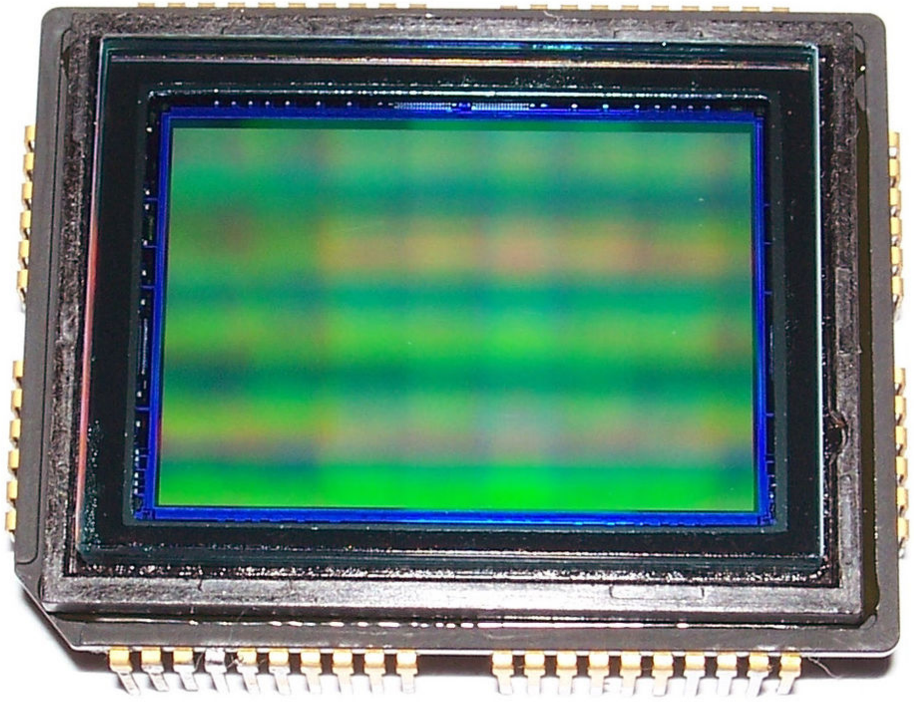
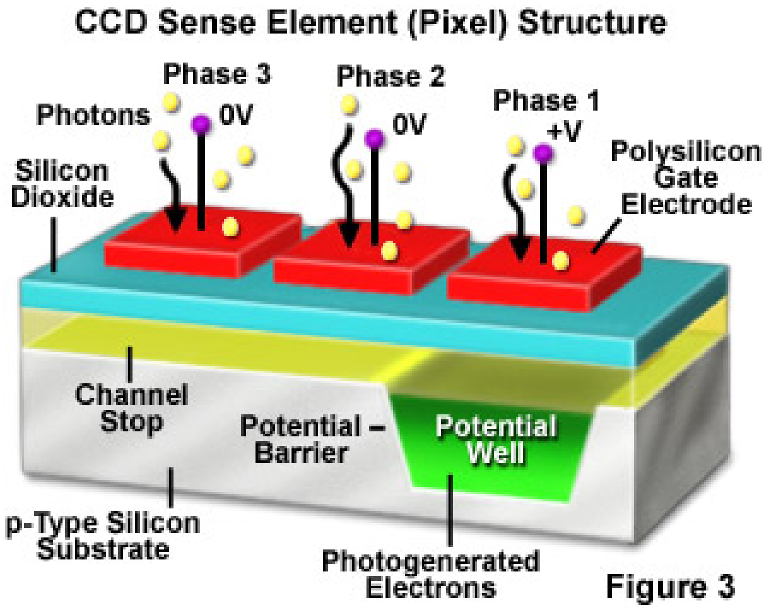
CCDs and sCMOS

- Last class
  - CCDs
  - Fancy CCDs
- This class
  - Camera specs
  - sCMOS





Fancy CCD cameras:

-Back thinned -> higher QE

-Unexposed chip -> frame transfer

-Electron multiplying -> higher SNR

-Fancy ADC -> higher frame rates

EMCCD specs

Key Specifications	
Active Pixels	1024 x 1024
Pixel Size	13 x 13 µm
Image Area (mm)	13.3 x 13.3
Image Area Pixel Well Depth	80,000 e-
Max. Readout Rate	30 MHz
Frame Rate	26 - 9690 fps
Read Noise	< 1 e- with EM gain
QE_{max}	> 90%

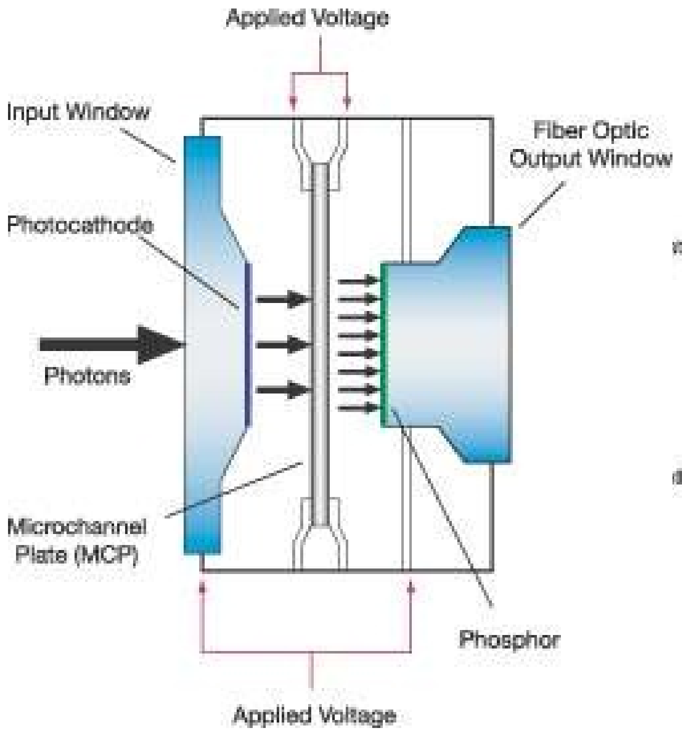
Dark current and background events [5,6]		
Dark current (e-/pixel/sec) @ -80°C	0.00025	
Dark current (e-/pixel/sec) @ -95°C	0.00011	
Spurious background (events/pix) @ 1000x gain / -85°C	0.005	
Active area pixel well depth	80,000 e^-	
Gain register pixel well depth [7]	730,000 e^-	
Pixel readout rates	Electron Multiplying Amplifier: 30, 20, 10 & 1 MHz	Conventional Amplifier: 1 & 0.1 MHz
Read noise (e^-) [8]	Without Electron Multiplication	With Electron Multiplication
30 MHz through EMCCD amplifier	130	< 1
20 MHz through EMCCD amplifier	80	< 1
10 MHz through EMCCD amplifier	40	< 1
1 MHz through EMCCD amplifier	12	< 1
1 MHz through conventional amplifier	6	-
100 kHz through conventional amplifier	3.5	-
Linear absolute Electron Multiplier gain	1 - 1000 times via RealGain™ (calibration stable at all cooling temperatures)	
Linearity [9]	Better than 99.9%	
Vertical clock speed	0.6 to 4.33 µs (user selectable)	
Timestamp accuracy	10 ns	

