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
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 $\eta$

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

Quantum Efficiency $\eta$

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

$\zeta$ 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.

{graphics taken from: Saleh & Teich, Fundamentals of Photonics}
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.

{graphics taken from: A. J. P. Theuwissen, Solid-State Imaging with Charge-Coupled-Devices}
Quantum Efficiency Curve

quantum efficiency [%]

wavelength [nm]
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

{graphics taken from: http://www.microscopyu.com/ & PCO}
CCD Image Sensors
architecture – frame transfer CCD

[+] 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)

{graphics taken from: www.microscopyu.com & A. J. P. Theuwissen, Solid-State Imaging with Charge-Coupled-Devices}
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…*

{graphics taken from: www.microscopyu.com & A. J. P. Theuwissen, Solid-State Imaging with Charge-Coupled-Devices}
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

{graphics taken from: www.microscopyu.com}
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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>Interline CCD; Progressive Scan</td>
</tr>
<tr>
<td>Total Number of Pixels</td>
<td>2404 (H) x 1800 (V)</td>
</tr>
<tr>
<td>Number of Effective Pixels</td>
<td>2360 (H) x 1776 (V)</td>
</tr>
<tr>
<td>Number of Active Pixels</td>
<td>2336 (H) x 1752 (V)</td>
</tr>
<tr>
<td>Pixel Size</td>
<td>5.5 μm (H) x 5.5 μm (V)</td>
</tr>
<tr>
<td>Active Image Size</td>
<td>12.85 mm (H) x 9.64 mm (V)</td>
</tr>
<tr>
<td></td>
<td>16.06 mm (diag) 1” optical format</td>
</tr>
<tr>
<td>Aspect Ratio</td>
<td>4:3</td>
</tr>
<tr>
<td>Number of Outputs</td>
<td>1, 2, or 4</td>
</tr>
<tr>
<td>Charge Capacity</td>
<td>20,000 electrons</td>
</tr>
<tr>
<td>Output Sensitivity</td>
<td>34 μV/e</td>
</tr>
<tr>
<td>Quantum Efficiency Pan</td>
<td>46%</td>
</tr>
<tr>
<td>R, G, B (+CBA, -PBA)</td>
<td>29%, 37%, 39%</td>
</tr>
<tr>
<td>Read Noise (f= 40MHz)</td>
<td>12 electrons rms</td>
</tr>
</tbody>
</table>
CCD Image Sensors
Bininning 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

Methods to reduce smear:
Reduction of light signal, extension of exposure time, faster readout
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!

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.
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.

{graphics taken from: http://www.microscopyu.com/ & PCO}
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
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

Diagram showing the readout process for a rolling shutter CMOS image sensor over time, with stages labeled as:
- Reset
- Exposure start
- Exposure stop
- Readout stop

The diagram illustrates the sensor's operation from left to right, showing the transition of the exposure and readout phases.
CMOS Image Sensors
readout schemes: global shutter

reset
exposure start

exposure stop
readout

time
CMOS Image Sensors

readout schemes: global shutter & rolling shutter

Global shutter

Rolling shutter

CMOS Image Sensors
Flash Illumination

- Rolling Shutter
  - Rolling reset until last row
  - Rolling readout with rolling exposure stop

- Global Reset - Rolling Shutter
  - Global reset
  - If available!
  - Rolling readout with rolling exposure stop

- Global Shutter
  - Global reset
  - Rolling readout with global exposure stop

If available!
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.
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

<table>
<thead>
<tr>
<th></th>
<th>CIS2521</th>
<th>CIS1910</th>
<th>CIS1210</th>
<th>CIS2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>resolution [pixel]</td>
<td>2560 x 2160</td>
<td>1920 x 1080</td>
<td>1280 x 1024</td>
<td>2048 x 2048</td>
</tr>
<tr>
<td>readout noise</td>
<td>1.1_{med} e-</td>
<td>&lt; 1.2 e-</td>
<td>&lt; 2 e-</td>
<td>0.9_{med} e-</td>
</tr>
<tr>
<td></td>
<td>1.3_{rms} e-</td>
<td></td>
<td></td>
<td>1.5_{rms} e-</td>
</tr>
<tr>
<td>frame rate [fps]</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(full)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rolling shutter</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>global shutter</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>QE_{peak}</td>
<td>60 %</td>
<td>&gt; 52 %</td>
<td>-</td>
<td>70 %</td>
</tr>
<tr>
<td>dynamic (rs)</td>
<td>1 : 27 000</td>
<td>1 : 25 000</td>
<td>1 : 25 000</td>
<td>1 : 33 000</td>
</tr>
<tr>
<td>fullwell capacity</td>
<td>30 000 e-</td>
<td>30 000 e-</td>
<td>30 000 e-</td>
<td>30 000 e-</td>
</tr>
</tbody>
</table>
sCMOS Image Sensors

how the low readout noise is achieved...

This capacity as dominant noise contribution must be sufficiently small.

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.

