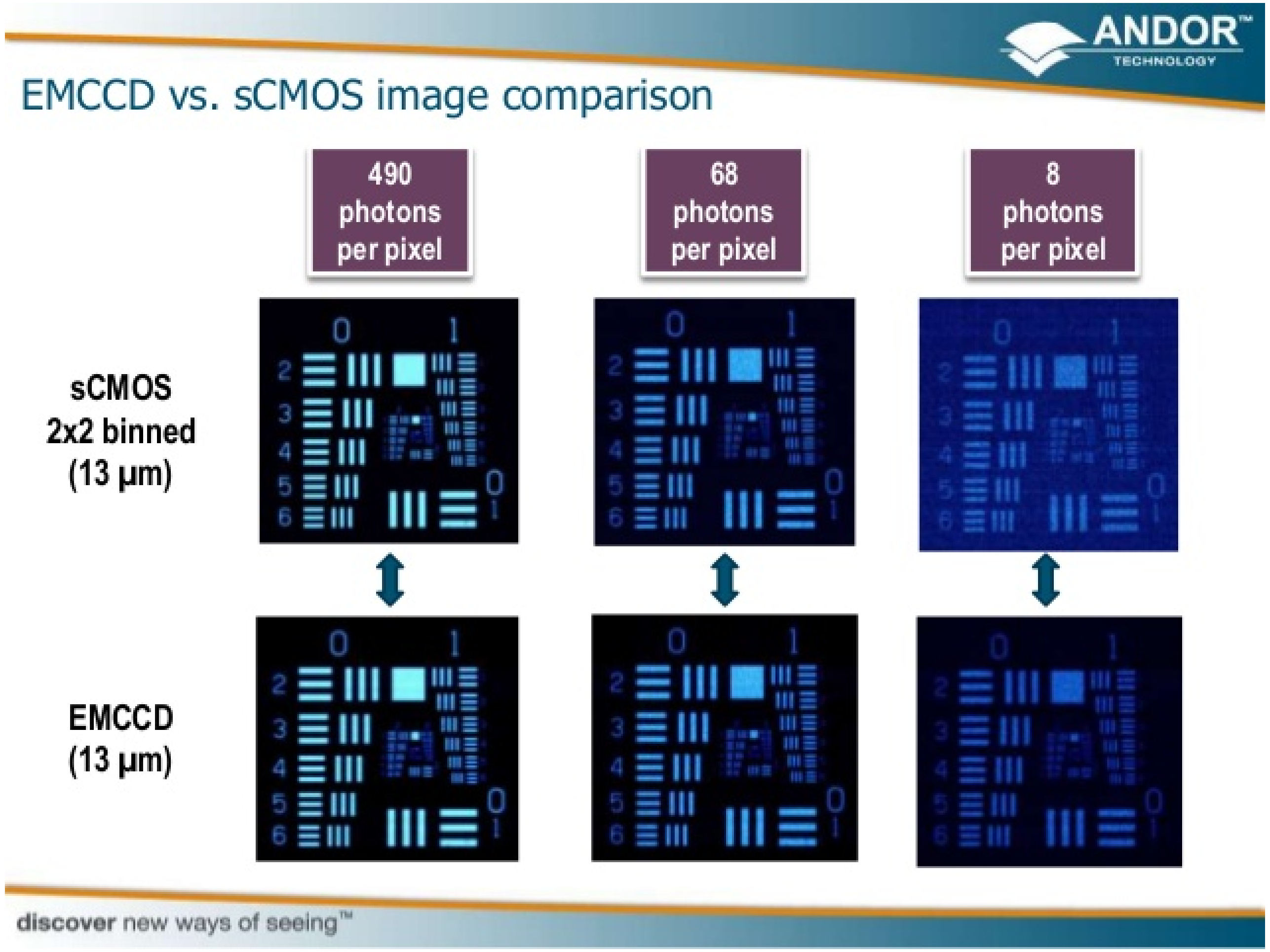
Camera parameters and single element detectors

- Last class
  - CMOS
  - sCMOS
- This class
  - Think about your imaging
  - Single element detectors

sCMOS vs EMCCD

- Both are expensive
- EMCCD does better at very low light levels
- EMCCD has bigger pixels
- sCMOS has more pixels
- sCMOS can run larger fields of view, faster
- sCMOS has better dynamic range



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EMCCD vs. sCMOS image comparison

	490 photons per pixel	68 photons per pixel	8 photons per pixel
sCMOS 2x2 binned (13 µm)			
EMCCD (13 µm)			

