Electron Microscopy: The Basics

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Abstract

Since its invention, electron microscope has been a valuable tool in the development of scientific theory and it contributed greatly to biology, medicine and material sciences. This wide spread use of electron microscopes is based on the fact that they permit the observation and characterization of materials on a nanometer (nm) to micrometer (µm) scale. This paper presents the basic theory for electron microscopy, focusing on the two basic types of Ems; SEM, TEM.

1. Introduction

Electron Microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects on a very fine scale. This examination can yield information about the topography (surface features of an object), morphology (shape and size of the particles making up the object), composition (the elements and compounds that the object is composed of and the relative amounts of them) and crystallographic information (how the atoms are arranged in the object).

Electron Microscopes were developed due to the limitations of Light Microscopes which are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 micrometers. In the early 1930's this theoretical limit had been reached and there was a scientific desire to see the fine details of the interior structures of organic cells (nucleus, mitochondria...etc.). This required 10,000x plus magnification which was just not possible using Light Microscopes. The Transmission Electron Microscope (TEM) was the first type of Electron Microscope to be developed and is patterned exactly on the Light Transmission Microscope except that a focused beam of electrons is used instead of light to "see through" the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931.

The first Scanning Electron Microscope (SEM) debuted in 1942 with the first commercial instruments around 1965. Its late development was due to the electronics involved in "scanning" the beam of electrons across the sample.

Electron Microscopes (EMs) function exactly as their optical counterparts except that they use a focused beam of electrons instead of light to "image" the specimen and gain information as to its structure and composition. The basic steps involved in all Ems are the following: A stream of electrons is formed in high vacuum (by electron guns). This stream is accelerated towards the specimen (with a positive electrical potential) while is confined and focused using metal apertures and magnetic lenses into a thin, focused, monochromatic beam.
The sample is irradiated by the beam and interactions occur inside the irradiated sample, affecting the electron beam. These interactions and effects are detected and transformed into an image.

The above steps are carried out in all EMs regardless of type. A more specific treatment of the workings of two different types of EMs (SEM, TEM) as well as the function of an electron gun and the theory of electron-specimen interaction is described in more detail below.

2. Electron Gun

The first and basic part of the microscopes is the source of electrons. It is usually a V-shaped filament made of LaB6 or W (tungsten) that is wreathed with Wehnelt electrode (Wehnelt Cap). Due to negative potential of the electrode, the electrons are emitted from a small area of the filament (point source). A point source is important because it emits monochromatic electrons (with similar energy). The two usual types of electron guns are the conventional electron guns and the field emission guns (FEG). Figure 1 illustrates the geometry of an electron gun.

In conventional electron guns, a positive electrical potential is applied to the anode, and the filament (cathode) is heated until a stream of electrons is produced. The electrons are accelerated by the positive potential down the column, and because of the negative potential of cap, all electrons are repelled toward the optic axis. A collection of electrons occurs in the space between the filament tip and Cap, which is called a space charge. Those electrons at the bottom of the space charge (nearest to the anode) can exit the gun area through the small (<1 mm) hole in the Whenelt Cap and then move down the column to be later used in imaging.

A field emission gun consists of a sharply pointed tungsten tip held at several kilovolts negative potential relative to a nearby electrode, so that there is a very high potential gradient at the surface of the tungsten tip. The result of this is that the potential energy of an electron as a function of distance from the metal surface has a sharp peak (from the work function), then drops off quickly (due to electron charge traveling through an electric field). Because electrons are quantum particles and have a probability distribution to their location, a certain number of electrons that are nominally at the metal surface will find themselves at some distance from the surface, such that they can reduce their energy by moving further away from the surface. This transport-via-delocalization is called 'tunneling', and is the basis for the field emission effect. FEGs produce much higher source brightness than in conventional guns (electron current > 1000 times), better monochromaticity, but requires a very good vacuum (~10^{-7} Pa).

![Figure 1. Illustration of the electron gun](image-url)
3. Electron-specimen interactions

When an electron beam interacts with the atoms in a sample, individual incident electrons undergo two types of scattering – elastic and inelastic (Figure 2). In the former, only the trajectory changes and the kinetic energy and velocity remain constant. In the case of inelastic scattering, some incident electrons will actually collide with and displace electrons from their orbits (shells) around nuclei of atoms comprising the sample. This interaction places the atom in an excited (unstable) state. Specimen interaction is what makes Electron Microscopy possible. The interactions (inelastic) noted on the top side of the diagram are utilized when examining thick or bulk specimens (Scanning Electron Microscopy, SEM) while on the bottom side are those examined in thin or foil specimens (Transmission Electron Microscopy, TEM).

