МЕХАНІЗАЦІЯ, АВТОМАТИЗАЦІЯ ТА РОБОТИЗАЦІЯ
MECHANIZATION, AUTOMATION AND ROBOTICS

УДК 621.316.13

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METHOD FOR INCREASING THE RESOLVING POWER OF AN OPTICAL MICROSCOPE

Purpose. *Increasing the resolution and magnification of the optical microscope to expand the possibilities of observation and research in various fields of science and technology.*

Research methods. *Theoretical, experimental.*

Results. *A comparative analysis of the human eye as an optical system and the optical system of a microscope was carried out. It was found that these systems have a similar nature, namely, a separate image of two closely located objects. If we consider the human eye as a light-sensitive matrix, then to determine the resolution of the eye, it is necessary to use the ratio of the average value of the number of light-sensitive elements (rods and cones) that fall on the retina to the area of the retina itself (for the human eye, it is 0.1...0.2 mm). For an optical microscope, it is necessary to separate the concepts of "resolution" and "magnification".*

We also mentioned the physical basis of the resolution of optical microscopes. It was shown that for a dry lens the resolution limit for λ = 555 nm is 0.3 μm.

One of the ways to increase the resolution is the use of immersion fluids. However, in certain studies its use is inappropriate or impossible. If instead of conventional illuminators (maximum intensity at λ = 555 nm) you use ultraviolet ones (λ = 380…410 nm), then the resolution can be increased several times, and the increase is up to 2000. The disadvantage of using UV illuminators is that it is necessary to use a light filter with direct observation or a monitor with an indirect method to avoid negative effects on the observer's vision.

Scientific novelty. *The increased resolution and magnification of the optical microscope when using coherent light sources instead of immersion fluids provides more detailed scientific information and facilitates its processing.*

Practical value. *Using the results of our research allows you to get a more informative image when using coherent light sources.*

Key words: *resolution, microscope optical system, immersion lens, LED, camera matrix, blood preparation.*

Introduction

To date, increasing the resolution of the microscope remains an urgent task in modern science and technology. With the development of medicine, biology, nanotechnology and other fields, the ability to observe objects and phenomena on a small scale and with higher detail is needed. Increasing the resolution of the microscope helps expand the boundaries of observation and research, opening up new opportunities for analyzing structures and processes at the micro- and nanoscale. In this context, the development and improvement of methods and technologies aimed at improving the resolution of optical microscopes remains an important area of research.

Thus, the purpose of this research is to increase the resolution of the optical microscope to expand the possibilities of observation and research in various fields of science and technology.

Comparative analysis of the human eye as an optical system and the optical system of a microscope

Before we introduce a method for increasing the separation power of an optical microscope, let us recall such a natural optical system as the human eye. The human eye is a natural optical system. And this system is characterized by a certain resolution. What is the resolution of an optical system? This is the smallest distance between the elements of the observed object, at which these elements can still be distinguished from each other (elements of the object are usually understood as points or lines).

If the object is removed to the so-called distance of best vision, which for a healthy person is 250 mm, then for a normal human eye the minimum resolution is about 0.1mm, and for many people – about 0.20 mm, which is approximately the thickness of a human hair [1–3]. Where do these values come from? If we imagine the eye as a light-sensitive matrix, then to determine the resolving

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DOI 10.15588/1607-6885-2024-3-10

power of the human eye we use Considering that the number of light-sensitive elements in the human eye is within the range of $(96...137)\cdot10^6$, and the area of the retina: 700...800 mm^2, we obtain these values [4–6, 14].

The sizes of objects such as microorganisms, most plant and animal cells, small crystals, details of the microstructure of metals and alloys, etc., are significantly less than 0.1 mm. We will call such objects micro-objects. Microscopes of various types are intended for observing and studying such objects. With the help of a microscope, the shape, size, structure and many other characteristics of micro-objects are determined. An optical microscope makes it possible to distinguish structures with a distance between elements up to 0.20 μm, i.e. the resolving power of such a microscope is about 0.20 μm or 200 nm [7, 8].

