Practical 2

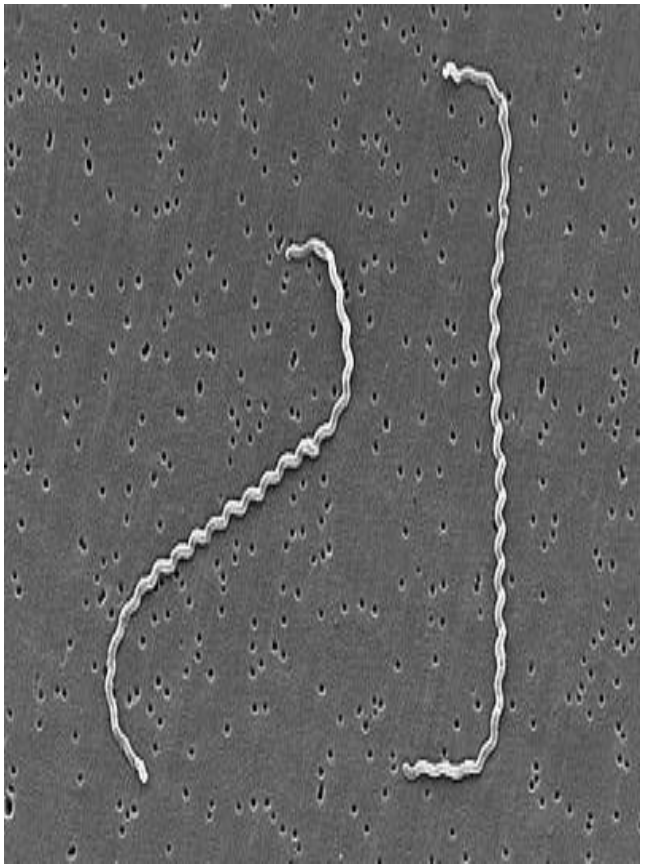
- Microscopy
- Wet mount smear
- Simple stain

Light microscope

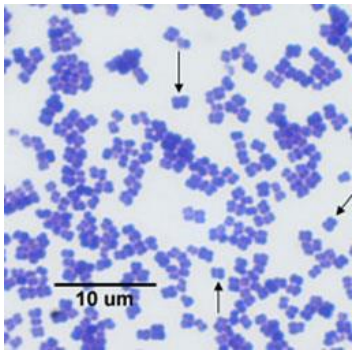
Index of light of the background and of the bacteria is almost the same = the discrimination is bad – native smear – living bacteria (movement, budding - stained, colored smears (the contrast between microbe and bacgound is greate) – better discrimination + identification of some subceluluar structures

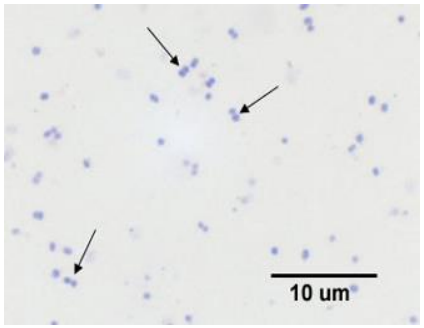
Darkfield microscopy – the sample is visualised by the light beam entering from the perifery – large angle (0,1/um – 0,2/um) – Treponema, Borrelia, Leptospira

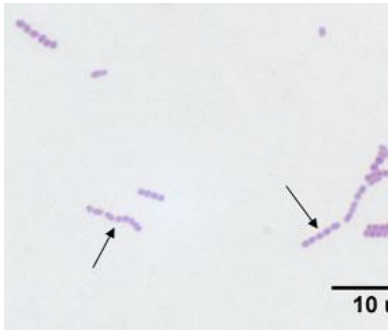
Microscopy with interference – filtration system visualise the phase differences of the light beam after its passing through objects with different density
The system anable 3 dimensional picture

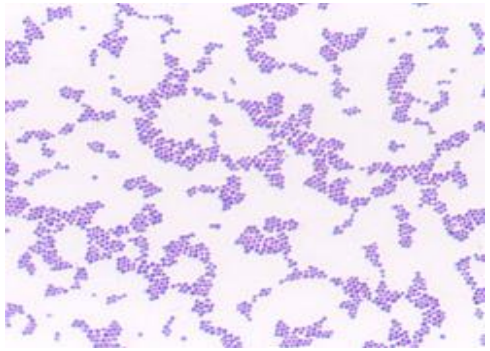


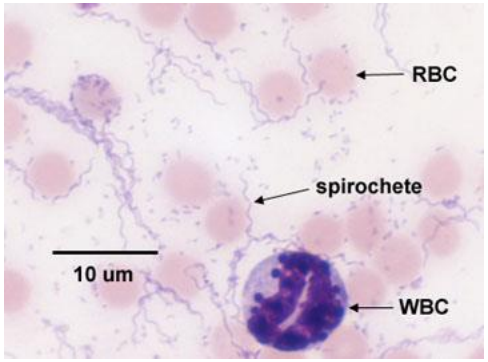
Improoving the discrimination properties of light microscope

