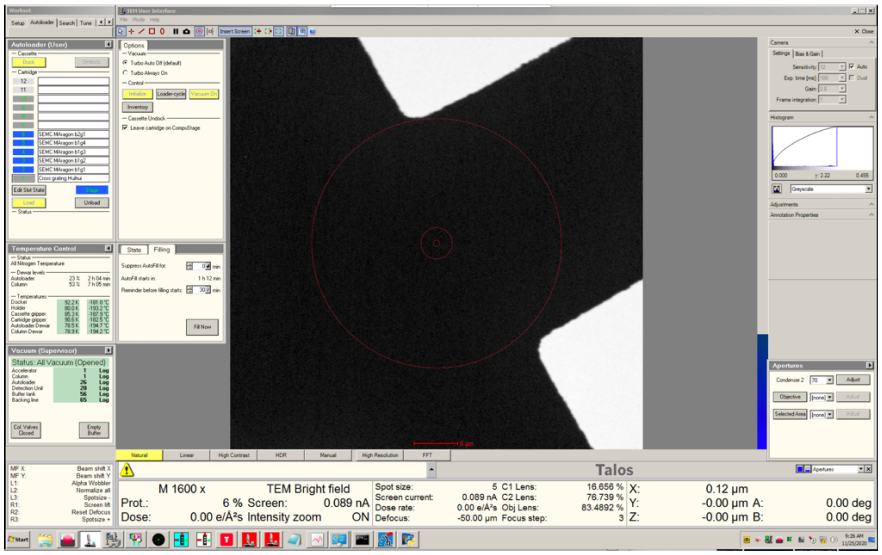
1. Setup a Leginon Session

A. Go to the microscope computer. Under the "**Autoloader**" tab, make sure the cross-grating is loaded (usually position 1 in the cassette). If it is not do the following:

- Make sure the column valves are closed ("Col Valves Closed" button should be yellow)
- Make sure the objective is out. Go to the "**AAM**" tab or select "**Apertures**" on the right side menu ("Objective" button should be gray).
- Select "insert screen" in the TEM User Interface to make sure the screen is inserted, not retracted.
- Click the number position the cross-grating is in and click the "**Load**" button.



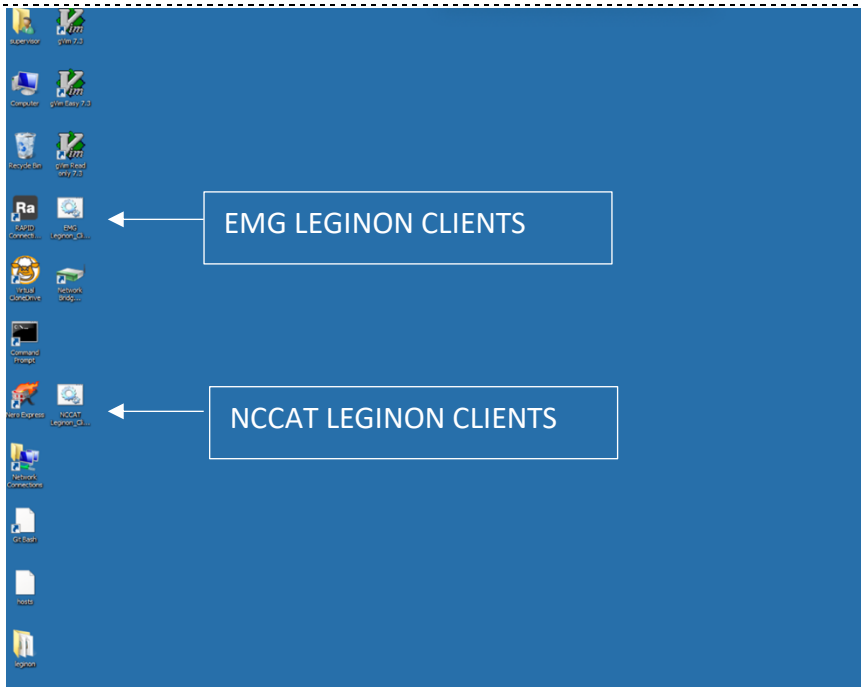
B. Start up the Leginon Clients on both the Microscope and Leginon computers.

For NCCAT:

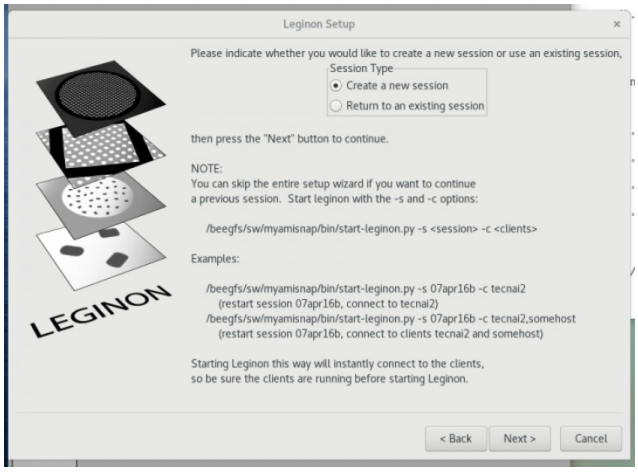
- Startup the **NCCAT Leginon Client** on desktop of Microscope Computer
- On the Leginon computer open a terminal and type in: **nccatleginon**
- if the user is not there in the terminal type in: **/beegfs/sw/bin/change_user.sh <username>; nccatleginon**

For EMG:

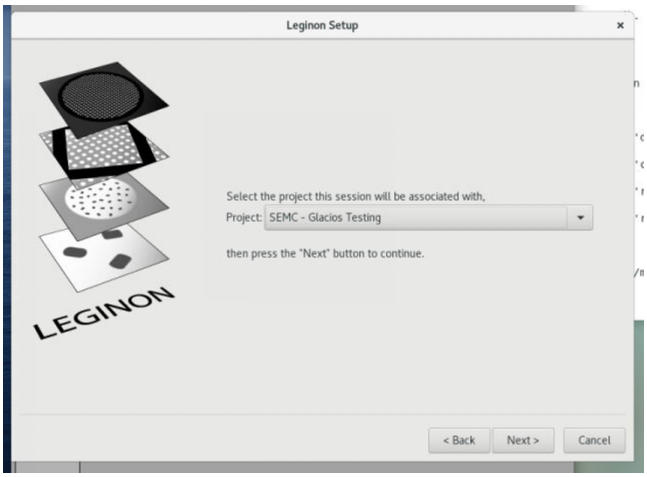
- Startup the **EMG Leginon Client** on the desktop of Microscope Computer
- On the Leginon computer open a terminal and type in: **betaleginon**
- if the user is not there in the terminal type in: **/gpfs/sw/bin/change_user.sh <username>; betaleginon**



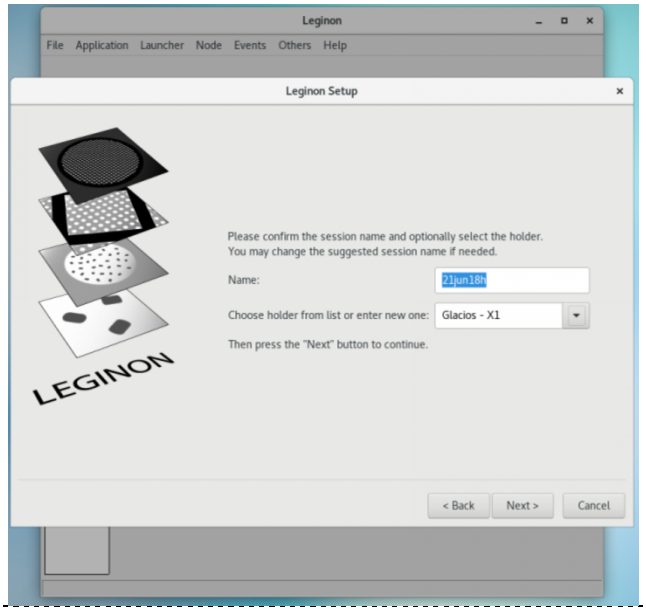
C. Select "**Create a new session**", then click "**Next>**"



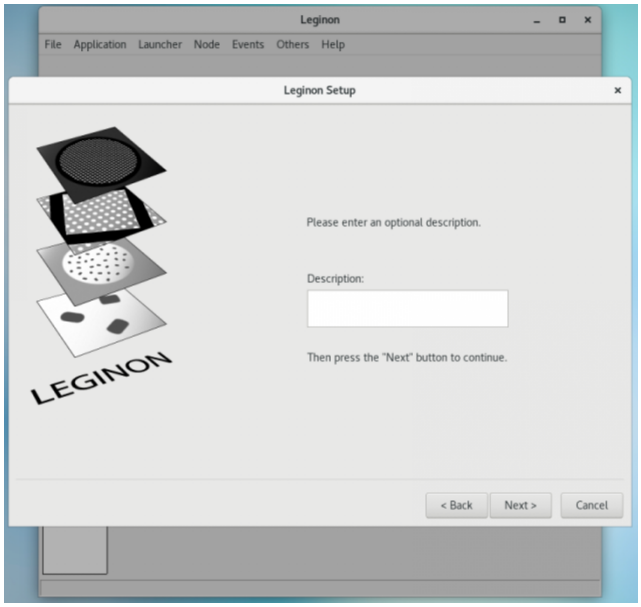
D. Select a project, then click "**Next>**". If there is no project listed, please submit a project here for NCCAT https://nccatdeon.nysbc.org OR here for EMG https://deon.nysbc.org



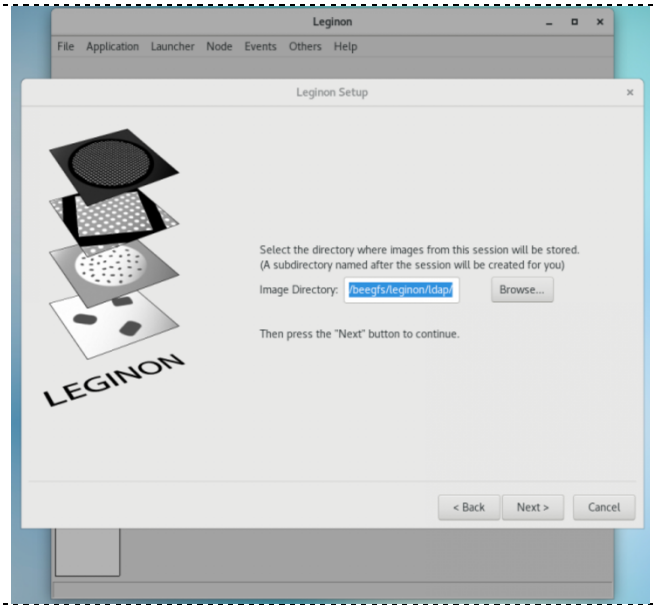
E. Please do not change the given session name. For holder, choose "**Glacios-X1**",then click "**Next>**".



