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Review Article

Scanning Electron Microscopy

Majid Hameed Nada*

*Department of Chemistry, University of Iowa, USA

Abstract

A scanning electron microscopy (SEM) is a type of electron microscope that uses a focused beam of high-energy electrons to produce images of a sample by creating a variety of signals at the surface of the sample. The signals are generated by the electron-sample interaction. These signals provide great information about the sample such as external morphology, crystalline structure, and chemical composition. In this review paper, the history, principles, capacities, major components, typical applications, and types of SEM are illustrated in a clear and concise manner. The major components explain how the SEM works beginning from producing the electron beam by an electron gun, going through a series of lenses to control the beam, and ending with creating the SEM images by capturing the signals produced from electron-sample interaction.

Introduction

In 1935, Stylizing and Knoll from Germany invented the first Scanning Electron Microscopy (SEM).After that, in 1942, Zworykin from USA developed the first version of SEM, but SEM still had some problems relating to signal to noise ratio at this stage. In 1950, Nixon and Oatly designed the modern SEM in Cambridge. Later, Stewart and Snelling built the first commercial SEM in 1965. The computer program has been attached to the recent SEM in order to increase its analysis ability and to achieve some other problems found in the previous versions like signal-to-noise ratio [1,2].

The recent scanning electron microscope (SEM) is considered one of the most powerful analytical techniques. SEM has been used for many purposes, for example, studying surface topography, properties of component, composition, and crystallography. SEM has been used a lot in topographic imaging researches and chemical composition studies of a sample [1,2].

Principles and Capacities of SEM

Scanning electron microscopy is a machine that is used to characterize objects with size between 1 micron to 1 nanometer. The basic idea of the SEM is the use of a focused beam of electrons. By using the beam of high-energy electrons, SEM can reveal levels of detail and complexity that are inaccessible by using light microscopy. In addition, SEM has the ability to magnify objects from about 10 times up to 300,000 times with high resolution [1,3]. SEM can produce images with high resolution at high magnification. Comparing to the light microscopy, the high resolution in SEM is about 10 nm while a typical light microscopy can produce images with the best resolution about 200 nm. Moreover, SEM has depth of field, which is the height of a sample that appears in focus in an image, approximately 300 times more than the light microscopy. This feature can be used to obtain great topographical information [1-5].

SEM works by creating an electron beam with high energy produced by an electron gun. The electron beam will be controlled by a series of lenses and apertures. When the electron beam arrives to the surface of the specimen, electron-sample interaction will occur and several types of signals will be generated. Different types of detectors to produce a SEM image can detect these signals. SEM machine operates at a high vacuum level to avoid the contamination. Overall, the way in which the SEM works can be compared to a person stands alone in a dark room and uses a fine beamed torch to scan all of objects on a wall systematically side-to-side by moving the torch up and down along the wall and observing images by eyes. After that, the person can build up an image of the objects by using the memory. In the same manner, an electron beam is used in SEM instead of the torch, and the electron beam scans the specimen systematically to create an image. Detectors are used to capture the signals instead of eyes, and a camera is used as memory to produce the SEM image [1,2,4,5].

The Major Components of SEM

Before explaining how the SEM works, we should know the main parts of SEM and their function. SEM contains five major parts that work together to analyze a sample. Each one of these five components has its specific function in the SEM system (Figure1).

***Corresponding author:** Majid Hameed Nada, Department of Chemistry, University of Iowa, USA, Tel: 319-471-5475; E-mail: majidhameed50@yahoo.com

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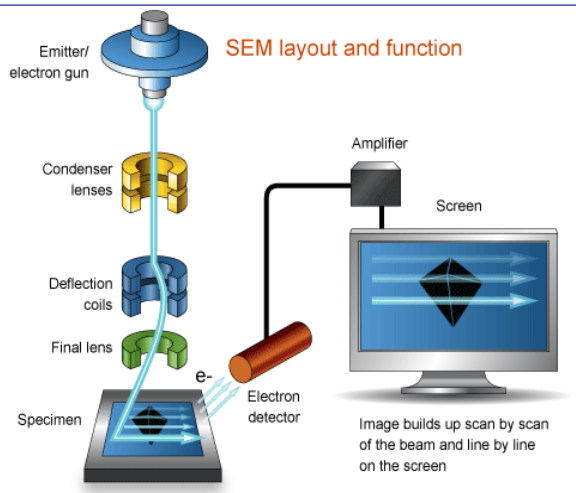

Figure 1: shows the layout of major components of SEM like electron gun, lenses, and detectors. In addition to their position in SEM [2].

