Unit I
Microscopy

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- Difference between Electron Microscope & Light Microscope

MICROSCOPE

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

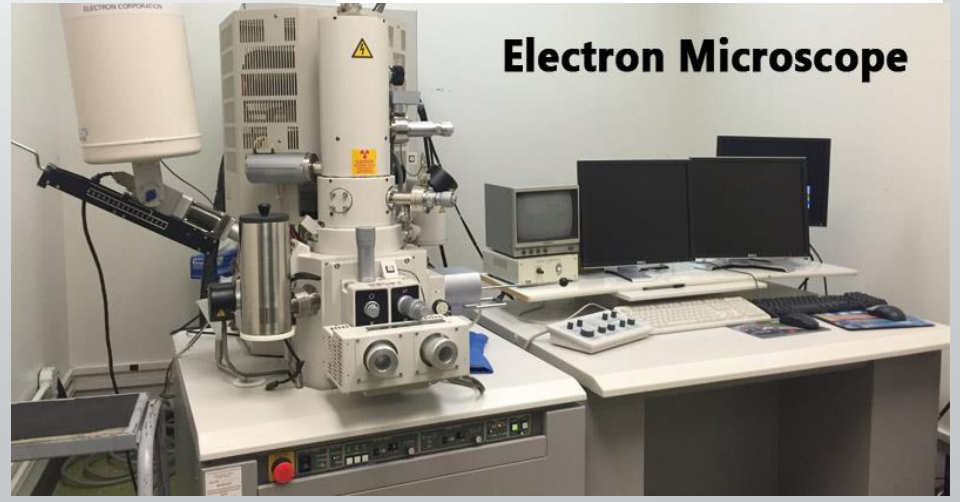
Types of Microscopy

LIGHT MICROSCOPE

- Bright field microscope.
- Dark field microscope.
- Phase contrast microscope.
- Fluorescence microscope.

ELECTRON MICROSCOPE

- Transmission electron microscope.
- Scanning electron microscope.



https://microbenotes.com/electron-microscope-principle-types-components-applications-advantages-limitations/

LIGHT MICROSCOPE

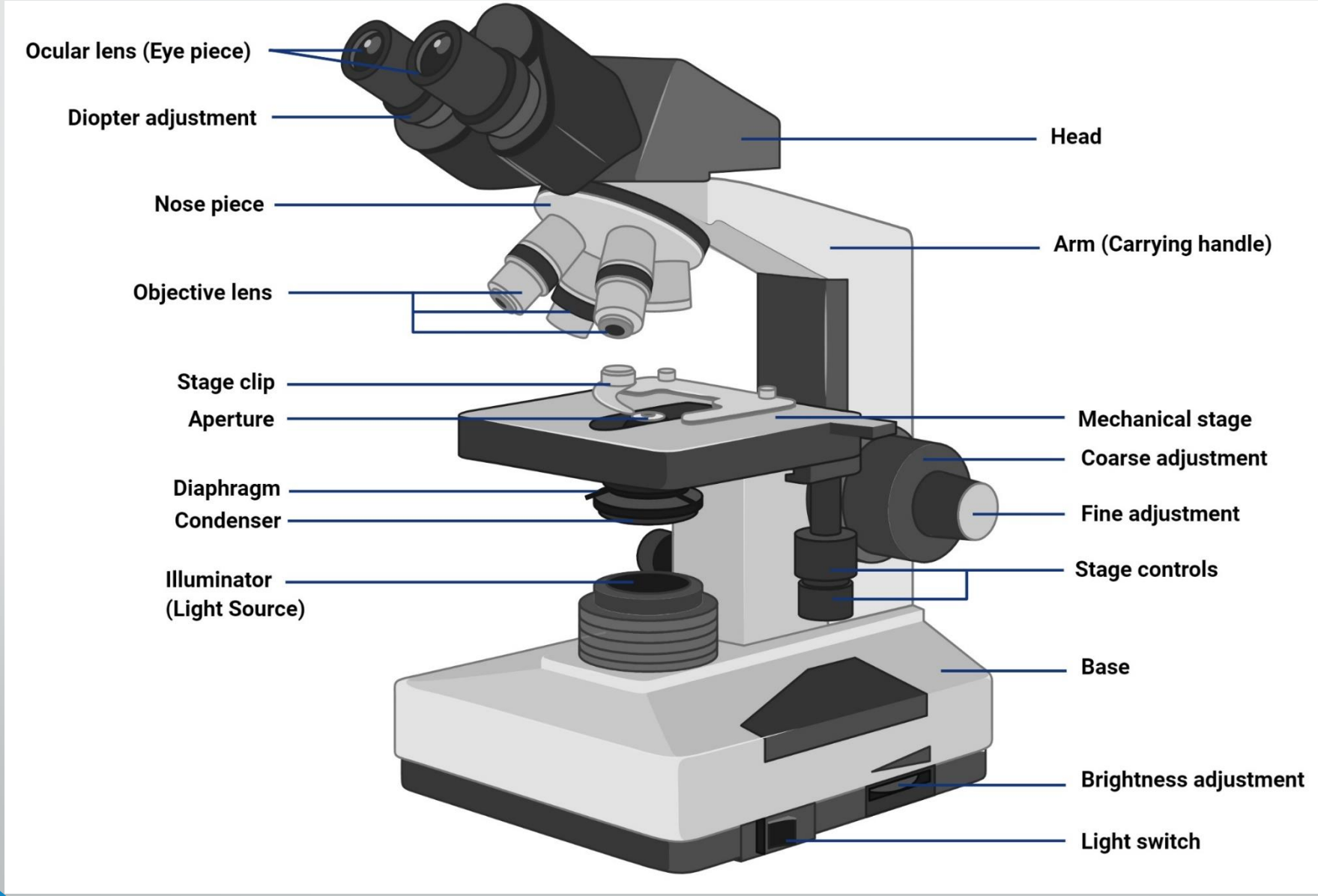
- use sunlight or artificial light.
- light typically passes through a specimen and then through a series of magnifying lenses.
- Light microscopes are simplest of all microscopes.
- Light microscopes use lenses to bend and focus light rays to produce enlarged images of small objects.

BRIGHT-FIELD MICROSCOPE

- also known as the **Compound/Light Microscope**
- uses light rays to produce a dark image against a bright background.
- it is important that the specimen should be stained first so that they can be properly viewed under this type of a microscope.
- used to view fixed and live specimens stained with basic stains which gives a contrast between the image and the image background.
- SIMPLE -Contain a single magnifying lens COMPOUND • Series of lenses for magnification
- Staining is achieved with the use of a chemical dye.
- By applying it, the specimen would be able to adapt the color of the dye.

