MICROSCOPY & STAINING.

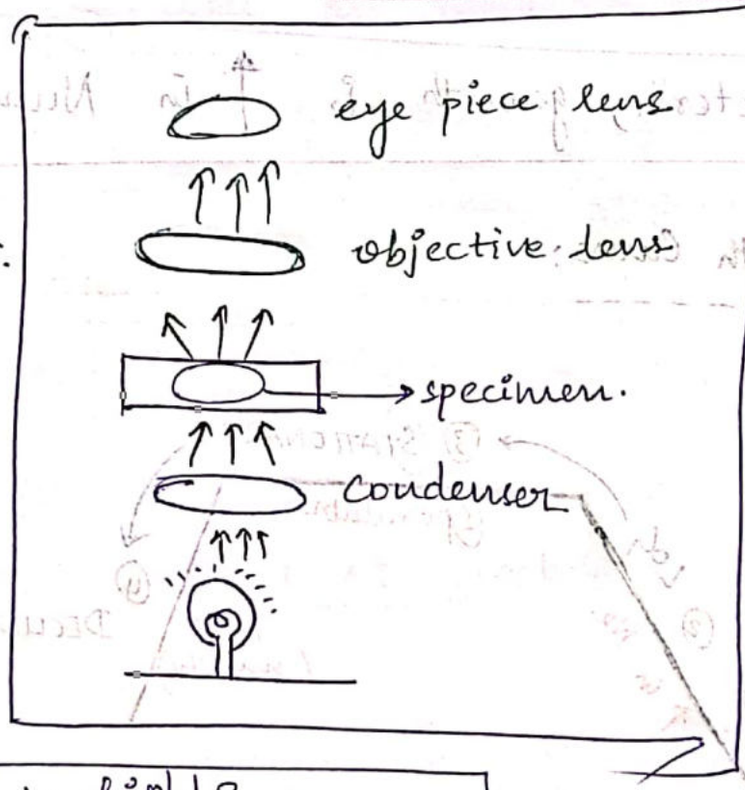
⑤ Types of Microscopes:

(i) Bright Field Microscope: (aka light micros./compd. micros)

Source: Normal light source (Tungsten/LED bulb).

Staining: (A)niline ((B)asic) dyes.

Principle:



Why Bright field?