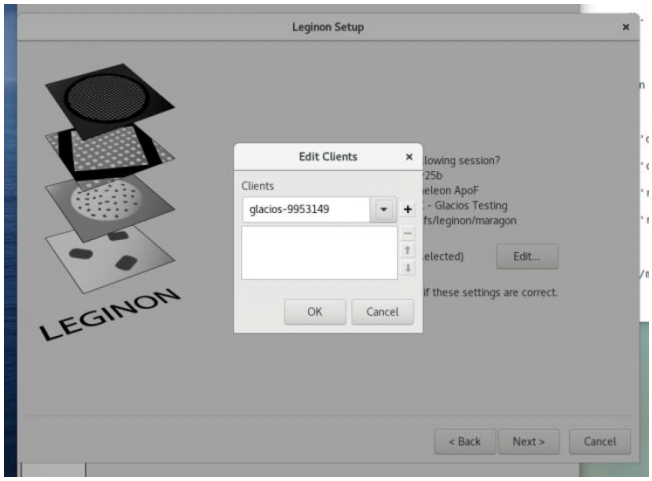
F. Enter a description of your session (ex: Glacios – ApoF), then click “**Next>**”.



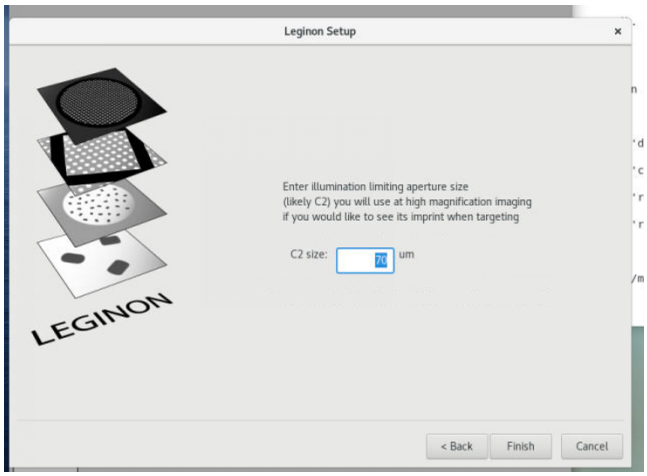
G. If the user is on NCCAT, make sure the directory is **/beegfs/leginon/username**. If the user is on EMG, make sure the directory is **/gpfs/leginon/username**, then click “**Next>”**



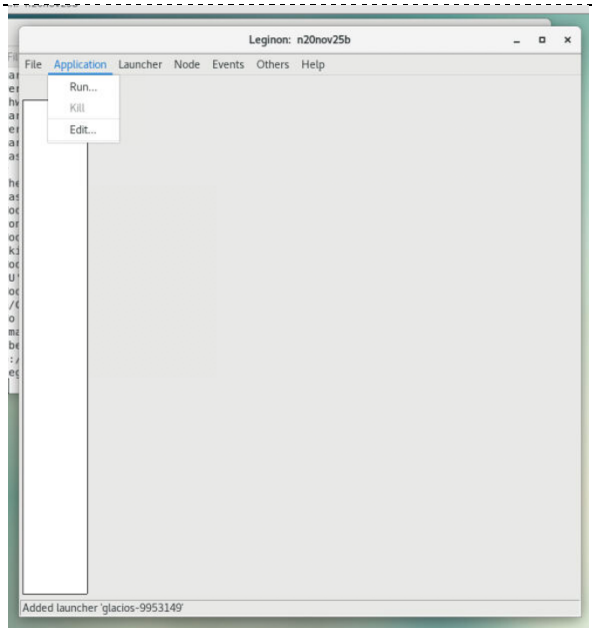
H. Click “**Edit**”. Select “**glacios-9953129**” from the dropdown menu, then click **+** sign to add it. Click “**OK**“, then click “**Next>”.**



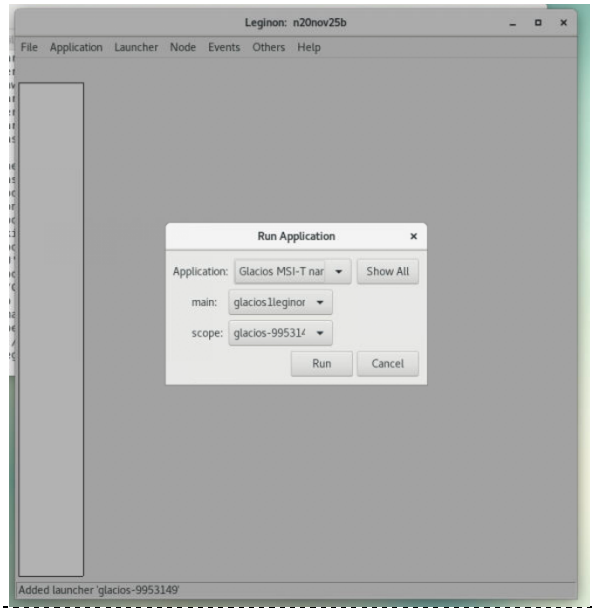
I. Check that the C2 size is set to **70**, then click “**Finish**”.



J. Select “**Application**” then “**Run**”



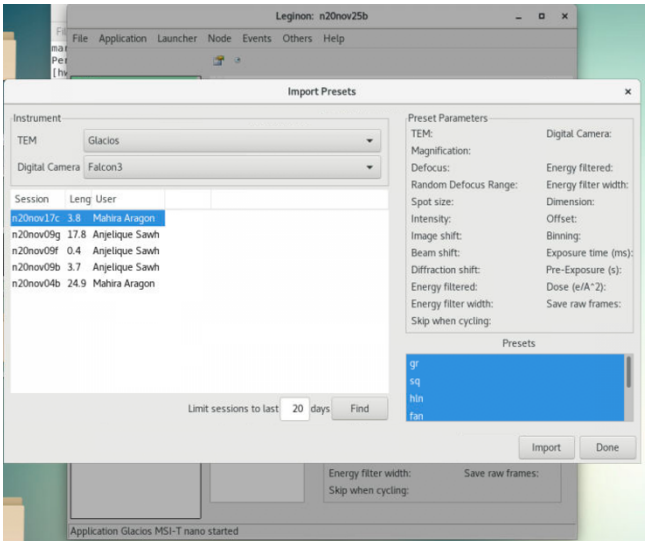
K. Application = “**Glacios MSI-T nano**”. If the option is not visible click “**Show All**”, then search the dropdown again.



L. In the *KPresets_Manager* Node, click the load preset icon  .

- TEM = "**Glacios**"
- Digital Camera = "**Falcon3**"

M. Click "**Find**". Select the most recent session. Select all preset (gr, sq, hl, fan, fcn, enn, and tune), then click "**Import**".



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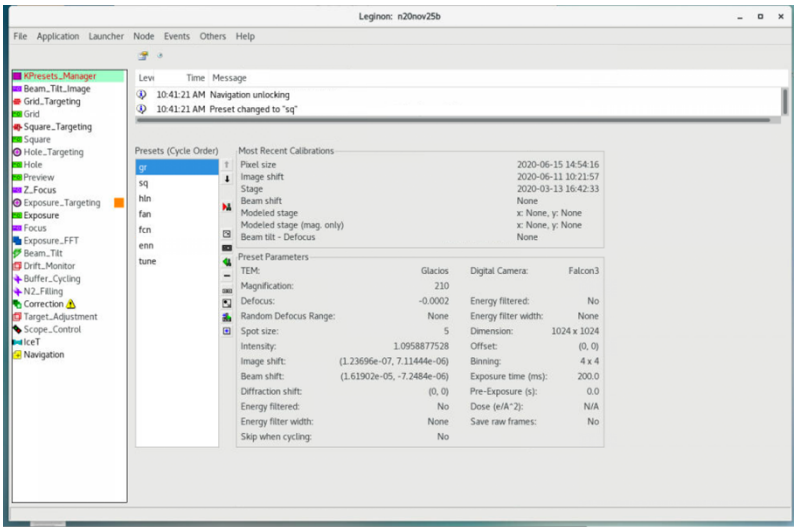
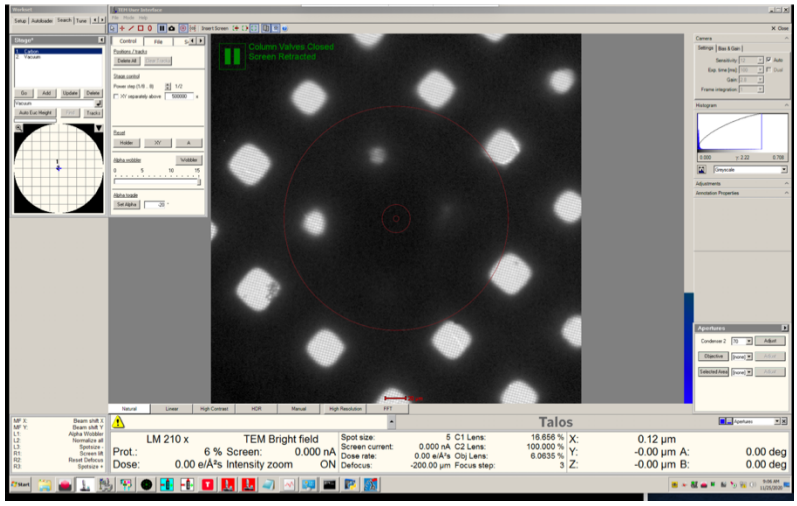
2. Saving Vacuum and Carbon Location

A. In the *KPresets_Manager* Node, select the *gr* preset, then click  to send to scope.



B. On the Microscope computer, lower the screen (hand panel **R1** button) Try to move to an empty square using the stage track ball on the hand panel.

