Microscope Design

Volume 2: Practice

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Chapter 1 Synthesis of Optical Systems for Mass-produced Budget Microscopes

To begin this chapter, I would like to remember my "production" mentor, Ivanova Tatiana Aleksandrovna. After graduating from the Institute of Precision Mechanics and Optics, now called the University of Information Technology, I joined the Optical Design Bureau in the Department of Computational Optics of the optical—mechanical association LOMO in Russia. I was very fortunate to be a part of the optical design group for light microscopes. Tatiana Aleksandrovna was the main specialist in this group. She was a complicated and highly ambitious woman with whom I worked closely for just three years. Despite there being very few women professors in optics, she became a professor. Unfortunately, her health was undermined; working in a production plant can be a very difficult place for the implementation of innovative ideas and projects. She had a bright and short life.

1.1 Achromatic Correction Objectives

Currently, it is not possible to trace the original method of constructing circuit solutions for achromatic objectives used by Abbe. Analyzing the construction of classical achromats, Berek [1] proposed that the construction of small-magnification objectives be considered according to the type of the known Petzval objectives in the reverse course of the rays. The construction of high-magnification objective designs should be carried out in such a way that all surfaces of the system have the smallest possible values of the first two third-order aberration coefficients.

This condition is satisfied by constructions built on the principle of synthesis of frontal elements containing surfaces close to aplanatic, and elements forming the design of the Petzval objective. When constructing circuit solutions for achromatic objectives of the classical type, the system can

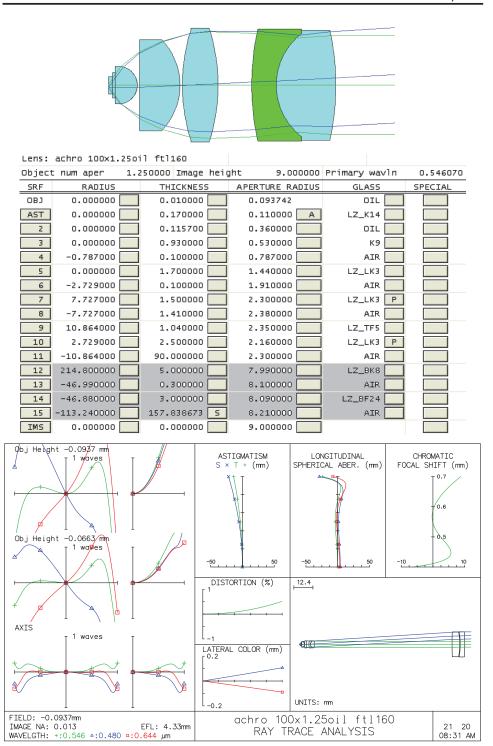
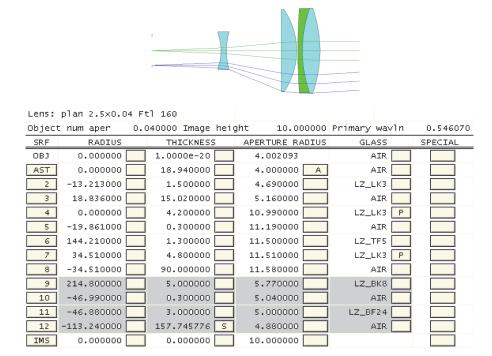


Figure 1.13 Optical design and graphs of aberration correction of a 100×1.25 oil immersion infinity achromatic objective ($F'_{ts} = 160 \text{ mm}$).



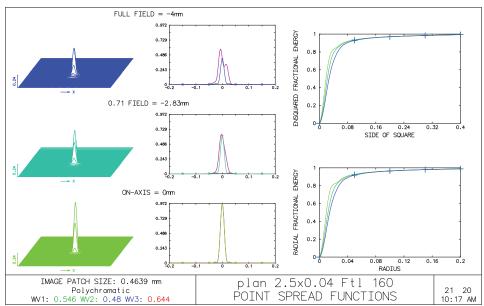


Figure 1.30 Optical design and graphs of aberration correction of a $2.5 \times /0.04$ infinity LCF plan achromatic objective ($F'_{ts} = 160 \text{ mm}$).

