

JEOL JSM-IT500HR: Electron Imaging Standard Operating Procedure (SOP)

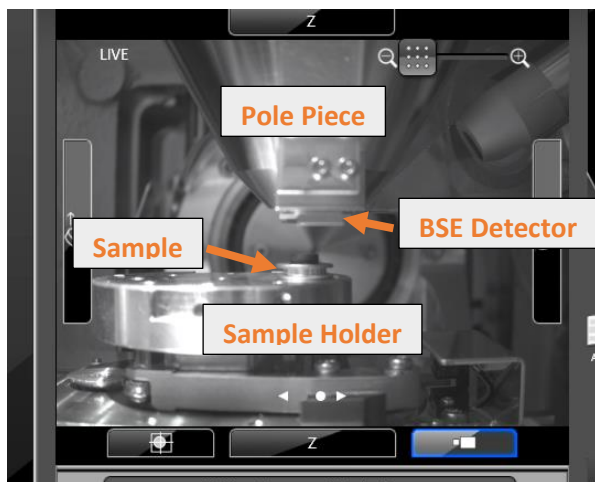
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This document provides step-by-step instructions for the basic operation of the JEOL JSM-IT500HR scanning electron microscope (SEM). This is designed to serve as a general guide for electron imaging; more advanced analyses including energy-dispersive spectroscopy (EDS) and cathodoluminescence are covered in separate, more detailed documents. During most of the steps outlined in this document the SEM user does not have sufficient control to cause significant mechanical damage to the SEM. However, when exchanging samples, it is possible to cause a collision between the sample and the backscattered electron detector. **Please read the section below prior to using this document.**

Sample height and specimen-detector collision

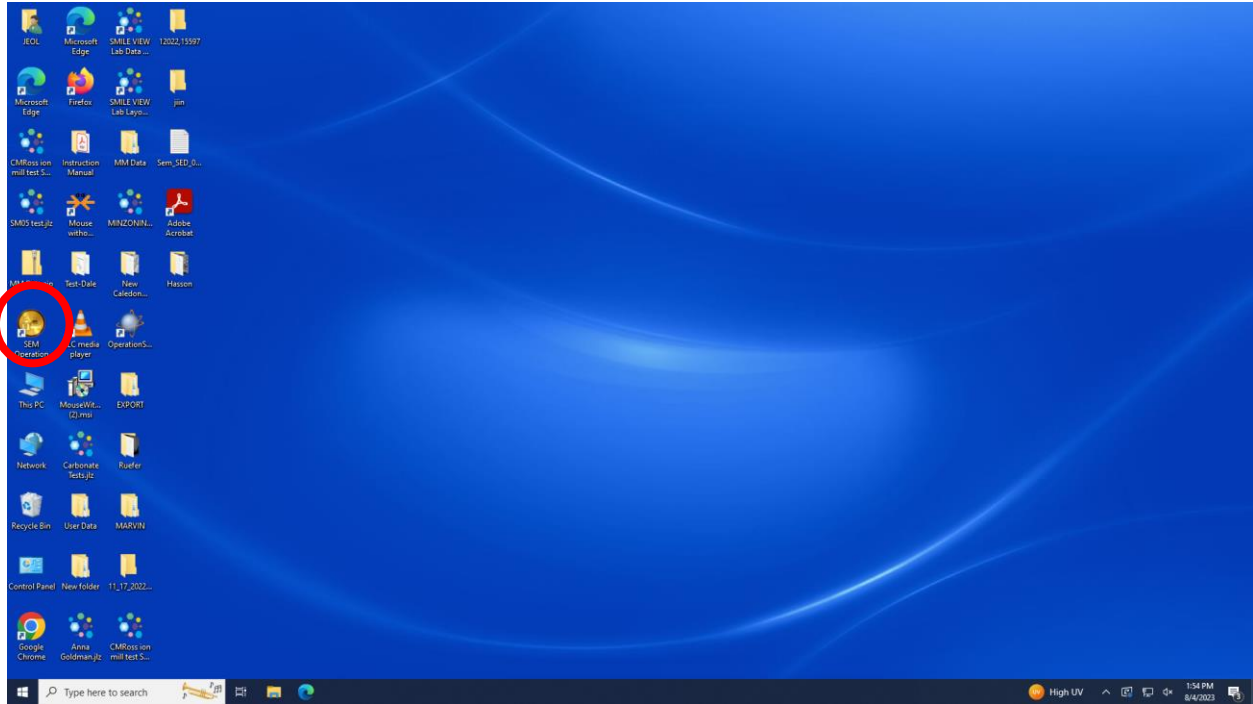
Once a sample is loaded onto the SEM stage, the sample must be moved to the proper location beneath the bottom of the electron column (i.e., the pole piece). Adjusting the X and Y coordinates is straightforward and poses very little risk on JSM-IT500HR. However, if the sample height is entered incorrectly, the sample and/or sample holder can collide with the backscattered electron detector mounted on the bottom of the pole piece (Figure 1). These collisions can destroy the backscattered electron detector and result in damages exceeding \$15k.



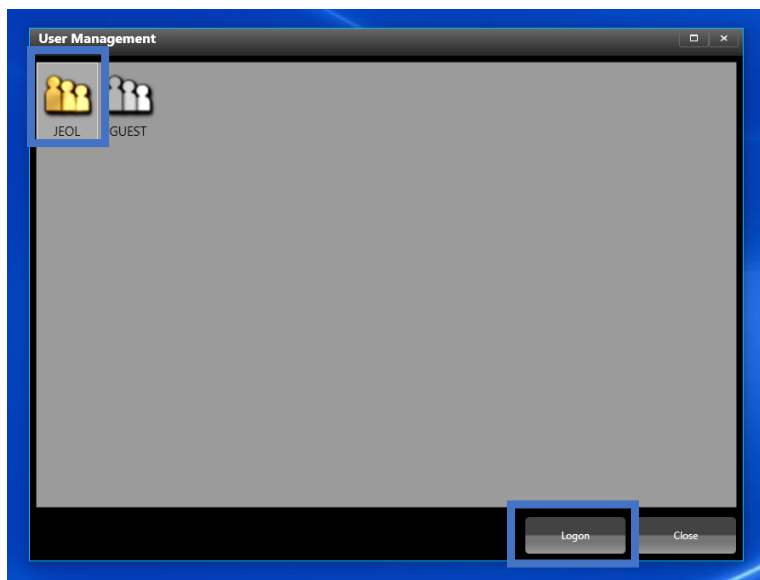
When exchanging samples, extreme caution must be exercised to ensure a collision does not occur. To prevent these collisions, it is critical to measure sample height correctly. In addition, while adjusting the sample/stage height it is crucial to use the side-mounted live camera and bring the sample into position slowly in multiple steps. The methods for measuring sample height and adjusting the stage/sample height are discussed in step 13. Similar precautions arise when tilting samples. Please contact the laboratory manager if sample tilting is required.

Section 1: Sample Loading and SEM Set-up

Step 1: Start the SEM operation software by clicking on the gold SEM Operation button on the left side of the screen.



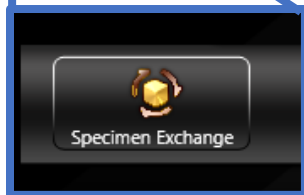
Step 2: When the User Management window appears, select the JEOL user group and click **Logon**.



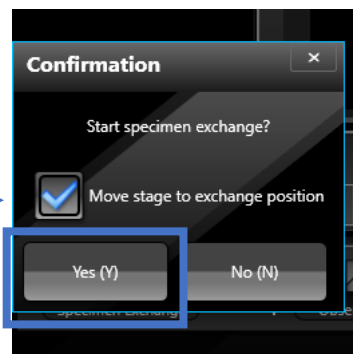
Step 3: Once the SEM operation software opens (this will take ~1-2 minutes) **click on the Specimen Exchange button** in the lower left corner of the screen (3A). When the exchange options appear, **select Specimen Exchange (3B)**, and then **choose Yes(Y) in the specimen exchange confirmation box (3C)**. Make sure the box is checked in the confirmation box.