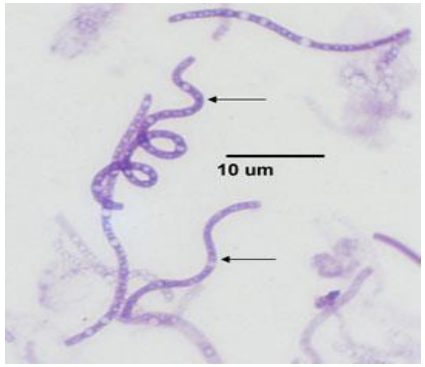




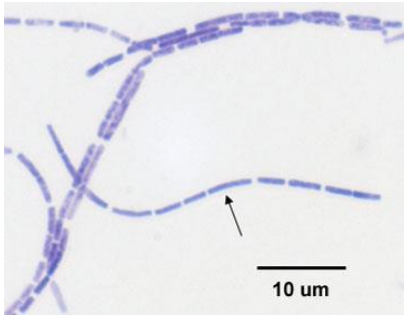


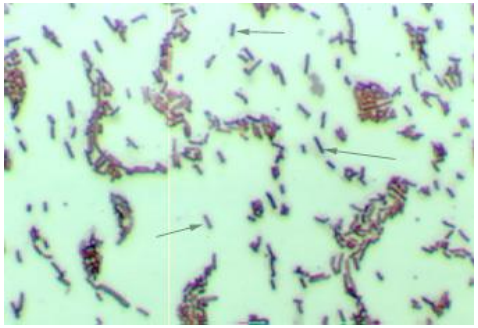


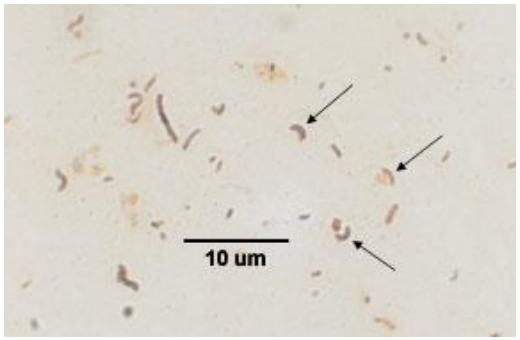




Vibrio, rod with spores, sprial filamentous rods, spirochets, staphylococci, streptobacili – rods in chain, streptococci, tetrades, diplococci



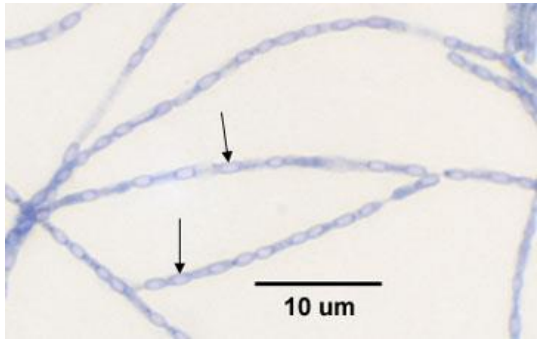


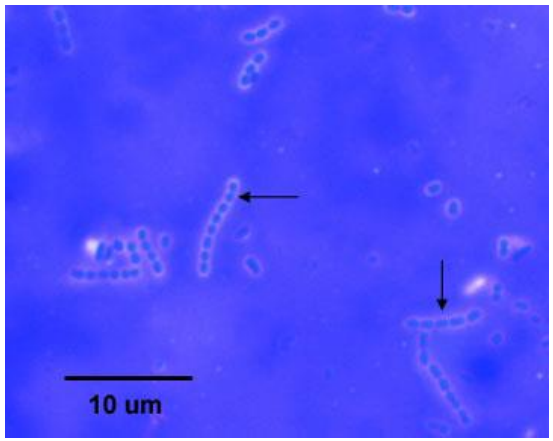


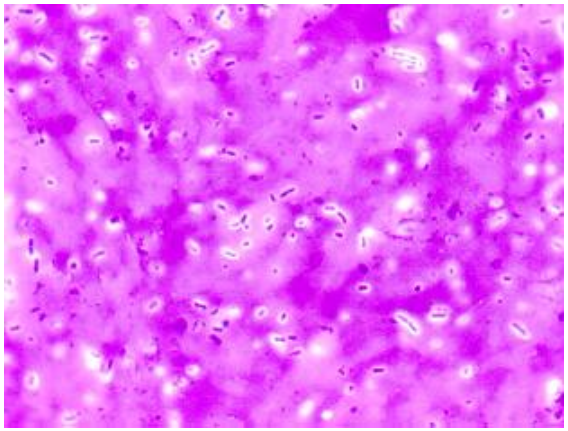
Negative staining

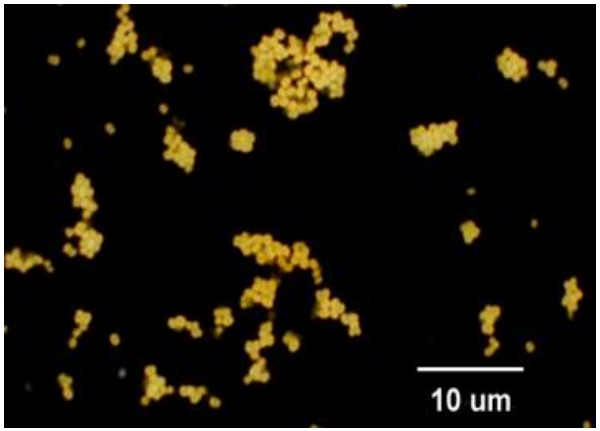
amelioration of the contrast against the dark background - Burri method – capsule is uncolored,

