SIR WILLIAM DUNN
SCHOOL OF PATHOLOGY

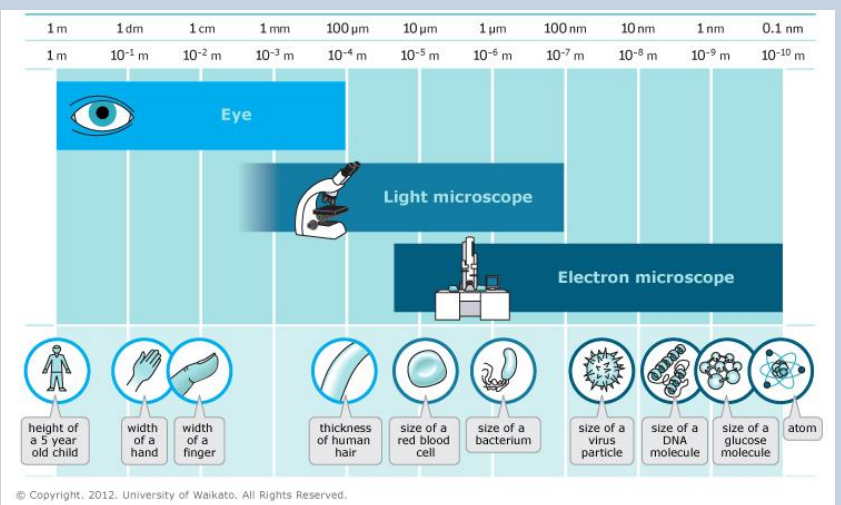
UNIVERSITY OF OXFORD

BIOLOGICAL ELECTRON MICROSCOPY

Dr Errin Johnson
EM Facility Manager

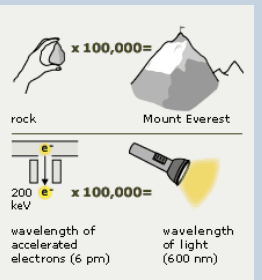
November 19, 2014

Electron microscopy
Resolution

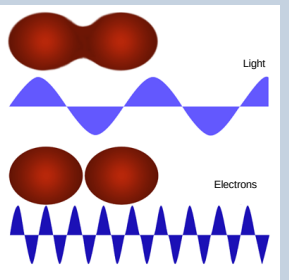


Electron microscopy
Resolution

- Resolution is the smallest distance at which two neighbouring points can be distinguished and is dependent on wavelength
- The wavelength of electrons is MUCH shorter than that of light
  - Confocal microscope resolution = 200 nm
  - Electron microscope resolution < 1 nm

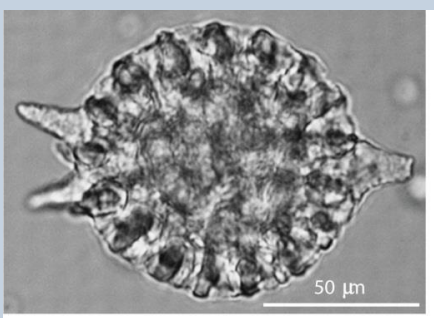


www.nobelprize.org

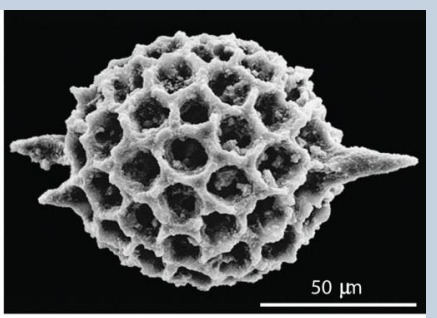


www.ammrf.org

Electron microscopy
Resolution



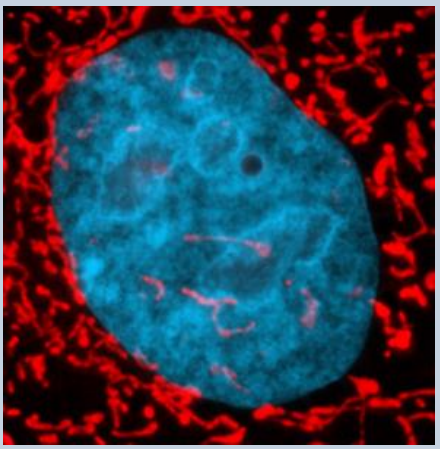
(a) Radiolarian under light microscope



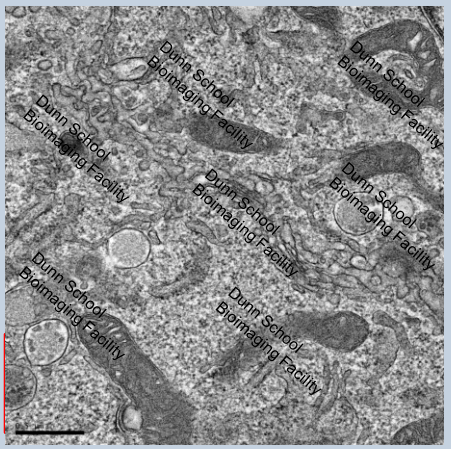
(b) Radiolarian under electron microscope

General Chemistry: Principles, Patterns, and Applications, B. Averill & P. Elderege

Electron microscopy
Resolution

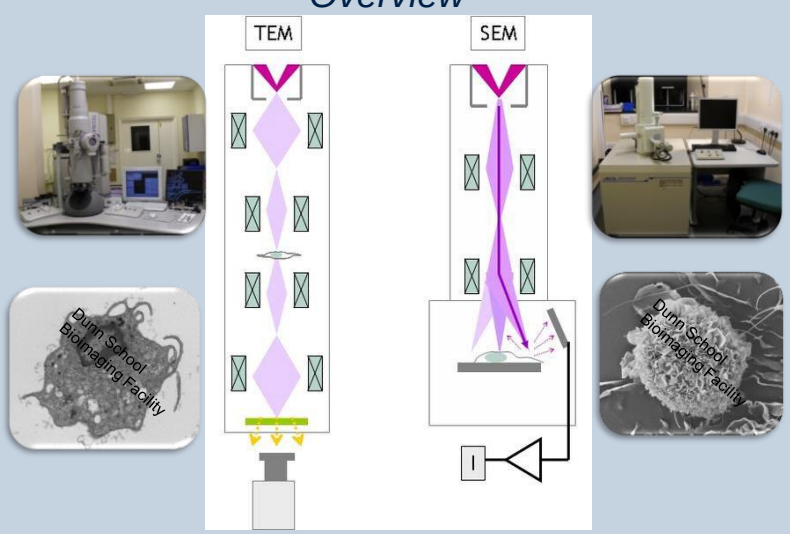


Confocal image of a kidney cell stained with DAPI and MitoTracker (Hammamatsu.magnet.fsu.edu)



