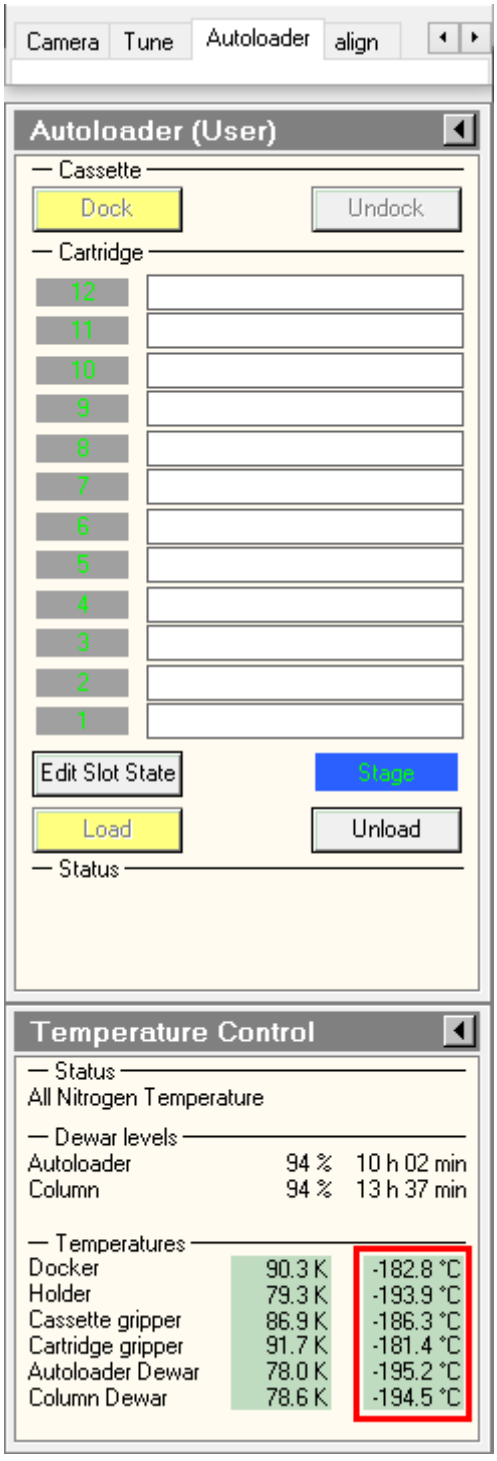
The Screening Pipeline

1. Load the Grids

1. Introduce your grids in the cassette
    - Cassette position 1 is dedicated to the Cross Grating Grid
    - Grids are loaded with the C-clip toward position 1

2. Load the cassette in the NanoCab. Make sure no contaminant (fiber, hair) entered the NanoCab.
3. Check Autoloder temperatures, then load the NanoCab in the microscope



4. Remove the NanoCab

2. Setup Serial-EM

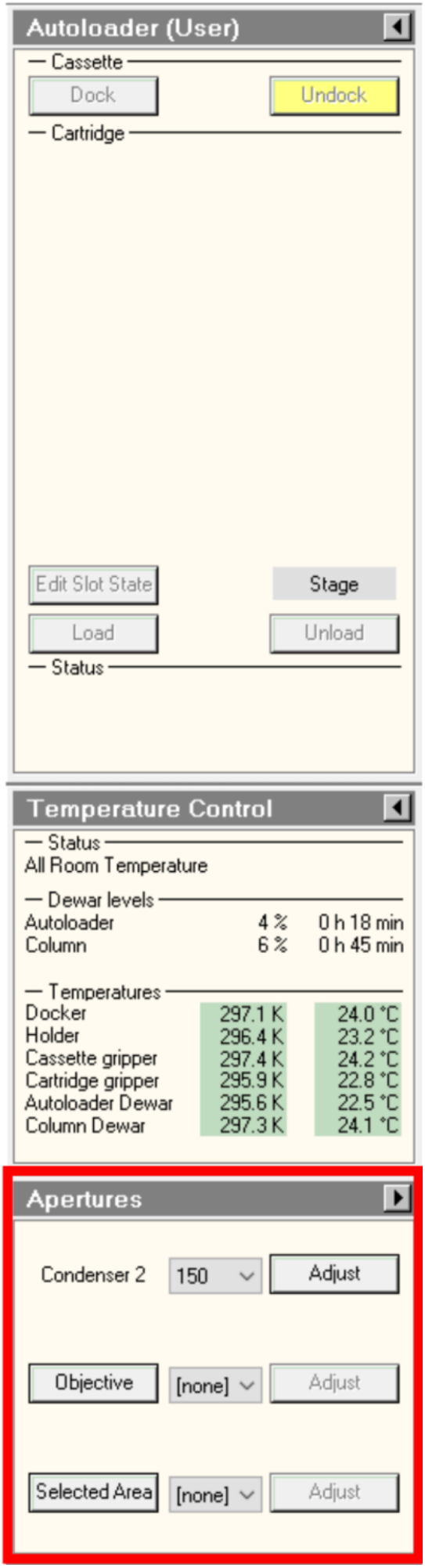
1. Start Serial-EM software
   - On the microscope computer, turn on the SerialEM server (if not already running) .
   - On the camera computer, turn on the serialEM install that match your method (SPA or uED) SerialEM -... .

2. Run the master script
    - In serial-EM: script tab > run > Master (starts automatically with serial-EM).
        - Select your directory in /data/users/your_directory/ and create your working directory
        - **Position of the first grid** = position in the cassette where you loaded your first grid (This should be at least position 2, since position 1 is occupied by the Cross Grating Grid)
        - **Do you want to screen ?** Yes
        - **Number of Grids** = the number of grids you have loaded in the cassette
        - Provide all grid names one by one
    - Menu: *Navigator > Open*
    - If the Imaging states window does not open with the Navigator window go to *Navigator > Open imaging states*

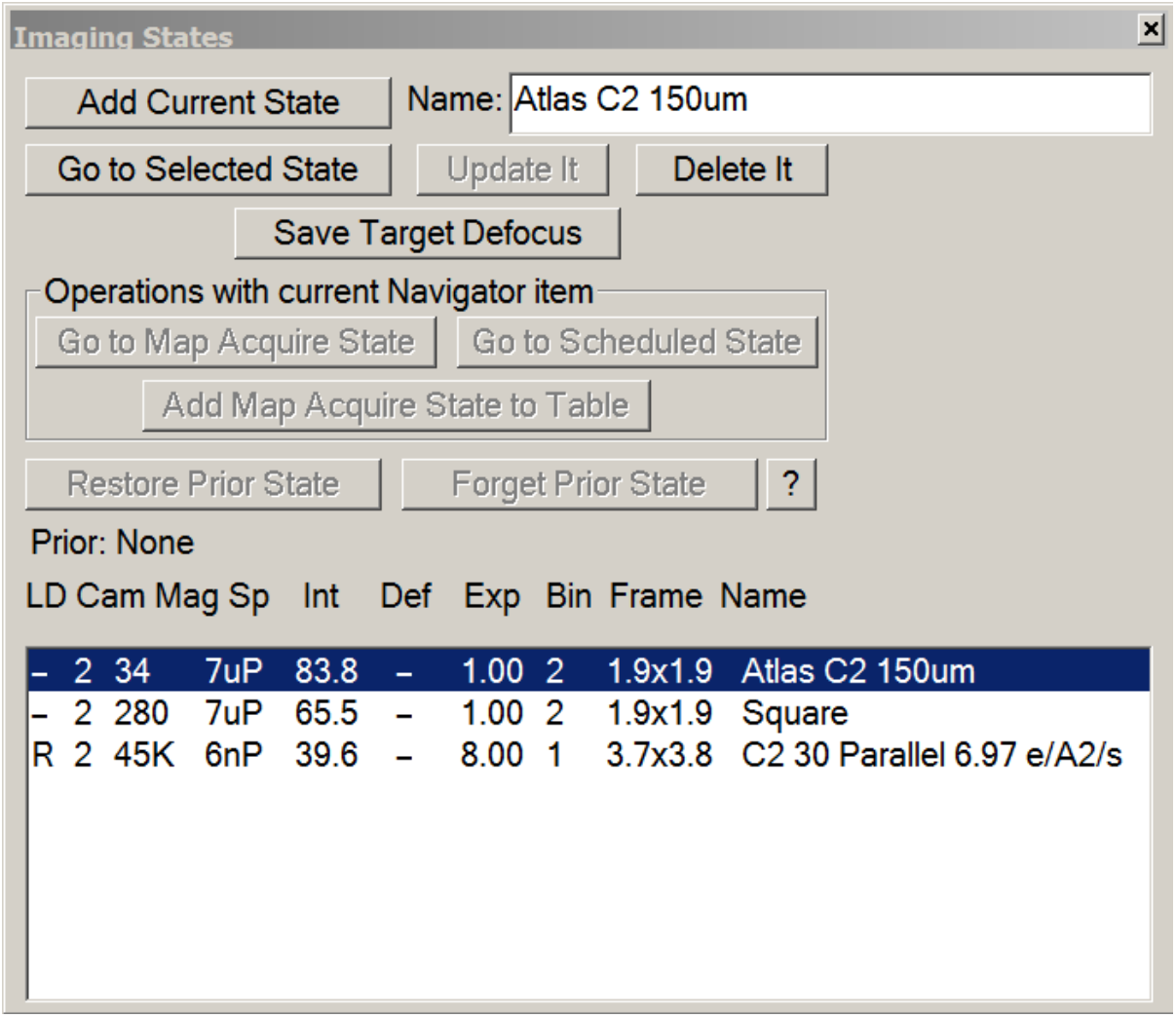
3. Load platform or user settings
    - Menu: *Settings > Open > SerialEM_Settings_SPA*

3. Record Atlases

1. Setup the microscope for recording the Atlases
    - On TEM user interface (**TUI**).
    - Autoloader or Tune tab > Apertures panel : select condenser 2 150µm. Make sure Objective and selected area apertures are out (None status).

