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APPLICATION OF ELECTRON MICROSCOPY IN ANIMAL SCIENCE RESEARCH

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Abstract

Electron microscopy is a seminal research technique that facilitates analysis of structures at the atomic level. The development of the electron microscope can be traced back to the 1930s, when Ernst Ruska and Max Knoll constructed the first such instrument, utilizing a beam of electrons in lieu of visible light. Since that time, significant advancements have been made in the field, resulting in the development of a variety of microscopes, including TEM (transmission), SEM (scanning), STEM (scanning-transmission), SBF-SEM (serial block-face scanning electron microscope), FIB-SEM (focused ion beam SEM), and SEM-AT (scanning electron microscopy array tomography). The operating principle of electron microscopes is based on the emission of electrons, which are accelerated and focused by electromagnetic lenses. In the transmission electron microscope (TEM), electrons are permitted to pass through the specimen, thereby creating an image of its internal structure. In the scanning electron microscope (SEM), reflected electrons are analyzed, thus enabling the generation of three-dimensional images of the surface. A STEM microscope is a sophisticated instrument that integrates the capabilities of a transmission electron microscope (TEM) and a scanning electron microscope (SEM), employing both transmitted electron and scanning of the sample surface techniques. This approach enables not only the observation of the sample's structural arrangement and the imaging of individual atoms, but also the analysis of its chemical composition. SBF-SEM, FIB-SEM, and SEM-AT microscopes facilitate the spatial imaging of the material under examination, predominantly based on the principle of electron reflection. The preparation of samples for electron microscopy is an exacting process that requires the application of specialized methodologies. These include ultrathin sectioning, deposition on metal grids, chemical contrasting and sputtering of conductive layers. The implementation of these techniques is essential to ensure the acquisition of high-quality imaging data. In the field of animal science, electron microscopy is employed in the study of the structure of animal cells, the analysis of the composition of feed,

and the diagnosis of diseases. The technology under discussion enables the precise determination of tissue microstructures, the identification of pathogens, and the assessment of the quality of biological materials utilized in the context of animal breeding. This technique also supports the development of biotechnology in animal science, enabling genetic research and control of animal health at the cellular level. Electron microscopy remains a pivotal instrument in both natural and technical sciences, with its ongoing development generating new possibilities in the domains of biological, material and animal science research.

Keywords: electron microscope, ultrathin sections, electrons, microscopic imaging

Introduction

The ability to observe objects at magnification in order to reveal details difficult for the human eye to discern has enabled the development of science, particularly in medicine and broadly understood biology. Technical progress over the centuries has led to an increasingly detailed understanding of the world, the description of new organisms, and the discovery of mechanisms operating in living systems. There should therefore be no doubt that the invention of microscopes was one of humanity's most important achievements, without which the development of science would not have been possible.

At the end of the sixteenth century, the Dutch opticians Hans and Zacharias Janssen built the first microscope, which made it possible to magnify an observed object tenfold. It consisted of two lenses: one called the eyepiece (the lens through which the object was viewed) and the other the objective lens (the lens directed toward the specimen) (Croft, 2006; Pluta, 1982). At the beginning of the seventeenth century, Galileo constructed a microscope consisting of three lenses; however, because it lacked a light source, observing the dark image was extremely difficult (Murphy and Davidson, 2013). Robert Hooke improved the device, among other things by adding a light source (La Berge, 1999). By observing the structure of cork, which reminded him of the arrangement of monks' cells in a monastery, he introduced the term "cell" into science. He described his observations in Micrographia, published in 1665 (Korczyński and Gacek, 2015). After reading Hooke's work, Antoni van Leeuwenhoek also began improving the microscope. By using transmitted rather than reflected light, he was able to observe objects at magnifications of up to 300× (Croft, 2006). This enabled him to observe protozoa, bacteria, erythrocytes and spermatozoa. In subsequent years, research on lenses and the properties of light contributed to improved resolution of observed objects. In 1893, August Köhler developed principles for specimen illumination, which made it possible to determine the theoretical resolution limit of the light microscope (La Berge, 1999; Croft, 2006; Bradbury and Bracegirdle, 1998; Mertz, 2009).

Discoveries at the turn of the nineteenth and twentieth centuries, such as X-rays, electrons and the theory of electron lenses, contributed to the invention of electron microscopes. In the 1930s, Ernst Ruska developed the transmission electron microscope (TEM), for which he later received the Nobel Prize (Datye, 2003). A few years later, Max Knoll constructed the scanning electron microscope (SEM) (Barbacki, 2003). In the late 1930s, Manfred von Ardenne developed an instrument combining elements of both microscopes, the scanning transmission electron microscope (STEM), which offered scientists new possibilities (Haguenau et al., 2003). Electron microscopes began to be produced commercially in the 1960s. Because they enable observation of objects smaller than 1 μm, electron microscopy became a key research tool in many fields of science.