Electron gun

Unlike other optical microscopy that uses light, SEM uses an electron gun. In SEM, an electron gun is located at the very top or bottom position of the SEM. Electron gun produces a steady and strong electron stream, which is necessary for operating SEM system because the resolution of SEM image depends on the stability of the electron beam when it interacts with the specimen. Two types of electron guns are used in SEM. The first one is called a thermionic gun in which thermal energy will be applied to a filament to generate a strong electron beam toward the specimen. Another type is a field emission gun that uses an electrical field to pull out electrons from associated atoms with it [1,2,6,4]. The main point of SEM operation is to produce an electron beam by using electron gun. What happens exactly is that the electron source has the filament (emitter) which is made from Lanthanum Hexaboride (LaB6) crystal, tungsten crystal for (field emission gun FEG), and tungsten wire. The filament is placed in Wenhnelt Cylinder that controls the electron beam [2,6,7] (Figure 2)

In a thermal emission system, heating will be applied to the filament to excite electrons off the filament. The filament current control knob will control the heating system of the filament because there is a point called the saturation point in which an increasing in the temperature will destroy the filament [2] (Figure 3).

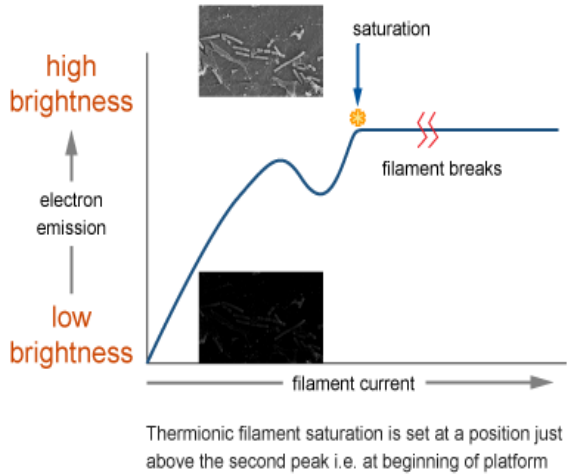

Figure 3: Explains how any increase of temperature after the saturation point of the filament will destroy the filament. In the figure above, the current of filament has been gone because the high temperature destroys the filament; therefore, the current become linear means there is no current [2].

The idea is that increasing the temperature will increase the temperature of the filament and then increase the number of emitted electrons. However, this process has a limit point in which, if the temperature increases, the number of emitted electron will not increase, but the filament temperature will still increase, which will destroy the filament; therefore, the filament current control knob is used to control the heating in SEM [2,6]. After the electron beam is generated, an anode placed under the exit of the electron gun system will attract the beam [2] (Figure 2c).

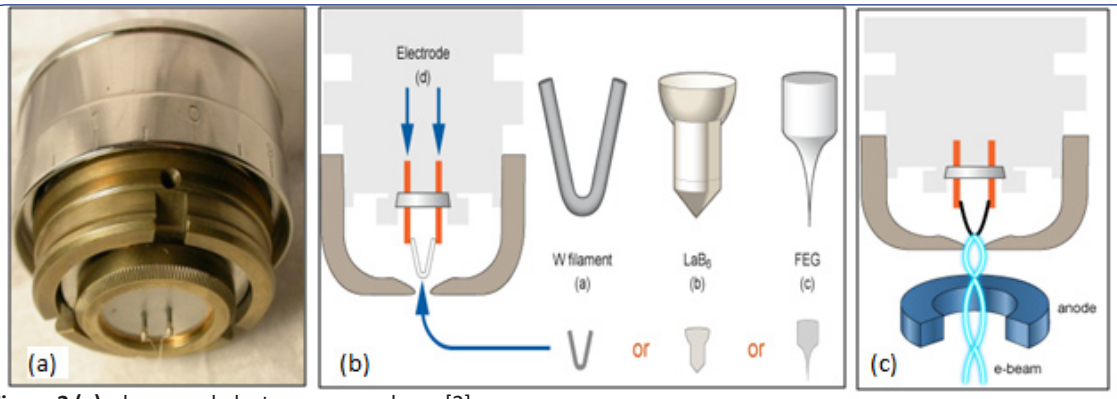

Figure 2 (a): shows real electron gun producer [2].

(b): Shows the key components of the electron gun like the filament and its types (could be LaB6, FEG, or W filament) and Wenhnelt Cylinder of the electron gun [2].