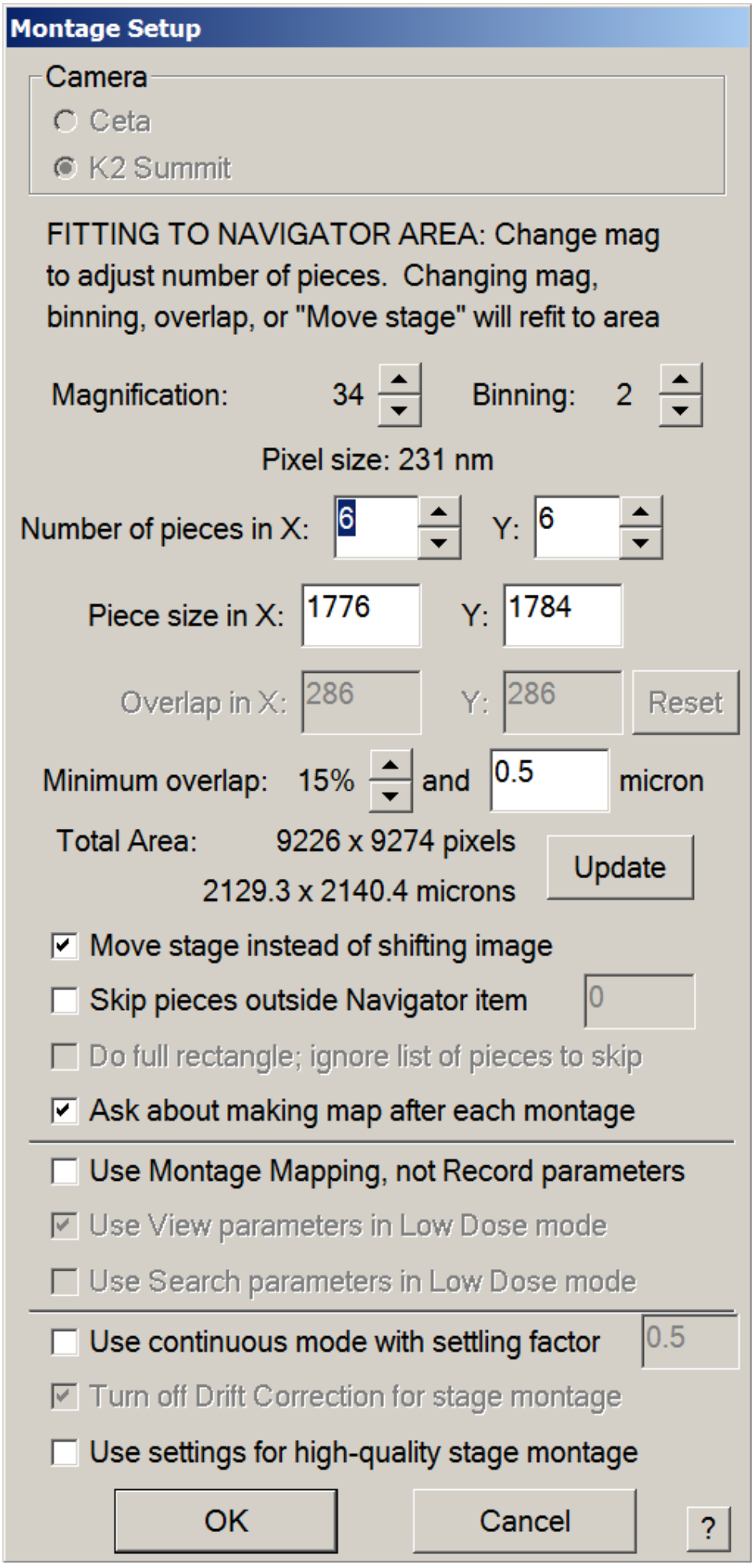

Autoloader (User)
Cassette
Dock
Undock
Cartridge
Edit Slot State
Stage
Load
Unload
Status
Temperature Control
Status
All Room Temperature
Dewar levels
Autoloader 4 % 0 h 18 min
Column 6 % 0 h 45 min
Temperatures
Docker 297.1 K 24.0 °C
Holder 296.4 K 23.2 °C
Cassette gripper 297.4 K 24.2 °C
Cartridge gripper 295.9 K 22.8 °C
Autoloader Dewar 295.6 K 22.5 °C
Column Dewar 297.3 K 24.1 °C
Apertures
Condenser 2
150
Adjust
Objective
[none]
Adjust
Selected Area
[none]
Adjust

- Camera tab > CCTV/Camera panel > Shutter: make sure “Standalone Camera” is yellow. Otherwise, press on it.
- On Serial-EM.
- Imaging States window > **double click** on *Atlas C2 150 µm*.

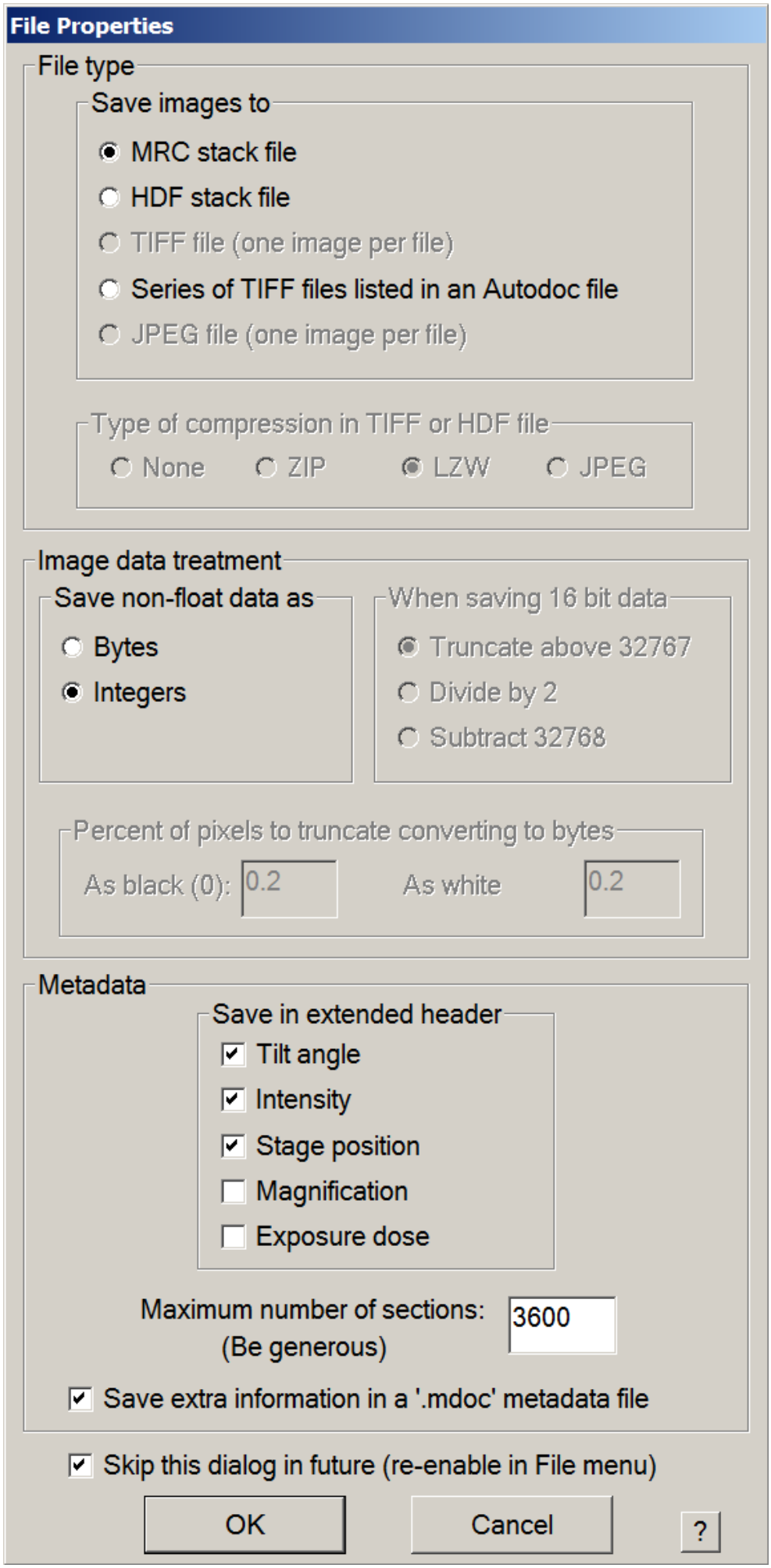


- On the microscope computer, make sure beam settings were updated according with Atlas imaging state.

2. Setup montage parameters
   - in *Navigator* **tab** > *Montaging & Grids* > *Setup Full Montage*
     - Make sure magnification is set to 34X.
     - Make sure binning is set to 2.
     - On Glacios + K2,number of pieces should be 6*6 with an overlap of 15%-20%.
     - Make sure other settings are in agreement with the *Montage setup* window bellow.

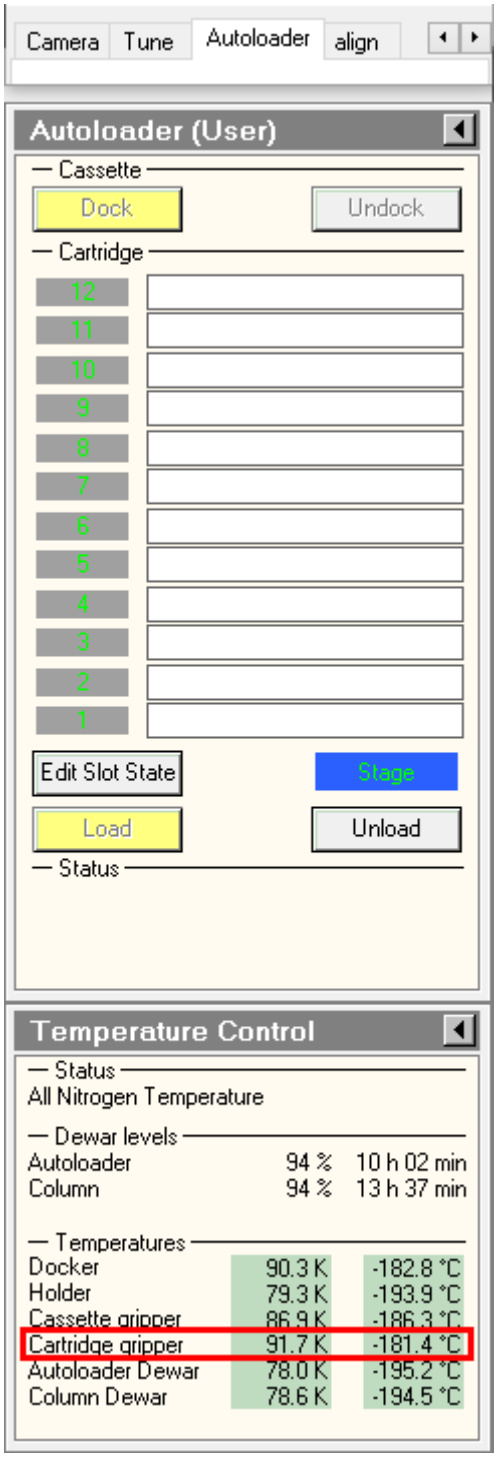

Montage Setup
Camera
Ceta
K2 Summit
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area
Magnification: 34
Binning: 2
Pixel size: 231 nm
Number of pieces in X: 6
Y: 6
Piece size in X: 1776
Y: 1784
Overlap in X: 286
Y: 286
Reset
Minimum overlap: 15% and 0.5 micron
Total Area: 9226 x 9274 pixels
2129.3 x 2140.4 microns
Update
Move stage instead of shifting image
Skip pieces outside Navigator item
0
Do full rectangle; ignore list of pieces to skip
Ask about making map after each montage
Use Montage Mapping, not Record parameters
Use View parameters in Low Dose mode
Use Search parameters in Low Dose mode
Use continuous mode with settling factor
0.5
Turn off Drift Correction for stage montage
Use settings for high-quality stage montage
OK
Cancel
?

- Press OK > The *file Properties* window will open.
- If you are screening more than 10 grids, change 360 to 3600 in *Maximum number os sections*.
- Make sure settings are in agreement with the image of the window bellow.

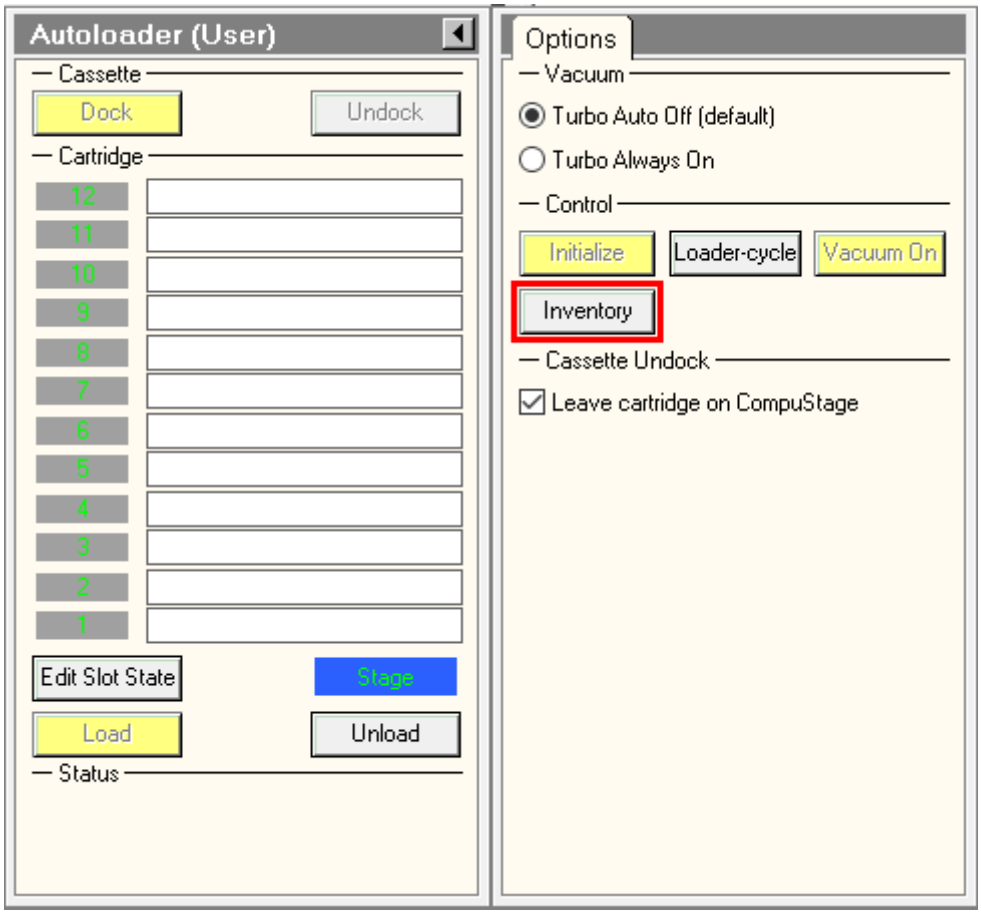

File Properties
File type
Save images to
MRC stack file
HDF stack file
TIFF file (one image per file)
Series of TIFF files listed in an Autodoc file
JPEG file (one image per file)
Type of compression in TIFF or HDF file
None
ZIP
LZW
JPEG
Image data treatment
Save non-float data as
Bytes
Integers
When saving 16 bit data
Truncate above 32767
Divide by 2
Subtract 32768
Percent of pixels to truncate converting to bytes
As black (0):
0.2
As white
0.2
Metadata
Save in extended header
Tilt angle
Intensity
Stage position
Magnification
Exposure dose
Maximum number of sections:
(Be generous)
3600
Save extra information in a '.mdoc' metadata file
Skip this dialog in future (re-enable in File menu)
OK
Cancel
?

