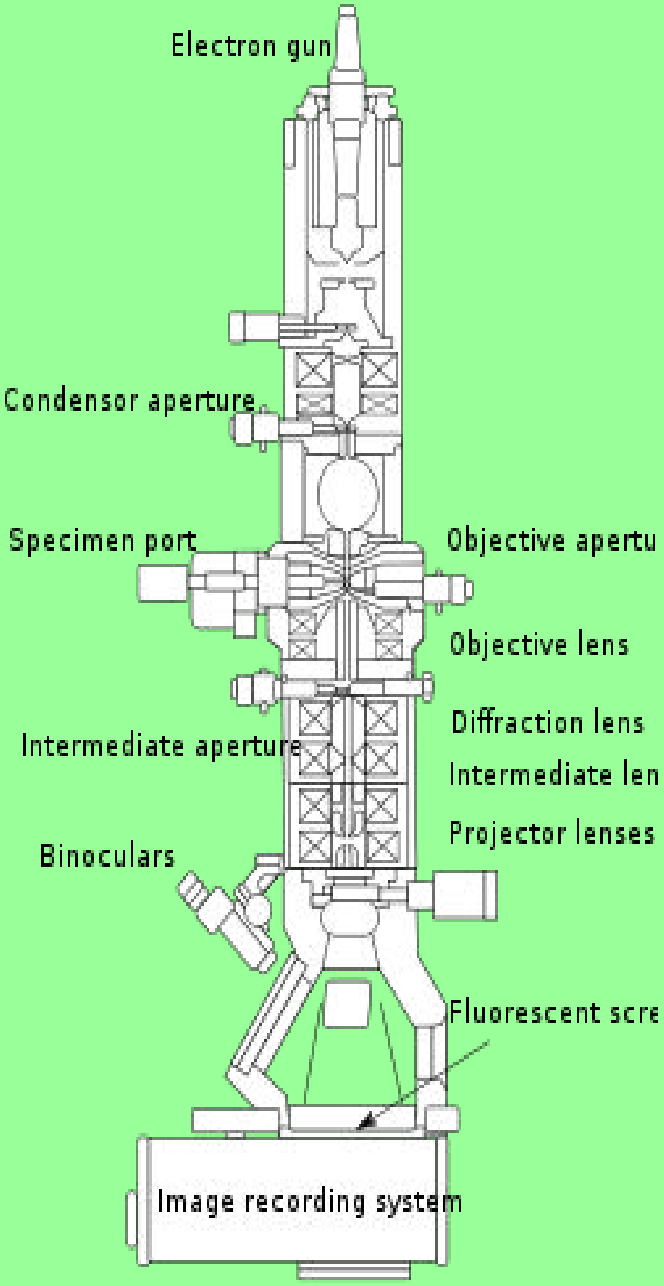
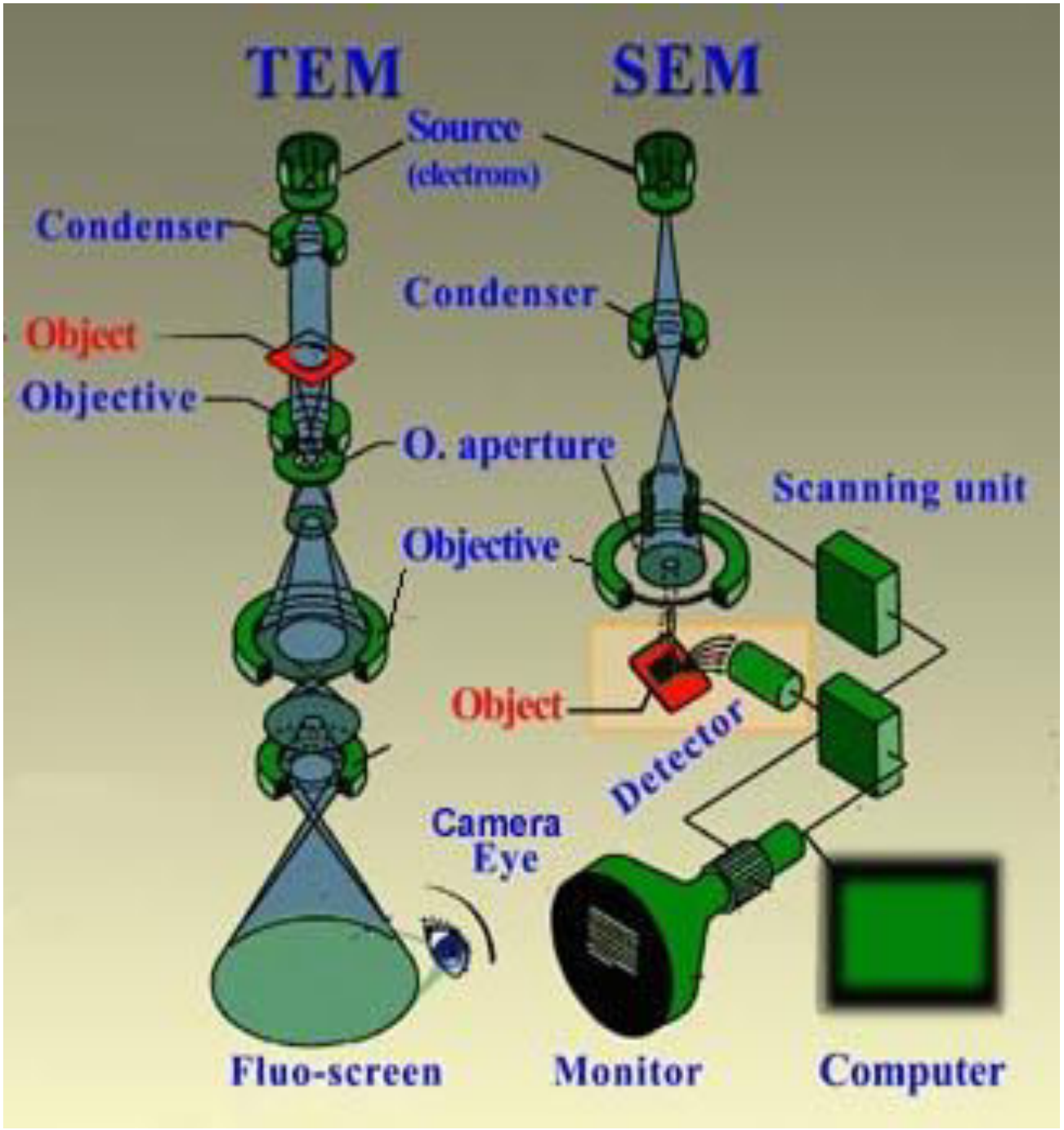
TRANSIMISSION ELECTRON MICROSCOPE