  Light passes through specimen into objective lens

Compound/Light Microscope



Compound/Light Microscope: Parts

- **Eyepiece (Ocular lens)** – lenses at the top of the microscope which focuses the image from the objective lenses. We see the formed image with your eyes.
- **The objective lenses** which make a clear image from the specimen
- **Two focusing knobs** -fine adjustment knob and the coarse adjustment knob, found on the microscopes' arm, which can move the stage or the nosepiece to focus on the image.
- **Stage** -the specimen is placed over it ,this allow the movement of the specimen around for better viewing with the flexible knobs
- **The condenser**: is mounted below the stage which focuses a beam of light onto the specimen.
- **The arm**: This is a sturdy metallic backbone of the microscope, used to carry and move the microscope from one place to another. They also hold the microscope **base** which is the stand of the microscope. The arm and the base hold all the microscopic parts.
- **Light illuminator** or a **mirror** found at the base or on the microscope's nosepiece.
- **Nosepiece** has about two to five objective lenses with different magnifying power. It can move round to any position depending on the objective lens to focus on the image.
- **Aperture diaphragm** It controls the diameter of the beam of light that passes through the condenser.

BRIGHT-FIELD MICROSCOPE

ADVANTAGES

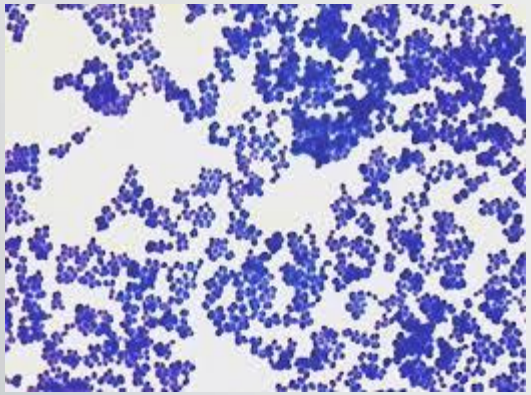
- Used to view live or stained cells.
- Simple setup with very little preparation required.

DISADVANTAGES

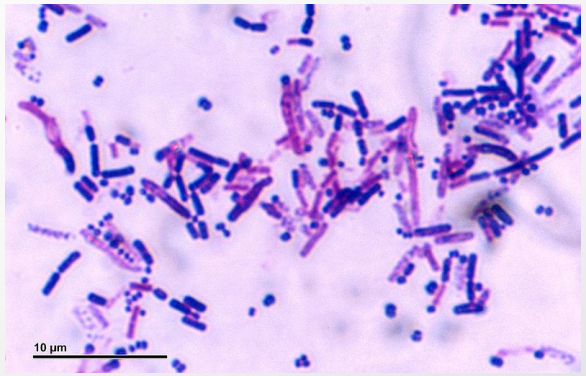
- Biological specimen are often of low contrast and need to be stained.
- Staining may destroy or introduce artifacts.
- Resolution is limited to 200nm.

IMAGE FORMED BY BRIGHT FIELD MICROSCOPY (unstained)

BRIGHT-FIELD MICROSCOPE: Images



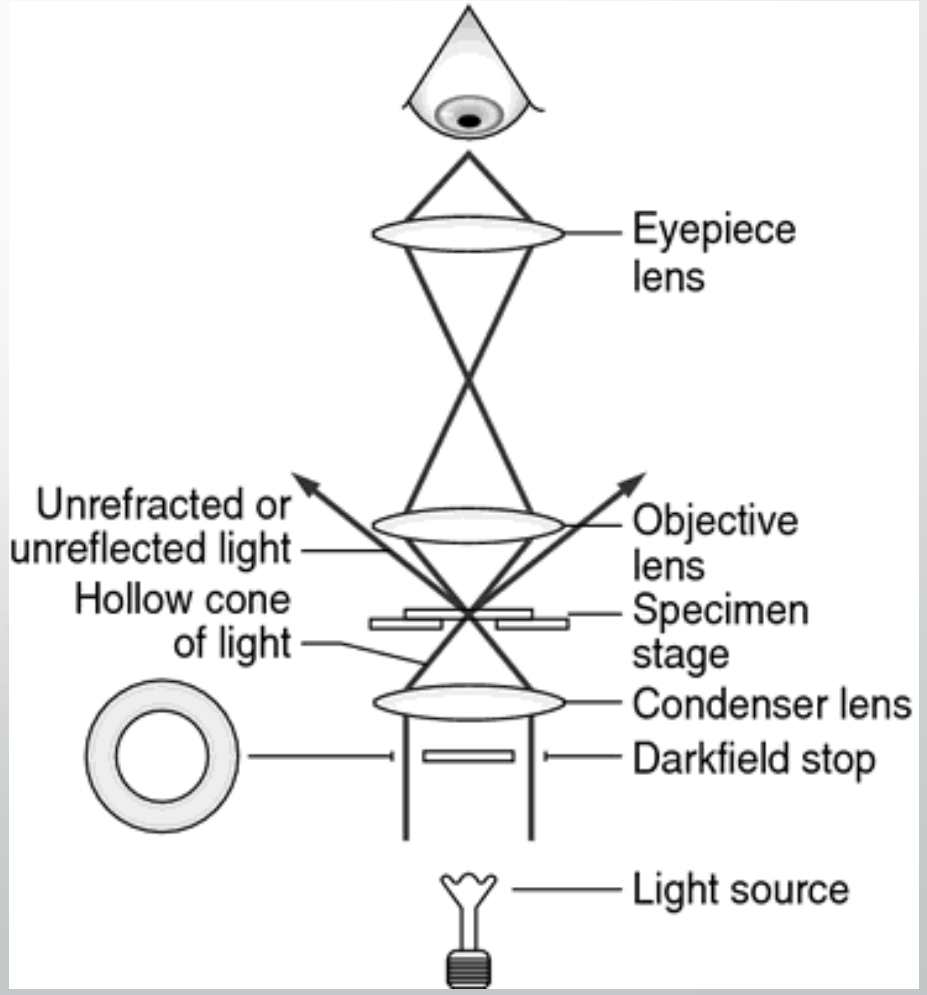
https://homepages.wmich.edu/~rossbach/bios312/LabProcedures/Simple%20stain%20results.html



https://commons.wikimedia.org/wiki/File:Bacteria_(248_28)_Airborne_microbes.jpg

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.



