MICROBIOLOGIA GENERALE

Staining bacteria cells

Staining bacteria cells for microscopic examination makes it possible:

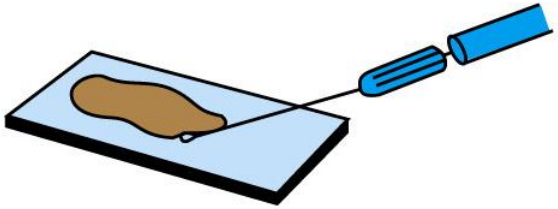
- to define their cell size, shape, arrangement;
- to study their chemical properties,and structures.

These characteristics can be use for bacterial identification

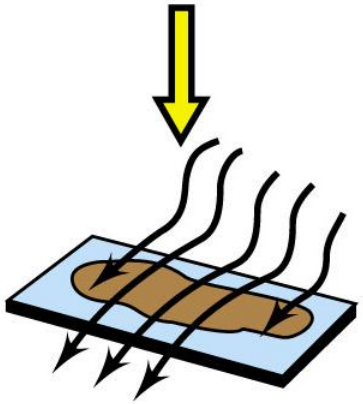
Staining bacteria cells: outline of the procedure

1. Preparing cells for staining
2. Simple stain
3. Differential staining
   - Gram
   - Acid-fast stain
4. Microscopic observation

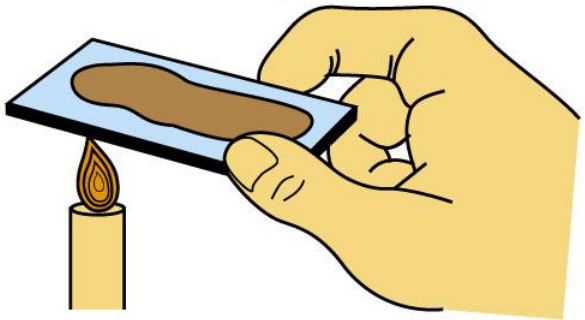
Overview of a bacterial staining procedure



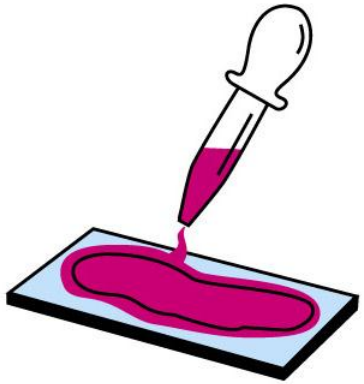
Spread culture in thin film over slide



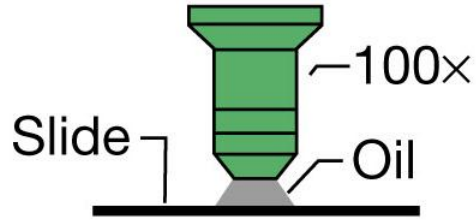
Dry in air



Pass slide through flame to fix

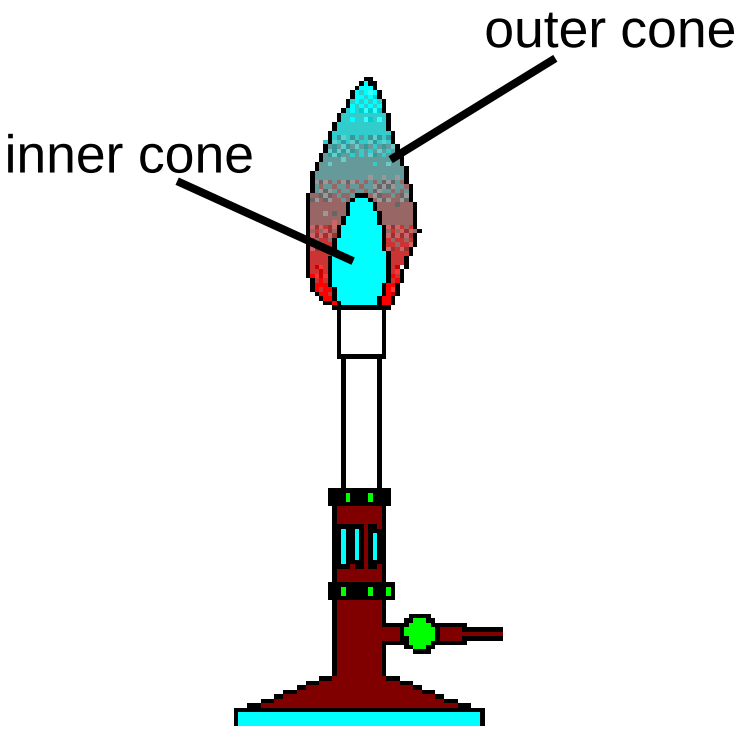


Flood slide with stain; rinse and dry

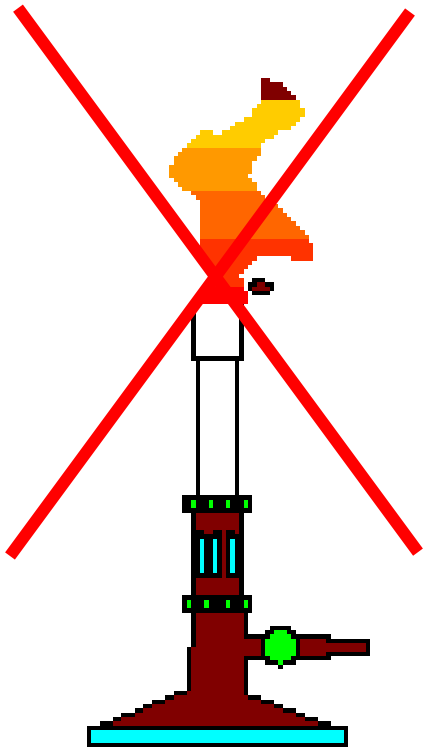


Place drop of oil on slide; examine with 100× objective

Aseptic transfer and the Bunsen burner flame

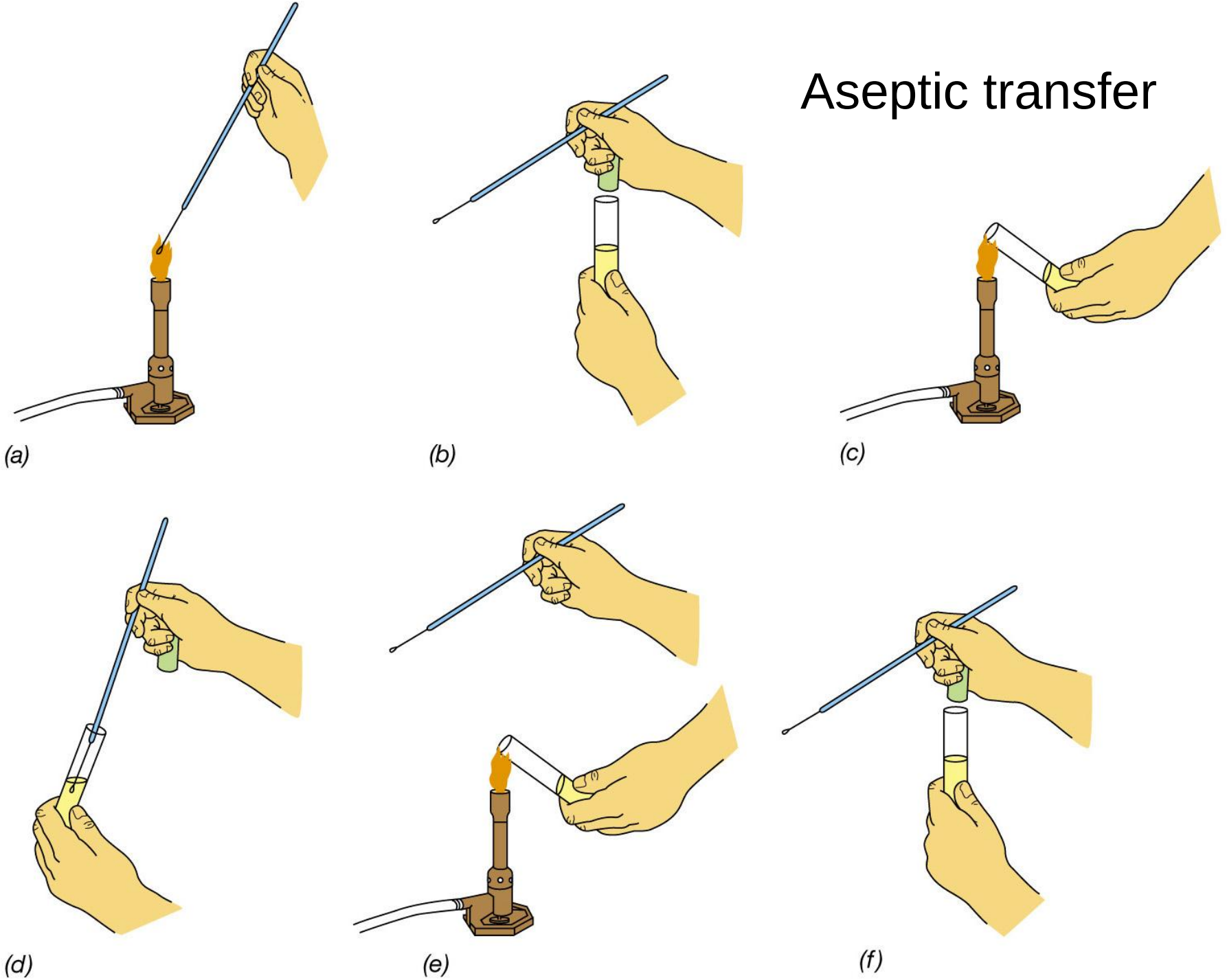


reductant flame

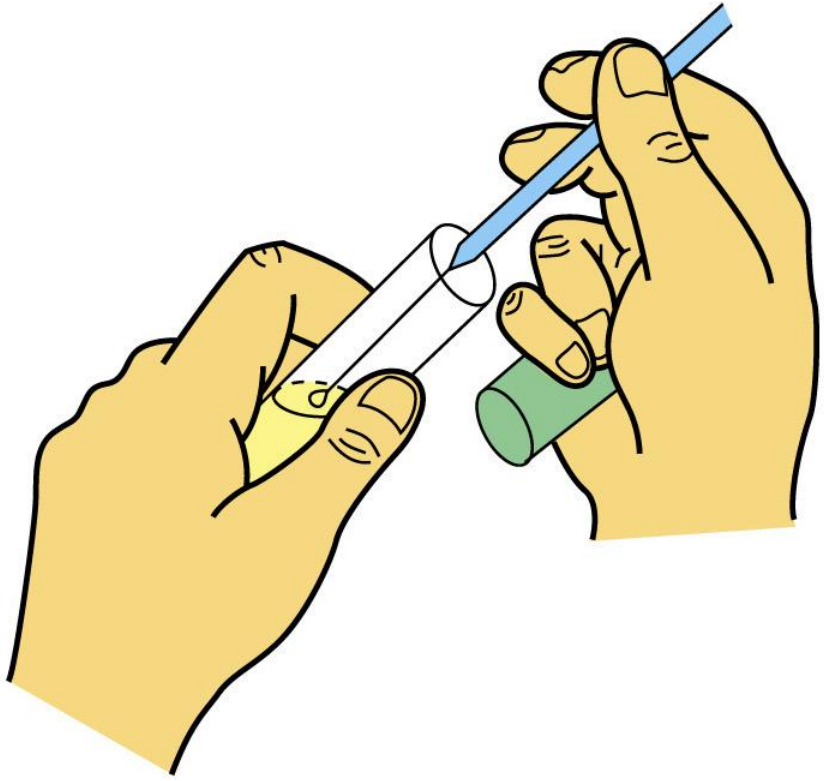


oxidant flame

Aseptic transfer



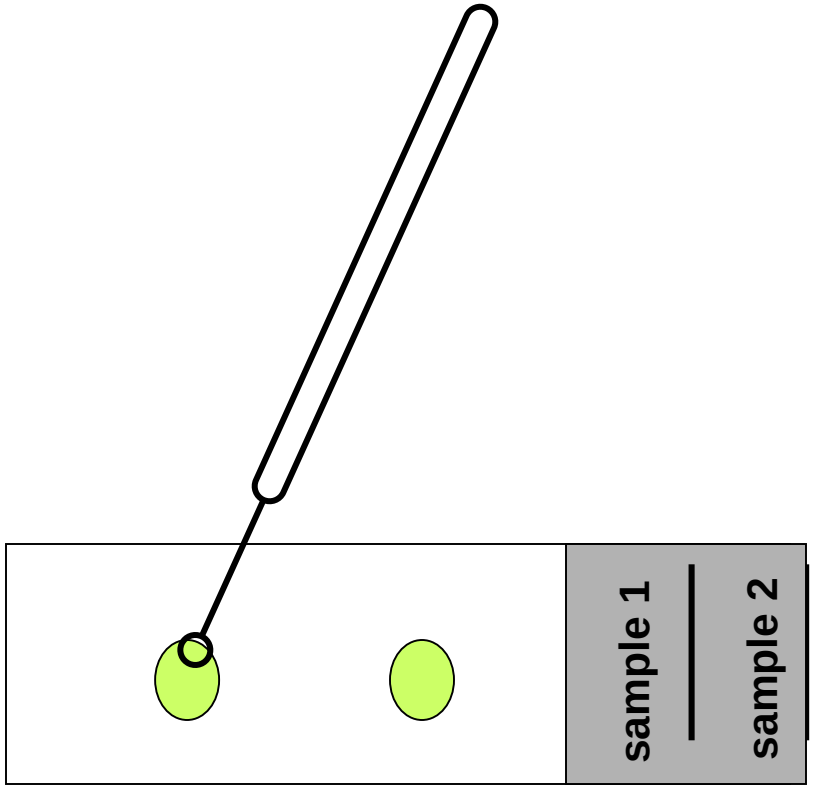
Aseptic transfer

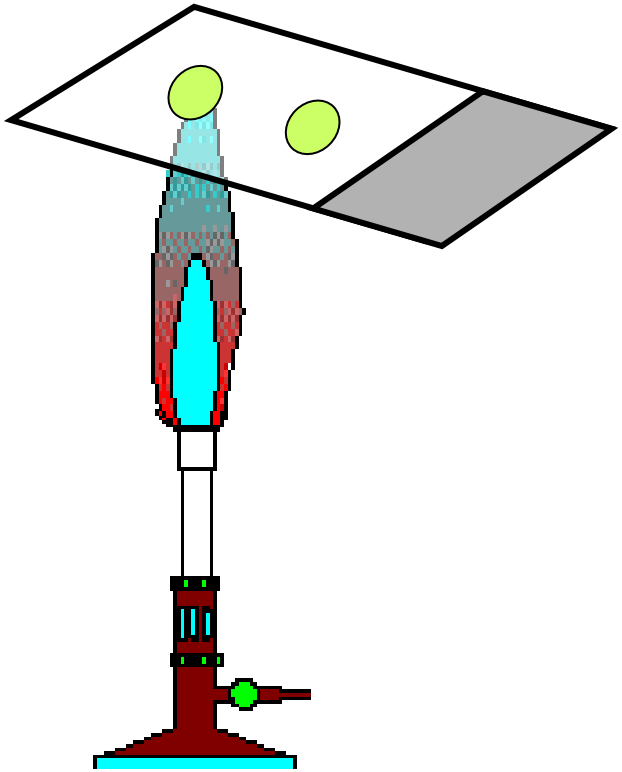


(a)

(b)

Preparation of the heat-fixed smear

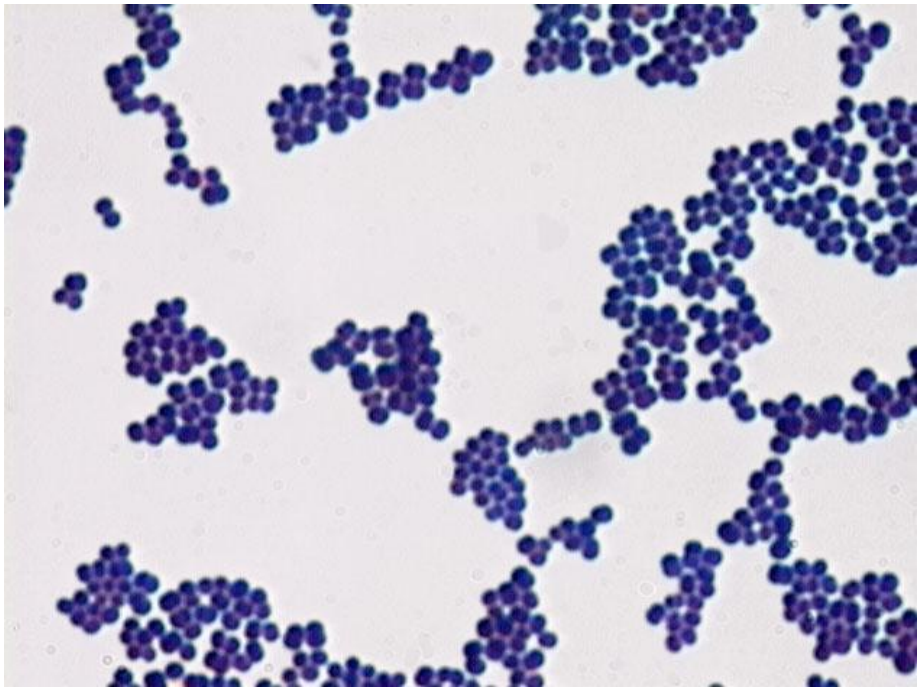


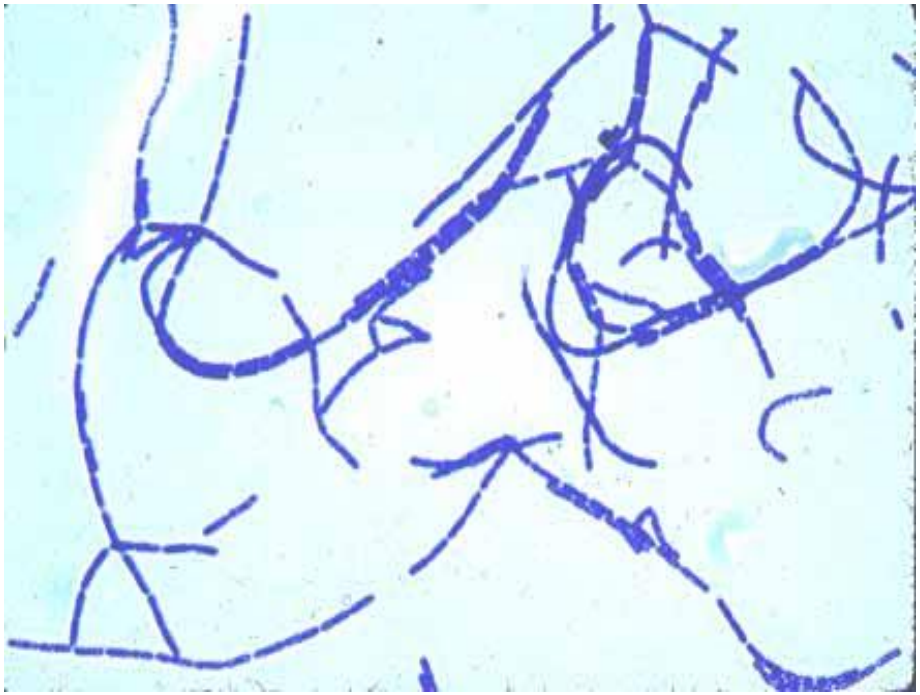


Staining bacteria cells:

simple staining

Simple stains use a single basic dye (e.g. crystal violet, methylene blue, safranin) to color bacterial cells so that their size, shape and arrangement can be observed





Staining bacteria cells:

differential stain

•Differential stains, such as the Gram stain and the acid-fast stain, differentiate bacteria based on the chemical composition of their cell wall.

•Differential stain use two dyes instead of one: the first stain is the primary stain, the second is the counterstain.

•A decolorization step occurs between the application of the primary stain and counterstain.

•Depending on the composition of the cell wall, bacteria will either retain the primary stain during decolorization or lose the primary stain and take up the counterstain.

Staining bacteria cells:

the Gram stain

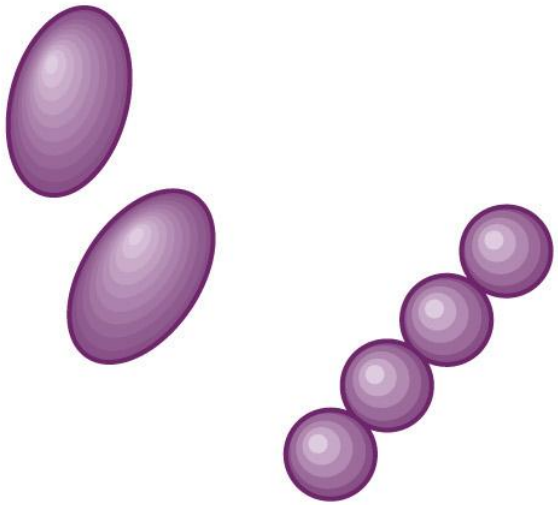
History of the Gram stain

- Hans Christian Gram was a Danish bacteriologist.
- He developed the Gram stain as a means to differentiate pneumococci from *Klebsiella pneumonia* in 1884.
- It remains one of the most important staining techniques in microbiology today.
- The Gram stain is often the first test performed in the identification of bacteria.

GRAM STAIN PROCEDURE

1. Stain with crystal violet 2%…… ……1 min.
2. Gram's iodine (Lugol)……1 min.
3. Wash off with tap water
4. Decolorizer (Alcohol 50%-Acetone 50%)…20 sec.
5. Wash off with tap water
6. Safranin 0,25%……1 min.
7. Wash off with tap water
8. Blot dry with bibulous paper

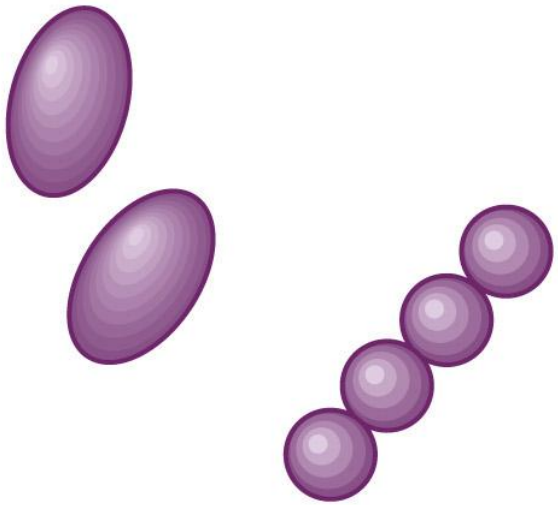
Step 1



Flood the heat-fixed smear with crystal violet for 1 min

All cells purple

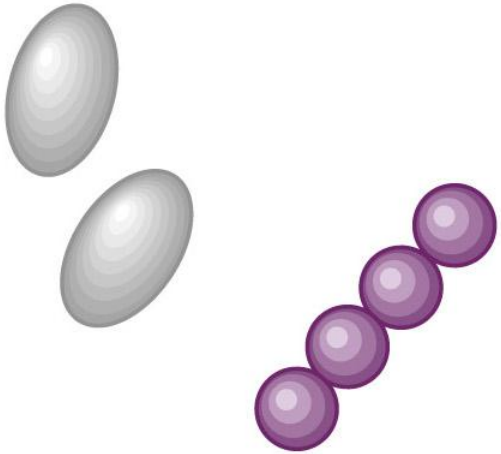
Step 2



Add iodine solution for 1 min

All cells remain purple

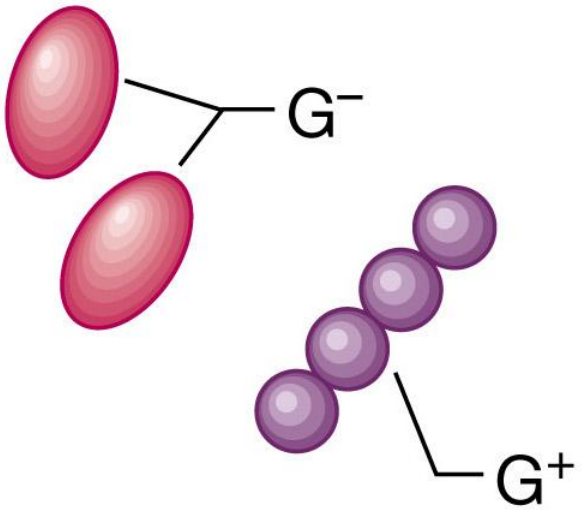
Step 3



Decolorize with alcohol briefly — about 20 sec

Gram-positive cells are purple; gram-negative cells are colorless

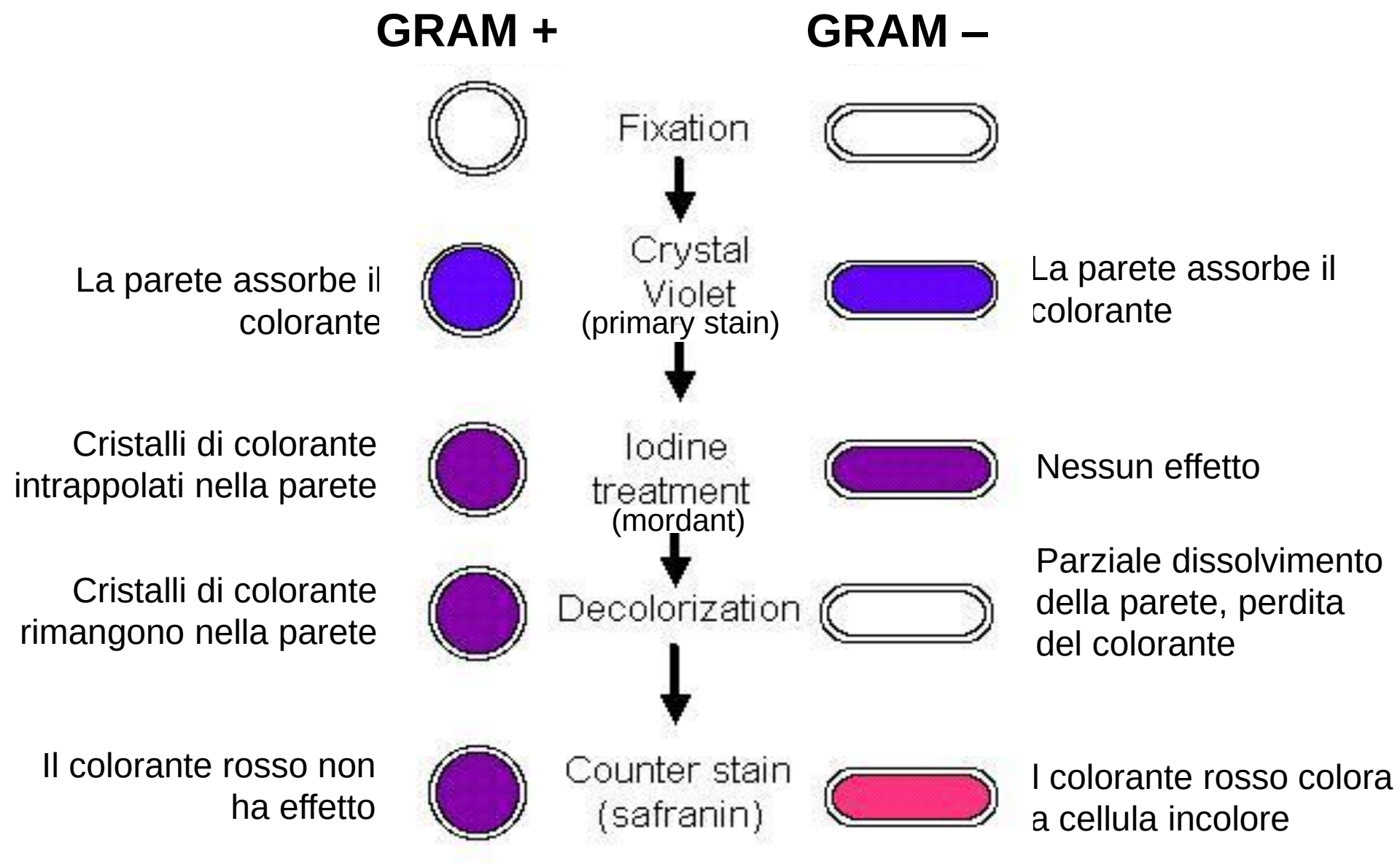
Step 4



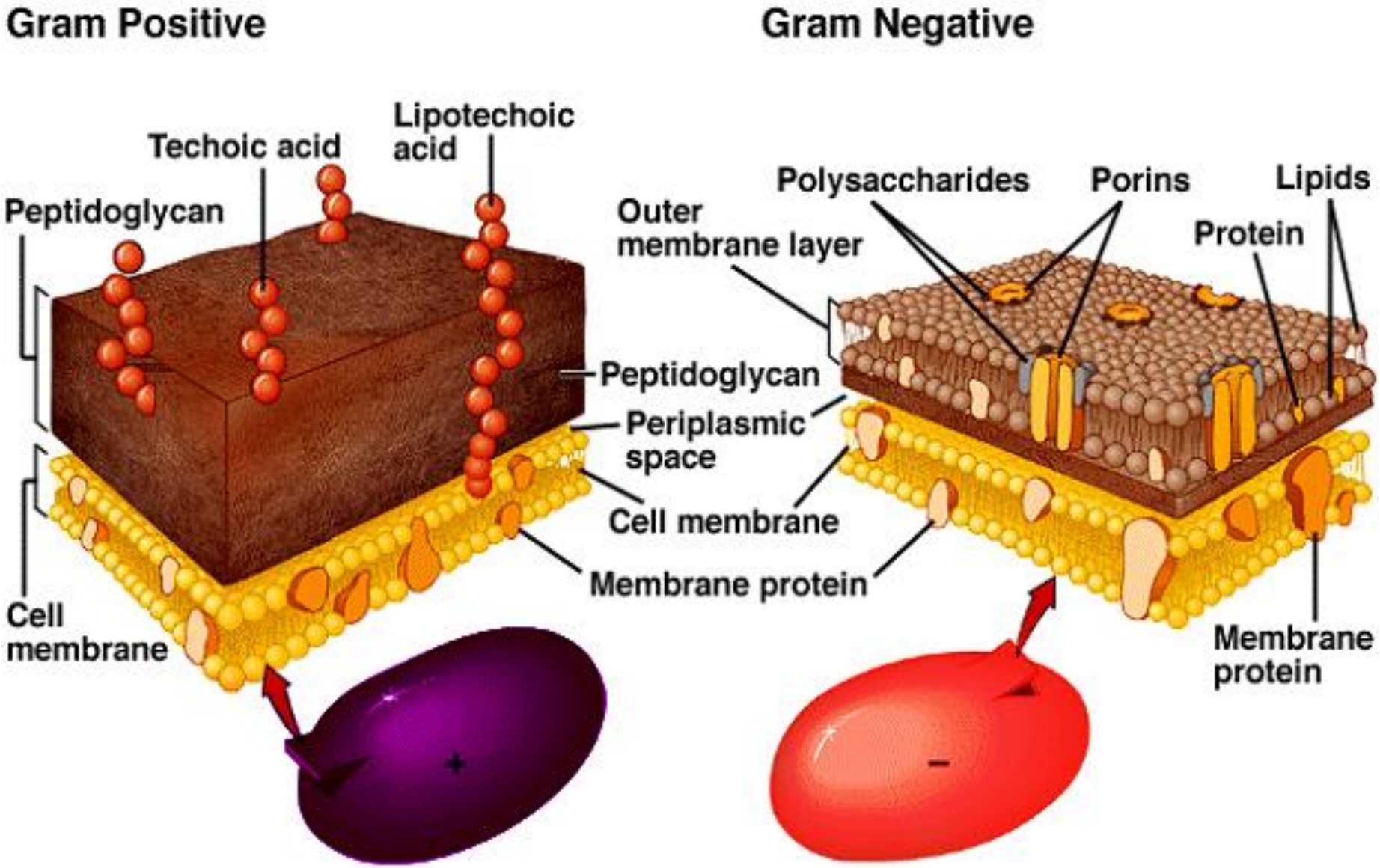
Counterstain with safranin for 1–2 min

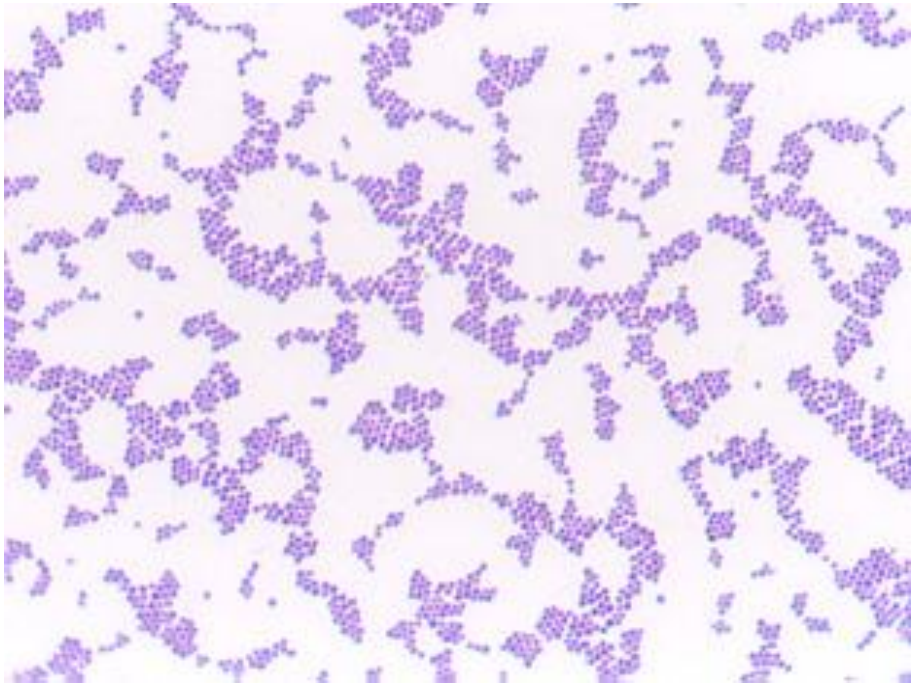
Gram-positive (G^+) cells are purple; gram-negative (G^-) cells are pink to red

Overview of the Gram stain



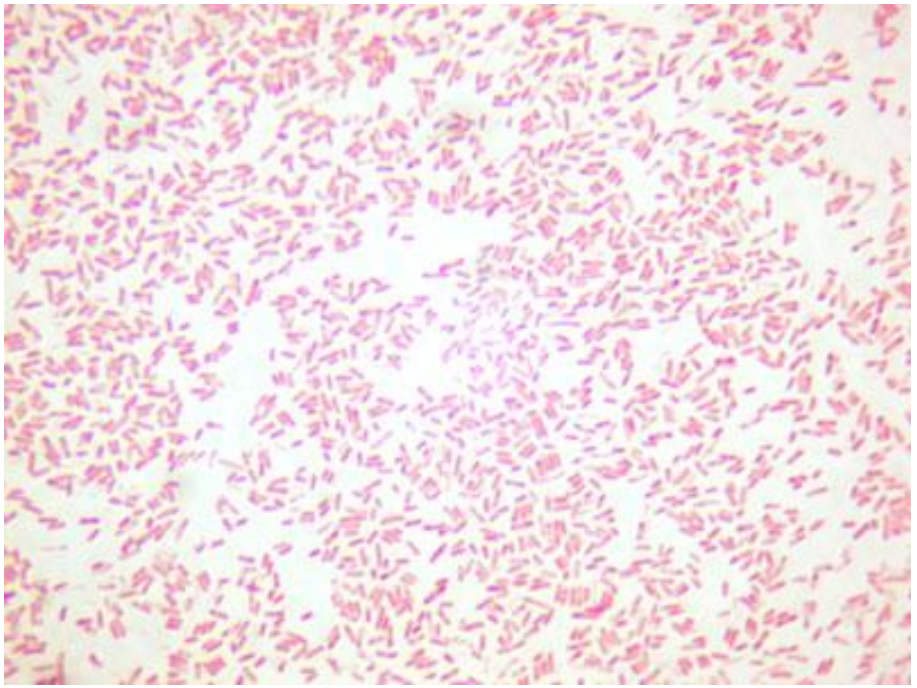
Gram positive and Gram negative reactions



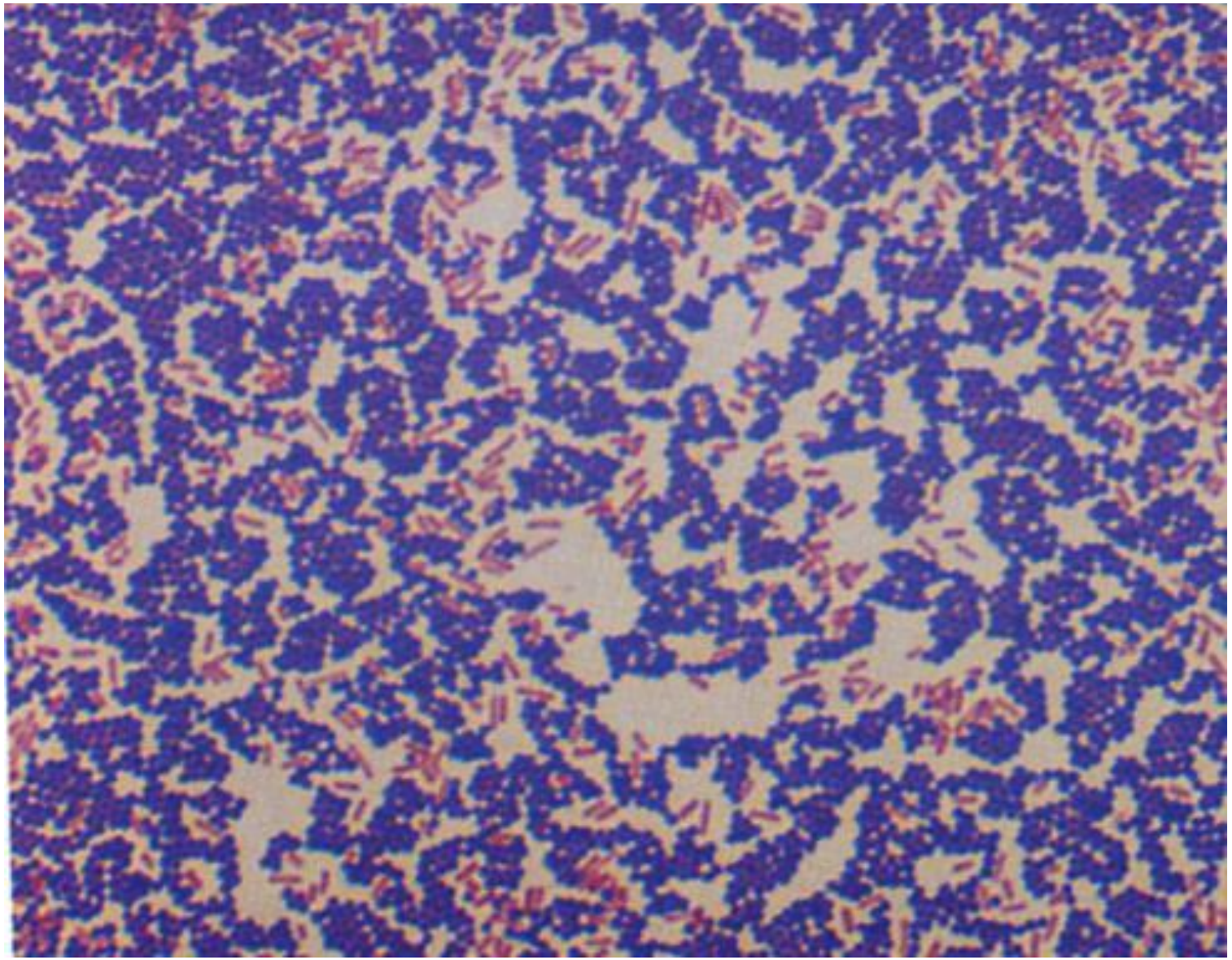


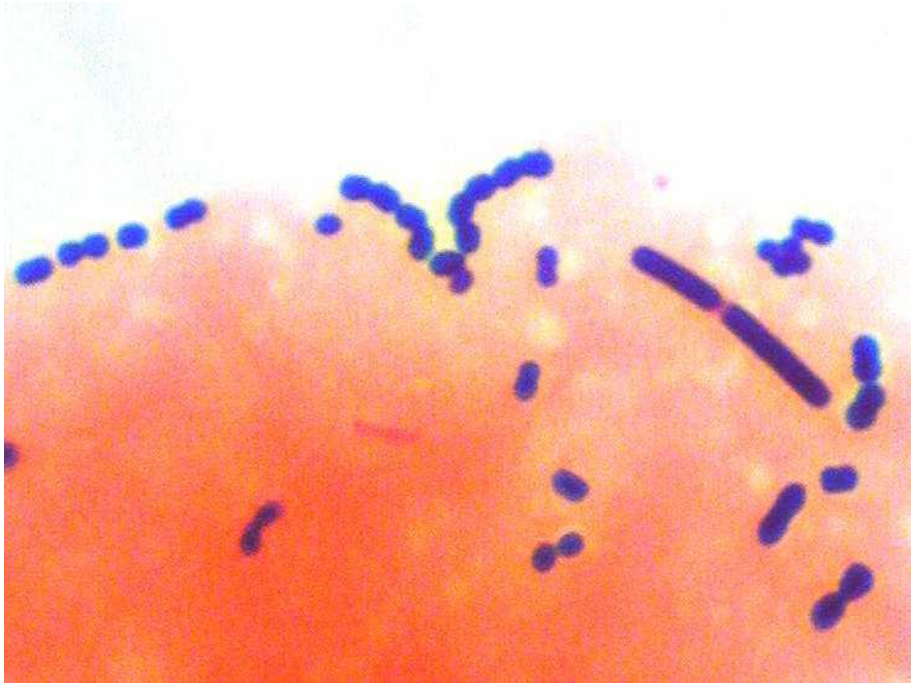
Staphylococcus aureus, 1μm

Escherichia coli, 1x3 μm

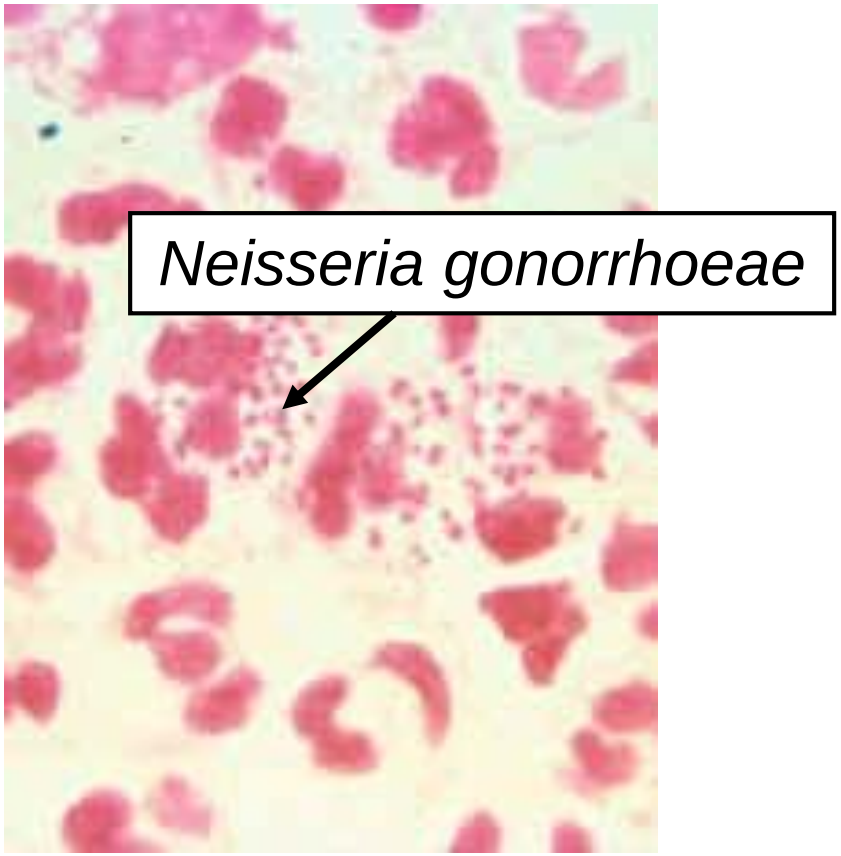


Gram stain of a mixture of *Staphylococcus aureus* and *Escherichia coli*





Gram stain of yogurt



Gram Stain of pus smear

Staining bacteria cells:

the acid-fast stain

History of the Acid-fast stain

- Paul Ehrlich was a German physician.
- He developed the acid-fast stain in 1882 as a means of staining the tubercle bacillus, *Mycobacterium tuberculosis*.
- His original method has undergone modifications by Ziehl and Neelsen that are still used today.

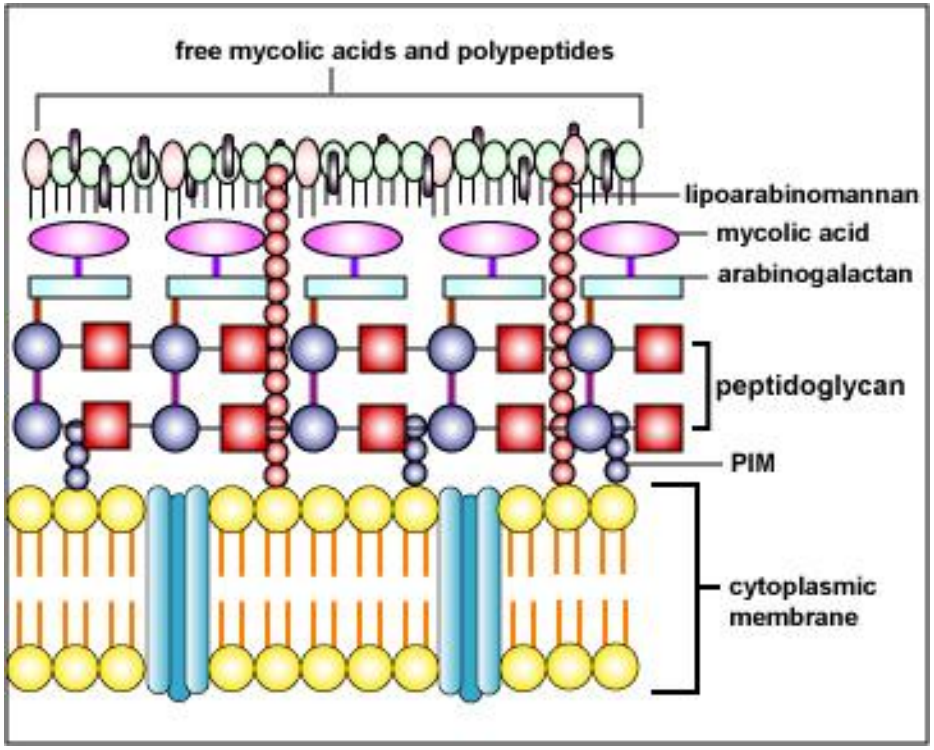
•The acid-fast stain distinguishes different types of bacteria based on the wax content of their cell wall.

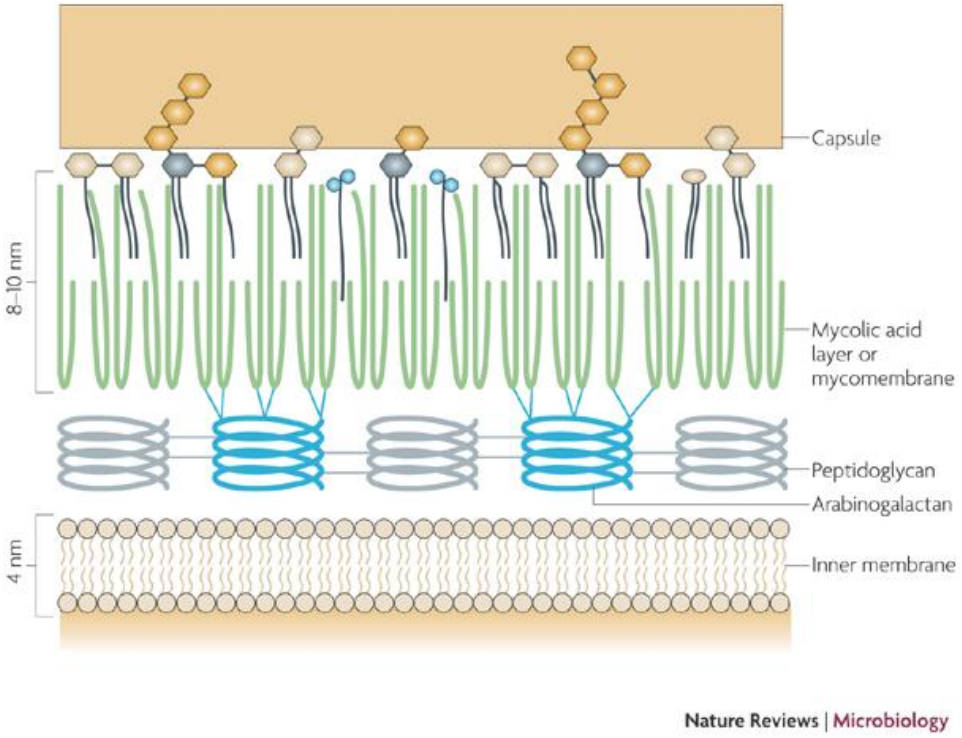
•Bacteria with a high wax content retain the primary stain carbolfuchsin when decolorized with acid-alcohol. These are acid-fast bacteria.

•Bacteria with a low wax content lose carbolfuchsin when decolorized with acid-alcohol and take up the counterstain methylen blue. These are non acid-fast bacteria.

•This stain is important in distinguishing acid-fast bacteria of the genus *Mycobacterium.*

Cell wall of *Mycobacterium tuberculosis*

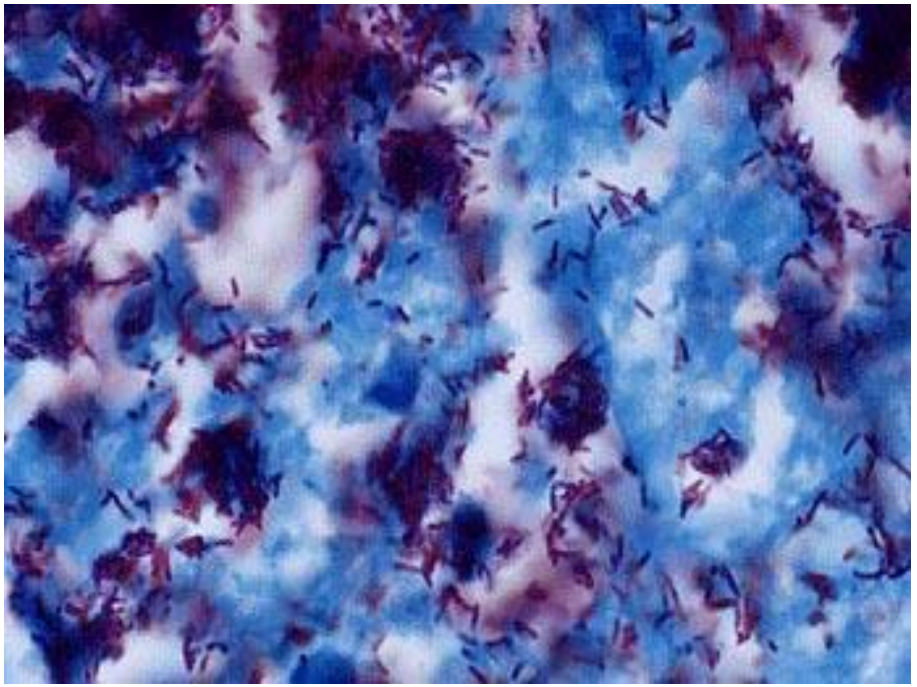


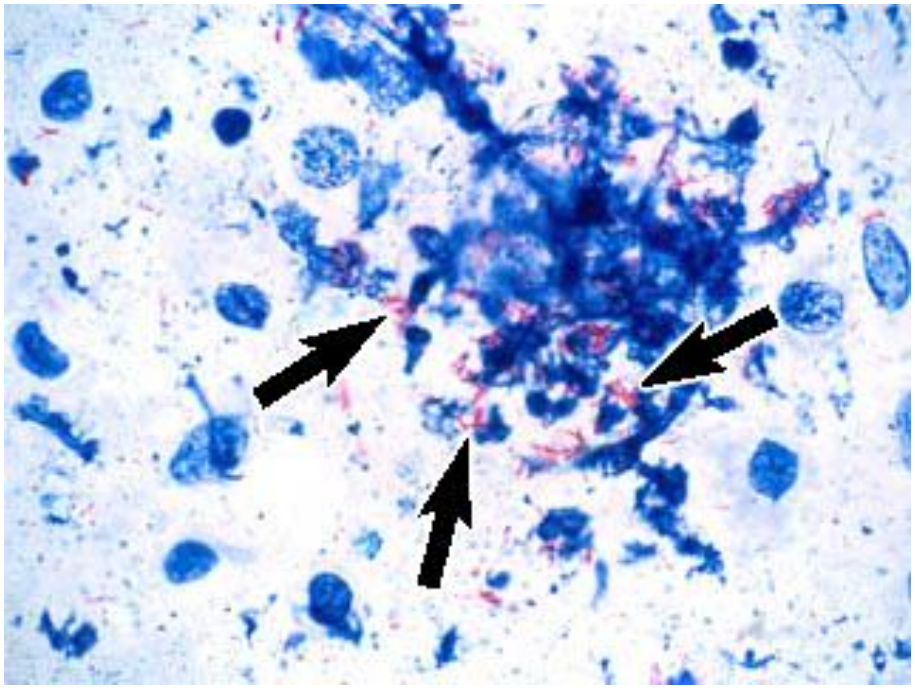


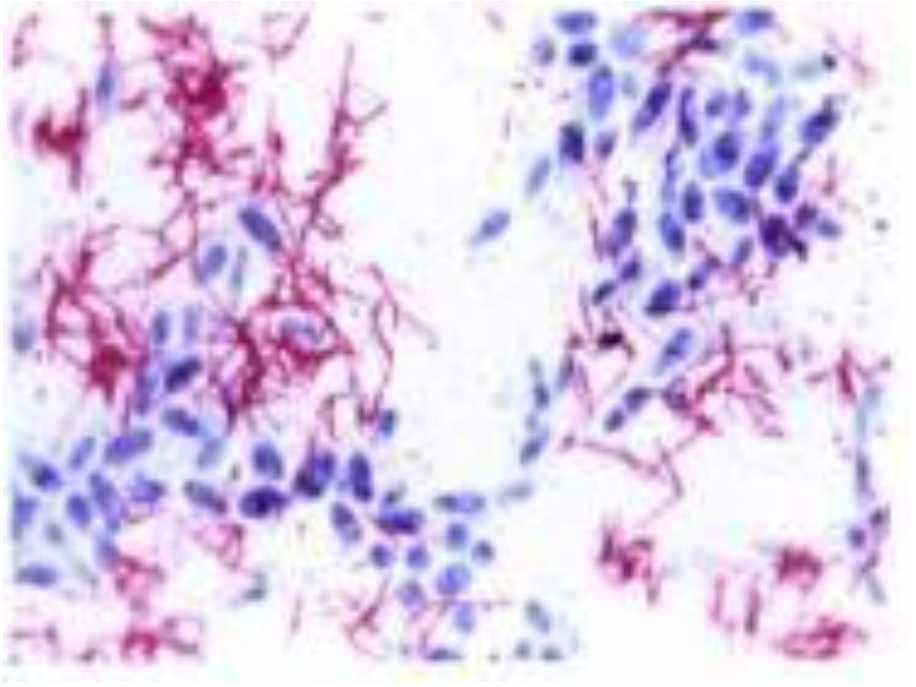
ACID-FAST STAIN PROCEDURE

1. Stain with carbolfuchsin……5 min. with heat
2. Wash off with tap water
4. Decolorizer Acid-Alcohol (3% HCl-Ethanol 95%)
5. Wash off with tap water
6. Counterstain with methylene blue……2 min.
7. Wash off with tap water
8. Blot dry with bibulous paper

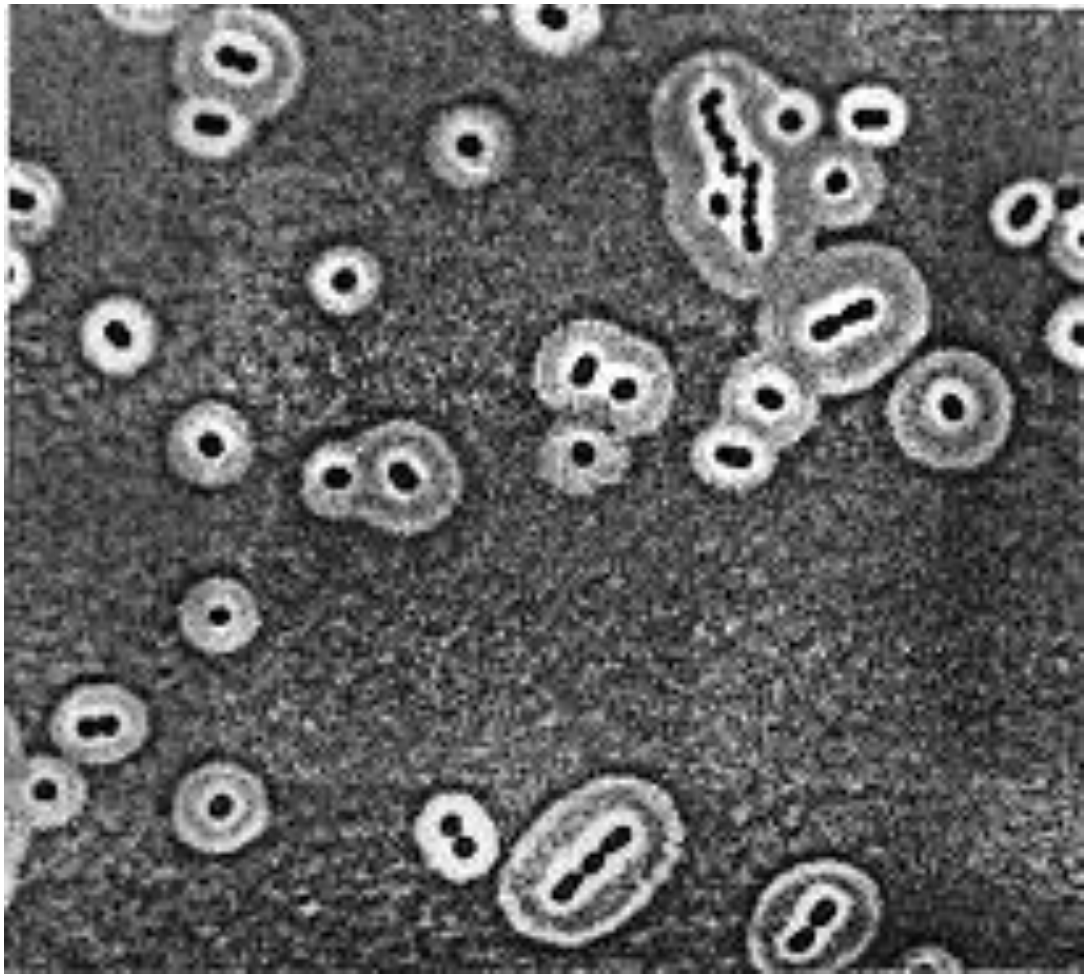
Acid Fast staining of Mycobacterium



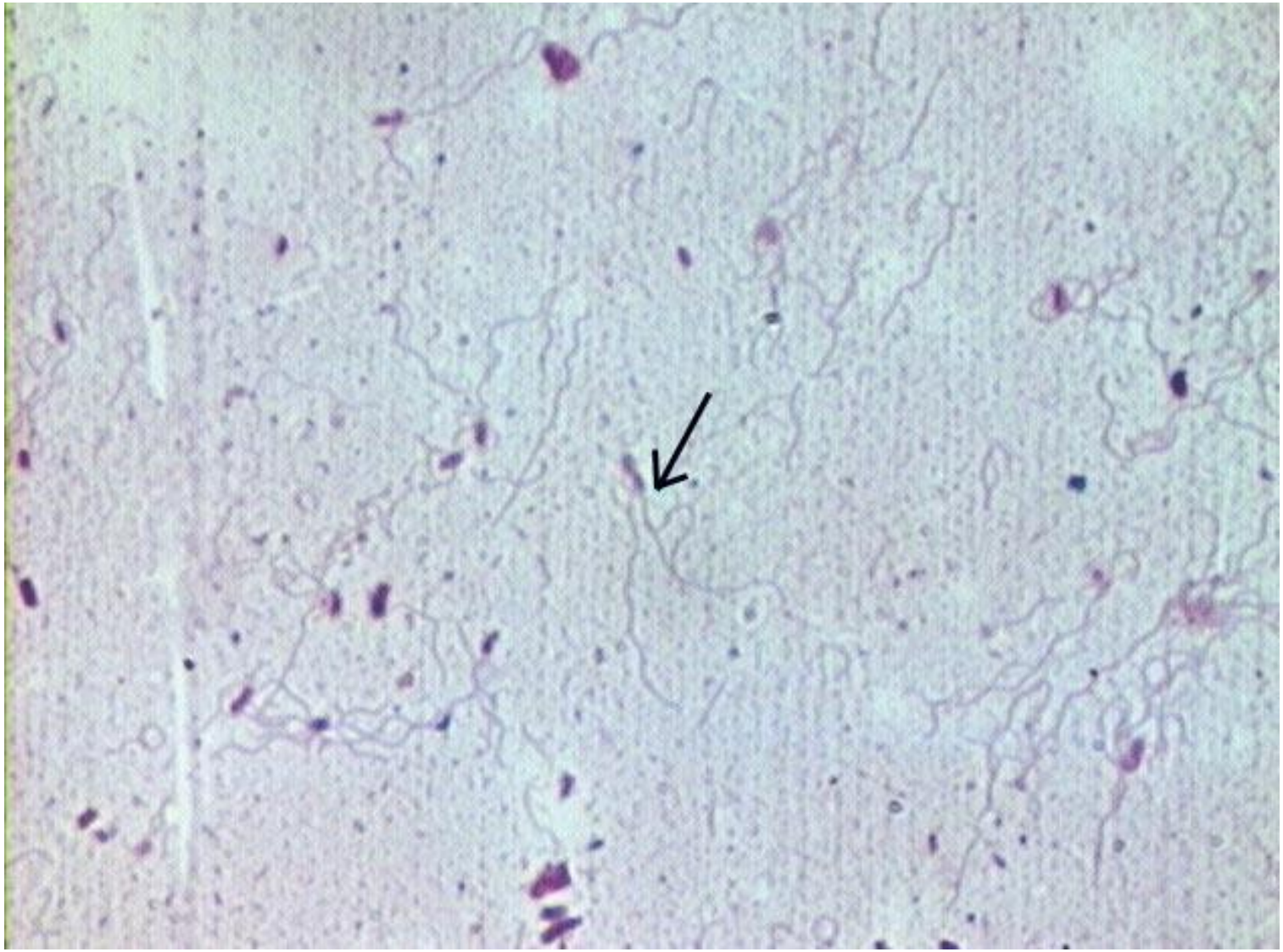




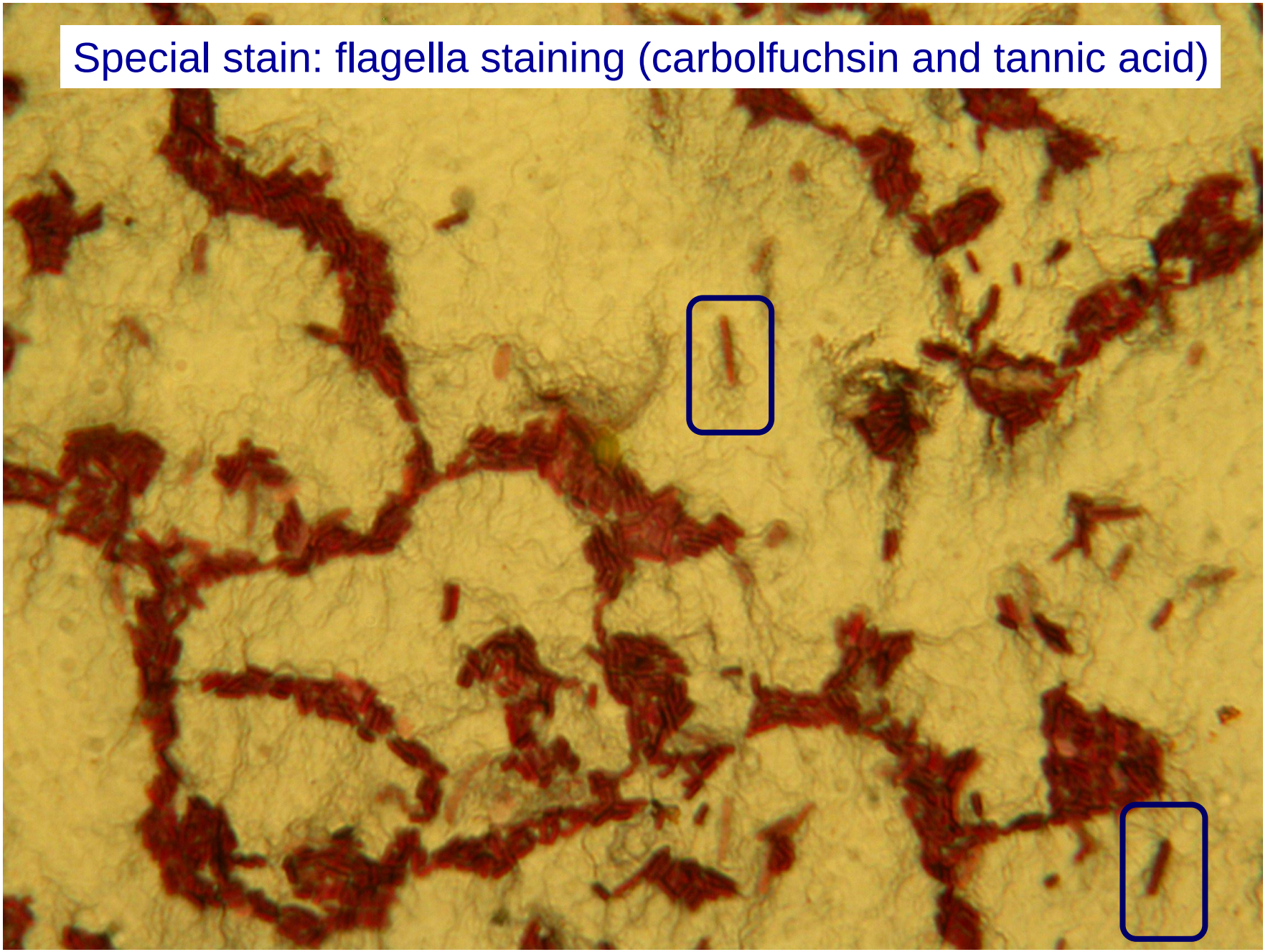
Special stain: negative staining for capsule with India ink



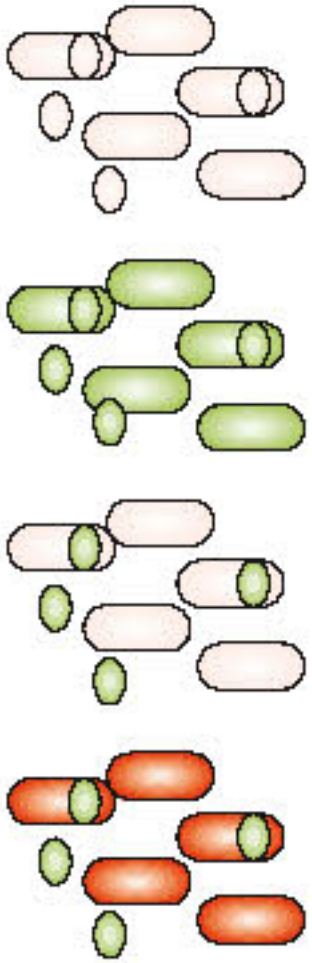
Special stain: flagella staining (carbolfuchsin and a mordant)



Special stain: flagella staining (carbolfuchsin and tannic acid)



Staining bacterial endospores (Schaeffer-Fulton - malachite green)



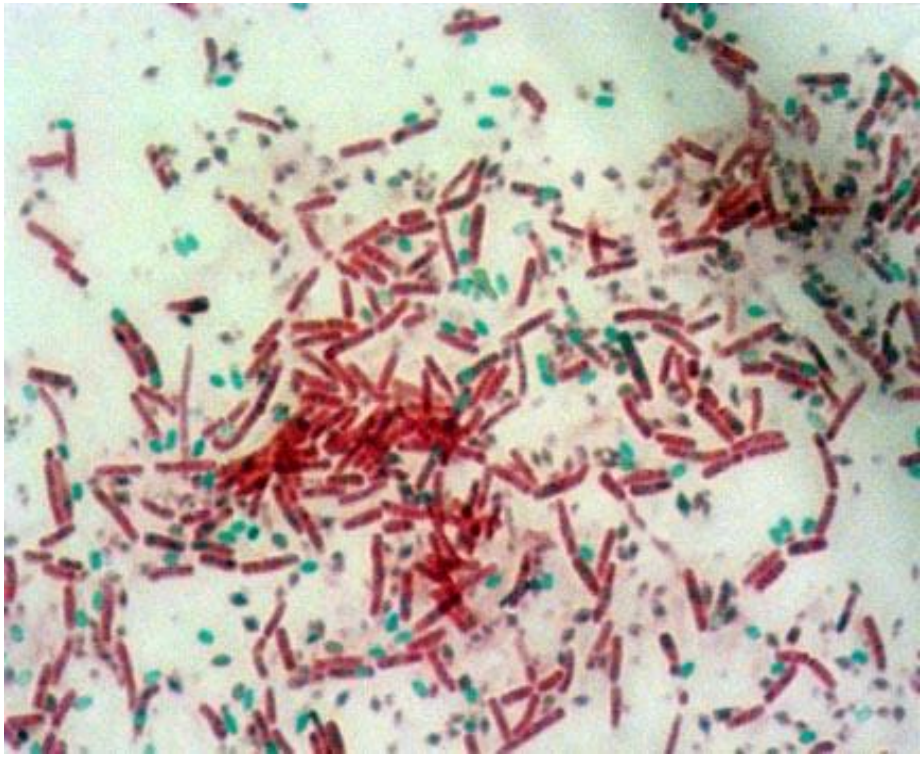
Batteri sporigeni

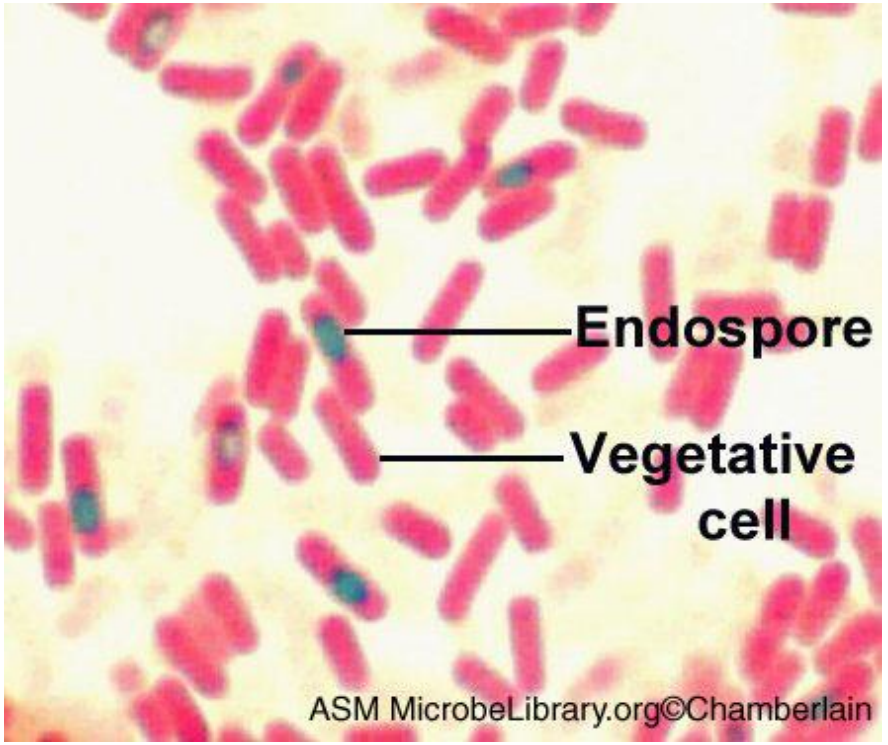
Colorazione a caldo con verde malachite

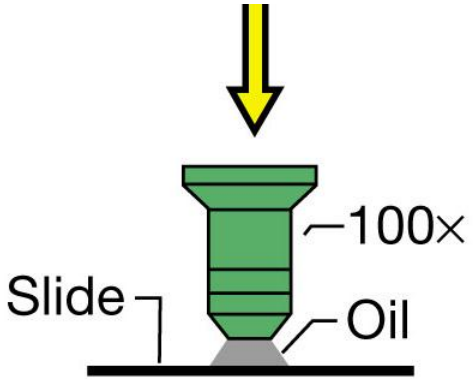
Lavaggio con acqua

Colorazione di contrasto (**safranina**)

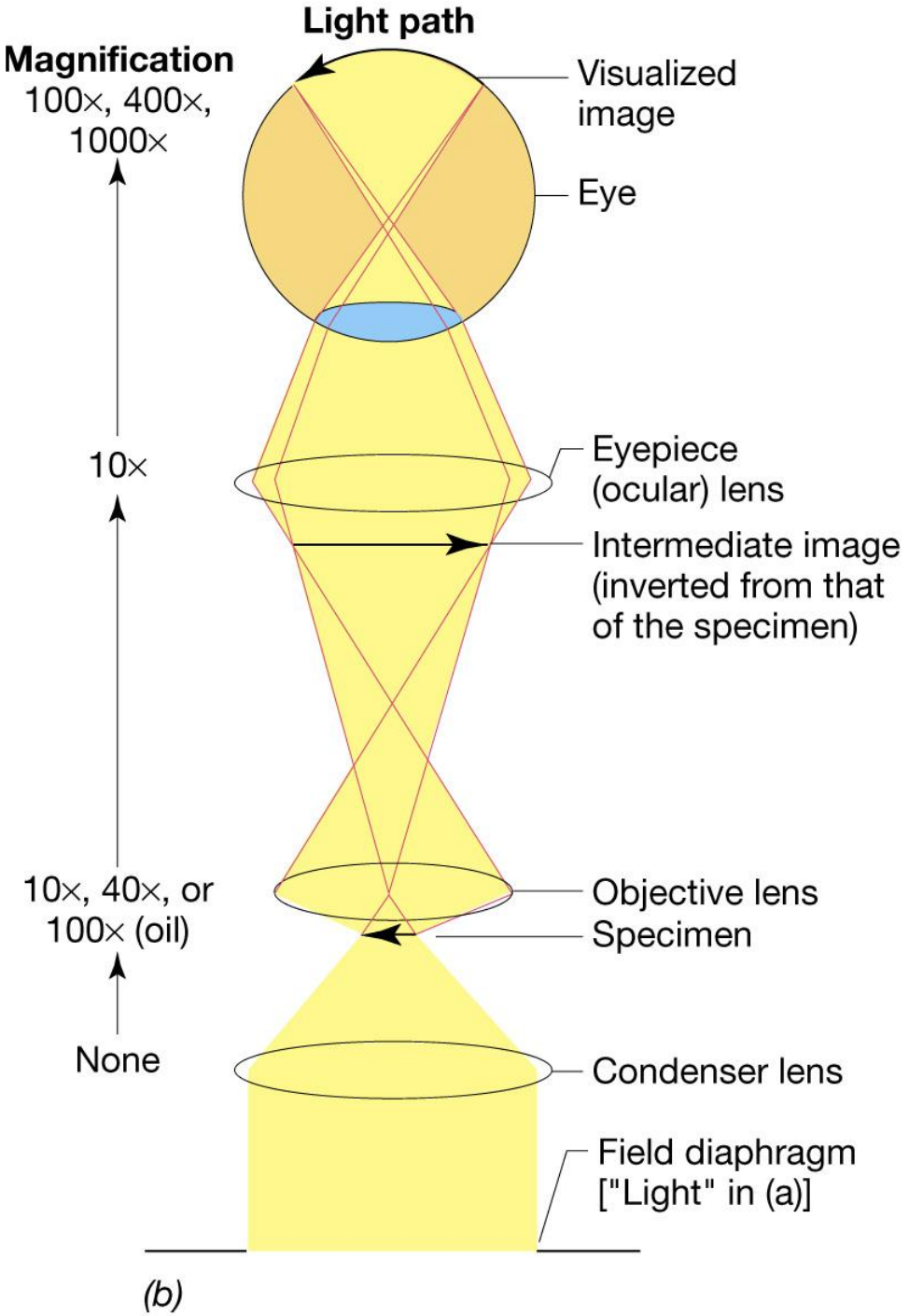
Special stain: endospore staining (malachite green)







Place drop of oil on slide;
examine with 100× objective


Light path
Magnification
100×, 400×,
1000×
Visualized
image
Eye
10×
Eyepiece
(ocular) lens
Intermediate image
(inverted from that
of the specimen)
10×, 40×, or
100× (oil)
Objective lens
Specimen
None
Condenser lens
Field diaphragm
["Light" in (a)]

(b)