Microscopy

Observing Microorganisms Through a Microscope

Need for a Microscope

- Our eyes have a limit to see up-to millimeter range and after that we require an aid to visualize the small objects
- From this need, different visualizing aids has been developed which are collectively comes under a common term known as “microscopy”
- Microscopy is the term used to describe the use of lenses to reveal details of an object
- A microscope (from Greek mikrós “small" and skopeîn, "to look") is an instrument used to visualize objects that are too small to be seen by the naked eye

Historical perspectives

- The microscope was first built in 1595 by Hans and Zacharias Janssen in Holland
- It was perfected in the 17th century in several countries, including by Robert Hooke
- In 1660s, Antony van Leeuwenhoek made a simple microscope using powerful magnifying glass
- Leeuwenhoek's simple microscope could magnify an object up to 200 times and he recorded minute details of various natural samples of animal and plant tissues, blood, fossils etc.
- The limitations including poor image resolutions and colour distortion were removed in the subsequent years
- Later in 19th century, Ernst Abbe discovered that oil-immersion lenses could prevent light distortion at highest magnification power that is still used with 100X objective microscopes

- **The technology of making very small things visible to the naked eye**
- **It is one of the tools essential for studying organisms that are too small to be seen**
- **The metric system is used to measure microorganisms**
- **Metric system: Basic unit of length: Meter**

❖ **kilometer (km) 1 km = 1000 m**

❖ **meter (m) 1 m = 39.37 in**

❖ **decimeter (dm) 1 dm = 0.1 m = 10^{-1}m**

❖ **centimeter (cm) 1 cm = 0.01 m = 10^{-2}m**

❖ **millimeter (mm) 1 mm = 0.001 m = 10^{-3}m**

❖ **micrometer (um) 1 um = 0.000001 m = 10^{-6}m**

❖ **nanometer (nm) 1 nm = 0.000000001 m = 10^{-9}m**

❖ **picometer (pm) 1 pm = 0.000000000001 m = 10^{-12}m**

Terms to be Remember

- **Magnification:** The process of enlarging the image of an object
- **Resolution/ Resolving power:** The ability of lens system to distinguish two adjacent objects as separate and distinct image
- **Wavelength:** The distance between two successive crests of a wave (λ)
- The lens system of human eye has a resolving power of approximate 0.2 mm, which means that eye cannot see any thing that is smaller than 0.2 mm

- The resolving power of a microscope is dependent on the wavelength of the beam of light used and the optical quality of lenses
- Shorter wavelength give better resolution

$$RP = \frac{\lambda}{2 * NA}$$

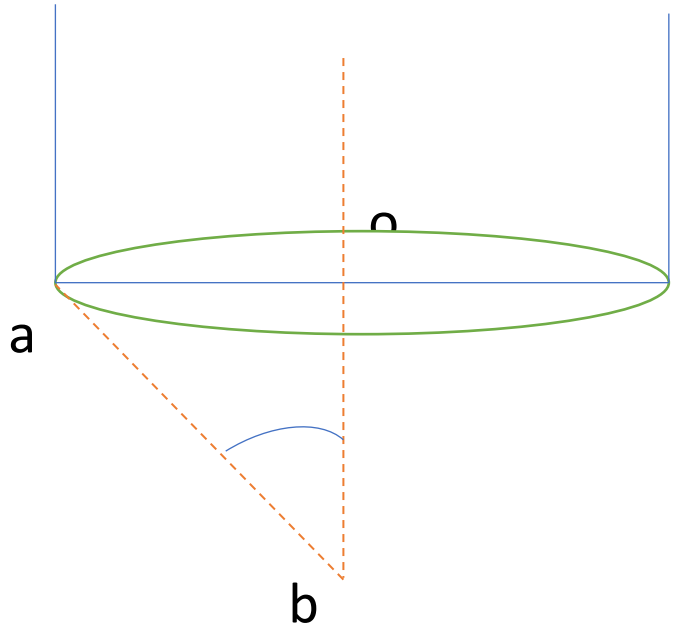
- λ usually set at 550nm for better results

