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

CCD vs. CMOS

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)

http://meroli.web.cern.ch/meroli/lecture_cmos_vs_ccd_pixel_sensor.html

Detectors in Electron Microscopy

A. Optically coupled
B. Fiber-optic coupling
C. Direct detection
D. Transmission

direct detection

e^-

Traditional Indirect Detection

1. Convert
electrons to light
Scintillator
2. Transfer light to
detector
3. Detect light and
Light transfer convert to signal
Light sensitive CCD or CMOS

e^- Direct Detection

1. Convert
electrons to light
2. Transfer light to
detector
3. Detect light and
convert to signal
Radiation hard CMOS

e^- Transmission Direct Detection

1. Convert
electrons to light
2. Transfer light to
detector
3. Detect light and
convert to signal
Radiation hard, thinned CMOS

Minimize back scattered electrons that add noise

Frequency

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

Noise floor ***Signal from incident electrons***

Electrons or Counts

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

Journal of Microscopy, Vol. 200, Pt 1, 2000, pp. 1±13.

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 x 20 = 163,800 counts of dynamic range

Journal of Microscopy, Vol. 200, Pt 1, 2000, pp. 1±13.

How Many Pixels are Enough?

14.4 Mpixels

23.6 Mpixels

Always Consider Magnification with Field of View

Side-mount camera

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 Rio™ 16: 4k x 4k, 9 µm Rio 9: 3k x 3k, 9 µm

Electron Counting Makes All the Difference

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

read-noise, the variability and from restores scattering, the DQE

rejects the electronic Same frame after counting

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.

Electron enters detector.
Electron signal is scattered.
Charge collects in each pixel.
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 enters detector.
Electron signal is scattered.
Charge collects in each pixel.
Event localized to sub-pixel accuracy.

Electron Counting Requires That Electrons Don’t Overlap on the Sensor

Lower beam intensity

Both methods allow counting, but the effect is not equivalent!

Faster frame rate

120
100
80

60 K3 40 20 K2 0 0 20 40 60 80 100 120

Measured Dose Rate (electrons/pixel/second

Input Dose Rate (electrons/pixel/second)

Perfect Detection: DQE of 1.0 with no coincidence loss

K3 TF20: K3 coincidence loss curve

K2: Original coincidence loss curve as measured at UCSF for Li et al Nature Methods 2013 publication

Cameras working at 40 fps are very
limited in the range of conditions they can be used "Counting camera" at 40 fps

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"
- NPS: Noise power spectrum
- Noise transfer function
- Noise as a function of spatial frequency

DQE s= SNR_{out} s SNR_{in} s

= MTF(s)2

T NPS_{out}(s) $Dose_{in}$(s)

DQE Challenges

DQE s• **Signal challenges:** Edge image non-ideality

- Charging and edge cleanliness

- Scale = SNRSNR$_{out\ in}$ s s
- Edge dose
- Motion
- Fields

= NPS^{SPS}$_{out_{out}}$(s) (s) T T SPS$NPS_{in_{in}}$(s) (s)

- Scatter
- **Noise challenges**
- Fixed pattern noise

• Calibration of noise power = NPS_{out} MTF(s) (s) T $Dose^2$

$_{in}(s)$

• Measurement of incoming beam level

• **Counting-related challenges**

• Spatial effects of coincidence loss: high-pass filtering

• Non-linear calibration. counting due to coincidence loss – • Background

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

400 nm 3 µm
Canted edge
Wire

K2 super-pixel (2.5 µm)
UltraScan® pixel (15 µm)

Measuring MTF with a Physical Edge (1)

Measuring MTF with a Physical Edge (2)

Motion – Edge creep Noise

Motion – Fields

Good: Difference between two 20 s edge images showing no "motion fringe"

At low dose rates, need long exposures to get enough dose → have to be careful about edge creep.

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.

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.

Measuring Image Performance Using Thon Rings

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

-

Counted Gain Correction Scheme

Electron Counting

Linear Image Correction Counted Image Correction

Electron Counting

-

Checking the Quality of Image Correction

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

FPN = 0.0106
#

Improved Noise Also Allows Us to Improve Electron Countability

False negatives ***False positives***

Readout noise ***Complete absorption of electron by detector (only for low E electrons or very large pixels)***
Traversing electrons
E
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.

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

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.

Camera Heating/Annealing

Future Directions for Electron Detection

2019* 2016 2013 2010 2007 2004 2001 1998 1995 1992

NMR X-ray EM

Cryo-EM and Structural Biology

- Cryo-EM is still growing at an exponential pace

2019* 2016 2013 2010 2007 2004 2001 1998 1995 1992

Cryo-EM and Structural Biology

- Cryo-EM is still growing at an exponential pace
- Efficient use of each microscope is that much more important

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

Image Shift to Improve Throughput

K2 = 1.4 Mpix/s **K3** = 11.8 Mpix/s

Longer range image shift/beam shift will increase this trend

Surpassing the physical Nyquist limit to produce super-resolution cryo-EM reconstructions J. Ryan Feathers, Katherine A. Spoth, J. Christopher Fromme

doi: https://doi.org/10.1101/675397

Electron enters detector

- Super-resolution should let you get bigger filed of view?
- What about reconstruction quality?

Electron signal is scattered.
Charge collects in each pixel
Event localized to sub-pixel accuracy

Standard Magnification

- 49kX magnification
- 1.66 Å/pixel
- 0.8 Å/super-pixel
- 233 particles per frame

- 240 collected
- 2.7 Å resolution

doi: https://doi.org/10.1101/675397

Low Magnification

- 39kX magnification
- 2.10 Å/pixel
- 1.0 Å/super-pixel
- 427 particles per frame
- 260 collected
- 3.0 Å resolution

doi: https://doi.org/10.1101/675397

Imaging conditions

- **Nominal magnification** **49kX** **39kX**
- **Physical pixel size** 1.66 Å 2.10 Å
- **Super-res pixel size** 0.83 Å 1.05 Å

Micrographs in dataset 240 260

Particles used for final model **56,038** **111,111**

0.143 FSC (masked) 2.77 Å 3.06 Å

Model-map 0.5 FSC **2.9 Å** **3.1 Å**

K3 Gives Much Faster High-Resolution Reconstructions

*“The take-home message of our work is that if you have a **K3**, you can lower your mag, get more particles in less time, and still generate buildable reconstructions.”*

Cornell University 6/19/2019

Thank You

K3

GAIAN