- Press OK, then save the mrc file in the work directory with a meaningful name i.e. “Atlases.mrc”.

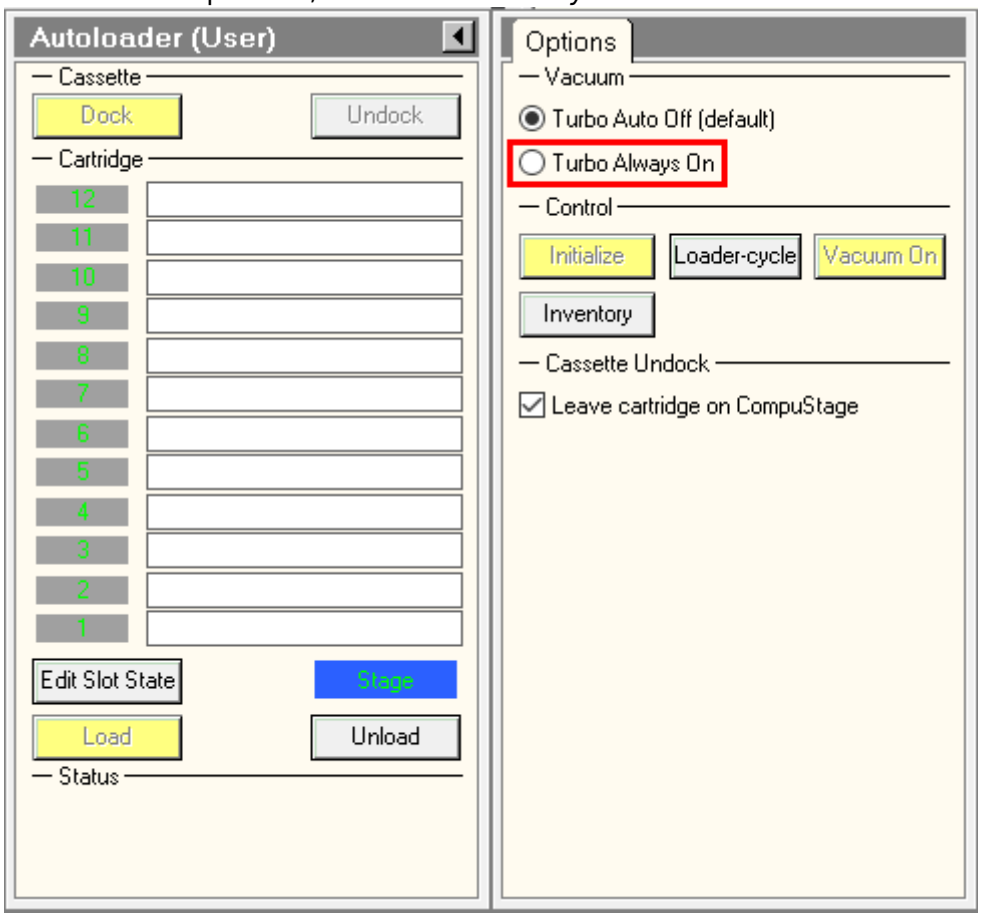
3. Manually collect atlases or run the gridmaps script
    1. Optional: Manually run cassette inventory
        - *After grid introduction, the autoloader initialization takes a few minutes.*
        - in the TUI, Make sure Autoloader elements temperatures are **colder** than **-170°C**. This is particularly important for the **cartrige gripper**, the autoloader element which grap the specimens.



- in the TUI, press on *Inventory* button and wait until autoloader finishes the inventory. If you did not load a full cassette, you can press on *stop inventory* once the last loaded position was mapped.

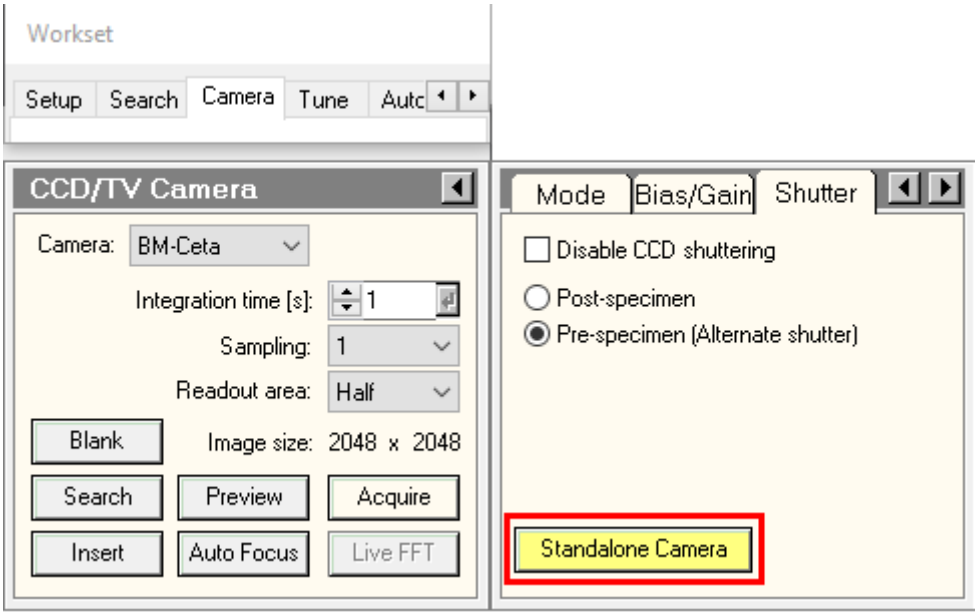


2. Automatically collect atlases with the gridmaps script
   - To fasten Atlases acquisition, turn on *turbo always on* in TUI.



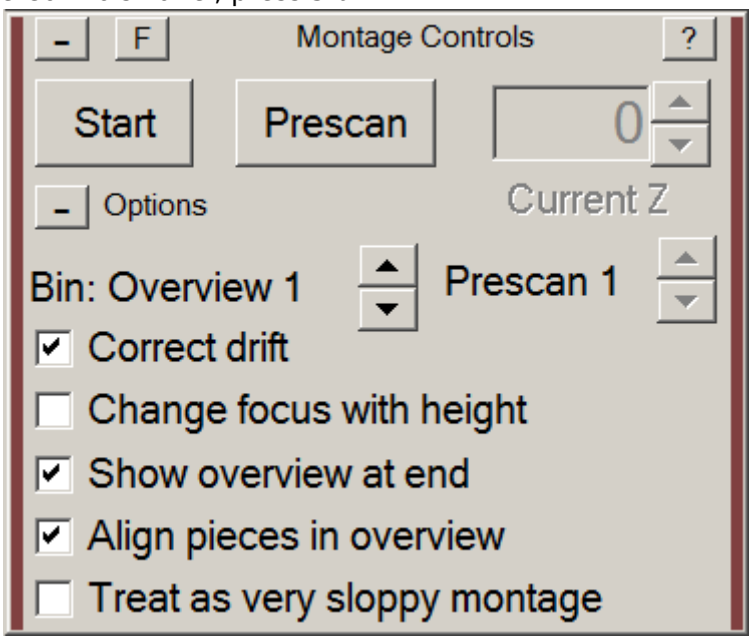
   - In Serial-EM *Script* **tab** > *run* > gridmaps.

- The interface will ask three questions:
  1. *Did you insert 150 µm C2 aperture ?* : **if not, refers to section 3.1**, then press Yes.
  2. *Please switch on the 'Standalone camera' in the camera/shutter menu in - only press 'OK' if it's done*: In TUI, **buttom must be yellow**. Otherwise, press on it.

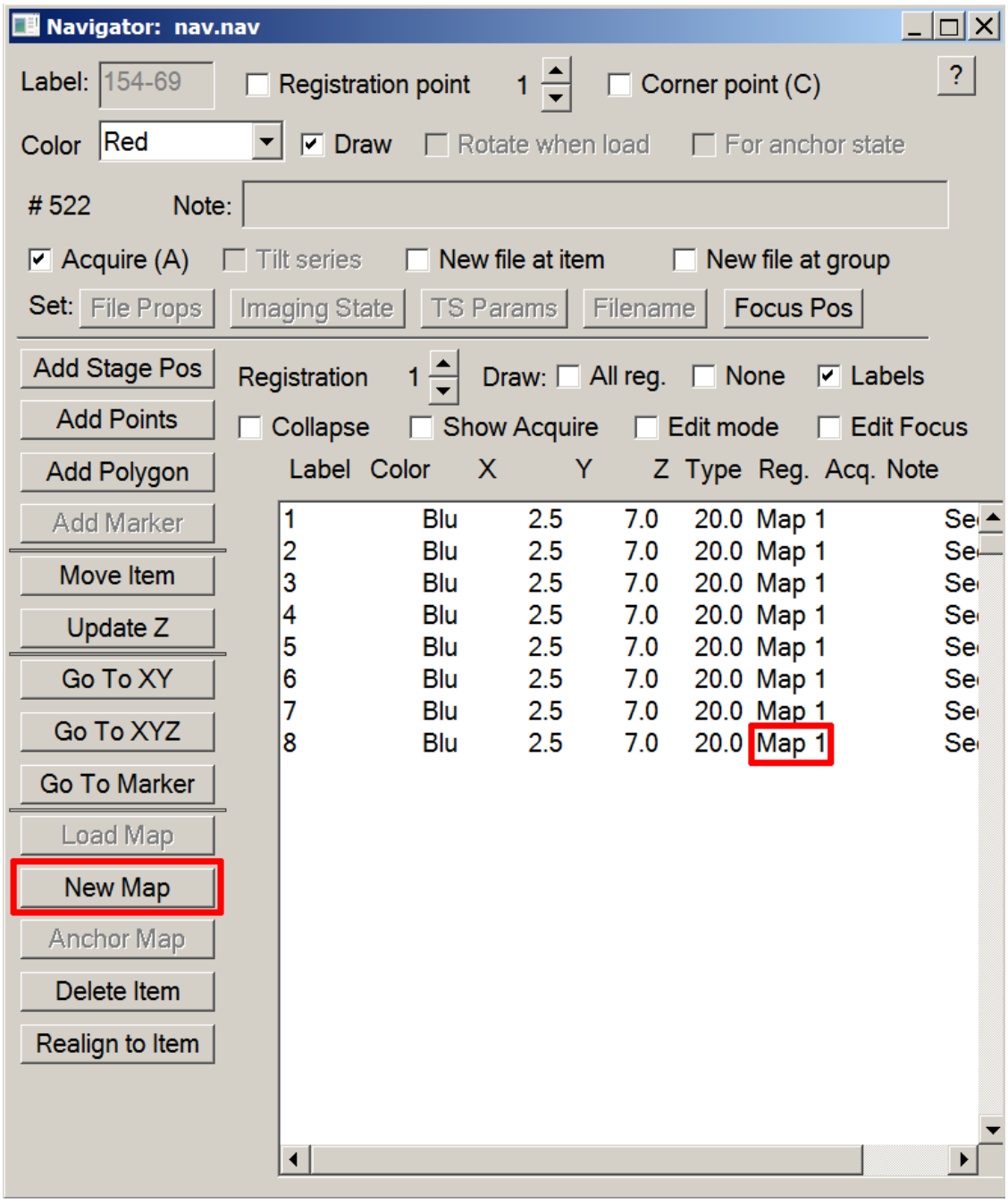


  3. *Do you want to launch the inventory with a 16min delay ?* : Look at the autoloader temperatures as indicated in section 3.1.a. If Cartridge gripper is **colder than -170°C**, press *No*. Otherwise, wait for temperature to be cold enough **OR** press *Yes*.
- Wait to see the few first Atlas tiles.
- Doing the Atlas for one grid takes approximately 10 minutes.

