TRANSMISSION ELECTRON MICROSCOPY

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The article presents transmission electron microscopy, arguably the most versatile technique for characterization of different materials on a scale down to less than a nanometer. Different parts of the microscope are described and the fundamental physical processes that make this technique possible are explained. Lastly, two interesting examples from the science of electrocatalysis, where measurements using transmission electron microscopy were extremely valuable, are discussed.

PRESEVNA ELEKTRONSKA MIKROSKOPIJA

V članku je predstavljena presevna elektronska mikroskopija, ki je ena najbolj vsestranskih metod za karakterizacijo različnih materialov z ločljivostjo pod enim nanometrom. Opisani in razloženi so različni sestavni deli mikroskopa ter fizikalni procesi, ki omogočajo njegovo delovanje. Na koncu sta predstavljena dva zanimiva primera iz področja elektrokatalize, kjer je bila uporaba te tehnike še posebej v pomoč.

1. Introduction

The historical motivation behind developing transmission electron microscopy (TEM) is the limited resolution of visible light microscopy, which is about 300 nm. In the 1932 paper by researchers Knoll and Ruska (who received a Nobel Prize in 1986), an electron microscope was proposed for the first time [1]. The paper introduced electron lenses and demonstrated first images. In the first years after introducing the technique, devices achieved a resolution of a few tens of nanometers, whereas today it is possible to achieve a resolution of only about 50 picometers [2].

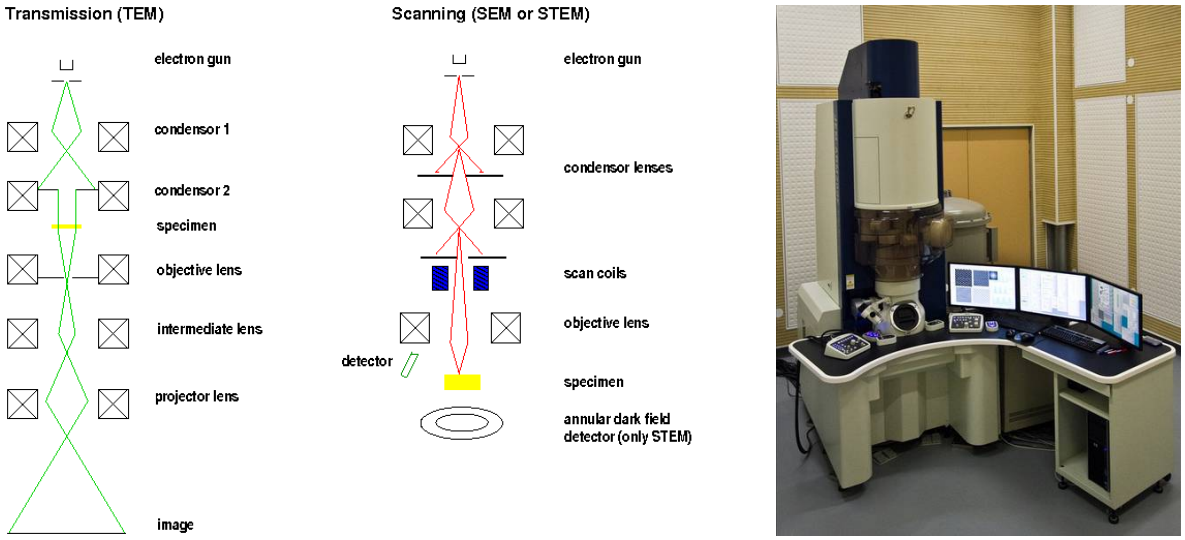
Today TEM devices are the go-to tool for observing different materials on different scales, from atomic resolution to micrometers and beyond. Other techniques for characterizing samples on the nanoscale, such as scanning electron microscopy (SEM), scanning tunneling microscopy (STM) and atomic force microscopy (AFM), offer valuable information about different surface properties of samples, whereas TEM is the superior technique for obtaining information about the inner structure.

In order to understand the principles of TEM, one needs to understand how electrons are affected by a magnetic field, how they interact with a specimen and how we can detect them. Since the transmission electron microscope is a complex device, this article does not explain all the components and modes of operation in full detail, but rather focuses on a few aspects.