field → Bright

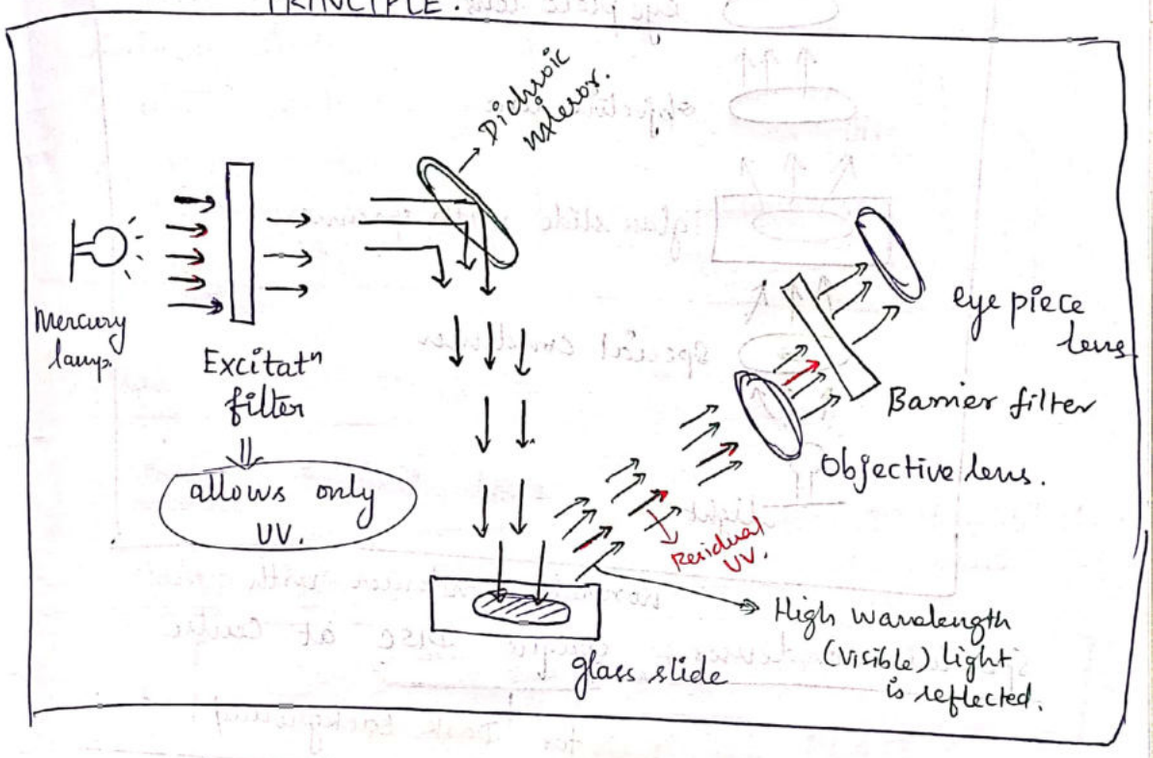
Bacteria → dark

(ii) Fluorescent microscope:

Source: UV light (Mercury ~~bulb~~ lamp).
(short wavelength).

Staining: fluoroscent (acridine/acidic) dye.

PRINCIPLE:



Mercury lamp → produces 'UV' + other waves.

Excitat^n filters: → filters ~~UV~~ shorter wavelength light except UV.

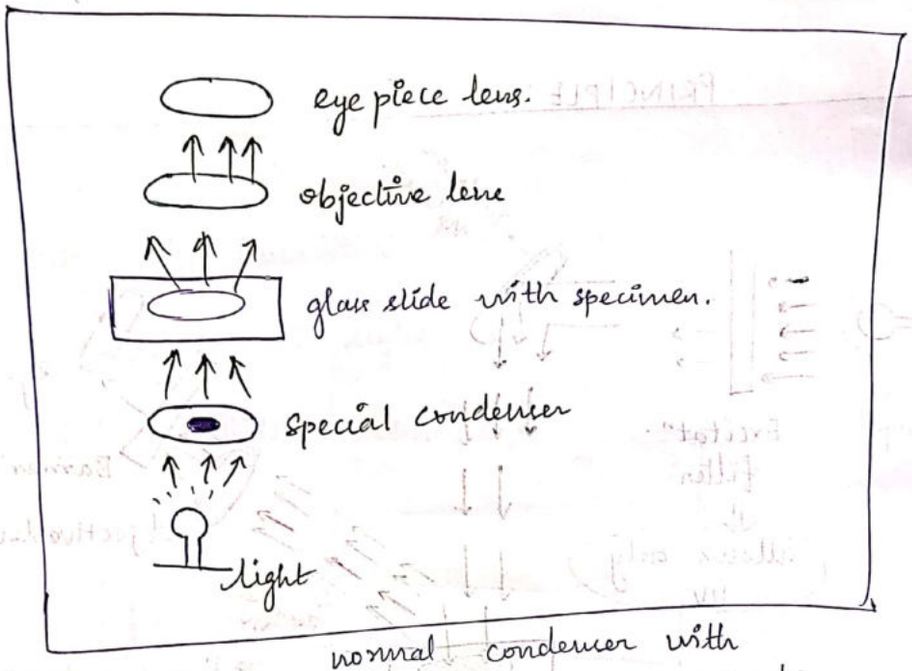
Barrier filter: → filters Residual UV reflected which can damage our eyes.

Bright field	Fluoroscent
(i) light — Normal	• Mercury lamps — UV.
(ii) No filter	② Filters.
(iii) Dye → Aniline/Basic	Acridine/Acidic/Fluoresc...

(iii) Dark field microscope:

Source: Normal light.

