Structured Notes According to

MICROBIOLOGY

Revision friendly **Fully Colored Book/Structured Notes**

For Best results, watch the video lectures along with reading notes

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1 SCIENTISTS AND STAINS

Louis Pasteur

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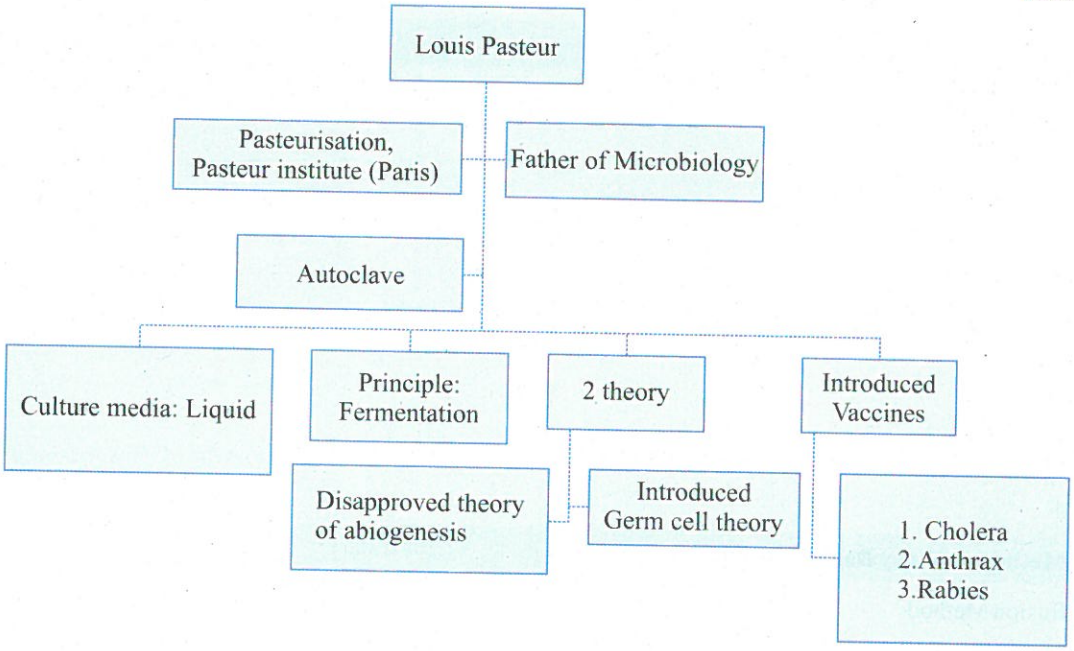

Robert Koch

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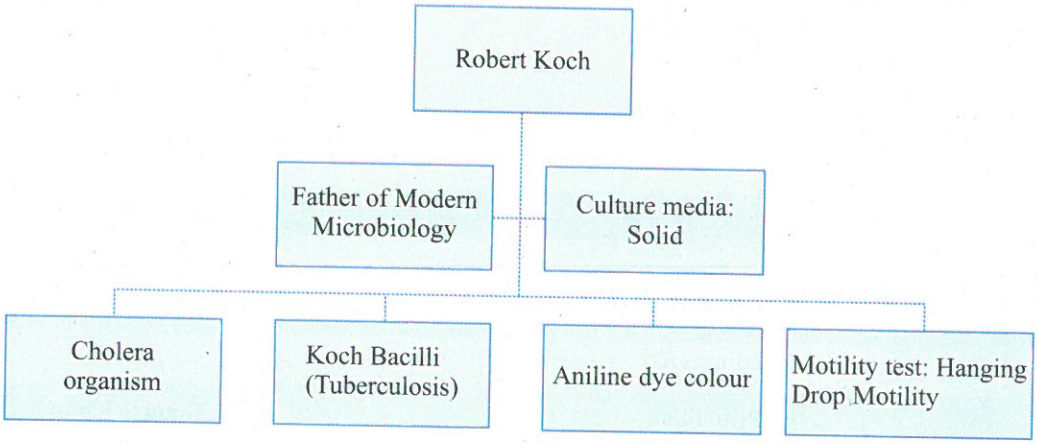


