

## 1. INTRODUCTION

A microscope can be defined as an instrument that uses one or several lenses to form an enlarged (magnified) image. The term microscope comes from the Greek "mikros" = small; and "skopos" = to look at. Microscopes can be classified according to the type of electromagnetic wave employed and whether this wave is transmitted or not through the specimen. In the transmission microscopes the electromagnetic wave passed through the specimen differentially refracted and absorbed. The most common type of transmission microscopes are transmitting light microscopes, in which visible spectrum or selected wavelengths passed through the specimen, and Transmission Electron Microscopes (TEM, Fig. 1A) where the source of illumination is an electron beam. Electron beams can also be passed over the surface of the specimen causing energy changes in the sample. These changes are detected and analyzed to give an image of the specimen. This type of microscope is called Scanning Electron Microscope (SEM, Fig. 1B). The optical paths of the illumination beam in light microscopes and TEMs are nearly identical. Both types of microscopes use a condenser lens to converge the beam onto the sample. The beam penetrates the sample and the objective lens forms a magnified image, which is projected to the viewing plane. SEM is nearly identical to TEM regarding the illumination source and the condensing of the beam onto the sample. However, significant features differ SEM and TEM. Before contacting the sample, the SEM beam is deflected by coils that move the beam in scan pattern. Then a final lens (which is also called objective lens) condense the beam to a fine spot on the specimen surface. The signals produced by the effect of the beam on the sample are interpreted by specialized signal detectors.

Electron microscopy, as it is understood today, is not a single technique but a diversity of different ones that offer unique possibilities to gain insights into morphology,

structure and composition of a specimen. The observable samples include biological specimens as well as inorganic and organic materials whose characterization need various imaging and spectroscopic methods acopled to the basic electron microscopy instrumentation

### **1.1 History of the development of the instrumentaton**

Hans Busch theoretically showed in 1927 that electron beams can be focused in an inhomogeneous magnetic field. He also predicted that the focal length of such a magnetic electron lens could be changed continuously by varying the coil current. In 1931 Ernst Ruska and Max Knoll confirmed this theory constructing a magnetic lens of the type that has been used since then in all magnetic electron microscopes leading to the construction of the first transmitted electron microscope instrument. The main limitation of their microscope was that electrons were unable to pass through thick specimens. Thus it was impossible to utilize the instrument to its full capacity until the diamond knife and ultra-microtome were invented in 1951. In 1938, Manfred von Ardenne (1907-1997) constructed a scanning transmission electron microscope (STEM) by adding scan coils to a transmission electron microscope. Vladimir Kosmo Zworykin (1889-1982), J. Hillier and R. L. Zinder developed in 1942 the first scanning electron microscope (SEM) without using the transmitted electron signal. Charles Oatley and his PHD students of the University of Cambridge provided many improvements incorporated to subsequent SEM models that finally in 1964 resulted in the first commercial SEM by Cambridge Instruments. In 1986 E. Ruska (together with G. Binnig and H. Rohrer, who developed the Scanning Tunneling Microscope) obtained Nobel Prize.

## 2. FUNDAMENTALS OF ELECTRON MICROSCOPY

### 2.1 Properties of electron beams

Electron microscopy is based on the use of a stable electron beam that interacts with the matter. Electrons are elementary particles with negative charge. These particles were discovered by J. J. Thompson in 1897 (Nobel Prize 1906), who deduced that the cathode rays consisted of negatively charged particles (corpuscles) that were constituents of the atom and over 1000 times smaller than a hydrogen atom.

