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Department of Mechanical Engineering

THESIS OF FINAL STUDY

To obtain the diploma of:

License

In Mechanical Engineering

Option: MATERIALS ENGINEERING

Theme

Scanning Electron Microscopy (SEM)

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Thanks

I would like to thank everyone who supported me in completing this work.

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Dedications

I dedicate this work to my dear mother, for her unconditional support, for her love, for all the sacrifices made and for all her precious advice, for all her assistance and her presence in my life.

My father, who was always by my side and proud of me and the result I achieved after years of sacrifice and deprivation and for helping me move forward in life.

This work is the result of his noble values, education and constant support.

All my family, my sisters, my friends

Contents

ملخص

الماسح المجهري الإلكتروني (SEM) هو تقنية تصوير قوية تستخدم لفحص سطح العينات بتفصيل كبير. إنه يعمل عن طريق مسح حزمة مركزة من الإلكترونات عبر سطح العينة، وتوليد إشارات توفر معلومات حول تكوين العينة وهيكلها وتضاريسها. يتفاعل شعاع الإلكترون مع العينة، وينتج إلكترونات ثانوية، وإلكترونات مبعثرة، وأشعة سينية مميزة. يستخدم الإلكترونات الثانوية لإنشاء صور عالية الدقة، بينما توفر الإلكترونات المتناثرة تباينًا تركيبيًا. يمكن أن يحقق SEM تكبيرات تتراوح من بضع مرات إلى مئات الآلاف من المرات، اعتمادًا على قدرات الجهاز. لمنع آثار الشحن، يجب أن تكون العينة موصلة للكهرباء أو مغلفة بطبقة رقيقة من مادة موصلة. يستخدم SEM على نطاق واسع في مختلف المجالات مثل علوم المواد وتكنولوجيا النانو والبيولوجيا والجيولوجيا والطب الشرعي لتحليل السطح المفصل. إنه يتيح فحص ميزات السطح والتشكيل والتوزيع الأولي بدقة استثنائية. يسمح SEM أيضًا برسم الخرائط الأولية، والتحليل الطيفي للأشعة السينية المشتتة للطاقة (EDS)، وحيود التشتت الخلفي للإلكترون (EBSD) لمزيد من التوصيف. بشكل عام، يعد SEM أداة أساسية للبحث العلمي والتحليل على المقياس الدقيق والنانوي

ABSTRACT

Scanning Electron Microscopy (SEM) is a powerful imaging technique used to examine the surface of specimens in great detail. It works by scanning a focused beam of electrons across the sample's surface, generating signals that provide information about the sample's composition, structure, and topography. The electron beam interacts with the sample, producing secondary electrons, backscattered electrons, and characteristic X-rays. Secondary electrons are used to create high-resolution images, while backscattered electrons provide compositional contrast. SEM can achieve magnifications ranging from a few times to hundreds of thousands of times, depending on the instrument's capabilities. To prevent charging effects, the sample must be conductive or coated with a thin layer of conductive material. SEM is widely used in various fields such as materials science, nanotechnology, biology, geology, and forensics for detailed surface analysis. It enables the examination of surface features, morphology, and elemental distribution with exceptional resolution. SEM also allows for elemental mapping, energy-dispersive X-ray spectroscopy (EDS), and electron backscatter diffraction (EBSD) for further characterization. Overall, SEM is an essential tool for scientific research and analysis at the micro- and nanoscale.

RESUME

La microscopie électronique à balayage (SEM) est une technique d'imagerie puissante utilisée pour examiner la surface des spécimens de manière très détaillée. Il fonctionne en balayant un faisceau focalisé d'électrons sur la surface de l'échantillon, générant des signaux qui fournissent des informations sur la composition, la structure et la topographie de l'échantillon. Le faisceau d'électrons interagit avec l'échantillon, produisant des électrons secondaires, des électrons rétrodiffusés et des rayons X caractéristiques. Les électrons secondaires sont utilisés pour créer des images haute résolution, tandis que les électrons rétrodiffusés fournissent un contraste de composition. Le SEM peut atteindre des grossissements allant de quelques fois à des centaines de milliers de fois, selon les capacités de l'instrument. Pour éviter les effets de charge, l'échantillon doit être conducteur ou recouvert d'une fine couche de matériau conducteur. Le SEM est largement utilisé dans divers domaines tels que la science des matériaux, la nanotechnologie, la biologie, la géologie et la criminalistique pour l'analyse détaillée des surfaces. Il permet l'examen des caractéristiques de surface, de la morphologie et de la distribution élémentaire avec une résolution exceptionnelle. Le SEM permet également la cartographie élémentaire, la spectroscopie à rayons X à dispersion d'énergie (EDS) et la diffraction par rétrodiffusion d'électrons (EBSD) pour une caractérisation plus poussée. Dans l'ensemble, le SEM est un outil essentiel pour la recherche scientifique et l'analyse à l'échelle micro et nanométrique.

LIST OF FIGURES

INTRODUCTION

1.1 Types of Microscopy

Optical microscopy (OM) and scanning electron microscopy (SEM) are the two main forms of microscopy. The first is the oldest and has been in use for the past 200 years as a straightforward gadget with constrained functionality. It is also known as light microscopy. Following are some characteristics and qualities where OM and SEM diverge: In contrast to SEM, the main working principle in OM is the light. which rely on the emission of electrons. While compound OM has two lenses, simple OM only has one lens. To enlarge the pictures, the lenses must bend light. Modern OM's magnification only reaches 400–1000 times its original sizes, which is incredibly low when compared to SEM, whose magnification may reach 300,000x. OM can analyze both solid things and live cells. Small solid pieces and a very small number of small organics can be seen, though. This is because the OM can only analyze thin and tiny samples. SEM, on the other hand, offers a gray-scale picture field that is more detailed. Therefore, SEM is reported to be more costly than OM and more difficult to maintain. Images created with OM display the studied object's actual colors. optical microscopy with compound lenses is shown. The stereo zoom optical microscope, petro graphic microscope, and automated optical microscope are the three commercially available forms of OM [1].

Scanning electron microscopy (SEM), which is also known as SEM analysis or SEM technique, is employed globally in many academic fields. On a nanometer to micrometer (nm–m) scale, it can be regarded as an efficient method for the analysis of organic and inorganic materials. With a high magnification that may reach 300,000x and even 1000000 (in some recent versions), SEM creates extremely exact pictures of a variety of materials. Together with SEM, Energy Dispersive X-ray Spectroscopy (EDS) produces qualitative and semi-quantitative results. The analysis is done through SEM equipment [2].

1.2 Definition of SEM

The word microscope is derived from Greek micros (small) and skopeo (look at). The scanning electron microscope (SEM), like any other microscope, is primarily used to magnify microscopic details or objects that are otherwise undetectable to the human eye. This is accomplished by employing an electron beam rather than the light that optical light microscopes utilize to create pictures. Images are acquired by scanning a high-energy electron beam across

the sample surface. the scanning electron microscope name. Due to its shorter wavelength, compared to optical light, electrons can distinguish finer characteristics and details of materials to a far larger extent. A contemporary SEM can resolve features as fine as 1 nm in dimension and magnify objects up to a million times their original size. Similar to this, when an electron beam interacts with a specimen, it releases x-rays with a certain energy that may be detected to identify the makeup of the object being studied. As a result, the SEM is a tool for materials characterization that offers details on the structure, composition, and surface or near-surface properties of the material. and flaws in large-scale materials. In order to expound on material qualities, it enables scientists to view surfaces at the submicron and nanoscale. It has become one of the most potent and adaptable tools, useful to both materials scientists and life scientists working in a variety of sectors [3].

1.3 History of SEM

The early 20th century saw the development of the electron microscope as a result of the limitations of the light microscope in terms of seeing tiny details of organic cells. German scientists Max Knoll and Ernst Ruska created the first electron microscope in 1931. It was a transmission electron microscope. Although it utilized a working model akin to a light microscope, a beam of electrons, Instead of using visible light, a sample's body was made to pass through it in order to create an image on a fluorescent screen. A light microscope can only achieve a resolution of 200 nm; using electrons as the imaging medium allowed for a resolution of 10 nm. The actual breakthrough was the first time in history that electrons have been used to successfully build pictures of matter. The resolution attained at the time would appear low now. Atomic resolution was attained in the following few decades thanks to advancements in accelerating voltage, lens technology, vacuum systems, electron guns, power sources, and microscope architecture as a whole. Ernst Ruska was awarded the 1986 Nobel Prize in physics for his "fundamental work in electron optics and for the design of the first electron microscope."

Max Knoll, a German scientist, proposed the idea of a scanning electron microscope in 1935.[4]. He suggested that a sample's surface may be scanned with a precisely focused electron beam to create a picture. Manfred von Ardenne, a different German scientist, elaborated on the technique's working principles and beam-specimen interactions. In 1937, he went on to create the first scanning electron microscope.

Later, in 1942, American scientists Zworykin, Hillier, and Snijder created the SEM, which had a resolution of 50 nm. The SEM was then created in 1952 at the University of Cambridge by Professor Sir Charles W. Oatley and his postgraduate student D. McMullan. Everhart and Thornley created the scintillator-based secondary electron detector in 1960. In 1965, Cambridge Scientific Instruments created the first commercial SEM under the name "Stereoscan" as a result of further technological advancements. The SEMs built in the 1960s had resolutions between 15 and 20 nm. The resolution was increased to 7 nm and 5 nm (at 1 kV), respectively, in the 1970s and 1980s.In the following two decades, resolution was improved to 3 nm and eventually to 1 nm. Currently, manufacturers advertise SEM resolutions of 0.5 nm. Despite being created later than the transmission electron microscope, the scanning electron microscope quickly gained favor for its user-friendliness, straightforward sample preparation, and capacity to produce 3-D-like images of the sample topography [3].

CHAPTER I

FUNDAMENTAL PRINCIPLES OF SEM

Fundamental Principles of SEM

The scanning electron microscope (SEM) operates based on several fundamental principles that allow for high-resolution imaging of specimens. Here are the key principles of SEM:

1 Principles of SEM Imaging

The definition of scanning electron microscopy (SEM) in the current study was given in terms of the main instrument part and a step-by-step breakdown of the SEM system. For a clear and accurate knowledge of the work technique, schematic drawings with photos of SEM components were provided. SEM variations were also given and discussed. Also, the power of the energy-dispersive spectrometer (EDS) was demonstrated, together with its historical context and SEM-compatibility. For both qualitative and quantitative analyses of any specimen, the presence of EDS capabilities with SEM equipment is crucial. SEM can only generate information about the specimen's surface topography in the absence of EDS. The two most potent SEM picture characteristics are introduced [5].

