Microscopy and Staining

Introduction

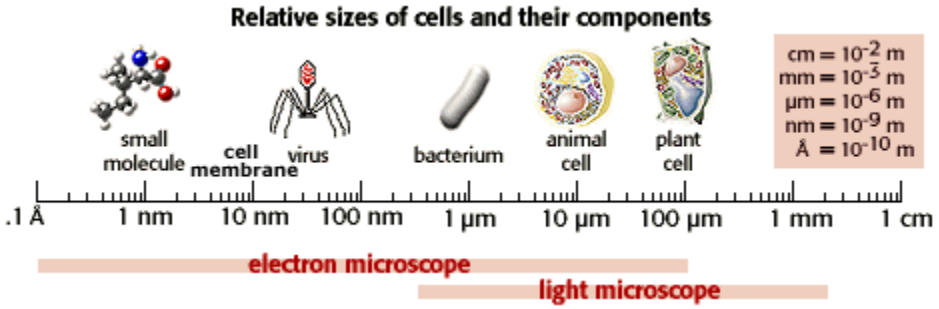
Development of Microscopy: Anton Van Leeuwenhoek (1632-1723)

"In the year of 1657 I discovered very small living creatures in rain water"

Principles of Microscopy

Metric Units:

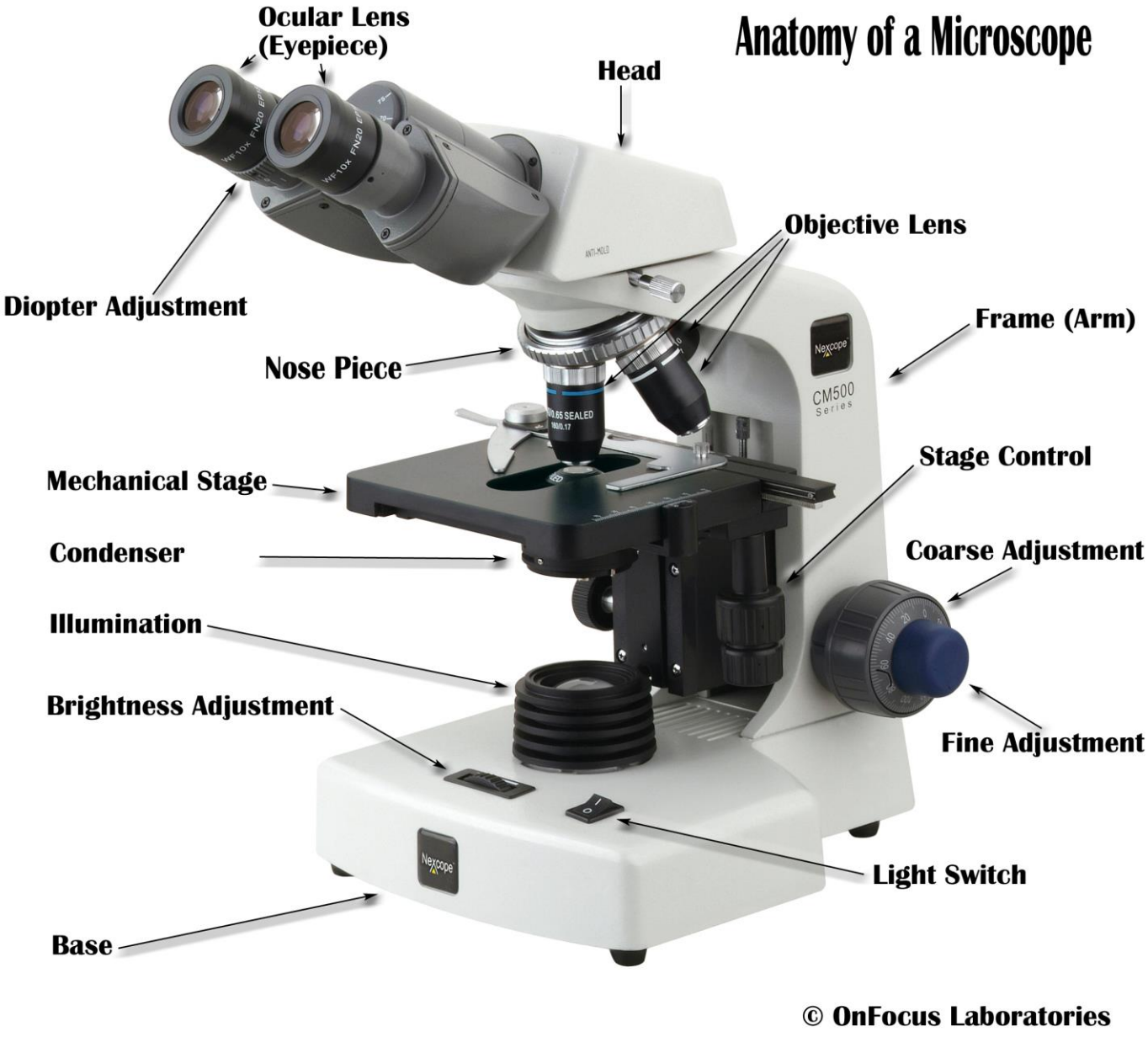
Micrometer (um) 1um = 10^{-6} m (or 0.000039 in)
Nanometer (nm) 1nm = 10^{-9} m (or 0.000000039 in)
Angstrom (A) 1 A = 10^{-10} m (or 0.0000000039 in)



Most prokaryotes (Bacteria) range from 0.5 – 2.0 um (1-10 um)
Viruses range from 10 nm – 100nm
Yeast cells: 10 um

