The Scale of Things – Nanometers and More

Things Natural

Dust mite

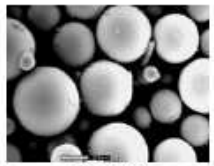
200 μm

Ant

~ 5 mm

Human hair

~ 60-120 μm wide

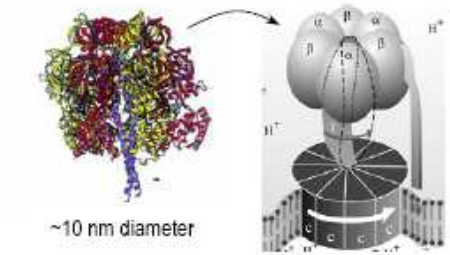


Fly ash

~ 10-20 μm

Red blood cells

(~7-8 μm)

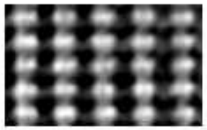


~10 nm diameter

ATP synthase

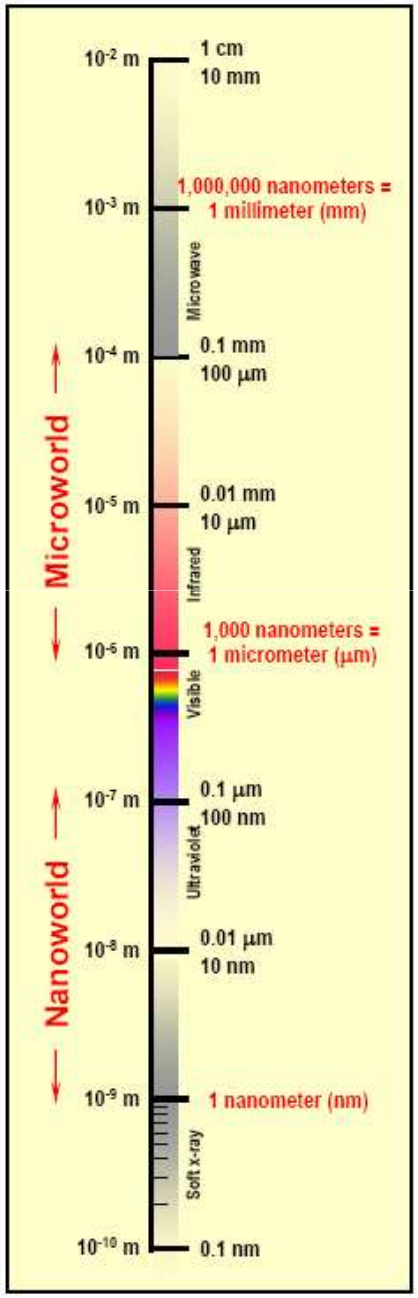
DNA

~2-1/2 nm diameter



Atoms of silicon

spacing 0.078 nm



Things Manmade

Head of a pin

1-2 mm

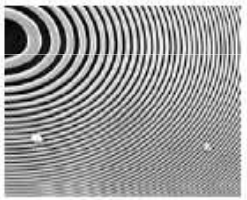
MicroElectroMechanical

(MEMS) devices

10 -100 μm wide

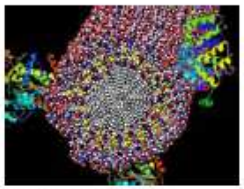
Pollen grain

Red blood cells



Zone plate x-ray "lens"

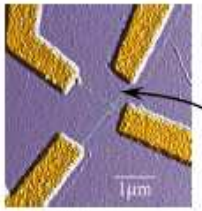
Outer ring spacing ~35 nm



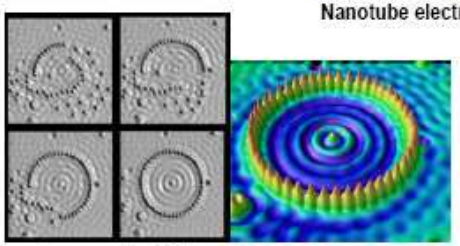
Self-assembled,

Nature-inspired structure

Many 10s of nm



Nanotube electrode

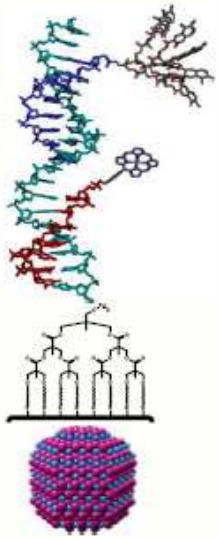


Quantum corral of 48 iron atoms on copper surface

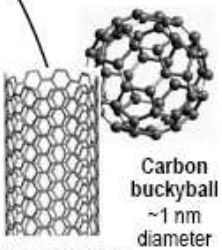
positioned one at a time with an STM tip

Corral diameter 14 nm

The Challenge



Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.



Carbon buckyball

~1 nm diameter

Carbon nanotube

~1.3 nm diameter

Office of Basic Energy Sciences
Office of Science, U.S. DOE
Version 05-26-06, pmd

Ultra structure

The electron microscope and the technique of cell fractionation may be used to study ultrastructure

- Magnification → increases the size of an object
- Resolution/resolving power → ability to distinguish between adjacent points

Table 1-10-2: Microscopes[1]

Feature	Optical microscope	Electron microscope
Radiation	Light	Electrons
Magnification	400x (max1500)	≈500 000x
Resolution	2µm	1nm / 0,001µm Electrons have a small wavelength ∴ Higher resolution
Vacuum in microscope	Absent	Present
Specimen is	- Alive or dead - Stained	- Dead (vacuum!) **Transmission microscope:** Electrons pass through internal structure of specimen **Scanning microscope:** Beams of electrons are reflected off specimens surface. Allows a three dimensional view

