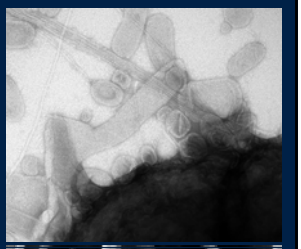
SIR WILLIAM DUNN
SCHOOL OF PATHOLOGY

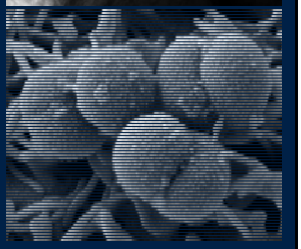
UNIVERSITY OF OXFORD

Basic Principles of Electron Microscopy (EM)

Dr Errin Johnson

Head of the Dunn School EM Facility





Basic Principles
Brief history of EM

1873 – Hermann von Helmholtz & Ernst Abbe show that the wavelength of light affects optical resolution

1924 – Louis de Broglie theorised the wave/particle duality of electrons

1926 – Hans Busch demonstrated that magnetic lenses can manipulate the path of electrons in the same way as optical lenses do with light

1932 – Ernst Ruska & Max Knoll invent the TEM

1934 – Ladisalus Marton publishes the first biological EM micrograph

1937 – Manfred von Ardenne builds the first SEM

1951 – Erwin Muller develops the field emission microscope for atomic resolution

1951 – Albert Claude *et al* publish the first TEM image of an intact cell

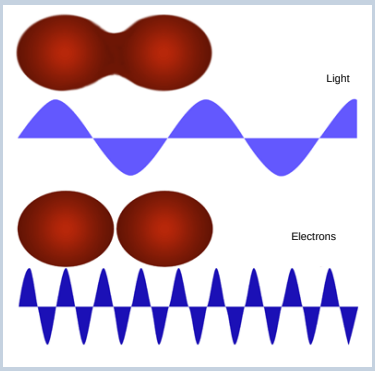
For a detailed history of EM, see:

1. Haguenau et al. (2003) Key events in the history of electron microscopy. *Microscopy & Microanalysis*, 9(2): 96-138.

2. Masters, B (2009) History of the electron microscope in cell biology. *eLS*, Wiley.

Basic Principles
Resolution

- Resolution is the smallest distance at which two neighbouring points can be distinguished and is dependent on wavelength
- The wavelength of accelerated electrons (6 pm) is several orders of magnitude shorter than that of light (600 nm)



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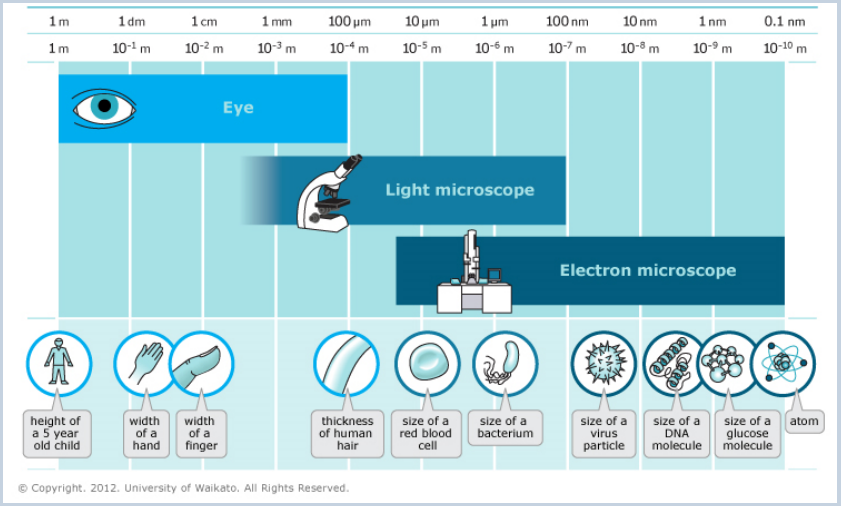
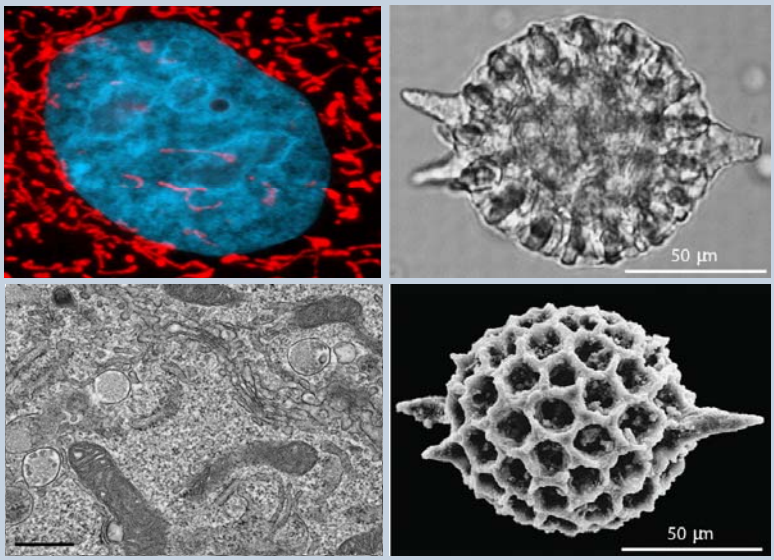
Basic Principles
Resolution



