UNIT - I

Culturing and microscopy

Culturing Microbes

The Five "I's

Innoculation: Producing a pure culture

Isolation: Colony on media, one kind of microbe, pure culture

Incubation: growing microbes under proper conditions

Inspection: Observation of characteristics (data)

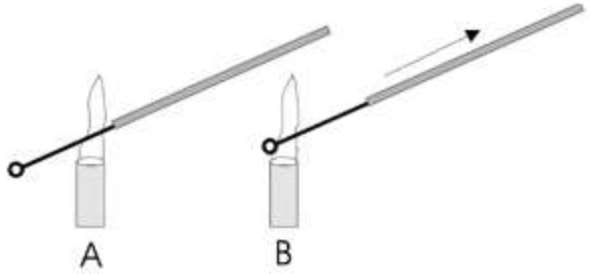
Identification: use of data, correaltion, to ID organism to exact species

Culturing Microbes

The Five "I's

Innoculation: Producing a pure culture

Introduce bacteria into a growth medium using "aseptic technique" to prevent contamination. Tools: Bunsen burner, loop. Needle, etc.




C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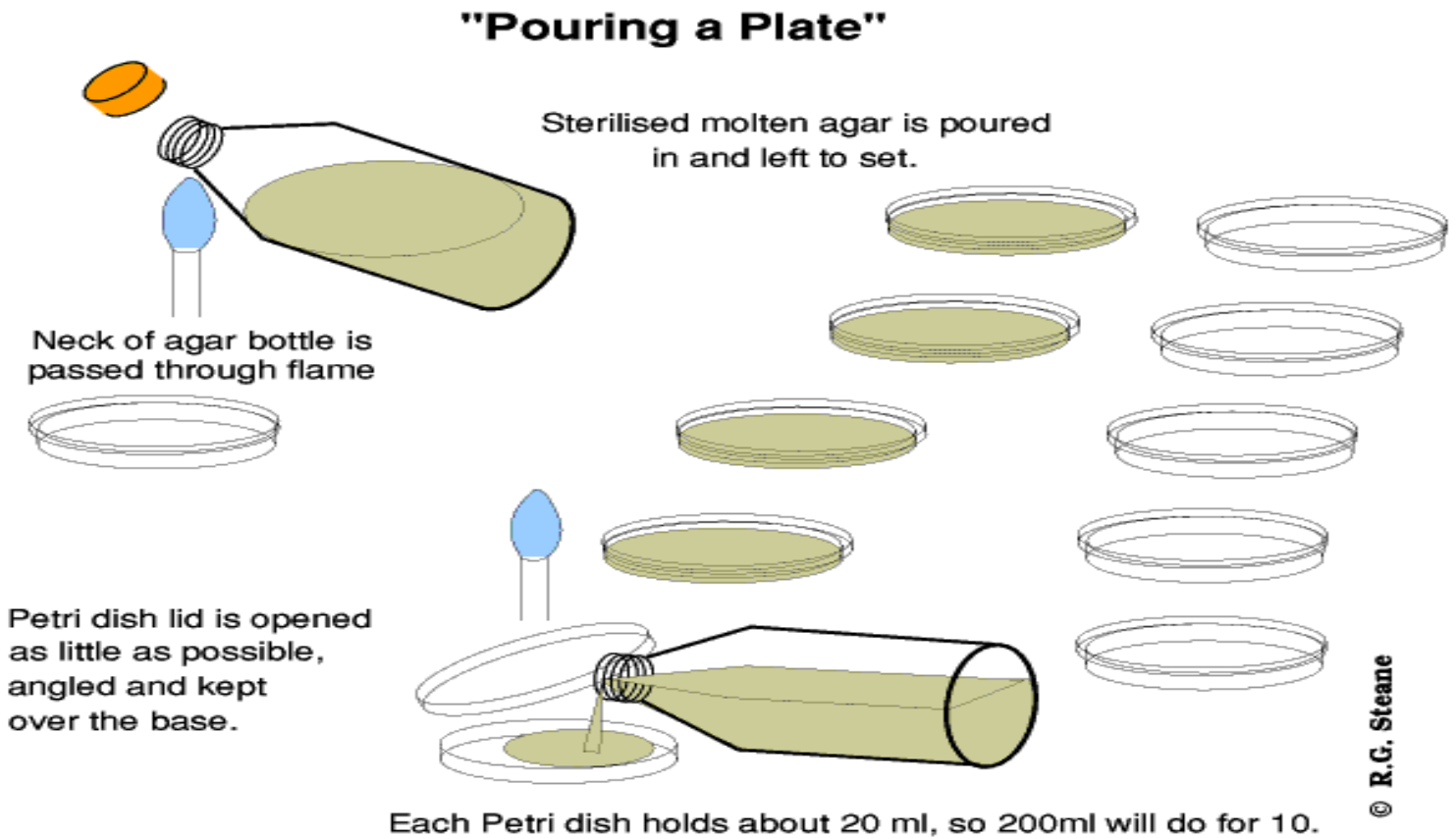
Innoculation: Producing a pure culture

Introduce bacteria into a growth medium using “aseptic technique” to prevent contamination. Tools: Bunsen burner, loop. Needle, etc.

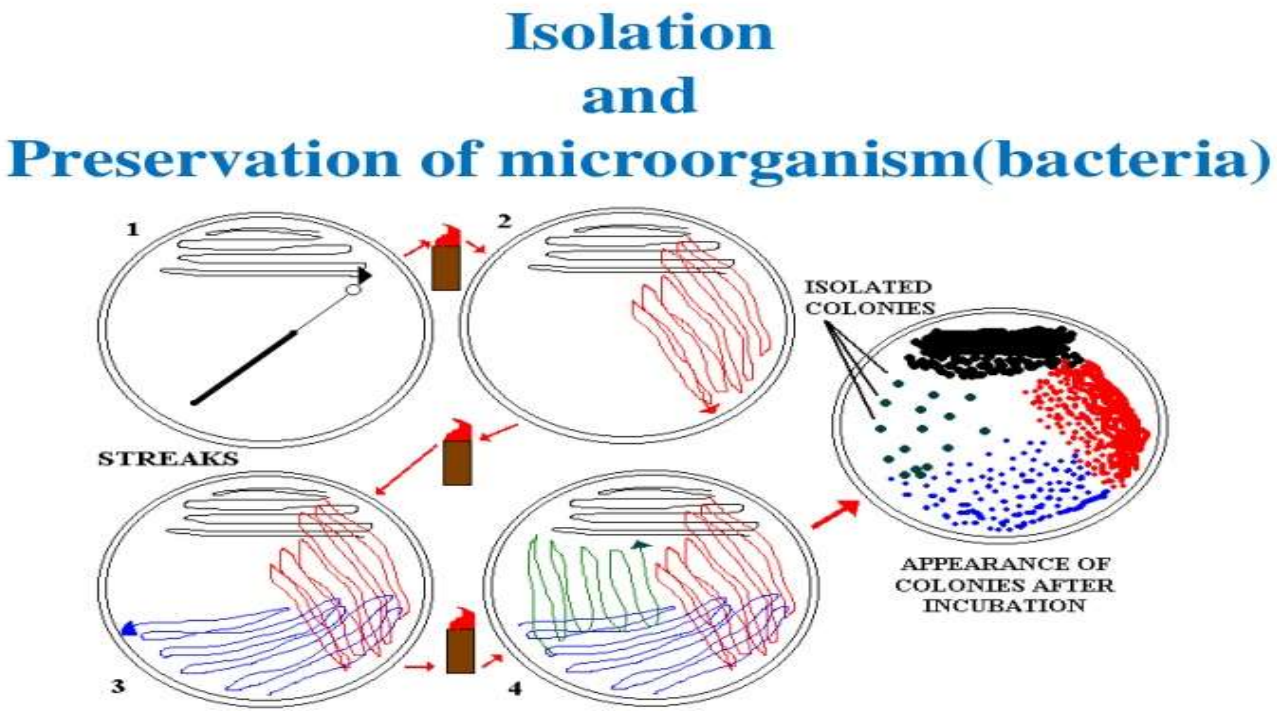


Innoculation

Isolation: Colony on media, one kind of microbe, pure culture: isolation on general and special “differential media”

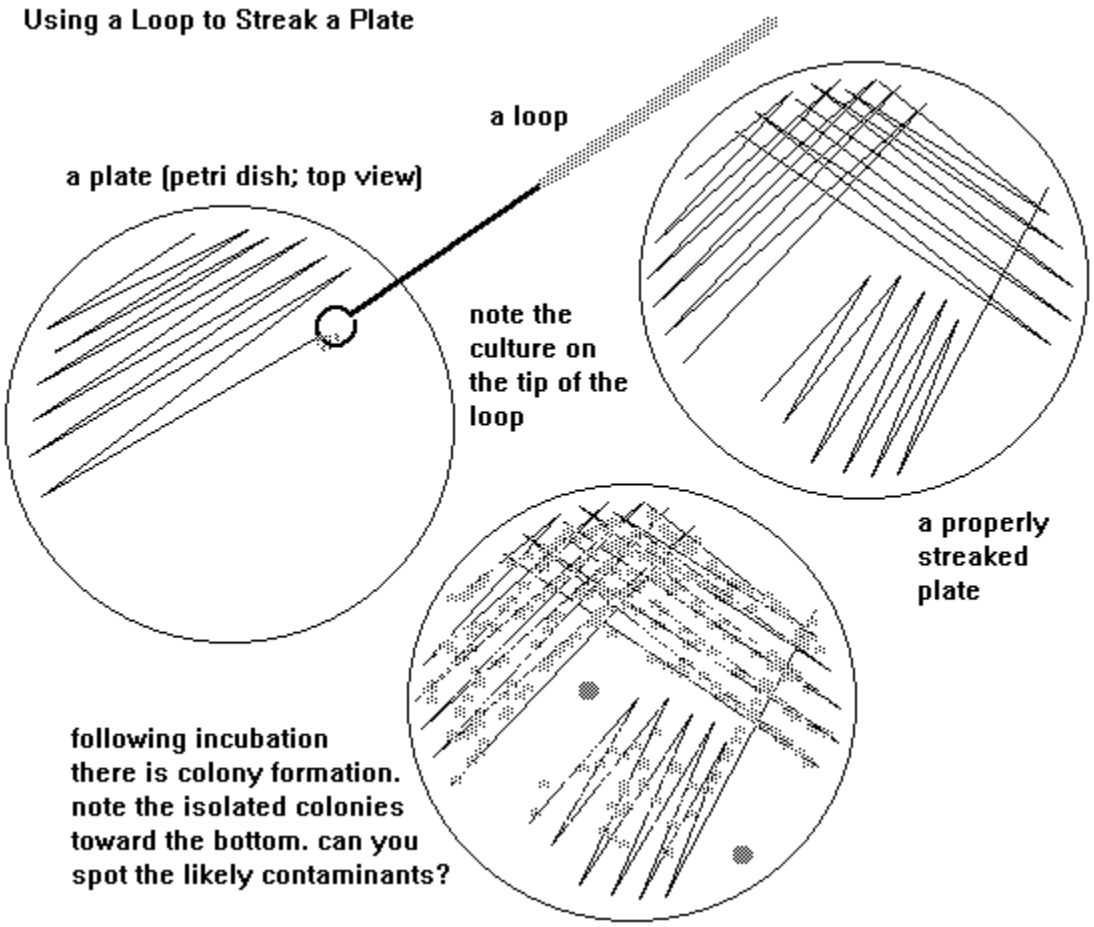
General growth media: NA, TSA/ Differential: Mac, EMB, SS

These have dyes, salts, inhibiting agents : see differences on plates.



