

MyScope

training for advanced research

Transmission Electron Microscope

Training module



THE UNIVERSITY OF
NEW SOUTH WALES



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Introduction - aims and learning outcomes

Welcome to the online learning module for transmission electron microscopy. Through self-instruction, this unit will help you learn:

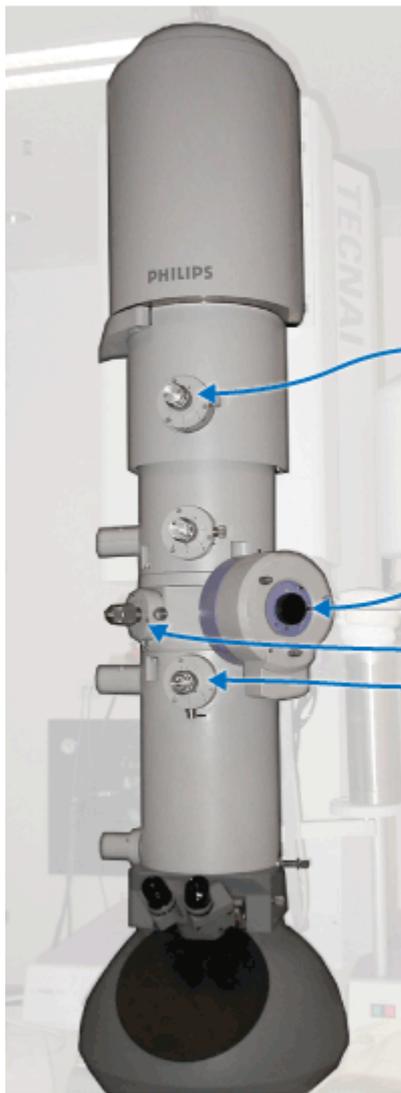
- the basic principles of TEM operation;
- the origin of images and their formation;
- to understand electron diffraction and its applications;
- to understand procedures for collecting and interpreting images;
- to develop routines for preparing samples for investigation; and
- how to work safely in an electron microscope laboratory.

You will also get the opportunity to “play” with a virtual transmission electron microscope by following the guided instructions. This module will not make you an expert overnight, but it will provide the background needed to begin experimentation and provide the scaffolding necessary to eventually become a fully-fledged electron microscopist through practice and perseverance.

At the end of the module menu is an online multiple choice test. You can do this at any time to determine how much you know about TEM, and repeat it as many times as you like. The result can be saved and sent to your teacher or supervisor if you wish.

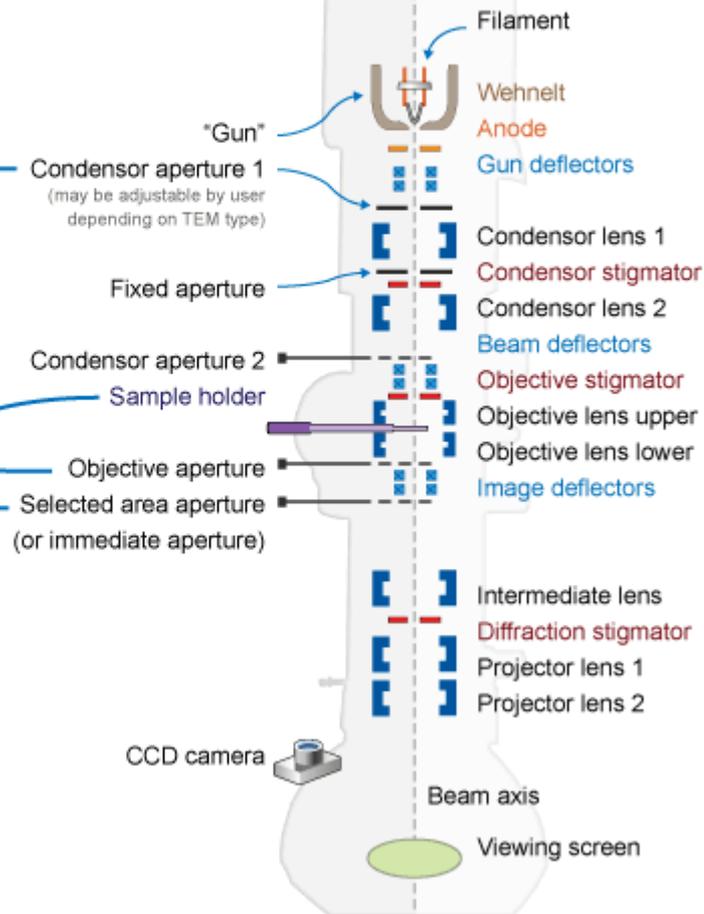
If you do not wish to start at the beginning and go all the way through the site, the screens following this one give you the opportunity to tailor the site to your specific learning needs.

We wish you an enjoyable learning experience.



Example TEM schematic

One of many types of TEMs



A generalised cut-away diagram of the internal structure of a transmission electron microscope alongside an example of a modern instrument.

Frequently asked questions

General:

What information does the TEM provide? Why would I use it?

Why are electron images not in colour?

Why does the TEM need to have a vacuum inside?

What is electron diffraction?

What kinds of images can be produced with a TEM?

What is dark field imaging?

What is the eucentric?

What is STEM?

Samples:

Why do samples need to be dry or frozen?

How do I prepare a sample?

How does the specimen preparation method vary with specimen type?

What are the safety issues involved with using transmission electron microscopy?

Using a TEM:

What steps are involved in using a TEM?

I don't have access to a TEM so how can I practice using a TEM?

What is the function of the apertures (condenser, objective and diffraction)?

Equipment/machinery:

What is the layout of a TEM?

Where does the electron beam come from?

How is a specimen loaded and inserted?

- The sample chamber

-
- Step-by-step procedures for using TEMs
 - Virtual microscope

How do I turn the beam on and saturate the filament?

- Step-by-step procedures for using TEMs
- Virtual microscope

How is the sample height set to the eucentric position?

- The eucentric
- Step-by-step procedures for using TEMs
- Virtual microscope

How do I obtain a focused bright field image?

- Step-by-step procedures for using TEMs
- Virtual microscope

How do I align the condenser aperture and Wobbler?

- Step-by-step procedures for using TEMs
- Virtual microscope

How is an image recorded?

- Step-by-step procedures for using TEMs
- Virtual microscope

How is EDS or EELS data acquired in the TEM?

What content is right for me?

TEM for simple imaging purposes

For basic image generation, where staff are on site to help with trouble-shooting and alignment issues, it is generally only important to understand:

- how to prepare a sample, including knowledge of both health and safety concerns.
- the general parts of the machine.
- how to insert the sample into the TEM [see Virtual TEM].
- basic background regarding image formation (i.e. bright field).
- how to use the apertures (both the condenser and objective) to enhance contrast [see Virtual TEM].
- how to operate the controls to adjust focus and correct for stigmation [Focus/stigmation & Virtual TEM].
- how to change the magnification, converge and diverge the beam [see Virtual TEM].
- how to judge if an image is properly adjusted, in order to take a photograph of the region of interest [Focus/stigmation & Virtual TEM].

Knowledge self test

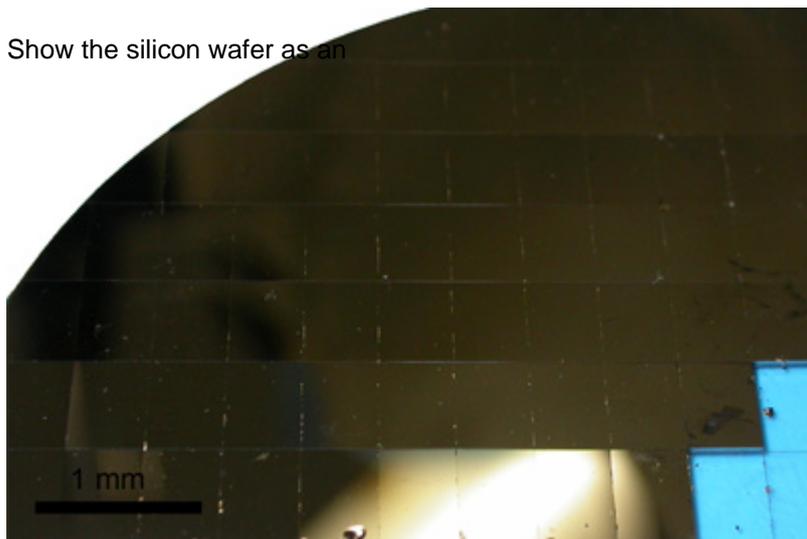
Test yourself with our self test to see how much you know, and what areas of knowledge you can improve on!

Background information - What is transmission electron microscopy?

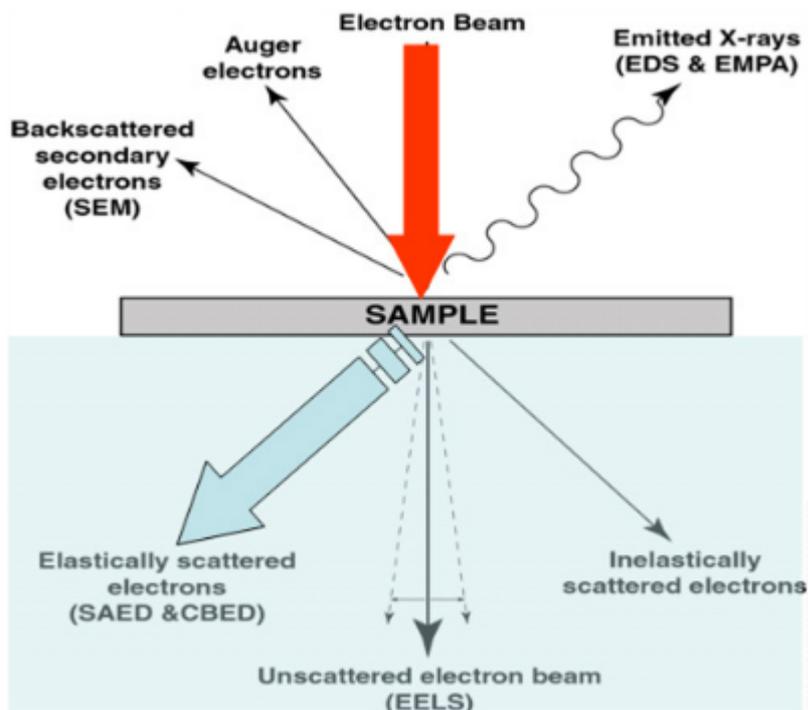
A transmission electron microscope (TEM) is an analytical tool allowing visualisation and analysis of specimens in the realms of microspace (1 micron/ $1\mu\text{m} = 10^{-6}\text{m}$) to nanospace (1 nanometer/ $\text{nm} = 10^{-9}\text{m}$). The TEM reveals levels of detail and complexity inaccessible by light microscopy because it uses a focused beam of high energy electrons. It allows detailed micro-structural examination through high-resolution and high magnification imaging. It also enables the investigation of crystal structures, specimen orientations and chemical compositions of phases, precipitates and contaminants through diffraction pattern, X-ray and electron-energy analysis.

Transmission electron microscopy is used to produce images from a sample by illuminating the sample with electrons (i.e. the electron beam) within a high vacuum, and detecting the electrons that are transmitted through the sample. Ultimately, using a TEM we can see the columns of atoms present in crystalline samples.

Show the silicon wafer as an



The word “transmission” means “to pass through”. Essentially, the way the transmission electron microscope creates a conventional image (usually termed a bright field image) of a sample can be compared to shadow puppetry. Imagine a torch beam shone through a lattice on a window. The light passes through the transparent parts of the window, but is stopped by the lattice bars. On a wall beyond, we see the lattice bars as shadows. The TEM uses a beam of highly energetic electrons instead of light from a torch. On the way through the sample some parts of the material stop or deflect electrons more than other parts. The electrons are collected from below the sample onto a phosphorescent screen or through a camera. In the regions where electrons do not pass through the sample the image is dark. Where electrons are unscattered, the image is brighter, and there are a range of greys in between depending on the way the electrons interact with and are scattered by the sample.



Magnifications of up to 1, 000,000x and resolution below 1 nm are achieved routinely. A scale bar is essential on a TEM image. From this the actual size of structures in the image can be calculated. For more on scale bar use visit: <http://scalebar.emu.usyd.edu.au/main.html>

Quantitative and qualitative elemental analysis can be provided from features as small as 1 nm. For crystalline phases the crystal structure, lattice constraints and specimen orientation can be determined.

Electrons are negatively charged particles within the atom. Unlike light photons, electrons cannot be focussed by glass lenses; instead, electromagnets are used to focus the electrons.

Advantages of the TEM over a light microscope

The transmission electron microscope (TEM) provides the user with advantages over the light microscope (LM) in three key areas:

1. *Resolution at high magnification.* Resolution can be defined as the smallest distance between two closely opposed points, at which they may be recognized as two separate entities. The best resolution possible in a LM is about 200 nm whereas a typical TEM has a resolution of better than 1 nm.
2. *Structural information.* If the material being viewed has a periodic structure like a crystal then the beam can interact with that structure in such a way that it diffracts. This provides information on crystal structure, symmetry and orientation of materials.
3. *Microanalysis* i.e. the analysis of sample chemical composition can be performed in the TEM.

Parts of the machine

The typical transmission electron microscope laboratory contains a machine with these components:

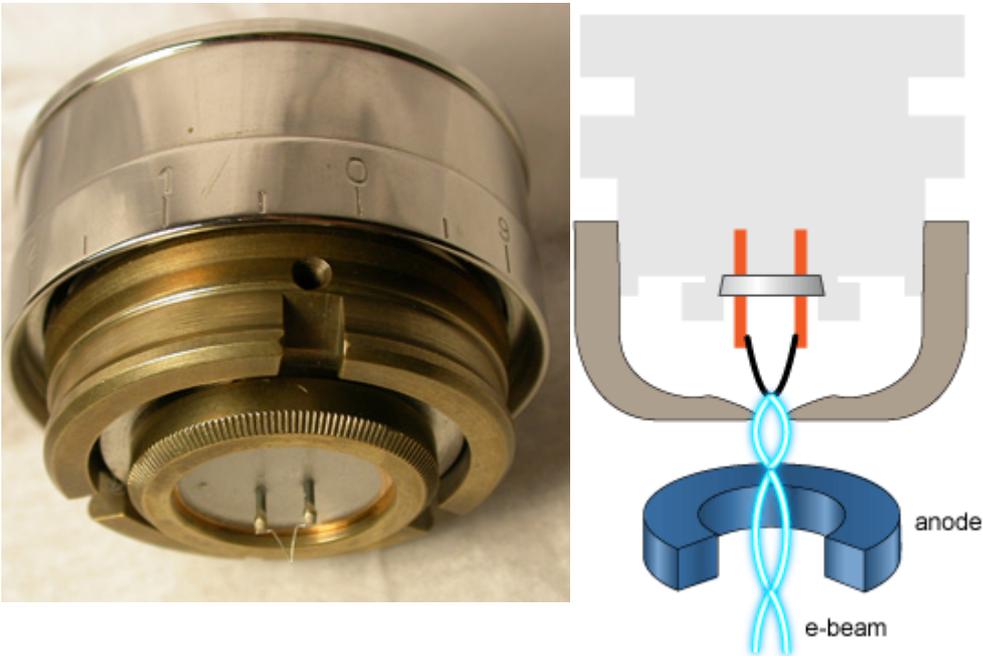
- Electron gun
- Electron column
- Electro-magnetic lens system
- Detectors
- Water chilling system
- Specimen/sample chamber
- Main control panel and operational controls
- Image capture





Electron gun

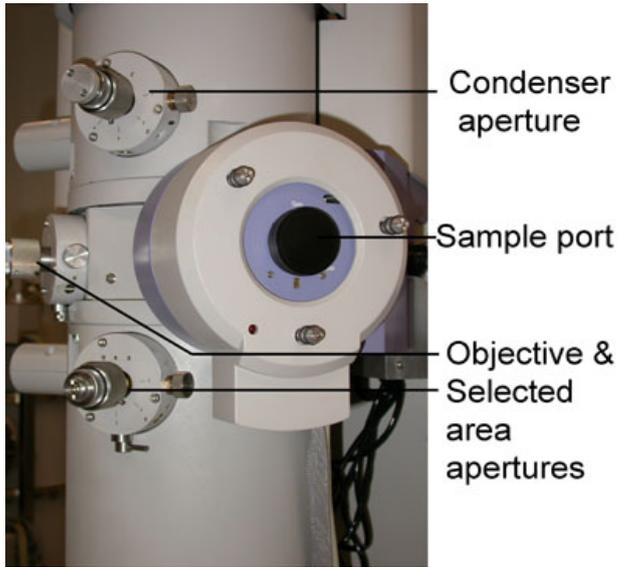
The electron gun generates the electron beam. It is usually positioned in the top of the instrument column. In the image sequence below the gun assembly is lifted off and moved aside to show how the electron emitter is replaced. The emitter is seated within a cone-shaped Wehnelt cylinder and the beam travels out of the small central hole shown in the apex of the cone. Details about the electron gun can be found in the SEM module [here](#) and [here](#).



This is an electron gun with the Wehnelt cylinder removed to show the filament within. This is a tungsten filament.

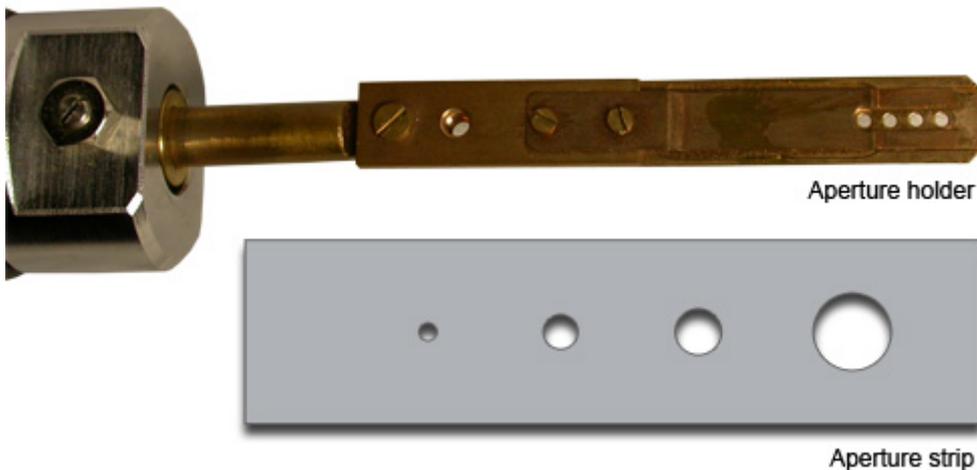
Electron column

The electron column is made up of the gun assembly at the top, a column filled with a set of electromagnetic lenses, the sample port and airlock, and a set of apertures that can be moved in and out of the path of the beam. The contents of the column are under vacuum.



The apertures can be easily removed from the beam path by the user. This is important for operation of the objective and selected area diffraction apertures during imaging.

The apertures are located within apertures strips, typically consisting of a strip of molybdenum containing a sequence of different sized holes, that allow modulation of the beam to different degrees of precision.





Adjacent to the sample port is a 'cold trap'; this consists of a liquid nitrogen dewar containing a conductive metal tassel which is joined to a rod at the top (see arrow path in image). The rod penetrates the column and sits near the sample. This cold area acts as a condensation site for material which leaves the sample. Such material can contaminate the chamber or affect the vacuum status of the machine.

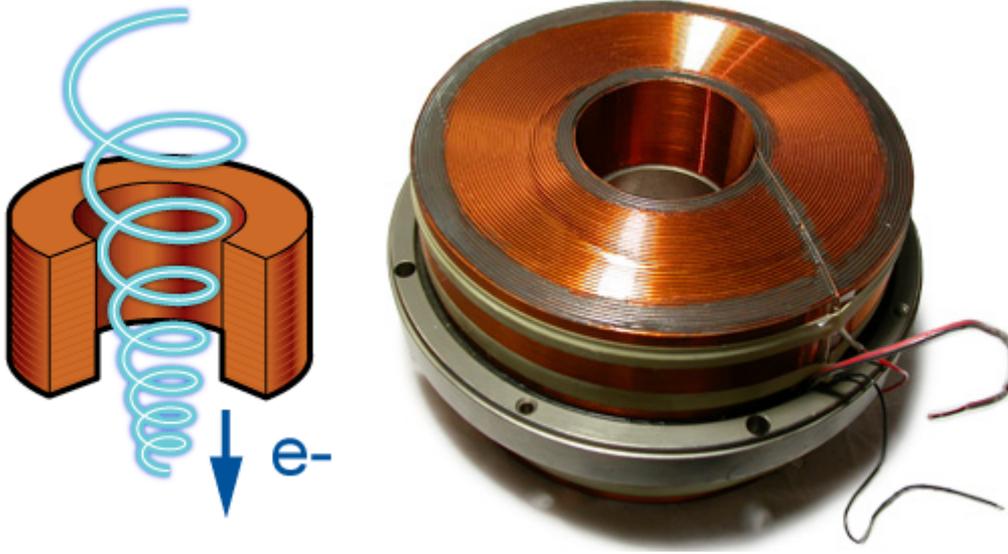


At the base of the column is the sample viewing chamber. This has a screen which produces an image via fluorescence when impacted by the projected electrons. A set of binoculars is attached to the column and can be swung around to focus on a small, movable screen within the chamber. This screen is used for finely focusing the image. A camera is positioned either above (removable: Veleta brand in this image) or below the screen (accessed by raising the large round white screen via a lever).

The viewing port can be blocked with a light-retardant cover. It is often important to do as the phosphorescent screen is sensitive to fluorescent light sources, and can degrade when exposed to them.

Magnetic lens system

Within the column the electromagnetic lenses shape the electron beam, which travels in a spiral trajectory. Each lens is constructed of a coil of copper wire through which a current runs. There is a hole in the center through which the beam travels.



The inside of the column is maintained under vacuum so that the density of molecules which can interfere with the electron beam are minimised. To achieve this, a system of vacuum pumps are attached to the TEM column.

