ПРИЛОЗИ, Одделение за природно-математички и биотехнички науки, МАНУ, том **43**, бр. 1–2, стр. 27–37 (2022) CONTRIBUTIONS, Section of Natural, Mathematical and Biotechnical Sciences, MASA, Vol. **43**, No. 1–2, pp. 27–37 (2022)

Received: August 30, 2022 Accepted: November 3, 2022 ISSN 1857–9027 e-ISSN 1857–9949 UDC: 537.533.35-027.22 DOI:10.20903/masa/nmbsci.2022.43.13

Original scientific paper

APPLICATION AND IMPORTANCE OF SCANNING AND TRANSMISSION ELECTRON MICROSCOPES IN SCIENCE AND TECHNOLOGY

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Recent developments in nanoscience and technology have increased the importance of electron microscopes, specifically the scanning electron microscope (SEM) and transmission electron microscope (TEM), in almost all fields of science and technology. Electron microscopes provide information such as morphology, composition, crystal structure, and crystal phase. They have the capability to confirm the formation of required products and also provide information on any defects that may occur in the product. SEM and TEM have been used in various disciplines including chemistry, physics, biology, pharmacy, archaeology, materials science, agriculture, soil science, environmental science, forensic sciences, civil engineering, and electronic engineering. All these features and advantages of electron microscopes contribute to their importance in academic as well as industrial research, thereby enriching the development and growth of nanoscience and nanotechnology.

Key words: SEM; TEM; nano-materials; nano-science; nanotechnology

INTRODUCTION

Nano-science, nanotechnology, nanostructure, and nanoparticles are now very common and frequently used terms in the literature of all fundamental sciences and technologies. Nano-science and nanotechnology pertain to the creation, study, and application of systems, materials, and devices that can control matter in the range of 1–100 nm for the betterment of the human future. The concept of nanotechnology can be referred to as the construction of miniature machines, ultra-precision processing, robots, supercomputers, molecular devices in the nanometric range, and their application in science and technology. This nanorange strongly affects their properties and behaviour [1]. Thus, in the current era, nanotechnology is considered the superior technology for all fields of study. In this regard, the investigation of materials at the nano level is highly recommended to understand their properties. However, there are serious challenges in the production of nanotechnology-based products, such as controlling size, distribution, uniformity, crystal structure, lattice type, morphology, and confirming these aspects, which require highly sophisticated characterization techniques [2, 3]. The scanning electron microscope (SEM) and transmission electron microscope (TEM) are highly sophisticated techniques in science and technology that provide morphological, elemental, and crystallographic information about a sample. They have been developed into vital tools for a better understanding of material properties and interpreting their behavior [4, 5].

The scanning electron microscope is actually based on the surface scanning of a sample with the help of a focused electron beam, while the analysis is made from the signals obtained from the interaction of electrons with the sample [4], a schematic diagram is shown in scheme 1. The electronic interaction in the scanning electron microscope helps to develop images with high magnification and large resolution, surpassing those achieved by a light microscope due to the distinct wavelengths of light and electrons. SEM also possesses the capability to analyze the chemical composition of a sample, enabling qualitative and quantitative measurements of elements [6, 7]. In the SEM, electrons are generated in an electron gun and then focused on the sample with the help of a condensing lens and beam-defining aperture to fall on the sample. Once these electrons strike the sample with their high energy, they eject secondary electrons from the sample. These secondary electrons are captured by a detector and converted into an image with the help of a computer. They provide very important information about the sample's surface morphology, crystallography, composition, and other properties. The SEM has high resolution, which may be due to the electron beam diameter and excitation volume. Similarly, electrons produced with high energy experience elastic collisions with the atomic nuclei of the sample, resulting in backscattered electrons. Backscattered electrons are extremely high-energy negatively charged particles that are usually used to produce images with greater pixel density and resolution, showing the dispersion of numerous elements contained in a specimen. Consider an exoplanet that rotates around a planet but then starts moving back into space again without being affected by the planet's gravitational effects. Similarly, whenever the specimen is exposed to high-energy electrons, a portion of these negatively charged subatomic particles will not be absorbed by the positively charged nucleus due to the influence of its centripetal force and outward pull of the atom. Instead, they will be back-reflected, resulting in "backscattered" electrons from the specimen. The amount of scattering electrons produced varies with the specific atomic mass of each element. Therefore, elements with larger atomic weight and larger nuclei, which correspond to denser materials, significantly deflect incident electrons more strongly than smaller components. As a consequence, heavy nuclei such as silver (Ag) with an atomic number (Z=47) appear brighter in SEM micrographs compared to light nuclei, such as silicon (Si) with an atomic number (Z=14), even though a large number of backscattered negatively charged particles are emitted from the surface of the specimen. These electrons are detected by a specialized detector and provide information about the local crystallographic structure of the sample. Additionally, when electrons are ejected, they also generate X-rays, which can be utilized for quantitative and qualitative

