

Laboratory Equipment and Procedures

2:1 The Light Microscope

MICROSCOPE: any tool with a lens to magnify and observe tiny details of specimens

Micro – tiny, small

Scope – to see

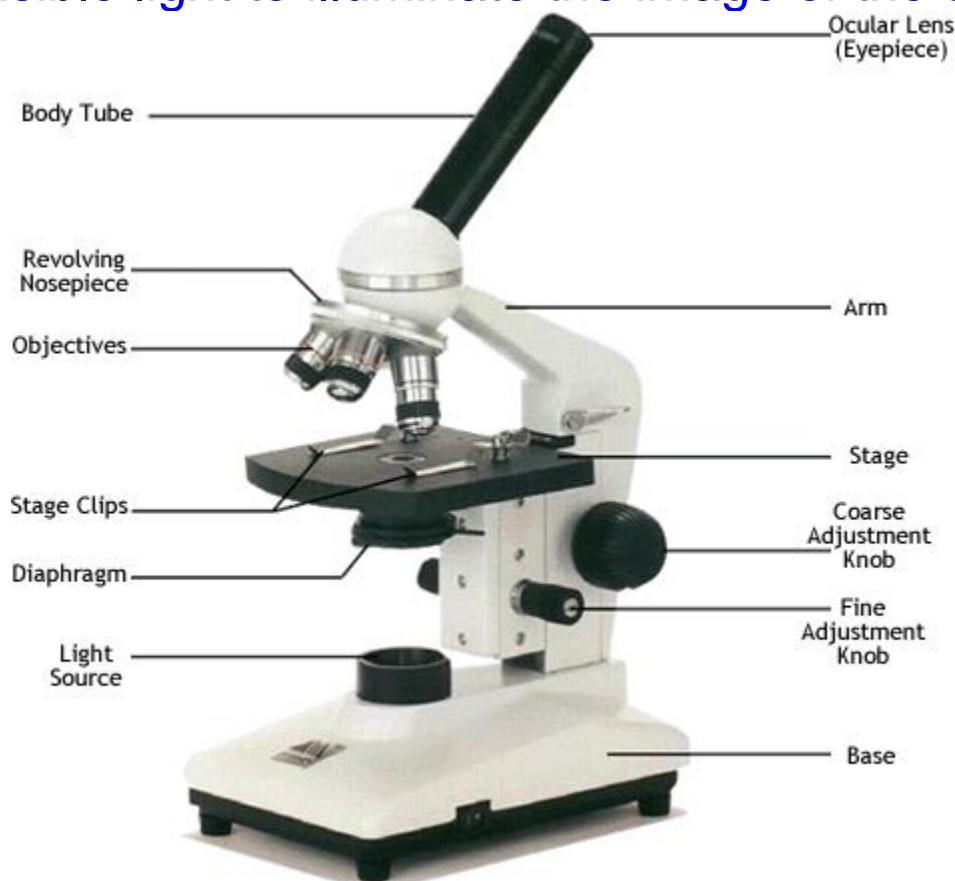


SIMPLE MICROSCOPE: a microscope with a single lens

e.g. magnifying glass

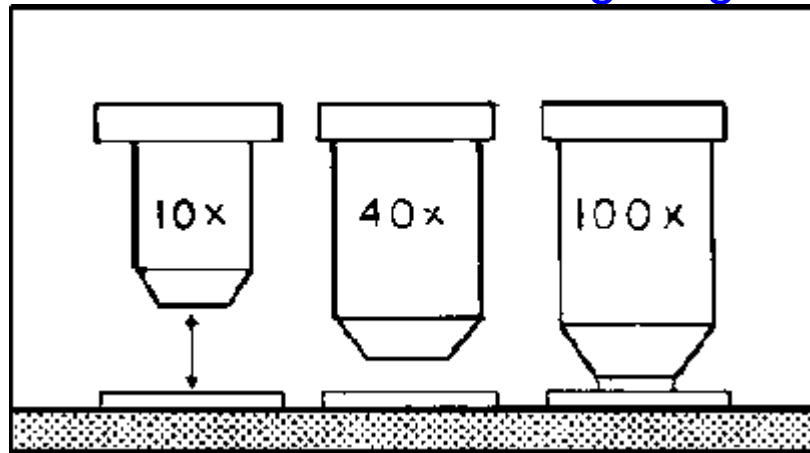
COMPOUND MICROSCOPE: a tool that magnifies using two lenses, one mounted on each end of a tube, magnification of one lens increases that of the other lens