3. Manual Atlas collection
   - This is to run a grid map on a single grid.
   - In the Montage Controls Panel, press *Start*.

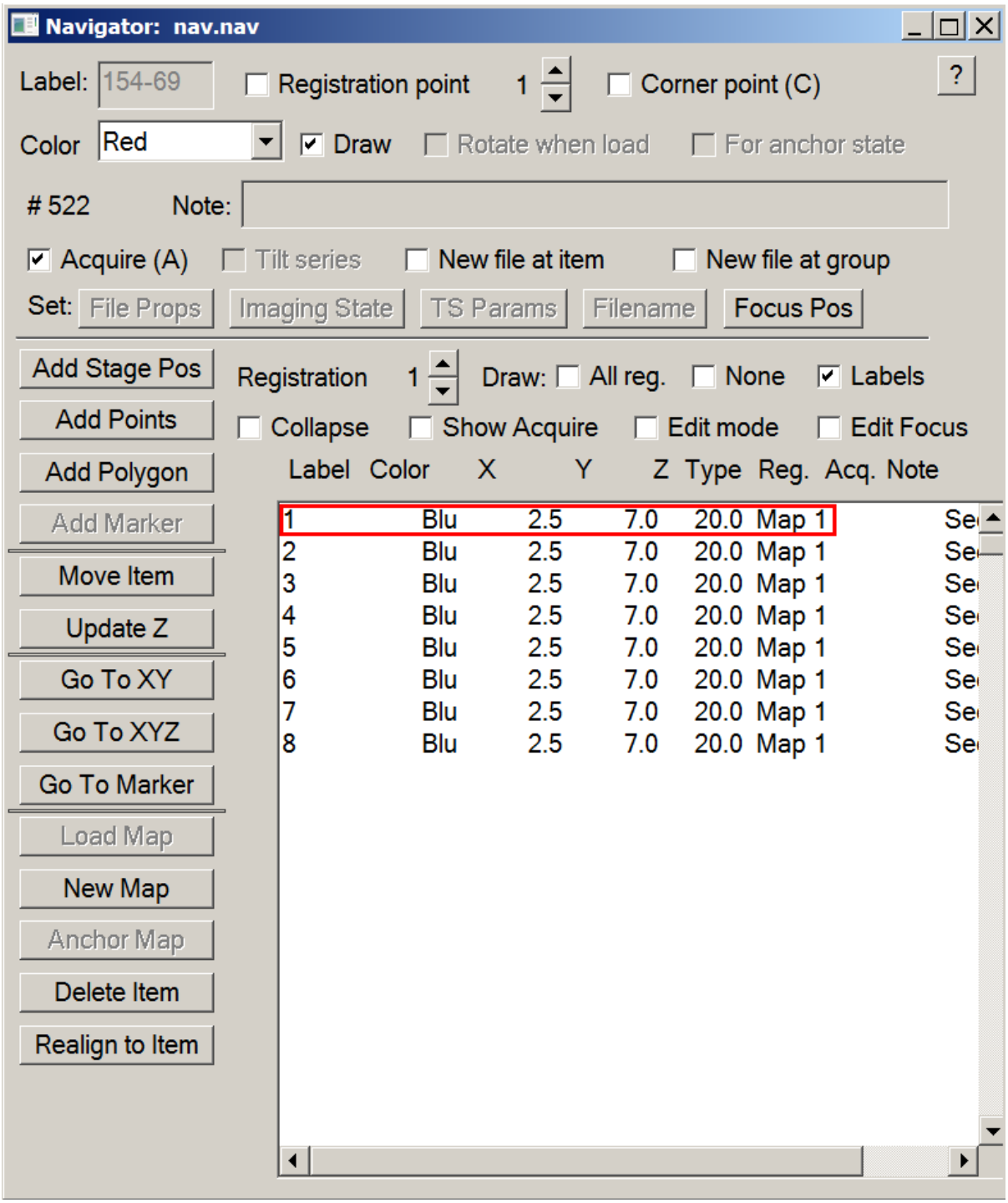


   - Once the atlas run is done, make sure it had been saved as a map in the navigator windows. Otherwise, press *New Map* in the navigator windows.



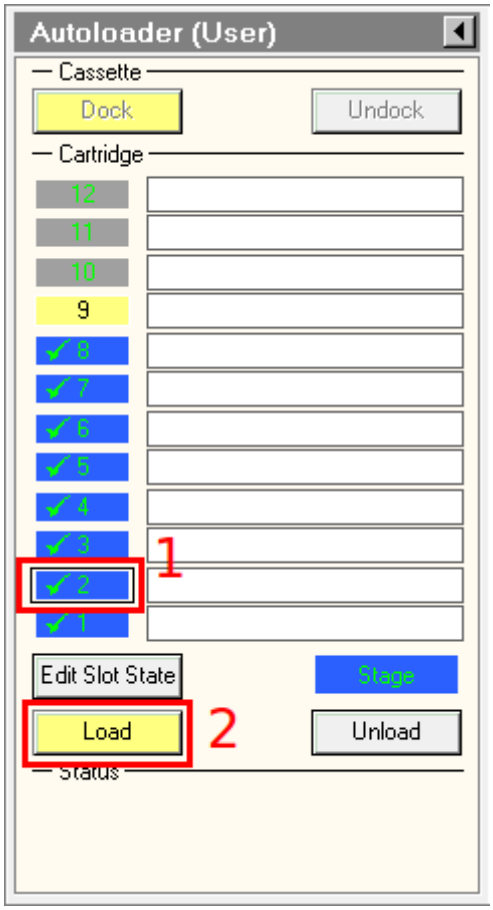
4. Select and Load a Grid

1. Look at your Atlases and select the first Grid to screen
    - **Double click** on each grid map in the navigator **window**

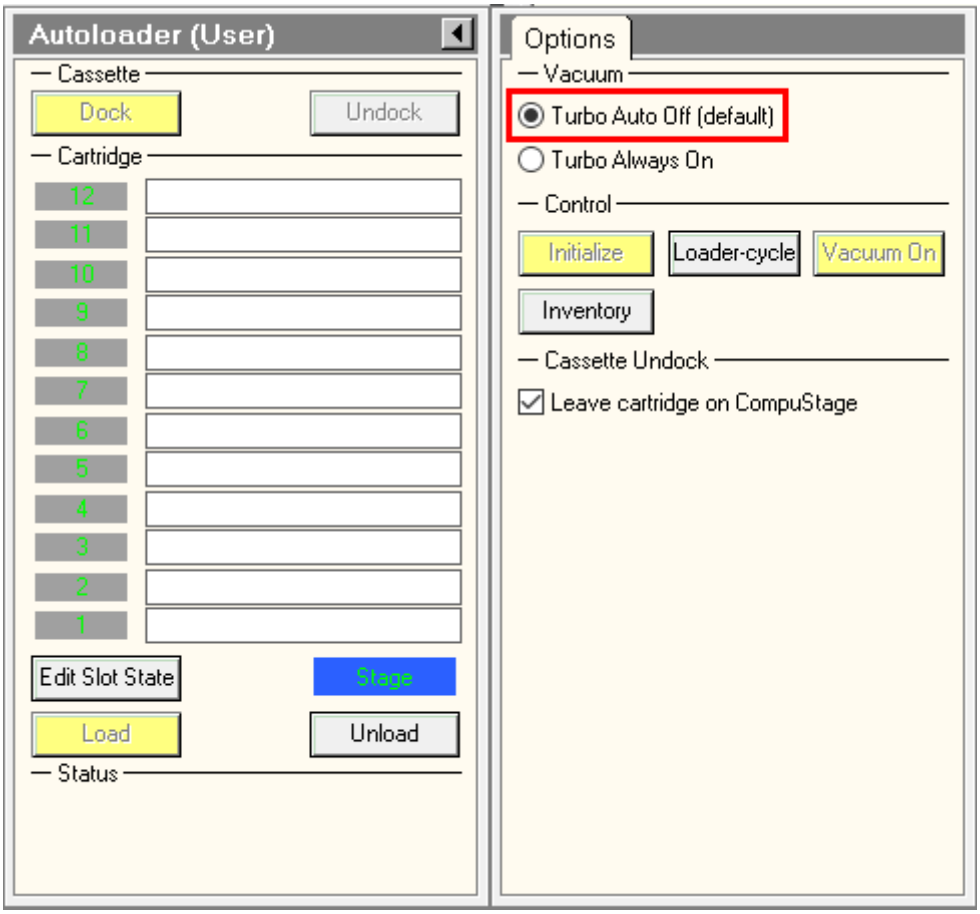


- Decide the grid you wish to screen first

2. Introduce the Grid in the column
   - In TUI, select the cassette position of the grid to be loaded, then press on Load.

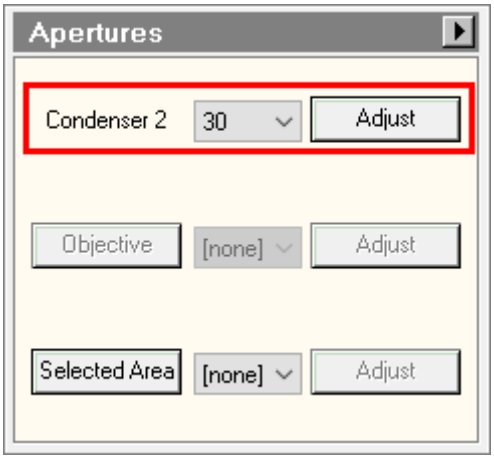


3. In TUI, select "Turbo Auto Off (default)"

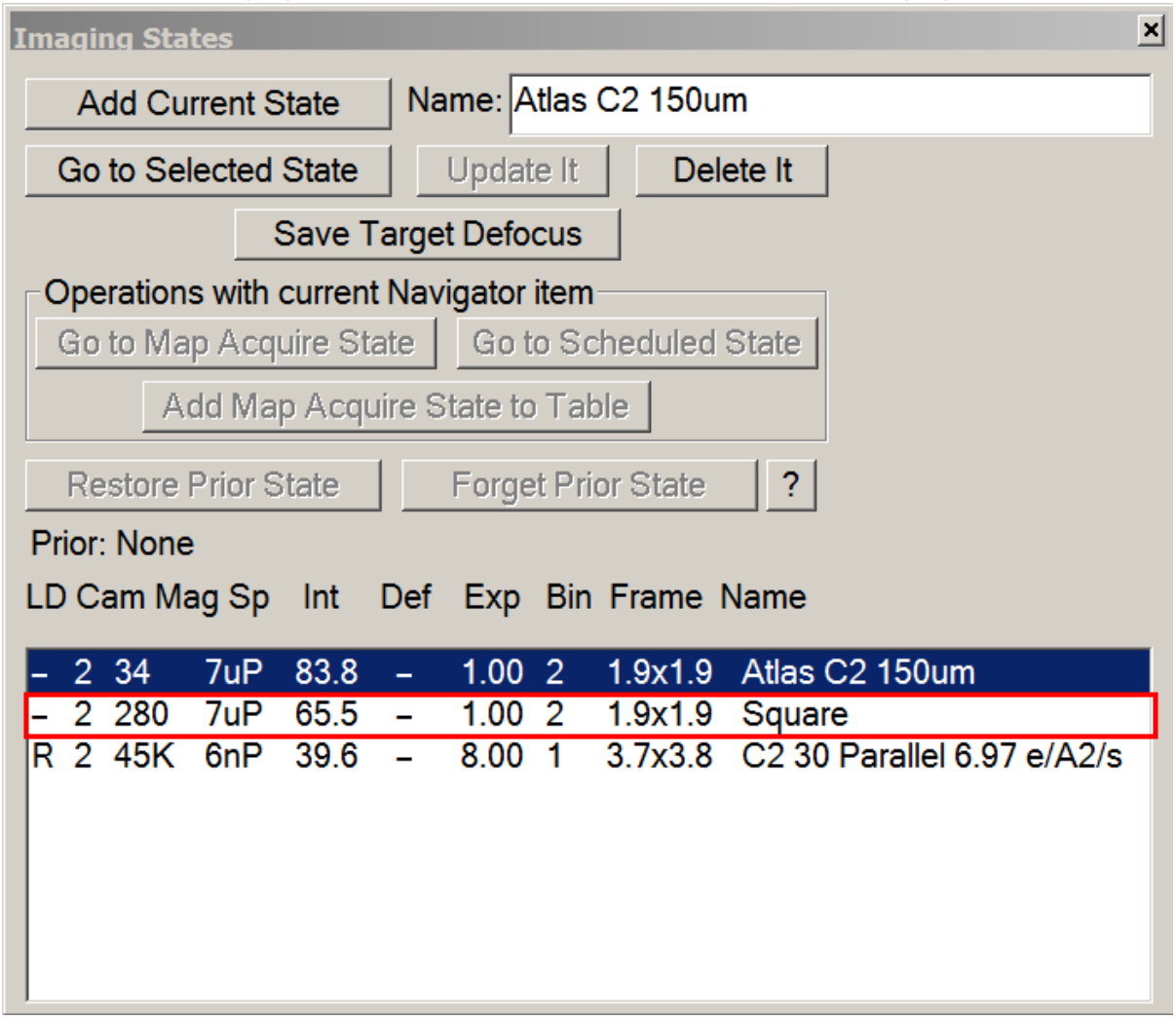


5. Record square maps

1. Setup the microscope for Squares imaging
    - On TEM user interface (**TUI**) : Autoloader or Tune tab > Apertures panel : select condenser 2 30µm or 50µm.



