MICROSCOPE OBJECTIVES

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Microscope objectives are key components of optical microscopes, but they also equip experimental set-ups to focus laser beams or to collect photons emitted from any physical events to analyze or to diagnose. There are properties to take into account about their specifications and their usage for selecting the right microscope objective. They are used in a wide range of applications, from life sciences to material sciences, in laboratories and in industries and each application requires a specific microscope objective.

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The microscope objective is a complex multi-lens assembly that collects light from the sample. It plays a key role for the primary image formation and determines the quality of the image that the microscope, or the optical set-up, provides.

Although the first compound microscope was invented in the 17th century [1], microscope objectives are still high-technology items and among the most sophisticated optical components to design and to assemble. High-class microscope objectives can be composed of a set of individual lenses, lens doublet and triplet groups that are cemented together in order to reach the optical specifications that are designed for. Some lenses are still manually manufactured and result from a high level of know-how and skills. The microscope manufacturers offer a wide range of objectives providing different features as regards the optical aberrations correction, the illumination conditions and most generally the applications and the conditions of operations. Their costs can vary according to those characteristics, from hundreds to dozens of thousands of Euros.

Microscope objectives specifications can easily be found *via* the writings and the signs engraved on their barrel (see Fig. 3). But finding the right objective for each specific application is not obvious. According to the specimen type and the set-up, the requirements may change. There are microscope objectives for a wide range of applications such as live sample imaging, metallography, petrography...

A first point to consider is to select the right type of objective according to the device, the equipment or the set-up which will accommodate it. Microscope objectives feature different sizes, weights but also different optical configurations that can limit their integration.

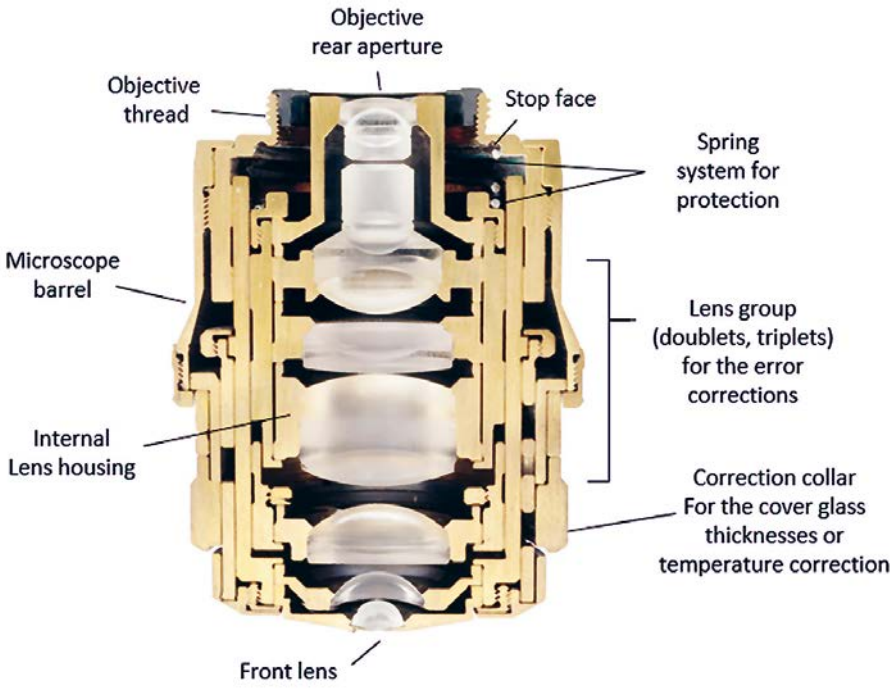


Figure 1. Cross-section of an apochromat objective showing the lenses and the main parts of the item.

A FINITE-CONJUGATE OR INFINITY-CORRECTED MICROSCOPE OBJECTIVES?

