Electron Microscopy

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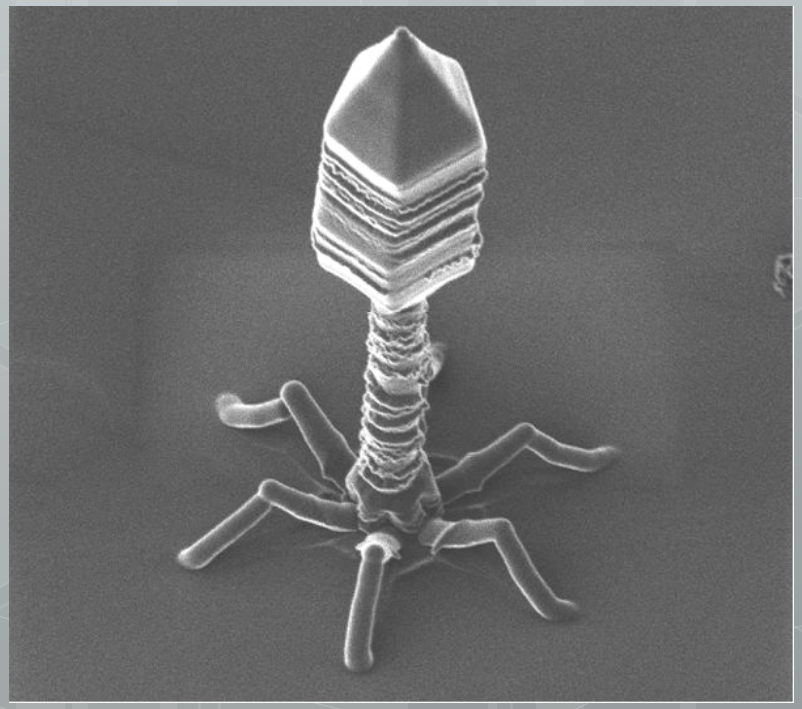
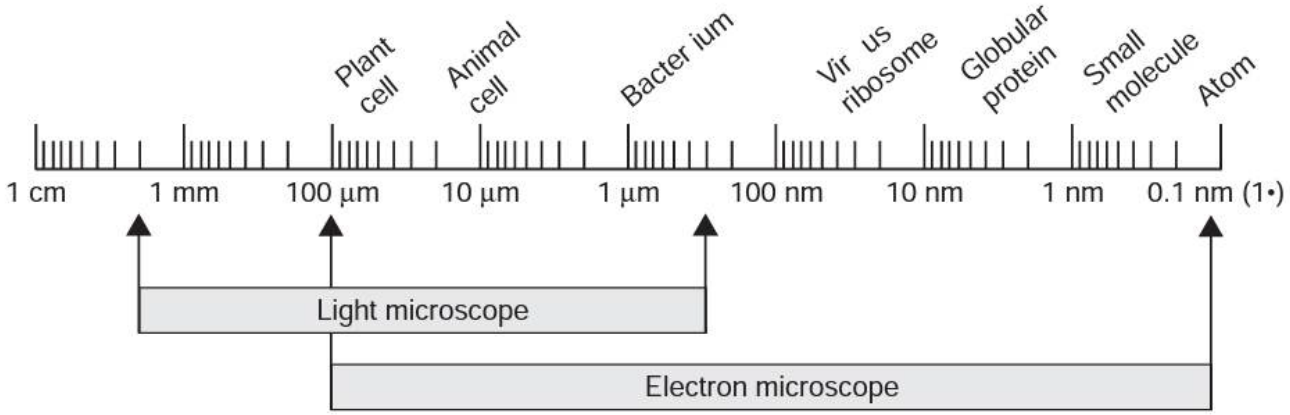


http://www.zyvexlabs.com/ElPBNuG/ElPBN%20images/05Ion.jpg

Intro to Electron Microscopy



- Similar to optical microscopy except with electrons rather than photons
- Used to image samples with a resolution of 10 Å
  - Can image many different structural geometries
- Mostly limited by radiation damage from the electron beam

