Electron Microscopy Shared Resource
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# Protocol for Glacios cryo-EM imaging (SerialEM)

**Location:**
DE-788 (Glacios room)

**General rules:**
- Do not use the Glacios or automated software unless you are accompanied by an EM core staff member or have been cleared as an independent user
- Facility staff will clip, load, and unload all autogrids
- Use computers and programs only for their intended purposes
- Respect your reservation time
- Report any problems to Caleigh ASAP

**Sign-up protocol:**
- Use iLab (base rate, $60/hr) to reserve Glacios for the entire time you will be using the Glacios
- Book supported use unless you confident in setting up collection completely
- Staff collection can be booked through “Services” on iLab
- Door access will be granted after training

**Training plan:**
- Please contact Caleigh at cazumaya@fredhutch.org to schedule training
    - Already trained on Leginon screening
    - Two 4-hour introduction and training in SerialEM
    - If returning >30 days after last Glacios use, **please** contact Caleigh (phone or email) for refresher and book “supported use time”

**Training objectives:**
- Perform startup and shutdown procedures responsibly
- Find eucentric height, focus, and set appropriate imaging conditions
- Setup and run automated data collection using SerialEM software

**User provided materials:**
- Frozen cryo-EM grids
- Storage space for movies and micrographs

**Shared resources tools list:** if anything is missing/out of the ordinary, please contact Caleigh ASAP
- Clipped and loaded cryo-EM grids
- Data stored for 30-days after collection

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**Screening (usually only for gold grids):**

**Setup**

1. Open correct settings file – 10eps_36kx is what most people use
2. Open navigator file
3. Put in 100um objective aperture (on microscope PC)
4. Turn on Low Dose

**Targeting**

5. Load in atlas and "Add Marker" on a good square
6. "Go to Marker"
7. "View" to find the spot that you were targeting
8. Move stage so you are actually in the square (**shift+right click drag**)
9. Run Eucentric – Rough task

**Focus/Record parameters**

1. Move to hole you want to image (shift + right click and drag)
2. Set focus spot
3. Setup View, Focus, and Record parameters
4. "Autofocus" x2
5. "Record"
6. "View"
7. Move to new hole you want to image
8. Repeat 4-7 until you want to move to a new square (back to targeting) or a new grid (back to setup)

**WARP**

1. Start "transfer_serialEM.bat" on desktop
2. Start WARP/Confirm WARP is running

**Collection:**

**Setup**

10. Open correct settings file – 10eps_36kx is what most people use
11. Open navigator file
12. Put in 100um objective aperture (on microscope PC)

**Grid-Square alignment**

13. Load in 155x imaging state
14. Load in atlas and "Add Marker" on a noticeable spot
15. "Go to Marker"
16. Run Eucentric – Rough task
17. Update Z of your atlas (in navigator)
18. "View" to find the spot that you were targeting
19. Click on the spot and "Shift to Marker"
20. Repeat "Add Marker" "Go to Marker" "View" to make sure marker is in the right spot

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**Square maps**

1. Load in atlas and "Add Points" to all of the squares that you want to collect
    a. ~20-25 full squares (1.2/1.3 300mesh) = 24hrs
2. Turn "Acquire" on for all of these points
3. New Montage (1x1)
4. Make sure PrepMMM script isn't set to tilt/is set to the tilt angle you want (**ctl+left click**)
5. Run PrepMMM through "Acquire at Items"

**Square-View alignment**

1. Turn on Low Dose
2. "Add Marker" on a noticeable spot in a square map
3. "Go to XYZ"
4. "View" to find the spot you were targeting (**shift+right click drag** to move the stage if you need to)
5. Click on the spot and "Shift to Marker"
6. Repeat 2-5 on 3 different squares

**P template**

1. Center on the middle of your image shift pattern (middle of a hole for odd numbers, middle of four holes for even numbers)
2. "Setup" your View parameters to
    a. Bin = 8, Area size = *just the middle of your template* Exposure = 2s
3. "View" and save in buffer P

**IS template**

1. "Setup" View parameters to
    a. Bin = 8, Area size = Full Exposure = 0.2s
2. "Setup" Record parameters to
    a. Bin = 1, Area size = Full Exposure = 0.5s
3. "Add Points" on the inner edges of your pattern corners
4. Select first point in navigator
5. Set Multi-Shot Parameters
    a. Choose X and Y numbers
    b. For Corners…
    c. IS to Nav Point
    d. "Record" to make sure you don't have carbon in your images (**right click drag** to move the image if you need to)
    e. Save Image Shift
    f. Repeat c-e for the rest of the corners
    g. OK
6. "Reset Image Shift"
7. "View" to make sure that you're hovering over the last point you added

**Astigmatism, coma (will not work on gold grids), collection setup**

1. Correct astigmatism and coma
2. Choose focus spot
3. Setup View, Focus, and Record parameters
4. Save Settings

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**Add points (pt1) and start**

1. "Add Polygon"
2. "Add Points" to identify x and y spacing
3. Add Grid of points
4. Delete bad holes in "Edit Mode"
5. "Combine points for Multi-Shot"
6. Save Navigator
7. Check Acq-…-IS script for defocus range
8. Run Acq-…-IS through "Acquire at Items"

**Motion correction**

3. Start "transfer_serialEM.bat" on desktop
4. Start WARP/Confirm WARP is running

**Add points (pt2 – DUMMY)**

1. Open DUMMY SerialEM
2. Open settings file
3. Open navigator file and Save As nav_d.nav
4. Add points to all squares
5. Save navigator throughout!!
6. "End Acquire" in main SerialEM program
7. Open nav_d.nav and save over nav.nav
8. Remove "Acquire" from the items that have already collected
9. Run Acq-…-IS through "Acquire at Items"
    a. Set for column valves to close at end and Email at end