



AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 1.

Medical microbiology and immunology, its goals and objectives. Systematics and classification of microorganisms. Classification of bacteria. The structure of the microbiological laboratory, the mode of operation there. Microbiological examination methods. Microscopic method. Microscopes, working procedure with immersion lens

FACULTY: General Medicine
SUBJECT: Medical microbiology - 1

Discussed questions:

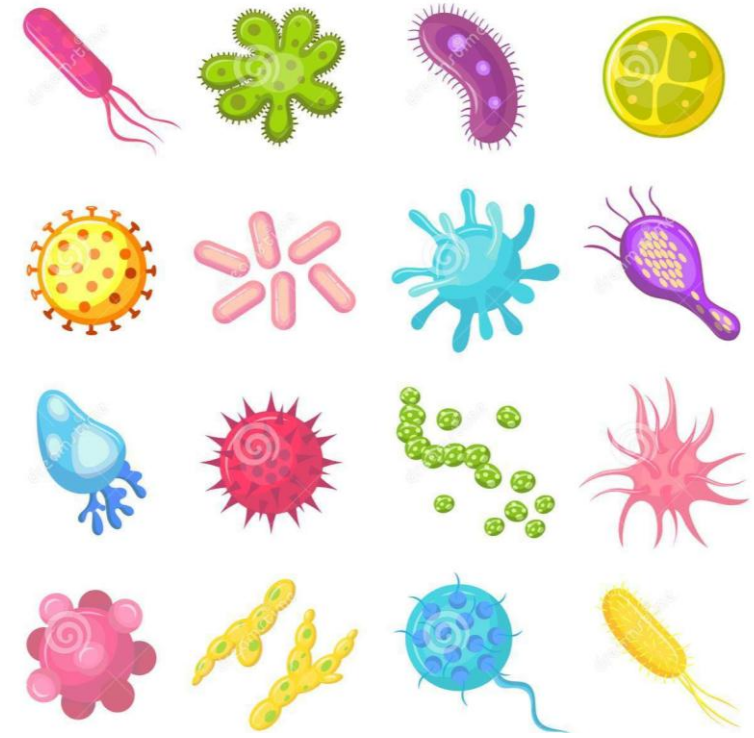
1. Types and structure of microbiological laboratory (bacteriological, mycological, parasitological, virological, immunological, molecular-genetic, especially dangerous).
2. Equipment and devices.
3. Mode of operation in the microbiological laboratory.
4. Methods of microbiological examination.
 - microscopic
 - cultural
 - biological (experimental)
 - immunological (serological reactions, skin-allergic reactions)
 - molecular-genetic methods.
5. The importance of the microscopic method.
6. Types and techniques of microscopes (light, dark field, contrast phase, luminescent, electron, scanning microscopes). The magnification of the microscope.
7. Types of lenses. How to work with an immersion lens.

Purpose of the lesson:

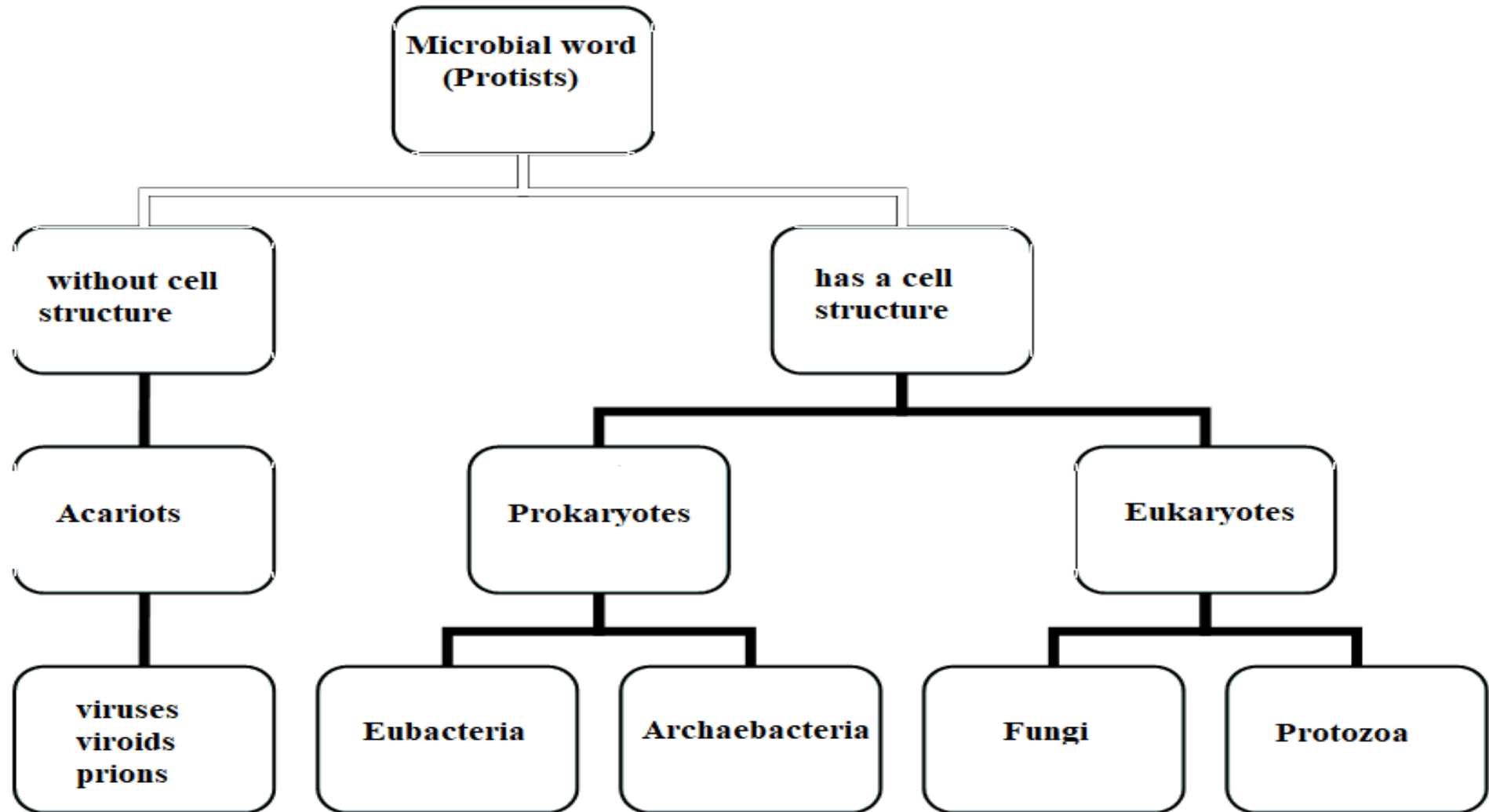
- To explain to students the role of microbiological laboratory in the diagnosis of infectious diseases. To give information about the types and structure of the microbiological laboratory, the mode of work there. Explain the methods of microbiological examination. To acquaint them with the method of microscopic examination, types of modern microscopes, the procedure for working with immersion lenses.

