

HITACHI HT-7700 TEM SOP

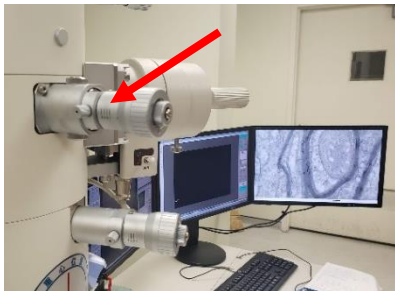
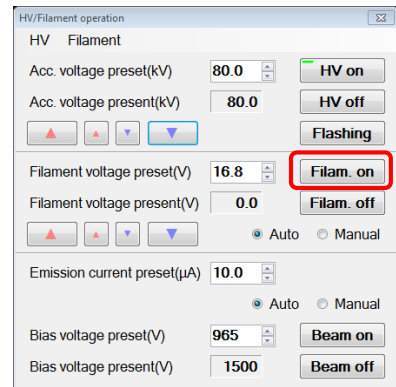
OCTOBER 2021

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.
2. Turn on the remaining two computer Monitors. (These will be referred to as Monitor #1, #2, #3 going from left to right.)
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**. It will take 15 to 20 minutes for the filament to power up. The filament is ready when **Filament Voltage Present** is the same value as **Filament Voltage Preset**. A small green bar will also appear on the **Filam. On** button.
5. Check the Aperture and make sure it is set to **3**.



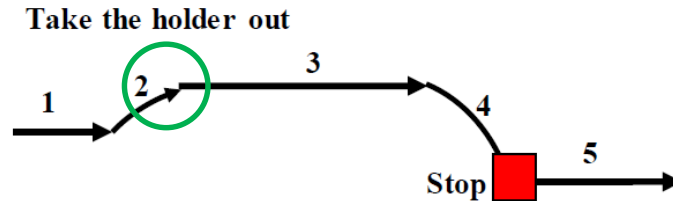
6. Determine which specimen holder to utilize.

HT7700-SS – Single slot specimen holder, **HT7700-MS2** – Three slot specimen holder



UNLOAD THE SPECIMEN HOLDER

1. While the TEM is not in use the specimen holder should be in position 2 or the **STANDBY** position, as noted by the green circle. To remove the specimen holder, gently pull it straight out until it stops or position 3. Then turn the holder **30-degrees counter-clockwise** to reach the **STOP** position.



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 anywhere in the designated box below or past the O-ring.**



4. Make sure a white cloth is under the specimen holder where the grids are placed in the holder. This will help capture lost grids if they come out of the specimen folder.

5. With tweezers/forceps **GENTLY** open the lid on the tip of the specimen holder while holding the handle. The lid is spring loaded and will try to snap backwards. Guide it carefully open to prevent it from snapping backwards.

6. Using tweezers, carefully select a grid **by the edge** from the grid box and place into the specimen holder's grid housing. Make sure the grid sits flat and the tissue is facing upwards. You can place up to 3 grids into this particular specimen holder. **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.

DO NOT force the specimen holder at ANY point in this procedure. Doing so will cause costly damage to the specimen holder, the TEM or both.

LOAD THE SPECIMEN HOLDER

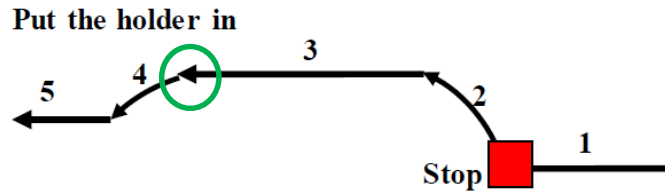
1. **CAREFULLY** guide the specimen holder into the exchange chamber and aligning the position of the pin. Gently push the holder into the chamber until it stops. Make sure that the specimen holder is fully inserted until you see the red light turn on.



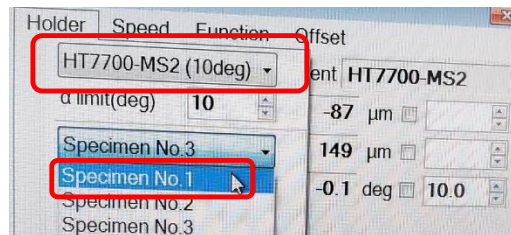
2. Flip the **EVAC-AIR** switch to the **EVAC** position to activate vacuum in the exchange chamber. Once the vacuum level reaches the appropriate level for insertion, the green light will switch ON and a continuous beep will sound.



