

Microscopy

MICROSCOPE (*n.)*

(from the Greek *mikrós*, "small" and *skopeîn*, "to look" or "see")
Def: An instrument used to see objects too small to see with the naked eye.

• First light microscope (essentially a magnifying lens) believed to have first been developed in the 1200s.

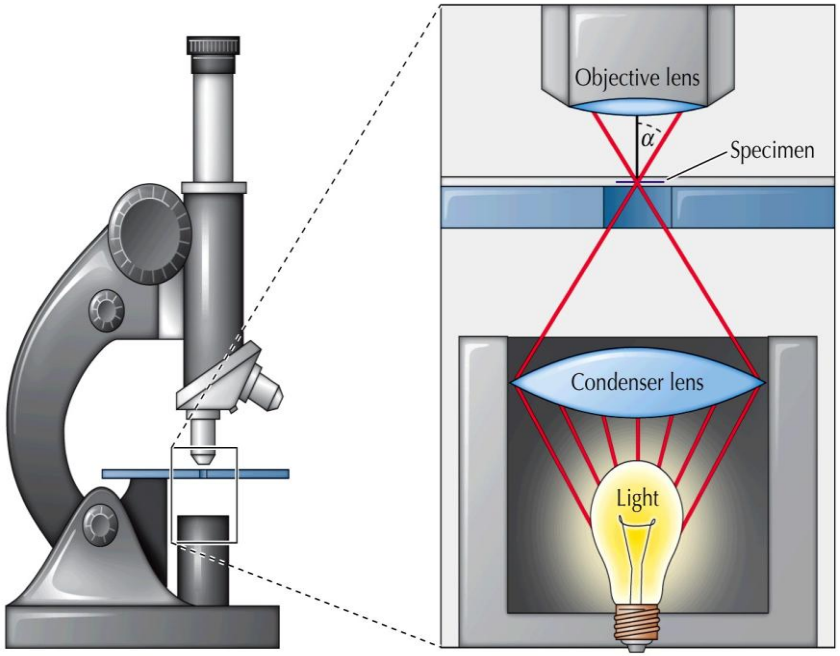
• Designed in their current **compound** form (use of multiple lens to magnify) in the 1600s. The inventor is unknown, however, Galileo Galilei and Antonie van Leeuwenkoek are often credited.



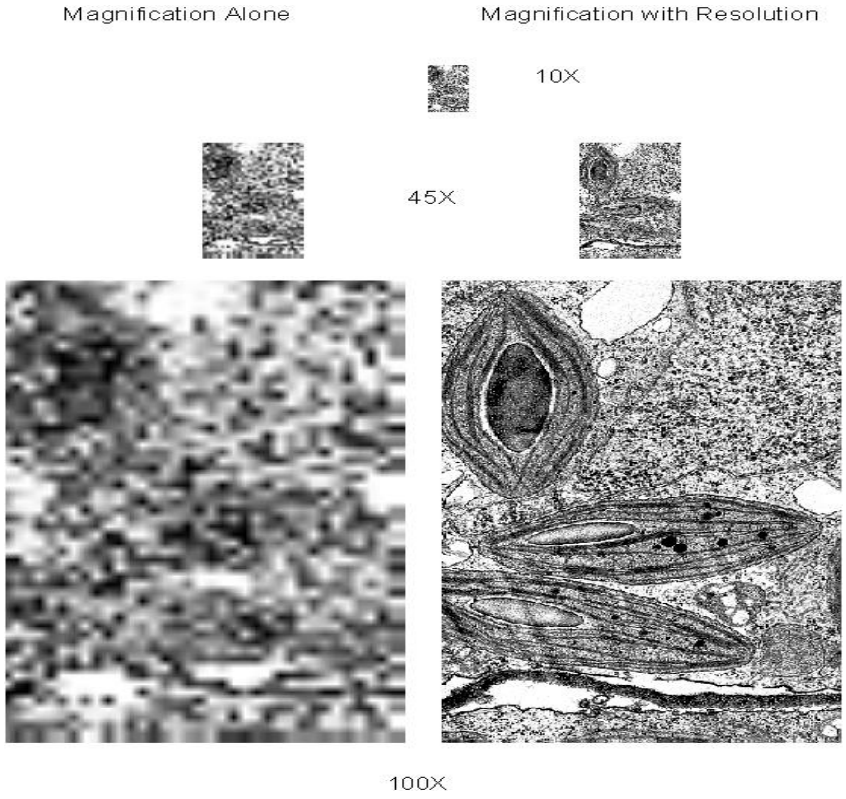
English: 18th century microscopes from the Musée des Arts et Métiers, Paris

LIGHT MICROSCOPY

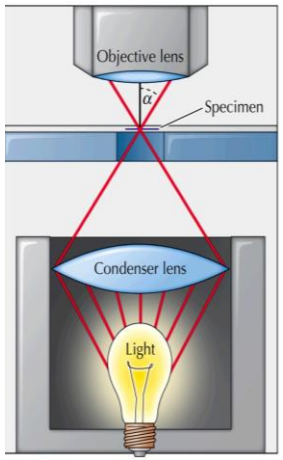
• Able to **magnify** objects up to 1000x. Sufficient for objects such as cells and large organelles (nuclei, mitochondria). The **resolution** power, however, of the light microscope limits its ability to observe the fine details of cell structures.