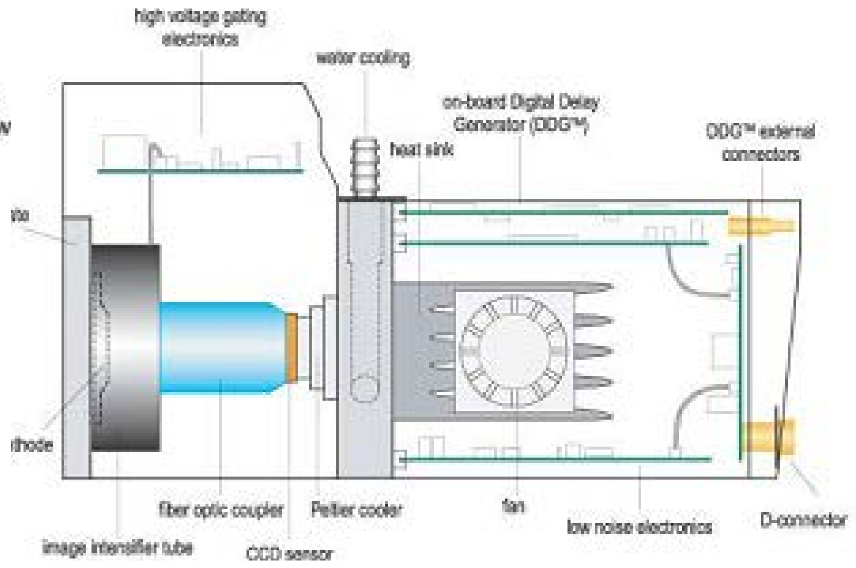
FRAME RATES - STANDARD MODE [3,10]

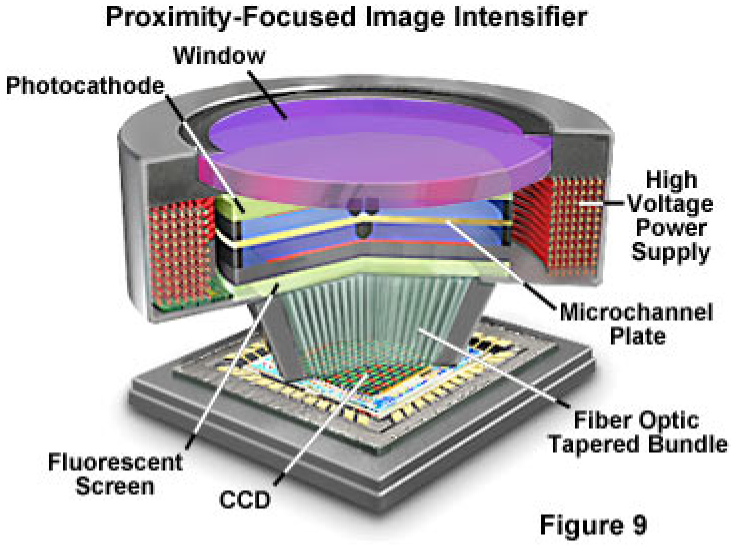
	Array size				
Binning	1024 x 1024	512 x 512	256 x 256	128 x 128	1024 x 100
1 x 1	26	50	95	171	220
2 x 2	50	94	170	285	368
4 x 4	92	167	281	426	552

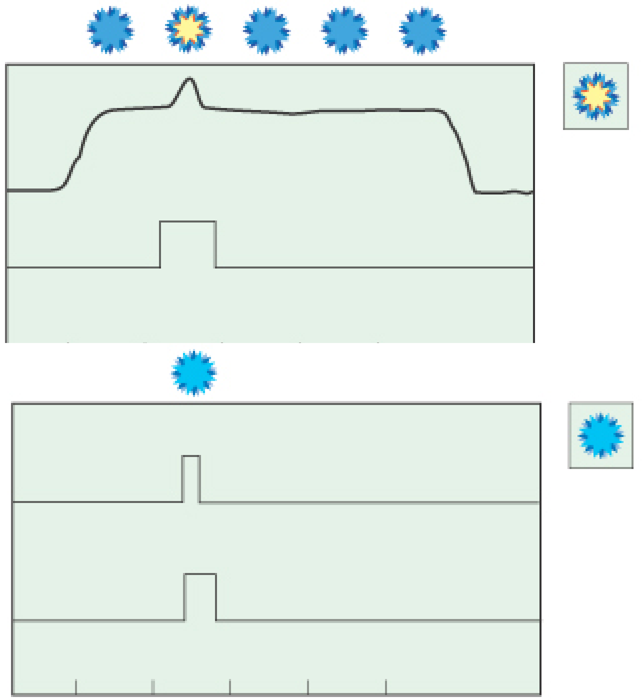
Intensified CCDs

- Intensified CCDs are the marriage between photomultiplier tubes and CCDs
- Photocathode array converts incoming light to electrons
- Electrons travel down high voltage gain
- Electrons crash into phosphor screen which creates photons
- CCD camera detects photons
- Application of high voltage sets whether or not you'll see anything on the camera
- ICCDs can be gated at very high time resolutions
- Still takes normal amount of time to read them out





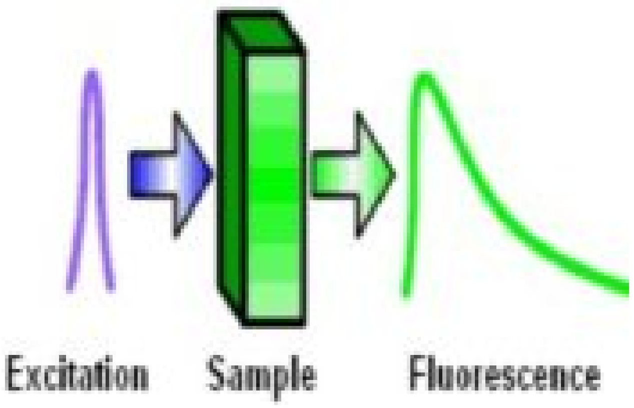




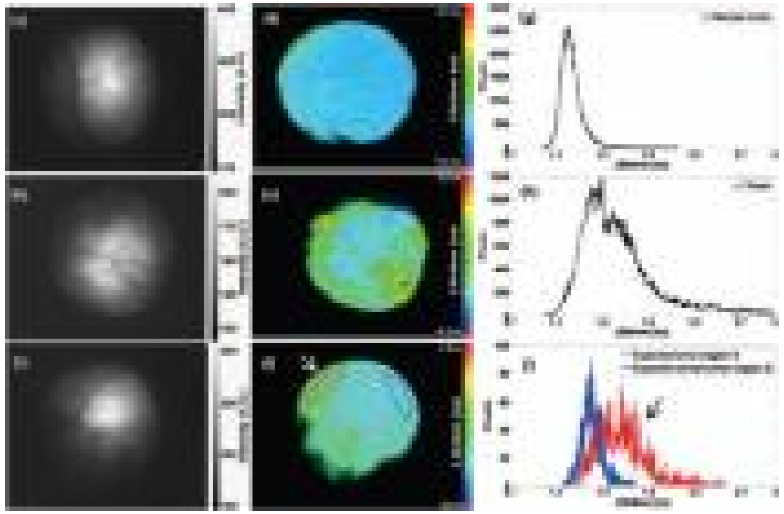
ICCD applications

- Good for applications needing very high time resolution
- Also useful for extreme low light imaging