When we talk about the resolving power of a microscope, we mean, just like the resolving power of the human eye, a separate image of two closely located objects. It should be remembered that resolving power and magnification are not the same thing. For example, if we use visualization systems to obtain photographs of two lines located at a distance of less than 0.20 μm (i.e. less than the resolving power of the microscope) from a light microscope, no matter how much we magnify the image, the lines will still merge into one. That is, we can get a high magnification, but we will not improve its resolution. The total magnification of a microscope is equal to the product of the linear magnification of the objective and the angular magnification of the eyepiece. Magnification values are engraved on the frames of the objectives and eyepieces. Let's consider a flat field microscope (not stereoscopic). These are biological microscopes, metallographic, polarizing. Usually, the objectives of such a microscope have magnifications from 4 to 100 times, and the eyepieces – from 5 to 16. Therefore, the total magnification of an optical microscope lies in the range from 20 to 1600 times. Of course, it is technically possible to develop and use objectives and eyepieces in a microscope that will provide a total magnification significantly exceeding 1600 times (for example, there are eyepieces with a magnification of 20 times, which, when paired with a 100 times objective, will provide a magnification of 2000 times). However, this is usually impractical. High magnifications are not the end in themselves of optical microscopy. The purpose of a microscope is to ensure the distinction of the smallest possible elements of the structure of a specimen, i.e., to maximize the use of the microscope's resolving power. And it has a limit due to the wave properties of light. Thus, a distinction is made between useful and unuseful magnification of a microscope. Useful magnification is when it is possible to reveal new details of the structure of an object, and unuseful magnification is a magnification at which, by magnifying an object hundreds of times or more, it is impossible to discover new details of the structure of an object [9, 10].

Let us dwell once again on the concept of resolving power. The resolving power of optical instruments (also called resolving power) characterizes the ability of these instruments to produce separate images of two close points of an object. The smallest linear or angular distance between two points, starting from which their images merge, is called the linear or angular limit of resolution. The existence of a limit of resolving power affects the choice of magnifications that we obtain with a microscope. Magnifications up to 1250 times are called useful, since with them we distinguish all the elements of the structure of the object. In this case, the capabilities of the microscope in terms of resolving power are exhausted. This magnification is obtained using a 100x objective lens working with oil immersion and a 12.5x eyepiece (the useful magnification of eyepieces ranges from 7.5 to 12.5 times). At magnifications over 1250 times, no new details of the structure of the preparation are revealed. However, sometimes such magnifications are used – in microphotography, when projecting images onto a screen and in some other cases [13, 14].

When significantly higher useful magnification is required, an electron microscope is used. This microscope has a significantly higher resolution than an optical microscope. An electron microscope is a device for observing and photographing a repeatedly (up to 10^6 times) enlarged image of objects, in which beams of electrons accelerated to high energies (30 ... 100 keV and more) in deep vacuum conditions are used instead of light beams.

It is technically possible to create optical microscopes whose objectives and eyepieces will give a total magnification of 1500–2000 or more. However, this is impractical, since the ability to distinguish small details of an object is limited by diffraction phenomena. As a result, the image of the smallest details of the object loses sharpness, a violation of the geometric similarity of the image and the object may occur, neighboring points will merge into one, and the image may disappear completely. Therefore, in optics, there are the following concepts that characterize the quality of a microscope:

The resolving power of a microscope is the property of a microscope to give a separate image of small details of the object under consideration.

The resolution limit is the smallest distance between two points that can be seen separately in a microscope.

The smaller the resolution limit, the higher the resolving power of the microscope! The resolution limit determines the smallest size of details that can be distinguished in a specimen using a microscope [13, 14].

Physical basis of the resolution of optical microscopes

The theory of the resolving power of the microscope was developed by the director of the K. Zeiss plant in Jena, professor of optics E. Abbe (1840–1905) [15]. As the simplest microscopic preparation, he took a diffraction grating (Fig. 1), studied the mechanism of image formation in the microscope and showed the following.

A beam of secondary light waves after diffraction on the object DD enters the objective and creates a diffraction pattern as a result of interference in its focal plane FF – a system of main maxima M_1, M_0, M_1 and minima.