The possibility to develop a microscope that utilized electrons as its illumination source began in 1924 when De Broglie (Nobel Prize 1929) postulated the wave-particle dualism according to which all moving matter has wave properties, with the wavelength  $\lambda$  being related to the momentum  $p$  by:

$$\lambda = h / p = h / mv$$

( $h$  : Planck constant =  $6.626 \times 10^{-34}$  Js;  $m$  : mass;  $v$  : velocity)

It means that accelerated electrons act also as waves. The wavelength of moving electrons can be calculated from this equation considering their energy  $E$ . The energy of accelerated electrons is equal to their kinetic energy:

$$E = eV = m_0v^2/2$$

$V$ : acceleration voltage;  $e$ : elementary charge  $1.602 \times 10^{-19}$  C;  $m_0$ : rest mass of the electron  $9.109 \times 10^{-31}$  kg;  $v$ : velocity of the electron

These equations can be combined to calculate the wavelength of an electron with a certain energy:

$$p = m_0v = (2m_0eV)^{1/2}$$

$$\lambda = h / (2m_0eV)^{1/2} (\approx 1.22 / V^{1/2} \text{ nm})$$

At the acceleration voltages used in TEM, relativistic effects have to be taken into account according to the following equation:

$$\lambda = h / [2m_0eV (1 + eV/2m_0c^2)]^{1/2}$$

Rest mass of an electron:  $m_0 = 9.109 \times 10^{-31}$  kg

Speed of light in vacuum:  $c = 2.998 \times 10^8$  m/s

Resolution in a microscope, defined as the ability to distinguish two separate items from one another, is related to wavelength of illumination. Table 1 shows several electron wavelengths at some acceleration voltages used in TEM.

Table 1. Electron wavelengths at some acceleration voltages used in TEM.

$V_{acc}^{SEP}/$ kV	B / pm	A / pm
100	3.86	3.70
200	2.73	2.51
300	2.23	1.97
400	1.93	1.64
1000	1.22	0.87

$V_{acc}$ : Accelerating voltage; A: Non relativistic wavelength; B: Relativistic wavelength.

Electron waves in beams can be either coherent or incoherent. Waves that have the same wavelength and are in phase with each other are designated as coherent. On contrast, beams comprising waves that have different wavelengths or are not in phase

are called incoherent. Electrons accelerated to a selected energy have the same wavelength. The generation of a highly monochromatic and coherent electron beam is an important challenge in the design of modern electron microscopes. After interacting with a specimen, electron waves can form either incoherent or coherent beams which interact with each other producing either constructive or destructive interferences that can lead extinguish waves.

## 2.2 Electron-Matter Interactions

When an electron encounters a material, different interactions occur producing a multitude of signals (Fig. 2). The different types of electron-specimen interaction are the basis of most electron microscopy methods. These effects can be classified into two main different types: elastic and inelastic interactions.

In the elastic interactions, no energy is transferred from the electron to the sample and the electron leaving the sample still conserves its original energy ( $E_{el} = E_0$ ). An example of this is the case of the electron passing the sample without any interaction which contributes to the direct beam leaving the sample in direction of the incident beam (Fig. 2). In thin samples, these signals are mainly exploited in TEM and electron diffraction methods, whereas in thick specimens backscattered electrons are the main elastic signals studied.

In the inelastic interactions, an amount of energy is transferred from the incident electrons to the sample, causing different signals such as X-rays, Auger or secondary electrons or cathodoluminescence.

Figure 3 shows the electron energy spectrum of several signals produced during the interaction of an electron beam with a specimen. Low-energy peaks, such as the large secondary electron peak, correspond to inelastic interaction whereas high-energy peaks;

those of the backscattered electron (BSE) distribution correspond to cases where the primary electron loses no energy or, to be accurate, only a negligible amount of energy is lost. Signals produced by inelastic electron-matter interactions are predominantly utilized in the methods of analytical electron microscopy.

The volume of material affected by the electron beam depends on many factors. The volume of interaction is controlled by energy loss through inelastic interactions and electron loss or backscattering through essentially elastic interactions. Factors controlling the electron penetration depth and the interaction volume are the angle of incidence, the current magnitude and the accelerating voltage of the beam, as well as the average atomic number ( $Z$ ) of the sample. The resulting excitation volume is a hemispherical to jug-shaped region with the neck of the jug at the specimen surface (Fig. 2). For an electron beam incident perpendicular to the sample, electron penetration generally ranges from 1-5  $\mu\text{m}$ .

