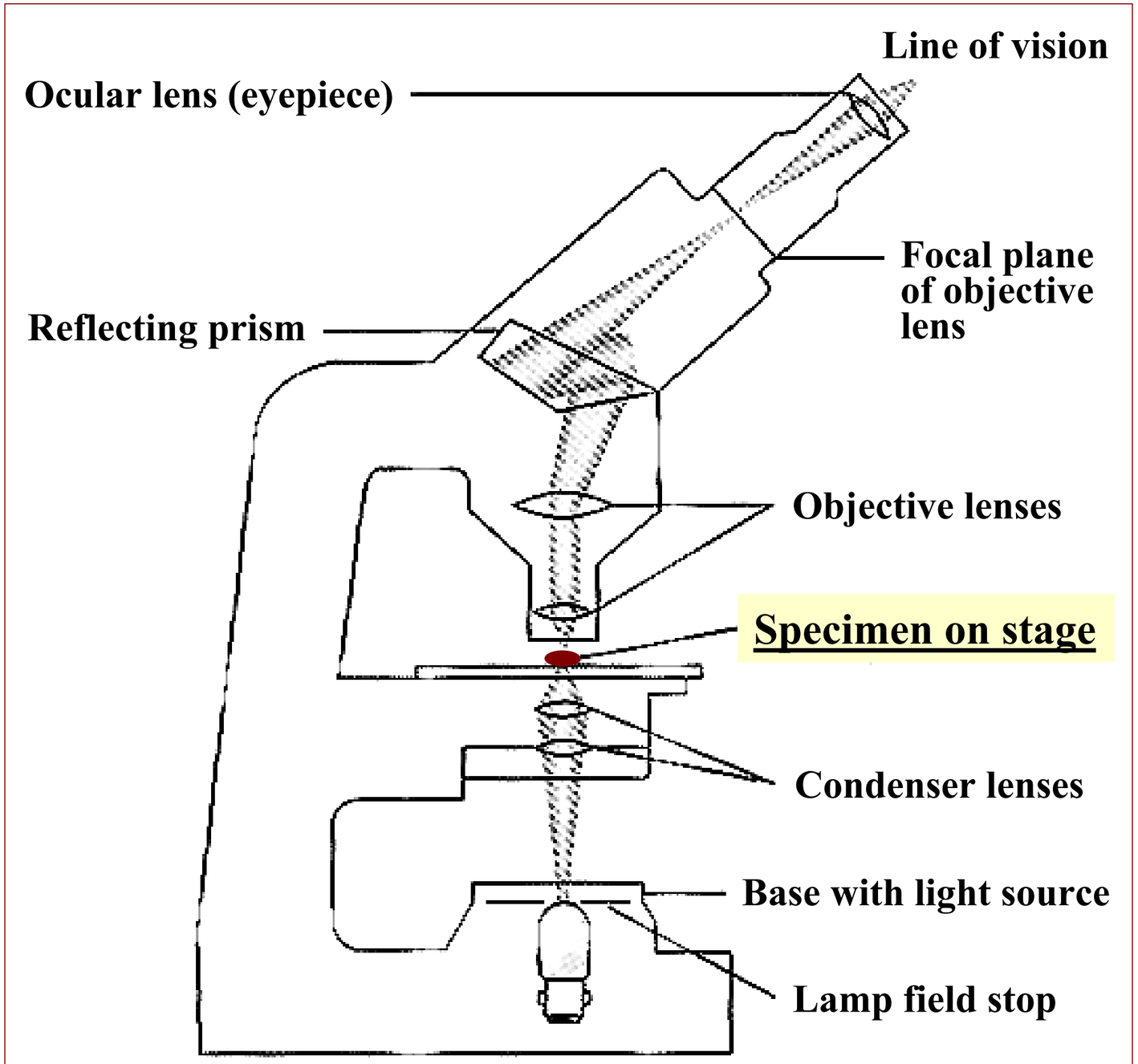
BIOL 266 – CELL BIOLOGY

Lecture 3:

How cells are studied (I)

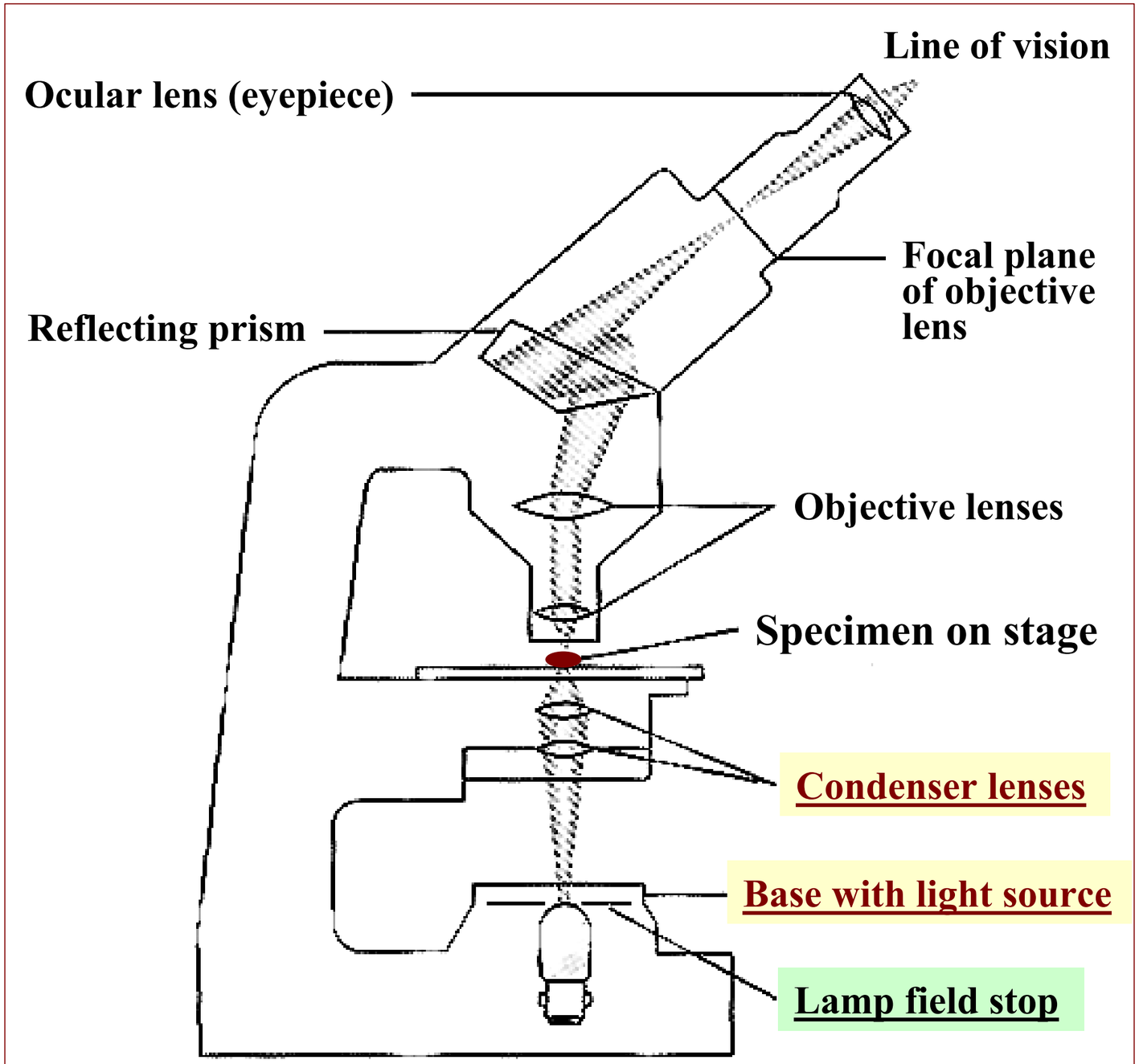
- **The compound microscope, the most common microscope in use today, contains several lenses that magnify the image of a specimen under study**

The optical pathway in a modern compound optical microscope



The specimen is mounted on a transparent glass slide

The optical pathway in a modern compound optical microscope

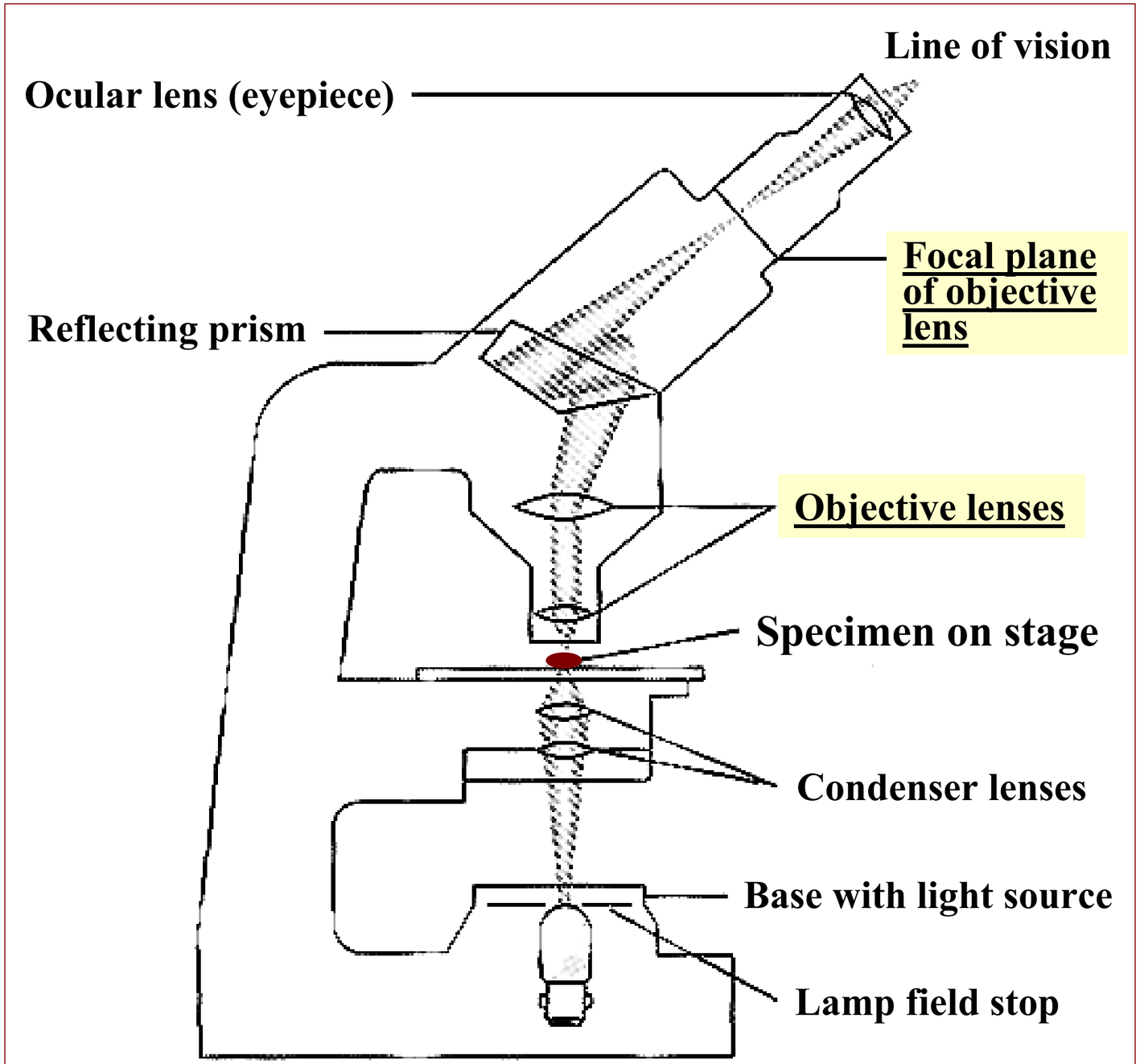


The condenser lenses <u>do not</u> <u>create a magnified image</u> of the specimen !

