Microscopy

322 Histological Techniques

Agenda for today

- What is Microscope, Facts and structures ?
- What is it use for?
- How is it function?
- What type of Microscope?

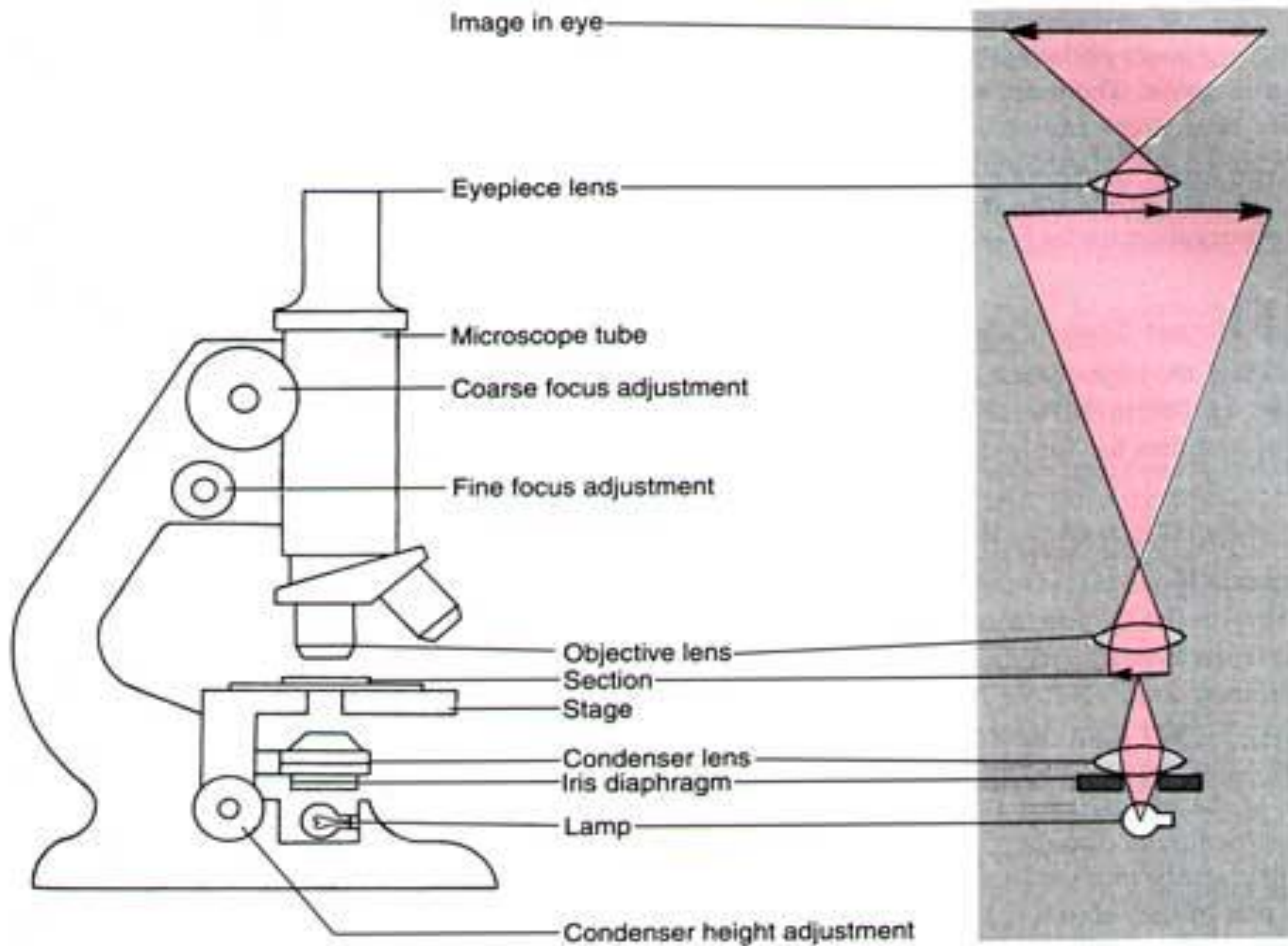


Image in eye
Eyepiece lens
Microscope tube
Coarse focus adjustment
Fine focus adjustment
Objective lens
Section
Stage
Condenser lens
Iris diaphragm
Lamp
Condenser height adjustment

Fact About Microscopes

Light microsocopes are used to see details, and enlarged images of small objects.

Magnification is enlarging an image

Resolution is the amount of fine detail that can be seen

Light is focused onto the specimen (i.e. the histology slide) by a condenser.

About Microscopes con.

The image produced is magnified by a combination of the objective lens and the eyepiece lens.

Usually, the eyepiece lens gives a x10 magnification.

Three objective lenses are usually used: x10, x40 and x100

The x100 lens is usually an oil-immersion lens - you need to view the sample through a drop of oil.

Disadvantage of Light Microscopy

- The resolving power is limited:

The resolving power (resolution) of a light microscope is.

Resolution = 0.61 x lambda /NA

λ Lambda is the wavelength of the illuminating radiation,

NA the numerical aperture of the lens.

For a light microscope, the highest practicable NA is around 1.4. For white light (lambda is approximately 0.53 m, the resolving power is 0.231 m, or 231nm.

