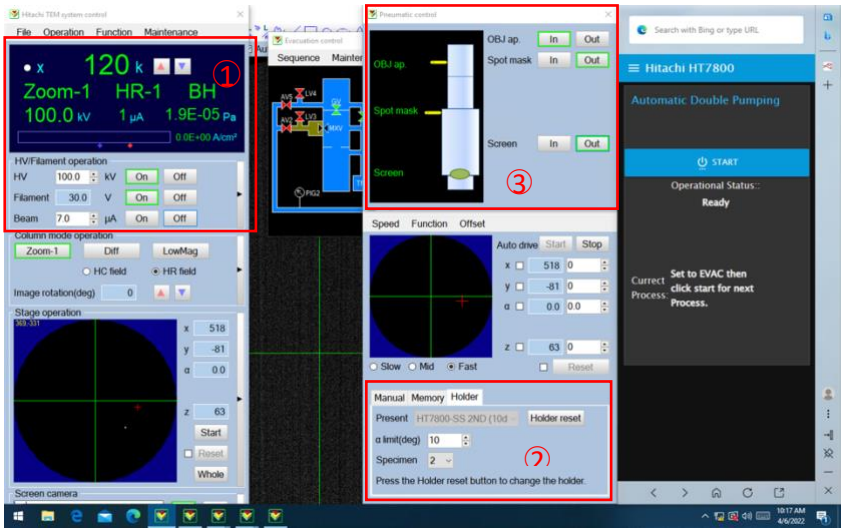
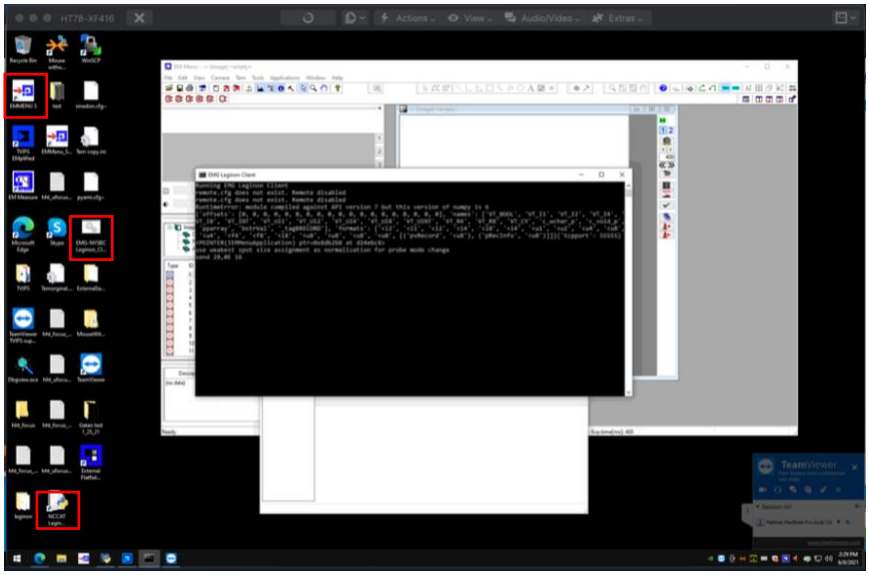
Microscope Startup

1. On the Microscope PC, make sure:
   - In area ① outlined in red, the HV is at "**100.0 kV"** as seen in the window. If it is not, in the "**HV Filament operation"** turn HV "**On**"
   - the magnification is 120 k.
   - the Filament is "**Off"**
   - the Beam is "**Off"**
   - In area ② highlighted red, the stage is centered by clicking "**Holder reset**"
   - In area ③ highlighted red, the screen is **"In"**

