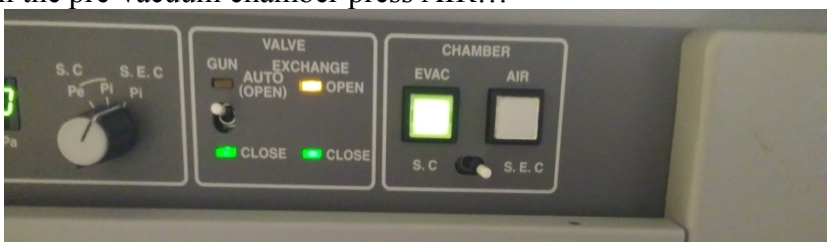
Hitachi S-4700 SEM Operation

1. Flash scope about 1 hour before use, repeat after 6 hours.
    a. Click on kV in top right – click flashing – execute.

2. Loading the Sample
    a. Put the sample onto one of the 2 holder bases provided.
    b. Double check the height with the measuring tool.
        i. Sample on holder must NOT be taller than the measuring tool.
        ii. If samples on multi-holder are too tall you must load them one at a time.
    c. To open the pre-vacuum chamber press AIR…

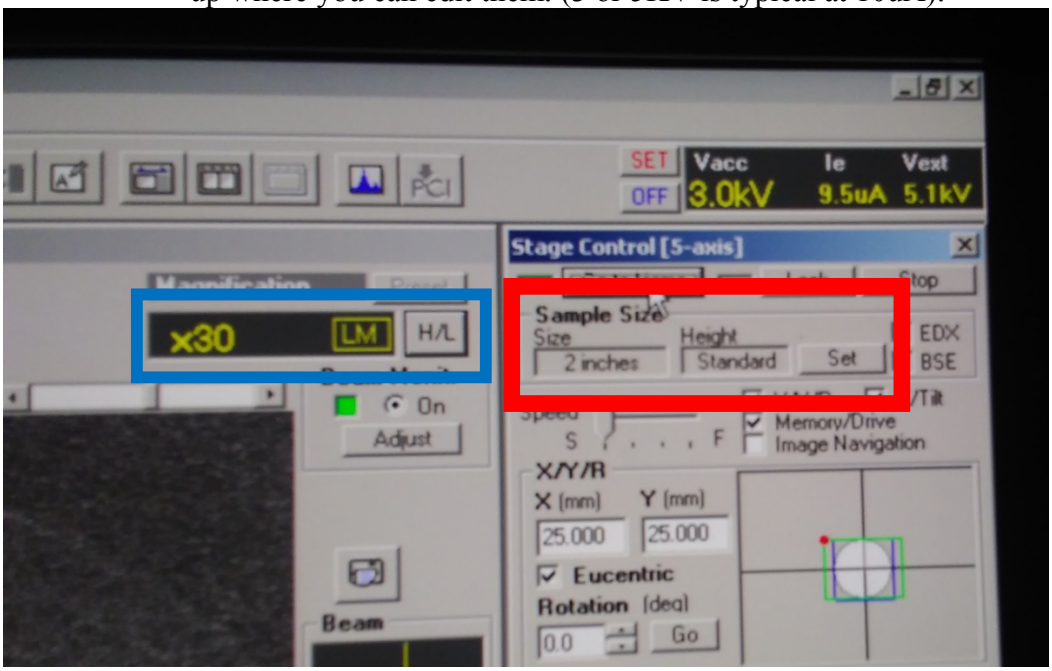


        i. Wait a few seconds and the chamber will release the vacuum.
        ii. Fully open the chamber (90º)
            1. Make sure to pull on the chamber itself not the rod.
        iii. With the door open, push the rod slightly forward so you can see it.
            1. Screw the sample holder base onto the rod till you can't turn the rod anymore.
            2. Pull the rod back as far is it will go.
            3. Close the chamber door (again don't pull on the rod to close).
    d. With one hand apply pressure to the door, with the other hit EVAC.
        i. Wait about 30 seconds.
        ii. Once you hear a clicking sound, and the yellow light is flashing by open, you can open the inside door to the vacuum chamber.
    e. Open the door by pushing the lever up (smiley face is open).
        i. You can see the door open on the internal camera.

