CHAPTER 16

Fundamental Differences Between Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

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Introduction

The word microscope is a combination of the two ancient Greek words μικρός (mikros), meaning "small", and σκοπεῖν (skopein), meaning "to see" (Liddell & Scott, 1996). Thus, the word microscope can be used to describe any instrument used to magnify small objects, regardless of whether it uses light or electrons. The first known microscope was invented in the late 16th century by Hans and Zacharias Janssen, two Dutch spectacle-makers. Their microscope was a simple compound microscope with two lenses, one objective lens and one ocular lens. It could magnify objects up to 9 times. Over the next century, microscopes were improved and refined by many different scientists. In 1665, Robert Hooke published his book Micrographia, which contained illustrations of many different objects as seen under a microscope. Hooke was the first person to observe and describe cells, and he is credited with coining the term "cell". In the 18th century, Antoni Philips van Leeuwenhoek built microscopes that could magnify objects up to 270 times. He was the first person to observe and describe bacteria, protozoa, and other microorganisms (Sanders et al. 2023).

In the 19th century, many new types of microscopes were developed, including the phase contrast microscope, the interference microscope, and the electron microscope. The electron microscope was invented in the early 20th century by Ernst Ruska and Max Knoll (Knoll & Ruska, 1932). Electron microscopes (EM) use a beam of electrons instead of light to magnify objects. This allows them to achieve much higher magnifications than light microscopes, up to millions of times. Since the discovery of the electron, imaging techniques have reached an advanced level and have rapidly accelerated scientific progress. After the discovery of the wave-like nature of the electron, using electrons instead of light in microscopes significantly increased the resolving power, paving the way for higher magnifications. Thus, with the help of EM, the resolution increased from 1/1000 meters, visible, to 1 nanometer (nm). EM has revolutionized many fields of science, including biology, chemistry, and materials science. They allow us to see objects that are too small to be seen with the naked eye, such as individual atoms and molecules. Over the past hundred years, EM has become the most important window into the nanoworld for scientists and researchers. The development of image

processing technology for advanced material production and characterization has driven the technology forward at an unprecedented rate. Evaluating a single atom now enables us to scale down the properties obtained at the macroscale to the nanoscale, enabling inventions that can be considered revolutionary for humanity. In general, electron microscopes can be divided into two categories: scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Both SEM and TEM microscopes provide the opportunity to examine samples with high precision by sending a focused electron beam through a vacuum chamber. However, SEM microscopes are designed to examine material surfaces, while TEM microscopes are designed to obtain information about the internal structure of samples. This study will discuss SEM and TEM, two imaging techniques that underpin advanced research, and reveal the fundamental differences between these two microscopes.

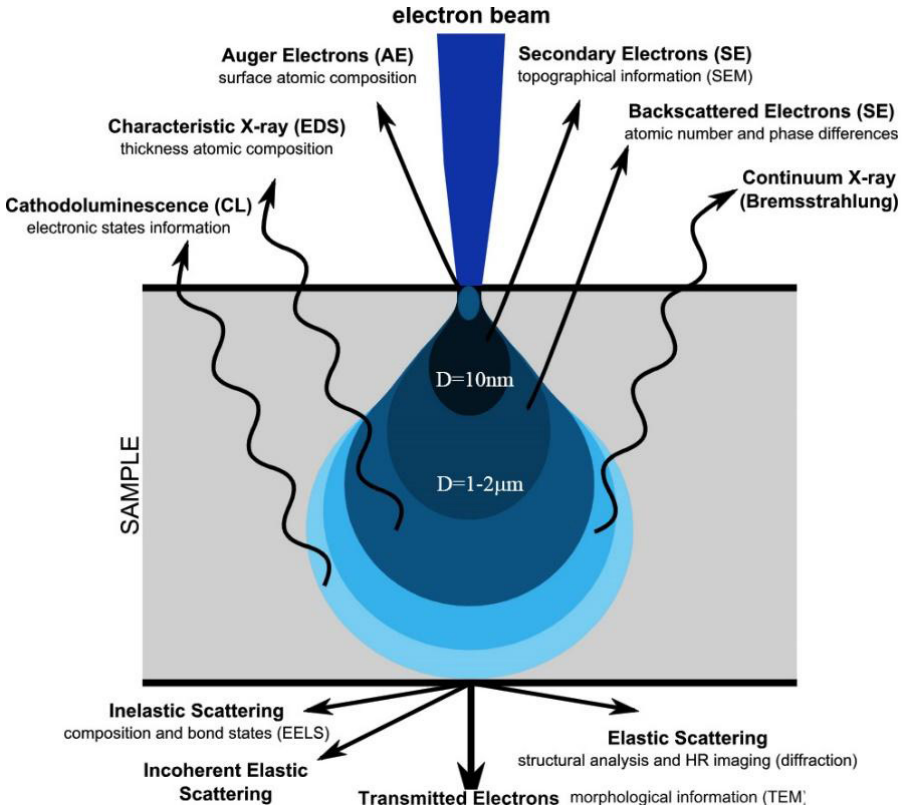
Scanning Electron Microscope (SEM)