C. Under the "**Search**" tab type in the label vacuum or empty, then click "**Add**". Repeat this for a carbon square, label it carbon.



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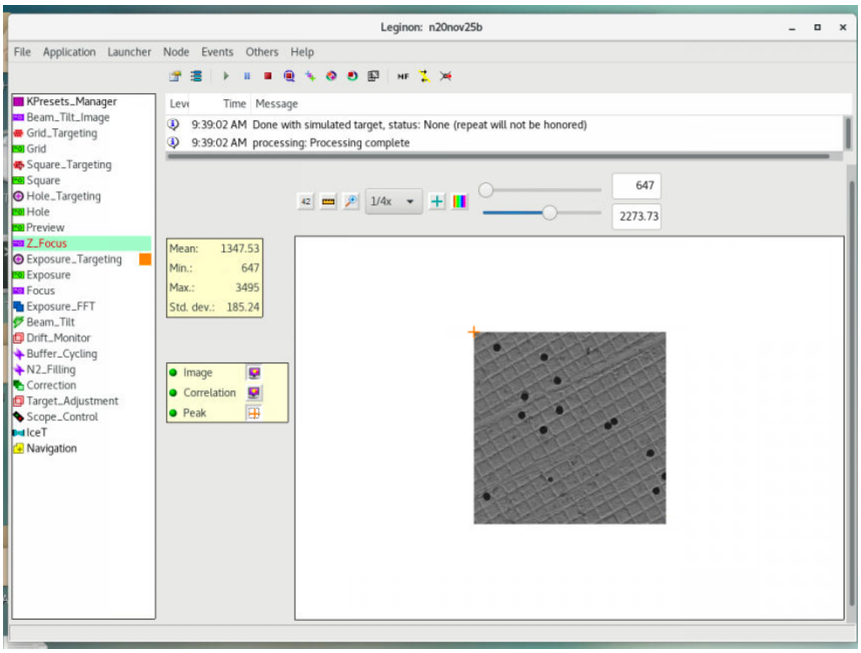
3. Eucentric Height/Focus

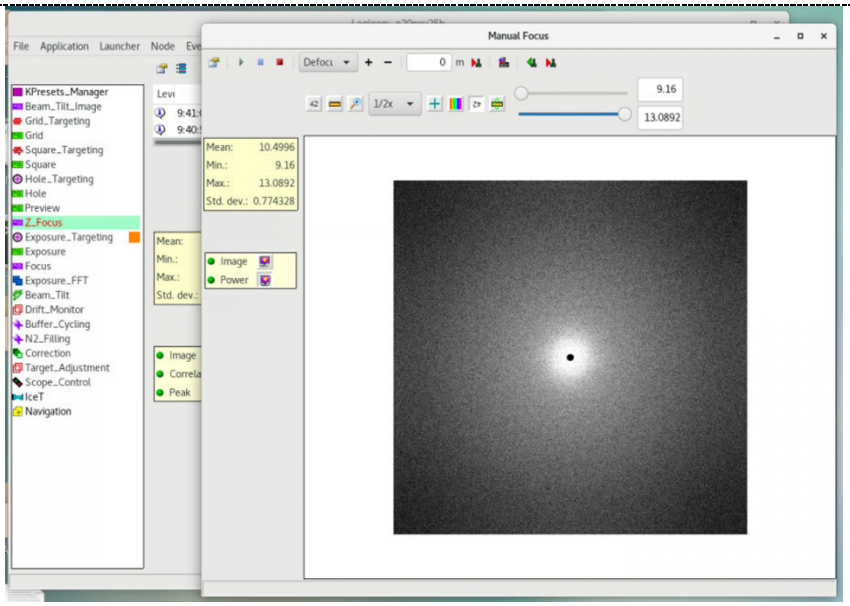
A. Must be on carbon area. If not, on the Microscope computer under the "**Search**" tab, select the label for the carbon square, then click "**Go**".

B. Go to the *Z_Focus* Node, then click the simulate target icon 



C. Select the manual focus icon  to verify zero focus.

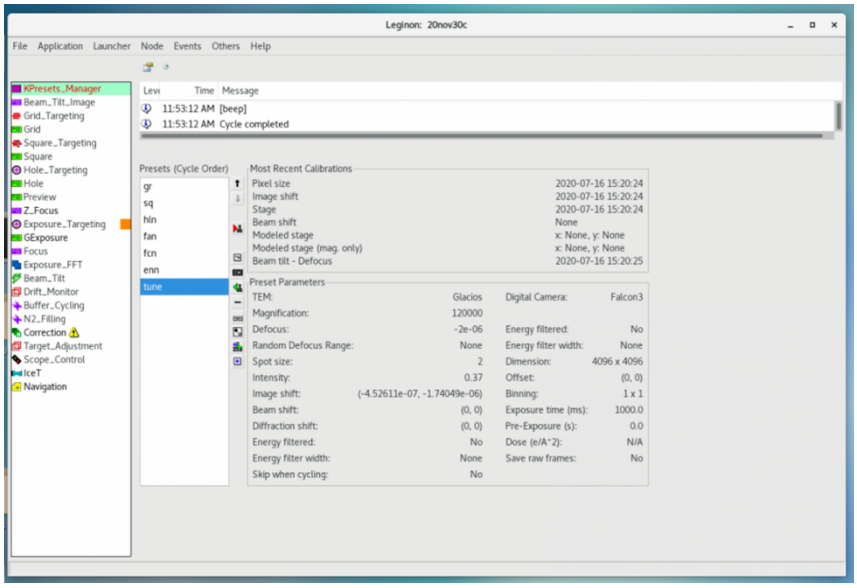
- Type in 0 m then click  to set the defocus to 0 m.
- If the FFT is not zero at this stage, click  and hover your mouse over the first zero to take note of the value. Left click on the first zero, then use + or – buttons next to 'Defocus' to increase or decrease the focus respectively. Repeat until you are at focus, as in the image on the right.
- Once at zero defocus, click the reset defocus icon  . Then click  to close the window.



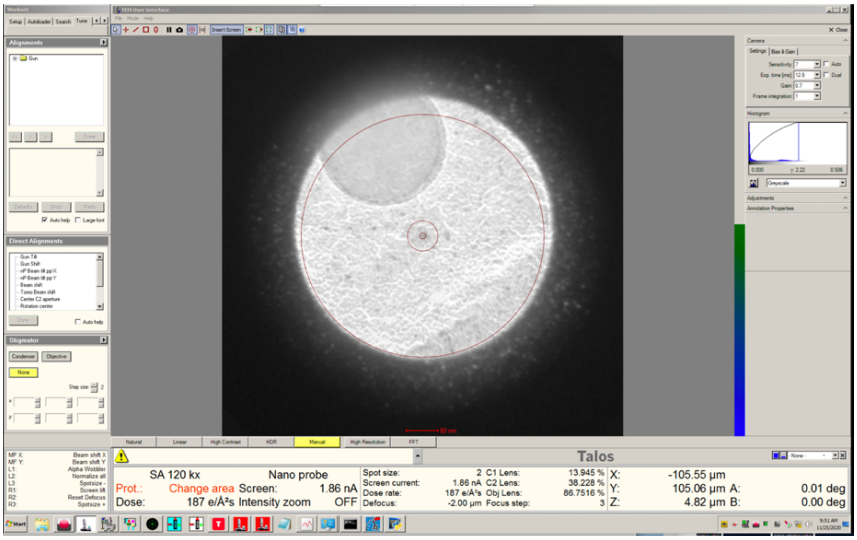
4. Beam tilt pivot point and rotation centering

A. Must be on carbon area. If not, on the Microscope computer under the "**Search**" tab, select the label for the carbon square and click "**Go**". Must be at Z height. If not refer to step 3.

B. Go to the *KPresets_ Manager* Node, select the *tune* preset and click  .



C. Put the screen down (**R1** button on hand panel). On the Microscope computer, under the "**Tune**" tab, in "**Direct Alignments**" check pivot point X, pivot point Y, rotation center, and beam shift to center beam.