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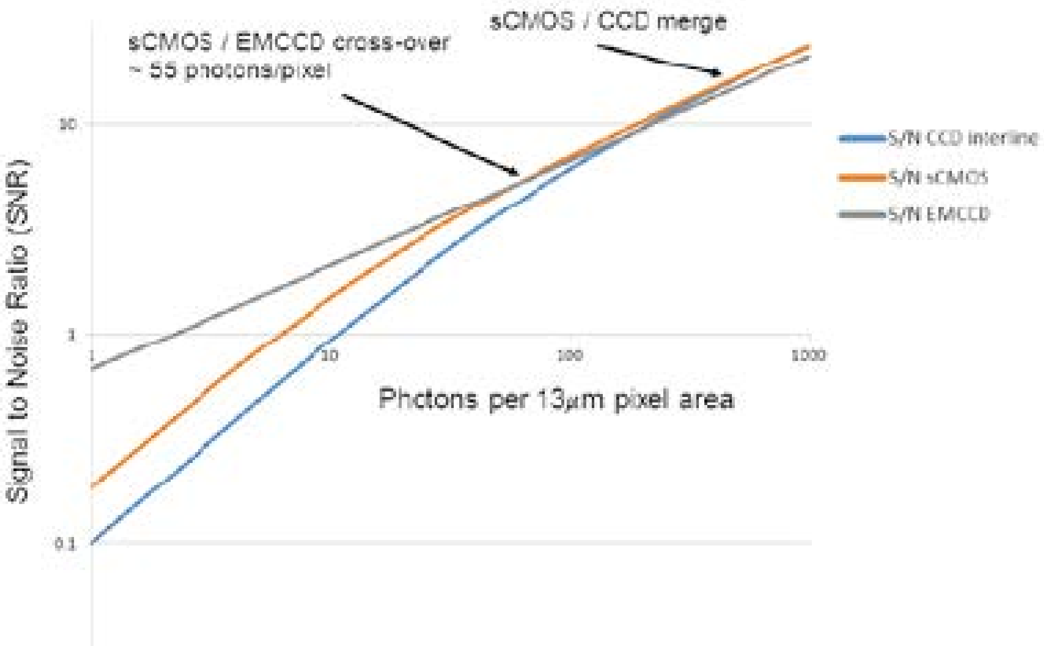
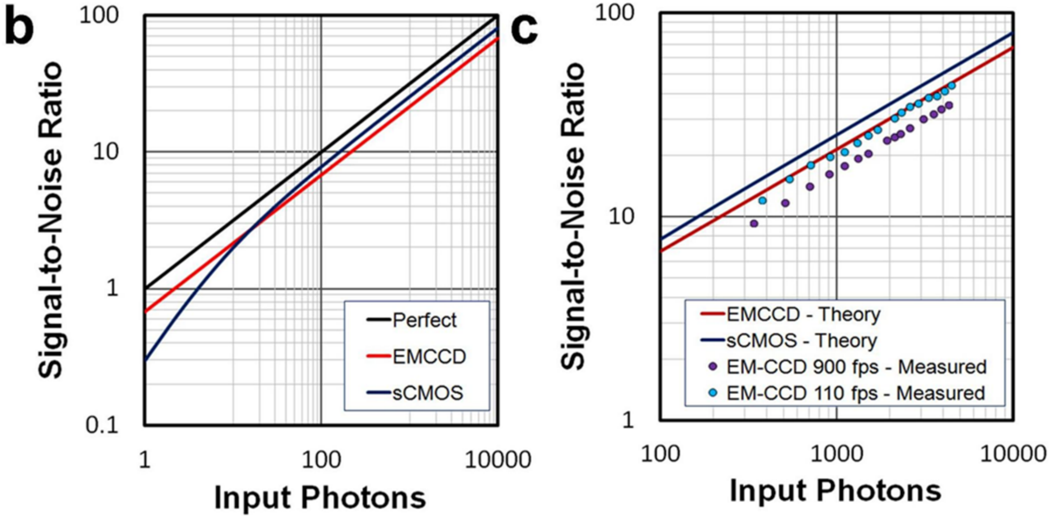
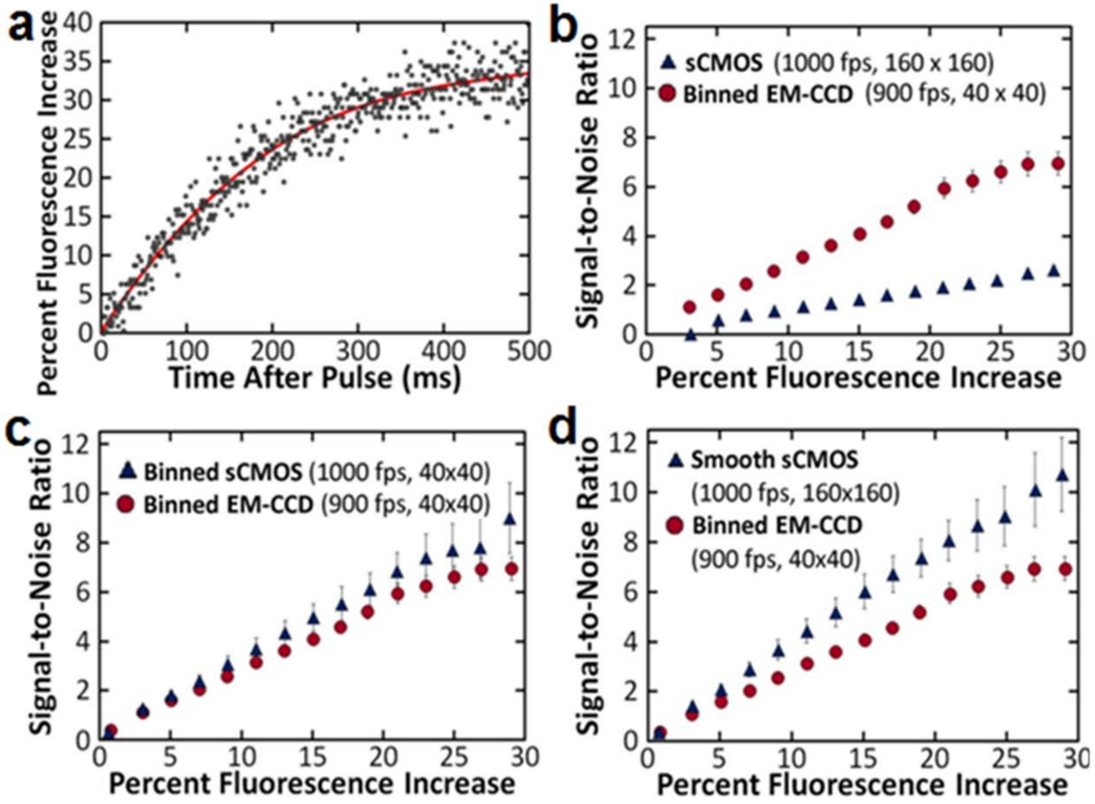
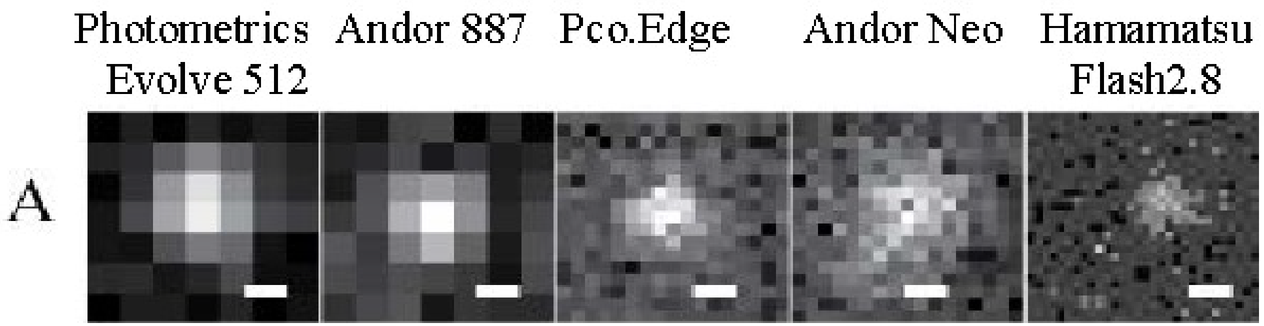


a

	Active Pixels	Peak QE	Read Noise	Max Full Frame Rate	Pixel Size	Dark Current (e⁻/pixel/sec)
EMCCD	512 x 512	90%	< 1 e⁻ with EM	56 fps	16 µm x 16 µm	0.001 @ -85°C
sCMOS	512 x 512	65%	< 2 e⁻	1000 fps	15 µm x 15 µm	0.5 @ -30°C