THE CELL: A MOLECULAR APPROACH 6e, Figure 1.23
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RESOLUTION



$$\text{Resolution} = 0.61\lambda/\text{NA}$$

λ= the wavelength of visible light (approx. 0.5um)
NA = numerical aperture
• The size of the cone of light that enters the microscope lens after passing through the specimen.

$$\text{NA} = \eta \sin \alpha$$

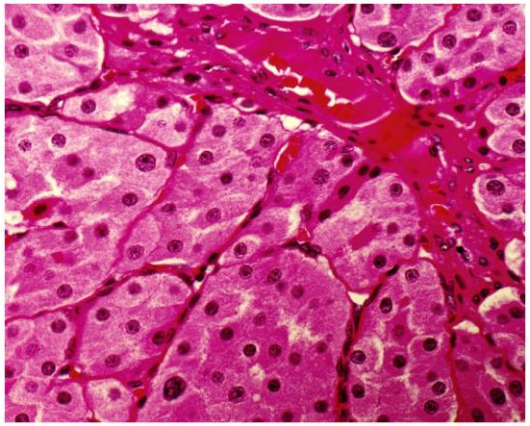
η= refractive index of the medium through which light travels between the specimen and the lens. ηfor air is 1.0, ηin oil immersion is 1.4
α= the angle of the cone of light collected by the lens

Limit of resolution for light microscopy is 0.22um

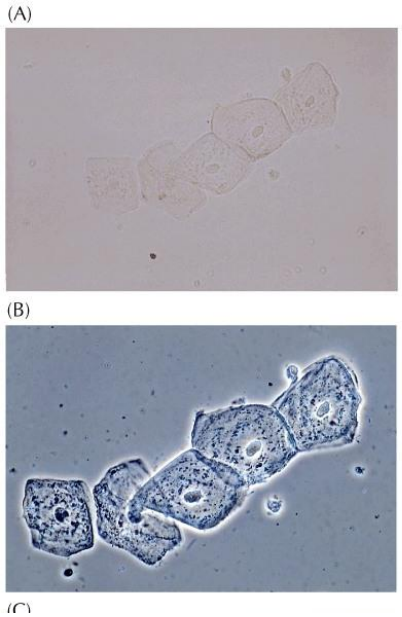
LIGHT MICROSCOPY

Bright-field microscopy: light passes through the cell and ability to see depends on the absorption of visible light by different cell components

- difficult to see cells (poor contrast) without fixative and staining



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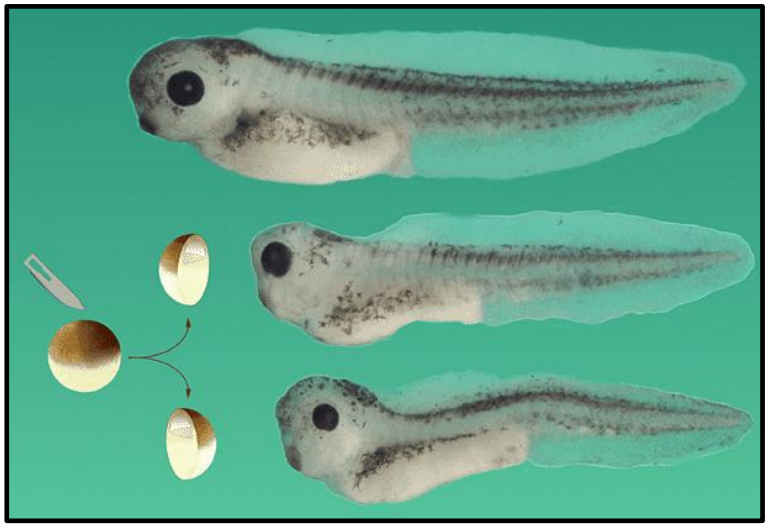
Phase-contrast microscopy: converts variations in thickness to differences in contrast that can be seen in the final image

- allows for imaging of live, unstained cells

LIGHT MICROSCOPY

Dissecting microscope: designed for low magnification of a specimen with three dimensional detail. Light is reflected off the surface, rather than passing through it, and an image is generated through two optical paths – one for each eye. This produces a three-dimensional, or stereoscopic image.