Types of electron microscopes and their operating principles

The principal types of electron microscopes are the scanning electron microscope (SEM) and the transmission electron microscope (TEM), while modifications of these instruments enable three-dimensional reconstruction of tissues or cells and are collectively referred to as Volume Electron Microscopy (Volume EM or VEM) (Collinson et al., 2023). This group includes, among others, scanning transmission electron microscopy (STEM), SBF-SEM (Serial Block-Face Scanning Electron Microscope), FIB-SEM (Focused Ion Beam SEM) and SEM-AT (Scanning Electron Microscopy Array Tomography) (Peddie et al., 2022). The common feature of all electron microscopes is that imaging is based primarily on electron beams and their interaction with matter (Kelsall et al., 2008; Zhou et al., 2012).

The properties of electrons determine the specific design of an electron microscope, which differs from that of a light microscope (Table 1). A key requirement is the use of a vacuum of at least 10^{-4} Pa, because electrons interact with matter much more strongly than photons, i.e. quanta of visible light; therefore, light microscopes do not require a vacuum. High-vacuum conditions allow electrons to move freely. The resolving power of an electron microscope, which is much greater than that of a light microscope, results from the much shorter wavelength of accelerated electrons compared with visible light and ultraviolet photons. Another important property of electrons is their electric charge, which makes it possible to focus the beam using a magnetic or electric field (Kelsall et al., 2008).

Table 1. Comparison of selected features of light and electron microscopes

Feature	Light microscope	Electron microscope
Radiation source	Visible light	Electron beam
Lenses	Glass optical lenses	Electromagnetic lenses
Image	Viewed directly through the eyepiece	Displayed on a screen or recorded digitally
Operating environment	Air	Vacuum
Magnification	Up to approx. 1500×	More than 1,000,000×
Resolving power	Approx. 200 nm	Down to 0.1 nm
Objects observed	Cells, tissues, microorganisms	Subcellular structures, viruses, nanoparticles
Cost and availability	Inexpensive, widely available	Expensive, requires a specialized laboratory

The basic design of electron microscopes uses a cathode as the electron source (formerly, the electron gun most commonly used a tungsten cathode filament; today it is often replaced by a lanthanum hexaboride cathode) and an anode that attracts electrons (Hałas and Szymański, 1965). In the region between the cathode and anode, electrons are accelerated and the beam acquires energy equal to the product of the electron charge and the voltage between the cathode and anode (Van Meerssche and Feneau-Dupont, 1984). The beam is then focused in the so-called Wehnelt cylinder, which acts as an electrostatic lens. The focused beam is concentrated in the condenser and directed onto the specimen. Primary electrons originating from the electron gun interact with the sample, causing emission of different types of electrons that can be used to form an image or analyze the chemical composition of the material (Kelsall et al., 2008).

Scanning electron microscope (SEM)

In a scanning electron microscope, the image is produced by raster scanning the beam across the sample surface (Table 2). Electrons striking the specimen may be backscattered, producing a backscattered electron (BSE) signal. Inelastic interactions between the primary electron beam and the sample cause emission of secondary electrons (SE), which have lower energy than backscattered electrons (Barbacki, 2003; Kelsall et al., 2008). Scanning electron microscopy can also use electron backscatter diffraction (EBSD), absorbed electrons (AE) and X-rays,

which may be detected by measuring either wavelength (WDS) or energy (EDS) (Barbacki, 2003). The electron signal is converted by detectors into a digital signal and then into an image. The key detector components are the scintillator, which converts the energy of secondary electrons into light pulses, and the photomultiplier, which amplifies these pulses (Barbacki, 2003). The resulting image has a three-dimensional appearance; therefore, SEM is used to analyze surface topography and, owing to the emitted X-rays, the chemical composition of the sample (Table 2). SEM can achieve magnifications of several hundred thousand times. The accelerating voltage typically used in SEM ranges from 1 to 30 kV.

Transmission electron microscope (TEM)

In a transmission electron microscope, part of the electron beam passes through the sample (Table 2), while part is diffracted by the crystal lattice (Egerton, 2016). This takes place under high-vacuum conditions maintained by rotary and diffusion pumps connected to vacuum valves.

As in SEM, the electron source is a cathode. As electrons move toward the anode, they are accelerated and then formed into a beam by the so-called Wehnelt cylinder (Barbacki, 2003). The beam is subsequently shaped by electromagnetic lenses. Condenser lenses alter the beam diameter and focus, while the condenser aperture blocks scattered electrons. Below these components is the specimen stage, with the specimen positioned between the objective lenses. The adjacent objective aperture removes scattered electrons and is used to adjust contrast, whereas the selected-area aperture limits the region subjected to diffraction. After passing through the lens system (diffraction, intermediate and projector lenses), the beam reaches the projection chamber, where the image is formed and displayed on a screen by the projector system (Egerton, 2016).