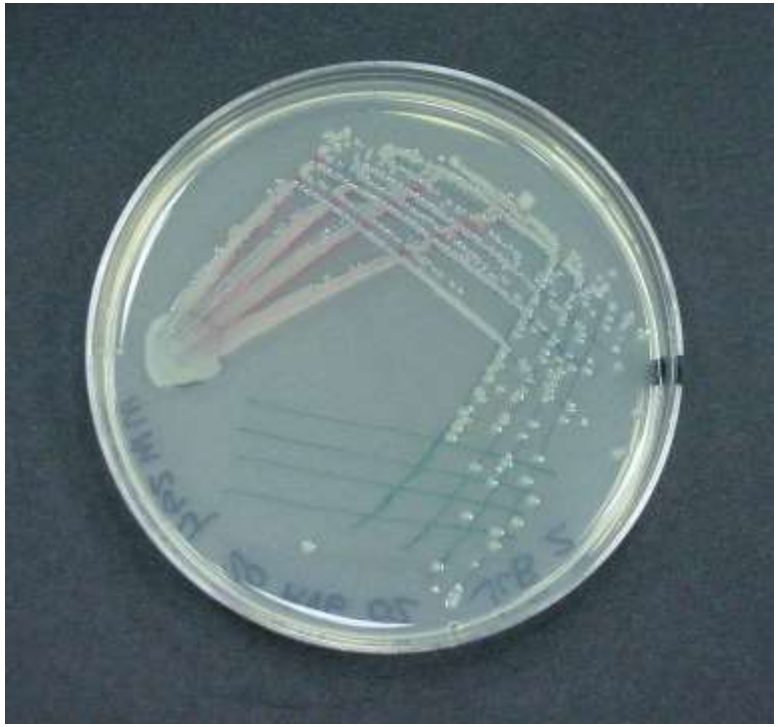
Isolation

Isolation: Colony on media, one kind of microbe, pure culture – Streak Plates



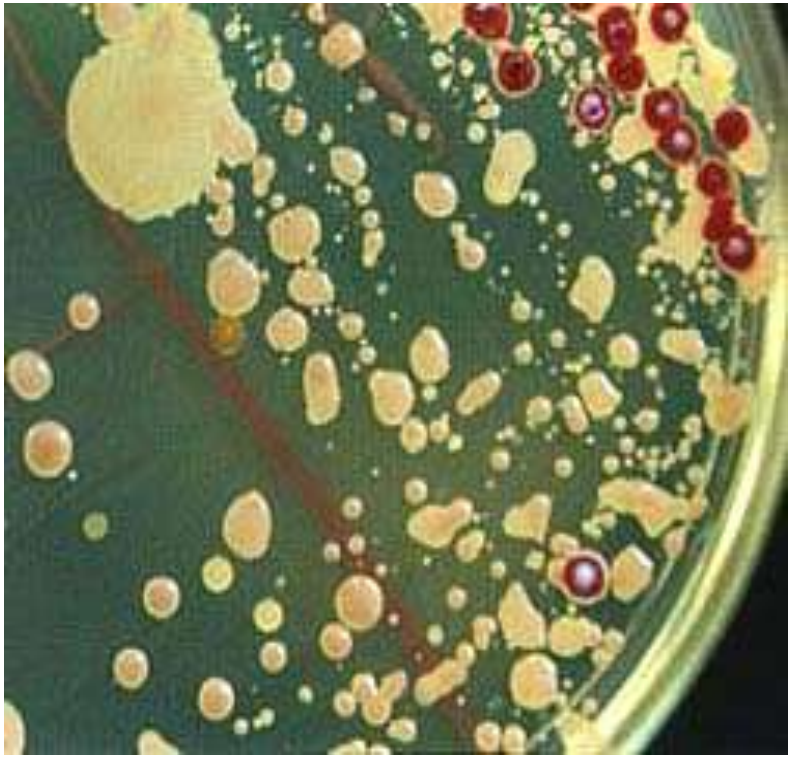
Microbiology

Isolation: Colony on media, one kind of microbe, pure culture



Isolation

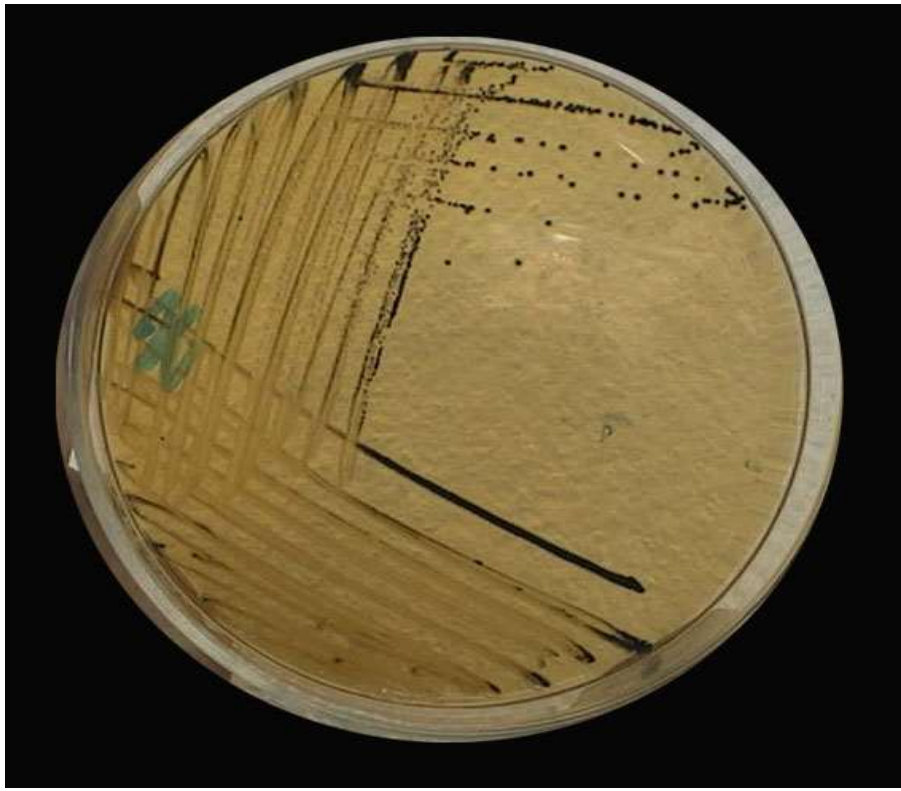
Isolation: Colony on media, one kind of microbe, pure culture. Many colonies? Use a needle, pick one, and redo streak plate



Culturing

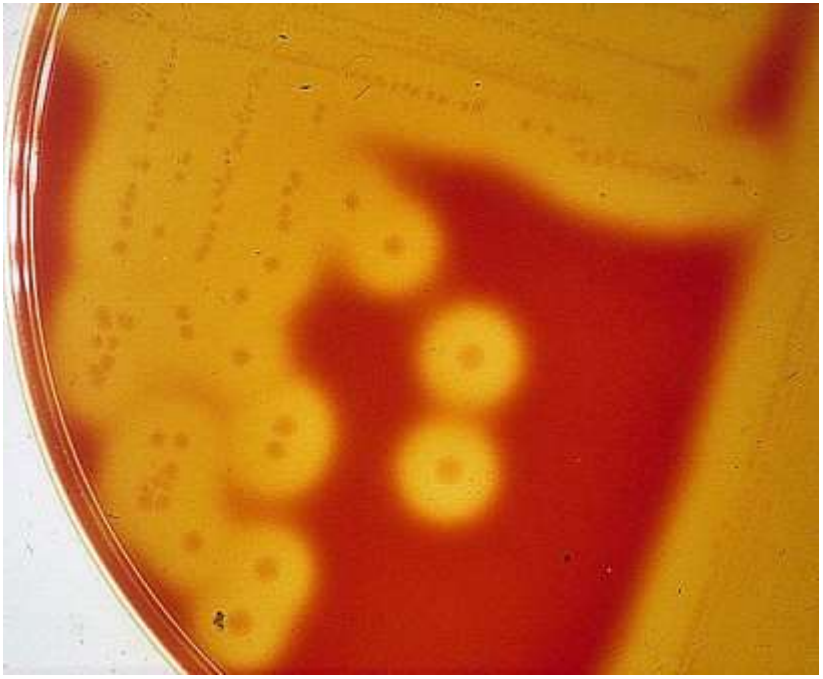
Differential: Mac, EMB, SS

These have dyes, salts, inhibiting agents : see differences on plates



culturing

- Blood agar : rich with nutrients, can see a difference, thus differential; much more later



Incubation

- Incubation: Allow organisms to grow under the optimal conditions
- Temperature, with or without oxygen etc

Incubation

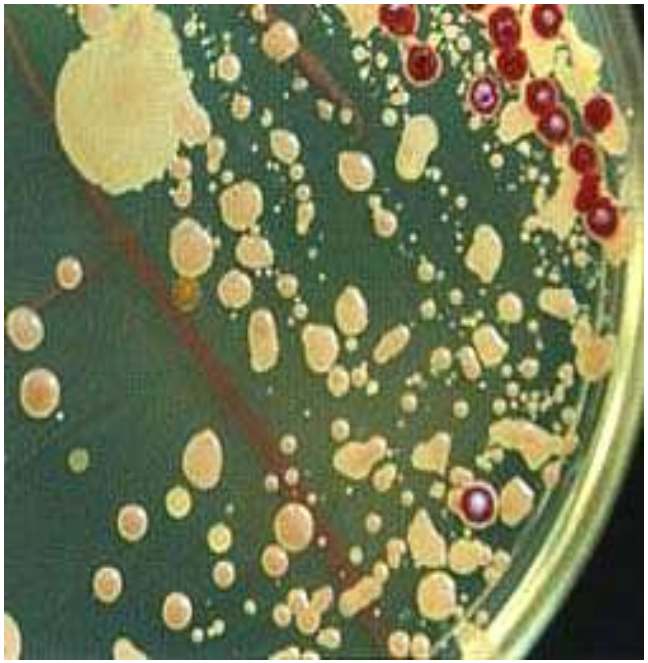
- Incubation: Allow organisms to grow under the optimal conditions
- Temperature, with or without oxygen etc
- Candle jar reduces oxygen

