# Advanced Engineered Solutions

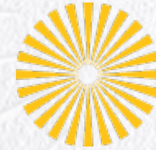
## A Global Leader in

Specialty Chemicals

Surface Finishing Equipment

Engineered Powders

Analytical Controls



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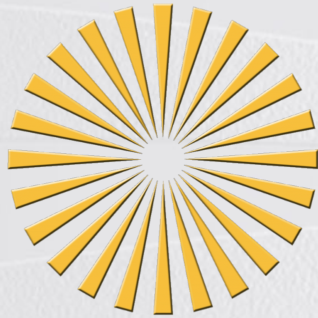

# TECHNIC INC

## Microscopy

## LAYOUT OF PRESENTATION

- Introduction
- Historical Background
- Variables Used In Microscopy
- Parts of microscope
- Micrometry
- Types of Microscope & Uses

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# Introduction

- A microscope (Greek: mikron = small and scopeos = to look).
- MICROSCOPE: Is an instrument for viewing objects that are too small to be seen by the naked or unaided eye.
- MICROSCOPY: The science of investigating small objects using such an instrument is called microscopy

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# History

- 590 - Hans Janssen and his son Zacharias Janssen, developed first microscope.
- 1609 - Galileo Galilei - occhiolino or compound microscope.
- 1620 - Christian Huygens, another Dutchman, developed a simple 2-lens ocular system that was chromatically corrected.

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Anton van Leeuwenhoek
(1632-1723)

Robert Hooke
(1635-1703)

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# Variables used in microscopy- MAGNIFICATION

- Degree of enlargement.
- No. of times the length, breadth or diameter, of an object is multiplied
- It depends upon – Optical tube length Focal length of objective Magnifying power of eye piece
- TOTAL MAGNIFICATION: magnification of the eyepiece x magnification of the objective.

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# Variables used in microscopy- RESOLUTION

- Ability to reveal closely adjacent structural details as separate and distinct.
- LIMIT OF RESOLUTION (LR): The minimum distance between two visible bodies at which they can be seen as separate and not in contact with each other.

$$LR = \frac{0.61 \times W}{NA}$$

W = Wavelength

NA = Numerical aperature

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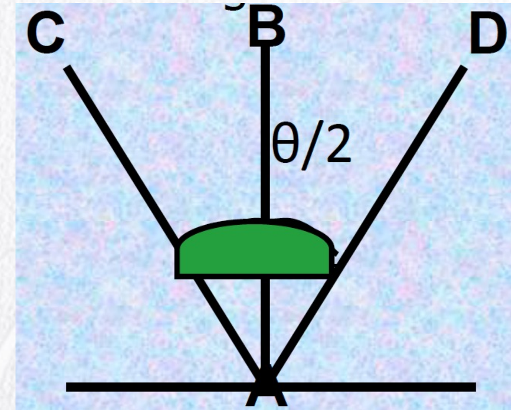
## NUMERICAL APERTURE(NA)

- Ratio of diameter of lens to its focal lengt
- NA = n Sin θ/2

  n = refractive index,

  θ = angle of aperture (CAD)




## DEFINITION

Capacity of an objective to render outline of the image of an object clear and distinct.

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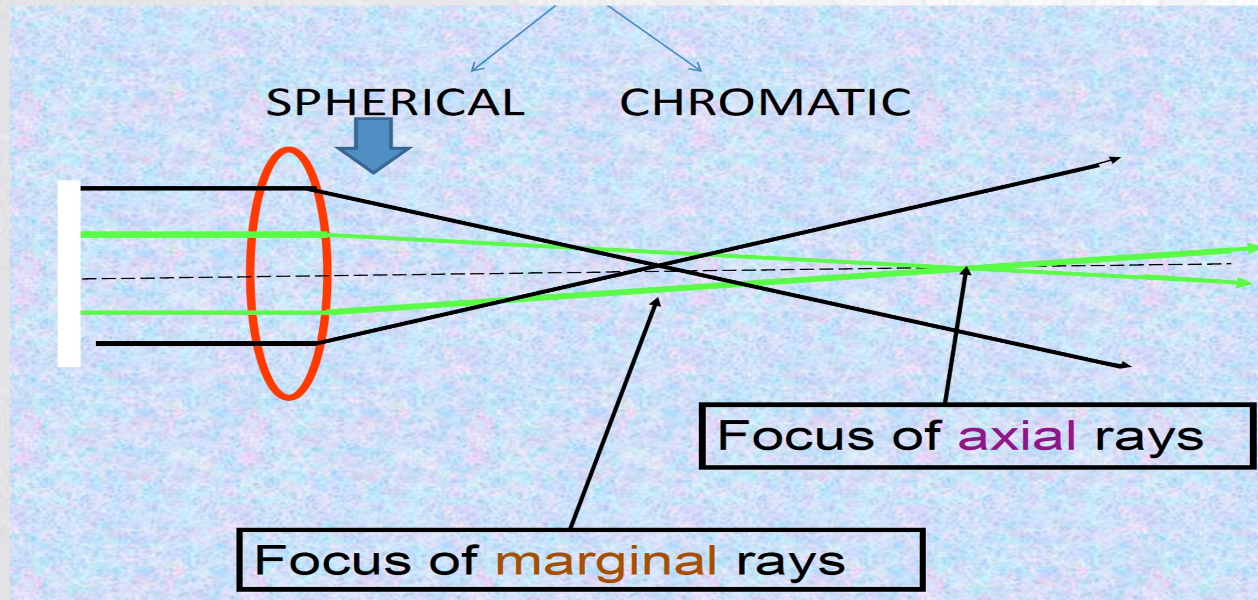
# ABBERATION




- Chromatic aberration, caused by differences in refractive index for different wavelengths of light, in contrast with monochromatic aberration, which occurs for all frequencies of light.
- Spherical aberration, which occurs when light rays pass through a spherical lens

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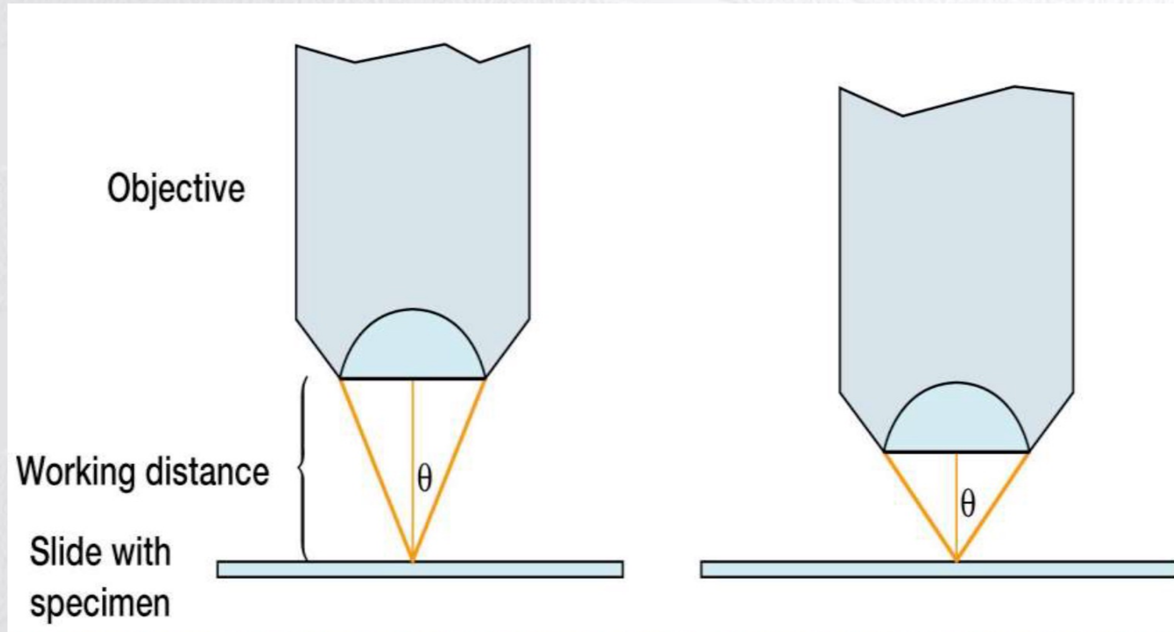
# WORKING DISTANCE

