Detection Technologies for Cryo-Electron Microscopy

S^2C^2 Workshop – Cryo-EM Training for Beginners

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Key Concepts in Detecting Electrons

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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

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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

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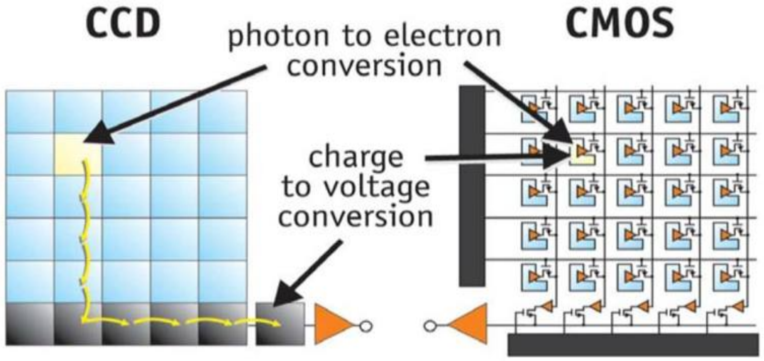
CCD vs. CMOS

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)



CCDs move photogenerated charge from pixel to pixel and convert it to voltage at an output node. CMOS imagers convert charge to voltage inside each pixel.

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

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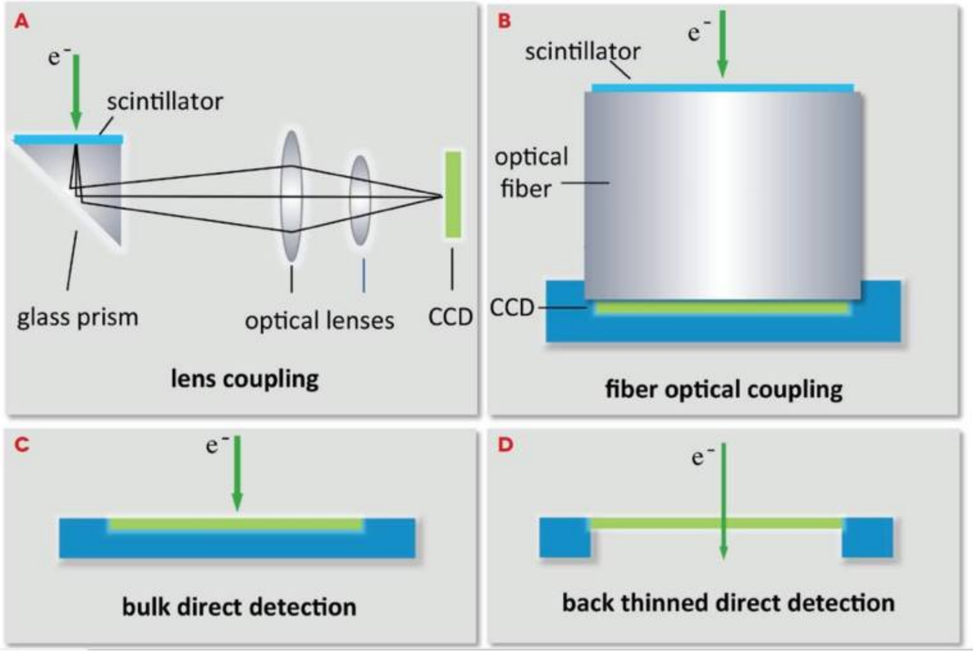
Detectors in Electron Microscopy

A. Optically coupled

B. Fiber-optic coupling

C. Direct detection

D. Transmission direct detection



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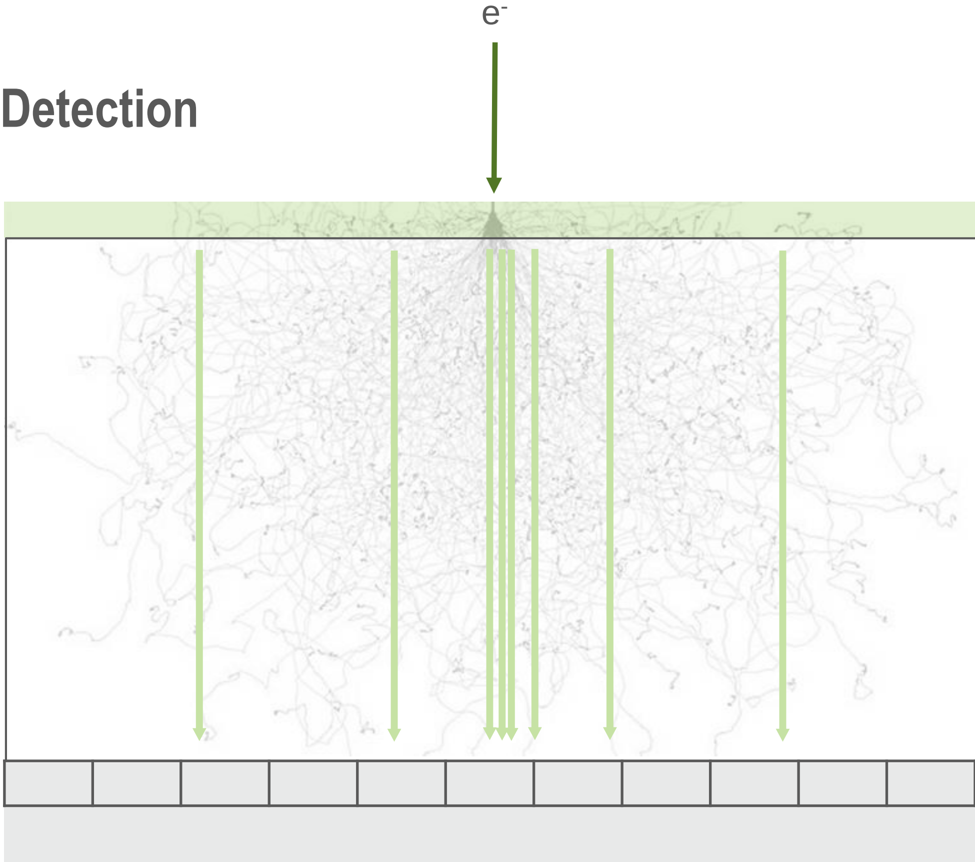
Traditional Indirect Detection

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

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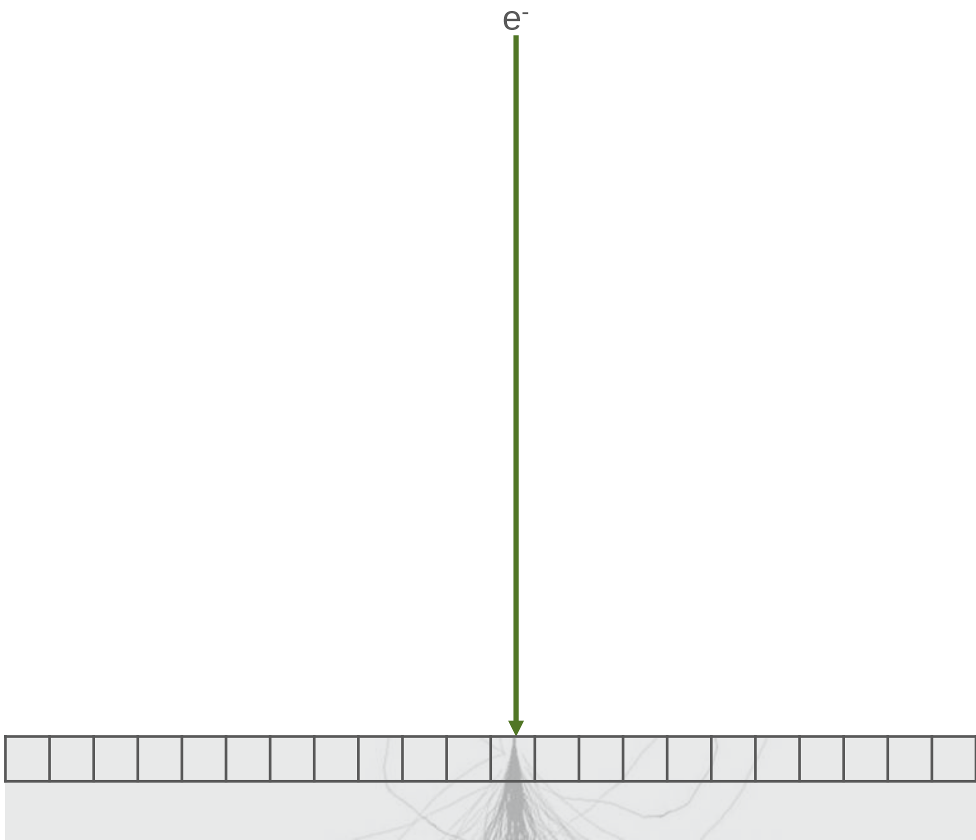
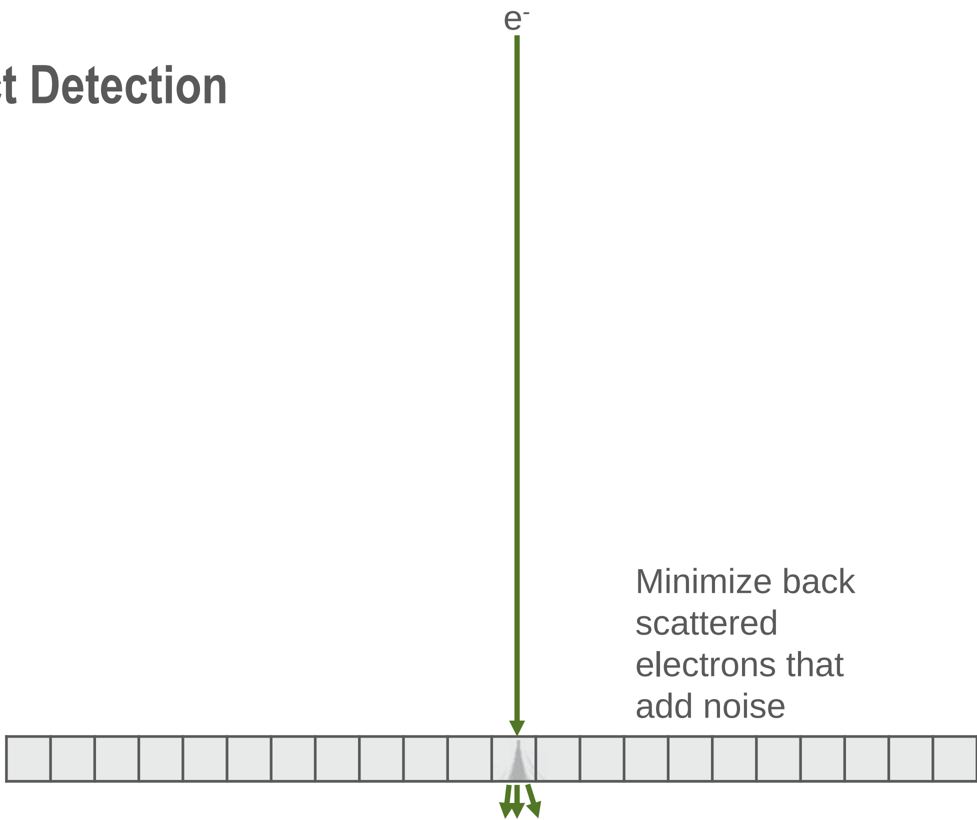


Transmission Direct Detection

1. ~~Convert electrons to light~~

2. ~~Transfer light to detector~~

3. Detect electron and convert to signal



Radiation hard, thinned CMOS

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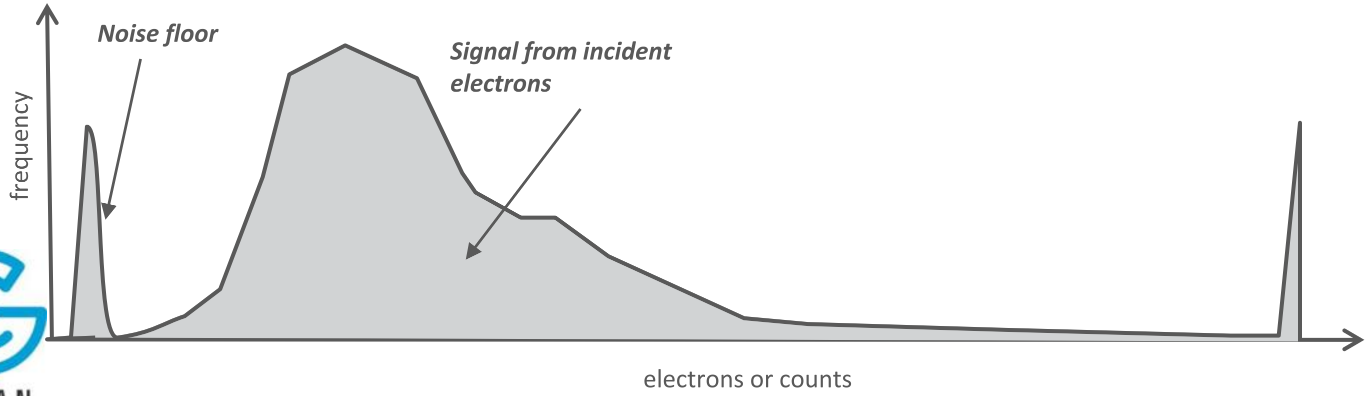
Sensitivity

- 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



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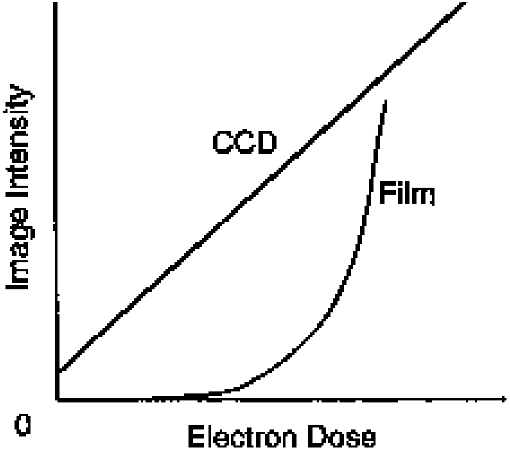
Linearity

- 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



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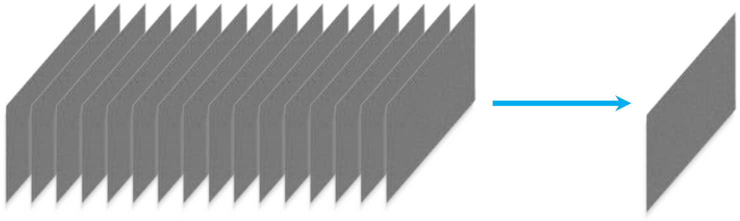
Journal of Microscopy, Vol. 200, Pt 1, 2000, pp. 1±13.