LIGHT MICROSCOPE: a compound microscope that uses ordinary visible light to illuminate the image of the specimen



Parts of the Compound Microscope

1. EYEPIECE: (ocular) lens at the top of the tube, closest to your eye, usually magnifies 10X
2. BODY TUBE: cylinder with the lenses on either end, focuses light up to the eyepiece
3. OBJECTIVES: lenses at the bottom of the tube, closest to the object being magnified, most microscopes have several objective lenses with differing magnifications



4. NOSEPIECE: holds objectives and rotates to change magnifying power
5. ADJUSTMENTS: knobs which focus image by changing distance between objectives and specimen, COARSE moves larger distances than FINE
6. STAGE: platform on which specimen or slide is placed
7. STAGE CLIPS: hold specimen or slide in place
8. DIAPHRAGM: (iris) allows varying amount of light to enter the lenses
9. MIRROR or LAMP: directs light into the lenses
10. BASE and ARM: support the microscope

2:2 Magnification and Resolution

MAGNIFICATION: the amount a microscope enlarges an image

TOTAL MAGNIFICATION = EYEPIECE POWER X OBJECTIVE POWER

e.g. eyepiece power = 10x objective power = 4x

$$TM = EP \times OP$$

$$TM = 10x \times 4x$$

$$TM = 40x \quad \text{image is enlarged 40 times}$$



MAGNIFIED IMAGE: the enlarged image that reached the eye through the compound microscope

In a compound microscope the magnified image will appear upside down, backward, and larger.

RESOLUTION: (resolving power) the ability of a microscope to distinguish two objects as being separate, the capability to deliver a visible image with clear details

The resolving power of light microscopes is limited by the wavelength of light.

