
Scanning Electron Microscope (SEM): Quick Start Guide

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Sample Preparation

1. Push the 'power switch' on the left-hand side of the microscope (Figure 1.2).

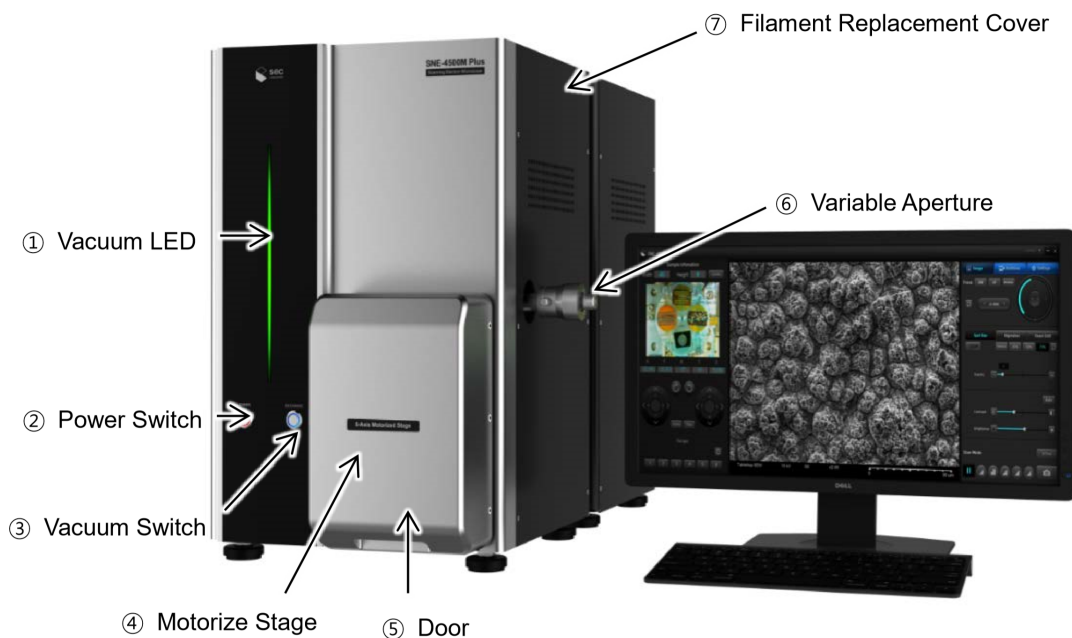


Figure 1. external image of SNE-4500M Plus annotating the key interactions for a user.
Image from the SNE-4500M Plus user manual at www.seceng.co.kr

2. Launch the **Nanoeye** software icon (Figure 2) on the desktop to launch the program

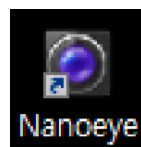


Figure 2. Nanoeye software icon

- Using the jig (Figure 3), measure the size of the specimen including mount. Use the gradations of the horizontal grid on the jig to measure the diameter of the specimen and the vertical grid for the specimen height. If you are using older 3.2mm style stubs with a narrow attachment post, there is an adapter available to fit into the stage. Make sure to measure dimensions with the adapter attached.

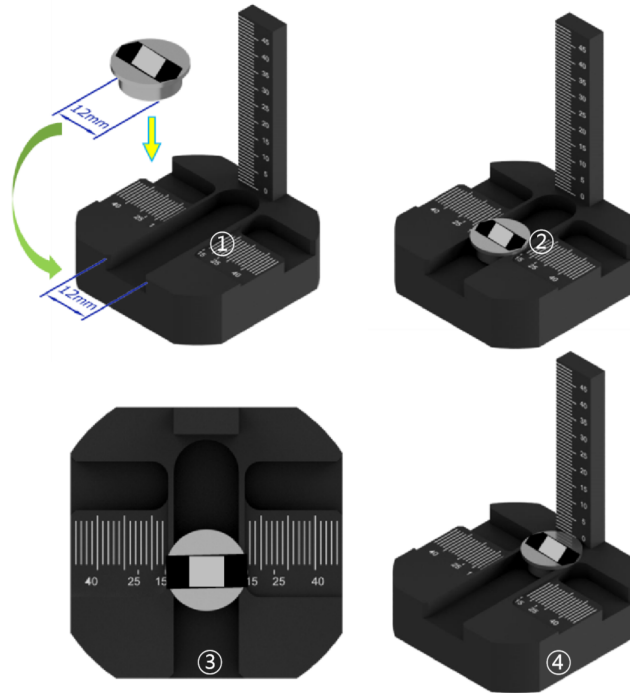


Figure 3. The mounting jig used to measure the width and height of the sample.