Depending on the voltage used, two types of transmission electron microscopes can be distinguished: low-voltage instruments, in which the accelerating voltage reaches a maximum of 500 kV, and high-voltage instruments, in which it can reach up to 3 MV (Reimer and Kohl, 2008). Low-voltage TEM enables observation of specimens thinner than 0.1 μm, whereas high-voltage TEM can accommodate specimens slightly below 10 μm thick. TEM can be used to observe the internal structure of cells, including organelles, the cytoskeleton and intercellular junctions, as well as viruses and bacteria, and the structure of technical materials such as crystal structures, nanoparticles, layers and coatings.

Scanning transmission electron microscope (STEM)

A scanning transmission electron microscope is an advanced type of electron microscope that combines features of transmission and scanning microscopy. Its operating principle is based on scanning a thin specimen with a focused electron beam, enabling detailed imaging at the atomic scale (Table 2) (Nellist, 2019).

A STEM microscope uses a high-energy electron beam generated by an electron gun, for example a thermionic or field-emission source. The beam is focused by condenser lenses to provide precise irradiation of the specimen. Unlike conventional TEM, in which electrons pass through the entire sample simultaneously, in STEM the beam is focused to a very small probe and moved across the specimen line by line. This provides high-resolution images. Electrons can interact with the material in different ways, and different detectors therefore provide different types of information. Transmitted electrons yield mass and structural contrast in a bright-field detector; electrons scattered to high angles provide dark-field contrast and information on chemical composition; and emitted X-rays enable EDX spectroscopy for elemental identification using EDX X-ray detectors (Nellist, 2019).

SBF-SEM

SBF-SEM is a powerful technique for imaging the three-dimensional ultrastructure of biological tissues. It allows the automatic collection of hundreds to thousands of serial images, enabling highly precise spatial reconstruction of microanatomy. The method derives from a concept proposed by Leighton in 1981, who integrated a microtome with a scanning electron microscope (Courson et al., 2021).

An SBF-SEM combines a conventional scanning electron microscope with a precise microtomy system that automatically images successive layers of the sample (Table 2) (Courson et al., 2021). Electrons are emitted from the cathode, focused by electromagnetic lenses and guided by deflection coils that scan the beam across the specimen surface. The focused beam strikes the freshly exposed surface cut by the microtome. Electrons penetrate the specimen and interact with its atoms, producing backscattered electrons (BSE) (Denk and Horstmann, 2004). Thus, unlike in TEM, the electrons do not pass through the specimen but interact with its surface. Detectors record the resulting signals and convert them into a digital image. After each scan, the microtome removes the next layer, exposing a new surface for imaging (Courson et al., 2021). Images are generated from secondary and backscattered electrons recorded after each cut. The resulting stack of section images is computationally assembled into a three-dimensional reconstruction of the sample structure (Denk and Horstmann, 2004).

FIB-SEM

FIB-SEM is an advanced dual-beam system that combines electron microscopy with precise material removal using an ion beam (Table 2) (Kizilyaprak et al., 2019). The electron source is usually a field-emission gun (FEG) or tungsten source, whereas the ion source is a liquid metal ion source (LMIS), most often based on gallium. The electron beam generated by the SEM column scans the sample surface and forms an image from backscattered electrons. The ion beam generated by the FIB column then precisely mills away material, exposing successive layers for imaging (Kizilyaprak et al., 2013). The electrons do not pass through the sample; they interact with its surface and generate detectable signals. Electron detectors record the backscattered electrons from which the image is produced (Kizilyaprak et al., 2019).

SEM-AT / FM/SEM-AT

SEM-AT is an alternative to automated volume methods such as SBF-SEM and FIB-SEM and offers greater analytical flexibility. In this approach, serial ultrathin sections are prepared manually, similarly to TEM, and placed in the electron microscope (Burel et al., 2018). The method can be combined with fluorescence microscopy (FM/SEM-AT) to obtain additional functional information. FM/SEM-AT therefore uses two microscope types, fluorescence and electron microscopy (Table 2) (Burel et al., 2018; Koike and Yamada, 2019; Micheva et al., 2024). The sample is imaged in both systems, often using the same sections. In this workflow, SEM-AT is the second stage of analysis, while fluorescence is the first. Initially, the section is labeled with fluorophores. A light source (LED or laser) then emits light of a defined wavelength onto the specimen, causing the fluorophores to emit light. Excitation and emission filters select wavelengths specific to the fluorophore, and the detector records a fluorescence image showing the location of selected molecules, for example proteins (Koike and Yamada, 2019; Micheva et al., 2024). In the second stage, the same sections are transferred to an electron microscope and imaged in SEM, usually with backscattered electrons to provide high ultrastructural contrast. The fluorescence and electron images are then digitally overlaid. Correlating the two microscopy modes makes it possible to identify molecular structures in their ultrastructural context, for example a receptor in a synapse or a protein in a mitochondrion, substantially increasing the analytical value of the data (Burel et al., 2018; Micheva et al., 2024).