    f. Then push the rod all the way into the chamber until the white plastic piece is pushed up against the outside of the pre-vacuum chamber.
        i. Unscrew the rod.
            1. As you do this you will see the white plastic piece move backwards.
            2. Unscrew until the white plastic piece stops moving backwards, then keep going for a few seconds.
    g. Carefully watch the internal camera and slowly pull the rod out.
        i. Once you are sure the rod is no longer connected to the sample, you can pull it out faster.
    h. Finally close the door (lever down, upside down face).
        i. Now your sample is in and you are ready to image.

Operating the SEM

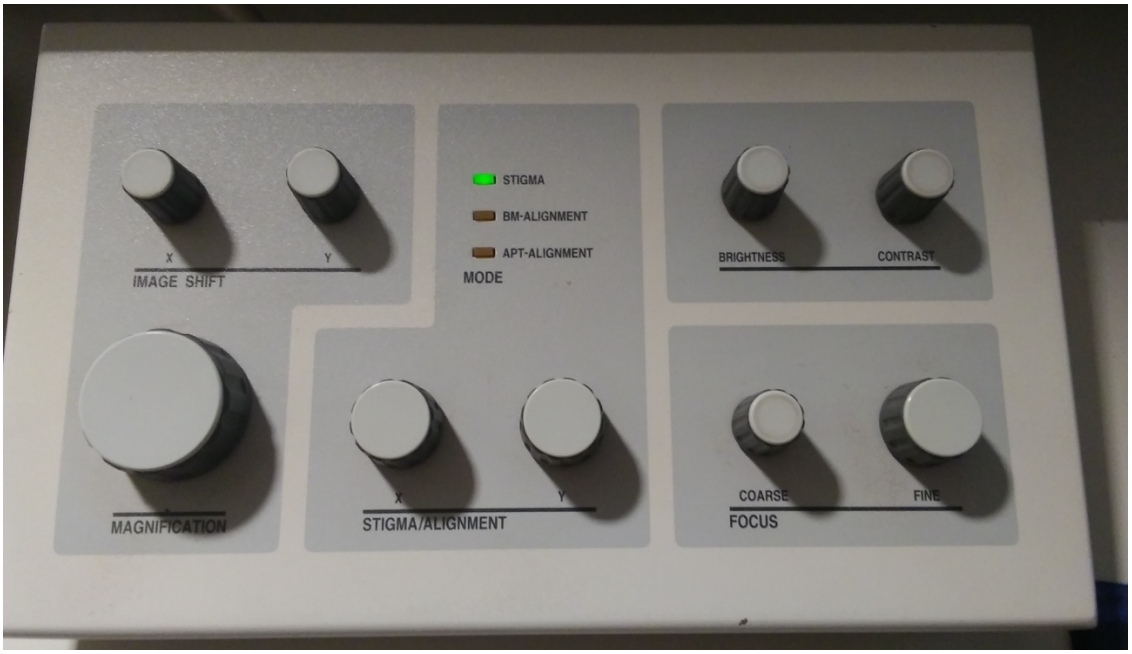
3. Things to check before you turn the beam on.
    a. Go to Setup – Image – make sure the imaging settings are what you want.
    b. Go to Setup – Column – make sure you're in Normal mode (NOT Analysis).
    c. Check your sample size (red box).
        i. Height should always be standard (unless you have a really tall sample).
        ii. Size = 2 inches for multi-sample holder, 15mm for individual sample.
    d. Make sure the beam is at the kV and uA you want.
        i. If you want to change it, click on the kV number and a window will pop up where you can edit them. (3 or 5KV is typical at 10uA).