(c): Shows how the components of the electron producer work to produce the electron [2].

Lenses

SEM uses lenses to correct the path of the electron beam and direct it toward the specimen. Two types of lenses are used in the SEM, and both of them should be presented at SEM to focus the electron beam because they work together for different purposes.

An electromagnetic lens

(Condenser lens) comes after the electron gun. It is made from a coil of wire with current flows in it to produce a magnetic field at right angle, which has the ability to make the electron beam in a suitable shape to pass through the hole of the coil wire toward the next lenses [2,6,4] (Figure 4).

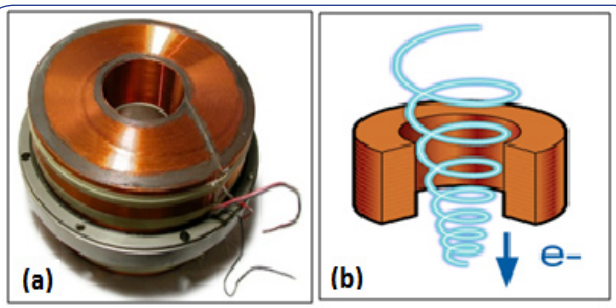

Figure 4 (a): Shows the real electromagnetic lenses [2].

(b): Shows how electromagnetic works to direct the electron beam (the blue color) when it passes through it [2].

After the electron beam passes through the exit of electron gun, it will pass through the first set of lenses called "condenser lens" [2,6,4]. In general, the motion of electron beam can be controlled by a magnetic field; therefore, passing a direct current through a coil of electric wire will form the magnetic field. This field will be used in the condenser lens to control the intensity of the electron beam, which makes the size of the beam's cross-over spot narrower than the input beam. The idea is that the first convergence of the electron beam passed through condenser lenses will be at different heights, the loser the beam is to the lens the smaller the diameter, and the farther away it is to the lens the larger the diameter (Figure 5a). Another type of lenses is *focus lens* (objective lens), it works to change the path of the electron beam and direct it to an interesting direction. It is made from ferromagnetic materials that can bend the path of the electron beam by generating a magnetic field [2,6,4]. This type of lens represents the second set of lenses called "objective lens" that are used in SEM to focus the electron beam on the specimen. This is a very important part of the SEM because it will determine the final diameter of the electron beam, and by determining the diameter of the electron beam, the produced image can be different, the narrower beam the more depth of focus, and the larger beam angle the smaller depth of focus [1,2,6] (Figure5b).

Between the two sets of lenses exactly above the objective lens, there is an *aperture* is made from a metal to stop any electron that is off-axis or off-energy from going down to the objective lens, and it helps to narrow the beam because it has four different sizes of holes [1,6,4,7] (Figure 6).

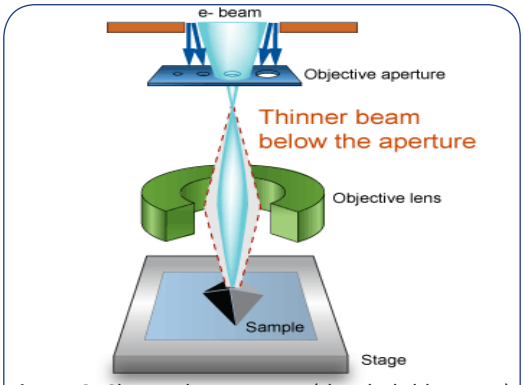

Figure 6: Shows the aperture (the dark blue part) it has four holes, which can be used to determine to diameter of the electron beam, depend on which hole is used the produced beam could be narrow or wide [2].

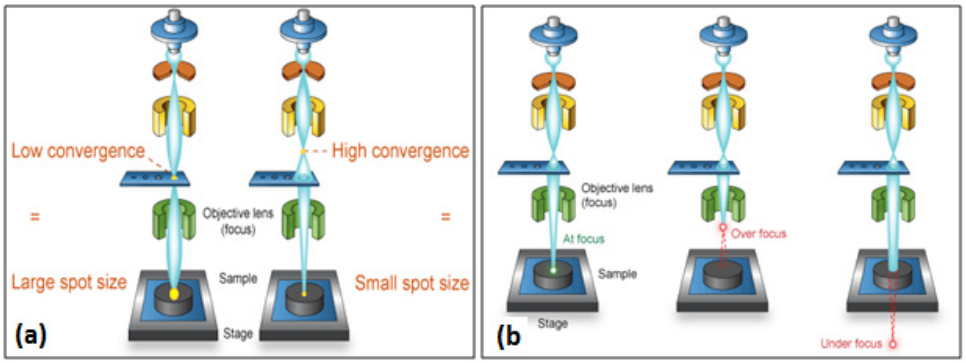

Figure 5 (a): shows how the condenser lens works. When the electron beam (the blue line) passes through the condenser lenses (the yellow part), it will be directed to the next lenses set. In addition, it will control the diameter for the beam [2].