The Electron Materials Interaction

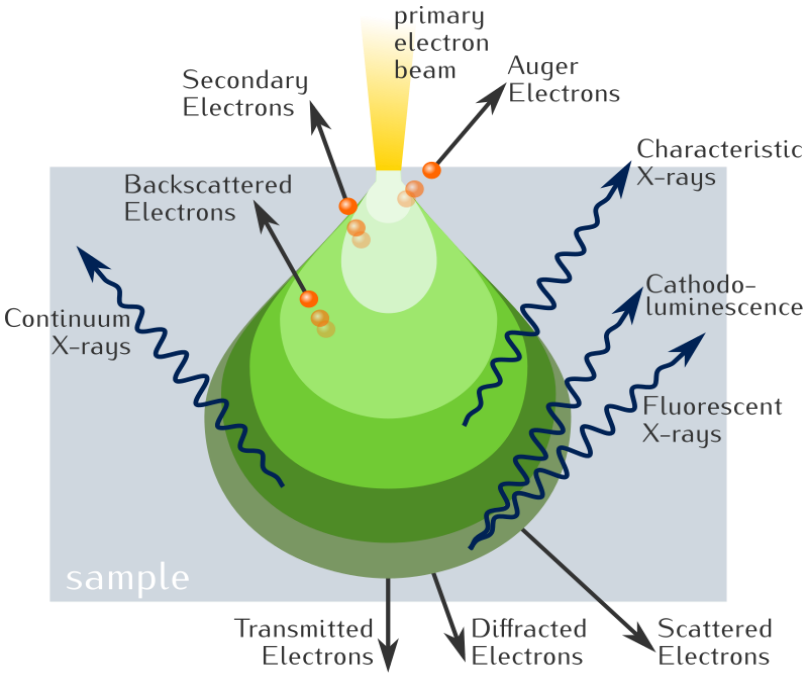

Figure I.1:Electron–matter interaction [6].

1.1 Electron beam

Focused ion beam (FIB) microscopy is widely used in conjunction with scanning electron microscopy (SEM) for high precision site-specific lamella sample preparation for transmission electron microscopy (TEM), 2D piezoelectric materials production, nano-milling, and 3D FIB tomography. Nevertheless, soft materials face difficulties due to their higher sensitivity to ion/electron irradiation and lower thermal conductivity. To produce high-quality FIB-prepared samples and milling processes for 3D tomography, a better knowledge of the damage mechanisms involved in the FIB-SEM system must be researched. Prior study discovered that the electron beam is the primary cause of chemical damage in soft materials. when compared to Researchers have studied electron beam (e-beam) damage in perovskite materials, nanomaterials, and polymers and have had some success in understanding the damage features in these materials and the underlying mechanisms. Due to the wide variability of soft materials, few solid conclusions have been drawn to explain the damage mechanisms prescriptively enough for practical applications in the FIB-SEM. The substance used in this investigation was Embed 812 epoxy resin, a common polymer for embedding biological samples for TEM and FIB-SEM 3D tomography. Bubbling and chemical degradation caused by electron beam damage frequently impede high-quality sample preparation or grinding of biomaterials. Minimal research has been conducted on the chemical alterations caused by electron beam irradiation of epoxy resin embedding polymers. In this study, we investigated the effects of beam voltage and dose on low-energy electron generated damage to 100 nm thick epoxy resin thin films. A scanning electron microscope was used to construct the damaged spots on the resin thin film in a 33-scanning pattern. The irradiated areas were later analyzed using near-edge X-ray absorption fine structure (NEXAFS) in a scanning transmission X-ray microscope (STXM) at the Canadian Light Source's 10ID1 beamline. (CLS). We discovered that even at low beam current, the electron beam can trigger chemical changes and carbon contamination on the resin thin film. We also discovered that, at the same electron dose, the degree of electron beam damage is dependent on the quantity of inelastic scattering occurring within the electron beam [7].

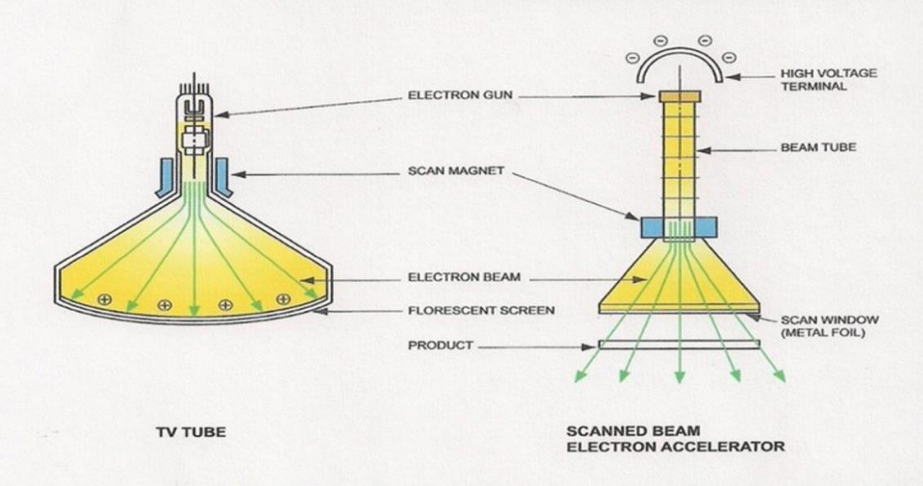

Figure I.2:Image courtesy of IBA Industrial [8].

1.2 Auger Electron Spectroscopy (AES)

The average depth of analysis for an AES measurement is approximately 5 nm. Spatial distribution information is obtained by scanning the micro focused electron beam across the sample surface. Depth distribution information is obtained by combining AES measurements with ion milling to characterize a thin film structure. The information AES provides about surface layers or thin film structures is important for many industrial and research applications where surface or thin film composition plays a critical role in performance including: nanomaterials, photovoltaics, catalysis, corrosion, adhesion, semiconductor devices and packaging, magnetic media, display technology, and thin film coatings used for numerous applications. AES is accomplished by exciting a sample's surface with a finely focused electron beam which causes Auger electrons to be emitted from the surface. An electron energy analyzer is used to measure the energy of the emitted Auger electrons. From the kinetic energy and intensity of an Auger peak, the elemental identity and quantity of a detected element can be determined. In some cases, chemical state information is available from the measured peak position and observed peak shape. Physical Electronics AES instruments function in a manner analogous to SEM/EDS instruments that use a finely focused electron beam to create SEM images for sample viewing and point spectra or images for compositional analysis. In contrast to SEM/EDS which has a typical analysis depth of 1-3 m, AES is a surface analysis technique with a typical analysis depth of less than 5 nm and is therefore better suited for the compositional analysis of ultra-thin layers and nanoscale sample features [9].

1.3 Characteristic X-ray (EDX)

X-ray Photoelectron Spectroscopy (XPS) is a surface sensitive technique frequently relied upon for studying the composition and short-range chemical bonding environments of the outermost atomic layers of solid materials. Sensitivity to the top ~5 nm of a surface makes XPS ideal for a variety of applications where the materials' surface composition and chemistry are extremely critical. Applications where XPS is commonly used include catalysts, oxidation state and oxide thickness of metals and alloys, bonding and adhesion issues, surface cleanliness and contamination, corrosion, discolorations, polymer surface functionalization, and composition vs. depth of optical and other thin films [10].

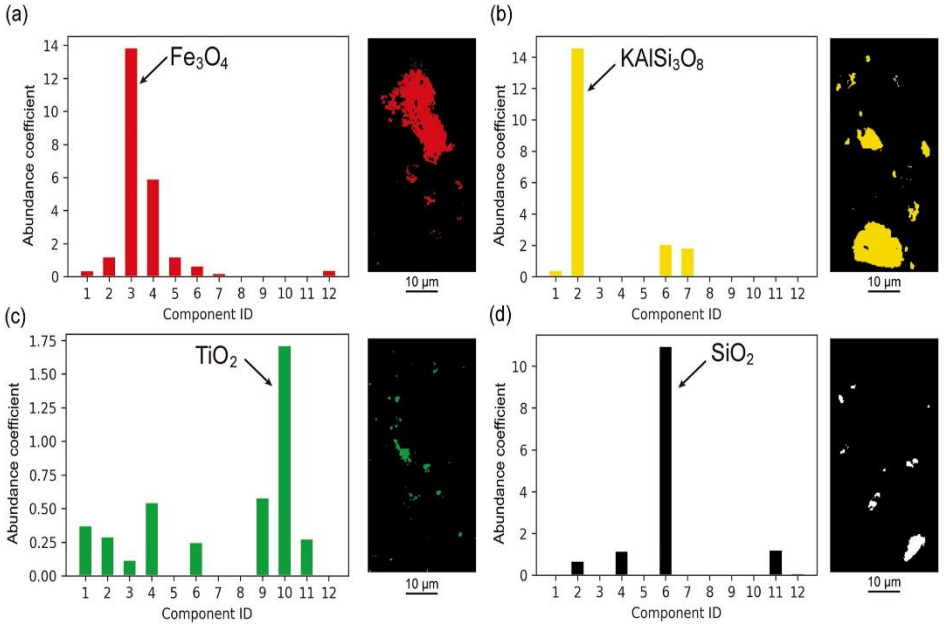

Figure I.3: Bar charts of abundance coefficients and pixel distributions showing the importance of NMF components for each cluster [11].

1.4 Cathodoluminescence (CL)

An insulating solid material (such as quartz or calcite) can be visualized as having a valence band and a conduction band with an intervening band gap (forbidden gap). EM-cathodoluminescence (SEM-CL) is the emission of photons of characteristic wavelengths from a material that is under high-energy electron bombardment produced in a scanning electron microscope. The nature of CL in a material is a complex function of composition, lattice structure, and superimposed strain or damage on the structure of the material.If a

crystal is bombarded by electrons with sufficient energy, electrons from the lower-energy valence band are promoted to the higher-energy conduction band. Most of the photons fall in the visible portion of the electromagnetic spectrum (wavelengths of 400-700 nm) with some falling in the ultraviolet (UV) and infrared (IR) portions of the electromagnetic spectrum. When the energetic electrons attempt to return to the ground state valence band, they may be temporarily trapped (on the scale of microseconds) by intrinsic (structural defects) and/or extrinsic (impurities) traps [12].

- Photon energy <EGap
- Recombination with impurity
- eA0: electron in CB – hole of neutral acceptor
- D0h: electron of neutral donnor – hole in VB
- DAP: electron of neutral donnor – hole of neutral acceptor

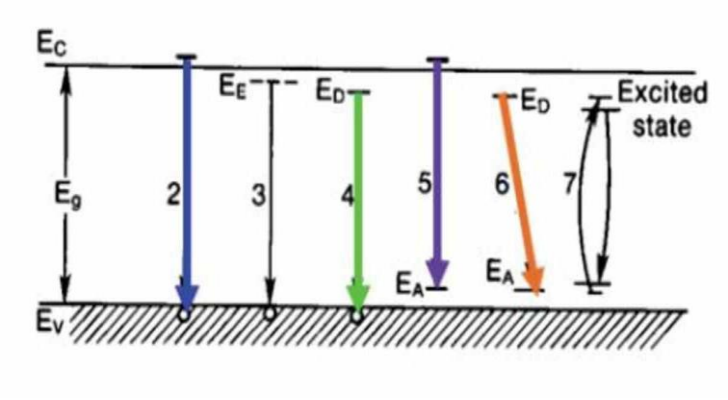

Figure I.4:SEM-CathodoLuminescence

1.5 The advantages of SEM-CL

1.5.1 High sensitivity:

SEM-CL analysis offers the advantage of indicating variations in chemical composition at a lower level than techniques based on X-ray analysis. Therefore, it is advantageous over conventional SEM-EDX and SEM-WDX analyses for detecting trace rare-earth elements. However, CL is so sensitive to a wide range of factors like temperature, chemical composition, defects, strain, and crystal structure that its interpretation is complex [12].

