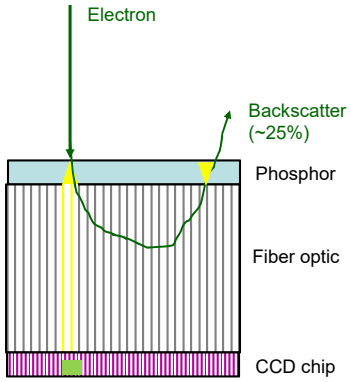
Using Direct Detectors with SerialEM

What Was Wrong with CCD Cameras?

- Spreading of signal in phosphor
- A bit more spreading and loss in fiber optic
- Variability in number of photons caught by CCD
  - Reduces ability to know how many primary electrons contributed to integrated signal
- At higher voltages, 20-30% of electrons backscatter and give strong signal at wrong place
- Readout is slow: accumulate whole image and read out once



Camera Efficiency: DQE

- DQE (detector quantum efficiency) is a factor (0 to 1) measuring how well a camera detects electrons compared to an ideal detector
- Inverse of DQE is how much extra dose is needed to get the same signal-to-noise ratio (SNR) as an ideal detector would give
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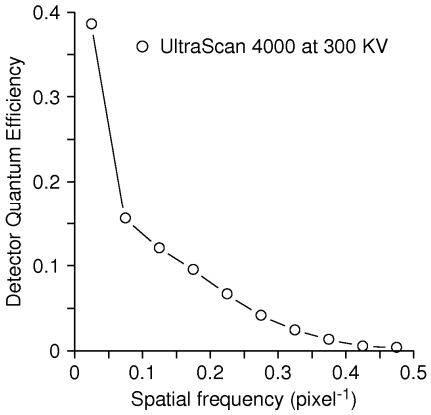
- DQE is actually a function of spatial frequency



Direct Electron Detection with CMOS "Monolithic Active Pixel Sensors"

- Primary electron generates 100-300 electrons in P- epilayer
- Electrons collect in closest well and have to be read out frequently
- Backscatter still a big problem unless substrate is severely thinned (from 700 μm to 30-50 μm

