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EDUCATIONAL AND METHODICAL MANUAL

BASIC INFORMATION NECESSARY FOR WORKING WITH A LIGHT MICROSCOPE

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Educational and methodical manual

This manual is intended for laboratory assistants and young professionals who are beginning to work with a light microscope. The manual discusses the basic theoretical concepts of optics used in microscopy. The device and the principle of operation of the microscope are described. Recommendations for setting up the microscope and taking care of it are given.

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THE HISTORY OF THE CREATION OF THE MICROSCOPE

With great inventions, it often happens that it is very difficult to unambiguously name their authors. As a rule, such inventions do not arise out of nowhere, their appearance is preceded by other discoveries and inventions that create the necessary material and scientific basis. Thanks to this, many inventors can claim authorship, and in order to determine the authorship, it is necessary to trace the entire history of the invention. In this case, it is extremely difficult to talk about one inventor and it is only possible to trace the main stages during which various people made this or that invention.

This rule also applies to the invention of the microscope. But in the literature, despite the fact that there are works showing the complexity of this problem [Katsnelson, 1963; Gurikov,1980], they often, sometimes undeservedly, try to name a specific author of the invention [Sobol, 1943; Sobol, 1957; Vinogradova, 2012], Therefore, the purpose of this article is to try to understand the question of authorship for the invention of the microscope and to tell about the stages of creating this optical device.

Stage 1 – the creation of lenses and the study of the main optical phenomena.

To create a light microscope, lenses are necessary, and the history of lens production goes back to the very distant past. So an ancient large flat-convex lens made of rock crystal in Nineveh 2500 years BC has come down to our times. Glass lenses began to be manufactured around 600-400 years before the Birth of Christ, in Mesopotamia [Vinogradova, 2012]. In Europe, in Sweden, a double lens (convex on both sides) with a diameter of 5 cm was also found made in 500 AD. The list of discovered ancient lenses can be continued for as long as you want, but now, alas, you can only guess about the scope of their application at that time. The first

and rather detailed description of lenses as an object for image magnification appears only in the works of Roger Bacon in the 13th century [Tolansky, 1971; Gurikov, 1983]. However, the first works on optics appear in antiquity. It is known that Euclid and Aristotle experimentally established the main optical phenomena — rectilinear propagation of light, independence of light beams, reflection from a mirror surface and refraction of light at the boundary of two transparent media. But although ancient scientists were interested in the nature and properties of light, nothing is yet known about the creation of optical devices by them. Despite the existence of a sufficient number of theoretical works on optics, practical optics, especially in terms of the use of lenses, developed extremely poorly until the late Middle Ages [Gurikov, 1980]. And only in the 13th century the first glasses appeared in Italy.

Stage 2-creation of complex optical devices.

The first ideas about creating complex optical devices from lenses to increase the image are found in the manuscripts of Leonardo da Vinci. In his manuscripts there are many graphic constructions of the course of rays in lenses, an experimental method for determining optical aberrations is given. From the works of the scientist, it can be learned that he may be the creator of not only a single-lens, but also a two-lens (with two convex (collective) lenses) optical device for image magnification [Gurikov, 1980]. There are drawings of such a device, but whether it was created, unfortunately, is unknown.

The business started by Leonardo da Vinci was continued by his compatriot Giovanni Battista de la Porta (1535-1615). Thus, in the book "De refractione" ("On Refraction"), he tries to study optical systems consisting of a combination of convex and concave lenses, i.e., the scheme of the telescope,which will later be created by Galileo [Dorfman, 1974; Gurikov, 1980]. Unfortunately, in the literature devoted to the creation of a microscope, neither Leonardo Davinci nor Giovanni Battista are almost never mentioned. It is generally believed that the first devices that can be called

microscopes, if one wishes, were invented either by the Dutch master of glasses Zachariah Jansen, or by the Italian scientist Galileo Galilei [Sobol, 1945; Katsnelson, 1963; Tolansky, 1971; Gurikov, 1983; Vinogradova, 2012]. But they cannot definitely be considered the inventors of the microscope. First of all, these inventors did not seek to create a microscope at all, and were not engaged in subsequent research of the microcosm. They simply created optical devices that could be used for microscopy if desired. Moreover, they have competitors who claim to be the first in the creation of two-lens devices for image magnification. There is information that Johann (Hans) Lippersgey, (a master of making glasses) who lived next door to the Jansen family, also created at the same time an optical device very similar to the device of Master Jansen. But he tried to use it not as a microscope, but as a telescope and tried to patent it, but he did not receive a patent because the right to invent similar devices at the same time was claimed by masters - Zachariah Jansen and Jacob Metius from Alkmaar [Borellus, 1665; Sobol, 1941; Sobol, 1945; Katsnelson, 1963; Gurikov, 1983]. Information about the invention and existence of the telescope reached Galileo Galilei. Thanks to this information, Galileo created his own optical instrument, but at the same time using the scientific knowledge accumulated in optics by this time. Later (1609-1610), Galileo, improving the telescope he designed, tried to use it as a kind of microscope by changing the distance between the concave eyepiece and the convex lens [Tolansky, 1971; Gurikov, 1983]. However, his device was extremely inconvenient to handle and was subsequently forgotten and lost.

Thus, four people at once claim the right to be called the inventors of an optical device that could be used as a microscope. But the instruments of Galileo, Zachariah Jansen, Lippersgey and Metius, unfortunately, have never been used to study the microcosm. However, thanks to them, the stage of active development of optics began, and devices designed specifically for

the study of the microcosm were invented, but these were devices of a different design.

Stage 3-creation of optical devices intended for microscopy.