Staining: No.



normal condenser with

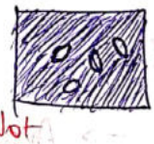
Special condenser: opaque DISC at centre
↓ for Dark Background

Bright field.	Dark field microscope.
Stain: Aniline/Basic.	— No —
Condenser: Normal	Special → Normal Condenser with opaque disc @ centre

Why? No stain in Dark field

⇓

Here organism will be seen as white in a dark background.



⇓

Similar to negative Staining "BUT" Not

In One stain, only the Capsule is Not stained, the org. is stained

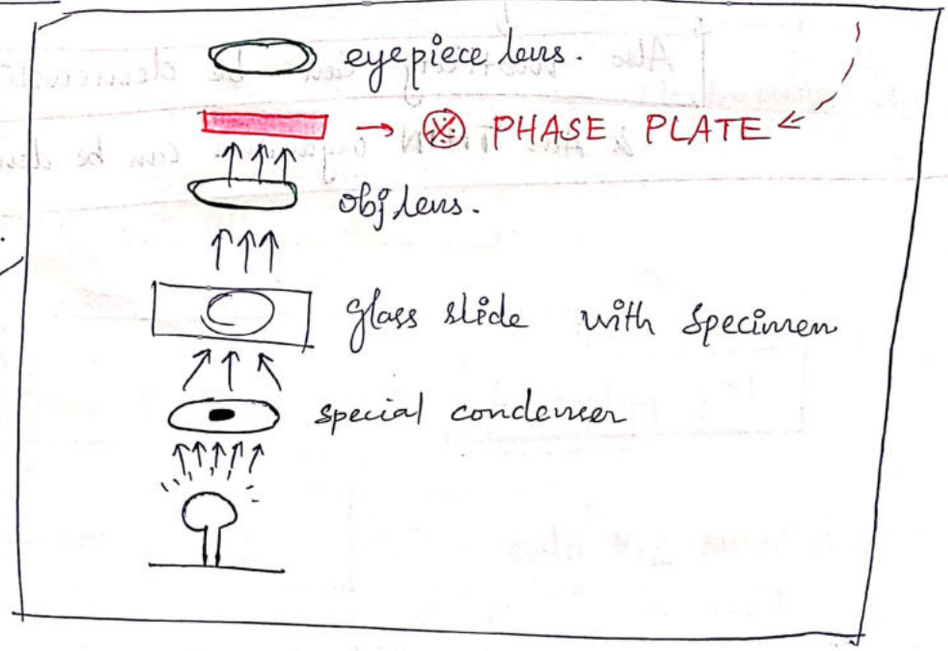
Negative staining	Dark field M.
organism is seen against a dark backgd.	"
Stain is used	— No Stain —
The surrounding environment is stained dark. Capsule – not stained Cytoplasm – Stained.	Special Condenser → opaque central disc. → environment is dark

iv) phase Contrast Microscope:

Source: Normal light
Stain: No

III law to Dark field M. except phase plate

Principle:



Concept of phase plate:

Difference in the Density & Refractive index of the org. & the surrounding media → is converted into → different intensities of light. Thats why environment is light & org is dark

Dark field	phase contrast
	
environment – dark, organism – light	env. – light org. – dark

ADVANTAGE OF DARK FIELD & PHASE CONTRAST

No stain is used in both

↓

so No organism is killed. (staining kills)

↓

live demonstⁿ possible.