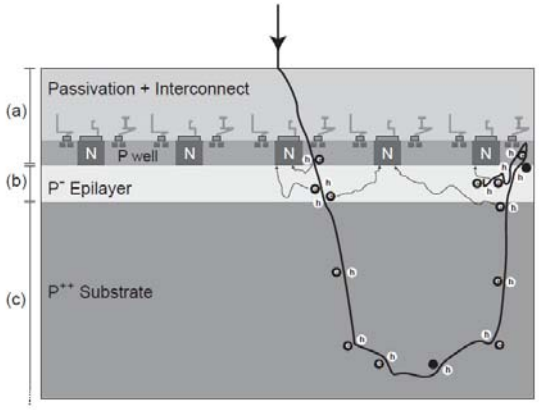
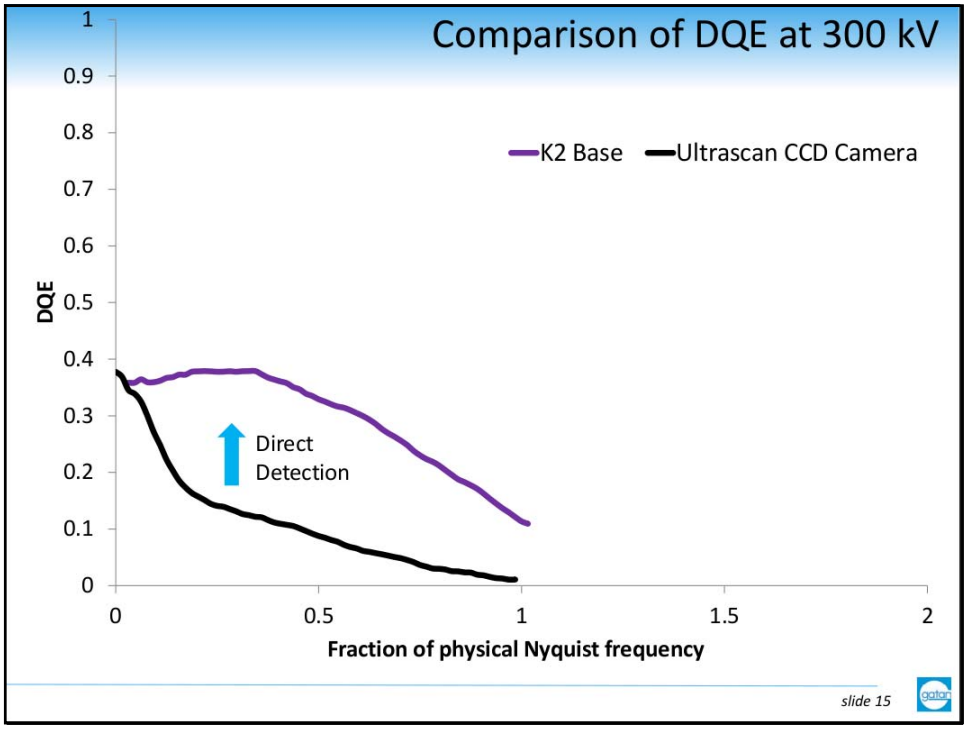
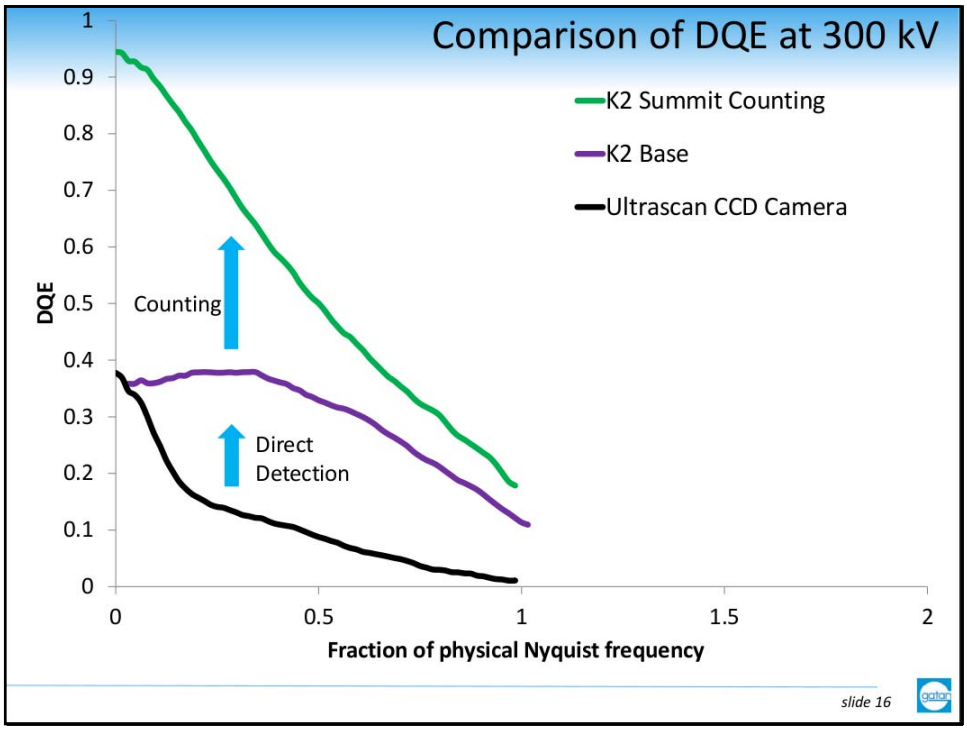


Fig. 1. Schematic of MAPS detector shown in cross-section. The detector has three main regions: (a) about 5-μm-thick passivation layer plus interconnections for readout electronics in the P well, (b) a few microns of lightly doped epilayer where the useful signal is generated, and drifts on to N wells prior to being read out, and (c) the main bulk of the detector, the substrate, which is heavily doped and which does not play a significant role in the detection process. A possible path for a single incident high-energy electron is shown to illustrate the problem with backscatter from the substrate.

