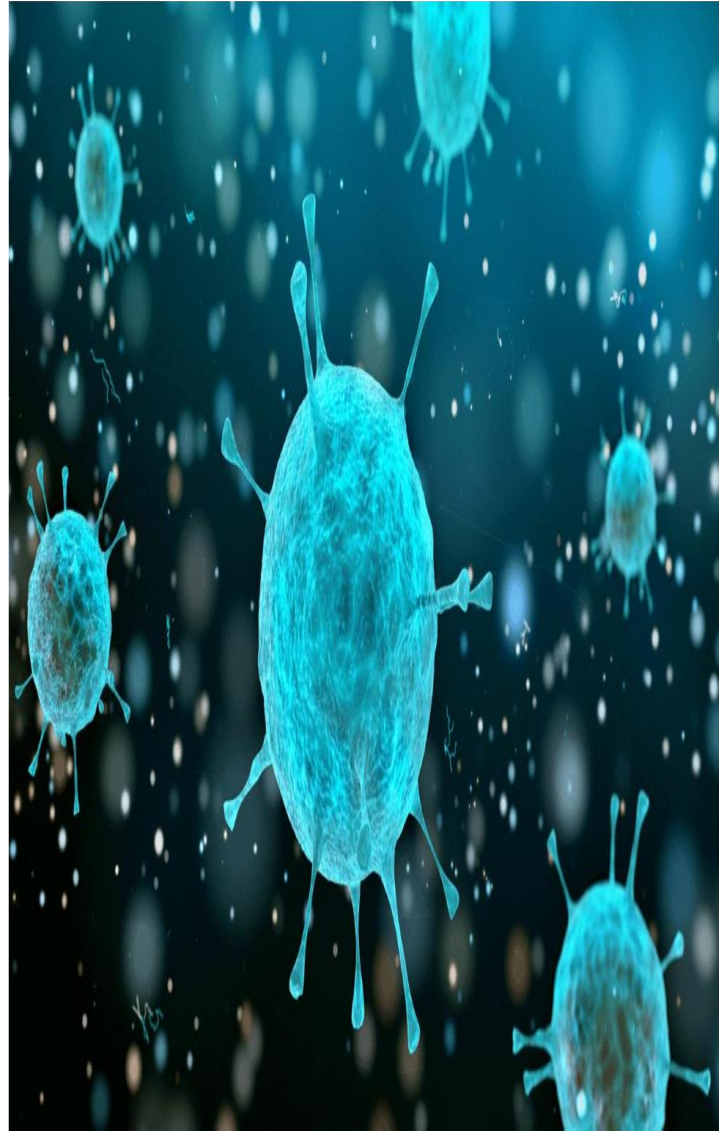


Second Year
Passion Batch



MICROBIOLOGY

LECTURE 3

ABDULLAH ABDULMONEM

Microscopy



**DR. WALEED AL MOMANI, MLT,
PHD**

Introduction



- Microbes are said to be microscopic
- In most cases, a type of microscope is required to see them
- The sizes of bacteria are expressed in micrometers, whereas the sizes of viruses are expressed in nanometers.

approximately the size of bacteria ranges from 1-3 μ m

the largest virus ranges about the smallest bacteria

mycoplasma is a very small bacterium (0.15-0.3 μ m) that was classified as a virus because of its size

Microscopes



- A microscope is an optical instrument that is used to observe tiny objects, often objects that cannot be seen at all with the unaided human eye (the “naked eye”).

