Scanning Electron Microscope
JSM-6380/LV

Scanning electron microscopy background and a user procedure for the SEM JSM-
6380/LV located in the Microscopy Center

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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-6380/LV can guarantee 3.0nm resolution at the accelerating voltage (Acc V) 30kV and work distance (WD) 8 mm in the SEI 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), X-Rays, 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.

![Optical Micrograph A](image1.png) ![Optical Micrograph B](image2.png)

**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 type 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 phenomena 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-6380/LV SEM” is composed of Electron optical column unit and an Operation desk (Fig. 4).

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.** The SEM chamber operates in high vacuum to ensure that electrons don’t scatter or disperse due to collisions with other molecules from the air. Atmospheric pressure at sea level is equal to 760 millimeters of mercury. A pressure of 1 millimeter of mercury is call a Torr. The minimum vacuum requirement for SEM operation is
$10^{-4}$ Torr, although most microscopes operate at $10^{-6}$ Torr or greater vacuum. The higher the vacuum (the lower the pressure), the better the microscope will function.

**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 thermionic emission gun composed of three parts: tungsten filament, Wehnelt cylinder and anode. 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. 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 10 kV, while 20 kV would be preferable for non-biological or metal samples.

**Filament current and its saturation point.** 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 life time and cause filament burn out. This is why the filament current should be carefully adjusted to maximize the number of emitted electrons without increasing the temperature hotter than necessary. Selecting filament current below the saturation point results in unstable beam current and poor image. Adjusting of the filament current is aided in our microscope with an automatic adjustment option, however it should be performed only by trained lab manager.

**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 copper 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. 6** shows the effect of astigmatism on the image containing fine random detail.

![Fig. 6](image) (Taken from (Scanning electron microscopy and X-Ray microanalysis. J. Goldstein and other. 3\textsuperscript{rd} 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.
Specimen preparation and observation technique.

(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 cause 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.

Sample mounting procedure:

- Specimens are mounted on the surface of the stub using double sided carbon adhesive tabs (tape). Sample surface should be as flat as possible. 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. Specimen can be adhered to 10 mm or 25 mm diameter stubs. Specimens of odd shape or greater than 25 mm can be mounted onto large sample holder.

- If there are four different samples, then they can be mounted onto 4 small stubs and placed into 4 stub sample holder.

Wear gloves when handling anything that will go inside the SEM instrument. Keep surfaces clean and dust free.

Following material 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
JEOL JSM6380-LV Scanning Electron Microscope Operating Instructions

1-Electron gun
2-Movable aperture
3-Z stage control knob
4-Specimen chamber
5-Specimen stage
6-Main control panel
7-EDS system/EDAX
8-Secondary electron Detector
9-Backscattered electron detector
Machine Setup.

1. From the computer screen open the JSM5000* software – (arrow 1 in the image).
2. Turn on IR Camera (to be sure that nothing there in the chamber and that it is clear to open chamber door) – (arrow 2 in the above image). You will see IR camera viewing window.

1. Make sure the stage $Z$ is lowered to at least 25 to 30mm as indicated on the right of the Z stage control.
2. Vent the system on front **main control panel** -press and hold VENT until the button lights up, it will flash until the system is vented and then the light will be solid. Or you can click on Sample in blue in the software and click on Vent.

3. Open the chamber door by grasping it from the sides and pulling out.

4. Mount your **sample holder** on the **stage**. Making sure the **dovetail** on the sample holder slides firmly onto the **mount holder** and is pushed on completely to make sure center on the sample is the same as center on the stage. The large flat side of the **sample holder** should be at the top when sliding onto the **mount holder**. Making sure the sample holders on correctly is very important because this helps ground your sample.
5. Take a picture of your sample at this stage, what your sample looks like to the naked eye is much different than when looking through the scanning electron microscope. This will help you to better visualize where on the sample you are when looking under the electron gun.

6. When the sample is in place gently push the chamber door closed –WATCH the stage as it moves to see where it is with respect to the detector. If any part of the sample will hit a detector – it will cause major damage! The highest point on the surface of your sample must be at least 10 mm below the detector.