3A

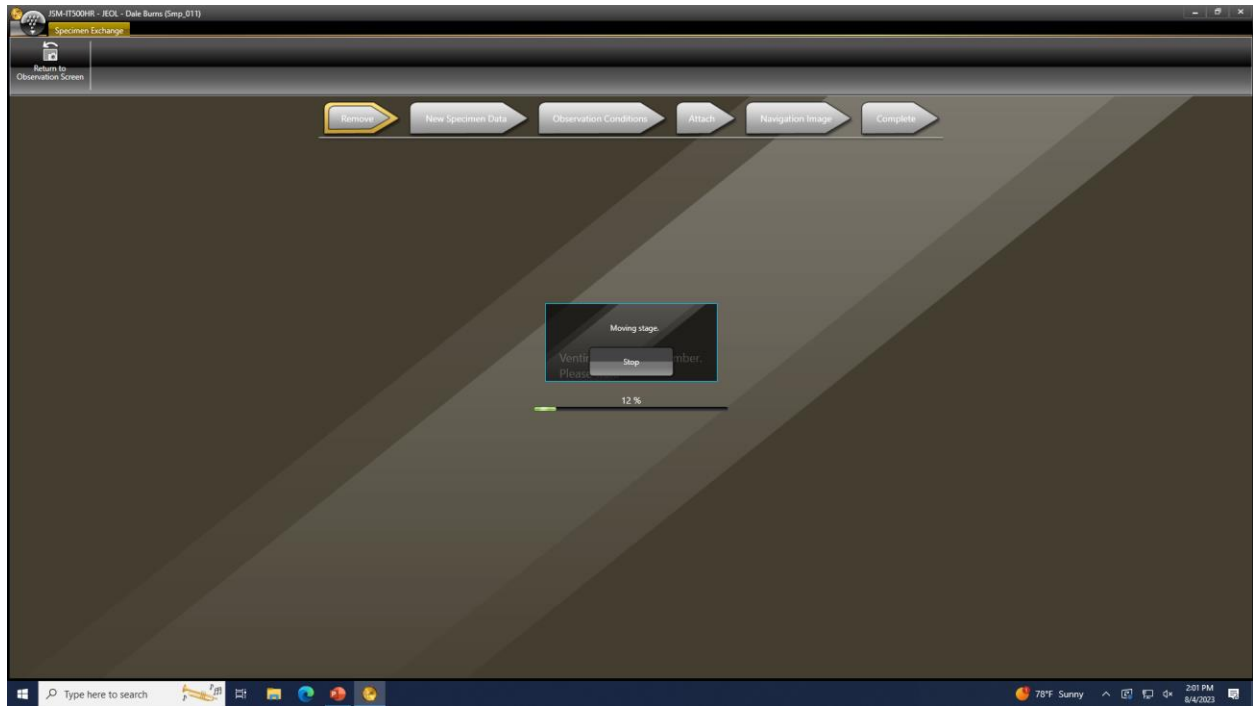


3B

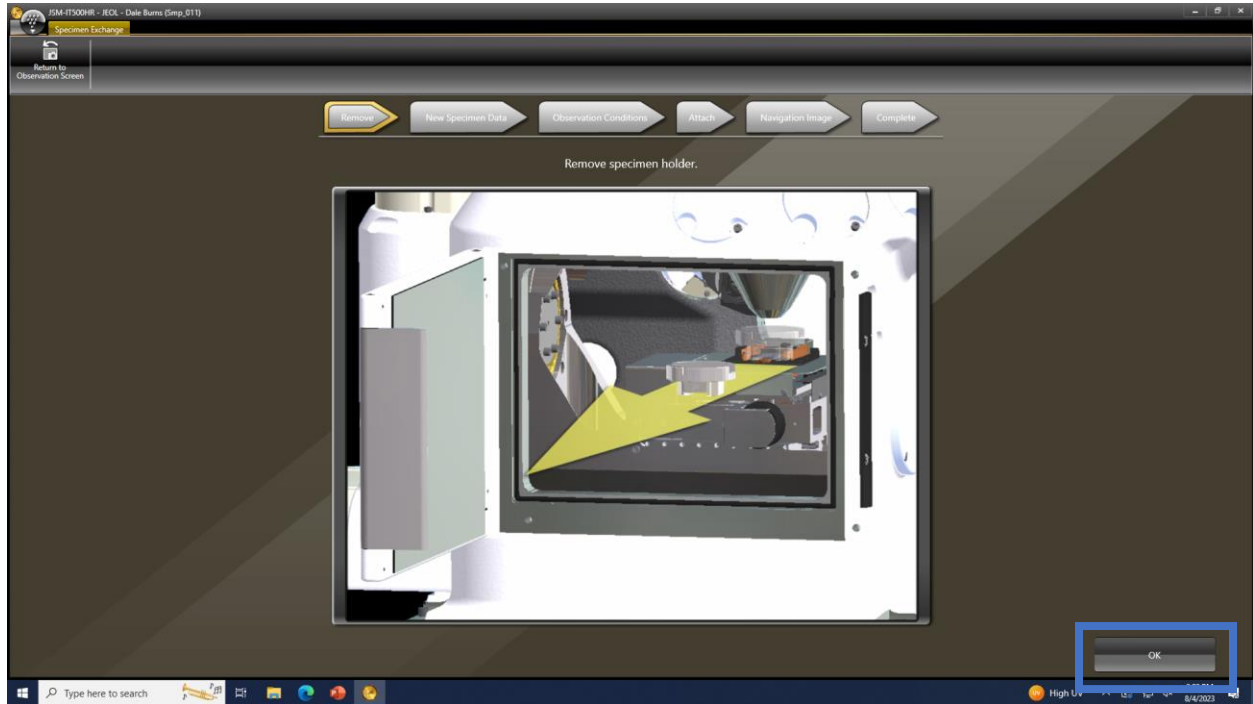


3C

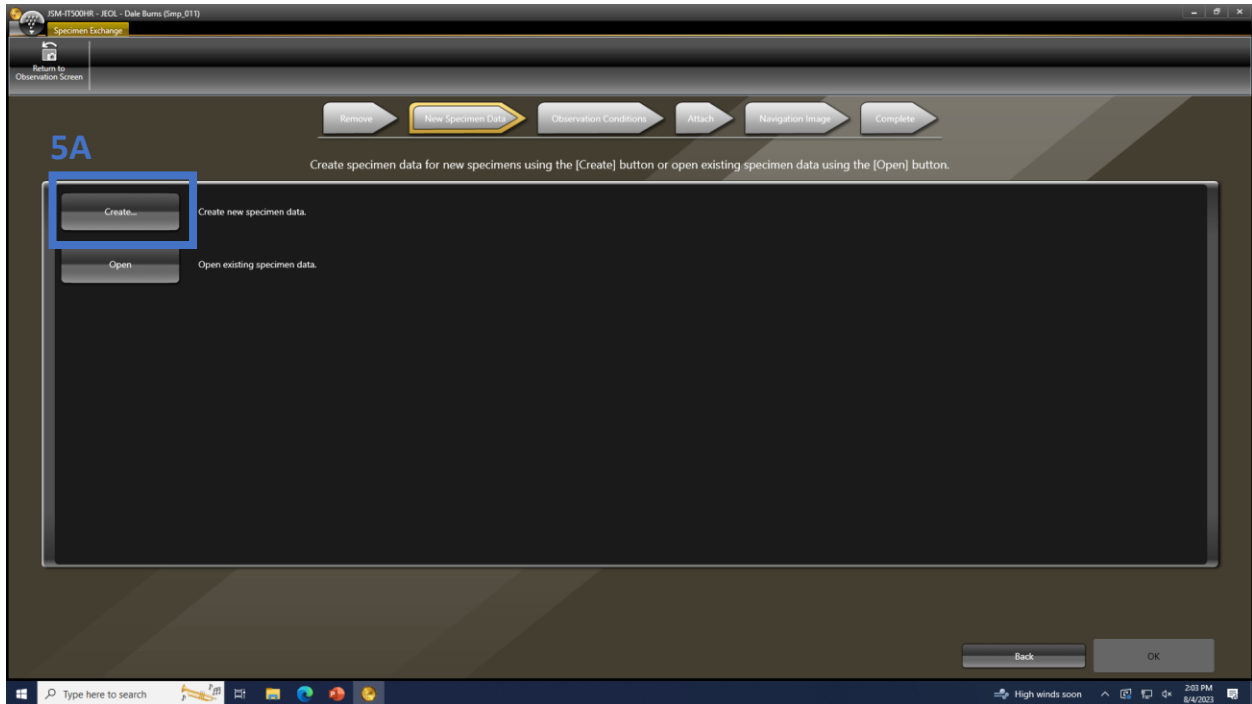
Note: Once the Specimen Exchange option is clicked, the stage will drive to the exchange position and the venting process will initiate. The progress bar shows the vacuum progress. Once the desired vacuum is achieved, the software will show an illustration of the SEM door opening. When this occurs, move to step 4.



Step 4: Detach and remove the sample holder from the stage by pulling the sample holder straight back (the sample holder is secured by two spring-loaded arms). Often, there will not be a sample holder attached to the stage. If this is the case, **click OK** and move to step 5.



Step 5: Click on the Create button to create a new specimen (5A). This opens a dialogue box in which the user enters information about their sample and project.



