JEOL JSM-6360 LV Operating Instructions (Scanning Electron Microscope)

A. Standby Settings for All Controls (Leave at these settings when not in use)

1. Turn off **HT**
2. Remove **Specimen** as described in section D below.
3. Click on **SEI mode** in **Data display** area at the bottom of the screen.
4. Set the **Magnification** @ 40,000 X
5. Set **Z control** at approximately **40 mm**
6. Click on **Scan 3**
7. Turn off the **monitor**

B. Start-up From a Cold Start

1. Check that small **Haskris water chiller** in C-109 is on and water temperature is approximately 66° F. The indicator light should be lit, showing that there is sufficient water in the recirculating water tank.
2. Turn on the SEM by momentarily turning the **key** on the console to **start.**
3. Wait approximately 1 min, and then turn on the **computer and monitor.**
4. When the Windows XP operating system finishes booting up, click on the **SEM Main Menu** icon to start up the graphical user interface (GUI).
5. Wait until the **HT** icon indicates **HT ready**. This indicates that operational vacuum has been reached.

C. Loading a Specimen

1. Make sure that **HT** is off.
2. Press and hold the **VENT** button for several secs, until the button lights up.
3. When the **VENT** button light quits blinking, the **chamber** is vented.
4. Open the **Chamber drawer** gently.
5. Insert the **Specimen holder**onto the stage dovetail, making sure that the flat edge is up against the raised left edge of the stage. The **specimen image** is 90° clockwise from the **specimen position** on the stage.
6. Make sure that the stage **Z knob** is set low enough that the sample cannot contact the **BEI detector** at the bottom of the **objective lens.** ***A new BEI detector will cost approximately $2,000.00 and that cost will be billed to the investigator responsible for breaking it.***

   a. The working distance indicated on the GUI screen does not take into account that the **BEI detector** is 3 mm thick. The **WD** indicated on the GUI indicates the distance between the top of the **Specimen holder** and the bottom of the **Objective lens**. Thus, if the WD indicates 10 mm, but the highest point on the specimen protrudes 4 mm above the specimen holder, there is only 3 mm

clearance between the top of the specimen and the **BEI collector** (10 mm indicated WD, minus 3 mm thickness of BEI collector, minus 4 mm of specimen above specimen holder equals 3 mm clearance).

b. Turn on the power supply for the **Infra-red camera** and click on the ***** **icon** to view the specimen in relation to the **BEI collector**.

c. ***If the BEI collector and specimen come into contact, both can be damaged (note the comment under C.6 above).***

6. Select the appropriate **Specimen Holder** (single 10 mm; 51 mm for large stubs or four 10 mm stubs; 76 mm for the cryostub of the holder for eight 10 mm stubs).
7. Gently close the **Specimen chamber drawer.**
8. After venting, **before pumping down**, push on **LV** button if planning to do LV work.
9. Apply gentle pressure to the **Specimen chamber drawer** and press the **EVAC** button for several secs until the button lights up.
10. The microscope is ready to use when the **HT ready** indication appears in the **HT** icon.
11. Click on the **Stage** icon and select the **Specimen Holder** from the **Holder** menu.

D. Unloading the Specimen

1. Press and hold the **VENT** button for about 1 sec until the button lights up.
2. When the **VENT** button quits blinking, the **Chamber** is vented.
3. Gently open the **Specimen chamber drawer.**
4. Remove the **Specimen holder** from the stage dovetail.
5. Close the **Specimen chamber drawer.** Press **LV** button, followed by **Evac** button if planning to do LV work, otherwise, proceed directly to D.6.
6. Press and hold the **EVAC** button for about 1 sec until the button lights up.
7. The microscope is ready to use when the **HT** button indicates **HT ready.**

E. Typical Operation For Secondary Electron Imaging (SEI)

1. Once the microscope is on and warmed up and the GUI is showing on the monitor, click on **Recipe** icon. Select **General**, and click on **OK.** This should bring up the setup parameters for standard Secondary Electron Imaging **(SEI)** work.
2. Load the **Specimen** into the **Specimen chamber** as indicated above.
3. **Generate the beam based on previously determined saturation conditions:**
   a. The **Accelerating Voltage** should be set at **5 kV.** If it is not, double-click on **5 kV** in **Data Display** to reveal pull-down menu of kV choices. Click on **5 kV.**

b. Click on **HT ready** icon. It will change to reading **HT on.**
c. **The filament is now saturated based on the previous user's saturation value.**

