Syllabus for Cryo EM training at CEMF (UTSW)

The objective of this training is to provide new users with the required knowledge to safely and skillfully operate the CEMF cryo-electron microscopes and to become an independent Cryo-EM user. This training aims to teach all the necessary hardware and software needed to operate the instruments and collect high resolution, high quality electron microscopy data. This training will also emphasize basic EM theory, so that the new user not only complies with procedure and protocol, but understands the reasoning behind procedure and protocol.

What you will learn by the end of this training:

- General safety rules for the CEMF
- How to safely operate the CEMF Talos Arctica 200KV Electron Microscope and associated software/hardware, and/or the CEMF Titan Krios 300KV Electron Microscope and associated software/hardware.
- How to clip autogrids to prepare them for insertion into the microscope autoloader (insertion done by specially trained "super-users").
- Basic EM theory with special regard to microscope alignments and imaging conditions to yield highest quality data.

Prerequirement: online tutorial

Though your instructor will attempt to answer any and all questions related to the training and electron microscopy in general, a helpful online introduction to cryo-electron microscopy is offered by Grant Jensen's group ("Getting Started in Cryo-EM"). It is not required to view the course in its entirety, but it is **required** that new trainees review the following tutorial videos **before** the hands-on training begins, because they present basic principles of cryo-EM:

Course URL:

http://cryo-em-course.caltech.edu/

Required Viewing
3. Introduction: Why electrons?
4. Basic anatomy of the EM
5. Electron Guns
6. Electron Lenses
7. The Column
8. The Sample Chamber
12. Summary and Safety
29. Part4: Sample prep methods involving freezing
30. Sample Prep: Grids
32. Dose Limitations
33. Part 5: Tomography
38. Part 6: Single Particle Analysis

Approximate total viewing Time: 3h 42m;

Understanding of these principles is necessary to take full advantage of the training offered at CEMF.

Training schedule overview:

Your training schedule for the upcoming weeks can be viewed in PPMS at the following link:
(https://ppms.us/utsw/login/?pf=3)

If you do not have a PPMS account, the facility manager will help you to set one up.

You will also be provided with documentation on microscope and camera operation, as well as safety and general CEMF policies. All documentation can be accessed via BioHPC at:
/project/cryoem/cryoem_doc_exchange/

The training is organized into 8 modules culminating in a practical application (test sample). The number of sessions required for a user to complete all 8 modules will vary depending on prior experience, and how comfortable and proficient the user is aligning the microscope and acquiring data.

Module 1. General safety procedures and anatomy of the microscope:

1.1 General Safety procedures:
Flip rules, Overview of alarms and sensors, proper handling and filling of liquid nitrogen dewars. Emergency protocols, shut off switches, oxygen sensors and alarms, pressure gauges for liquid nitrogen filling. Initial microscope state assessment.

1.2 Anatomy of the electron microscope and associated equipment:
Gun, column, Lenses and apertures, stage, auto loader, projectors, various vacuum pumps, cameras, digitizers, Energy filter, HT tank and lines/cables, processors, computers, Emi Cage, air tables/platforms, work station(s) (more on the work stations when auto grid clipping is covered), Ethernet and Wi-Fi connections, and room environment (humidity and pressure)

1.3 Operation basics:
User interface startup and layout basics. What programs need to communicate. Standard voltage and vacuum settings, digital micrograph, TIA, Column valves, Beam path, projector screen and flu camera.

1.4 Cameras:
Ceta camera and K2 camera properties, how to insert and retract, best practices to avoid damaging cameras. What is binning and what are its advantages and disadvantages? Linear/counting/super resolution modes. Dose fractionation and its advantages.

1.5 How to manipulate the electron beam:
What is Spot size, how is it changed, and what are the effects of changing spot size? What are Microprobe and Nano probe, and which should I use? What is beam intensity and how is it changed? What is meant by crossover and what “side” of the crossover you should work on. What is beam shift and how is that changed? What is focus and how is that changed? What is magnification, how is that changed, and what is happening in the microscope when magnification is changed. WHAT PARAMETERS NOT TO ADJUST***

1.6 Proper log off procedures.

Module 2. Microscope alignment part 1:

Proper microscope alignment is essential for collecting high quality & resolution data. This process needs to be performed prior to every data collection session for optimal image quality and resolution.

2.1 Alignment files and FEG resister files:
How to load alignment/feg resister files, what information is contained within the files and how to load a backup copy in cases of bad initial alignment.

2.2 Autoloader basics and stage basics:
LN2 Filling. how to load and unload grids, best practices. How to move in X, Y, Z and tilt with the compustage. How to find and define a hole vs. carbon. What is meant by the term mesh? Grid square? Hole?