Definitions

- Microbiology from Greek μῑκρος, mīkros, "**small**"; βίος, bios, "**life**"; and -λογία, -**logia**
- Medical microbiology
 - is a branch of medical science
 - concerned with the prevention, diagnosis and treatment of infectious diseases
- There are four kinds of microorganisms that cause infectious disease:
 - bacteria,
 - fungi,
 - parasites
 - viruses



Classification of microbes



Classification of prokaryotes

- The modern classification of prokaryotes is based on the Bergey's Manual. This classification, first given by the American bacteriologist D. Bergey in 1923, is periodically updated by the International Committee on Bacterial Systematics.
- In its ninth edition, all prokaryotes were divided into four major categories according to the structure of the cell wall.
- Each category consists of numerous groups.

The modern Bergey's Manual of prokaryotes

- Gram-negative bacteria that have a cell wall
- Gram-positive bacteria that have a cell wall
- Eubacteria without cell walls
- Archaeobacteria

In the modern classification of prokaryotes There are 24 types and 33 classes.

1. Gram-negative eubacteria with a thin cell wall - Gracilicutes - 16 groups

2. Gram-positive eubacteria with a thick cell wall - Firmicutes - 13 groups

3. Non-cell wall eubacteria - mycoplasmas -
Tenericutes Mollicutes class - 1 group

4. Archaeobacteria do not have a cell wall and peptidoglycan. Does not cause disease in humans - 5 groups

Prokaryotes

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graph TD; Prokaryotes[Prokaryotes] --> Eubacteria[Eubacteria]; Prokaryotes --> Archaeobacteria[Archaeobacteria]; Eubacteria --> Gracilicutes((Gracilicutes  
Gram negative)); Eubacteria --> Firmicutes((Firmicutes  
Gram positive)); Eubacteria --> Tenerecutes((Tenerecutes  
Mycoplasma)); Archaeobacteria --> Mendosicutes((The cell wall is  
defective  
(Mendosicutes)));
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Eubacteria

Archaeobacteria

Gracilicutes
Gram negative

Firmicutes
Gram positive

Tenerecutes
Mycoplasma

**The cell wall is
defective
(Mendosicutes)**

Taxonomy

- Kingdom
 - (American system has six: Animalia, Plantae, Fungi, Protista, Archaea, Bacteria)
- Phylum
- Class
- Order
- Family
- Genus
- Species
- Subspecies

TAXONOMIC CATEGORIES

Kingdom	Prokaryotae
Division	Gracilicutes
Class	Scotobacteria
Order	Eubacteriales
Family	Enterobacteriaceae
Genus	Escherichia
Species	coli
Subtype	Escherichia coli O157:H7

- For high-ranking taxa, the name "Type" (Phylum) is more appropriate than "Division"

Identification of microorganisms:

- *morphology*
- *tinctorial*
- *cultural*
- *biochemical*
- *antigen*

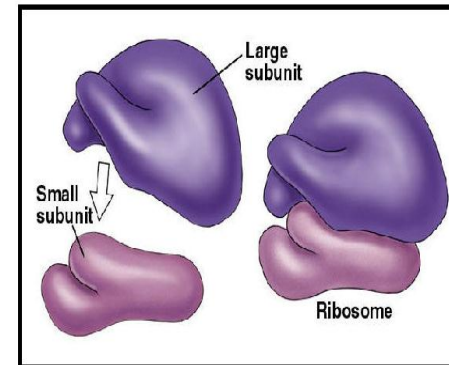
Phenotypic

- *$Q + S$ as a percentage,*
- *DNA hybridization,*
- *sequencing,*
- *restriction enzyme*
- *Due to polymorphism in the length of DNA, etc.*

Genotypic

- *16S ribosome RNA conservatism*
- *Genes encoding RNA and ribosomal proteins*

Phylogenetic



50s
30s(16s)

Nomenclature of microorganisms:

- The binomial nomenclature proposed by K. Linnaeus is used for the nomenclature or naming of microorganisms (except viruses).
- In this case, the first word indicates gender and is written in capital letters, and the second word indicates the name of the species and is written in lower.
- For example,
 - ✓ *Mycobacterium tuberculosis*
 - ✓ *Francisella tularensis*
 - ✓ *Staphylococcus aureus* and others.

- **A species** is a group of individuals who have a common origin and genotype, who are similar in biological characteristics, who have inherited a strong, qualitatively determined process under standard conditions.
- **A strain** is a pure culture of a species of microorganism obtained at different times from different sources (or from the same source).
- **A clone** is a culture that develops from a microbial cell.
- **A colony** is a collection (population) formed by bacteria in a solid nutrient medium.
- **Pure microbial culture** is a population formed by a single species of microorganism in a solid nutrient medium.

Subtype or variant

- Morphovars – differ morphologically
- Biovars – differ biochemically and physiologically
- Serovars – differ in antigenic properties
- Phagovars - differ bacteriophage susceptibility
- Chemovar - differ biochemically properties
- Resistovar - differ in sensitivity to antimicrobial drugs.

The role of the microbiological laboratory in the diagnosis of diseases

Research conducted in microbiological laboratories is important for early and accurate diagnosis of infectious diseases.

Microbiological laboratories operate under the following:

- ✓ Centers of hygiene and epidemiology
- ✓ Polyclinics
- ✓ Hospital
- ✓ Scientific-research institutes

Medical microbiology laboratories

- The Clinical Microbiology Laboratory is a full laboratory including
 - Bacteriology,
 - Mycology,
 - Parasitology,
 - Virology
 - Immunology
 - Molecular Microbiology laboratories

STRUCTURE OF MICROBIOLOGICAL LABORATORY:

THE MICROBIOLOGICAL LABORATORY CONSISTS OF SEVERAL ROOMS:

1. Examination materials (sample) reception room
2. Preparation room - nutrient media, materials, color solutions, etc. for examination. is prepared
3. Autoclave room - sterilization devices (autoclave, air sterilizer) are located
4. Washroom - Petri dishes, test tubes, flasks, used pipettes are disinfected and washed in a disinfectant solution
5. Examination room - examination materials taken from patients - pus, sputum, blood, urine, feces, spinal fluid, etc. liquid is examined by different methods.
6. Vivarium - animals needed for the experimental method are kept.

Modern microbiological laboratory



Specially dangerous laboratory



Microbiology Lab Tools And Equipment

- Light microscope
- Incubator
- Oven
- Autoclave
- Fridge and refrigerator
- Hood (safety cabinet)
- Electronic balance
- pH meter
- Hot-plate
- Electronic micropipette
- Water bath
- Distillator
- Flasks, beakers
- Petri-dishes
- loops
- Vortex mixer
- Centrifuge

Microbiology Lab Tools And Equipment

Colony counter

Magnetic stirrer

Spectrophotometer

High Performance Liquid Chromatography (HPLC)

Gas Chromatography (GC)

Gel Electrophoresis

VITEK 2

BacT/ALERT 3D system

Polymerase Chain Reaction

Microbiological laboratory equipment:



Petri dish



tampon



loop



needle



spatula



Tube



menzur



flame

Microbiological laboratory equipment:



Incubator



Paster oven



Autoclave



Fridge and refrigerator



Centrifuge



Gaspak system

Bacteriological analyzers



***Identification of microorganisms, antibiotics analyzers
for studying sensitivity and hemoculture***

Work regime in the microbiological laboratory

Since work with pathogenic microorganisms is carried out in the microbiological laboratories of medical institutions, in order to prevent re-infection and the spread of microbes, the following rules, i.e. regime, must be followed:

1. You cannot enter the laboratory without a gown and cap. It is imperative to use a mask if necessary.
2. Do not enter the laboratory with outerwear, walk around the room and talk too much.
3. Eating, drinking tea and smoking are not allowed in the laboratory.
4. Pathological material is accidentally spilled on a gown, table, floor, etc. if it falls on the ground, it should be treated immediately with a disinfectant.
5. Used pipettes, spatulas, test tubes, Petri dishes should be thrown into the disinfectant solution.
6. At the end of the work, the work table should be cleaned and disinfected, planted Petri dishes should be placed in the thermostat, museum strains and the remaining nutrient media should be placed in the refrigerator.
7. The object table of the microscope and the 90x magnification objective should be cleaned of oil and a filter piece should be placed under the objective. In order not to be contaminated, the microscope must be covered with a special cover;
8. At the end, hands should be wiped with a towel soaked in disinfectant solution and washed with soap.

