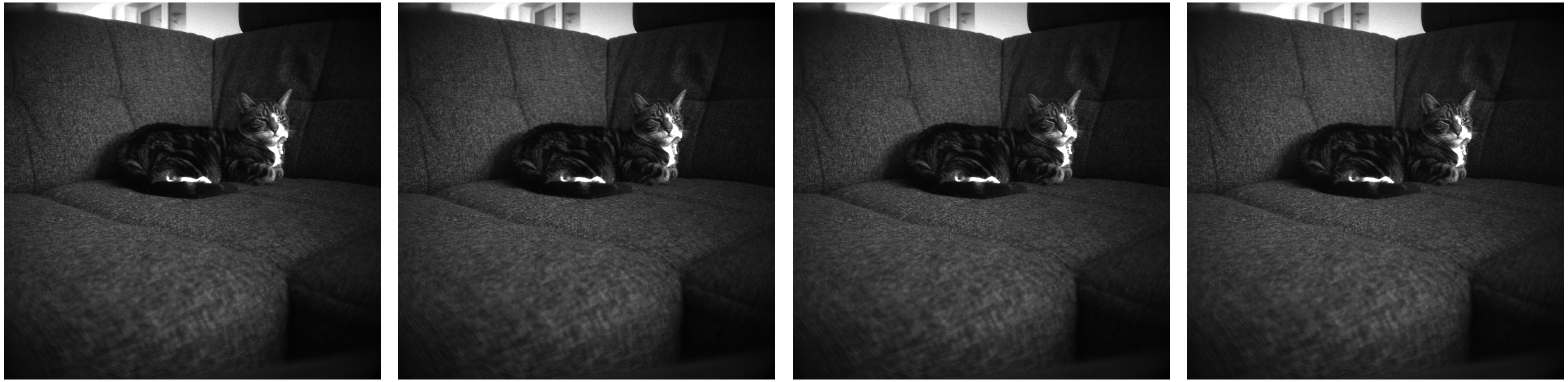
$$\text{Photoelectrons} = (\text{Grey levels} - \text{Offset}) \times \text{Gain}$$

- Knowing signal in **photoelectrons allows us to:**

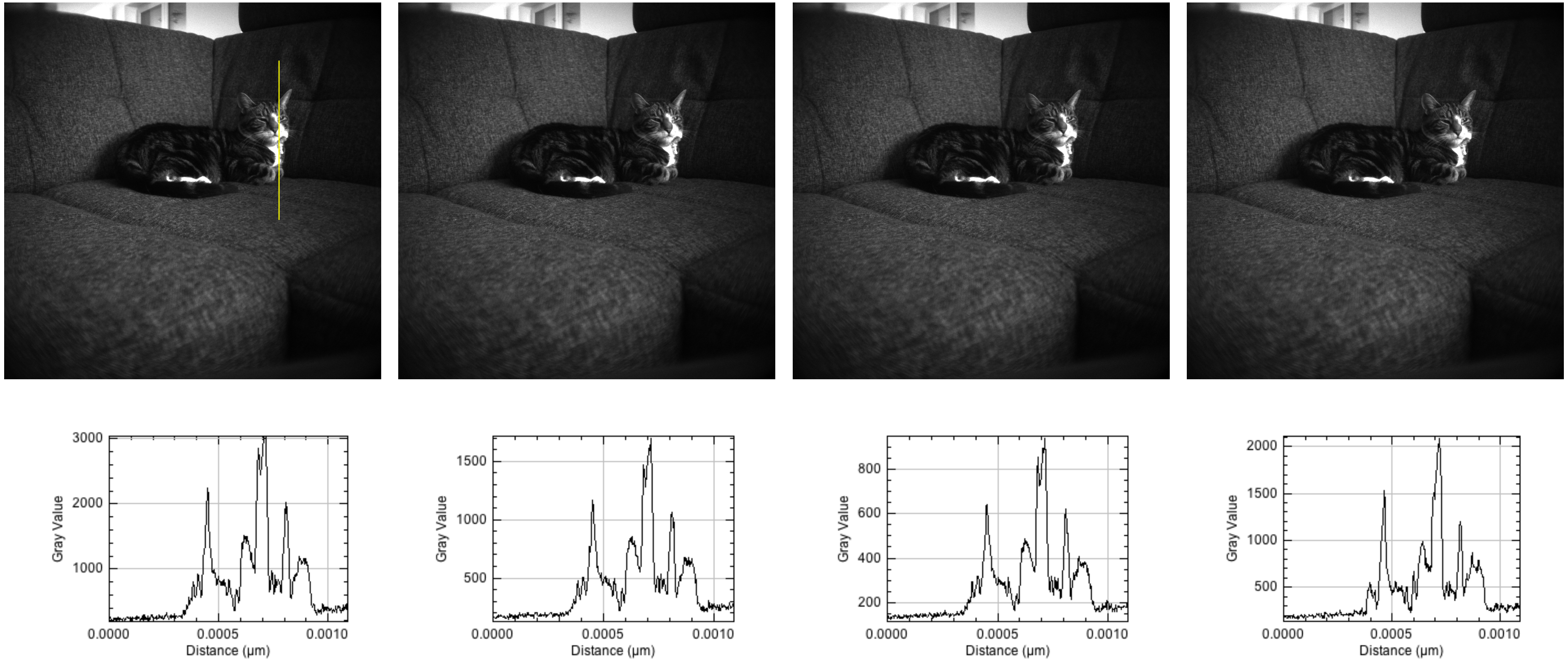
1. Compare intensity information between different cameras / camera settings
2. Estimate Signal to Noise Ratio
3. Understand your light level

