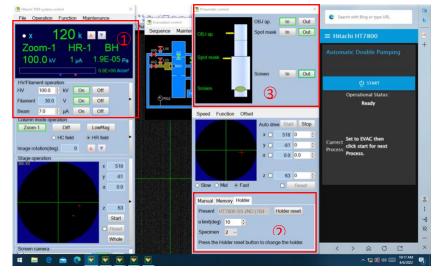
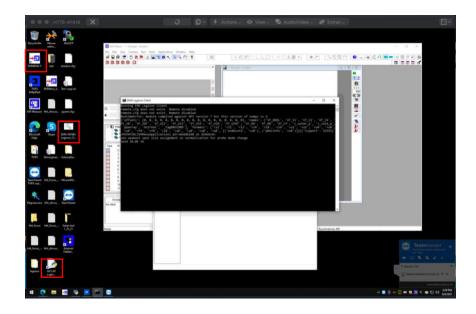
### Microscope Startup

- 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



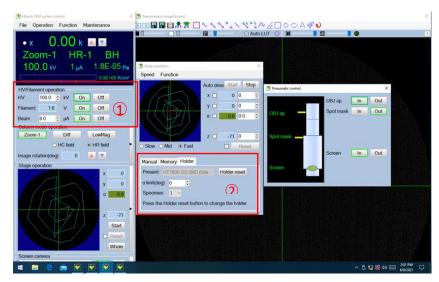
# **Microscope Operation**

### 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".

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	Phaamatic control	Stage operation X     × Speed Function
100.0 kV 9 μA 2.4E-05 ρa 0.0E-00 Acom HVF/Kament operation Fament 31.0 V 0n Off Fament 31.0 V 0n Off Column mode operation 200m-1 Deff LowMag O HC field ■ HR field	OBJ ap. OBJ ap. In Ox OBJ ap. Spot mask In Ox Spot mask Screen In Ox	x 0 0 2 y 1000 0 3 a 00 0 0 3
Shape operation x 0 y 1000 a 000 z 0 State Protect		a limit(dog) 0 2 Specimen 3

- 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 area, 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 >"

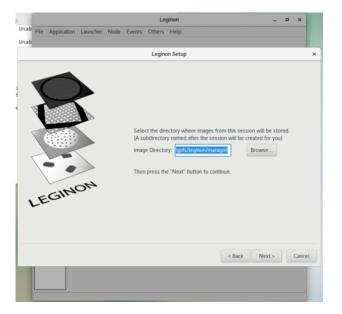


 Please do not change the given session name. Select the holder "HT7800-MS2", then click "Next >"

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4					Choose h	older from	m list or enter new one	HT7800-1	4S2	*	
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- Enter a description of your session (ex: Hitachi– Testing), then click "Next >"
- Leginon \_ = × File Application Launcher Node Events Others Help Leginon Setup Please enter an optional description. Descrip Hitachi - Testing LEGINON Then press the "Next" button to continue < Back Next > Cancel Legino \_ 0 × Unab File Application Launcher Node Events Others Help Leginon Setup Select the project this session will be associated with, Project: SEMC - Testing \* then press the "Next" button to continue LEGINON < Back Next > Cancel
- Select a project, then click "Next >". If there is no project listed, please submit a project here for NCCAT <u>https://nccatdeon.nysbc.org</u> OR here for EMG <u>https://deon.nysbc.org.</u>, then click "Next >"

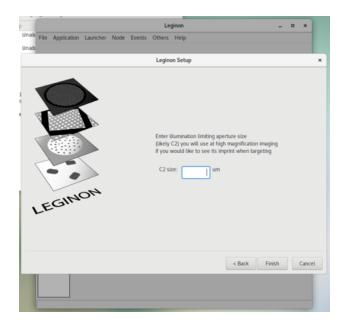
- 5. Check the directory path, then click "Next>":
  - If the user is on NCCAT, make sure the directory is **/beegfs/leginon/username**.
  - If the user is on EMG, make sure the directory is **/gpfs/leginon/username**
  - If the directory is not correct, please let the assisting staff member know.