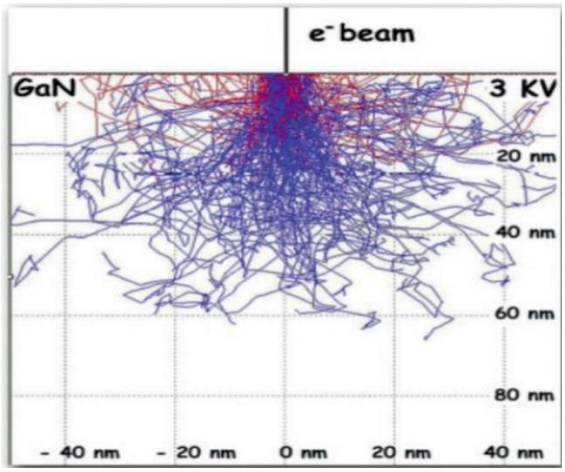

Figure I.5:Monte-Carlo simulation of electron paths

1.5.2 High spatial resolution:

The volume inside the specimen in which interactions occur depends on several factors the greater the angle of incidence for the electron beam, the smaller the volume. Higher-atomic-number materials absorb electrons. Higher accelerating voltages for the electron beam penetrate further into the sample and generate larger interaction volumes [12].

1.6 Secondary Electrons (SE)

Unlike BSEs, SEs originate from the surface or near-surface regions of the sample. Secondary electrons are very useful for the inspection of the topography of the sample's surface. The SE detector is placed at an angle at the side of the electron chamber to increase the efficiency of detecting secondary electrons. BSEs and SEs are the most commonly used signals by SEM users for imaging. Because researchers often seek different kinds of data, having multiple detectors makes SEM a very versatile tool that can provide valuable information for many different applications [13].

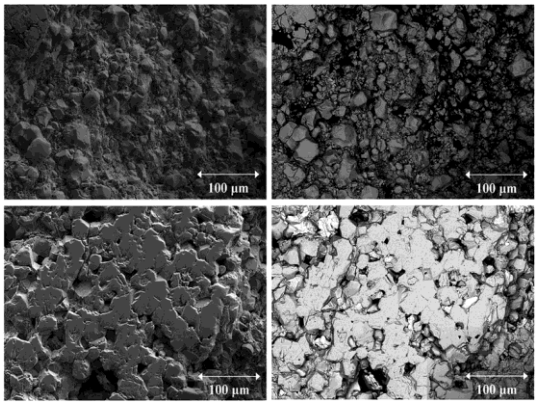

Figure I.6: SEM images [14].

1.7 Backscattered Electrons (SE)

A new Combined System for High-Efficiency Detection of Secondary and Backscattered Electrons (CSSBE) in the ESEM consists of three detectors: an ionization SE detector, an improved scintillation BSE detector, and a new ionization secondary electron detector with an electrostatic separator (ISEDS). High-efficiency detection of the ISEDS is demonstrated by imaging a low-atomic-number sample under a reduced beam energy of 5 KV, very low beam currents of up to 0.2 PA, and a gas pressure of hundreds of Pa. The ISEDS optimizes conditions for electron-gas ionization phenomena in the ESEM to achieve a strongly amplified signal from the secondary electrons with a minimal contribution from backscattered and beam electrons [15].

1.8 Continuum X-ray (Bremsstrahlung)

The continuous spectrum is due to bremsstrahlung, while the sharp peaks are characteristic X-rays associated with the atoms in the target. For this reason, bremsstrahlung in this context is also called continuous X-rays. The shape of this continuum spectrum is approximately described by Kramer's' law [16]. Non-invasive beam monitoring tools are required to guarantee the delivered dose during ion irradiation. Therefore, the bremsstrahlung signal gives access to the deposited dose and beam energy with precision, depending on the experimental uncertainties. In addition, the bremsstrahlung spectrum shape contains information about the beam energy [17].

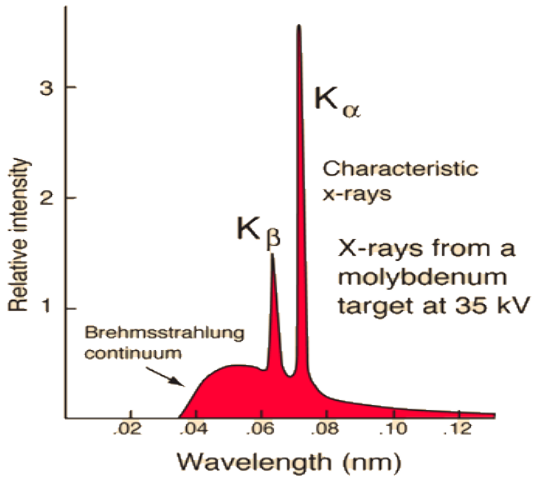

Figure I.7:Typical x-ray spectrum showing the Bremsstrahlung continuous spectrum and the characteristic x-rays [18].

When heavy elements' electrons move between their lower atomic energy levels, distinctive x-rays are released. When vacancies form in the atom's n=1 or K-shell, electrons fall from above to fill the void, producing the distinctive x-ray emission that is depicted as two sharp peaks in the illustration at left. K-alpha x-rays and K-beta x-rays are terms used to describe the x-rays generated by transitions from the n=2 to n=1 level, respectively. L x-rays are transitions to the n=2 or L-shell (n=32 is L-alpha, n=42 is L-beta, etc.). Bremsstrahlung radiation is the name for the constant x-ray distribution that serves as the foundation for the two distinct peaks at left. In order to produce X-rays, high speed electrons that have been accelerated by tens to hundreds of kilovolts of potential are typically fired at a metal target inside an X-ray tube. Electrons from the metal target's atoms' inner shells may be ejected by the bombarding electrons. Electrons falling from higher levels will quickly fill those vacancies, emitting x-rays with sharply defined frequencies connected to the difference between the atomic energy levels of the target atoms. The Bohr model allows for the prediction of the characteristic x-ray frequencies. A plot known as a "Moseley plot" was created by Moseley using measurements of the frequencies of the distinctive x-rays from a significant portion of the elements in the periodic table. Characteristic x-rays are used for the investigation of crystal structure by x-ray diffraction. Crystal lattice dimensions may be determined with the use of Bragg's law in a Bragg spectrometer [19].

1.9 Inelastic Scattering composition and bone states (EELS)

Since its origin, electron energy loss spectroscopy (EELS) has been recognized as a particularly sensitive technique for the detection of lighter elements because those atoms have favorable inelastic scattering cross sections for inner shell excitations. It was therefore understood at the outset that EELS had potential for analyzing the composition of biological specimens, which are predominantly composed of lighter atoms[20, 21]. In addition, early studies in Albert Crewe’s laboratory at the University of Chicago showed that EELS could distinguish between biological molecules, such as different amino acids and different nucleic acid bases, by analyzing details of the EELS fine structure caused by excitation of valence or core shell electrons [22, 23].Extraction of chemical information from fine structure in the valence or core-edge spectra requires consideration of the radiation sensitivity of biological compounds and the subtle differences in EELS fine structure associated with compositional variations(Figure 4a) [24].To map the distributions of biological compounds in cells therefore entails a highly efficient and precise collection of spectral data at each pixel in an image, i.e., parallel detection of the spectrum in the STEM-EELS mode(Figure 4a). shows that there are

significant differences between the valence loss spectra of frozen water and protein, which can be exploited to derive water maps. For example, a study on cry sectioned rat hepatocytes showed that it is feasible to quantify water content in different sub cellular organelles [24].Only cutting-related compression artifacts are visible in (Figure 4b) dark-field STEM picture of the frozen hydrated segment. The distribution of water and protein, as shown in (Figure 4c), was obtained by acquiring a 128 128-pixel STEM-EELS spectrum-image, deriving the single scattering distributions, and fitting reference spectra at each pixel using a least squares method. Regions containing mitochondria, cytoplasm, red blood cells, and plasma are all clearly visible. Each pixel's water content may be determined and represented as a histogram (Figure 4d), which indicates values of around 75% in the cytoplasm and about 55% in the mitochondria. Using this method, other laboratories have also determined water distributions [25, 26].

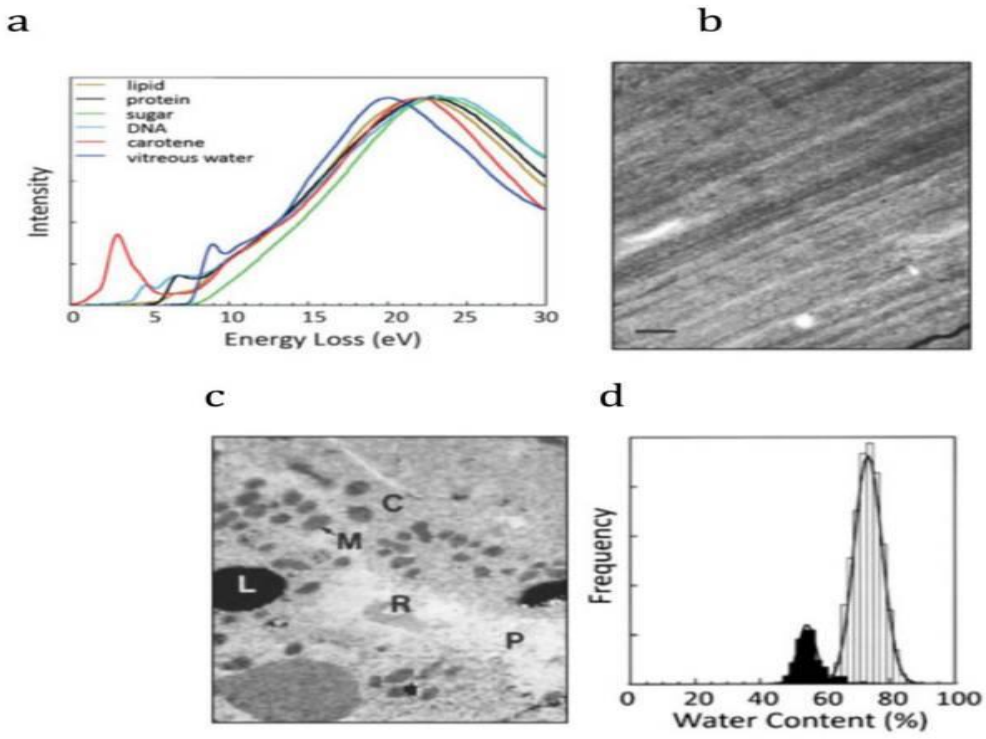

Figure I.8:STEM-EELS imaging [24].

