

# **Microscope Design**

**Volume 1:  
Principles**

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This book is dedicated to my children,  
Elena and Alexey, as well as my grandchildren,  
Violetta, Olesya, Dmitry and Alexander,  
whom I love so much.

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# Preface

This book is based on almost 40 years of practice in the field of optical instrument making, when there were regular working days, as well as heavy workloads and sleepless nights. It shares an approach, methodology and practice for designing microscope systems and other optical instruments. I consider this approach to be original and efficient. At each stage of the design,, we can follow “consistently by adding” for the theoretical level of quality of the image given both by individual elements (systems) and the instrument as a whole. In accordance with the traditional layout of the microscope, when the lighting, projection and “registering” system is connected in it, we can make engineering calculations of each of these systems separately and then the “cross-total” calculations when all systems are connected. For example, an approach to the design of the “observational” microscope system, when the objective system, visual head (including prisms) and the eyepiece are considered as a whole. Such “cross-total” calculations of the optical microscope scheme can give a lot of information about the real (albeit theoretical) image. Unfortunately, sometimes the objective is “good” (the visual head, too, and the eyepiece “likewise”), but a satisfactory image quality cannot be achieved. The ability to analyze system performance is vital. Some recommendations are also offered to achieve predicted high-quality characteristics.

Engineers and researchers “talk in different languages”, so I made sure that in my new work I made microscope operation available for researchers in the lab. Typically, this requires special “certified” engineers, but they do not have access to the project documentation, as they are not developers. Direct communication with researchers not only presented me with very valuable experience operating microscopes but also gave me an opportunity to appreciate and optimize the “technical policy” in their design. I hope that this new vision is reflected in the book, where an attempt was made to “look at the microscope” through the eyes of engineers and researchers. It is my hope that the book will spark new discussions between those groups. Finally, this book could encourage dialogue between highly specialized engineers in the field of optical calculation and scientists and engineers working in the adjacent areas of instrument making.

The book also attempts to connect the “empirical” parameters of microscope systems (such as the objectives, condenser, eyepiece, registration system, etc.) with the “theoretical” image quality to compare the real quality of the image with the image that the microscope produces. Therefore, the chapters include many constructive parameters of real objectives and other microscope systems, as well as photographs of real microscopic objects obtained using these systems. For example, various objectives can be used that change the characteristics of the image quality and produce photographs with different details. The book discusses which systems and techniques can provide the desired level and depth of research. Moreover, this information should be sufficient strictly “for the object that is investigated under the microscope”, since the system and the effective techniques may differ significantly for various types and kinds of microscopic objects.

As a rule, photographs of real microscopic objects given in the book were not subjected to any editing and are shown “as is” due to the requirements of the proposed microscope design methodology. However, readers can always improve the aesthetic qualities of the produced images by using modern digital methods and specialized software.

I confirm once again that in this text, all opinions and reasoning belong exclusively to me and do not reflect the point of view of any other organization or individual. All errors, inaccuracies, shortcomings and uncertainty are attributed to me exclusively as the author. Any feedback and discussions, including negative ones, I welcome, because I would like to improve the book. Also, all references, examples of designs and photos are not intended as propaganda or advertising.

I would be happy if the book was useful for professionals to expand their horizons. But also (and maybe even more) I would like to share information and experience with “non-professionals”, for whom natural science and microscopy is a hobby, a fascinating journey into the world of knowledge and personal discoveries.

May the book encourage readers to continue their own research.

I would like to express my gratitude to many people with whom I had the benefit of working and who, ultimately, helped me realize my need to write this book. First, the staff of the Russian Lomo factory, where I worked for more than 20 years: General Director A.M. Aronov, who was favorable to me and gave me opportunities for creative development; and co-workers, such as T.F. Kalinina, N.L. Freidberg, O.I. Litinskaya, O.V. Egorova, E.V. Lobacheva, O.N. Nemkova, E.N. Sergeev, E.N. Orlova, S.A. German, and I.R. Petruchenko. My colleagues and teachers shared the results of their research with me. For me, it was a great honor to become their co-author, and some results of such research became part of this book.

I extend my personal gratitude to my friend Dr. A.G. Tabachkov; my brother and co-author of some articles, Vladimir, and his wife, Svetlana; my

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I want to say a big thanks to the developers of the Translate.Google service. They were a huge help with translating the manuscript into English; moreover, this service is constantly developing and improving.

**Dmitry Frolov**  
October 2021

# **Chapter 1**

## **Non-modern Modern Microscopes**

“... all those features of the image observed in the microscope, which are not due to the simple absorption of light in the object, but by the interaction of diffraction beams, that is, all the details of the structure, in general, do not reproduce the true state object – do not form an image, geometrically similar to the object...” (Abbe).

This, of course, is a job for historians, people who are professionally engaged in the search and proof of certain facts. Like any science, microscopy developed along with the development of humans, the improvement of their ability to study and understand the world. As the need arose for the study of the nature of phenomena and substances, as well as the development of new technological capabilities, a person came up with more and more new techniques for research under a microscope; they looked for and found new technical solutions that allowed (as it seemed to them) to bring the microscope to perfection. Today, we think that those modern microscopes, which were developed by the evolution of more ancient optical devices, are their logical continuation and have significant advantages. We think that modern microscopes are quite perfect and allow us to implement all the numerous techniques and fully realize the experience of all scientists and engineers who invented and designed microscopes before. Probably, it would be wise to direct creative potential not only to search for answers—who, when, and under what circumstances invented and perfected the microscope. It is more important to understand what discoveries and achievements have become possible with a microscope, how this optical instrument can be useful in the future development of science and practice of human existence. The theory and practice of building a microscope as an instrument intended for the study of the surrounding world should be only a tool, a means to ensure comfortable and efficient work of people who study this world. In this sense, it is very important to try to understand the psychology and logic of the

aperture of 1.50 became feasible only after the outstanding work of the German optician Ernst Abbe (1840–1905), who discovered the law of sinuses to allow the elimination of coma within a small object (within a small linear field), which is very important for large apertures. In addition, based on his theory of image formation in a microscope, he clarified the question of the resolution of a microscope. Under his leadership in 1872, a series of first-rate objective achromats was designed and manufactured by Carl Zeiss in Jena with different apertures up to 1.50. In 1886, Carl Zeiss, led by Abbe, produced a series of eight apochromats (with compensating eyepieces), and in 1888 it created an apochromat with monobromonaphthalene immersion with an aperture of 1.60. In 1938, H. Bohehold finished his work on the design of a series of planachromat objectives, featuring the flat surface of the image.

Describing the role of Abbe's work, the academician D. S. Rozhdestvensky wrote,

"For the first time, Abbe clearly showed that its own limit of capacities corresponds to the sharpness of every tool. You can not handle even a soft material with rough fingers with a hundredth-millimeter accuracy, instead you need fine tools to do so. The finest of any tools is the wavelength. You can not see an object less than half the length of the wave as Abbe's diffraction theory states, and you can not get an image less than half the length of the wave, that is, less than  $1/4 \mu\text{m}$  ... Thus, Abbe's genius established conscious creativity in microscopy and the limits of possible were reached."