Light from a bright source <u>is</u> <u>focused by the condenser lenses</u> onto the specimen

The lamp field stop <u>restricts the amount of light entering a lens</u>

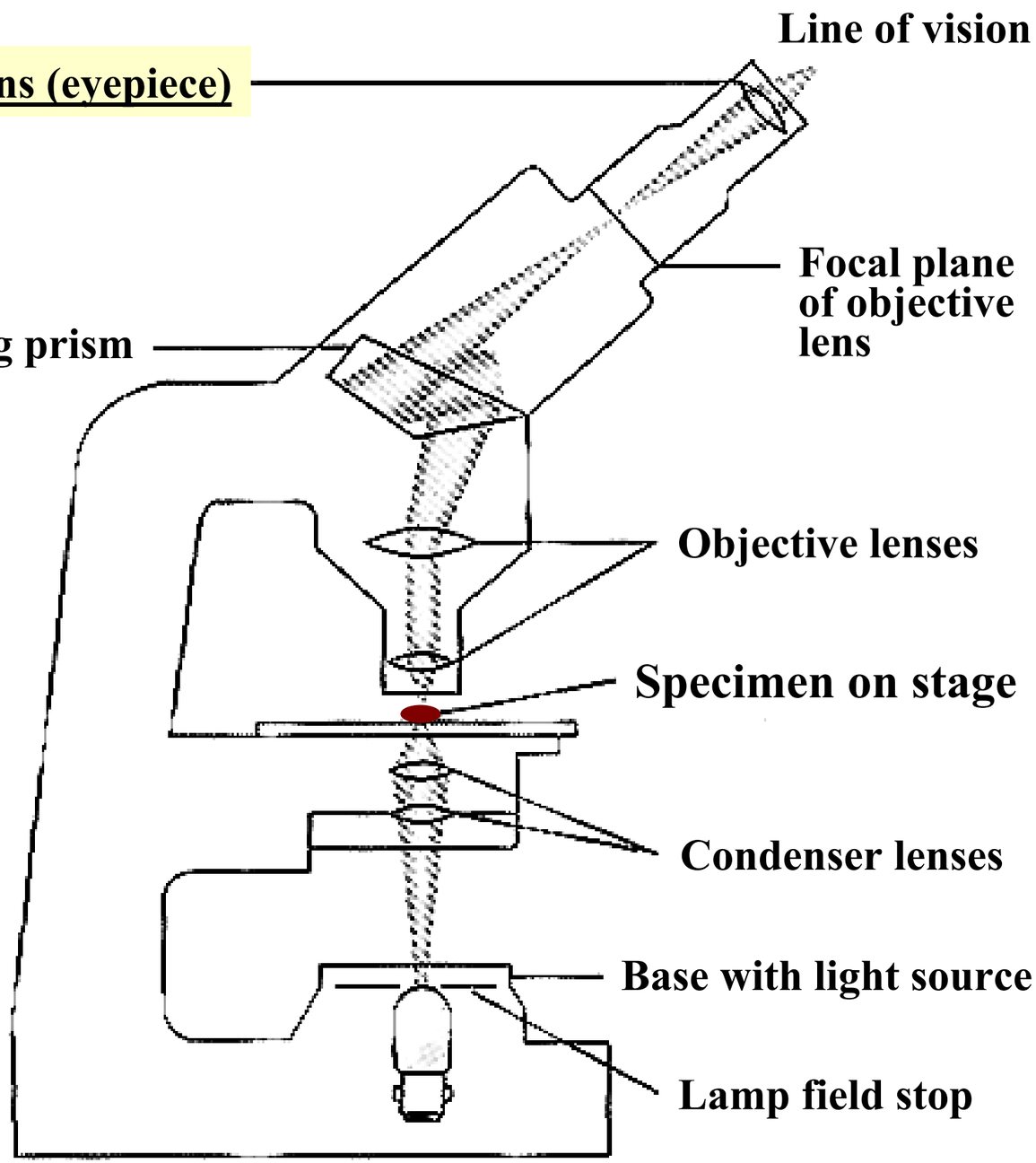
The optical pathway in a modern compound optical microscope



The objective lenses pick up the light transmitted by the specimen and focus it on the focal plane of the objective lens, creating a magnified image of the specimen

YES magnification!

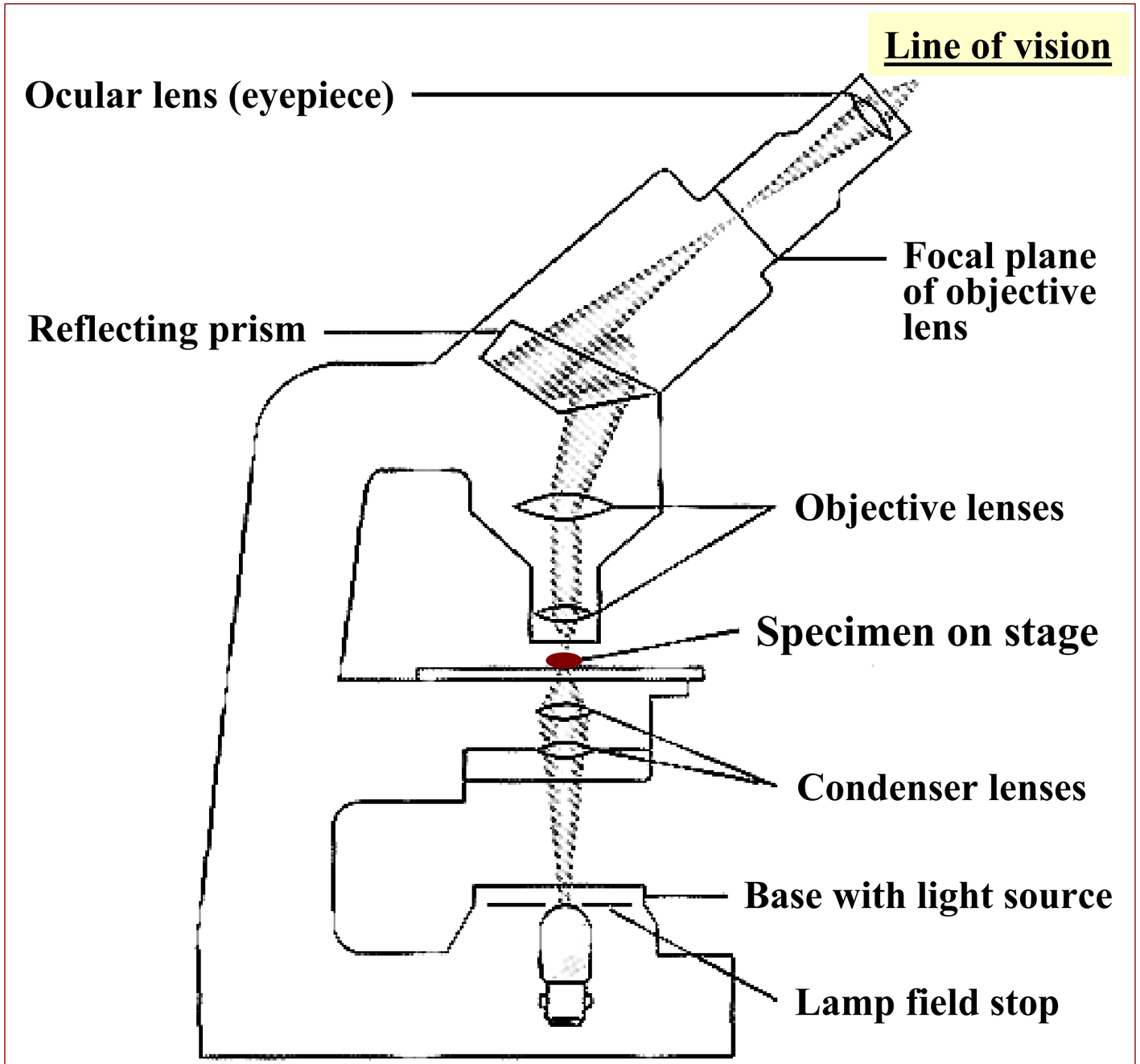
The optical pathway in a modern compound optical microscope



The image on the objective focal plane is magnified by the ocular lens, or eyepiece, which is focused on the objective focal plane

YES magnification!

The optical pathway in a modern compound optical microscope



The ocular lens picks up the light emanating from the already magnified image of the specimen and projects it onto the plane of the human eye

- **The total magnification is a product of the magnification of the individual lenses:**

If the objective lens magnifies 100-fold (a 100X lens) and the ocular lens magnifies 10-fold (a 10X lens), the final magnification will be 100 x 10 = 1000-fold

- **However, <u>the most important property of any microscope is not its magnification but its resolving power, or resolution</u>**

- **<u>Resolution</u> – the ability of a microscope to distinguish between two very closely positioned objects**

Microscope 1 **Microscope 2**

Resolution 1 > Resolution 2

(<u>better</u>)

Resolution = D

- **D - the minimum distance between two distinguishable objects**

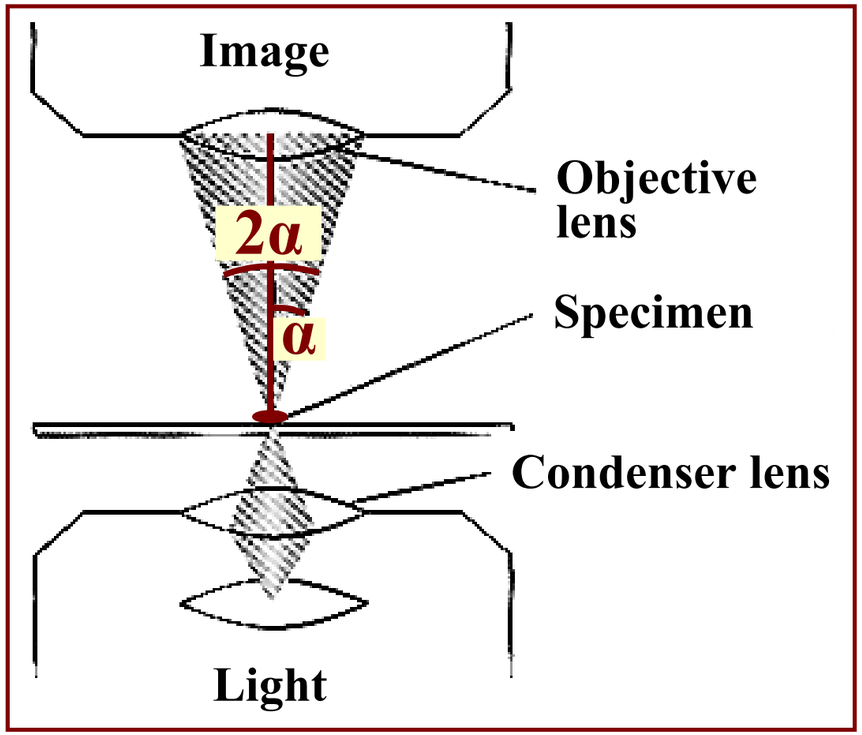
- **The smaller the value of D, the better the resolution**

Microscope 1 **Microscope 2**

1 µm **5 µm**

D 1 < D2

Resolution 1 better then Resolution 2



$$D = \frac{0.61 \cdot \lambda}{n \cdot \sin \alpha}$$

- λ - <u>wavelength</u> of incident light in nm
- n - <u>refractive index</u> of the air or fluid medium between the specimen and the objective lens
- α - <u>angular aperture</u>, a half-angle of the cone of light entering the objective lens from the specimen

$$D = \frac{0.61 \cdot \lambda}{n \cdot \sin \alpha}$$

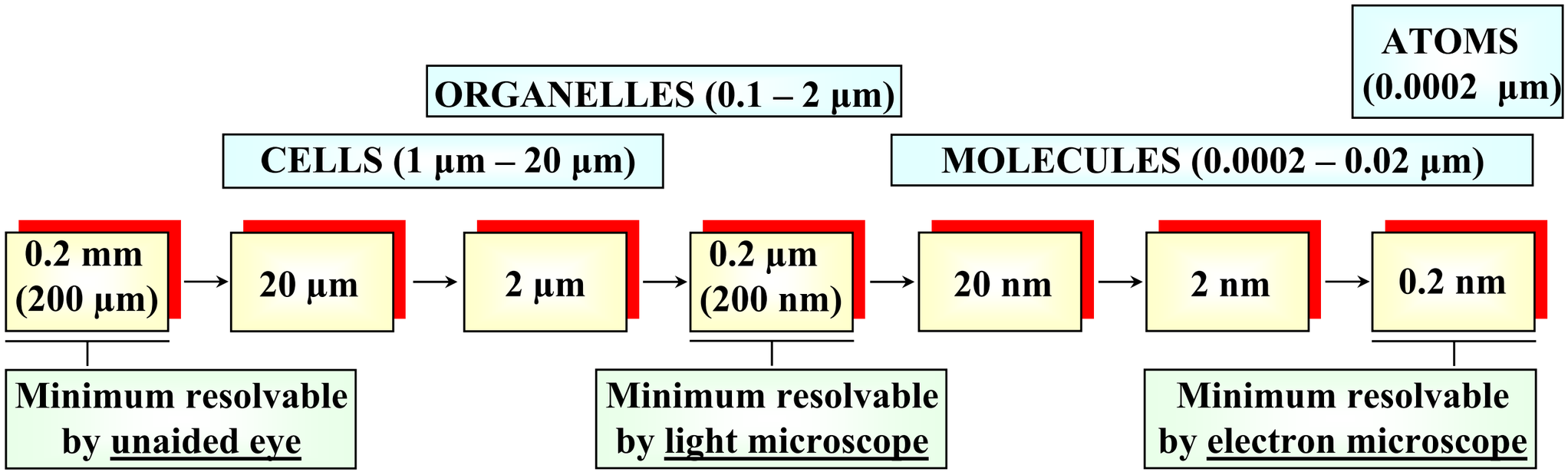
- **The shorter the wavelength, λ, of incident light, the lower will be the value of D and the better the resolution**
- **Visible light of shortest wavelength (blue): λ = 450 nm**
- **An electron in an electron microscope with an accelerating voltage of 100,000 V: λ = 0.004 nm**
- **In theory the resolution of such an electron microscope should be 100,000 times greater than that of the light microscope**

- **A fundamental limitation of all microscopes:**

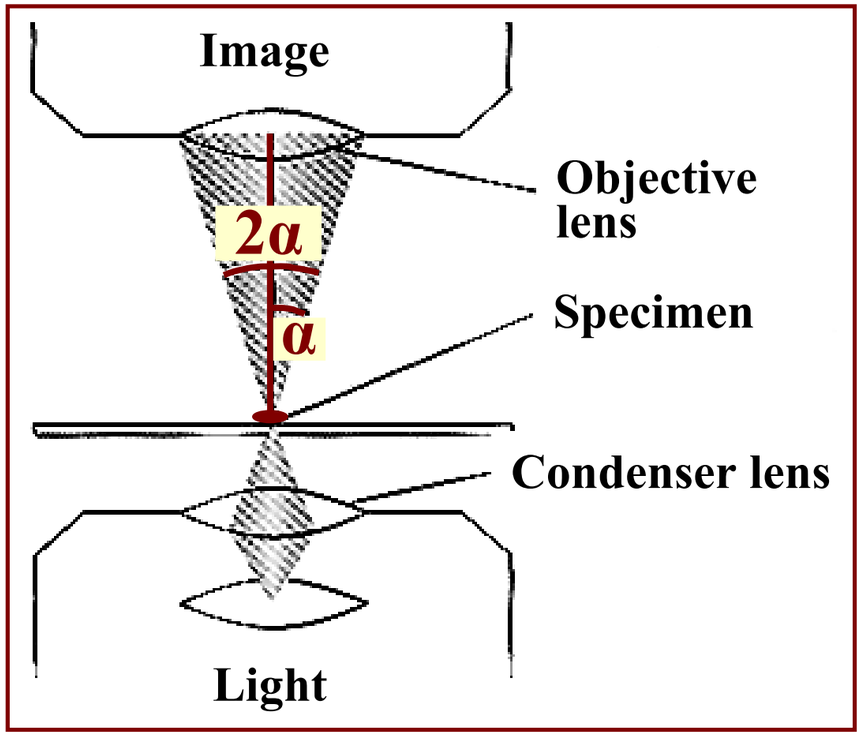
A given type of radiation cannot be used to probe structural details much smaller than its own wavelength, λ

