Only recommended for gold/gold grids

SerialEM Protocol cryo screening Fred Hutch Glacios

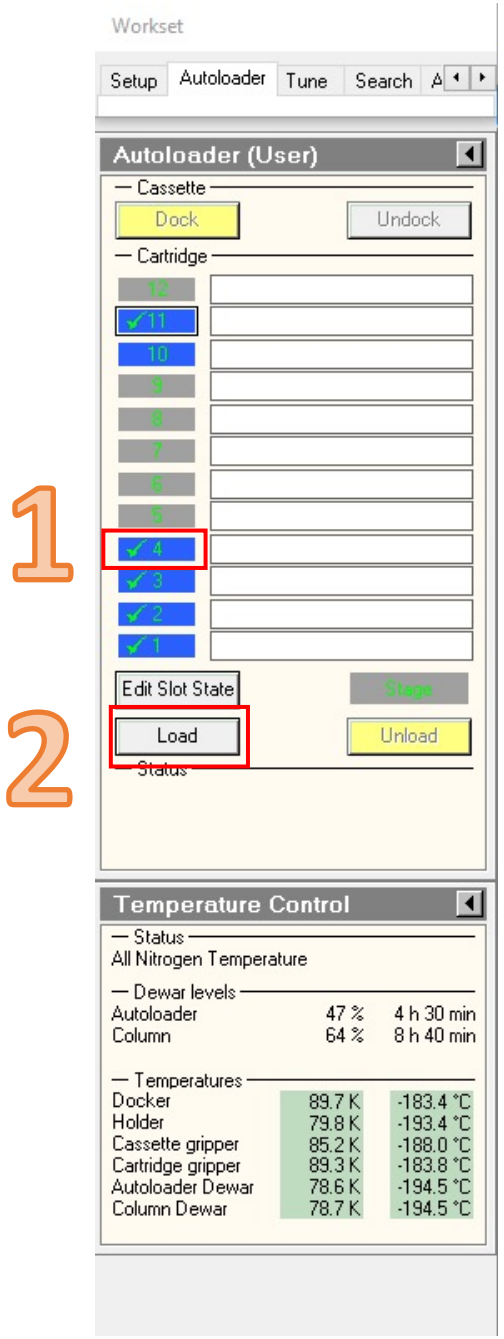
What you're starting with

- You have performed the "Glacios Start Up Checklist" and have:
  - A cold, vacuum-stable microscope
  - The Turbo is on "Auto Off"
  - You know the grid you want to screen
  - WARP is ready to launch

Load your first grid

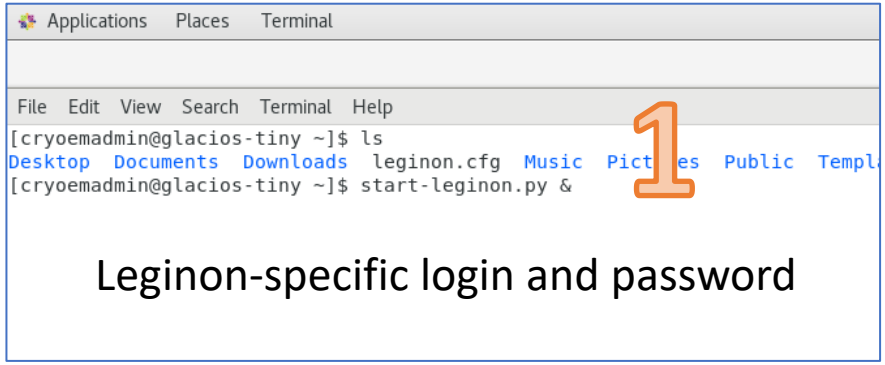
On the Autoloader tab of the microscope UI

1. Click the number grid you want to load
2. Click Load



Make fake Leginon session

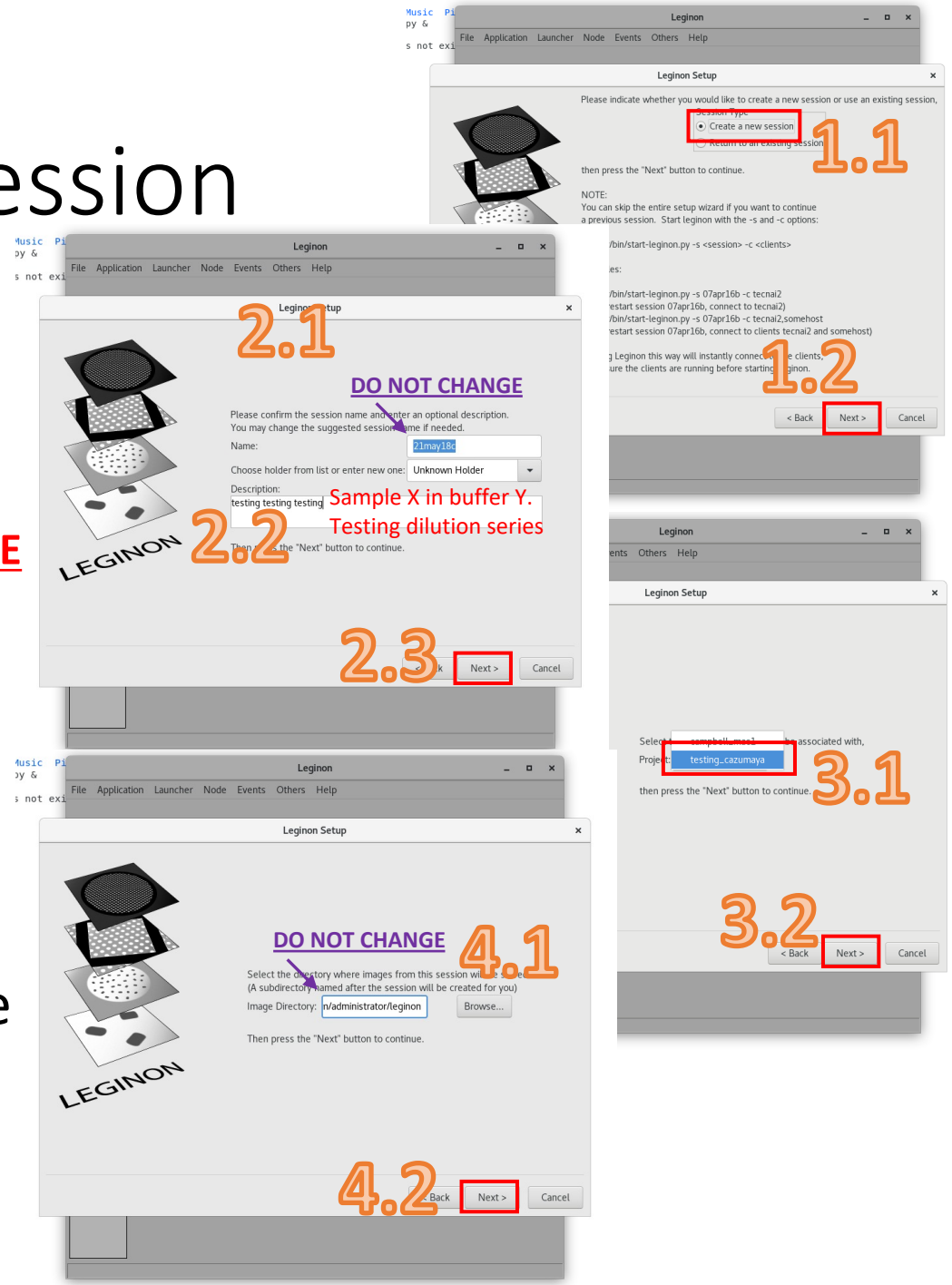
1. Login to Leginon computer
    1. Open terminal (right click on background
    2. start-leginon.py



