Light sources and detectors

McGill Systems Biology Program
Second Annual
Introduction to Light Microscopy
December 8th 2010

Judith Lacoste, Ph.D.
Light Microscopy: beginning and end

Criteria for light sources:
- Intensity
- Color
- Coherent
- Polarized
- Collimated

Criteria for detectors:
- Sensitivity
- Resolution
- Speed
Fluorescence

Ability of a molecule to absorb light energy, get excited, and release light energy when returning to the ground state of energy.

- Fluorochromes
- Fluorophores
- Fluorescent proteins
Sources and qualities of light

- Non-laser sources:
  - Tungsten--Halogen
  - Mercury
  - Xenon
  - Metal halid
  - Light emitting diodes (LEDs)
  - Monochromator

- Lasers:
  - Gas
  - Helium-based
  - Diode
  - IR

http://www.olympusmicro.com/primer/lightandcolor/electromagintro.html
Tungsten-Halogen lamp

- Cheap long lasting bulbs.
- Used mainly for TL illumination.
- Can be used for fluorescence excitation above 400 nm.
- Ideal for live cell imaging because lower power and no UV component.

http://micro.magnet.fsu.edu/primer/techniques/fluorescence/fluorosources.html
http://www.olympusmicro.com/primer/anatomy/sources.html
Mercury (HBO) Lamp

Two electrodes sealed under high pressure in a quartz glass envelope which also contains mercury.
Mercury (HBO) lamp: facts

Pros:
- 10-100x brighter than tungsten-halogen.
- 50 watts to 200 watts.
- Very bright intensity peaks at specific wavelengths for many standard fluorophores.
- Readily available.

Cons:
- Bulb life of 200 hours.
- Requires bulb alignment.
- Bulb are a hazardous waste.
- Lamp intensity decays over time.
- Intensity is not uniform but contains intense peaks.
- Can flicker in intensity especially as bulb ages.

http://www.olympusmicro.com/primer/techniques/fluorescence/fluorosources.html

C. Brown, Imaging Facility, McGill University
Xenon lamp

Two electrodes sealed at high pressure in quartz glass with xenon gas.
Similar to mercury lamps.

Xenon lamp: facts

Pros:
- Relatively even intensity across the visible spectrum.
- 75-150 watts.
- Readily available.

Cons:
- Bulb life of 200 hours.
- Requires bulb alignment.
- Bulb are a hazardous waste.
- Lamp intensity decays over time.
- Weaker intensity in the UV.
- Generate a lot of heat in the IR region.
Light from the lamp passes through a liquid light guide coupled to the microscope to ensure homogeneous illumination.

Metal halide lamp: facts

- **Pros:**
  - Brighter peaks than mercury bulbs.
  - Brighter intensity between peaks than mercury bulb.
  - No bulb alignment.
  - Improved lamp stability over time – minimal decay.
  - Bulb lifetime 1500 hours.
  - More uniform field of illumination.
  - Feedback controls for stable power output.
  - Considerable reduction of mercury waste.

- **Cons:**
  - Expense upfront cost.
  - Expensive bulbs.
  - Expensive replacement of liquid light guides.
LED light sources

Individual light emitting diodes (LEDs) of various colours are optically combined and coupled to the microscope by a liquid light guide.

http://www.coolled.com/images/3intens.jpg
LED light sources

**Pros:**
- Discrete colour peaks.
- No excitation filters needed.
- No shutters needed.
- Fast (ms) switching.
- No intensity decay.
- Lifetime $\sim$10,000 hours.
- No heat.
- Precise control of intensity.

**Cons:**
- Not enough power for some applications at certain wavelengths.
- Expensive upfront cost.

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Zeiss Colibri

http://zeiss-campus.magnet.fsu.edu/tutorials/colibri/index.html
Monochromator: selectable wavelengths

DeltaRAM X™

Pros:
- Expensive upfront cost.
- Fast (ms) wavelength switching.
- No excitation filters needed.
- Variable band width excitation.

Cons:
- Expensive upfront cost.
- Still depends on mercury or xenon lamp supply
- Expensive upfront cost.
- Software not usually integrated with the microscope.

RatioMaster™

http://www.obb1.com/MicroscopeAccessories/DeltaRAM.html
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Laser sources: high resolution microscopy

http://www.olympusmicro.com/primer/lightandcolor/electromagintro.html
Laser: facts

Pros:
- Single wavelengths for excitation.
- No excitation filters needed.
- Fast (ms) switching.
- No intensity decay.
- Lifetime ~10,000 hours.
- Precise control of intensity.
- Can get multiple lines in a single laser.
- Can get lots of power.

Cons:
- Can be very expensive.
- Can generate heat.
- Limited Lifetimes.

http://www.sciencebuddies.org/
Gas lasers

Gas lasers:
- Argon Ion (Ar)
- Krypton (Kr)
- Krypton-Argon

Pros:
- Single wavelengths for excitation.
- Can get multiple lines in a single laser.
- Can get lots of power.