2. The instrument

Transmission electron microscopy uses electrons to make images of specimens and thus requires a few specific parts of equipment to make that possible, namely electron guns, magnetic lenses, sample holders and detectors. Two different combinations of the listed parts can be assembled to form a transmission electron microscope or a scanning transmission electron microscope (STEM) as shown in Figure 1a. A TEM device uses electron beam paths analogous to light paths in an optical microscope. It can roughly be divided into three components: the illumination system (electron gun and two condenser lenses, transfers electrons from the source to the specimen), the objective lens/specimen stage (where the beam-specimen interactions take place, the heart of the device), and the imaging system (several lenses for magnification and focus, and the detector) [2]. These three components are called the column, because they are placed on top of each other. A STEM device is similar, but includes a scanning component which allows to record pictures in a serial

fashion. Additionally, the entire system is under vacuum, which we control with vacuum pumps (not pictured). Figure 1b shows an example of a STEM device.



(a) scheme **(b)** instrument

Figure 1. (a): schematic depictions of the most important parts in TEM and STEM with respective beam paths [3]. (b): the scanning transmission electron microscope at the National Institute of Chemistry in Ljubljana, Slovenia [4].

The difference between TEM and STEM lies in the electron beam encountering the sample. In a TEM device, the electron beam is parallel and coherent, which enables the creation of a diffraction pattern like in Figure 4. Afterwards, one part of a diffraction pattern is chosen with an aperture to create a real image of the sample by dispersing the signal. In a STEM device, the electron beam is focused and incoherent as it encounters the sample. The lack of coherency cannot lead to a diffraction pattern, nor can we observe a useful image of the specimen, which is why the beam needs to be scanned across the sample to see an image. This difference can be observed in Figure 1a. The left scheme shows the TEM configuration in imaging mode with a parallel electron beam and the right scheme shows the STEM configuration with a focused electron beam.

2.1 Electron guns

It is important that the source of electrons, part of an assembly called the electron gun, produces an electron beam with desirable brightness (current density per unit solid angle of the source), coherency (both temporal - narrow energy spread, and spatial - small source size), and stability (electric current is stable in time). There are two types of guns, thermionic and field-emission guns. Table 1 lists their basic properties.

Property	Thermionic sources	Field-emission sources
used material	lanthanum hexaboride ($LaBr_6$) crystals or (rarer) tungsten(W) filaments	fine tungsten needles with a tip radius of about 100 nm
production of electrons	material is bonded to a metal wire, which is resistively heated	when a large electric potential is applied between the source and an anode
energy spread of electrons	$\approx$ 2 eV	0.4 - 0.8 eV

Table 1. A table containing the most important distinctions between two types of electron guns.

In a thermionic source, electrons gain their energy by heating the $LaBr_6$ crystal or W filament to 2000 K or 3000 K, respectively, so they escape the source and form a beam after passing two condenser lenses (Figure 1a). In newer devices, $LaBr_6$ crystals are more commonly used than W due to a lower work function (energy required to remove an electron out of material; $\sim$ 2.4 - 2.6 eV compared to $\sim$ 4.5 eV for W) and a higher current density at a lower temperature ($\sim$ 100 A/cm^2 at 2000 K compared to $\sim$ 14 A/cm^2 at 3000 K for W), which leads to better spatial resolution. Brightness for thermionic sources is of the order of magnitude of $\sim 10^9$ A/m^{2}sr [2].

In a field-emission source, we apply a voltage of several kV (extraction voltage) between a tungsten wire (cathode) and an anode and thus generate an electric field, which lowers the work-function barrier, so the electrons can tunnel out of the material. They are then accelerated with a help from a second anode with a voltage of 100 kV or more (accelerating voltage) and form a beam. The strength of the electric field, E, if a voltage, V, is applied to a point of radius r, is $E = \frac{V}{r}$ and equals $\sim 10^{10}$ V/m for $V \sim 1$ kV and $r < 0.1$ μm. For the field emission to occur, the surface has to be free of contaminants, which we can achieve with the help from vacuum. If the material is contaminated, the work-function barrier gets higher and the emission current at the same extraction voltage gets lower. Brightness for field emission sources can be up to the order of magnitude of $\sim 10^{13}$ A/m^{2}sr [2].

The scanning transmission electron microscope at the National Institute of Chemistry is equipped with a field-emission gun with an energy spread of only 0.33 eV, and is operated at the accelerating voltage of 200 kV.