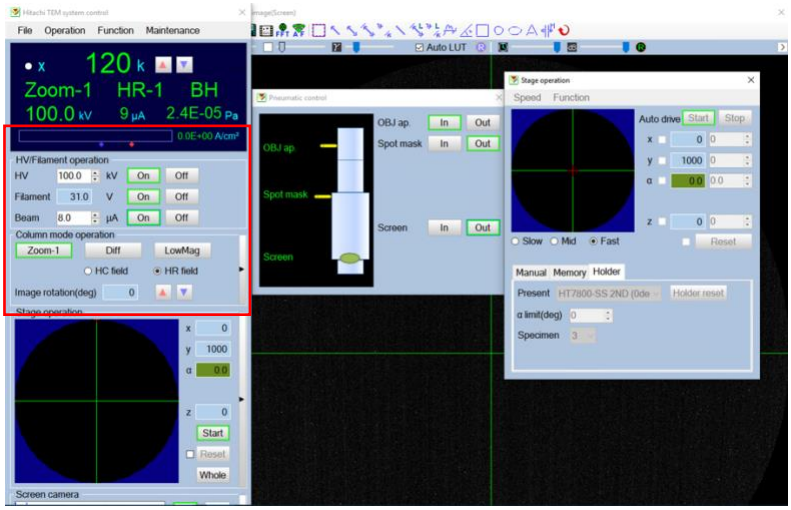


1. On the Camera PC, make sure:
   - "**EMMENU**" is open. If it is not open, click on "**EMMENU5**" (highlighted in red).
   - For EMG, open the "**EMG-NYSBC Leginon Client"** (highlighted in red).
   - For NCCAT, open the "**NCCAT Leginon Client"** (highlighted in red).
2. *Please look for the icons as they may have moved*

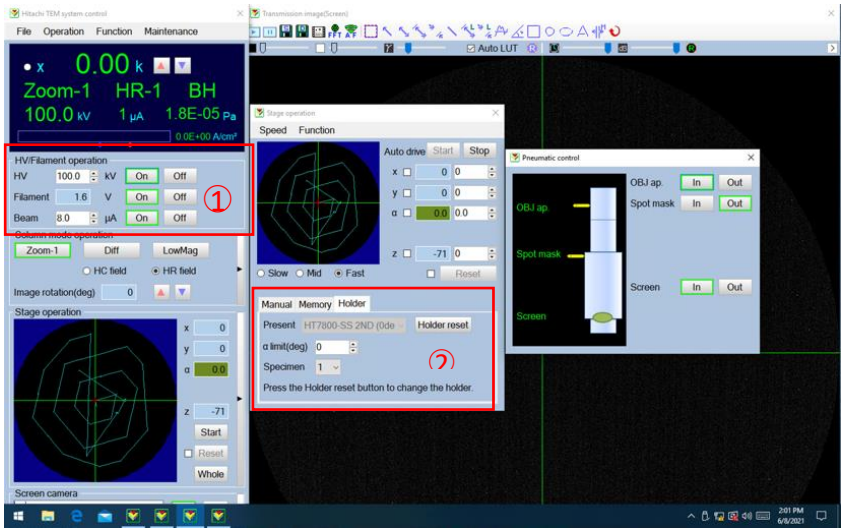


Microscope Operation

1. After insertion:
   - In the area outlined in red, wait for vacuum to read at least 4.0E-05 Pa.
   - Then click “On” for the filament, when ready it will read “30 V” (takes ~15 min).
   - Once the filament is ready, click “On” for the Beam, it should read “7.0 uA”.