Table 2. Comparison of selected features of different types of electron microscopes

Technique	Operating principle	Sample type	Examples of applications
SEM (SCANNING ELECTRON MICROSCOPE)	Electrons scan the sample surface; secondary electrons are recorded	Conductive or coated with a conductive layer, e.g. gold	Surface topography, chemical composition
TEM (TRANSMISSION ELECTRON MICROSCOPE)	Electrons pass through a very thin sample	Resin-embedded, ultrathin sections	Internal structure of organelles, viruses and nanoparticles
STEM (SCANNING TRANSMISSION ELECTRON MICROSCOPE)	Point-by-point scanning of the sample with an electron beam	Resin-embedded, ultrathin sections	Structural and morphological analysis; quantitative and qualitative elemental analysis
SBF-SEM (serial block-face **SEM)**	Automatic block sectioning and surface imaging after each layer	Resin-embedded	Three-dimensional imaging of biological structures
FIB-SEM (focused ion beam **SEM)**	An ion beam removes material while SEM records images of successive layers	Resin-embedded	Three-dimensional reconstructions; semiconductor studies
SEM-AT (scanning electron microscopy array tomography)	Serial imaging of ultrathin sections	Resin-embedded, serial ultrathin sections	Visualization of ultrastructural details; localization and distribution of cellular components; analysis of material porosity and deformation
FM/SEM-AT (fluorescence/scanning electron microscopy array tomography)	Serial imaging of fluorescently labeled ultrathin sections	Resin-embedded, serial fluorescently labeled ultrathin sections	Localization of proteins in the context of cellular ultrastructure

Preparation of samples for electron microscopy

Tissue fragments intended for SEM analysis must be fixed to preserve their structural integrity and protect them from degradation (Fig. 1). A standard fixation method is incubation in 3% glutaraldehyde in phosphate buffer (pH 7.4) or in formalin, although depending on the sample type, acetone, alcohols of different concentrations or osmium tetroxide, which enhances imaging contrast, may also be used (Mehdizadeh et al., 2014).

After fixation, samples are dehydrated through an alcohol series of increasing concentration (25%, 50%, 75%, 96%, 100%), which gradually removes water and minimizes the risk of morphological changes (Fig. 1). The samples are then placed in acetone to remove residual moisture and prepare them for subsequent analytical stages (Boyde and Wood, 1969; Mehdizadeh et al., 2014).

Dehydrated samples placed in dedicated holders are subjected to critical-point drying using carbon dioxide (CO_2), which removes liquid without causing morphological changes (Fig. 1). If smaller sample fragments are required, controlled fragmentation by splitting or crushing may be carried out to obtain a size appropriate for the specific research requirements (Jones, 2012). The samples are then mounted on specialized holders (Fig. 1) covered with conductive carbon tape or carbon tabs, providing stability and suitable conditions for further analysis (Boyde and Wood, 1969).

To enable electron conduction through the sample, its surface is coated with a thin layer of a noble metal (Fig. 1), most commonly gold, platinum or palladium, which substantially improves imaging quality in SEM. Prepared specimens are stored in a desiccator to protect them against moisture and potential structural changes (Mehdizadeh et al., 2014).