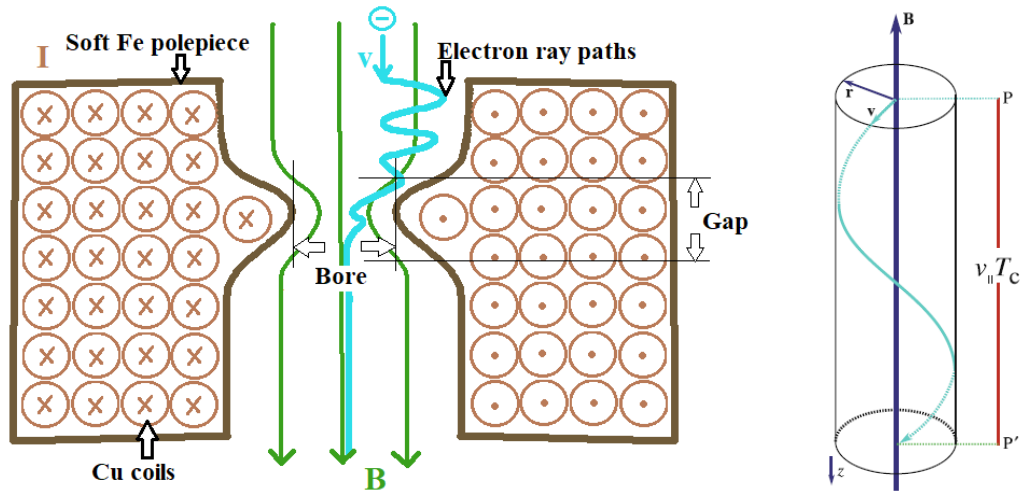
All sources emit electron beams with an energy spread of only up to 2 eV, which is very narrow compared to the total energy of the beam in the range of several hundred keV. With such sources we can achieve a current in the range of several microamps. However, by the time the electrons reach the specimen, the current decreases by three to six orders of magnitude, due to electrons passing through a set of condenser lenses and an aperture as shown on Figure 1a. Parallel beams, reaching the specimen in TEM, are typically several micrometers broad, but can be focused to very small diameters, typically <5 nm and at best <0.1 nm in STEM devices.

2.2 Lenses

Electron lenses are equivalent to the glass lenses in visible light microscopy. We use them to focus the electron beam. Assumptions when dealing with glass lenses are also applicable to electron lenses. In most cases they are magnetic and we can change their strength, simultaneously affecting also their focus, where the image is created, intensity of illumination, and magnification. Typical focal lengths for electron lenses are of the order of magnitude of a few milimeters, stronger lenses having shorter focal lengths.

To make a magnetic electron lens we need two parts, as shown in Figure 2a. A polepiece, the first part, is a cylindrically symmetrical core of soft magnetic material(can be easily magnetized and demagnetized) such as soft iron, with a hole drilled through it (called a bore). In most lenses there are two polepieces with a gap between them and the bore-to-gap ratio is an important characteristic of lenses, controlling the focusing action of the lens. The second part is a coil of copper wire which surrounds each polepiece. The entire lens is usually the size of a fist and has a bore of the size of a finger [2].

We can change the strength of the lens by changing the current through a coil which changes the strength of the resultant magnetic field in the bore. The current in the objective lens can be as high as $\sim$ 0.1 microamps and should be stable in time, varying about $\pm$1% per hour in thermionic sources and up to $\pm$5% per hour in field-emission sources, otherwise quantitative measurements would be unreliable. The speed at which the lens adapts to new currents is limited by properties of the material the lens is made of. Lens response can be improved with employing a feedback loop [5].



(a) lens **(b)** path

Figure 2. (a): A depiction of magnetic lens, used in a TEM. Magnetic field lines are shown in green. The specimen is placed in the bore of the upper polepiece of the objective lens. The electron trajectory is shown in teal. Electric current through the coils is shown in brown. (b): A depiction of an electron trajectory in a magnetic field [2].

The field is axially symmetric, but inhomogeneous along the length of the lens. Electromagnetic lenses can generate a field of up to 2 T. Its strength and direction control the electron trajectories, which are helical due to the velocity being neither parallel nor perpendicular to the field, so the electrons spiral through the lens as shown in Figure 2b. This can be summarized with introducting the Lorentz force as

$$\vec{F} = e\vec{v} \times \vec{B}, \tag{1}$$

where e is the charge of the electron, $\vec{v}$ its velocity and $\vec{B}$ the magnetic field experienced by the electron. The Lorentz force causes electrons passing through point P on the optic axis to spiral through the field and intersect the axis again at P'. The distance between the two intersections is the product of the period of rotation, T_c, and the parallel component of electron velocity, $v_{\parallel}$, which is constant in both speed and direction.

