Scanning Electron Microscope
JSM-IT710HR/LV

Scanning electron microscopy background and a user procedure for the SEM JSM-IT710HR/LV located in the High Resolution & Advanced Imaging Microscopy Center

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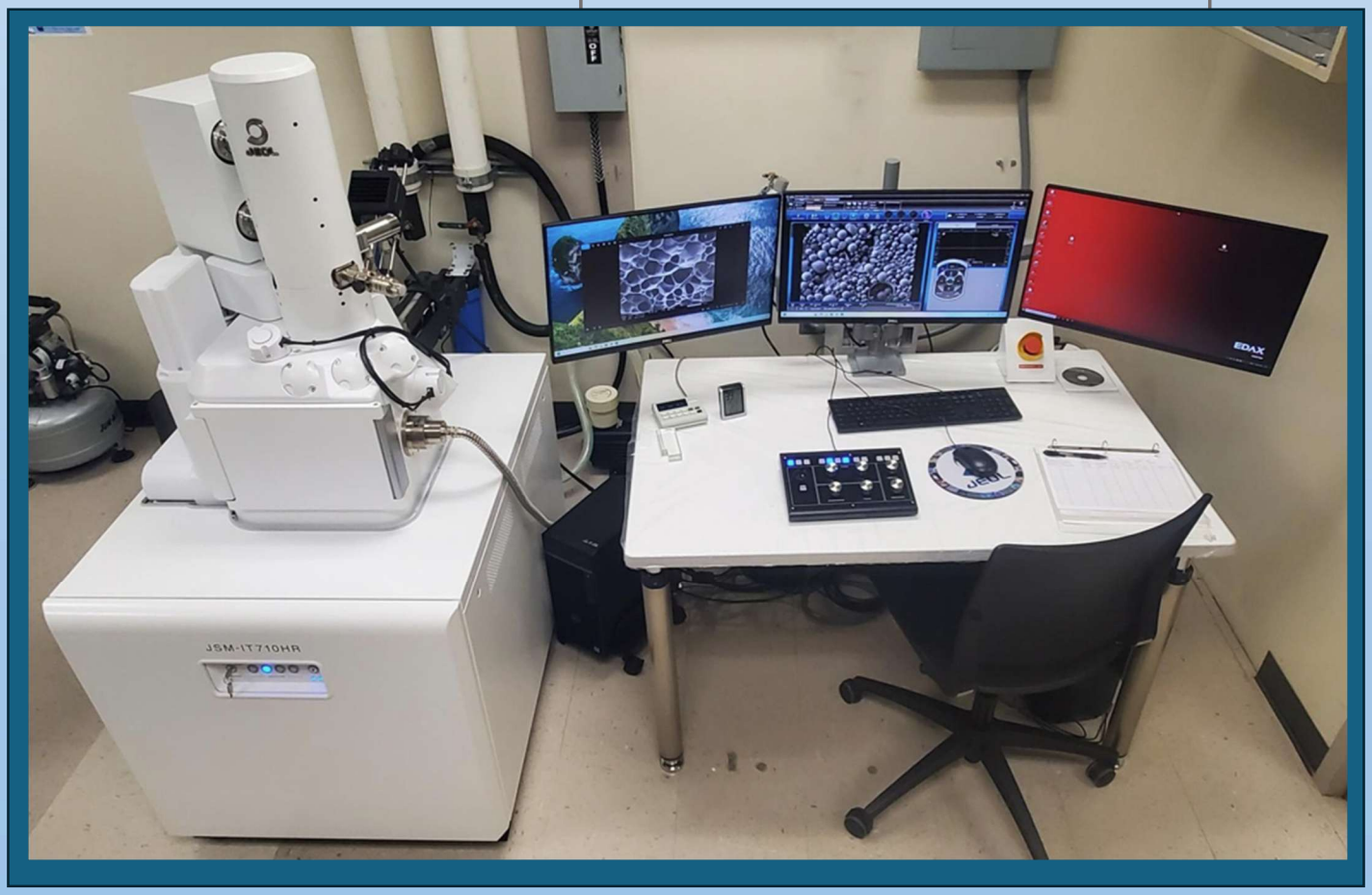

Safety precautions.

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⚠ DANGER

- **Effect of the magnetic field**

  This instrument generates a magnetic field leakage of about 1 mT. Keep magnetic products, such as iron, away from the instrument, or if you are wearing a medical device or implantable medical device that is affected by a magnetic field, do not go near the magnetic field generating part of the instrument shown in the figure below.

⚠ WARNING

For the parts that might be hazardous due to electrical, thermal, or radiative properties, do not remove the shielding parts to expose them, remove the parts to modify them, or disassemble them in a way that is not described in the instruction manual.

⚠ CAUTION

- **If you find any abnormality of the instrument (abnormal noise, abnormal odor, natural disaster, etc.), immediately turn the main power key switch to off (○) to stop the instrument and contact your local JEOL service office.**

- **When opening or closing the specimen chamber front door, be careful not to pinch your fingers between the door and the specimen chamber.**

- **Be careful not to touch the protruding part of the EDS detector attached to the main unit of the electron microscope.**

  There is a risk of personal injury or damage to the instrument.

- **If you continue to stare at the monitor in the same position for long periods of time, your eyes and body will become fatigued.**

  For your health, take a 10 to 15 minute break every hour to rest your eyes and body.

I. Theory of Scanning Electron Microscopy

A scanning electron microscope (SEM) allows the observation of heterogeneous organic and inorganic materials on a nanometer to micrometer scale. SEM uses electron beam just like an optical microscope uses light rays to visualize the object. The interaction of electrons with the object can result in different effects which are utilized in imaging and chemical analysis performed by SEM.

Electron microscope was developed to overcome detection limits of optical microscope. Since microscopy is the study of objects illuminated by radiation or particles the wave length of illuminating electron or light beam limits the resolving power of the microscope. Electrons have the wavelength much shorter than the wavelength of light rays resulting in that the resolving power of electron microscope is orders of magnitude better than for the optical microscope. Resolving power is the smallest distance between two points that microscope can observe separately.

Fig. 1. (Taken from https://www.nanoscience.com/techniques/scanning-electron-microscopy/). Limits of detection. The electron microscope was developed when the wavelength became the limiting factor in light microscopes. Electrons have much shorter wavelengths, enabling better resolution.

The smallest features that you can resolve by SEM are 1 to 20 nm apart, while theoretical limit of resolution (not visibility) of the light microscope in white light of about 200 – 250 nm. In theory an electron has an equivalent wavelength less than 1 nanometer, which can enable the resolving power of ordinary electron microscope to be 0.1 nm, however the construction details of the microscope and specimen characteristics diminish the resolving power. Our SEM model JSM-IT710HR/LV can guarantee 1.0nm resolution at the accelerating voltage (Acc V) 30kV in a high vacuum mode.

In scanning electron microscopy signal detection begins when electron beam enters a specimen. The interaction of an electron beam and the atoms composing the specimen produce various kinds of information. Right panel in Figure 2 shows the different types of information produced and the regions from where the information is produced.

When the primary electron enters a specimen it will penetrate into it for some distance before colliding with another particle. After colliding with an electron, nucleus or other particle, the primary electron will follow a new trajectory, known as scattering. It is the components of scattering events that are detected by SEM, some of them presented in figure 2 (A-E) as various modes of electron emission.

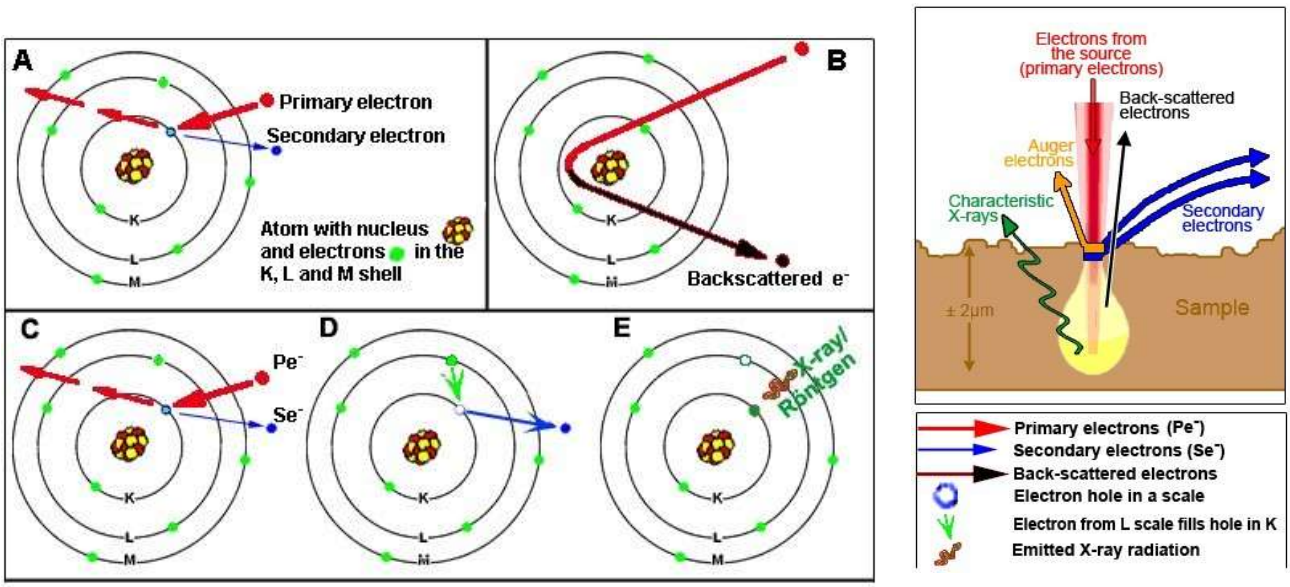
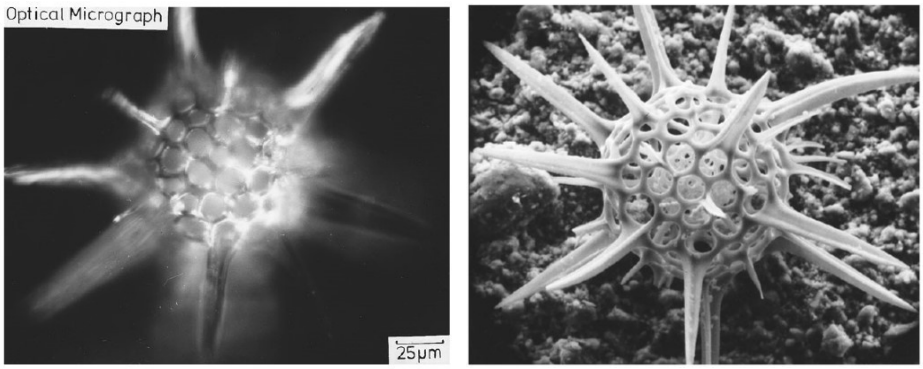