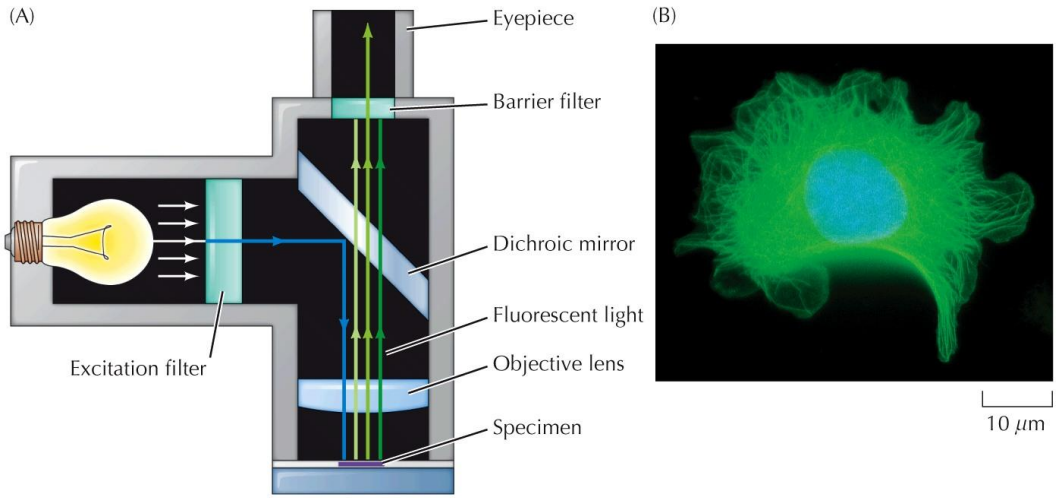
- Used extensively in micro-dissection





LIGHT MICROSCOPY

Fluorescence microscopy: use of a fluorescent dye to label molecules of interest within a cell. The dye absorbs light at one wavelength and emits at a second. The specimen can then be illuminated by the "excitation" wavelength and filters use to detect the "emission" wavelength.



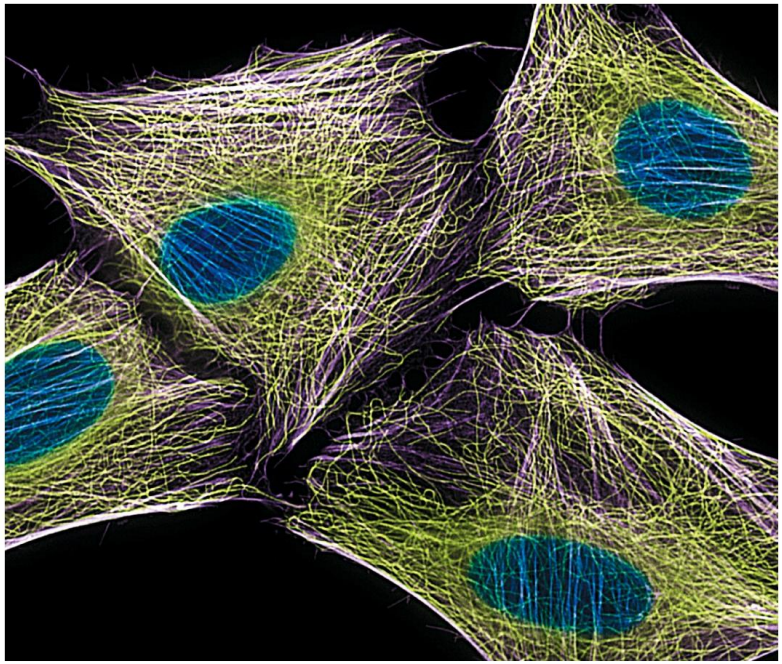
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Green fluorescent protein (GFP)

Protein expressed by the jellyfish *Aequorea victoria* and first used as a fluorescent marker in the 1990s. GFP can be used as a reporter of expression of a protein of interest using recombinant DNA techniques.

LIGHT MICROSCOPY

Confocal microscopy: allows images of increased contrast and detail to be obtained by analyzing fluorescence from only a single point in the specimen. Produces a much sharper image than standard, widefield, fluorescence microscopy