- **Numerical aperture:** Function of the diameter of the objective lens in relation to its focal length

$$NA = \eta \sin\theta$$

where η = refractive index of the medium through which light passes before entering the object

$\sin\theta$ is the trigonometric sign of one half the angle formed by the light rays coming from the condenser and passing through the material



$$\sin\theta = \frac{ao}{ab}$$

- **With dry objective the value of η is 1, since the refractive index of air is 1, with the use of immersion oil η is 1.56**

If θ is 58^{o} then NA = 1.56 X sin58

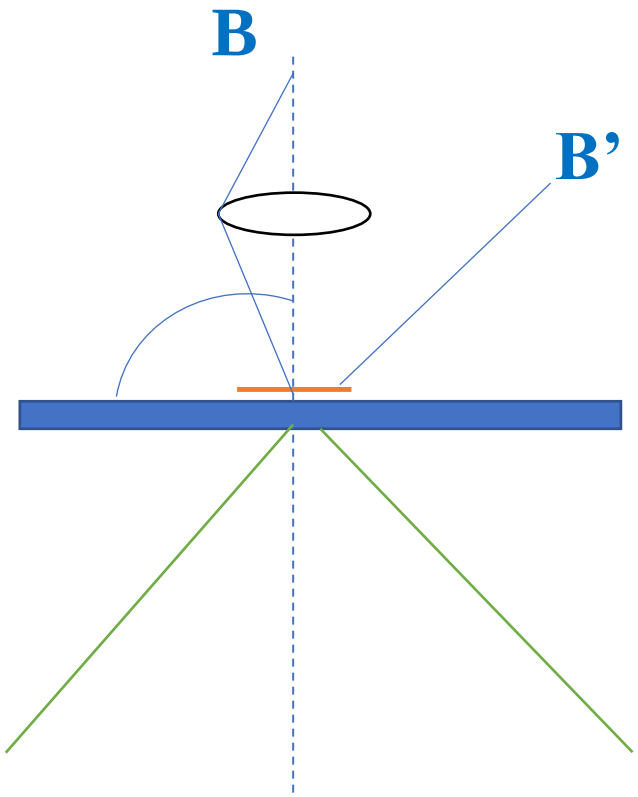
= 1.56 X 0.85 = 1.33

For low power objective, a common NA is 0.25 and for high power objective 0.65 and for oil immersion it is 1.25