- Gram staining – negative staining of staphylococci or spores that are not stained



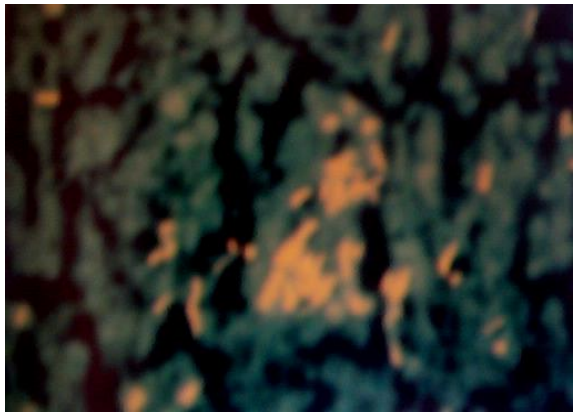






Fluorescent microscoy

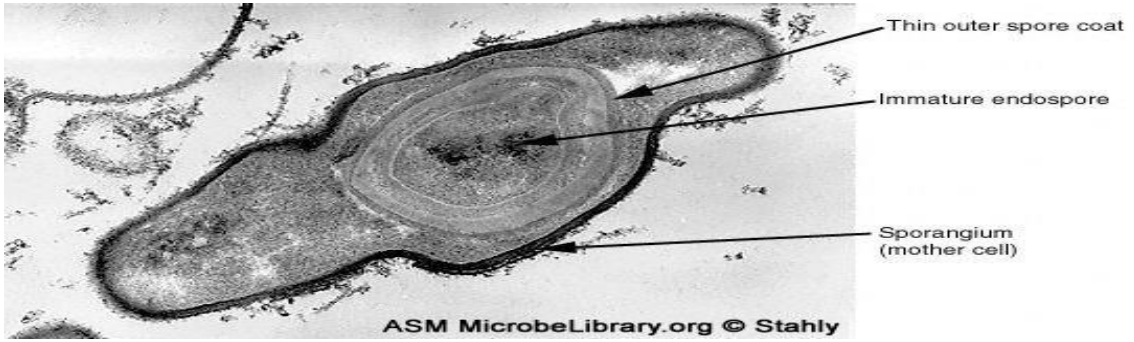
- Mercury vacuum lamp emits the light of shorter wavelenght
- The smear is prepared with fluorochromes – compositions that can absorbe the short lenght´s ultraviolet or ultrablue light and can emit energy of higher wavelenght
  Fluorochromes that stain the smear – fluorescence staining – that after the beam touch it will emit the green fluorescein light
- Highly sensitive



Electron microscop

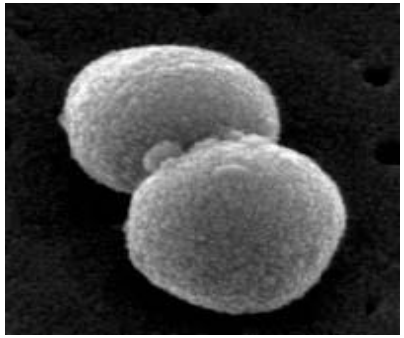
- Uses the magnet – not lenses – to direct the beam of electrons throught speciment to the screen. This process uses the shorter wavelenghts of light.Higher discrimination
- Visualisation of viruses and subcellular structures

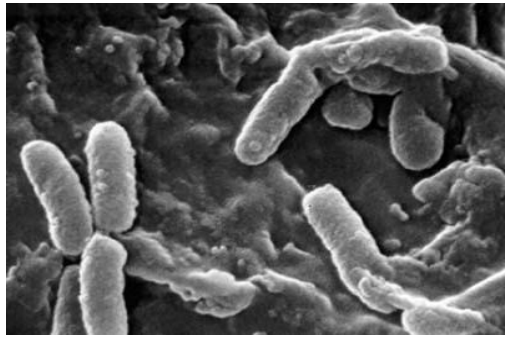
- 2 types :



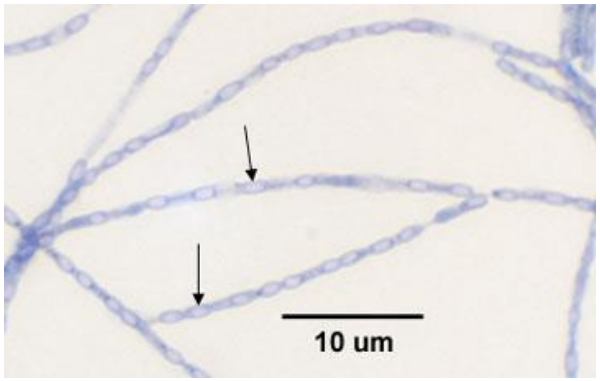
– transmission – beams enter directly throught the sample