## **2.2.1 Elastic Interactions**

### **2.2.1.1 Incoherent scattering, the backscattered electron signal**

When an electron penetrates into the electron cloud of an atom is attracted by the positive potential of the nucleus deflecting its path towards the core. The Coulombic force  $F$  is defined as:

$$F = Q_1 Q_2 / 4\pi\epsilon_0 r^2$$

with  $r$  being the distance between the charges  $Q_1$  and  $Q_2$  and  $\epsilon_0$  the dielectric constant. The closer the electron comes to the nucleus, the larger is  $F$  and consequently the scattering angle (Fig. 4). Scattering angles range up to  $180^\circ$ , but average about  $5^\circ$ .

### *Backscattered electrons*

In some cases, even complete backscattering can occur in an individual interaction (Fig. 5A). In thick samples, many incident electrons undergo a series of such elastic events that cause them to be scattered back out of the specimen (Fig. 5B). Backscattered electrons (BSE) are high-energy primary electrons that suffer large angle ( $> 90^\circ$ ) scattering and re-emerge from the entry surface of a specimen. Individual backscattering events are generally elastic, where a negligible amount of energy is lost by the primary electron in the process. Most BSE have energies slightly lower than that of the primary electron beam,  $E_0$ , but may have energies as low as  $\sim 50$  eV. Because of its dependence on the charge, the force  $\mathbf{F}$  with which an atom attracts an electron is stronger for atoms containing more positive charges. Thus, the Coulomb force increases with increasing atomic number  $Z$  of the respective element. Therefore, the fraction of beam electrons backscattered from a sample ( $\eta$ ) depends strongly on the sample's average atomic number,  $Z$  (Fig. 6). However, in thick samples it must be taken into account that an electron that has undergone inelastic scattering may subsequently escape the sample surface as a BSE, and thus the energy of a backscattered electron depends on the number of interactions that it has undergone before escaping the sample surface.

#### **2.2.1.2 Coherent scattering, the electron diffraction (ED) signal**

When electrons are scattered by atoms in a regular array, collective elastic scattering phenomenon, known as electron diffraction (ED), occur. The incoming electron wave interact with the atoms, and secondary waves are generated which interfere with each other. This occurs either constructively (reinforcement at certain scattering angles generating diffracted beams) or destructively (extinguishing of beams) which gives rise

to a diffraction pattern (Fig. 2). The scattering event can be described as a reflection of the beams at planes of atoms according to the Bragg law, which gives the relation between interplanar distance  $d$  and diffraction angle  $\Theta$ :

$$n\lambda = 2d\sin\Theta$$

Since the wavelength  $\lambda$  of the electrons is known, interplanar distances can be calculated from ED patterns.

### **2.2.2 Inelastic interactions**

Most electrons of the incident beam follow complicated trajectories through the sample material, losing energy as they interact with the specimen atoms producing a number of interactions. Some of the most significant effects are shown in figure 2. Several interaction effects due to electron bombardment emerge from the sample and some, such as sample heating, stay within the sample.

#### **2.2.2.1 Secondary electrons**

Secondary electrons are produced by inelastic interactions of high energy incident electrons with valence electrons of atoms in the specimen causing the ejection of the electrons from the atoms (Fig. 7) which can move towards the sample surface through elastic and inelastic collisions until it reaches the surface, escaping if its energy exceed the surface work function,  $E_w$ . The strongest region in the electron energy spectrum is due to secondary electrons (SE), which are defined as those emitted with energies less than 50 eV (Fig. 3).

The mean free path length of secondary electrons in many materials is  $\sim 1$  nm ( $10 \text{ \AA}$ ). Although electrons are generated in the whole region excited by the incident beam, only



those electrons that originate less than 1 nm deep in the sample are able to escape giving rise to a small volume production. Therefore, the resolution using SE is effectively the same as the electron beam size. The shallow depth of production of detected secondary electrons makes them very sensitive to topography and they are used for scanning electron microscopy (SEM).

