Unit 1: Bright field microscopy

Instrumentation and Biotechniques,
DSE-7, Sem VI

Introduction

- Microorganisms are much too small to be seen with the unaided eye; they must be observed with a microscope
- Latin word ***micro (small)*** *and the Greek word* ***skopos (to look at)***
- Robert Hooke –compound microscope, cells
- van Leeuwenhoek – Animalcules, simple microscopes, 300X magnification

Units of Measurements

Unit	Abbreviation	Value
1 centimeter	cm	10^{-2} meter or 0.394 inches
1 millimeter	mm	10^{-3} meter
1 micrometer	μm	10^{-6} meter
1 nanometer	nm	10^{-9} meter
1 Angstrom	Å	10^{-10} meter

Light Microscopy

- **Light microscopy refers to the use of any kind of microscope that** uses visible light to observe specimens.
- Types of light microscopes are:

1. Bright-field,
2. Dark-field,
3. Phase-contrast, and
4. Fluorescence microscopes.

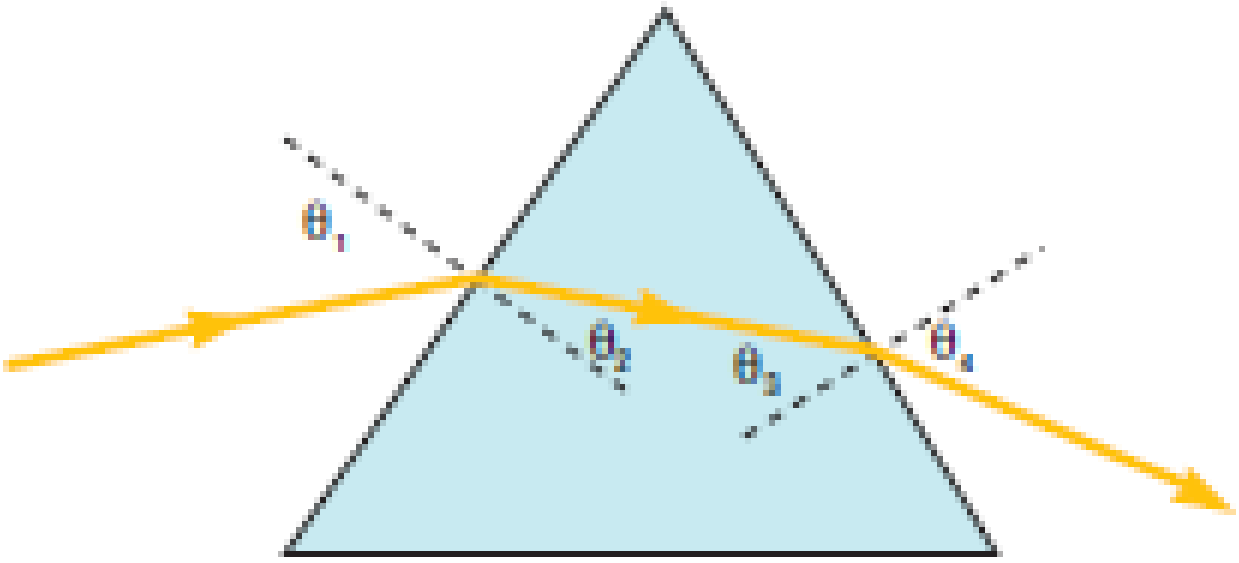
   Each yields a distinctive image and may be used to observe different aspects of microbial morphology.