Electron Properties

- Since electrons exhibit wave and particle behavior, the de Broglie relationship applies:

$$h = \lambda_e \mathrm{p}$$

- Since the electron is charged, when introduced to an electric potential difference, it accelerates to its equilibrium momentum:

$$q\varphi = \frac{1}{2}\frac{p^2}{m} \rightarrow p = \sqrt{2m_e e\varphi}$$

- So particle momentum is only dependent on the electric potential difference

Electromagnetic Lenses

- Used to focus the electron beam
- We can relate wavelength to accelerating voltage:

$$\lambda_e = \frac{h}{\sqrt{2m_e e\varphi}} \approx 12\varphi^{-\frac{1}{2}}$$

  - Electron wavelengths are 5 times smaller photons
- Maximum resolution (d) of a lens is related to the aperture angle and the wavelength by:

$$d = \frac{\lambda_e}{\sin\alpha}$$

- However due to aberration in the lens, the resolution is also limited by:

$$d = (C_s\lambda_e^3)^{1/4}$$

  - Where C_s is the spherical aberration coefficient

Signal vs. Noise

- Largest issue is radiation damage to the specimen
- Image is generated from elastic scattering while noise is generated from the inelastic scattering
  - Inelastic scattering deposits energy on the sample which damages the sample (occurs 3-4 times more often than elastic scattering events)
- Signal-to-noise ratio is described by the Rose model:

$$S/\sigma_{Rose} = C\sqrt{An_{background}}$$

  - Where C is the contrast ($C = \left|{}^{n_{object}-n_{background}}/_{n_{background}}\right|$)
  - Ratios between 5-7 are required to identify features with good enough confidence
- Can either use an energy filter (remove certain energies) or higher accelerating voltage

Effect of the Microscope on Electron

- When a wave is passed through a samples, it interacts and is released as a phase shift of the wave in. This is represented by:

$$\tau_{out}(x,y) = \tau_{in} e^{i\varphi_P(x,y)} \approx \tau_{in}(1 + i\varphi_P(x,y))$$

- This is then adjusted to account for electron absorbance:

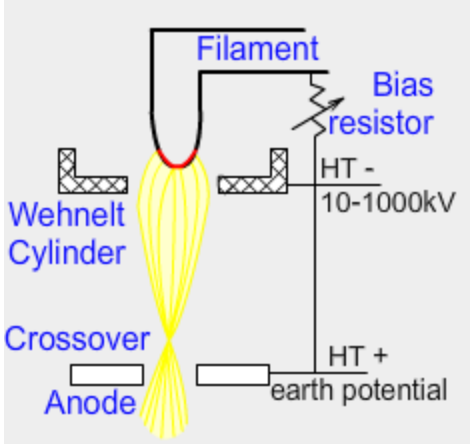
$$\tau_{out}(x,y) = \tau_{in}(1 + i\varphi_P(x,y) + \mu(x,y))$$

- The microscope then observes the phase shift due to this change which can be represented by the Fourier Transform:

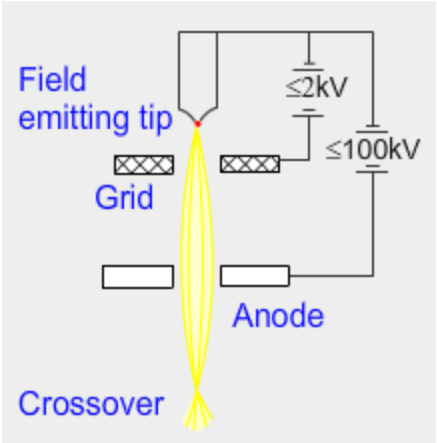
$$T_{out}(u,v) = \tau_{in}[\delta(u,v) + i\Phi_P(u,v) + \mathrm{M}(u,v)] \times P(u,v)$$

