

INSTRUCTIONS FOR ZEISS EVO 50VP SCANNING ELECTRON MICROSCOPE

A. TURNING ON THE INSTRUMENT:

1. Make sure EVO water chiller is on. (Note: this should be left on but check it just to be sure)
2. Open main nitrogen tank valve. **DO NOT** adjust the pressure regulator.
3. If the EVO is in the **STOP** position (**RED** button lit on column console), first go to the **STANDBY** position by depressing the **YELLOW** button and then go to the **ON** position by depressing the **GREEN** button. If the EVO is already in the **STANDBY** position (**YELLOW** button lit on the column console), then go to the **ON** position by depressing the **GREEN** button.
4. Computer should boot up automatically. If it does not, depress the computer's "on" button which is located on the front of the main CPU.
5. From the main desktop, double click on the **Zeiss SmartSEM** icon.
6. At the **EM Server Log On** box, for **User Name** type in MMiller and for a **Password** type in millier.
7. When EM Server is completely loaded, a black window will appear on the left-hand monitor. Maximize to fill the screen.
8. From the top menu tool bar, select **Vacuum** and then select **Vacuum Status** from the drop-down menu. A **SEM Control** panel should now appear on the right-hand monitor.
9. From the top menu tool bar, select **Stage** and then select **Navigation** from the drop-down menu. A **Stage Navigation** panel will appear on the right-hand monitor.

B. INSERTING A SPECIMEN:

1. From the **SEM Control** panel (right-hand monitor), select the **Vacuum** tab and then select **Vent**. As an alternative way to vent the chamber, you may click on either the word **Vac, Gun, or EHT** which located on the lower left-hand monitor tool bar and then select **Vent** when prompted. Select **Yes** on the confirmation box.
2. Wait until the specimen chamber has come to room pressure; this will take approx. 2-3 minutes.
3. Once the chamber is at room pressure, open the chamber by pulling the chamber door toward you.
4. Using forceps (NO HANDS/FINGERS) seat your specimen stubs in to the specimen holder.
5. **Gently** tighten the corresponding stage set screw. **Caution: Do not over-tighten the screw.**
6. Slowly and carefully close the chamber door.
7. Once you are sure the door is closed, either click on the **Pump** button located on the **Vacuum** tab of the **SEM Control** panel (left-hand monitor) OR click on either the word **Vac, Gun, or EHT** which are located on the lower left-hand monitor tool bar and then select **Pump** when prompted.
 - Under **Vac Status**: =**Waiting Turbo Speed** (means turbo pump is coming up to speed)
=**Waiting Penning** (means high vacuum penning gauge is activated)
=**Pumping**
 - Under **System Vac**: at a vacuum of approx. 1.0e-004 (i.e. 1.0×10^{-4}) mbar a **GREEN** check mark ✓ will appear next to the word **Vac** at the bottom of left-hand monitor when the vacuum is sufficient.
8. Wait until there is a **GREEN** check mark ✓ next to the word **Vac**. You might also have a **GREEN** check mark ✓ next to the word **GUN** and a **RED X** next to the word **EHT** (i.e. **Vac: ✓ Gun: ✓ EHT:X**) or in some cases you might have two **RED Xs** next to the words **GUN** and **EHT** (i.e. **Vac: ✓ Gun:X EHT:X**).

C. TURNING ON THE BEAM:

1. Click on either the word **Vac**, **Gun** or **EHT** at the bottom of the left-hand monitor and select **Beam On**. The beam will “run up” slowly as shown by an indicator meter at the bottom of the screen.
2. Once the beam is on completely, the box will read **All:** ✓

D. GUN/BEAM ALIGNMENT (Gun Aperture Alignment and Beam Alignment):

1. From the **SEM Control** panel (right-hand monitor), select the **Aperture** tab.
2. Click on the **Emission** button. A **GREEN** target pattern (*) should appear on the left-hand monitor.
- 3 Adjust contrast with the **Contrast Knob** so that a large white spot is visible in the middle of the screen.
4. Adjust position of gun aperture with the **SHIFT** button. Using the left mouse button, click and hold on the small **red** ball (•). While holding down on the left mouse button and while watching the image on the left-hand monitor, move the ball around to center the image of the aperture.
5. Adjust position of beam with the **TILT** button. Using the left mouse button, click and hold on the small **red** ball (•). While holding down on the left mouse button and while watching the image (bright spot) on the left-hand monitor, move the ball around to center the beam.
6. Click on the **Normal** button on the **Aperture** tab to return to normal imaging.