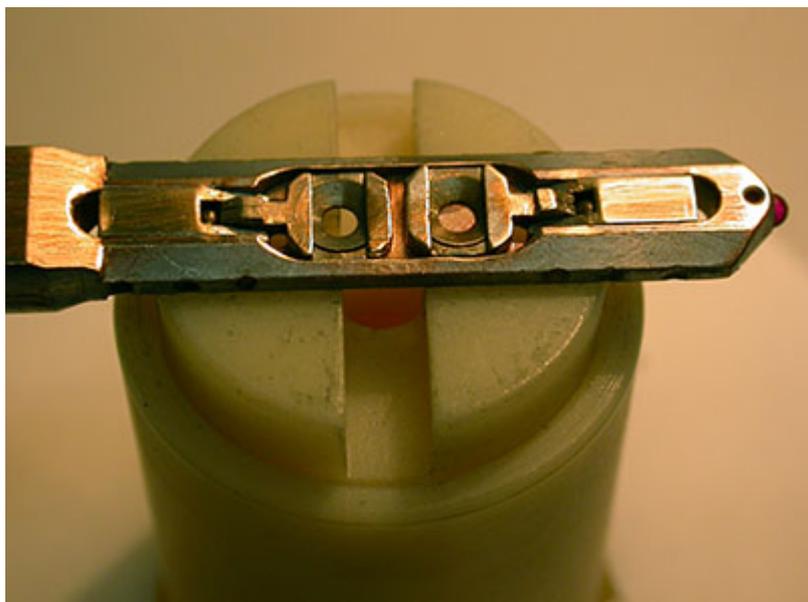
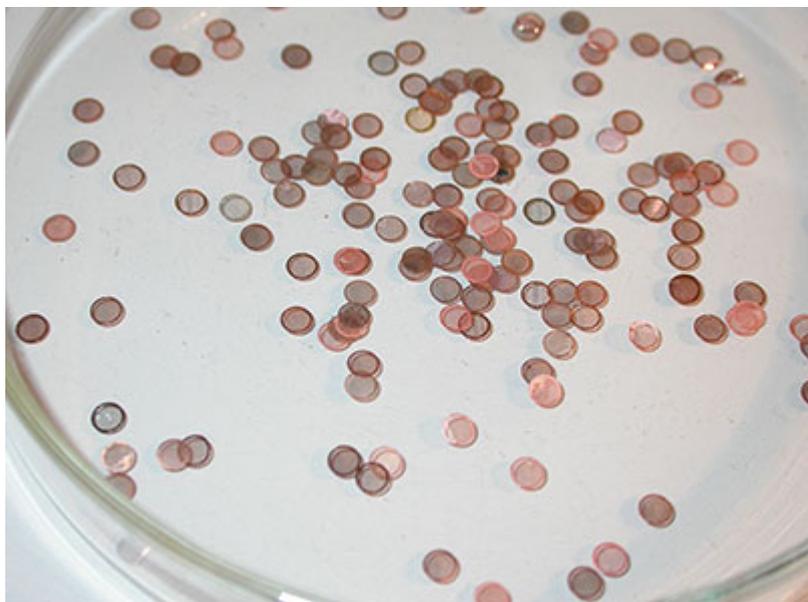
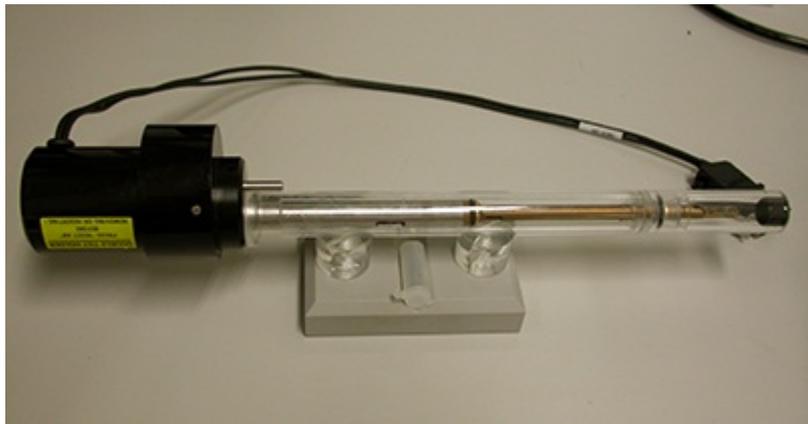
Detectors

One of the most common detectors seen on a transmission electron microscope is the x-ray energy dispersive spectroscopy (EDS or EDX) system. This typically involves a large dewar for liquid nitrogen (to keep the detector cold), an arm on which the equipment sits, and a solid state detector that penetrates the column (arrow) so it is located near the sample.



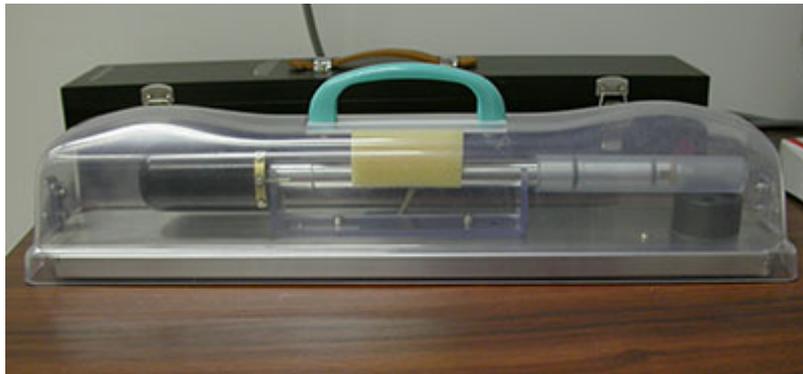
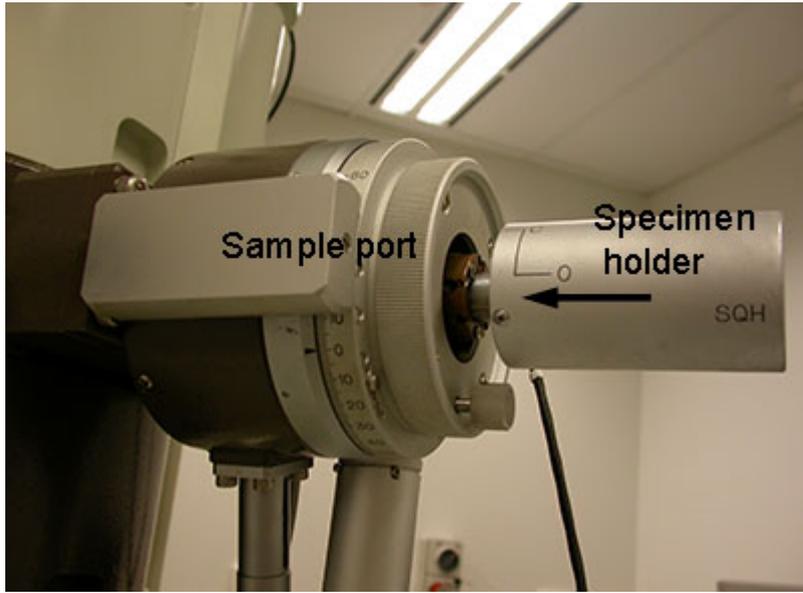
Specimen/sample chamber

The specimen holder has one or two wells at the end. The sample is loaded into this via a flange or a ring that screws into the well to hold it securely in place. It is imperative that the grid is secured so that it does not fall out of the specimen holder.



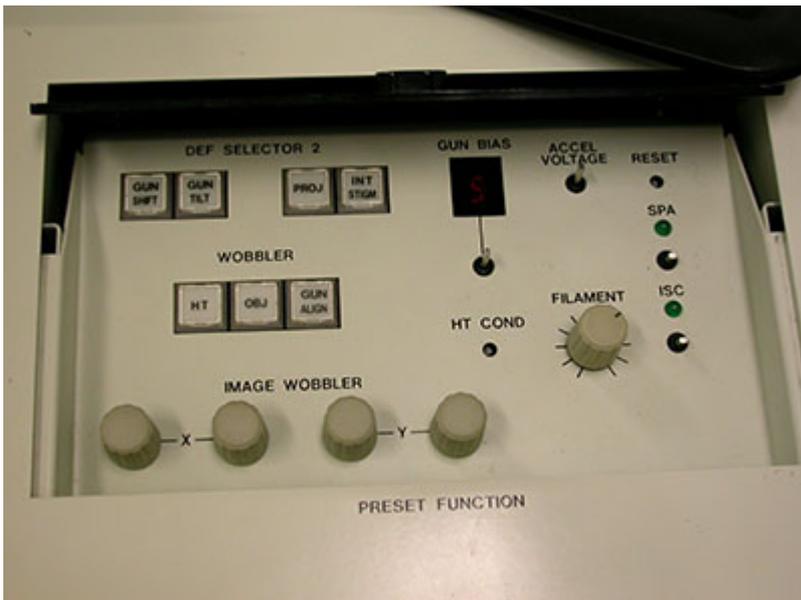
The holder is then inserted into the column. During this process the sample airlock is evacuated which can take a few

minutes. It is important to keep the O-ring on the holder free from lint or dust and properly greased or it can interfere with the vacuum. To keep it clean, the holder is stored in a covering sleeve when outside of the machine. A cable can be observed on some specimen holders. The cable is plugged into the column to enable electronically-controlled tilting of the holder and therefore the sample. This is important for obtaining precisely oriented diffraction patterns and high resolution images.



Main control panel and operational controls

The instrument can be controlled through external panels (as shown below), which consist of buttons, toggles and knobs. Foot operated tilt-pedals may be positioned on the floor. The virtual TEM steps a user through how they are used.



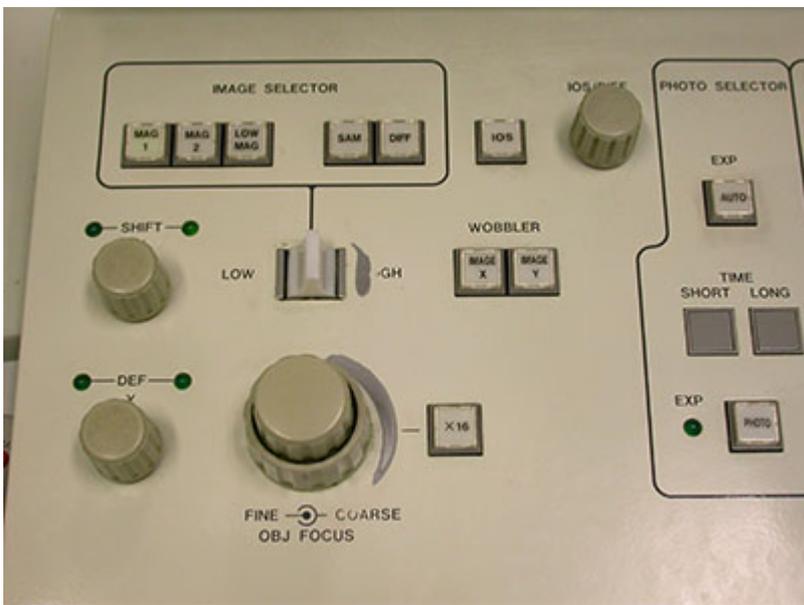
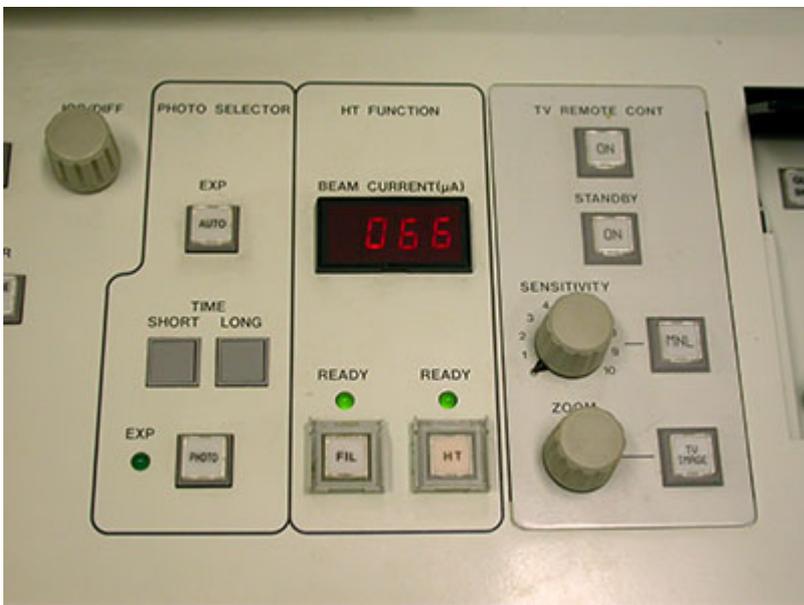
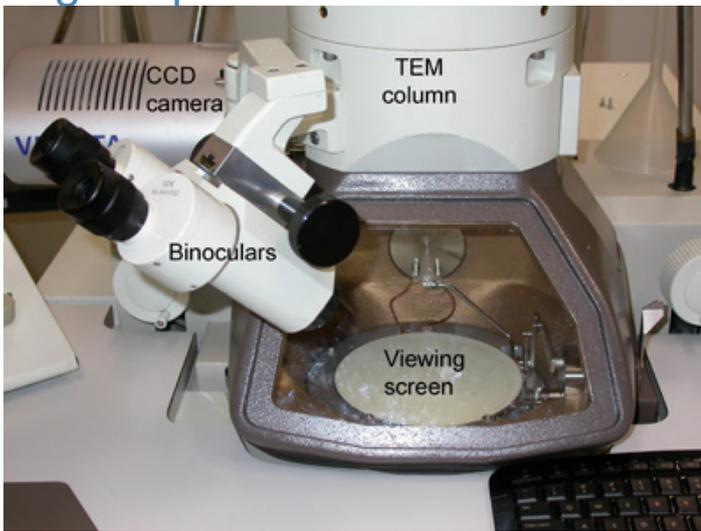




Image capture



At the base of the column is a viewing chamber with a window port and an adjustable pair of binoculars. The image is projected onto the screen in the viewing chamber. The binoculars are available for focusing the image. The screen in the chamber is only for producing a temporary image. To collect a permanent image a CCD camera is inserted into the path of the beam. This allows the image to be collected in a digital form.

The exposure time – the length of time that the beam is directed at the collection device – can be adjusted to suit beam parameters and to control the quality of the desired image.

Applications and practical uses - what the TEM can do

The transmission electron microscope (TEM) is used to examine the structure, composition, and properties of specimens in submicron detail. Aside from using it to study general biological and medical materials, transmission electron microscopy has a significant impact on fields such as: materials science, geology, environmental science, among others.

The investigation of the morphology, structure, and local chemistry of metals, ceramics, and minerals is an important aspect of contemporary materials science. It also enables the investigation of crystal structures, orientations and chemical compositions of phases, precipitates and contaminants through diffraction pattern, characteristic X-ray, and electron energy loss analysis.

Transmission electron microscopy **can**:

- Image morphology of samples, e.g. view sections of material, fine powders suspended on a thin film, small whole organisms such as viruses or bacteria, and frozen solutions.
- Tilt a sample and collect a series of images to construct a 3-dimensional image.
- Analyse the composition and some bonding differences (through contrast and by using spectroscopy techniques: microanalysis and electron energy loss).
- Physically manipulate samples while viewing them, such as indent or compress them to measure mechanical properties (only when holders specialised for these techniques are available).
- View frozen material (in a TEM with a cryostage).
- Generate characteristic X-rays from samples for microanalysis.
- Acquire electron diffraction patterns (using the physics of Bragg Diffraction).
- Perform electron energy loss spectroscopy of the beam passing through a sample to determine sample composition or the bonding states of atoms in the sample.

What the TEM can't do

There are some things TEM can't do:

- TEM cannot take colour images. Colour is sometimes added artificially to TEM images.
- TEM cannot image through thick samples: the usual sample thickness is around 100-200nm. Electrons cannot readily penetrate sections much thicker than 200nm.
- A standard TEM cannot image surface information.
- The TEM cannot reliably image charged molecules that are mobile in a matrix. For example, some species (e.g. Na⁺) are volatile under the electron beam because the negative electron beam exerts a force on charged material.

Where the TEM is not suitable, other techniques should be used. If in doubt consult the Technique Finder on the AMMRF web site.

Images from electrons

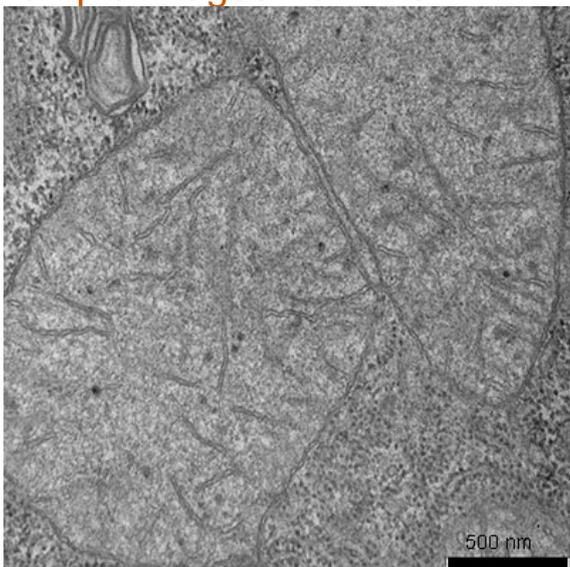
Electron images from the TEM can be used to achieve different information, for example for morphological, crystallographic or compositional studies.

It is also possible to label molecules with electron dense particles (e.g. nano-sized gold spheres that attach to molecules through immunolabelling techniques) or construct 3-dimensional images of particle, structures or cells through tomography.

Various types of microanalysis are also able to be undertaken using TEM equipped with electron energy loss spectroscopy (EELS) or x-ray energy dispersive spectroscopy (EDS or EDX) systems. These are described in the Microanalysis module.

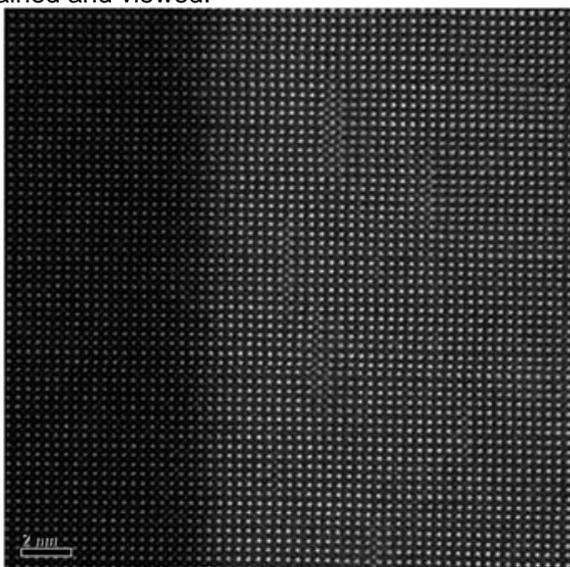
Click on any of the images on this page to see them at their full resolution.

Example images from the TEM



Bright field electron micrograph of mitochondria from a rat's liver. The

tissue was chemically fixed, mounted on a grid, sectioned with a room-temperature ultramicrotome and the section was stained and viewed.

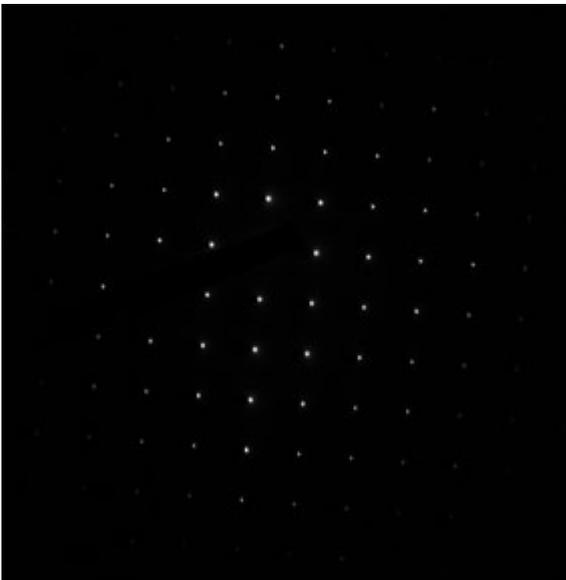


High resolution electron microscope image of lanthanum strontium

manganate on left, bismuth ferrite on right.



Dark field image of a zinc oxide crystal.

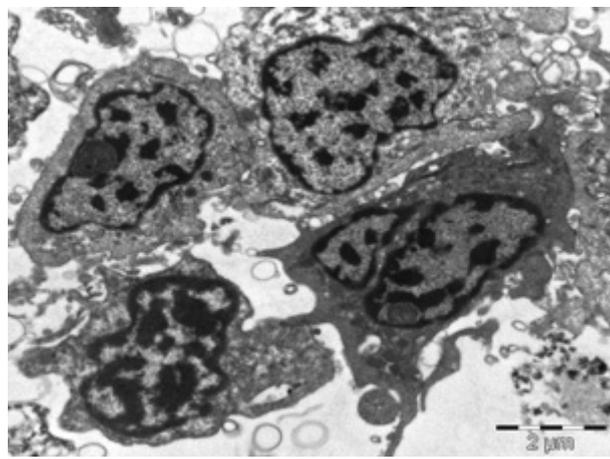
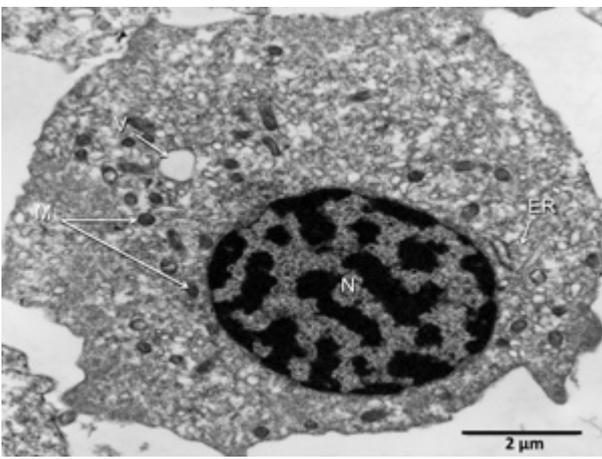


Selected area diffraction pattern of Nd₁₃CaO₇.

This video is a sequence of 3 serial electron tomograms of the golgi region of an HIT-T15 cell (a hamster-derived pancreatic beta cell). Such videos are constructed from a series of images taken through a thick tissue section.

Tomograms courtesy of Dr. Brad Marsh

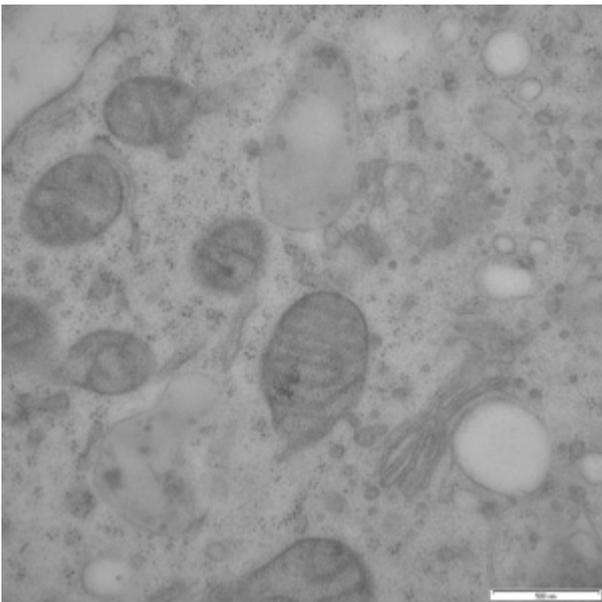
[A range of bright field images related to biology \[see sample preparation for background on procedures\]](#)



Haemocytes from

Detail on samp

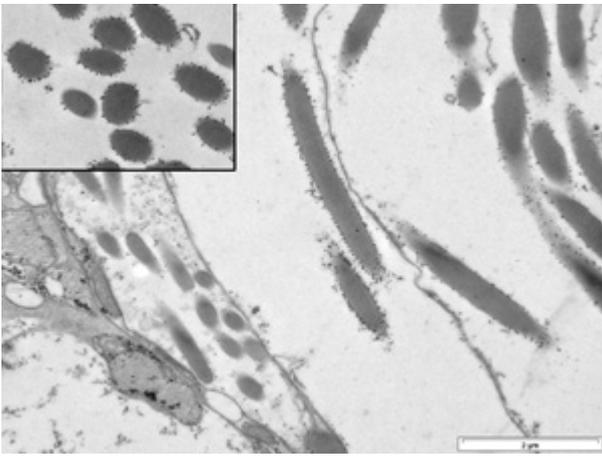
The samples have
graded series of e
strained, dried an



Baby Hamster Kidney (BHK) cells. This tissue was fixed by high pressure freezing followed by freeze substitution.

Detail on sample preparation

This tissue was fixed by high pressure freezing followed by freeze substitution. It was then low temperature embedded in the resin HM20. The block was then sectioned at room-temperature by ultramicrotomy, picked up on a grid, stained, dried and viewed.