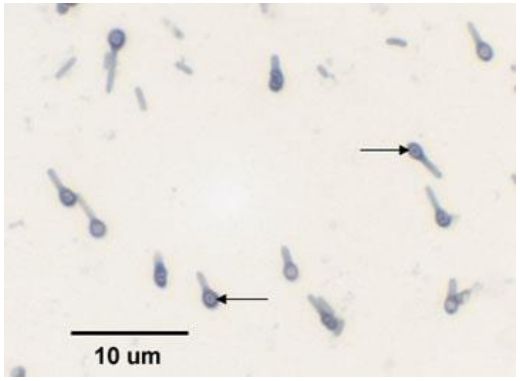
– scanning – beams enter the sample in the angle 3 dimmensional picture

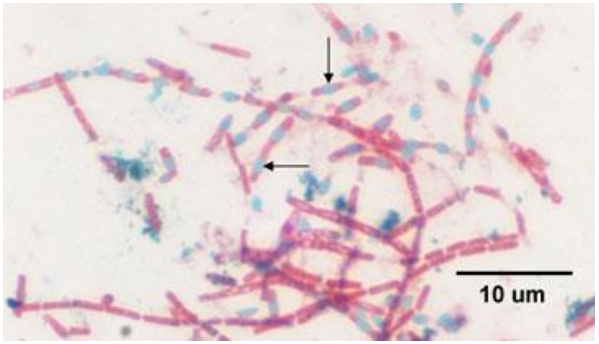


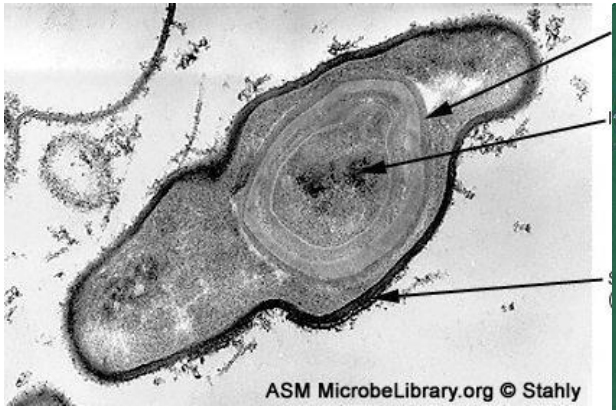


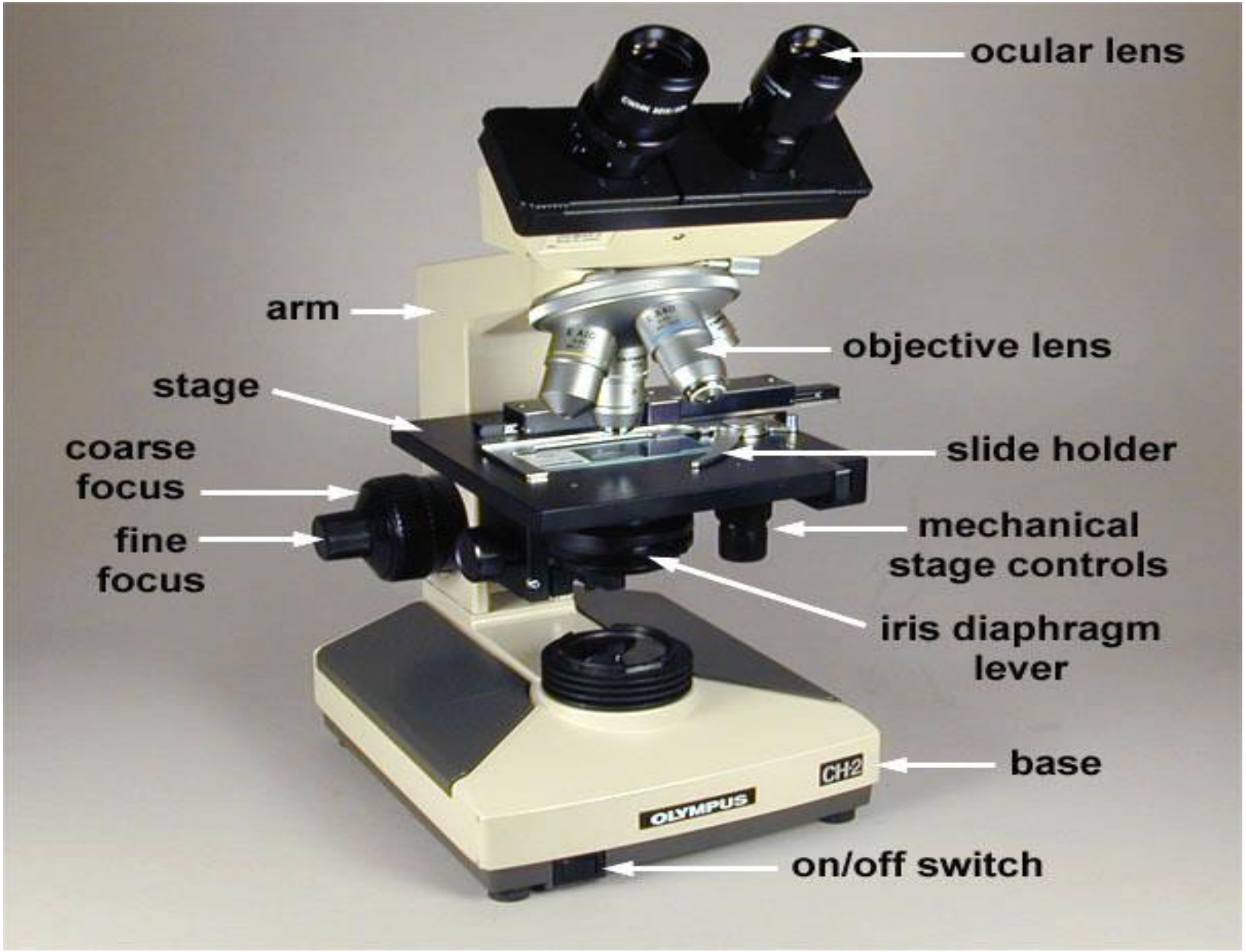
Possibilities in visualisation of bacterial spores negative staining, WirtzConcklin, transmission electron microscopy, scanning electron microscopy