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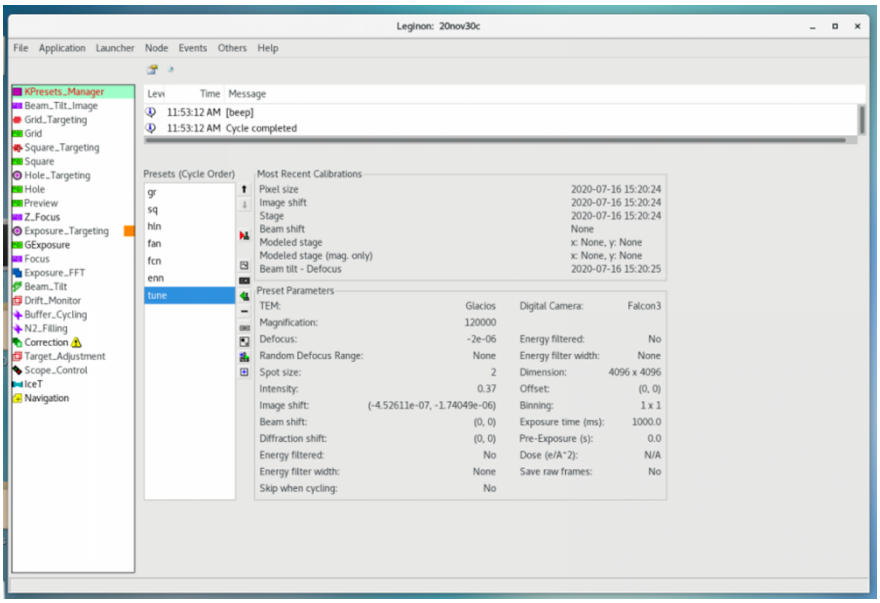
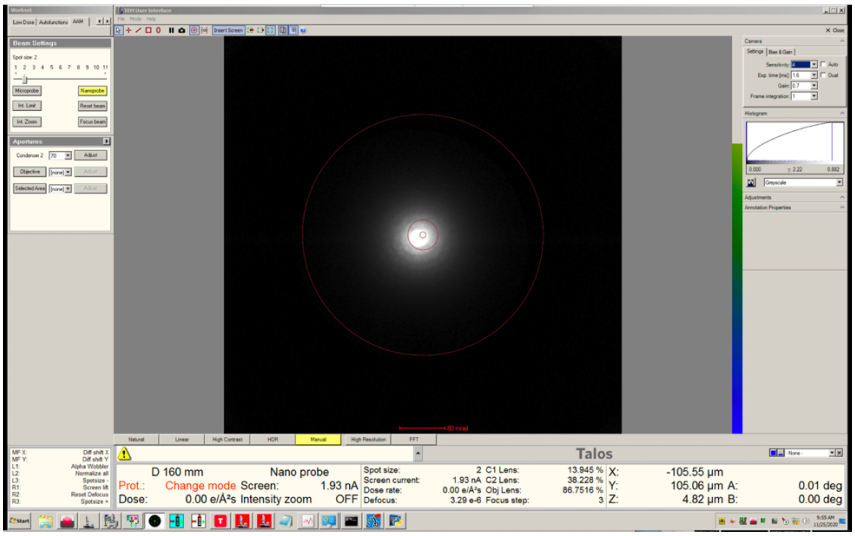
5. Objective Centering

A. Must be on carbon area. If not, on the Microscope computer under the "**Search**" tab, select the label for the carbon square and click "**Go**". Must be at Z height. If not refer to step 3.

B. Go to the *KPresets_ Manager* Node, select the *tune* preset and click the send to scope icon  .

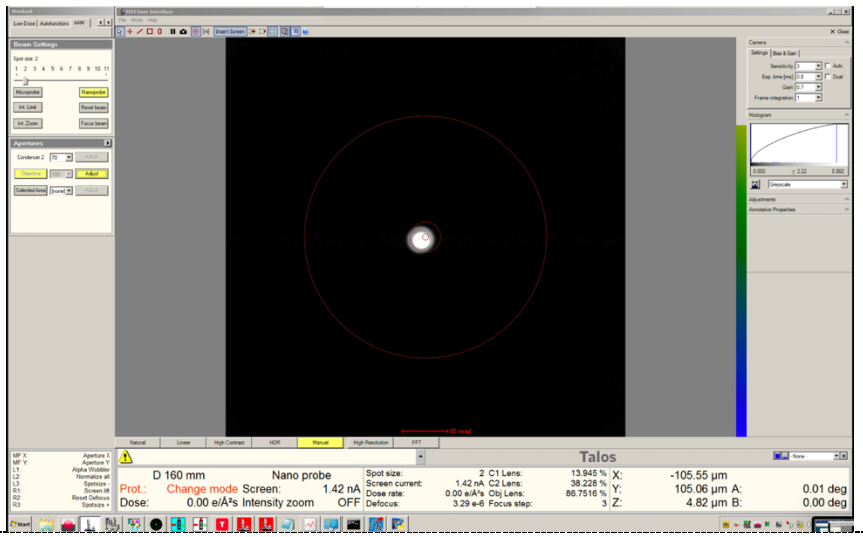


C. On the Microscope computer, lower the screen by pressing **R1** button on the hand panel. Next click the "**Diffraction"** button on the hand panel.



D. Under the "**AAM**" tab insert the objective, by clicking "**Objective**" button (will turn yellow)

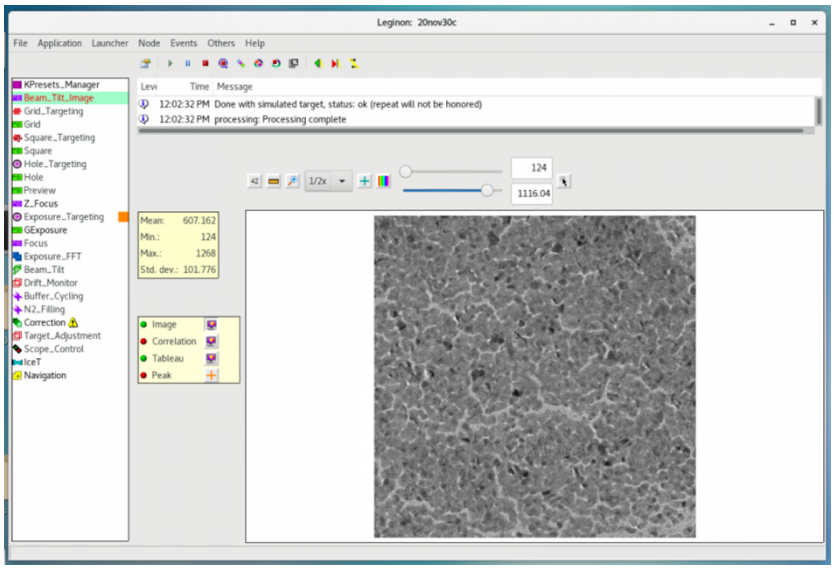
- To adjust click "**Adjust**" button (will turn yellow), use **MF X** & **MF Y** on the hand panels to center the objective, when done click "**Adjust**" button again (will turn gray).
- Make sure to click the "**Diffraction**" button on the hand panel to exit diffraction mode.



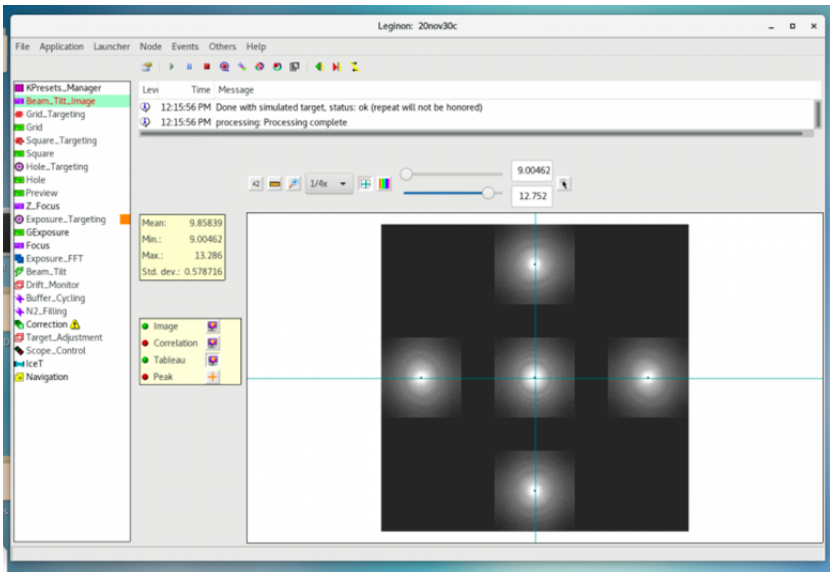
6. Coma Correction

A. Must be on carbon area. If not, on the Microscope computer under the "**Search**" tab, select the label for the carbon square and click "**Go**". Must be at Z height. If not refer to step 3.

B. Go to the *Beam_Tilt_Image* Node and select the simulate targe icon  . Select  next to "**Tableau**" to view the Tableau Image.



C. To adjust select the crosshairs  and the cursor  , click toward the more defocused image until the top and bottom images are symmetric and the left and right images are symmetric.