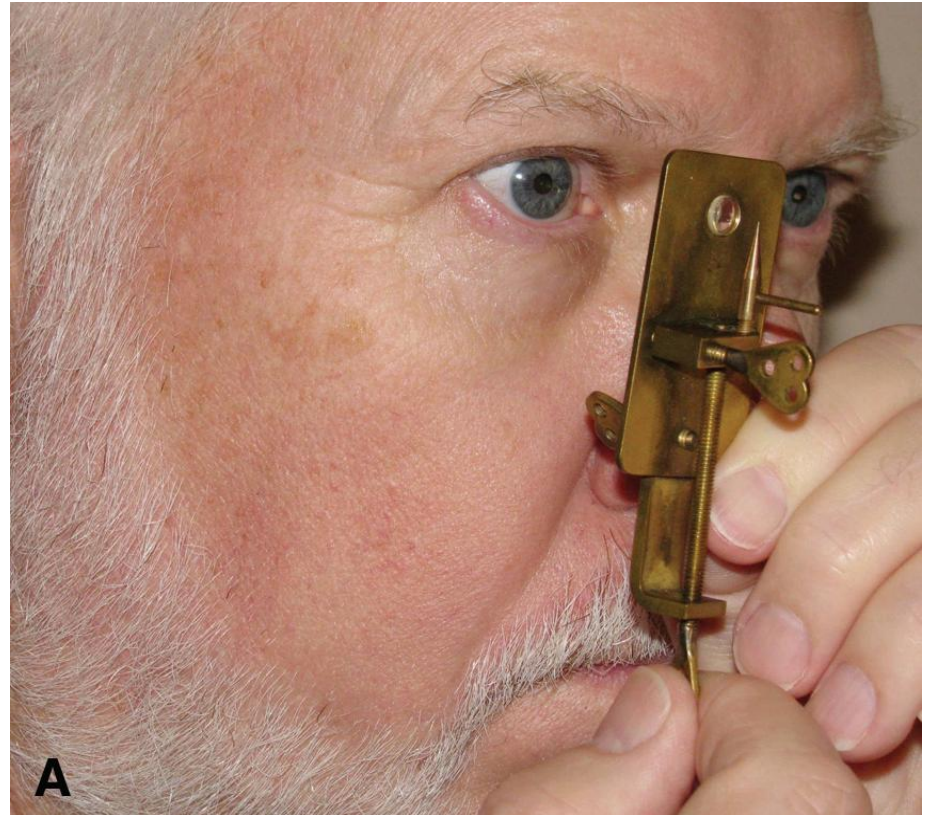
Simple Microscopes

- A simple microscope is defined as a microscope containing only one magnifying lens

it was first used by Van leeuwenhoek

VAL made a simple microscope with magnifying power of 300times (single magnifying lens normally magnifies by 20 times)

simple microscope has no ability to have a variable magnification(one lens is used)