Distance between the front surface of lens and surface of cover glass or specimen.

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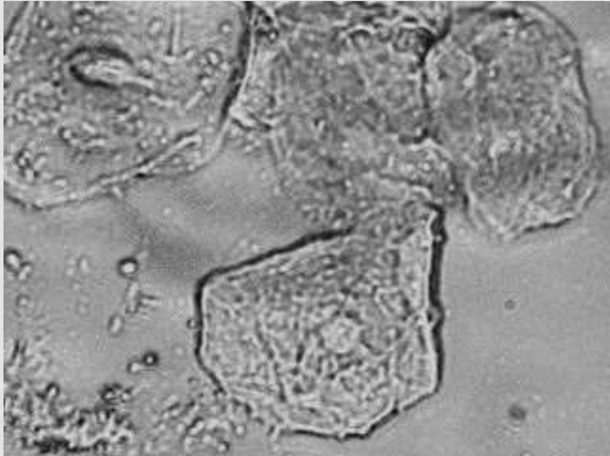
# CONTRAST

Differences in intensity between two objects, or between an object and background.



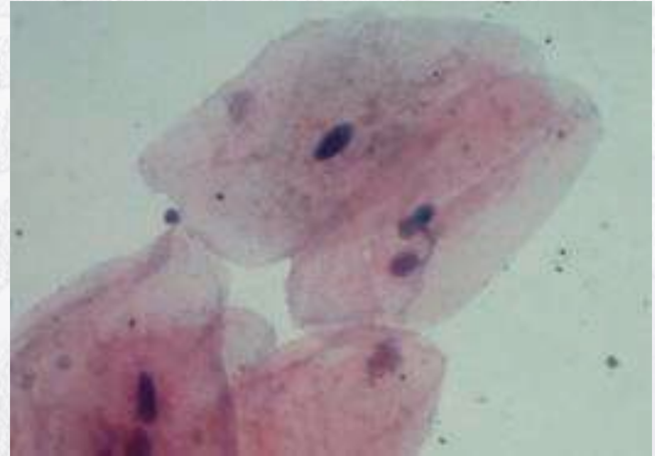


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**Table 2.2** The Properties of Microscope Objectives

| | *Objective* | | | |
|---|---|---|---|---|
| **Property** | **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 |

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# TYPES OF MICROSCOPE & USES

LIGHT MICROSCOPE : use sunlight or artificial light.

A. Bright field microscope.
B. Dark field microscope.
C. Phase contrast microscope.
D. Fluorescence microscope.

ELECTRON MICROSCOPE : use of electron.

1. Transmission electron microscope.
2. Scanning electron microscope.

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## A: BRIGHT-FIELD MICROSCOPE

- Produces a dark image against a brighter background.
- Has several objective lenses.

2 types: Simple  AND Compound

## SIMPLE

- Contain a single magnifying lens.

## COMPOUND

- Series of lenses for magnification.
- Light passes through specimen into objective lens
- Oil immersion lens increases resolution.
- Have one or two ocular lenses.
- Resolution=200nm

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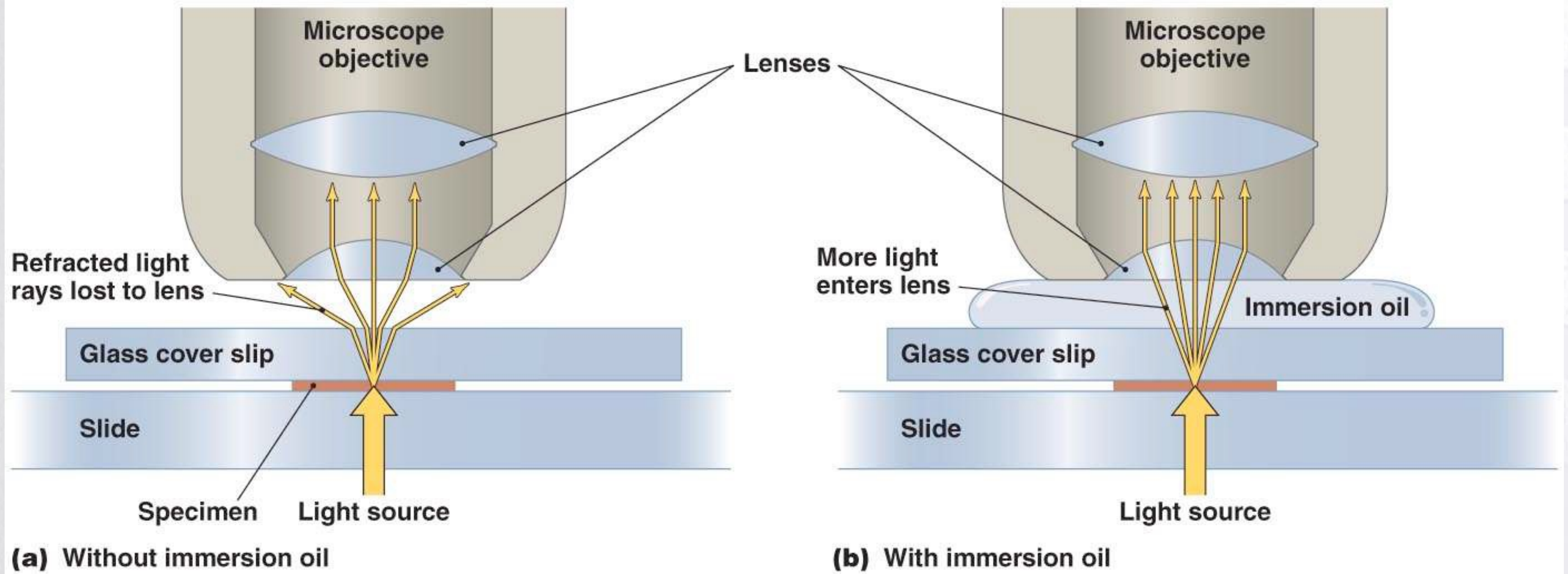
# THE EFFECTS OF IMMERSION OIL ON RESOLUTION