The fraction of secondary electrons produced,  $\delta$ , is the average number of SE produced per primary electron, and is typically in the range 0.1 to 10.

#### **2.2.2.2 Other significant inelastic signals**

##### *Characteristic X-rays*

When an electron from an inner atomic shell is displaced by colliding with a primary electron, it produces a vacancy in that electron shell. In order to re-establish the proper balance in its orbitals, an electron from an outer shell of the atom may fall into the inner shell and replace the spot vacated by the displaced electron. In doing so this falling electron loses energy and this energy is referred to as X-rays. The characteristic X-ray of a given element can be detected and identified, therefore information about the chemical composition of different points of the sample can be obtained.

##### *Auger Electrons*

Auger electrons are produced when an outer shell electron fills the hole vacated by an inner shell electron that is displaced by a primary or backscattered electron. The excess energy released by this process may be carried away by an Auger electron. Because of their low energies, Auger electrons are emitted only from near the surface. The energy of an Auger electron can be characteristic of the type of element from which it was

released and thus Auger Electron Spectroscopy can be performed to obtain chemical analysis of the specimen surface.

### *Cathodluminescence*

The interaction of the primary beam with the specimen in certain materials release excess energy in the form of photons. These photons of visible light energy can be detected and counted forming an image using the signal of light emitted.

## **3. INSTRUMENTATION**

All electron beam instruments are built around an electron column containing an electron gun that produces a stable electron beam and a set of electromagnetic lenses that control beam current, beam size and beam shape, and raster the beam (in the SEM and STEM cases, see [Fig. 1](#)). Electron microscopes also have a series of apertures (micron-scale holes in metal film) by which the beam passes through controlling its properties. Electron optics are a very close analog to light optics, and most of the principles of an electron beam column can be understood by thinking of the electrons as rays of light and simply the electron optical components as their optical counterparts.

### **3.1 Electron gun**

The electron gun is located at the upper part of the column and its purpose is to provide electrons to form a stable beam of electrons of adjustable energy. This is carried out by allowing electrons to escape from a cathode material. The total energy required for a material to give up electrons is defined by the equation:

$$E = E_w + E_f$$

where,  $E$  is the total amount of energy needed to remove an electron to infinity from the lowest free energy state,  $E_f$  is the highest free energy state of an electron in the material (which must be achieved), and  $E_w$  is the work function or work required to achieve the difference.

Electron beams can be produced by two different electron gun types (Fig. 8): thermoionic guns (electron emission through heating) and Field Emission Guns (electron emission through the application an extraction voltage).

In the thermionic sources (Fig. 8A), electrons are produced by heating a conductive material to the point where the outer orbital electrons gain sufficient energy to overcome the work function barrier and escape. Most of the thermoionic electron guns have a triode configuration consisting of a cathode, a Wehnelt cap and an anode.

The cathode is a thin filament (about 0.1 mm) wire bent into an "V" to localize emission at the tip, yielding a coherent source of electrons emitted from a small area which in many cases is not perfectly circular. There are two main types of thermionic sources: tungsten metal filaments and LaB<sub>6</sub> crystals. These two types of sources require vacuums of  $\sim 10^{-5}$  and  $\sim 10^{-7}$  torr, respectively. Tungsten filaments resist high temperatures without melting or evaporating, but they have a very high operating temperature (2700 °K), which decreases their lifetime due to thermal evaporation of the cathode material. The electron flux from a tungsten filament is minimal until a temperature of approximately 2500 K. Above 2500 K, the electron flux will increase essentially in an exponential mode with increasing temperature, until the filament melts at about 3100 K. However, in practice, the electron emission reaches a plateau termed saturation. Proper saturation is achieved at the edge of the plateau; higher emission currents serve only to reduce filament life. LaB<sub>6</sub> cathodes yield higher currents at lower cathode temperatures than tungsten, exhibiting 10 times the brightness and more than 10 times the service life

of tungsten cathodes. Moreover, its emission region is smaller and more circular than that of tungsten filaments, which improves the final resolution of the electron microscope. However, LaB<sub>6</sub> is reactive at the high temperatures needed for electron emission.