Bright field microscopy

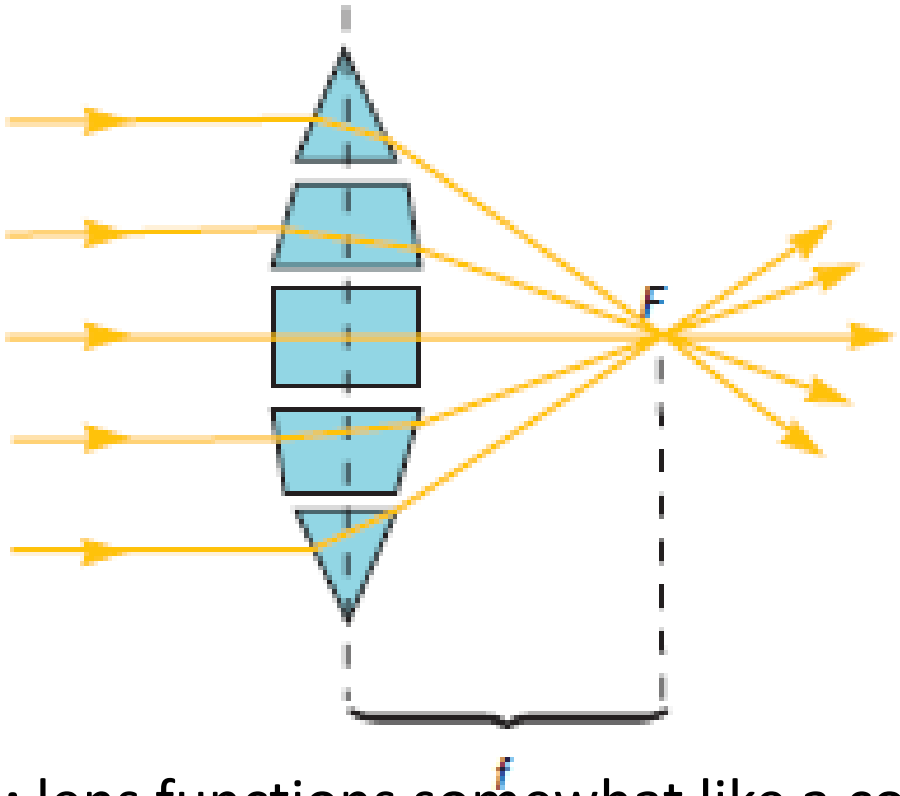
- The ordinary microscope is called a **bright-field microscope because** it forms a dark image against a brighter background.
- Most microorganisms are colorless and therefore not easily seen in the bright-field microscope, they are usually fixed and stained before observation.
- Either simple or differential staining can be used to enhance contrast.
- Specific bacterial structures such as capsules, endospores, and flagella also can be selectively stained.

Refractive index

- When a ray of light passes from one medium to another, **refraction occurs—that is, the ray is bent at the interface**
- The **refractive index** is a measure of how greatly a substance slows the velocity of light, and the direction and magnitude of bending is determined by the refractive indexes of the two media forming the interface.



Bending of light rays in a prism



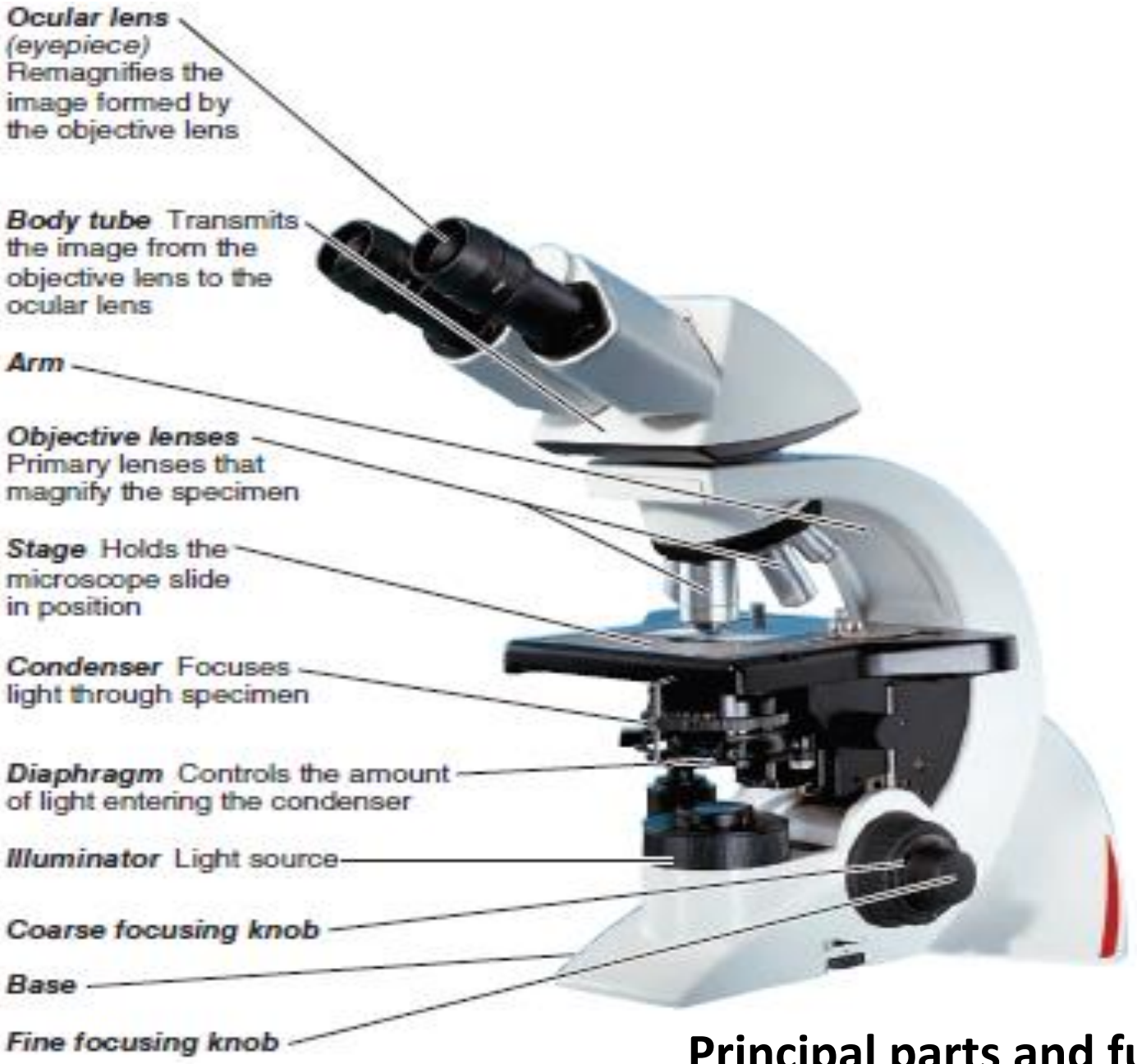
Lens function: lens functions somewhat like a collection of prisms. Light rays from a distant source are focused at the focal point *F.* *The focal point lies a distance f, the focal length, from the* lens centre.