D (the minimum distance between two distinguishable objects) for a given type of radiation (visible light or an electron) = λ of this type of radiation

$$1\ m = 10^3\ mm = 10^6\ \mu m = 10^9\ nm$$



- **The limits of resolution, the sizes of cells and of their component parts, and the units in which they are measured**



$$D = \frac{0.61 \cdot \lambda}{n \cdot \sin \alpha}$$

- **Increasing the value of angular aperture, α, will decrease the value of D and thus improve the resolution**

- **The maximum angular aperture, α, for the best objective lenses = 70° (sin 70° = 0.94)**

Compare: α = 90°, sin 90° = 1 vs. α = 70°, sin 70° = 0.94 vs. α = 45°, sin 45° = 0.71 for visible light of shortest λ = 450 nm (blue light)

$$D = \frac{0.61 \cdot \lambda}{n \cdot \sin \alpha}$$

- **Increasing the value of refractive index, n, will decrease the value of D and thus improve the resolution**
- **n (air) = 1.0 n (immersion oil) = 1.5**
- **D (air) = 100% > D (immersion oil) = 67%**
- **Use of immersion oil is a simple way to reduce D by 33% and thus to improve the resolution**

Compare: n = 1 (air) vs. n = 1.5 (immersion oil)
for visible light of shortest λ = 450 nm (blue light)

$$D = \frac{0.61 \cdot \lambda}{n \cdot \sin \alpha}$$

- **$n \cdot \sin \alpha$ is called the <u>numerical aperture</u>, NA, of the lens and is a function of its light-collecting ability**

- **<u>The higher the NA</u>, the lower will be the value of D and the <u>better the resolution</u>**

<u>Compare</u>:

(1) n = 1 (air), sin 70^o = 0.94, NA = 0.94 <u>vs</u>. n = 1.5 (oil), sin 70^o = 0.94, NA = 1.41 for visible light of shortest λ = 450 nm (blue light)

(2) n = 1 (air), sin 45^o = 0.71, NA = 0.71 <u>vs</u>. n = 1 (air), sin 70^o = 0.94, NA = 0.94 for visible light of shortest λ = 450 nm (blue light)

- **The limit of resolution of a light microscope:**

With the visible light of shortest wavelength (blue, $\lambda = 450$ nm), an immersion oil ($n = 1.5$) and the best objective lens ($\alpha = 70^o$, $\sin \alpha = 0.94$):

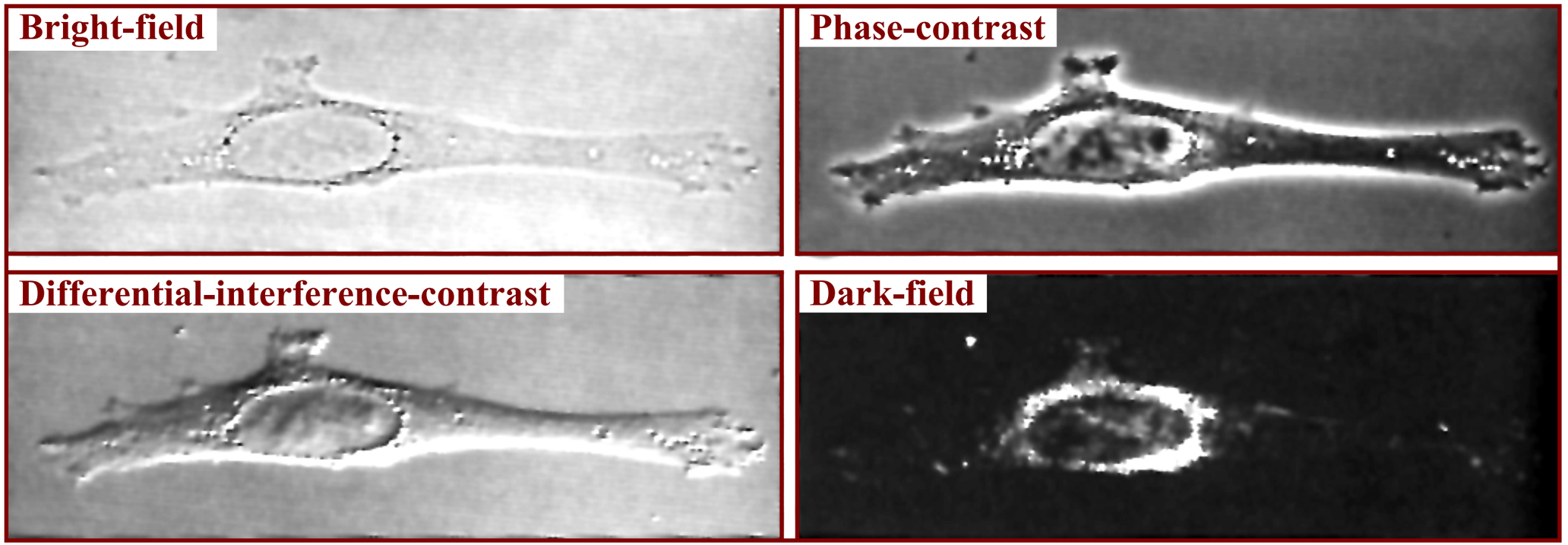
$$D = \frac{0.61 \cdot 450 \text{ nm}}{1.5 \cdot 0.94} = 194 \text{ nm} \sim 0.2 \text{ µm}$$

- **No matter how many times the image is magnified, the light microscope can never resolve objects that are less than ~ 0.2 µm in size**

Four types of light microscopy:

- **Bright-field microscopy**
- **Phase-contrast microscopy**
- **Nomarski differential-interference-contrast microscopy (*Nomarski microscopy*)**
- **Dark-field microscopy**

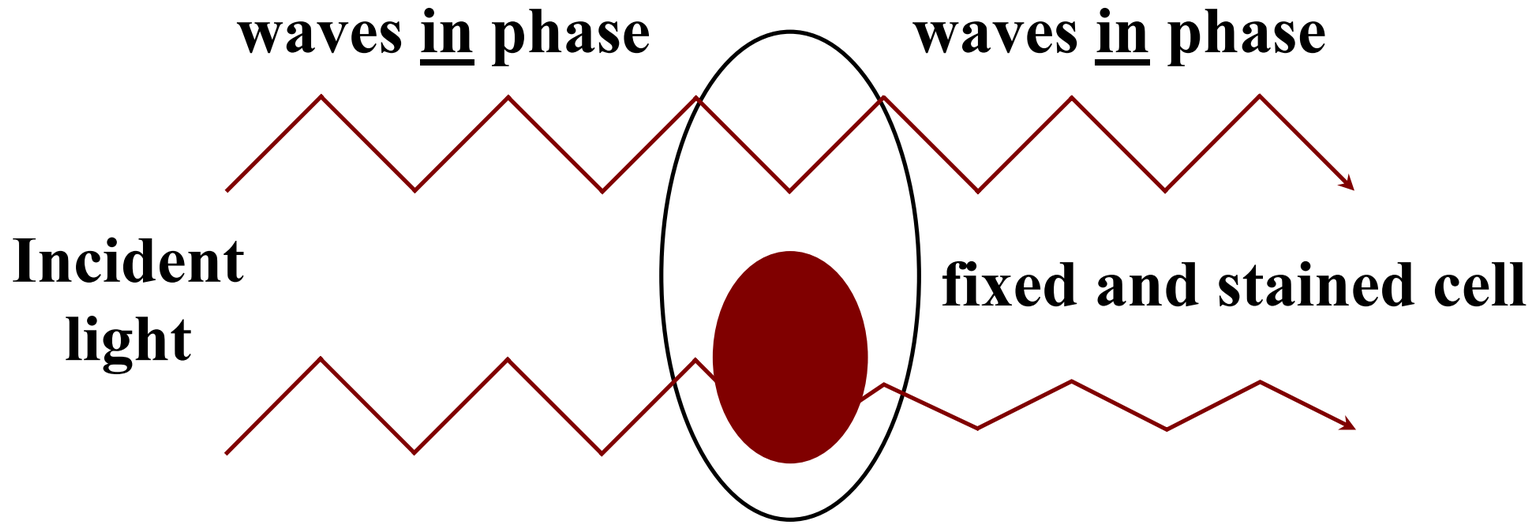
Four types of light microscopy



A fibroblast cell

- All four types of image can be obtained with most modern microscopes simply by interchanging optical components

First way to obtain contrast in light microscopy (*is used in bright-field microscopy only*):



- **The light is not slowed down = the phase of the light is not altered**

- **The stained portions of the cell absorb the light =**

  the amplitude of light waves of particular wavelengths passing through them is reduced

First way to obtain contrast in light microscopy (*<u>is used in bright-field microscopy only</u>*):

Many chemical stains bind to molecules that have specific features:

- ***<u>Hematoxylin</u>* *acidic* side chains of amino acids (aspartate and glutamate) of proteins as well as to DNA and RNA**

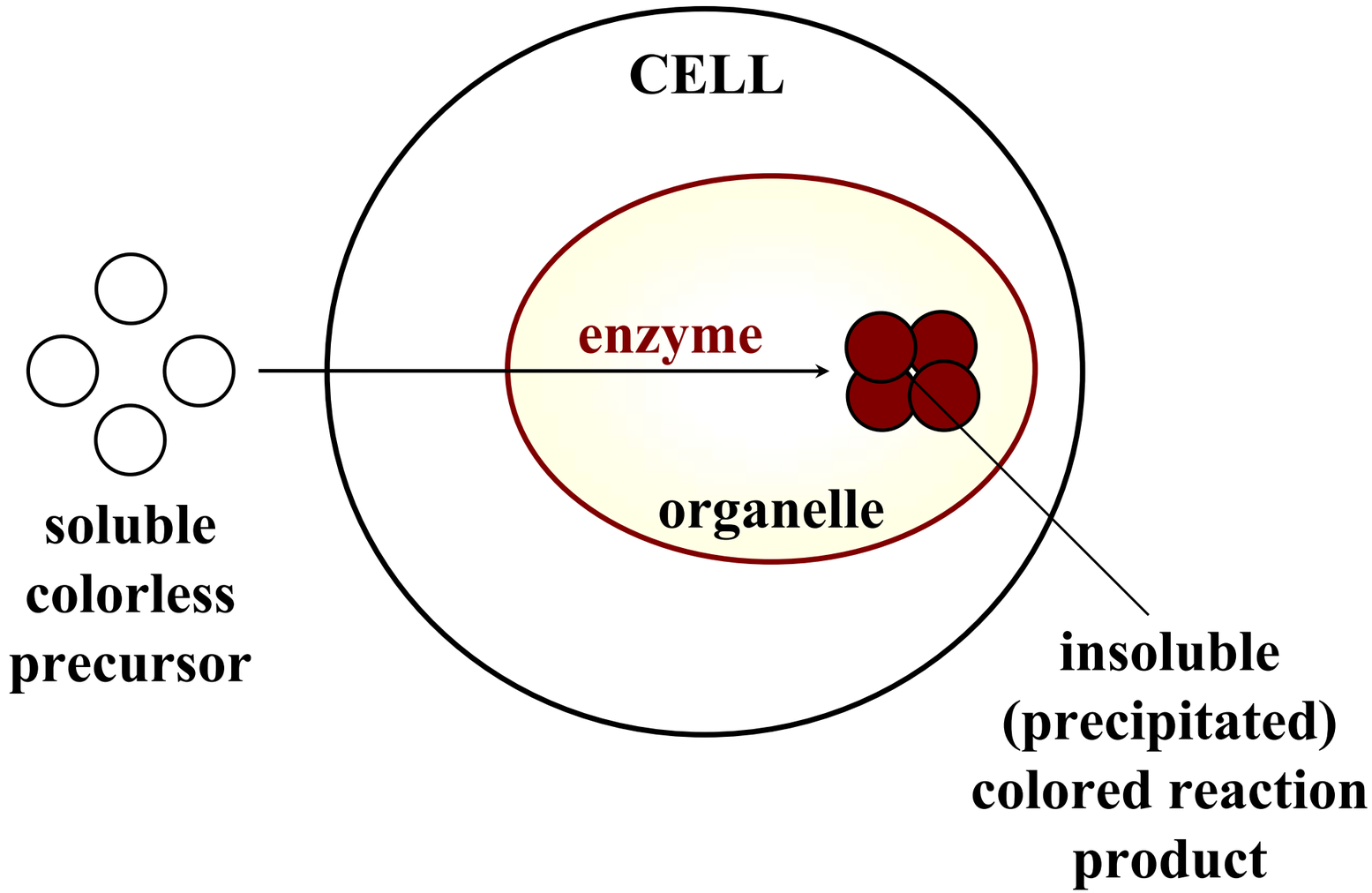
- ***<u>Eosin</u>* binds mostly to *basic* side chains of amino acids (lysine and arginine) of proteins**

N.B.