Just once per session

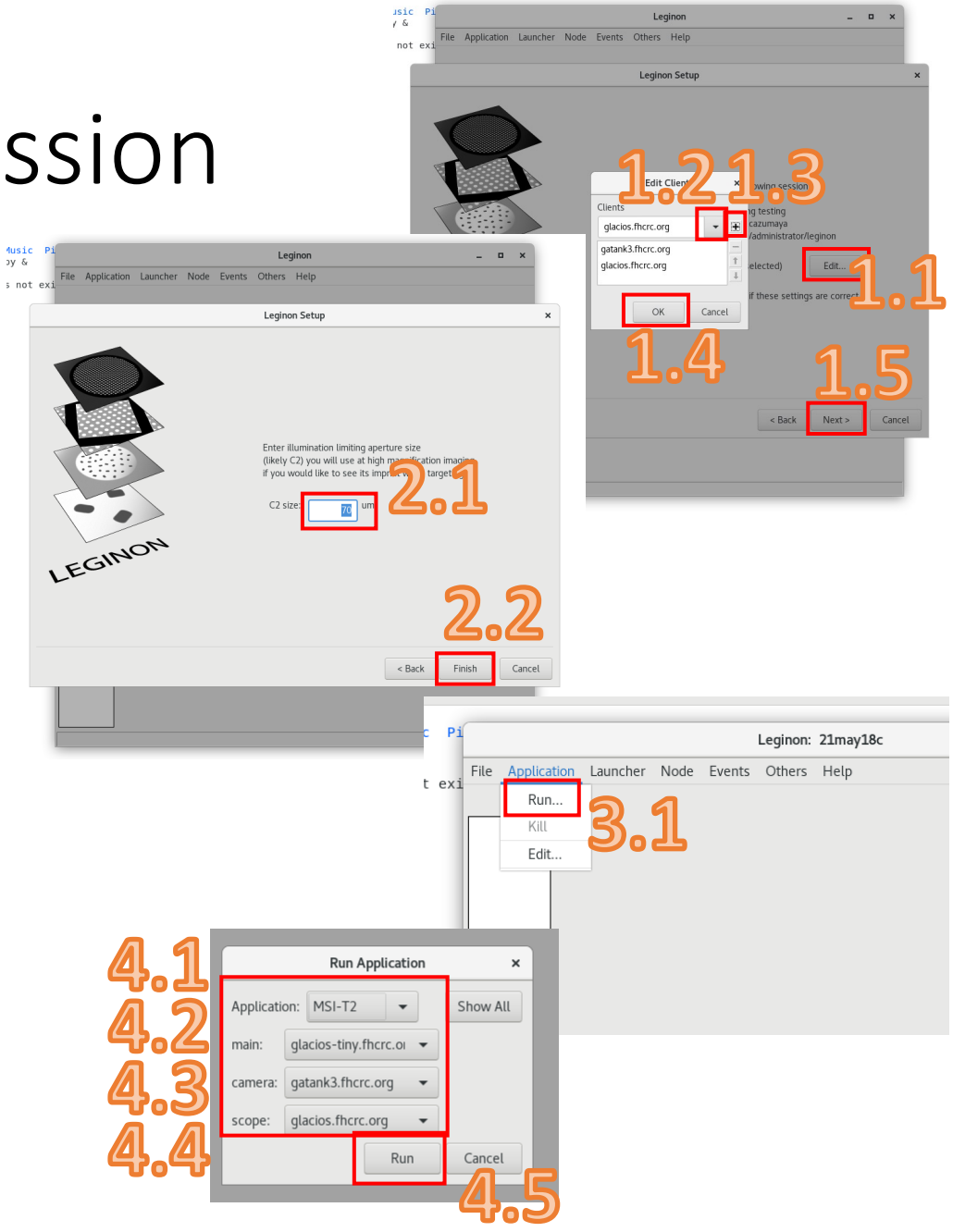
Fake Leginon session

1. Choose session
    1. Create a new session
    2. Next
2. Define session
    1. Name: **DO NOT CHANGE**
    2. Description: for your whole session
    3. Next
3. Pick project
    1. Project: Pick from dropdown
    2. Next
4. Choose where to save
    1. Image directory: **DO NOT CHANGE**
    2. Next



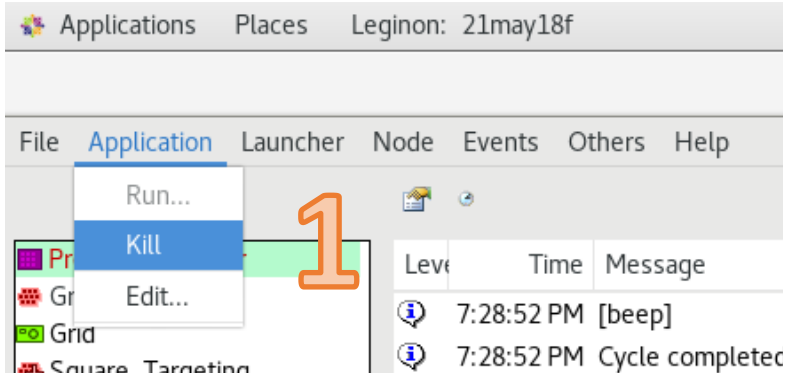
Fake Leginon session

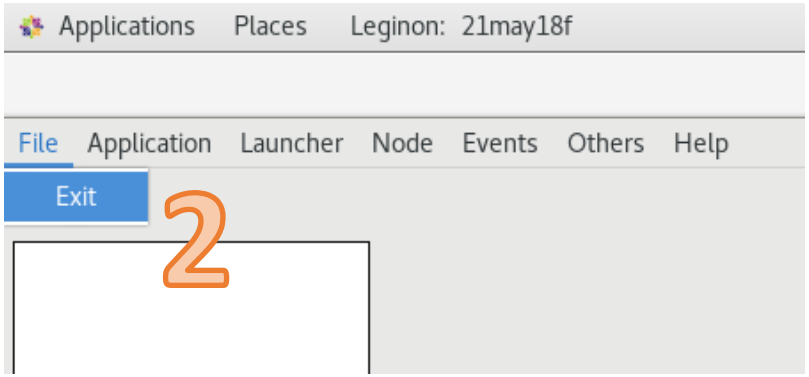
1. Add clients
   1. Edit
   2. Choose glacios.fhcrc.org from dropdown
   3. Click + !!
   4. Choose gatank3.fhcrc.org from dropdown
   5. Click + !!
   6. OK
   7. Next
2. Define C2 aperture
   1. 50um
   2. Finish
3. Start session
   1. Application -> Run
4. Choose application
   1. Application: MSI-T2
   2. Main: **glacios-tiny.fhcrc.org**
   3. Camera:**gatank3.fhcrc.org**
   4. Scope: **glacios.fhcrc.org**
   5. Run