The theoretical basis for the creation of such microscopes was laid by the work of Johannes Kepler. He suggested using an eyepiece and a lens in the form of single convex lenses, which gave an inverse (inverted) image [Sobol, 1945; Katsnelson, 1963]. The first device for studying the microcosm according to the Kepler scheme was created by Cornelius Jacobson Drebbel — a Dutch inventor who was engaged in research in the field of chemistry and optics. His optical device was first presented to the public in 1619 in London, and brought him wide fame. Many modern devices for microscopy also work on the principles proposed by Drebbel. The microscopes made by him spread in Europe, having penetrated from England to France and Italy [Sobol, 1943; Sobol, 1957]. Due to the fact that it was his model of the microscope that became widely distributed for the first time, he is also considered one of the inventors of the microscope. In any case, Christian Huygens wrote: "In 1621, Drebel, a Dutchman who lived in London, was known as the owner of such complex microscopes and was considered by everyone to be their inventor" [Sable, 1945]. Christian himself was also interested in optics. He is the author of the "Treatise on Light" (the wave theory of light), which was published in 1678. He also wrote the theory of reflection, refraction and double refraction. And it is Huygens who is considered by many to be the father of the principles of modern microscopy [Sobol, 1945; Katsnelson, 1963]. But, despite his great contribution to the development of optical devices, Huygens still did not invent the microscope. But thanks to his theory of the undulating propagation of light in 1665, Robert Hooke, an English naturalist, created his own microscope. Moreover, he supplemented the theoretical scientific basis necessary for the creation of microscopes by creating a hypothesis about the transverse nature of light waves, which he outlined in the book "Micrography". The main postulates of this theory were

subsequently confirmed by experience, and are used in modern microscopy. R. Hooke also owns the first successes associated with the use of the microscope in scientific biological research [Sobol, 1945; Katsnelson, 1963; Vinogradova, 2012].

A special and very noticeable trace in the history of the development of microscopy was left by Anthony Van Leeuwenhoek, who lived in the Netherlands, in the city of Delft from 1632 to 1723 [Churilovsky, 1966; Vinogradova, 2012]. He is often called the inventor of the microscope. But the merit of Leeuwenhoek is not the creation of a microscope. I must say that not only the device itself, but also the name of the device appeared even before the birth of Leeuwenhoek. It was proposed in 1625 by I. Faber, a member of the Roman "Academy of the Sharp-Sighted" ("Akudemia dei lincei") [Vinogradova, 2012]. The merit of Leeuwenhoek is that he independently made and used simple (single-lens) microscopes in his research, which gave an image magnification of up to three hundred times. It was Anthony Van Leeuwenhoek who first, based on the experience of his observations, compiled a description of the kingdom of microscopic organisms and bacteria. He actively popularized his discoveries and because of this he is often called the inventor of the microscope. In the future, the light microscope was improved many times: In 1668 Eustachy Divini, having attached a field lens to the eyepiece, invented an eyepiece of a modern type [Vermel, 1970; Vinogradova, Zakharov, 1918]. In 1673, Jan Hevelius introduced a micro-screw, in 1716, G. Hertel proposed placing a small mirror under the object table to direct light rays into the microscope tube [Vinogradova, Zakharov, 1918]. As a result, microscopes began to be made from five main parts, which are currently part of the modern optical microscope. These are: 1. the housing; 2. the light source, the beam from which is focused on the lens; 3. the object table; 4. the lens; 5. the eyepiece.

THE MAIN TYPES OF LIGHT MICROSCOPES USED FOR BIOLOGICAL RESEARCH

To date, the design of the microscope has become somewhat more complicated, although the basic scheme remains the same. A conventional light microscope used in laboratories for biological research usually has several interchangeable lenses installed in a revolver. A pair of eyepieces is also used to provide binocular perception of the studied drug. Projection or photo eyepieces designed for projecting images onto the screen or photographing can be attached to it as options. The design of light microscopes largely depends on the methods of light microscopy, determined by the goals of research and the characteristics of the objects under study. Therefore, in addition to the usual universal optical microscopes found in schools and laboratories, there are a large number of specialized microscopes:

- Comparison microscopes. They provide a visual comparison of the two drugs.
- Contact microscopes. They are used for microscopic studies of the structures of individual sections of biological tissues. To do this, the microscope lens is pressed against the object under study.
- Stereo microscopes allow you to examine an object from different angles of view. Thanks to this, a person perceives the image three-dimensionally.
- Ultraviolet and infrared microscopes designed to study objects in the ultraviolet or infrared part of the light spectrum that is invisible to the naked eye. To do this, they are equipped with a fluorescent screen, on which an image of the studied drug is formed. A camera or an electron-optical converter can also be used.
- Polarizing microscopes. They allow us to detect inhomogeneities (anisotropy) of the structure of objects by studying the contrast of the image or color changes. With their help, it is possible to

analyze molecular compounds of organic and synthetic types, as well as to study various natural objects with a crystal structure.

- Interference microscopes that make it possible to study objects with low refractive indices of light and extremely small thickness. In an interference microscope, the light beam entering the microscope is bifurcated. Part passes through the object under study, and the other passes by. In the ocular part, both beams connect and interfere, which allows you to see the structure under study.
- Luminescent microscopes. They use the effect of luminescence of biological objects that occurs under the influence of ultraviolet radiation. These microscopes examine the structure of the objects under study, which is actively used in microbiology and immunology.
- Operating microscopes. They can have a fairly complex mechanical device and are used for microsurgical operations.
- Comparison microscopes that allow visual comparison of two drugs;
- Contact microscopes for microscopic studies of the structures of individual sections of biological tissues (for this, the microscope lens is pressed against the object under study;
- Ultraviolet and infrared microscopes designed to study objects in the ultraviolet or infrared part of the light spectrum invisible to the naked eye, and many other microscopes designed for a narrow range of purposes.