RP of low power = $\frac{550}{2\ X\ 0.25}$ = 1100 nm = 1.1 µm

RP of high power = $\frac{550}{2\ X\ 0.65}$ = 423.07 nm = 0.42 µm

RP of oil immersion = $\frac{550}{2\ X\ 1.25}$ = 220 nm = 0.22 µm

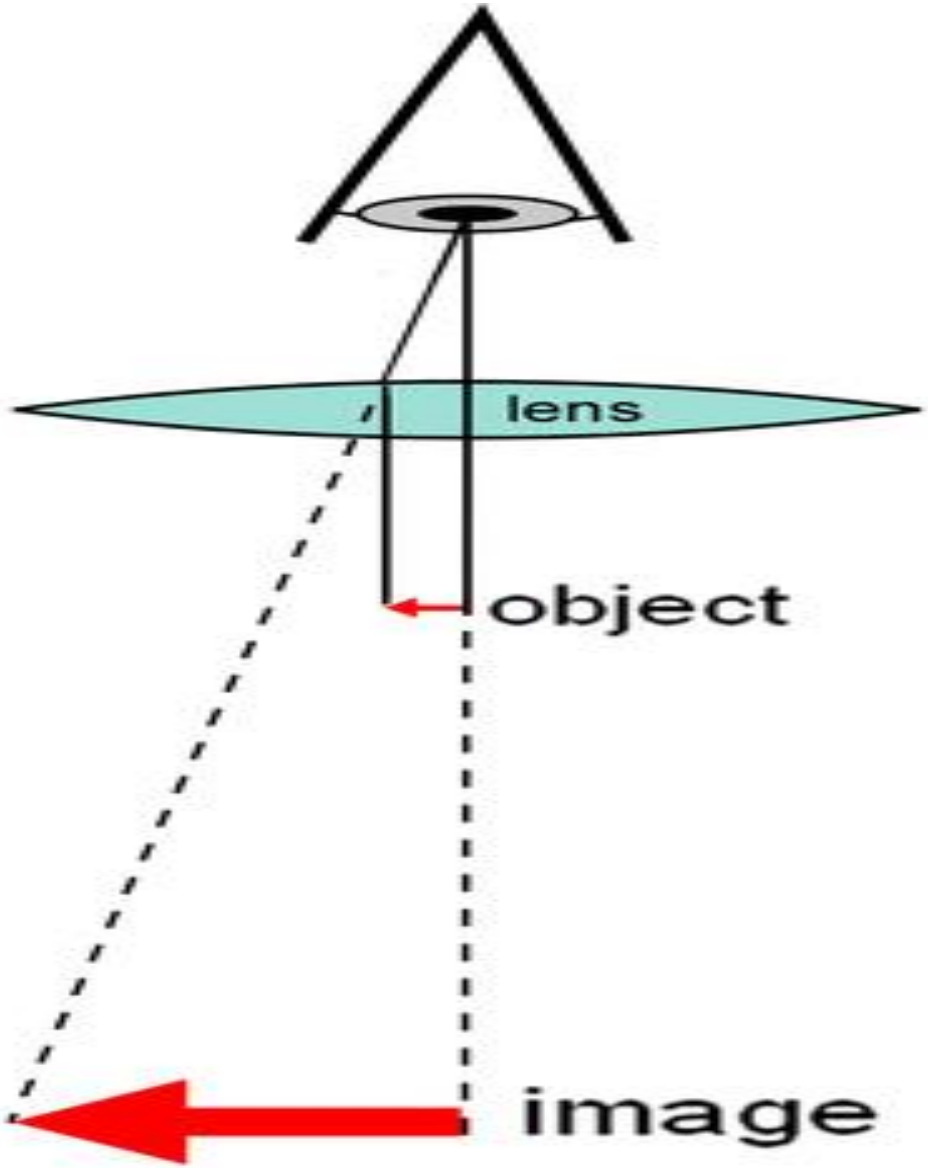


Light rays saved due to oil immersion A → B

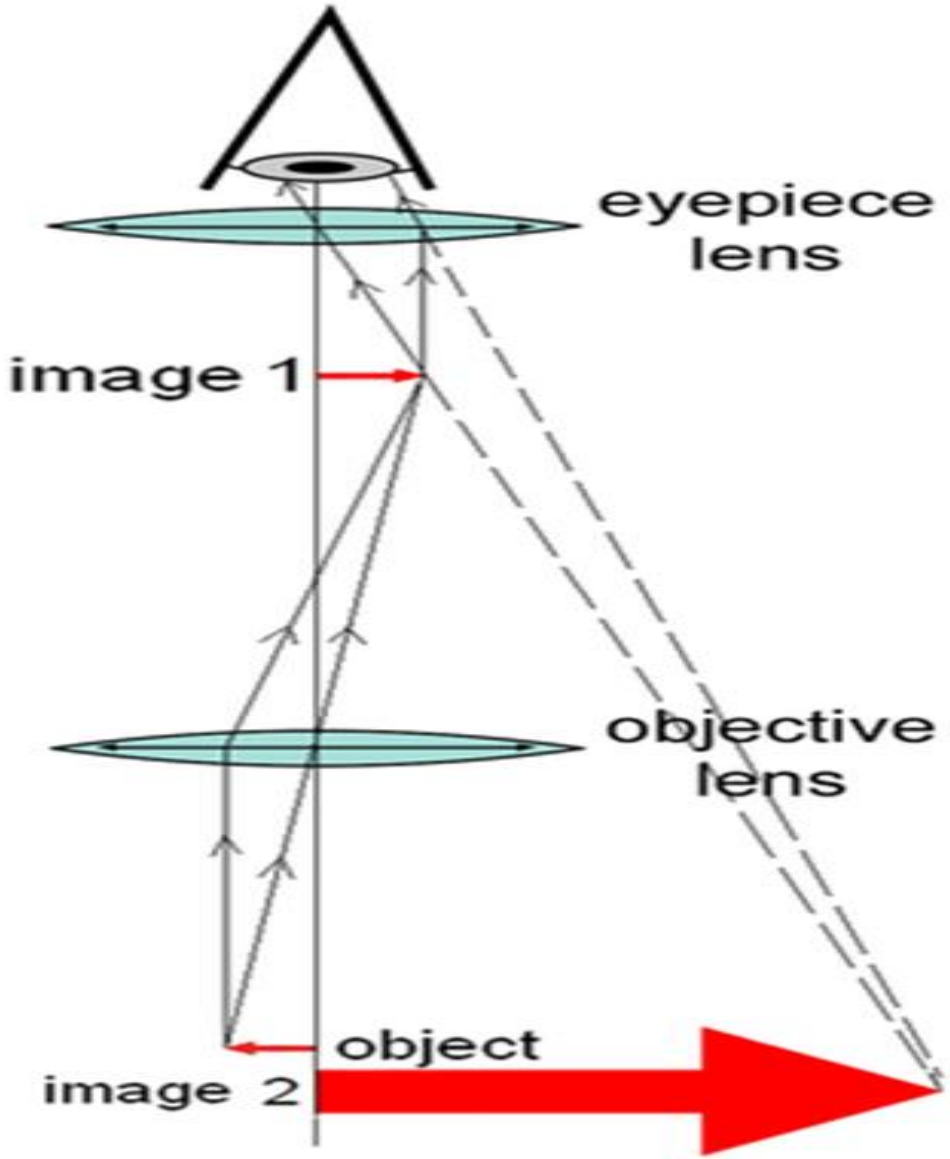
Light rays lost A' → B' showing low magnification

A A'

Simple microscope



Compound Microscope



Instruments of Microscopy

1. Simple Microscope:

- Only have one lens, similar to a magnifying glass
- Magnify images from 100 to 300 X
- First developed by Leeuwenhoeck

2. Compound Light (CL) Microscope:

❖First developed by Zaccharias Janssen, Dutch spectacle maker in 1600

✓Poor quality and could not see bacteria

❖ Joseph Jackson Lister (Lister's father) developed improved compound light microscope in 1830s

✓Basis for modern microscopes

❖Use visible light as a source of illumination

Compound Light Microscopy…….

- ➢ Have several lenses:

- Light originates from an illuminator and passes
- through condenser lenses, which direct light onto the specimen.
- Light then enters the objective lenses, which magnify the image
- These are the closest lenses to the specimen:
  - Scanning objective lens: 4 X
  - Low power objective lens: 10 X
  - High power objective lens: 40-45 X
  - Oil immersion lens: 95-100 X
- The image of the specimen is magnified once again by the ocular lens or eyepiece (10 X)

Compound Light Microscopy

❖ **Total magnification: Obtained by multiplying**

- **Objective lens power by ocular lens power**
- **Condenser lenses do not magnify image**

Lens	Magnification	Ocular Mag.	Total Mag.
Scanning	4 X	10 X	= 40 X
Low power	10 X	10 X	= 100 X
High power	45 X	10 X	= 450 X
Oil immersion	100X	10 X	= 1000 X

