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4 Imaging with the SEM

The scanning electron microscope is routinely used to characterize wide-ranging materials due to its ease of operation and relatively straightforward sample preparation as well as due to simple image interpretation. New users can readily obtain images after little practice. However, high-resolution microscopy and examination of "difficult" samples require experience and know-how of the principles of image formation in the SEM. This chapter describes the role of various operational parameters used during microscopy in more detail. The effect of these parameters on contrast, resolution, and depth of field depicted by images is discussed. Pros and cons of microscopy conditions that have a direct bearing on the quality of images, type of information obtained, and image interpretation are elaborated. Guidelines for operation and upkeep of the SEM instrument are also summarized in this chapter.

4.1 Resolution

Theoretical resolution in a perfect optical system is given by Abbe's equation [1]:

$$d = 0.612 \frac{\lambda}{n \sin \propto} \tag{4.1}$$

where d is the resolution, λ is the wavelength of the radiation used for imaging, n is the index of refraction of the medium between the point source and lens, and α is the half-angle of the cone subtended by the specimen plane to the objective lens in radians. The term $n \sin \alpha$ is known as numerical aperture, NA. It is not possible to focus light perfectly due to diffraction and interference effects. Diffraction changes parallel wave front that interacts with an aperture into a spherical wave front. Similarly, light gets focused not as a spot but as a set of concentric circles with

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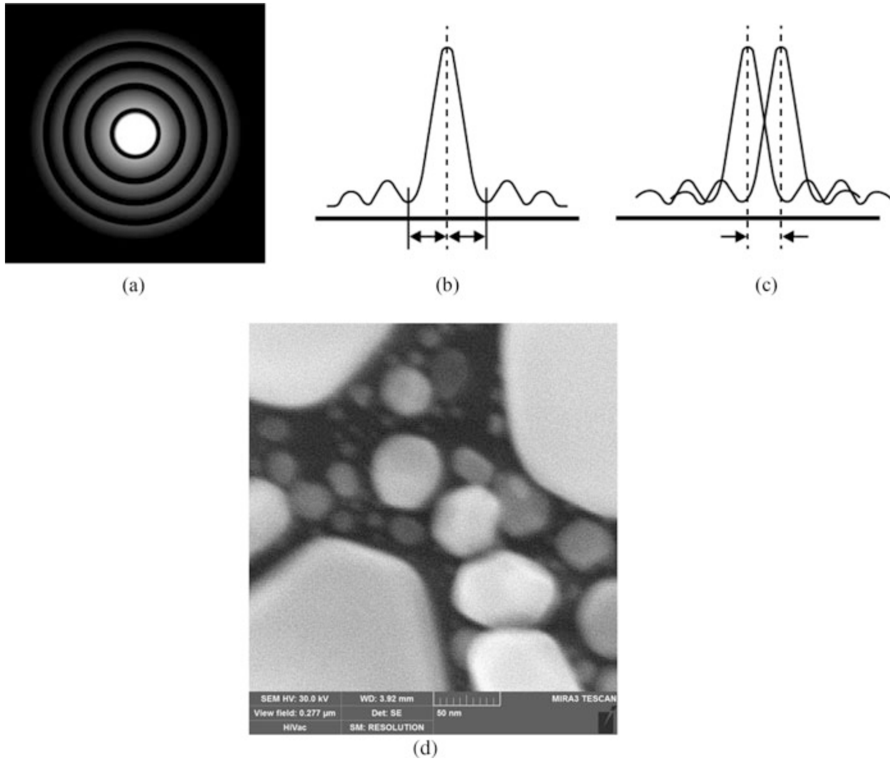


Fig. 4.1 Illustration of resolution in (**a**) airy disc and (**b**) wave front form where resolution is the distance between the first-order peak and trough. (**c**) When the distance between the first-order peaks from two points is smaller than the distance between first-order peak and trough, the points are not resolved. (**d**) An example of a high-resolution SEM image taken at a magnification of ×2,000,000. (Image courtesy of TESCAN)

diminishing intensity due to interference effect. This is known as the *airy disc* as shown in Fig. 4.1a. Wave front form is shown in Fig. 4.1b, c. An example of a high-resolution SEM image is shown in Fig. 4.1d.

The resolution is the distance between the first-order peak and the first-order trough as indicated by the width of the region bound by arrows in the waveform shown in Fig. 4.1b. If the resolution is defined as the minimum spacing between two features at which they are recognized as separate and distinct, then two features are not distinguished if the first-order peaks generated by them are separated by a distance smaller than the distance between the first-order peak and trough (see Fig. 4.1c). Lack of resolution will make them appear as a single feature. This can be considered as the resolution of an optical system that is free of lens aberrations and is limited by diffraction effects only.

4.1.1 Criteria of Spatial Resolution Limit

The ability of the SEM to distinguish fine details of a specimen is determined by its spatial resolution. Several definitions or criteria have been described for spatial resolution limit, and these are summarized in the literature [2–6]. Some of these criteria are listed below.

4.1.1.1 Rayleigh Criterion

According to the Rayleigh criterion, two point sources are resolved if the central maxima of the diffraction pattern generated by one point coincides with the first zero of the diffraction pattern generated by the second point source. Effectively, the Rayleigh's resolution limit is the distance between the first zero and the maxima of point spread function of the system. This criterion can be extended to points with non-zero sources around it. This is done by describing the resolution limit as the distance for which the intensity at the central dip in the composite image is 81% of the maximum. This criterion is based on presumed resolving abilities of the human eye.

The Rayleigh limit or the point resolution of an imaging system is given by the following equation:

$$p(r) = \frac{1}{2\,\pi\,\rho^2}\exp\left(-\frac{x^2+y^2}{2\,\rho^2}\right) - \frac{1}{2\,\pi\,\rho^2}\exp\left(-\frac{r^3}{2\,\rho^2}\right) \tag{4.2}$$

where ρ is the width of the Gaussian function, r is the absolute value of a two dimensional vector $t = (x\ y)^T$, T is for the transposition, $\rho_{\mathrm{p}'}$ is the smallest distance over which two points can be resolved, which is given by the necessity that the value of the composite intensity distribution located at half-way between two points is 0.8 times the value of the maxima,

$$2\exp\left[-\frac{\rho_{\mathrm{p}}^2}{8\,\rho^2}\right] = 0.8 \tag{4.3}$$

which results in

$$\rho_{\mathrm{p}} \approx 2\,\sqrt{2}\,\rho \tag{4.4}$$

The definition proposed by the Rayleigh criterion is applicable to point-like specimen structures which are smaller than the diameter of the probe. Due to this reason, they show separation in the distribution inside the range of the order of the spot size. Resolution limits of 10–15 nm were obtained when using probe diameter of 5–7.5 nm with a LaB_6 electron gun. Resolution of 5 nm was obtained with a probe diameter of 3 nm. The distance between two points is taken to be larger than the electron probe diameter.

4.1.1.2 Sparrow Criterion

According to sparrow criterion, the smallest distance that can be resolved is when the minimum in the composite image intensity distribution just disappears. Using this criterion:

$$\rho_s = \frac{\sqrt{2}\,\rho_p}{2} \tag{4.5}$$

4.1.1.3 Schuster's Criterion

Shuster's criterion states that two point sources can be resolved when no fraction of the central diffraction pattern of one point coincides with the central diffraction pattern of the other. This criterion gives a resolution limit which is twice as large as the Rayleigh criterion.

4.1.1.4 Houston Criterion

Two point sources are resolved if the distance between the central maxima of two points is equal to the half maximum of the diffraction pattern of any one of the point sources.

4.1.1.5 Buxton Criterion

This is similar to the Rayleigh criterion, but instead of intensity, the criterion is based on amplitude diffraction pattern. Amplitude diffraction pattern is the square root of the intensity diffraction pattern. According to this criterion, two points are resolved when the components of the amplitude patterns intersect their points of inflection.

4.1.1.6 Edge Resolution

If an electron beam scans at an angle perpendicular to a sharp edge, the transmitted intensity becomes a smoothed step function.

$$I(x) = \int_{-\infty}^{x} \left[\int_{-\infty}^{\infty} j\left(\sqrt{x^2 + y^2}\right) dy\right] dx \tag{4.6}$$

The distance between points that correspond to 0.25–0.75 fraction of the maximum intensity can be defined as the edge resolution.

4.1.1.7 Radial Intensity Distribution

The radial intensity can be defined as:

$$I(r) = \int_0^r \frac{j(r)\, 2\,\pi\, r}{I_p}\, dr \tag{4.7}$$

With this equation, the size of the electron probe or the resolution can be defined as the diameter of the circle that contains 50–75% of the total probe current, I_p.

4.1.1.8 Maximum Spatial Frequency

The maximum spatial frequency is given as,

$$q_{\max} = \frac{1}{T_{\min}} \tag{4.8}$$

The spatial resolution is defined by the maximum spatial frequency given above, at which the contrast level drops so low that the periodicity $T_{\min}$ cannot be detected by the micrograph.

Other advanced theories about the resolution limit have been proposed; an in-depth review can be found in the works of Dekker and Bos [2].

According to the Rayleigh resolution criterion, the ability to distinguish between two closely spaced objects is linked to the wavelength of its illumination [3]. Since the wavelength of the electrons is much less than 1 Å, the theoretical resolution of an electron microscope is much higher than that of an optical microscope. The wavelength of electrons is given by De Broglie equation:

$$\lambda = \frac{h}{mv} \tag{4.9}$$

where λ is wavelength, h Planck's constant (6.6×10^{-27} J seconds), m mass of the particle (9.1×10^{-28}), and v velocity of the particle.

The energy of an electron is:

$$ev = \frac{1}{2}mv^2 \tag{4.10}$$

where ev is energy in electron volts ($e = 4.8 \times 10^{-10}$), m mass of the particle, and v velocity of the particle.

By substituting the values of h and m in Eq. 4.2, the wavelength λ can be expressed in terms of the accelerating voltage V as follows:

$$\lambda = \frac{1.23\ \text{nm}}{\sqrt{V}} \tag{4.11}$$

The wavelength of electrons at an accelerating voltage of 30 kV is 0.0071 nm. Above value for λ is substituted into Abbe's equation. Beam convergence angle α is small in electron microscopy; so sinα is replaced by α. The value of refractive index n is taken as 1. Therefore, the equation for the resolution limit of the SEM can be given as:

$$d = \frac{0.753}{\propto \sqrt{V}} \tag{4.12}$$

where d is the resolution in nm, α is half aperture angle, and V is accelerating voltage. At electron beam energy of 30 keV and α of 0.01 radian, the theoretical resolution

limit is calculated to be 0.435 nm. At lower beam energy, it will deteriorate. At 1 keV, the limit is 2.38 nm. However, due to the presence of aberrations in electromagnetic lenses used for focusing and image formation, the attained resolution is relatively inferior. The main challenge is to be able to focus the beam into a small probe. At low accelerating voltage, electron charge repulsion tends to widen beam diameter. Use of advanced technology such as beam monochromators, beam deceleration, and immersion optics, where specimen in immersed in the magnetic field of the lens, has enhanced the attainable resolution considerably. Commercial manufacturers have claimed to attain a resolution of 0.5 nm at 30 kV and 0.9 nm at 1 kV with the SEM.

4.1.2 Imaging Parameters That Control the Spatial Resolution

The spatial resolution of the SEM is controlled by manipulating four important parameters of the electron beam, namely, probe size, beam current, convergence angle, and accelerating voltage.

4.1.2.1 Probe Size

Spatial resolution of an SEM is primarily dependent upon the probe size of the beam that falls on the specimen. Secondary electrons do not have sufficient energy to travel distances larger than 10 nm in solid specimens. Only those close to the surface are able to escape. These SE_1 electrons are stimulated from the vicinity of the beam. The spatial resolution of an image is determined by the SE_1 signal which is dependent on the beam diameter or the spot size on the specimen. The resolution of the SEM cannot be better than the diameter of the probe; however, it can be worse depending upon the level of magnification used.

It can be seen in Table 3.1 that the size of the picture element on the specimen at a magnification of 100,000× is 1 nm only. If the probe size of 1 nm is achieved in the SEM through careful use of operational parameters, it is still likely that signal delocalization will produce a much larger excitation volume within the specimen (see Sect. 3.1.2). Materials with a lower atomic number will produce large excitation volumes. Once the sampling region becomes larger than the picture element, signals from adjacent picture elements overlap, and the image may appear blurred. Thus the ability to resolve fine features is affected not only by the probe size but also by the degree of delocalization of the probe within the specimen, the magnitude of which depends on the beam energy and the atomic number of the material examined. However, it is a common observation that features are actually resolved in the SEM at much higher magnifications than mentioned above. This is due to the fact that the high-resolution SE_1 signal is superimposed on low-resolution (SE_2, SE_3, and BSE) signal when detected using E-T detector, thereby making it possible to resolve fine objects.

So, to obtain a high-resolution image, the spot size should be kept as small as possible, in order to resolve the smallest feature of the specimen. This is achieved by using a suitable choice of operating parameters.

Table 4.1 Comparison of the electron source and final spot size of W, LaB_6, and field emission guns

	Thermionic, W	Thermionic, LaB_6	Schottky FE	Cold FE
Source diameter	15 μm	5 μm	15 nm	2.5 nm
Spot diameter	7 nm	4 nm	1 nm	1 nm

The spot size is reduced by decreasing the working distance or using the lenses in the SEM. These lenses help demagnify the beam emanating from the electron gun. It is helpful if the diameter of the beam emanating from the gun is small to start with, i.e., the source radius is small. Field emission guns have small source size and produce fine probes as seen in Table 4.1.

Small probe size while enhancing the resolution also decreases the probe current. To resolve two points of a specimen, there must be a distinct difference between the signals emanating from these points to produce contrast.

The contrast level C in a specimen in terms of critical current I_c is given by Rose criterion [7–9]:

$$I_c > \frac{4 \times 10^{-12}}{q \times F \times C^2} \tag{4.13}$$

where q is the detector efficiency and electron yield product F is the frame scan time.

4.1.2.2 Beam Current

It can be seen that for a given detection system, at a fixed scan time, there is a critical beam current required to observe a particular contrast level, beyond which it is difficult to distinguish the signal from the noise.

The spot size for a thermionic emission filament can be calculated as:

$$d = d_{\min} \left[7.92 \times 10^9 \left(\frac{I\,T}{j}\right) + 1\right]^{\frac{3}{8}} \tag{4.14}$$

where T is the temperature and j is the current density at the surface of the filament.

$$d_{\min} = K\,\lambda^{\frac{3}{4}}\,C_s^{\frac{1}{4}} \tag{4.15}$$

From the two equations, it can be seen that to properly discern the features in an SEM, a minimum current is required which corresponds to a minimum spot size (see Sect. 2.4.4.5). However, as suggested by Eq. 2.3 for any given beam energy, smaller currents result in smaller probe sizes. For this reason, it is common practice to employ currents in the order of tens of picoamps during high-resolution imaging.

4.1.2.3 Convergence Angle of the Probe

Probe convergence angle is defined as the half-angle of the cone of electrons converging onto the specimen. The spot size can also be reduced by manipulating the convergence angle. One way is to use apertures which prevent off-axis electrons

from passing through; the lens then focuses the beam into smaller spot size. This also reduces the probe current as the number of electrons allowed to pass through decrease. Another way is to manipulate the working distance; having a small working distance increases the angle of convergence, thereby decreasing the spot size and increasing the resolution. Large working distance increases lens aberrations and needs strong lenses to help focus the image. Small working distance decreases the probe size increasing the resolution, but it also reduces the depth of field in an SEM image.

4.1.2.4 Accelerating Voltage

High accelerating voltage produces high brightness and smaller spot size. The highest resolution is achieved at high beam energies. For samples with a high atomic number, the high beam energy is ideal as interaction volume within the specimen stays within acceptable limits. Similarly, for thin samples (with small interaction volume), such as nanoparticles, use of high beam energy produces high-resolution images. However, for the bulk sample with a low atomic number that exhibits high excitation volume, generation of SE_2, SE_3, and BSE signals adds to the noise component. This contributes to the lowering of the signal-to-noise ratio (SNR). However, high brightness at high beam energy serves to compensate for the lowered SNR. The inclusion of SE_2, SE_3, and BSE in the total signal serves to degrade the spatial resolution. For low Z materials, therefore, the low beam energy is preferred in order to reduce the generation of low-resolution SE_2, SE_3, and BSE signals.

