Detectors for microscopy - CCDs, sCMOS, APDs and PMTs

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“Bio-Imaging”

- when making decisions about the right sensor for an imaging application, there are many variables to consider
    - Light level
    - Integration times
    - Optical configurations of your microscope
    - Accurate morphological representation of your specimens
    - Appropriate spatial resolution
    - Appropriate signal levels relative to noise
    - Accurate capture of dynamic events
    - Minimum perturbation of specimens

Detectors/Sensors in general…

…are devices that detect events or changes in quantities (intensities) and provide a corresponding output, generally as an electrical or optical signal

- What do we expect of a sensor while taking an image?
  - speed – as fast as possible
  - work within a wide range of light levels
  - good dynamic range
  - work at different emission wavelength
  - enough resolution to see details
  - low noise level (good signal-to-noise ratio)

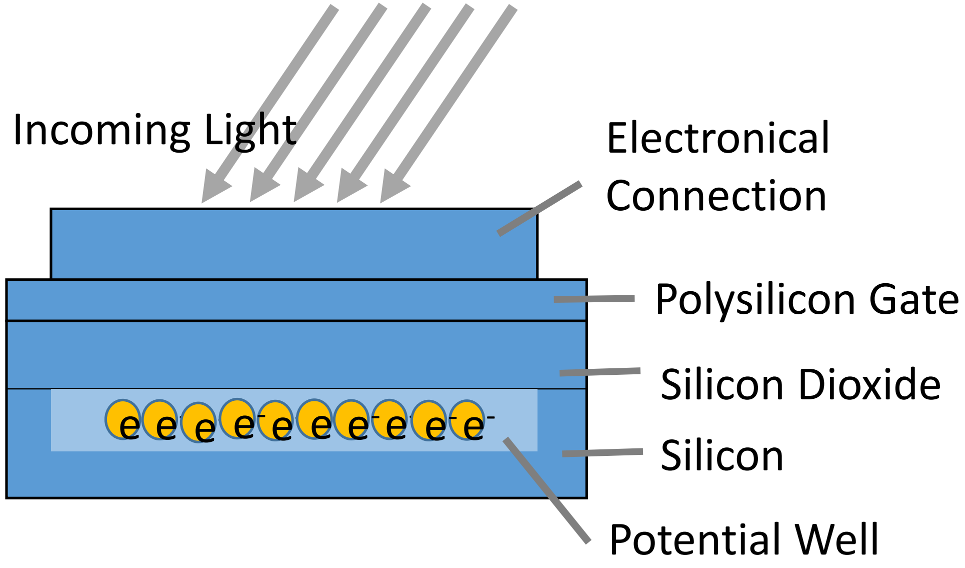
Sensitivity

“A sensor's sensitivity indicates how much the sensor's output changes when the input quantity being measured changes (ratio between output signal and measured property)”

- Sensitivity is a horrible word which is often confused with **Quantum Efficiency**, **Pixel Size**, **Signal** and **Signal to Noise**

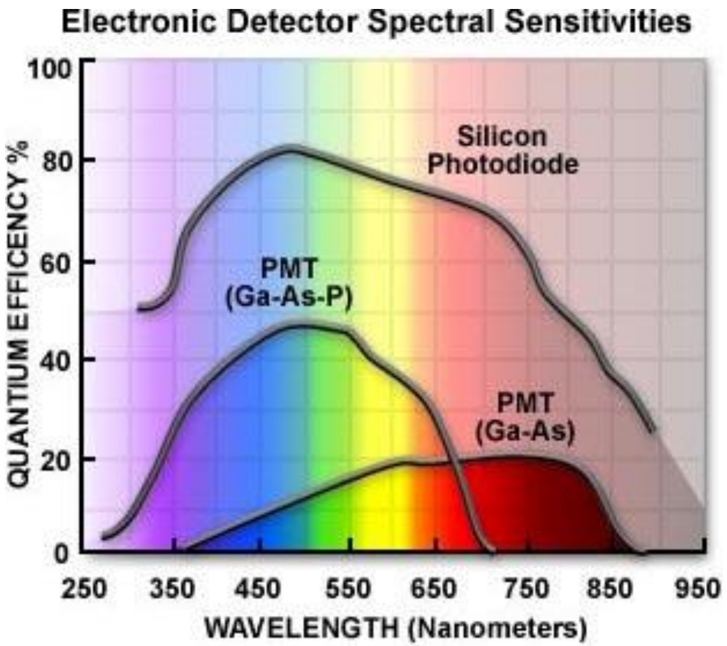
- some key facts:
  - Photons convert to electrons in sensors and they can then be measured – this conversion rate is defined as **Quantum Efficiency**
  - Sensors convert photons of some wavelengths better than others
  - The number of photons that interact with a pixel depend on the physical size of the pixel
  - We can have a sensitive sensor but if our signal to noise is low we get a noisy image with data we cannot decipher

Quantum Efficiency (QE)



- QE is a measure of the effectiveness of an imager to produce electronic charge from incident photons

- The wavelength of incoming light and photon absorption depth are directly related; the shorter the wavelength, the shorter the penetration depth into the silicon



Different Detector Types

- CCD – Charged Coupled Device
- EMCCD – Electron Multiplying CCD
- CMOS – Complementary Metal Oxide Semiconductor
- sCMOS – scientific grade CMOS