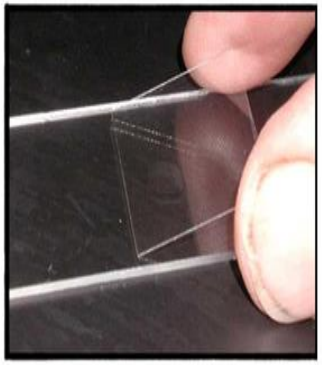

ocular lens
arm
objective lens
stage
slide holder
coarse focus
fine focus
mechanical stage controls
iris diaphragm lever
base
on/off switch
OLYMPUS
CH2

Native smear- Wet mount slide

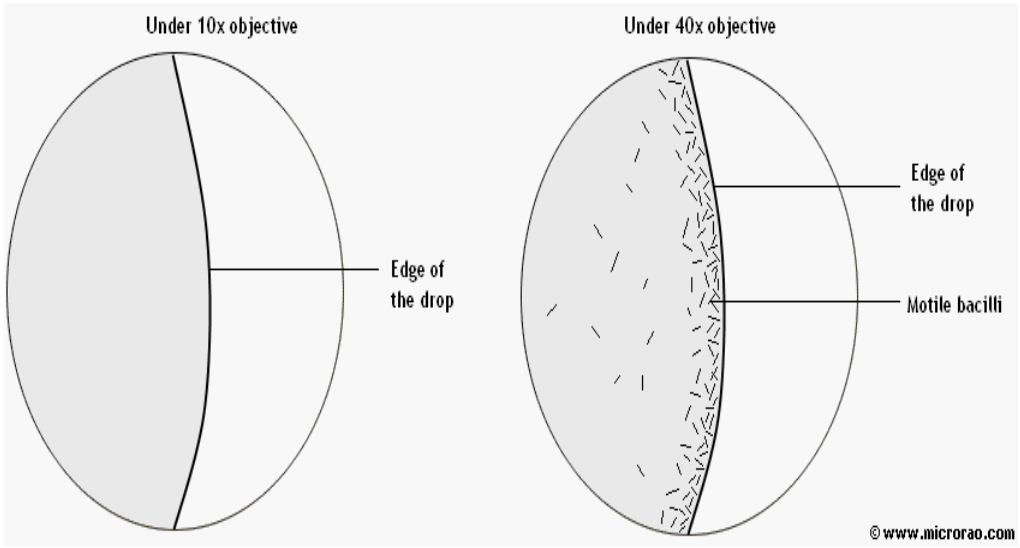
- – suspension of bacterial culture is prepared on slide and is covered by cover slide without staining.
- We can identify movement and morphology, in parasites also structure