But the most common is the classical optical microscope, the basics of work on which this material is devoted.

BASIC METHODS OF LIGHT MICROSCOPY

There are the following main methods of research using light microscopes:

- The method of the light field in the flow of passing light. It is based on the principle of passing a stream of light through a sample, which partially absorbs and scatters the rays falling on it, and thus forming an image obtained in the eyepiece. The most common method used to study colored tissues of plants and animals, thin sections, cuts, etc.
- The method of oblique lighting. The light flow is directed at a large angle to the test sample. It is used to identify the relief of the sample under study and to increase the contrast of the resulting image.
- The method of the light field in reflected light. The subject of the study is illuminated from above, and the image is formed due to the different reflectivity of the object's surface. Allows you to study the surfaces of opaque objects.
- The dark field method. The light rays are directed by the condenser so that they form a hollow cone, in the center of which is the lens. Due to this, some of the rays do not fall into the microscope lens, and the observed object looks like it is illuminated in a dark field. The method is intended for the study of transparent samples that do not absorb light.
- Ultramicroscopy method. Bright rays of light are directed perpendicular to the object table. The wave scattering effect makes it possible to detect very small particles less than half the wavelength in size. It is used for observation, analysis and counting of small objects.
- Phase-contrast method. When passing through the sample, the light wave acquires a phase relief, which is then fixed by a special lens. The image is visible as elements with different brightness. Allows you to study transparent and unpainted samples, the structures of which have different optical densities. For phase-contrast microscopy, special eyepieces and a condenser, or a specialized phase-contrast microscope, are used.

- The polarization method. The analysis of anisotropic materials is carried out in light passed through a special filter, as a result, when passing through the sample, the plane of polarization of the rays changes. Thanks to this, it is possible to analyze and study objects that have the properties of double refraction. It is intended for the formation of images of unpainted anisotropic structures (for example, collagen fibers and myofibrils).
- Interference method. It is based on the interference of light, when each beam is bifurcated, entering the microscope. One of the received rays is directed through the observed object, and the other is directed past it. In the ocular part of the microscope, both beams are connected again by interfering. It is used to study living tissues and cells.
- The fluorescent (luminescent) method. It is used for detecting fluorescent (luminescent) objects. In a fluorescent microscope, light from a powerful source passes through two filters. One filter passes only the light of the wavelength that excites the fluorescence of the sample. Another filter passes light of the wavelength emitted by a fluorescent object. Thus, fluorescent objects absorb light of one wavelength and emit another wavelength. It is widely used in materials science and biomedical research.

BASIC CONCEPTS OF OPTICS USED FOR LIGHT MICROSCOPES

In order to talk about the device of the microscope, let's consider some concepts that determine the principles of its operation.

The main task of the microscope is magnification. But the concepts of magnification are different. The generalized magnification is understood as the ratio of the size of the object to the size of the image. Visible magnification refers to the ratio of the linear or

angular dimensions of the image and the object. In the case of a conventional laboratory microscope, its magnification is determined in krats. Krats show how many times (i.e., how many times) one value is greater than another. Thus, the magnification of the microscope is determined by multiplying the magnification of the lens by the magnification of the eyepiece. Sometimes there are models of microscope tubes with additional lenses, then their magnification is also taken into account.

There is also a distinction between useful and useless magnification. If when you zoom in on an object, you can see new details of its structure, then this is a useful increase. If new details of the structure are not detected when the object is enlarged, then this is a useless increase. If we take a drop of turbid water and look at it through a microscope, then as we zoom in, we can first see small dots, then large single-celled organisms, and with even greater magnification, small ones. But if a drop of water is simply photographed with an ordinary camera and viewed on a computer monitor with different magnifications, then no matter how much we increase the image of the drop, it will not be possible to see microorganisms (Fig. 1).

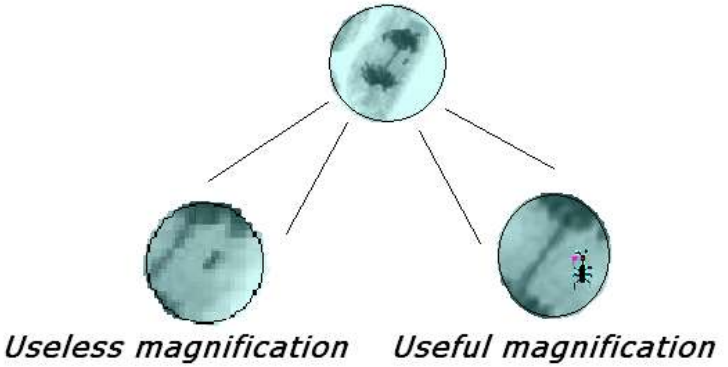

Figure 1. Types of magnification.

The useful magnification depends on the resolution of the microscope (the linear limit of the resolution of the microscope), i.e. the ability to give a separate image of two lines close to each other. A person with good eyesight is able to distinguish about 5-10 lines per 1 mm. That is, the eye can distinguish a line with a thickness of about 100 microns (1 micron). A good microscope has a resolution of about 0.2 microns and on a segment of 1 mm long, you can already distinguish 5000 lines [Hubel, 1990].