4. Turn the beam on – press the ON button in the top right of your screen.

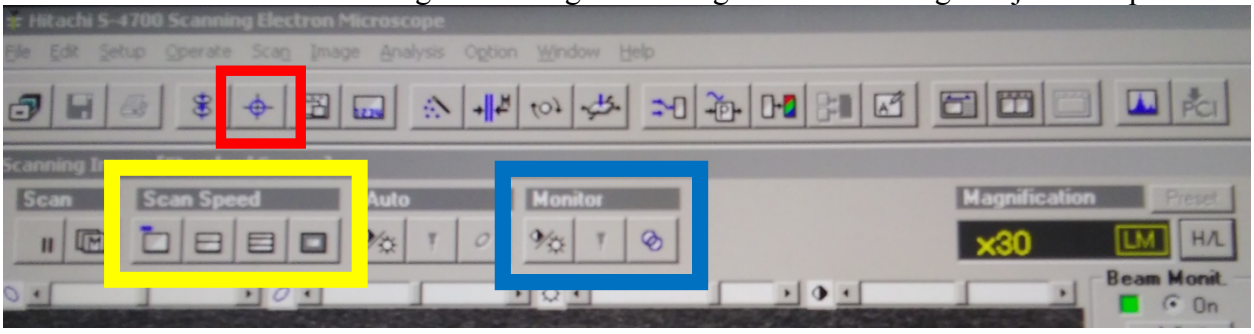
5. Magnification
    a. You usually want to start in Low Mag mode.
    b. Magnification is shown in the blue box.
        i. To change from low to high mag press the H/L button.
        ii. If you are in low mag it will say LM to the right of the magnification.
            1. Low Mag goes from 30x to 2,000x
            2. High mag goes from 250x up to about 300,000x

6. Control Panel



   a. Do Not touch the image shift knobs.
   b. There is a track ball (not pictured) that allows you to move the sample around.
   c. The large knob on the bottom left is for magnification.
   d. The X & Y knobs are for stigmation and alignment.
      i. If you haven't clicked on the alignment menu they default to stigmation.
   e. There are coarse and fine focus knobs (bottom right).
   f. There are brightness and contrast Knobs (top right).

7. Adjustments (do at 5,000x to start)
   a. Once you have located the area you want to image, you will want to check the adjustments (Don't do adjustments on area of interest, could burn it out).
   b. All adjustments are done with the X & Y knobs on your control panel.
   c. To do this click the target icon highlighted in red.
      i. For beam alignment – align the image in the center of the target.
   d. For the X & Y adjustments – if the image is shaking move the corresponding knob until the image is still.
   e. Aperture is the most important alignment (can only be done in high mag mode).
      i. You want the image to be bouncing in and out, not bouncing sideways.
      ii. If the image is moving left and right while focusing – adjust the aperture.

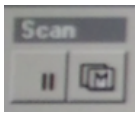


8. Scan Speeds (Yellow Box) 
    a. The one on the left (blank square) is the fast scan speed.
        i. Use this one when moving around and focusing.
    b. The next one over (box with 2 bars) is the medium scan speed.
        i. You can still move around with this one but there is a lag.
    c. The next one (box with multiple lines) is the slow scan speed.
        i. Think of this like print preview – it shows you what your picture will actually look like.
        ii. You only use this mode once you have done your adjustments and focused.
        iii. This is the mode you take pictures in.
    d. The one on the right (box within a box) is the narrow field scan mode.
        i. It shows you a smaller portion of the image.
        ii. This is good for focusing.

9. Brightness/Contrast (B/C)
    a.  To adjust the B/C hit this icon under Monitor (Blue Box).
    b. A screen will appear with a fast-moving wave pattern.
    c. You want to adjust the waves so they're within the lines.
        i. You do this with the brightness contrast knobs
            1. Generally, the contrast knob condenses or expands the wave.
            2. And the brightness knob moves the wave up and down.
    d. You can hit auto contrast (to the right of the blue box in the picture).
        i. But it is usually fairly off.

10. Focusing
    a.  When focusing it is best to use the reduced area scan mode.
    b. You also want to focus at twice the magnification you are trying to image at.
    c. Use the coarse and fine focus knobs to focus.
    d. You should also check the astigmatism before taking a picture.
        i. Do this with the X & Y knobs while in reduced area mode.
        ii. Sometimes this will help the image be more in focus.

