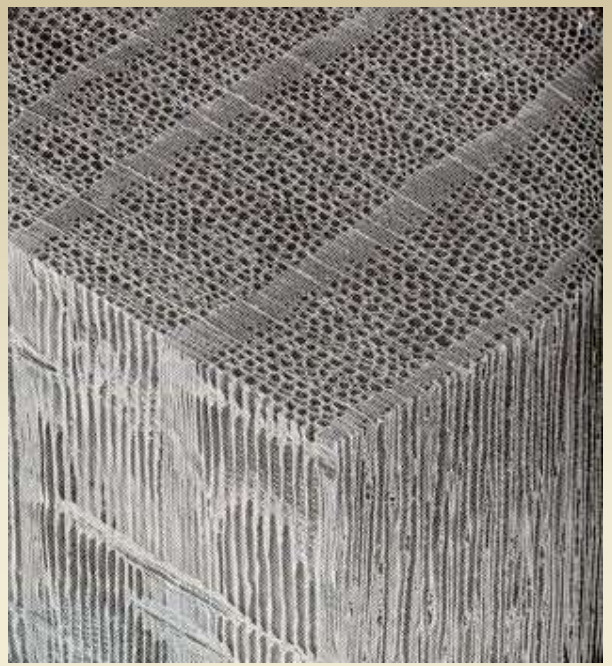
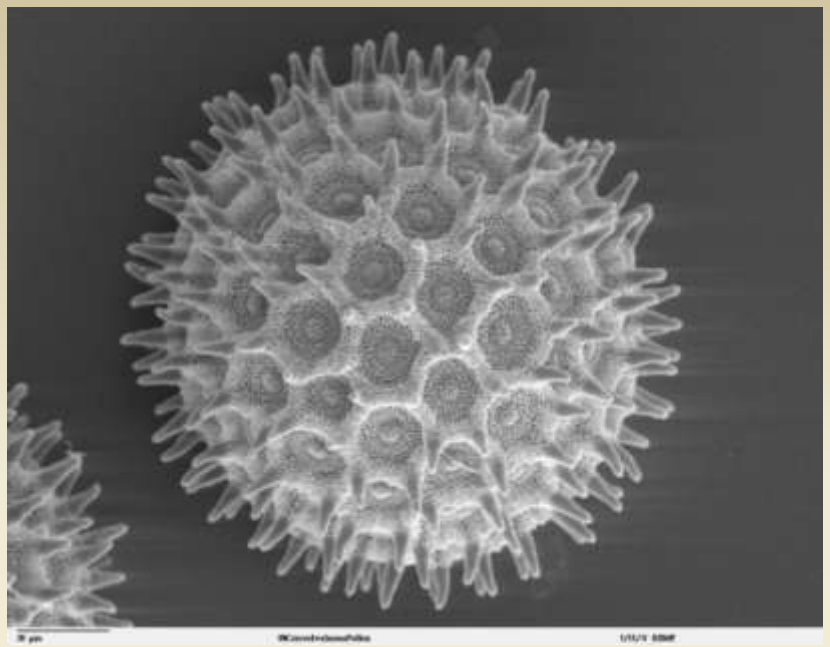
Scanning Electron Microscopy - MBG

JEOL 5000 Neoscope





Light Microscope

Resolution - The best resolution of the light microscope is 0.2 µm or 200 nm.

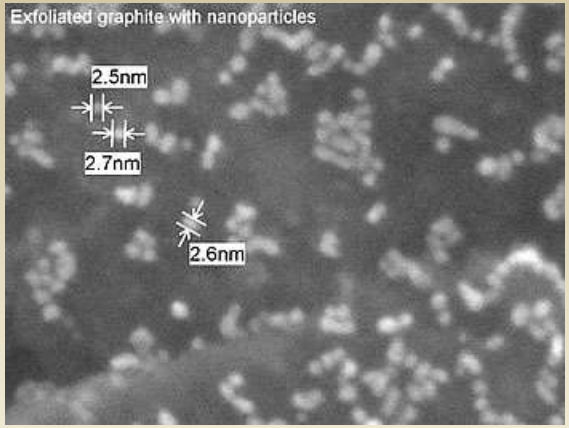
Magnification - to the magnification, in addition to the resolution. The highest useful magnification of an objective lens in the light microscope is 100 times. When you look through the eyepiece this often adds another 10 times magnification. Thus, the highest magnification of the light microscope is 1000 times.

SEM

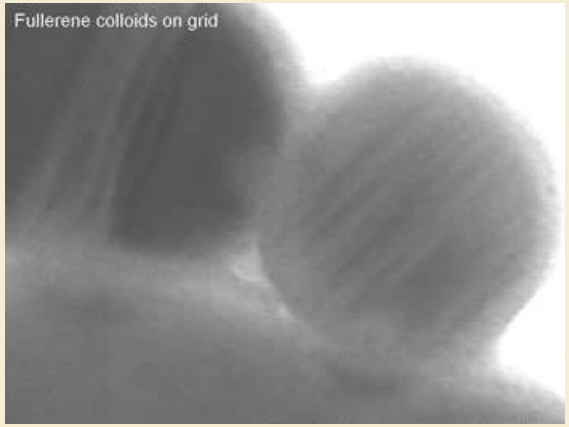
Resolution -The resolution of our SEM (Neoscope) is 3 to 6 nm. That's almost **100 times better than the light microscope**. This is why we can see so much more detail with electron microscopes than light microscopes.

Magnification - The highest magnification of our scanning electron microscope is 20,000 times! That's 20 times more than the light microscope. They go up to 1,000,000X.

JEOL SEM from 2008



Exfoliated graphite with nanoparticles, 1,000,000X



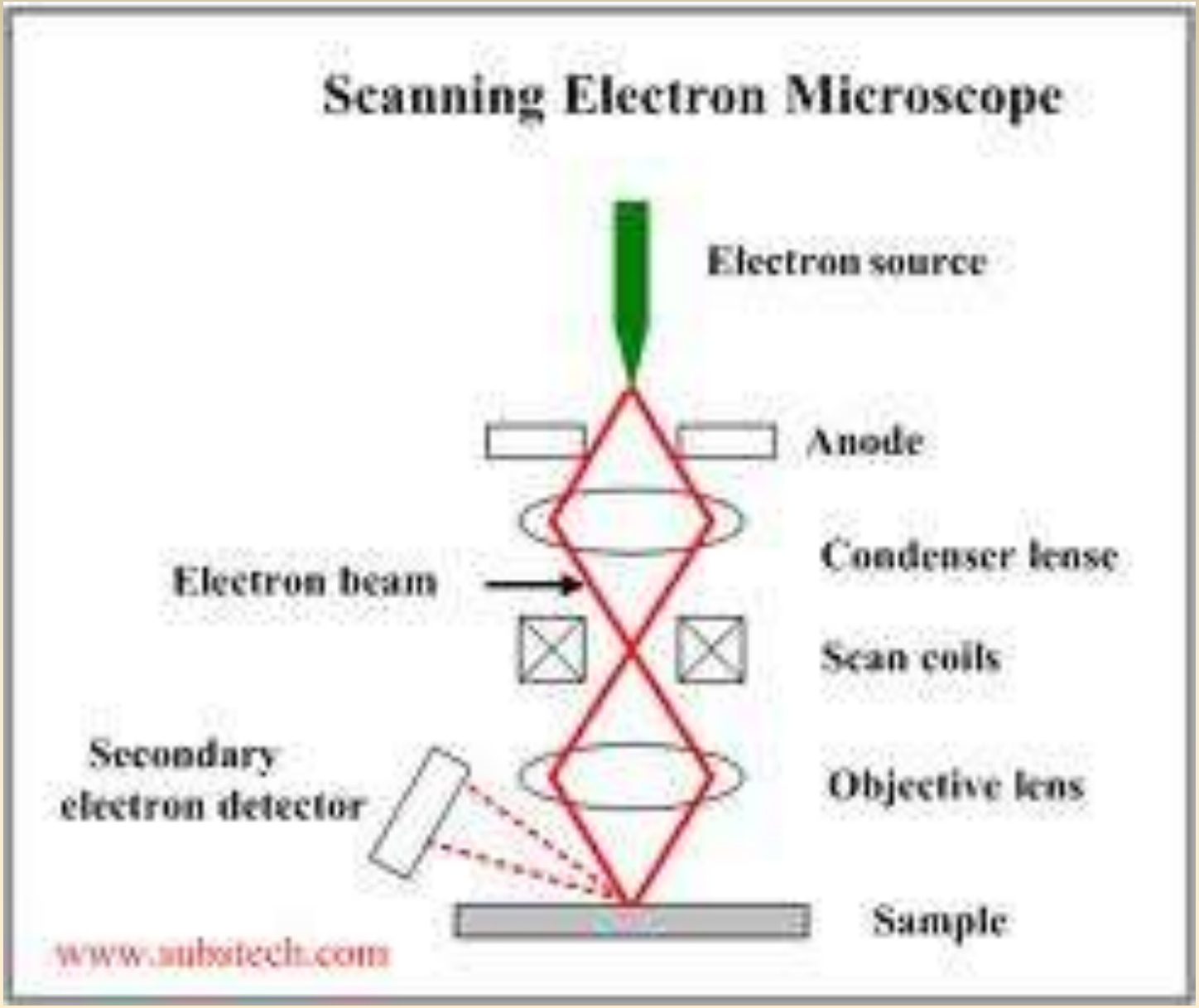
Fullerene (Buckyball) colloids, 1,000,000X

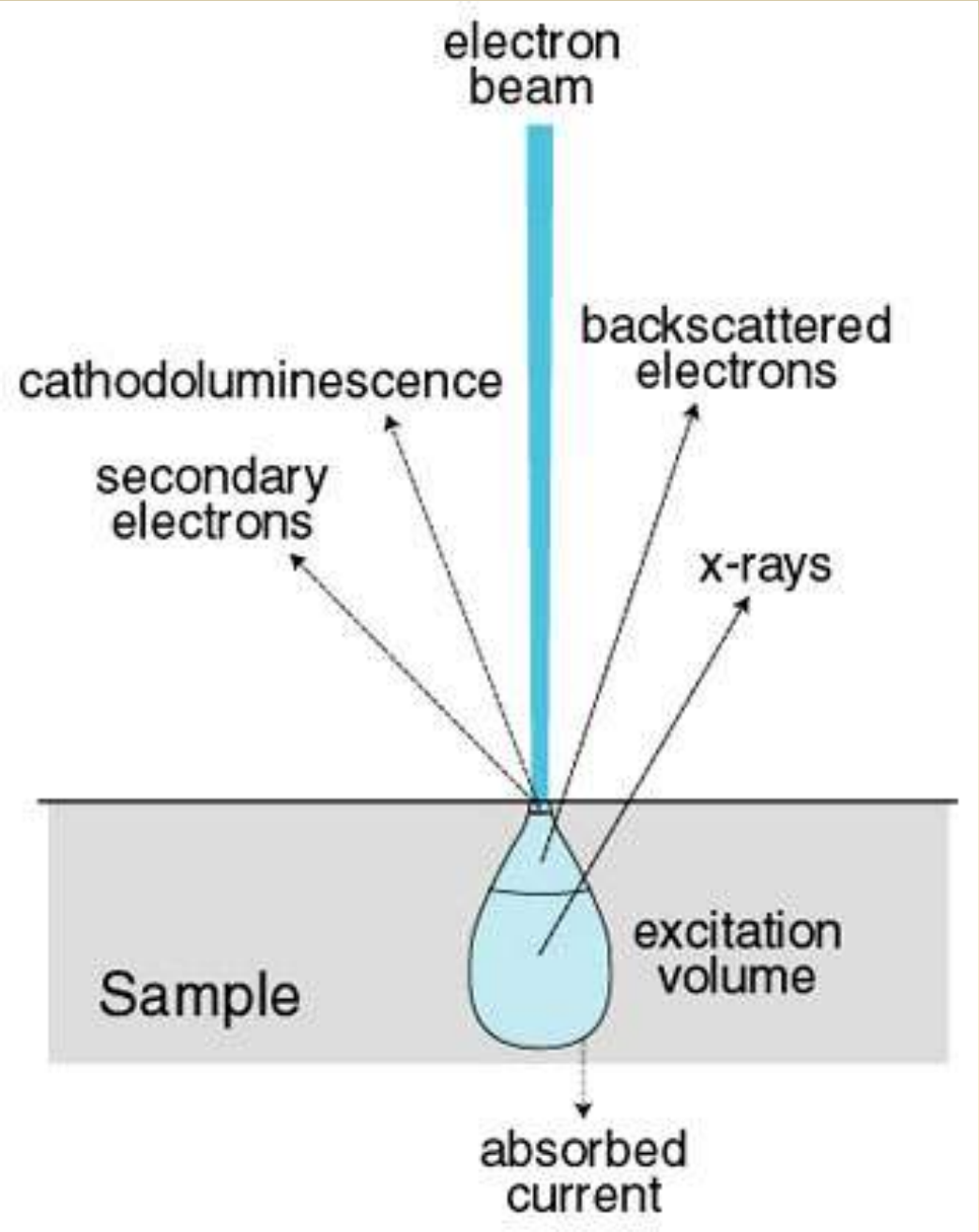
Scanning Electron Microscopy - MBG

Neoscope SEM Gun and Vacuum Column

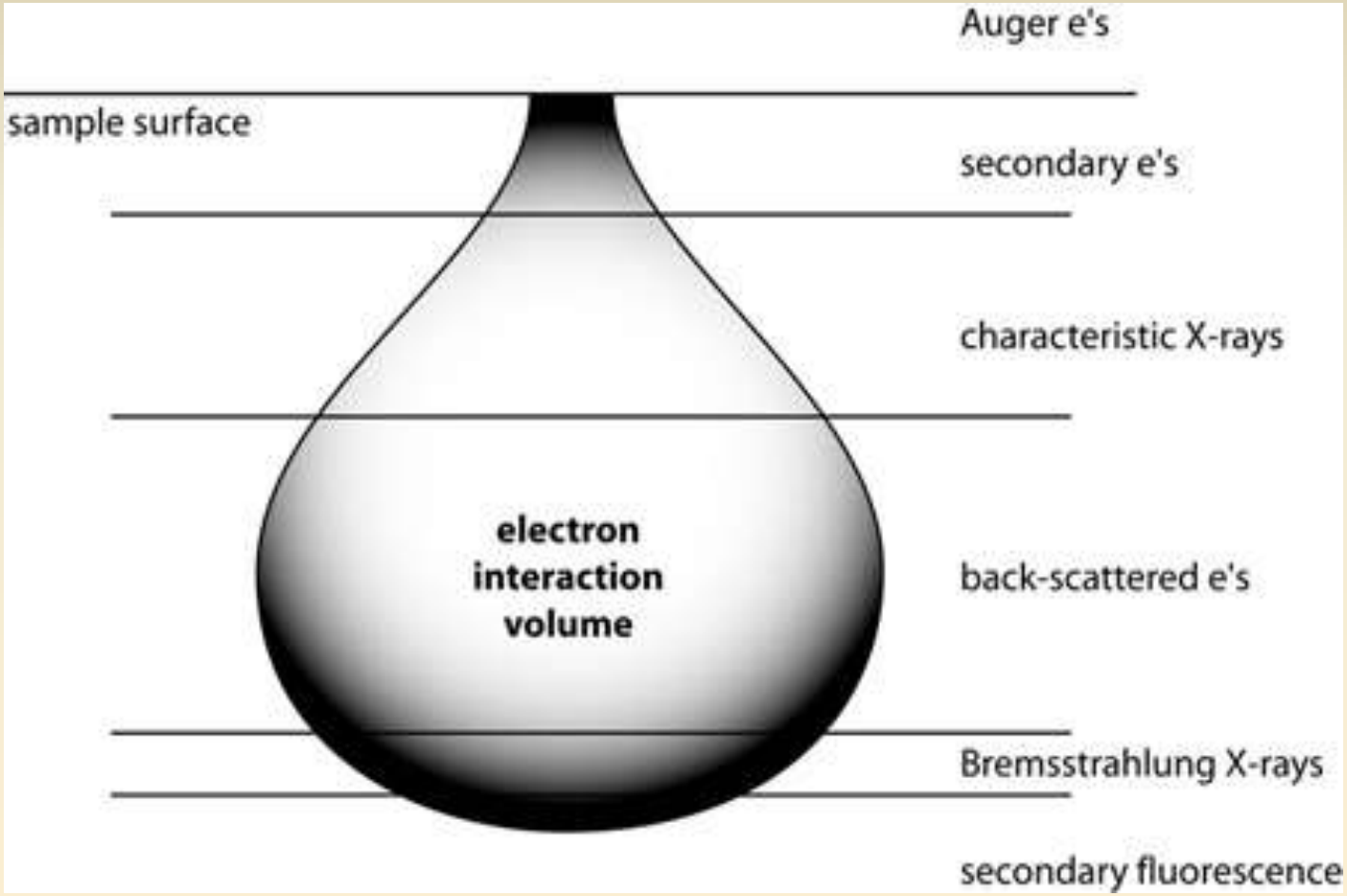
Neoscope Sample Chamber, X-Y Controls

Scanning Electron Microscope

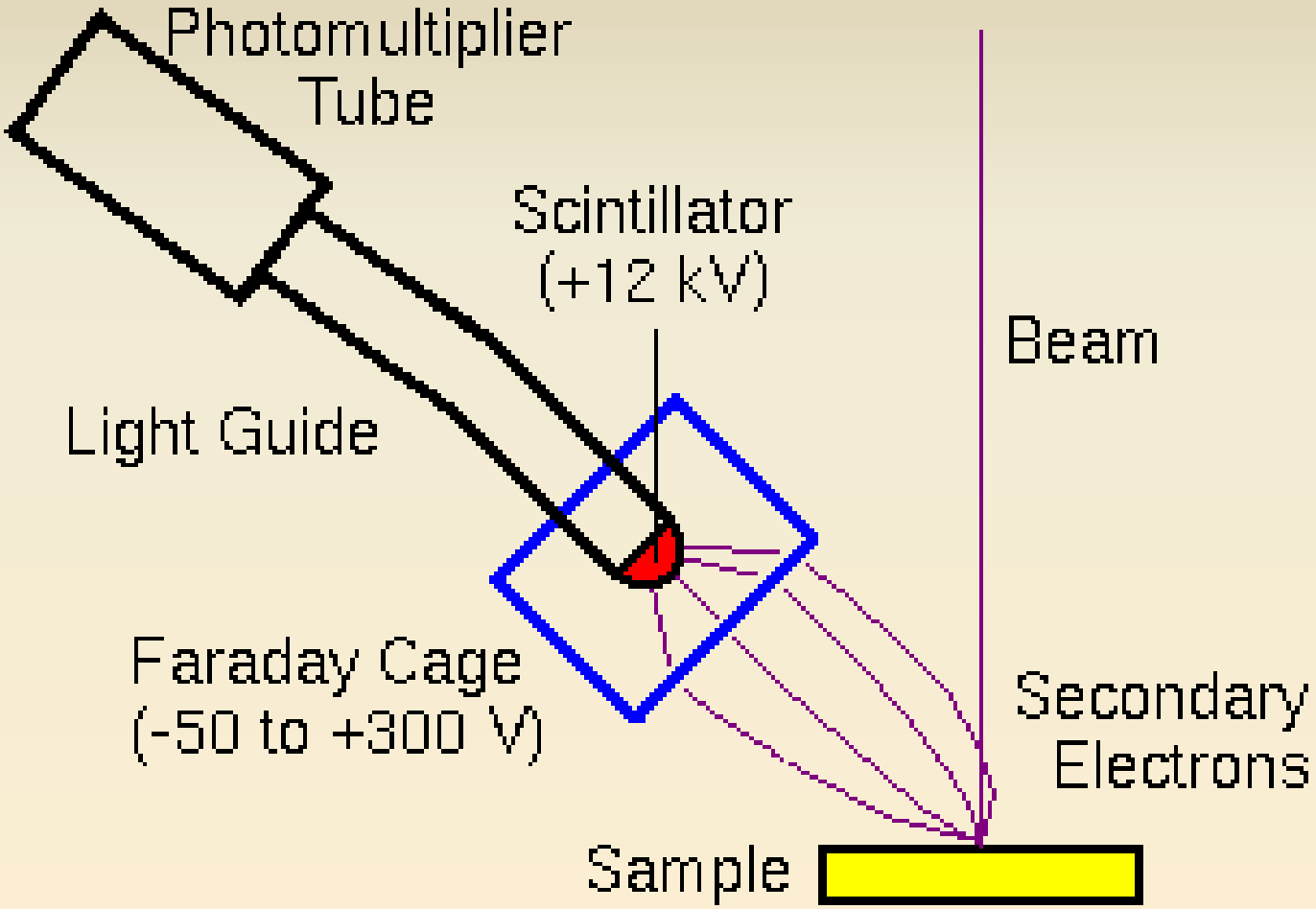



electron
beam
backscattered
electrons
cathodoluminescence
secondary
electrons
x-rays
Sample
excitation
volume
absorbed
current

Volumes within the specimen where signals are generated

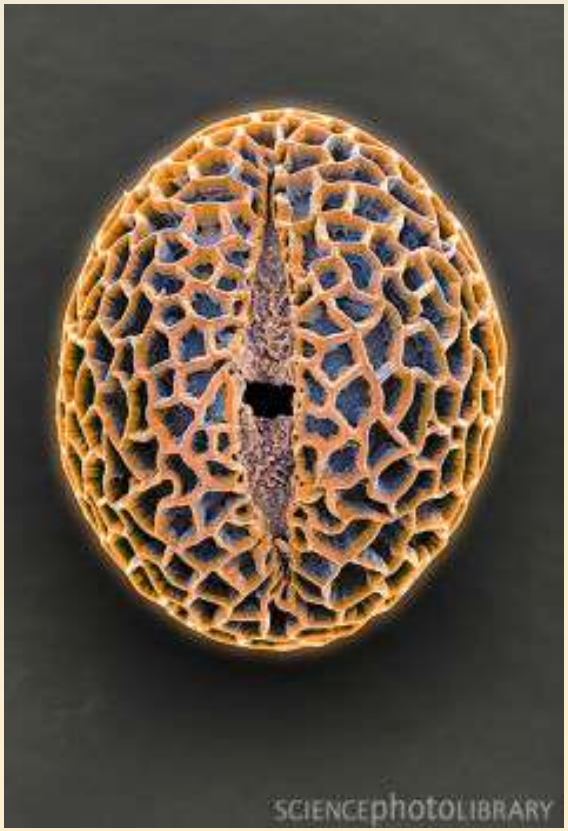


Detecting Secondary Electrons



The SEM provides two outstanding improvements over the optical microscope: it extends the resolution limits and improves the depth-of-focus resolution more dramatically (by a factor of ~300).

The SEM is also capable of examining objects at a large range of magnifications. This feature is useful in forensic studies as well as other fields because the electron image complements the information available from the optical image.



History of SEM

1920s – particle wave theory
1931 – first TEM built, Ernst Ruska and Max Knoll
1935 – SEM developed by Knoll
1938 – first SEM built – Von Ardenne
1956 – electromagnetic lens improved
1960 – improved SEM detector
1965 – first commercial SEMs, Cambridge
1982 – first CCD devices used with SEM

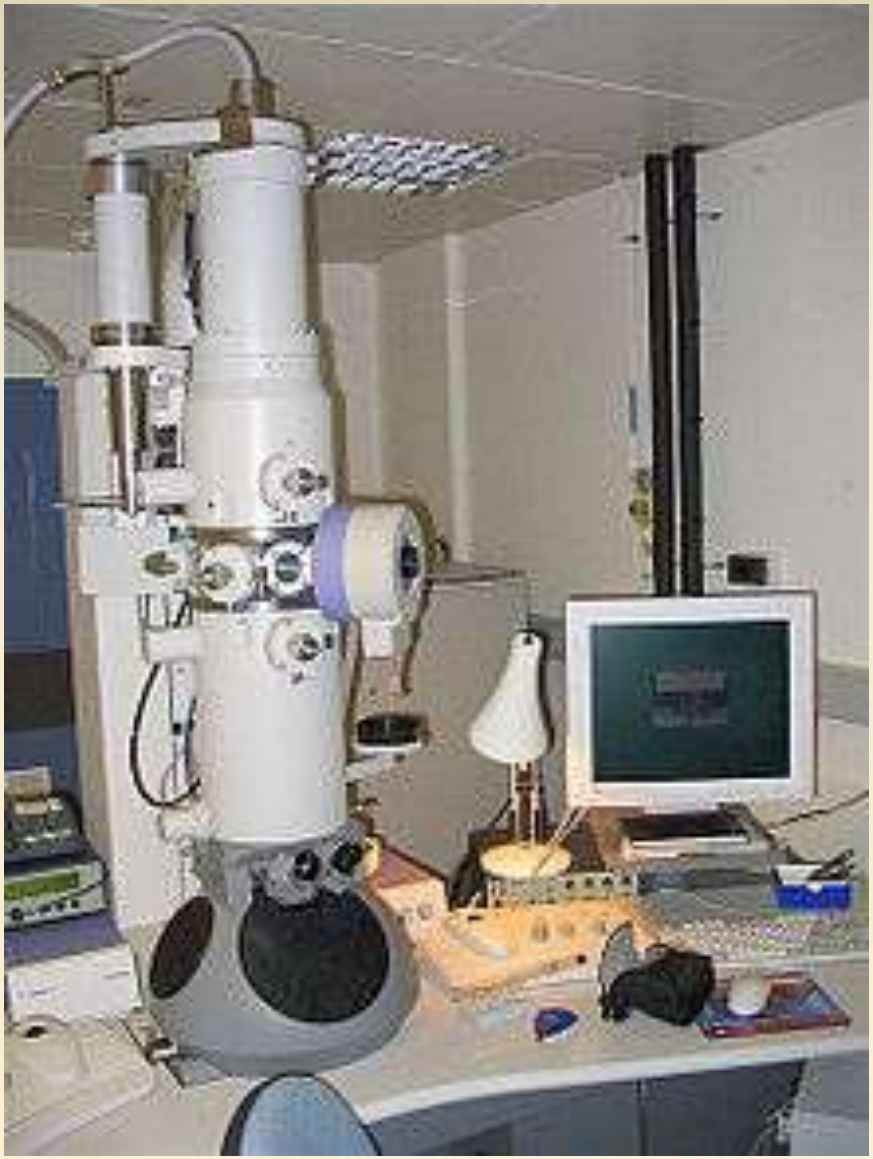
History of SEM

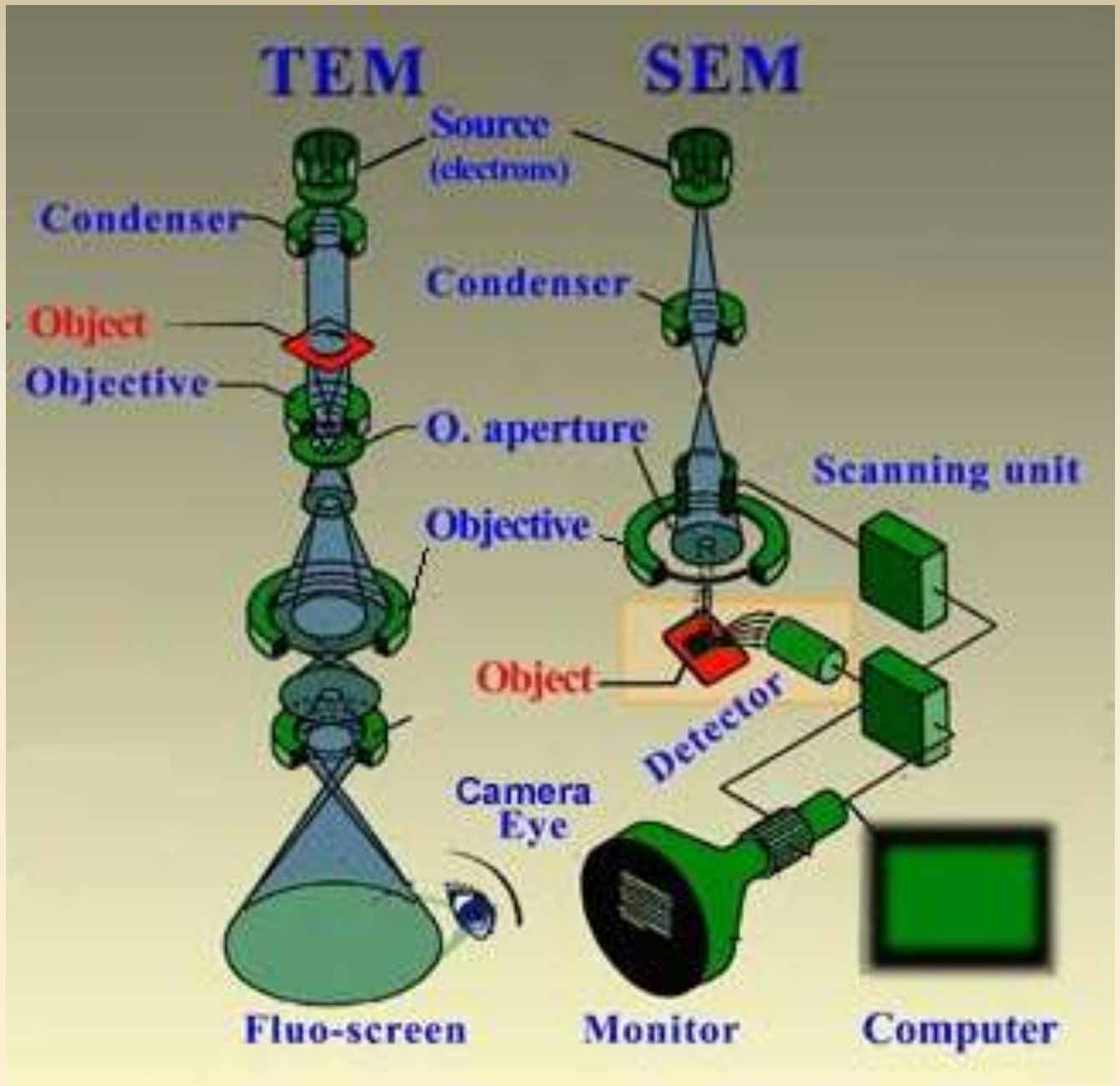
1931 – first TEM
Ernst Ruska
Max Knoll

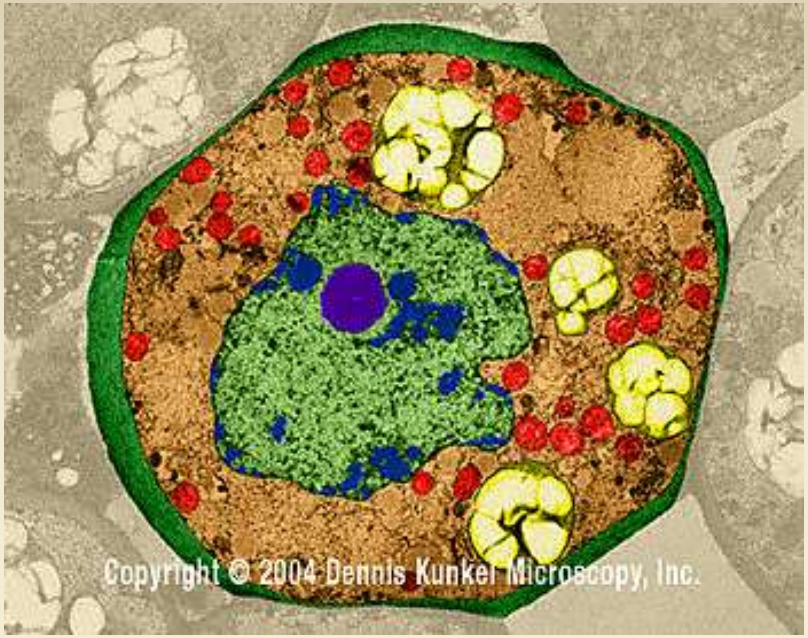
used two magnetic lenses, and three years later a third lens was added, demonstrating a resolution of 100 nm, twice as good as that of the light microscope

TEM Microscopes
Model built by Ruska in 1933

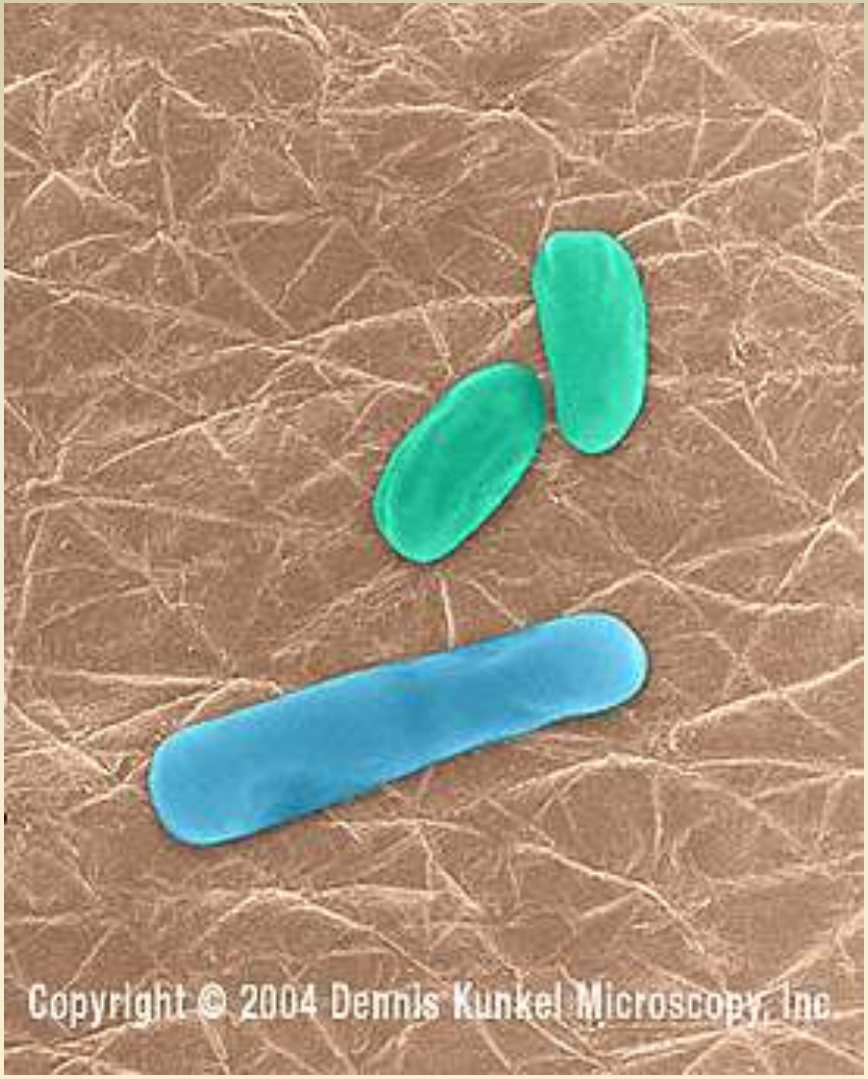
Current model




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

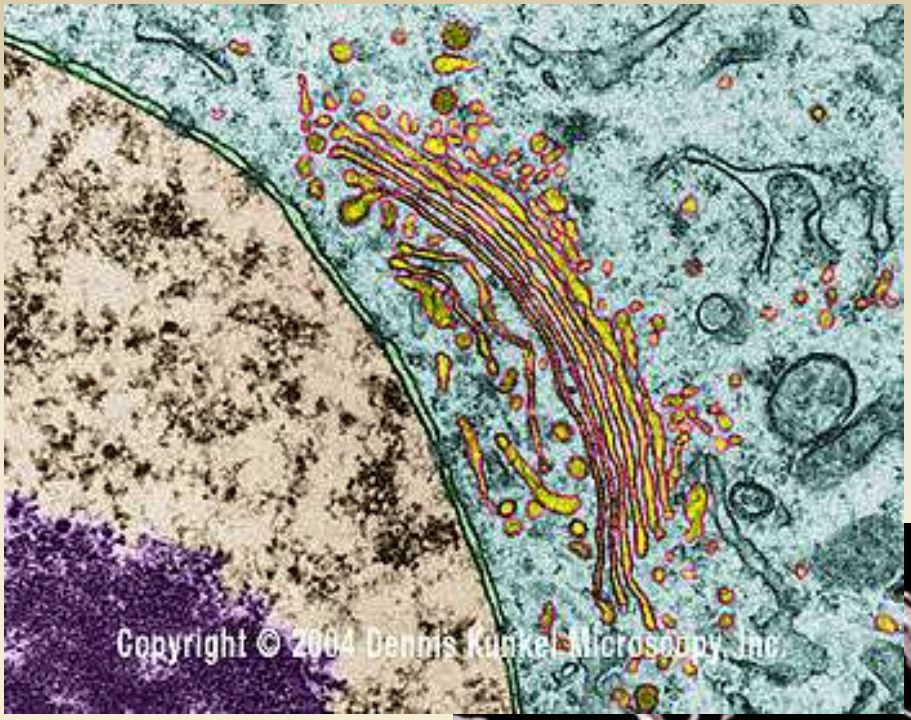


Sauromatum guttatum
Araeceae, TEM
Parenchyma cell

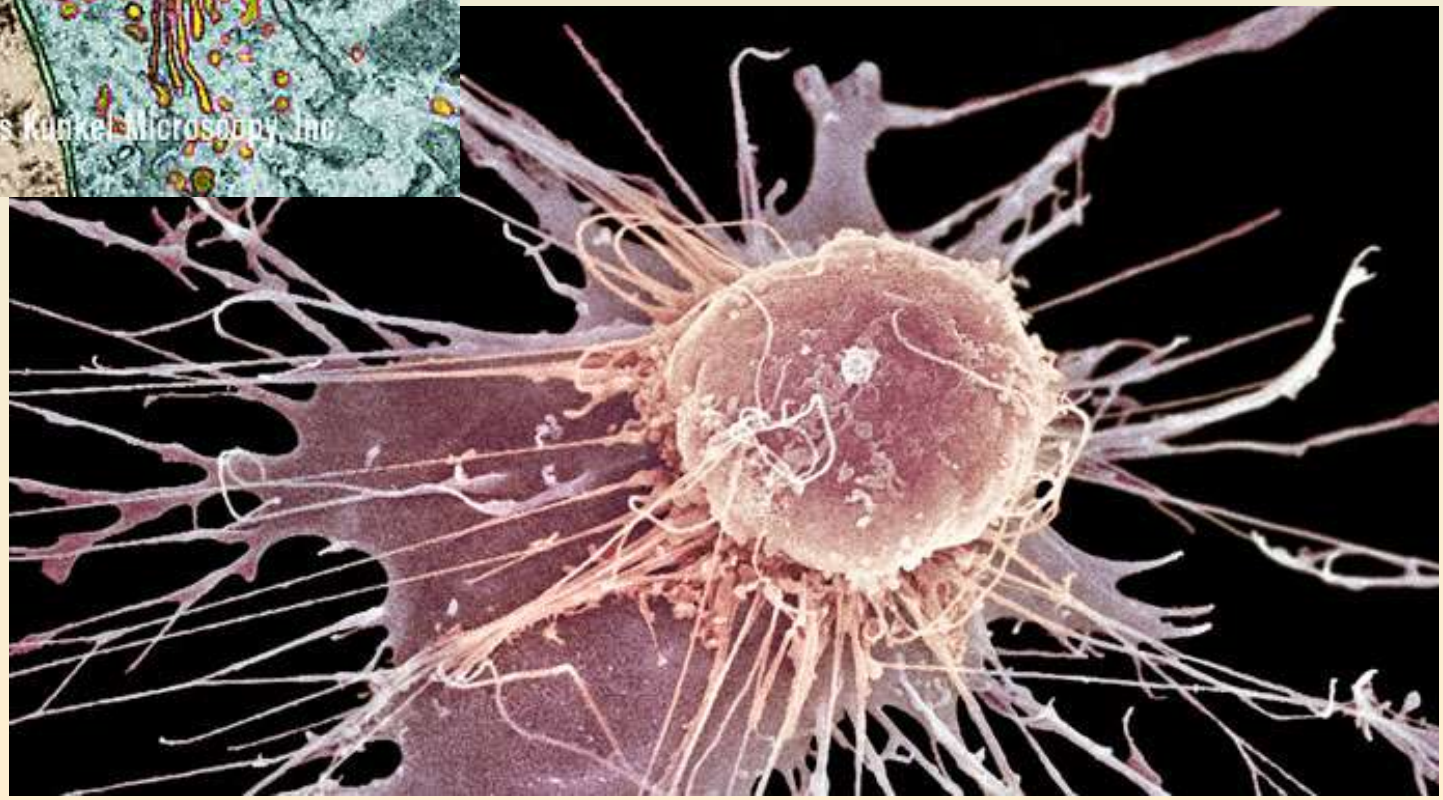


Bacillus anthracis – Anthrax, SEM

TEM – Golgi apparatus



SEM – stem cell



Max Knoll's Electron Beam Scanner

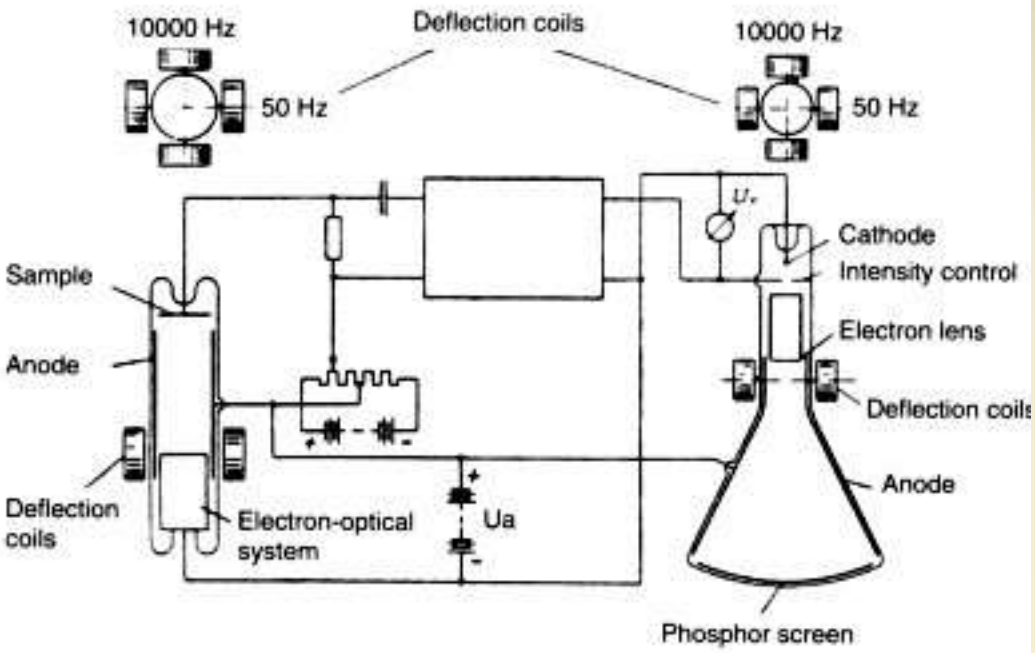


FIG. 2 Schematic diagram of Knoll's (1935) electron-beam scanner.

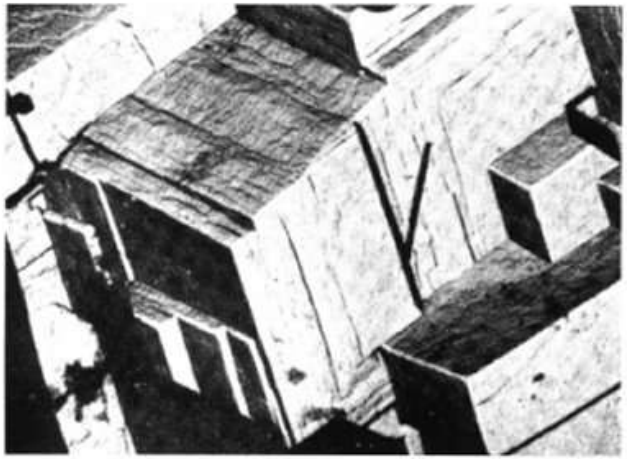


FIG. 1 TEM image of an early oxide replica of etched aluminum (Mahl 1941); horizontal field width = 9 μm.

Manfred von Ardenne – SEM design

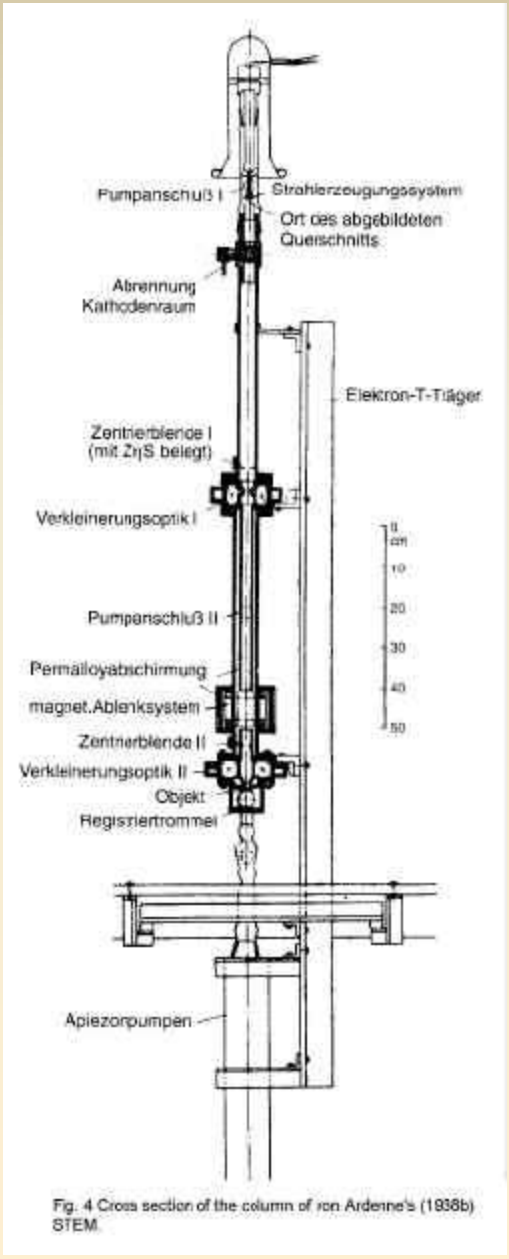


Fig. 4 Cross section of the column of von Ardenne's (1938b) STEM.

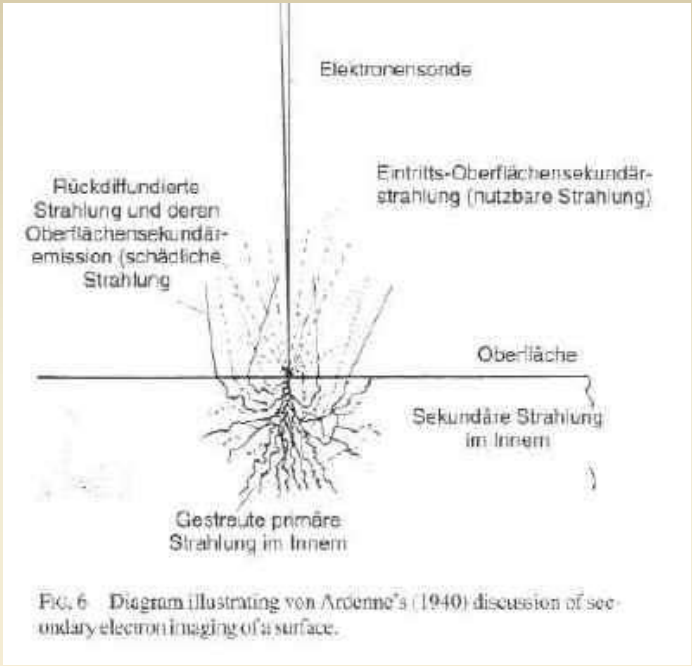


FIG. 6 Diagram illustrating von Ardenne's (1940) discussion of secondary electron imaging of a surface.

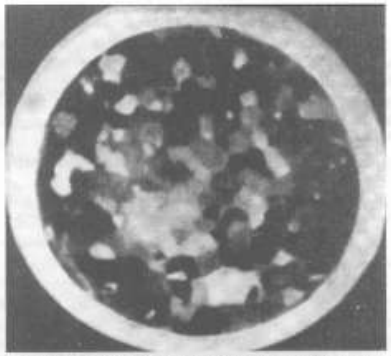


FIG. 3 Electron-beam scanner image of silicon iron showing electron channeling contrast; horizontal field width = 50 mm. (Knoll 1935).

Prof. Oatley's Group SEMs – 1950's and 1960's

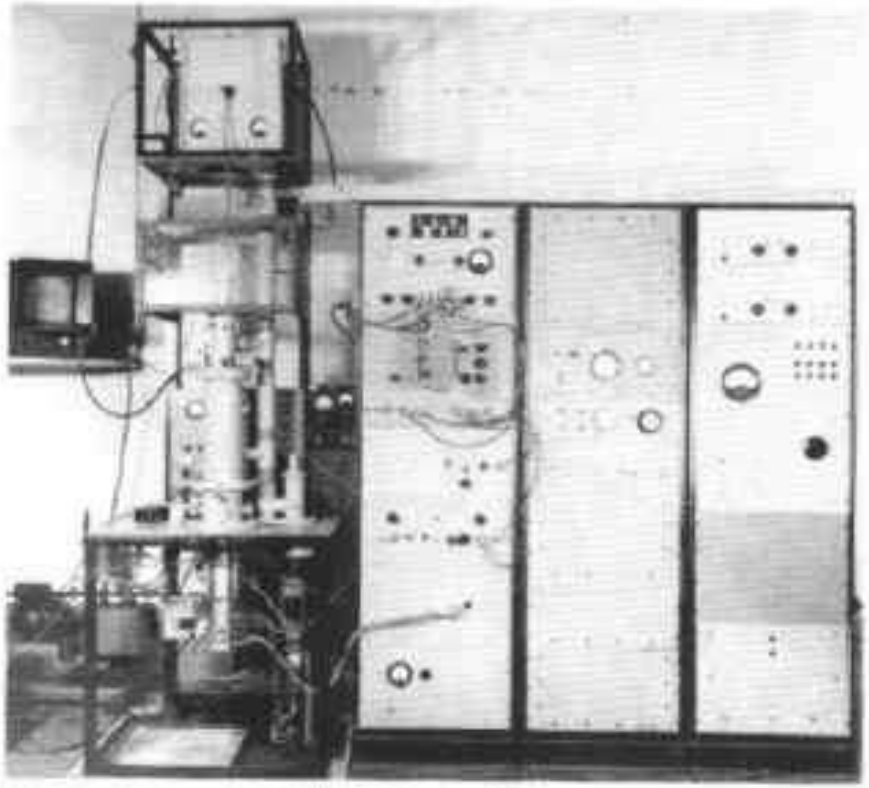


FIG. 12 Photograph of SEM 1 taken in 1953.

Photograph device added

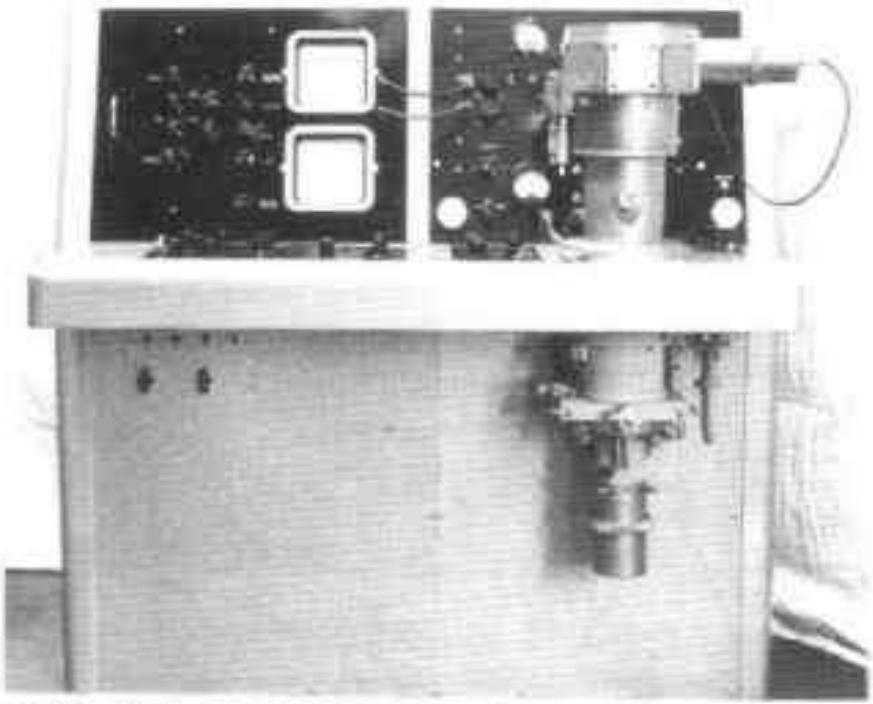


FIG. 13 The first magnetically focussed scanning electron microscope (SEM 3) built by K.C.A. Smith for the Pulp and Paper Research Institute of Canada (Smith 1959, 1961).

Magnetic focus added

1967 - Stereoscan Mark VI – Cambridge Instruments

An early commercial SEM

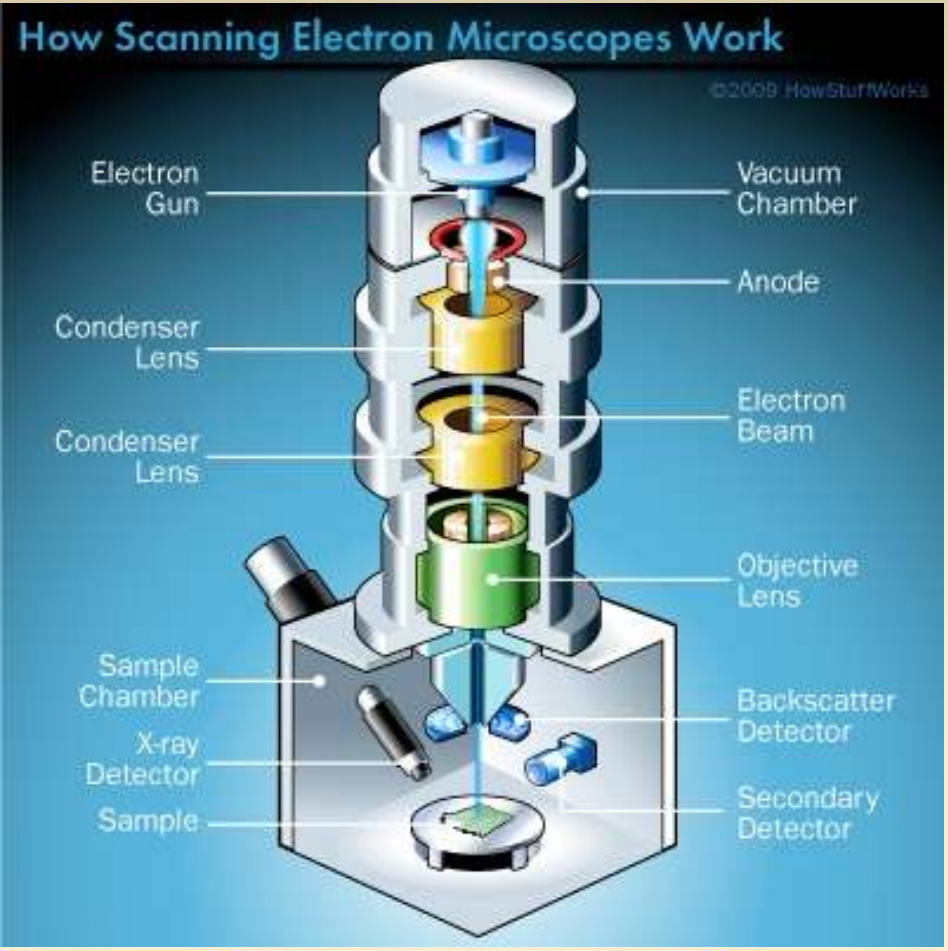
JEOL – first commercial SEM, 1966

The JSM (now known as the JSM-1 was JEOL's first commercially produced Scanning Electron Microscope. The JSM -1 was made commercially available in 1966. Among its advanced features was a Eucentric Stage.

Resolution: 250Å (at 25kV)

Magnification: 100 - 30,000

Scan area: 1x1 mm (at 25kV)


How Scanning Electron Microscopes Work
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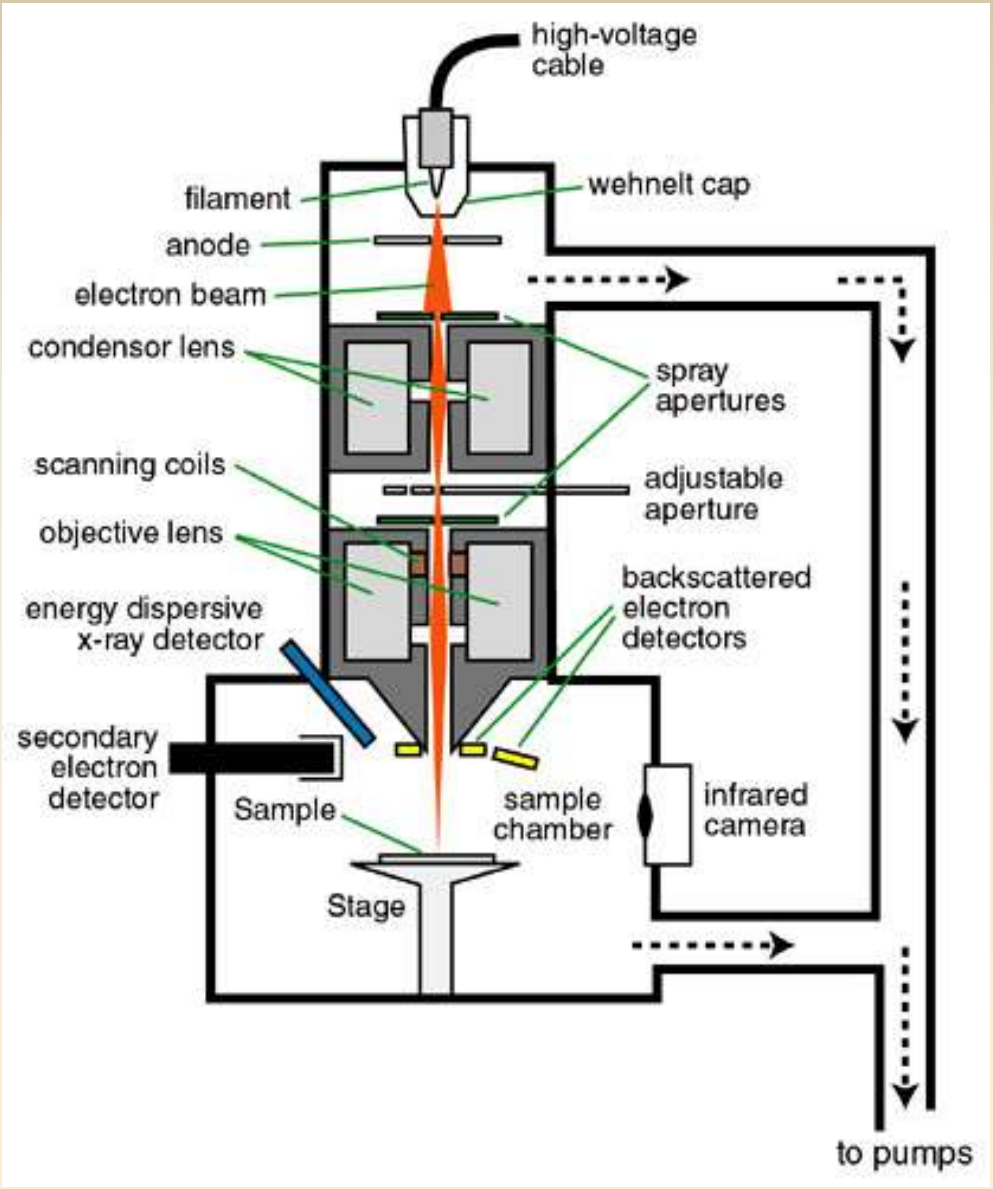
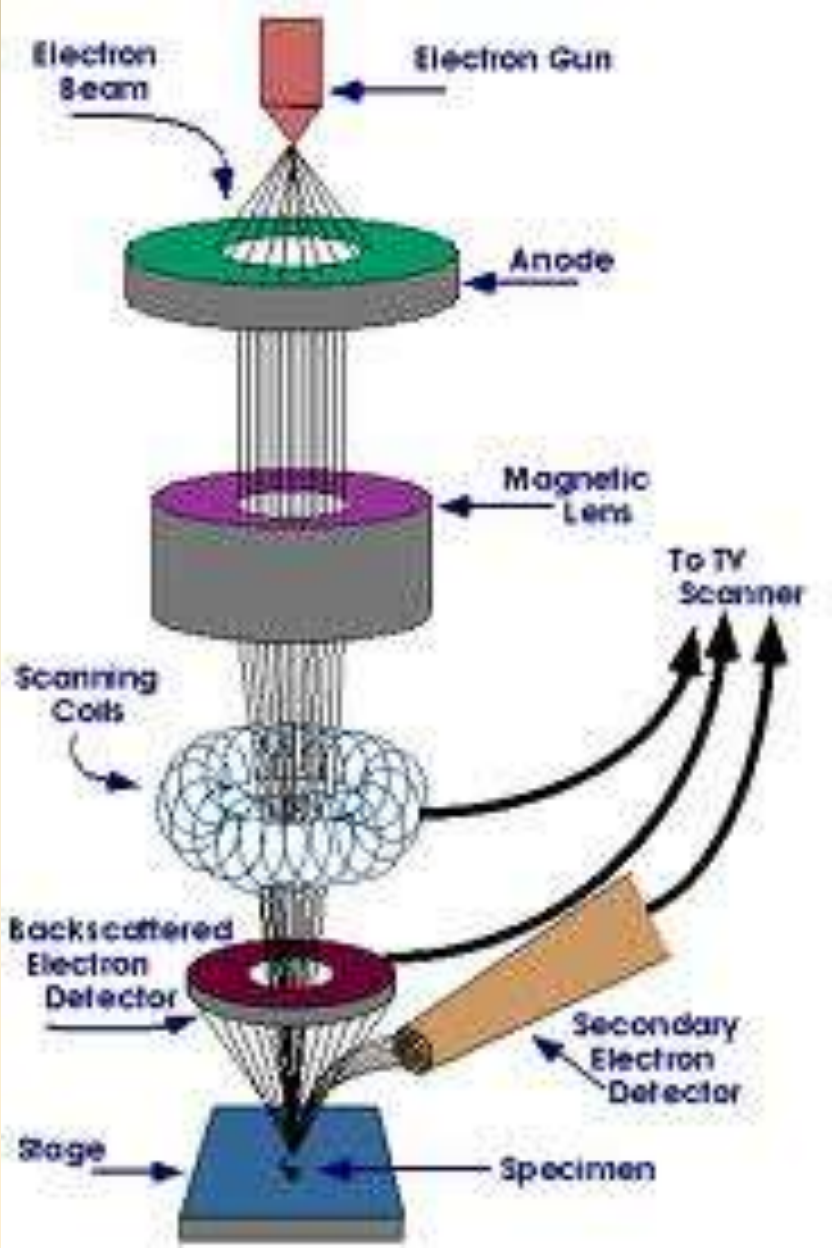
Electron Gun
Vacuum Chamber
Anode
Condenser Lens
Electron Beam
Condenser Lens
Objective Lens
Sample Chamber
Backscatter Detector
X-ray Detector
Secondary Detector
Sample


DELL



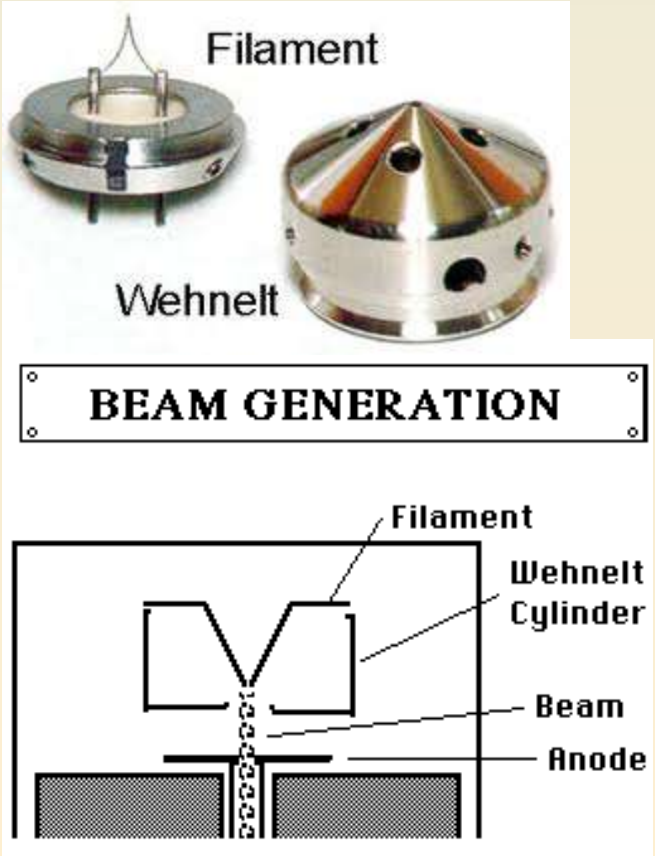
Vacuum System

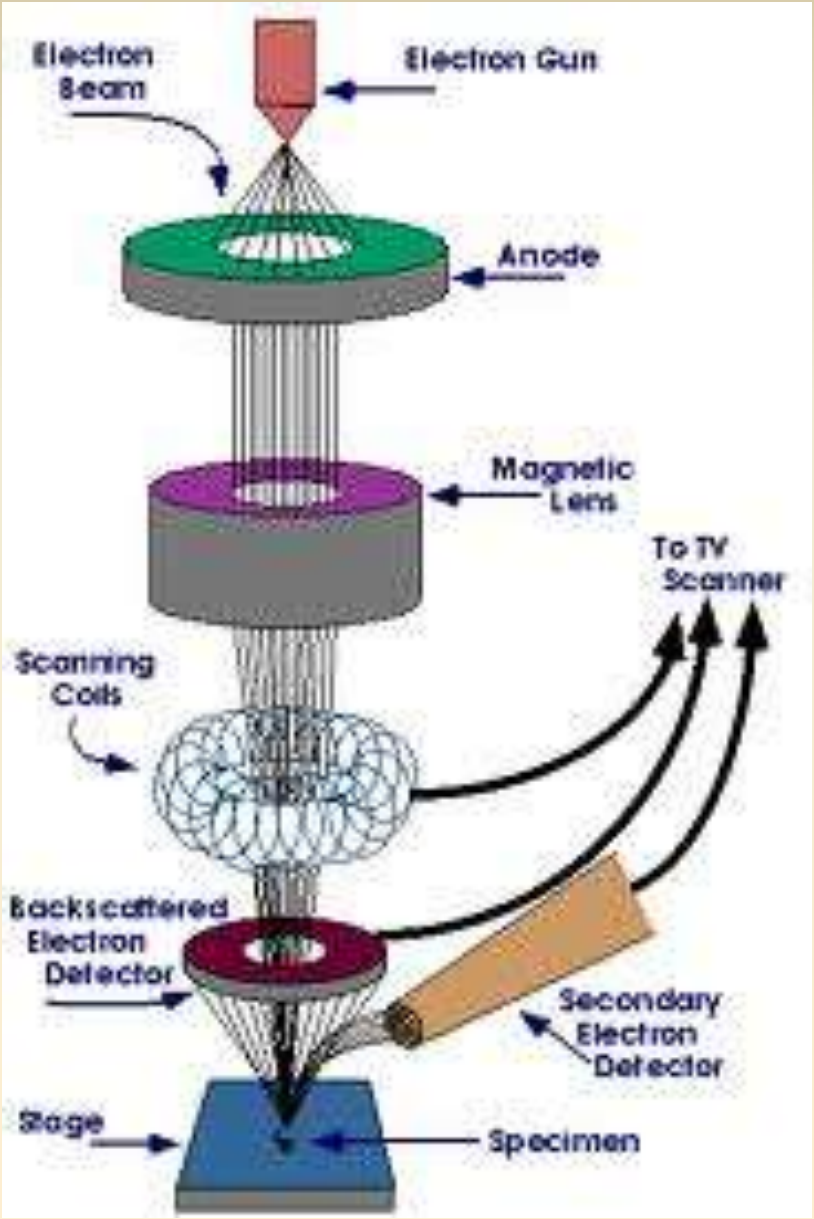
A vacuum is required when using an electron beam because electrons will quickly disperse or scatter due to collisions with other molecules.



Electron beam generation system.

This system is found at the top of the microscope column, and generates the "illuminating" beam of electrons known as the primary (1o) electron beam.



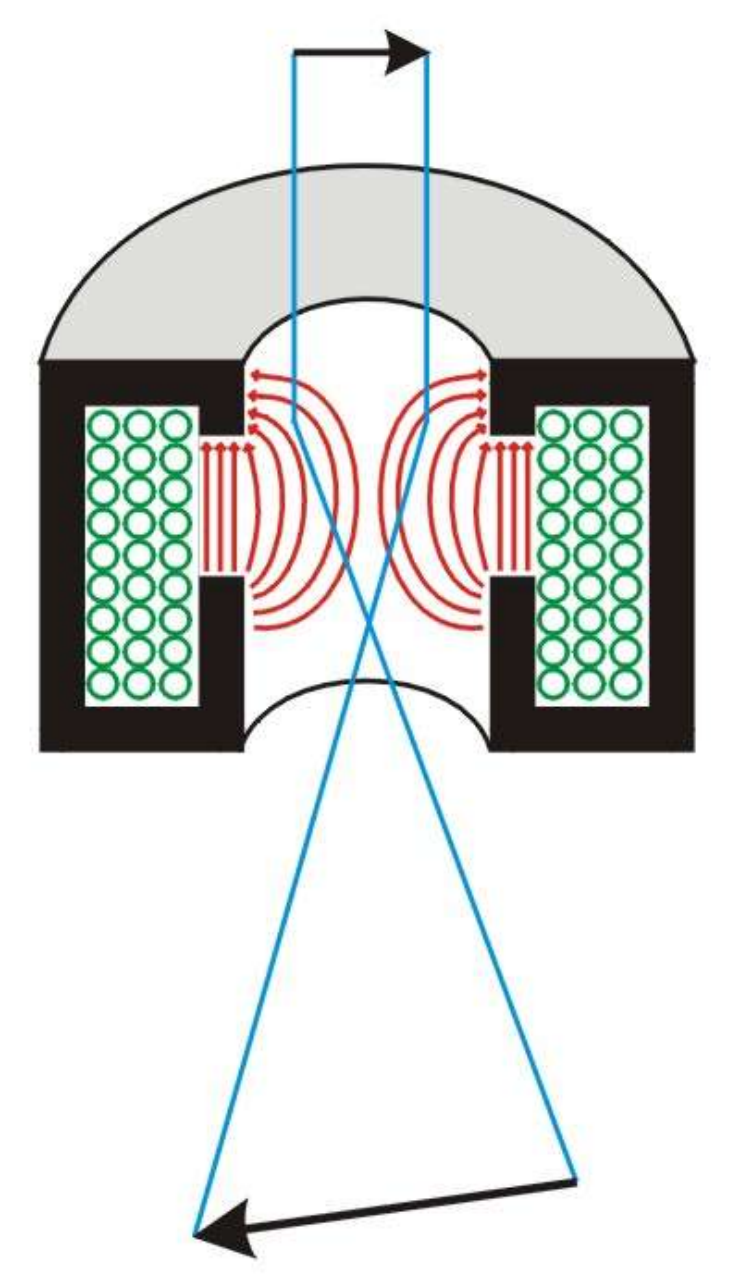


Electron beam manipulation system.
This system consists of electromagnetic lenses and coils located in the microscope column and control the size, shape, and position of the electron beam on the specimen surface.

Condenser lens converges the electron beam generated from the electron gun to a fine electron beam

Scanning coils generate the “raster” beam that scans back and forth on the specimen. The electron beam is scanned across the specimen by scan coils while a detector measures the radiation emitted from the specimen.

Magnetic Lens



A magnetic lens consists of a coil of copper wires inside the iron pole pieces.

A current through the coils creates a magnetic field (symbolized by red lines) in the bore of the pole pieces.

The rotationally symmetric magnetic field is inhomogeneous in such a way that it is weak in the center of the gap and becomes stronger close to the bore.

Electrons close to the center are less strongly deflected than those passing the lens far from the axis.

<u>The overall effect is that a beam of parallel electrons is focused into a spot</u> (so-called cross-over).

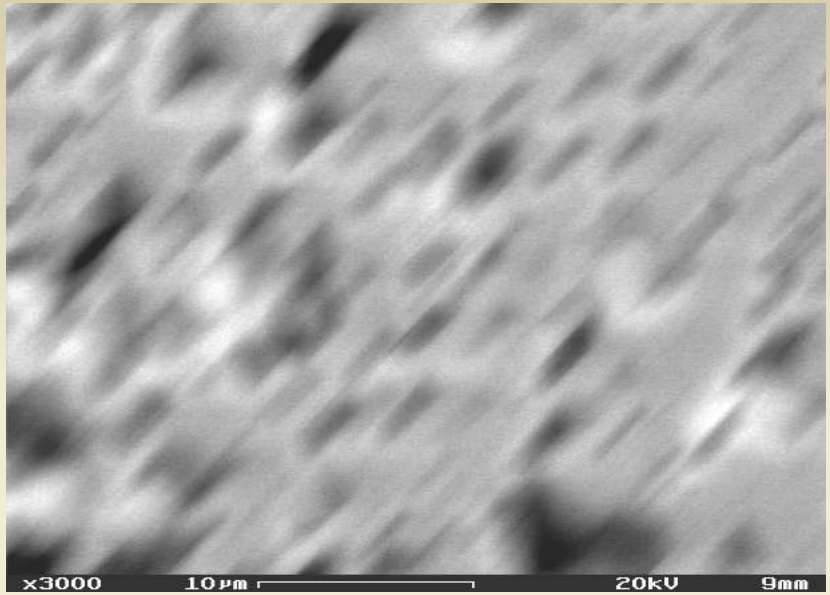
Lens Aberrations - Astigmatism:

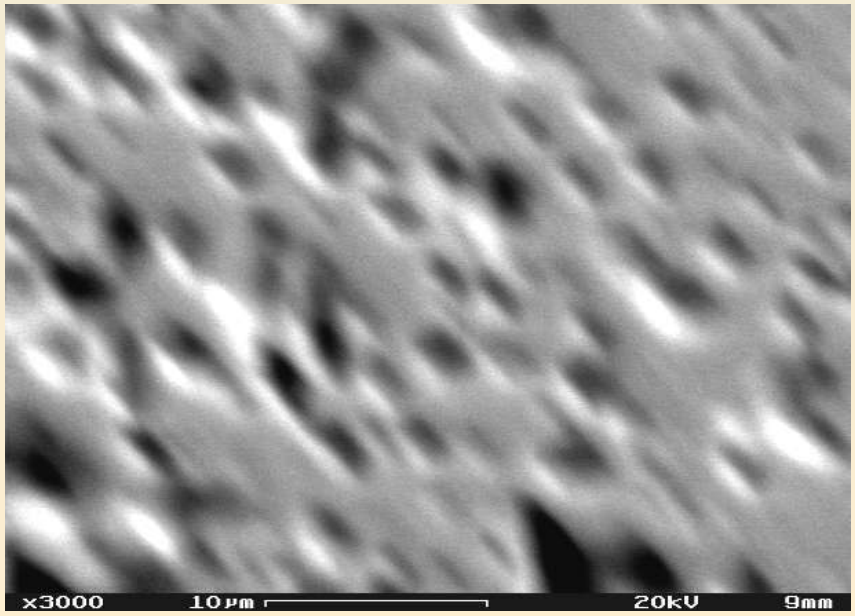
Strength of lens is asymmetrical; it is stronger in one plane than another.

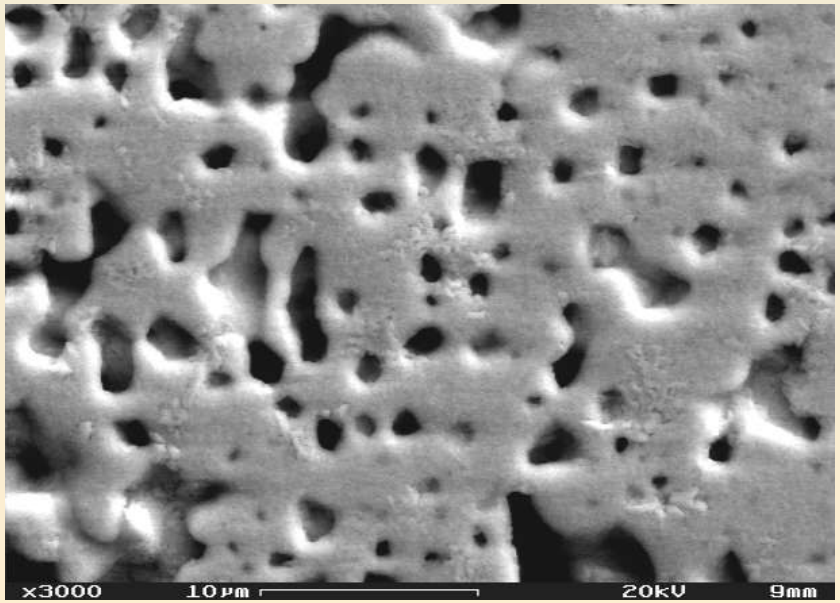
Caused by machining errors, non-homogeneous polepiece iron, asymmetrical windings, dirty apertures.

Results in out-of-focus “stretched” image.

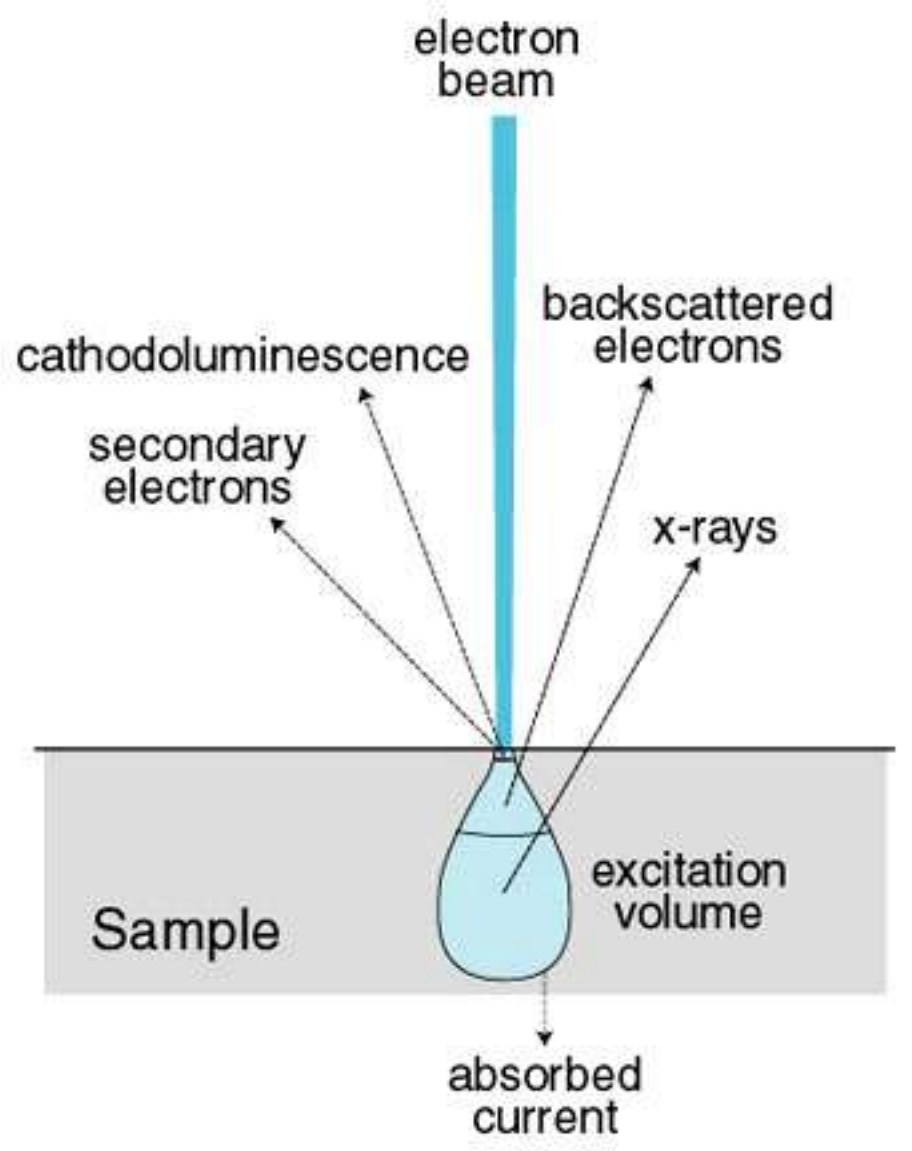
Corrected with stigmator coils.







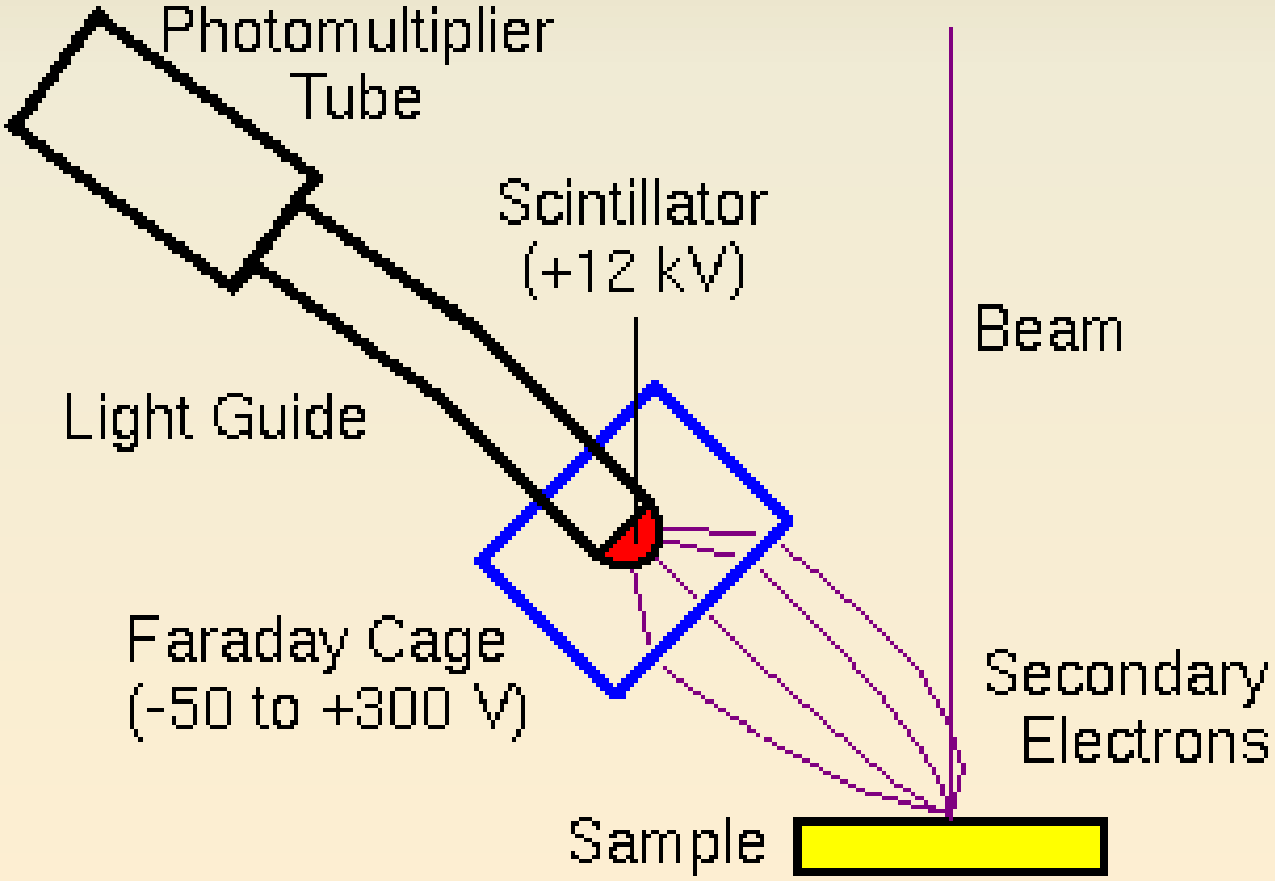
Beam specimen interaction system.

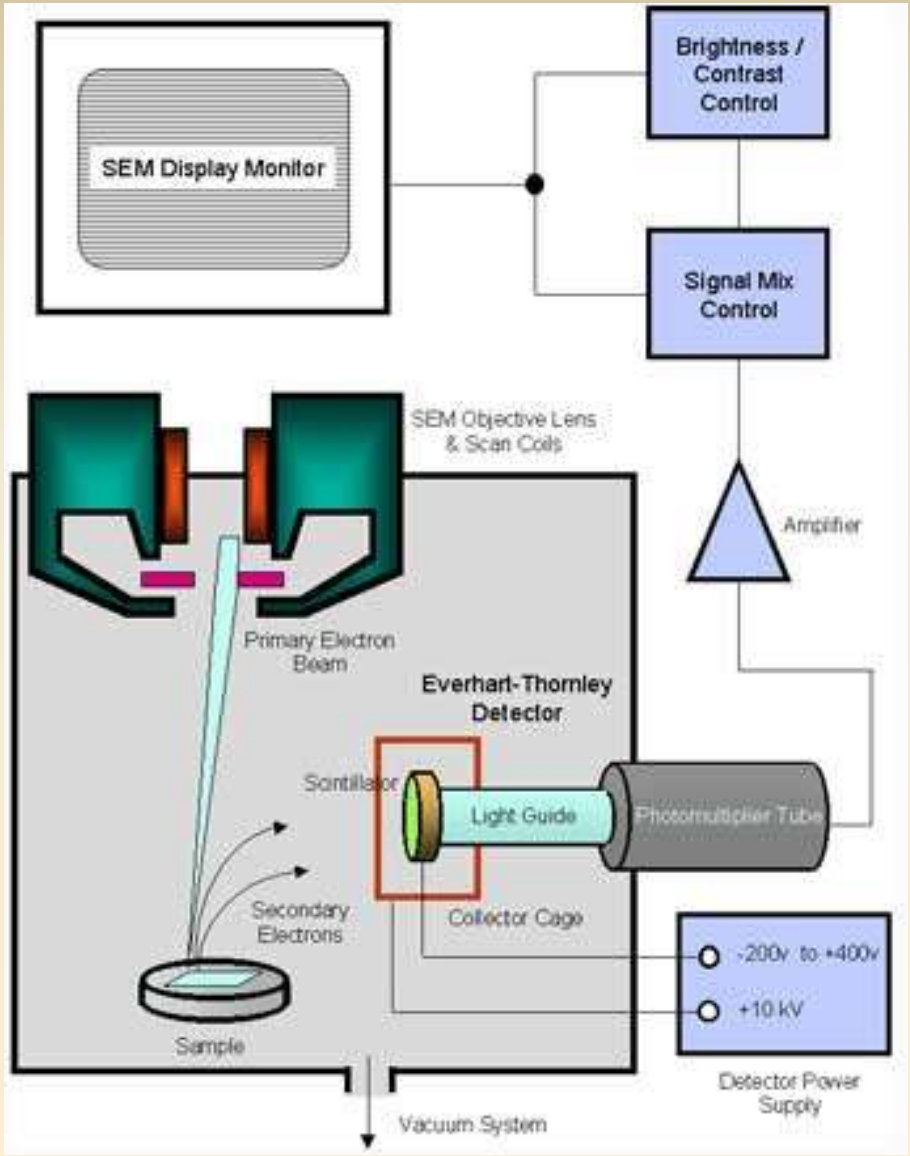


This system involves the interaction of the electron beam with the specimen and the types of signals that can be detected.

Detection system.

This system can consist of several different detectors, each sensitive to different energy / particle emissions that occur on the sample.





Signal processing system.

This system is an electronic system that processes the signal generated by the detection system and allows additional electronic manipulation of the image.

Signal manipulation begins with the amplifier in the detector and ends with the image on the viewing screen. All controls associated with the changing the way the image is viewed in terms of brightness and contrast is considered part of the signal manipulation system

Display and recording system.

This system allows visualization of an electronic signal using a cathode ray tube and permits recording of the results using photographic or magnetic media.

Factors that determine the quality of a micrograph

Brightness – value of pixels in image

Contrast – difference between highest and lowest pixel

Resolution - size of the beam spot, working distance, aperture size, beam bias current, voltage, and how cylindrical the beam is

Magnification - function of area scanned and viewing size

Depth of field - region of acceptable sharpness

Noise - any level of brightness observed in a micrograph, white or black, that is not a result of the planned interaction of the beam with the specimen

Composition - all the above characters plus the way the subject is framed

Sample Preparation

Size -Maximum size approximately 1 cm in diameter
Clean – free of oil, resins, loose parts, debris, dust
Dry - completely dehydrated prior to being placed in the microscope chamber
Conductive or non-conductive
Biologicals
Powders, loose parts
Plane, angularity, shape, parts sticking out

SEM Stubs

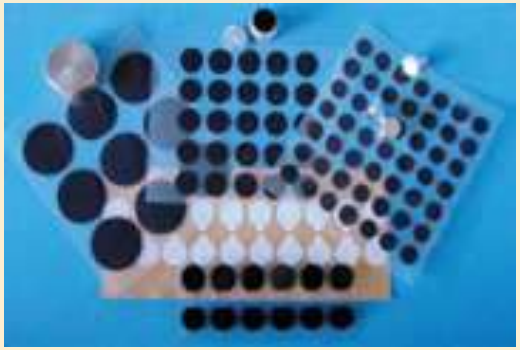
Carbon Paint

Carbon Tape

Stub Adhesives

Silver Paint

Copper Tape

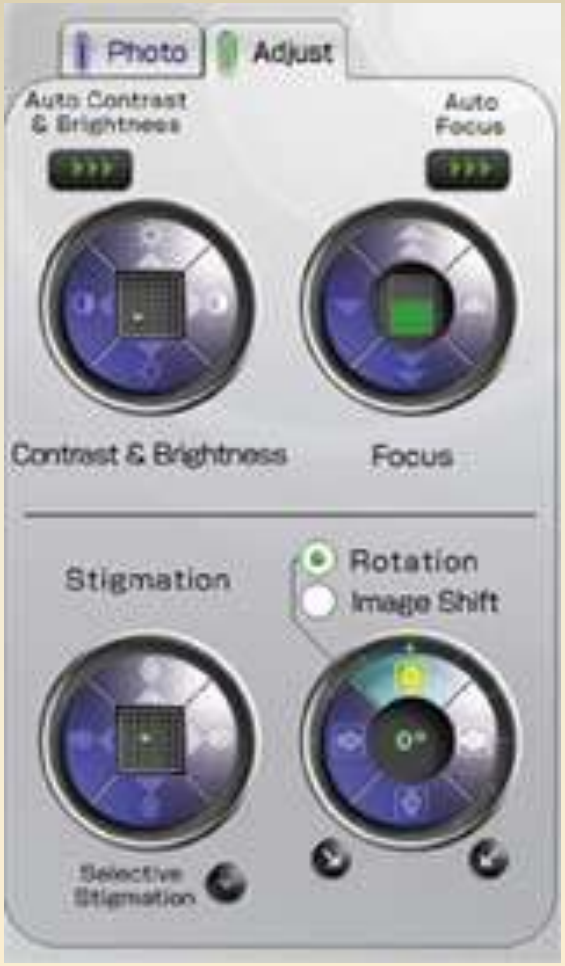


Pelco Adhesive Pads

Scanning Electron Microscopy - MBG

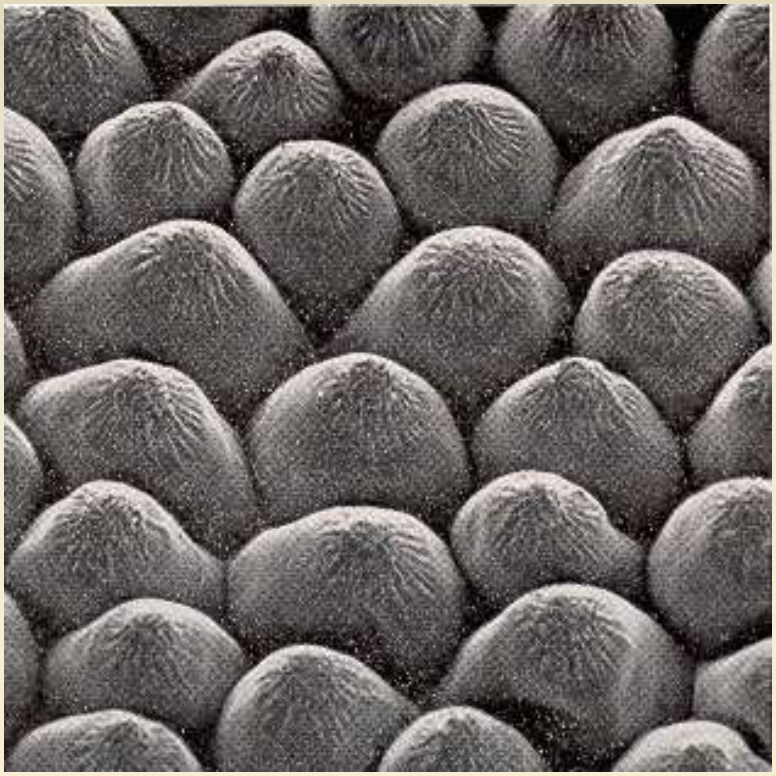
Sharon Carter – 2012 REU

Neoscope SEM Control Panel

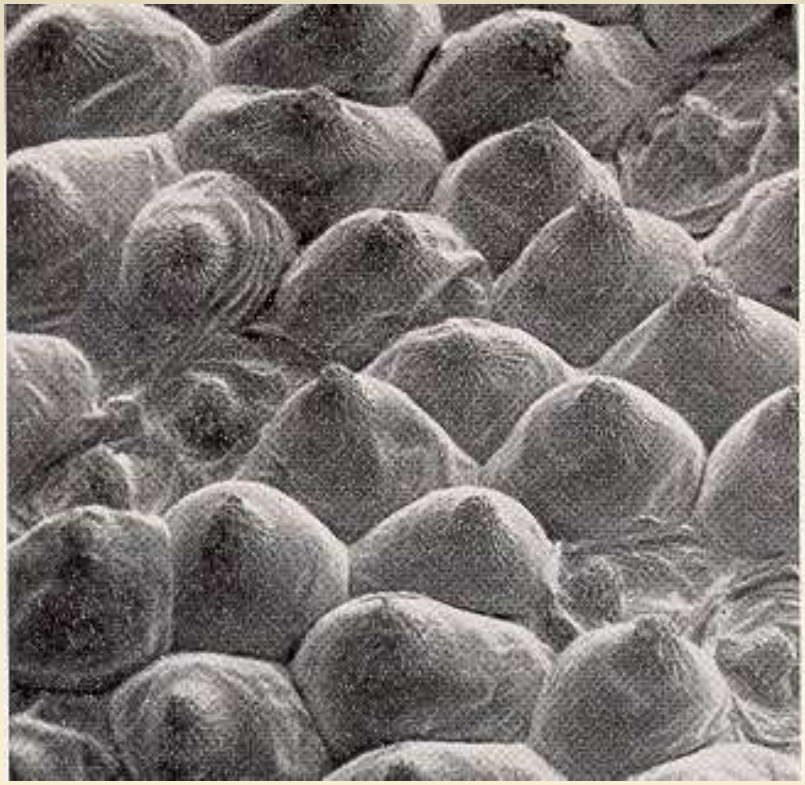


Rose Petal – sample prep

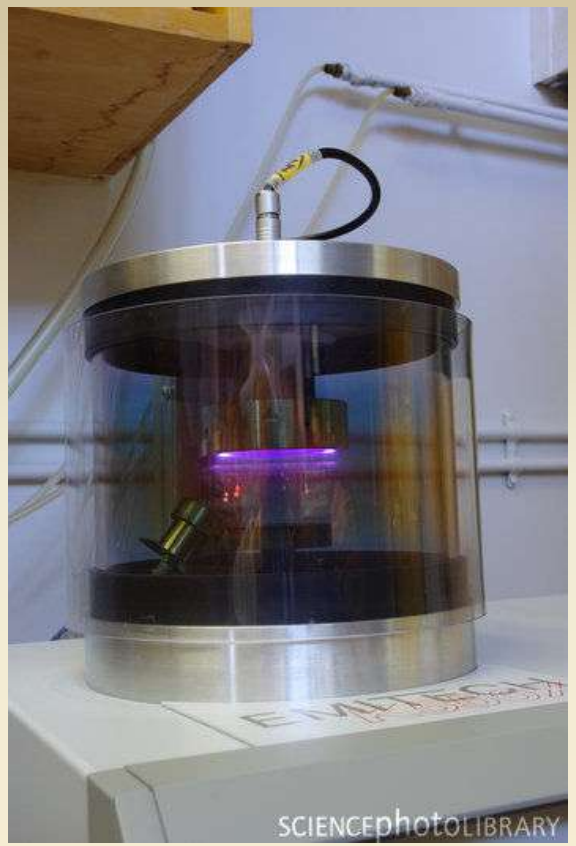
CPD



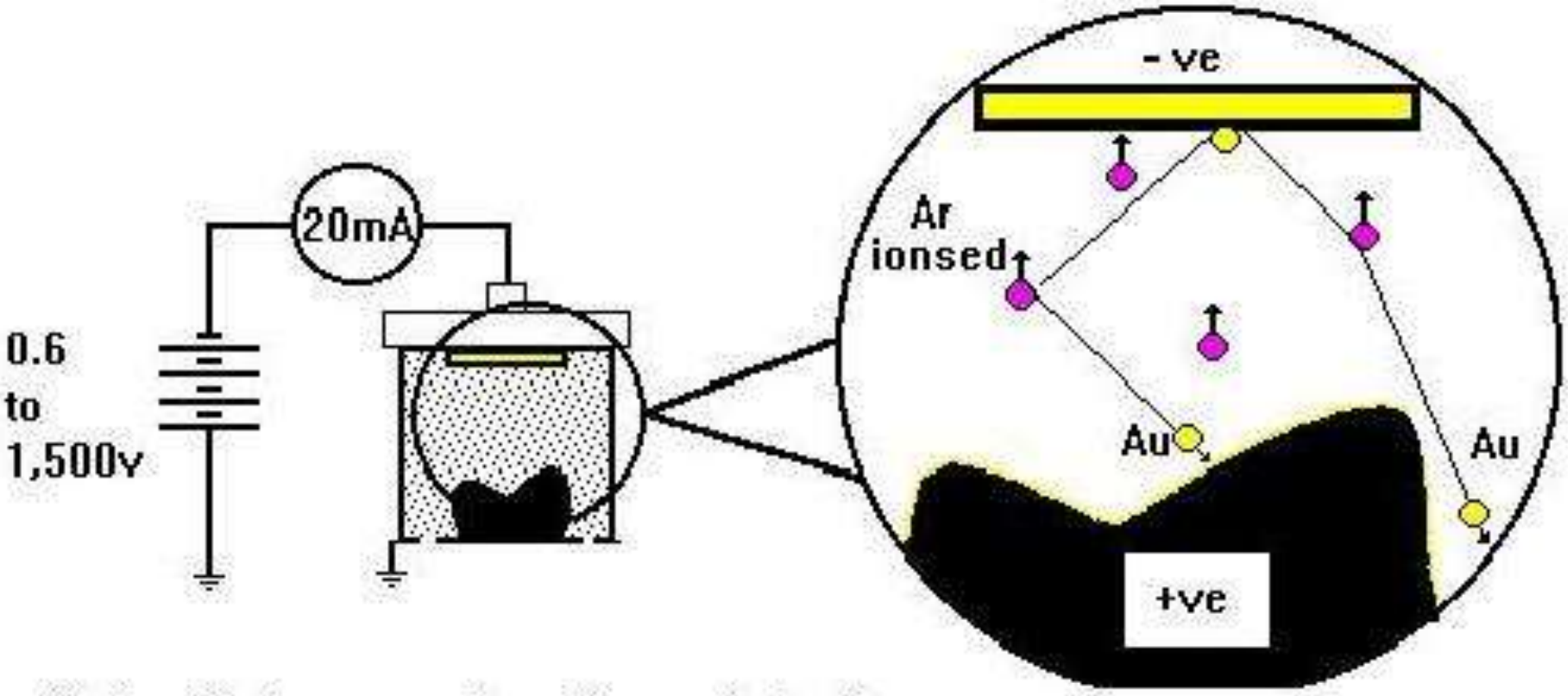
Fixed and Air-Dried



Denton Desk V Sputter Coater

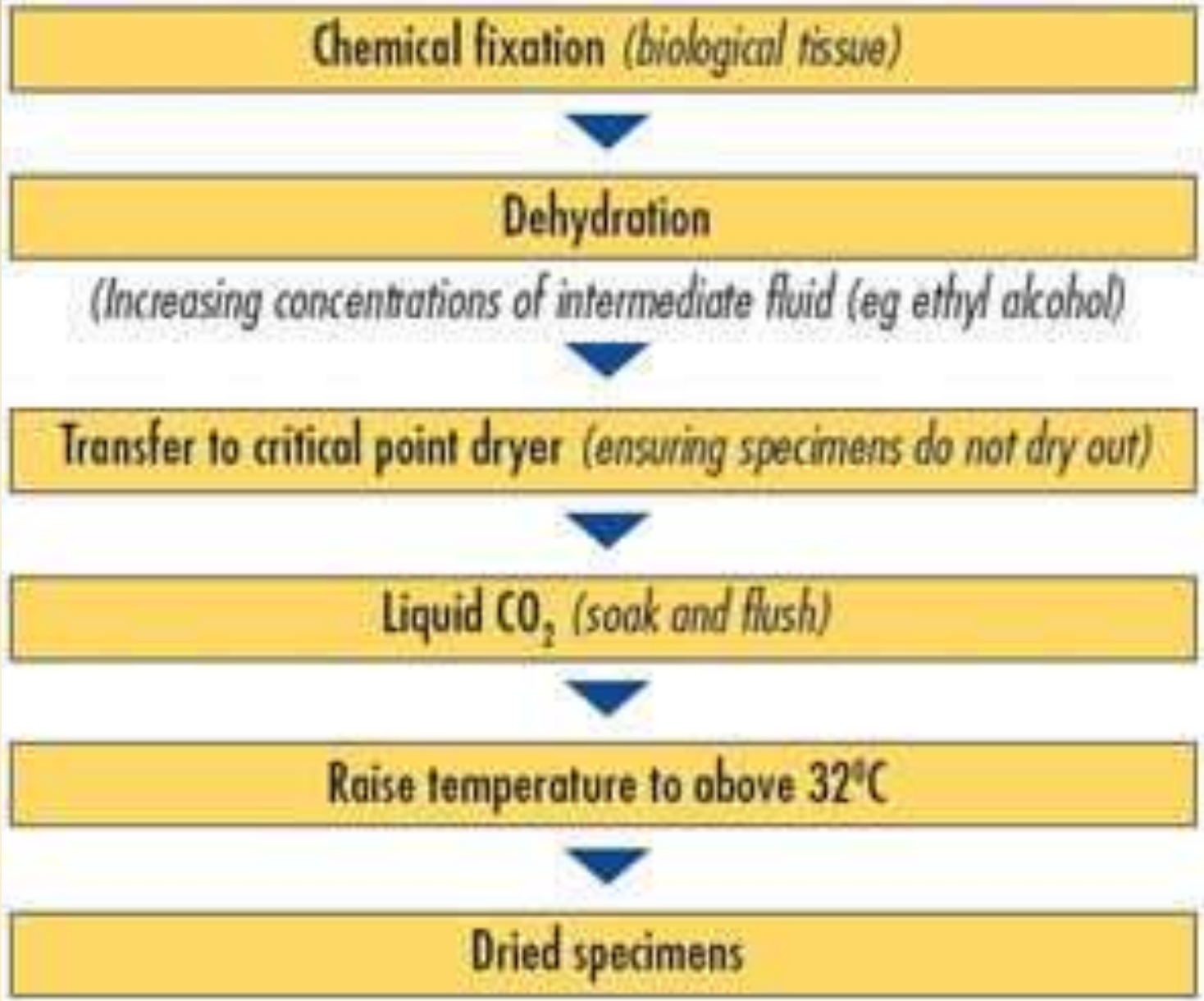


Sputter Coating

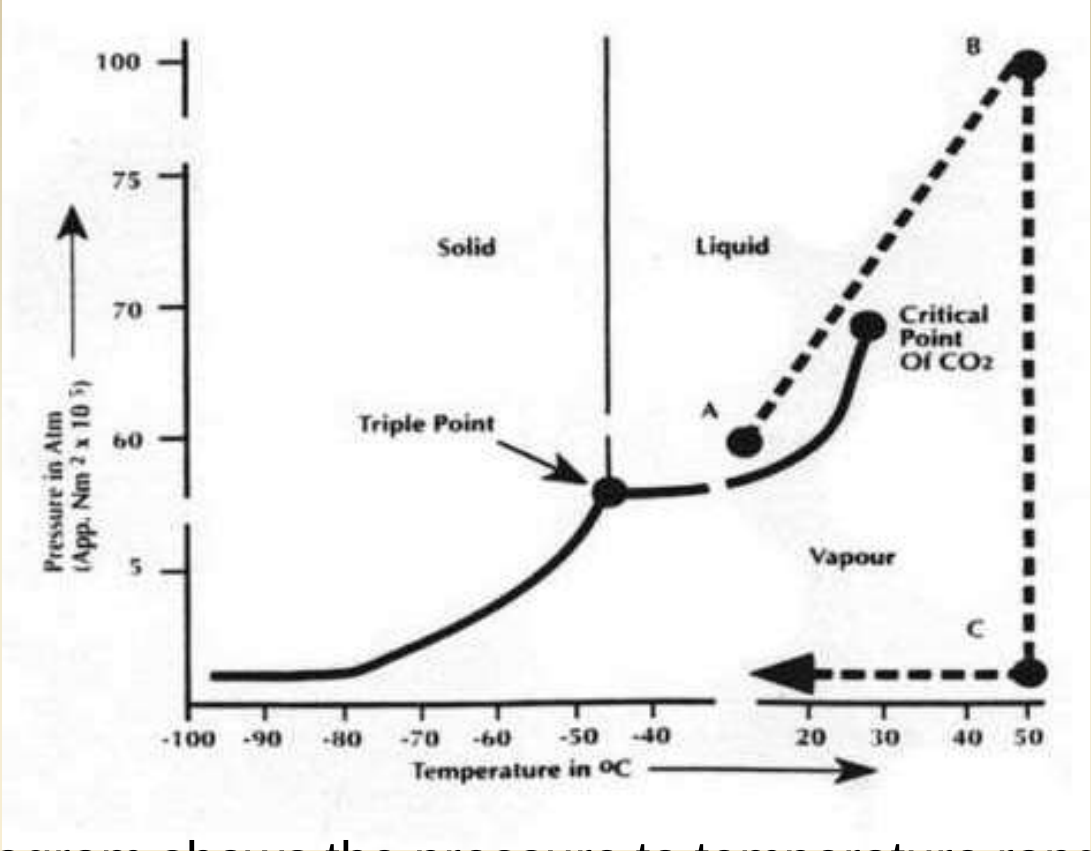


Modern Coaters use a deposition control or the gas flow to adjust the current at a low sputtering voltage. Older style coaters use a higher sputtering voltage the current being controlled by a voltage regulator or the gas flow.

Summary of the Critical Point Drying Technique



Critical Point



The phase diagram shows the pressure to temperature ranges where solid, liquid and vapor exist.

The boundaries between the phases meet at a point on the graph called the triple point.

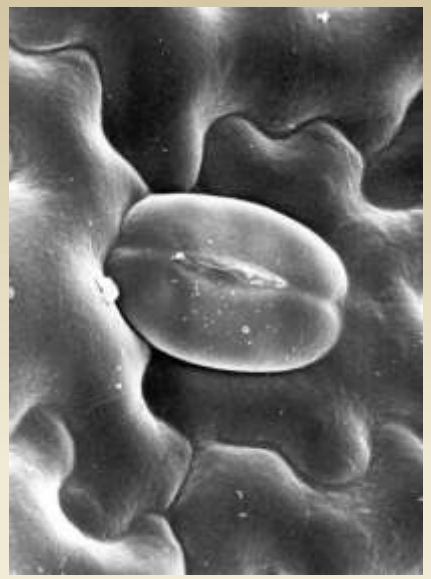
Along the boundary between the liquid and vapor phases it is possible to choose a particular temperature and corresponding pressure, where liquid and vapor can co-exist and hence have the same density. This is the critical temperature and pressure.

EMS 850 - features built-in chamber cooling and heating,

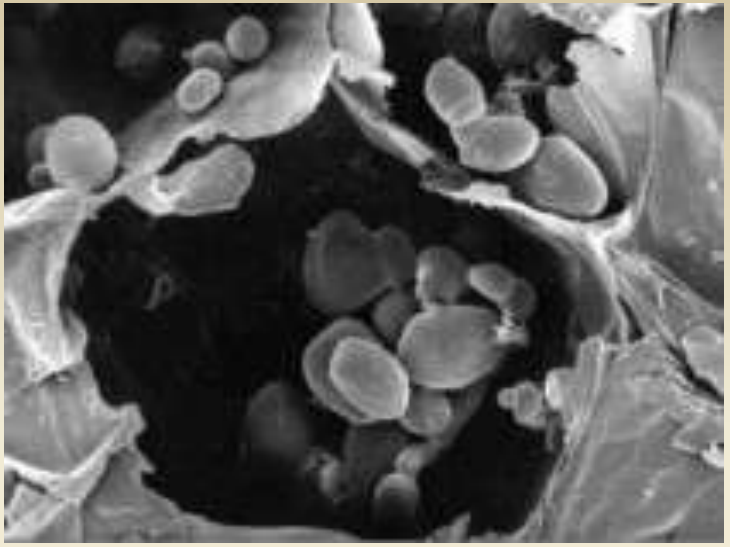
Specimen holder

12 Chambers

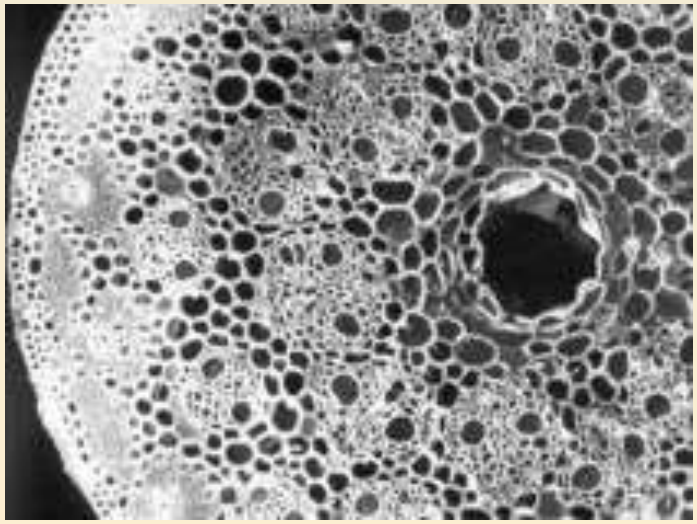
12 Porous pots



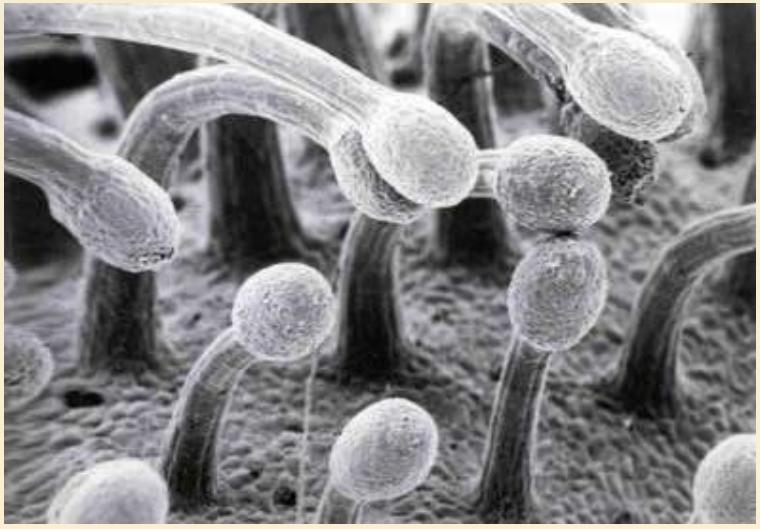
Raised stomatal pore



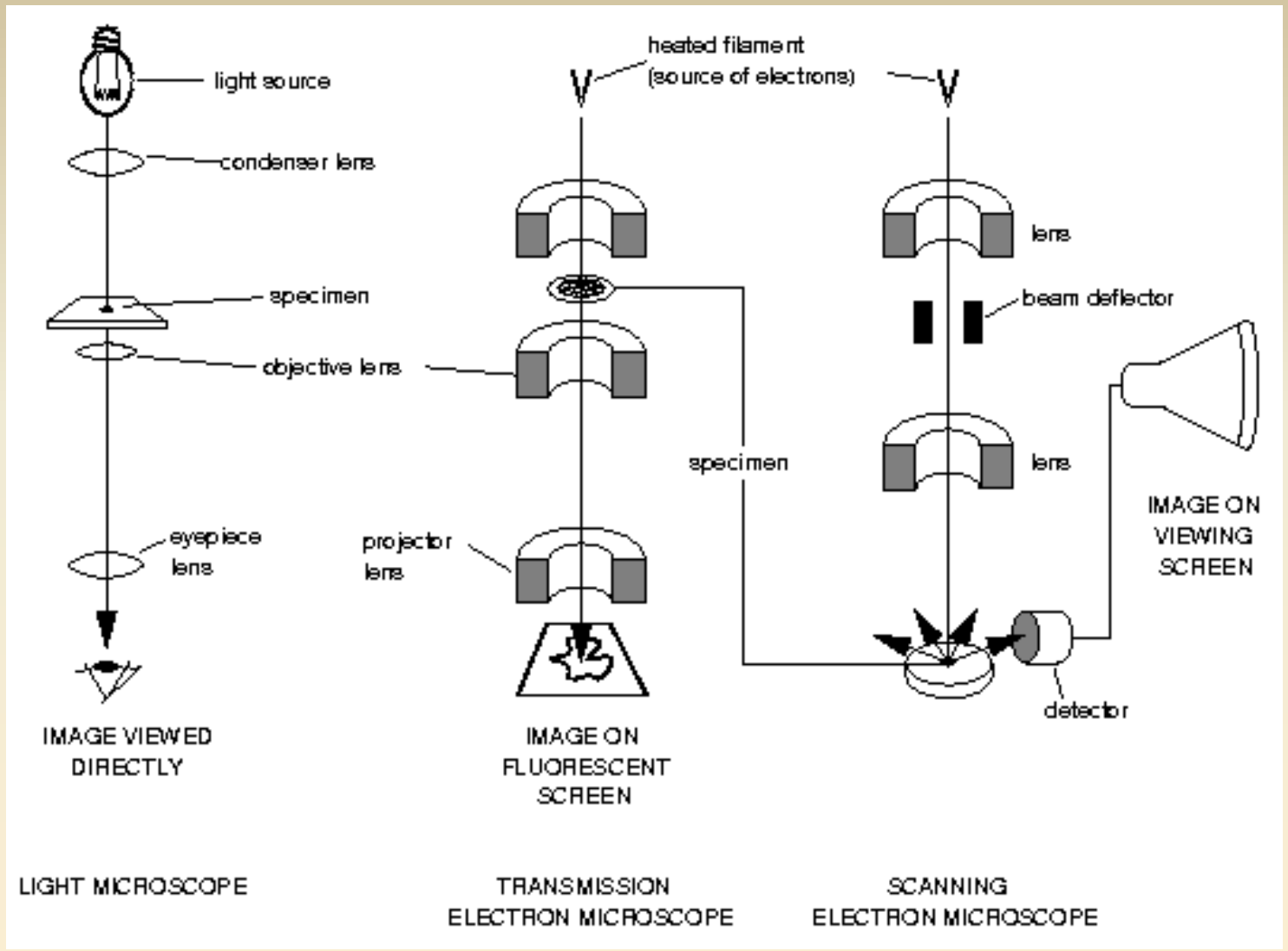
CPD – starch amyloplasts



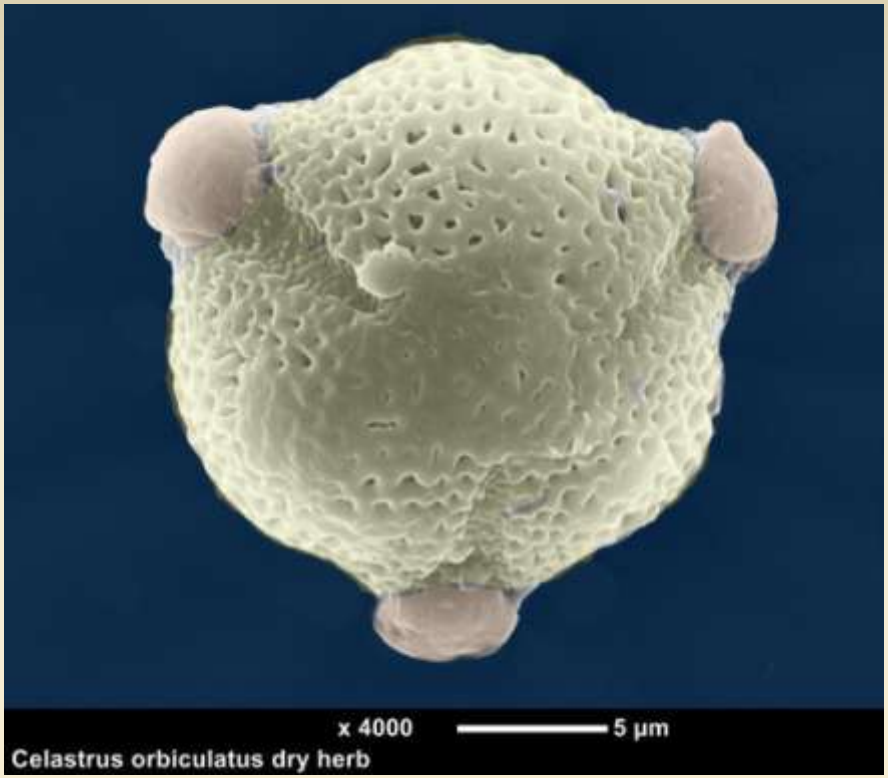
Bamboo Stem



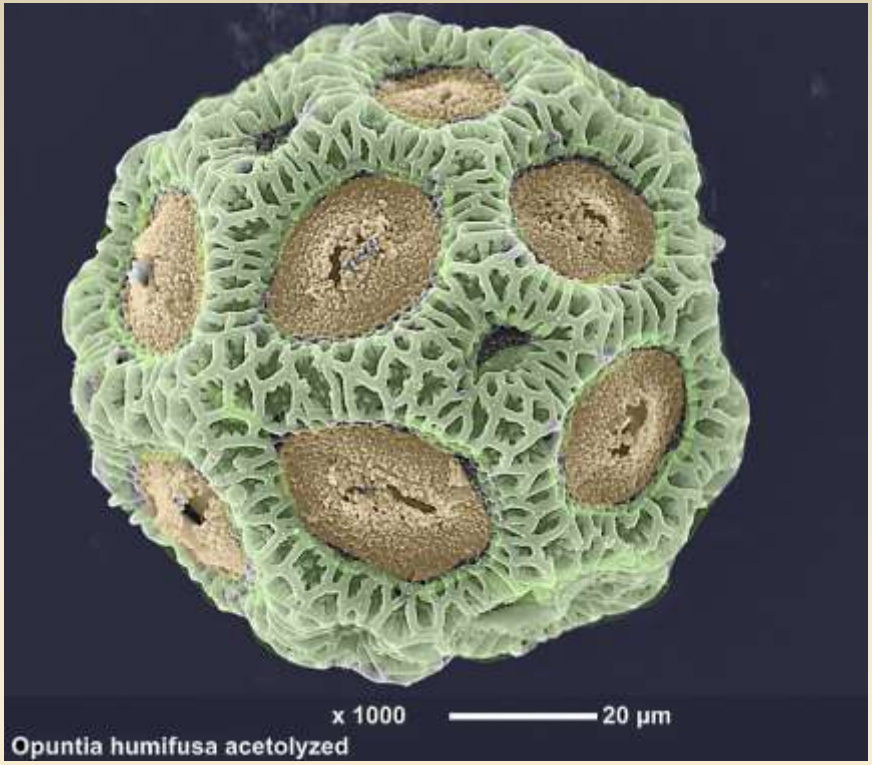
CPD - glands



Principal features of a light microscope, a transmission electron microscope (TEM), and a scanning electron microscope (SEM), drawn to emphasize the similarities of overall design. The TEM and SEM require that the specimen be placed in a high-vacuum environment.



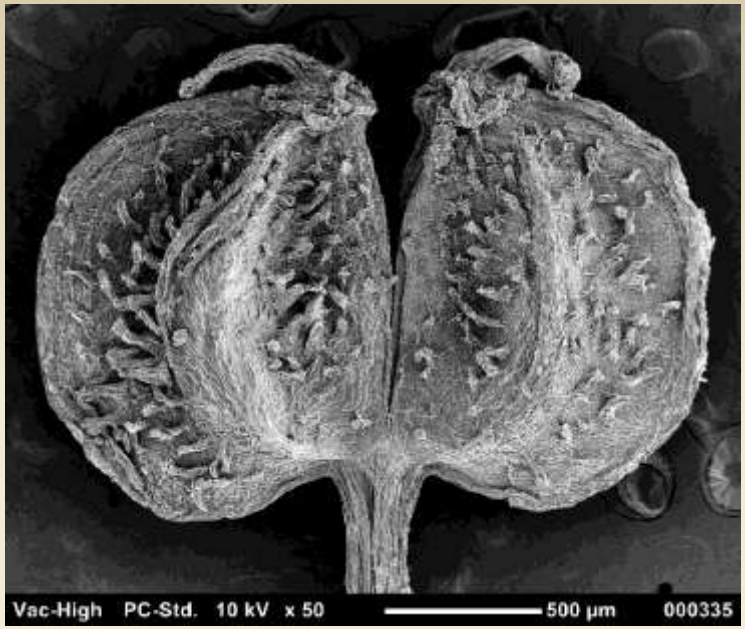
Celastrus orbiculatus



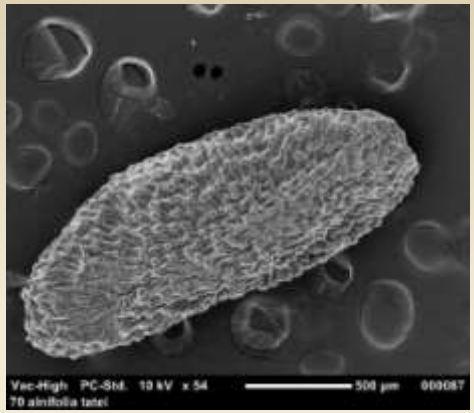
Opuntia humifusus

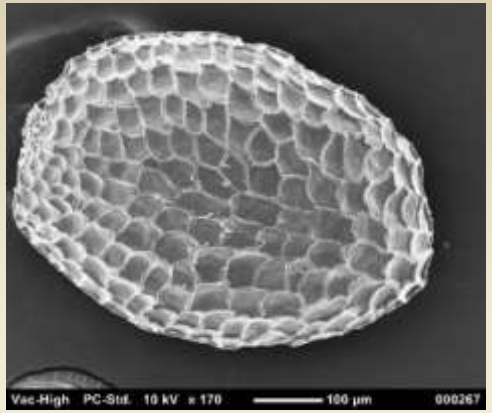
Rhododendron sp.

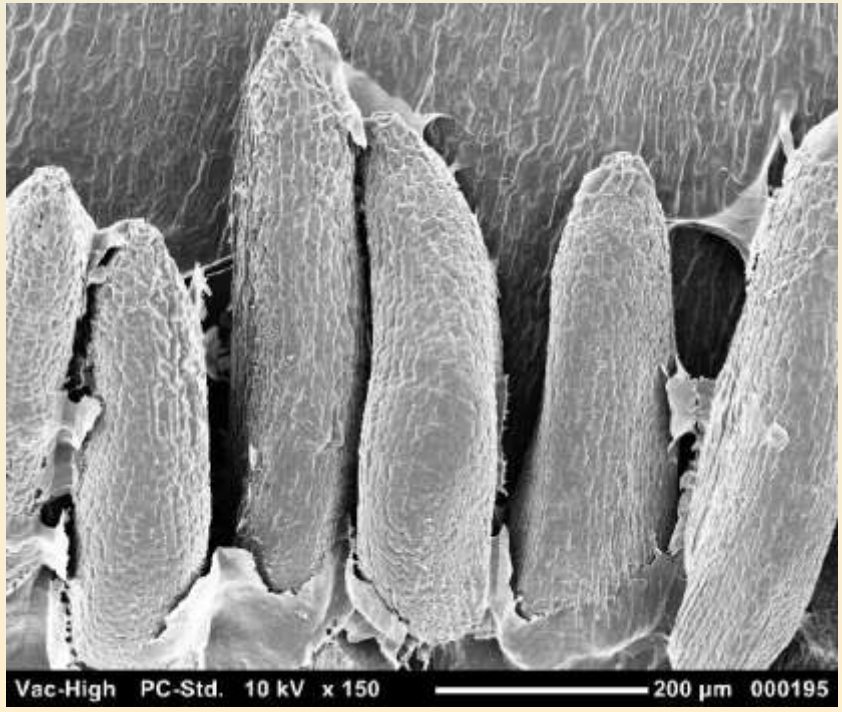
Mendoza – *Hydrocotyle* fruits



Nallarett Davila – Rubiaceae Parque Virua

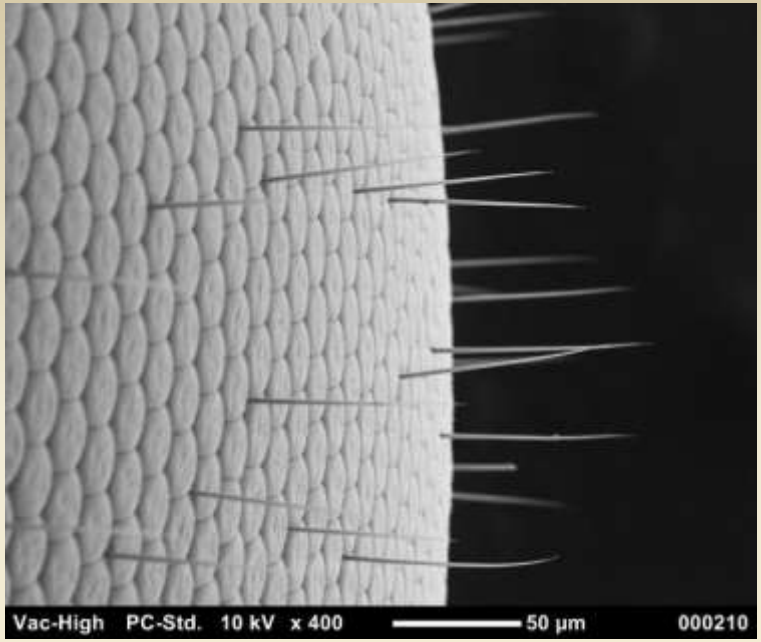


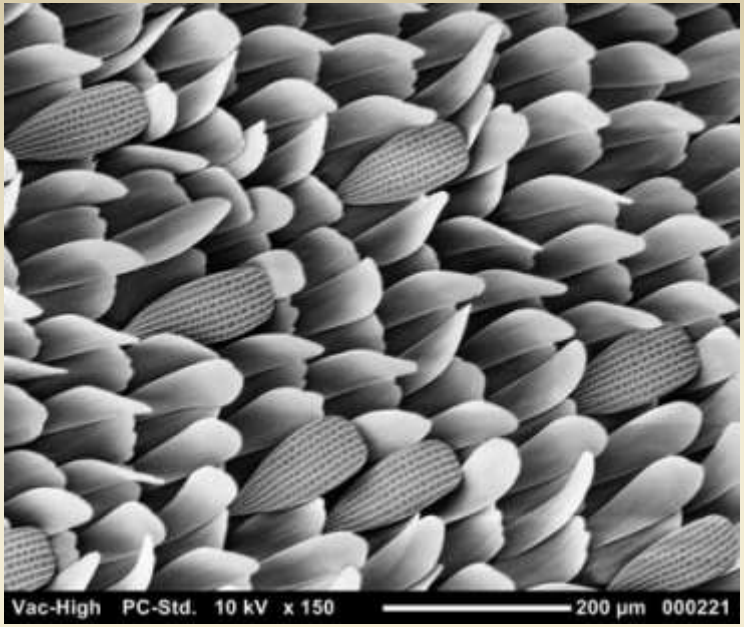


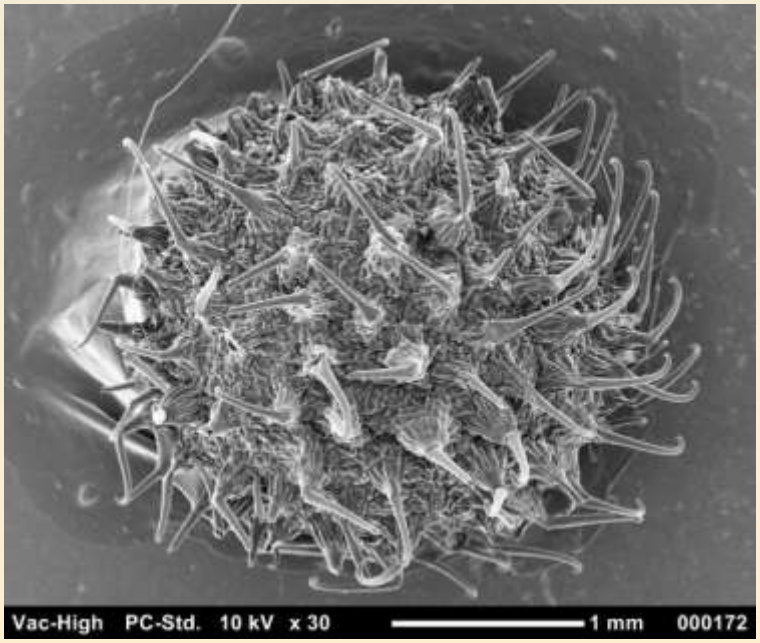


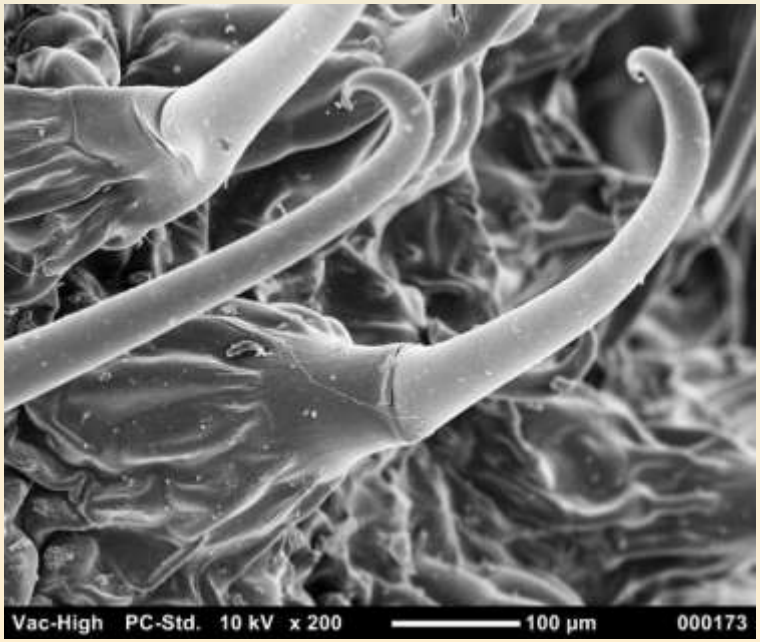
Colleters

Shaw Institute for Field Studies - High School Students

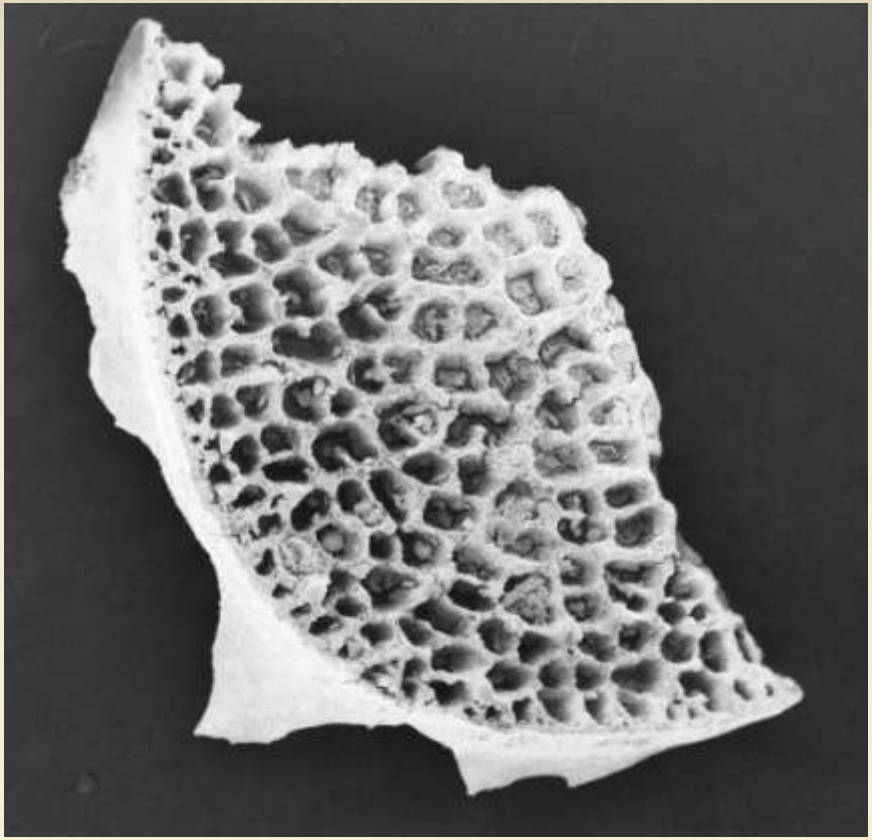


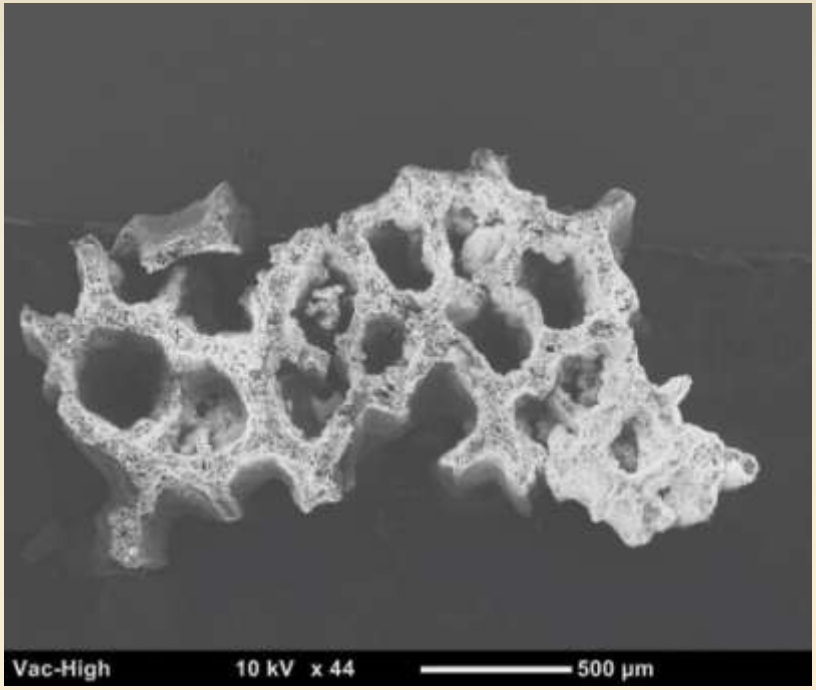




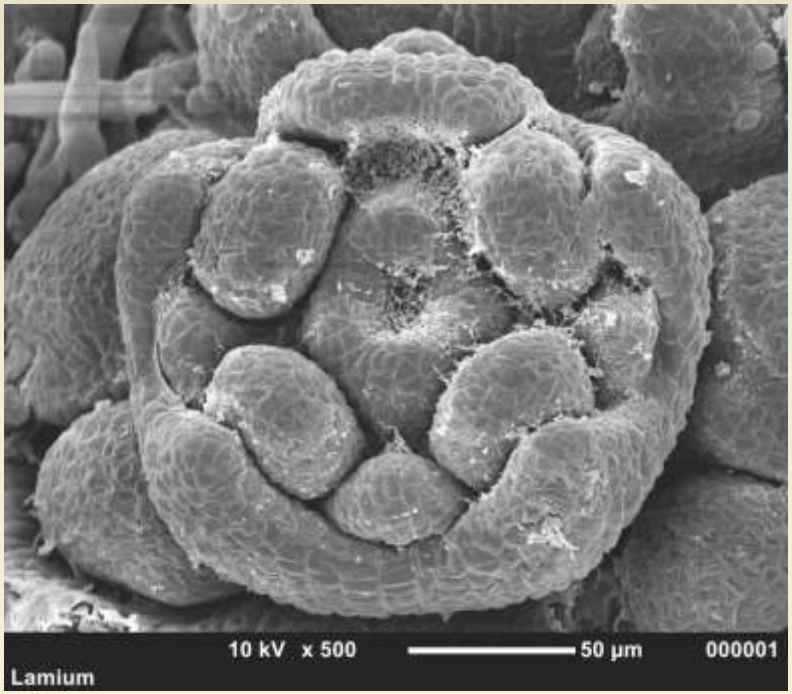


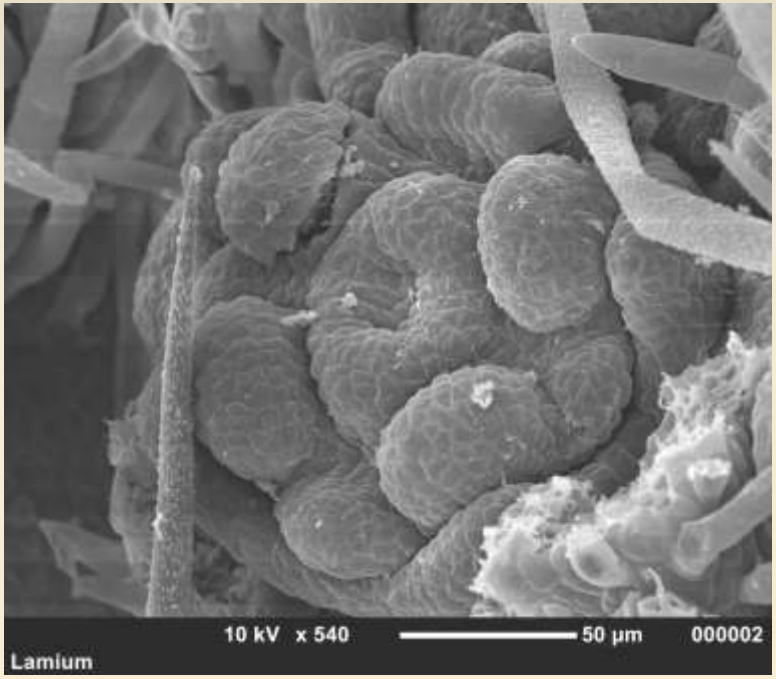
BrieAnna Langlie – carbonized potato, 3,000 bp

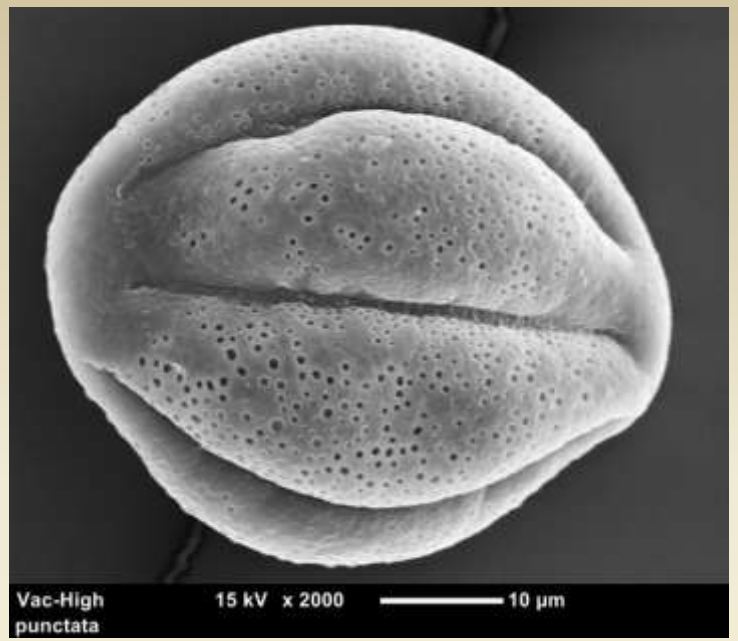




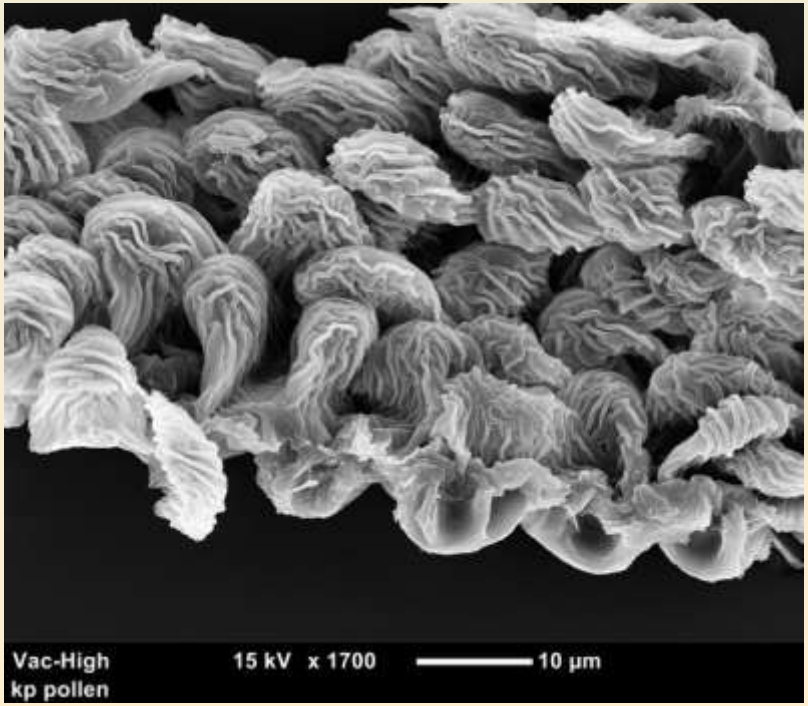
Jinshun Zhong – *Lamium* apices

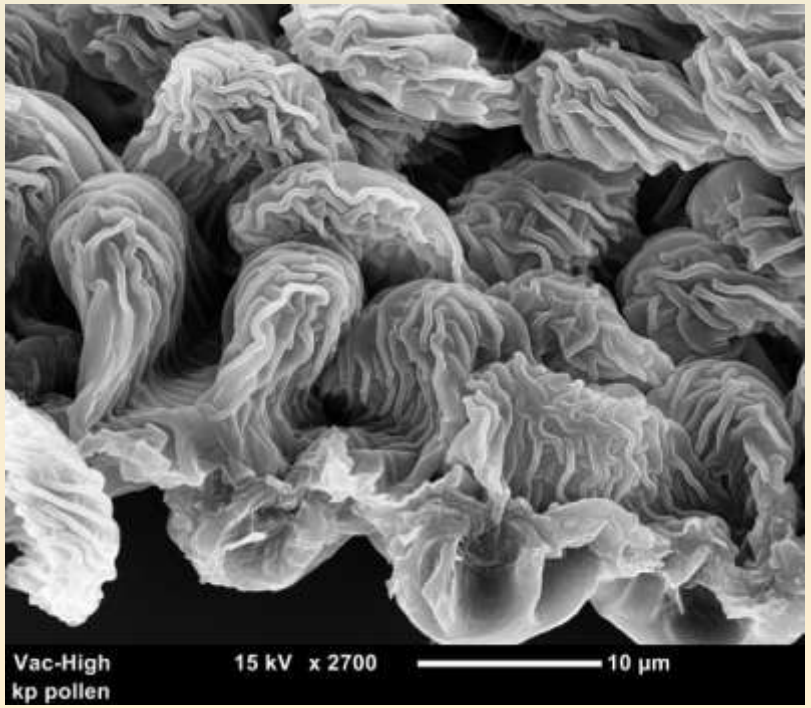






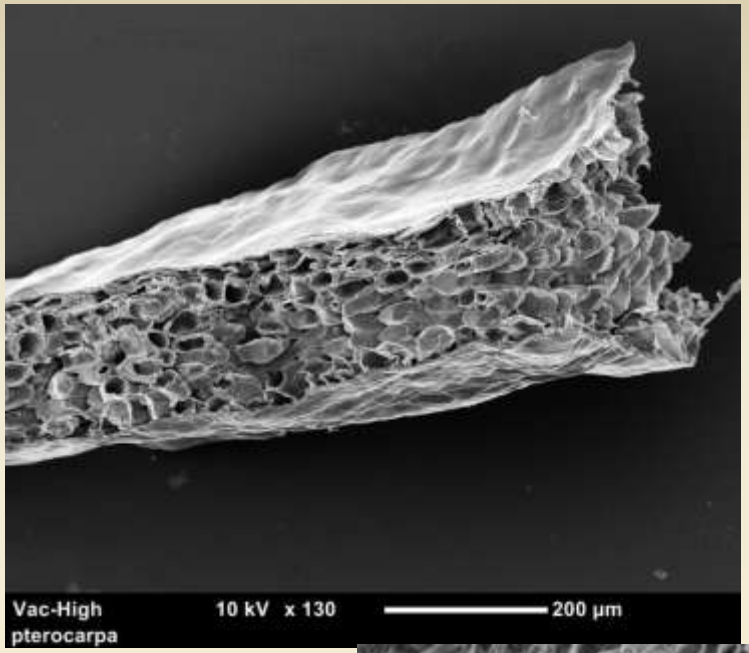
Katie Parks
Monarda

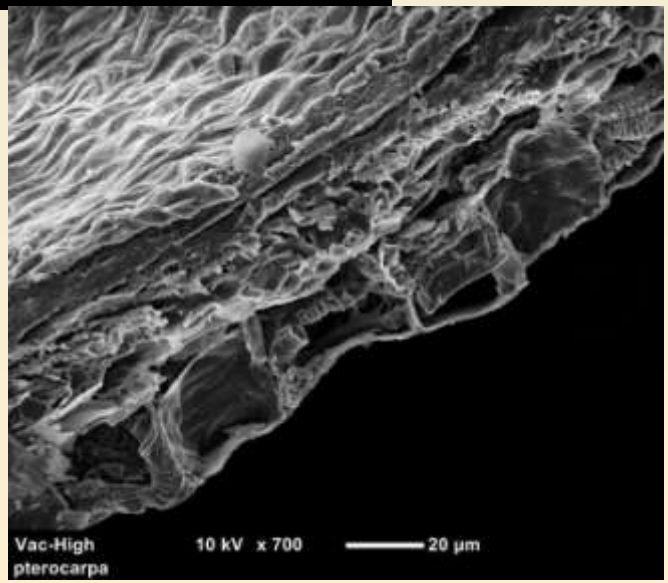




Peris Kamau – *Pteris* spores from Africa

Rachel Hillabrand – Lythraceae Seed Walls





End