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



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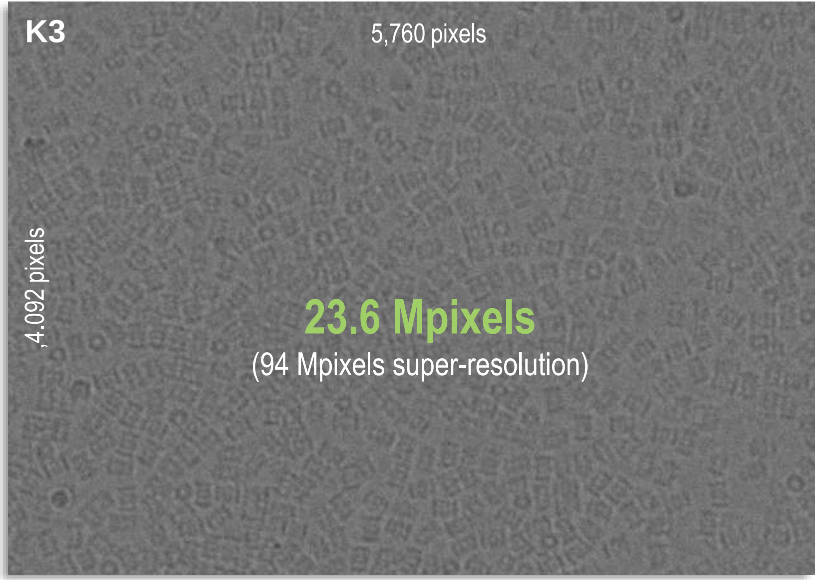
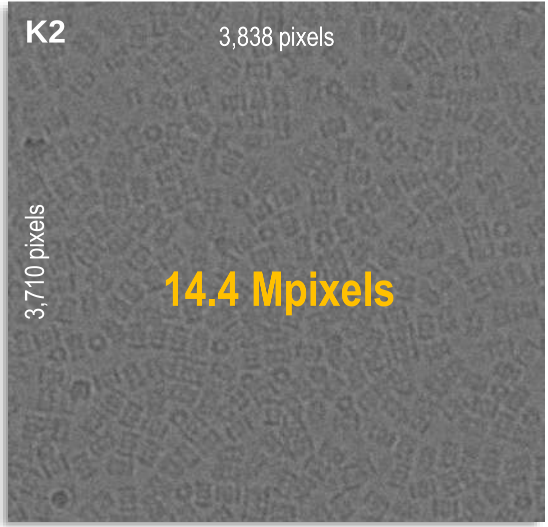
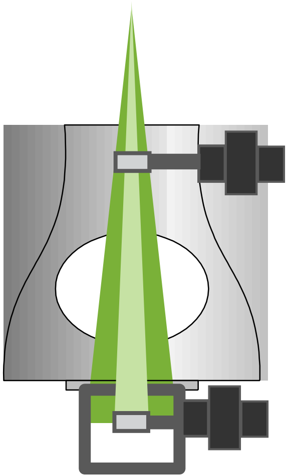
How Many Pixels are Enough?





How Important is FOV?

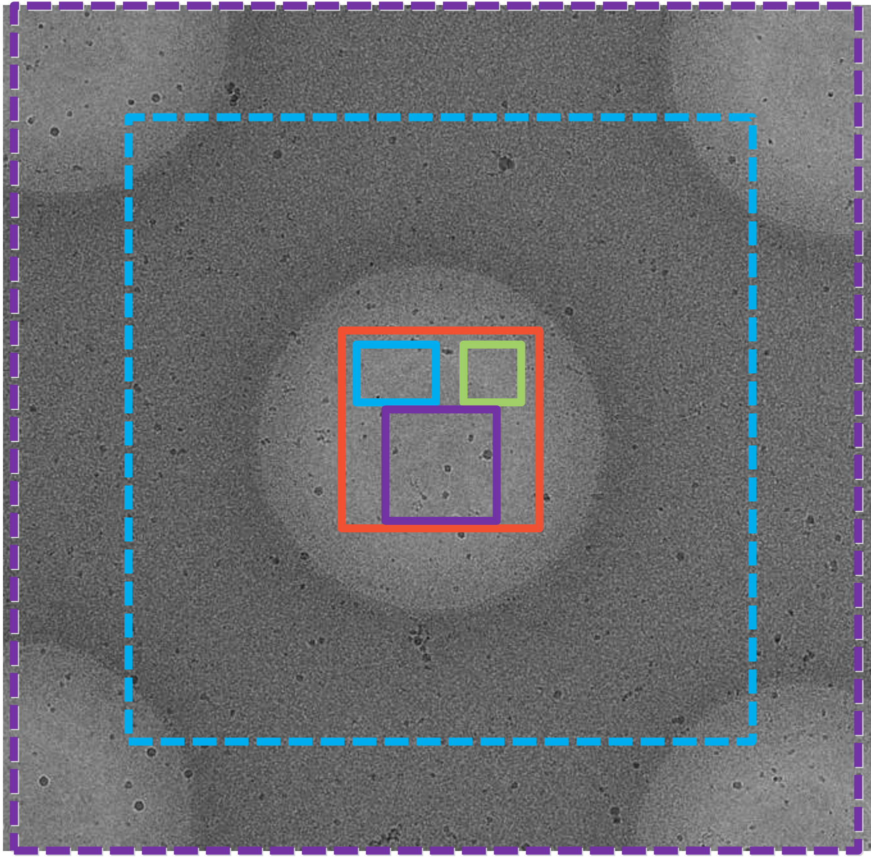


Side-mount camera

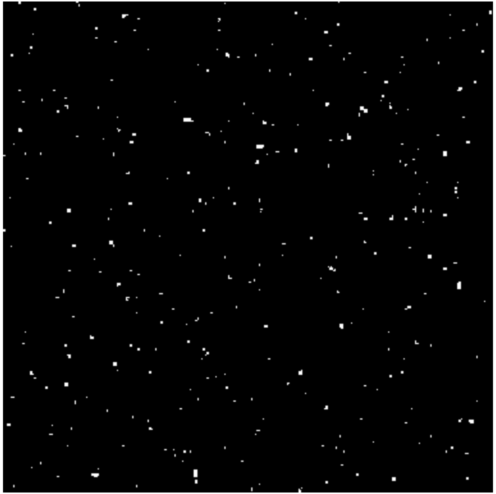
- Rio™ 16: 4k x 4k, 9 µm
- Rio 9: 3k x 3k, 9 µm

Bottom-mount camera

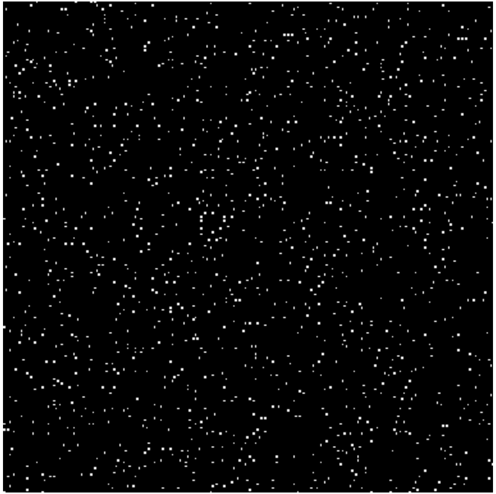
- OneView®: 4k x 4k, 15 µm
- Rio 16: 4k x 4k, 9 µm
- K3™ : 6k x 4k, 5 µm
- K2®: 4k x 4k, 5 µm



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.

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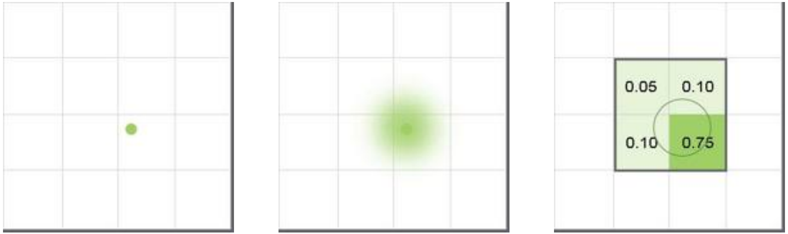
Traditional Integration

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.

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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.

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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.

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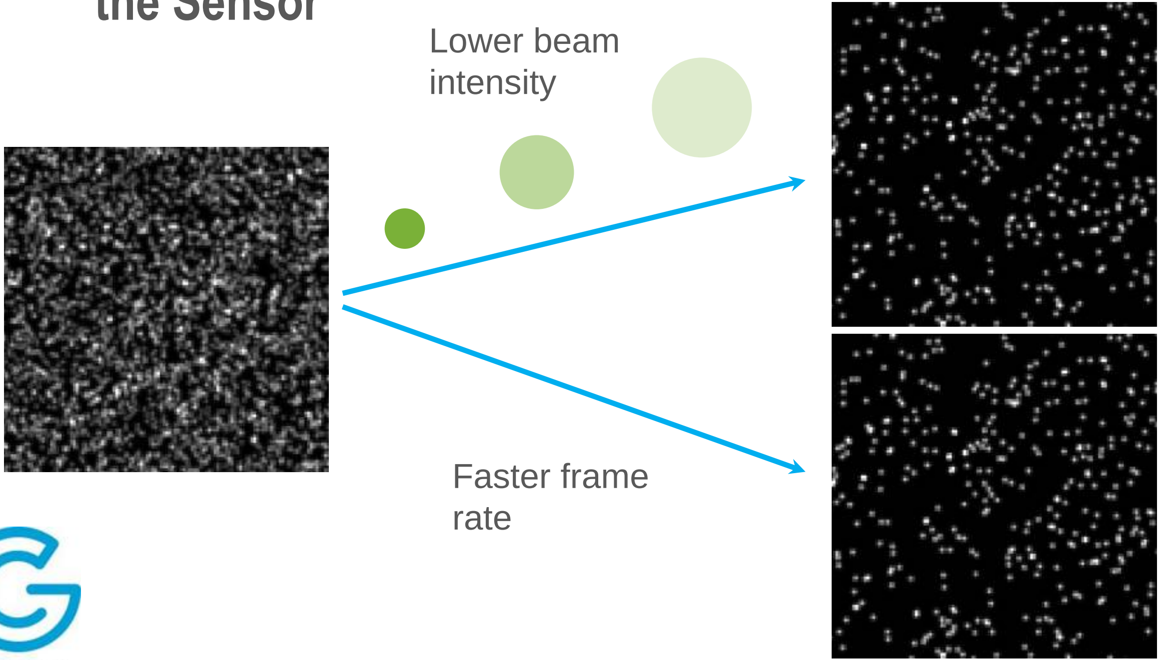
Electron Counting Requires that Electrons Don't Overlap on the Sensor



