JSM-6480 TYPICAL OPERATION

1. Load the specimen into the specimen chamber: See SPECIMEN EXCHANGE. (Page 6)

2. Generate the beam based on the previously determined Saturation conditions:

NOTE: If the specimen is beam sensitive, pre-select an Accelerating Voltage low enough to protect the specimen (1 – 5 kV) ..... DATA DISPLAY: Click [Acc.Volt]: Double click desired kV)

1. Turn Accelerating Voltage On .... ICONS: Click [HT] (Status changes from READY to ON)

THE FILAMENT IS NOW SATURATED based on the *PREVIOUS* Saturation

3. Obtain an image:

1. Select a fast scan & a low mag .. ICONS: Click [Scan2], [Mag -] or Click [View]
2. Adjust Image Contrast and Brightness until the image is comfortable for viewing ..... ICONS: Click [ACB] or IMAGE BUTTONS: Click and hold [Contrast]/[Brightness] and move mouse up and down (or use knobs)
3. Focus the image if necessary ... IMAGE BUTTONS: Click and hold [Focus] and move mouse up and down (or use knob)

4. Choose Spot Size: ..... DATA DISPLAY: [Spotsize] (range = 0 - 99)

-for searching the specimen or other low mag operation: .. ~ 60
-for medium mag operation or imaging: ..... ~ 40
-for high mag operation or imaging: ..... ~ 10
-for BEI imaging or EDS : ..... ~ 60

5. If necessary -- Set the Working Distance with the Stage Z-control:

- BE MINDFUL OF THE ACTUAL DISTANCE BETWEEN THE SPECIMEN AND THE LENS!!
- AFT must be off: ..... MENU BAR: Tools: Click [Auto Focus Tracer]: [check mark off].

1. Pre-focus with the Coarse Focus control to desired WD as read out on DATA DISPLAY: WD.
2. Bring the image into focus by carefully moving the Stage Z with the Stage Z control.
   -for SEI or BEI or EDS: ..... ~20 mm WD
   -for better resolution or stronger BEI (esp. LV) & EDS: ... 10 mm WD
   -for best resolution, especially at Low kV : ..... 8 mm WD

6. Do whatever work is necessary on the specimen:

-including:
-Acquire images: ..... ICONS: Click [Scan4] (or use button)
-Change Accelerating Voltage ... DATA DISPLAY: Click [Acc.Volt]: Double click desired kV
-etc. .....

7. Turn off Accelerating Voltage: ..... ICONS: Click [HT] (Status changes from ON to READY)

8. Unload the specimen from the specimen chamber: See SPECIMEN EXCHANGE. (Page 6)

JSM-6480 IF NECESSARY

1. If necessary, -- *if present saturation or alignment is uncertain* -- verify Filament saturation and align the Gun using the signal from the specimen (image brightness):

1. Set Spot Size to ~60 .... DATA DISPLAY: [Spotsize]
2. Select fastest scan ..... ICONS: Click [Scan 1]
3. Select Gun Alignment Window ..... ICONS: Click GUN
4. To verify Saturation using the signal from the specimen (image brightness):
   a. Move the Filament Heating slider left until the image brightness starts to drop.
   b. Move the Filament Heating slider right until the image brightness does not increase any more.
   c. Select Scan 2 .... ICONS: Click [Scan 2]
   d. Scan for a few seconds to establish an image with the maximum brightness determined in b.
   e. Select Scan 1 .... ICONS: Click [Scan 1]
   f. Move the Filament Heating slider slightly left until the image brightness just starts to drop.
   g. Move the Filament Heating slider to the right until the image brightness just stops increasing. (Image brightness should now match brightness of surrounding Scan2 image)

   THE FILAMENT IS NOW SATURATED based on the *PRESENT* conditions.
5. Adjust Gun Bias, if necessary, for L.C.= 60-80uA ..... Set Bias: Coarse: < or >; Store; Exit.
6. Verify Gun Alignment using the signal from the specimen (image brightness):
   a. Adjust Gun Alignment-Tilt to ..... Alignment Tilt X, Y sliders maximize image brightness.