Low voltage microscopy is a common technique to get high-resolution images from the SEM. The voltage used in this technique is generally in the order of 500 V to 5 kV. Use of low voltage decreases the interaction volume of the beam with the specimen (i.e., electron range decreases as a function of $E_0^{1.67}$; see Sect. 3.2.6). Due to this, the SE_2 signals that emanated from a wider volume now emanate from a smaller volume close to that of the SE_1 signal. Typical spatial distribution of SE_1 and SE_2 signal is shown in Fig. 3.29. Decreasing the beam energy serves to localize generation of the SE_2 signal. The SE_2 signals now carry more relevant information from the vicinity of the beam footprint. This enhances the resolution as now most of the SE signal carries high spatial resolution information. This also eliminates the need of separating SE_1 signal from the SE_2 signal as it carries useful information rather than the background information. Consequently, the performance of the imaging technique is improved, and better resolution and contrast are attained in SE images. This also enhances the resolution of the BSE, which now gives resolution similar to that for SE imaging.

The SE signal at low beam energies increases more rapidly than the decrease in brightness at low voltage. Due to this the signal-to-noise ratio remains good even up to voltages of 500 V. Low voltage imaging also enhances the imaging for intermediate and high atomic number specimens, because the range of signal for strongly scattering materials decreases significantly at lower beam energies.

There are certain disadvantages to using low beam energy imaging technique. Firstly, the electron source brightness which is proportional to the beam energy

reduces linearly with accelerating voltage. Brightness is given by Eq. 2.3 where it can be seen that imaging at very low voltage might cause the beam current to fall below the threshold current for any useful contrast. This directly affects the feature visibility. At 500 eV the field emission gun has the same brightness as that of a tungsten emitter at 20 keV. The decrease in beam current is usually compensated by an increase in the SE signal. The performance of the SEM also degrades at a lower voltage due to chromatic aberration which is a result of the energy spread of the gun as can be seen from Eq. 2.10. This also causes the profile of the beam to change shape producing a broad skirt of intensity about the center of the probe, resulting in a reduction of the image contrast. These can be corrected by the use of beam monochromator which reduces the energy spread of the beam. Low-energy beams are also prone to degradation/deflection of stray electromagnetic fields, which necessitates the use of short working distances. Hence, cold field emission guns equipped with a snorkel or immersion lens are ideal for low voltage imaging. Another method of tackling the brightness problem at low accelerating voltage is the use of negative bias on conducting specimens. This slows down the incoming electrons from the beam, resulting in lower penetration; but also the number of electrons in the beam remains high, thus improving the brightness.

Another factor that can seriously impact low voltage microscopy is contamination of the specimen surface. The imaging is carried out at such low energies that signals from contamination might dominate over the actual signal from the specimen surface. Cleaning of specimen surface is therefore important which can be done with plasma beams. The rate of contamination buildup can be reduced by:

1. Avoiding high magnification.
2. Focusing and removing astigmatism in the image at an area other than that is essential for imaging.
3. Not using spot or reduced area raster mode.

In biological samples, the contamination grows due to the field-enhanced mobility of hydrocarbons across the surface of the sample. Using a thin film coating of metals can significantly reduce the growth of contaminants, as it eliminates fields from charged regions. When low atomic number specimens like biological samples are to be imaged, the lower amount of SE_1 signal makes the imaging difficult, due to low signal-to-noise ratio, which results in dark images. The signal-to-noise ratio can be improved by application of ultrathin coatings of metal. Such coatings have a thickness of 2–3 nm, to prevent distortion of surface features. Metals such as gold-platinum alloy, platinum, and tungsten pure metals are used to coat low atomic number/biological specimens. Such coatings can be easily deposited on specimens using sputter coater. At high magnifications such as 100,000×, grains are very fine and difficult to image. At high magnification, the individual particles can be difficult to distinguish from one another, due to which artifacts may arise in the images and the image might not be able to capture the actual surface detail. However, the ultrafine coating can also improve focus and make astigmatism defects easy to correct.

4.1.3 Guidelines for High-Resolution Imaging

The spatial resolution of SEM can be improved by adopting the following guidelines:

1. Sample preparation: Deficiencies in the samples prepared in haste are exposed at high magnifications. Poor polishing shows up as glaring scratch marks. Contamination at the sample surface hides surface features and leaves contamination marks in the images. Currently available commercial instruments claim a spatial resolution of 0.5 nm. At this high level of resolution, monolayer(s) of contamination becomes a limiting factor as it starts to hide structural details on the specimen surface. Such small levels of contaminants are unavoidable in the SEM chamber as currently available instruments do not operate under ultrahigh levels of vacuum. Use of thin samples in place of bulk samples can reduce the generation of low-resolution SE_2, SE_3, and BSE signals.
2. Sputtered coatings to reduce charge-up effects: The grain structure of metal coatings such as Au, Au-Pd, Pt, etc., sputtered to reduce charging effects may become visible at magnifications of roughly 100,000×. Coating thickness should be kept to a minimum by reducing the duration of the coating application. Coatings that exhibit fine grains such as those produced from Cr targets can be used. Application of thin coating also increases the secondary electron coefficient, thus contributing to the high spatial resolution.
3. Type of SEM used: Instrument equipped with advanced technology such as field emission gun, through-the-lens detector, immersion lens, beam deceleration, aberration corrector, filtering capability, high vacuum, stable anti-vibration platform, etc. serves to enable and enhance high-resolution imaging. For instance, SEM equipped with the latest lenses and detectors efficiently collects SE_1 electrons and leaves out a low-resolution signal. Entry level equipment with basic features is not used for high-resolution imaging.
4. SEM operation: SEM column should be properly aligned. Apertures should be clean and centered, and astigmatism should be removed completely. Deleterious effects of external factors such as electromagnetic interference, acoustic/electronic noise, floor vibrations, etc. become apparent at increased magnifications and need to be addressed before high-resolution imaging is undertaken [10].
5. Use of small spot size: Small electron beam diameters are responsible for high image resolution. One way to control the size of the electron probe is by varying the strength of the condenser lens (i.e., through the use of spot size). Condenser lens demagnifies the beam emanating from the electron gun. The higher strength of condenser lens results in finer probe size which can result in higher resolution, but at the same time, it lowers the probe current that may result in noisy images. Therefore, small probe size gives high spatial resolution but at the same time reduces the ability to actually distinguish features of the object under observation. For high-resolution imaging, the smallest probe size is desirable as long as enough signal-to-noise ratio is generated to get adequate contrast from the features of interest.

6. Selection of final aperture: Use of small aperture diminishes the size of the probe and also minimizes the spherical aberration. However, it can limit resolution due to diffraction effects. Intermediate aperture suitable for the beam energy and working distance employed should be selected.
7. Good signal-to-noise ratio: The objective here is to maximize the generation of a signal from the specimen. This can be achieved by using more beam current, but this also increases the spot size, so an optimal balance needs to be reached. Another way is to use a slower scan rate. Longer scan time will generate more secondary electrons from the spot being scanned. However, this may also damage the specimen. Fast scan rate can be used in combination with frame averaging.
8. Accelerating voltage: High accelerating voltage produces fine beam probes which in turn can result in high resolution. However, high beam voltage also results in large interaction volume with increased contribution from low-resolution SE_2, SE_3, and BSE signals which degrade the overall quality of images. If high resolution is to be achieved at high beam energy, then microscopy conditions are set in a way that low-resolution signal needs to be separated from the high-resolution SE and BSE signal that originates from near the surface of the specimen.

   The alternative is to use low accelerating voltage (i.e., small beam penetration) where both the elastic and inelastic mean free paths rapidly decrease. This limits the interaction volume in the specimen to a point where all the signals are generated from near the surface of the specimen. Low beam energy encourages the generation of high-resolution SE_1 signal from the specimen by confining the interaction volume close to the beam impact point. Under these conditions, low-resolution (SE_2, SE_3, and BSE) signal is restricted. This eliminates the need to separate low- and high-resolution signal as SE_2 is generated from near the beam impact point and becomes part of the high-resolution signal. Surface features become prominent at low beam energy. The drawback is that low accelerating voltage decreases brightness; therefore optimum probe current to produce an acceptable signal-to-noise ratio is required. Low atomic number matrices such as polymers may suffer from low signal-to-noise ratio which is normally overcome by coating the specimen surface with a thin conductive coating. Low accelerating voltage also increases the deleterious effects of chromatic aberration.

   The decision to use either high or low beam energy is made by taking into consideration the type of sample and the nature of information required from the specimen. High beam energy can be used for high-density materials where signal delocalization within the specimen is comparatively smaller. It is also suitable for thin specimens. For low Z and bulk materials and where surface features are of prime interest, low beam energy can be employed. Low voltage SEM is used at 500 V to 5 kV or less where use of high brightness field emission gun and a high-resolution lens system makes it possible to get nanometer scale resolution. Small working distance (i.e., 2 mm) is used. Spot size is 2 to 3. Field emission guns and through-the-lens (TTL) detector is normally employed for

high-resolution imaging. Also, beam deceleration technique helps to reduce the beam interaction area within the specimen.

9. Working distance: Use of shortest possible working distance will reduce lens aberrations and result in fine probe size improving resolution. The close proximity of the specimen to the column maximizes SE_1 collection by the TTL detector.
10. Type of signal used for imaging: The highest-resolution signal is SE_1 since it originates from a small area whose diameter is comparable to the probe size itself. SE_1 should be used to achieve the highest possible spatial resolution. SE_2 and SE_3 are generated away from the probe making them unsuitable for high-resolution imaging. BSE signal is produced from a region almost as large as the excitation volume generated within the sample, therefore rendering it unsuitable for resolving fine details at high magnifications. However, if BSE image is required, high-energy BSE are preferable for imaging since they undergo fewer interactions within the sample keeping the sampling volume small. Low-resolution signal can be excluded by using low beam energy and also by employing a through-the-lens (TTL) detector.

4.1.4 Factors that Limit Spatial Resolution

The factors that primarily limit the spatial resolution include probe diameter, size of excitation volume, and poor signal-to-noise ratio. Spatial distribution of SE signal within the specimen ultimately establishes the resolution of the image. Excitation volume depends on the beam energy and probe diameter as well as on the specimen density and feature topography. Attainable resolution is therefore not an instrument constant and can vary with specimen and application. Instrument manufacturers test an ideal standard sample such as small Au particles dispersed on a low Z film to measure spatial resolution. The quoted value shows the capability of the equipment and does not suggest the range of information that might be extracted from various types of samples analyzed in the same instrument. Samples that need to be imaged at low voltage or current as well as low Z samples that show poor SE yield may not achieve this level of resolution. High-resolution capability might be able to spatially resolve two features, but poor signal-to-noise ratio may not provide sufficient contrast necessary to examine the topography of the specimen. When the SEM is used in EDS or environmental mode, the resolution will be limited by the diameter of the excitation volume which can be a few tenths of a micrometer (e.g., large) depending on the density of the sample material and the electron beam energy. Use of high beam current and energy degrades image resolution. In low voltage SEM, the electron range and the escape depth of SE are of comparable size. Under such conditions, the probe size may no longer remain an indication of the measure of the resolution limit. The user, therefore, needs to determine the optimum operating parameters that can extract the required information from a sample at the optimum resolution.

4.2 Depth of Field

One of the most important advantages of SEM is the large depth of field. It is the ability of a microscope to focus different depths simultaneously such that the specimen surfaces at different distances from the lens remain in focus. The sample appears focused not only at the plane of optimum focus but also at some distance above and below it. When a specimen with large depth is observed, focusing the upper region may result in blurring of the lower region and vice versa. In such case, if the range of upper and lower features that are in focus is large, the depth of field is considered to be large [11]. SEMs have the ability to focus large depths simultaneously making it one of the most effective tools for 3-D imaging at the micro- and nano-levels. The ability of the SEM to convey three-dimensional information is largely due to its large depth of field. This feature is especially useful to image as-received rough specimens such as fracture surfaces, corrosion deposits, solids in powder form, etc.

The reason for this large depth of field is the geometry of beam optics as shown in Fig. 4.2 where the electron beam scans the surface of a rough sample at steady focus. The objective lens of SEM focuses the electron beam to a crossover at the plane of optimum focus. During the process of scanning, sample region labeled “a” coincides with the plane of optimum focus and displays the sharpest focus. At this plane, the probe diameter (or more specifically sampling region diameter) is smaller than the sample pixel size. The diameter of the probe increases both above and below this plane due to beam divergence. At some distance above and below the plane of optimum focus, if the diameter of the beam is less than 2× sample pixel size, the plane remains in focus, and any feature within this range will appear focused in the SEM image. Therefore, regions labeled “b” and “c” in Fig. 4.2 located at some distance above and below this plane, respectively, appear focused. Beyond points

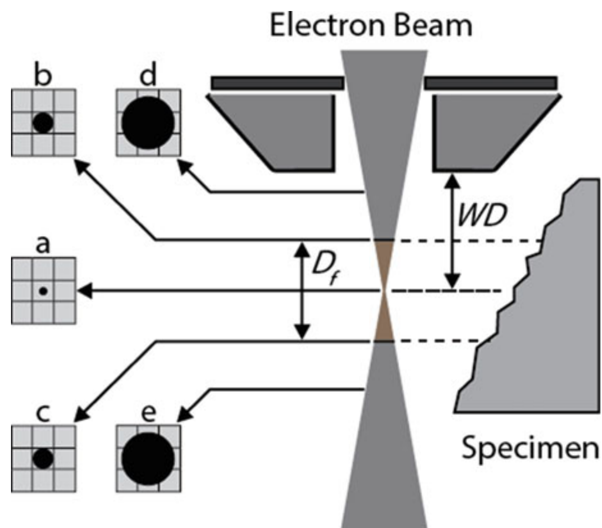


Fig. 4.2 The geometry of beam optics. The probe diameter is overlaid on a pixel at different heights of the specimen to indicate the point where the image goes out of focus. Points (**a**–**c**) will be in focus while points (**d**–**e**) will be out of focus Adapted from [12]

"b" and "c," the diameter of the probe becomes larger than 2 pixels. As a result, information from adjacent pixels overlaps and the image becomes out of focus (see Sect. 3.1.2). Regions labeled "d" and "e" will be out of focus because the probe diameter that scans these regions will be 2× pixel diameter at the selected magnification. At these regions, signals from adjacent pixels will overlap to create blurriness in the image.

The probe size within the distance labeled "D_f" in Fig. 4.2 will remain adequately small to be able to focus regions of the sample that coincide with the probe. This distance along the vertical height of a sample where all features are in sharp focus concurrently is called the *depth of field*, D_f. Features remain in focus as long as the probe diameter is <2× the pixel size at that particular magnification. Blurriness will occur at a distance of one-half of the depth of field (i.e., 1/2 D_f) both above and below the optimum focus plane where the diameter of the probe becomes too coarse to provide adequate focus. Regions of a specimen, therefore, that are scanned by coarse probe will appear blurred in the SEM image.

From the schematic in Fig. 4.3, it can be seen that:

$\tan \propto = \frac{r}{D_f/2}$ (where α is the half-angle of convergence and r is the radius of the probe)

$$\tan \propto = \frac{2r}{D_f}$$

$$D_f = \frac{2r}{\tan \propto}$$

Since α is small, $\tan\alpha$ is taken as α.

$$D_f \approx \frac{2r}{\propto}$$

Now, $r = 1$ pixel $= \frac{100\ \mu\text{m}}{M}$ where $M =$ magnification

$$D_f \approx \frac{200\ \mu\text{m}}{\propto M}$$

Now, $\propto = \frac{R_{ap}}{\text{WD}}$ where R_{ap} is the radius of final aperture and WD is the working distance

$$D_f \approx \frac{200\ \mu\text{m} \times \text{WD}}{R_{ap} \times M} \quad (4.16)$$

The depth of field is dependent upon the electron beam divergence angle, which in turn is characterized by the radius of the final lens aperture and the working distance as shown in Eq. 4.16. It follows that large depth of field can be obtained by reducing the beam divergence which in turn can be achieved by using small objective aperture and a large working distance as shown in Fig. 4.3. In Fig. 4.3a, the effective focus region as defined by the depth of field is the smallest since a large-sized aperture is

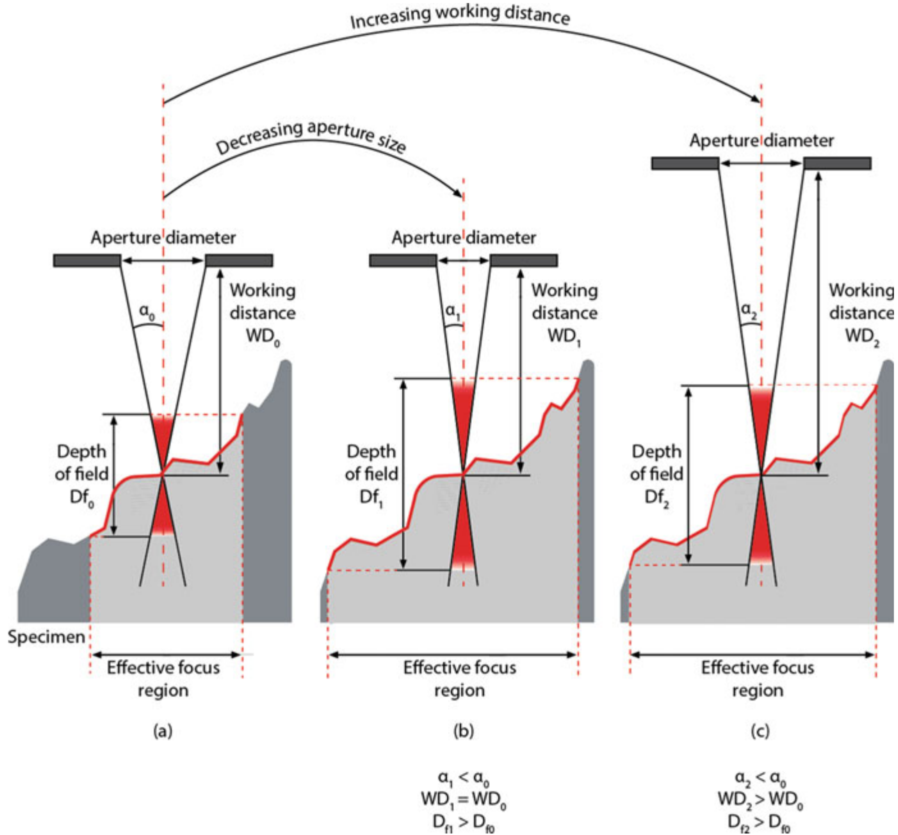


Fig. 4.3 (**a**) Large aperture gives small D_f. (**b**) Use of small aperture increases D_f and effective region in focus. (**c**) Increased working distance also results in larger D_f

used which creates greater beam divergence. As the aperture is changed to a smaller size (Fig. 4.3b), the beam is converged further to increase the depth of field resulting in an increase in the area of the specimen which is now in focus. The same effect can be obtained by increasing the working distance as shown in Fig. 4.3c.