Linear resolution limit according to the Abbe diffraction theory, it depends on the wavelength of light (λ) that illuminates the object and the numerical aperture of the microscope (NA)

$$R = \lambda / 2NA$$

Therefore, in order to increase the resolution of the microscope, it is necessary to reduce the wavelength of light and increase its numerical aperture (numerical aperture is a characteristic of microscope lenses, showing their ability to collect light without diffraction blurring of image details). You can reduce the wavelength by illuminating the object with short-wave ultraviolet light. An increase in the numerical aperture can be achieved [Skvortsov et al., 1969]:

- Choosing a larger angle of the light cone, from the lens side and from the lighting source side. This allows you to collect more refracted rays of light from very thin structures in the lens. To do this, a condenser is used on the microscope;
- By increasing the refractive index. For this purpose, immersion fluids are used between the front lens of the immersion lens and the cover glass. This allows you to increase the value of the numerical aperture.

Immersion fluids they are used in conjunction with immersion lenses specially designed to work with these liquids

and, accordingly, marked. Due to the higher refractive index compared to air, immersion liquids allow light rays deflected by the smallest details of the object not to disperse, leaving the drug, but to get into the lens. This helps to increase the resolution of the microscope.

Distilled water is used as an immersion liquid for water immersion lenses, and cedar oil or a special synthetic immersion oil is used for oil immersion lenses. The use of synthetic immersion oil is preferable, since its parameters are more accurately normalized, and unlike cedar oil, it does not dry out on the surface of the front lens of the lens. For lenses operating in the ultraviolet region of the spectrum, glycerin is usually used as an immersion liquid. Most often, immersion lenses are designed to work with a certain immersion liquid, but there are also lenses that can work with any immersion medium. Since immersion oils differ somewhat, when using them, you should follow the recommendations of the manufacturer of optics. It is especially important to avoid mixing different oils. If there are traces of oil on the lens, then the image quality may deteriorate when using another oil, so the lenses must be cleaned immediately after applying the immersion liquid.

In rare cases, to increase the aperture of the condenser, an immersion liquid (more often distilled water) can be placed between the condenser and the preparation.

Many lenses produce an image in which the central part and the periphery cannot be focused simultaneously (Vinogradova, Zakharov, 2018). The degree of such discrepancy is determined by the depth of field.

Depth of field (GRIP) The image is such a range of distances in the resulting image, in which objects are perceived as sharp (Fig. 2). The sharpness of the image changes gradually. Since there is no clear boundary for determining the maximum blur of an object, after which it is perceived as unsharp, a more accurate term

is used - "circle of non-sharpness". When the circle of non-sharpness becomes discernible with our eyes, this area is considered to have gone beyond the depth of field and is not "acceptably clear" [Churilovsky, 1966; Begunov, 1966; Ivanova, 1984].

Figure 2. Depth of field

To solve this problem, manufacturers produce special lenses with a minimum curvature of the field of view.

Contrast – the distinctness of the object of observation against the surrounding background. It is necessary that the details of the image differ in brightness or color, so that the human eye can distinguish them from each other. In the microscopy of transmitted light, the contrast of the sample is usually provided by different levels of light absorption by individual parts of the preparation.

The aperture is the value that characterizes the light transmission of the lens, i.e. the degree to which the luminous flux

weakens after hitting the lens on the way to the eyepiece. This indicator is influenced by the opening diameter of the diaphragm, and the characteristics of the optics used (lens transparency, etc.).

When working with a microscope, it is also important to know the disadvantages inherent in optical devices. A modern lens consists, as a rule, of several glass lenses. The first, the main lens is designed to obtain an enlarged image, and all other lenses eliminate optical aberrations (Churilovsky, 1966; Begunov, 1966; Ivanova, 1984). In particular:

- Spherical aberration caused by a stronger refraction of light rays falling on the edge of the lens than rays passing through its center. This leads to the fact that the rays that are variously removed from the axis are collected at different foci and the image of the point in the eyepiece is visible as a blurry spot. Spherical aberration is eliminated with the help of scattering lenses because their aberrations are of the opposite nature. Sometimes, for the same purpose, lens diaphragming is used, i.e. limiting the width of the light beam. The aperture can be an opening in an opaque screen placed in front of the lens, or the lens frame itself.
- Comatic aberration (coma) – which is a special case of spherical aberration, when image distortions are visible, for example, in the form of dots with a blurred "tail", similar to the tail of a comet. These aberrations are corrected, as well as spherical ones.
- Astigmatism. When the rays propagating in the same direction and along the same straight line, in perpendicular planes, have different focal lengths, which is why the image is blurred in one of the planes (horizontally or vertically). It is corrected – by precise sharpening of the lens.
- Chromatic aberration. Due to the dispersion of light, rays with different wavelengths, for example, red and green, are refracted differently in the lens and the foci for them do not coincide. As a

result, the outline of the object in the eyepiece will be visible spectrally colored. To correct chromatic aberration, a system of lenses made of special grades of glass is selected. Such a system (in which the spherical aberration is also compensated) is called an achromate.

- The simplest monochromatic aberrations – defocusing and distortion on an inclined plane, are corrected by shifting the lens along the optical axis in order to align the focal plane of the lens with the image plane.

OPTICAL LIGHT MICROSCOPE DEVICE

The principle of operation of an optical microscope is based on the fact that a diverging beam of light passes through the sample and, falling on the lens, forms an enlarged image, which, passing through the tube, falls on the eyepiece, where it is enlarged again. After that, the light entering the retina of the eye forms the image we see.

An optical microscope consists of two main parts - mechanical and optical (Fig. 3). The mechanical part does not directly participate in the image creation process, only the optical part directly participates in this.

The mechanical part consists of:

1. housing (tripod);
2. the object table;
3. tubes;
4. revolvers with lens sockets;
5. Macro and micrometer screws for coarse and fine tuning.