Fig.2. (Taken from https://www.vcbio.science.ru.nl/en/fesem/eds/) A –Secondary electron emission. The secondary electrons represent valence electrons that are dislodged by bombarding with primary electrons. Secondary electrons are formed by inelastic scattering. B – Backscattered electron emission. The backscattered electrons are formed by elastic scattering, when larger atoms deflect trajectory of primary electrons. C,D,E – X-Rays formation. When an ejection of inner shell electron (C) is compensated for by an electron moving from outer shell and filling a vacant energy level (D). Simultaneously with electron transition from a higher orbital level to a lower orbit an excess energy will be released and generate characteristic X-Rays (E).

Scattering components include secondary electrons (SE), backscattered electrons (BSE), Auger electrons (AE), XRays, cathode luminescence and other phenomena. They arise from different depth of the specimen and provide microscopic information about the specimen. This forms a basis for another advantage of the SEM over the OM described as depth of focus or an ability of a microscope to bring the features of an object at different depths into focus. Figure 3 shows that in the light microscopy image (A) only a portion of the radiolarian is in focus, while in the SEM image (B) the whole sample is in focus.

A 

B

Fig. 3. (Taken from J.I. Goldstein et al., eds., Scanning Electron Microscopy and X-Ray Microanalysis, (Plenum Press,NY,1980).)

The most commonly used electron scattering components in SEM is the secondary electron. As shown in figure 2 A secondary electron is generated when a primary electron penetrates the electron shells and dislodges a valence electron. Secondary electrons can only be detected when they are ejected near the surface of the sample, because they possess low energy level of only a few electron volts. If they are formed deeper in the sample, they cannot escape from that distance to the surface and become absorbed. Operating in a secondary electron imaging (SEI) mode allows to obtain detailed topographical information of the sample surface with high resolution. Imaging in the secondary electron mode remains most popular because the contrast and soft shadows of the image retain close similarity with that of an optical microscopy image. Thus, image interpretation is easier since the images look more familiar.

Backscattered electron emission occurs due to the scattering of primary electrons in such a way that they escape back from the sample without going through the sample (Fig. 2 B). Since backscattered electrons are original primary electrons, they possess high energy level near that of a gun voltage. These types of electrons conserve their energy, but their trajectory has been modified by size of the atoms as well as by density of the atoms. The regular arrangement of atoms in crystals can influence the backscattering of electrons compared to those when same atoms randomly distributed in an amorphous material. Thus, this BEI mode is used in characterization of crystalline specimens.

X-rays are generated due to the de-energizing of the atom in the sample after secondary electron emission occurred. Soon after the ejection of secondary electron the newly formed vacant orbit is filled by the electron dropping down from a higher energy orbital level (Fig. 2, C-E). During this process an excess energy is released in the form of characteristic X-rays with the wavelength and energy specific for elemental atom from which it originated. This signal is extremely important for analytical purposes since analytical X-Rays enable qualitative and quantitative analysis of the specimens. This phenomenon allows determination of elemental composition of the specimen and known as Wavelength dispersive spectroscopy (WDS) or energy dispersive spectroscopy (EDS).

Specimen current is important characteristic of electron- sample interaction. Most of the time the primary electrons undergo multiple scattering events and lessen their energy level to the point where the electrons are absorbed by the sample, phenomena known as specimen current. Changes in specimen current can influence imaging substantially and can be regulated to improve the imaging quality. In most electro-conductive samples the induced current is just led to the ground. In the non-electro-conductive samples like most ceramics and polymers, the region being irradiated by the electron beam will build up a negative charge. This is known as charging and can be alleviated or completely prevented by properly mounting of the samples using grounding practice, electro-conductive tape or carbon paint as well as coating the samples with a conductive material such as gold.

SEM is a capital equipment, it is expensive, requires maintenance and a substantial laboratory space. “JSM-IT710HR/LV SEM” system comprises an Electron optical column unit, an Operation desk, ion pump, rotary pump, an air compressor, cryo-SEM unit and UPS (Fig. 4). The SEM column includes secondary electron detector, backscattered electron detector, EDS and EBSD detectors.

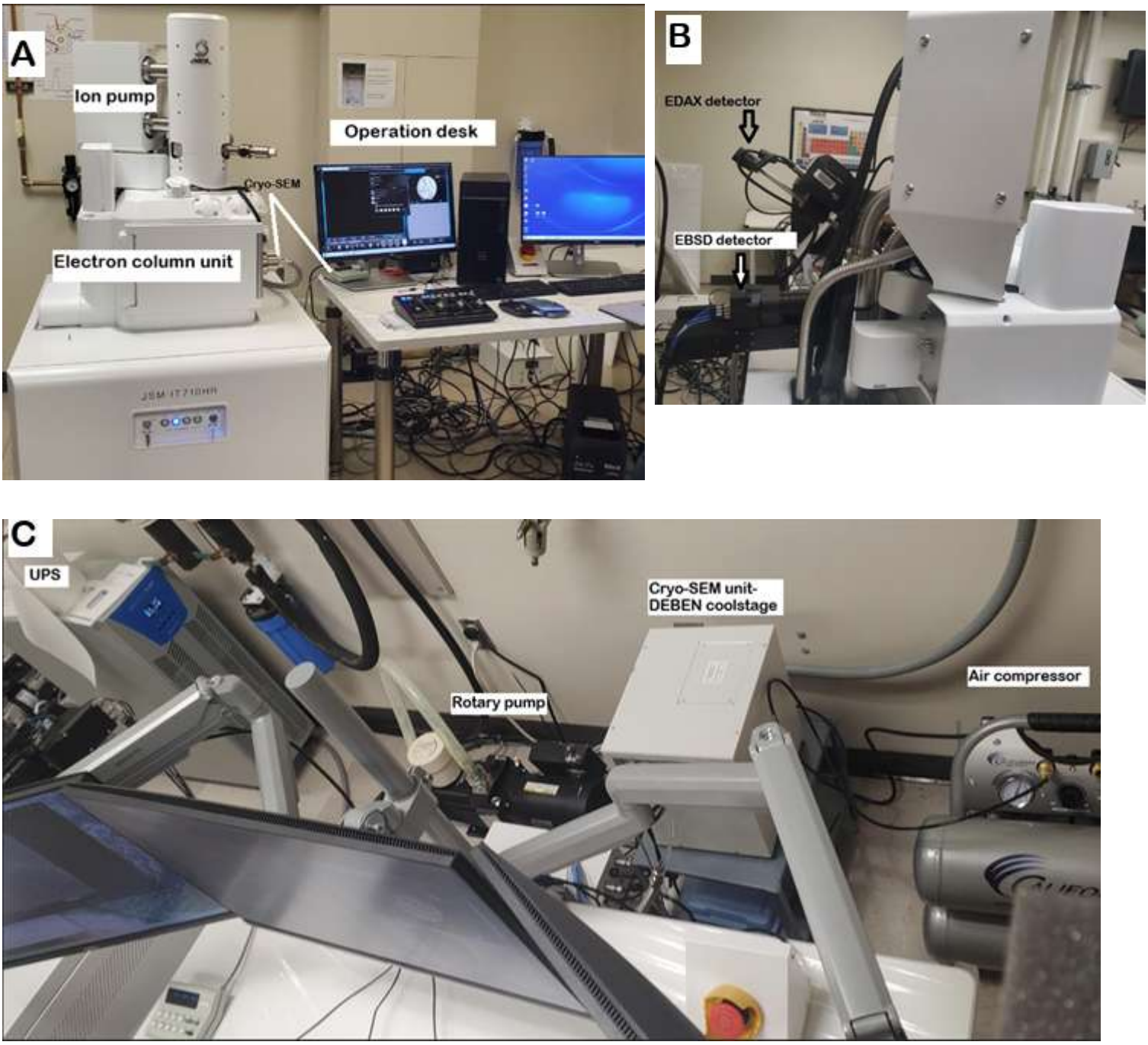


Fig.4. Front view (A), side view (B) and back view (C) of the SEM.