4. **Obtain an image**
   a. Click on **Scan 2** icon and select a low magnification **(20-40 X).** Alternatively, click on the **View** icon.
   b. Click on **ACB** icon (automatic contrast and brightness) or click on the contrast and brightness buttons at the top of the image screen and increase or decrease their values by moving the slider at the left of the viewing screen up and down while depressing the left mouse button.
   c. Focus the image by using the **Focus knob** or by selecting the **Focus button** at the top of the viewing screen and using the mouse to focus as it was used to adjust contrast and brightness.
      1. The coarsest focus control is the knob on the control pad.
      2. The next coarsest is achieved by clicking on the **Focus** button on the **Image screen** with the right mouse button.
      3. The finest focus step is achieved by clicking on the **Focus** button with the left mouse button.
   d. When comparing **Images**, clicking on the **Image screen** produces a frozen **Snapshot** on the right side of the screen.

5. **Choosing Spot Size**
   a. Click on **Spot size** in **Data display** area below image screen.
      1. For **searching the sample** or other low magnification work, select a spot size of **approximately 60**, using the slider or the up and down buttons.
      2. For **medium magnification** work (1,000-5,000X), use a **spot size of approximately 40**.
      3. For **high magnification** work, use a **spot size of approximately 10**.
      4. For **Backscatter electron imaging (BEI),** use a **spot size of approximately 60**.

6. **Setting the Working Distance with the Z Control**
   a. After determining that there is enough room between the **top of the specimen** and the **BEI** detector, as explained in **Section c.6** above, pre-focus to the desired **WD** as read out in the **Data Display WD** window.

b. Bring the **Image** into focus by carefully moving the **Stage Z control**. It is probably best to use the **Infra red camera** to make sure that the **Specimen** and the **BEI** detector do not come into contact.
c. **For SEI or BEI work**, 20 mm WD is a good starting point.
d. **For better resolution or stronger BEI, especially in the LV mode,** 10 mm WD is suggested. For the best resolution, particularly at **low kV**, 8 mm WD is suggested.

7. **Acquiring an Image Using Visual Observation of the Monitor to Set Brightness and Contrast**
   a. Use **Scan 2** to frame the image to be acquired.
   b. Select **Scan 3.** Adjust **Contrast and Brightness** while observing image.
   c. Select **Scan 4** to acquire the image. It will freeze at the end of the scan.

8. **Acquiring an Image Using the Exposure Marker to Set Contrast and Brightness**
   a. Use **Scan 2** to frame the image to be acquired.
   b. Select **Scan 1** to bring up the **Exposure Marker** and to reduce the scanning area (and increase scan speed).
   c. Adjust **Contrast and Brightness** with the **Markers** near the middle of the image screen.
   d. Select **Scan 3** to check the image to be acquired.
   e. Select **Scan 4** to acquire the image.

9. **To center item of interest in specimen**, double-click on item.

F. Typical Operation in Backscattered Electron Imaging (BEI)

1. First, use the **SEI** mode to choose the sample location. **The BEI amplifier is slower than the SEI amplifier, so it is better to set up in SEI before switching to BEI.**
2. Adjust **Magnification, Focus, and Astigmatism** (as necessary).
3. Adjust **Brightness and Contrast** to get good **Image** on **Image screen**.
4. Go to **Data Display** at bottom of screen and select **BEIW**.
5. Depress **ACB** button on **Control pad** or use **BEI Contrast and Brightness sliders** for coarse adjustments, or normal **Brightness and Contrast** controls at top of **Image screen** for fine adjustments. Go to **Scan 1** and use **Exposure Marker** as a guide.

6. If no **Image** is seen or the **Contrast** is too weak, it may be necessary to:
   a. Increase **Brightness** and/or **Contrast**.

b. Increase the **Probe Current** by going to **Data Display** area, selecting **Spot size**, and increasing **Spot size**.
c. Increasing the size of the **OL aperture** (use OL#1).
d. Decreasing the working distance (**WD**). Observe the **Specimen** with the **Infra red camera** to make sure that the height and/or tilt will not allow the specimen to come into contact with the **BEI detector**.

7. If the **Image** is too bright:
   a. Decrease the **Brightness** and/or decrease the **Contrast**.
   b. Decrease the **Probe Current** by selecting a smaller **Spot size** with the **Spot size** control in the **Data Display**.
   c. Decrease the **Objective aperture** size. Use **Aperture #1 or 2**.

8. More **Probe current** is needed for good contrast in BEI mode if:
   a. The **Working Distance (WD)** increases. **Shorter WD catches more of the diverging backscattered electron signal.**
   b. The **kV** is decreased.

