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Structure of BF male and Female Leaf by SEM.

Physics

KEYWORDS:

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1. INTRODUCTION

The investigations of different materials and objects in the material science and in other fields and technologies require the use of scanning electron microscope (SEM). Electron microscopy is claiming more and more in process of observation of small objects. SEM is indispensable when observation of objects with the size less then visible light wave ($\lambda 0<5*10-7 m$) is requiring. As example it can be shown the use of SEMs in chemistry, material physics, biology and nanotechnology. Electron impacting a solid is slowed down largely through "inelastic" collisions with outer shell electrons in the atoms of the solid. These inelastic interactions are of three main types:

1. With bound orbital electrons, such as ionization or band transitions, including x-ray and Auger emission,
2. Plasmon's, which are collective oscillation in weakly bound or delocalized electrons. This Plasmon's may be transverse surface waves (a few ev) or volume Plasmon's, (10-30ev),
3. Phonons which are vibrations of the crystal lattice and result in heating of the sample (mostly <2ev)

Electron microscopy

Electron Microscopy (EM) can be defined as a specialized field of science that employs the electron microscope as a tool and uses a beam of electrons to form an image of a specimen In contrast to light microscopy (LM) which uses visible light as a source of illumination and optical (glass) lenses to magnify specimens in the range between approximately 10 to 1,000 times their original size, EM is operated in the vacuum and focuses the electron beam and magnifies images with the help of electromagnetic lenses. The electron microscope takes advantage of the much shorter wavelength of the electron (e.g., λ = 0.005 nm at an accelerating voltage of 50 kV) when compared to the wavelengths of visible light (λ = 400 nm to 700 nm). When the accelerating voltage is increased in EM, the wavelength decreases and resolution decreases. In other words, increasing the velocity of electrons results in a shorter wavelength and increased resolving power.

Research development of electron microscopes began in the 1920s. Under the guidance of Max Knoll,Ernst Ruska began work on the development of electron lenses at the Technical University of Berlin,Germany, in 1928 His work was fundamental for the subsequent creation of an electron microscope in that these lenses were needed to channel the electrons of the beam. The first functional transmission electron microscope was developed in the early 1930s by Ruska who constructed a two stage electron microscope with three magnetic lenses, condenser, objective, and projector. Early electron microscopic studies were primarily focused on the study of the optical behaviour of electron beams under various conditions. Thus, no biological applications were initially envisioned. However, it soon became clear that the superior magnifying power of an electron microscope could be applied to the study of the structure of various specimens, including those from plants, animals, microorganisms, and viruses. EM is considered today by many scientists as one of the most significant and useful developments for the ultra structural investigation of specimens in the life sciences as well as in physics and material science Because of the broad applications of the electron microscope to the fields of biology, medicine, and material science, Ruska received the prestigious Nobel Prize in Physics in 1986 for his fundamental work in electron optics and for the design of the first electron microscope.

The two basic types of electron microscopes are the scanning electron microscope and the transmission electron microscope. Although both types were invented within the same decade (the scanning electron microscope was invented by Manfred von Ardenne in 1938]), they differ fundamentally in their uses. In brief, the scanning electron microscope generates an image with the help of secondary electrons that gives the viewer the impression of three dimensions, while the transmission electron microscope projects electrons through an ultrathin slice of the specimen and produces a two dimensional image. There is also a third, less used type of electron microscope, the scanning transmission electron microscope which has features of both and uses a scanning electron beam to penetrate thin specimens. Depending on the instrument used, specimens can be magnified roughly between 10 and 100,000 times in scanning electron microscopes and between 500 to 500,000 times in transmission electron microscopes. Extreme high magnifications above 200,000 are rarely used by biologists. However; the relatively high magnification range of both basic types of EM allows investigators to detect in specimens much greater detail than in those examined by LM. This makes electron microscopes extremely valuable tools for the ultra structural examination of any kind of object, but in particular for specimens of very small size.

2. METHOD

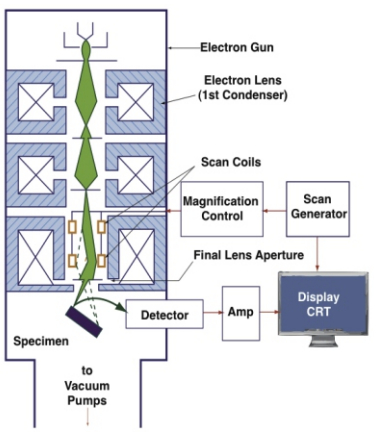

Figure 1: Cross section of a generic SEM(model no.3700N)