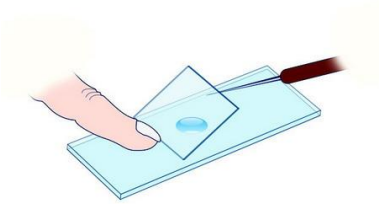
Light Microscopy

- The compound light microscope
- Parfocal Microscope
- Dark-Field Microscopy
- Phase-Contrast Microscopy
- Electron Microscopy

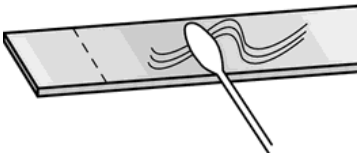


Techniques of Light Microscopy

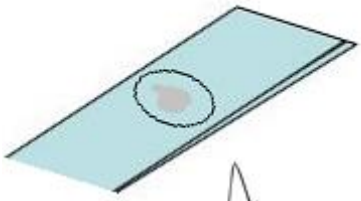
- Wet Mounts



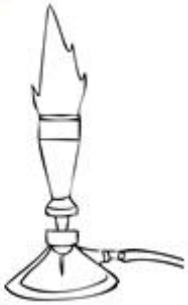
- Smears
- Heat fixing



- Simple Staining
- Differential Staining: Gram Stain



- Special Staining: i.e. Endospore staining

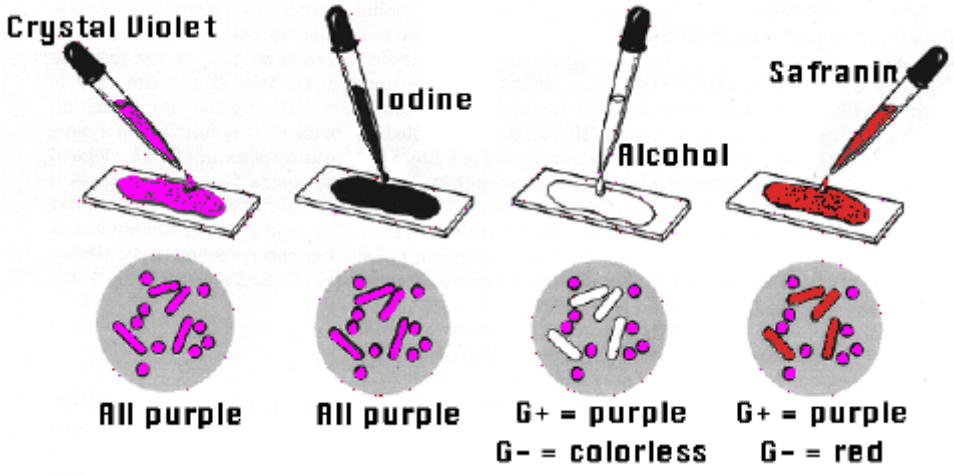


Differential Staining – **Gram Staining**

Developed by Hans Christian Gram in 1884

Gram Positive – Bacteria stain purple by crystal violet

Gram Negative – Bacteria stain red by safranin and do not retain crystal violet stain



Protocol

Materials Needed:

1.) Clean glass microscope slide
2.) Sterile inoculating loop
3.) Water dropper or pipette
4.) Beaker of water
5.) Bunsen burner
6.) Forceps
7.) Marker or wax pencil
8.) Culture of bacteria
9.) Gram stain solutions

Slide Preparation:

- Obtain a clean glass slide.
- Draw a circle on the center of the slide where you will place your bacterial culture.
- Place a droplet of water into the circle.
- Remove one colony of bacterial culture using a sterile inoculating loop and smear the bacteria into the water droplet.
- Holding the end of the slide with forceps, heat fix the bacteria by passing the bottom of the slide through the Bunsen burner flame for 5 seconds.
- Let the slide cool before staining.

Gram Staining Procedure:

- Flood the smear with crystal violet (primary stain) until is completely covered. Leave it on for 30 seconds and then discard into the sink.

- Rinse the slide with water from a wash bottle of distilled water. Drain off excess water.

- Flood the slide with Gram's iodine solution (mordant) and let it stand for 30 seconds and wash with water.

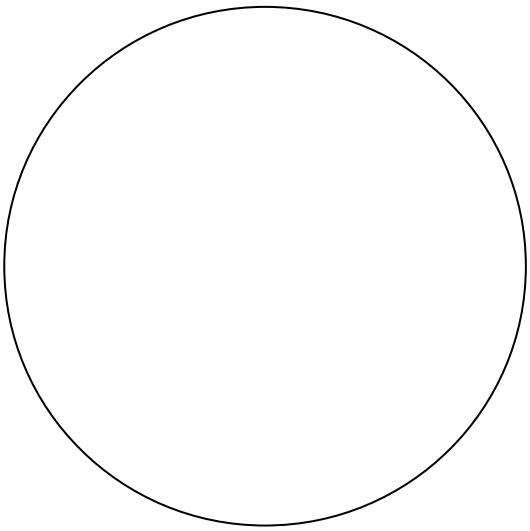
- Hold slide at 45^0 angle and slowly add 95% ethyl alcohol over for 15 seconds, until no more crystal violet runs off, (timing is critical in this step). Immediately wash with water for few seconds.

- Cover the smear with safranin (counter stain) for 30 seconds, and wash with water.

- Blot the slide carefully with bibulous paper, and air dry.

- Examine the slide using the phase contrast microscope using immersion oil.

- Describe the color and morphology of your stained bacteria:



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Yeast Study

If time permits,

Saccharomyces cerevisiae will be examined.

- Living culture: Place two or three loopfuls of *S. cerevisiae* on the slide with a drop of methylene blue stain. Place a cover slip and examine under high-dry or immersion oil.

- Look for the shape of the cells, nucleus, vacuoles, blastospore or bud.