  - Where P(u,v) is the transfer function of the microscope

Electron Generation



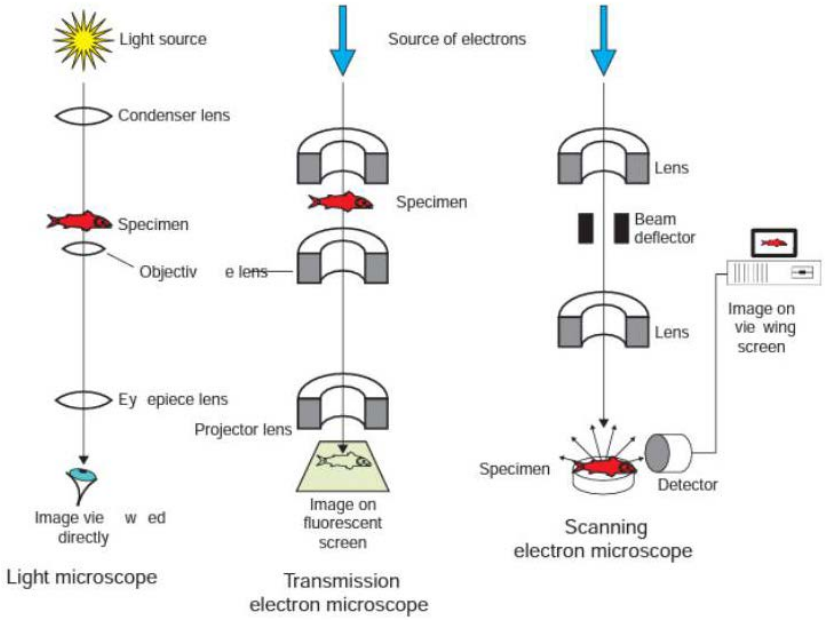
- Thermionic Electron Gun
  - Heated filament produces electrons
    - Typically made of Tungsten or Lanthanum hexaboride
  - Electrons drawn towards an anode
  - An aperture in the anode creates a beam



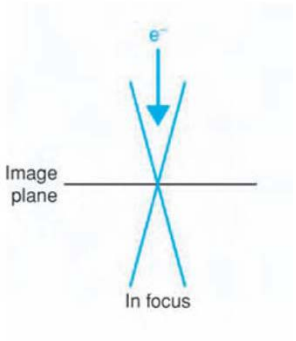
- Field Emission Gun
  - A very strong electric field is used to extract electrons from a metal filament
    - Filament typically a single tungsten crystal
  - Requires a vacuum
  - Similar anode setup

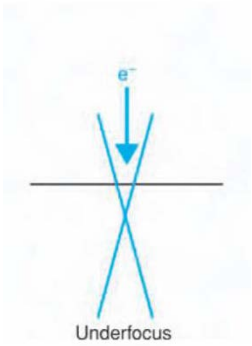
Microscope Setup

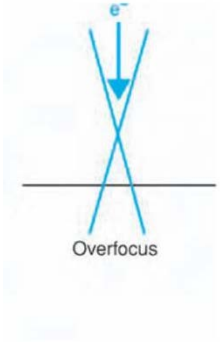
- Transmission Electron Microscope
  - Phase contrast Image is formed by the interference between electrons that passed through the sample and ones that did not
- Scanning Electron Microscope
  - Electron beam is scanned across the sample
  - The reemitted electrons are measured in order to form the image



Focusing



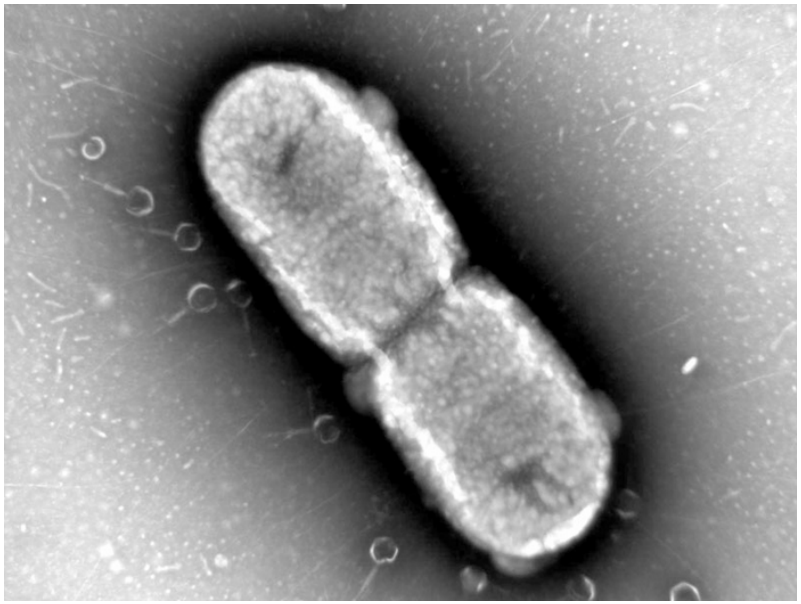




- When the image is in focus, there is very low contrast due to the electron loss around the objective
- By imaging underfocus or overfocus, a phase shift and amplitude contrast are created
- This creates a dark image with a white ring around or a white image with a dark ring (respectively