When conducting research in a microbiological laboratory, the following rules should be followed:

1. Infected materials should be handled only with tools (tweezers, loops, etc.). It is forbidden to touch the cultured material and condensate in the Petri dishes.
2. Before starting the work, the integrity of the glass containers, the passage of the needles, the reliability of the diving of the syringes should be carefully checked.
3. When the material is cultivated, the date of the month and the number of the analysis are recorded on the test tubes, Petri dishes, flask, vials.
4. The material should be transferred to the test tubes, Petri dish near the flame, the spatula, the edge of the test bottles should be passed through the flame, and the loop should be flaming.
5. When working, culture dishes should be placed in tubs or trays, and test bottles should be placed on a tripod.
6. The solution containing pathogenic microorganisms should be collected in a rubber flask with a Pasteur pipette.
7. Solutions should not be sucked from the mouth and poured into a container other than a broken bowl.
8. After the work is finished, it is forbidden to leave unfixed preparations, Petri dishes, test tubes, other containers contaminated with infected material on the work tables.

Microbiological examination methods

1. Microscopic method

2. Culture (bacteriology, virology, serology, mycology, parasitology) method

3. Biological method

4. Immunological method: - serology
 - skin-allergic tests

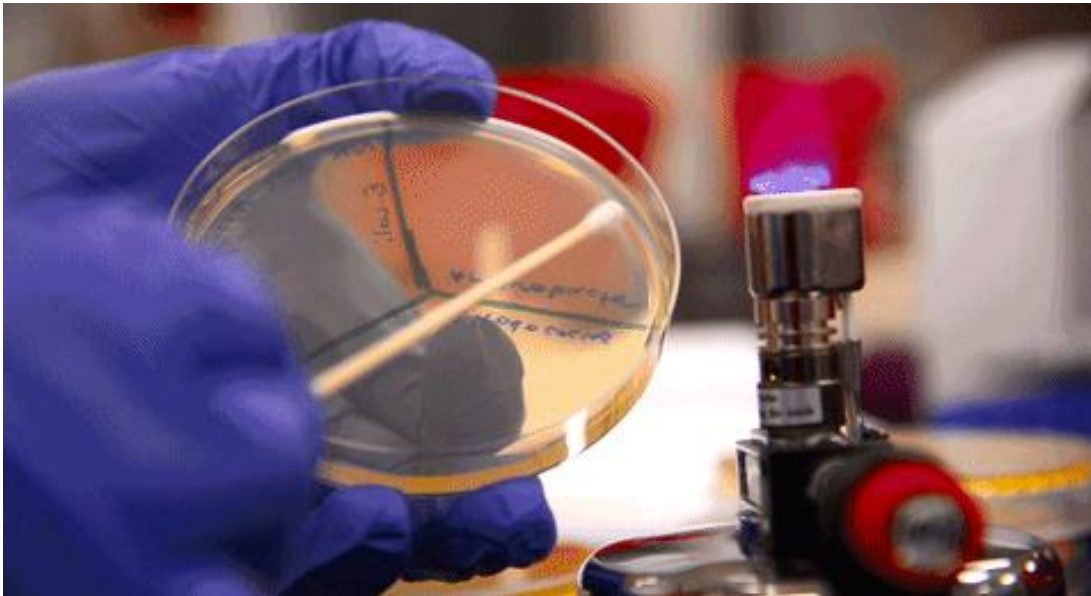
5. Molekular-genetic method

Microscopic method

- With the help of a microscopic method, the presence of microorganisms in the examination material and their morphology is determined.
- Additional elements - capsule, spore, flagella, others - volutin granules are also determined.
- Since many microorganisms cannot be determined based on their morphological and tinctorial characteristics, the microscopic method is an approximate diagnostic method.

Culture (bacteriology) method

- *When conducting an examination with this method, the pathological material is planted in appropriate nutrient media and cultivated, cultured and identified.*
- *It is the "golden method" in microbiological diagnostics, which allows to accurately determine and identify the causative agent.*



Biological or experimental method

- *It is done by infecting laboratory animals with pathological material.*
- *If it is not possible to obtain a pure culture by the bacteriological method, the biological method is used.*
- *Pathogenicity, virulence and toxigenicity of the microbe are studied.*
- *Experimental tests of new medicines are being conducted.*



Immunological method (serological and skin-allergic)

***Serological method** - antigens of the causative agent or antibodies formed against them are determined in the blood serum, as well as the type and serovar of the unknown microbe is determined with the help of a known immune serum (serological identification).*



Skin-allergic test

- Since the antigens of many causative agents have a sensitizing effect, allergic reactions are also used in the diagnosis of infectious diseases.
- In tuberculosis – Mantoux test
- In brucellosis - Burne test
- In tularemia - Tulyar's test, etc.