Basic Principles
Resolution and Contrast

Light microscopy

Electron microscopy



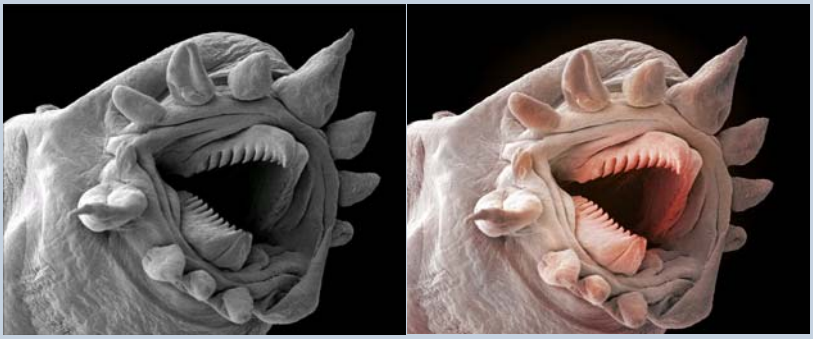
TEM image of fibroblast cell (E Johnson). Bottom: Confocal image of a kidney cell stained with DAPI (blue) and MitoTracker (red) (Hammamatsu.magnet.fsu.edu)

Radiolarian imaged with both SEM (top) and light microscopy (bottom). From: General Chemistry: Principles, Patterns, and Applications, B. Averill & P. Elderege

Basic Principles
Signal detection

- EM images are monochromatic and are essentially intensity maps of the number of electrons that are detected from a given point. False colour may be added during post-processing of the image, if desired.

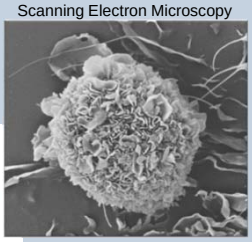
Original SEM image *False-coloured SEM image*

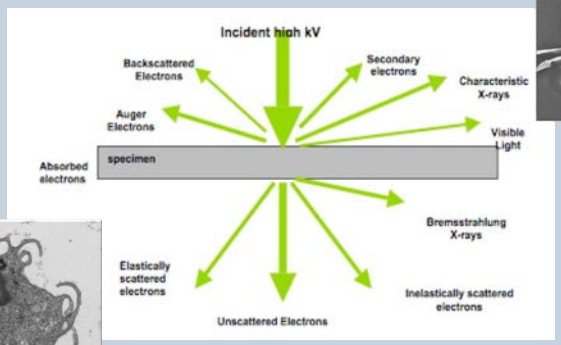


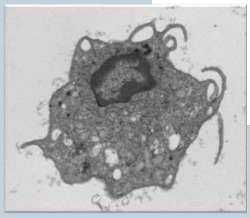
Hydrothermal worm (x525) by Philippe Crassous, FEI.com

Features of Electron Microscopes
Overview

Scanning Electron Microscopy

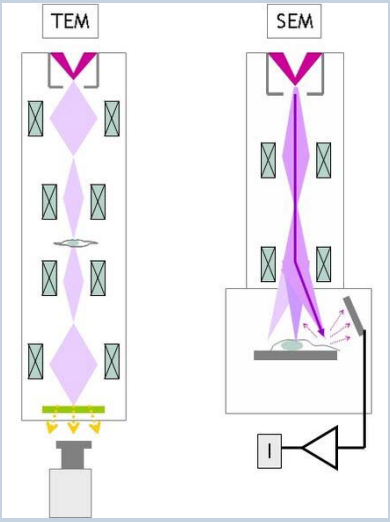






Transmission Electron Microscopy

Features of Electron Microscopes
Overview

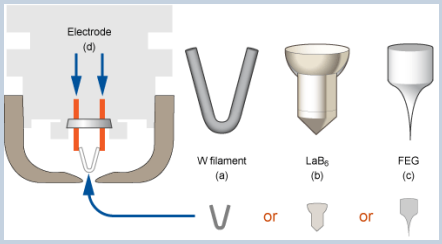


The main components of an electron microscope are:

- An electron gun
- Electromagnetic lens system
- Vacuum system
- Camera/detector
- Computer

Features of Electron Microscopes
The Electron gun

- The gun consists of an electron source, electrode, Wenhelt assembly and anode
- Electron sources are typically Tungsten or LaB6 and can be thermionic or field emission (FEG). A current is run through the filament/crystal to heat it or, a field is applied to the tip, resulting in the emission of electrons from the tip. The high voltage difference between the cap and the anode causes the electrons to accelerate and form a beam