Why is the presence of a bore in a magnetic lens so important? If there was no bore, the magnetic field inside the lens would be homogeneous (magnetic field lines would be parallel to the optic axis). A parallel beam of electrons entering the lens would have a velocity parallel to the magnetic field, which means that according to equation (1), there would be no Lorentz force acting on the electrons. Without the Lorentz force, there would be no way to impact the electron trajectories and thus focus the beam.

The cyclotron radius, r, can be defined from the perpendicular component of velocity and the period of rotation through the field as

$$r = \frac{mv_{\perp}}{eB}, \tag{2}$$

where $v_{\perp}$ is the perpendicular component of velocity, B the resultant magnetic field density and m the mass of the electron. This also gives the cyclotron frequency $\omega_c = \frac{2\pi}{T_c} = \frac{eB}{m}$. Those equations are useful expressions for estimates, but they neglect relativistic effects which unfortunately cannot be ignored at energies greater than about 100 keV because the velocity of the electrons becomes greater than half the speed of light. To be exact we must begin with the energy-momentum equation

$$E^2 = p^2c^2 + m_0^2c^4 = (eV + m_0c^2)^2, \tag{3}$$

where $m_0 = 9.109 \times 10^{-31}$ kg is the rest mass of an electron. From this equation we express

momentum, p,

$$p = \frac{((eV + m_0c^2)^2 - m_0^2c^4)^{1/2}}{c} \tag{4}$$

$$= \frac{(2m_0c^2eV + e^2V^2)^{1/2}}{c}, \tag{5}$$

which we can then plug into the expression for radius (equation (2)) to give

$$r = \frac{(2m_0eV(1 + \frac{eV}{2m_0c^2}))^{1/2}}{eB}. \tag{6}$$

For electrons with a kinetic energy of 100 keV and a magnetic field of 1 T, the radius is ~ 1 mm and the period of rotation, T_c, is ~ 1 ps.

The expression for radius reveals its dependence on the magnetic field strength. The perfect spiral in Figure 2b is thus not an accurate representation of electron trajectories inside a lens, where the magnetic field is inhomogeneous and at different angles with respect to the symmetry axis depending on how close to the bore we observe it. Again, let us focus onto the equation (1) describing the Lorentz force, which acts on electrons. An electron travels along the helical path as soon as there is a magnetic field present, as described before. As it travels, it approaches the optic axis, where the magnetic field lines are at a lower angle with respect to the electron's velocity. The Lorentz force diminishes because of that, which leads to the electron staying closer to the optic axis and its path is not as helical as before. As the electron eventually does not experience an external force anymore, it remains at the optic axis and the electron beam is thus focused. A more accurate schematic representation of electron trajectory inside a magnetic lens is depicted in Figure 2a.

The problem with electron lenses are spherical and chromatic aberrations (different focal length for rays away from the optic axis and for rays of different wavelengths, respectively), which limit the resolution of a TEM. Aberrations can be controlled by inserting limiting apertures to select electrons nearest to the optic axis and by utilizing modern aberration correctors, which significantly reduce aberrations.

2.3 Vacuum pumps

The previously mentioned elements are usually placed one on top of the other, creating a column. The entire column is permanently placed under vacuum to maximize the average distance between impacts and minimize scattering of electrons on anything but the specimen. To lower pressure we first use a roughing pump (100 - 0.1 Pa) and then a high-vacuum/ultrahigh-vacuum (HV/UHV) pump (10^{-4} to 10^{-7} Pa and below 10^{-7} Pa, respectively). Typically, the pressure inside the column is around 1.3×10^{-5} Pa [2]. Not only the column is under vacuum; the camera chamber has a similar pumping system. To achieve a low enough pressure ($\sim 1.3 \times 10^{-5}$ Pa for a typical TEM or below 10^{-7} Pa for a UHV-TEM) the pumping system includes a vacuum reservoir, which allows the roughing pump to be turned off once the pressure inside the reservoir is under 0.1 Pa, and turned on again after the pressure becomes higher due to the HV/UHV pump exhausts.

2.4 Detectors

Because our eyes are not sensitive to electrons, it is important to have a viewing screen, which converts electron intensity into light intensity, as TEM images are just images of electron density variations.

A historic interface between electrons and our eyes was cathodoluminiscence which is a result of a luminescent material (emits light spontaneously not resulting from heat) being bombarded by

electrons and converts energy of the electrons into light. The viewing screen in a TEM device is coated with a material that emits visible light, for example with ZnS.

Today, charge-coupled device (CCD) cameras, equally sensitive to visible light and high-energy electrons, are a popular choice for viewing and recording images. Numerous software options exist for researchers to process images, enhance the contrast, reduce the noise etc.