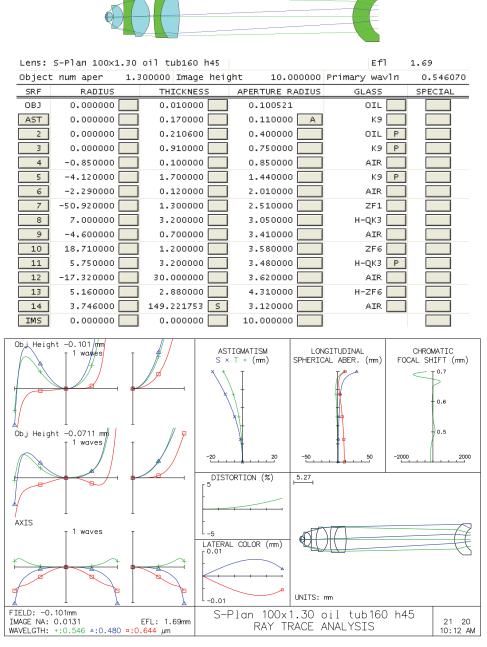


Figure 1.57 Optical design and graphs of aberration correction of a 100×/1.30 oil immersion finite LCF semi-plan achromatic objective.

By the way, it is precisely for polarization studies that an objective that can work with various types of immersions (water, glycerin, oil, etc.) can be used most effectively. Finally, to achieve high contrast in conoscopy and, especially, in orthoscopy, attention must be paid to reducing the influence of spurious illumination that occurs when reflections from planar *mirror* fragments of the object under study. Optical lens systems should also reduce the likelihood of a false birefringence effect.

When working with some objectives, the use of an immersion is expected. For example, with an immersion oil, the refractive index is close to 1.518 for a wavelength of 546 nm; the Abbe number is about 48. In the proposed set below, optical systems were created, including the *twin* objectives, in which for each objective working without immersion there is a corresponding objective with the same linear magnification and numerical aperture operating in an oil immersion. Such objectives are necessary for the study of both polished minerals those of weakly reflecting objects, for example, coals. The fine structure of charcoal grinding, which is poorly observed when viewed with *dry* objectives, is of higher contrast with the use of immersion.

Another big problem is that the presence of a significant amount of scattered light and reflexes from the lens surface reduces image clarity. Grammatin et al. [12] and Arlievsky [13] have studied the possibility of reducing scattered light in objectives operating on reflection. They concluded that the most effective way to reduce scattered light is to perform an optical design based on the use of lenses with a certain configuration. For objectives operating in a polarizing microscope, these conclusions are also valid. The use of immersion in objectives (especially small magnifications) is also one way to reduce scattered light. In addition, with the use of immersion, there are several other qualitative changes in the image due to the enhancement in the immersion of a number of optical contrasts: reflectivity, the color of a mineral in white light, the effects of anisotropy, the strength and color of internal reflexes, etc. Figures 1.140-1.142 show the design parameters and graphs of aberration correction of budget oil immersion objectives intended for use in mass segment polarizing microscopes. Figures 1.143–1.149 are photographs of a real sample taken on a polarizing microscope with a 5x linear magnification objective at various rotational positions of the stage. Figure 1.150 is a photograph of a real sample taken on a polarizing microscope with 2x, 4x, 20x, and 40x linear magnification objectives.

1.7 Topical Reasoning about Optics for Budget Microscopes

This may seem strange, but despite the advances made in motorization, for example, the sensory and *stepping* mechanisms used in many consumer devices, the light microscope of the mass-market segment is still a very primitive *optical*—*mechanical* device that uses technologies from the 19th

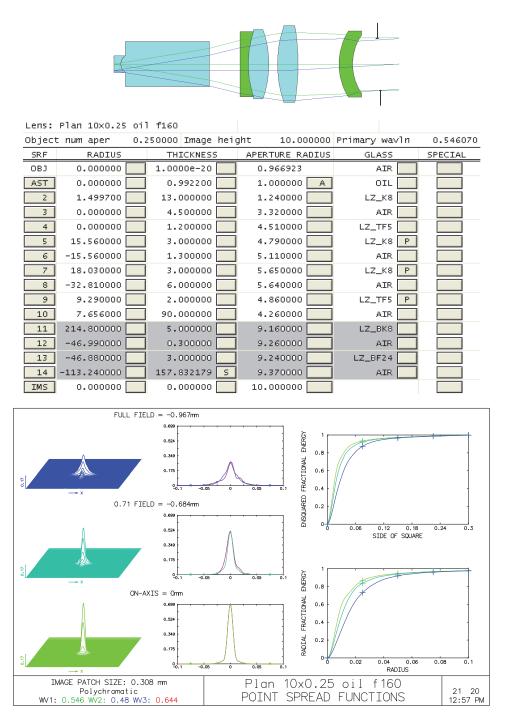


Figure 1.140 Optical design and graphs of aberration correction of a $10 \times / 0.25$ oil immersion infinity LCF plan achromatic objective ($F'_{ts} = 160 \text{ mm}$).

contrast devices. In addition, these technologies can be successfully applied to build the so-called *dark field* lighting in transmitted light microscopes. LED technology in lighting systems can give some impetus to the development of new techniques and practice in microscopy. For example, the effects of strobe, modulation, colorimetry, and others.