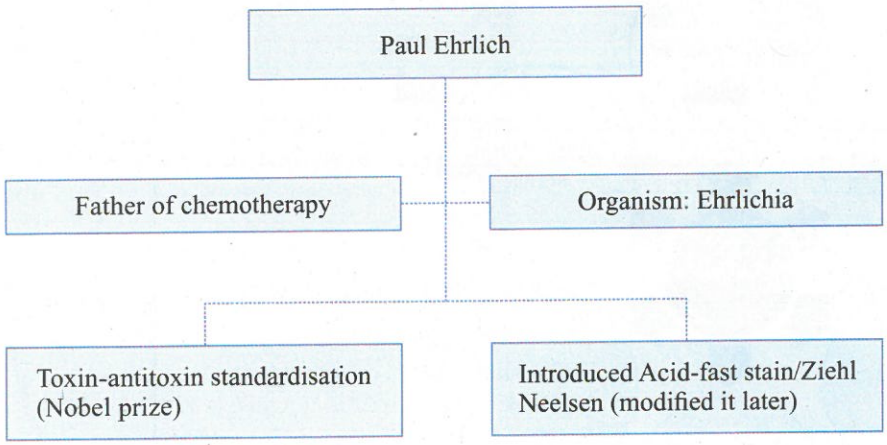
- **Koch postulates (4+1):**
  - Constant association of causative organisms with the disease (Mycobacterium TB always causes tuberculosis)
  - Isolation in culture media possible
  - Culture growth inoculated in animals should produce the same lesion.
  - Re-isolation from the experimental animals is possible.
  - Whenever there is an antigen, the human should be able to produce antibodies in serum
  - Exception from postulates:

    → **L-Mycobacterium leprae grown in armadillo**

    → **P-Treponema Pallidum**

    → **G-Gonococci**

Paul Ehrlich



Scientist	Found/Known as
Joseph Lister	• Father of antiseptic surgery • First used carbolic acid
Anton Von Leeuwenhoek	• Father of the light microscopy (Unilocular) • The first thing he visualised under his microscope was Animalcules. • Scientist: Jansen brothers invented the compound microscope (Bilocular)
Ernst Ruska	• Father of electron microscopy
Edward Jenner	• First vaccine- smallpox o Prepared using cowpox
Karry B Mullis	• PCR
Frederick Sanger	• Sanger sequencing
H C Gram	• Gram staining
Kleinberger	• L forms (cell wall deficient)
Alexander Fleming	• Penicillin
Barbara McClintock	• Transposons (Jumping genes)

Nobel prizes during Covid Era

- Hepatitis C virus- Michael Hougton, Harvey J. Alter, Charles M. Rice
- CRISPR Cas9- Emmanuelle Charpentier and Jennifer A. Doudna

Stains

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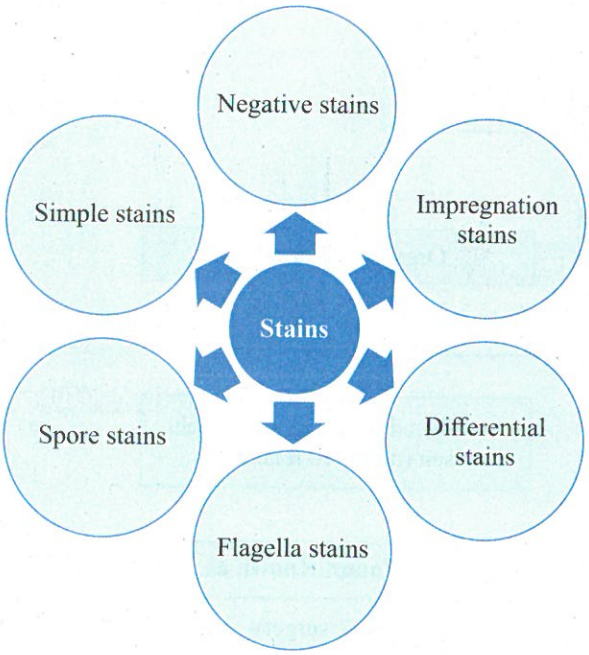
Fixation