How is gain measured? Use a **Mean Variance Test** (see our website) or ask your camera manufacturer for the gain values for **your specific camera**

Gain states – what it means?

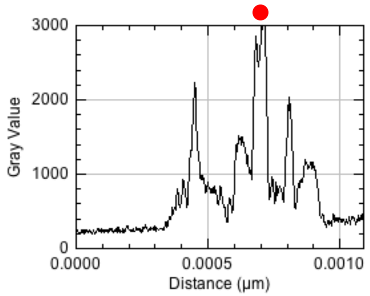


Gain states – what it means?



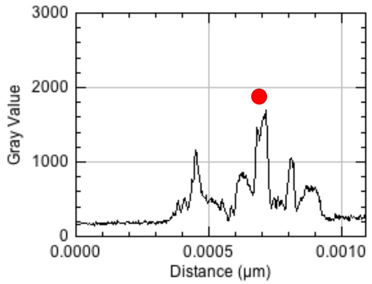
Gain states – what it means?

12-bit sensitivity
= 0.5 e^-/ADU



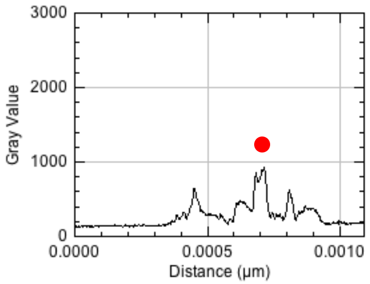
Max. grey 3400 ADU = 1700 e^-

12-bit balanced
= 1 e^-/ADU



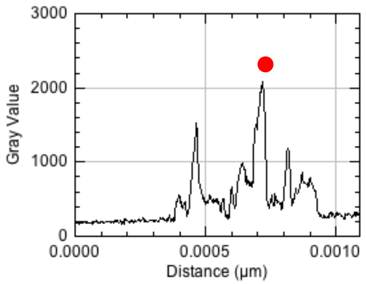
Max. grey 1700 ADU = 1700 e^-

12-bit full well
= 2 e^-/ADU



Max. grey 850 ADU = 1700 e^-

16-bit
= 1.2 e^-/ADU



Max. grey 2040 ADU = 1700 e^-

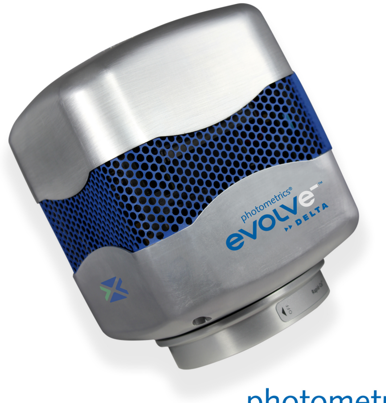
EMCCD

- EMCCD – Electron Multiplied CCD sensors have been in place for over 10 years and are used for extreme low light imaging applications

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

- EMCCD sensors are also **back illuminated** to give higher quantum efficiencies (>90%) and have **large pixels** (as much as 24x24 µm) for improved photon collection

- The real advancement comes from the addition of an **Electron Multiplication** register to **overcome read noise**