TEM image of fibroblast cell stained with no specific stain (E Johnson, Dunn School)

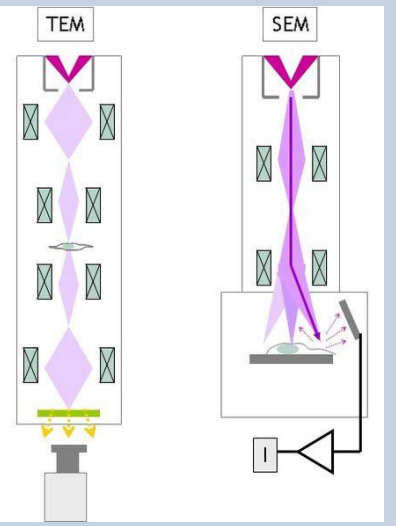
Electron microscopy
Overview



Electron microscopy
Overview

The main components of an electron microscope are:

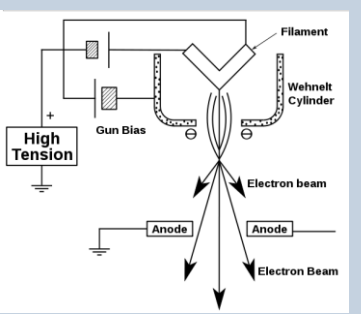
- An electron gun
- Electromagnetic lens system
- Vacuum system
- Camera/detector
- Computer



Electron microscopes
The Electron gun

- The gun consists of an electron source, electrode, Wenhelt assembly and anode
- A current is run through the filament/crystal to heat it, 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

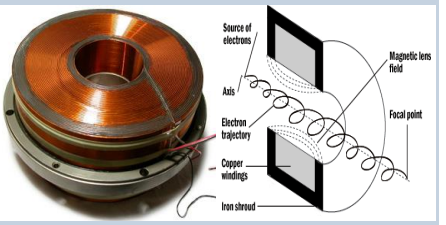
www.ammrf.org



www.wikipedia.org

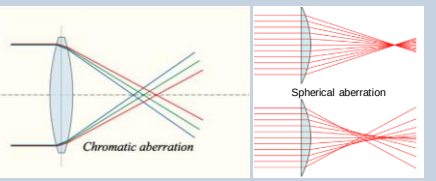
Electron microscopes
Electromagnetic Lenses

- TEM 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.uiowa.edu

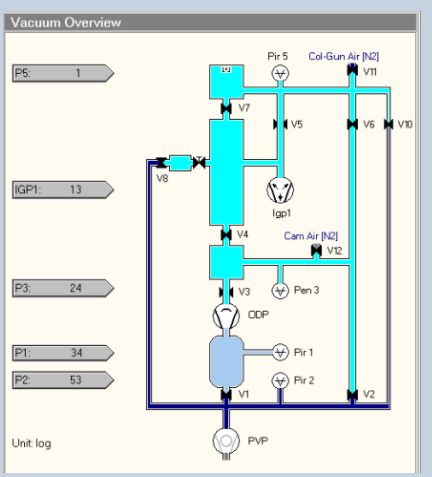
- Similarly to optical lenses, electromagnetic lenses are also susceptible to aberrations
  - Chromatic aberration
  - Spherical aberration
  - Astigmatism



A. Kach, University of Zurich

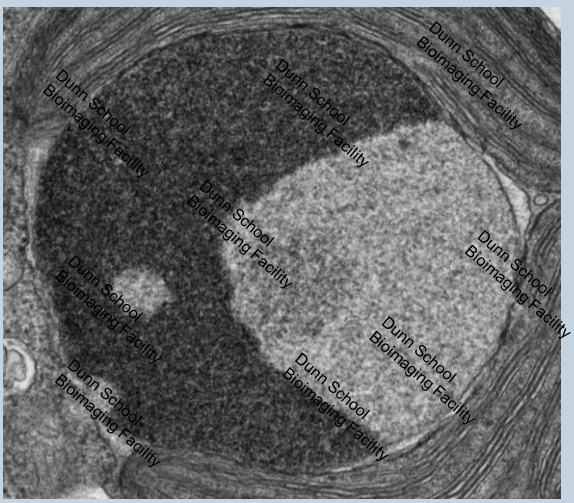
Electron microscopes
Vacuum systems

- 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)



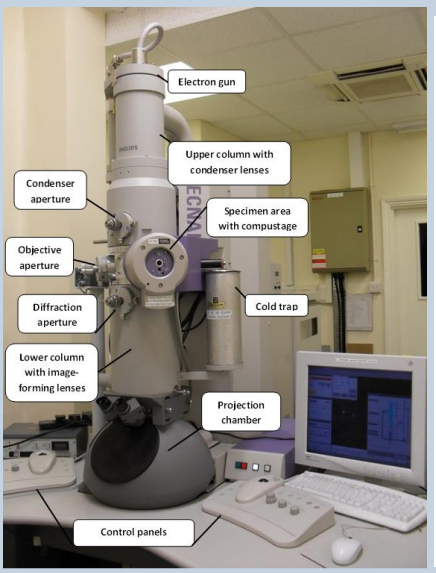
Overview of vacuum system on the Tecnai12 TEM