1.7.3 Independent correction terminology

The terminology used for independent objective correction should not address the fact that microscope objectives are designed to work with the infinite length of the tube. Terms such as infinite color system (ICS) or infinite color correction system (ICCS) mean that the objectives are designed to work under the conditions of an infinite length of the microscope tube, when the aperture rays propagate parallel to the optical axis after the objective and then are focused by a special tube system. The term *independent correction* should only refer to an objective quality parameter, such as aberration correction, regardless of whether it is intended for operation for the finite or infinite optical length of the tube. Nevertheless, the infinite length of the tube is gaining a more stable position in the construction of optical systems of modern microscopes, including mass-produced budget microscopes. Therefore, we developed a set of budget objectives for infinity for potential use in such microscopes. However, these objectives (as well as the objectives considered above for the finite length of the tube) also have high correction quality with respect to image curvature and lateral chromaticity. Table 1.11 shows the main technical parameters of a set of third-generation budget objectives for mass-produced microscopes of an infinite length of tube. All objectives are designed for a 45-mm parfocal distance standard and use the additional tube system F' = 200 mm. The calculated linear field in the image space was chosen equal to 2v' = 20 mm, but the real field reaches 22 mm without a noticeable deterioration in quality.

Some objectives are designed for use without a cover glass and are mainly intended to work as part of metallographic microscopes. Some objectives use a 0.17-mm cover glass while others can work equally well with or without a cover glass. The use of an infinite length of the tube for such objectives has become advantageous, since when focusing on an object, the optical length of the tube after the objective does not change and there are no additional defocus aberrations in the image area.

It is believed that the difference between using a 0.17-mm-thick cover glass and not using one cannot be detected when using objectives of weak magnifications. To what extent can the estimated image quality deteriorate if, for example, an objective designed for working with a cover glass is used without it? Conversely, to what extent can it deteriorate if an objective designed for working without a cover glass (d=0) is used with a 0.17-mm

Table 1.11 Main technical parameters and basic optical layout of a set of third-generation budget objectives for mass-produced microscopes of an infinite length of tube.

Magnification	N/A	WD	F' (*****)	R	DF (vers)	on object	FOV on image	Optical design at figure	The principal optical layout
Wiagiiiicatioii	IVA	(IIIII)	(IIIII)	(µIII)	(μιιι)	(IIIII)	(IIIII)	no.	The principal optical layout
5	0.10	20	40	3.33	33	4.4	22	1.32	
10×	0.28	15.5	20.0	1.2	4.25	2.2	22	1.35	
20×	0.40	8.0	10.0	0.84	2.1	1.1	22	1.37	
40×	0.60	2.9	5	0.56	0.93	0.55	22	1.42	
50×	0.60	2.4	4.0	0.56	0.92	0.55	22	1.47	
100×	0.80	0.52	2.0	0.42	0.52	0.22	22	1.51	
100×	0.90	0.15	2.0	0.37	0.41	0.22	22	1.53	
100×	1.30 oil	0.12	2.0	0.26	0.20	0.22	22	1.59	

one? It has been reported [17] that for small-magnification objectives (up to $16-20\times$, or more precisely for objectives with numerical apertures to about 0.40), the loss in image quality is insignificant; there is practically no difference in the study of an object with and without a coverslip. As an argument, formulas have been given for calculating the spherical aberration that arises when using an objective in the not quite normal mode, concluding that the magnitude of this spherical aberration is small, so there should not be milkiness in the image. Nevertheless, my practical studies [18] have shown that objectives designed and assembled in a traditional way (in performance only at d=0.17 mm or at d=0) noticeably lose their image quality when operating in not quite normal mode.

Based on the physiology of the eye, 0.5Δ per eye is painlessly overcome by the muscular apparatus of the eye:

$$0.5\Delta = \frac{0.5 \, cm}{1m} = \frac{5 \, mm}{1000 \, mm} = 0.005 \, radian \approx 17.24'.$$

Accordingly, in both eyes: $17.24' \times 2 \approx 35'$.