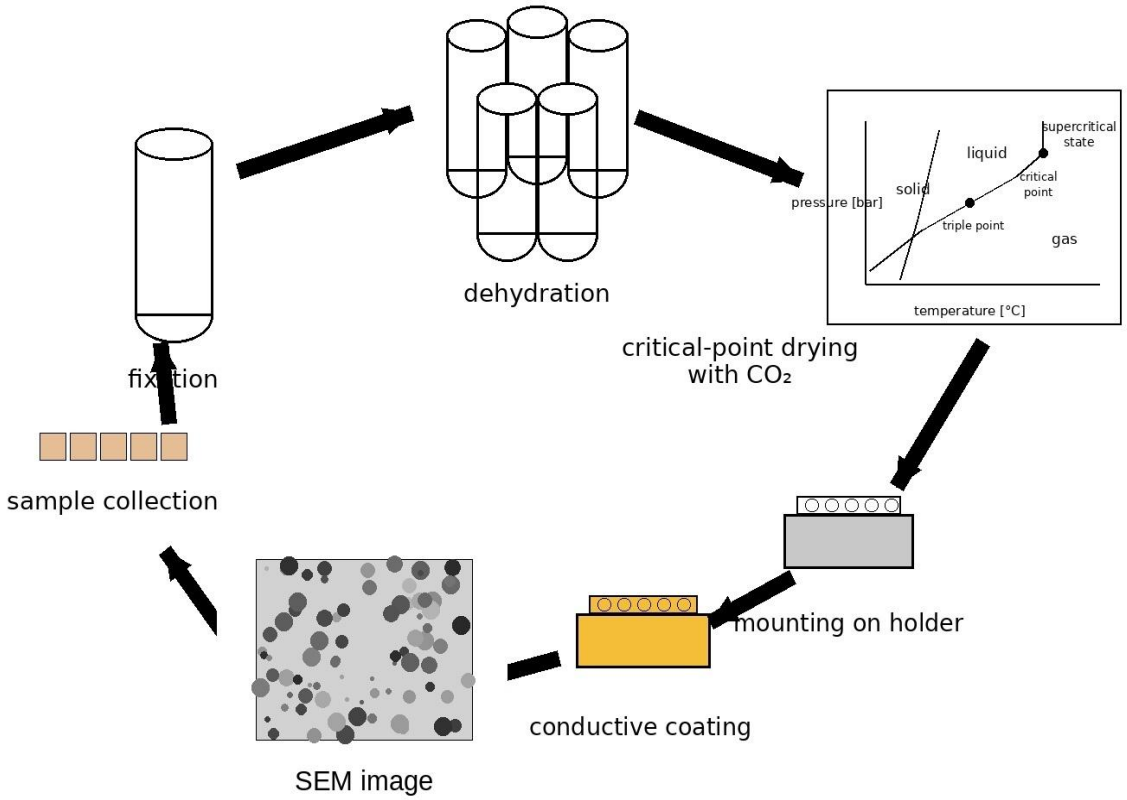


Figure 1. Schematic representation of the stages of sample preparation for analysis in a scanning electron microscope

Samples used for elemental analysis in SEM are cleaned in a solvent such as acetone to remove surface contamination. Harder materials must first be cut and polished (Goldstein et al., 2003). Non-conductive samples may require an additional conductive coating, usually sputtered gold, to improve electron-signal quality. During analysis, detection parameters and detector type must be appropriately selected for the material being examined (Ali et al., 2023).

Tissue samples intended for TEM, SBF-SEM, FIB-SEM and SEM-AT imaging should be divided into small fragments with a maximum diameter of about 3 mm and then fixed to preserve structural integrity (Fig. 2). The standard procedure is to incubate samples for 24 hours in 3% glutaraldehyde in phosphate buffer (pH 7.4). After fixation, the tissues are rinsed in phosphate buffer (pH 7.4) and then placed in 1% osmium tetroxide in the same buffer, providing further fixation and improved contrast (Tizro et al., 2019).

After fixation, the material is dehydrated through a graded alcohol series (25%, 50%, 75%, 96%, 100%) and then placed in propylene oxide, which prepares the sample for embedding in epoxy resin (Fig. 2). Specialized silicone molds are optimal for resin embedding because they allow precise formation of tissue blocks (Graham and Orenstein, 2007). Samples for SBF-SEM and FIB-SEM are prepared in a similar manner, and protocols dedicated to these techniques continue to be optimized to maximize visualization of different structures (Denk and Horstmann, 2004; Kizilyaprak et al., 2013; Kizilyaprak et al., 2019; McClain and Nowotarski, 2024; Sall et al., 2024). Blocks prepared in this way are ready for placement in the SBF-SEM or FIB-SEM chamber. For TEM and SEM-AT, further preparation is required, including trimming, i.e. mechanical removal of excess resin using grinders, files and razor blades. The final

top surface of the block should contain only the embedded tissue and be a few millimeters in diameter (Mielańczyk et al., 2015).

Before electron-microscope imaging, semithin sections are prepared for preliminary analysis under a light microscope (Fig. 2). This stage enables assessment of the tissue cross-section that will subsequently undergo more detailed ultrastructural analysis. Semithin sections are cut with an ultramicrotome equipped with glass knives. Tissue sections 0.5 µm thick are mounted on microscope slides and stained with an Azure II–methylene blue solution (1:1). After drying, the preparations are ready for light-microscope observation. If the semithin section provides satisfactory results and allows identification of structures intended for TEM or SEM-AT imaging, the block is processed further. A diamond knife is then used to cut ultrathin sections 20–60 nm thick, which are placed on specialized microscopy grids (Fig. 2). Different grid types may be used depending on sample characteristics and specific research requirements. The sections are contrasted with uranyl acetate and lead citrate to enhance the visibility of cellular structures. After these procedures, the sample is ready for TEM or SEM-AT imaging (Graham and Orenstein, 2007; Hayat, 2012; Mielańczyk et al., 2015).