Slide Preparation: Wet Mount

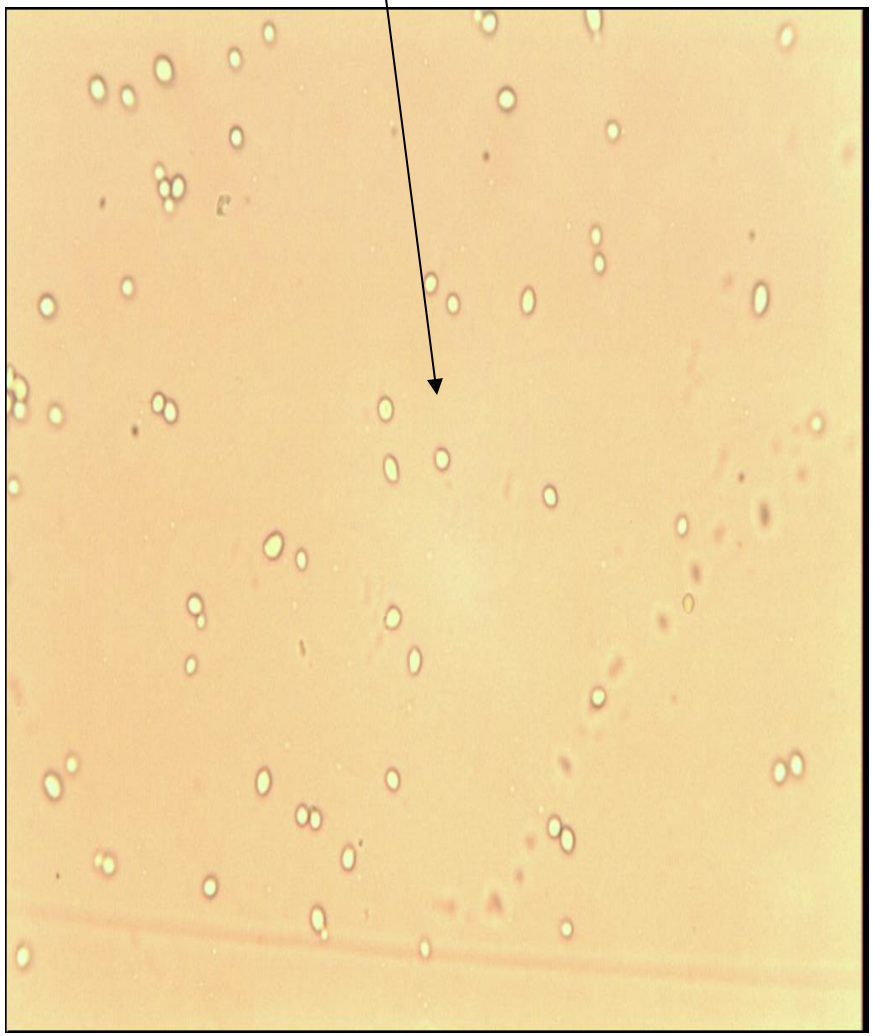
- Agar Culture :Place a loop full of water onto a clean glass slide.
- Flame your loop, pick up a small amount of culture (*Candida albicans, Escherichia coli*), and mix it into the drop of water without spreading it out too much.



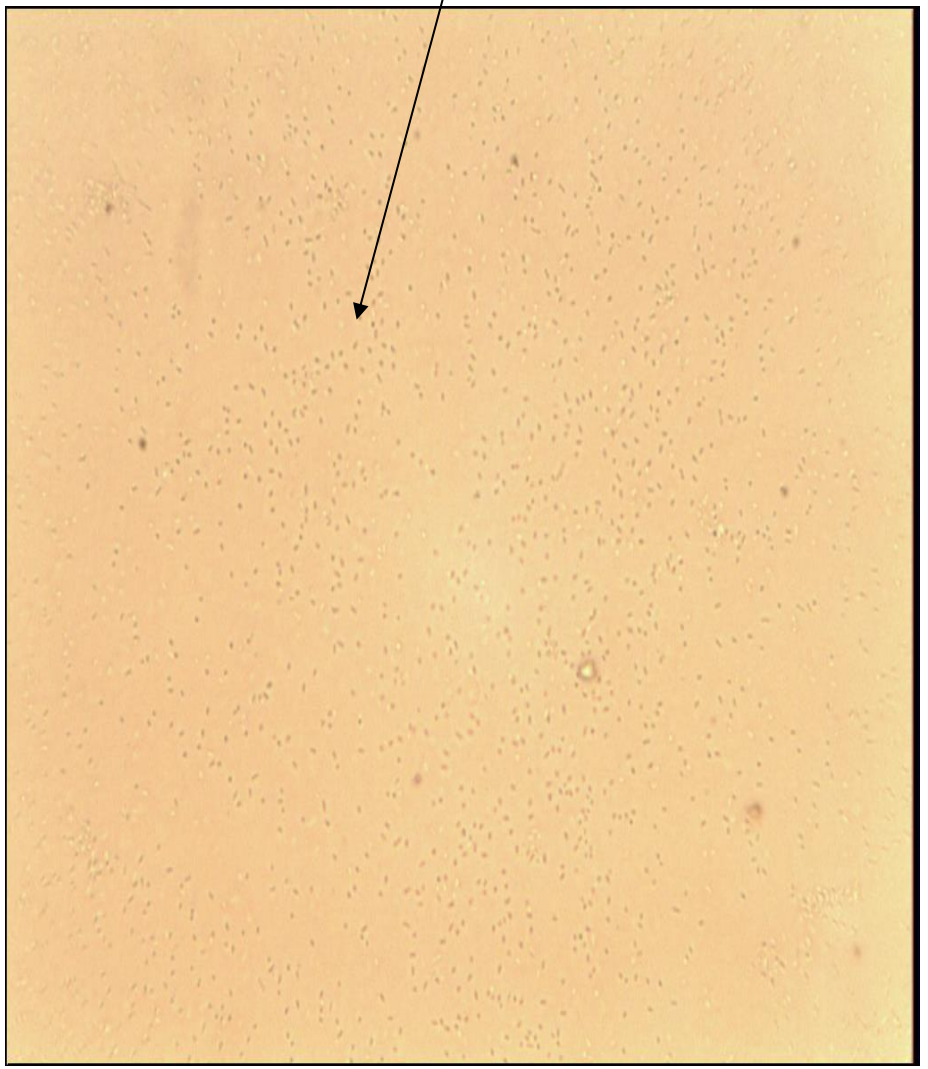
3. Place a cover slip over your sample. By angling the cover slip as you place it on the slide you remove air bubbles from the wet mount.