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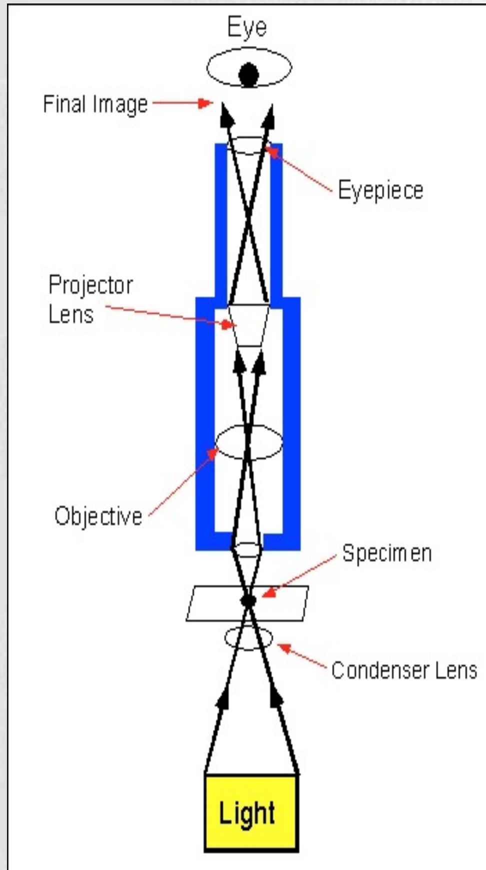
# OPTICS OF COMPOUND MICROSCOPE




## ADVANTAGES

- Used to view live or stained cells.
- Simple setup with very little preparation required.

## DISADVANTAGES

- Biological specimen are often of low contrast and need to be stained.
- Staining may destroy or introduce artifacts.
- Resolution is limited to 200nm.

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# IMAGE FORMED BY BRIGHT FIELD MICROSCOPY

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# B: DARK FIELD MICROSCOPE

- Produces a bright image of the object against a dark back ground.
- Optical system to enhance the contrast of unstained bodies.
- Specimen appears gleaming bright against dark background

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# REQUISITES FOR DARK FIELD MICROSCOPY

- Dark ground condenser
- High intensity lamp
- Funnel stop

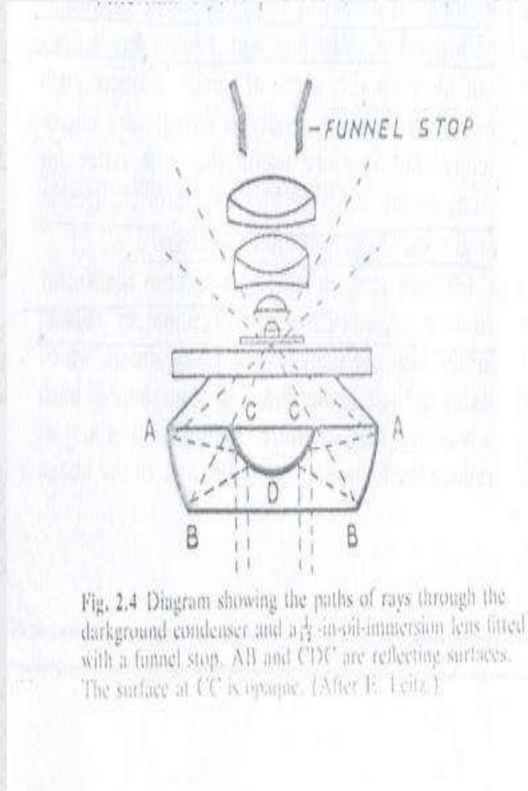


Fig. 2.4 Diagram showing the paths of rays through the darkground condenser and a $\frac{1}{12}$-in-oil-immersion lens fitted with a funnel stop. AB and CDC' are reflecting surfaces. The surface at CC is opaque. (After E. Leitz.)

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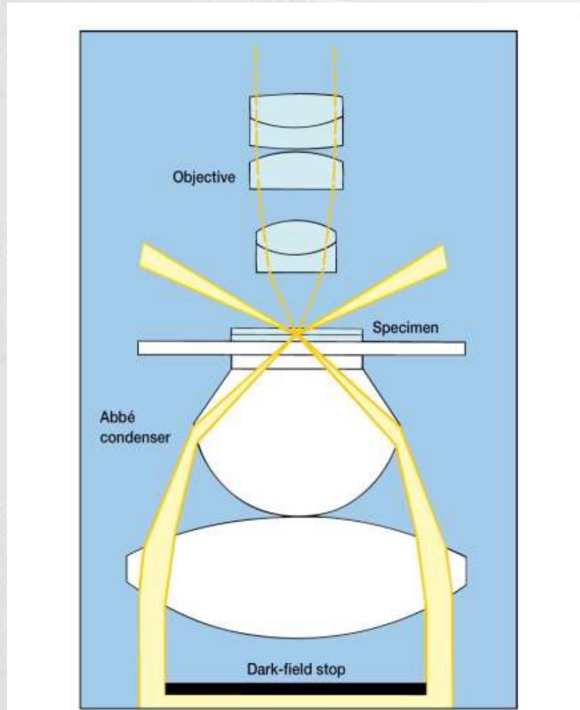
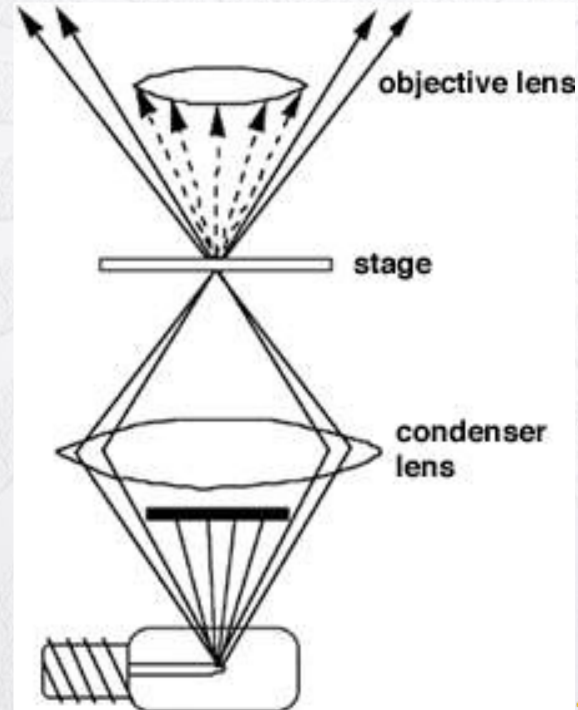
# OPTICS OF DARK FIELD MICROSCOPY

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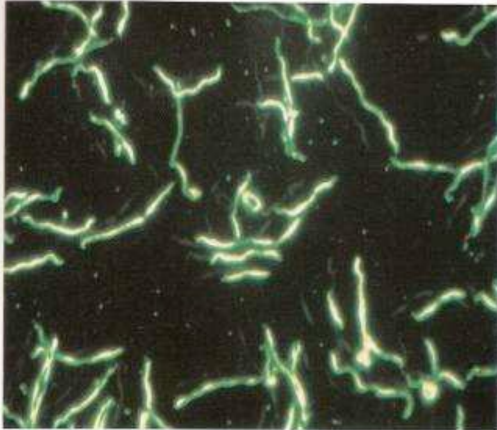
# USES OF DARK GROUND MICROSCOPY


(a) T. pallidum

Treponema pallidum

Useful in demonstrating

-Treponema pallidum
- Leptospira
- Campylobacter jejuni
- Endospore

## ADVANTAGES

- Simple setup
- Provides contrast to unstained tissue,so living cells can be viewed.