- For sputter coating, please see the **Sputter Coating Quick Start Guide**.
Important: use compressed air to ensure that blow off any loose material on the sample, that otherwise could be mobilized within the vacuum and damage the detectors.
- To do the specimen exchange, the SEM needs to be pumped with air. Press the **Exchange** button on the front of the SEM. The button LED light indicates the inner vacuum status of the chamber, and the signals are:
 - Light Off*: Vacuum is not applied
 - Light On*: Vacuum is applied
 - Slow blinking*: Vacuum is being released
 - Fast blinking*: Vacuum is being applied
- Fully open the door and click the 'calibration' button to initiate the stage motor calibration (Figure 4). This should take around 3 minutes.
- In the **Nanoeye** software, click the "**Sample Information**" text box (Figure 4, red rectangle), enter the height and width values, then press **Enter**.

Important: The command will not be registered if the user fails to hit enter.

After the height is entered, the Z-axis will automatically lower the stage to accommodate for the entered height to a distance of $Z = [\text{Entered height}]$.



Figure 4. **Nanoeye** window showing the sample preparation commands. Enter the height and width into the Sample Information (red box).

8. Insert the stub to the stage:
 - a. Use a 1.5 mm allen wrench to carefully loosen the set screw, then insert the mount with the specimen to the stage, and tighten the set screw (Figure 5).

Important: Ensure that stub (+/- adaptor) is seated fully into the stage mount. Use caution when loading samples to prevent damage to detectors. Wear gloves when handling any components that go into the vacuum chamber.

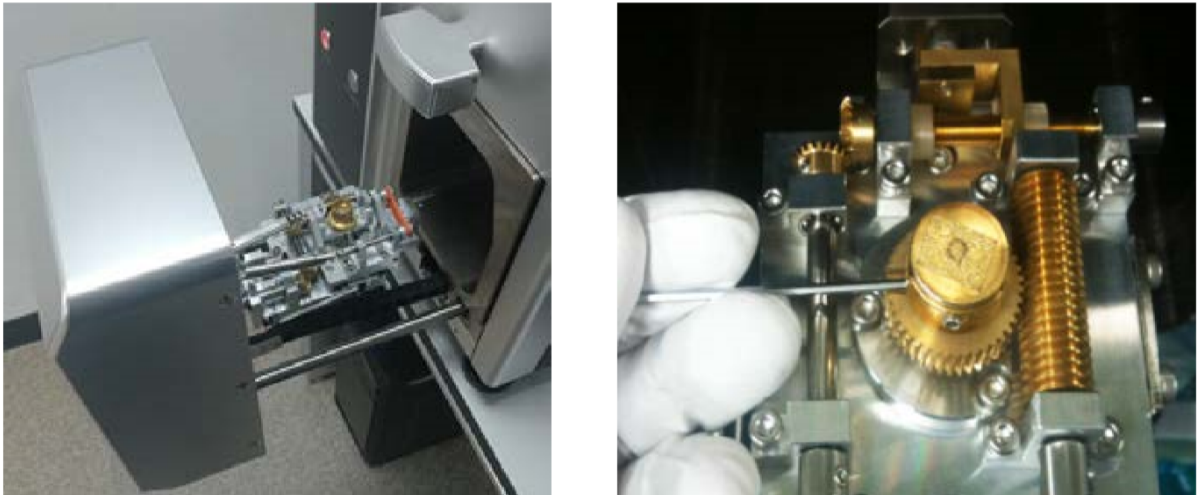


Figure 5. Mounting the stub to the stage.

