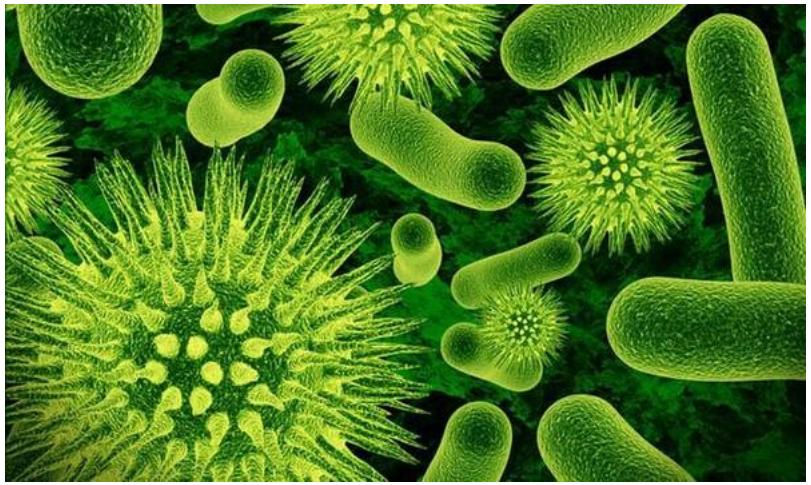
LESSON №1

MICROBIOLOGY
MICROBIOLOGICAL LABORATORY
SYSTEMATICS OF MICROORGANISMS
MORPHOLOGY OF MICROORGANIMS



SAFETY RULES

1. Always wear lab coats and caps
2. Don’t put your bags and personal things on lab table
3. DON’T EAT, DRINK AND SMOKE IN THE DEPARTMENT
4. Used reagents and materials or pipettes put in bottle with disinfectant
5. If dangerous material got on the table, floor or clothes tell about it immediately to professor or lab technician and strictly follow his recommendations
6. Table must be clean all time
7. Be careful with the equipment
8. Wash your hands with soap after lesson

IT IS STRICKTLY PROHIBITED

- to pump fluid into the pipette by mouth
- to move a burning spirit lamp
- to light up one burner from the other

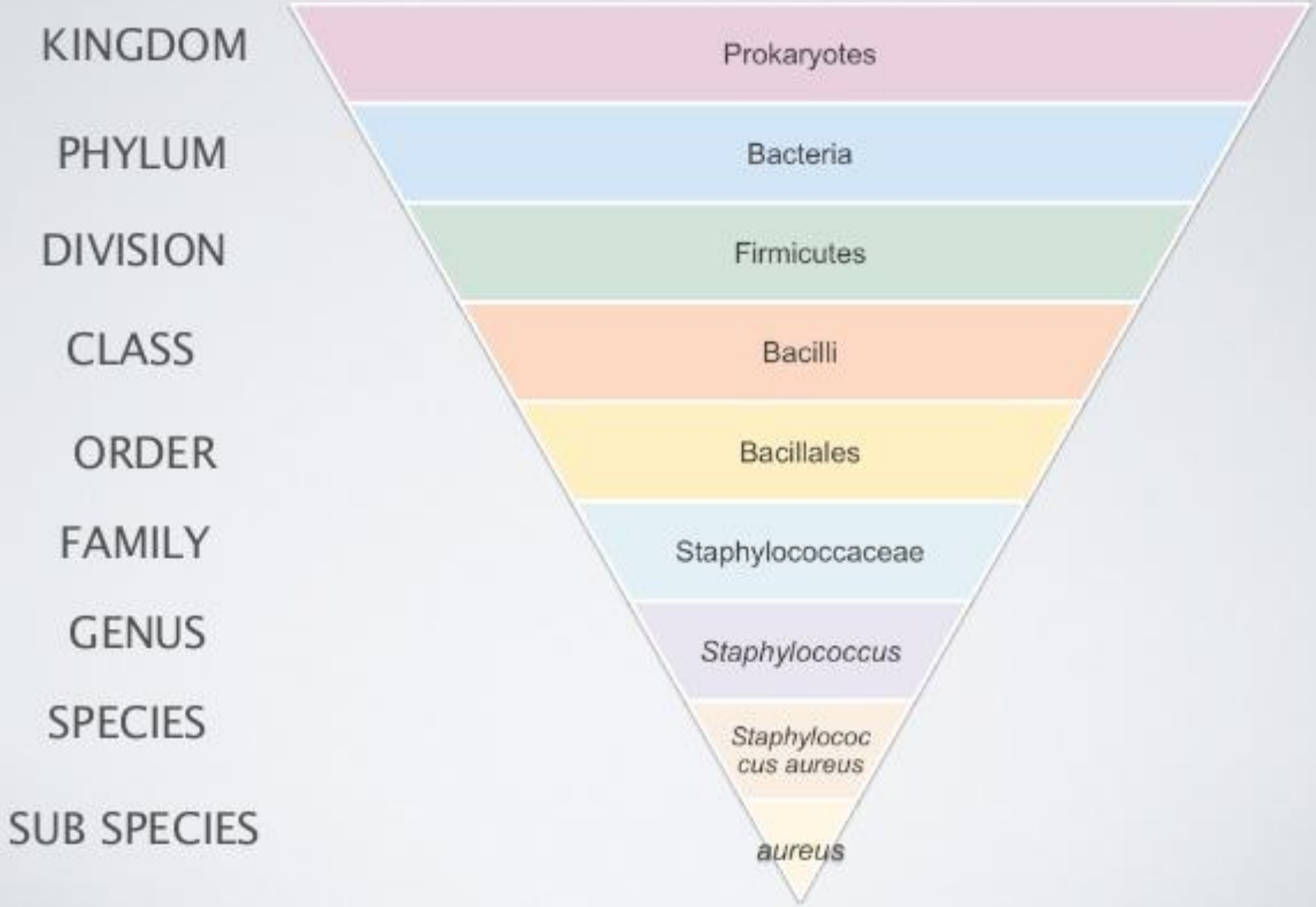
PURPOSES

- ✔ to get acquainted with principles of organization, equipment of microbiology laboratory and rules of work in it
- ✔ to get acquainted with main microscopic methods, preparation of bacterial smears, simple staining and immersion microscopy technique and main morphological forms of bacteria

MICROBIOLOGY

- ✔ **Microbiology** (from Greek μῑκρος, *mīkros*, "small"; βίος, *bios*, "life"; and -λογία, *-logia*) is the study of microorganisms, those being unicellular(single cell), multicellular (cell colony), or acellular (lacking cells)
- ✔ Microbiology encompasses numerous sub-disciplines
  including virology, parasitology,
  mycology and bacteriology

taxonomic hierarchy (Fig.3)