2. If necessary, -- *if the image shifts while focusing* -- align the Objective Aperture:

--- Choose OL Aperture 2 for most applications; OL Aperture 1 for higher resolution. ---

1. Set Spot Size to ~30 ..... DATA DISPLAY: [Spotsize]
2. Center a feature on the viewing screen at ~10000X. ..... STAGE: X/Y; ICONS: Click [Mag+/-]
3. Adjust Contrast and Brightness for good viewing of feature ..... IMAGE BUTTONS: Click & hold [Contrast],[Brightness] and move mouse up and down (or use knobs)
4. Focus the image ..... IMAGE BUTTONS: Click and hold [Focus] and move mouse up and down (or use knob)

   Correct astigmatism if necessary (see Section 3 below)
5. Turn On the OL Wobbler ..... MENU BAR: Tools: Click [OL Wobbler]
6. If image shifts, adjust OL Aperture knobs to stop image shifting. .. [COLUMN]: OL APERTURE X-Y knobs
7. Turn Off the OL Wobbler ..... IMAGE AREA: OL wobbler - Click [OFF]

   NOTE: If difficulty is encountered-Turn OL Wobbler off and repeat Steps 4 – 7 until centering is achieved.

3. If necessary, -- *if the image shows directionality of focus* -- correct the Astigmatism:

1. Using the Focus control (mouse or knob), check for astigmatism by going through over & under focus while looking for directionality of focus in the image (over and under focus directionality will be at right angles to each other).
2. Stop focus at the center of over and under focus (image may not be sharp but has no directionality of focus).
3. Adjust the Stig X & Stig Y controls (mouse or knobs) - as if they were each another focusing control - to obtain the sharpest image.
4. Check focus using the Focus control (mouse or knob). Repeat Steps 1 - 4 if necessary.

4. If necessary, -- *if beam is lost with spot size changes to large spot sizes* -- correct the coarse Condenser Lens alignment (Current Axis alignment):

(Gun Alignment-Tilt and Objective Aperture Alignment have already been done.)

1. Select fast scan ..... ICONS: Click [Scan 2]
2. Set Magnification to low mag ..... ICONS: Click [Mag -] or use Mag knob
3. Select Gun Alignment Window ..... ICONS: Click GUN
4. Select largest Spot Size ..... Gun Alignment Window: [Spotsize]:99
5. Maximize image brightness with ..... Alignment Shift X,Y sliders
   Gun Alignment Shift controls

   NOTE: If screen becomes dark as you move Spotsize toward 99:

   1.- move Spotsize back toward 0 until brightness is just seen again;

   2.- maximize image brightness with Gun Alignment Shift controls;

   3.- move Spotsize toward 99 again and maximize image brightness with Gun Alignment Shift controls as necessary until image brightness on screen is finally maximized at Spotsize 99.
6. Select medium Spot Size ..... Gun Alignment Window: [Spotsize]:~30
7. Maximize image brightness with ..... Alignment Tilt X,Y sliders
   Gun Alignment Tilt controls
8. Re-check Objective Aperture Alignment.
9. If necessary, repeat Steps 4 - 8.

JSM-6480 BACKSCATTERED ELECTRON IMAGING

SOLID STATE BEI DETECTOR

I. OPERATION:

1. First, using the SEI mode if possible:
   -choose sample location;
   -adjust Magnification, Focus and, if necessary, Astigmatism.
   -adjust SEI Contrast and Brightness to obtain a good image on the viewing screen.

2. Now select BEI. (DATA DISPLAY: Signal: BEIW: Compo)

3. Adjust the image by using ACB and/or:
   -BEI Contrast and Brightness sliders for coarse, medium adjustment
   and/or
   -the normal Contrast and Brightness controls for fine adjustment

   (View the image itself and/or use the Exposure Marker as a guide)

II. NOTES:

1. If no image appears or contrast is too weak, it may be necessary to:

   -Increase the Brightness and/or increase the Contrast.

   -Increase the Probe Current:
   by selecting a larger Spot Size (DATA DISPLAY: Spotsize)
   and/or
   by changing to a larger OL aperture.

   -If the SAMPLE is not already at short Working Distance,
   it may be necessary to go to a shorter Working Distance (minimum= ~8 mm).

2. If the image appears too strong, it may be necessary to:

   -Decrease the Brightness and/or decrease the Contrast.

   -Decrease Probe Current
   by selecting a smaller Spot Size (DATA DISPLAY: Spotsize)
   and/or
   by changing to a smaller OL aperture.