![Diagram of electron interactions](image)

Figure 2. Effects produced by electron bombardment of a material

3.1. Reactions Exploited In SEM

3.1.1. Secondary Electrons

When a sample is bombarded with electrons, the strongest region of the electron energy spectrum is due to secondary electrons. The secondary electron yield depends on many factors, and is generally higher for high atomic number targets, and at higher angles of incidence. Secondary electrons are produced when an incident electron excites an electron in the sample and loses most of its energy in the process. The excited electron moves towards the surface of the sample undergoing elastic and inelastic collisions until it reaches the surface, where it can escape if it still has sufficient energy.

Production of secondary electrons is very topography related. Due to their low energy (5eV) only secondaries that are very near the surface (<10 nm) can exit the sample and be examined. Any changes in topography in the sample that are larger than this sampling depth will change the yield of secondaries due to collection efficiencies. Collection of these electrons is aided by using a "collector" in conjunction with the secondary electron detector. Figure 3 presents two secondary electron images from SEM.
3.1.2. Backscattered Electrons

Backscattered electrons consist of high-energy electrons originating in the electron beam, that are reflected or back-scattered out of the specimen interaction volume. The production of backscattered electrons varies directly with the specimen's atomic number. This differing production rates causes higher atomic number elements to appear brighter than lower atomic number elements. This interaction is utilized to differentiate parts of the specimen that have different average atomic number. Figure 4 illustrates a backscattered electron image.

3.1.3 Relaxation of excited atoms

As was mentioned above, inelastic scattering, places the atom in an excited (unstable) state. The atom "wants" to return to a ground or unexcited state. Therefore, at a later time the atoms will relax giving off the excess energy. X-Rays, cathodoluminescence and Auger electrons are three ways of relaxation. The relaxation energy is the fingerprint of each element.

When the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimens surface. A resulting electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted to balance the energy difference between the two electrons. The EDS X-ray detector (also called EDS or EDX) measures the number of emitted x-rays versus their energy. The energy of the x-ray is characteristic of the element from which the x-ray was emitted.

In practice, EDS (or EDX) is most often used for qualitative elemental analysis, simply to determine which elements are present and their relative abundance. In some instances, however, the area of interest is simply too small and must be analyzed by TEM (where EDS is the only option) or high resolution SEM (where
the low beam currents used preclude WDS-Wavelength X-ray Dispersive Spectroscopy, making EDS the only option)[1].

Cathodoluminescence (CL) is the emission of photons of characteristic wavelengths from a material that is under high-energy electron bombardment. The electron beam is typically produced in an electron microprobe (EPMA) or scanning electron microscope (SEM-CL)

Auger electrons are electrons ejected by radiationless excitation of a target atom by the incident electron beam. When an electron from the L shell drops to fill a vacancy formed by K-shell ionization, the resulting X-ray photon with energy $E_K - E_L$ may not be emitted from the atom. If this photon strikes a lower energy electron (e.g. an M-shell electron), this outer electron may be ejected as a low-energy Auger electron. Auger electrons are characteristic of the fine structure of the atom and have energies between 280 eV (carbon) and 2.1 keV (sulfur). By discriminating between Auger electrons of various energies, a chemical analysis of the specimen surface can be made. Auger electrons are exploited in Auger Electron Spectroscopy tools (AES)

The volume inside the specimen in which interactions occur while being struck with an electron beam is called specimen interaction volume. Figure 5, illustrates the interaction volumes for secondary and backscattered electrons, as well as X-Rays.

Figure 5. Generalized illustration of interaction volumes for various electron-specimen interactions

3.2. Reactions Exploited In TEM

TEM exploits three different interactions of electron beam-specimen; Unscattered electrons (transmitted beam), elastically scattered electrons (diffracted beam) and inelastically scattered electrons.

When incident electrons are transmitted through the thin specimen without any interaction occurring inside the specimen, then the beam of these electrons is called transmitted. The transmission of unscattered electrons is inversely proportional to the specimen thickness. Areas of the specimen that are thicker will have fewer transmitted unscattered electrons and so will appear darker, conversely the thinner areas will have more transmitted and thus will appear lighter.

Another part of the incident electrons, are scattered (deflected from their original path) by atoms in the specimen in an elastic fashion (no loss of energy). These scattered electrons are then transmitted through the remaining portions of the specimen. All electrons follow Bragg’s Law and thus are scattered according to
\[ n \cdot \lambda = 2 \cdot d \cdot \sin(\theta) \]

where:
\( \lambda \) is the wavelength of the rays
\( \theta \) is the angle between the incident rays and the surface of the crystal and 
\( d \) is the spacing between layers of atoms.

All incident electrons have the same energy (thus wavelength) and enter the specimen normal to its surface. All incidents that are scattered by the same atomic spacing will be scattered by the same angle. These scattered electrons can be collated using magnetic lenses to form a pattern of spots; each spot corresponding to a specific atomic spacing (a plane). This pattern can then yield information about the orientation, atomic arrangements and phases present in the area being examined. Figure 6 shows the diffraction pattern of a monocrystalline sample.