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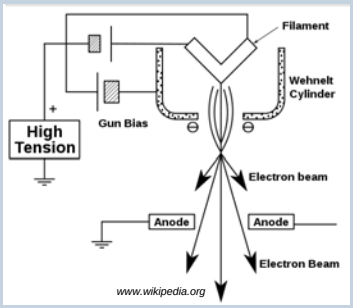
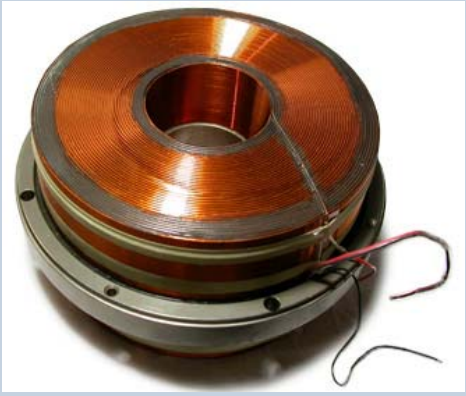
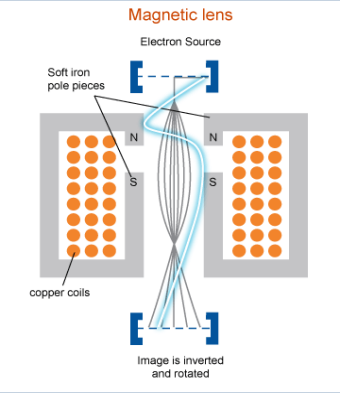


www.wikipedia.org

Features of Electron Microscopes
Electromagnetic Lenses

- EM lenses are electromagnetic, creating precise, circular magnetic fields that manipulate the electron beam, much the same way that optical lenses focus and direct light

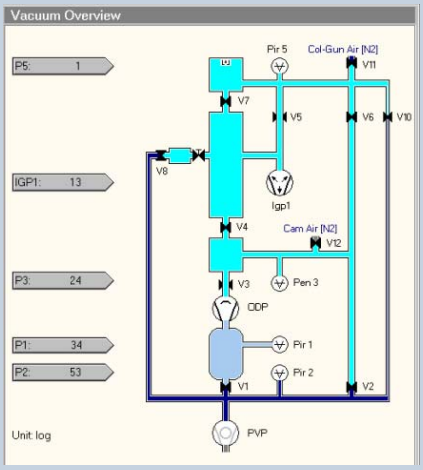

www.ammrf.org



www.ammrf.org

Features of Electron Microscopes
Vacuum systems

- EMs have elaborate pumping systems to ensure that the microscope is operated under a high vacuum (10^{-4} Pa)
  - Maintains the integrity of the electron beam, as any interaction with gas atoms will cause the beam to scatter
  - Avoids arcing between the cathode and ground (and damage to the filament)

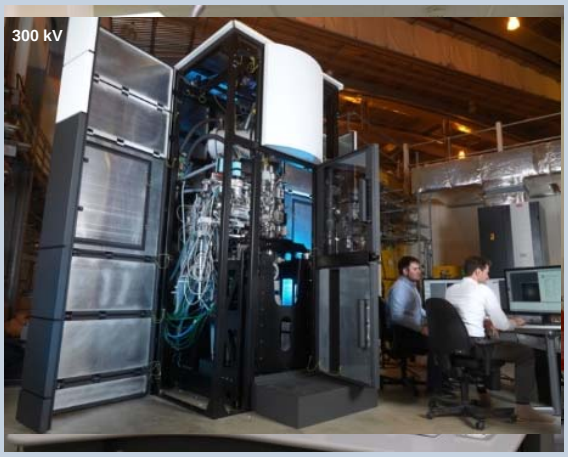


Overview of vacuum system on the Tecnai12 TEM

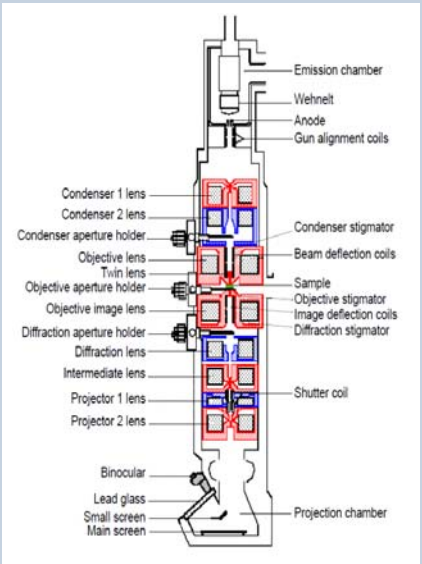
Transmission Electron Microscopy (TEM)

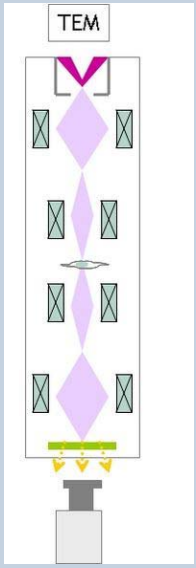
The TEM

- Resolution depends on a number of factors, including the accelerating voltage, the type of electron source used and how you setup the microscope
- Accelerating voltage (kV) is typically 80-300 kV for biological specimens



