

HITACHI HT-7700 TEM SOP

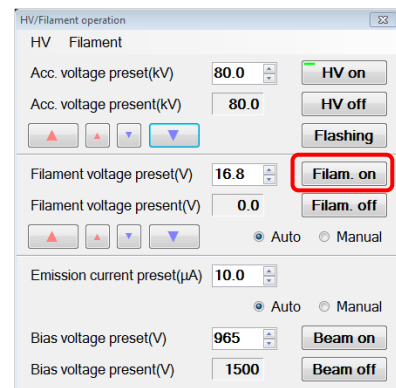
February 2023

The Hitachi HT7700 TEM is located in Room 526 in the MDR Building. Contact the MNTC at mntc@louisville.edu 24 hours in advance to gain access to the room for it is always locked.

NOTE: The voltage must never be changed or adjusted from **80kV** on the TEM for the filament voltage and beam alignment are configured for this setting.

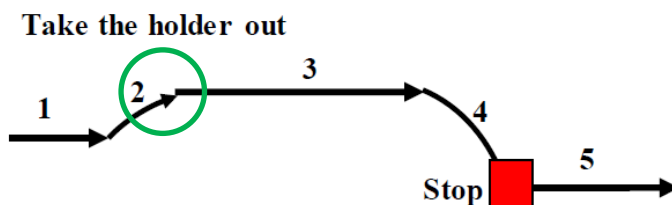
START THE TEM

1. Login into FOM to start an imaging session. If you do not log into FOM the center monitor will not turn on.
2. Turn on the left and right computer monitors.
3. Check the initial conditions. The vacuum level on the vacuum gauge next to the screen is in the 10^{-7} Torr range and High Voltage (HV) is on.
4. Turn on the filament by clicking **Filam. On**. A small green bar will also appear on the **Filam. On** button. It will take 15 to 20 minutes for the filament to power up. The filament is ready when **Filament Voltage Preset** and **Filament Voltage Present** are the same value.



UNLOAD THE SPECIMEN HOLDER

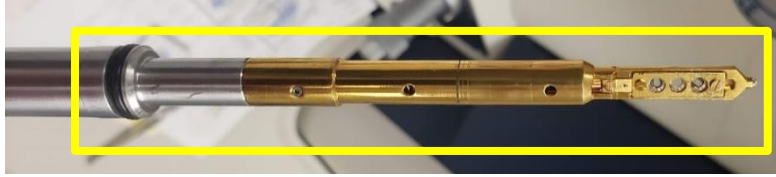
1. When the TEM is not in use, the specimen holder should be in the **STANDBY** position (green circle). To remove the specimen holder, gently pull it straight out until it stops at position 3. Turn the holder **30-degrees counter-clockwise** to reach the **STOP** position. *Scan the QR code to see the video.*



2. Set the **EVAC-AIR** switch to **AIR** and **WAIT** approximately 10 seconds until you hear 2 clicks. **It is extremely important to not pull out the specimen holder until you hear 2 clicks.**



3. **CAREFULLY** extract the specimen holder from the EM column. **DO NOT touch the specimen holder in the designated box below or past the O-ring.**



4. Make sure a white cloth is under the specimen holder to capture lost grids if they come out of the specimen folder.

5. With tweezers/forceps **GENTLY** open the tip of the specimen holder. The lid is spring loaded and

6.

7. Using tweezers, select a grid **by the edge** and place it into the specimen holder. Make sure the grid sits flat and the tissue is facing upwards. **CAREFULLY** close the lid using tweezers.

NOTE: Grid #1 is at the tip, #2 is in the middle, #3 is closest to the handle of the specimen holder.

LOAD THE SPECIMEN HOLDER FOR IMAGING

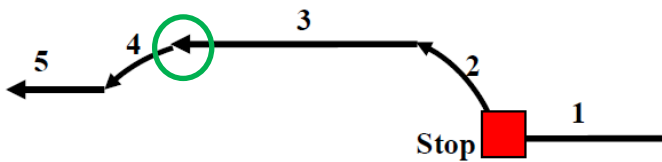
1. **CAREFULLY** align the pin and guide the specimen holder into the TEM until it stops.