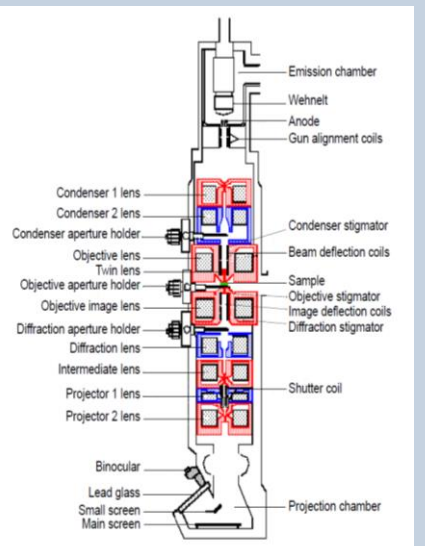
Transmission Electron Microscopy (TEM)



Nucleus of a single celled algae (M Eason-Hubbard & E Johnson)

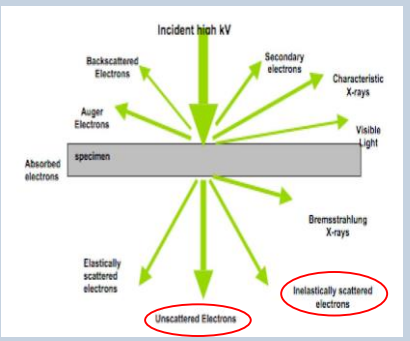
The TEM

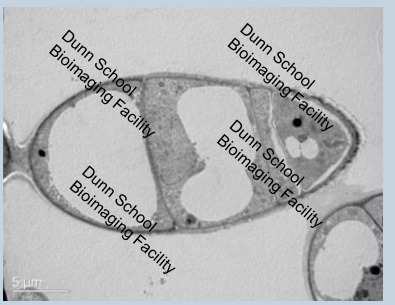




The TEM
Contrast

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



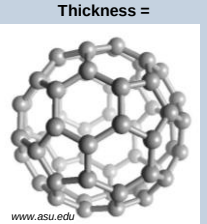


Lavender trichome, E Johnson

Specimen Preparation for TEM
Overview

- TEM specimens must be:
  - Very thin (typically 70 nm)
  - Well preserved
  - Electron dense
  - Stable in the vacuum

Thickness = **70 buckyballs** **7 million buckyballs!**



Buckyball ~1nm diameter

70 nm sections on a TEM grid

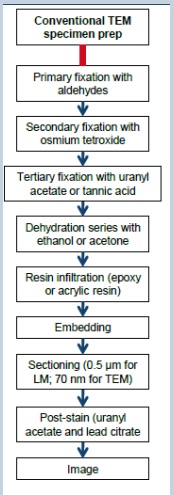
Mouse heart ~7 mm wide

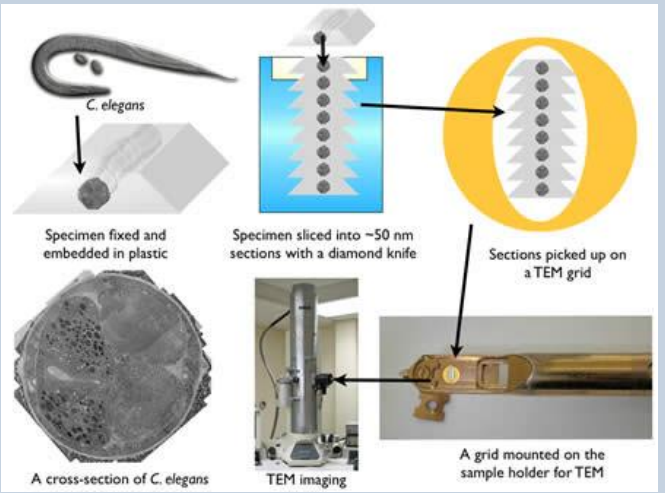
- The degree of specimen preparation for biological TEM depends on the specimen
  - Particulate samples (eg: protein and viruses) can be stained and viewed quickly
  - Cells and tissue samples require extensive preparation for TEM

Specimen Preparation for TEM

Cells & Tissue – Overview

Dunn School Bioimaging Facility

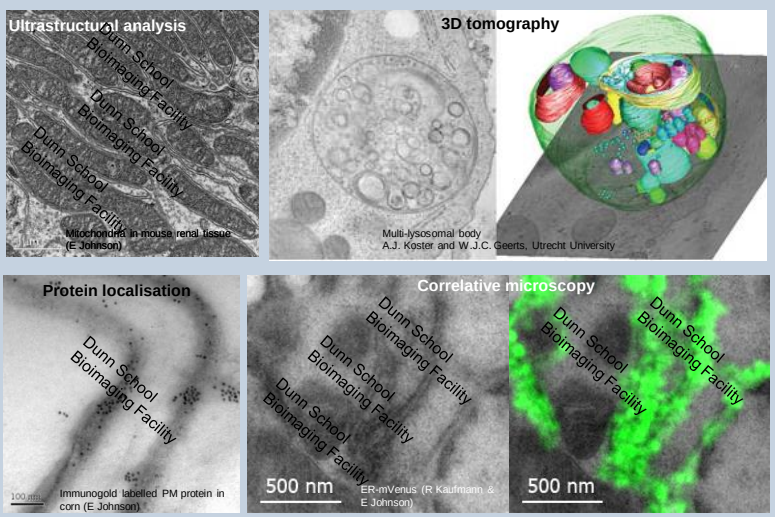




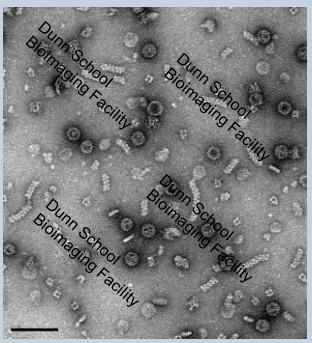
http://www.research.utah.edu/advanced-microscopy/education/electron-micro/index.html