DARK FIELD MICROSCOPE

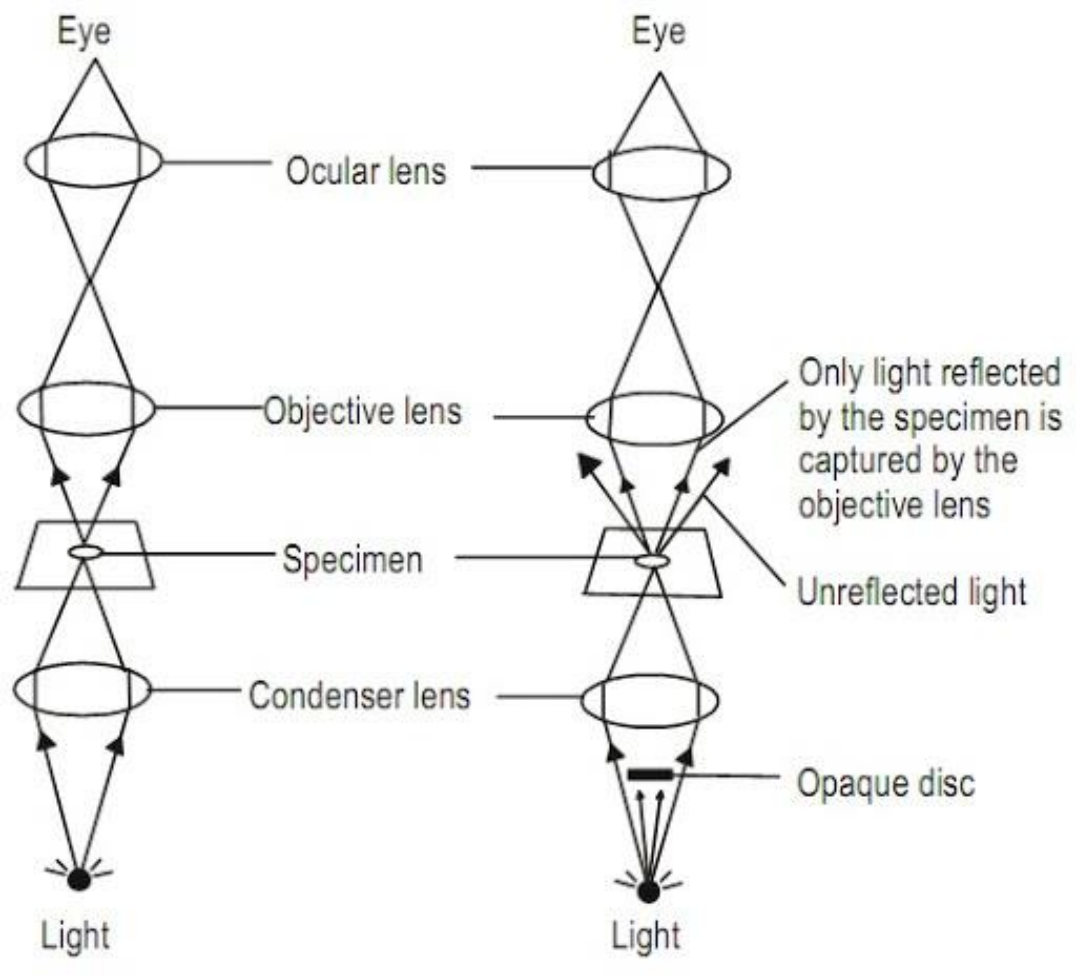
ADVANTAGES

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

DISADVANTAGES

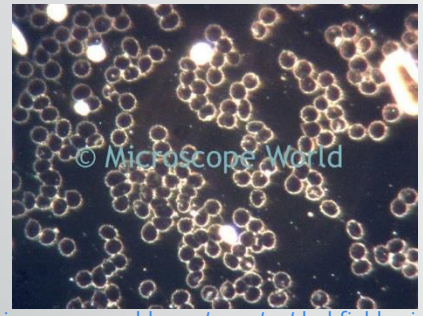
- Specimen needs to be strongly illuminated which can damage delicate samples.

BRIGHT FIELD
Vs
DARK FIELD MICROSCOPE



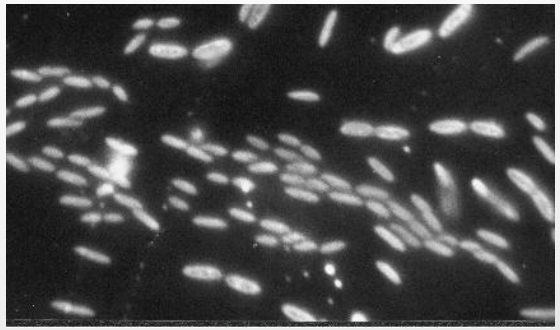
DARK FIELD MICROSCOPE: Images

Blood cells



http://blog.microscopeworld.com/2014/01/darkfield-microscopy-slide-preparation.html

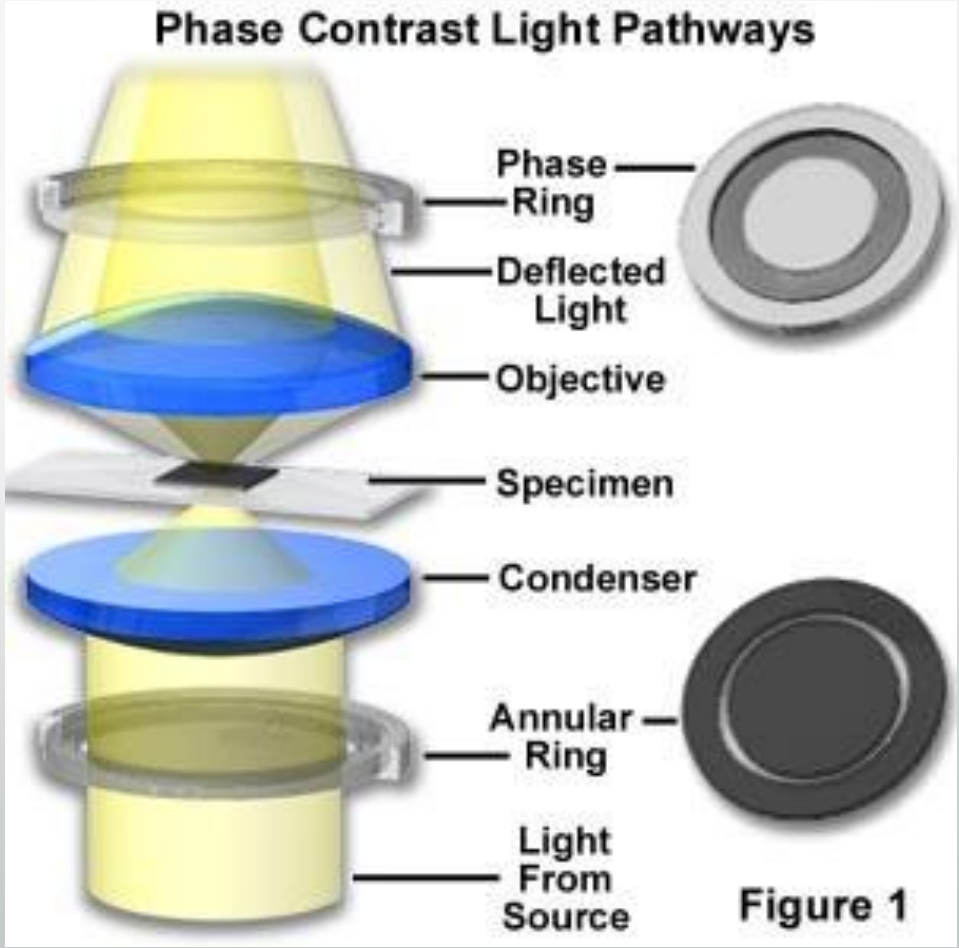
Bacteria cells



http://www.microscopy-uk.org.uk/index.html?http://www.microscopy-uk.org.uk/primer/special.htm

