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.
  - DQE of 33% => 3 times as much dose.
- 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)

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
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 to 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

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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

<table>
<thead>
<tr>
<th>K2 Mode</th>
<th>Dose Fractionation mode</th>
<th>Save frames</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Frame time 0.2 sec</td>
<td></td>
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<tr>
<td></td>
<td>Align frames</td>
<td>Set Up</td>
</tr>
<tr>
<td></td>
<td>Always reduce to &quot;Binned size&quot; with anti-aliasing</td>
<td></td>
</tr>
</tbody>
</table>

Align in Plugin with "4K default set"
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.

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### 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.
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
- 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

![Control Panel Example]

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

![Saving Frames Example]