Transmission Electron Microscopy

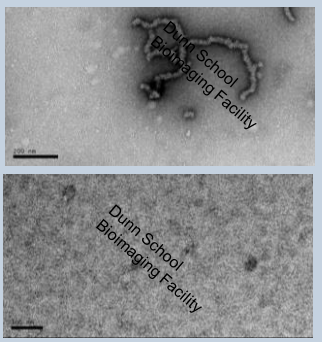
Biological Applications



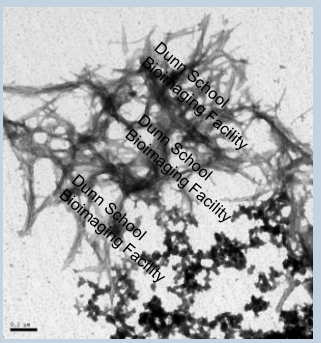
TEM Applications
Ultrastructural imaging – Particulate samples



Negatively stained virus-like particles
(Jenner Institute/E Johnson)

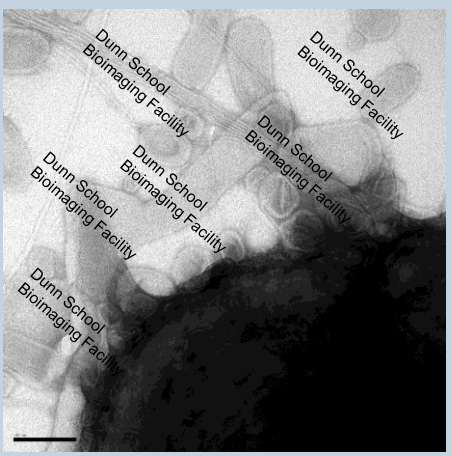


Negatively stained SAS-6 protein
WT aggregates, top; Mutant dimers, bottom
(M Cottee/E Johnson)

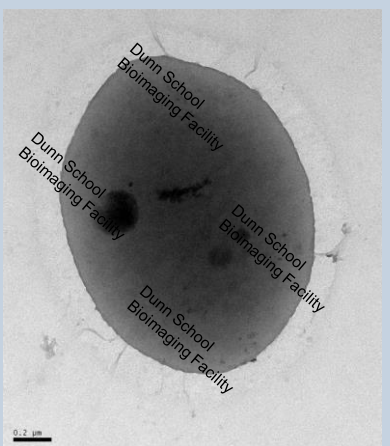


Positively stained amyloid fibrils
(E Johnson)

TEM Applications
Ultrastructural imaging – Particulate samples

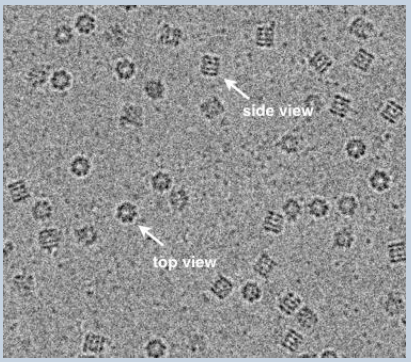


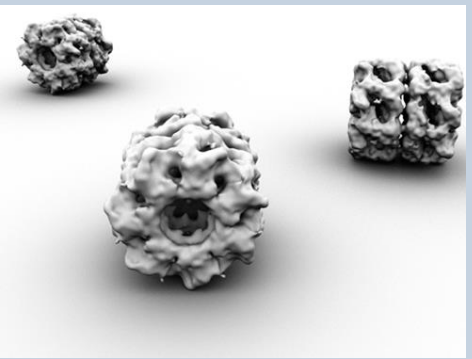
Negatively stained Neisseria meningitidis
(R Exley/EJohnson)



Rhodobacter sp.
(E Johnson/I Stuart, Biochemistry)

TEM Applications
Single particle imaging

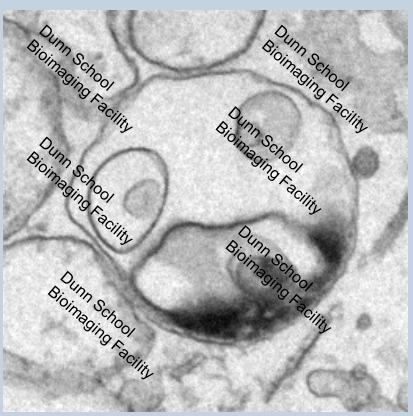




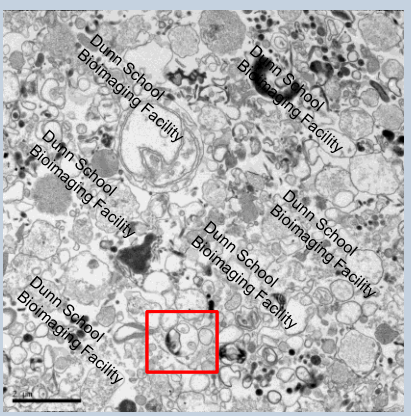
Cryo-TEM of the GroEL chaperonin (image: N Vossman), in a range of different orientations which can be averaged to reconstruct and render the complex in 3D to 1 nm resolution (image: D Nowakowski), both images from http://bsp.med.harvard.edu

Dunn School Bioimaging Facility

TEM Applications
Ultrastructural imaging – Particulate samples



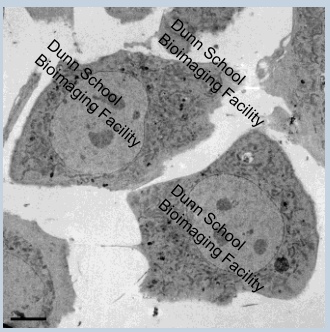
Resin embedded trypanosome flagellum isolation prep (T Benecke/E Johnson)



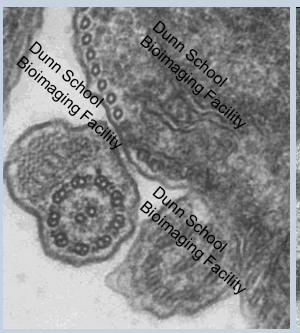
Resin embedded mitochondrial isolation prep (J Long)

Dunn School Bioimaging Facility

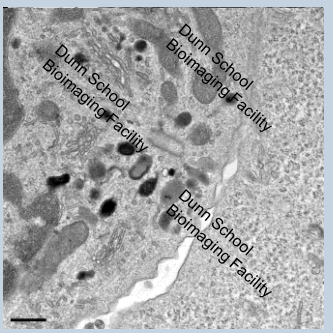
TEM Applications
Ultrastructural imaging – Cells