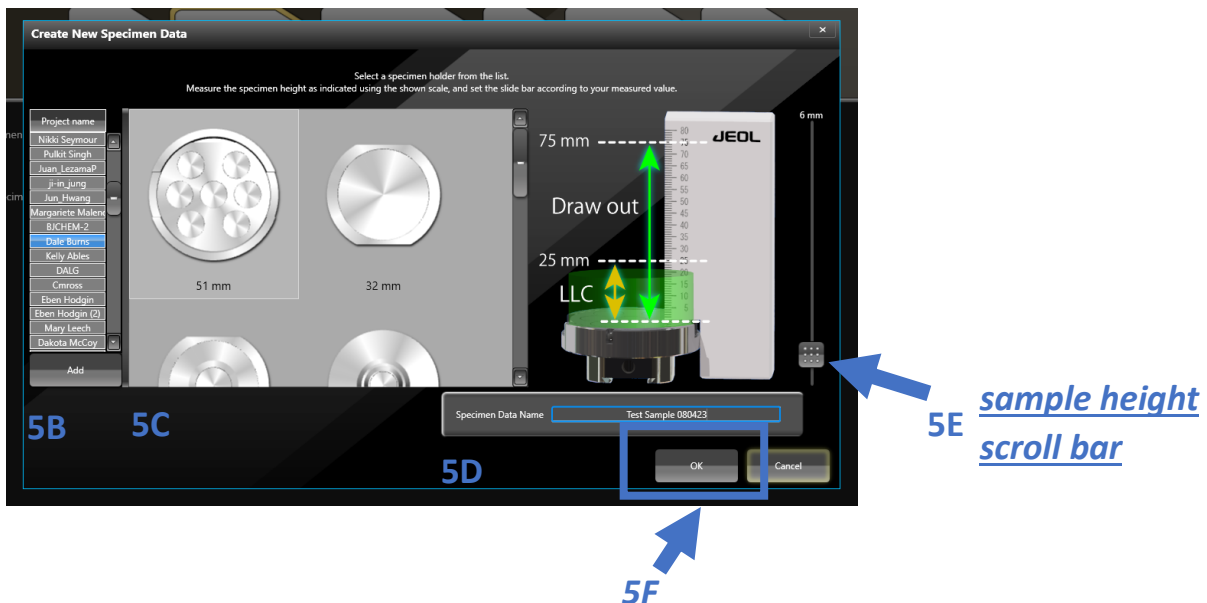
5B. Create a new project name or select a previously created project by either clicking the Add button or selecting a name on the list or (in this case, project refers to the username).

5C. Select the correct sample holder by clicking on the holder (note the 1" plug holder is not currently in the system).

5D. Enter a Specimen Data Name. This is typically the project name and the date.

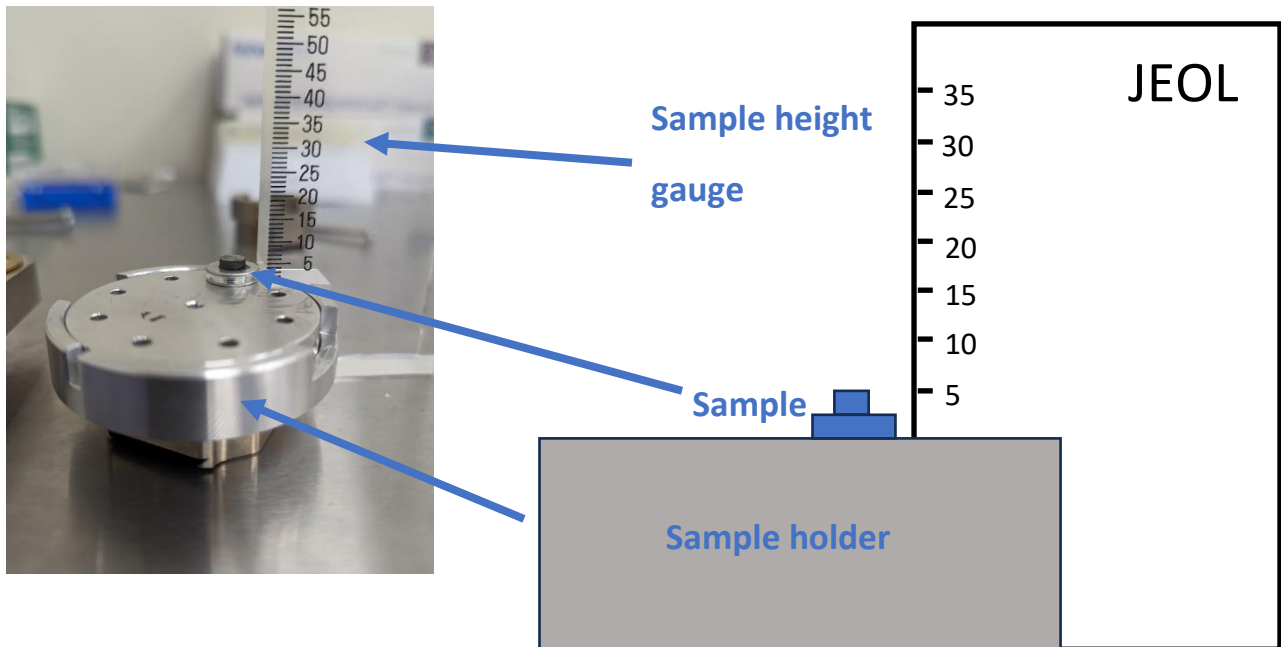
5E. Measure the height of your sample (see note on the next page)

5F. Click OK

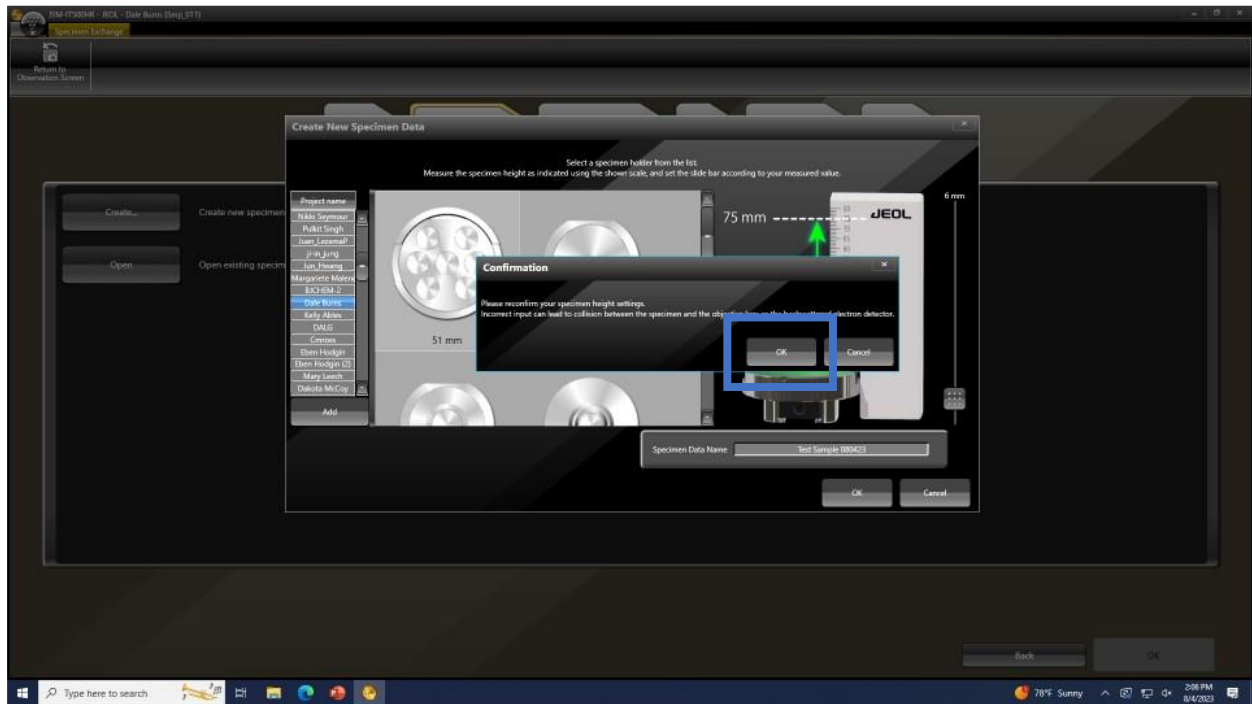


Note: measuring sample height

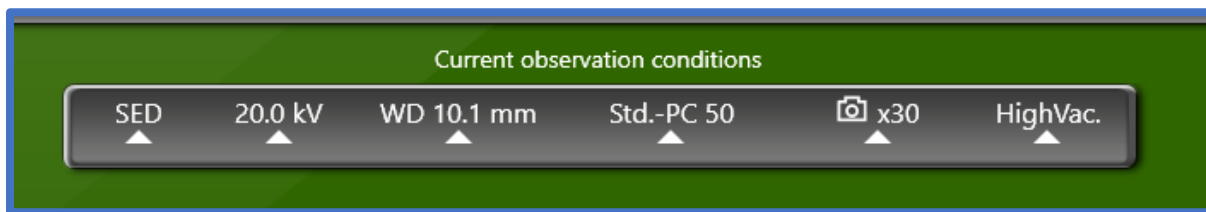
Prior to placing a sample in the SEM, it is critical to accurately measure the sample height. The images below show both an image and a diagram of the sample height gauge. The image on the left shows the correct way to measure the sample height. Note that the top surface of JEOL sample holder is at the 0 mm position. In the case of the image below, the sample height is 6 mm. The JSM-IT500HR can accommodate samples from <1mm to 75 mm tall.



