

Camera Tutorial – How to choose and use the right camera for a microscopy application – part I

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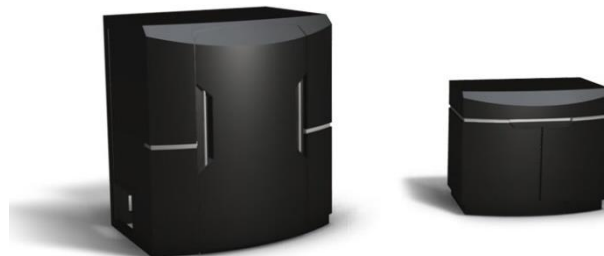
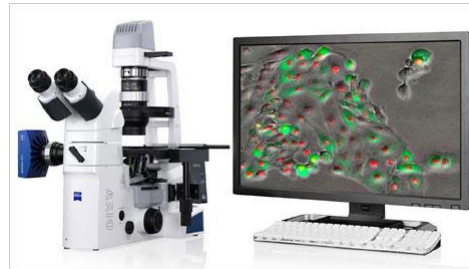
PCO develops and manufactures since 1987 high performance CCD, CMOS & sCMOS camera systems for scientific & industrial research, metrology, quality control and OEM applications.



Overview part I

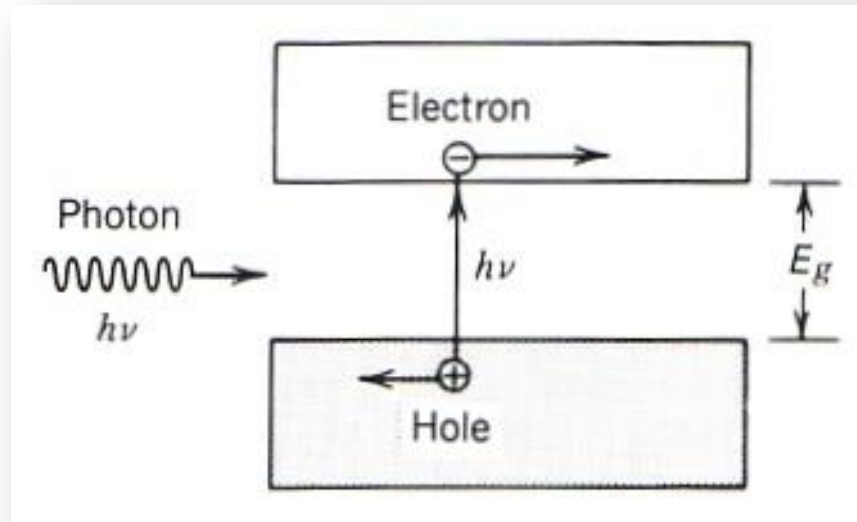
- **Photo - Physics**
- **Image Sensors – Basics**
 - CCD image sensors
 - CMOS & sCMOS image sensors
- **Image Sensors – Characteristical Features**
 - pixel size, noise, signal-to-noise-ratio, quantum efficiency, linearity, dark current, cooling, frame rate
- **Digital Cameras – Features**
 - raw & not so raw data, data transfer

Cameras in Microscopy



Light & Matter

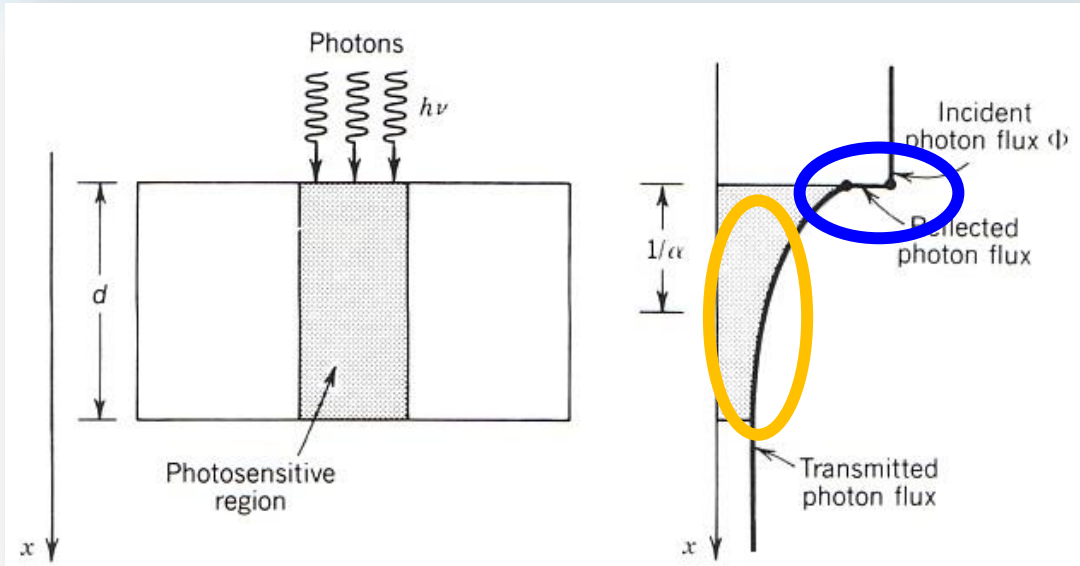
Inner Photoelectric Effect



The energy of the photon has to be large enough to raise an electron into the conduction band: $h\nu > E_g$

But there are various loss mechanisms, which interfere with this transition.

Quantum Efficiency η



$$\eta = (1-R) \cdot \zeta \cdot (1 - e^{-\alpha \cdot d})$$

Quantum Efficiency η

$(1-R)$ Reflection at the surface, which can be minimized by appropriate coatings

ζ Part of the Electron-hole-pairs, which contribute to the photo current and did not recombine at the surface.

$(1 - e^{-\alpha \cdot d})$ Part of the photon flux, which is absorbed in the semiconductor. Therefore the thickness d should be sufficiently large, to increase this part.

Light Impinges on Matter

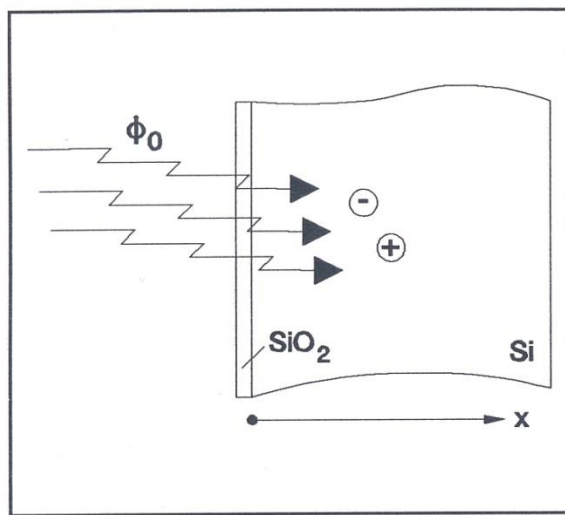


FIGURE 5.1. Schematic illustration of the generation of an electron-hole pair, due to the impinging photons, in the bulk of the silicon.

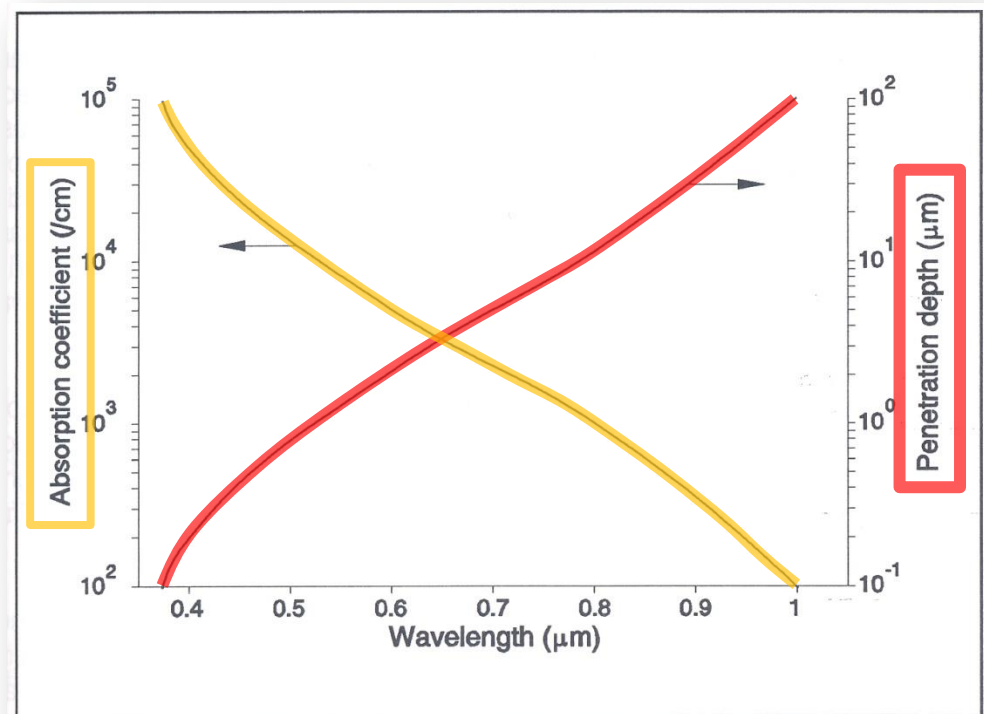


FIGURE 5.2. The absorption coefficient of silicon together with its corresponding penetration depth as a function of the wavelength of the incident light.

Quantum Efficiency Curve

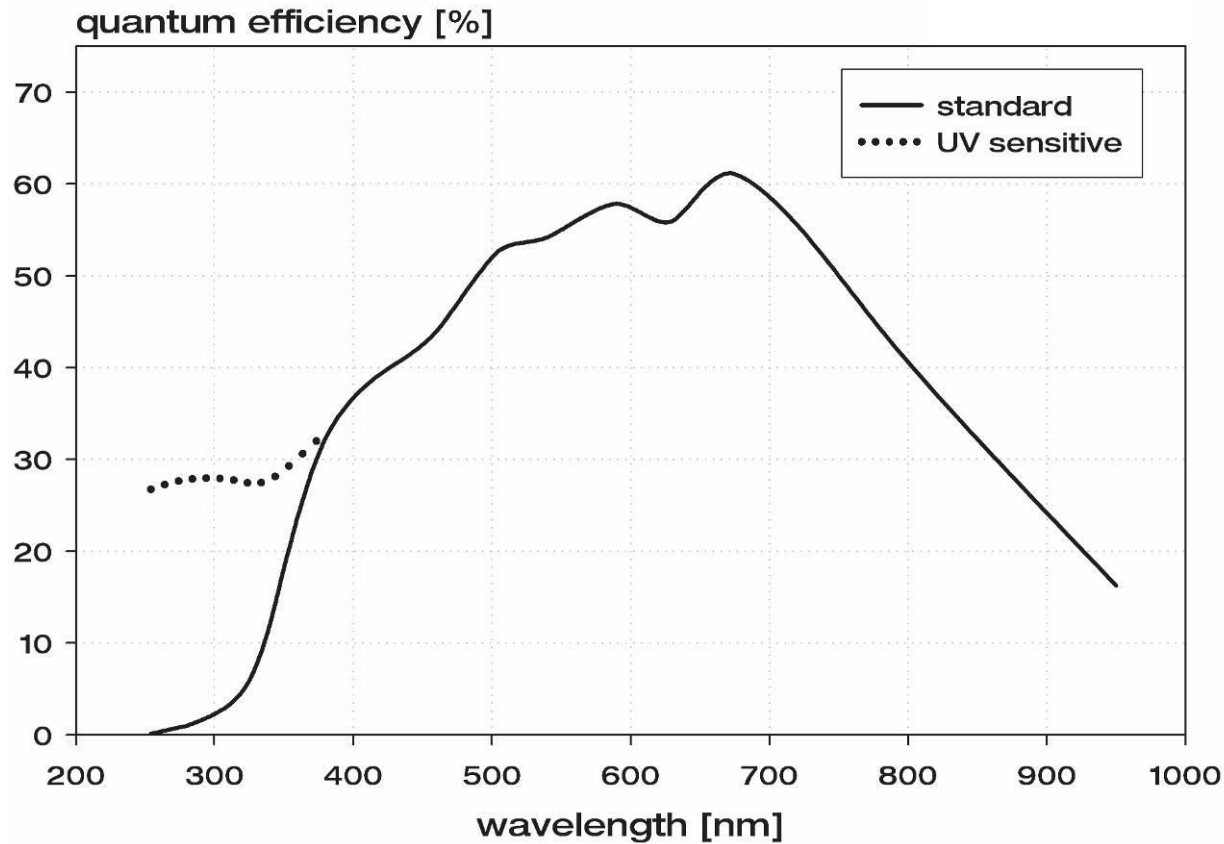


Image Sensors

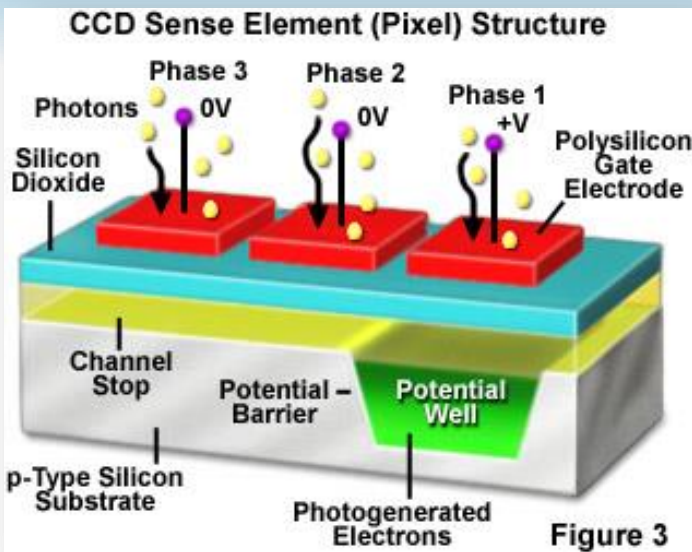
Overview

- **Multichannel-plate (MCP) image intensifier tubes**
- **CCD – charge coupled devices**
- **emCCD – electron multiplication charge coupled devices**
- **CMOS – complimentary metal oxide semiconductor**
- **sCMOS – scientific complimentary metal oxide semiconductor**

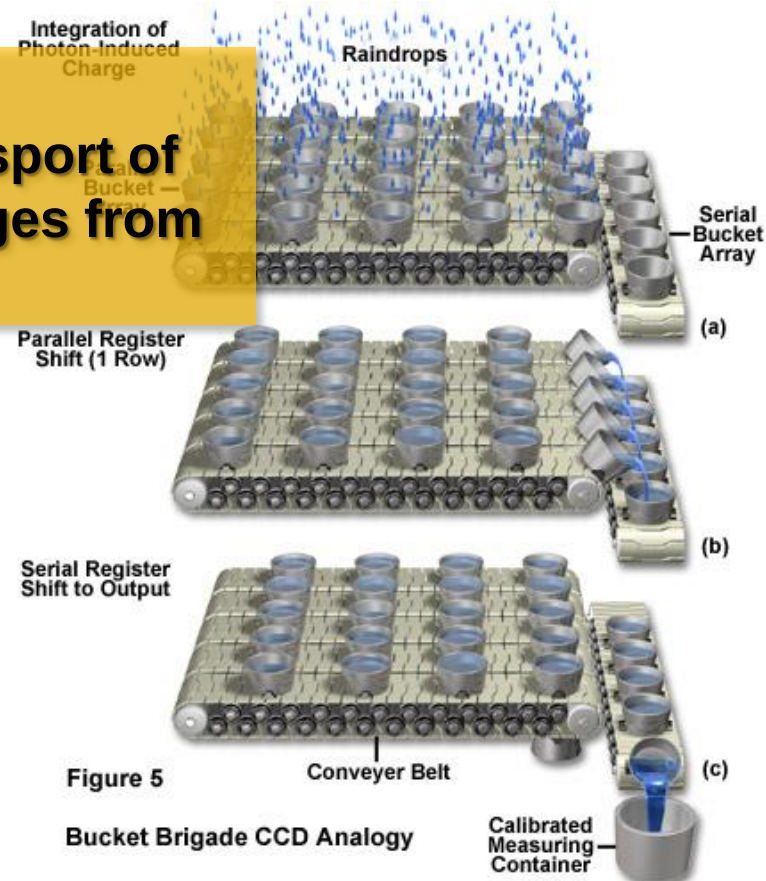
CCD

CCD Image Sensors

principle

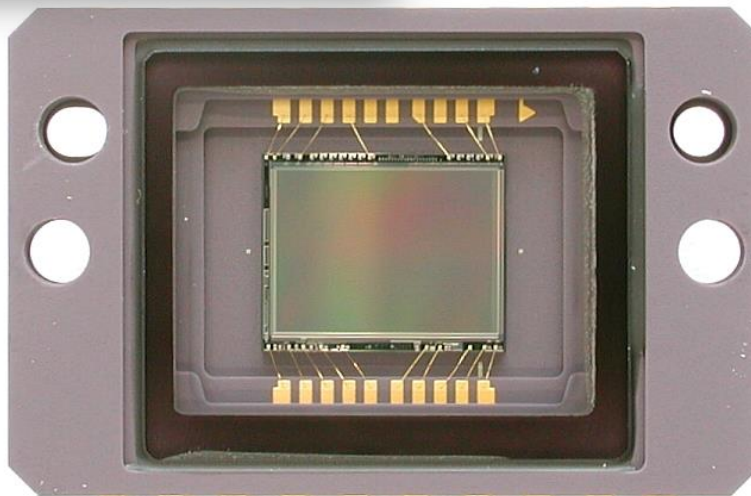
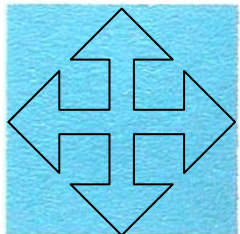


Advantage:
Lossless transport of
charge packages from
pixel to pixel!



ICX285AL

1cm



CCD Image Sensors

architecture – frame transfer CCD

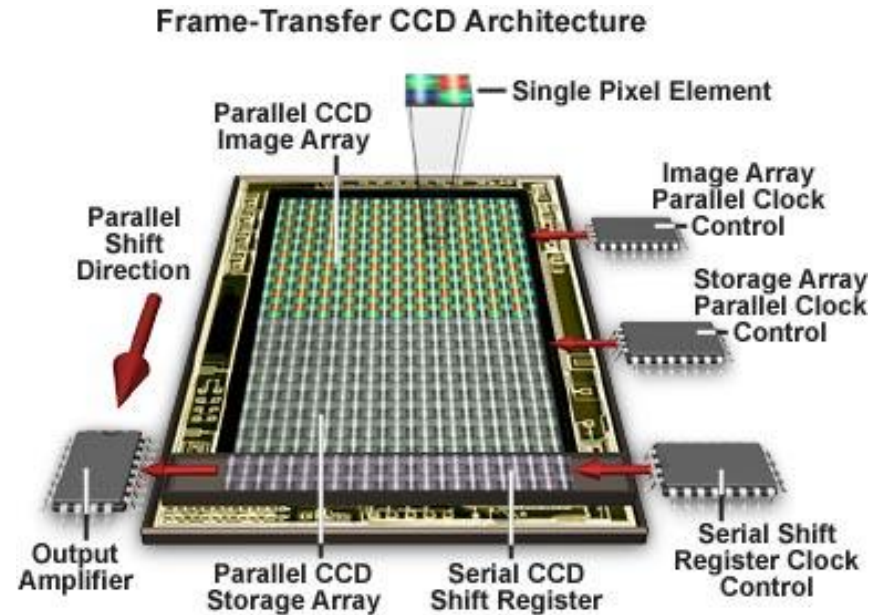
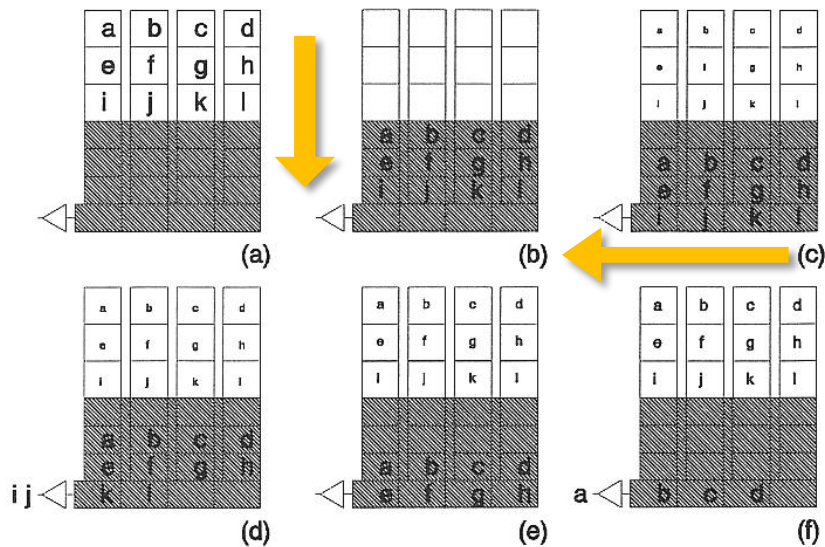
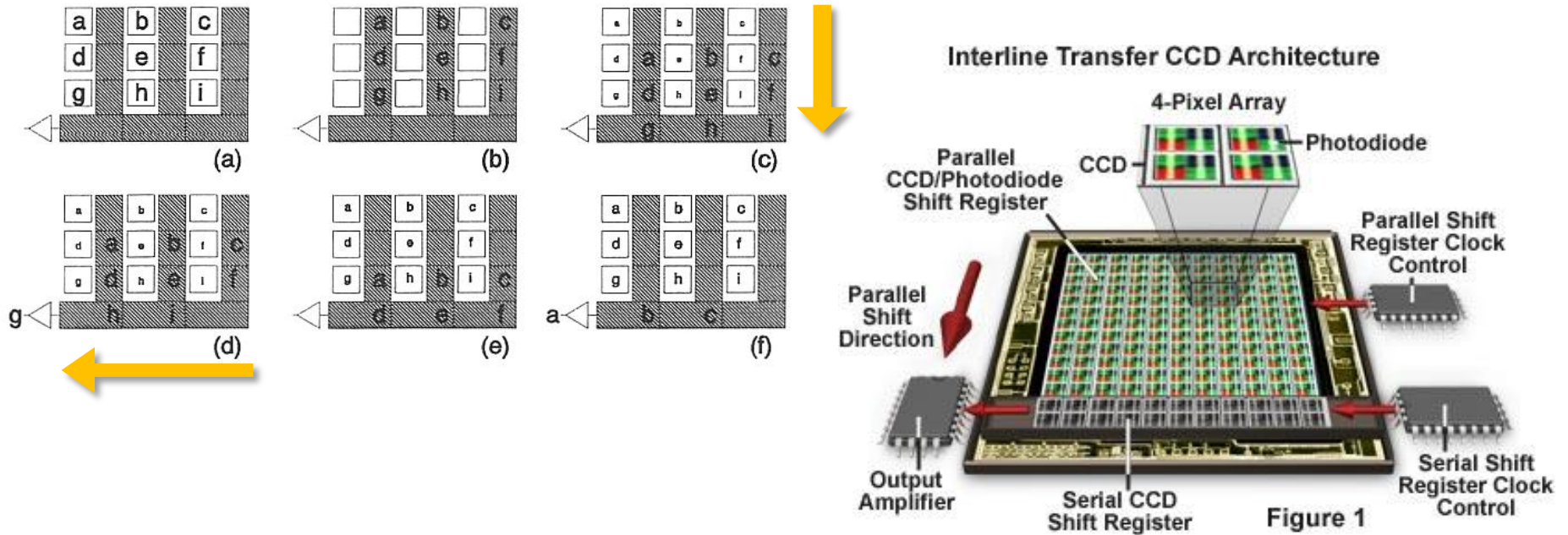


Figure 1

[+] good resolution, high fill factor, electronic shutter possible
 [-] large chip area, large smear, small total resolution (half area of chip for storage)
e.g. ImagEM X2, iXon 3 888, Evolve 512, sensicam em, Falcon (emCCD cameras)

CCD Image Sensors

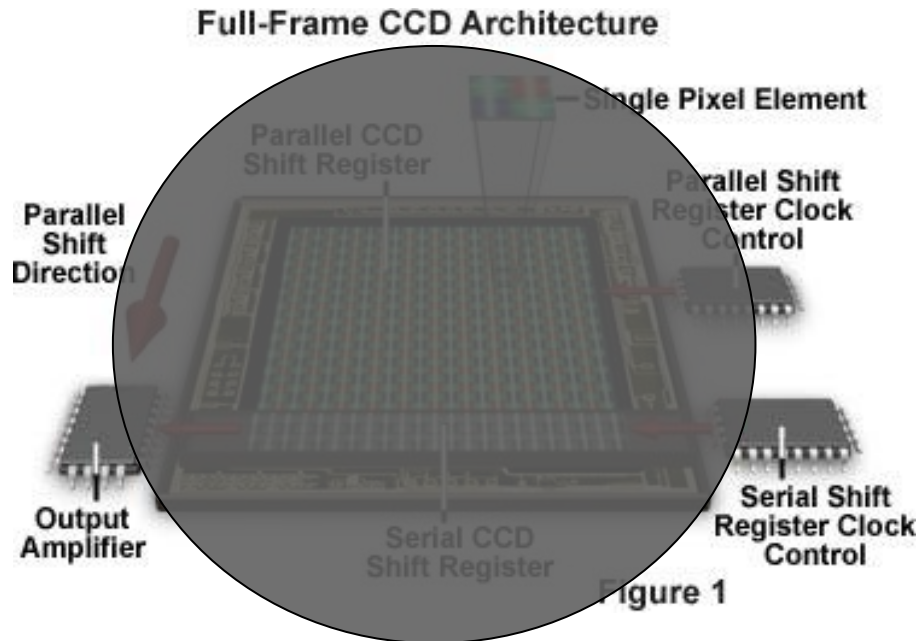
architecture – interline transfer CCD



[+] small chip size, shutter possible, high resolutions possible, progressive scan
[-] small fill factor, large smear, more complex processes in manufacturing
e.g. pco.2000, Orca-R2, Clara, CoolSNAP HQ...