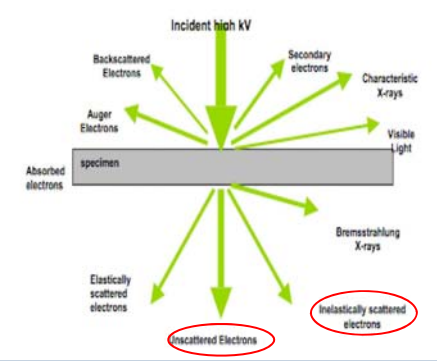
The TEM

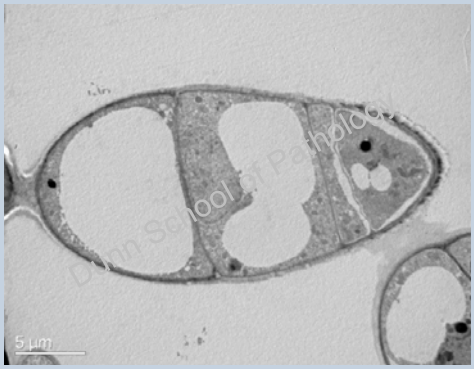




The TEM
Contrast

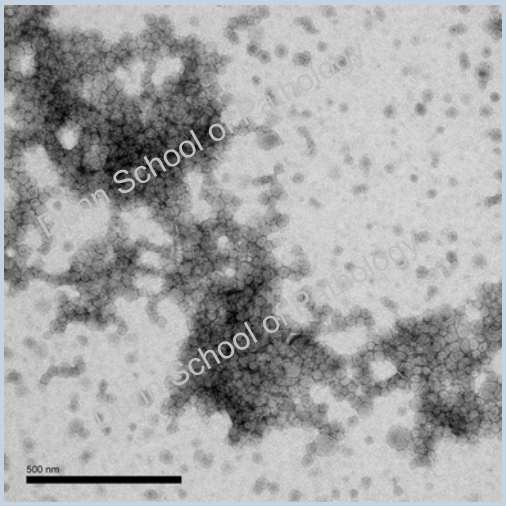
- Contrast is generated by density differences within the sample.
- Darker areas in the image are where few electrons have been transmitted through the sample, due to thickness or high atomic number.



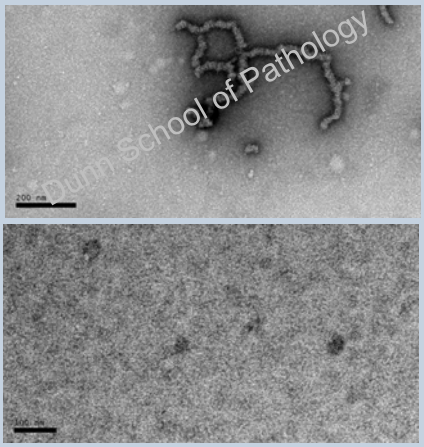


Lavender trichome, E Johnson

TEM applications
Particulate samples

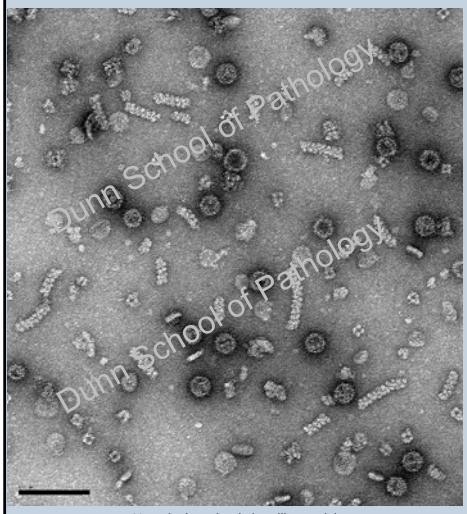


Negatively stained a-synuclein
E Johnson

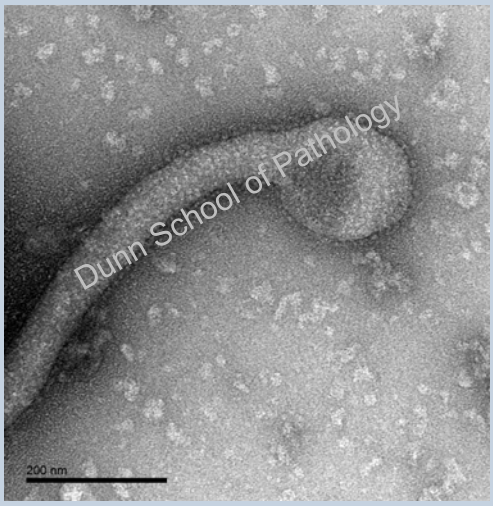


Negatively stained SAS-6 protein
WT aggregates, top; Mutant dimers, bottom
(M Cottee/E Johnson)

TEM imaging
Particulate samples - Viruses

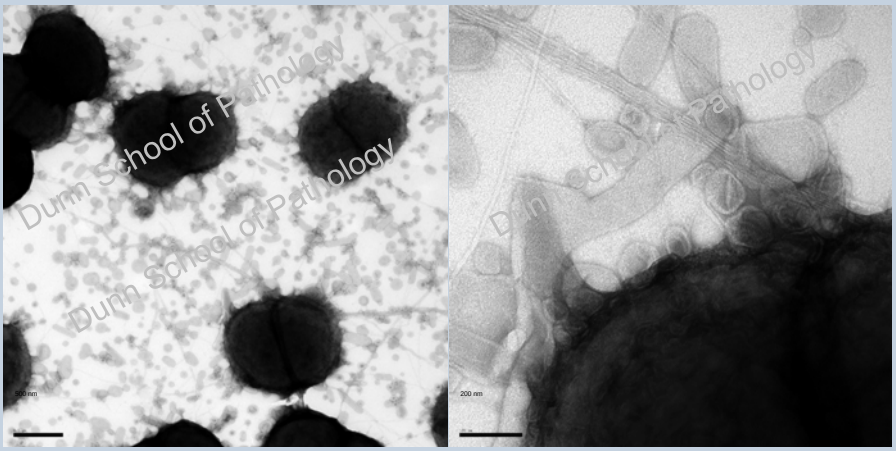


Negatively stained virus-like particles
(D Leneghan/E Johnson)