SEM is an indispensable tool in scientific research and materials analysis due to its high-resolution imaging capabilities and ability to provide valuable information about the surface morphology and composition of various materials. Unlike optical microscopes, which cannot provide sufficient magnifications, SEM microscopes can be produced as compact desktop devices and are widely used around the world for advanced studies in research laboratories. Basically, a SEM image is created by a focused electron beam accelerated from the anode to the cathode and interacting with the surface of the material. The microscope column is placed under vacuum at approximately 10 to $1x10^{-4}$ Pa. The electron source may vary depending on the device's characteristics, such as a tungsten filament, single crystal lanthanum hexaboride (LaB_6), or field emission gun (FEG) (Paredes, 2014). SEMs typically have accelerating voltage from 200 V to 30 kV. The electron beam is collected by the condenser electromagnetic lens (condenser lens) focused by the objective lens and then scanned across the sample surface using an electromagnetic deflector or coils. Image formation in a SEM is based on the collection and analysis of signals resulting from the physical interactions of the electron beam with the surface of the sample under study (elastic and inelastic scattered electrons, among others) (Inkson, 2016; Fuller et al., 2013; Spence & Zuo, 1992). Another group of electrons that emerge from the interaction of the electron beam and the material on the surface of the sample under examination are backscattered electrons (these electrons are scattered at an angle of approximately 180 degrees from the incident electron beam). Backscattered electrons (BSE) are higher-energy electrons that come from deeper regions of the surface (down to about 300 nm). Because electrons at this energy of appearing photons have too high energy to be detected by a photomultiplier tube, they are usually detected with the help of quadrant photodetectors (i.e., solid-state detectors) (Mi et al., 2021). As you know, such detectors give output signals proportional to the intensity of the electric current induced by the electrons incident on them. As a brief reminder, the energy of an electron scattered from a high-atomic-number atom in the sample under examination is higher than that scattered from a low-atomic-number atom. As a result, backscattered electrons provide information about the composition of the sample under examination. Another interaction of the incoming electron beam with the examined sample surface (at a depth of approximately 1000 nm) is the emission of characteristic X-rays (whose energy is in the order of keV). When an electron hitting the sample knocks an electron out of the inner orbit of an atom in the sample, an electron in an upper orbit transitions to that level to balance the energy, and as it does, it emits an X-ray photon into the environment. This is called a characteristic X-ray. For example, a Si-Li detector (semiconductor detector) with a diameter of 10 mm^2 detects this X-ray. The resulting signal is sent to the amplifier, then to the multi-channel analyzer, and finally to the computer of the SEM system. The resulting characteristic X-ray, whose energy is specific to each atom, helps to qualitatively and quantitatively determine the elemental composition of the material examined in the SEM (Silva & Ferri, 2017; Egerton, 2011; Williams & Carter, 2009; Bell & Garratt-Reed, 2003). In addition, information about the content of the sample

examined is obtained with the energy-dispersive X-ray spectrometer (EDS) integrated into the SEM. X-rays, in particular, are commonly used for examining materials, and they offer an image resolution of approximately 1 nm.

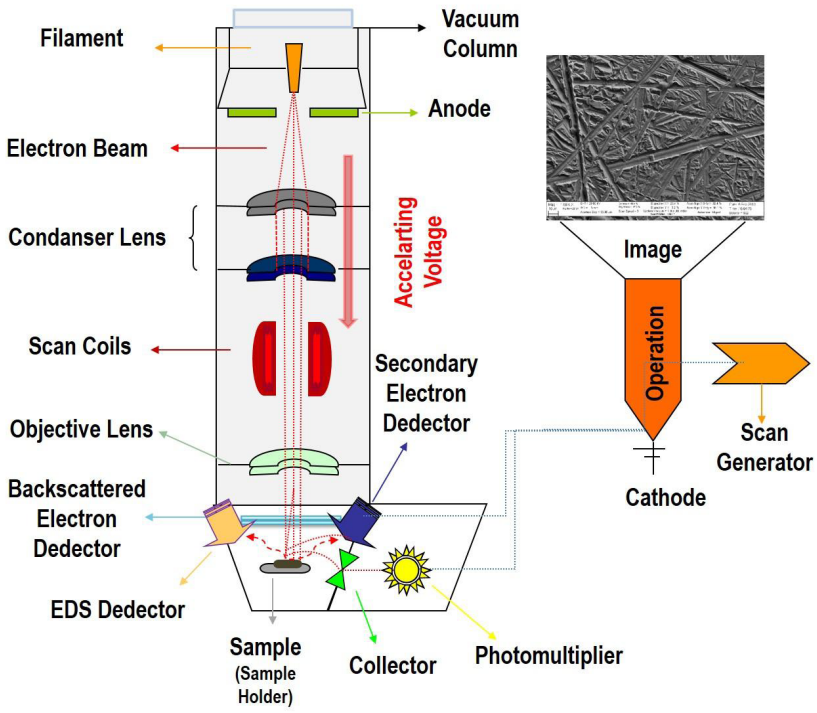
When electrons hit the sample, an interaction occurs between them. Figure 1 shows that the first electrons focused on the sample surface are called primary electrons, and secondary electrons are the electrons that result from the energy loss of primary electrons interacting with matter. Of these electron interactions, backscattered electrons (BSE), secondary electrons (SE), and X-rays undergo elastic scattering, whereas the others undergo inelastic scattering. Most secondary electrons are captured by detectors, called SE detectors or Everhart-Thornley (ET) detectors (Griffin, 2011).

Figure 1.
The Interaction Between Accelerated Electrons And Matter Is Illustrated Schematically (https://en.wikipedia.org/wiki/Electron_microscope)



Detectors that capture backscattered electrons are called BSED detectors, and detectors that capture backscattered X-rays are called EDS detectors (Goodhew et al., 2001; Inkson, 2016). Collecting backscattered electrons in the detector creates a contrast difference, which allows for the elemental discrimination of the atoms in the sample. Secondary electrons create the basic surface image, which helps to obtain a 3D image of the examined area. Additionally, collecting backscattered electrons in the detector creates a contrast difference, which allows for the elemental discrimination of the atoms in the sample. Figure 2 shows a schematic diagram of the colon structure of the SEM device. All the lenses mentioned here are magnetic and operate based on the Lorentz force principle [electromagnetic force equation is $\vec{F} = q(\vec{E} + \vec{\vartheta} \times \vec{B})\vec{F} = q(\vec{E} + \vec{\vartheta} \times \vec{B})$] (Jacson,1999). The charge q in this equation represents the electron. A dedicated EBSD detector collects the scattered BSEs over a large solid angle, which forms electron backscatter diffraction patterns made up of Kikuchi lines (Schwarzer et al., 2009).