Mouse fibroblast cells (E Johnson)



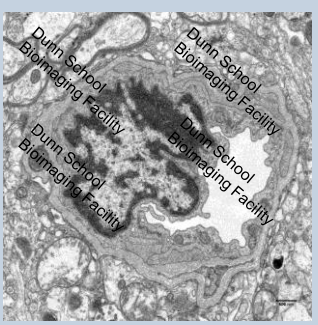
Cross-section of flagella in T. brucei (J Sunter)



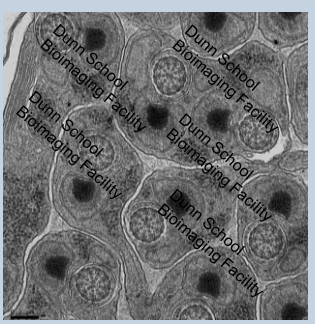
HRP labelled T-cell interacting with a melanoma cell (E Johnson)

Dunn School Bioimaging Facility

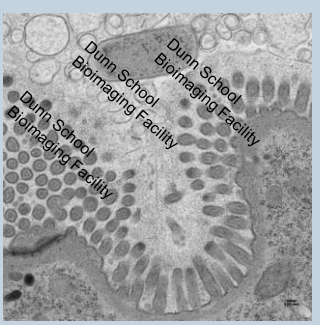
TEM Applications
Ultrastructural imaging – Tissue



Mouse blood/brain barrier (A Douglas)

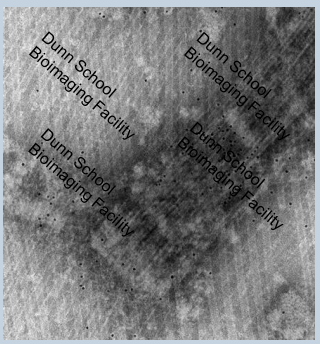


Centrioles in Drosophila spermatocytes (M Pratt)

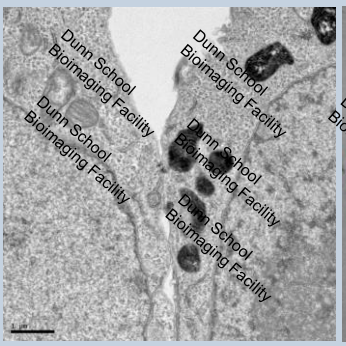


Bacterium in gut of cryo-fixed C. elegans (A Moloney/E Johnson)

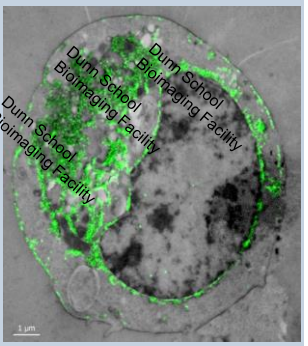
TEM Applications
Protein localisation



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



HEK cells untransfected (left) and transfected (right) with APEX protein tagged to a mitochondrial matrix protein (J Long, Dunn School)

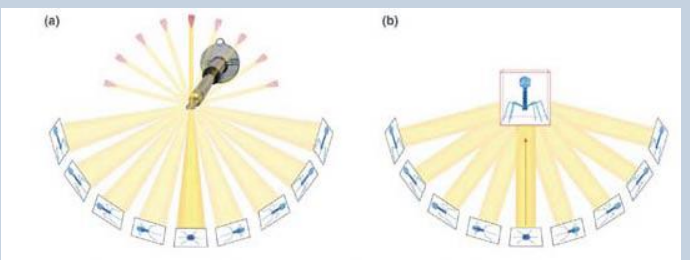


Correlative light & electron microscopy of HEK cells expressing mVenus (E Johnson & R Kaufmann, Micron)

Dunn School Bioimaging Facility

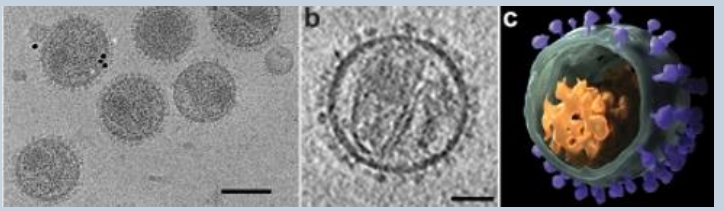
TEM Applications
Electron tomography

- Thicker sections (150-300 nm) on filmed slot grids with gold fiducial markers
- Use special tomography holder for dual axis tilting of the specimen
- Reconstruct using computer modelling (eg: IMOD)

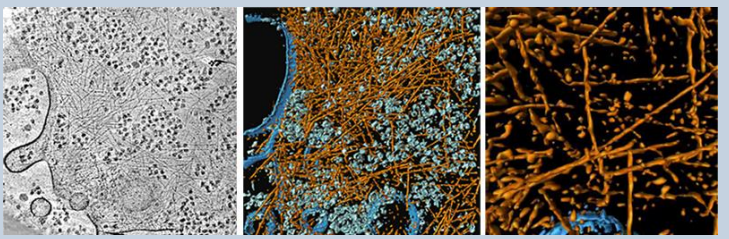


Principles of Electron Tomography. (a) A biological specimen, in this case a bacteriophage contained in an EM sample holder, can be imaged from several orientations by tilting the holder in the electron microscope. (b) Process of computed backprojection, in which each tilted view is used to reconstruct to three-dimensional information of the original structure. [McIntosh, et al. (2005) Trends Cell Biol. 15:43-51].

TEM Applications
Cryo-TEM tomography



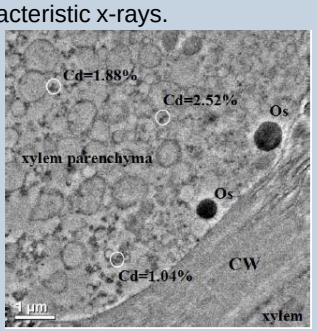
Cryo-electron tomography and modelling of trimeric SIV Env virions (White et al 2010, PLoS Pathog, 6(12): e1001249)



Cryo-electron tomography of the actin network in a slime mold (Wolfgang Baumeister lab, Max Planck Institute)

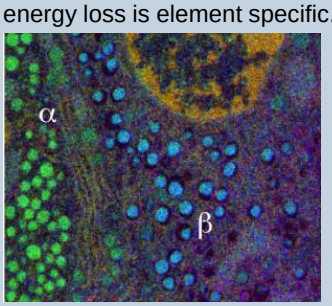
TEM applications
Chemical characterisation

Energy-dispersive x-ray spectroscopy (EDS) allows chemical characterisation of specimens, based on the emission of characteristic x-rays.



