Detection Technologies for Cryo-Electron Microscopy

S²C² Workshop – Cryo-EM Training for Beginners
Christopher Booth
Gatan Inc.

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Key Concepts in Detecting Electrons
Key Concepts in Detecting Electrons

- CMOS and CCD
- “indirect” vs direct detection cameras
- Sensitivity
- Linearity and dynamic range
- Dynamic range
- Pixel size and field of view
- Electron counting
- Co-incidence loss
Recording Images In Electron Microscopy
A little bit of history

- Oldest recording medium: photographic film
- 1970: Charge coupled device (CCD) was invented
- 1976: CCD camera was used for astronomy
- 1982: 100 x 100 CCD was *directly* exposed to 100 kV electrons…radiation damage
- 1988: 576 x 382 CCD used with scintillator and optical coupler
- 1990: **Gatan** made the world’s first commercial CCD camera
- 2002: 128 x 128 direct detection camera developed
- 2008 – 2009: commercial complementary metal-oxide semiconductor (CMOS) cameras and radiation hard CMOS cameras were introduced
Both CCD and CMOS use photo diodes to convert photons to electrons, the difference is how they store charge and transfer it.

- **CCD**: Charge is transferred between neighboring cells, and read-out
- **CMOS**: Charge immediately converted to voltage (read out with digital output)

Detectors in Electron Microscopy

A. Optically coupled

B. Fiber-optic coupling

C. Direct detection

D. Transmission direct detection
Traditional Indirect Detection

1. Convert electrons to light
2. Transfer light to detector
3. Detect light and convert to signal
Direct Detection

1. Convert electrons to light

2. Transfer light to detector

3. Detect electron and convert to signal
Transmission Direct Detection

1. Convert electrons to light
2. Transfer light to detector
3. Detect electron and convert to signal

Minimize back scattered electrons that add noise

Radiation hard, thinned CMOS
Sensitivity

- Minimum detectable signal in terms of the number of incident electrons.
- Single-electron sensitivity
  - if the gain of the system is such that the output of a single incident electron is above the noise floor
Linearity

- Relationship between output (image intensity in digital units) and the input (number of incident electrons).
- CMOS and CCDS are much more linear than film.
- Counted cameras have a special kind of non-linearity called co-incidence loss.
Dynamic Range

**Dynamic range**: The range of values that can be distinguished between a maximum level (saturation) and zero (noise)

- Driven by combination of max allowable charge in each and noise floor
  - One pixel can have 16 bit dynamic range (values between 0 – 16000)

- Used to be a very important factor for cameras, now frame rate is much more important
  - A camera with only 12 bit dynamic range (0 – 4095) might accumulate 40 frames in a second.
  - $4095 \times 20 = 163,800$ counts of dynamic range
How Many Pixels are Enough?

K3

5,760 pixels

4,092 pixels

23.6 Mpixels
(94 Mpixels super-resolution)

K2

3,838 pixels

3,710 pixels

14.4 Mpixels
How Important is FOV?

Side-mount camera
- Rio™ 16: 4k x 4k, 9 µm
- Rio 9: 3k x 3k, 9 µm

Bottom-mount camera
- OneView®: 4k x 4k, 15 µm
- Rio 16: 4k x 4k, 9 µm
- K3™: 6k x 4k, 5 µm
- K2™: 4k x 4k, 5 µm
Electron Counting Makes All the Difference

Single high speed frame using conventional CCD-style charge read-out

Same frame after counting

Counting removes the variability from scattering, rejects the electronic read-noise, and restores the DQE.
Traditional Integration

Similar to indirect detection cameras, direct detectors can integrate the total charge produced when an electron strikes a pixel.

Electron enters Detector.

Electron signal is scattered.

Charge collects in each pixel.
Counting

In counting mode, individual electron events are identified at the time that they reach the detector. To do this efficiently the camera must run fast enough so that individual electron events can be identified separately.

1. Electron enters Detector.
2. Electron signal is scattered.
3. Charge collects in each pixel.
4. Event reduced to highest charge pixels.
Super-Resolution

The theoretical information limit defined by the physical pixel size is surpassed when you use the K2 in super-resolution mode. The K2 sensor pixel size is slightly smaller than the area that the electron interacts with; as a result each incoming electron deposits signal in a small cluster of pixels. High-speed electronics are able to recognize each electron event (at 400 fps) and find the center of event with sub-pixel precision.
Electron Counting Requires that Electrons Don’t Overlap on the Sensor

- Lower beam intensity
- Faster frame rate

Both methods allow counting, but the effect is not equivalent!
Cameras working at 40 fps are very limited in the range of conditions they can be used. Perfect Detection: DQE of 1.0 with no coincidence loss.
Measuring Detector Performance
PSF, MTF, NTF and DQE?