Molecular-genetic method

- The principle of the polymerase chain reaction is to multiply (amplify) the nucleic acid of any causative agent in pathological material or pure culture.
- Molecular hybridization of DNA or RNA. It is based on the determination of genome fragments belonging to the progenitors.
- The main advantage of molecular-genetic methods is their high sensitivity and specificity.



Microscopes

- In microbiological laboratories, microscopes are used to examine microorganisms.
- Microscope (lat. micro-small, scopid-to look) is a device for magnifying the image of an object, as well as for measuring the part that cannot be seen with the naked eye.
- A modern biological microscope is a complex optical device, which allows studying objects passing through light rays in the light and dark field.
- The shape, structure, dimensions, etc. of microorganisms whose dimensions are larger than $0.2 \mu\text{m}$. properties are studied with a light microscope.

Types of microscope

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graph TD; A["Types of microscope"] --> B["Biological m. (light)."]; A --> C["Electron m."]; A --> D["Other m."]; B --> E["Dark field m."]; B --> F["Contrast phase m."]; B --> G["Luminescent m."];
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*Biological m.
(light).*

Electron m.

Other m.

Dark field m.

*Contrast
phase m.*

Luminescent m.

Microscope

- Main parts of microscope:
 - ocular lens,
 - objective lens,
 - arm,
 - stage,
 - stage clips,
 - coarse adjustment knob
 - fine adjustment knob,
 - light source,
 - condenser
 - base

revolving
nose piece
(to hold multiple
objective lenses)

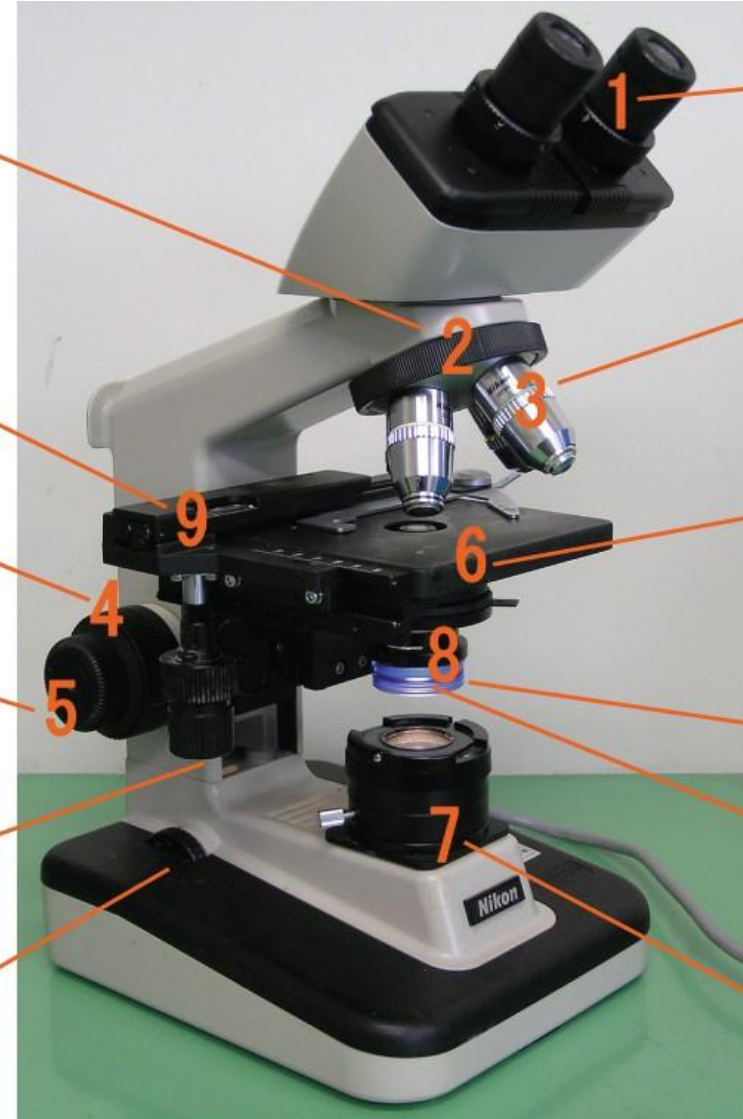
mechanical
stage

coarse focus
(larger knob)

fine focus
(small knob)

x-y mechanical
stage knobs
(to move slide)

rheostat
(to adjust light
intensity)



eyepiece
(ocular lens)

objective
lenses

stage
(to hold the
specimen)

diaphragm

condenser

illuminator

Stage (to hold the specimen)



The stage refers to the mechanical part of the microscope.

The preparation is placed on the microscope and it is the studied part.