Cd Distribution in roots of Arabis paniculata
(Y. Tang, R. Qiu et al. Sun Yat-sen University. PR China)

Electron energy loss spectroscopy (EELS) measures the amount of energy lost by inelastically scattered electrons as they pass through the sample. The energy loss is element specific.

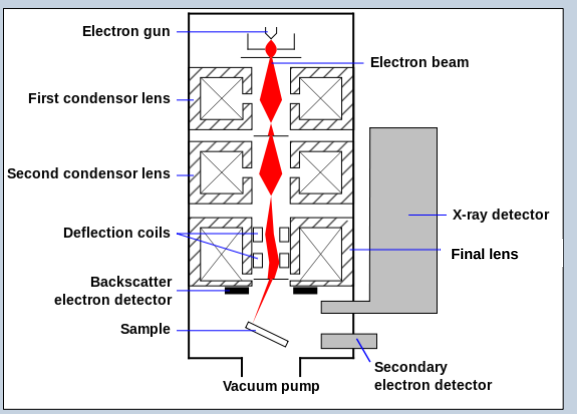


Unstained mouse pancreas with elemental contrast using EELS, NIH

Scanning Electron Microscopy (SEM)

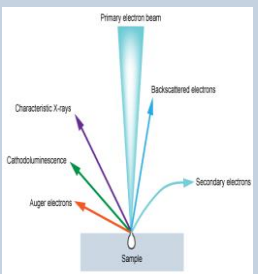
Penicillium (E Johnson)

The SEM

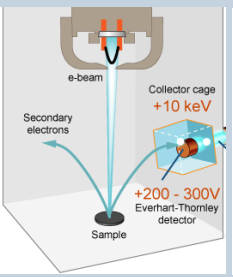


How the SEM works
Signal detection

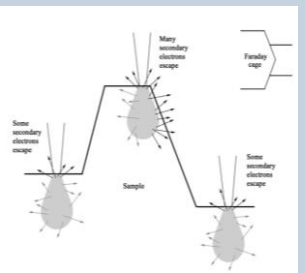
- Secondary electrons (SEs) provides surface morphology and topology information.
- SEs are captured by the Everhart-Thornley detector



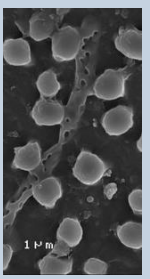
www.ammrf.org



www.ammrf.org



Dept Biological Sciences, Smith College Northampton USA



www.ammrf.org

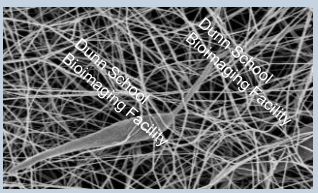
Sample Preparation for SEM
Overview

- SEM specimens must be:
  - Well preserved with no surface contamination or damage
  - Stable in the vacuum
  - Conductive
  - Composed of high atomic number elements
- The conventional preparation for SEM samples is similar to that for TEM, although the resin and sectioning steps are omitted.
- There are less size restrictions on SEM samples compared to TEM. Some samples (eg: pollen, insects) can be imaged without much sample prep at all.

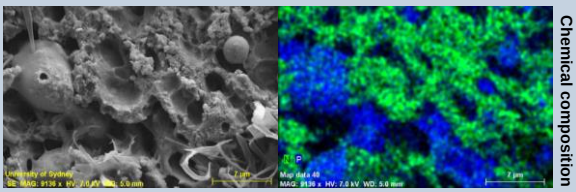
Bottom: SEM of a caterpillar (E Johnson)

Scanning Electron Microscopy
Biological Applications

Topography



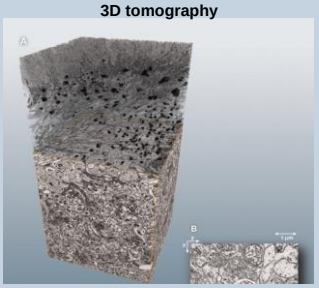
Leishmania on collagen (R Jain/E Johnson)



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

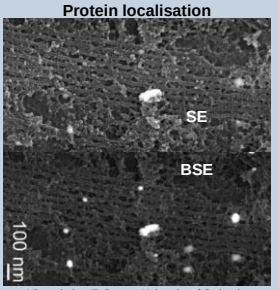
Chemical composition

3D tomography



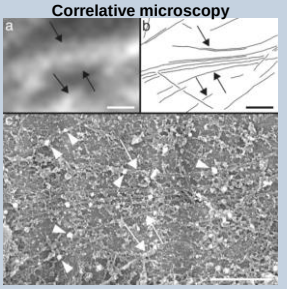
Moritz Helmstaedter, Max-Planck Institute for Medical Research

Protein localisation



Microtubules (D Barton, University of Sydney)

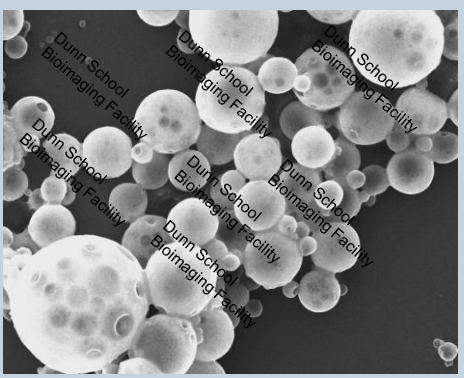
Correlative microscopy



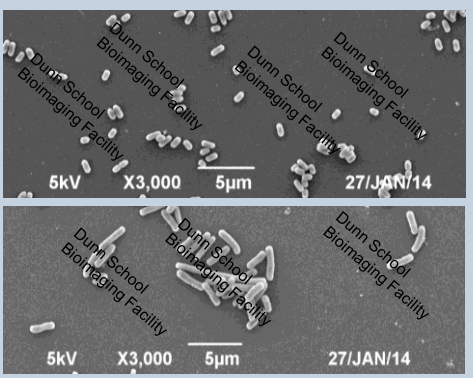
Microtubules (D Barton, University of Sydney)