Negative Staining

- Biological samples are often imaged using negative staining
- The elements of biological molecules do not interact strongly with the electron beam
- Instead they are seated in a material that does and then the negative space of the sample is imaged in this material



http://www.izw-berlin.de/electron-microscopy.html

Cryo-Microscopy

- Samples are often frozen in order to preserve the structure against radiation damage from the electron beam
- In order to not damage the structure when freezing, the sample is flash frozen
  - If ice crystals were allowed to form they would damage the sample
- Samples are typically dunked into liquid ethane or propane (~11° K)

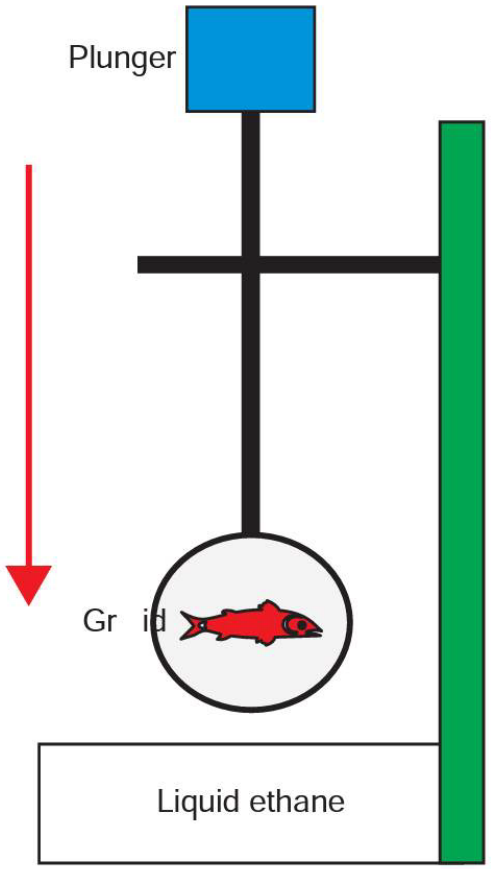


Image Options

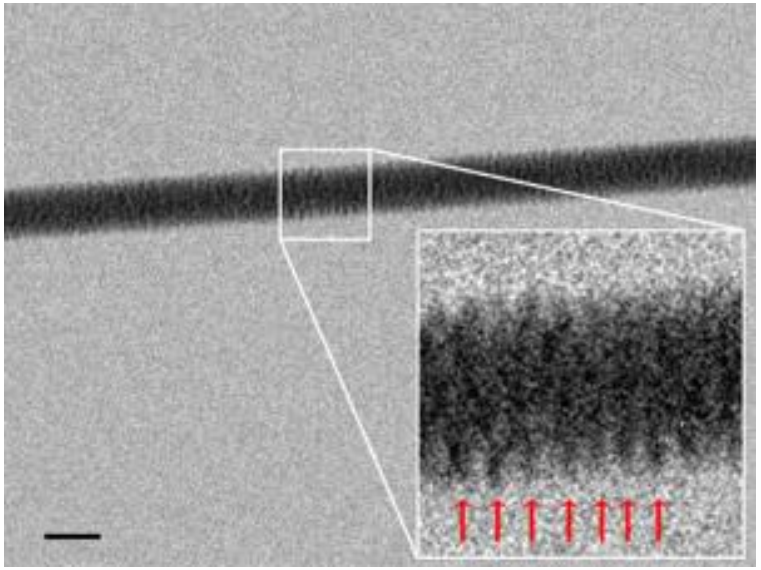
- EM can magnify a sample between 1000 and 200,000 times
  - However due to limitations, macromolecules are usually imaged between 40,000 and 60,000 for a resolution of 10 – 20 Å
  - Image intensity decreases as magnification goes up with $1/M^2$
- Protein concentration is typically around 1 mg ml^{-1} to ensure sufficient particle density without being overcrowded
- Biological samples can only be exposed to 10-15 electrons per $Å^2$
  - Using a stain allows increased exposure
  - Lower temperatures can similarly protect the sample

Data Collection Protocol

- Search/Focus/Exposure
  - Use low-dose/magnification to find area of interest to magnify
  - Specific defocus is picked and drift is checked
  - Sample is exposed to a high-does to image the sample