analysis of the elements present in the sample. This technique is known as X-ray microanalysis, also referred to as wavelength-dispersive spectroscopy (WDS) or energy-dispersive spectroscopy (EDS). In SEM, structural defects and trace element composition can be confirmed by examining the light emitted from the sample, which is a result of the cathodoluminescent phase. This analysis is known as cathodoluminescent (CL) imaging analysis. High resolution and nanoscale investigation are the most attractive benefits of SEM. However, SEM is not recommended for samples that require high pressure or special preparation protocols due to their composition. The environment inside the column of SEM and TEM plays a crucial role in these electron microscopes. The production or monitoring of the electron beam in TEM and SEM relies on maintaining adequate vacuum. The presence of oxygen, nitrogen, or other particles can significantly reduce the operating life of the filament. This can be likened to allowing air into a tungsten filament of a light bulb, which would cause it to burn out. Although these gaseous particles in the SEM and TEM vacuum chamber can also serve as samples, they diffuse the intensity of the electron beam when struck by the negatively charged electrons. Recently, environmental SEM (ESEM) has been developed to address this issue for sensitive samples, such as biological samples. ESEM has the capability to maintain the sample at moderate pressure while keeping the electrons at high pressure, allowing for easier study of the sample. This requires special protocols in SEM [6, 8].



Scheme 1. Schematic diagram of the core components of an SEM

The transmission electron microscope (TEM) is designed similarly to a light transmission microscope, with the only difference being that light is replaced by electrons [9]. In TEM, electrons are generated in an electron gun and then focused on the sample using condenser lenses and an aperture. The electron beam strikes the sample and transmits through its thin surface, creating an image on the objective lens. This image is further magnified by passing it through the projector lens, which adjusts its strength. In the case of a crystalline sample, a diffraction pattern is formed in the lens known as the back focal plane [7]. A schematic diagram illustrating these concepts is shown in Scheme 2. Dark field (DF) and bright field (BF) imaging techniques form the basics of TEM. High-resolution TEM (HRTEM) allows for the examination of atomic structures and crystal information through the interaction of electrons with the crystal planes of the sample. Additionally, selected area electron diffraction (SAED) provides valuable insights into the crystal structure and the presence of crystalline or amorphous regions in the sample.



Scheme 2. Schematic diagram of the core components of a TEM

In order to define the sample area, scanning transmission electron microscopy (STEM) can be applied, and energy-dispersive X-ray spectroscopy (EDS) and Electron Energy Loss Spectroscopy (EELS) can provide comprehensive information for qualitative and quantitative elemental analysis of the sample, similar to SEM. Similarly, chemical analysis can be performed using energy-filtered TEM (EFTEM), where electrons with specific kinetic energies are employed for investigative study. To utilize these techniques effectively, a complete and detailed understanding of the sample is necessary in order to better comprehend its composition, texture, structure, morphology, and properties for various applications [7, 9–11]. TEM has the capability to serve as a complementary characterization technique to X-ray diffraction (XRD) and provide valuable crystal information that can support XRD data. However, TEM can be considered superior to XRD due to its additional features. In addition to crystal information, TEM can provide comprehensive information about the morphology and chemical composition of a sample, both qualitatively and quantitatively. It can provide insights into crystalline, pseudo-crystalline, and amorphous samples, similar to XRD [12–15]. Additionally, electron tomography is a technique that utilizes TEM to generate a threedimensional image of a targeted sample by capturing images at different tilt angles around a single axis. This technique is particularly significant in paleontology as it enables the reconstruction of the internal structure of microorganism fossils [8]. The primary distinction between SEM and TEM lies in their imaging mechanisms. SEM generates images by scanning the surface of the specimen and detecting reflected, bounced-back, or knocked-off electrons. On the other hand, TEM generates images by measuring the density of transmitted electrons that pass through the specimen. As a result, TEM provides crucial information about the internal composition of the specimen, including lattice parameters, topography, and stress conditions. In contrast, SEM provides insights into the external surface and stoichiometry of the specimen.