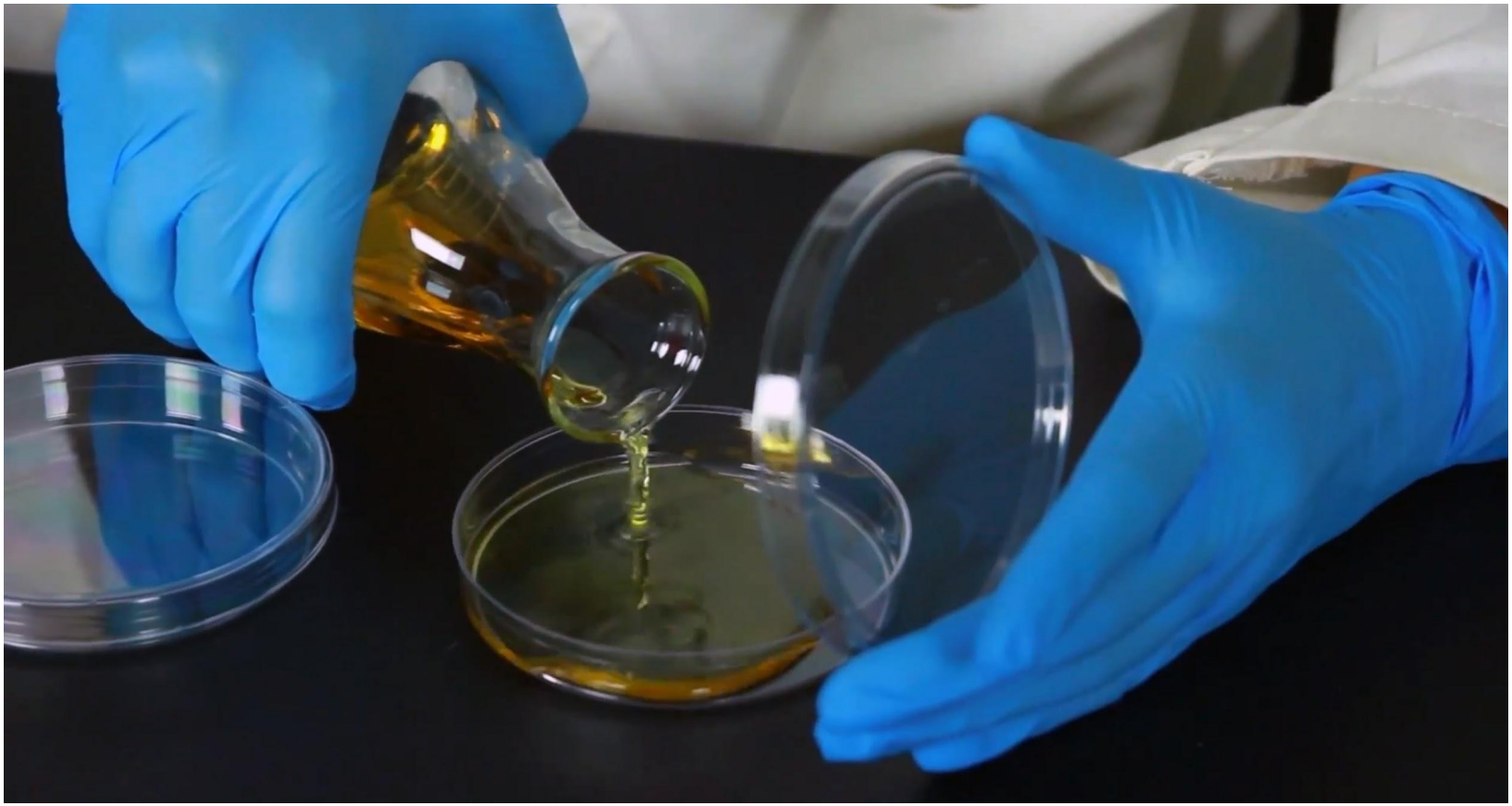


Nomenclature

- *International code for Nomenclature of Bacteria*
- Uses two-word naming system: Binomial Nomenclature
  - First name is the Genus, capital
  - Second name is the species, lower case
  - Both are italicized
  - Example: *Escherichia coli,* or *E.coli*
  - Strains; minor differences with in species:
    - *E. coli* strain B or *E.coli* strain K-12

MICROBIOLOGICAL LABORATORY

Laboratory rooms and laminar flow cabinets are designed for specific activities in aseptic conditions



Room for preparation of nutrient media

Table automatic boiler for the preparation of small volumes of nutrient media

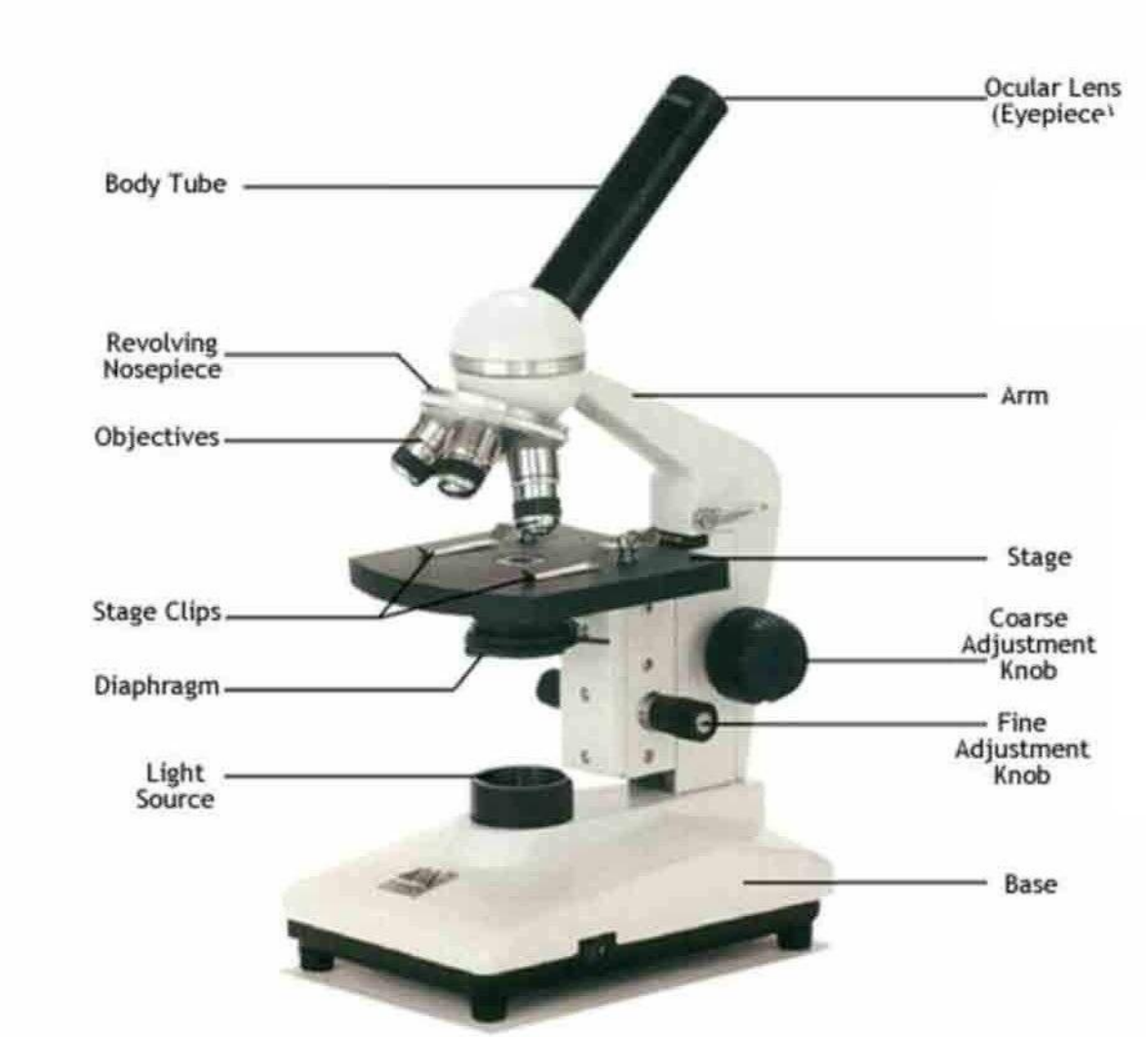
Specially equipped rooms for sterilization of nutrient media, laboratory glassware, disinfection of infectious material

Vivarium for laboratory animals

LABORATORY EQUIPMENT

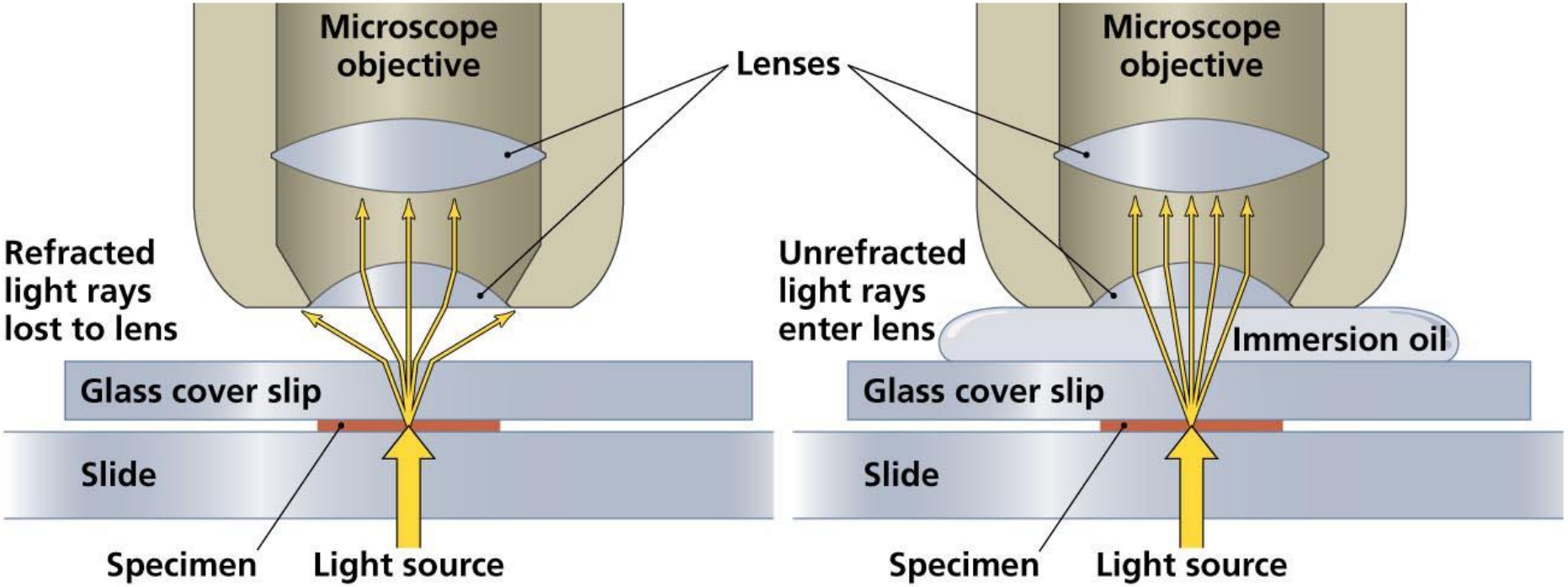
- ✔ Biological immersion microscope
- ✔ Instruments: inoculation loops, spatulas, tweezers, spirit lamps, etc
- ✔ Laboratory glassware: tubes, Petri dishes, flasks, pipettes, etc
- ✔ Devices for sterilization of glassware, nutrient media, reagents, pH meters, distillers, centrifuges, technical and analytical balances, filtering equipment, etc
- ✔ Other fire and chemical safety equipment (fire extinguishers, disinfectants, etc)