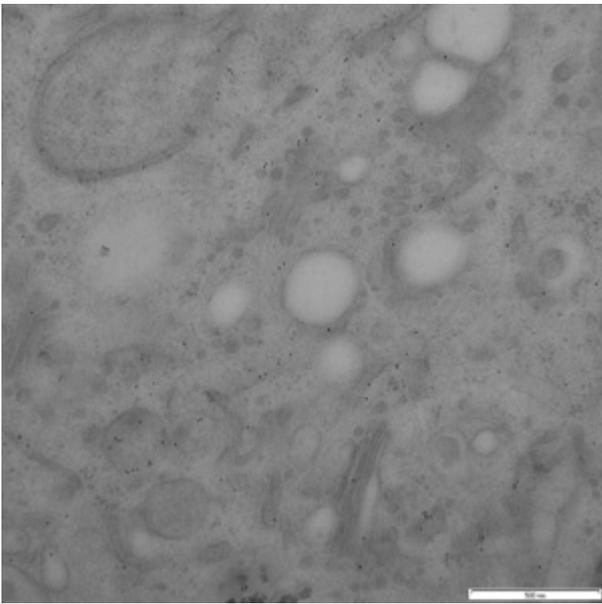


The rod-shaped secretion used in the temporary glue of *Troglcephalus rhinobatidis* marine parasites. These external parasites of stingrays look like small worms. The black dots show sugar chemistry on the surface of the secretions (cross sections and longitudinal sections of rod-secretions are shown).

Detail on sample preparation

Molecules within cells can be labelled using a variety of methods but to see them using electron microscopy the labels must be electron dense or coupled to electron dense material such as silver or gold particles. This tissue has been labelled using a modification of the periodic acid-Schiff (PAS) histochemical method with silver as the visible marker under electron microscope: a periodic acid-thiosemicarbazide-silver proteinate reaction. PAS is used to detect polysaccharides such as glycogen, glycoproteins and glycolipids.

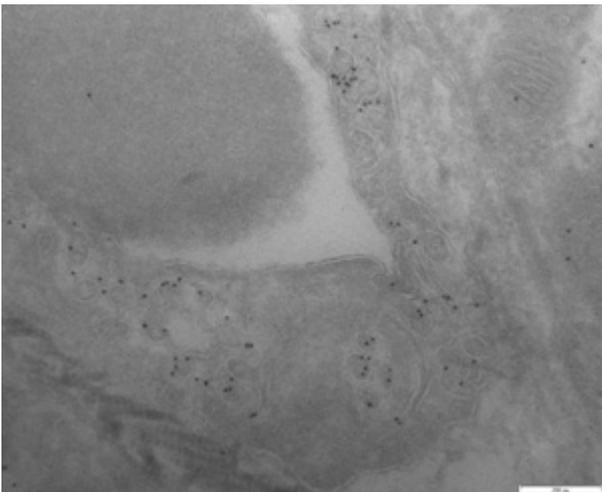
Here, ultrathin sections prepared from chemically fixed tissue were placed on nickel grids, treated with 0.5% periodic acid in water for 20 min, rinsed with water, then reacted with 1% thiosemicarbazide in 10% acetic acid for 30 min. They were then rinsed in a series of water washes. The sections were reacted with 1% silver proteinate in water in darkness for 30 min, rinsed and dried. The labelled sections on grids were then stained with uranyl acetate and lead citrate and viewed.



Baby Hamster Kidney (BHK) cells labeled for GFP-Rab18: immunolabeled with anti-GFP. The labels are the small black dots. GFP=Green Fluorescence Protein.

Detail on sample preparation

This tissue was fixed by high pressure freezing followed by freeze substitution. It was then low temperature embedded in the resin HM20. The block was then sectioned at room-temperature by ultramicrotomy, picked up on a grid, stained, dried and viewed.



Endothelia in mouse muscle, immunolabeled for cavin1 using the Tokuyasu method. The labels are the small black dots.

Detail on sample preparation

The process used followed the steps: chemical fixation; cryoprotection; high pressure freezing; cryo-ultramicrotomy; thawing; mounting on grid; immunolabeling; staining; drying; and viewing.

Concepts - introduction

The fundamental basis of electron microscopy is the use of an electron beam. It is for this reason that the TEM requires a vacuum. If air were present the molecules would cause the beam to scatter. It is also imperative to understand the interactions between the electron beam and the sample so as to interpret the resulting images. The use of electrons has an impact on image resolution and absence of colour, and explains the two dimensional nature of micrographs (images/photographs).

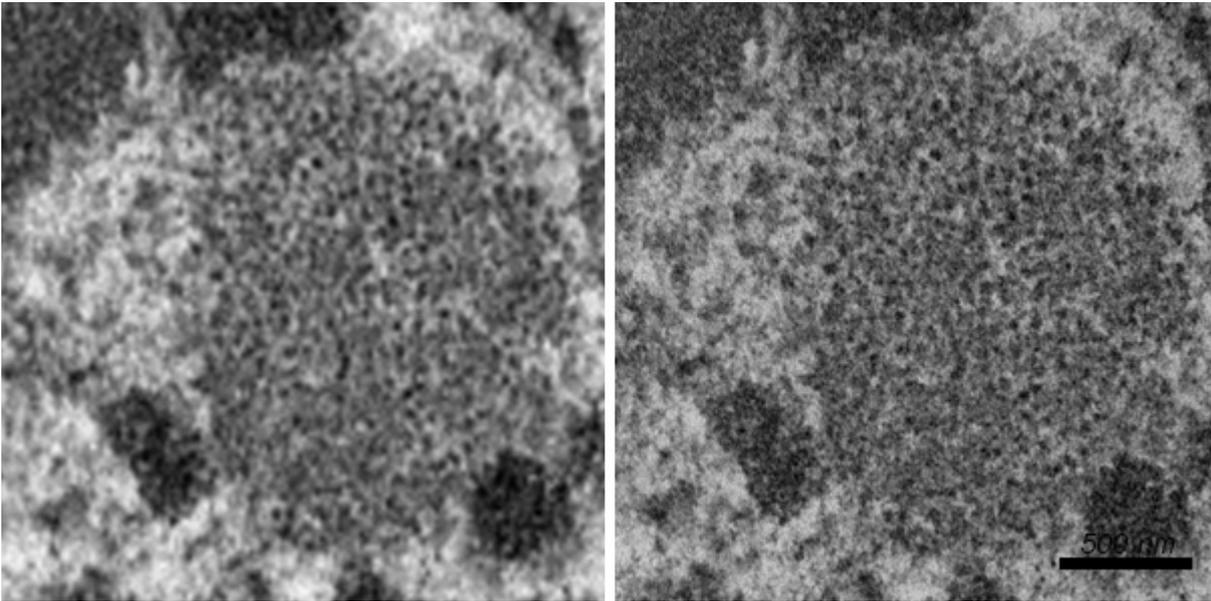
Resolution

Definitions

- **Resolution** the ability to distinguish closely spaced points as separate points.
- **Resolution Limit** smallest separation of points which can be recognized as distinct.
- **Resolving Power** resolution achieved by a particular instrument under optimum viewing conditions.

The concepts of resolution and magnification are often confused. Any image can be magnified without limit, but most will become blurry if we do this. A crisp image is what is wanted. This means:

- the image must be properly focused, and
- the image must have adequate resolution



The left image shows low resolution and the right image shows high resolution. The left image is an enlargement of a low magnification image whereas the right image is taken at the higher magnification. Both images are of a cell nucleus (rat's liver cell).

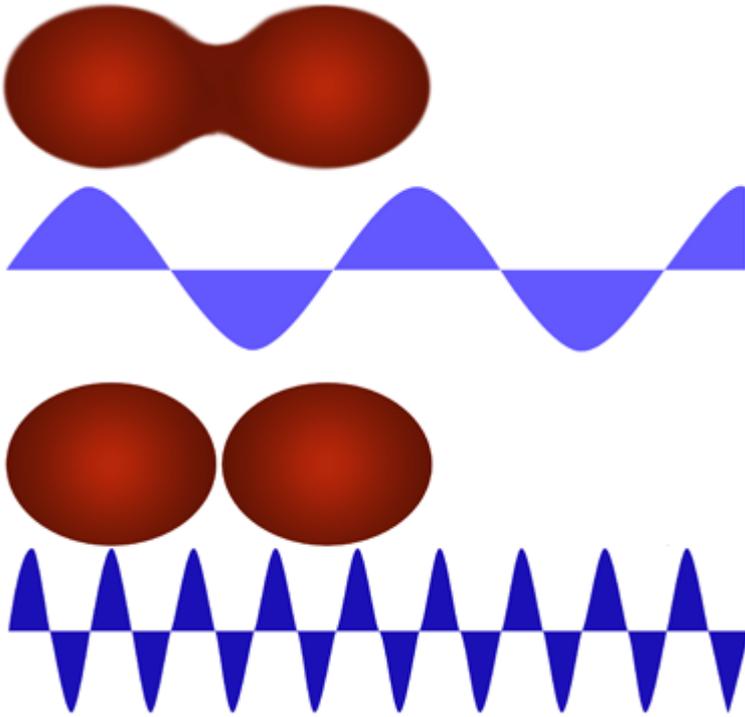
Magnification is simply the process of enlarging an image. Once a TEM is calibrated then it is possible to determine exactly how much enlargement has occurred. This can be recorded on an image as a scale bar. The use of 'times magnification', e.g. 50,000x, will only be accurate for an image of set/ fixed dimension and so can lead to errors.

Higher magnification will not necessarily give higher resolution. Unless a microscope is equipped to deliver higher resolution images, higher magnification will only achieve 'empty' images.

Resolution in a microscope is determined primarily by the wave nature of light or electrons according to Abbe's equation:

$$d = \frac{0.61\lambda}{n \sin \alpha}$$

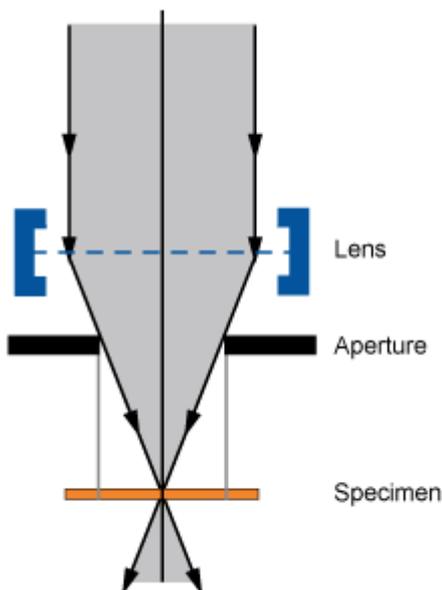
Illumination with a smaller wavelength results in better resolution (the two spots can be seen as distinct) and this is why the electron microscope produces higher resolution images than the the light microscope; because the wavelength of an electron is smaller than visible light.



Illumination with a smaller wavelength results in better resolution.

Where	Equals
d	resolution (minimum resolvable distance)
λ	wavelength of the energy source
n	refractive index of the medium
θ	aperture angle

Note: The term $n \sin \theta$ is named numerical aperture.



For more on resolution see the Scanning Electron Microscope (SEM) module.

Theory of TEM image generation

- Wavelength
- Image types
- Image formation basics
- Object/Image planes
- Diffraction basics
- Diffraction images
- Combining images
- Imaging mode setup
- Focus/stigmatism

The electron wavelength

The wavelength of an electron is dependent upon accelerating voltage and is given by:

$$\lambda = \frac{h}{\sqrt{2meV}}$$

Where	Equals	
h	Planck's constant (6.626 x 10 ⁻³⁴ J s)	
m	electron mass (9.109 x 10 ⁻³¹ kg)	
e	electronic charge (1.60 x 10 ⁻¹⁹ C)	
V	accelerating voltage (0.5 – 3 x 10 ⁴ V)	
Voltage (kV) of	Becomes	? (nm)
5	?	0.0173
10	?	0.0122
200	?	0.0027
Light	?	400 - 700

$$\lambda = \sqrt{\frac{1.5}{V}} \text{ nm}$$

can be approximated to:

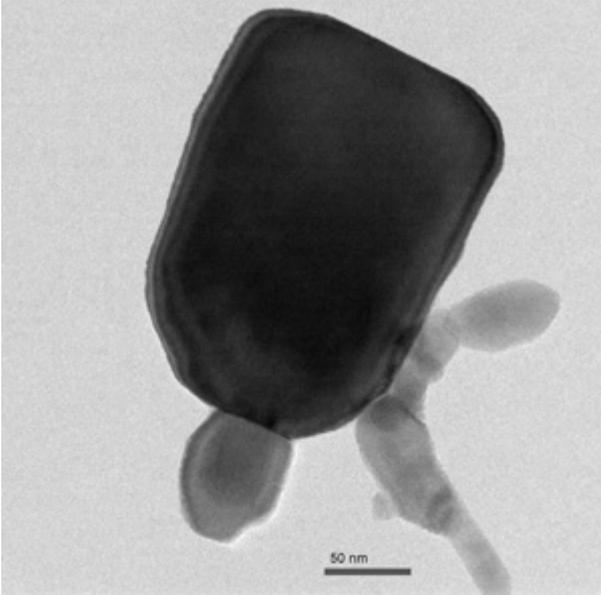
The higher the accelerating voltage, the smaller the wavelength of the electrons and the higher the possible achievable resolution.

Examples from this equation

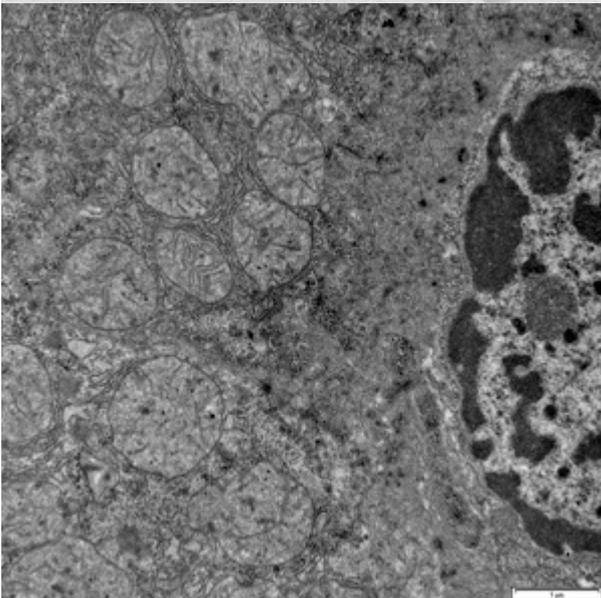
Image types

Bright field, HREM and diffraction images

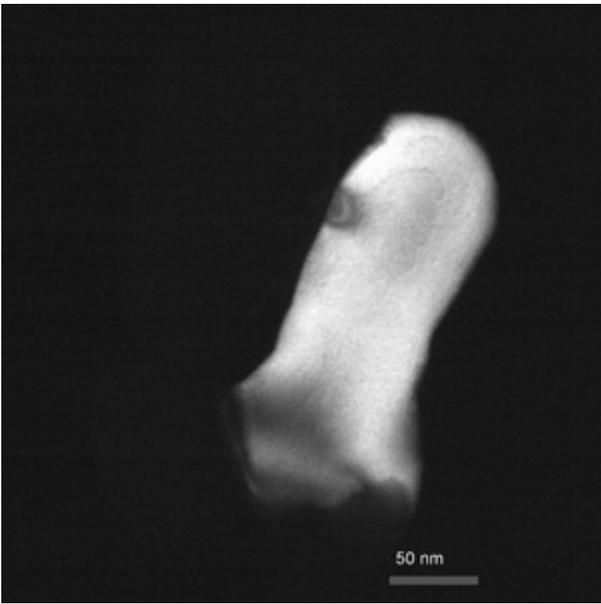
The most common image generated using a TEM is a bright field image. Some areas of the sample scatter or absorb electrons and therefore appear darker. Other areas transmit electrons and appear brighter. In simple terms the bright field image appears as a shadow of the specimen. In the bright field image the objective aperture is used to select the unscattered electron beam. In doing so, the scattered electrons are excluded from forming the image. This aperture enhances the contrast in the image.



Zinc oxide crystals: bright field image.

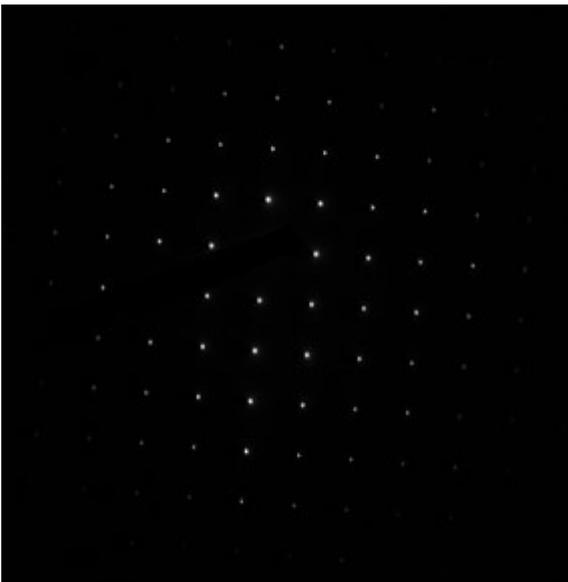


Rat's liver: bright field image.



Zinc oxide crystals: dark field image.

Dark field images are produced by excluding, using the primary aperture, the primary (unscattered) beam from the image collected below the sample. The image is produced by scattered electrons (i.e. only selected electrons are used to form the image). Regions where no scattering occurs, such as where the primary electron beam passes straight through the sample, appear black (e.g. in areas around the sample). This kind of imaging is useful in studying crystal defects, and for the imaging of specific crystallographic phases.



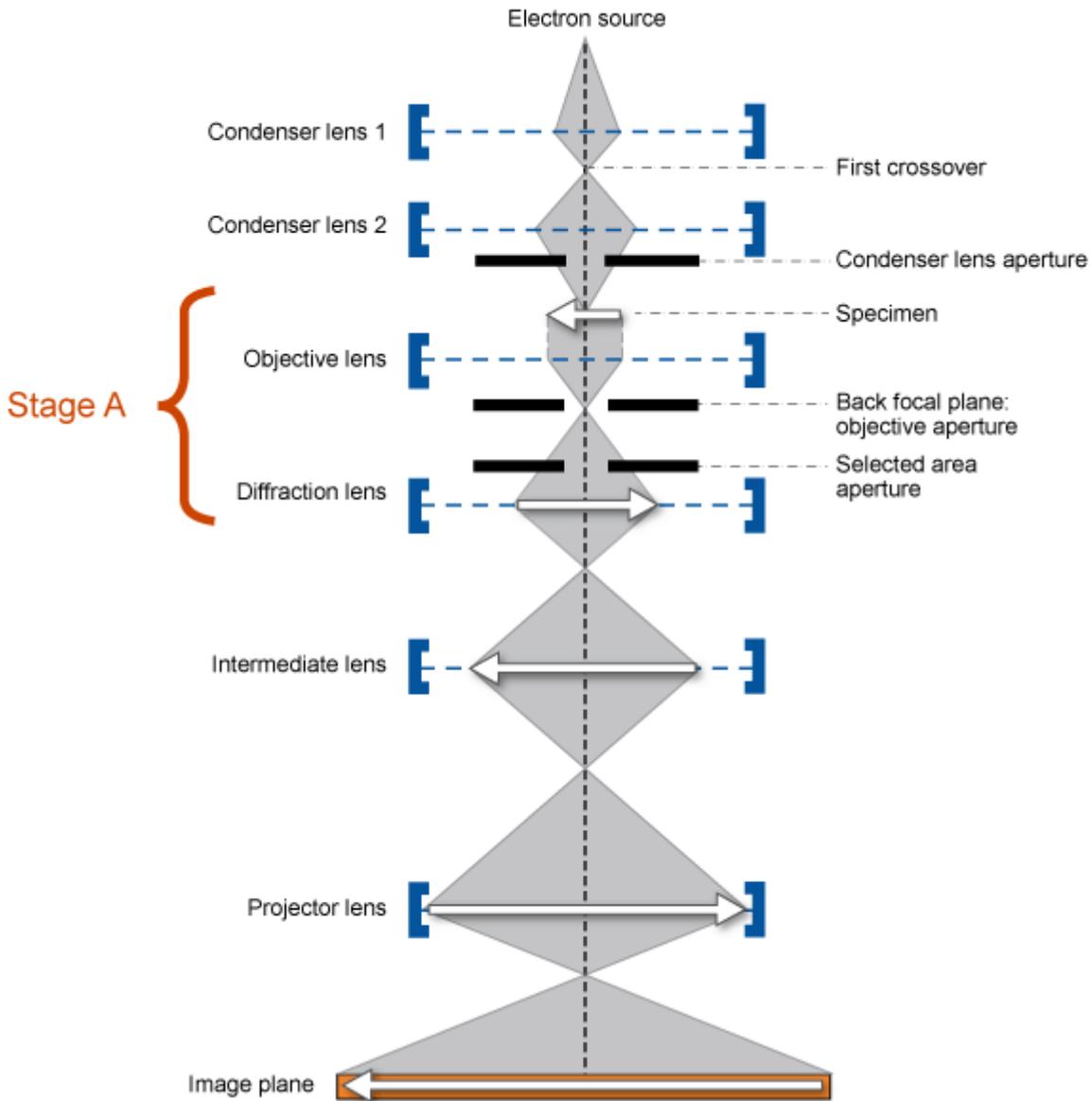
Spot diffraction pattern of $\text{Nd}_{13}\text{CaO}_7$

Diffraction images are the result of Bragg scattering as the beam passes through a crystalline sample. If a “selected area diffraction aperture” is inserted to delimit the region of interest, then an image created below the sample (in the region called the back focal plane) is seen as an array of dots (or a set of diffuse rings). This informs the viewer about the crystal structure of the sample.

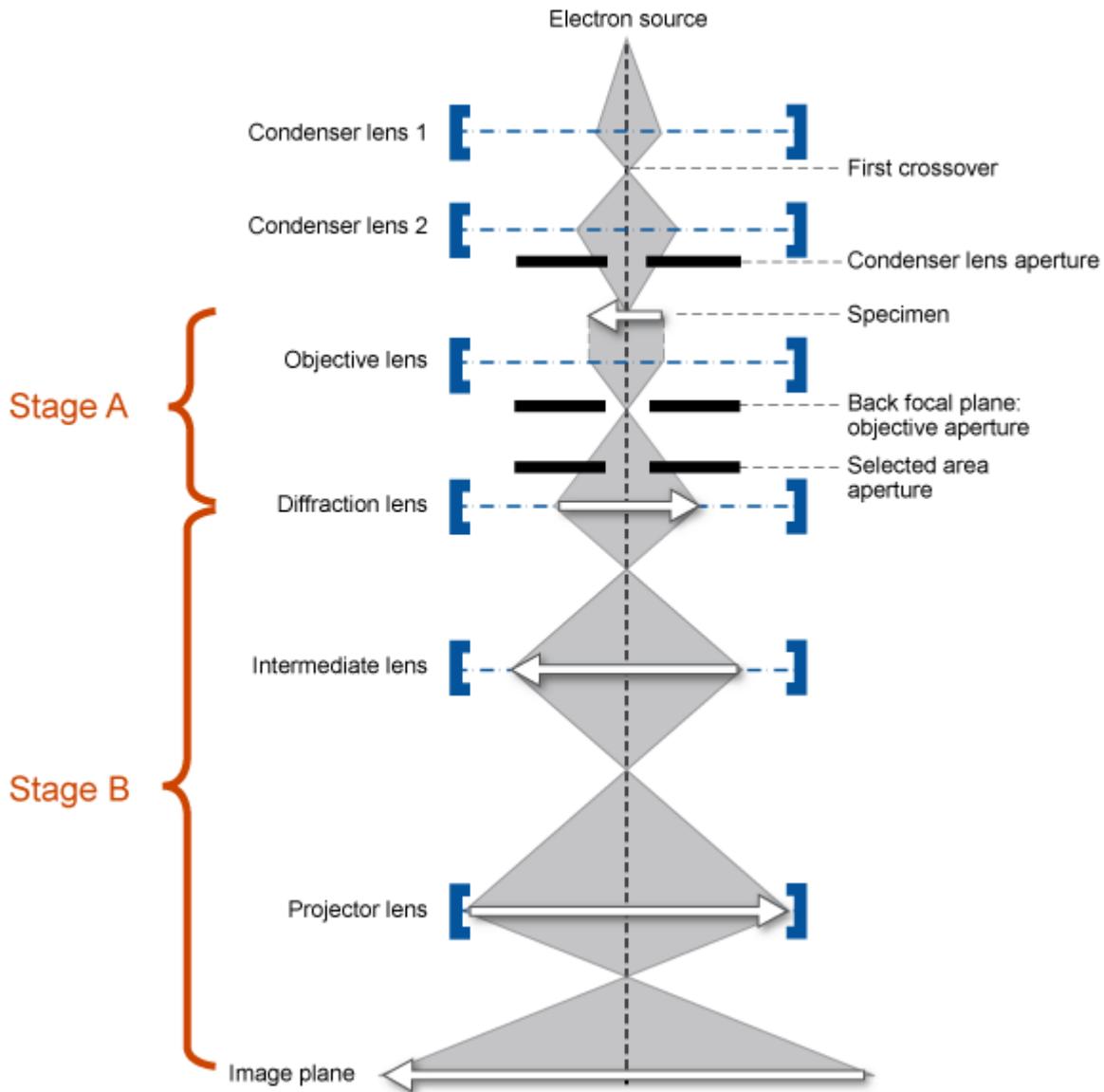
Image formation basics

The TEM images are formed in two stages:

A. **Stage A** is the scattering of an incident electron beam by a specimen. This scattered radiation passes through an objective lens, which focuses it to form the primary image.