Multiple systems required to support operation of SEM. Fig.5 shows major components of the SEM, which include among others a vacuum system, generation of electron beam, electron beam manipulation lenses and coils, detection system, signal processing and recording parts. Well-coordinated operation of these systems enables to obtain high quality images that can be tuned in its magnification, resolution, depths of field, contrast and brightness.

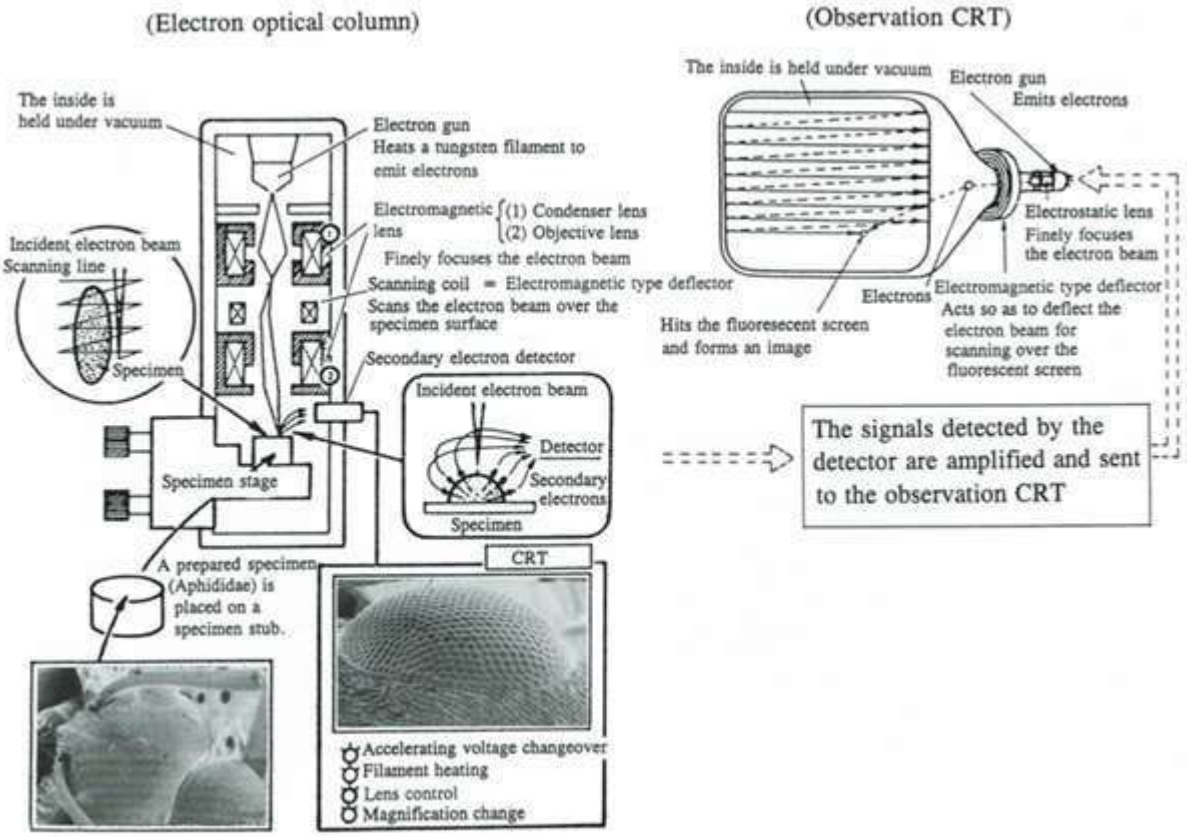


Fig. 5. (Taken from the booklet "Invitation to the SEM World", JEOL LTD). Operation of main components in SEM. The electron beam generated from the electron gun is finely focused and illuminated on the specimen. And as the beam is scanned over the specimen surface in both the X- and Y-directions, secondary electrons and backscattered electrons are detected. By amplifying these electron signals and modulating their brightness on the observation CRT (Cathode Ray Tube), a specimen image is displayed on the CRT. Generating a fine electron beam and detecting electron signals efficiently make it possible to obtain high resolution.

Vacuum system. Our SEM has three chambers, including electron gun chamber, intermediate chamber and sample chamber. All three chambers are operating in high or ultra-high vacuum of $10^{-7}$and 10^{-8} Pa. For comparison atmospheric pressure at sea level is equal to 760 millimeters of mercury or 10^{4} Pa. Vacuum in the sample chamber ensure that electrons don't scatter or disperse due to collisions with other molecules from the air. The sample chamber can be vented to an atmospheric pressure for the sample exchange procedure. Once the samples are placed into the chamber closing the chamber 's door will start the pumps to evacuate the air and generate the operational vacuum in the sample chamber which takes 1 to 3 mins. Two other chambers; the electron gun chamber and the intermediate chamber maintain vacuum constantly. Electron gun chamber need vacuum to avoid contamination of the filament due to residual gases.

Electron beam generation. The electron beam is generated by the electron gun, which could be one of two types: thermionic emission gun and the field emission gun. Our SEM equipped with field-emission electron gun (FEG).

Thermionic emission gun composed of three parts: tungsten filament, Wehnelt cylinder and an anode (Fig. 6). In thermionic emission process first the thermoelectrons are emitted from the filament, and then the electron beam is produced by giving an energy to the thermoelectrons by applying an accelerating voltage between the filament and anode. During this process, the thermoelectrons are collected at one point by the bias voltage applied between the filament and Wehnelt. This point is called "crossover point". Two types of thermionic filaments are the tungsten filament and the LaB6 cathode filament. The last one has smaller crossover point and can generate higher brightness.

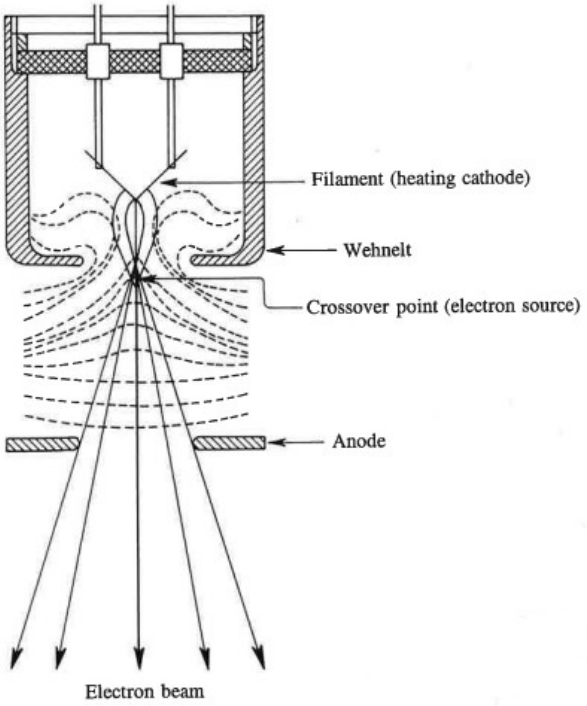


Fig. 6. The principle of thermionic gun.

Saturation point of the filament. Electron beam generation by thermal emission gun uses heat to excite the electrons off the filament. Electrical current in the filament causes filament heating and with increasing its temperature the number of electrons emitted by the filament will increase up to a point. After this point, called the saturation point, the filament current will only increase the filament temperature while will be no increase in emitted electrons. Continuing increase of the filament current beyond the saturation point will significantly reduce filament lifetime and cause filament burn out

The field emission gun (FEG) uses a single crystal tungsten (W) filament with a pointed tip of about 100 nm, which is not heated by filament current. Instead, electrons are emitted from the cold filament by a strong electric field generated around the very small emitter tip. This field emission phenomenon is based on the tunneling effect: When a strong electric field is applied to the surface of a solid, the potential barrier which encloses the electrons in the solid becomes low and thin, and the electrons are released into vacuum. Advantages of FEG over the thermionic filament include high brightness, high resolution and longer lifetime however the emission current is not stable due to the room temperature of gun operation (so it is called a cold emitter). FEG is superior in terms of resolution capabilities among electron sources. Because of a smaller tip emitting diameter of FEG the emitted electrons possess a narrower energy spread allowing the improved ability to be focused into a small probe and image fine features. For advanced applications of nanostructural analysis or low- kV imaging choosing the high-brightness FEG is the best option. Brighter field emission source can efficiently emit electrons even at low accelerating voltage. This gives field emission-SEMs the unique ability to support low-kV imaging, which generally refers to acquiring SEM images at an accelerating voltage of less than 5 kV. Low voltage mode has benefits of reduced beam damage and improved imaging of non-conductive samples. These can be particularly advantageous when imaging biological samples. Therefore, FEG is preferred for high resolution imaging but disadvantageous for energy dispersive X-ray analysis due to the current fluctuation.

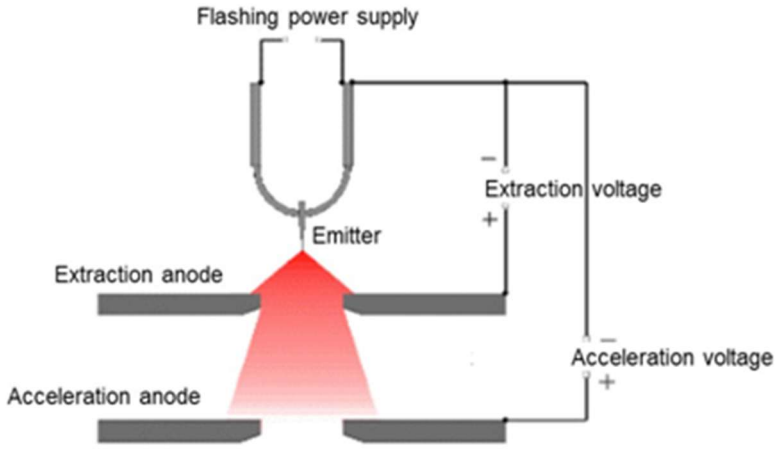


Fig. 7 (Taken from https://www.jeol.com/words/semterms/20121024.062458.php#gsc.tab=0).
Basic structure of Field Emission Gun.

