DIGITAL IMAGING

Internal Course 2015
January, 27\textsuperscript{th}
DIGITAL IMAGING OUTLOOK

1600
1700
1800
1900
2000

Digital Camera (Kodak, 1975)
**HUMAN EYE**

**Visual Pigments**
- Protein + Retinal
- 13 cis/trans Photoreaction (Isomerization)
- Signal transduction pathway
  - Amplification steps
  - Hyperpolarization
  - Stimulated cells release less Neurotransmitter
- System is noiseless
PHOTON DETECTION
Physical Principles
WHAT IS LIGHT
PHOTOELECTRIC EFFECT

Albert Einstein
Nobel price 1921

Experiments by Lennart 1902

- Maximal energy of the photo electrons is independent of the intensity of the light
- Slope is the same for different cathode materials
- Light can be described as particles called photons or light quants.

Detection of Photons
PHOTOMULTIPLIER

Diagram showing the components of a photomultiplier tube:
- Incoming Photon
- Photocathode
- Window
- Dynodes
- Anode

Graph showing the relationship between dynode number and PMT gain:
- 14 Stage
- 12 Stage
- 10 Stage

Voltages: 1000, 1500, 2000, 2500
PHOTOMULTIPLIER
SUMMARY

• Photons “produce” electrons which are multiplied by acceleration and detected afterwards.
• Gain (=multiplication factor) can be varied.
• Large linear range.
• Quantum efficiency of the photocathode up to 30% in the visible range.
• “no” spatial resolution, i.e. photomultipliers can only be used as point (scanning) detectors.
DIGITAL IMAGING DEFINITION

• Array of photosensitive elements
• Signal is dependent on the number of detected photons; ideally linearly dependent = high dynamic range
• Easy read-out procedure
• Reusable
• Appropriate size, comparable to an analogue film
CCD CAMERAS PRINCIPLE

- Conductor (metal)
- Semi conductor
- Isolator

- Conduction band
- Band gap
- Valence band
**CCD CAMERAS**

**Principle**

- Internal photoelectric effect.
- Energy of the photon is used to transfer an electron from the valence band to the conduction band (semiconductors).
- Photodiodes
PHOTON DETECTION
Practical Aspects
CCD ARCHITECTURE

- Silicon based integrated circuits
- Dense matrix of photodiodes
- Electrons are stored in a potential well
- Electrons can be transported across the chip (=read out)
CCD CAMERA
FULL-WELL CAPACITY

photons

QE

photo-electrons

Read out
AD conversion

pixel value
CCD ARCHITECTURE

Front illuminated QE: ~65%

back illuminated QE: ~95%
CCD CAMERA QUANTUM EFFICIENCY

<table>
<thead>
<tr>
<th>Wavelength (Nanometers)</th>
<th>Penetration Depth (Micrometers)</th>
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</thead>
<tbody>
<tr>
<td>400</td>
<td>0.19</td>
</tr>
<tr>
<td>450</td>
<td>1.0</td>
</tr>
<tr>
<td>500</td>
<td>2.3</td>
</tr>
<tr>
<td>550</td>
<td>3.3</td>
</tr>
<tr>
<td>600</td>
<td>5.0</td>
</tr>
<tr>
<td>650</td>
<td>7.6</td>
</tr>
<tr>
<td>700</td>
<td>8.5</td>
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<td>750</td>
<td>16</td>
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<td>800</td>
<td>23</td>
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<td>850</td>
<td>46</td>
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<td>900</td>
<td>62</td>
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<td>950</td>
<td>150</td>
</tr>
<tr>
<td>1000</td>
<td>470</td>
</tr>
<tr>
<td>1050</td>
<td>1500</td>
</tr>
<tr>
<td>1100</td>
<td>7600</td>
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</table>
CCD READ OUT

Figure 1

Full-Frame CCD Architecture

Frame-Transfer CCD Architecture

Interline Transfer CCD Architecture
SPECTRAL DETECTION

Bayer Color Filter Mosaic Array and Underlying Photodiodes

Bayer Filter Transmission Spectral Profiles

Figure 2

Figure 4

Wavelength (Nanometers)

Absolute Quantum Efficiency (%)
DIGITAL DETECTION DEVICES meet Microscopy
RESOLUTION/PIXEL SIZE

