Optical design of custom objectives for biological light microscopy

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Abstract:
The results of the optical design of lenses for biological light microscopy, including those that require using water immersion.

Introduction

Various research methodologies using light biological microscopes require a whole Gama various elements of the optical components. The main element is optical lens, which has the most advanced optical correction. Proceeding from the conditions in which working microscope, which solves the problem, identifies the need for the using of certain lenses with varying degrees and nature of the correction of aberrations using one or another immersion.

The most widely used lens microscope lenses because of universality, the possibility of unification for use in various types of microscopes. They have advantages in technological opportunities, lack of screening, and other undesirable effects.

Analysis of major trends in optical systems of lenses shows that solved the problem increasing the resolving power and intensity, increasing the linear field, to achieve an increasingly high degree of aberration correction across the field, expanding the spectral range. A new direction was the development of lenses that work with digital receivers, which requires obtaining a flat field images, with apochromatic correction of aberrations throughout the linear field. In [1] shows the results of theoretical and practical research to develop new lenses for microscopes, most of them used standard techniques. The possibility of implementing non-standard methods and micro-studies is a complex scientific and technical task, requires the creation of original elements of the optical element base microscopes, namely lenses.

Increased informativity

Developers lenses for biological light microscopes are trying to solve the optical problems that increase informativeness systems.

The most important component of the increase of the information is to increase the numerical aperture microobjectives. The numerical aperture of the lens in the object space determines the resolution of the microscope, the image space significantly affects the energy parameters of the microscope. Of particular interest is the possibility of obtaining low magnification lens with a large input numerical aperture. In lenses with larger numerical apertures especially laborious spherochromatic aberration correction, as its higher-order non-linear increase with the relative aperture of the lens.

The problem of optimal correction of chromatic aberrations in the microscope is discussed for the past 40 years. Improvement of chromatic aberration influence on improving the color, determines the use of microscopes, analog and digital receivers image. Along with the traditional requirements for aberration correction, special attention is given to correction microobjective chromatic aberration (SFO). The concept of constructing the optical system of the microscope compensation method (when the residual aberration of the lens offset eyepiece) now in the past, as when a microscope linear fields 25-30 mm high-quality mutual compensation of aberrations over the field almost impossible. Which came to replace the system of the microscope optical elements with independent aberration correction improves the reproducibility and convenience in operation, creates conditions to further increase the linear field microscope.

To increase the information content of the image of an object by virtue of the specificity of any of its qualitative characteristics are insufficient or increasing numerical aperture microscope objective, no increase in the linear field. In microscopy (especially in biological light microscopy) are often used original methods, such as staining or other methods, to enhance the quality of research through the use of phase, or other interference phenomena in optics.

Synthesis of optical circuits is not the standard lenses for biological light microscopy

Table 1 shows the specifications and optical circuit of standard lenses, which are intended for use in biomedical optical microscope.

Improving the technology of optical calculation avoiding the use of triple gluing in high-aperture immersion stigmafluar (positions 1, 2). In this case the lenses with a linear increase of 100x achieved values of the numerical apertures of 1.24

and 1.40 for the variations of water and oil immersion, respectively.

Optical scheme of lens positions 3, 4 illustrate the development planachromatic lenses with larger numerical apertures, where there are no glued parts that is a prerequisite to "non-detuning" lens, with sharp differences of working temperature, which occurs when fluorescent microscope working in the field, rather than laboratory conditions. The same concept of responsible kvartzfluar lenses, built on the optical circuit shown in positions 7-12. Developed lenses in accordance with technical requirements for fluorescence analysis (including anti-Stokes) provide a flat field image of the object. It is well corrected by for a "green" region of the spectrum used for the study of luminescence. However, these objectives through the use of only two optical materials, fluorite and quartz, have a very good transmittance over a wide spectrum from 200nm to 1300nm, which allows their use in the work of the microscope on the methods of dual and multi-luminescence. These optical circuits may be the basis for calculating super apochromatic lenses are equally well corrected "in all of the spectral range that can be achieved through the use of optical materials such as lithium fluoride, sodium chloride, potassium bromide, etc.