Figure 6. Diffraction pattern of a monocrystalline sample

Finally, another way that incident electrons can interact with the specimen is inelastically. Incident electrons that interact with specimen atoms in an inelastic fashion, loosing energy during the interaction. These electrons are then transmitted through the rest of the specimen. Inelastically scattered electrons can be utilized in two ways; Electron Energy Loss Spectroscopy (EELS) and Kikuchi Bands.

Elemental composition and atomic bonding state can be determined by analyzing the energy with the spectroscope attached under the electron microscope (Electron Energy Loss Spectroscopy). Because the analyzing region can be selected from a part of the enlarged electron microscopic image, one can analyze very small region. Moreover, by selecting electrons with a specific loss energy by a slit so as to image them, element distribution in specimen can be visualized (Elemental Mapping) [2].

Kikuchi lines appear in transmission electron diffraction patterns of relatively thick crystals due to Bragg reflection of the inelastically scattered electrons. They are alternating light and dark lines that are related to atomic spacings in the specimen. These bands can be either measured (their width is inversely proportional to atomic spacing) or "followed" like a roadmap to the "real" elasticity scattered electron pattern [3].
4. SEM-TEM

For the purpose of detailed materials characterization, two potent instruments are used: the Scanning Electron Microscope (SEM) and the Transmission Electron Microscope (TEM). Their operation is described below.

4.1 SEM

4.1.1 Operation

In SEM, a source of electrons is focused in vacuum into a fine probe that is rastered over the surface of the specimen. The electron beam passes through scan coils and objective lens that deflect horizontally and vertically so that the beam scans the surface of the sample (Figure 8).

As the electrons penetrate the surface, a number of interactions occur that can result in the emission of electrons or photons from or through the surface. A reasonable fraction of the electrons emitted can be collected by appropriate detectors, and the output can be used to modulate the brightness of a cathode ray tube (CRT) whose x- and y- inputs are driven in synchronism with the x-y voltages rastering the electron beam. In this way an image is produced on the CRT; every point that the beam strikes on the sample is mapped directly onto a corresponding point on the screen [2]. As a result, the magnification system is simple and linear magnification is calculated by the equation:

\[
M = \frac{L}{l}
\]  

where L is the raster’s length of the CRT monitor and l the raster’s length on the surface of the sample.

SEM works on a voltage between 2 to 50kV and its beam diameter that scans the specimen is 5nm-2µm. The principle images produced in SEM are of three types: secondary electron images, backscattered electron images and elemental X-ray maps. Secondary and backscattered electrons are conventionally separated according to their energies. When the energy of the emitted electron is less than about 50eV, it is referred as a secondary electron and backscattered electrons are considered to be the electrons that exit the specimen with an energy greater than 50eV [4]. Detectors of each type of electrons are placed in the microscope in proper positions to collect them.

Figure 7. The Kikuchi lines pass straight through the transmitted and diffracted spots. The diffracting planes are therefore tilted at exactly the Bragg angle to the optic axis.
4.1.2 Advantages and Disadvantages

Electrons in scanning electron microscopy penetrate into the sample within a small depth, so that it is suitable for surface topology, for every kind of samples (metals, ceramics, glass, dust, hair, teeth, bones, minerals, wood, paper, plastics, polymers, etc). It can also be used for chemical composition of the sample’s surface since the brightness of the image formed by backscattered electrons is increasing with the atomic number of the elements. This means that regions of the sample consisting of light elements (low atomic numbers) appear dark on the screen and heavy elements appear bright. Backscattered are used to form diffraction images, called EBSD, that describe the crystallographic structure of the sample. In SEM, X-rays are collected to contribute in Energy Dispersive X-Ray Analysis (EDX or EDS), which is used to the topography of the chemical composition of the sample.

Consequently, SEM is only used for surface images and both resolution and crystallographic information are limited (because they’re only referred to the surface). Other constraints are firstly that the samples must be conductive, so non-conductive materials are carbon-coated and secondly, that materials with atomic number smaller than the carbon are not detected with SEM.

4.1.3 SEM Today

As time goes on, the ultimate resolution of the SEM levels out near 0.6nm at 5kV. In Scanning Transmission Electron Microscopy in which internal microstructure images of thin specimens are obtained, achieved resolution is up to 1.5nm at 30kV.

4.1.4 Environmental SEM (ESEM)

The major growth of SE Ms is in the development of specialized instruments. Environmental SEM uses differential pumping to permit the observation of specimens at low-pressure gaseous environments (e.g. 1-50 Torr), at high relative humidity (up to 100%) and at higher pressures. In this type of SEM, there’s no need for conductive coating, the secondary electron detector operates
in the presence of water vapour, and in the microscope’s column there are pressure-limiting apertures. The ESEM is ideal for non-metallic surfaces, such as biological materials, plastics and elastomers [4].