Same area
Less pixels
Larger pixel size (in the image)
Less sampling frequency

Lateral resolution

\[ \delta^R = 0.61 \frac{\lambda}{NA} \]

Nyquist theorem
Undersampling/oversampling

Spatial Resolution Effect on Pixelation in Digital Images

(a) 175 x 175
(b) 88 x 88
(c) 58 x 58
(d) 44 x 44
(e) 22 x 22
(f) 11 x 11

Figure 4
## RESOLUTION/PIXEL SIZE

<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>
CCD CAMERA BINNING

Binning:
- Increases effective pixel size
- Decreases sampling frequency
- Increases read-out speed
CCD CAMERA
NOISE/BACKGROUND

Sources of noise:
• Detector noise
• Dark noise
• Read noise
• Photon noise (shot noise)

\[
N_{tot} = N_{Sig}^2 + N_{Cam}^2
\]

\[
N_{Sig}^2 = QE \times P
\]

\[
N_{Cam}^2 = N_{Dark}^2 + N_{Read}^2
\]
CCD CAMERA SIGNAL TO NOISE RATIO

Photonflux: $10^4$ photons/s  
Exposure time: 0.1 s  
SNR: ~ 5 for a typical CCD camera
## CCD Camera Noise/Background

<table>
<thead>
<tr>
<th></th>
<th>C8484</th>
<th>OrcaER</th>
<th>CoolSnap</th>
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</thead>
<tbody>
<tr>
<td><strong>P</strong></td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td><strong>Q</strong></td>
<td>0,6</td>
<td>0,6</td>
<td>0,6</td>
</tr>
<tr>
<td><strong>T</strong></td>
<td>0,1</td>
<td>0,1</td>
<td>0,1</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>1</td>
<td>0,1</td>
<td>0,05</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>10</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>SNR</strong></td>
<td>4,74</td>
<td>5,39</td>
<td>5,39</td>
</tr>
</tbody>
</table>

**P**: Photons; **Q**: Quantum yield; **T**: exposure time/s; **N**: read noise/e–
CCD CAMERA
SIGNAL TO NOISE RATIO

Figure 2

Dark Noise versus Temperature

Figure 4

Signal-to-Noise Variation with Integration Time

Photon Flux (P) = 1000
Quantum Efficiency (QE) = 0.70

Read Noise (N_r) = 10
Dark Current (D) = 0.1

0.15 sec

Figure 5

Signal-to-Noise Improvement with Binning

Photon Flux (P) = 40
Quantum Efficiency (QE) = 0.65

4 Pixels

16 Pixels

No Binning

Read Noise (N_r) = 10
Dark Current (D) = 0.1
DIGITAL DETECTION DEVICES
Comparison
EM CCD

Figure 1

Figure 3
# DIFFERENT CAMERA TYPES

<table>
<thead>
<tr>
<th></th>
<th>CCD Sony Interline</th>
<th>EM CCD</th>
<th>sCMOS</th>
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</thead>
<tbody>
<tr>
<td>Sensor Format</td>
<td>1.4 MP</td>
<td>1 MP (max.)</td>
<td>5.5 MP</td>
</tr>
<tr>
<td>Pixel Size / µm</td>
<td>6.45</td>
<td>8-24</td>
<td>6.5</td>
</tr>
<tr>
<td>Frame Rate</td>
<td>12 fps @ 20 MHz</td>
<td>&gt; 30 fps</td>
<td>100 fps</td>
</tr>
<tr>
<td>Read Noise/</td>
<td>4-8 e⁻</td>
<td>Negligible &lt; 1 e⁻</td>
<td>1 e⁻ @ 30 fps</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.4 e⁻ @ 30 fps</td>
</tr>
<tr>
<td>QE</td>
<td>60 %</td>
<td>65 % - 90 %</td>
<td>57 %</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>3.000:1</td>
<td>8.500:1</td>
<td>25.000:1</td>
</tr>
<tr>
<td>Dark current</td>
<td>0.0003 e/pix/sec</td>
<td>0.001 e/pix/sec</td>
<td>0.07 e/pix/sec</td>
</tr>
</tbody>
</table>
SNR OF CCD AND EMCCD

CCD CAMERA
SUMMARY

• Photons “elevate” electrons from the valence to the conduction band
• High Quantum efficiency
• Large linear range.
• Spatial resolution is influenced by the pixel size of the camera
• EM CCD cameras for “low light” applications