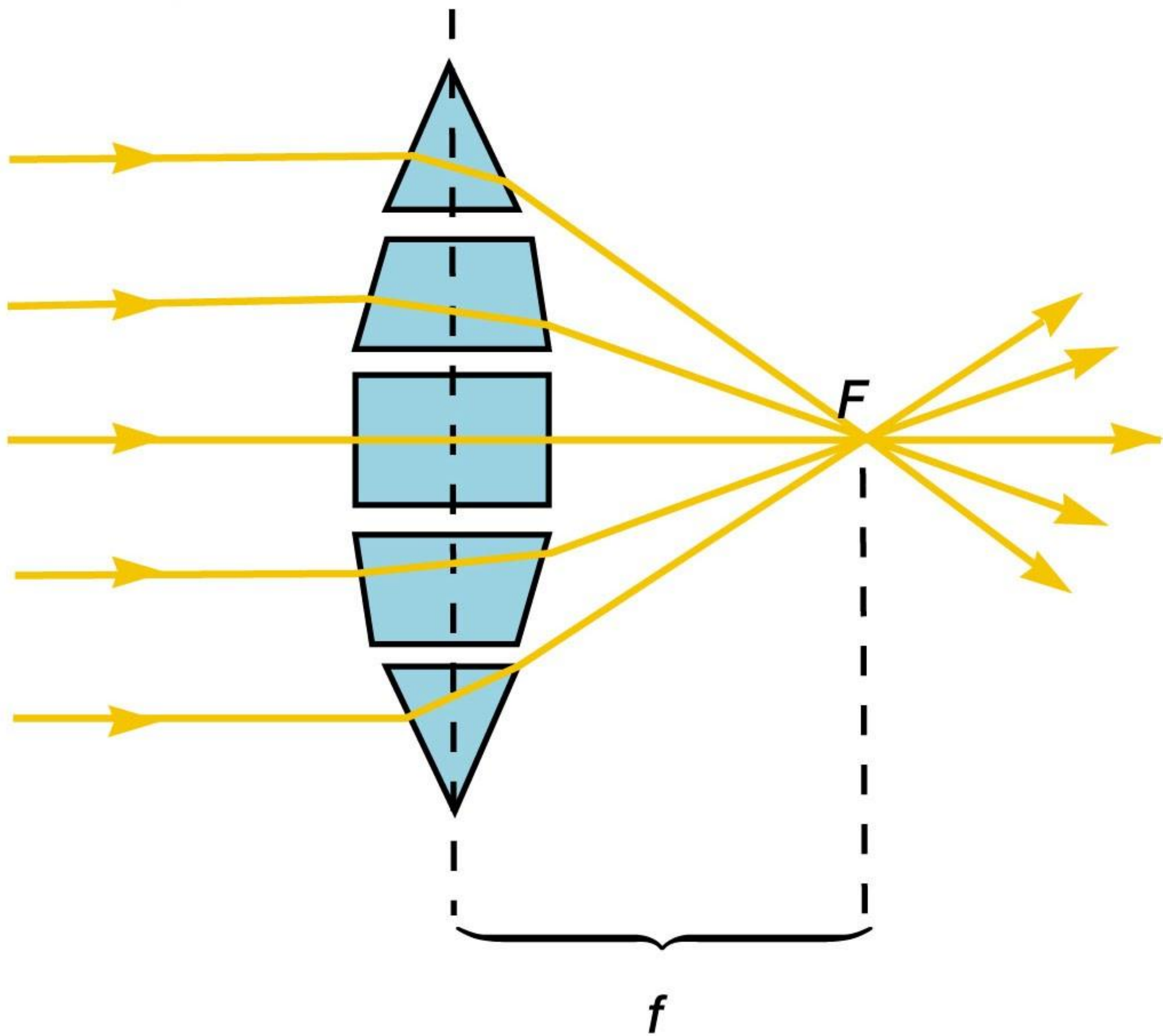
Under optimal conditions, with a high numerical aperture lens, you can only resolve, or see as separate particles, two particles that are more than 200 nm apart.

Lenses and the Bending of Light

- light is refracted (bent) when passing from one medium to another
- refractive index
  - a measure of how greatly a substance slows the velocity of light
- direction and magnitude of bending is determined by the refractive indexes of the two media forming the interface

Lenses

- focus light rays at a specific place called the focal point
- Focal length is the distance between center of lens and focal point.
- The strength of lens related to focal length
  - short focal length $\Rightarrow$ more magnification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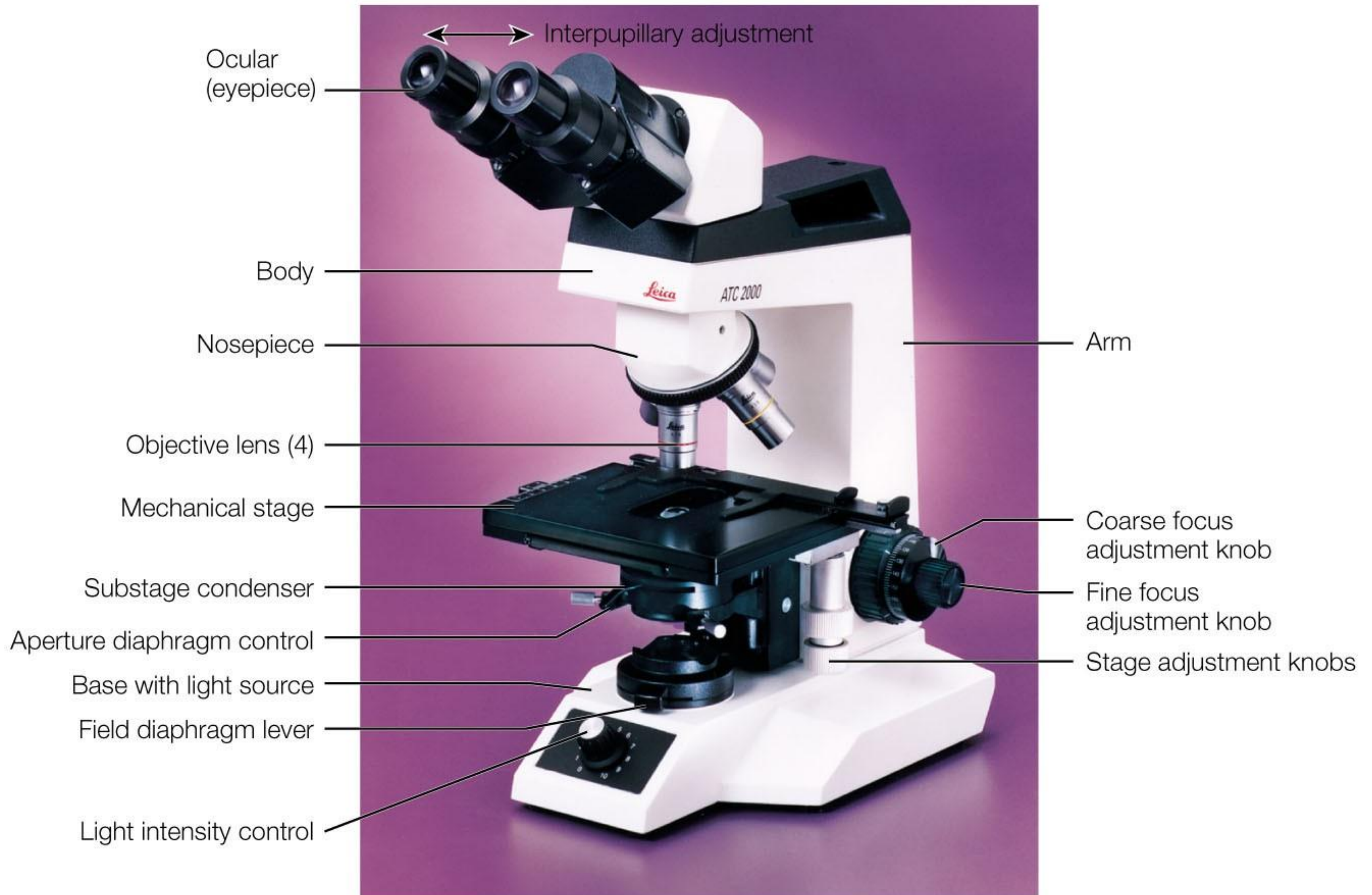
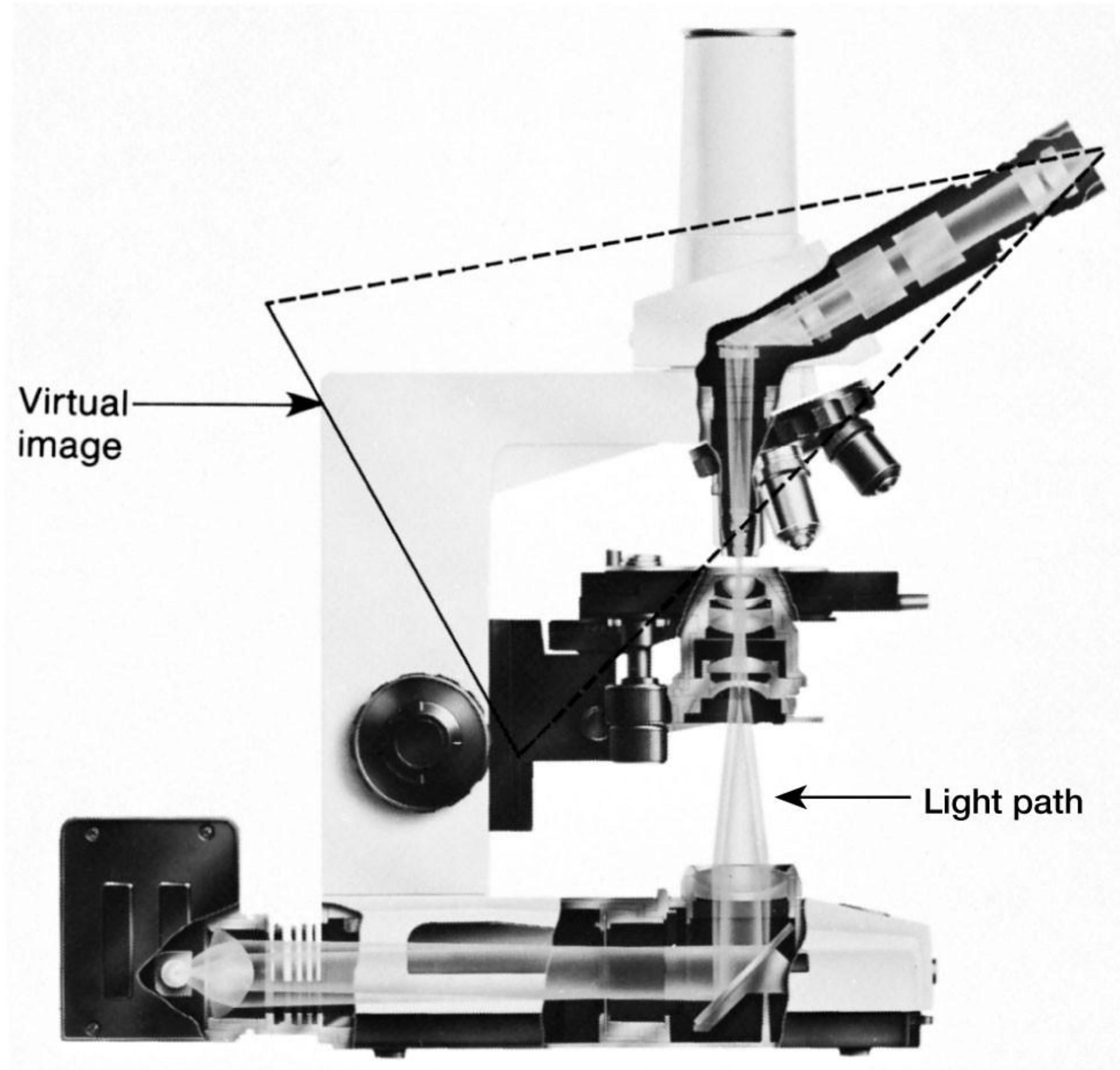
The Light Microscope