CCD Image Sensors

architecture – full frame CCD



[+] high fill factor, simple process in manufacturing
[-] always external shutter required for readout of image
e.g. SensoCam HR-830, Aspen CG8300

CCD Image Sensors

How to increase frame rate - multiple readouts & consequences

- Introduction of more readout channels increases the frame rate and at the same time the inhomogeneity

KAI-04050 Image Sensor

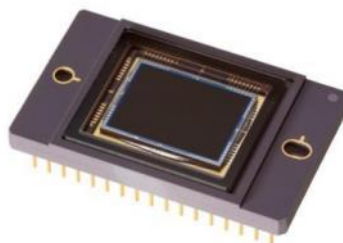
DESCRIPTION

The KAI-04050 Image Sensor is a 4-megapixel CCD in a 1" optical format. Based on the TRUESENSE 5.5 micron Interline Transfer CCD Platform, the sensor features broad dynamic range, excellent imaging performance, and a flexible readout architecture that enables use of 1, 2, or 4 outputs. The sensor supports full resolution readout up to 32 frames per second, while a Region of Interest (ROI) mode enables partial readout of the sensor at even higher frame rates. A vertical overflow drain structure suppresses image blooming and enables electronic shuttering for precise exposure control.

The sensor is available with the TRUESENSE Sparse Color Filter Pattern, a technology which provides a 2x improvement in light sensitivity compared to a standard color Bayer part.

The sensor shares common pin-out and electrical configurations with other devices based on the TRUESENSE 5.5 micron Interline Transfer CCD Platform, allowing a single camera design to support multiple members of this sensor family.

FEATURES

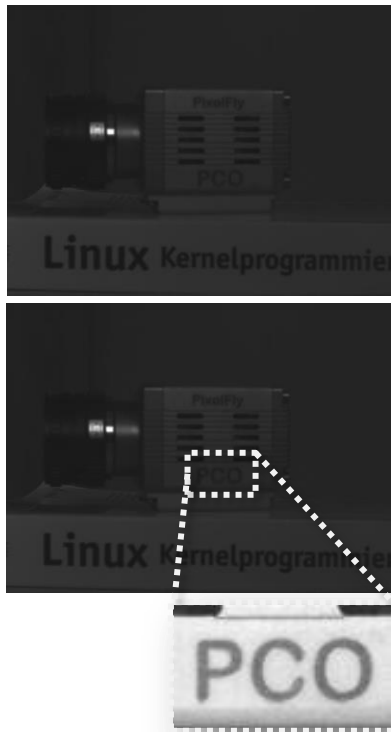


Parameter	Typical Value
Architecture	Interline CCD; Progressive Scan
Total Number of Pixels	2404 (H) x 1800 (V)
Number of Effective Pixels	2360 (H) x 1776 (V)
Number of Active Pixels	2336 (H) x 1752 (V)
Pixel Size	5.5 μm (H) x 5.5 μm (V)
Active Image Size	12.85 mm (H) x 9.64 mm (V) 16.06 mm (diag) 1" optical format
Aspect Ratio	4:3
Number of Outputs	1, 2, or 4
Charge Capacity	20,000 electrons
Output Sensitivity	34 $\mu\text{V}/\text{e}^-$
Quantum Efficiency Pan (-ABA, -PBA) R, G, B (-CBA, -PBA)	46% 29%, 37%, 39%
Read Noise ($f=40\text{MHz}$)	12 electrons rms

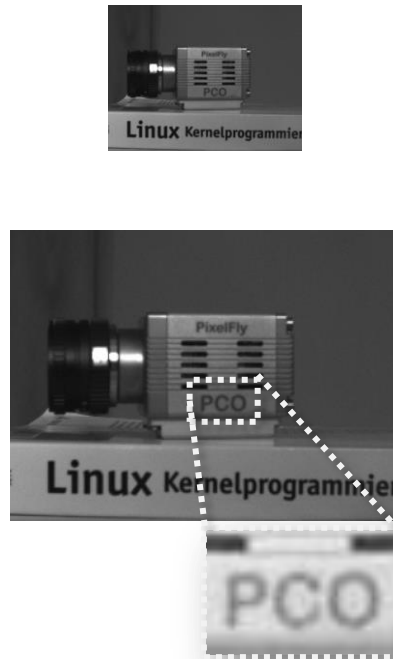
CCD Image Sensors

Binning of Pixels

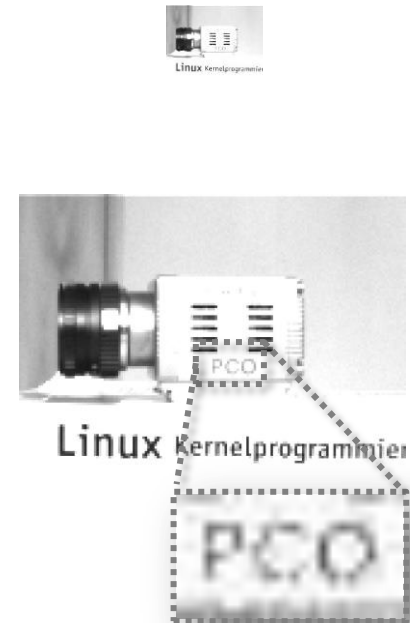
without binning
=> 640x480 pixel



2x2 binning
=> 320x240 pixel



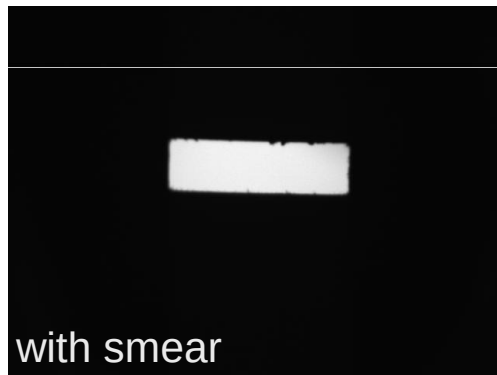
4x4 binning
=> 160x120 pixel



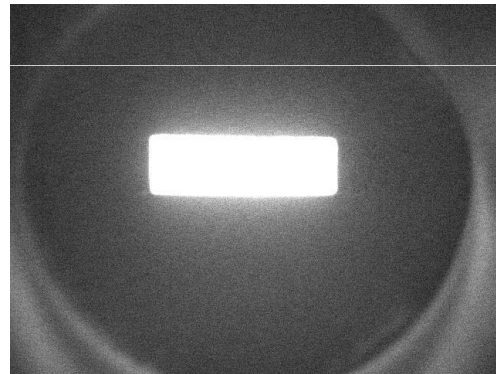
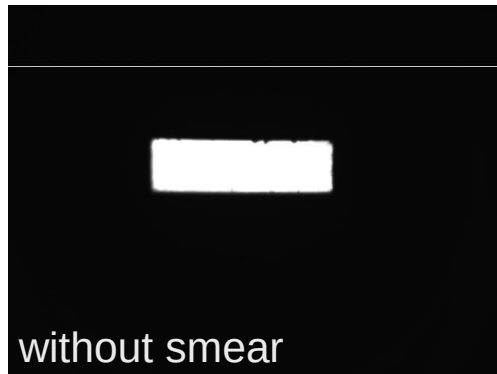
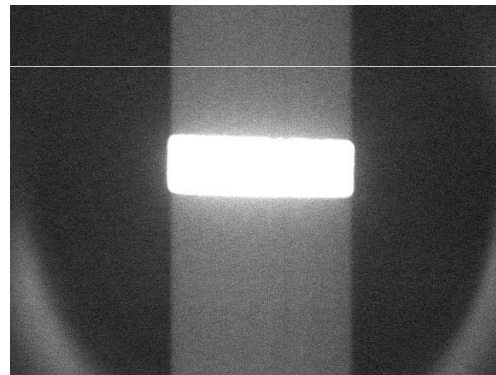
- [+] Improvement of SNR and higher frame rate
- [-] Reduction of resolution and pixel dynamic

CCD Image Sensors

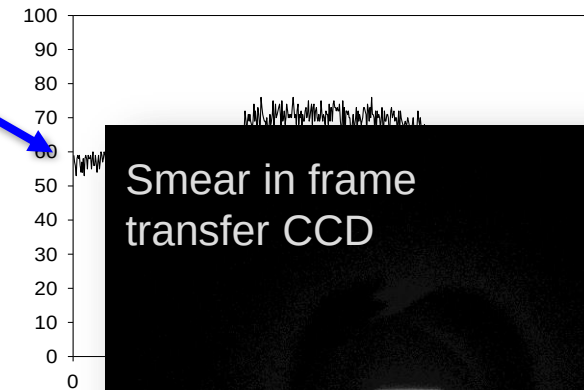
Smear



fs=0-2500

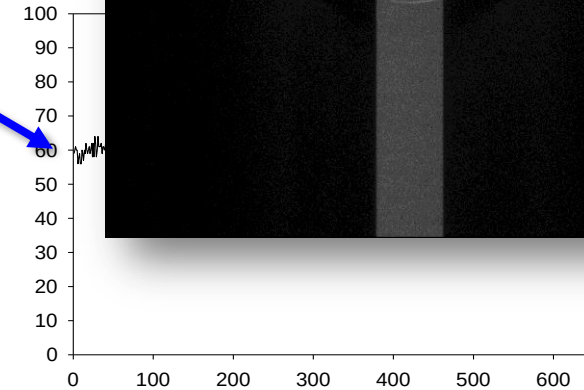


intensity [counts]



Smear in frame
transfer CCD

intens



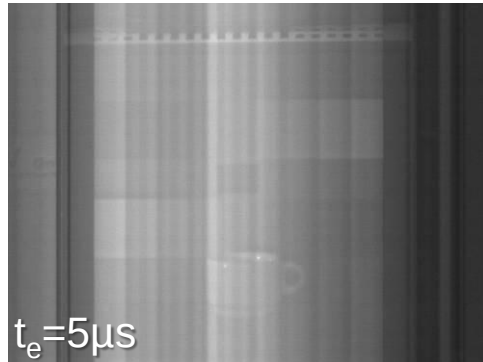
Methods to reduce smear:

Reduction of light signal, extension of exposure time, faster readout

CCD Image Sensors

Smear

cont. illumination

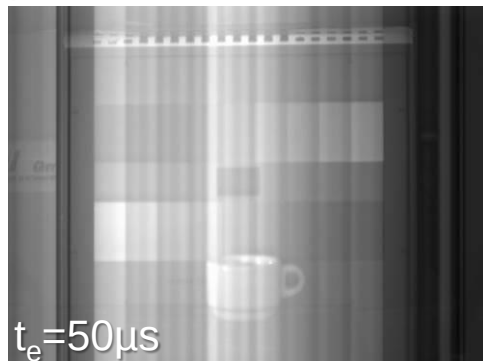


flash illumination

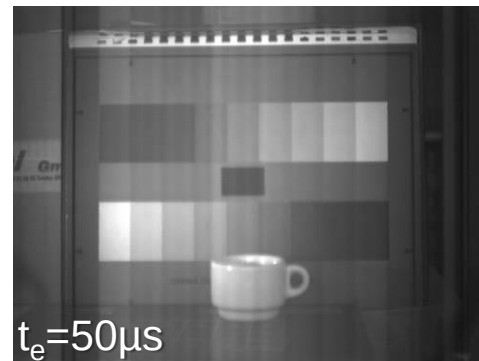


The most important relationship for the estimation of smear is the ratio of the readout time of the chip t_r and the exposure time t_e , if $t_e < t_r$, smear is increasing with respect to the signal!

pixel clock = 1x10MHz



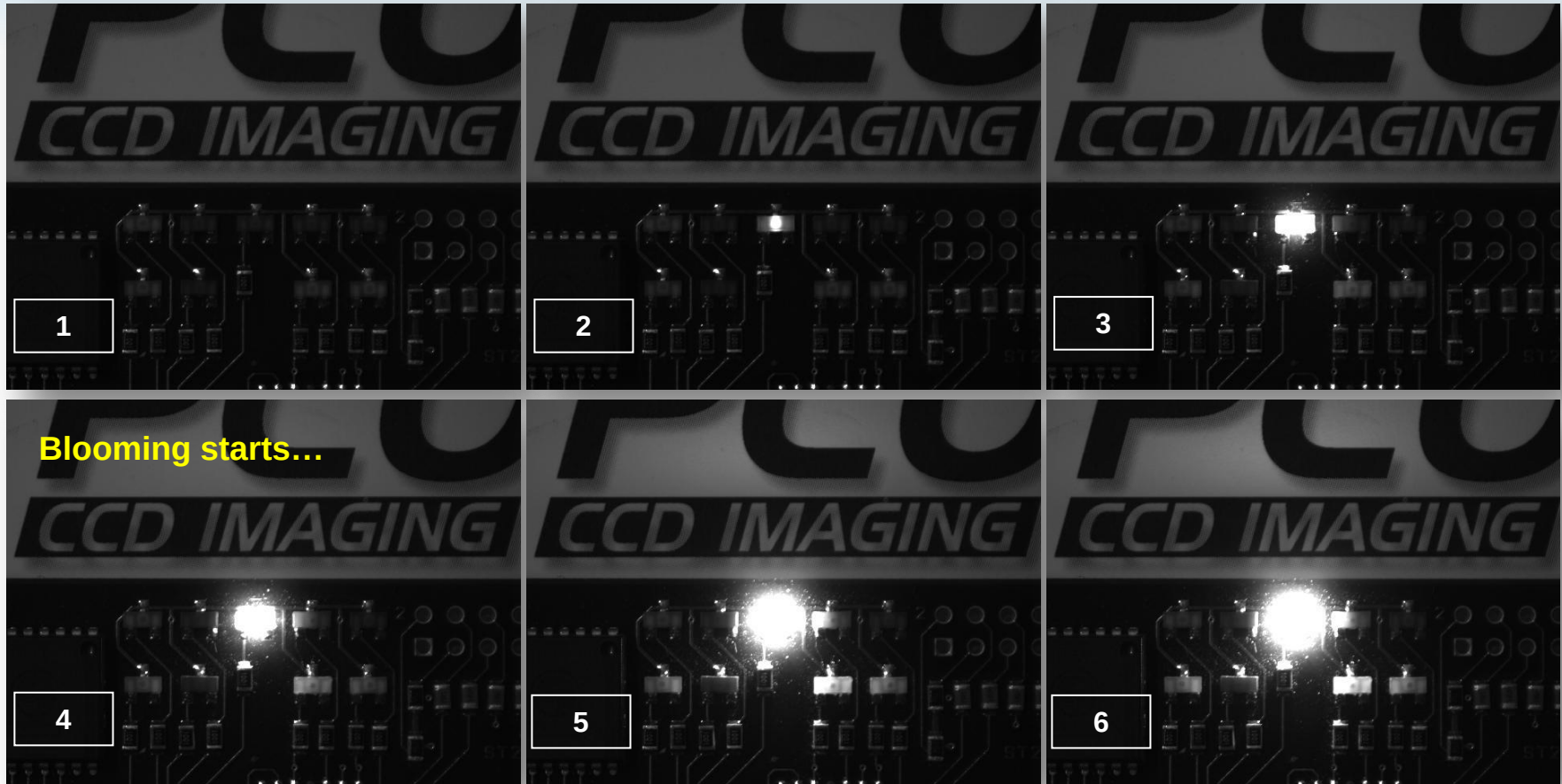
pixel clock = 2x40MHz



If the readout time of the chip is reduced at constant exposure time, also smear is reduced. Higher pixel resolutions cause longer readout times and therefore more smear.

CCD Image Sensors

Blooming



A sequence of images, at constant exposure time and full scale (0-4095 counts), shows the influence of the light signal, which causes blooming. From image to image the light output of the LED is increased and from image 4, blooming occurs and is visible.

CCD Image Sensors

general features

■ Advantages

- Low spatial noise (high homogeneity)
- Low dark current
- High linearity
- Binning
- Excellent image quality

■ Disadvantages

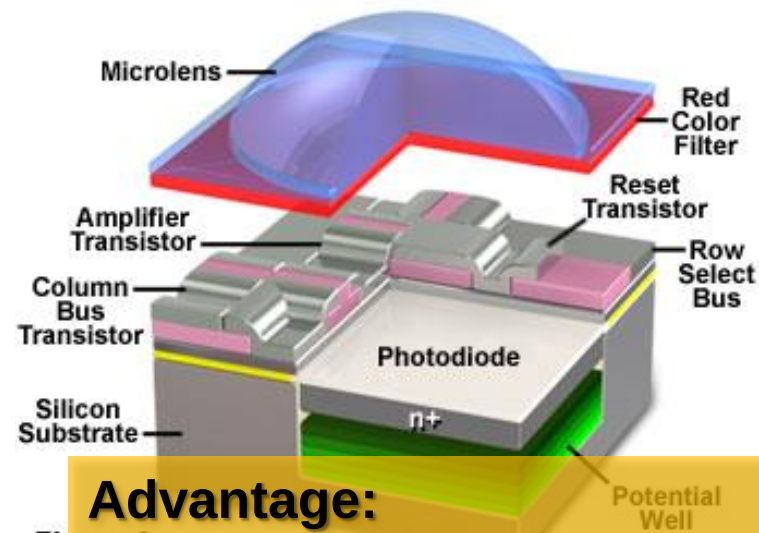
- Low frame rates (serial readout process)
- smear and blooming
- Expensive manufacturing process

CMOS

CMOS Image Sensors

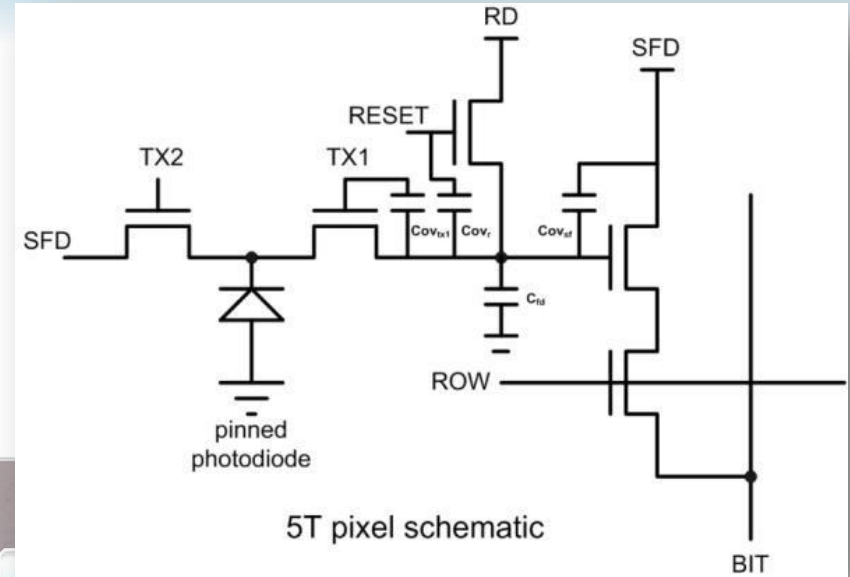
principle

Anatomy of the Active Pixel Sensor Photodiode

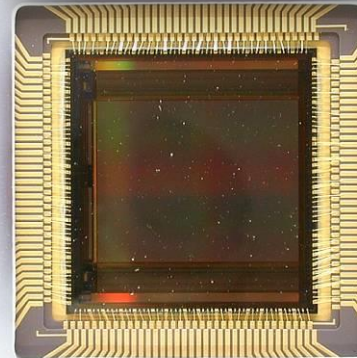


Advantage:
Many parallel readouts,
therefore high frame rates
possible.

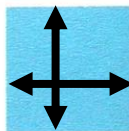
Figure 3



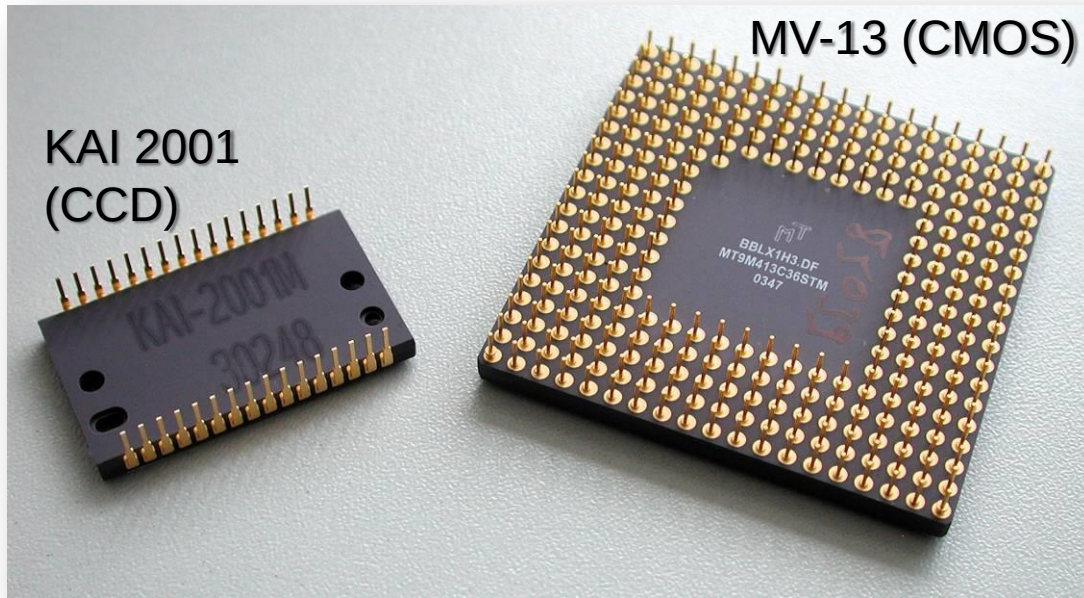
5T pixel schematic



1cm



CMOS Image Sensors

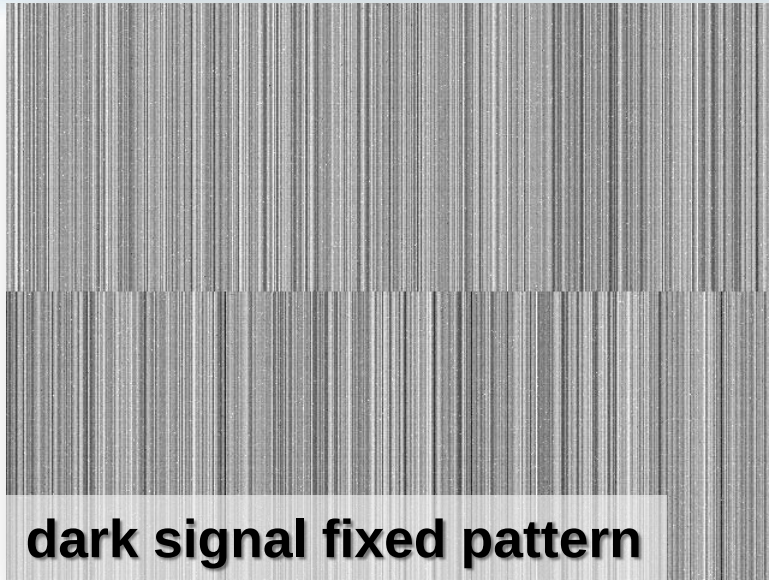


In general due to the higher parallel readout process of CMOS image sensors, they always have a much larger pin-count.