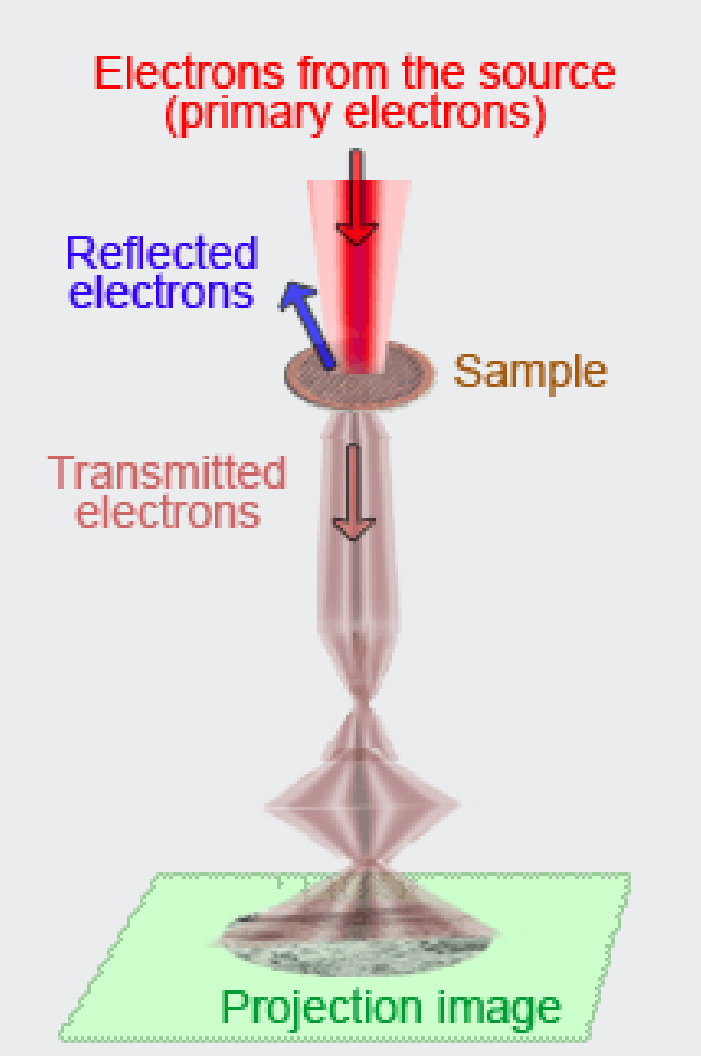
Transmission electron microscopy (**TEM**) is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer ofphotographic film, or to be detected by a sensor such as a CCD camera.