## DISADVANTAGES

- Specimen needs to be strongly illuminated which can damage delicate samples

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## C: PHASE CONTRAST MICROSCOPE

- First described in 1934 by Dutch physicist Frits Zerni
- Produces high-contrast images of transparent specimens.
- Advantage - Living cells can be examined in their natural state.

## PRINCIPLE OF PHASE CONTRAST MICROSCOPY

- It is an optical illumination technique in which small phase shifts in the ligh passing through a transparent specimen are converted into contras changes in the image.
- Light rays in phase produce brighter image.
- Light rays out of phase form darker image.
- Contrast is due to out of phase rays.

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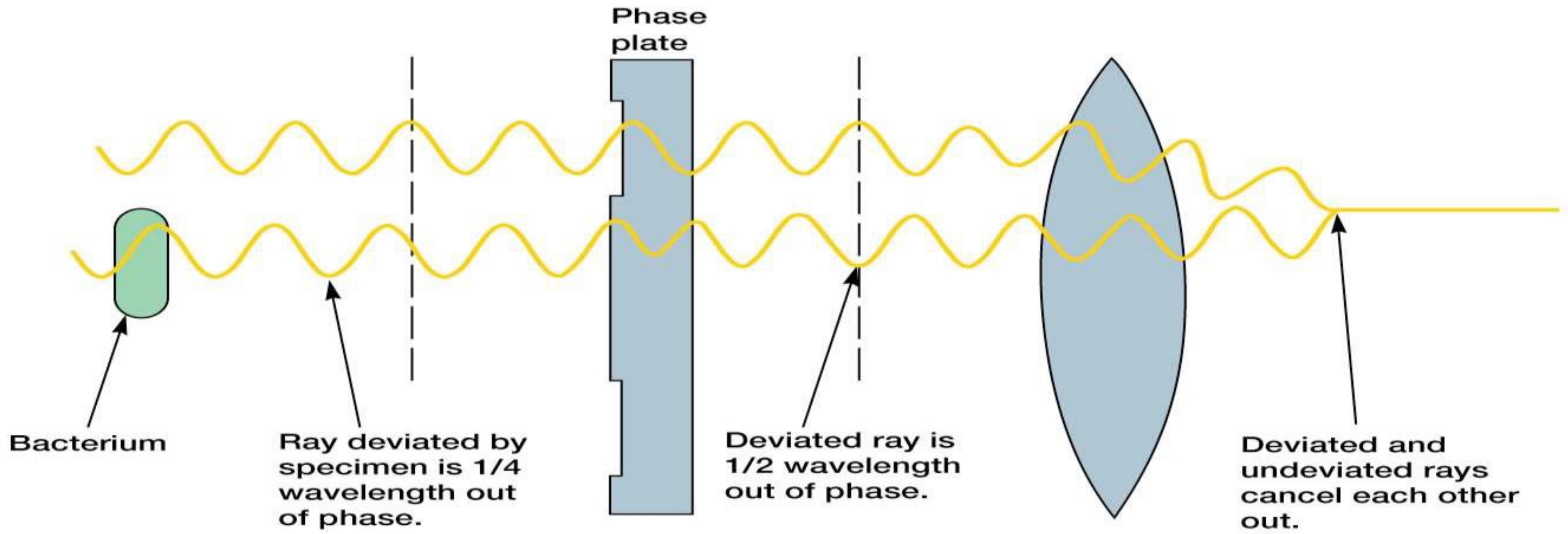


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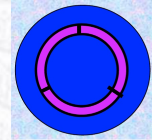
# REQUISITE FOR PHASE CONTRAST MICROSCOPY

- Annular Diaphragm
- Phase Plate

## CONDENSER ANNULUS

- The condenser annulus or annular diaphragm is opaque flat-black (light absorbing) plate with a transparent annular ring.
- Produces hollow cone of light.



## PHASE PLATE

Placed in back focal plane of objective.

- Function:

1. Enhances phase difference by retarding diffracted wave front by one quarter of wavelength .

2. Reduces intensity of direct rays and equalizes it with diffracted ray intensity

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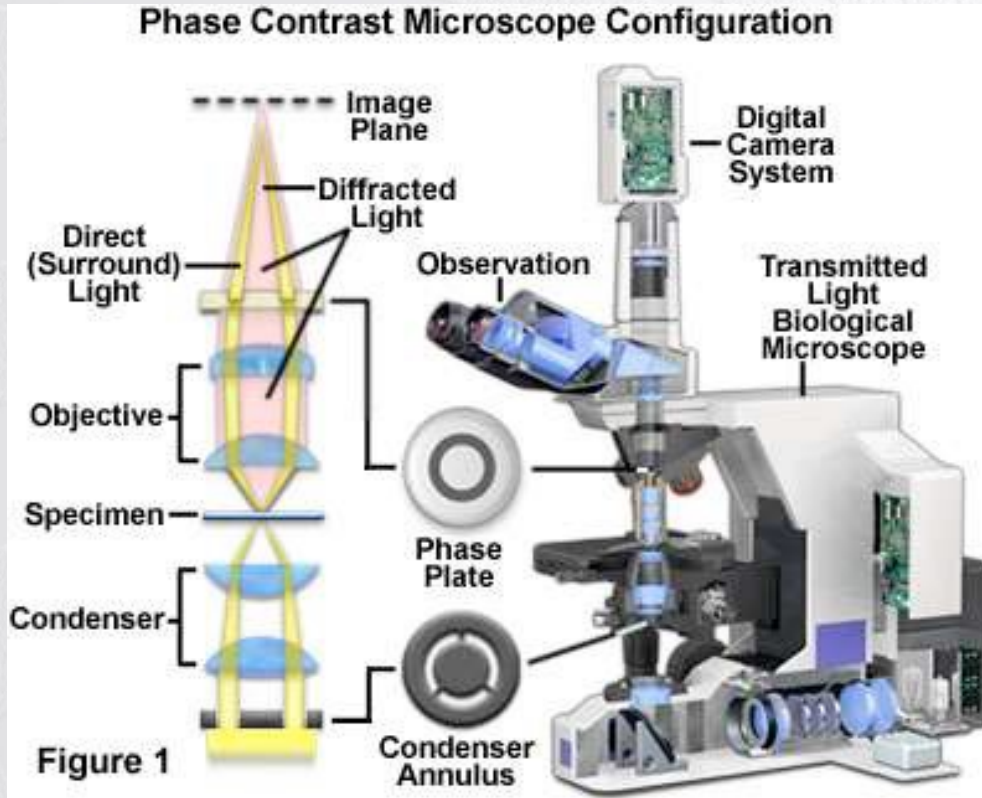
# OPTICS OF PHASE CONTRAST MICROSCOPE

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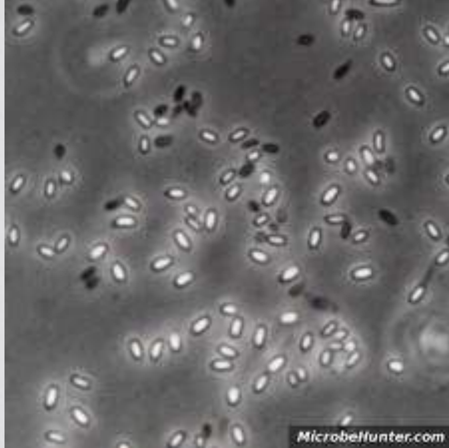
## IMAGES OF PHASE CONTRAST MICROSCOPY




### ADVANTAGES

- Phase contrast enables visualization of nternal cellular components.
- Diagnosis of tumor cells .
- Examination of growth, dynamics, and behavior of a wide variety of living cells in cell culture.
- Ideal for studying & interupting thin specimen.