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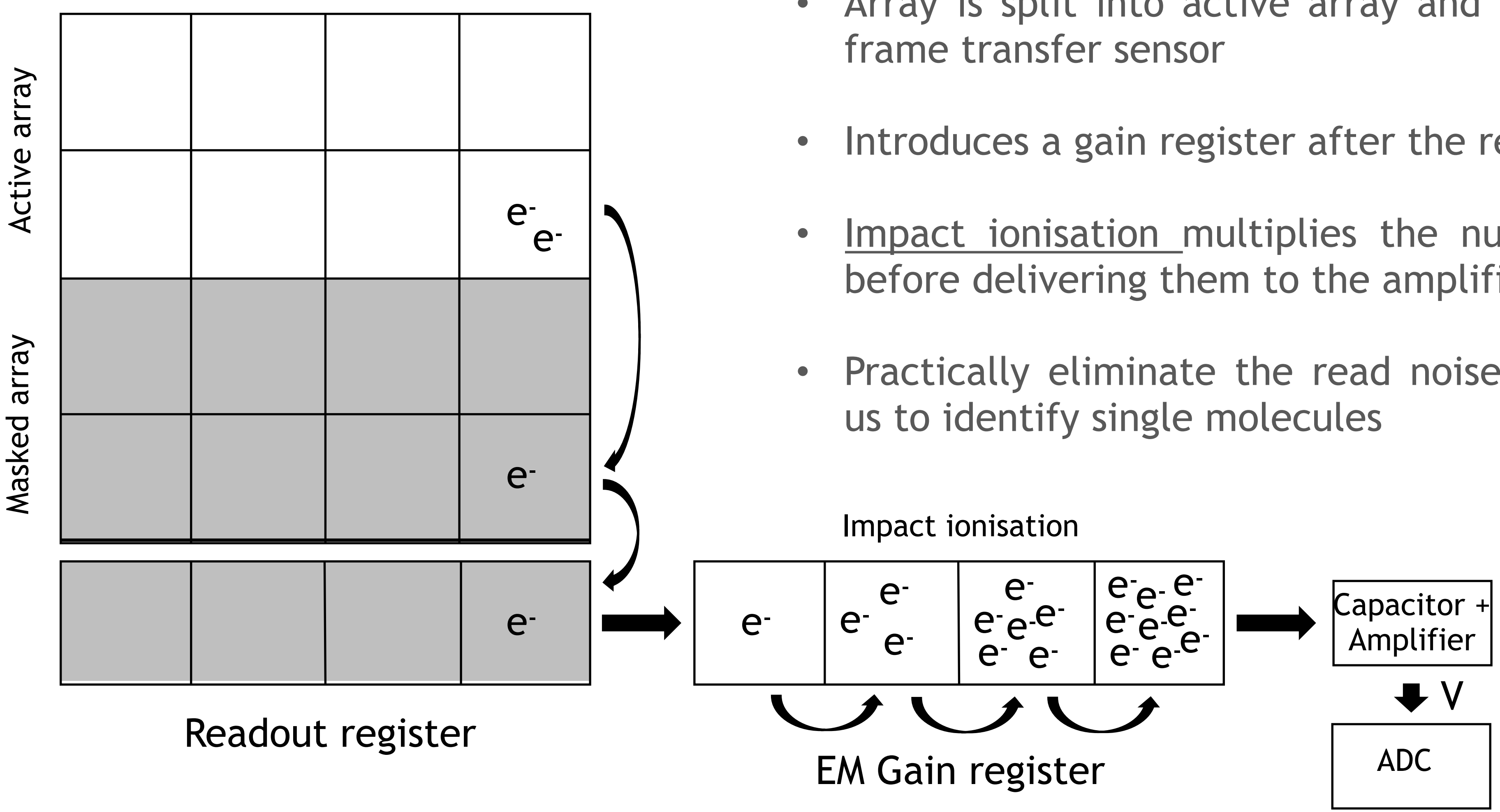
EMCCD Fundamentals



- Array is split into active array and masked array on a frame transfer sensor
- Introduces a gain register after the readout register
- Impact ionisation multiplies the number of electrons before delivering them to the amplifier
- Practically eliminate the read noise floor and enables us to identify single molecules

SNR for EMCCD: The new equation

EMCCD SNR:

$$SNR = \frac{S}{\sqrt{[S * F2] + [D * F]2 + [\sigma R/G]2}}$$

S	=	Signal in Photoelectrons
D	=	Dark Current Noise = $\sqrt{}$(Dark Current * Exposure)
σR	=	Read Noise
F	=	Excess noise factor , = $\sqrt{2}$ = 1.41
G	=	EM Gain

All values must be compared in units of **electrons,** not grey levels!

Excess Noise Factor (ultra-low signal)

- Consider a very weak signal of **2 photoelectrons** in a pixel, at the same image pixel size

- **Back-illuminated CMOS Sensor:**

  Photon Shot Noise = ± 1.4 e-
  Read Noise = ± 1.5 e-
  Signal to Noise = 2 : 2.05 = **0.97 : 1**

- **EMCCD Sensor:**

  Photon Shot Noise x **E.N.F** = ± 2.0 e-
  Read Noise = ± 0.25e- (at 300 EM Gain)
  Signal to Noise = 2 : 2.01 = **0.99 : 1**

Note: only a back-illuminated CMOS can collect the same number of photons as the EMCCD due to its high quantum efficiency

Excess Noise Factor (typical signal)

- Consider a typical signal of **200 photoelectrons** in a pixel, at the same image pixel size

- **Back-illuminated CMOS Sensor:**

  Photon Shot Noise = ± 14 e-
  Read Noise = ± 1.5 e-
  Signal to Noise = 200 : 14.2 = **14 : 1**

- **EMCCD Sensor:**

  Photon Shot Noise x **E.N.F** = ± 20e-
  Read Noise = ± 0.25e- (at 300 EM Gain)
  Signal to Noise = 200 : 20 = **10 : 1**

Note: only a back-illuminated CMOS can collect the same number of photons as the EMCCD due to its high quantum efficiency