9. **The Solid-state BEI detector consists of an A section, a B section, and a C section for the Shadow Mode**.
   a. **A + B signals provide a Compo image** (Giving information about atomic composition of the specimen. Lower atomic number areas are darker).
   b. **A – B signals provide a Topo image** (Giving information about topographic details of specimen).
   c. **A + B + C signals provide a Shadow image**, combining aspects of topography and composition in one image. This is most useful in the **LV mode**, where SEI information cannot be generated.

G. Typical Low Vacuum (LV) Operation

1. **Initial Operating Conditions**
   a. Use #2 **Objective aperture**.
   b. When the **LV mode** is selected, the **BEIW detector** is automatically selected to avoid having arcing with the Everhart-Thornley SEI collector.
   c. Use **Working Distance (WD) of 8-12 mm**. Visually estimate the WD to make sure that the specimen surface cannot hit the BEI detector. The **Infra red camera** also is useful to avoid collisions.
   d. Center the specimen on the stage by clicking on the **Stage** icon. Select 0 values for X and Y and click on ok.
   e. Close the **Specimen Chamber Door**, press **LV** button and then the **EVAC** button. With typical fresh biological specimens, do not go to high

vacuum before going to low vacuum, because the high vacuum can cause distortions in the specimen.

f. Set **kV** at **20**.

g. Set **Spot size** at **50-60**.

h. Set **Pressure** to **10-20 Pa**. The pressure will appear in the **Data Display** area at the bottom of the screen and the **Pressure** button in the **Data Display** area will quit blinking when the pressure has been reached. Do not let in any more air than necessary, since higher pressures cause less signal.

i. **Low Vacuum Controls**
   1. **Manual Mode:** adjust arrows to get pressure desired as shown on **Data Display** area under **Pressure**.
   2. **Auto Mode:** scroll through list of target pressures and click on the one desired. The **Start** button begins the **Auto Mode**, while the **Stop** button interrupts it. **Chamber pressure** reads out under **Pressure** in the **Data Display** area.

j. **Shadow Mode:** The higher the **Shadow** setting in the **Data Display** area, the less signal, so in a low-signal situation, a lower **Shadow** setting would be indicated. Otherwise, set **Shadow** to **10**, which will produce more directional shadow, and most topographic information.

k. **Tips:**
   1. If charging occurs, adjust air pressure and judge visually when the charging has stopped. Enough air is needed to neutralize the charge (to decrease overly bright areas), but too much air can result in “black charging” (too little signal).
   2. If charging still occurs, adjust probe current and judge visually when charging has stopped. Less probe current (smaller spot size) generates less charging, but too little probe current may produce too little signal.
   3. If charging still occurs, adjust the kV and judge visually when charging has stopped.
   4. Think of approximately **10,000X** as the practical high magnification.
   5. Think of approximately **5 kV** as the practical low kV.
   6. There is more evidence of **Contamination** when working at low kV, because of less beam penetration.

H. Producing Stereo Pairs

1. The stage tilts to the left.
2. Find a feature at relatively high magnification.
3. Center feature by double-clicking on it.

4. Do normal procedures to prepare image for recording at the magnification desired.
5. Click on **Save**. Name file and identify it as the **0-tilt image**.
6. Tilt specimen approximately 6°, go to high magnification previously used, double click on feature to center feature previously centered. Refocus image.
7. Go to magnification previously used for recording image.
8. Click on **Save**. Give file same name as previous image, except add **6° tilt** to file name.
9. Print both images, put the 0° tilt image on left and observe with stereo glasses.
10. The two images can be imported into PhotoShop and can be layered, with one green and one red for viewing with red/green glasses (**anaglyph is produced**).

I. Electron Mirror Effect (Which can be used to Check Backscatter Detector)

1. Put non-conductive specimen in **Specimen Chamber**. A good specimen is a round polystyrene ball, similar to the ones used on the hinge assemblies of SEM specimen boxes. Pump down to high vacuum.
2. Select SEI mode.
3. Select **20 kV**, **Spot size 50** and look at the specimen until it charges significantly.
4. Drop **kV to 20 kV**.
5. You will now see the **BEI detector**. The charged specimen has become an electron source (the 2 kV electron beam scan is reflected from the specimen surface with a 20kV charge. The reflected beam scan scans the BEI detector.
6. Turn the **BEI detector** on. Select **Compo**, and both halves of the detector should become light. Select **Topo**, and one half of the detector should turn dark.
7. Select **Shadow and set to 10**. The square **Shadow chip** should be half lit, while the rest of the **BEI detector** should be lit.
8. Set **Shadow at 1** and all of A, B, and C portions of BEI detector should be lit.