KEY APPLICATIONS OF ELECTRON MICROSCOPES

Electron microscopes possess the capability of high resolution, allowing for the determination of crystal structures as well as the aggregation of atoms in various nanostructures such as nanowires, clusters, nanoparticles, and thin films. They are also adept at detecting and characterizing phenomena such as cracking, dislocations, and surface contamination in samples. Furthermore, electron microscopes provide valuable information about polycrystalline and ceramic materials [16].

SEM can be applied to conduct a detailed topography study of a sample, providing microstructural information such as material size, distribution,



and density. It also offers valuable insights into morphology, particularly in terms of 3D morphology, including the number and shape of materials, as depicted in Figures 1a, b, 2a, b, and 3a-c.

Fig. 1. (a) SEM (b) TEM and (c) HRTEM images of Ni-GCS. Reproduced with permission from Ref. [17] (Copyright 2020, Springer.)



Fig. 2 The morphology and crystal structure of CMO@NC/450 sample: (a, b) SEM, TEM and HR-TEM images (inset showed corresponding SAED pattern); images; (d-e) and (fei) EDS elemental mapping (Scale bar: 2 mm). Reproduced with permission from Ref.[18] (Copyright 2020, Elsevier B.V.).

Moreover, SEM facilitates the examination of porosity within the sample, including its size, tortuosity, and distribution. It is also useful for investigating cracks, including their size, length, shape, and interconnection. Additionally, SEM, aided by energydispersive spectroscopy (EDS) as shown in Figures 2f-i, enables qualitative and quantitative analysis of elements present even in trace amounts in the sample. electron backscatter Furthermore, diffraction (EBSD), a specialized technique in SEM, is instrumental in determining crystal structures, orientations, morphologies, and sizes of individual grains, as well as in alloys and composites, and their crystallographic relationships with other phases. [16].

Similarly to SEM, TEM also enables chemical and structural analysis of samples with high magnification and resolution. While SEM is limited to a pixel density and resolution power of 0.5 nm, TEM has confirmed the capture of photos with ultrahigh resolution as low as 50 pm. Moreover, the magnification power of TEM surpasses that of SEM significantly. Transmission electron microscopes can enlarge specimen sizes by over 500 million times, whereas the optical zoom of SEM is restricted to 1–2 million times. Techniques with atomic resolutions such as HAADF, STEM, and HRTEM are employed in the analysis of crystal structures, interfaces of different phases, and grain boundaries, as illustrated in Figures 1c, d, 2c-e, 3d, e, and 4.



Fig. 3 (a-c) SEM image of the Ni encapsulated CNTs at 600, 650 and 700 _C
(d) TEM image of the Ni encapsulated CNTs at 650 _C (e) HRTEM image of the Ni encapsulated CNTs at 650 _C Reproduced with permission from Ref. [19](Copyright 2020, Elsevier B.V.).

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Fig. 4. (a-b) TEM image of CMC/750SA; (b) Inset: Particles size distribution of CMC/750SA; (c-f) HR-TEM image of CMC/750SA catalyst and distance between lattice fringes; (g-h) HAAF elemental mappings; (i) molybdenum; (j) cobalt; (k) oxygen; and (l) carbon (Scale bar = 200 nm). Reproduced with permission from Ref. [20] (Copyright 2020, Elsevier B.V.).

Related electron diffraction techniques can be utilized to quantitatively analyze the crystal structure and corresponding lattice parameters, as demonstrated in Figures 3e, 4e, and 5e, f. These techniques also aid in identifying imperfections such as dislocation cores and stacking faults. Additionally, EDX and EELS are employed to assess homogeneities and associated modulations, including amorphous layers in grain boundaries, precipitation, and surface impurities. Elemental mapping provides comprehensive information about the presence of all elements and their distribution, confirming the homogeneity within the sample, as shown in Figures 4g-l. TEM has the capability to determine surface morphology, including carbon sheets/graphene, nanoparticles, differentiation of carbon nanotubes and nanowires, as well as particle sizes in the sample, as depicted in Figures 1c, d and Figures 3d.