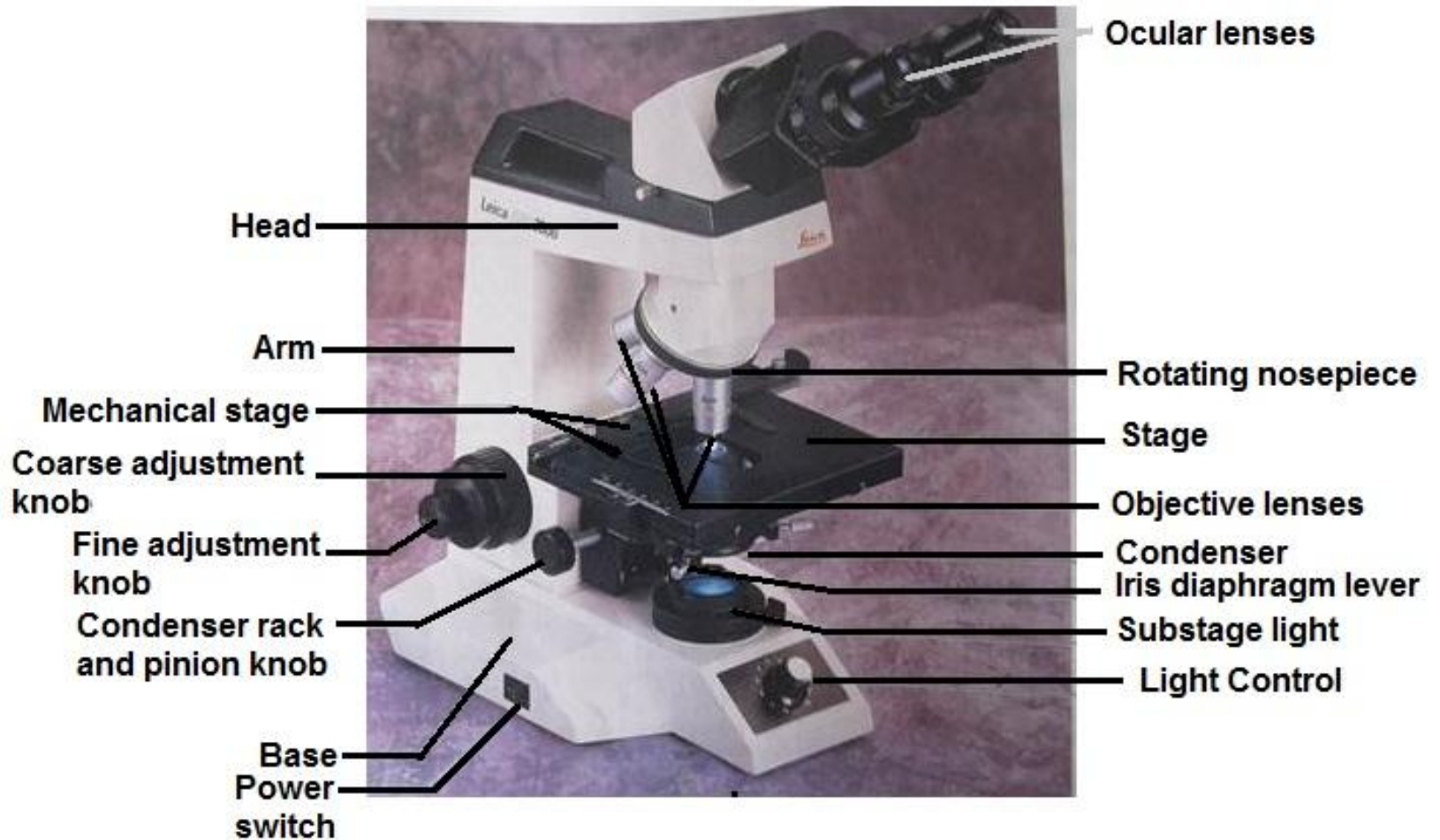
Compound Microscopes

also called Bright field M or teaching microscope



compound microscopes are also called teaching microscope

- The most commonly used instrument for observing any cell
- Visible light illuminates the object being studied
it can magnify for about 1000 times
- Has two sets of magnifying lenses
 1. an objective lens there is 3-4 objective lenses in the LM
 2. an ocular lens
ocular lens is fixed



- The resolving power of a microscope is defined as the minimum distance that can exist between 2 objects such that the points are observed as separate entities

shortest distance between two distinguishable adjacent points

- The max resolving power of the best light microscope is 0.2 micrometer

- The resolving power of a microscope can be increased by ways to improve RP:

1. using oil rather than air as the material between the specimen being viewed and the objective lens

2. using a shorter wavelength than that of the visible light

like using UV light that is characterized by very short wavelength



- The objective lens that is designed to be used with oil is called an **oil immersion lens**
- The total magnification of a microscope is the product of the magnification of the ocular lenses and the objective lenses
$$M = O_c L * O_b L$$
- **Empty magnification:** Increasing magnification without increasing the resolving power.