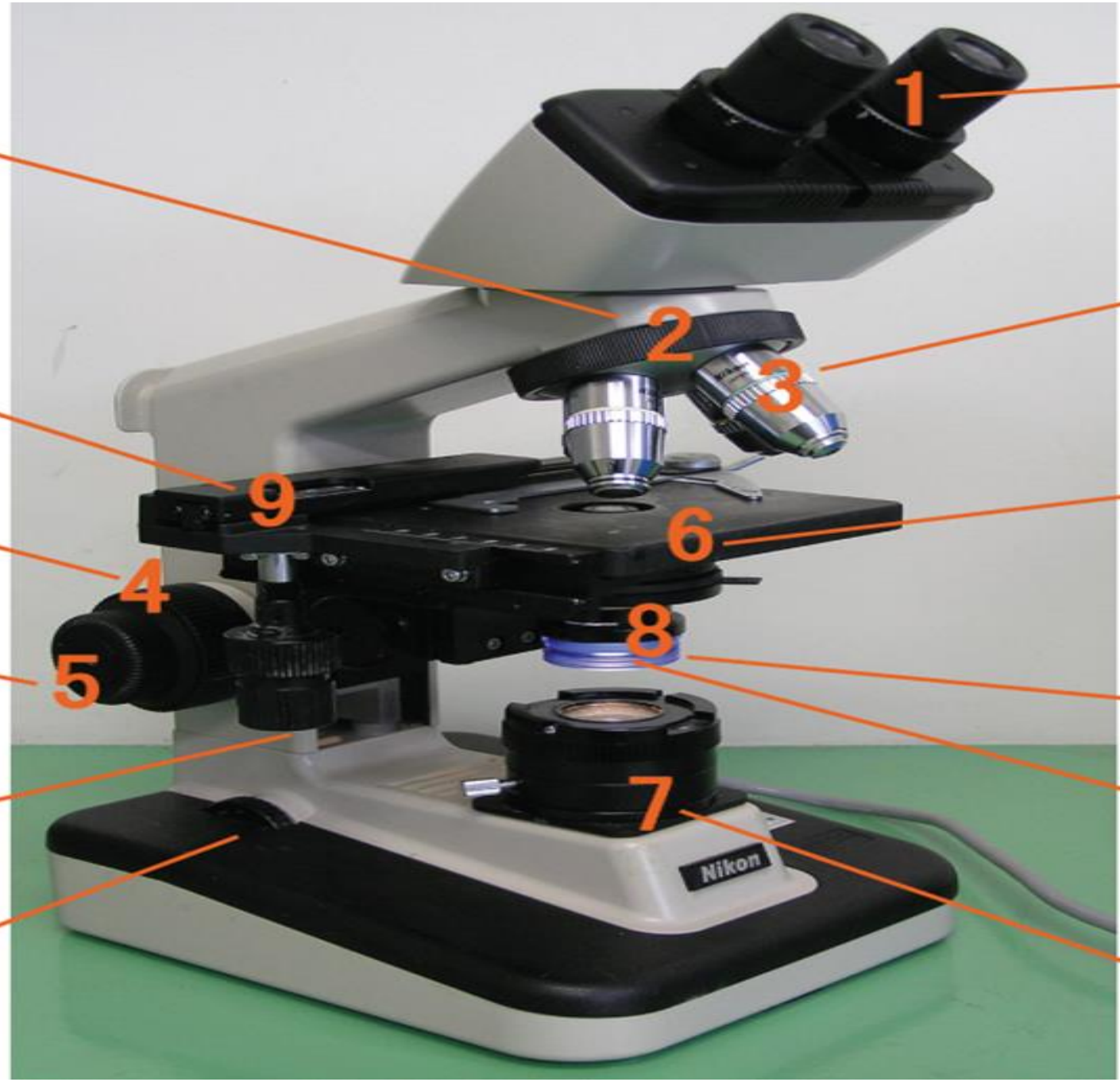
- **Highest possible magnification with CL microscope is about 2000 X**

Compound Light Microscopy…..