B. **Stage B** uses the primary image obtained in stage A and magnifies this image using additional lenses to form a highly magnified final image.

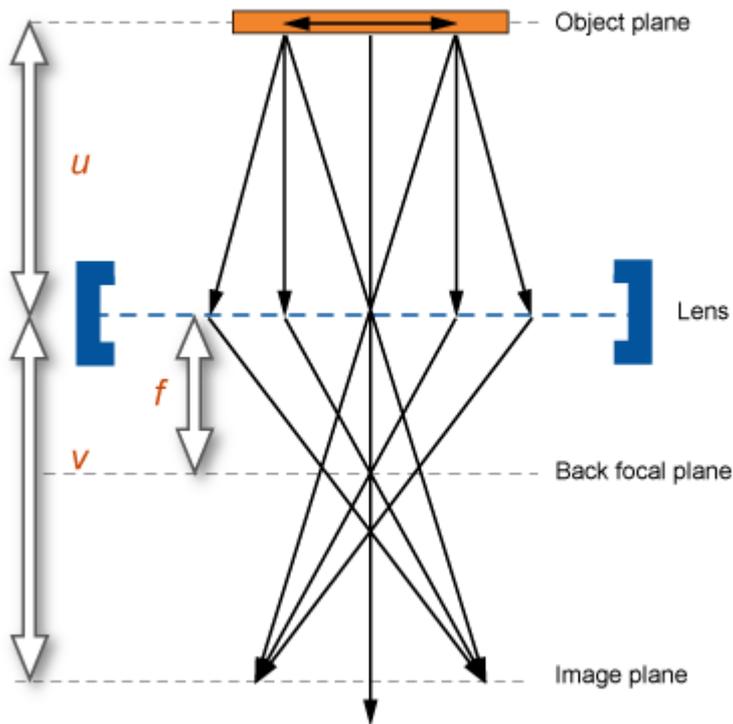


In the process of forming the primary image the objective lens produces a diffraction pattern at its back focal plane. The diffraction pattern is a Fourier transform of the scattered electron wave. The primary image is the Fourier transform of the diffraction pattern.

This two-step process forms the basis of image formation during high-resolution transmission electron microscopy (HRTEM).

The high-resolution image is, in effect, an interference pattern of the beams formed at the back focal plane of the objective lens.

Object/Image planes



Definition of "Planes"

- The object plane contains the object point and is always located above the lens.
- The image plane contains the image point and is always located below the lens.
- The focal plane of the lens is where parallel rays are brought to a focus.

Definition of "Distances"

- The object distance (u) is the distance from the object plane to the lens.
- The image distance (v) is the distance from the lens to the image plane.
- The focal length (f) is the distance from the lens to the back of the focal plane. If the lens is symmetric in strength on either side of the lens plane, the following basic equation known as Newton's lens equation can be written:

$$\frac{1}{u} + \frac{1}{v} = \frac{1}{f}$$

Diffraction basics

Samples viewed using the TEM can provide information about its structure, in particular its crystalline nature. This is because a crystal lattice acts as a diffraction grating: interference patterns are produced in the electron beam as it travels out from the lattice and these can be projected as an image of regular dots or rings.

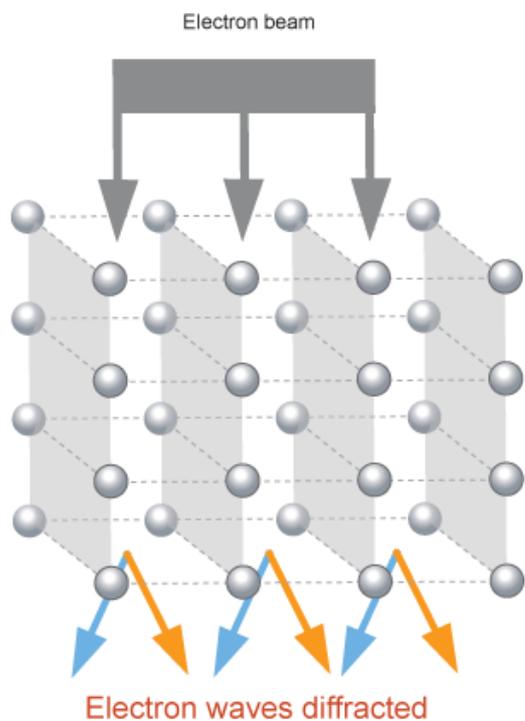
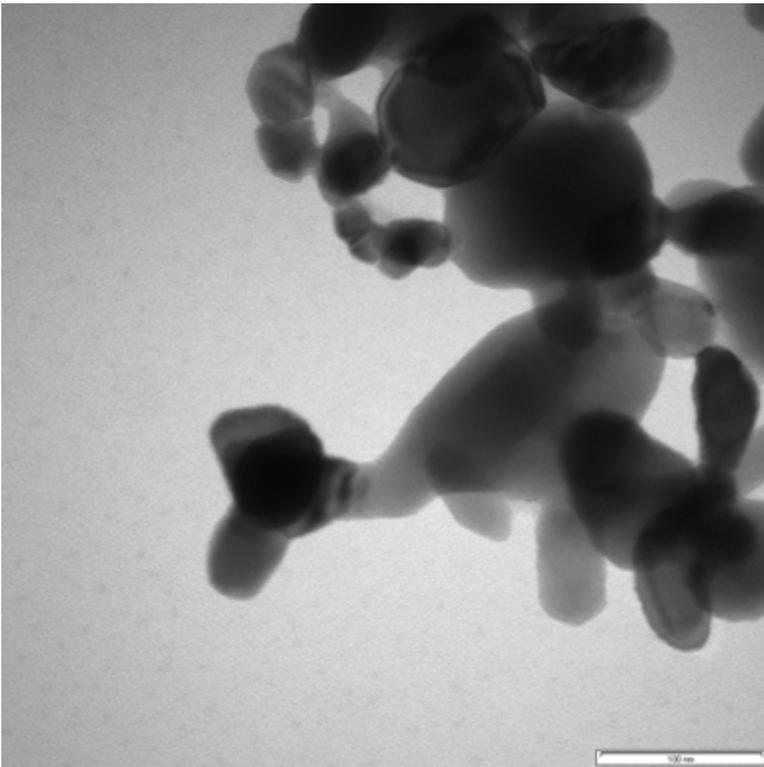


Image appearance

At high magnification, crystals may exhibit diffraction contrast. This can occur for one of two reasons:

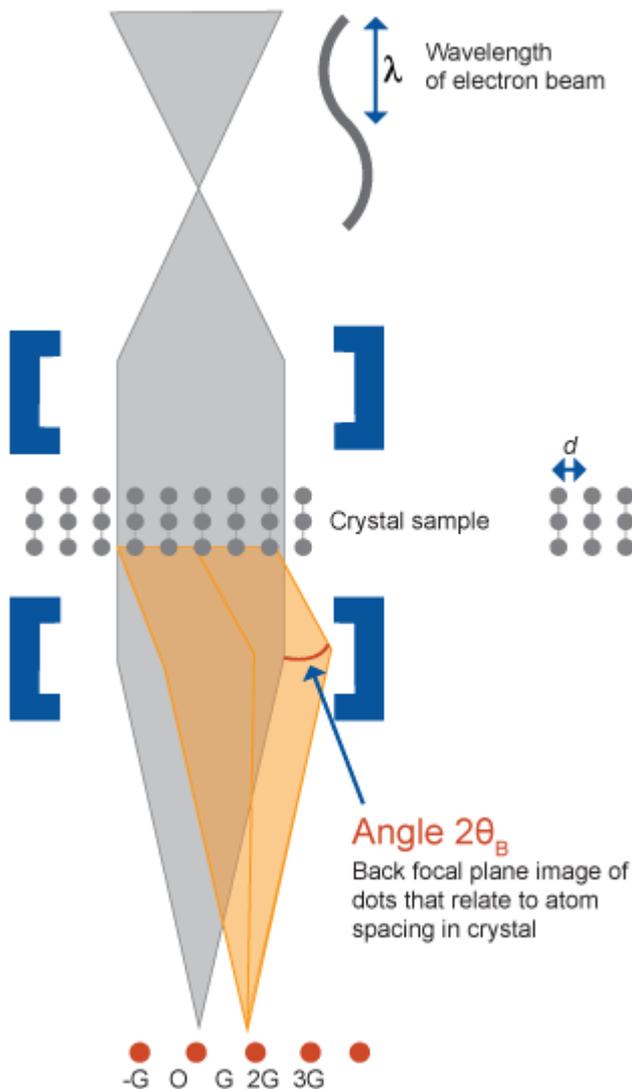
1. Strongly diffracting regions of crystals can appear darker because there are fewer electrons transmitted along the primary beam. These crystals may be sitting on the grid holder at a tilted angle so that a lattice of the crystal lines up parallel to the beam.
2. Thicker regions can also appear darker due to greater scattering. This can be seen in the image here, where some crystals lie on top of one another.

As the sample is tilted it will change in appearance to darker or lighter contrast depending on how the beam is interacting with the internal lattice. When strong diffraction conditions are achieved, the image will appear darker as more electrons are scattered outside of the objective aperture.



The diffracted beam

When the electron beam passes through the thin crystalline sample, it is diffracted by the atomic planes in the sample when the Bragg condition is satisfied. These waves interact constructively and are brought to focus at the back focal plane of the objective lens (see Planes) to form the diffraction pattern.



Unscattered electrons continue through to **O** to produce a central spot. The beam diffracted by angle $2\theta_B$ produces a spot, marked **G**. The distance between a diffracted (**G**) and transmitted (**O**) spot is inversely proportional to the corresponding lattice spacing in the sample.

The beam deflection angle and electron beam wavelength are important.

Bragg's law describes the interaction:

$$\lambda = 2d \sin \theta_B$$

This equation can be used as long as the wavelength is less than the crystal interplanar spacing (d). This works for a TEM where the accelerated electron beam describes a wavelength of a few pm. This means for most crystalline materials that the

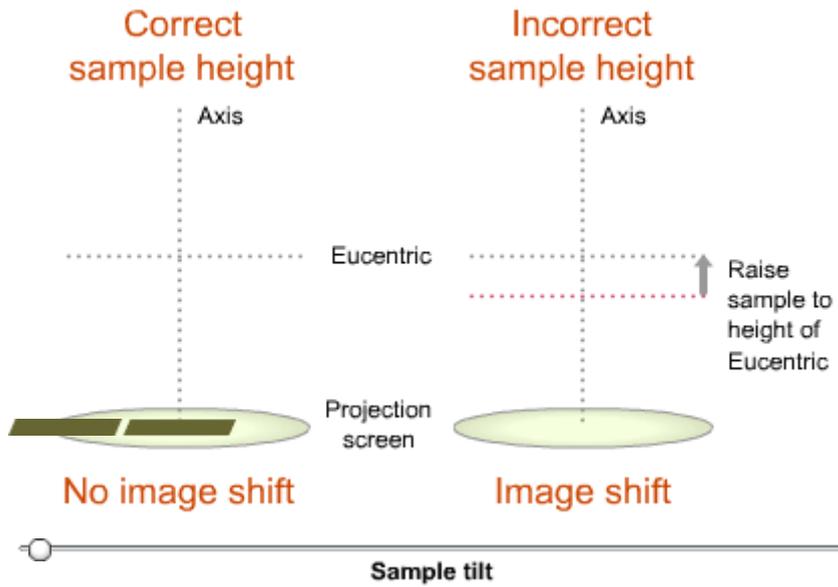
Bragg angle is much less than 1° .

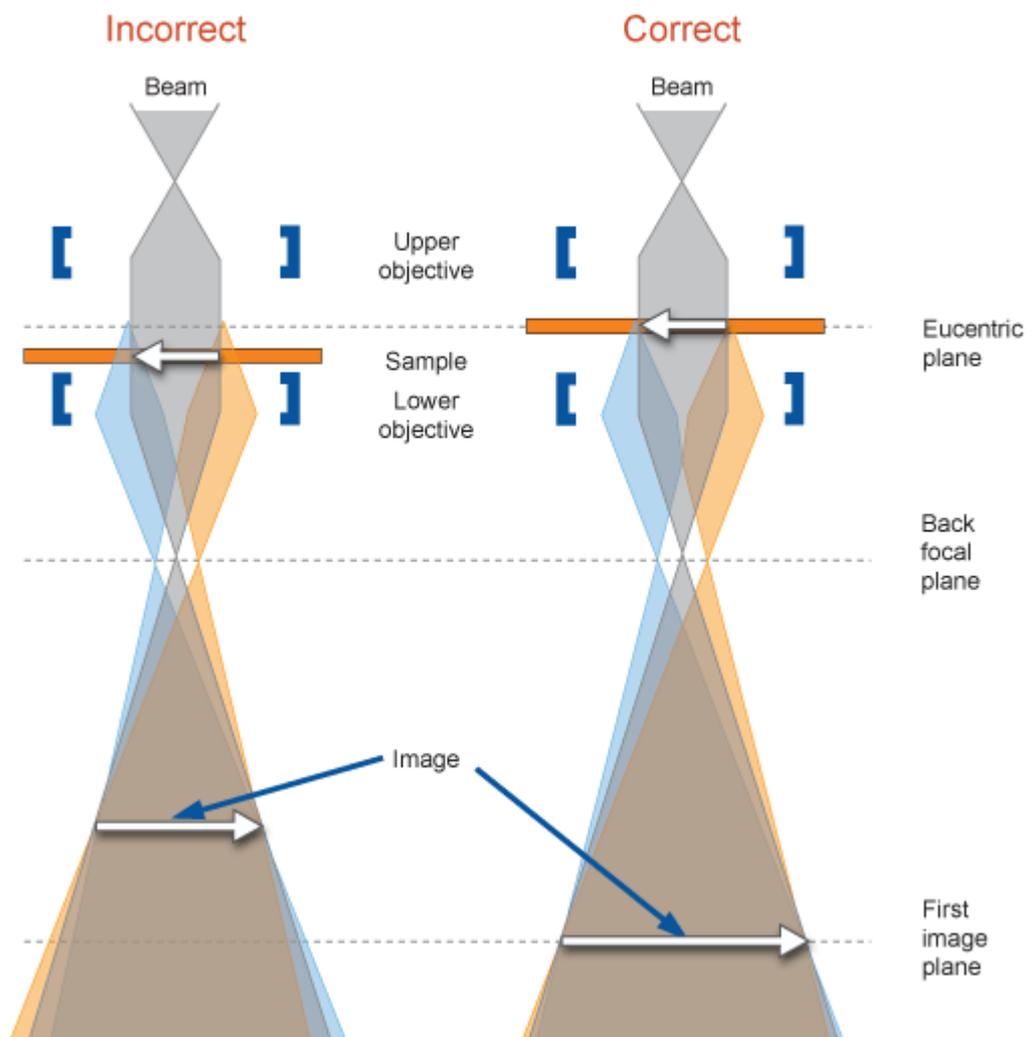
The Camera length (projection distance) is also important to know in order to calculate details about the sample. It can be set when photographing a diffraction pattern.

The Eucentric Position

The eucentric position is the center of the objective lens. The sample must be set to this position. To do this the entire sample holder is raised or lowered.

TEMs are set up so that magnification, camera length, and correct focus are set to this reference position. When the sample height (in the Z-direction) is set at the eucentric position, one can tilt the sample around its axis without the image of the sample moving across the projection screen.





The eucentric height can be set by adjusting the objective lens current to a specific known setting for a specific voltage (e.g. 80kV, 100kV or 200kV), and then raising or lowering the Z-height until the image is in focus. The "wobbler" button can be used as a focus aid where one brings the vibrating split image together into one static image.

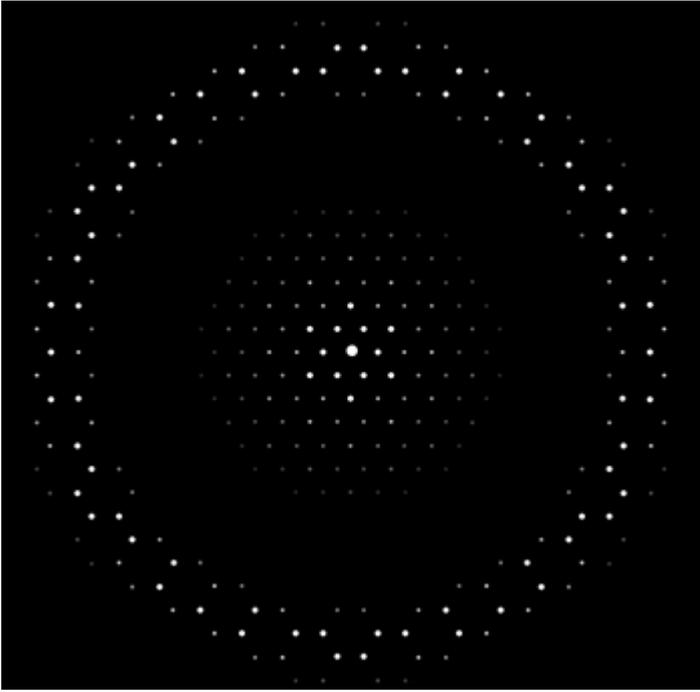
Alternatively, if the objective lens current is not known for the correct sample height, the sample Z-height is raised or lowered until, when tilted, the axis of the sample remains centered.

This is how the beam path looks when set up for selected area diffraction (SAD) and the sample height is incorrectly set (left) and correctly set (right). Observe what happens to the magnification and focus.

Tilting

The appearance of a diffraction pattern will depend on the orientation of the specimen to the electron beam. If the specimen is tilted so a plane of atoms or crystallographic direction satisfies the Bragg condition, distinctive diffraction patterns will be obtained with diffraction maxima (i.e. spots - often called reflections) in arrangements which reflect the crystal structure of the specimen.

To achieve this, samples in a TEM can be tilted. There are both single-tilt and double-tilt specimen holders. A double-tilt holder is superior since the tilting of the sample can be achieved in two axes (X and Y). It is common to try and tilt a sample so that a crystal zone axis is, in effect, parallel to the electron beam. Under these conditions a predictable arrangement of reflections will be present in the diffraction pattern - see the pattern below for ZnO.



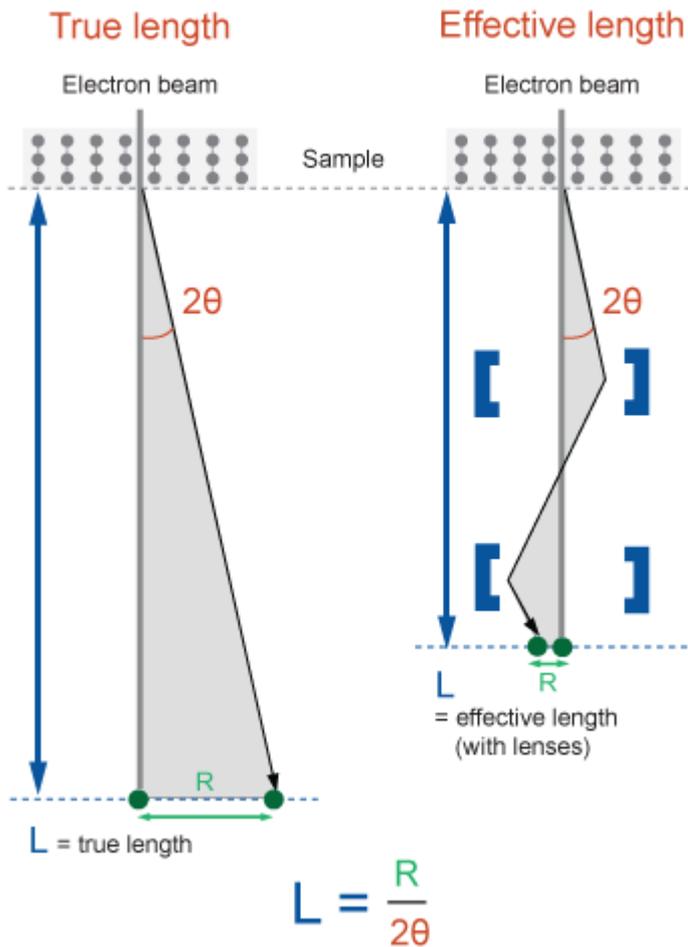
This diffraction pattern has been simulated and tilted using the jems program by Pierre Stadelmann.

You can practice tilting a sample using the virtual TEM .

Camera length

In order to calculate the lattice spacing in our sample we need to know the 'camera length'.

Camera length = the distance from the sample to the projected image.



The diffraction pattern (formed in the back focal plane) forms the projected image on the screen and can be recorded, hence the term 'camera length' since this is where the camera is positioned.

The projection process enlarges the distance between the reflections in the diffraction pattern.

To obtain precise measurements from a diffraction pattern using a TEM, one must precisely know the camera length.

A small camera length provides a pattern with little space between the reflections and a large camera length provides a pattern with large spaces between the reflections.

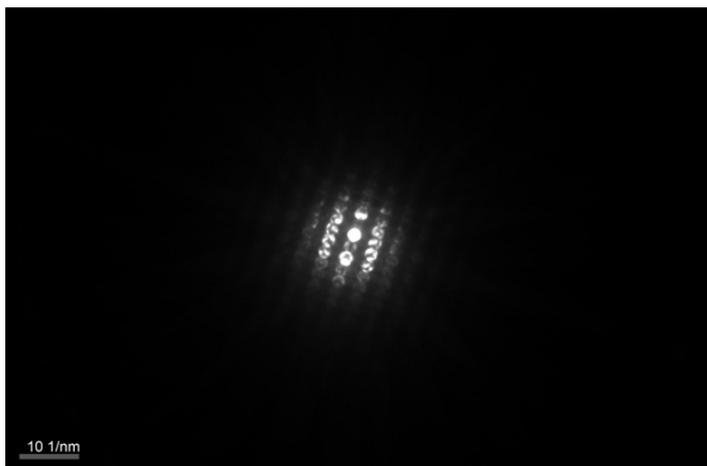
So, too small a length and the diffraction image only fills up a small region of the projection screen. Too large and part of the diffraction pattern can be lost at the edges beyond the projection screen.

It must be remembered that the camera length must be calibrated for accurate measurements. This can be done by using a calibration standard sample for which the lattice spacing is known.