We will take into account the special working conditions associated with using binocular microscopes (large values of linear magnifications, location and small diameters of exit pupils, resolution of objects, large linear fields of view, etc.). Even with a theoretically ideal microscope optical system, prolonged use causes fatigue and discomfort. You can set the following requirements for the values of permissible deviations from the parallelism of the rays emerging from the eyepieces:

- The convergence of beams of rays in a horizontal plane of not more than 20',
- Beam divergence in the vertical plane of not more than 15', and
- Beam divergence in the horizontal plane of not more than 60'.

1.8.3 Design of eyepieces

It is the eye—or, more precisely, its optical characteristics—that should determine the parameters of the optical and mechanical design of the eyepiece. Indeed, the whole theory of light microscopy, which has not yet been questioned, is based on the fact that the microscope is an observational device; i.e., it is intended for observation by eyes. At the same time, in light microscopy theory, eye parameters such as resolution, spectral sensitivity (there is no point in correcting in the eyepiece, for example, an aberration such as the secondary spectrum), the eye's base, the location of the pupil, and some others are taken into consideration. However, in the optical design of eyepieces (and in a broader sense of light microscopes), parameters such as the intrinsic aberrations of the observer's eye, the eye's ability to accommodate and converge, and the degree of tension or relaxation of the eye when observing at different distances are not always considered. As a rule, the initial numerical aperture of the projection system (the rear numerical aperture of the objective or the output aperture of the tube system), as well as the linear field in the so-called intermediate image plane on the microscope, is taken as the initial data for calculating the eyepiece. The diameter of the exit pupil for the eyepiece depends on these parameters; it is believed that the eye is able to adapt to this size (changing aberrations and lighting parameters are not taken into account).

It should be noted that there is some inconsistency between the same parameters for different parts of a light microscope. For example, a projection system is characterized by relatively high numerical apertures and small angular



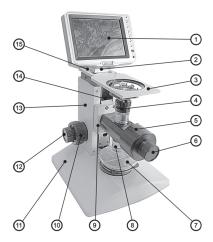


Figure 1.264 Photograph and technical drawing of a model of a digital telecentric microscope without a visual channel. 1, liquid crystal display; 2, mechanism of display rotation; 3, upper subject stage; 4, a camera with a projection objective; 5, LED illuminator of reflected light; 6, the handle of rotation of the illuminator; 7, lower subject stage; 8, interchangeable objective; 9, the mechanism of rotation of the illuminator; 10, coarse focus handle; 11, a stand; 12, handle fine focus; 13, case; 14, guide rail of focusing mechanism; 15, the handle of the rotation mechanism.

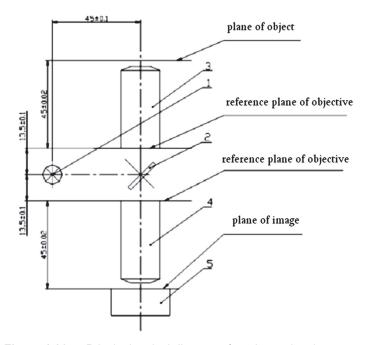


Figure 1.265 Principal optical diagram of a telecentric microscope.



Figure 1.271 Photographs of the MICROVISOR and MicroScreener digital microscopes.



Figure 1.272 Set of metallographic LCF plan achromatic objectives for an infinite length of the tube.

The MicroScreener appears to offer a preferable technical solution than the MICROVISOR because the MicroScreener user may not have to think about matching the parameters of the visual channel with the digital one; there is an optimal balance of optical dimensions and aberration correction. The system does not introduce shading, vignetting, or other image artifacts associated with the need to coordinate the location of the entrance and exit pupils of individual elements of the optical system.

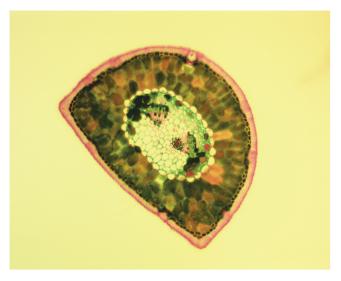


Figure 1.311 Photograph of a real object using a standard 10x objective (LED panel instead of a stage).

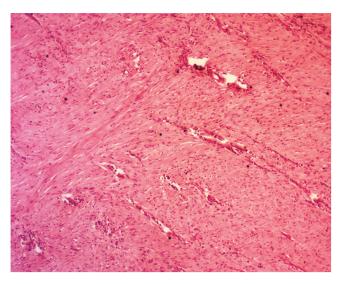


Figure 1.312 Photograph of a real object using a standard $10\times$ objective (LED panel instead of a stage).