The theory of image generation by Abbe was further developed in the works of the Russian scientists L. I. Mandelstam and academician D. S. Rozhdestvensky; he introduced the concept of the relative incoherence of lighting, expressed as the ratio of the numerical apertures of the lighting device (condenser) and the objective of the microscope. To create optimal lighting conditions in a microscope, an employee of Zeiss, R. Richter, as early as in 1939 patented a lighting device containing a pancractical system, with the goal of smoothly changing the aperture of the lighting beam while changing the size of the lighted observation area. [4] And, nevertheless, the problem of reconciling the parameters of the microscope's lighting system with the parameters of interchangeable objectives is still very difficult today.

## 1.2 Non-modern Modern Microscopes

Of course, everything is relative. It is probably difficult to substantiate the distinction between the concepts of "a long time ago" and "relatively recently." The company Zeiss was probably one of the first international optical companies, which began selling its instruments around the world. Let us turn to their catalog of 1937, that is, more than 80 years ago. We can try to

### 1.2.3 Achromats and apochromats water immersion objectives

Also in the production program of Zeiss 80 years ago a rather wide range of water immersion objectives was offered. Old masters and microscopists knew that the use of water immersion in comparison, for example, with objectives of oil immersion has many clear advantages. First of all, such objectives allow you to carry out lifetime studies of objects without harming them or causing “injury.” Oil immersion is a rather poisonous and aggressive environment that kills many living organisms that would need to be examined under a microscope. On the other hand, water is a natural substance, since in each of the living organisms it is contained in an absolutely greater quantity than other components. This is a completely natural and logical desire: to explore micro-organisms in the environment of “their natural habitat.”

For the comfort of researchers working under a microscope, water immersion also has advantages over oil immersion, because it is odorless, not aggressive. In addition, water, unlike oil, is not a viscous and non-sticky substance. Air bubbles, which always appear in the immersion layer when applied to the coverslip and the front lens of the objective, are “pushed out” by water, unlike oil, and do not interfere with observation. Microscopists, who work a lot with oil immersion objectives, know that comfort and convenience of work very much depend on the quality of the oil used. If it is not a very-high-quality oil (or old oil that has been “on the shelf” for a while or stored in an inappropriate way), when focusing the objective, it often gives the effect of “sticking” of the sample and the object glass (cover glass) to the front part of the objective. The sample on the slide is “shifted and detached” from the stage, following the objective. In this case, it is very difficult to focus accurately on the object. After finishing the work on the microscope, in order to clean the water from the front lens of the objective, you just need to “wipe” it with a cotton swab (in general, but not necessarily, since the water evaporates naturally).

To clean the oil from the front lens, you must use a special solution. In fact, this is a big problem in cleaning the front lens after using oil. This is also related to the design of the frontal component of the objective, where the lens frame protrudes a little before lens to protect it. Some of the researchers do not know how to clean the lens properly and thoroughly. In many cases, the oil remains on the objective and dries out, significantly affecting the image quality during subsequent work with the objective. Statistics show that dried oil on the front of the objective is often the main cause of poor image quality of the objective. Quite often (after some time), the oil immersion objective “loses” the front lens; it is unglued from the frame due to the action of the oil, and the image quality of the objective becomes unsatisfactory. Repair of such a lens is possible only in a specialized workshop. All the listed problems have no relevance if an objective is used in which water is used as an immersion. Even if the researcher “not very qualitatively” cleaned the front lens with a



**Figure 1.25** Photographs of some Russian mass-produced objectives for water immersion.

immersion objectives, in the Soviet Union the production of such objectives in the mass-production sector was arranged. We present the design parameters and graphics of aberrations of some of these objectives. Figure 1.25 presents photographs of some Russian mass-produced water immersion objectives.

Figure 1.26 presents the parameters of optical design and graphs of aberration correction for soviet 30x water immersion achromatic objective, which has 0.90 numerical aperture in a water immersion.

Figure 1.27 presents the parameters of optical design and graphs of aberration correction for a Soviet 60x water immersion apochromatic objective, which has a 1.0 numerical aperture in water immersion. This objective is designed to work without a cover glass.

Figure 1.28 presents the parameters of optical design and graphs of aberration correction for a Soviet 65x water immersion apochromatic objective, which has a 1.1 numerical aperture in water immersion.

Figure 1.29 presents the parameters of optical design and graphs of aberration correction for a Soviet 85x water immersion achromatic objective, which has a 1.0 numerical aperture.

## 1.2.4 Eyepieces, condensers, and other components

The inventors and manufacturers of microscopes were well aware that there is one contradiction in the concept of the light microscope that must be considered. This is a contradiction between the linear field of the microscope on the object under study and the resolution of the microscope, that is, the ability to distinguish the minimum fragments of the details of the object. In a philosophical sense, this is a well-known contradiction between the general and the particular. Indeed, there has always been the problem of obtaining



**Figure 1.69** Photograph of shell marine foraminifera *Baculogypsina sphaerulata* (by Mikhaltsov).

In some cases, it is advisable to use simultaneously the methods of reflected light and transmitted when the object under study is transparent or translucent, so-called “mixed lighting.” There are various options for sharing different methods of lighting in transmitted light and reflected light. For example, in transmitted light, one can use a bright field with oblique illumination, while in reflected light one can use a dark field. Or in the transmitted light the dark field, and in the reflected bright field; or dark field in both lighting systems. The number of different combinations can be significant, and their effective use depends entirely on the imagination and success of the experiments, i.e., the person working with the microscope. At the same time, different objectives can be used, designed to work both with the cover glass and without it. Figures 1.71–1.75 show photographs of different real objects.

### 1.2.6 Old mass-produced Soviet microscopes and devices

The old mass-produced Soviet light microscopes were the same as Zeiss microscopes in both appearance and configuration. These were, for example, such models as the MBI-1, MBI-3, and others. Later, those mass-produced

- $3.7 \times 0.11$  lens: 0.0025 mm
- $10 \times 0.30$  lens: 0.001 mm
- Micrometer screw scale graduation: 0.01 mm
- Division value of the glass scale: 1 mm
- Limits of changing the focus of the eyepiece:  $\pm 5$  diopters

When using the MPV-1 device, it is more convenient to calculate the sizes of fragments in the image of an object as follows: determine what corresponds to moving the crosshair in the plane of the object when the screw rotates one division of the drum, according to the formula  $\epsilon = 0.01/\beta$ , where  $\epsilon$  is the scale mark of the drum in the plane of the object; 0.01 is the magnitude of the crosshair movement of the eyepiece when the screw is rotated by one division of the drum scale; and  $\beta$  is the linear magnification of the objective. The value of the measured object is calculated by the formula  $(II - I)$ , where  $(II - I)$  is the difference of the readings on the scales of the micrometer (in absolute divisions of the drum).