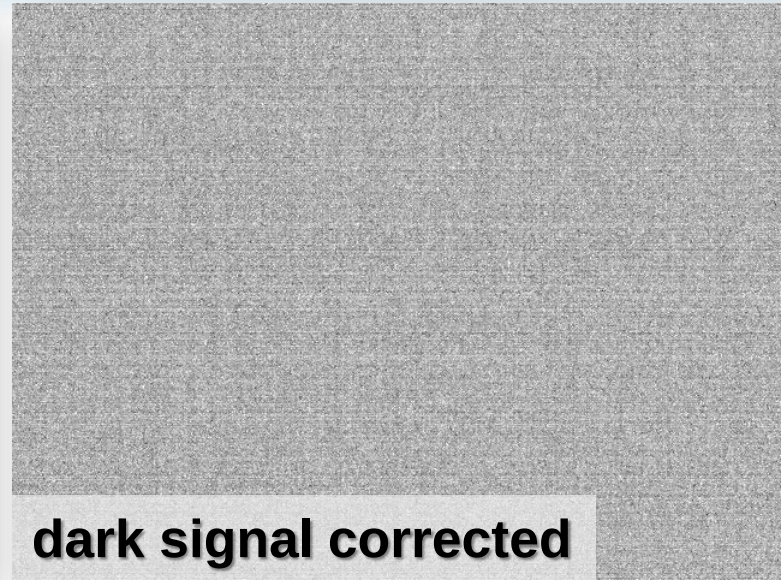
[+] high degree of parallel processing, faster frame rates, better & cheaper manufacturing process
[-] larger spatial inhomogeneity, higher dark current due to process
e.g. pco.dimax, Orca Flash 2.8, Osprey OS4MPc

CMOS Image Sensors

Fixed Pattern Noise



dark signal fixed pattern

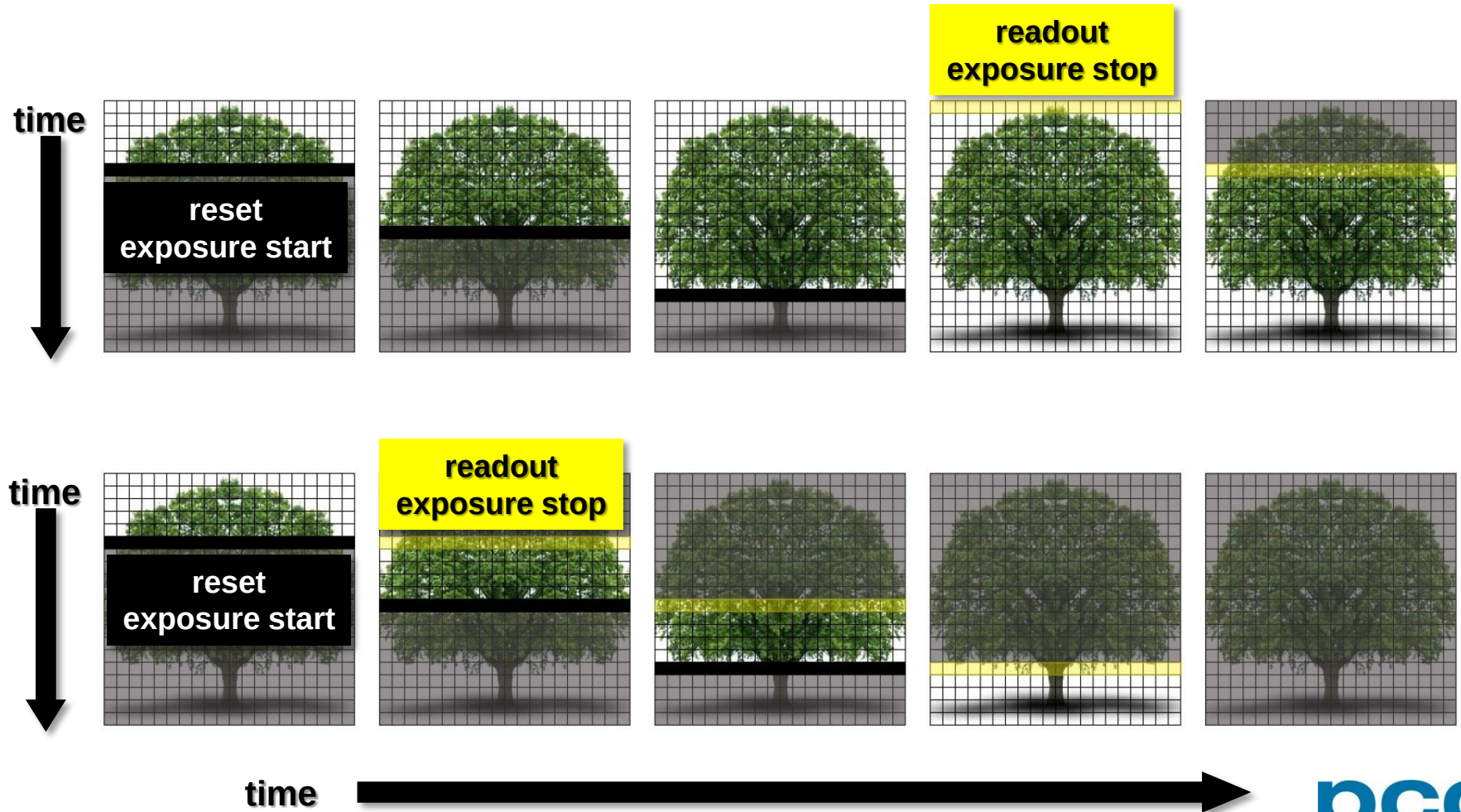


dark signal corrected

The „fixed pattern“ noise describes the spatial inhomogeneous behavior of the column amplifiers, A/D converters etc. It can be found as spatial variance in the dark image (offset variances) and in the bright image (gain variances). This is the price to be paid by the higher degree of parallel processes to speed up the readout.

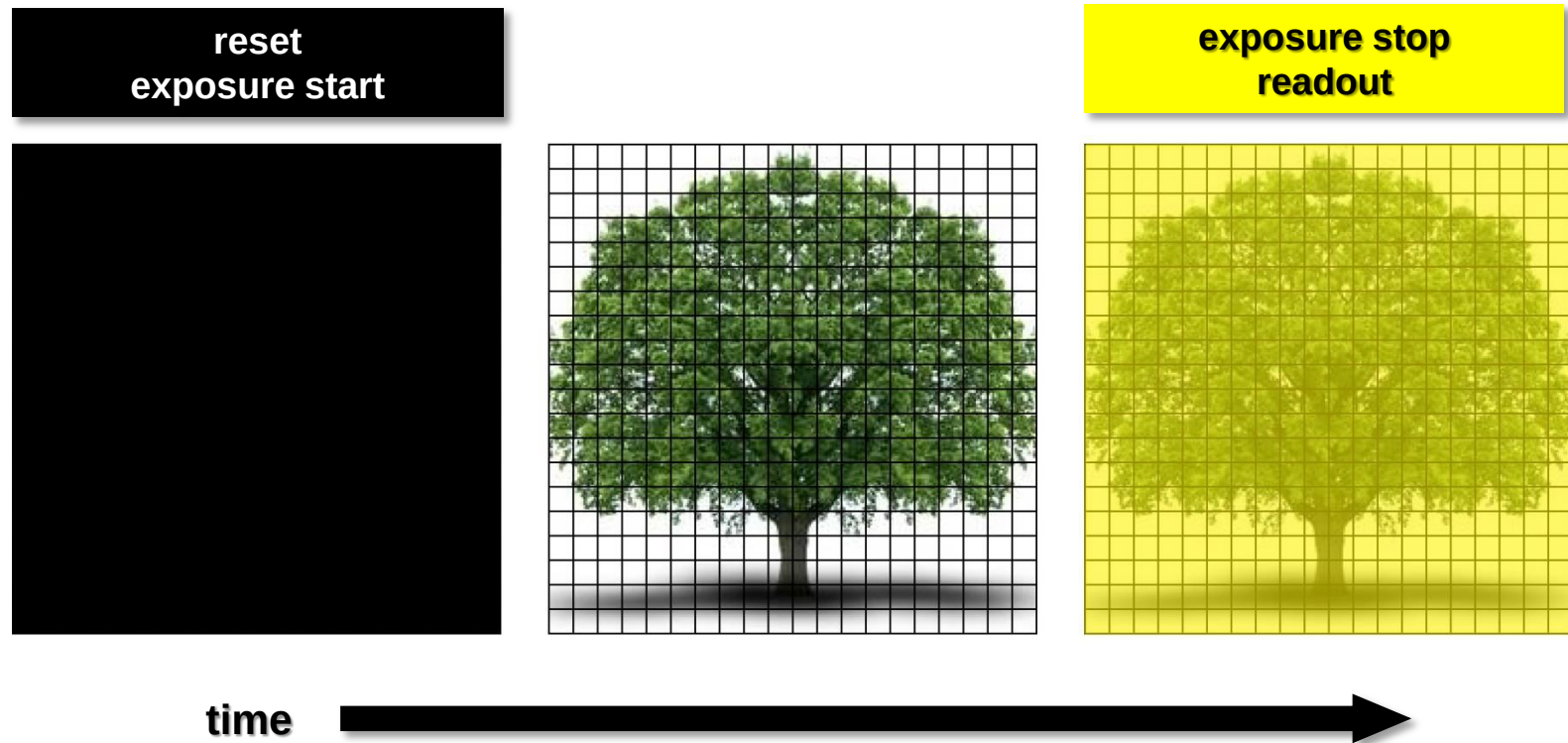
CMOS Image Sensors

readout schemes: rolling shutter



CMOS Image Sensors

readout schemes: global shutter



CMOS Image Sensors

readout schemes: global shutter & rolling shutter



Global shutter

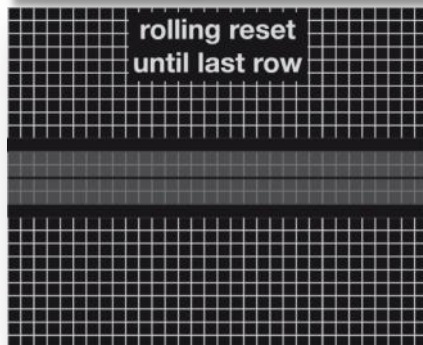


Rolling shutter

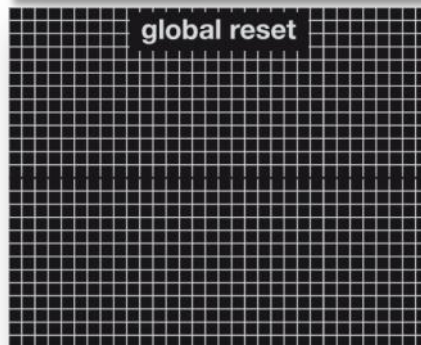
CMOS Image Sensors

Flash Illumination

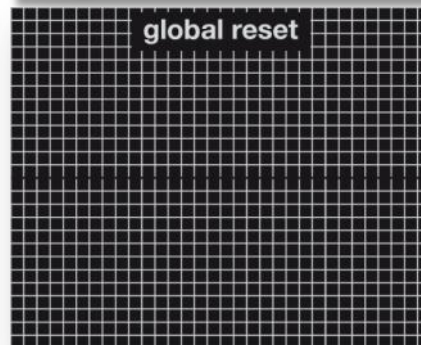
rolling shutter



global reset - rolling shutter



global shutter



If available!

rolling readout
with rolling
exposure stop

A diagram showing a sensor array grid with a rolling readout. The grid is divided into horizontal bands of different shades of grey, representing the rolling exposure and readout process. The bottom portion of the grid is black, representing the active exposure period.

rolling readout
with rolling
exposure stop

A diagram showing a sensor array grid with a rolling readout. The grid is divided into horizontal bands of different shades of grey, representing the rolling exposure and readout process. The bottom portion of the grid is black, representing the active exposure period.

rolling readout
with global
exposure stop

A diagram showing a sensor array grid with a rolling readout. The grid is divided into horizontal bands of different shades of grey, representing the rolling exposure and readout process. The bottom portion of the grid is black, representing the active exposure period.

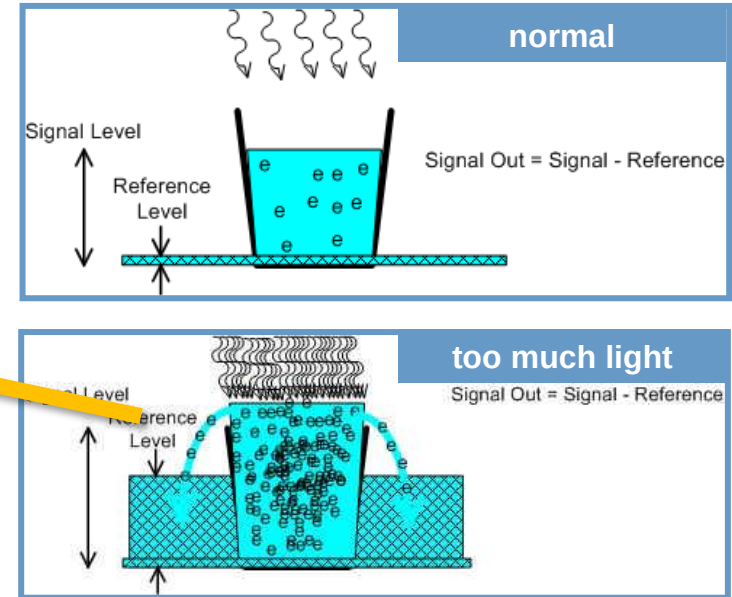
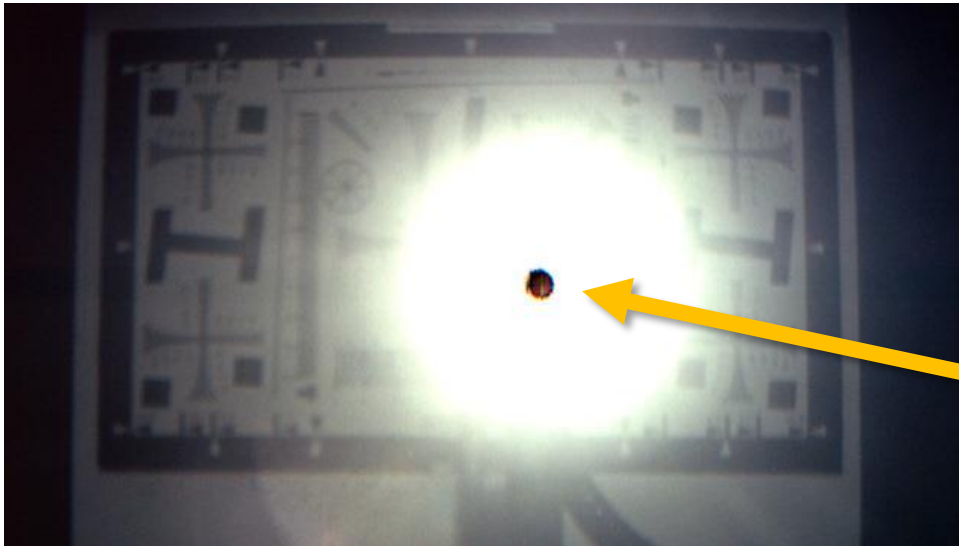
CMOS Image Sensors

readout schemes: global & rolling shutter

- Depending on the complexity of the pixel architecture, the size of the pixel, the application area and the available IP of the sensor designer, there are two different readout schemes used for CMOS image sensors: global shutter and rolling shutter
- In a global shutter readout mode, all pixel are reset at once, therefore the exposure starts for all pixel at the same time, and it is as well stopped for all pixel at the same moment. The advantage is, that moving objects are not distorted. The cost is a more complex pixel structure and smaller light sensitive area.
- In a rolling shutter readout mode, usually a row of pixel is reset, and for this row immediately the exposure starts. Then this row moves further up or down, creating a moving exposure slit. A second stop row follows to interrupt the exposure. The advantage of rolling shutter sensors is a less complex pixel structure and due to that a larger light sensitive area. If there are moving objects in the image, they can be distorted due to the row serial exposure.

CMOS Image Sensors

"Black Sun" Effect



If too much light impinges on the pixel of some CMOS image sensors, here as well the potential pot can spill over (called blooming for CCDs) and cause an increase of the reference level. This in turn causes due to the subtraction of signal and reference negative values appearing as „black spots“ in the bright areas.

CMOS Image Sensors

general features

■ Advantages

- High frame rates due to high degree of parallel processing
- Inexpensive manufacturing process
- Additional processing on-chip possible

■ Disadvantages

- Non-Linearity
- High dark current
- Low fill factor
- „Black Sun“ & parasitic light sensitivity
- No binning possible

sCMOS

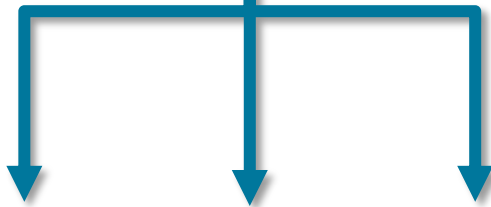
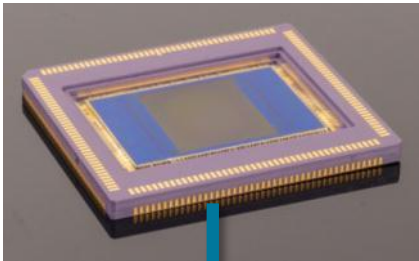
Scientific CMOS image sensors

[+] fast frame rates, low readout noise, high dynamic

[-] spatial inhomogeneity, dark current

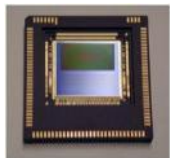
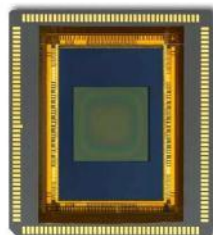
e.g. pco.edge 5.5, Orca Flash 4.0, Neo

CIS2521



CIS1210

CIS2020



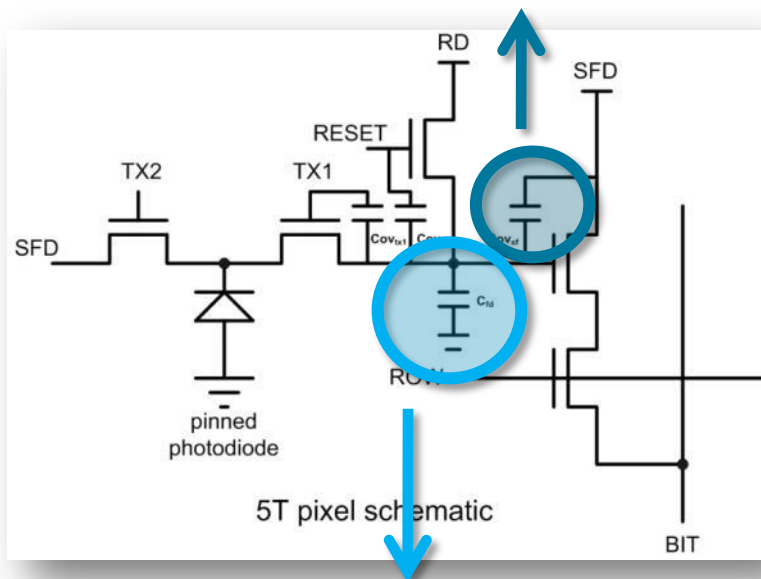
CIS1910

	CIS2521	CIS1910	CIS1210	CIS2020
resolution [pixel]	2560 x 2160	1920 x 1080	1280 x 1024	2048 x 2048
readout noise	1.1 _{med} e- 1.3 _{rms} e-	< 1.2 e-	< 2 e-	0.9 _{med} e- 1.5 _{rms} e-
frame rate [fps] (full)	100	100	100	100
rolling shutter	yes	yes	yes	yes
global shutter	yes	yes	yes	no
QE _{peak}	60 %	> 52 %	-	70 %
dynamic (rs)	1 : 27 000	1 : 25 000	1 : 25 000	1 : 33 000
fullwell capacity	30 000 e-	30 000 e-	30 000 e-	30 000 e-

sCMOS Image Sensors

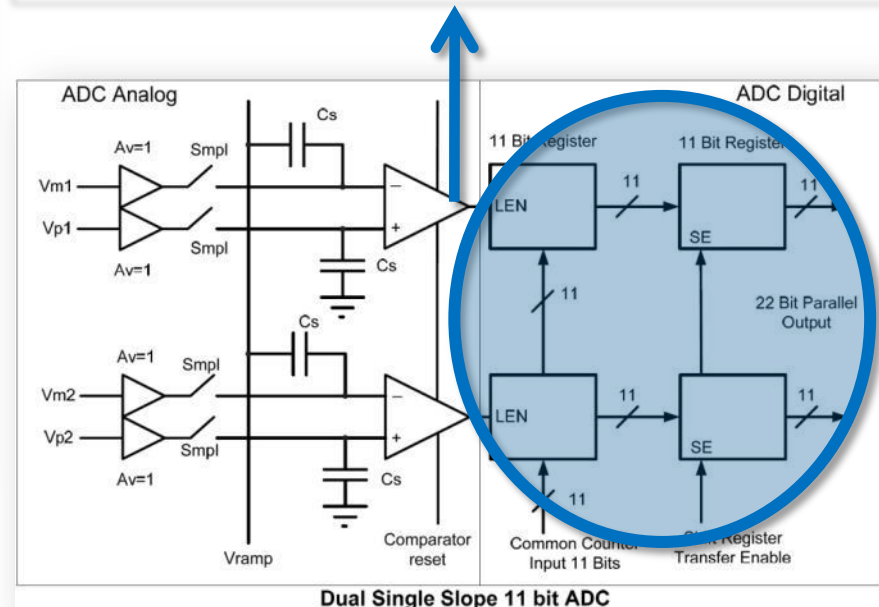
how the low readout noise is achieved...

This capacity as dominant noise contribution must be sufficiently small



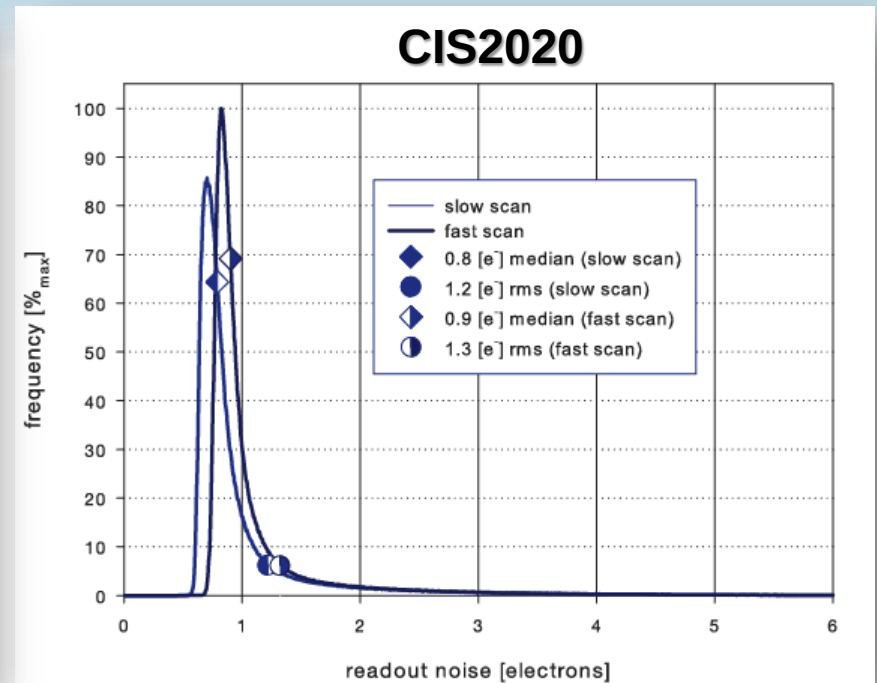
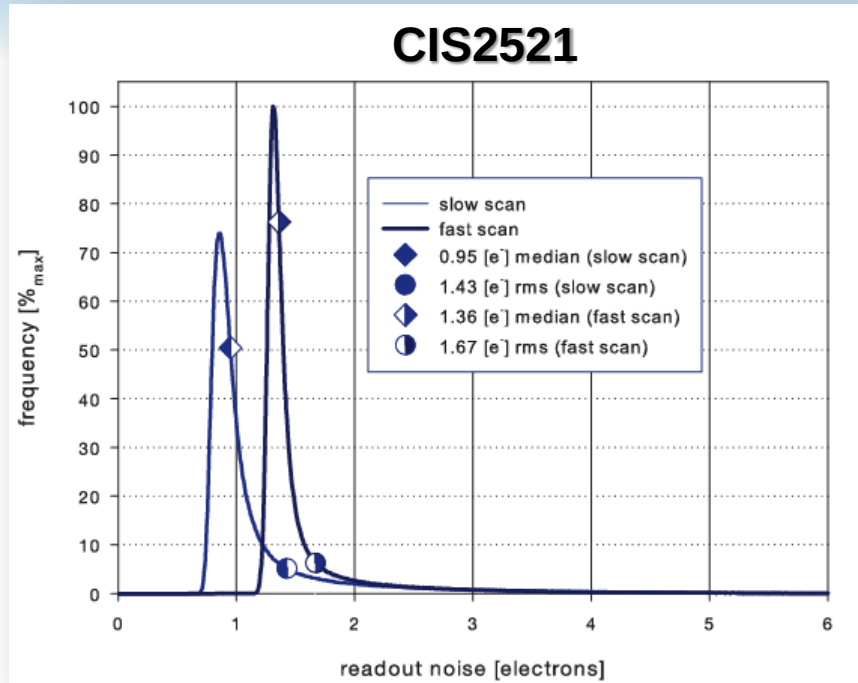
Reset (kTC) noise must be minimized by correlated double sampling (CDS)

Two 11bit A/D converters can be operated at a lower frequency than one 16 bit A/D converter (at constant output frame rate), which results in a lower $1/f$ -noise of the converters.