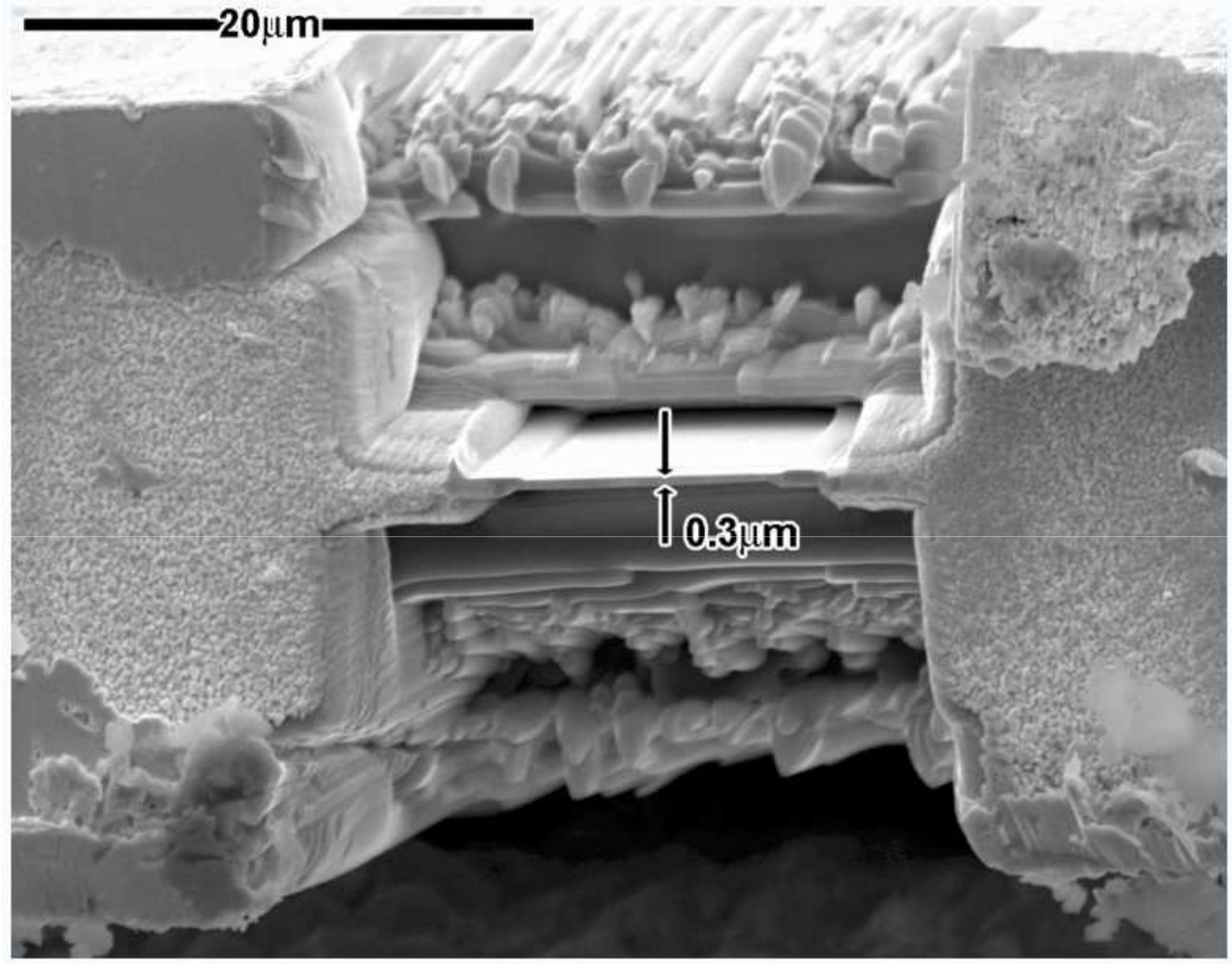
Different types of Electron Microscopy

Transmission Electron Microscope (TEM)
Scanning Electron Microscope (SEM)
Reflection Electron Microscope (REM)
Scanning Transmission Electron Microscope (STEM)