Reducing magnification also increases the depth of field. Conversely, depth of field decreases with an increase in magnification (Eq. 4.16 and Fig. 4.4).

In the SEM, the divergence angle formed is very small (in milliradians) compared to that in an optical microscope. Due to this reason, the change in probe size with distance (depth) from the lens is very small. Small probe size keeps the sampling volume restricted over a large range of depth. All features in the sample along that depth will appear in focus where the diameter of sampling volume is smaller than 2× picture element of the sample. The remarkable depth of field obtained in SEM images is as important as high resolution. In fact, most of the SEM images are taken to observe the topography and morphology of specimen surface which looks so enriching due to the large depth of field. This characteristic alone is responsible for

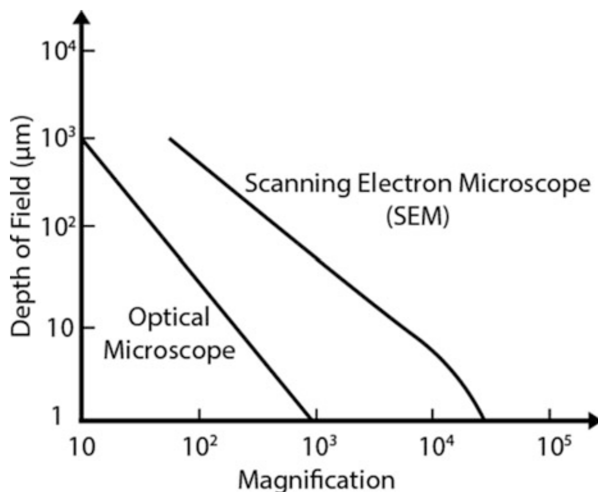


Fig. 4.4 Depth of field decreases as magnification increases [11]

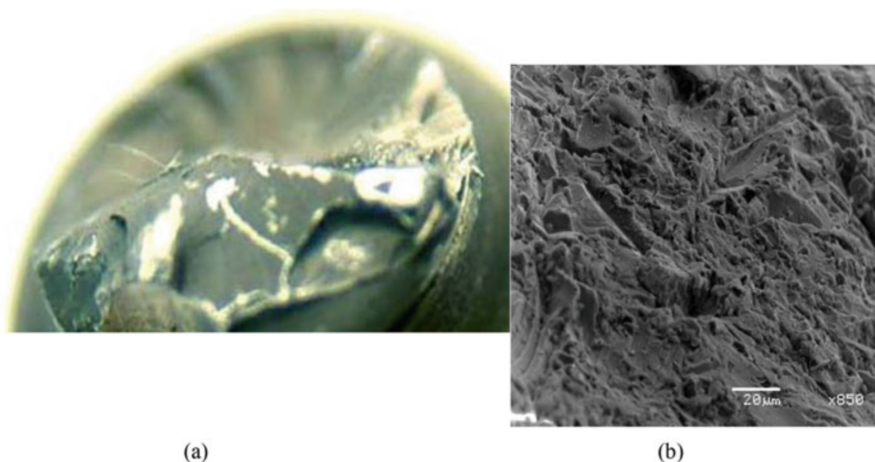


Fig. 4.5 Images taken using (**a**) optical microscope and (**b**) SEM. The optical image is suitable for images at low magnifications but displays small depth of field resulting in blurring of some detail. SEM provides fully focused images of rough samples such as fracture surface

such wide usage of SEM technique across so many diverse fields of applications. The depth of field in the SEM can be between 10% and 60% of the field width depending on the selected magnification. The depth of field in the SEM is tens of times than that of the light microscope. A comparison between the two techniques is provided in the optical and SEM images shown in Fig. 4.5a, b, respectively.

The ability of the SEM to exhibit large depths of field is displayed in Fig. 4.6 where a screw sample is imaged while held in an upright position. Unfortunately, the imaging conditions that promote high depth of field concurrently reduce attainable

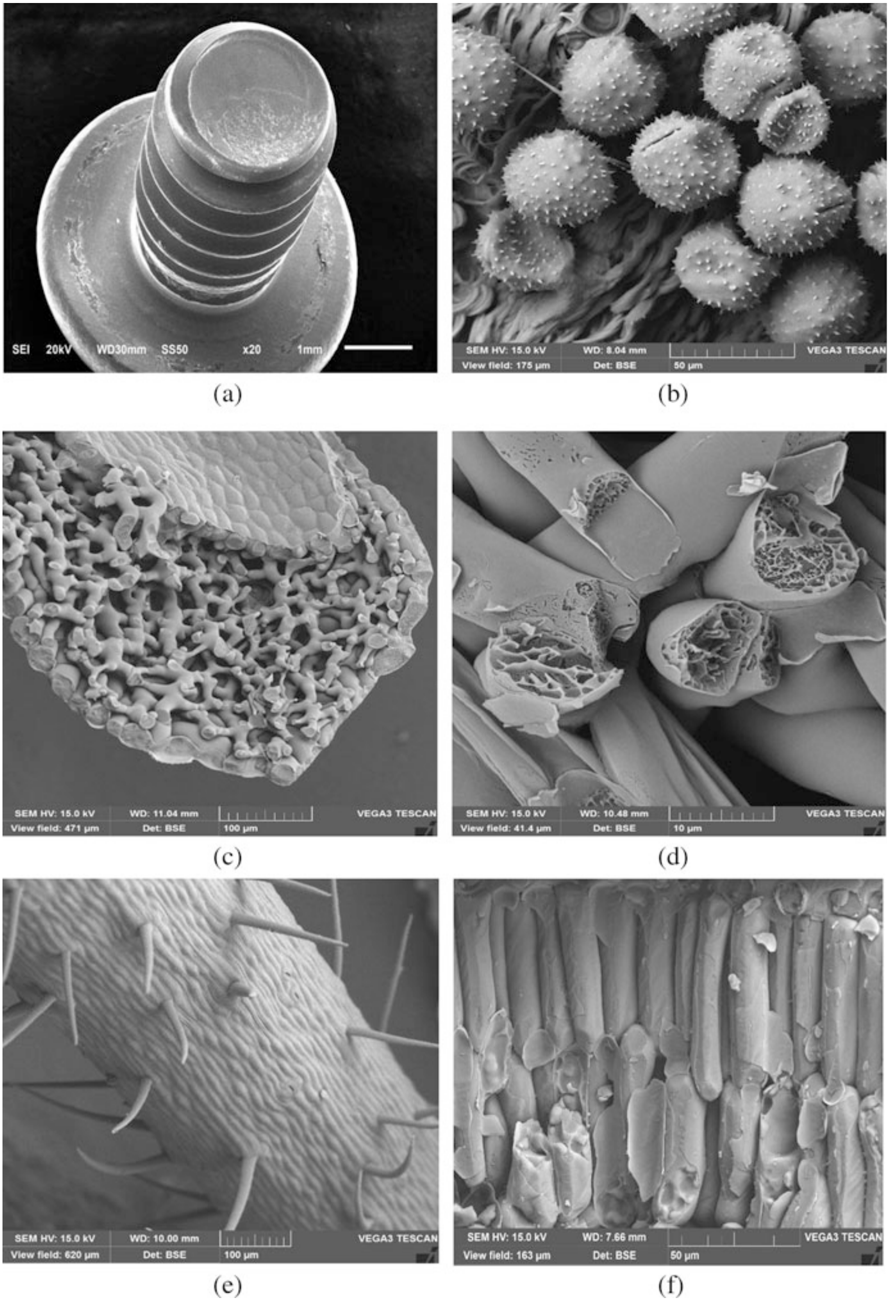


Fig. 4.6 Secondary electron SEM images showing the large depth of field capability of the SEM. (**a**) Screw sample fully focused from the top to the base. (**b**–**f**) Various biological samples. (**b**–**f** images courtesy of TESCAN)

resolution since small convergence angle α results in large focal lengths making images susceptible to aberrations.

The depth of focus refers to the ability of a lens to focus an image at varying heights relative to the image plane. In the SEM, the image is formed in an indirect manner using the signals emitted from the specimen. There is no lens that forms an image directly at any plane in the beam optics. Due to this reason, it is not appropriate to use the term "depth of focus" with regard to the SEM. The concept is more aptly defined by the phrase "depth of field" which refers to the z-range within an image where various features of a specimen remain focused within a field of view.

4.3 Influence of Operational Parameters on SEM Images

The scanning electron microscope is a powerful tool that images microstructural features of materials in significant detail. Microscopy at low magnification and imaging of conductive samples is fairly straightforward. However, during high-resolution microscopy, the knowledge of optimum imaging conditions and operational parameters is required to produce high-quality images that can reveal fine surface structure. In addition, necessary know-how helps the user to interpret SEM images and identify and differentiate between related factors that influence the results. Important imaging parameters are summarized in the following sections.

4.3.1 Effect of Accelerating Voltage (Beam Energy)

Accelerating voltage is the difference in potential between the filament and the anode. The magnitude of accelerating voltage used during microscopy has a direct bearing on the extent of surface features resolved, spatial resolution, brightness, chromatic aberration, interaction volume, edge effect, charge buildup, beam contamination, and damage and strength of analytical x-ray signal.

High accelerating voltage produces a smaller electron probe diameter, thus enabling higher spatial resolution (Eq. 4.12). It also produces brighter images (Eq. 2.4). Chromatic aberration degrades image resolution at accelerating voltages below 10 kV (Eq. 2.10). So, use of high beam energy reduces the deleterious effect of chromatic aberration. High beam energy is also required to excite x-rays from heavy elements during microchemical analysis. Apart from this, high accelerating voltage is not suitable for imaging since it results in greater beam penetration into the specimen resulting in larger excitation volume (Sect. 3.2.5.1) and generation of low-resolution signals. These signals, such as backscattered electrons, reduce image contrast and tend to hide fine features at the specimen surface. High accelerating voltage also tends to enhance edge effect, charge buildup, and beam contamination/damage of the specimen.

Operation of the SEM at low accelerating voltages ($\leq$5 kV) leads to confined specimen-beam interaction restricted close to the surface producing an image with rich surface detail. This is the basis for low voltage microscopy employed for high-

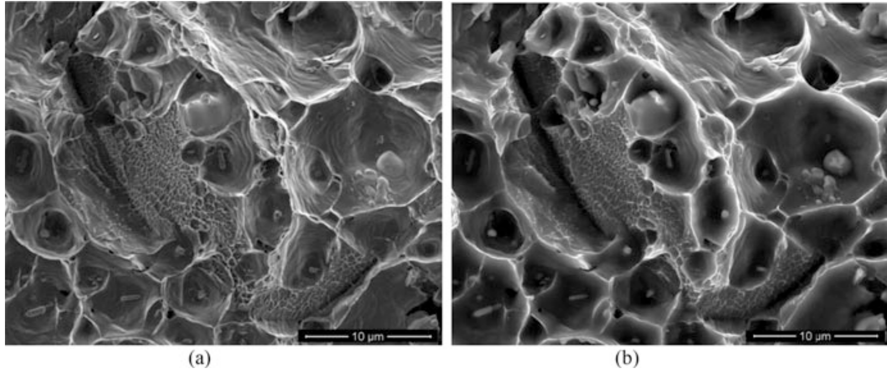


Fig. 4.7 Secondary electron SEM images of the fracture surface of Al showing typical microvoid coalescence structure. (**a**) At beam energy of 2 keV, surface details are prominent, while at (**b**) 15 keV, the surface features are not as clearly discernible, void pits are dark, and sharp fracture edges are brighter due to the enhanced edge effect

resolution imaging in field emission scanning electron microscopes. Lower accelerating voltages are suitable for examining fragile/soft samples (such as cells and polymers) and features present in low quantity and also for revealing fine surface structures since the electron beam penetration in the sample is limited. It can be seen in secondary electron SEM images shown in Fig. 4.7a, b that fine features visible at the specimen surface at 2 keV are lost when beam energy of 15 keV is used.

Another example is shown in Fig. 4.8a–c where surface features visible in a polymer sample at 5 kV are obscured when the accelerating voltage is increased to 20 kV.

The overall effect of accelerating voltage on the image quality is summarized in Fig. 4.9.

4.3.2 Effect of Probe Current/Spot Size

The current that impinges upon the specimen and results in the generation of various signals is called the probe current. For any given beam energy, smaller current results in smaller probe size (Eq. 2.16). The spatial resolution of an SEM is primarily dependent upon the probe size of the beam that falls on the specimen. The smaller the electron probe diameter the higher is the image resolution attained in the SEM. For high-resolution imaging, maximum probe current is sought for a small probe diameter by using a suitable choice of operating parameters. However, the larger the probe current the bigger is the probe diameter. The relationship between the probe current and probe diameter is depicted in a plot shown in Fig. 4.10. It is clear that the probe size increases with increasing probe current. The final probe diameter that interacts with the specimen is determined primarily by the electron source size, degree of demagnification by the electron lenses, and the extent of spherical

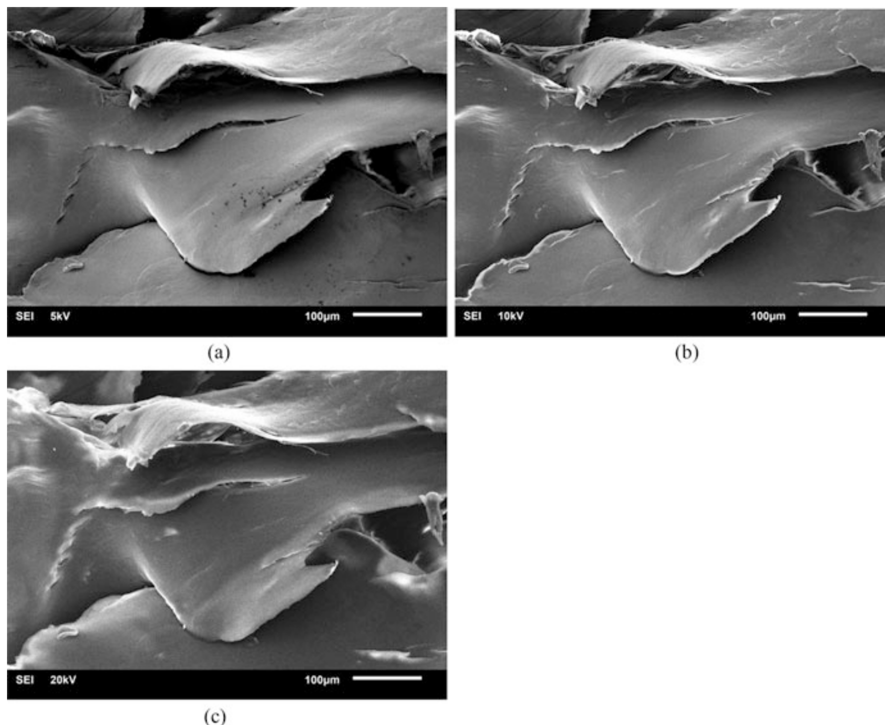


Fig. 4.8 (**a**–**c**) Secondary electron SEM images obtained from the polymer sample. Increase in accelerating voltage from 5 to 20 kV obscures surface features such black spots visible in (**a**)

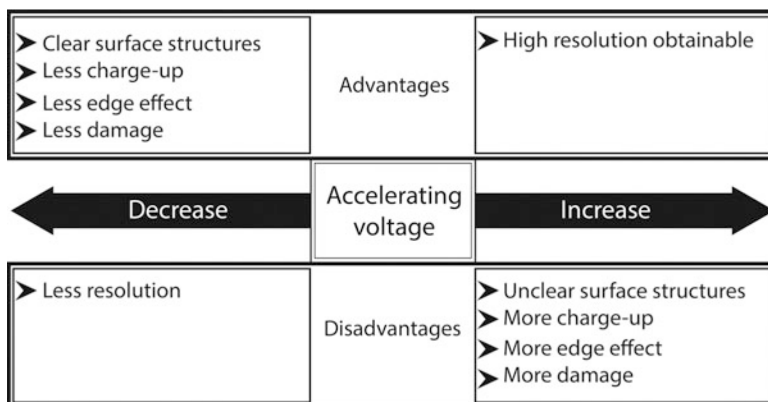


Fig. 4.9 Schematic illustrating the effect of accelerating voltage on the image quality

aberration. For thermionic emission W or LaB_6 filaments, focusing the beam into small probe results in very low beam currents. On the other hand, field emission electron guns can concentrate a large amount of current in the small probe.