A so-called *Beck plate* (or similar reflector) is also used in the lighting system of metallographic microscopes, for example, to increase the dimensions of the illuminator itself. Bright field objectives initially have a smaller diameter where the thread of the mount to the nosepiece is located. The goal of using such an illuminator is to "dress" it on a bright field objective but use only the

Chapter 2 Synthesis of Optical Systems for Medium Series Budget Microscopes

The classification of light microscopes into mass-produced, *medium series*, and other categories is conditional. The production of optical, optical—mechanical, and optical—electronic products is primarily based on market demand. It is not always true that there is less demand for more expensive and complex products. It is often the case that modules and accessories are designed to be interchangeable, allowing them to be used in products of various price points and technical specifications. This applies to microscopes, in which most of the nodes and accessories are unified and interchangeable. The optimal choice of the configuration of a particular microscope for solving specific research problems and the effectiveness of the research itself depend on the qualifications of the engineer completing the microscope as well as the experience of a researcher working with the microscope.

Often, the use of a medium series mass-produced device can successfully solve a research problem without the need for more expensive equipment. For example, using objectives of a different class on the tripods of ordinary microscopes can improve the consumer qualities of these standard instruments. The same applies to the retrofitting of conventional microscopes with additional accessories, such as those using contrast methods for research (polarization, dark field, phase contrast, and others), as well as equipping imaging systems with digital receivers. Many researchers follow this path, gradually equipping their microscope with various additional accessories and complicating its original basic configuration; the sequence of the microscope's improvement by the trial-and-error method makes it possible to obtain the optimal configuration for solving a research problem. In this context, the main task for microscope engineers and developers is to ensure the possibility of such modernization and customization of standard microscopes with additional equipment. Another task is to develop and offer a wide range of

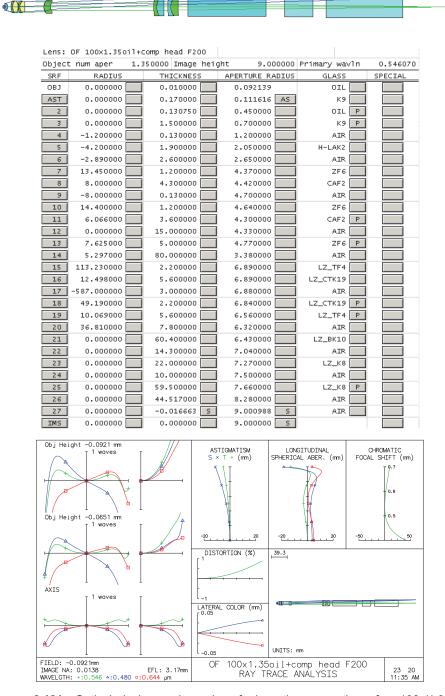


Figure 2.131 Optical design and graphs of aberration correction of a 100×1.35 oil immersion infinity LCR MicroFluar objective combined with a (LC compensate) visual head ($F'_{ts} = 200$ mm).

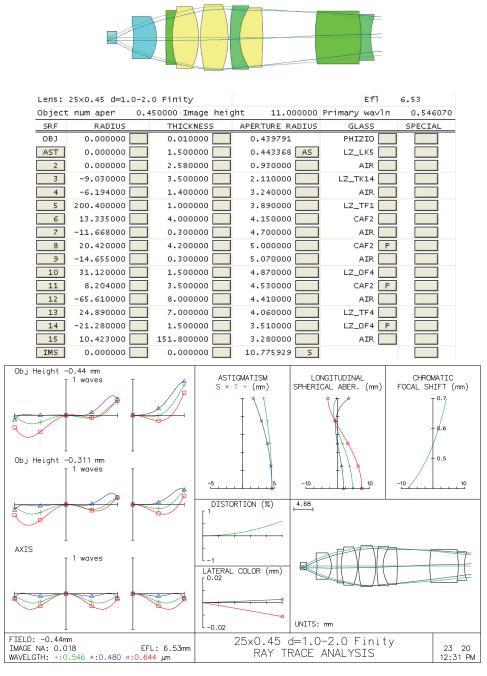


Figure 2.167 Optical design and graphs of aberration correction of a 25×0.45 finite plan MicroFluar objective (d = 1.5 mm; nominal).