- PMT – Photon Multiplier Tube
- APD – Avalanche Photodiode

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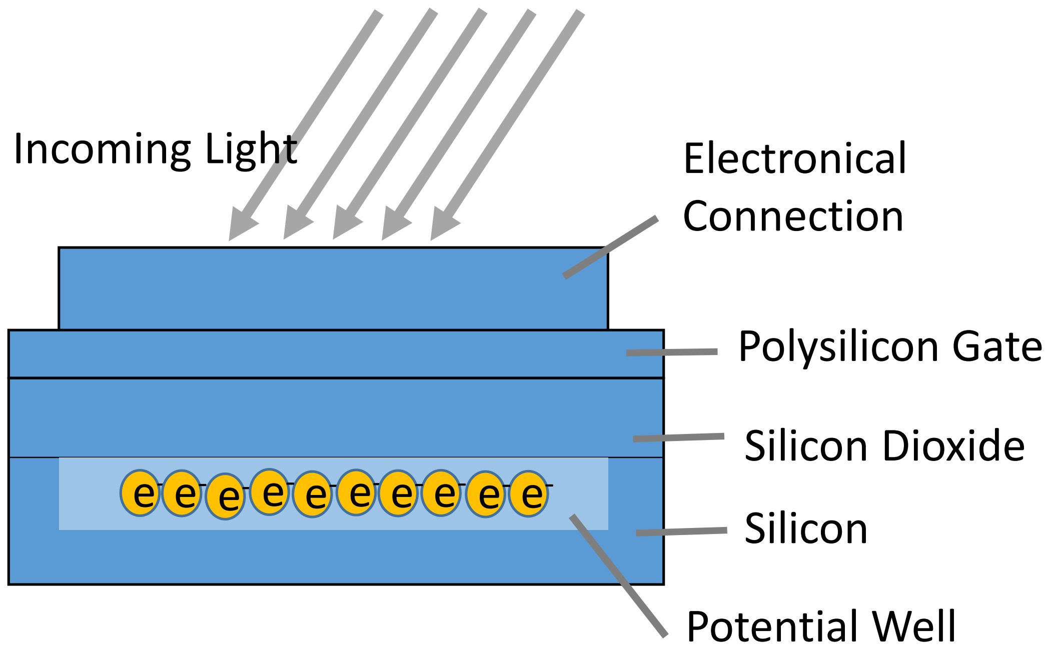
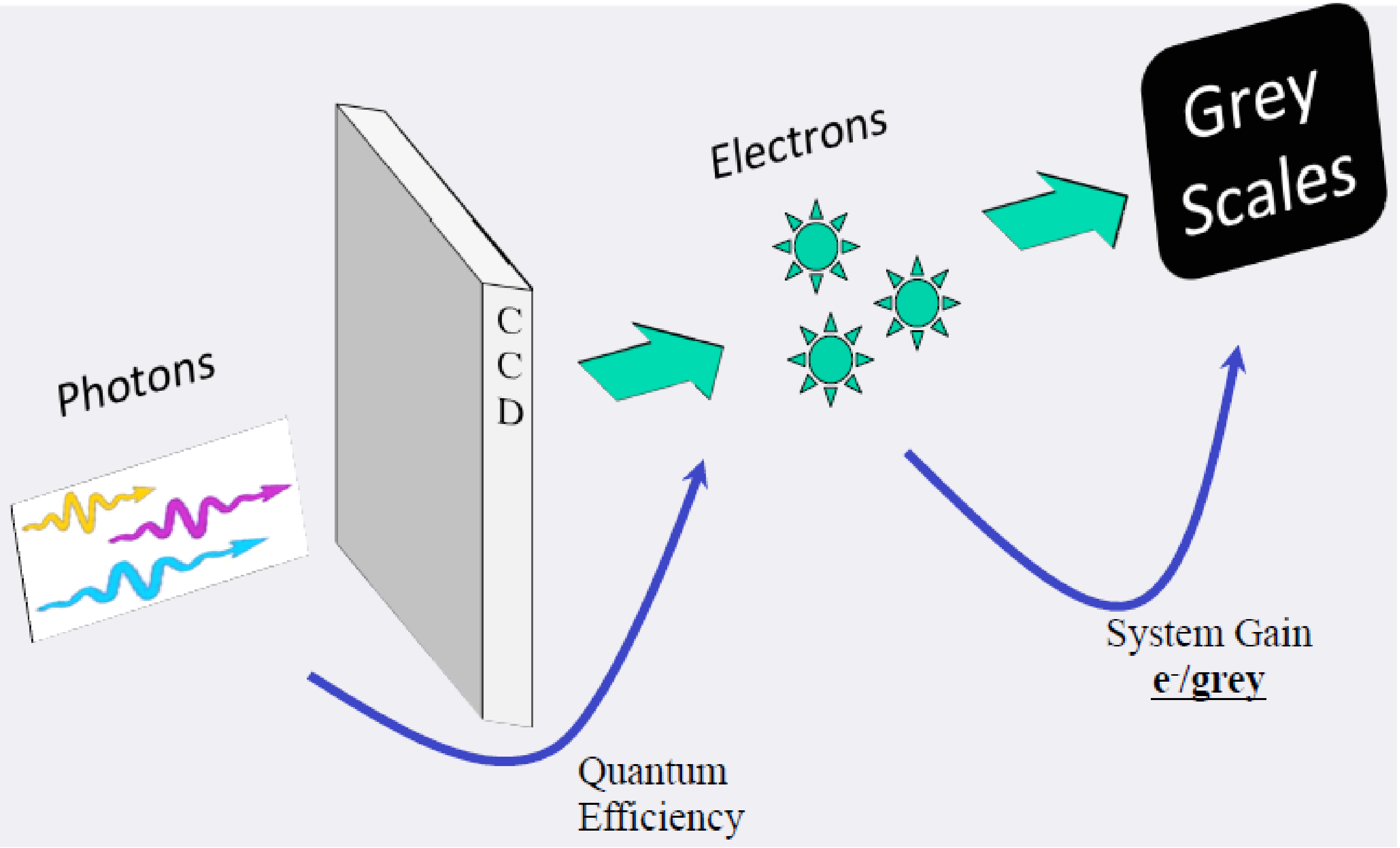
CCD Fundamentals

- Invented in 1970 at Bell Labs
- A silicon chip is structured as an array of photosensitive pixels
- converts light into an electrical signal (≈ incident light)
- Widely used in TV cameras and consumer camcorders



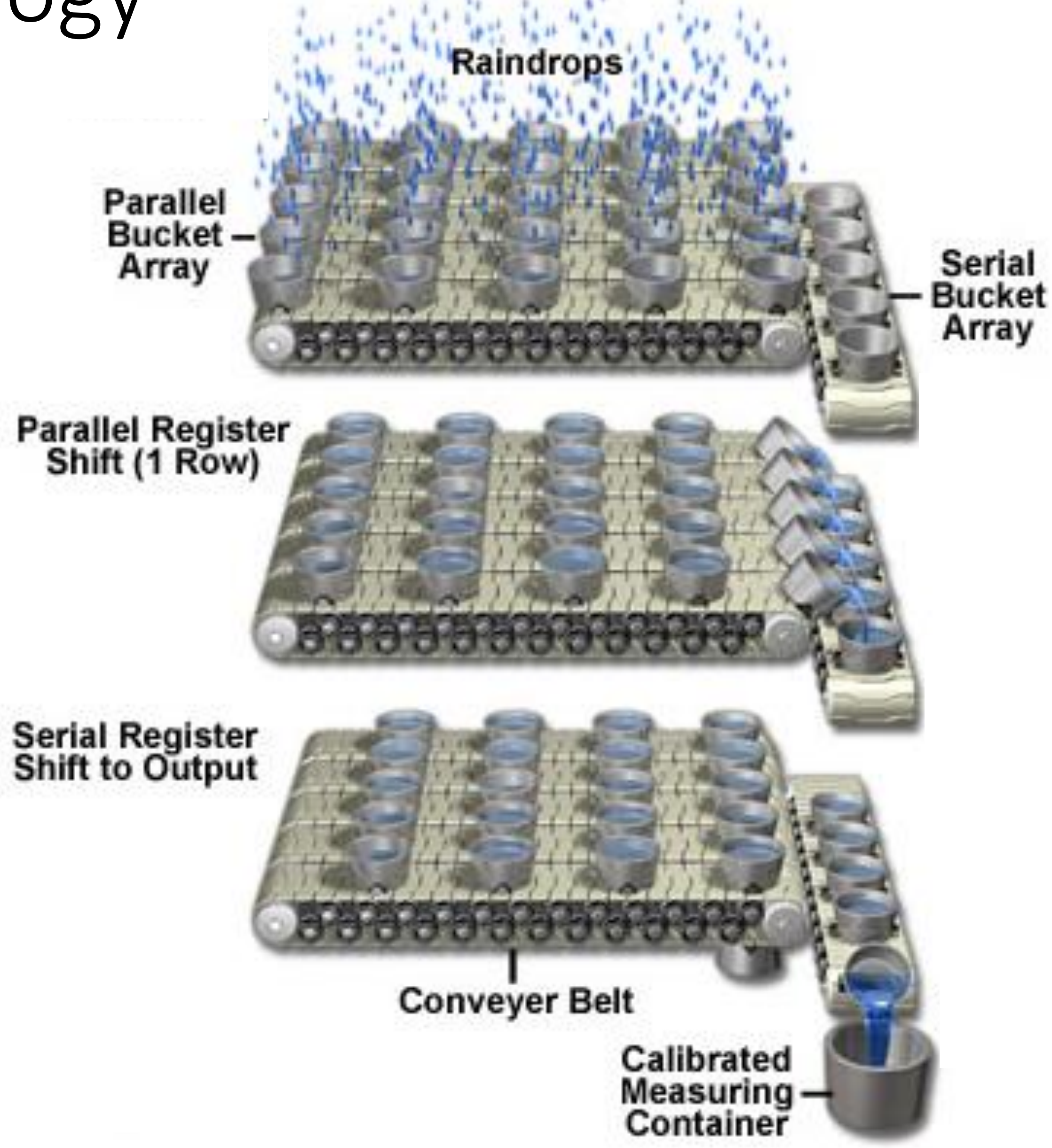
What is actually happening at each pixel?