I. Maintenance Procedures

1. **Changing the Filament**
   a. Move the **Filament Heating Slider** all the way to the left.
   b. Turn off **HT**.
   c. Depress **VENT** button for 1-2 sec.
   d. When **VENT** button quits blinking, open **Gun Chamber**.
   e. Use special **Wehnelt Removal Tool** to remove the **Wehnelt**. ***Do Not Touch with Bare Hands as it will be Extremely Hot.***
   f. Quickly install pre-assembled **Wehnelt**, make sure “o” ring and groove are free of dust, and close **Gun Chamber**.

g. Depress **EVAC** button. Wait about 10 min before proceeding.

2. **Saturating the new Filament**
   a. Select desired **Accelerating Voltage** from **Data Display** at the bottom of the screen.
   b. Select **Spot Size** on **Data Display** area of screen and set **Spot Size** at approximately **60**.
   c. Insert **Specimen**.
   d. Click on **Gun** icon.
   e. Move **Filament Slider** all the way to the left.
   h. Click on **HT** icon, which will change from **HT Ready** to **HT on**.
   i. Select a relatively low magnification for **Image** such as 40-100 X.
   j. Select **Scan 2**.
   k. Move the **Filament Slider** to the right. The image will become apparent and will get brighter and then get dimmer, before getting brighter again. Move the **Filament Slider** to the right slowly until the second brightness does not get any brighter. The **Filament Slider** should not be under the **Orange bar**. If it is, the filament is oversaturated. **If the change in image brightness is not obvious on the Image screen, increase the contrast setting.** If too bright, decrease the contrast.
   l. Select **Scan 1**.
   m. Move the **Filament Slider** to the left until the small image **(Scan 1 image)** in the center of the **Scan 2** image (the rest of the screen) becomes visibly darker than the rest of the **Scan 2 image**.
   n. Move the **Filament Slider** gradually to the right until the **Scan 1** image borders are just as bright as the rest of the **Scan 2** image. **The filament is now saturated for the present conditions.**
   o. If necessary, click on the **Bias** button at the bottom of the **Gun** menu and use the **Coarse arrows** to set the **bias** at **45-50 µA** (for 5 kV) or **65 to 80 µA** (for 15-20 kV). Press **Store**.
   p. **Align the gun** by selecting the **Tilt X and Y Sliders** on the **Gun menu**. Move the sliders to the right and left to determine the point of maximum brightness for both X and Y.
   q. **Auto Gun Control** fully saturates the **Filament** at 30 kV and leaves it there. ***Do not use this mode.***
   r. **Semi-auto Gun Control** automatically saturates the **Filament** at the kV selected by the user.

3. **Cleaning the Wehnelt Assembly**

a. When the **Wehnelt Assembly** has cooled to room temperature, use lint-free gloves to loosen the three allen screws securing the spacer and filament and remove the filament and spacer.
b. Carefully polish the **Wehnelt Cap** with **Pol** polish and a clean swab until all the discoloration around the hole in the center is gone. Clean off the polish with a clean swab until none can be seen.
c. Place **Wehnelt Cap and Spacer** in a beaker of clean 100% acetone, with the **Wehnelt Aperture** pointing up to avoid abrading it on the glass beaker.
d. Sonicate for about 5 min.
e. Drain and add fresh 100% acetone to the beaker and sonicate for about 5 min.
f. Drain the acetone and replace with Freon 112 or 100% methanol and sonicate about 5 min.
g. Drain the methanol and replace with fresh Freon 112 or 100% methanol and sonicate about 5 min.
h. Remove **Wehnelt Cap and Spacer** from beaker with a clean applicator stick and place on lint-free cloth.
i. Handle only with lint-free gloves while drying with an air duster.
j. Examine carefully for evidence of remaining polishing compound.
k. Place **Spacer ring** and new pre-centered **Filament** into **Wehnelt**, and tighten three allen screws to secure. Check that both the ring and filament are flat by wiggling them clockwise and counter-clockwise slightly against the rotating screw before securing them with the allen screws.
l. Examine the **Wehnelt Assembly** with a dissecting microscope to make sure that filament is centered. If the tip is more than one tip-width away from the center of the **Wehnelt Aperture**, center the filament with the three allen screws on the filament base.
m. Wrap assembled **Wehnelt Assembly** in aluminum foil and place in storage box.

