Agenda of Cryo EM Basic Training with Glacios

The Yale CryoEM Resource

> This training is designed for users who aim at independently preparing cryo samples and screening them using Glacios with SerialEM.

> It consists of 3 full-day training sessions, which must be completed within 10 weeks after the first training session. To ensure training quality, trainees are required to complete all the 3 sessions, although YCR will try to be flexible based on trainees' requests. For example, the training can start from grid clipping as user requested. The skipped and optional parts are indicated in gray text.

> To ensure training quality and avoid wasting user's samples, user's grids will not be loaded into the Glacios in the 1st and 2nd sessions. Trainees can use their grids on the 3rd session, but no more than 4 grids.

> To gain permission of independent usage of corresponding Glacios, trainees will be required to pass a certification (practical test) during the 3rd session or later user's session.

> Users are responsible for the cost of C-clip rings, C-clips, and grid boxes. Although the YCR provides these consumables with original prices from TFS, users are encouraged to order them directly from TFS.

> Advanced training on specific technique or application (e.g., Advanced training on SPA with SerialEM) is available from YCR upon request.

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**Session 1: Cryo sample preparation and 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
   - Overview of the entire freezing workflow with Vitrobot
   - Proper handling of liquid nitrogen (LN2) and ethane
   - Freezing a real biological sample
   - Hands-on practice

3. **Autogrid Clipping:**
   - Introduction to c-clip and c-clip rings, and tools used for clipping
   - Clip a real cryo grid
   - Hands-on practice

4. **Electron microscope and Software:**
   - System and optics
     - Explain the microscope from top to bottom
     - Differences between nano-probe and micro-probe modes
     - Parallel illumination
   - Hand panels
   - Microscope Software Launcher
   - TEM User Interface (UI): show and explain each tab
   - FluCam: show and explain each mode
   - Hands-on practice
Session 2: Microscope Operation, Detector, and SerialEM

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

1. Setting up the microscope for daily operation
   - Condenser stigmation
   - Center C2 aperture
   - Eucentric height and Eucentric focus
   - Direct alignments
     - Beam tilt Pivot point
     - Rotation center
     - Coma free alignment
   - Select and Center objective aperture
   - Objective stigmation
   - Cryo Cycle (introduction only)
   - Hands on practice

2. CETA camera (for bench marking):
   - Introduction to CETA camera and TIA
   - Setting up the CETA for acquiring images in TIA
   - Acquiring Gain/Bias references
   - Discuss what settings/parameters are commonly used/desired
   - Hands on practice

3. K2 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

4. 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 & Macro Controls
   - Camera Parameters
     - Record
     - Preview
     - Focus
     - View
     - Search
   - Macros
   - 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
   - High magnification images acquisition for evaluating the grid
     o C2 aperture selection and centering
     o Objective aperture selection and centering
     o Set up Low Dose illuminations at SerialEM
     o Direct Alignments of Record Beam using SerialEM
     o K2 gain/dark references acquisition
     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 Macros editing and Automated data acquisition
   - Switch grids and screening
   - Autoloader cassette undocking (only for Superuser candidate upon request)
     o Undocking cassette out from the Autoloader
   - Data saving and transferring
   - 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.