CHAPTER II

COMPONENTS OF SEM

Components of SEM

Scanning Electron Microscopy (SEM) is a type of Electron Microscopes (EM) which is widely used to study the morphology and composition of nanostructures. In this chapter, we show you the components of scanning electron microscopy.

1 COMPONENT OF SCANNING ELECTRON MICROSCOPY

Recently, new technology has been developed that allows for higher resolution images than ever before. These microscopes are called high-resolution scanning electron microscopes. A cold field emission gun is used to create a cold beam of energy. This beam is then used to form a small-gap immersion probe-forming lens. Finally, a clean dry-pumped vacuum is used to remove the unwanted particles from the probe. Some key characteristics, including as a spatial resolution that routinely exceeds 1 nm in secondary electron mode on solid objects, characterize the performance of these microscopes. a probe current density incidental of the order of 106 A/cm^2; This means that the camera can take pictures at a range of different voltages, from 1 volt up to 30 volts[27]. Computers are used to operate SEMs in the modern day. However, the quality of an image depends on the settings you use. by the operator .This calls for a knowledge of SEM, its numerous components, and how it might be applied to provide excellent pictures and trustworthy analytical results[3]. The elements of a typical SEM are shown in (**Figure II.1)**. As it is seen, the top of the device has an electron cannon that produces an electron beam. The beam travels via the magnetic lenses which produce magnetic field to deflect the electrons to concentrate them onto the sample. Every SEM compartment requires to be in a vacuum [28].

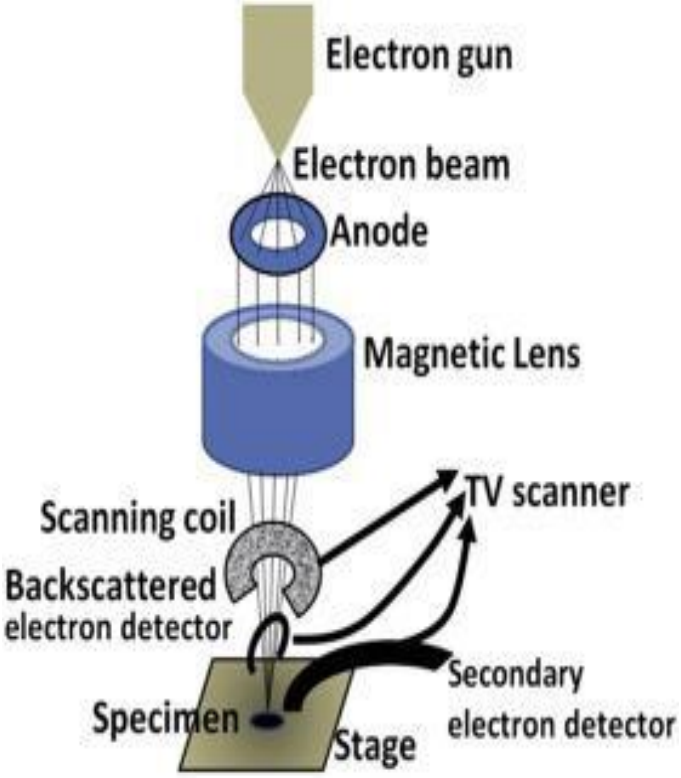

Figure II.1:Scanning electron microscope components [28].

1.1 Electron Gun

The assembly of the cathode and related electrodes known as the electron gun occupies the highest portion of the column, this is attached to a high-voltage (30–40 kV) power line from the outside, the electron cannon is situated in the uppermost portion of the electron column, as shown in a schematic depiction of the inner section of an electron column in FigureII.2. A generator of electrons with variable magnitude and acceleration, the electron gun, The electron gun's main purpose is to produce electrons, which are subsequently propelled downward through the column by a potential difference that occurs within the cannon assembly, The accelerating voltage employed determines the force with which the electrons move through the column (and eventually impact the sample), which, depending on the sort of sample being studied, might range from 2 to 30 kV, Analytical methodology, and the necessary details, In order to prevent the dispersion of released electrons [3].

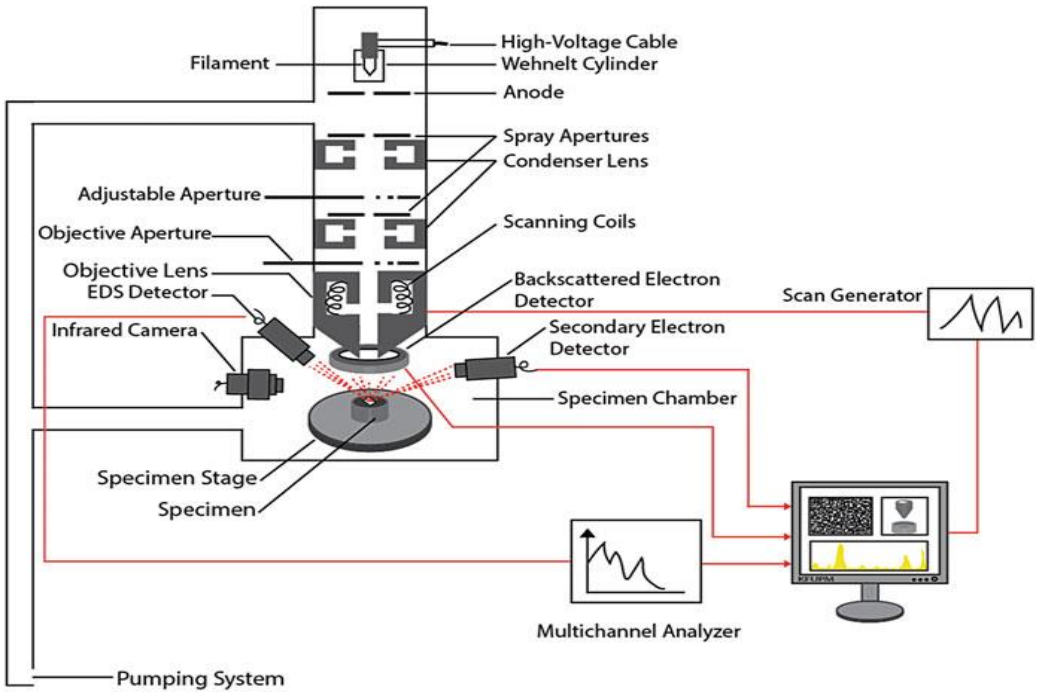

Figure II.2:diagram illustrating the design and operation of different parts found in the SEM electron column and specimen chamber [3].

1.2 Condenser Lenses

Two condenser lenses that are used after the anode cause the beam to converge and pass through a focal point [29]. A magnetic lens typically consists of two iron pole pieces that are symmetrical in rotation and a copper winding that generates a magnetic field. The electron beam can pass through a hole in the middle of the pole pieces. The two pole parts are separated by a lens-gap, when the electron beam is impacted by (focused by) the magnetic field. Adjusting the condenser lens current can change where the focus point is located. Condenser opening, generally, connects to the condenser lens, and the electron beam focus point is above the aperture. As long as the proper aperture size is selected, many of the dispersed and inhomogeneous electrons are eliminated. Considering current electron microscopes, For more electron beam control, a second condenser lens is frequently employed[30].

1.3 Apertures

Microscopes can have different apertures that let light pass through the microscope. This allows different types of objects to be seen. The apertures on the lens help to reduce and exclude unwanted electrons from the lens. The size of the beam that is seen by the scanning coils is determined by the final lens aperture. The resolution and depth of field will be partially influenced by the size of the spot on the specimen. Reducing the spot size will reduce brightness while increasing resolution and depth of field [31].

1.4 Scanning System

Deflection coils within the objective lens are used to raster the electron beam over the material to create images. The stigmator, also known as an astigmatism corrector, is a component of the objective lens that employs a magnetic field to lessen electron beam aberrations. The stigmator works to solve this issue since the electron beam, which should impact the specimen with a circular cross section, typically has an oval cross section [29].

1.4 Specimen Chamber

Controls are situated at the lowest part of the column, which is where the specimen stage is. The stage is fixed with mountings and fastened, and a goniometer controls its movement. A positive charge on the detector draws the secondary electrons from the material. For x-y-z movement, manual stage controls are located on the front side of the specimen chamber[29].

1.5 Electron Detectors

electrons are the signal that secondary electron detectors most frequently gather (Everhart–Thornley) Backscattered electrons are detected using a solid-state detector for backscattered electrons, while X-ray signals are detected using an EDS detector [29].

1.6.1 Secondary Electron Detector

Secondary electron detector. Nowadays, the majority of SEMs use an Everhart-Thornley detector. A combination of a scintillator and a secondary electron photomultiplier. The specimen's surface emits low-energy secondary electrons, which are accelerated toward the scintillator by a cylindric net around the scintillator with a voltage of one to several hundred volts. It is crucial to note that the shape of the detector causes electrons to be accelerated in a variety of directions towards the scintillator. When the electrons have passed the cylindric net, a second electrostatic field accelerates them to an energy of roughly 10 kev, and they are then further accelerated in the direction of the scintillator [32].

1.6.2 Backscattered Electrons

Backscattered electrons (BSEs) are electrons from the primary beam that have been so strongly deflected by atom collisions that their route actually leads them back up through the sample surface. They may emanate from the sample's inside. Depending on prior collisions, Primary beam energy E0 and secondary electron energies are both possible BSE energy levels [33].

1.6.3 X-Rays Radiation

Element-characteristic Inelastic incident electron scattering across the material produces X-rays. While the inner-shell electrons of the sample atoms are stimulated, Transient outer electrons generate X-rays that include distinctive elemental information. EDS (X-ray energy dispersal spectroscopy) is a technique that, Ingredients and compositional data for micro-areas can be inspected. Through line scan and mapping, EDS can offer qualitative results of components, semi-quantitative or quantitative compositions of samples, and the distribution of elements in the sample [34].

1.7 Multichannel Analyzer

The multichannel analyzer's job is to gather, classify, archive, and display the pulses that come in from the main amplifier. In actuality, an analog-to-digital converter (ADC) interfaced to a hard-wired computer memory is used to do this. To check if pulses fall within a predetermined range of acceptability and to see whether any internal events are to blame for the system being busy, pulses are first subjected to an acceptance test utilizing an upper and lower-level discriminator. The common technique of conversion, if they are accepted, is from pulse amplitude to time. To do this, a particular stretcher capacitor is charged to a voltage proportionate to the input signal's peak value, and the number of clock pulses that happen when the capacitor returns to its initial level is counted [35].