↓

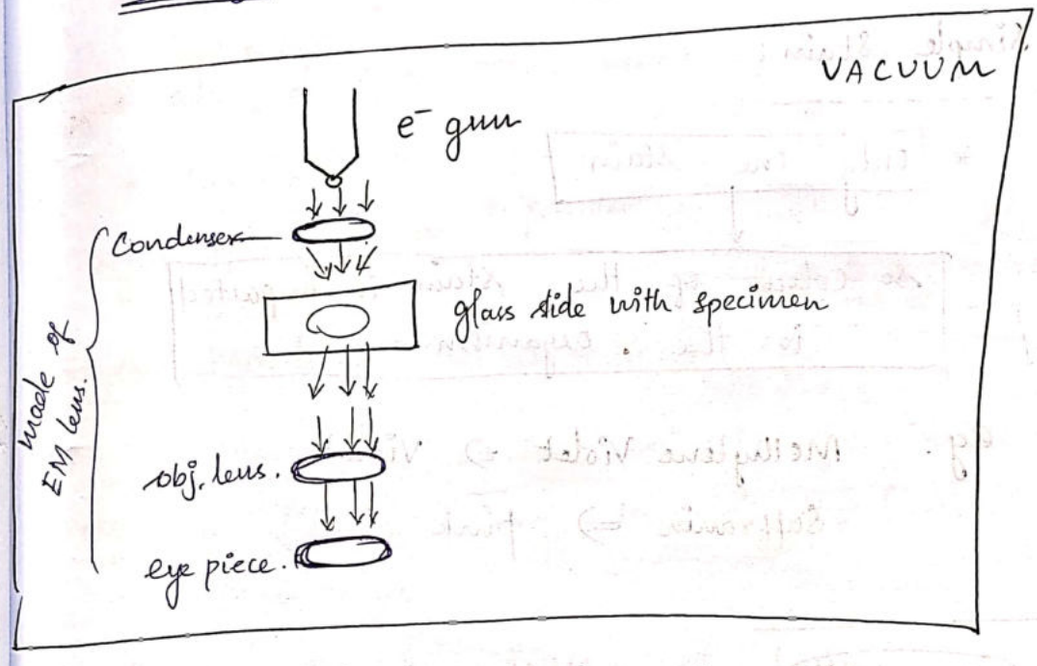
Also motility can be demonstrated

& Also THIN organisms can be demonstrated

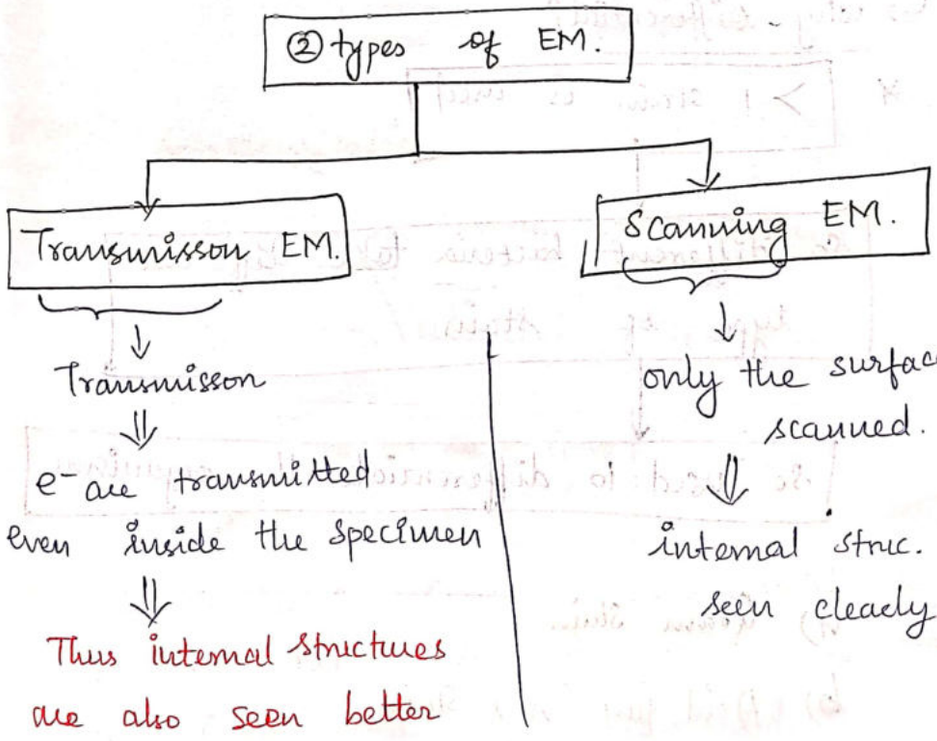
V) ELECTRON MICROSCOPE

Source: * Electrons (from electron gun).
* e^- scattered from org. gets recorded on film

Staining: HEAVY METAL STAINS. (Pb.).