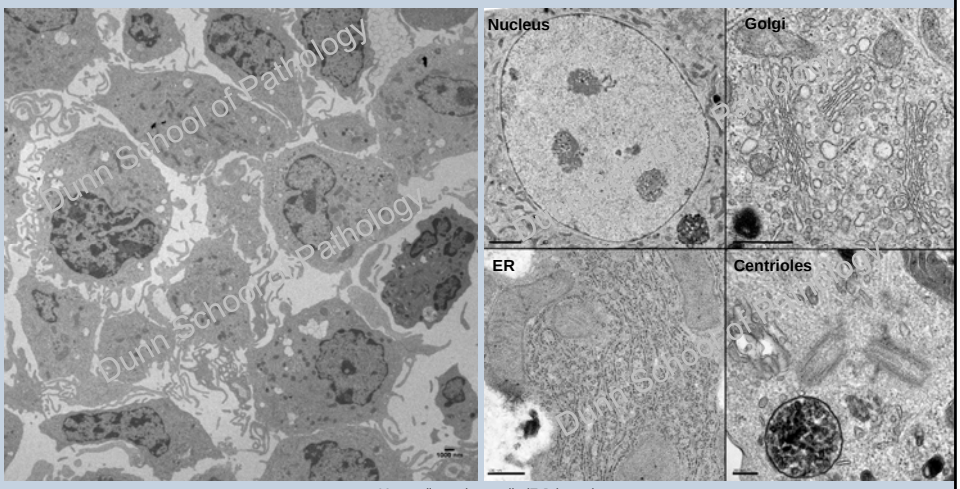
Negatively stained virus-like particles (Ebola)
(E Johnson)

TEM imaging
Particulate samples - Bacteria



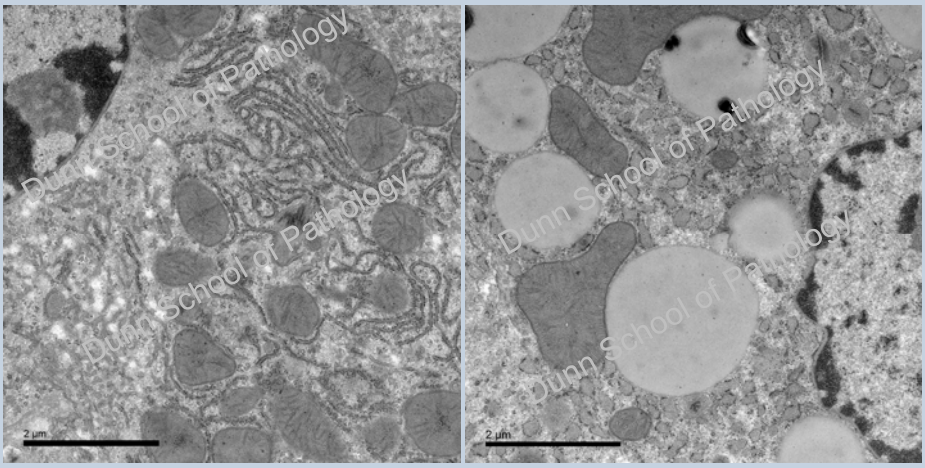
Negatively stained Neisseria sp. (R Exley/EJohnson)

TEM Applications
Ultrastructure - Cells



Mammalian culture cells (E Johnson)

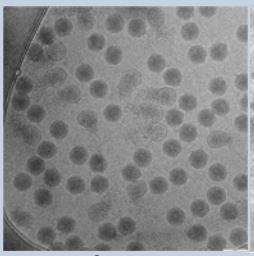
TEM Applications
Ultrastructure - Tissue



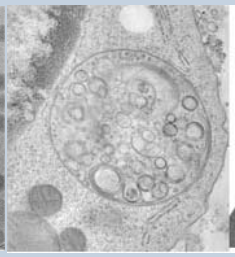
Mouse liver tissue, untreated (left) and under ER stress (right) (V Liebe & E Johnson)

TEM Applications
Advanced techniques

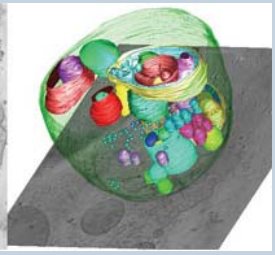
Cryo-EM



Gatan.com



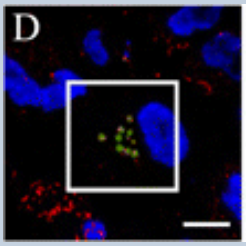
Multi-lysosomal body (A.J. Koster and W.J.C. Geerts, Utrecht University)