Limit of resolving power

$$= \frac{1}{2} \text{ wavelength of light}$$

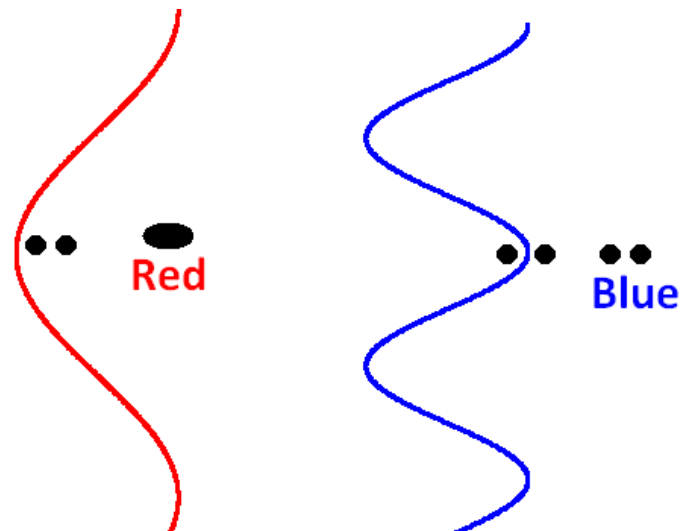
Wavelength of visible light

$$= 400\text{-}800 \text{ nm}$$

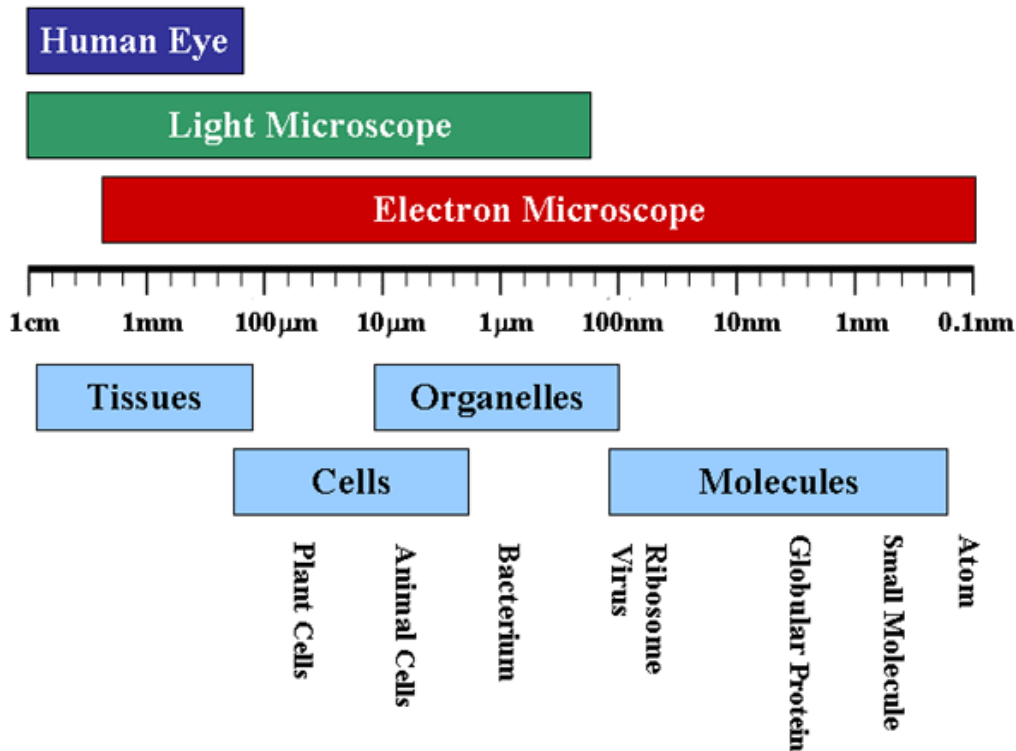
Resolving power light microscope

$$= 200 \text{ nm}$$

Diameter of bacteria=300-1000 nm



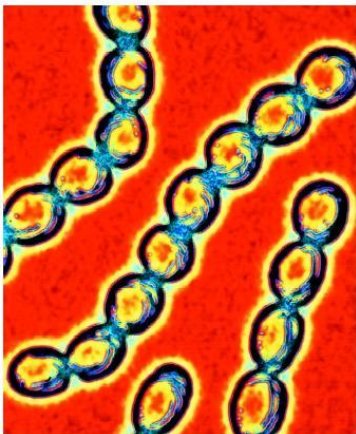
What structure do you want to resolve?



WORKING DISTANCE: space between the objective lens and the specimen when the specimen is in focus.

2:3 Other Types of Microscopes

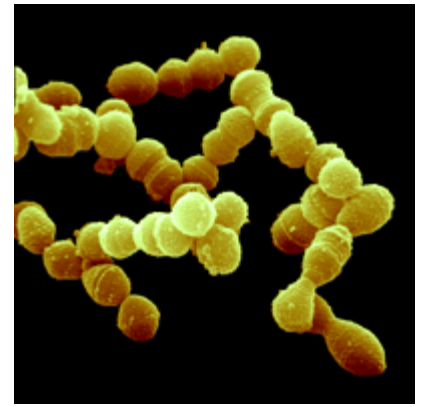
ELECTRON MICROSCOPE: microscope using a beam of electrons to illuminate specimen, yielding greater magnification and resolution



TRANSMISSION ELECTRON MICROSCOPE: stream of electrons pass through small structures, illuminating parts