(b) shows how the objective lens works. The object lenses (the green part) determines the focus point (three different location could be for the produced beam from this set) for the electron beam passes to the sample [2].

Sample chamber

When researchers want to place the specimen in the SEM, they will put them in the sample chamber. The role of the sample chamber is to hold the specimen during the analysis procedure. In addition, it is used to rotate the sample into different angles in order to scan the whole area of the specimen [1,6]. Sample chamber must be stable and isolated from any vibration because SEM is very sensitive to vibrations, and any kind of vibrations could lead to noise in the SEM image because it will change the direction for the electron beam and the focus point on the surface of the specimen [1,6,4,5]. That means the results will not be accurate. Some samples cannot be used in the SEM without doing some sample preparation. For example, if the sample has some volatile components such as water, they must be dried from these components before the sample is entered to the sample chamber [2]. In addition, non-conducting sample must be coated with some conducting layer such as Iridium to help to produce the secondary electrons to improve the quality of the image [2].

Detector

Four types of detectors are involved the SEM. When the electron beam hits the specimen, it will interact with the specimen. The result of the interaction between the electron beam and the specimen will be four major components secondary electrons, backscattered electrons, X-rays, and visible light [3] (Figure 7a). Each one of these four types gives different information about the specimen, but the main key on which the SEM depends it is the secondary electrons. The electron beam, which will hit the specimen, is called the primary beam. This beam will interact with the specimen in a shape like a teardrop sometime called a teardrop shaped reaction vessel in which all the scattering events of the four types of components are taking place [1,6] (Figure 7b). The resolution and the number of signals will depend on the reaction vessel; large reaction vessels give more signals, while small reaction vessels give better resolution. The topography, atomic density of the specimen, and the energy of the primary electron beam will control the volume of the reaction vessel. That means if we have a sample with low density and electron beam with high energy, the reaction vessel will be larger because the electron beam will penetrate deeper into the sample [1,4] (Figure 7b). For better understanding, we need to know the roles of the four scattered events above in SEM system and how they are formed.

Visible light

Because of some specimen's molecules has the ability to emit light photons when they are exposed to an electron beam, the visible light will be produced in this case. Some information about the compound and the structure of the specimen can be obtained from this event. Cathode luminescence is used as a visible light detector in some SEM [2, 8,6,4,5].

Backscattered electrons

When the primary electron beam hits the surface of the specimen and cannot go through the specimen; therefore, it will be reflected back to produce a backscattered electrons with high energy level approximately the same as the gun energy. This event will provide great information about the chemical component information of the sample in addition to some information relates to the topographical analysis [1,6,4,5]. For backscattered electron, the detector will be the same as for the secondary electron detectors