The finite-conjugate microscope objectives are designed to project a diffraction-limited image at a fixed plane, the intermediate image plane. They are mainly used for simple compound microscopes or OEM integration, or as focusing objectives. Because they are generally composed of two or three lenses, including an achromatic lens, they have a smaller size and a lower weight than higher classes of objectives [2]. However, they are limited as regard the color and the chromatic error corrections. The infinity-corrected objectives are designed to project incoming rays to infinity and they require a specific

MANUFACTURER	TUBE LENS FOCAL LENGTH (MILLIMETERS)	PARFOCAL DISTANCE (MILLIMETERS)*	THREAD TYPE	INFINITY-CORRECTED DESIGNATION
Leica	200	45	M25	HC system (Harmonic Compound System)
Nikon	200	60	M25	CFI (Chrome-Free Infinity).
Olympus	180	45	RMS	UIS (Universal Infinity System)
Zeiss	165	45	RMS and M27	ICS (Infinity Color Corrected System)
Mitutoyo	200	95	M26	

Table 1: Common manufacturer's specifications standard on the objective design. Adapted from [4]. For home-built optical set-up, microscope objective threading adapters exist.

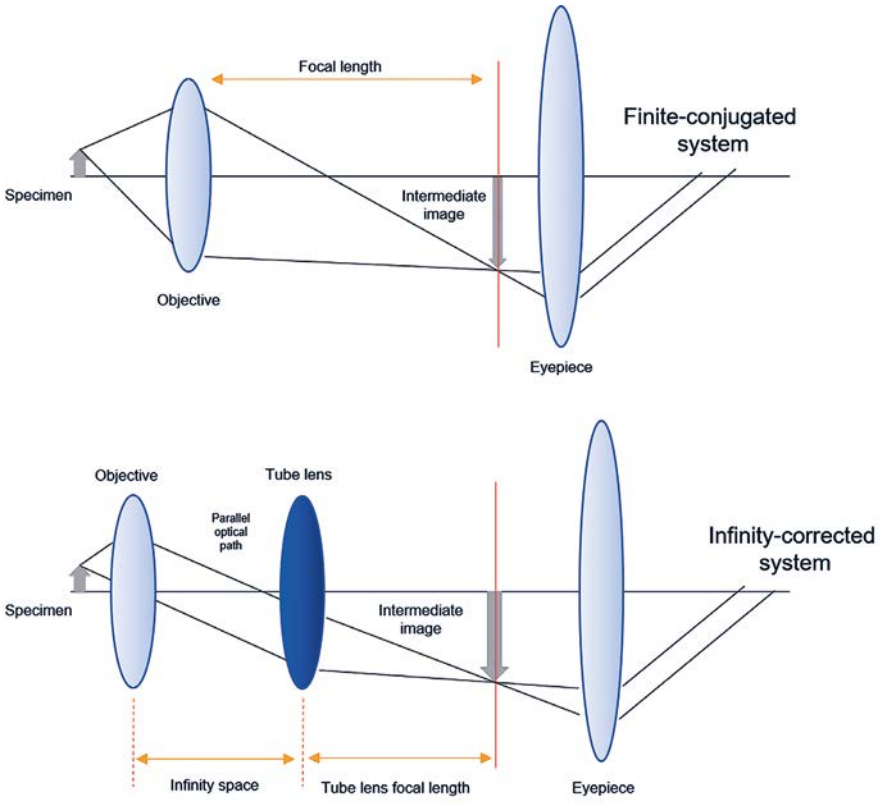
*The parfocal distance is the distance between the objective lens mounting plane and the specimen.

RANGE	OBJECTIVE MAGNIFICATION	MAIN COMMENTS
Low	1× to 5×	It gives a large overview image of the sample. The illumination homogeneity can be compromised.
Medium	10× to 40×	It offers a good trade-off between the field of view and the spatial resolution.
High	60× to 100×	It is used for small samples and to image fine structures. Image brightness can be weak.