### DISADVANTAGES

- Annuli or ring limits the apperature to some extents which causes decrease in resolution.
- Not ideal for thick specimen.
- Shade off and Halo effect may occur.

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# D: 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.

## PRINCIPLE OF FLUORESCENCE MICROSCOPY

- Certain dyes,called as fluorochrome after absorbing UV rays raised to a higher energy level
- When the dye molecules return to their normal state,they release excess energy in the form of visible light(fluoroscence).

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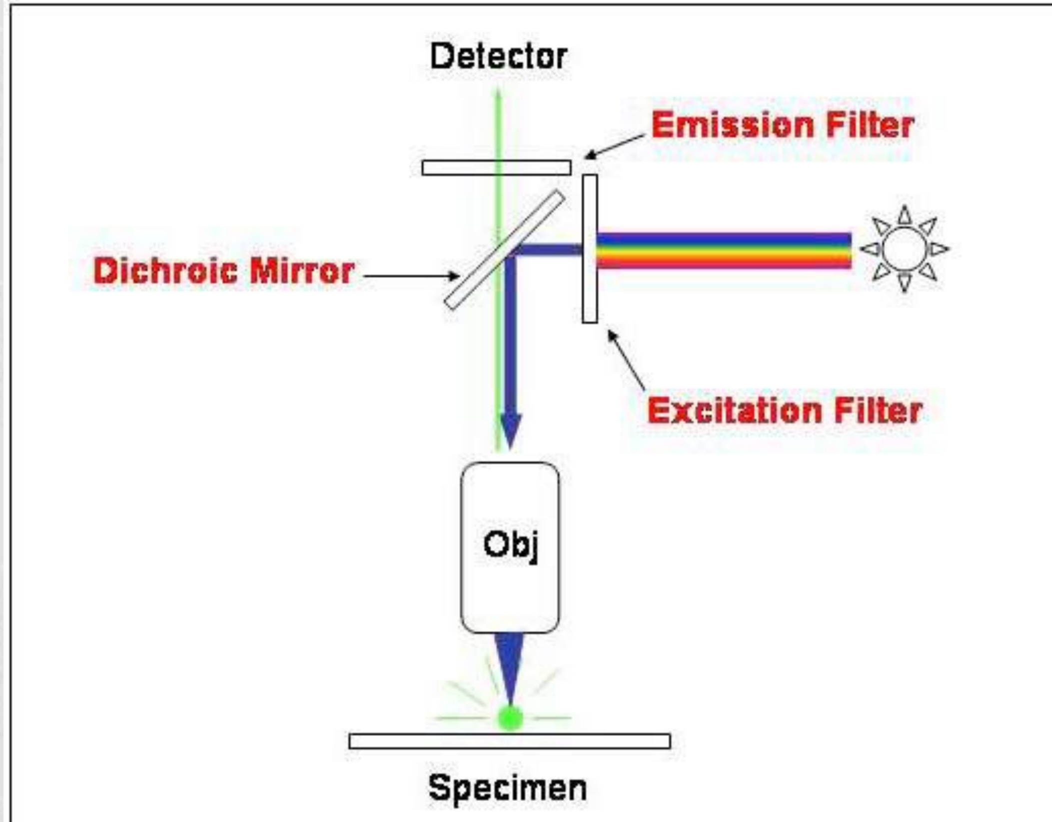
# OPTICS OF FLUOROSCENT MICROSCOPE

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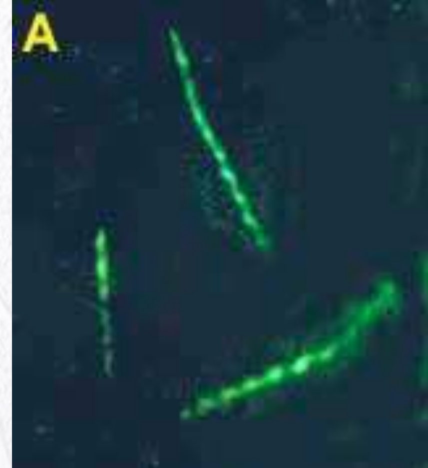
# USE OF FLUORESCENCE MICROSCOPY

- Auramine Rhodamine – Yellow fluorescence Tubercle bacilli
- Acridine Orange R - gives orange red fluorescence with RNA and yellow green fluorescence with DNA
- QBC
- IMMUNOFLUORESCENCE

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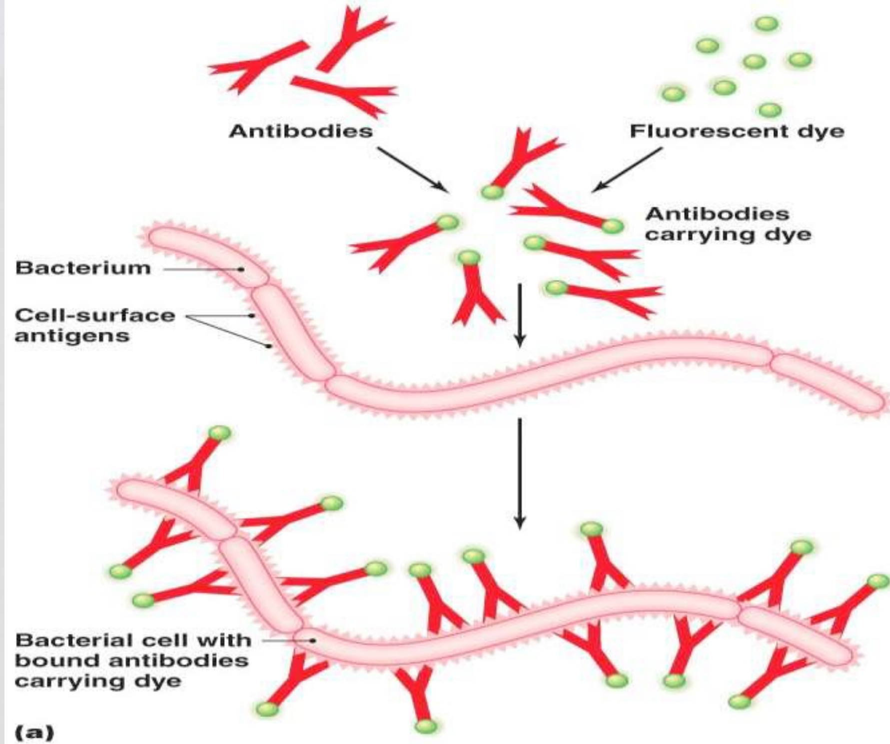
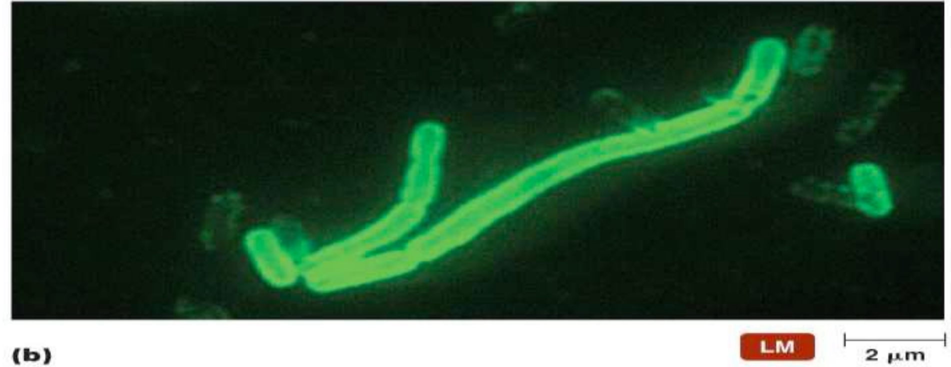
# IMMUNOFLUORESCENCE