It helps in understanding the chemistry of 3D morphology and the encapsulation of materials, as demonstrated in Fig. 1d and Fig. 3e, which is particularly beneficial in minimizing leaching of active materials and in drug delivery applications. Additionally, it provides comprehensive analysis of electronic, polymeric, and semiconductive devices, as well as their related chemistry, to assess their functionality [16]. In

addition, SEMs generate highly detailed 3D images of the external surface of the specimen, while TEM analysis provides 2D views of the specimen. This difference in imaging can sometimes make it challenging for researchers to correlate their findings between the two techniques, as depicted in Fig. 1 and 2. However, all characterization techniques, including SEM, TEM, and XRD, are complementary to each other and offer mutual support. SEM, TEM, and XRD provide similar information, but with different emphases. XRD primarily provides information on the composition and crystal structure of the sample, while SEM and TEM excel in providing detailed information about the topography and morphology of the sample. However, these techniques still benefit from cross-validation and support from one another to confirm sample characteristics and enhance the understanding of the sample's chemistry for its application in the field of science and technology.

Importance of electron microscopes in science and technology

In the last two decades, great advancements in nanoscience and nanotechnology have been made, which have greatly increased the importance of electron microscopes due to their effective application in nanoscience and technology.

Electron microscopes in biological and medical science

The current advancement of nanoscience, technology, and their long-term effects has also attracted the biomedical field for the synthesis of nanodrugs/nanomedicine and their application in medical sciences. In this regard, recently, 22,000 new articles focusing on nanomedicine for cancer treatment have been published, and all of these articles have utilized electron microscopes to study the medicines and their effects on cell death and necrosis. The investigative studies primarily concentrate on the morphology and structure of nanomedicine, its relationship with cytoplasmic organelles, and its impact on cell death or damage. TEM has the capability to determine the physiochemical characterization of freshly synthesized samples and assess the effects of nanomedicine on biological organisms, providing valuable information for effective therapeutic and diagnostic strategies. Thus, histochemists and biologists have always supported physicists, chemists, and pharmacologists in the synthesis of nanostructures/nanomedicine. This technique is convenient for investigating nanovector-based therapies aimed at treating affected cells that require preservation and functional repair. Following drug delivery, ultrastructural cytochemistry describes any changes in morphofunctional features, providing evidence of negative effects or restoration of healthy characteristics. All of this information requires an appropriate cell system and the support of biologists for those involved in nanoscience/technology [21]. Electron microscopes can be used to investigate histopathology and determine abnormalities in cells and tissues. Additionally, techniques such as EDX and EELS can be applied to identify significant issues in biological samples [22]. Colloidal gold particles function as specific complementary DNAs when attached to DNA [23], and DNA microchip can be used for mapping genetic information in RNA and DNA. Nanosensors can be applied to understand the effects of different stimuli on living organisms and to determine various environmental issues and their effects [24]. In addition, these techniques can be used to investigate the topography, morphology, crystallinity, and composition of newly developed drugs. They can also help in identifying any abnormalities in the new drug and quantitatively determining its ingredients, even in trace amounts. The importance and application of electron microscopes remain significant in this area of study. Sensitive (biological) samples can be treated specially and studied under electron microscopes to examine abnormalities, depositions, functions, treatments, improvements in health, side effects, and cell death, among other factors.

Electron microscopes in archeology

SEM has the capability to investigate various materials for archaeological applications, such as faience, stone, metals, glass, soil particles, pottery, bone, fingernails, hair, teeth, skin, eggshell, mollusks, wood, insects and parasites, plant remains, pollen, fibers, pigments, and more. Electron microscopes can be used to qualitatively and quantitatively determine the composition of these materials and identify decay or other structural and compositional defects. Electron microscopes can quickly determine the crystal structure and phase of the materials in a nondestructive manner. In particular, SEM does not require extensive sample preparation protocols and can provide sufficient qualitative information about the constituents of the sample. TEM can also be used to investigate the internal structure of samples, especially thin fossils or samples collected from archaeological sites, without damaging them [25].