Candida albicans



Escherichia coli



Simple staining

- The use of a single stain or dye to color a bacterium is called a simple stain.
- These types of dyes, called basic dyes, are positively charged, containing cationic chromophores.
- Because the bacterial cells are slightly negatively charged, there is an attraction between the positive dye and the negative cell.

Some common basic dyes used in staining are:

methylene blue,

crystal violet,

basic fuchsin.

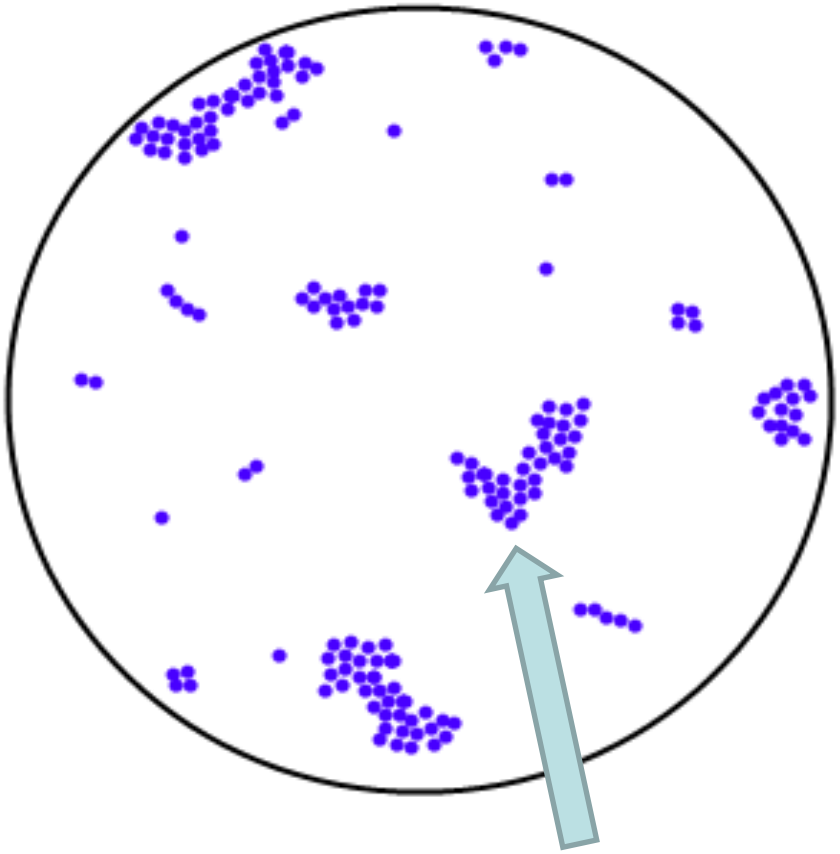
Stained smears

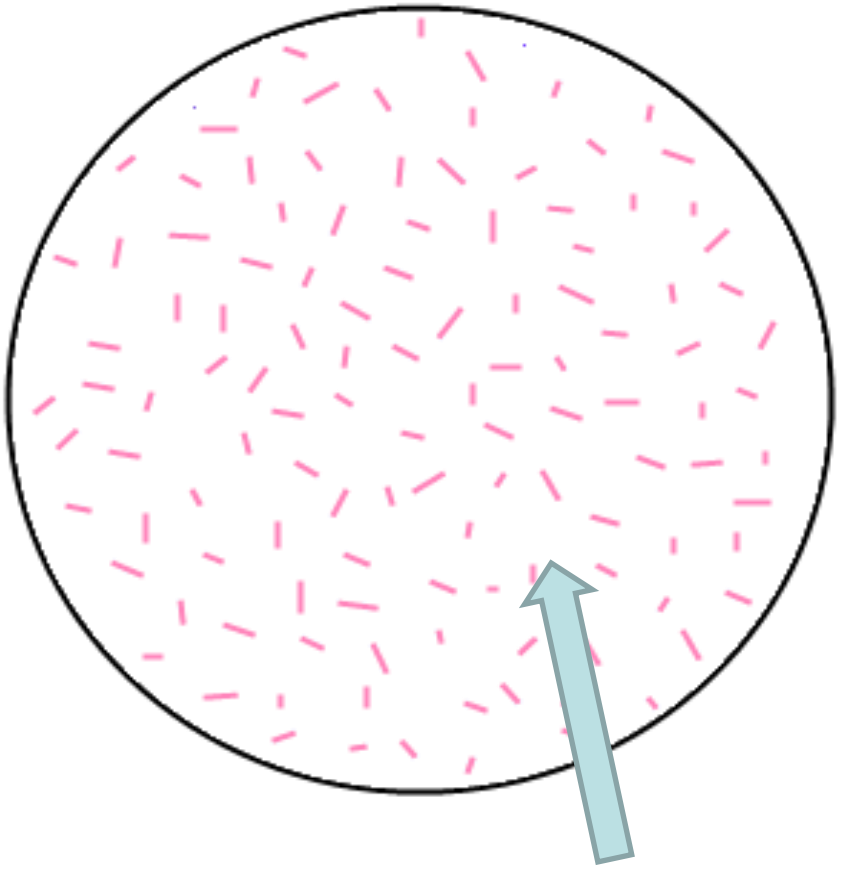
- – simple (orientation) staining – application of one color on fixed smear - identification of morphology and alignement

- After application of basic stain, this is bound on negatively charged parts in protoplasma (phosphates of nucleic acids)

Microbiology Lab Slide Prep: Simple Smear

- Prepare bacterial smears for the microscopic visualization of bacteria.
- 2. Perform a simple staining procedure.
- 3. Compare the shapes and arrangements of bacterial cells.





