Onsite Applications Training Agenda

Glacios cryo-EM Sample prep, screening and data collection

University of Nebraska-Lincoln, March 17-20, 2025

Disclaimer: The following agenda is suggested and subject to change. Application personnel will try to be flexible based on customer experience and requests.

Format: Four on-site training days for Sample prep and Glacios cryo-EM screening
Instructor: Helen Donelick

Day 1: (3/17/2025) Sample preparation with Vitrobot mk4

Start: 9am (CST)
Lunch: ~12pm
End: 5pm

Sample preparation:

- Discussion and planning for sample vitrification.
  - User sample or apoferritin standard.
- Using the Vitrobot (demonstration and hands-on practice).
  - Setup and shutdown.
  - Grid and sample preparation.
    - Room temp then cryogenic.
  - Freezing samples and common considerations for parameters.
- Grid clipping (demonstration and hands on practice).
  - Clip grids at both room and cryogenic temperatures.
  - Save grids for screening.

Loading grids:

- Loading station and cassette.
  - Tutorial and hands-on practice loading the cassette for the autoloader.
  - Load grids into Glacios via nanocab.
  - Inventory grids and atlas.

Atlasing grids:

- Check atlasing presets in EPU.
- Begin atlasing grids prepared earlier in the day.
- As atlases become available talk about what we are looking for, and where we might screen

Learning Objectives:

- By the end of the day, the participants will be able to:
  - Understand the freezing and clipping process
  - Understand how to load grids into cassette.
  - Be able to start and atlasing session in EPU
  - Have a basic understanding of EPU.

Day 2: (3/18/2025) Glacios operations, EPU for screening/data collection

Start: 9am (CST)
Lunch: ~12pm
End: 5pm

Starting a Glacios session:

- Load grids via autoloader and inventory (if needed).
    - Should have been completed yesterday
- Select xg to start discussion of EPU presets

Creating EPU presets:

- Inventory and atlas grids.
- Screen a grid.
    - Load grid into TEM and atlas
    - Go through EPU presets
        - Common considerations for each preset, save after completing.

Common alignments/tuning for Glacios

- Direct alignments (beam shift, C2 aperture, pivot points)
- Optimize optics (stig and coma)
- Tune Selectris (EPU and Sherpa)
- When to take gain references

Setting up Automated EPU data acquisition

- Connect to CryoFLOW
- Square selection.
- Hole selection.
- Template definition.
    - Setting up focus, drift, and exposure area.
    - Defocus values.
- Template execution.
    - Multi-shot
- Automated data acquisition.
- Viewing results.
- Data management.
- Troubleshooting.

Learning objectives:

- By the end of the day, the participants will be able to:
    - Use the microscope/EPU to screen sample quality.
    - Direct alignments and Selectris tuning
    - Start data collection and use CryoFLOW to analyze results.

Day 3: (3/19/2025) Glacios operations, EPU for screening/data collection: user operated

Start: 9am (CST)
Lunch: ~12pm
End: 5pm

Decide if more grids should be made- ApoF or user samples.
Starting a Glacios session:

- Load grids via autoloader and inventory (if needed).

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    - Should have been completed yesterday
- Select xg to start discussion of EPU presets

Creating EPU presets:

- Inventory and atlas grids.
- Screen a grid.
    - Load grid into TEM and atlas
    - Go through EPU presets
        - Common considerations for each preset, save after completing.

Common alignments/tuning for Glacios

- Direct alignments (beam shift, C2 aperture, pivot points)
- Optimize optics (stig and coma)
- Tune Selectris (EPU and Sherpa)
- When to take gain references

Setting up Automated EPU data acquisition

- Connect to CryoFLOW
- Square selection.
- Hole selection.
- Template definition.
    - Setting up focus, drift, and exposure area.
    - Defocus values.
- Template execution.
    - Multi-shot
- Automated data acquisition.
- Viewing results.
- Data management.
- Troubleshooting.

Learning objectives:

- By the end of the day, the participants will be able to:
    - Use the microscope/EPU to screen sample quality.
    - Direct alignments and Selectris tuning

Start data collection and use CryoFLOW to analyze results

Day 4: (3/20/2025) Glacios operations, EPU for screening/data collection: user operated

Start: 9am (CST)
Lunch: ~12pm
End: 5pm

Decide if more grids should be made- ApoF or user samples.

Starting a Glacios session:

- Load grids via autoloader and inventory (if needed).
    - Should have been completed yesterday
- Select xg to start discussion of EPU presets

Creating EPU presets:

- Inventory and atlas grids.
- Screen a grid.
    - Load grid into TEM and atlas
    - Go through EPU presets
        - Common considerations for each preset, save after completing.

Common alignments/tuning for Glacios

- Direct alignments (beam shift, C2 aperture, pivot points)
- Optimize optics (stig and coma)

- Tune Selectris (EPU and Sherpa)
- When to take gain references

Setting up Automated EPU data acquisition

- Connect to CryoFLOW
- Square selection.
- Hole selection.
- Template definition.
  - Setting up focus, drift, and exposure area.
  - Defocus values.
- Template execution.
  - Multi-shot
- Automated data acquisition.
- Viewing results.
- Data management.
- Troubleshooting.

Learning objectives:

- By the end of the day, the participants will be able to:
  - Use the microscope/EPU to screen sample quality.
  - Direct alignments and Selectris tuning

Start data collection and use CryoFLOW to analyze results
Feel comfortable operating microscope independently.

Applications support summary.

Estimate of days used for this session: 4.