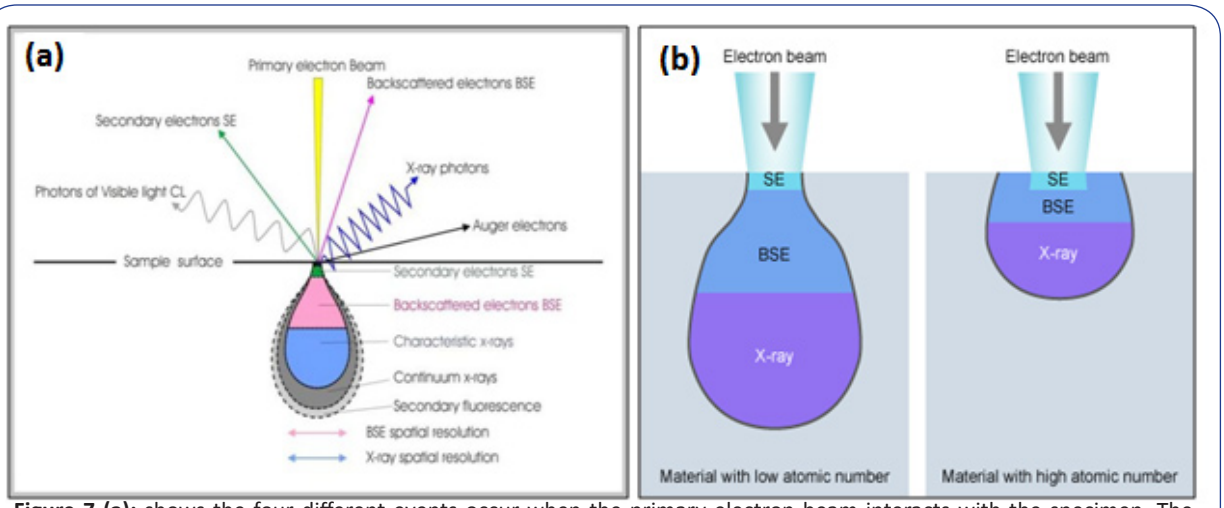

Figure 7 (a): shows the four different events occur when the primary electron beam interacts with the specimen. The secondary electron, backscattered electron, visible light, and X-ray photos all these are results from the surface of sample when the electron beam hits it.

(b) Explains how the atomic density of the specimen effects on the reaction vessel volume. In the left, the materials with low density allow for deeper interaction between the beam and the surface of the materials. However, in the right side, the high-density materials will be less interaction with the beam [2].

(will be discussed in the next section) with one exception: there is no positive charge needed to attract the electron. This detector will be a solid-state diode, and it will be placed around the optic axis [2,6,5] (Figure 8).

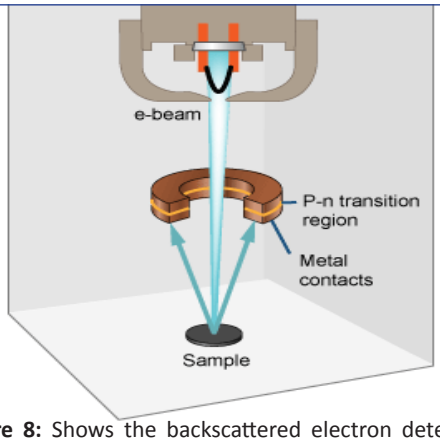

Figure 8: Shows the backscattered electron detector as a solid-state diode around the optical axis to attract the scattered electron from sample's surface with high-energy level [2].

X-rays

When the primary electron beam hits the sample some electrons will be removed from some orbits in the sample, these electrons will form X-rays. Obtaining X-rays will be helpful to get elemental information about the specimen [1,2,6]. The energy dispersive spectrometer (EDS) or wavelength dispersive spectrometer (WDS) are used as X-rays detectors [6,4].

Secondary electrons

This is the main key for the SEM. When the primary electron beam hit the surface of the specimen, some electrons will be dislodged from the surface with a low energy level. Secondary electrons can be generated just from the electron in the surface of the specimen; the electrons in the deep area of the sample cannot create a secondary electron. The real topographical information can be obtained by secondary electrons imaging mode. The principle of SEM work depends on secondary electron events to analyze the topographical information, which is the goal for SEM, of the specimen [1,2,8,6,4]. Therefore, using a suitable detector to detect secondary electrons is very important because the SEM image is generated by these electrons and any loss in these electron will give less accurate images. Secondary electrons have a low energy level; therefore, they will be attracted by a ring around the detector with +200V called "Faraday Cup" [2,6]. When the secondary electrons pass through the ring, they will be accelerated a scintillator (fluorescent substance) with 10KV applied on it. Light will be generated after the secondary electrons hit the scintillator. Then, by using a light guide, the light will be directed to a photo-multiplier tube (PMT), which will increase and amplify the original signal (Figure 9). Then, the light will be converted to electrons to produce electric signals. These signals will be converted to an image by an amplifier to project the specimen's image on a monitor. The image will provide great topographical information about the specimen [1,2].

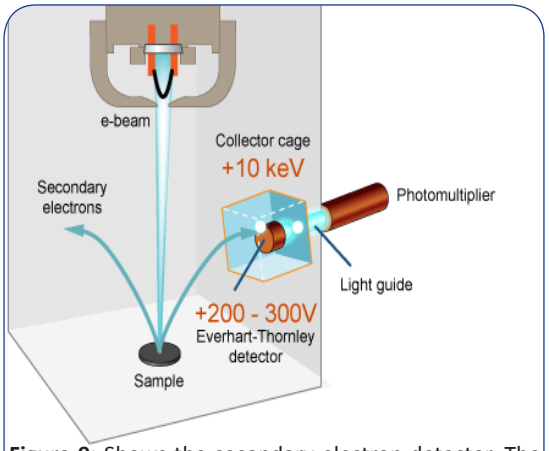

Figure 9: Shows the secondary electron detector. The secondary electron is scattered from the sample's surface with low-energy level, then it will be attracted by a positive charge at the beginning of PMT detector [2].