1. Photon hits the CCD sensor
2. is then converted to an Electron
3. and digitised using an Analogue to Digital converter (ADC)
4. Electron value is now converted to a grey scale
5. User measures grey scale (ADU)



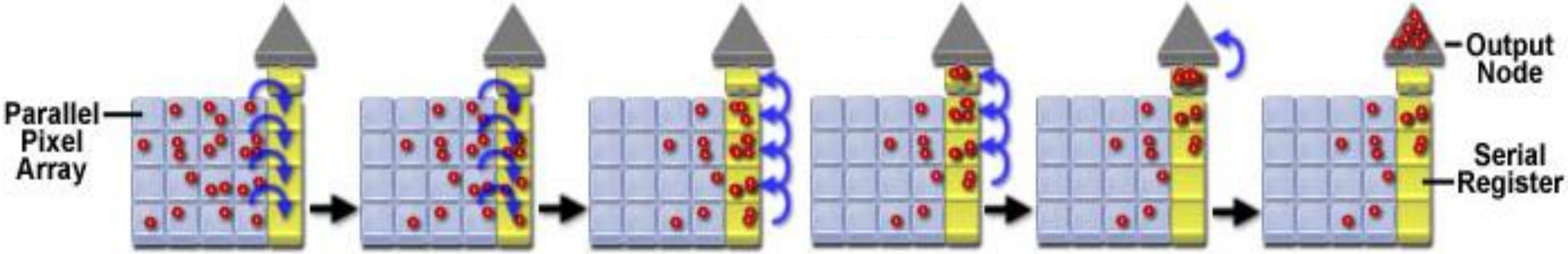
Bucket Brigade Rainfall Analogy

- rain intensity may vary from place to place
- Collection time = integration time
- parallel buckets on a conveyor belt transported stepwise to a row of empty serial buckets
- serial buckets move on a second conveyor oriented perpendicularly to the first
- accumulated rainwater in each bucket is transferred sequentially into a calibrated measuring container (= CCD output amplifier)
- process is repeated until all parallel buckets are shifted to the serials



Readout of a CCD

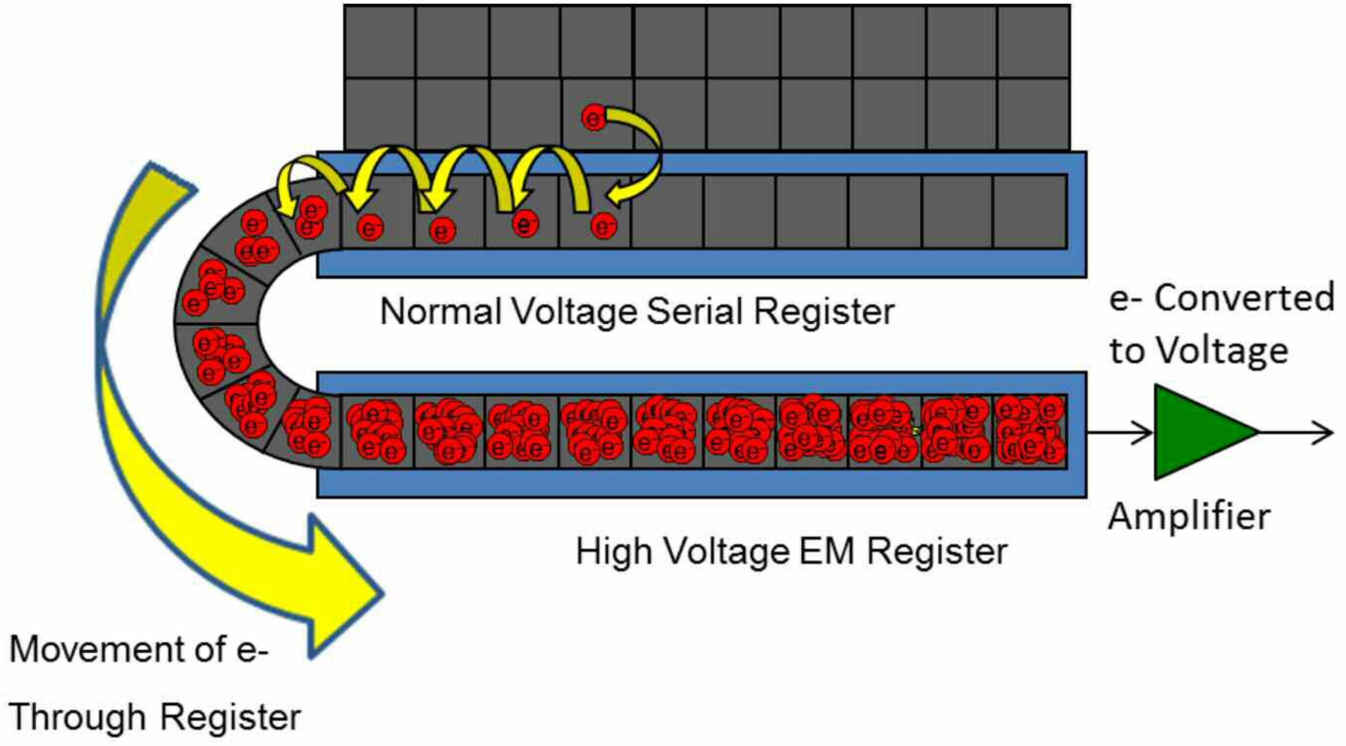
- camera shutter is opened to begin accumulation of photoelectrons
- end of the integration period = shutter is closed
- Shift of accumulated charge
- an ADC assigns digital value for each pixel according to its voltage
- each pixel value is stored in computer memory or camera frame buffer
- serial readout process is repeated until all pixel rows of the parallel register are emptied
- CCD is cleared of residual charge prior to the next exposure



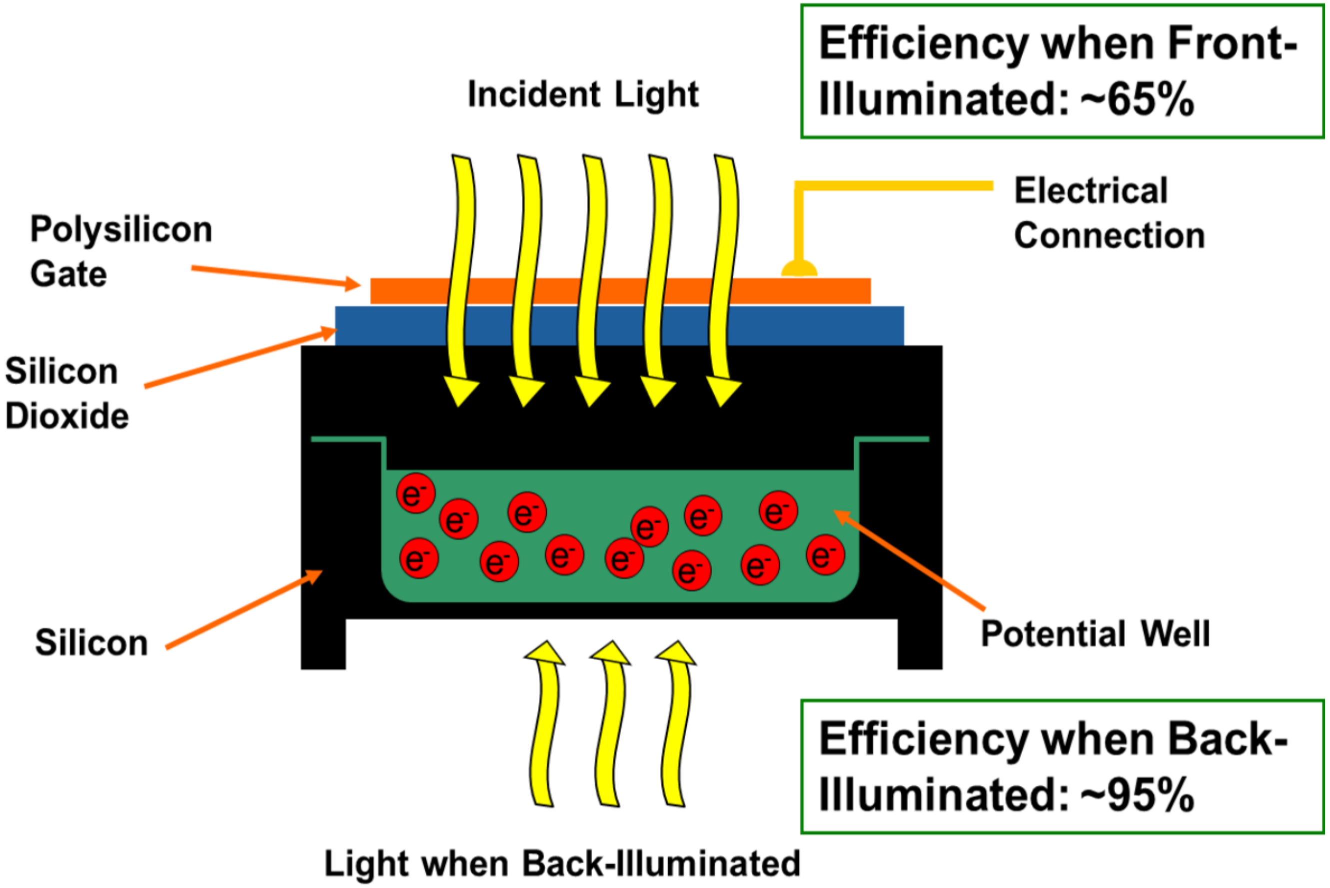
EMCCD- Electron Multiplying CCD

- addition of an **Electron Multiplication register** (‘gain register’ between the usual serial shift register and the output amplifier)
- enabling higher signals relative to the fixed camera noise