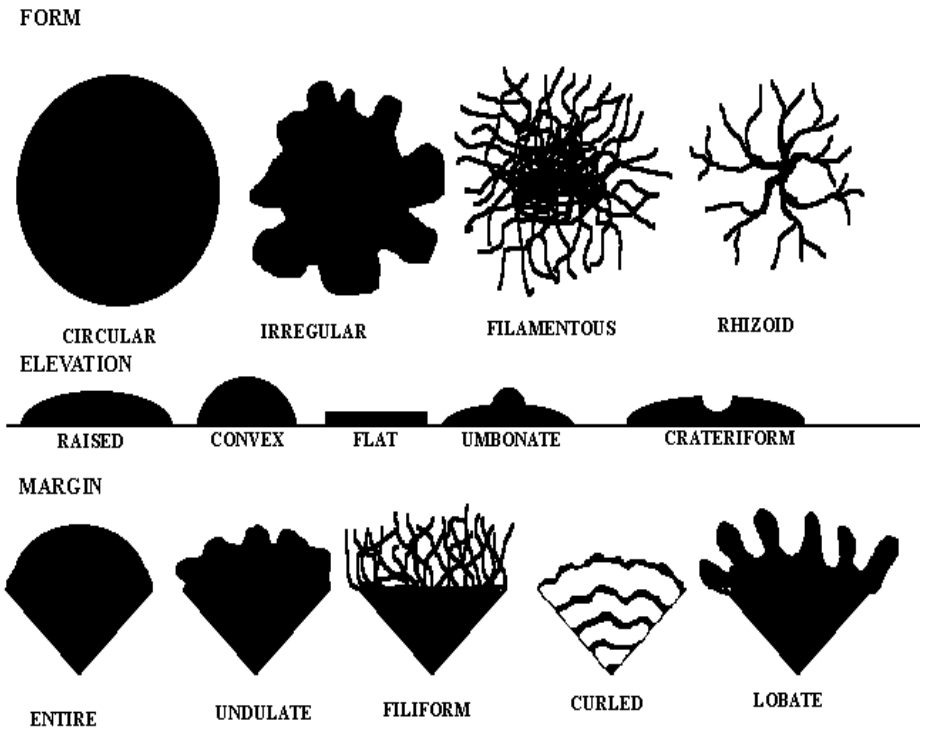
Innovative method and traditional

Incubation chamber

Incubation

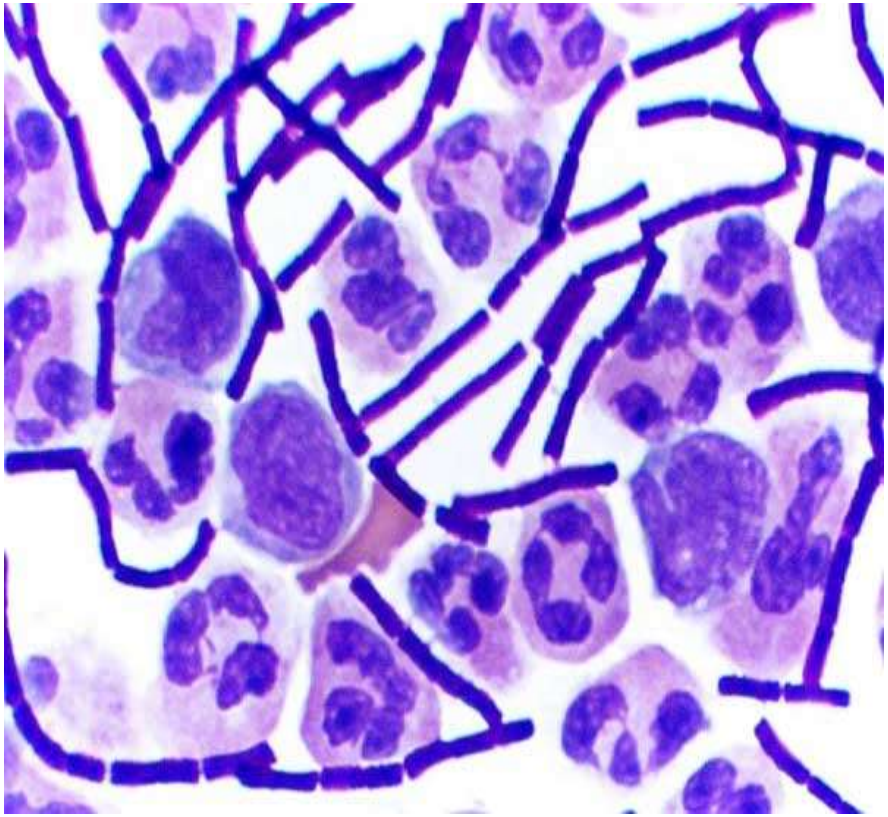
- Incubation : Observation, description
- Colony Morphology, Microscopic examination (grams stain)
- Systematic recording of “DATA”

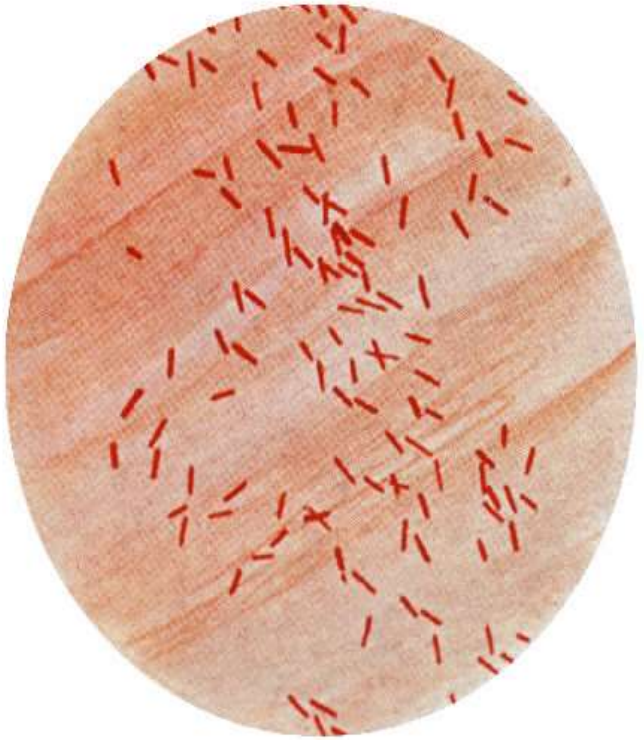




Microscopy

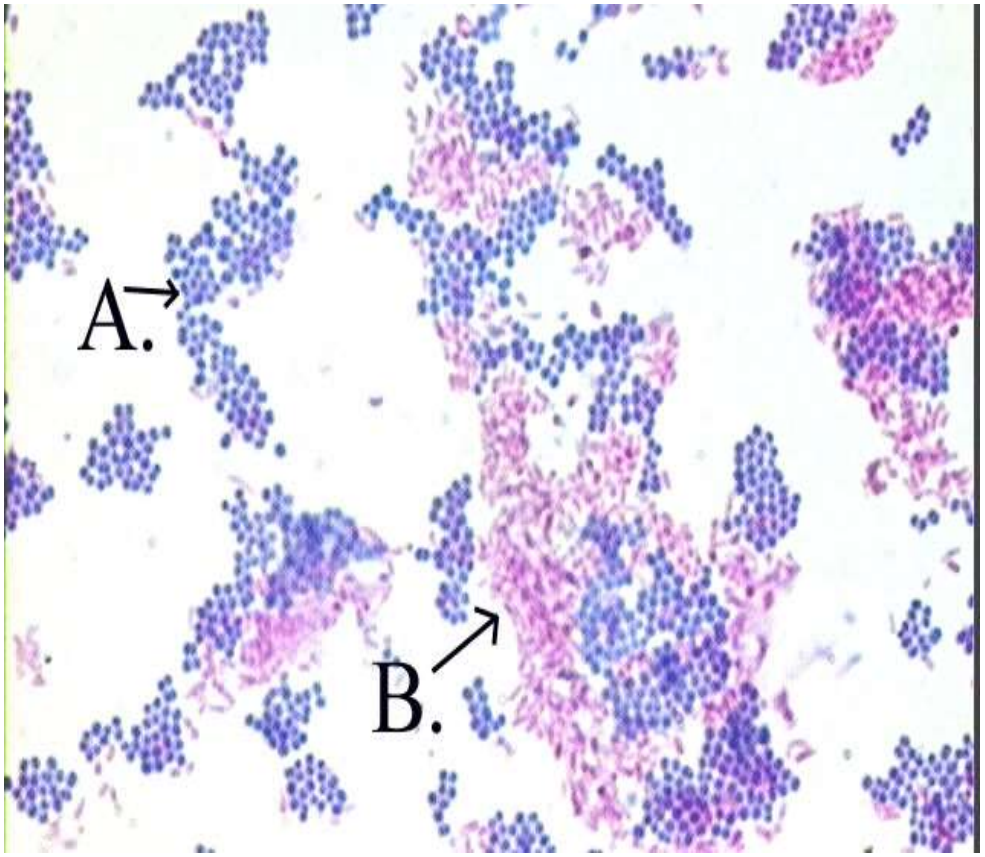
- Microscopic study: Gram + bacilli, Gram - bacilli