Figure 2.
The Schematic Diagram Illustrates the Column Structure Of The SEM Device



Transmission Electron Microscope (TEM)

TEM is important because it allows us to see the internal structure of materials in unprecedented detail. This information is essential for understanding the properties of materials and for developing new materials with improved properties. TEM is also important for studying the fundamental physics and chemistry of materials, as well as the structure and function of biological cells and organelles (Spence, 2013; Williams & Carter, 2009; Reimer & Kohl, 2008; Smith, 2005; Goodhew et al., 2001). TEM provides information about the internal structure of the examined sample. The electron beam sent directly to the sample passes through it (at high vacuum). Operating voltage values for a TEM device typically range from 30 to 300 kV, but can be adjusted depending on the type of material being examined (Inkson, 2016; Kleebe et al. 2010). The filaments, electromagnetic lenses, condenser lenses, objective lenses, detectors, and surface scanning coils used in a SEM device are also used in a TEM device. When the high-speed electrons hit the sample, they interact with it. The microscope column is placed under vacuum at approximately $1x10^{-6}$ Pa levels to prevent electron scattering caused by atmospheric conditions. All of these lenses and coils are used to ensure that the electrons fall on the sample being examined. The number of accelerated electrons that leak into the sample depends on the number of electrons in the electron beam, the diameter of the electron beam, the energy of the electron (acceleration voltage), and the atomic number of the sample. Using a TEM device, magnifications from 20 x to 2,000,000 x can be obtained, as well as opposing lattice points. The objective lens is a particularly significant component in the TEM setup. This component is paramount, as its inherent aberrations directly impact the resolution capabilities of the microscope. Additionally, an electron diffraction pattern is generated in the back focal plane of the objective lens (Paredes, 2014). A removable aperture is strategically positioned in this plane to facilitate the selection of distinct electron beams, thereby enabling the formation of diverse images and the variation of image contrast. This precise control over electron beams and contrast is a fundamental feature of TEM analysis.

Electrons are emitted from an electron gun and accelerate them through an illumination system. The imaging system consists of magnetic (objective and projector lenses) lenses, and the resulting image is visualized and recorded. Within the TEM setup, the objective lens plays a crucial role in determining the resolution capabilities of the microscope. The back focal plane of the objective lens generates an electron diffraction pattern, and a removable aperture allows for the selection of distinct electron beams and the manipulation of image contrast. This level of control over electron beams and contrast is vital for TEM analysis. Overall, the objective lens in a TEM is crucial to determine the resolution and contrast of the microscope. By manipulating the size of the objective aperture, different electron beams can be selected, allowing for the visualization of various microstructural features in the specimen. The choice between classical diffraction contrast imaging and phase contrast lattice imaging depends on the desired level of resolution and contrast in the final image. Figure 3 shows a schematic diagram of the colon structure of the TEM device. When an accelerating voltage of 200 kV is applied to a TEM device, the wavelength of the electron will be approximately 0.025 Å (Angstroms). This value allows for interaction at the atomic scale, so the incoming wave will interact with the atoms of the material being examined. The electron wave interacts with the material through a wave function that undergoes Fourier transformations. Using this wave function operation, the system wave function can also be obtained as an inverse Fourier transform (Karlik, 2001). This results in a diffraction pattern on the fluorescent screen. The resulting diffraction pattern contains information about the crystal structure of the examined sample, and reciprocal lattice parameters can also be found using wave function transformations.

The basic design of the electron microscope has remained essentially unchanged since its inception in the 1930s. Despite this unwavering design, the resolution capability of TEMs has increased by over 1000-fold compared to its initial value. This remarkable progress began approximately 20 years ago with the development of electromagnets capable of correcting distortions in the electron beam. By the late 2000s, long-awaited aberration correctors had empowered advanced TEMs to achieve sub-angstrom resolution. Figure 4 shows the improvement in resolution dimensions of the TEM microscope over the years (Courtland, 2018).

Figure 3.
The Schematic Diagram Illustrates The Column Structure Of The TEM Device

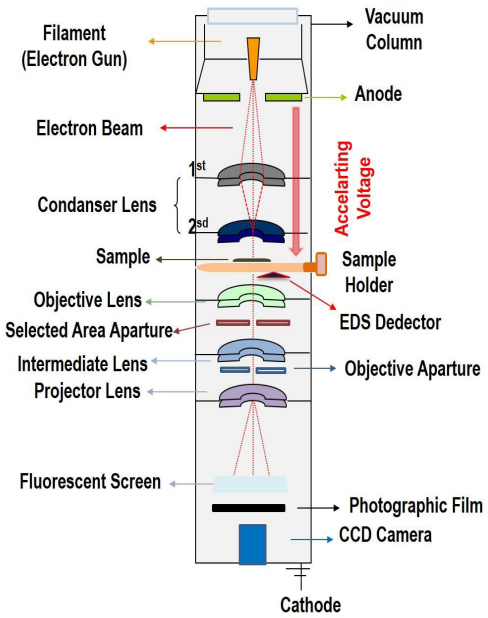
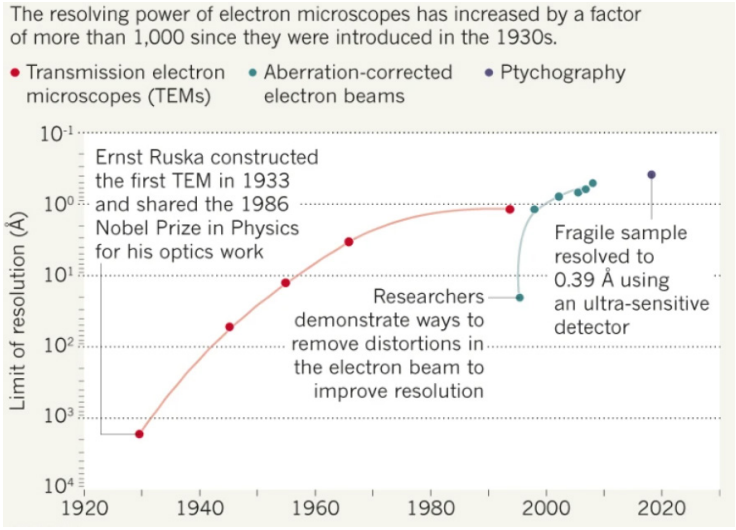


Figure 4.
Evolution Of TEM Microscope Resolution Dimensions (Courtland, 2018)



How to Choose the Right Microscopy Technique for Your Needs: SEM vs TEM

SEM and TEM are two widely used techniques in the field of electron microscopy, each specifically designed for different aspects of specimen analysis. These methodologies differ fundamentally in their imaging principles, applications, and the nature of the information they provide.