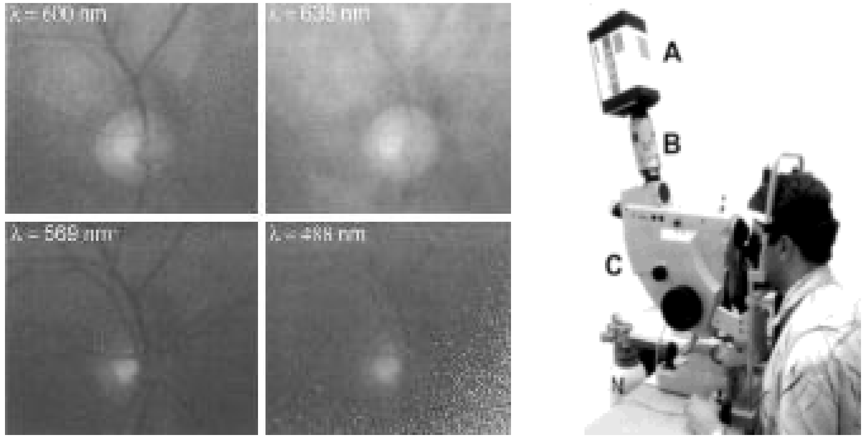
Wide field FLIM



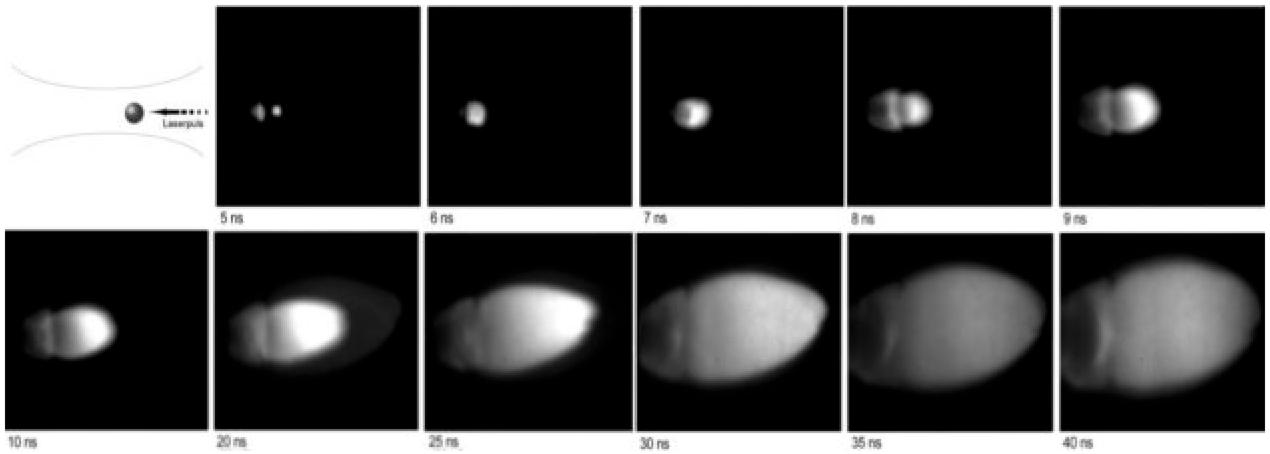
FLIM surgery



Reflectance oximetry

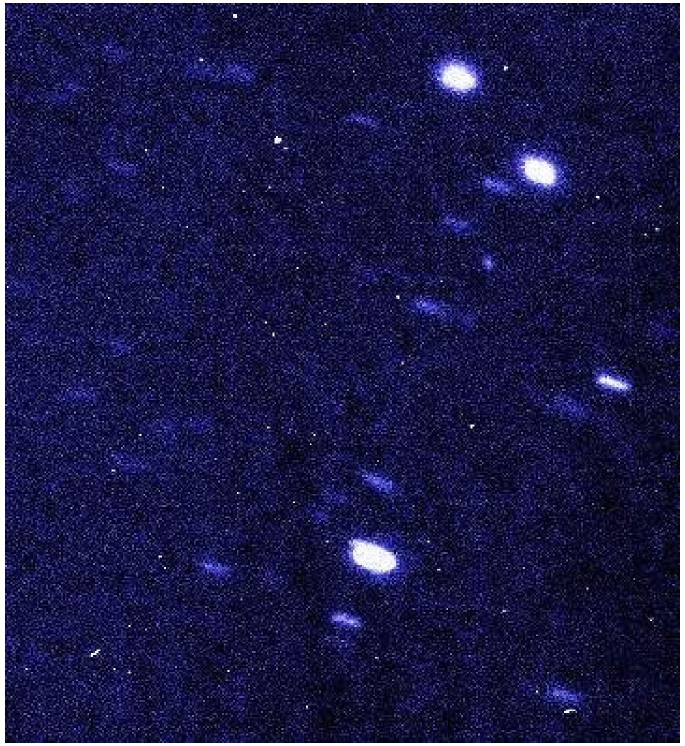


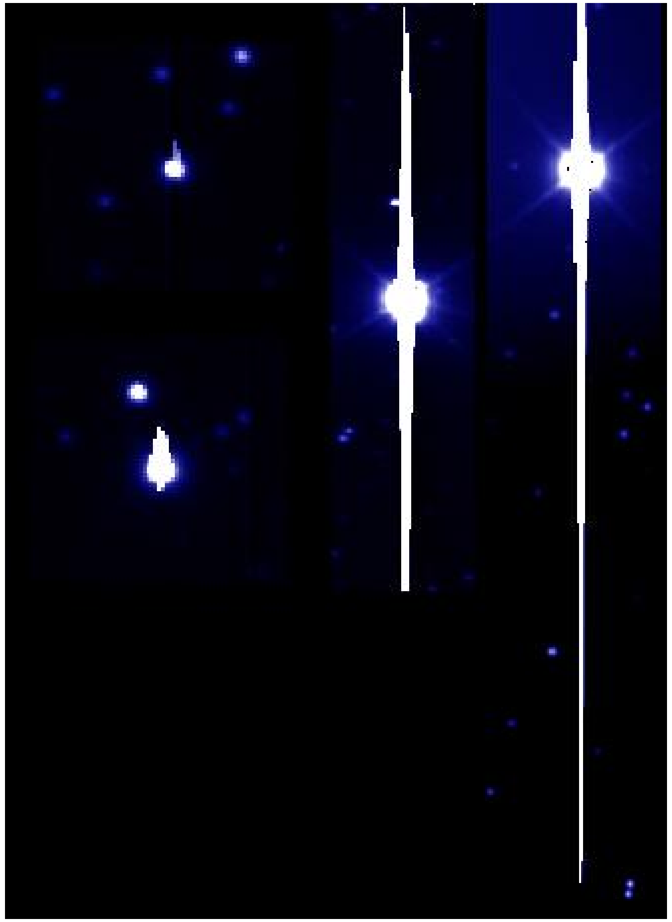
Plasma dynamics



CCD artifacts

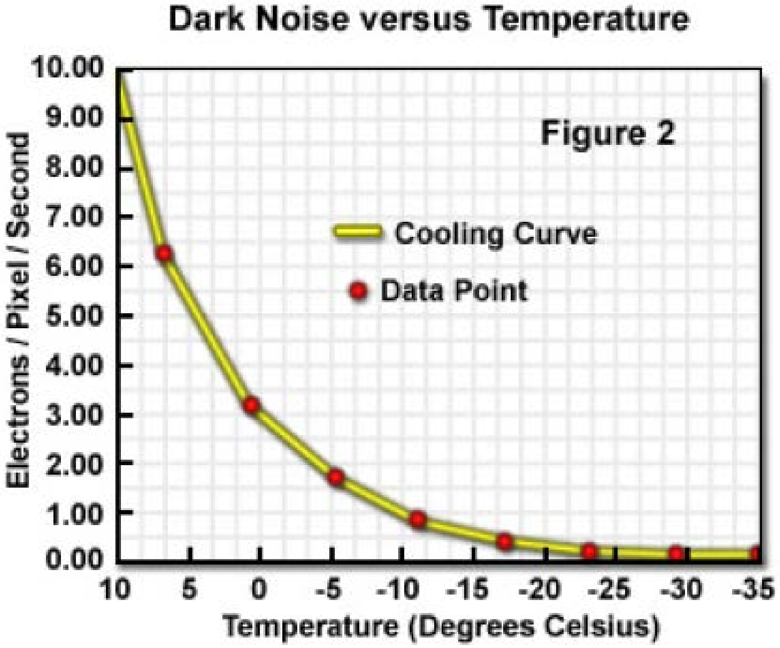
- Cosmic rays – usually light up as a few pixels. Noticeable because they're completely saturated. Good software filters exist to get rid of them
- Dead pixels – some pixels might not respond to light any more
- Blooming – oversatured pixel will bleed into vertical neighbors during charge readout





Sources of noise

- Dark current – Thermally generating electrons
  - Cool sensors down to -80 C
- Readout Rate – Electronic noise during conversion of electrons to votlages
  - Electron multiplication gain
- Pixelation error – Non-uniform pixel sizes