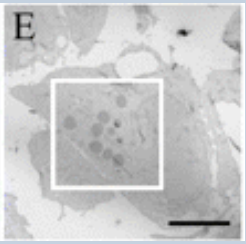


3D tomography

Protein localisation

Whole mount immunolabelled Trypanosome cytoskeleton (S Dean, Dunn School)

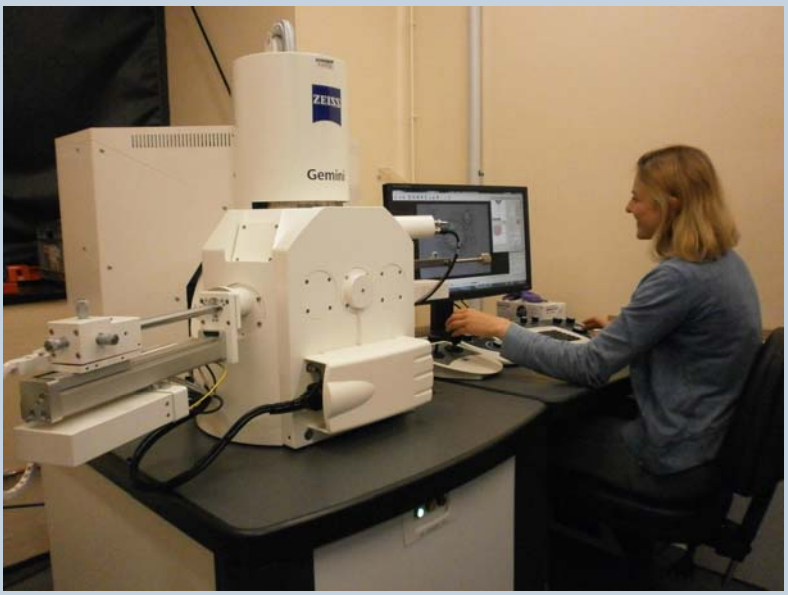




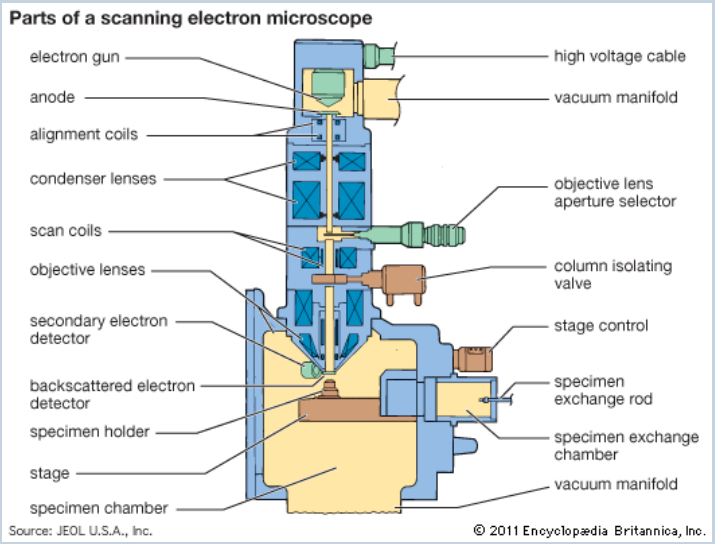
Correlative light and electron microscopy of cryo-sections (Vicidomini et al. Traffic 9:1828–1838, 2008)

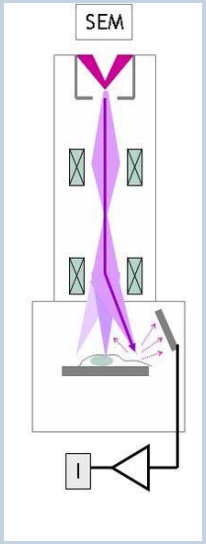
Correlative microscopy

Scanning Electron Microscopy (SEM)



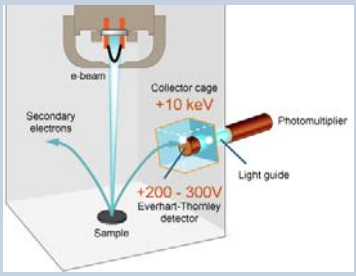
The SEM





The SEM
Signal detection

- Secondary electrons (SEs) provides surface morphology and topology information.
- SEs are captured by the Everhart-Thornley detector



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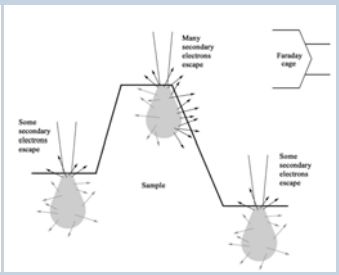
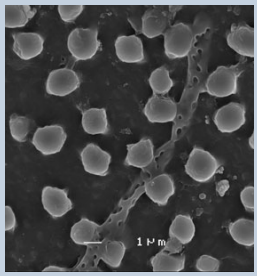