1. Imaging Principle:

SEM employs a focused beam of electrons that scans the surface of the specimen. The interaction between the beam and the sample generates signals, producing high-resolution three-dimensional surface images.

In contrast, TEM involves passing an electron beam through the specimen. This transmission allows for the visualization of internal structures in high resolution.

2. Depth of Field:

SEM provides a greater depth of field, enabling the examination of surface topography in three dimensions. This is particularly useful for studying surface features and morphological details.

TEM yields images with a shallow depth of field, limiting the three-dimensional details but facilitating the detailed examination of internal structures. This is ideal for studying the arrangement and organization of subcellular components, nanoparticles, and crystal lattices.

3. Sample Thickness:

SEM is suitable for studying thicker samples and material surfaces, as the electron beam can penetrate to a certain depth. This is advantageous for examining bulk materials and fracture surfaces.

TEM is ideal for ultra-thin sections and provides insights into the internal structures of specimens. Thin sections are required for optimal transmission of the electron beam, allowing for high-resolution imaging of internal features.

4. Resolution:

SEM offers slightly lower resolution compared to TEM but excels in producing detailed surface images. This is particularly useful for studying surface morphology and elemental distribution.

TEM provides exceptionally high resolution, allowing for the visualization of structures at the nanoscale. This is essential for studying the fine details of internal structures, such as the arrangement of molecules and the crystal structure of materials.

5. Applications:

SEM is particularly advantageous for morphology studies, compositional analysis, and elemental mapping of surfaces. This is useful for characterizing the surface topography, chemical composition, and elemental distribution of materials.

TEM is indispensable for investigations requiring detailed information on internal structures, including organelles, nanoparticles, and crystal lattices. This is essential for studying the structure and function of cells, the properties of nanomaterials, and the arrangement of atoms in materials.

6. Operational Considerations:

SEM is well-suited for examining conductive or non-conductive specimens without the need for special sample preparation techniques. This makes SEM a versatile and widely applicable technique.

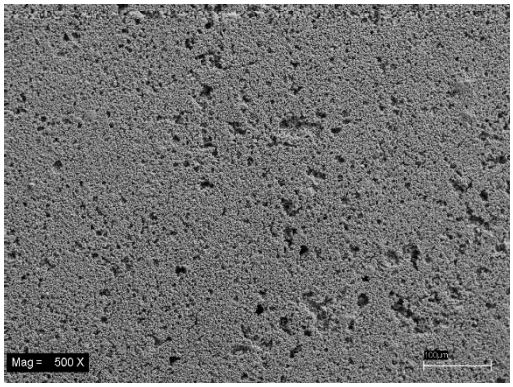
TEM often demands thin sample sections (or nanosize particles) and necessitates intricate specimen preparation, including the creation of ultra-thin slices for optimal transmission. This can be a time-consuming and challenging process, but it is essential for achieving high-resolution TEM images.

In conclusion, while SEM and TEM share a common basis in utilizing focused electron beams, their distinctive imaging principles and applications make them complementary tools in the realm of electron microscopy. Researchers strategically choose between SEM and TEM based on the specific aspects of the specimen they aim to investigate and the type of information required for their studies.

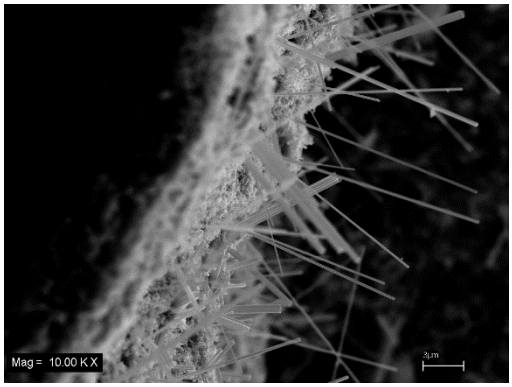
Sample Images Taken on a SEM Device to Illustrate Its Working Principle

Figure 5.

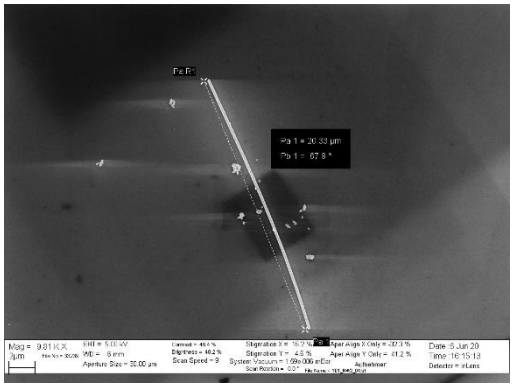
(a, b, c, d, e, f, g, h, I, j ,k ,l) SEM Images Of Specially Prepared Materials



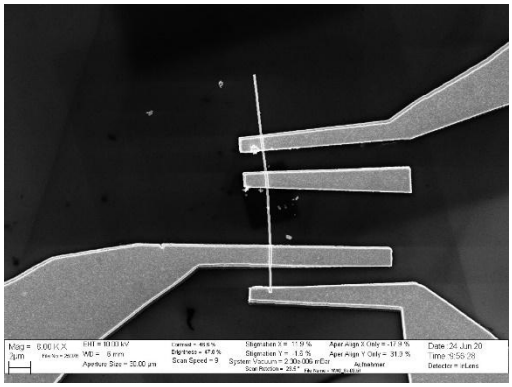
(a)