- To ensure the sample stays on the slide
- Heat fixation
- Chemical fixation (methanol)

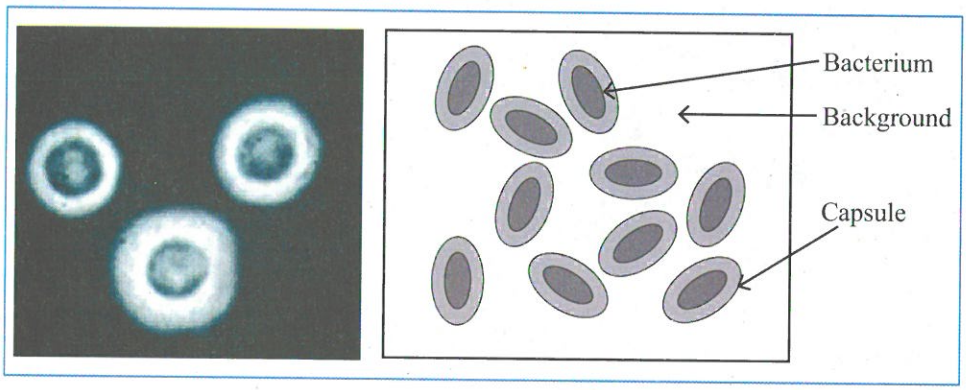


Simple stains (one colour)

- Methylene blue
- Basic fuchsin (red)

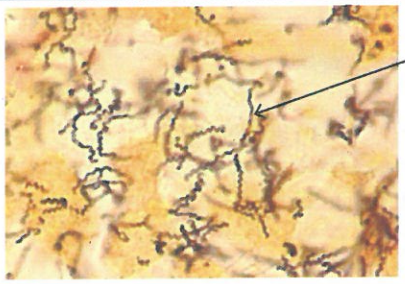
Negative stains (b/w)

- Staining the backgroundd to highlight the organisms
- Cryptococcus causes cryptococcal meningitis Sample : CSF
  - Capsulated fungi, that that doesn't take up any colour
- E.g., India ink stain, Nigrosin stain
- Nigrosin



Impregnation Stains

- Deposit stain on the surface of the object to make it look thicker.
- Silver stains- black colour
- For thin structures
  - Flagella
  - Spirochetes (Eg: Treponema caused syphilis)
    → Syphilis patient with Genital ulcers
- Fontana stain- fluid samples
- Levaditi's stain-tissue samples



Differential Stains

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- **Gram stain (+/-):**

PYQ: AIIMS 2021

Stains	Gram +ve	Gram –ve
Crystal/ Methyl/ Gentian Violet	Purple	Purple
Iodine (Mordant)	Makes Crystal violet stick	
Alcohol/ Acetone (decolouriser most crucial step)	Purple	Colourless
Safranin	Purple	Red/Pink

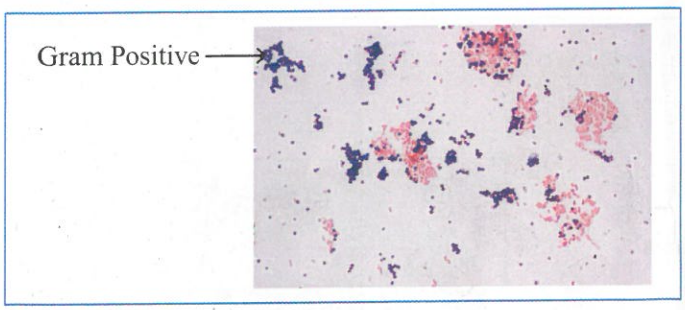
  - Game positive cell wall contains a lot of peptidoglycans, which retain the dye longer
  - Gram-negative cell wall contains lipopolysaccharides (LPS) making it permeable to the secondary dye after decolorization (as alchohol/acetone dissolves & washes away all the lipids in bacterial cell wall).
  - Poorly gram staining:(gram stain doesn't work well)