- issues with EMCCDs
  - EM Gain decay
  - Bias Stability
  - EM Gain Stability (aging)
  - Back ground events – dark current
  - Excess Noise Factor (uncertainty due to unpredictable multiplication of electrons)

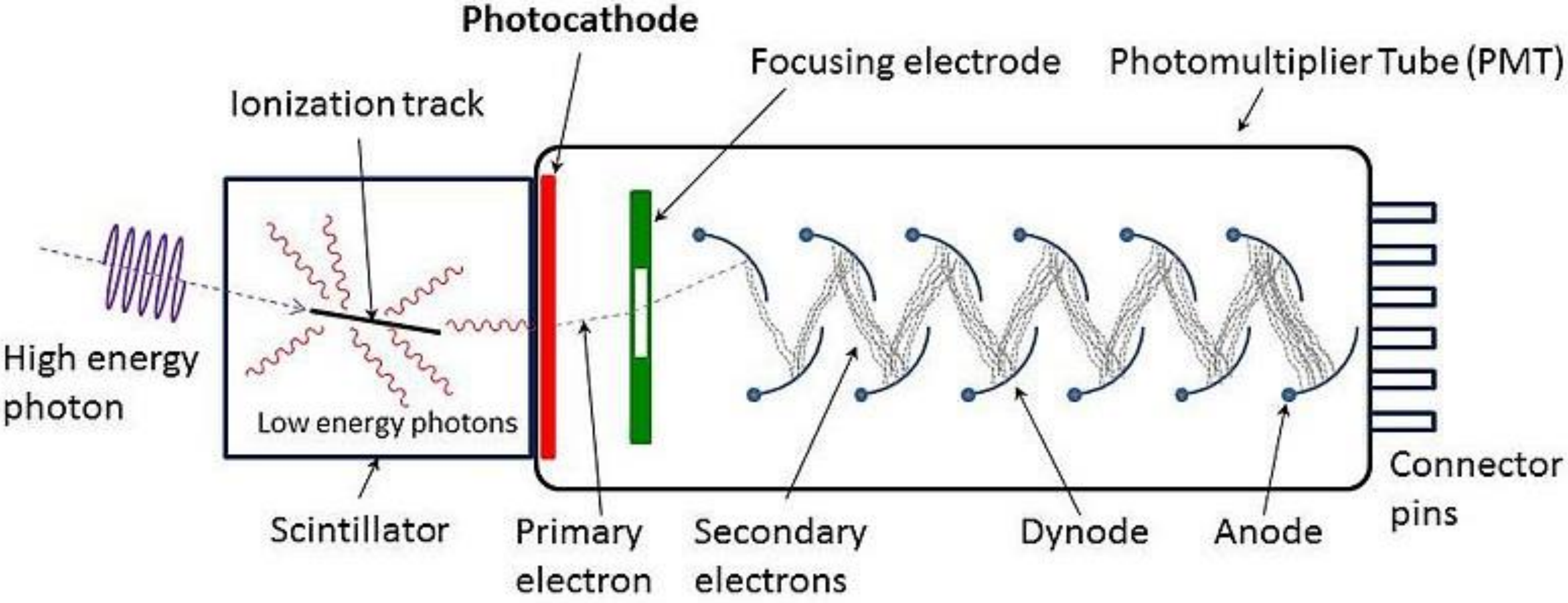


Front- and Backside-Illumination, Intensified CCD



- **Front-side illumination**: light must pass through several layers before reaching the silicon --> light loss
- **Back-illuminated sensor** orientates the wiring behind the photocathode which improves the chance of an input photon being captured from about 60% to over 90%

Excursus: Photomultiplier Tube (PMT)

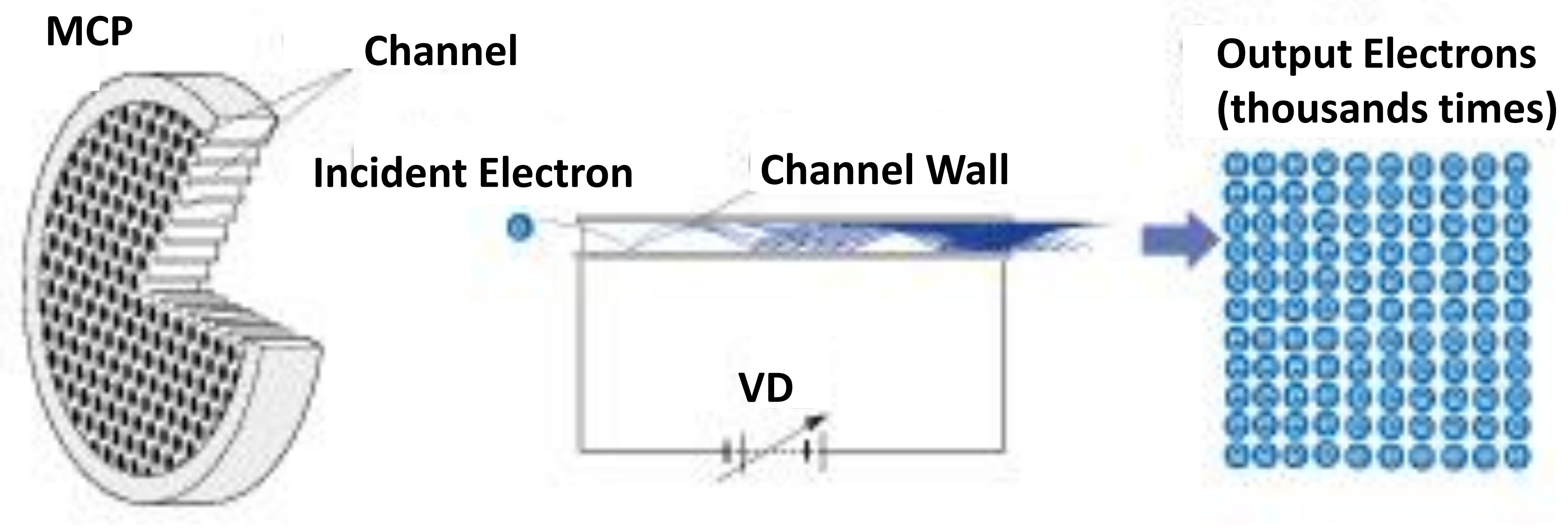


- constructed from a glass envelope with a high vacuum inside, which houses a photocathode, several dynodes, and an anode
- photons produce electrons at the photocathode (photoelectric effect)
- electrons are multiplied by the process of secondary emission (amplification $\approx 10^8$)