Accelerating voltage that can be used to image different samples varies from 0.5 to 30 kV. Choosing the appropriate voltage depends on the type of specimen and a desired resolution. Higher voltage provides better resolution but the heat that is generated on the specimen is higher and may damage the sample. Thus, for heat sensitive (rubber or plastics) and biological samples the optimal voltage would be around 5 kV, while 15 -20 kV would be preferable for non-biological or metal samples.

Electron beam manipulation by lenses. Most SEMs use one to three magnetic lenses to reduce size of electron beam. The lenses operate by passing electric current through a coper wire. These are known as condenser lenses and since they possess spherical aberration, they can limit microscope resolution similar to optical microscope lenses. There are other set of lenses that correct astigmatism and alignment. Astigmatism drastically impacts the image quality and decrease the resolution by causing the electron beam entering the sample in the form of noncircular spot. A few components may contribute to astigmatism, first of all is the elliptical shape of the beam formed by the filament, and secondary, an accumulation of dirt in the column or on the aperture may distort the beam. Fig. 8 shows the effect of astigmatism on the image containing fine random detail.

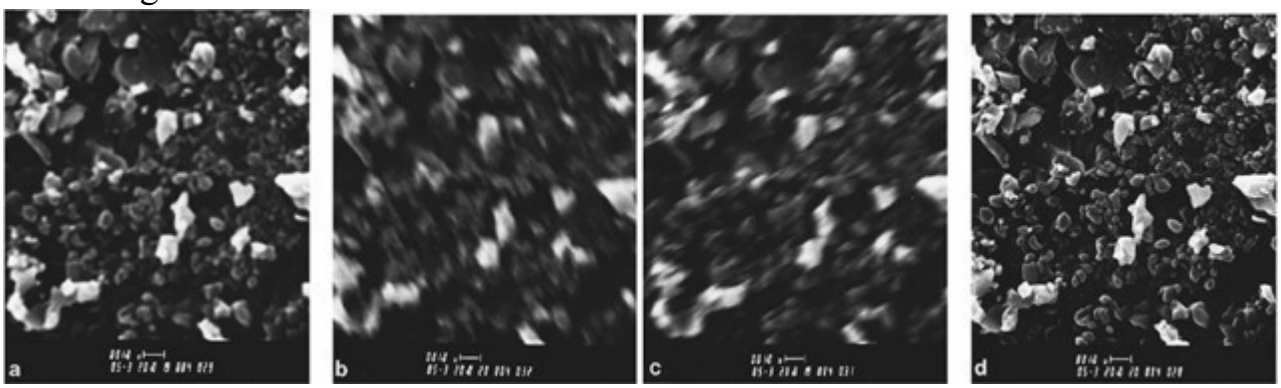


Fig.8 (Taken from (Scanning electron microscopy and X-Ray microanalysis. J. Goldstein and other. 3rd edition, Springer Science+ Business Media, LLC). Effect of astigmatism on the image containing fine random detail. a) Initial situation, b) Underfocus, c). Overfocus, d) image corrected for astigmatism.

Beam manipulation allowing a raster sample scanning. The electron beam is scanned over the specimen surface in both the X- and Y- directions with the help of magnetic field that is generated within the final condenser lens. Two sets of magnetic scanning coils move the beam to allow a raster scan pattern. The raster pattern images the specimen by starting in the upper left corner and continuing to the right, then transferring to one line down with each line scanned. That it is these coils that give the SEM its name.

II. Specimen preparation.

(From the booklet "Invitation to the SEM World", JEOL LTD)

Because of wide variety of SEM application field, there many kinds of specimen that are observed. Therefore, many different specimen preparation techniques have been developed depending on the object and the purpose of the research. In every case though, there are major points that must be taken into consideration during specimen preparation.

1. The specimen surface must be clean.
   A SEM observes the surface of a specimen. Therefore, it is essential that the specimen surface be clean. In order to observe the inner structure, fracturing, polishing and cutting are commonly performed. In some cases, ion etching, or chemical etching are done to remove an unwanted film coating on the specimen surface.
2. The original morphologic construction must be maintained.
   If a specimen containing water or gas is brought into a vacuum, the specimen may shrink or deform. Therefore, for biological specimens, fixation, dehydration and drying are done prior to observation.
3. The specimen must not acquire an electrostatic charge.
   When the specimen is irradiated with an electron beam, some electrons are emitted from the specimen as secondary electrons and backscattered electrons. The rest of the irradiated electrons may be absorbed in the specimen. However, if the specimen has no electric conductivity, the absorbed electrons can charge the specimen. This charge causes many errors in observations. Metal coating, observation with low accelerating voltage, or observation under low vacuum are done to prevent a specimen from acquiring an electric charge.

Wear gloves when handling anything that will go inside the SEM instrument.

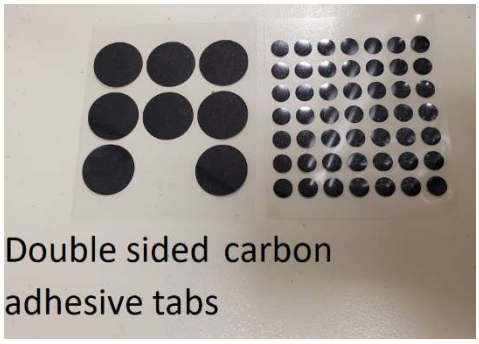
Following materials must never be placed in the SEM instrument:

- Wet samples either from water or a solvent.
- Porous materials that outgas water vapor or toxic gases.
- Magnetic materials can misalign electro-magnetic lenses and can not be analyze with SEM.

Keep specimen surfaces clean and dust free. Enclose the samples in the suitable container when transferring it between the labs. Use electrical blow dryer to clean the specimen surface. Blowing away loosely attached particles off the sample is mandatory when mounting powder samples. Powder particles can cause ballistic damage to delicate surface of the EDS and EBSD detectors that are located inside the sample chamber.

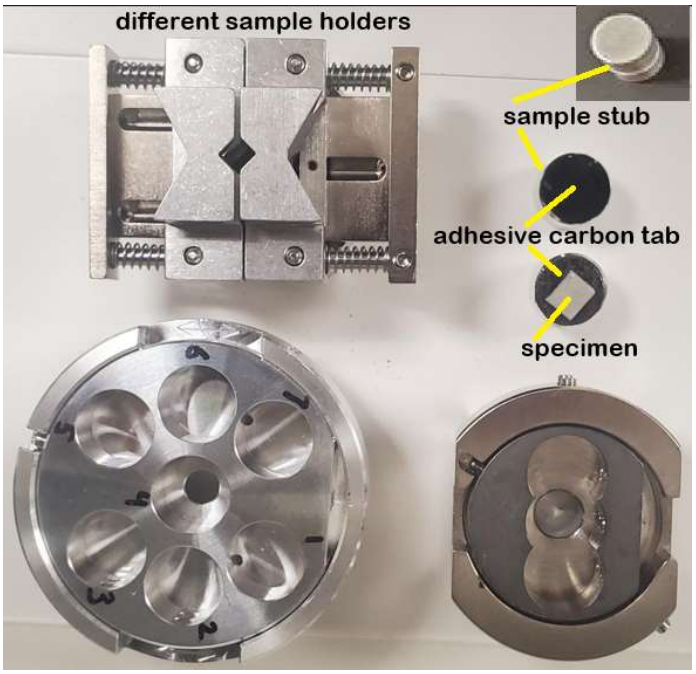
Specimens are mounted on the surface of the aluminum stub using double sided conductive carbon adhesive tabs (tape). Sample surface should be as flat as possible. Specimen can be adhered to 12 mm or 25 mm diameter stubs.

Sample stubs; specimens are mounted onto them using double sided carbon adhesive tabs

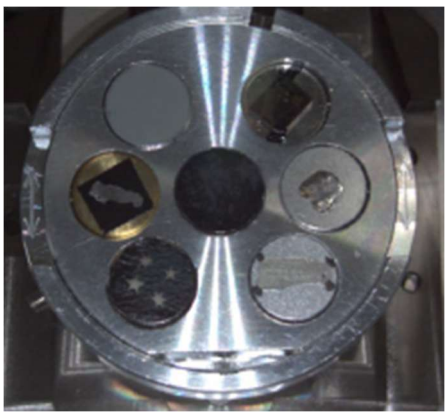


Double sided carbon adhesive tabs

Specimens must be mounted onto the standard sample holders and never should be mounted directly onto specimen stage. There are variety of sample holders available.

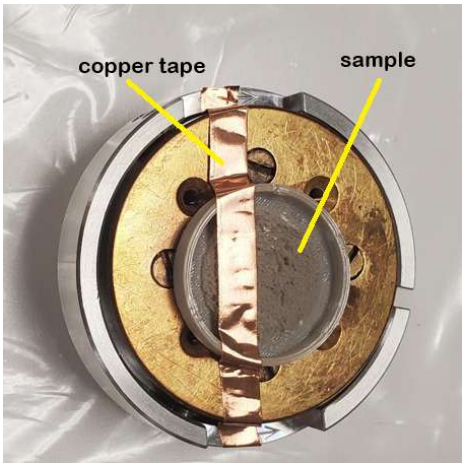


If there are seven different samples, then they can be mounted onto 7 small stubs and placed into 7 stubs sample holder.