- many types
  - bright-field microscope
  - dark-field microscope
  - phase-contrast microscope
  - fluorescence microscopes
- are compound microscopes
  - image formed by action of ≥2 lenses

The Bright-Field Microscope

- produces a dark image against a brighter background
- has several objective lenses
  - parfocal microscopes remain in focus when objectives are changed
- total magnification
  - product of the magnifications of the ocular lens and the objective lens


Interpupillary adjustment
Ocular (eyepiece)
Body
Leica
ATC 2000
Nosepiece
Arm
Objective lens (4)
Mechanical stage
Coarse focus adjustment knob
Substage condenser
Fine focus adjustment knob
Aperture diaphragm control
Stage adjustment knobs
Base with light source
Field diaphragm lever
Light intensity control


Virtual image
Light path

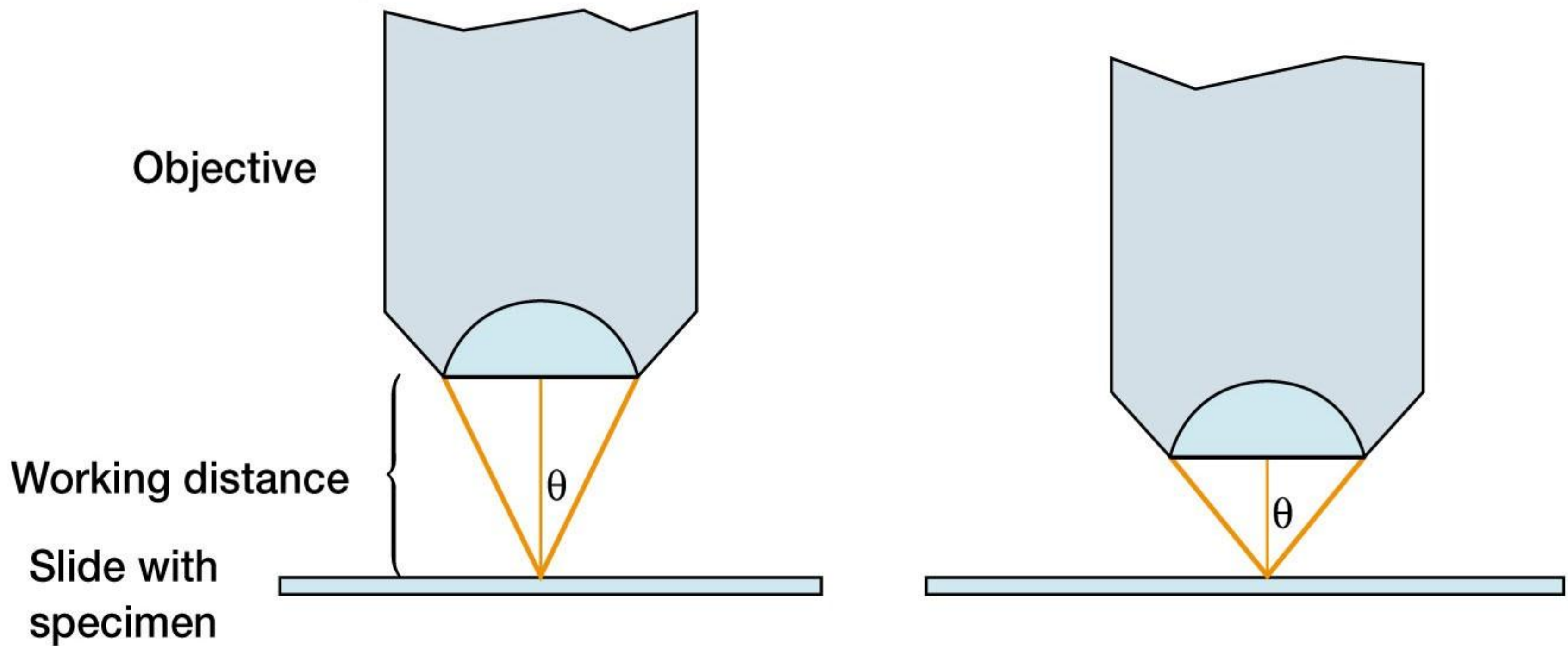
Microscope Resolution

- Ability of a lens to separate or distinguish small objects that are close together
- Wavelength of light used is major factor in resolution