  - Buy expensive camera
- Shot noise – Fundamental noise of light collection – Goes as $\sqrt{N}$
  - No solution, use bright samples



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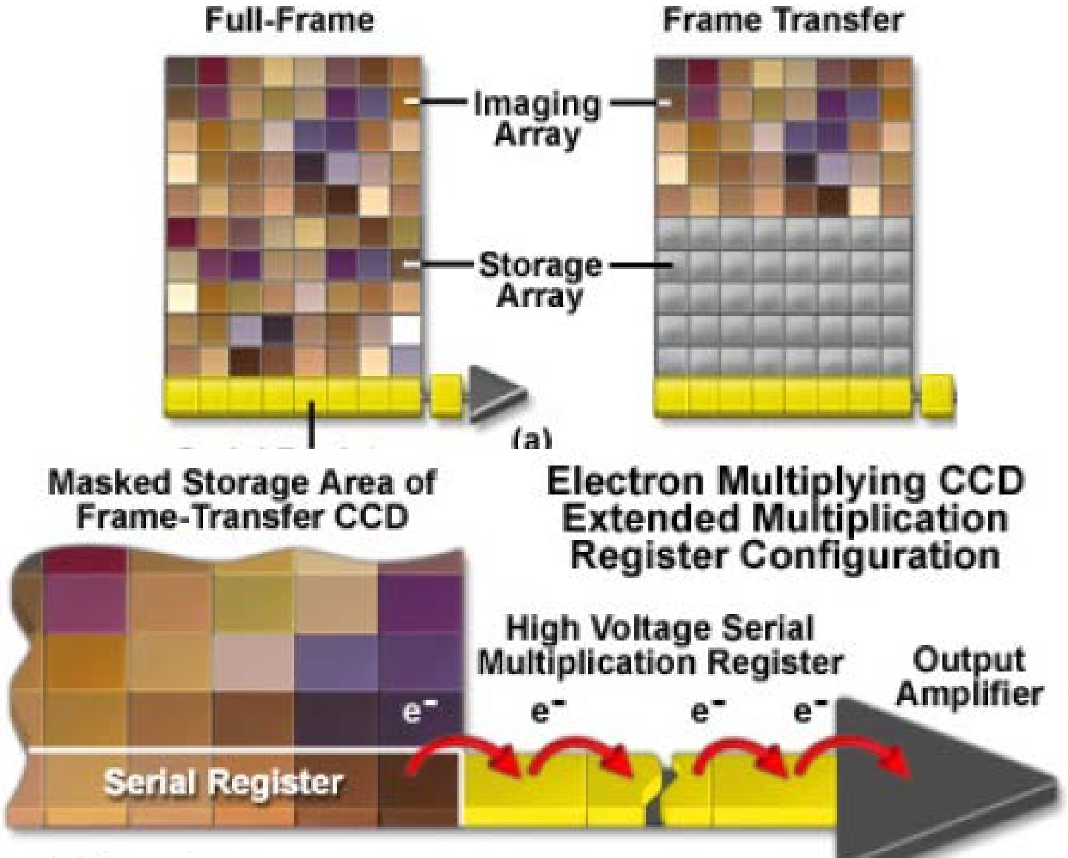


Figure 3

Frontside and Backside Illuminated CCDs

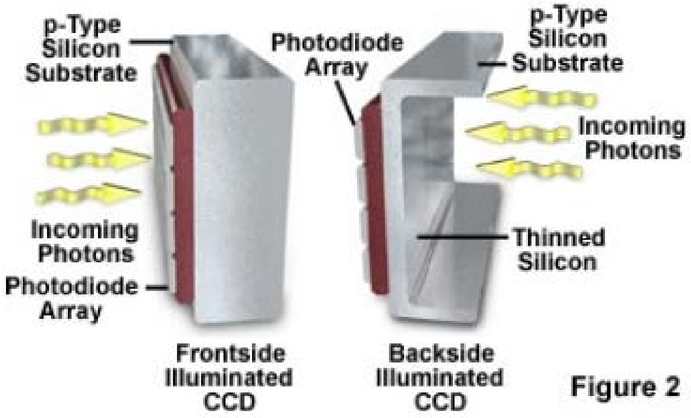


Figure 2

Signal to noise ratio - redux

- At very dim light levels, the read noise becomes the dominant source of noise, but we get over that hump with EM
- Binning on EMCCD gets around read noise. Since a 2x2 region (4 pixels) gets summed together, and then read out, increases overall signal to noise
- Binning also reduces image time – fewer pixels means less time with ADC
- Binning reduces single pixel dynamic range

$$Signal = QE * N_{ph}$$

$$Shot\ noise = \sqrt{QE * N_{ph}}$$

Ideal, noiseless camera:

$$SNR = \frac{QE * N_{ph}}{\sqrt{QE * N_{ph}}} = \sqrt{QE * N_{ph}}$$

More realistic camera

$$SNR = \frac{QE * N_{ph}}{\sqrt{(QE * N_{ph}) + Dark + \delta_{read}^2}}$$

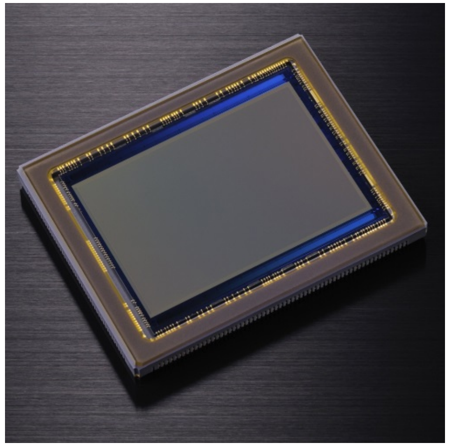
Camera parameters

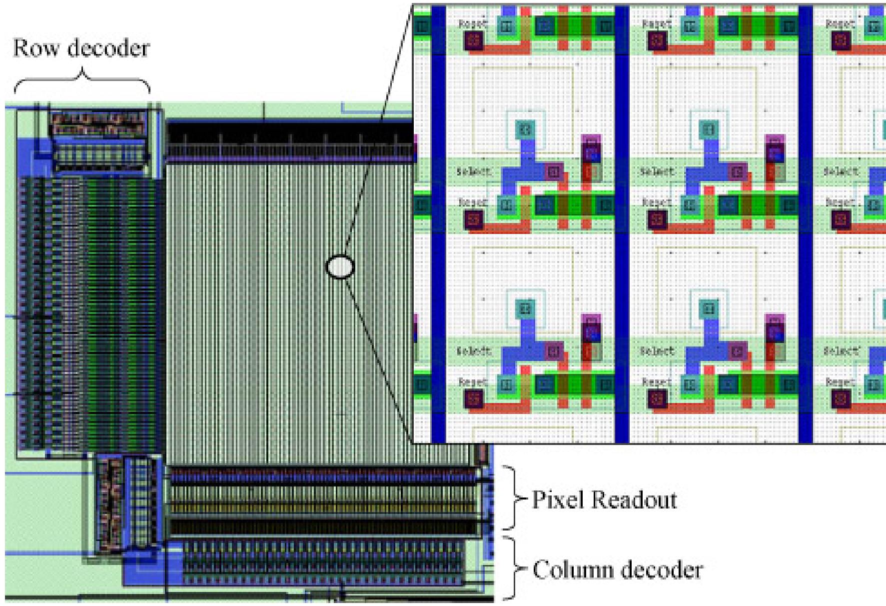
Quantum Efficiency: Probability of generating photoelectron out of incoming photon.
Dark Current: Spontaneous generation of electron due to thermal noise.
Spatial Resolution: Determines the ability to capture fine specimen details without pixels being visible in the image.
Effective Pixel Size: Actual camera pixel size divided by magnification. Nyquist Criterion, 100 nm-160 nm for optimum resolution and brightness.
Signal-to-Noise Ratio: Determines the visibility and clarity of specimen signals relative to the image background.
Dynamic Range: Defines the dynamic range or number of gray levels that are distinguishable in the displayed image. 16 bit ADC gives 0-65384.
Time Resolution: The sampling (frame) rate determines the ability to follow live specimen movement or rapid kinetic processes.
Readout Rate: Acquisition speed in serial registry. 10MHz camera can take 30 msec images with 512*512 pixels. Faster readout increases the electronic noise.
Region of Interest: Subarray image provides faster acquisition (whole rows are read regardless of the image size).
Binning: Combining the pixels, improves time resolution with poorer spatial resolution.

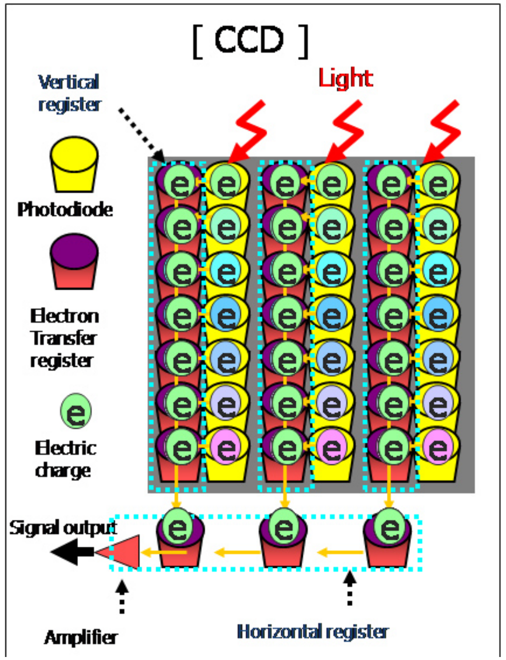
CMOS cameras

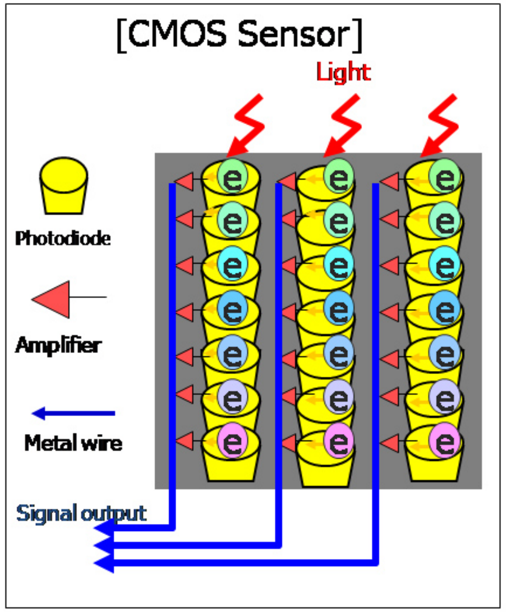
CMOS cameras
Complimentary Metal Oxide Silicon

- Each pixel has it’s own amplifier
- Each row of pixels has it’s own ADC converter
- Images can be read much faster
- Many ADCs allow for higher number of pixels (like your cell phone)
- 20 megapixel is no problem (it would take 700 ms to read out with fancy EMCCD)









CMOS limitations

- Extra circuitry takes up physical room, reducing effective QE (lower fill factor)
- Each amplifier has it’s own characteristics, leads to fixed gain variations across chips
- Many cheap amplifiers mean that the dynamic range is often lower
- Data transfer to ta computer has to occur REALLY fast

Feature	CCD	CMOS
Sensitivity	Higher fill factor means higher light sensitivity. The light sensitive elements are in the surface of the sensor. Electronic shutters reduce the aperture only slightly.	Lower fill factor means lower light sensitivity, because circuits are integrated in between the pixels. The required transistors for global shutters reduce the aperture.
Image Quality	Better uniformity as the same electronic generates the value for each pixel. Only a few transistors are involved and generate lower noise.	Each circuit has its own characteristics, which generates fixed pattern noise in the image. Several transistors with individual differences generate higher noise.
Windowing	Always the whole sensor needs to be read out.	Single pixels can be addressed.
Speed	Higher read out clock reduce image quality of a CCD chip. Image processing is done outside the chip.	Clocking and digitization is done in the CMOS-chip, image processing can be done also inside.
System design	Good electronics around the CCD is necessary to get good images. Electronic affects the image quality directly.	Digitization is done in the CMOS-chip itself. You need less and easier components around. Image quality is mainly influenced by the chip.