Close Leginon

1. Application -> Kill
2. File -> Exit
3. Logout of computer
    1. Power logo ->
       Username -> Sign out



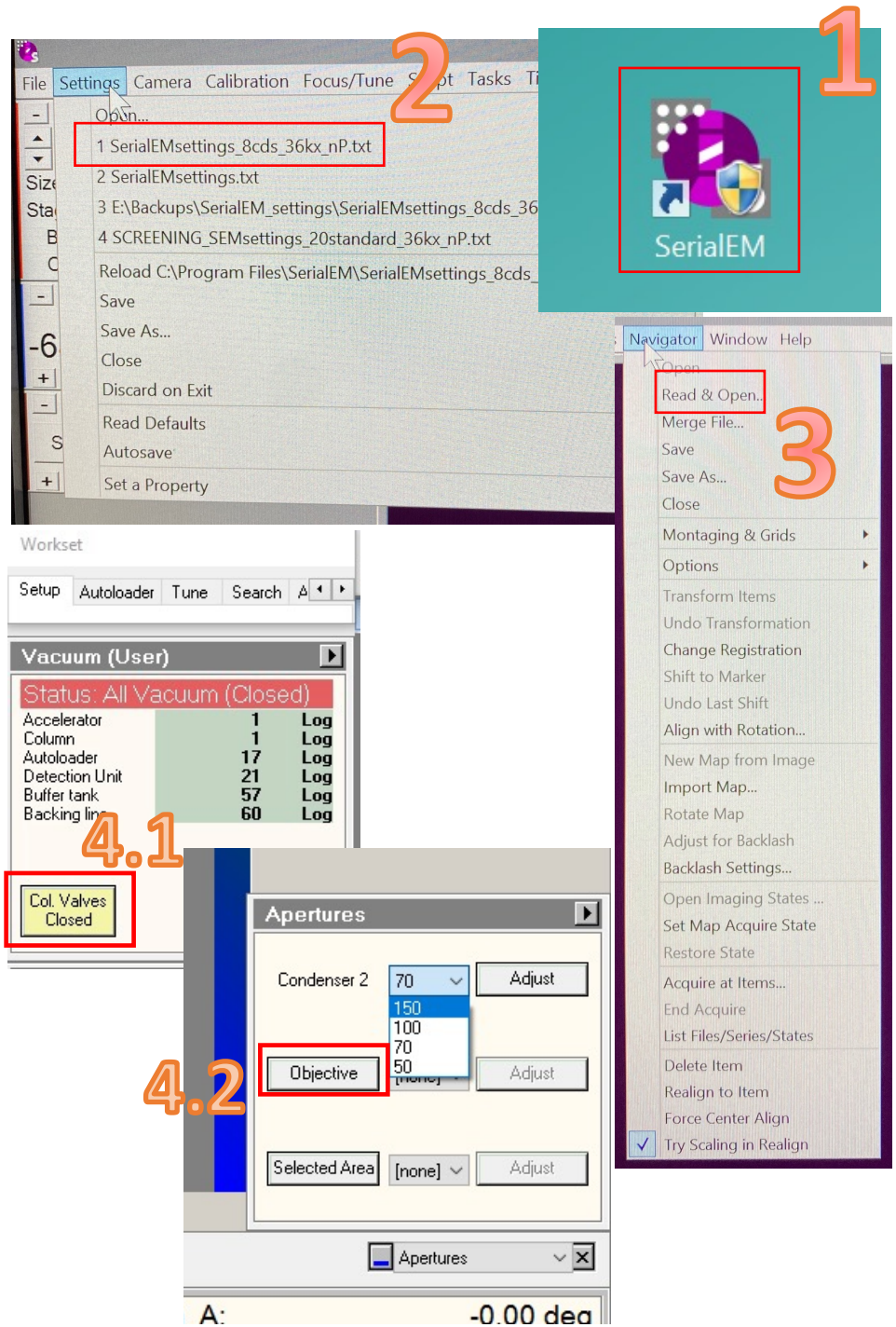


Create folder for movies

1. Go to File Explorer (on K3 computer)
2. Go to M:/cryoem/username/frames/
3. Right click -> new folder (name of your fake leginon session)
4. Go to fake session
5. Right click -> new folder (rawdata)