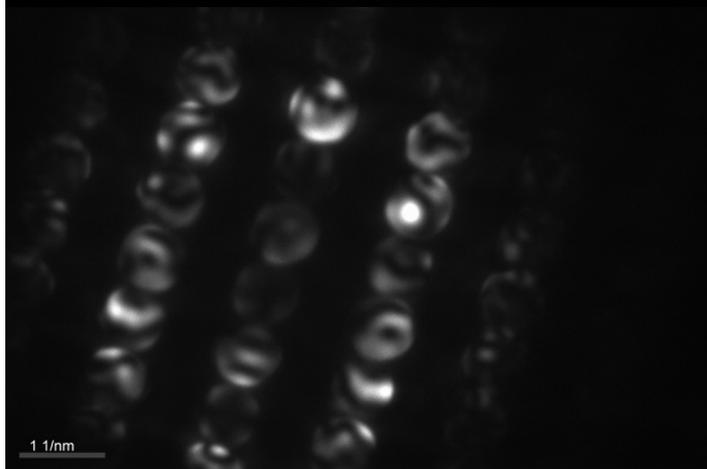
Once an accurately calibrated diffraction pattern is achieved, the information in the pattern is used to determine lattice

planes and in the indexation of diffraction patterns [see useful links for information on how to index diffraction patterns].

The images are convergent beam electron diffraction patterns (CBED) from a ZnO crystal. With a larger camera length we see more detail from the discs.



The image at a camera length of 8cm

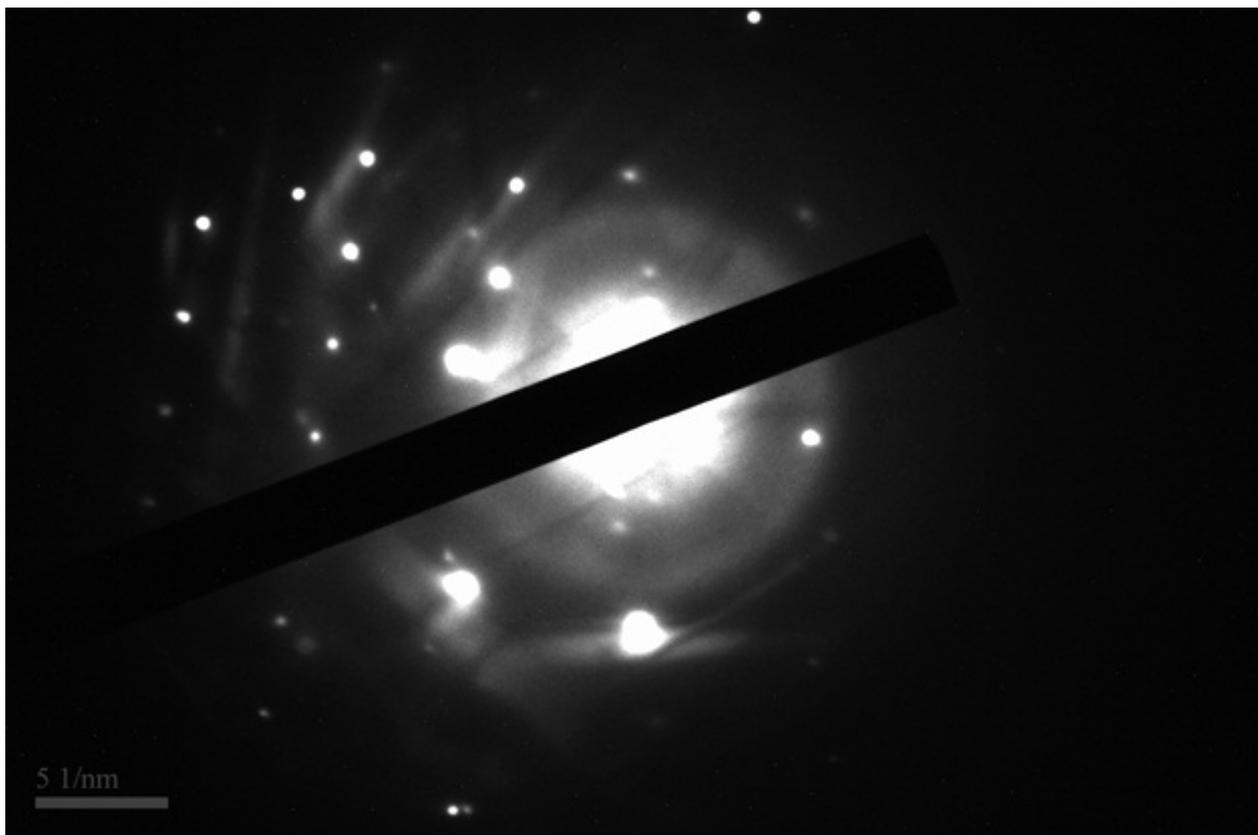


The image at a camera length of 50cm

Kikuchi Patterns

Bragg scattering, that is diffraction of inelastically scattered electrons, can lead to the formation of pairs of parallel lines in the diffraction pattern called Kikuchi lines. For each plane of atoms in the sample there exists a pair of parallel lines, rather like train lines. The various sets of Kikuchi lines intersect in diffraction space in a manner which represents the arrangement of crystal planes in real space. This is called a Kikuchi map. These lines can help an operator to tilt a crystal around to find different crystal planes. Kikuchi Patterns are a useful phenomenon to use when initially learning how to tilt crystals because they form regular intersecting lines over a zone axis.

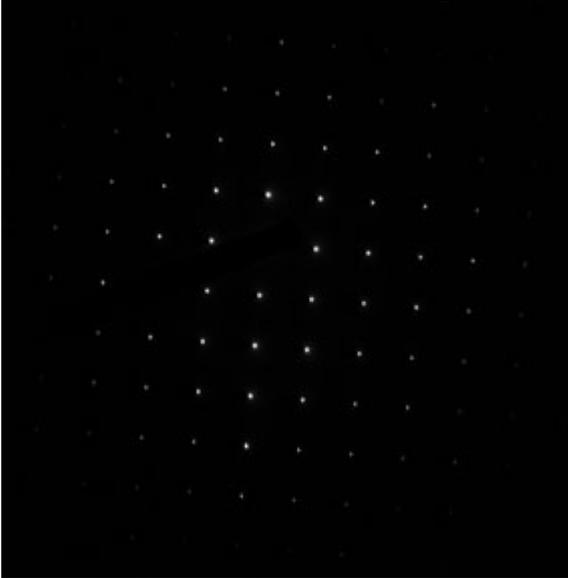
This spot diffraction pattern shows Kikuchi lines on the left side of the pattern. This pattern is in the process of being tilted and not yet on the zone axis.



Diffraction patterns

The appearance of the diffraction pattern can reflect the nature of the crystalline phases in the specimen. For example, if the material is microcrystalline or amorphous the diffraction pattern consists of a series of concentric rings rather than spots/discs.

Spot patterns



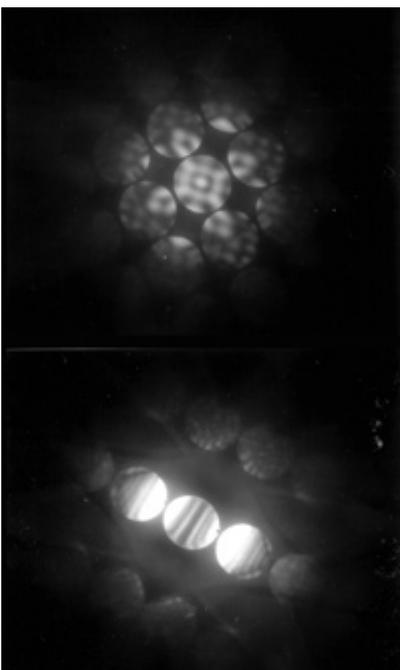
When the electron beam interacts with the sample when the sample is

oriented with a zone axis pattern parallel to the electron beam, then the diffraction pattern form in the back focal plane of the objective lens is a regular array of reflections. This is seen projected onto the viewing screen as an array of reflections organized in a predictable manner based on the crystal structure of the sample.

The image shows a classic spot diffraction pattern obtained from $\text{Nd}_{13}\text{CaO}_7$.

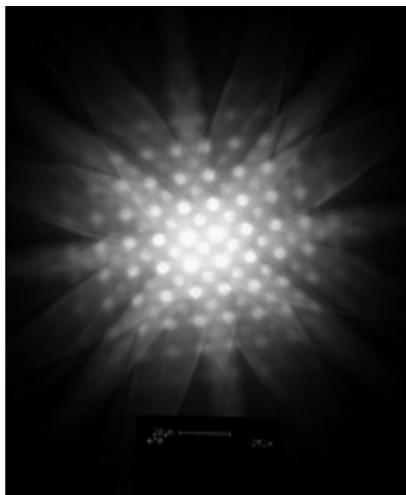
[Click here](#) to find out how to set up the TEM lenses in order to obtain such an image.

Convergent Beam Electron Diffraction (CBED)



When the electron beam is converged on the sample to a point (method = convergent beam), instead of using a parallel stream of electrons through the sample, the diffraction pattern forms discs instead of spots in the back focal plane of the objective lens.

These discs can contain detail that provides information about the crystal structure of the specimen. In these images we see almost perfect mirror symmetry in the patterns within the discs. These symmetries can be used to determine the point and space group of the crystal.

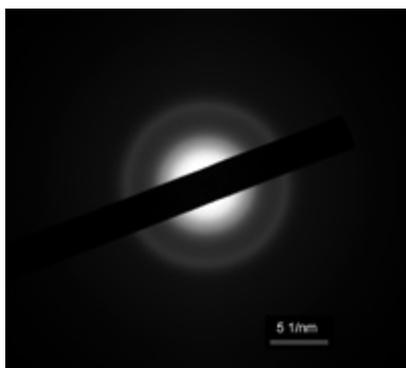


This image shows Kikuchi Patterns with a convergent beam diffraction pattern. The star-like peaks show mirror symmetry in a regular crystal matrix that is well aligned along the zone axis.

Ring patterns

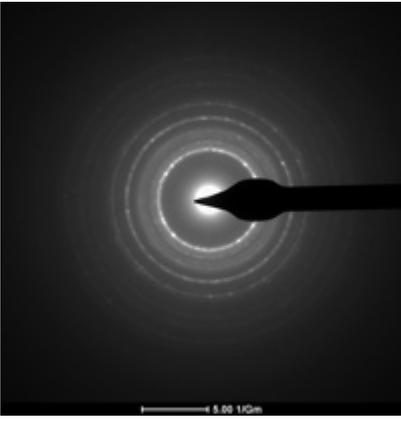
Sometimes, instead of intensity spots, the electron diffraction pattern is composed of concentric rings.

Materials that contain no long-range order in the atomic lattice produce diffuse ring diffraction patterns with no discrete reflections and one or possibly two diffuse rings of maximum intensity. Amorphous samples, e.g. polymers and metallic glasses, produce this kind of pattern.



Some thin films used as substrates to hold samples are made of materials such as amorphous SiN. This is the ring pattern from such a film.

The bar across the pattern is the "beam stop" used to cover the bright central beam spot so that the more diffuse rings can be captured as a digital image.

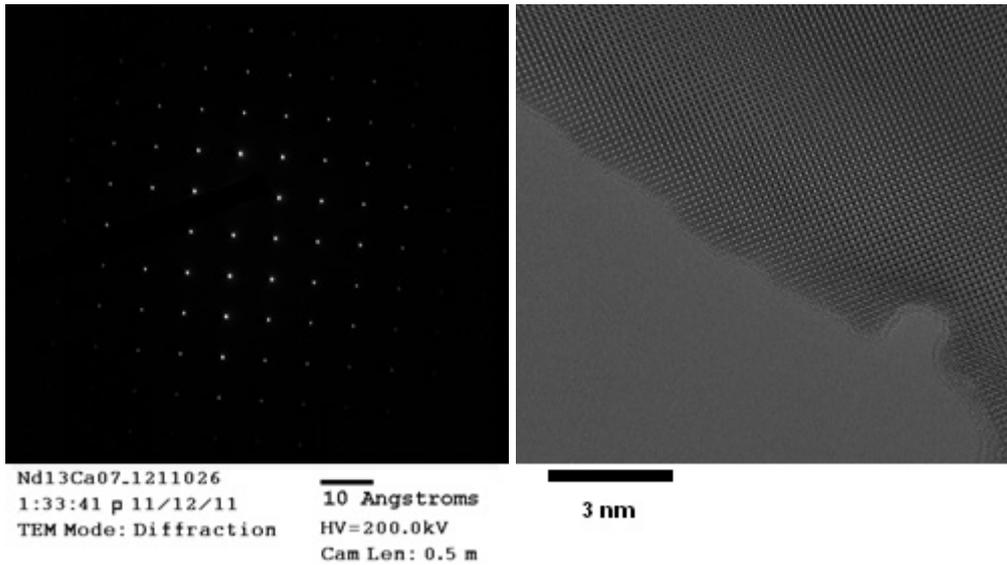


If the material is a collection of a large number of crystals, with different orientations, then individual reflections are seen within the rings.

This image is a selected area diffraction (SAD) pattern from a Zr/Ni/Cu-based alloy and zirconia

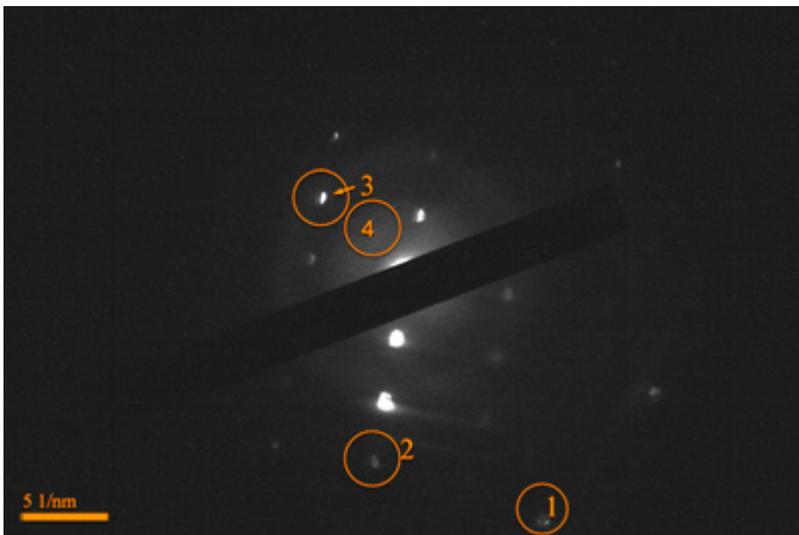
Combining images

Images can be combined to get the most information out of a sample. Here is a diffraction pattern on the left and a high resolution electron image on the right for the same material: $\text{Nd}_{13}\text{CaO}_7$



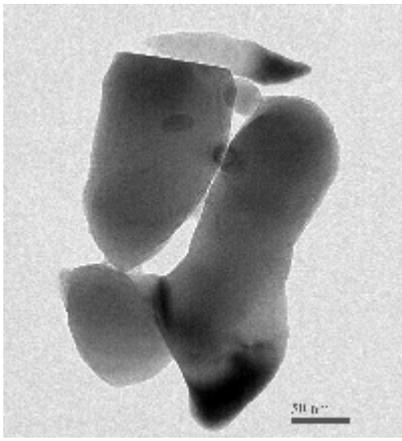
But more is achievable. Diffraction patterns can be combined with information from bright field and dark field images.

Here is the diffraction pattern from a cluster of minute crystals of zinc oxide (a hexagonal crystal structure).



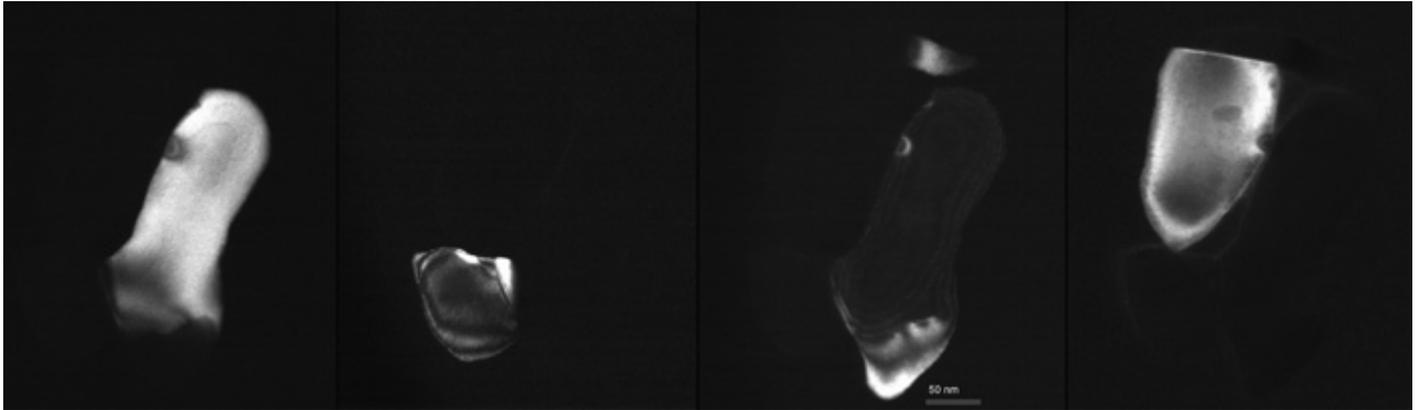
The diffraction pattern is a mixture of information from closely positioned crystals that are all fairly similarly aligned so the patterns are being gained from three of the crystals at the same time.

In one sense it is not an ideal diffraction pattern because it contains multiple sets of information. But if we were looking at material containing different phases, or different alignments of the lattice within a material, this is the sort of image we might see.



Bright field image of the ZnO crystals.

Dark field images of the ZnO crystals (from left to right, 1 to 4, as in the diffraction pattern).

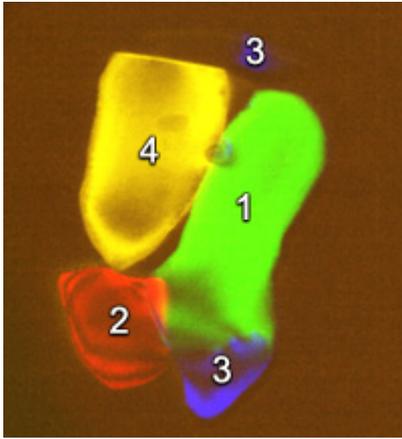


In dark field imaging, notice that only one crystal at a time produces an image and it is a 'white' image with a black background.

The reason only one crystal or region is seen in each dark field image is because the electron beam is lined up on a discrete intensity spot in the diffraction pattern, hence only electrons diffracted by that lattice arrangement are being imaged.

For basic information on how to align a beam for bright field or dark field imaging click [here](#).

The process of selecting a spot in the diffraction pattern can be repeated for different intensity spots of interest and the dark field images gathered and coloured, then overlaid so we can see all the information in one image.



Notice that the fourth colour belongs to a crystal alignment that does not show up in the diffraction pattern shown (see circle number 4). The circle placement for 4 in the diffraction pattern shows where the beam has been positioned to collect the image for this fourth crystal.

To understand more about what diffraction patterns are and how they are generated, [click here](#).

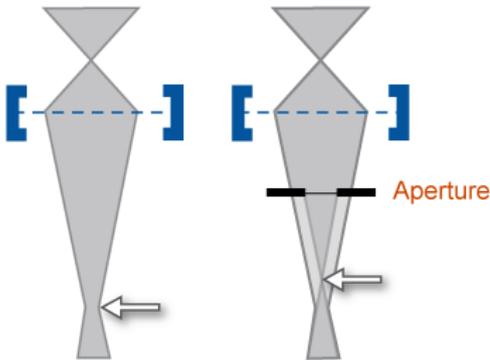
Using this approach of combining bright field, dark field and diffraction pattern imaging is useful to pin-point the presence of a phase in material because a diffraction dot that does not 'fit' with the overall pattern can be 'interrogated' by discovering exactly where that phase occurs in the material using the dark field image.

Imaging mode setup

There are a number of concepts that need to be mastered when setting up the electron column for suitable imaging modalities. The first is that the column should be properly aligned.

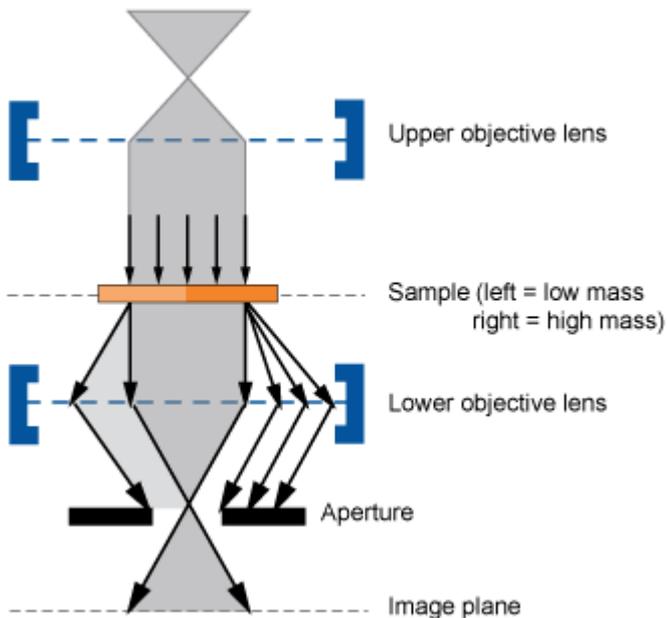
The first component to consider are apertures. There are a number of movable (adjustable) apertures in the TEM column.

The condenser lens aperture, situated below the condenser lens apparatus, can be used to reduce the spot size of the beam and reduce aberration.



There are two other apertures to be concerned with when imaging. The objective aperture is the first aperture situated below the sample plane. It is used to increase contrast and is introduced into the column for bright field and dark field imaging.

Sitting below the sample, the objective aperture is designed to improve the contrast of the image since it excludes scattered electrons that serve to reduce contrast.



There are a range of sizes to choose from: the smaller the aperture, the greater the contrast and darker the image (because more electrons are excluded from the image).

Note that the greater the mass in a sample or the thicker the sample, the more the beam scatters as it passes through the

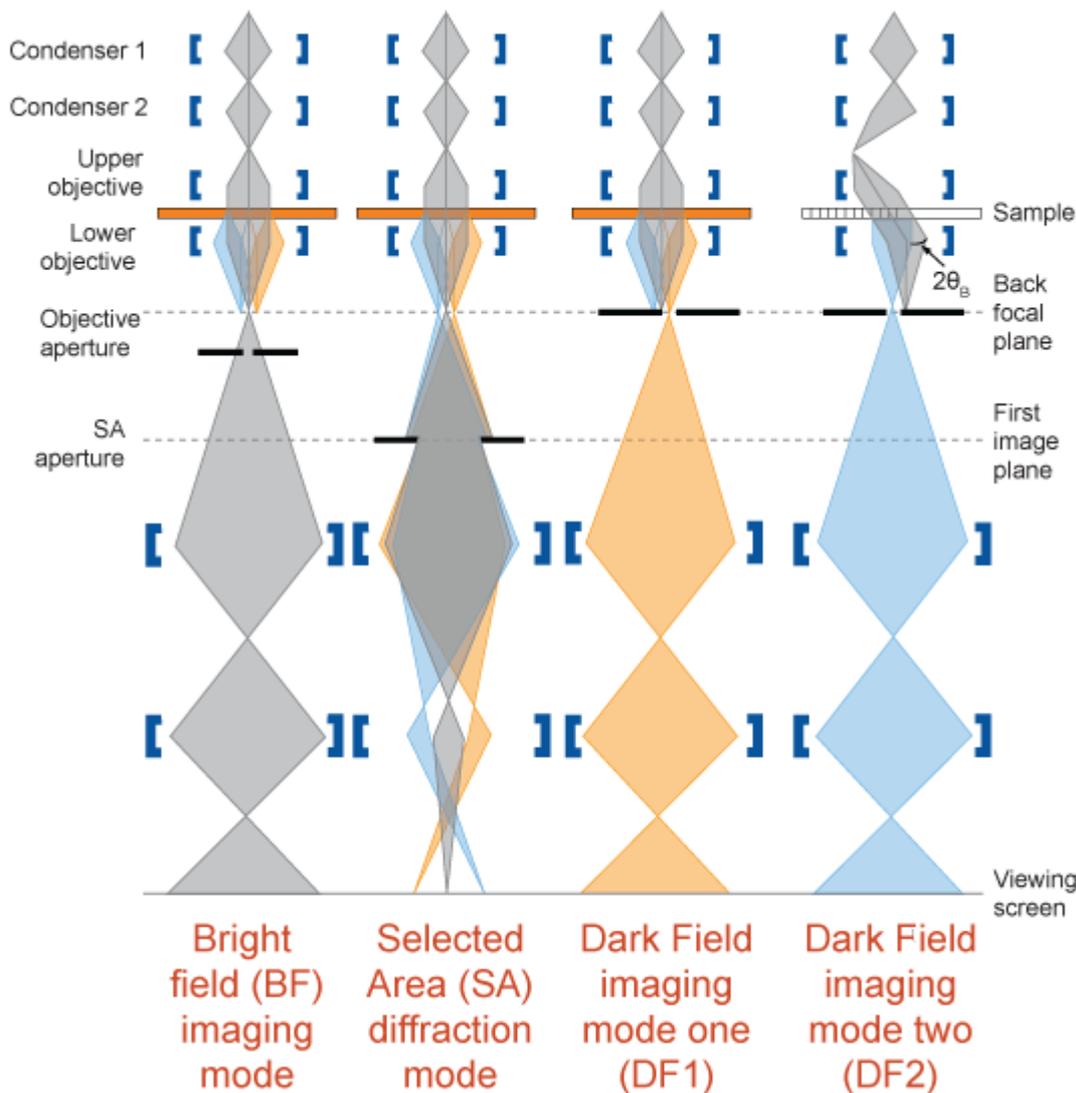
sample.