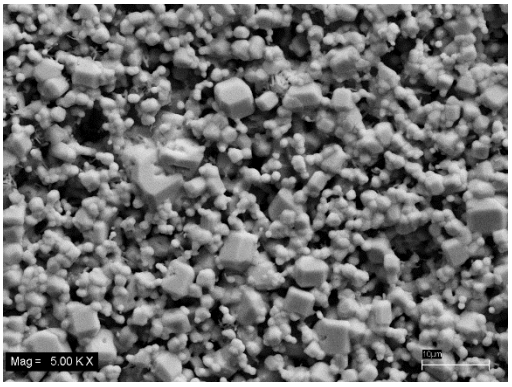
(b)



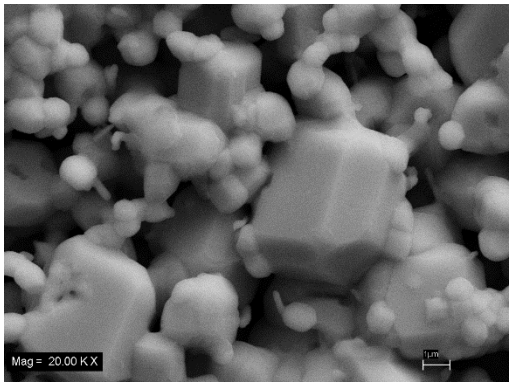
(c)



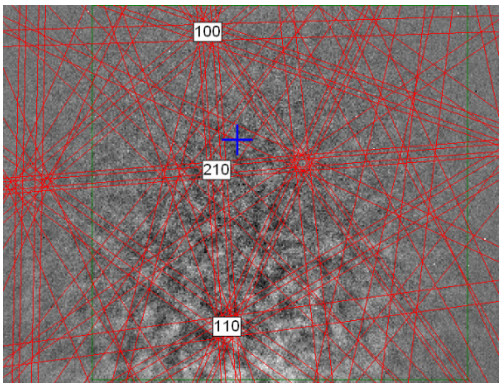
(d)


Mag = 5.00 K X

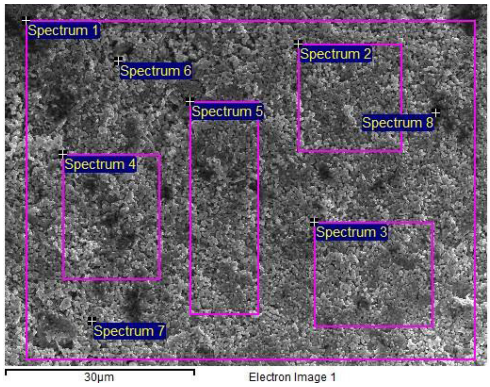
(e)


Mag = 20.00 K X

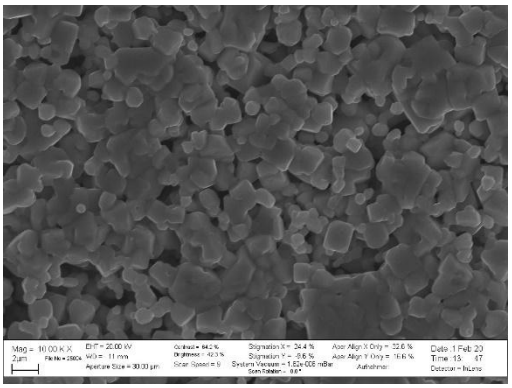
(f)


100
210
110

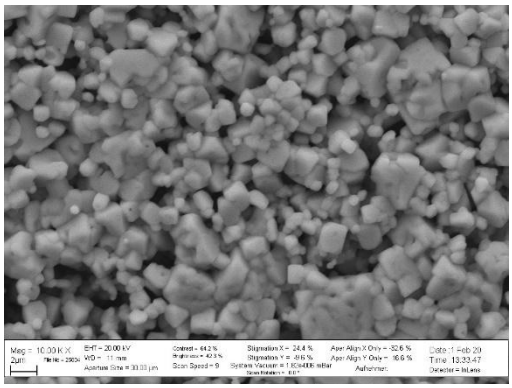
(g)


Spectrum 1
Spectrum 6
Spectrum 2
Spectrum 5
Spectrum 8
Spectrum 4
Spectrum 3
Spectrum 7
30µm
Electron Image 1

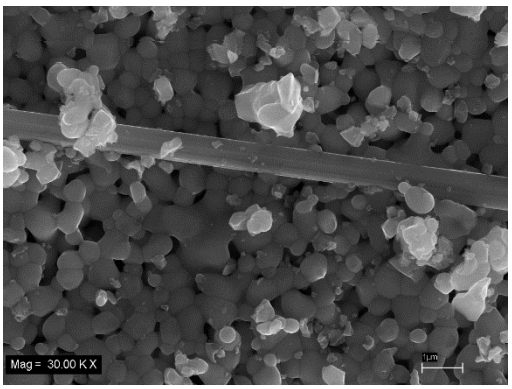
(h)



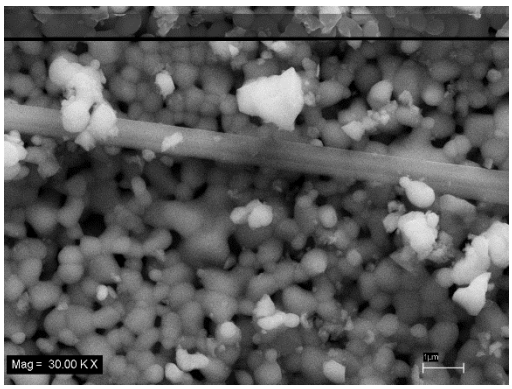
(i)



(j)


Mag = 30.00 K X

(k)


Mag = 30.00 K X

(l)

When examining the SEM images above, it becomes evident that each image possesses distinct visual properties. However, immediately identifying the exact material type and obtaining clear information about its composition is challenging.