3. More Probe Current will be needed for good contrast in the Backscattered Electron Image:

   -If the Working Distance is longer
   and/or
   -If the Accelerating Voltage (kV) is lower

JSM-6480 LV OPERATION

I. SET UP THE INITIAL OPERATING CONDITIONS:--

1. LV Orifice: ..... 400 um (installed at factory)

2. OL Aperture: ..... #2

3. Working Distance: .... 10 mm for EDS & Imaging; 8 mm - 10 mm for Imaging only.
   Visually estimate the Working Distance position (and the X & Y position) of the Sample before pumping down.
   (Determine the actual Working Distance after obtaining the image. Adjust Sample position then if necessary)
   (Optimum EDS Working Distance is 10mm. Most EDS companies provide a detector for this W. D. but check to see what geometry you have and set SAMPLE there.)

4. Press LV button and then press EVAC button or Data Display: Vacuum Mode: [LV] & [Sample] icon.

5. kV: ..... 20 kV (less if the sample may need less dosage or less depth of penetration)

6. Spot Size: ..... 50 - 60

7. Pressure: ..... 10 - 20 Pa (133 Pascal = 1 Torr)

II. LOW VACUUM CONTROLS:--

1. Manual Mode:
   - Adjust arrows (up and down arrows) adjust air flow into specimen chamber.
   - Specimen chamber pressure is read on DATA DISPLAY: Pressure.

2. Auto Mode:
   - Scroll through the list of target pressures.
   - Click on the target pressure you want to reach. Target pressure chosen is read in 'Set' readout.
   - [Start] button starts the Auto Mode. -[Stop] button interrupts the Auto Mode.
   - Specimen chamber pressure is read on DATA DISPLAY: Pressure.

3. Shadow Mode:
   - Use as necessary to emphasize topography. (Setting #10 is the most shadowing)

III. OPERATION: CHANGE THE INITIAL OPERATING CONDITIONS AS NECESSARY:--

1. If charging occurs: Adjust air and judge visually when charging has stopped.
   - Enough air is needed to neutralize the charge; but too much air may result in 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 charge; but too little probe current may result in too little signal.

3. If charging still occurs: Adjust kV and judge visually when charging has stopped.
   - Lower kV generates less charge; but too low kV may result in too little signal.

IV. NOTES:

1. Think of ~ 10,000X as the practical high magnification. However higher mags are attainable with many samples.

2. Think of ~ 5 kV as the practical low kV. However lower kV's are attainable with many samples.

JSM-6480 SPECIMEN EXCHANGE

I. LOADING THE SPECIMEN:

1. Undo the specimen chamber door clasp.
   Press and hold the VENT button for about 1 second until the light in the button lights up.
   or [Sample] icon: [VENT].

2. When the chamber is vented (VENT light will stop blinking), open the specimen chamber door.

3. Make sure the stage Z is set low enough so the sample won't hit the lens or BEI detector.

4. Using the Specimen Exchange Tool or a gloved hand, (the flat on the specimen holder goes in first) insert the specimen holder straight in onto the stage dovetail.
   Remove the Specimen Exchange Tool straight out.

5. Close the specimen chamber door and close the clasp.

6. Press and hold the EVAC button for about 1 second until the light in the button lights up.
   or [Sample] icon: [EVAC].

7. Wait until the HT READY indication appears in the HT icon.

8. Suggested optional step for safety and convenience:
   Choose the correct holder setting. [Stage] icon: [Holder]: [Correct holder diagram]

II. UNLOADING THE SPECIMEN:

1. Undo the specimen chamber door clasp.
   Press and hold the VENT button for about 1 second until the light in the button lights up.
   or [Sample] icon: [VENT].

2. Make sure the stage Tilt is at 0° and the stage Rotate is at 0°.

3. When the chamber is vented (VENT light will stop blinking), open the specimen chamber door.

4. Using the Specimen Exchange Tool or a gloved hand, remove the specimen holder from the stage dovetail. Pull straight out.

5. Close the specimen chamber door and close the clasp.

6. Press and hold the EVAC button for about 1 second until the light in the button lights up.
   or [Sample] icon: [EVAC].

7. Wait until the HT READY indication appears in the HT icon.

Skidmore Microscopy Imaging Center (SMIC)

January 19, 2005

Variable Pressure Scanning Electron Microscope (VPSEM)

Guidelines and Protocols.

Introduction

1.) The ***Variable Pressure Scanning Electron Microscope*** (VPSEM) is a state-of-the-art system that allows one to perform conventional high vacuum SEM as well as low vacuum imaging of wet or "dirty" samples. With proper use, one can obtain highly magnified and resolved images of many different types of specimens. The associated computer system also allows for easy image capture and quantitative analysis.