For bright and dark field imaging, it is necessary to adjust the objective lens (focus knob) to produce a crisp image of what is in the plane of the sample (specimen). This means that the electron beam is adjusted so that the beam cross-over occurs at the level of the sample.

When undertaking diffraction imaging, the focus point drops below the sample to the back focal plane. So for diffraction imaging the cross-over of the beam needs to occur in the back focal plane. When this happens, the objective aperture, if left in place, is seen to be in focus. It becomes obvious as a hole in a black platform with a crisply focused edge.

Often, in diffraction mode, it is necessary to isolate a local region so that only this region produces a diffraction pattern. This is achieved by introducing the selected area diffraction (SAD) aperture into the column. When the selected area diffraction aperture is used to limit the area which is used for obtaining a diffraction pattern, this is called selected area diffraction (SAD).

The beam paths for the different imaging modes are shown in the diagram.



Dark Field imaging is achieved by blocking the unscattered beam and only allowing scattered electrons to form the image.

There are a few ways this can be achieved. Methods 1 and 2 involve putting the objective area into to column.

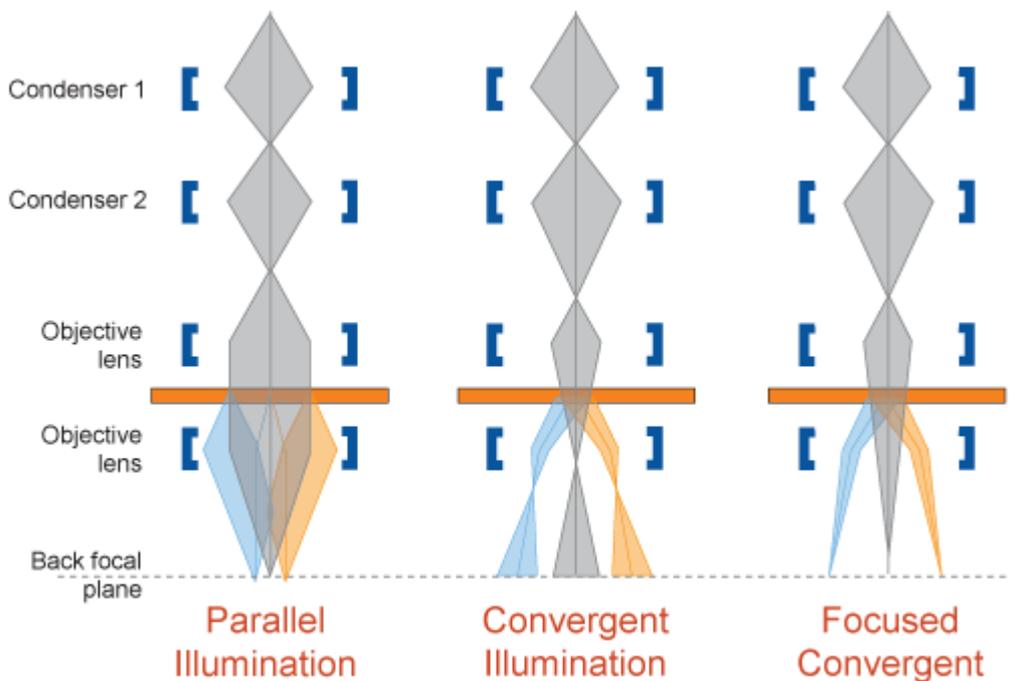
Method 1: (DF1 in the image) is where an objective aperture is introduced and moved so that it excludes the unscattered beam but allows some signal to pass through from a specific area of interest in the diffraction pattern, for example a specific intensity spot.

Method 2: (DF2 in the image) is where the beam is tilted so that the unscattered beam path is blocked by the objective aperture. In this way a particular intensity spot in a diffraction pattern can be centred as the new “main beam”. The dark field image will be produced only from those electrons being diffracted along this axis. The image is then focussed in the sample plane (on the sample).

Convergent Beam Electron Diffraction (CBED)

When the electron beam is converged on the sample to a point (method = convergent beam), instead of using a parallel stream of electrons through the sample, the diffraction pattern forms discs instead of points ("Focused Convergent" in the image below). These discs can contain detail that provides information about the crystal symmetry of the specimen.

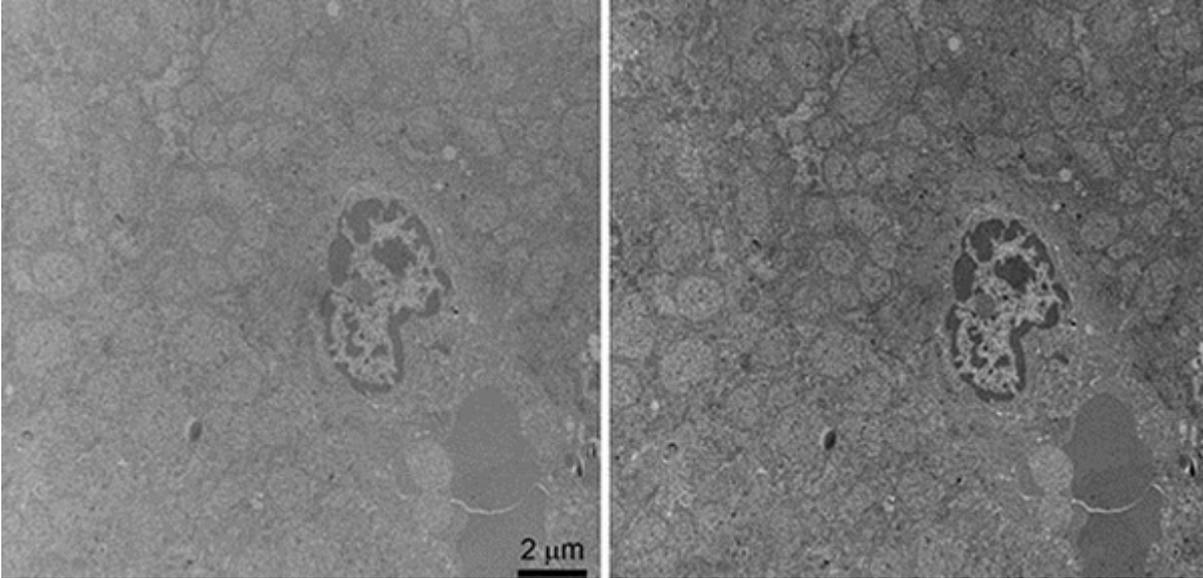
For this type of imaging the focus (objective lens) is adjusted so that the sample is in focus (not the objective aperture).



Focus/stigmation

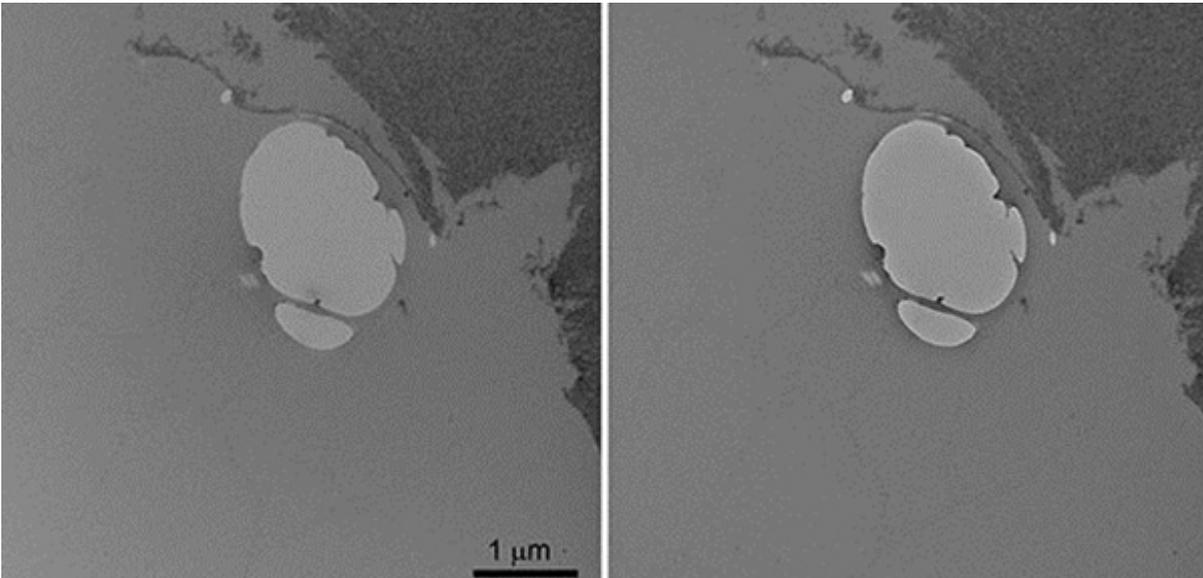
Micrograph quality is affected by aperture size, focus, stigmation, and use of image collection software.

The image of rat's liver on the left was taken without an objective aperture inserted. The image on the right had an objective aperture inserted. Note the increased contrast in the right image.



Achieving correct focus of an image is important.

The image on the left shows a hole in a resin support film that is close to focus (suitable for collection). On the right the image is out of focus: note the added white ring (also known as a Fresnel fringe) on the inside edge of the hole that provides extra contrast which is appealing to the eye but incorrect.



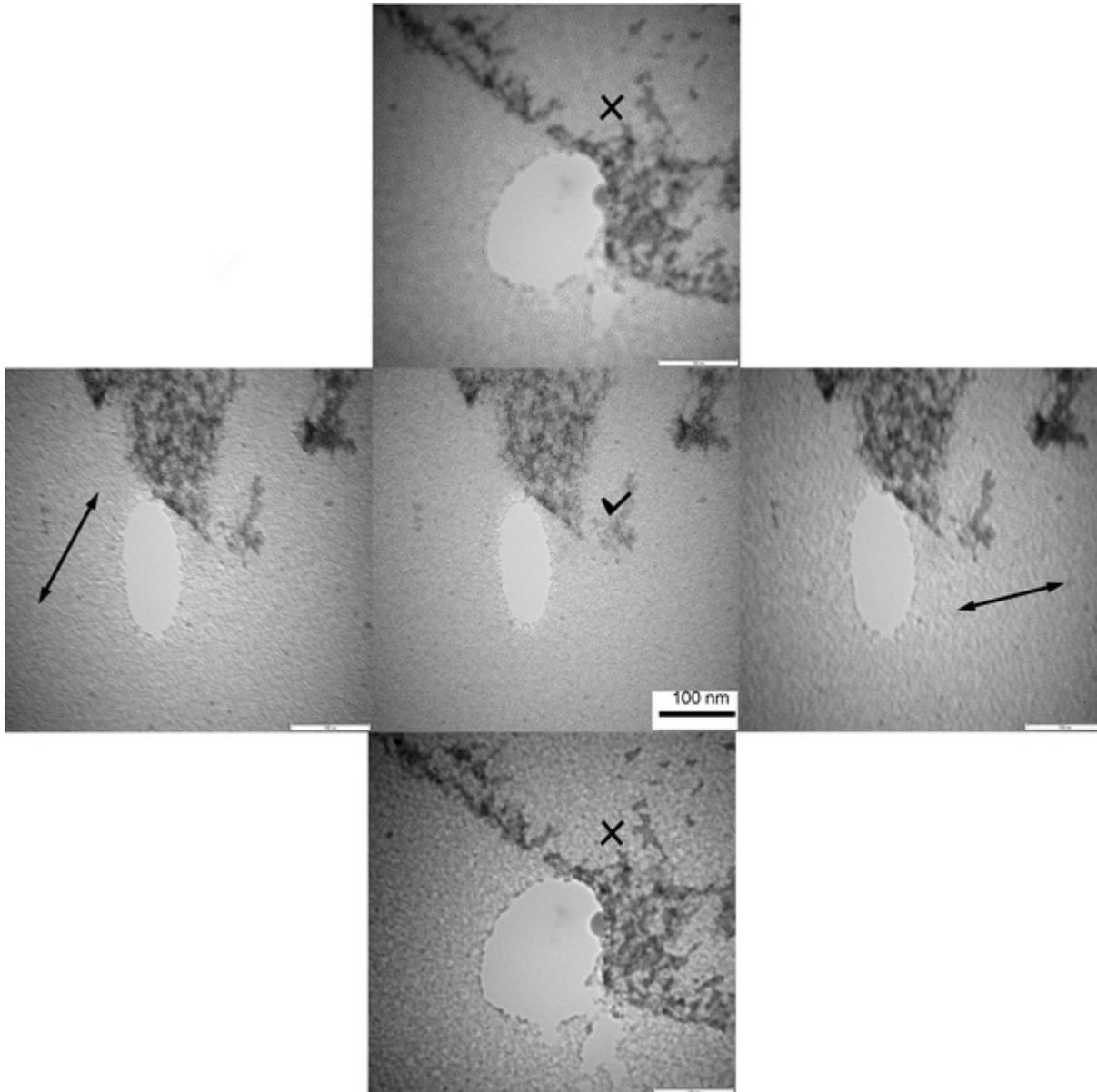
To achieve good focus by eye, at high magnifications, it is helpful to focus on the 'grain' of the sample support film. This supplies information about both focus and stigmation. The presence of stigmation results in a streaking or stretching of the normally roundish grains (see arrows on the left and right in the image) which makes achieving focus more difficult.

Correcting stigmation involves rounding the cross-section of the beam with the X and Y stigmators. Often both X and Y

stigmation need adjustment, however, each results in a stretching of the grains along a different axis.

In the image the top and bottom images are out of focus.

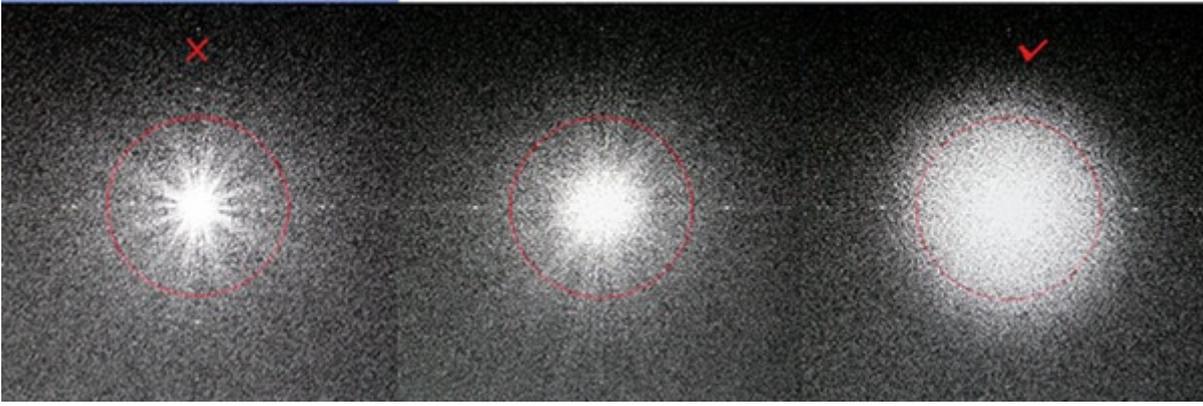
When the beam cross-over point is slightly above the sample or slightly below the sample, the beam is not at focus (see top and bottom frames in the image for out-of-focus images). The contrast rings around the grains will be negligible at focus but as we move through focus from over to under, the contrast ring colour will invert (white to black).



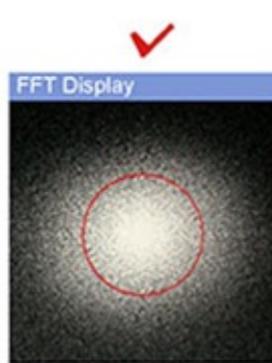
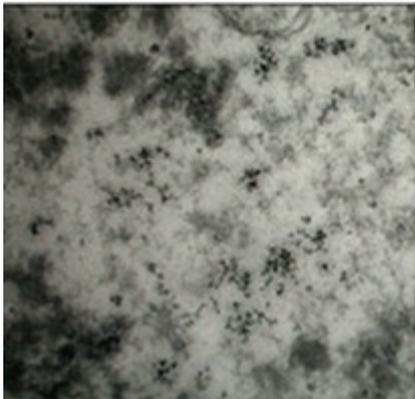
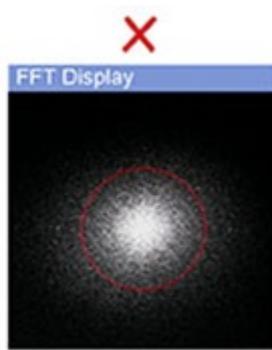
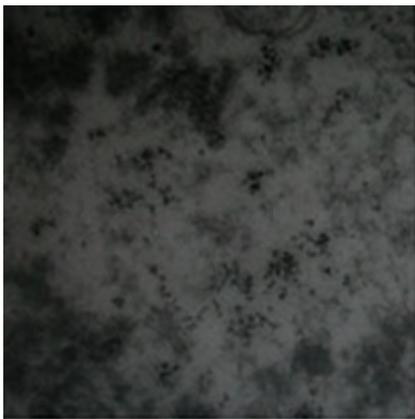
An image can be brought into focus either optically, as discussed above (by using binoculars attached to the TEM to observe the viewing screen) or by using the Fast Fourier Transform (FFT) application available in imaging software packages for TEMs equipped with digital image collection equipment.

Detail on using FFT

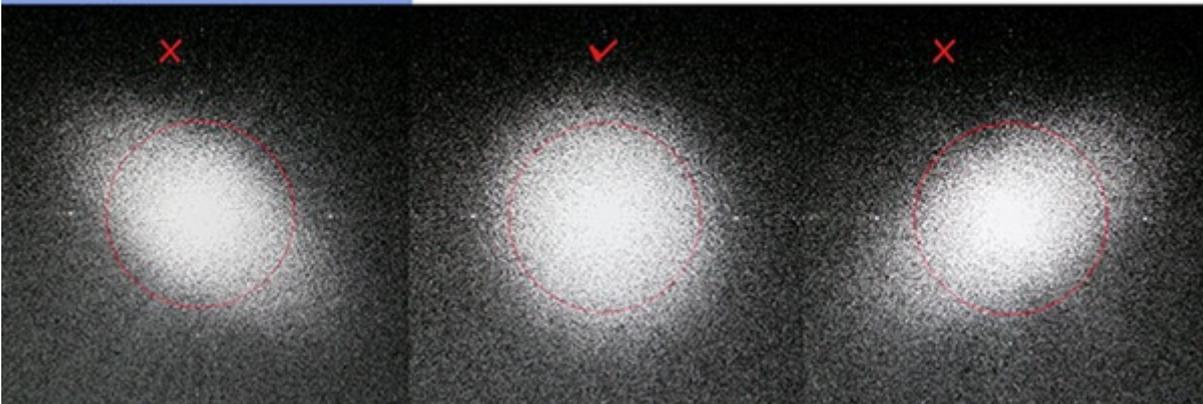
FFT Display



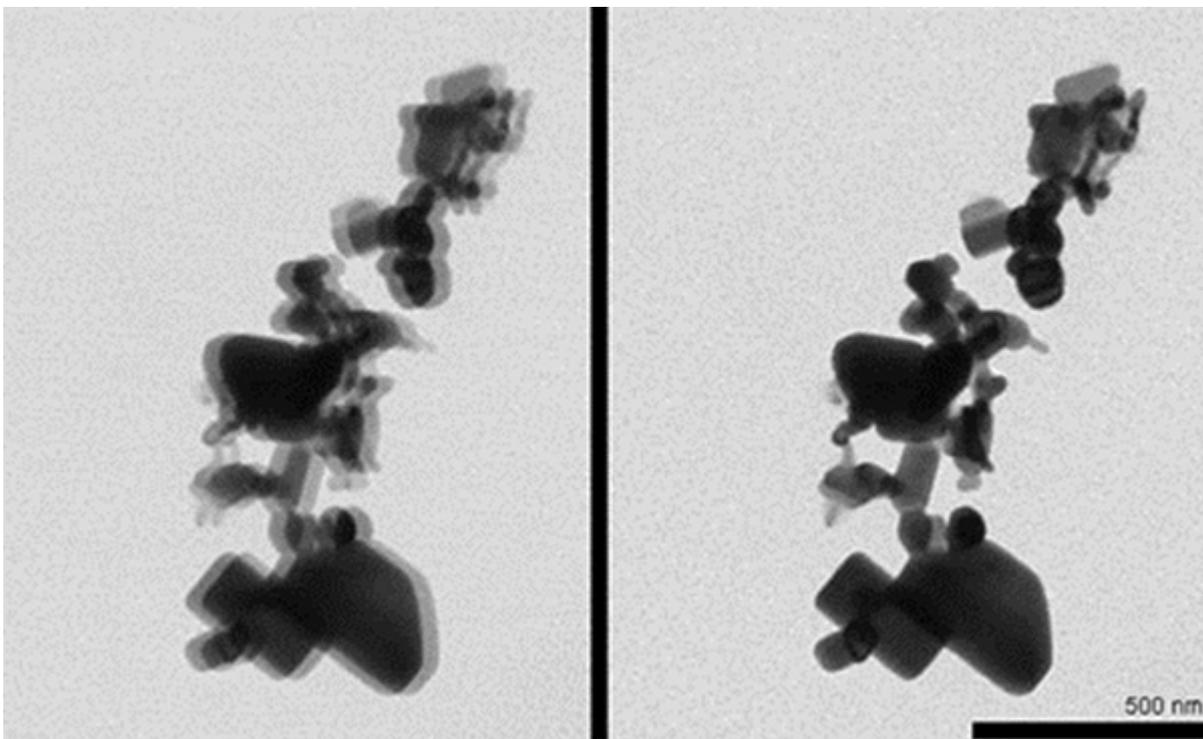
The image is a series of examples from a version of software that uses FFT. The right panel shows the signal from a biological sample with the image in focus; the left panel is from the same biological sample image but it is not in focus.