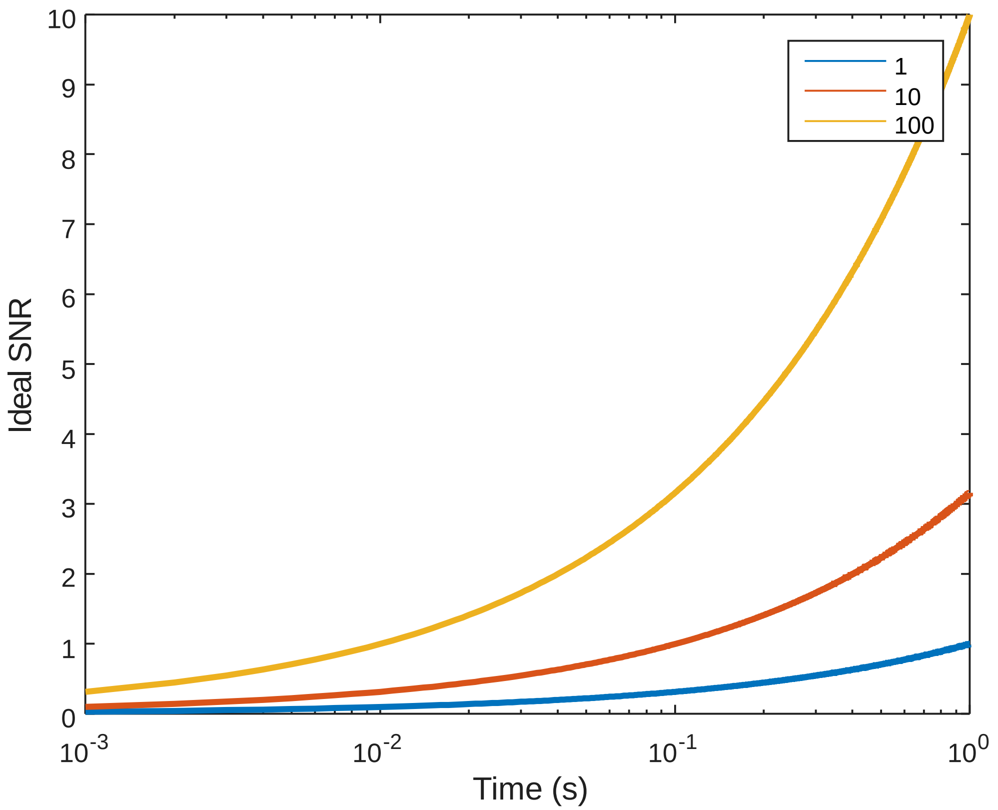


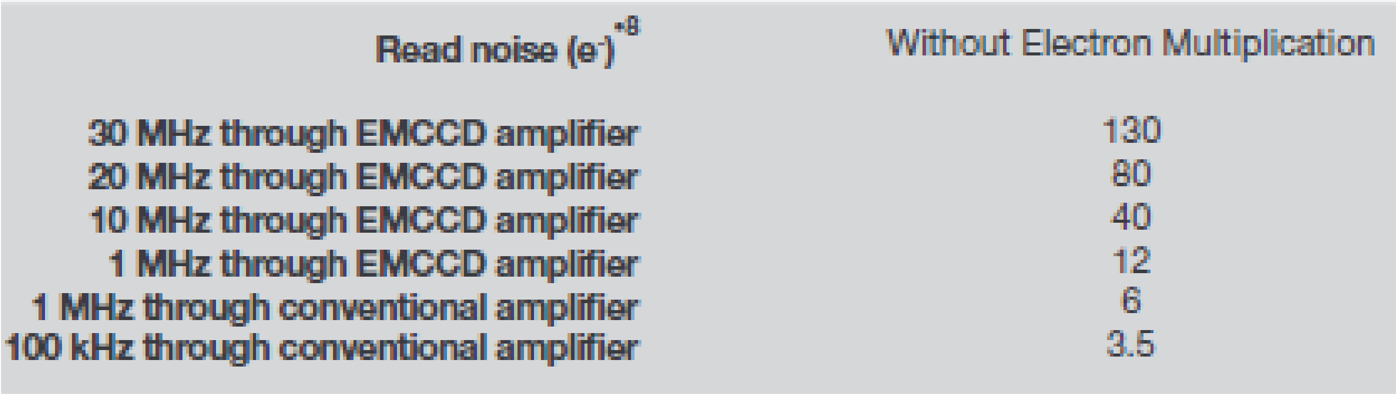
6 parameters for image quality

- Temporal resolution
- Spatial resolution
- Quantum efficiency
- Noise
- Dynamic Range
- Signal to noise ratio

Temporal resolution

- How fast are the processes you need to measure?
- Sample at least 2x (Nyquist frequency)
- Timing will help determine which camera is necessary
- More pixels -> slower times
- Dynamic range -> slower times
- Shot noise goes as √N





Read noise (e$^-$)^{*8}	Without Electron Multiplication
30 MHz through EMCCD amplifier	130
20 MHz through EMCCD amplifier	80
10 MHz through EMCCD amplifier	40
1 MHz through EMCCD amplifier	12
1 MHz through conventional amplifier	6
100 kHz through conventional amplifier	3.5

Spatial resolution

- Maximum resolution set by the NA of the objective and wavelength
- Pixels should ideally be spaced at 2x density (1/2 of max resolution)
- On small pixel cameras, often binning at 2x2 still satisfies Nyquist
- Image size also can become an issue

Consider 100x, 1.3 NA objective
6.5 μm sCMOS pixel size

$$d = \frac{\lambda}{2NA} = \frac{650\ nm}{2 * 1.3} = 250nm$$

$$s_{pix} = \frac{6.5\mu m}{100x} = 65nm$$

Lose no resolution by binning at 2x2, but decrease noise

Consider 60x, 1.49 NA objective
6.5 μm sCMOS pixel size

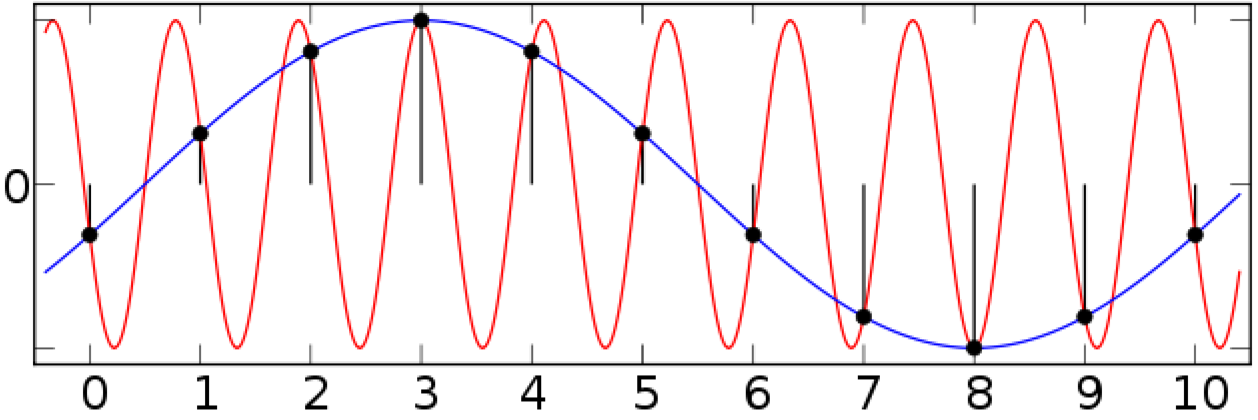
$$d = \frac{\lambda}{2NA} = \frac{520\ nm}{2 * 1.49} = 174nm$$

$$s_{pix} = \frac{6.5\mu m}{60x} = 108nm$$

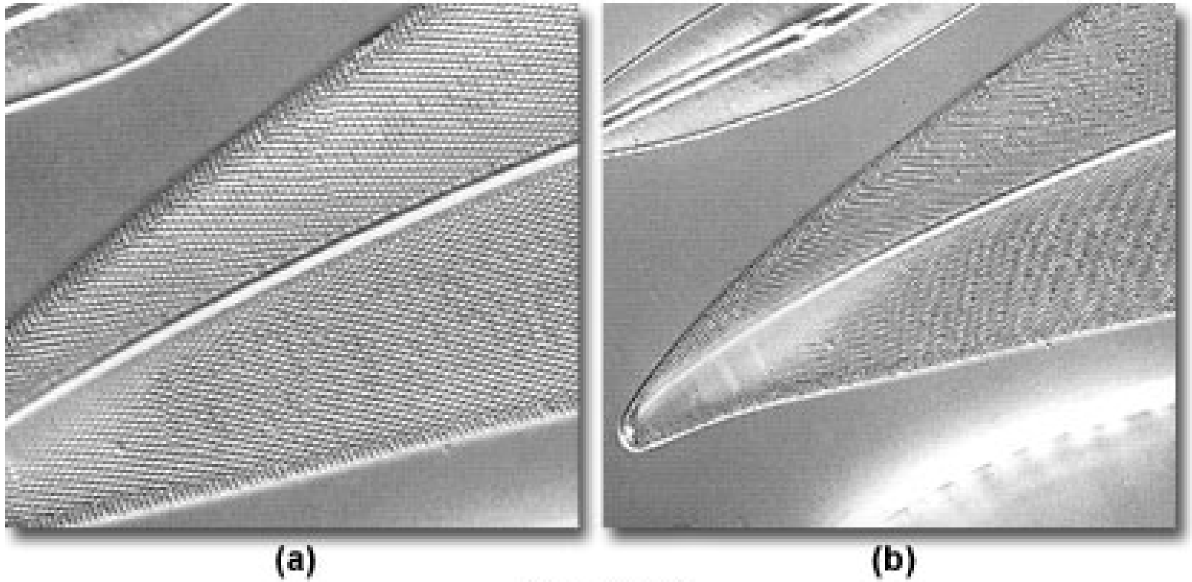
On this objective, you can maintain max resolution at 1x1