Figure 5-a: The image captured at 500 x magnification reveals a spongy structure. This sample is actually an SEM image of a polycrystalline sample with a porous structure, obtained at the lowest magnification setting. In the image of this highly electrically conductive material, gaps can be observed in certain areas.

Figure 5-b: In the image captured at 10000 x magnification, two structurally distinct structures are clearly visible. The first consists of long, thin rod-like structures, while the second resembles a spherical shape. EDS analysis revealed that the rod-like structure is a mineral that formed independently of the underlying base material during its formation. Since understanding this situation at first glance is not possible, EDS detector signal analysis and backscattered electron (BSE) reflection analysis can be explained.

Figure 5-c: The SEM image captured at approximately 10kx magnification depicts a single-crystal material. This material is placed on a conductive substrate coated with a thin layer of conductive organic material. Upon examination in the SEM, the slightly darker rectangular-shaped region represents the area deformed by the electron beam. Utilizing the software integrated into the SEM, various paths are drawn by focusing the electron beam on these areas, and electronic circuits are designed.

Figure 5-d: The SEM image captured as a continuation of the previous image shows that Au (gold) was coated on the single crystal by evaporation, and contacts were placed on the paths drawn in the SEM. Now, a current or voltage difference can be applied to the obtained electronic circuit element. Simultaneously, with the help of the SEM device, a crucial step in scientific research or the production of new-generation nanoscale sensors can be achieved.

Figure 5-e: When focusing on the middle part of the image at 5kx magnification, as shown in figure 5-f, increasing the magnification of the same region to 20kx reveals that the grains within the structure vary in size, both large and small, and are tightly interconnected. The SEM image clearly illustrates that some grains within this tightly interlocking structure range between 1µm and 10 µm.

Figure 5-g: This image depicts a Kikuchi pattern obtained from the image at approximately 1kx magnification. In this image, the signals received by the electron backscatter diffraction (EBSD) detector from backscattered electrons are processed in the computer, and a diffraction pattern is formed by collecting the reflections of the crystal structure.

Figure 5-h: Information about the material's composition is obtained by collecting signals from point and area (spectrum) scans taken from the surface of the sample examined in the SEM image captured at the lowest magnification (Low Mag Mode) with the help of the EDS detector. As a result of analyzing the signals obtained by collecting characteristic X-rays, the elemental content of the sample is determined by obtaining the values of the elements in atomic percentage or weight ratios.

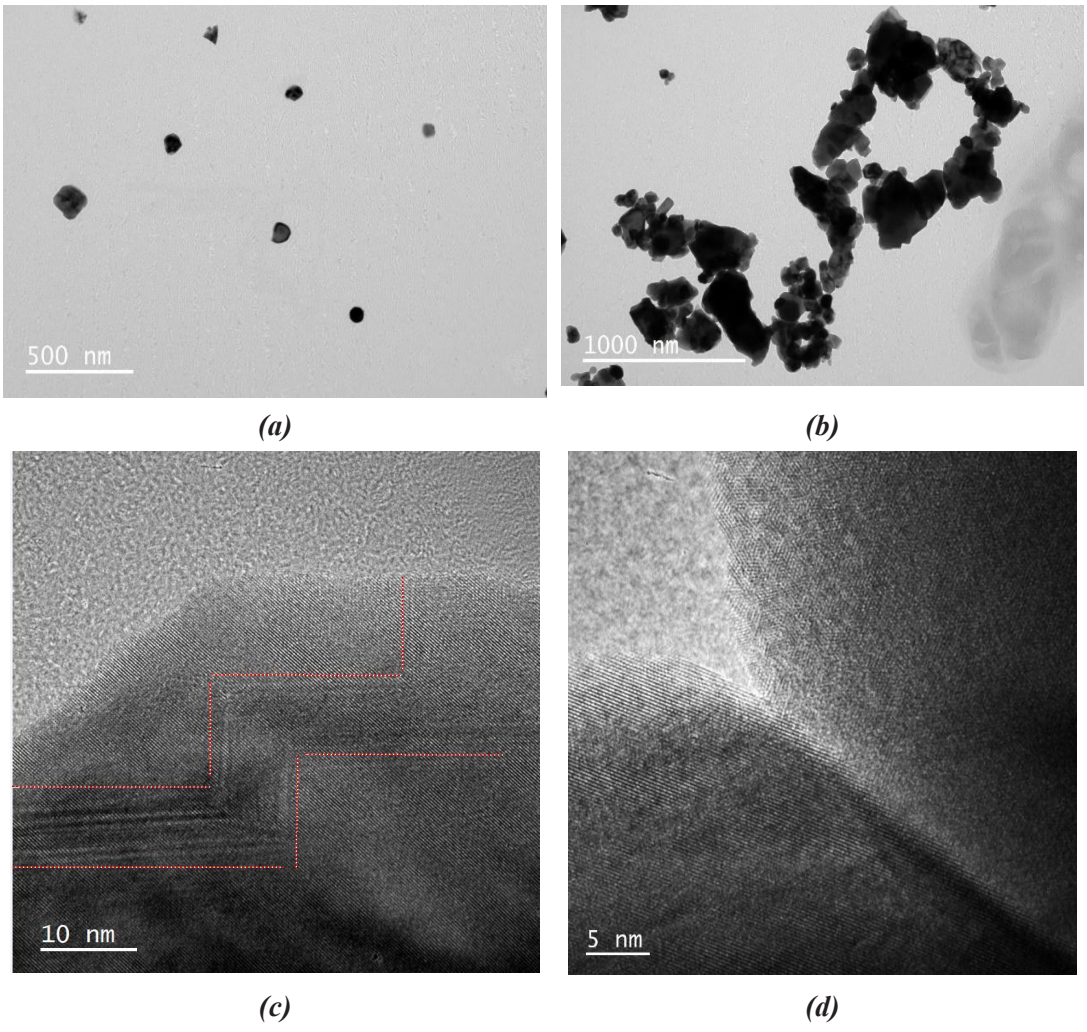
Figure 5-i shows that the surface image obtained by collecting secondary electrons (SE) scattered from the surface of a conductive polycrystalline material at 10kx magnification is given. To determine whether any impurities were present in the elemental analysis of this material, the image of the same area in the figure j shape at the same magnification was detected by commissioning the BSE detector. As can be seen from the SEM image, it is observed that the color tone is consistent throughout the image. The absence of different colored atoms anywhere indicates that the same atoms are present throughout the material, suggesting a compositionally stable material consisting of a single structure. If brightly colored regions or contrastingly different colors (grains) were observed in this image, it would indicate the formation of a separate phase, independent of the structure of these grains.