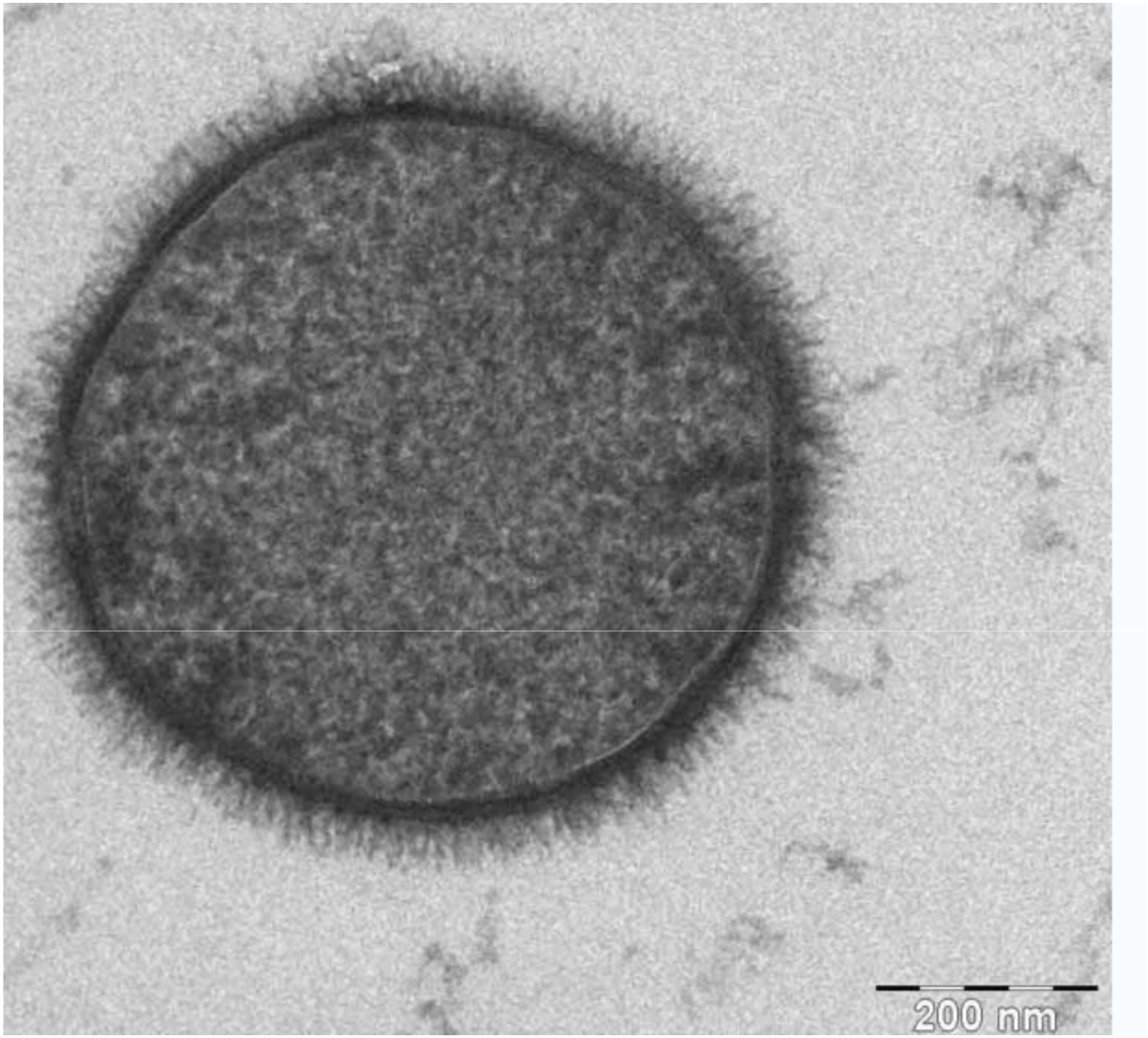
Transmission electron microscopy (**TEM**) is an imaging technique whereby a beam of electrons is transmitted through a specimen, then an image is formed, magnified and directed to appear either on a fluorescent screen or layer of photographic film (see electron microscope), or to be detected by a sensor such as a CCD camera. The first practical transmission electron microscope was built by Albert Prebus and James Hillier at the University of Toronto in 1938 using concepts developed earlier by Max Knoll and Ernst Ruska.

The TEM is used heavily in both material science/metallurgy and the biological sciences. In both cases the specimens must be very thin and able to withstand the high vacuum present inside the instrument.

For biological specimens, the maximum specimen thickness is roughly 1 micrometre. To withstand the instrument vacuum, biological specimens are typically held at liquid nitrogen temperatures after embedding in vitreous ice, or fixated using a negative staining material such as uranyl acetate or by plastic embedding. Typical biological applications include tomographic reconstructions of small cells or thin sections of larger cells and 3-D reconstructions of individual molecules via Single Particle Reconstruction.



SEM micrograph of a wide-bandgap semiconductor prepared for TEM by focused-ion-beam milling



The Bacterium Bacillus subtilis taken with a Tecnai T-12 TEM. Taken by Allon Weiner, The Weizmann Institute of Science, Rehovot, Israel. 2006.

Scanning Electron Microscope (SEM)

Unlike the TEM, where electrons of the high voltage beam form the image of the specimen, the Scanning Electron Microscope (SEM) produces images by detecting low energy secondary electrons which are emitted from the surface of the specimen due to excitation by the primary electron beam.

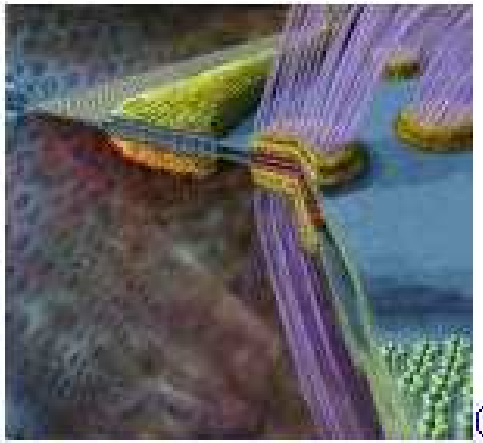
In the SEM, the electron beam is rastered across the sample, with detectors building up an image by mapping the detected signals with beam position. Generally, the TEM resolution is about an order of magnitude greater than the SEM resolution, however, because the SEM image relies on surface processes rather than transmission it is able to image bulk samples and has a much greater depth of view, and so can produce images that are a good representation of the 3D structure of the sample.

Scanning Transmission Electron Microscope (STEM)

The STEM combines the high resolution of the TEM with the beam rastering functions of the SEM, allowing a range of analytical techniques to be used that are not possible with conventional TEM.

The inner side of a typical animal cell

Generalised Animal Cell Structure Diagram



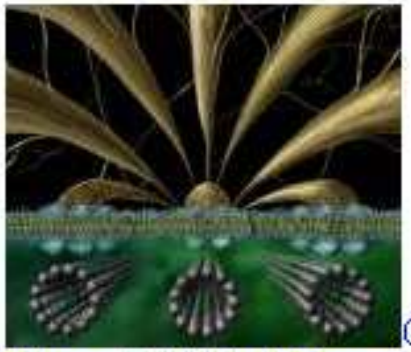
Cell Junctions Simplified diagram of tight junction, adherens junction, desmosome and gap junctions.