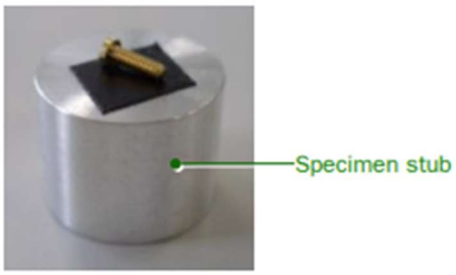
Specimens of odd shape or greater than 25 mm can be mounted onto large sample holder.

Copper tape may be attached across the non-conductive sample to reduce charging effect and improve conductivity.

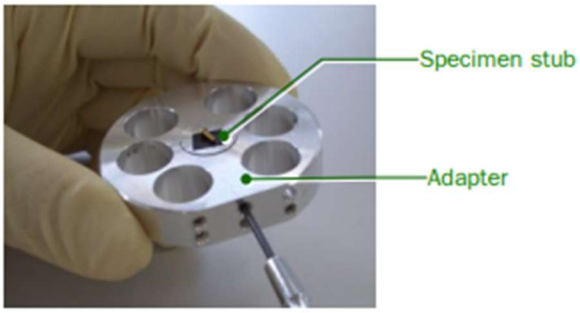


III. Specimen mounting procedure.

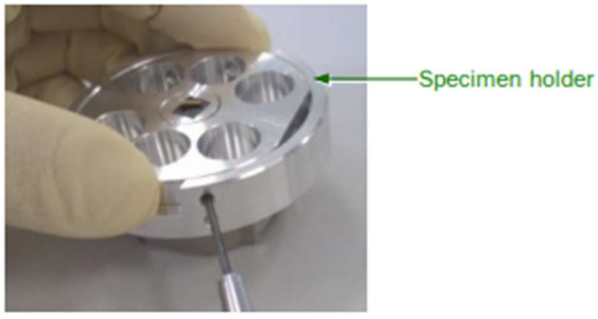
1. Using carbon adhesive tabs secure the specimen on the sample stub.



2. Using the Allen Wrench tighten the screws to secure the specimen stub inside the adapter.

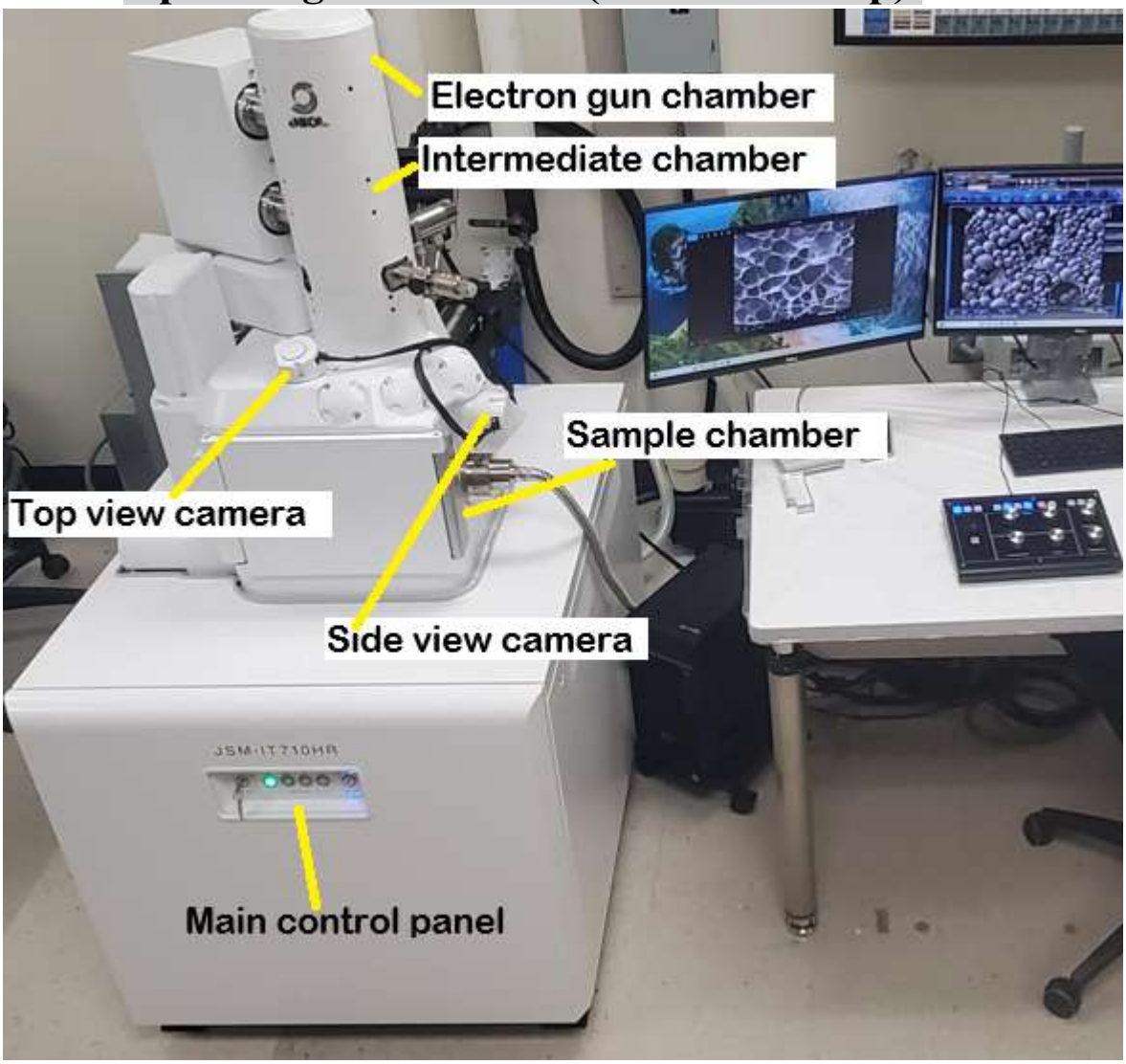


3. Attach the adapter to the specimen holder and tighten the screws with the Allen Wrench.



4. Measure the amount of specimen protrusion using the specimen height scale. You will use the value of the specimen height at the time of specimen exchange process while creating specimen data. It is very important to measure this value accurately since it prevents damage to the objective or specific detectors due to collision. If any part of the sample will hit a detector – it will cause major damage!

IV. JEOL JSMIT710-HR/LV Scanning Electron Microscope Operating Instructions (Machine Setup).



1. To start the SEM control program, open the InTouchScope software. This software usually remains open, in case it is closed go ahead and open it. The monitor remains lit, there is no screen time out.

2. After the SEM control program starts, the User Management window appears. Select the Student user and click the Logon button.

3. Prepare for the image observation. Currently the sample chamber is under the vacuum.

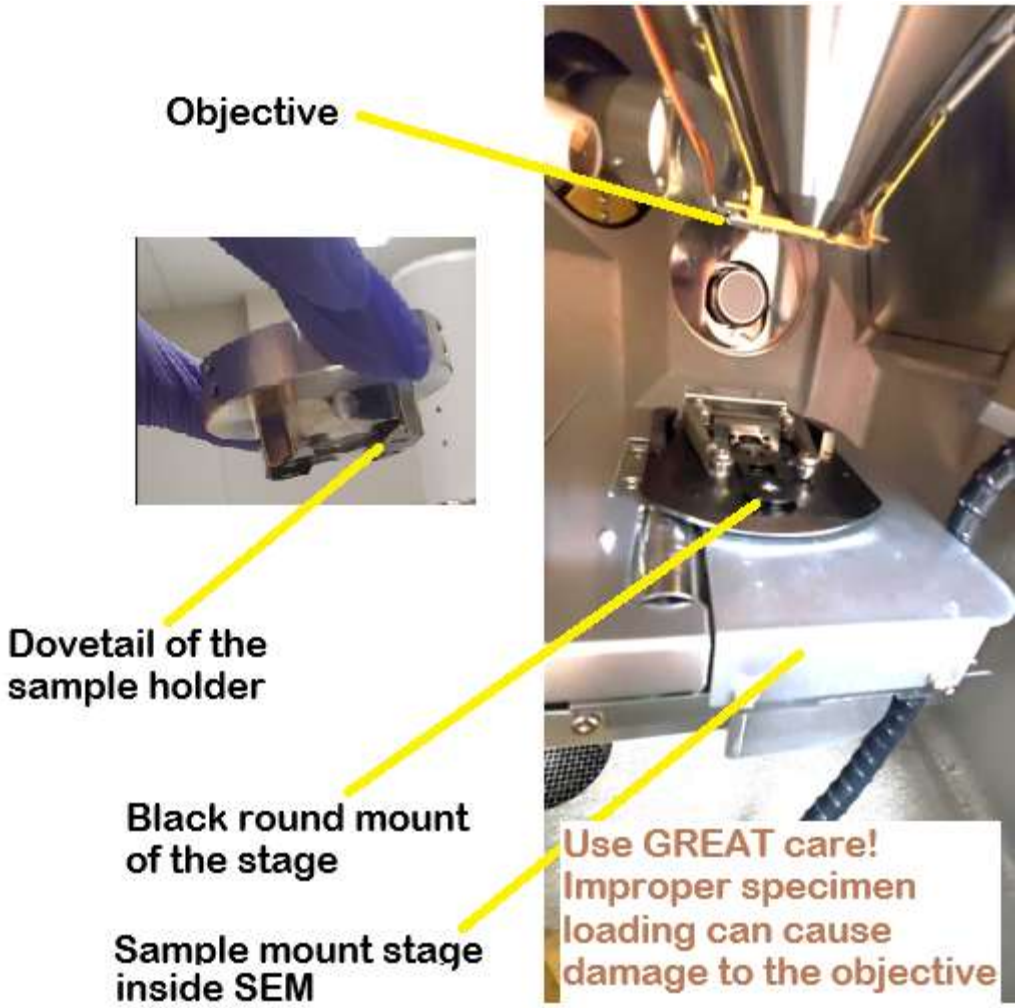
Fig.9. Main control panel on the electron column unit shows that EVAC button is lit with blue color - Sample chamber is under the vacuum.