Further, only the rays that form the main maxima participate in the formation of the image. They intersect in the corresponding plane and produce an image of the object D'D'.

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DOI 10.15588/1607-6885-2024-3-10

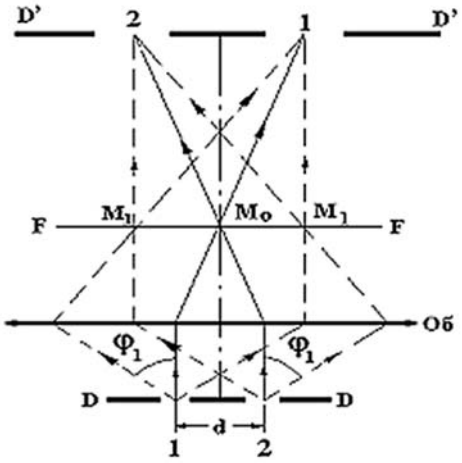

Figure 1. Mechanism of image formation in a microscope

To create an image, that is, to resolve the object, it is sufficient for the rays that form only the maxima of the zeroth and first order to enter the objective at least on one side.

The participation of rays from a greater number of maxima in the formation of the image increases the quality of the image, its contrast. Therefore, the rays that form these maxima must be within the aperture angle of the objective.

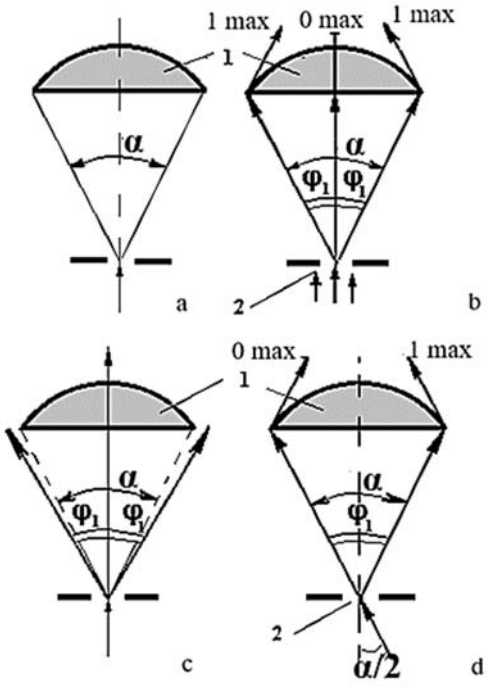

Figure 2. The image produced by a diffraction grating: 1 – front lens of the lens, 2 – objective

Thus, if the object is a diffraction grating with a period d and the light falls on it normally (Fig. 2*b*), then the rays forming the maxima of the zero and first orders on both sides must necessarily participate in the formation of the image, and the angle φ_1 – the angle of deviation of the rays forming the maximum of the first order, accordingly, must be, in the extreme case, equal to the angle α/2.

If we take a grating with a smaller period d', then the angle φ'1 will be greater than the angle α/2 and the image will not appear. This means that the grating period d can be taken as the limit of the microscope resolution Z. Then, using the formula for a diffraction grating, we write for k =1:

$$d = \frac{\lambda}{sin\,\varphi_1}.$$

Replacing d with Z and φ1 with α/2, we obtain

$$Z = \frac{\lambda}{sin(\alpha/2)}. \quad (1)$$

If we use the wavelength of light λ = 555 nm, to which the eye is most sensitive, in the calculation, then the resolution limit of a dry lens will be 0.30 μm.

Methods of increasing the separation capacity of an optical microscope

Today, the most widely used method of increasing the resolving power of optical microscopes is the use of immersion liquids. For example, consider the following situation: we use a red laser (λ = 620 nm) as a source of illumination and find the resolution of an optical microscope without and with immersion liquid (Fig. 3). Let's use the following Helmholtz formula:

$$l_{min1} = \frac{0{,}61\cdot\lambda}{n\cdot sin\alpha}. \quad (2)$$

where λ is the wavelength, n is the refractive index of the immersion liquid, α is the aperture angle.

