Agenda of CryoEM Trainings

Yale CryoEM Resource

> This agenda is prepared for the ***CryoEM basic training on Glacios*** (Sessions 1-3). It is designed for users who aim at independently preparing cryo samples and screening them using Glacios with SerialEM.

> To ensure training quality, trainees must complete all three sessions of ***CryoEM basic training on Glacios***, though YCR will strive to be flexible in response to trainees' requests. For example, training can begin with grid clipping if requested. Optional parts are indicated in gray.

> Trainees can vitrify their samples in the first session. However, to ensure training quality and minimize sample waste, a **maximum of 4 EM grids** can be prepared. In addition, trainees' grids will not be loaded into the Glacios during the 1st and 2nd sessions, but **up to 4 grids** may be used in the **3rd session**.

> To gain permission for independent usage of the corresponding Glacios, trainees will be required to pass a certification (practical test) during the third training session or a later regular session.

> Trainees are responsible for EM grids, C-clip rings, C-clips, and grid boxes. YCR provides these items but encourages independent purchase.

> Advanced training on other specific techniques or applications (e.g., Advanced training on SPA with SerialEM or MicroED) are available upon request.

> For more details, please refer to YCR-Policies at https://cryoem.yale.edu/policies-and-rates. Questions and comments are welcome to Jianfeng.lin@yale.edu.

Session 1: Cryo sample preparation & TEM Basics

Date: TBD
Morning: 9am-12am
Afternoon: 1pm-5pm

1. **Introduction to plunge freezing of biological samples:**
   - Review training agenda
   - Introduction to EM grids and proper handling, including glow discharge of EM grids
   - Overview of plunge freezing of biological samples

2. **Vitrobot Training and Practice:**
   - Introduction to the Vitrobot and an overview of the freezing workflow
   - Proper handling of liquid nitrogen (LN_2) and ethane
   - Freezing cryoEM sample grids
   - Hands-on practice

3. **Autogrid Clipping:**
   - Introduction to c-clip, c-clip rings, and clipping tools
   - Clipping cryoEM sample grids
   - Hands-on practice

4. **Electron microscope and Software:**
   - Microscope Software Launcher
   - TEM User Interface (UI): composition and editing
   - Hand panels
   - FluCam: demonstration and explanation of its usage.
   - System and Optics
     - o 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

1. **K3 Introduction (for Sample screening and/or Data collection):**
   - Calculating dose, dose rate, and pixel size
   - Camera gain reference/dark reference
   - Setting up for image acquisition
   - Discussing commonly used and desired settings/parameters
   - Hands-on practice

2. **Introduction to SerialEM:**
   - Overview of the software
   - Introduction to the software interface
   - Low dose control
   - Eucentric rough/fine/both/by focus
   - Autofocus
   - Navigator
   - LMM (Low Magnification Map) and MMM (Medium Magnification Map)
   - Camera & Script Controls
   - Camera Parameters
     - Record
     - Preview
     - Focus
     - View
     - Search
   - Script
   - Hands-on practice

3. **Glacios Daily Alignments:**
   - Condenser astigmatism correction
   - Centering a C2 aperture
   - Eucentric height and Eucentric focus
   - Direct alignments:
     - Beam tilt Pivot point
     - Rotation center (optional)
     - Coma free alignment
   - Selecting and centering an objective aperture
   - Objective astigmatism correction
   - Hands-on practice

Session 3: Cryo sample screening using Glacios with SerialEM

Date: TBD
Morning: 9am-12am
Afternoon: 1pm-5pm

1. **Workflow of Cryo sample screening:**
   - Autogrid clipping (completed by trainee before the 3rd session)
   - Autoloader cassette docking (only for Superuser candidates upon request)
     - Loading Autogrids into an Autoloader cassette
     - Docking the cassette into the Autoloader and creating a sample inventory
   - SerialEM startup and setting file loading
   - Automated multiple grid screening
     - Acquiring LMMs of multiple selected grids
     - Checking LMMs and evaluating grid quality
   - Semi-automated high magnification images acquisition for ice and sample evaluation
     - Selecting and inserting C2 and Objective apertures
     - Setting up Low Dose illuminations in SerialEM
     - Calibrating image shifts
       - Search vs View
       - View vs Record
     - Square selection and MMMs acquisition
     - Template definition
       - Setting up focus and exposure (record) area
     - Target selection
     - Script editing and Automated data acquisition
   - Switch grids and screening individual grids
   - Autoloader cassette undocking (only for Superuser candidate upon request)
     - Undocking the cassette 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 microscope use.
   - Trainees can request certification once they feel confident in their skills.
   - Certification can be conducted in this session or a later user's session, and will be supervised by a YCR Director/Site-Director.
   - During certification, users must demonstrate proficiency in the full workflow of cryo sample screening, including but not limited to:
     - Clipping autogrids
     - Full cryo sample screening procedure
     - Basic troubleshooting
   - Trainees who independently (i.e., without referring to manual or notes) complete the test and demonstrate good judgement on issues that require immediate notification of the YCR staff will be approved by the YCR Director or Site-Director as a Certified user (or Superuser) of corresponding Glacios.
   - After Introductory training on microscope- or site-specific knowledge, users can also become Certified users (or Superusers) of the other Glacios of YCR. To become a Certified user of Krios, the users must also complete Advance training on (SPA or Tomography) data collection on Glacios and an Introductory training on Krios, and pass the certification on Krios.