PHASE CONTRAST MICROSCOPE

- First described in 1934 by Dutch physicist Frits Zernike and awarded Nobel prize in physics in 1953
- Produces high-contrast images of transparent specimens
- Diffraction of light



https://www.olympus-lifescience.com/de/microscope-resource/primer/techniques/phasecontrast/phase/

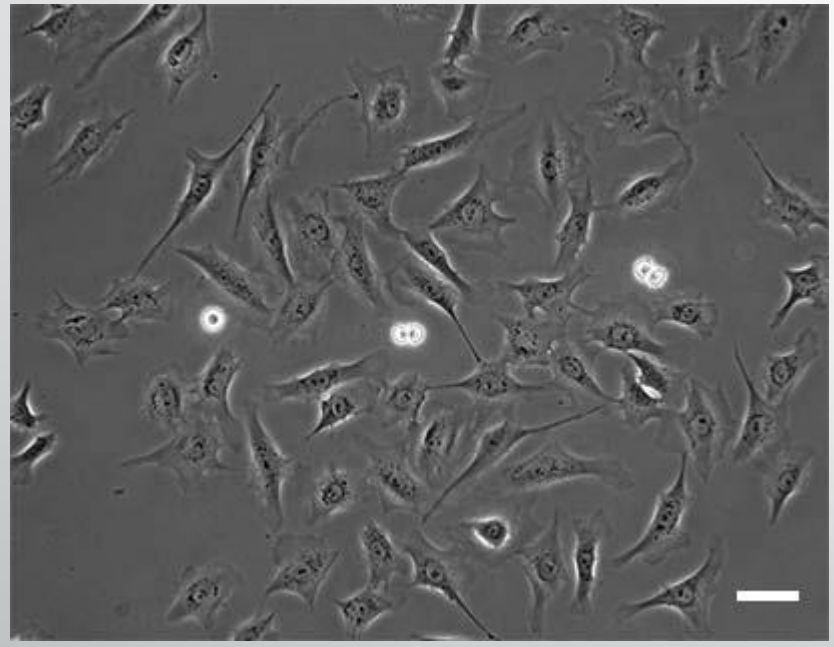
PHASE CONTRAST MICROSCOPE

PRINCIPLE

- It is an optical illumination technique in which small phase shifts in the light passing through a transparent specimen are converted into contrast changes in the image.
- small **phase** changes in the light rays, induced by differences in the thickness and refractive index of the different parts of an object, can be transformed into differences in brightness or light intensity.
- Phase contrast microscope employs an optical mechanism to translate minute variations in phase into corresponding changes in intensity of image.
- Unstained bacteria have constituents of different refractive index
- Light rays in phase produce brighter image.
- Light rays out of phase form darker image.

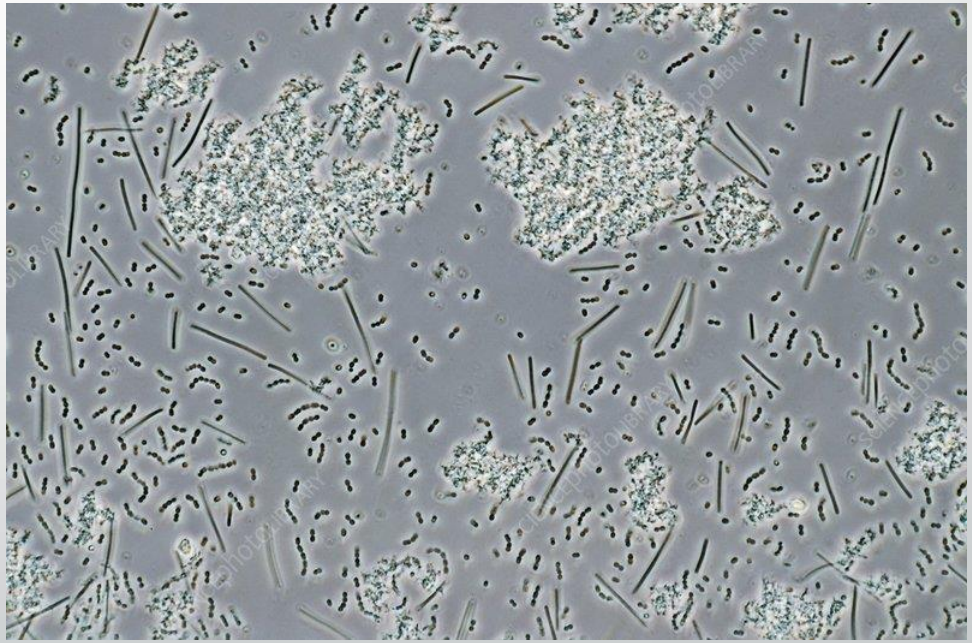
Contrast is due to out of phase rays.

Rat cell



https://ibidi.com/content/213-phase-contrast

Lactic acid bacteria



https://ibidi.com/content/213-phase-contrast

PHASE CONTRAST MICROSCOPE

Advantage –

- Living cells can be examined in their natural state
- Phase contrast enables visualization of internal cellular components.
- Diagnosis of tumor cells
- Examination of growth, dynamics, and behavior of a wide variety of living cells in cell culture.

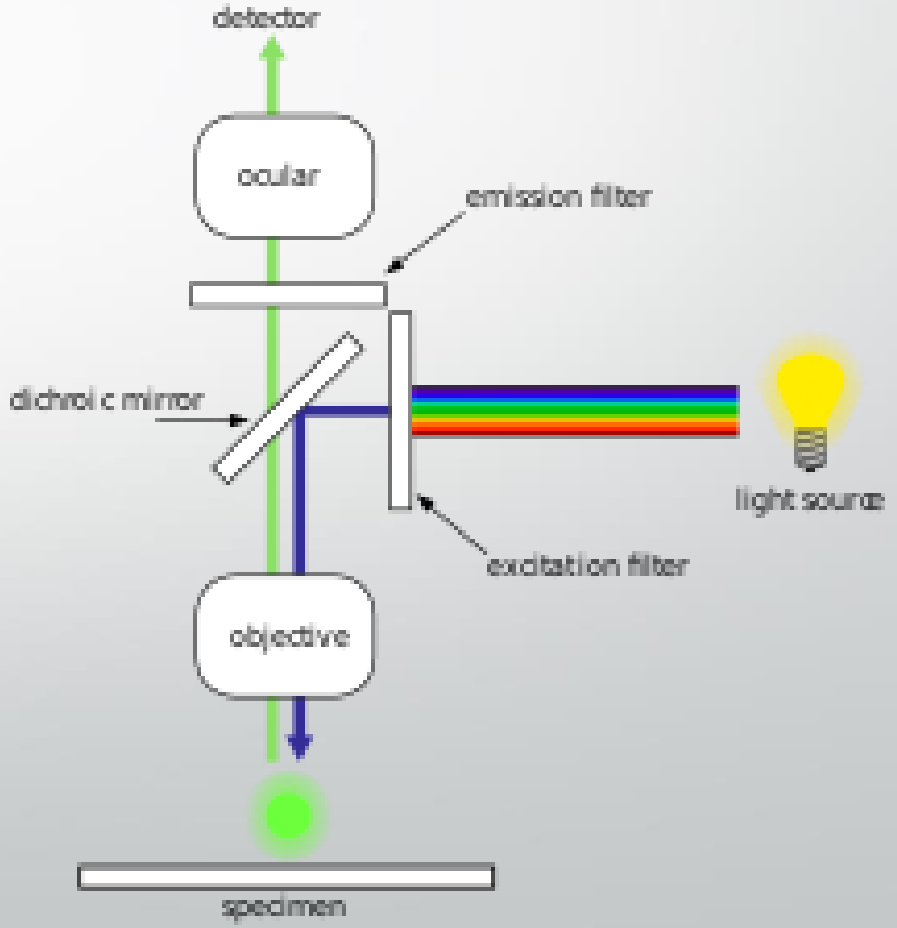
DISADVANTAGES

- 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.