E. CHANGING VOLTAGE:

If you find it necessary to adjust the voltage, then:

1. Double click on the word **EHT** on the **data zone** and type in the desired **kV** value. Hit **Enter** and the new **kV** value will be displayed.
2. Alternatively, from the **SEM Control** panel (right-hand monitor), select the **Gun** tab.
3. Change **kV** by moving slider bar or use arrows under **EHT Target =**.
4. **You may need to realign beam** (see **Section D** above).

F. ADJUST WORKING DISTANCE: (*Only Accurately Determined on a Focused Specimen*)

1. Adjust brightness and contrast in order to see an image. First click on the **Levels** button located on the upper menu bar of the left-hand monitor. This will display the brightness and contrast values at the bottom of the left-hand monitor. Adjust brightness to approximately **50%** with the **Brightness knob** located on the right-hand side of the hard panel (keyboard panel). Next adjust the contrast to approximately **25-35%** with the **Contrast knob** also located on the right-hand side of the hard panel (keyboard panel)
2. **Focus** on your specimen with the **FOCUS knob** to determine the current working distance. The working distance will be displayed on the **data zone** (white data field at the bottom of the viewing screen on the left-hand monitor)
3. If you need to change working distance, turn on chamber camera with the **CAMERA** button which is located on the upper portion of the hard panel/keyboard. You will now be able to see inside the chamber.
4. Using the motorized **Z direction lever**, carefully raise or lower the specimen stub and stage. Be sure to refocus often (you will need to turn off the camera to focus on your image). Repeat until you have reached the desired working distance.

5. It is important to focus on the **highest point** on your specimen especially on specimens with a dynamic topography AND/OR on the specimen stub with the **tallest sample**.
6. A proper working distance depends on your desired magnification range, your focus and imaging needs, and the overall topography of your specimen. **However, the working distance should never be less than 6 mm.** Shorter working distances run the risk of hitting the detectors inside the chamber. If the working distance is below 6 mm, use the **Z drive lever** to adjust to 6mm or greater. Refocus with the **FOCUS knob** and the new working distance will appear on the **data zone**. Remember to focus on the **highest point on your specimen** especially on specimens with a highly variable surface or on the **tallest specimen**. Focusing in a “valley” may place a “peak” a few millimeters closer to the detectors than the 6mm.

G. CALIBRATING STAGE NAVIGATION (this will ensure you are viewing the correct sample):

1. Start out on a ‘known’ hole on the SEM stage. This can either be an empty hole on the stage or you can use position #2 as a reference since it is covered with tape and always blank. Take note of which position you are actually viewing.
2. From the **Stage Navigation** panel (right-hand monitor), see if the stage navigation graphic (pink and grey image) is correct and corresponds to the actual image/position you are seeing on the left-hand monitor. If it is correct, the stage navigation graphic is correct and calibrated. If it does not correspond to what you are seeing, then proceed to step 3.
3. Click on the **Settings** button on the **Stage Navigation** panel. A **Stage Nav Settings** panel will appear. On the right-hand side of this panel, find the **Holder Rotation Offset Calibration** slider.
4. Using either the **slider** or the **() arrows** make adjustments until the graphic on the **Stage Navigation** panel corresponds to the actual image position you see on the left-hand monitor.
5. When finished, close the **Stage Nav Settings** panel by clicking on the small red **x**. Be sure to leave the **Stage Navigation** panel open.

H. FINDING AN IMAGE:

1. Once the working distance is properly adjusted, begin imaging your specimen. It is probably best to begin with the following settings:

Lower Magnification	I Probe Current (pA) Found under Gun tab	Scan Mode (Speed) Found under Scanning tab	Focus & Stigmation
less than 1000x	Large (~700-900pA)	Fast Scan Speed (~1-5)	Coarse

2. As you increase magnification or need higher resolution, make the following setting changes:

Higher Magnification	I Probe Current (pA) Found under Gun tab	Scan Mode (Speed) Found under Scanning tab	Focus & Stigmation
~1000-5000x ~5000-10,000x Over 10,000x	Small (~200-700pA) Smaller (less than 200pA) Smallest (~25-100pA)	Slower Scan Speed (~3-6) Slower Scan Speed (~3-6) Slower Scan Speed (~3-6)	Coarse or Fine