SCANNING ELECTRON MICROSCOPE:

stream of electrons is reflected off surface of small structures, yielding a 3-dimensional image



Other Types of Microscopes

1. ULTRAVIOLET MICROSCOPE: greater resolving power because it uses UV light to illuminate the specimen

- Wavelength of UV = 200 – 300 nm
- Resolving power of UV = 100 – 150 nm

2. FLUORESCENCE MICROSCOPE: uses UV light and fluorescent dyes to stain specimens

- Fluorescent dyes absorb UV light and emit it as visible light
- Greater resolving power and results which are visible to humans



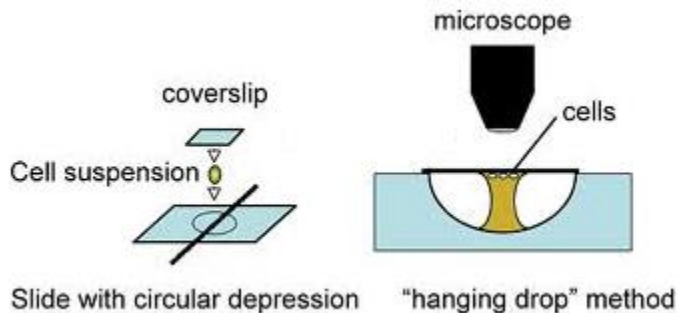
3. DARK FIELD MICROSCOPE: condenser reflects light away from the objective, light waves touching the specimen will be directed through the objective

- Specimen looks white against the dark field
- No specimen – field is black

4. PHASE CONTRAST MICROSCOPE: translates the phase difference (different speed of light through various cell structures) into a difference in the brightness of the light that can be seen

2:4 Techniques for Microscopic Study

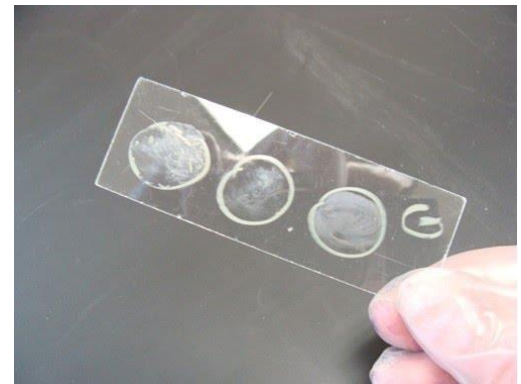
HANGING DROP PREPARATION: using a thick slide with a depressed center to suspend a drop of liquid live bacterial culture, used to view living bacteria



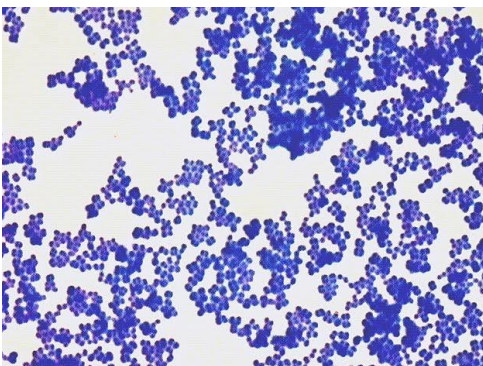
- Hanging drop preparation is the only way to view live bacteria; all other techniques kill the bacteria before viewing the specimen.

STAINING BACTERIA: coloring microorganisms with chemical dye to make them easier to see

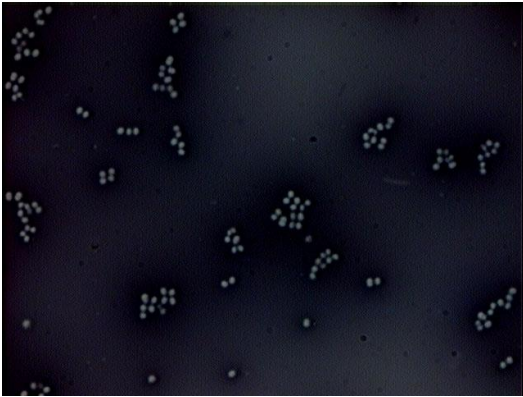
SMEAR: small amount of bacterial culture spread on a slide and heat fixed to the slide



- Bacteria have an overall negative charge due to large amount of nucleic acid in the small cell.
- Basic dyes (pH above 7) adhere to bacteria because their chromophore (colored ion) has a positive charge.
- Acidic dyes (pH below 7) are repelled by bacteria because their chromophore has a negative charge.