BIOLOGICAL IMMERSION MICROSCOPE

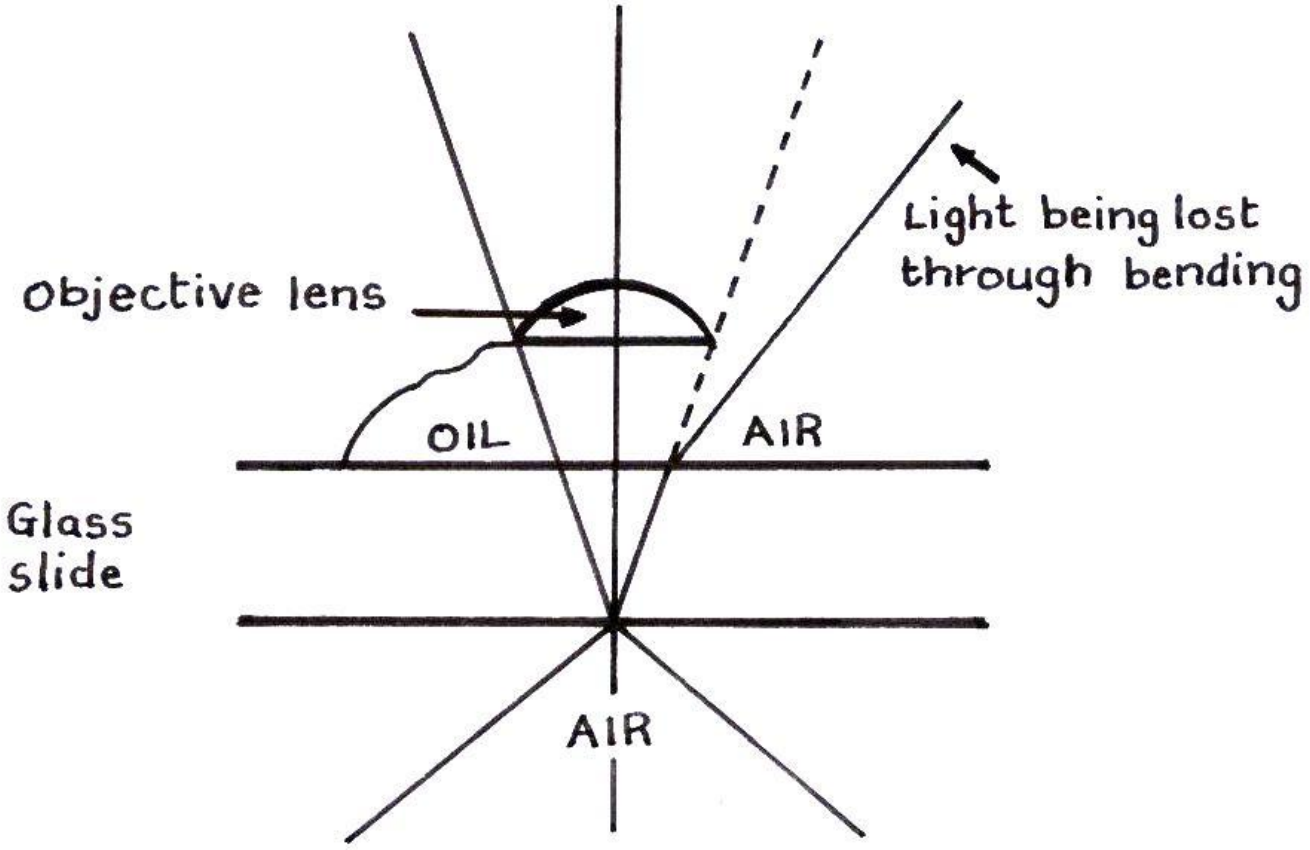


TASK 1 (P. 13) NAME PARTS OF LIGHT MICROSCOPE

IMMERSION MICROSCOPY



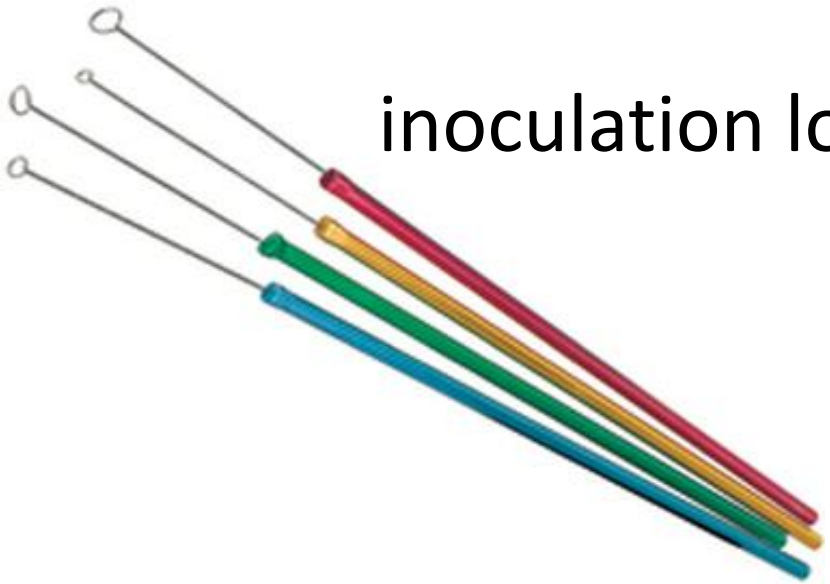
IMMERSION MICROSCOPY



TASK 2 (P. 13) DRAW WAY OF LIGHT IN IMMERSION SYSTEM

INSTRUMENTS

inoculation loops

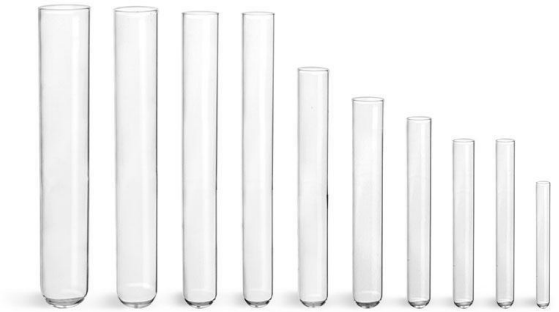


spatula

tweezers

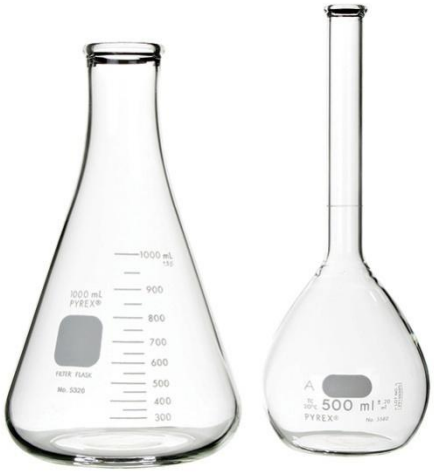
spirit lamp

LABORATORY GLASSWARE

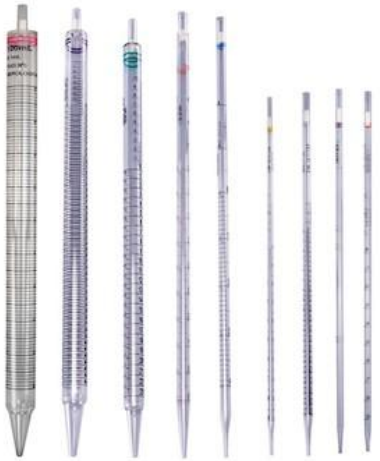


test tubes

Petri dish



flasks



pipettes

DEVICES FOR STERILIZATION

autoclave

Pasteur oven

NUTRIENT MEDIA

1- SIMPLE MEDIA
b- Nutrient agar

Nutrient Broth
+
2% agar-agar

Blood agar

Endo media

REAGENTS

pH Meters


OMEGA