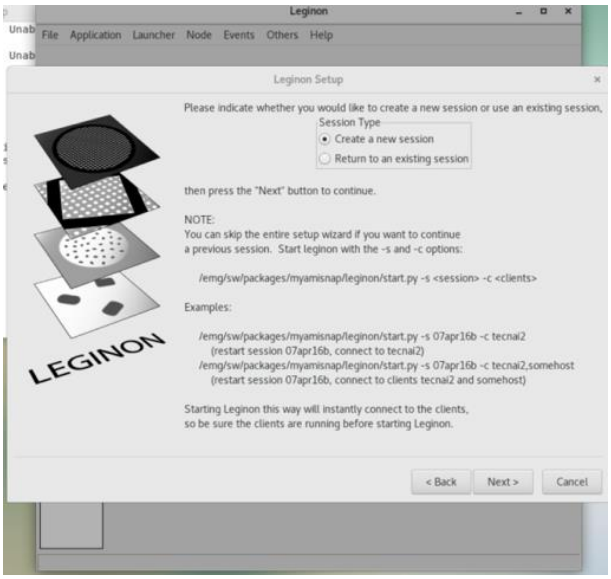


2. Please follow diagram for insertion and retraction and note:
   - In the highlighted area ①,make sure the beam is “**Off**”, when inserting and retracting.
   - In the highlighted area②, make sure holder is rest, by clicking “**Holder Reset**”
   - To change the specimen position, use the dropdown labeled “**Specimen**”. If this does not appear as an option, make sure for “**Present**” the holder selected is “HT7800-SS 2ND”

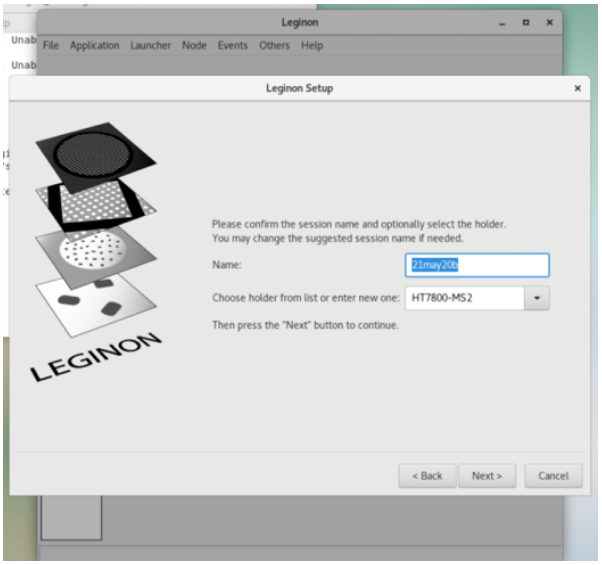


Starting a Leginon Session

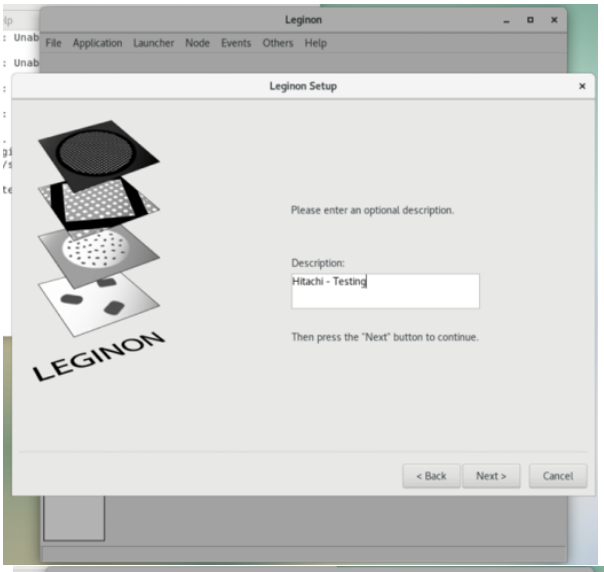
1. On the Leginon PC. Open a Terminal window.
   - For EMG, type in the command *betaleginon.*
   - For NCCAT, type in the command *nccatleginon.*
   - Once the Leginon window appears, select "**Create a new session**", then click "**Next >**"