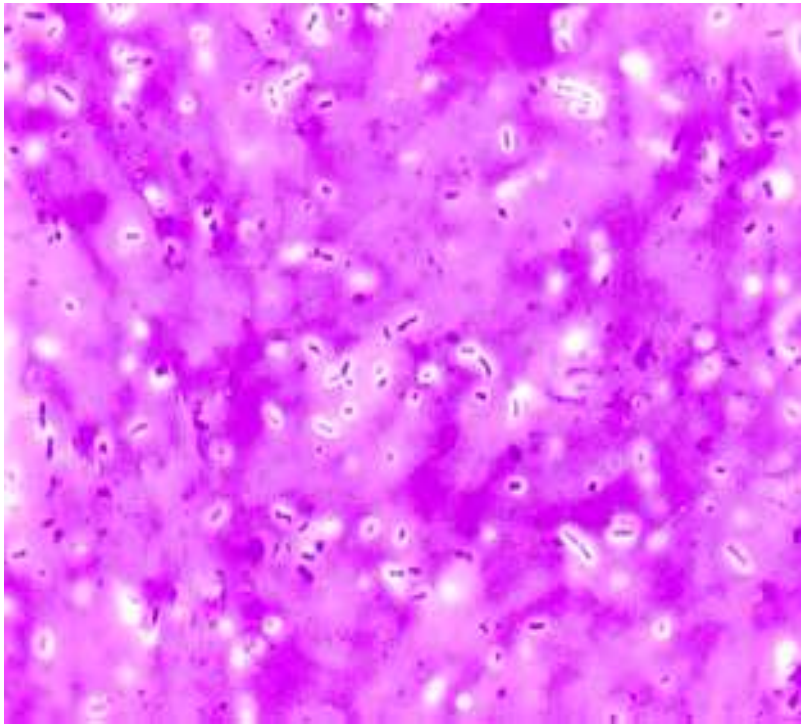




Microscopy

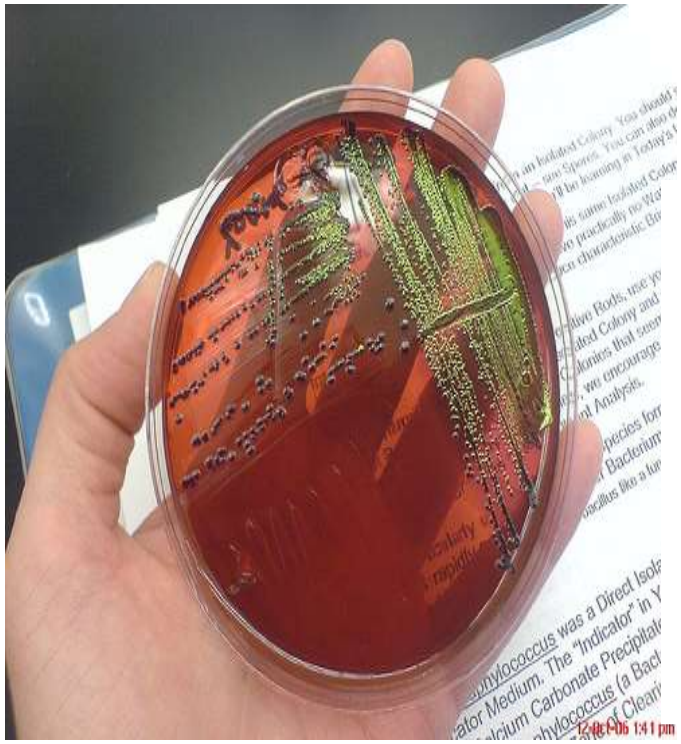
- Microscopic study: Acid fast, and capsule

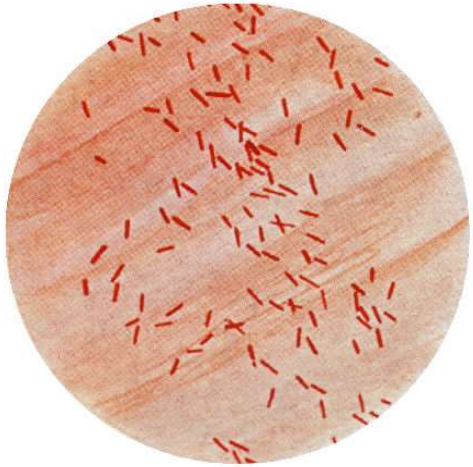




Incubation

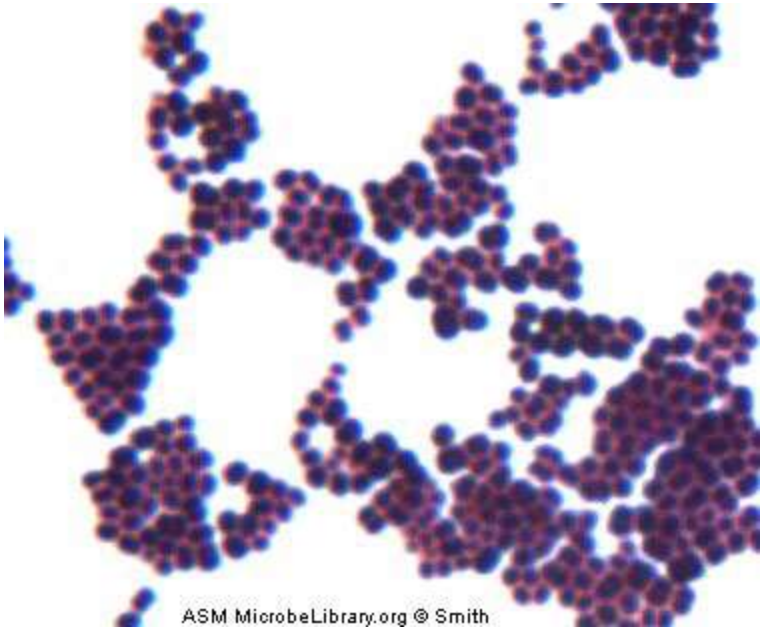
- Identification: Correlating data from all observations to ID organism to species
- Resources: flow charts, Bergey's manual etc.
- Ex. Gram – bacilli, ferments lactose, green sheen on EMB: E.coli





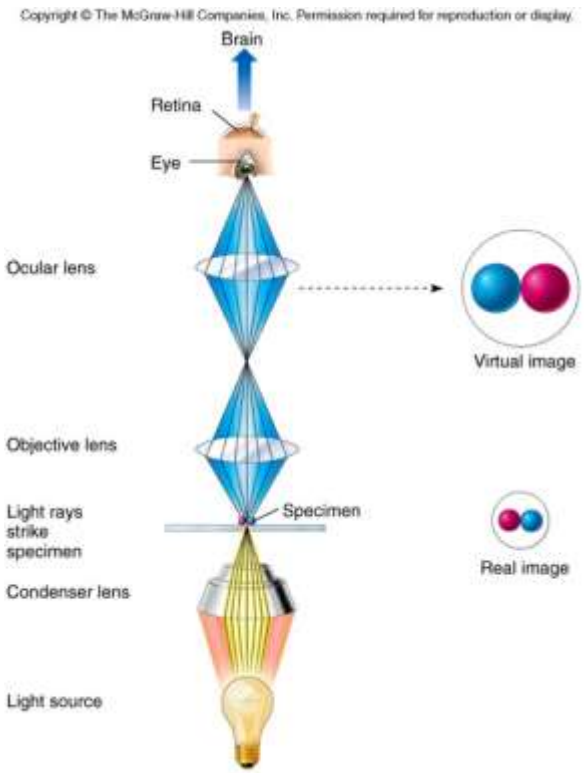
Identification

- Identification: Correlating data from all observations to ID organism to species
- Gram + cocci, grape like clusters, golden yellow colonies, catalase +, coagulase +, resistant to Methicillin (MRSA)
- Staphylococcus aureus



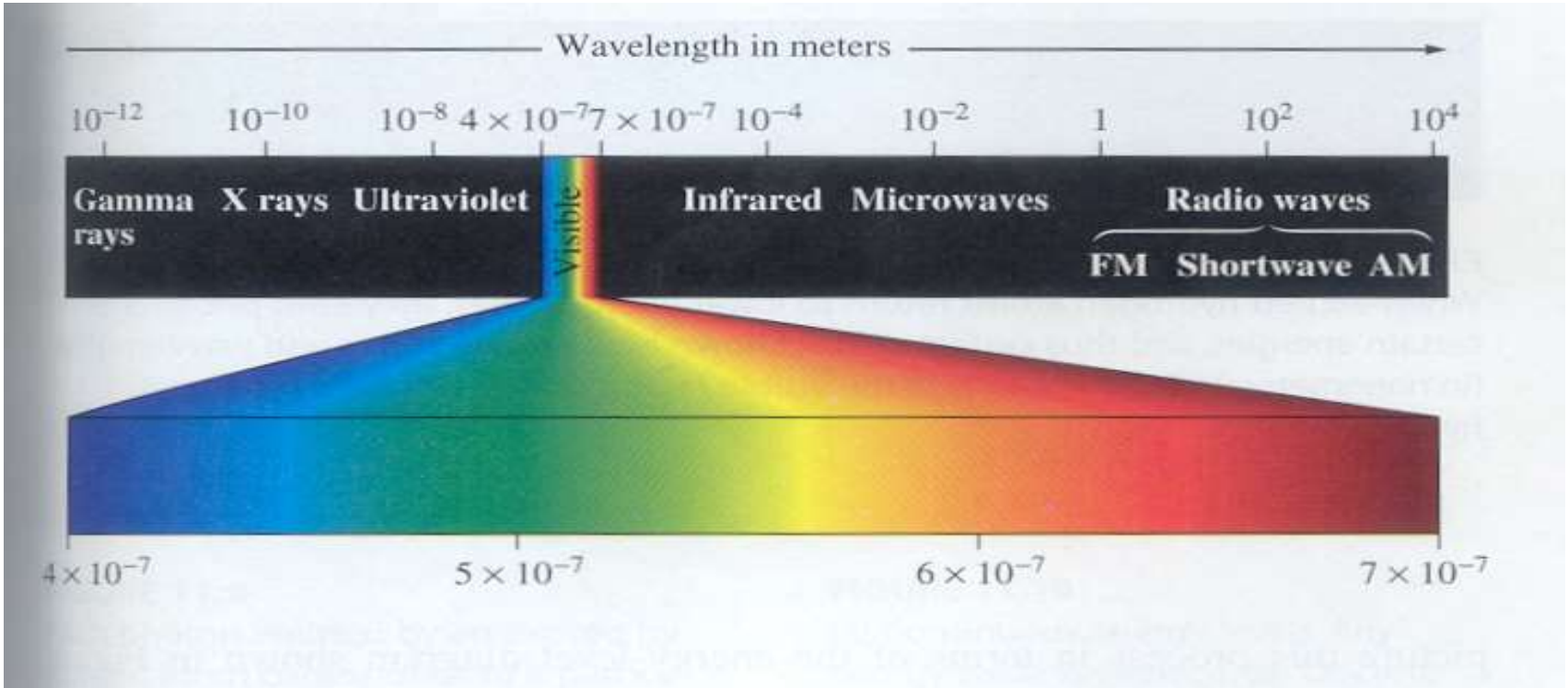
Microscopy

Light microscope: Visible Light is the energy source



Microscopy

Light can be described as a form of energy that moves in “waves” .
Wavelengths of light in the visible spectrum are used in most microscopes.
Remember the “prism”? Light is composed of different colors of light. Each color has different wavelength. Longer wavelengths have less energy (red end). Shorter; more energy (violet to UV).