1.8 Vacuum System

To increase the mean free path of electrons and enable them to flow through the electr on column and specimen chamber without being dispersed by air molecules, the SEM must be kept under vacuum. Water vapor and organic contaminants must be evacuated out of the specimen chamber because they deposit on the surface of the specimen and interfere with the electron beam ability to see tiny surface details. Furthermore, vacuum is necessary to prevent oxidation damage to the electron source. It avoids high-voltage discharges that can cause the filament to fail between the anode and the filament. The electron column is kept at a high

vacuum level of around $\mathbf{10^{-4}}$Pa.when there is around$\mathbf{10^{-3}}$ Pa of vacuum in the specimen chamber. Both portions of the chamber the electron column and the specimen chamber can be evacuated independently of one another thanks to an isolation valve. The very high vacuum of $\mathbf{10^{-6}}$–$\mathbf{10^{-7}}$ Pa is maintained in the columns of field emissions SEM.A pump-based vacuum system is used., valves, airlocks, vacuum gauges, pipes, valves, etc. Initially, A rotational mechanical pump is used to create vacuum within the chamber

Figure II.3:of Common Rotary Pumps [3].

that pushes air out through a pipe attached to a chamber exit (**Figure II.3**). The scroll pump and the diaphragm pump are additional low vacuum pumps that might be utilized. Once the first vacuum (around 101 Pa) is set up, in order to achieve a final high vacuum ($\mathbf{10^{-3}}$- $\mathbf{10^{-4}}$ Pa) in the chamber, an oil diffusion pump is turned on. The diffusion pump has no moving parts and a rather straightforward construction. In Figure II.4, a picture of a rotary pump and a schematic for a basic vacuum system utilized in the SEM are displayed.

Instead of a diffusion pump, which might introduce oil vapor into the specimen chamber owing to potential back pressure, the oil-free turbo molecular pump is employed. Although the vacuum ranges of the turbo molecular and diffusion pumps are comparable, the turbo molecular pump produces a "cleaner" vacuum.as contrasted with a diffusion pump. This has led to a gradual replacement of diffusion pumps in the SEM. The turbo molecular pump has a more intricate construction and blades that spin at >20,000 rpm. Up to two ion getter pumps (IGP) attached to the column can be used to create an ultra-high vacuum inside the field emission SEM column. For independent venting and evacuation of the gun during filament replacement, a gun-isolation valve is incorporated. It is possible to interchange specimens without venting the room when

there is a specimen exchange airlock chamber (load lock). The specimen chamber can be vented with dry nitrogen gas instead of air to minimize the introduction of water vapor and the recontamination in the chamber and to reduce the duration of the pump down. The vacuum system is automated and has inbuilt safeguards against accidental-evacuation, power failure, stoppage of water supply, contamination of chamber due to back pressure, etc. Vacuum levels are measured using a cold cathode gauge or a Pirani gauge [3].

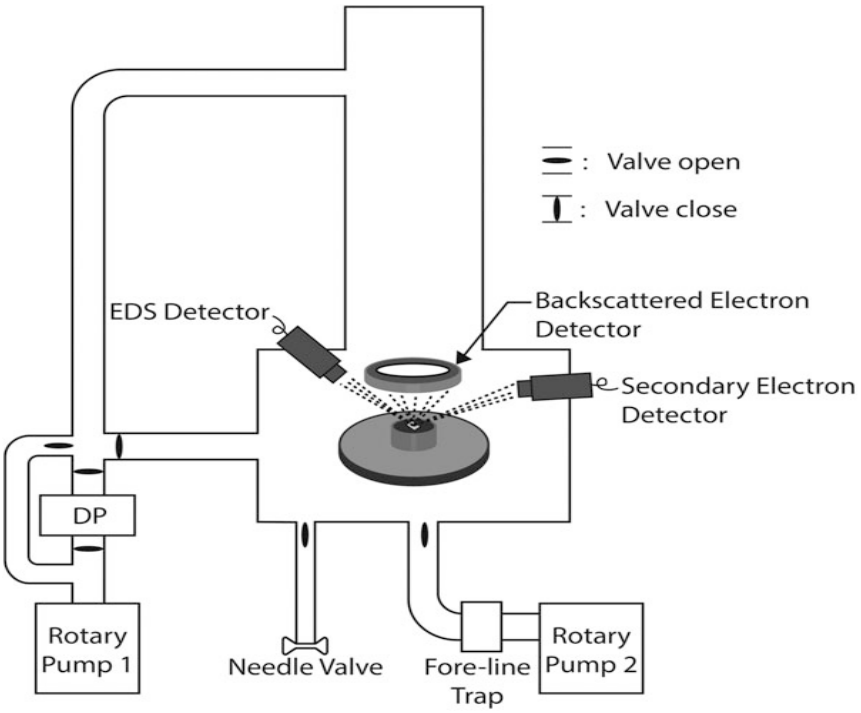

Figure II.4:A rotary pump is used to pump rough down [3].

CHAPTER III

TYPES OF SEM

TYPES OF SEM

In this chapter, we show you the types of scanning electron microscopy which is conventional scanning electron microscopy and environmental scanning electron microscopy and Low Vacuum Scanning Electron Microscopy.

1 TYPES OF SCANNING ELECTRON MICROSCOPY

The term "SEM" stands for a broad device that includes the high-vacuum traditional scanning electron microscope. (CSEM), the low-vacuum environmental scanning electron microscope (ESEM) and the environmental SEM (ESEM) (LVSEM). Broken fragments, slabs, and other concrete objects are frequently used for electron microscope observations. parts that are narrow and polished. blocks with a high polish and narrow parts, If an object is going to be analyzed using a CSEM or in "CSEM mode" with an LVSEM or ESEM. it must have a conductive covering. For inspection by the ESEM or LVSEM at low pressure, typically no coating is needed [36].

1.1 Conventional Scanning Electron Microscopy (CSEM)

In a conventional SEM (**Figure III.1**), In a high vacuum, the electron beam interacts with the specimen. (10-6 torr), whose pressure is measured in torr (1 torr = 133.32 Pascal) [37]. enabling low collision rates between electrons and the gas molecules in the chamber (high vacuum, few gas molecules in the chamber) and transmission of the input electron beam and emission of the low energy secondary electrons from the sample. This makes it possible to operate under ideal circumstances with a well-defined incident electron beam and the highest possible yield of electrons from the specimen. However, this extreme dehydration and cracking of concrete are caused by the high vacuum, directly see cracking without being affected by indirect observations. In contrast to the concrete that was sampled, the CSEM essentially creates a picture of changed concrete. Numerous times, this is unimportant. Based on their cation ratios, ASR reaction products and secondary deposits may be quickly detected. Other components, such aggregates, are not impacted by the vacuum [36].

Figure III.1:Conventional Scanning Electron Microscopy [38].

1.2 Environmental Scanning Electron Microscopy (ESEM)

The environmental scanning electron microscope (**Figure III.2**), is a novel instrument that is currently offered for sale and has the ability to establish itself as a tool for research and development across many disciplines. With this lens, objects can be examined while in a gaseous atmosphere. It has opened up new opportunities, such as the study of barriers, and liquid samples that have not been pretreated or modified. Generally speaking, reactions between the solid, liquid, and vapor, and other systems can now be examined under steady or dynamic circumstances. The standard scanning electron microscope (SEM) has several sensing modes that have been modified by the ESEM to function in the presence of gas. Additionally, it has sparked the creation of entirely novel instruments for electron microscopy, Specifically, the use of specific types of gaseous instruments linked to those created in other scientific disciplines [39].

Environmental SEM has positive and negative effects.

1.2.1 Positive effect

- Prevents or reduces exhaustion (In general, 4.6 torr is the minimal pressure needed to maintain flowing water).

- Additionally, the increased gas pressure has advantages; Ionization of the gas molecules will result in the discharge of any surface charges. This lessens the requirement for conductive coatings and results in better concrete imagery.
- Gas molecules are ionized as a consequence of impacts between the sample's released electrons and the beam electrons. This ionization will increase the strength of electron signal and this is appositive effect.

1.2.2 Negative effect

- The electron stream is scattered and defocused as a result of these impacts, which ultimately caused doubt regarding the location of the electron beam on the object.
- This is not a problem for imaging, but it is a significant issue in x-ray microanalysis because the assumed analysis point may actually not contain the element that is being evaluated in the analyzed spectrum [37].

Figure III.9:Environmental Scanning Electron Microscopy (ESEM)[40].

1.3 Low Vacuum Scanning Electron Microscopy (LVSEM)

Soft tissue can be observed using low-vacuum scanning electron microscopy (**Figure III.3**), which has been created, unlike traditional scanning electron microscopy, it can be used on materials that are wet, electrically inert, and without any preparation, which calls for firm, desiccated, and typically electrically conductive samples [41]. by obtaining secondary electron pictures of untreated, sample without conductivity. By permitting a tiny gas pressure in the

specimen chamber, this accomplishment is accomplished. Primary electrons and electrons released from the object both ionize gas molecules. The sample's charge is then dissipated with the help of these electrons. However, , the atoms' reactions with one another, These devices' specific contrast processes are created by the specimen and secondary electrons [42].

Figure III.3:Low Vacuum Scanning Electron Microscopy(LVSEM) [43].

CHAPTER IV

IMAGING WITH THE SEM

Imaging with the SEM

The scanning electron microscope is routinely used to characterize wide-ranging materials due to its ease of operation, relatively straightforward sample preparation, and simple image interpretation. This chapter describes the role of various operational parameters used during microscopy in more detail. Guidelines for the operation and upkeep of the SEM instrument are also summarized in this chapter.

1 SPECIMEN PREPARATION FOR SCANNING ELECTRON MICROSCOPY

There are many kinds of materials. The preparation of specimen related to its physical phase and conductivity. Scanning Electron Microscopy (SEM) specimen preparation is a crucial step in obtaining high-quality images and accurate data using a scanning electron microscope. The process involves several key steps to ensure that the specimen is properly mounted, preserved, and suitable for imaging under the electron beam[44].

1.1 Liquid specimen

Preparing liquid specimens for SEM imaging can be challenging due to the volatile nature of liquids and the need to immobilize them for analysis. However, there are several techniques available for the preparation of liquid specimens for SEM.