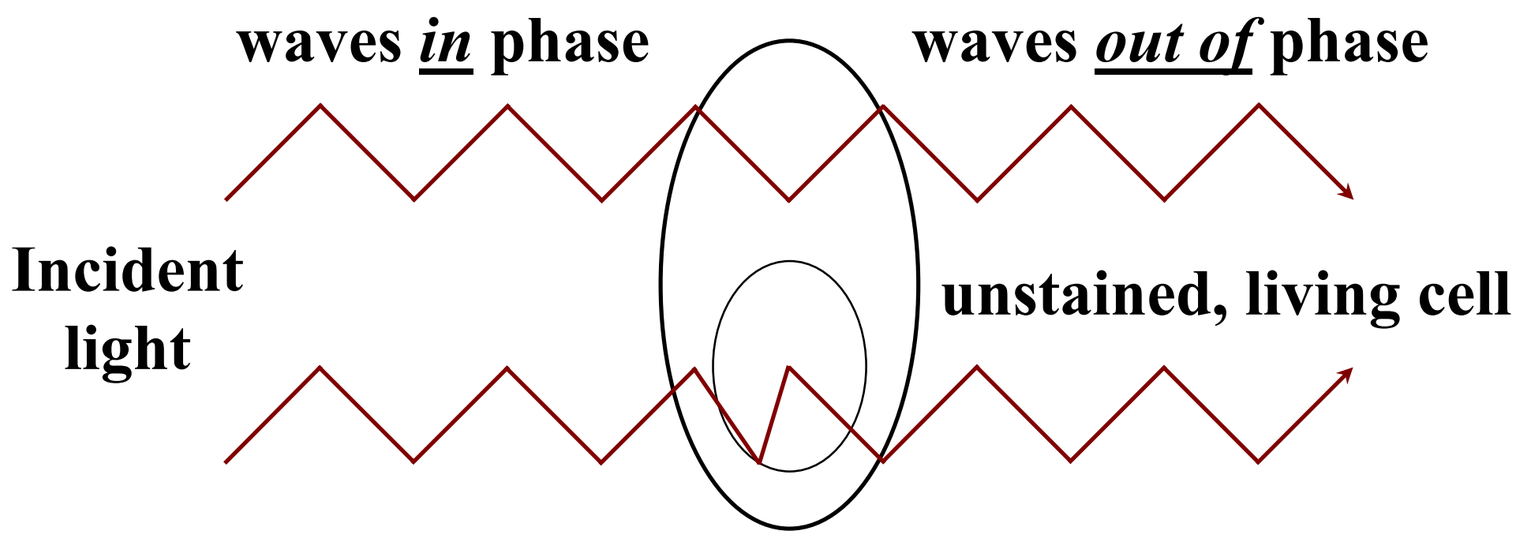
Don't believe in what is written in the "Molecular Cell Biology" textbook by Lodish *et al*. published by W.H. Freeman & Co. in 2008 (as well as several earlier editions of this textbook)

First way to obtain contrast in light microscopy
(*<u>is used in bright-field microscopy only</u>*):

Cytochemical staining:



Second way to obtain contrast in light microscopy
(is used in *phase-contrast and Nomarski microscopy*)

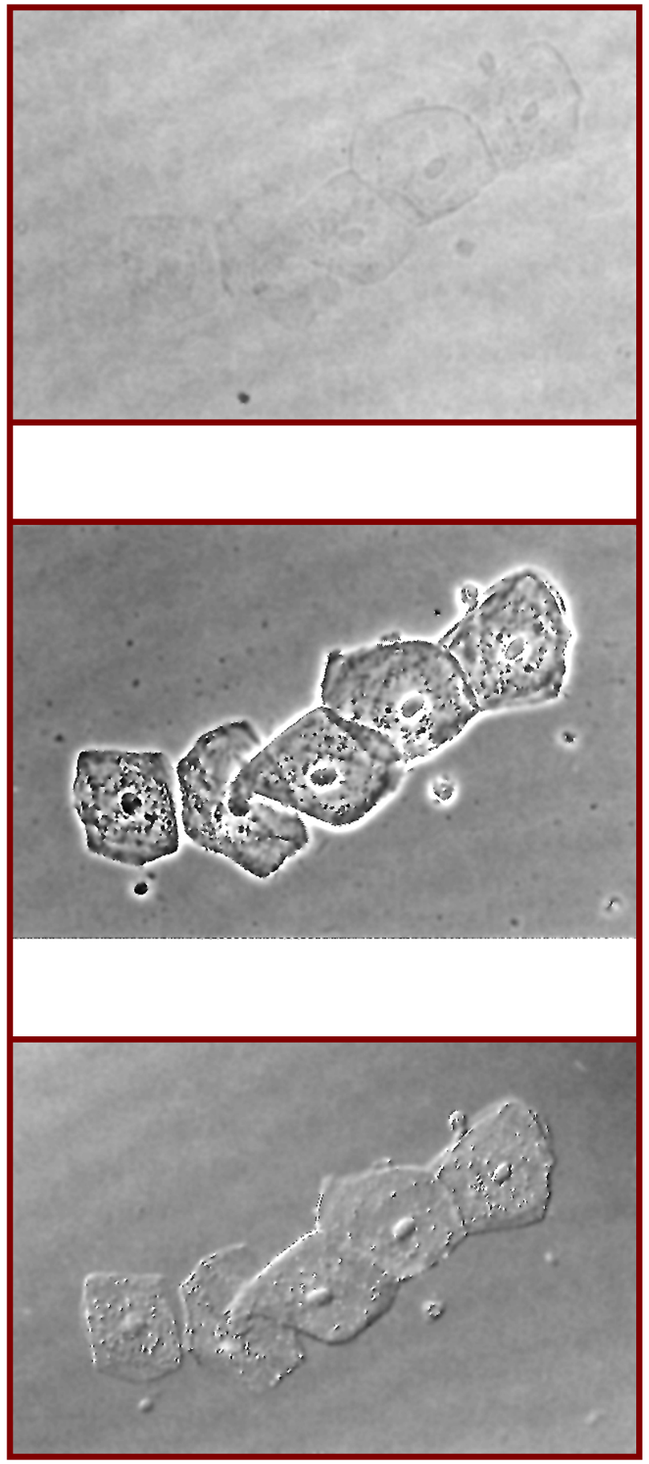


- **The light is slowed down = *the phase of the light is altered***

- **The unstained portions of the cell absorb the light poorly = *very little change in the amplitude of the light***

- ***Special optical systems* are used to convert these differences in phase to differences in contrast**

Microscopic observation of *living cells*



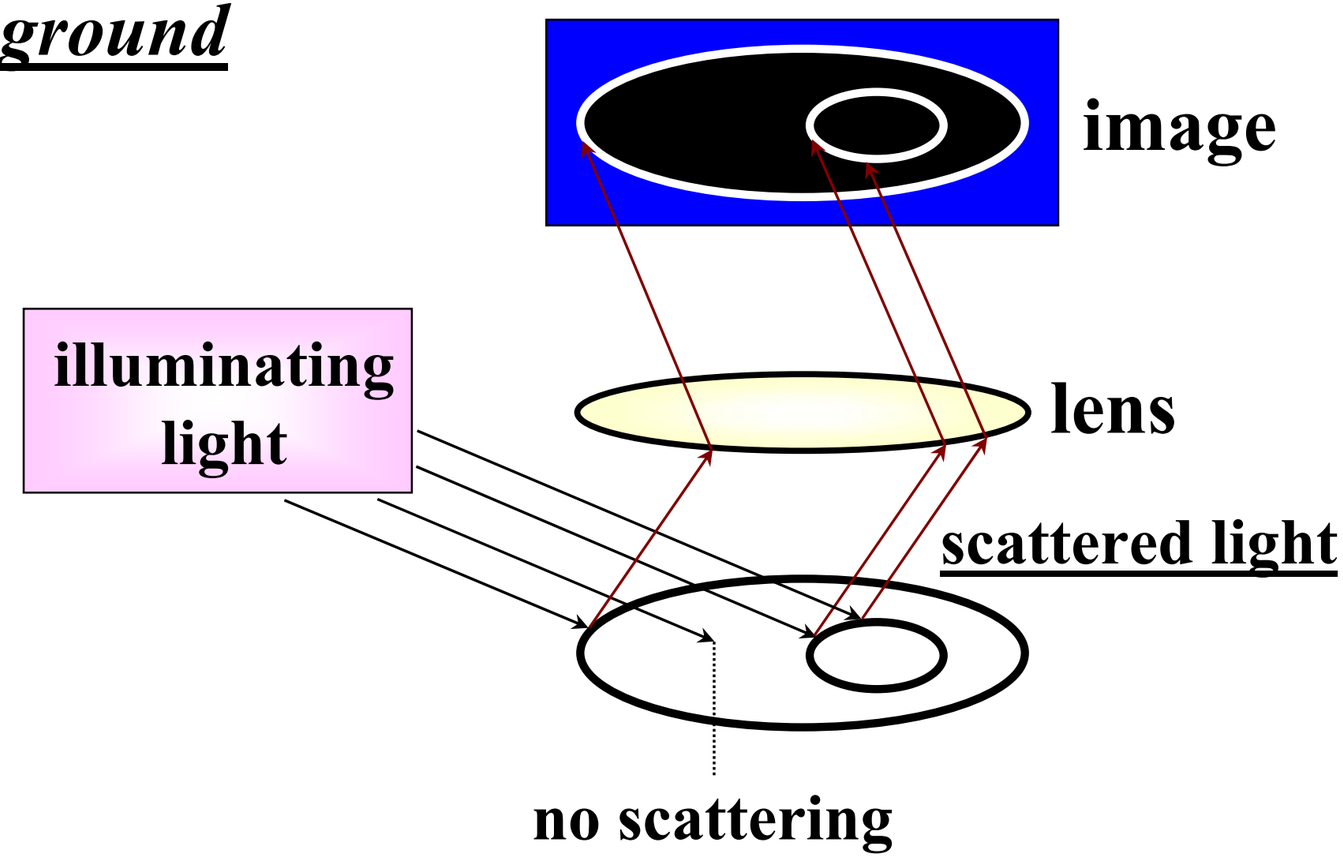
Bright-field (*no staining*)

Phase-contrast (*improved, sharp image*)

Nomarski (*improved, sharp image*)

Dark-field microscopy:

- Observes the light that is *scattered* by various components of a living cell
- The illuminating light is directed *from the side* so that only scattered light enters the microscope lenses
- The cell appears as *an illuminated object against a black background*

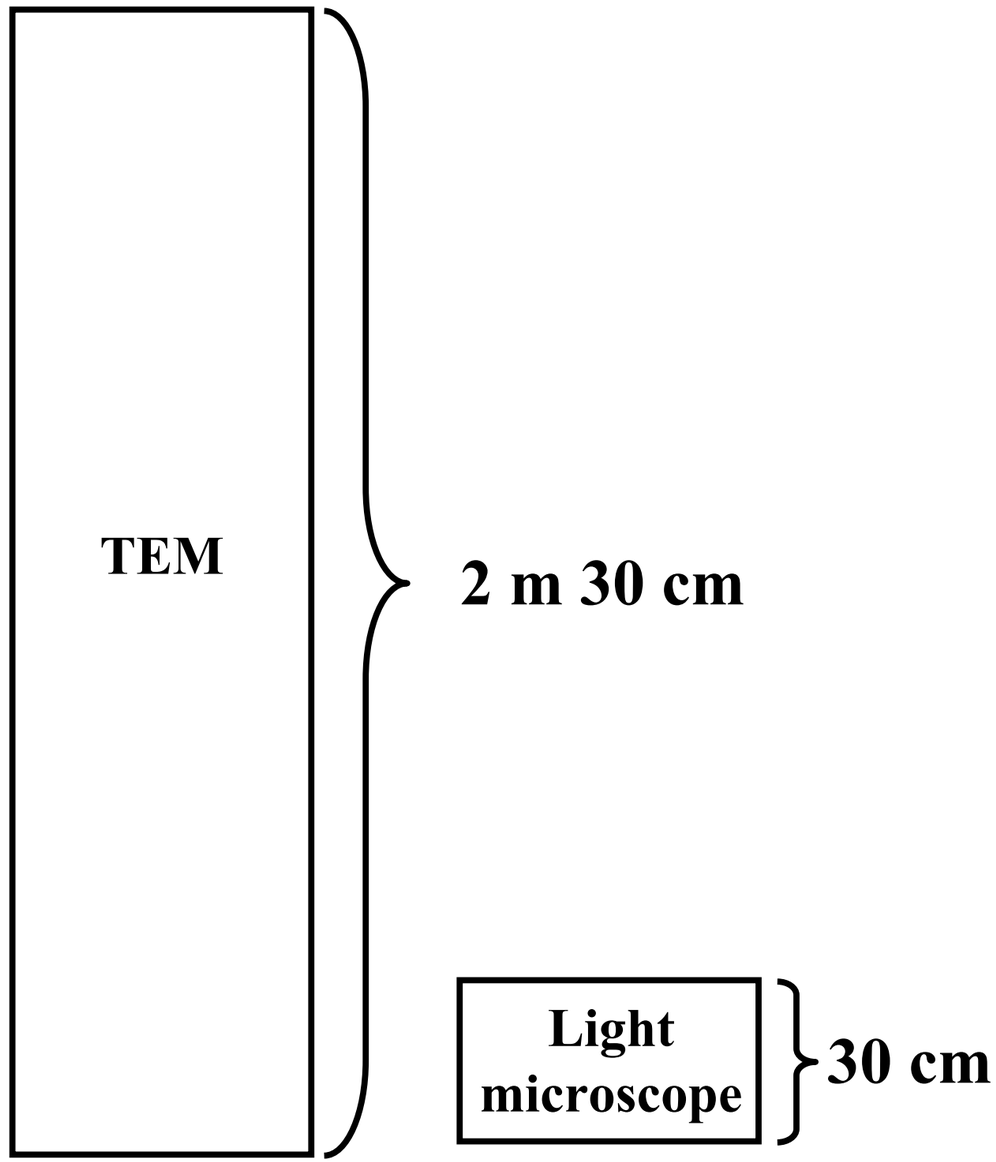


$$D = \frac{0.61 \cdot \lambda}{n \cdot \sin \alpha}$$

- **The shorter the wavelength, λ, of incident light, the lower will be the value of D and the better the resolution**
- **Visible light of shortest wavelength (blue): λ = 450 nm**
- **An electron in an electron microscope with an accelerating voltage of 100,000 V: λ = 0.004 nm**
- **In theory the resolution of such an electron microscope should be 100,000 times greater than that of the light microscope**