Interaction as individual events entail relatively small energy in comparison the energy of the beam electrons so we may make a reasonable approximation that the beam electron decelerates as function of the distance traveled (in a sample of constant composition. Scanning Electron Microscopy (SEM) is a powerful method for the investigation of surface structures of molecules. This technique provides a large depth of field, which means, the area of the sample that can be viewed in focus at the same time is actually quite large [3]. SEM has also the advantage that the range of magnification is relatively wide allowing the investigator to easily focus in on an area of interest on a specimen that was initially scanned at a lower magnification. Furthermore, the three-dimensional appearing images may be more appealing to the human eye than the two-dimensional images obtained with a transmission electron microscope. Therefore, an investigator may find it easier to interpret

SEM images. Finally, the number of steps involved for preparing specimens for SEM investigation is lower and thus the entire process is less time consuming than the preparation of samples for investigation with a transmission electron microscope. However, SEM specimen preparation harbors various risk factors that can easily distort the integrity and ultra structure of the molecules. The basic steps involved in SEM sample preparation include surface cleaning, stabilizing the sample with a fixative, rinsing, dehydrating, drying, mounting the specimen on a metal holder, and coating the sample with a layer of a material that is electrically conductive Because each of these steps are crucial and will affect the outcome of the study, they will all be described individually in more detail below.

2.1 Cleaning the surface of the specimen

The proper cleaning of the surface of the sample is important because the surface can contain a variety of unwanted deposits, such as dust, silt, and detritus, media components, or other contaminants, depending on the source of the biological material and the experiment that may have been conducted prior to SEM specimen preparation. If these deposits are not removed prior to fixation, this material may get permanently fixed to the specimen surface and it will be almost impossible to remove later. Robinson et al. as well as Bozzola and Russell suggested that the specimen should be quickly rinsed in a suitable buffered solution of the appropriate pH, temperature, and osmotic strength close to the milieu from which the specimen has been removed. Perhaps the best way to clean the surface of molecules is to carefully rinse them three times for 10 min in 0.1 M cacodylic acid buffer (pH 7.3) at room temperature.

2.2 Stabilizing the specimen

There are various ways of stabilizing a biological specimen. Stabilization is typically done with fixatives. Fixation can be achieved, for example, by perfusion and microinjection, immersions, or with vapours using various fixatives including aldehydes, osmium tetroxide, tannic acid, or thiocarbohydrazide . For mollicutes, a simple chemical fixation by immersing the specimen in a 1.5% glutaraldehyde solution prepared in 0.1 M cacodylic acid buffer (pH 7.3) and incubated at 4oC overnight appears in most cases sufficient. The use of a postfixative (e.g., osmium) has been described to improve bulk conductivity of the specimen but does not necessarily provide a better stabilization of mollicutes prepared for SEM.

2.3 Rinsing the specimen

After the fixation step, samples must be rinsed in order to remove the excess fixative. Perhaps the best protocol for mollicutes is to rinse the specimens in 0.1 M cacodylic acid buffer (pH 7.3), starting with one time for 10 min, and then three times for 20 min at 4 oC. Some of the samples can be +stored in this EM buffer for several months because the buffer contains arsenic which inhibits the growth of unwanted microorganisms in the specimen container. However, I strongly suggest changing the cacodylic acid buffer at least monthly if the samples are to be stored in this buffer for longer periods of time.

2.4 Dehydrating the specimen

The dehydration process of a biological sample needs to be done very carefully. It is typically performed with either a graded series of acetone or ethanol. The protocol that proved most suitable for dehydrating mollicutes for SEM includes the immersion of the s specimens in 50% acetone for 5 min, 70% acetone for 10 min, 80% acetone for 10 min, 90% acetone for 15 min, and 100% acetone (dried with CaCl2) twice for 20 min at 4 oC. This process allows the water in the samples to be slowly exchanged through liquids with lower surface tensions

2.5 Drying the specimen

The scanning electron microscope (like the transmission electron microscope) operates with a vacuum. Thus, the specimens must be dry or the sample will be destroyed in the electron microscope chamber. Many electron microscopists consider a procedure called the Critical Point Drying (CPD) as the gold standard for SEM specimen drying. I would recommend this procedure and have performed it numerous times using liquid carbon dioxide as the transitional fluid. Carbon dioxide is removed after its transition from the liquid to the gas phase at the critical point, and the specimen is dried without structural damage. It is very important to exactly follow the instructions of the manufacturer of the CPD apparatus or the ultrastructure of the sample may be significantly altered.

In some of my experiments, I tried a specimen drying process called Simple Desiccation (SD). This technique is essentially a simple air-drying procedure after fixation, rinsing, and dehydration of the mollicutes. SD is not easy to do and there is the risk that specimens collapse, flatten, or shrink uncontrollable under these conditions Although SD is faster and cheaper, this method is like "walking on a tight rope." For the EM novice investigating mollicutes, I would suggest the safer method of CPD.

2.6 Mounting the specimen

After the mollicutes have been cleaned, fixed, rinsed, dehydrated, and dried using an appropriate protocol such as the one outlined above, specimens must be mounted on a holder that can be inserted into the scanning electron microscope. Samples are typically mounted on metallic (aluminum) stubs using a double-sticky tape. It is important that the investigator first decides on the best orientation of the specimen on the mounting stub before attaching it. A re-orientation proves difficult and can result in significant damage to the sample.