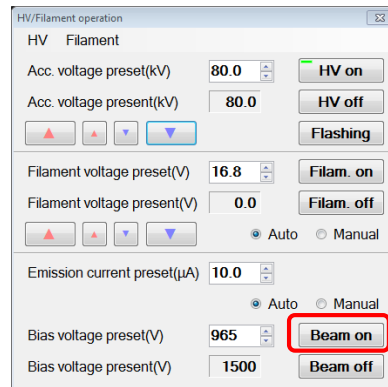
3. Once the green light is on turn the specimen holder **30-degrees clockwise**. The holder will insert itself up to the standby point due to the vacuum. Once it stops, the holder is now in the **STANDBY** position as indicated by the **green circle**. Below is a visual summary at the end of **Step 3** at the **STANDBY** position.



4. From the center Monitor, on the **Stage operation** window select the **Holder** tab and specify the specimen holder that was loaded into the scope and the **Grid location**.



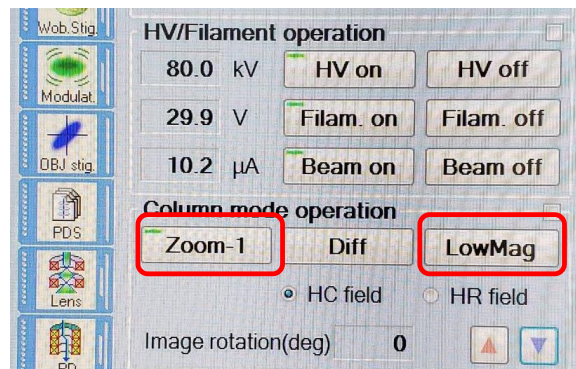
5. Turn on the **Beam On** and confirm a green bar is showing on the button (Monitor #1).



6. To place the grid samples into the beam path turn the specimen holder **15-degrees counter-clockwise** (end of **Step 4**). The scope's vacuum will pull the holder into the center of the column and will stop (**Step 5**). Do not push the holder towards the microscope.

IMAGING THE SAMPLE

1. 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 Monitor #1 and reduce magnification (knob on desktop controller) to 0.5K.



2. 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**.

3. Pick out a zone that you want to view via the track ball control on the desktop.

4. Increase magnification to 1.0K using the magnification knob on the desktop controller.

DO NOT EXCEED 1.0K or you will damage your sample.

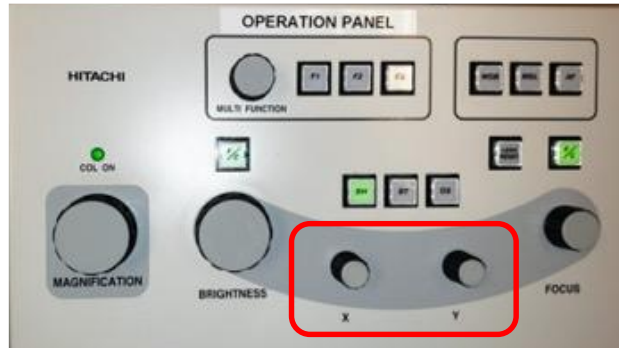
5. Click on **Zoom-1** on Monitor #1.

6. Gently place the aperture back **3** by turning it clockwise.

7. If illumination of the image does not look centered, then adjust in the aperture in the X and Y directions using the aperture control on the column.



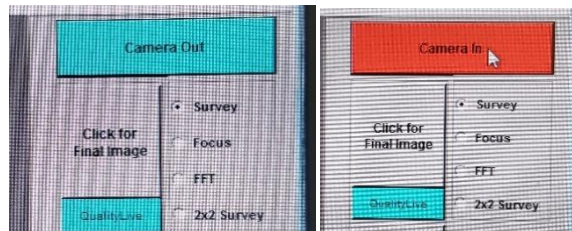
8. Increase the magnification. The light halo will be off center and will likely appear out of focus. This can be remedied by adjusting X & Y knobs on the desktop controls.



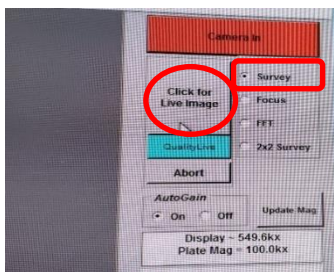
9. Once you have achieved the desired magnification turn on the high resolution camera by clicking the **AMT700** icon at the bottom of Monitor #2.



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



11. Click for **Live Image** followed by **Survey** on Monitor#2 to see your sample.

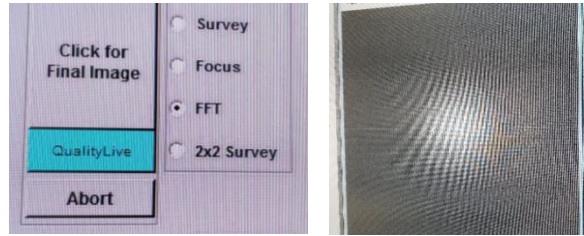


12. Use the **Brightness** knob on the desktop controller as needed. The F/C button will switch between fine and coarse brightness adjustments (**green = coarse focus**).