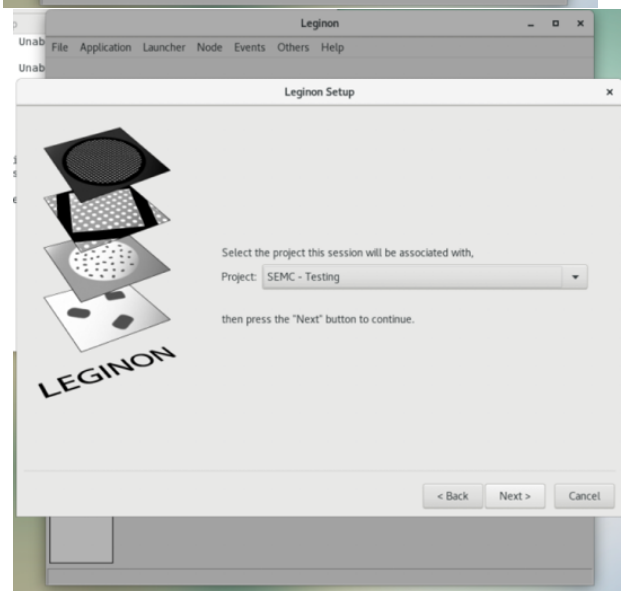
2. Please do not change the given session name. Select the holder "**HT7800-MS2**", then click "**Next >**"



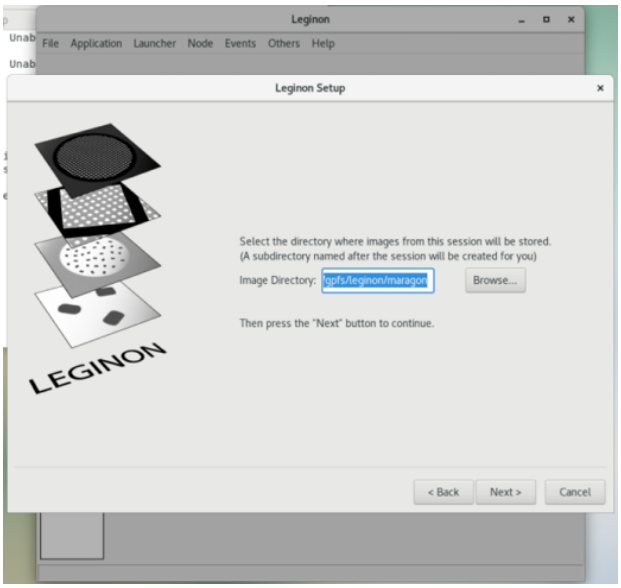
3. Enter a description of your session (ex: Hitachi– Testing), then click “**Next >**”



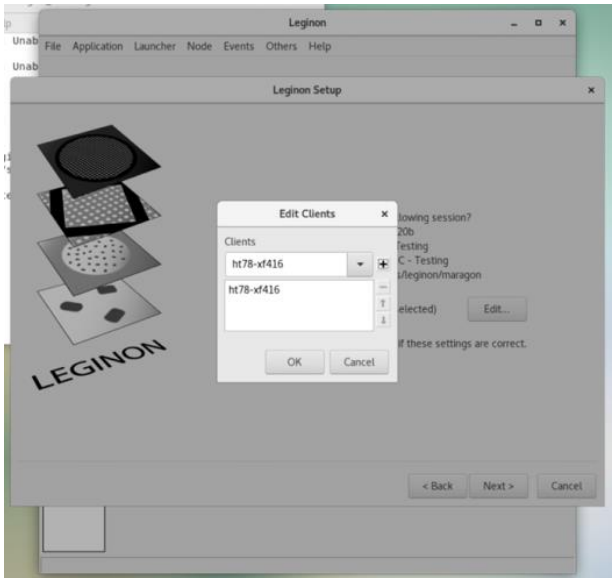
4. Select a project, then click “**Next >**”. If there is no project listed, please submit a project here for NCCAT https://nccatdeon.nysbc.org OR here for EMG https://deon.nysbc.org., then click “**Next >**”