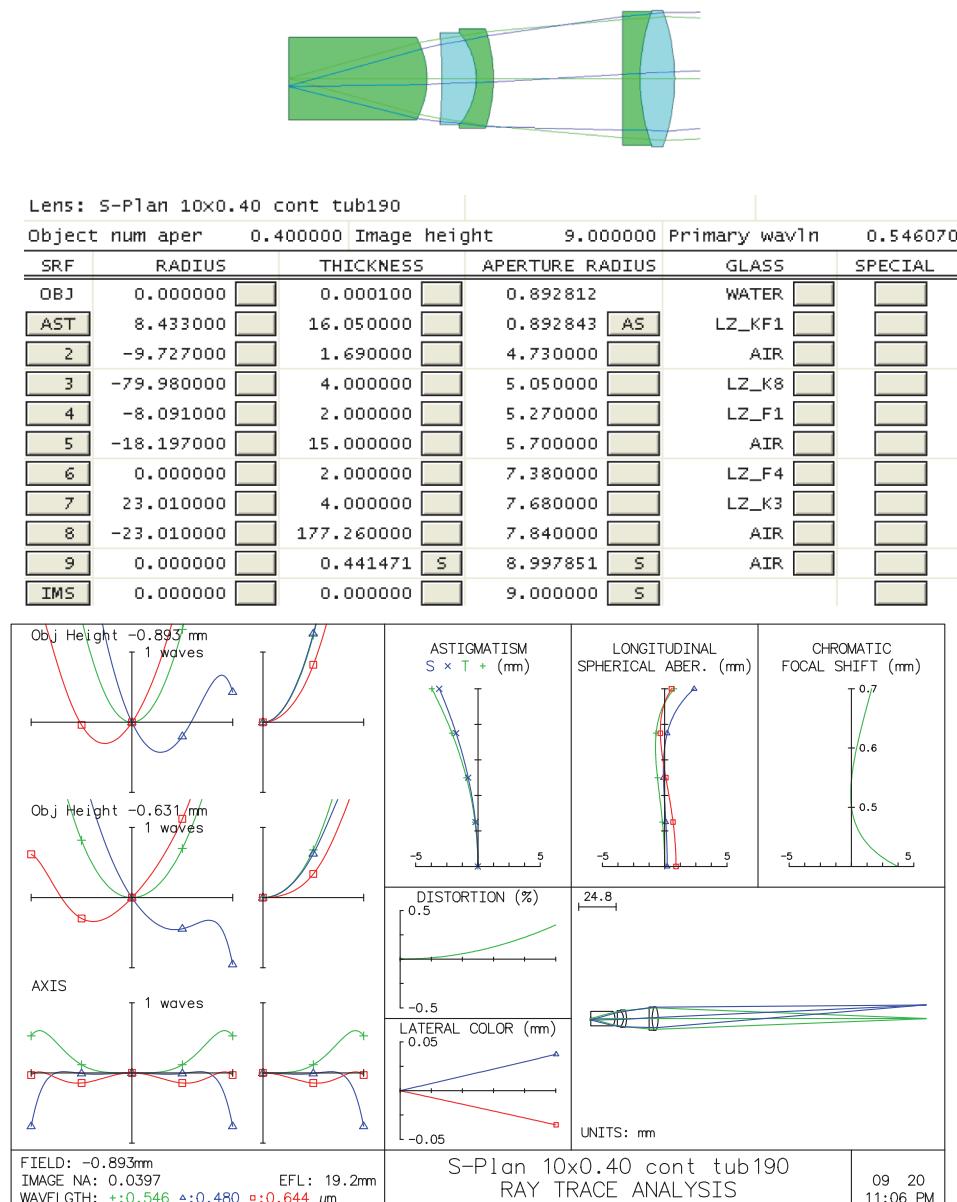
#### **1.2.6.5 Polarizing microscopes and devices**

We must take into account the methods and conditions for research on polarization microscopes to take them into account when designing optics. First of all for optical and mechanical design of objectives. Polarization microscopes can be used in almost all fields of natural science, when double cross refraction and interference colors are determined using crossed polarization filters. On the basis of this analysis, a conclusion is making about the structures of the objects under study. In medicine, protoplasm, cell nuclei, fibers of connective tissue, bones, etc. Are investigated. In botany, one can study the structure of plants. In the chemical industry, crystals and gels are studied. In the food industry, this method is used in the study of food products. For example, it is possible to determine the double refraction of starch grains.

##### **1.2.6.5.1 Traveling microscope MPD-1**

The MPD-1 traveling polarizing microscope is intended for field research of transparent or semi-transparent objects in the form of thin sections or powdered minerals and rocks. Investigations can be carried out in ordinary and polarized light in the orthoscopic and conoscopic rays. The design of the microscope allows the use of phase contrast devices, lighting devices of the dark field, oblique lighting, as well as other devices designed for use in budget microscopes of the mass-produced.

The MPD-1 microscope is manufactured for work in laboratory rooms and for a not long time in the open air in conditions. One of the distinctive devices of this microscope is a precision stage, which has the ability to turn around  $0-360^\circ$ ; the stage is graduated with a scale whose accuracy is



**Figure 1.107** Parameters of optical design and graphs of the aberration correction of a 10 × 0.40 (contact) S-plan achromat objective.

### 1.2.8 Old mass-produced American microscopes

The Spencer Lens Company has some very interesting models of light microscopes. As in the case of the old Zeiss catalog, we refer to the old

# **Chapter 2**

## **Abstracts and Reviews**

The beginning of this chapter presents some photos taken by a Russian scientist who has been studying various types of biological objects for many years: Anatoly Mikhaltsov, who lives in the city of Omsk. But most importantly, he is actively engaged in teaching children of different ages the basics of light microscopy.

In the end, the task of developing and creating a microscope is precisely to obtain photographs of real objects examined under a microscope. The higher the quality of such photographs, the more successful the project for creating a microscope on which these photographs are made can be considered. Of course, we can and should look through a microscope with our eyes, but at the same time, a summary of the object under study will be a reflection of the subjective opinion of a particular researcher. A photograph of an object, on the contrary, makes it possible to objectively evaluate the object under study as the result of discussion by many researchers. Of course, photographs of real objects should make it possible to solve “research” tasks; but many of the photos can also be of artistic value, because they look very beautiful. Figures 2.1–2.7 show some of these photos.

### **2.1 Group Design of Optical Instruments**

The concept of the so-called group design of optical instruments for several decades was the main “argument” of R. M. Raguzin, PhD and engineer, who realized this concept in many Russian light microscopes. (I was very fortunate to work with him for a long time.) This section summarizes the main theses of group design; the following provisions of the system approach have the greatest methodological significance:

1. Separation and connection of components into a whole, and the study of the relationships between them.
2. Fulfillment of the requirements of integrity, invariance, hierarchy, functionality, structure, and development.

stages are carried out. In addition, due to increased serialization, more efficient technology and metrology are used. However, in group design, an appropriate organization of the work of developers is necessary in accordance with the essence of group design.

During the initial stages when drawing up structural-functional schemes (identifying the basic decisions regarding the design of optical schemes and other components), as many options as possible should be considered. If necessary, conduct research work in advance. However, after making a decision, the conditions of integrity, hierarchy, invariance, compliance with basic parameters, and other requirements arising from the theory of group design should be strictly observed. The disadvantages of group design include

1. The greater inertia of a group of optical devices than a single device;
2. Additional time spent on preparatory analysis at the initial stage and a significant increase in design complexity, due to a sharp increase in the number of links; and
3. The complexity of the issues associated with the quantization of parametric series of various components (nodes, parts, sizes, etc.) and the determination of the optimal type of device.

With the rational organization of group design, these difficulties can be significantly reduced. For this, the development process should be continuous, that is, at intervals of 10–15 years (corresponding to the time intervals of the generation of devices), the previously developed generation of optical devices should be replaced with a new one. Subject to this sequence, obsolete devices are replaced with improved ones. At the same time, part of the results obtained earlier are either used in full or require only partial refinement.