FLUORESCENCE MICROSCOPE

PRINCIPLE OF FLUORESCENCE MICROSCOPY

The specimen is illuminated with light of a specific wavelengths which is absorbed by the fluorophores or fluorescent chemical compound that can re-emit light upon light excitation, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light).

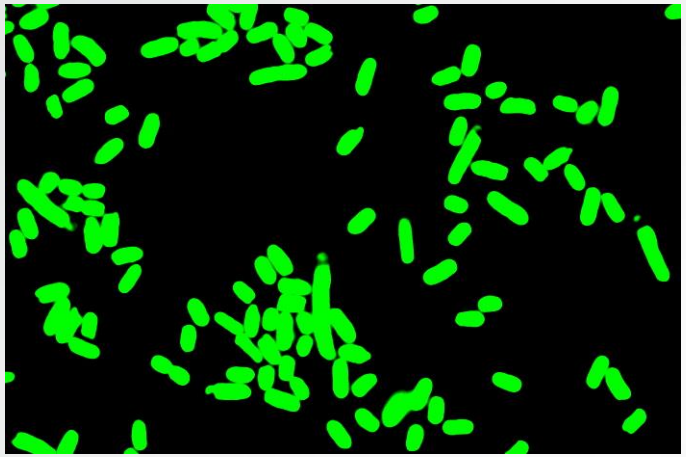


https://en.wikipedia.org/wiki/Fluorescence_microscope

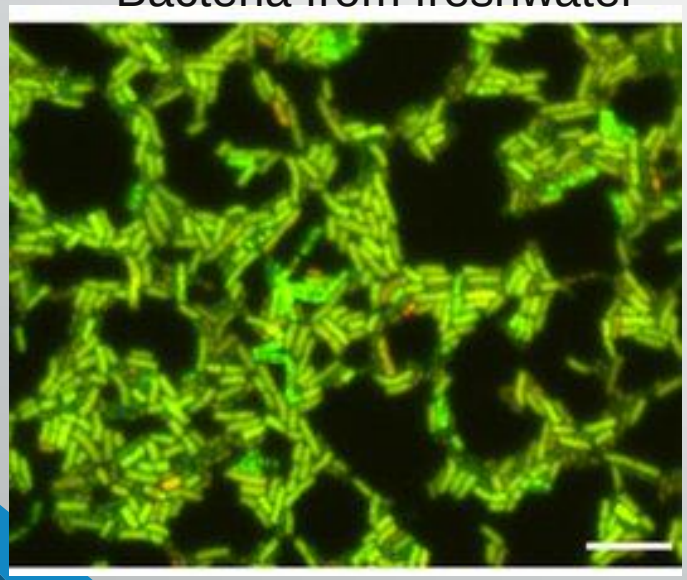
FLUORESCENCE MICROSCOPE

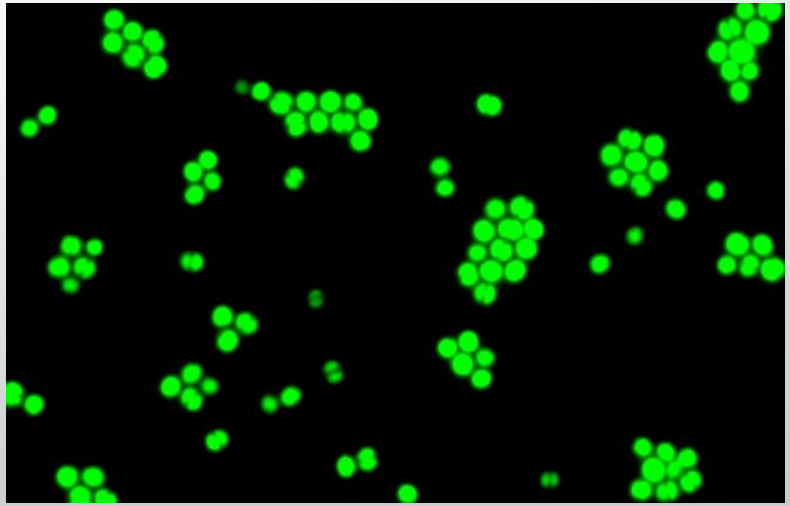
- Exposes specimen to ultraviolet, violet, or blue light.
- Specimens usually stained with fluorochromes.
- Shows a bright image of the object resulting from the fluorescent light emitted by the specimen.
- Certain dyes, called as fluorochrome after absorbing UV rays raised to a higher energy level.
- When the dye molecules return to their normal state,they release excess energy in the form of visible light(fluoroscence).