- **Resolution (Resolving power): Ability of microscope to see two items as separate and discrete units**
  - **The smaller the distance between objects at which they can be distinguished as separate, the greater the resolving power**
  - **Light must pass between two objects in order for them to be seen as separate.**
  - **Depends on light wavelength. If wavelength is too long to pass between objects, they will appear as one.**
    - ✓**White light has a relatively long wavelength (550 nm), and cannot resolve structures less than 220 nm (0.2 um) apart**
    - ✓**Ultraviolet (UV) light has a shorter wavelength (100 to 400 nm), and can resolve distances as small as 110 nm**

➢**Refraction: Bending of light as it passes from one medium to another of different density**

- **Index of refraction: A measure of the speed at which light passes through a material**
- **Can be changed by staining, which increases contrast between specimen and surrounding medium**
- **Immersion oil has the same index of refraction as glass slide, preventing light loss from refraction**



revolving nose piece (to hold multiple objective lenses)

mechanical stage

coarse focus (larger knob)

fine focus (small knob)

x-y mechanical stage knobs (to move slide)

rheostat (to adjust light intensity)

eyepiece (ocular lens)

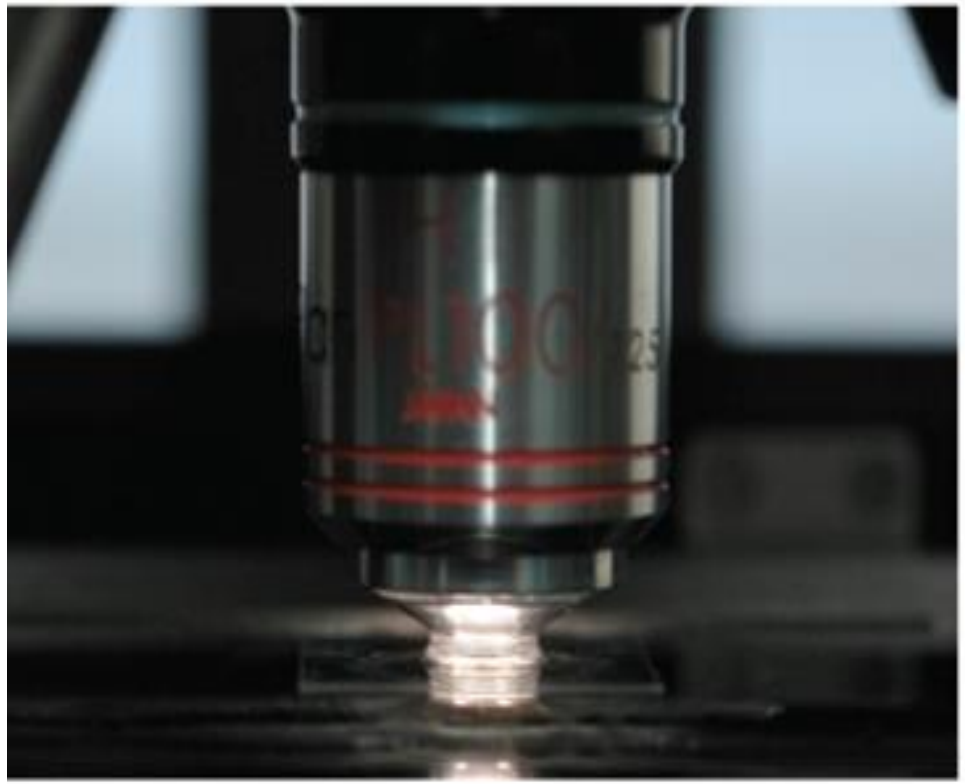
objective lenses

stage (to hold the specimen)