2.5 Sample preparation

There exist numerous procedures for sample preparation, which usually follow the rule that thinner is better. It is nonetheless important to keep in mind that those procedures alter microstructure and microchemistry and that artifacts such as variable thickness and defects (impurities, grain boundaries, voids, ...) can be produced. Depending on the average atomic number of the specimen, thin means electron transparent. This usually means biological samples have a thickness of up to several hundred nanometers or one micrometer, whereas samples from material science have a thickness of up to one hundred nanometers due to the higher average atomic number of the sample.

3. Principle of operation

A thin specimen is illuminated with a beam of electrons with a uniform intensity over the illumination area (the parallel incident beam in a TEM device). Electrons are following well-defined paths. After exiting the specimen, they are separated into those that have the same direction as electrons entering the specimen, and those that were scattered through different angles. Their energy can either be the same as the incident beam, which means the electrons were elastically scattered, or lower, which means they were inelastically scattered. Elastic scattering provides contrast in images and most of the useful information in diffraction patterns (more on them in section 3.2), while inelastic scattering is the source of spectroscopic signals, which are not a part of this article. The end result is a non-uniform distribution of electrons after interacting with the specimen, which contains all structural, chemical, and other information about the specimen. This distribution can be displayed as a spatial or angular distribution of scattered electrons. The first is observed as an image of a specimen and the latter as a diffraction pattern.

3.1 Electron scattering

Scattering of electrons on a specimen (Figure 3) is a process which makes TEM feasible. Only when electrons are scattered in some way when they interact with the nucleus and the electron cloud through Coulomb force we can see an image. Scattering is strongly connected to structural properties of the specimen. Electrons can be scattered through different angles, but in TEM we are mostly interested in forward scattered electrons, because they give us the information we seek about the internal structure and the chemistry of the specimen. The ones, which are parallel to the incident beam, form a so called direct beam, which is used to construct bright field images (for example Figure 5). Electrons, scattered at higher angles (outside of direct beam), construct dark field images (for example Figure 6).

Electrons can also be scattered more than once, but in TEM we approximate all scattering with a single scattering, which is a reasonable assumption for thin enough specimens. The major source of contrast are elastically scattered electrons, which do not lose a significant amount of energy.

The probability for scattering can be described with a scattering cross section, σ. It does not represent a physical area, but rather a probability for scattering on an atom if σ is divided with the area of the atom. It can be determined with the help of the differential cross section $\frac{\mathrm{d}\sigma}{\mathrm{d}\Omega}$, which describes the angular distribution of scattering, and can be empirically determined for different

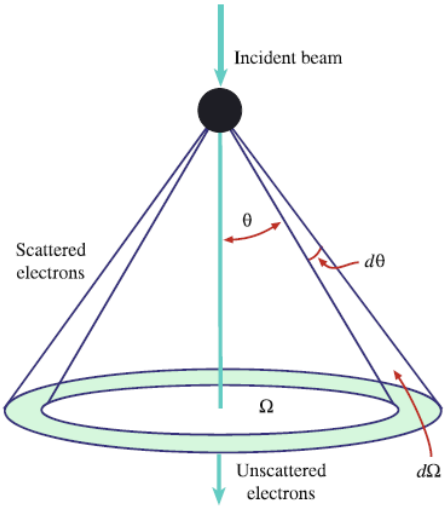


Figure 3. A schematic depiction on how electrons can be scattered on an atom. Θ is the angle of scattering and Ω is the total solid angle of scattering. Incremental increases in angles $\mathrm{d}\Theta$ and $\mathrm{d}\Omega$ are the basis for determining the scattering cross section [2].

elements. The relationship between the solid angle of scattering, Ω, and the angle of scattering, Θ, can then be written as

$$\Omega = 2\pi(1 - \cos\Theta), \tag{7}$$

so their incremental increases are connected as

$$\mathrm{d}\Omega = 2\pi \sin\Theta \mathrm{d}\Theta. \tag{8}$$

The differential cross section can be written as

$$\frac{\mathrm{d}\sigma}{\mathrm{d}\Omega} = \frac{1}{2\pi \sin\Theta} \frac{\mathrm{d}\sigma}{\mathrm{d}\Theta}. \tag{9}$$

The scattering cross section for one atom can be calculated by integrating the differential cross section over chosen angles

$$\sigma_{atom} = \int_{\Theta}^{\pi} \mathrm{d}\sigma = 2\pi \int_{\Theta}^{\pi} \frac{\mathrm{d}\sigma}{\mathrm{d}\Omega} \sin\Theta \mathrm{d}\Theta \tag{10}$$

and the total scattering cross section can be written as

$$\sigma = n\sigma_{atom} = \frac{N_A \sigma_{atom} \rho}{M}, \tag{11}$$

where n is the number of atoms per unit volume, N_A is Avogadro's number, ρ is the density and M is the atomic weight of atoms in the specimen.

