Detector systems for light microscopy

The human eye – the perfect detector?

- Resolution: 0.1-0.3mm @25cm object distance
- Spectral sensitivity ~400-700nm
- Has a dynamic range of 10 decades
- Two detectors: rods for night vision (~120 mega pixels), cones for daylight (~6-7 mega-pixel)

Basic elements of a CCD chip

- A CCD chip is a 2-dimensional array of light-sensing elements
- The light sensing unit of a CCD is a metal oxide semiconductor capacitor
- Each unit corresponds to an individual picture element (pixel)
- Smallest Pixelsize about 4.2 µm x 4.2 µm

Chip readout
Chip readout

Full-well capacity:
maximal number of electrons that can be stored in each photodiode
\( e_{\text{max}} = 1000 \times A \ [\mu \text{m}^2] \)
(FWC: 10µm × 10µm = 100,000 e⁻)

Three basic variations of CCD architecture

Close to 100 % fill
+ Large sensitive area
- Shutter required
- Slow readout

Close to 100 % fill
+ Large sensitive area
+ No shutter required
+ Fast readout
- Smear while reading out

Only about 25 % fill
70% - 90% (micro-lenses)
+ No shutter required
+ Fastest readout
+ No smear while reading out

Analog to Digital Units (ADU)

The analog signal is converted to a digital signal. Analog to Digital Units (A.D.U.) defines the conversion efficiency:

\[ A.D.U. = \frac{FWC(e)}{\text{Bitdepth} - \text{digitizerOffset}} \]

\( e_{\text{max}} \)

X bits = \( 2^x \)

Camera: up to 16 bit, Monitor: 8 bit, Human eye: 5-7 bit
You need more than 8 bit digitization depth:
• for low light level applications
• when use images for calculations

Special chip types

front / back-illuminated CCDs
CCDs with UV coatings
Low light imaging – overcoming noise

In microscopy you often work with low signals. In such low light applications, the camera noise becomes crucial.

Two sensor technologies exist for low light applications:

**Signal integration (Long exposure) sensors:**
- Cooled CCD (Chip cooled by peltier elements)

**Signal Multiplication (Rapid exposure) sensors:**
- EB-CCD
- EM-CCD
- I-CCD

**Intensified CCD camera (I-CCD)**

Photons are converted into electrons at a photocathode. These electrons hit a microchannel plate (MCP). Those passing through the MCP are multiplied several thousand times and strike a phosphor coating. Thereby they are converted back into photons which are then focused on a CCD by a lens.

**Strong Points**
- Larger gain than EB-CCD and EM-CCD
- Enough gain for photon counting

**Weak points:**
- Higher multiplication noise than EB-CCD
- Lower resolution than EM-CCD
- Overlight protection necessary

**Electron Bombardment CCD camera (EB-CCD)**

Photons are converted into electrons at a photocathode. These electrons are accelerated by a high voltage into a back thinned CCD. The additional energy by this electron bombardment creates a direct gain of several hundreds electrons in the CCD.

**Strong points:**
- Higher spatial resolution than I-CCD
- Lower multiplication noise than EM-CCD and I-CCD

**Weak points:**
- Lower resolution at low gain, because photo-electrons can hit adjacent pixels
- Overlight protection necessary!

**Electron Multiplication CCD camera (EM-CCD)**

Similar to a frame transfer camera, but a special multiplication register is added. Voltages up to 50 Volts accelerate the signal electrons and generate occasional extra electrons via impact ionization.