sCMOS Image Sensors

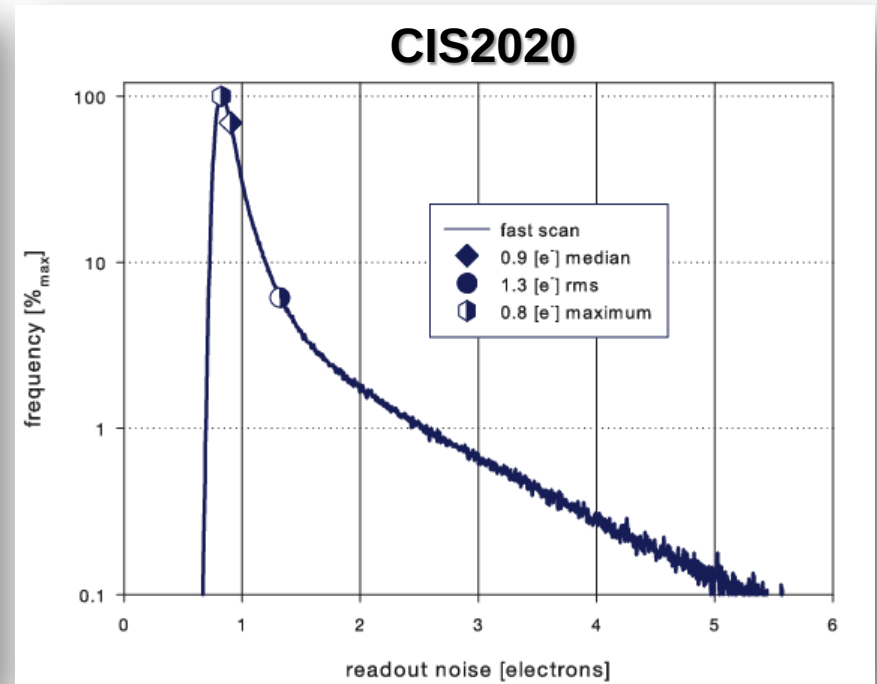
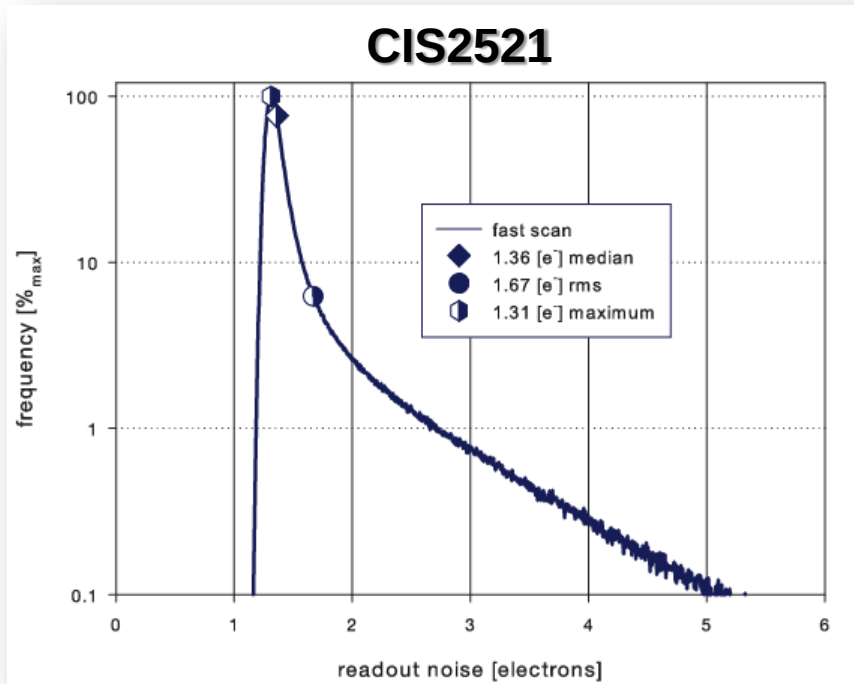
Readout Noise CIS2521 & CIS2020, rolling shutter, linear scale



A large sequence of images has been recorded and for each pixel the variance has been determined. All pixel variances are displayed in the above histograms (including the influence of the „blinking“ pixels). The noise distribution in the sCMOS sensors is not symmetrical, therefore both approximations, Gaussian (rms) and Median, are equally justified or not.

sCMOS Image Sensors

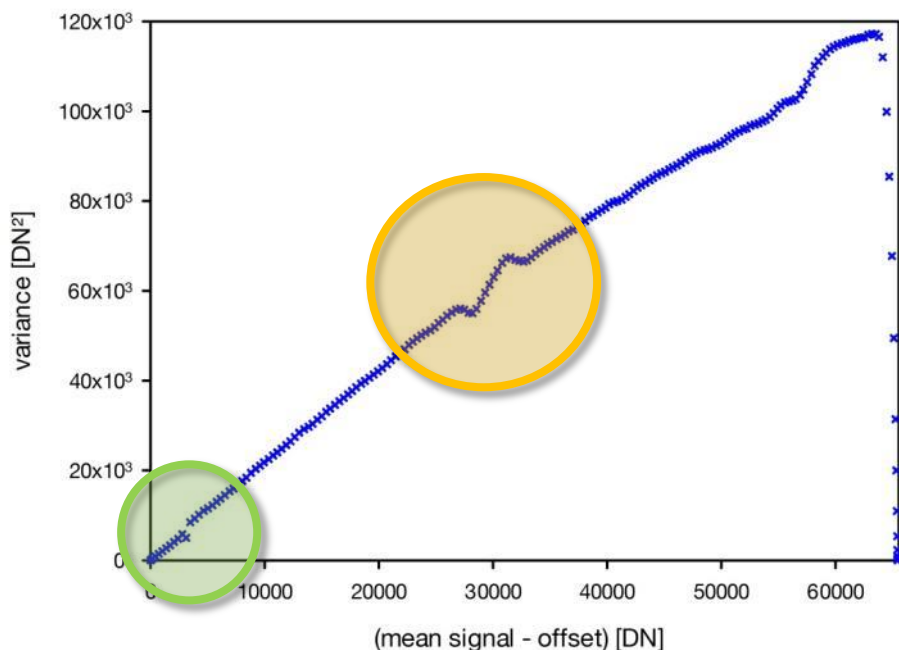
Readout Noise CIS2521 & CIS2020, rolling shutter, logarithmic scale



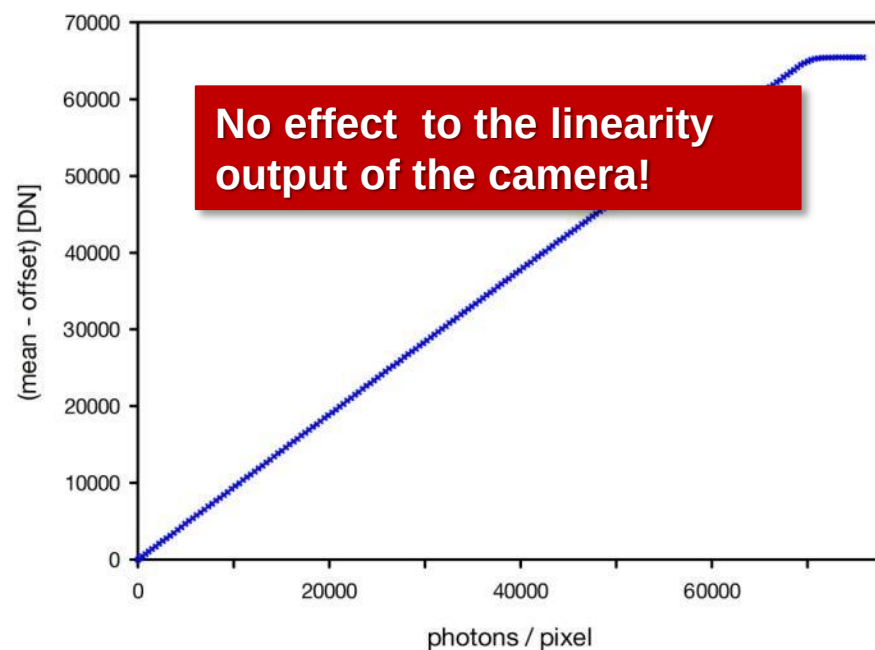
Keep in mind that 50% of all pixels have smaller readout noise values than the median value!

sCMOS Image Sensors

Photon Transfer Curve (PTC) & Linearity



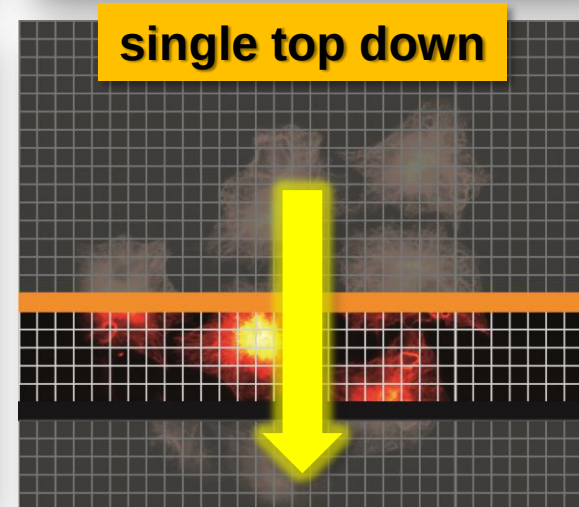
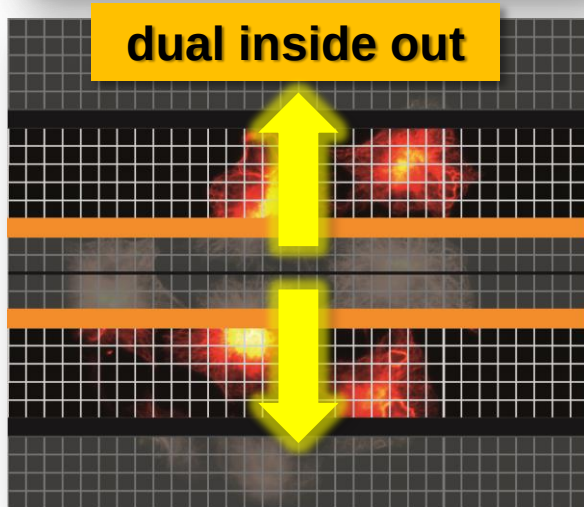
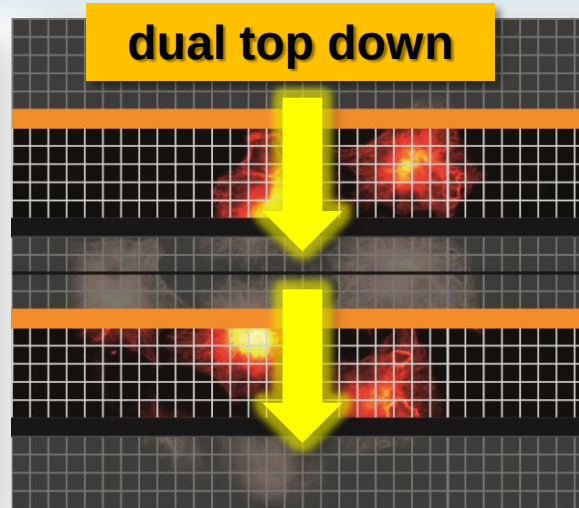
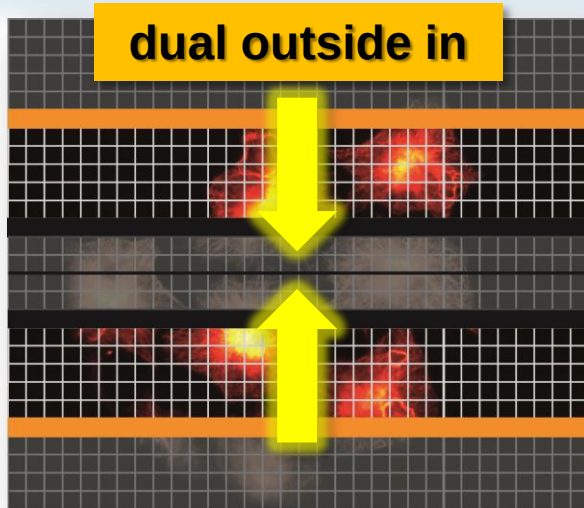
This little step is caused by the merging of the high gain and the low gain 11 bit values.



The switching between the S&Hs of reset and light signal value causes this „kink“. It could be removed by changing the switching to the moment after the A/D-conversion is finished, but this would reduce the frame rate to 50%.

sCMOS Image Sensors

Readout Schemes for Rolling Shutter Mode



The various readout modes for rolling shutter are suitable for synchronization of microscopes and scanning applications (together with the precise line trigger signal).

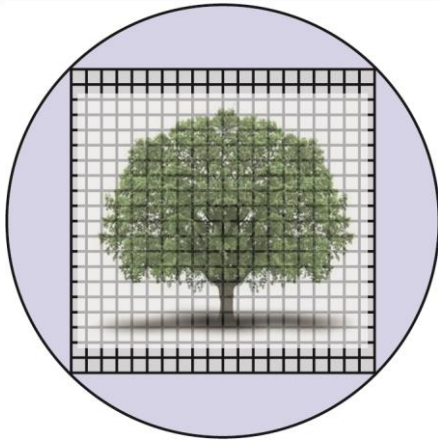
Pixel Size

Pixel Size & Sensitivity

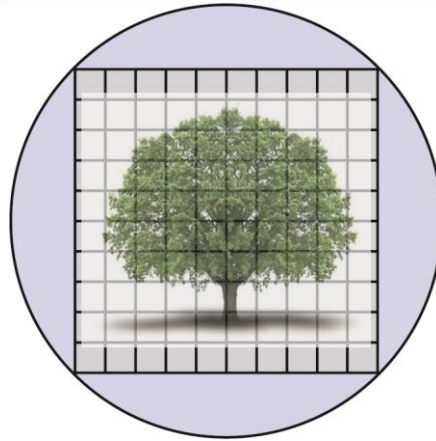
- Unfortunately there exists a very persistent believe, that „large pixel are always more sensitive than small pixel“ (because they can collect more photons)
- In many cases this is the result of a non appropriate comparison of cameras with lower and higher resolution, by using the same optics for comparison.
- Nevertheless the pixel size determines the maximum possible modulation transfer function (MTF).
- Together with the ratio of light sensitive area vs. electronics area it influences with the „fill factor“ the effective sensitivity.

Cameras with Different Pixel Size

Comparison with same optics (constant area)



sensor A

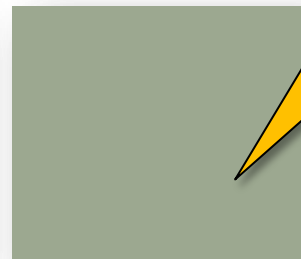


sensor B

pixel size A = $\frac{1}{4}$ pixel size B

table 3: consideration on signal and SNR for different pixel sizes same total area

pixel type	signal	readout noise	SNR low light	SNR bright light
small pixel	m	r_0	s_0	s_1
large pixel	$4 \times m$	$> r_0$	$> s_0$	$2 \times s_1$
4 small pixels	$4 \times m$	$2 \times r_0^{iv}$	$2 \times s_0$	$2 \times s_1$



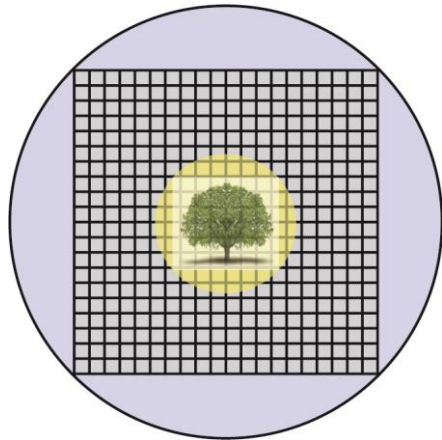
Best possible SNR due to largest area, but no information !

increasing pixel size

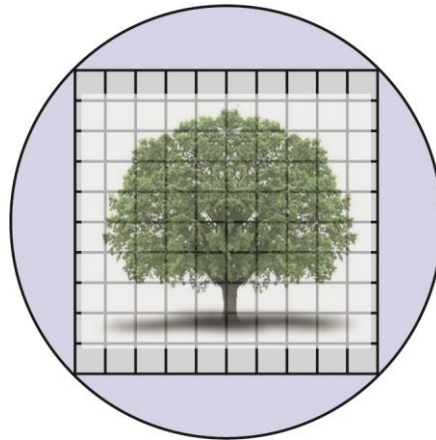


Cameras with Different Pixel Size

Comparison with different optics (constant resolution) i



sensor A



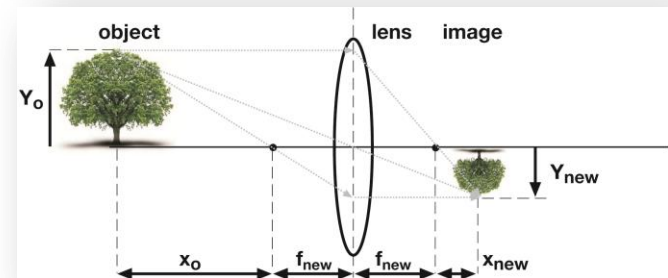
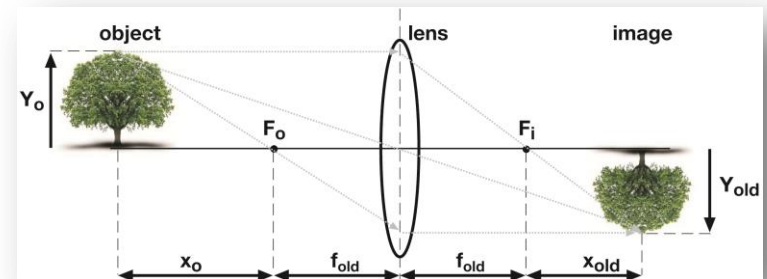
sensor B

pixel size A = $\frac{1}{4}$ pixel size B

table 4: consideration on signal and SNR for different pixel sizes same resolution

pixel type	signal	readout noise	SNR low light	SNR bright light
small pixel	m	r_0	s_0	s_1
large pixel	m	$> r_0$	$< s_0$	s_1

original lens imaging to sensor B

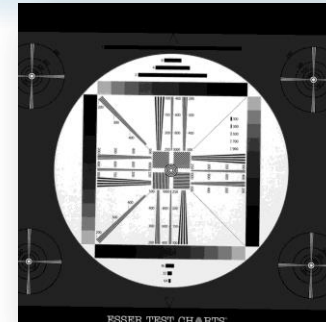
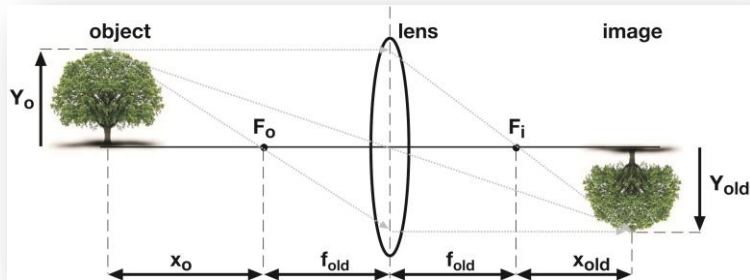


new lens imaging to sensor A

Cameras with Different Pixel Size

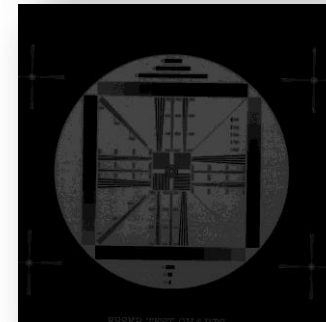
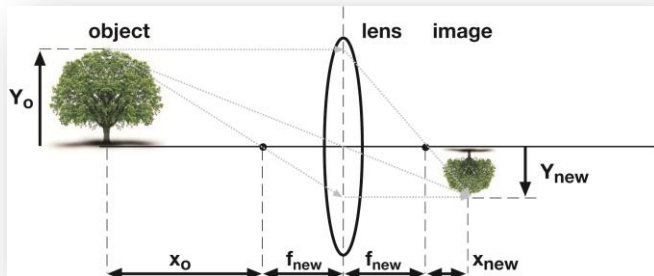
Comparison with different optics (constant resolution) ii

original lens imaging to sensor B



large pixel
 $f = 100 \text{ mm}$
 $f\text{-stop} = 16$

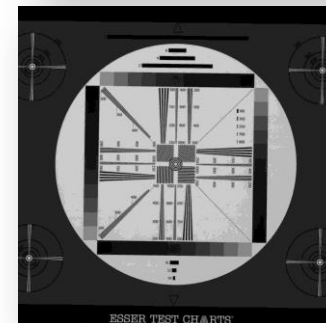
pixel size A = $\frac{1}{4}$ pixel size B



small pixel
 $f = 50 \text{ mm}$
 $f\text{-stop} = 16$

new lens imaging to sensor A

The new focal length e.g. from 100 mm to 50 mm achieves the same depth of field at a larger aperture or f-stop.



small pixel
 $f = 50 \text{ mm}$
 $f\text{-stop} = 8$

MTF Modulation Transfer Function

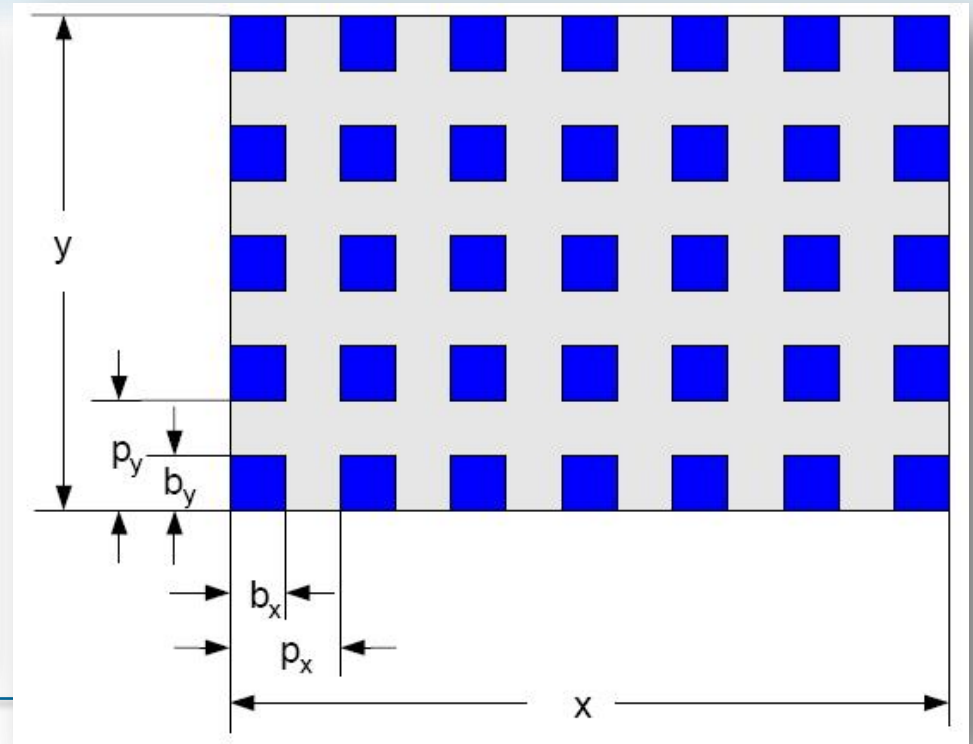
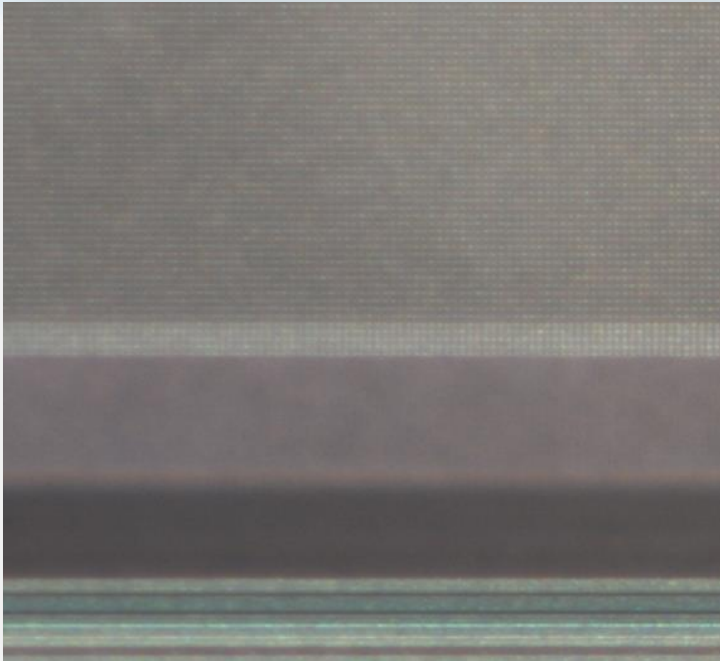


image resolution (e.g. ICX285):

axial

$$1 / (2p) = 1 / 13 \mu\text{m} = 76.9 \text{ [line pairs / mm]}$$

diagonal

$$1 / (\text{sqrt}(2) (2p)) = 1 / 18.38 \mu\text{m} = 54.4 \text{ [line pairs / mm]}$$

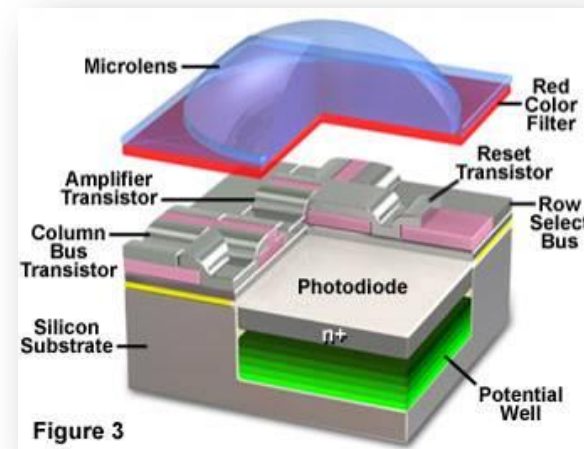
Some MTF Data

**Pixel Size Requirements for Maximum Resolution
in Optical Microscopy**

Objective (numerical aperture)	Resolution Limit (microns)	Projected Size on CCD (microns)	Required Pixel Size (microns)
4x (0.20)	1.5	5.8	2.9
10x (0.45)	0.64	6.4	3.2
20x (0.75)	0.39	7.7	3.9
40x (0.85)	0.34	13.6	6.8
40x (1.30)	0.22	8.9	4.5
60x (0.95)	0.31	18.3	9.2
60x (1.40)	0.21	12.4	6.2
100x (0.90)	0.32	32.0	16.0
100x (1.25)	0.23	23.0	11.5
100x (1.40)	0.21	21.0	10.5

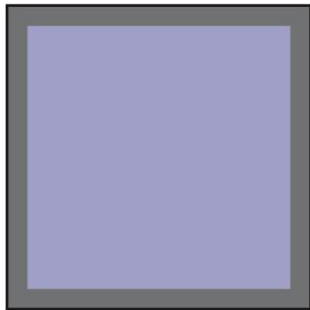
Pixel Size

- In most modern image sensors (except full frame or frame transfer CCDs) only a partial area of the total pixel area is light sensitive, the remaining area is occupied by circuitry (CMOS) or registers (interline transfer CCDs)
- The fill factor describes the ratio of light sensitive area to total area of the pixel ($< 100\%$).
- As common counter measure, microlenses are applied, such that the incident light is best possible focussed to the light sensitive area.