4. Press VENT button on the main control panel: VENT button will light with green color and start blinking; soon the light become solid (Fig.10). At this time, the sample chamber is at atmospheric pressure and the door can be open.

Fig.10. VENT button is lit with green and not blinking – the sample chamber is at atmospheric pressure.

5. Open the sample chamber door:

6. Mount your sample holder on the stage. Making sure the dovetail of the sample holder slides firmly around the black circular mount of the stage and is pushed forward gently until you feel resistance (See video “Sample holder mounting in the sample chamber”). Take great caution not to touch or collide with the objective or backscattered detector. Damage to the objective or detectors will require very expensive repair.



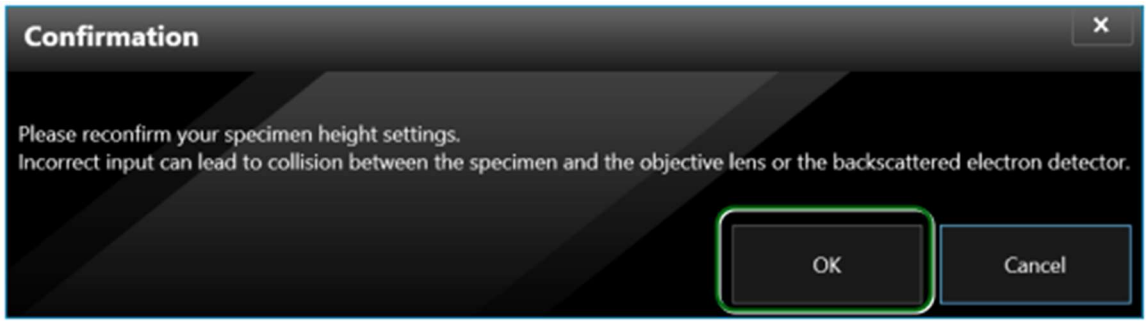
7. When the sample is in place gently close the chamber door, this will automatically start vacuum pumps to evacuate the air from the sample chamber. EVAC button will start blinking and become solid when the pressure in the chamber reaches an operational vacuum level. It takes 1 to 3 mins to get to the operational vacuum.

8. The window “Create new specimen data” (Fig. 11) will pop up simultaneously with the completion of the step 4 (at the time when the sample chamber reaches atmospheric pressure). Click “create” and in the next window (Fig. 12) select appropriate sample holder, then using the sliding button select correct value for the sample height that was measured in the step III_4. Enter the specimen name and click “OK” button.

Fig. 11

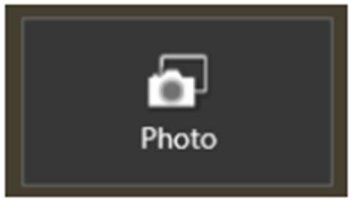
Fig. 12

9. In the next window check the message, make sure you entered the correct sample height and click OK button.



10. Take the photo of the sample holder. For this look in the navigation window and select top-view camera by clicking on the dots (Fig. 13)  .

11. Click on the photo camera icon



in the top-view camera window and the sample stage will start moving towards the top-view camera position to acquire the image. This image will help you to navigate within the sample or between the samples. X, Y, Z coordinates at the bottom of the window are changing when the stage is moving.

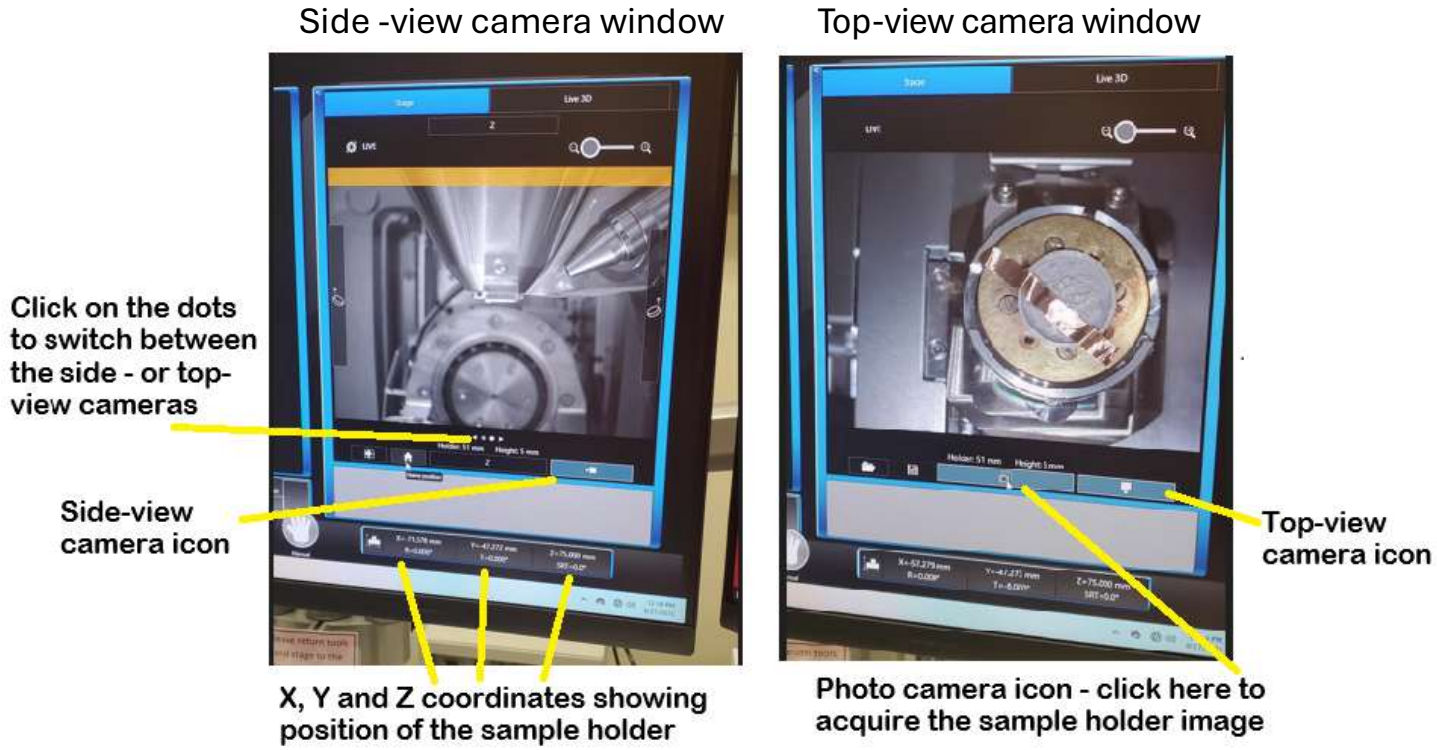
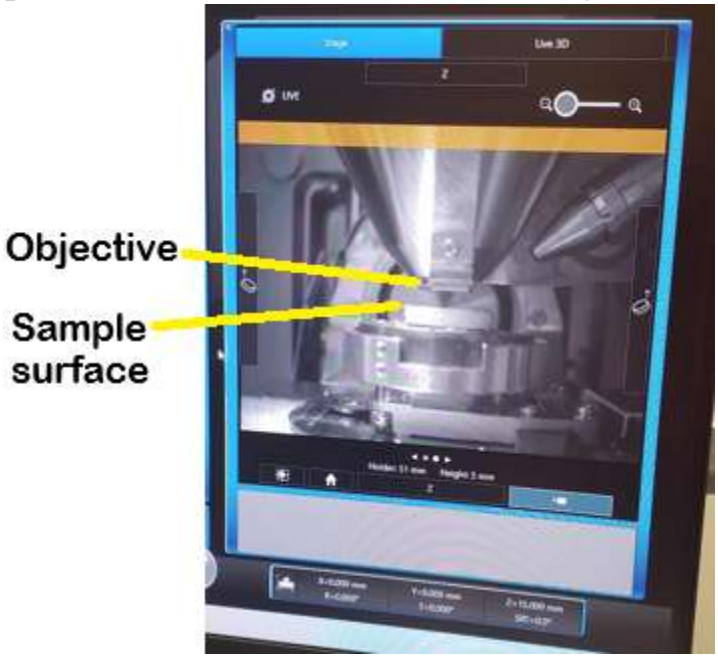


Fig. 13

12. After taking the sample holder image change navigation panel to side-view camera window by switching dots, then click on the home button.

Clicking the home button will move the stage to the observation position and lift it up to the distance of 10 mm from the objective.