Figure 2.198 Microphotograph of Actinoptychus heterostrophus (taken by A. Mikhaltsov).



Figure 2.199 Microphotograph of Xylem Pteridium aquilinum (taken by A. Mikhaltsov).



Figure 2.200 Microphotograph of Auliscus oamaruensis (taken by A. Mikhaltsov).

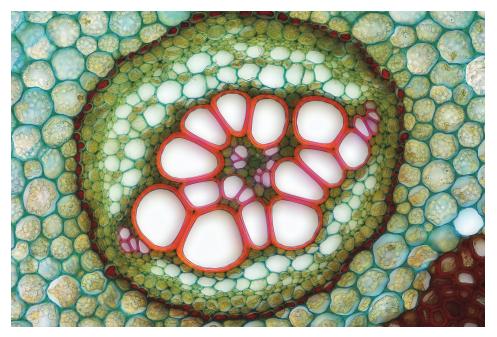


Figure 2.201 Microphotograph of a root of a fern (taken by A. Mikhaltsov).

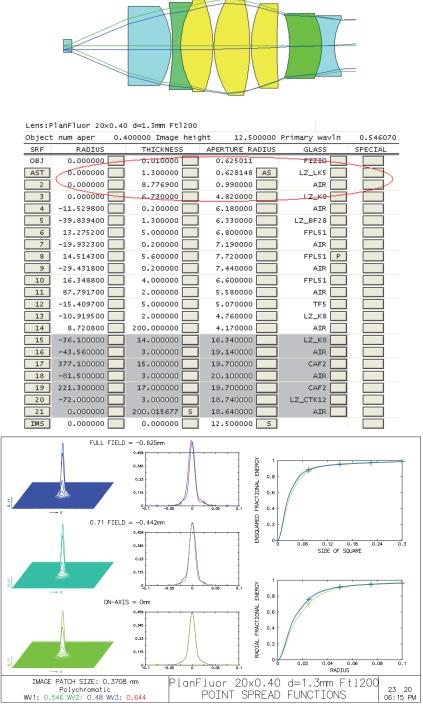


Figure 3.153 Optical parameters and graphs of aberration correction of a 20×0.40 (d=1.3 mm) LCF plan MicroFluar objective ($F'_{ts}=200$ mm).

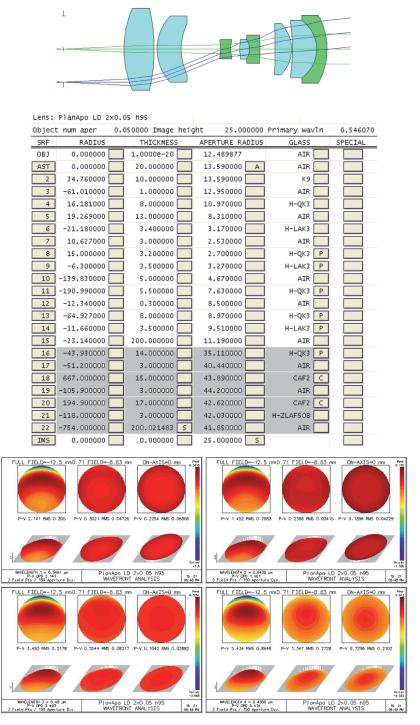


Figure 3.170 Optical design and graphs of aberration correction of a $2\times /0.05$ CCF plan apochromatic objective ($F'_{ts} = 200$ mm; h = 95 mm; 2y' = 50 mm).

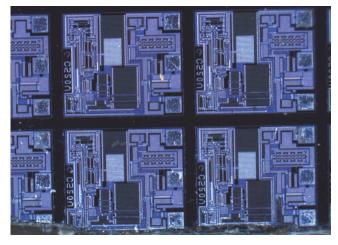


Figure 3.190 Photograph of a real object using a $5 \times$ CCF plan apochromatic objective (using the dark field method; h = 95 mm).



Figure 3.191 Photograph of a real object using a $10 \times$ CCF plan apochromatic objective (h = 95 mm).

spectral range. The observation of fluorescence in this case is possible in the visible region of the spectrum; however, this can substantially limit the effectiveness of the studies. A microscopist may wish to study fluorescence outside the visible spectral range. For example, some researchers would like to more effectively apply spectral ranges that extend into the shorter blue region of the spectrum [the near-UV (NUV) region in the wavelength range of 360 to

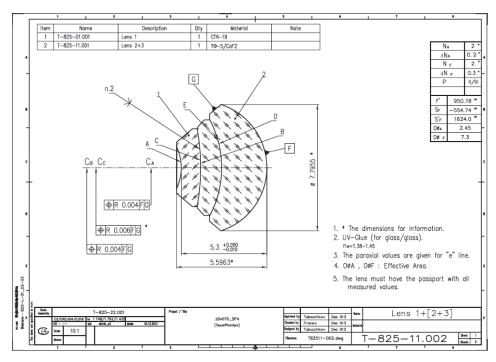


Figure 3.256 A drawing of the first, second, and third lenses (no. 1).