    - On Serial-EM : Imaging States window > **double click** on *Square* imaging state.



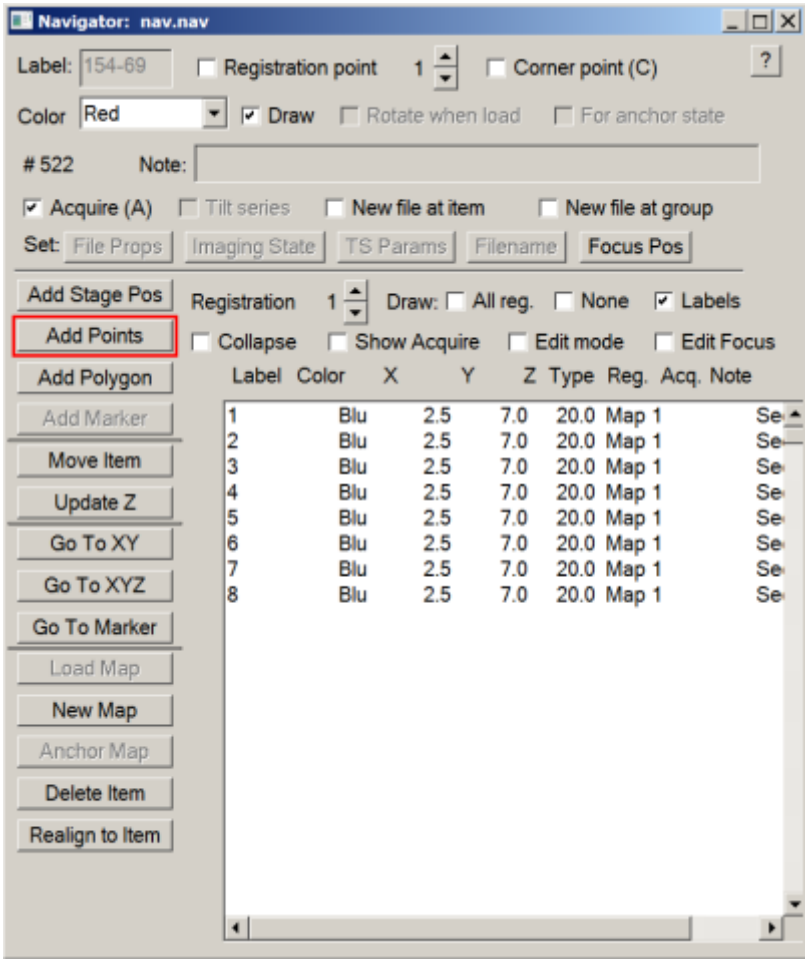
    - On the microscope computer, make sure beam settings were updated according to Atlas imaging state.

2. (Optional) Determine the overall eucentric height.
    - In Serial-EM, center a square in the middle of the grid.
        1. add a marker (green cross) by **left-clicking** on a square.

2. in the navigator **windows** press the *go to marker* button. Wait for the stage to reach the specified position.
3. take a *record* image.
4. if the square is off-center: keep pressed the right mouse button, grab the center of the square to the center of the screen, and release the button. Wait for the stage to reach its position.

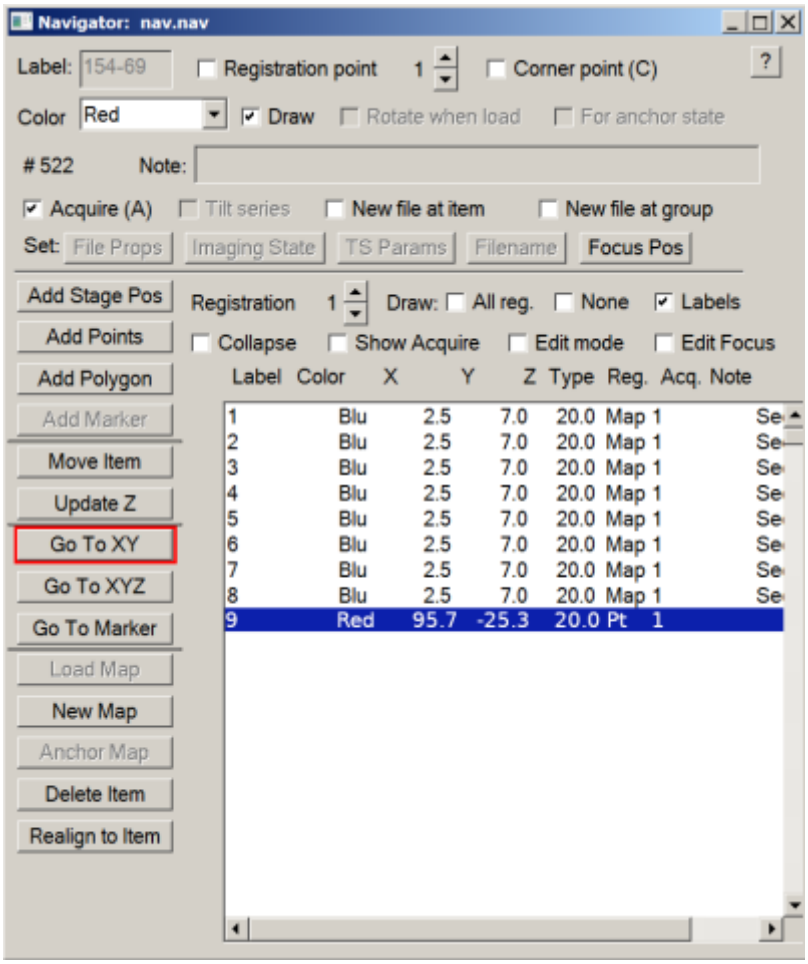
- In *Tasks* **tab** > *Eucentricity* > press on *Rough Eucentricity*.
- Once eucentric height was determined, in navigator **windows**, select the line of the corresponding atlas and press on *Update Z* button.

3. Correct for the coordinate discrepancy between Atlas and Square magnifications
   - In the Navigator **windows**, double click on the Atlas line to load it.
   - On the Atlas, look for the feature which is easily recognizable. It is recommended to choose a feature in the vicinity of the area of interest.
   - Add a Point on the feature
     1. In the navigator **windows**, click on *Add Points*. The button Will change to *Stop Adding*.

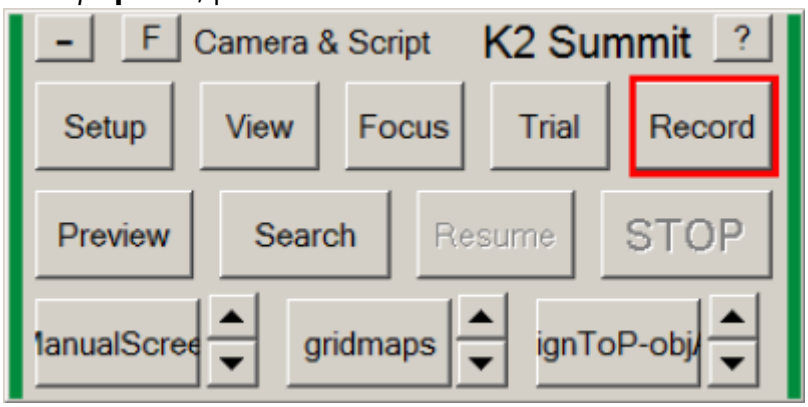


     2. Click on the chosen feature.
     3. In the navigator **windows**, click on *Stop Adding*.

   - Make sure the Point is selected in the Navigator **windows**.
   - In the Navigator windows, press on Go To XY, then wait for the stage to reach the feature position.



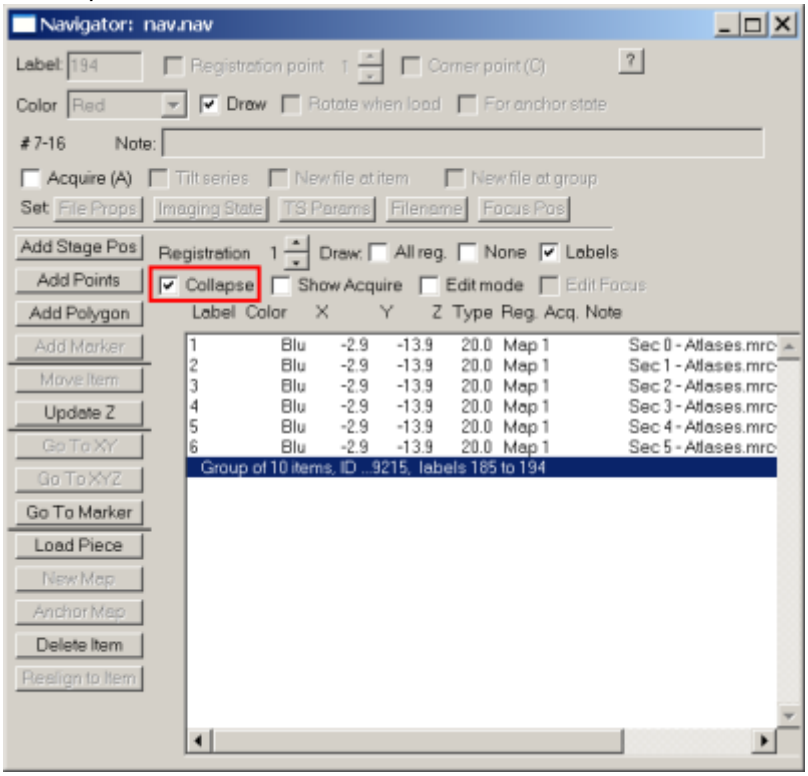
- In the *Camera & Script* **panel**, press on *Record*.



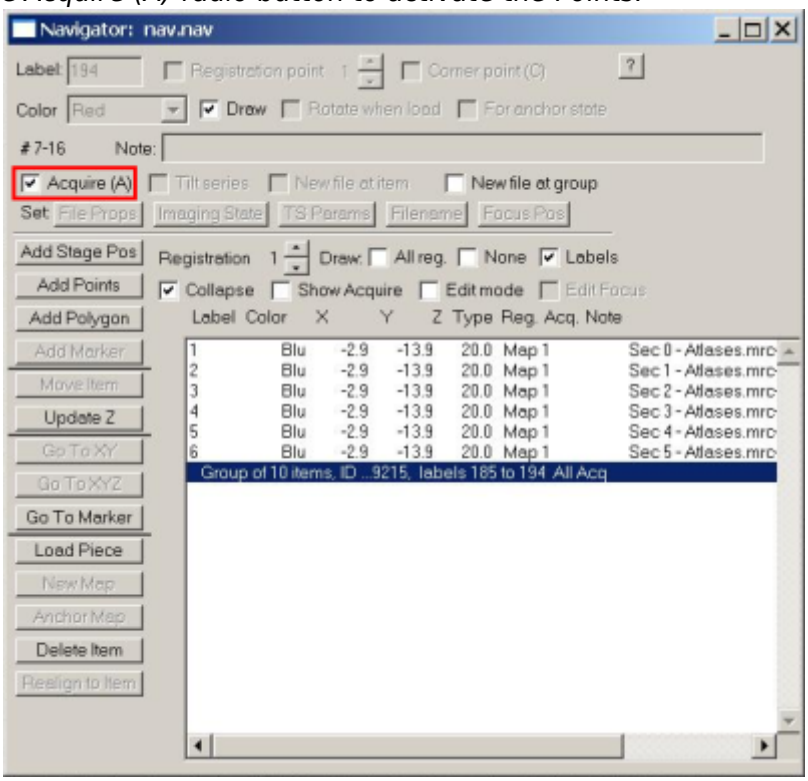
- Find the feature in the newly recorded image. If necessary, recenter the feature with the same method presented in section 5.2.d.
- Add a **Marker** on the chosen feature, i.e. simply do a left click which will draw a green cross.
- Make sure the **Point** is still selected in the Navigator **windows**.
- In Navigator **tab**, click on *Shift To Marker...*. A new windows will open, indicating the X and Y translation (in um) to apply to align the feature in at square magnification to the one at Atlas magnification. If this value make sens, i.e X and Y shifts are in the 10 → 50 um range, press OK.
- If you did a mistake, like applying the shift with an atlas/square map selected instead of the point, the sift can be deleted in Navigator **tab** > *Undo last Shift*.