Spatial resolution
Aliasing

- Aliasing occurs when there are frequencies in your image higher than your sampling rate
- Higher frequencies can be mapped into lower frequencies detected by the camera
- Often seen in periodic structures like muscle fiber
- Ensure that your pixelation is at least 2x the periodic frequency in your sample



Aliasing in Digital Images



(a)

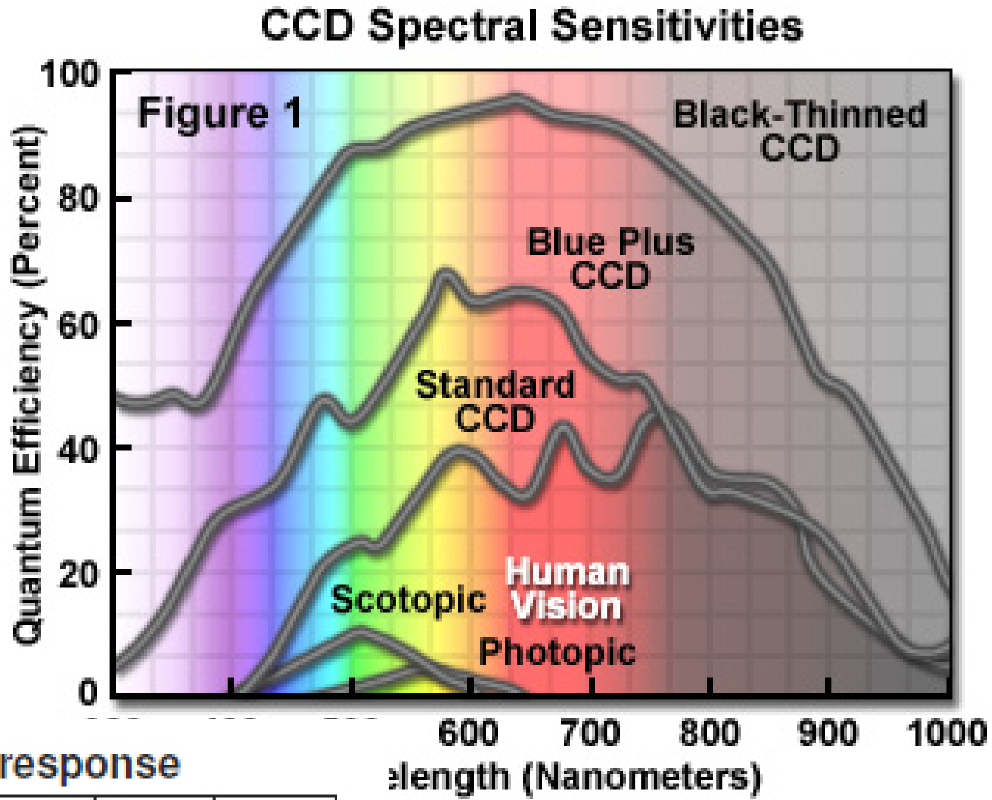
(b)

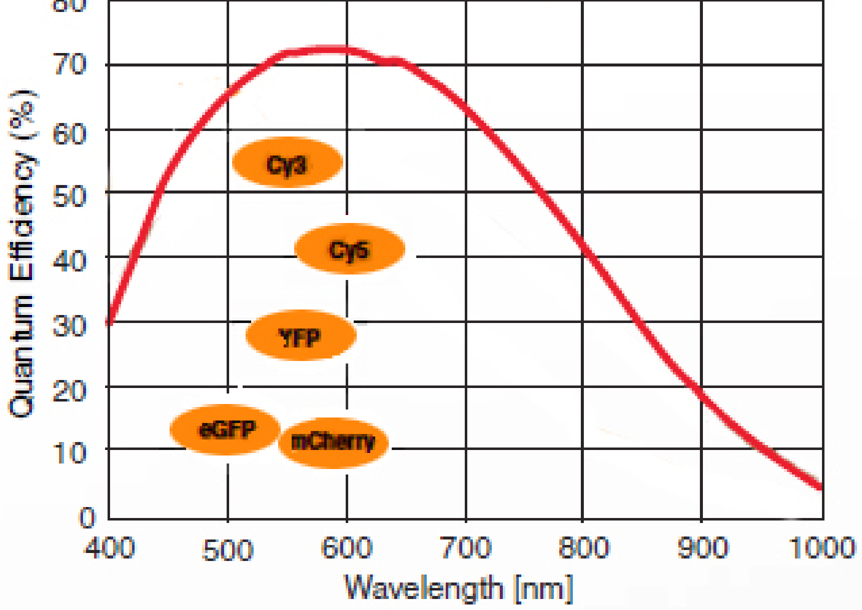
Figure 1

$$f_{alias} = |f - N * f_s|$$

Quantum efficiency

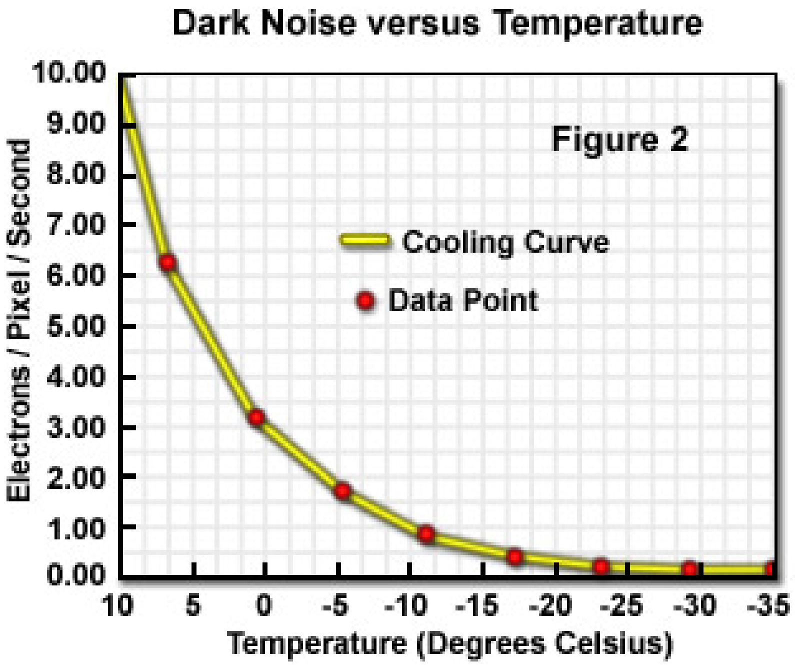
- Front illuminated CCD – 50%
- Back illuminated CCD – 90%
- CCD and sCMOS cameras are peaked in the visible (500 – 650 nm), but if your dye falls outside that region, you may need to think carefully