Prep SerialEM

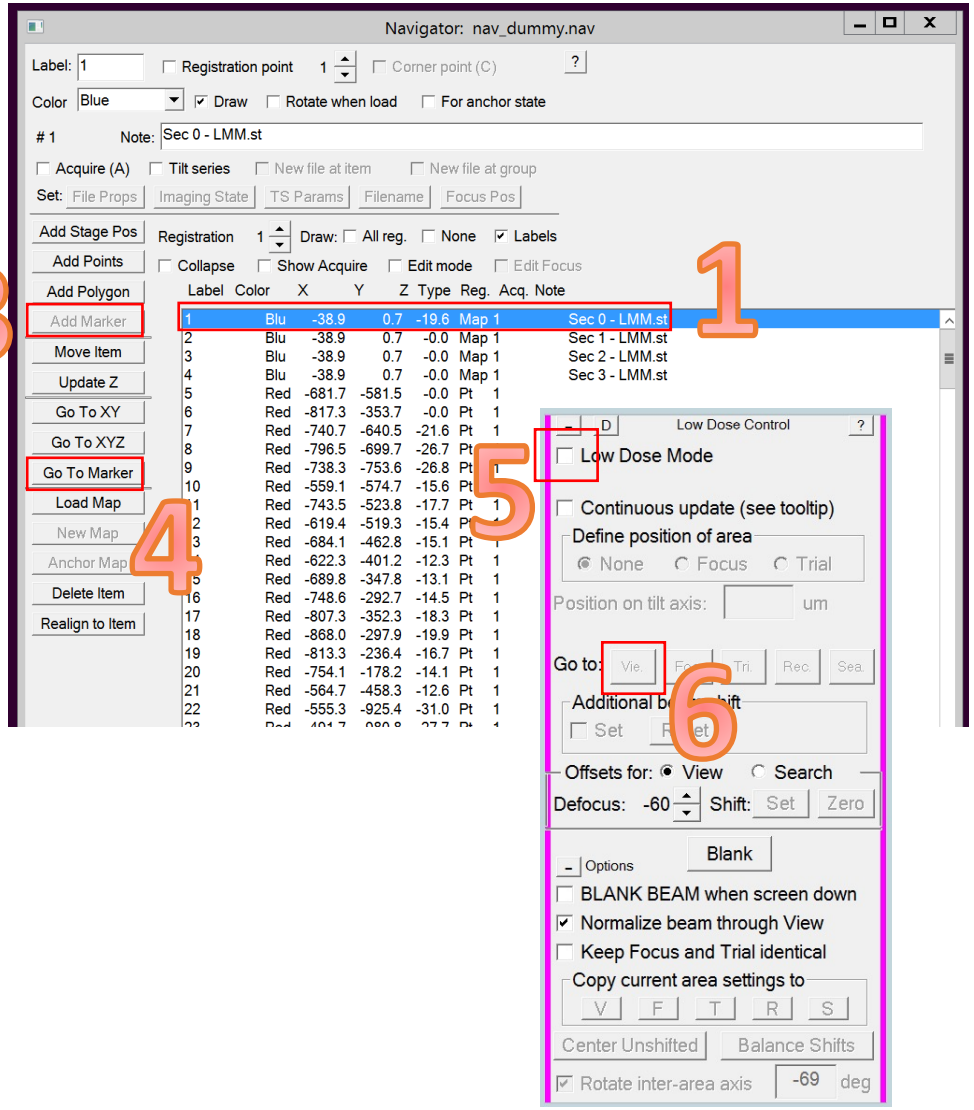
1. Open SerialEM
2. Load Settings file
    1. Settings -> SerialEMsetting_10cds_36kx_nP.txt
3. Load Navigator
    1. Navigator -> Read & Open -> nav.nav (in your today folder)
4. When grid is loaded and ready!!
    1. **Open column valves**
    2. **Put in 100um objective aperture**



Go to square of interest

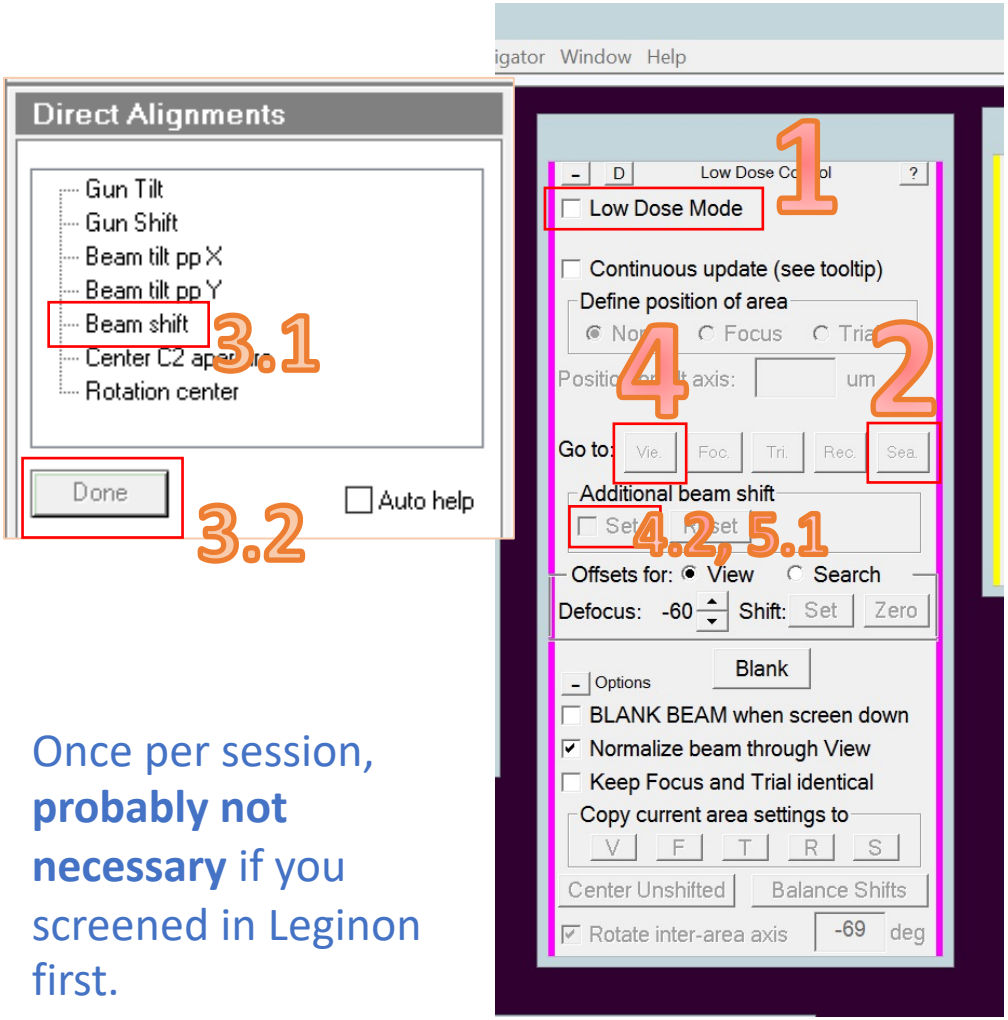
Are your column valves open?

1. Double click on atlas in Navigator window
2. Left click in the middle of a square of interest
3. Click “Add Marker”
4. Click “Go to Marker”
5. Check “Low Dose” in LowDose
6. Go to “View” in Low Dose
7. Insert screen and scoot yourself into the square with joystick