Generalised Animal Cell Plasma Membrane Structure Diagram

Protein Synthesis Simplified diagram of protein synthesis in the cell

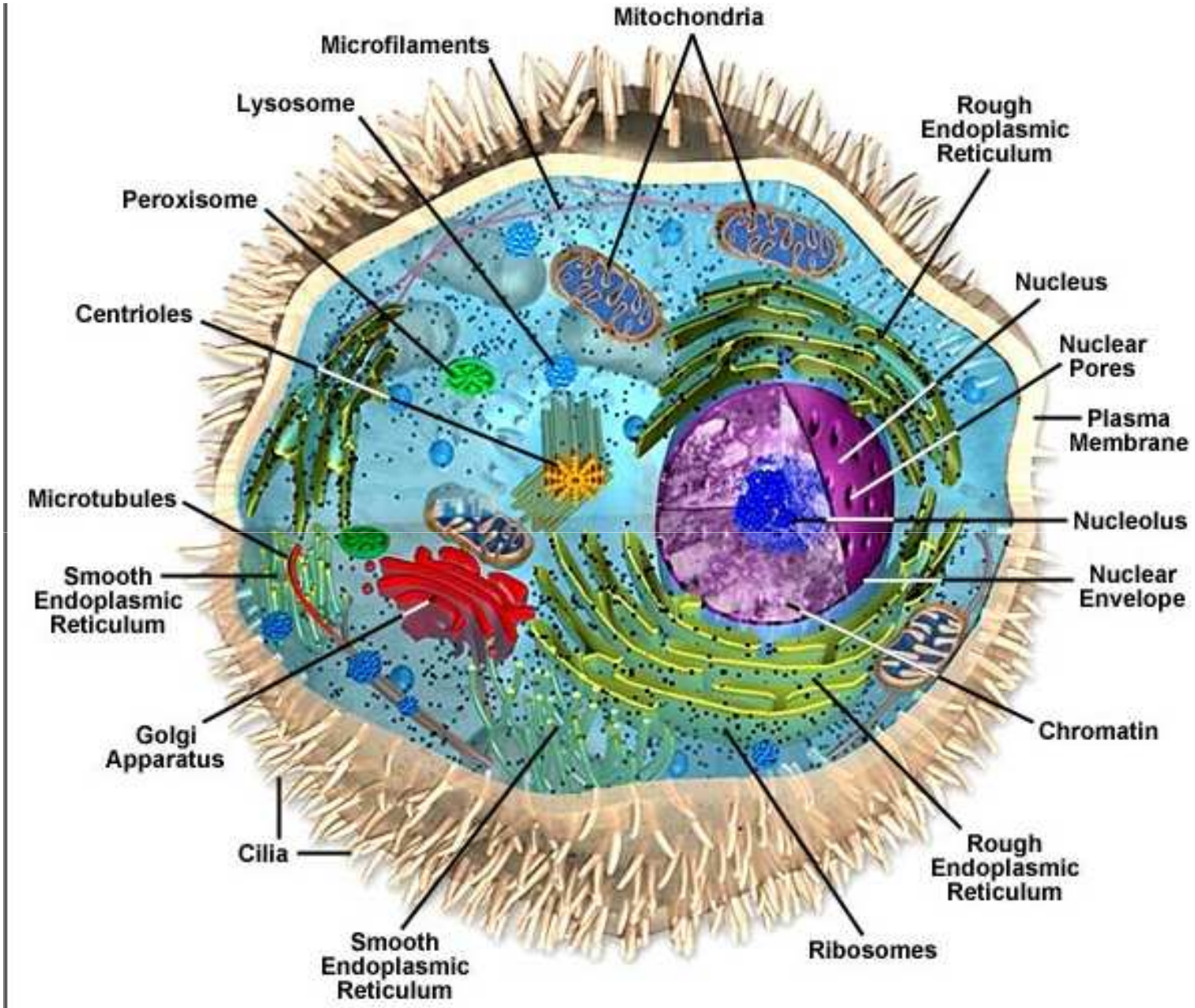
Generalised Plant Cell Structure Diagram