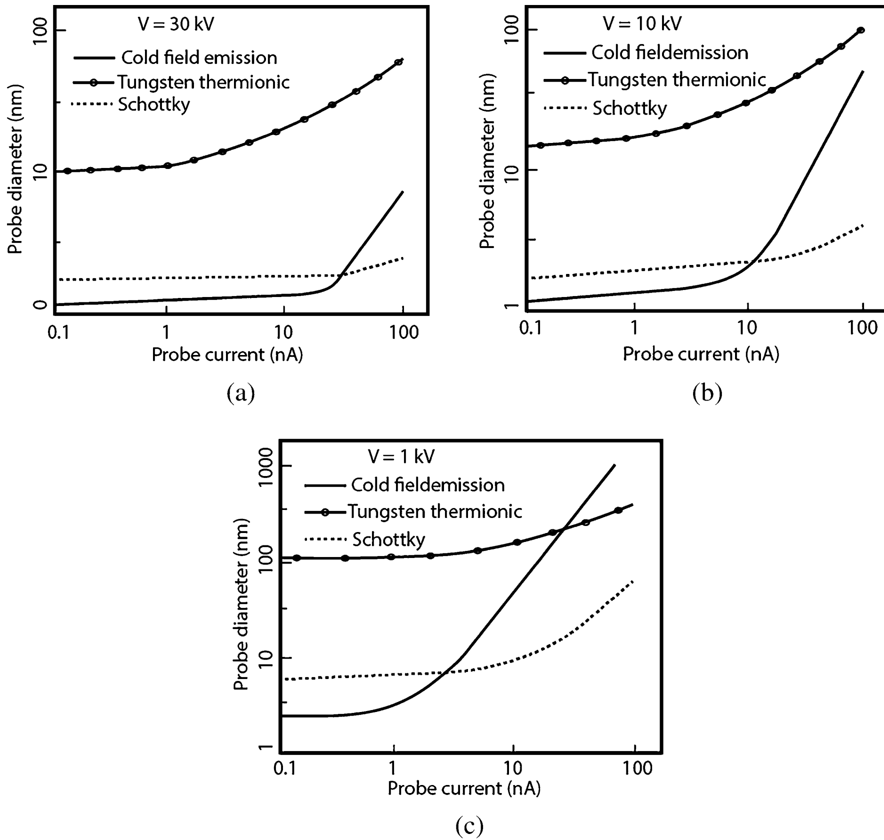
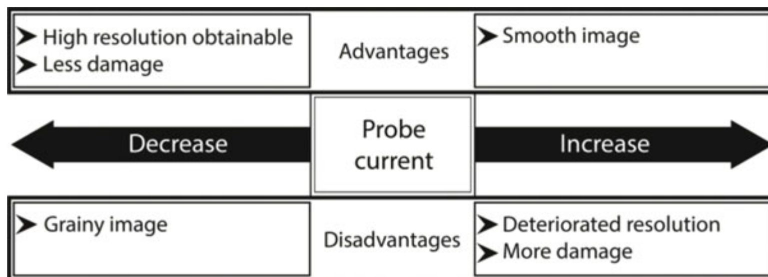
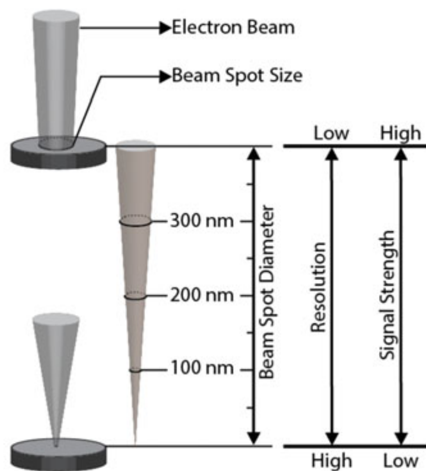


Fig. 4.10 (**a**–**c**) Plots to illustrate the relationship between the probe current and probe diameter. Probe diameter increases with probe current at all accelerating voltages from 30 to 1 kV. At higher accelerating voltage, the probe diameter is smaller. Probe size increases with decreasing accelerating voltage. Cold field emitter has the smallest probe size followed by Schottky, LaB_6, and W filament at all accelerating voltages and probe currents used [9]

High probe current results in smooth images but degraded image resolution. It can also induce beam damage. Low probe currents realize high image resolution while the specimen is susceptible to less beam damage. Very low probe currents give rise to grainy images which tend to hide surface details. A critical level of probe current is required to achieve an acceptable contrast in the image (Eq. 4.13). The magnitude of such current corresponds to the minimum spot size. Optimum probe current is selected based on magnification, accelerating voltage, specimen type, etc. and is usually in the order of a few picoamps. Schematic in Fig. 4.11a shows the effects of probe current on SEM images. High beam current results in greater signal strength. During imaging at low magnifications where very high spatial resolution is not required, use of large spot size is recommended. Any increase in spot size (by weakening the condenser lens) is accompanied by an increase in the beam current by a magnitude that is roughly square of the beam diameter (Eq. 2.21).



(a)



(b)

Fig. 4.11 (**a**, **b**) Schematics illustrating the effect of probe current and spot size on the image quality. Small spot size or probe current gives high spatial resolution and low signal strength. Large spot size or probe current gives high signal strength but the resolution is degraded. For imaging at low magnification, large probe diameter (≈100 nm) can be used

A large-sized beam diameter is preferred during low magnification imaging as long as the spatial resolution is not affected noticeably. At low magnifications, the size of a picture element in the sample is large and accommodates delocalization of the signal well resulting in focused images even at large beam diameters. Schematic in Fig. 4.11b illustrates the effect of spot size on the signal strength and resolution.

The effect of spot size on the image quality in the SEM is demonstrated in Fig. 4.12. It is seen that at a small spot size (small probe current), the image is noisy. With an increase in spot size (or probe current), the signal-to-noise ratio increases and the image becomes sharp.

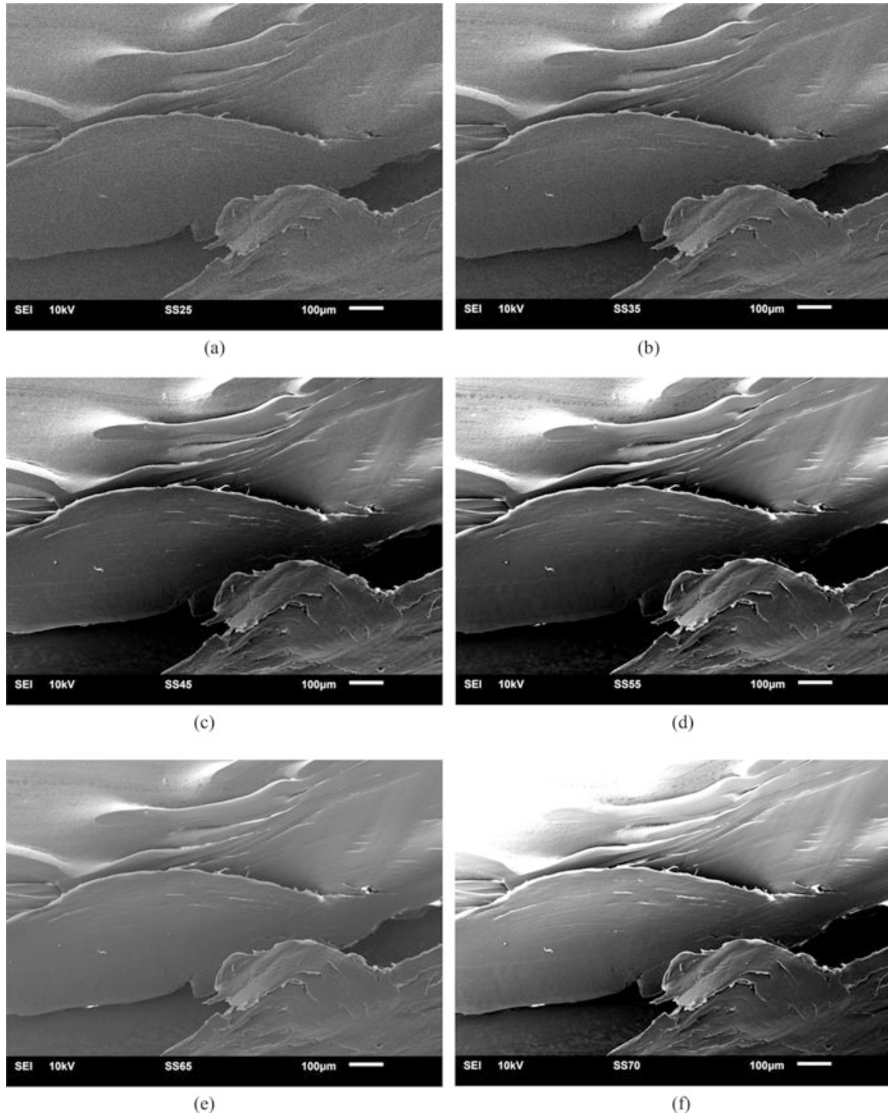


Fig. 4.12 (**a**–**f**) SEM images of polymer sample with increasing spot size (e.g., probe current). The image is noisy at small spot size. As the spot size is increased, the signal-to-noise ratio increases and the image becomes sharper. At the largest spot size, charging is observed as the polymer sample is unable to discharge the accumulated current at its surface

4.3.3 Effect of Working Distance

Working distance (WD) is the distance between the pole piece of the objective lens and the plane of best focus. Working distance is adjusted by moving the sample stage

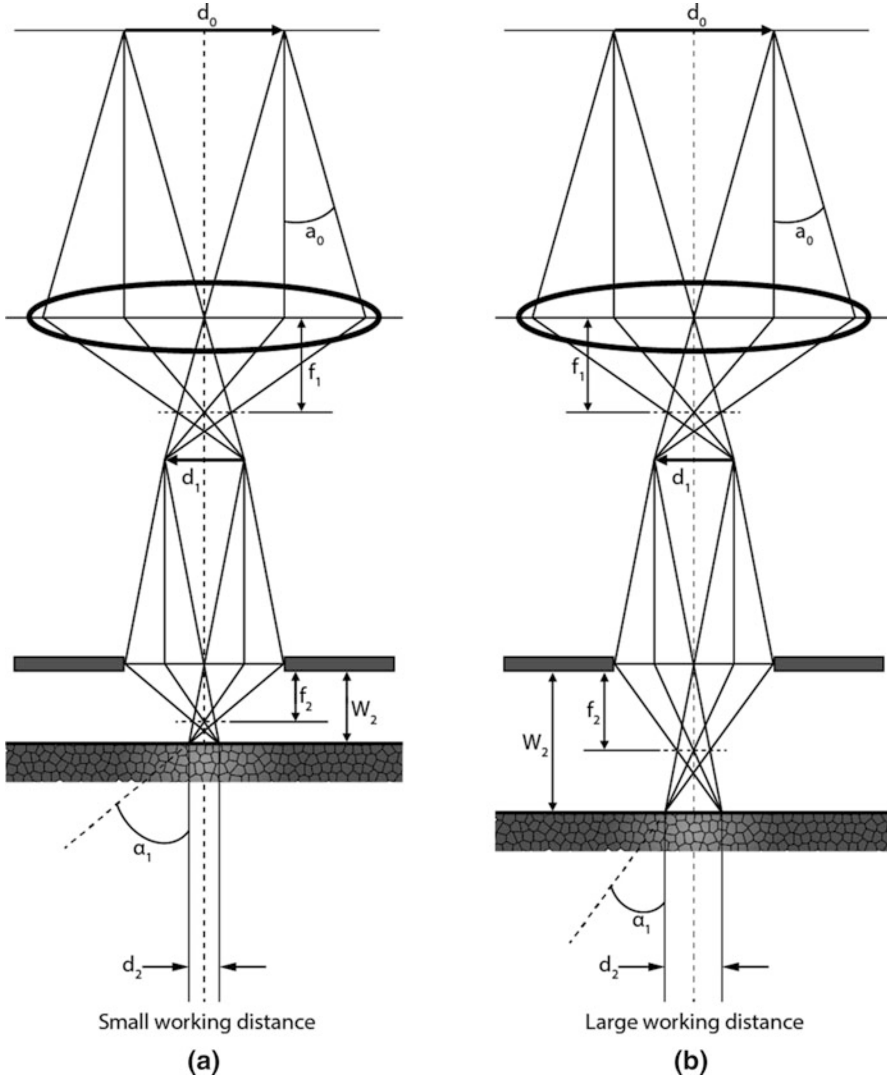


Fig. 4.13 Effect of working distance on the probe size. (**a**) Short working distance gives small probe size due to large convergence angle and small focal length, while (**b**) large working distance increases the probe size (Adapted from [9])

along the *z*-axis and focusing the beam on the sample surface. In order to image at a set WD, the sample is brought into optimal focus by moving the sample stage along *z*-axis while keeping objective lens current constant. Positioning the sample close to the lens enables high image resolution but decreases the depth of field (Eq. 4.16). The resolution is improved as the probe size becomes smaller at short WD as shown in Fig. 4.13a, b. Use of immersion lens can avail 2–5 mm WD for a specimen with

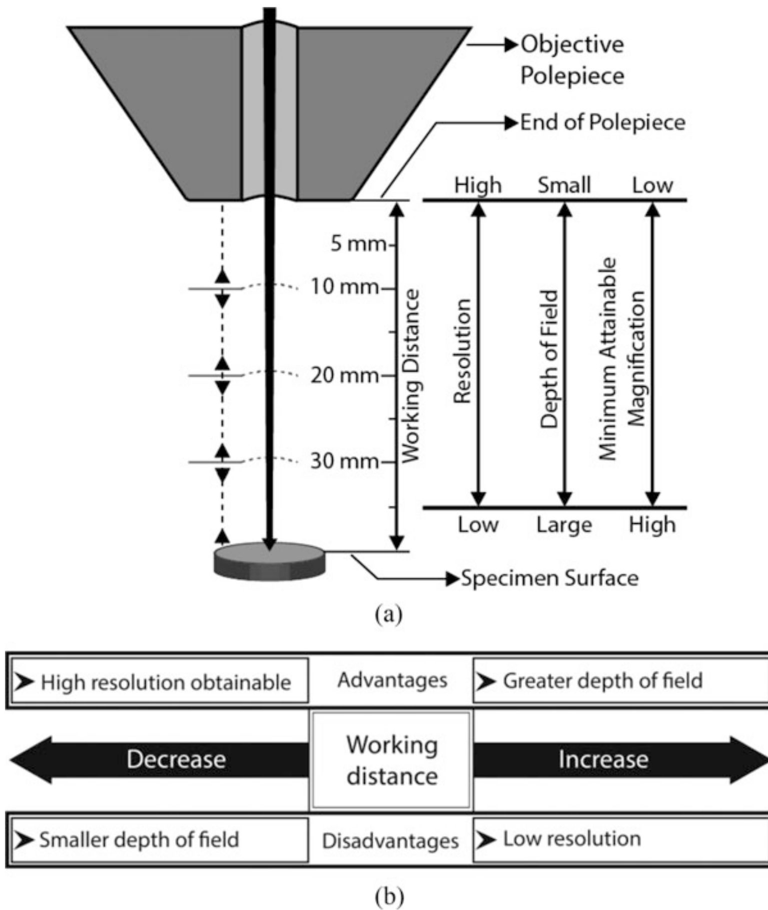


Fig. 4.14 (**a**, **b**) Schematics illustrating the effect of working distance on the image quality. Large working distance increases the depth of field and enables the specimen to be viewed at low magnification (i.e., increases field of view). Short working distance is utilized for high-resolution work

small size (<5 mm) located directly inside the lens gap. Short WD will result in small focal length which helps reduce spherical aberration.