CMOS

- CMOS as a technology is as old as CCD but was not considered as a sensor capable of light detection until 1992 by Dr. Eric Fossum, a scientist at NASA's Jet Propulsion Laboratory

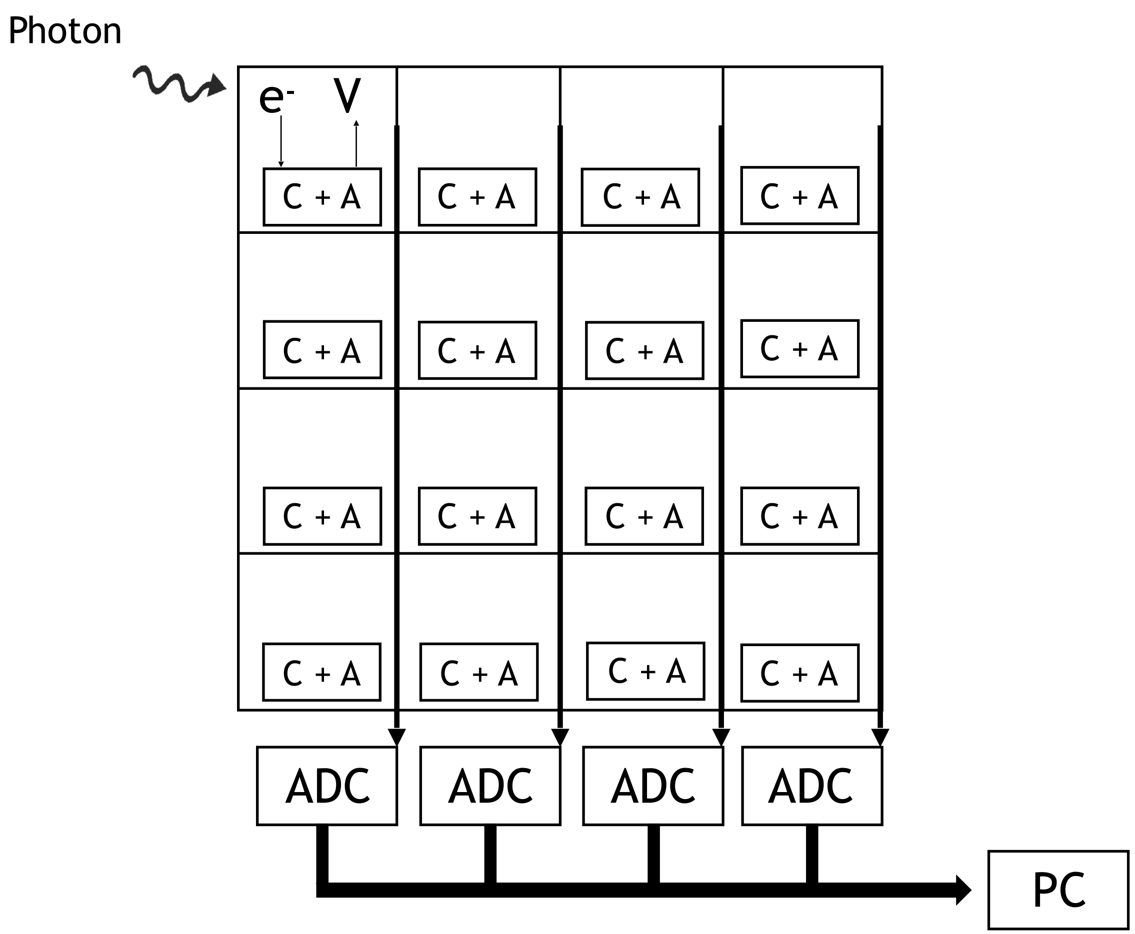
- CMOS technology differs by amplifying signals at the individual pixel level, and digitising the values of all columns in **parallel**.

  Parallelisation → Speed!

- CMOS sensors also, by nature, require around 100x less power than CCD making them the perfect choice for camera phone sensors

- As sensors are mass produced for mobile phone imaging and also for non-imaging applications, the pricing has been driven low by the market

CMOS Fundamentals



- Photons hit the pixels and create electrons
- Capacitor and amplifier are on every pixel
- Voltages down the whole column are sent to the analogue to digital convertor (ADC) and the digital signal is read by a computer

- CMOS cameras are much faster because they have one amplifier per pixel and one ADC per column