Before you plan to use the VPSEM, be sure that this is the instrument that you need for your research or teaching. Search the literature for specimen preparation, techniques, SEM working conditions and other factors that may direct you to proper application of this system to your work. If you have a project in mind, please discuss your project with David Domozych (Biology). Before being allowed to use this instrument independently, please fill out the "***User application form***" and send it to David Domozych, Biology. He will then contact you to discuss specific needs and goals. Presently, there is no charge for Skidmore users. However, you will need to bring your own forceps, stubs, CDs, zip disks,..., photographic paper for the printer and your own materials for specimen mounting. EM forceps and stubs can be purchased from EMS of Ft. Washington, PA. A catalogue is available in Dana 100.

For the first three years of the VPSEM project (2004-2007, the duration of the NSF-MRI grant), there is a priority list of users in this order: 1.) NSF-MRI faculty (Domozych, Lindemann, Fidopiastis, S. Frey, Bender). 2.) Skidmore EM based classes (e.g. BI 311), 3.) other users. *Please note that there should be plenty of time for all Skidmore users.*

2.) <u>TRAINING:</u> ***Before you can use the VPSEM independently, you will need to attend the training session(s) and be checked out.***

3.) <u>SIGN-UP SHEETS/SEM RESERVATION TIME</u>: Be sure to sign-up for the instrument well in advance. Use the ***sign-up sheet*** provided for you in Dana 100 and include your extension or e-mail. Please note that if you are more than 15 min late for your scheduled time, another user can claim your time. If you have to cancel your time, please cross your name off the sign-up sheet asap.

4.) When you are finished using the microscope, please be sure to fill in the log book.

5.) If you experience trouble when using the microscope, please call 5075 (David Domozych) or e-mail at ddomoz@skidmore.edu. If you cannot find anyone to help you, be sure that the High Voltage is "off" and you can leave the microscope. Or, if you notice vacuum pump malfunctions or other major problems, turn the key on the front panel to "off" (leave the key in the microscope).

6.) Please note that if the microscope needs to be serviced, you may be bumped off you scheduled time.

7.) Images, images, images!! It is highly recommended that you transfer your images to your own computer and/or to the Imacs in SMIC image analysis lab (the first room as you enter the Dana 100 lab). In fact save your files to multiple computers. Because of space problems on the VPSEM's computer, it will be deeply appreciated if you remove your images to other computers every 6 months and then erase your images for the VPSEM computer.

8.) **Important features of the JEOL 6480LV VPSEM**: Resolution at high vacuum- 3 nm (at 30kV and WD of 8 mm); Resolution at low vacuum- 4 nm (at 30 kV and WD of 5 mm); magnification range- 8x to 300,000x; vacuum range- 10-270 pa, apertures: 1= 20 μm (high resolution), 2= 30 μm (normal use); 3= 100μm (for large current); WD= 5-48 mm.

VPSEM Operating Instructions

1.) When you enter the VPSEM lab, you should note the following conditions: the microscope is on, the computer is "asleep" (i.e., the monitor is not lit) and the specimen chamber door is closed/latched with the chamber in high vacuum.

2.) Touch any key on the computer keyboard to "awaken" the computer. The VPSEM software should already be activated or if not, double click the icon to turn it on. You should note that the ***HIGH TENSION*** icon (***HT***- upper left) is "off" and is colored blue.

3.) **Set your parameters for the working conditions of the microscope**. This includes: High Vacuum or Low Vacuum, accelerating voltage (kV), Secondary Electron mode or Backscattered mode, working distance,...,. You may want to save your working conditions on the computer and return access them when using the microscope.

3a) **IMPORTANT:** If you have a specimen of significant height (it stands up above the specimen stub or holder, you must enter its height into the computer. To do so, go to ***Set-up*** and click on ***Fundamental Set up*** and then ***Auto Function***. Enter a new height from 0-75mm. Then hit **Close**.

4.) Click on the ***SAMPLE*** icon on the upper menu bar. Click on ***Vent***. The microscope will vent and you will be able to open the front latch/door.

5.) ***Carefully remove the specimen holder***. Slide the holder straight out. *WARNING: BE VERY CAREFUL NOT TO TOUCH THE DETECTOR- IF IT IS COMPROMISED, IT WILL COST $3,000 TO REPLACE!!*

6.) Place your sample in the appropriate holder and be sure to gently tighten the holder in place with the small wrench. For the cryostub, please contact David D. for instructions.

7.) Carefully slide the specimen holder into the microscope. Close and latch the door. Click on the ***Evac*** button on the screen. It will take about 3 minutes to pump down the microscope. You can now click on ***Close*** on the screen to return to the main screen.