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


K3
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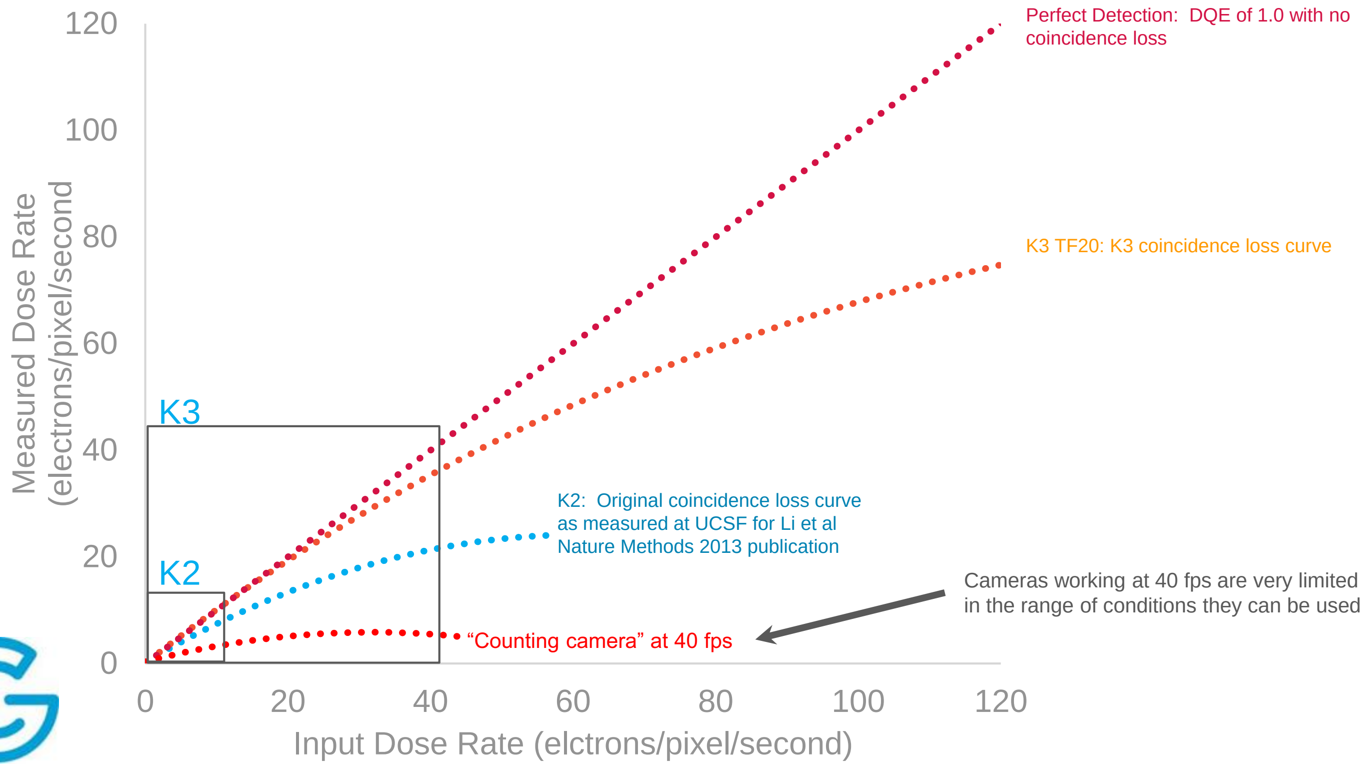

Perfect Detection: DQE of 1.0 with no coincidence loss
K3 TF20: K3 coincidence loss curve
K2: Original coincidence loss curve as measured at UCSF for Li et al Nature Methods 2013 publication
Cameras working at 40 fps are very limited in the range of conditions they can be used
"Counting camera" at 40 fps
K3
K2
Measured Dose Rate (electrons/pixel/second
Input Dose Rate (elctrons/pixel/second)
0
20
40
60
80
100
120

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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

$$\mathrm{DQE}(s)$$

$$= \frac{\mathrm{SNR}_{\mathrm{out}}(s)}{\mathrm{SNR}_{\mathrm{in}}(s)}$$

$$= \frac{\mathrm{MTF}(s)^2}{\mathrm{NPS}_{\mathrm{out}}(s)/\mathrm{Dose}_{\mathrm{in}}(s)}$$

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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

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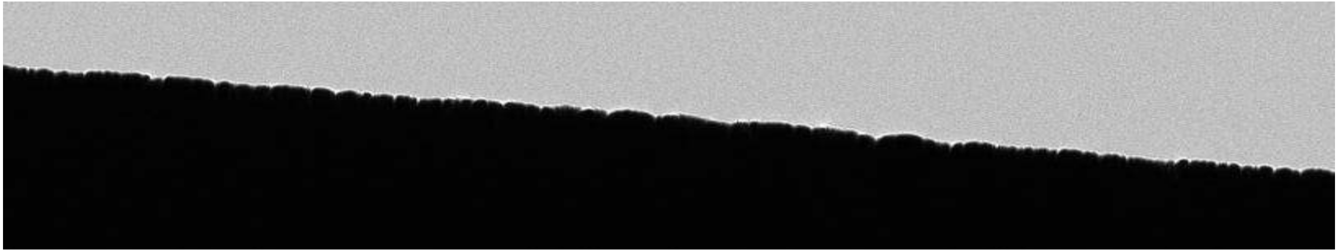
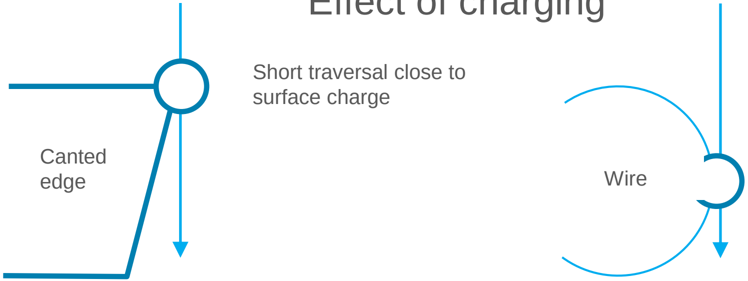
Measuring MTF with a Physical Edge (1)

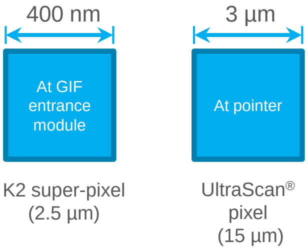


AuPd-coated and plasma cleaned edge with canted face mounted in the entrance aperture of a GIF Quantum® imaged on the K2 at the end of the GIF in super-resolution mode at 200 kV. (particularly bad example – not always this bad)

Effect of charging



Scale at edge



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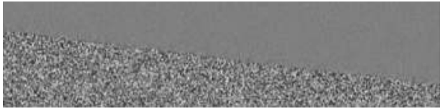
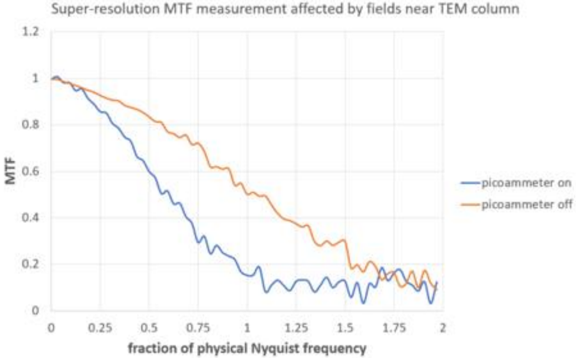
Measuring MTF with a Physical Edge (2)

Motion – edge creep

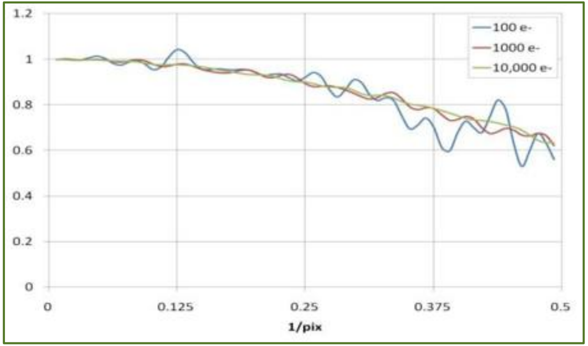


Good: difference between two 20 s edge images showing no “motion fringe”.

Motion – fields



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.

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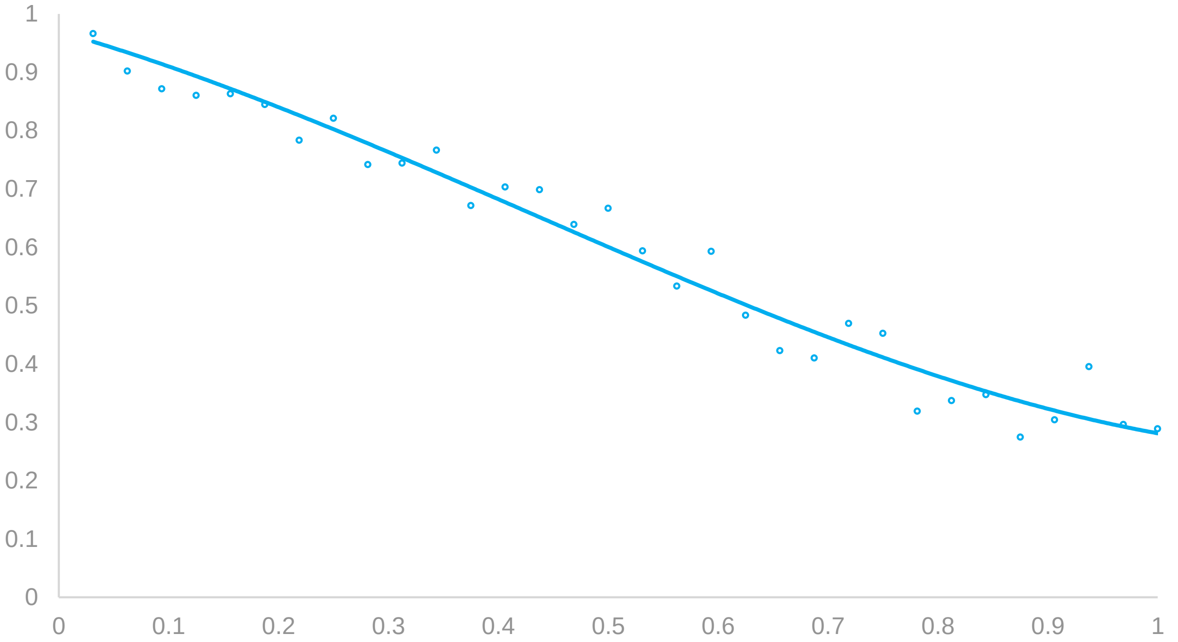
DQE and Binning



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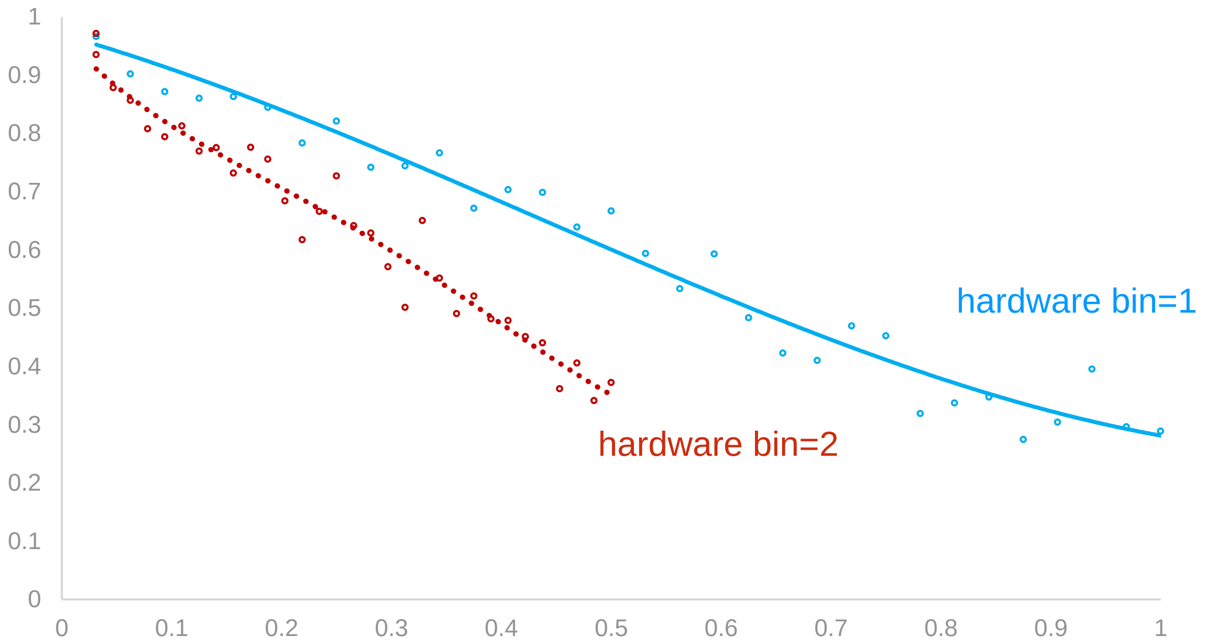
DQE and Binning



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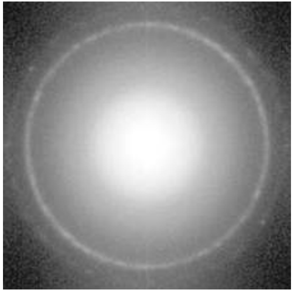
39kx