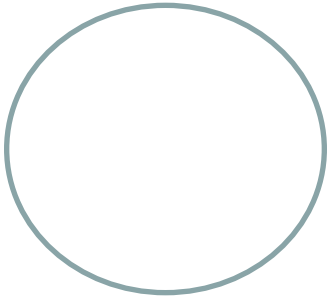
- **simple staining with methylen blue showing cocci**

Simple staining with dilute carbol fuchsin showing bacilli

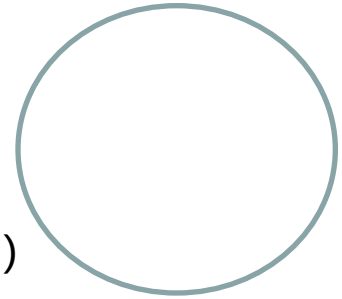
1. Prepare native smear : *a.) Candida albicans* – passive movement

b.) Escherichia coli- active movement

a.)



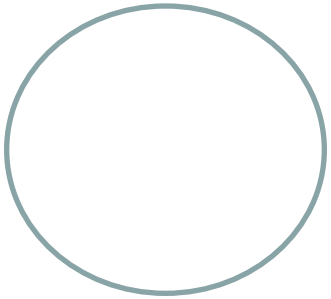
b.)



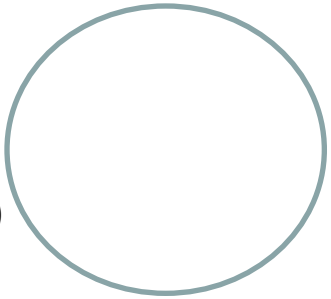
2. Prepare fixed simple smear: a.) *Staphylococcus epidermidis* – methylene blue

b.) *Escherichia coli* – methylene blue

a.)

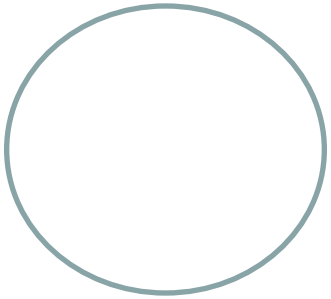


b.)

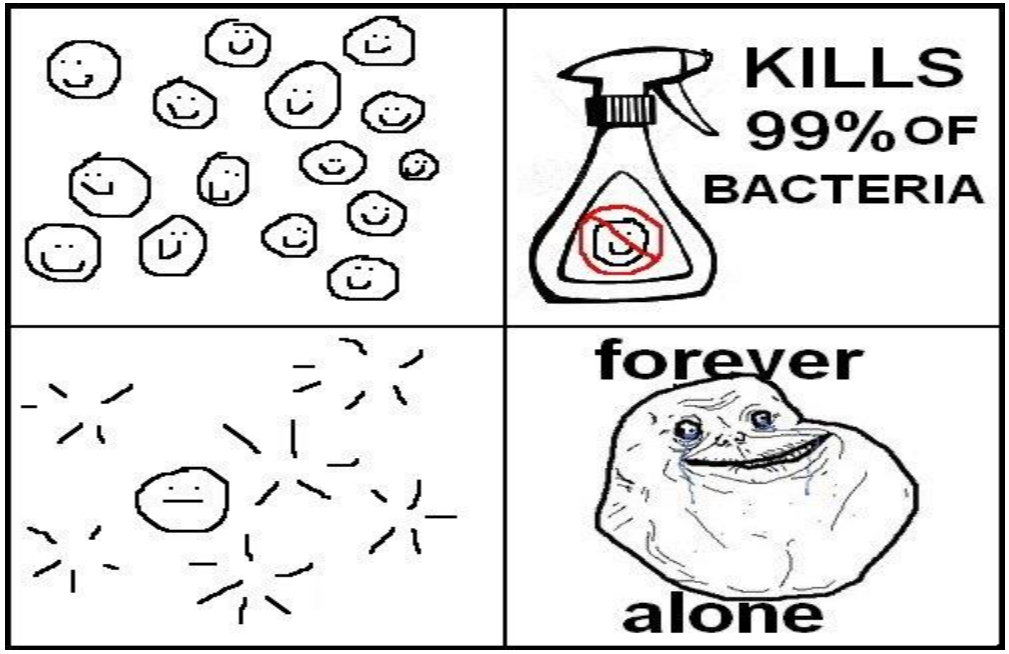


3. Prepare fixed simple smear (mixed):

Bacillus cereus (blood agar culture) and *Streptococcus pyogenes* (broth culture)-carbol fuchsin



THE END



Wash your hands, please ☺