Microscopy

When light strikes an object the light can be:

Reflected – Bounces off (Mirror)

Transmitted – Passes through (GLASS)

Absorbed – Soaked (black colored paper)

Diffracted – Scattered as it passes through

(bugs on a dirty windshield)

Refracted – Bent as it passes (objects seen under water) Glass lenses

Refractive index: degree of bending, based on lens material and shape of lens

Microscopy

So What? It is a big deal. When light in a scope strikes an object (stained bacteria on a slide) some of the light is:

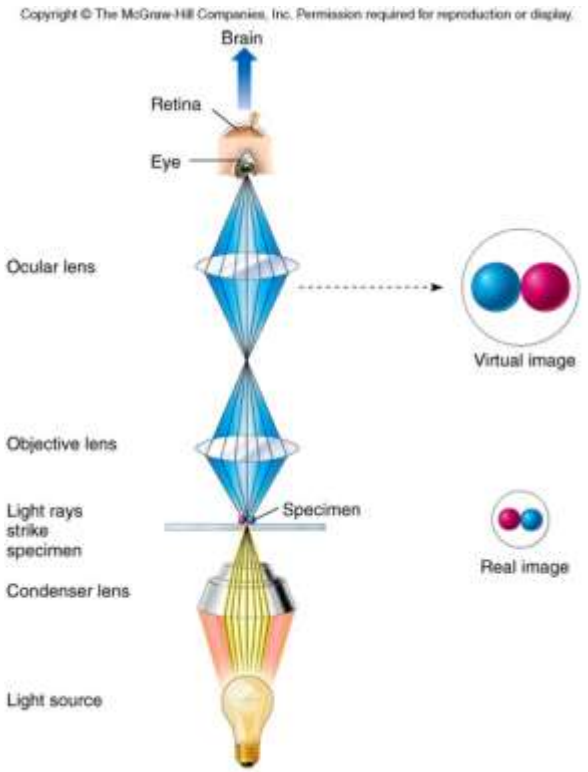
Absorbed A pattern is collected by the lenses and our

Refracted eyes see a magnified “object”

Diffracted

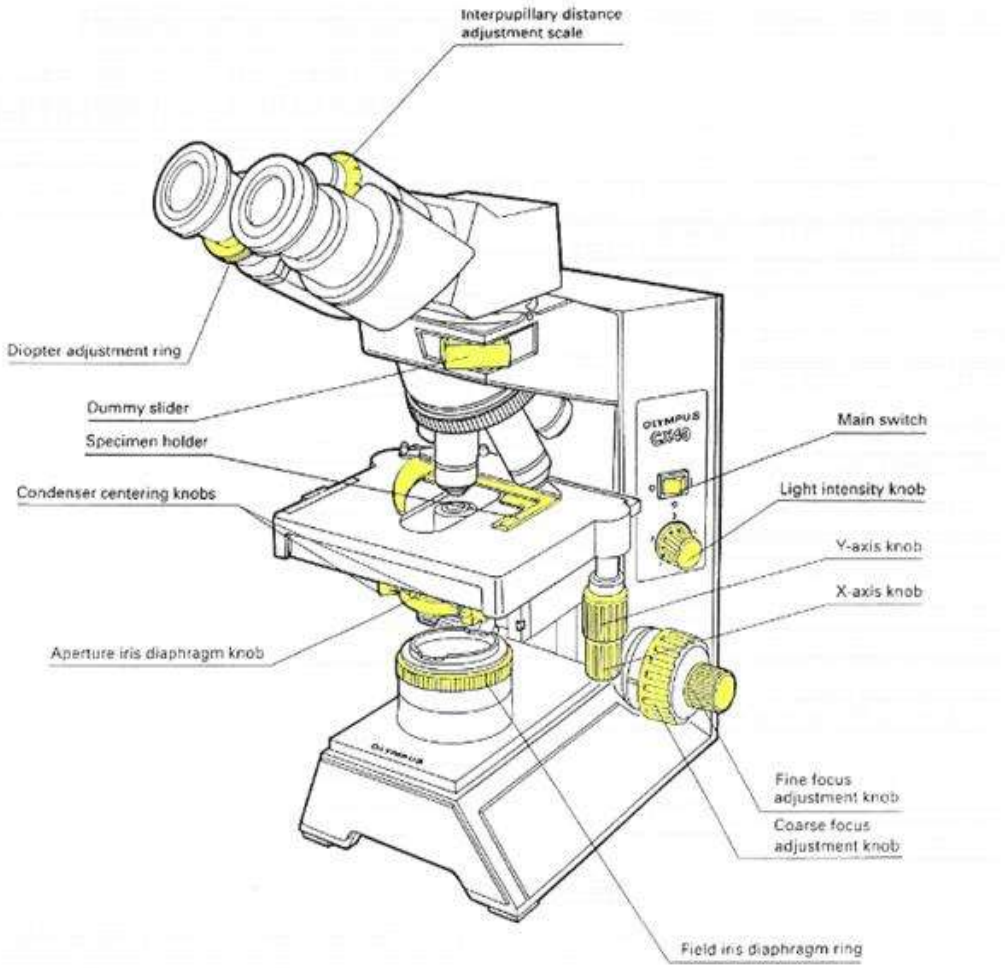
Reflected

Transmitted



Microscopy

Compound Light Microscope: Lens system with two magnifying lenses, magnification is calculated by multiplying the power of the two lenses (10 X 10 = 100 power)



Microscopy

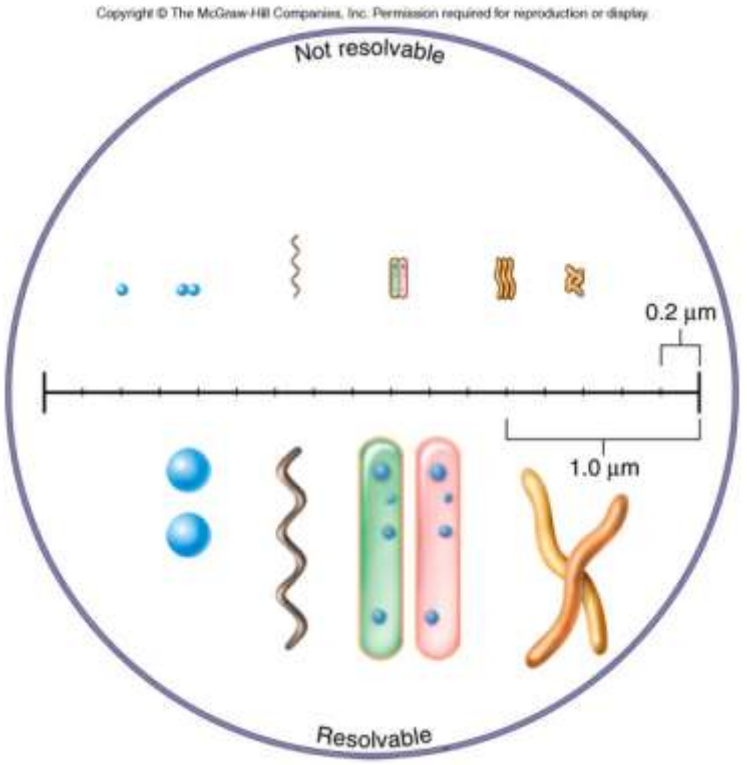
Technicality

Contrast: Bacteria have little contrast unstained. Light is only slightly refracted – diffracted – reflected etc. as it passes through the cells. To see them we usually stain them. Stains are colored dyes (chromophores) that increase contrast. Without stains, special expensive microscopes are needed.

Resolution: aka “resolving power” The ability of a lens system to allow an observer to see fine detail. Quality of lens systems (fine quality of glass and special lens coatings). The best lens systems allow one to see two points as distinct points eve when they are tiny and very close together.

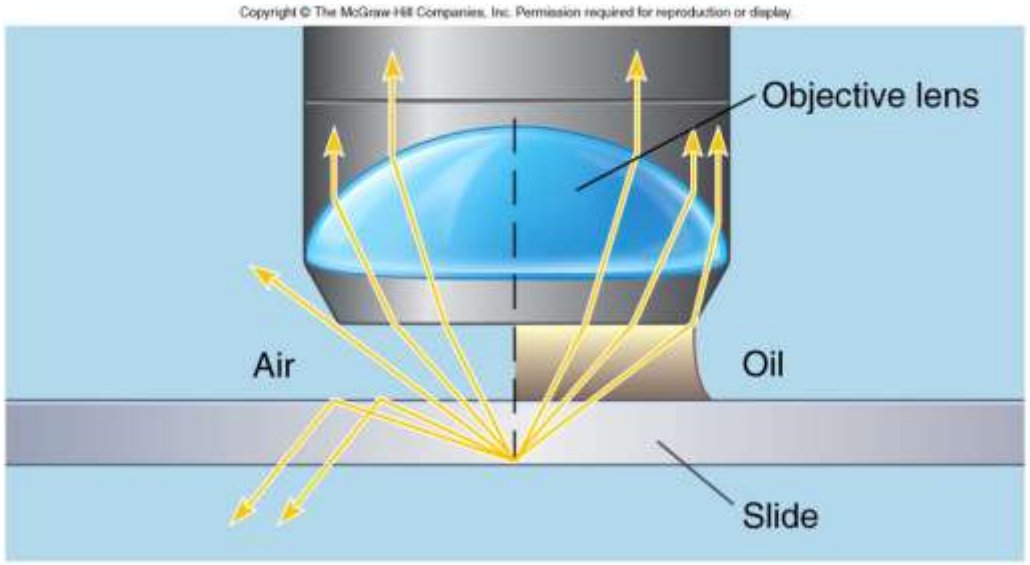
Microscopy

The best light microscopes can resolve objects to only about 0.2 – 0.5 microns. It is a function of the energy of visible light and its wavelength (we make really good lenses). To increase resolving power we need and energy source with more energy (shorter wavelength) thus the electron microscope.



Microscopy

The best magnification on our scopes is achieved with the “oil immersion” objective. Oil is used with the lens because it has “the same refractive index as glass”. We can see objects with clarity at about 1000X magnification. Less light is refracted away from the tiny lens and objects are “clearer”. No oil = fuzzy poor quality image.



Microscopy

- Types of Light Microscopes
  - Brightfield – most common, objects are dark against a bright background
  - Darkfield - special condenser, objects are light against a dark background – used to see live microbes unstained (spirochetes in fluid)
  - Phase contrast – expensive condenser and internal lens components, change “phase of light”, so live specimens appear with more internal contrast
  - Fluorescence – fluorescent dyes and UV light

- **Light or optical microscope**
- They are of two types namely Simple and Compund Microscope
- Simple Microscope consists of a single lens. A hand lens is an example of a simple Microscope.
- Compound Microscope consists of two or more lenses in series. The image formed by the first lens is further magnified by another lens.
- Bacteria may be examined under the compound microscope, either in the living state or after fixation and staining. Examination of wet films or hanging drops indicates the shape, arrangements, motility and approximately size of the cells. But due to lack of contrast details cannot be appreciated**.**
- **Phase contrast microscope**

  This imposes the contrast and makes evident the structure within the cells that differ in thickness or refractive index. The difference in the refractive index between bacteria cells and the surrounding medium makes them clearly visible. Retardation, by a fraction of a wavelength, of the rays of light that pass through the object, compared to the rays passing through the surrounding medium, produces phase difference between the two types of rays.
- **Dark field / Dark ground microscope**
- Another method of improving the contrast is the dark field microscope in which reflected light is used instead of the transmitted light used in the ordinal microscope. The contrast gives an illusion of increased resolution, so that very slender organisms such as spirochete, not visible under ordinary illumination, can be clearly seen under the dark field microscope.
- **Electron Microscope**
- Beams of electron are used instead of beam of light, used in light microscope. The object which is held in the path of beam scatters the electrons and produces an image which is focused on a fluorescent viewing screen. Gas molecules scatter electron, therefore it is necessary to examine the object in a vacuum.

Quick quiz

- Match the following

- **Microscopes**

1. Light microscope
2. Phase contrast microscope
3. Dark field microscope
4. Electron microscope

Properties:

(a) reflected light (3)

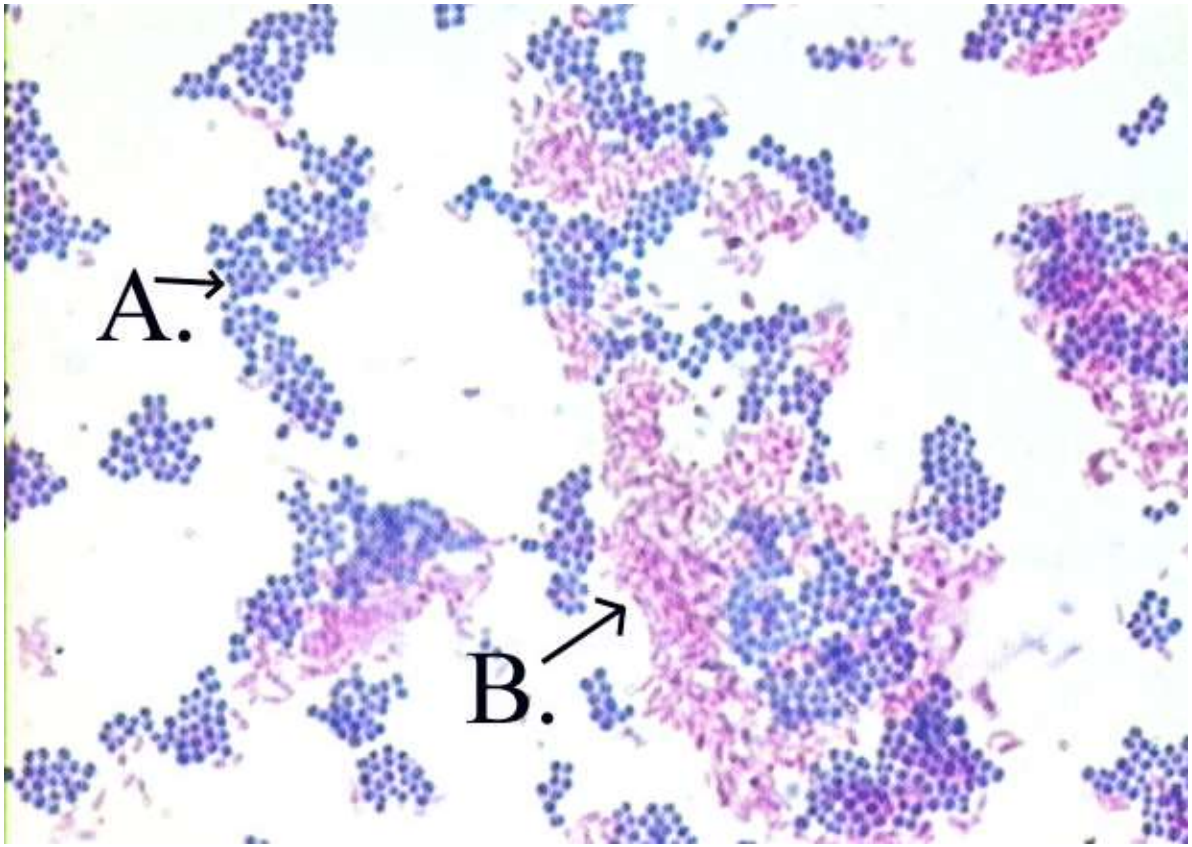
(b) electron beam (4)

(c) light beam (1)

(d) refractive index (2)