The optical part consists of:

6. the lens;
7. eyepieces;
8. lighting system.

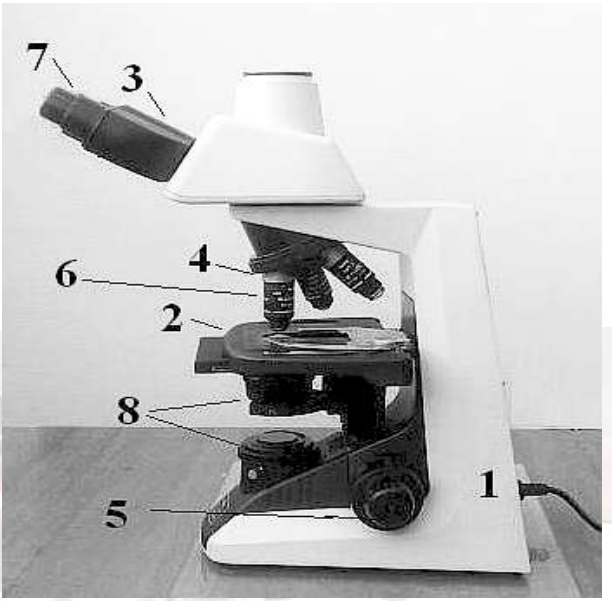

Figure 3. The device of the microscope

MECHANICAL DEVICE OF THE MICROSCOPE

The body (tripod) of the microscope is the basis for the installation of optical and mechanical parts of the microscope. By design, tripods are divided into two main groups — straight and inverted (Fig. 4). In straight tripods, the lenses and eyepieces are located above the table, the condenser is below the table, and in inverted microscopes, vice versa.

Figure 4. Straight & Inverted microscope

The slide table is designed for fixing or fixing in a certain position of the object of observation. Tables can be fixed, coordinate and rotating (centered and not centered) (Fig. 5). Even the simplest object tables allow you to move an object in two coordinate planes, and more complex ones provide movement along three axes and rotation at a certain angle. Motorized tables can also be used in research microscopes, which allow you to automate the shooting process and track the drug at certain coordinates at certain time intervals.

Figure 5. Subject tables

The tube is designed to combine the lenses and eyepieces of a microscope into a single optical system. On one side of the tube is a revolver with lenses, and on the other an eyepiece.

The revolver is designed for quick change of lenses that are screwed into its sockets. Usually 3, 4 or 5 lenses can be installed on the revolver. The lens is changed by simply turning the revolver head. Microscope lenses are characterized by nominal magnifications, therefore, as a rule, a set of lenses is selected from the following series of magnifications: 5; 10; 20; 40 times for dry lenses and with an increase of 50; 60; 90; 100; 120 times for immersion lenses.

Macro and micrometer screws for coarse and fine tuning. The macro screw is designed for coarse focusing and performs a smooth movement of the tube or the object table for a sufficiently large distance, depending on the design of the microscope (Fig. 6). The micro screw is designed for precise adjustment of the lens to the sharpness of the image. The value of the exact movement usually does not exceed 2 mm with the price of dividing the drum scale of 2 microns.

Sometimes there may be a third screw next to the macro and micro screws – a screw that regulates the torque of the macro screw. It can be used to reduce or increase the force when rotating the macro screw. Excessive weakening can lead to the fact that the object table will fall down under its own weight.

Figure 6. Screws: 1-macro 2-micro; 3-adjusting the torque of the macro screw

OPTICAL DEVICE OF THE MICROSCOPE

Lenses are optical systems for constructing a microscopic image in the image plane with the appropriate magnification, resolution, reproduction accuracy in the shape and color of the object of study. Lenses are the key and often the most expensive part of a microscope. They are usually located in the lower part of the tube in the sockets of the revolver.

There are several categories of lenses:

- Achromatic (achromats) are the most common. They bring the blue and red rays into one focus. The image they create may have slightly noticeable colored rings, green or purple. Achromats are corrected for spherical aberration only for green rays.
- Semi-apochromatic or fluorite lenses (so named because they contain lenses made of the mineral fluorite). Chromatic aberration is better corrected in them than in achromatic lenses. Due to this, they are produced with a relatively larger (at a given magnification) aperture and give a higher-quality and contrasting image. The large aperture makes fluorite lenses convenient for fluorescence microscopy. They can also be successfully used for photomicrography.
- Apochromatic-unlike achromatic lenses, they almost do not distort the natural color of the object. Apochromats are the most corrected lenses, in which chromatic aberration is almost completely corrected, and spherical aberration is corrected not for one, but for two colors. Such lenses are difficult to manufacture, therefore, in microscopes of many companies, they achieve correction of secondary chromatic aberration with the help of special "compensating" eyepieces.

- Planachromatic lenses almost completely eliminate spherical aberration and have a minimal curvature of the field of view. Such lenses are marked with the prefix "Plan" (Plan), for example, Planachromat or Planapochromat (Plan-Aro). Planapochromatic lenses allow you to get a sharp and not distorted image across the entire field. In addition, some modifications of flat-field lenses correct chromatic aberrations.
- There is also a distinction between dry and immersion lenses. When using dry lenses, there is air between the cover glass covering the object and the front lens of the lens. During the transition from glass to air, due to differences in the refractive coefficients, some of the rays are deflected to the side, which worsens the illumination of the object.

Immersion lenses should be used in cases where high image clarity is needed. Immersion (from Lat. immersion is a liquid that fills the space between the object of observation and a special immersion lens (a condenser and a slide). There are the following types of immersion lenses:водной иммерсии (ВИ, W);

- oil immersion (Oil);
- glycerin immersion (Glyc).

