

FAQ:

1) How can I create an account on PPMS?

The cryo-EM facility benefits from the established PPMS system of the EM Core Facility directed by Dr. Kate Luby-Phelps. Therefore, please follow these steps:

- a) Please create an Account on PPMS by following this guide: www.utsouthwestern.edu/labs/emcf/services/quick-start.pdf
- b) Please make sure to provide a valid subledger # in your account (otherwise you will not be able to sign-up for instruments); if you have not provided the subledger during account creation or the number has changed, please update the subledger in the PPMS system (or email it to the facility manager/director).
- c) Once you have submitted the form and the EM Core Facility staff has accepted your account creation request, please check that you can log-on and email the Facility Manager to be added to the “cryo-EM user group”.
- d) As soon as you are a cryo-EM group member, please log-on and click on the tab “Documents”, then open the **Document #8: Policies, Rules & Rates for the Cryo-EM facility at UT Southwestern Medical Center.**



- e) All **users** of the cryo-EM facility and **their Pls** have to confirm online that they have **read, understand and agree to the Policies, Rules & Rates for the Cryo-EM facility at UT Southwestern Medical Center**, before the certified user will use the instruments in the cryo-EM facility. Therefore, after reading this document, please confirm at the end of the document that you understand and agree to it (check the box and submit). This will be recorded with time stamp and constitutes your electronic signature.

2) How do I sign-up for instrument usage on PPMS?

Once a user has been certified for usage of an electron microscopes or accessory instrument in the cryo-EM facility, the facility manager or director will assign the respective booking calendar(s) to the user, who can then access the calendar via their “Book” tab in PPMS. When booking the Krios or Talos instrument, please follow the slot-organization given in the policies.

Users have to be signed-up online BEFORE using the respective instrument – including for “grabbed” sessions. Please note that users cannot make changes to the sign-up calendar retroactively, and not being signed up for the full time of usage is a policy violation.

- a) If you don’t see a particular instrument on your “Book” tab, please let the facility manager or director know, so they can assign the instrument to the user’s account
- b) If you see the instrument, but cannot book a session, likely the system does not have a (valid) subledger number from your lab for charging in the system. Please either request online to change/add a subledger number, or email the subledger number to the facility manager or director, so that they can add it to the user account.

3) Do I need a BioHPC account to use the CEMF?

Yes, you need a BioHPC account to store and access your cryo-EM images (see point #7).

- a) If your department is a member of the BioHPC, please sign up for the “Introduction to BioHPC - New User Training” usually 1st Wednesday each month [see <https://portal.biohpc.swmed.edu/content/>]. As soon as you have your account name please email it to the Facility Manager.
- b) If your department is NOT member of the BioHPC, please contact Diana Tomchick to apply for a SBL-sponsored BioHPC account. [more information will be provided by Diana; you will also need the New User Training] .

4) When and how do I acknowledge the cryo-EM facility?

If a publication contains data that were recorded in the CEMF, you should acknowledge the UTSW cryo-EM facility in your publication. Once your study is published, please send your paper to the facility director, so that we can occasionally report about your successes on our facility webpage in the “News & Images” section.

5) If I encounter computer or software glitches, can I reboot the computer or software?

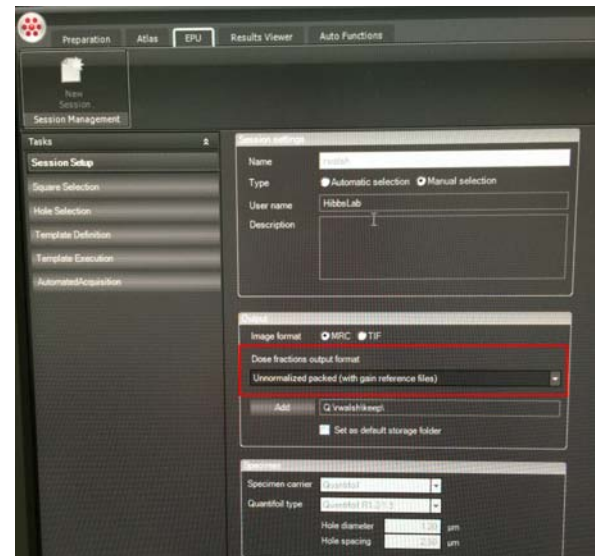
Please **NEVER shut-down or restart the microscope computers, or kill/reboot software (e.g. via Task-Manager)! All the computers and software that control the microscopes are direct extensions of the microscopes.** During training, all certified users were instructed in which sequence to start various programs, otherwise the microscopes might not work properly. Regular users are neither allowed to shut-down the microscope itself, nor are they allowed to shut-down or restart any of the computers, or kill/reboot individual software using the “Task Manager” (even if the Task Manager says “program not responding”) – UNLESS the facility manager has specifically instructed them to do so! Please inform the facility manager if you encounter errors, frozen computers or other computer/software problems. Also it is always helpful if you can take a screenshot (with your cell phone) and provide it to the facility manager.