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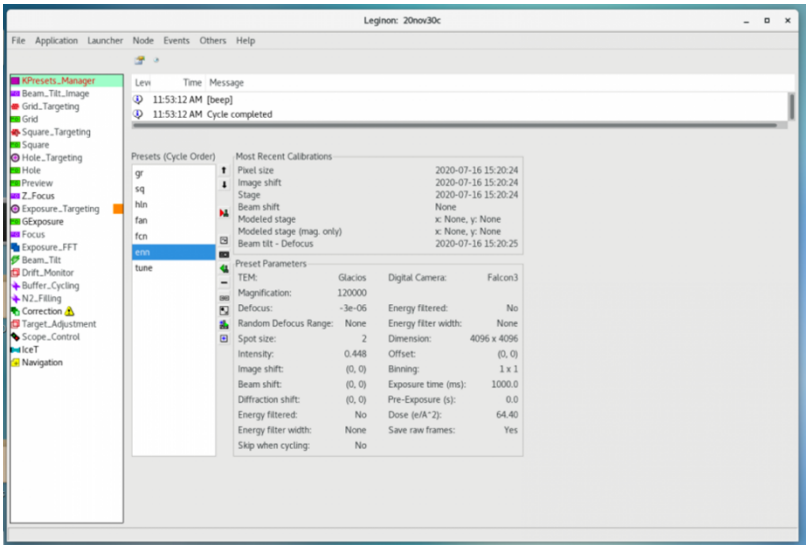
7. Leginon Gain Reference

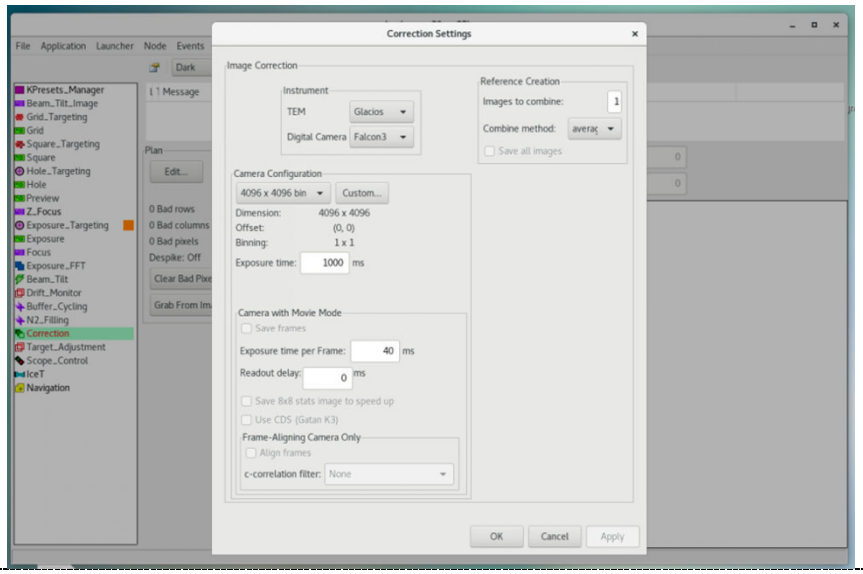
A. Must be on vacuum area. If not, on the Microscope computer under the "**Search**" tab, select the label for the vacuum square, then click "**Go**".

B. Go to the *KPresets_Manager* Node, select the *enn* preset, then click  .



C. In the *Correction* Node, select the settings icon and input the following information:

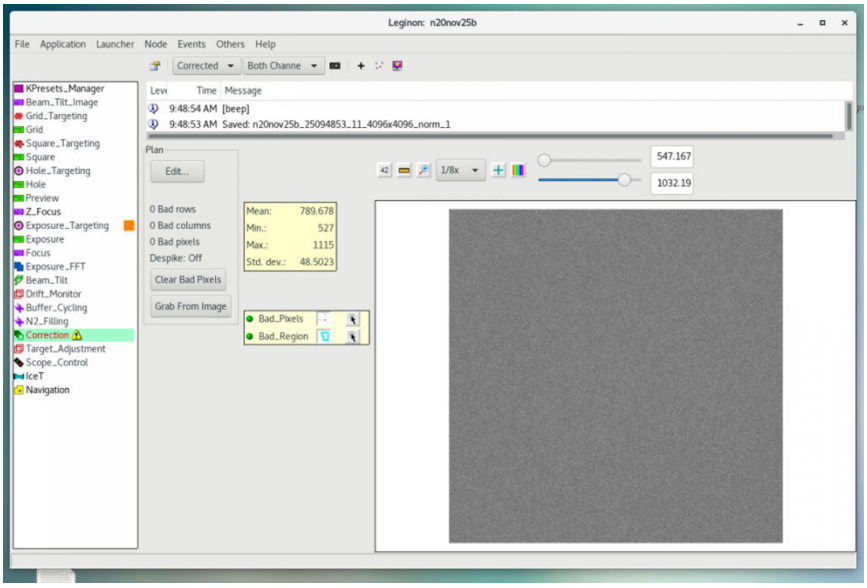
- TEM = "**Glacios**"
- Camera = "**Falcon3**"
- Select the dimensions "**4096 x 4069 bin 1**"
- Exposure time = **1000** ms
- Exposure time per Frame = **40** ms
- Images to combine = **10**
- Click "**OK**"



D. Select "**Dark**" and "**Both Channels**", then click the camera icon .

E. Once it is done select "**Bright**" and "**Both Channels**", then click .

F. Once it is completed select "**Corrected**" and "**Both Channels", then** click .

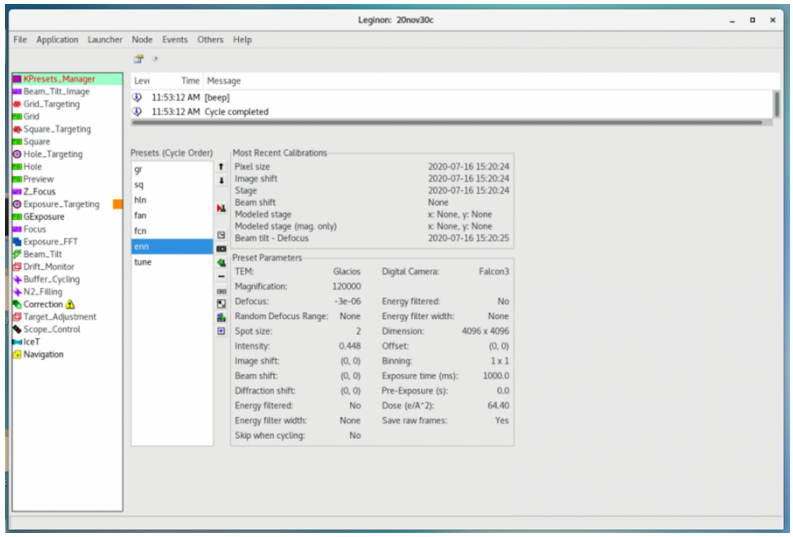


8. New Dose for exposure

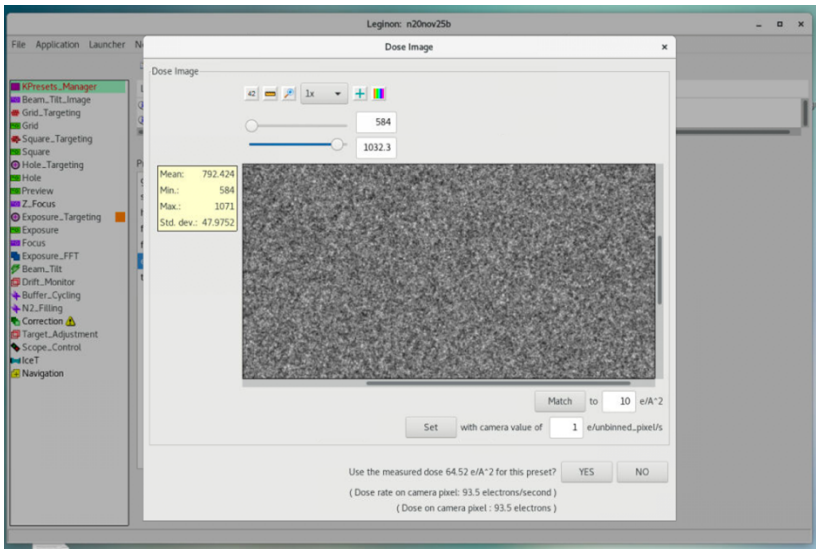
A. Must be on vacuum area. If not, on the Microscope computer under the "**Search**" tab, select the label for the vacuum square, then click "**Go**".

B. Go to the *KPresets_ Manager* Node Select the *enn* preset and send to scope .

C. On the Microscope computer, lower the screen (hand panel **R1** button) to confirm that the beam is present and centered.