Why Is Electron Counting Good?

- Integrating variable-sized packets reduces the DQE
- Packets spread over several pixels and this reduces the resolution of an integrated signal
- Deducing where each electron occurs eliminates variability in measured events and spread of signal
- Using counting also reduces effects of noise and crap in signal read out from chip


Comparison of DQE at 300 kV
K2 Base
Ultrascan CCD Camera
DQE
Direct
Detection
Fraction of physical Nyquist frequency
slide 15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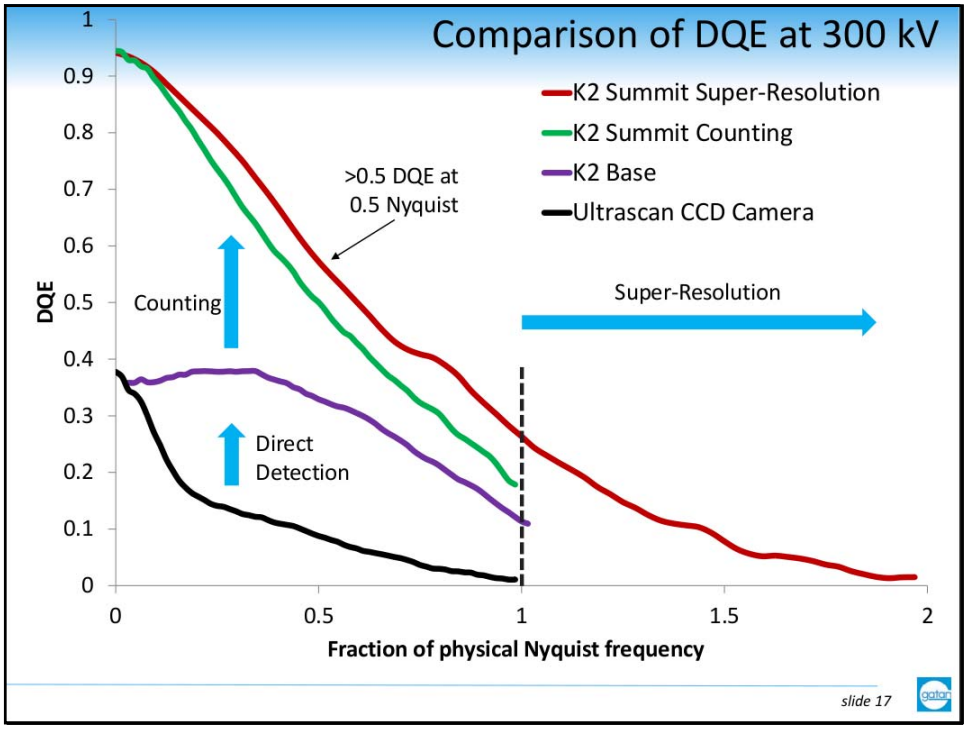
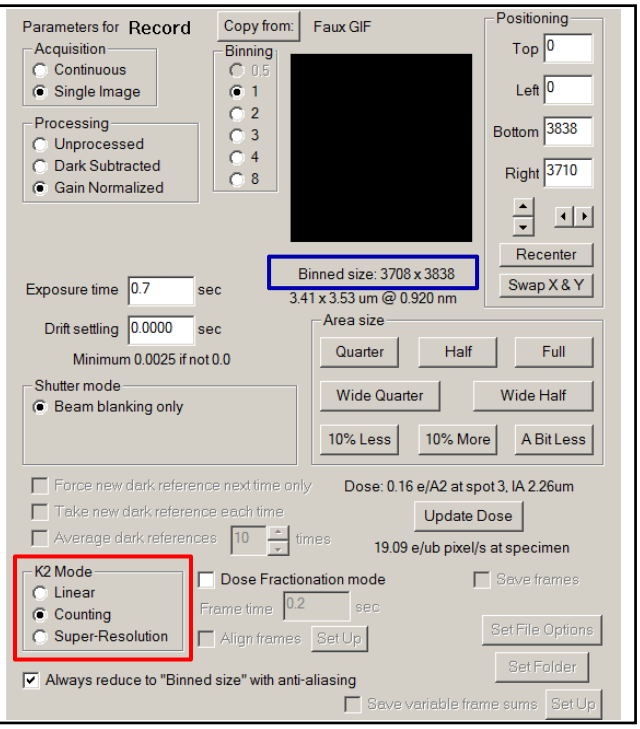