6) What is the most space efficient format to save cryo-EM data (K2 movie files)?

The files size of images recorded with the K2 direct electron detector – especially when recorded in super-resolution (counting) mode (file size x4), are very large, making it difficult to store and transfer the files. Therefore, SerialEM and now also FEI’s EPU software use a trick to keep the file sizes smaller for storage and transfer (while maintaining full resolution): instead of Gain-normalizing the 4-bit unsigned integer movie stack on the microscope, which would require file conversion to 32-bit floating points format (large), the unprocessed image and the Gain-reference file are kept separate until image processed (e.g. by for motion-correction); this also allows loss-less LZW-compression of the image stack. **To use the trick in EPU:**

- A. Choose “Unnormalized packed (with gain reference files)” in EPU
- B. In the output folder, it will generate a gain reference file for each movie stack (gain reference file is highlighted in red box)

```
File Edit View Search Terminal Help
FoilHole_1637721_Data_1645616_1645617_20170704_0006-11255.mrc
FoilHole_1637721_Data_1645616_1645617_20170704_0006-gain-ref.dm4
FoilHole_1637721_Data_1645625_1645626_20170704_0007-11256.mrc
FoilHole_1637721_Data_1645625_1645626_20170704_0007-gain-ref.dm4
FoilHole_1637758_Data_1645607_1645608_20170704_0011-11257.mrc
FoilHole_1637758_Data_1645607_1645608_20170704_0011-gain-ref.dm4
FoilHole_1637758_Data_1645616_1645617_20170704_0012-11258.mrc
FoilHole_1637758_Data_1645616_1645617_20170704_0012-gain-ref.dm4
FoilHole_1637758_Data_1645625_1645626_20170704_0013-11259.mrc
FoilHole_1637758_Data_1645625_1645626_20170704_0013-gain-ref.dm4
FoilHole_1637759_Data_1645607_1645608_20170704_0014-11260.mrc
FoilHole_1637759_Data_1645607_1645608_20170704_0014-gain-ref.dm4
FoilHole_1637759_Data_1645616_1645617_20170704_0015-11261.mrc
```



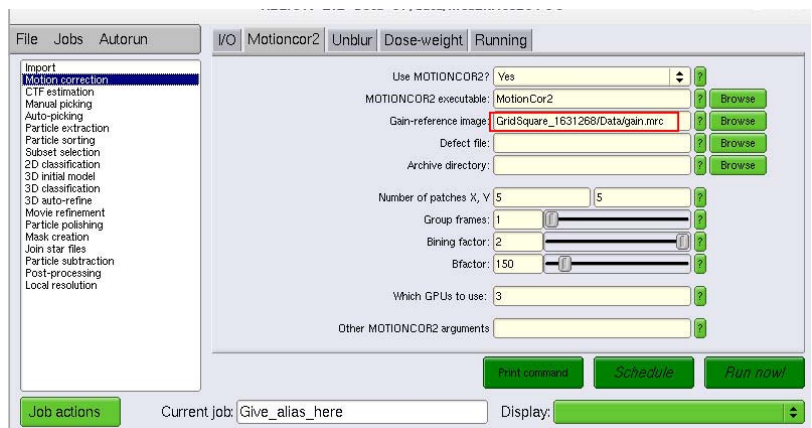
- C. Convert the gain reference file from DM4 format to MRC format e.g. using EMAN2