D. Select the camera icon to take a dose (~65 e/A^2), then click "**YES**".



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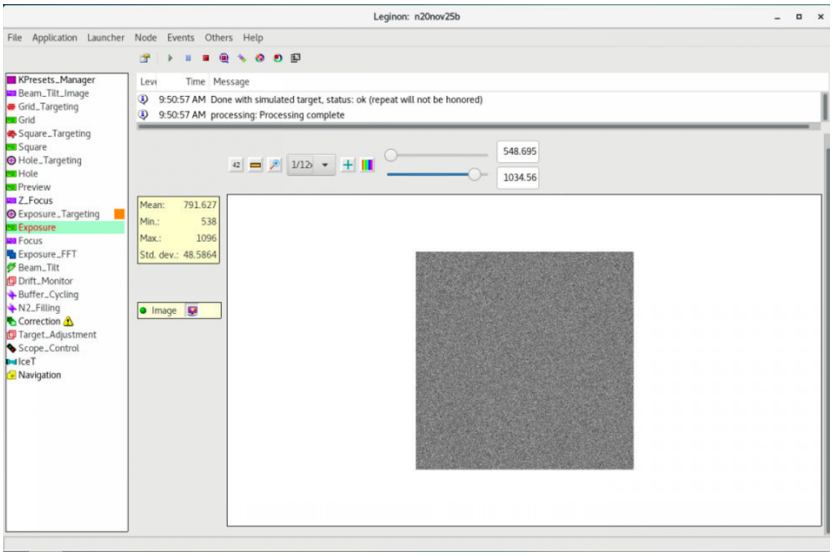
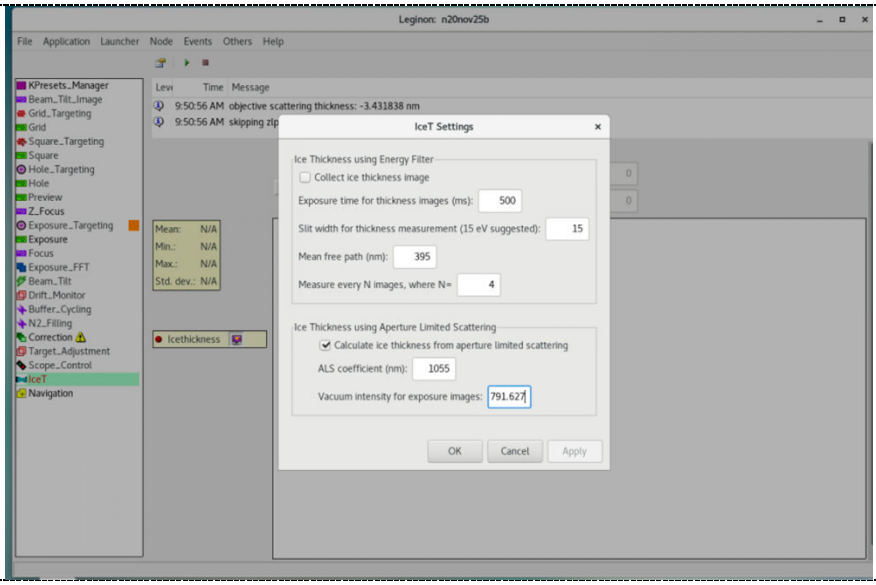
9. Ice Thickness

A. Must be on vacuum area. If not, on the Microscope computer under the "**Search**" tab, select the label for the vacuum square, then click "**Go**".

B. Go to the *Exposure* Node and click the simulate target icon  OR go to the *Navigation* Node, select the *enn* preset, click  and then click  to take an image. After the image is taken, note down the "**Mean**" value.



C. Go to *Ice_T* Node and select the setting icon  . Enter the "**Mean**" value in the space next to "**Vacuum intensity for exposure images**". Click "**OK**".

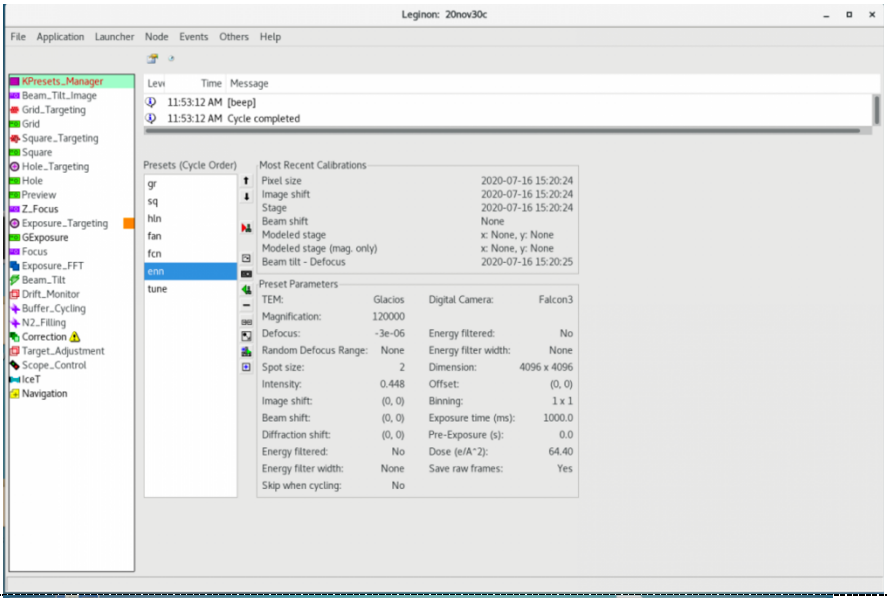


10. Preset alignments

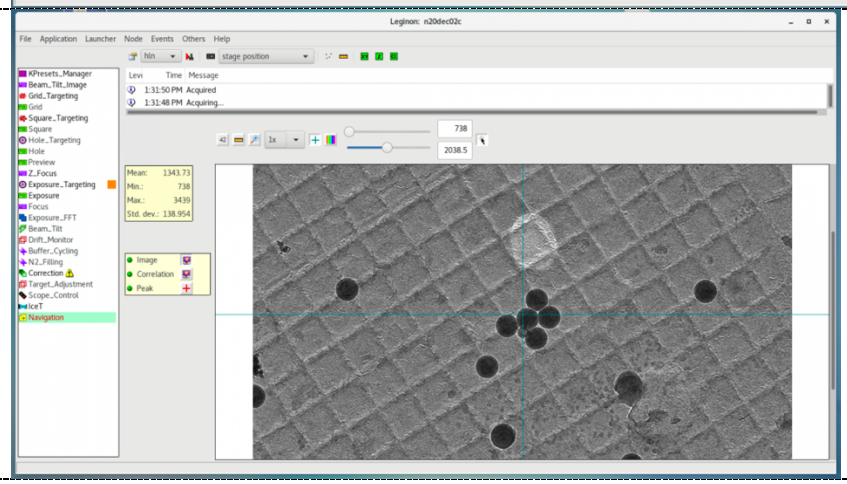
A. In the *KPresets_Manager* Node, make sure that *fan, fcn* and *enn* presets have:

- **Image shift: (0,0)**
- **Beam shift: (0,0)**

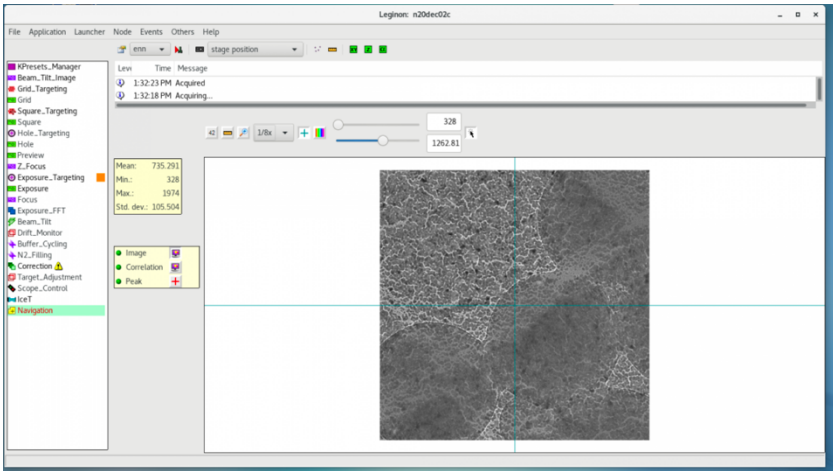
B. Must be on carbon area. If not, on the Microscope computer under the "**Search**" tab, select the label for the carbon square and click "**Go**". Must be at Z height. If not refer to step 3.



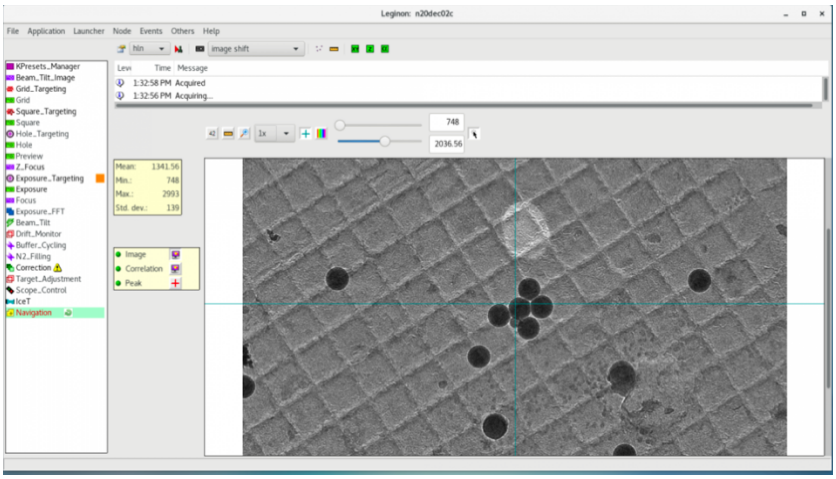
C. Go to the *Navigation* Node. Select the *sq* OR *hln* preset then click  . Select "**stage position**", then click  . Select  and  to center over a feature.



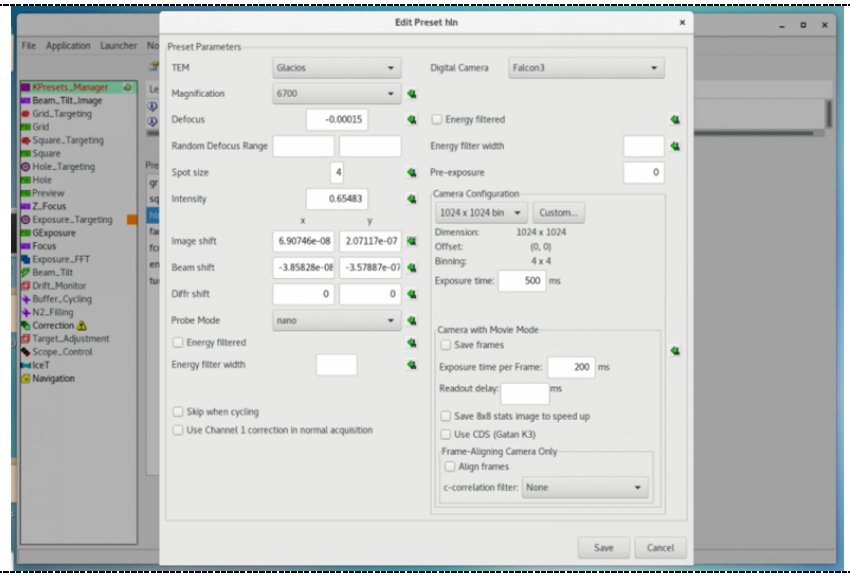
D. Once centered select the *enn* present then click  . Select "**stage position**", then click  . Note the position of the feature in the center of the crosshairs.