7. While continuing to push gently on the chamber door (this makes sure the vacuum pump does not pull on room air and backflow oil into the chamber) press the EVAC button on the front panel until it lights. Hold the door closed until you hear the pump come on.

8. Let the system pump down for 15 minutes to insure a stable environment and better imaging results.

9. Set the accelerator voltage, ACC VOLT, Spot size, and Aperture according to Appendix 1. Generally you can start with an Aperture of 2, accelerating voltage of 5kV, and spot size of 35.

10. Make sure the Magnification is set to the lowest magnification.

11. Turn HT on (in the upper left corner). If the image is either too bright or too dark, press ACB (auto contrast brightness) to make viewing the sample easier.

12. Open the Stage Icon in the set of icons on the right in blue. Select sample holder you are using, generally one of the offerings on the top row. If using the single holder press STAGE - INITIAL POSITION - GO to center the sample. Remember that the image
you see is rotated 90 degree clockwise in relation to where it was when putting your sample in the chamber. So the left side of your sample is now at the top when moving the stage.

13. Click on **WD** in the bottom of your screen towards the left. Select 10mm for your working distance. Ideally the working distance is between 8mm and 10mm for the best resolution and EDAX detection. Selecting 10 mm does not move the stage in the Z direction, it only sets the lens focal length to 10 mm, you will need to turn the Z knob counter-clockwise to bring the stage up to meet that 10 mm focal length. If at any point the image is too bright or dark, select **ACB** along top of screen, or the two **Brightness** and **Contrast** knobs on the **manual adjustment pad**.

**Position of software buttons on the software screen window.**
Description of the Manual Adjustment Pad.

Preparing for Imaging.

14. There are seven scan modes but generally only 4 or 5 that are used.
   a. **Scan 1** is used for focusing your sample. It creates a small window within the **Image Display Area** that has a real-time imaging. Fine Focus should be done in Scan 1 only.
   b. **Scan 2** is the entire view of the Image Display Area based on your magnification. This scan is used to move around on your sample. It should never be used for fine Focus.
   c. **Scan 3** is a 10 second line by line scan of your sample. This will give you a much cleaner image than **Scan 2** but is difficult to move around the sample when using it. Use Scan 3 to get a better idea how well your image will look before committing to Scan 4 or Photo.
   d. **Scan 4** and **Photo** are for taking and saving images. **Scan 4** is a line by line scan that takes 80 seconds and gives a high resolution image. Photo is also a line by line scan but takes 180 seconds and gives even higher resolution image.
   15. Starting in **Scan 2**, do a coarse **Focus** on your sample using the right mouse button and clicking on **Focus**, keeping **Focus** and the right mouse button depressed while moving your mouse up and down until you achieve the best focus (or use the knob on the manual
adjustment pad). Look for an area on your sample to do a fine Focus. Look for small distinct edges to do a fine Focus, soft edges or spheres do not make for good focal points. Cracks, points, or distinct holes are ideal for fine focus. Once you've chosen an area double click on it in the Image Display Area to center it. Use coarse focus to bring the sample into focus. (Coarse Focus can only be done on low magnification). Once you have chosen the object to focus on click on Scan 1 and bring your magnification up as high as you can while still being able to see the object you are trying to focus on. Ideally you will focus at 15 times the magnification you will be imaging at. Now you will click on Focus using the left mouse button and keeping the mouse button depressed while you move the mouse up and down to find the best focus.  

**Note:** especially during fine focus, if the focus becomes better when moving in one direction but you hit the limit of focus in that direction.Let go of the mouse button at the edge of the window and then click on Focus with the left mouse button again and continue movement in that direction until your focus improves.