4.2.1 TEM

Transmission Electron Microscopy (TEM) is a technique where an electron beam interacts and passes through a specimen. The electrons are emitted by a source and are focused and magnified by a system of magnetic lenses. The geometry of TEM is shown in figure 9. The electron beam is confined by the two condenser lenses which also control the brightness of the beam, passes the condenser aperture and “hits” the sample surface. The electrons that are elastically scattered consist the transmitted beams, which pass through the objective lens. The objective lens forms the image display and the following apertures, the objective and selected area aperture are used to choose of the elastically scattered electrons that will form the image of the microscope. Finally, the beam goes to the magnifying system that is consisted of three lenses, the first and second intermediate lenses which control the magnification of the image and the projector lens. The formed image is shown either on a fluorescent screen or in monitor or both and is printed on a photographic film.

![Figure 9. Transmission electron microscope with all of its components](image)

4.2.2 Operation

The operation of TEM requires an ultra high vacuum and a high voltage. The first step is to find the electron beam, so the lights of the room must be turned off. Through a sequence of buttons and adjustments of focus and brightness of the beam, we can adjust the settings of the microscope so that by shifting the sample holder find the thin area of the sample. Then tilting of the sample begins by rotating the holder. This is a way to observe as much areas as we can, so we can obtain as much information.
Different types of images are obtained in TEM, using the apertures properly and the different types of electrons. As a result, diffraction patterns are shown because of the scattered electrons. If the unscattered beam is selected, we obtain the Bright Field Image. Dark Field Images are attained if diffracted beams are selected by the objective aperture. Also in TEM, analysis is done with EDX (Energy Dispersive X-ray), EELS (Electron Energy Loss Spectrum), EFTEM (Energy Filtered Transmission Electron Microscopy), etc data.

In transmission microscopy, we can actually see the specimen’s structure and its atomic columns, thus compositional and crystallographic information is attained. However, it is a very expensive technique, expertise is needed and the sample preparation phase is too difficult so that very thin samples are achieved.

4.2.3 Sample Preparation

The first step is to decide whether the sample is useful to be observed and in which view, plan or cross-section. Due to the strong interaction between electrons and matter, the specimens have to be rather thin, less than 100nm. This is achieved with several methods, depending on the material. In general, mechanical thinning is used to thin and polish the sample. Then it is glued with epoxy glue on a really small and round holder. Whereas TEM data come from the edges of a hole in the centre of the specimen, in sample preparation, the hole is created by the method of ion thinning. Ion thinning is a method where a specimen is irradiated with beams of Ar ions (usually), and after a period of time a hole is created. To minimize the damage created during focus ion beam milling, the embedded sample can first be coated with a metal deposition layer [5].

Consequently, sample preparation is a precise and a severe procedure, which may affects the results of the microscopic analysis and study.

4.2.4. Main difficulties in the exploitation of TEM

Transmission Microscopy provides several types of images, as reported above. The diffraction patterns show dots, regions or circles originating from the sample area illuminated by the electron beam that depend on the material's structure. Monocrystals show distinguished dots in diffraction patterns, polycrystalline materials common centred circles and amorphous materials diffused circles. Distortions and defects are visible in bright and dark field images, but expertise is needed in order to interpret whether they are defects or artifacts. Electron or ion beam damages must be considered in TEM analysis, because of the sensibility of the sample and its really low thickness.

Additionally, there’s always the problem of calibration and alignment of the instrument. Both of them require experience and skills so the resulting images and data that emerge are reliable and free of objective astigmatism. These works have to be done in order to keep the instrument in excellent working condition.

4.2.5 Important Technological Challenges

TEM provides accurate measurements and studies in different types of materials, given that observations are in atomic scale in HRTEM. This is due to technology that reduces the errors and corrects more and more the interferences in formed images.

In order to improve the results of TEM, ultra high vacuum with no vibrations is needed, fact that emerge the production of different types of pumps such as mechanical pumps, oil diffusion pumps, ion getter pumps, cooled stage. Higher
voltage up to 3MV and small probe size were developed, and methods to assure monochromaticity and coherency of the electrons. This is a way to avoid « chromatic aberration » and «spherical aberration», the most usual errors in electron microscopy. Lastly, stability of the beam and sample position to vibrations, drift etc, are achieved.

Today’s transmission electron microscopes offer resolutions up to 0.1nm at 300kV and probe diameters up to 0.34nm. Thus, future trends include the use of ultrahigh vacuum TEM instruments for surface studies and computerized data acquisition for quantitative image analysis.

5. References