Alignment of Multiple Frames

- The other major feature of direct detectors is the ability to break one image acquisition into multiple frames
- When these frames are aligned, the effects of drift can be reduced or eliminated and higher-resolution information is preserved
- There are several options for frame alignment
  - Motioncorr and Motioncor2 from UCSF
  - Unblur from Grigorieff group
  - Alignframes in IMOD
    - Incorporates features from Motioncorr and Unblur
    - Should be good for tilt series data AND more convenient

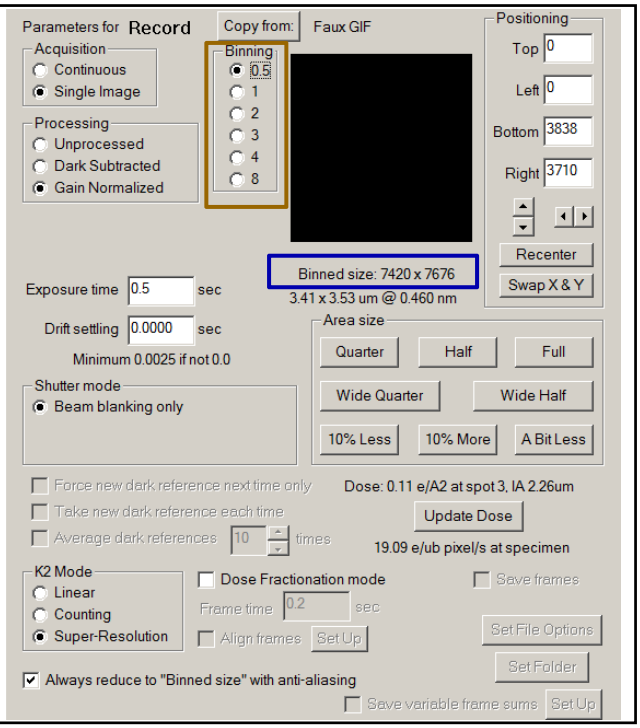
Counting Mode

- The mode can be set to Linear, Counting, or Super-resolution
- In counting mode, the full size is the number of physical pixels



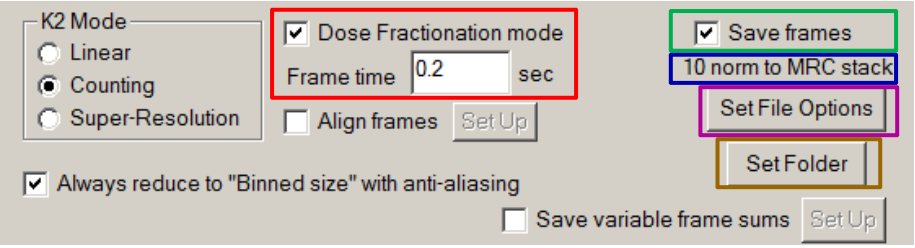
Super-Resolution Mode

- The binning is shown as 0.5 for the full super-resolution image.
- Now the size is twice as big as the physical number of pixels.