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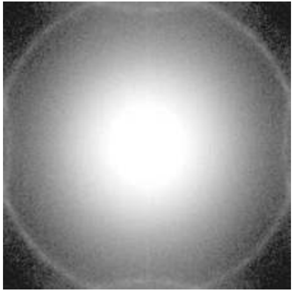
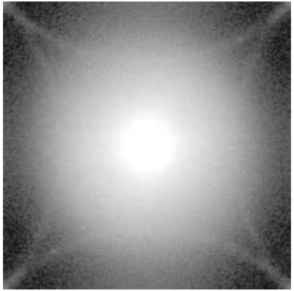
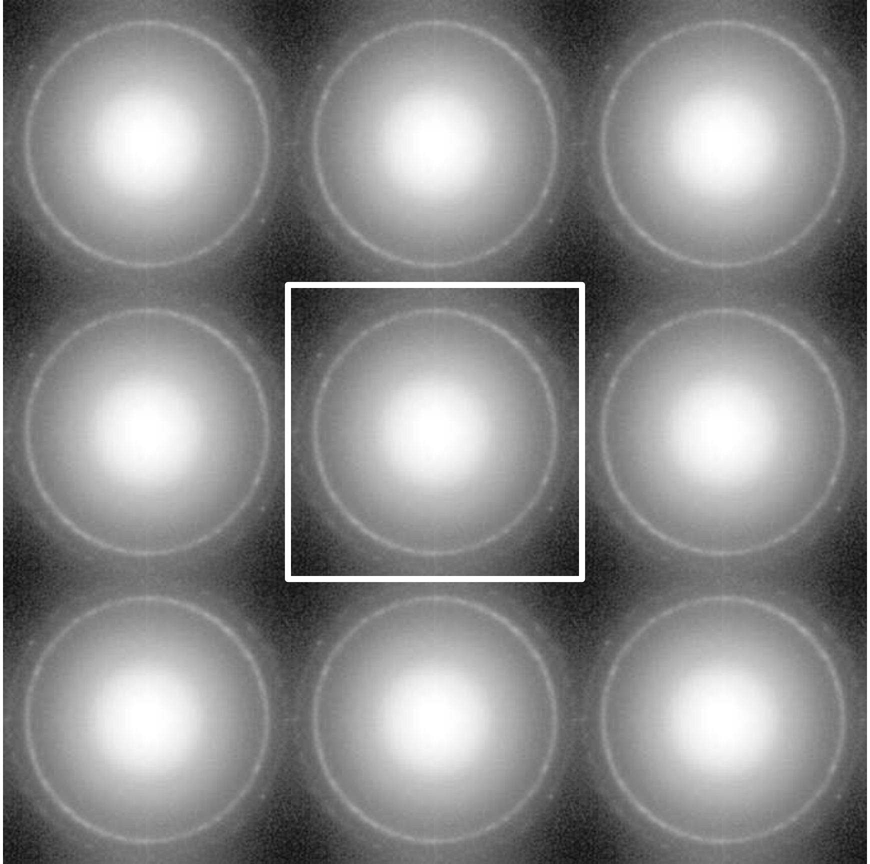
31kx



23kx



39kx



An FFT is calculated as though each image were bordered by an infinite number of neighbors

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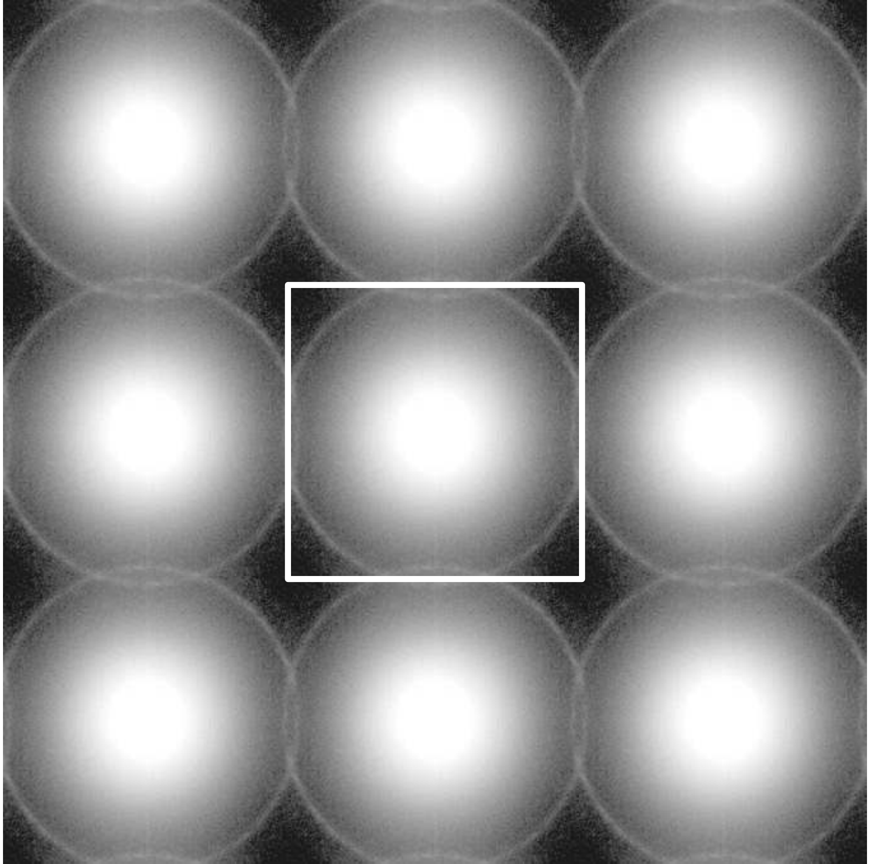
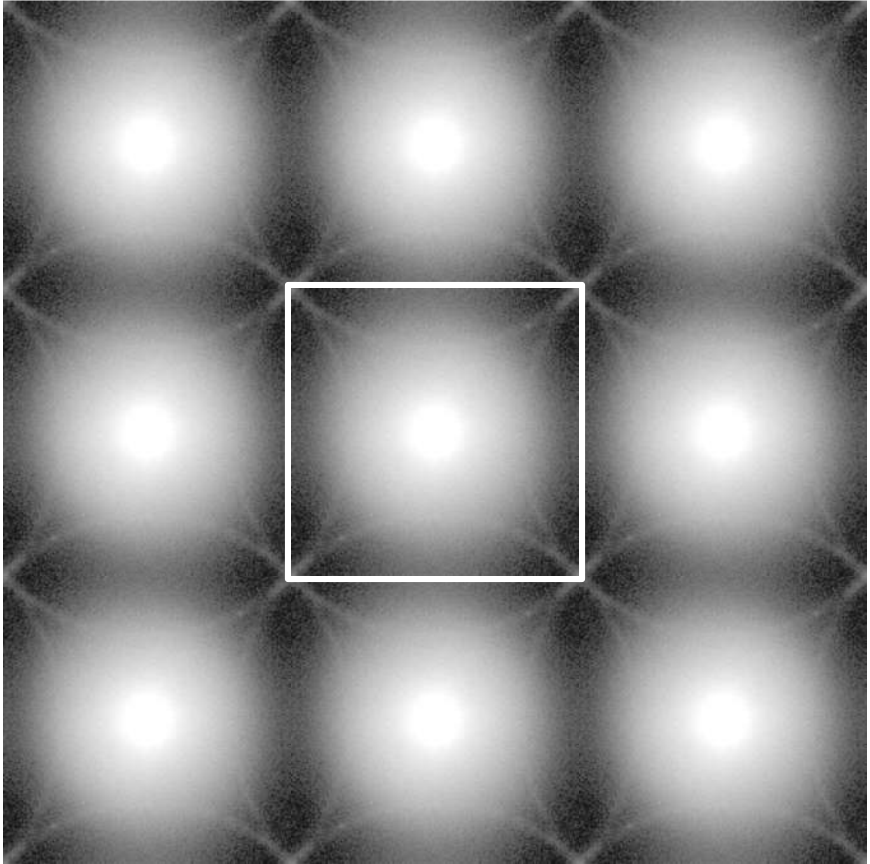


31kx

As you drop the magnification, the information from one region begins to show up in the neighboring region

23kx



It becomes very obvious with a strong signal at low magnification

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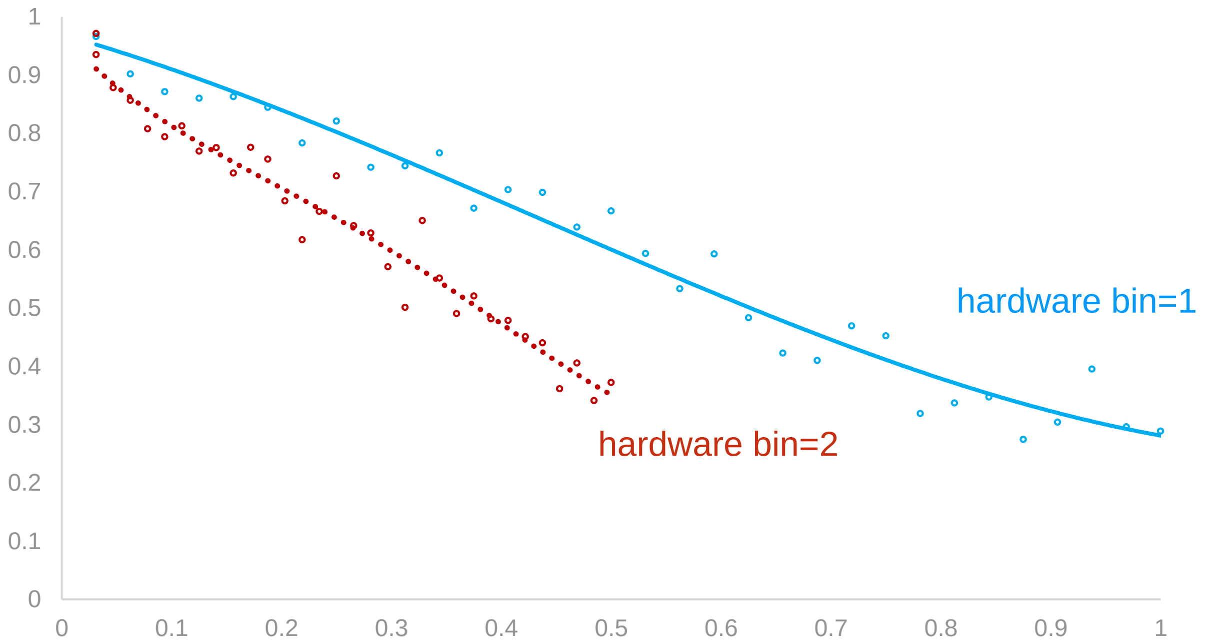
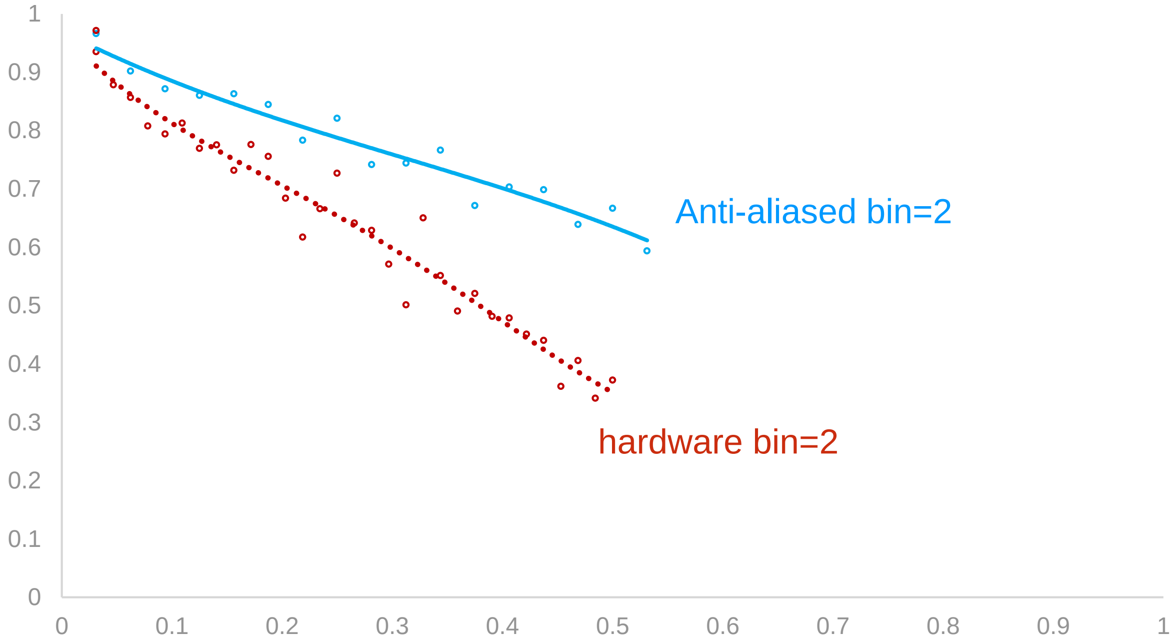
DQE and Binning