Large WD increases the depth of field due to smaller convergence angle. It also allows the specimen to be observed at small magnifications (e.g., at 5×) encompassing the large field of view (see Fig. 4.14a, b). Large WD lowers the spatial resolution due to increased probe diameter. The signal strength at large WD decreases and the image can appear relatively noisy. Figure 4.15(a-c) shows the large depth of field achieved in the SEM. It shows the effect of working distance and aperture size on the depth of field.

4.3.4 Effect of Objective Aperture

The SEM has a set of objective lens apertures (holes in a strip) ranging from 50 to 500 μm in diameter. The final aperture controls the beam convergence angle, size of electron probe, and the amount of current in the final probe. Use of small final aperture gives rise to small beam convergence angle resulting in a large depth of field. Small aperture allows fewer electrons to pass through, thus allowing the formation of fine probe resulting in higher resolution. However, lower probe current can result in grainy images. Small aperture blocks off-axis electrons and serves to

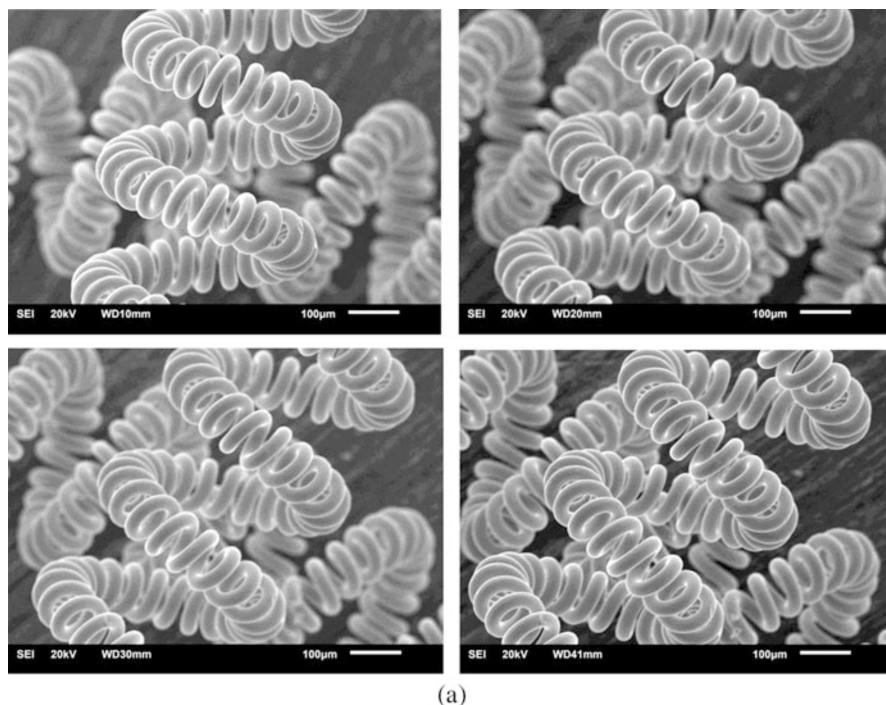
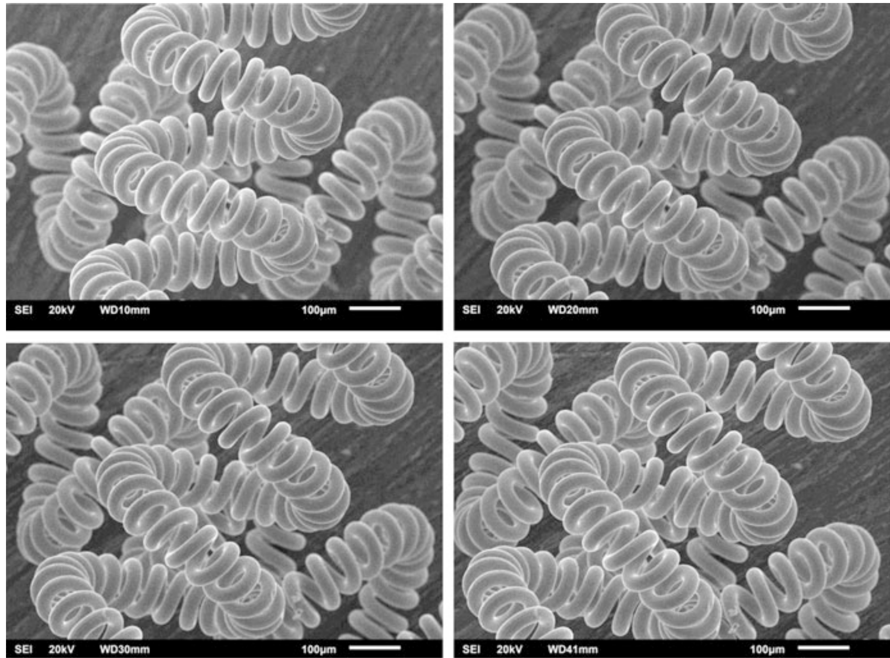
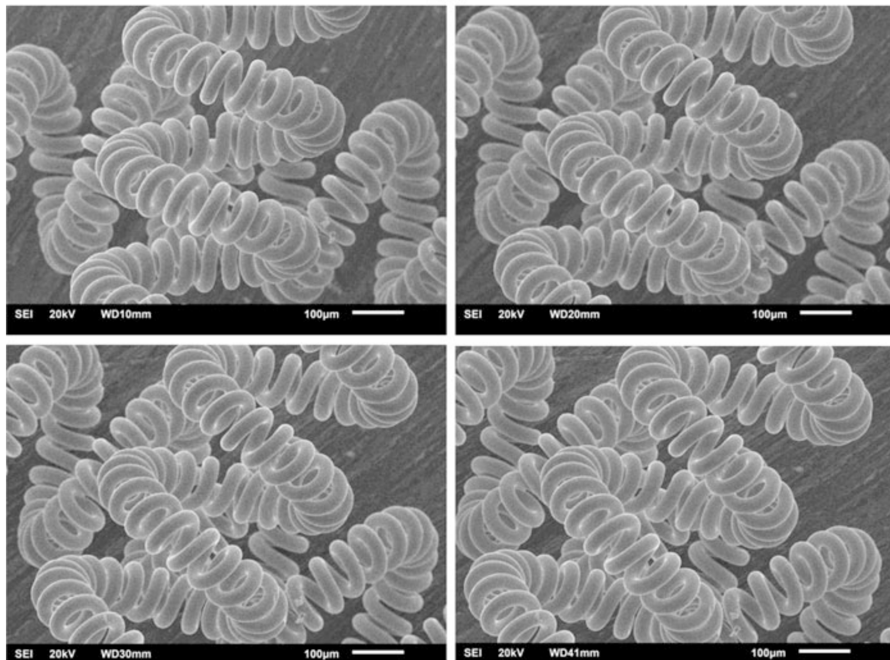


Fig. 4.15 Secondary electron SEM images of W filament retrieved from a light bulb. (**a**) Large aperture: as the WD increases from 10 to 41 mm, blurred regions of the filament come into focus. However, at WD of 41 mm, filament at the bottom surface is still not fully focused. This is due to the large size of the aperture used. (**b**) Medium aperture: as the WD increases from 10 to 41 mm, blurred regions of the filament come into focus. At WD of 41 mm, all regions of the filament are in focus. This is due to the medium-sized aperture used to take these images. (**c**) Small aperture: as the WD increases from 10 to 41 mm, blurred regions of the filament come into focus. At WD of 30 mm, all regions of the filament are already in focus. This is due to the small size of the aperture used to take these images. Also, note that the signal-to-noise ratios of images decrease from large to small apertures as the current that passes through them decrease. Edges of the filament are bright due to higher secondary electron emission for images taken with a large aperture



(b)



(c)

Fig. 4.15 (continued)

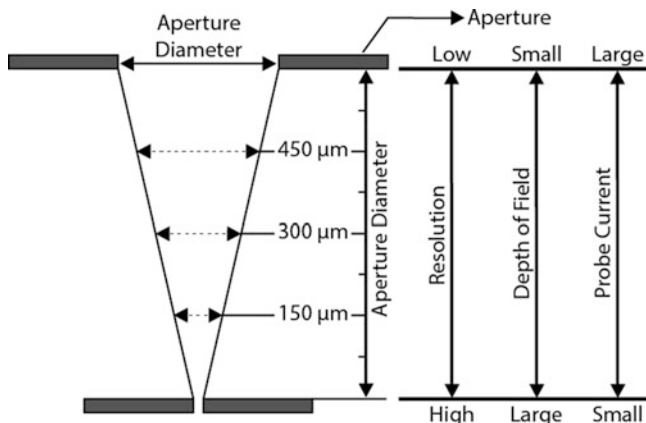


Fig. 4.16 Small final aperture results in high resolution, large depth of field and small probe current. It also reduces the effects of spherical aberration by blocking off-axis electrons

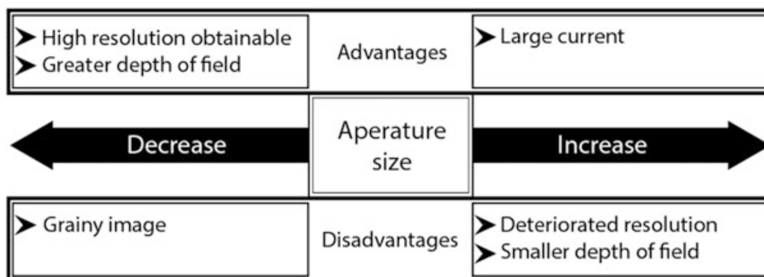


Fig. 4.17 Use of large final aperture results in large probe current which is required for x-ray microanalysis and backscattered imaging. Small aperture reduces the beam convergence angle giving large depth of field

minimize the detrimental effects of spherical aberration. Large apertures allow a larger amount of current which is required for backscattered imaging and x-ray analysis; however, it results in lower image resolution and a smaller depth of field. Schematic in Fig. 4.16 summarizes the effect of final aperture on various imaging outcomes. Appropriate aperture is selected based on the desired information keeping in mind the relative benefits of various sizes as shown in Fig. 4.17. Effect of aperture size on the quality of SEM images obtained for a steel fracture surface is shown in Fig. 4.18. It is clear that the use of small apertures helps to focus larger regions of the coil due to increased depth of field.

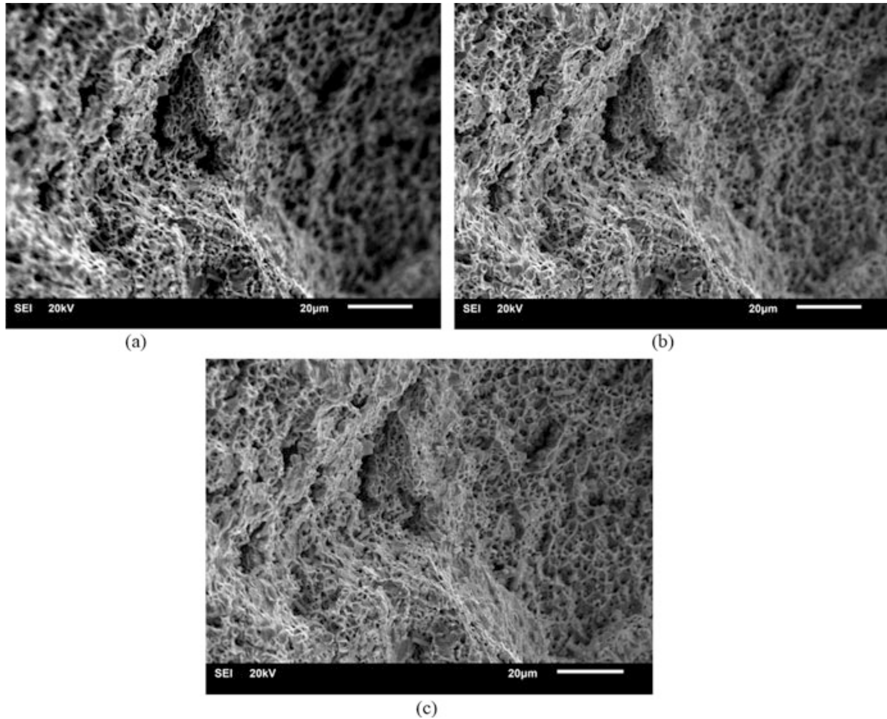


Fig. 4.18 SEM images of a steel fracture surface obtained with (**a**) large, (**b**) medium, and (**c**) small objective aperture. The image with large aperture shows the highest proportion of out-of-focus regions, while the image with small aperture shows regions at all depths focused. This shows that the depth of field increases as the size of the final aperture is decreased

4.3.5 Effect of Specimen Tilt

The specimen is sometimes tilted at certain angles in order to highlight specimen features otherwise not prominent. This can include surface topography features and side or cross-sectional views of the specimen. Tilting is also undertaken to obtain stereo micrographs (SEM images that give 3-D visual impression). Displayed magnifications are no longer valid during tilt and need to be corrected or taken at zero tilt angles when the sample lays flat perpendicular to the beam. This is apparent in the SEM image of Fig. 4.19 where a grid is tilted 45° resulting in an image which is demagnified in the horizontal direction (perpendicular to the tilt axis) by $\frac{1}{\cos 45^{\circ}}$.

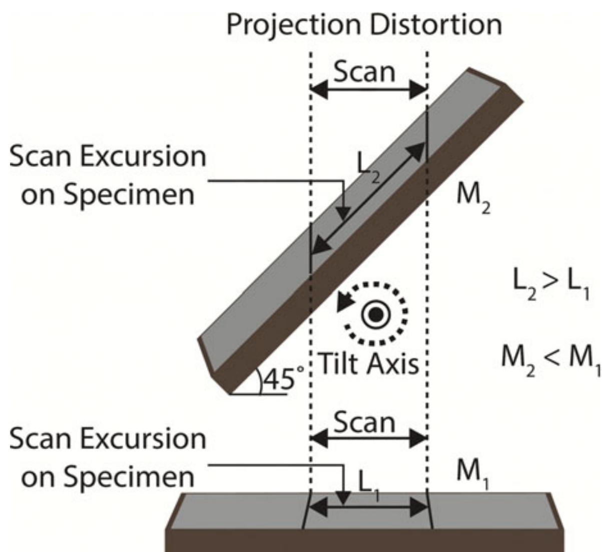


Fig. 4.19 Displayed magnification is not valid when the specimen is tilted as the scan length on a tilted sample (L_2) is greater than that on the untilted sample (L_1). Actual magnification of a tilted surface, therefore, will be less than the displayed number

Specimen tilt can introduce distortion in an image. This occurs because the beam scans a greater length of the specimen due to tilt while projecting it onto the same length of scan. Magnification of features will appear smaller perpendicular to the tilt axis and larger parallel to it. As a result, features appear to change shape and dimensions as seen in Fig. 4.20 where grid size appears to have become substantially smaller when the specimen is imaged at 45° tilt.

Another effect of the tilt is the change in the spot size as the beam scans from the top to the bottom of the specimen surface. *Dynamic focusing* is employed to change the focal length of the lens as the beam scans over the tilted surface. This serves to keep the spot size constant along the *z*-axis. Tilting also results in the formation of asymmetrical interaction volume (Sect. 3.2.5.3) influencing the BSE (Sect. 3.4.1.5) and SE yield (Sect. 3.4.2.7). Also, since the location of specimen features change due to tilting, images can display *shadowing contrast* depending on the orientation of the features with respect to the detector. Detector position with respect to the specimen surface is a critical factor in producing shadowing contrast. At zero-degree specimen tilt angle, the E-T detector is located at the top of the specimen which allows for the effective collection of SE signals. However, if the specimen surface is tilted to the opposite side, the E-T detector will not be able to collect adequate SE signal since the latter have momenta in the opposite direction which results in shadowing contrast.