Dept Biological Sciences, Smith College Northampton USA



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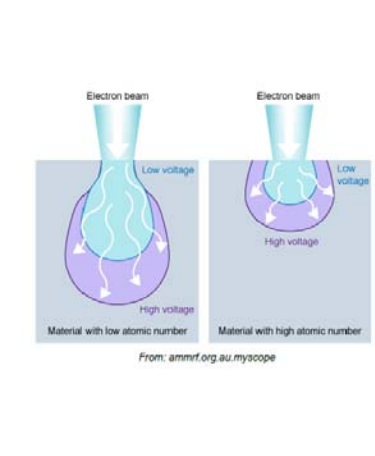
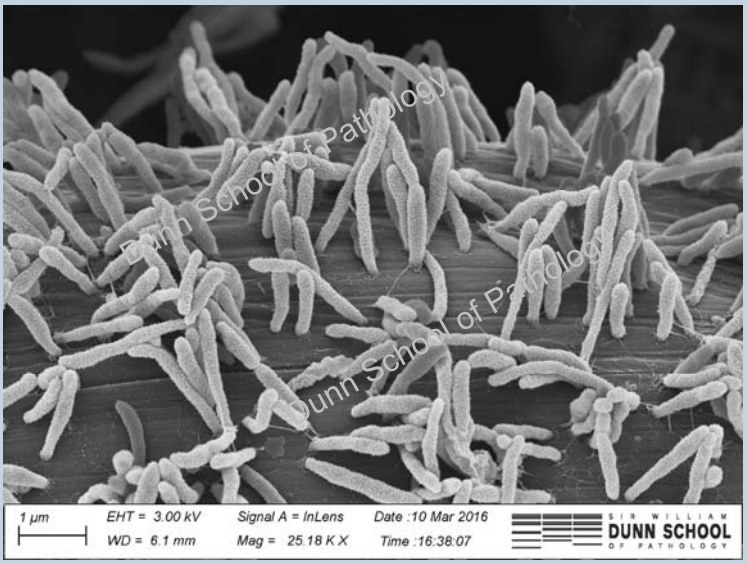
The SEM
Accelerating voltage

- Resolution depends on a number of factors, including the accelerating voltage, the type of electron source used and how you setup the microscope and the scan
- Accelerating voltage (kV) is typically 1-10 kV for biological specimens

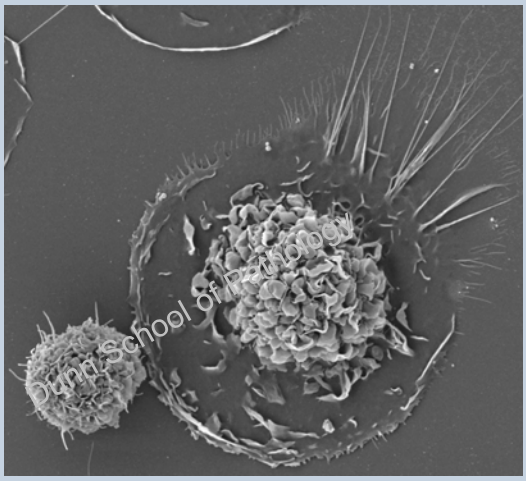


SEM Applications
Topography - Cells

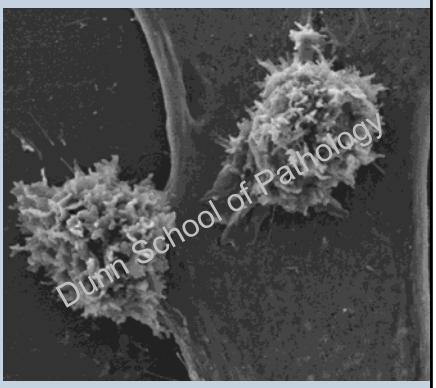


Bacteria on carbon electrodes
(s Putra/E Johnson)

SEM Applications
Topography - Cells

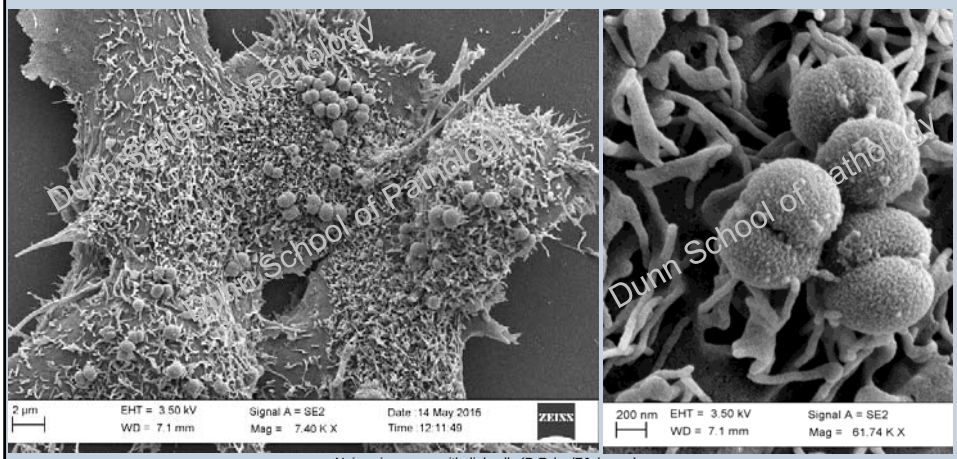


Monocyte and macrophage
(B van Wilgenburg/E Johnson)