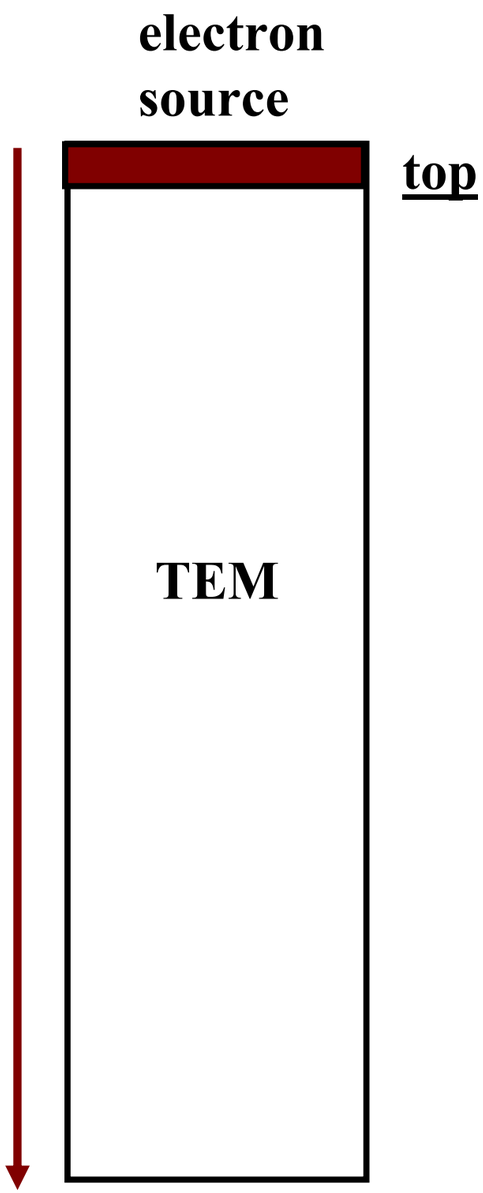
<u>The transmission electron microscope</u> (TEM) is similar to a light microscope, but:

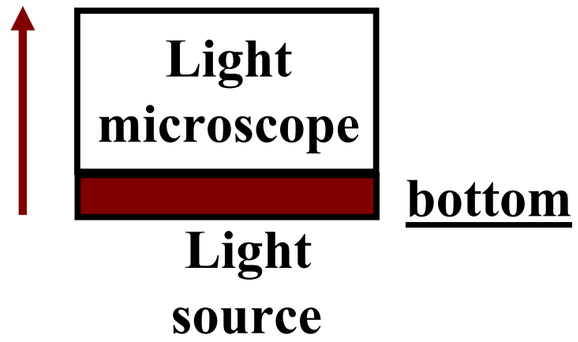
- **(1) it is much larger (about 2 m 30 cm high)**



<u>The transmission electron microscope</u> (TEM) is similar to a light microscope, but:

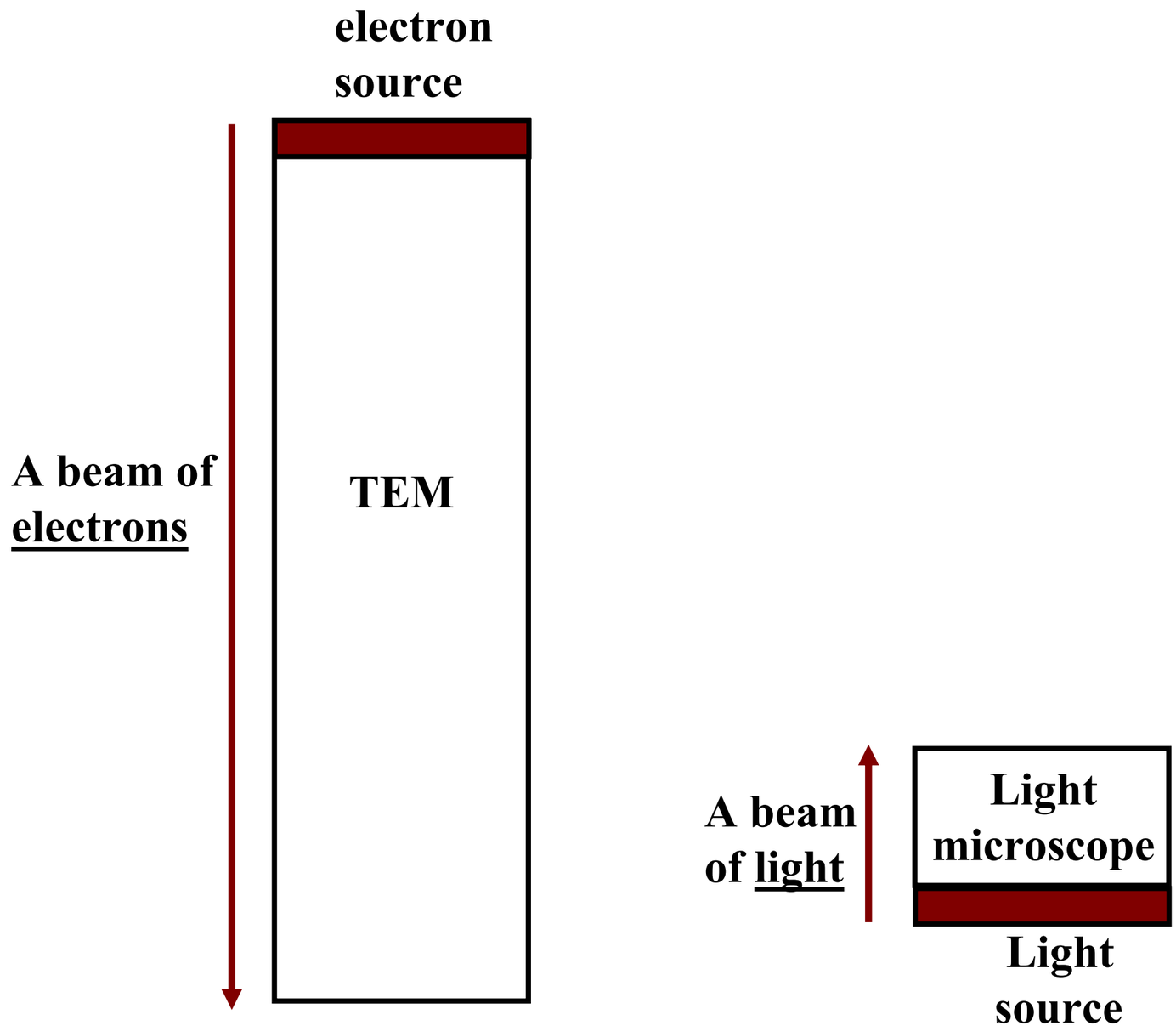
- **(2) it is upside down**





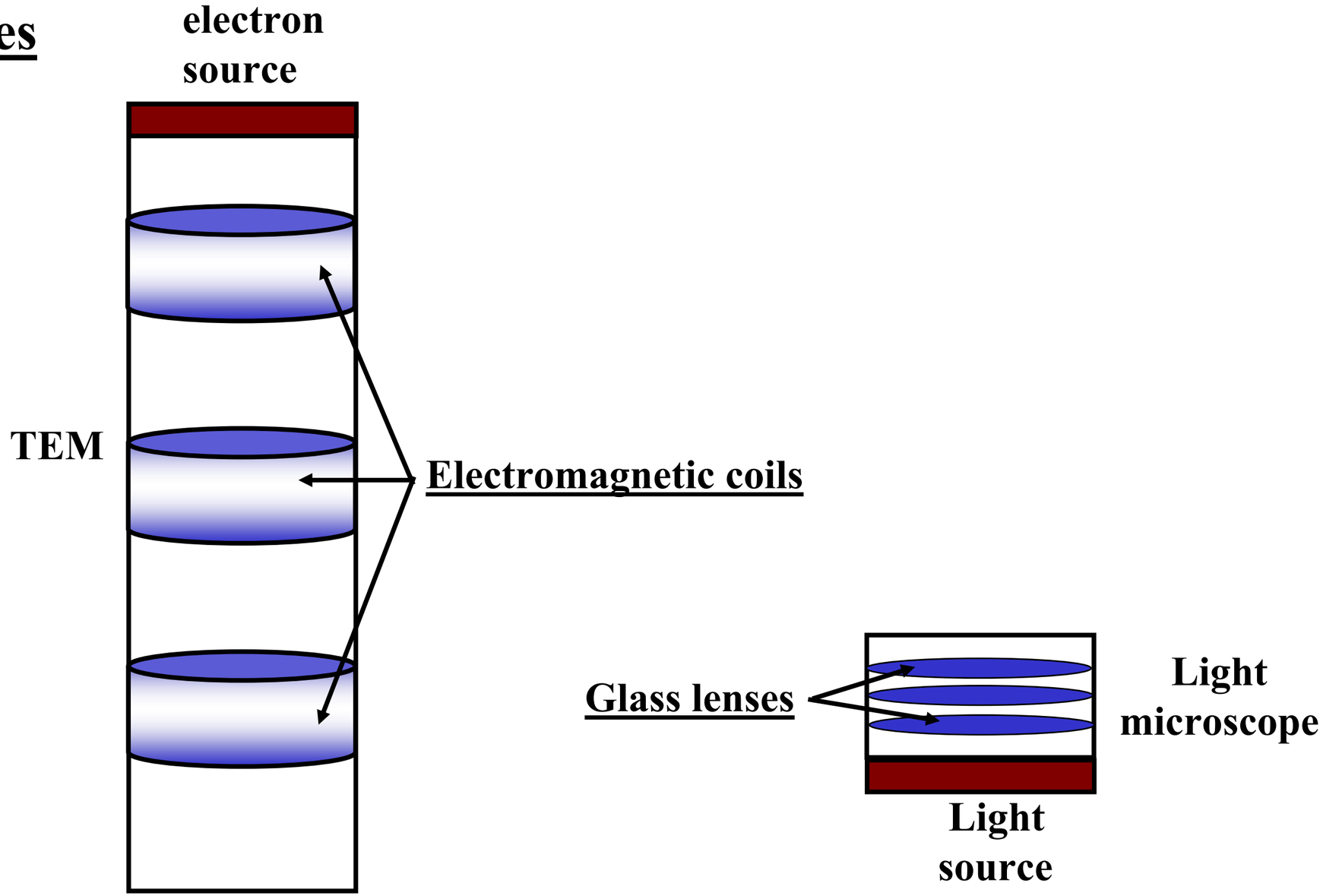
<u>The transmission electron microscope</u> (TEM) is similar to a light microscope, but:

- **(3) it uses <u>a beam of electrons</u> instead of a beam of light**



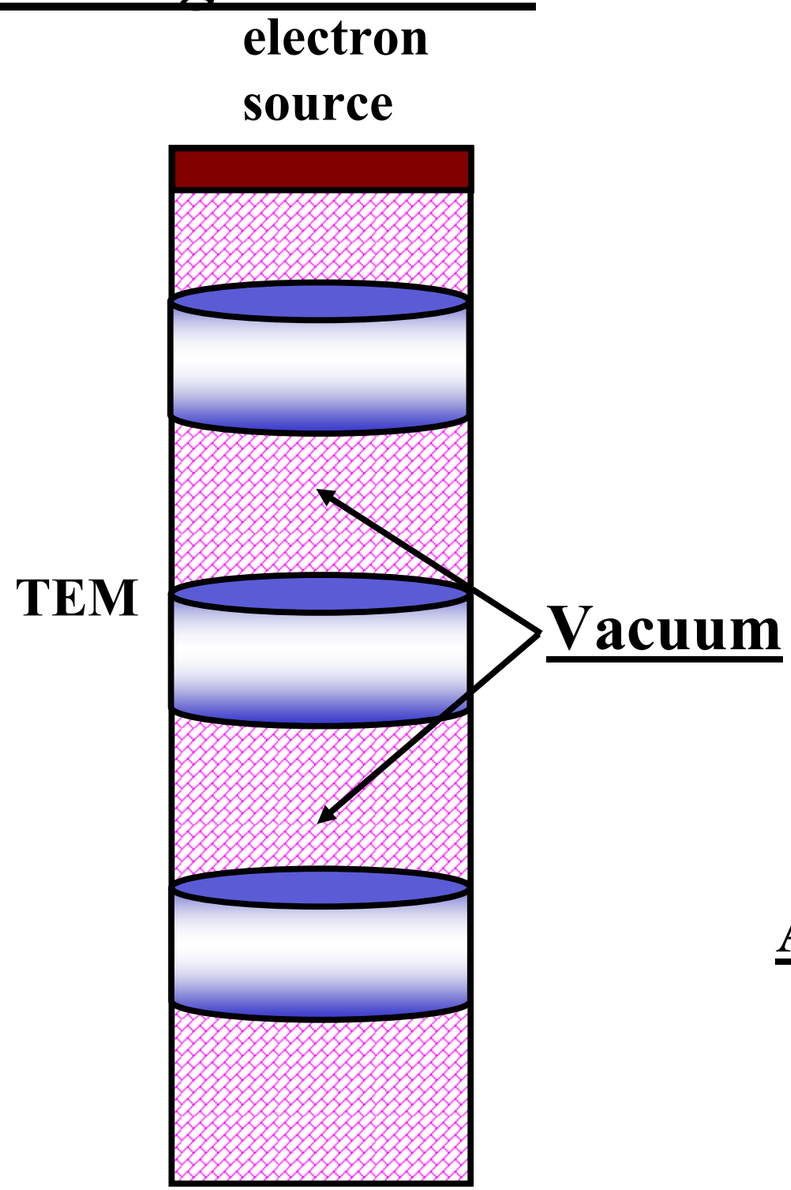
The transmission electron microscope (TEM) is similar to a light microscope, but:

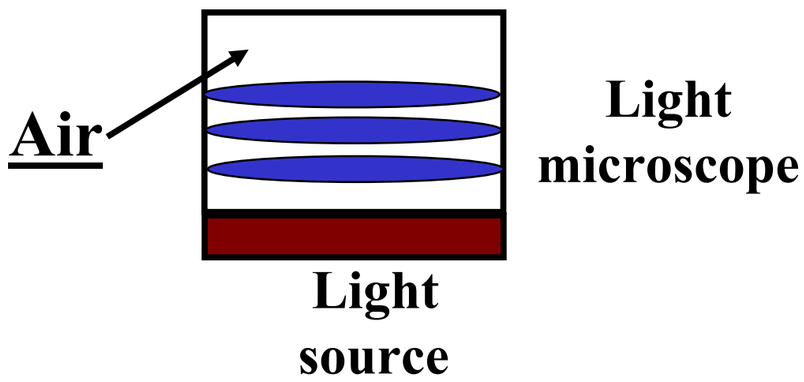
- **(4) it uses magnetic coils (to focus the beam of electrons and to create a magnified image of the specimen) instead of glass lenses**

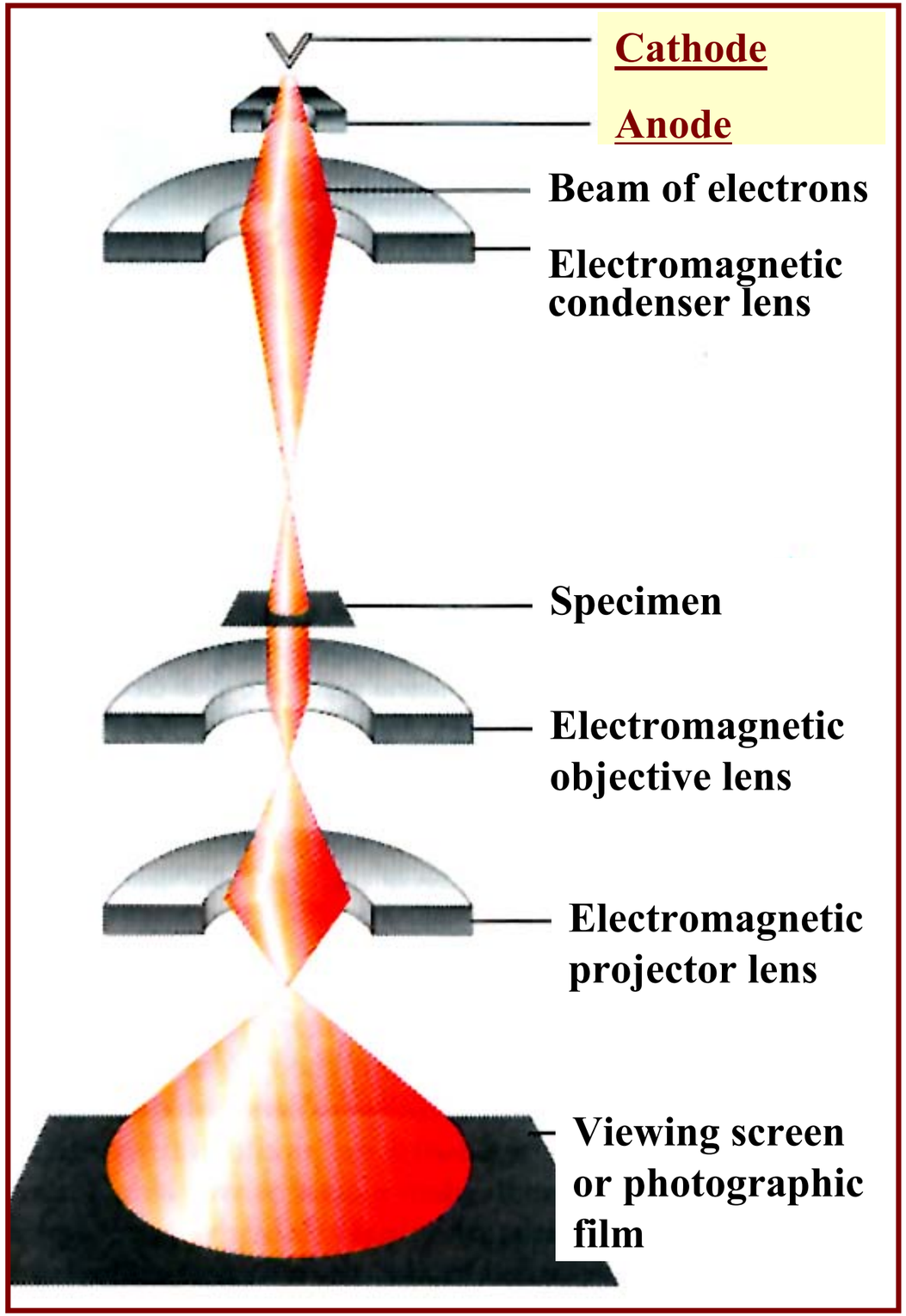


<u>The transmission electron microscope</u> (TEM) is similar to a light microscope, but:

- **(5) the entire column of the TEM is maintained under an <u>ultrahigh vacuum</u>**



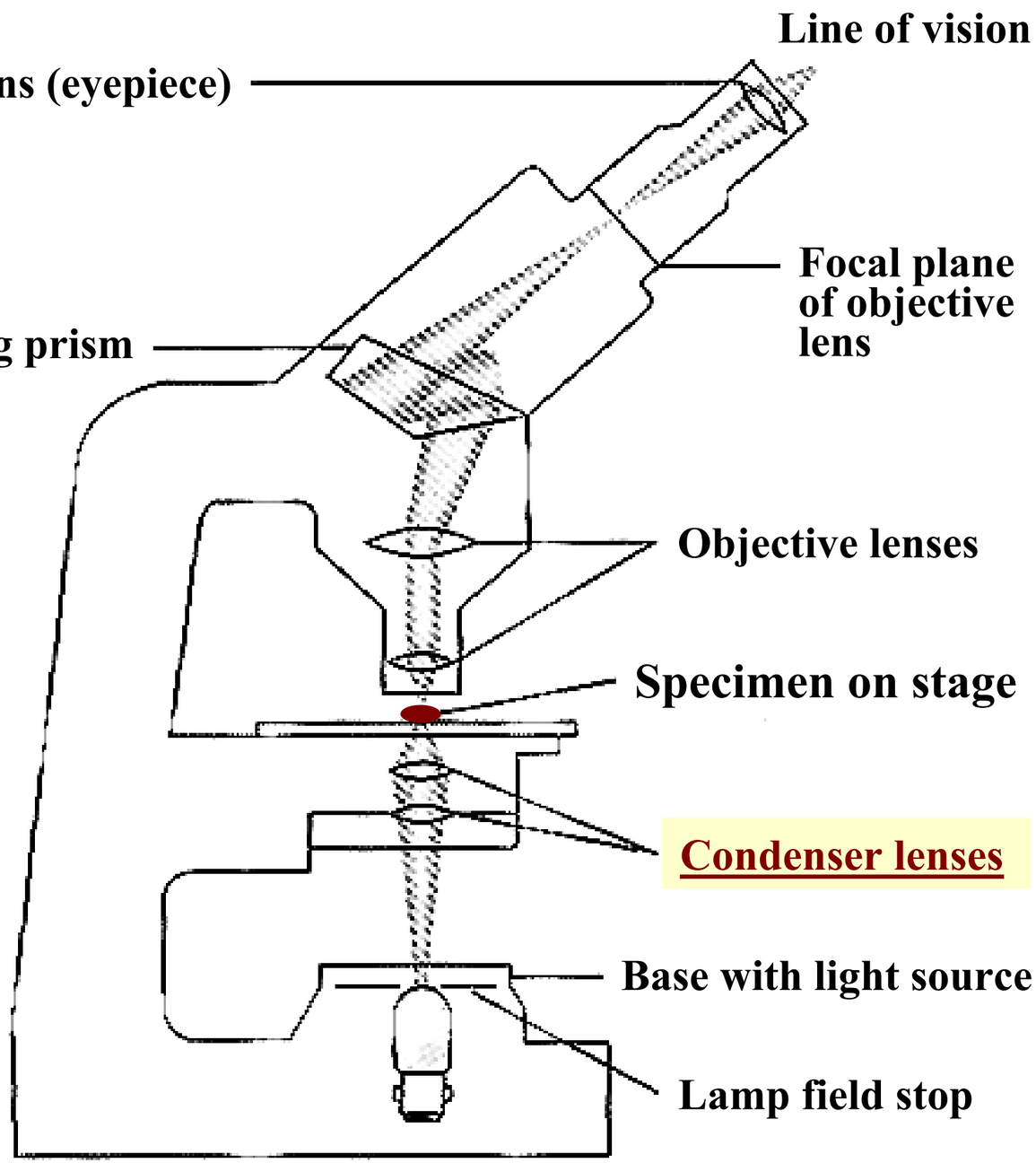




The optical path in a *transmission electron microscope*:

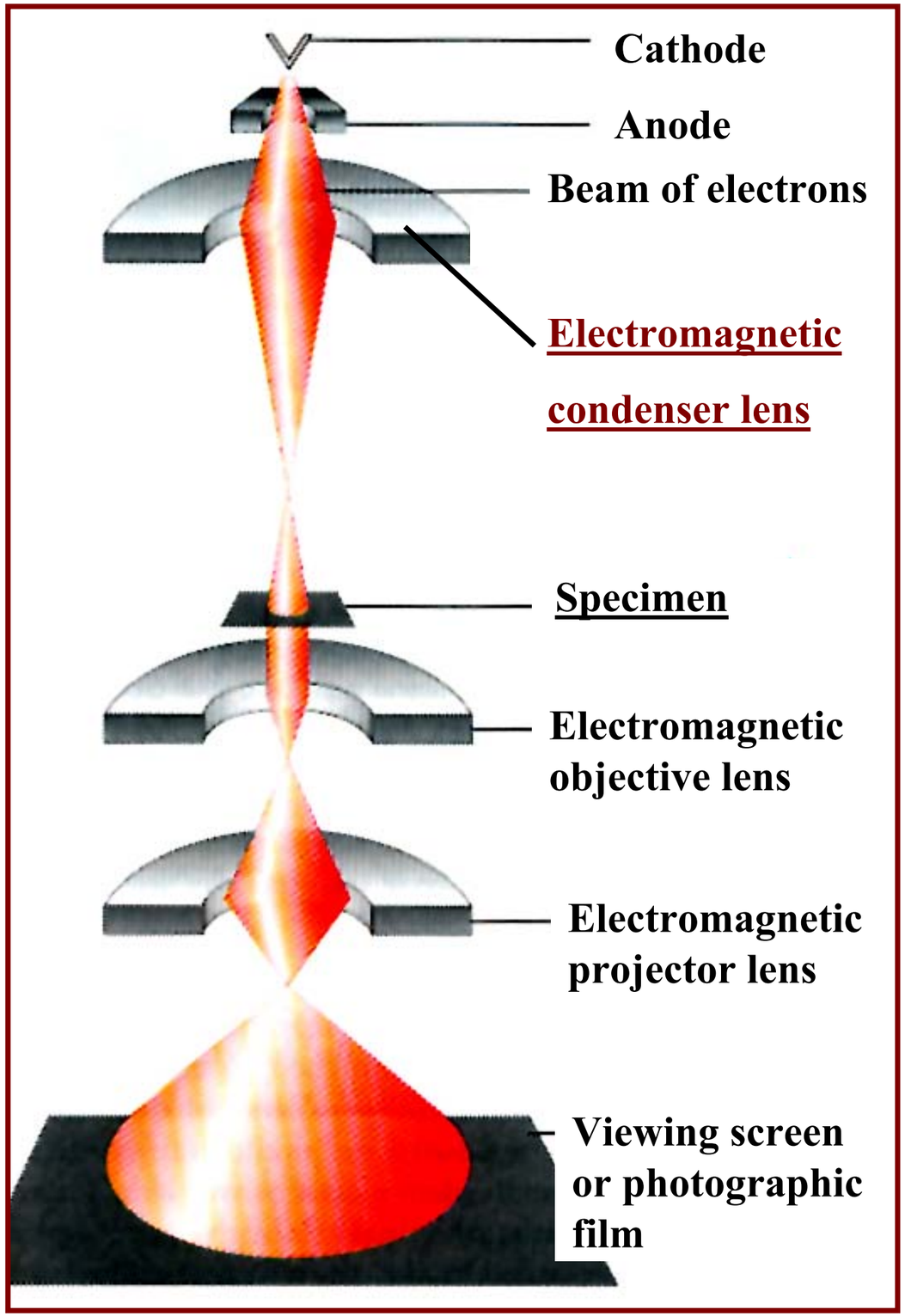
- Electrons are emitted by a cathode when it is electrically heated
- The electric potential of the cathode is kept at 50,000 - 100,000 volts
- The electric potential of the anode is *zero*
- This drop of voltage causes the electrons to *accelerate* as they move toward the anode