Plasmodesmata Diagram



Generalised Plant Cell Wall Structure Diagram

Chloroplast Diagram

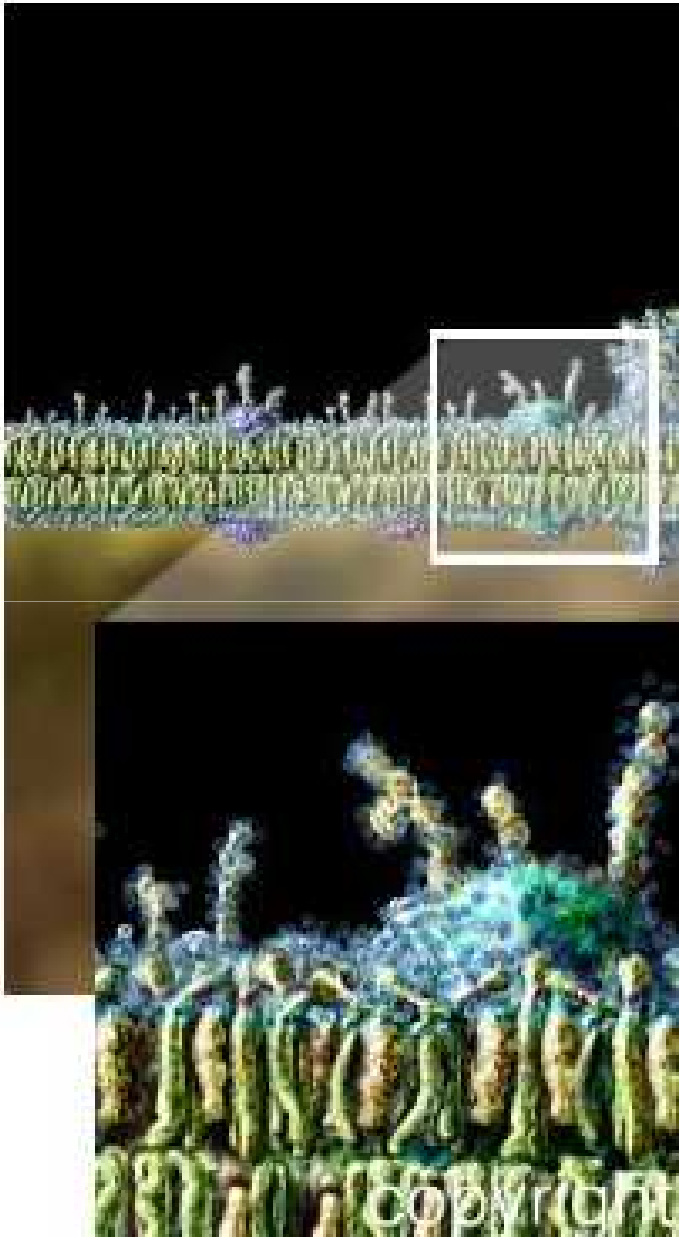

Mitochondria
Microfilaments
Lysosome
Rough Endoplasmic Reticulum
Peroxisome
Nucleus
Centrioles
Nuclear Pores
Plasma Membrane
Microtubules
Nucleolus
Smooth Endoplasmic Reticulum
Nuclear Envelope
Golgi Apparatus
Chromatin
Cilia
Rough Endoplasmic Reticulum
Smooth Endoplasmic Reticulum
Ribosomes

CELL TYPES: CANCER CELLS + RED BLOOD CELLS + FAT CELL

Generalised Cancer Cells

Red Blood Cells or Erythrocytes

Obesity: Fat Cells or Adipocytes



The cellular membrane

Materials to be viewed under an electron microscope may require processing to produce a suitable sample.

The technique required varies depending on the specimen and the analysis required:

Cryofixation – freezing a specimen so rapidly, to liquid nitrogen or even liquid helium temperatures, that the water forms vitreous (non-crystalline) ice. This preserves the specimen in a snapshot of its solution state. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy of vitreous sections (CEMOVIS), it is now possible to observe virtually any biological specimen close to its native state.

Dehydration – replacing water with organic solvents such as ethanol or acetone.

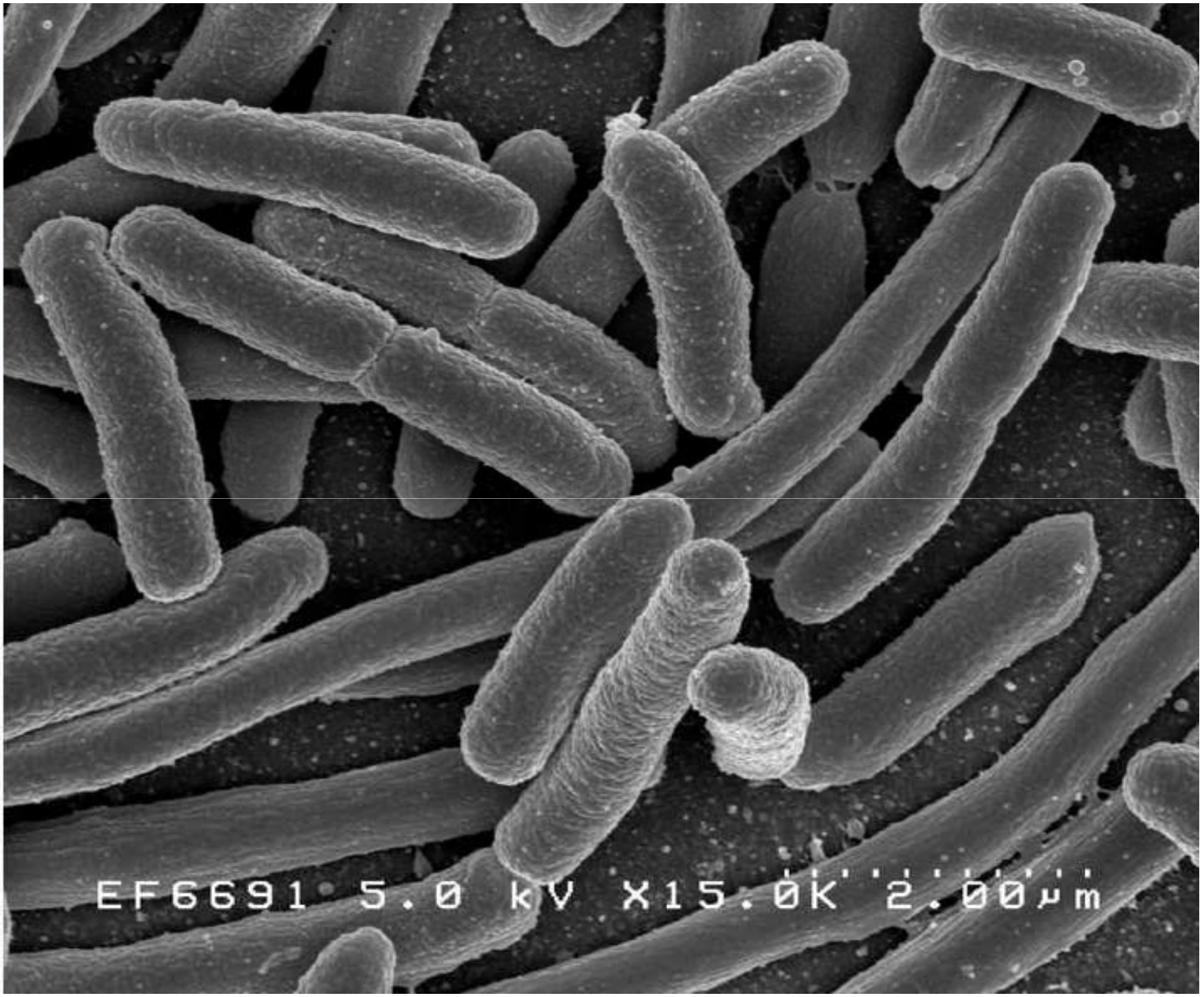
Embedding – infiltration of the tissue with a resin such as araldite or epoxy for sectioning. After this embedding process begins, the specimen must be polished to a mirror-like finish using ultra-fine abrasives. The polishing process must be done accordingly, or it may lead to scratches imposing on the image quality.
Sectioning – produces thin slices of specimen, semitransparent to electrons. These can be cut on an ultramicrotome with a diamond knife to produce very thin slices. Glass knives are also used because they can be made in the lab and are much cheaper.