FFT Display

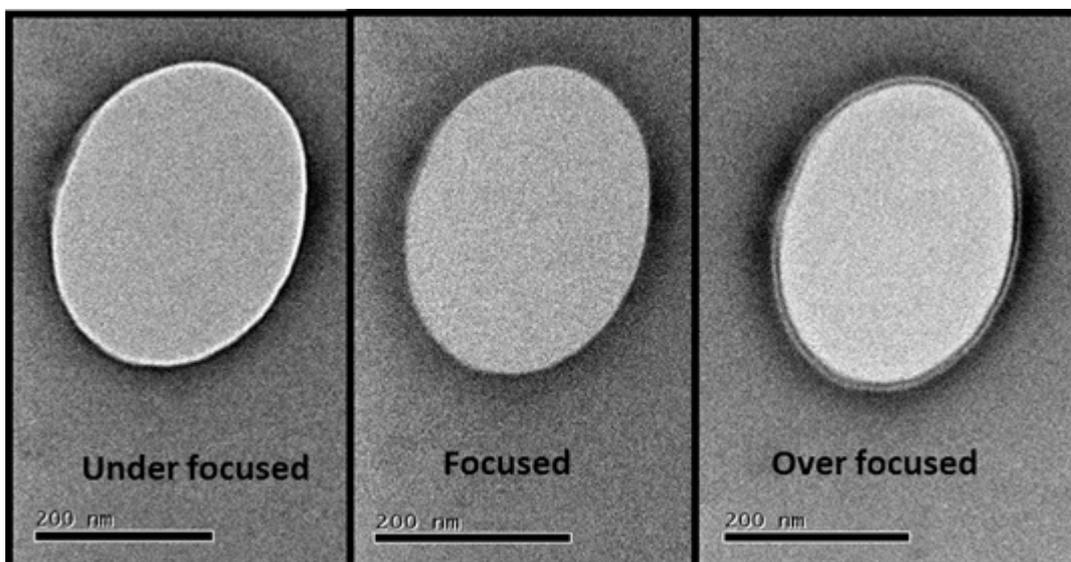


The image shows an FFT display from a biological sample where the image in the central panel has correct stigmation. To the left and right the image needs correcting.



Alternatively, an image at focus will have the least contrast, as was seen in the image of the hole above. The selected area diffraction aperture is used to enhance contrast when working in the low magnification mode, in the same way that the objective aperture is used at higher magnification. By taking the selected area diffraction aperture out, the point of lowest contrast can be seen more clearly as the focus is changed. This can be used as a guide to when focus is reached.

The presence of an artifact known as a Fresnel fringe can be used as a guide to help focus an image. The image below shows this in practice, where the inner fringe denotes an under focused image, and a thicker outer fringe shows an over focused image.



Images of a holey film on a 200Cu grid. Image by Rhiannon P. Kuchel.

Lenses: electromagnetic lenses



This is an image of the copper coil of an electromagnetic lens from an electron microscope. The electron beam travels through the central hole. Current runs through the coiled copper wire creating a magnetic field.

The electron beam present within a TEM requires manipulation by lenses as it travels down the column and through the sample in order to examine the right areas of the sample.

In an optical microscope this is achieved using glass lenses, but the electron microscope requires electromagnetic lenses instead.

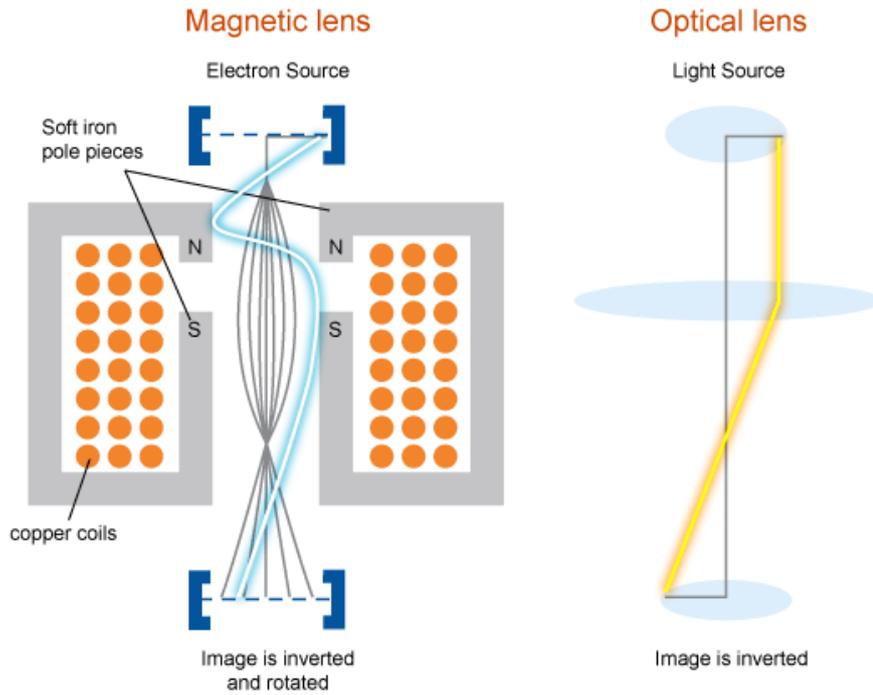
Since the electron beam is charged (negative), an electromagnetic force can be used as a lens. Wire coils surround the beam and produce such a field which generates a deflecting force on the electrons.

Pole-pieces and Coils

A magnetic electron lens has 2 parts:

- a pole-piece: a cylindrically symmetrical core of soft iron with a hole drilled through it (bore)
- a coil of copper wire which surrounds each pole-piece.

When we pass a current through the coil, a magnetic field is created in the bore. The strength of the field in a magnetic lens controls the ray paths, bringing off axis rays back to focus.



Comparing the action of an electromagnetic lens with an optical lens we see that the image is rotated, to a degree that depends on the strength of the lens. Focal length can be altered by changing the strength of the current.

The resistive heating of the coil means that the lenses have to be cooled and a water recycling system is an essential part of TEM lenses.

Problems with lenses: aberrations

Over the last 300 years, glass lenses have developed to near perfection, while electromagnetic lenses remain quite imperfect. There are at least 10 kinds of defects for electromagnetic lenses but we will emphasize the ones that limit microscope performance in substantial ways.

There are:

- Spherical aberration
- Chromatic aberration
- Astigmatism

Spherical and chromatic aberrations limit the resolution of conventional electron microscopes. Both these defects are unavoidable when using static rotationally symmetric electromagnetic fields. It is necessary to learn how to use the microscope to minimise them.

Spherical aberration is the type that is most significant in defining the performance of the objective lens.

Chromatic aberration worsens for thicker samples. To reduce this problem it is good to make thin samples (e.g. thin foils for physical science preparation).

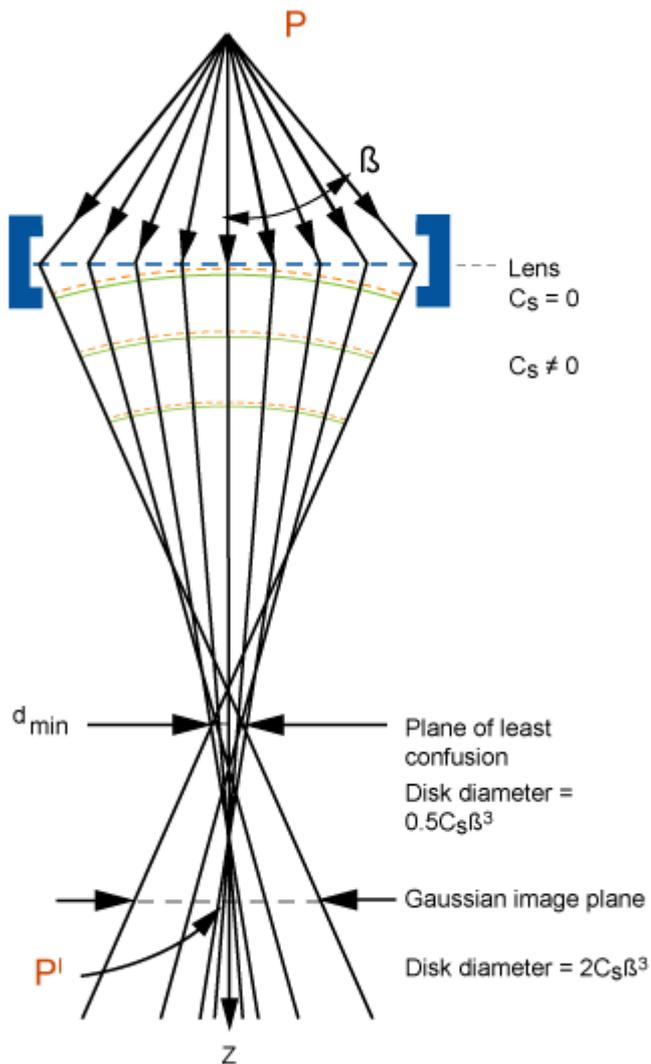
Astigmatism affects the ability to focus an image but is totally correctable.

Spherical aberration

The spherical aberration is caused by the lens field acting inhomogeneously on the off-axis rays. In other words, the rays which are "parallel" to the optic axis but at different distances from the optic axis fail to converge at the same point. The further off-axis the electron is, the more strongly it is bent back toward the axis.

As a result, a point object is imaged as a disk of finite size, which limits the ability to magnify detail, because features are degraded by the imaging process.

The figure shows the effect of spherical aberration. A point P is imaged as a disk with a minimum radius in the plane of "least confusion" and as PI with an intense central bright region with a surrounding halo in the image plane.

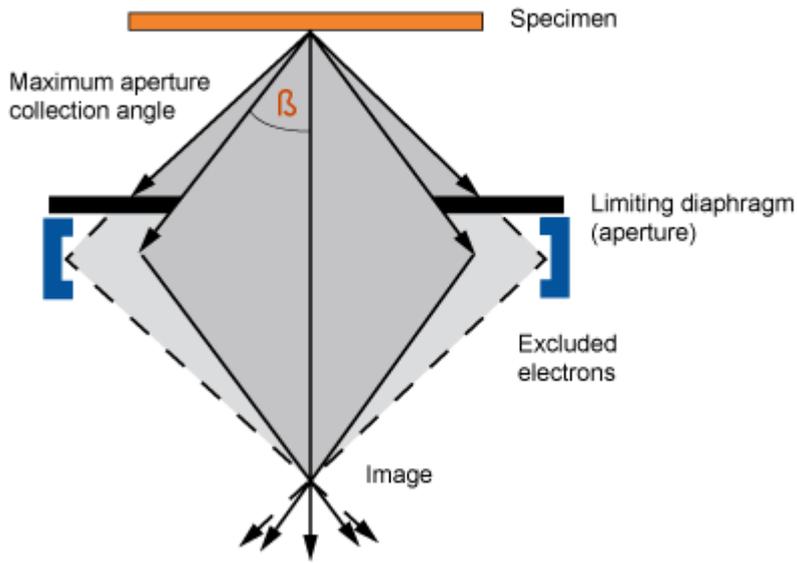


An expression for calculating the radius of the spherical aberration disk (r_{sph}) in the image plane use:

$$r_{sph} = C_s \beta^3$$

Where C_s is a constant for a particular lens called the spherical aberration coefficient and β is the maximum semi-angle of collection of the objective lens aperture.

From this derivation, C_s has the dimensions of length; typically, it is approximately equal to the focal length which in TEM is normally about 3 mm but in HRTEM is well below 1 mm. One way to minimize this aberration is to use a short focal length lens (i.e. small spherical aberration coefficient).



Examples

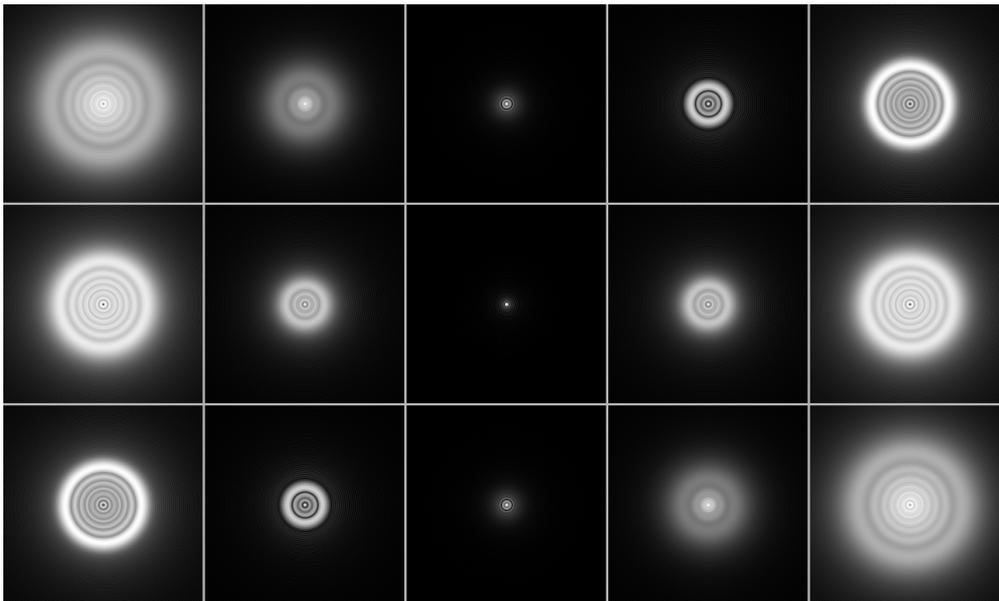


Image by Mdf, available under a Creative Commons Attribution-Share Alike 3.0 Unported license.

This is an example of a point source imaged by a system with negative (top), zero (center), and positive (bottom) spherical aberration. Notice only the central point is a dot; the image above and below it appears as a disc.

Images left of the center column are defocused toward the inside; images right of the center column are defocused toward the outside.

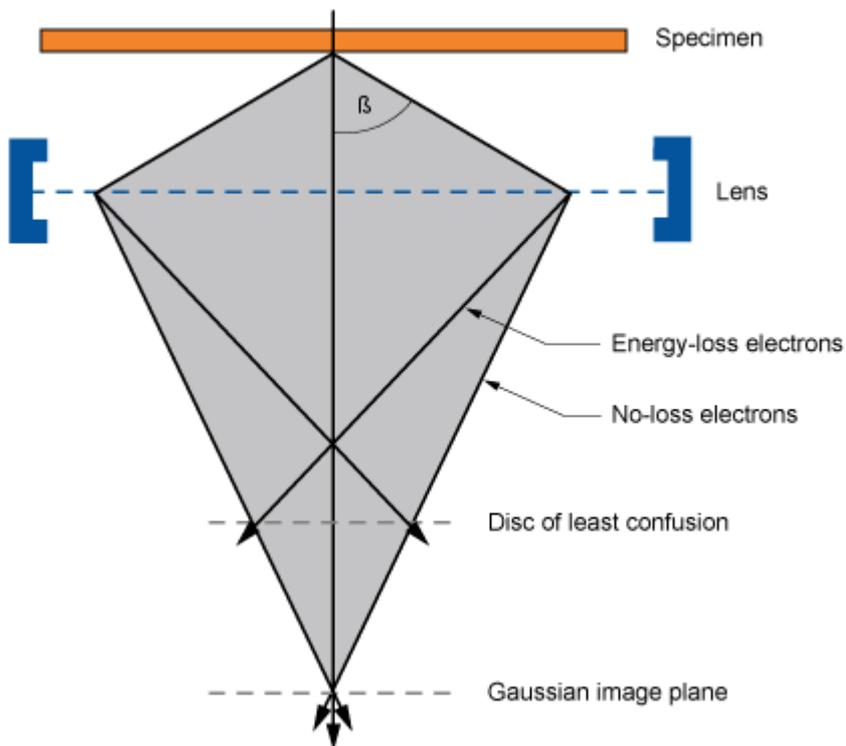
Chromatic aberration

The term chromatic aberration is related to the energy of the electrons.

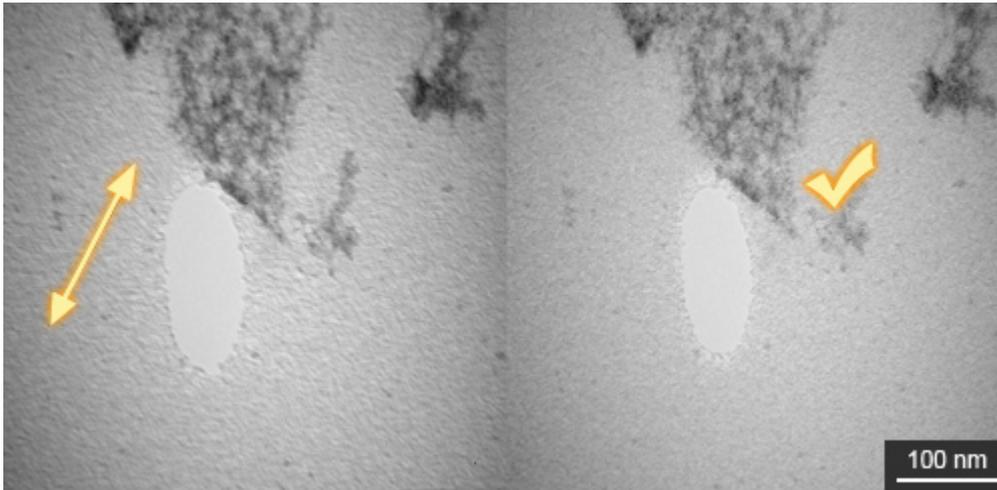
Electrons are not monochromatic. Electrons emerge from the gun at a whole range of energies and are bent by the objective lens to different degrees; electrons that have lost energy are bent more strongly. Thus, once again, electrons from a point on the specimen form a disk image, as for spherical aberration. The radius (r_{chr}) of the disk is given by:

$$r_{chr} = C_c \frac{\Delta E}{E_0} \beta$$

Where C_c is the chromatic aberration coefficient of the lens (length), ΔE is the energy loss of the e, E_0 is the initial beam energy, and P is the semiangle of collection of the lens. While ΔE in the incident electron beam is < 1 eV. It is typically 15-25 eV for a good fraction of the electrons coming through thin foil 50–100 nm thick. Chromatic aberration gets worse for thicker foils as this leads to a higher fraction of inelastically scattered electrons which may be subject to such effects.



Astigmatism



The left image of this sample film shows astigmatism

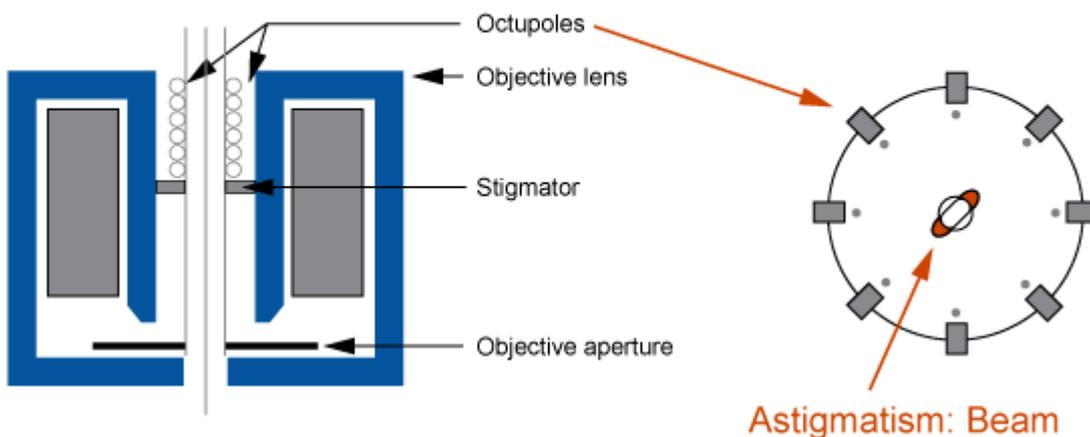
The aberration called astigmatism occurs when the electrons sense a non-uniform magnetic field as they spiral round the optic axis. It arises because the soft iron pole pieces comprising the electromagnetic lens cannot be fabricated with perfect cylindrical symmetry. The soft iron may also have micro-structural inhomogeneities which cause local variations in the magnetic field strength.

The apertures introduced into the lens may disturb the field if they are not precisely centered around the axis. Furthermore, if the apertures are not clean, contamination causes charge accumulation and deflects the beam in unexpected ways.

There are a variety of factors which contribute to form an astigmatism, which distort the image by an amount $r_{ast} = \Delta f$, where Δf is the maximum difference in focus induced by astigmatism.

Fortunately, astigmatism is "easily" corrected using stigmators. These are small octupoles that introduce a compensating field to balance the inhomogeneities causing the astigmatism. Stigmators are present both in the illumination system (condenser lenses) and in the imaging system (objective lens).

Astigmatism: Non-spherical electron beam



Transmission electron microscopy in practice

- Virtual transmission electron microscopy
- Sample preparation
- Step-by-step operating procedures

Virtual TEM

- Basic Imaging
- Diffraction and advanced imaging

Virtual TEM - Basic imaging

Adobe Flash player is required to use the simulator.