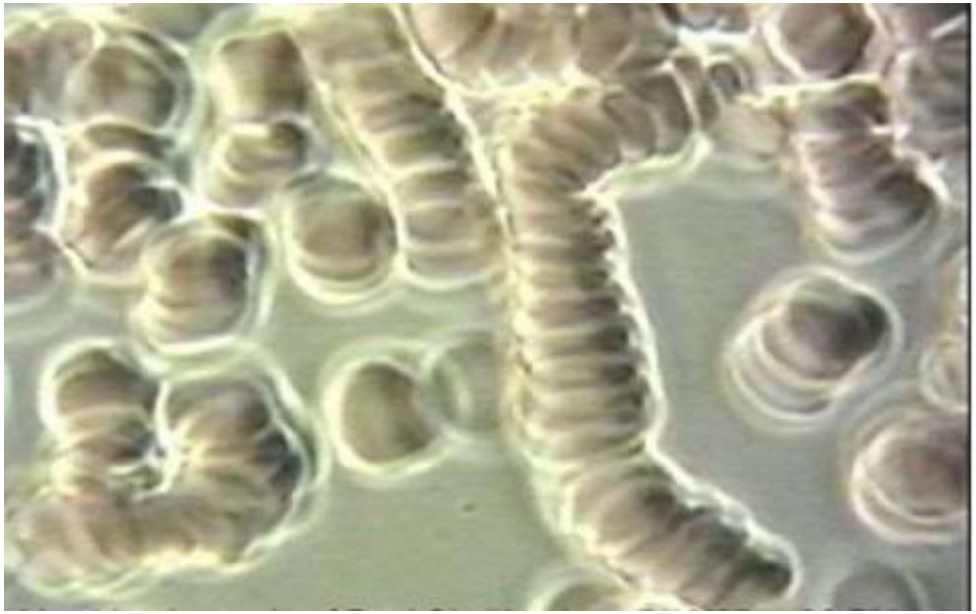
Microscopy

- Brightfiled



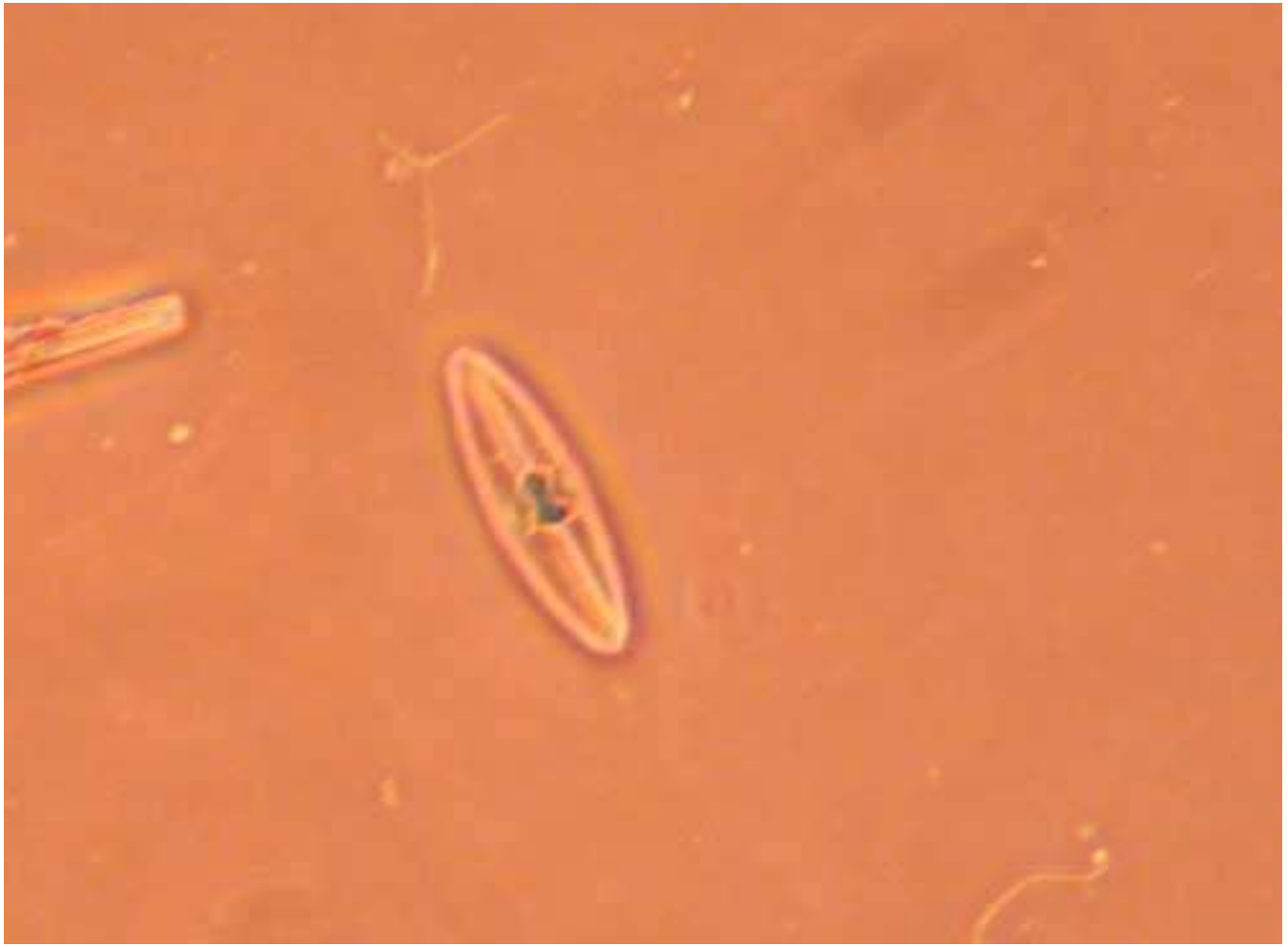
Microscopy

- Darkfield



Microscopy

- Phase contrast

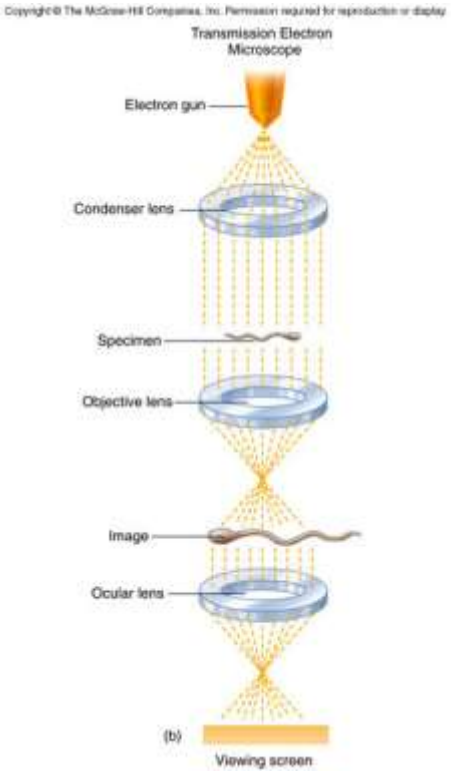


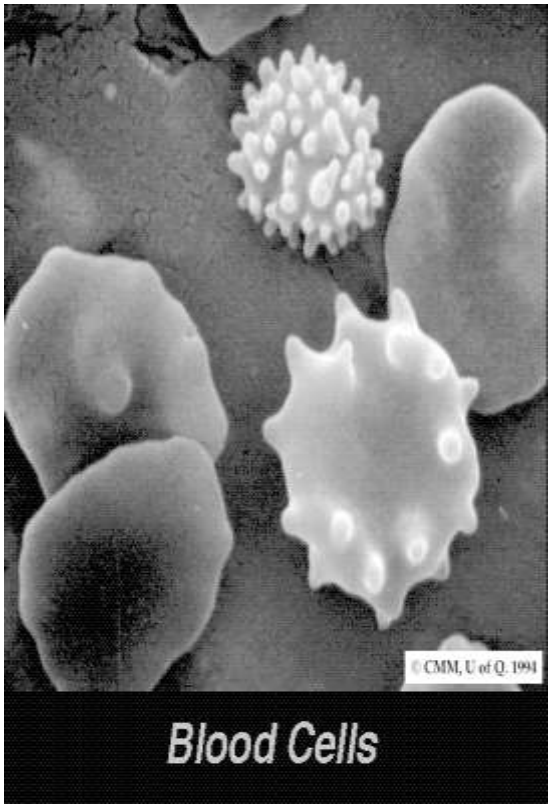
Microscopy

- Fluorescence Microscope

Microscopy

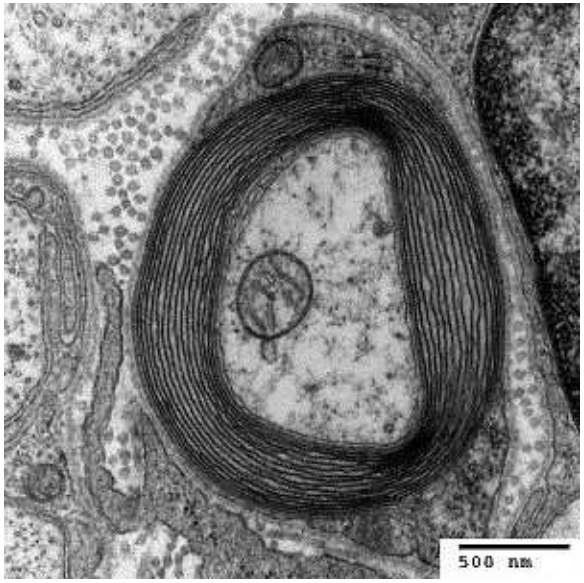
- Electron Microscope: energy source for magnification is a beam of electrons (negative charged subatomic particles

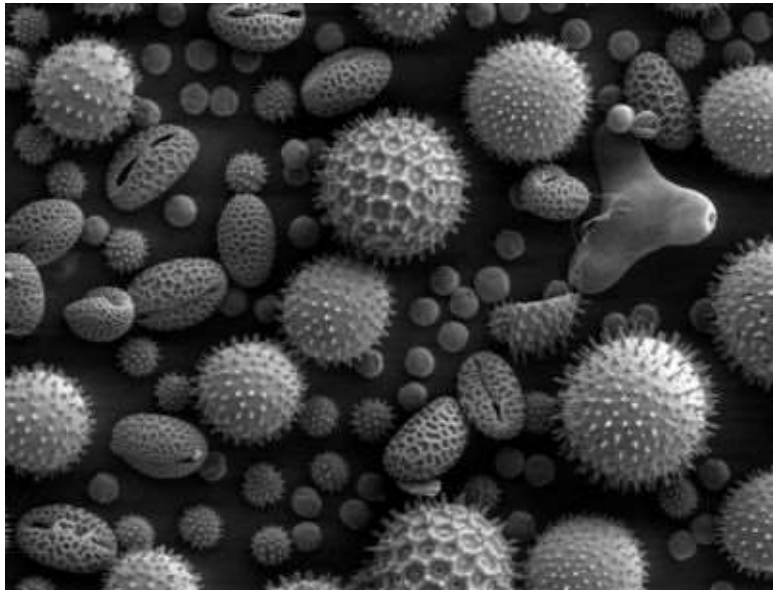




Microscopy

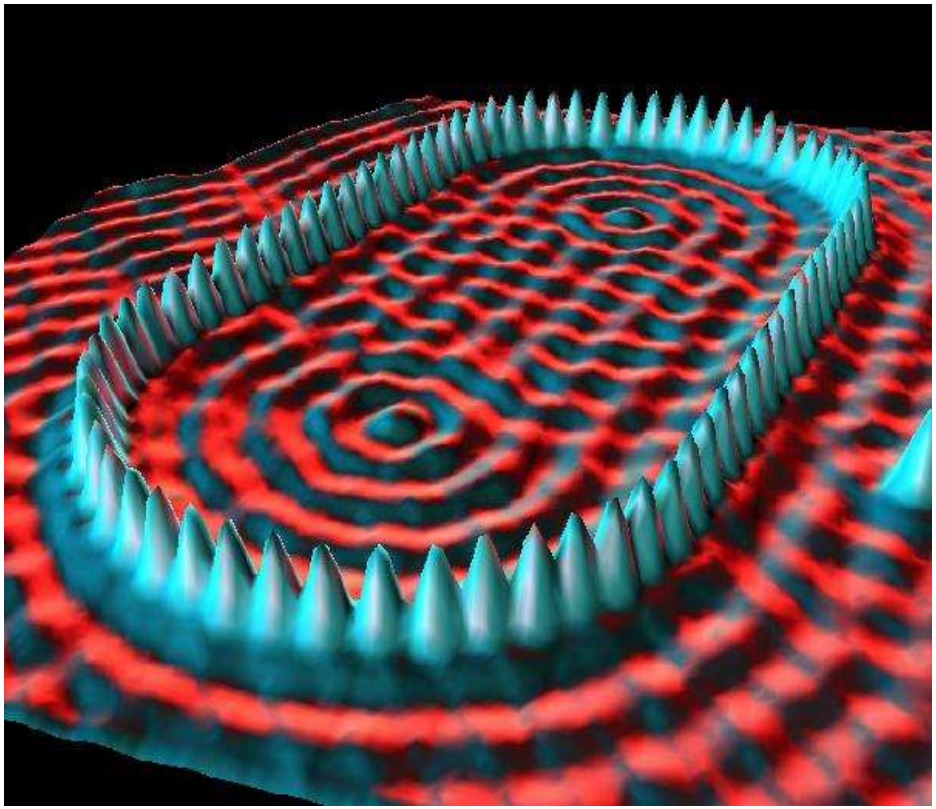
- Transmission electron microscope – very high magnification (100,000 X)
- Scanning: tremendous surface detail
- Transmission Scanning





Microscopy

- Tunneling scanning electron microscope
- Molecular and atomic level? Research

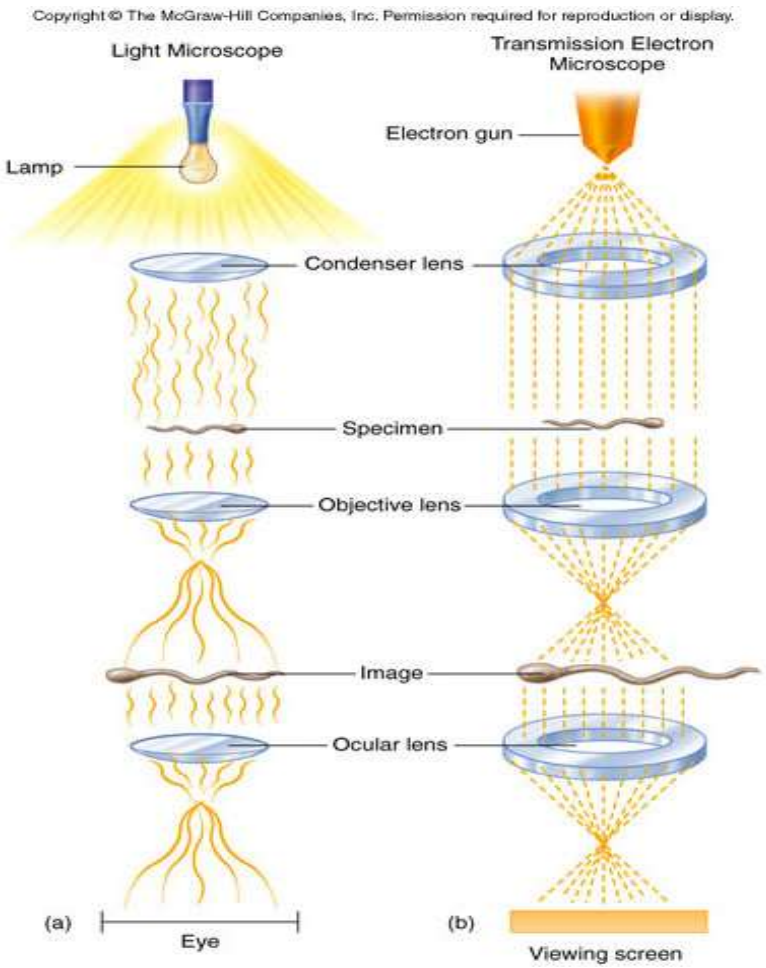


Microscopy

- Compare and contrast Light and Electron Microscope
- Light — Electron
- Energy – light — Energy – electron beam
- Cost - $1200 — Cost – $120,000
- Simple to use — Complex processes. trained technician
- Magnification – 1200X — Magnification – 100,000X
- Viewed by eye, camera — Viewed with CRT, photos