**Strong points:**
- Gain: up to few thousands
- No photocathode
- Wide range of sensitivity
- Good resolution
- No damage from excess light
- High frame rates possible

**Weak points:**
- Higher multiplication noise than EB-CCD
- Smaller gain than I-CCD
- Higher darknoise
- Gain is temperature dependent!
Other ways of increasing signal

- **Optimize light collection efficiency**
  - Typically only 10% of light arrives to CCD (lenses, mirrors, filters, dust, etc.). For QE=0.5 only 5% detection efficiency
- **Longer integration time**
  - Reduction of temporal resolution
- **Binning**
  - Electronic coupling of pixel groups to one pixel
  - Reduction of spatial resolution
  - Cameras available with up to 8x8 binning (not really relevant in microscopy)

Sampling

- Test Target imaged with a 40x/0.9 objective and over-sampling (left) and with under-sampling using 8x8 binning (right)
- Strong aliasing occurs on the right picture.

Image sampling

- Nyquist- sampling theorem:
  - The sampling frequency must be greater than twice the highest frequency of the input signal.
  - \( v_{\text{cutoff}} = \frac{2\text{NA}}{\lambda M} \)
  - To ensure adequate sampling for high-resolution imaging, a sampling of 2.5 to 3 for the smallest resolvable feature is suggested.

Practical implementation

- Pixel size defined by camera

  \[ p_x \leq \frac{\lambda \cdot M}{4\text{NA}} \]

  Defined by objective

- Example 1: \( p_x = 6.4\mu m; \text{NA} = 1.4; M = 100; 6.4\mu m \leq 8.9\mu m \) oversampling
- Example 2a: \( p_x = 24\mu m; \text{NA} = 1.4; M = 100; 24\mu m > 8.9\mu m \) undersampling/aliasing
- Example 2b: \( p_x = 24\mu m; \text{NA} = 1.4; M = M_{\text{obj}} \cdot M_{\text{optovar}} = 100 \cdot ?; 24\mu m \leq 8.9\mu m \cdot ? \)

- Use Optovar to match magnification with resolution

- To prevent aliasing: make pixel size smaller or magnification bigger
Signal to noise ratio (SNR) and Dynamic Range

\[ (S / N)_{db} = 20 \log(S / N) \]

Dynamic Range \( DR = FWC / N_{Camera} \)

Example: \( FWC = 18000e; N_{Camera} = 8e; \)
\( DR = 2250 = 67dB = 3.35 \) orders of magnitude (\( \log(2250) \))

Shot noise (Signal Noise)

The Signal Noise is poisson distributed, therefore it is equal to the square root of the number of Photo-electrons in a particular region of the image.
As the signal increases the signal to noise ratio becomes better since the square root becomes a smaller percentage of the Signal.

\[ \sqrt{4} = 2 \] (50%)
\[ \sqrt{100} = 10 \] (10%)
\[ \sqrt{500} = 22 \] (4%)
\[ \sqrt{20'000} = 141 \] (0.7%)

Readout noise

The faster the noisier!

As a rule of thumb:
- 50kHz ~ 5 e-
- 1MHz ~ 20 e-
- 20MHz ~50 e-

Frame-rate and pixel readouts

\[ FR \sim \frac{P[MHz]}{\#\text{Mpixels}} \]

With binning: for example 4 pixels become one pixel; faster readout and more signal collected without increasing readout noise.

Examples
- Hamamatsu ORCA IIER: 10 MHz (7-8e-); 1.25 MHz (3-5e-)
- Kappa DX3: 10 MHz (18e-)
- Medium quality video camera: 1 MPixels; 7 x 7 \( \mu \text{m}^2 \);
\( (S/N)_{db} = 50 \text{ db} \ S/N = 316; e_{\text{max}} = 50'000; 160 e^- \)

Dark noise

The longer integrated, the more!

\[ N_{\text{Dark}} = \sqrt{D \cdot t} \]

D: Dark current
\( t \): Integration time

with
\[ D = c \cdot T \exp(-E/kT) \]

c: Constant (system-dependent)
\( T \): Temperature
E: Kinetic energy of collected electrons

To obtain dark noise in the range of the readout noise requires cooling of the chip!
Typical specification sheet