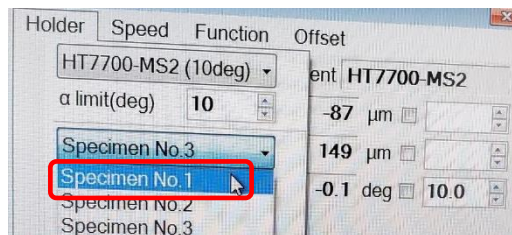
2. Switch the **EVAC-AIR** toggle to the **EVAC** and **wait** until the green light is illuminated along with a continuous beep.

3. Turn the specimen holder **30-degrees clockwise**. The holder will pull itself into the standby position (green circle) due to vacuum. *Scan the QR code to see the video.*

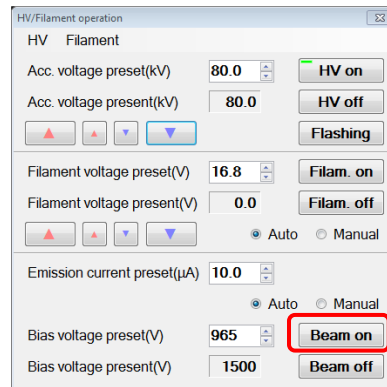
Put the holder in



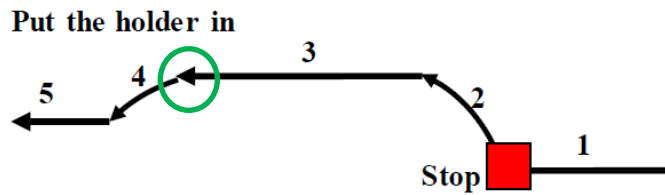
4. From **Stage Operation** window on the left monitor select the **Holder** tab. Specify the grid you wish to image by selecting **Specimen No. 1, 2 or 3.**



- Click **Beam On** and confirm a green bar is showing on the button.

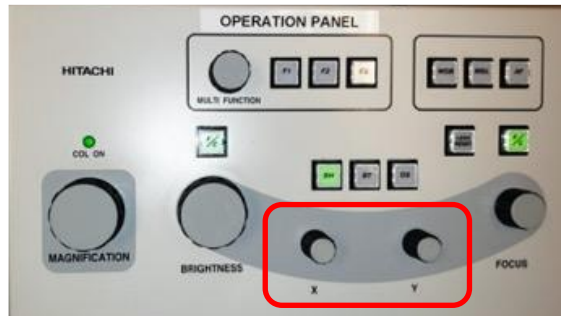


- Turn the specimen holder **15-degrees counter-clockwise** from the standby position (green circle). Vacuum will pull the holder into the center of the column and will stop at the end of **Step 5**.



IMAGING THE SAMPLE

- Increase magnification to 20k and brightness, so you can slightly see a spot. Adjust the X & Y knobs on the desktop controls to center the spot on the monitor.



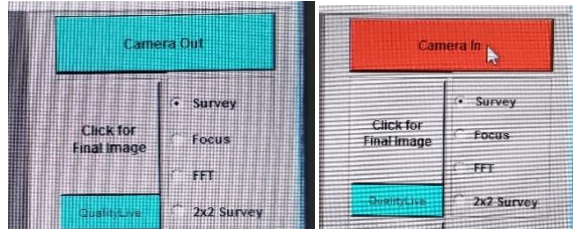
- Reduce brightness and magnification to 1 k.
- Make sure the **F/C button** is green (**green = coarse focus, no green = fine focus**).