11. Taking a Picture
    a. Before taking a picture make sure
        i. You are at your desired magnification.
        ii. You have gone through the adjustments (unless you are at a similar magnification as the last picture, then you don't have to adjust every time).
        iii. Your brightness/contrast looks good.
        iv. Your image is in focus.
    b.  Now go to the slow scan speed.

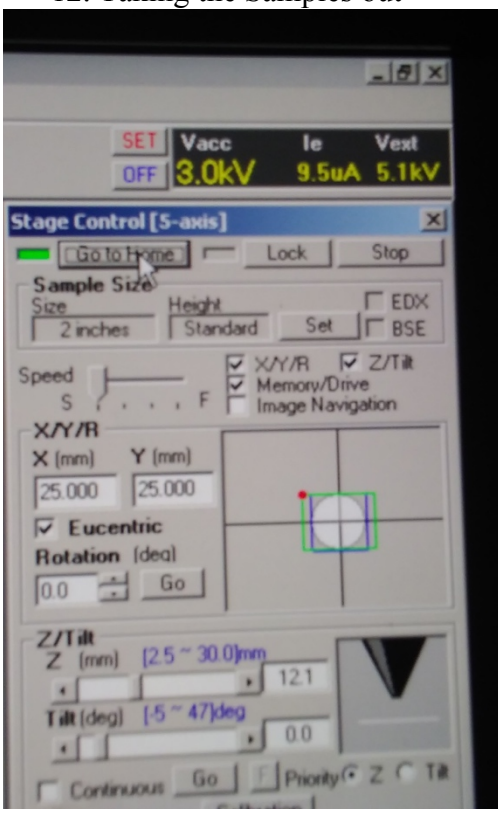
c. 

Then under Scan hit the box on the right with the M (this is Capture).
d. It takes a minute and a half to take a picture.
e. Once the picture has been taken an image window should pop up.
f. Click the floppy disc icon to save your picture.

    i. Make a folder if you haven't.
    ii. Name your picture.
    iii. Hit Save. – It also takes a few seconds to save.
g. If you want to do this in batches you can hit "all save" to save all the images in the window at once.
h. Your image will be saved with all the information on the bottom of the screen
    i. This includes accelerating voltage (5kV), working distance (11.5mm), magnification (x2.5k), the date and time, and scale bar.
    ii. The number under the scale bar is for the whole thing, not each section of the dashed line.

i. Once you have saved your image click the play button next to the capture button.
    i. Then go back to fast scan mode so you can move around.

12. Taking the Samples out



a. First under the Stage Control Menu hit – Go to Home. This will re-center your sample.
b. If you changed the WD reset it to 12mm both in the Column Control and Stage Control Z-knob.
c. Then turn the beam off by clicking OFF.
d. Now open the internal door with the lever and insert the sample rod until it hits the sample holder.
e. Tighten the rod until the white plastic piece hits the wall of the door and you can't turn it anymore.
f. Pull the sample all the way out.
g. Close the internal door.
h. Hit AIR. (make sure door is closed before you hit air or you will evacuate the whole chamber!)
i. Open the pre-vacuum chamber.
j. Push the rod forward so you can see the sample.
k. Unscrew the rod from the sample.
l. Take the sample off the base.
m. Leave the base in the container by the computer.
n. Pull the rod all the way back and close the door.
o. Apply a little pressure and press EVAC.