Some Facts about PMTs

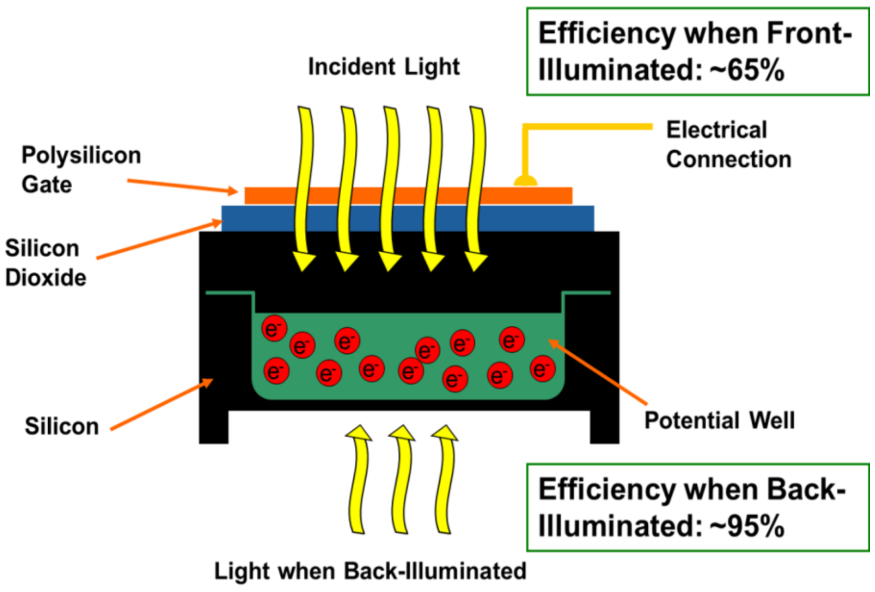
- release electrons with a peak quantum efficiency of about 40 %
- the photocathode active area ranges in size from a few millimeters to a half meter in diameter, depending upon the application.
- commonly used in applications without spatial resolution
- Photomultiplier tubes (**PMTs**) are widely used in confocal microscopes and high-end automatic exposure bodies for film cameras as well as in spectrometers
- because PMTs do not store charge and respond to changes in input light fluxes within a few nanoseconds, they can be used for the detection and recording of extremely fast events
- typically generate low noise values (and dark current) resulting in a huge dynamic range over which electrical current output still accurately reflects the photon flux

MCP – Microchannel Plate

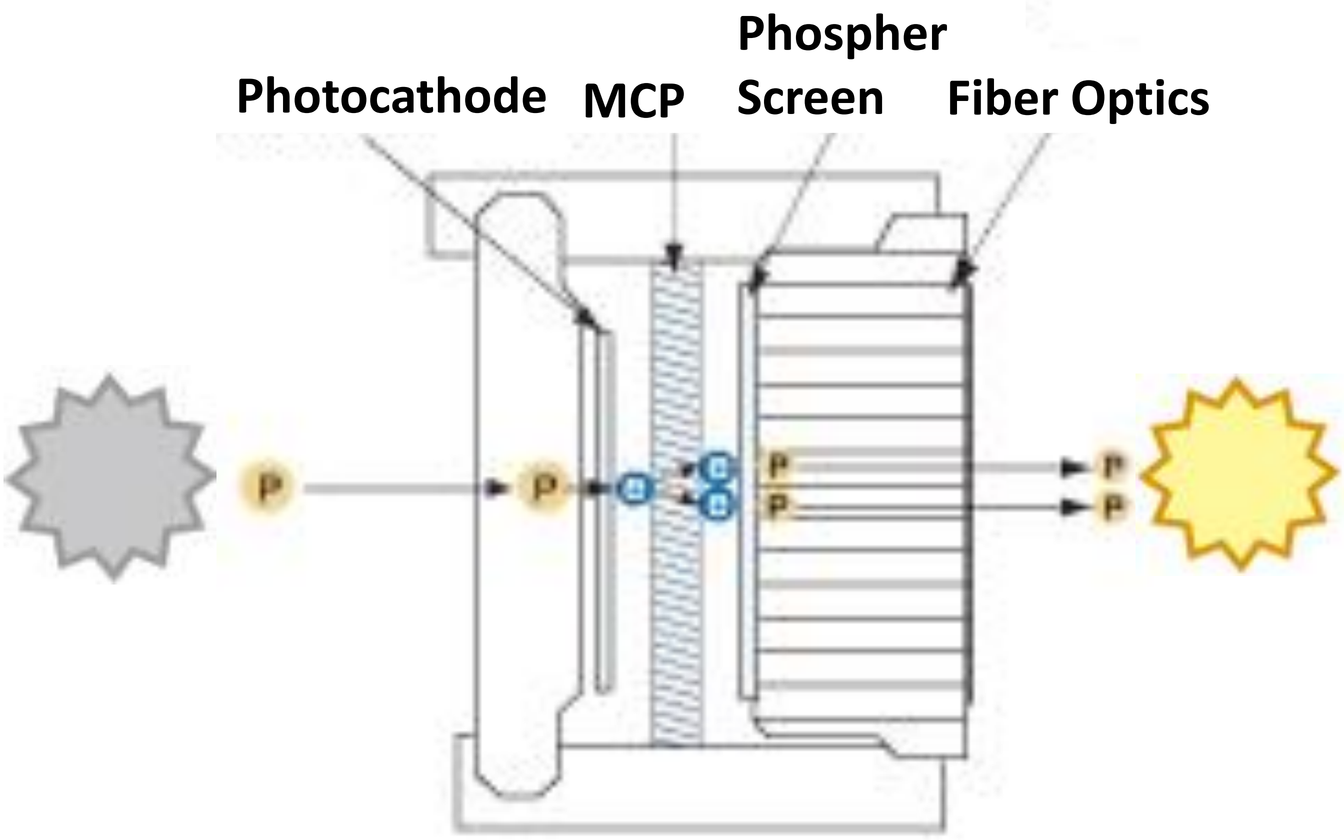


Each channel in the MCP is a secondary electron multiplier, multiplying electrons with each bounce off the channel wall

Front- and Backside-Illumination, Intensified CCD



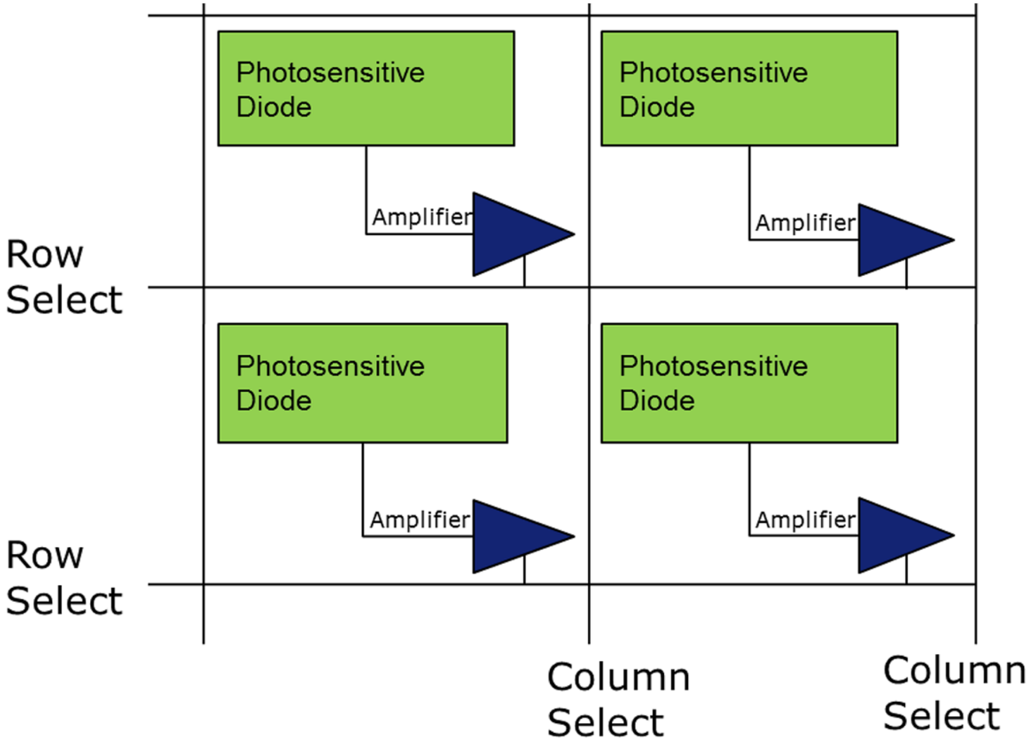
A back-illuminated sensor orientates the wiring behind the photocathode which improves the chance of an input photon being captured from about 60% to over 90%.