Table 2. The main magnification ranges. Adapted from [5].

tube lens (or a secondary lens) to focus the image at the intermediate image plane. Infinity-corrected and finite objectives are consequently not interchangeable and can only be accommodated in specific microscopy systems, according to the focal length of the tube lens and the parfocal distance used by the manufacturer. A secondary lens is not needed for laser focusing applications. Infinity-corrected objectives are composed of a higher number of optical elements (see Fig.1) and have larger sizes and weights than finite-conjugate objectives. However, they provide better optical performances. Another consideration is the objective pupil diameter. The effective exit pupil diameter (D) necessary to achieve a given numerical aperture is D= 2NA×f where NA is the numerical aperture and f is the objective focal length. The limited factor within the objective is the thread size which differs from the manufacturer. There are two categories: the RMS having a thread size of 20.32 millimeters with a pitch of 0.71 mm, and the M25 & M27 having

Figure 2. Simplified diagram of the finite-conjugate and infinity-corrected optical configurations. Adapted from [3].



OBJECTIVE CLASS	SPHERICAL ABERRATION	CHROMATIC ABERRATION	FIELD CURVATURE	TYPICAL DESIGNATIONS
Achromat	1 Color	2 Colors	No	Achro
Plan Achromat	1 Color	2 Colors	Yes	Plan Achro
Fluorite	2-3 Colors	2-3 Colors	No	Fluor, FL
Plan Fluorite	2-3 Colors	2-3 Colors	Yes	Plan Fluor, FL Plan
Plan Apochromat	2-3+ Colors	3+ Colors	Yes	Plan Apo

Table 3. Summary of the different types of optical aberration corrections. Adapted from [3-5]

respectively thread sizes of 25mm and 27mm with a pitch of 0.75mm. Some microscope objectives are designed with a larger thread size (M32 or M40) in order to provide a longer working distance and a wider field of view. The parfocal distance is also longer than conventional objectives.

Manufacturers employ different combinations of parfocal distance, tube lens focal length and thread size to overcome the optical limitations which may occur along the optical path and to meet the requirements for the design of new models of objectives. Current specifications used by the main microscope objective manufacturers are summarized in Table 1.

READING THE OBJECTIVE BARREL: A CLOSER LOOK TO THE WRITINGS

Resolution versus magnification

The linear magnification M and the numerical aperture NA are the two main specifications needed when seeking for an objective. The first gives the ability of the objective to magnify small objects while the second is related to the spatial resolution. The objective magnification is usually classified in three ranges as indicated in Table 2.

Another important parameter is the numerical aperture (NA) which indicates the incoming light acceptance angle of the microscope objective. This parameter determines the light collection, the resolution and the depth of field of the objective. The resolving power or the spatial resolution (in microscopy) is the minimal distance at which two distinct points of the object can still be discriminated from each other. In other words, it estimates the lateral resolution that can be calculated in conventional light microscopy by the formula presented by Ernst Abbe in 1873 : Abbe resolution = $\lambda/2NA$, where λ is the illumination wavelength. The higher the NA, the better the resolution. But it is important to know that the specimen and refractive index of the medium between the objective's front lens and the sample surface can also influence the resolution.

CORRECTION OF THE OPTICAL ABERRATIONS

Light is composed of different wavelengths which are not associated with the same refractive index of lenses. Color aberrations may therefore occur when light is transmitted through a lens. There are different degrees of color corrections which lead to different types of objectives (Table 3).

The achromatic objective is the least expensive type. Those objectives are corrected for axial chromatic aberrations in two wavelengths (typically blue and red). They are additionally corrected for spherical aberrations in the green color.

The next level of corrections and type is the fluorite or semi-apochromat objective. They are, axially corrected for two colors. They are additionally corrected of spherical aberrations for two or three colors. They usually have higher NA and yield brighter images and higher contrasts.

Apochromat objectives provide the highest level of spherical and chromatic corrections. They are corrected for at least three colors, typically blue, green and red, and spherically corrected for two or three wavelengths. Images are free of color fringes. They are the most complex and expensive objectives as they result from specific combinations of different glass materials with opposite dispersion properties.

Lenses in microscope objectives are curved, convex or concave, resulting in image curvature. The higher the magnification, the worse this curvature effect. To eliminate the field curvature, a specific optical design must be considered. Objectives with flatness correction are called "plan" or "planar".