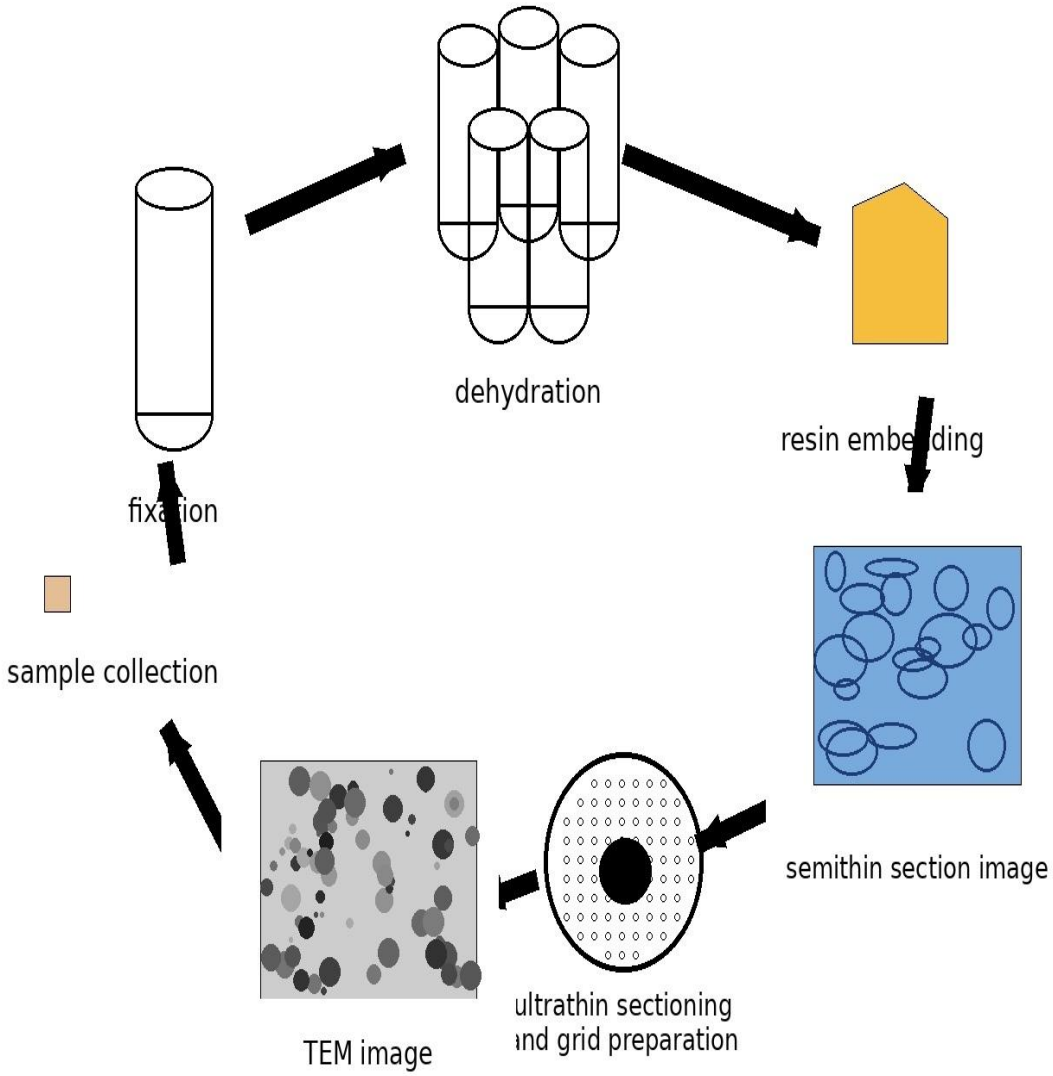


Figure 2. Schematic representation of the stages of sample preparation for analysis using a transmission electron microscope as an example

Applications of electron microscopy in animal science research

Electron microscopy is an extremely valuable tool in animal science research, enabling detailed analysis of cellular structures and microorganisms present in livestock. Key applications include: 1) analysis of feed and feed-additive quality; 2) ultrastructural studies of cells and tissues to investigate pathological and adaptive changes in animal organs, especially in the context of disease or the effects of environmental factors; 3) diagnosis of animal diseases, because electron microscopy enables identification of pathogens such as viruses, bacteria and parasites that may affect livestock health; 4) animal reproduction studies, in which analysis of sperm and oocyte quality is important for breeding programs and artificial insemination; and 5) assessment of environmental effects, including analysis of the impact of toxic agents, pollutants and housing conditions on animal organisms (Fig. 3) (Mebus and Underdahl, 1977; Graham and Orenstein, 2007; Orlova and Saibil, 2011; Sharma and Bhardwaj, 2019; Curry, 2000).