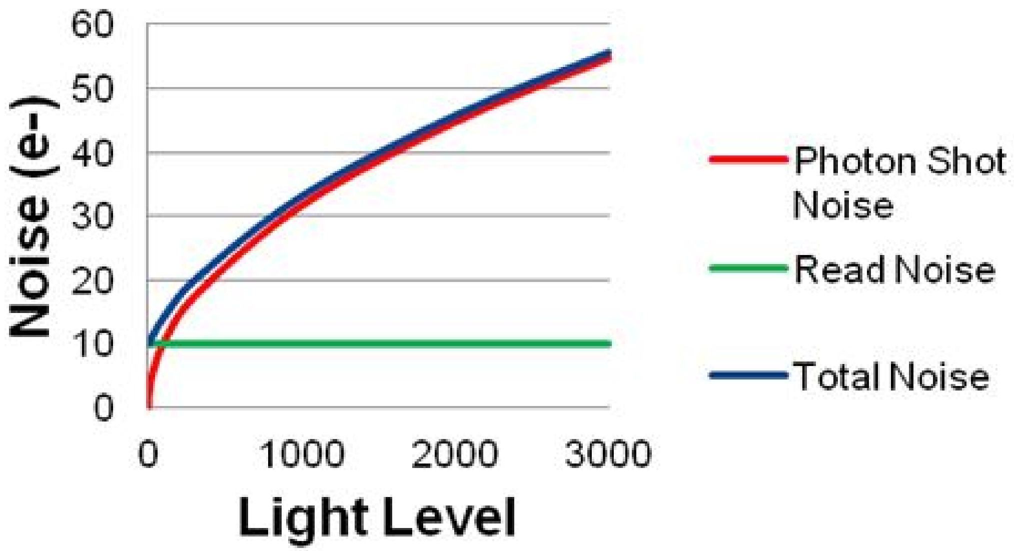

CCD Spectral Sensitivities


Spectral response

Noise

- Photon noise (shot)
- Read noise
- Dark noise
- Other sources of noise, but they are all smaller than read or shot noise
- Each pixel will carry its own noise with all 3 components contributing
- Only in CCDs, binning will reduce the read noise (1 read for 4 pixels in 2x2 bin)
- Will be affected by exposure time, number of photons, gain

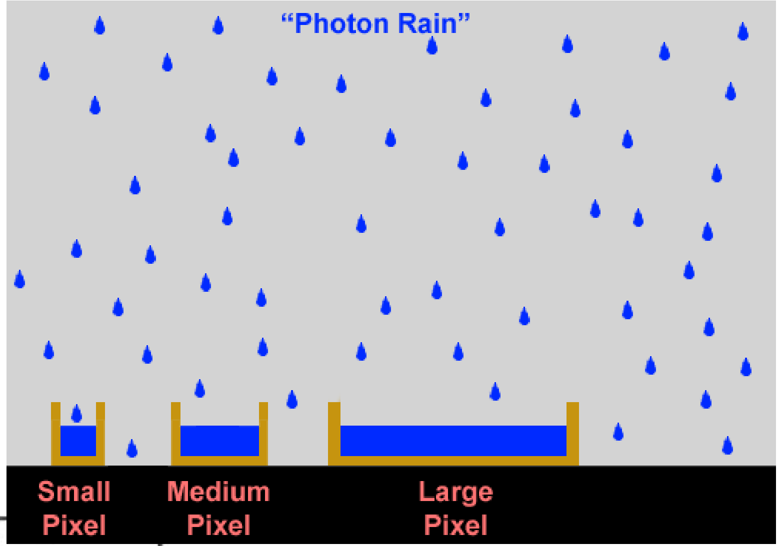


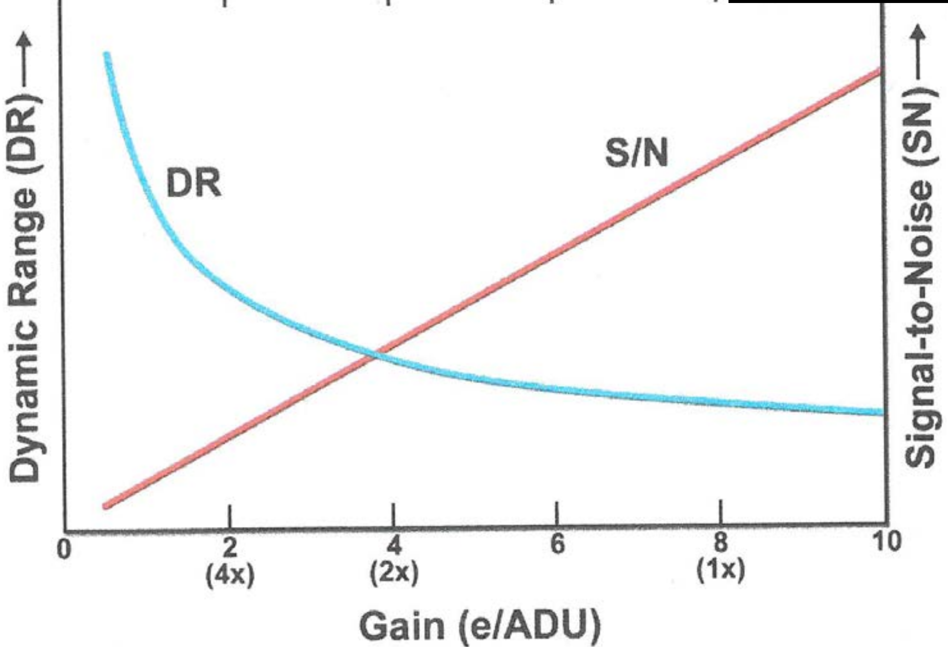


Dynamic range

- Dynamic range is number of gray levels from completely empty to completely full
- Factors of electron well size, analog to digital converter, and gain
- 8- to 16-bit cameras out there, most scientific will be at least 12 bit
- 8 bit = 256 gray levels
- 16 bit = 65535 gray levels
- Also plays a role in image size (bytes)
- Note: Printers will often only be able to handle (on 8 bit scale) from 30 – 235 (everything < 30 will be black, everything > 235 will be white)

8 bit, 2 MP image = 2 MB - 200 fr movie = 400 MB
16 bit, 2 MP image = 4 MB - 200 fr movie = 800MB

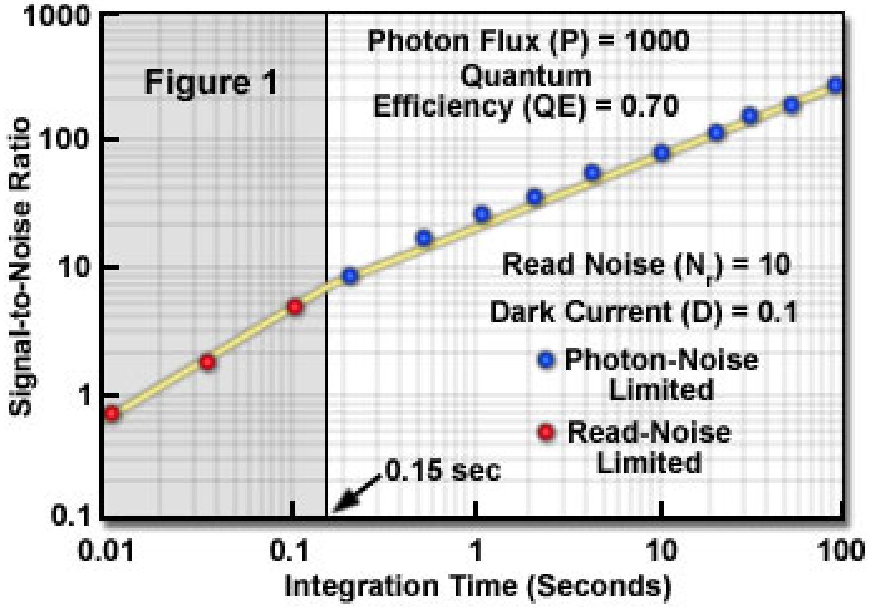




Signal to noise ratio

- Signal to noise is calculated for cameras based on single pixel
- Calculation based on #electrons, not #photons
- Your goal is to lower read noise (it's the only thing you can change) and to raise the signal
- If you're imaging slowly, use a slow ADC read speed
- Shot noise is unavoidable, will always limit SNR