    → Mycoplasma

    → Rickettsia

    → Chlamydia

    → Spirochetes



- **Acid-fast Stain/Ziehl Neelsen Stain:**

00:39:12

  - Carbol Fuschin (primary stain) - red color
  - Heat (mordant)
  - Acid/ Acid-alcohol is added to decolorize -Sulphuric acid (most commonly)

    → Mycobacteria- 20% H_2SO_4

      – TB - Alcohol (95% alcohol) and Acid fast (20% H_2SO_4)

      – Atypical TB (M.avium)- Acid fast (20% H_2SO_4)

      – Lepra- Acid Fast (5% H_2SO_4): Fite foracco stain

    → Nocardia, Legionella- 1% H_2SO_4

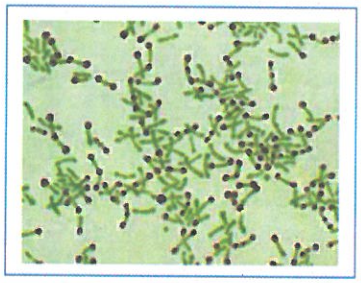
    → Coccidian parasite family- COLD ZN stain (5% H_2SO_4)

      – Isopora, Cyclospora, Cryptosporidium

→ Spores, Head of sperm: 0.25-0.5%H_2SO_4
→ Hooklet of Hydatid
→ Eggs: Tenia Saginata

- Methylene blue or Malachite green (secondary stain) added -Background colour
- COLD ZN stain- Kinyoun Stain/Gabbet stain(modification of ZN stain)
  → Instead of heating, increase concentration of phenol in carbon fuchsin.
  → Cold ZN stain is used for the family of coccidian parasites

- **Albert Stain:** 00:52:58
  - Albert solution 1
    → Malachite Green- Organism
    → Toludine blue - stains volutin granules (Metachromatic stain- 2 colours)
      – Actual colour- blue
      – On sample- purple
    → Glacial Acetic acid
  - Albert solution 2: Iodine
  - Used for Volutin / Babes Ernt granules
    → present in C-diphtheria
    → located at 2 poles, it is also called bipolar Granules
    → Metachromatic Granules-stained by Toluidine blue.
  - Stains for Volutin granules:
    → Ponder's Stain
    → Loeffler methylene Blue (best)
    → Albert Stain
    → Neisser stain
  - Volution granules also seen in Spirillum, Gardnerella, Yersinia pestis, yeast, MTB

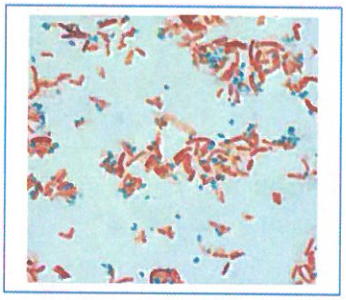


Flagella Stains

- Silver stains (impregnation method)
- Leifson and Ryu stain
  - Dye: Basic Fuchsin for flagella - Red
  - Mordant- Tannic acid
  - Dye: Methylene blue for cell - Blue

Spore Stain

- Spore- resting/ dormant form of bacteria.
- Schaeffer and Fulton stain/ Modified Ashby stain
  - Malachite green- spore
  - Heat- Mordant
  - Water- decolorizer
  - Safranin Red- bacteria/organism



MCQs

Q. In Gram staining, the mordant is?
A. Tannic acid
B. Loeffler's mordant
C. Lugol's iodine
D. Bovine's fixative

Q. After the primary strain and mordant has been added but before the decolourizing agent has been used, gram-positive organisms are stained _____, and gram-negative organisms are stained _____.