NOTE: Be careful that you don't get a **LOW SIGNAL** warning from Monitor #2 for you can only go so far with the brightness level. If you see an **OVERSATURATED** warning for the camera, **IMMEDIATELY REDUCE BRIGHTNESS**. Failure to do so could damage the camera.

13. 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 #2.



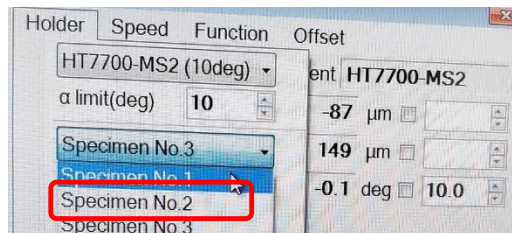
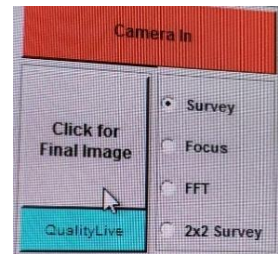
HELPFUL HINTS:

- 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.

14. To take a picture click on survey and **Click for Final Image** on Monitor #2. Click the **Save with Caption** option for annotative data embedded within the picture.

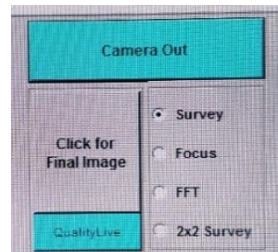
15. Click **Show Live Image** on Monitor #2 to resume imaging and to take additional images. Repeat as many times as you wish.

16. To image another sample, select the preferred specimen number on Monitor #1 and repeat the previous steps for locating items of interest, focus and taking images.



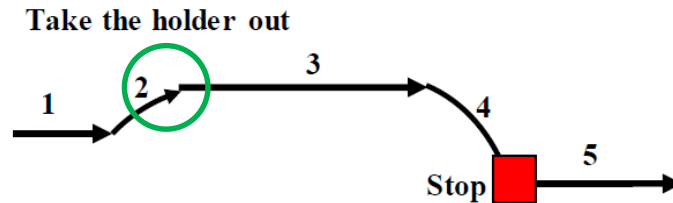
NOTE: When you want to switch out samples in the specimen holder, but are not done with your imaging session perform the following steps.

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. Proceed with the unload procedure of the specimen holder, exchange the samples and reload the specimen holder into the EM column.
4. Click **Beam On** and confirm a green bar is showing on the button.
5. Proceed with the imaging procedure described above.

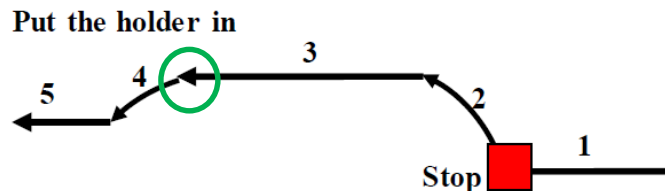


WHEN YOU ARE DONE

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 EM, 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 Monitor #2.
4. In Monitor #1, 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.
6. Unload the specimen holder and remove your grids from the holder.



7. Place the specimen holder back into the EM column in the standby position (green circle). **DO NOT leave the Specimen Holder on the desktop.**



8. Click **Zoom-1** button on Monitor #1 and adjust magnification to 200K to help preserve the coils of the EM.
9. Log out of imaging session in FOM.
10. Turn off the remaining two Monitors. (#2, #3)
11. If you are the last TEM user of the day, turn off the lights in the room and make sure the door is closed when you leave.

THE FOLLOWING STEPS WILL BE PERFORMED BY DESIGNATED PERSONNEL

REMOVAL AND CLEANING OF A SPECIMEN HOLDER IN THE PLASMA CLEANER

1. The specimen holders need to be cleaned periodically via the Plasma Cleaning device located on the bench top behind the TEM.
2. Before extraction press the **STOP** button to remove vacuum on the specimen holder.
3. You will see a timer on the electronic screen above the buttons count down from 60 seconds.
4. When the timer has achieved 0 **CAREFULLY** extract the specimen holder from the cleaner.

BE VERY CAREFUL FOR THE PLASMA CLEANER IS LINED WITH GLASS FIXTURES.

5. If you wish to clean a specimen holder, **CAREFULLY** remove the specimen holder with the holder's notch is approximately at the 1 o'clock position.
6. Press the **START** button.
7. A blue clean light will illuminate for 1 minute.
8. When finished the plasma cleaner will ask you to rotate the specimen holder counter-clockwise to the 7 o'clock position to clean BOTH sides of the specimen holder.
9. The blue clean light will illuminate again for 1 minute. When finished the specimen holder will be clean.