* condenser & lens ⇒ made of Electromag. lens.



STAINING. methods:

Why? staining:

↑ contrast.

But kills the organisms.

③ imp. staining Methods:

1) Simple stain:

* only one stain
  ↓
  so colour of the stain is imparted to the organism

eg: Methylene Violet ⇒ violet
Saffranin ⇒ pink

2) Differential stain:

↳ why differential?

* > 1 stain is used
  ↓
  so different bacteria take different type of stain
  ↓
  so used to differentiate the organisms.

Eg: a) Gram Stain
b) Acid fast (AF) Stain.

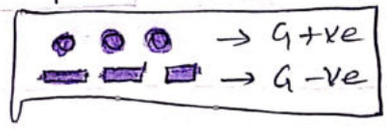
a) Gram stain:

* introd- by Hans Christian Gram.

④ steps : Pre-requisites: In a glass slide, a smear is prepared

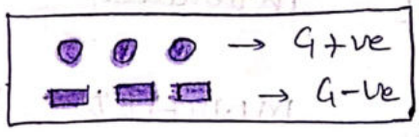
Step ①: 1° (primary) staining

* m/c used 1° stain → Methyl Violet (or) Gentian violet
* add & leave for a time period of 1min.
* Look at the Microscope, Both gram +ve & -ve org. appear Violet. (1111ar to simple staining).



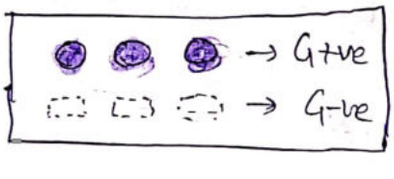
Step②: Mordant (Gram's iodine)

* Why mordant? 1° stain should get inside the org. & fill the org., this is the function of mordant.
* leave for a time of 1min.



Step③: Decolourisation

Decolourisation / acetone ⇒ 2-3 sec (leave for a Time)
\ alcohol ⇒ 10-30sec.

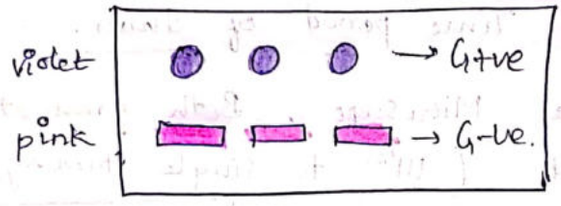


* After decolorizn, G+ve remain violet bcoz of their thick cell wall.
But G-ve org. are decolourized.

Step (4): Secondary (or) Counter staining (2°)

2° stain: → (i) saffranin (or)
→ (ii) Dil. carbol fuschin

* Add & leave for 1min



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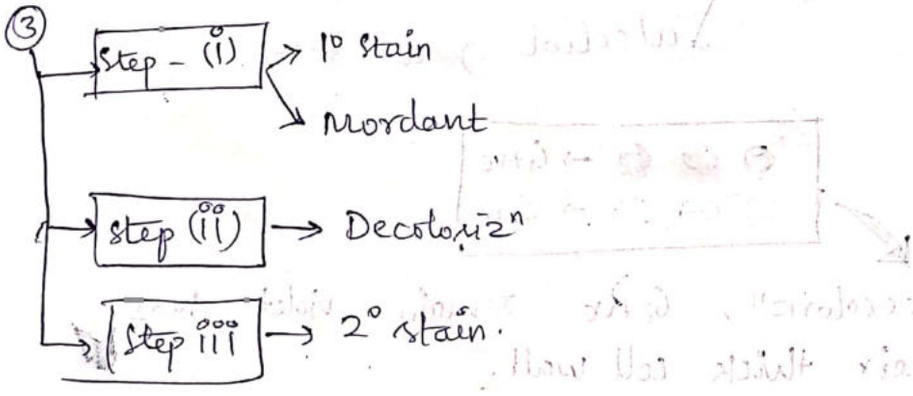
b) Acid fast staining:

* It is also a differential stain bcoz it helps to differentiate org. into Acid fast org. & Non acid fast org.