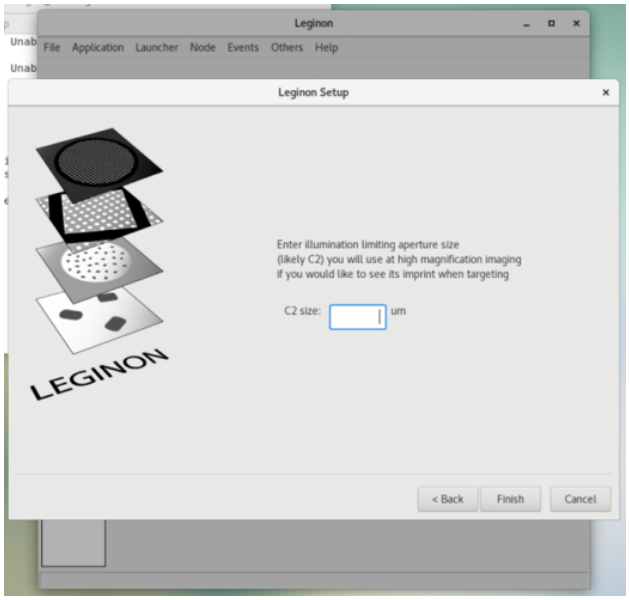
5. Check the directory path, then click “**Next>”**:
   - If the user is on NCCAT, make sure the directory is **/beegfs/leginon/username**.
   - If the user is on EMG, make sure the directory is **/gpfs/leginon/username**
   - If the directory is not correct, please let the assisting staff member know.



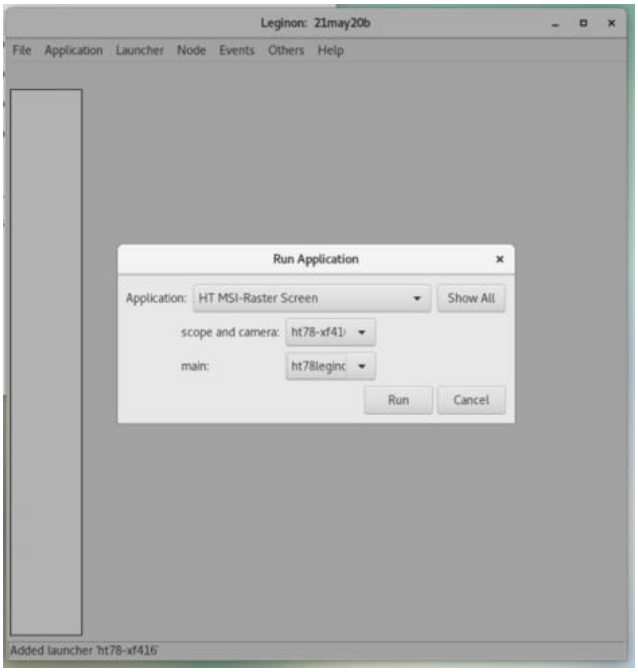
6. Click “**Edit**”. Select “**ht78-xf416**” from the dropdown menu, then click **+** sign to add it. Click “**OK**“, then click “**Next>”.**



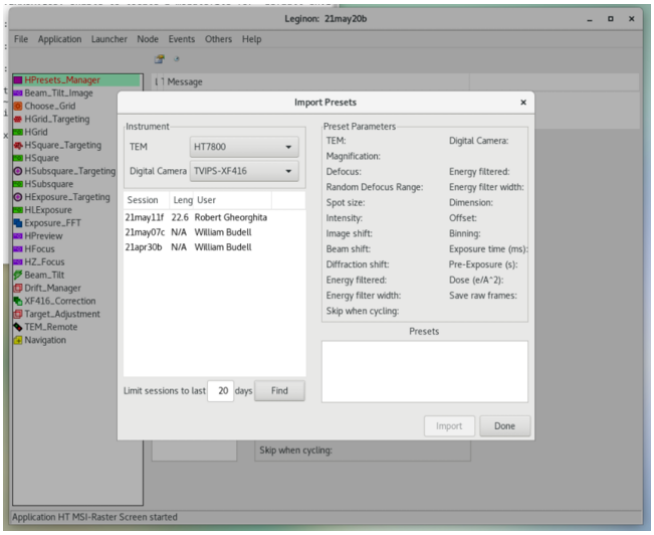
7. Check that the C2 size is set to **100**, then click “**Finish**”.