Electron microscopes in earth sciences

Electron microscopes have the advantage of high resolution, allowing for in-depth investigation of samples. This is particularly beneficial in the field of earth science, as electron microscopes enable direct analysis of non-conductive samples without the need for pre-treatment. Electron microscopes provide detailed imaging of the sample surface, as well as analysis of its crystal structure and composition. The direct examination of samples in their natural state eliminates the requirement for pretreatment. When combined with energy-dispersive X-ray spectroscopy (EDX) and backscattered electron imaging (BSE), electron microscopes can provide accurate information about the presence and concentration of all elements in the sample. Line scanning and mapping techniques further aid in the determination of migration patterns, mineral alteration, and growth conditions. EBSD can determine phase changes in minerals, grain orientation, and the crystal structure of the sample. Electron microscopes have been instrumental in investigating zircon and providing essential information for in-situ U-Pb dating of zircon. These microscopes have demonstrated excellent accuracy in analysis. Advanced features, such as cathodoluminescence (CL), can be employed in electron microscopes to study

luminescent samples under electron irradiation. This technique provides more detailed information about trace elements, their distribution, and lattice defects. [26]. Similarly, the electron microscope has been applied to investigate the composition and quality of coal. Thanks to its capabilities, the electron microscope can be used to determine the qualitative and quantitative composition of each ingredient in coal, which serves as a basis for assessing coal quality. This qualitative and quantitative analysis is crucial in determining the production of pollutants that occur after coal combustion [8]. In addition, electron microscopes can be utilized for qualitative and quantitative analysis of various minerals to determine structural defects, composition, and assess their quality. These powerful instruments provide valuable insights into the internal structure and characteristics of minerals, enabling researchers to study their properties in detail and make informed assessments of their quality.

Electron microscopes in engineering

Nanotechnology is an emerging field of science and technology that finds applications in civil engineering. In civil engineering, nanotechnology can be utilized to reduce concrete segregation, incorporate copper nanoparticles in low heat Portland cement, develop nanosensors for monitoring earlyage concrete properties, and design granules for water purification [27-29]. Nanotube-based transistors and nanomaterial-based semiconductors serve as the foundation for advanced microchips, which are fundamental to current technology [30]. Additionally, nanotubes can function as intermolecular wires in electronic devices[31]. Nano base semiconductor especially III-IV nitride such as InGaN as laser diods and LEDs are impressive technology while quantum dots and wires have numerous uses [32]. To better understand the shape, morphology, surface feature, structure, composition, and its relation with its properties is superior side of civil engineering. To get deep information about structure, morphology, composition and its relationship with application can be done with electron microscope. In addition, electron microscopes can be applied to study the microchips, transistor and diodes which are the prime part of electronic engineering in current society. Electron microscopes can determine the composition and structure of microchips, transistor, diodes, and their defect if it happens to these chips during development [33]. Basically, the study of materials for the construction of these chips, transistor and diodes can be done with electron microscopes and provide its structure, composition, crystallinty, and its relationship with conductivity for electronics application.

Electron microscopes in food/agricultural science

Electron microscopes have been applied to study the strength of eggshells and their relationship with palisade (directly proportional) in the field of food science. SEM and TEM have confirmed that frozen and concentrated egg yolk has an open surface structure compared to normal egg yolk, and any change in concentration leads to a change in its shape and organization. The freezing process may aggregate lipoproteins in low and high-density egg yolk, and this aggregation may develop a 3D structure and increase its viscosity. Electron microscopes have confirmed that tomato production can be increased with increased potassium, and potassium is independent of magnesium and calcium, showing an inverse effect. It has also been confirmed that starch gelatinization takes place inside cell units, other cell components disperse, and cells separate without rupture, and denaturation of proteins causes food swelling during the cooking process. Furthermore, it has been confirmed that urea-containing food supplements are toxic if they contain 50g of urea per 100 kg of supplement [34]. In addition, the electron microscope can also confirm the composition and concentration of synthetic or natural food, thereby assessing its structure, morphology, and effects on taste and quality. The study of the relationship between different substances and the quality and quantity of food, as well as the effects of various supplements on food production, is also a part of this scientific field. In addition, the electron microscope can also confirm the composition and concentration of synthetic or natural food, thereby assessing its structure, morphology, and effects on taste and quality.