- Our eyes cannot focus on objects nearer than about 25 cm or 10 inches

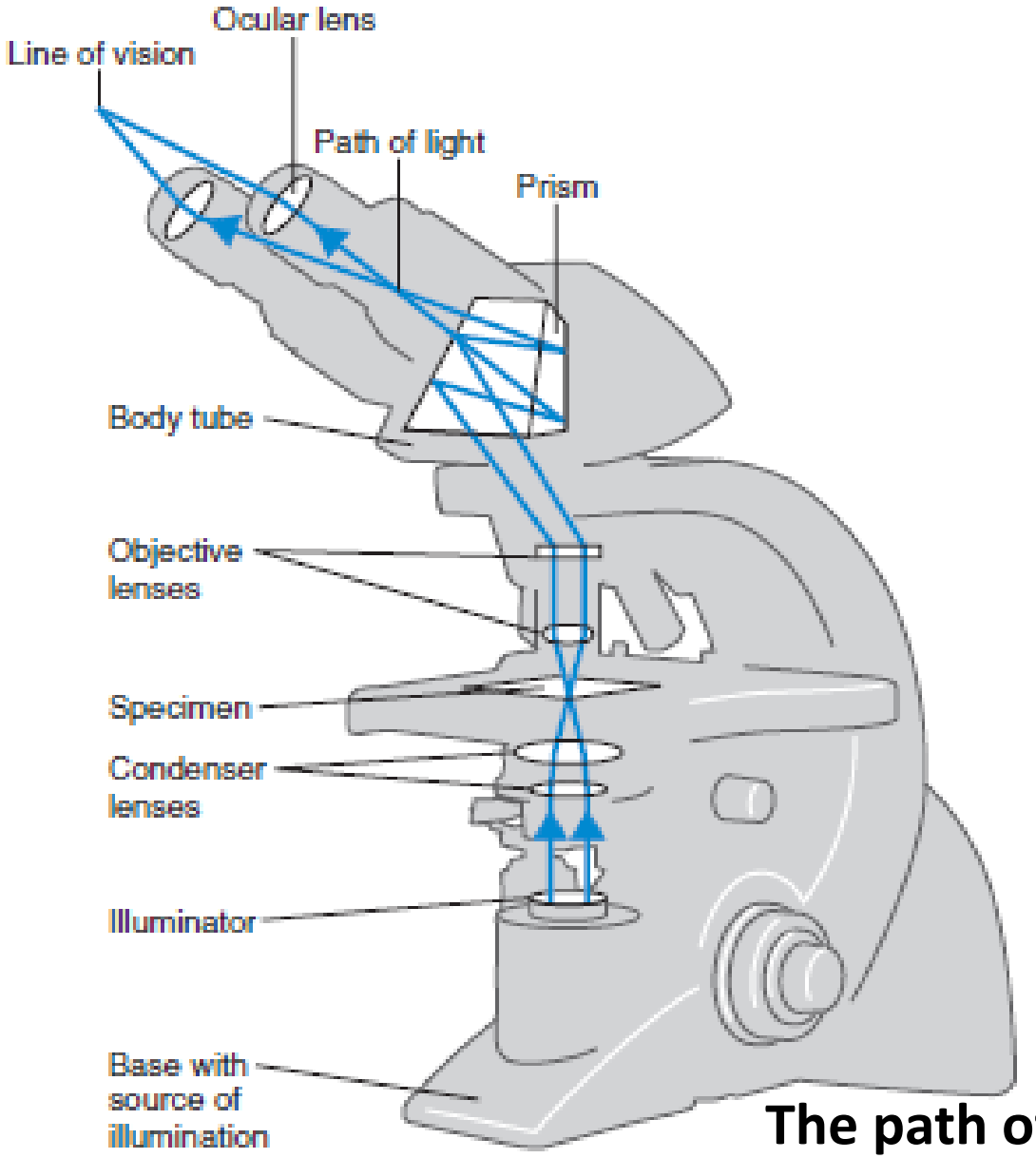
- Lens strength is related to focal length; a lens with a short focal length will magnify an object more than a weaker lens having a longer focal length.