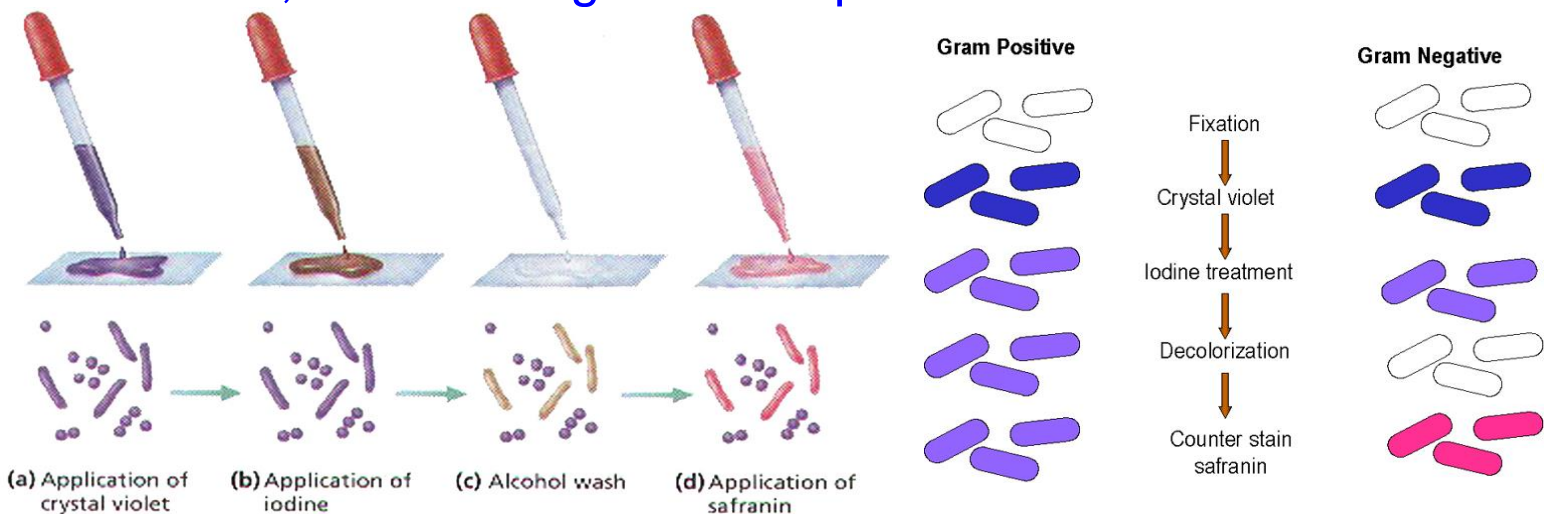


SIMPLE STAIN: using a basic dye to stain bacterial smear, bacteria will be colored against the white background



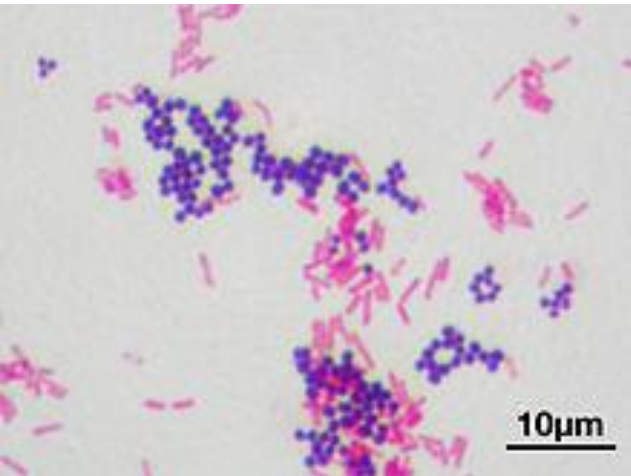
NEGATIVE STAIN: using an acidic dye to stain the background of a bacterial smear while the bacteria remain colorless, used to observe the overall shape of very small cells