IMMERSION MEDIA	REFRACTIVE INDEX (N)	ABBREVIATIONS	CONVENTIONAL LINEAR MAGNIFICATION RANGE	CONVENTIONAL NUMERICAL APERTURE RANGE
Air	1.00	Dry	1× – 100×	0.04 – 0.9
Water	1.33	W, Water	10× – 100×	0.3 – 1.27
Silicon oil	1.40	Sil	25× – 100×	1.05 – 1.35
Glycerol	1.47	Glyc	10× – 93×	0.5 – 1.3
Oil	1.515	Oil	20× – 100×	0.4 – 1.49

Table 4. Main properties of a set of immersion media. Multi-immersion objectives exist with a specific adjustable collar to select the refractive index of the immersion media.

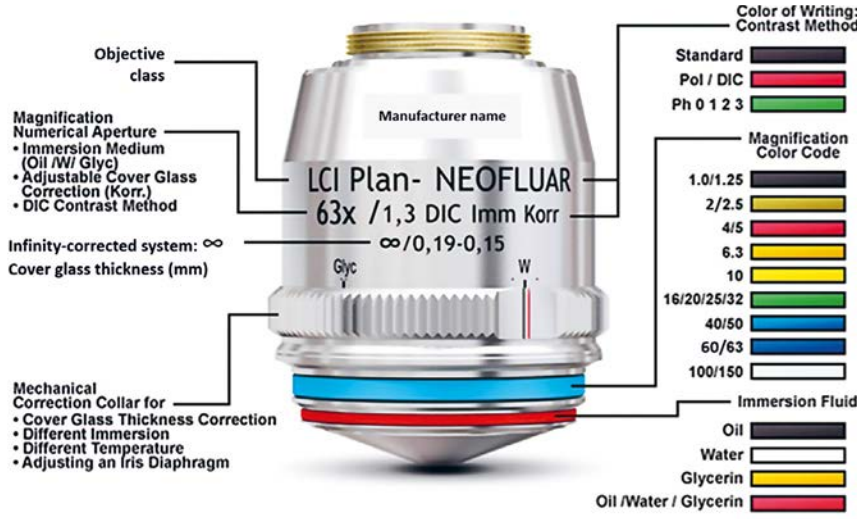


Figure 3 – Color Codes – The microscope manufacturers label their objectives with color codes which allow for a rapid identification of the magnification and the requirements of any specialized immersion media. The figure lists the current magnification and imaging media color codes. Other specifications marked on the objective barrels can be also found.

IMMERSION OBJECTIVE: HOW TO REACH A NA HIGHER THAN 1?

The typical maximum achievable numerical aperture for dry objectives is 0.95. It is possible to increase the numerical aperture by including an immersion media between the objective lens and the specimen. To increase the resolving power, typical immersion liquids are used such as synthetic immersion oil, glycerol, water or silicone oil (Table 4).

WORKING DISTANCE

The working distance is the distance between the objective front lens and the top of the specimen (or the cover glass) when the specimen is in the focus plane. The working distance decreases as the magnification (and the numerical aperture) increases. This specification is not always engraved on the objective barrel. It is mentioned when the objective provides specific working distance characteristics. Three categories of working distances can be identified: conventional working distance (not indicated on the objective barrel), long working distance (abbreviated as L, LL, LD, and LWD) and extra-long working distance (abbreviated as ELWD (extra-long working distance), SLWD (super-long working distance), and ULWD (ultra-long working distance).

Those features will affect the size and consequently the weight of the microscope objective. A higher magnification will make a longer and larger objective. At a given linear magnification, the diameter will not be identical according to the color correction and the working distance characteristics.

PHOTON EFFICIENCY

A high photon efficiency (or transmittance) can be required, *e.g.* in fluorescence microscopy techniques, at a given range of the optical spectrum, for instance at the extrema of the visible spectrum and even in the near-infrared (NIR) and near-ultraviolet (NUV) domains. That is why manufacturers offer specific objective series with specific photon efficiency performances, depending on the antireflective coatings and materials used for the lenses.

