Options in SerialEM for Efficient Handling of K2 Camera Data

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SerialEM

- SerialEM is used for both tilt series collection and acquisition of data for single-particle reconstruction

- SerialEM supports acquisition of movie frames from all 3 direct detectors, but options for more efficient handling of frames currently exist only for the K2

- All of these options are implemented in SerialEM's plugin to DigitalMicrograph
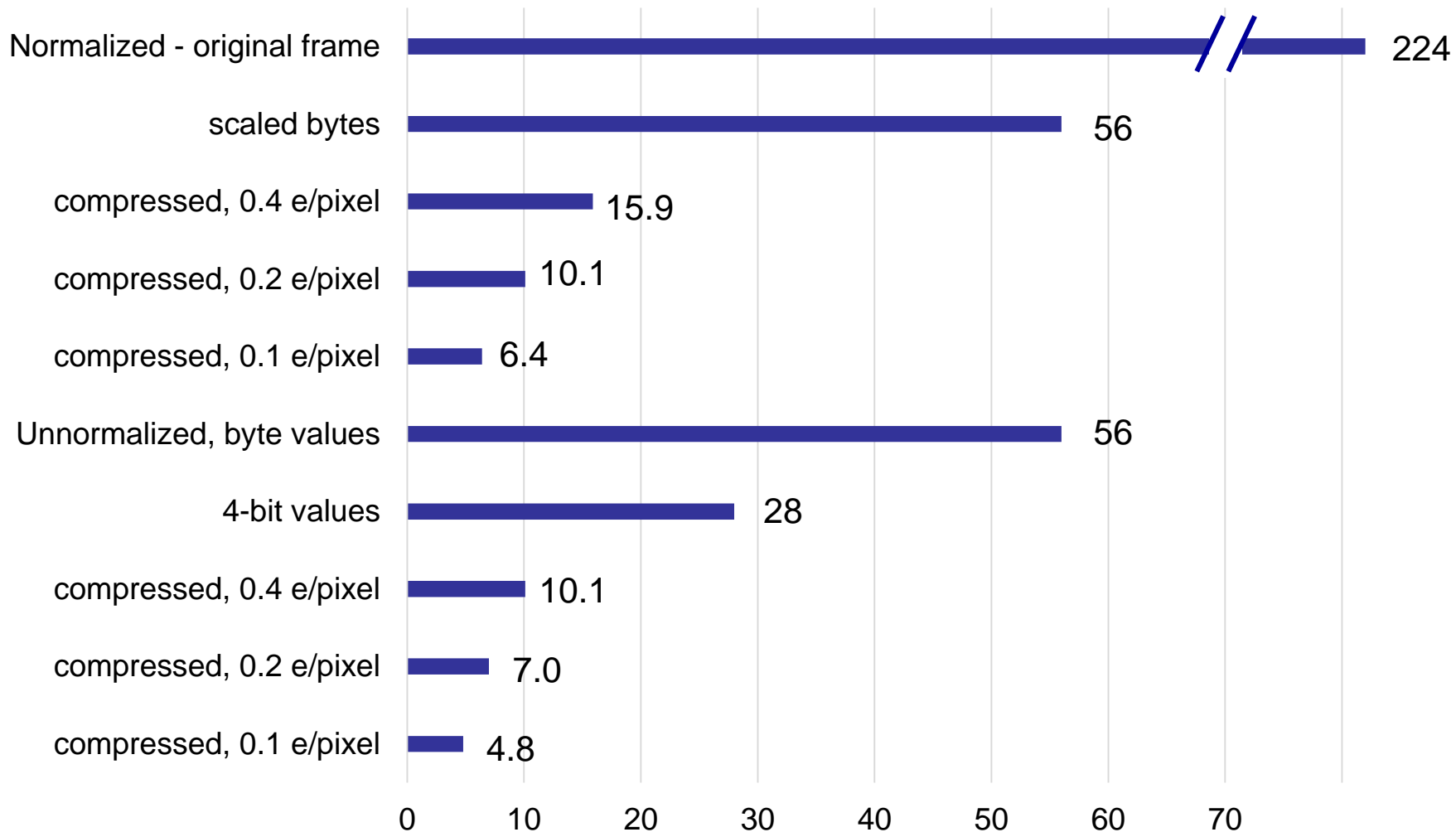 - Leginon also uses this plugin
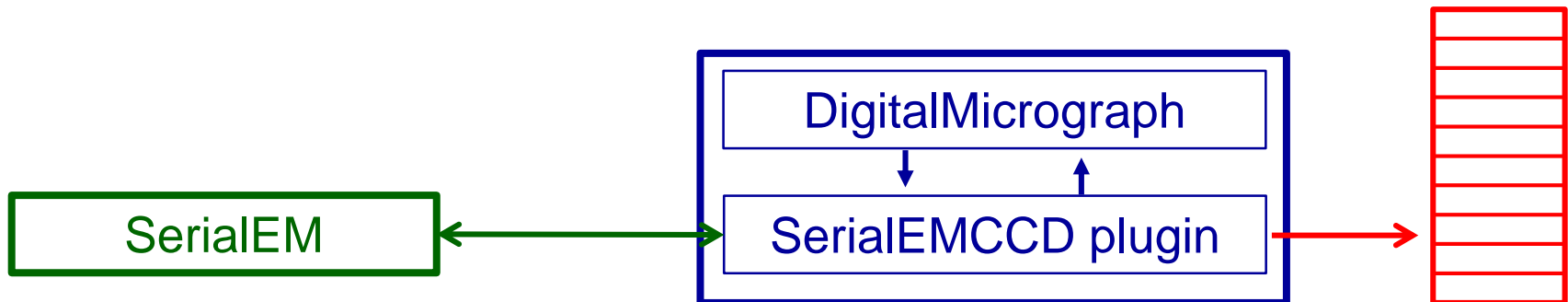
Options for Saving Gain-Normalized Frames from the K2