GRAM STAIN: differential staining procedure used to classify unknown cultures of bacteria based on their cell wall structure, used to diagnose and provide disease treatment



- **GRAM POSITIVE**: organisms with thick cell walls, appear purple after Gram staining procedure, and are easily killed with penicillin
- **GRAM NEGATIVE**: organisms with thin, lipid filled cell walls, appear pink after Gram staining procedure, and are easily killed with streptomycin

ACID-FAST STAIN: differential stain used to stain and diagnose disease producing microbes of the genus *Mycobacterium*, including tuberculosis and leprosy



2:5 Growth and Preparation of a Pure Culture

PURE CULTURE: culture containing only one species of microorganism



MEDIUM: nutrient substance used to grow microorganisms

COLONY: visible growth of bacteria on medium, all the progeny of a single preexisting bacterium

INOCULATION: the implantation of microbes or infectious material onto culture media

AGAR: gelatinous substance prepared from seaweed, used as a base for solid culture media

STREAK PLATE: Petri plate containing solid culture media on which microbial specimen is spread and grows



2:6 Culture Media and Sterilization

INFUSION MEDIA (aka nutrient broth or nutrient agar): commonly used bacterial media, made from a meat broth base

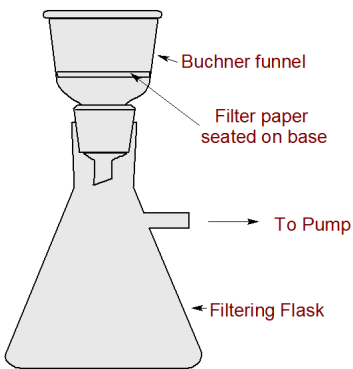
- Agar may be added to nutrient broth to produce solid nutrient agar

Other types of media (carbohydrate, selective, differential, enrichment) may be used to identify unknown cultures, diagnose diseases, and inhibit growth of certain species.

Media and tools used in bacterial procedure must be sterile (free from bacteria) to insure that pure cultures are not contaminated.

Sterilization Methods

1. AUTOClave: steam pressure sterilizer that raises the boiling point of water to a temperature at which all forms of life are killed



2. FILTRATION: passing a liquid through a filter with pores (holes) so small that bacteria can not pass through

3. HOT AIR STERILIZATION: sterilizing material in a hot air oven

2:7 Microbial Cultivation Requirements

Conditions Needed for Microbial Growth

1. Food: any substance that provides nutrition to microbes
2. Water
3. Proper temperature: disease producing bacteria must live at or near normal human body temperature (37° C), other bacteria may live at various temperatures
4. Proper pH: most bacteria grow best at or near neutrality
5. Darkness: most bacteria grow best without the presence of light

Classifying Bacteria by Oxygen Requirement

1. STRICT AEROBES: require free oxygen to grow, at least the 20% found in air
2. STRICT/OBLIGATE ANAEROBES: will not grow or may be killed by the presence of oxygen
3. MICROAEROPHILIC: grow best in the presence of low levels of oxygen
4. FACULTATIVE AEROBE/ANAEROBE: grow in the absence of oxygen but do not normally do so
 - Aerobically oxidize carbohydrates into water and carbon dioxide
 - Anaerobically ferment carbohydrates into fermentation products (alcohol, lactic acid, etc.)
5. AEROTOLERANT: grow in the presence of oxygen but do not oxidize carbohydrates, ferment (anaerobic respiration) even in the presence of oxygen