* 1st introduced by "Paul Ehrlich"

But MODIFIED by Zeihl Neelsen

* Similar to Gram staining but only ③ steps bcoz 1st ② steps are combined.



Prerequisties - Prepare by a specimen

Step (i) 1° staining.

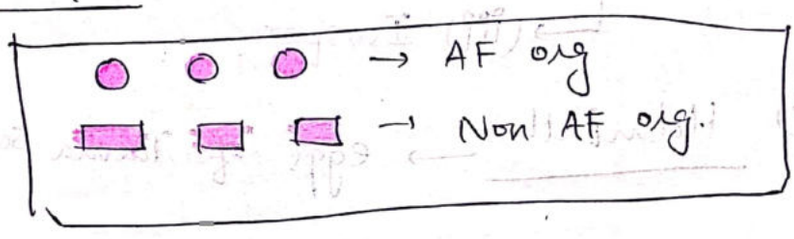
1° stain – Conc. Carbol fuschin.

* Leave for **5 mins**

*  → intermittent Heating
  ↓
  stop once fumes start appearing

  } acts as MORDANT
  ↓
  helps to fill the org. with 1° stain.

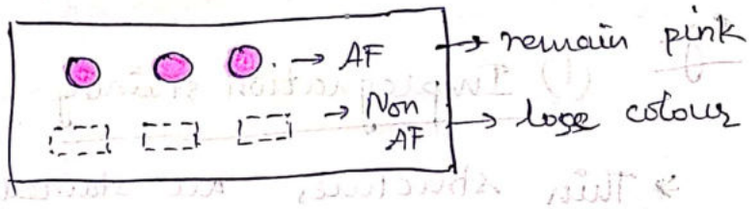
After step (i)



Step (ii) Decolourisat°

Decolorizer – H_2SO_4 for **1 min.**

After (ii)



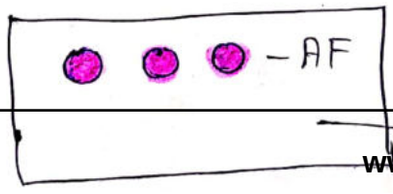
Step (iii) 2° staining

2° stain – Methylene blue for **1 min.**

↓

Stains the backgd. as "blue"

Why? Normally AF org. are light pink & blue backgd. makes the pink to stand out from



Examples of AF structures:

1) Spores.
2) Sperm's head.
3) Mycobacteria
4) Nocardia. (Bacteria).
5) Parasites (3)
   - (i) Cryptosporidium
   - (ii) Cyclospora
   - (iii) Isospora.
6) Helminth → eggs of Taenia saginata

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3) SPECIAL STAINING

* used to demonstrate a particular structure

eg: (i) Impregnation staining

* Thin structures are stained & are easily seen
* m/c used → silver impregnation stain

(i) Warthin starry stain → demonstrate → Helicobacter pylori.

(ii) Fontana stain → demonstrate → SPIROCHETES.

* Another impregnation method is

TANNIC acid impregn stain

↓

Leifson stain.

↓ demonstrate

FLAGELLA.

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ii) Albert staining

↓ demonstrate

Metachromatic granules

↓ m/c present in.

Corynebacterium Diphtheria.
Corynebacterium xerosis
Gardenella Vaginalis.
Spirillum.

⇓

(4) imp bacteria which contain metachrom. ~~stain~~ granules & stained by Albert stain.

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other than (i) Albert's stain;

AMP
Mnemonic

ii) Misser's stain

iii) Ponder's stain

} can also be used to demonstrate metachr. granules in bacteria

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(iii) Negative stain → demonstrate CAPSULE

eg: India Ink & Nigrosin (Burri)

* Negative staining
  - organism & background both are stained but not the capsule.
  - & so Capsule appears as clear Halo.

Summary