- **PSF**: Point spread function
  - Blurring of a single point in the camera

- **MTF**: Modulation transfer function
  - PSF as a function of spatial frequency
  - Most often estimated using a “knife edge”

- **NTF**: Noise transfer function
  - Noise power spectrum
  - Noise as a function of spatial frequency

\[
\text{DQE}(s) = \frac{\text{SNR}_{\text{out}}(s)}{\text{SNR}_{\text{in}}(s)} = \frac{\text{MTF}(s)^2}{\text{NPS}_{\text{out}}(s)/\text{Dose}_{\text{in}}(s)}
\]
DQE Challenges

\[ DQE(s) = \frac{SNR_{out}(s)}{SNR_{in}(s)} \]

\[ = \frac{SPS_{out}(s)/SPS_{in}(s)}{NPS_{out}(s)/NPS_{in}(s)} \]

\[ = \frac{MTF(s)^2}{NPS_{out}(s)/Dose_{in}(s)} \]

- **Signal challenges:** Edge image non-ideality
  - Charging and edge cleanliness
  - Scale
  - Edge dose
  - Motion
  - Fields
  - Scatter

- **Noise challenges:**
  - Fixed pattern noise
  - Calibration of noise power
  - Measurement of incoming beam level

- **Counting-related challenges:**
  - Spatial effects of coincidence loss: high-pass filtering
  - Non-linear counting due to coincidence loss – calibration.
  - Background
Measuring MTF with a Physical Edge (1)

AuPd-coated and plasma cleaned edge with canted face mounted in the entrance aperture of a GIF Quantum® imaged on the K2 at the end of the GIF in super-resolution mode at 200 kV. (particularly bad example – not always this bad)

Effect of charging

- Short traversal close to surface charge
- Long traversal close to surface charge

Scale at edge

- At GIF entrance module: 400 nm (K2 super-pixel: 2.5 µm)
- At pointer: 3 µm (UltraScan® pixel: 15 µm)
Measuring MTF with a Physical Edge (2)

Motion – edge creep

Good: difference between two 20 s edge images showing no “motion fringe”.

Motion – fields

Super-resolution MTF measurement affected by fields near TEM column

Noise

A noise-tolerant method for measuring MTF from found-object edges in a TEM, Paul Mooney, Microscopy and Microanalysis 15:1322-1323 CD Cambridge University Press (2009). Figure 4: Simulated MTF with various amounts of shot noise added.

At low dose rates, need long exposures to get enough dose → have to be careful about edge creep.
Other Things to Avoid with DQE Measurements

- Missing dose in a Faraday cup holder: overestimates DCE and therefore DQE (in same proportion)

- Using the TEM screen calibration

- Drifting beam current
  - Over- or under- values MTF(0) or DCE

- Leaving specimen holder inserted during MTF measurement
  Charging of specimen and/or holder can move image of edge shadow.
DQE and Binning
DQE and Binning

- Hardware bin = 1
- Hardware bin = 2
A visual representation of Aliasing:

39kx
An FFT is calculated as though each image were bordered by an infinite number of neighbors.
As you drop the magnification, the information from one region begins to show up in the neighboring region.
It becomes very obvious with a strong signal at low magnification.
DQE and Binning

hardware bin=1

hardware bin=2
DQE and Binning

Anti-aliased bin=2

hardware bin=2
DQE and Binning

Anti-aliased bin = 1.5

Hardware bin = 2
Measuring Image Performance Using Thon Rings

Thon rings are a indicator of performance. However, they are a system test and really hard to compare quantitatively.
Practical Considerations in Data Collection
Dark Subtraction

- Removes the noise baseline from the image
- New dark references are often taken once a day
Gain Correction

• Gain correction normalizes the response of each pixel to an electron
• This is why images are often floating point values
• In K3 we are allowing integer gain normalization
  • Each electron is 32 counts

• Usually collected once per week
Defect Correction

- Removes poorly performing pixels
  - Hot
  - Dark
  - Unstable

- Defect pixels contribute to fixed pattern noise

- Usually updated with Gain Reference
Typical Gain Correction Scheme

Uncorrected image - Gain map

Dark image

Defect map (enlarged detail)