Find your beam in Low Dose

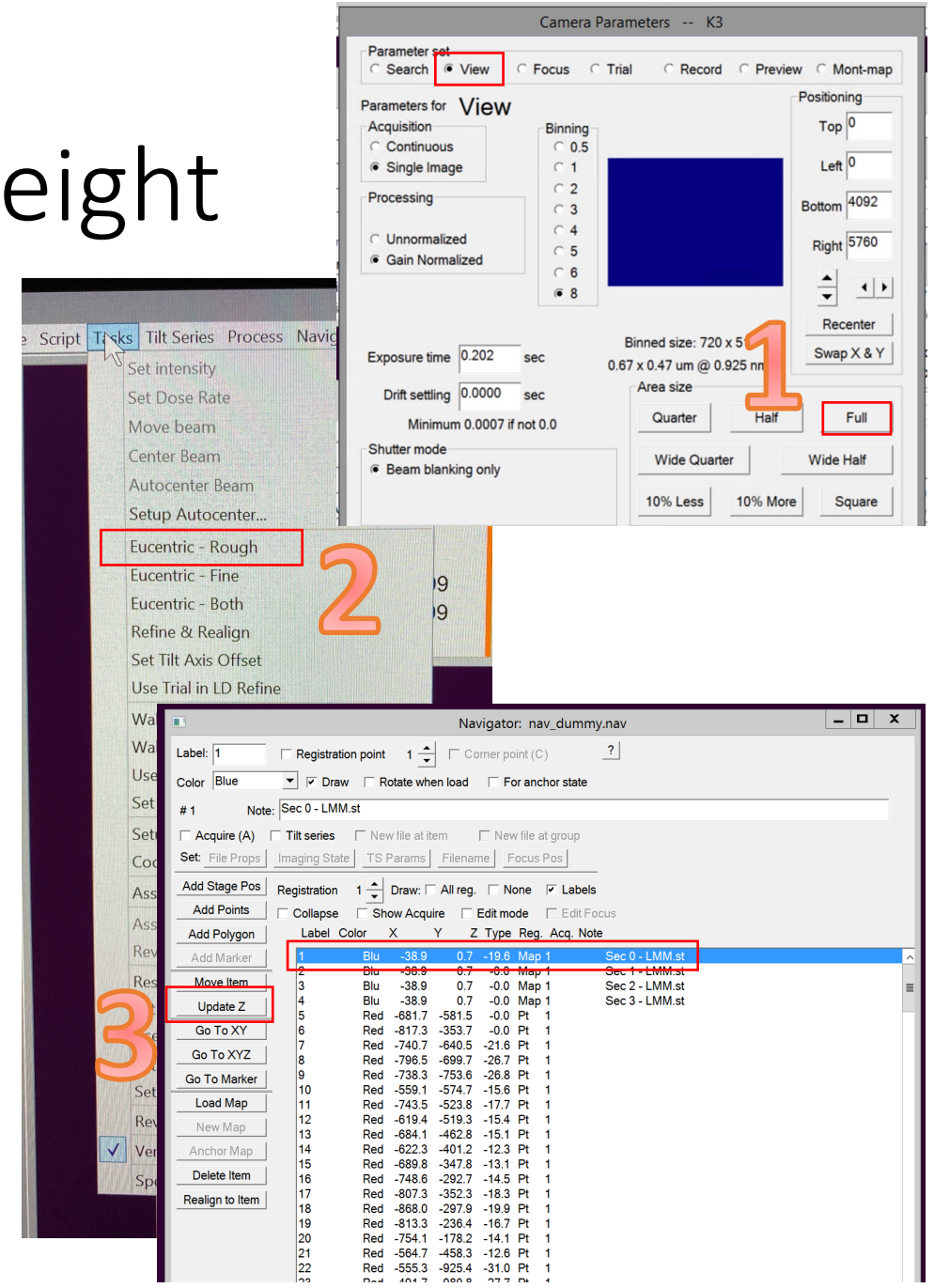
1. Go to: Rec in LowDose
2. Insert screen and
   1. Center ‘beam shift’ in Direct Alignments
   2. Click Done
3. Go to: View in LowDose
   1. Make sure that defocus on microscope is -80, if not change defocus to -80 with hand panel focus knob
   2. If beam is not centered:
      1. Check Set
      2. Center beam with track ball
      3. Uncheck Set
4. Go to: Focus in LowDose
   1. If beam is not centered:
      1. Check Set
      2. Center beam with track ball
      3. Uncheck Set



Once per session, **probably not necessary** if you screened in Leginon first.

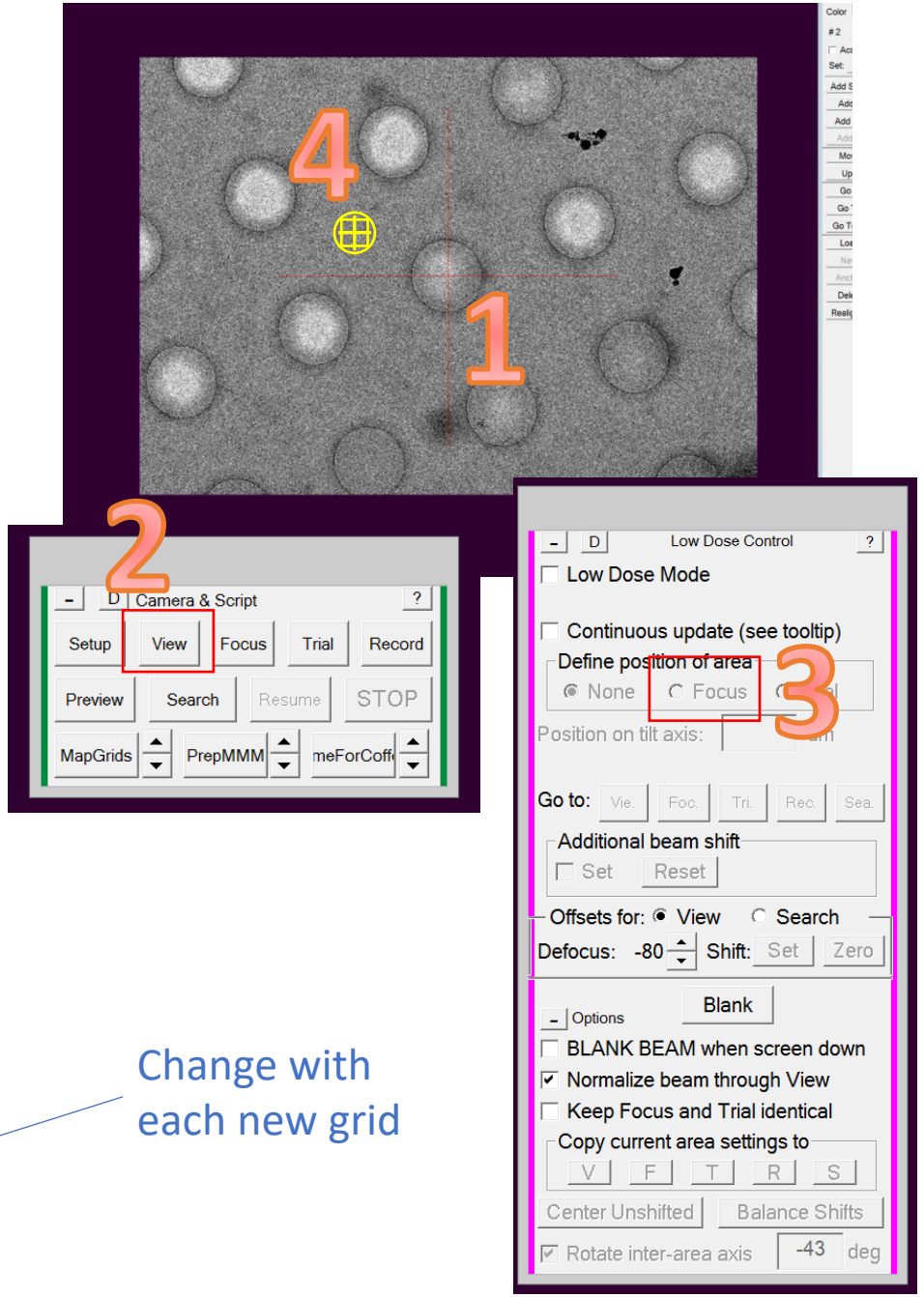
Find eucentric height

1. In Camera “Setup” make sure View has area size = Full
2. Tasks –> Eucentric-Rough
3. Select atlas in Navigator window and click “Update Z”
4. Take a ”View” image in Camera