Scientific CMOS (sCMOS) enables new imaging

- sCMOS is a number of new features that are branded into one new name
- Use microlenses to increase effective fill factor (and QE)
- Use fancy electronics to tune each amplifier to give consistent gain across the entire image
- Fancy electronics also give higher dynamic range
- Fancy electronics also reduce read noise

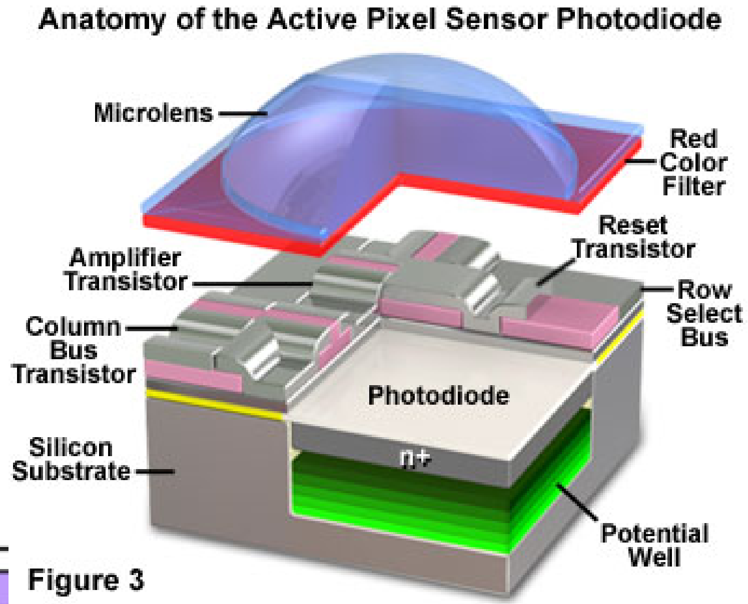
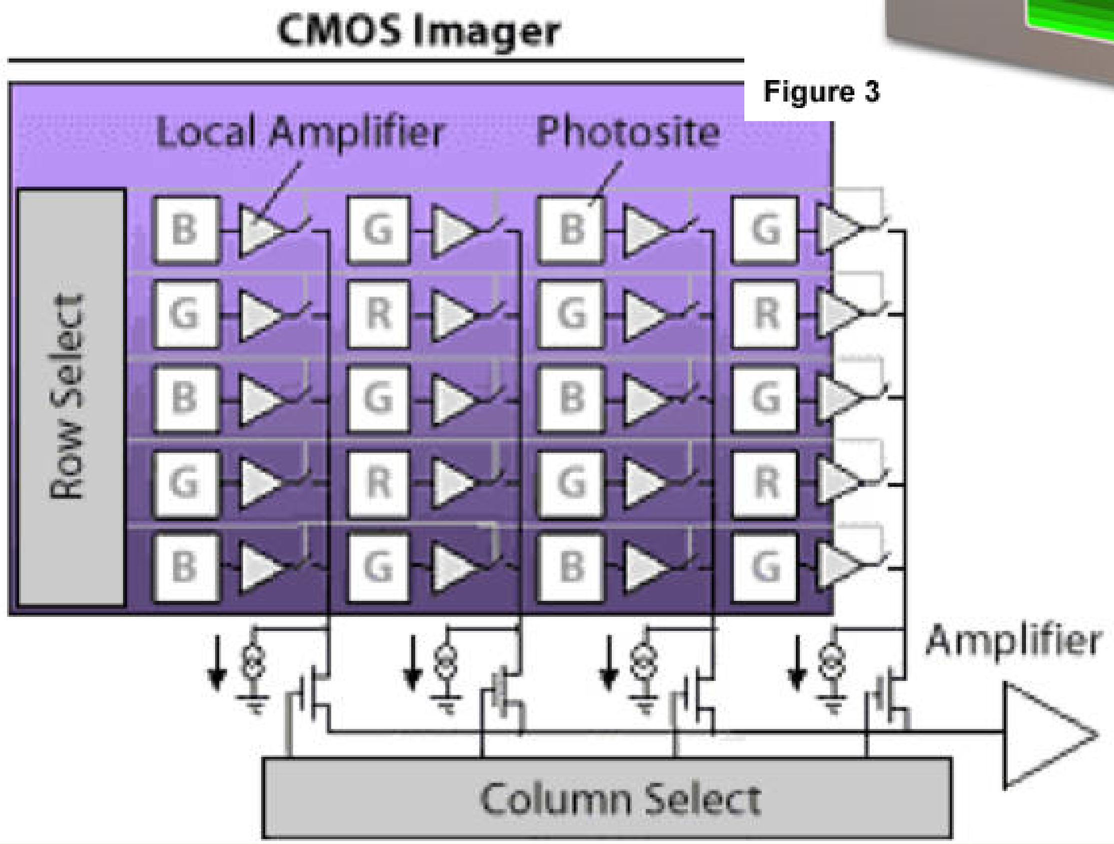
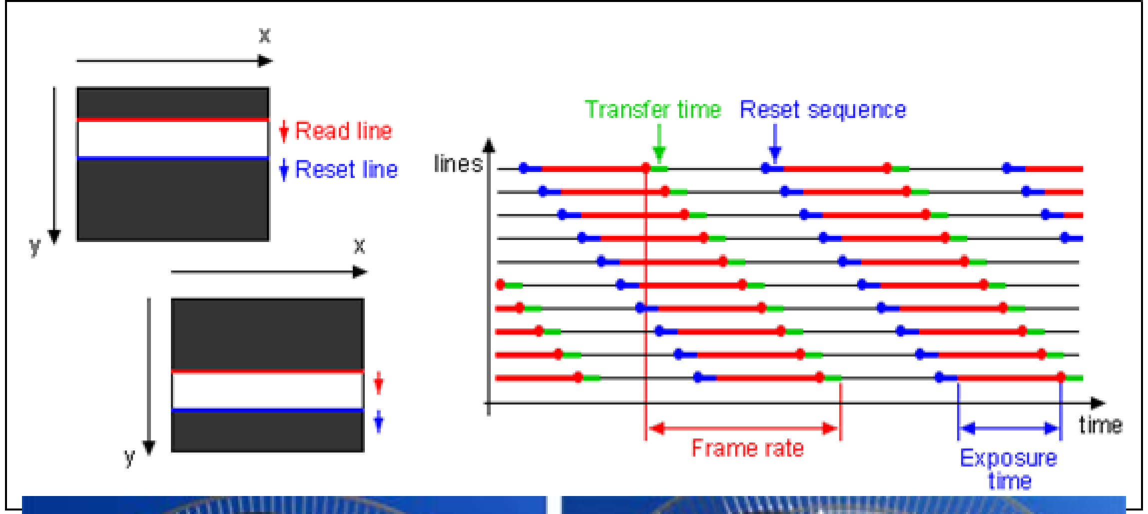