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# 1: ELECTRON MICROSCOPE

- Co-invented by Max knoll and Ernst Ruska in 1931.
- Electron Microscopes uses a beam of highly energetic electrons to examine objects on a very fine scale.
- Magnification can upto 2million times while best light microscope can magnify up to 2000 times.

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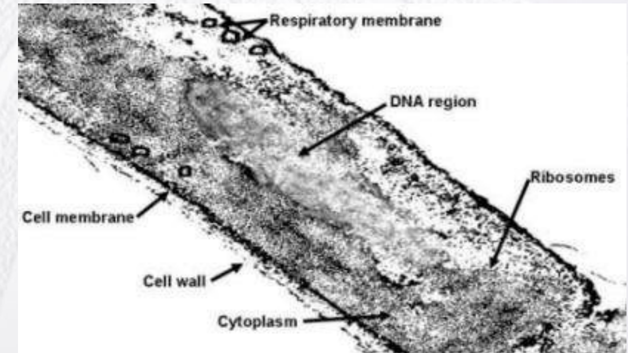
# TRANSMISSION ELECTRON MICROSCOPE (TEM)

- Stream of electrons is formed
- Accelerated using a positive electrica potential.
- Focused by metallic aperture and Electro magnets.
- Interactions occur inside the irradiated sample which are detected and transformed into an image.

- Projector Lens forms image on Fluorescentviewing screen
- 2D Image
- Magnification 10,000 X to 100,000 X

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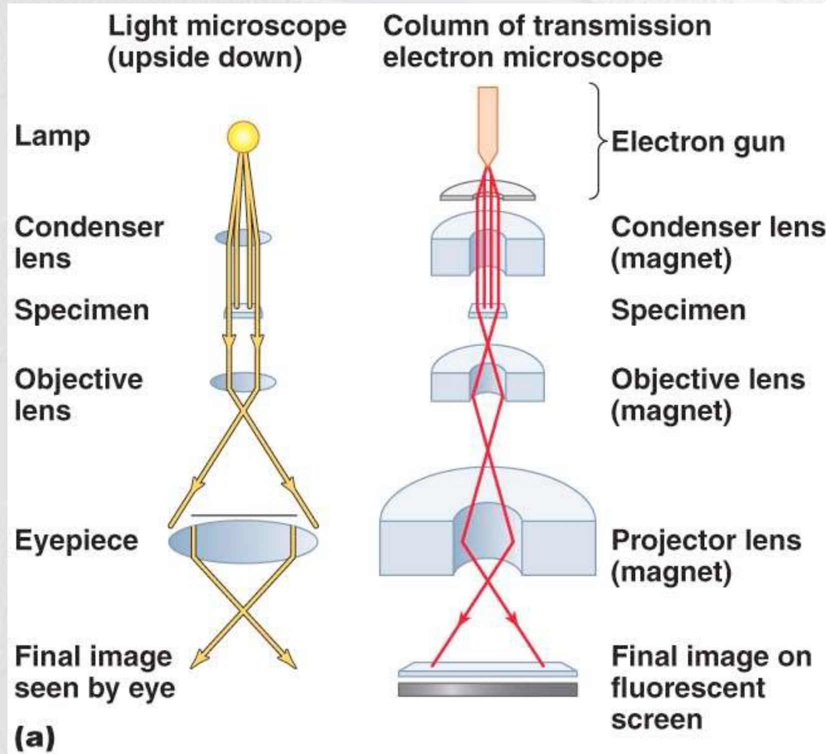
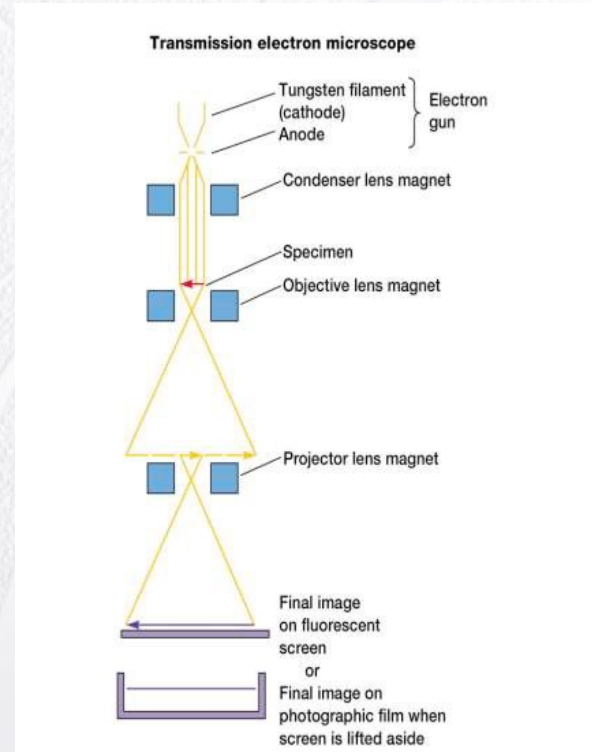
# OPTICS OF TEM



## 2: SCANNING ELECTRON MICROSCOPE

- Scan a gold-plated specimen to give a 3-D view of the surface of an object which is black and white.
- Used to study surface features of cells and viruses.
- Scanning Electron microscope has resolution 1000 times better than Ligh microscope.