Signal-to-Noise Variation with Integration Time



$$SNR = \frac{Signal}{std(noise)}$$

Signal-to-Noise Ratios in Fluorescence Microscopy

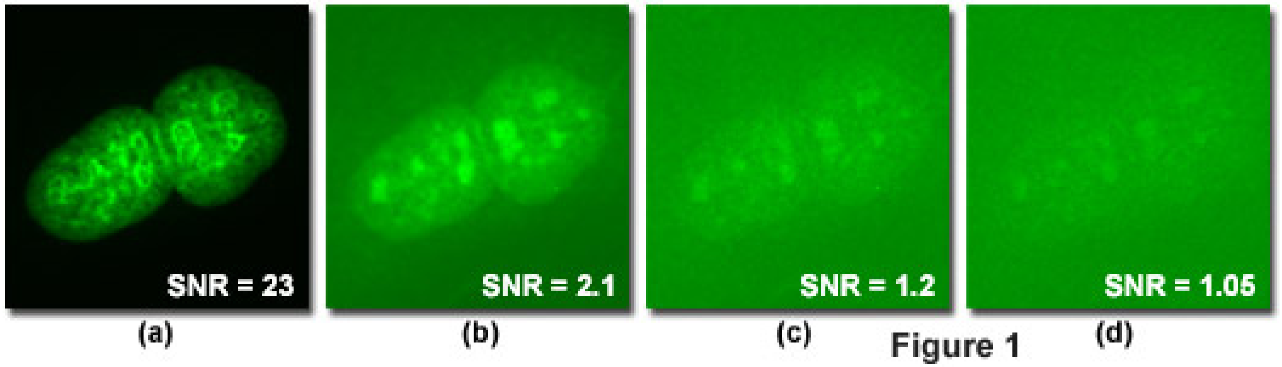
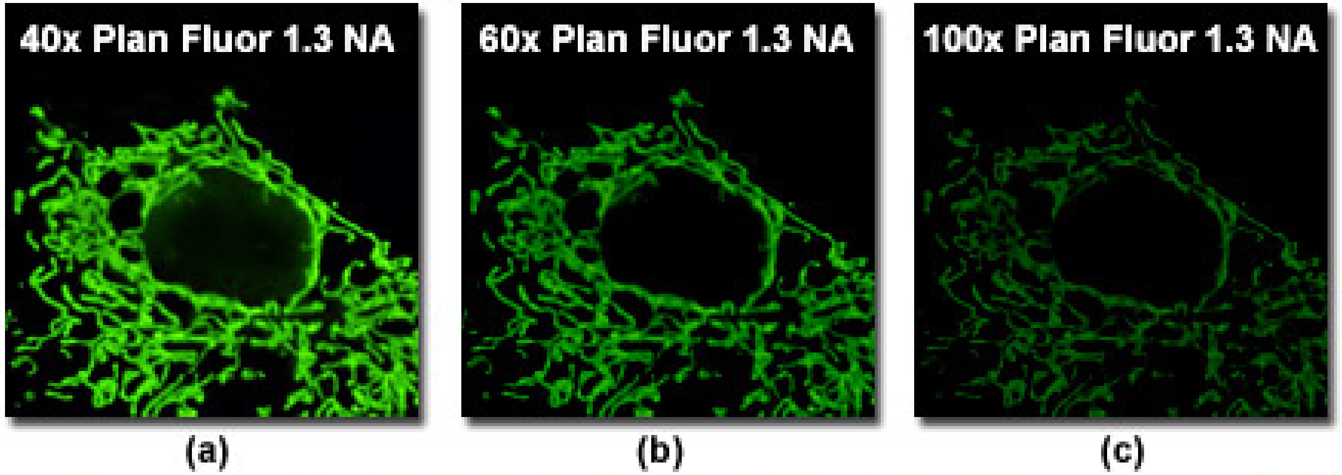


Figure 1

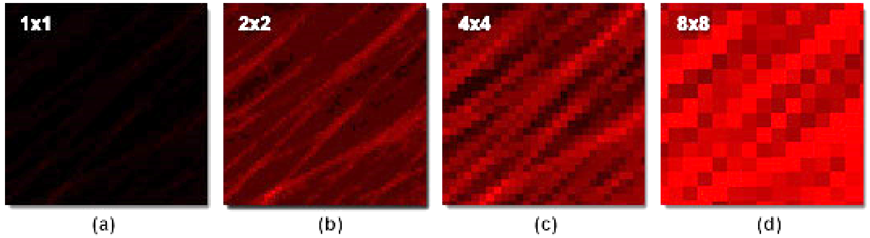
Thinking carefully about experiments

- We know that magnification is not the only number when thinking about objectives, but it will still affect signal
- Each pixel has noise (read, dark, shot), so the more spread out your signal, the higher the noise
- Each sample emits a set number of photons, when they are spread out over more pixels, lower signal to noise

Objective Numerical Aperture Effects in Fluorescence Imaging



Effects of Binning on Spatial Resolution and Final Image Dimensions



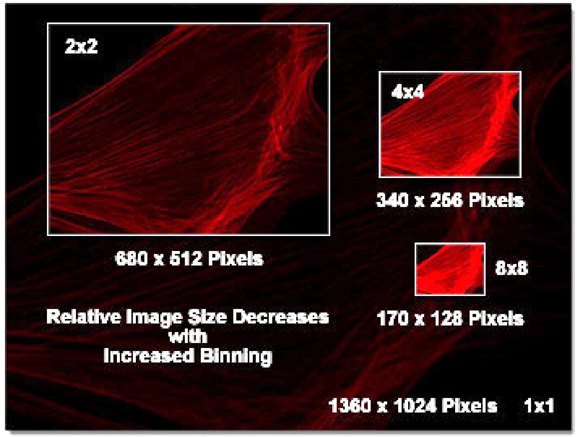


Figure 3

Go to .xls worksheet

Single element detectors

- Good to think about for confocal and 2 photon imaging

Photodiode

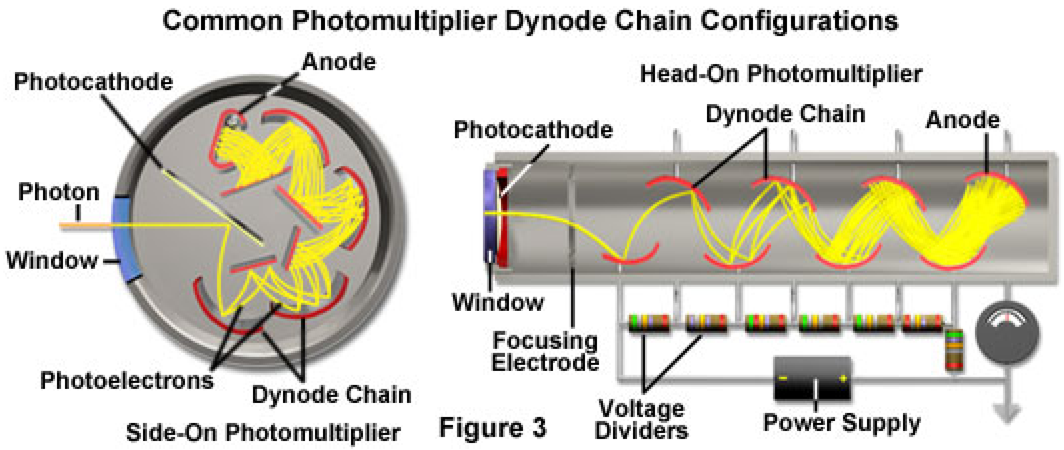
- Photodiode is a single element detector typically made of semiconductor
- It’s similar to a PMT, except there is no amplification
- Very fast and linear response to light, but it is not sensitive to low level applications
- Often used to measure excitation intensity (power meters, or real time measurements)
- Only reports instantaneous voltage, does not store charge