**SPECIFICATIONS**

<table>
<thead>
<tr>
<th><strong>Type number</strong></th>
<th>C4742-08-DAG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Imaging device</strong></td>
<td>Progressive scan interline CCD with micro-lens</td>
</tr>
<tr>
<td><strong>Effective size</strong></td>
<td>14 μm x 8.5 μm (square format)</td>
</tr>
<tr>
<td><strong>Effective area</strong></td>
<td>0.53 × 0.65 mm (2.7 x 2.7 inch format)</td>
</tr>
<tr>
<td><strong>Readout time</strong></td>
<td>1.3 μs/pixel</td>
</tr>
<tr>
<td><strong>2 K x 2 binning</strong></td>
<td>10 frames/sec</td>
</tr>
<tr>
<td><strong>4 K x 2 binning</strong></td>
<td>27 frames/sec</td>
</tr>
<tr>
<td><strong>8 K x 2 binning</strong></td>
<td>66 frames/sec</td>
</tr>
<tr>
<td><strong>Readout noise (rms)</strong></td>
<td>10 electrons</td>
</tr>
<tr>
<td><strong>Full well capacity</strong></td>
<td>15,800 electrons</td>
</tr>
<tr>
<td><strong>Dynamic range</strong></td>
<td>256:1</td>
</tr>
<tr>
<td><strong>Cooling method</strong></td>
<td>Liquid at ambient cooling with hemispherical vacuum-sealing</td>
</tr>
<tr>
<td><strong>Cooling temperature</strong></td>
<td>-50°C at 30°C ambient temperature</td>
</tr>
<tr>
<td><strong>Dark current</strong></td>
<td>0.4 e-/sec/pixel/sec</td>
</tr>
<tr>
<td><strong>A/D converter</strong></td>
<td>12 bit</td>
</tr>
<tr>
<td><strong>Exposure time</strong></td>
<td>10 μsec to 2.500 sec</td>
</tr>
<tr>
<td><strong>Sub-array</strong></td>
<td>Single</td>
</tr>
<tr>
<td><strong>Contrast enhancement</strong></td>
<td>Analog Gain (10-bit area) and Offset function</td>
</tr>
<tr>
<td><strong>Environmental</strong></td>
<td>Specifications</td>
</tr>
<tr>
<td><strong>Lens mount</strong></td>
<td>C-mount</td>
</tr>
<tr>
<td><strong>Interface</strong></td>
<td>IEEE1394-1988</td>
</tr>
<tr>
<td><strong>External control</strong></td>
<td>1394-based Digital Camera Specifications Ver. 1.30</td>
</tr>
<tr>
<td><strong>Line voltage</strong></td>
<td>99Vx117V±20% (240V AC, 50/60Hz)</td>
</tr>
<tr>
<td><strong>Power consumption</strong></td>
<td>40W</td>
</tr>
<tr>
<td><strong>Ambient storage temperature</strong></td>
<td>-60°C to +60°C</td>
</tr>
<tr>
<td><strong>Ambient operating temperature</strong></td>
<td>0°C to +40°C</td>
</tr>
<tr>
<td><strong>Ambient storage humidity</strong></td>
<td>7% max. (40°C)</td>
</tr>
</tbody>
</table>

*Calculated from the ratio of the full well capacity and average maximal noise*

**Camera:**

ORCA ERG (Hamamatsu)

Cameras summary

- Many different cameras are now available
  - Every camera has benefits and limitations
- No camera can deal with all applications
  - Very flexible cameras (e.g. digital CCD camera Orca ERG)
  - Cameras for special applications (e.g. photon counting camera C22741-32)
- Important for camera selection
  - Requirements of the application
  - Benefits and limitations of a camera
- Cameras are components of workstations
  - Image quality depends not only on the camera
  - Images are processed and analyzed by application specific software
  - Enormous amount of data has to be recorded and saved

Literature


- http://www.microscopyu.com


- http://micro.magnet.fsu.edu