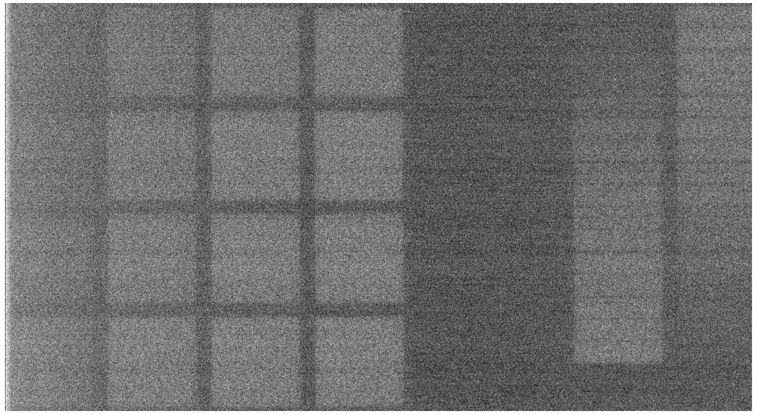
- Low enough read noise floor to detect weak fluorescence, but early CMOS couldn't rival EMCCD

sCMOS vs CCDs

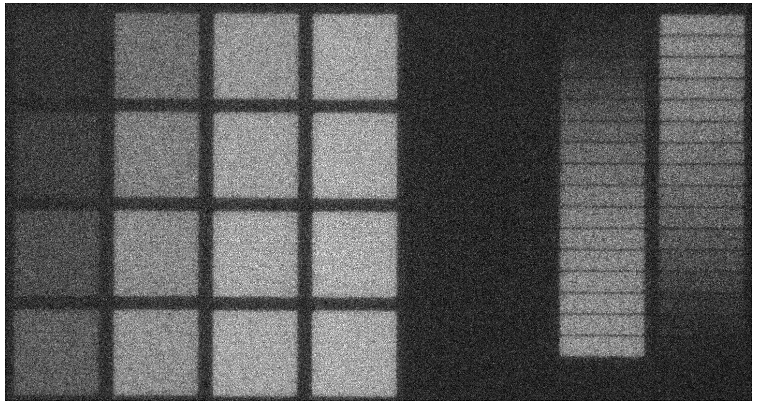
sCMOS was an obvious improvement over the dominant CCD cameras of the time

Since then, sCMOS has largely taken over as the dominant camera with all camera manufacturers offering their version

But how are we taking CMOS devices forward?



CCD Camera
CoolSNAP EZ
10ms Exposure
$8e^-$ Read Noise
8 mm diagonal
10 fps max



sCMOS Camera
Prime
10ms Exposure
$1.3e^-$ Read Noise
18.8 mm diagonal
100 fps max

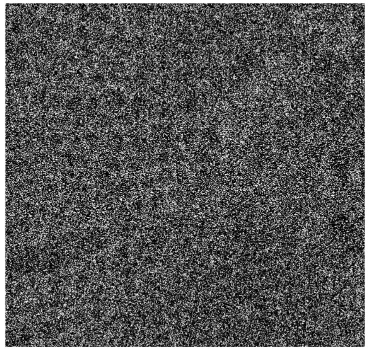
Competing with EMCCD Sensitivity

EMCCD was the standard for low light imaging:

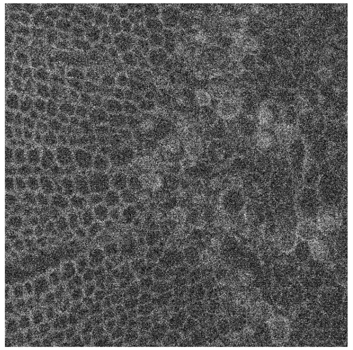
- Back illumination for up to 95% QE
- Large 16 µm pixels for high photon capture
- Electron multiplication eliminated read noise

sCMOS couldn't compete at low signal levels:

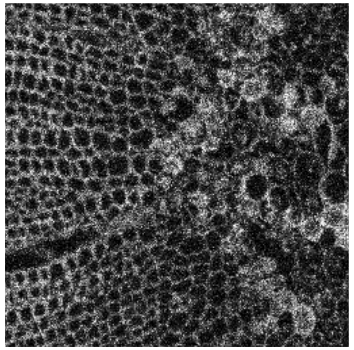
- Lower QE
- Higher noise
- Problems with patterns & artefacts
- Smaller pixels



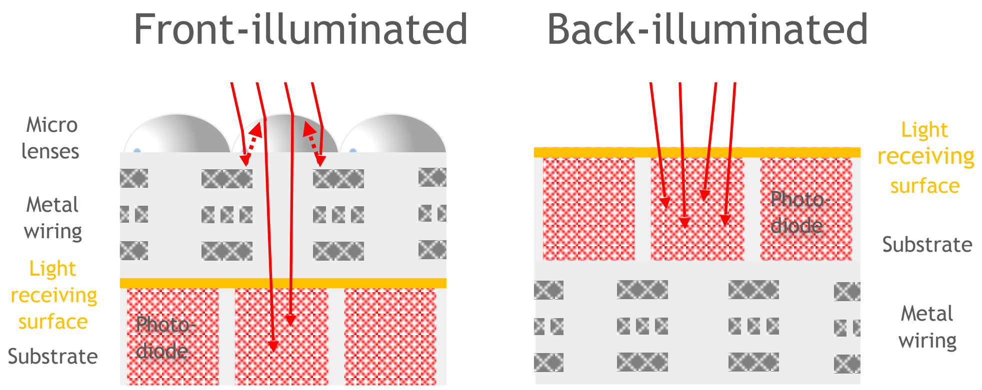
Interline CCD ($5e^-$ read noise)



sCMOS ($1.5e^-$ read noise)