Also, to achieve better brightness and clarity of the image, a special anti-reflective coating can be applied to the lenses of the lenses, which reduces the amount of reflected light and, as a result, increases the light transmission coefficient of the optical system, which, in turn, leads to the formation of a brighter and more contrasting image. Such optics are often called enlightened.

LENS DEVICE

The lens has a complex opto-mechanical design, which includes several single lenses and components glued together from 2 or 3 lenses. The number of lenses is determined by the

range of tasks solved by the lens. The higher the image quality given by the lens, the more complex its optical scheme is.

In the front part of the lens located opposite the object being studied, there is a front lens facing the drug. It is the main lens for building an image of the appropriate quality. The front lens determines the working distance and the numerical aperture of the lens. The subsequent part of the lens in combination with the front one provides the required magnification, focal length and image quality.

The degree of magnification of the image of the object being studied is one of the main parameters of optical lenses. According to the degree of magnification, the lenses are divided into:

- small magnification – up to 10x;
- average magnification - from 10x to 50x;
- large magnification – from 50x to 100x;
- lenses with ultra-large magnification-over 100 x.

The lens, in addition to magnification, also provides the resolution of the microscope, which is characterized by the numerical aperture of the lens. According to the size of the aperture, the lenses can be divided into:

- lenses with a small aperture-up to 0.25;
- with an average aperture - from 0.25 to 0.65;
- with a large aperture-more than 0.65.

The larger the NA of the lens, the finer details it can resolve. Magnification and aperture are not strictly related to each other. For example, there may be lenses with the same magnification of 40 times, but different apertures-0.65 and 1.3. Both give images similar in size, but the second allows you to distinguish smaller details (Bradbury et al., 1992; Vinogradova, V. V. Zakharov, 2018).

When working with a microscope, the working distance of the lens is also of great importance, i.e. the distance from the

lower (front) lens of the lens to the cover glass. The thicker the cover glass and the greater the magnification of the lens, the smaller the working distance. It can range from a few millimeters for weak magnifications and up to fractions of a millimeter for strong ones. Therefore, the front lenses of lenses with high magnification can be equipped with a spring-loaded telescopic mechanism that prevents the cover glass from crushing and damaging the lens.

Some lenses are designed to work with an uncovered cover glass preparation, others, on the contrary, can be used when working with culture vials and are designed for the thickness of their walls.

Small deviations in the thickness of the cover glass are usually insignificant for lenses with an aperture of less than 0.65, but they are important for dry lenses with a large aperture (0.75-0.95).

MARKING OF LENSES

Data about each lens is marked on its body (Bradbury et al., 1992; Pritychenko et al., 2010) (Fig. 7). The

magnification is indicated by numbers often with an "x" sign indicating the magnification factor - 10x, 40x, 100x. According to the international marking system, it is duplicated by color rings: black (1x, 1, 5 x); brown (2x); red(5x); yellow (10x) , green (20x, 25x, 30x); blue (40x, 50x); white (100x, 150x).

The numerical aperture is usually written together with the magnification values in the form of digits-0.20; 0.65. Such an entry may look like this-40/0, 65 or 40x/0.65.

The correction for the infinite length of the tube is indicated by the infinity sign -∞.

Working with or without a cover glass – 0 - without glass, – - does not matter

The type of optical correction is marked as follows:

- ARO (APO) - apochromatic lenses;
- S-Plan, Semi-Plan-Semi-planachromatic lenses;
- PL, Plan (PLAN) - planachromatic lenses;
- Plan-Aro (PLAN-APO) - planapochromatic lenses;
- **Achrostigmat, CP-achromat, Achroplan**

There may be an additional letter marking if the lens is used for various methods of research and contrast:Рп2 (Ф) - фазовый;

- Pol - polarizing;
- L - luminescent;
- PhL - phase-luminescent
- Epi, HD — an epi lens for working in reflected light using the dark field method;
- * DICK - differential interference contrast.

Figure 7. Lenses.

THE DEVICE OF EYEPIECES

The eyepieces are placed in the upper part of the tube. They consist of two lenses forming the image as one convex collecting lens. Eyepieces give an increase in the image, but do not contribute to increasing the resolution of the microscope. The most common

are eyepieces with magnification of 7, 10, 15 and 20 times (Bradbury et al., 1992; Pritychenko et al., 2010).

In general, eyepieces consist of two groups of lenses: the eye — the closest to the observer's eye — and the field-the closest to the plane in which the lens builds an image of the object under consideration.

Distinguish between eyepieces:

- compensatory (K — compensate for the chromatic difference in magnification of lenses over 0.8%) and non-compensatory action;
- regular and flat fields;
- wide-angle (with an ocular number-the product of the magnification of the eyepiece by its linear field-more than 180);
- ultra-wide-angle (with an ocular number greater than 225);
- for working with and without glasses;
- for observation, projection, photo eyepieces, gamals;
- with internal aiming (with the help of a movable element inside the eyepiece, the adjustment is made to a sharp image of the grid or the image plane of the microscope, as well as a smooth, pankratic change in the magnification of the eyepiece) and without it (Fig. 8).

Figure 8. Eyepieces.

MARKING OF EYEPIECES.

The magnification on the eyepieces is indicated as well as on the lenses - 10x, 15x, 20x. The size of the field of view of this eyepiece 18, 20, 22 (in mm) can be indicated next to it through a slash. Such a record may look like this-10x / 18 (Fig. 5). Work with glasses is indicated by an additional symbol in the form of glasses, for example - ∞.

