On-The-Fly-Processing Manual UTSW CEMF

What is 'On-The-Fly-Processing' doing:

On-The-Fly-Processing will gain normalize, motion correct, and dose weight data recorded on the Krios and Talos in real time (usually ~10 min processing time), and output the corrected images to individual user accounts in the CEMF's BioHPC transfer directory.

What Users need to do:

1) Every time a user collects data that are transferred to BioHPC ('Images-Disc*'), users have to provide a small text file, called "**para.txt**", specifying two parameters: pixel size & dose (see details below). Before collecting data, users should copy the para.txt template from the desktop, edit the parameters to their needs and then safe the file in their current data collection directory on the K3 computer.

2) IMPORTANT:

- Please provide the para.txt file *BEFORE* starting data collection: the on-the-fly-processing cannot automatically start (in the background on BioHPC) until the file is provided; therefore, providing the file late creates a large queue of unprocessed data that will delay processing of your data and the data of users that follow you!
- Don't forget to also place the gain reference file in your folder *BEFORE* starting data collection, because on-the-fly-processing cannot start without the gain reference file.
- Even if you do not want your data processed "on-the-fly", users have to provide the para.txt (specifying "cancel", see details below), to avoid queues of unprocessed data.

Information about the para.txt file:

The name must be para.txt and the file should be saved to the images-disk/basename directory *before transfer of recorded images starts. Also, there must be at least one space between the text for the* parameter (i.e. "pixel") and the corresponding value in the same line.
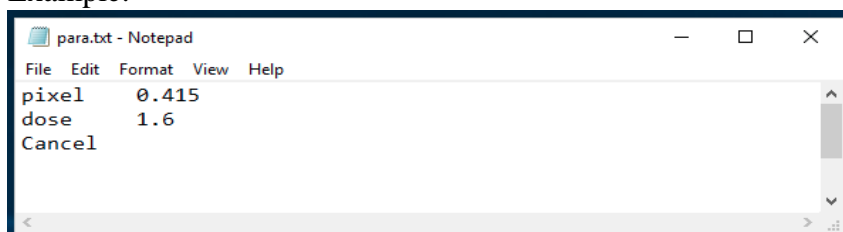
`pixel`: is the pixel size in nanometers. If super resolution mode is used, please use the super resolution pixel size (i.e. ½ of counted mode pixel size).

`dose`: is the dose/frame (e^-/A^2); in the example below, the input value is 1.6 e^-/A^2/frame (default value), and the total dose will be automatically calculated as 1.6 X number of frames.

If you don't want your data to be processed "on-the-fly", just add "Cancel" in the para.txt file.
(e.g. at the moment "on-the-fly-processing" does not work for cryo-electron tomography data)

User only needs to change the value(s), and add or remove "Cancel" in the para.txt;
Example:

Input and output files:

input files:

Basename.tif   (original stack)

Gainfile.dm4   (original latest gain file in the folder)

output files:

The original raw image stacks will still be transferred to BioHPC as before; however, in addition a sub-directory called 'biohpc_onfly_process' will be generated in the same folder into which the raw data are copied. The following output file are copied into this new sub-directory:

biohpc_onfly_process/Basename.mrc   (corrected stack)

biohpc_onfly_process/Basename.log   (process log)

biohpc_onfly_process/Basename-gainfile-Gainfile.mrc (converted gain file by using e2proc2d.py command)

biohpc_onfly_process/Basename.mrc   (Motion corrected image without dose weighting)

biohpc_onfly_process/Basename_DW.mrc   (Motion corrected image with dose weighting; ideal

for image processing)

What to do and who to contact in case of problems:

If users encounter any errors with on-the-fly processing, please contact the CEMF facility manager: Daniel Stoddard (518) 932-3971 and initiate a ticket with BioHPC help by emailing: [BioHPC-Help@UTSouthwestern.edu] with Daniel CC'd.

For general questions about on-the-fly processing, please also contact Daniel Stoddard.

Detailed command that is running in the background:

Krios:

MotionCor2 -InTiff "$1" -OutMrc "$outfile" -LogFile "$outdir" -Bft 150 -PixSize "$pixel" -Patch 5 5 -Gain "$gainfile" -FmRef 0 -Ft Bin 2 -Kv 300 -FmDose "$dose" -InitDose 0 -Gpu "$gpuid" > "$outdir/$3.log"

Talos:

MotionCor2 -InTiff "$1" -OutMrc "$outfile" -LogFile "$outdir" -Bft 150 -PixSize "$pixel" -Patch 5 5 -Gain "$gainfile" -FmRef 0 -Ft Bin 2 -Kv 200 -FmDose "$dose" -InitDose 0 -Gpu "$gpuid" > "$outdir/$3.log"