  shorter wavelength $\Rightarrow$ greater resolution

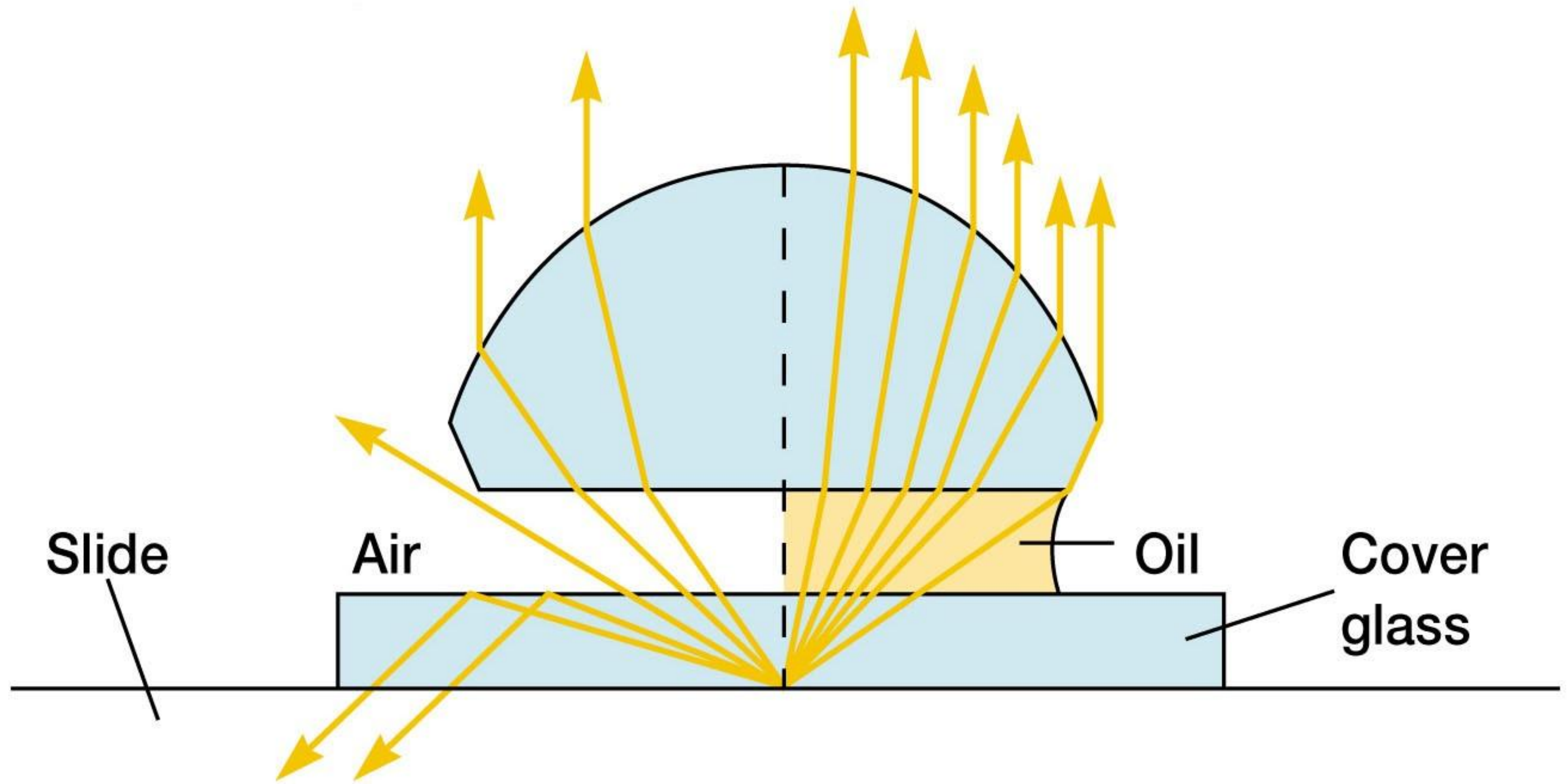
Table 2.2 The Properties of Microscope Objectives

Property	*Objective*			
	Scanning	**Low Power**	**High Power**	**Oil Immersion**
Magnification	4×	10×	40–45×	90–100×
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4
Approximate focal length (*f*)	40 mm	16 mm	4 mm	1.8–2.0 mm
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm
Approximate resolving power with light of 450 nm (blue light)	2.3 µm	0.9 µm	0.35 µm	0.18 µm



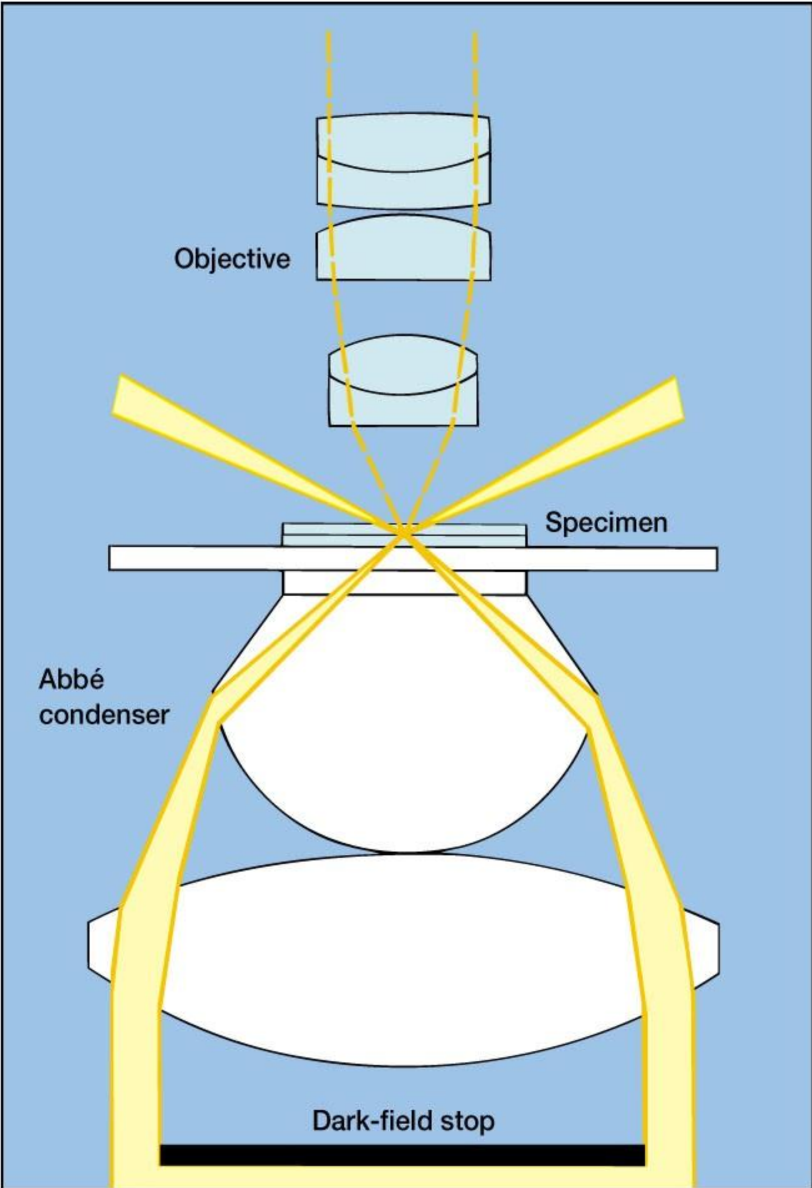
working distance

— distance between the front surface of lens and surface of cover glass or specimen