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

No effect to the linearity output of the camera!
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
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
pixel size A = 1/4 pixel size B

sensor B

increasing pixel size

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

Table 3: Consideration on signal and SNR for different pixel sizes same total area

<table>
<thead>
<tr>
<th>pixel type</th>
<th>signal</th>
<th>readout noise</th>
<th>SNR low light</th>
<th>SNR bright light</th>
</tr>
</thead>
<tbody>
<tr>
<td>small pixel</td>
<td>m</td>
<td>r₀</td>
<td>s₀</td>
<td>s₁</td>
</tr>
<tr>
<td>large pixel</td>
<td>4 x m</td>
<td>&gt; r₀</td>
<td>&gt; s₀</td>
<td>2 x s₁</td>
</tr>
<tr>
<td>4 small pixels</td>
<td>4 x m</td>
<td>2 x r₀̅</td>
<td>2 x s₀</td>
<td>2 x s₁</td>
</tr>
</tbody>
</table>
Cameras with Different Pixel Size
Comparison with different optics (constant resolution) i

**sensor A**

**sensor B**

**pixel size A = ¼ pixel size B**

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

<table>
<thead>
<tr>
<th>pixel type</th>
<th>signal</th>
<th>readout noise</th>
<th>SNR low light</th>
<th>SNR bright light</th>
</tr>
</thead>
<tbody>
<tr>
<td>small pixel</td>
<td>m</td>
<td>$r_0$</td>
<td>$s_0$</td>
<td>$s_1$</td>
</tr>
<tr>
<td>large pixel</td>
<td>m</td>
<td>$&gt; r_0$</td>
<td>$&lt; s_0$</td>
<td>$s_1$</td>
</tr>
</tbody>
</table>
Cameras with Different Pixel Size
Comparison with different optics (constant resolution)

pixel size A = \( \frac{1}{4} \) pixel size B

original lens imaging to sensor B

\[
\begin{align*}
Y_o & \quad F_o \\
x_o & \quad f_{\text{old}} \\
 & \quad f_{\text{old}} \\
x_{\text{old}} & \quad Y_{\text{old}}
\end{align*}
\]

new lens imaging to sensor A

\[
\begin{align*}
Y_o & \quad F_i \\
x_o & \quad f_{\text{new}} \\
 & \quad f_{\text{new}} \\
x_{\text{new}} & \quad Y_{\text{new}}
\end{align*}
\]

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.

large pixel
\[
\begin{align*}
f & = 100 \text{ mm} \\
f-\text{stop} & = 16
\end{align*}
\]

small pixel
\[
\begin{align*}
f & = 50 \text{ mm} \\
f-\text{stop} & = 16
\end{align*}
\]

small pixel
\[
\begin{align*}
f & = 50 \text{ mm} \\
f-\text{stop} & = 8
\end{align*}
\]
MTF Modulation Transfer Function

image resolution (e.g. ICX285):
axial
\[ \frac{1}{(2p)} = \frac{1}{13 \text{ µm}} = 76.9 \text{ [line pairs / mm]} \]
diagonal
\[ \frac{1}{(\sqrt{2} \cdot (2p))} = \frac{1}{18.38 \text{ µm}} = 54.4 \text{ [line pairs / mm]} \]
Some MTF Data

Pixel Size Requirements for Maximum Resolution in Optical Microscopy

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

{graphics taken from: http://www.microscopyu.com/}
**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% + µlens
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.
A high resolution is useful for:

- a larger field of view
- more details

but...

- higher data load
- same light / pixel

- higher data load
- less light / pixel
Noise
Which camera shows more noise?

**Image 1**

**Image 2**
Which camera shows more noise?

Image 1

Image 2

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

Mean: 3506.19 ± 24.18 std

Mean: 227.57 ± 5.16 std
Noise – EMVA 1288 Camera Model

A number of photons ...
... hitting the pixel area during exposure time ...
... creating a number of electrons ...
... forming a charge which is converted by a capacitor to a voltage ...
... being amplified ...
... and digitized ...
... resulting in the digital gray value.

Light input

Sensor & Camera

Grey Value output
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 \( \mu_y \) 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

\[
\mu_y = \frac{1}{2N} \sum_{i,j} (y^A_{ij} + y^B_{ij})
\]

(28)

averaging over all rows and columns. \( \mu_y \) is the mean gray value of dark images, \( \mu_y_{dark} \) 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

\[
\sigma_y^2 = \frac{1}{2N} \sum_{i,j} (y^A_{ij} - y^B_{ij})^2.
\]

(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 responsivity \( R = K \eta \). A linear regression curve will be fitted to the responsivity data and the equation of the regression line will be determined.