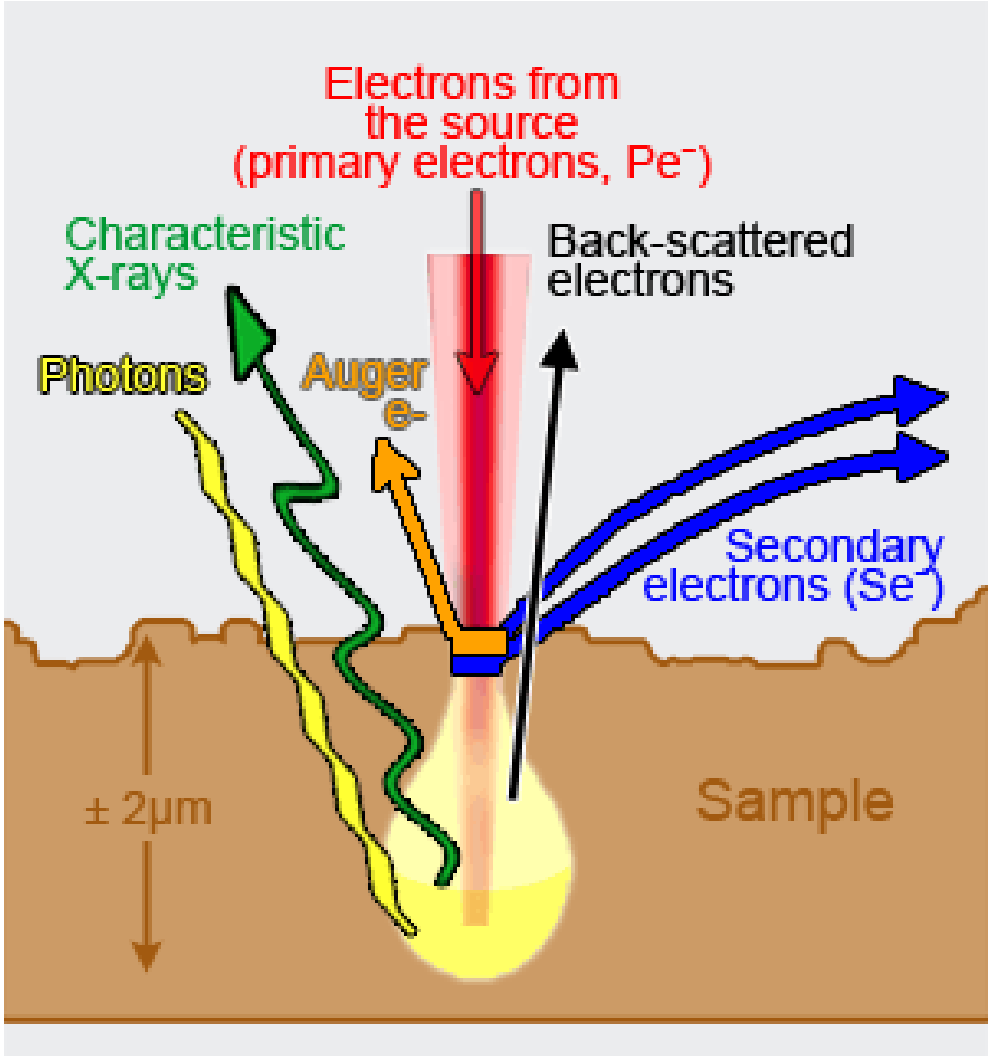
Electron microscopy (EM) is basically utilized to study structures which are unvisible to the naked eye, or are too small to be well revealed with a light microscope.