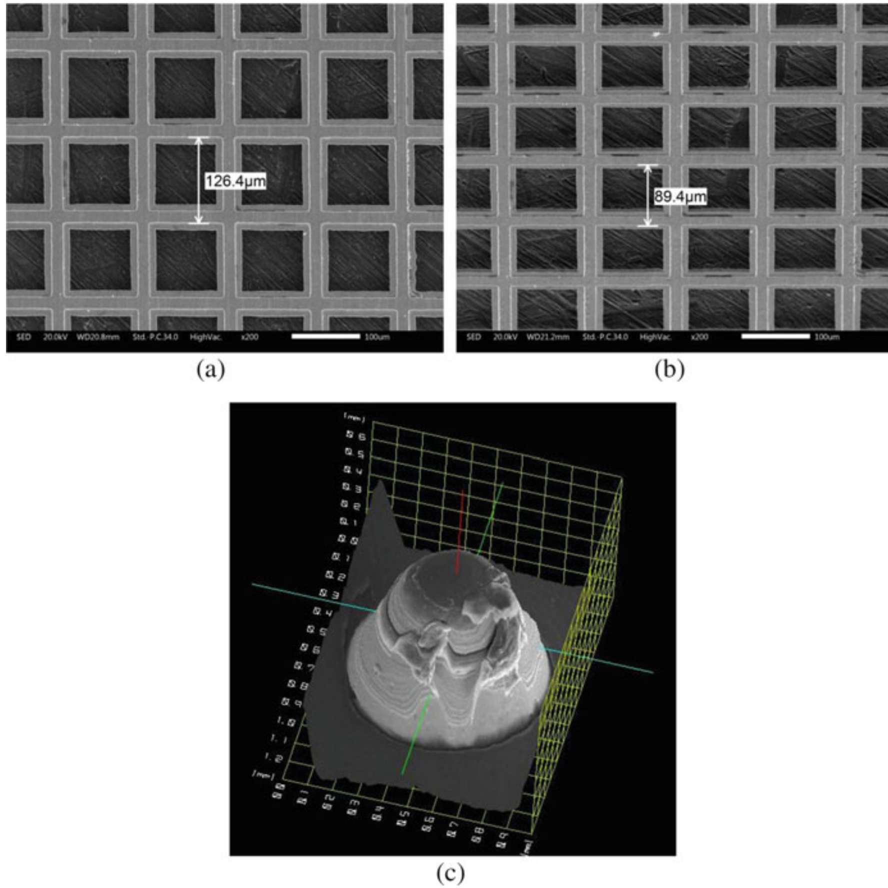


Fig. 4.20 Secondary electron SEM images showing grid sample at (**a**) 0° tilt and at (**b**) 45° tilt. The dimensions of the grid appear smaller when the sample is tilted. (**c**) SEM image showing a tilted sample. (Images courtesy T. Siong, JEOL Ltd)

4.3.6 Effect of Incorrect Column Alignment

Good image resolution can only be obtained when the microscope is properly aligned. Alignment of the microscope column enables the electron beam to fall onto the specimen surface in the most effective manner. Whenever an SEM user undertakes mechanical and/or electrical maintenance like filament change or cleaning of the column, it is important to check and adjust the SEM column alignment. During alignment, the gun, lenses, and apertures are positioned such that these are concentric about the optic axis which is an imaginary line running down the center of the microscope column. It is also not possible to remove small misalignments from within the electron column completely. Shortcomings due to imperfections in mechanical alignment pointed above are overcome by aligning the

beam along the optic axis using electromagnetic coils. Alignment is obtained by means of both electrical and mechanical adjustments.

Ideally, upon generation, the electron beam should be uniformly concentric about the optic axis. For this, the filament tip should be concentric about and leveled with the aperture of Wehnelt cylinder. However, this is not likely to be achieved with flawless precision. Moreover, the position of the filament may also change slightly during SEM operation. Over the course of SEM usage, the gun will need adjustment to restore its alignment. In this procedure, gun shift and tilt are aligned to obtain the brightest image on the screen. Misaligned filament gun tip will affect the emission current passing through the Wehnelt cylinder aperture which in turn affects the probe current.

The position of the objective aperture is adjusted to make sure the electron beam passes through the center of the objective lens. The objective aperture is set at the center of the objective pole piece. Any shift from this position results in high levels of astigmatism that in turn deteriorates resolution. At optimum aperture position, there is no lateral movement of the beam as the current in the objective lens (i.e., focus) is varied. *Wobbler* control is used to oscillate the focus during aperture alignment. Aperture alignment is necessary when very high-resolution imaging is undertaken or if there is a large change in the gun alignment, probe current, accelerating voltage, or working distance. The conventional aperture type (real aperture) is located close to the SEM chamber, while the virtual aperture is located away from the SEM chamber. For this reason, the virtual aperture can serve longer without the need for cleaning which in turn reduces the requirement to align it after cleaning.

Stigmators are also aligned along the optic axis and their strength adjusted for the sharpest image that does not stretch when the focus is changed. Variation in beam current with time can introduce errors in x-ray microanalysis. This is mainly caused by the movement of the filament tip during operation. This can be corrected by adjusting the beam alignment coils. The alignment settings can be stored in a computer file available for retrieval at a later date.

4.4 Effects of Electron Beam on the Specimen Surface

4.4.1 Specimen Charging

Primary beam current i_B entering the specimen is equal to the specimen current i_{sp} flowing out of the specimen into the ground plus backscattered and secondary electron current (i_{BSE} and i_{SE}, respectively) ejecting out of the specimen as shown in the following equation:

$$i_B = i_{sp} + i_{BSE} + i_{SE} \tag{4.17}$$

Rearrangement of the above equation gives specimen current I_{sp} as:

$$i_{sp} = i_B - i_{BSE} - i_{SE} \quad (4.18)$$

or

$$i_{sp} = i_B - \eta - \delta \quad (4.19)$$

where η and δ are the BSE and SE yield, respectively.

During the scan process, i_B remains constant while η and δ vary. At accelerating voltages typically used for imaging (>5 kV), the number of electrons leaving the specimen in the form of SE and BSE combined (i.e., total electron coefficient, $\eta + \delta$) falls significantly short of those entering it as beam current i_B. For example, pure Cu target imaged at 20 kV exhibits total electron coefficient of 0.4 only, which means that 60% of the beam electrons entering the specimen need to leave through electrical contacts to avoid accumulation within the specimen. Specimen stage is grounded for this purpose. For a metal target like Cu which is conductive, beam electrons reach the specimen stage by passing through the specimen and the specimen holder. A continuous conductive path connecting the specimen surface to the ground needs to exist for this purpose. When an uncoated insulating specimen is scanned, conductive path that serves to ground the specimen current i_{sp} does not exist. Electrons in the beam that strike the specimen surface do not find a conductive path to dissipate and thereby fail to reach the grounded specimen stage. As a result, they accumulate within the specimen in the form of a localized negative charge known as charge buildup or specimen charging. This kind of electrostatic charging increases local potential which disrupts the normal secondary electron emission from the specimen and severely degrades the imaging capability of the SEM. It could deflect the beam to another area of the specimen to generate an excessive amount of secondary electrons. Charging effect may present itself in many forms such as unusual contrast (fluctuation in image intensity such as excessive brightness/darkness in images), horizontal lines on images, beam shift and image distortion (spherical objects appear flat), etc. Whether an insulating specimen is going to acquire an electric charge will depend on the number of incident electrons impinging upon the specimen (i_B) compared to those leaving the sample ($i_{sp} + \eta + \delta$). If a balance between the incident and emitted electrons is achieved, then specimen shall not charge. If the number of incident electrons is higher than the emitted electrons, the sample shall charge. Accumulated charge at localized specimen surface could well be positive as indicated by Eq. 4.19; however, this does not pose as much difficulty as an amassed negative charge. Any positive charge created at the specimen surface is neutralized by SE emitted from the specimen and pulled back toward the surface. Various forms of charging encountered during imaging are shown in Fig. 4.21a–f.

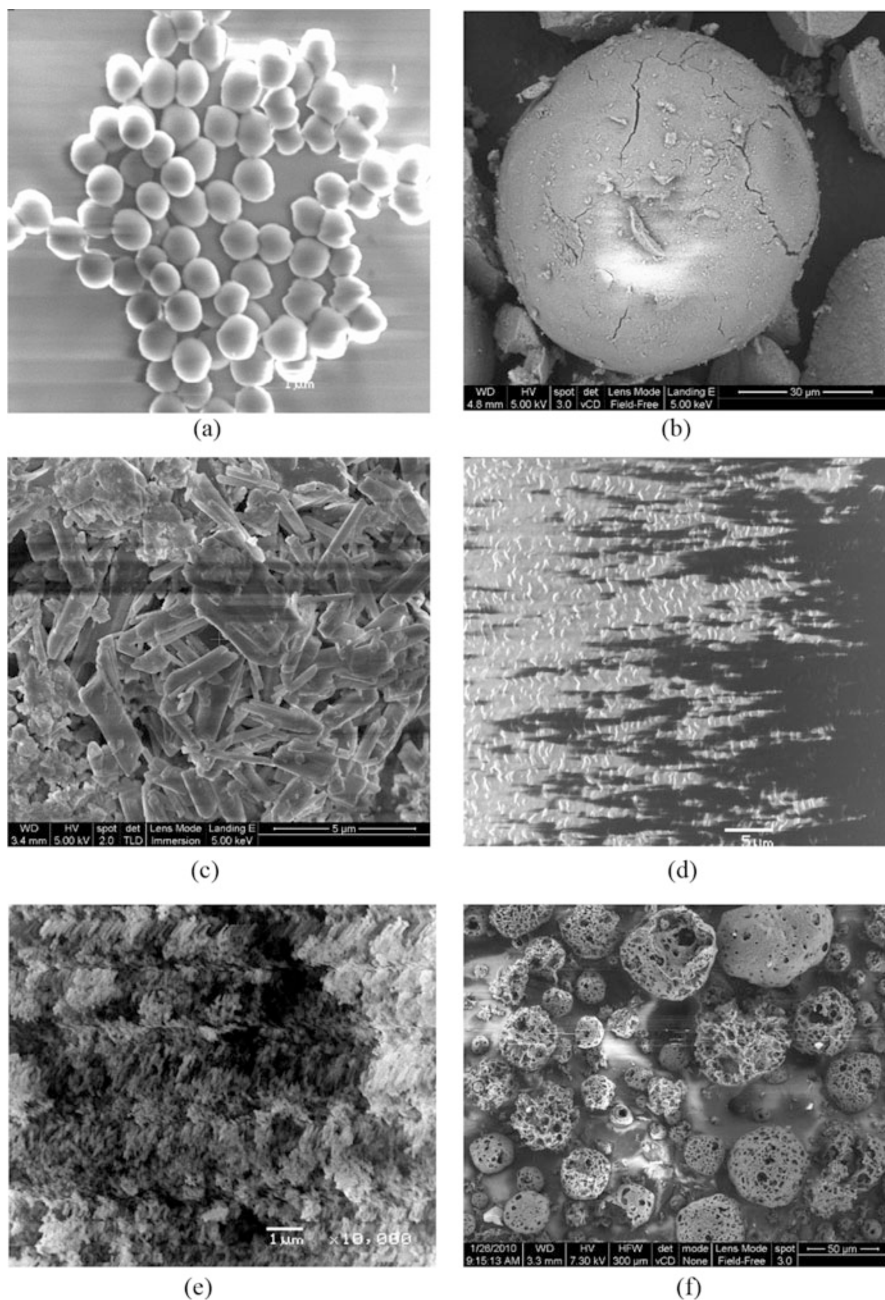


Fig. 4.21 SEM images showing various charging effects. (**a**) Colloid particles showing extremely bright regions, amplifier overloading due to saturation of signal (horizontal lines), and image distortion (spherical particles appear flat). (**b**) Catalyst particle showing bright spot at the top region. (**c**) Decreased SE collection due to changes in potential at localized regions makes some areas appear dark. (**d**) Nanoclay-polyethylene composite showing bright and dark regions due to variation in potential at the specimen surface. Dark regions represent areas where SEs are recollected due to local variation in the potential field. (**e**) Zeolite showing scan discontinuities (horizontal lines). (**f**) Carbon ash specimen showing bright regions and thin intense horizontal lines

4.4.1.1 Methods to Reduce Charge Buildup

(a) *Coating the specimen surface*

For conventional imaging in the SEM, specimen surface must be electrically conductive and electrically ground to prevent the buildup of electrostatic charge at the surface. In order to avoid charge buildup, the surface of a nonconductive specimen is generally coated with a thin conductive film such as gold, carbon, gold-palladium, tungsten, etc., prior to examination in the SEM (see Sect. 8.1.10 for details). This film has small grain size (e.g., few nanometers) and is low in thickness (few nanometers) depending on the duration of deposition. It does not interfere with the examination of the surface morphology of specimens at low to high magnifications. However, during ultrahigh-resolution imaging (~100,000×), extra care needs to be taken to ensure that the actual specimen features are being imaged and not the coating grains itself. For high-resolution imaging, a thin fine sub-nanometer coating of chromium is preferred. It is also customary to use conductive paint/tape to establish electrical contact of the coated specimen with the specimen holder and the stage.

Polymer specimens generally exhibit significant charge buildup during microscopy. While it is usually necessary to coat a polymer with conducting metal layer, it is important to keep its thickness to a minimum in order to make sure it does not mask specimen features. This is especially important during low voltage imaging where the beam penetration into the specimen is small. This could result in contrast due to the top coated layer rather than the underlying specimen surface. An example is shown in the SEM image of Fig. 4.22 where the surface of the polymer composite is buried under the thick layer of gold sputter coating deposited to eliminate charge-up. Low magnification imaging is possible with thick coatings. However, for high-resolution imaging, thin coatings need to be employed, or accelerating voltages need to be increased appreciably to allow penetration into the specimen.

It is common practice to coat specimens even if the specimen exhibits adequate conductivity. This is undertaken to increase signal strength and surface resolution, especially when samples with light elements are examined. The improvement in resolution takes place because secondary electron emission near the surface is enhanced. Instead of coating, it is more effective to stain biological samples (e.g., impregnate them with osmium or its variants) to increase the bulk conductivity of the analyzed material.

(b) *Use of low accelerating voltage, beam deceleration, and small probe current*

Effects of charging can be diminished by using low accelerating voltage/beam deceleration and probe current during imaging. These techniques restrict charge buildup by reducing the number of electrons entering the specimen, I_p. At high accelerating voltage, the beam penetration in the specimen is deep. A large proportion of SE and BSE generated cannot leave the specimen which decreases the total ($\eta + \delta$) electron yield/coefficient resulting in an accumulation of negative charge within the specimen. The decrease in accelerating voltage increases δ reaching a

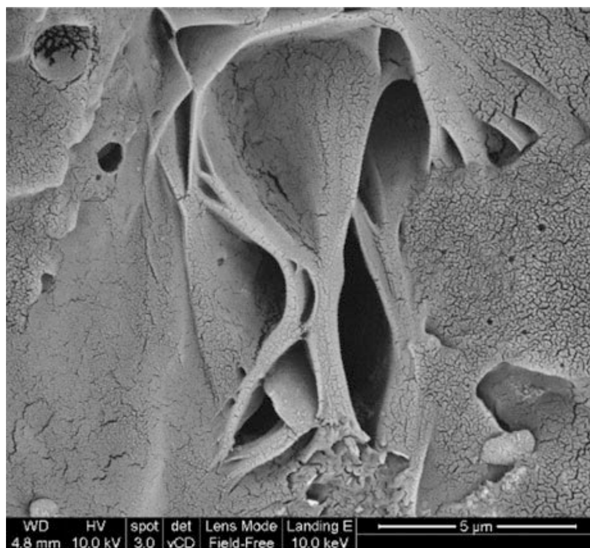


Fig. 4.22 Polypropylene specimen sputtered with Au coating for 120 s resulting in a thick coat

point where the number of electrons emitted out of the specimen due to backscattering and secondary electron emission surpasses those supplied by the beam (see Fig. 3.30a). At this point, the specimen current I_p, which maintains charge neutrality, falls to zero. At such low beam energy, the specimen will not charge. The region of zero charging exists between beam energies indicated as E_1 and E_2 in Fig. 3.30a. The optimal accelerating voltage varies with the type of material. It is in the range 0.5–2 keV for organic and 2–4 keV for inorganic materials. However, optimum beam energy where charging is reduced to zero value is found by experimentation. One disadvantage of using low beam energy is an increase in chromatic aberration effect. Therefore, field emission source is generally used for low voltage microscopy to counter chromatic aberration to a certain extent.

Semiconductor materials are prone to charging and thermal damage and are routinely examined at low kV and small probe current using field emission SEM. Samples with rough surfaces can develop complicated electric fields and interrupt charge removal. The large variation in surface topography impacts charge distribution at the surface and makes it inhomogeneous. Also, it is difficult to prevent charge buildup in pure insulator materials by means of these techniques only.

(c) *Use of samples with small dimensions*

SEM chamber can accommodate large specimens. However, it is preferred to use specimens with small dimensions as long as it adequately represents the material or component under investigation. Small-sized specimens are free of extra material and can be well prepared to avoid charge buildup.