The cathode is surrounded by a slightly negative biased Wehnelt cap (200-300 V) to localize the cloud of primary electrons emitted in all directions from the entire heated filament at one spot at the tip above an aperture in the Wehnelt. The filament must be centered to the proper distance in relation to the opening of the Wehnelt cap. Otherwise, an off-center beam will be produced which is either weak/condensed or bright/diffuse.

The electrons emitted from the cathode-Wehnelt assembly are drawn away by the anode plate, which is a circular plate with a hole in its center. A voltage potential between the cathode and the anode plate is used to accelerate the electrons down the column, which is known as the accelerating voltage. Thus, the anode plate serves to condense and roughly focus the beam of primary electrons.

In the field emission guns (**Fig. 8B**), the cathode consists of a sharp metal tip (usually Tungsten) with a radius of less than 100 nm. A potential difference ( $V_1$ = extraction voltage) is established between the first anode and the tip. The result is an electric field, concentrated at the tip, which produces electron emission (emission current). The potential difference between the tip and the second grounded anode determines the accelerating voltage ( $V_0$ ) of the gun. The higher the accelerating voltage the faster the electrons travel down the column and the more penetrating power they have.

There are two types of field emission guns: cold and thermally assisted. Both types of field emission require that the tip remain free of contaminants and oxide and thus they require ultra high vacuum conditions ( $10^{-10}$  to  $10^{-11}$  Torr). In the cold FEG the electric field produced by the extraction voltage lowers the work function barrier and allows

electrons to directly tunnel through it and thus facilitating emission. The cold FEGs must periodically heat their tip to be polished of absorbed gas molecules. The thermally assisted FEG (Schottky field emitter) uses heat and chemistry (nitride coating) in addition to voltage to overcome the potential barrier level.

### 3.2. Electromagnetic Lens

Electromagnetic lenses are made of a coil of copper wires inside several iron pole pieces (Fig. 9). An electric current through the coils creates a magnetic field in the bore of the pole pieces. The rotationally symmetric magnetic field is strong close to the bore and becomes weaker in the center of the gap. Thus, when an electron beam passes through a electromagnetic lens, electrons close to the center are less strongly deflected than those passing the lens far from the axis. The overall effect is that a beam of parallel electrons is focused into a spot. In a magnetic field, an electron experiences the Lorentz force  $F$ :

$$F = -e (E + v \times B)$$

$$|F| = evB\sin(\nu, B)$$

$E$ : strength of electric field.  $B$ : strength of magnetic field.  $e/v$ : charge/velocity of electrons

The focusing effect of a magnetic lens therefore increases with the magnetic field  $B$ , which can be controlled via the current flowing through the coils. As it is described by the vector product, the resulting force  $F$  is perpendicular  $v$  and  $B$ . This leads to a helical trajectory of the electrons and to the magnetic rotation of the image (Fig. 9).

Electromagnetic lenses influence electrons in a similar way as convex glass lenses do with light. Thus, very similar diagrams can be drawn to describe the respective ray paths. Consequently, the imaginary line through the centers of the lenses in an electron microscope is called optical axis as well. Furthermore, the lens equation of light optics is also valid in electron optics, and the magnification is defined accordingly:

$$1/u + 1/v = 1/f$$

Magnification  $M = v/u$ ;  $f$ : focal length;  $u$ : object distance;  $v$ : image distance

As in glass lenses, magnetic lenses have spherical (electrons are deflected stronger the more they are off-axis) and chromatic aberrations (electrons of different wavelengths are deflected differently). Moreover, the iron pole pieces are not perfectly circular, and this makes the magnetic field deviating from being rotational symmetric. The astigmatism of the objective lens can distort the image seriously. Thus, the astigmatism must be corrected, and this can fortunately be done by using octupole elements, so-called stigmators. These stigmators generate an additional field that compensates the inhomogeneities causing the astigmatism.