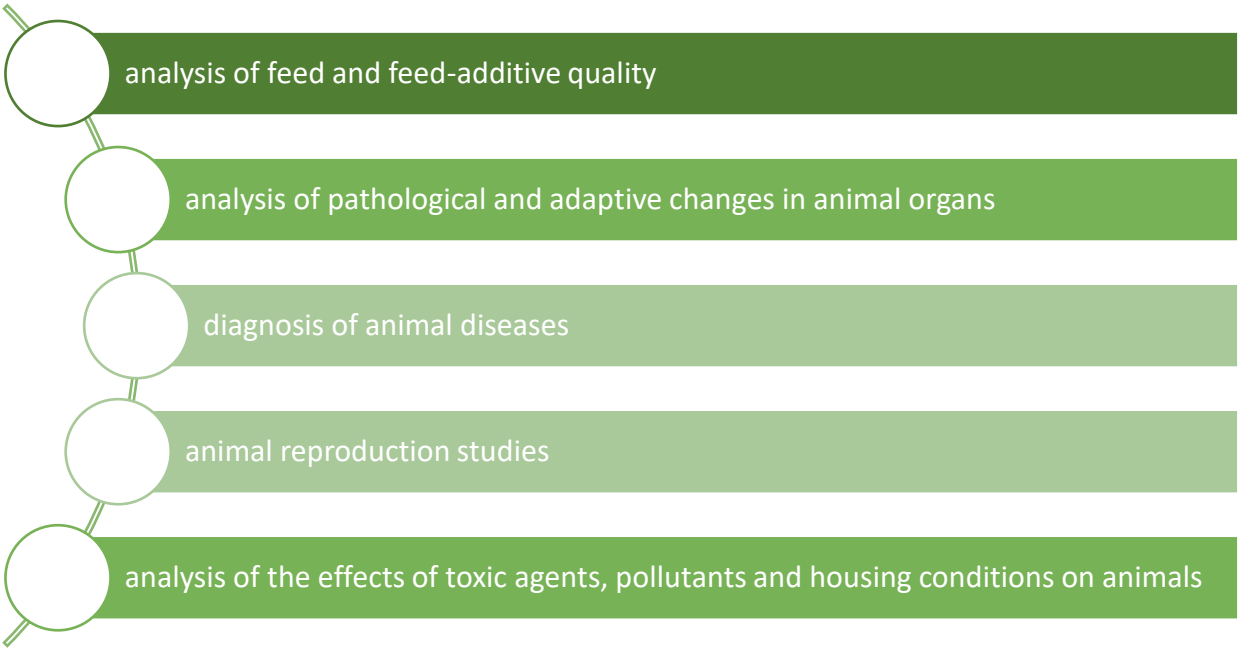


Figure 3. Key applications of electron microscopy in animal science

Analysis of feed and feed-additive quality using electron microscopy is an extremely precise method that enables assessment of both the chemical composition and molecular structure of components used in livestock nutrition. Electron microscopy permits detailed examination of feed microstructure, which is important for nutrient availability and digestibility (Sharma and Bhardwaj, 2019). It can be used to analyze particle size and shape, which influence digestive processes in animals, and to identify undesirable structures such as contaminants or mycotoxins. Electron microscopy also allows evaluation of protein quality, including denaturation and the effects of processing on biological value, and fat quality, including oxidation and the presence of undesirable substances (Orlova and Saibil, 2011). It can also identify microorganisms and pathogens that may be present in feed, such as pathogenic bacteria (e.g. Salmonella or Escherichia coli), fungi and molds, and viruses (Curry, 2000). In addition, electron microscopy can be used to assess how technological processes such as extrusion, drying or pelleting affect feed microstructure, which is important for evaluating nutrient stability and bioavailability.

Ultrastructural studies of cells and tissues using electron microscopy play a key role in disease diagnosis and in the analysis of adaptive mechanisms in animal organisms in response to environmental factors. The technique enables detailed examination of microstructures that are not visible using conventional light microscopy. It can reveal, among other things, cell degeneration and necrosis, cell-membrane damage, accumulation of pathological substances, changes in mitochondrial structure and alterations in immune cells (Graham and Orenstein, 2007).

Electron microscopy is an exceptionally precise method for diagnosing animal diseases, allowing detailed analysis of cellular structure and identification of pathogens at the ultrastructural level (Mebus and Underdahl, 1977). A particular application is the examination of samples collected from animals for epidemic monitoring and research on new therapies. Because of its precision and reliability, electron microscopy is a foundation of modern veterinary diagnostics, contributing to more effective treatment and improved animal health protection (Graham and Orenstein, 2007).