The optical pathway in a modern compound *light microscope*



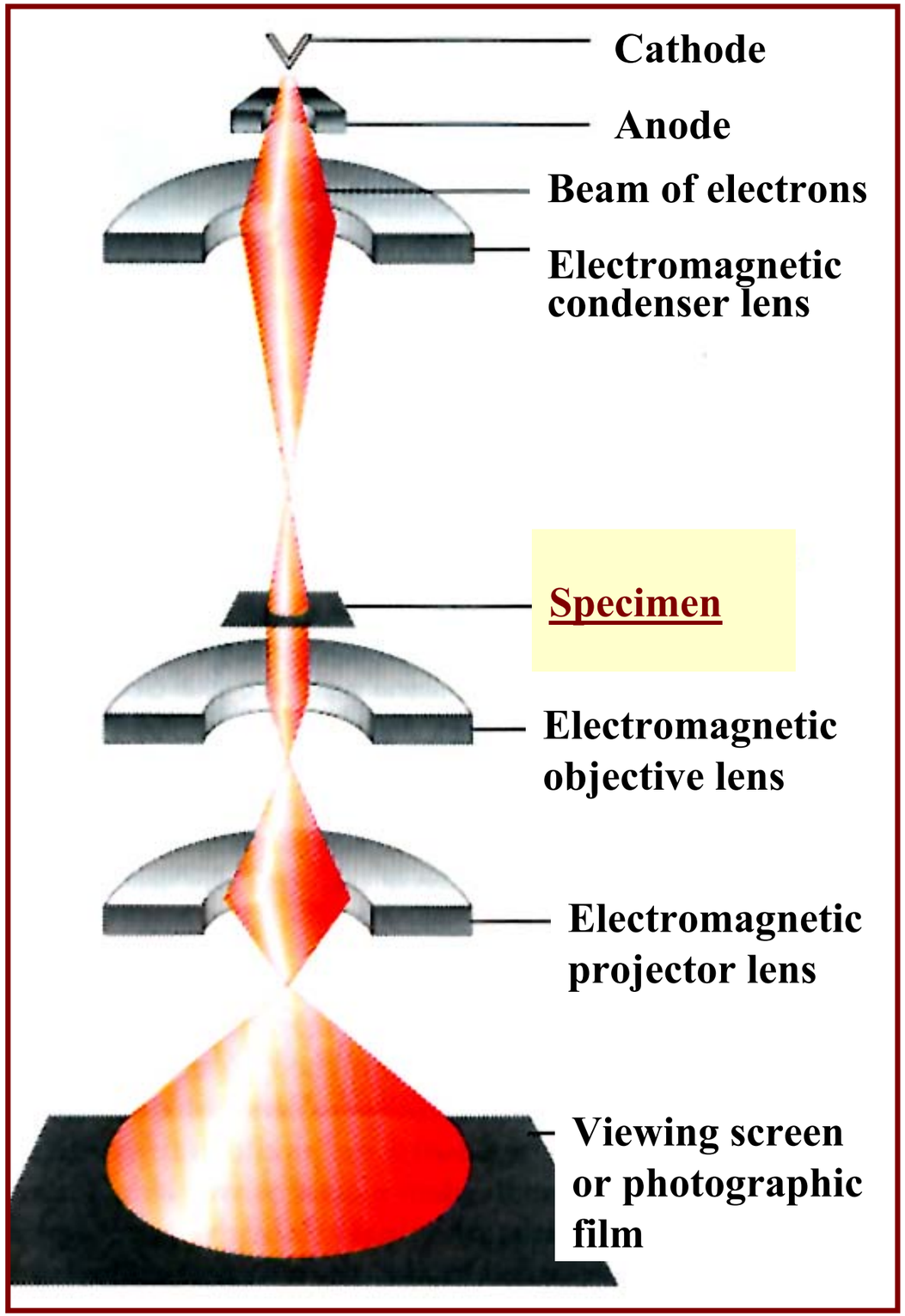
The condenser lenses <u>do not</u> <u>create a magnified image</u> of the specimen !

Light from a bright source <u>is</u> <u>focused by the condenser lenses</u> onto the specimen



The optical path in a transmission *electron microscope*:

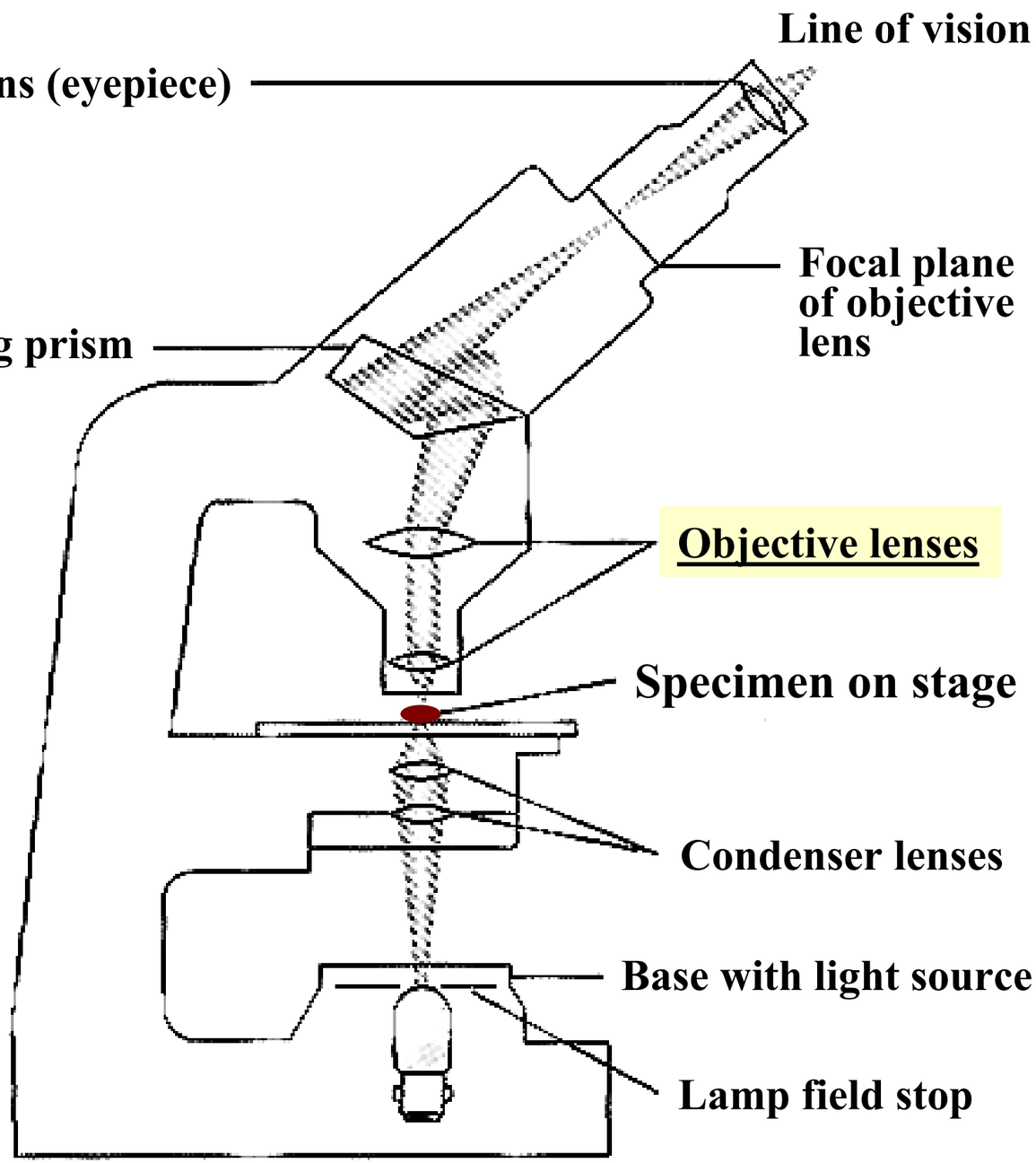
- (1) A beam of electrons is focused onto the specimen plane by the electromagnetic condenser lens
- (2) The electromagnetic condenser lens does *not* create a magnified image of the specimen!



The optical path in a transmission *electron microscope*:

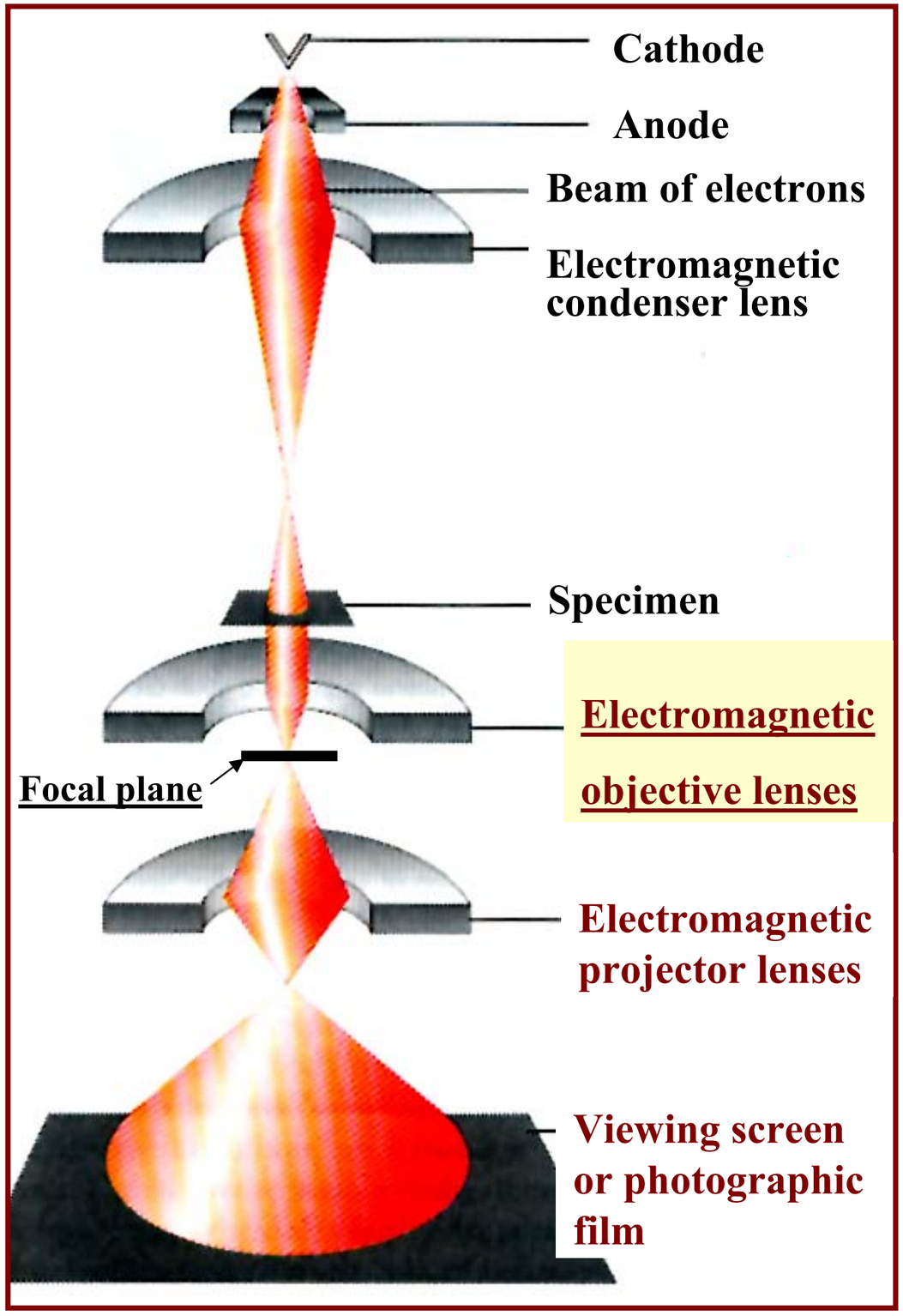
- The specimen must be *extremely thin* – sections are 50 to 100 nm thick

The optical pathway in a modern compound *light microscope*



The objective lenses **pick up the light transmitted by the specimen and focus it on the focal plane of the objective lens, creating a magnified image of the specimen**

YES magnification!