At this time the window “Moving Stage” will pop and there will be a **Stop** button visible (Fig. 14). Monitor the stage moving up and in case the sample is getting too close to the objective click stop button. Another way to stop the stage movement is to touch the joystick (yellow arrow) on the manual operation panel (Fig.15).

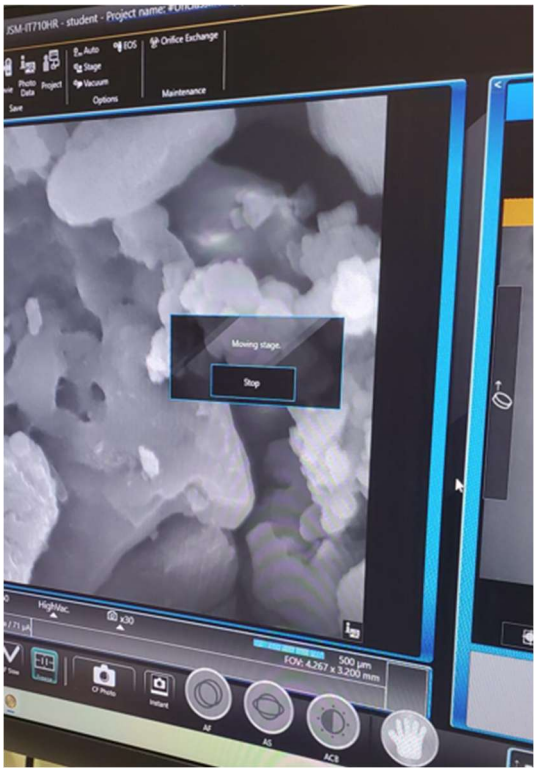


Fig. 14

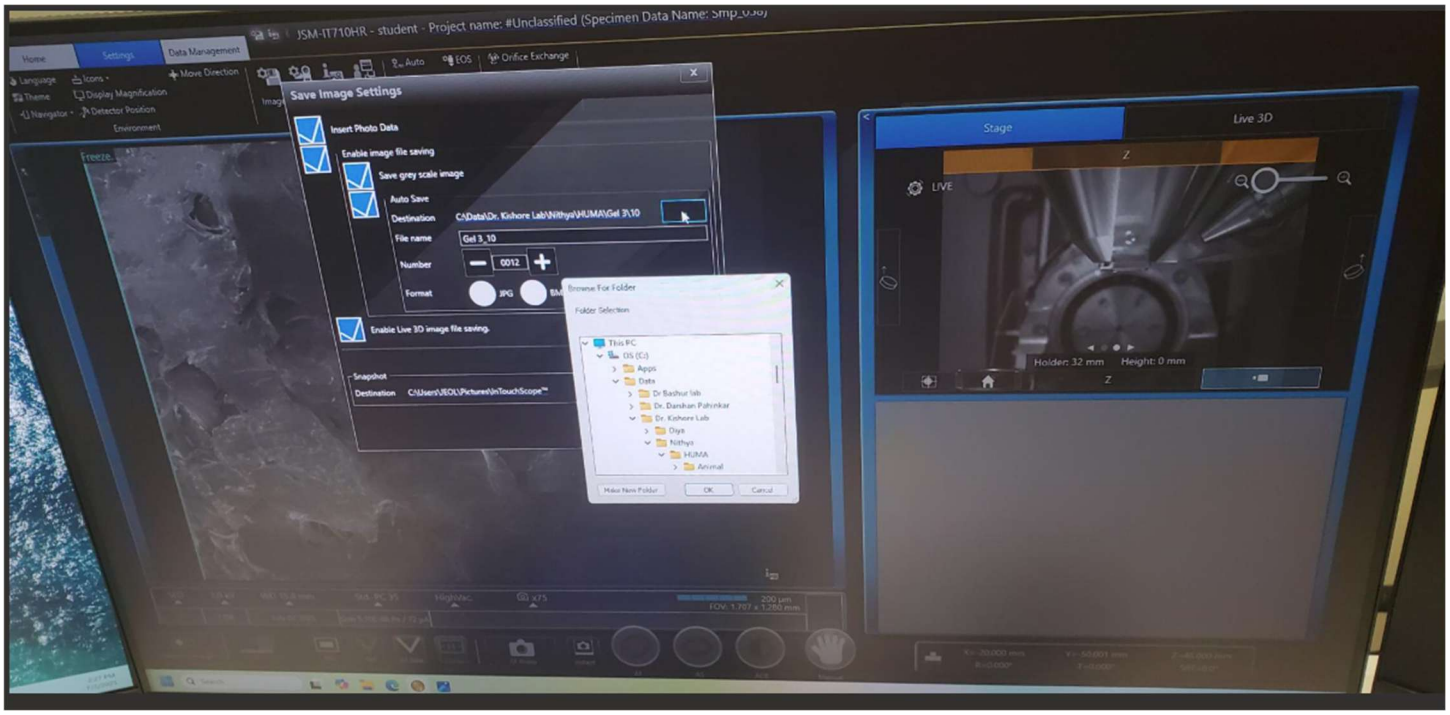
Fig.15

13. To set the directory for saving SEM images under **Setting** choose **Image** icon:

"Save image setting" window will open, from this window select **destination** path and choose/type the folder for saving your SEM images. All images are saved under

My computer
C/OS
-Data
- PI lab name
-Investigator name (student)

In the **"File name"** bar type the name that you want your images to be saved with. Every next image will have the same name plus succeeding number.



v. IMAGE OBSERVATION – basic steps.

14. Start imaging by turning ON the observation button.

15. Select **Fast** scan mode from the following window (scan mode menu):

Fig.16.

16. In the navigation window find the photo image of the holder with your samples that was taken in the step IV. 11

Double click with the mouse pointer on the sample or the area of the sample that you want to image, and it will move under the beam for imaging.

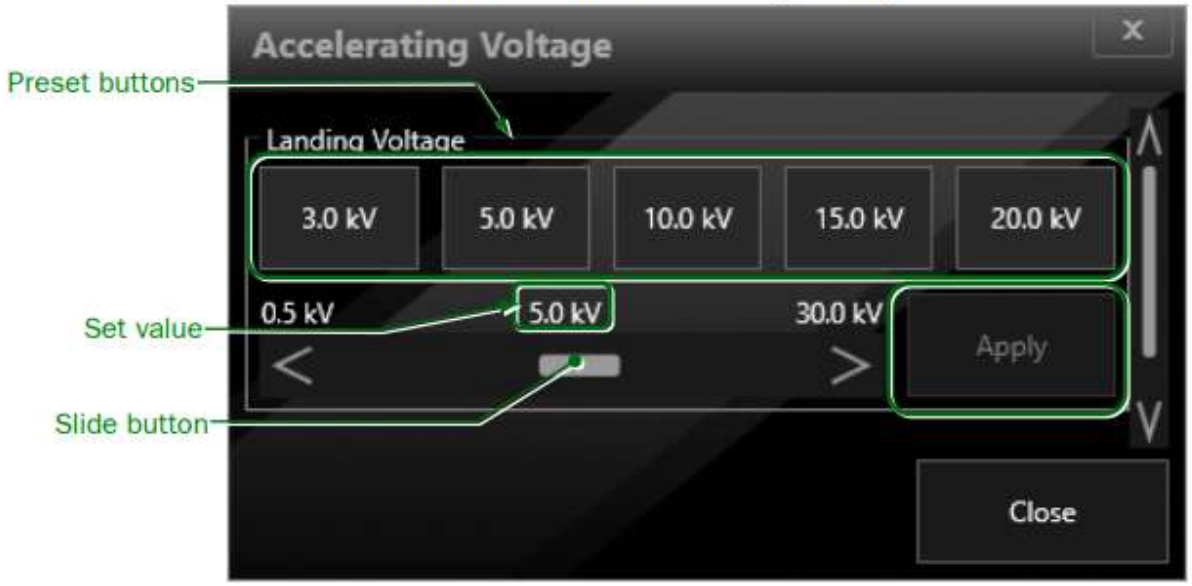
17. Set the observation conditions, such as accelerating voltage and probe current.

Setting the Accelerating Voltage

Click the △ symbol for the accelerating voltage in the Photo data.
The Accelerating Voltage window appears.

Set the accelerating voltage.

Preset buttons	Click a preset button to change the accelerating voltage.
Slide button	Move the slide button to the desired value and click the Apply button to set the accelerating voltage.



The SEM offers a range of accelerating voltage, starting below 1kv and expands to 30kV. Generally, start with 5 kV for biological samples and 10kV for metals. Keep in mind that higher accelerating voltage may damage beam sensitive samples like biological samples or biomaterials.

Setting the Probe Current

Click the △ symbol for Std.-PC in the Photo data.

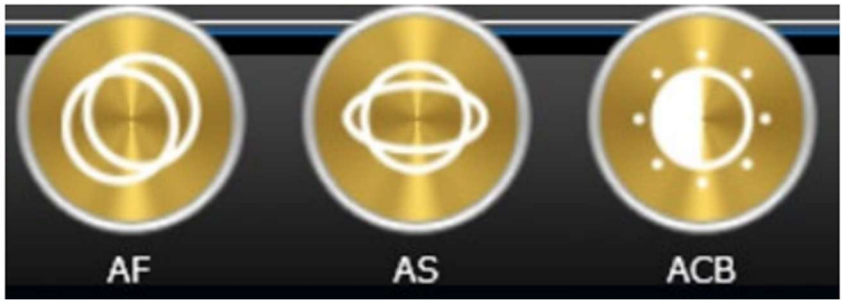
The Probe Current window appears.