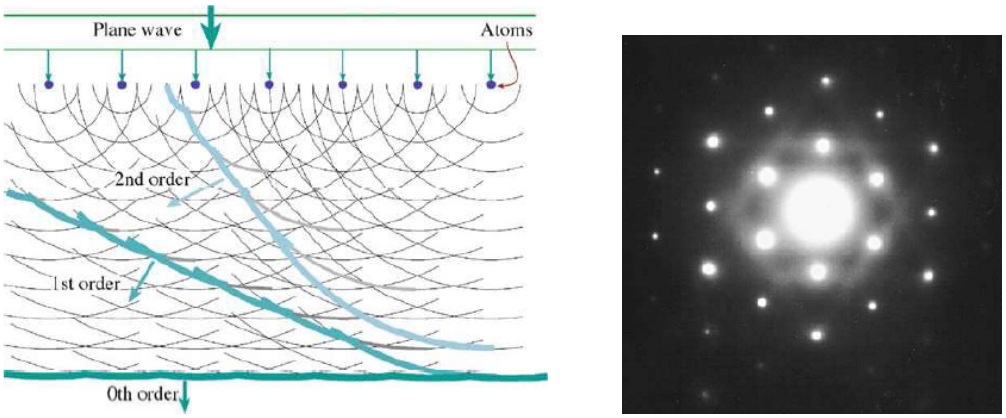
A typical cross section for elastic scattering on transition metals is $\sim 10^{-22}$ m^2, whereas for inelastic scattering it is generally smaller and ranges from $\sim 10^{-22}$ m^2 to $\sim 10^{-26}$ m^2, all values given for 100 keV electrons. It is worth noting that values for σ are generally imprecise. Scattering can be manipulated with the accelerating voltage, V. As the electron energy increases, the scattering cross section decreases (less scattering at a higher voltage), which means a more narrow selection of angles through which electrons can be scattered [2].

3.2 Diffraction

Diffraction is an interaction between a wave and an object. Just like with light, electrons can be also treated with both the particle and the wave approach. When dealing with electrons and specimens,

the same principles apply as when discussing light and slits/holes. A wave generates secondary wavelets after encountering atoms, which then interfere and result in beams of different orders at different angles, as shown in Figure 4a. The result is a diffraction pattern, where distances on the film correspond to inverse distances in the specimen, unlike in images, where distances on the film correspond to distances in the specimen.

Most of the intensity is in the center of the pattern, which means that most electrons travel straight through the specimen. The intensity decreases with distance from the center, which is consistent with the decrease in the scattering cross section (probability for scattering) for higher angles. Figure 4b shows an example of a diffraction pattern on crystalline aluminium. Each of the intensity maxima can be attributed to a set of parallel crystal planes in real space and can be characterized by Miller indices of crystal planes, which represent the orientation of a plane in a crystal.



(a) diffraction of a plane wave on atoms **(b)** aluminium

Figure 4. (a): A depiction of how a plane wave is diffracted on periodically spaced atoms and how resultant waves of different orders are formed. (b): An example of a diffraction pattern on crystalline Al [2].

Diffraction is arguably the most useful aspect of TEM for material scientists, because diffraction patterns contain information about crystal structure and particularly crystal defects, and they can always be related to images. An advantage over X-ray diffraction is that it offers a better resolution.

3.3 Resolution

Resolution can be described with a parameter called the minimum resolvable spacing, r. The generally accepted criterion for minimum resolvable detail is the Rayleigh criterion which says that imaging is limited by diffraction when the first minimum of one source coincides with the maximum of another and it can be summarized in the equation

$$r = 0.61\frac{\lambda}{NA}, \tag{12}$$

where λ is the wavelength of electrons traveling through the sample and NA is the numerical aperture. NA is the product of refractive index of the medium between the lens and the specimen, n, and the sine of the half angle between the optical axis and the objective lens, $\sin\alpha$. In TEM the refractive index equals $n = 1$ and the half angle equals $\alpha = 0.01$ rad, so the small-angle approximation is justified and the numerical aperture is essentially just the half angle between the specimen and the objective lens, $NA \approx \alpha$.