### **2.3 Brief Classification of Microscopic Objects**

All objects of research in microscopy can be attributed to two groups: transparent and opaque (see Figure 2.8). Transparent objects include biological objects (bacteria, cellular structures), some minerals and crystals, emulsion preparations; opaque objects include ores, coals, minerals, and metals.

The objects included in each of these groups can be divided into amplitude and phase. Amplitude objects change the amplitude of the light transmitted or reflected from them and therefore can be seen through a microscope without additional optical devices. Phase preparations do not change the amplitude of the light transmitted through them or reflected from them but only change its phase, to which the eye is not sensitive; therefore, such objects are not visible in an ordinary microscope, and to observe them requires the use of special devices, such as phase contrast, interference, etc.

the lens of surface objective. It was experimentally established that the magnitude of the spread of the exit pupils of the objectives should not exceed 3–5 mm, in this case; for example, the focusing mechanism of the Bertrand lens system does not require high precision of performance, and the dimensions of the optics are optimal. Note that for objectives with small magnifications, fulfilling the requirement of unifying the position of pupils with strong lenses leads to a considerable complication of the optical structures. The decisive role in this case is played by technical and economic characteristics.

We should also consider the rational location of the intermediate image plane on the microscope and, therefore, the determination of the optimal value of the height of the eyepiece. Previously, it is assumed that the intermediate image plane is located 13 mm below the reference plane of the eyepiece. Today, when switching to the production of microscopes with enlarged fields of view, more companies have reduced this size to 10 mm without changing the fitting diameters of the eyepieces. The resulting defocusing of the microscope is eliminated by refocusing the objectives.

This innovation, however, does not solve the problem comprehensively. The appropriate solution would be to place the intermediate image plane on the cut of the eyepiece, defining the reference end of the eyepieces, or, better yet, the extension 10–15 mm higher. Such a solution not only provides a solution to the problem of increasing the field of view but also allows you to work effectively with an intermediate image; for example, it is convenient to set various scales, grids, pointers, etc. in the field of view.

Thus, the modern development of microscopy has led to the need to revise or supplement individual norms and standards. Most of the above findings are based on the practice of developing modern microscopes.

## 2.7 Optical System of a Modern Microscope

A modern microscope system is constructed according to the principle of modular design, in which the designer, as well as the user, can assemble from available optomechanical elements the device that most completely corresponds functionally to given properties [2]. The system can be reconstructed using various sets of standard optomechanical components to implement a wide range of studies in transmitted light by means of the methods of direct and indirect illumination, contrast microscopy, conoscopy, etc. The possibility of using microphoto attachments and video projectors for CCD arrays makes it possible to document the studies as they are carried out.

In order to make it possible to use the devices in many modifications, the optical elements have independent spatial-aberrational correction, and this guarantees that they are completely compatible. As a result, when the investigator uses various techniques of microscopy, the resulting optical image

optical elements are significant. However, the chief obstacle to obtaining high-quality illumination is the presence of a certain zone where light tubes of similar intensity but different functional purpose intersect (and interact).

Figures 2.9(b) and (c) show three-component and multicomponent illuminator systems. They implement a new approach to the organization of light fluxes in the illuminator systems of microscopes. A modified Keller method is used, with a telecentric path of the principal rays, and this allows full use of the advantages of partially collimated light to enhance the local contrast of the illuminated elements of structures. The light source is located in the front focal plane of the collector, the front focal plane of each subsequent component is located in the back focal plane of the preceding one, and the object to be illuminated is located in the back focal plane of the condenser [4]. Such an arrangement has obvious advantages when making adjustments and allows one to achieve achromatic aberration correction of the illuminator beams. However, the main thing is that this satisfies the principle of independent spatial-aberrational correction of the separate components. This means that the collector systems, the condenser systems, and also the scale-matching systems of the illuminator can be replaced by other functionally analogous ones with no detriment to the resulting quality with which the object of interest is illuminated. It is this that provides for change, in particular, of the lamp-plus-collector system (for example, when a more powerful light source is used) and the condenser system (allowing various investigative techniques to be used with the microscope - phase contrast, indirect illumination, etc.). The optical systems of modern microscope illuminators provide for the presence of intermediate image planes, and this can be used to achieve additional illumination effects. The described method of constructing illuminator systems guarantees stability, high contrast, and good illumination uniformity. The use of special technologies in producing optical items, as well as nonspherical refracting surfaces, makes it possible to obtain high-contrast, uniform illumination of the structures of interest on a large object field with high numerical apertures of the illuminator beams.

## 2.7.2 The visual observation system

It determines the main user properties of a microscope as an observational device with diffraction-limited image quality. It consists of a magnifier part, which constructs a congruent image of the object, and an observational part, which works directly with the investigator's eye. The questions of how the ray path is constructed and how the image is formed in the magnifier part are of special interest. Reference [5] proposed rational dimensional layouts of microscopes. Figure 2.10 schematically shows how a finite tube length differs from an infinite length.

In microscopes with a finite tube length, parameters  $h$ ,  $S'$ , and  $L$  are rigorously standardized and depend on each other. In actually fabricated

**Table 2.4** Fundamental optical layouts of the simplest microscope objectives.

Technical parameters			
Magnification × numerical aperture	Working distance, mm	Optical layout	Description of the optical design
5 × 0.10	24.7		One component, made by gluing positive and negative lenses.
10 × 0.25	3.80		The first component is made in the form of a positive lens with a flat first surface or that is concave with a concavity facing the object; the second component is made by gluing positive and negative lenses.
20 × 0.40	0.58		The first component is made in the form of a positive lens with a flat first surface or that is concave with a concavity facing the object; the second component is a single positive lens; the third component is made by gluing positive and negative lenses.
40 × 0.65	0.65		The first component is made in the form of a positive lens with a flat first surface or that is concave with a concavity facing the object; the second and third components are a single positive lens; the fourth component is made by gluing negative and positive lenses.
25 × 1.0 oil	1.0		The first component is made in the form of a positive lens with a flat first surface or that is concave with a concavity facing the object; the second and third components are a single positive lens; the fourth component is made by gluing negative and positive lenses.
32 × 0.9 water	0.85		
50 × 1.0 oil	0.32		
63 × 0.85	0.15		
63 × 1.15 water	0.20		
100 × 1.20 water	0.12		
100 × 1.25 oil	0.11		
100 × 1.30 oil	0.12		

made from heavy flint and turned with its concave side toward image space is added to the objective after the main layout.

It then also becomes possible to correct partially or fully (depending on the number  $N$  of positive single lenses in the front part) the curvature of the field. To optimize the distribution of the aberrational loading, as well as to make it easier to fabricate the designs, one of the single positive components can be placed between the compound lens and the negative meniscus [12]. Based on the described principle, a set of microscope objectives has been designed as stigmachromats and planachromats, which are currently being produced commercially. The main technical characteristics and fundamental optical layouts of these microscope objectives are shown in Table 2.5.