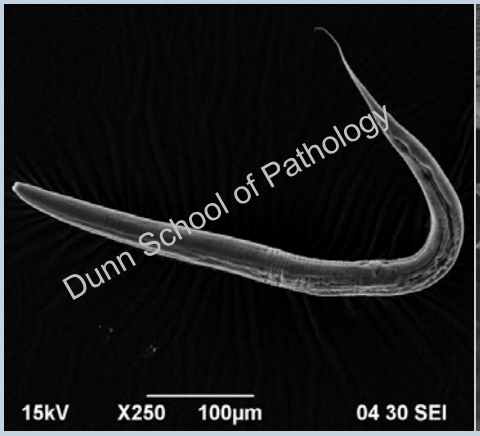
HEK 293 cell interacting with streptavidin coated dynabeads plus biotinylated anti-EPCAM (M Brenner/E Johnson)

SEM Applications
Topography - Cells

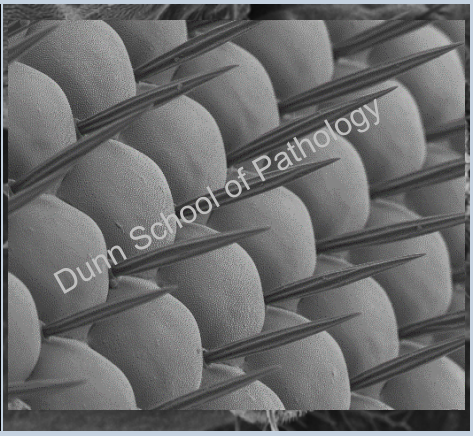


Neisseria sp. on epithelial cells (R Exley/EJohnson)

SEM Applications
Topography - Organisms

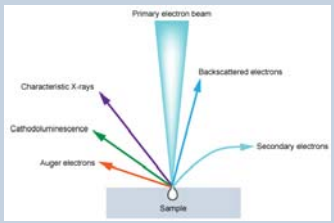


C. elegans
(E Johnson/A Moloney, Dunn School)



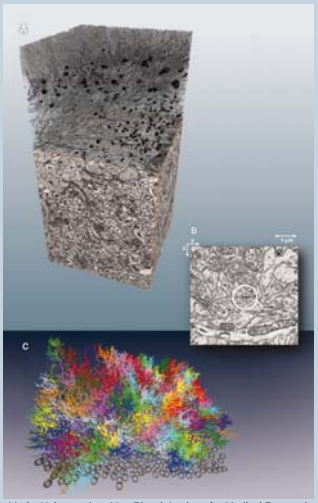
Drosophila rough eye phenotype
(M Elschami, NDCN)

The SEM
Diverse imaging capabilities

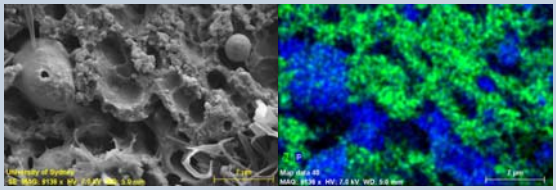


Biological Applications
SEM - Advanced

3D SEM



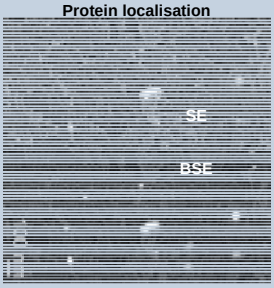
Moritz Helmstaedter, Max-Planck Institute for Medical Research



EDS analysis of cheese (M Foley, University of Sydney)

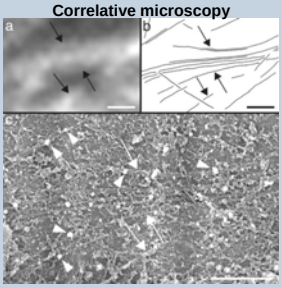
Chemical composition

Protein localisation



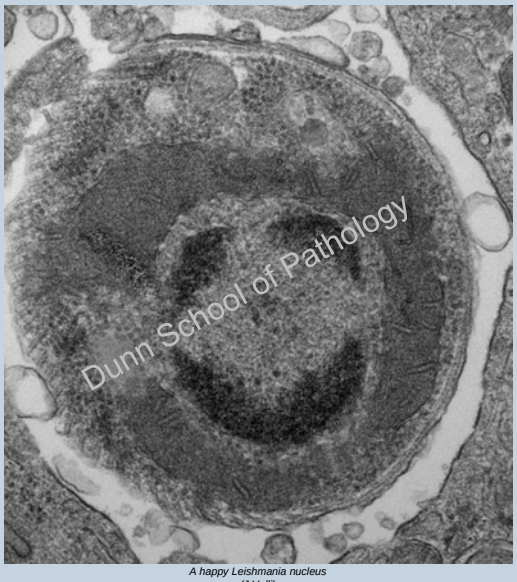
Microtubules (D Barton, University of Sydney)

Correlative microscopy



Microtubules (D Barton, University of Sydney)

Questions?



A happy Leishmania nucleus
(J Valli)