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ELECTRON MICROSCOPY

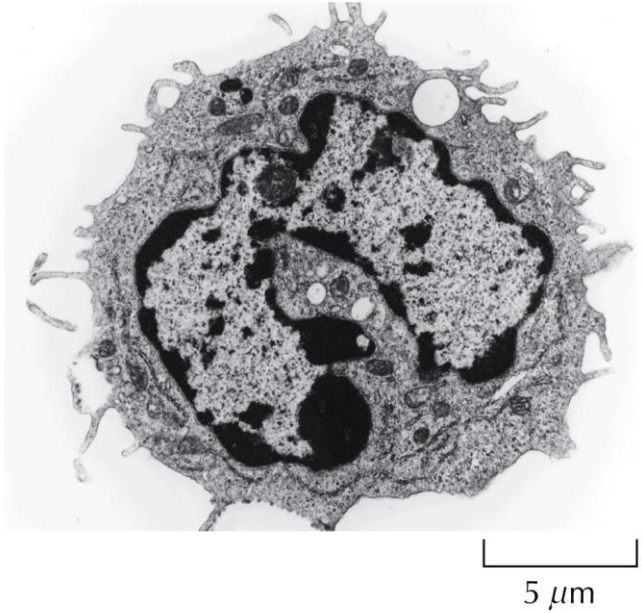
- Use of electrons instead of light to pass through the specimen

- Allows for much higher resolution due to the short wavelength of electrons (0.004nm – 100,000x shorter than visible light)

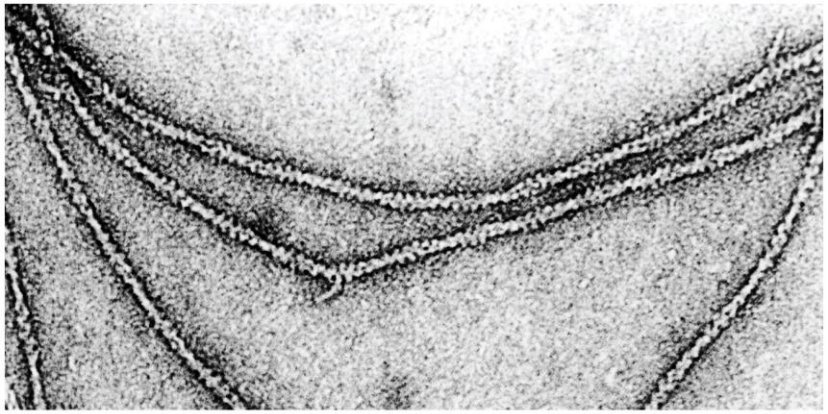
Limit of resolution for electron microscopy (with biological samples) is 1 to 2nm. ***More than 100x improvement over light microscopy.***

ELECTRON MICROSCOPY

Transmission electron microscopy: similar to the bright-field light microscope in concept, only using electrons instead of light. Specimen are stained with heavy metals which deflect electrons and will appear black.



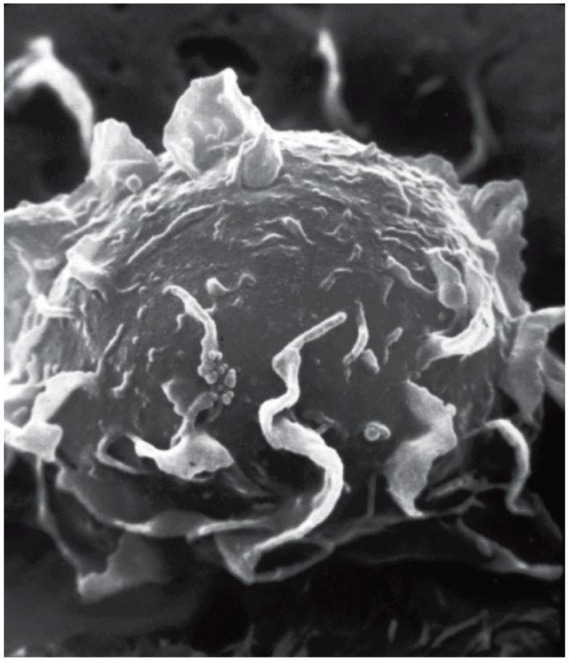
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ELECTRON MICROSCOPY

Scanning electron microscopy: The surface of the cell is coated with heavy metal and electrons are bounced off the specimen (like in a dissecting scope) to create a three-dimensional image.



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.38**
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USE AND CARE OF THE MICROSCOPE
inverted, phase-contrast

• Remember: magnification is the objective lens (4x, 10x, 20x, 40x) multiplied by the eye-piece (10x)

• Always turn off the light when done and replace the dust cover

• Use the appropriate phase shift ring as indicated on the objective lens (4x = Ph0 10x, 20x, 40x = Ph1 20x, 40x = Ph2)



USE AND CARE OF THE MICROSCOPE
EVOS

- Digital inverted microscope for use in bright-field, phase-contrast and fluorescence microscopy.
- Be sure that the phase annuli selector is in the correct position that corresponds to the selected objective and contrast method.
- Use the light shield box to reduce ambient light and improve image quality.
- Bring a USB flash drive if you'd like to save images for lab reports.
- Always remember to turn off the microscope and replace the dust cover when done.