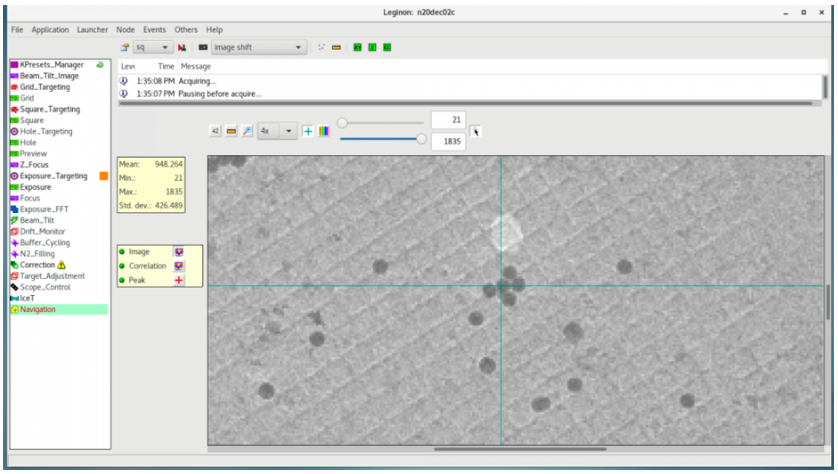
E. Select the *hl* preset then click  . Select "**image shift**", then click  . If the center of the crosshairs is not at the same location as in the *enn* image. Use the  to move it to the same location.



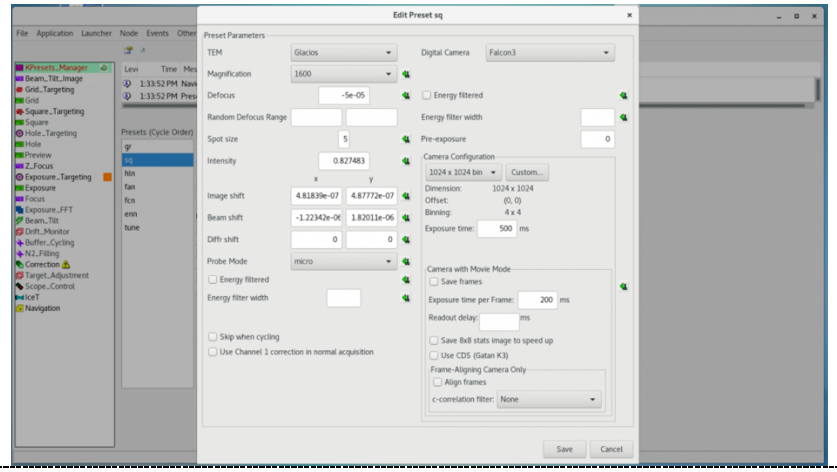
F. Once moved, go to the *KPresets_Manager* Node, select the *hln* preset, click the edit settings icon  , next to "**Image Shift**" click from scope icon  . Click "**Save**".



G. Select the *sq* preset then click  . Select "**image shift**", then click  . If the center of the crosshairs is not at the same location as in the *hln* image. Use the  to move it to the same location.



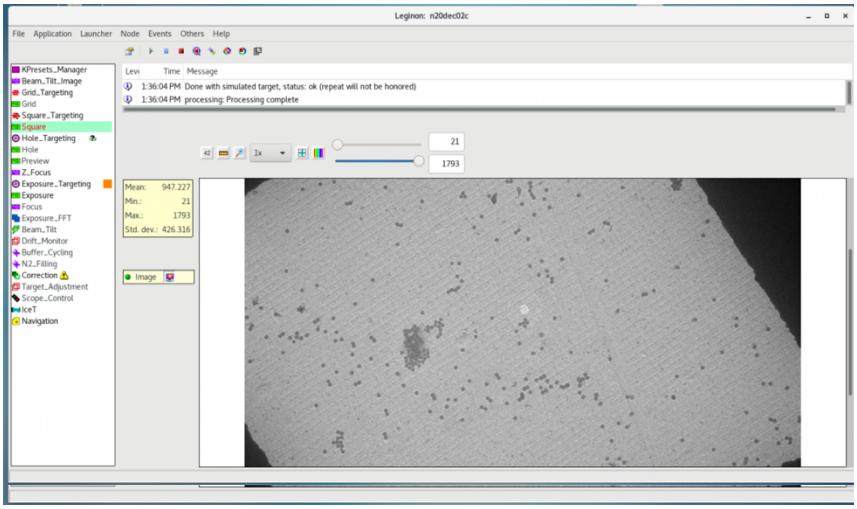
H. Once moved go to the *KPresets_Manager* Node, select the sq preset, click the edit settings icon , next to "**Image Shift**" click from scope icon . Click "**Save**".



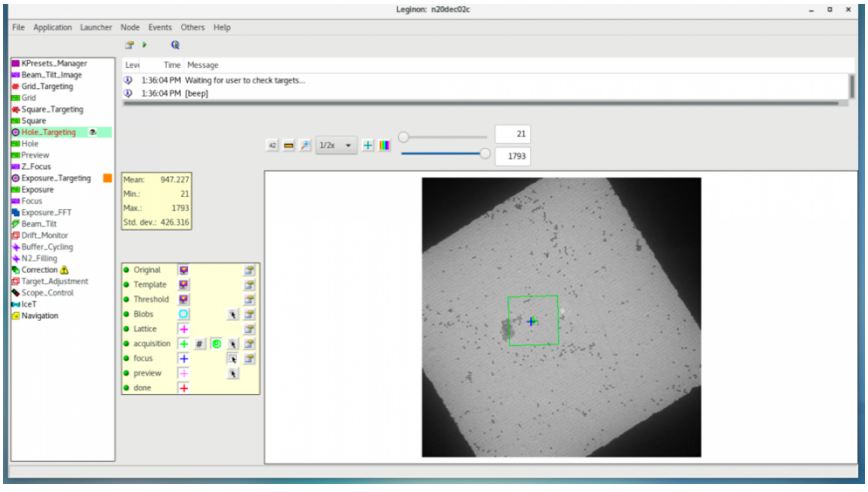
11. Test Images

A. Must be on carbon area. If not, on the Microscope computer under the "**Search**" tab, select the label for the carbon square and click "**Go**". Must be at Z height. If not refer to step 6.

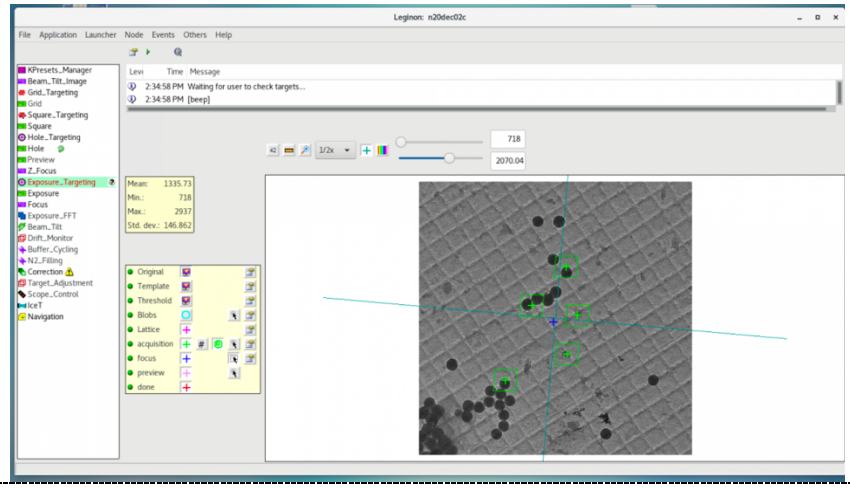
B. Go to the *Square* Node, click .



C. Go to the *Hole_Targeting* Node, select the cursor next to "**acquisition**". Select one area to image. Select the cursor next to "**focus**", place a focus target near the acquisition target. Click .



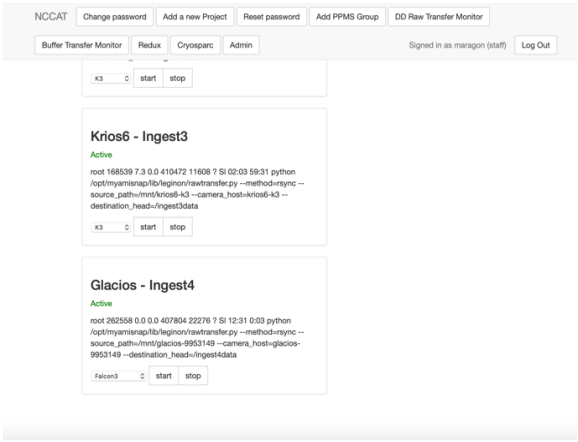
D. Go to the *Exposure_Targeting* Node, select the cursor next to "**acquisition**". Select a few areas to image. Select the cursor next to "**focus**", place one focus target in the center of the acquisition targets. Click to submit.