PHH222
3.78
250
PH METER


8.6
pH · °C/°F
waterproof
pHep®
by HANNA

DISTILLERS

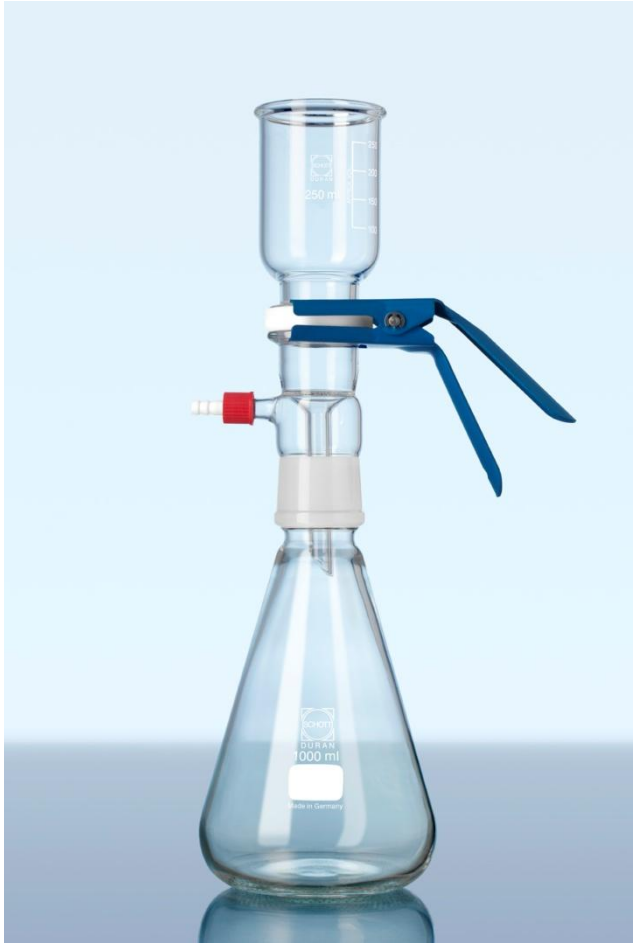
CENTRIFUGES

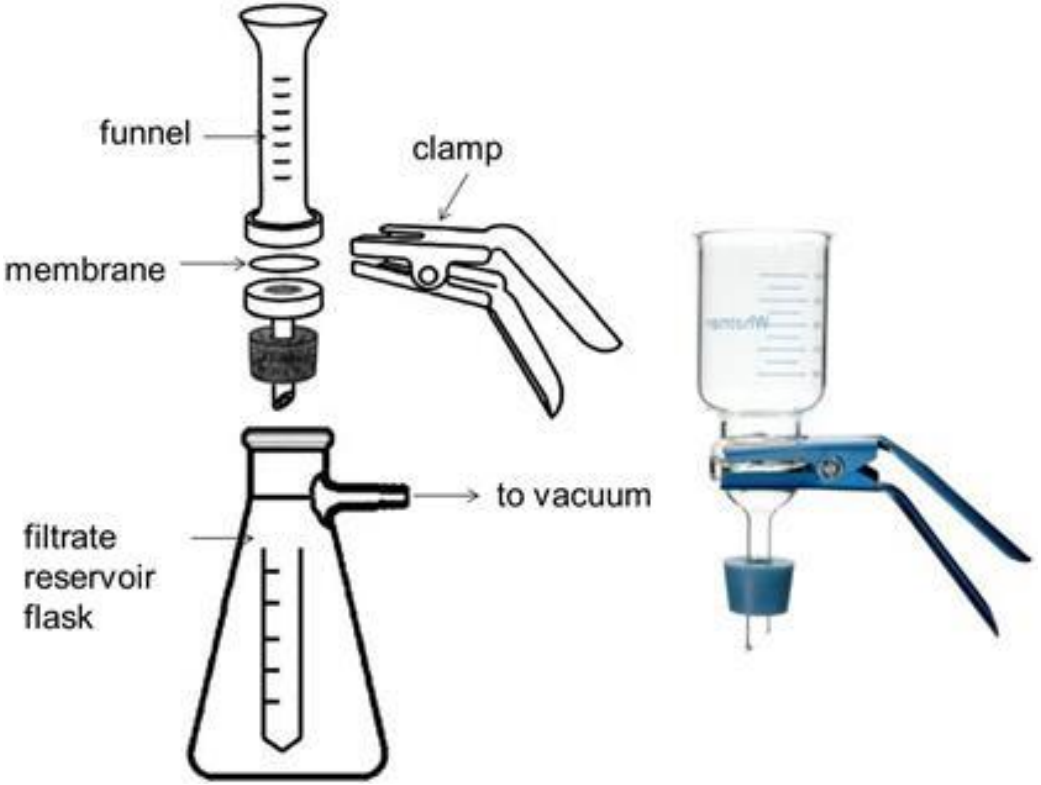
BALANCES

technical

analytical

FILTRATION EQUIPMENT





DISINFECTANTS

STUDENT'S LABORATORY EQUIPMENT

- Microscope
- Immersion oil
- Inoculating loop
- Burner or spirit lamp
- Staining kits
- Water for washing smears
- Slides
- Stands for tubes
- Crystallizer and bridge
- Tweezers for collecting slides
- Filter paper for drying smears
- Flask for used slides

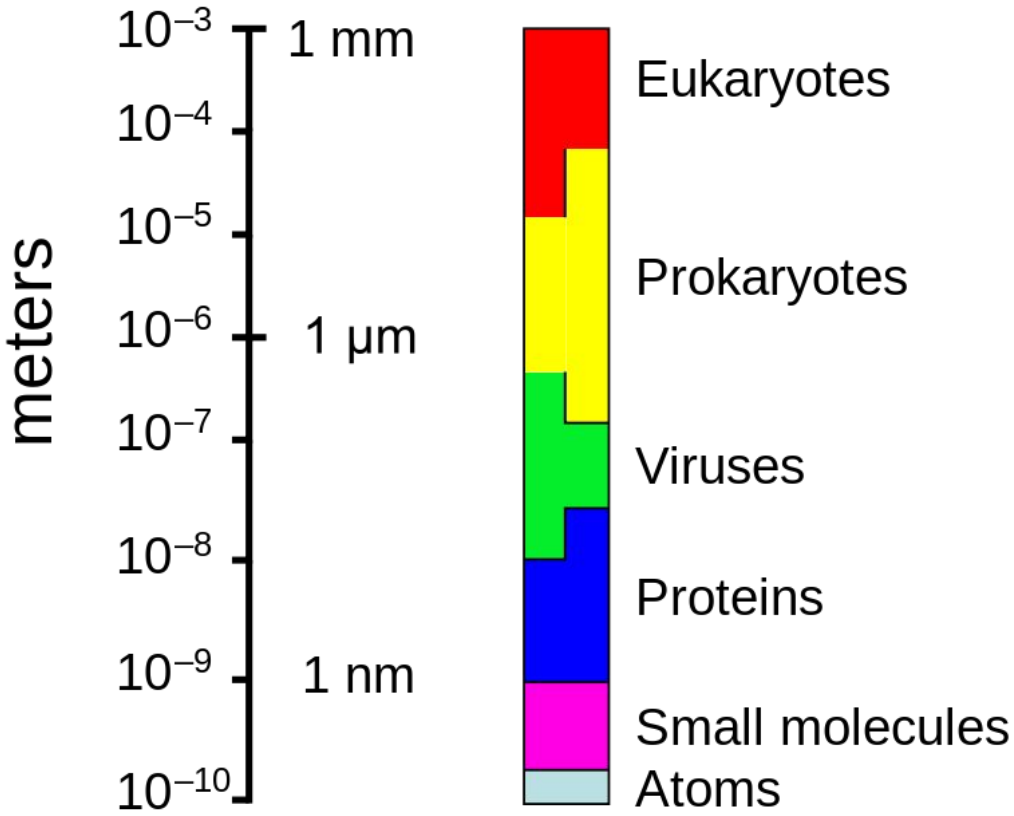
MORPHOLOGY OF MICROORGANISMS

- ✔ Size of microbial cells
- ✔ Shape of microbial cells
- ✔ Arrangement of microbial cells