An ICCD is a CCD that is optically connected to an image intensifier sitting in front of the CCD

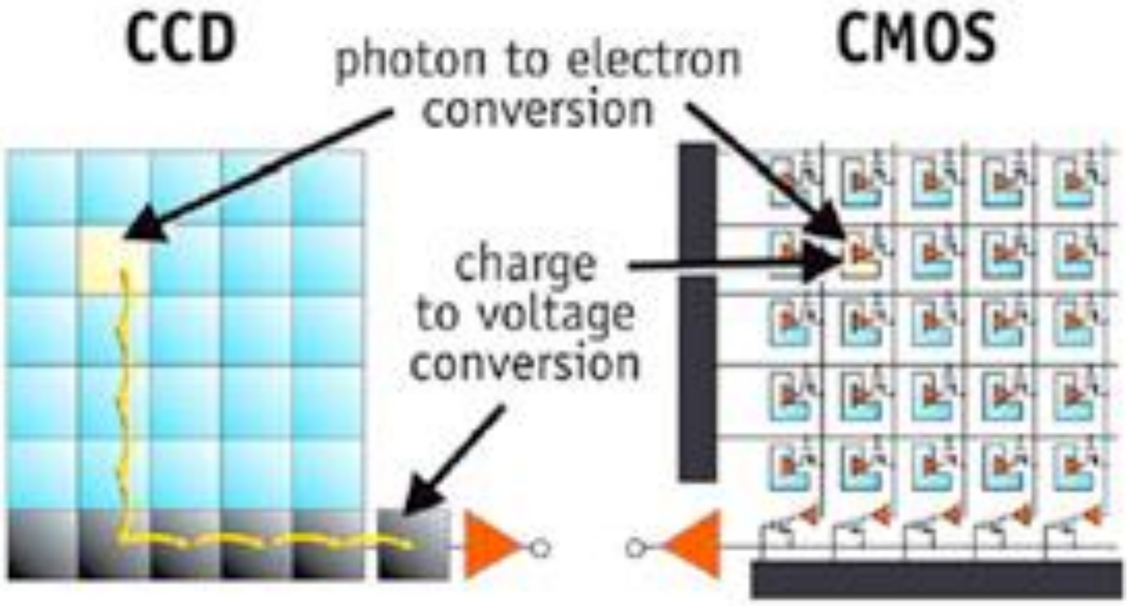
CMOS - Complementary metal-oxide-semiconductor

- CMOS technology uses an array of light sensitive pixels to collect full area image
- CMOS technology differs by completing all digitisation at the pixel point (faster)
- each pixel has its own amplifier
- CMOS sensors require around 100x less power than CCD making them the perfect choice for camera phone sensors
- low costsA
- disadvantages: small pixels so low dynamic range, high noise level, Rolling shutter, lower QE



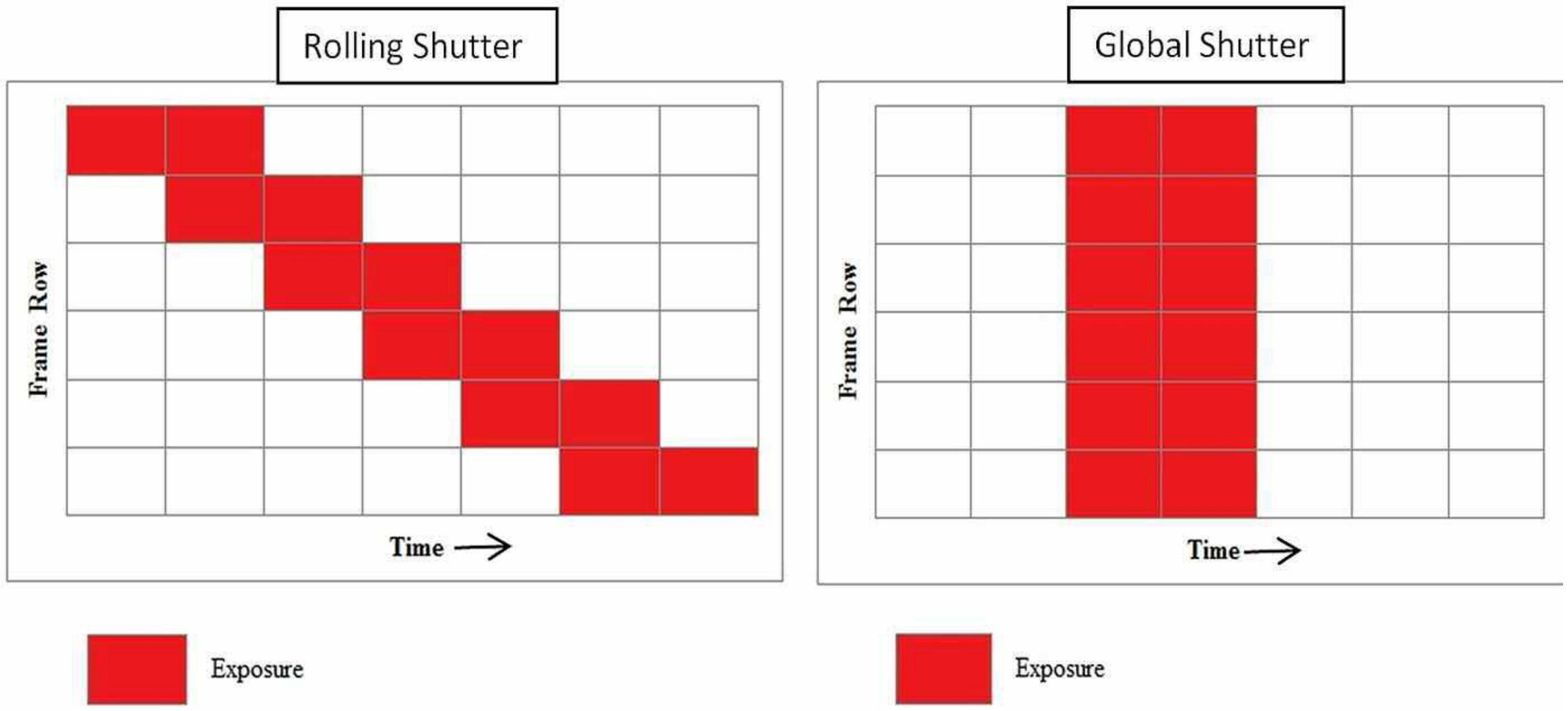
CMOS goes Scientific (sCMOS)

- in 2009 manufacturers launched a camera technology called SCMOS (scientific CMOS)
- a new sensor type with
  - low noise – less than 2e read noise
  - high speed – > 100 fps
  - high QE – 55-70%
  - high resolution – 2-5 million pixel



- **BUT**: Noise/Uncertainty occurs with the readout and digitization of each pixel's signal
- noise is not longer Gaussian distributed (random telegraph noise)
- rolling shutter (distortion of moving objects, poor synchronization with changing illumination experiments)

Imaging Modes: Rolling/Global Shutter



- CCD used in a basic ‘snapshot’ capture mode = all pixels are simultaneously exposed and then read out (slower, more noise)
- sCMOS with ‘rolling shutter’ = capturing each row at a slightly different time (distortion)

Chip Layouts

- Pixel size contributes to sensitivity: the larger the pixel the lower the resolution
- EMCCD designed for high sensitivity = has larger pixel than CCD and sCMOS

	Use	Active Pixel	Pixel Size	Frame Rate	QE	Read Noise
EMCCD iXON Ultra 897	Low light Applications	512x512	16x16µm	56 fps	> 90%	< 1e$^-$
EMCCD iXON Ultra 888		1024x1024	13x13µm	26 fps	> 90%	< 1e$^-$
sCMOS Zyla 4.2	Speed, big field of view	2048x2048	6.5x6.5µm	100 fps	max 72%	1.1e$^-$
sCMOS ORCA Flash 4		2048x2048	6.5x6.5µm	100 fps	max 70%	1.6e$^-$