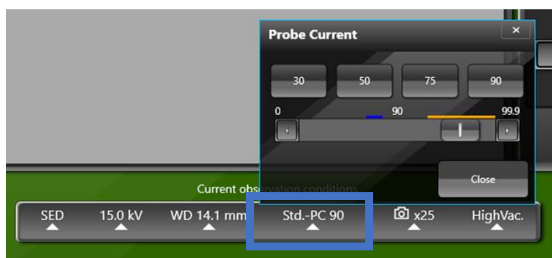
Step 7: Once you click Ok, the software will ask for the sample height to be confirmed. This is to minimize the chance of a sample-detector collision. **DOUBLE CHECK** the sample height. Click OK.



Step 8. In this step, the user chooses the conditions/settings for the electron gun. This will vary between users and the type of analysis. There are multiple preset options, in addition to user-specified stored conditions. To choose/change conditions, click on the white triangles on the Current observation conditions bar (an example is given below with the Std-PC setting). At this stage, it is only important to specify the detector type, accelerating potential, beam current, and vacuum mode. The working distance (WD) and the magnification will be changed in the upcoming steps. For the initial beam alignments use SED (secondary electron detector) mode. Once the conditions are set, click the OK button in the lower right corner of the screen.

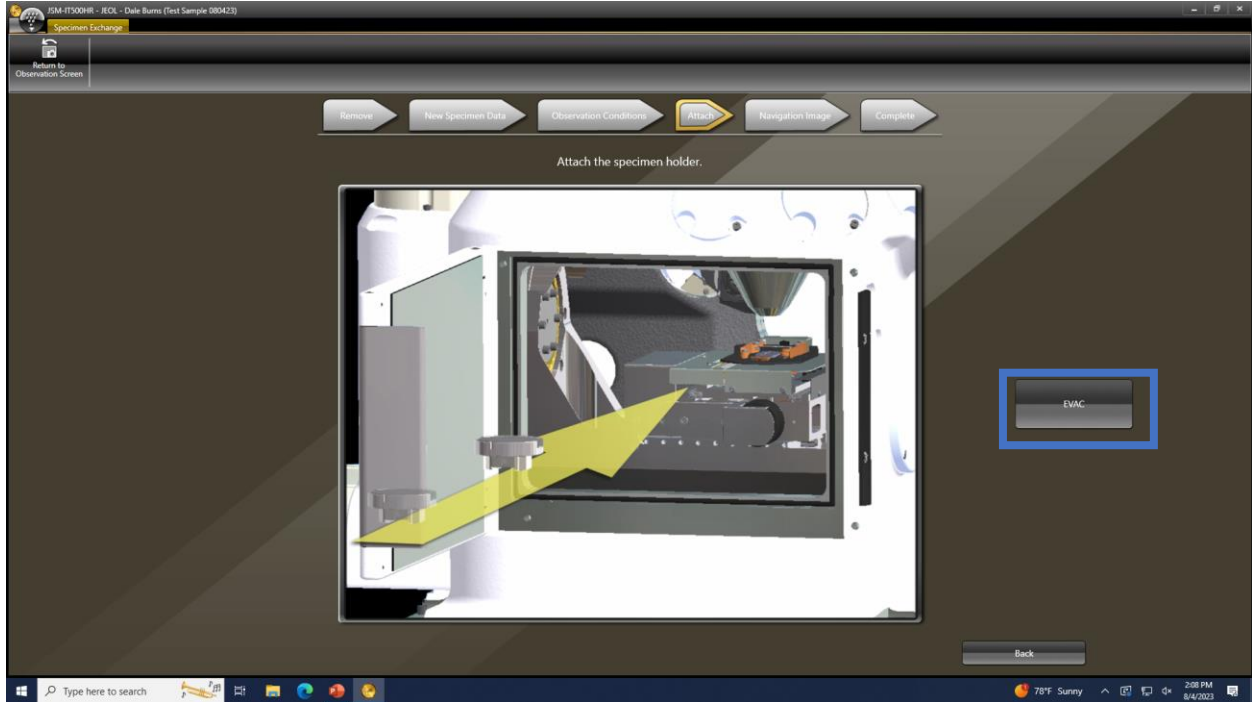


Active detector (i.e., BED vs. SED) Accelerating Voltage Working Distance Beam Current Magnification Vacuum Mode

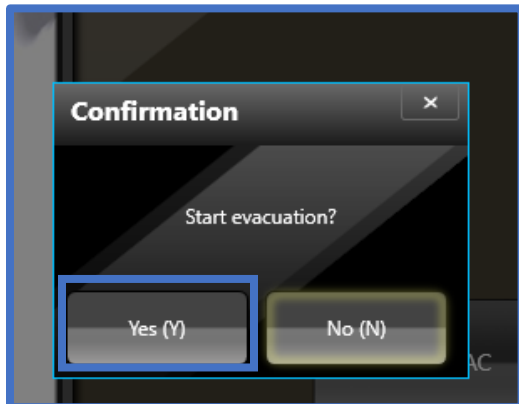


Note: Clicking on the white triangle below Std.-PC opens a box in which the user can adjust the probe current (PC). The PC can be adjusted manually with the scroll bar, or By clicking on a numerical value (i.e., 90). All the conditions/ Parameters on this this bar can be adjusted this way. Remember WD and magnification will be adjusted in the upcoming steps.

Step 9: Attach the sample holder, with your sample loaded, onto the SEM stage (see sample attaching notes on next page). Once the sample holder is attached, **close the SEM door**, and **click the EVAC button (8A)**. When the confirmation box appears, **click YES (8B)**. This will initiate the vacuum system.

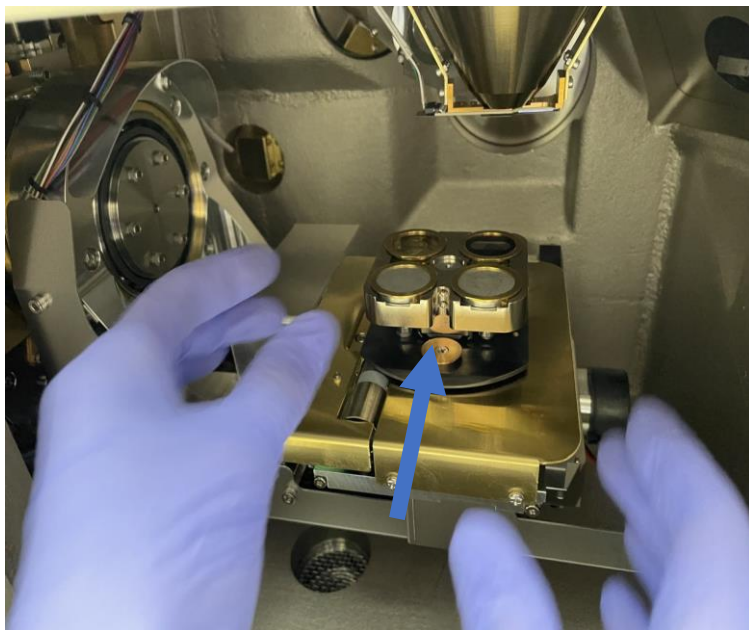
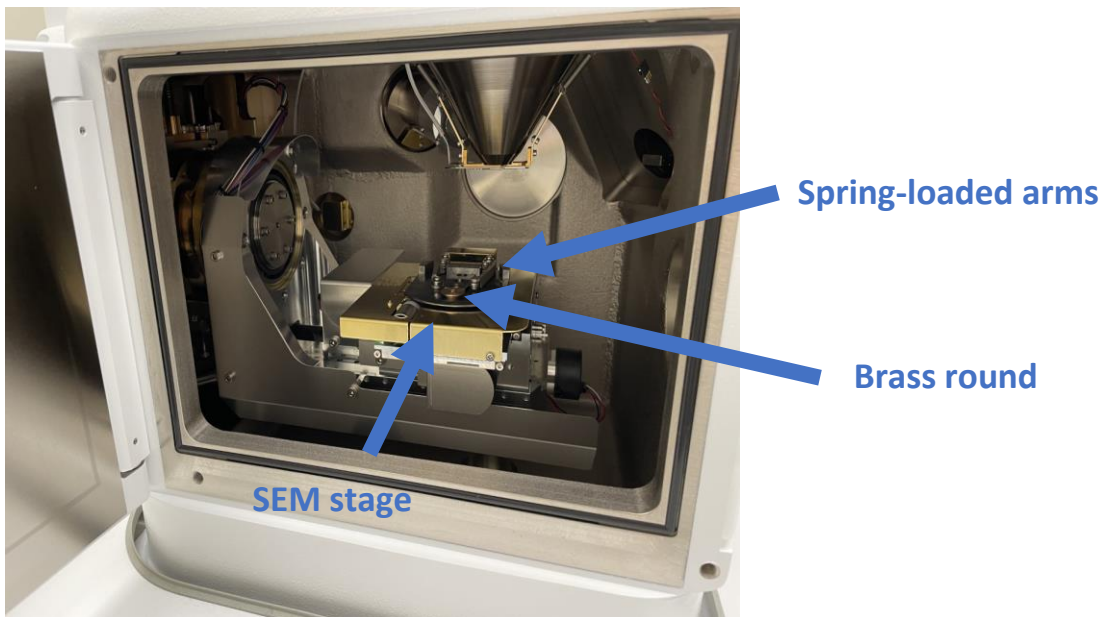