To reduce the effects of spherical aberration, apertures are introduced into the beam path (see [Fig. 1](#)). It must be taken in account that the introduction of apertures reduces beam current and can produce diffraction effects.

In electron microscopes, magnetic lenses perform two different tasks:

1. Beam formation and focusing (condenser lenses in TEM and SEM and objective lens in SEM):

Condenser lenses are located at the upper part of the column ([Fig. 1A and 1B](#)) ensuring that the electron beam is symmetrical and centered as it passes down the electron column of the microscope. These lenses allow making variations of the beam intensity,

controlling the amount of current that passes down the rest of the column. This is accomplished by focusing the electron beam to variable degrees onto a lower aperture of fixed size. The sharper the focus, the less of the beam intercepted by the aperture and the higher the current. Moreover, in the TEM, condenser lenses focus the illuminating beam on the specimen.

In the SEM, the objective, or "probe-forming", lens is located at the base of electron column just above sample (Fig. 1B). The beam is divergent after passing through the condenser lens and must be refocused. The objective lens focuses the electron beam onto the sample and controls its final size and position.

An objective aperture of variable size is also located under the lens. Decreasing the diameter of the aperture will decrease lens aberrations, increasing resolution, and decreases the probe current and the convergence angle of the beam.

Hosted within it are scanning coils. Scanning (or "deflector") coils raster the beam across the sample for textural imaging. The coils consist of four magnets oriented radially that produce fields perpendicular to optical axis of the electron beam. The beam is rastered by varying the current through these magnets.

## 2. Image formation and magnification (objective, diffraction, intermediate, and projective lenses in TEM).

The TEM has a complex imaging system located under the sample chamber which consists of a group electromagnetic lenses. The objective lens focuses the beam after it passes through the specimen and forms an intermediate image of the specimen. Electrons, which come from the condenser system of the TEM, are scattered by the sample, situated in the object plane of the objective lens. Electrons scattered in the same direction are focused in the back focal plane, and, as a result, a diffraction pattern is

formed there. Electrons coming from the same point of the object are focused in the image plane.

An objective aperture is situated within the beam path just below the objective lens, allowing select only some beams to contribute to the formation of the image. Subsequent lenses (eye piece/intermediate and projector lenses) magnify portions of the intermediate image to form the final image.

### **3.3 Sample chamber**

Samples involved in the TEM and SEM techniques have different characteristics. TEM samples are usually small and must be as thin as possible, whereas, in contrast, SEM samples are far more variable ranging from small specimens, similar to those observed in the TEM, to large pieces of specimens. The sample chambers of the two instruments reflect this difference. TEM has a sample chamber just large enough to permit observation of specimens included in a ring or grid of few mm. In contrast, SEM has a sample chamber that permits large and complex movements of the sample stage; for example, the SEM can accommodate samples up to 200 mm in diameter and ~80 mm high. Moreover, SEM sample chambers are designed to accommodate a wide set of signal detectors that allow to develop their imaging and analytical facilities.

### **3.4 Detection of signals**

#### **3.4.1 Backscattered electron detection**

The backscattered electron signal is mainly used in SEM instruments. Backscattered electrons are detected using diodes, acting as semiconductors, located at the base of the objective lens (Fig. 10). When backscattered electrons strike these semiconductors chips, imparting energy, a current flows. The size of the current (signal) is proportional to the number of electrons hitting the semiconductor. The BSE detectors are sensitive to



light and cannot be used if a sample illumination device is on at the chamber.

The BSE detector systems commonly consist of either two semicircular plates or four semiconductor plates mounted at the base of the bottom of the electron column.