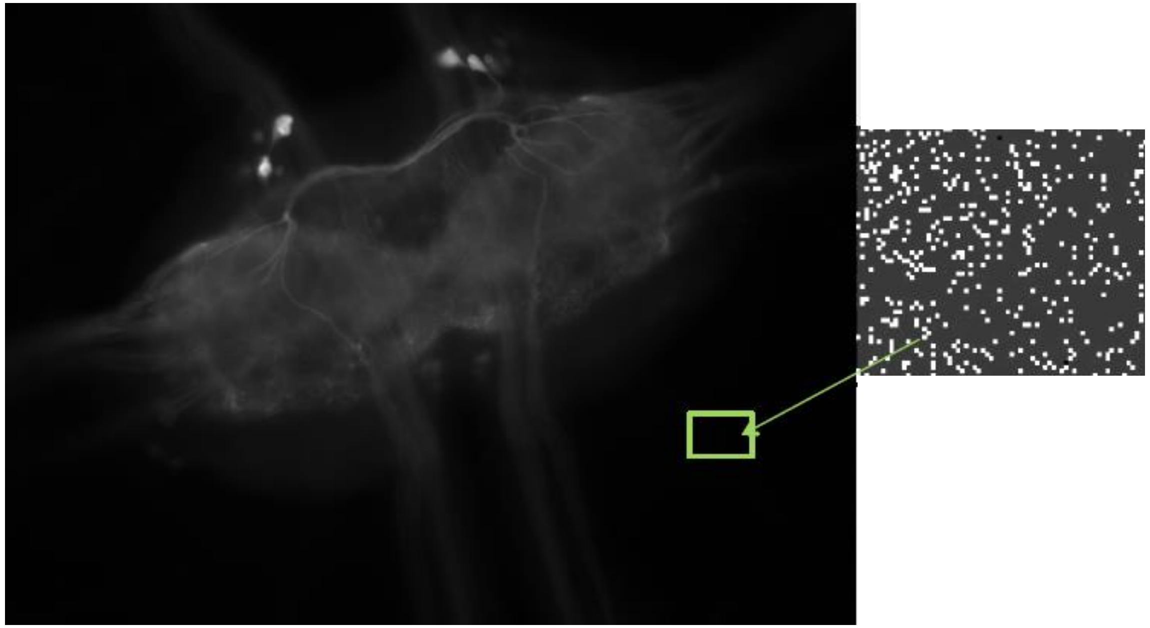
Summary

- **CCD** cameras have been the standard for general microscopy applications for many years and will continue to be the best choice for a variety of applications from colour imaging and fixed sample fluorescence to ‘long stare applications’

- **EMCCD** cameras continue to offer the best solution when imaging at very low light levels with speed, for example single molecule fluorescence

- **SCMOS** is a new addition to the sensors available for microscopy when speed is key. Combining this with great sensitivity a large field of view and low noise.

Camera Noise



- … is uncertainty
- … is plus or minus (not additive)
- … is driven by statistics
- … can be calculated
- … is not background
- standard deviation is an easy way for us to measure noise

- noise exists on every camera and in every measurement
- dependent on the image scale used you may or may not see it
- noise distorts measurements and increases the uncertainty in measurements.

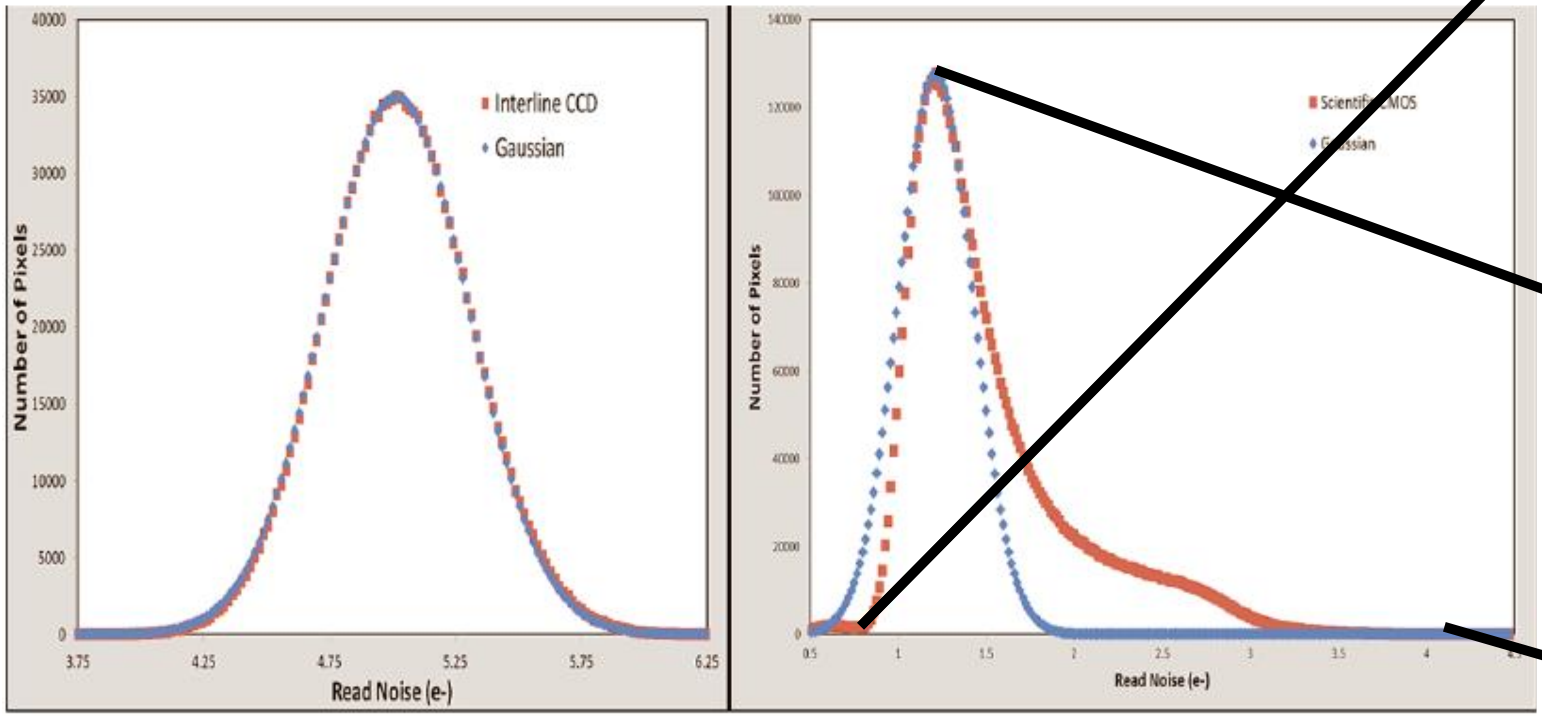
Noise Sources

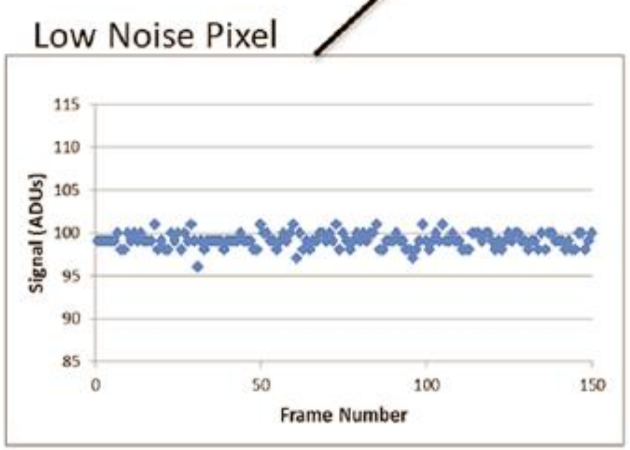
1. ***Dark Current*** – noise from heat and cosmic noise – time/exposure dependent (less important)
2. ***Read Noise*** – noise of reading the signal (from the amplifier during conversion from analog signal to digital signal) – fixed
3. ***Photon Shot*** – signal fluctuations due to the quantum properties of photons – signal dependent (square route of signal, Poission distributed)

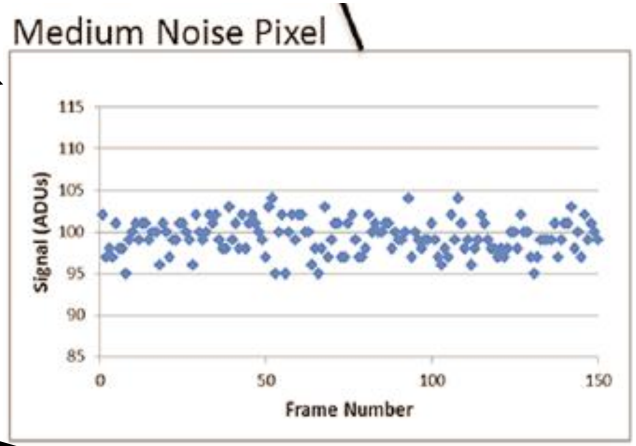
$$SNR = {S * QE}\Big/{\sqrt{(S * QE)^2 + DC + \sigma_R^2}}$$