4. Select squares to be mapped.

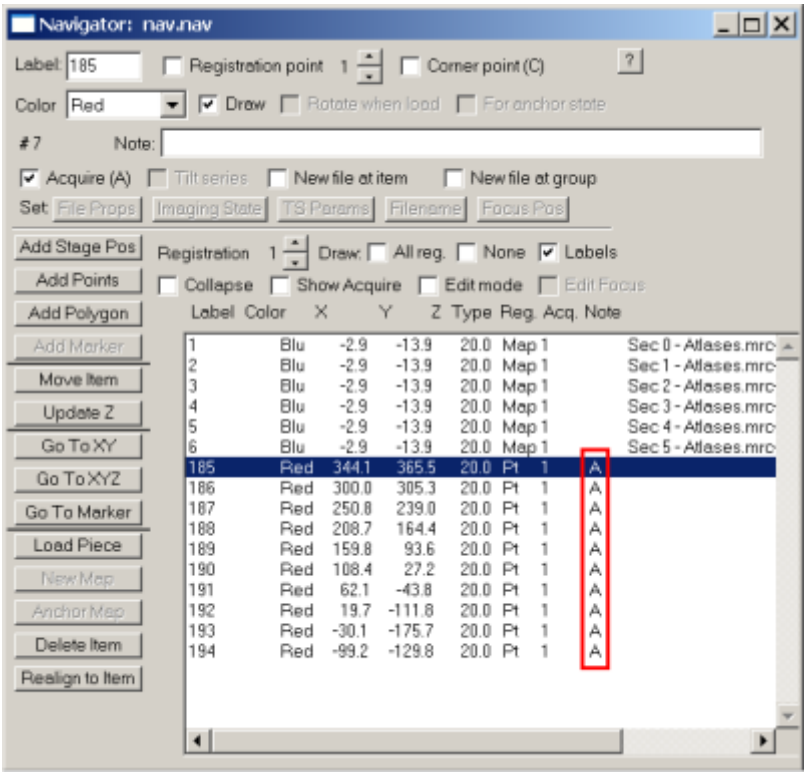
- Load the Atlas.
- Add Points in the middle of all the squares that you wish to map.
- Change Points status to Acquire.
  - In Navigator **windows**:
    1. Check the *Collapse* radio button.



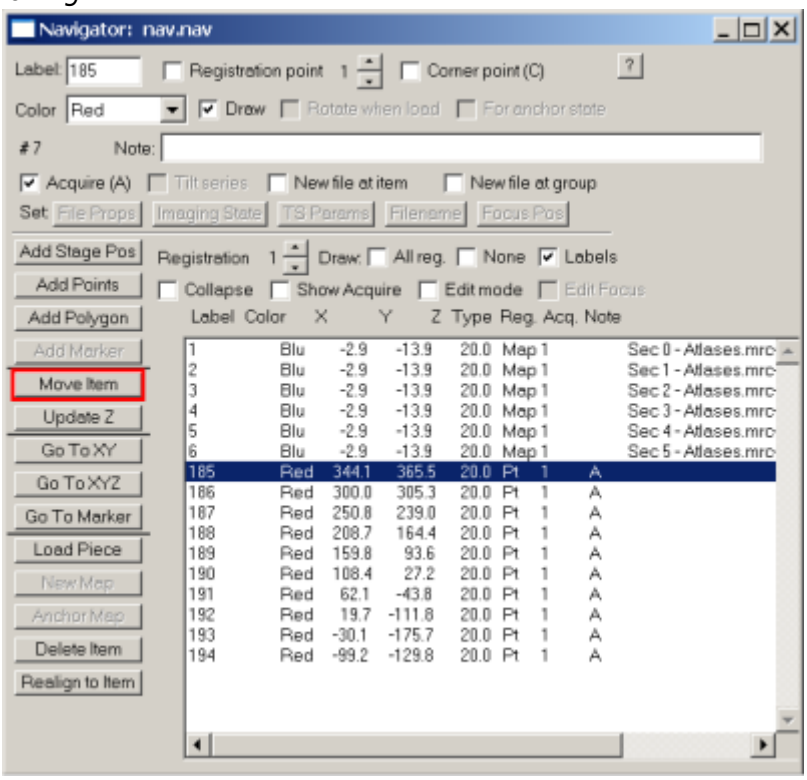
    2. Select the group of items.
    3. Check the *Acquire (A)* radio button to activate the Points.



    4. Uncheck the *Collapse* radio button. All Points should display a *A*.



- (Optional) Go through all the points to correct for centering.
  - In the navigator windows:
    1. Select the first Square Point.
    2. Press *Go to XY* button, then wait for the stage to reach the square.
  - In the Camera & Script Panel, take a *Record*.
  - If the Point if off-centered:
    1. Make sure the Point which is selected is the one you want to move.
    2. click on *Move Item* button in the Navigator **windows**. The button will change to *Stop Moving*.

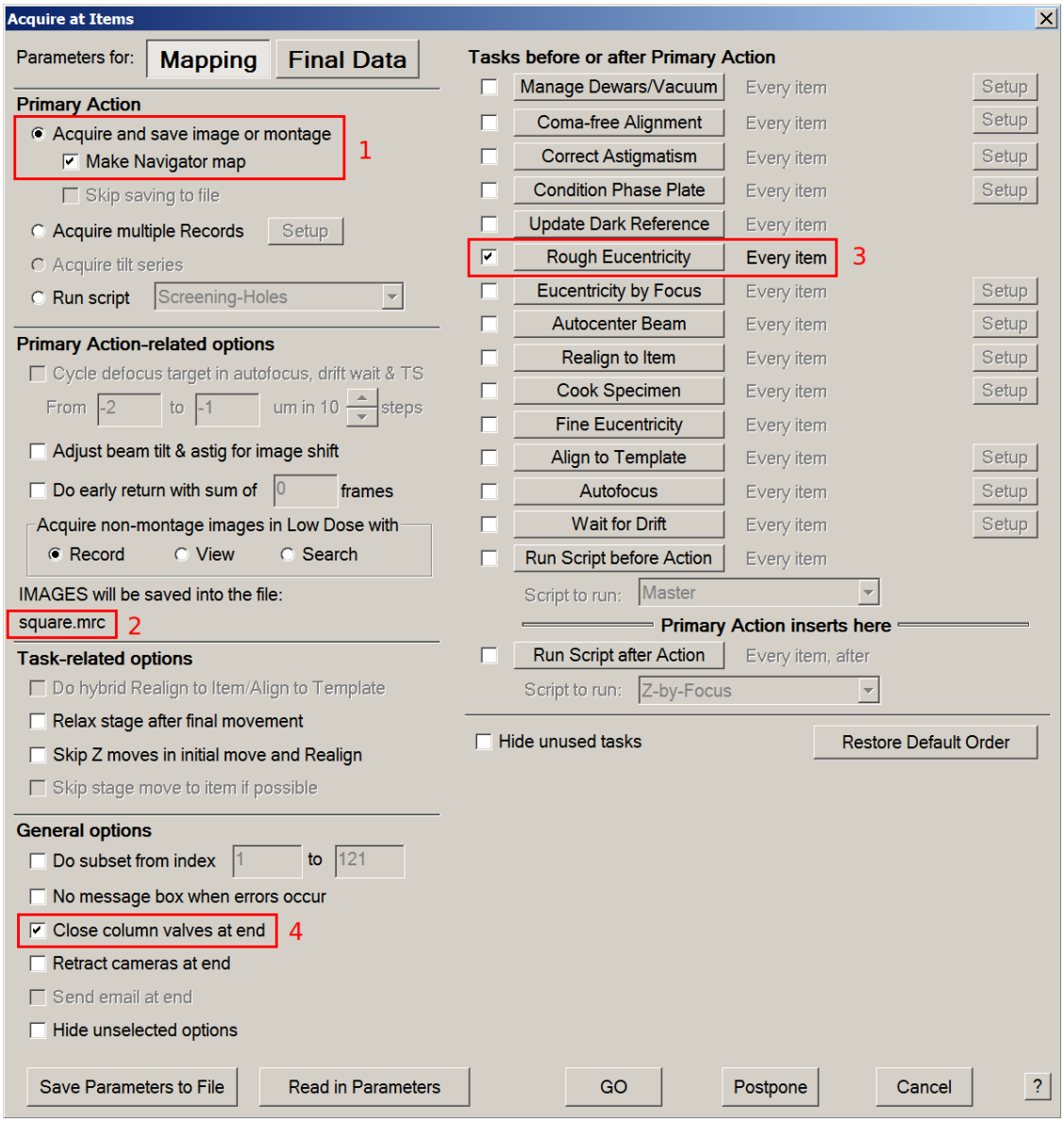


    3. Click in the middle of the Square.

4. Click on the *Stop Moving* button.

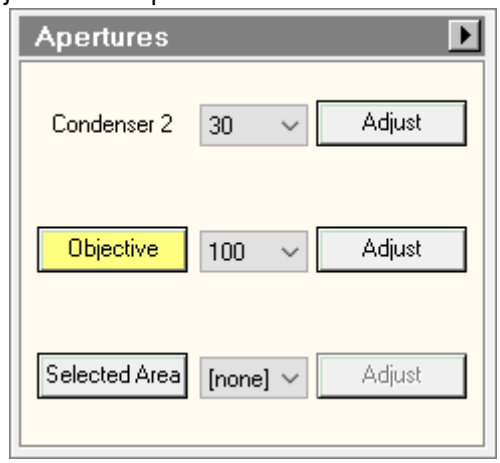
- Repeat these steps for all the Square Points.

5. Automatically collect squares maps and determine their eucentric height.
   - Menu > File > *Open New*. Save a mrc file wherein Square maps will be saved.
   - In menu > Navigator tab > Acquire at Items: start square mapping
     1. Tick *Acquire and save image or montage* & *Make Navigator map* radio buttons.
     2. Make sure that *IMAGES will be saved into the file:* directs toward the previously saved mrc file.
     3. Tick the Rough Eucentricity option and make sure it will be performed at every item.
     4. If you plan to leave the microscope room while it records squares maps, tick the *close column valves at end* option.
     5. Press GO.