### **3.4.2 Secondary electron detection**

Due to the low energy of the secondary electrons (Fig. 3) emitted from the sample in all directions, in a first stage of the detection process, they have to be drawn from the sample surface toward the detector. They are gathered by a charged collector grid, which can be biased from -50 to +300 V (Fig. 11). Secondary electrons are then detected using a scintillator-photomultiplier detector. The amplified electrical signal is sent to further electrical amplifiers, which increase the electrical signal thus increasing brightness.

### **3.5 Low-vacuum mode**

SEM has the added capability to also work with a relatively poor vacuum in the specimen chamber. Air (or another gas) is admitted into the sample chamber to neutralize the build-up of the negative electrostatic charge on the sample. In "low-vacuum (LV) mode", the sample chamber pressure, typically 20-30 Pa, is maintained at a specifiable value by a separate extra pump with a large foreline trap. The electron column is separated from the chamber by a vacuum orifice (aperture) that permits the column and objective lens apertures to be maintained at a higher vacuum pressure. LV mode allows the study of samples with poor or no electrical conductivity. Normally, these materials would be either coated with an electrically conductive coating, which

takes time and hides the true surface, or studied at low voltages, which do not give backscatter or X-ray information. Additionally, samples containing volatile substances (water) can often be studied directly.

An environmental SEM utilizes relatively low vacuum pressures (up to 50 torr ~ 6700 Pa) not only to neutralize charges, but also to provide signal amplification. The electron column is gradually pressured by a series of pumps and apertures.

A positively charged detector electrode is placed at the base of the objective lens at the top of the sample chamber (Fig. 12). Electrons emitted from the sample are attracted to the positive electrode, undergoing acceleration. As they travel through the gaseous environment, collisions occur between electrons and gas particles, producing more electrons and ionization of the gas molecules and effectively amplifying the original secondary electron signal (electron cascade). This form of signal amplification is identical to that which occurs in a gas-flow X-ray detector. Positively charged gas ions are attracted to the negatively biased specimen and offset charging effects.

## **4. IMAGING IN ELECTRON MICROSCOPY**

### **4.1 Imaging in TEM**

Image formation in the TEM is carried out by the objective lens, which physically performs:

- A Fourier transform (FT, Fourier analysis) that creates the diffraction pattern of the object in the back focal plane of the objective lens
- an inverse FT (Fourier synthesis) that makes the interference of the diffracted beams back to a real space image in the image plane.

Therefore, the selection of the focusing plane and the beams allowed to pass through the aperture of the objective lens play critical roles in the TEM image formation.

#### **4.1.1 Diffraction patterns**

The Fourier transform of periodic structures give rise to sharp spots at well-defined positions in the resulting diffraction pattern. Each set of parallel lattice planes of the periodic structure is represented by spots which have a distance of  $1/d$  ( $d$ : interplanar spacing) from the origin and which are perpendicular to the reflecting set of lattice planes. The diffraction can be described in reciprocal space by the Ewald sphere construction (Fig. 13). A sphere with radius  $1/\lambda$  is drawn through the origin of the reciprocal lattice. For each reciprocal lattice point that is located on the Ewald sphere surface, the Bragg condition is satisfied and diffraction arises. Such diffracted patterns provide useful information about the nature of the periodic structure.

#### **4.1.2 Contrast images**

The focus in the image plane of the electrons coming from the same point of the object produces images whose points have different contrast. Basic contrast mechanisms in TEM are controlled by the local scattering power of the sample. The interaction of electrons with heavy atoms having a high charge ( $Q$ ) is stronger than with light atoms so that areas in which heavy atoms are localized appear with darker contrast than those with light atoms (mass contrast). In thick areas, more electron scattering events occur and thus thick areas appear darker than thin areas (thickness contrast). In particular, this mass-thickness contrast is important in bright and dark field imaging.