Terms used in this activity

- Magnification
- Focus
- Sample height

Sample insertion videos:

1. Place sample in holder
2. Insert holder halfway
3. Switch on vacuum pump
4. Insert holder fully

Sample removal videos:

1. Remove holder halfway
2. Switch off vacuum pump
3. Wait for pump
4. Remove holder fully

Virtual TEM - Diffraction and advanced imaging

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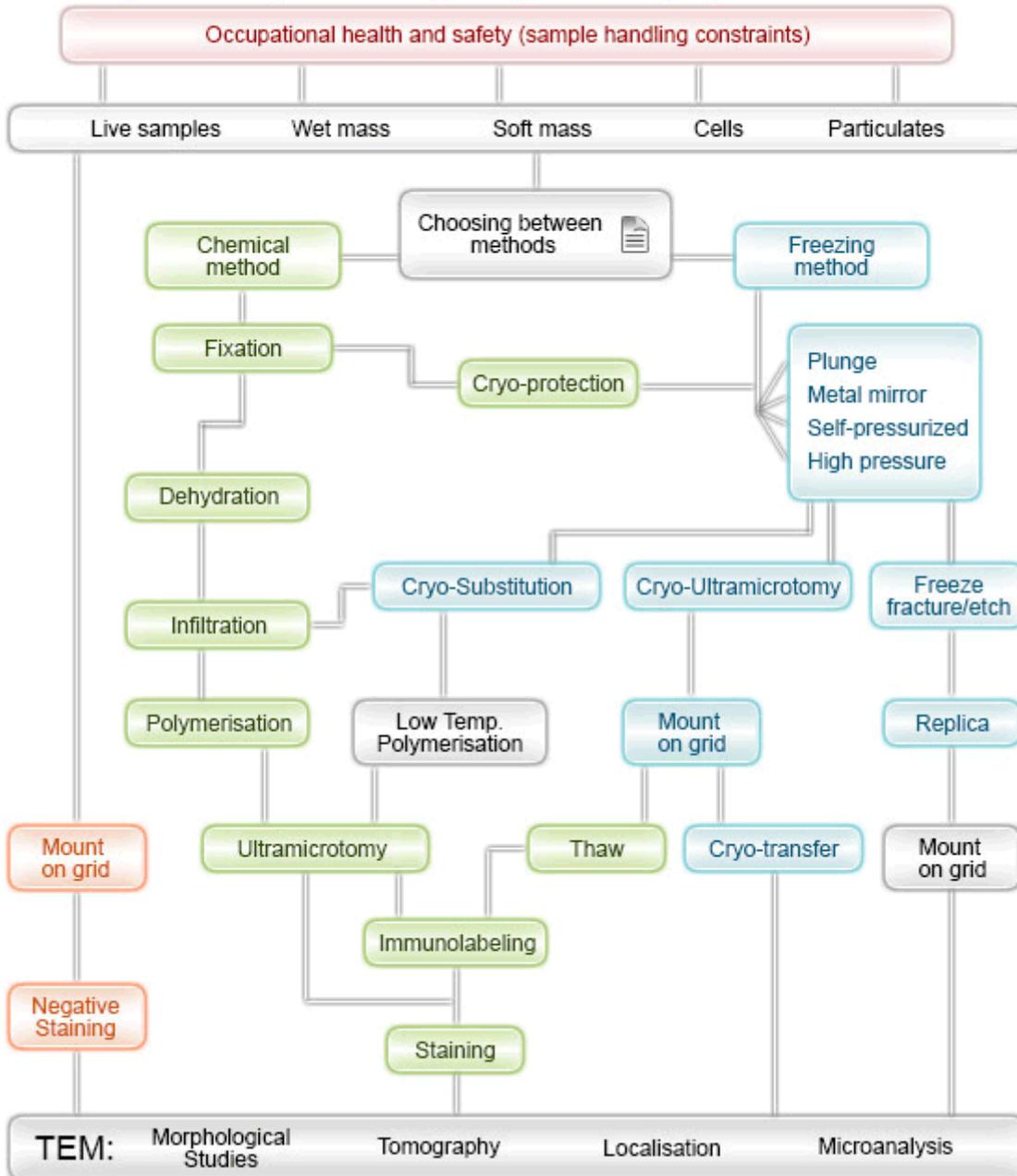
Sample preparation

- Chart for organic or biological samples
- Chart for inorganic or physical science samples
- Artifacts
- Fixation

Organic sample/physical science specimen preparation for TEM

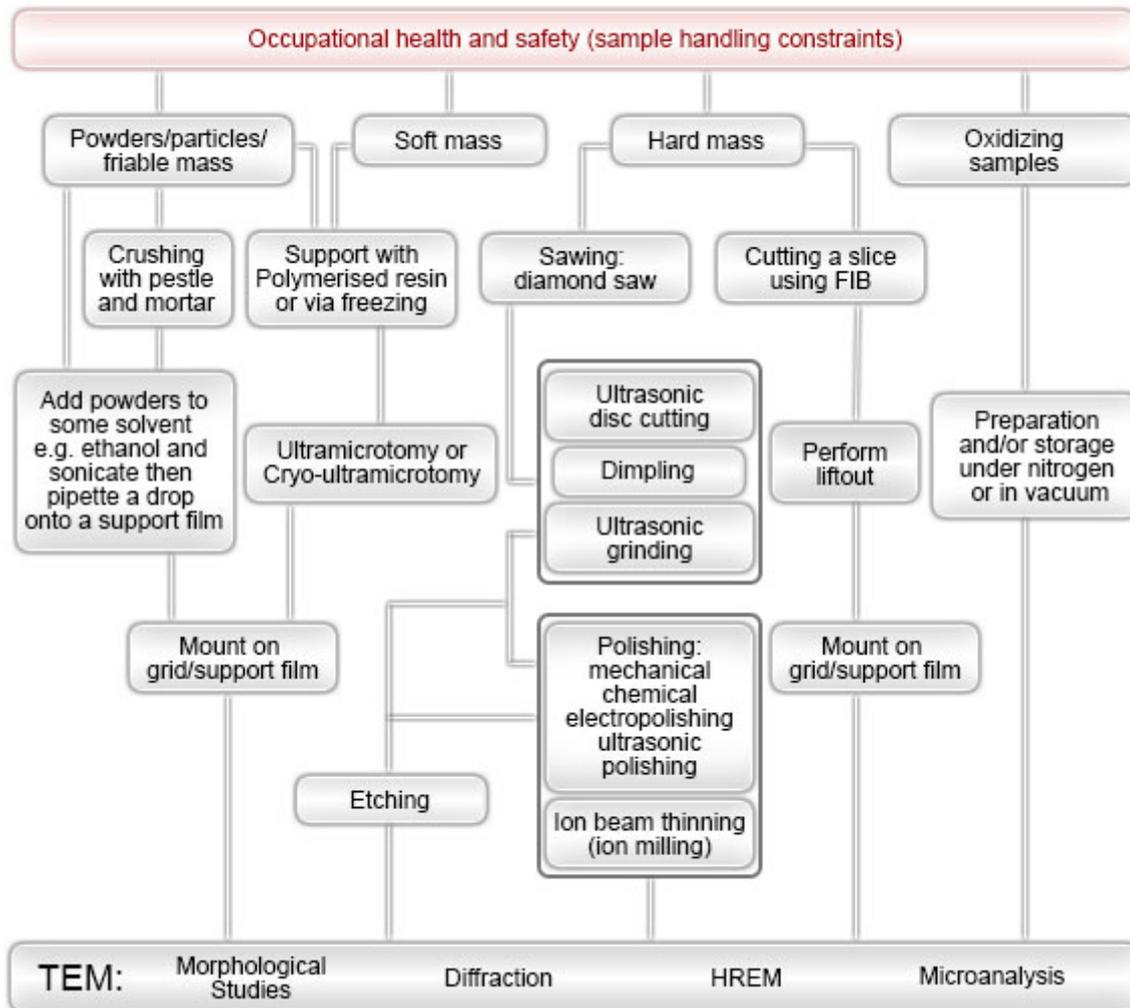
This is vital to getting good quality information from your sample. A poorly processed sample can lead to viewing artefacts. This section is designed to help you choose appropriate preparation processes. Text and video help guide you through processes.

Click the linked items below to read information or watch videos about each process.



Inorganic sample/physical science specimen preparation for TEM

Click the linked items below to read information or watch videos about each process.



Artifacts

Artifacts are often found in TEM specimens. These can be the result of problems with the initial sample that was chosen or can develop during preparation. Since the aim is to produce a sample for viewing that is artifact free it is important to be able to recognize artifacts and determine what has caused them.

In physical science preparations artifacts may arise from several sources. For example, during ion milling/ thinning, argon ions can be implanted into the sample and this can result in amorphization and phase transformations. This is caused by using too high an acceleration voltage leading to energetic ions damaging the specimen. Chemical preparation procedures can leave contamination on the sample. Poor mechanical polishing can produce uneven surfaces and scratches, leave residues or introduce dislocations into the sample. Even preparation of particulate on a sample grid can result in artifacts if the samples are sonicated for too long or the wrong solvent chosen. When using cryo-techniques the sample must be able to tolerate the low temperatures involved without compromising the structure.

For biological samples fixation can introduce artifacts. Sample degradation can occur as a result of too much time elapsing between harvesting a sample and fixation. Mechanical damage can be introduced by poor harvesting procedures. Osmotic damage can occur by not matching the fixation solution to the sample conditions. Poor washing between fixation steps can later result in the appearance of "pepper" in the sample from precipitation.

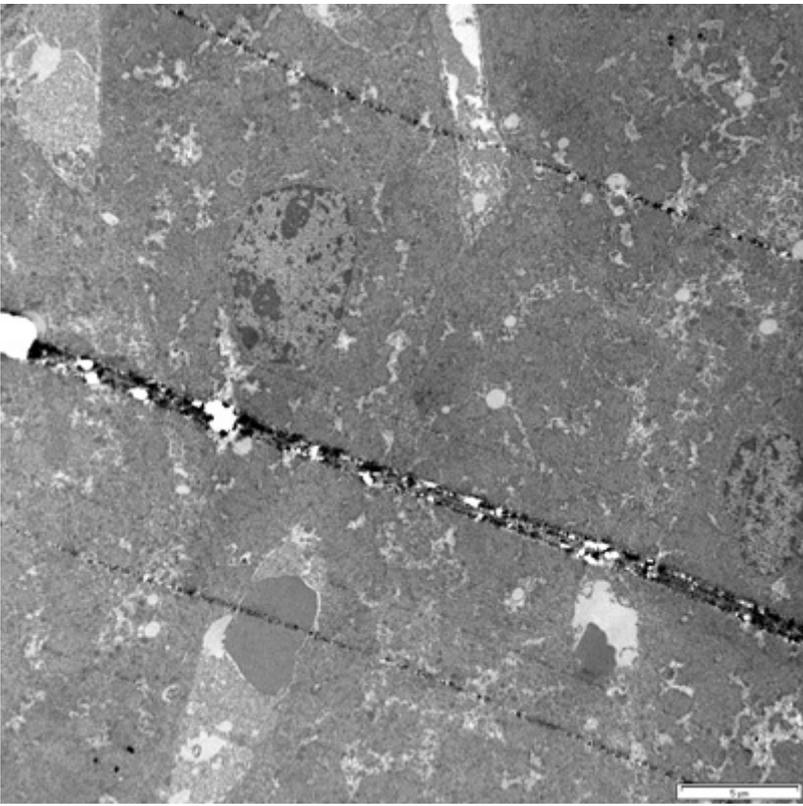
Cryo-fixation has its own range of artifacts related to sample thickness and too slow a cooling rate.

Dehydration and resin infiltration can result in artifacts. Removing water too quickly can result in shrinkage artifacts. Poor resin infiltration and polymerization can result in holes in a sample.

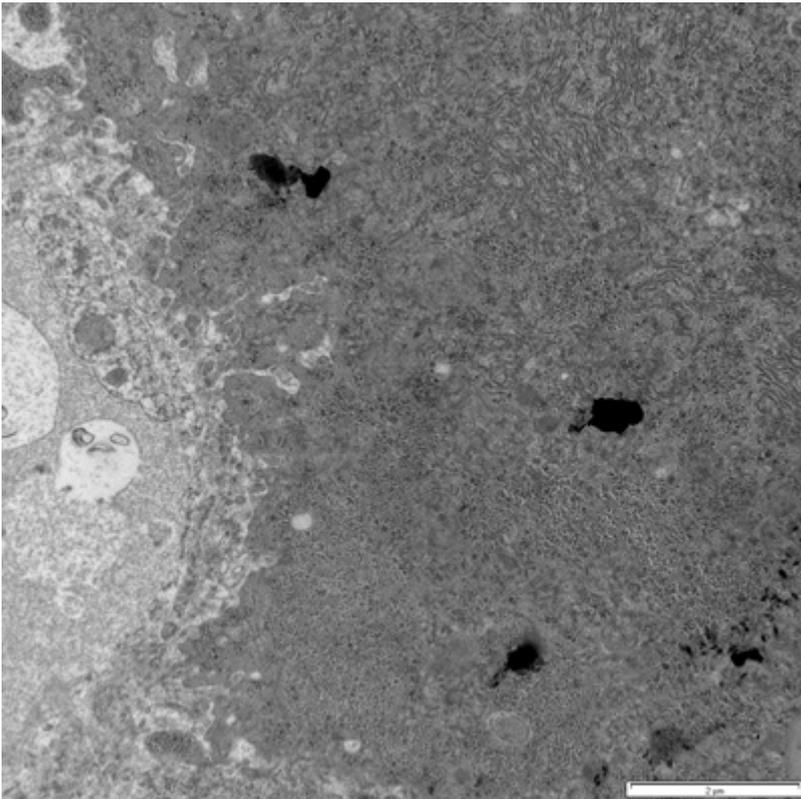
The sectioning of material introduces another set of possible artifacts: tearing, compression, or scratches.

Staining samples can result in precipitation on the surface. Even breathing too heavily on samples during staining with lead citrate will result in lead carbonate precipitation. Poor cleanliness of equipment (forceps) and bench can result in oils and contamination ending up on the sample surface.

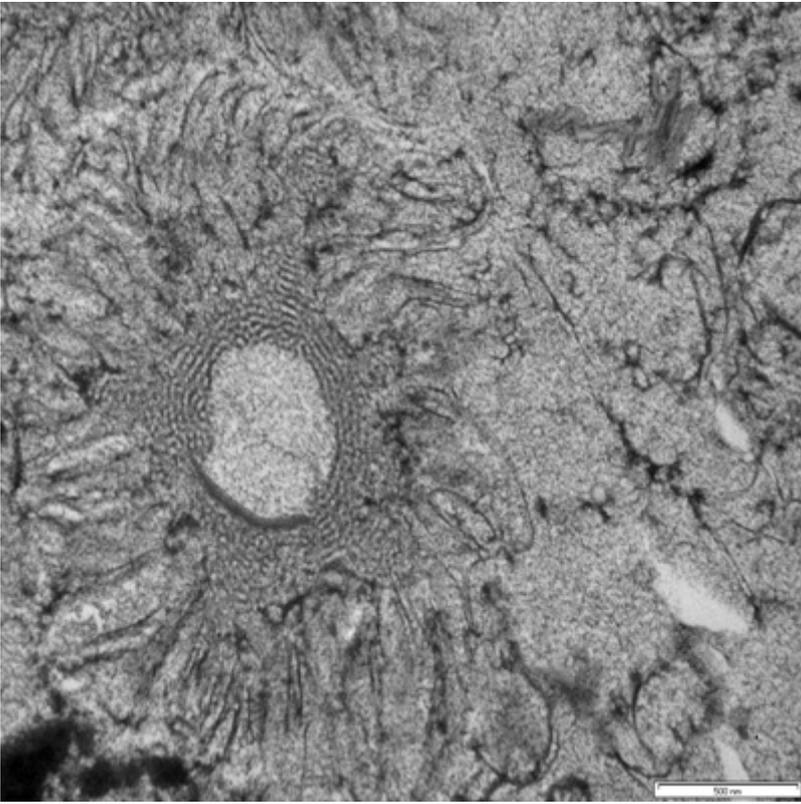
Condensing the beam too rapidly onto the sample can also cause damage to the specimen or the film on the grid.



Section of rat's liver showing scratches caused by a damaged knife edge.



Section of rat's liver showing precipitation on the surface that occurred during staining and handling.



Section through the venom gland of a honeybee fixed with high pressure freezing. Notice the poor detail within the cell caused by ice crystal formation during freezing. This sample was too thick for rapid enough freezing to the depth required.

Fixation

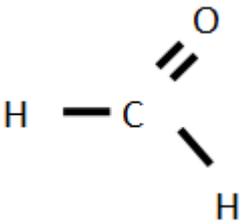
Why chemically fix samples? Organic and living materials usually need to be preserved before further preparation for viewing. Biological samples are alive. When they die changes occur to their fine structure quite rapidly. It is necessary to hold the sample in a form as close to living as possible using a process referred to as fixation. Chemical fixation involves cross-linking proteins and stabilizing some lipid components.

The initial step usually involves aldehydes since they stabilize the proteins in the sample.

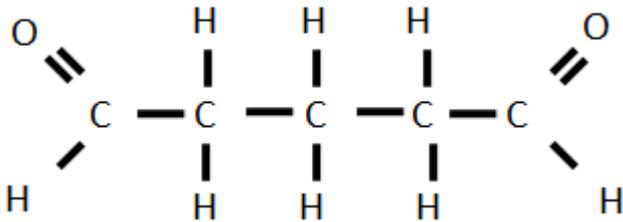
Formaldehyde is a smaller molecule than glutaraldehyde and fixation is relatively reversible. It can be washed out with water. However formaldehyde fixation is rapid. Glutaraldehyde is slower to penetrate but not reversible in its bonding. It is usually preferred alone or in combination with formaldehyde. A mixture of the two is known as Karnovsky's fixative.

Tip: always cut up the sample under fluid to stop drying; e.g. under buffer or fixative.

Formaldehyde molecule



Glutaraldehyde molecule



Tip: A technique used to fix a sample while it is still within an animal is to perfuse the fixative through the sample using the circulatory system to carry the chemicals. This is not a common technique and needs special ethical clearance.

Glutaraldehyde is the most commonly used primary fixative. It is an uncharged molecule in solution so it can penetrate biological membranes. It forms large three dimensional networks of cross-linked molecules throughout the cytoplasm of cells.

The initial reaction releases protons and the result is a drop in pH, hence the need to buffer the fixative. Often used is a solution of 3% glutaraldehyde in 0.1M buffer (e.g. sodium cacodylate or Sorrenson's phosphate buffer). Another protocol uses 4% paraformaldehyde, 2% glutaraldehyde, 0.1M PIPES buffer and 0.3M sucrose.

The pH chosen is dependent on the natural tissue acidity. Usually pH 6.8 is suitable for plants and pH 7.2-7.4 for animal tissues. Phosphate buffer is not toxic whereas cacodylate contains arsenic. Other buffers such as PIPES and HEPES are often used for cell culture material.

Safety: aldehyde fluid and fumes will fix the tissue of the handler as well as the sample so ensure exposure does not occur.

Osmolarity: When fixing marine samples osmotic pressure may be a problem as the tissue will be most stable when in seawater rather than in distilled water. To avoid swelling of the sample it may be necessary to make up the chemicals in

seawater or equivalent. Increasing the concentration of the buffer is an alternative.

Time and temperature: Tissue is generally immersed in the fixative for 1- 2 hours at 4 degrees or sometimes at room temperature.

Microwaves: The fixation process can be sped up by using a specifically constructed laboratory microwave (e.g. the BioWave microwave) that is fitted with a heat sink. In this way the tissue is exposed only to the effect of the microwave and not to excessive heat generated in the tissue. It is thought that the radiation flips dipoles in the tissue rapidly, allowing faster infiltration by the fixative and enhancing bonding. At 80 Watts and with mild vacuum, fixation of a 1 mm² of tissue occurs in 12 minute (with 2 minutes of microwaves on, 2 minutes off and 2 more minutes on, and repeated sequence). Larger or particularly dense blocks of tissue will take longer.

Washing: After fixation the tissue is washed in buffer (or water) to remove extraneous fixative. This can be done in a fume hood on the bench with 5 to 10 minute washes or faster, in the specialist microwave.

Post-fixation: Tissue is usually post-fixed with 1% osmium tetroxide in buffer (e.g. 0.1M cacodylate buffer) for 1 to 2 hours at room temperature (in a fume hood!). Penetration is slow at about 0.5 mm per hour at room temperature.

The fixative bonds with fatty acids, locking them into position within cell membranes. The reaction turns the tissue black so progress can be observed as it occurs. Usually tissue is left for 1 hour if small (1 mm²).

Safety again: A fume hood or air extraction device is absolutely necessary for use of osmium because the vapour (and fluid) can fix eyes and turn the user blind.

Mixing fixatives: Aldehydes and osmium when mixed together react with one another. So any aldehydes remaining in the tissue after the wash can cause a precipitation. Under the TEM this may be seen as black “pepper”. It is also useful to remember that osmium reacts with acetone and alcohol so these must be kept away from the sample till after it is fixed. Once fixed, the tissue can be washed in water to remove extraneous osmium solution.

General handling procedures:

- Use gloves, lab coat, safety glasses and a fume hood when undertaking chemical fixation.
- Always keep the sample covered with solution so it does not dry.
- Use a volume of fixative that is 10 times the volume of the sample or more.
- Keep specimens in vials. Ideally vials should be labeled but can be placed in well-labeled containers. Labels must contain date, name, chemical in use and hazard information. An example can be seen in the image below.



Machine settings

Thin Sections/Biological Samples	High Resolution
80-120 kV	200-300 kV
Condenser aperture: 1 (biggest)	Condenser aperture: 2 or 3 (small)
Spot size: 1 (unless sample is "drifting", 5 or 6)	Spot size: 6 to 8
Objective aperture: 3 (smallest)	Objective aperture: 1 (biggest)

An increase in voltage = increased brightness = increased resolution = thicker sections can be used = reduced contrast

The condenser aperture is the top one on the column **A smaller condenser aperture** = increased resolution = decreased beam damage to sample = decreased brightness

The objective aperture is the middle one (just below the specimen holder) **A smaller objective aperture** = increased contrast = decreased brightness

Spot size on the JEM-1010 gets smaller with increasing number **A smaller spot size** = increased resolution (for some imaging modes) = decreased brightness = decreased beam damage to the sample = decreased sample "drift" (stability improved)

Alignment

The beam travels from the electron gun at the top through the sample and down to the viewing screen. If it is off axis or suffers from astigmatism then poor imaging can be expected. The path needs to be adjusted depending on viewing parameters. This process is called alignment. TEM alignment procedures involve a number of systems and parts of the instrument:

1. Illumination system: beam tilt; beam shift
2. Condenser aperture alignment
3. Condenser astigmatism correction
4. Alignment of illumination with respect to the objective lens
5. Objective aperture centering
6. Objective astigmatism correction
7. Alignment of intermediate lens

Additional material

Links

Physics behind Electron Microscopy

- http://www.snaggledworks.com/em_for_dummies/
- <http://www.mih.unibas.ch/Booklet/Lecture/>
- <http://em-outreach.ucsd.edu/web-course/toc.html>
- <http://www.matter.org.uk/tem/>

Basics and Protocols

- <http://treefrog.cvm.uiuc.edu/Methods.html>
- <http://www.biologymad.com/cells/microscopy.htm>
- <http://pathcuric1.swmed.edu/emboCourseManual/CourseManual.pdf>
- <http://web.uct.ac.za/depts/mmi/stannard/negstain.html>
- <http://www.hei.org/research/aemi/tec.html>
- http://www.nanoprobes.com/tech_help/TechIEM.html
- <http://www.udel.edu/biology/Wags/b617/b617.htm>
- <http://www.uga.edu/caur/temindex.htm>
- http://bama.ua.edu/~hsmithso/class/bsc_656/websites/sample_tem.html
- <http://www.microstartech.com/index/diamondknives/diamondmanual.htm>
- PowerPoint on general physical science sample prep:
 - <http://www.docstoc.com/docs/67848693/TEM-Specimen-Preparation-Pips>
- A pdf on dimple grinding and ion milling: <http://www.ims.uconn.edu/~micro/Dimple%20Grinding2.pdf>
- Ultrathin cryo-sectioning and immuno-gold labeling:
<http://www.umcutrecht.nl/subsite/cmc-utrecht/Courses/course-manual.htm>
- Electron diffraction: http://www.matter.org.uk/diffraction/electron/electron_diffraction.htm
- Diffraction and HREM simulation package: <http://www.microscopy.cen.dtu.dk/computing/jems/index.html>
- Theory on diffraction: <http://www.microscopy.ethz.ch/ED-1.htm>
- Interactive tutorial about diffraction by Proffen and Neder: <http://www.lks.physik.uni-erlangen.de/diffraction/>

Galleries and Atlases

- <http://www.uni-mainz.de/FB/Medizin/Anatomie/workshop/EM/EMAtlas.html>
- <http://www.einstein.yu.edu/iag/samples/files/TEM.html>
- <http://www.uq.edu.au/nanoworld/>
- <https://www.biomedica.cellbiology.ubc.ca/cellbiol/>
- http://www.bu.edu/histology/m/t_electr.htm

Additional Links

- <http://www.unl.edu/CMRAcfem/glossary.htm>
- <http://www.hei.org/research/aemi/book.htm>

- <http://www.fmhs.auckland.ac.nz/sms/biru/links/tem.aspx>
- <http://www.ou.edu/research/electron/www-vl/>
- SiMPore TEM Grids: www.TEMwindows.com

General Reading List

- **Ayache, J., Beaunier, L., et al.** Sample Preparation Handbook for Transmission Electron Microscopy. New York: Springer Science+Business Media, LLC, 2010. Google Books...
- **Belk, J. A. (ed.)**. Electron microscopy and microanalysis of crystalline materials. London : Applied Science Publishers, 1979.
- **De Graef, Marc**. Introduction to conventional transmission electron microscopy. Cambridge : Cambridge University Press, 2003.
- **Eberhart, J. P.** Structural and chemical analysis of materials: x-ray, electron and neutron diffraction; X-ray, electron and ion spectrometry; electron microscopy. New York : Wiley, 1991.
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- **Loretto, M. H.** Electron beam analysis of materials (2nd ed.). New York : Chapman & Hall, 1994.
- **Pennycook, S. J. & Nellist, P. D. (eds.)**. Scanning transmission electron microscopy [electronic resource]: imaging and analysis. New York: Springer, c2011.
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- **Williams, D. B., C. Barry Carter, C. B.** Transmission Electron Microscopy [electronic resource] : A Textbook for Materials Science (2nd ed.). Boston, MA : Springer US, 2009.
- **Zewail, A. H., Thomas, J. M.** 4D electron microscopy [electronic resource]: imaging in space and time. London : Imperial College Press ; Singapore : Distributed by World Scientific Pub. Co., c2010.
- **Zou, X., Hovmöller, S., Oleynikov P.** Electron Crystallography: Electron Microscopy and Electron Diffraction. Oxford : Oxford University Press, 2011.<http://www.ims.uconn.edu/~micro/>