(d) *Proper specimen mounting*

Carbon tape is widely used as a mounting material for samples in the SEM. Carbon tape may outgas in the SEM chamber and create an environment conducive to charge buildup in the specimen. On the other hand, it also serves to enhance the conductivity of the specimen. These contradictory effects dictate the use of a small quantity of tape only and keep microscopy sessions as short as possible. In addition, the sample should be secured to the underlying tape with carbon or silver tape to ensure proper conductivity. Charge buildup in powder samples is uneven and may make some portions of the specimens look very bright and others dark. Powder samples should be distributed evenly in the form of a single thin layer on SEM stub, and excess material should be blown off.

(e) *Tilting of the sample*

As discussed in Sects. 3.4.1.5 and 3.4.2.7, tilting a specimen changes the BSE and SE yield. This phenomenon can be used to advantage to reduce the charging effect by tilting the specimen to an angle that enhances emission of electrons from the surface.

(f) *Use of BSE detector*

Emission pattern of energetic BSE is not disturbed by the change in local potential at specimen surface. BSE emitted from the specimen have adequate energy not to be attracted back to the localized positive potential regions created at the specimen surface due to charging. Therefore, imaging with the BSE detector can eliminate the effects of low-intensity charging.

(g) *Fast scan and frame averaging*

Images are obtained at fast (TV rate) scan to reduce the dwell time of the beam at any pixel to reduce charge accumulation. This, however, results in noisy images. A series of images or frames of a single field of view are taken, and their pixel intensity is averaged to increase the signal-to-noise ratio and thereby eliminate the effects of charging.

(h) *Use of low vacuum or environmental SEM*

In a low vacuum or environmental SEM, gas or water molecules are injected above the specimen surface. These are ionized by the electron beam on its way to the specimen producing a mass of positive charge. If a specimen starts accumulating a negative charge at its surface, this positive mass is attracted toward it and serves to neutralize the charge buildup. This technique is discussed in more detail in Sect. 5.2.

(i) *Energy filtering*

Charging is primarily produced by lowest energy secondary electrons. Electron energy filters (such as E × B filter and r-filter) separate electrons based on their energy and suppress the role of low-energy SE which serves to reduce charging effects. The working principle of energy filters is discussed in Sect. 5.1.

4.4.2 Surface Contamination

If the surface of a specimen is scanned for long durations, it may cause loss of sharpness in the image with an accompanying dark rectangular smudge at its surface as shown in Fig. 4.23. This mark is caused by carbon deposition which occurs due to the interaction of the electron beam with residual gas molecules present in the vicinity of the specimen surface. Usually, this residual gas is volatile hydrocarbon molecules that are ionized by the electron beam and deposited on the specimen surface as nonvolatile carbon. This phenomenon is known as specimen *contamination* which occurs at the point of beam impact. This thin film of carbon is deposited on the area that is scanned with the beam for a considerable amount of time and can be observed by zooming out during live imaging. This contaminant layer serves to obscure and blur the surface details of the specimen with an accompanying darkening of the scanned area.

Despite the presence of vacuum in the specimen chamber of the SEM, some degree of gas molecules is present in the environment, which results in specimen contamination. Source of these contaminants could be the hydrocarbons introduced by the specimen itself due to outgassing, the organic material used to prepare/mount specimens, instrument surfaces or grease, backpressure from rotary oil pump used to evacuate the SEM chamber, etc. These hydrocarbons are broken down into its

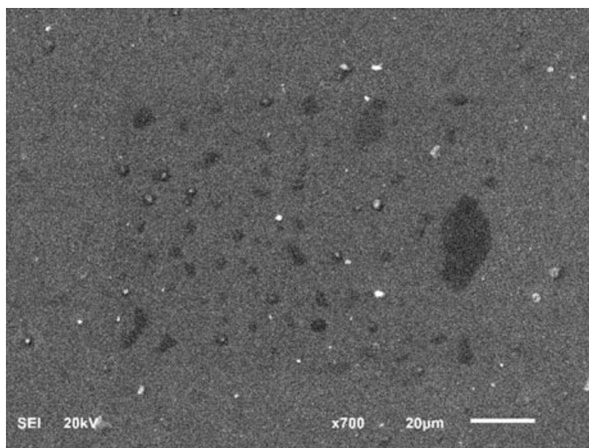


Fig. 4.23 Secondary electron SEM image of Ni-based alloy specimen showing contamination effect after long exposure to beam scan. The scanned area contains large dark spots and also loses sharpness due to contamination buildup

constituent materials and while nitrogen and oxygen are pumped out by the vacuum system; carbon deposits on the specimen surface.

Residual hydrocarbon molecules can also be present on various components of the SEM column such as apertures. Beam interaction with these residuals can produce contaminants on these component surfaces that can result in beam instability. Contamination can become a serious issue, while imaging at very low accelerating voltages and probe currents as the electron beam is not energetic enough to penetrate the deposited contaminant layer. In this case, the contaminants may be imaged instead of the underlying specimen surface. Moreover, low-energy x-rays emanating from the specimen may be absorbed in the contamination layer and introduce error in the EDS microanalysis.

Contamination from the instrument is reduced by employing dry pumps or installing a vapor trap in the pump backing line that can control hydrocarbon contamination originating from vacuum pumps. In addition, cold traps can be employed to seize contaminants, and the SEM chamber is purged with dry nitrogen gas during specimen exchange. Contamination from the specimens can be reduced by proper handling (e.g., use of gloves and completely dry specimens) and use of minimum amount of adhesive tapes or conductive paints. Size of outgassing biological or hydrocarbon volatile specimens that need to be imaged should also be kept to a minimum. Embedding agents and resins used for sample preparation should be carefully selected as some might give off a high amount of gas. Also, since organic gas is given off when the resin surface is irradiated with an electron probe, use of the smallest possible surface area is recommended for imaging, or surface is to be coated with a conductive material.

4.4.3 Beam Damage

During electron beam-specimen interaction, heat is generated due to ionization at the irradiated spot as energy is transferred from the beam to the specimen. The magnitude of heat generated or level of temperature achieved depends on the accelerating voltage, probe current, time of exposure, specimen area, and the ability of a specimen to dissipate heat. Beam damage can occur due to ionization and subsequent chemical reaction at the specimen surface due to the incident beam. The extent of the damage varies with the nature of the specimen material. Conductive specimens such as metals and alloys can dissipate heat effectively and therefore are more resistant to beam damage. Polymers and biological specimens, on the other hand, are poor conductors of heat and thereby more prone to beam damage.

Radiation damage in organic materials occurs as a result of inelastic scattering which disturbs the valence electron configuration and introduces permanent changes to the chemical bonds of the solid. The effects of damage in these materials may result in specimen heating, structural damage, mass loss, reduction in crystallinity, and contamination. Susceptibility to this type of damage makes it all the more difficult to undertake high-resolution microscopy and microchemical analysis of polymeric, biological, and life science specimens [13].

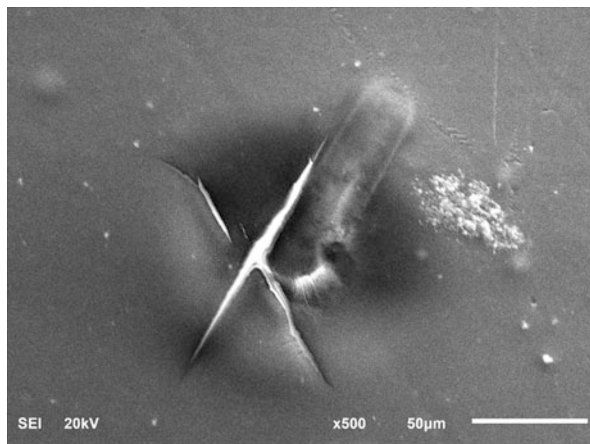


Fig. 4.24 Secondary electron SEM image showing beam radiation damage induced in a polyethylene specimen during microscopy

Some polymers are more sensitive to the radiation damage than others. Aliphatic and amorphous compounds are more prone to damage than aromatic and crystalline compounds. Presence of oxygen in materials results in peroxide formation enhancing damage. Radiation damage exhibits itself in the form of cracks, bubbles, holes, depressions, and dimensional changes. An example of the damage caused by the beam is shown in a polyethylene sample in Fig. 4.24.

Beam damage is an irreversible process. It can occur fairly quickly and sometimes is difficult to judge whether a feature is part of a specimen or a consequence of beam damage. During imaging of sensitive specimens, certain steps can be taken to contain beam damage, i.e., use low accelerating voltage, decrease probe current, reduce exposure time, use low magnifications/large scan areas, and apply conductive coatings such as of gold, carbon, etc., at the specimen surface to improve thermal conductivity.

The phenomenon of radiation damage is utilized in electron beam lithography in the manufacturing of integrated circuits. The electron beam is scanned over the surface of a thin polymer film to introduce *controlled damage* at the surface in the form of a pattern. The pattern is then exposed to a mild etch, such that the regions damaged by the electron beam react at a different rate than the unexposed regions. The pattern is thus used as a mask for subsequent deposition.

4.5 Influence of External Factors on SEM Imaging

External factors that originate from the environment and poor maintenance of the SEM can influence the quality of SEM images as discussed below.

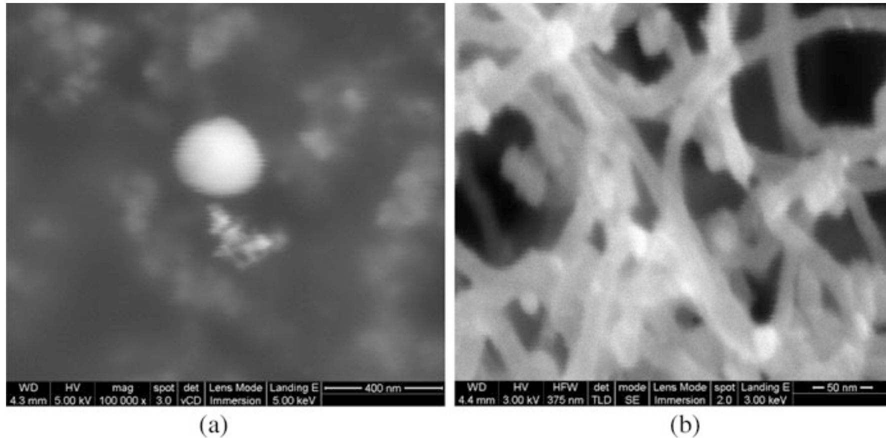


Fig. 4.25 High magnification secondary electron SEM images showing image distortion introduced due to electromagnetic interference effect. The edges of (**a**) Ca-YSZ particle and (**b**) carbon nanotubes appear jagged in the horizontal direction due to EMI

4.5.1 Electromagnetic Interference

Image distortion can be produced due to the presence of external electromagnetic interference (EMI) effects in the area where the SEM is operated. This interference is caused by electrical equipment located in the vicinity of the SEM. These could be transformers, distribution boards, high tension cables, lights, improper electrical grounding, and other lab equipment. The source of this equipment needs to be identified, and if their removal is not possible, their effects should be canceled by installing EMI cancelers. This equipment eliminates or minimizes EMI by applying magnetic screening/shielding or applying a field of similar magnitude in the opposite direction that serves to cancel the stray field. SEM images distorted due to electromagnetic interference are shown in Fig. 4.25a, b. It could be seen that the edge of imaged feature exhibits sharp spikes. Such an effect is more visible at higher magnification. Also, the lower the beam energy the greater is the interference effect. Use of high accelerating voltage and short working distance can reduce the effects of EMI.

4.5.2 Floor Vibrations

The location where the scanning electron microscope is installed has to meet certain specifications regarding mechanical vibrations and stray magnetic fields. Vibrations can arise due to mechanical vacuum pumps, motors, etc., and also if the microscope is installed on higher floor levels in a building. This is why most microscopes are equipped with anti-vibration mounts/table or soft extension springs and installed in the basement or ground floor of the building. Image distortion produced due to floor vibrations is similar to that produced by EMI

such as features exhibiting jagged edges. These distortions are more visible at large magnifications during high-resolution imaging. SEM image showing the effect of floor vibrations is shown in Fig. 4.26.

4.5.3 Poor Microscope Maintenance

The scintillator of E-T detector and HT tank are supplied with very high voltages of up to 10 kV. Poor vacuum, bad electrical connections, contamination, and dust can lead to electrical discharge that appears as horizontal lines and a bright spot in the image as shown in Fig. 4.27.

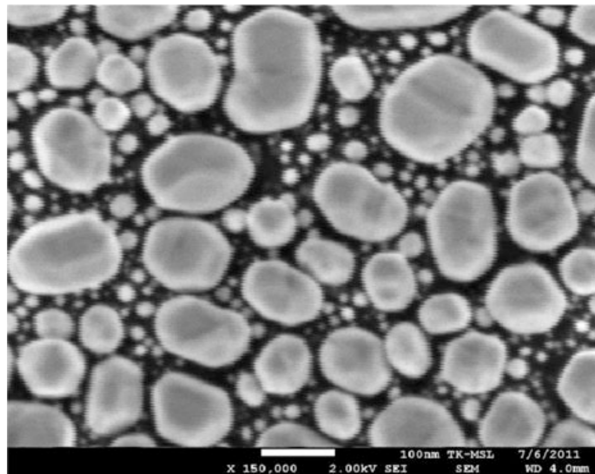


Fig. 4.26 SEM image showing the effect of floor vibrations. The edges of particles show jagged appearance

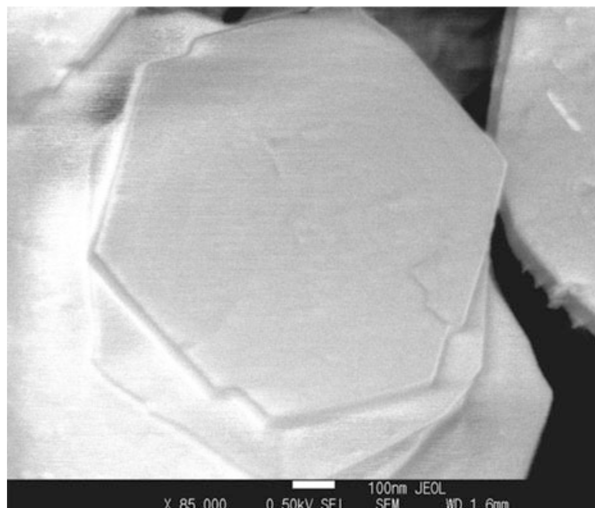


Fig. 4.27 Secondary electron SEM image showing the probable effect of electrical discharge brought about due to poor microscope maintenance

4.6 Summary of Operating Conditions and Their Effects

Effects of different SEM operating conditions on image quality are summarized in the following table.

Operating conditions	Effects
Misaligned microscope	Images lack sharpness and focus
Coated specimen	Eliminates charge buildup Better contrast Coating grains visible at magnifications > ×100,000 Not suitable for low voltage microscopy
Uncoated specimen	Specimen charging Low contrast Suitable for low voltage microscopy
High beam energy	High resolution Good for bulk surfaces Required for EDS microchemical analysis Lacks surface details due to high beam penetration and large interaction volume Specimen prone to charging Specimen prone to beam damage Specimen prone to contamination Pronounced edge effects
Low beam energy	Low resolution Good for surface morphology examination due to low beam penetration and small interaction volume Not adequate to measure the chemistry of heavy elements Less charge buildup Fewer edge effects Less specimen damage
Large probe current (large spot size)	Broad beam probe Strong signal Low resolution Smooth image Increased surface damage Required for microchemical analysis
Small probe current (small spot size)	Small beam probe High resolution Grainy image Less surface damage Weak signal Not adequate for microchemical analysis
Large working distance (WD)	Large field of view Small minimum magnification High depth of field Low resolution
Small working distance (WD)	Small field of view Large minimum magnification Small depth of field High resolution

(continued)

Large objective aperture	Low resolution Small depth of field Smooth image Weak contrast Suited to microchemical analysis
Small objective aperture	High resolution High depth of field Grainy image Strong contrast Not suited for microchemical analysis
Secondary electron imaging	Suitable for surface morphology examination High resolution Prone to specimen charging
Backscattered electron imaging	Suitable for contrast based on the composition Low resolution Less prone to charging
Energy dispersive x-ray detector (EDS)	Qualitative and quantitative microchemical analysis Spot (point analysis), area analysis, line profile, 2D area profile
Low vacuum mode	Backscattered electron images Reduces charge buildup Low resolution Low contrast
Low voltage imaging	Backscattered electron imaging Suitable for examination of surface features Reduces charge buildup
STEM imaging technique	Used for detection of electrons transmitted through the sample
Beam deceleration	Reduces charge buildup Makes use of high accelerating voltage with low penetration depths Reduces chromatic aberration Surface study due to low interaction volume

4.7 SEM Operation

Electron gun generates an electron beam with an accelerating voltage that can range from 500 V to 30 kV. The beam is focused into a fine probe of approx. 1 nm to 10 nm by electromagnetic condenser lenses located within the column. The fine electron probe is then rastered over specimen surface in a rectangular area by scan coils. The sample sits in the SEM chamber. The electron beam penetrates into the sample in the form of a teardrop/hemisphere extending from 100 nm to 5 μm depending on accelerating voltage and sample density. This interaction produces a variety of signals including secondary and backscattered electrons and x-rays which are collected and used to produce images as well as to determine the elemental composition of the specimen material. Images are digitally processed, displayed on computer screens, and saved on hard drives.