Slide
Air
Oil
Cover glass

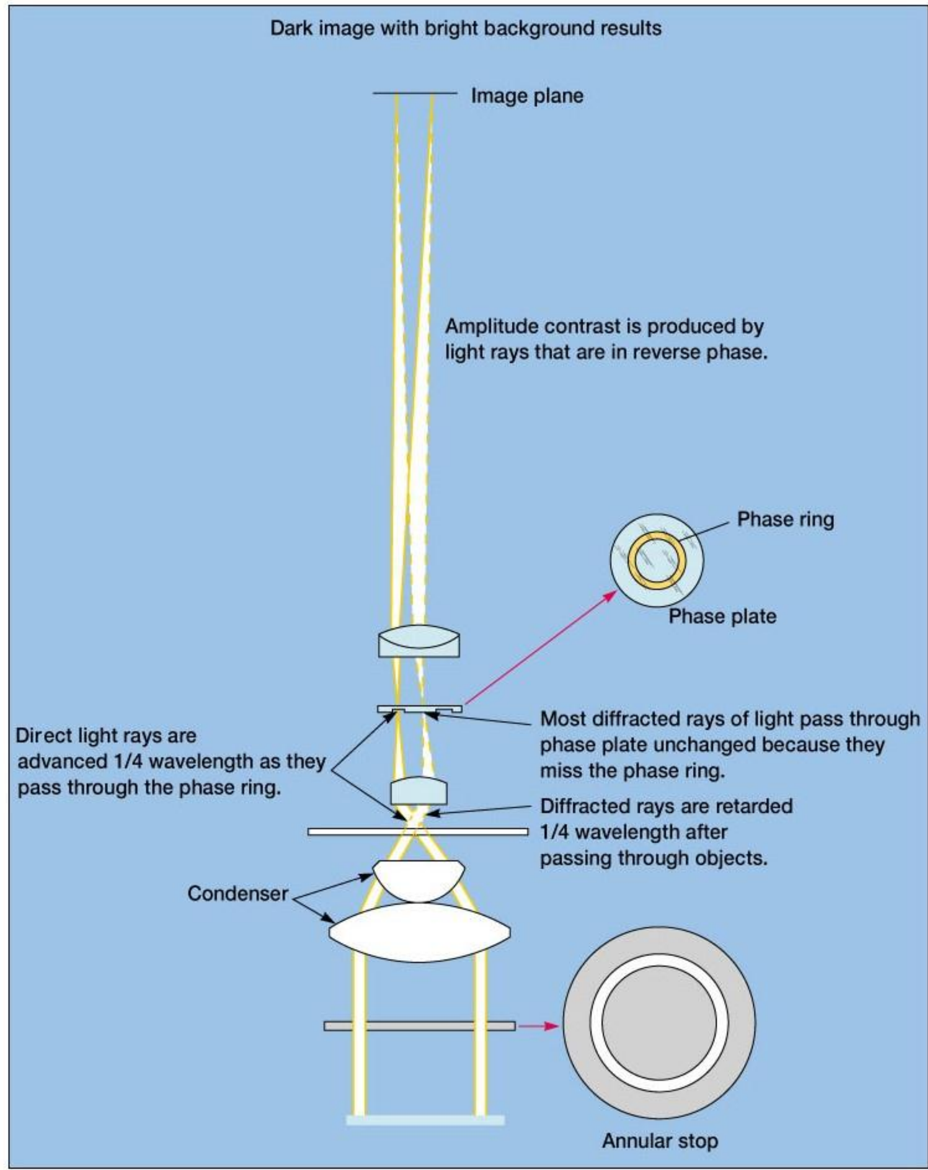
The Dark-Field Microscope

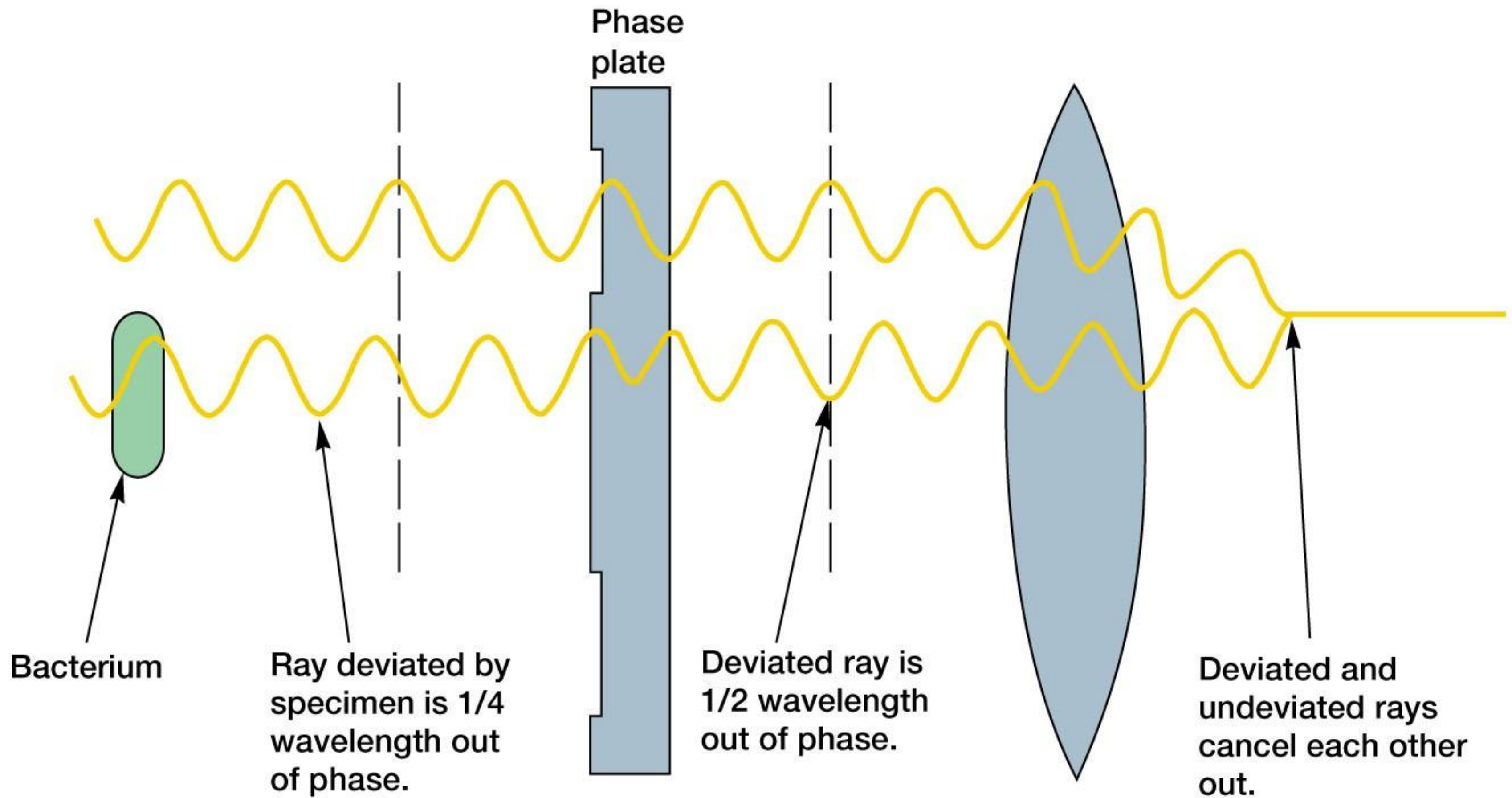
- Produces a bright image of the object against a dark background
- Used to observe living, unstained preparations