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



Competing with EMCCD Sensitivity

Aside from sensitivity, sCMOS devices have multiple advantages over EMCCD:

Speed

- EMCCDs - 67 fps full frame
- **sCMOS - 100 fps full frame**

FOV

- EMCCD - 11.6 mm diagonal
- **sCMOS - 18.8 mm diagonal**

Pixel size

- EMCCD – 16 µm pixels, 150x magnification for Nyquist sampling
- **sCMOS - 6.5 µm pixels, 60x magnification for Nyquist sampling**

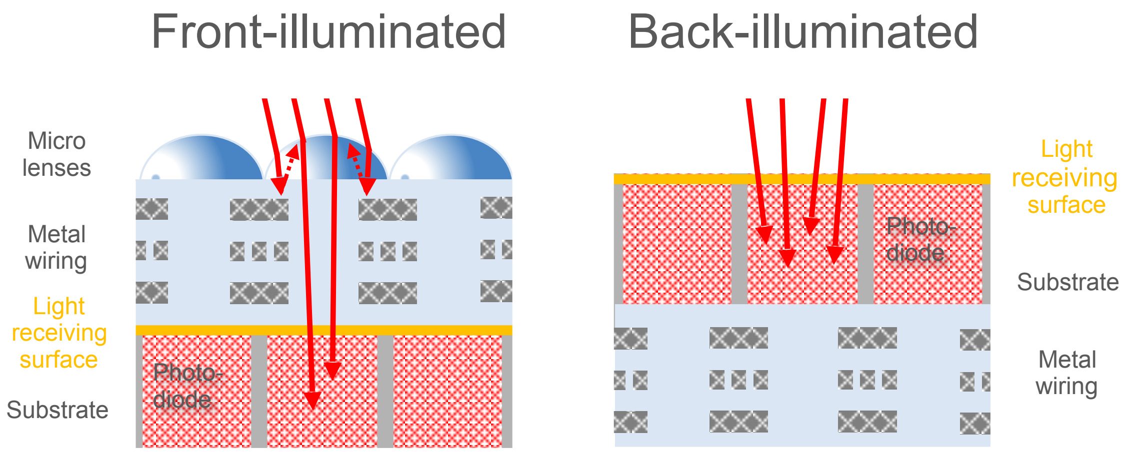
Other EMCCD downsides:

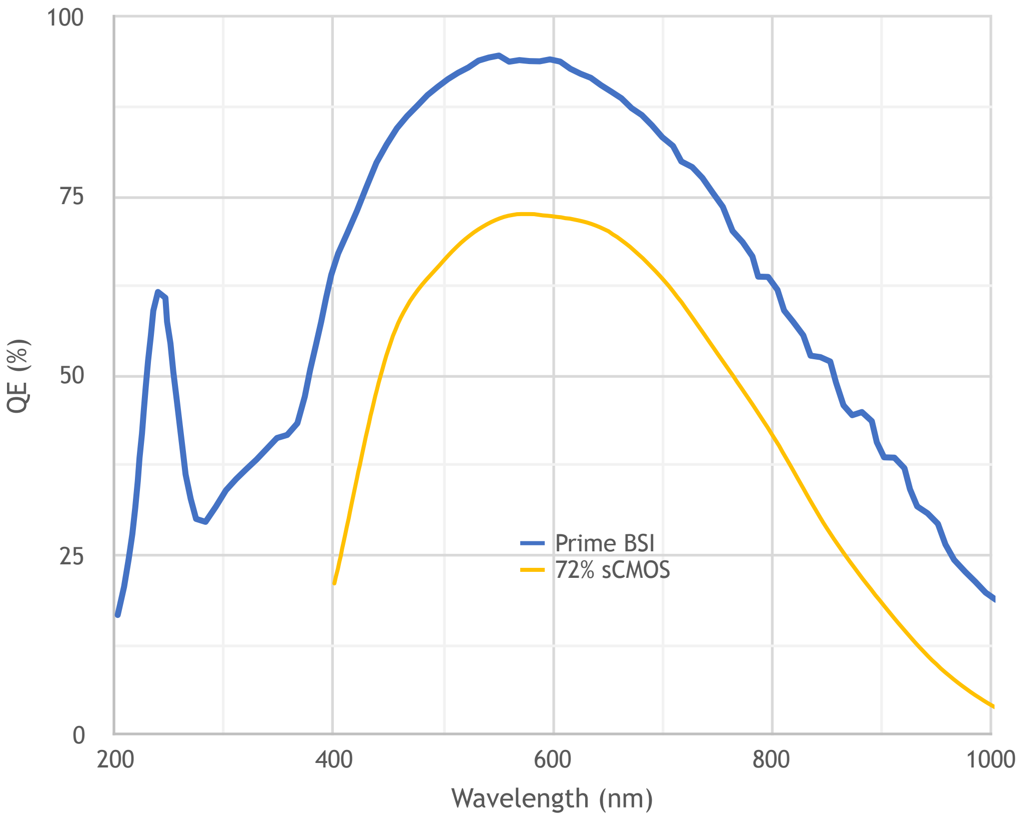
- Excess noise factor
- EM-gain decay
- Regular calibration by user
- Expensive

…But for many applications, the sensitivity of sCMOS is not enough.