Vacuum chamber

SEM must be operated under a vacuum because the electron beam must be stable and without any interference. Operating SEM without vacuum chamber will lead to make the electron beam contaminate with other interferences from the air. These interferences could block the electron beam or distort the specimen's surface by interacting with it, which gives incorrect results about the sample [2,6,4].

Typical Applications of SEM

SEM is used in all scientific fields such as archaeology, engineering, medicine, industry, technology, and art. SEM is used whenever information about the surface or near-surface area of a sample is required [2]. However, there are some limitations of SEM. For example, samples of SEM must be solid and dry; living cells, soft bodies, and tissues cannot be used unit some sample preparation done on them. In addition, all samples must be stable under the high vacuum system of SEM because the vacuum system can destroy some unstable samples [3]. By using the secondary electron events, SEM can be used for morphological investigations of organic and inorganic specimens. Secondary electrons provide great information about the structure of the specimen with a resolution down to the nanometer range [8,6,5]. Moreover, using backscattered electrons gives compositional images for the sample which are used to know the chemical components and bonding differences in the specimen. Determining the chemical composition also can be done in SEM by using X-rays event from the specimen, andopt electronic behaviors are another study can be done by using the visible light in SEM [2,9]. There are many researches done in different field by using SEM technique. In this section, I will show two studies has been done by using the SEM technique. For instance, studying the antibacterial activity of extracts from *Ocimum basilicum* plants, commonly known as basil, on microorganisms (bacteria) has been done by using SEM [10]. The effect of the extracts from *O. basilicum* with different solvents such as methanol, chloroform, and acetone was studied

by taking SEM images and see the morphological changes in the bacterial cell wall. The size appears to be shrinking [11] (Figure10). Furthermore, here was obvious damages in the bacterial cell wall when methanol extracts applied [10] (Figure 11).

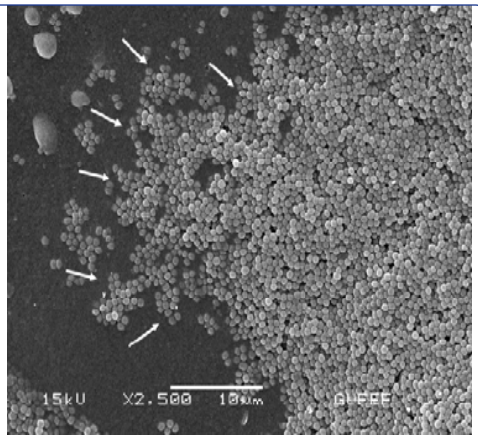

Figure 10: Shows SEM image for the region of inhibition zone (arrows) of *S. aureus,* which is kind of the microorganisms used in this study, appears to be shrinking after treatment with methanol extracts of *O.basilicum*

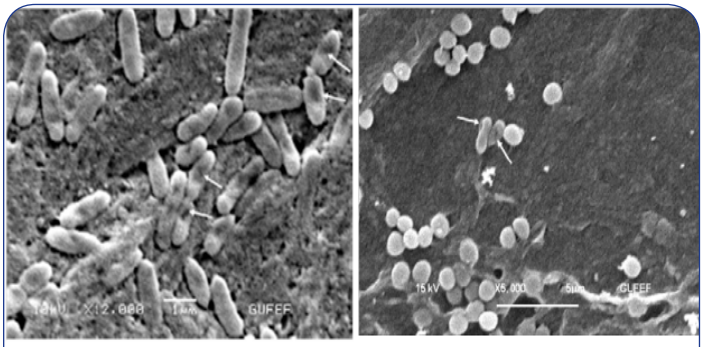

Figure 11(a): Scanning electron microscope image of damaged *P. aeruginosa* cells (arrows), kind of bacteria used in this study after treatment with methanol extracts of *O. basilicum* plant [10].

(b) Scanning electron microscope image of damaged *S. aureus* cells (arrows), kind of bacteria used in this study after treatment with methanol extracts of *O. Basilicum* [10].