Cons:
- Moderately expensive.
- Generate heat.
- Limited Lifetimes.
- Power fluctuations.

http://micro.magnet.fsu.edu/primer/anatomy/sources.html
Helium-based lasers

- Helium-based lasers:
  - Helium Neon (He-Ne)
  - Helium Cadmium (He-Cd)

- Pros:
  - Affordable.
  - Well shaped focal beam.
  - Do not generate heat.
  - Long Lifetime.

- Cons:
  - Limited power.

http://www.plasmalabs.com/production/HeNe_lasers
Diode lasers

Pros:
- Compact.
- No cooling needed.
- Long lifetimes.
- Higher powers now available.

Cons:
- Expensive.
- Sensitive to electrostatic charges.

IR lasers

Pros:
- Tunable to multiple wavelengths (600-1000 nm).
- High Powers.
- Can penetrate tissues up to 1 mm.

Cons:
- Requires a pump laser.
- Extremely expensive (>150k).
- Can be difficult to lock at specific wavelengths.
- Generate a lot of heat.

http://www.gammadatainstrument.se/Productlist.aspx?MID=847
Eternal triangle of detectors

- Resolution:
  - Number of Pixels
  - Numerical Aperture
  - Magnification
  - Couplers/Adapters
  - Field of View
  - Wavelength

- Speed:
  - Frame Rate
  - Read-Out Rate
  - Hz / MHz
  - Pixels per Second
  - Bit Depth

- Sensitivity:
  - Quantum Efficiency Curve
  - Noise (Read Noise, Dark Current...)
  - Full-Well Capacity
  - Dynamic Range
Creating pixel values

**Geometry:** array or scanning.

**Light intensity:** photons to voltage to grey level (integer)

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Davidson, Microscopy primer.
Complementary metal oxide semiconductor (CMOS) sensors

- Fast – electronics attached to each pixel.
- Affordable – basis for consumer cameras.
- Moderately Sensitive – not as sensitive as CCD cameras.
- Usually colour – lower resolution than CCD cameras.
- Scientific grades are now available.

http://cpn.canon-europe.com/files/education/infobank/capturing_the_image/cmos.jpg
Basic architecture – CCD pixels

- Basic architecture
- CCD pixels
- Electrical Connection
- Potential Well
- Overflow Gutter (for anti-blooming)
- Incident Light
- Light when Back-Illuminated

Max Efficiency when Front-Illuminated: 65%
Max Efficiency when Back-Illuminated: 95%
Basic Architecture – Interline CCD

- Opaque Mask (Parallel Shift Register)
- A/D Converter
- Readout Amplifier
- Serial Register
- Micro-Lenses
- Photoactive Pixels
- Computer

David Hitrys, QImaging
Basic Architecture – Interline CCD

Expose

Photo-Active Pixels

Opaque Mask

Serial Register

A/D Converter

Readout Amplifier

Computer

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Basic Architecture – Interline CCD

Shift Horizontal

- Photo-Active Pixels
- Opaque Mask
- Serial Register
- A/D Converter
- Readout Amplifier
- Computer

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Basic Architecture – Interline CCD

Shift Vertical

Serial Register

A/D Converter

Readout Amplifier

Computer

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Basic Architecture – Interline CCD

Read

Serial Register

Computer
A/D Converter
Readout Amplifier

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Basic architecture – interline CCD

Read / Expose

Serial Register

Computer

A/D Converter

Readout Amplifier
Read / Expose

Read noise is introduced at the Readout Amplifier.

David Hitrys, QImaging
Camera specification terms

- **Quantum Efficiency:**
  - percentage of photons that hit the camera that produce a photoelectron.

- **Full Well Capacity:**
  - how many electrons can fit in the pixel.

- **Bit Depth**
  - how many grey levels the signal is assigned to.

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Frame transfer back illuminated CCD

Serial Register

Readout Amplifier & ADC

>90% QE!
Electron multiplication (EM)-CCD cameras

Back-illuminated, frame-transfer CCD (for >90% QE)

Normal Voltage Serial Register

High Voltage Serial Register

Readout Amplifier STANDARD

Readout Amplifier & ADC
Quantum efficiency comparison

http://micro.magnet.fsu.edu/primer/digitalimaging/concepts/emccds.html
Detector noise sources

- **Read Noise**
  - Depends on camera electronics and read speed.

- **Dark Current**
  - Electrons produced from thermal noise. Reduced by decreasing temperature of the camera chip.

- **Clock-Induced Charge**
  - Spurious Noise – Seen only in EM-CCDs – due to impact ionization events during charge transfer.