Back-Illuminated CMOS

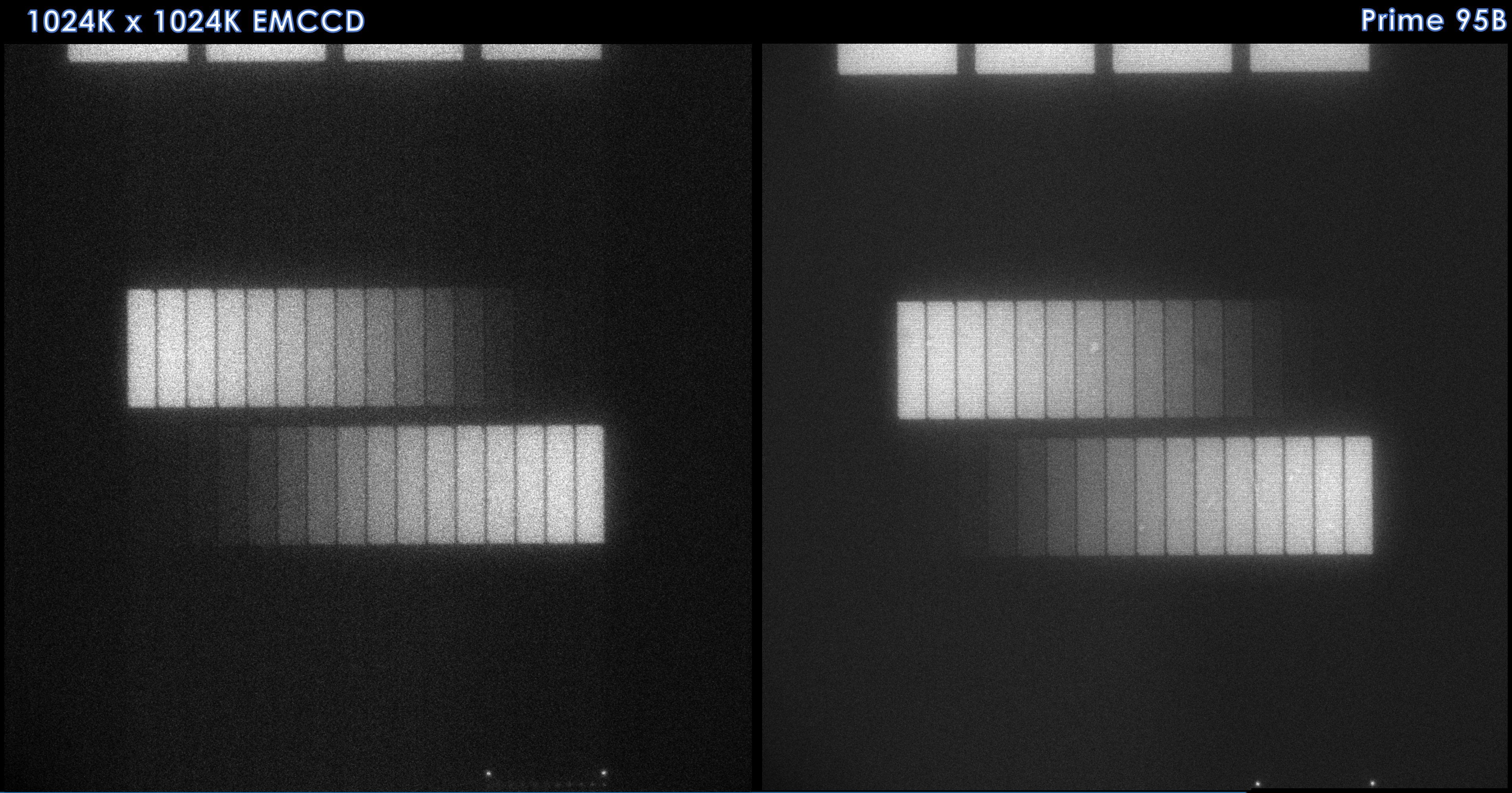
- Avoid the reflecting layers completely by bringing the light in from the back of the sensor





- Achieve real 95% QE
- Maintain 1.0 - 1.6e- Read Noise

Sensitivity comparison EMCCD vs 95B – 30 ms



Which one to choose?