- Modern microscopes are all compound microscopes.
- In Compound microscope, a magnified image formed by the objective lens is further enlarged by one or more additional lenses.

A Bright- field Compound Microscope



Principal parts and functions



The path of light (Bottom to top)

- The **substage condenser** is mounted within or beneath the stage and focuses a cone of light on the slide
- The nosepiece holds three to five **objectives with lenses of differing** magnifying power and can be rotated to position any objective beneath the body assembly.
- **parfocal—that is, the image should remain in focus when objectives** are changed

- The objective lens forms an **enlarged real image** within the microscope, and the eyepiece lens further magnifies this primary image.

- When one looks into a microscope, the enlarged specimen image, called the **virtual image**, appears to lie just beyond the stage about 25 cm away.

- The total magnification is calculated by multiplying the objective and eyepiece magnifications together.

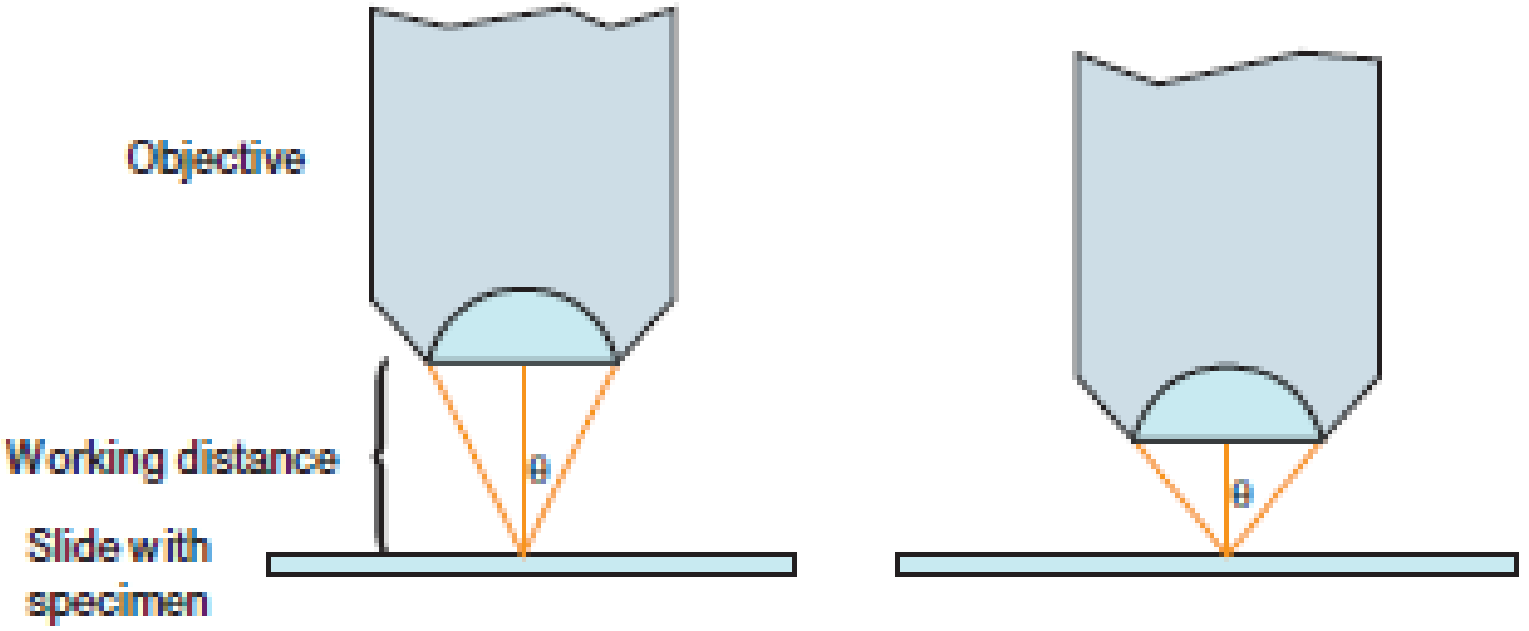
Resolution:

- **Resolution** is the ability of a lens to separate or distinguish between small objects that are close together
- German physicist Ernst Abbe in the 1870s.
- The minimum distance (*d) between two objects that reveals them* as separate entities is given by the Abbe equation, in which lambda (λ) is the wavelength of light used to illuminate the specimen and *n sin* θ *is the numerical aperture (NA).*

$$d = \frac{0.5\lambda}{n \sin \theta}$$

- As *d becomes smaller, the resolution increases, and finer detail* can be discerned in a specimen
- The wavelength must be shorter than the distance between two objects or they will not be seen clearly. Thus the greatest resolution is obtained with light of the shortest wavelength, light at the blue end of the visible spectrum (in the range of 450 to 500 nm).

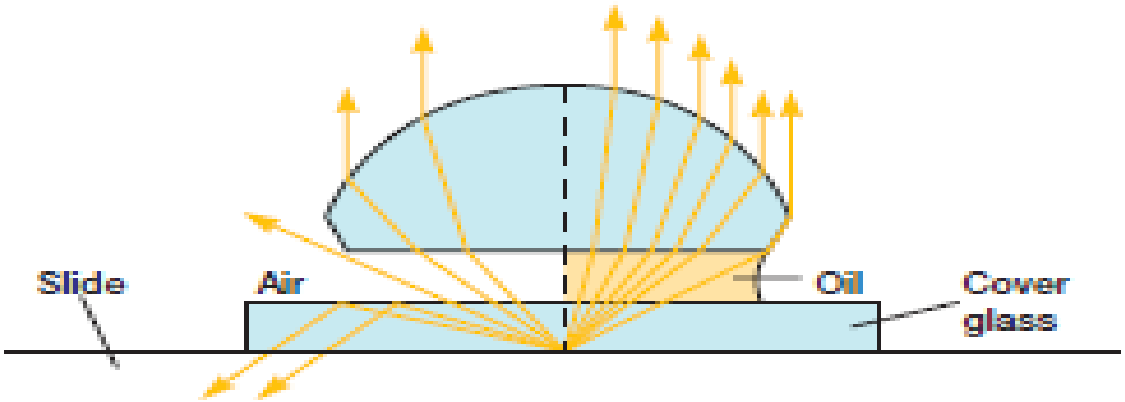
Numerical aperture in Microscopy



Numerical aperture in Microscopy

- ***n sin θ, where* Theta is defined as 1 /2 the angle of the cone of light entering an objective**
- The angle of the cone of light that can enter a lens depends on the refractive index (*n) of the medium in which the* lens works, as well as upon the objective itself
- The refractive index for air is 1.00. Since sin θ cannot be greater than 1 (the maximum is 90° and sin 90° is 1.00),
- No lens working in air can have a numerical aperture greater than 1.00. The only practical way to raise the numerical aperture above 1.00, and therefore achieve higher resolution, is to increase the refractive index with **immersion oil**, a colorless liquid with the same refractive index as glass

Oil immersion objective



The total power of magnification of the final image formed by the combined lenses is a product of the separate powers of the two lenses:

Power of objective	×	*Power of ocular*	=	*Total magnification*
10× low power objective		10×	=	100×
40× high dry objective		10×	=	400×
100× oil immersion objective		10×	=	1,000×

Depending on the power of the ocular, the total magnification of standard light microscopes can vary from 40 X with the lowest power objective (called the scanning objective) to 2,000 X with the highest power objective (the oil immersion objective).