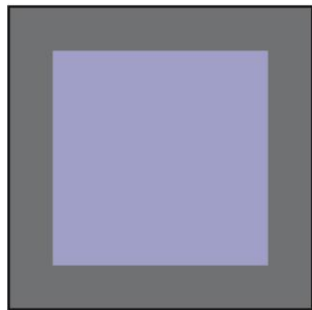
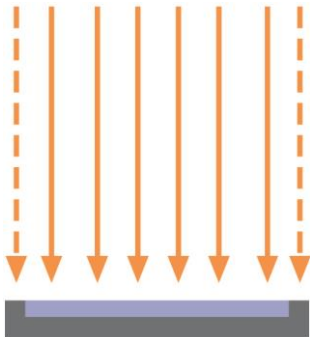


Fill Factor

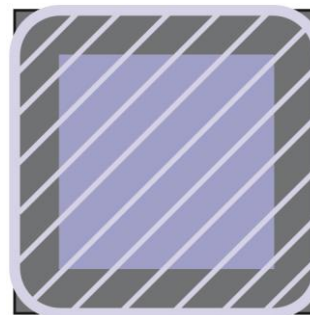
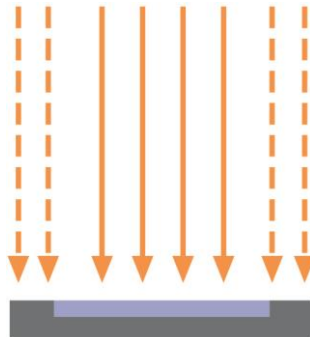
3 pixels with different fill factors and microlens



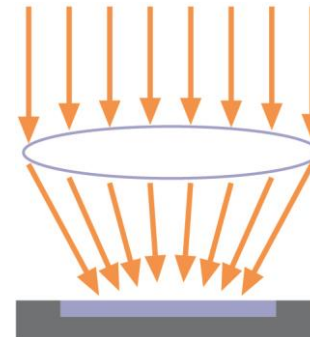
**fill factor
75%**



**fill factor
50%**

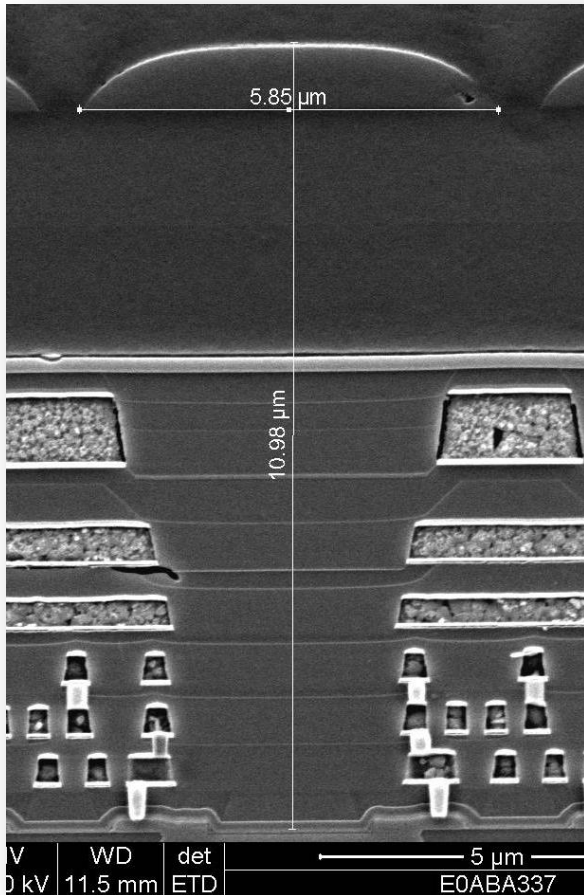


**fill factor
50% + μ lens**



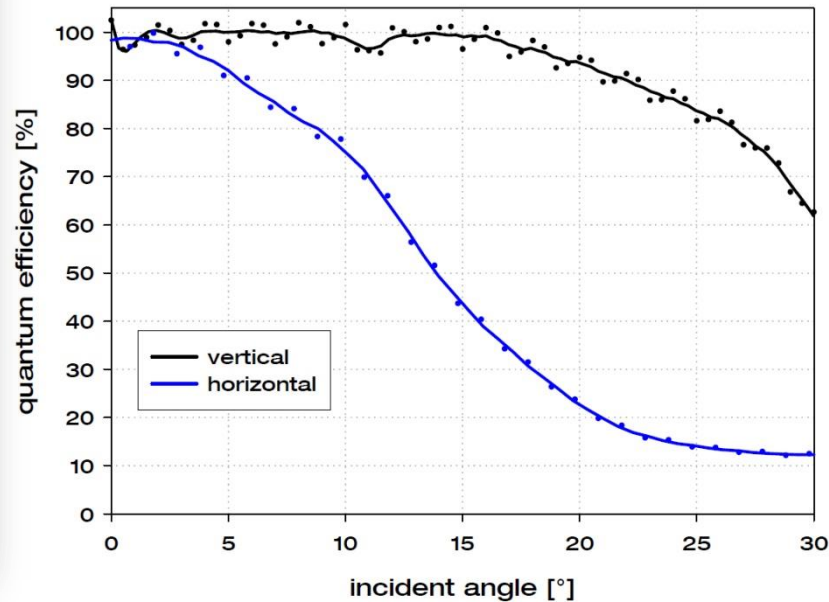
Microlenses on Pixel

To Improve the Fill Factor and therefore the effective QE



It should be noted that the micro lens always adds an angular dependence to the quantum efficiency.

QE curves of KAI-2001 monochrome angular dependence



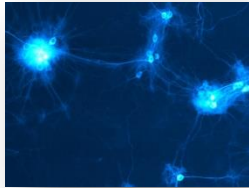
Resolution

A high resolution is useful for:



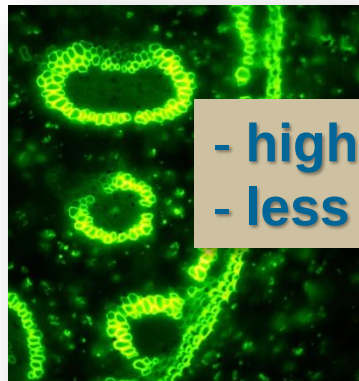
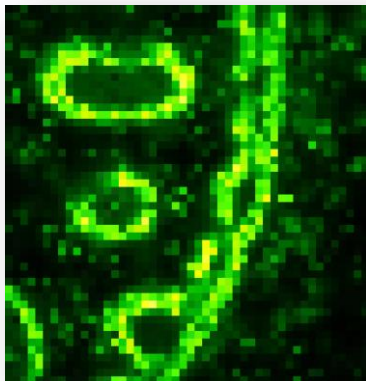
but...

a larger field of view



- higher data load
- same light / pixel

more details



- higher data load
- less light / pixel

Noise

Which camera shows more noise?

Image 1



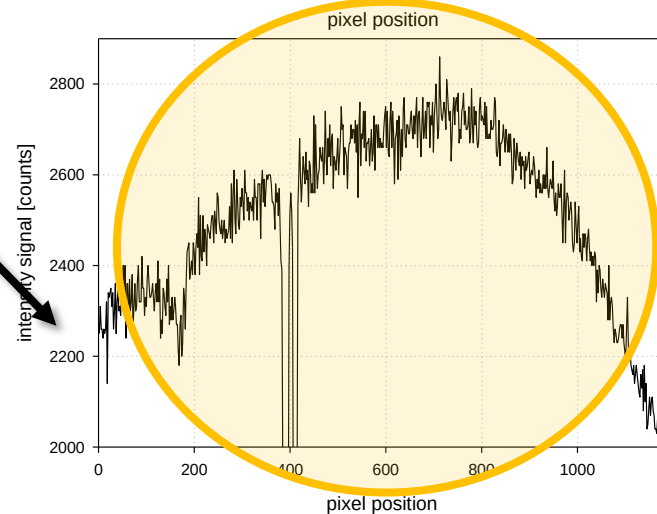
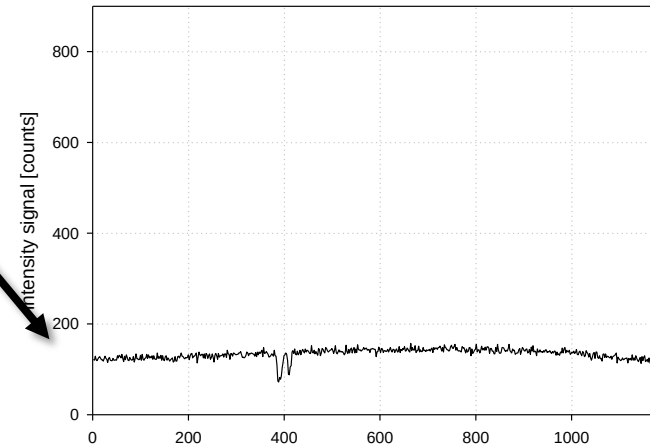
Image 2



Which camera shows more noise?



y=460

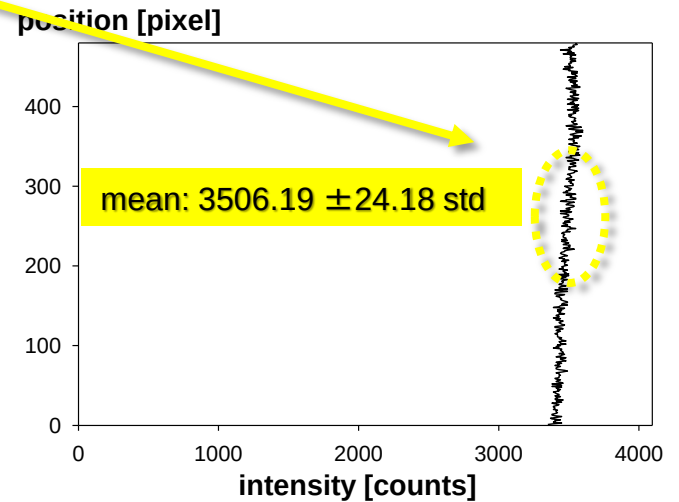
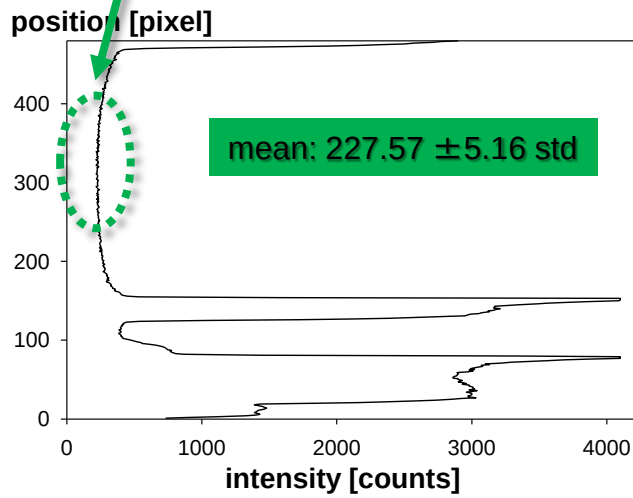
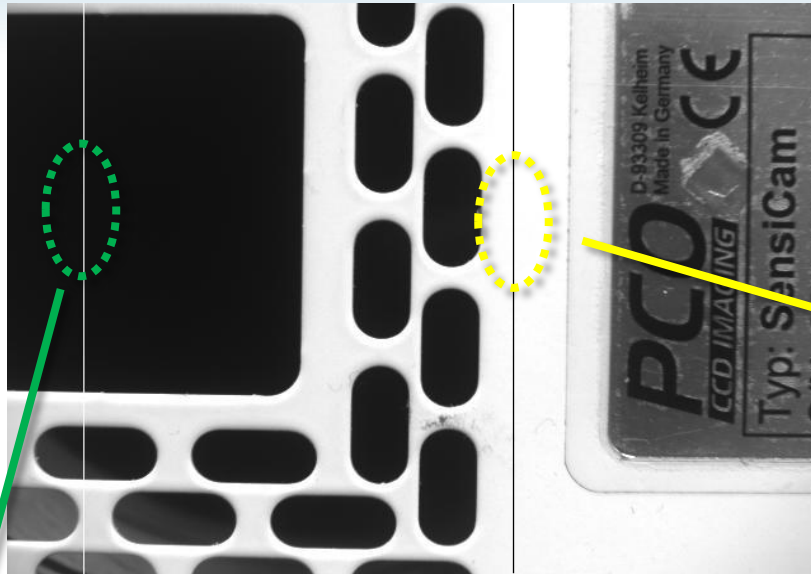


Which camera shows more noise?

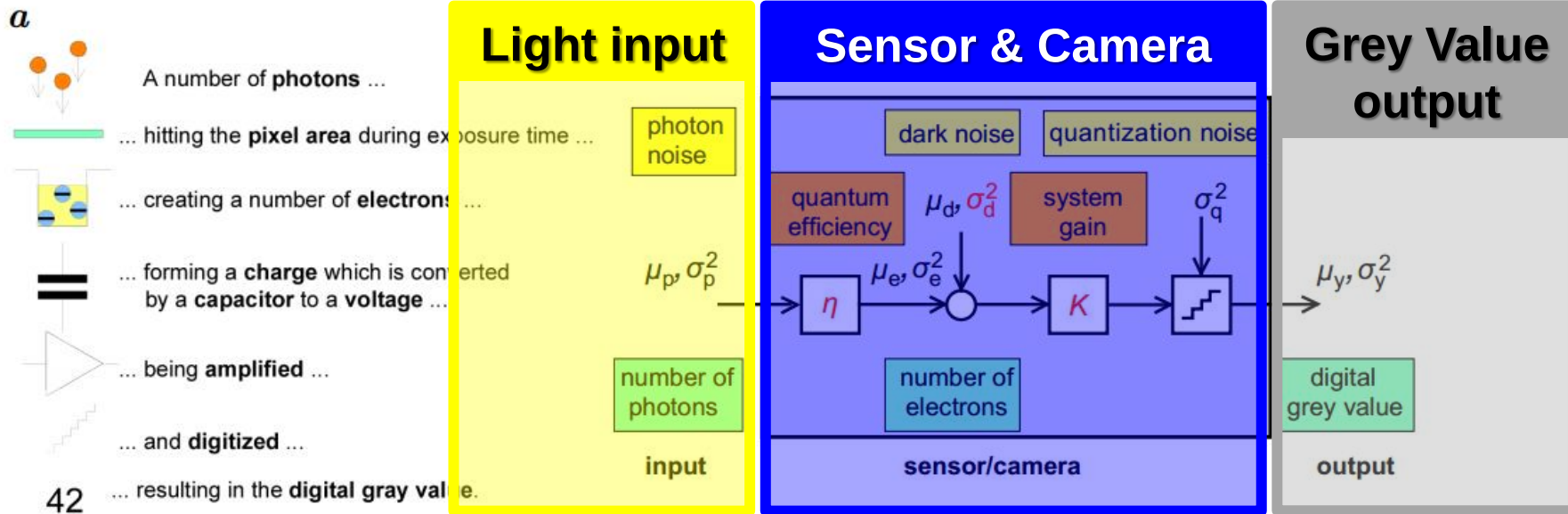
Answer:

None - image 1 was recorded at 1 ms exposure time at an aperture of $f = 11$ and image 2 was recorded at 15 ms exposure time at an aperture of $f = 5.6$ with the same camera, the same lens and the same illumination. The difference was the amount of light falling onto the same interline transfer CCD image sensor. With other words, Image 1 has less total noise but also less signal-to-noise-ratio.

Photon or Shot Noise



Noise – EMVA 1288 Camera Model



Readout Noise

EMVA1288, linear camera model

6.3 Evaluation of the Measurements according to the Photon Transfer Method

As described in section Section 2, the application of the photo transfer method and the computation of the quantum efficiency requires the measurement of the mean gray values and the temporal variance of the gray together with the irradiance per pixel in units photons/pixel. The mean and variance are computed in the following way:

Mean gray value. The mean of the gray values μ_y over *all* N pixels in the active area at each irradiation level is computed from the two captured images y^A and y^B as

$$\mu_y = \frac{1}{2N} \sum (y_{ij}^A + y_{ij}^B) \quad (28)$$

averaging over all rows

readout noise

mean gray value of dark

images, $\mu_{y,\text{dark}}$, is computed.

Temporal variance of gray value. Normally, the computation of the temporal variance would require the capture of many images. However on the assumptions put forward in Section 1, the noise is stationary and homogenous, so that it is sufficient to take the mean of the squared difference of the two images

$$\sigma_y^2 = \frac{1}{2N} \sum_{i,j} (y_{ij}^A - y_{ij}^B)^2. \quad (29)$$

Because the variance of the difference of two values is the sum of the variances of the two values, the variance computed in this way must be divided by two as indicated in Eq. (29).

The estimation of derived quantities according to the photon transfer method is performed as follows:

Responsivity $R = K\eta$. According to Eq. (6), the slope of the relation $\mu_y(\mu_p)$ gives the

system gain = 1 / conversion factor

Overall system gain K . According to Eq. (9), the slope of the relation $\sigma_y^2(\mu_y - \mu_{y,\text{dark}})$ gives the absolute gain factor K . Select a contiguous range of measurements where this relation show a sufficiently linear correspondence. It should be the same range as for the estimation of the responsivity (see above).

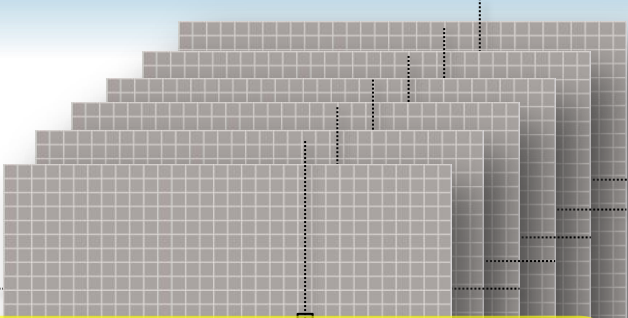
Compute a least-squares linear regression of σ_y^2 versus $\mu_y - \mu_{y,\text{dark}}$ over the selected range and specify the gain factor K and its statistical error.

The stationary and homogenous noise distribution works well for CCD image sensors. BUT!

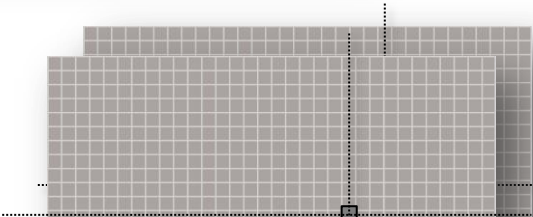
Here is as well a linear relationship assumed, therefore a linear regression is used. BUT!

Readout Noise

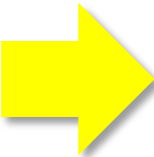
Temporal noise



First, a large sequence of dark images is measured and for each pixel the mean value and the variance are determined.



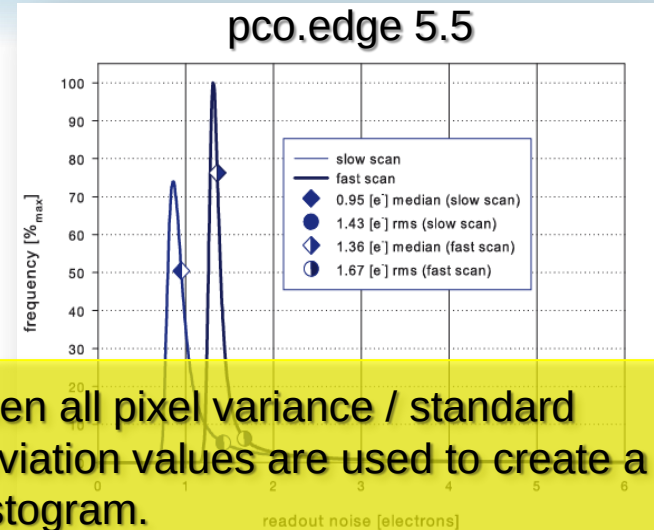
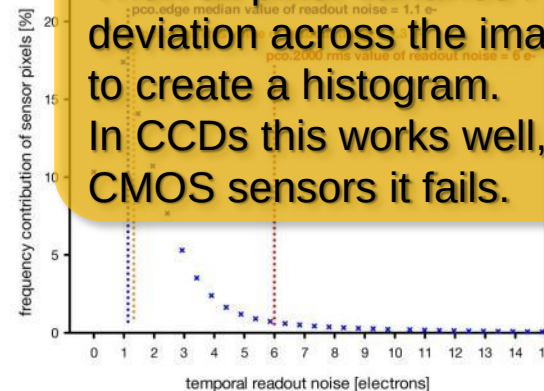
EMVA1288: 2 dark images are measured and for each pixel the mean value and the variance are determined.



Then all pixel variance / standard deviation values are used to create a histogram.

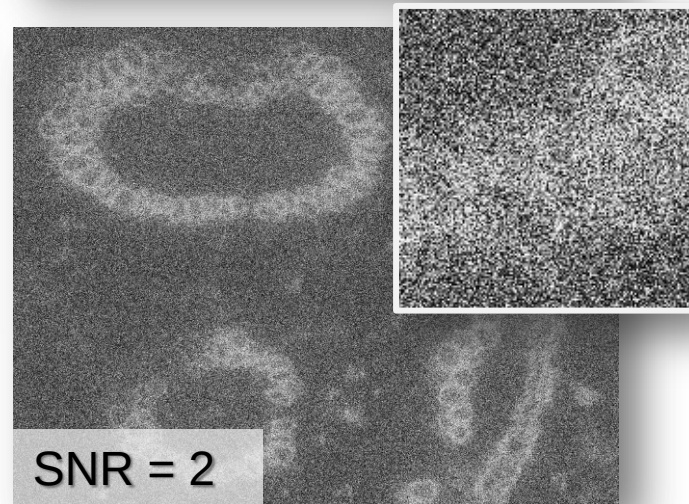
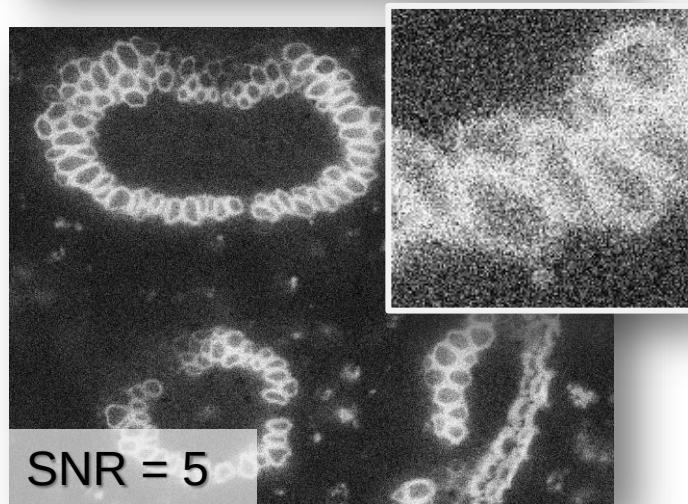
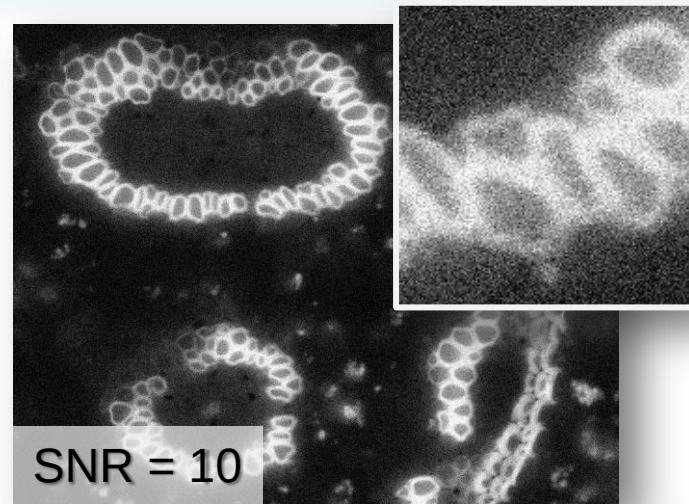
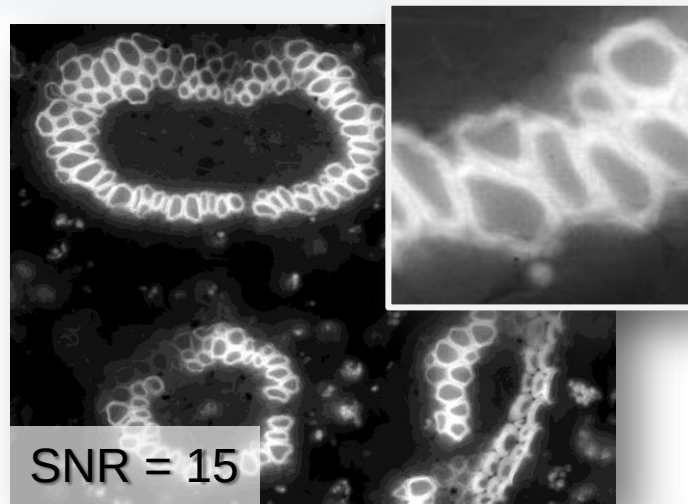


Then all pixel variance / standard deviation across the image are used to create a histogram. In CCDs this works well, but in many CMOS sensors it fails.