Dunn School Bioimaging Facility

SEM Applications
Topography – Particulate samples



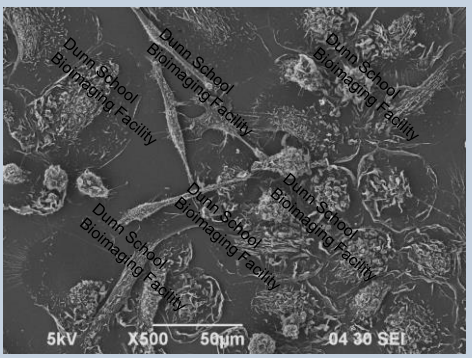
Plastic beads conjugated to vaccine particles (A Walters/E Johnson)



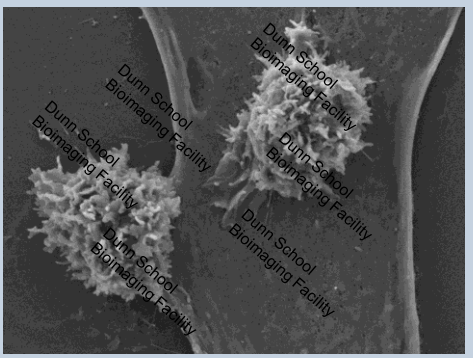
E coli (WT at top, +vector at bottom) (R Harding/E Johnson)

Dunn School Bioimaging Facility

SEM Applications
Topography – Cells



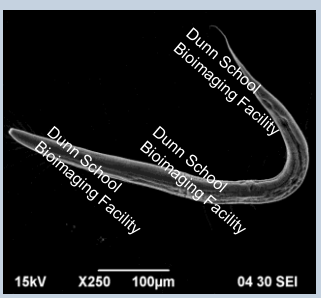
Monocytes and macrophages (B van Wilgenburg/E Johnson)



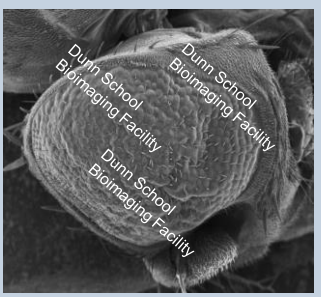
T-cells interacting with a cancer cell (E Johnson, Dunn School)

Dunn School Bioimaging Facility

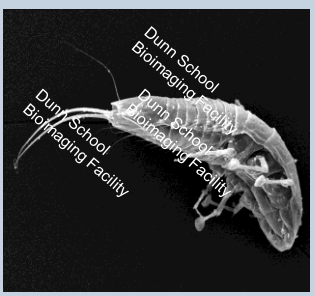
SEM Applications
Topography – Whole organisms



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



Drosophila rough eye phenotype
(M Elschami, NDCN)



Exotic arthropod (E Johnson)

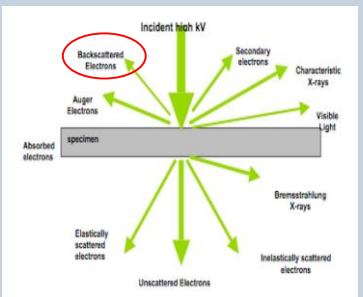
Dunn School Bioimaging Facility

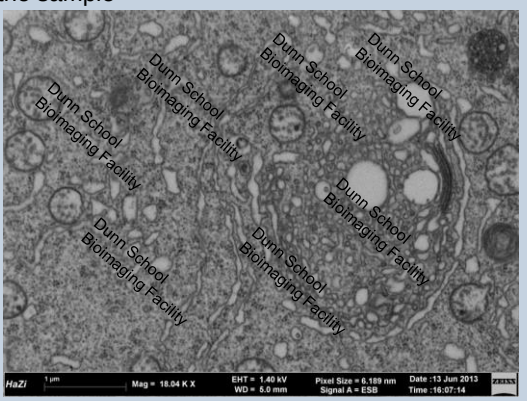
SEM Applications – 3D

Serial Block Face Sectioning – 3View

OXFORD BROOKES UNIVERSITY

SEM can be used to generate 'TEM' images by detecting backscattered electrons, beam electrons that have been elastically scattered/deflected by high atomic number elements (heavy metals) in the sample





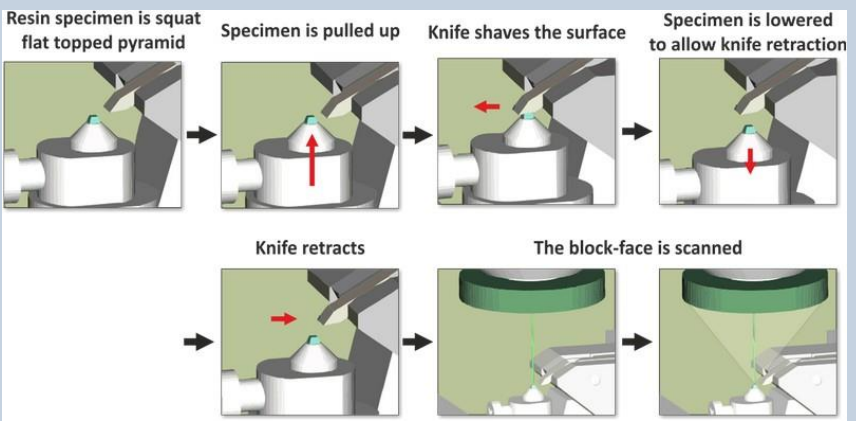
Dunn School Bioimaging Facility

SEM Applications – 3D

Serial Block Face Sectioning – 3View

OXFORD BROOKES UNIVERSITY

One method for generating a 3D high resolution image stack is to use serial block face sectioning with the Gatan 3View system