A. Purple, Purple
B. Purple, colourless
C. Purple, pink
D. Pink, pink

Q. Metachromatic granules stained by all except?

A. Albert stain
B. Neisser
C. Ponder
D. Kinyoun

Q. Silver Impregnation method of staining is used to demonstrate?

A. Mycobacteria
B. Spirochaetes
C. Both of the above
D. None of the above

Q. Which of the following is acid-fast with 20% H_2SO_4?

A. M. avium
B. M. Leprae
C. Actinomyces
D. Nocardia

Q. In the ZN staining procedure, the secondary stain is?

A. Crystal violet
B. Safranin
C. Methylene blue
D. Alcohol

Q. Correct order of gram staining?

A. Carbol fuchsin-iodine-Acetone-methyl violet
B. Crystal violet-iodine-Acetone-Safranin
C. Methyl violet-Acetone-iodine-Safranin
D. Crystal violet-Carbol fuchsin-Acetone-iodine

2 MICROSCOPES

Features of Microscopes

00:00:26

- **Magnification:** Uses lens
- **Resolution:** The ability to differentiate two points as separate
  - Human eye: 0.2 mm
  - Light microscope: 0.2 micron
  - Electron microscope: 0.2-0.5 nm
- **Contrast**: Dyes

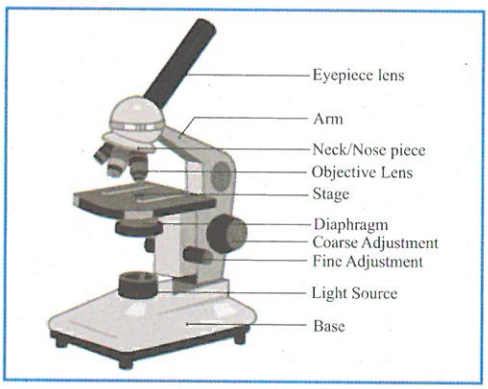
Light Microscope

00:03:06

Components of Microscope

PYQ: AIIMS 2018

- Source of Light: Transmitted light.
- Slides are kept on Stage
  - Light should go only to the slide through condenser (with iris/diaphragm), which regulates the light.
- The condenser is placed below the stage.
- Two knobs:
  - Big knob is for coarse adjustment
  - Small knob is for fine adjustment
- Two lenses
  - Eye Piece lens: 10x
  - Objective lens
    - → Scanner: 4x
    - → Low Power: 10x
    - → High Power: 40x
    - → Oil Immersion: 100x
- Lenses revolve around the neck piece

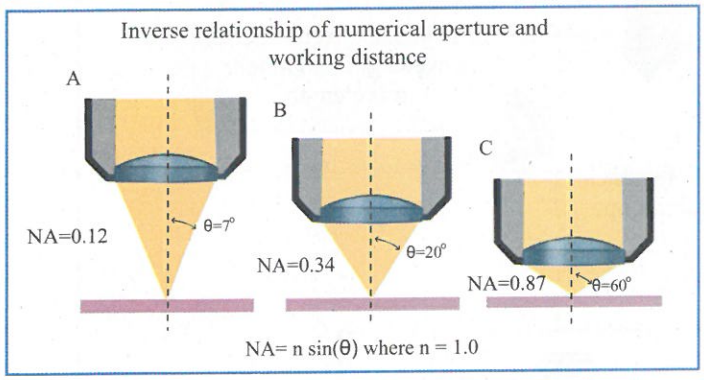


Lens	Magnification	Eyepiece	Total Magnification	Numerical Apertures
Scanner	4x	10x	40	0.12
Low Power	10x	10x	100	0.22
High Power	40x	10x	400	0.65
Oil Immersion	100x	10x	1000	1.25

Important Information

- Maximum magnification offered by light microscope is 1000x.
- As the lenses increase, numerical apertures also increase.

Numerical Aperture:

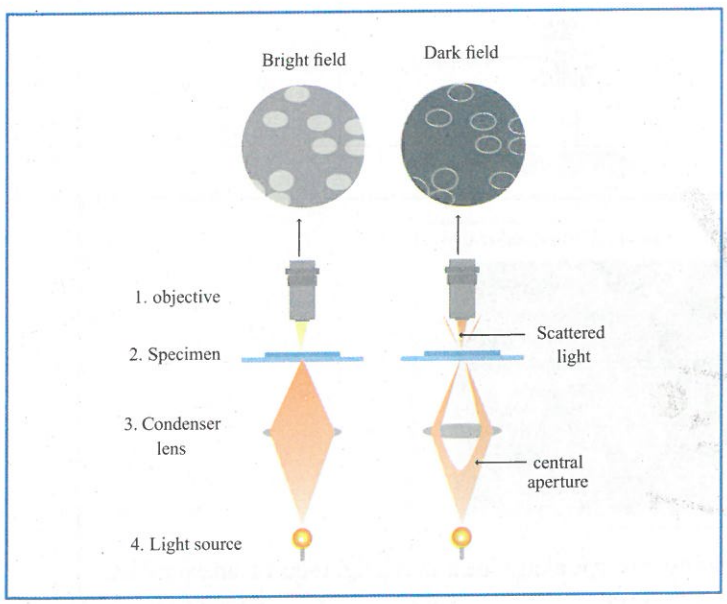


- Numerical aperture is the angle by which light falls on the slide and gets reflected.
- NA = n (sin θ)
  - Where, n is the refractive index, θ is half of the angle between the object and lens.

Dark Field Microscope

00:15:30
PYQ: FMGE 2021
PYQ: NEET PG 2021

- Reflected Light which illuminates the objects.
- Used for Thin structure.
  - Flagella
  - Spirochetes - spiral structures

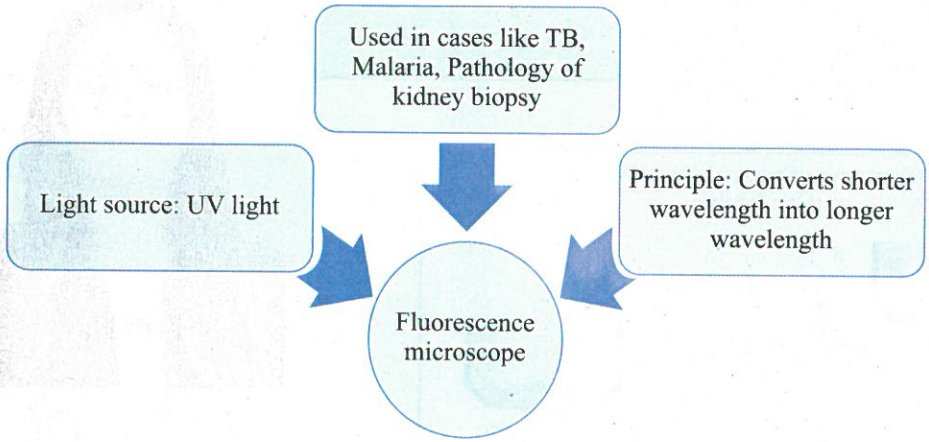


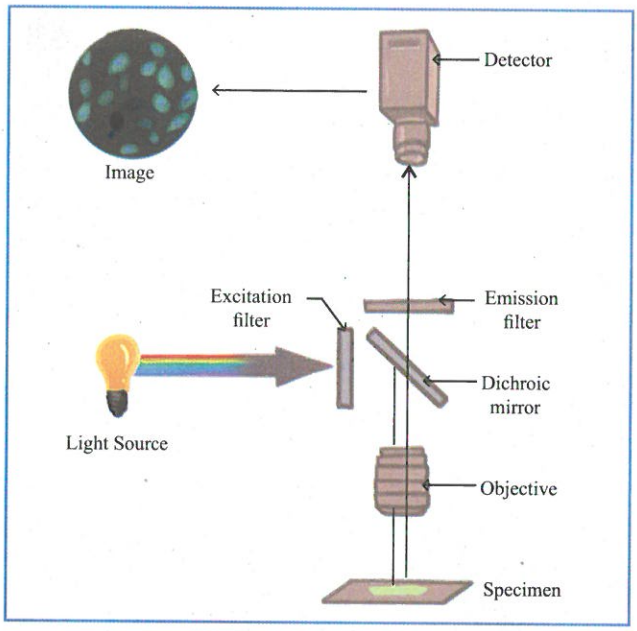
Interference Contrast Microscope

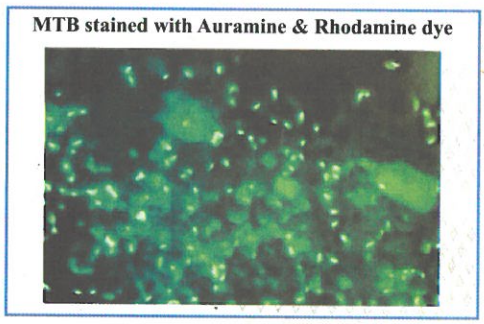
- Reveals cell organelles
- Measurements of chemical constituents of cells, such as
  - Lipids
  - Proteins
  - Nucleic acids