E coli



Bacteria from freshwater

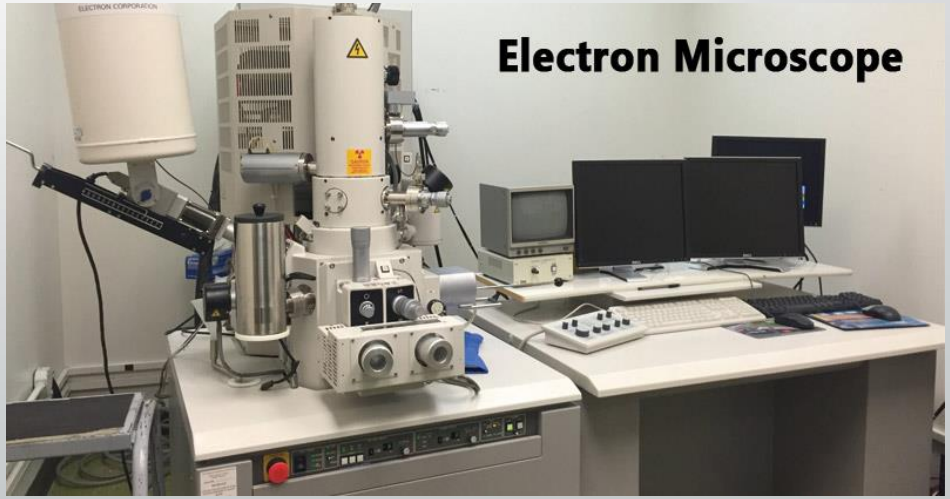




FITC-Fluorescein isothiocyanate

ELECTRON MICROSCOPE

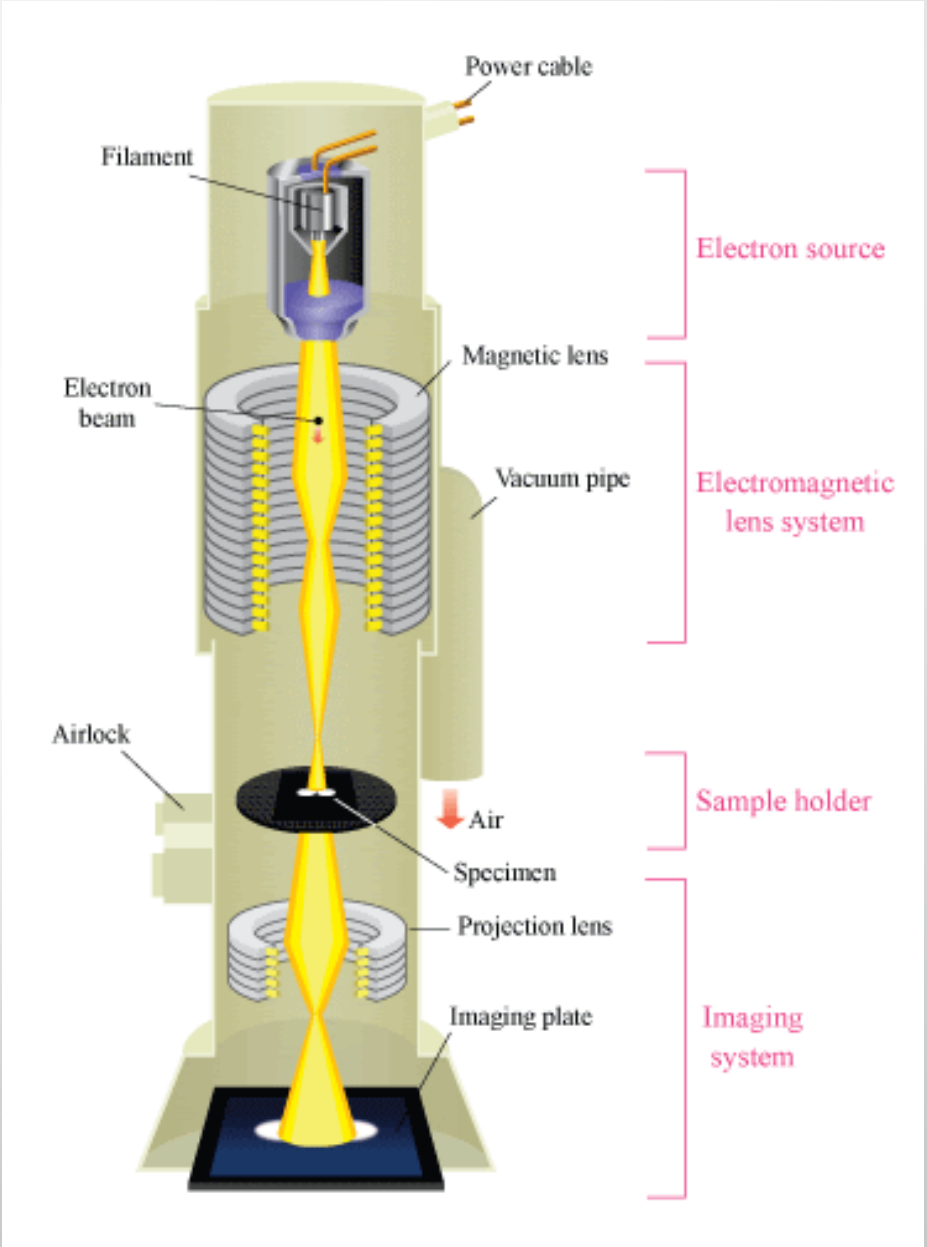
- Co-invented by Max knoll and Ernst Ruska in 1931
- Electron Microscopes uses a beam of highly energetic electrons to examine objects on a very fine scale.
- Magnification can upto 2million times while best light microscope can magnify up to 2000 times.
- Transmission electron microscope.
- Scanning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



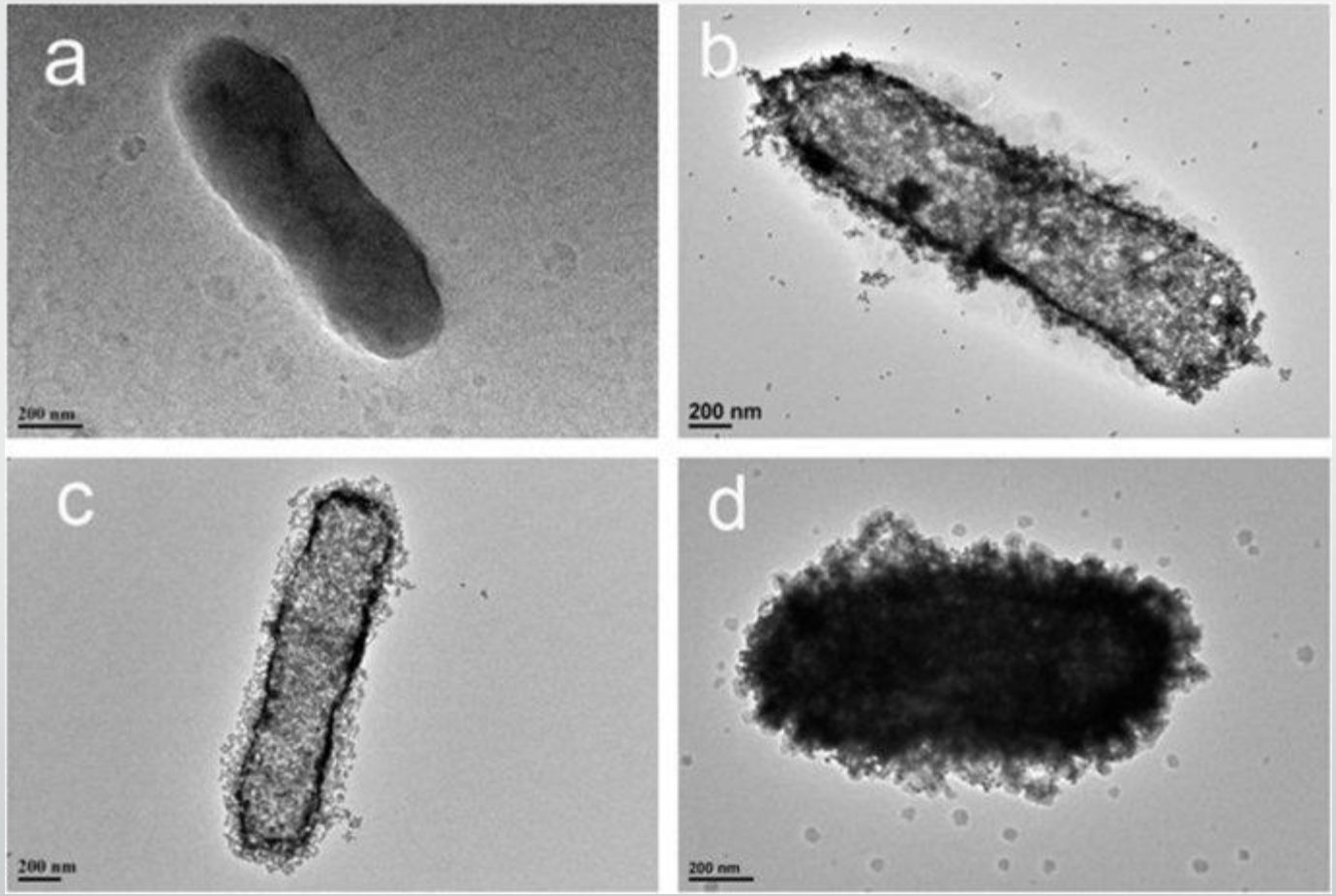
https://microbenotes.com/electron-microscope-principle-types-components-applications-advantages-limitations/