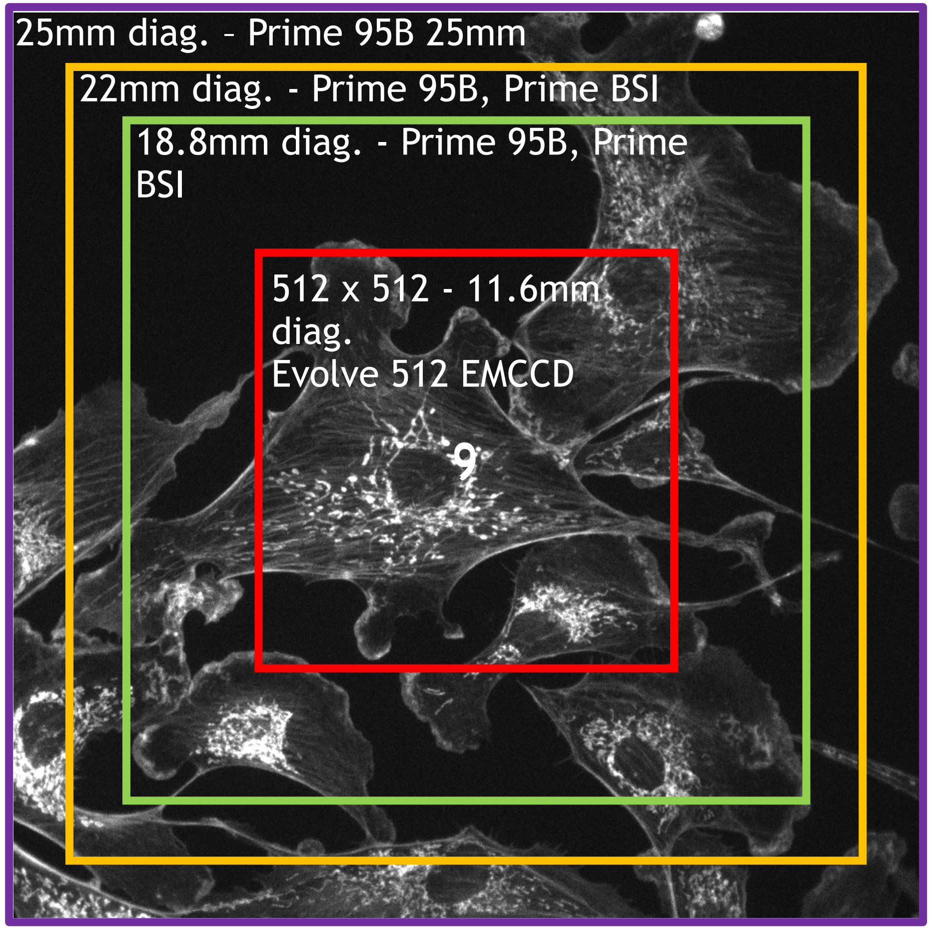
CCD	EMCCD	Scientific CMOS
Plenty of light	Very low light	(Very) low light
High read noise	Very low read noise	Low read noise
Slow processes	Fast processes	Very fast processes
Small pixels - Lots of detail with low mag objectives	Very large pixels – poor resolution or needs high mag	Balancd pixel sizes - Optimized for Nyquist (40x/60x/100x)
Small FOV	Small FOV	Large FOV
Cheap(ish)	Very expensive	Affordable/expensive
➔ Documentation purposes ➔ Long exposure time ➔ Colour	➔ Single Molecule Tracking, Photon Counting	➔ Single Molecule Tracking, TIRF, Spinning Disk, FRET, Super Resolution, Light Sheet, Widefield, Calcium Imaging...

Thank you!

Field of View: Sensor Size

Example Camera	Pixel size (µm)	XY (pixels)	Diagonal (mm)
Evolve Delta	16x16	512x512	11.6
Prime BSI	6.5x6.5	2048x2048	18.8
Prime 95B	11x11	1200x1200	18.66
Prime 95B 22mm	11x11	1400x1400	22.0
Prime 95B 25mm	11x11	1608x1608	25.0

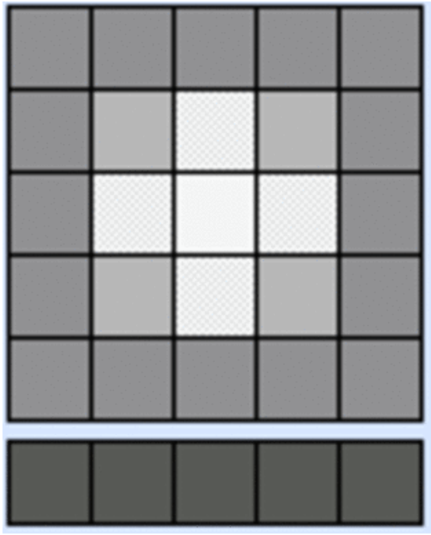
Field of view depends on **sensor size** and **magnification**



Rolling Shutter

- CCD's shutter all pixels at an identical time leading to single snap shot in time
- CMOS sensors are read/shutter different pixels at different time meaning you have a chance for a distortion.
- This shuttering process is known as "Rolling Shutter"
- Possible distortion of <u>very</u> fast moving objects
- Poor synchronization with changing illumination experiments
- Some experiments may require a "Global" shutter

CCD



CMOS