Phase contrast microscope

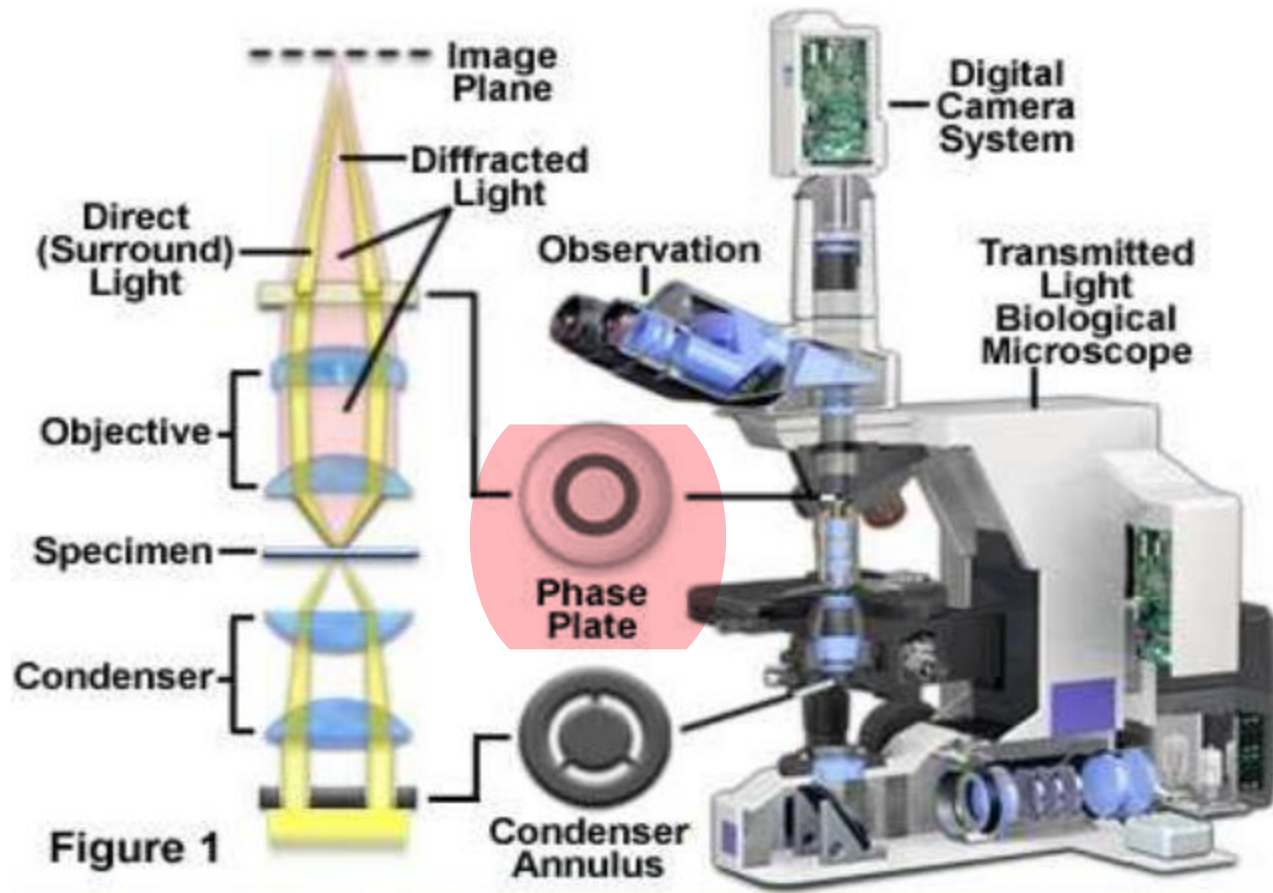


- A special kind of microscope commonly used in research labs for observing **living microorganisms**
- It has optical devices that increase the contrast between the microbes and the surrounding medium, so light passing through a cell's components is slowed down more than it is by the surrounding medium

PCM:used to observe the and monitor the living microbe

in PCM light is passed through the organism slowly while through the medium light passes very fast that makes a contrast(تباين) between the organism and the medium

Phase Contrast Microscope Configuration



condenser is located above the light source and below the specime(it concentrates light on the specimen)



- Since different cell structures slow down the light in varying degrees, some of the structures of the cell can be discerned by phase contrast microscope
- It is possible to observe living organisms clearly and study their movements in the medium

Dark-field microscope



- Used to develop a marked contrast between living organisms and the background here the background is dark in color

we use Dark field condenser instead of normal condenser(normal condenser makes the specimen bright)

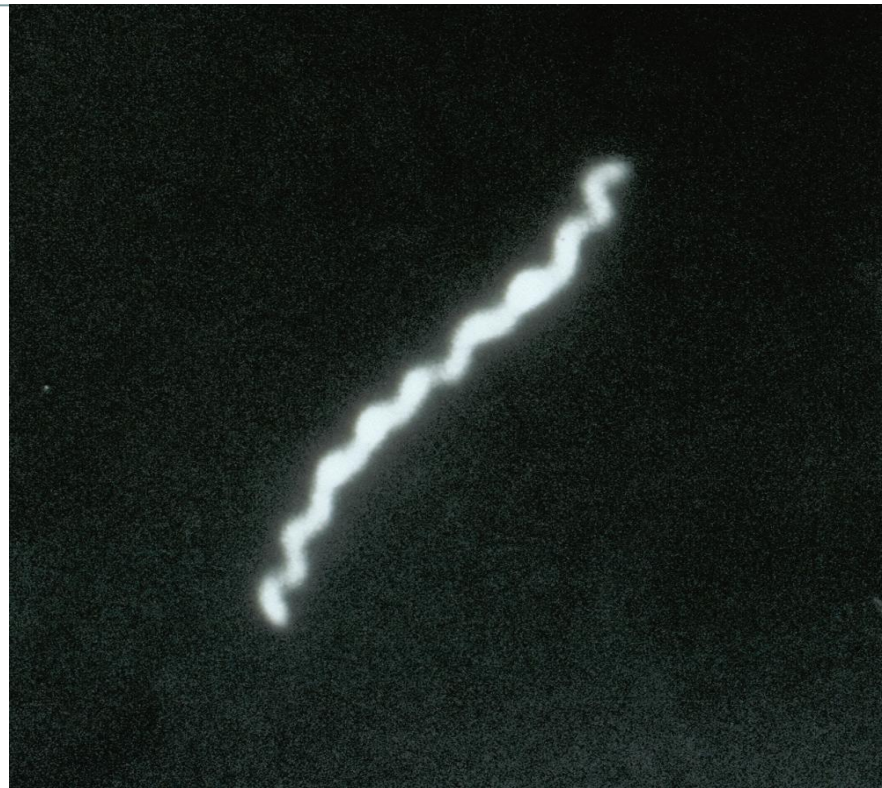
- Light is directed toward the specimen at an **angel**, so that only light that is scattered by the specimen enters the objective lens and is seen

used in cases when the specimen doesn't appear under light microscope (compoundM)