TRANSMISSION ELECTRON MICROSCOPE (TEM)

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



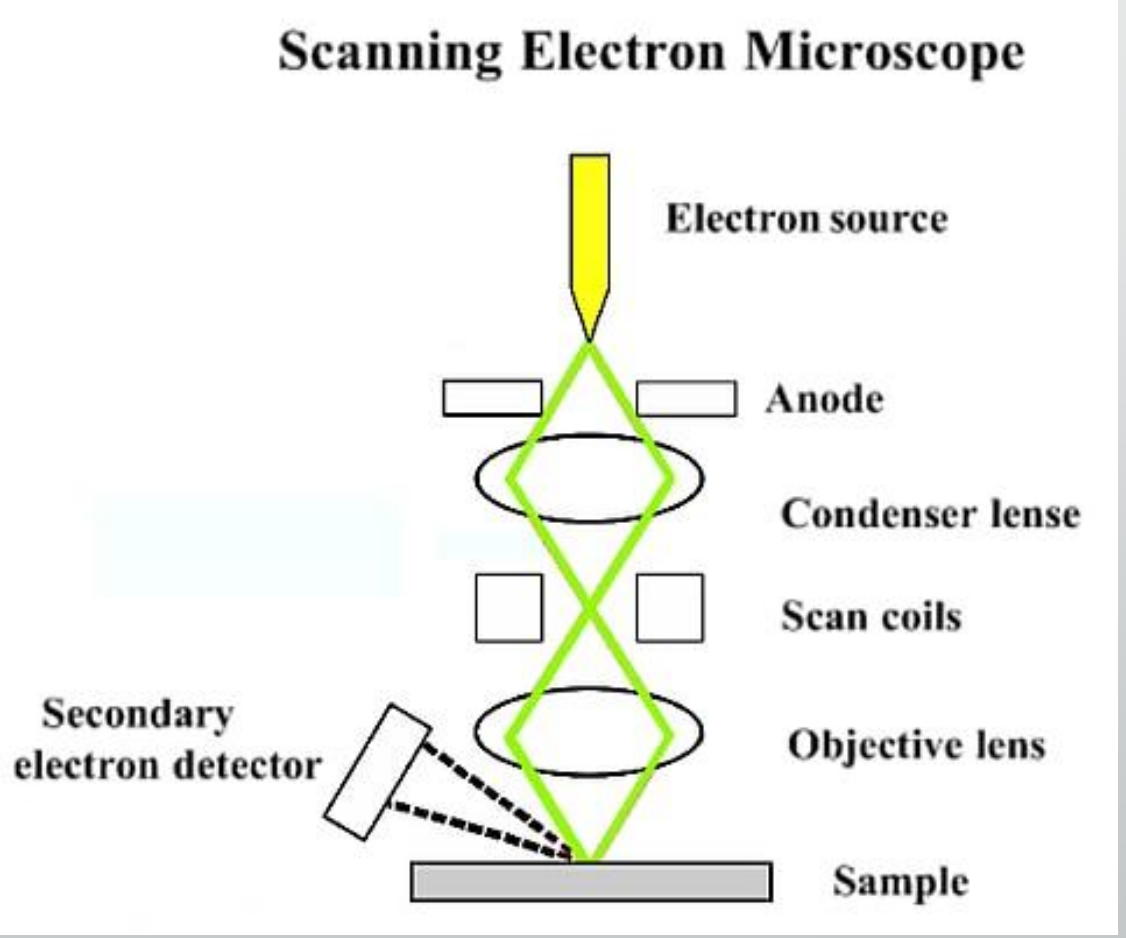
TEM Images



https://www.researchgate.net/figure/Transmission-electron-microscopy-images-of-a-E-coli-O157H7-bacteria-b_fig4_291555538

SCANNING ELECTRON MICROSCOPE

- SEM use the electrons that are reflected or knocked off the near-surface region of a sample to create an image.
- the resolution of SEMs is superior to that of a light microscope



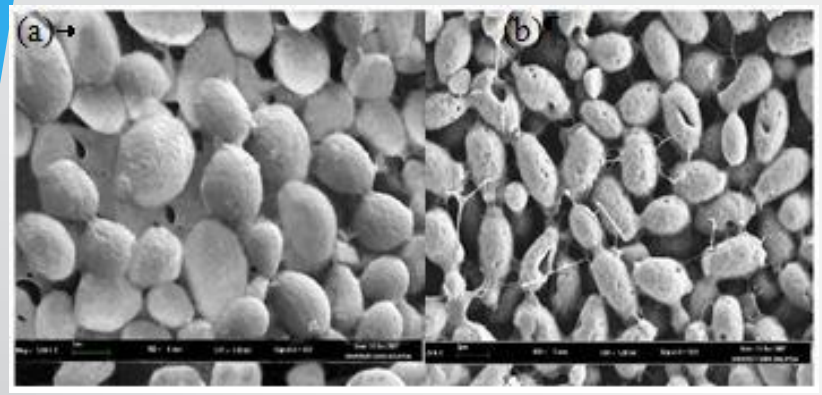
https://www.thermofisher.com/blog/microscopy/what-is-sem-scanning-electron-microscopy-explained/

Characteristics viewed by SEM

1. Topography-The surface features of an object or "how it looks", its texture;
2. Morphology-The shape and size of the particles making up the object; direct relation between these structures and materials properties
3. Composition-The elements and compounds that the object is composed of and the relative amounts of them; direct relationship between composition and materials properties
4. Crystallographic Information- How the atoms are arranged in the object; direct relation between these arrangements and material properties