#### 2.10.4 The simplest microscope objectives with correction for two wavelengths or spectral regions “dualchromat” type

For microscopic tasks involving the focusing of laser radiation in a spectral region outside the visible, microscope objectives are required that are very well corrected with respect to spherical aberration for the maximum apertures. A feature of the calculation of such objectives is that the spherical aberration

**Table 2.13 (Continued)** Descriptions of the optical design of some types of condensers.

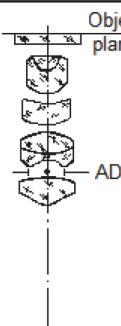
Nº	Optical layout	Characteristic	Description of the optical design
5	 Object plane AD FD	Aplanatic achromatic condenser NA = 1.44 oil immersion	The first component is a positive lens, glued together from positive plano-convex and negative lenses; the second component is a positive meniscus, facing its concavity to the plane of the object; the third component, a negative meniscus convex to the plane of the object, is made of glued positive and negative lenses; and the fourth component is a positive lens. As a special case, the surface facing the field diaphragm of the illuminator is non-spherical, for example, parabolic.

Table 2.13 shows the description of the optical design of some types of condensers.

The proposed approach allows to unify and standardize the overall parameters of the condensers and illumination systems of the microscope, as well as to carry out a freely completing set of microscopes by various types and designs of condensers.

## 2.12 Conditions for Obtaining Uniform Light Distribution, Generated by Lighting Devices

Professor S. N. Natarovsky consistently adapted the best results of Russian scientists and researchers to the theory and practice of light microscopy. He is also an outstanding theoretician and practitioner of microscopy who made great contributions to the use of new technologies in microscopy, and his theoretical studies are often used in the practice of modern science of microscopes. Some joint projects were implemented.

The task of any lighting device is to provide high-quality lighting of the object. By quality characteristics, we mean the illumination sufficient for registering an image of an object, the light distribution acceptable for satisfactory perception of the image, and a number of other characteristics, which are discussed below. G. G. Slusarev argued [14] that a centered optical system can provide uniform light distribution in any of the planes if there is a uniform light distribution in any section of the light beams going through this system. If there is none, then a centered system will not create uniform light distribution, but nevertheless, such systems form the main part of various kinds of lighting devices.

The following well-known formula [7]

$$E \approx \tau \cdot \pi \cdot B \sin^2 \sigma' \cos^4 \omega \quad (2.1)$$

- Smaller than the incandescent lamp, the luminous flux of the LED can be attributed to its shortcomings (temporary), but this also allows for high lighting characteristics to eliminate the heating of the lamp housing.
- The geometry of the luminous body of the LED, the design of the cap allows to obtain a light beam of a limited in space solid angle less than  $4\pi$ , which makes it possible to realize in the microscope all types and methods of illumination and all types of microscopic examination.
- The use of color filters allows, as in the case of an incandescent lamp, to change the spectral composition of the illuminating beams, including the allocation of narrow spectral regions.
- The spectral characteristics of colored LEDs, while using three or more LEDs while controlling their intensities, can receive illumination in white light, or in any other color spectrum, in almost the entire XYZ color space.
- It is very promising to use lines or matrices of LEDs, which allows one, by actively controlling the luminescence of individual LEDs of the matrix, to realize various forms of illuminating apertures with an “instantaneous” transition from one type of lighting to another (from a bright field to a dark field), as well as the possible implementation of the synthesized aperture. The latter allows as to actively influence the resolution and, last but not least, reduce the requirements for correcting the aberrations of the lighting device.
- Single LEDs can be recommended for use as a light source in mass segment microscopes.
- LED matrices and lines can be recommended for use in more complex microscope models, and they can be installed, for example, directly in the aperture diaphragm of the condenser.
- It is advisable to consider the use of LED luminous panels.

## **2.18 Technique for Calculating the Designs of Filled-type Objectives**

Engineer L.I. Krynin, PhD, who has been involved in the design and testing of camera objectives for many years, has made a significant contribution to the development of light microscopy as well. In the design of new objectives for microscopes, our many disputes and discussions were very useful, the experience and recommendations of this person have provided significant assistance in our work. It was a pleasure working several years together when some joint projects were implemented.

objective's front focus. With the help of an additional tube optical system, the image of the test object is projected onto the receiver, which is located strictly in the plane of the back focus of the tube system. In this setting, in contrast to the device for the checking of components requires a very precise focus on the test object, an algorithm for such should have developed autofocus, providing several iterations when focusing. Further, as in the component cheking device, the real and theoretical images are compared; or the real image is quantitatively analyzed. If the image quality is not satisfactory, in automatic mode (without disassembling the objective) using the second "rough" stepper motor, some components are moved along and perpendicular to the axis. Then the focusing is again on the test object. Simultaneously with the assembly process, a special algorithm is used to monitor image quality as a function of feedback. When two stepper motors work alternately – assemblies of objective can be achieved within a few minutes, and the main time costs of such an automated assembly fall on the first iterations of focusing. When the image quality becomes satisfactory, the components are fixed, the working body is inserted in the finishing body, the microscope objective is adjusted in height to automatic mode.

Automation of assembly and control of objectives for microscopes in the process of their manufacture is a very difficult task. There is no doubt that it will be resolved, including due to the improvement of the element base of microelectronics and system engineering products, which currently do not fully meet the quality criteria set by optical systems. The principle of operation of the main optical-mechanical systems remains unchanged – the projection of the image of the test object on the receiver for further research.

## **2.21 The Concept of an Automatic Assembly Line for Microscope Objectives, Based on Adaptive Selection of their Components**

In connection with the topic of this discussion, I would like to talk a little about assistant professor K. P. Zocher from Ilmenau University in Germany. This is a rather cheerful and cheerful person who at some time helped our specialists adapt to "Western social values." Of course, he is an excellent specialist and expert in engineering; it was he who proposed the concept of "adaptive selection," which was then translated into the field of the optical-mechanical industry. It was a great success to work together for several years and implement joint projects.

The automation of the assembly of many optomechanical and optoelectronic functional devices and subassemblies is a very complex problem. The reason for this is that deviations of the characteristics of the materials of optical items and errors in their dimensions and the shape of the working and

and it follows from the quenching condition that

$$\gamma_2 + \gamma_1 = \frac{\alpha_c + \alpha_{ob}}{2}.$$

When there is only one half-wave plate, located in front of the condenser, we find for its azimuthal angle

$$\gamma = \frac{\alpha_c + \alpha_{ob}}{2},$$

and, if it is behind the objective,  $\gamma = -\frac{\alpha_c + \alpha_{ob}}{2}$ .

One way to install the half-wave plate is inside the system – for example, between  $\varphi_c$  and  $\varphi_{ob}$ . Then, for  $\gamma$  we have

$$\gamma = \frac{\alpha_{ob} - \alpha_c}{2}.$$

This case is interesting in that the half-wave plate can be installed inside the microscope objective, in accordance with Figure 2.106. It is noteworthy that a segment including a half-wave plate and the last component of modern microscope objectives of type OCX, is similar to the compensator shown in Figure 2.107 (b). By rotating the plate, compensation can be obtained under different conditions, for example, for various rays.

As a result, we can draw the following conclusions:

- An algorithm and program have been developed that make it possible to calculate the angle of rotation of the plane of vibration for any ray, the degree of quenching in a polarization microscope, and afocal compensators (according to S. Inoue).
- Calculations using the indicated program showed that it is not expedient to use afocal compensators to compensate the rotation of the plane of vibration, and the authors propose that a half-wave plate should be introduced into the composition of the microscope objective or the condenser for this purpose.
- It has been shown analytically and checked by a numerical method that the maximum angle of rotation of the plane of vibration corresponds to an angle between the planes of vibration and the plane of propagation of the ray not of 45° but somewhat larger.