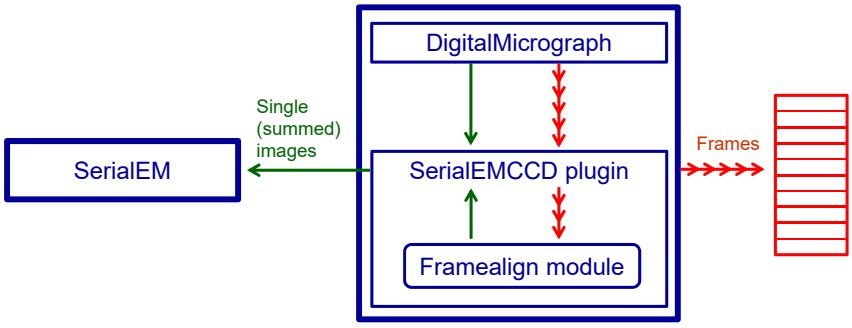
Dose Fractionation Mode and Saving Frames

- Dose Fractionation mode must be turned on to have multiple frames with the given frame time
- Save frames can then be turned on have them saved
- The summary line shows the number of frames and how they will be saved
- Set File Options opens a dialog for file name and format control
  - Unnormalized counting mode data (small integer counts) can be saved in TIFF files with good compression
- A folder must be defined: anywhere accessible from K2 computer



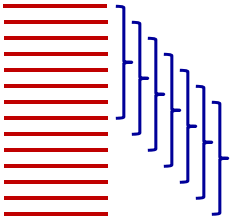
K2 Frame Handling in Dose Fractionation Mode

- All frames are handled by the SerialEMCCD plugin to DigitalMicrograph; SerialEM receives only single images (sums of frames)
- The plugin can save frames and/or align them with the Framealign module shared with IMOD



The Framealign Module in Alignframes and SerialEMCCD

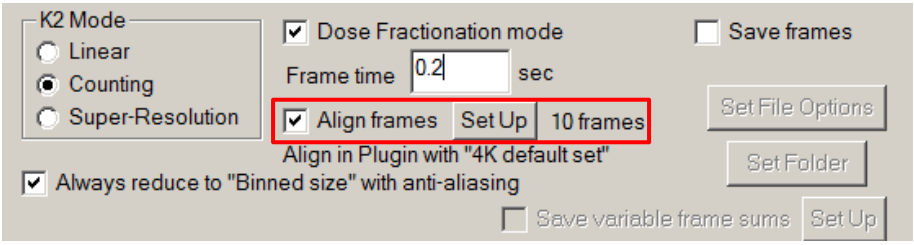
- Alignment is found by correlating many pairs of frames with each other and solving for best shifts of individual frames
  - Robust regression is used to reject effects of some bad alignments
  - All pairs are aligned in successive subsets of frames to avoid dependence on square of number of frames
- It processes data as it is available, leaving as little computation as possible until the end
- It can use the GPU of an NVIDIA card
- Aligning is slightly faster than saving frames even without a GPU



In each subset of 8, all 28 pairs
are aligned to solve for 7 shifts

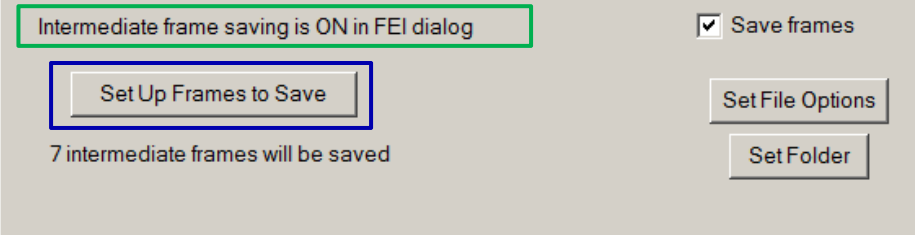
Aligning K2 Frames

- When Align frames is selected, you can then set the parameters and options for alignment
- Frames can be aligned in plugin and aligned image returned to SerialEM, or a command file can be written for running Alignframes