8. Select “**Application**” then “**Run**”
   - Application = “**HT MSI-Raster Screen**”.
   - If the option is not visible click “**Show All**”, then search the dropdown again.
   - Scope and camera = “**ht78-xf416**”
   - Main = “**ht78leginon”**

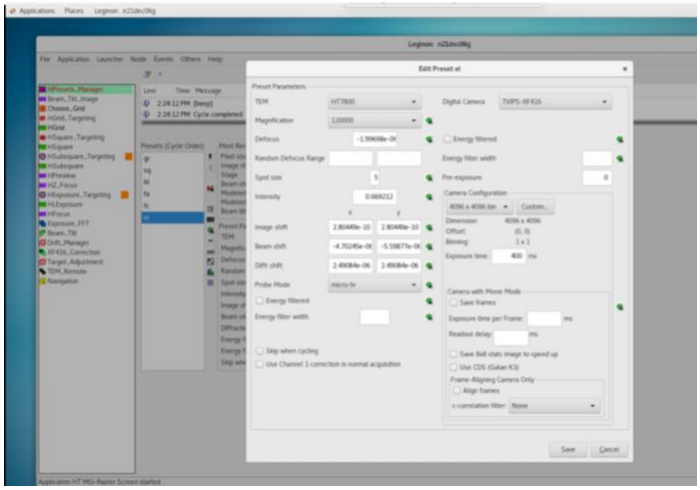


9. In the *HPresets_Manager* Node, click the load preset icon  .
    - TEM = “**HT7800**”
    - Digital Camera = “**TVIPS-XF416**”
    - Click “**Find**”. Select the most recent session. Select all presets (gr, sq, hl, fa, fc, and el), click “**Import**” and then “**Done**”

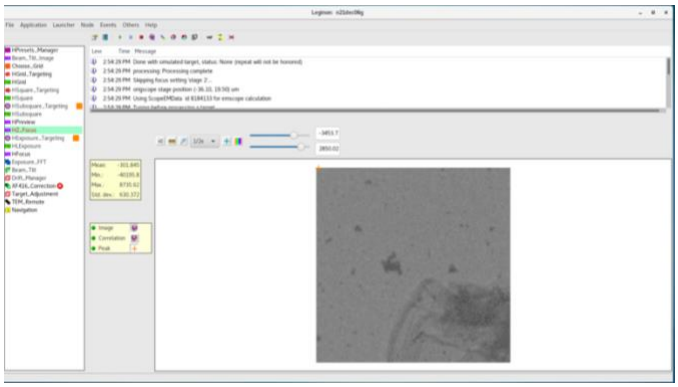


Screening

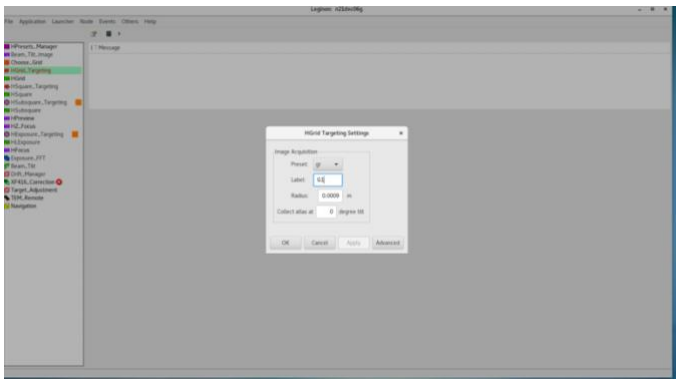
1. Go to the *HPresets_Manager* Node, select the *gr* preset and click  .
    - On the Microscope PC, click Screen **“In”.** On the hand panel use the **Stage Control** track ball to center on a square.
    - Make sure the beam is centered. If it is not centered, use the **Alignment** knobs on the hand panel to center the beam. Then click on  , then next to “**Beam Shift**” click  . Then click “**Save**”.
    - Repeat these steps for the sq, *hl* and *el* preset. For the *el* preset save any changes made to the Beam shift for the *fc* and *fa* preset as well.