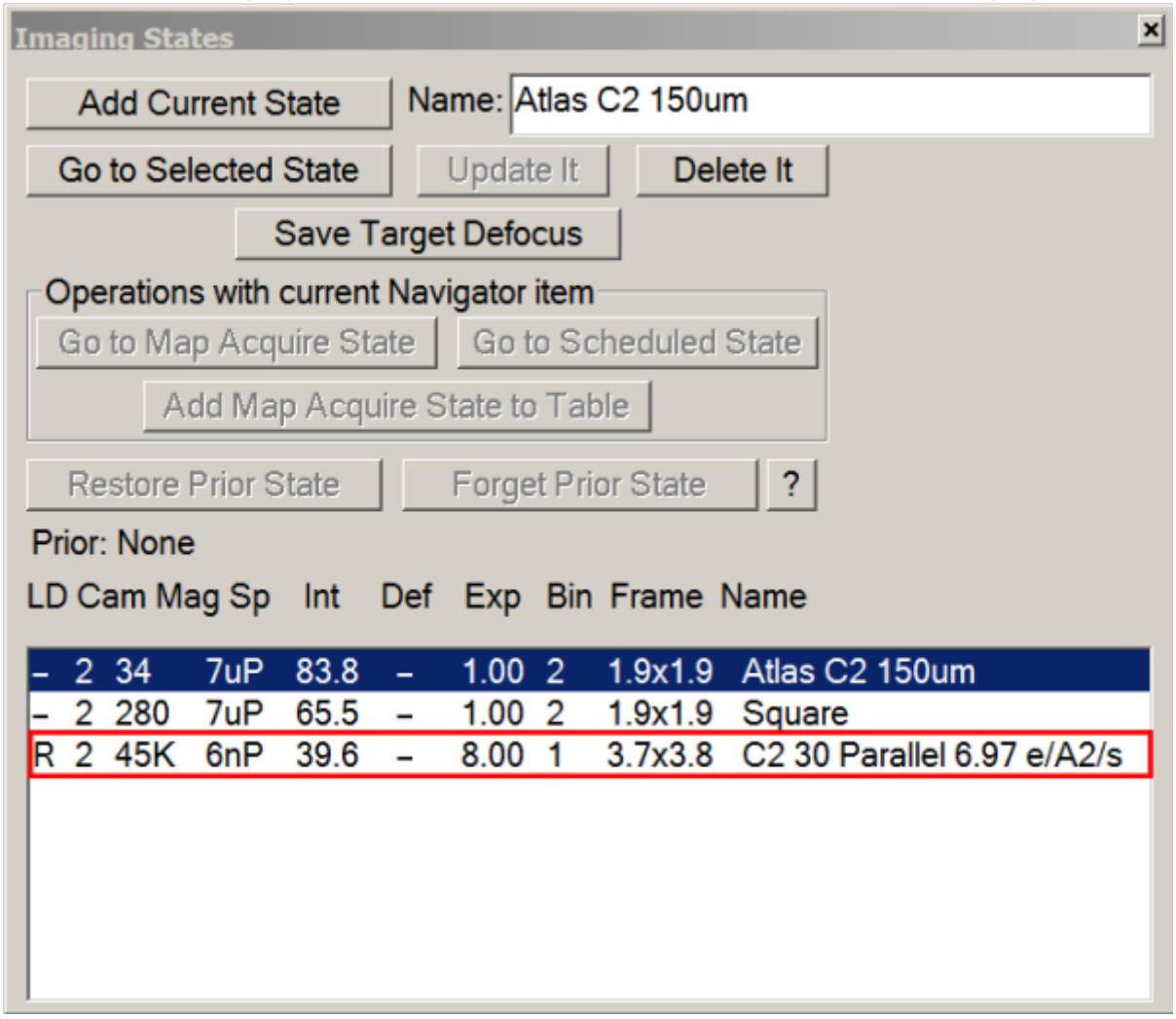


6. Prepare Serial-EM for screening

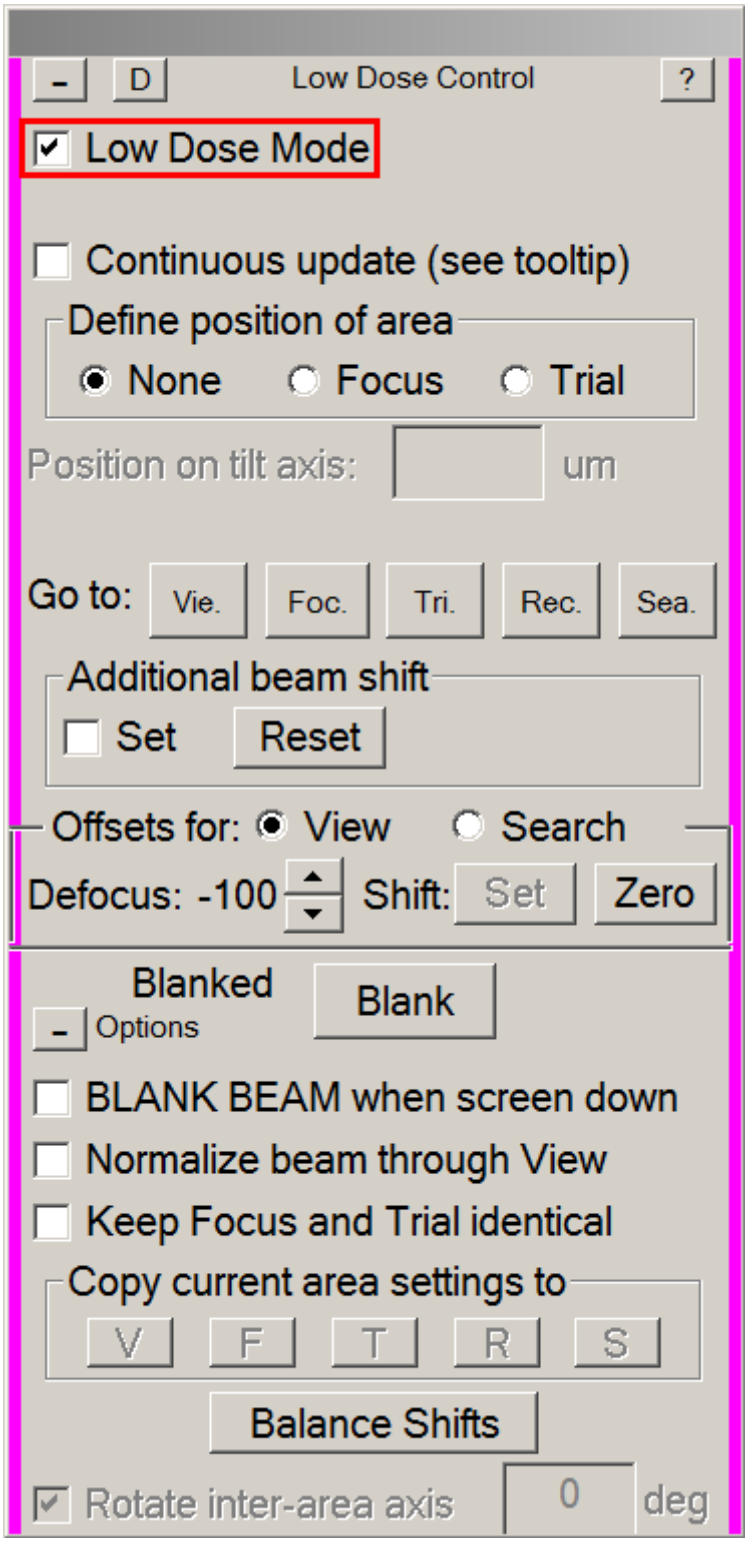
1. Setup the microscope for Low Dose imaging
   - On TEM user interface (**TUI**) : Autoloader or Tune tab > Apertures panel : select condenser 2 30µm and Objective 100µm.



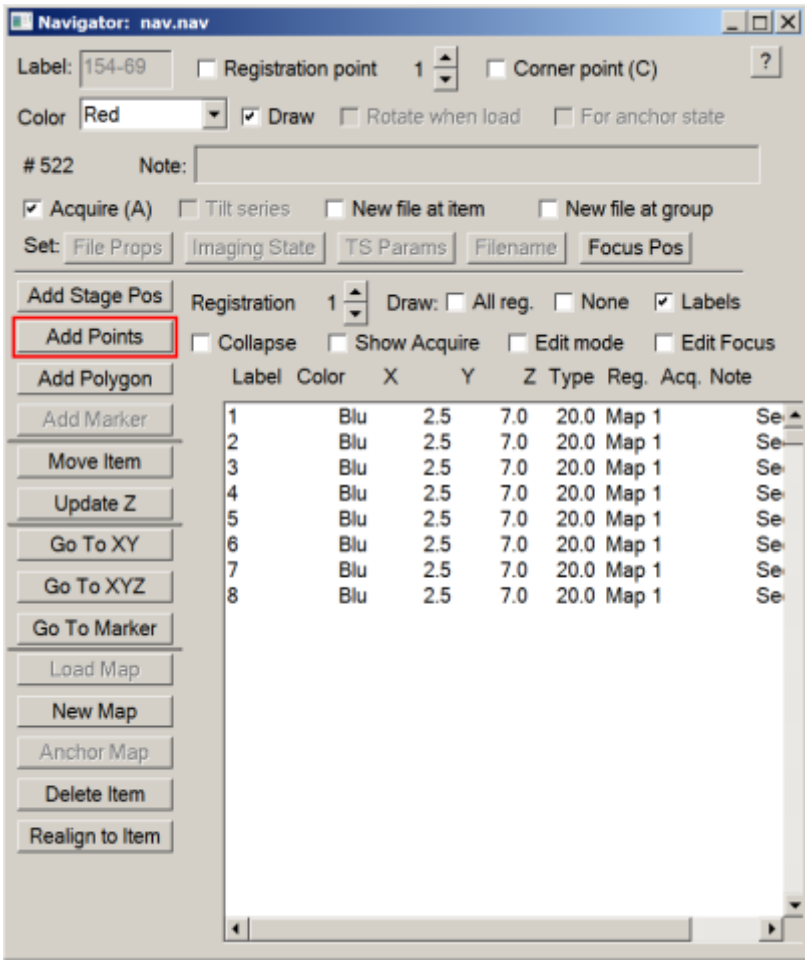
   - On Serial-EM : Imaging States window > **double click** on *C2 30 Parallel* imaging state.



   - Make sure the Low Dose panel is activated.

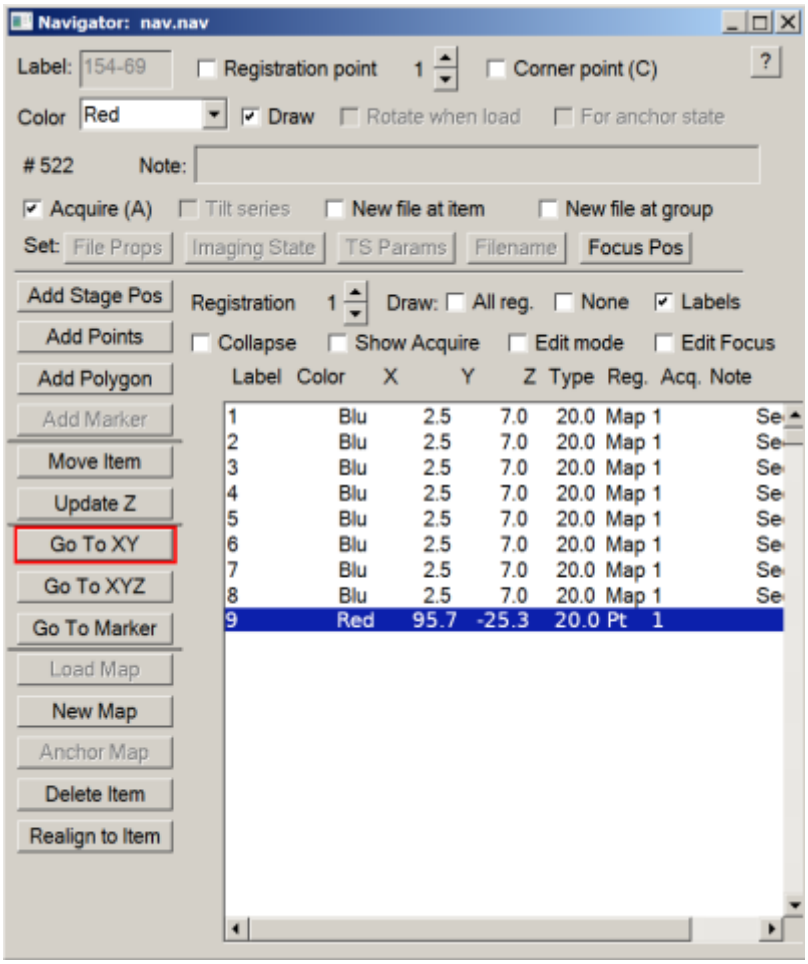


2. Correct for the coordinate discrepancy between Square and Low Dose View magnifications
    - In the Navigator **windows**, double click on a Square line to load it.
    - On the square, look for the feature which is easily recognizable.
    - Add a Point on the feature
        1. In the navigator **windows**, click on *Add Points*. The button Will change to *Stop Adding*.