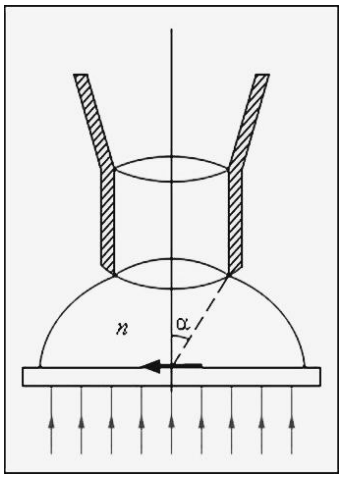

Figure 3. The immersion liquid in front of the microscope lens [16]

The dimension n·sin α is called the numerical aperture. In good microscopes, the aperture angle α is close to its limit: α ≈ π/2, we will consider the index of refraction of the immersion liquid to be 1.5.

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DOI 10.15588/1607-6885-2024-3-10

$$l_{min1} = \frac{0{,}61 \cdot 620}{1 \cdot sin90^{\circ}} = 378{,}2 \text{ nm},$$

$$l_{min1} = \frac{0{,}61 \cdot 620}{1{,}5 \cdot sin90^{\circ}} = 252{,}13 \text{ nm}.$$

Calculations show that the use of immersion fluid reduces the size of objects that we can observe by 1.5 times.

Increasing the resolving power of an optical microscope by decreasing the wavelength of light is achieved by using ultraviolet radiation. For this purpose, there are special ultraviolet microscopes with quartz optics and devices for observing and photographing objects. Since these microscopes use light with a wavelength approximately two times shorter than that of visible light, they are capable of resolving structures of a preparation with dimensions of about 0.1 μm. Ultraviolet microscopy has another advantage – it can be used to examine unstained preparations. Most biological objects are transparent in visible light, since they do not absorb it. However, they have selective absorption in the ultraviolet region and, therefore, are easily distinguishable in ultraviolet rays.

The useful magnification of a microscope is limited by its resolving power and the resolving power of the eye.

Let us recall once again that the resolving power of the eye is characterized by the smallest angle of view at which the human eye can still distinguish two points of an object separately. It is limited by diffraction on the pupil and the distance between the light-sensitive cells of the retina. For a normal eye, the smallest angle of view is 1 minute. If the object is at the best visual distance of 25 cm, then this angle corresponds to an object of 70 μm. This value is considered the resolution limit for the naked eye Zr at the best visual distance. However, it has been previously shown that the optimal value of Zr is 140...280 μm. In this case, the eye experiences the least strain.

The useful magnification of a microscope is its maximum magnification, at which two lines are clearly distinguishable, as separate lines of a detail, it is also the resolution of the microscope.

Using known values – the wavelength of light 555 nm, the optimal values of the limits of eye resolution 140...280 μm, we will find the range of values of the useful magnification of the microscope

$$500\,A < Kn < 1000\,A\,.$$

For example, when using the best immersion objectives with a numerical aperture of 1.43, the useful magnification will be 700 ... 1400, hence it is clear that it is usually impractical to design optical microscopes with high magnification. All of the above is typical for the spectra of incandescent lamps with a predominant sodium spectrum of 586 nm. Most of the lighting elements in microscopes have such technical characteristics.

Modern illuminators can be built on a clearly defined spectrum, using LED elements. For example, by making an illuminator from LEDs with an ultraviolet spectrum of 380 nm, it is possible to obtain magnifications exceeding 2000. The main thing in using such lighting is not in increasing the magnification factor, but in the detail and clarity of the resulting image. It becomes possible to obtain images of elements that were not available when using standard illuminators.

Fig. 4 shows images of a human blood sample prepared for examination, obtained using different illuminators: *a* – neutral cold-color LED (Samsung LH351B) and *b* – ultraviolet LED. For the studies, we used an MBS-10 microscope with Karl Kays optics. Fig. 4*b* reveals blood structures that have not been observed before (Fig. 4*a*), such as the structure of red blood cells and biological markers that can be used to detect some diseases at early stages of development.