DQE and Binning



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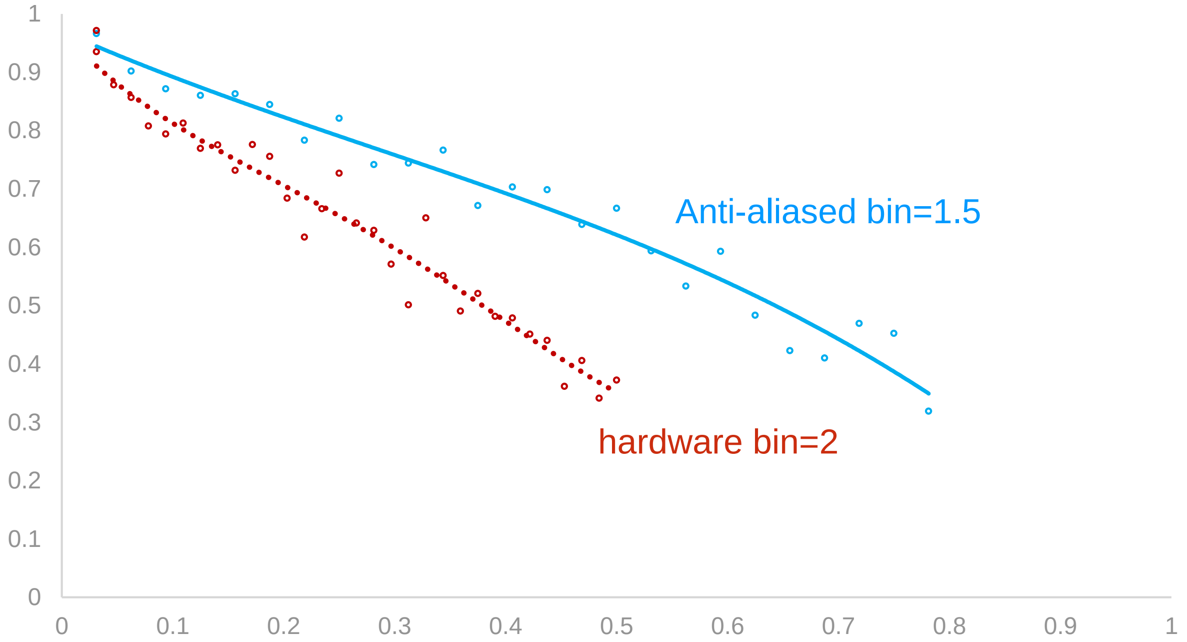
DQE and Binning



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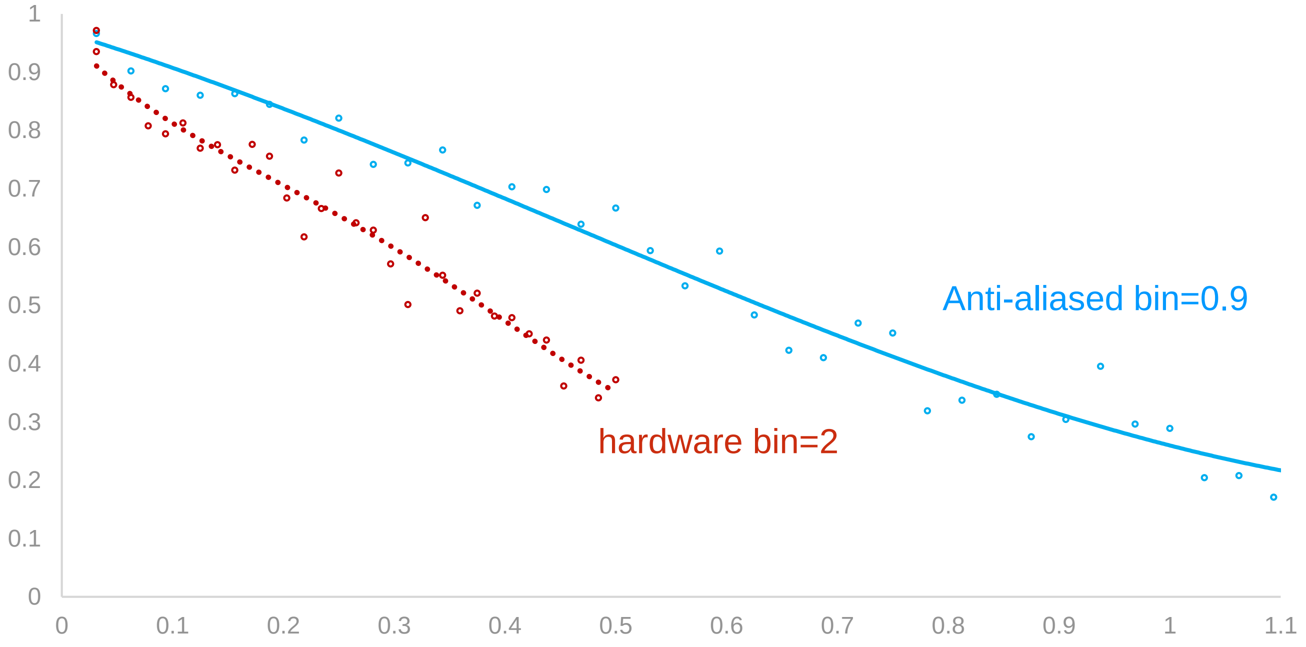
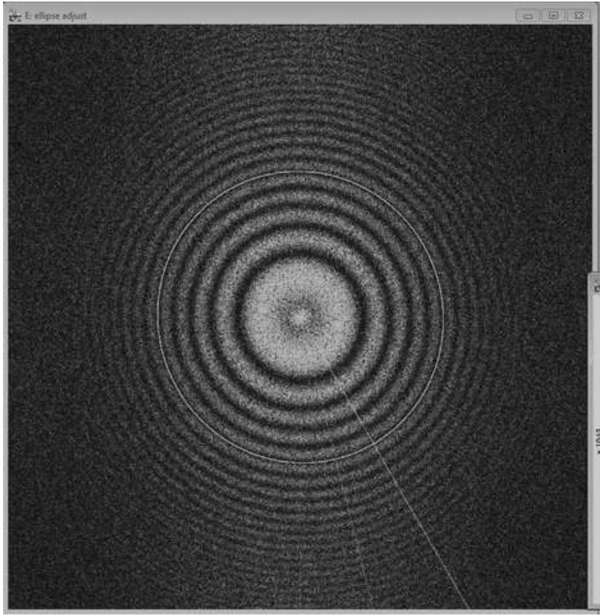
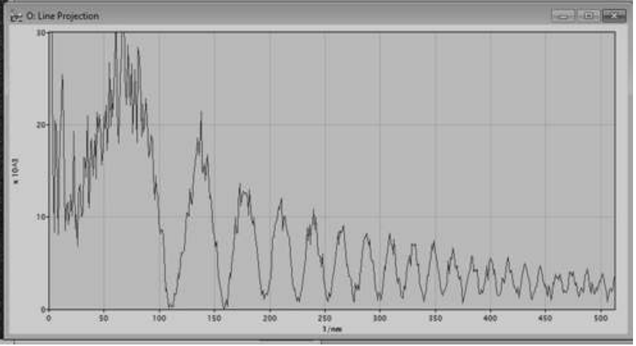
DQE and Binning



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.



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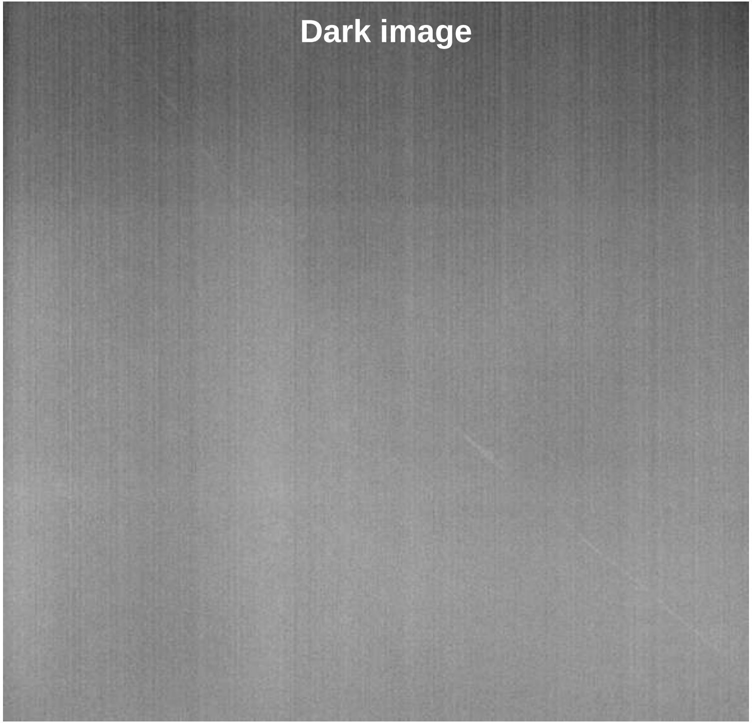
Practical Considerations in Data Collection

Dark Subtraction

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



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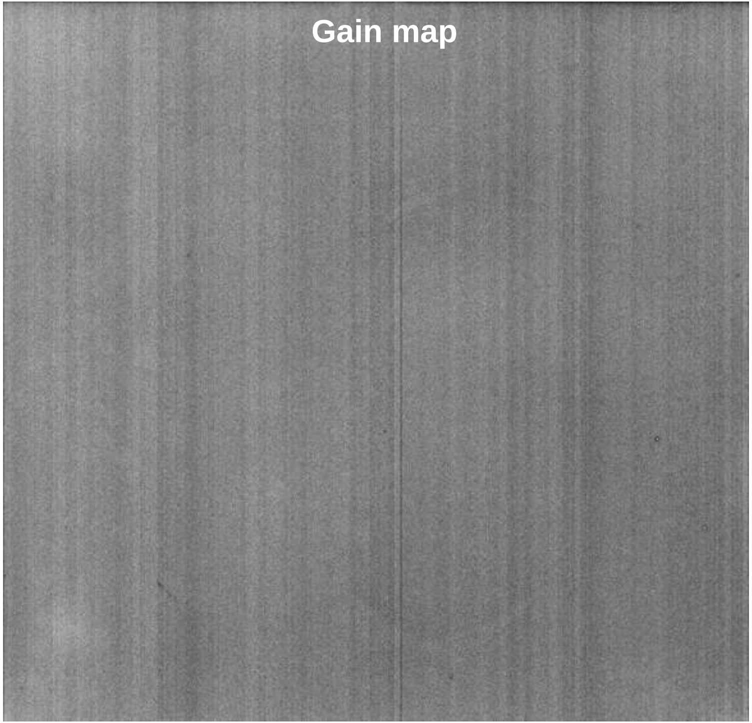
Gain Correction

- 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



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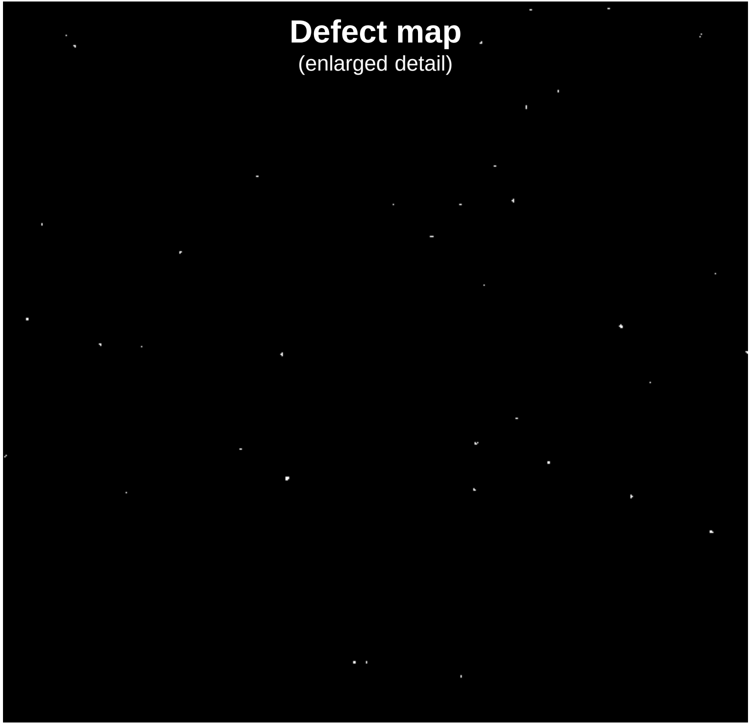
Defect Correction

- Removes poorly performing pixels
  - Hot
  - Dark
  - Unstable
- Defect pixels contribute to fixed pattern noise
- Usually updated with Gain Reference