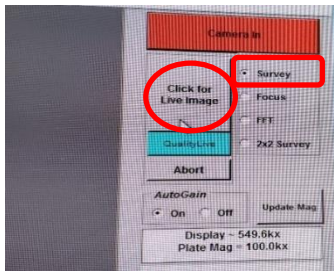
4. Turn on the high-resolution camera by clicking the **AMT700** icon at the bottom of the center monitor.



5. Click on the blue **Camera Out** button and it will turn red.



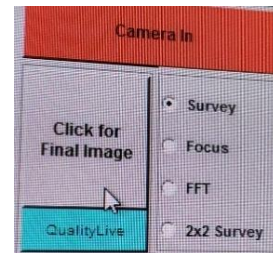
6. Click for **Live Image** followed by **Survey** to see your sample.



7. Use the trackball to move to different sections of the grid while adjusting the magnification and brightness.

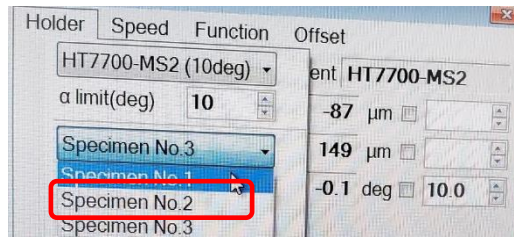
NOTE: Be careful to not get a **LOW SIGNAL** warning when lowering the brightness level. If you see an **OVERSATURATED** warning for the camera, **IMMEDIATELY REDUCE BRIGHTNESS**. Failure to do so can damage the scintillator.

8. To take a picture click on survey and **Click for Final Image** on the center monitor. Click the **Save with Caption** option for annotative data to be embedded within the picture and change the annotative data per your preferences.



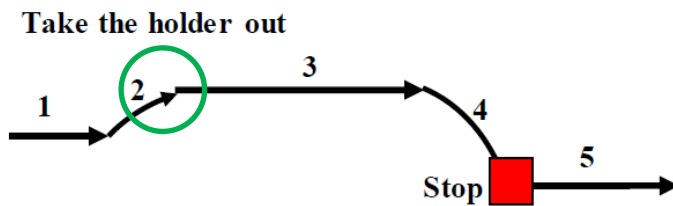
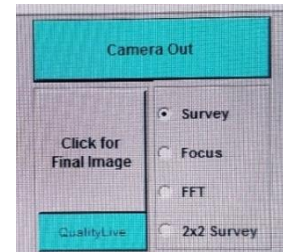
9. To resume imaging, select **Show Live Image**.

10. To image another sample, select the preferred specimen number on the left monitor and repeat the previous steps for locating items of interest, focus and taking images.



CHANGING SAMPLES AND REMOVING THE SPECIMEN HOLDER

1. Turn off the High Resolution Camera by clicking the red **Camera In** button. It will turn blue and change to **Camera Out**.
2. Click **Beam Off** and confirm a green bar is not showing on the button.
3. Pull the specimen holder to the end of 1 and rotate clockwise 15 degrees to the standby position (green circle).
4. Pull the specimen holder to the end of 3.
5. Rotate the specimen holder 30° counter-clockwise to the STOP position.
6. WAIT until you hear the beep and the green light comes on.
7. Toggle the EVAC-AIR switch to AIR.
8. Wait 10 seconds or until you hear 2 clicks.
9. Carefully pull out the specimen holder. Scan the QR code to



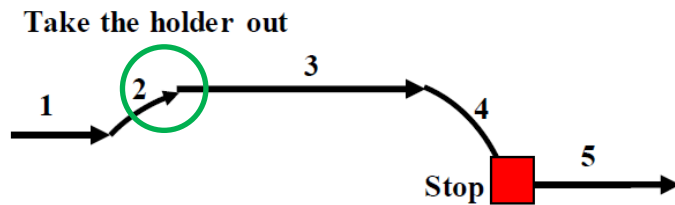
10. Exchange the samples and reload the specimen holder into the TEM column.
11. Click **Beam On** and confirm the green bar is showing on the button.
12. Turn on the High Resolution Camera by clicking the blue **Camera Out** button. It will turn red and change to **Camera In**.