There are fixing springs on it. These clamping springs are elastic and press the object under examination to the stage.

Tube



✓Between the eyepiece and the revolver is a tube or sight tube.

✓The tube has a guiding function. In other words, it directs the light waves from the ghost towards the eye.

✓The distance between the objective and the eyepiece is called the optical length of the tube.

Ocular

- The word ocular comes from the word "oculus" and means "eye".
- It is located at the top of the eyepiece tube.
- The main magnifying glass of the microscope is one of the two parts and performs the function of a magnifying glass.
- When we observe an object in a microscope, we lean our eyes on the eyepiece.
- The eyepiece consists of 2 lenses and a frame that holds them.
- The one farthest from the tube is called the superior lens or "eye lens" and the other is called the inferior lens.



TYPES OF LENS:

Belongs to the optical part of the microscope and consists of convex lenses on both sides:

- frontal lens in front
- the corrective lens is located at the back (above).

Biological microscopes: (x8, 10, 40, 60) – dry (x90, x100) - equipped with an immersion lens.



Magnification of a microscope:

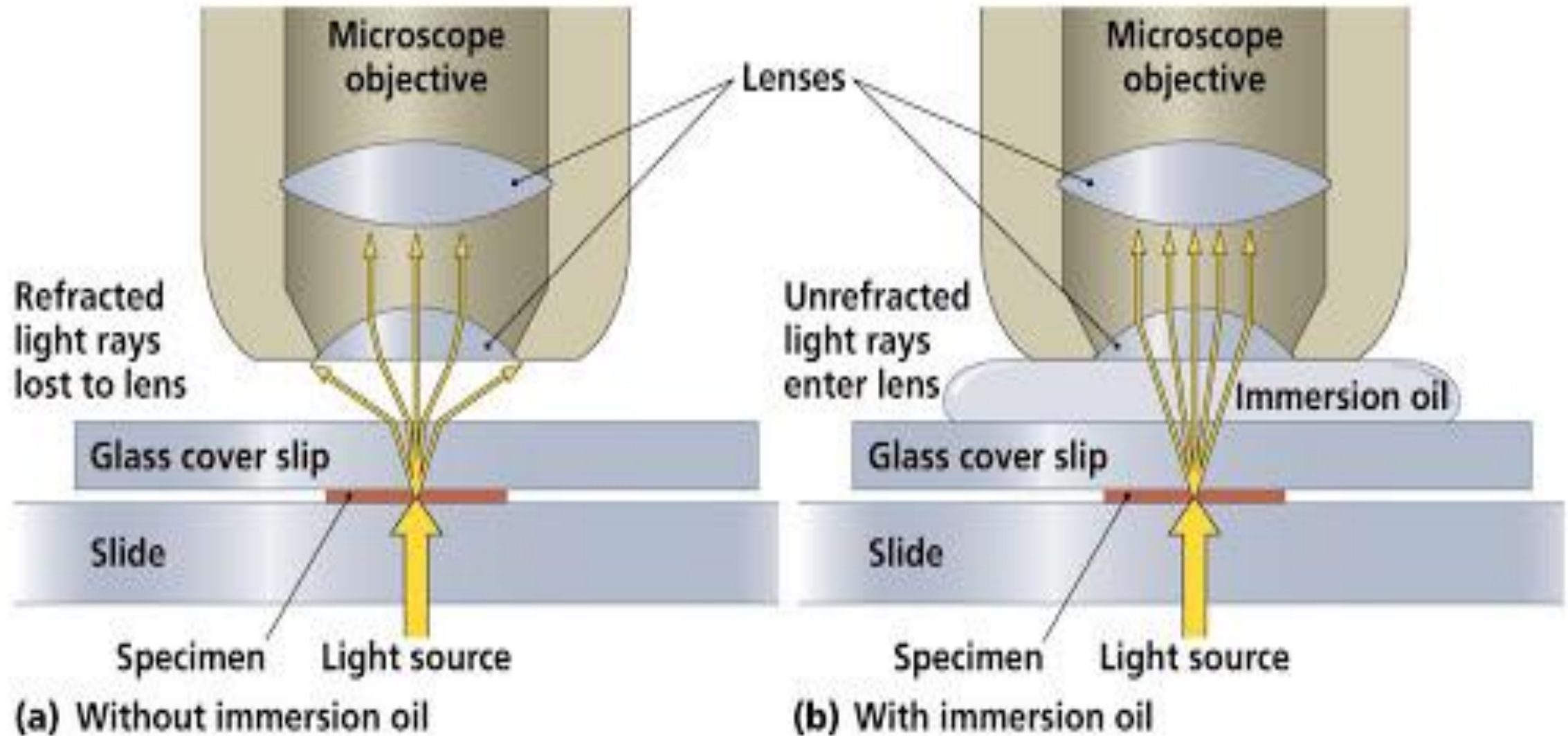
➤The magnification of a microscope is equal to the product of the magnification of the objective and the eyepiece.

Objective X eyepiece = full zoom

➤If the objective magnifies 100 times and the eyepiece 10 times, the total magnification of the microscope is $100 \times 10 = 1000$ times.