## 2.24 Interferometric Quality Control of Lenses and Objectives

Professor V.K. Kirillovsky is also one of the teachers. He also made a great contribution to the development of light microscopy, mainly developing and introducing into the production of some original methods for testing optical

The change in the optical path difference corresponding to the shift of the zero band in the transparent film can be determined by the formula

$$\Delta_{tr} = N_{tr}\lambda.$$

The transparent film thickness equals

$$h_{tr} = \frac{N_{tr}\lambda}{2(n-1)} = \frac{c_{tr}\lambda}{2b(n-1)},$$

where  $n > 1$ . When measured with a MII-4 double beam micro-interferometer, the maximum film thickness is limited only by the sharp image depth, since within this depth the device provides a satisfactory band contrast. Considering that in an opaque film, bands are localized on surfaces  $P$  and  $E$ , we have

$$h = T = \frac{\lambda}{2A^2},$$

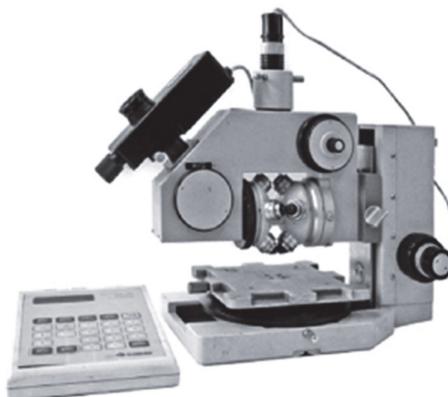
where  $T$  is the depth of the sharp image.

In a transparent film, band systems are localized on the  $E$  and  $E'$  planes. Since the plane  $E'$  is located at a distance  $h(n-1)/n$  over the plane  $E$ , then in this case

$$h_{\max} = \frac{\lambda n}{2A^2(n-1)}.$$

## 2.26 Light Section Microscope PSS-3

This microscope is another interesting device that allows measurements in the “micrometric range.” In fact, this is an original double microscope, it is also manufactured in the Russian company Lomo [84]. The engineering solution of the principal system of this microscope deserves a more detailed description. Figure 2.123 shows general view of the PSS-3.



**Figure 2.123** The general view of the PSS-3 microscope.

This microscope is designed to measure the roughness parameters  $R_z$  and  $R_{max}$  in the range from 0.5 to 400  $\mu\text{m}$  by a non-contact method, and can also be used to measure the heights of individual irregularities and the thickness of opaque films. Measurement and processing of the measurement results are carried out using a photoelectric ocular micrometer with interchangeable eyepieces and carried out by equipped with a processor unit with original software. The microscope is used in engineering, instrument-making industries, in laboratories of research institutes and in workshop conditions. The microscope provides the output of the image of the test object through a special adapter to a digital receiver up to 1/2" in size. Also, object research and measurements can be performed using the visual channel when observed through the eyepiece.

Main technical specifications:

Linear magnification of the microscope..... from 25 $\times$  to 640 $\times$

Linear magnification of objectives..... 2.5 $\times$ ; 4 $\times$ ; 16 $\times$ ; 40 $\times$

A linear magnification in the eyepieces ..... 10 $\times$ ; 12.5 $\times$ , 16 $\times$

The linear field of view of the eyepieces, mm ..... 20, 14, 11

Light source: LED illuminator with a power of 3W.

Overall dimensions of a microscope in mm:

width ..... 210

length ..... 420

height ..... 460

The mass of the device, kg ..... 10

The microscope includes the following main parts:

- head with basic optical elements;
- stand with a focusing mechanism;
- object stage;
- micrometer; and
- LED illuminator with a secondary power source.

### **2.26.1 Principle of operation**

The principle of operation of the device is based on the application of the method of lightsection of the investigated surface.

The device is a system of two microscopes: a microscope projecting an image of a luminous slit onto a rough surface (MP) and an observation microscope (MO).

The essence of the lightsection method is as follows:

An image of a narrow, illuminated gap is projected onto a rough surface at an angle  $\alpha$  to the normal. This intermediate image of the slit takes the form of a surface profile and is observed using a microscope, the optical axis of which makes an angle  $\beta$  with the normal to the surface.

$$V_{ob} = \frac{\sin \sigma_p - \sin \sigma_{p0}}{\sin \sigma_{p\max} - \sin \sigma_{p0}} V_{ob\max}.$$

In this case,

$$J = n_p y_p \sin \sigma_p = n_p \frac{y'_p}{V_{ob}} \sin \sigma_p = n_p y'_p \frac{\sin \sigma_{p\max} - \sin \sigma_{p0}}{\sin \sigma_p - \sin \sigma_{p0}} \frac{\sin \sigma_p}{V_{ob\max}}.$$

When  $\sin \sigma_p = \sin \sigma_{p\max}$ ,

$$J = J_0 = n_p y'_p \frac{\sin \sigma_{p\max}}{V_{ob\max}}.$$

Let  $\Delta J = J - J_0$ . Then,

$$\frac{\Delta J}{J_0} = \frac{\sin \sigma_{p0}}{\sin \sigma_{p\max}} \frac{\sin \sigma_{p\max} - \sin \sigma_p}{\sin \sigma_p - \sin \sigma_{p0}}. \quad (37)$$

It follows from Eq. (37) that, when  $\sigma_{p0} = 0$ , we have the ratio  $\Delta J/J_0 = 0$ . Thus, to satisfy the condition  $J = \text{const}$ , the interconnection of the parameters  $V_{ob} = V_{ob}(n_p \sin \sigma_p)$  needs to be linear; the line in the  $(V_{ob}, n_p \sin \sigma_p)$  coordinate system can have any slope but must pass through point  $(0, 0)$ .

## 2.28 Development of “Micron Resolution Microscopes” for Reducing Photolithography

This section discusses some technical solutions for the reduction of optical photolithography systems based on the principle of obtaining a scaled-down object image. Dioptric reduction system design trends are discussed and applied technical solutions for building such systems are proposed. Some original optical systems are offered which comply with the requirements mentioned above.

The projection photolithography in the general case can be performed by simultaneously transferring all topological layer elements to a plate. The common transfer technique for the reduction photolithography is the element-wise projection of separate fragments or the direct patterning in a photoresist layer by means of focused light beam, therefore a high aperture of objectives is the most essential condition of reduction photolithography. It can be accepted that in such a case only a small part of a template image is obtained, the full image can be obtained by scanning or moving the template and the plate synchronously, or by moving the objectives itself. The example of so-called “Reduction Stepper” can identify the of projection photolithography

now. It shall be noted that the immersion technique application significantly lowers the non-defective unit output, as the immersion is not a quasi-stable substance as per the chemical composition and it will always contain or generate some inclusions such as, for example, atmospheric gases. Besides, despite the noticeable advancements in the immersion photolithography, the fluid presence significantly complexifies the imaging process technology, introduces specific mistakes, is rather costly and requires the application of additional precise equipment.

Therefore, switching to shorter wavelength of 121.6 nm and applying a non-immersion system seems to be logically relevant. Alongside it is hoped that immersion fluid technologies being developed at present (differing from water which loses transparency at 185 nm in the same manner as air) will be successfully implemented for a shorter spectrum, and that will provide new opportunities for further increase of objectives numerical aperture and resolution power.