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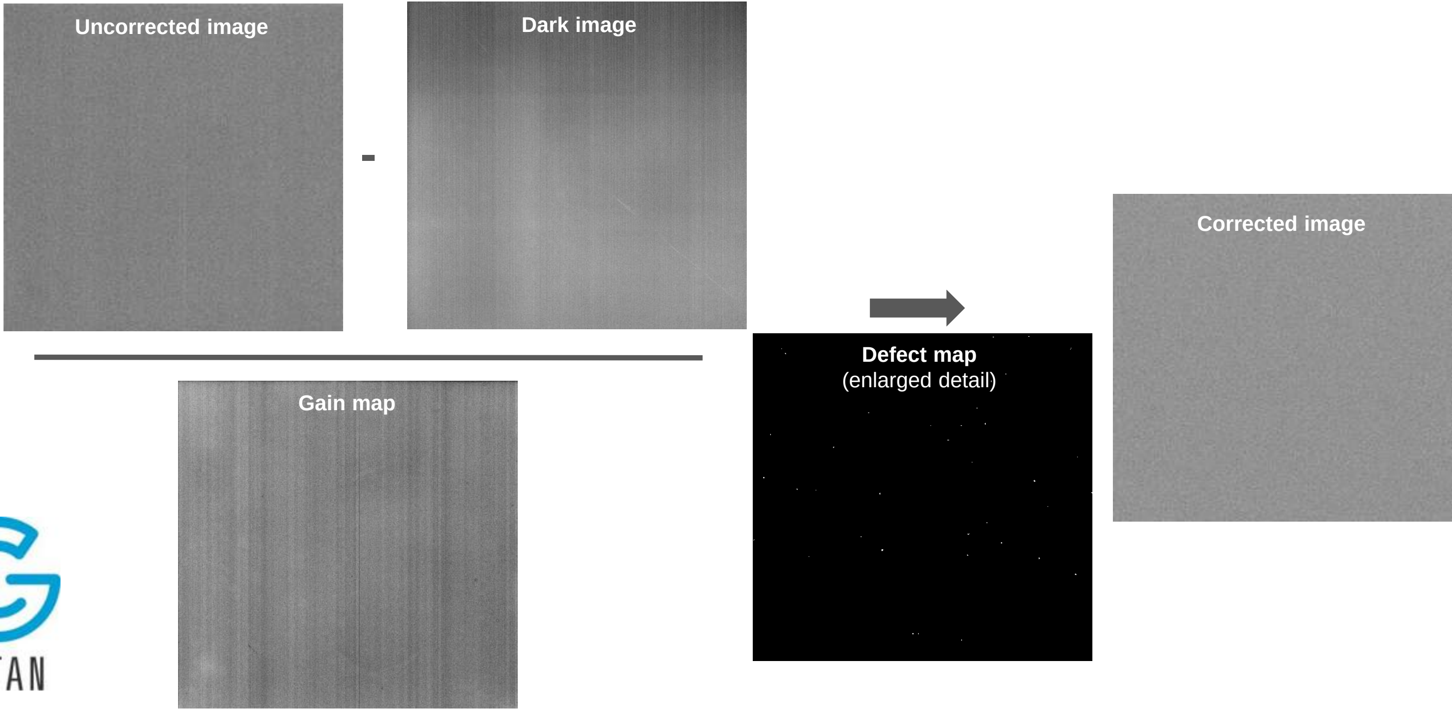
Typical Gain Correction Scheme

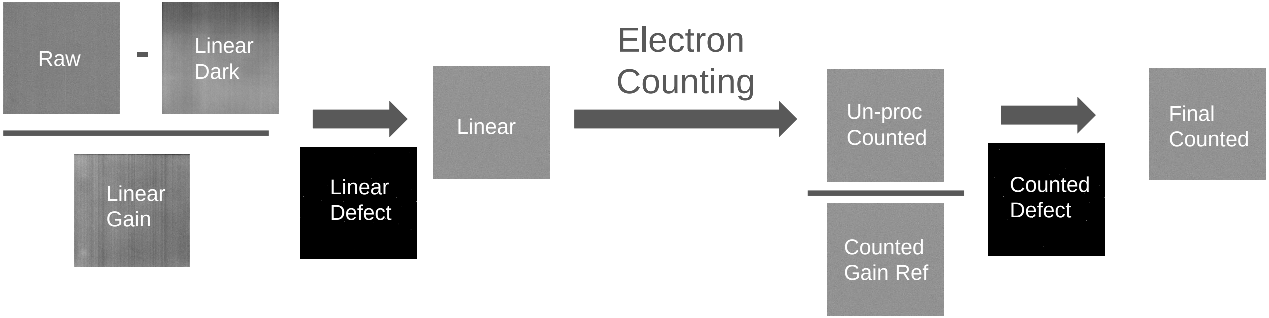


Counted Gain Correction Scheme

Linear Image Correction

Electron Counting

Counted Image Correction



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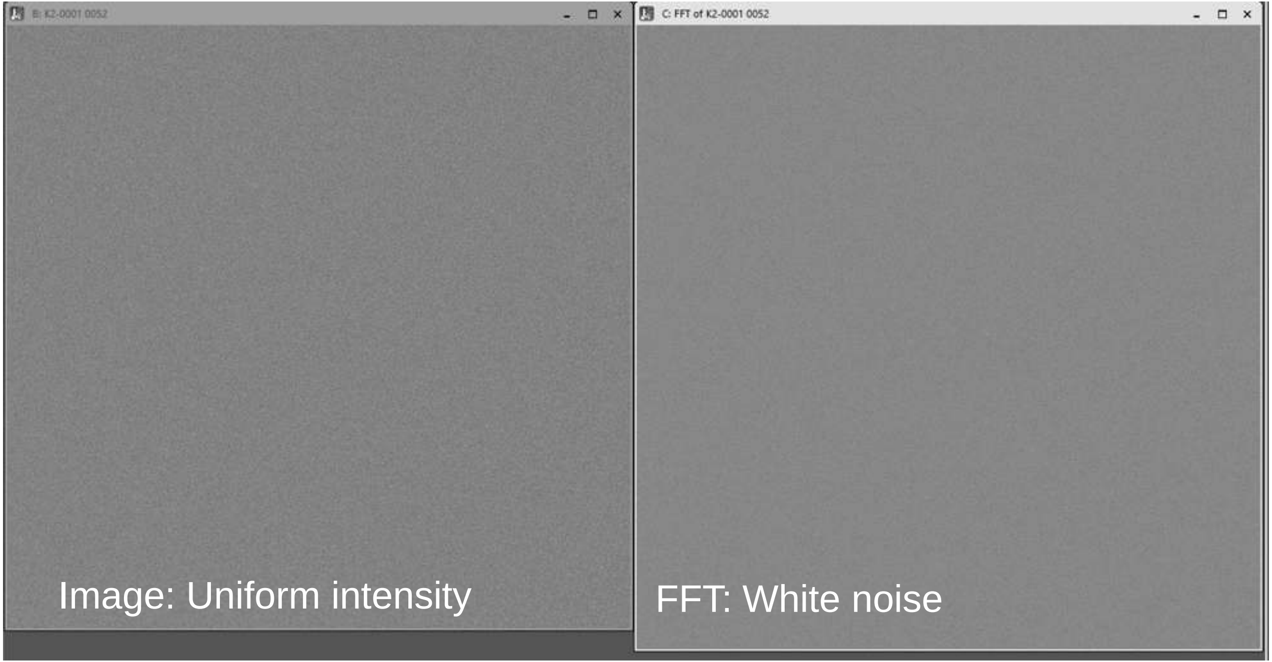
Checking the Quality of Image Correction



Measurement of Fixed Pattern Noise (FPN)

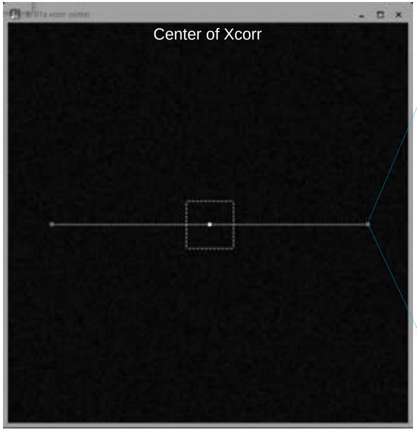
Uniform illumination

Common defects, dark image and gain image

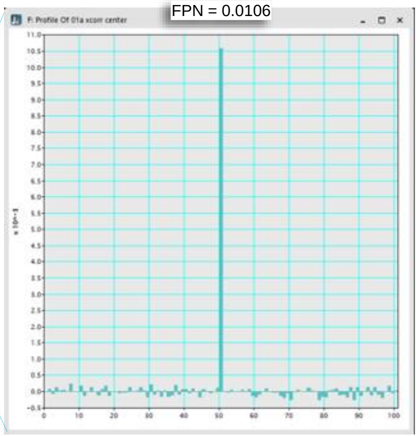
Frame rate = 75 fr/s, (0.0133156 s/fr), all images.