`e2.proc2d.py FoilHole_1637762_Data_1645616_1645617_20170704_0006-gain-ref.dm4 gain.mrc`

or IMOD

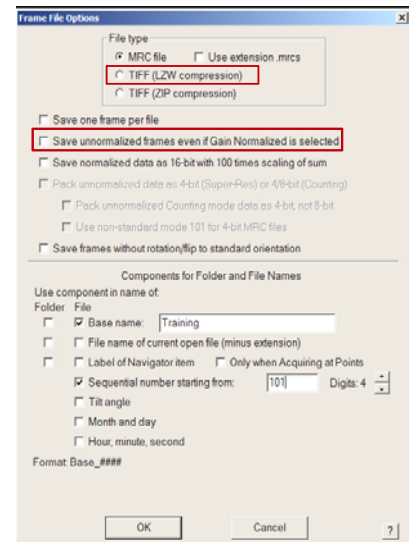
`dm2mrc inputname.dm4 outputname.mrc` (IMOD man page: <http://bio3d.colorado.edu/imod/doc/man/dm2mrc.html>)

- D. Input the gain reference file into RELION for motion correction



To use the trick in SerialEM:

- In the Frame File Options window when setting up your image stack, select “TIFF (LZW compression)” (whereas MRC file is default). This is lossless compression for counting and superresolution mode of K2. You must also select “Save Unnormalized frames even if gain normalized is selected” in the same window.
- These settings will output the gain reference as a .DM4 file (same as in EPU) and the data images separately.
- The images can then later be gain normalized as described above for EPU.



7) How do I get the data that I have recorded off the microscope computer to my personal data storage space/device? And are there any naming conventions or is there anything else I need to know about Data handling/transferring/deleting in the Cryo-EM Facility?

Please note that personal storage devices of any kind are not permitted on the microscope computers and the computers are not on the public network for microscope security. Therefore a protocol is set-up that automatically transfers the user data to dedicated temporary storage on BioHPC from where users can access and copy their data. To allow smooth operations of this data transfer and deletion protocol it is critical that users familiarize themselves with the following naming and data handling instructions:

a) *Synchronization from CryoEM Machines to BioHPC*

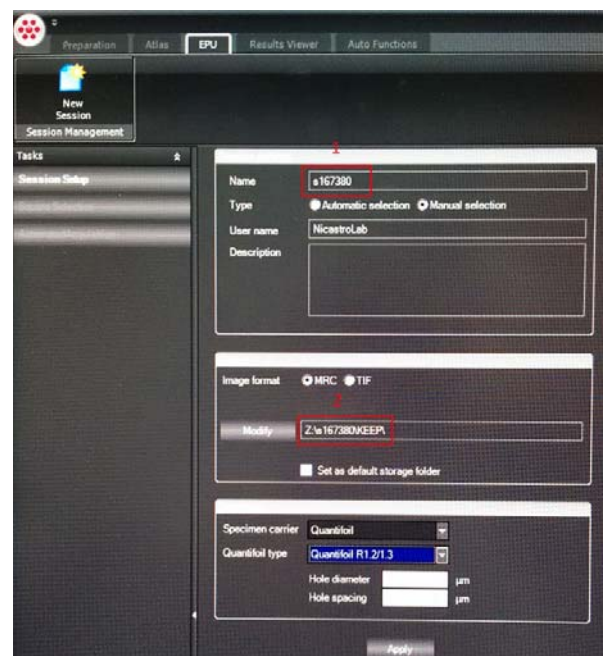
- Users will acquire all data under the folder `X:\DoseFractions\` on the camera PC, where `<username>` is the user's BioHPC username.
- For EPU users only: Please note that the `X:\DoseFractions\` folder is mounted as drive `Z:`; therefore, users who want to use EPU for the data acquisition, should create a folder with their BioHPC username under the drive Z, e.g. `Z:\<username>`

- Krios data will be synced to BioHPC - `/project/cryoem/cryoem_transfer/krios/<username>`
 - Arctica data will be synced to BioHPC - `/project/cryoem/cryoem_transfer/arctica/<username>`
- Please note: a) users have 4 days to copy their data from `/project/cryoem/` to their own space or storage device; files older than 4 days might be automatically deleted as space is needed). b) Only the person whose**

BioHPC username (e.g. `s123456`) was used on the TEM computer when the data were collected, will be able to see and copy the data from the CEMF space on BioHPC. c) Users do not have permission to delete data from `/project/cryoem/` to void system confusion; data deletion will be performed automatically.