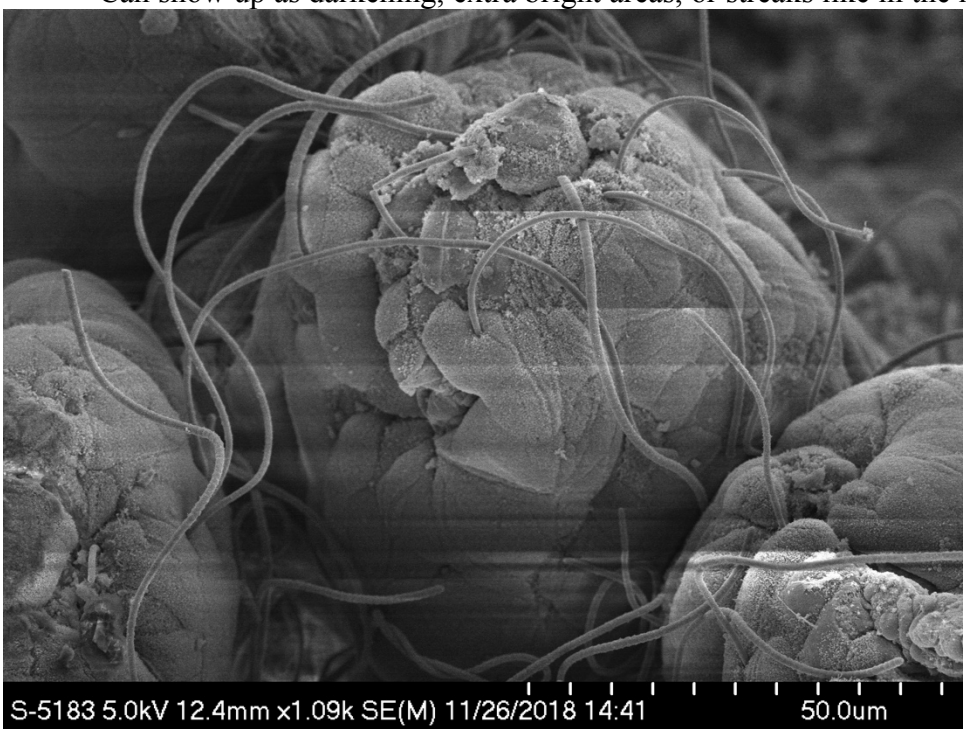
With the microscope beam OFF, the door closed, and the scope under vacuum, you are done. Leave the SEM software up and the computers on.

13. Retrieving your pictures off the computer:
    a. If you have a USB or hard drive you can take your pictures.
        i. If not let the facility staff know and we can email them to you.
    b. To do this use the computer on the right (not the one with the imaging software).
        i. This computer is hooked up to wifi and has virus protection software.
    c. Click on the Users folder on the desktop.
        i. Your folder should be in this.
        ii. Drag your images to your drive, then eject and remove.

Problem Solving:

Charging:

- A buildup of negative charges due to non-continuous grounding (non-conducting areas), this can cause deflection of the beam or discharge.
- Causes higher negative surface potential making it hard for SE's to escape, but BSE's are less effected.
- Can show up as darkening, extra bright areas, or streaks like in the image below.



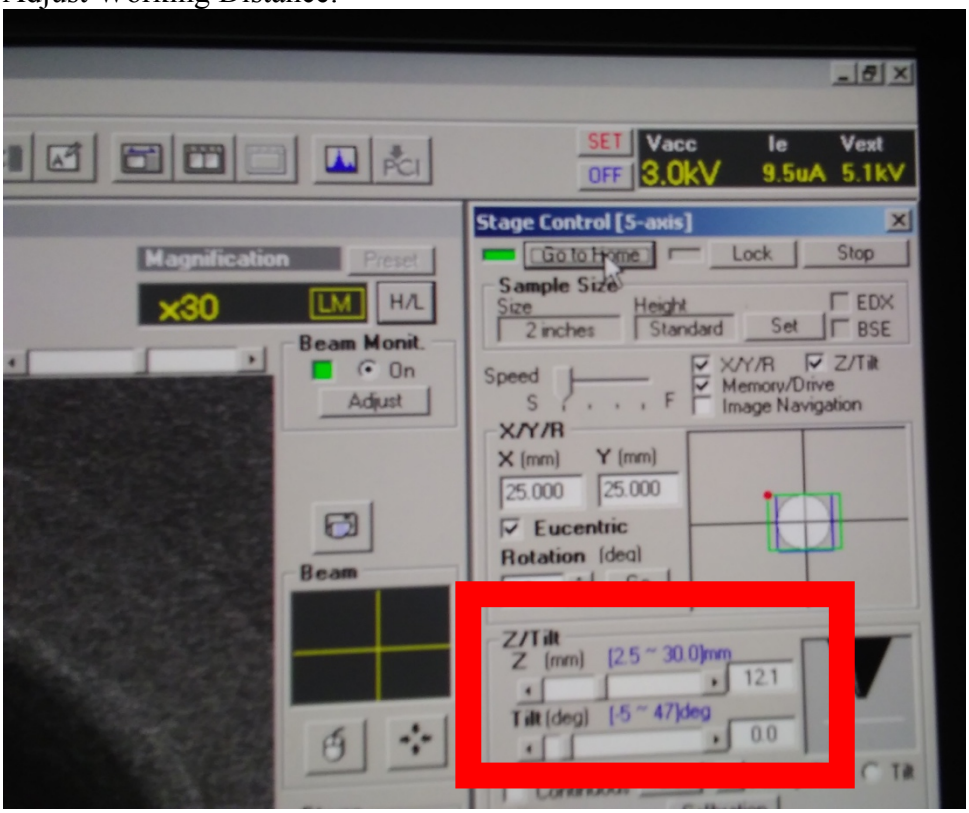
How to Fix Charging:

1. First – smaller spot size **(check if we can change this)**
2. Second – lower the accelerating voltage (kV)
3. Third – recoat & check grounding
4. You can also try…
    - Frame averaging – Image with the fast scan speed.
    - Imaging with just the lower detector – instead of mixed (can only change in high mag mode).
        - **Go to column - select or signal select and click lower detector.**
5. Reduce the probe current (uA)

If image still seems out of focus…

1. Try a different accelerating voltage kV.
2. Try adjusting stigmation while focusing (not just in adjustment window).
3. Make sure you are not in a lower, or shadowed area.
4. Adjust Working Distance.
    a. If you have a taller sample and want it all in focus you need a longer working distance
    b. If your sample is fairly flat use a shorter working distance.

Adjust Working Distance:



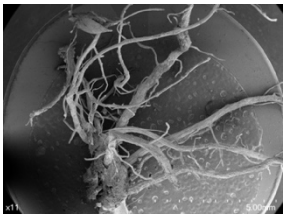
Adjust Working Distance:

1. Use the Z/Tilt window in the Stage control window.
2. Type the working distance you want under Z (where it says 12.1 in the picture.
    a. Hit enter, and watch the internal camera.
    b. Hover mouse over <u>stop</u> in case your sample gets too close to the pole piece.
    c. Adjust focus so WD at the bottom of the screen matches what you entered.
3. 8mm is recommended, 6mm is the lowest you can go. **– check this**

If You want a long Working Distance: **(check this on our scope)**

1. Long Working Distance
    - Focus on the top, then focus on the bottom of your sample.
    - DOF is above and below – focus on the middle
    - Drop working distance – lowers the magnification.

    Picture of a whole root taken at a long WD →



Adjustments for Composition:

1. Raster Rotation – rotates image (detector)
2. Tilt compensation
3. Orient top of sample toward detector (like sun)
4. **Dynamic Focus - set tilt, focus structure, tilt compensation on – double check**

Parameters for High Magnification & High Resolution:

- Shorter working distance < 5mm **(was told scope can't go below 6mm?)**
- Small spot size or low probe current.
- Small final aperture (#3 or #4).
- Accelerating voltage depends on the microscope and sample
    - 3kV or 5kV usually works best

Noises:

1. If you hear a beeping noise coming from the scope this means the nitrogen tank needs to be changed.
    a. Please contact the facility if you change the tank yourself.
    b. If you don't know how to change the tank, get someone in the facility to help you.
2. If you hear a sustained high-pitched tone coming from below the computer…
    a. Shutdown the computer and turn off Display Power.
        i. The Display Power switch is located in a panel right under the monitor table to the left.
        ii. Open the door and you will see these 2 switches below and the computer tower.
    b. Then turn Display Power back on and restart the computer.

If S.C vacuum is not happening after restart.

1. Turn obj apt heater to OFF
2. Under 'chamber' switch from S.E.C to S.C
3. Turn lever on sample chamber from C (closed) to O (open) position (but do not vent!)
4. Both s.c and s.e.c go to 'air'
5. After a few minutes Magic happens !
6. Both sc and sec will go to HIGH in a few min
7. Do not use scope for about 1 hr since Obj apt was off and needs to re-heat!