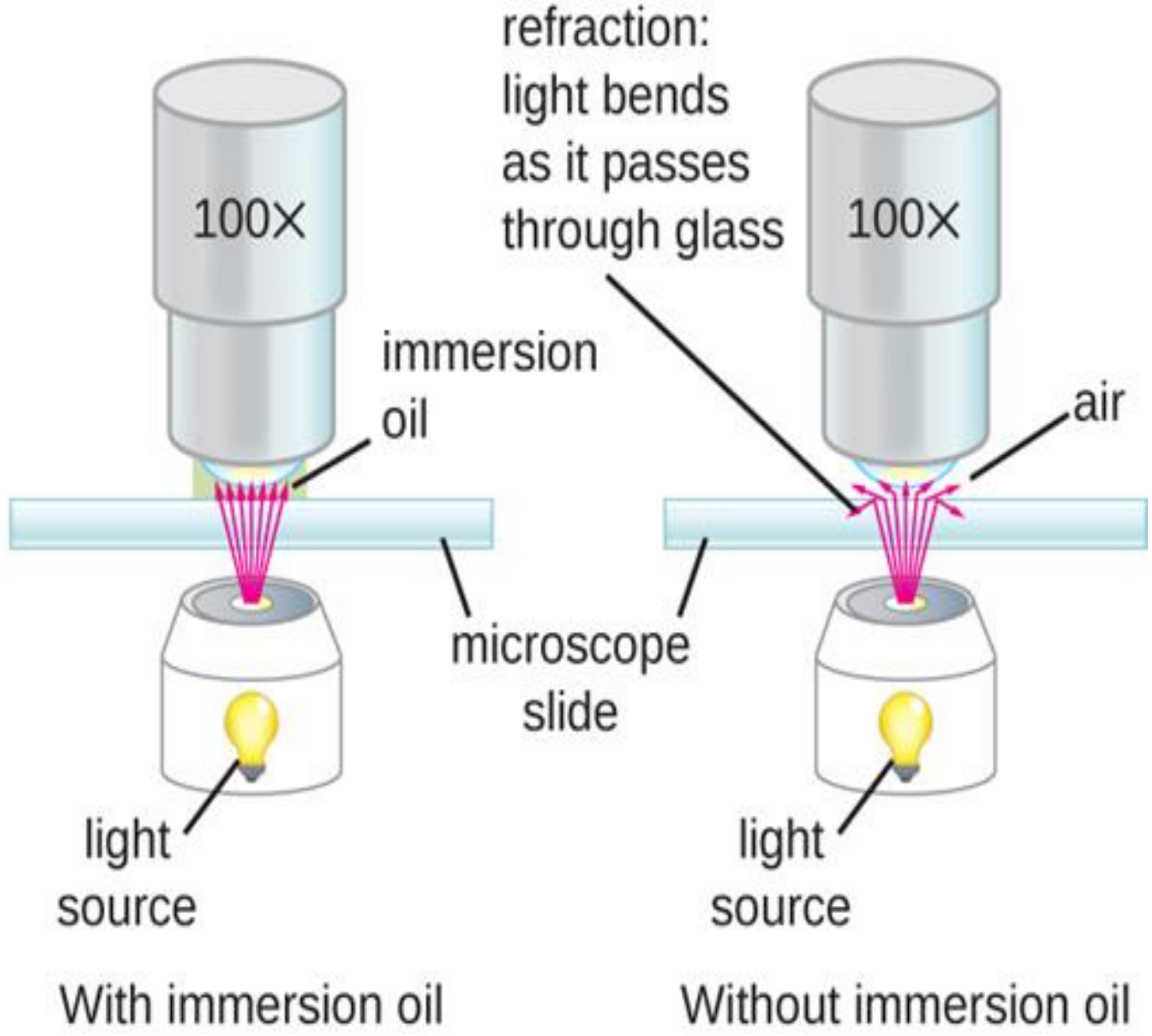
diaphragm

condenser

illuminator



(a)


refraction:
light bends
as it passes
through glass
100X
100X
immersion
oil
air
microscope
slide
light
source
light
source
With immersion oil
Without immersion oil

(b)