Electron microscopes

in materials/chemical/physical/natural sciences

It is well known that the science and technology of nanomaterials is an interdisciplinary field of study encompassing materials science, chemistry, physics, biology, and engineering. These disciplines are interconnected, and scientists from these backgrounds must collaborate in the fields of nanoscience, nanomaterials, and nanotechnology. This technology finds applications in various sectors such as electronics, space exploration, industry, chemicals, medicine, and healthcare, benefiting humanity. Material chemists play a significant role in the design, assembly, synthesis, and catalysis of these materials [32]. The sol-gel and aerogel methods have been developed to create materials with high surface areas and exceptional absorbent properties, making them effective in the removal of toxic substances [35, 36]. Nanocomposites have resulted in the production of materials with ultrahigh toughness, strength, and ductility, which find applications in various fields such as cement, ceramics, plastics, textiles, and novel magnets. Nanomaterials, characterized by their nanosize and unique morphology, exhibit exceptional catalytic properties and are utilized in capacitors, metal-ion batteries, and other industrial applications [37]. Nanoporous materials, polymers, and nanotubes exhibit excellent applications in catalysis, storage, purification, and lubricant applications [30, 32, 38]. In order to gain a better understanding of nanomaterials, including their surface topography, morphology, and size, electron microscopes such as SEM and TEM are crucial. These electron microscopes are necessary for investigating the crystalline and amorphous structures of materials and understanding their applications in catalysis, adsorption, capacitance, and batteries. Electron microscopes also enable quantitative and qualitative analysis of materials, allowing researchers to determine their effects on various applications. They can easily investigate the agglomeration of active materials, which can impact their performance. Additionally, electron microscopes are capable of studying hetero-junctions, the interaction between metals and supporting materials, and their effects on applications. Furthermore, electron microscopes can provide valuable insights into the crystal structure, crystal phase, and atomic arrangement within crystals.

Electron microscopes play a crucial role in forensic science by allowing for in-depth investigation of samples to gather evidence. They have the capability to analyze even trace amounts of samples and provide valuable information about compounds, compositions, and concentrations. This makes electron microscopes an indispensable tool in forensic investigations. The significance of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) extends beyond forensic science and extends to all fields of science and technology. Their importance cannot be denied in today's society, as they enable researchers and scientists to gain a deeper understanding of materials, structures, and phenomena at the nanoscale. Electron microscopes have revolutionized scientific research and continue to play a vital role in advancing various disciplines.

CONCLUSION

The emerging field of nanomaterials has opened new avenues in science and technology, leading to significant advancements in recent years. One crucial aspect of this field is the confirmation of required materials, and in this regard, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have gained extensive attention. These techniques provide sophisticated information by enabling investigations at the subnanoscale range and detecting trace elements in the sample. Moreover, they offer advanced insights into morphology, composition, crystal structure, and defects of the desired products. The applications of SEM and TEM span across various disciplines, including chemistry, physics, biology, pharmacy, archaeology, materials science, agriculture, soil science, environmental science, forensic science, civil engineering, and electronic engineering. Importantly, the data obtained from electron microscopy can be compared with results from other techniques for confirmation, as these techniques are complementary. The numerous features and advantages of electron microscopy highlight its significance in both academic and industrial research, contributing to the development and growth of nanoscience and nanotechnology.

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ПРИМЕНА И ВАЖНОСТ НА СКЕНИРАЧКАТА И ТРАНСМИСИОНА ЕЛЕКТРОНСКА МИКРОСКОПИЈА ВО НАУКАТА И ТЕХНОЛОГИЈАТА

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Најновиот развој на т.н. нанонауки и технологии ја истакнаа важноста на електронската микроскопија, особено на скенирачката и трансмисиона електронска микроскопија во многу области на науката и технологијата. Електронската микроскопија нуди информации за морфологијата на материјалите, нивниот состав, кристалната структура и постоењето различни кристални фази. Со оваа техника може да се докаже дека се синтетизирани нови материјали со посакувани особини, но и да се откријат дефекти во нивниот состав и структура. Скенирачките микроскопии се користат во многу научни полиња, како што се хемија, физика, биологија, фармација, археологија, наука за материјали, земјоделство, наука за почвата, наука за животната средина, форензичка наука, градежништво, електронска индустрија итн. Многуте предности на овие техники придонесуваат за нивната важност и неопходност во светот на академските истражувања, а и во индустријата, што сè заедно овозможува особен развој на нанонауката и нанотехнологијата.

Клучни зборови: скенирачка електронска микроскопија; трансмисиона електронска микроскопија; наноматеријали; нанонаука; нанотехнологија