OBJECTIVE LENSES BEYOND THE UV AND THE NIR

Specific objectives designed in specific glass materials can be manufactured to be compatible with the X-ray and the IR domains. UV microscope objectives can be found in the semiconductor industry, X-ray objectives in X-ray microscopes or facilities like synchrotrons. IR objectives can be found in laser processing systems and used for laser beam focusing.

CLEANING AND MAINTENANCE

Microscope objectives are very sensitive materials. They must be carefully manipulated to maintain their performances. It is important to follow the manufacturer recommendations for the microscope objective cleaning and their conditions of usage and storage. It is also important to pay attention to the type of reagent the microscope objective can be in contact with. Some reagents might be aggressive and dissolve the glue used to seal the lenses within the microscope objective. Besides, it is advised to store microscope objectives in their dedicated casing when they are not mounted in order to prevent any shocks which could move lenses within the objective and affect their quality. Some objectives can be equipped with a spring-loaded assembly that protects the front lens elements and the specimen from collision damage. With the correct conditions of usage and storage, the performances of the microscopy objective can be maintained for a very long period as it has no wearing parts.

CONCLUSION

The main microscope objective specifications have been introduced in this article. That list is not exhaustive and other specifications can be used to characterize other significant features in link with the application. The microscope objective, as a fundamental component in the laboratory, is an optical component that demands continuous R&D efforts, motivated by the development of new imaging and microscopy techniques as well as the specific end-users' requirements.

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[2] https://www.edmundoptics.fr/knowledge-center/application-notes/microscopy/understanding-microscopes-and-objectives

[3] Zhang, Yueqian & Gross, Herbert, Advanced Optical Technologies 8 (2019), 10.1515/aot-2019-0002.

[4] https://www.microscopyu.com/microscopy-basics/infinity-optical-systems

[5] https://application.wiley-vch.de/Microscopy_for_Dummies/

For further pieces of information about the microscope objectives and the microscopy techniques, please consult the educational resources made by the manufacturers:

https://www.leica-microsystems.com/science-lab/science-lab-home/ | Leica

https://www.microscopyu.com/ | Nikon

https://www.olympus-lifescience.com/en/microscope-resource/ | Olympus

http://zeiss-campus.magnet.fsu.edu/ | Zeiss

MANUFACTURER	APPLICATION / PRODUCT	DEALER	CONTACT
Nikon	Life Sciences (LS)	Nikon Healthcare	https://www.nikon.com/products/microscope-solutions/inquiries/index.htm
	Materials Science (MS)	Nikon Metrology	https://www.nikonmetrology.com/en-gb/contact-us/find-a-sales-or-distributor
	Industrial Microscopy (IM)		
Olympus	Life Sciences (LS)	Olympus Life Science	https://www.olympus-lifescience.com/en/contact-us/
	Materials Science (MS)	Olympus IMS	
	Industrial Microscopy (IM)		
Leica	Life Sciences (LS)	Leica Microsystems	https://www.leica-microsystems.com/contact/contact-us-online/
	Materials Science (MS)		
	Industrial Microscopy (IM)		
Zeiss	Life Sciences (LS)	Zeiss Industrial Quality & Research	https://www.zeiss.com/microscopy/int/service-support/microscopy-contact.html
	Materials Science (MS)		
	Industrial Microscopy (IM)		
Mitutoyo	Industrial Microscopy	Mitutoyo Europe GmbH	https://mitutoyo.eu/en_us/contact
Thorlabs	Microscope objectives (LS, MS and IM), reflective objectives, UV, visible & NIR focusing objectives.		https://www.thorlabs.com/locations.cfm
Edmund Optics	Microscope objectives (LS, MS and IM), reflective objectives, UV, visible & NIR focusing objectives.		https://www.edmundoptics.com/contact-support/
OptoSigma	Microscope objectives (LS, MS and IM), reflective objectives, UV, visible & NIR focusing objective.		https://www.optosigma.com/eu_en/contact-us?___from_store=us_en
Jenoptik	Customized and Standardized Objective Lenses (LS, MS and IM)		https://www.jenoptik.com/contact