12. Frame Alignment and CTF Estimation

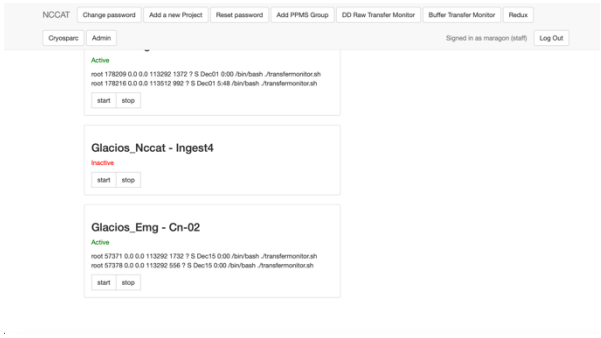
A. For EMG betaleginon session:

- If switching from a nccatleginon session to a betaleginon session. Go to nccatdeon.nysbc.org.
- Click "**DD Raw Transfer Monitor**". Under the section for "**Glacios – Ingest4**", click "**stop**". Select "**Falcon3-Emg**" and click "**start**".
- Click "**Buffer Transfer Monitor**". Under the section for "**Glacios _EMG – Cn-02**", click "**start**". It should show the word "Active". In the "**Glacios_Nccat-Ingest4**" section click "**stop**", it should show the word "Inactive".



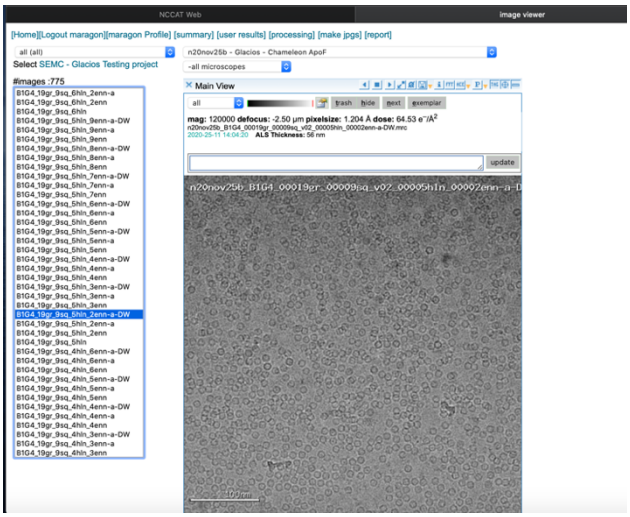
B. For NCCAT nccatleginon session:

- If switching from a betaleginon session to a nccatleginon session. Go to nccatdeon.nysbc.org
- Click "**DD Raw Transfer Monitor**" Under the section for "**Glacios – Ingest4**", click "**stop**". Select "**Falcon3**" and click "**start**".
- Click "**Buffer Transfer Monitor**". Under the section for "**Glacios _EMG – Cn-02**", click "**stop"**. It should show the word "Inactive". In the section "**Glacios_Nccat-Ingest4**" click "**start**" it should show the word "Active".



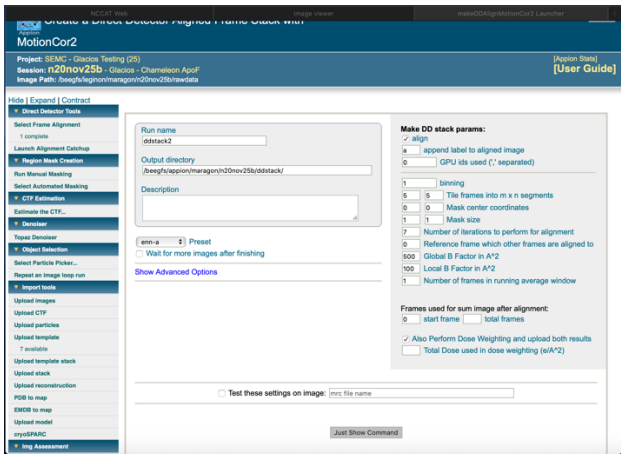
C. Open ~5 terminals and ssh the user into ingest04 (~2 for Frame Alignment and ~3 for CTF)

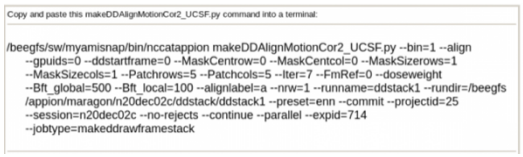
- For NCCAT sessions go to http://nccatweb.nysbc.org OR for EMG sessions go to https://emgweb.nysbc.org
- Select the correct session from the dropdown
- Click "**[processing]**"



D. From the Appion menu, choose "**Select Frame Alignment**", then select "**MotionCor2**".

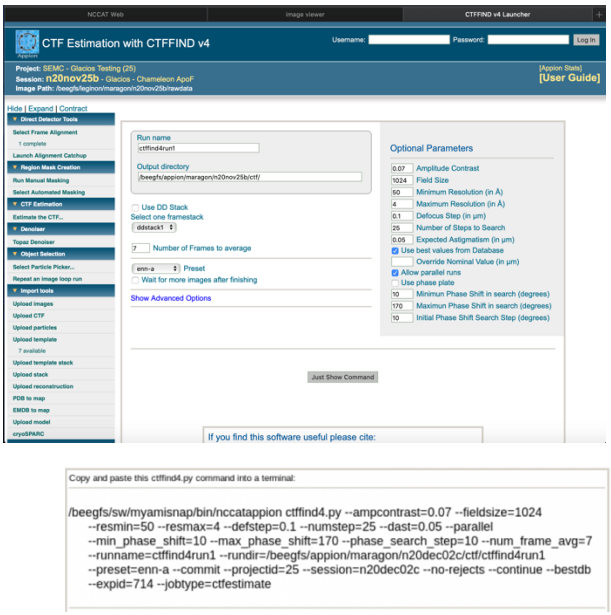
- Make sure the selected preset is *enn* (will only appear once enn have been taken*)*. Click "**Just Show Command**".
- For a betaleginon EMG session only, before pasting the command in a terminal replace **/opt/myamisnap/bin/appion** with **/opt/myamisnap_gpfs/bin/glacios_test_appion**
- Enter the command in the ingest04 terminal.
- With the same command change **gpuid=0** to **gpuid=1,** and enter the command in another ingest-04 terminal.





E. From the appion menu, select "**Estimate the CTF**" then select "**CTF Find v4**".

- Make sure the selected preset is *enn-a* (will only appear once Frame Alignment starts). Click "**Just Show Command**".
- For a betaleginon EMG session only, before pasting the command replace **/opt/myamisnap/bin/appion** with **/opt/myamisnap_gpfs/bin/glacios_test_appion**
- To the end of the command add the flag "**--nproc=5**" before entering into the terminal.



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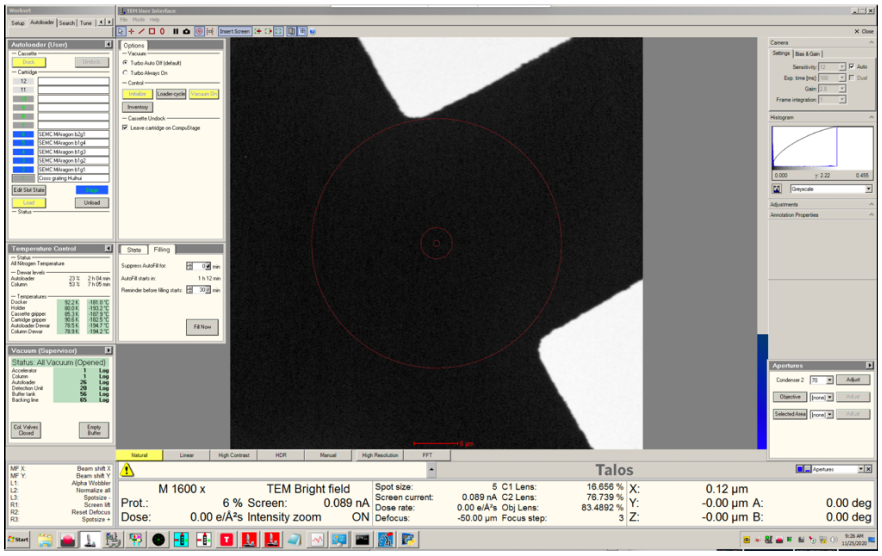
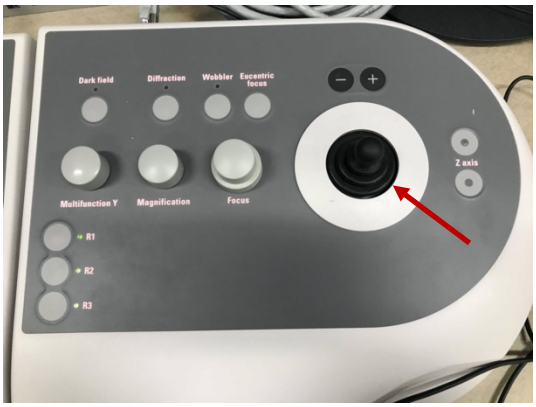
13. Switch to user's grid and collect an atlas

A. Go to the microscope computer. Under the "**Autoloader**" tab:

- Make sure the column valves are closed ("Col Valves Closed" button should be yellow)
- Make sure the objective is out. Go to the "**AAM**" tab OR select "**Apertures**" in the right side menu click the "**Objective**" button (will turn gray).
- Click the position number to be loaded. Then click the "**Load**" button (will turn yellow once loaded).



B. On the Leginon computer, go to the *KPresets_Manager* Node. Select the *sq* preset then click  . Then on the Microscope computer, lower the screen (hand panel R1 button). Try to move to a square using the stage joystick on the hand panel. Go to the *Z_Focus* Node, then click the simulate target icon  .



C. Go to the *Grid_Targeting* Node. Click on the settings icon  .

- Enter a label for the grid and the radius. Largest atlas is 0.009 m (43 targets). Then click  and then  .
- Go to the *Square_Targeting* Node to view the atlas.