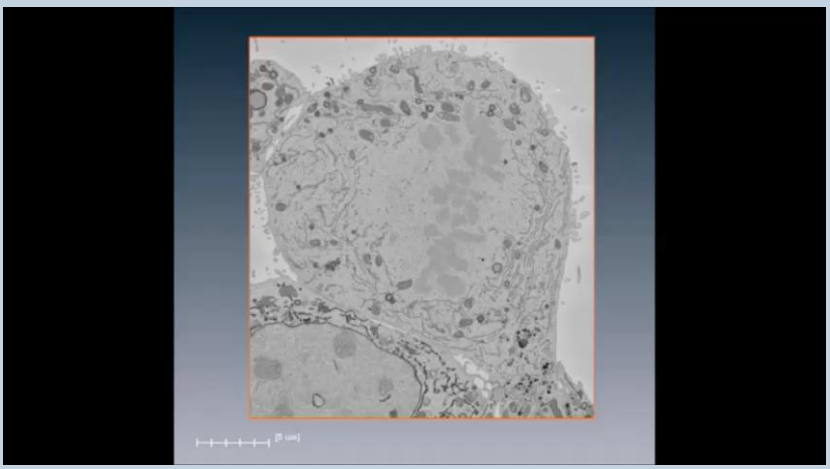


http://www.biocenter.helsinki.fi/bi/em/emu_methods_3view.html

Dunn School Bioimaging Facility

SEM Applications – 3D
Serial Block Face Sectioning – 3View

OXFORD BROOKES UNIVERSITY

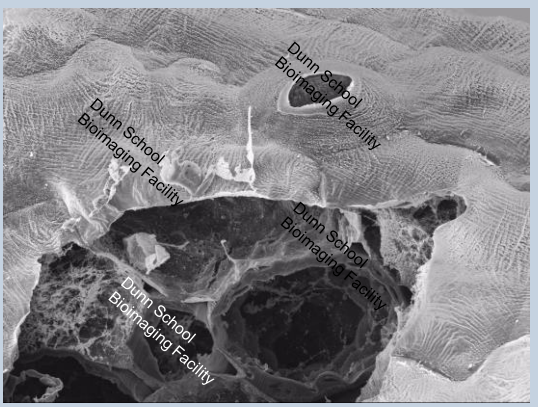


Huh-7 cell in early-metaphse with chromosomes and ER segmented and modelled - Puhka et al (2012) Mol Biol Cell 23(13)

OxfordMaterials

SEM Applications – 3D
Focussed ion beam

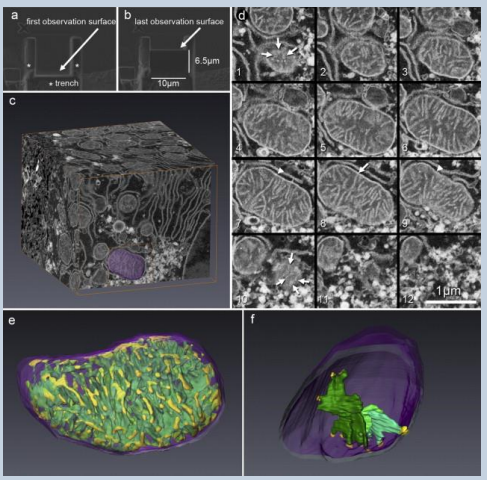
Oxford Physics



Arabidopsis leaf, Zeiss Auriga FIB (S Moody/E Johnson)

SEM Applications – 3D

Serial Block Face Sectioning – Focussed Ion beam

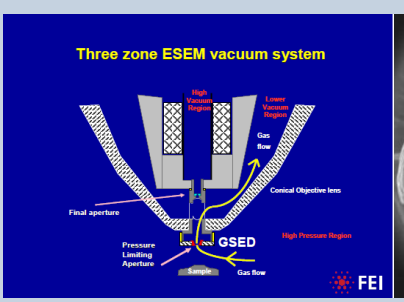


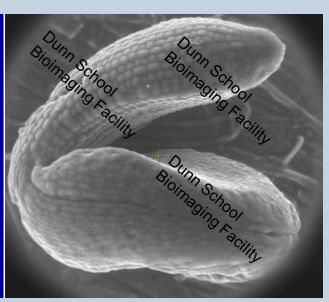
FIB serial-sectioning of resin-embedded hepatocyte (Ohta et al (2012) Micron, 43(5): 612-620)

SEM Applications – 3D

Environmental SEM

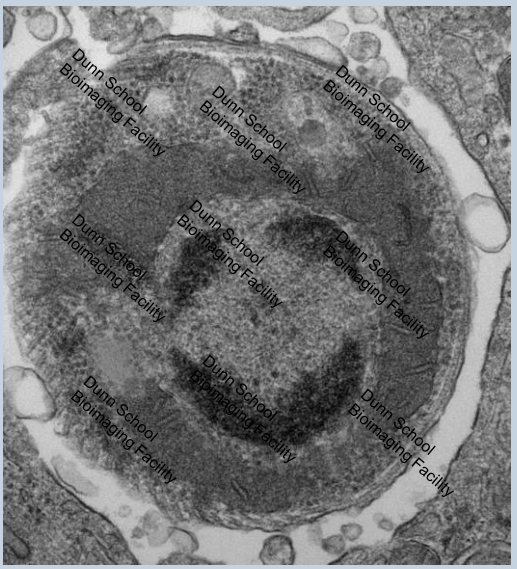
- Variable pressure and environmental SEM (ESEM) allows untreated, hydrated specimens to be imaged at high resolution.
- Utilises a specialised detector and vacuum system that enables imaging under low pressure conditions (ie; not a vacuum!).





ESEM of an Arabidopsis embryo, E Johnson

Lunch time!


Dunn School Bioimaging Facility
Dunn School Bioimaging Facility
Dunn School Bioimaging Facility
Dunn School Bioimaging Facility
Dunn School Bioimaging Facility
Dunn School Bioimaging Facility
Dunn School Bioimaging Facility
Dunn School Bioimaging Facility
Dunn School Bioimaging Facility