Ability to use a microscopic studies of water immersion lenses cannot be overemphasized. Water is perhaps the only one used by immersion, to enable a lifetime of research facilities on the microscope. Benefits high-aperture water immersion lens in front of lenses, for example, oil immersion obvious. Water, not aggressive and do not "sticky" medium, using water immersion is no toxicity, easy to clean the front lens, the lens is less susceptible to collapse, it means more reliable and durable. All these circumstances are particularly relevant for fluorescent light microscopy. However, calculation and design of water immersion lenses are associated with certain difficulties in the theoretical and practical plan. Thus, the typical water wetting properties of the requirements for the form of a lens with which the water is in contact, this in turn imposes limitations on the correction capabilities of this lens and the lens as a whole. Refractive index and dispersion characteristics of water as an optical material, "not like other immersion environments. The front lens water immersion lens should be made of optical materials resistant to "damp atmosphere. Calculation of water immersion lens, especially for fluorescence analysis is a laborious task, which requires developers not only expertise, but also practical experience. Calculating lens stigmaflyuarov positions 13-16 is made in the light of such experiences, using the original technique of optical calculation, thus attaining high values of numerical apertures, with significant operating segment.

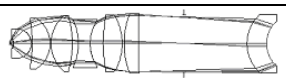
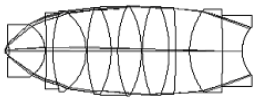
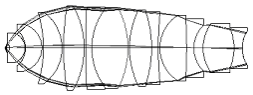
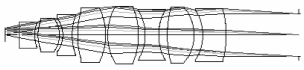
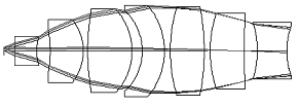
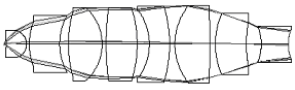
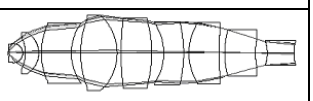
The advantage of these lenses over other water immersion lenses is the possibility of using microscopic methods, in particular, fluorescence analysis, based on the use of micromanipulators, microinjections devices, etc. Such lenses can also be used in ophthalmic confocal microscope, systems, genetic engineering, etc.

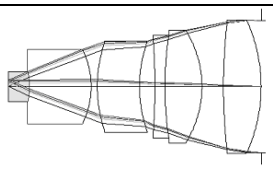
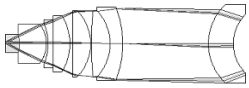
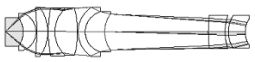
Conclusion

Classic microscope as the instrument of visual observation will never lose its value to researchers. The quality of research is constantly improving, expanding the scope of research, new techniques, which requires the development of new optical components.

References

D.N. Frolov “Synthesis of optical systems of lens microobjectives” Journal of Optical Technology, Volume 69, № 9, 2002, p. 16-20

Characteristics		Principal optical structure
increasing x aperture, type of correction	Free working distance, mm	
height 45mm, infinity tube length, F’tl=160mm, 2y’≈20mm		
1) 100x1.24wi 2) 100x1.40oi stigmafluar	0.15 0.10	
3) 20x0.75 4) 40x0.85 planachromat	0.88 0.45	
5) 32x0.85 planapochromat	0.74*	
6) 63x1.20oi planachromat	0.13	
7) 2.5x0.03 quartzfluar	8.04	
8) 10x0.20 quartzfluar	2.18	
9) 20x0.40 quartzfluar	1.40	
10) 40x0.65 11) 63x0.80 quartzfluar	0.60* 0.30*	
12) 100x0.90 quartzfluar	0.20*	

13) 10x0.55 wi LD stigmafluar	2.7*	
14) 20x0.65 wi 15) 40x0.87 wi LD stigmafluar	2.7* 2.7*	
16) 100x0.95 wi LD stigmafluar	2.7*	
* taking into account the deflection of the front lens microscope objective		

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