For instance, figures k and l demonstrate, with the help of BSE images, that three structures in different phases are formed on the surface of the material. The very light-colored particles represent one phase, the light-colored rods represent the second phase, and the interlocking structure at the bottom represents the third main phase. EDS analysis

performed while examining this material may not provide clear elemental values for these three phases. This is because the incident electron beam passes through the top particle and descends to a certain depth; as a result, the detector will collect various reflections from the main phase at the bottom. The most suitable analysis method to determine the composition values of these three phases would be either X-ray diffraction pattern analysis or repeating the EDS analysis by separately collecting these three phases from the surface of the material with an extremely thin focused-ion beam (FIB) integrated another SEM device.

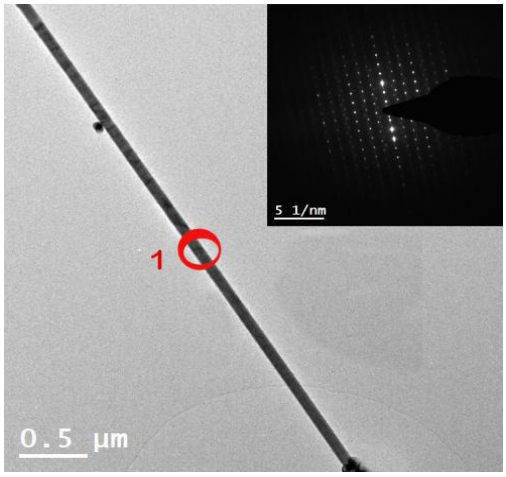
Sample Images Taken on a TEM Device to Illustrate Its Working Principle

Figure 6.
TEM Images (a, b, c, d, e, f, g, h) Of Specially Prepared Materials

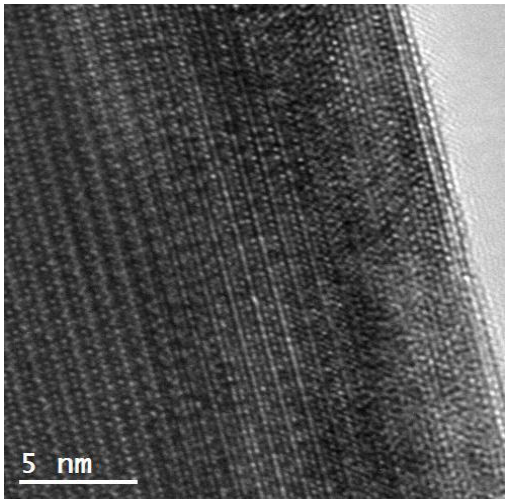


(a) *(b)*

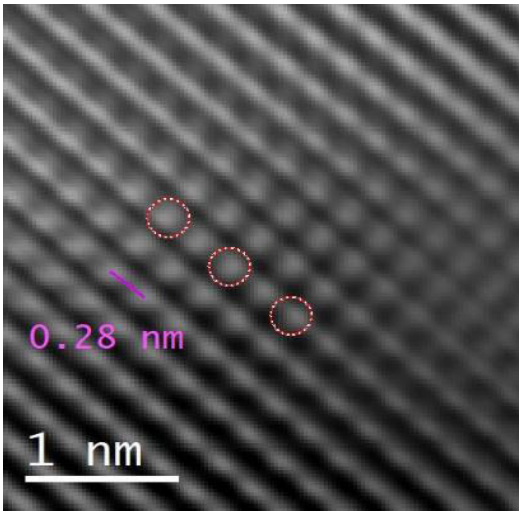
(c) *(d)*



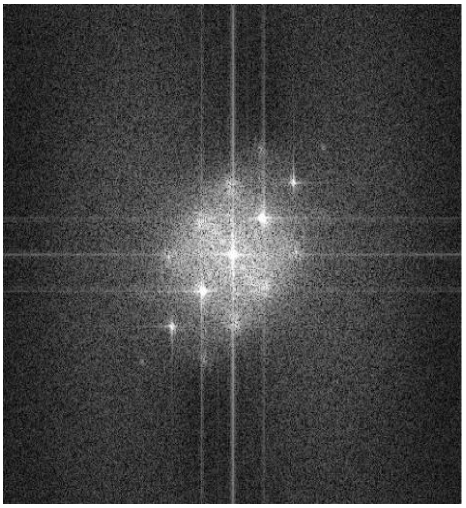
(e)



(f)



(g)



(h)