Center on a hole

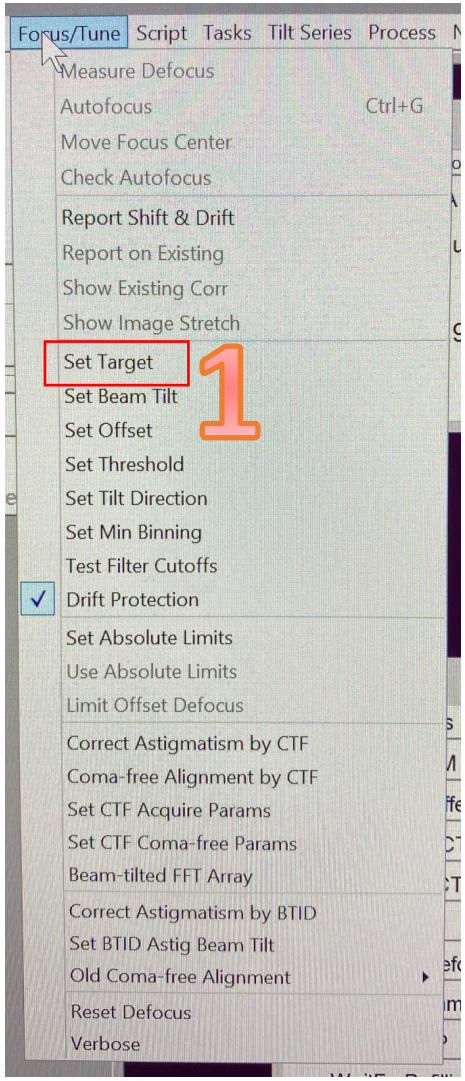
1. Shift + right click and drag to move your stage until you are centered over a hole
2. Click ”View” in Camera a few times to make sure your stage isn’t drifting away from this position
3. Select “Define position of area: Focus” in Low Dose
4. Left click to drop focus position (yellow) onto carbon next to the hole