Corrected image
Counted Gain Correction Scheme

Linear Image Correction \rightarrow \text{Electron Counting} \rightarrow \text{Counted Image Correction}

- Raw - Linear Dark
- Linear Gain
- Linear Defect

- Linear
- Un-processed Counted
- Counted Defect
- Counted Gain Ref
- Final Counted
Checking the Quality of Image Correction

Image: Uniform intensity

FFT: White noise
Measurement of Fixed Pattern Noise (FPN)

Uniform illumination
Common defects, dark image and gain image
Frame rate = 75 fr/s, (0.0133156 s/fr), all images.
Total dose = 14 e/pix, all images

Uniform A ⊗ Uniform B
Cross-correlation map
FPN = peak pixel value
Improved Noise Also Allows us to Improve Electron Countability

- **False negatives**
- **False positives**
- **Complete absorption of electron by detector (only for low $E$ electrons or very large pixels)**
- **Traversing electrons**
- **Readout noise**
Dose rate on the detector
Mean number of electrons hitting a detector pixel per unit time.

Total dose at the sample
Number of electrons that traverse a unit area of the sample during the exposure of this image frame.

5.317 e-/px/s, 0.743 e-/Å²

5.317 e-/px/s, 0.743 e-/Å²
Keeping track of Pixel Saturation in K3

At 8-bit/pixel, gain-corrected data saturates with a value of 255. The saturation monitor reports the percentage of pixels that have reached saturation in a single frame.
How Frame Alignment Works

Raw counted frame

Final aligned image
Raw counted frames are summed

Sub-frames are aligned and summed

1 sub-frame

1 final image
<table>
<thead>
<tr>
<th>Sensor Frame Rate</th>
<th>Dose Rate</th>
<th>Counted Frame</th>
<th>Sub-frame</th>
<th>Summed/Aligned Frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other</td>
<td>40</td>
<td>0.8 e/pix/s</td>
<td>0.025 s</td>
<td>1 s (1 fps)</td>
</tr>
<tr>
<td>K2</td>
<td>400</td>
<td>8 e/pix/s</td>
<td>0.0025 s</td>
<td>0.1 s (10 fps)</td>
</tr>
<tr>
<td>K3</td>
<td>1500</td>
<td>30 e/pix/s</td>
<td>0.00066 s</td>
<td>0.027 s (37 fps)</td>
</tr>
</tbody>
</table>

- No motion correction
- Motion correction
MotionCor2 on the K3

MotionCor2 - InMrc Stack.mrc - OutMrc CorrectedSum.mrc

-Patch 5 5 - FtBin 1.2 - Iter 10 - FmDose 1.2 - bft 1.1 - Tol 0.5
Annealing Prevents Contamination Buildup

- A cold sensor is essentially a vacuum pump. Contamination builds up on its cold surface and, if left unchecked over prolonged times, will accumulate to the point of degrade data quality.
- Severe contamination may even become evident on the gain reference images, as in the example below of a K2 sensor.
• Regular annealing of the sensor reduces background levels and surface contamination
• It can also help to repair some radiation damage
• If the camera is used for a prolonged time without warming, the electron beam can harden any contaminants, making the CMOS detector difficult to clean

• Annealing should be done at the end of every session one day long or longer
• Every time you do a cryo-cycle is a good idea
Future Directions for Electron Detection
Throughput is Going to be Critical

- We should be aiming to collect enough data in a few hours
- K3 + Latitude + Titan Krios

2.3 Å ApoF in less than an hour
Improving Throughput: Larger Sensors

- One chance to expose a specimen area

- If the pixel quality is high, larger sensors reduce the number of images needed
Improving Throughput: Faster Sensors

• Reduce exposure times during counting
  • Exposure times: 100 s to 10 s to 1 s
• Reduce time for non-data images during automation
  • Focusing
  • Centering
Improving Throughput

• Reducing exposure time and increasing image size gives you the biggest benefit

• K2: 6 images × 10 s = 60 s

• K3: 5 images × 2.5 s = 12.5 s
• K3: area is 25% larger
Better Data Through Motion Correction

- Sample damage increases during the exposure
- The first frames have the least damage but the most drift
Better Data Through Motion Correction

- Sample damage increases during the exposure
- The first frames have the least damage but the most drift
- Today the first 2–3 frames are excluded
- The K3 should be fast enough to let us use frames 1–3!
Thank You