8.) When the microscope has pumped down, the ***HIGH TENSION OFF*** (or ***HT OFF***) icon will be blue. At this time, click on the ***HT OFF*** icon and it will turn green and read ***HT ON***. Click on ***Scan 3*** to see your image. It may be necessary to lower the magnification in order to orient yourself on the specimen holder. Hint: Go to lowest magnification to find your specimen if you are having trouble.

9.) For gross orientation/positioning, click on the ***STAGE*** icon on the upper menu bar. Click on the position that you desire and click on "Go". The specimen motor drive will bring you to that position on the holder.

Note: To match appropriate holder: to computer: Go to ***STAGE*** and click on ***Holder***. Choose the appropriate holder and click on ***Close***.

10.) In order to get rough Contrast and Brightness, click on the ***ACB*** (Automatic Contrast Brightness) button on the upper menu bar. In a few seconds, the microscope will provide "optimal" contrast and brightness. However, each sample has different qualities and you may have to adjust brightness and contrast on the screen. You may also click on ***Scan 1*** and adjust contrast and brightness to the mid-position.

11.) To focus, click on the ***AFB*** icon (Automatic Focus). The microscope will get you close to a focused image. However, you will want to focus either with the manual control or computer control on the screen. Also, be sure to recognize the importance of stigmation and how to properly adjust for it.

12.) You now have an image and you want to rotate it. To do so: Go to ***TOOLS*** icon and click on ***Scan Rotation***. Select On and then select angle of rotation. You can photograph your specimen. When finished, click on the ***Off*** button and close.

13.) You will now want to experiment with magnification, and re-adjust brightness, contrast and focus. When you have the image that you desire, click on the **PHOTO** icon. This will begin image capture. Or you can click on **Freeze-** this will allow you to adjust various settings.

14.) After the image is captured, go to ***FILE*** and click on ***Save image file***. Choose the appropriate file and format for your image (BMP, TIFF, JPEG,...). Type in a name for the image.

15.) **TURNING OFF THE MICROSCOPE**: Bring the magnification down to 30x and click off the HT. You can vent the microscope (Go to ***Sample-Vent***) and remove your specimen. After removing your specimen, close and lock the specimen chamber door and evacuate (***Evac***) to High Vacuum.

15.) **Printing**: Click on **Report**. Next choose your format. Next choose your image (blue folder icons). You can also add text as well. Go to **File** and click on **Print**. Choose the appropriate printer and click on **Print**.

Extra steps after you learn the basics:

1.) **Snapshot**: Get your image in the center of the screen. Next go to the small box on the upper right side. **Right click** and then click on ***Snapshot***. This will take you image and place it in this smaller box. Now choose any part of your image in the small box. This part will be brought to the center of your viewing screen. This is great for orientation.

2.) **ZOOM**: Get your image in the center of the screen. Next go to the small box on the upper right side. **Right click** and then click on ***Snapshot***. Use your left click to draw a rectangle around the part of the image that you wish to enlarge.

Image analysis:

1.) From any saved image or a frozen image you can do the following:

a.) **Zoom**: Go to ***Image*** and select ***Digital Zoom***. Place rectangle (left click) around area of interest. Click on ***x2*** or ***x4***, then click on ***Zoom In***. You can now save your image. To cancel the zoom, click ***Zoom out***.

b.) **Scaler**: Go to ***Image*** and select ***Scaler***. Choose ***X***, ***Y*** or ***D***. Change the borders by dragging your lines to wherever you like. You can now save your image.

c.) **Measuring distances**: Go to ***Image*** and select ***Mulipoint Measure.*** Choose either ***line*** or ***circle***. Draw line using left click or with circle, choose diameter. You can now save your image.

d.) **Adding Text**: Go to ***Editor*** and select ***Text***. A cursor appears on your image. Type your information and close. You can save your image. To cancel: be sure to click on ***Clear***.

There are many other functions for this microscope- feel free to read the instruction manual.

Observation guide in the LV mode

Image observation in the low vacuum mode

1. Vent the specimen chamber to atmospheric pressure and mount the specimen you want to observe.
2. Change the [Vac. mode] shown on the active data display to [LV] and evacuate the specimen chamber.

   Wait until the text icon changes to [HT OFF]. (It takes about 3 minutes 30 seconds in the case of JSM-6480 series. It takes about 1 minute 30 seconds in the case of JSM-6380 series.)
3. Click [HT OFF] button to turn it [HT ON].
4. Set the accelerating voltage to [15kV], and the pressure in the specimen chamber to [30Pa].
5. Set the spotsize to [30 to 40] and the Shadow level on the BEIW menu to [1].