In the TEM images above, we see that there are different magnifications and different materials. In this section, we will briefly discuss what the images mean and their features. Figure 6-a: It can be seen that there are circular particles that stand out at first glance and that these particles are located on a 500 nm scale. This situation is also seen in the image in Figure 6-b. The 1000 nm scale image was taken with the help of a TEM device to obtain information about the initial general structure of the examined material. The difference between Figure 6-b and 6-a is that there is an obvious clustering. Since this clustering occurs mostly in magnetic materials, it reveals the necessity of distributing such nanosized particles evenly on the grid and performing the drying process properly while taking the TEM image. You can think of a grid as a very small sieve with certain properties. Alcohol etc., the sample to be examined is placed in liquid and dropped onto the TEM grid, and after drying, it is carefully placed in the vacuum system with the help of a sample holder. Since the electron beam passes directly through the sample, cooling is carried out with the help of liquefied nitrogen to prevent deformations in the sample holder part. As you may remember from the previous sections, electrons accelerated by applying very high voltage are used in TEM. (Figures 6-a and 6-b correspond to a magnification ratio in the corresponding SEM range of 50 kx-100 kx)
Figure 6-c: Here we see the atomic dislocations (a different atom entering the structure and disrupting the structure while the atoms are arranged in the crystal structure) obtained when examining a polycrystalline material. If you pay attention, the arrangements of the crystals at the atomic level are clearly understood with the 10nm scaling scale. When

we take a high-resolution (HR) image on the TEM device, the locations of the atoms and the arrangement in which these atoms are located can be determined.Figure 6-d: The grain boundaries of two nanosized particles are shown on the HR-TEM image. This image, which we cannot see at maximum magnification with macro-scale SEM analysis, provides clear information about the new material created. The HR-TEM image helps us make interpretations of how these grain boundaries affect the electron transition in the structure. Likewise, by determining the distance between atoms, information about the crystal structure of the produced material can be obtained.
Figure 6-e: A single nanorod stands out. After this nanorod was produced under special conditions, its diffraction pattern was taken on the TEM device (black image white dots) to obtain information about its crystal structure. Region 1 was selected for the diffraction pattern (with the help of the selected area aperture). Each of the points in the resulting image corresponds to a crystal plane in the diffraction pattern. That is, they are reciprocal lattice points. The real image of how the atoms are arranged for the same nanorod is shown by taking the HR-TEM image in Figure 6-f. What should be noted here is the formation of atomic planes and the symmetrical repetition of the dimensions of some planes. When the material is produced, the selected elements are bonded to each other to form a new composite, and atomic arrangements in the form of large atoms or small atoms can be distinguished due to the difference in the radius of the atoms. This allows us to perform nanoscale engineering at the scale we want. Figure 6-g: A high-resolution image taken on the TEM device is processed with the help of a computer program to create a Fast Fourier Transformation (FFT) image. Figure 6-h shows the masking process of this image. If you notice in this FFT image, the lengths of the interatomic planes and the distances between the atoms can be measured. Each atom depicted as a circle interacts with another atom, and the distance between atoms was determined to be approximately 0.28 nm. This information provides the most accurate understanding of the material's internal structure or crystal structure.

Conclusion

Microscopy is a powerful tool developed to perform analyses at a scale that the human eye cannot see (micro-nanometer level). Light microscopes are limited by the resolution of light, which is constrained by its wavelength. In pursuit of higher resolution, scientists turned to electrons, which could replace light rays and provide atomic-level resolution. By accelerating electrons under high vacuum, they achieved unprecedented resolution. The acceleration voltage of the electron beam varies depending on the applied voltage value, and the wavelength of the resulting electron beam also changes accordingly. While SEM can utilize acceleration voltages of 3-30 kV, TEM can employ voltages up to 200 kV. While SEM provides information about the surface of a material, TEM reveals its internal structure. Analyzes can be made with the help of many detectors in the SEM device. The most important condition for performing these analyses is that the material examined is conductive. The most important condition for conducting these analyses is that the material under examination is conductive. When examining an insulator material, the electron beam scanning the surface is discharged, making image acquisition extremely challenging. Therefore, if the materials to be examined exhibit insulating properties, they are rendered conductive by coating them with various conductive metals. In contrast, the TEM device allows for adjusting the particle size and acceleration voltage of the electron beam according to the type of material being examined, regardless of its conductivity or insulation.

The energy of the electron beam is crucial for both microscopes. When tungsten filament is chosen as the electron source, a stable electron beam is formed, but its lifespan is short (about 3 months). Additionally, it provides low brightness (contrast value) and low resolution (maximum magnification of 80kx). If a single crystalline LaB_6 filament is selected for the electron source, it offers a long working time (3-4 years working interval) along with a stable electron beam. It also delivers high contrast value and high resolution. In microscopes utilizing FEG filaments, a more stable electron beam

is produced. This enhanced stability is particularly beneficial for examining specific proteins in TEM devices, enabling the analysis of single particles and the acquisition of 3D and tomographic images. As you are aware, higher-energy electrons are more likely to damage the sample. Therefore, when examining biological samples in both SEM and TEM devices, it is essential to employ FEG to achieve a more stable electron beam at lower energies.

Humankind's insatiable curiosity, fueling the path to innovation, drives the continuous creation of groundbreaking inventions and novel devices. This unwavering quest has led to the creation of ever-more useful tools, devices, and electronic structures, highlighting the importance of microscopy in various fields, from healthcare and food production to textile manufacturing and mineral processing. Technology, empowered by microscopy, has permeated every aspect of our lives, facilitating progress and enhancing our understanding of the world around us. Detector technologies, developed to elucidate the interactions between electrons and matter, play an integral role in our daily lives, from mobile devices to automotive engineering and logistics. This innate human desire to explore first turned its gaze towards the heavens, leading to the invention of the first telescope, which brought distant celestial objects within our reach. Similarly, the advent of microscopes marked the beginning of an extraordinary journey of exploration into the microcosm, igniting a profound curiosity about the wonders that lay hidden within the realm of the infinitely small. As we strive to unravel the intricacies of the world at the atomic level, to validate theoretical calculations against empirical observations, and to decipher the mechanisms that govern the natural world in its intricate detail, our reliance on microscopy will continue to grow. With each advancement in resolution, the universe unveils an ever-expanding tapestry of scientific knowledge, beckoning us to delve deeper into its boundless mysteries.

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