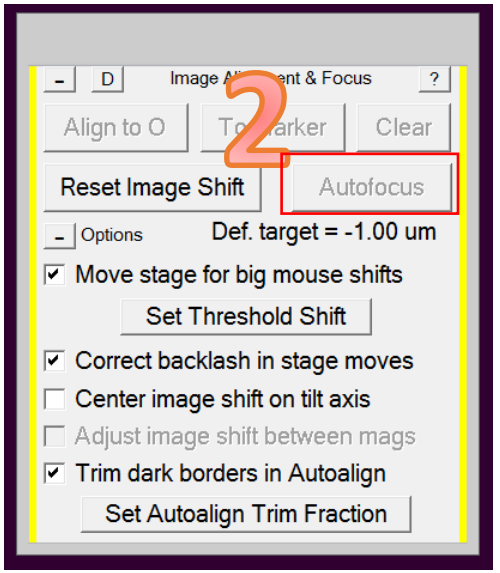


Change with each new grid

Autofocus

1. Focus/Tune -> Set Target (-2.0)
2. Click Autofocus in Image Alignment and Focus

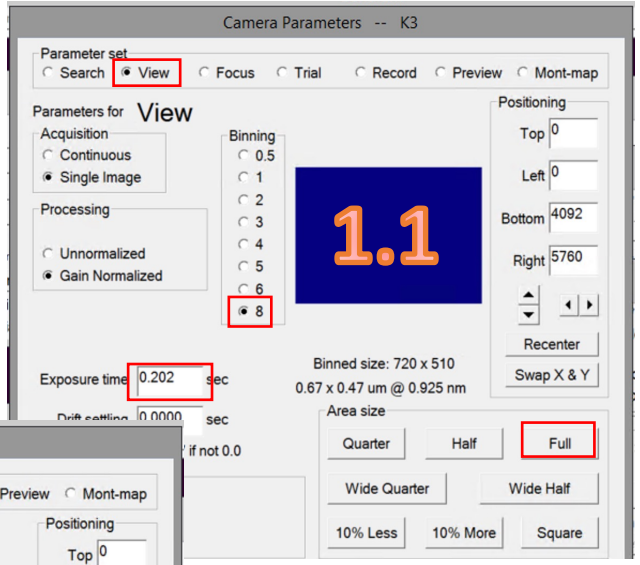


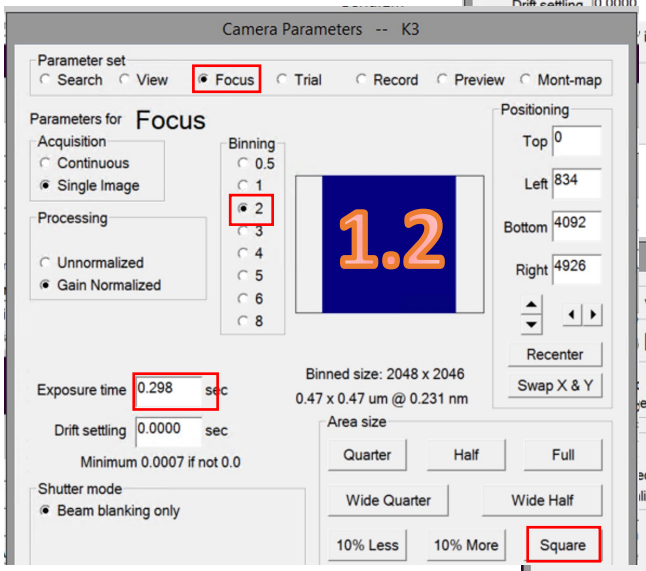


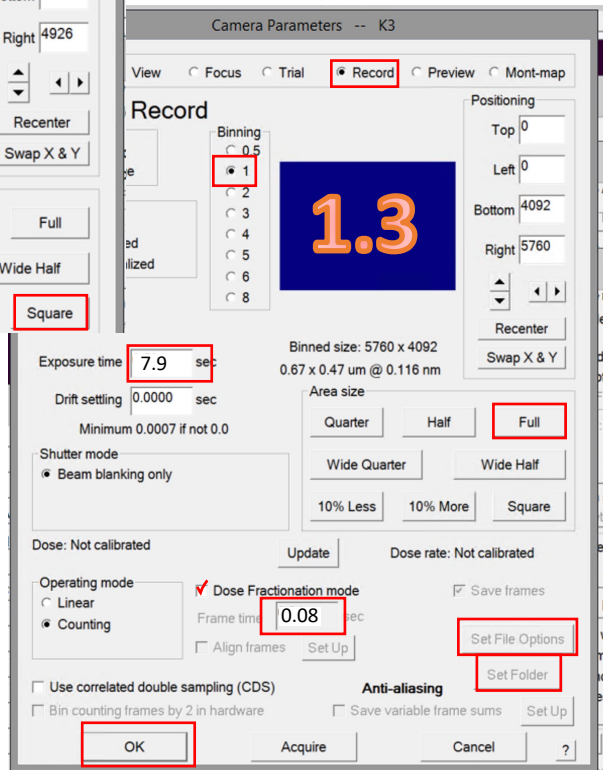
Set imaging settings

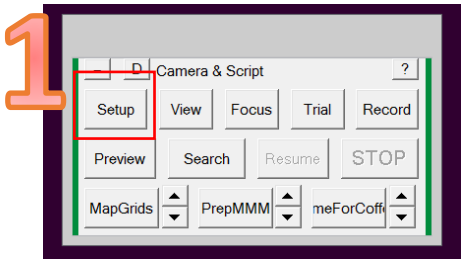
1. Click “Setup” in Camera and set parameters
   1. View: bin = 8, area size = full, exposure = 0.2s
   2. Focus: bin = 2, area size = square, exposure = 0.2-0.5s
   3. Record: bin = 1, area size = full, exposure = (from settings sheet), dose fractionation ON – frame time: (from settings sheet), Set File Options: (YYYYMMDD_username_sample_grid), Set Folder (X:/SEM_frames)