9A



9B

Notes: The bottom of the sample holder consists of two rails with beveled ends. When loading the sample holder make sure that the beveled ends are facing the back of the SEM and slide the sample holder over the brass round and straight back into the spring-loaded arms. It's critical that the sample is at level with the stage and completely flat when sliding over the brass piece. If the sample is not level, it will not attach to the stage.

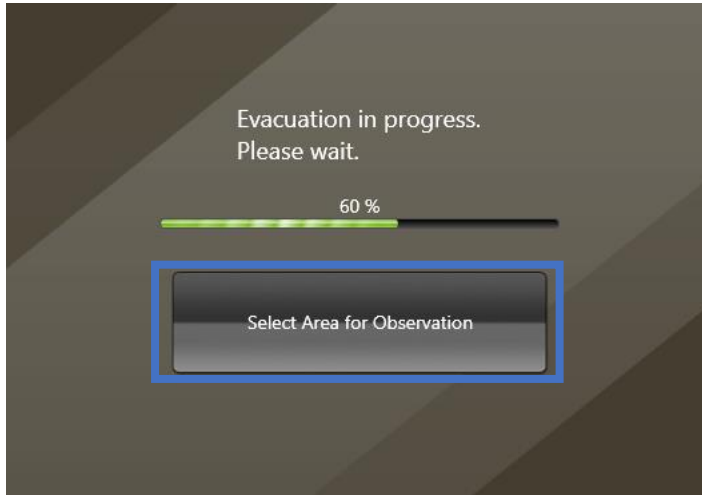


Sliding sample onto the stage

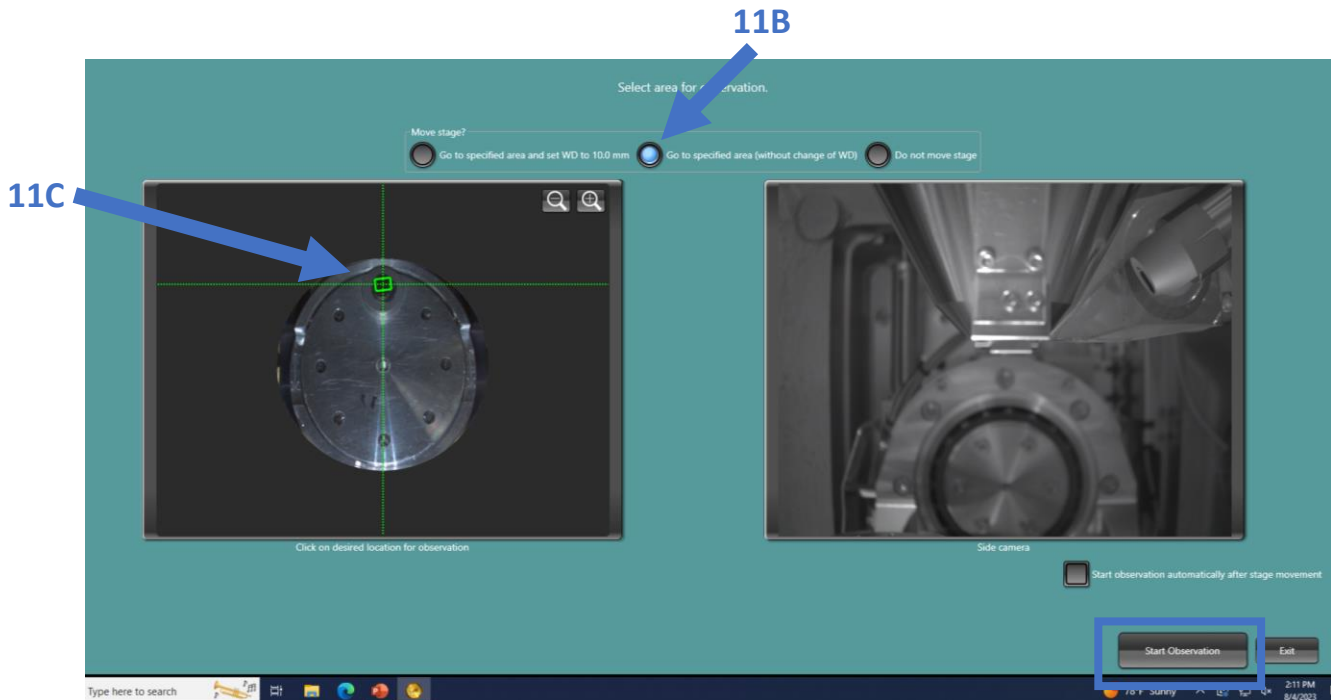
Step 10: While the system is pumping down, the loaded sample can be imaged using the integrated specimen camera. **Click on the Reacquire Photo button (10A).** This will drive the stage to beneath the camera and take a photograph (10B). Once the sample is photographed a box will appear indicating that the process is complete and asking if the user would like to move stage to horizontal position. **Click OK.**



Step 11: Click on the Select Area for Observation button to set stage coordinates (11A). Select the option Go to specified area (without changing the WD)(11B). In this case, the X-Y coordinates will be set, but not the Z. Double click on the image on the left to set the X-Y coordinates for the stage (11C). Click on the Start Observation button in the lower right corner of the screen (11D).

