

Agenda of CryoEM Trainings

Yale CryoEM Resource

- > This agenda is prepared for the **Cryo EM basic training on Glacios**. It is designed for users who aim at independently preparing cryo samples and screening them using the Glacios with SerialEM.
- > To ensure training quality, trainees must complete all three sessions of **CryoEM basic training on Glacios**, although YCR will strive to be flexible in response to trainees' requests. For example, training may begin with grid clipping as requested by the trainee. *Skipped or optional parts are indicated in gray text.*
- > To ensure training quality and avoid wasting trainee's samples, trainee's grids will not be loaded into Glacios during the 1st and 2nd sessions. However, **trainees may use their grids (no more than 4 grids) on the 3rd session.**
- > To gain permission of independent usage of corresponding Glacios, trainees will be required to pass a certification (practical test).
- > Trainees are responsible for the cost of C-clip rings, C-clips, and grid boxes, and are encouraged to purchase them independently despite YCR providing these items.
- > Advanced training on other specific technique or application (e.g., Advanced training on SPA with SerialEM or MicroED) is available from YCR upon request.
- > Please refer to the YCR-Policies (available in <https://cryoem.yale.edu/policies-and-rates>) for more details about training & rules. Questions and comments are welcome to Jianfeng.lin@yale.edu.

Session 1: Cryo sample preparation & TEM Basics

Date: TBD

Morning: 9am-12pm

Afternoon: 1pm-5pm

1. Introduction to plunge freezing of biological samples:

- Review training agenda
- Introduction to EM grids, and the proper handling and glow discharge of EM grids
- Introduction to plunge freezing of biological samples

2. Vitrobot Training and Practice:

- Introduction to Vitrobot and overview of the entire freezing workflow with Vitrobot
- Proper handling of liquid nitrogen (LN₂) and ethane
- Freezing cryoEM sample grids
- Hands-on practice

3. Autogrid Clipping:

- Introduction to c-clip and c-clip rings, and tools used for clipping
- Clip cryoEM sample grids
- Hands-on practice

4. Electron microscope and Software:

- Microscope Software Launcher
- TEM User Interface (UI): the composition and editing of UI
- Hand panels
- FluCam: show and explain its usage.
- System and optics
 - o A comprehensive overview of the main components of Glacios
 - o Parallel illumination
 - o Nano-probe and micro-probe modes
 - o Imaging and Difference modes
- Hands-on practice

Session 2: Microscope Operation, Detector, and SerialEM

Date: TBD

Morning: 9am-12pm

Afternoon: 1pm-5pm

1. K3 Introduction (for sample screening and/or data collection):

- Calculating dose, dose rate, and pixel size
- Camera gain reference/dark reference
- Setting up to acquire images
- Discuss what settings/parameters are commonly used/desired
- Hands on practice

2. Introduction to SerialEM:

- Overview of the software
- Show and explain software Interface
- Eucentric rough/fine/both
- Autofocus
- Navigator
- Low dose control
- LMM (low magnification map) and MMM (medium magnification map)
- Camera & Script Controls
- Camera Parameters
 - o Record
 - o Preview
 - o Focus
 - o View
 - o Search
- Script
- Hands on practice

3. Glacios daily alignments:

- Condenser stigmatism
- Center an C2 aperture
- Eucentric height and Eucentric focus
- Direct alignments
 - o Beam tilt Pivot point
 - o Rotation center (optional)
 - o Coma free alignment
- Select and Center an objective aperture
- Objective stigmatism
- Hands on practice

Session 3: Cryo sample screening using Glacios with SerialEM

Date: TBD

Morning: 9am-12pm

Afternoon: 1pm-5pm

1. Workflow of cryo sample screening:

- Autogrid clipping (completed by trainee before the 3rd session)
- Autoloader cassette docking (only for Superuser candidate upon request)
 - o Loading Autogrids into an Autoloader cassette
 - o Docking cassette into the Autoloader and creating a sample inventory
- SerialEM startup and setting file loading
- Automated multiple grid screening
 - o Acquire LMMs of multiple selected grids
 - o Check LMM stitching and quality of the grids
- Semi-automated high magnification images acquisition for evaluating the ice and samples
 - o C2 and Objective apertures selection and inserting
 - o Set up Low Dose illuminations at SerialEM
 - o Image shifts calibration
 - Search vs View
 - View vs Record
 - o Square selection and MMMs acquisition
 - o Template definition
 - Setting up focus and exposure (record) area
 - o Target selection
 - o Script editing and Automated data acquisition
- Switch grids and screening individual grids
- Autoloader cassette undocking (only for Superuser candidate upon request)
 - o Undocking cassette out from the Autoloader
- Data saving and transferring
- Cryo Cycle (introduction only)
- Finish up

2. Hands on practice

3. Certification (optional, upon request)

- Certification is required for independent usage of the microscope.
- Certification can be requested by trainees once they feel confident about their skills.
- Certification can be carried out in this session or later user's session, and will be supervised by YCR manager or director.
- During the certification, individual users must demonstrate proficiency in performing the full workflow of cryo sample screening, including but not limited to:
 - o Clipping autogrids
 - o Full cryo sample screening procedures
 - o Basic troubleshooting
- The trainees who independently (i.e., without referring to manual or notes) complete the test and demonstrate good judgement on problems that require immediate notification of the YCR staff will be approved by YCR manager or director as a Certified user of corresponding Glacios.
- After Introductory training on microscope- or site-specific knowledges, the users can also become a Certified user of the other Glacios of YCR. To become a Certified user of Krios, the users must also complete an Advance training on (SPA or Tomography) data collection on Glacios and an Introductory training on Krios, and pass the certification on Krios.