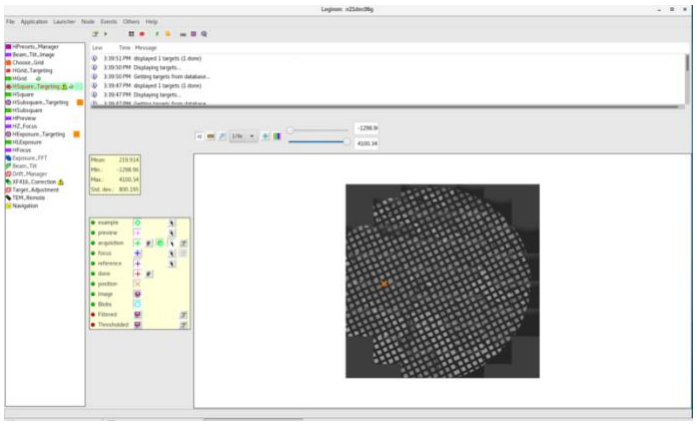
2. Go to the *HZ_Focus* Node, then click the simulate target icon  . Wait for it to complete.



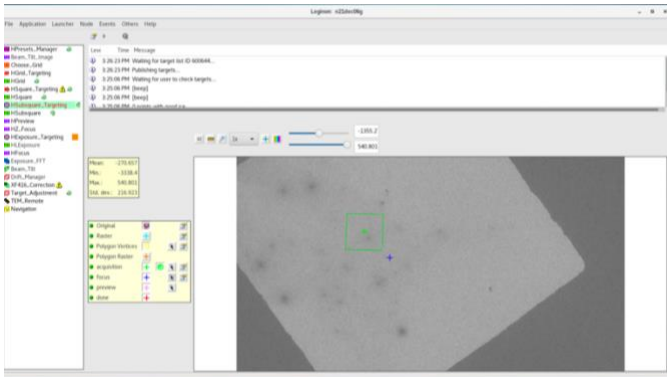
3. Go to the *HGrid_Targeting* Node. Click on the settings icon  .
    - Enter a label for the grid and the radius. Largest atlas is 0.009 m (33 targets). Then click  and then  .



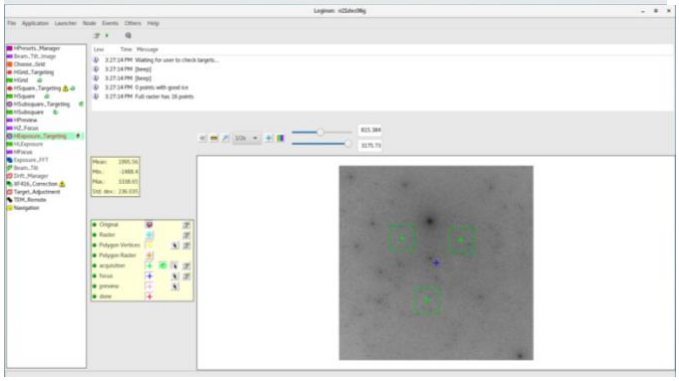
4. Go to the *HSquare_Targeting* Node to view the atlas.
   - select the cursor next to “**acquisition**”. Select square(s) to image.
   - Click



5. In the *HSubsquare_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 . If there is a queue click after selecting targets for each image, when done click .



6. Go to the *HExposure_Targeting* Node
   - select the cursor next to “**acquisition**”. Select a few areas to image. S
   - elect the cursor next to “**focus**”, place one focus target in the center of the acquisition targets.
   - Click to submit. If there is a queue click after selecting targets for each image, when done click .



Microscope Shutdown

1. On the Microscope PC, make sure:
   - In area ① outlined in red, the HV is at "**100.0 kV"** as seen in the window. If it is not, in the "**HV Filament operation"** turn HV "**On**"
   - the magnification is 120 k.
   - the Filament is "**Off"**
   - the Beam is "**Off"**
   - In area ② highlighted red, the stage is centered by clicking "**Holder reset**"
   - In area ③ highlighted red, the screen is **"In"**
2. On the Leginon PC, make sure to log off.