Objective
Specimen
Abbé
condenser
Dark-field stop

The Phase-Contrast Microscope

- Enhances the contrast between intracellular structures having slight differences in refractive index
- Excellent way to observe living cells


Dark image with bright background results
Image plane
Amplitude contrast is produced by
light rays that are in reverse phase.
Phase ring
Phase plate
Most diffracted rays of light pass through
phase plate unchanged because they
miss the phase ring.
Direct light rays are
advanced 1/4 wavelength as they
pass through the phase ring.
Diffracted rays are retarded
1/4 wavelength after
passing through objects.
Condenser
Annular stop

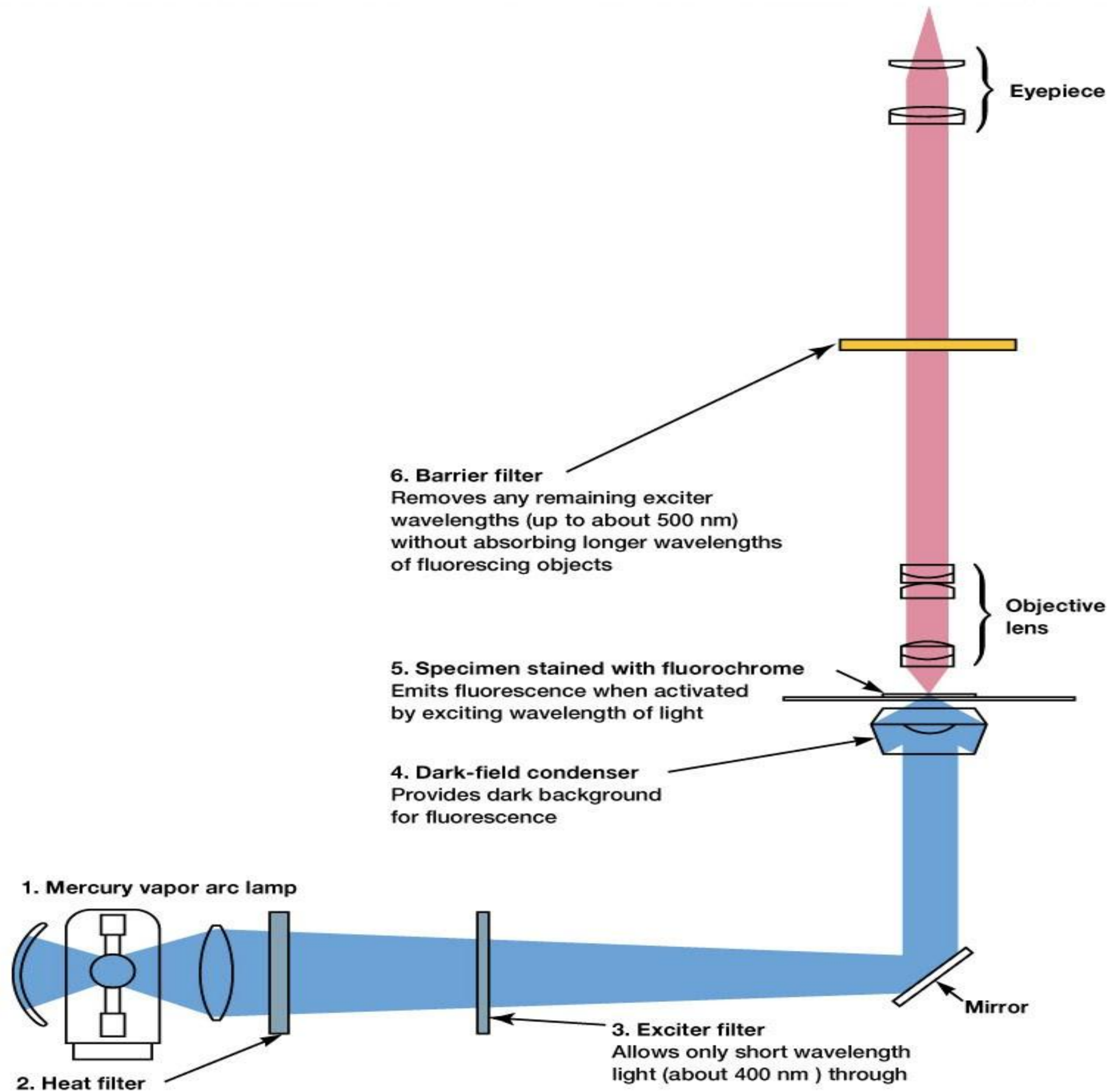

Phase plate
Bacterium
Ray deviated by specimen is 1/4 wavelength out of phase.
Deviated ray is 1/2 wavelength out of phase.
Deviated and undeviated rays cancel each other out.

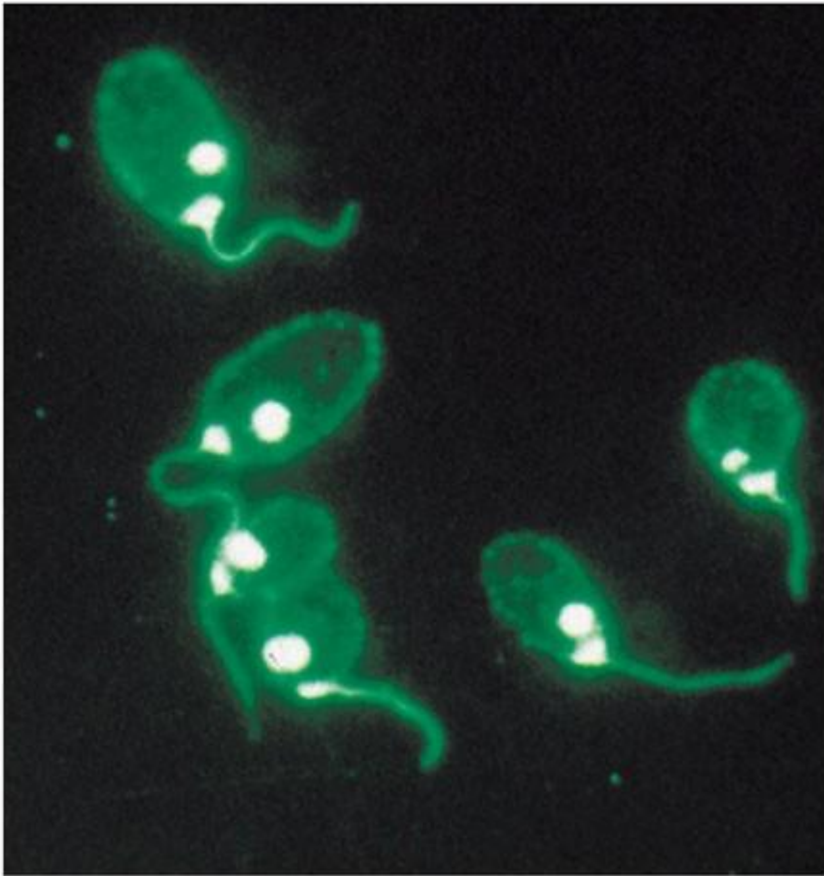
The Differential Interference Contrast Microscope