- For EPU, the unique `GridSquareXXXXX` data folders will ensure that data acquired at different times does not overwrite previously synchronized data at BioHPC. However, files that the EPU user needs for longer than 5 min. for automatic data collection (e.g. atlases and images of each square) need to be stored in a folder called “KEEP”, because only files downstream of a KEEP-folder are guaranteed to be protected from rolling deletion (see below); e.g.

`Z:\s123456\2017-03-15-MyExperiment\KEEP\`



- For SerialEM, and other applicable software (like DM), users are responsible for acquiring data into a unique subdirectory named with a date-stamp to prevent overwrite at BioHPC, e.g.

[X:\DoseFractions\s123456\2017-03-15-MyExperiment](#)

- The synchronization script will be modified so that ***all*** files are synchronized from camera PC to BioHPC, when they are at least 5 minutes old, and re-synced whenever they change.
- This change will be implemented after the CryoEM user community has been informed.

b) Deletion of synchronized files from the camera PC - there will be 2 modes of file deletion from the camera PC:

(i) Rolling Deletion of Image Data – Large Image Data (e.g. frequently recorded movie-stacks .mrc during data collection) must be acquired into a folder named in the pattern ‘**Images-Disc***’. This is the default for EPU; for other software these folders need to be created & chosen as storage location. => An automatically running script at the camera PC (of each microscope) will delete files within folders named ‘**Images-Disc***’ on a continuous rolling basis, after confirming by checksum hashes that the file is successfully transferred to BioHPC. **EXCEPTION:** EPU users should store the files that are necessary for automatic data collection (e.g. Atlas, images of each square...) in a folder whose name contains “KEEP” to prevent unwanted deletion!

Important: to avoid accidental data loss, users must not store ANY files that are “touched/used/needed” by the software more than once or to which data might be added after a pause of >5 min. (see examples below) inside a folder named “Images-Disc*” (except if the upstream path contains \KEEP);

ET users must – at the moment - not align tilt series frames (“framewatcher”) on the microscope PC.

(rational: movie-stacks .mrc never change once they are fully saved, and the automated continuous rolling deletion is essential so that the non-expandable, limited local storage space does not limit data acquisition or require manual file deletion every 2 hours)

(ii) Periodic Deletion of Other/Accessory Files at least 7 days old – At daily intervals, the script will identify all other/accessory files (e.g. Atlas, LMM/MMM, nav.nav, tilt-series.st, ...) under [X:\DoseFractions \(Z:\\)](#) that are at least 7 days old. Files 7 days or older will be deleted, after confirming by checksum hashes that the file is successfully transferred to BioHPC.

(rational: according to our calculations these “other files” should not accumulate more than 100 GB (SP)–200 GB (ET) per week, so max 10% of disc space and age of 7 days ensures that atlases or so remain available during long data collection sessions; user only copy (not move/delete) from cryoem_transfer on BioHPC (data removal will be automated), and checksums of successfully transferred files are kept in log files, therefore the checksum hashes comparison should not be an issue even after 7 days).

For EPU users the Images-Disc folders should be automatically created correctly, but the “KEEP” folder must be created. Users of all other software (e.g. SerialEM) need to create at least 2 folders, e.g.:

[X:\DoseFractions\s123456\2017-03-15-MyExperiment\Images-Disc*](#) (for rolling deletion)

[X:\DoseFractions\s123456\2017-03-15-MyExperiment\KEEP](#) (for weekly deletion)

Important: there is no need to be alarmed, if data transfer to BioHPC and rolling deletion do not initiate within the first 5 minutes of data collection. Especially on weekends and during network maintenance outages the time for transfer initiation may increase, however anything greater than 25 minutes is unusual and if your data have not appeared on BioHPC after one hour please do the following:

- Check the folder names, file names and extensions to be sure that they adhere to the naming guidelines described above and that the files did not get transferred into an unexpected location. (*Note:* you need the password for the respective BioHPC account from the person who started data collection to login and see files).
- If data transfer and rolling deletion has not initialize within one hour, please contact the BioHPC Helpdesk by emailing to BioHPC-Help@UTSouthwestern.edu to report the problem (with cc. to the facility manager and

facility director). Although there is no guarantee, BioHPC staff will often generously try to troubleshoot issues also after-hours and on weekends.

- c) If there is an error presented on the microscope computer screen(s), please take a screen shot or cell phone picture and include the picture in your email to the BioHPC Helpdesk.