The optical path in a transmission *electron microscope*:

- **The electromagnetic objective lenses:**

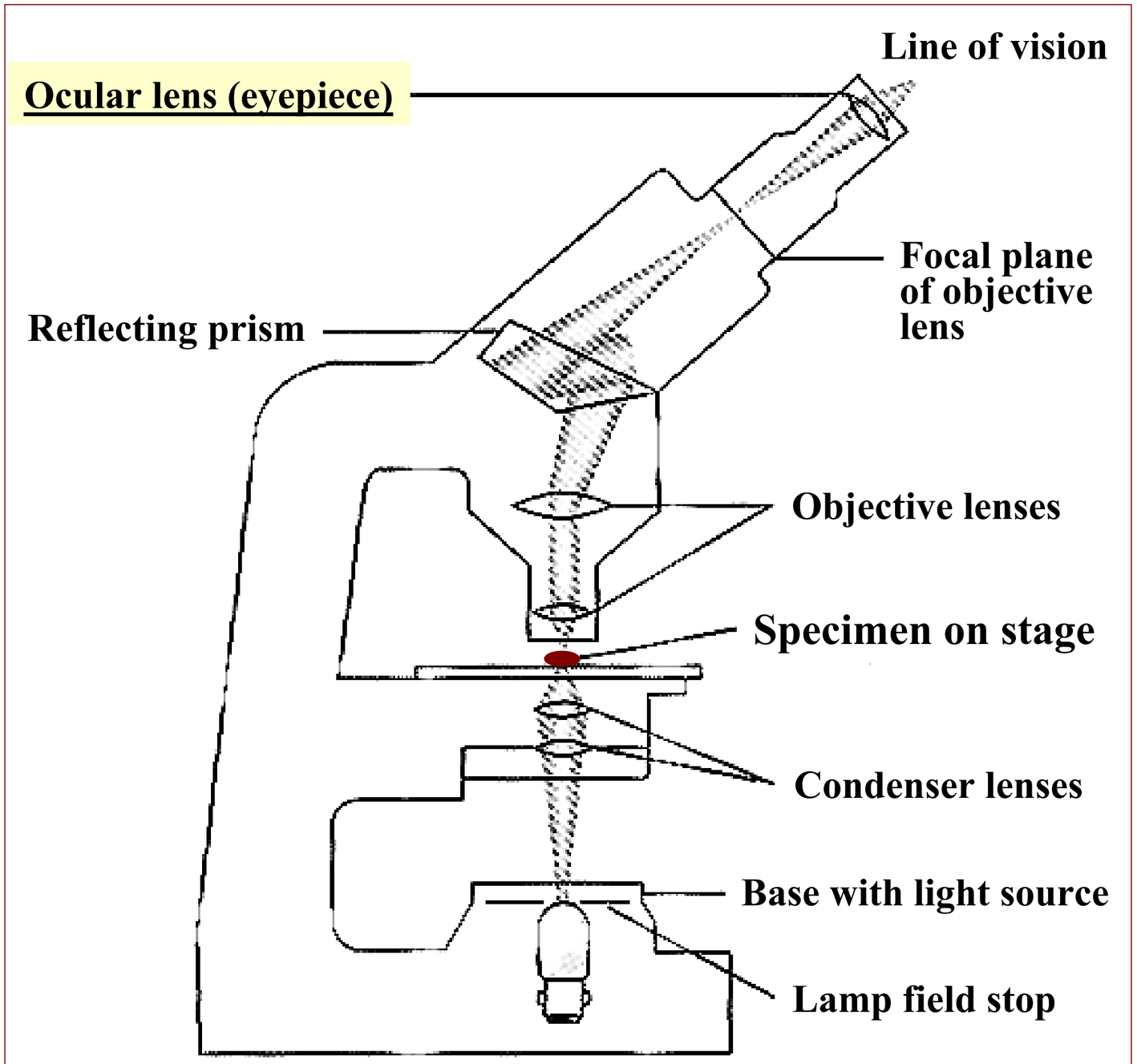
  (1) pick up electrons passed through the specimen

  (2) focus these electrons on the focal plane of the objective lenses and

  (3) create a magnified image of the specimen on the focal plane

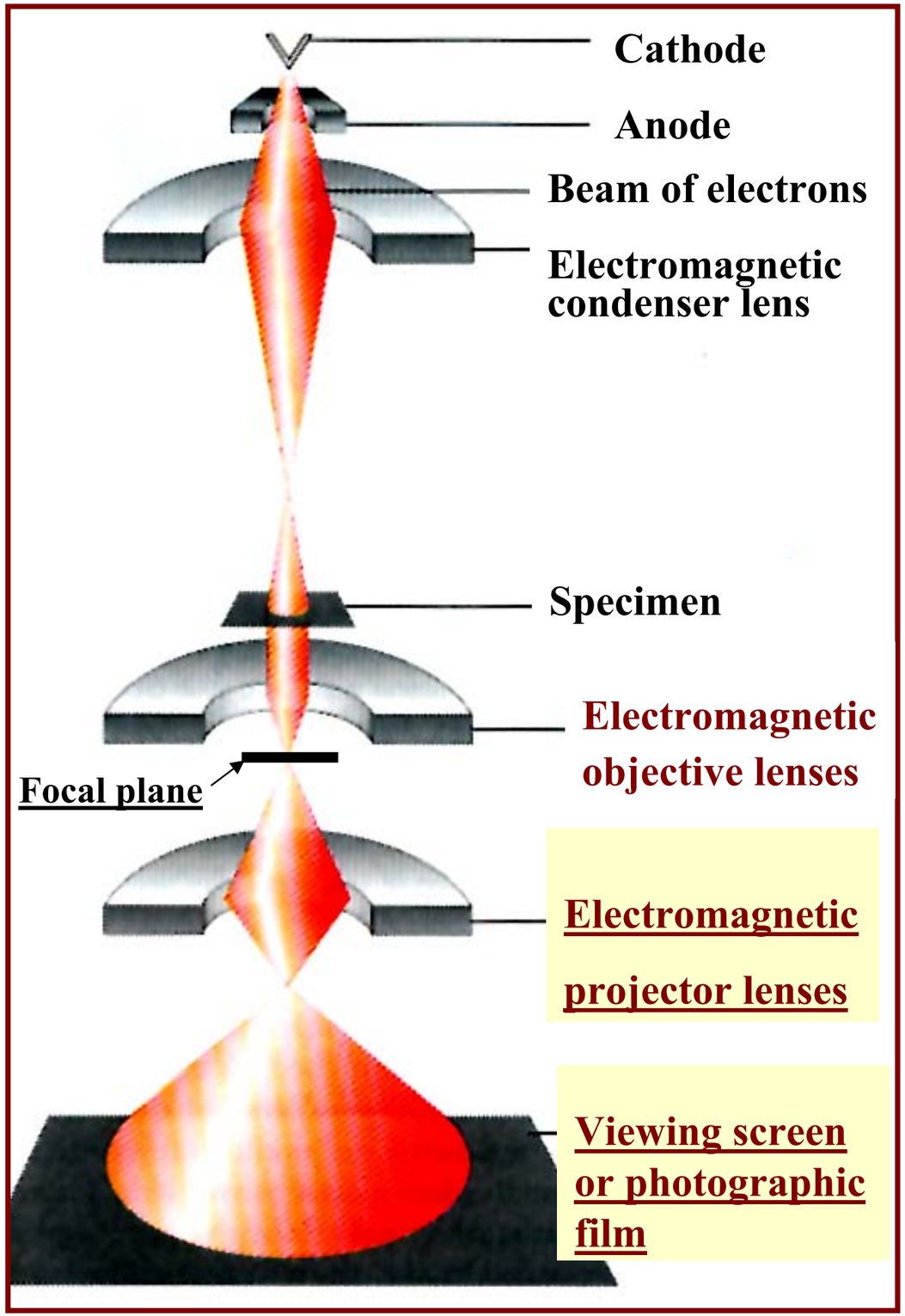
YES magnification!

The optical pathway in a modern compound *light microscope*



The image on the objective focal plane is magnified by the ocular lens, or eyepiece, which is focused on the objective focal plane (line of vision)

YES magnification!



The optical path in a transmission *electron microscope*:

- **The electromagnetic projector lenses (are equivalent to the ocular lenses in a light microscope):**

(1) pick up electrons focused on the focal plane of the objective lenses

(2) focus these electrons on a viewing screen or a piece of photographic film and

(3) create a magnified image of the specimen on the viewing screen or photographic film

YES magnification!

$$D = \frac{0.61 \cdot \lambda}{NA}$$

Light microscope:

λ (blue light) = 450 nm

NA (immersion oil) = 1.4

D = 0.2 µm (= 200 nm)

TEM:

λ (electron) = 0.004 nm

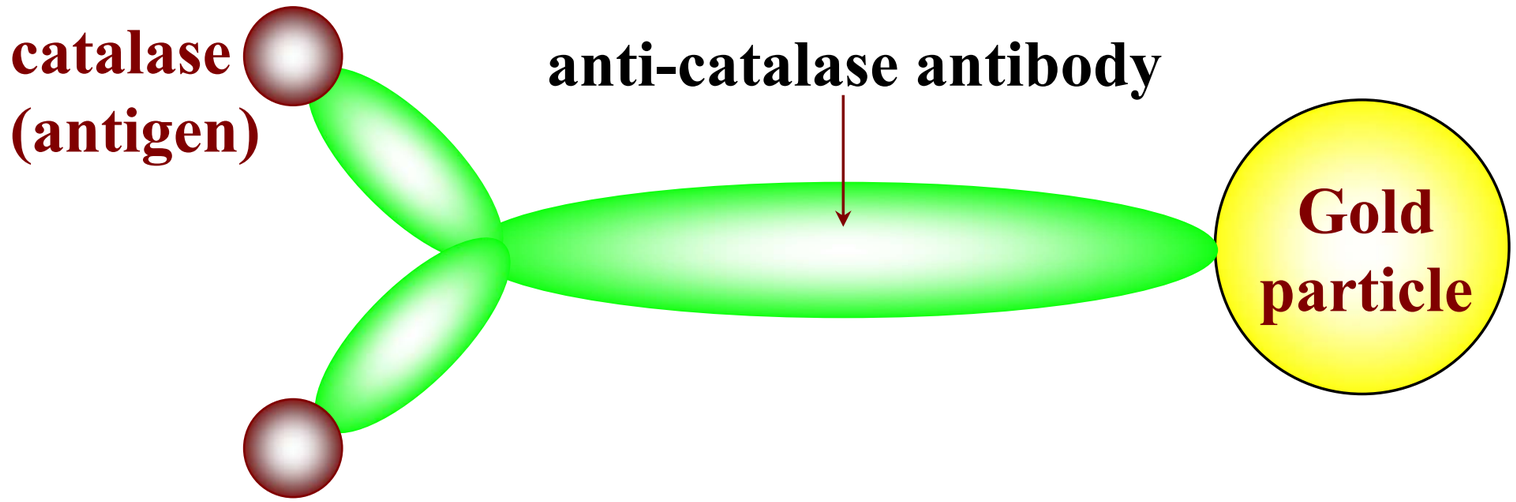
NA (electromagnetic lens) = 0.01

D = 0.2 nm

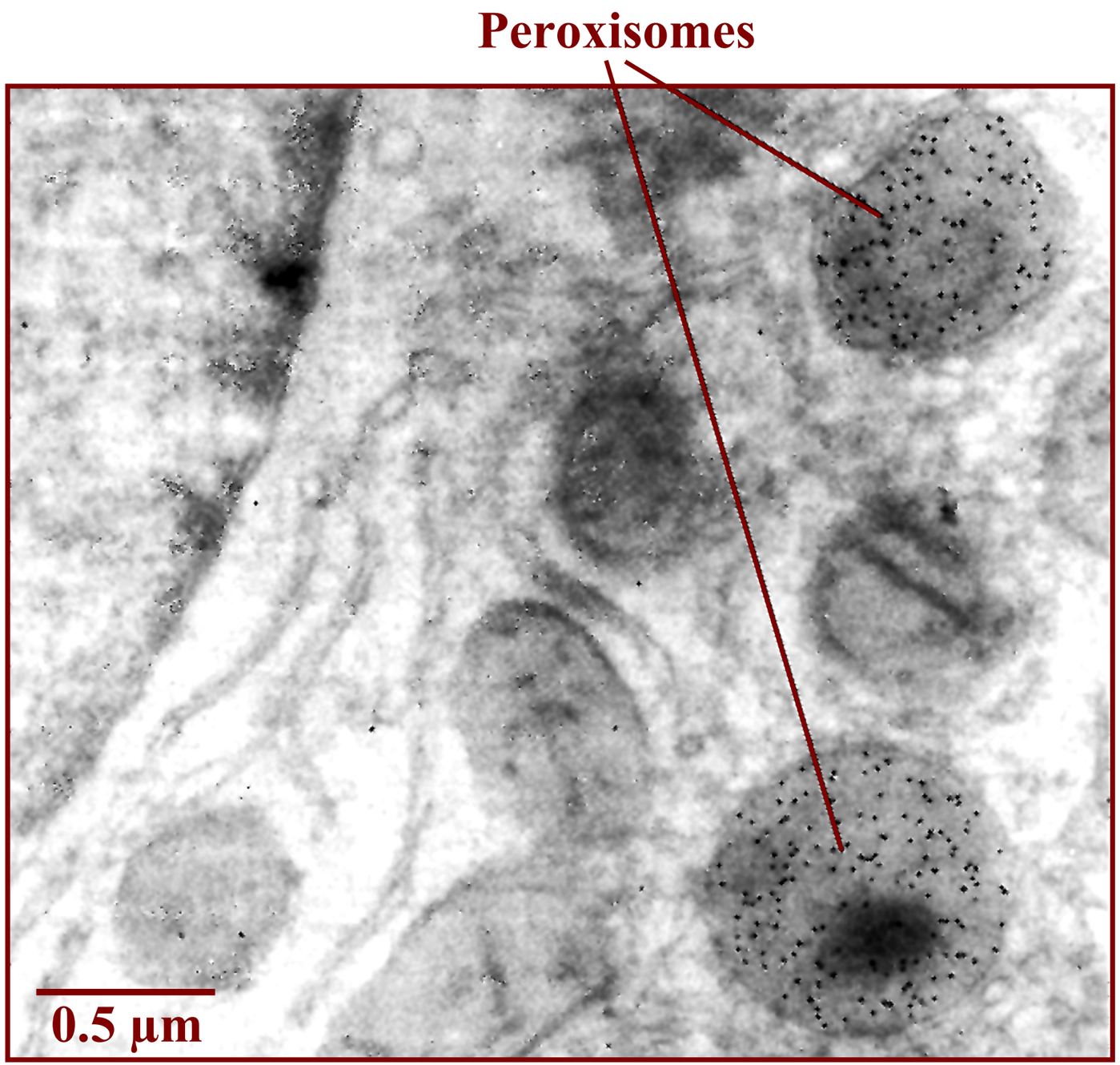
$$\frac{D \text{ (light microscope)}}{D \text{ (electron microscope)}} = 200 \text{ nm} : 0.2 \text{ nm} = 1000$$

Immunogold electron microscopy:

- Attach antibodies directed against a specific protein, catalase, to electron-dense colloidal gold particles (5-20 nm in diameter)
- These antibodies interact only with their specific antigen, catalase



- Treat thin sections of glutaraldehyde-fixed cells or tissues with these gold-labeled anti-catalase antibodies
- Determine the *subcellular location* of catalase in the electron microscope



The gold particles (black dots) indicating the presence of catalase are located exclusively in distinct organelles, called peroxisomes

The subcellular location of catalase in a rat liver cell