Here are a few common methods:

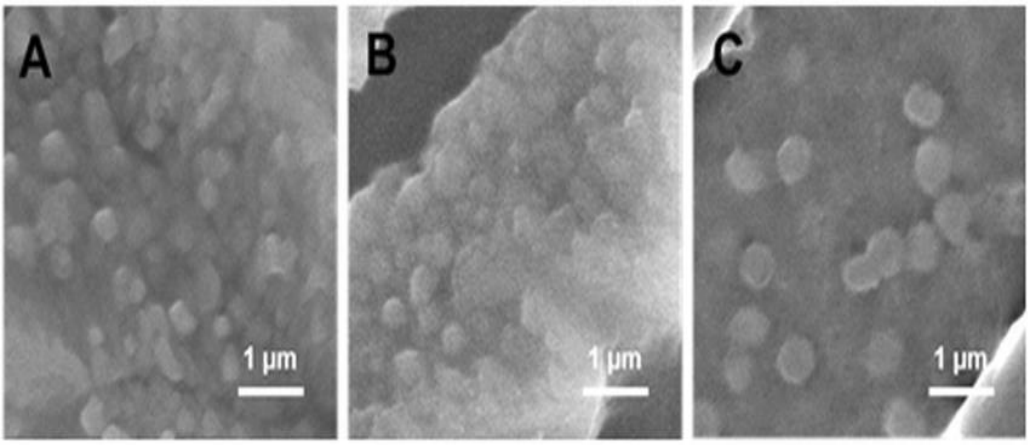

Figure IV.10:SEM images of liquid samples under deferent pH [18].

1.1.1 Freeze-Fracture:

This technique involves freezing the liquid specimen rapidly, typically using liquid nitrogen, to create a solid frozen sample. The frozen sample is then fractured, revealing the internal structures. The fractured surface can be coated with a thin layer of metal (e.g., platinum) to enhance conductivity before imaging.

1.1.2 Critical Point Drying (CPD):

CPD is a technique used to remove liquid from a specimen without causing significant structural damage. It involves replacing the liquid with a solvent that can be easily evaporated, such as liquid carbon dioxide, under carefully controlled temperature and pressure conditions. The liquid is then gradually replaced with a transitional fluid that has a lower surface tension to allow for complete drying. Finally, the transitional fluid is evaporated at a critical point, where it transitions from a liquid to a gas without passing through the liquid-vapor phase boundary. The dried specimen can then be mounted and coated for SEM imaging.

1.1.3 Air-Drying and Fixation:

Some liquid specimens can be air-dried directly onto a suitable substrate, such as a glass slide or a specialized SEM stub. This method is commonly used for samples containing solid particulates suspended in a liquid. The liquid is evaporated by leaving the sample to air-dry naturally or by using controlled heating. Fixation may be required to stabilize the sample and prevent deformation during drying.

1.1.4 Micro encapsulation:

In this technique, liquid specimens are encapsulated in a solid matrix to immobilize them for SEM imaging. The liquid is typically mixed with a polymer material, such as epoxy resin or embedding media, and allowed to cure or solidify. The resulting solid block can then be sectioned to expose the internal structures for SEM analysis[45].

1.2 Gaz specimen

Preparing gas specimens for SEM imaging is not a conventional practice since scanning electron microscopy (SEM) is primarily designed for imaging solid samples. The high vacuum environment and electron beam interaction with gases make imaging gas specimens challenging. However, there are specialized techniques that allow for the visualization of gas samples in SEM under certain conditions. Here are a couple of methods:

1.2.1 Environmental SEM (ESEM):

ESEM is a modified version of conventional SEM that allows imaging of specimens in a variable-pressure or low-vacuum environment. This technique can be used to examine hydrated or gaseous samples without the need for extensive sample preparation. In an ESEM, a gaseous specimen is introduced into the chamber, and the pressure is controlled to maintain a suitable environment for imaging. However, it's important to note that the resolution and quality of images obtained with ESEM may not match those achieved with traditional high-vacuum SEM.

1.1.2 Cryogenic SEM:

Cryo-SEM involves freezing a gaseous sample in a cryogen, such as liquid nitrogen or liquid helium, to solidify it. The frozen sample is then transferred to the SEM chamber while maintaining low temperatures. The low temperature helps preserve the sample's structure, and it also reduces the vapor pressure of the gas, preventing it from evaporating or spreading too much during imaging. Cryo-SEM is commonly used to study samples that are inherently gaseous at room temperature, such as volatile compounds or biological specimens.

It's important to remember that gas specimens in SEM require specialized equipment, modifications, or techniques, and the resulting images may have limitations in terms of resolution, contrast, or sample preservation. If you're specifically interested in analyzing gases, techniques such as gas chromatography coupled with mass spectrometry (GC-MS) or Fourier transform infrared spectroscopy (FTIR) are more commonly used for gas analysis due to their compatibility and sensitivity to gaseous samples[46].

1.3 Solid specimen:

Preparing solid specimens for SEM imaging is a more common practice and involves several steps to ensure proper sample mounting, preservation, and imaging.

Here is a general outline of the process:

1.3.1 Sample Collection:

Obtain the solid specimen of interest for SEM imaging. This could be a material, a geological sample, a metal, a semiconductor, or any other solid object.

1.3.2 Sample Preparation:

Depending on the nature of the sample, you may need to perform some preliminary steps to prepare it. This can include cutting, polishing, grinding, or sectioning the sample to obtain a

suitable surface for SEM imaging. The goal is to expose the region of interest and achieve a flat and smooth surface.

1.3.3 Mounting:

The prepared sample needs to be mounted on a suitable substrate or sample holder to ensure stability during imaging. Common methods include using adhesive carbon tabs, conductive adhesives, or specialized sample holders. The choice of mounting technique depends on the nature of the sample and the desired analysis.

1.3.4 Coating (Optional):

Some non-conductive samples may require a conductive coating to prevent charging during SEM imaging. A thin layer of metal, typically gold, gold/palladium alloy, or carbon, is deposited on the sample surface using techniques such as sputter coating or carbon coating. The conductive coating helps to improve image quality and reduce charging artifacts.

1.3.5 Vacuum Chamber Preparation:

SEM imaging requires a high-vacuum environment to allow electron beam interactions with the sample. Before introducing the sample into the SEM chamber, it is necessary to ensure proper vacuum conditions, which may involve evacuating the chamber and reaching a stable vacuum level.

1.3.6 Sample Insertion:

Carefully place the mounted sample into the SEM chamber. Depending on the SEM instrument, there may be a specific loading mechanism or sample stage for this purpose. Ensure that the sample is properly positioned and secured.

1.3.7 Imaging Parameters:

Set the desired imaging parameters, such as accelerating voltage, beam current, working distance, and detector settings, based on the specific requirements of your analysis. Adjust these parameters to achieve optimal imaging conditions for your sample.

1.3.8 Imaging and Analysis:

Start the SEM imaging process and acquire images of the sample at various magnifications and angles as needed. Additionally, you can perform other analyses like elemental analysis using energy-dispersive X-ray spectroscopy (EDS) or mapping techniques to gather additional information about the sample's composition and structure[47].

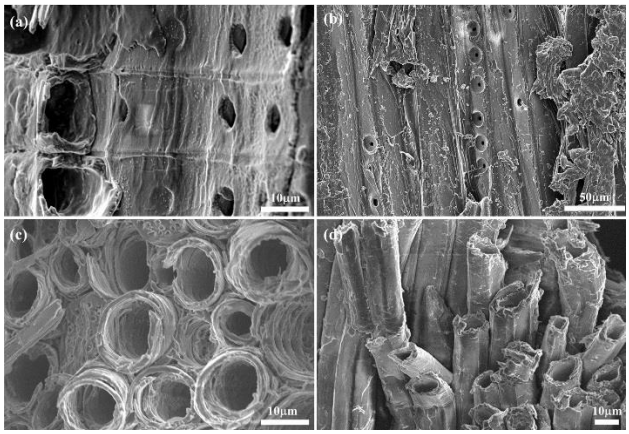

Figure IV.11:SEM images of various types of wood [48].

1.4 Powder specimen

Preparing powder specimens for SEM imaging involves a different set of techniques compared to solid specimens. The challenge with powder samples lies in achieving proper sample dispersion and mounting to ensure accurate imaging.Here are the steps involved in preparing powder specimens for SEM:

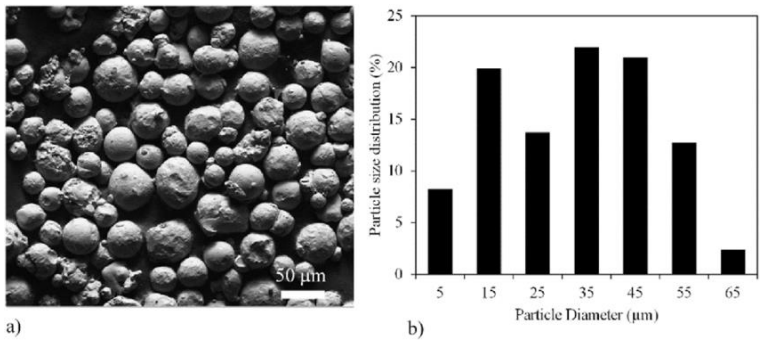

Figure IV.12:Gaz atomized stainless steel powder, and b [49].

1.4.1Sample Collection:

Obtain the powder specimen you wish to analyze. Powders can include various materials such as minerals, ceramics, pharmaceuticals, or finely ground samples.

1.4.2 Sample Dispersion:

Powders tend to form agglomerates or clumps, which can hinder imaging. It's crucial to disperse the powder particles for a more representative analysis. One common method is ultrasonic dispersion, where the powder is suspended in a suitable liquid (e.g., ethanol or water) and subjected to ultrasonic waves to break up agglomerates. Alternatively, other dispersion techniques such as vertexing or sonication can be used depending on the sample and its characteristics.

1.4.3 Filtration (Optional):

If the powder sample contains particles of different sizes and you want to analyze a specific size fraction, filtration can be employed. Use a suitable filter with the desired pore size to separate the desired particle size range. The collected particles on the filter can then be further processed for SEM analysis.

1.4.4 Mounting:

The dispersed powder sample needs to be mounted onto a suitable substrate or sample holder for SEM imaging. There are different methods for mounting powder samples:

- **Carbon Tape:**

Place a small piece of conductive carbon tape on a sample stub or holder. Gently spread a thin layer of the dispersed powder onto the adhesive surface, ensuring good contact.

- **Conductive Adhesive:**

Apply a conductive adhesive, such as silver paste or conductive epoxy, onto a sample stub or holder. Carefully place the dispersed powder onto the adhesive and let it dry or cure, following the manufacturer's instructions.

- **Agar or Wax:**

Mix the dispersed powder with a suitable embedding material, such as agar or wax, and allow it to solidify. The resulting solid block can be trimmed, polished, and mounted onto a sample holder.

1.5.5 Coating (Optional):

Non-conductive powder samples may require a thin conductive coating to prevent charging during SEM imaging. Apply a conductive coating, such as gold, gold/palladium, or carbon, using a sputter coater or carbon coater. Ensure a uniform and thin coating is achieved to enhance image quality.

1.5.6 Vacuum Chamber Preparation:

Before inserting the mounted powder sample into the SEM chamber, ensure that the vacuum conditions are properly established.

1.5.7 Sample Insertion:

Carefully place the mounted powder sample into the SEM chamber, ensuring it is securely positioned and properly oriented for imaging.