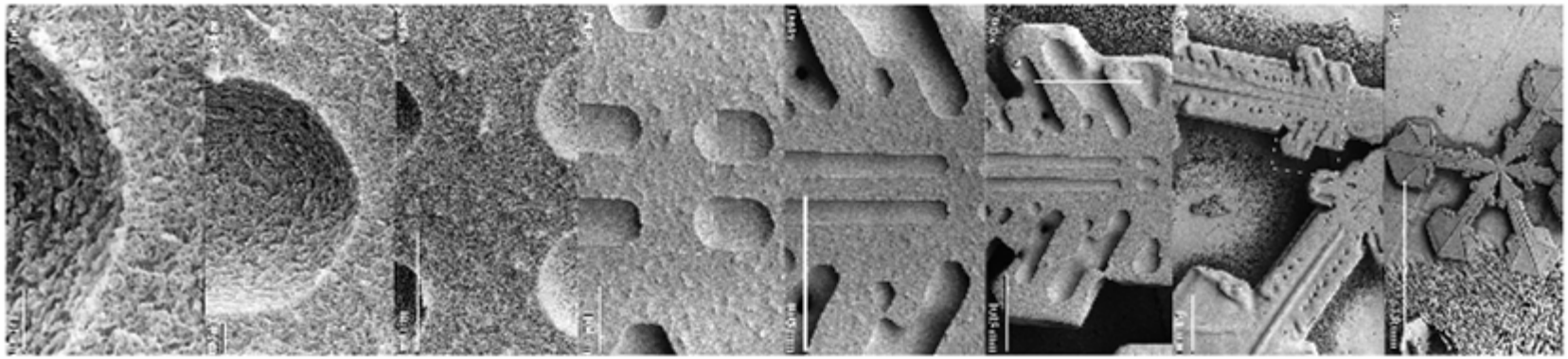
Staining – uses heavy metals such as lead, uranium or tungsten to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). In biology, specimens are usually stained "en bloc" before embedding and also later stained directly after sectioning by brief exposure to aqueous (or alcoholic) solutions of the heavy metal stains.

Freeze-fracture or freeze-etch – a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The fresh tissue or cell suspension is frozen rapidly (cryofixed), then fractured by simply breaking or by using a microtome while maintained at liquid nitrogen temperature. The cold fractured surface (sometimes "etched" by increasing the temperature to about -100℃ for several minutes to let some ice sublime) is then shadowed with evaporated platinum or gold at an average angle of 45° in a high vacuum evaporator. A second coat of carbon, evaporated perpendicular to the average surface plane is often performed to improve stability of the replica coating. The specimen is returned to room temperature and pressure, then the extremely fragile "pre-shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The still-floating replica is thoroughly washed from residual chemicals, carefully fished up on EM grids, dried then viewed in the TEM.

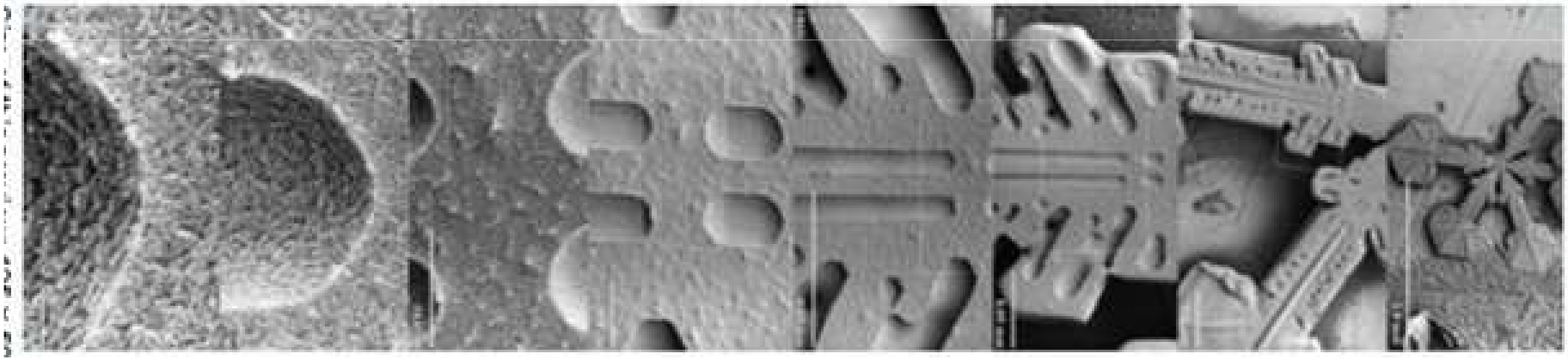
Ion Beam Milling – thins samples until they are transparent to electrons by firing ions (typically argon) at the surface from an angle and sputtering material from the surface. A subclass of this is Focused ion beam milling, where gallium ions are used to produce an electron transparent membrane in a specific region of the sample, for example through a device within a microprocessor. Ion beam milling may also be used for cross-section polishing prior to SEM analysis of materials that are difficult to prepare using mechanical polishing.

Conductive Coating – An ultrathin coating of electrically-conducting material, deposited either by high vacuum evaporation or by low vacuum sputter coating of the sample. This is done to prevent the accumulation of static electric fields at the specimen due to the electron irradiation required during imaging. Such coatings include gold, gold/palladium, platinum, tungsten, graphite etc. and are especially important for the study of specimens with the scanning electron microscope. Another reason for coating, even when there is more than enough conductivity, is to improve contrast, a situation more common with the operation of a FESEM (field emission SEM). When an osmium coater is used, a layer far thinner than would be possible with any of the previously mentioned sputtered coatings is possible


EF6691 5.0 kV X15.0K 2.00µm



(900 × 3994 pixel)



Biology Brookhaven National Laboratory

Scanning Transmission Electron Microscope

An Outside User Facility at the Biology Department

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U.S. Department of Energy, BER

Facility

STEM is a custom-built electron microscope optimized for imaging unstained biological molecules with minimal radiation damage. The group at Brookhaven operates STEM as a User Facility with partial DOE and partial fee-for-service support.