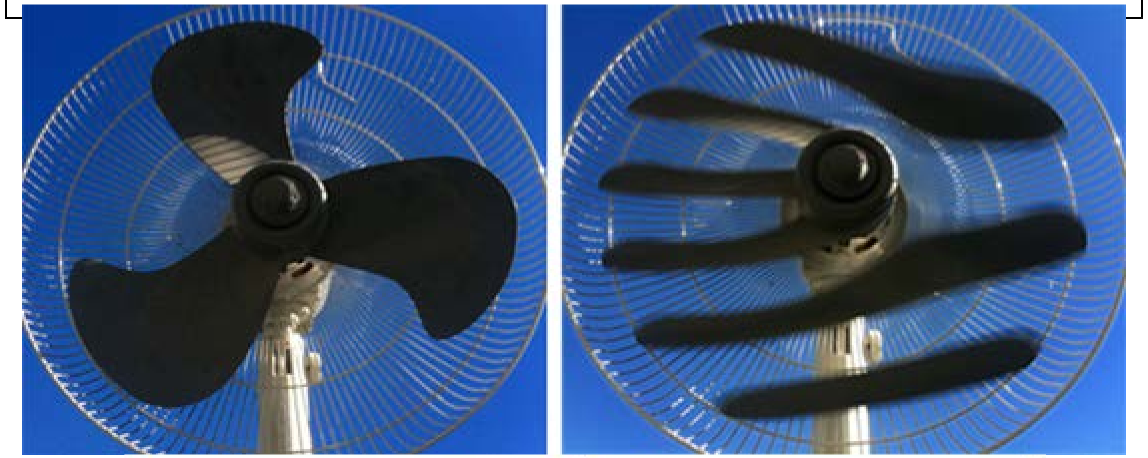
Figure 3

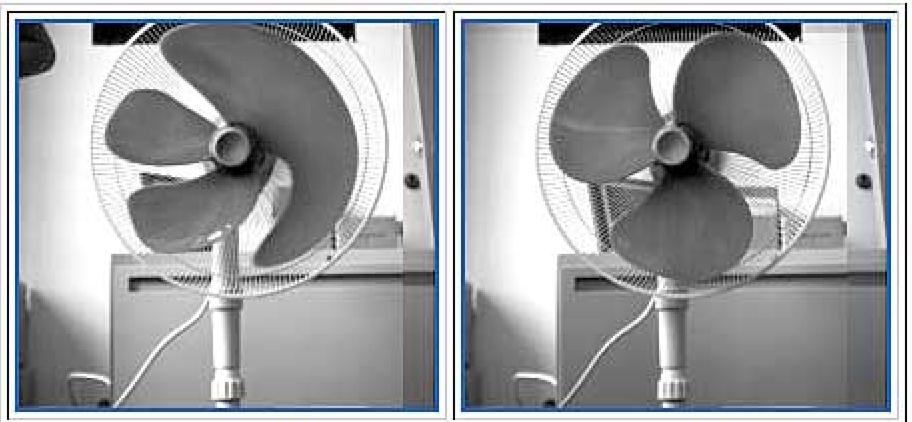


Rolling shutter

- sCMOS cameras use a rolling shutter to read out pixels.
- This decreases the total read time, and reduces dead time
- Each line is read out, and before it finishes, the next one starts
- Entire read out of sCMOS camera takes < 1 ms
- Imaging really fast things will result in error



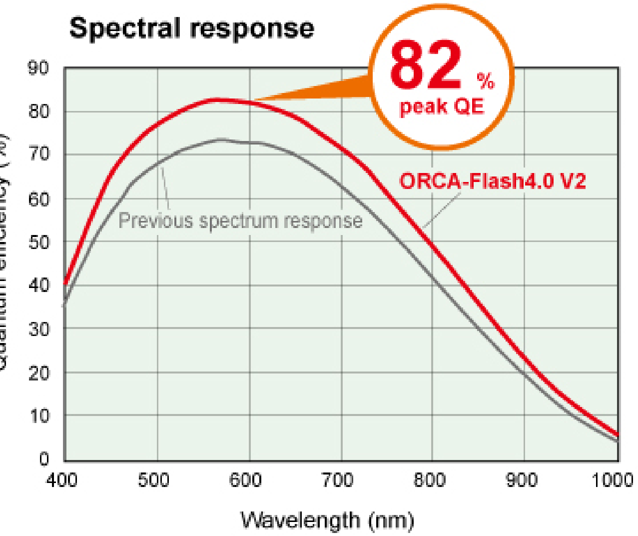




Rolling Shutter

Frame (Global) Shutter

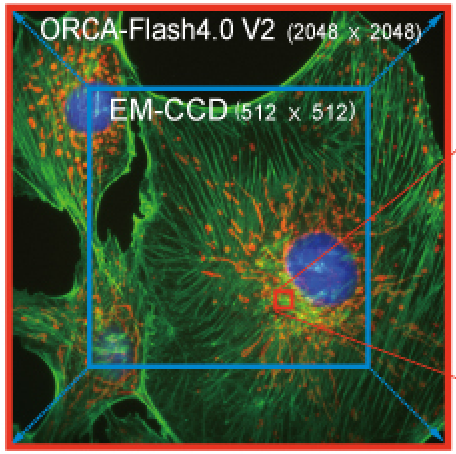
State of the art sCMOS cameras



Quantum efficency	82 % peak QE
Imaging device	Scientific CMOS Sensor
Effective no. of pixels	2048 (H)×2048 (V)
Cell size	6.5 µm×6.5 µm
Effective area	13.312 mm×13.312 mm
Full well capacity	30 000 electrons (typ.)
Readout speed	100 frames/s (Full resolution, Camera Link) 30 frames/s (Full resolution, USB 3.0)
Readout noise	Standard scan (at 100 frames/s, typ.):1.6 electrons rms (1.0 electrons median) Slow scan (at 30 frames/s, typ.): 1.4 electrons rms (0.8 electrons median)
Exposure time	Internal trigger mode: 1 ms to 10 s (at full resolution)*1 Internal trigger mode with sub-array readout: 38.96 µs to 10 s External trigger mode with sub-array readout: 1 ms to 10 s
Cooling method	Peltier cooling
Cooling temperature	Forced air (Ambient at +20 °C): -10 °C Water (+20 °C): -20 °C Water (+15 °C): -30 °C
Dark current	0.06 electrons/pixel/s (-10 °C) (typ.) 0.02 electrons/pixel/s (-20 °C) (typ.) 0.006 electrons/pixel/s (-30 °C) (typ.)

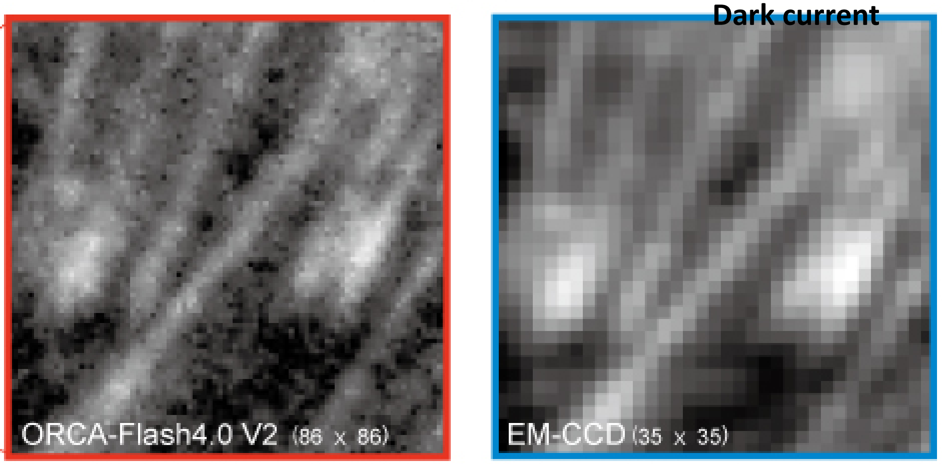
● Comparison of field of view

Field of view is 2.5x larger than that of a standard EM-CCD camera.



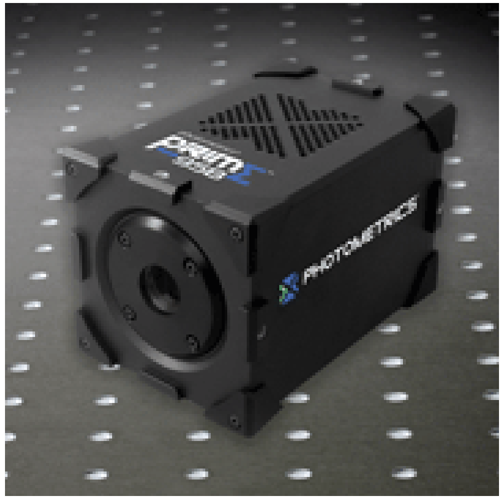
● Comparison of resolution

The 6.5 μm x 6.5 μm pixels of the ORCA-Flash4.0 V2 enable much finer detail to be resolved when compared to the 16 μm x 16 μm pixels of an EM-CCD camera.