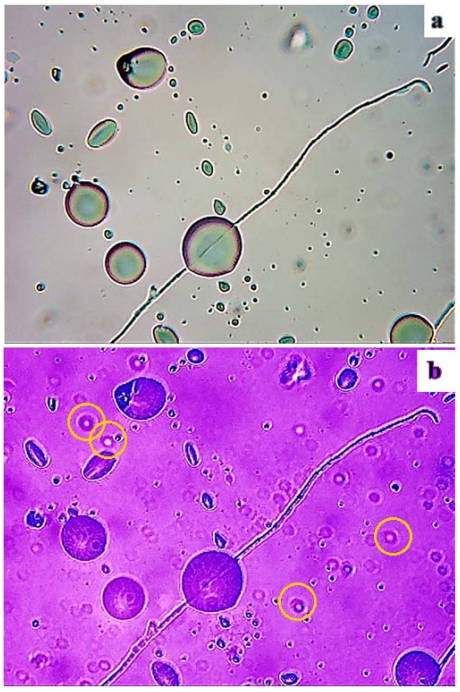

Figure 4. Blood preparation: *a* – λ = 590…600 nm, *b* – λ = 380…410 nm, ○ – biological markers

Conclusions

The resolution of an optical microscope can be increased by using monochromatic light sources instead of conventional lamps.

Reducing the wavelength of the source and the transition from the visible range of light waves to the ultraviolet allows without the use of an immersion liquid to increase the resolution of the optical microscope up to 3 times.

The disadvantage of this method is the need to observe either through a light filter during direct observation, or on a monitor during an indirect observation method to avoid a negative impact on the observer's vision.

The results of research are significantly influenced, especially during indirect observation, by the image receiver – the sensitive matrix of the photo receiver (sensors of the camera matrix).

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DOI 10.15588/1607-6885-2024-3-10

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Одержано 12.10.2024

СПОСІБ ЗБІЛЬШЕННЯ РОЗДІЛЬНОЇ ЗДАТНОСТІ ОПТИЧНОГО МІКРОСКОПА

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Мета роботи. *Збільшення роздільної здатності та збільшення оптичного мікроскопа для розширення можливостей спостереження та дослідження в різних галузях науки та технологій.*

Методи дослідження. *Теоретичний, експериментальний*

Отримані результати. *Проведено порівняльний аналіз ока людини як оптичної системи та оптичної системи мікроскопа. З’ясували, що ці системи мають подібну природу, а саме роздільне зображення двох близько розташованих об’єктів. Якщо розглядати око людини як світлочутливу матрицю, то для визначення роздільної здатності ока необхідно використати відношення усередненого значення кількості світлочутливих елементів (палички та колбочки), які припадають на сітківку, до площі самої сітківки (для ока людини це 0,1…0,2 мм). Для оптичного мікроскопа необхідно розділяти поняття «роздільна здатність» та «збільшення».*

Також ми згадали фізичні основи роздільної здатності оптичних мікроскопів. Показали, що для сухого об’єктива межа роздільної здатності для $\lambda = 555$ нм складає 0,3 мкм.

Одним із способів підвищення роздільної здатності є використання імерсійних рідин. Однак, у певних дослідженнях її використання є недоречним або неможливим. Якщо замість звичайних освітлювачів (максимум інтенсивності при $\lambda = 555$ нм) використовувати ультрафіолетові ($\lambda = 380…410$ нм), то роздільну здатність можна збільшити у декілька разів, а збільшення – до 2000. Недоліком використання УФ освітлювачив є те, що необхідно використовувати світлофільтр при прямому спостереженні або монітор при опосередкованому методі, щоб уникнути негативного впливу на зір спостерігача.

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DOI 10.15588/1607-6885-2024-3-10

Наукова новизна. *Збільшення роздільної здатності та збільшення оптичного мікроскопа при використанні когерентних джерел світла замість імерсійних рідин дає більш деталізовану наукову інформацію та полегшує її обробку.*

Практична цінність. *Використання результатів нашого дослідження дозволяє отримати більш інформативне зображення при використанні когерентних джерел світла.*

Ключові слова: *роздільна здатність, оптична система мікроскопа, імерсійний об'єктив, світлодіод, матриця фотокамери, препарат крові.*

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DOI 10.15588/1607-6885-2024-3-10