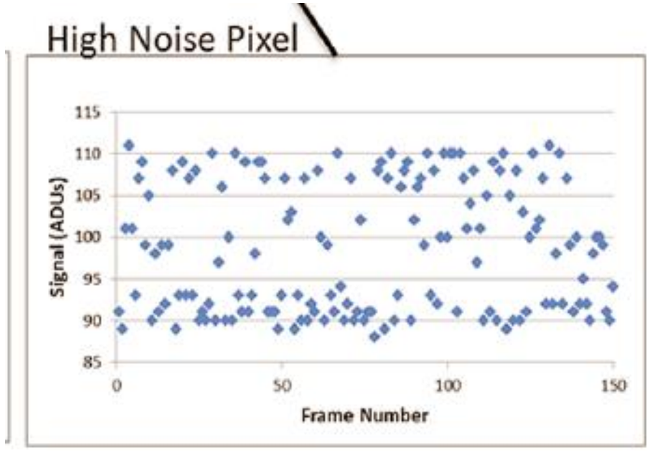
- S = Signal in Photons (converted to electrons by * QE)
- QE = Quantum Efficiency of light at that emission
- D = Dark Current Noise = Dark Current * Exposure Squared
- σ_R = Read Noise

Read Noise Distributions





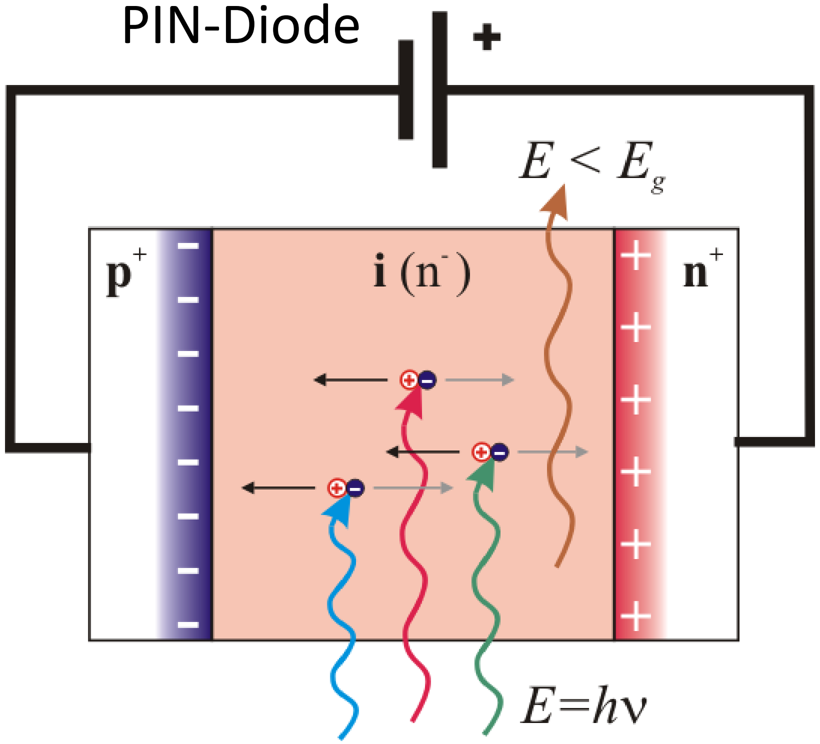




- Read Noise Distribution for CCD and sCMOS
- Noise follows a Gaussian distribution across all pixels for CCD
- sCMOS: follows a non-Gaussian skewed distribution of read noise values across a chip

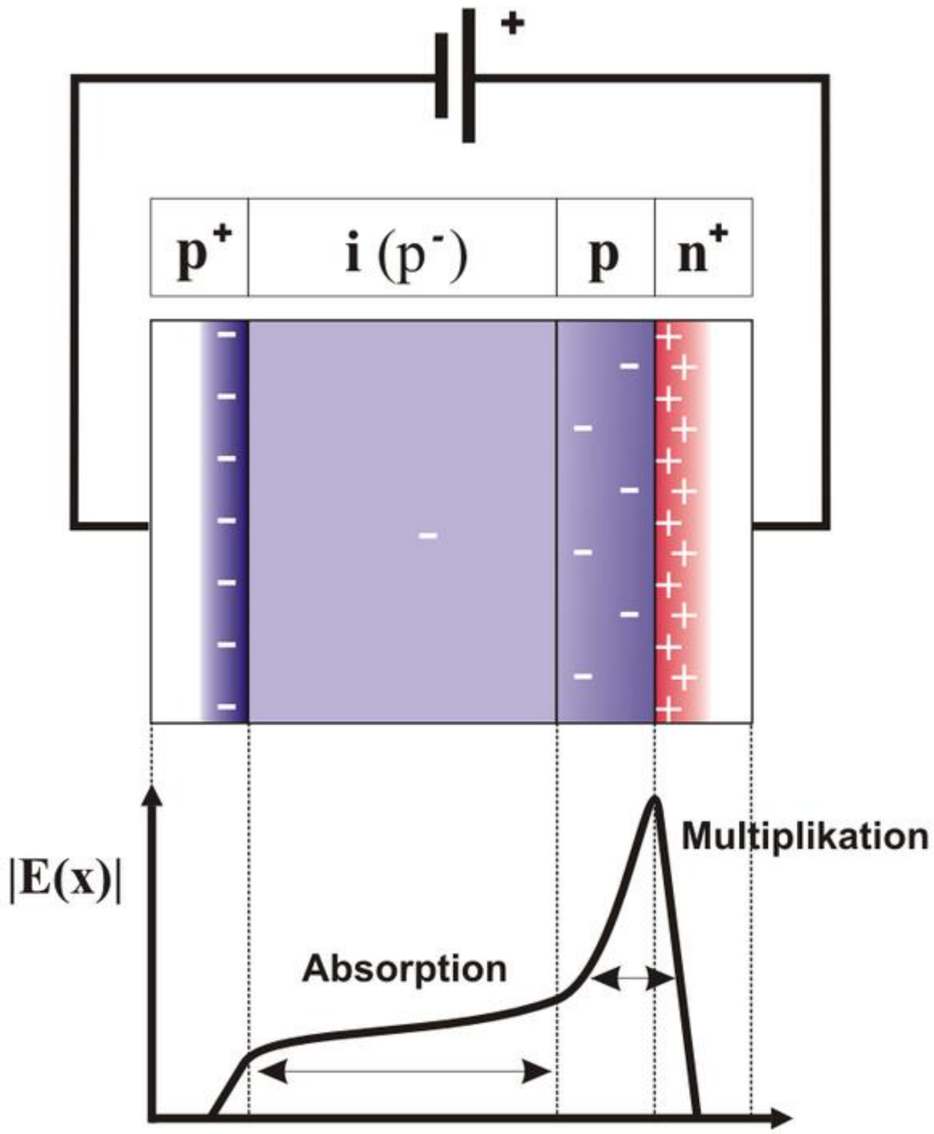
PIN - Diode

- Incident photon creates an electron-hole pair (inner photoelectric effect)
- holes move toward the anode, and electrons toward the cathode, and a photocurrent is produced. The total current through the photodiode is the sum of the dark current
- PIN = **high-level injection =** electric field extends deeply into this region



APD – Avalanche Photodiode

- APDs are similar to regular PIN diodes but operate with much higher revers bias
- have an additional heavily doped p- or n-region which allows an amplification (avalanche multiplication)
- acceleration of charge carriers in depletion region and generation of new secondary charge carriers via impact ionization (like PMTs)
- because of high revers voltage (close to breakdown voltage) of several 100V high magnifications of primary charge carriers
- for voltages bigger then the breakdown voltage = avalanche effect = amplification ≈ 10^6



APD – Some Key Facts

- active area ≤ 1 mm^2 and ≤ 10 µm x 10 µm for a high-speed APD. It is therefore difficult to focus the fluorescence onto the APD, so the sensitivity is too low for most measurements.
- dead times of ≈ 100ns, wavelength dependent, event rates ca. 10MHz
- routinely used for TCSPC, especially in applications where the emission can be tightly focused, such as single-molecule detection (SMD) and fluorescence correlation spectroscopy (FCS)
- APDs have high quantum efficiencies at real wavelengths, and are the detector of choice for these applications.

The End!