As λ decreases, the minimum resolving spacing decreases, so the resolving power improves. To understand what is the wavelength of electrons, we can turn to de Broglie's ideas of the wave-particle

duality. The particle momentum, p, can be related to its wavelength, λ, through Planck's constant, h, thus

$$\lambda = \frac{h}{p}. \tag{13}$$

In the microscope we give momentum to the electron by accelerating it with voltage, V, giving it the energy eV, where $e = 1.602 \times 10^{-19}$ C is its charge. Here we can recycle the equation (4) for momentum from section 2.2, which already takes into account the relativistic effects. Equations (4) and (13) define the relationship between the electron wavelength, λ, and the accelerating voltage of the electron microscope, V,

$$\lambda = \frac{h}{(2m_0eV(1 + \frac{eV}{2m_0c^2}))^{1/2}}. \tag{14}$$

The inverse relationship between λ and V introduces a very important concept: by increasing the accelerating voltage we decrease the wavelength of the electrons and thus improve resolution [2]. Wavelengths of electrons are typically of the order of magnitude of a few picometers. The effect of relativity is greater for higher accelerating voltages. At 100 kV, the non-relativistic wavelength is 4 % larger than the relativistic one, whereas at 400 kV it is already 18 % larger.

According to formulas (12) and (14) the theoretical resolution for images by electrons with energies between 100 and 300 keV is several picometers. However, the classical transmission electron microscope cannot attain a resolution better than 200 picometers mainly due to spherical aberration and other limitations of the lens. A spherical aberration corrector can improve the resolution to around 50 picometers.

4. Limitations

There are a few limitations to TEM. One of them is that the operator can only look at a very small part of the specimen due to high magnifications. It is therefore recommended to first use a tool which offers poorer resolution but better sampling, before doing TEM imaging. Another problem is that although we are imaging 3D samples, all images are in 2D and thus lack depth. It is difficult to determine false defects in an image because our brain is not used to recognizing such images so it is easily fooled. This can be solved with combining 2D images from different points of view into a more intuitive 3D image, known as TEM tomography. It is also important to keep in mind that with a voltage up to 400 kV, the beam can damage specimens, particularly soft ones like polymers.

5. Examples

There are countless examples of TEM images being a very important part of a study. Here, I would like to single out two publications([6],[7]) on the topic of electrocatalysis. Electrocatalysts are an important material in a proton exchange membrane fuel cell (PEMFC) which is a promising alternative to current internal combustion engines. TEM does not only provide images of the material, but can also perform in-situ experiments such as heating and electrochemical shaping and deliver images of every step of the process.

5.1 In-situ heating

A 2019 study by researchers from the National Institute of Chemistry, published in Nano Energy, gives an insight into thermal annealing of highly-active $PtCu_3$/C electrocatalyst [6]. The catalyst features PtCu nanoparticles embedded in a carbon matrix. Usually, thermal annealing processes are developed based on empirical findings by ex-situ investigation([8],[9]).

In this study, particle growth mechanisms during heat treatment were directly observed with high resolution TEM imaging in combination with carefully designed in-situ heating protocol which allows for confident explanations of each step. The procedure included imaging at room temperature (RT), in-situ heating to 500 °C, rapidly cooling back to RT and imaging, heating to 800 °C and again rapidly cooling back to RT and imaging.

One can see in Figure 5 that during heating particles experienced reshaping and a gradual decrease in their number as a consequence of inter-particle sintering.

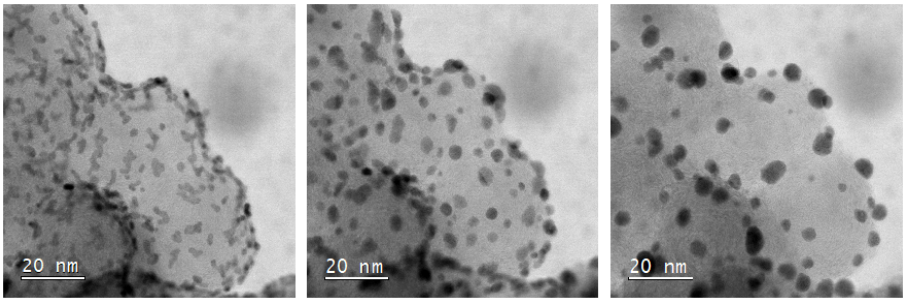


Figure 5. Scanning transmission electron microscopy of a catalyst at different temperatures on identical location. First image is taken at RT, second after heating to 500 °C and cooled down to RT and the third after heating to 800 °C and cooled down to RT [6].