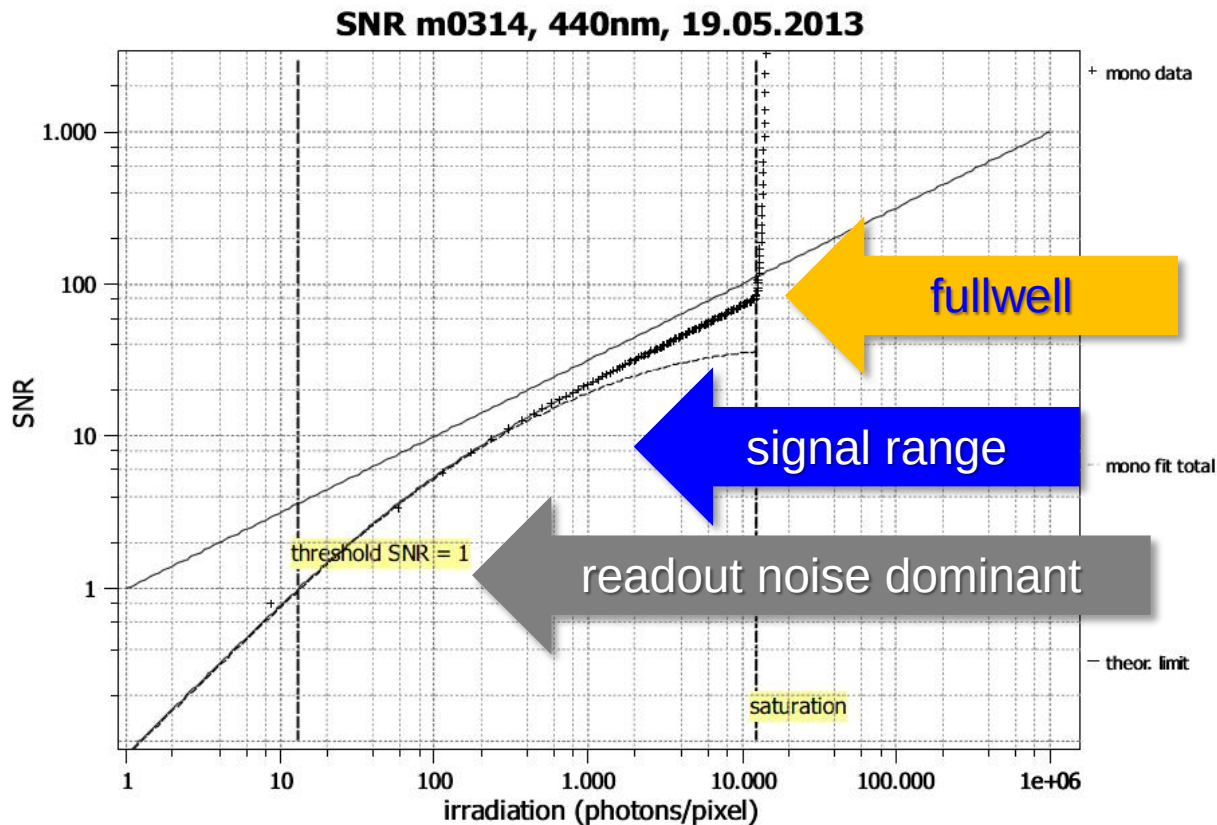


Signal-to-Noise- Ratio

Signal-to-Noise-Ratio (SNR)



Signal-to-Noise-Ratio (SNR)



EMVA1288 measurement

Quantum Efficiency

Quantum Efficiency (QE)

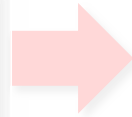
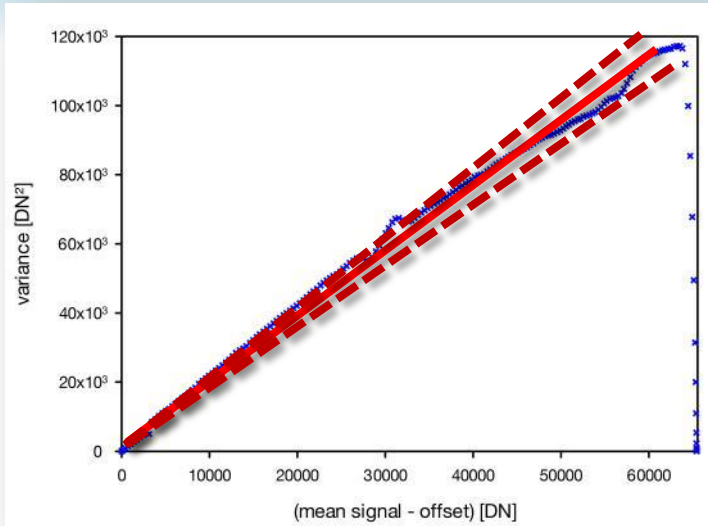
The quantum efficiency of a photo detector is defined as:

$$QE = \frac{\text{generated charge carriers, electrons}}{\text{incident photons}}$$

It is usually given in [%], and best possible values are above 90 %. For QE measurements of image sensors or cameras the impacts of fill factor, microlenses, reflection and transmission losses at glass surfaces are included and an effective quantum efficiency is measured, but it is often still QE called.

How QE is measured...

The photon transfer curve way

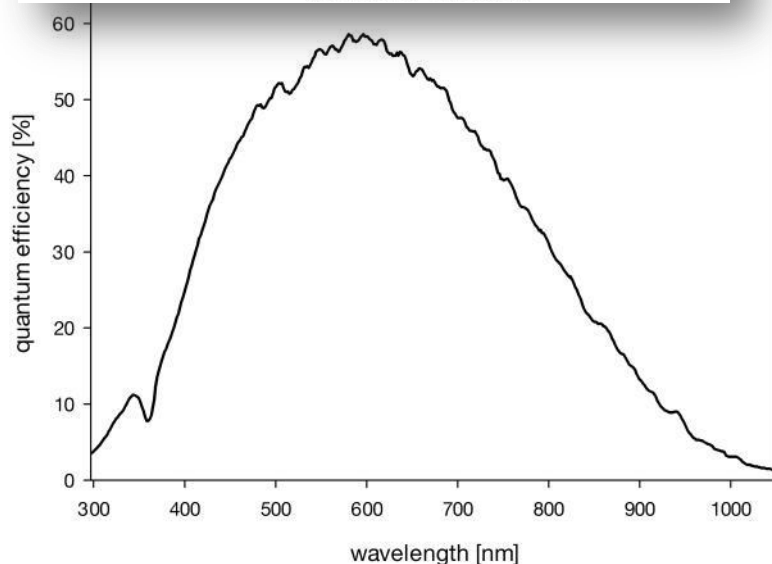


slope = system gain
(conversion factor)

If the slope changes due to variability in the noise behavior the QE results will change accordingly!?



with a calibrated light detector comparison, measured digital values can then be back-calculated into photons from counts => QE



How QE is measured...

The Fe⁵⁵ way

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CHAPTER 2

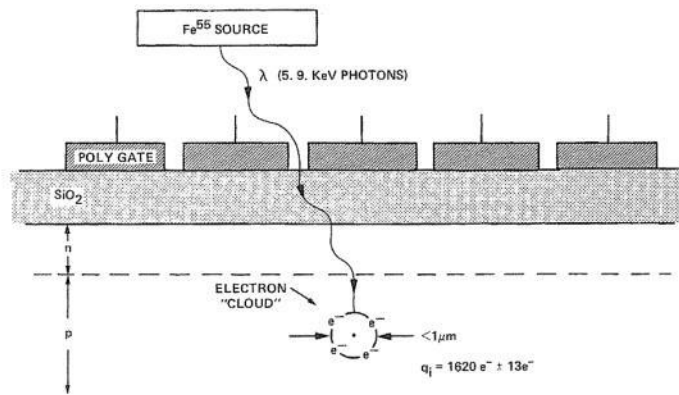
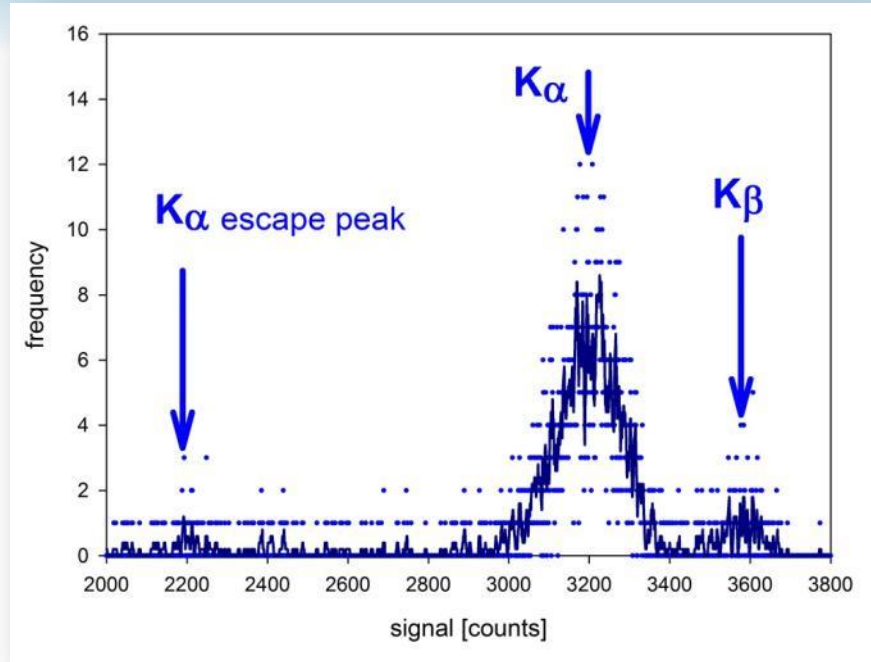


Figure 2.17 Illustration showing an Fe⁵⁵ x-ray event interacting with a CCD.

The Fe⁵⁵ soft x-ray source produces five different signals in silicon image sensors:
K _{α} (1620 e-), K _{β} (1778 e-), K _{α} escape peak (1133 e-), K _{β} escape peak (1291 e-) and a silicon line (487 e-).



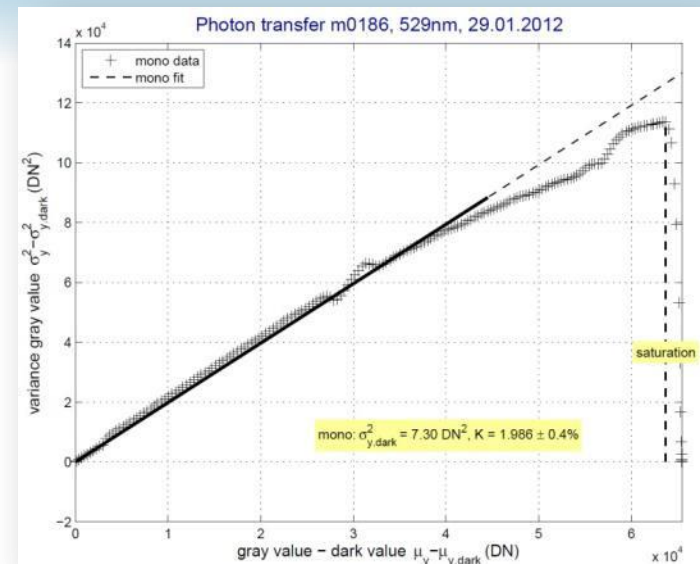
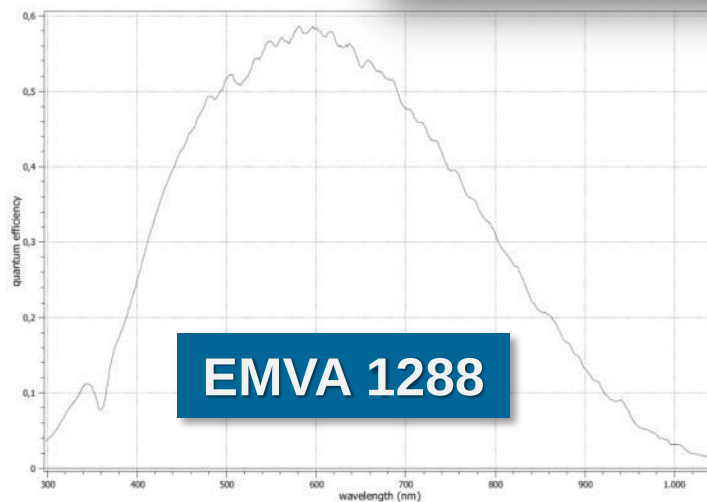
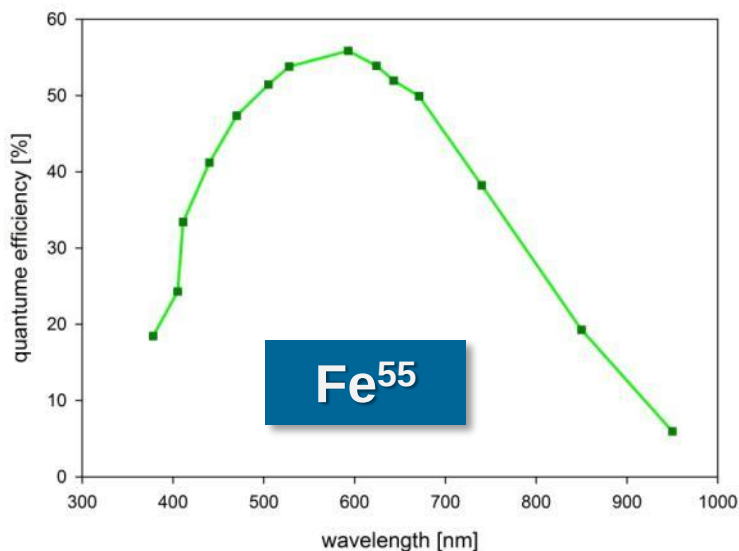
	K _{α} esc	K _{α}	K _{β}
signal [count]	2180	3208	3560
conv. factor [e-/count]	0.519	0.505	0.499

QE measured completed

Fortunately in this case the EMVA1288 determination of the system gain or conversion factor delivers the same results like the Fe⁵⁵ determination:

conv. factor_{EMVA1288} = 0.503 [e-/count]

conv. factor_{Fe55} = 0.505 [e-/count]



QE Improved Micro Lens

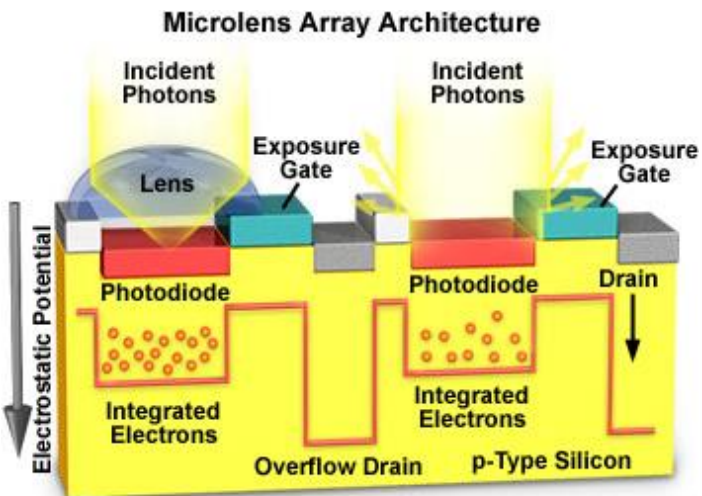


Figure 2

CFA & MLA for fine pitch pixels – 1.1um -
Lens shape and form factor programmable
Using grey-scale masks and proprietary process

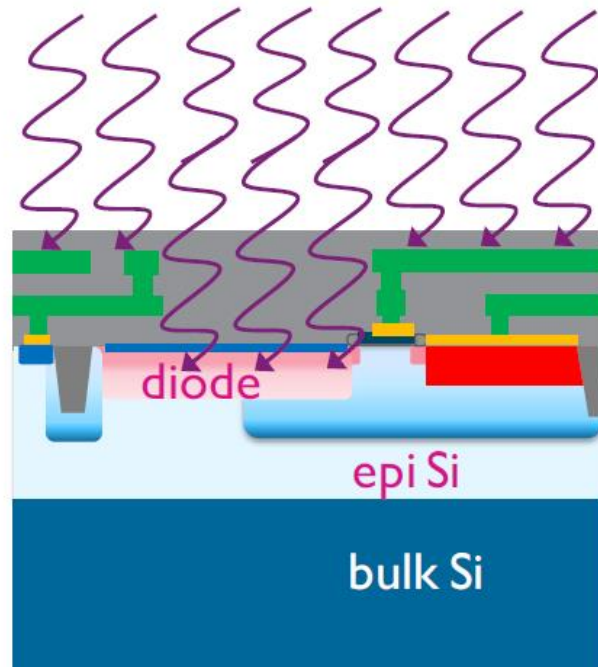
20120629 update

Lens Type	Double-Lens	New-EBL	Conventional-EBL
SEM			
cross-sectional shape (image)			
Cell size	1.4~4.0um	0.9~4.0um	> 2.2um
Lens gap (diagonal)	0.2~0.35um	0.1~0.35um	0.6~1.0um
Lens shape	spherical lens	spherical lens	aspherical lens
area ratio	≥ 95%	≥ 95%	≥ 95%
spectral sensitivity ratio (B/G, R/G)	▲	○	○

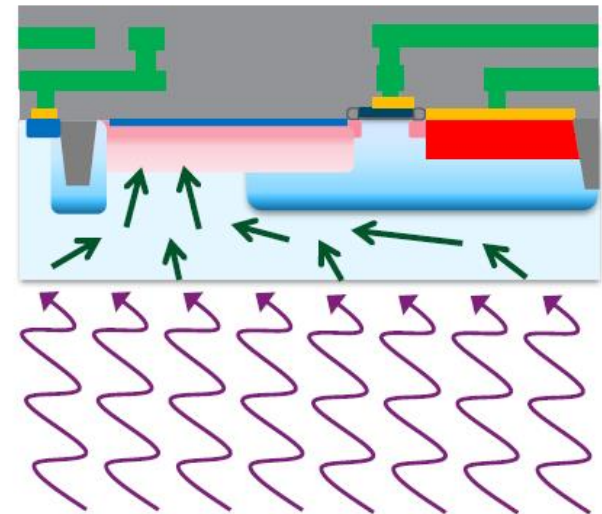
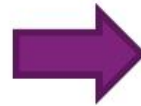
Nowadays even QE values of 70 % and higher are achieved due to optimization of microlenses and optical stack.

QE Improved

Front Illuminated & Back Illuminated Image Sensors



frontside illuminated



backside illuminated

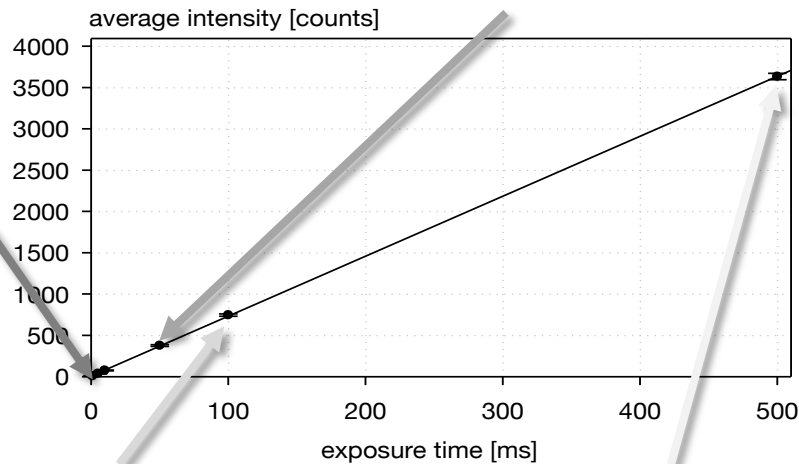
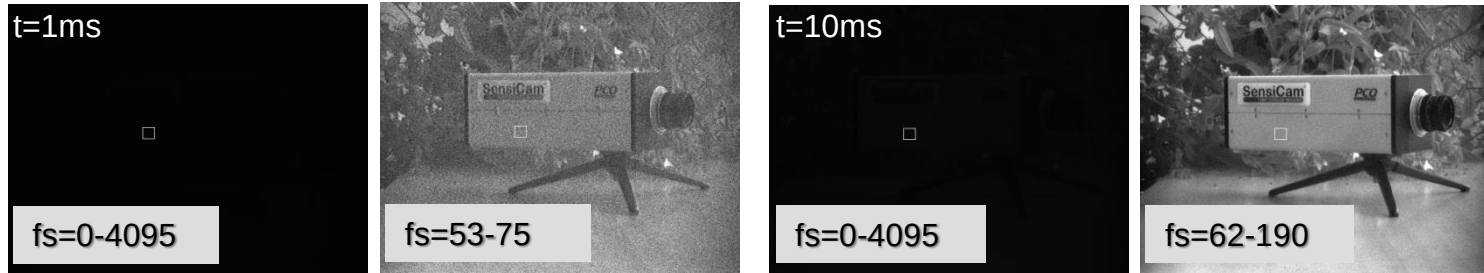
Linearity

Linearity

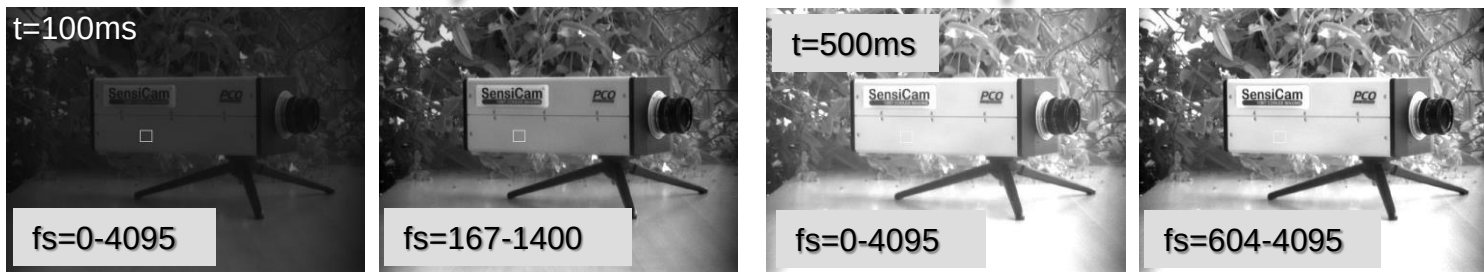
- In principle it should be expected, that the gray level in the image doubles if the light input has been doubled, because that is a natural experience. Unfortunately CMOS image sensor exhibit a non-linear relationship.
- For structure recognition in the microscope images, like cell counts or connectivity of cells etc. it is not relevant, but for ratiometric evaluation (like Ca^{+} measurements) it is.
- Therefore for some applications the linearity of a camera can be very important.

Linearity

CCD image sensor example



$$\begin{aligned}\text{intensity} &= m \cdot t + n \\ m &= 7.26 \\ n &= 6.77 \\ r^2 &= 1.000\end{aligned}$$



Dark Current & Cooling

Dark Current

- The dark current are thermally induced electrons. This dark signal of an image sensor increases with the exposure time.
- Further the dark current roughly increases exponentially with the temperature.
- Due to structure and process parameters the dark current of CCD image sensors is always a lot smaller compared to CMOS image sensors, but CMOS image sensors are continuously improved. Therefore modern CMOS image sensors, like sCMOS, reach good dark current levels.

Cooling Is Good For ...?

Temperature Control

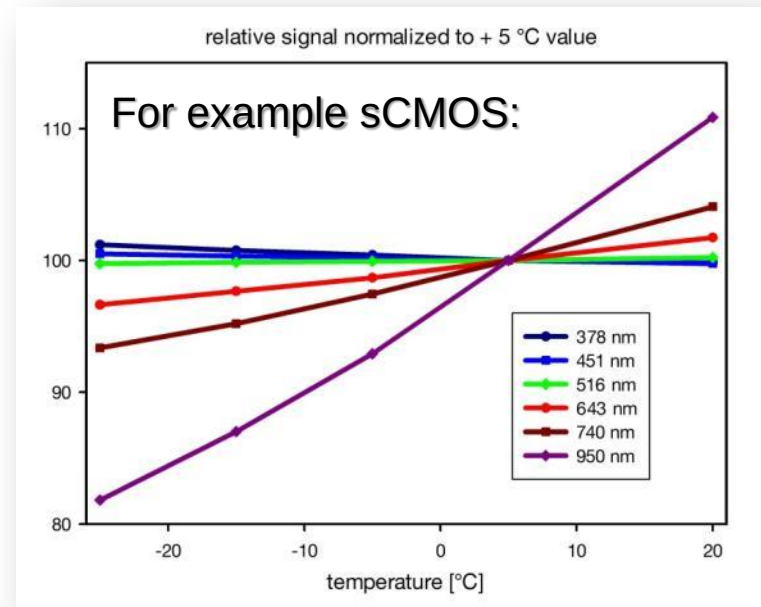
Independent of absolute value, but very important to keep the offset constant and prevent drift phenomena, when the camera records images

Reduction of dark current noise contribution in image sensors

Relevant only for long exposures (> 1 s exp. time), since the dark current is proportional to exposure time

But, cooling changes the spectral sensitivity...