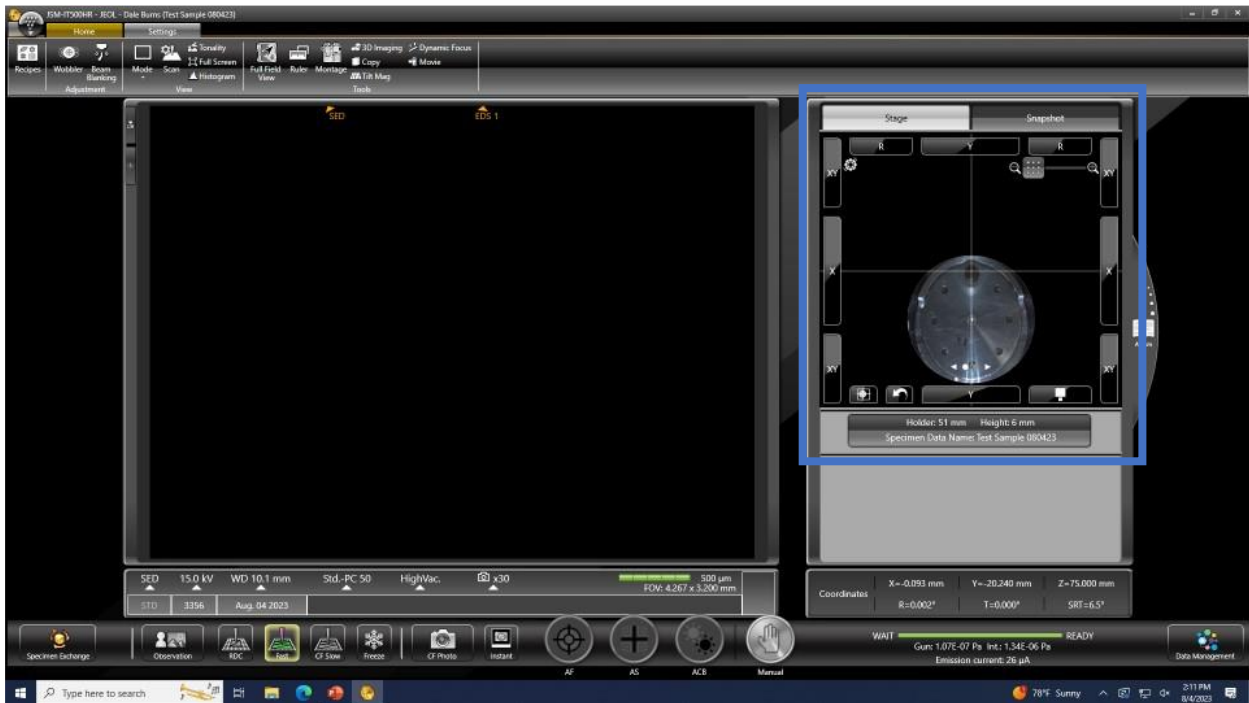


11A

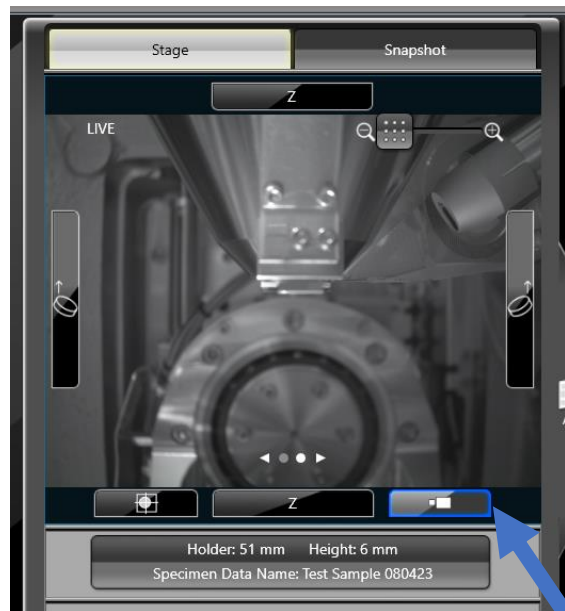


11D

Step 12: Once the vacuum system is ready, the SEM operation screen will open. The sample will be at the assigned X-Y position but will still be 75 mm beneath the bottom of the pole piece. Prior to moving the stage, **click the small, right pointing white arrow beneath the specimen camera image (12A)**. This will turn on the live camera on the right side of the SEM and allow for the stage a pole piece to be viewed while adjusting the stage height.



Specimen cam saved image



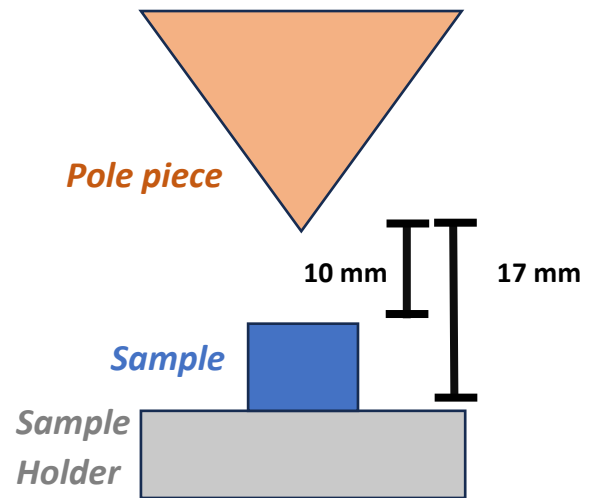
LIVE camera view

Toggle live view and schematic

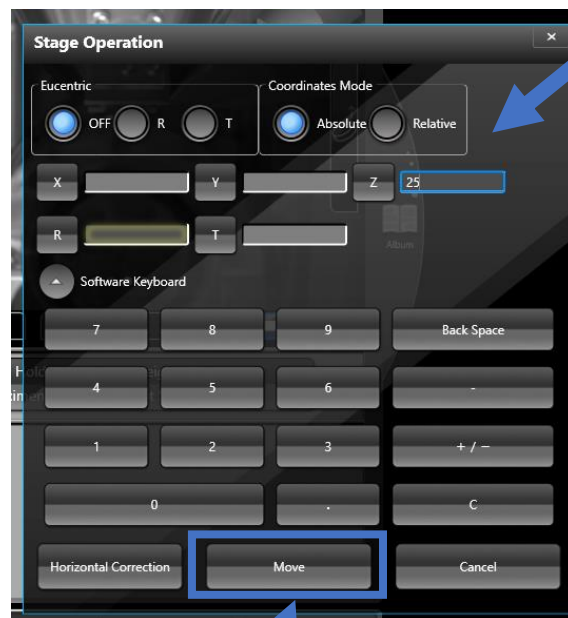
Step 13: To adjust the sample/stage height, click on the Z value (i.e., Z=75.000 mm) in the stage coordinates box (13A). When Stage Operation box appears, a numerical value can be added into the box next to the letter Z (13B). **The target distance between the top of the sample and the bottom of the pole piece is 10 mm for standard imaging.** This IT500HR is typically left in absolute coordination mode meaning that to achieve a 10 mm distance between the pole piece and a 7 mm sample, the Z positions would be 17 mm. **Enter the desired Z value and click Move button (see note below).**

Alert: If a mistake is made and a collision is going to occur after clicking the move button, push the joystick on the SEM operation console (i.e., controls on the table). This will override all stage movement and stop the stage.

Note: While driving the stage to the correct Z position, it's recommended to drive in at least two steps. The first step being ~10-15 mm short of the final stage height (in this case ~25 mm). This allows for a visual inspection prior to moving the final Z position.