The advantage over other techniques, which have a high enough resolution to see nanoparticles, such as SEM, is that they are surface sensitive and do not provide information about what is inside the 3D specimen. TEM images are 2D projections of 3D structures and movement of nanoparticles can also be observed inside the carbon matrix rather than only on its surface.

5.2 Electrochemical shaping

Catalystic properties of novel materials are greatly influenced by their morphology. Their structural properties are changing depending on the reaction conditions. A 2019 study by researchers from the National Institute of Chemistry, published in Nano Letters, shows how electrochemical treatment which influences structural properties of a catalyst, can be observed on the atomic scale using the TEM [7].

Figure 6 shows three different sections of nanoparticles before and after electrochemical activation (EA; submerging the catalyst in an electrolyte and varying the potential applied to it). Different artefacts can be observed, such as pores and dents originating from etching, size reduction of facets, surface reshaping and nonuniform etching. Observing differences at such a small scale on identical location is possible only in a TEM.

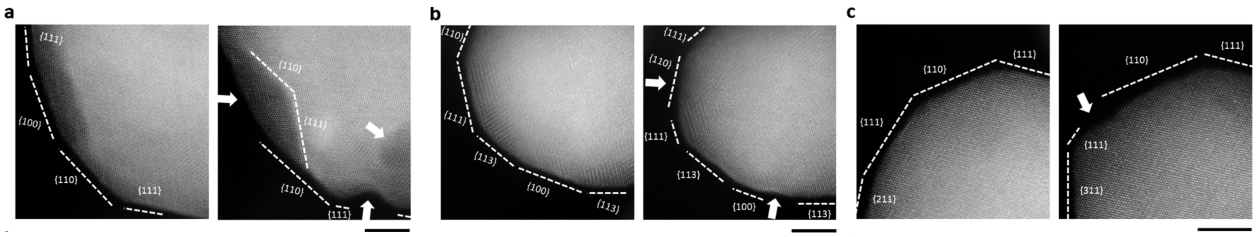


Figure 6. Identical location images of nanoparticles. Left image was recorded before EA whereas the right image was taken after EA in all images. Scale bar is 5 nm [7].

6. Conclusion

Transmission electron microscopy is a versatile technique that combines simple physical principles into a complicated machine capable of wide characterization of materials on the nanoscale. With few limitations and countless advantages, it has already greatly shaped the field of materials science and as it continues to develop, it will enable us to gain an even deeper qualitative and quantitative understanding of various materials.

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REFERENCES

[1] M. Knoll and E. Ruska, *Das Elektronenmikroskop*, Zeitschrift für Physik **78** (1932), 318–339.

[2] D. B. Williams and C. B. Carter, *Transmission Electron Microscopy: A Textbook for Materials Science*, Springer, 2009.

[3] *Basics - Microscope types*. http://www.ceos-gmbh.de/en/basics/microscope-types (accessed on 10 Feb. 2020)

[4] *Elektronska mikroskopija*. https://www.ki.si/odseki/d10-odsek-za-kemijo-materialov/elektronska-mikroskopija-in-katalizatorji/elektronska-mikroskopija/ (accessed on 6 Jan. 2020)

[5] J. T. L. Thong and F. Li, *High-speed operation of scanning electron microscope lenses*, Scanning **19** (1997), 275–280.

[6] M. Gatalo, F. Ruiz-Zepeda, N. Hodnik, G. Dražić, M. Bele and M. Gaberšček, *Insights into thermal annealing of highly-active $PtCu_3$/C Oxygen Reduction Reaction electrocatalyst: An in-situ heating transmission electron microscopy study*, Nano Energy **63** (2019), 2211–2855.

[7] F. Ruiz-Zepeda, M. Gatalo, A. Pavlišič, G. Dražić, P. Jovanovič, M. Bele, M. Gaberšček and N. Hodnik, *Atomically Resolved Anisotropic Electrochemical Shaping of Nano-electrocatalyst*, Nano Letters **19** (2019), 4919–4927.

[8] M. K. Jeon and P. McGinn, *Carbon supported Pt-Y electrocatalysts for the oxygen reduction reaction*, Journal of Power Sources **196** (2011), 1127–1131.

[9] K. C. Neyerlin, R. Srivastava, C. Yu and P. Strasser, *Electrochemical activity and stability of dealloyed Pt–Cu and Pt–Cu–Co electrocatalysts for the oxygen reduction reaction (ORR)*, Journal of Power Sources **186** (2009), 261–267.