Figure 2.142 demonstrates the optical design for objective with monochromatic aberrations correction for the wavelength of 121.6 nm. The description includes the lens optical layout and substantial residual aberration charts. This objective was designed for operating with the exit numerical aperture of 0.90 and the linear field in image space of 1.25 mm. The objective focal length is 10 mm. The optical material is MgF<sub>2</sub> crystal.

It was made a prototype of the optical design of the high-end segment objective. This is a monochromat using dioptic optics from a magnesium fluoride crystal to operate at a wavelength of 121.6 nm. The numerical aperture of the objective is 0.90, the linear field is 23 mm, the reduction factor is 5× (linear magnification is -0.2×). The objective contains 26 single lenses, the maximum diameter of some reaches 500 mm. Figure 2.143 shows the design parameters and the optical layout of this objective. This optical design is not final and requires increasingly more accurate calculation. However, the principle of creating such an objective is shown.

## 2.29 Building Lithography Optics by Mirrors

There were many expectations around how the conversion to X-ray optics can become a revolution in the applied Hi-End lithography. There were plenty of preconditions for such revolution: primarily, a conversion to the wavelength ten times shorter than the wavelength used today promises a significant increase in the resolving power. The second important factor was the decision (although necessitated) to cease using the lenses that are giant. That supposed the significant decrease of the equipment cost. There seemed an opportunity for a higher yield due to significant increase in efficiency of new equipment against existing equipment.

# **Chapter 3**

# **Principles of Constructing Microscope Optics**

I would like to begin by saying some kind words about my closest teacher, Professor V.A. Zverev. Previously, he was a major leader at the Soviet enterprise “Lomo,” where he showed himself as a unique engineer, who participated, among other things, in the creation of a 6-meter telescope - the pride of Soviet optical instrumentation. Then he became a teacher at a specialized institute, wrote several books and taught optical disciplines. He was my supervisor for my dissertation; we worked hand in hand and very informally. We met quite often at his home, discussed not only “optical issues” but also various topics. Professor Zverev is not just a teacher but a person who has managed to convey a lot of soul, creative energy and desire to achieve a result. He is an excellent optics theorist and an outstanding engineer of the highest qualification. The basis of his “track record” is a galaxy of engineers and scientists whom he taught some aspects of optical skill. Of course, it is impossible to describe all the facets of his talent, let alone take away from him even a small part of his knowledge and experience. But he shared his talents free of charge with his students and simply to all those people who worked and lived next to him; he passed on to many engineers and scientists a love for optics and design. Thanks to this man, Russian optical science is developing and strengthening; he is one of the “legends” of Russian optical instrumentation.

## **3.1 Element Base and Principles of the Composition of the Optical Systems of the Microscope**

### **3.1.1 Aberrational properties of a spherical surface**

On one of the lines passing the center  $C_v$  of the spherical refracting surface and adopted as the optical axis is the axial point  $A_{vo}$  of the object, as shown in Figure 3.1.

The minimum distance  $\rho$  between two resolvable strokes of the test object of the Foucault in the plane of the object can be defined by the formula of the form

$$\rho = \psi \frac{\lambda}{2n \sin \sigma}. \quad (3.20)$$

With  $n' = 1$ , the diameter of the exit pupil of the microscope is

$$d' = 2f'_{oc} \sin \sigma' = 2f'_{\mu} n \sin \sigma. \quad (3.21)$$

The diameter of the exit pupil of the microscope determines the diameter of the entrance pupil of the eye.

The pupil of the eye is a hole in the opaque iris, through which the luminous flux penetrates the eye. The diameter of the pupil of the eye is impermanent and depends mainly on the brightness of the picture perceived by the eye. Changes to the size of the pupil of the eye are due to the reduction of some and the relaxation of other muscles of the iris and occurs without the participation of the will of the person. The pupillary reflex can be caused by various reasons, even emotions, but primarily by a change in the brightness of the background. As the background brightness increases, the pupil diameter decreases. In the general case, this dependence is determined by the formula [7]

$$d_{eye} = 5 - 3th(0.4 \cdot \lg L), \quad (3.22)$$

where  $d_{eye}$  is the diameter of the pupil of the eye in mm;  $L$  is the background brightness in  $\text{cd}/\text{m}^2$ ; and  $th$  is the hyperbolic tangent  $thx = \frac{e^x - e^{-x}}{e^x + e^{-x}}$ .

It is easy to make sure that in extreme cases, when  $L = 0$ , that

$d_{eye} = 5 - 3 \cdot (-1) = 8$  mm, and if  $L = \infty$ , that  $d_{eye} = 5 - 3 \cdot 1 = 2$  mm.

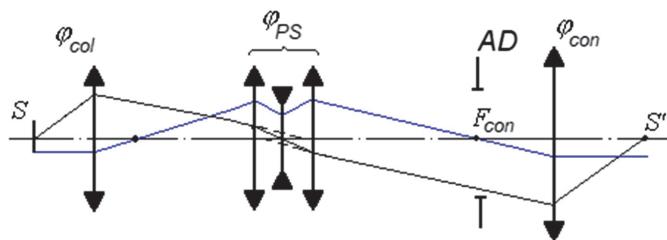
As shown by N.I. Pinegin [8], the diameter of the pupil of the eye depends not only on the brightness of the background but also on its size: a decrease in the angular size of the field with a constant brightness of the background leads to an increase in the pupil of the eye. However, with constant background brightness, an increase in the field above  $5-10^\circ$  has practically no effect on the size of the pupil of the eye. To determine the resolution of a microscope in combination with an eye, one must obviously know the properties of the eye and know the effect of its defects.

### 3.3 The Quality (Q-factor) of the Microscope Optical System

The luminous stream forming the image will be approximately equal to

$$F = \pi \cdot L \sin^2 \sigma' \cdot ds' = \pi \cdot L_0 n^2 \sin^2 \sigma' \cdot ds',$$

where  $L_0$  is the radiation brightness reduced to air; and  $ds'$  is the image area.



**Figure 3.15** Scheme of the illumination device of the microscope with the optical system variable magnification with critical lighting method (first version).

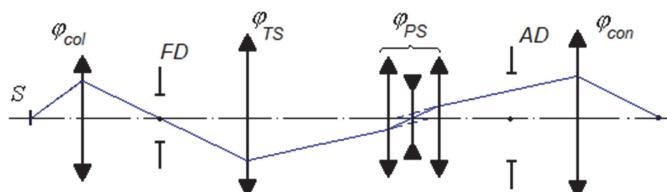
A possible disadvantage of such a scheme is that the contour of the illuminated field is determined by the contour of the surface of the radiation source. To eliminate this drawback, we can supplement the scheme in Figure 3.15; in this case, the system  $\varphi_{TS}$  of the transfer image of the radiation source can be used, as shown in Figure 3.16.

It is obvious that the use of a variable magnification system leads to a complication of the optical scheme of the lighting device into two lenses with a single-component basic variable magnification scheme and four lenses with a three-component basic scheme. However, the requirements for the manufacture of these lenses are rather low, which consequently lowers the complexity of their manufacture. However, the efficiency of the use of the luminous flux can be increased by an order.