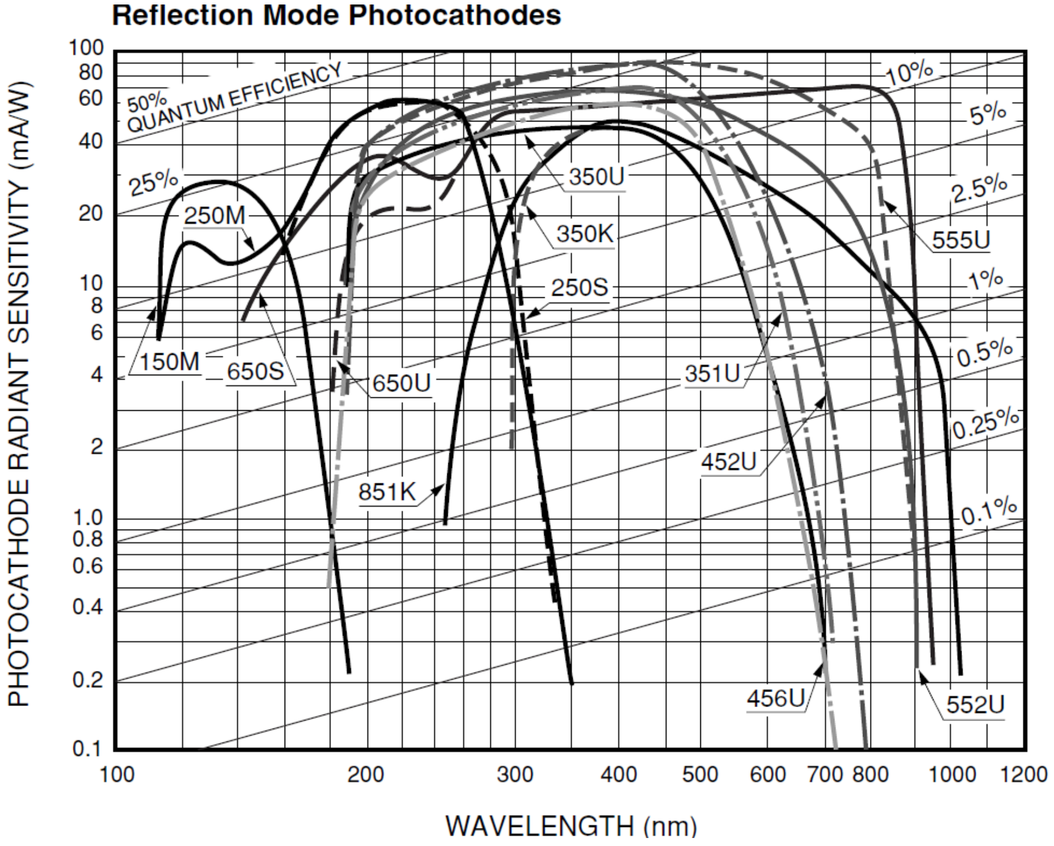
Material	**Range (nm)**
Silicon	190–1100
Germanium	400–1700
Indium gallium arsenide	800–2600
Lead(II) sulfide	<1000–3500
Mercury cadmium telluride	400–14000

Item #	Active Area	Wavelength	Rise Time[a]	(NEP)	Dark Current	Capacitance[b]	Bias Voltage[c]
DET10A	0.8 mm^2	200 - 1100 nm	1 ns	1.2 x 10^{-13} W/Hz$^{1/2}$	0.3 nA (Typ.) 2.5 nA (Max)	6 pF	10 V
DET36A	13 mm^2	350 - 1100 nm	14 ns	1.6 x 10^{-14} W/Hz$^{1/2}$	0.35 nA (Typ.) 6 nA (Max)	40 pF	10 V
DET100A	75.4 mm^2	350 - 1100 nm	43 ns	2.07 x 10^{-13} W/Hz$^{1/2}$	100 nA (Typ.) 600 nA (Max)[d]	300 pF	10 V

Photomultiplier tubes

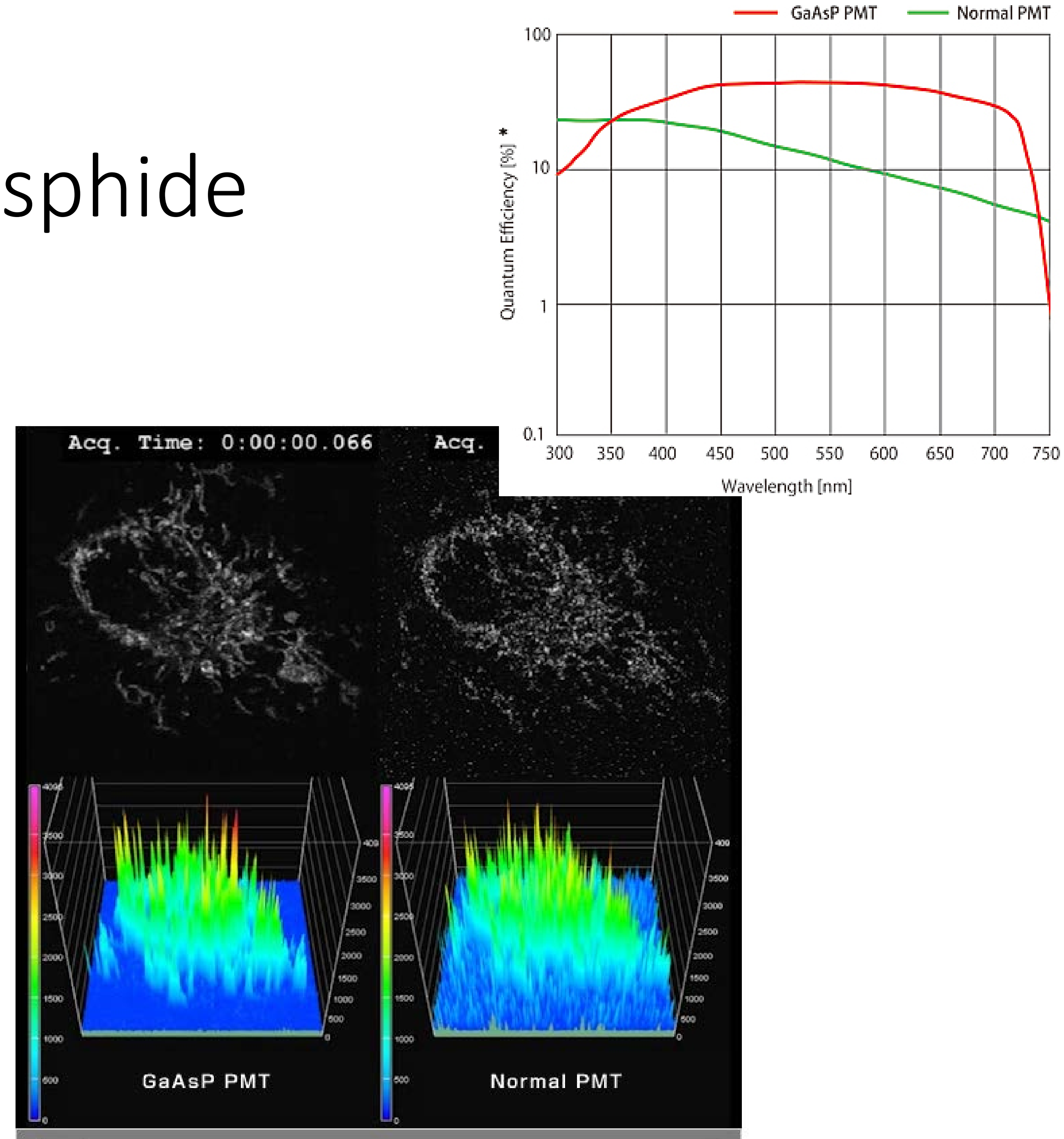
- Single element detector
- Photons incident on photocathode turn into electrons
- Electrons accelerated and increase
- Voltage is read out as signal
- Quantum efficiency is determined by photocathode material
- Photocathodes are often semiconductors