1.5.8 Imaging and Analysis:

Set the desired imaging parameters, such as accelerating voltage, beam current, working distance, and detector settings, based on the requirements of your analysis. Start the SEM imaging process and acquire images of the powder sample at various magnifications and orientations.

When working with powder specimens, it's important to note that achieving a representative analysis can be challenging due to potential particle settling or agglomeration during sample preparation and imaging. Multiple areas of the sample should be imaged to capture the overall characteristics [44].

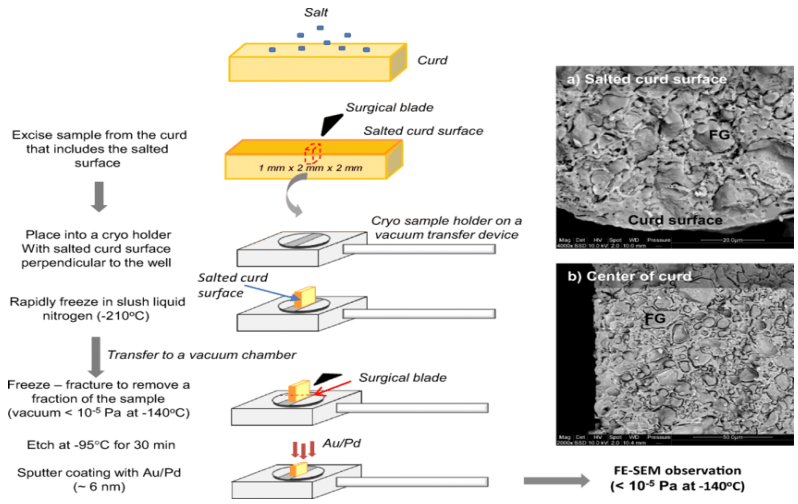

Figure IV.13: Sample preparation technique for Cory SEM observation of a milled and salted curd chip [50].

2 SEM images interpretation

SEM (Scanning Electron Microscopy) images are obtained by scanning a sample with a focused beam of electrons. The interaction of the electrons with the sample generates various signals, which are then detected and used to create the final image. Interpreting SEM images involves understanding the features, structures, and composition of the sample based on the image's characteristics.

Here are some general guidelines for interpreting SEM images:

2.1 Surface Topography:

SEM images provide high-resolution details of the surface of the sample. The brightness or contrast variations in the image can reveal surface features such as bumps, ridges, pits, or

cracks. By examining the morphology, shape, and size of these features, you can infer information about the sample's surface texture and roughness.

2.2 Magnification and Scale:

SEM images are typically taken at various magnifications. Pay attention to the scale bar or any indicated magnification factor to understand the size of the features observed. The higher the magnification, the finer the details you can observe.

2.3 Composition and Elemental Analysis:

SEM can provide elemental information through energy-dispersive X-ray spectroscopy (EDS). EDS allows the identification and mapping of elements present in the sample. Look for areas with different colors, indicating the presence of different elements. EDS spectra can also be obtained to analyze the elemental composition quantitatively.

2.4 Contrast Modes:

SEM images can be obtained using different contrast modes, such as secondary electron (SE) imaging or backscattered electron (BSE) imaging. SE imaging provides information about the sample's topography and surface characteristics, while BSE imaging reflects differences in atomic number, density, and composition. BSE imaging can be particularly useful for examining variations in material composition or detecting heavy elements.

2.5 Sample Preparation Artifacts:

Keep in mind that sample preparation techniques can introduce artifacts in SEM images. These artifacts can include charging effects, specimen damage, contamination, or artifacts caused by the deposition of conductive coatings. Consider the possibility of such artifacts when interpreting the image.

Remember that interpreting SEM images is highly dependent on the specific sample and the objectives of the analysis. It is often necessary to combine SEM observations with additional analytical techniques and prior knowledge to gain a comprehensive understanding of the sample's characteristics.

3 IMAGEJ SOFTWARE

Imagej is a popular open-source image processing and analysis software developed by the National Institutes of Health (NIH). It provides a wide range of tools and functionalities for scientific and technical image analysis.

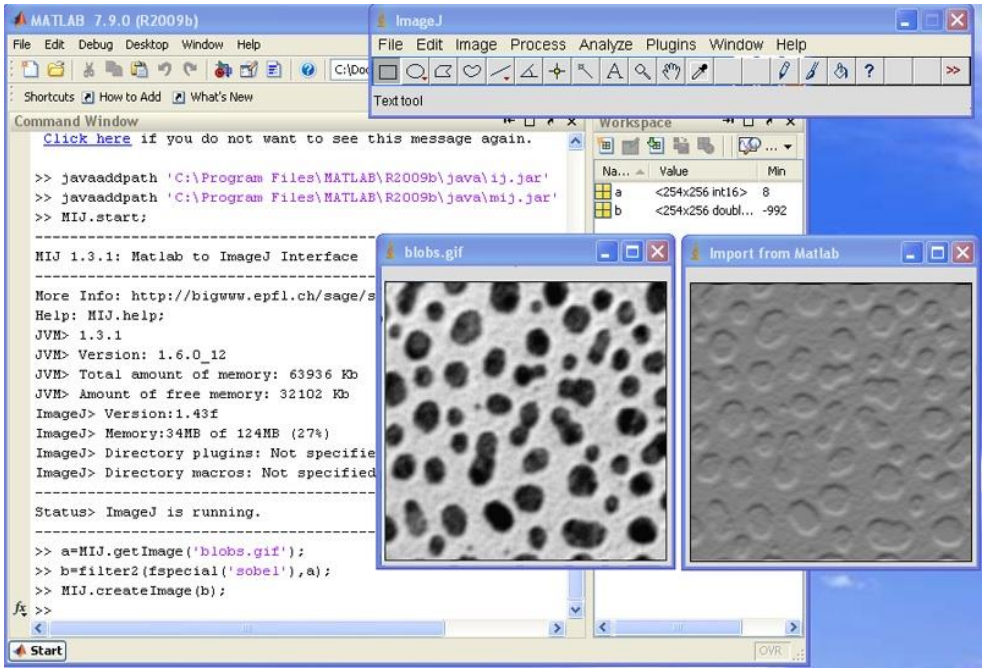

Figure IV.14:Using fast MATLAB routines and display results on ImageJ[51].

Some key features of ImageJ include

❖ **Image Processing:**

ImageJ offers a variety of image processing functions, such as filtering, enhancement, segmentation, and geometric transformations. These tools allow users to manipulate and improve the quality of their images.

❖ **Measurement and Analysis:**

ImageJ enables users to measure various parameters within images, such as lengths, areas, intensities, and angles. It also provides tools for statistical analysis and graphing of the measured data.

❖ **Plugins and Extensions:**

ImageJ has a vast collection of plugins and extensions contributed by the user community. These plugins extend the functionality of the software and allow users to perform specialized image analysis tasks.

❖ **Macro Recording and Scripting:**

ImageJ supports macro recording, which allows users to automate repetitive tasks by recording a series of steps and replaying them later. It also provides a scripting interface using the Java-based ImageJ Macro language, enabling users to write custom scripts for complex image analysis workflows.

❖ **3D Image Processing:**

ImageJ supports the analysis of three-dimensional (3D) images, allowing users to stack, visualize, and analyze volumetric data.

❖ **Image Visualization:**

ImageJ provides a range of visualization options, including different color maps, overlaying images, and creating 3D renderings. These features aid in the interpretation and presentation of image data.

❖ **Image Stacks and Time-Series Analysis:**

ImageJ can handle image stacks, which are a series of images captured over time or as a Z-stack (multiple focal planes). It allows users to navigate through the stack, extract individual frames, and perform analysis on each frame or across the stack.

❖ **Image Restoration and Deconvolution:**

ImageJ offers plugins for image restoration and deconvolution, which can help improve image quality, remove noise, and enhance details. These techniques are particularly useful in microscopy and imaging applications.

❖ **Batch Processing:**

ImageJ supports batch processing, allowing users to apply a series of image processing steps or analyses to multiple images simultaneously. This feature saves time and improves efficiency when working with large datasets.

❖ **Machine Learning and Deep Learning:**

ImageJ has plugins and integrations that enable users to apply machine learning and deep learning techniques for image analysis tasks. These include tools for image classification, object detection, and image segmentation using pre-trained models or custom-trained models.

❖ **Image File Formats:**

ImageJ supports a wide range of image file formats, including common formats like JPEG, PNG, TIFF, and BMP, as well as specialized formats used in scientific imaging, such as DICOM, LSM, and OME-TIFF.

❖ **Plugins and Scripting Languages:**

ImageJ provides a plugin architecture that allows users to extend its functionality by developing and installing custom plugins. Additionally, it supports scripting in various languages, including Java, JavaScript, Python, and others, enabling users to automate tasks and customize workflows.

❖ **Community and Resources:**

ImageJ benefits from an active user community that contributes to its development, shares plugins, provides support, and collaborates on image analysis techniques. There are also online resources, forums, and documentation available to help users learn and utilize ImageJ effectively.

ImageJ versatility and flexibility make it a powerful tool for image analysis in research, education, and various industries. Its open-source nature and extensive plugin ecosystem contribute to its popularity and continual growth.

4 EXAMPLES

Here is an example of an experiment for preparing and imaging a glass powder sample in a scanning electron microscope (SEM):

4.1 Sample selection:

We have a sample of glass powder that we want to study using SEM. Glass powder can be a finely ground substance obtained from a particular glass composition (**Figure IV.6**).

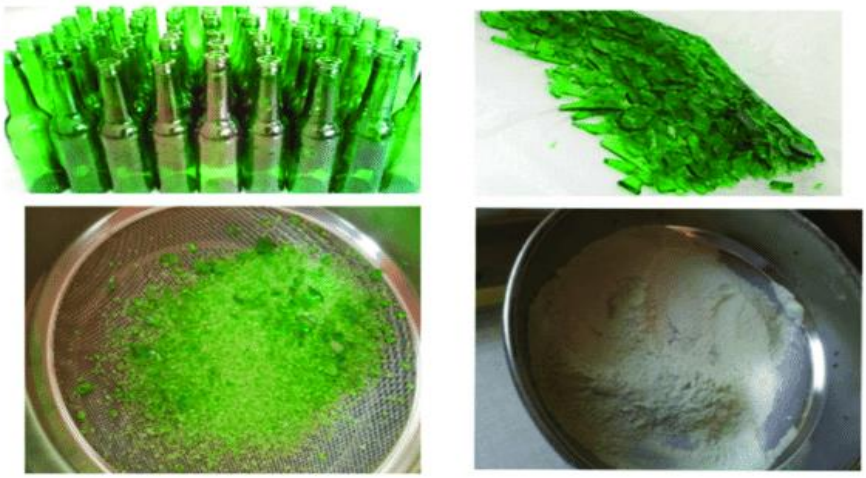

Figure IV.15:The raw material of glass before and after grinding [51].