### 3.5 The Construction of Frontal Components of Objectives for Microscope: Optical Design

It is known that in the light microscope system the objective is the most important part. This is recognized by many researchers, as designers of optical systems of microscopes, and those who use a microscope in their work for solving applied problems. That is why in the arsenal of a microscopist, as a rule, there are a number of objectives that differ both in consumer properties and in the degree of aberration correction. The well-founded choice of a particular objective for specific types of research is an integral part of the work of any microscopist.

However, we almost never think about what parts (lenses and mechanical elements) used for this or that objective for a light microscope. At the same



**Figure 3.16** Scheme of the illumination device of the microscope with the optical system variable magnification with critical lighting method (second version).

work “through glass” up to 2 mm thick. Another change in the frontal component makes it possible to obtain an objective (3) for studies using the interference contrast method (MIRO configuration).

With all these variants of the frontal lens design, the design quality of the aberration correction of the objective as a whole remains unchanged.

In modern microscopy, there have been steady trends toward a broad unification of objectives for microscopes. In the development of objectives with unified parameters, it became necessary to change the methods and approaches to optical design, design and technology of manufacturing parts, assembly of objectives for microscopes. To achieve the goal, the search for the most rational designs is under way, new optical materials are being developed and introduced into production, the methods of construction are being improved, and the qualifications of specialists are being improved.

We propose to carry out multilevel unification of optical systems of objectives for microscopes already at the stage of dimensional and aberration optical calculation and development of optical design. It is shown that by using the methodology of the basic components and the base optical scheme, it is possible to obtain a whole gamut of objectives intended for use in various applications and for implementing various research techniques on a microscope. It is shown that the basis of unification of optical systems of objectives for microscopes is the use of optical design as a tool for creating a composition of elements with known dimensional, aberration properties. It is shown that it is possible to provide interchangeability of objectives for the assembly of microscopes of various manufacturers.

### **3.7 An Example of the Optical and Mechanical Design of a Microscope Objective**

Suppose we want to perform a lens objective optical design, the technical and consumer parameters of which correspond to those specified in a certain “Technical Assignment.”

#### **3.7.1 Terms of reference for the design**

1. Overall characteristics:

- Linear magnification  $V = -20\times$ .
- The numerical aperture in the space of objects is at least  $NA = -0.75$ .
- Linear field in the space of objects  $2y = 1.0$  mm.
- Infinity optical tube length.
- Rear focal length of the objective  $F' = 8$  mm.
- The position of the entrance pupil at infinity.
- The position of the exit pupil (from the plane of the object) is arbitrary.

their control and certification. Also, the identification of defective parts, optical-mechanical components and fully assembled objectives can be formalized; for example, the existing ASA concept may allow such control and “sorting” of low-quality details, while achieving maximum objectivity of this process.

If it is impossible to “integrate” a specific “optical design” using the existing range of mechanical parts, other sizes can be developed, but it is necessary, taking into account the framework of the described engineering solution; the range of parts will simply be expanded. This approach, in turn, makes it possible to use standard technology for the manufacture of mechanical parts, the most complete use of special equipment and control equipment.

We assume that the new  $20 \times 0.80$  objective under development refers to the budget objectives of the medium series; it also requires the use of the mechanical design described here.

### **3.7.13 Description of the design of the developed objective**

The designed objective consists of four lenses components. Each lenses component is mounted in a separate frame on the glue; in this way an optical-mechanical component is obtained. Then each optical-mechanical component is subjected to mechanical processing using the so-called autocollimation method; this achieves the combination of the optical axis of the lens with the mechanical axis of the frame. All optical-mechanical components are installed in a common through (inner) housing and are clamped on both sides by threaded rings. The developed mechanical design of this objective provides for spacer rings between the end ring and the first node and between the first and second node. With their help, the lens is corrected for spherical aberration, and the size of the parfocal height of the lens is  $45 \pm 0.015$  mm.

The third opto-mechanical component of the structure has a “smaller” diameter, and there are four diametrically located holes in the housing, with the help of which lateral displacement (perpendicular to the optical axis of the system) of this opto-mechanical component can be carried out, and thereby the optical part of the objective is centered relative to the connecting thread and reference plane. For reliable fixation of the third node, the gap between this node and the body is filled with sealant through the same holes. After the final assembly of the objective, a decorative cap is screwed onto the outer casing.

## **3.8 An Example of the Design of a Stereo Microscope**

### **3.8.1 Physiological and geometric factors of stereoscopic vision**

A person has two eyes, the fields of vision in which almost coincide. Therefore, both human eyes see the same objects at the same time. In this case,

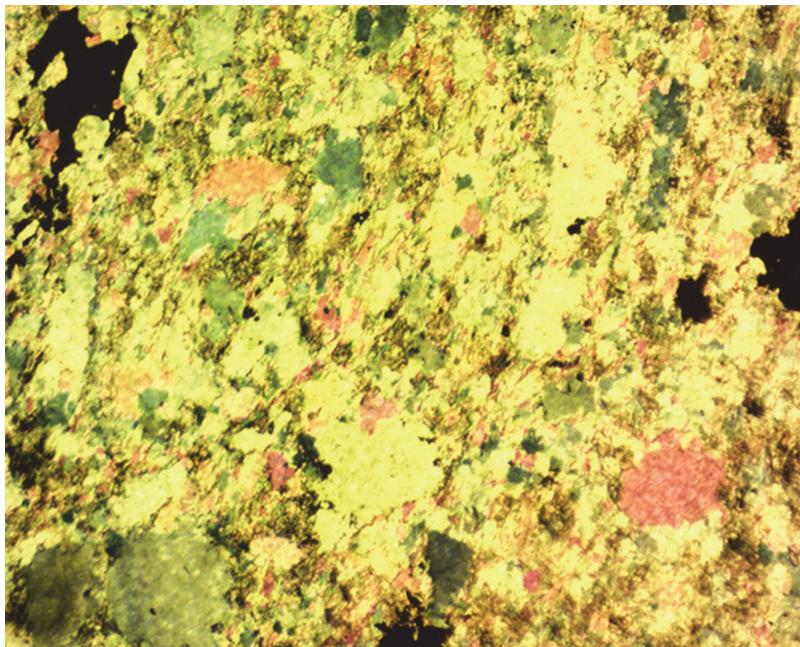


Figure 3.89 Photographs of some real objects (MPS-2).

### 3.10 Comparison Microscopes

The purpose of comparison microscopes is determined by their name, they are intended to identify images of compared objects under study. A distinctive feature of comparison microscopes is the presence of a special optical-mechanical module, which allows two images obtained by two microscopes to be combined into a single visual space. We can say that this optical-mechanical module is the main element of comparison microscopes. But two microscopes that form independent primary images of two objects to be identified should not only be functionally similar, but should have the same optical and mechanical structures, be equipped with exactly the same optical-mechanical components, such as objectives, lighting and other systems. The quality and reliability of the research conducted to identify the objects to be studied depends on how carefully calibration and adjustment of comparison microscopes, adjustment of their lighting systems, objectives assembly, and adjustment of focusing mechanisms (even careful selection of light sources according to spectral and other characteristics) are performed.

#### 3.10.1 MS-51 comparison microscope

Here are some parameters of this device to describe the principle of operation of a standard comparison microscope. The MS-51 microscope is a special microscope designed to study two compared objects similar to each other by



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