Fluorescence Microscope

00:18:51







- A dichroic mirror is used in a fluorescence microscope alongside a strong light source, an excitation filter, and an emission filter.

Autofluorescence

- Fluorescent without dye (Specimen which has their own shine, kept under UV lights)
- Spora Brothers
  - Cyclospora
  - Isospora

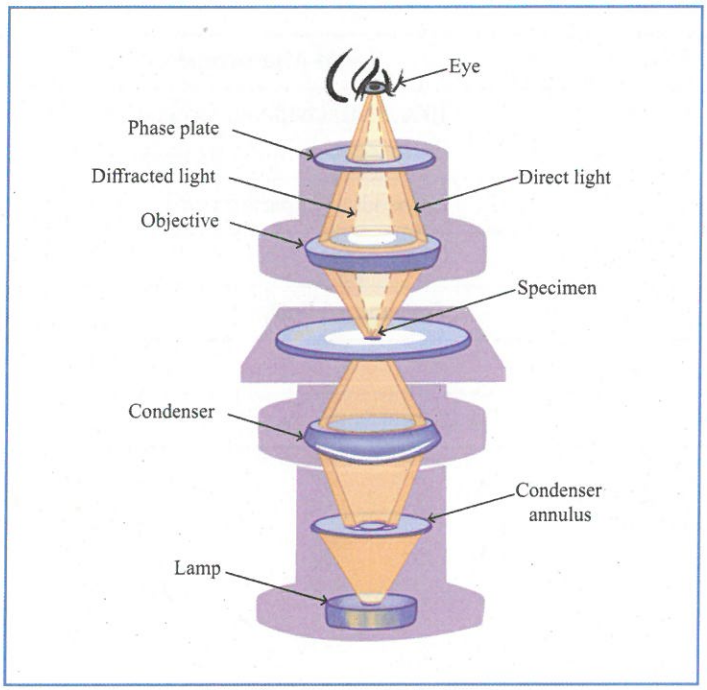
- Formalin
  - Skin biopsy for immunofluorescence in NS not formalin
- NADPH - Used in fluorescent spot test in G6PD deficiency
- Wood lamp: Ultraviolet light

Phase Contrast Microscope

00:29:38

PYQ: AIIMS 2022

- Added at the bottom: Annular diaphragm (below the condenser)
- Added at the top: Annular phase plate
- Differences in refractive indices, it will be shown in different phases
  - Converts into a change in the amplitude of light



Electron Microscope

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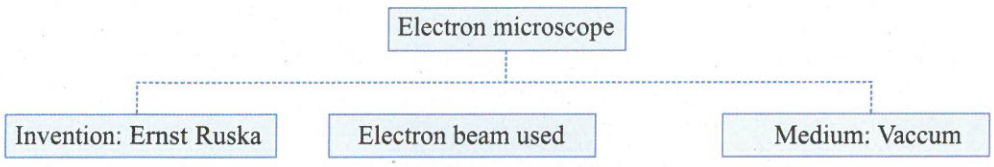
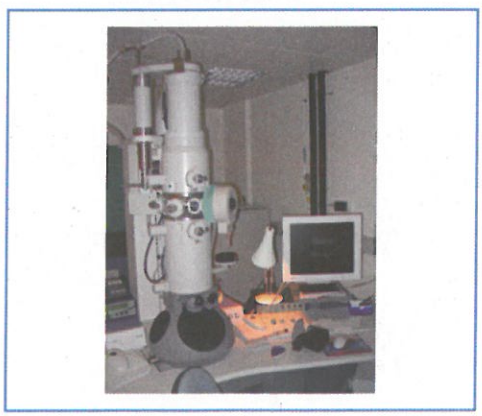