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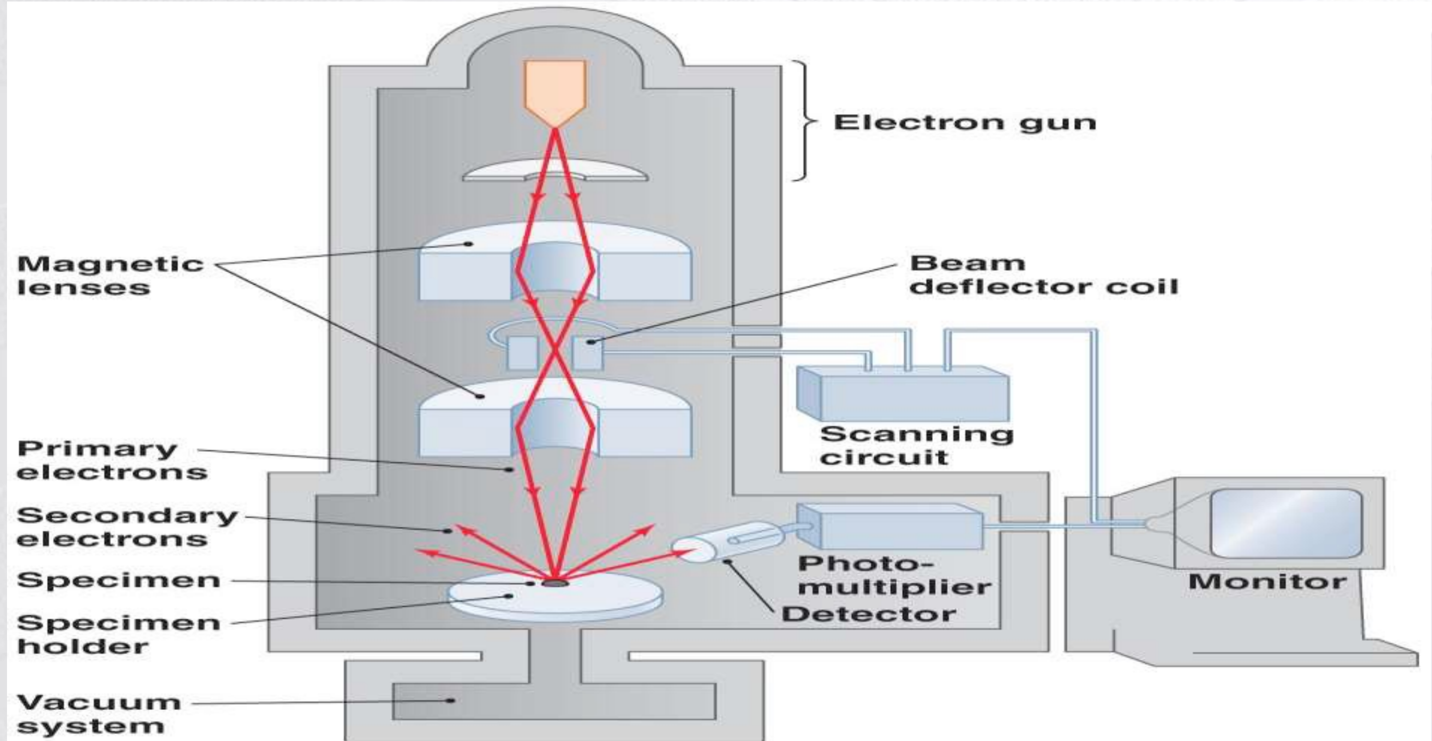
# OPTICS OF SEM



# COMPARING SEM AND TEM

| | TEM | SEM |
|---|---|---|
| *Electron Beam* | Broad, static beams | Beam focused to fine point; sample is scanned line by line |
| *Voltages Needed* | TEM voltage ranges from 60-300,000 volts | Accelerating voltage much lower; not necessary to penetrate the specimen |
| *Interaction of the beam electrons* | Specimen must be very thin | Wide range of specimens allowed; simplifies sample preparation |
| *Imaging* | Electrons must pass through and be transmitted by the specimen | Information needed is collected near the surface of the specimen |
| *Image Rendering* | Transmitted electrons are collectively focused by the objective lens and magnified to create a real image | Beam is scanned along the surface of the sample to build up the image |

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# Other microscopes and uses

- **CONFOCAL LASER SCANNING MICROSCOPE**
  - Uses a laser beam to illuminate a specimen whose image is then digitally enhanced for viewing on a computer monitor.
  - Laser beam scans single plane of 1µm thickness.

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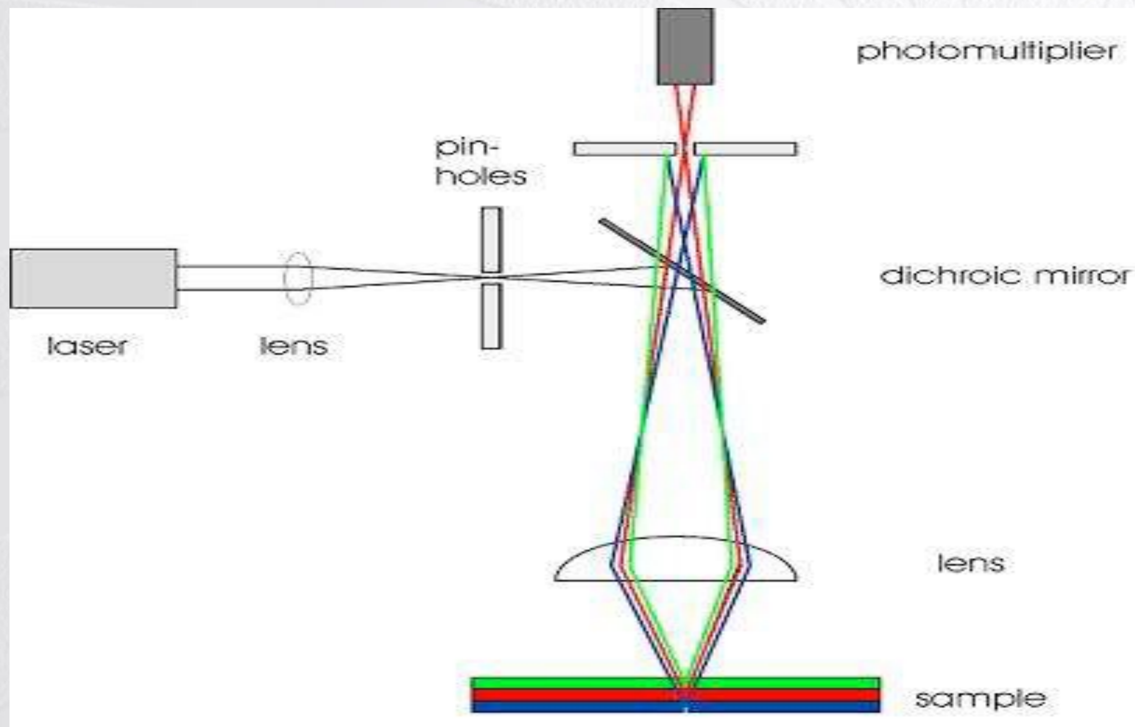
# OPTICS OF CONFOCAL MICROSCOPY