2.3 Eucentricity:
What is eccentricity and why is it important for data collection? How to set the eucentric height at low and high magnification. Where this information is shown on the user interface.

2.4 C2 aperture:
How to center the C2 aperture, how to avoid changing the C3 aperture. (free control for Krios only), what happens if the aperture is not centered correctly?

2.5 Stigmatisim:
What is stigmatism? How to correct astigmatism. What is happening inside the microscope when astigmatism is corrected?

2.6 Image planes and crossovers:
Crossover and plane locations, why the objective lens is so important, and why we don't align the shift of the objective lens.

2.7 True focus:
Why it's important to be at 0 focus during direct alignments. How to find the true focus, why this is important, and how we visualize this adjustment.

2.8 Beam tilt Pivot point:
What is Beam tilt? What is a pivot point? How to adjust pivot point in X and Y. What the microscope is doing during pivot point adjustment, and how the Z height affects the pivot point alignment.

2.9 Rotation center:
What rotation center is, why this is important, what is happening inside the microscope when tuning the rotation center.

2.10 Coma-free alignment:
What coma is, how it distorts the image, how to visualize coma, how to correct for coma in X and Y, what is happening inside the microscope during coma-free alignment.

2.11 Centering the objective aperture:

What is diffraction mode and why use it? How to visualize the objective aperture. How to adjust the position of the objective aperture.

Module 3. Microscope alignment part 2:

3.1 TEM/EFTEM modes:

What is Energy filtered TEM. What is camera length and how does it affect magnification and illumination area.

3.2 Energy filter (Krios):

What is an energy filter and how does it function? Elastic vs. inelastic electron scattering. How filtering energy improves contrast. How to move the slit in and out of the beam path. What is zero loss? How to change the slit width. How to refine the zero loss peak (ZLP). Tuning the GIF. Mag distortion and chromatic abortion, and how the GIF corrects for these.

3.2 Dark reference:

What is a dark reference? Why do we need one? How to collect/update a dark reference. When to take a gain reference.

3.3 Gain reference:

What is a gain? How can we correct for gain on our camera?

How to take/update gain reference. When should this be done and who should do it. Is there a difference between linear and counted mode?

3.4 Parallel beam conditions:

What does is mean for the beam to be parallel? Why are parallel beam conditions ideal for collection of high resolution data and how is this related to beam tilt? How to reach parallel beam conditions.

3.5 Volta phase plate (VPP):

The relationship between defocus and contrast. The optimal focus while using VPP. What the VPP is and what it does. When to use the VPP. How to insert and retract VPP. How to adjust the VPP position in the back focal plane to find the blow up point, and how to properly stigmate it (Krios only). How to calculate the .5 nano coulomb charge required for charging the phase plate at your specific imaging condition. How to charge the VPP. How and when to move to the next spot on the VPP. Logging VPP usage.

3.6 Low dose image acquisition in the User Interface:

The meaning of “Low dose mode” and how to implement this imaging strategy. How to search, focus, and acquire.

Module 4. Guided practice of microscope alignment procedures.

4.1 Users will practice aligning the microscope with guidance from their alignment protocol and the instructor until they are comfortable aligning the microscope on their own using the protocol, with minimal input from the instructor.

****The number of practice sessions will vary based on the user****

Module 5a. Data collection software for single particle data acquisition on the Krios:

5a.1 Single Particle data collection:
Introduction to EPU for single particle users on the Krios

5a.2 Opening EPU:
Must be in EFTEM mode. Explaining EPU the user interface. Functions of the SET and GET buttons.

5a.3 Setting Beam conditions:
What are the different beam conditions needed for imaging (Atlas, grid square, hole, focus, drift, record, tune GIF.)

5a.4 Atlas:
What is an Atlas and how to acquire one. How to estimate ice Thickness. The differences between good and bad ice (vitreous, cubic, hexagonal). How to identify and select grid squares worth imaging.

5a.5 Grid square images.
How to acquire individual grid square images. How to refine local Eucentricity before acquiring grid square images and why this is important.

5a.6 holes.
How to define holes with optimal ice thickness and clearance. Using the brush tool to add and subtract holes. How to discriminate between good and bad acquisition areas.

5a.7 Templating:
How to set up image acquisition areas, how many per hole, where in the hole. Where to autofocus and acquire images for drift correction. How to set defocus parameters for each exposure set. How to execute the template one time to test for accuracy.

5a.8 Image shift:
Image shift vs stage shift, and how to adjust the image shift calibration within EPU.