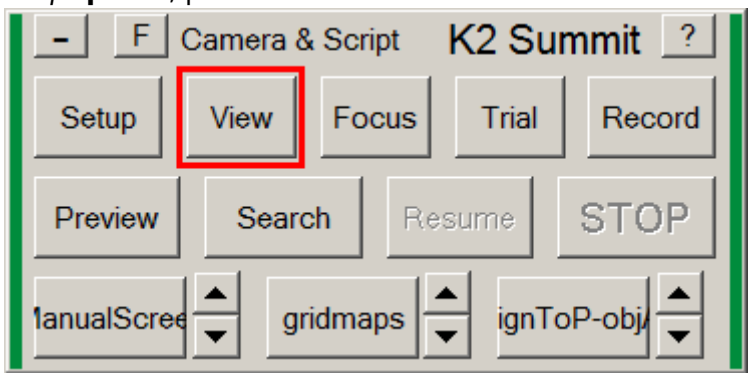


2. Click on the chosen feature.
3. In the navigator **windows**, click on *Stop Adding*.

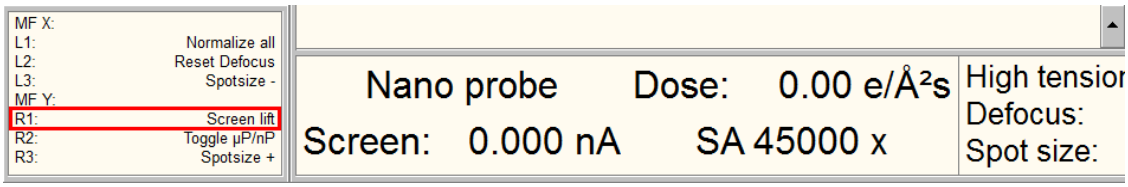
- Make sure the Point is selected in the Navigator **windows**.
- In the Navigator windows, press on Go To XYZ, then wait for the stage to reach the feature position.



- In the *Camera & Script* **panel**, press on *View*.



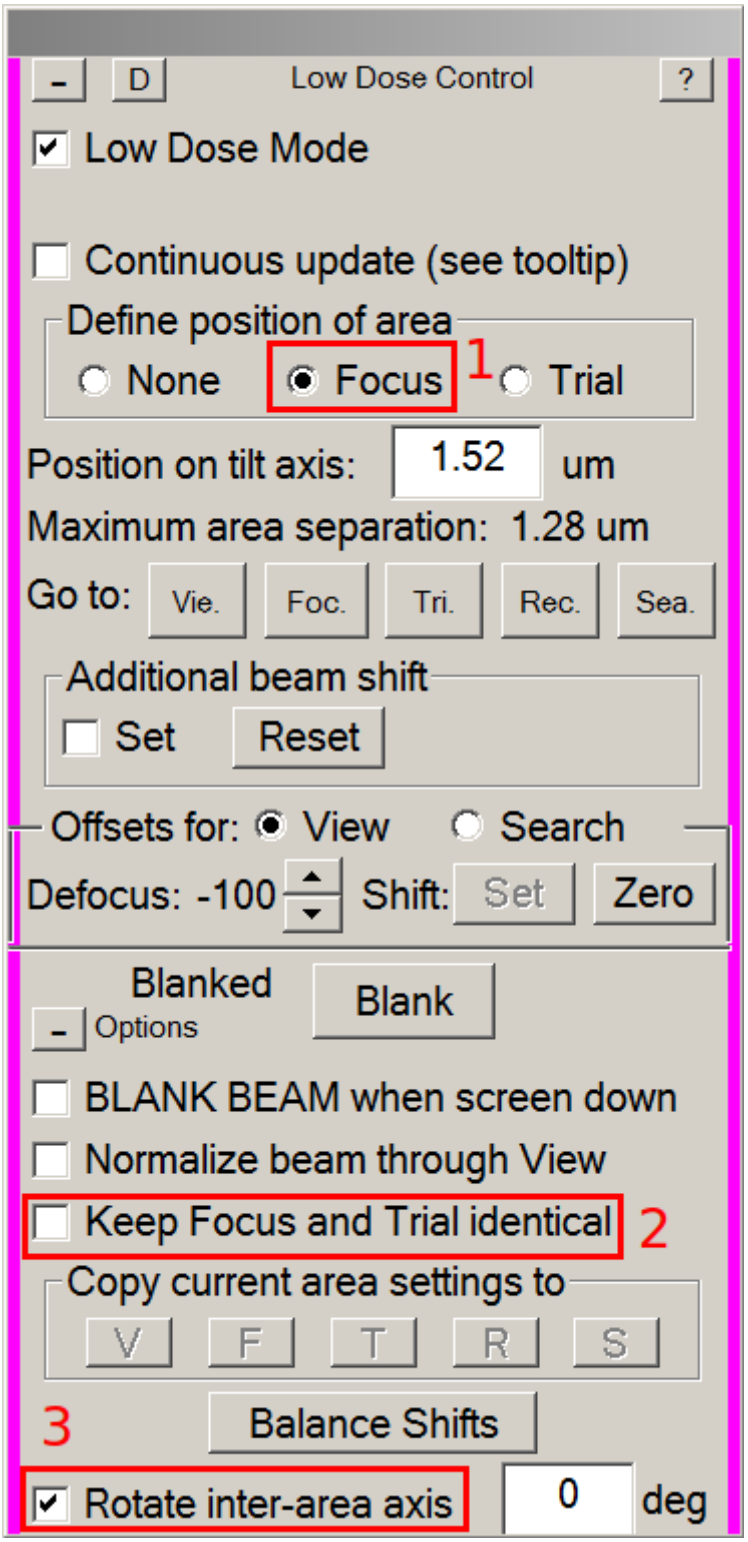
- Find the feature in the newly recorded image. If necessary, recenter the feature with the same method presented in section 5.2.d.
- If you have trouble finding the feature, use the fluo-screen and the joystick:
  1. lower the fluorescent screen with the console button indicated in the lower panel of the TEM User Interface.



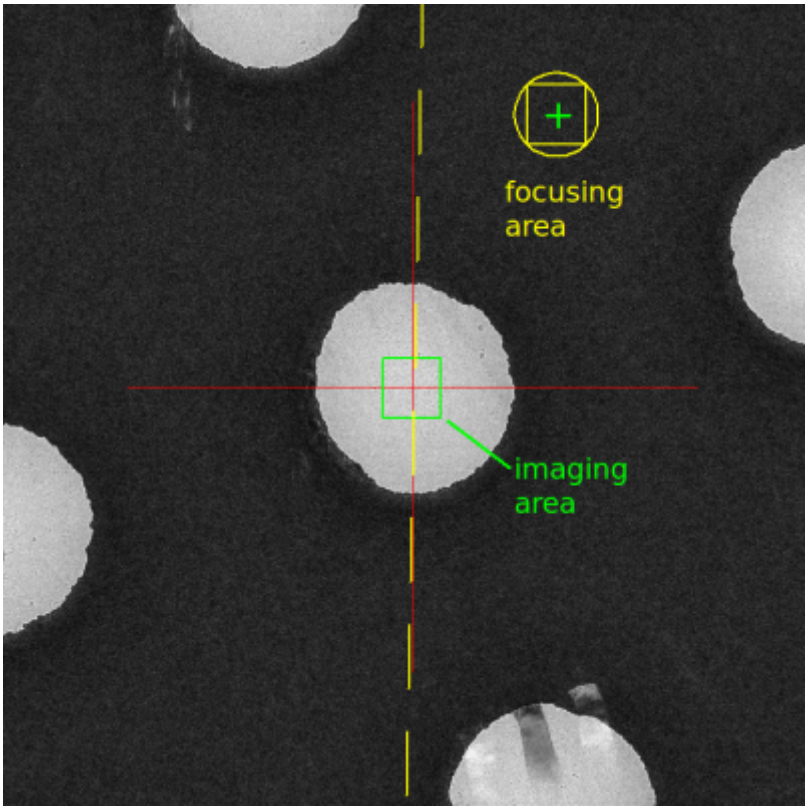
  2. with the joystick, move the stage so that the desired feature is in the green square (K2 area) on the fluorescent screen.
  3. Lift up the fluorescent screen with the same console button.

- Add a **Marker** on the chosen feature, i.e. simply do a left click which will draw a green cross.
- Make sure the **Point** is still selected in the Navigator **windows**.
- In Navigator **tab**, click on *Shift To Marker…*. A new windows will open, indicating the X and Y translation (in um) to apply to align the feature in at square magnification to the one at Atlas magnification. If this value make sens, i.e X and Y shifts are likely lower than 10um, press OK.
- If you did a mistake, like applying the shift with an atlas/square map selected instead of the point, the sift can be deleted in Navigator **tab** > *Undo last Shift*.

3. Select the focusing area
    1. Center a hole. If you plan to record images at the hole periphery, center the camera closer to a hole edge.
    2. Take a View and correct position if necessary.
    3. In the Low Dose control panel:
        - Tick the *Focus* **radio button** (1).
        - Make sure *Keep Focus and Trial Identical* is **not** thicked (2).
        - Make sure *Rotate inter-area axis* is ticked (3).



4. On the *view* image, **left-click** between four holes. This will move the focusing area to this place.



5. Tick the *None* **radio button** to leave the focus display.

4. Create a reference hole
    1. Center a hole. It must be non-empty, with an ice thickness representative of your grid, and free of ice contamination. If you plan to record images at the hole periphery, center the camera closer to a hole edge.
    2. Take a View and correct position if necessary.
    3. Adjust the camera field of view to capture a single hole in the image.
        - **Note:** For gold grids, have a little piece of the surrounding holes help in downstream alignment.
        1. Press Setup in the *Camera & Script* **panel**.
        2. Tick the *View* radio button.
        3. Adjust the Camera field of view with the *Area Size* buttons.
        4. Press Acquire to take a snapshot and Adjust the area size if necessary.

    4. In the *Buffers Control* **panel**, press on **P** to save the reference hole image in this buffer.

5. Center the Objective aperture.
    - **Note:** Be very careful ! for this step, you will need to work in **diffraction mode**. Direct exposure of the camera sensor with the direct beam may result in damages of the electron detector.
    1. Center on carbon or gold support film.
    2. In the *Low Dose Control* **panel**, press the *Rec.* button to switch the beam from View to Record setup.
    3. **Lower the fluorescent screen** as described in **section 6.2**.
    4. On the microscope Console, press the *Diffraction* button.
    5. Recenter the direct beam in the small red circle of the fluorescent screen with **multifunction X & Y knobs**.

6. With the mouse knob, change the sensitivity of the fluorescent screen to make the objective aperture shadow visible.
7. In the TEM User Interface, on the *Apertures* panel, press the *Adjust* button next to the Objective aperture selector.
8. With **multifonction X & Y knobs**, center the objective aperture around the direct beam.
9. Deselect the *adjust* button in the *Apertures* panel.
10. **Turn off diffraction mode.**
11. Lift the fluorescent screen.

6. Center the low-dose beams on the camera.
    1. Move to a empty area (empty hole or brocken square)
    2. In the *Low Dose Control* panel, tick *Continuous Update*.
    3. Switch to **record beam** with the *Rec.* button.
    4. On the console, press the **Eucentric Focus** button.
    5. Lower the fluorescent screen.
    6. In TEM User Interface > *Tune or Autoloader* tab > *Direct Alignments* panel: press on **beam shift**.
    7. With **multifunction X & Y knobs**, center the beam on the fluorescent screen, then press *Done*.
    8. Switch to **View beam** with the *Vie.* button. Tick *Set* in *Additional beam shift*. **View beam only need to be centered once**.
    9. With the **trackball**, center the view beam on the fluorescent screen.
    10. Center the Record beam again as described above.
    11. Switch to **Focus beam** (*Foc.* button) and change its diameter with the intensity knob according with the desired focus surface (depends on your hole spacing).
    12. Center focus beam as explained for the view beam (with *Set* ticked and trackball).
    13. Switch to **Trial beam** (*Tri.* button) and change its diameter with the intensity. Trial beam will be used for auto-centering and its diameter should fit in the camera field (green square) on the fluorescent screen.
    14. Repeat Record (Beam Shift + M X/Y), Focus and Trial (*Set* ticked + Trackball) centering until all beams stay centered.
    15. Untick *Continuous update* on the *Low Dose Control* panel.

7. In an empty hole or a broken square: Menu > Scripts > run > DatasetDefineVacuumIntensity
8. measure the dose rate and set up record parameters.

7. Start screening and analyze the results

1. Select a few target holes on each square
    - **Add Points** on the desired holes
    - **Stop adding** and **Collapse**
    - Press **A** key to define acquisition at all points and **Collapse** again

2. Manually screen your targets or run the script Screening Holes
    - Menu: *Navigator > Acquire at Items > Screening-Holes* (uncheck Rough Eucentric)

8. Load the next grid

1. Double-click on the desired grid in the TUI
2. Go back to step 4

9. End your screening session

1. Save your Navigator
2. Close your Navigator
3. Load the Cross Grating Grid in the column (usually in position 1 of the cassette)
4. Remove the cassette with your grids from the autoloader in the NanoCab
5. Remove the cassette and your grids from the NanoCab