

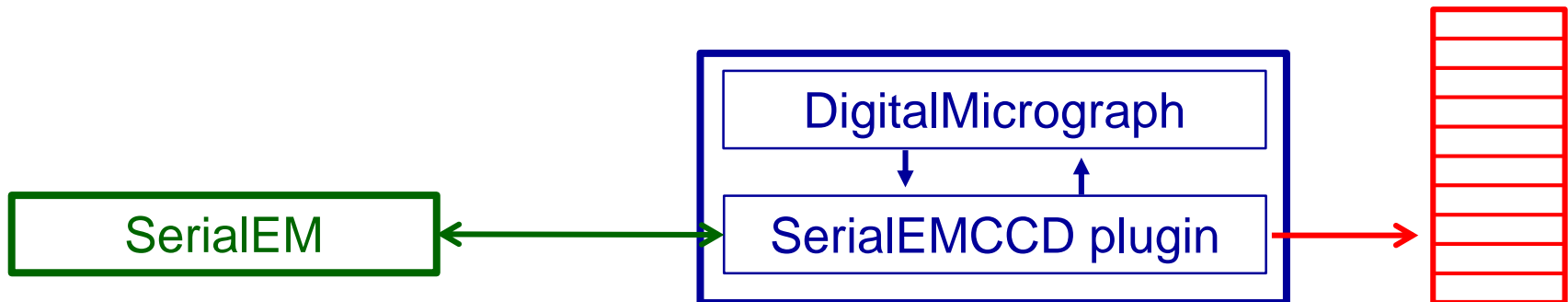
Options in SerialEM for Efficient Handling of K2 Camera Data

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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)
 - Option to **scale by 100** was added before I did analysis showing that scaling by 16 reduce DQE by 0.05% or less.
- Saving as **TIFF with LZW compression** can save an additional 2.5-4x for typical doses and up to 9x for lower doses

File type

MRC file Use extension .mracs

TIFF (LZW compression)

TIFF (ZIP compression)

Save one frame per file

Save unnormalized frames even if Gain Normalized is selected

Save normalized data as 16-bit with 100 times scaling of sum

Pack unnormalized data as 4-bit (Super-Res) or 4/8-bit (Counting)

Pack unnormalized Counting mode data as 4-bit, not 8-bit

Use non-standard mode 101 for 4-bit MRC files

Save frames without rotation/flip to standard orientation

Unnormalized Frames Can Be Saved More Efficiently

- Unnormalized frames consist of small integer electron counts
 - Option to **save unnormalized frames regardless** is best way to make sure it always happens
- Super-resolution frames can be saved as **4-bit values**; **counting** mode frames as bytes
 - **Counting** can even be saved as **4-bit values**, but this is risky
 - **4-bit MRC files** can be saved as **mode 101** – not needed with current versions of MotionCor2

The image shows a screenshot of a software interface with several options for saving frames. The options are as follows:

- File type** (grouped in a box):
 - MRC file Use extension .mracs
 - TIFF (LZW compression)
 - TIFF (ZIP compression)
- Save one frame per file
- Save unnormalized frames even if Gain Normalized is selected (highlighted with a red box)
- Save normalized data as 16-bit with 100 times scaling of sum
- Pack unnormalized data as 4-bit (Super-Res) or 4/8-bit (Counting) (highlighted with a blue box)
- Pack unnormalized Counting mode data as 4-bit, not 8-bit (highlighted with a green box)
- Use non-standard mode 101 for 4-bit MRC files (highlighted with a purple box)
- Save frames without rotation/flip to standard orientation