- The ordinary condenser is replaced by dark-field condenser that doesn't allow the illuminating light to pass through the specimen directly

used for samples that are shiny that wouldn't appear under normal light microscope (like T.pallidum)





Spiral-shaped *T. pallidum* bacterium. *The causative* agent of syphilis, as seen by darkfield microscopy.

treponema pallidum
syphilis:sexually transmitted infection that has
four stages



- This microscope makes it possible to see invisible objects by the light microscope
- We can see the same object at magnification power lower than that of the light microscope

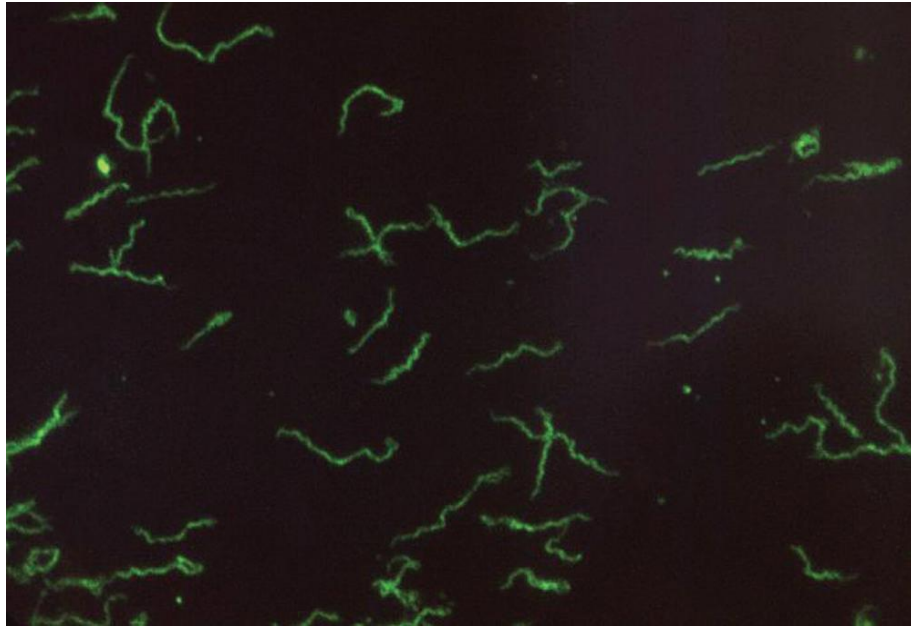
Fluorescence microscope:



- Used to visualize objects that fluoresce (emit light when light of different wavelength strikes them) by themselves (naturally)
- The fluorescence may be a natural property of the specimen being viewed or it may result from the attachment of fluorescent compound

green and colors appear for the specimen

there is special type reflector (protector) for UV light that affects the eye badly



Photomicrograph of *T. pallidum spirochetes* using immunofluorescence.
A fluorescent dye is first attached to anti-*T. pallidum antibodies*

treponema pallidum spirochetes (ملتويية)



- The objects stained with a fluorescent material can be seen when it is illuminated by **ultraviolet light** supplied by a special lamp
- A special filter on the microscope allows the yellowish green light from the source of fluorescence to pass, but it **blocks the passage of the ultraviolet light**

Electron microscope



- Transmission electron microscope was developed to increase the resolving power of the microscopes
- **TER has much higher resolution than SER**
- Electrons rather than the visible light illuminate the specimen being viewed
- **RP is 0.2nm of TER**
- Electrons have a wavelength 100,000 times shorter than the visible light, the resolving power increases about 100,000 folds to about 0.2 nanometer

the shorter the wavelength the higher the resolution

the disadvantage of EM we cannot deal with living organisms



Scanning electron microscope.



- Magnetic fields focus the beam of electrons, so function as the condenser lens
- TEM has an objective lens, some electron pass through the specimen, others are scattered and some cannot pass
- The electrons hit an electron sensitive screen and thereby creating an image that is determined by the ability of the electrons to pass through various objects



- Scanning electron microscope: the beam of electrons scans back and forth over the surface of the specimen being viewed
- The surface is coated with a thin film of metal
- As the beam moves over the specimen electrons are released from the specimen and reflected back into the viewing chamber



- SEM is useful for observing surface details but not internal structures of cells
- SEMs have a resolving power of about 20 nm—about 100 times less than the resolving power of TEMs.

atomic force microscope has very high resolution and used to observe living microbes

atomic force microscope used to study samples in 3D not like ER