Applications

STEM mass measurements can:

1. Determine stoichiometry and homogeneity of a complex
2. Compare modified or re-assmebled vs native structures
3. Determine filament symmetry from mass per unit length.

In addition, STEM is the best instrument for visualizing small metal clusters used as heavy aom labels.

Video

The role of the STEM and cryo-EM in structural biology is summarized by Joe Wall in this 3:30 minute STEM Video clip viewable with Real Player.

Scanning Transmission Electron Microscopy Facility
STEM Operating Parameters

BNL STEM 1

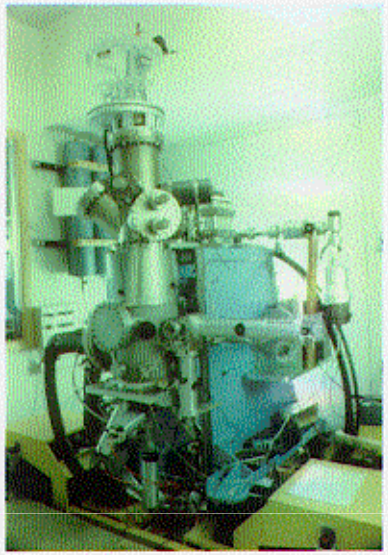
V0	40 keV
Probe	0.25 nm
Beam Intensity	10^3 el/pixel
Dark Field	15 - 40 mR or 40 - 200 mR
Specimens on X,Y Stage	6 Samples in Vacuum at 20° or -180°C
Data Aquisition	Windows PC

Probe

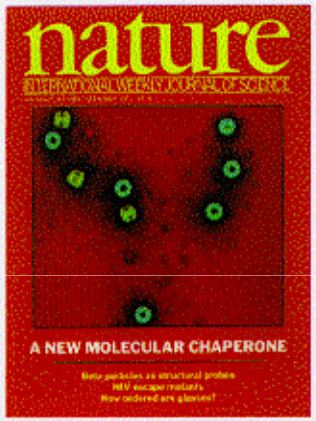
The STEM is operated at 40 keV with a probe focussed to 0.25 nm. The sample is maintained at -150°C to eliminate contamination and to reduce mass loss.

Scanning Transmission Electron Microscope Images Molecular Assemblies

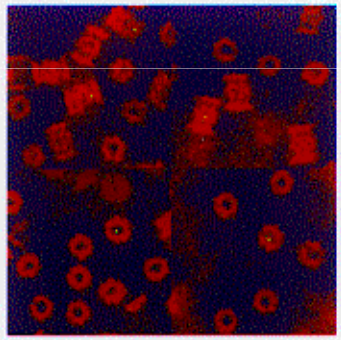
Many of the human cellular functions are carried out efficiently by large molecular complexes. These organelles are assembled from proteins, or proteins and nucleic acid, and are too small for observation in the light microscope and too large, too flexible, and too few in numbers for x–ray crystallography.



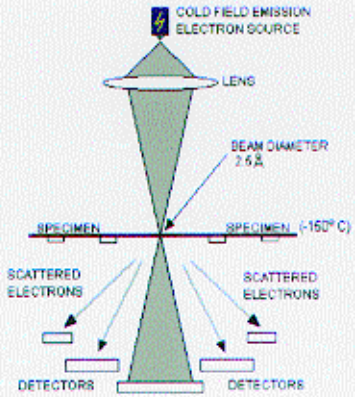
The Scanning Transmission Electron Microscope (STEM) is ideally suited to reveal architecture and function of macromolecular machines. Its low–dose electron beam yields a quantitative image of mass distribution in biomolecules, and it provides a precise mapping of heavy atom labels as well as elemental distributions.



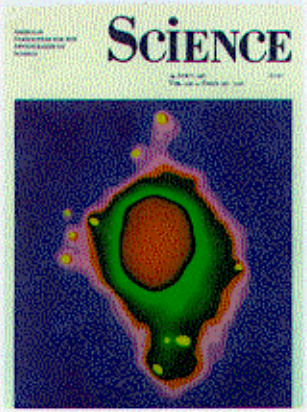
Molecular Chaperones
assist in the folding of nascent proteins. TF55 was discovered to consist of two stacked rings each containing 9 identical proteins.