Bacteria are of about 0,5—5 µm in size

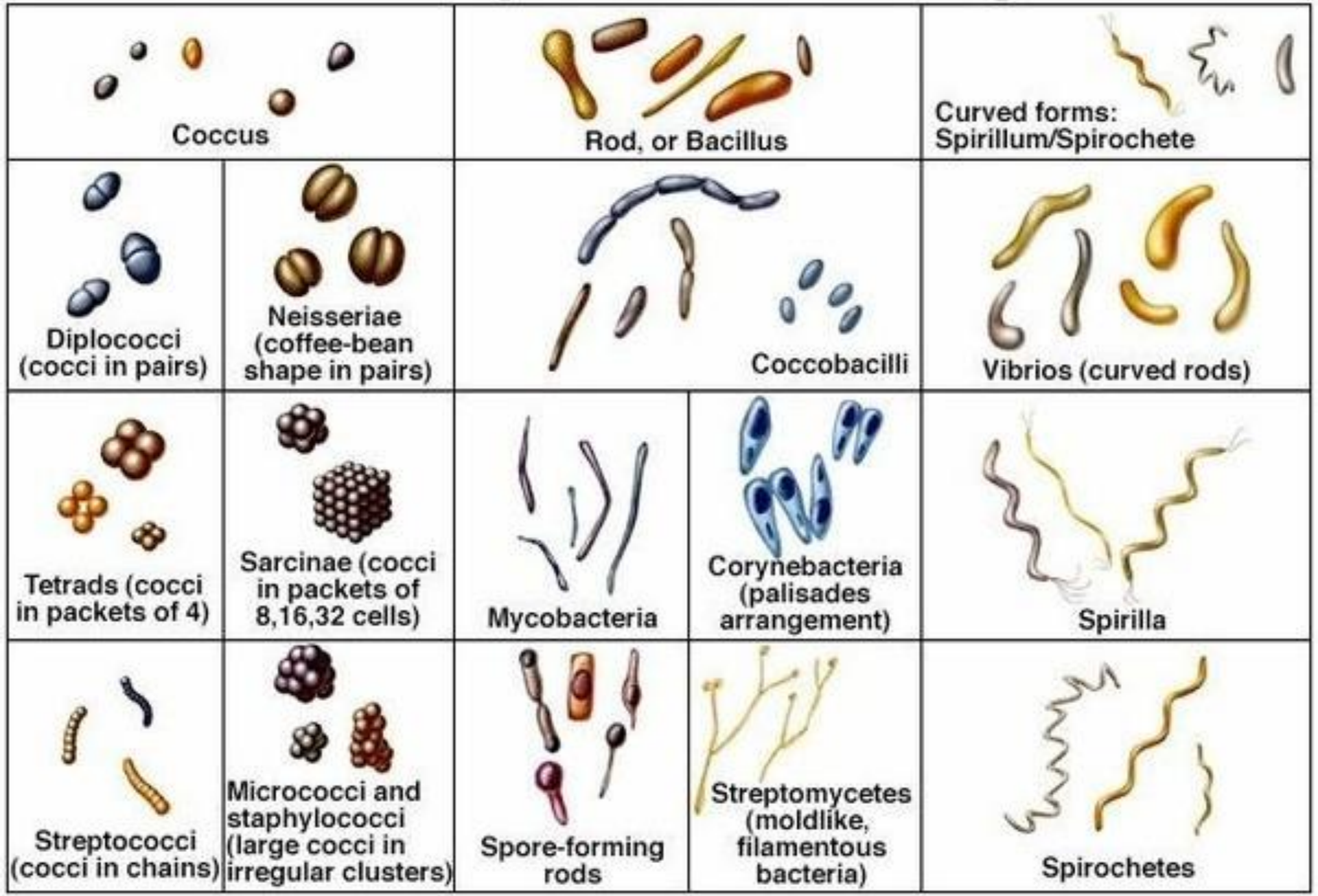
SIZE OF MICROORGANISMS

Bacteria are of about 0,5—5 µm in size

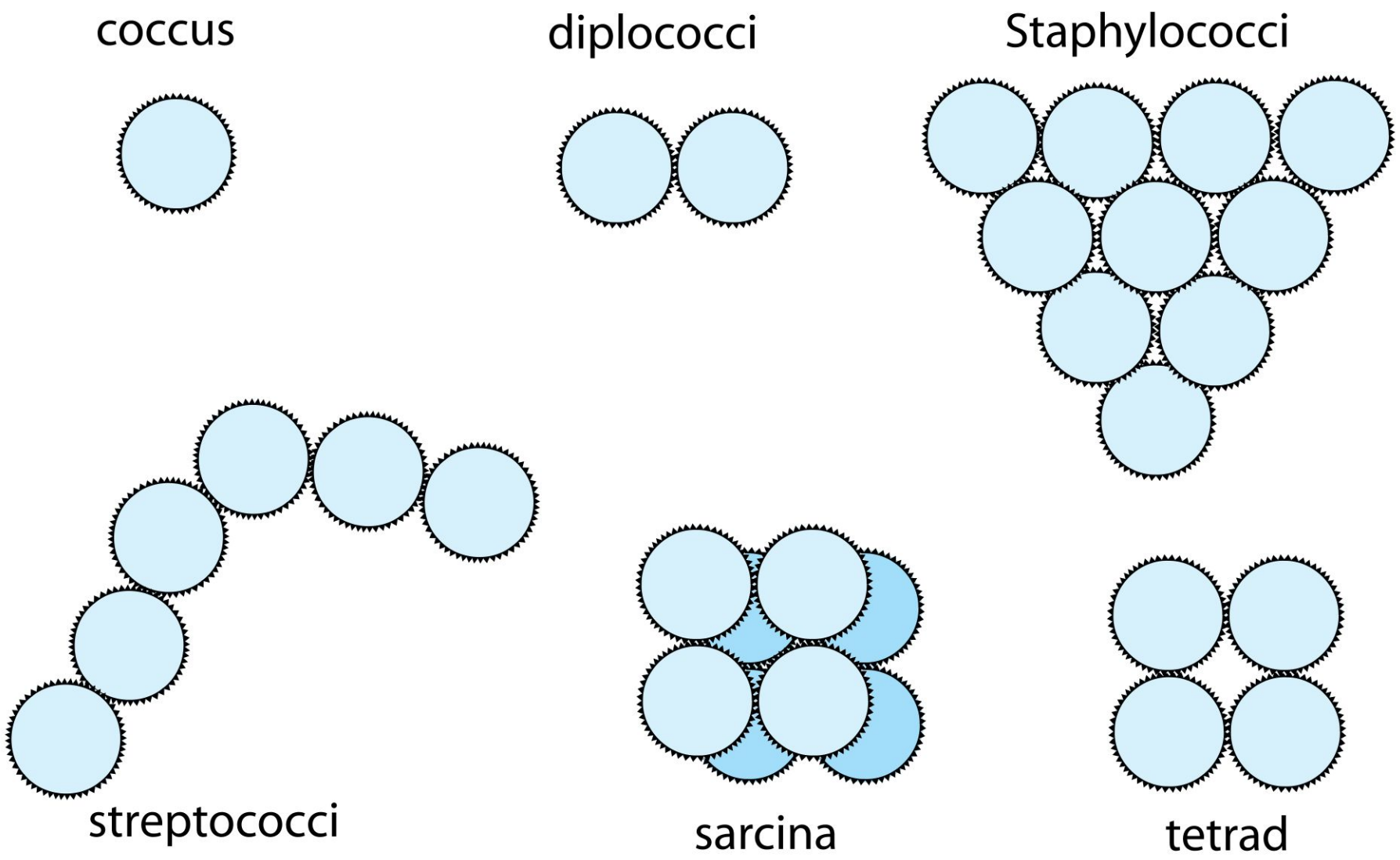


The range of sizes shown by prokaryotes, relative to those of other organisms and biomolecules

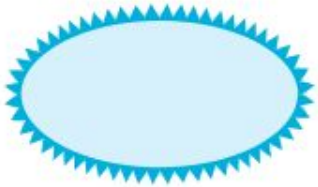
Bacterial shapes and arrangements



Arrangements of Cocci

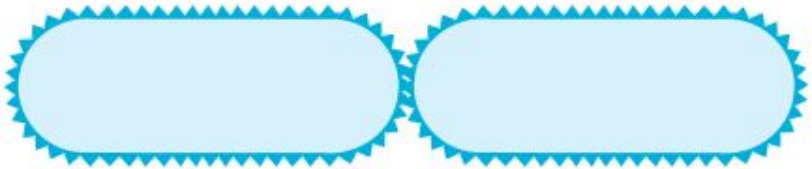


Arrangements of Bacilli

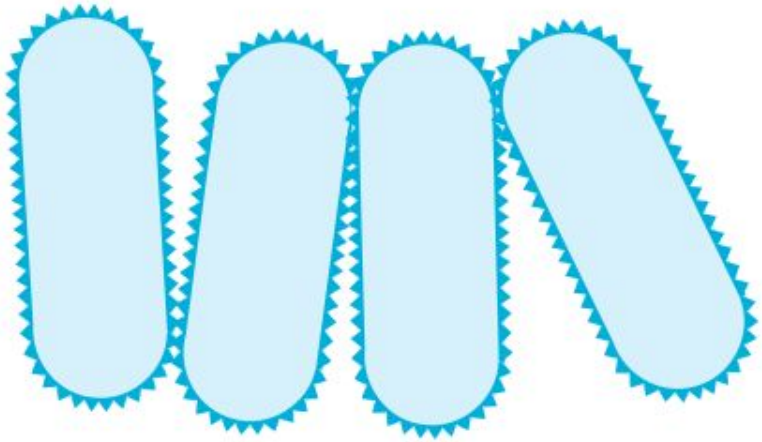


coccobacillus.

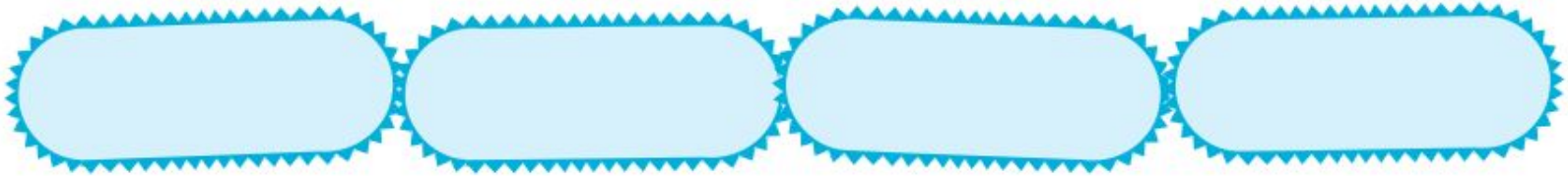
bacilli



diplobacilli



palisades



Streptobacilli

STAINING

- Because microbial cytoplasm is usually transparent, it is necessary to stain microorganisms before they can be viewed with the light microscope. In some cases, staining is unnecessary, for example when microorganisms are very large or when motility is to be studied, and a drop of the microorganisms can be placed directly on the slide and observed
- **Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image**

Types of staining techniques

Simple staining
(use of a single stain)

For visualization of morphological shape & arrangement.