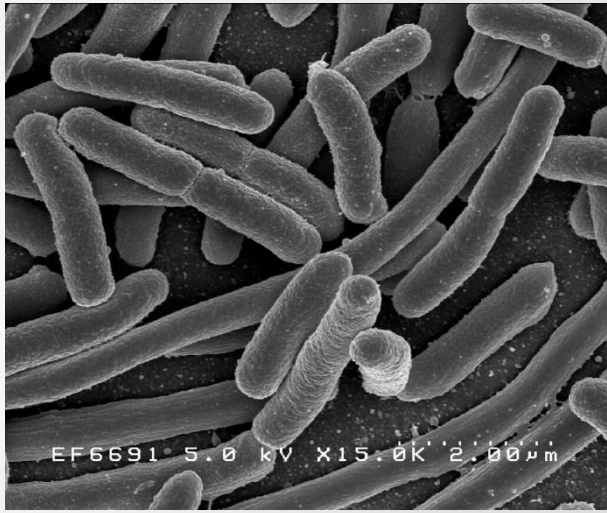
SEM Images

Candida Albicans



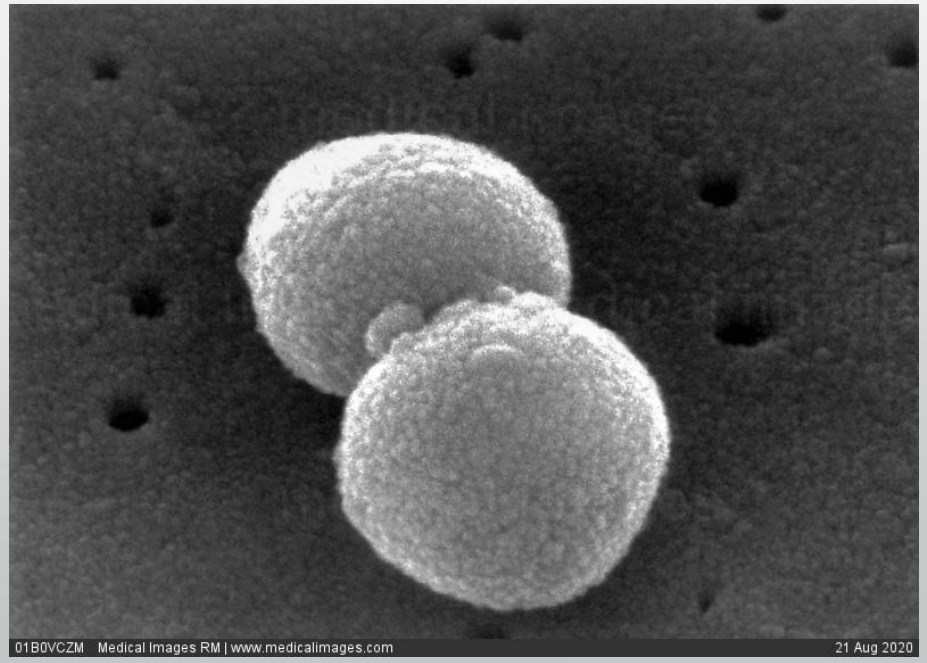
https://www.intechopen.com/books/scanning-electron-microscopy/scanning-electron-microscopy-sem-and-environnmental-sem-suitable-tools-for-study-of-adhesion-stage-a

E coli



https://www.flickr.com/photos/niaid/7316101966

Streptococci



gefotostock.com/age/en/Stock-Images/Rights-Managed/GMS-ag65372final

Difference between Electron Microscope & Light Microscope

Light Microscope

1. Uses light (400-700 nm) as an illuminating source
2. Lower magnification than an electron microscope
3. Specimen preparation is easy and takes about a few minutes
4. Both live and dead specimen can be seen
5. Low resolution
6. Inexpensive and requires low maintenance cost

Electron Microscope

1. Uses electron beams (approx. 1 nm) as an illuminating source.
2. Higher magnification
3. Specimen preparation is difficult and takes time
4. Only dead and the dried specimen can be seen
5. High resolution
6. Expensive and requires high maintenance cost

Magnification Power

- **Magnification** is the ability to make small objects seem larger, such as making a **microscopic** organism visible.
- The total magnification of microscope is calculated by multiplying the magnifying power of the objective lens by that of eye piece.

Light Microscope

	Objective		
PROPERTY	**LOW POWER**	**HIGH POWER**	**OIL IMMERSION**
Magnification of objective	10x	40-45x	90-100x
Magnification of eyepiece	10x	10x	10x
Total magnification	100x	450 – 450x	900 – 1000x
Numerical aperture	0.25 – 0.30	0.55 – 0.65	1.25 – 1.4
Mirror used	Concave	Concave	Plane
Focal length (Approx)	16 mm	4 mm	1.8 – 2 mm
Working distance	4 – 8 mm	0.5 – 0.7 mm	0.1 mm
Iris diaphragm	Partially closed	Partially opened	Fully opened
Position of condenser	Lowest	Slightly raised	Fully raised
Maximum resolution (Approx)	0.9 µm	0.35µm	0.18µm

https://www.slideshare.net/sarathy4/microscope-10905635

Resolution Power

- It is the ability to differentiate two close points as separate.
- Resolution power of microscope is function of
  - wave length of light &
  - Numerical aperture of lenses
- The resolving power of human eye is 0.25 mm
- The resolving power of light microscope is 0.25µm.
- The electron microscope can separate dots that are 0.5nm apart.
- Light **microscopy** has limits to both its **resolution** and its **magnification**.

Limit of resolution(d)

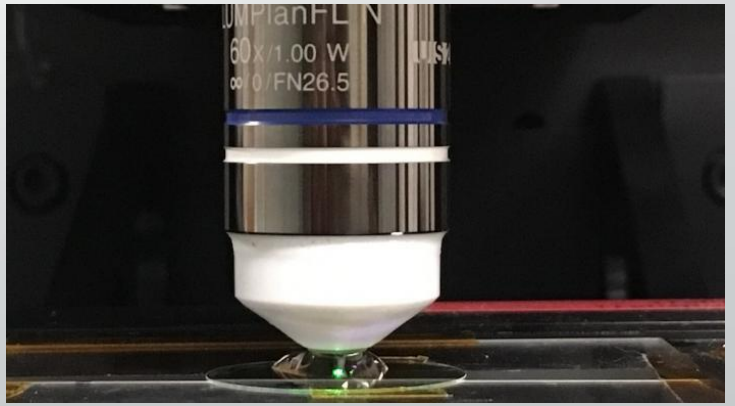
- It is the minimum distance between two points to identify them separately.
- It is calculated by Abbé equation.
- Limit of resolution is inversely proportional to resolution power.
- The **reciprocal of limit of resolution** is known as the resolving power.

$$d = \frac{\lambda}{2NA}$$ Where d = Limit of resolution

- $\lambda =$ wavelength of light and NA $=$ numerical aperture of lenses
- **NA =** $n\ sin\Theta$
- Where n = refractive index and $sin\Theta$ = value of half aperture angle
- Greater resolution is obtained by
  - Using shorter wave length light
  - Lenses with maximum numerical aperture

Oil Immersion Technique

- In light microscopy, to increase the resolving power of a microscope this technique is used.
- This is achieved by immersing both the **objective** lens and the specimen in a transparent **oil** of high refractive index, thereby increasing the numerical aperture of the **objective** lens
- With dry objective lense n = 1 since refractive index of air is one
- With oil immersion n = 1.56

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