Another study is to determine the gunshot residue (GSR) for a person who has handled, loaded, or discharged the fire arm. The main technique in this research was voltammetric technique coupled with SEM and X-rays detector (EDX), which has been explained above in this paper, SEM/EDX is used to confirm the gunshot residue (GSR) by observing Ba, Sb, and Pb elements in

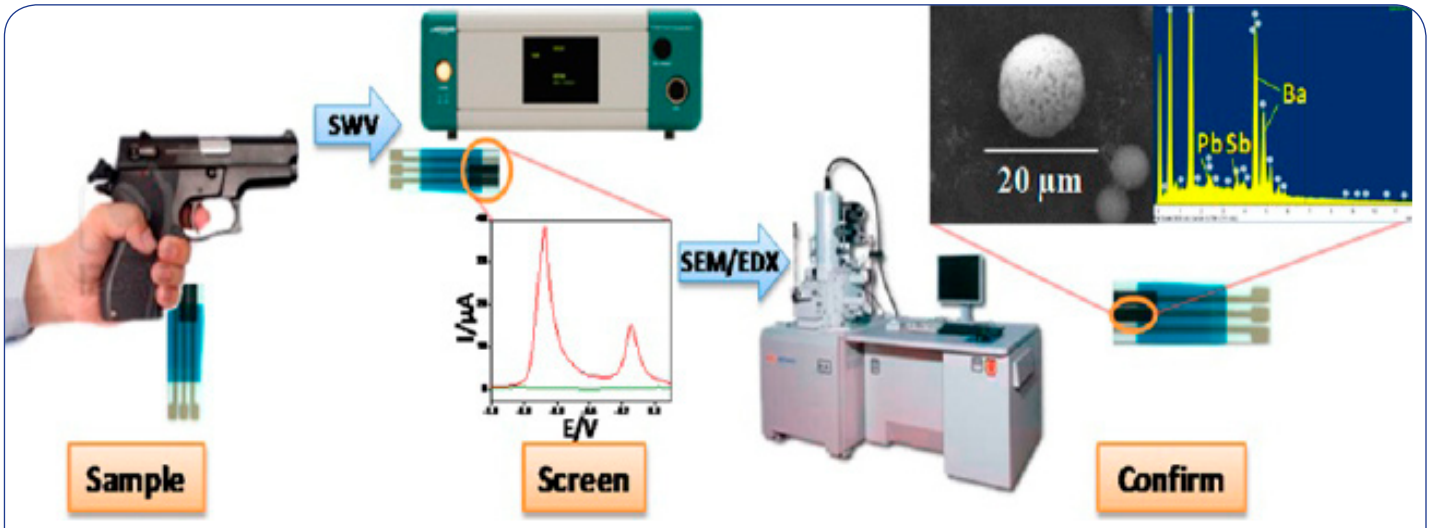

Figure 12: Explains the gunshot residue detection sequences involving voltammetric analysis, the first step to detect the elements such as Pb, Ba, and Sb, which indicate to be GSR, then, the same samples will be used with SEM/EDX to confirm the result. The SEM gives an image for the residue elements, and EDX screen shows the found elements as a peak signal [11].

the SEM screen [2] (Figure 12). By using Carbon screen-printed electrodes (CSPEs) to collect samples from different location on the suspect's body, the samples should be prepared to be suitable with SEM chamber. The carbon tape on the electrode will collect the residues from the body by using a stubbing mode of sample collection. It was a successful way to confirm the GSR and discriminate between a person who has any contact with the firearm and who has no contact with it (Figure 12). The residuals for a person who had any contact with the firearm will appear as a shepherd "cracked shell" shape (Figure13). In addition, the EDX detector will give obvious peaks for Pb, Sb, and Ba elements in its screen [2] (Figure14).

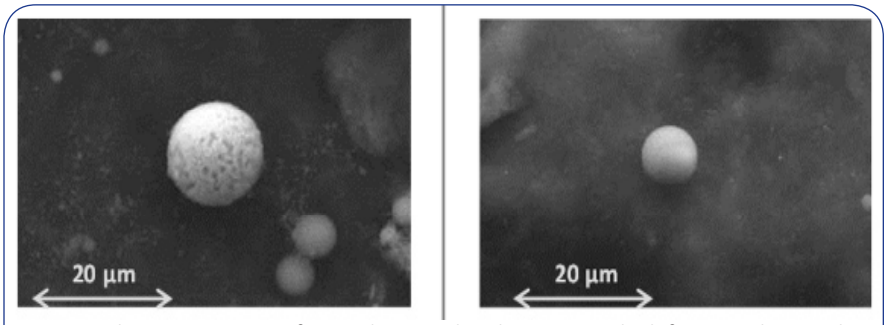

Figure 13: Shows SEM images for residues used to detect GSR. The left image shows sphere "cracked shell" shape, which indicates that there is a contact with a firearm. The right image shows normal size for normal residue, which indicates there is no contact with any firearm. These images are matched with standard image for residues indicate GSR [11].