Cooling changes the mobility of the charge carriers in silicone, therefore it has an influence on the quantum efficiency



Cooling

- In most cooled cameras the cooling of the image sensors is done thermoelectrically by Peltier-coolers. The waste heat of the cooler is either dissipated by a fan or by a cooling liquid.
- Deep cooling requires special constructive efforts to prevent thermal feedback, therefore the image sensors are either gas- or vacuum tight packed, and to reach the deepest temperatures always the waste heat of the cooler is dissipated by a cooling liquid.
- If not actively cooled the cameras should at minimum apply a software compensation of the temperature change of the image sensor.

More efforts in manufacturing usually mean, you have to pay more!

Dynamic

Pixel Dynamic

The pixel dynamic is given by the signal-to-noise-ratio in the pixel. Once the signal is a lot larger than the readout noise, the readout noise can be neglected, and the SNR is determined by the ratio of the number of photons divided by the photon noise (square root (number of photons)). Obviously the maximum value is limited by the fullwell capacity of the image sensor.

$$\text{pixel dynamic} = \frac{\text{number of electrons [e-]}}{\text{sqrt}(\text{number of electrons [e-]})}$$

If for example the sCMOS sensor with a fullwell capacity = 30 000 [e-] is taken, it means its maximum pixel dynamic amounts to 1 : 173, which is a lot smaller than the intra-scene dynamic of 1 : 22 000.

Intra-Scene Dynamic

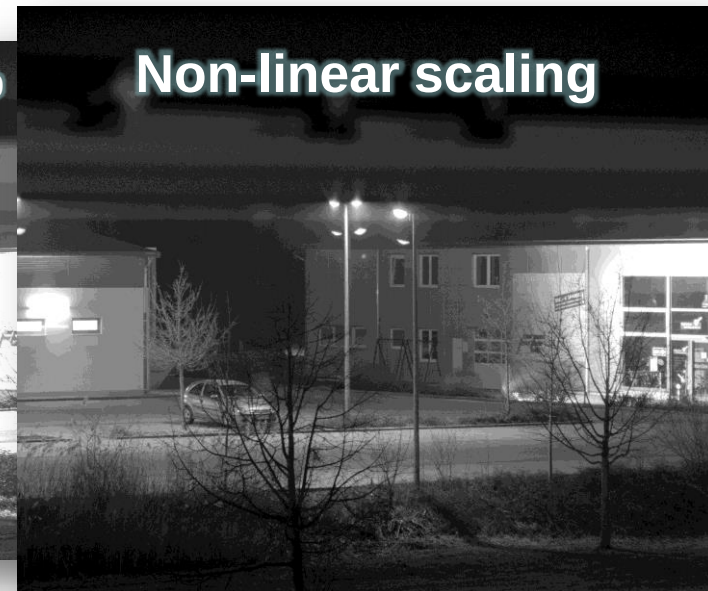
The intra-scene dynamic is usually the parameter, which is given in data sheets of image sensors or cameras as dynamic. It describes the ratio of the maximum possible signal (saturation/fullwell capacity) divided by the smallest discriminable signal (readout noise).

$$\text{intra-scene dynamic} = \frac{\text{saturation / fullwell capacity [e-]}}{\text{readout noise [e-]}}$$

If a sensor with large fullwell and noise is compared to a sensor with small fullwell and noise but same dynamic range, it is important to know whether the application is at low or bright light conditions.

Intra-Scene Dynamic

- **A high intra-scene dynamic can result in a larger information content of the image. This can be used to:**
 - get enough gray levels to analyze structures in the low and bright light parts of the image
 - to process non-linearly the image such that shadows and lights show the required structures for a beautiful image
 - to process afterwards and decide which range of levels should be used



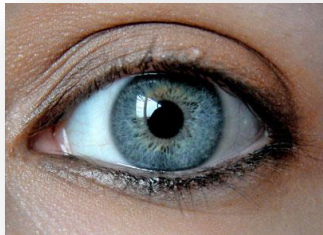
Dynamic Ranges of Image Sensors

sensor	resolution [pixel]	pixel size [μm x μm]	readout noise [e-] (rms)	fullwell capacity [e-]	dynamic
IA-DJ-02084 CCD	2048 x 2048	24 x 24	17	170 000	1 : 10000
Lupa 4000 CMOS	2048 x 2048	12 x 12	40	80 000	1 : 2000
MT9M413 CMOS	1280 x 1024	12 x 12	70	63 000	1 : 900
TC253 CCD	680 x 500	7.4 x 7.4	42	26 000	1 : 619
KAI-4020 CCD	2048 x 2048	7.4 x 7.4	12 / 25	40 000	1 : 3333 / 1600
sCMOS CIS2051	2560 x 2160	6.5 x 6.5	1.4	30 000	1 : 21429
ICX285AL (IT CCD)	1392 x 1040	6.45 x 6.45	7	18 000	1 : 2571
CMV2000 CMOS	2048 x 1088	5.5 x 5.5	13	13 500	1 : 1038
ICX267AL CCD	1434 x 1050	4.65 x 4.65	6	15 000	1 : 2500
MT9P013 CMOS	2592 x 1944	1.75 x 1.75	-	-	1 : 2238
OV14810 CCD	4416 x 3312	1.4 x 1.4	-	-	1 : 3162
MT9E013 CCD	3264 x 2448	1.4 x 1.4	-	-	1 : 1000

Dynamic Range

We are living visually mainly in a 8 bit world

human eye



printer



PC screen



TV screen

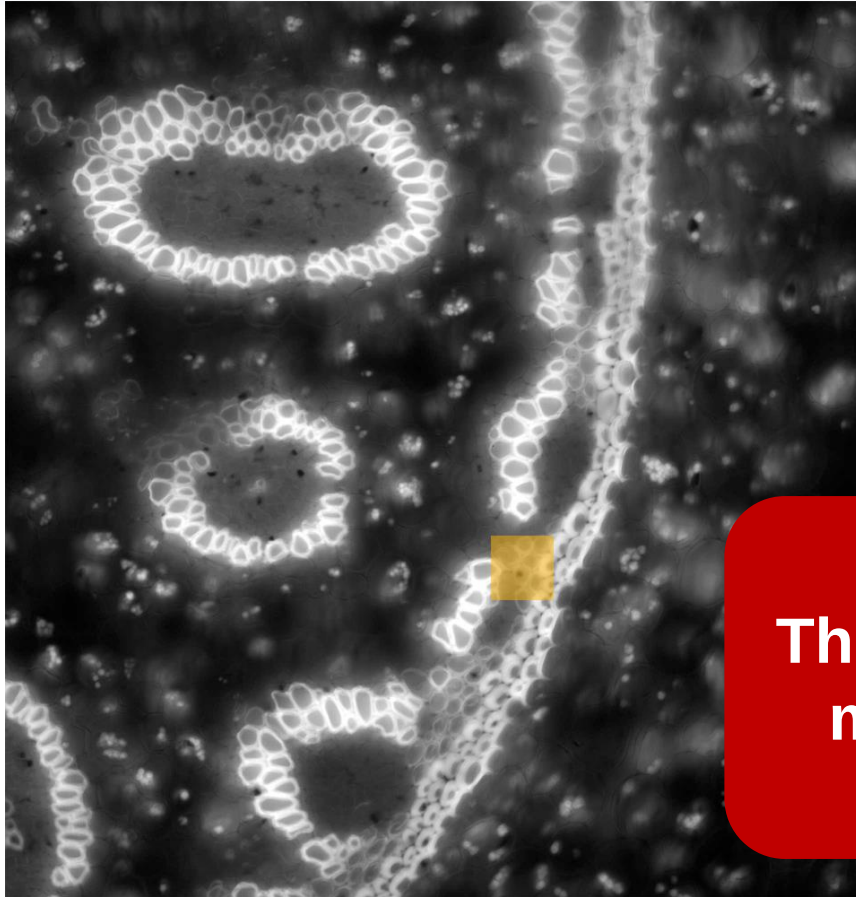


So, what's the point in using high dynamics?

exception: radiologists say, that they are able to distinguish up to 10 bit gray levels



Dynamic Range

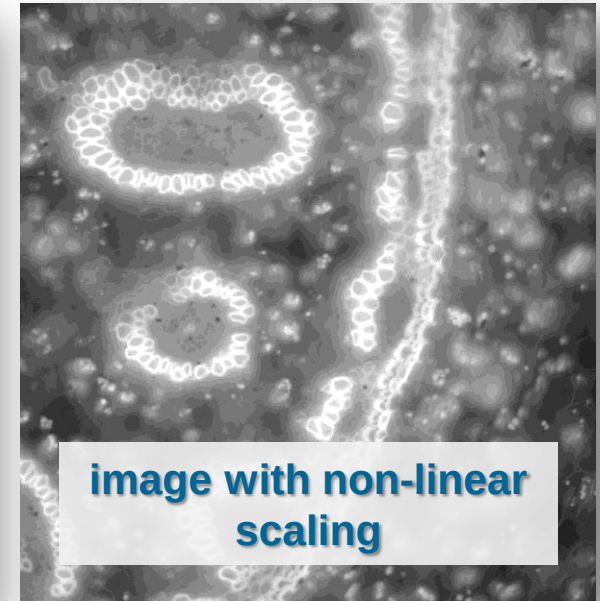
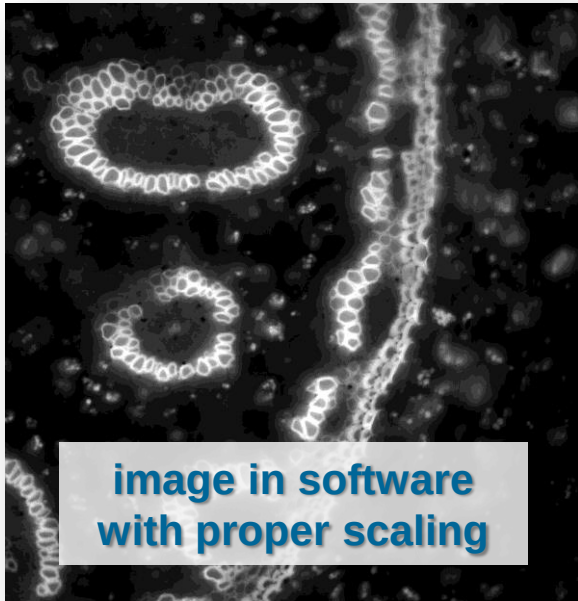


Therefore the point is
more information!

Scaling: min - max

Dynamic Range

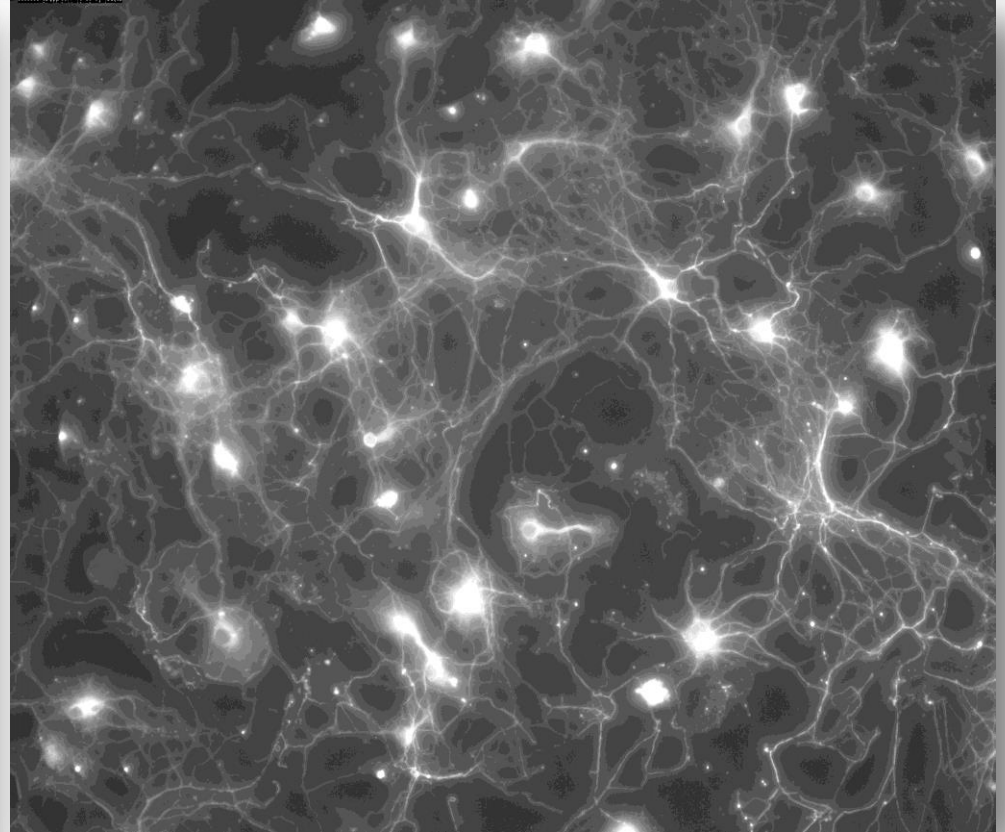
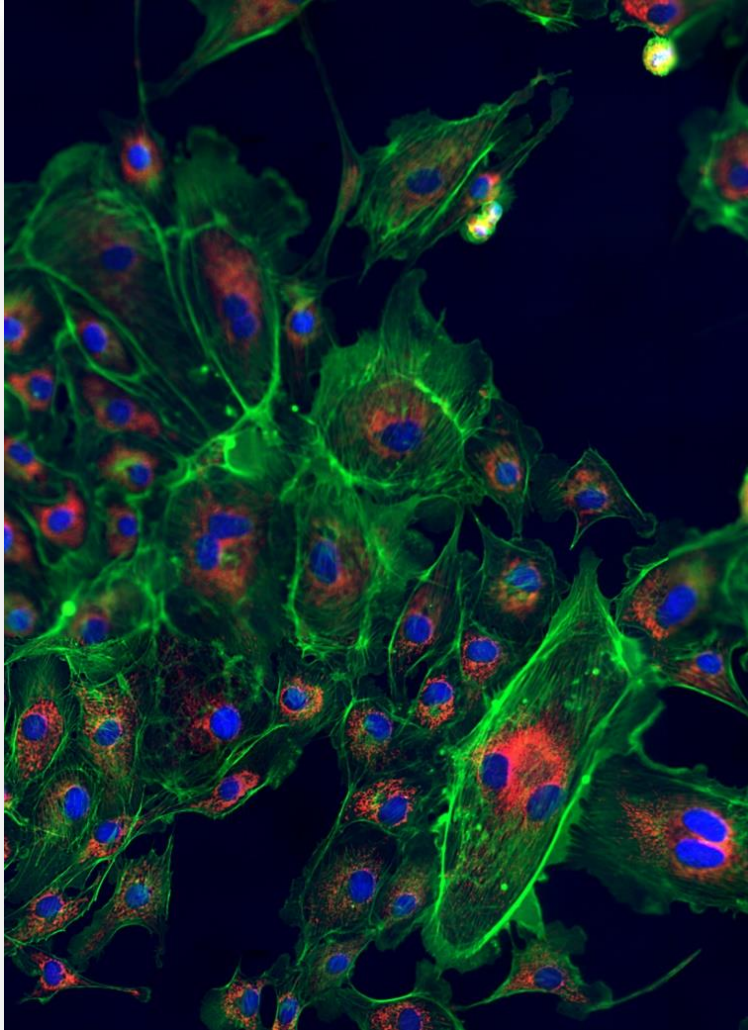
Be aware, that it might be not so easy to look at high dynamic images...



Frame Rate

Frame Rates

Single Images



Frame Rates

Time Lapse Series



Frame Rates

Slow Motion



Frame Rates of Image Sensors

Name	Type	Resolution [pixel]	Max. Full Frame Rate [frames/s]	Transfer Data Rate [Mbyte/s]
ICX285AL	CCD (IT)	1392 x 1040	16 (12 bit)	46.3 (17.4)
CCD87	emCCD (FT)	512 x 512	34 (12 bit)	17.8 (6.7)
CCD201-20	emCCD (FT)	1024 x 1024	9 (12 bit)	18.9 (7.1)
KAI-4022	CCD (IT)	2048 x 2048	14.7 (14 bit)	123.3 (53.9)
CMV4000	CMOS	2048 x 2048	180 (10 bit)	1509.9 (471.9)
VITA 5000	CMOS	2592 x 2048	75 (12 bit)	796.3 (298.6)
CIS2521	sCMOS	2560 x 2160	100 (16 bit)	1105.9
CIS2020	sCMOS	2048 x 2048	100 (16 bit)	838.9
MT9 M413	CMOS	1280 x 1024	636 (12 bit)	1667.2 (625.2)
ON-Semi	CMOS	2016 x 2016	1279 (12 bit)	10396.1 (3898.6)

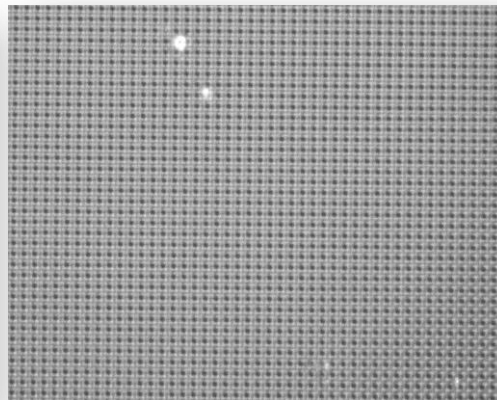
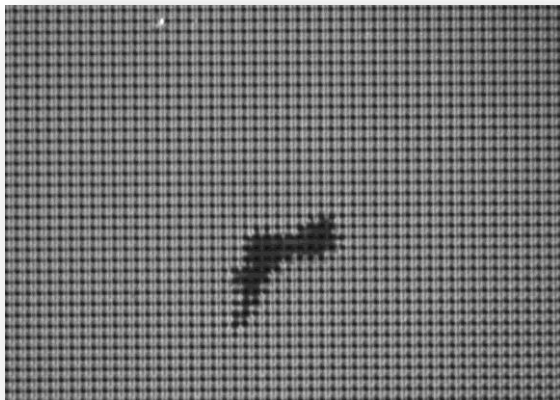
Frames (dynamic)

TIFF (data)

Raw & not so Raw Data

Pre-Processing – Why?

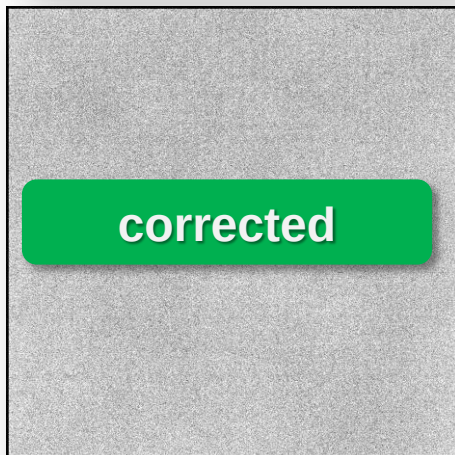
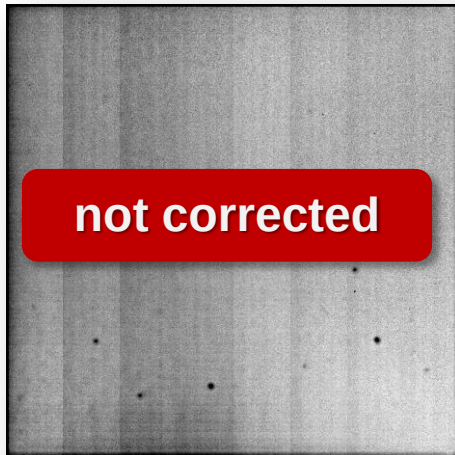
- A flawless image sensor for a reasonable price does not exist. For most image sensors there are acceptance criteria, which allow a certain amount of blemishes (dead pixel, cluster etc.).
- In contrary to CCD image sensors, the CMOS image sensors always have a non linear relationship between light input signal and digital output.
- Therefore in most of the relevant cameras for microscopy, the „raw image“ is pre-processed to deliver linear images without visible blemishes.



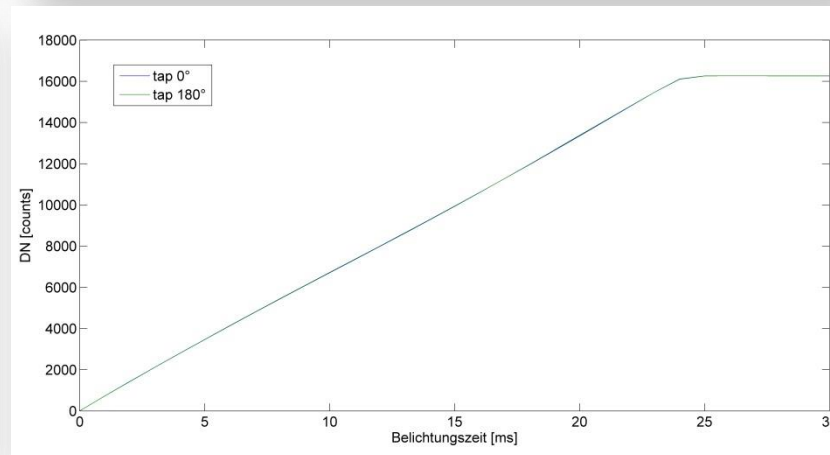
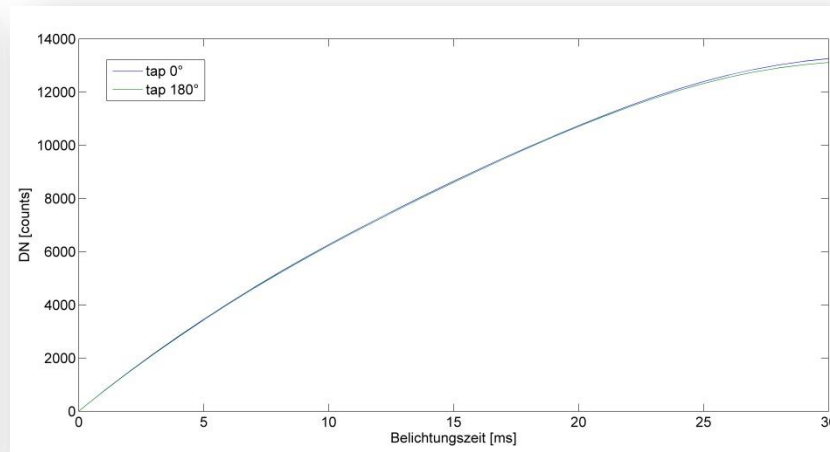
Pixel Correction

Fixed Pattern Correction (DSNU & PRNU) & Linearization

Homogeneity

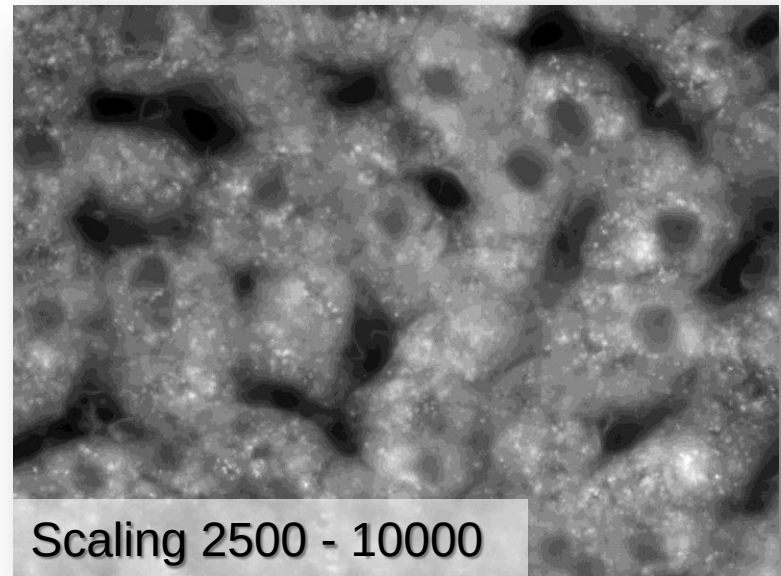
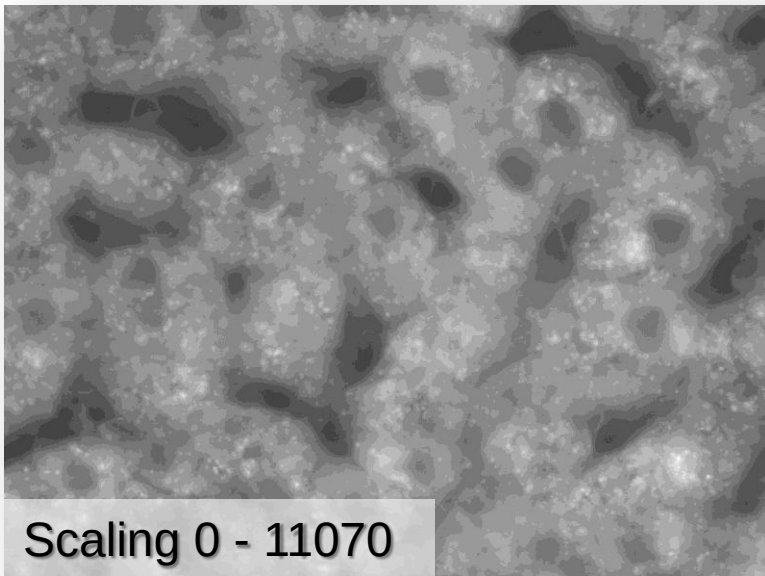