This section focuses on the practical aspect of the technique. It includes a stepwise guide to the use of SEM with an aim to get useful images. The way the controls and software user interface is laid out differs from one model of the microscope to the other. Nomenclature used may also vary depending on the manufacturer. It is not the intention of this chapter to describe specific details for use of instrumentation and software of a particular model. This information can be found in the relevant user manual of the microscope. The aim here is to explain practical steps to undertake scanning electron microscopy irrespective of the model in use. This can serve as a source of guidance to a new or casual user.

4.7.1 Sample Handling

4.7.1.1 Sample Size

The sample should be of an appropriate size to fit in the SEM chamber. The sample is generally mounted on a holder whose size can vary from 10 mm to 30 mm. Generally, various sizes of holders are available for use with a particular microscope. The holders are placed on the specimen stage located within the SEM chamber. Still bigger specimens can be accommodated since the dimensions of the stage can generally be in the order of 150 mm to 250 mm. Figure 2.28 shows pictures of various types and sizes of holders available for mounting samples. Specimen holders are exposed to vacuum in the SEM chamber. Therefore, gloves are used to handle specimens and specimen holders to minimize contamination that might cause problems during imaging.

4.7.1.2 Sample Preparation

Polished specimens of metals and alloys prepared using metallographic sample preparation techniques are mounted in epoxy or Bakelite in appropriate sizes of mounts to fit into the available holders. Loose powders are placed on C tabs or Cu/Al adhesive tapes that are attached to Al stubs which are in turn inserted into specimen holders for examination in the SEM. As-received specimens such as broken metal pieces are held down on the holder with the help of adhesive tape. Conductive tapes, paints, and tabs are available to dissipate current and reduce accumulation of electrostatic charge on the specimen surface during observation in the SEM. Metal, alloy, ceramic, and glass samples do not require any preparation except for coating. It is normal practice to coat samples in order to reduce charging effects during imaging. Nonconductive samples like polymers, rocks, glass, etc. need to be coated; however, conductive samples like metals and alloys are also coated to get good imaging results.

Usually, the SEM is operated in a high vacuum which necessitates the use of dry specimens. If the specimens are wet (e.g., rocks, soils, corrosion deposits), they can be dried in an oven or by simply leaving them out in the air for an appropriate length of time. Polymeric samples charge significantly and therefore need to be coated prior to the examination. In some cases, it is desirable to dip polymer samples in liquid nitrogen to make it brittle and then smash it to reveal fresh fracture surface. This

procedure allows imaging of certain features otherwise not visible in as-processed surfaces. Samples and sample holders are stored in dry and dust-free environments to minimize contamination of the SEM chamber.

Some samples (e.g., biological samples or tissues) might change their shape or structure as a result of drying. These specimens are subjected to techniques such as freeze drying or critical point drying. They are dried slowly in a controlled fashion in order to secure fine details of their structure. These sample preparation techniques are discussed in more detail in Chap. 8. Cryo-SEM has been used to examine wet specimens such as plants, oily rocks, etc., in a high vacuum environment. Currently, variable pressure SEM instruments are available to examine wet specimens without any preparation.

4.7.2 Sample Insertion

Specimen should be of a correct size to fit in the SEM chamber. It is held or mounted on an appropriate stub or holder. It is usually coated with gold or carbon to improve its conductivity. A conductive path between the specimen and stub/holder is ensured to dissipate electron current and prevent the buildup of excessive charge. The microscope should be in a ready state. Electron beam should be off. In microscopes where the chamber is purged with dry N_2 gas during sample exchange, the gas supply is turned on. Sample insertion procedure is started by pressing the appropriate control button on the SEM console or clicking the button in the computer software interface. Once the specimen chamber is appropriately vented with air or N_2 gas, the chamber door is opened, and the specimen which is already held in a holder is placed onto the specimen stage. Some microscopes use specimen exchange airlock (load lock) system to keep the vacuum within the chamber intact during specimen exchange. Once the specimen is placed onto the stage, the door is closed, and the chamber is evacuated by pressing or clicking the appropriate button. In order to reduce the level of contamination in the chamber and keep good vacuum, any components exposed to the inside of the chamber including the holders, stubs, and specimens should be handled with lint- and powder-free gloves. For high vacuum operation, the specimen should not outgas or get damaged. The specimen is brought under the objective lens by clicking appropriate buttons in the software program. Some microscopes require aligning of the specimen stage. The specimen is brought up to the correct working distance (WD). This could be 10, 5, 2 mm, etc., depending on the type of imaging required. High-resolution imaging is undertaken at short WD, while large WD is used for high depths of field and low magnification microscopy. Movement of specimen stage can be controlled manually as well as through the software. Evacuation normally takes 1–2 min during which time the electron beam cannot be switched on.

4.7.3 Image Acquisition

Once the vacuum is in the ready state, the HT button is turned on. For a microscope equipped with tungsten filament, the filament heating knob is slowly turned clockwise to gradually increase the current in order to heat the filament. This is done to the point where the screen reaches its maximum brightness; after this point, the brightness starts to decrease. This is the point of maximum saturation. Using a filament beyond this point will drastically reduce the service lifetime of the filament which can last up to 100 h of usage or more. Some users keep filament knob set at the saturation point. In this case, only the HT needs to be turned on, and the filament reaches the set saturation point by itself. In modern field emission microscopes, only the HT needs to be turned on by clicking the appropriate button in the software. Once the HT is on and the filament is saturated, a secondary electron image of the specimen should appear on the screen.

Magnification is kept low (e.g., 100× or so) and scan rate is set to a fast raster scan. Brightness and contrast are adjusted. Contrast is set at minimum value and brightness is adjusted to show a slight change in intensity to the screen. Contrast is then increased to get a reasonable image on the screen. The auto brightness contrast feature can be used to get an appropriate image. Magnification is increased to 1,000× or so and focus adjusted. If the sample starts to charge up or show beam damage, a faster scan speed is used.

Appropriate spot size is selected. Spot size dictates the amount of current in the beam. The smaller the spot, the lower is the beam current. Smaller spot size will reveal finer details in the specimen but the image will get noisier. So, a balance needs to be achieved for a current setting that reveals as much as detail of the specimen without rendering the image too noisy. A noisy image is improved by lowering the scan speed. Spot size is controlled through a condenser lens which is the first lens beneath the electron gun. Spot size is the actual area on the specimen where the beam is focused. Focused beam area and the beam current both increase with increasing spot size. Smallest spot size is selected for ultrahigh-resolution imaging (e.g., >200,000×), intermediate size is used for standard imaging, while bigger spot size is required for microchemical EDS analysis, cathodoluminescence, EBSD, etc. Larger than required spot size gives out of focus images, while smaller size produces grainy images due to low signal strength. Good focus and astigmatism correction are indicative of optimum spot size.

The focus is controlled through the objective lens which is the last lens in the SEM column. Microscopes usually have coarse and fine control knob for adjusting the focus. Usually, a feature of interest with distinct edges on a specimen is used for focusing. Appropriate scan rate (about 0.1 μs to 3 μs dwell time) is selected. Different areas of interest can be examined using x and y stage controls operated through manual knobs provided on the door of the SEM chamber or through the software using a handheld device such as a mouse. The specimen can also be rotated using rotation controls in order to align particular features in the specimen.

The next step is to remove astigmatism. In order to check for astigmatism, the image is magnified to 10,000×, and the focus knob is turned to positions of under-

and overfocus. If the image stretches to opposite directions 90° apart during this operation, the image is deemed to be astigmatic. To remove astigmatism, the image is set midway between under- and overfocus, and one of the knobs for astigmatism correction is used to sharpen the image as much as possible. The image is refocused again followed by adjustment through the second knob provided for astigmatism control. This procedure can be repeated to get a sharp image. Astigmatism needs to be corrected when there is a change in imaging conditions, objective lens aperture, or after specimen exchange. Astigmatism in the image is usually better visible at higher magnifications (3,000× or more). It is not possible to carry out astigmatism correction fully if the objective lens aperture is dirty or if the magnification is too high for the beam spot size in use or if the sample is charging.

Magnification is modified to the proper level to take an image. Brightness and contrast are also adjusted. High brightness and low contrast produce soft images, while high contrast and low brightness produce sharp images. Image quality is enhanced by adjusting contrast, brightness, magnification, and focus with an aim to maximize the image quality. Care is taken not to scan the area of interest for too long in order to avoid contaminating or damaging the sample before the final image is taken. The usual practice is to move away from the feature of interest onto the adjacent area using x and y stage controls and focus until the image is sharp. Focusing is performed at a higher magnification than the one at which the image is taken. For example, for an image required at 10,000×, focusing is performed at 30,000× or so. An image is taken by pressing the appropriate button on the control or clicking the button in the computer software. Modern microscopes provide filtering functions which improve image quality by averaging two or more frames. It can be used to decrease the high noise level generated during fast scans. Different frames can be added into a single averaged frame. However, it is necessary to ensure that the specimen isn't charging and the beam is stable for this function to produce good results. Images can be saved in many formats including TIF, BITMAP, JPEG, GIF, PNG, etc.

Advanced microscopes have more than one lens including immersion or semi-immersion lens for high-resolution microscopy. The specimen is usually placed very close to this lens at a short working distance so that the specimen is immersed in the high magnetic field created by this lens. The correct lens mode needs to be selected, and the specimen (if magnetic) is to be held securely in its position to avoid being pulled by the field.

4.7.4 Microscope Alignment

It is necessary to align the microscope column after each filament change. The SEM needs to be properly aligned during microscopy to get optimum imaging. The purpose of alignment is to get the gun, lenses, and apertures concentric about the optic axis which can be considered as an imaginary line passing through the center of the SEM column. Gun alignment procedure may vary depending on the microscope model. A general guideline to align a microscope is as follows:

A conductive specimen is placed at 10 mm working distance and focused at a magnification of 10,000× with an accelerating voltage of 30 kV. Objective aperture used is large, and condenser lens strength (beam current/spot size) is relatively high (i.e., large spot size).

Firstly, the objective aperture is aligned by activating the focus wobbler which starts to change the focus of the objective lens automatically from over- to underfocus positions. Due to aperture misalignment, periodic change of focus results in the translation of the image. Aperture misalignment is corrected by adjusting *X* and *Y* knobs provided on the column near the aperture. Once the aperture is aligned, image translation diminishes and the image appears to wobble in one position.

Secondly, the gun tilt is aligned by adjusting the *X* and *Y* controls provided on the SEM console. The brightest image on the screen is obtained at the correct alignment. The same procedure is adopted to correct the gun shift.

Thirdly, stigmators need to be aligned along the optic axis. For each of the *X* and *Y* stigmators, image movement or stretching is minimized by using *X* and *Y* controls. The focus is adjusted every time a stigmator control is used. The adjustment should result in a sharp image which should not stretch or elongate when the focus is changed.

Checklist for acquiring good quality images can be summarized as follows:

- The microscope is aligned with correctly mounted and properly cleaned filament assembly.
- The specimen is prepared and mounted on the holder. It is preferably coated and electrically ground to specimen holder.
- Proper objective aperture size (typical range 30–100 μm) is selected, i.e., for high-resolution microscopy (30 μm) and for general imaging and EDS (40–50 μm).
- The appropriate accelerating voltage is selected.
- Optimum working distance is selected.
- Appropriate probe current is selected.
- The specimen is focused.
- Astigmatism is removed.
- Brightness and contrast are set at an optimal level.

4.7.5 Maintenance of the SEM

Maintenance of the SEM is essential to keep it at an optimum operable condition and to realize its maximum useful service lifetime. Both preventive and corrective maintenance on a regular basis are important. Most of the complicated maintenance and regular servicing of the SEM is carried out by qualified service engineers. The tasks required for an operator to undertake for the upkeep of instrument are kept to a minimum. Reliable instrumentation used in the microscope ensures long uptimes

and renders frequent servicing unlikely. Usually, service engineers are contracted to pay 6-monthly visits for regular servicing and maintenance of the instrument. Some of the maintenance activities are summarized as follows:

All parts exposed to the electron beam are kept clean and highly polished. The aim is to free them from dirt, scratches, or any media which can charge-up and degrade the image. During operation of the SEM, some contamination builds up in the column and chamber. These are cleaned and polished. Components such as removable detectors are also cleaned. Lint-free cloth with a small amount of soft scrub is used for cleaning. A cotton swab or toothpicks can be used for inner and small holes, respectively. Lint-free nylon or latex surgical gloves are worn during the cleaning operation. Cleaned parts (not detectors) are washed with deionized or distilled water in an ultrasonic bath to remove any contamination or polishing residue. It is again cleaned with alcohol or isopropanol. Threaded parts are not polished as they are not exposed to the beam. They can trap cleaning material and become a source of contamination.

Specimen stage is inspected periodically and cleaned of any residue samples that might have fallen during specimen exchange. Small vacuum cleaner or small bursts of dry nitrogen gas are used for this purpose. Abrasives and solvents are not used to clean stage components. Care is taken not to cause harm to the pole piece or detectors within the chamber.

Sample holders are cleaned using a lint- free cloth and mild abrasive cleaner. They can be rinsed in tap water and ultrasonically cleaned in distilled water or alcohol. Parts should be washed separately. The Water chiller is checked on a regular basis to make sure there are no leaks and water temperature and pressure is within prescribed limits.

Components such as emitter, anode aperture, standard apertures, extractor aperture, pre-vacuum pump, etc. need to be serviced at regular intervals or changed when required. O-ring seals should be replenished. Bake out of the SEM column should be performed at regular intervals (after several months) to keep vacuum at an optimum level. Circuit breakers should be checked. Protective covers should be kept in good condition. Safety labels should be legible. Hard drives of the computer should be defragmented and cleaned. The computer system should be protected by an antivirus system.

A logbook that contains the complete record and history of any maintenance done on the equipment should be kept by the custodian of the equipment or lab. An up-to-date inventory of the spares should be managed.

4.8 Safety Requirements

4.8.1 Radiation Safety

The SEM produces ionizing radiation (x-rays) when high voltage electron beam strikes the specimen surface or any walls of the SEM column or chamber. High energy BSE emanating from the specimen can also produce x-rays when they

interact with the SEM components. Exposure to x-rays can produce permanent damage to the human body such as skin burn, pigment alteration, dermatitis, and tumor. Hence, safety regulations need to be enacted and safe practices are to be followed to minimize radiation hazards and ensure personal safety.

The SEM manufacturers provide proper shielding to prevent any radiation leakage. Radiation safety tests should be conducted at the time of purchase and upon installation. The joints between different sections of the SEM column and the points where apertures are located at the column are the sensitive points. Interlocks of the machine should be checked and the ports within the SEM chamber need to be secured against any x-ray leakage.

Radiation should be checked at high beam voltage and current and with all apertures removed. Radiation leak check should be conducted at least every 1–2 years. Radiation level should be comparable to the background. Radiation limit of 5 μSv/r is considered safe. Operators/users should be educated and made aware of the radiation hazards associated with the equipment. The warning label should be put at the door of the room where the SEM is located. A similar label should be posted on the microscope itself clearly stating that it is a radiation generating equipment.

4.8.2 Safe Handling of the SEM and Related Equipment

The SEM should be operated by authorized trained personnel only. Operating procedure should be prepared and made available to all interested personnel. Proper training sessions should be organized. Start-up and shutdown procedures should be summarized. The microscope usage should be password controlled. The logbook should be available to accurately record personnel and usage data. User operational manual of the SEM should be at hand. Safety devices should not be allowed to be tempered with. Select personnel should have the clearance to override interlocks or warning devices. Rules for electrical safety should be followed to avoid high voltage shocks from equipment such as sputter coaters. Electrodes in vacuum evaporators should be handled carefully to avoid burns. Eye protection should be worn, and direct observation of the heated bright filament should be avoided to prevent eye damage. Pressurized gas cylinders are to be handled with established safe work practices.

4.8.3 Emergency

Record of all SEM machines in an organization should be kept complete with serial number, model, manufacturer, date of installation, and contact information. Standard emergency procedures should be in place. Emergency contacts should be available. First aid kits should be kept stocked.

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