**a.** If this is the first sample you have done or if you changed the aperture you must do a Wobble, Stigma X and Stigma Y to get the best focus. Wobble should only be attempted by someone who has been trained, ask for help from the microscopy center manager or approved representative if you need help. Click on Wobble the image should appear to be breathing in and out, if it is moving side to side or up and down you will need to adjust the X-direction fine adjustment knob and the Y-direction fine adjustment knob to stop the movement. These fine adjustment knobs are located on the column along with the Aperture selection knob. The movements on the X & Y-fine adjustment knobs are very minor movements. If you move these knobs too much you will lose the aperture hole and the Microscopy Center Manager or a representative will need to be called to fix it. Once you have stopped the x and y movement press Wobble again to close the window. Then immediately press Lens Reset.

**b.** Now that the stigma has been corrected mechanically you will now need to refine it electronically. If your sample is really out of focus from the Lens Reset process, you can do a coarse focus followed by a fine focus. Once you have completed the fine Focus you will use the same procedure on the Stigma X as you do when doing Focus, pressing the left mouse button while clicking on either Stigma X or Stigma Y. Once the image is as good as you can get it do the Focus one more time. This should be performed in Scan 1.

16. Capturing the Image

**a.** Consistency is a key for research and deciding the magnification(s) in which you will taking images is crucial. Standard suggestion is to take a low magnification image, an intermediate magnification and a high magnification. Of course you can decide how many images and at what magnifications are important for your research.

**b.** Frame your image as you would like to save it. Move the stage so that the object you want to image is centered horizontally and up slightly vertically (this is to make room for the image information on the bottom). Press Scan 4 or Photo to take the image. When the scan is complete the screen will freeze and the scanning information will be on the bottom of the image display screen. This will display across the bottom of your image.
Included from left to right is the voltage used, the magnification, the scale bar for the image, the number of images (if set up to record), the working distance, the spotsize, secondary electron (other option is backscatter).

17. To finish, turn HT OFF (top left corner).
18. Lower Z to 25 or greater.
19. Wait 10 minutes for the chamber to cool before opening the chamber, introducing cold air immediately can cause condensation on the chamber.

Appendix 1.
Choosing Operating setup

1. Choose the correct aperture on the column for the sample you are working with.
   Note the beam at higher kv damages the aperture so for Apertures 1 and 2 use 10kv or lower.
   a. Aperture 1 is 20 µm which gives higher resolution and a greater depth of field but can give you a much grainier image.
   b. Aperture 2 is 30 µm and decreases the resolution and depth of field slightly. This is the most used aperture on the machine. Use this aperture if your image using Aperture 1 is too grainy.
   c. Aperture 3 is 100 µm and is used for using higher kv or EDS x-ray analysis. The disadvantages of the higher kv are: unclear surface structures, sample damage, increased charge-up, and more edge effect.
   d. Typical suitable diameters for example are as follows.
      - For high resolution 20 µm
      - For routine observation 30 µm
      - For EDS analysis or work with large probe currents 100 µm

2. Selecting the kv and spotsize is more subjective and here are a few general suggestions. When working with samples that are easily damaged by the ion beam it is suggested that you start with 5 kv and a spotsize of 30. For metals and samples that are less affected by the ion beam you can start with 10 kv or higher and again, start at a spotsize of 30.
   a. From the JEOI Operating Manual for accelerating voltage - When you use a lower accelerating voltage, the details of microscopic structure of the specimen surface appear more clearly. When using a high accelerating voltage, the illuminating electrons can reach deeper inside the specimen and as a result unnecessary signals generated from the inside of the specimen (backscattered electrons, for example) lower the contrast, thus hiding the details of microscopic structure of the specimen surface. For this reason, especially for observing a specimen of lower density material, a low accelerating voltage is desirable.
From the JEOL Operating Manual for **spotsize** - As the electron probe diameter (the **spotsize**) becomes smaller, higher magnification and so higher resolution are obtained. However, image smoothness, that is, the SIN (signal/noise) ratio of the image, depends on the illuminating current. When you try to reduce the probe diameter, the probe current reduces as a result. Therefore, an appropriate illuminating current must be selected according to magnification, observation conditions (including accelerating voltage, tilting of specimen and others) and the specimen itself.