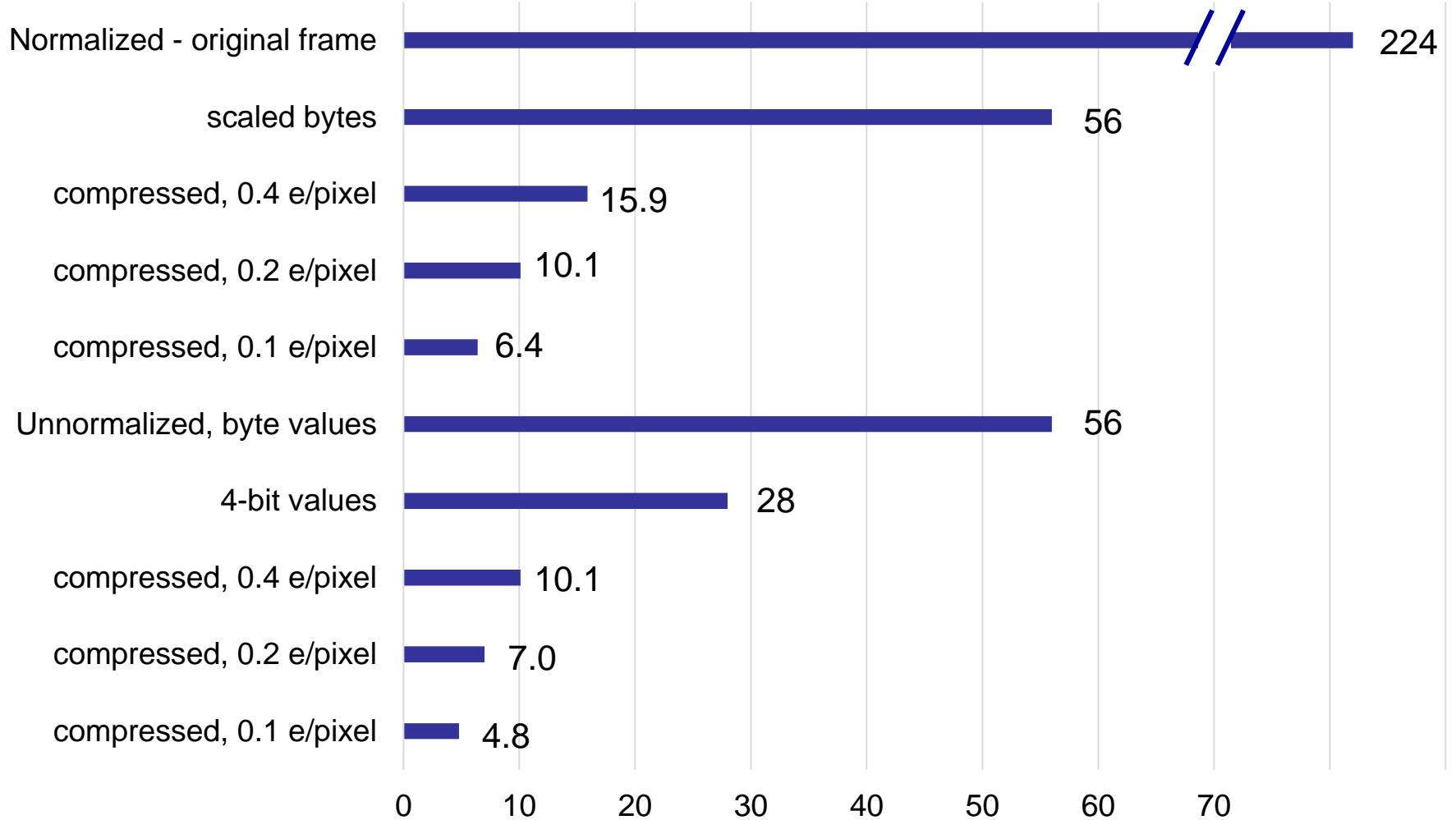
Unnormalized Frames Can Be Saved More Efficiently

- 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 advantage, so skip it if downstream will have trouble with it
- Saving **without rotation/flip** will save time, not space, if there is +/-90 rotation
 - Flipping is quick but +/-90 degree rotation costs 0.25/super-res frame
 - For tilt series, be sure to apply the proper rotation/flip afterwards

The image shows a software interface with the following elements:

- File type** section:
 - MRC file Use extension .mrcs
 - TIFF (LZW compression)** (highlighted with a red box)
 - TIFF (ZIP compression)
- Checkboxes:
 - Save one frame per file
 - Save unnormalized frames even if Gain Normalized is selected
 - Save normalized data as 16-bit with 100 times scaling of sum
 - Pack unnormalized data as 4-bit (Super-Res) or 4/8-bit (Counting)
 - Pack unnormalized Counting mode data as 4-bit, not 8-bit
 - Use non-standard mode 101 for 4-bit MRC files
 - Save frames without rotation/flip to standard orientation (highlighted with a blue box)

Super-resolution Mode Storage Options, MB/frame



Considerations with Unnormalized Frames

- Gain reference and “defect” files need to be kept with data
 - Plugin makes sure there are current files in the directory
- Using Alignframes:
 - If saved in standard orientation, the gain reference needs rotating:
`alignframes -gain SuperRef_Jun04_07.11.24.dm4 -def defects_Dec02_21.35.34.txt -rot -1`
 - If saved without reorientation, a tilt series needs RotationFlip value in camera properties
`alignframes -gain CountRef_Dec14_20.05.13.dm4 -def defects_Dec02_21.35.34.txt -rfsum 7`
 - The default is to output 16-bit integers scaled by 30; you can output floats with “-mode 2” and/or specify a different scaling with “-scale”
 - “Align in IMOD” will write command file with all appropriate options

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 - “Align in IMOD” will write command file with all appropriate options
- If using software other than IMOD for aligning, data may need to be preprocessed with IMOD for unpacking compressed 4-bit data and applying corrections
 - `clip norm -D defects_Dec02_21.35.34.txt -R -1 -m 1 frameFile SuperRef_Jun04_07.11.24.dm4 output`
 - The default is scaling by 16; add “-n scalingFactor” or output floats with “-m 2”
- MotionCor2 now takes all of this directly except that it requires a defect “map” (image) instead of the text file. With IMOD 4.10.7 or higher:
`clip defect -D defects....txt -f tif anyFileWithFrames defects....tif`

More Details on Processing Unnormalized Frames

- All of this is described in the SerialEM help section on **Image Acquisition from CCD Cameras and Direct Electron Detectors** - Gain-normalizing frames during post-processing
- A continually updated version is online at http://bio3d.colorado.edu/SerialEM/hlp/html/about_camera.htm#directDetectors