Total dose = **14 e/pix**, all images

Uniform A $\otimes$ Uniform B

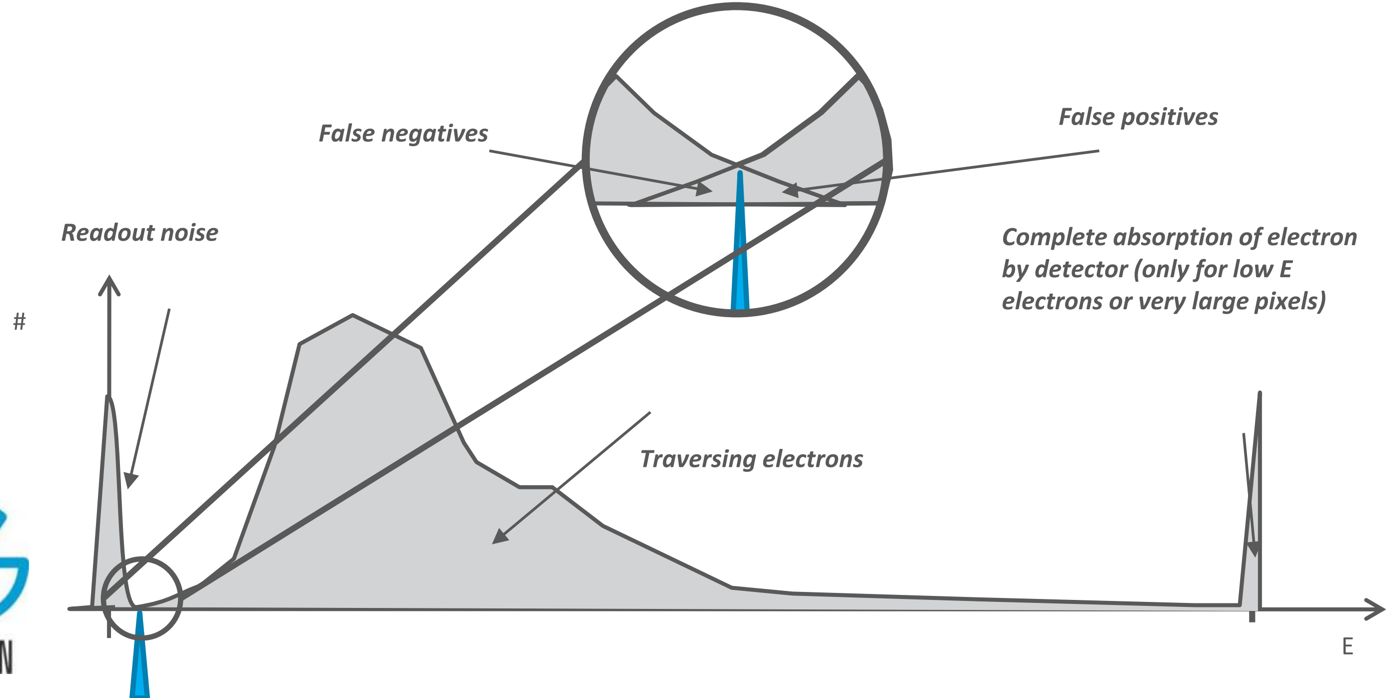


Cross-correlation map



FPN = peak pixel value

Improved Noise Also Allows us to Improve Electron Countability



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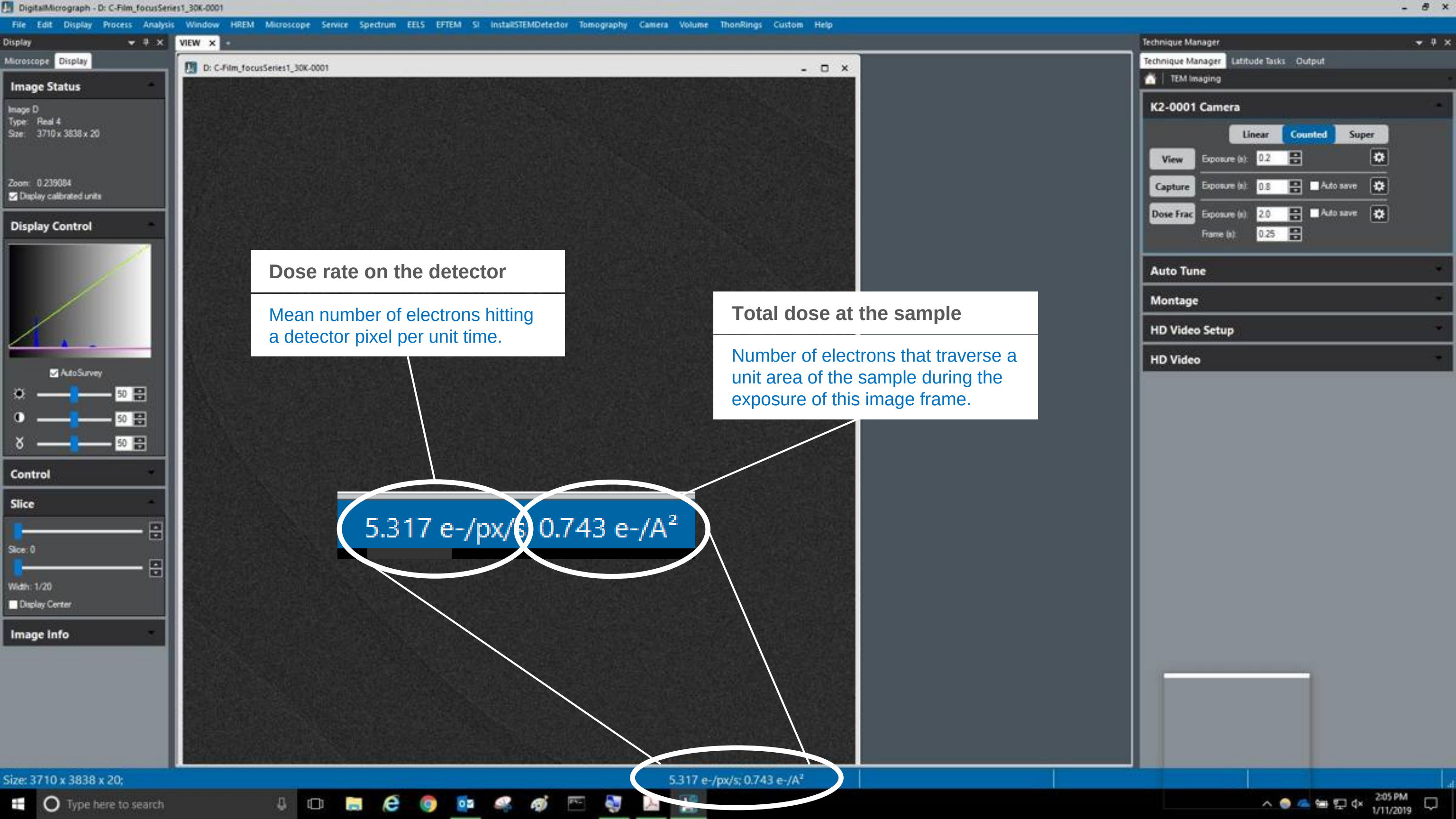
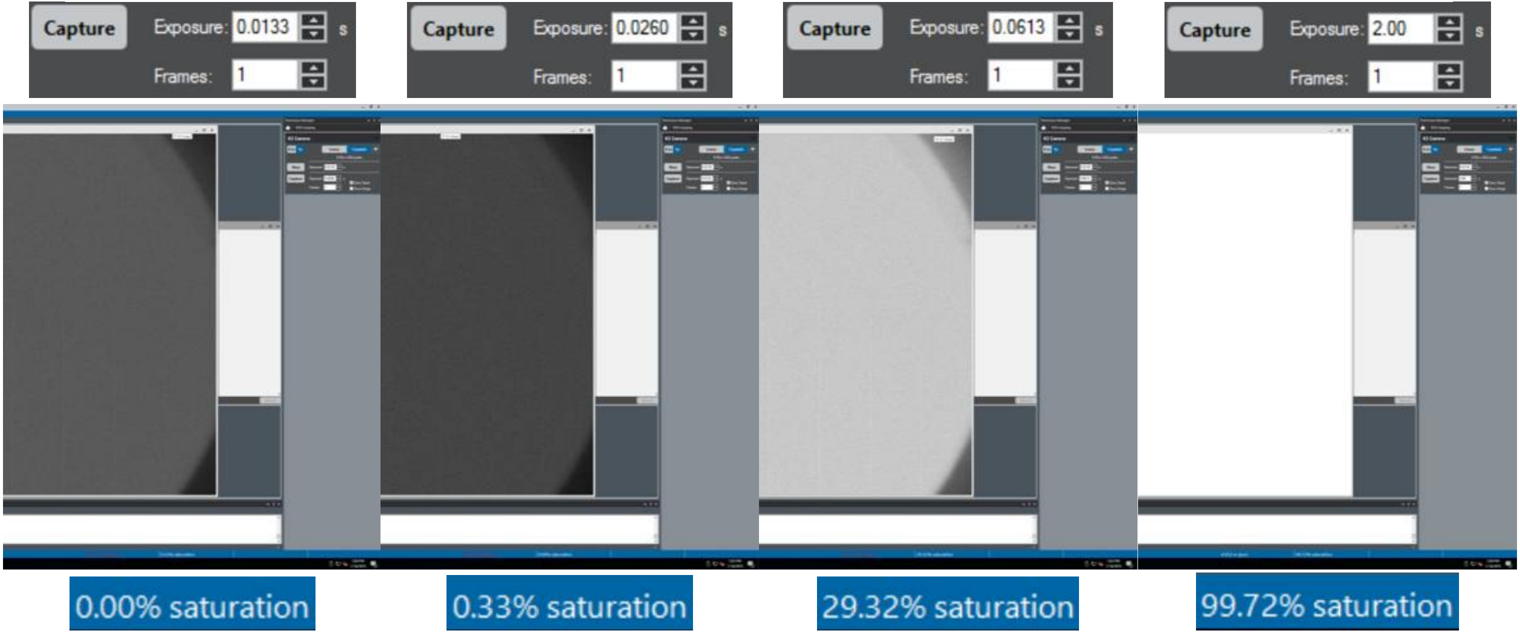
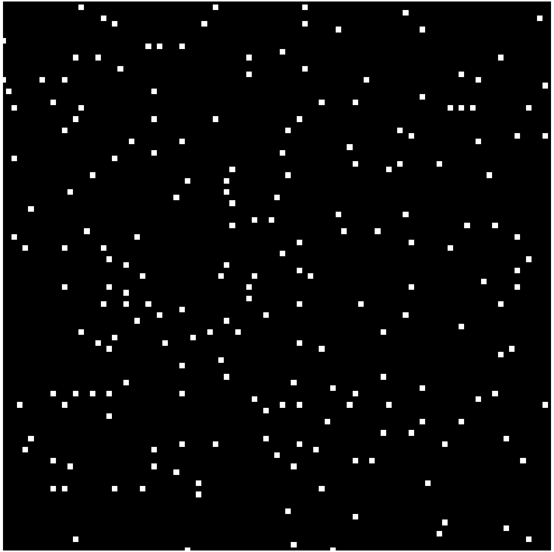

DigitalMicrograph - D: C-Film_focusSeries1_30K-0001
D: C-Film_focusSeries1_30K-0001
Image Status
Image D
Type: Real 4
Size: 3710 x 3838 x 20
Zoom: 0.239084
Display calibrated units
Display Control
AutoSurvey
Control
Slice
Slice: 0
Width: 1/20
Display Center
Image Info
Dose rate on the detector
Mean number 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-/A²
Technique Manager
Latitude Tasks
Output
TEM Imaging
K2-0001 Camera
Linear
Counted
Super
View
Capture
Dose Frac
Exposure (s):
Frame (s):
Auto save
Auto Tune
Montage
HD Video Setup
HD Video
Size: 3710 x 3838 x 20;
5.317 e-/px/s; 0.743 e-/A²

Keeping track of Pixel Saturation in K3

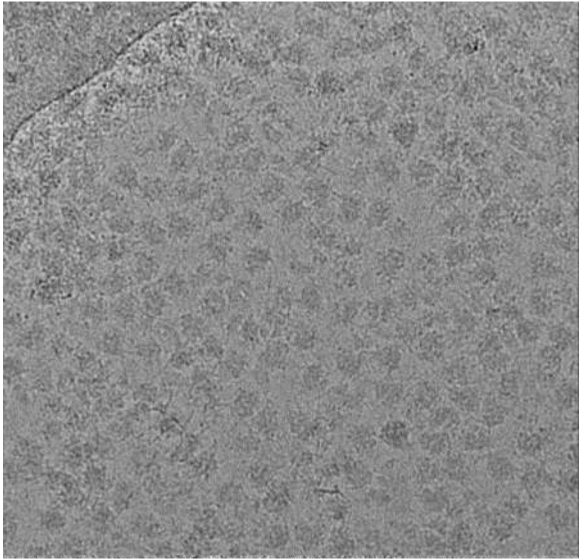
At 8-bit/pixel, gain-corrected data saturates with a value of 255.
The saturation monitor reports the percentage of pixels that have reached saturation in a single frame.



How Frame Alignment Works



Raw counted frame



Final aligned image

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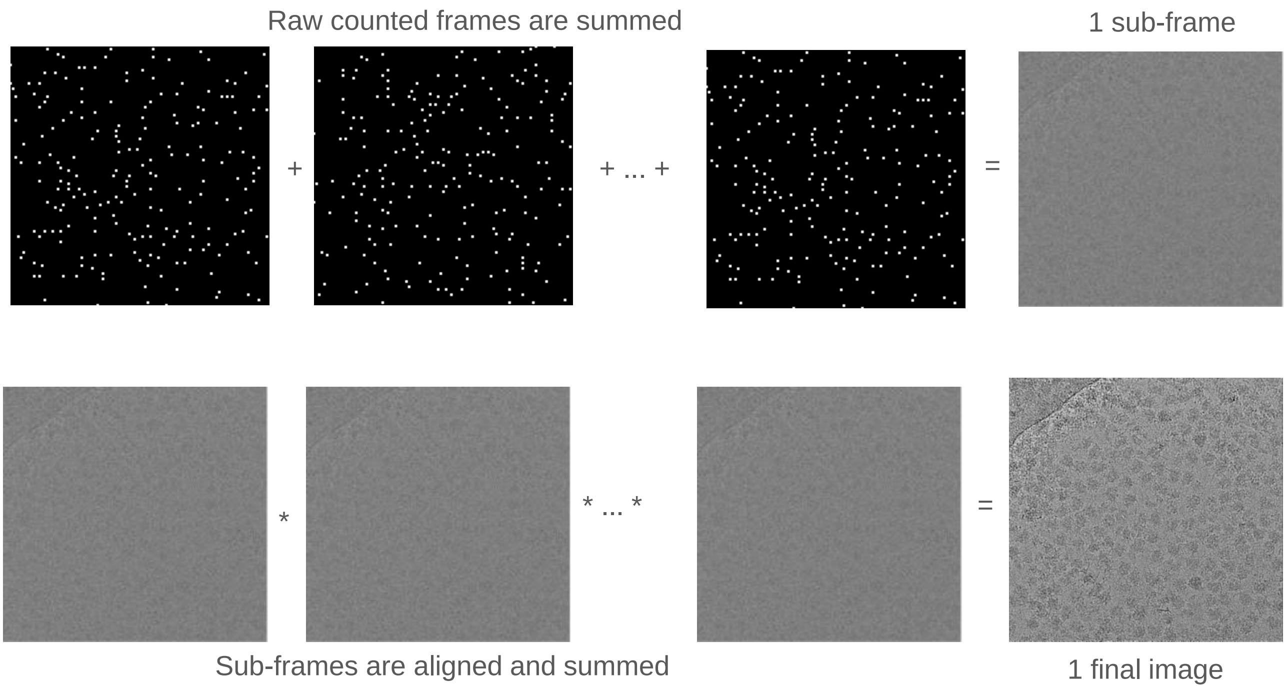
Raw counted frames are summed
1 sub-frame
+
+ ... +
=
*
* ... *
=
Sub-frames are aligned and summed
1 final image
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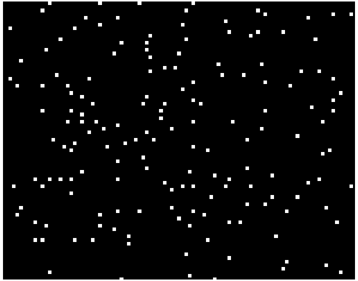
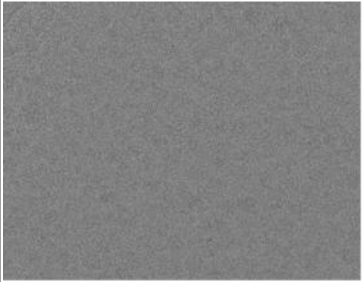
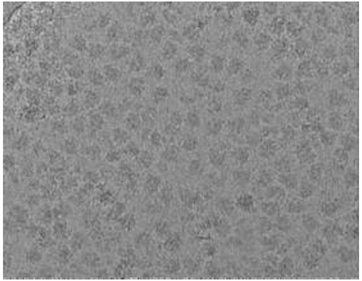
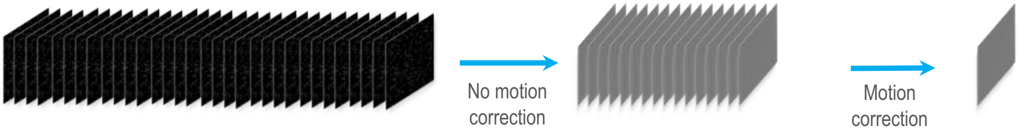
	Sensor Frame Rate	Dose Rate	 Counted Frame	 Sub-frame	 Summed/Aligned Frame
Other	40	0.8 e/pix/s	0.025 s	1 s (1 fps)	100 s
K2	400	8 e/pix/s	0.0025 s	0.1 s (10 fps)	10 s
K3	1500	30 e/pix/s	0.00066 s	0.027 s (37 fps)	2.7 s



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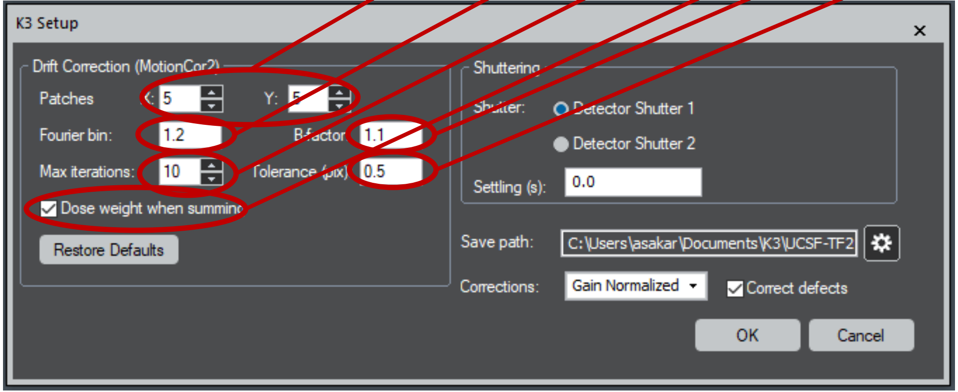
MotionCor2 on the K3