Linearity



Offset

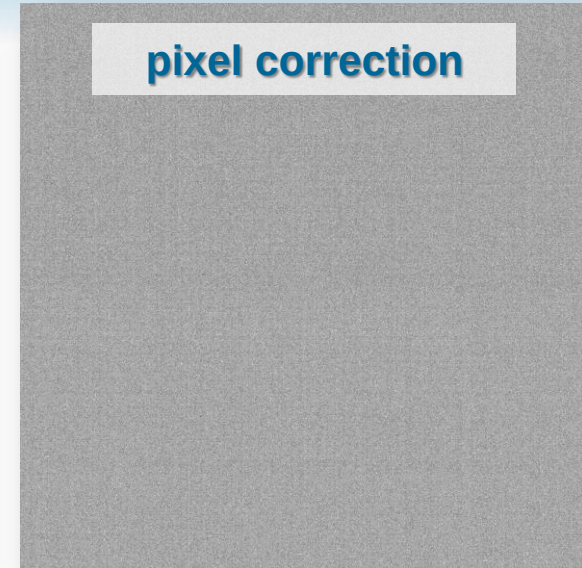
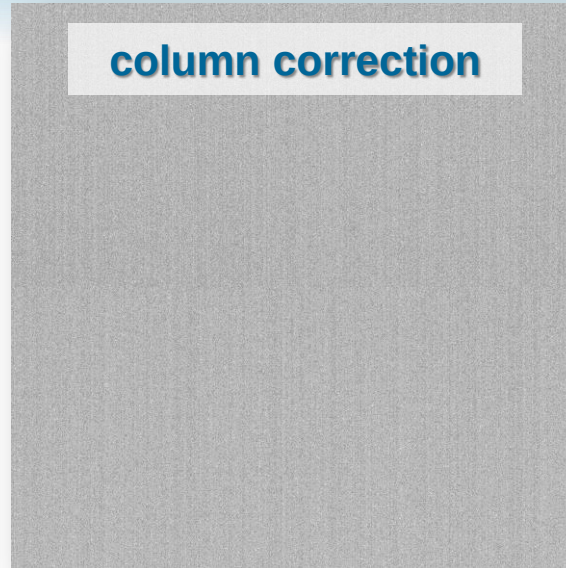
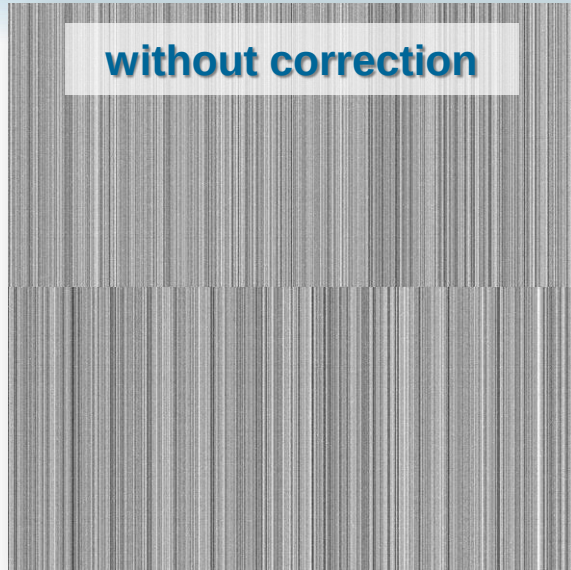
The offset is a constant small signal, which is added to the light induced signal to allow the access of the complete readout noise.



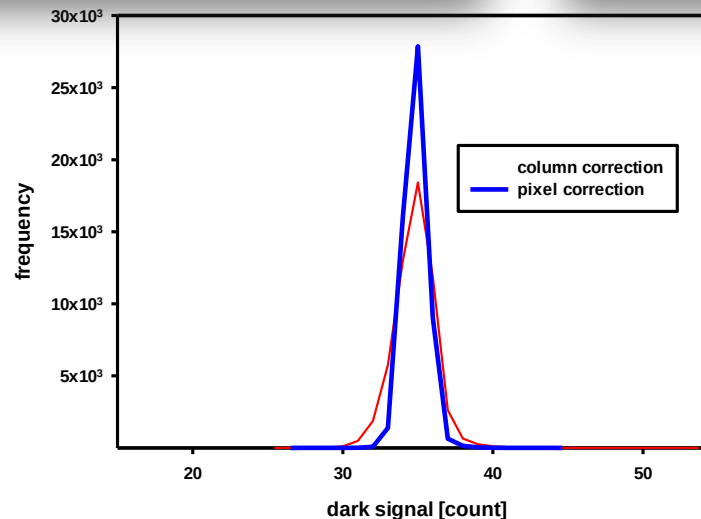
If the offset is suppressed (for a nicer image), an important piece of information is missing, if the images should be quantitatively evaluated for example in ratio measurements. Even the positioning of the dark noise level to the zero line, halves the noise contribution and should be avoided.

Fixed Pattern Noise (Spatial Noise)

dark signal



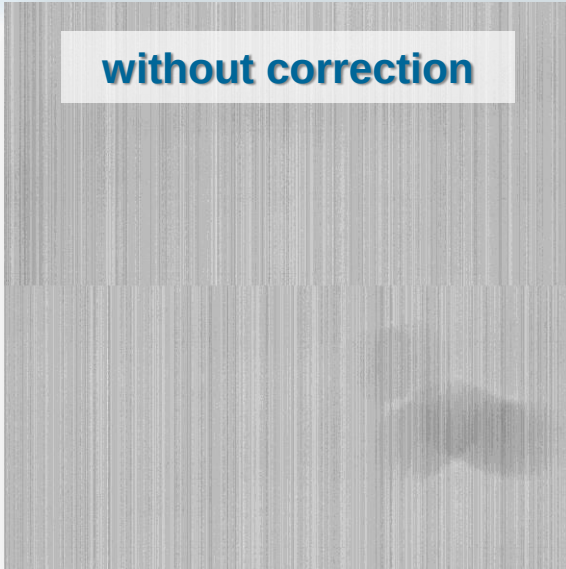
dark signal non-uniformity (DSNU) of a sCMOS sensor
256 dark images averaged
and a central 1k x 1k part
of the total image
extracted...



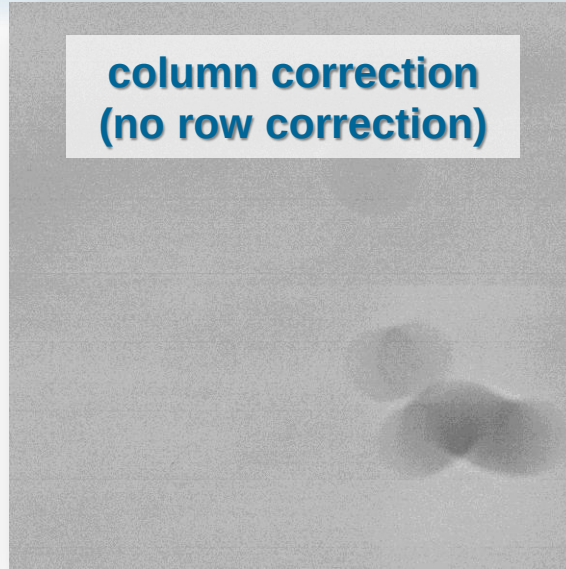
Fixed Pattern Noise (Spatial Noise)

bright signal

without correction



**column correction
(no row correction)**



pixel correction

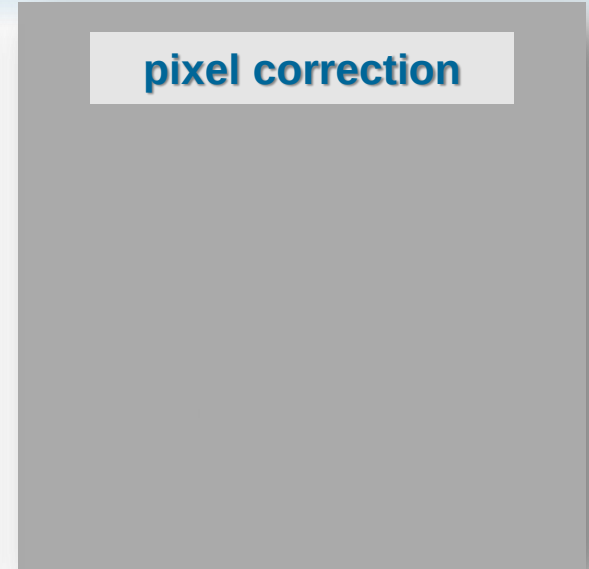
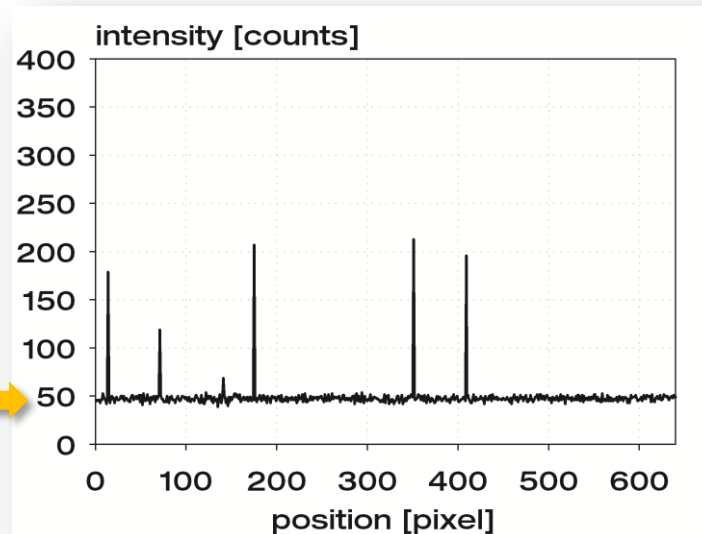


photo response non-uniformity (PRNU)
of a sCMOS sensor
256 dark images (mean = 33 000 counts,
scaling 29 000 – 37 000 counts) averaged
and a central 1k x 1k part of the total
image extracted...

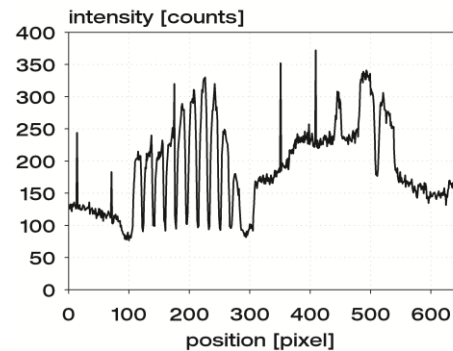
Hot Pixel, Warm Pixel, Blinker

- The term „hot pixel“ usually describes pixel, which are always „white“ or „black“, which means they don't respond to light, they are damaged.
- „Warm pixel“ always show a significantly higher signal than the normal pixel, meaning they always appear to be brighter than the neighbourhood.
- Blinkers are pixel, which are not permanently „hot“ or „warm“, but most of the time.
- All of these can be considered as locally fixed defects or blemishes.

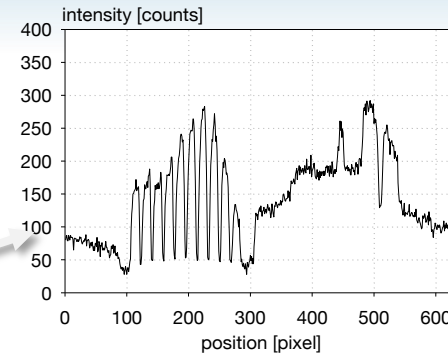
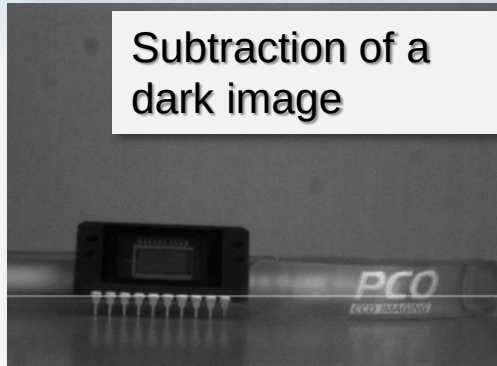


...How to Remove Warm & Hot Pixel

Before...

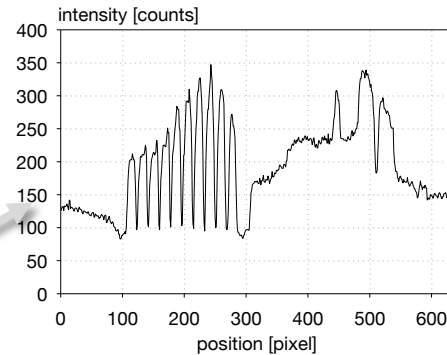
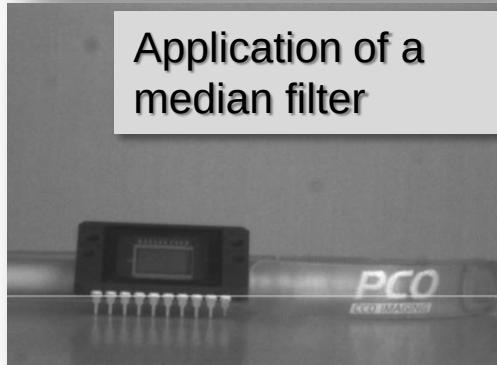


Subtraction of a dark image

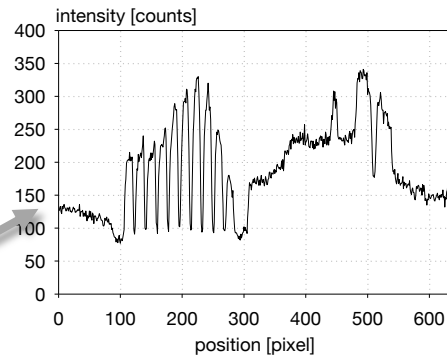
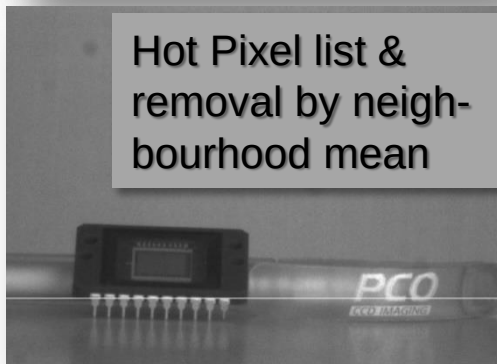


After...

Application of a median filter

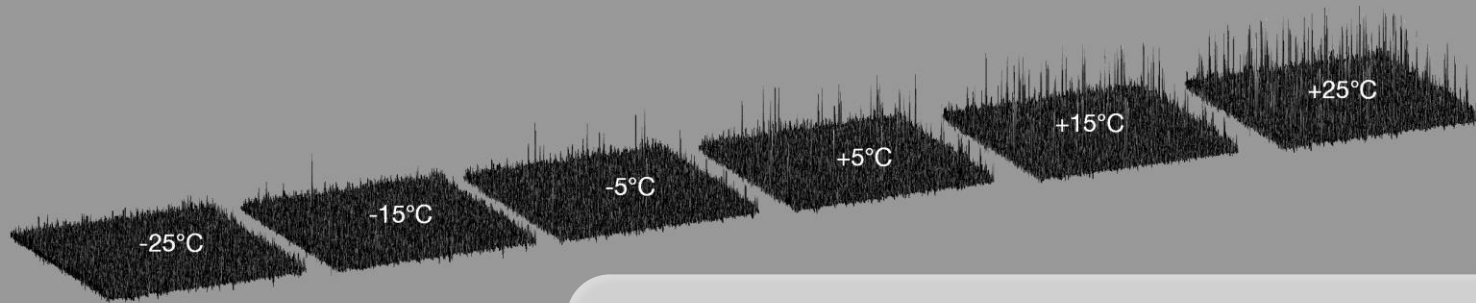


Hot Pixel list & removal by neighbourhood mean



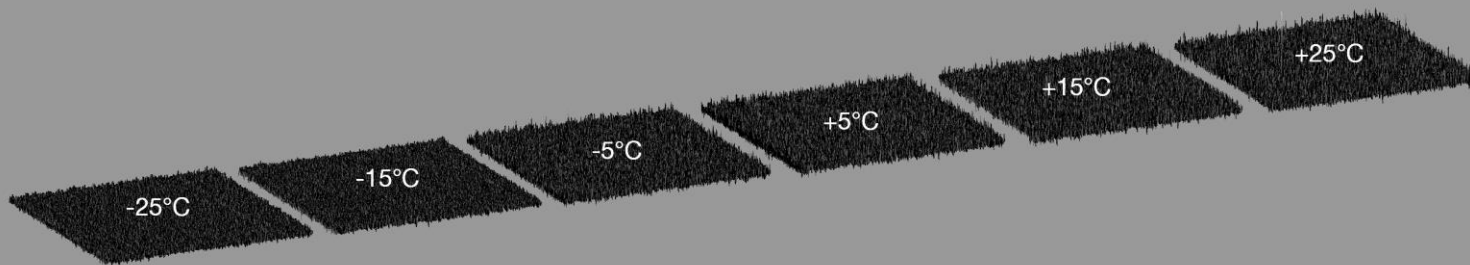
„Blinkers“ – non permanent warm pixels

without filtering



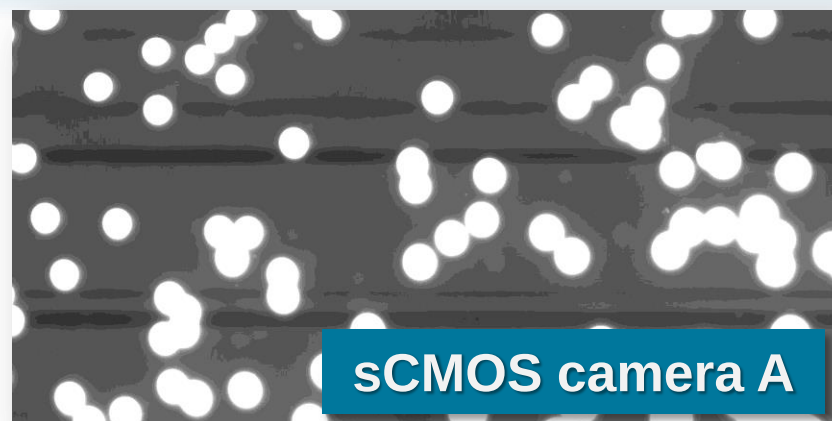
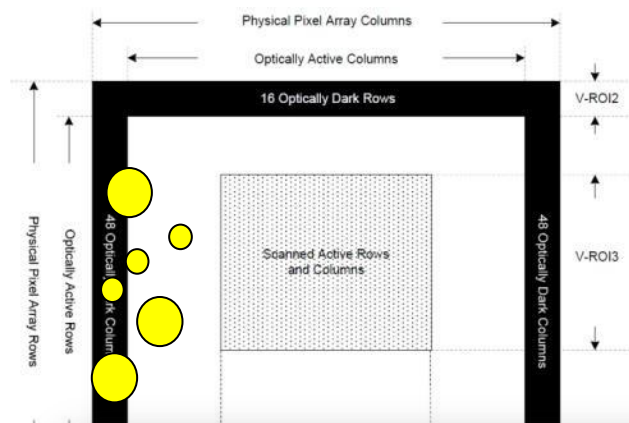
The warm pixels can be reduced by cooling, while the „blinkers“ are less improved by cooling. In case of a sophisticated (= noise & signal aware) filtering the cooling influence is hardly visible.

with filtering



„Banding“

When correction goes wrong...



Data Transfer

Data Transfer Rates of Interfaces

Interface	Medium	Cable Length	Theoretical max. Data Rate
Firewire	TwistedPair / Fiberoptic	4.5 m / 100 m	125 Mbyte/s
USB 2.0	TwistedPair	5 m	50 Mbyte/s
USB 3.0	TwistedPair	3 m	450 Mbyte/s
USB 3.1	TwistedPair	2 m	1100 Mbyte/s
1 Gigabit Ethernet	TwistedPair / Fiberoptic	100 m / 10 km	118 Mbyte/s
10 Gigabit Ethernet	TwistedPair / Fiberoptic	100 m / 10 km	1183 Mbyte/s
Thunderbolt	TwistedPair	3 m	PCIe Gen2 x2 DP (515 Mbyte/s)
HD-SDI	Coaxial Cable	100 m	297 Mbyte/s
CoaXPress	Coaxial Cable	200 m / 40 m	116 Mbyte/s / 580 Mbyte/s
CameraLink	Special Cables	15 m	806 Mbyte/s
CameraLinkHS	CX4 / Fiberoptic	15 m / 10 km	2051 Mbyte/s / 1187 Mbyte/s

Data Transfer vs. Frame Rates

Interface	Theoretical max. Data Rate
Firewire	125 Mbyte/s
USB 2.0	50 Mbyte/s
USB 3.0	450 Mbyte/s
USB 3.1	1100 Mbyte/s
1 Gigabit Ethernet	118 Mbyte/s
10 Gigabit Ethernet	1183 Mbyte/s
Thunderbolt	PCIe Gen2 x2 DP (515 Mbyte/s)
HD-SDI	297 Mbyte/s
CoaXPress	116 Mbyte/s / 580 Mbyte/s
CameraLink	806 Mbyte/s
CameraLinkHS	2051 Mbyte/s / 1187 Mbyte/s

Name	type	Data Rate [Mbyte/s]
ICX285AL	CCD (IT)	46.3 (17.4)
CCD87	emCCD (FT)	17.8 (6.7)
CCD201-20	emCCD (FT)	18.9 (7.1)
KAI-4022	CCD (IT)	123.3 (53.9)
CMV4000	CMOS	1509.9 (471.9)
VITA 5000	CMOS	796.3 (298.6)
CIS2521	sCMOS	1105.9
CIS2020	sCMOS	838.9
MT9 M413	CMOS	1667.2 (625.2)
ON-Semi	CMOS	10396.1 (3898.6)

Visual lossless compression

A valuable method to reduce the data load

The sCMOS image sensor generates pixel data with a full frame rate of maximum 1 GB/s. The Camera Link Full interface can transfer 650 MB/s, therefore a compression is required to transfer the image data at full speed.

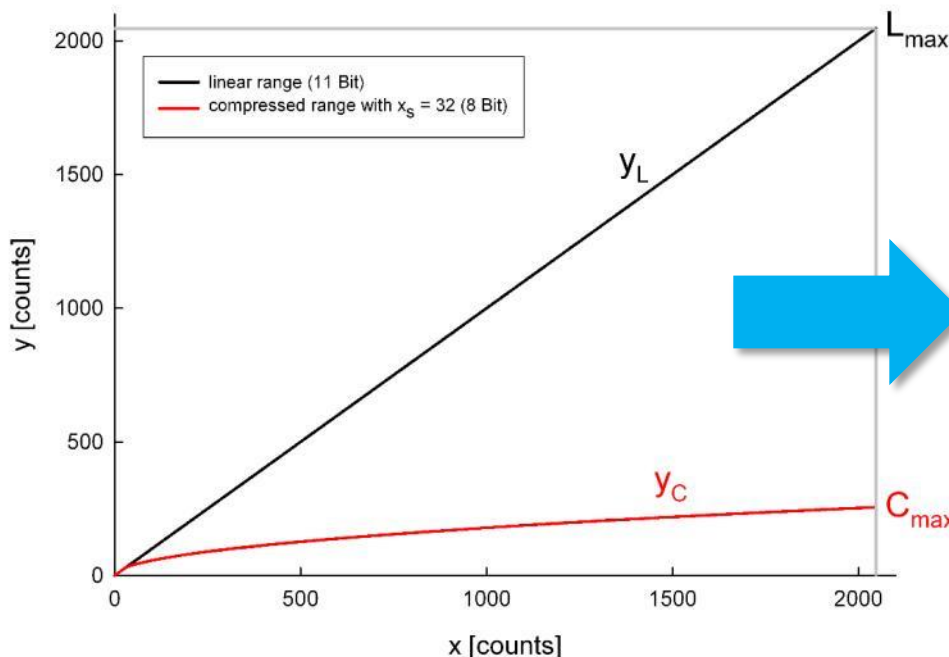


figure 1: data compression with square root function

A square root like type of look-up table is used to convert the signal with a compression, which doesn't compress small signals but large signals. This compression is done in a way that the introduced error always is smaller than the photon noise - therefore it is visually lossless¹.

¹Bernd Jähne, Digitale Bildverarbeitung und Bildgewinnung, 7th edition, Springer Vieweg, 2012, chapter 7.2.4

**End of part I
Have a break...**