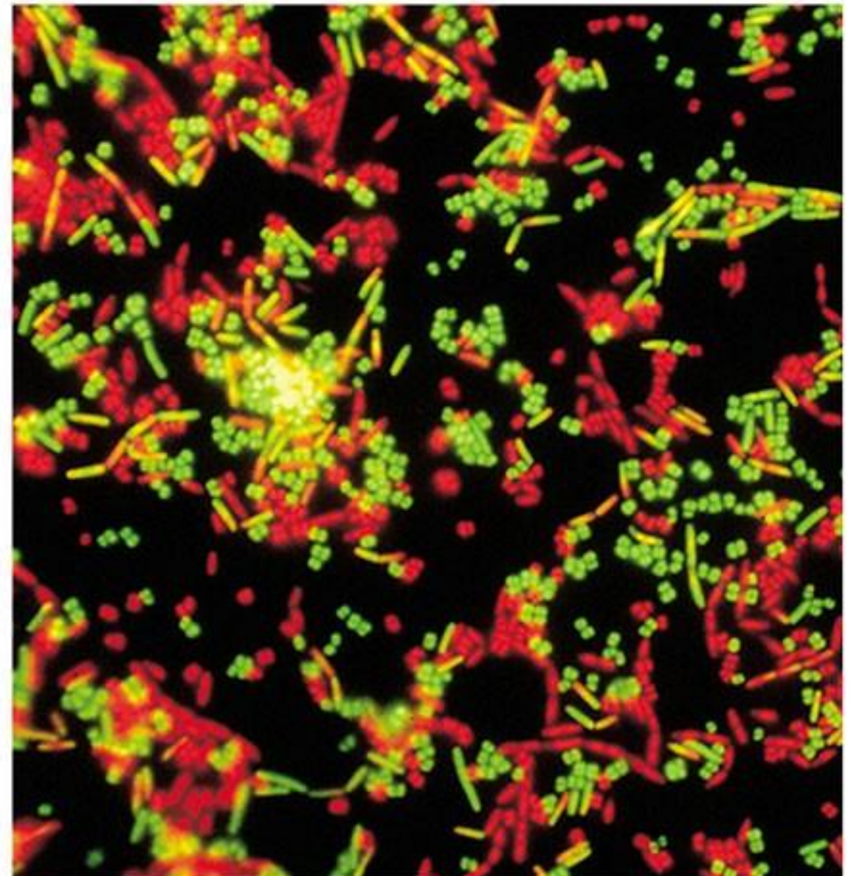
- Creates image by detecting differences in refractive indices and thickness of different parts of specimen
- Excellent way to observe living cells

The Fluorescence Microscope

- Exposes specimen to ultraviolet, violet, or blue light
- Specimens usually stained with fluorochromes
- Shows a bright image of the object resulting from the fluorescent light emitted by the specimen

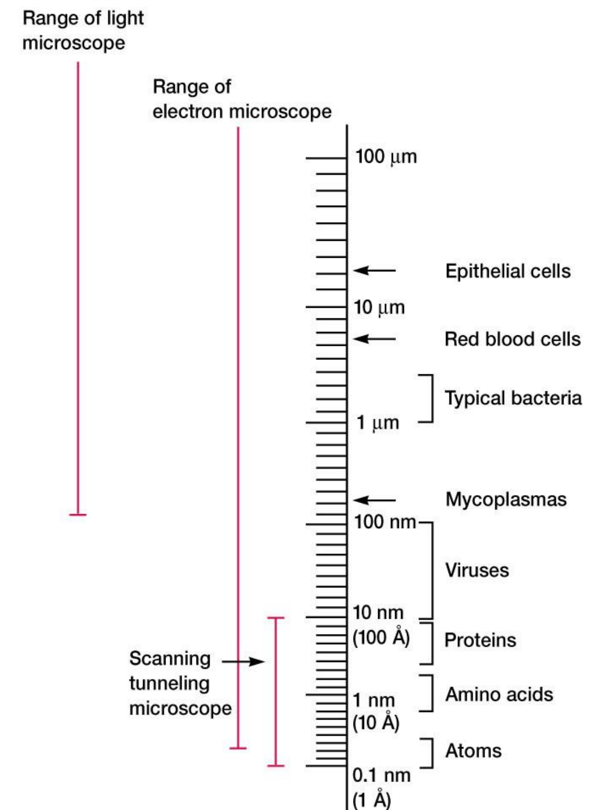

Eyepiece
6. Barrier filter
Removes any remaining exciter
wavelengths (up to about 500 nm)
without absorbing longer wavelengths
of fluorescing objects
Objective
lens
5. Specimen stained with fluorochrome
Emits fluorescence when activated
by exciting wavelength of light
4. Dark-field condenser
Provides dark background
for fluorescence
1. Mercury vapor arc lamp
Mirror
3. Exciter filter
Allows only short wavelength
light (about 400 nm) through
2. Heat filter





Newer Techniques in Microscopy

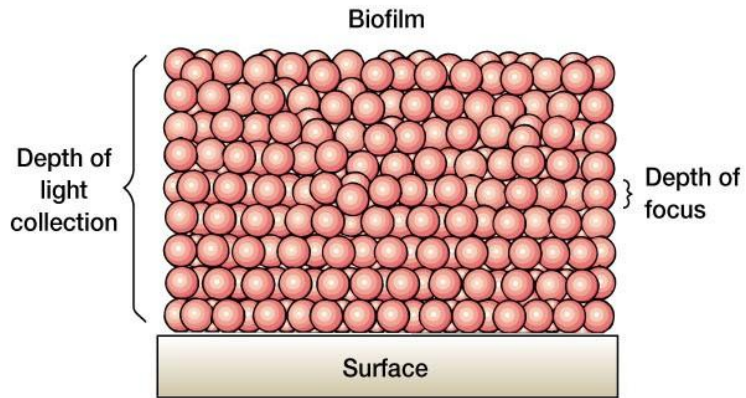
- confocal microscopy and scanning probe microscopy
- have extremely high resolution
- can be used to observe individual atoms