In the bright field (BF) mode of the TEM (Fig. 14), the aperture placed in the back focal plane of the objective lens allows only the direct beam to pass. In this case, the image results from a weakening of the direct beam by its interaction with the sample and

therefore mass-thickness contrast contribute to image formation: thick areas and areas enriched in heavy atoms appear with darker contrast. If a specimen with crystalline structure is appropriately oriented, many electrons will be strongly scattered to contribute to the reflections in the diffraction pattern, then only a few electrons pass without interactions and therefore this specimen appears with dark contrast in the BF image (diffraction contrast). In real specimens, all contrast mechanisms, namely mass-thickness and diffraction contrast, occur simultaneously, making interpretation of TEM images sometimes difficult. In dark field (DF) images (Fig. 14), the direct beam is blocked by the objective lens aperture while one or more diffracted beams are allowed to pass, resulting an image where the specimen is in general weakly illuminated and the surrounding area that do not contain sample is dark.

#### **4.1.3 Lattice images**

The lattice images are formed by the interference of various diffracted beams. A larger objective aperture has to be selected to obtain this type of images, allowing much beams, including, or not, the direct beam to pass. If the resolution of the microscope is sufficiently high and a suitable sample is oriented along, then high-resolution TEM (HRTEM) images, showing the crystalline periodicity, are obtained.

#### **4.2 Imaging in SEM**

In the SEM images, there is a conjugate correspondence between the rastering pattern of the specimen and the rastering pattern used to produce the image on the monitor. The signal produced by the rastering is collected by the detector and subsequently processed to generate the image (Fig. 1B). That processing takes the intensity of the signal coming from a pixel on the specimen and converts it to a grayscale value of the corresponding

monitor pixel. The monitor image is a two-dimensional rastered pattern of grayscale values.

Interestingly, no lens is directly involved in the image forming process. The magnification is then simply the ratio of the length of the scan  $C$  on the CRT to the length of the scan on the specimen. Changing magnification does not involve changing any lens current and therefore, focus does not change as magnification is changed and the image does not rotate with magnification change.

For obtaining images in sharp focus, the area sampled by the incident electron beam must be at least as small as the pixel diameter for the selected magnification. Due to the scattering of the incident electrons, the signals produced are actually generated from a larger area (perhaps 1.5 to 2 times) than the beam diameter. Therefore, the beam must be focused to a diameter between 0.5 to 0.7 times the diameter of the magnification.

Contrast  $C$  involves the signals produced by the detector for two points A & B on the sample, which depend on the number of signal electrons emitted from the sample, the number of these electrons reaching the detector, and its efficiency to record them. The signal produced by the detector can be modified to change the appearance of the image, however such change does not alter its information content.

#### **4.2.1 Imaging with BSE**

BSE provide valuable information because of their sensitivity to atomic number variations. However, it must be taken into account that BSE are produced from the entire upper half of the interaction volume (Fig. 2) and, therefore, the spatial resolution of BSE images is poor (usually  $\sim 1 \mu\text{m}$ ; at best  $\sim 0.1 \mu\text{m}$ ).

The BSE signal is mainly used to obtain information on the specimen composition (average  $Z$ ). SEM uses paired detectors to receive BSE (Fig. 15). Addition of signals

received by each detector highlight compositional characteristics of the sample, providing a composition image (COMPO mode).

#### **4.2.2 Imaging with SE**

SE provide morphological information of the specimen. The topographical aspects of a secondary electron image depend on how many electrons actually reach the detector. Given that, SE yield depends on the angle of tilt of the specimen relative to the primary-electron beam,  $\phi$ ; when the incident electron beam intersects the tilted edges of topographically high portions of a sample at lower angles, it puts more energy into the volume of secondary electron production, producing more secondary electrons and generating a larger signal. Therefore, places where the beam strikes the surface at an angle have larger volumes of electron escape than the perpendicular incidence ( $\phi = 0$ ). These effects cause tilted surfaces to appear brighter than flat surfaces, and edges and ridges to be markedly highlighted, in images formed with secondary electrons.

Faces oriented towards the detector also generate more secondary electrons. Secondary electrons that are prevented from reaching the detector do not contribute to the final image. Therefore, faces oriented towards the detector will be brighter, whereas those in the opposite direction will be dark.