Electron microscopy plays a key role in animal reproduction research, particularly in evaluating sperm and oocyte quality. Its extremely high resolution allows detailed analysis of gamete structure and morphology, which is highly important for breeding programs and artificial insemination technologies. Electron microscopy enables assessment of many parameters affecting male fertility, including sperm morphology, acrosome integrity, cell-membrane condition and mitochondrial status, as well as female reproductive-cell characteristics such as the structure of the zona pellucida and the condition of the cytoplasm and cellular organelles (Baylor et al., 1943; Sotelo and Porter, 1959; Van Der Horst et al., 1991; Suzuki et al., 1996; Pesch et al., 2006). Such detailed analysis makes it possible to select the highest-quality gametes for artificial insemination, increasing the likelihood of healthy offspring and improving the efficiency of breeding programs. Electron microscopy is also used to study the effects of environmental factors on fertility and to monitor the effectiveness of gamete cryopreservation methods (Khalil et al., 2018).

Electron microscopy is also a valuable tool for studying environmental effects on animals, allowing precise analysis of the consequences of toxic substances, pollutants and housing conditions at the cellular and subcellular levels. It can be used to investigate the accumulation of heavy metals in tissues, which causes organelle damage and impaired cell function; DNA damage resulting from exposure to environmental mutagens, which may lead to neoplastic changes or reproductive problems; changes in cell-membrane structure caused by pesticides or other chemicals; and the presence of micro- and nanoplastics in animal and plant tissues and cells and their effects on cellular metabolism (Soyer and Bayram, 2007; Bell et al., 2012; Mankos et al., 2014; Miserli et al., 2023).

Summary

Electron microscopy is one of the most important research tools in modern science, enabling analysis of structures at the atomic level. Different types of electron microscopes, including TEM, SEM and STEM, allow detailed investigation of biological, nanotechnological and engineering materials. The technique is applied in many fields, from medicine to industry, contributing to the development of new technologies and scientific discoveries.

In animal science, electron microscopy plays an important role in studying the structure of animal cells, analyzing feed composition and assessing the quality of biological materials used in breeding. It enables detailed analysis of microorganisms affecting animal health and examination of tissue ultrastructure to improve understanding of physiological and pathological processes. Electron microscopy also supports the development of biotechnology in animal

breeding by enabling precise genetic studies and disease diagnostics. Progress in the field continuously improves imaging resolution and analytical capabilities, opening new perspectives for future research. Modern techniques such as SBF-SEM, SEM-AT and FIB-SEM enable three-dimensional reconstruction of tissues and cells. Electron microscopy therefore remains a key tool for understanding micro- and nanostructures and for the continued development of science and technology.

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ELECTRON MICROSCOPY IMAGING METHODS IN ZOOTECHNICAL RESEARCH

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SUMMARY

Electron microscopy is a seminal research technique that facilitates analysis of structures at the atomic level. The development of the electron microscope can be traced back to the 1930s, when Ernst Ruska and Max Knoll constructed the first such instrument, utilizing a beam of electrons in lieu of visible light. Since that time, significant advancements have been made in the field, resulting in the development of a variety of microscopes, including TEM (transmission), SEM (scanning), STEM (scanning-transmission), SBF-SEM (serial block-face scanning electron microscope), FIB-SEM (focused ion beam SEM), and SEM-AT (scanning electron

microscopy array tomography). The operating principle of electron microscopes is based on the emission of electrons, which are accelerated and focused by electromagnetic lenses. In the transmission electron microscope (TEM), electrons are permitted to pass through the specimen, thereby creating an image of its internal structure. In the scanning electron microscope (SEM), reflected electrons are analyzed, thus enabling the generation of three-dimensional images of the surface. A STEM microscope is a sophisticated instrument that integrates the capabilities of a transmission electron microscope (TEM) and a scanning electron microscope (SEM), employing both transmitted electron and scanning of the sample surface techniques. This approach enables not only the observation of the sample's structural arrangement and the imaging of individual atoms, but also the analysis of its chemical composition. SBF-SEM, FIB-SEM, and SEM-AT microscopes facilitate the spatial imaging of the material under examination, predominantly based on the principle of electron reflection. The preparation of samples for electron microscopy is an exacting process that requires the application of specialized methodologies. These include ultrathin sectioning, deposition on metal grids, chemical contrasting and sputtering of conductive layers. The implementation of these techniques is essential to ensure the acquisition of high-quality imaging data. In the field of animal science, electron microscopy is employed in the study of the structure of animal cells, the analysis of the composition of feed, and the diagnosis of diseases. The technology under discussion enables the precise determination of tissue microstructures, the identification of pathogens, and the assessment of the quality of biological materials utilized in the context of animal breeding. This technique also supports the development of biotechnology in animal science, enabling genetic research and control of animal health at the cellular level. Electron microscopy remains a pivotal instrument in both natural and technical sciences, with its ongoing development generating new possibilities in the domains of biological, material and animal science research.

Keywords: electron microscope, ultrathin sections, electrons, microscopic imaging