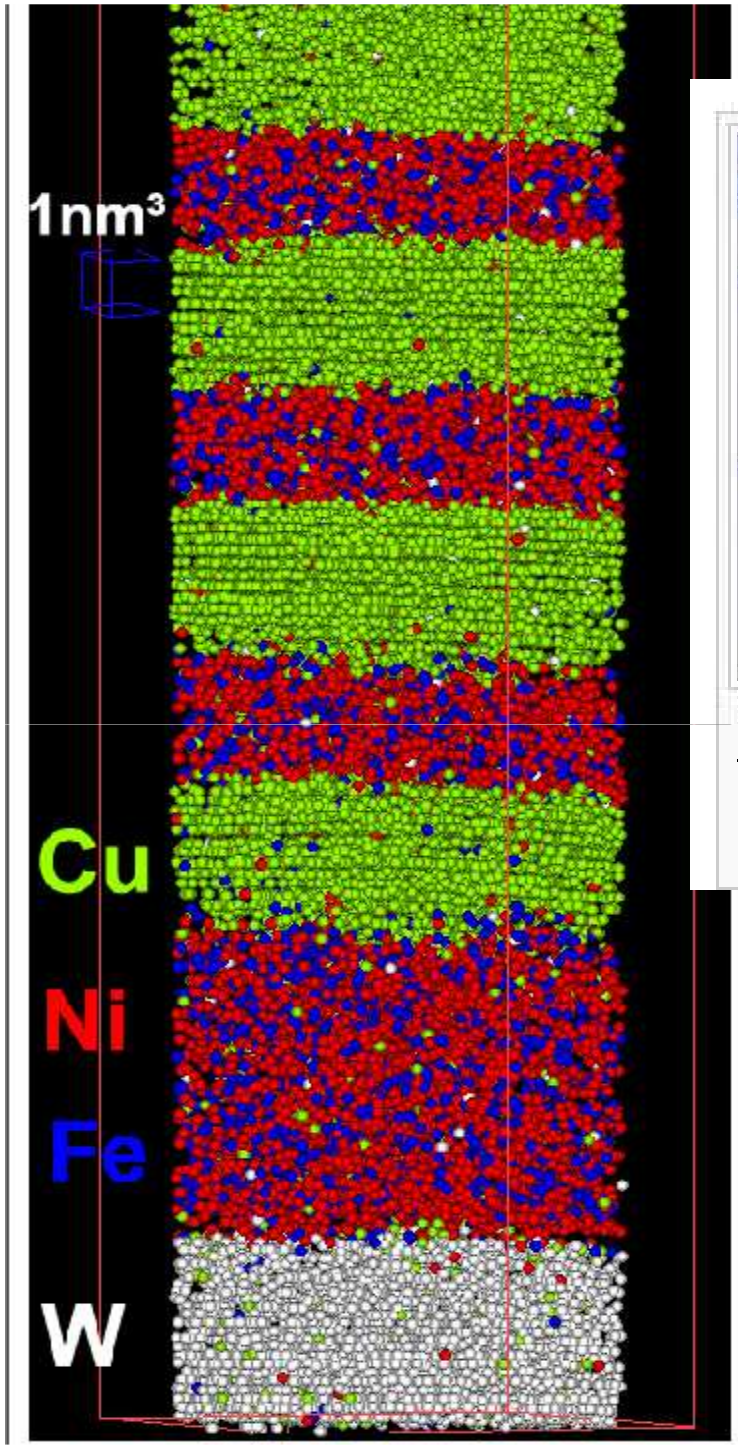
Alzheimer's Disease
results in elevated amyloid beta proteins in the brain. These images show gold labeled amyloid (yellow dots) to interact with the inside of proteasomes (red rings), which degrade proteins.

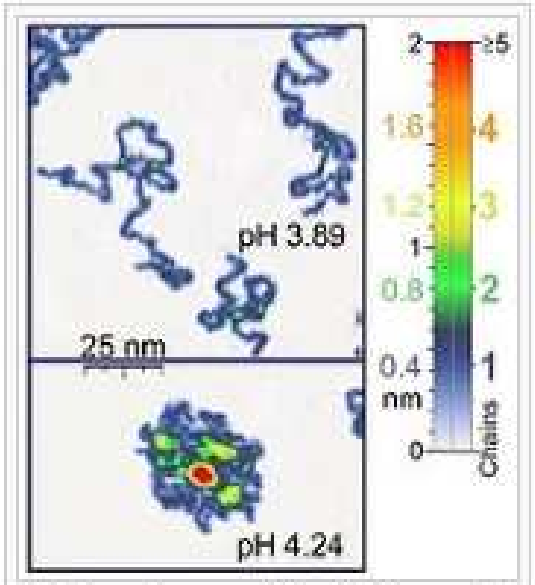


STEM Diagram
The electron beam is focused on the specimen. Its image is collected point by point in a raster scan. Wide–angle detectors are deployed for mass mapping and a spectrometer can be included for elemental analysis at each raster point.

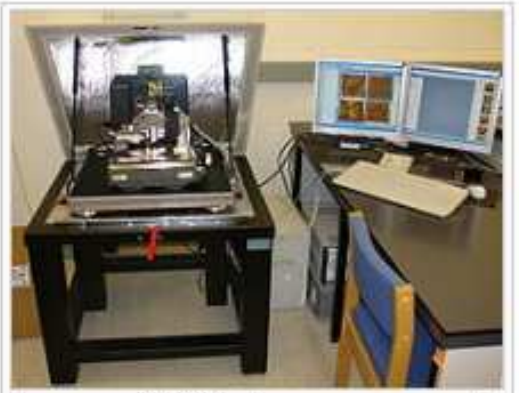


Gold–labeled Antibodies
Gold clusters (yellow) when bound to antibodies provide a powerful probe to locate antibodies and sites of immune reaction. The image shows a ferritin molecule loaded with iron (red central mass) decorated with gold labelled antibodies.

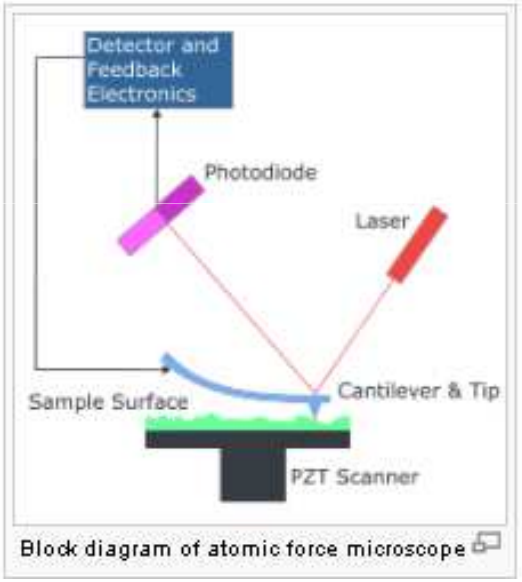




Single polymer chains (0.4 nm thick) recorded in a tapping mode under aqueous media with different pH.[5]



A commercial AFM setup



Block diagram of atomic force microscope

Atomic force microscopy