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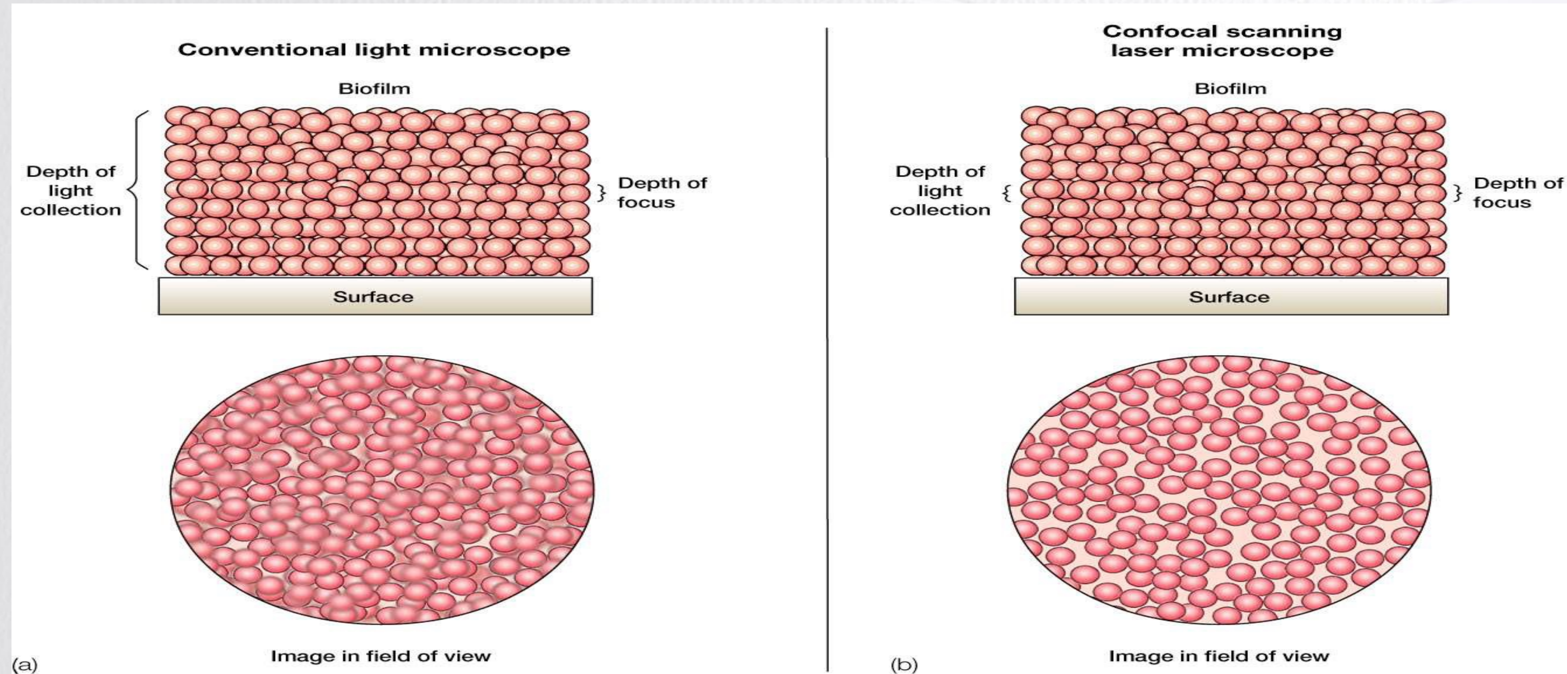
# USES OF CONFOCAL MICROSCOPE

Observing cellular morphology in multilayered specimen.
- Eg. used in diagnosing Ca cervix
- Evaluation and diagnosis of basal cell carcinoma of skin.



# ADVANTAGE OF USING A CONFOCAL MICROSCOPE

- By having a confocal pinhole, the microscope is really efficient at rejecting out of focus fluorescent light so that very thin section of a sample can be analyzed.

- By scanning many thin sections through a sample, one can build up a very clean threedimensional image .

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## INVERTED MICROSCOPE

- Used in metallurgy
- Examination of cultures in flat bottom dishes
- Micro dissection
- Examination of parasites
- Observation of agglutination in serology

## STEREO MICROSCOPE

- Double Microscope
- Produces 3D images

## POLARIZING MICROSCOPE

- Uses two Polariser
- Gives information about Birefringence of a body
- Used in Crystallography, Urine examination
- Apple Green Birefringerence in AMYLODOSIS

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# SCANNING PROBE MICROSCOPE

Class of Microscope that measures surface features by moving a sharp probe over object surface.
Used to visualize atoms and molecules

- scanning tunneling microscope (stm)
- atomic force microscope (afm)

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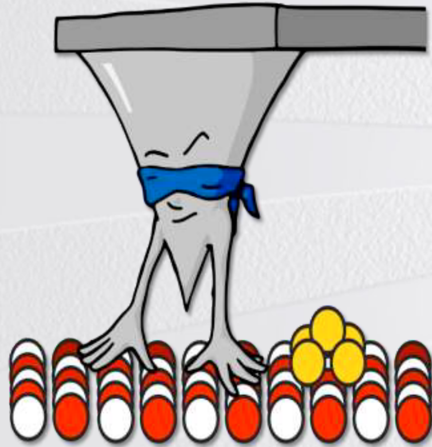
## SCANNING TUNNELING MICROSCOPE

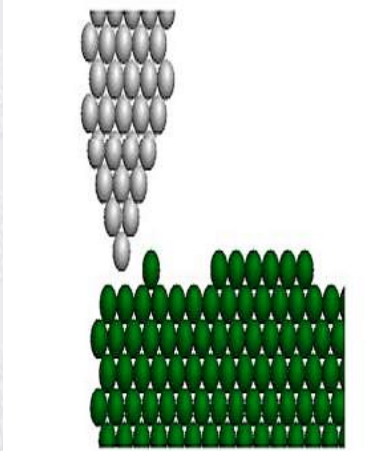
- Steady current (tunneling current) maintained between microscope probe and specimen.
- The arrangement of atoms on the specimen is determined by moving probe tip back and forth over specimen keeping a constant height.

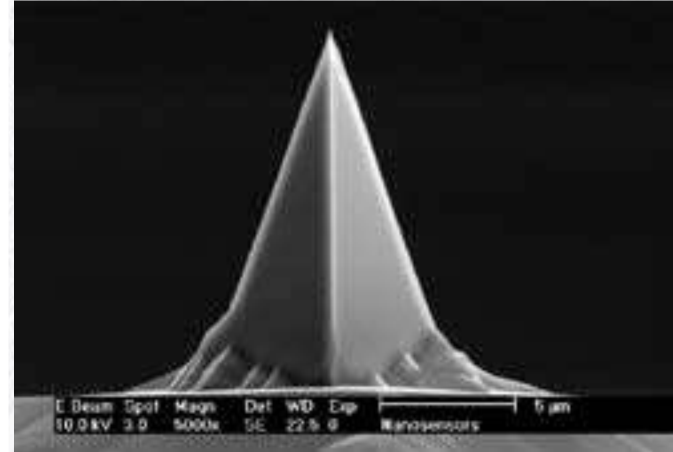
## ATOMIC FORCE MICROSCOPE

- 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.

# SCANNING PROBE MICROSCOPES








Scanning probe microscope “feel” changes in surfaces o the sample. They use ultrasharp tips to measure changes in electric currents, electrostatic forces, or magnetic forces coming from the surface.
The tips of the microscopes have to be incredibly sharp to allow them to collect information about individual atoms or molecules

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# Many Thanks for your attention

Henk Verlind