   The spot size set to [30 to 60] in the case of JSM-6380 series.
6. Click [View] button.
7. Set up the specimen stage to the specimen center.

   For JSM-6480 series, set X= 0mm and Y= 0mm.

   For JSM-6380 series, set X= 23mm and Y= 25mm.
8. Click [ACB] to activate automatic contrast and brightness.
9. Click [AFD] to activate Autofocus.

   Use the exposure marker displayed by [Scan] as a guide for appropriate brightness setting.

   Brightness is approximately appropriate when the marker has come to the center. However, the optimum brightness varies slightly with the specimen.

   If it is not easy to check the image when the marker is in the center, increase the spot size and click the [ACB] button again.

If no image is displayed as a result of the above operations, perform the following operation and try again.

1. Vent the specimen chamber to atmospheric pressure, mount an appropriate specimen (an empty specimen holder will do) and evacuate the chamber in the [HV] vacuum mode.
2. Set the working distance to 10mm.

   For JSM-6480 series, set the Z-coordinate position to 10mm

   For JSM-6380 series, turn the Z-knob of the specimen stage to set the Z-scale to 10
3. Click [WD ※※mm] of the active data display and then double-click [WD 10mm] in the list. (This accomplishes coarse adjustment of the focus)
4. Click [HT OFF] button so that [HT ON] appears.
5. Click the [Gun] button.
6. Select [Semi Auto] and click the [Start] button.

   If the error message is displayed, check the filament.
7. Upon completion of Auto Gun Alignment, click the [HT ON] button so that [HT OFF] appears.

【Guide for observing conditions】

Specimen	Pressure	Spotsize
Biological specimen containing much moisture	50 to 70Pa	30 to 60
Dry paper, cloth and resin, etc.	20 to 30Pa	30 to 40

Whenever inserting a specimen containing much moisture, insert the specimen in the low vacuum mode.

Some specimens are susceptible to damage by electron beam irradiation. Therefore, increase the spot size slowly starting from about [30] while checking the image quality.

Observation guide in the LV mode

Checking charge-up

Bombarding a nonconductive specimen surface with electron beams electrically charges the specimen. This charge buildup will be observed as unusual nonuniformity of brightness in the area being observed or as a shift of the field of view during slow scanning at Scan3 or more. The amount of charging varies with the electron energy (the accelerating voltage) of the electron beam with which the specimen is bombarded, beam current, scanning area (magnification), scanning speed and other factors. For instance, the higher the magnification, the higher the electron beam irradiating and the amount of charge buildup also increases. As a result, the specimen may be charged during photography, even though it is not charged during observation. Eliminate and check charge buildup according to the following procedure.

1. Increase magnification by about four steps over the current magnification.
2. Click [Scan1] or [Scan3] button and check if the specimen charges.
3. If the specimen charges up, regulate the pressure using the adjustment button in the Low Vacuum Control tool. Increasing the pressure will reduce charge buildup.

Observation at the lowest magnification

～ Setting parameters for observation and photography of entire specimen ～

1. Set the working distance to 48mm.

   For JSM-6480 series, set the Z-coordinate position to 48mm

   For JSM-6380 series, turn the Z-knob of the specimen stage to set the Z-scale to 48
2. Click [WD ××mm] of the active data display and then [WD 48mm] in the list. (This accomplishes coarse adjustment of the focus)
3. Set the accelerating voltage to [10 to 20kV] and click [View] button.
4. Set the pressure in the specimen chamber, referring to **Guide for observing conditions.**

To obtain a sharp image at high magnification

a) Use a short working distance (WD).

WD20 to 15mm	Resolution is not to poor, but focal depth can be assured.
WD15 to 10mm	Resolution improves and specimen can be tilted slightly.
WD10 to 8mm	At ×10,000 or when the highest resolution is required. **With WD 8mm, the specimen cannot be tilted. Take care not to allow the specimen observation surface to protrude above the specimen holder surface.**

b) Decreasing the working distance will increase the intensity of the detected signal of the backscattered electron detector, allowing the spot size to be reduced. Decrease the spot size while checking the exposure meter displayed after Scan 1.

c) With a short working distance (WD), charge buildup increases compared with a long working distance. Therefore, if the working distance is decreased, increase the pressure in the specimen chamber as required while checking the charge buildup condition.