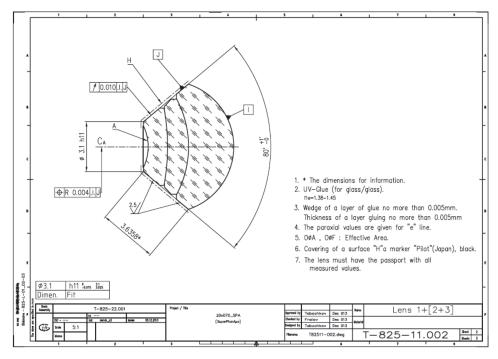


Figure 3.257 A drawing of the first, second, and third lenses (no. 2).

Table 3.12 Main technical parameters and basic optical layout of plan poly-apochromatic objectives (365–1100 nm).

Magnification	on <i>NA</i>	WD (mm)	F' (mm)	R (µm)	DF (μm)	FOV on object (mm)	FOV on image (mm)	Optical design at figure no.	The principal optical layout
4×	0.13	3.2	50	2.56	19.7	6.3	25	3.305	
4×	0.13	2.4	50	2.56	19.7	6.3	25	3.306	
10×	0.30	4.1	20	1.11	3.7	2.5	25	3.307	
10×	0.30	3.2	20	1.11	3.7	2.5	25	3.308	
20×	0.75	0.42	10	0.44	0.59	1.25	25	3.309	
40×	0.80	0.33	5.0	0.42	0.52	0.63	25	3.310	
50×	0.50	3.0	4.0	0.67	1.33	0.50	25	3.311	
100×	1.2 water	0.13	2.0	0.28	0.23	0.20	20	3.312	

microscope should allow these contrast and fluorescent methods to be implemented. Implementing these methods should be possible since they mainly involve adapting the microscope's optical components.

Maximum numerical aperture and ultrahigh values of the linear magnification of the objective must also be achieved. This is due to at least two distinctive characteristics of the studied objects: their linear dimensions and their motor ability, that is, their speed of movement in the medium. Most likely, it will not be possible to use an ordinary microscope with which researchers only observe the obtained image with their eyes. To study the details of the most important cell processes, such as division, phagocytosis, cytoplasmic currents, and others, we will probably need a digital image receiver, which is quite versatile. Such a receiver should be able to record both fast and slow processes.

Thus, the usual block diagram of a light microscope, which contains such basic parts as the illumination, projection, and registration systems, can

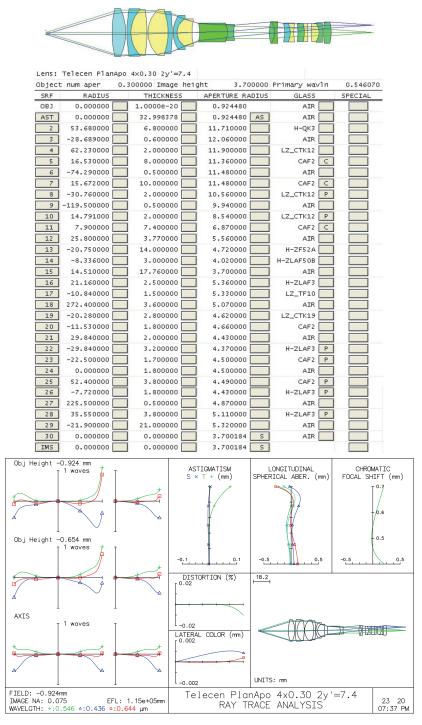


Figure 3.430 Optical design and graphs of aberration correction of a 4×/0.30 CCF plan apochromatic telecentric objective for direct digital receiver projection.



Figure 4.3 Photograph taken with a 10×/0.28 objective, a 0.17-mm-thick specimen glass, a 0.17-mm standard cover glass, and an LED panel used as a condenser.