MotionCor2 -InMrc Stack.mrc -OutMrc CorrectedSum.mrc -Patch 5 5 -FtBin 1.2 -Iter 10 -FmDose 1.2 -bft 1.1 -Tol 0.5



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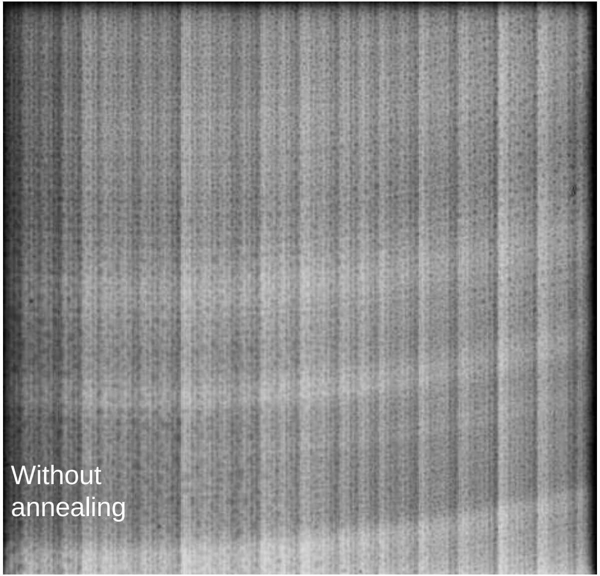
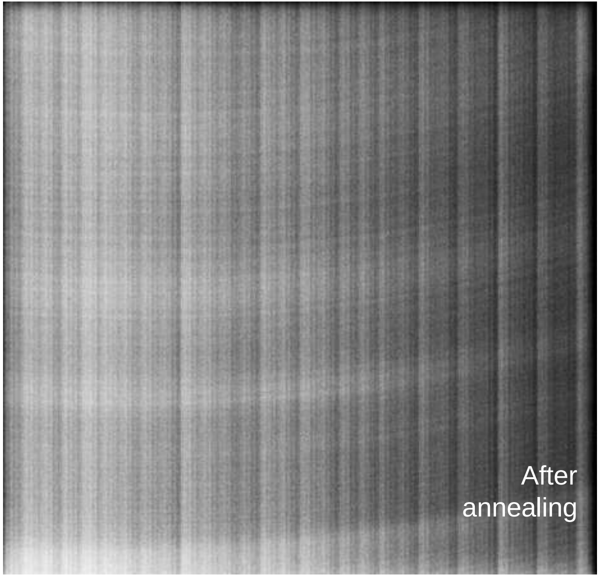
Annealing Prevents Contamination Buildup

- 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





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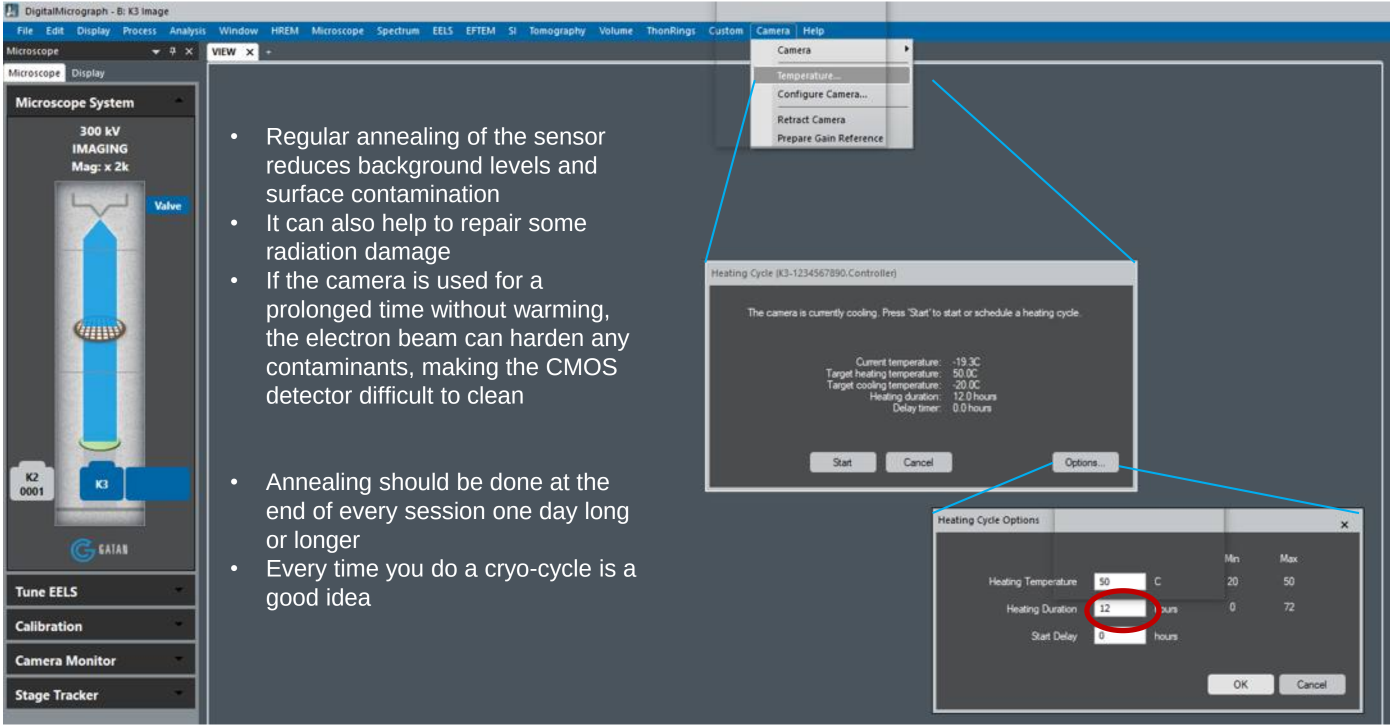
Camera Heating/Annealing



- 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

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Future Directions for Electron Detection

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Throughput is Going to be Critical

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

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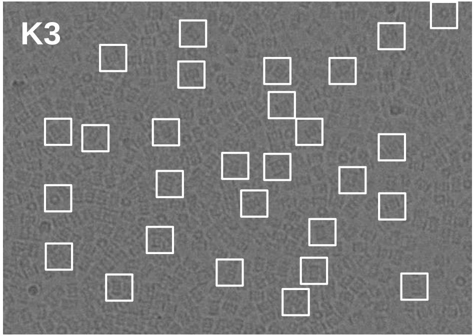
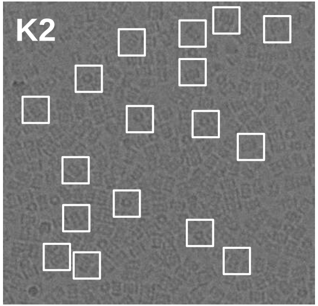
Improving Throughput: Larger Sensors

- One chance to expose a specimen area
- If the pixel quality is high, larger sensors reduce the number of images needed





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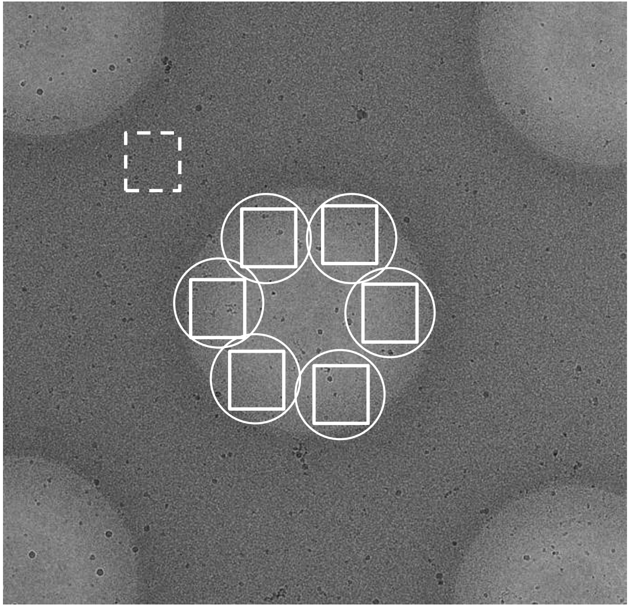
Improving Throughput: Faster Sensors

- 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

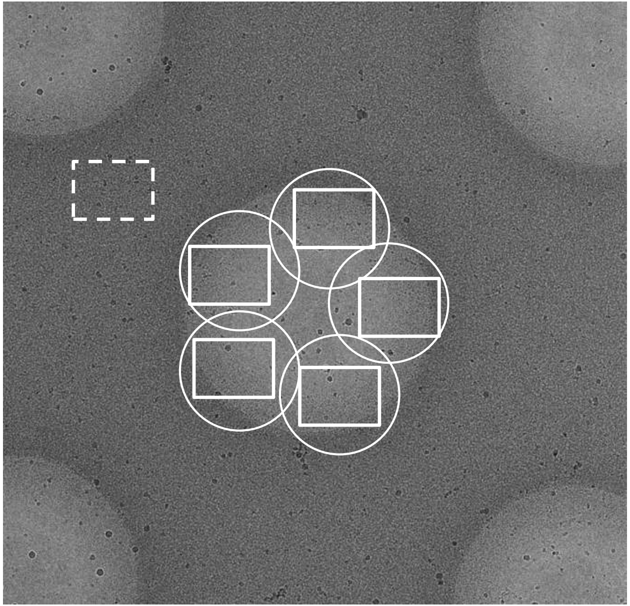


Improving Throughput

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

- K2: 6 images x 10 s = 60 s

- K3: 5 images x 2.5 s = 12.5 s
- K3: area is 25% larger



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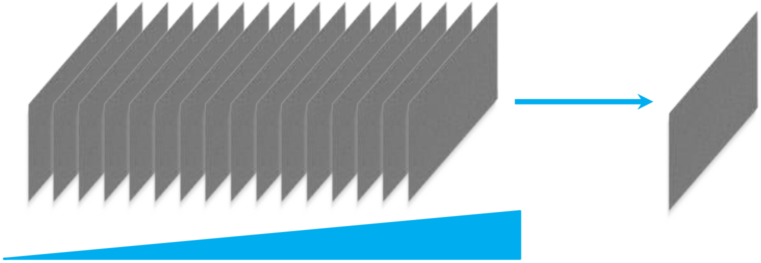
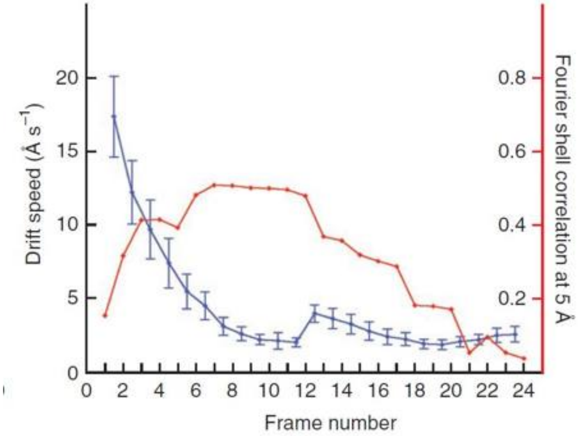
Better Data Through Motion Correction

- Sample damage increases during the exposure
- The first frames have the least damage but the most drift





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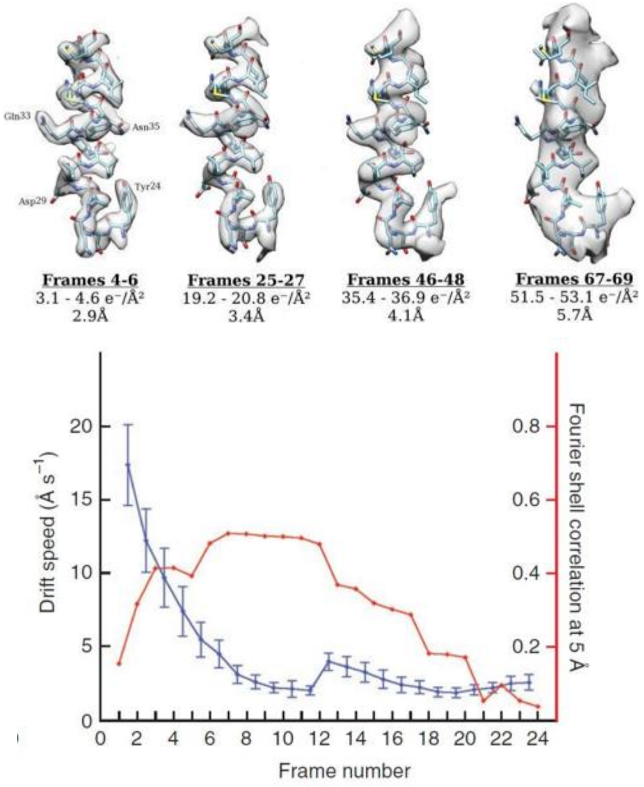
Better Data Through Motion Correction

- 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 !



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Thank You

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