The focusing (mobile) element inside the eyepiece for focusing the image of the eyepiece grid is designated-foc. The type of correction is Pl. Compensation of the chromatic difference of magnification-K. In the eyepieces of Nikon microscopes, the CFI UW symbols indicate the presence of eyecups.

LIGHTING SYSTEM

Despite the fact that the lighting system is not involved in the formation of the image, it is one of the most important factors determining the image quality in the microscope, by creating the best illumination of the drug. It generates a luminous flux that is applied to the object in such a way that the subsequent parts of the microscope perform their functions as accurately as possible to build an image.

Depending on the model of the microscope, the following lighting systems are distinguished:

- Illuminator with a mirror. It is used, as a rule, for field and children's microscopes.
- "Critical" or simplified lighting. It is used in budget microscopes that are used in biology and medicine.

A uniformly bright light source is located directly behind the field diaphragm and with the help of a condenser, its light is projected on the plane of the object. In this light, the size of the field aperture is selected so that its image is precisely limited by the field of view of the eyepiece (at low magnification of the lens). Due to the fact that

critical illumination does not give a direct path of rays through the entire optical path, the resolution at critical illumination is lower than when illuminated by the Keller (Keller) method.

Lighting by Koehler. It is used in microscopes of the laboratory class and higher. The principle of Koehler illumination consists in setting the direct path of the beam along the entire optical axis of the microscope. This gives:

- uniformity of illumination in the plane of the drug (no darkening at the edges);
- removing artifacts - the user sees the surface of the object without dust from the illuminator and cover glass;
- due to the matching of the apertures of the illuminator and the lens, the maximum image resolution of the object under study is achieved;
- * Koehler illumination is also necessary for working with such contrast methods as phase contrast, dark field, polarization, fluorescence, etc.

Without adjusting the Koehler illumination, these methods will not work in principle, since they assume a completely straight course of rays along the entire optical path.

In direct microscopes, the lighting system is located under the object (for example, laboratory, polarizing, etc.) and above the object in inverted ones. It includes (Fig. 9):

1. A light source (a halogen lamp or LED and an electric power supply);
2. Optical-mechanical system:

- collector;
- condenser;
- field and aperture adjustable/iris diaphragms.

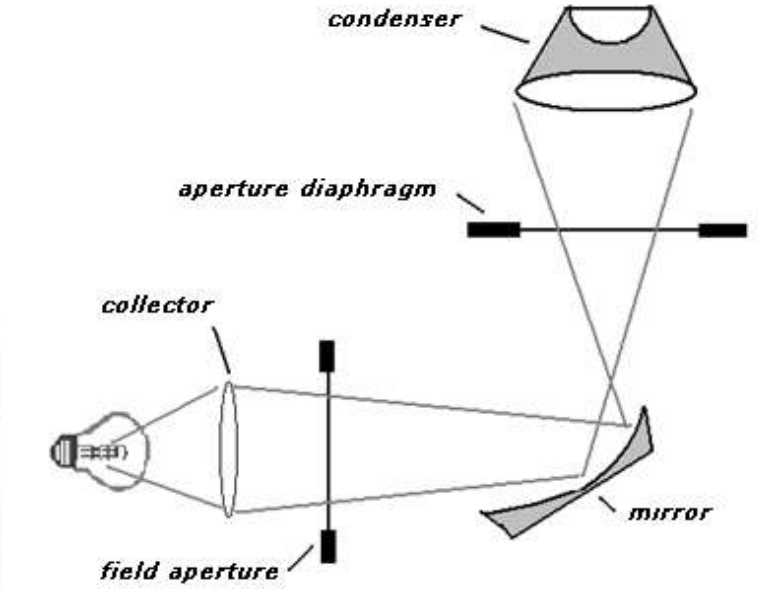

Figure 9. Lighting system

The light source can be either ordinary halogen lamps, or xenon, mercury lamps for fluorescent (luminescent microscopy). Also, LED illuminators with a long service life and lower energy consumption are gaining more and more popularity.

The collector is located in close proximity to the light source. It forms an enlarged image of a luminous body in the plane of the aperture diaphragm of the condenser (or, in the case of reflected light, in the plane conjugated to the output pupil of the lens).

In laboratory models of a microscope, the illuminator (lamp, collector and field diaphragm) is usually placed on the tripod of the microscope. In simpler models, the illuminator is not connected to the microscope tripod.

A condenser is an optical system consisting of two (sometimes three) lenses. It is located between the object and the light source. The condenser increases the amount of light entering the microscope to illuminate the object with a widely diverging

beam of rays. To obtain the correct illumination, the condenser is installed so that the aperture of the diaphragm is clearly visible in the plane of the drug. To do this, you can use a not very dense sheet of paper placed on the slide table. The adjustment is made by a light spot visible on a piece of paper. When adjusting the illumination (adjusting the microscope), the condenser moves along and perpendicular to the optical axis. Usually, the condenser should be located close to the object of observation.

Figure 10. The condenser

At the bottom of the condenser there is an aperture iris diaphragm, which affects the image contrast and resolution.

The iris diaphragm (from Lat. iris "iris" (the flower of the same name has nothing to do with the name) serves to regulate the number of rays entering the lens. The diaphragm, together with the ring for frosted glass located under it, is fixed in a frame that moves up and down in common with the condenser. The diaphragm is designed to limit the amount of light only in the part of the drug that is being studied at a given time. This is especially useful when working with large magnifications, when it is necessary to

highlight only a small area of the sample (Fedin, 1961; Bradbury et al., 1992; Pritychenko et al., 2010).

An open field aperture increases the width of the light beam. This setting is used when working with small magnifications (a larger field of view). With increasing magnification, the aperture becomes narrower.