David Hitrys, QImaging
## Comparison of CCD specifications

<table>
<thead>
<tr>
<th>Feature</th>
<th>EM-CCD</th>
<th>Interline-CCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light-Sensitive Pixels</td>
<td>912 x 912</td>
<td>1392 x 1040</td>
</tr>
<tr>
<td>Binning Modes</td>
<td>1, 2, 4, 8, 16, 32, 64 (horizontally, arbitrary vertically)</td>
<td>2, 4, 8</td>
</tr>
<tr>
<td>ROI (Region of Interest)</td>
<td>From 1x1 pixels up to full resolution, continuously variable in single-pixel increments</td>
<td>10μs to 17.9μs</td>
</tr>
<tr>
<td>Sensor Type</td>
<td>42H-3LPVision CCD™, back-illuminated device</td>
<td>Sony® ICX285 front-illuminated interline CCD</td>
</tr>
<tr>
<td>Pixel Size</td>
<td>12μm x 12μm</td>
<td>6.45μm x 6.45μm</td>
</tr>
<tr>
<td>Linear Full Well</td>
<td>800,000e- (EM mode); 100,000e- (conventional)</td>
<td>18,000e- (20MHz); 18,000e- (10MHz)</td>
</tr>
<tr>
<td>Read Noise</td>
<td>&quot;SM gain&quot; amplifier</td>
<td>6.5e- (10MHz); 7.2e- (20MHz)</td>
</tr>
<tr>
<td>Dark Current</td>
<td>0.3 m/σ/pix/s</td>
<td>0.15 e-/pix/s</td>
</tr>
<tr>
<td>Cooling Technology</td>
<td>Three-stage Peltier cooling, chamber back-filled with nitrogen at atmospheres, assembled in a classroom unacidized</td>
<td>0°C (regulated)</td>
</tr>
<tr>
<td>Cooling Type</td>
<td>Down to -20°C, regulated, with software control in 1°C increments</td>
<td></td>
</tr>
<tr>
<td>Digital Output</td>
<td>14 bits</td>
<td>8 bits/14 bits</td>
</tr>
<tr>
<td>Readout Frequency</td>
<td>10, 50MHz (EM mode); 5, 1.25MHz (normal mode)</td>
<td>20, 10MHz</td>
</tr>
<tr>
<td>Frame Rate</td>
<td>10μs full resolution @ 14 bits (1000 max speed with binning and ROI functions)</td>
<td>10.9fps full resolution @ 14 bits (20MHz)</td>
</tr>
</tbody>
</table>


## Comparison of CCDs

<table>
<thead>
<tr>
<th></th>
<th><strong>Interline-CCD</strong></th>
<th><strong>EM-CCD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Resolution</strong></td>
<td><em>(0.1 µm at 60x)</em></td>
<td>*Lower Resolution <em>(0.3 µm at 60x)</em></td>
</tr>
<tr>
<td><strong>60-70% Quantum Efficiency</strong></td>
<td></td>
<td><em>90% Quantum Efficiency</em></td>
</tr>
<tr>
<td><strong>Slow</strong></td>
<td></td>
<td><em>Fast</em></td>
</tr>
<tr>
<td><strong>Low read noise</strong></td>
<td></td>
<td><em>High read noise</em></td>
</tr>
<tr>
<td><strong>Low spurious noise</strong></td>
<td></td>
<td><em>High spurious noise</em></td>
</tr>
</tbody>
</table>

Claire Brown, McGill University Imaging Facility
Color cameras

- RGB filter
- 3CCD chips
- Bayer mask
  - Loss of resolution
  - Loss of sensitivity
  - Avoid for fluorescence images
Bayer mask
Monochrome vs colour CCDs
Monochrome vs colour CCDs

Bayer Mask

Three shot
Images acquired monochrome, pseudo-coloured and overlaid.
Monochrome cameras: more sensitive

Monochrome

Color
Monochrome cameras: higher resolution

Monochrome

Color
Photomultiplier tubes

Great amplifiers.
Noisy – especially at high sensitivity.
Only 20-30% quantum efficiency.

http://micro.magnet.fsu.edu/primer/digitalimaging/concepts/photomultipliers.html
Spectral detectors

Slit Based Single Detector

32 Array Detector

http://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/introduction.html
Sources for light microscopy

- **Non-laser sources:**
  - Tungsten--Halogen
  - Mercury
  - Xenon
  - Metal halid
  - Light emitting diodes (LEDs)
  - Monochromator

- **Lasers:**
  - Gas
  - Helium-based
  - Diode
  - IR
Detectors for light microscopy

- Complementary metal oxide semi-conductor (CMOS) Cameras
- Interline-charged coupled device (CCD) Cameras
- Electron Multiplied (EM)-CCD Cameras
- Colour Cameras
- Photomultiplier tubes (PMT)
- Spectral Detectors
THANK YOU!

- Colleagues and users:
  - Cellular Imaging and Analysis Network (CIAN)
  - Imaging Facility
  - MIA Cellavie
  - Canadian Cytometry Association members
  - “Commercial faculty”

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