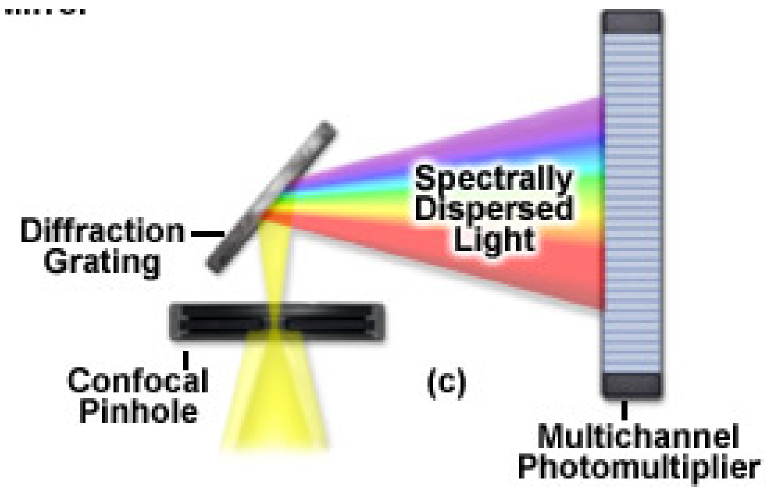
GaAsP –
Gallium Arsenide Phosphide

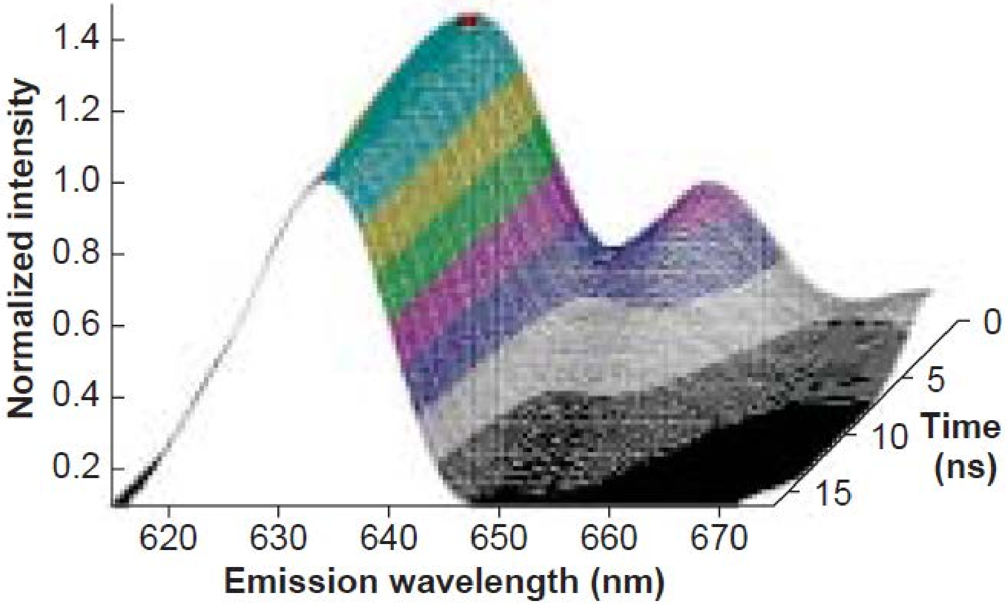
- GaAsP is a very sensitive photocathode
- Quantum efficiencies in the visible are much higher than standard PMTs
- Exotic semiconductor that is hard to make – expensive
- Enables turning down gain to get same signal – less background noise



PMT arrays

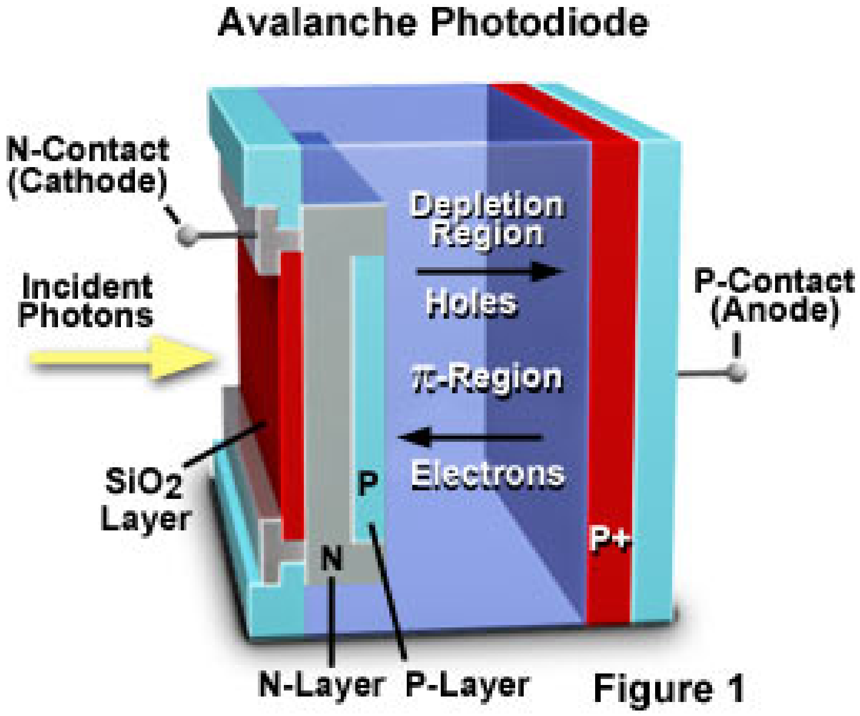
- Up to 32 linear elements with accessible electronics for each channel
- Like a very low resolution, linear camera
- Maintains all the gain benefits of having a PMT

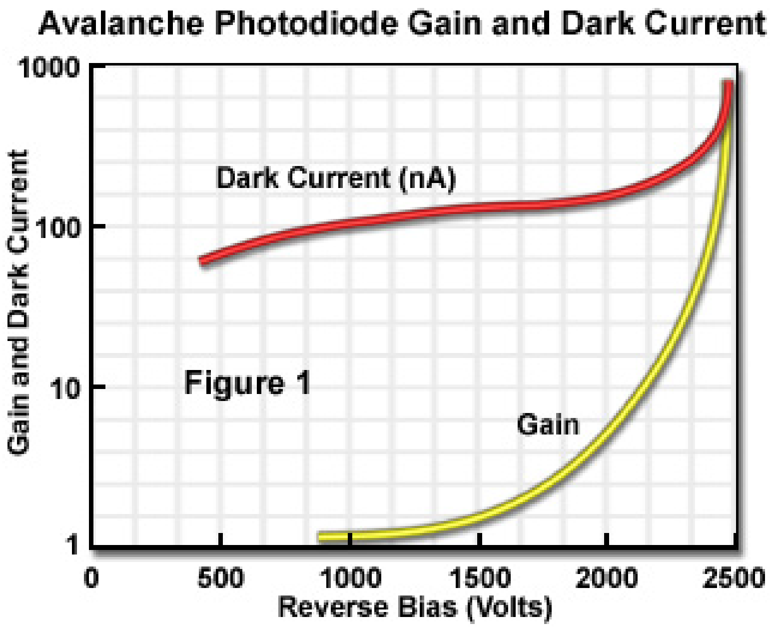




Avalanche photodiode (APD)

- It’s a photodiode with a region of very high electric field to produce high gain of electrons and holes
- Fancy doping of seminconductors enables high voltages to be applied – 1000V -> gains of ~1200x
- A single photon that hits the photodiode will induce the avalanche, and trigger current
- Act as single photon counters





APD issues

- Very useful for low light applications
- Semiconductor absorber, so it is possible to get QEs around 90%
- Single photon resolution
- Dark noise can become an issue at very high gains
- Age degradation of gain

Fluorescence correlation spectroscopy