Overall system gain \( K \). According to Eq. (9), the slope of the relation \( \sigma_y^2(\mu_y - \mu_{y,dark}) \) gives the absolute gain factor \( K \). Select a contiguous range of measurements where this relation shows 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 \( \sigma_y^2 \) versus \( \mu_y - \mu_{y,dark} \) over the selected range and specify the gain factor \( K \) and its statistical error.
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)

SNR = 15

SNR = 10

SNR = 5

SNR = 2
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 Fe$^{55}$ way

The Fe$^{55}$ soft x-ray source produces five different signals in silicon image sensors: $K_\alpha$ (1620 e-), $K_\beta$ (1778 e-), $K_\alpha$ escape peak (1133 e-), $K_\beta$ escape peak (1291 e-) and a silicon line (487 e-).
Fortunately in this case the EMVA1288 determination of the system gain or conversion factor delivers the same results like the Fe$^{55}$ determination:

\[
\text{conv. factor}_{\text{EMVA1288}} = 0.503 \text{ [e-/count]}
\]

\[
\text{conv. factor}_{\text{Fe55}} = 0.505 \text{ [e-/count]}
\]
Nowadays even QE values of 70 % and higher are achieved due to optimization of microlenses and optical stack.

{graphics taken from: www.microscopyu.com & presentation by Toppan}
QE Improved
Front Illuminated & Back Illuminated Image Sensors

diode
epi Si
bulk Si

frontside illuminated

backside illuminated

{graphics taken from: Conference Image Sensors 2014}
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

\[ \text{intensity} = m \cdot t + n \]

\[ m = 7.26 \]
\[ n = 6.77 \]
\[ r^2 = 1.000 \]
Dark Current & Cooling
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 (> 1s 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.

For example sCMOS:
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
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.

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

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.
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

Min=104 Max=65536
Min=104 Max=449
Non-linear scaling
## Dynamic Ranges of Image Sensors

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

We are living visually mainly in a 8 bit world

human eye

TV screen

PC screen

printer

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

So, what’s the point in using high dynamics?
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...

- Image in software with proper scaling
- Image in image viewer software with min/max scaling for 16bit images
- Image with non-linear scaling
Frame Rate
Frame Rates
Single Images
Frame Rates
Time Lapse Series
Frame Rates

Slow Motion
# Frame Rates of Image Sensors

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

not corrected

corrected

Linearity

![Graph showing linearity with and without correction]
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

Subtraction of a dark image

Application of a median filter

Hot Pixel list & removal by neighbourhood mean

Before…

After…
The warm pixels can be reduced by cooling, while the „blinders“ are less improved by cooling. In case of a sophisticated (= noise & signal aware) filtering the cooling influence is hardly visible.
„Banding“
When correction goes wrong…

sCMOS camera A

sCMOS camera B
Data Transfer
## Data Transfer Rates of Interfaces

<table>
<thead>
<tr>
<th>Interface</th>
<th>Medium</th>
<th>Cable Length</th>
<th>Theoretical max. Data Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firewire</td>
<td>TwistedPair / Fiberoptic</td>
<td>4.5 m / 100 m</td>
<td>125 Mbyte/s</td>
</tr>
<tr>
<td>USB 2.0</td>
<td>TwistedPair</td>
<td>5 m</td>
<td>50 Mbyte/s</td>
</tr>
<tr>
<td>USB 3.0</td>
<td>TwistedPair</td>
<td>3 m</td>
<td>450 Mbyte/s</td>
</tr>
<tr>
<td>USB 3.1</td>
<td>TwistedPair</td>
<td>2 m</td>
<td>1100 Mbyte/s</td>
</tr>
<tr>
<td>1 Gigabit Ethernet</td>
<td>TwistedPair / Fiberoptic</td>
<td>100 m / 10 km</td>
<td>118 Mbyte/s</td>
</tr>
<tr>
<td>10 Gigabit Ethernet</td>
<td>TwistedPair / Fiberoptic</td>
<td>100 m / 10 km</td>
<td>1183 Mbyte/s</td>
</tr>
<tr>
<td>Thunderbolt</td>
<td>TwistedPair</td>
<td>3 m</td>
<td>PCIe Gen2 x2</td>
</tr>
<tr>
<td>HD-SDI</td>
<td>Coaxial Cable</td>
<td>100 m</td>
<td>297 Mbyte/s</td>
</tr>
<tr>
<td>CoaXPress</td>
<td>Coaxial Cable</td>
<td>200 m / 40 m</td>
<td>116 Mbyte/s / 580 Mbyte/s</td>
</tr>
<tr>
<td>CameraLink</td>
<td>Special Cables</td>
<td>15 m</td>
<td>806 Mbyte/s</td>
</tr>
<tr>
<td>CameraLinkHS</td>
<td>CX4 / Fiberoptic</td>
<td>15 m / 10 km</td>
<td>2051 Mbyte/s / 1187 Mbyte/s</td>
</tr>
</tbody>
</table>
## Data Transfer vs. Frame Rates

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<td>10396.1 (3898.6)</td>
</tr>
</tbody>
</table>
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.

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 lossless\(^1\).

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\(^1\)Bernd Jähne, Digitale Bildverarbeitung und Bildgewinnung, 7th edition, Springer Vieweg, 2012, chapter 7.2.4
End of part I
Have a break...