Types of Electron Microscope

Scanning Electron Microscope (SEM)	Features	Transmission Electron Microscope (TEM)
Scattered Electrons	Principle	Transmitted electrons
3D	Dimensional	2D
More sample viewed in lesser time	View and Time	Less sample viewed in the same time
Surface details	Details	Internal Details

Differences between Electron and Light Microscope

Electron Microscope	Features	Light Microscope
2-2.5% Glutaraldehyde	Fixation	10% neutral buffered formalin (NBF)- causes watering of eyes
Resin	Embedding	Embedding in paraffin wax
Copper metal slides	Slide	Glass slides
Electron	Source	Transmitted light
Vacuum	Medium	Air
0.5 nm	Resolution	0.2 micron

MCQs

Q. Scanning Electron Microscope is used to reveal what?

A. Surface structure
B. Internal structure
C. Both of the above
D. None of the above

Q. Maximum magnification strength attained by a light microscope is?

A. 10x
B. 40x
C. 100x
D. 1000x

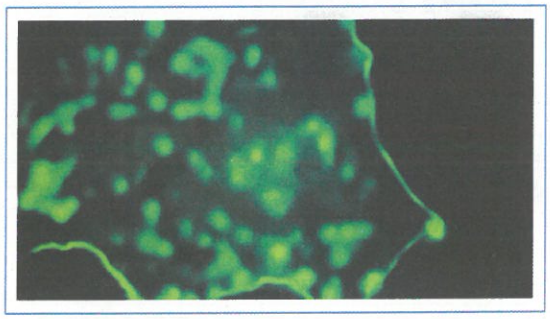
Q. In a light microscope, what function does a condenser serve?

A. Increase light intensity
B. Focus the light on sample
C. Focus the light on the eye
D. Reduced the glare

Q. A microscope that exposes specimens to UV LIGHT and forms an image with the emitted light at a different wavelength is called a _____ microscope?

A. Phase-contrast
B. Dark-field
C. Scanning Electron
D. Fluorescent

Q. Which of the following structures are required in the microscope for taking this type of image?



A. Dark field condenser
B. Phase plate
C. Dichroic mirror
D. Cathode ray tube

Q. A microbiologist intern wanted to study cells and microorganisms. His senior advised him to use a light microscope. What is the arrangement from eye to light source in a light microscope?
A. Objective lens ----- condenser ----- eyepiece lens
B. Condenser lens ----- objective lens ----- eyepiece lens
C. Eyepiece lens ----- objective lens ----- Condenser
D. None of the above

3 BACTERIAL ANATOMY

Capsule Layer

00:00:26

Capsule layer

Tough and demarcated
Prevents phagocytosis by preventing opsonization

Slime Layer

Slime layer

Loose and undemarcated

Capsule and Slime Layer

Bacteria has either slime or capsule layer

Exception: Only bacteria have both layers (Streptococcus salivarius)

Capsulated Organisms	
Mnemonic: Pretty NIce CApsule: • Streptococcus Pneumonia • Klebsiella Pneumoniae • Bordetella Pertussis • Vibrio Parahaemolyticus • Clostridium Perfringens • Yersinia Pestis -F1 peptide • Neisseria meningococcus (Lens-shaped) • Haemophilus Influenzae • Cryptococcus • Staphylococcus Aureus • Bacillus Anthracis	All capsules are made of polysaccharides **Except:** ○ Yersinia pestis - F1 peptide ○ Bacillus anthracis - polypeptide • S. aureus has microcapsule • S. pyogenes sometimes have capsule - made of hyaluronic acid

Demonstration of capsule

PYQ: FMGE 2019

McFadyen's reaction

Eg: Bacillus anthracis
Add Polychrome methylene blue stain
Turns capsule into Purple color

aka neufeld reaction

Eg: Pneumococcus
Bacteria capsule (Ag) + Antisera (Ab) = Swelling of capsule
Antigen antibody reactions occurs