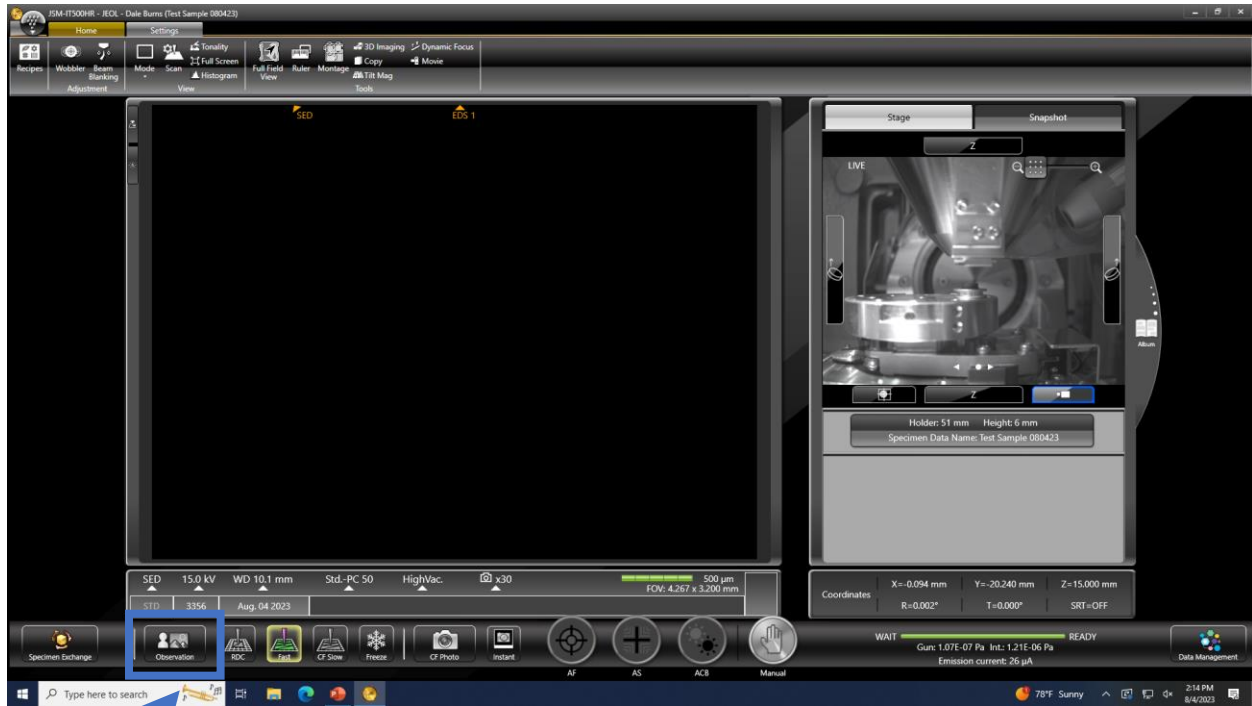


13A

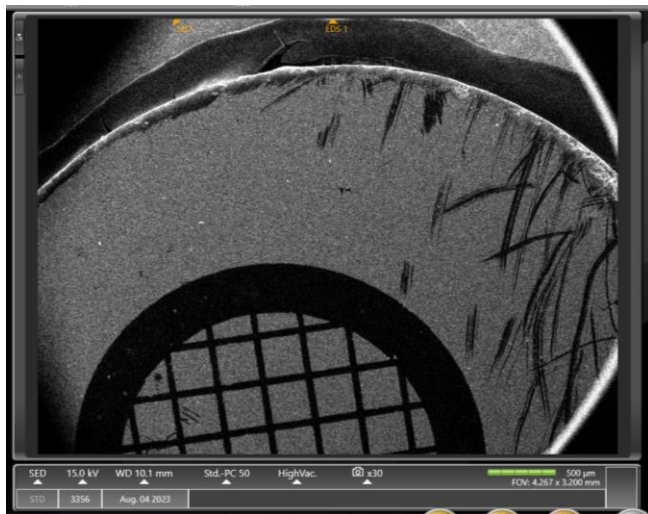


13B

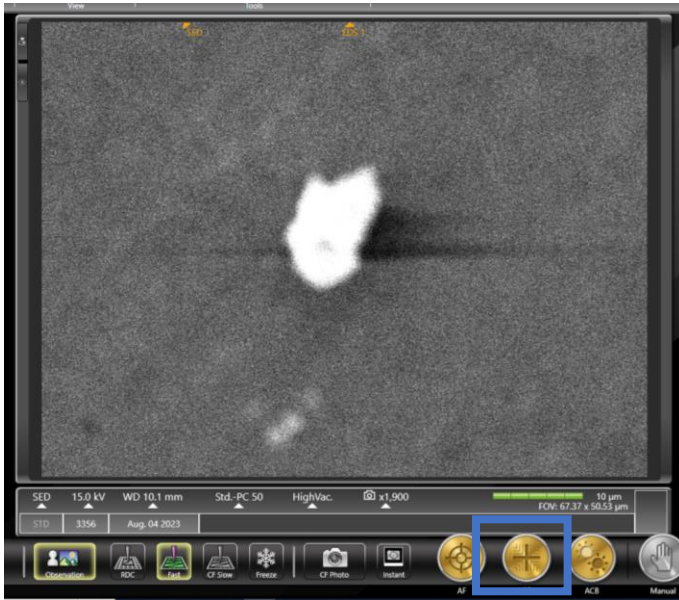
Step 14: Once the stage is in the correct position (left image), turn on the accelerating voltage by clicking on the **Observation** button on the bottom left side of the screen. Once Observation is clicked, an electron image should appear (see lower photo).



Observation



Step 15: In SED mode, zoom in on a small feature with relatively high contrast relative to the background. The images below show a small contaminant on the sample surface, viewed at ~2000x. Center the feature by double clicking or using the joystick on the SEM operation console. Zoom in using the scroll wheel on the PC-mouse, or the magnification knob on the console. **Click the gold AS button** beneath the image. This will run automatic focus (AF), astigmatism (AS), and contrast and brightness (ACB) corrections (for details see next page). Once this sequence is complete, the image will be much sharper with better contrast and brightness.



AF + AS + ACB

Original
Image



Focused
Image

Notes: Focusing and astigmatism corrections are crucial to generating high quality SEM images. Electron microscopes consist of an electron source, where electrons are emitted, a cathode, and a set of electromagnetic lenses that condense the electrons into a beam. **Focusing** the electron beam requires optimizing the distance between the bottom of the column (i.e., final lens) and the sample surface to deliver the smallest possible primary beam diameter to the sample surface. Importantly, the spatial resolution of an electron image is directly controlled by the ability to focus the primary electron beam. In the case of the IT500HR, these corrections are done automatically through the AF function. In most cases, the auto function works very well. However, at high magnification, or with samples that have considerable topography, manual focusing may be required. This can easily be achieved using the focus knob on the operation console. The second major correction that can be critical to generating high quality SEM images is astigmatism. **Astigmatism** refers to stretching and/or distortion of the electron spot due to a lack of magnetic uniformity in the lens material. This is typically due to a lack of precision during machining and/or assembly of the lens. Like the AF function discussed above, the IT500HR has an automatic astigmatism correction feature. Generally, this feature works well at correction for astigmatism. However, at high magnification, an experienced user may choose to fine-tune the corrections manually using the operation console.

Step 16: Another step that is sometimes required to obtain a high-quality SEM image is to adjust the the beam alignment at the objective lens (OL). To check the alignment at the OL, zoom in on a small feature (2000-3000x) and click the Wobbler button (Home tab) in the upper left corner of the screen (16A). Once the wobbler is activated, look for movement in the X and/or Y direction (s). If there is notable movement in either direction, the alignment is off. If the alignment is off, click the ABA button (16B). This initiates an automatic OL beam alignment. If the alignment works properly, the feature will be pulsing in and out of focus but will not translate in the X or Y direction. If the alignment was considerably off, the screen may brighten significantly. If this occurs, the contrast and brightness can be adjusted with the ACB button or manually on the operation console. Once the alignment is adjusted, repeat step 15. It is worth noting that small, high contrast feature that are roughly equant work the best for this alignment. Once the beam is aligned and focused, move forward to the next section.



Section 2: Electron Imaging

Note: The JSM-IT500HR is equipped with two types of electron detector; a secondary (SED) and backscattered (BSE) electron detector. This section provides a brief overview of the two detectors and provides instructions designed to help users generate and save high-quality electron images. This document DOES NOT cover cathodoluminescence imaging as it is less straightforward and requires additional training. This SOP is available upon request.

Secondary Electron Imaging

Secondary electrons are low energy electrons generated at the sample surface in response to either the incident electrons entering the specimen, or backscattered electrons emerging. Owing to their low energies, only electrons generated within a few nanometers of the surface can escape and reach the detector. The relationship between secondary electrons and sample surface is critical for secondary electron imaging. Secondary electron images can show topographic contrast and create images with a crisp 3-dimensional appearance.

Backscattered Electron Imaging

Backscattered electron (BSE) detectors are solid-state detectors that are typically placed above the sample surface and collect incident electrons that are elastically scattered (at high angles) during interactions with the atomic nuclei of the chemical constituents within the sample. Importantly, during these elastic interactions, electron pathways (i.e., amount of deflection) are heavily dependent on the atomic number of the nucleus. By measuring the number of backscattered electrons generated while scanning an electron beam over a sample surface, images can be generated that show atomic number variations within a sample.

Step 1: Choosing a detector

Once the electron beam is aligned and focused, choose the active detector by clicking on the white triangle beneath the signal type and select a signal. SED represents the secondary electron detector, and the various BED signals are types of backscattered electron signal (see note below for details).

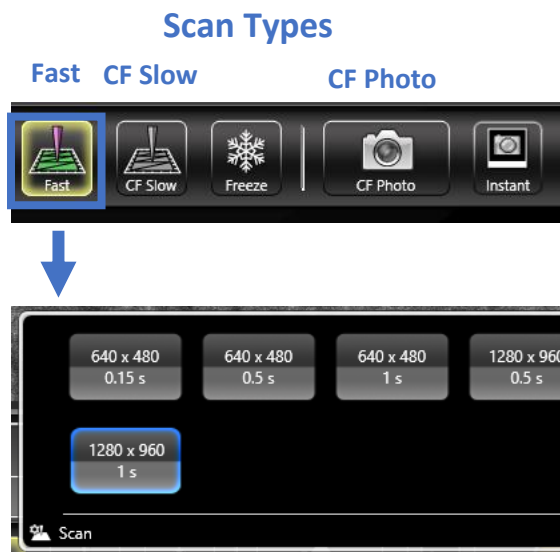


Signal Types

Note: The IT500HR is equipped with a multi-segment backscattered-electron detector. By manipulating signal between the detector segments, the user can generate traditional “compositional” BSE images (BED-C), highlight sample topography (BED-T), or utilize low angle backscattered electrons to emphasize shadowing and create stereoscopic images (BED-S).

Step 2. Scan rates and frame times

When generating electron images, two of the most important considerations are the scan rate and the frame time. For rapid imaging and sample exploration, faster scan rates and lower resolution allow for sample exploration to be done in near real time. ***It's important to recognize that blurry images may be related to low scanning resolution rather than electron beam focus.*** When attempting to image at higher resolution, higher resolution and longer count times are required. In addition, when the signal being imaged (particularly in BED images) is relatively low intensity, longer frame times greatly enhance the signal (sometimes the brightness and contrast need adjusted when drastically changing scan types). There are two general scan types built into the JEOL software, Fast and CF Slow. There are a variety of scan resolutions and frame time combinations that can be selected by right clicking on either the Fast or CF Slow button (see figure below). Once a scan rate is selected, the scan can be initiated by left clicking on either button. A table with recommended scans and frame times is included below. Both the Fast and CF Slow scan settings are intended for sample exploration and characterization. Collecting and saving high resolution images will be covered in Step 3.