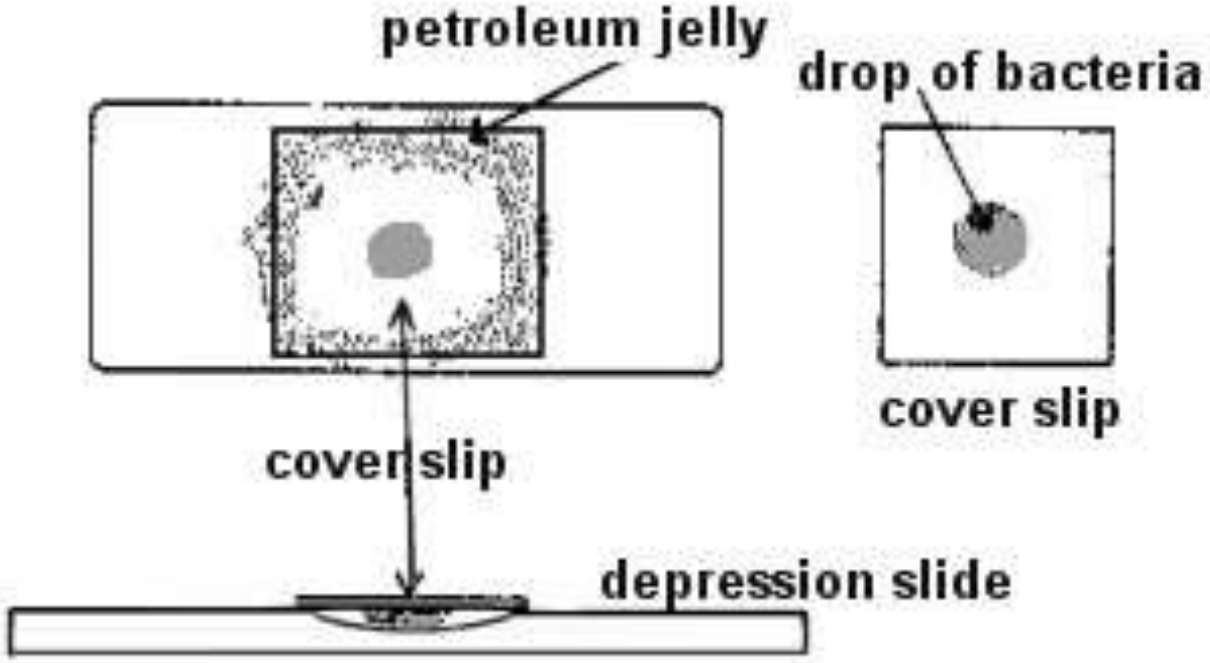
Microscopy

- Compare and contrast Light and Electron Microscope



Microscopy

- Preparation of samples for light microscope
- Wet mounts (ex. **Hanging drop method**) for live observation

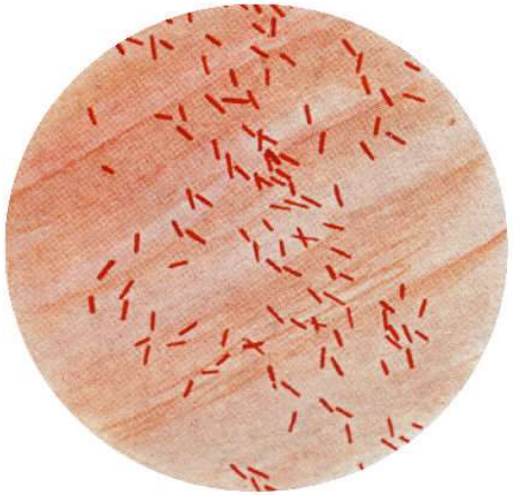


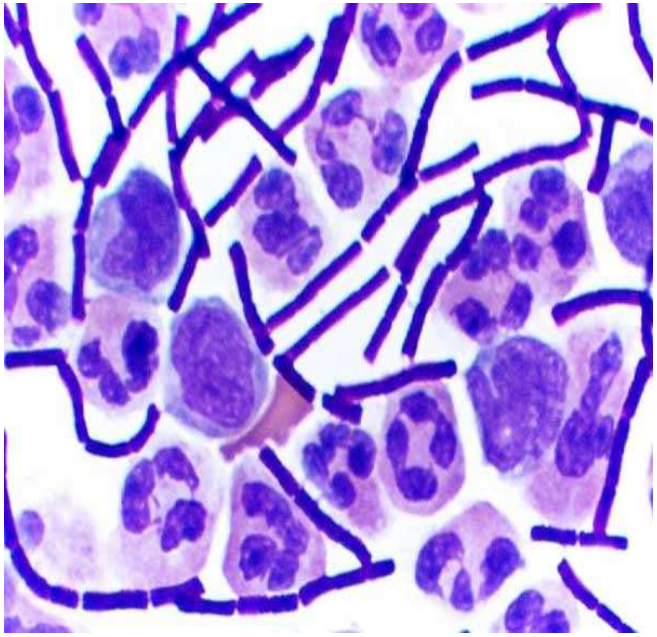
Microscopy

- Simple stain – one dye
- Differential stain – complex procedure, see difference between cells
  - Grams + and (-)
  - Acid fast + and (-)
  - Negative – acid dye stains background and cells are white (cell wall repels stain)
  - Capsule – modified negative stain to show capsule layer

Microscopy

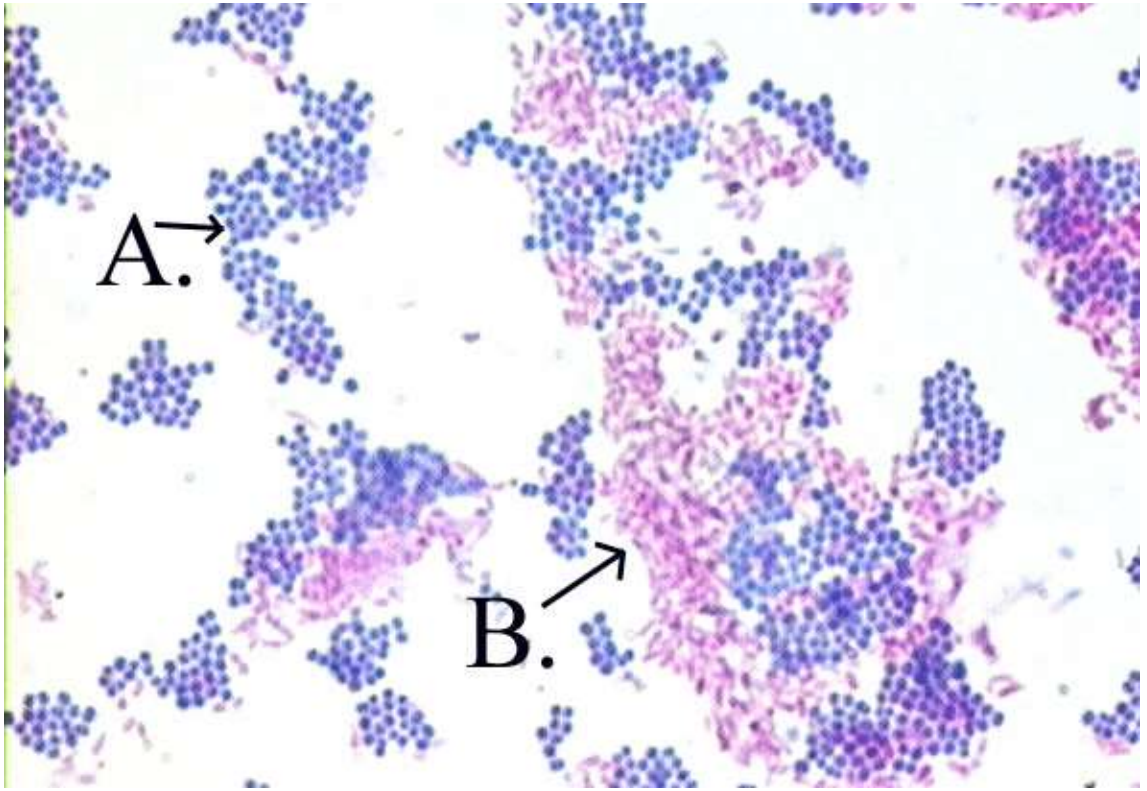
- Grams





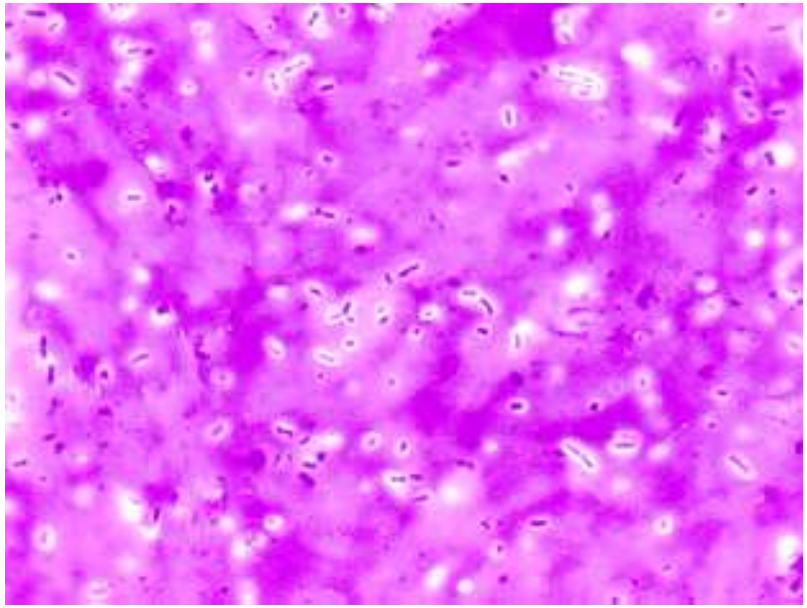
Microscopy

- Acid fast



Microscopy

- Capsule



Microscopy

- Negative stain