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 **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 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 **miller**.
- 7. When EM Server is completely loaded, a black window will appear on the left-hand monitor. You may need to maximize to fill the screen.
- 8. From the top menu tool bar on left-hand monitor, 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 on the left-hand monitor, 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**. 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 x 10⁻⁴) mbar a GREEN check

mark ($\sqrt{\ }$) 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 ($\sqrt{\ }$) next to the word Vac. You might also have a GREEN check mark ($\sqrt{\ }$) next to the word GUN and a RED X next to the word EHT (i.e. Vac: $\sqrt{\ }$ Gun: $\sqrt{\ }$ EHT:X) or in some cases you might have two RED Xs next to the words GUN and EHT (i.e. Vac: $\sqrt{\ }$ 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:** $\sqrt{}$

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 beam (white spot) with the **TILT** button. Using the left mouse button, click and hold on to 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.
- 5. If you need to adjust the position of the gun aperture, first move the beam around with the **TILT** button to locate the edge of the aperture. Next 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. Once the aperture is centered, select the **TILT** button again and recenter the beam (white spot) as you did in step 4.
- 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. 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 <u>brightness and contrast</u> 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 **20-35%** 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, <u>carefully</u> 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. The working distance should never be less than 6 mm.
- 5. It is important to focus on the <u>highest point</u> on your specimen especially on specimens with a dynamic topography AND/OR on the specimen stub with the <u>tallest sample</u>.
- 6. A proper working distance depends on your desired magnification range, your focus and imaging

needs, and the overall topography of your specimen. Again, 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 $\langle \rangle$ 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 and OPTIMIZING AN IMAGE:

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

Magnification	I Probe Current (pA)	Scan Mode (Speed)	Focus &
	Found under Gun tab	Found under Scanning tab	Stigmation
less than 1000x	Large (~700-900pA)	Fast Scan Speed (~1-5)	Coarse
~1000-5000x	Small (~200-700pA)	Slower Scan Speed (~3-6)	Coarse
~5000-10,000x	Smaller (less than 200pA)	Slower Scan Speed (~5-7)	Coarse
Over 10,000x	Smallest (~25-100pA)	Slower Scan Speed (~8+)	Fine

2. 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 as needed.
- 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.
- g. Adjust focus with the Focus knob.

- 3. **Hysteresis** (removing residual magnetic field from lenses): At some point during your SEM session you may want to perform this function.
 - a. Focus on a round object or particle 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 (turn focus knob clockwise).
 - 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, you 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):

For normal viewing: select any scan speed. Under Noise Reduction: select Frame Avg. N=1

<u>For photography:</u> any speed can be used (e.g. low mag: scan speed 3-6, medium mag: scan speed 5-7, higher mag: scan speed 8+. Under **Noise Reduction**: select **Line Avg** with a value of **N=4 or greater.**

- 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 or on the **FREEZE** button located on the computer screen. A **RED** dot (•) will appear in the lower right-hand corner of the image. This in 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 images 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. <u>Note: This is **NOT** the yellow standby button on the front of the microscope!</u>
- 2. Once the beam is off, there will be a **GREEN** check mark ($\sqrt{\ }$) next to the words **Vac** and **Gun** and there will be a **RED X** next to the word **EHT** (i.e. **Vac:** $\sqrt{\ }$ **Gun:** $\sqrt{\ }$ **EHT: X**).
- 3. From the **SEM Control** panel (right-hand monitor), select the **Vacuum** tab and then select **Vent**. 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 ($\sqrt{\ }$) next to the word **Vac**. Once the vacuum is sufficient, the indicator will appear as: **Vac:** $\sqrt{\ }$ **Gun:** $\sqrt{\ }$ **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:** $\sqrt{}$

K. END OF SESSION (during the daytime):

- 1. Turn off the beam by first clicking on **All:** \(\sqrt{\text{which}} \) 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**. 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).
- 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. 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**. 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, click on the **Pump** button located on the **Vacuum** tab of the **SEM Control** panel (left-hand monitor).
- 9. Close the black **SmartSEM** screen on the left-hand monitor by clicking on the small $\mathbf{red} \mathbf{x}$ 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.
- 10. 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.
- 11. Be sure to save your images and take them with you before shutting down the computer.
- 12. 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**.
- 13. Let Windows close completely (small blue lights on monitors will change to orange).
- 14. Place EVO in the **STANDBY** position by depressing the **YELLOW** button on the column console.
- 15. Log in your SEM time in the SEM logbook.