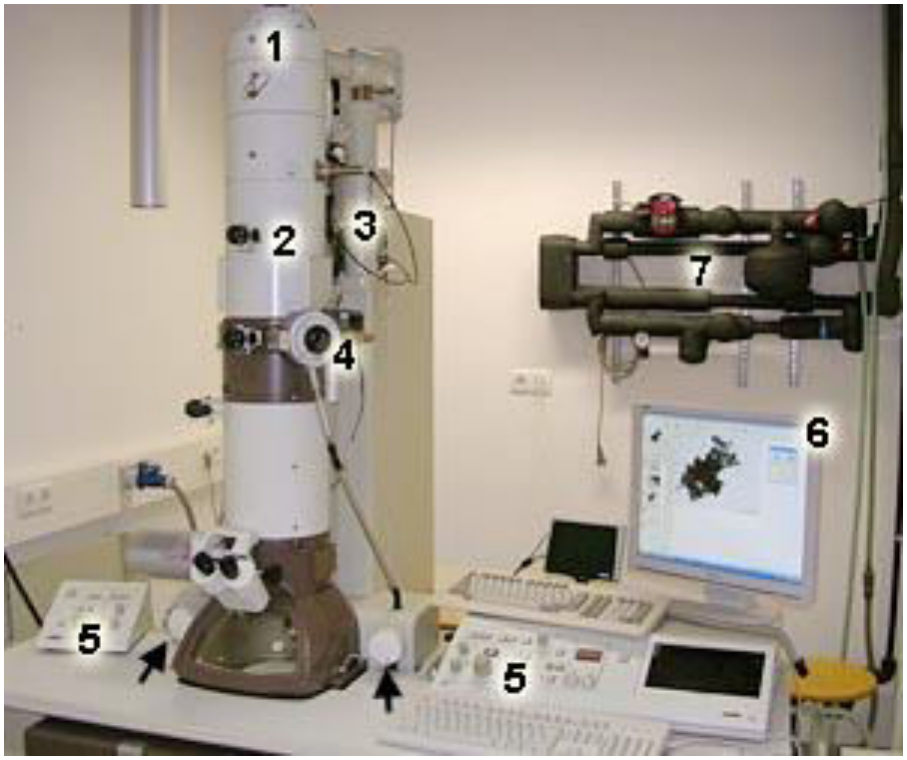
The two main types of EM techniques are
Transmission Electron Microscopy (TEM)
and
Scanning Electron Microscopy (SEM).


TEM
SEM
Source
(electrons)
Condenser
Condenser
Object
Objective
O. aperture
Scanning unit
Objective
Object
Detector
Camera
Eye
Fluo-screen
Monitor
Computer