9. Capture the specimen image to aid in navigation of the stub. To do so:
 - a. Slide the door halfway closed to the point where the door catches and clicks.
 - b. In **Nanoeye**, click the “**Camera**” button and the screen display will show the specimen (Figure 6).
 - c. With the camera activated, right click the “**Camera**” button to activate the brightness/contrast menu (Figure 6).
 - d. Click the camera button again to take an image.

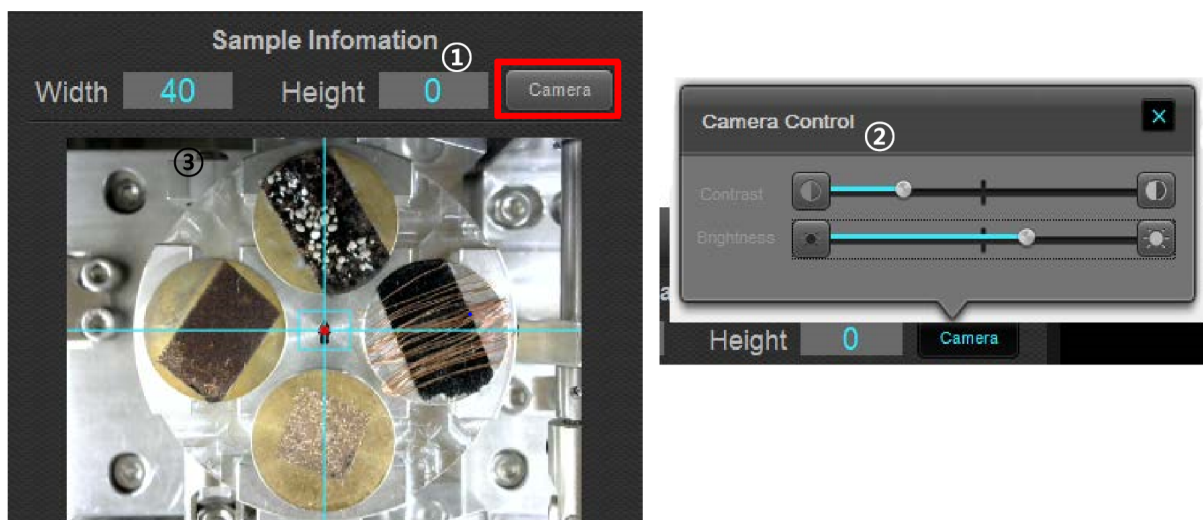


Figure 6. Display windows of the navigational camera operations.

10. Close the chamber door gently and push the **Exchange** button (Figure 1.3) to put the chamber under vacuum. Gently press the door into the SEM to aid the seal as the vacuum begins to pump down.

Turning on the beam

1. Once the machine is under vacuum (indicated by the SEM beeping twice), navigate to the Operation Panel (Figure 7). Select an accelerating voltage (range: 1 to 30 kV; see table 1 for working guide). Select a detector type: SE (secondary electrons) or BSE (backscattered electrons). Click the START button to generate an electron beam.

1-5 kV	Delicate or uncoated samples (e.g. microfossils)
5-10 kV	Coated biological samples (e.g. <u>Au coated microfossils, recommended</u>)
10-30 kV	Carbon-coated thin section samples

Table 1. Working guide for accelerating voltage

- a. Monitor the emission current, which should be around 110uA. If it has diverged by more than ± 20 uA, please notify a technician as this either means the filament is about to die or the beam is not stable, and image quality will be subpar.
- b. Once the beam is on, you can navigate around the sample in the x-y direction by double-clicking on the SEM display or sample map/camera screen. Magnification can be adjusted using the mouse wheel.

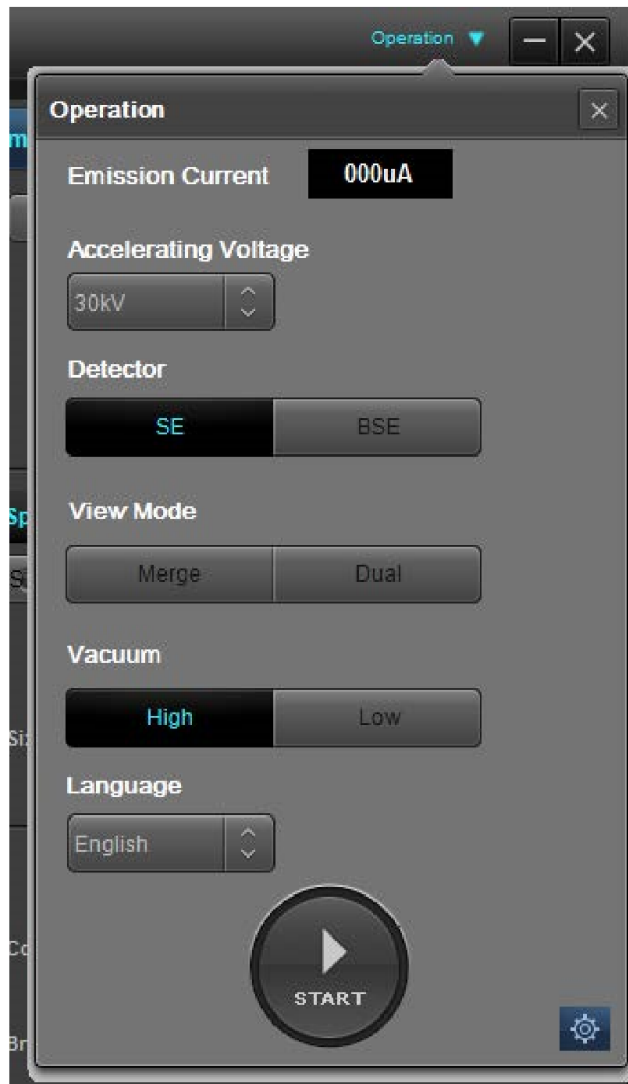


Figure 7. Operational start-up window

2. The adjustment, signal processing and scan rate controls will pop up on your left (Figure 8). These processes will be described in more detail as follows.



■ Focus Area

■ Beam Area

■ Image Area

■ Scan Area

Figure 8. The left-hand panel consists of the focusing, beam area, signal processing (Image area) and scan rates(Scan area) controls

3. Select the “Scan Mode” for adjustments and navigation (Figure 9). Numbers correspond to buttons in Figure 9.
 - a. 1= Fast Scan: Real-time screen (320*240). Adjust focus and move position.
 - b. 2= Slow Scan: View and edit image before save (640*480). View and edit the image before saving it.
 - c. 3= Fast Photo: Save image at high resolution (1280*960)
 - d. 4= Slow Photo: Save image at highest resolution (2560*1920)
 - e. 5= Slow Photo2: Save image at highest resolution (5120*3840)
 - f. 6= Freeze scan: note the beam is still on, just the image is frozen
 - g. 7= Save image: After selecting the option, the image will finish its scan through the window before automatically opening a save window. This should be selected when you start your slow scan.

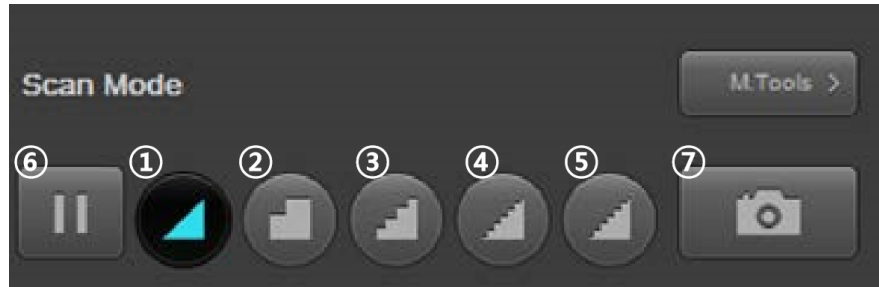
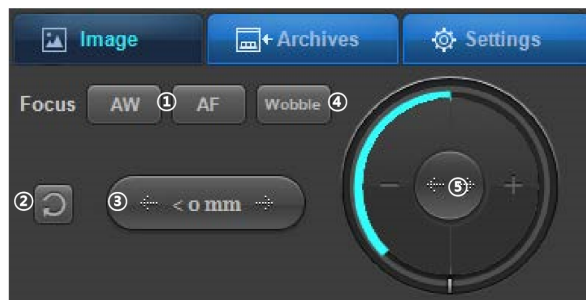


Figure 9. Various scanning speeds. Button in the top right is the measuring tool, where you can measure the distance and angle between two points, measure a marked area and annotate

Adjustments

1. Focus your specimen:
 - a. Click the buttons under “Focus” area to adjust the focus (Figure 10). Perform primary adjustment at >1,000x, rather than using a low magnification <500x.
 - b. Note, if the screen wobbles a lot, either horizontally or vertically during focus adjustment, let the technician know and they will adjust the aperture’s alignment using knobs on the side of the instrument.