3. Centering Objective Aperture:

- a. At each major magnification level, **Focus** on a round object or particle.
- b. Place a **Reduced box** around the object.
- c. From the **Apertures** tab, select **Focus Wobble**. You may also select **Fast Wobble** if preferred. Increase or decrease the **Amplitude** as needed. Recommend at least **75%**.
- d. Increase scan speed with the **Scanning Speed +** button go to the **Scanning** tab on the **SEM Control** panel and make changes.
- e. Adjust the **X** and **Y Centering Micrometers** on the **Objective Aperture** (located on SEM column) until the object remains centered or “pulses” around a center point on the screen. If the object shifts back and forth, the aperture is out of alignment.
- f. When finished, uncheck the **Focus Wobble** box to return to normal viewing.

4. Hysteresis (removing residual magnetic field from lenses): At some point during your SEM session you may want to perform this function.

- a. **Focus** on some object on your specimen.
- b. Place a **Reduced box** around the object.
- c. Once the object or structure is in focus, **depress both the Shift button and the F2 button on the keyboard**. This will initiate the **Hysteresis** step as indicated on the screen. Allow this process to finish.
- d. More than likely, the image will be out of focus after this process. Refocus the image using the **Focus** knob.
- e. Once the image is back in focus, perform the Hysteresis step again by **depressing both the Shift button and the F2 button on the keyboard**. Allow this process to finish. In most cases, your image should appear nearly in focus after this second step. However, if it is still out of focus, repeat the process. After the third attempt, the image should be nearly in focus after the **Hysteresis** process is complete.

I. PHOTOGRAPHY:

1. Adjust scanning speed with **Scanning Speed +/-** buttons or go to the **Scanning** tab on the **SEM Control** panel and make changes. Use a slower scan speed for high resolution and where an image of higher quality is desired.

Scan Speed Recommendations(found under **Scanning** tab on the **SEM Control** panel):

Normal viewing: select any scan speed. Under **Noise Reduction**: select **Frame Avg. N=1**
Photography: any speed can be used but I recommend a **scan speed of 8-12** . Under **Noise Reduction**: select **Line Avg** with a value of **N=4+**. However, you can also decrease the **scan speed to ~5** and increase the **Line Ave N value to 25+** for good results.

2. When the scan line reaches the bottom of the screen, press the **FREEZE** button which is located on the upper portion of the hard panel/keyboard. A **RED** dot (•) will appear in the lower right-hand corner of the image. This is an indication that the image is “frozen.”

3. On the upper tool bar (left-hand monitor), click on **File** and then select **Save Image** from the drop-down menu. Change the **Directory** to locate a specific file folder and then **name** your image file. Click the **Save [file].tif** button to save and then **close** the box.

4. Once your image is saved, a **BLUE** dot (•) will now appear in the lower right-hand corner of your image. This means your image file was saved but the image is still “frozen.”

5. Press the **FREEZE** button again to unfreeze your image and to resume normal scanning.

6. Be sure to take your image files with you when you are finished. The best way to do this is with a USB flash drive or jump drive. Insert your drive in to the **USB hub** located on the desk just behind the hard panel/keyboard. There is no guarantee your images will stay on the EVO computer.