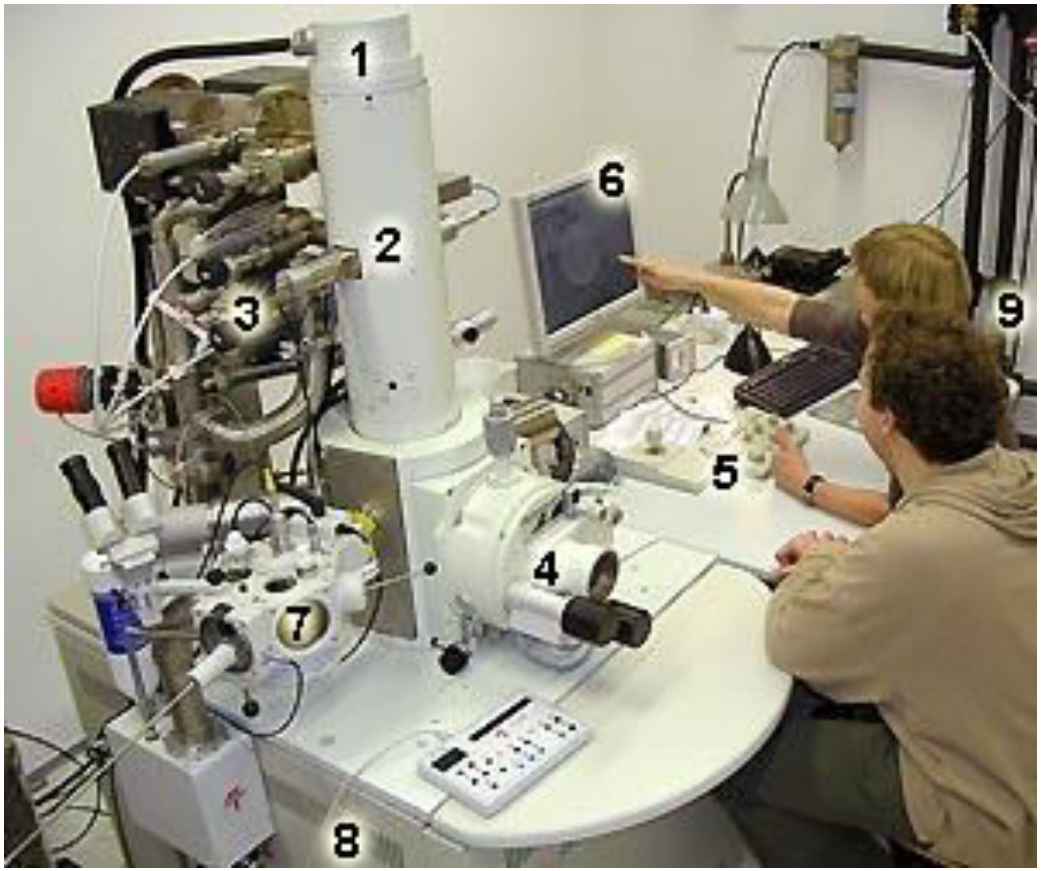
TEM - SEM


Electrons from the source
(primary electrons)
Reflected
electrons
Sample
Transmitted
electrons
Projection image


Electrons from
the source
(primary electrons, Pe⁻)
Characteristic
X-rays
Back-scattered
electrons
Photons
Auger
e-
Secondary
electrons (Se⁻)
± 2µm
Sample



1: Electron cannon i**Transmission Electron Microscope (TEM)** n the upper part of the column. 2 Electro-magnetic lenses to direct and focus the electron beam inside the column. 3: Vacuum pumps system. 4: Opening to insert a grid with samples into the high-vacuum chamber for observation. 5: Operation panels (left for alignment; right for magnification and focussing; arrows for positioning the object inside the chamber). 6: Screen for menu and image display. 7: Water supply to cool the instrument**.**



Scanning Electron Microscope (SEM)

1: Electron cannon in the upper part of the column (here a so-called field-emission source). 2 Electro-magnetic lenses to direct and focus the electron beam inside the column. 3: Vacuum pumps system. 4: Opening to insert the object into the high-vacuum observation chamber in conventional SEM mode. 5: Operation panel with focus, alignment and magnification tools and a joystick for positioning of the sample. 6: Screen for menu and image display. 7: Cryo-unit to prepare (break, coat and sublimate) frozen material before insertion in the observation chamber in Cryo-SEM mode. 8: Electronics stored in cupboards under the desk. 9: Technicians Mieke Wolters-Arts and Geert-Jan Janssen discussing a view.

Cryo-electron microscopy

Several difficulties are involved in imaging small organic objects such as proteins by TEM or SEM: many organic samples may not tolerate a bombardment of electrons, or removing them from their natural surrounding may destroy them.

Cryo-electron microscopy

The technique of cryo-electron microscopy overcomes these difficulties by quick-freezing the sample in, e.g., liquid ethane at -150 degr. celsius which forms a vitreous layer of ice around the sample. This layer decreases the damages inflicted by the electrons, and because the sample is not dehydrated it will not undergo significant conformational changes when transfered to the microscope.

John E. Heuser (born August 29, 1942)

Professor of Biophysics

Department of
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Physiology
Washington University
School of Medicine

Heuser explains what is done in his laboratory:

"Electron microscopic visualization of everything from whole cells to individual molecules is the work of this laboratory. Special emphasis is given to developing new methods of sample preparation that will achieve a more natural, life-like appearance of samples in the microscope.

To accomplish this, we have developed what is now called the "quick-freeze, deep-etch" technique for electron microscopy.

Currently, we use "quick-freezing" to capture several different cellular processes that are unusually fleeting, including membrane budding and fusion, synaptic vesicle discharge during neural transmission, movement of cilia and flagella on vertebrate andprotozoal cells, and muscle contraction. In each case, our aim has been to visualize the underlying molecular mechanisms occurring.

We also use "deep-etching" to visualize molecules adsorbed to inert substrates in order to study mechanisms ofmacromolecular assembly and disassembly in various processes, including remodeling of cytoskeletons, clathrin-mediated endocytosis, cell-to-cell recognition, and the formation of extracellular matrices."

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 (cryofixation), 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 °C 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 free from residual chemicals, carefully fished up on fine grids, dried then viewed in the TEM.

An insect coated in gold for viewing with a scanning electron microscope

PREPARATION OF MATERIALS FOR ELECTRON MICROSCOPE

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: