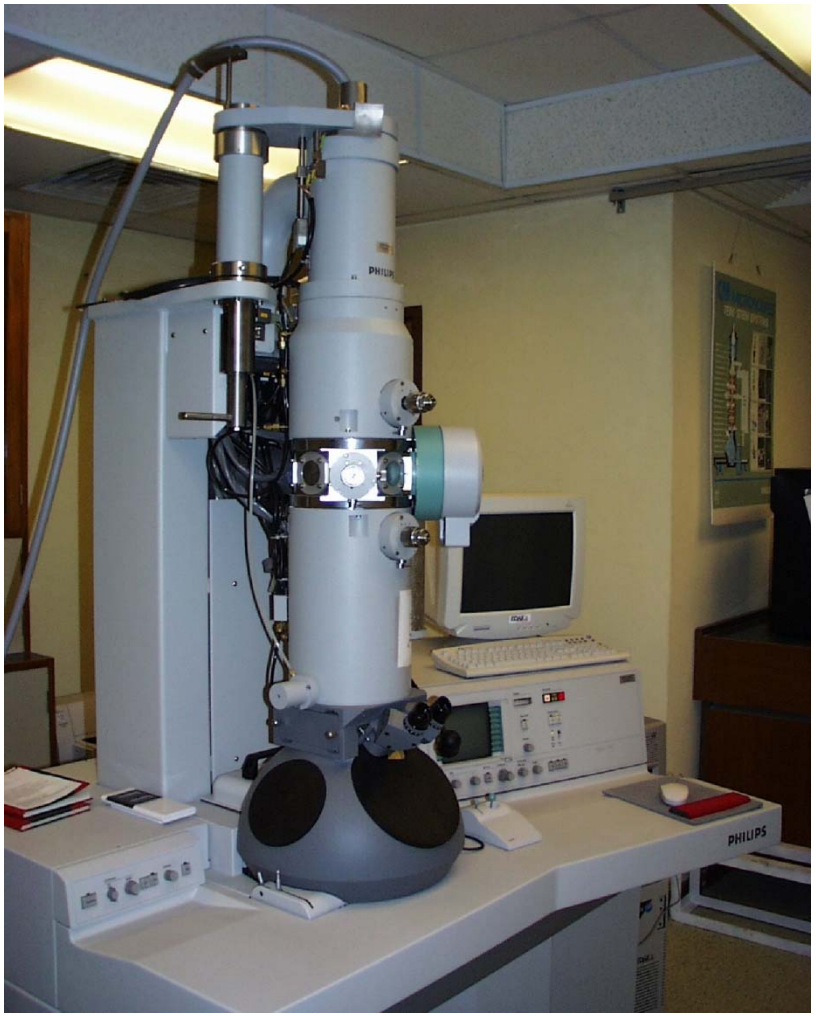
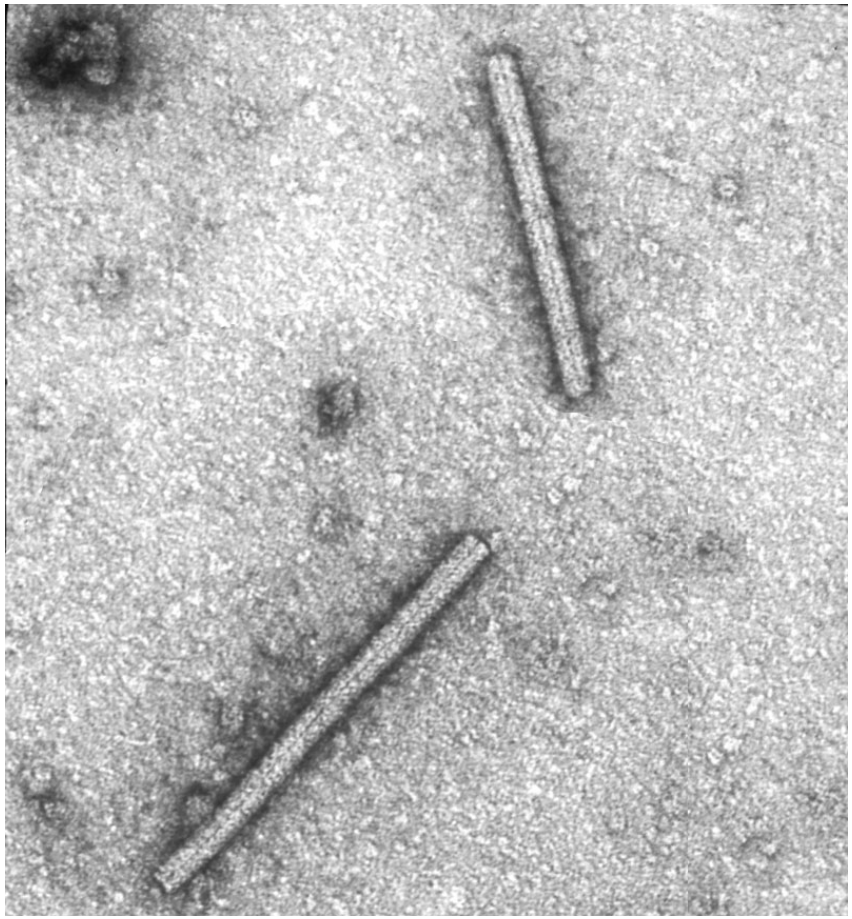
Laboratory 4:
Electron Microscopy





Objectives

- **Perform a simple negative staining of virus particles from a “leaf-dip” extraction**

- **Obtain images of possible virus particles associated with your “unknown” that you can use to help identify your virus**

Negative Staining and TEM

- a simple procedure to view virus particles
(also referred to as the 'leaf dip technique')

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History of the EM

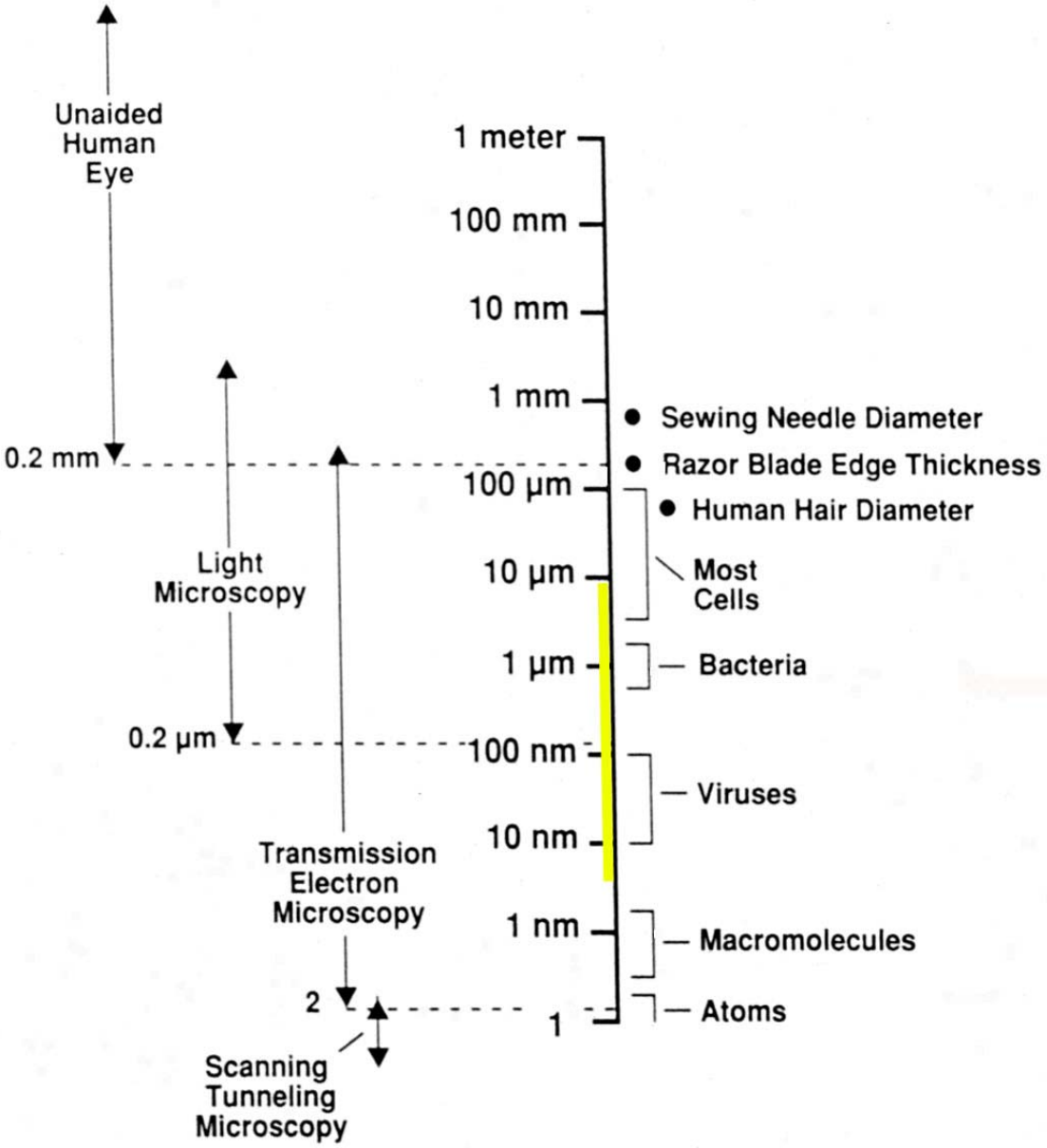
- Early developmental work of the instrument was done by physicists.

- The first operating EM was built in Germany in 1932 by the Ruska brothers.

  (What year did Takahashi observe virus particles for the first time ever?)

- The first commercial EM was available in 1938, and was primitive in comparison to modern EMs.

Limitations of imaging techniques



Units of Measurement

Common Units of Measure in Electron Microscopy

Prefixes:		Example
milli (m)	10^{-3}	millimeter (mm)
micro (μ)	10^{-4}	micrometer (μm)
nano (n)	10^{-9}	nanometer (nm)
Angstrom (Å)	10^{-10}	

1 nm = 10 Å

1000 nm = 1 μm

1000 μm = 1 mm

1000 mm = 1 m

Common Types of Biological Electron Microscopes (EM) and Their Applications in Plant Virology

Transmission Electron Microscopy (TEM)

* view particulate material such as viruses and bacteria

* view very thin sections of embedded material: high resolution enables fine detail of cell structure, organelles to be visualized

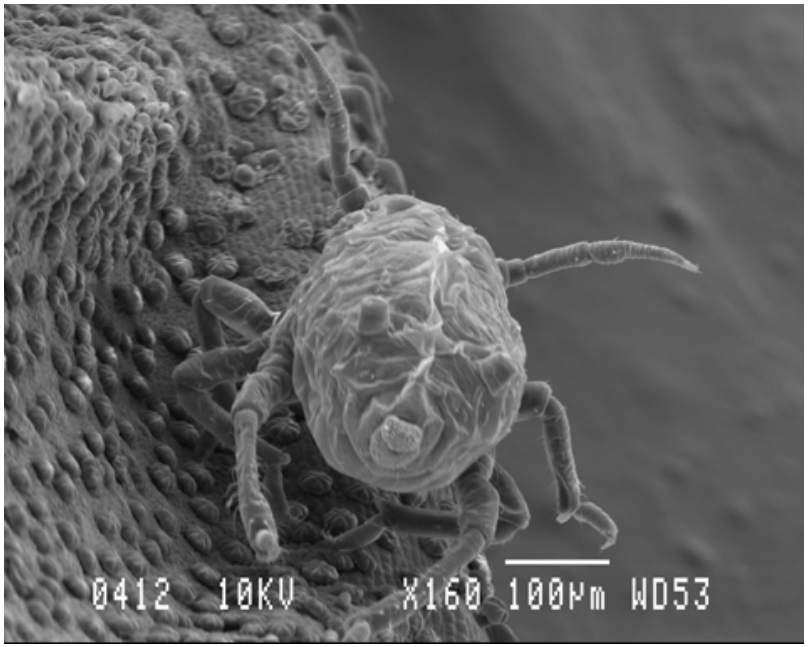
Scanning Electron Microscopy (SEM)

* view surfaces of three dimensional samples, with greater depth of field than with TEM or light microscopy, but lower resolution.

http://www.mos.org/sln/SEM/works.html

Examples of an SEM micrograph

- **Notice the three dimensional view SEM gives of the surface.**
- **Notice the great depth of field - all areas of the micrograph are in focus at the same time.**

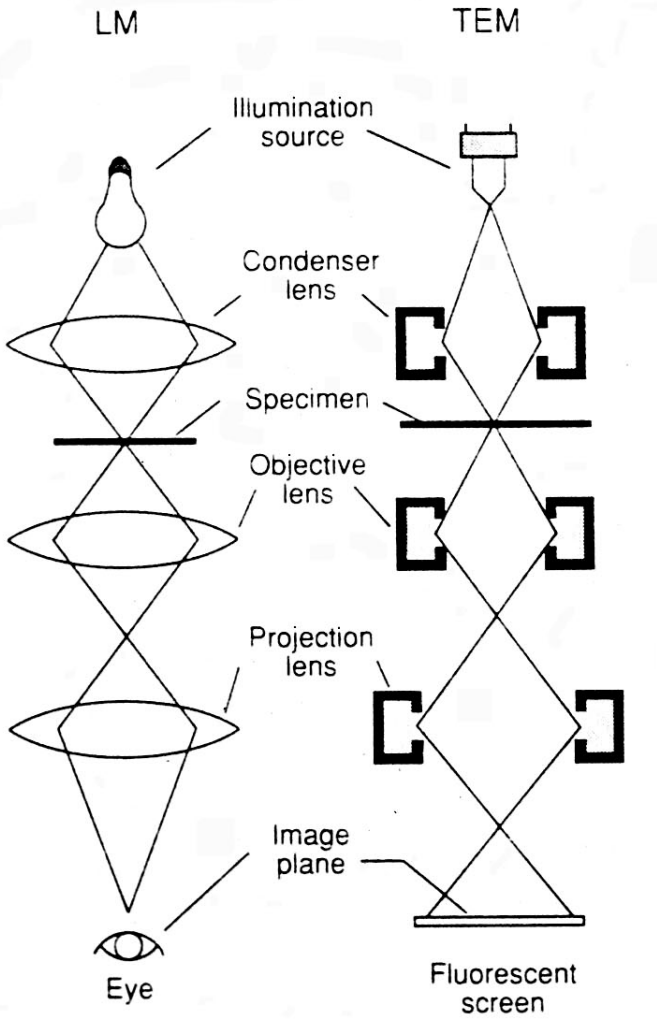


"flower" of silicon carbide and gallium

http://static.howstuffworks.com

Comparison of Optics of Light Microscopy with TEM

Beam of Visible Light



Beam of electrons

Diagram of a modern TEM

Major Systems:

Vacuum:

mechanical pump plus a high vacuum pump

Illuminating:

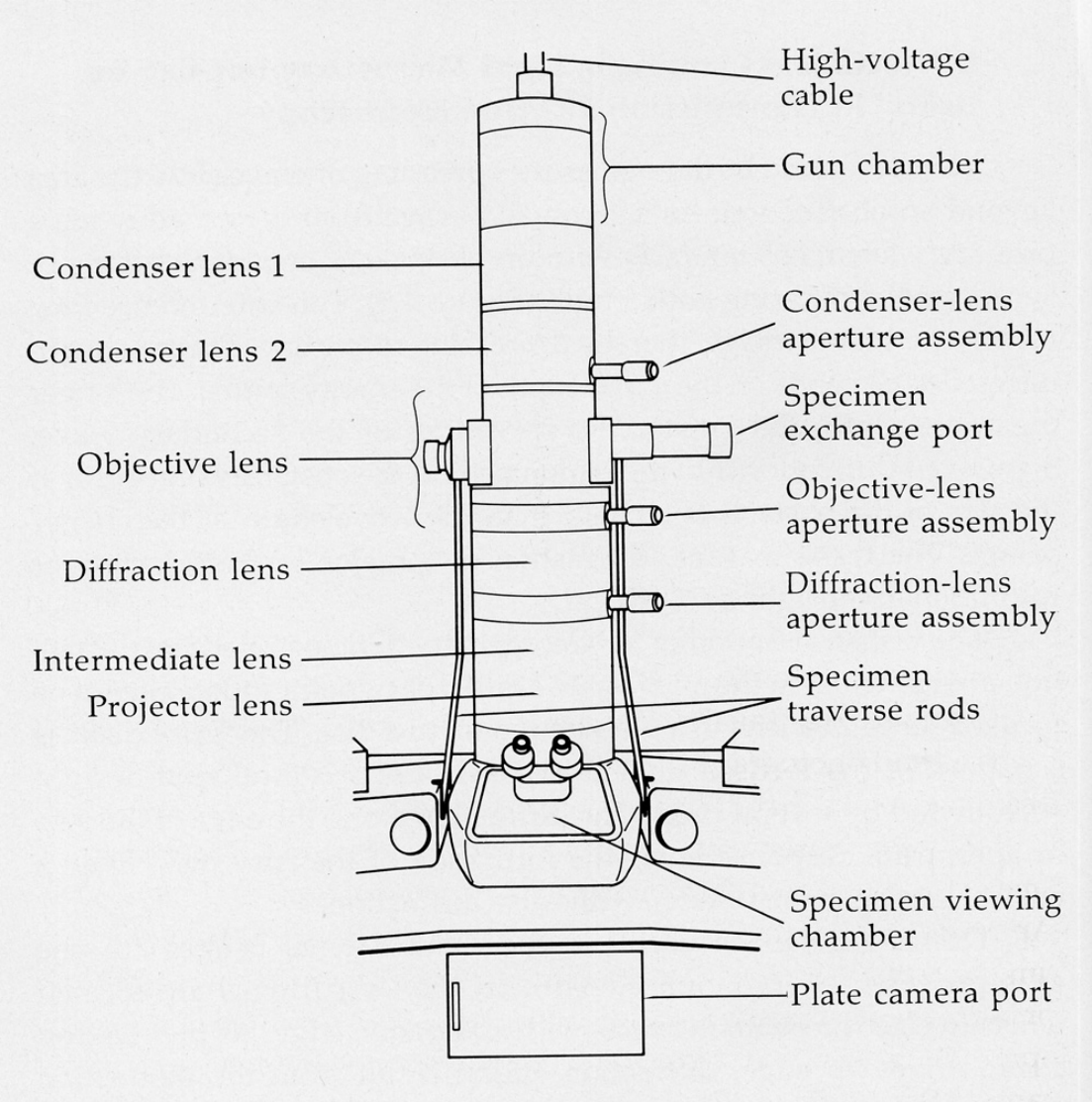
high voltage electron gun

Imaging:

Electromagnetic lenses

Viewing screen

Camera(s)



Good animation: http://www.doitpoms.ac.uk/tlplib/tem/index.php

Why use Negative Staining?
Improves contrast in the TEM

- Contrast depends on the atomic number of the atoms in the specimen; the higher the atomic number, the more electrons are scattered and the greater the contrast.

- Biological molecules are composed of atoms of very low atomic number (carbon, hydrogen, nitrogen, phosphorus and sulphur).

- Thin sections of biological material are made visible by selective staining.

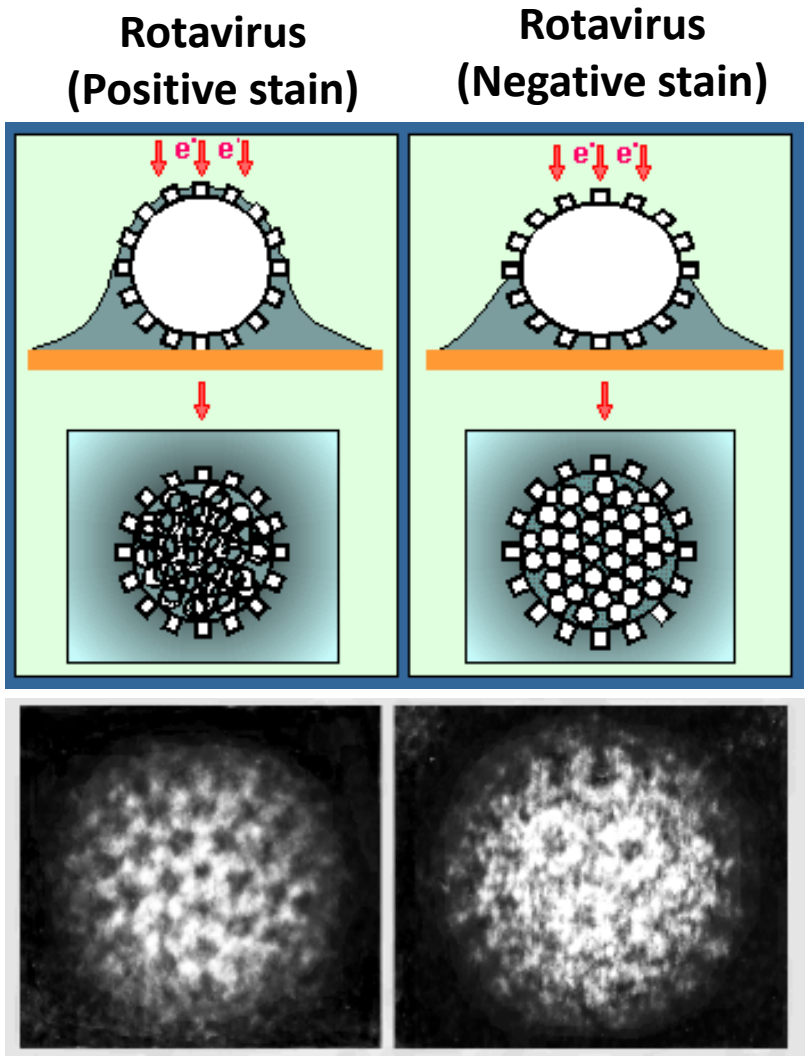
- This is achieved by exposure to salts of heavy metals such as uranium, lead, gold, and osmium, which are electron opaque.

How viruses can be seen in the EM

Viruses have no color and are transparent to electrons.

Electron-dense "stains“ (heavy metals) scatter electrons creating shadowing and contrast between viruses and the background (negative staining).

If virus particles are coated with stain (positive staining), fine detail may be obscured.



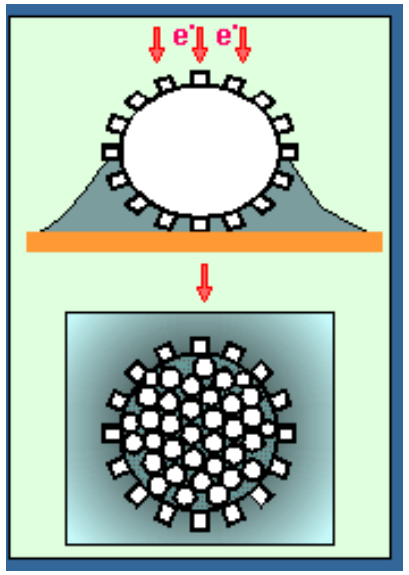
http://web.uct.ac.za/depts/mmi/stannard/negstain.html

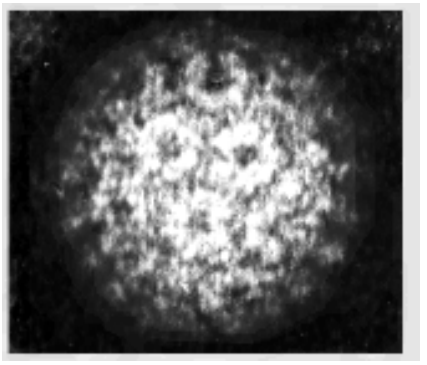
How viruses can be seen in the EM

Negative staining is used in transmission electron microscopy, and only electrons which pass through the specimen are involved in the formation of the final image.

The background is stained, leaving the actual specimen untouched, and thus visible.

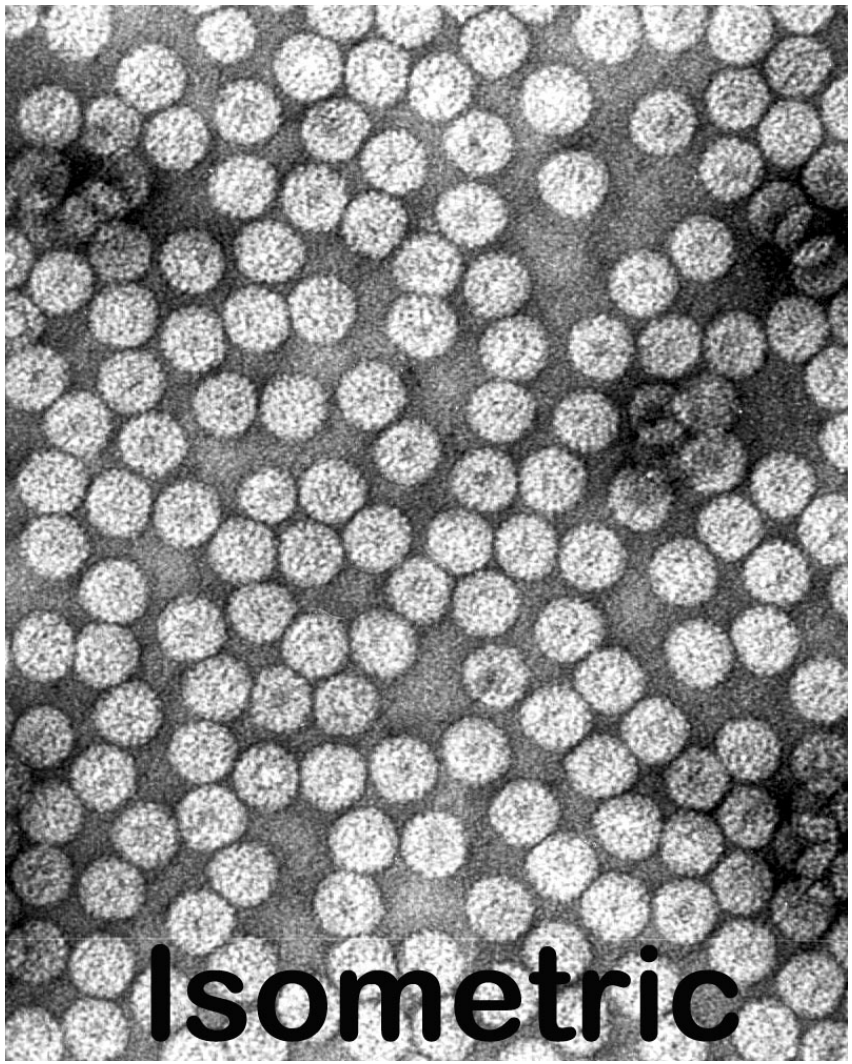
Negative staining stains the background and leaves the virus relatively untouched. The negative stain molds around the virus particle, outlining its structure, and is able to penetrate between small surface projections delineate them.
If there are cavities within the virus particle that are accessible to the stain, these will be revealed and some of the internal structure of the virus may be disclosed.

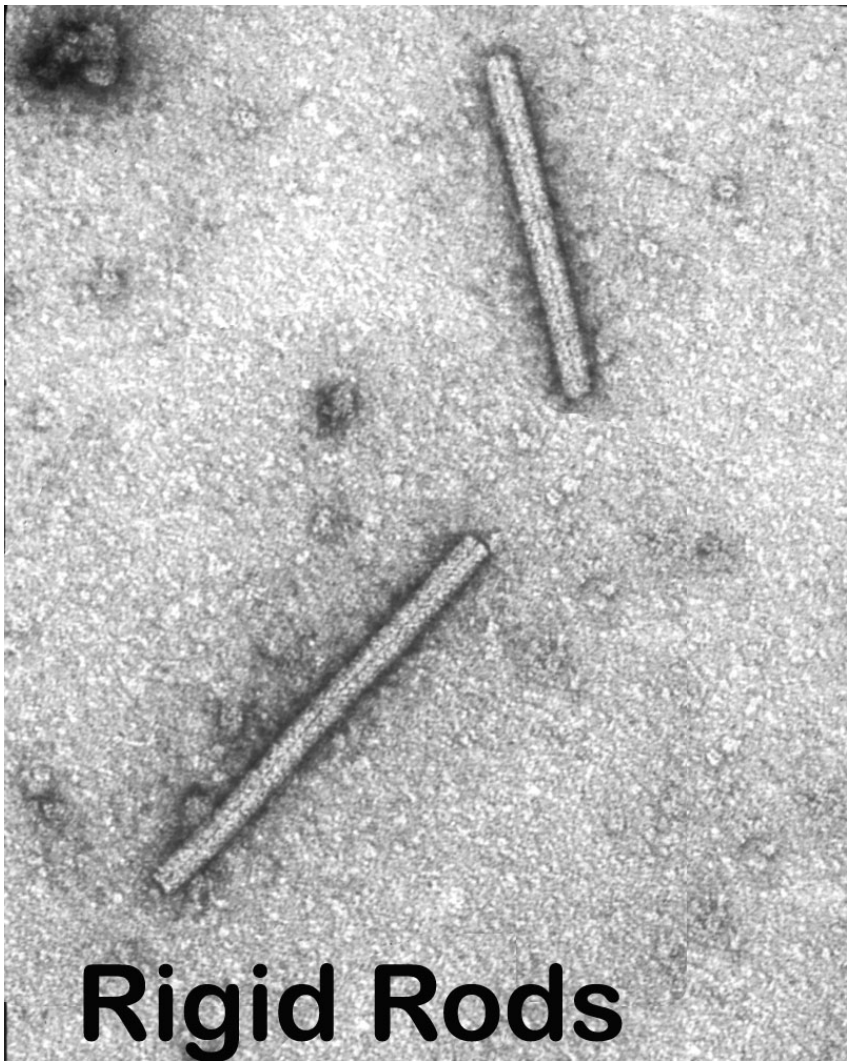




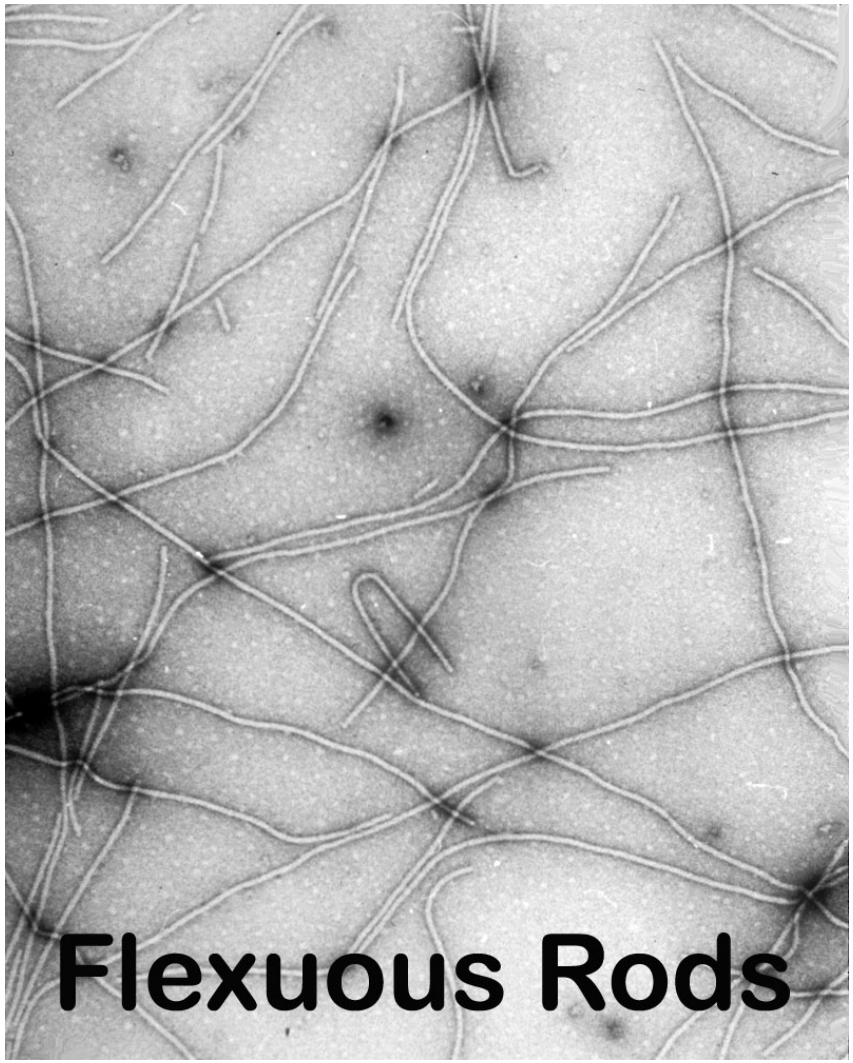
From: http://web.uct.ac.za/depts/mmi/stannard/negstain.html

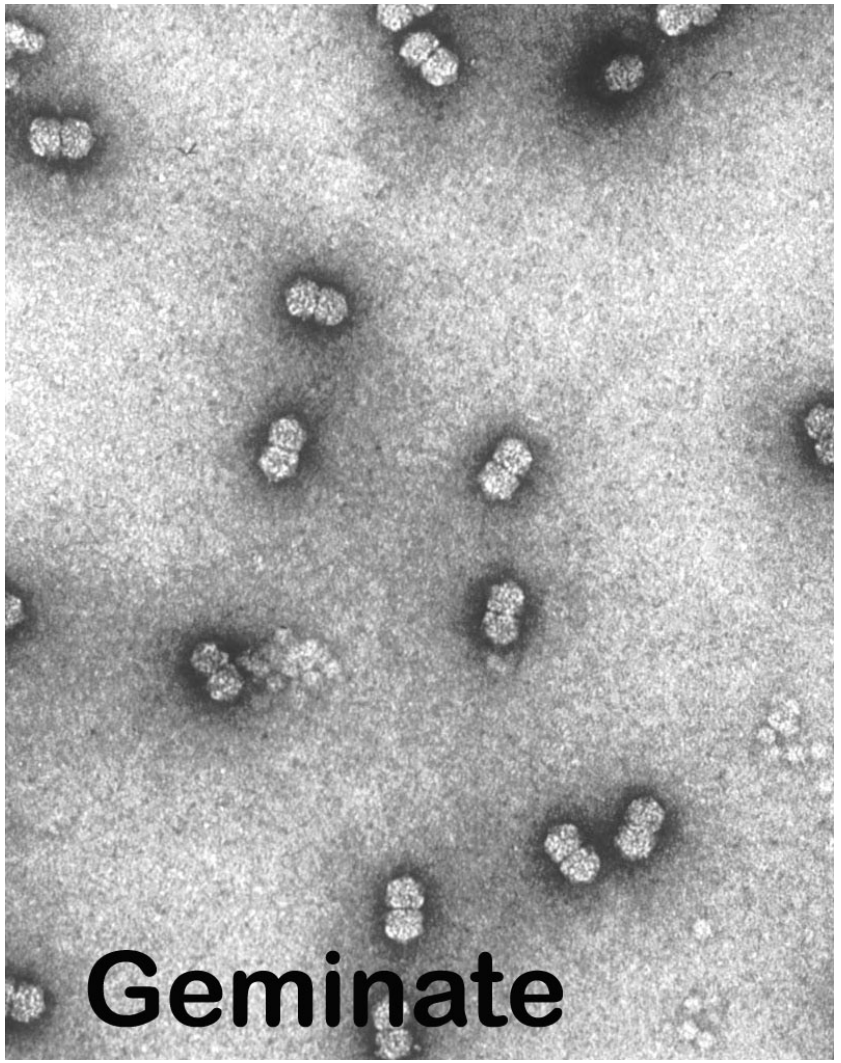
TEM: negative staining reveals the morphology of virus particles





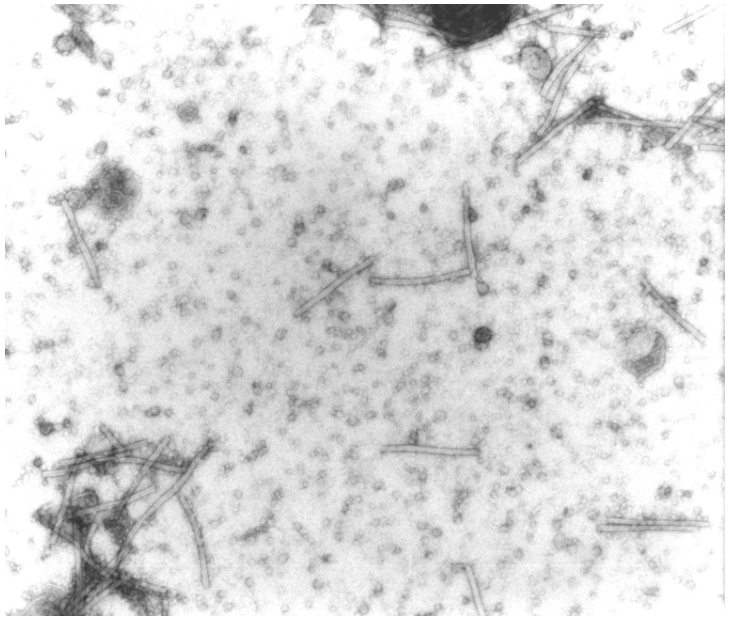
TEM: negative staining
reveals the morphology of virus particles





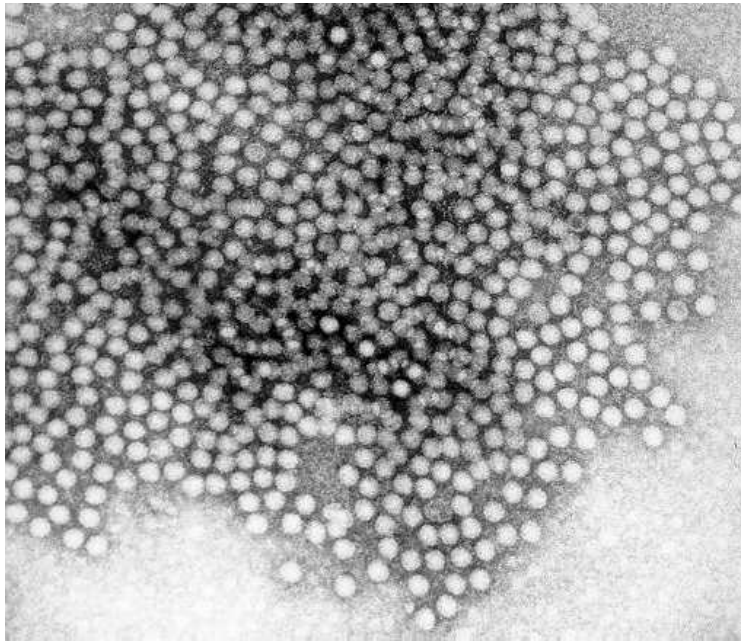
Negative stained virus particle (using ‘leaf dip technique’)

Notice the amount of background (cellular debris)



Negative stained virus particles from purified virus preparation

Low background and higher density of virus particles



Today:

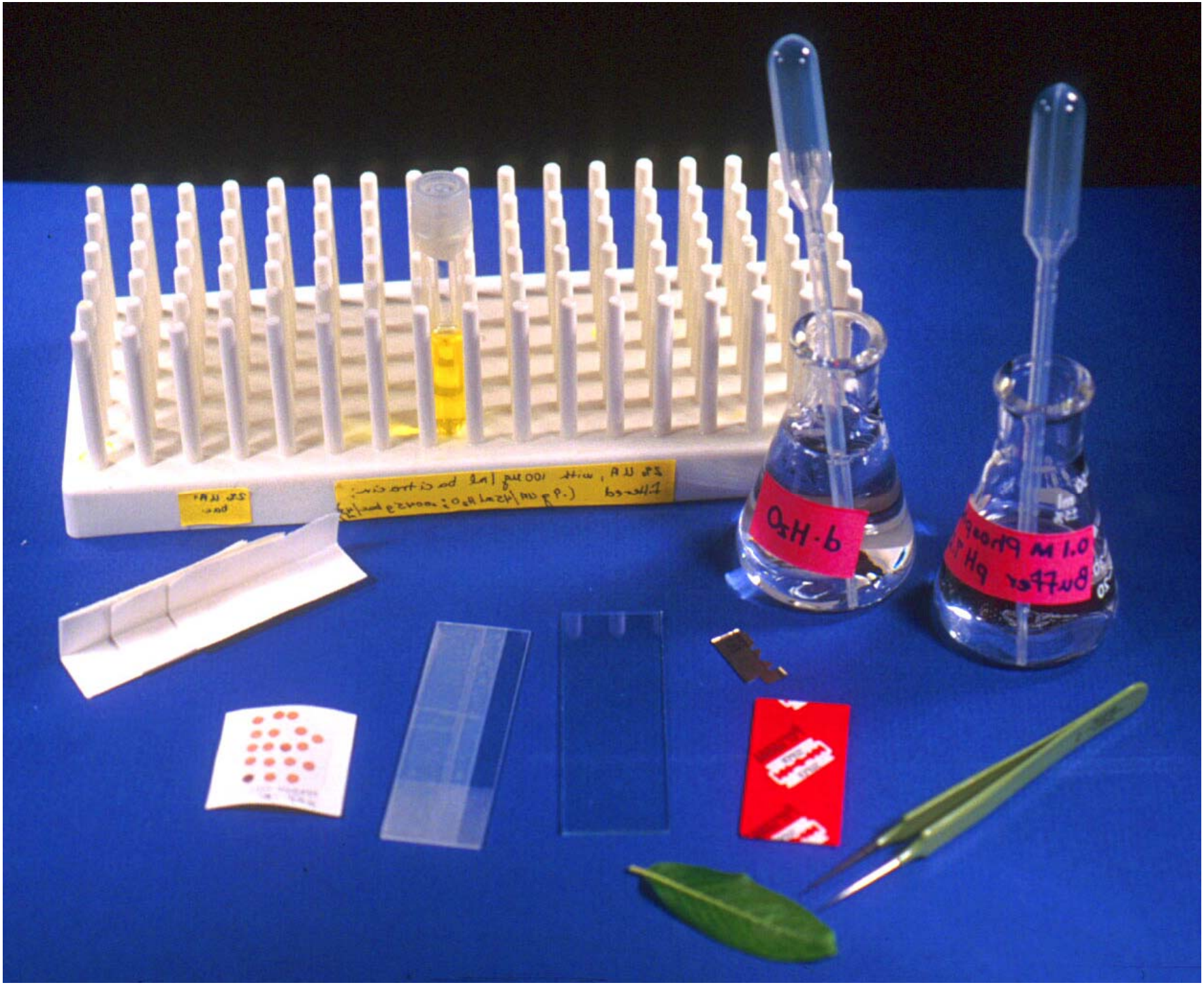
- Ms. Kelley will demonstrate and supervise you on the “leaf dip” technique

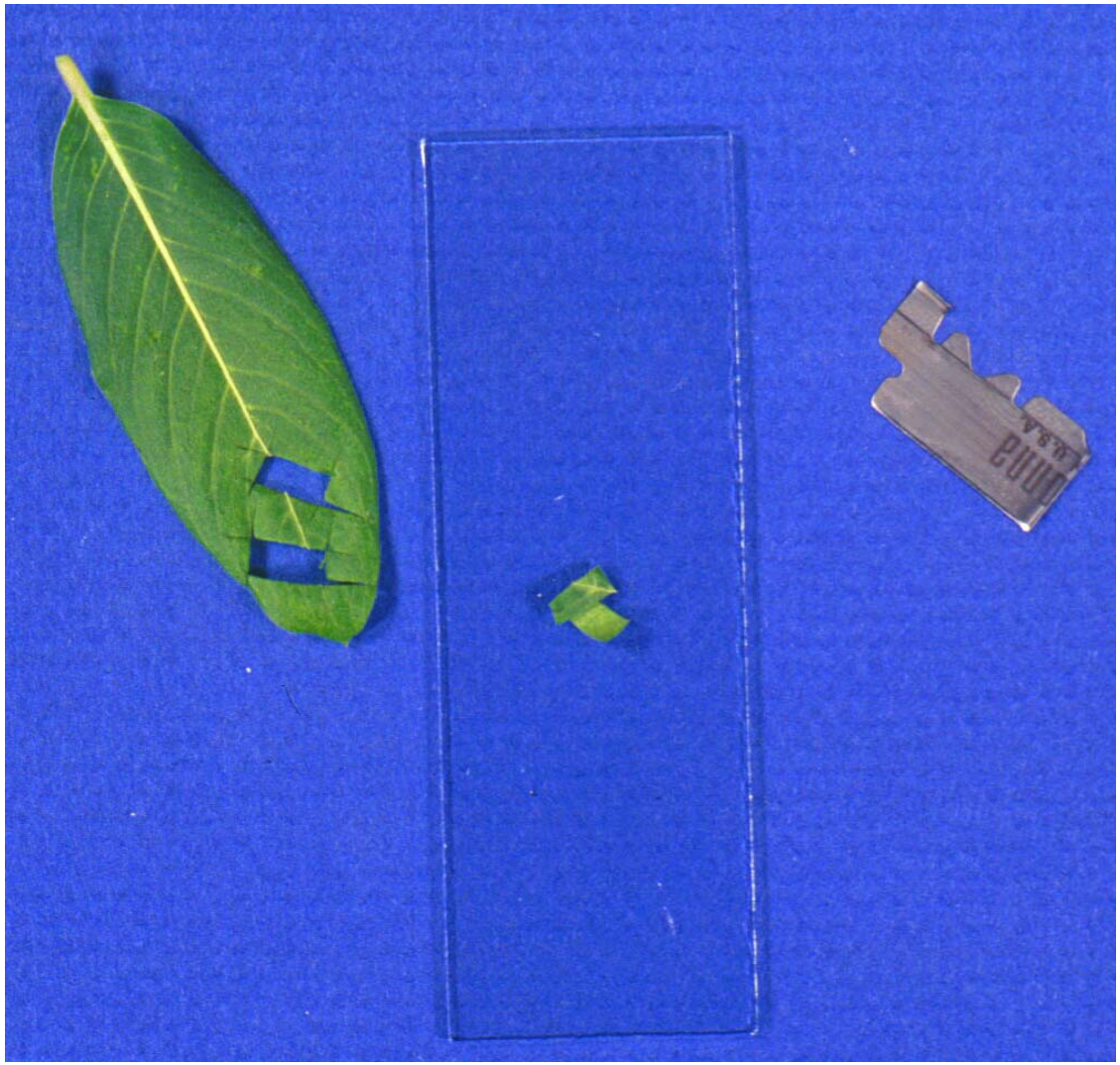
- You will prepare a grid with a sample of your unknown

- Each team has arranged for a time to view their grids on the EM at the ICBR (Dept. of Microbiology and Cell Science)

When you go to the EM:

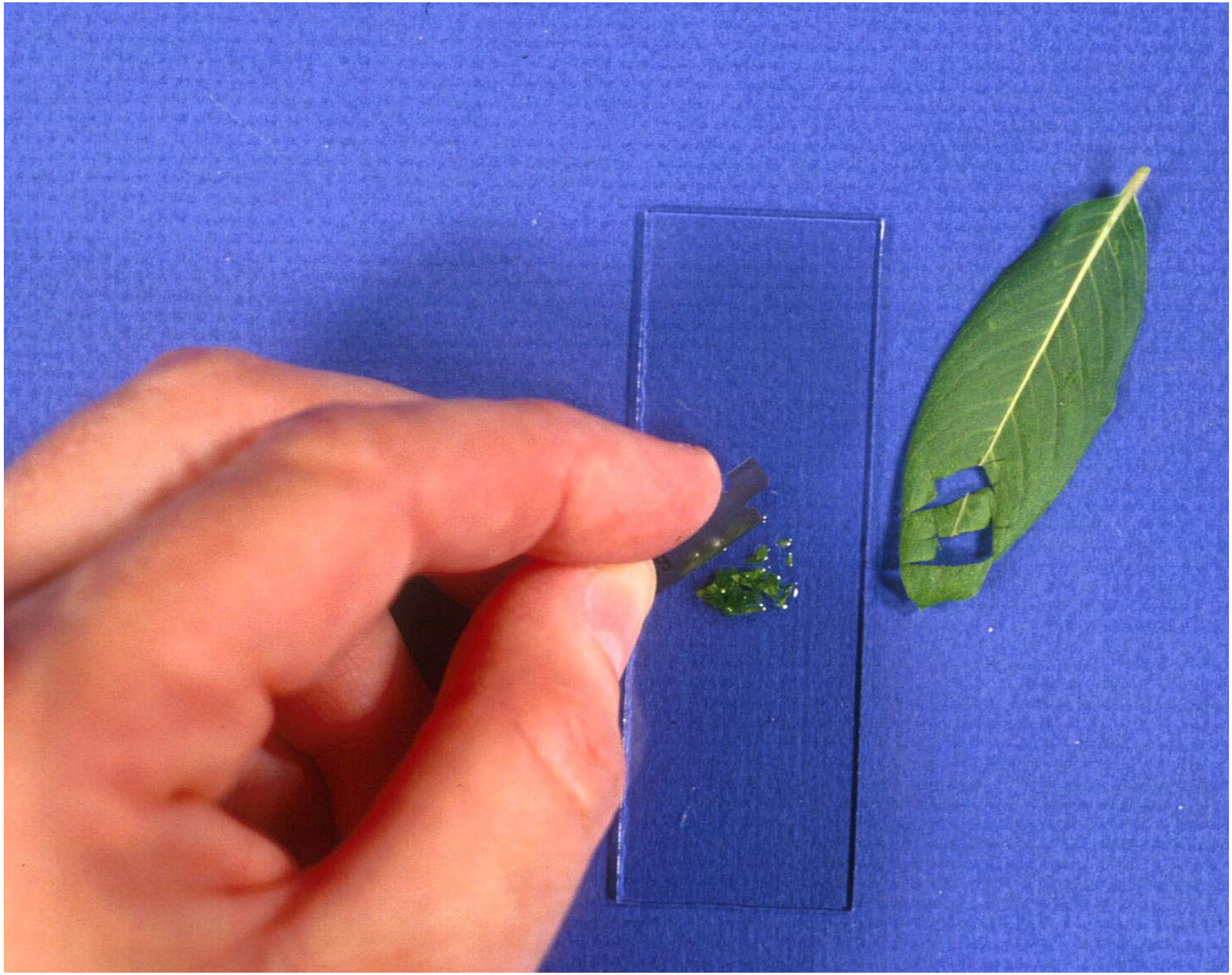
- Bring plant tissue (in case there is a problem with the grid)

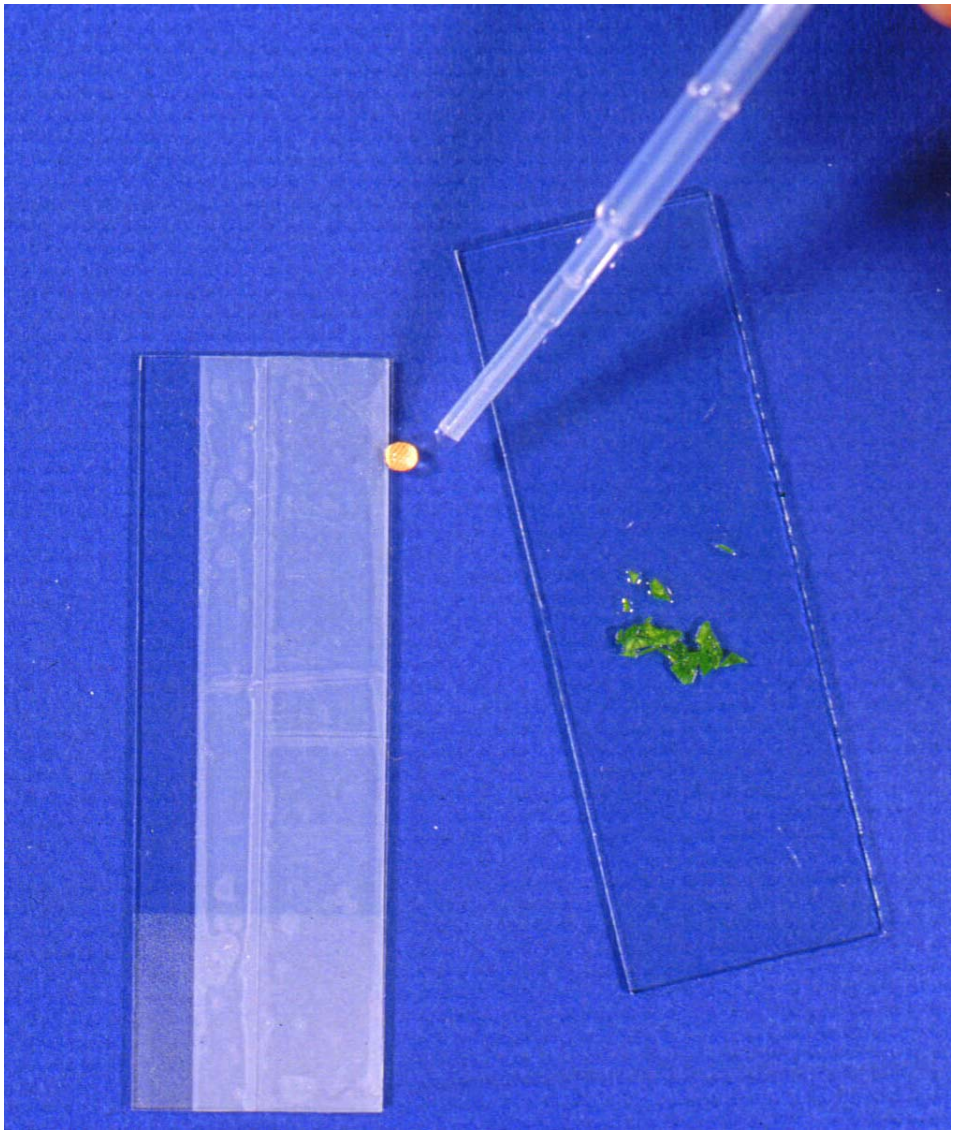

O2H-6



1. Select a young to middle age leaf showing typical symptoms

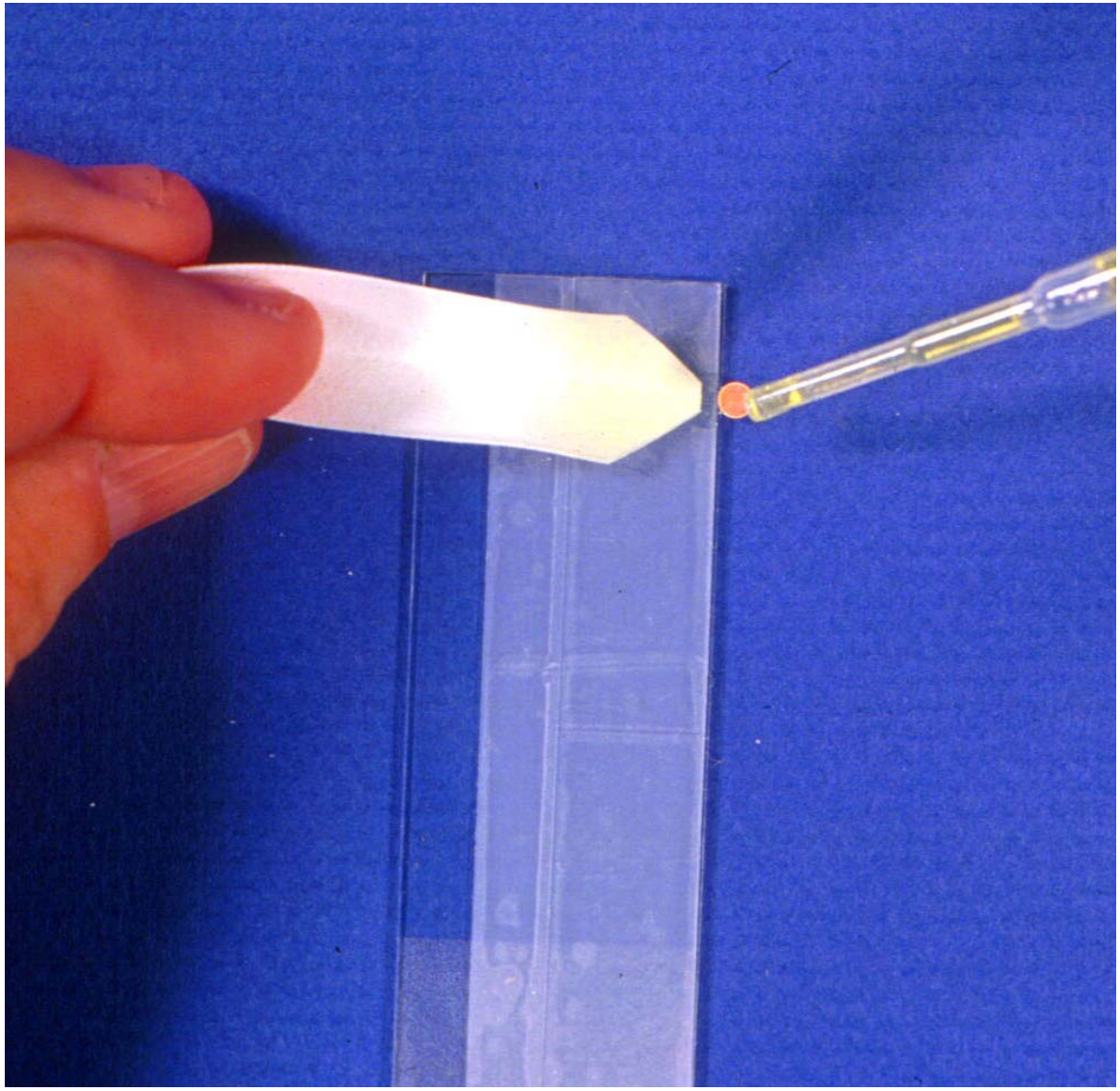
2. Chop plant tissue in a few drops of the buffer to prepare a crude extract.





3. Transfer a drop of extract to a formvar coated grid and allow to stand one to several minutes.

4. Blot extract with filter paper, then wash with buffer, and then wash with water.



5. Stain grid with two drops of uranyl acetate*

6. Transfer grid to a grid box or petri dish and allow to air dry several minutes before viewing in the TEM.

7. Insert grid into TEM and scan the grid for virus-like particles.

8. Take picture of image in the viewing chamber using cell phone/camera