➤Biological light microscopes allow you to magnify the object up to 2000-3000 times.

➤The resolution is 0.00027 mm.



Scheme of light microscope examination with dry (a) and immersion (b) objectives

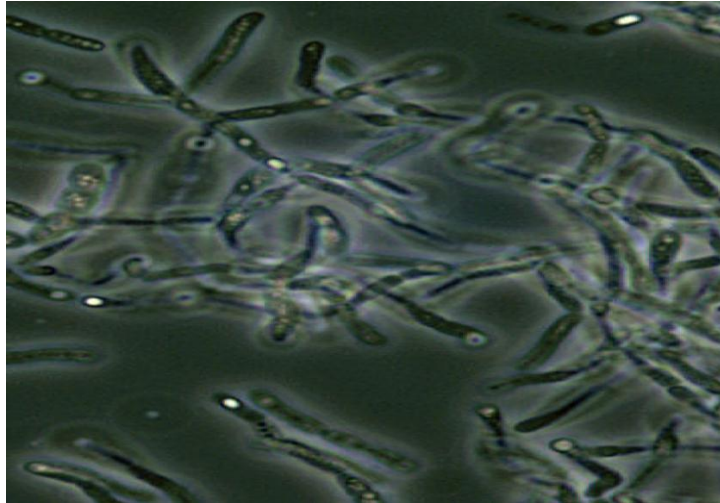
Dark field microscope

- It is obtained by replacing the condenser of the light microscope with a paraboloid or cardioid condenser.
- The edge of the upper lens of the paraboloid condenser is round and colorless, and the center is black. The rays falling on the black part are absorbed, the oblique rays passing through the transparent circular part are refracted when they encounter microorganisms and fall into the lens (diffraction). As a result, microorganisms appear that glow in the dark (Tyndal effect).
- It is mainly used in the unstaining study of hard-to-stain microorganisms such as spirochetes.

Phase contrast microscope

Light rays passing through the optical density area of any object lag behind other areas in phase. Such areas are transparent and cannot be seen under a microscope. Therefore, with the help of a contrast phase device, the phase variation of the light rays passing through the object is converted into the amplitude variation, and transparent objects are visible under the microscope to obtain a contrast image.

- This device converts wavelength magnitude to phase magnitude.
- It is made by placing a special diaphragm on the light microscope and a diffraction plate in front of it.
- Organelles light up differently and are easily distinguished under a microscope.
- Structural elements of bacteria, reproduction, sporulation, effects of chemicals are studied.

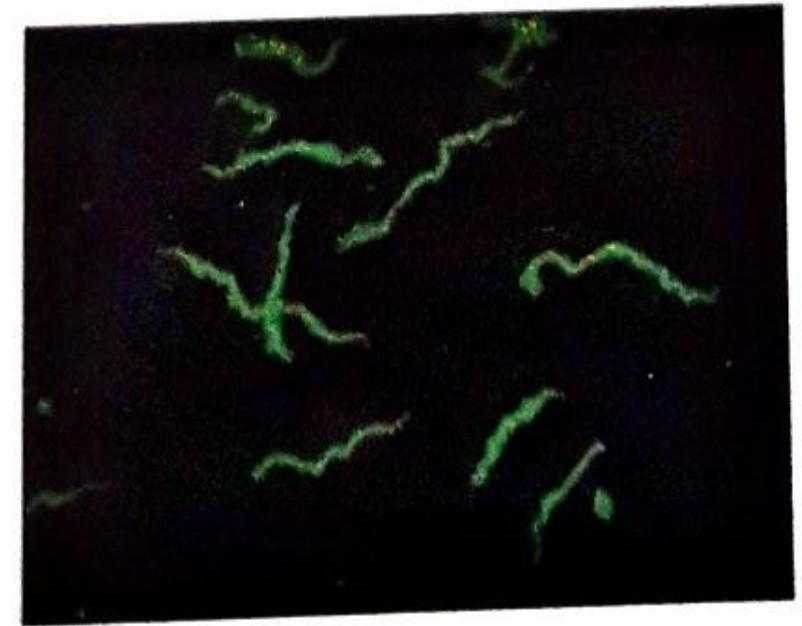


*B. anthracis spore appearance
in contrast phase microscope*



Luminescent (fluorescent) microscope

- Luminescence (lat. lumen means light) is the transformation of potential energy absorbed by matter into light energy and emitting light in a cold state.
- UVR is used. Since the human eye does not see these rays, the drug is first treated with a fluorescent dye (acridine, auramine, neutral red, fluorescein, etc.).
- Microorganisms appear as fluorescent bodies on a black field.