2.7 Coating the specimen

The idea of coating the specimen is to increase its conductivity in the scanning electron microscope and to prevent the build-up of high voltage charges on the specimen by conducting the charge to ground [1]. Typically, specimens are coated with a thin layer of approximately 20 nm to 30 nm of a conductive metal (e.g., gold, gold-palladium, or platinum). For the coating of mollicutes, I have used gold and gold palladium, and found both suitable. To guarantee best results (i.e., to achieve an even layer of metal coating over the sample), I recommend carefully following the instructions that come with the sputter coater apparatus. After all the steps described above have been performed, the investigator is ready to view the mollicutes in the scanning electron microscope. This is the moment when the mycoplasmologist will find out whether or not the multi-step sample preparation for SEM was successful. It is important to remember that each step has to be performed to perfection in order to achieve SEM images that can be interpreted without the influence of artifacts caused by specimen handling.

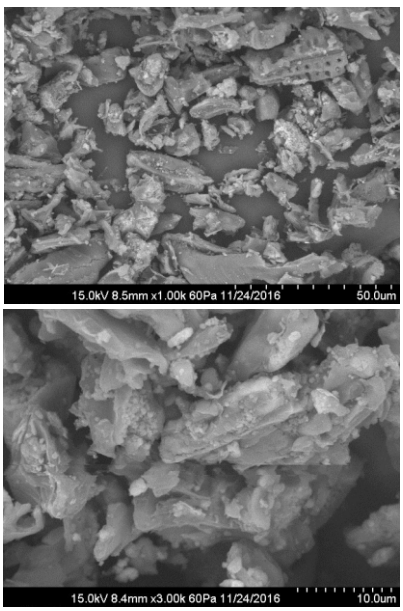

Figure 1: BF MALE at 50 µm and 10 µm

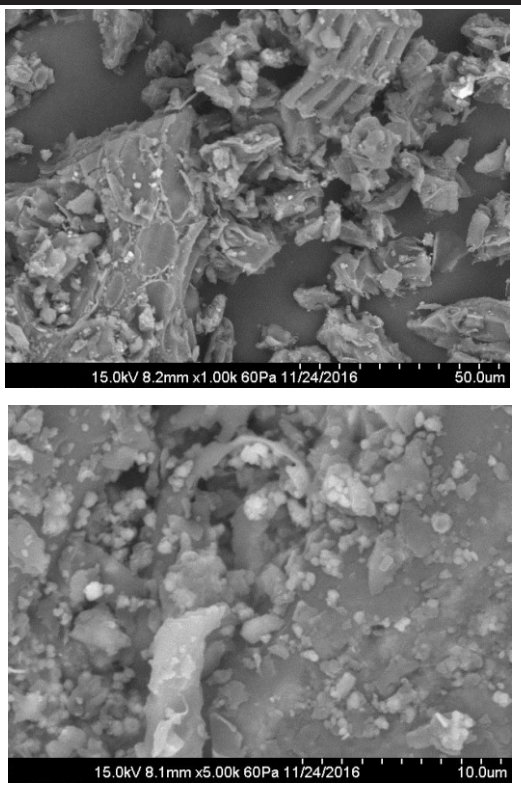

Figure 2: BF FEMALE at 50 µm and 10 µm

3. Result and Discussion

As has been mentioned in the introduction, scanning electron microscopy is essential to assessments of causes of damage due to fracture. Microscopic analysis has made it possible to distinguish between material defects and processing defects. Thus, considerable legal consequences may result with regard to liability for damage. From the above figures, one can clearly see flaky particles dominating the image. Various types of separation occur in brittle and tough material. These images, as described above, are characteristics of an illite's microstructure. In the case of trans angular brittle fracture, crystallites are split without. If the material is tough, sliding processes occur in crystallographically preferred plans; micro voids and cavities form themselves. The cavities widen, any metal remaining in between propagates and narrow edges are formed. The resulting microstructure is called dimple fracture.

Slag inclusion in welding seams, e.g., cannot be clearly identified using a light-optical microscope whereas owing to the fact that the conductivity of metal differs considerably from that of slag, contrasts become clearly visible when an electron microscope is used. In connection with x-ray analysis such slag inclusion can be clearly identified. Heavy expenses occur to insurance companies both in the commercial and in the private field for the evaluation of damage due to corrosion of water pipes etc. defective connections between different metals can be located.

4. Conclusions

The detailed structural examination of molecules as well as the characterization of the interaction .The between these microbes and host cells would not be possible without the electron microscope. The reason is that the preparation of specimens for SEM includes a series of steps; a single mistake in one of these steps will affect all remaining steps, and thus the outcome of the entire study. Some hexagonal shapes can also be seen in the upper left corner of the image.

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