Differential staining
(use of two contrasting stains separated by a decolorizing agent)

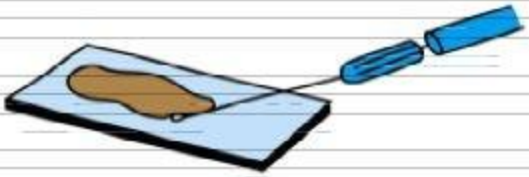
- Identification
  - Gram stain
  - Acid fast stain
- Visualization of structure
  - Spore stain
  - Capsule stain

STAINING

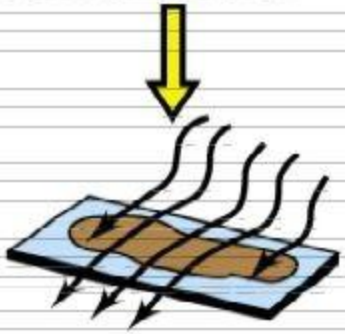
- **Simple stain techniques**

  Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Such a procedure is the **simple stain procedure**
- The **differential stain technique** distinguishes two kinds of organisms. An example is the **Gram stain technique.** This differential technique separates bacteria into two groups, Gram-positive bacteria and Gram-negative bacteria.

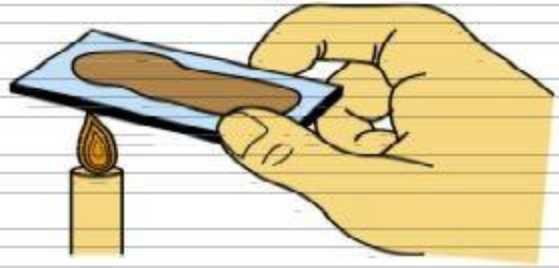
Summary of simple stain



Spread culture in thin film over slide

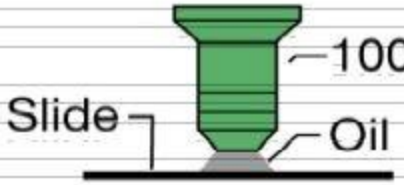


Dry in air



Pass slide through flame to fix

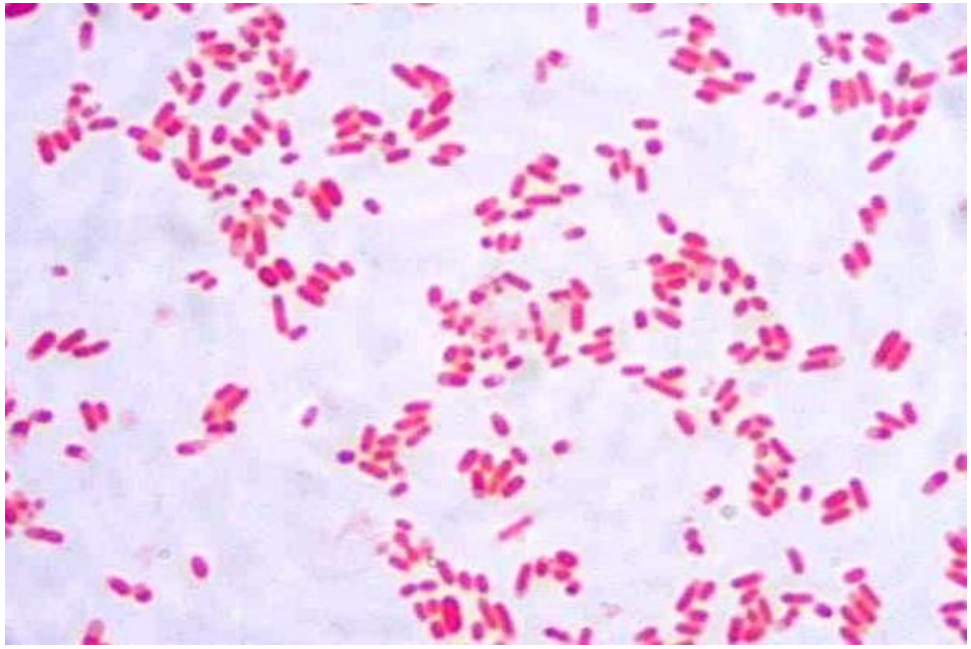
Flood slide with stain; rinse and dry



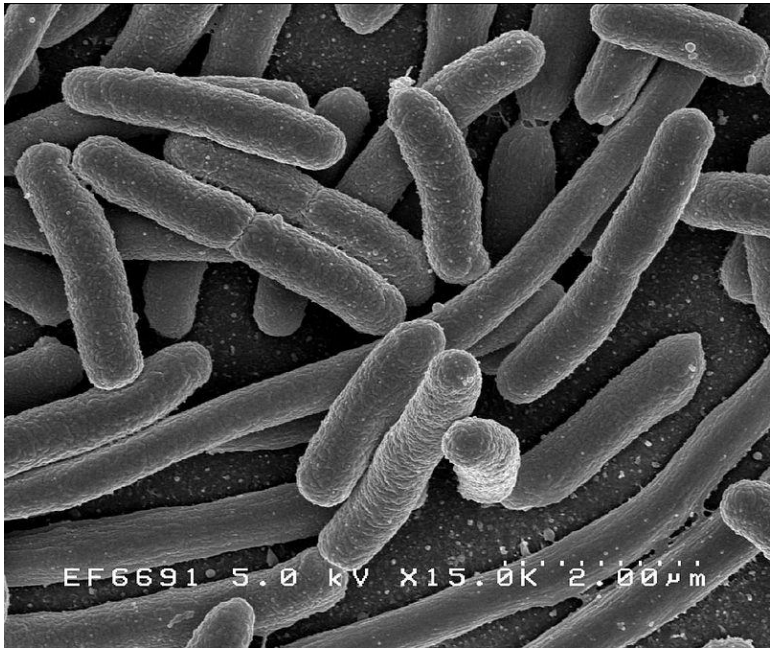
Place drop of oil on slide; examine with 100× objective

IMMERSION MICROSCOPY PROCEDURE

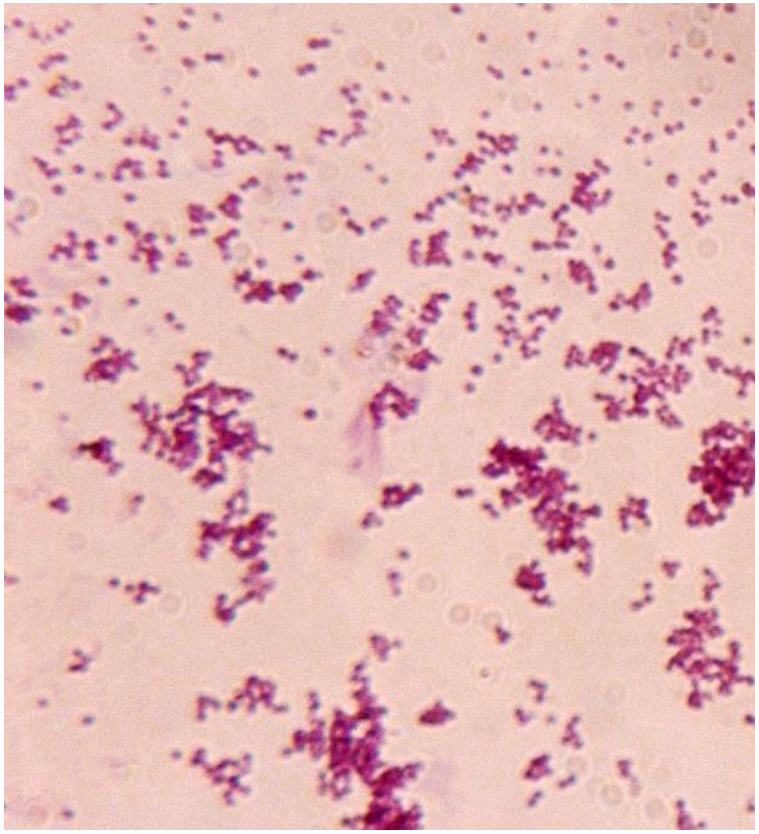
1. Work well seated.
2. Lift up the condenser to the level of an object table.
3. Set up the lightening looking through an ocular.
4. Place a preparation with a drop of immersion oil on an object table and fix it with clamps.
5. Install an immersion lens (with a magnification of 90 or 100). Work very carefully with an immersion lens. Be careful while immersing the lens into a drop of oil, because it can crush the glass.
6. Lower a tube under the control of the eyes using the macroscrew, immerse the lens into the oil, don't touch the glass surface.
7. Looking into an ocular, slowly raise a tube up with the macro-screw until an image appears (until something flashes in the field of view).
8. After that, turn the micro-screw to receive the clear image of an object. Both eyes should be open, using the left hand move a preparation in such way for general review.
9. After treating the preparation, raise a tube up with the macro-screw. Remove a preparation, lower a condenser, wipe the oil from an oil immersion lens with a soft napkin, then return a drawtube to its initial position.