Change with each new grid





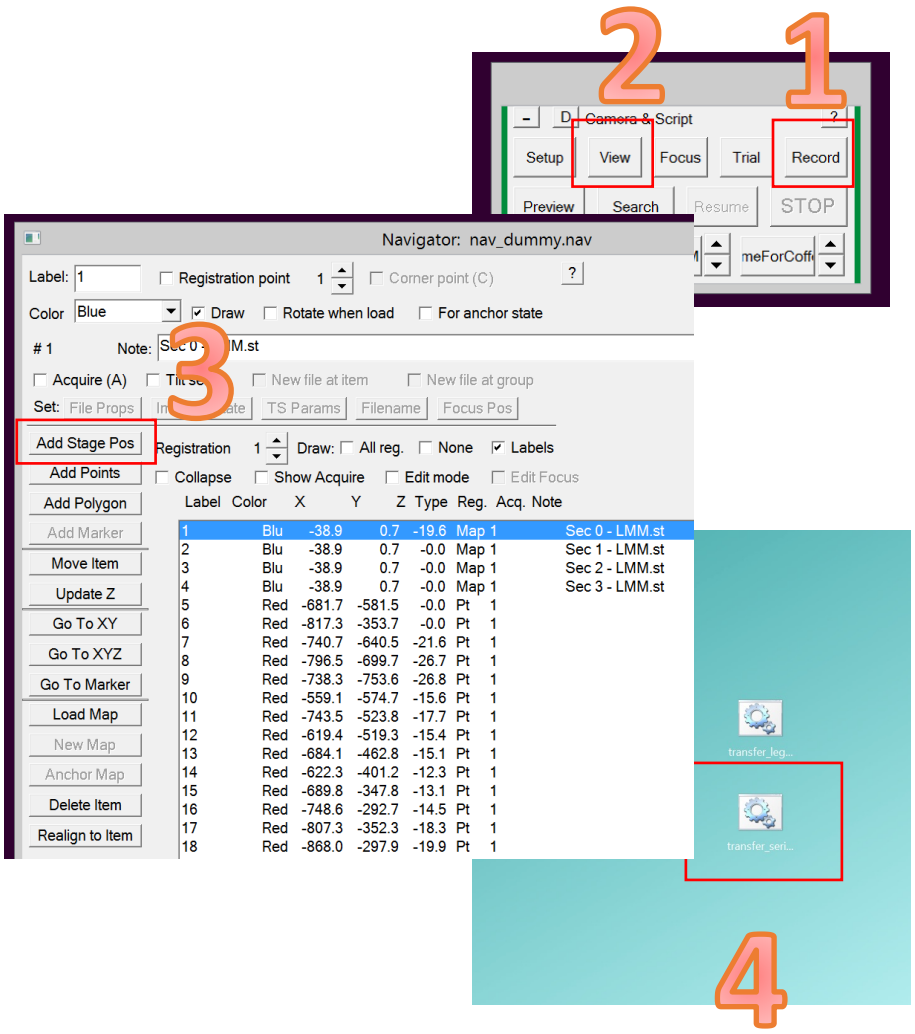




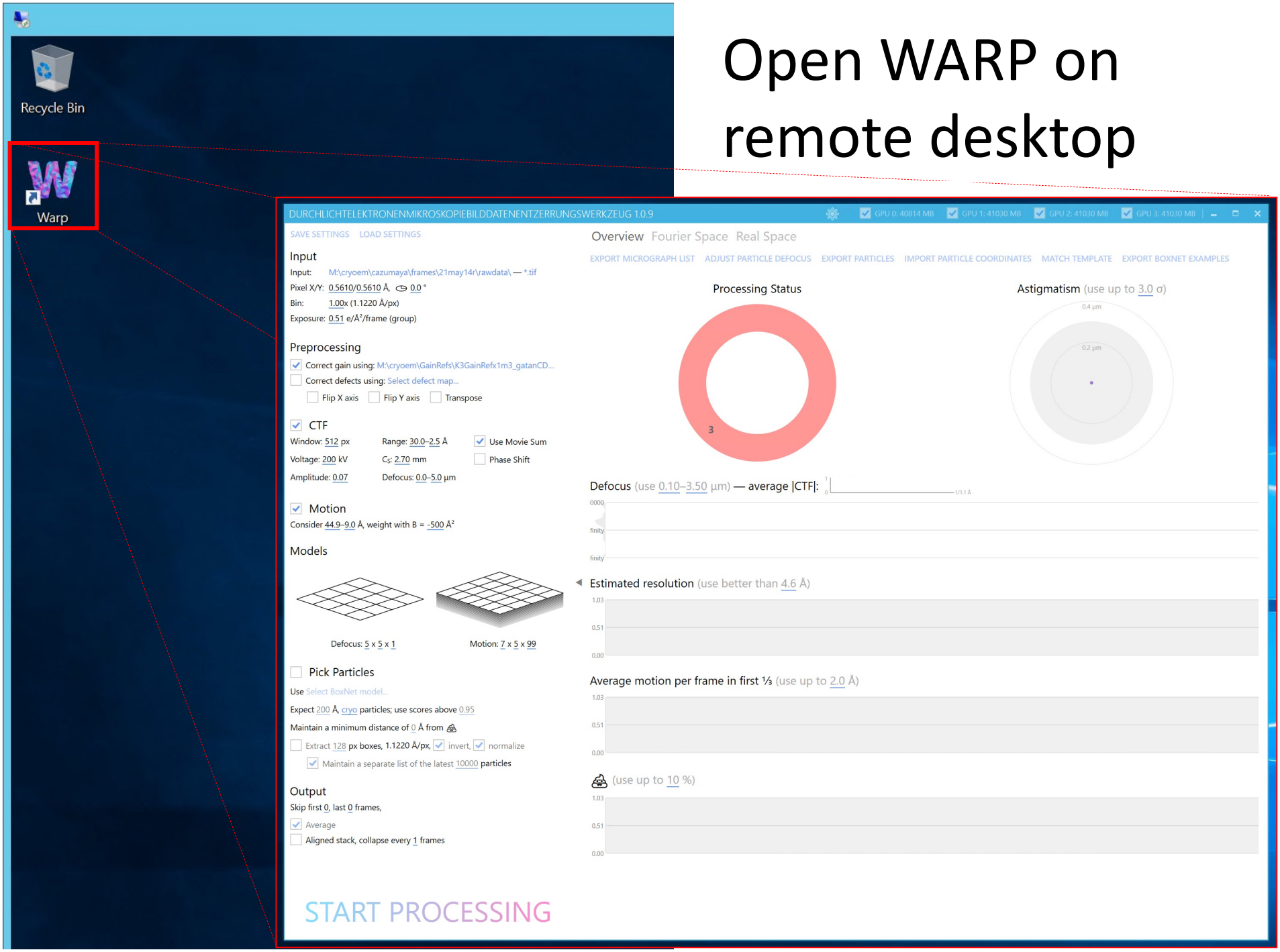
Take exposures and start data transfer

1. Click “Record” in Camera
2. Click “View” in Camera
3. Click “Add Stage Position” on Navigator
4. Double click on “transfer_serialEM.bat”
    1. Enter username
    2. Enter session name (from Leginon screening/fake session i.e. 21sep14f)

Just once per session

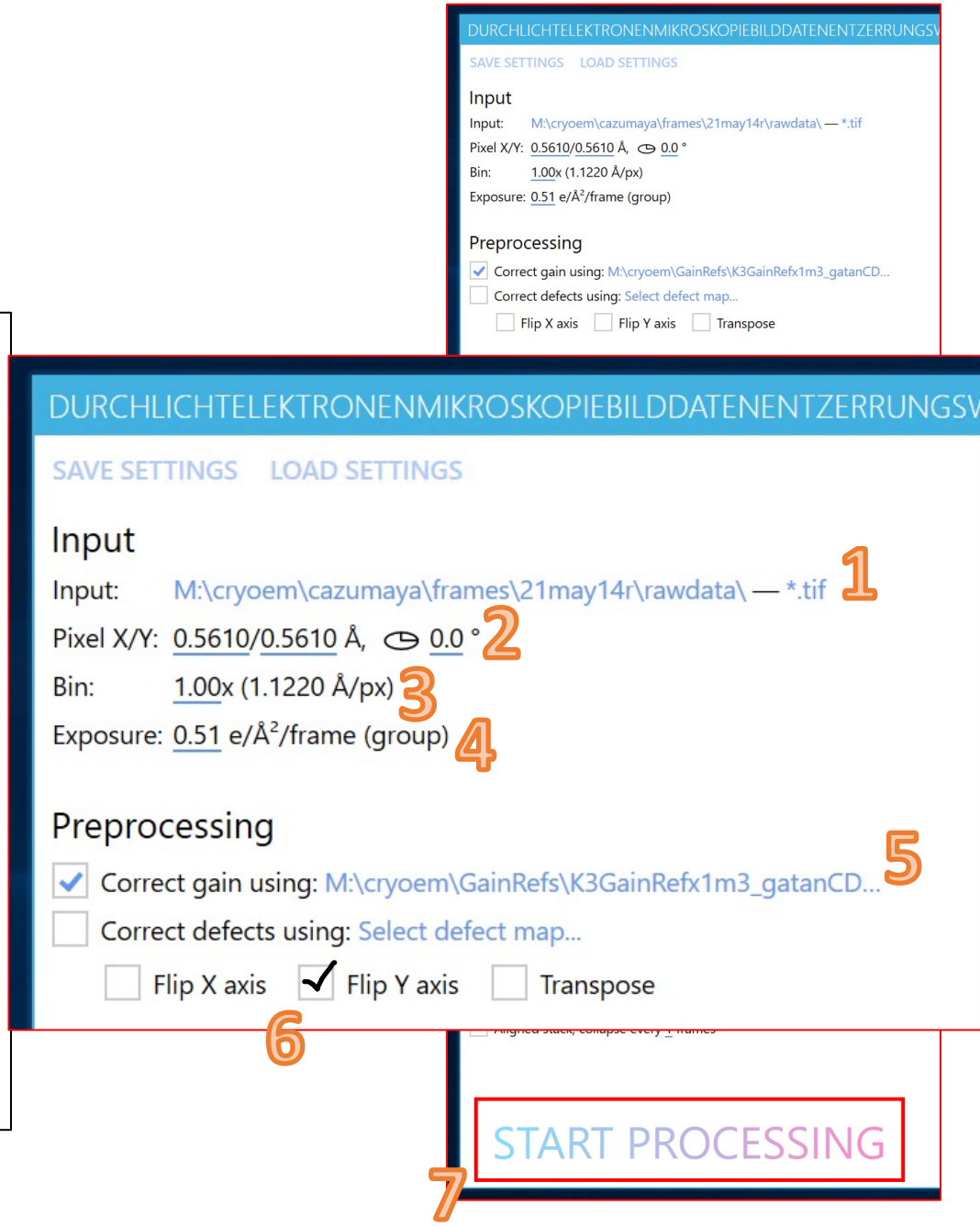


Open WARP on remote desktop



Setup WARP

1. Input: Browse for movie folder
2. Pixel size: (see Teams) usually 0.561
3. Bin: 1.00x (usually)
4. Exposure: (see Teams) usually 0.51
5. Correct gain using: Browse for gain image
6. Flip Y axis for gain
7. Start Processing



Setup collection on this grid (SerialEM Protocol)

OR

Screen more

(repeat slides 13,14,16 to screen *in same square*)

(repeat slides 10,12,13,14,16 to screen *in new square*)

(repeat slides 3,10,12,13,14,15,16 to screen *on new grid*)

OR

Shutdown

(next slide).

Do shutdown without collection Glacios check list!

End iLab time and sign out!