5a.9 EPU + VPP.
How to set up and use the VPP with EPU. when to use VPP

5a.10 Alignment refinement:

How to refine pivot points, rotation center, and coma in EFTEM mode on the K2/3 camera prior to data collection, and why this is important.

5a.10 Automated data collection

How to begin automated data collection and monitor the process to ensure high quality data collection.

5a.11 ZLP and Dark Reference:

When and how to refine ZLP during Data collection. When and how to update the dark reference during data collection.

5a.12 saving data:

What folders to save Atlas and grid square overviews vs. where to save your data. How data transfer works between CEMF and BioHPC. How to transfer your data from BioHPC storage to your lab storage space.

Module 5b. Data collection software for tomography (Krios, Arctica) or single particle data acquisition (Arctica):

5b.1 Starting Serial EM

How to start the serial EM server and the program

5b.2 The User Interface

A general description of the different panels on the user interface and what functions each panel contains. Especially the beam settings

5b.3 Montaging (LMM)

How to open and save the Navigator and log window. What the Navigator records, how to use the “go to XY”, “Go to XYZ” buttons, and the differences between the two. How to take a low magnification montage. How to estimate ice thickness. The differences between good and bad ice (vitreous, cubic, hexagonal). How to identify and select grid squares worth imaging.

5b.3 Linking image shift

How to find features at different magnifications and shifting to marker between LM and HM.

5b.4 Montaging (MMM)

How to setup and acquire medium mag maps. How to generate a polygon. How to identify and select grid squares worth mapping. How to take the eucentricity at each grid square while taking the montage.

5b.5 Screening:

For Tomography, how to screen individual holes and mark areas of interest. For single particle, how to select holes of interest to acquire images using the grid method to select holes.

5b.6 Low dose mode:

How to set up low dose mode for tomography or single particle imaging. Focus, Record, Preview, Trial, Search. Correct imaging parameters for each beam condition. How to properly offset (Image shift) Trial and Focus beams from preview, and record, and the reasons for this. How to set up dose fractionation and where to save the data files. How to autofocus in Serial EM. Various other aspects of the low dose window. How to calibrate dose in Serial EM.

5b.7A Single particle data acquisition

How to acquire and center holes. How to acquire images at all holes using macros.

5b.7B OR: Tilt series acquisition (Tomography):

How to set up a tilt series, and what each setup parameter means. What macros are, and how to use a macro to run the "Hagen Scheme". The pros and cons of other tilt schemes.

5b.8 ZLP and Dark Reference:

When and how to refine ZLP during Data collection (Krios only). When and how to update the dark reference during data collection.

5b.9 saving data:

What folders to save LMM/Atlas and grid square overviews vs. where to save your data. How data transfer works between CEMF and BioHPC. How to transfer your data from BioHPC storage to your lab storage space.

Module 6. Practicing Data acquisition with serial EM or EPU

6.1 Users will practice aligning the microscope and collecting Serial EM/EPU data with guidance from their protocol, and the instructor, until they are comfortable aligning the microscope and taking data on their own using the protocol, with minimal input from the instructor.

****The number of practice sessions will vary based on the user****

Module 7. Grid clipping

7.1 Users will be shown the working station, tools, and components of the autogrid assembly. Users will be instructed in the proper way to clip auto grids and place in grid boxes for long term storage. Users will be shown best practices to minimize ice damage, contamination, and grid damage. This will be done first with no liquid nitrogen. When the users are comfortable, clipping will be done under liquid nitrogen. If Users are ready they can even clip the grids for their certification.

Module 8. Certification

8.1 Ribosome practical for single particle users:

Users will align the microscope and acquire data in either EPU (Krios) or SerialEM (Arctica) from Quantifoil grids containing a standard single particle sample that behaves well, Ribosomes for instance. The user must demonstrate knowledge of all safety rules and procedures, as well as adequate proficiency (As judged by the instructor) in operation of the microscope for alignment and single particle data collection. Though there is no need to complete this practical entirely from memory, being practiced enough to refer minimally to protocols and manuals is expected.

8.2 Flagella practical for tomography users:

Users will align the microscope and acquire data in SerialEM from Quantifoil grids containing a standard tomography sample that behaves well, axonemes for instance. The user must demonstrate knowledge of all safety rules and procedures, as well as adequate proficiency (As judged by the instructor) in operation of the microscope for alignment and tomography data collection. Though there is no need to complete this practical entirely from memory, being practiced enough to refer minimally to protocols and manuals is expected.

For documentation and further reading access the BioHPC cryo EM document exchange in the following location: /project/cryoem/cryoem_doc_exchange/