Figure 11. Aperture aperture

RULES FOR WORKING WITH A MICROSCOPE

Preparation of the microscope for operation begins with its inspection and, if there is a need, removing dust from the surfaces of the slide table, lenses and eyepieces with a soft cloth or a cloth specially designed for the care of optics.

If necessary, before starting work, the lighting system is adjusted. On different models of the microscope, it can be carried out in different ways, depending on the design. Let's look at several ways to configure microscopes with a lighting system.

If this is a school microscope, or some other microscope that has a rotating mirror in the lighting system, then the following setup scheme may suit it:

- Turn on the illuminator lamp and direct its light to the flat side of the microscope mirror;
- Cover the microscope mirror with a piece of white paper and focus on it the image of the filament of an incandescent lamp or a clearly defined spot of an LED lamp. To do this, move the illuminator itself or its lamp;
- Remove the sheet of paper from the mirror;
- Close the aperture diaphragm of the condenser. By moving the mirror and slightly moving the lamp holder, focus the image of the filament of the incandescent lamp or the clearly defined spot of the LED lamp on the aperture diaphragm. The distance of the illuminator from the microscope should be such that the image of the lamp filament is equal to the diameter of the aperture diaphragm of the condenser (you can observe the aperture diaphragm using a flat mirror placed on the right side of the base of the microscope);
- Open the aperture diaphragm of the condenser, reduce the opening of the field diaphragm of the illuminator and significantly reduce the glow of the lamp;
- Place the finished product on the microscope slide table;
- At low magnification (10x), looking into the eyepiece, get a sharp image of the drug;
- By turning the mirror slightly, move the image of the field aperture, which has the form of a light spot, to the center of the field of view of the eyepieces. By lowering and raising the condenser, achieve a sharp image of the edges of the field diaphragm in the plane of the preparation (a colored border may be visible around them);
- Open the field aperture of the illuminator to the edges of the field of view, increasing the glow of the lamp thread and slightly (by 1/3) reducing the opening of the aperture diaphragm of the condenser;

- When changing the lens, it is advisable to check the light setting.

For modern laboratory microscopes, the following scheme is suitable

- Focus the image on a low-magnification lens (10x);
- Close the field diaphragm;
- Use the handle to raise the condenser to its working height. At the same time, you should see the edges of the field aperture;
- Center the field diaphragm with the alignment screws;
- Open the field aperture;
- Use the aperture scale on the condenser to set the aperture slightly smaller than the lens aperture.

After the end of the light adjustment, it is not necessary to change the position of the condenser, the opening of the field and aperture diaphragm in the future. The illumination of the drug can be adjusted only by neutral light filters or by changing the lamp glow using a rheostat. Thus, excessive opening of the aperture diaphragm of the condenser can lead to a significant decrease in image contrast, and insufficient opening can lead to a significant deterioration in image quality (the appearance of diffraction rings) (Cherkes et al., 1986).

After installing the lighting, you can install the object of observation, i.e. correctly position it under the microscope to get the best image. To do this, you need to put the drug so that the object in question is in a beam of light coming through the diaphragm.

After that, we bring the object closer to the eyepiece using a macro screw at a distance of several millimeters. Further configuration is carried out using a micro-screw. To do this, looking through the eyepieces, carefully bring the object under study closer to the lens until its clear, sharp image appears. Sometimes it is advised to make the adjustment without bringing it closer, but on the contrary, bringing the object of observation as

close as possible to the lens in the future, remove it from it. This can prevent unwanted contact of the lens with the observed object.

After that, we proceed to the observation. The study of an object always begins with a general acquaintance with it at low magnification, in the future, to consider the details of interest to the researcher, he transfers the microscope to large magnifications.

To study an object at high magnification, first you need to put the selected area in the center of the field of view of the microscope at low magnification. Then change the lens by turning the revolver so that it takes the working position. Using a micro-screw, we adjust the image of the object. If necessary, add immersion oil.

At the end of working with a large magnification, set a small magnification, raise the lens or lower the object table, remove the drug from the work table, wipe all parts of the microscope with a clean cloth and cover it with a cover.

RECOMMENDATIONS FOR THE CARE OF MICRO-OPTICS

Special attention should be paid to the cleanliness of the surfaces of optical parts. Do not touch the lenses of lenses, condensers and eyepieces with your fingers, since fingerprints are very difficult to remove from the surface of the optics. The lenses must either be screwed into the revolver, or packed in cases.

Dust and lint sometimes fall on the last lens surface of the lens (from the thread side). They should be removed with a rubber pear, blowing the surface with a jet of air. It is extremely difficult to remove contamination from this surface, so you should always leave the eyepiece in the microscope tube or put a special cap on the tube. In addition, if dust or plaque appears on the inner surfaces of the lens lenses, then in no case should the lens be disassembled for cleaning. This is one of the most expensive and complex parts of the microscope, which can only be repaired in special workshops that have devices for assembling and adjusting lenses.

Dust is also removed from other external optical surfaces using a rubber pear or a very soft clean brush. If this does not help, then the surface should be gnrt67 for optics. You can also use a swab slightly moistened with a special mixture of alcohols for cleaning optics, consisting of ether and isopropyl alcohol or ethanol.

After working with the immersion, the remnants of immersion on the front lenses of the lens and the condenser should be removed with filter paper or a cotton swab, and the surface should be carefully wiped with a small swab slightly moistened with an alcohol mixture. The individual fibers remaining on the surface after cleaning from a cloth or swab are removed using a rubber pear or a brush. Special attention should be paid to the cleanliness of the surfaces of the collector lens and light filters, since these surfaces are depicted near the plane of the drug and their dirt is inevitably visible in the field of view.

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