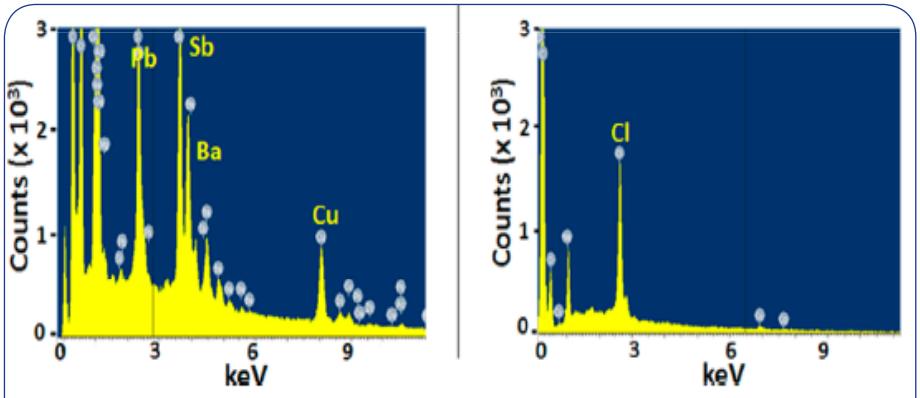

Figure 14: Obvious peaks for Pb, Sb, and Ba elements in its screen.

Types of SEM

Conventional (High Vacuum) scanning electron microscopy

In this type of SEM, sample preparation is required. That means the sample must be dry and conductive. Therefore, non-conductive samples must be coated with a thin layer of metal by using a technique called sputtering. In addition, the sample must have the ability to stand under the high vacuum condition, which is required for this type of SEM [1,2].

Environmental scanning electron microscopy (ESEM)

Sample preparation is not needed in this type of SEM. Samples with their natural states can be run by using ESEM. Nonconductive and biological materials can be analyzed by ESEM without the coating process, which is required in conventional SEM. In some cases, the coating process may make the analysis more difficult, especially for X-ray analysis, and the thin layer, which is used in coating, may conceal some of the surface features. Therefore, ESEM can be used to study the sample without doing the sample preparation [2,12].

Three dimensional scanning electron microscopy (3D SEM)

SEMs do not have the ability to produce 3D images naturally. Nevertheless, by using the eucenric tilt capability of the sample sage with especial software installed in the 3D SEM system, 3D images can be produced. The basic idea is that sets of stereoscopic images will be collected by using the eucentric tilt capability in the sample stage. After that, the 3D SEM software will determine symmetric points on the collected images which correspond to the same points on the sample surface to create the three dimensional image. 3D SEM can be used to study the defects on the surface and residues or stains on metals, ceramics, polymers, and glasses. In addition, 3D SEM is used to study the effect of different treatments on human skin [13].

Transmission scanning electron microscopy (TSEM)

TSEM is a combination between transmission electron microscopy (TEM) and SEM. In TSEM, the transmitted electron beam will be collected by using a transmission detector underneath the sample. Because of the electron-sample interaction, the energetic and angular distribution of the incident electron beam will be changed by elastic and inelastic scattering events, and a TSEM image can be generated by using these changes. Materials with low Z, polymers, biological samples, and nano particles can be characterized by using TSEM [2].

Conclusion

SEM is one of most powerful analytical techniques; it has been used in most of scientific fields such in medical, environmental, industrial, and forensic fields. SEM has the ability to take 3D image of the specimen through x-y-z directions with high resolution about 10nm [2,3]. The resolution in SEM depends on the width of electron beam and the interaction between the electron beam and the specimen's surface; the more focus beam with smaller width and large interaction volume with specimen, the highest resolution image [2,3]. However, SEM is so expensive machine, and it is very sensitive for any kind of vibrations [3]. In addition, not all samples can be measured with SEM. Some samples must be conductive, dry and solid [2,9]. All these requirements make some limitations of using SEM in some studies.

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