Imaging Symmetry

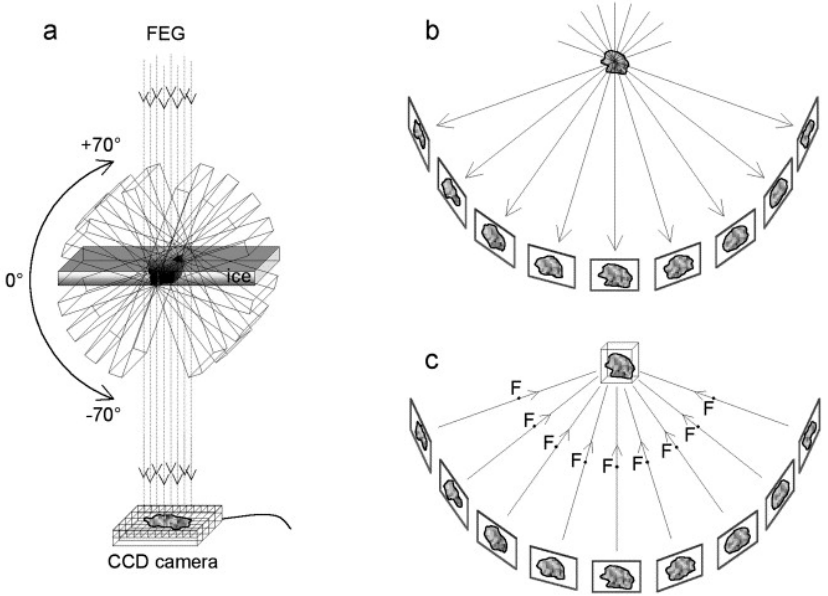
- When molecules have symmetry or are in helical structures, a two dimensional EM image can be used to reconstruct the 3D structure
- This information is often using in conjunction with X-ray crystallography in determined the crystal structure of molecules



http://www.newscientist.com/data/images/ns/cms/dn22545/dn22545-1_300.jpg

Electron Tomography

- Data is collected at multiple tilt angles
  - Typically every 1º – 2º over ± 70º
- Image is then compiled to determine the 3D structure of the image



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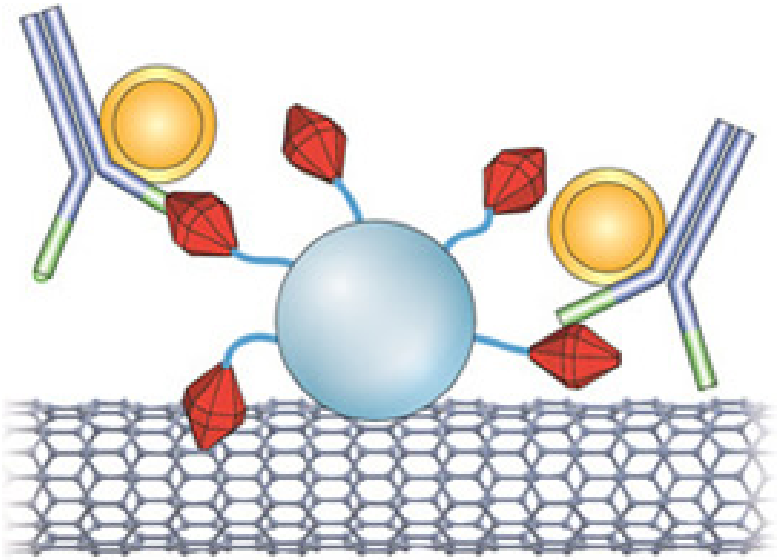
Immunochemical Applications

- It is very easy to image gold clusters with EM due to gold's properties
- Thus the use of gold labeled antibodies is particularly helpful in immunochemistry
- Labeled antibodies will bind to their antigen
- EM can then be used to identify the location of antibodies and by extension the antigens



http://www.nano.org.uk/news/images/imageL1282120449.jpg

Sources

1. Serdyuk, Igor N., Nathan R. Zaccai, and Joseph Zaccai. *Methods in Molecular Biophysics: Structure, Dynamics, Function*. New York: Cambridge University Press, 2007. Print.
2. "Introduction to Electron Microscopes." *Matter.org.uk*. University of Liverpool, n.d. Web. 20 Oct 2013. <http://www.materials.ac.uk/elearning/matter/IntroductionToElectronMicroscopes/SEM/electron-gun.html>.