No.	Description
①	Automatic focus adjustment
②	Reset (restores the default state.)
③	Focus adjustment (Coarse Focus)
④	Beam re-alignment (sets the variable aperture center)
⑤	Fine focus adjustment (Fine Focus)

Figure 10. the focus adjustment window, with descriptions of each button

2. Focus the beam area (spot size):
 - a. If the image looks dark, adjust the “Spot Size” (the lower the % value, the greater the amount of beam and the brighter the image).
 - b. Reduce the spot size of the beam when the amount of focused beam becomes larger. This option is suitable for ultra-fine images (the higher the % value, the tighter the beam focus, but the image will also become darker).

3. Correct the beam shape (Stigmatism tab). Perform this adjustment if you see streaking in your image.
 - a. The best procedure to correct for astigmatism is to set x and y stigmators to zero offset, then fine focus the sample as well as possible.
 - b. Then adjust either the X or Y stigmator controls (not both) for the best image and refocus the image.
 - c. When the best image has been obtained with one stigmator, use the other stigmator to get the sharpest possible image.
 - d. Note that astigmatism is usually negligible in an image at less than 1000x.

Signal processing

1. Adjust the contrast and brightness of your scan to achieve appropriate image levels.
 - a. The image becomes brighter or darker as the scroll bar moves right or left, respectively (Figure 8).
 - b. A good quality image has a gradation of greys with very little of the full image fully black or white.
 - c. Another mechanism to increase SE contrast in an image is to tilt the sample so that it is at an angle to probe (typically 30 ° to 60°). As a result of tilting, more SE are generated per unit of projected specimen area and this enhances contrast by making the distribution of light and dark areas more pronounced. Take care in tilting larger samples (e.g. thin sections, smear slides) to avoid collision between the sample and the EDS detector.
2. When you are satisfied with your scan, select the slower scan rate (Figure 9, button 5) and the camera icon (Figure 9.7)
 - a. Two file types are generated by the SEM:
 - .tif : This file is the image taken by the software.
 - .txt : This file contains the metadata for the image. This file includes parameters under which the image was taken, instrument specifics, filename, timestamp, and directory.
 - b. Naming convention for SEM files:
 1. EXP number
 2. Site
 3. Hole
 4. Core
 5. Section
 6. Sample
 7. Taxa/specimen/piece
 8. No. 1, 2, 3...

E.g.

349_U1431A_1H_1_10-11cm_Emiliana_huxleyi_1,
 349_U1431A_1H_1_10-11cm_Emiliana_huxleyi_2,
 349_U1431A_1H_1_10-11cm_Emiliana_huxleyi_3, ...

c. Storage convention for SEM files on the local PC:

1. SEM
2. Site
3. Hole
4. Sample

E.g.

Folder SEM

Folder 1341

Folder 1341A

Folder U1341A_1_H_1_1-11cm (all files for this SEM sample)

d. NOTE: All files need to be moved to USERVOL and DATA1 by the end of the expedition.

Finishing up SEM session

1. When you are finished scanning, turn the beam off by clicking the stop button in the Operation dropdown panel.
2. Push the vacuum button (Figure 1.3).
3. The door will be easily opened once pumped with air
4. Remove your specimen mount from the holder (Figure 5)
5. Fully open the door and click the 'calibration' button (Figure 4.2).
6. Close the door, push the vacuum button (Figure 1. 3) to pump out the air from the chamber and to create a seal with the door.
7. Once the vacuum has be reached, turn off the SEM (Figure 1.2)

Recommended workflow for obtaining the best possible SEM images