- The plugin receives gain-normalized frames as floating point

- By default, these values are scaled up and saved as integers (counting mode) or bytes (super-resolution mode)

- Saving as TIFF with LZW compression can save an additional 2.5-4x for typical doses and up to 9x for lower doses

Unnormalized Frames Can Be Saved More Efficiently

- Unnormalized frames consist of small integer electron counts
- Super-resolution frames can be saved as 4-bit values; counting mode frames as bytes or even 4-bit values (risky)
- Saving as compressed TIFF can save about 3x, or up to ~6x for lower doses/frame
  - With compression, saving as 4-bit data has little or no advantage

Super-resolution Mode Storage Options, MB/frame

Option	Value
Normalized - original frame	224
scaled bytes	56
compressed, 0.4 e/pixel	15.9
compressed, 0.2 e/pixel	10.1
compressed, 0.1 e/pixel	6.4
Unnormalized, byte values	56
4-bit values	28
compressed, 0.4 e/pixel	10.1
compressed, 0.2 e/pixel	7.0
compressed, 0.1 e/pixel	4.8

Considerations with Unnormalized Frames

- Gain reference file needs to be kept with data, and "defect" file is needed for later DigitalMicrograph versions
  - Plugin makes sure there are current files in the directory
- Even if using software other than IMOD for aligning, data may need to be preprocessed with IMOD for:
  - Unpacking 4-bit data
  - Reading compressed TIFF
  - Applying defect correction

Why Not Align the Frames instead of Saving?

- For typical tilt series, a fast and reliable alignment of frames during acquisition should be able to replace saving frames

- In pursuit of this goal, IMOD and SerialEM have a new GPU-capable module for frame alignment

Frame Alignment in SerialEM

- There are many options… that correspond to what is in IMOD

- Optimal parameters should be found in IMOD for a class of data, then applied in SerialEM

- Multiple parameter settings can be stored

Frame Alignment in SerialEM

- There are many options… that correspond to what is in IMOD
- Optimal parameters should be found in IMOD for a class of data, then applied in SerialEM
- Multiple parameter settings can be stored
- Alignment can be done with approach of "motioncorr" from UCSF (fitting to shifts between many pairs of frames) and allows refinement and smoothing as in "unblur" from Grigorieff
- Alignment in SerialEM costs little or no time relative to saving frames and can be done asynchronously

Frame Alignment Parameters

Where to align frames
- In DigitalMicrograph
- In SerialEMCCD plugin
- With IMOD

☑ Use the GPU
☐ Align only whole tilt series
Set Folder for Align Com Files

New Parameter Set   Delete

Counting
Super-resolution
Offline processing

Parameter set name: Super-resolution

☐ Truncate values above [0] electrons

Alignment strategy
- Fit pairwise shifts of 8 frames
- Fit pairwise shifts of HALF the frames
- Fit pairwise shifts of ALL frames
- Align to cumulative reference

Alignment binning 8   Filter cutoff 0.06

Maximum shift between frames 20 unbinned pixels

☐ Group frames by 2 (Requires at least 6 frames)
☐ Refine alignment with up to 5 iterations
☑ Smooth shifts if at least 15 frames

+ More parameters

OK   Cancel   ?

Prospects for Falcon and Direct Electron Cameras

- High camera counts from these cameras will not compress nearly as well as the electron counts from the K2
  - ZIP will compress somewhat better than LZW, but is slower
  - Lossy compression of high-noise data sounds difficult
- The frame alignment module could be incorporated into SerialEM
  - SerialEM already handles Falcon frames directly, so this would be the easier camera to do
  - First, need to get some feedback on effectiveness of this approach for the K2 (does it indeed replace saving of frames at least for typical cryoET?)