WHEN YOU ARE FINISHED IMAGING

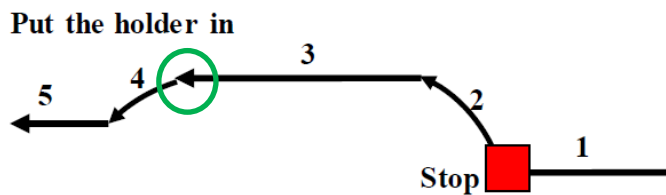
1. When finished imaging, turn off the High-Resolution Camera by clicking the red **Camera In** button. It will turn blue and change to **Camera Out**.
2. If you need to transfer photos or other documents from the TEM, insert a flash drive into the computer on the right side of the desk. **DO NOT** use the computer that is in the desk below the **Emergency Stop** button.
3. Close the window of the **AMT 700** application on the center monitor.
4. On the left monitor, click **Beam Off** and confirm the green bar is not showing on the button.
5. Turn off the filament by selecting **Filam Off**. Confirm the **Present Voltage** is 0.0 and the green bar is no longer seen on the button.

NOTE: DO NOT TURN OFF THE HV VOLTAGE.

6. Unload the specimen holder and remove your grids.



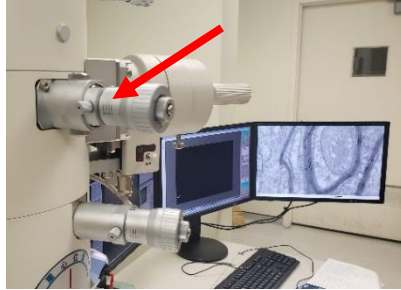
7. Place the specimen holder into the TEM column in the **standby position** (green circle). **DO NOT leave the Specimen Holder out of the TEM. Scan the QR code to see the video.**



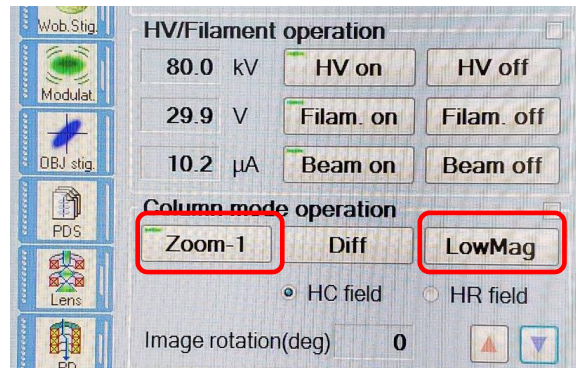
8. Adjust magnification to 200k.
9. Log out of your imaging session in FOM.
10. Turn off the left and right monitors.
11. Turn off the lights in the room and make sure the door is tightly closed when you leave.

TIPS IF YOU CAN NOT SEE AN IMAGE

1. Zoom out to 1k.
2. Adjust your brightness.
3. Make sure the beam is on.
4. Change grids.
5. Move the trackball.
6. Check the Aperture is set to **3**.



7. Use **Zoom-1** (Monitor #1) to see if you can find your tissue samples using 1.0K magnification. If you cannot find it, you may want to use the **Low Mag** option on the left monitor and reduce magnification (knob on desktop controller) to 0.5K.



8. If you cannot see anything you will need to remove the objective lens aperture from the column by **GENTLY** turning the aperture setting counter-clockwise from setting **3** to **0**.



HELPFUL HINTS:

- If the center screen does not display anything, click the “Auto LUT” button at the top of the center cross hair image.
- If you wish to see large cross hairs instead of small cross-hairs, click the Multi-View Button.
- Another focusing aid is to select **FFT** below survey to sharpen your image. After selecting **FFT** another screen will show a halo image. Turn the **Focus** knob on the desktop until the halo image is at its largest, diffuse and circular. Once satisfied click **Survey** on Monitor