Table 4.1 shows that when moving from the visible range to the shorter spectral range, the theoretical resolving power of a microscope can improve by a factor of 3–5. This is a strong argument for investigating the possibility of creating systems that operate in the NUV–DUV spectral ranges. Although some researchers' proposals for creating microscopes that operate in the DUV

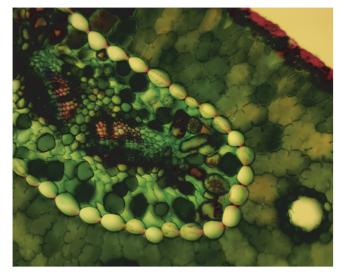


Figure 4.4 Photograph taken with a 20×/0.40 objective, a 0.17-mm-thick specimen glass, a 0.17-mm standard cover glass, and an LED panel used as a condenser.

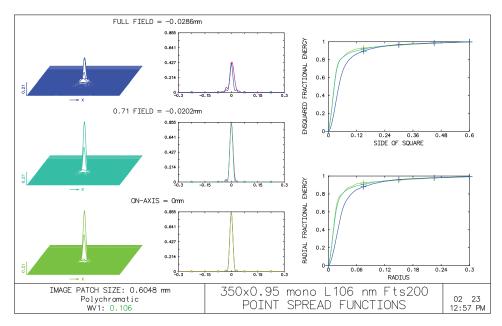


Figure 4.20 (*Continued*) Optical design and graphs of aberration correction of a 350×/0.95 plan monochromatic objective (106 nm).

software. Indeed, in our case, the layout of the optical system of the objective can be obtained by a similar method of determining the location of optical elements (lenses) within the framework of one optical scheme. However, when all the optical calculations have been completed and all the optical elements have taken their places, we find that the received optical systems turned out to be very logical and amenable to formal description. We can even talk about the peculiar beauty of such optical schemes.

Table 4.3 shows the main technical parameters and basic optical layout of some CCF plan multi-apochromatic objectives for the 200–1800-nm spectral range. This design is for an infinite length of the microscope tube, using an additional focusing system ($F_{ts} = 200 \text{ mm}$). The values of the resolution of R and the depth of sharpness DF are presented for the main wavelength $\lambda = 546.07 \text{ nm}$.

4.3.2 Optics of a microscope for working in the infrared range of the spectrum

A few decades ago, the infrared range was used quite rarely in studies using optical instruments, especially in microscopic applications. But modern photonics increasingly applies both classical and original methods of infrared visualization, particularly in applications such as data transmission, thermal visualization, and others. We will keep in mind that we are talking about a spectral range from approximately 0.75 to 15 μ m. First, we must classify

Lighting system

The lighting system of the so-called *transmitted light* for an underwater microscope can differ from the classical one used in standard light microscopes. In a conventional microscope, a lighting system comprises several elements, such as a light source (halogen lamp or LED), a collector optical system, and a condenser optical system. In general, all of these systems can be used to build an underwater microscope if, for example, they are placed in a sealed box that withstands high pressure and allows these systems to be

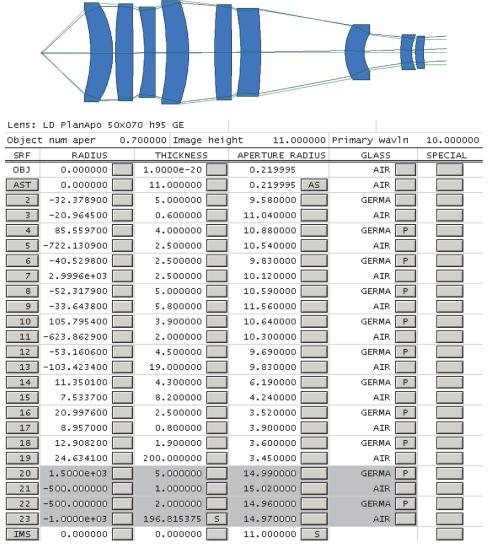


Figure 4.51 Optical design and graphs of aberration correction of a $50\times/0.70$ LWIR CCF plan poly-apochromatic objective ($F'_{ls} = 200$ mm).



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was promoted from an engineer to the head of the design bureau of complex instrument making. He has published more than 70 patents and more than 50 scientific articles. Currently, he works as a technical specialist for the project Labor-Microscopes. His primary field is microscopy, particularly the development of light microscopes and accessories; the design of optical systems of microscope objectives is his main professional skill. He has made more than 350 types of optical designs for microscope objectives, many of which were produced in different factories. He is a certified foreign expert in the field of optics and optical instrument making.