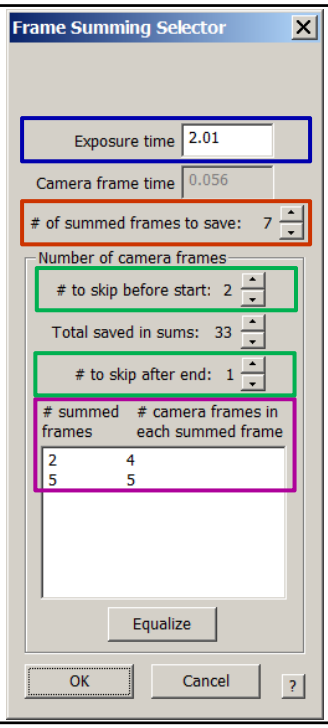
Saving Frames from Falcon 2

- SerialEM may or may not be able to control or even know whether frame saving is turned on in the separate FEI dialog, so there can be either a checkbox to tell SerialEM it is on, a status line like here, or no text at all
- The FEI software can save up to 7 (or up to 40) sums of sequential frames (camera readouts) and the Set Up dialog lets you control that



Controlling Which Frames Are Saved

- In the simplest case, you set the exposure time and set the selector to save 7 summed frames (or more with newer FEI versions)
- It is often necessary to skip a camera frame at the start and/or end, so there are selectors for that
- When you change exposure in the main dialog, the # of camera frames in each summed frame is automatically redistributed



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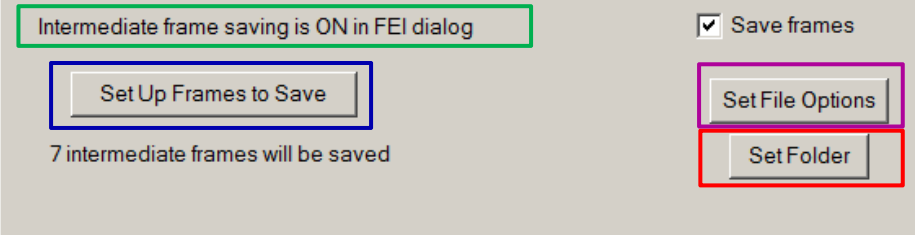
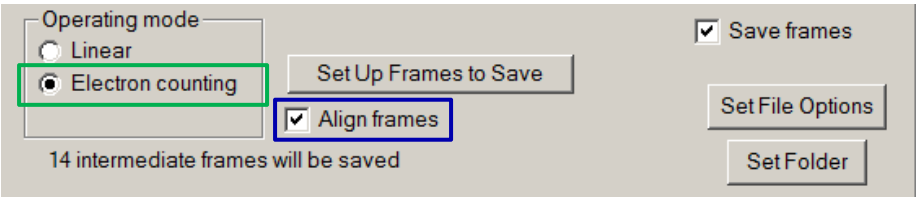
- A folder must be defined: anywhere accessible from SerialEM
- Set File Options opens a dialog just for file name and folder control



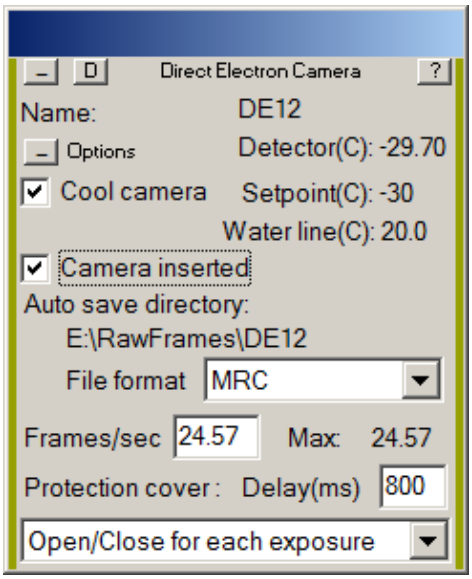
A Sneak Peek at Falcon 3

- Electron counting possible
- The processing hardware can align the frames



The Control Panel for DE Cameras

- It has controls for the frame file format, frame rate, and a few other features



Saving Frames from Direct Electron Cameras

- Frames are saved by the DE server and are not currently accessible from SerialEM
- Both raw frames and summed frames can be saved
- These frames need dark-subtraction and gain-normalization
- Set File Suffix opens a dialog just to control a portion of the file name