4.2 Sample handling:

Glass powder is susceptible to air pollution, so it is important to handle it in a clean environment. Wear appropriate personal protective equipment (PPE) such as gloves, lab coat, and face mask to prevent contamination from skin oils, dust, or other particles.

4.3 Sample dispersion:

glass powder tends to agglomerate, so it needs to be dispersed before shooting. You can disperse a small amount of glass powder in a suitable liquid medium, such as ethanol or isopropyl alcohol, to create a suspension. Ultrasonication can be used to enhance effective dispersion and prevent agglomeration.

4.4 Mounting:

To make it easier to shoot, we will need to transfer a small amount of dispersed glass powder to a suitable substrate or stand. A common method is to use a heel counter with conductive adhesive or carbon tape. You can carefully apply a drop of finely dispersed glass suspension powder to a stub and allow it to dry to form a thin layer of powder on the substrate.

4.5 Coating:

Depending on the conductivity of the glass powder and the desired imaging quality, you may choose to apply a conductive coating to the sample. The conductive coating helps improve sample stability and reduces charging effects. You can apply a thin layer of an electrically conductive material, such as gold-palladium, or carbon, using a spray coater.

4.6 Drying:

Once the powder glass has been installed and coated, if applicable, you ensure it is completely dry to remove any remaining liquid. The drying process can be accelerated by using a dedicated dryer or drying chamber.

4.7 SEM Imaging:

With the glass powder sample prepared, you bring the sample stub into the SEM chamber. Ensure that the chamber is at the proper vacuum level for imaging. Set the necessary imaging parameters, including the electron beam energy, spot size, and detector settings.

4.8 Focusing and Imaging:

Once the SEM is ready, you can select a representative area of the glass powder layer. Using the SEM phase controls, you can position the region of interest under the electron beam. We adjust focus, astigmatism, and stigma settings to get clear, focused images. He began to acquire SEM images of the glass powder at different magnifications to capture the required surface details and particle morphology.

4.9 Analysis:

After obtaining the SEM images (**Figure IV.16)**, you can perform various analyzes on the glass powder sample. You can measure the size and shape of individual glass particles, observe surface features with the help of software (ImageJ), or perform elemental analysis using an energy-dispersive X-ray spectrometer (EDS) if available on SEM.

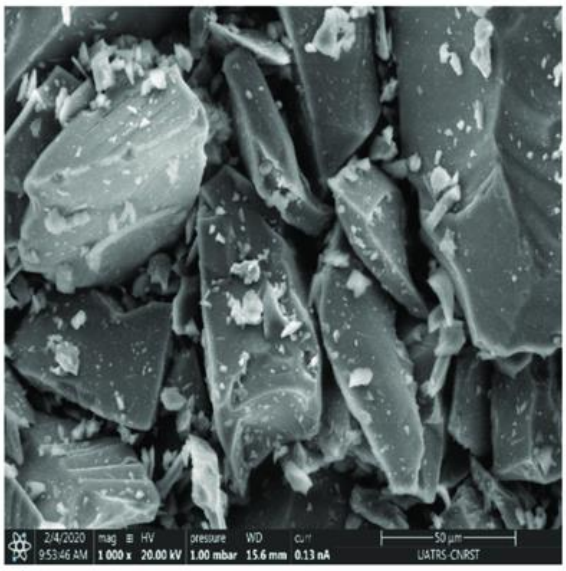

Figure IV.7:Scanning electron microscopy of Glass Powder [51].

4.10 Data Interpretation:

Based on the SEM images and analysis, you can draw conclusions about the particle size distribution, morphology, and other properties of the glass powder sample corresponding These results can be used for material quality control, material characterization, research purposes such as what we're working on in this experiment. In the present study of our example, The SEM micrographs analysis of the glass powder were done. In the Figure IV.7, as the author mentioned, the micrograph of the glass powder shows a sharp and varied smooth morphology, with a grain size ranging from 58 to 290 micrometer.

CONCLUSION

A Scanning Electron Microscope (SEM) is a type of microscope that uses a beam of electrons to create high-resolution images of the surface of a sample. Unlike a conventional optical microscope, which uses visible light to create an image, an SEM uses electrons to create a magnified image. In an SEM, the electron beam is generated by an electron gun and directed onto the surface of the sample, which is typically coated with a conductive material such as gold or carbon. As the electron beam scans across the sample, secondary electrons are emitted from the surface, which are detected by a detector and used to create an image. SEM can produce high-resolution images with magnifications of up to several hundred thousand times. It is commonly used in materials science, biology, and other fields to study the surface topography and structure of samples at a very high resolution. SEMs are also used for elemental analysis, allowing researchers to determine the chemical composition of a sample based on the energy and intensity of X-rays generated by the interaction of the electron beam with the sample. It provides a detailed three-dimensional view of the sample, allowing researchers to examine its topography and analyze the composition and structure of its surface.

Here's how an SEM works:

- **Electron Beam Generation**:

  The SEM contains an electron gun that produces a beam of high-energy electrons. This electron beam is accelerated and focused using electromagnetic lenses.

- **Sample Preparation:**

  The sample to be examined in the SEM needs to be properly prepared. It is typically coated with a thin layer of conductive material, such as gold or carbon, to prevent the buildup of static charge and improve the quality of the image.

- **Scanning the Sample:**

  The focused electron beam scans across the surface of the sample in a raster pattern. As the beam interacts with the sample, various signals are generated.

- **Signal Detection:**

  Different signals are collected to provide information about the sample. The primary signals in SEM include secondary electrons, backscattered electrons, and characteristic X-rays.

- **Secondary Electrons:**

  When the primary electron beam strikes the sample, it can dislodge secondary electrons from the surface. These low-energy electrons are collected and used to create an image with high spatial resolution. Secondary electron imaging is often used to observe surface topography.

- **Backscattered Electrons:**

  Some of the primary electrons interact with the atoms in the sample and are scattered backward. Backscattered electrons have higher energy than secondary electrons and are collected to provide compositional information about the sample.

- **X-ray Emission:**

  When the primary electrons interact with the atoms in the sample, they can also cause the emission of characteristic X-rays. These X-rays can be detected and analyzed to determine the elemental composition of the sample.

- **Image Formation:**

  The detected signals are amplified and converted into electrical signals. These signals are then used to create an image of the sample on a display screen. The image can be black and white, grayscale, or color-coded, depending on the specific configuration of the SEM.

In conclusion, scanning electron microscopy (SEM) is a powerful technique used to generate high-resolution images of the surface of a sample. It uses a focused beam of electrons to scan across the sample and collects various signals to provide information about its morphology, composition, and structure. SEM is widely used in many scientific fields and has numerous applications, including materials science, biology, geology, nanotechnology, and forensic analysis. Its ability to provide detailed three-dimensional views of the sample makes it a valuable tool for researchers seeking to understand the properties and behavior of various materials and specimens.

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ملخص

الماسح المجهري الإلكتروني (SEM) هو تقنية تصوير قوية تستخدم لفحص سطح العينات بتفصيل كبير. إنه يعمل عن طريق مسح حزمة مركزة من الإلكترونات عبر سطح العينة، وتوليد إشارات توفر معلومات حول تكوين العينة وهيكلها وتضاريسها. يتفاعل شعاع الإلكترون مع العينة، وينتج إلكترونات ثانوية، وإلكترونات مبعثرة، وأشعة سينية مميزة. يستخدم الإلكترونات الثانوية لإنشاء صور عالية الدقة، بينما توفر الإلكترونات المتناثرة تباينًا تركيبيًا. يمكن أن يحقق SEM تكبيرات تتراوح من بضع مرات إلى مئات الآلاف من المرات، اعتمادًا على قدرات الجهاز. لمنع آثار الشحن، يجب أن تكون العينة موصلة للكهرباء أو مغلفة بطبقة رقيقة من مادة موصلة. يستخدم SEM على نطاق واسع في مختلف المجالات مثل علوم المواد وتكنولوجيا النانو والبيولوجيا والجيولوجيا والطب الشرعي لتحليل السطح المفصل. إنه يتيح فحص ميزات السطح والتشكيل والتوزيع الأولي بدقة استثنائية. يسمح SEM أيضًا برسم الخرائط الأولية، والتحليل الطيفي للأشعة السينية المشتتة للطاقة (EDS)، وحيود التشتت الخلفي للإلكترون (EBSD) لمزيد من التوصيف. بشكل عام، يعد SEM أداة أساسية للبحث العلمي والتحليل على المقياس الدقيق والنانوي

Abstract

Scanning Electron Microscopy (SEM) is a powerful imaging technique used to examine the surface of specimens in great detail. It works by scanning a focused beam of electrons across the sample's surface, generating signals that provide information about the sample's composition, structure, and topography. The electron beam interacts with the sample, producing secondary electrons, backscattered electrons, and characteristic X-rays. Secondary electrons are used to create high-resolution images, while backscattered electrons provide compositional contrast. SEM can achieve magnifications ranging from a few times to hundreds of thousands of times, depending on the instrument's capabilities. To prevent charging effects, the sample must be conductive or coated with a thin layer of conductive material. SEM is widely used in various fields such as materials science, nanotechnology, biology, geology, and forensics for detailed surface analysis. It enables the examination of surface features, morphology, and elemental distribution with exceptional resolution. SEM also allows for elemental mapping, energy-dispersive X-ray spectroscopy (EDS), and electron backscatter diffraction (EBSD) for further characterization. Overall, SEM is an essential tool for scientific research and analysis at the micro- and nanoscale.

Résumé

La microscopie électronique à balayage (SEM) est une technique d'imagerie puissante utilisée pour examiner la surface des spécimens de manière très détaillée. Il fonctionne en balayant un faisceau focalisé d'électrons sur la surface de l'échantillon, générant des signaux qui fournissent des informations sur la composition, la structure et la topographie de l'échantillon. Le faisceau d'électrons interagit avec l'échantillon, produisant des électrons secondaires, des électrons rétrodiffusés et des rayons X caractéristiques. Les électrons secondaires sont utilisés pour créer des images haute résolution, tandis que les électrons rétrodiffusés fournissent un contraste de composition. Le SEM peut atteindre des grossissements allant de quelques fois à des centaines de milliers de fois, selon les capacités de l'instrument. Pour éviter les effets de charge, l'échantillon doit être conducteur ou recouvert d'une fine couche de matériau conducteur. Le SEM est largement utilisé dans divers domaines tels que la science des matériaux, la nanotechnologie, la biologie, la géologie et la criminalistique pour l'analyse détaillée des surfaces. Il permet l'examen des caractéristiques de surface, de la morphologie et de la distribution élémentaire avec une résolution exceptionnelle. Le SEM permet également la cartographie élémentaire, la spectroscopie à rayons X à dispersion d'énergie (EDS) et la diffraction par rétrodiffusion d'électrons (EBSD) pour une caractérisation plus poussée. Dans l'ensemble, le SEM est un outil essentiel pour la recherche scientifique et l'analyse à l'échelle micro et nanométrique.