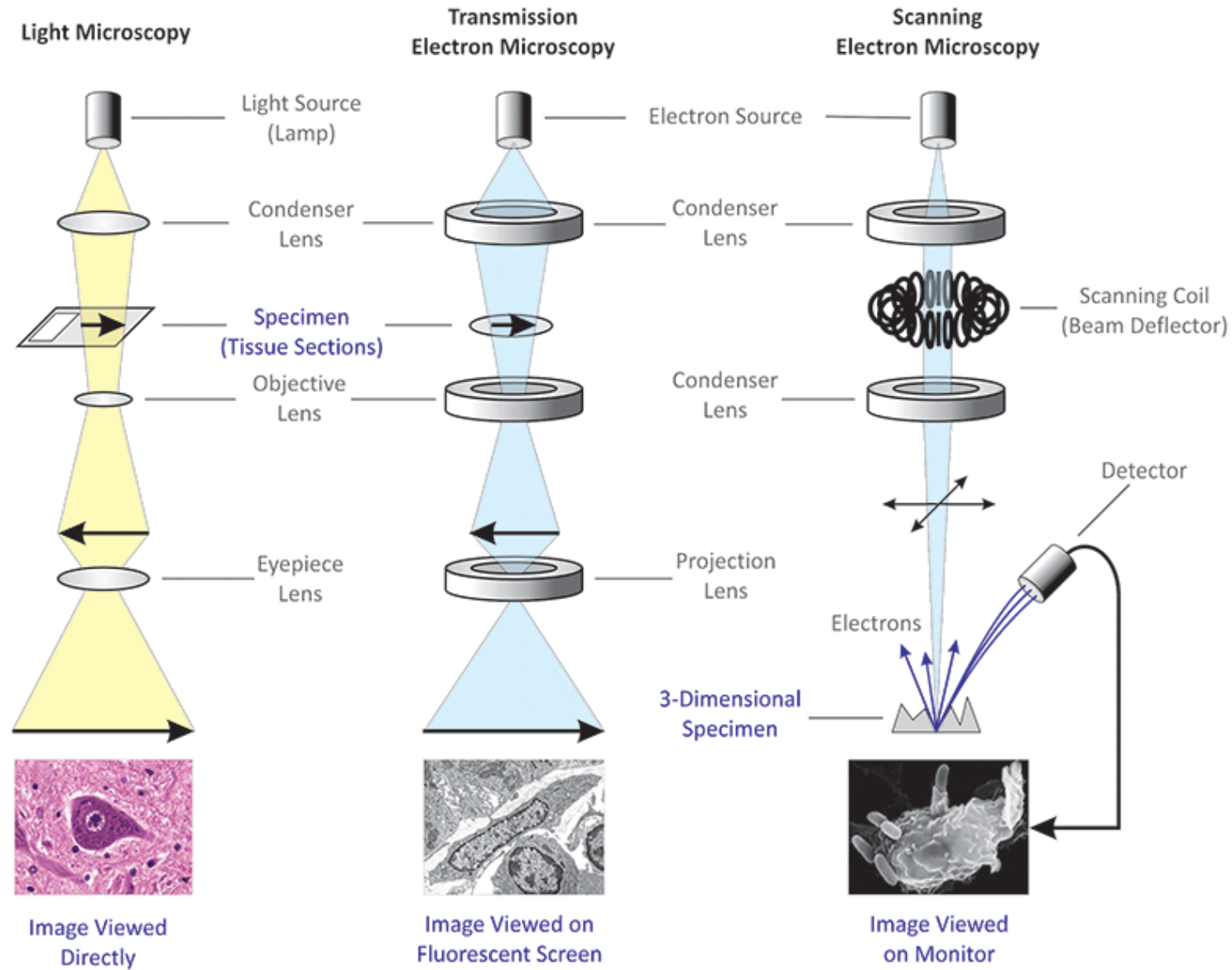
Electron microscope

- Electron flood is used instead of light rays.
- An electron microscope allows you to see very small objects - structural elements of viruses, bacteria and other microorganisms, macromolecules and other submicroscopic objects.
- The wavelength of the electron beam is about 0.005 nm, which is 200,000-300,000 times smaller than the wavelength of the light beam.
- Because the wavelength of electrons is shorter than that of light, the useful magnification in an electron microscope reaches its upper limit, providing 1000 times more magnification ($\times 1000000$) than a light microscope.

Electron microscope



The principle scheme of light and electron microscopes



Scanning electron microscope

Scanning Electron Microscope (SEM) is a device from the electron microscope class. It is a device designed to obtain an image of the surface of a large (up to 0.4 nm) object, as well as information about the composition, structure and some other properties of its surface layers. It is based on the principle of interaction of the electron beam with the studied object. The computer creates a picture from the obtained data.



How to work with an immersion lens:

- In microbiological studies, a wet (oily) immersion (immersio - lat. dip) system with a high magnification (90 times magnification) is used.
- During microscopy, when the rays falling on the preparation pass through the glass and fall into the air, a certain part of them is scattered, so it does not fall on the lens, and the display ability decreases.
- Therefore, immersion oil whose refractive index is close to the refractive index of glass (refractive index -1.52) is used to prevent ray loss.
- Immersion oil fills the space between the objective and the preparation, all the rays passing through the preparation fall on the objective, the magnification of the microscope increases.

How to work with a microscope

