Concise Overview of Common Electron Microscopy Modalities

The theory of image formation in electron microscopes is very similar to image formation in light microscopes. However, the practice and design are completely different: The most crucial difference being that the illumination is a beam of (charged) electrons rather than (neutral) photons. Since electrons have more mass than photons, their energy is higher and wavelength shorter, so much higher resolutions can be achieved (down to 1 nm). Unlike photons, electrons only travel directionally when they are accelerated through a voltage gradient in a high vacuum. The electrons must be focused using electric and magnetic fields, rather than refractive and reflective materials such as glass. Electrons also interact (scatter) appreciably only with heavy atomic nuclei (>20 amu). The 'expertise' in conventional biological electron microscopy is primarily related to preparing samples is such a way that they can be imaged in an electron beam. Biologists rarely push the resolution limits that an electron microscope can achieve. Please contact us (microscopy@umich.edu) to help you decide which sample preparation methods and microscopes are best for you project.

Transmission electron microscopy (TEM) is a wide-field imaging technique conceptually analogous to bright-field in light microcopy. A filament produces free electrons that are focused onto the sample using an electromagnetic condenser lens system. Electrons that pass through the sample are then used to form an image. Some of the transmitted electrons are scattered by metal atom stains in the sample. The more scattering that occurs, the darker a region will appear in the final image. Phase imaging based on electrons that (inelastically) interact with the sample is also possible but not common for biological applications. After passing through the sample, an electromagnetic objective lens system refocuses the electrons, typically onto a phosphor screen, which converts the electron image into light. Finally, light optics are used to focus the phosphorescence photons onto a standard CCD or CMOS camera.

Advantages:	Extremely high (< 1 nm) resolution
Disadvantages:	Sample prep requires a long and complex procedure of fixation, dehydration, and heavy metal staining, and ultrathin sectioning prior to imaging. Sections are extremely 2D (50-100 nm thick), making it difficult to appreciate complex 3D objects.
Common Applications:	Accelerating voltage at 60 - 80 keV: High magnification imaging for ultrathin section of cell monolayer or tissue specimen. Accelerating voltage at 100 keV: High magnification imaging for protein, nanoparticle, microorganism, and cell organelle. Accelerating voltage of 200 or 300 keV: High magnification imaging for TEM array tomography and high angle tilt tomography on thin sections.

TEM Tomography performs a series of TEM images on a sample at many different tilt angles. Using tomographic reconstruction methods, a 3D image can then be reconstructed from the tilt

series. The larger the range of tilt angles, the higher the z-resolution that can be achieved. Larger angles also mean the electron beam must pass through the sample at a highly oblique angle, so traverse a longer distance in the sample. A higher accelerating voltages are required to penetrate through more sample (e.g. 300 keV).

Scanning Electron Microscopy provides information about surface topography and in some cases surface composition as well. Unlike transmission electron microscopy, which illuminates the entire sample at once (wide-field), scanning electron microscopy is a point scanning technique that quickly scans a single focused point of electrons across the sample in a raster pattern to collect an image sequentially over time. (Analogous to confocal reflectance microscopy). The scanned electrons then interact with the sample and produce various signals that can be detected to create an image. Three types of signals are commonly used for biological SEM imaging applications:

Secondary electron (SE) imaging is based on electrons ejected from the sample after being hit with the electron beam. The SE signal originates from the very surface of the sample and mainly provides information about surface topography. Samples are often coated with gold prior to SE-SEM imaging.

Backscattered electron (BSE) imaging is based on electrons that penetrate into the sample and collide with an atomic nucleus. Thus, BSE images mainly provide information about differences in atomic number and density: the higher the atomic number, the brighter the material appears in the image since more beam electrons are backscattered.

Energy dispersive x-ray spectroscopy (EDS, EDX, or XEDS) imaging is based on detection of x-rays (high energy light) that are produced when the electron beam ejects electrons from the sample. When sample electrons from other energy levels fill these 'holes', characteristic x-ray energies are produced, similar to fluorescence in light microscopy. The measured energy of the x-rays then provides information about the local atomic energy levels and so is used for elemental analysis or chemical characterization of a sample.

Advantages:	High (2 - 10 nm) resolution information about the surface of the sample.
Disadvantages:	No information about the interior (>10 nm deep) of the sample.
Common Applications:	Any time surface information is desired.

Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM) is a serial block face imaging technique used to create 3D SEM images. An ion beam (Ga or Xe) is used to very finely mill away the sample surface (5-10 nm per cut). After each milling, the newly exposed block face is

imaged in SEM mode. This process is repeated over and over to slowly create a 3D image of the sample. Note this method is destructive since the sample is milled away during each step.

Advantages:	High (2 - 10 nm) resolution in xy and z.
Disadvantages:	Very expensive instrument, and milling means that the sample is destroyed during the imaging process. Practically limited to small (4 x 4 x 2 um) regions due to slowness of acquisition.
Common Applications:	When a high resolution 3D image is required. For even higher resolution but with much less depth, consider TEM tomography. For lower resolution, consider array tomography or knife-based serial block face imaging.

Array Tomography is really a sample serial sectioning technique, but it enables 3D EM imaging at low (500-100 nm) z-resolution. Using a special ultra-microtome, a resin embedded sample is serially sectioned for up to 100s of sections. The sections are then placed onto a silicon wafer for SEM imaging or slotted grid for TEM imaging. After 2D images of the sections have been acquired, software is used to aligned the images altogether into a 3D image.

Advantages:	Large field of view (100s um) in xy at lower resolutions. Serial sections are not destroyed during imaging.
Disadvantages:	Reliably cutting many serial ultrathin sections is very difficult and labor intensive. Image processing may also be difficult due to geometric distortions.
Common Applications:	Low resolution 3D image reconstruction.

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