4. **Aligning the Objective Lens (OL) Aperture**
a. This procedure is indicated when the image shifts when focusing.
b. Click on the **Spot size** button in the **Data Display** area at the bottom of the screen and set the Spot size to **about 30**.
c. Center a feature on the **Image** screen at approximately **10,000X**.
d. Adjust **Contrast and Brightness** with buttons at top of **Image** screen.
e. Focus the image using the button at the top of the **Image** screen.
f. Click on the **Tools** button on the **Menu bar** and select **OL Wobbler**.
g. If the image shifts, adjust the **X and Y knobs** on the **Objective Aperture Holder** until the object passes through focus without shifting from side to side.

h. Click the **Off** button in the upper right of the **Image** screen to turn **OL Wobbler** off.

5. **Correcting Objective Lens Astigmatism**
   a. Astigmatism should be checked when changing **kV**, after changing or realigning **Objective Apertures**, or changing **Spot size** at high magnifications.
   b. Go to a **Magnification** setting above where you intend to work.
   c. Use **Focus** control at top of **Image screen** or the knob on the **Control pad** to sweep through focus. Look for stretching of the image in a specific direction. This indicates the presence of astigmatism.
   d. Critically focus **Image**. Using **Scan 1** usually makes this easier.
   e. Use the **Stig X and Stig Y** controls on the **Image screen** or the knobs on the **Control pad** to make image sharper (one at a time).
   f. Refocus **Image**.
   g. Repeat **Stig X and Stig Y** adjustments, followed by **Focus** adjustments until maximum sharpness is obtaining in image and no directional smearing is seen when passing from above focus, through focus, to below focus.

6. **Centering Gun Alignment Shift Controls**
   a. This is indicated when changing to large **Spot size** leads to a loss of the **Beam spot.**
   b. Select **Scan 1**.
   c. Set **Magnification** at approximately **40X**.
   d. Click on **Gun** icon to open **Gun Alignment** window.
   e. Change **Spot size** to **99**.
   f. Use **Alignment Shift X,Y Sliders** to maximize **Image brightness**.
      1. If **Image** becomes dark as you move to **Spot size 99**, move **Spot size** back toward **0** until brightness increases.
      2. Use **Alignment Shift X,Y Sliders** to maximize brightness.
      3. Move **Spot size** toward **99** and use **Sliders** to maximize brightness.
      4. Continue moving **Spot size** toward **99** and adjusting brightness with sliders until maximum brightness is achieved at **Spot size 99**.
      5. Select **Spot size** of approximately **30** in **Gun alignment window**.
      6. Maximize brightness with **Alignment *Tilt* X,Y sliders**.

7. Re-check **Objective aperture** centering as described in Section 4 above.

8. **Lens Clearing (Hysteresis Removal)** is indicated whenever critical microscope alignments or calibrations are to be performed, particularly at high magnifications. High magnification images will be better if lens clearing is done before objective aperture centering and astigmatism corrections. Magnification calibrations or probe current calibrations will also be more accurate.
   a. Focus on a specimen.
   b. Go to **Tools** on the **Menu Bar**.
   c. Click on **Lens Reset**.
   d. Refocus.
   e. Repeat process 1-3 times or until no refocus is necessary.

8. **Rebooting Procedures:** Since the JSM-6360LV consists of a microprocessor-controlled system that is, in turn, controlled by a PC and a good deal of cross-talking takes place, the microscope occasionally gets confused. If microscope operation does not seem normal, the fault may lie in the Windows operating system software, the SEM operating program, or the microprocessors that control the microscope. Thus there are several approaches to eliminating the glitches that can be undertaken before calling JEOL service:

   a. **Reboot the JEOL 6360LV Program**
      1. Exit the JEOL 6360LV Program.
      2. Click on the **SEM MAIN MENU** icon to re-start the program.

   b. **Reboot the PC**
      1. Exit the JEOL 6360LV Program.
      2. Exit the Windows operating system to shut down the PC.
      3. Re-start the PC.
      4. Click on the **SEM MAIN MENU** icon to re-start the program.

   c. **Reboot the SEM**
      1. Exit the JEOL 6360LV Program.
      2. Exit the Windows operating system to shut down the PC.
      3. Turn off the SEM using the key on the lower left of the console.
      4. Wait about 15 sec.
      5. Restart the SEM by turning the key momentarily to **Start**.
      6. Wait about 1 min, or until the **EVAC** button quits blinking.
      7. Start the PC.
      8. Click on the **SEM MAIN MENU** icon to re-start the program.