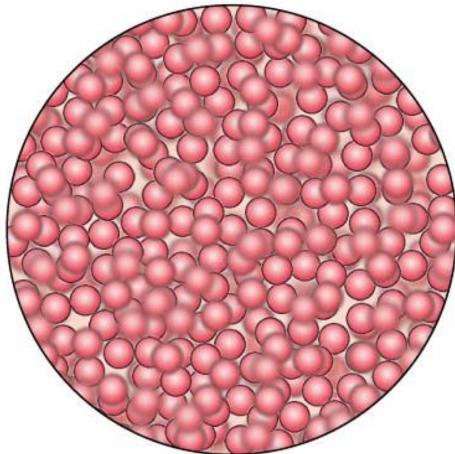


Confocal Microscopy

- confocal scanning laser microscope
- laser beam used to illuminate spots on specimen
- computer compiles images created from each point to generate a 3-dimensional image

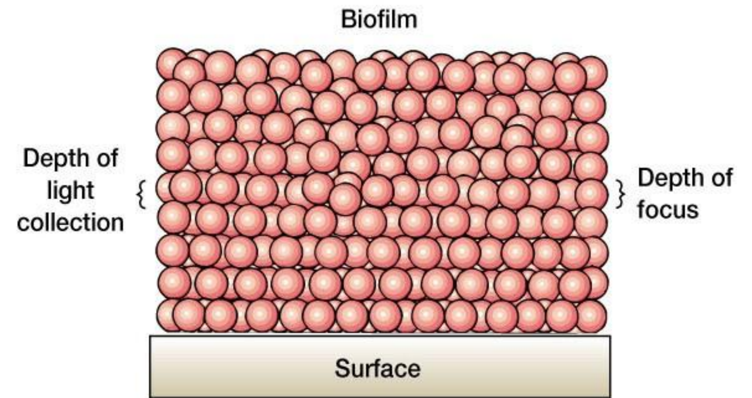
Conventional light microscope

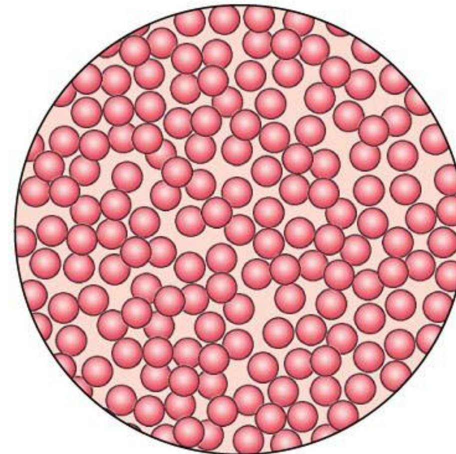




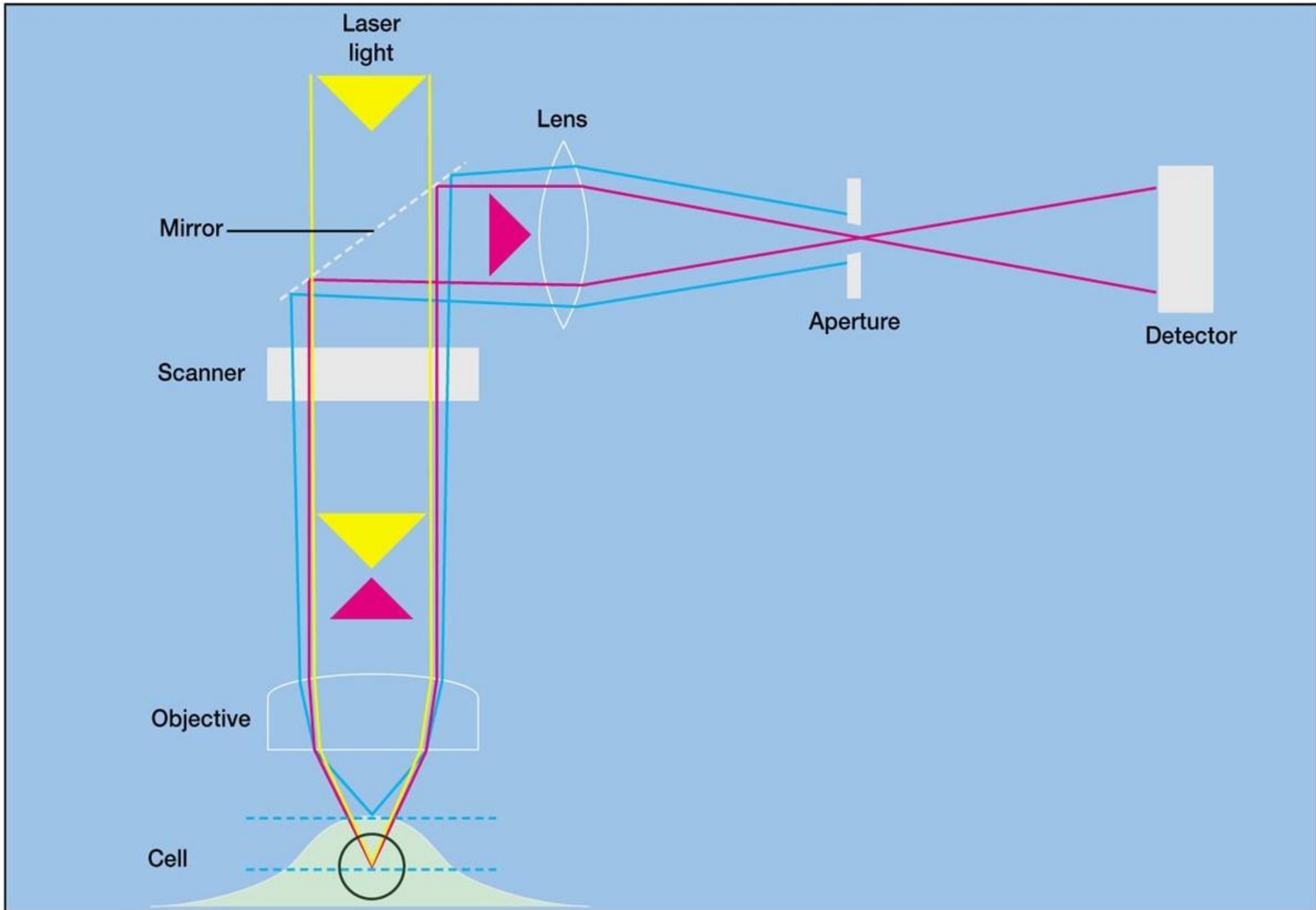
(a) Image in field of view

Confocal scanning laser microscope





(b) Image in field of view


Laser light
Lens
Mirror
Aperture
Detector
Scanner
Objective
Cell

Scanning Probe Microscopy

- Scanning tunneling microscope
  - steady current (tunneling current) maintained between microscope probe and specimen
  - up and down movement of probe as it maintains current is detected and used to create image of surface of specimen

Scanning Probe Microscopy

- Atomic force microscope
  - sharp probe moves over surface of specimen at constant distance
  - up and down movement of probe as it maintains constant distance is detected and used to create image