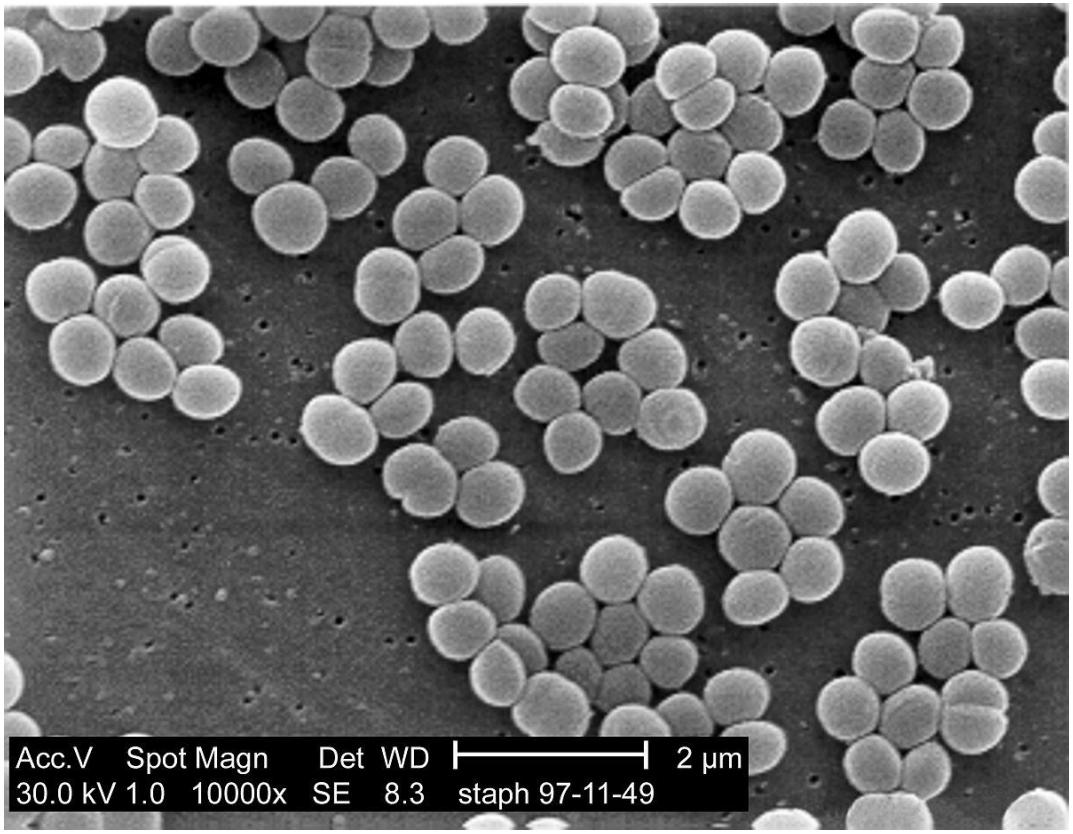
Escherichia coli
(simple stain by fuchsine)



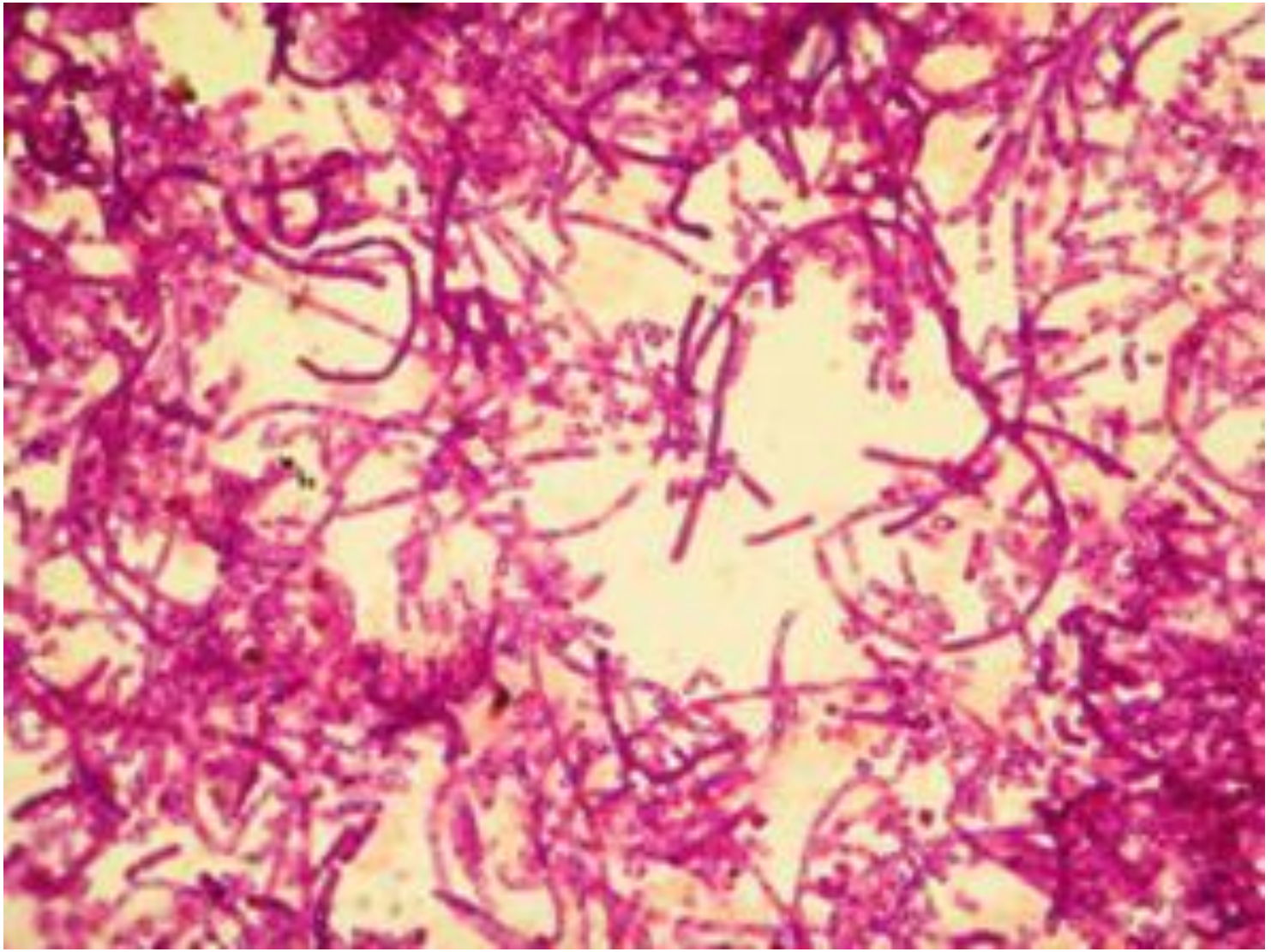
Escherichia coli
(scanning electron microscope)



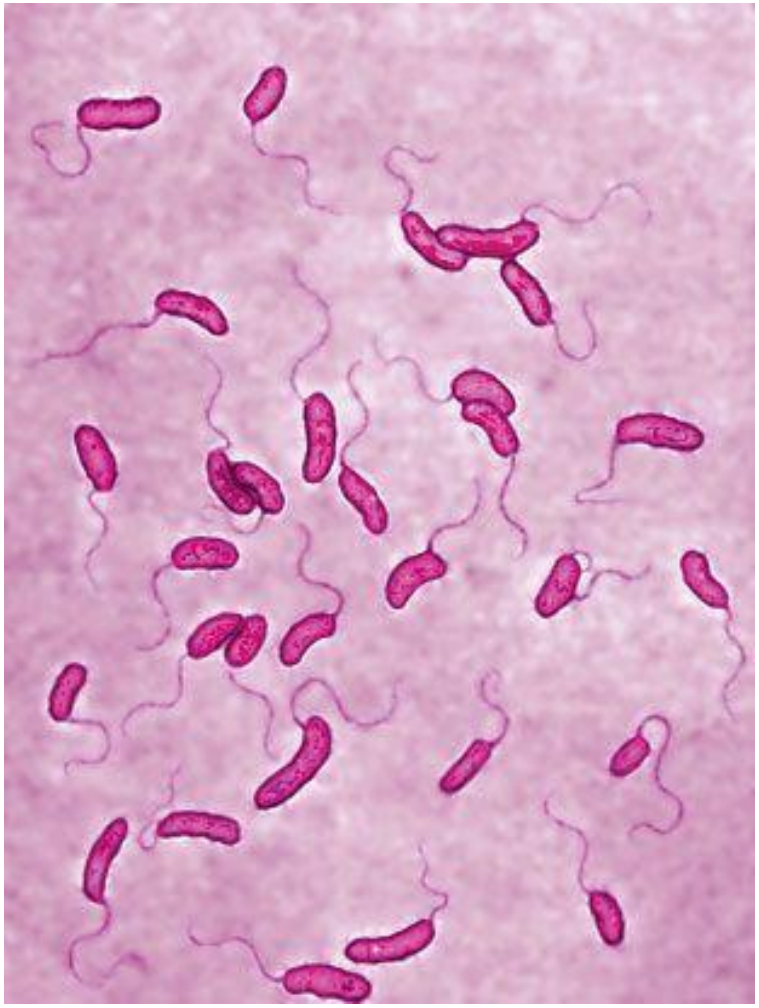
Staphylococcus aureus
(simple stain by fuchsine)