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

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LE	GIN	014			Clients	× T I I I I I I I I I I I I I	lowing session? 20b Testing C - Testing Lieganon/maragon elected) Edit If these settings are corre	ect.	
							< Back Next >		Cancel

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"

			Leg	inon: 21may20b			-	×
File	Application	Launcher No	ode Events Ot	hers Help				
			R	un Application		×		
			HT MSI-Raster		•	Show All		
			cope and camera: ain:	ht78-xf41/ • ht78leginc •	Run	Cancel		
Adde	d launcher 'ht	78-x1416						

- 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"

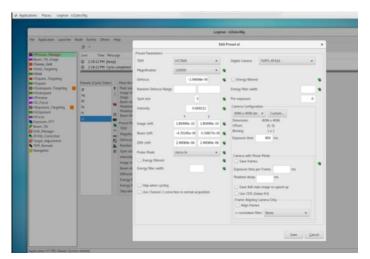
	Legino	on: 21may20b		
File Application Launch	er Node Events Others Help			
	<b>3</b> •			
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Beam_Tilt_Image Choose_Grid	Imp	ort Presets	×	
HGrid_Targeting HGrid HSquare_Targeting HSquare_Targeting HSubsquare_Targeting HExposure_Targeting HExposure_FT Exposure_FT HPreview HFCous	Instrument TEM H17800  Upgral Camera TVPS-XF416  Upgral Camera TVPS-XF416  User  Zimy117 Z26 Robert Cheorghta  Zimy070 R/A KMilliam Budell  Ziapr30b N/A William Budell	Preset Parameters TEM: Magnification: Defocus: Random Defocus Range: Spot size: Intensity: Image shift: Beam shift: Diffraction shift:	Digital Camera: Energy filtered: Energy filter width: Dimension: Offset Binning: Exposure time (ms): Pre-Exposure (h):	
Beam_Tilt Drift_Manager Tilt_Manager Tilt_Manager Tilt_Adjustment Target_Adjustment TEM_Remote TEM_Remote Tem_Semote Tilt_Semote Tilt_Sem		Energy filtered: Energy filter width: Skip when cycling: Preset	Dose (e/A^2): Save raw frames:	
	Limit sessions to last 20 days Find		Import Done	
	Skip when cy	rcling:		
Application HT MSI-Raster	Screen started			

#### Screening

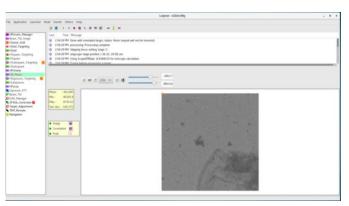
- 1. Go to the *HPresets\_Manager* Node, select the *gr* preset and click **M**.
  - On the Microscope PC, click Screen **"In"**. On the hand panel use the **Stage Control** track ball to center on a square.
  - Make sure the beam is centered. If it is not centered, use the Alignment knobs on the hand panel to center the beam. Then click on

then next to "**Beam Shift**" click <sup>44</sup>. 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.



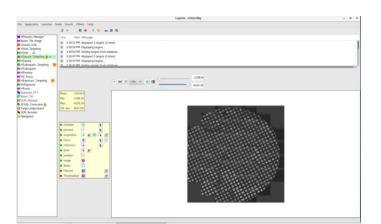
Go to the *HZ\_Focus* Node, then click the simulate target icon
 Wait for it to complete.



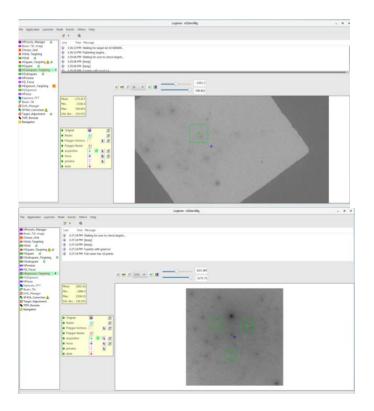
- 3. Go to the *HGrid\_Targeting* Node. Click on the settings icon<sup>27</sup>.
  - Enter a label for the grid and the radius. Largest atlas is 0.009 m (33 targets). Then click and then



- 4. Go to the *HSquare\_Targeting* Node to view the atlas.
  - select the cursor in next to "acquisition". Select square(s) to image.
  - Click 🕨



- 5. In the *HSubsquare\_Targeting* Node,
  - select the cursor in next to "acquisition". Select one area to image.
  - Select the cursor **1** 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 in next to "acquisition".
     Select a few areas to image. S
  - elect the cursor interval 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.