Right clicking on one of the scan type buttons (i.e., Fast vs. CF Slow) allows for specific resolutions and frame times to be selected.

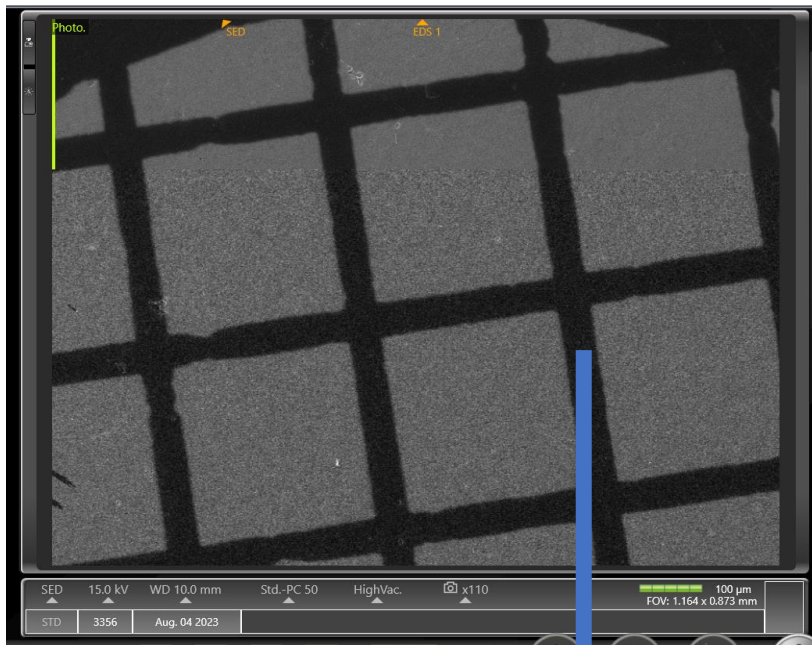
Scan Type	Resolution (pixels)	Frame time (s)
Fast	640 x 480	0.15
Fast	640 x 480	0.5
Fast	640 x 480	1
Fast	1280 x 960	0.5
Fast	1280 x 960	1
CF Slow	1280 x 960	10
CF Slow	1280 x 960	20
CF Slow	1280 x 960	40
CF Slow	2560 x 1920	40

Recommended Fast

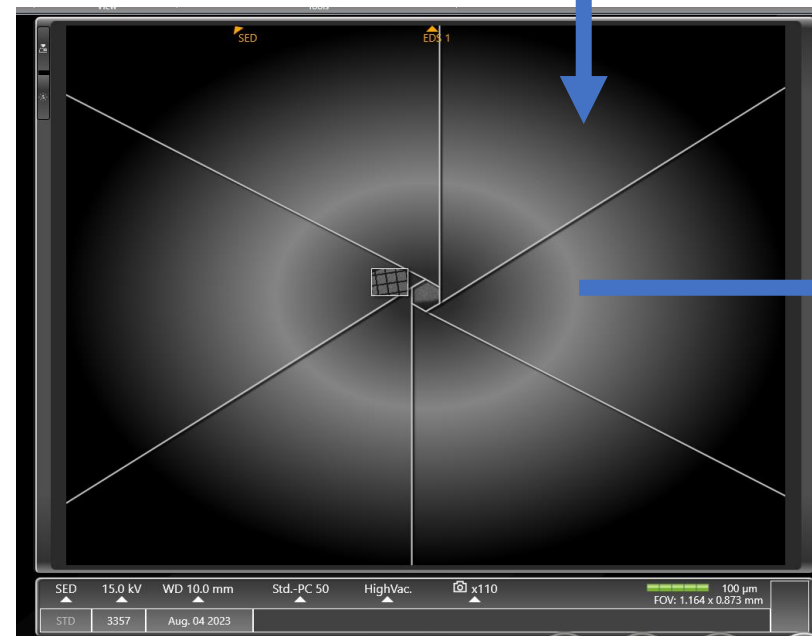
Recommended CF Slow

Step 3. Capturing images

To generate and save a high-resolution image, **right click on the CF Photo** button on the bottom of the screen. This will open a box displaying the available scan resolutions and frame times (see table below). The exact conditions will vary depending on user and the amount of signal needed to show the features/variations of interest. Recommended settings are provided below. After the scan resolution and frame time are selected, **left clicking on the CF Photo button** will initiate the scan. The SED image below shows a snapshot of an active photo scan (note the green progress bar). When the scan is finished, a graphic of a camera shutter will close, and an animation of a file moving into the Data Management tab in the lower right corner of the screen will play.



Active CF Photo acquisition



Camera shutter closing



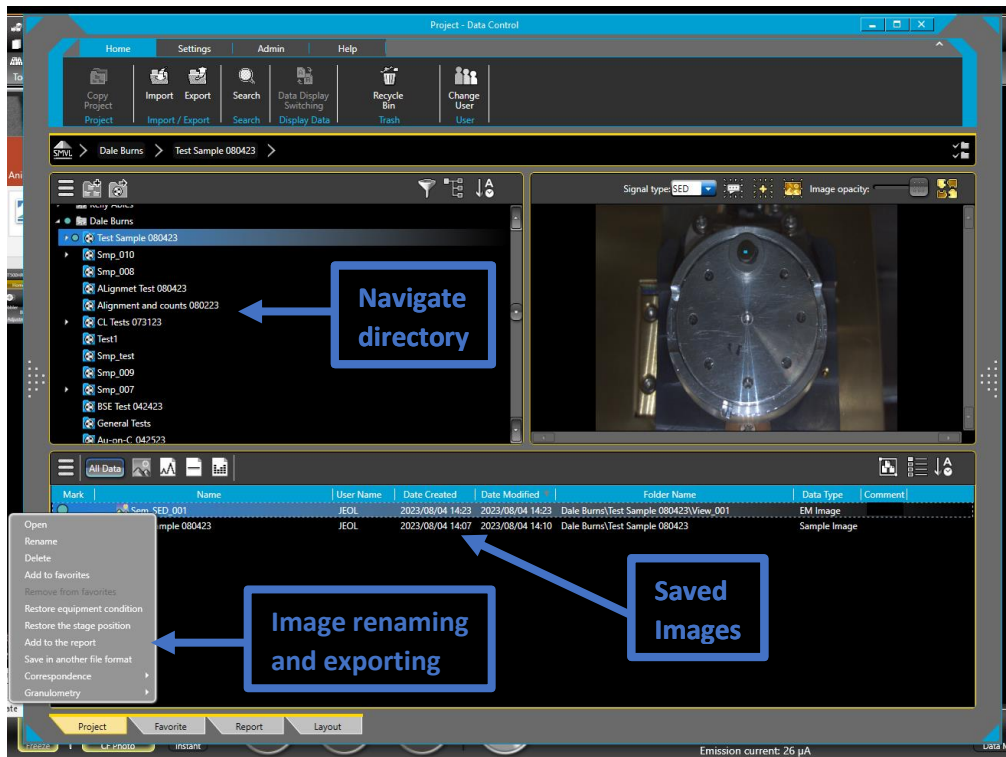
Scan Type	Resolution (pixels)	Frame Time (s)
Photo	1280 x 960	10
Photo	1280 x 960	20
Photo	1280 x 960	40
Photo	2560 x 1920	40
Photo	2560 x 1920	80
Photo	2560 x 1920	80
Photo	5120 x 3840	80
Photo	1280 x 960	160
Photo	2560 x 1920	160
Photo	5120 x 3840	160

Recommended standard CF Photo

Recommended high-resolution CF Photo

Step 4. Viewing and exporting images

To view your images, **click on the Data Management button** in the lower right corner of the screen. This will open a window to the user database. **Navigate to the appropriate username and project** in the lefthand screen. Once the correct project has been selected, the **saved images within the project will be visible in the lower portion of the window**. Note that there will be two types of images saved in the project directory: EM images and sample images. The EM images will have names that include the following: Sem_signal type_number (e.g., Sem_SED_001). These are the electron images. The other type of image, sample images, are images taken with the top-mounted camera during the sample exchange. **To change the name of an image, right click on the image and select Rename** in the drop-down menu. **To export images, right click on the image, or batch of images, and select the Save in another file format option**. This allows for the various types of images to be exported to physical or cloud storage. The system can also be set up to export images automatically during acquisition. See the note below for details.



Note: When starting an SEM session, the system can be set-up to automatically save the images in a user-defined location and format. This can be set by **clicking on the Settings tab** in the upper left corner of the screen, and then **clicking on the Image Setting button**. In the Image settings, file names, format, and export destination can be specified.