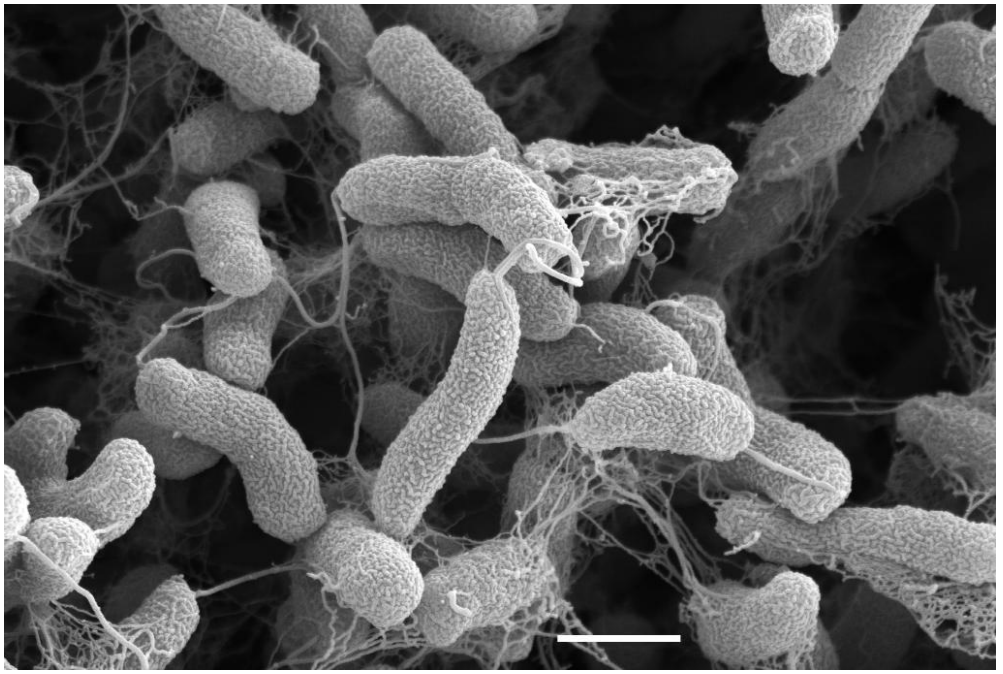
Staphylococcus aureus
(scanning electron microscope)



Bacillus anthracoides (simple stain by fuchsine)



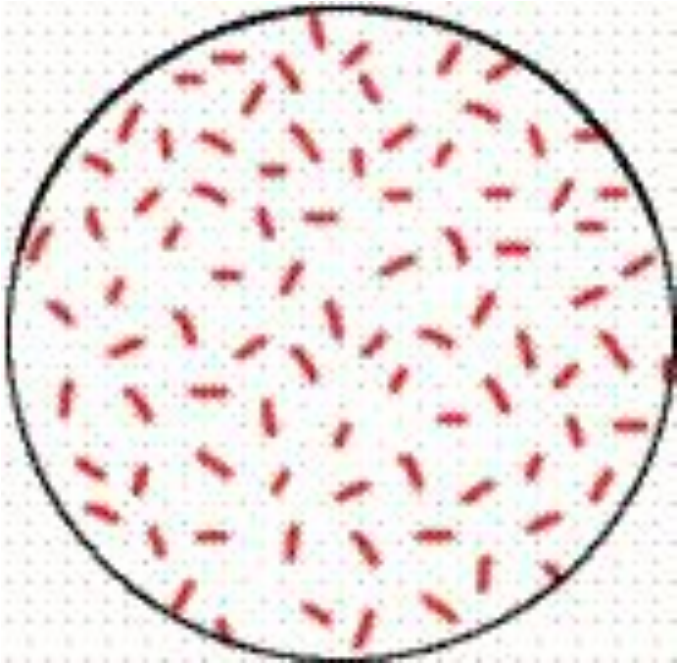
Vibrio cholerae
(simple stain by fuchsine)



Vibrio cholerae
(scanning electron microscopy)

TASK 3

DESCRIBE THE MORPHOLOGY OF YOUR PREPARATION



Species ***Escherichia coli***

Size **small**

Cell form **rods**

Cell location **single**

Cell arrangement **chaotically**

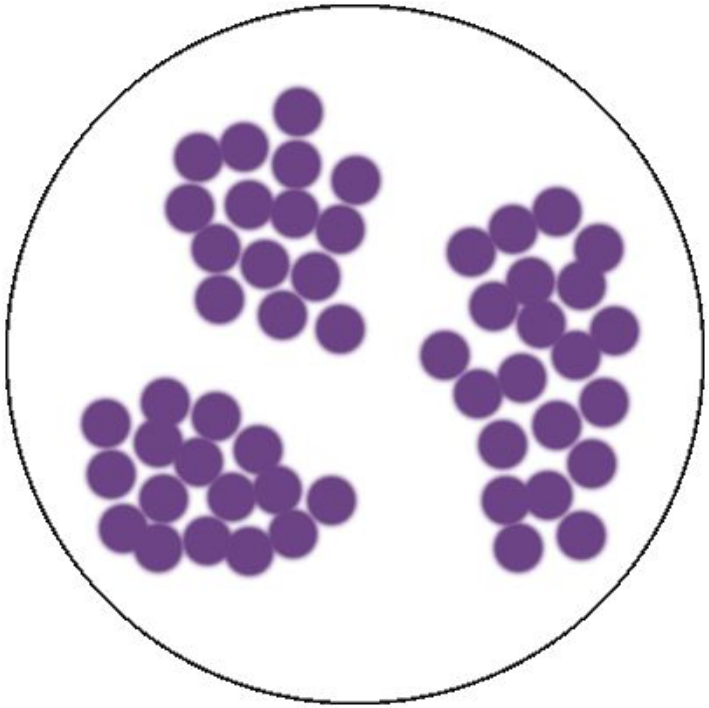
Form of cell edge **rounded edges**

Stain **simple stain by fuchsine**

Magnification **1000x**

TASK 3

DESCRIBE THE MORPHOLOGY OF YOUR PREPARATION



Species ***Staphylococcus aureus***

Size **large**

Cell form **coccus (spherical)**

Cell location **”grapes”-like clusters**

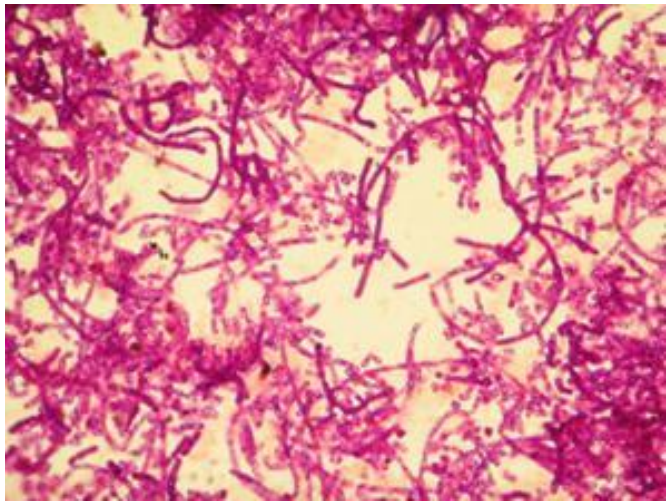
Form of cell edge –

Stain **simple stain by fuchsine**

Magnification **1000x**

TASK 3

DESCRIBE THE MORPHOLOGY OF YOUR PREPARATION



Species ***Bacillus anthracoides***

Size **large**

Cell form **bacillus**

Cell location **chains**

Form of cell edge **chopped edges**

Stain **simple stain by fuchsine**

Magnification **1000x**

TASK 3

DESCRIBE THE MORPHOLOGY OF YOUR PREPARATION

Species ***Vibrio cholerae***

Size **small**

Cell form **vibrio (curved comma-like rods)**

Cell location **single**

Cell arrangement **chaotically**

Form of cell edge **rounded edges**

Stain **simple stain by fuchsine**

Magnification **1000x**

Recommendations

1. Attend all lectures and lessons
2. Prepare home task for each lesson
3. At the end of each lesson show results in workbook and answer questions
4. Books, slides and other useful materials will be published in this public: *https://vk.com/pmedpharm_mb*
5. Our official web-page: https://www.pmedpharm.ru/departments/kafedra_biologicheskoy_himii_i_mikrobiologii/