J. EXCHANGING A SPECIMEN:

1. Place the beam in **Standby** mode by first clicking on **All:** ✓ which is located on the lower left-hand monitor tool bar and then select **Standby** from the drop-down menu. Note: This is NOT the yellow standby button on the front of the microscope!
2. Once the beam is off, there will be a **GREEN** check mark ✓ next to the words **Vac** and **Gun** and there will be a **RED X** next to the word **EHT** (i.e. **Vac:** ✓ **Gun:** ✓ **EHT:** X).
3. From the **SEM Control** panel (right-hand monitor), select the **Vacuum** tab and then select **Vent**. As an alternative way to vent the chamber, click on either the word **Vac, Gun, or EHT** which are located on the lower left-hand monitor tool bar and then select **Vent** when prompted. Select **Yes** on the confirmation box. Indication at the bottom will be **Vac:** X **Gun:** ✓ **EHT:** X.
4. Wait until the specimen chamber has come to room pressure; this will take approx. 2-3 minutes.
5. Once the chamber is at room pressure, open the chamber by pulling the chamber door toward you.
6. Before removing aluminum stub, loosen set screw by turning it counterclockwise one half a turn. **Do not loosen screw all the way.** Head of screw should not be showing.
7. Using forceps (**NO HANDS/FINGERS**), remove your specimen from the chamber and place another set of specimen stub(s) in to the stage specimen holder. **Gently** tighten the corresponding stage set screw. **Caution: Do not over-tighten the screw.**
8. **Gently** close the chamber door.
9. Once you are sure the door is closed, either click on the **Pump** button located on the **Vacuum** tab of the **SEM Control** panel (left-hand monitor) OR click on either the word **Vac, Gun, or EHT** which are located on the lower left-hand monitor tool bar and then select “**Pump**”.
10. Wait until there is a **GREEN** check mark ✓ next to the word **Vac**. Once the vacuum is sufficient, the indicator will appear as: **Vac:** ✓ **Gun:** ✓ **EHT:** X.
11. Turn on **EHT** by clicking on either the word **Vac, Gun, or EHT** which are located on the lower left-hand monitor tool bar and then select **EHT On**. The beam will “run up” slowly as shown by an indicator meter at the bottom of the screen.
12. Once the beam is on completely, the box will read **All:** ✓

K. END OF SESSION (during the daytime):

1. Turn off the beam by first clicking on **All:** ✓ which is located on the lower left-hand monitor tool bar and then select **Shutdown Gun** from the list of options. The beam will “run down” slowly as shown by an indicator meter at the bottom of the screen.
2. From the **SEM Control** panel (right-hand monitor), select the **Vacuum** tab and then select **Vent**. As an alternative way to vent the chamber, click on either the word **Vac, Gun, or EHT** which are located on the lower left-hand monitor tool bar and then select **Vent** when prompted. Select **Yes** on the confirmation box. Indication at the bottom will be **Vac:** X **Gun:**X **EHT:** X.
3. Wait until the specimen chamber has come to room pressure; this will take approx. 2-3 minutes.
4. Once the chamber is at room pressure, open the chamber by pulling the chamber door toward you.
5. Before removing aluminum stub, loosen set screw by turning it counterclockwise one half a turn. **Do not loosen screw all the way.** Head of screw should not be showing.

6. Using forceps (**NO HANDS/FINGERS**), remove your specimen(s) from the chamber.
7. **Gently** close the chamber door.
8. Once you are sure the door is closed, either click on the **Pump** button located on the **Vacuum** tab of the **SEM Control** panel (left-hand monitor) OR click on the word **Vac, Gun, or EHT** which are located on the lower left-hand monitor tool bar and then select "**Pump**" when prompted.
9. Leave the black **SmartSEM** screen open on the left-hand monitor
10. Leave the **EM Server** box open (located on the right-hand monitor).
11. Leave the computer on during the day.
12. Leave the EVO in the ON position (**GREEN** button lit).
13. Copy your images to a CD, portable drive or send them to yourself through the internet.
14. **Log in your SEM time in the SEM logbook.**

L. SHUTTING DOWN (FOR NIGHTS AND WEEKENDS)

1. If it is at the end of the day or if you turned on the scope in the case of it being at night or during the weekend, then in addition to the steps outlined in Section J above, also close the black **SmartSEM** screen on the left-hand monitor by clicking on the small **red x** in the upper right-hand corner of the this screen. Select **YES** when the prompt to log off appears. This will close the black imaging screen.
2. Close the **EM Server** box which is located on the right-hand monitor by clicking on the small **red x** in the upper right-hand corner of this box. When the "EM Server User Message: Are you sure you want to close EM Server?" prompt appears, select **YES**. This will close the **EM Server** box.
3. Be sure to save your images and take them with you before shutting down the computer.
4. Now shut down the computer. From the main Windows desktop, click on the **START** button (bottom of the left-hand monitor) and click on **Turn Off Computer**. Click on **Turn Off**.
5. Let Windows close completely (small **blue** lights on monitors will change to **orange**).
6. Place EVO in the **STANDBY** position by depressing the **YELLOW** button on the column console.