Set the probe current.

Preset buttons	Click a preset button with the desired value.
Slide button	Move the slide button to the desired value.

For normal image observation	Set PC between 30 and 40.
For high-resolution observation	Set PC to 30 or less.
For analysis	Set PC to 70 or more.

18. Use the ACB button for automatic brightness/contrast adjustments and the AF and AS buttons for focusing the image.



Tips for best focusing:

- Find a distinctive feature on the sample (ideally something spherical, that you know what shape it has to be when in the best focus)
- Increase magnification a lot higher than you need for your image. This way you will be able to observe even smaller features. The smaller features help to achieve precise focusing.
- When you achieved the focused image, then go ahead and reduce magnification to desired for your image. Now click on the **Slow** icon on the scan mode menu (Fig.16) and you will see how your image will look like when it will be saved.

19. To save the image click on the **Photo** icon in the scan mode menu (Fig.16), this will start image acquisition and automatically save the image to the folder that you set up in the step IV. 13. After image acquisition finishes, the screen displays a static image. Clicking the icon returns it to a live image.

20. To end the imaging session, reduce the **magnification** to 30x, check the **Fast** scan mode icon, click on the **Observation** icon to turn it off. Then press the **VENT** button on the main console panel of the SEM column. Once the light on the VENT button stops blinking, put on the gloves, open the sample chamber door and carefully without touching other components inside the chamber remove the sample holder.

VI. OPERATION DETAILS

21. Selecting the scan mode.

There are several different scanning modes available for imaging.

A. **RDC icon.** The electron beam scans a smaller field of view at very fast speed, and it takes 0.008 sec to 0.5 sec to complete the scan. The image looks very grainy, it has very low resolution. This mode is suitable for electron gun alignment or field of view search.

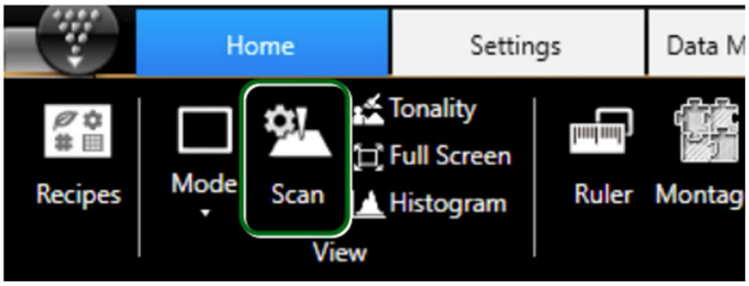
B. **Fast** icon. The live image is displayed at a high speed, the scan completes in 0.15 sec to 1 sec. This is suitable for searching the field of view and adjusting the image quality like focus, brightness and contrast. This **Fast** mode gives you faster feedback without a delay, which is convenient for adjusting the parameters of imaging.

C. **Slow** mode gives you a higher resolution image than Fast mode, but it is difficult to navigate around the sample or adjust focus when using it because of a delay between changing the imaging parameters and observing it's result. Use this **Slow** mode to get a better idea how well your image will look before committing to Photo scan. This Slow mode scan is completed in 10 sec to 30sec.

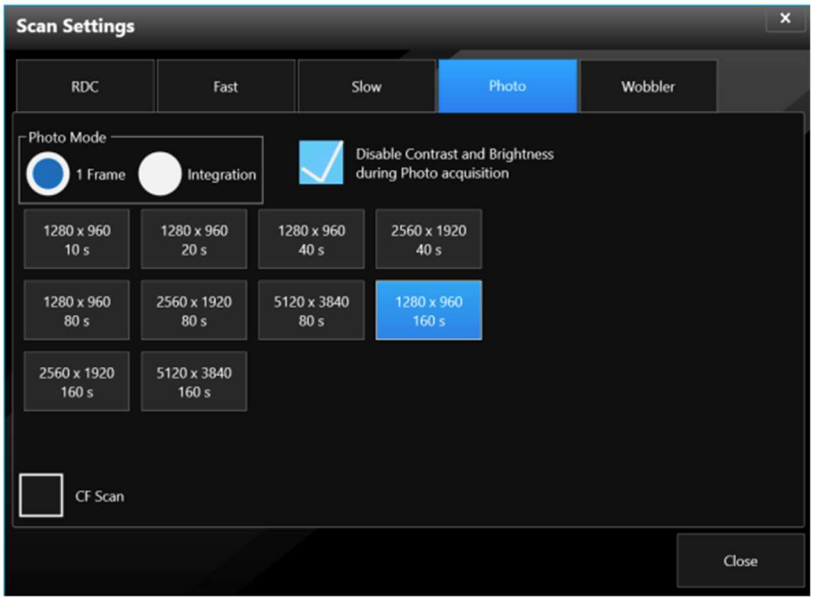
D. **Freeze** icon. The live image is stopped to display a static image.

E. **Photo** scan is for taking and saving images. It is line by line scan that takes usually 80 sec to 160 sec (depending on the resolution selected) and gives a higher resolution image.

You can modify the resolution and the scan speed under the Home and the Scan menu:



In the Scan Setting window under the Photo you will see the following screen where you can choose one of the ten combinations sufficient image resolution for most publications is 1280x 960 and it takes 80s:



Selecting the CF Scan check box reduces the specimen charge-up. (Using the CF scan in LV mode may deteriorate the image quality).

22. Focus adjustment

Focus can be controlled through software and hardware. You can do focus adjustment using a focus knob (3) on the Operation panel (hardware) or using the software tools.

Hardware – Operation panel (Knob set)

Software tools

Click the **Manual** icon.

Click the **Focus** button of the manual adjustment tool.

The image focus can be adjusted continuously by rotating the mouse wheel, while the mouse cursor is positioned over each button. In this case, both overfocus and underfocus adjustment can be performed on a single button.

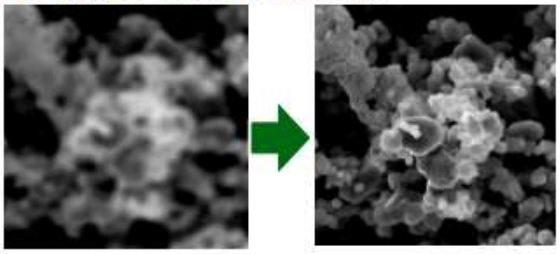
For example, hover **over the overfocus** button and move the mouse wheel.

Then, both overfocus and underfocus can be continuously adjusted.

Use the **underfocus** button for rough adjustment and the **overfocus** button for rough adjustment to adjust the image.

Use the **underfocus** button for fine adjustment and the **overfocus** button for fine adjustment to adjust the image.

Adjust the image so that it is the sharpest.



23. Astigmatism correction

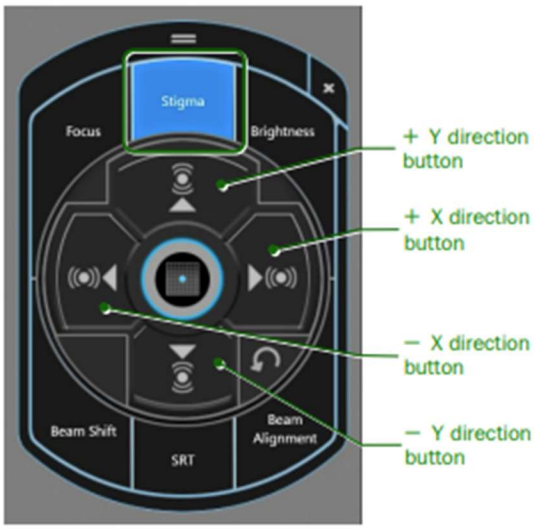
If the shape of the electron beam irradiated on the specimen surface is not circular, there is astigmatic aberration, and the adjustment of the shape is called astigmatism correction. Sharp images with high magnification cannot be obtained without astigmatism correction. To correct astigmatism manually, follow the procedure below.

Click the **Manual** icon.

Click the **Stigma** button.

The following description assumes that the astigmatism correction in the X direction is performed first.

(The result is the same even when the correction in the Y direction is performed first.)



The image focus can be adjusted continuously by rotating the mouse wheel, while the mouse cursor is positioned over each movement button. In this case, adjustments in both directions can be performed on a single button.

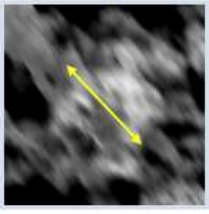
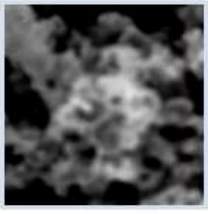
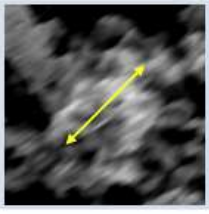
For example, hover over the **+ X direction** button and rotate the mouse wheel.

Then, both the + and - X directions can be continuously adjusted.

Keep pressing the **+ X** or **- X** button with your finger.

The image blur changes. Release the button when the outline of the image is clearer.

Press the opposite button (- X direction for the + X direction, and + X direction for the - X direction) repeatedly to adjust the outline as clearly as possible.

Over-corrected (in the - X direction)	Just-focus position	Over-corrected (in the + X direction)
Appears to flow in the direction of the arrow.	Still blurred due to astigmatism.	Appears to flow in the direction of the arrow.
		

Perform the same correction for the Y direction.

Repeat Steps 4 and 5 several times to correct the astigmatism in both the X and Y directions. Then, adjust the focus so that the image is as sharp as possible.