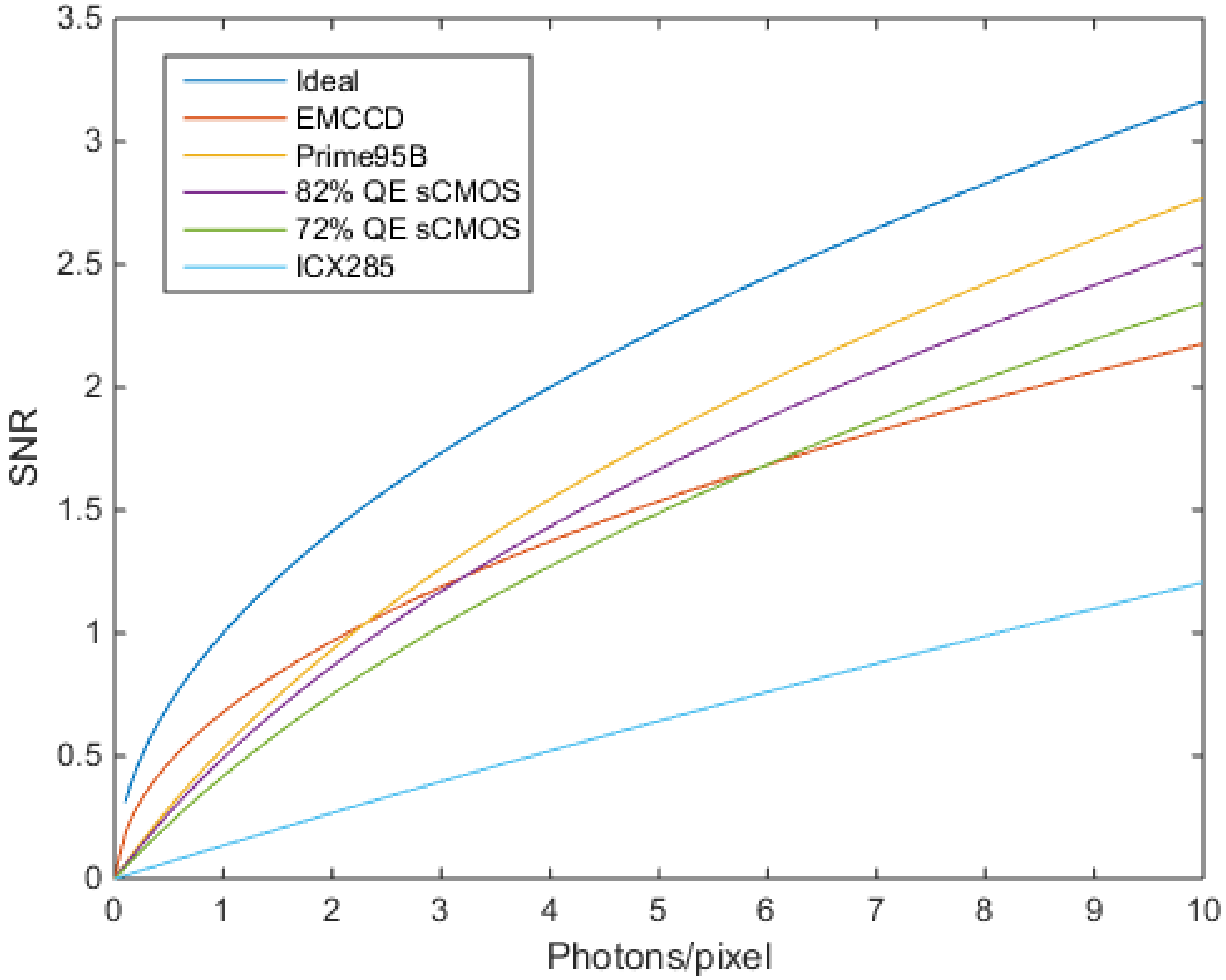


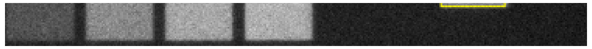
▲ Sample : FluoCells Prepared Slide #1, Objective lens : S Plan Fluor 100 x

Back thinned sCMOS



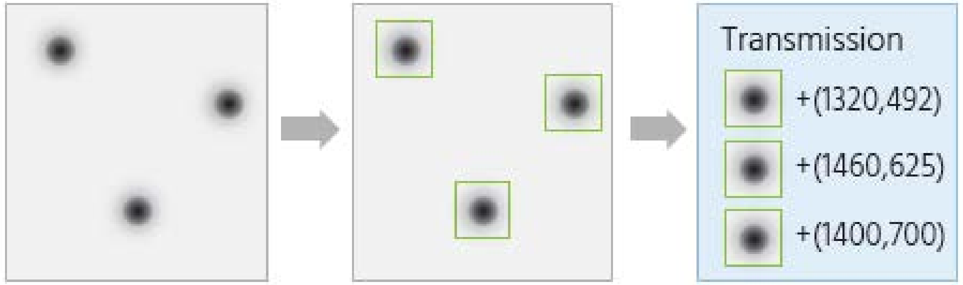
- 95% Quantum Efficiency
- 11µm x 11µm Pixel Area
- 1200 x 1200 array - 1.44 Megapixe
- 1.6e- Read Noise
- 80,000e- Pixel Full Well
- 61,500:1 Dynamic Range
- 41fps @ 16-bit
- 82fps @ 12-bit



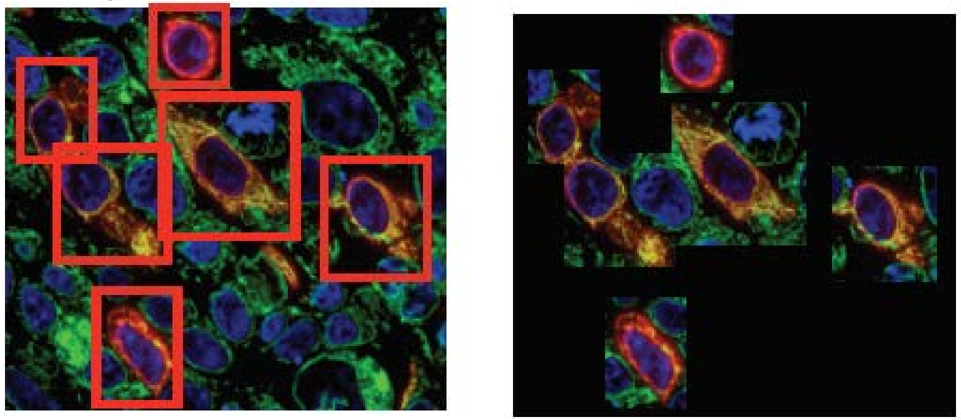


Large field of view and embedded signal processing

- 18 x 18 mm chip size – larger field of view
- Enables use of the full objective field number
- More pixels = more data
- Larger file size, and longer data transfer



- Only the data within the patches is transferred to the host computer



- Only the data within the user-defined ROIs is transferred to the host computer
- Select up to 15 unique regions