

Microscopy from Carl Zeiss

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## **Objectives from Carl Zeiss** **Exceeding Your Expectations**



**Brilliant Imaging for Research  
and Routine Work in Life Sciences**



We make it visible.

# When Your Research Pushes the Boundaries of What Is Visible, Only Performance Counts.

Maximum image information for the best possible result: in order to acquire meaningful images, choosing the right objective is a crucial criterion for success. To ensure reliable analysis, this applies to routine tasks as well as demanding high-end applications. The requirements of users always have one commonality – to achieve maximum resolution with extremely high contrast. Modern research demands the highest standard of optical performance from objectives – particularly in complex applications in which structural information has to be imaged with optimum quality. For over 130 years, objectives have been developed at Carl Zeiss according to scientific calculations which, consistently have set the standards in their class. Carl Zeiss has often redefined the boundaries of the technology in this area:

- First ever calculation of microscope objectives by Ernst Abbe
- Coating glass surfaces to minimize stray light
- Infinity Color Corrected System (ICS) optics
- Stray-light-minimized IC<sup>2</sup>S optics with improved contrast

At Carl Zeiss, together with you, experienced application experts define the criteria that are relevant to you. Starting from objective magnification and working distance to the selection of possible contrast techniques, numerous factors need to be taken into consideration. You will receive comprehensive support when selecting the objective that is right for your individual range of applications. In this way, you can always be sure of one thing: contrast-rich, brilliant images showing as much specimen detail as possible.



## 1872

*Introduction of the first calculated microscope objectives by Prof. Ernst Abbe*

## 1886

*Development of fully color-corrected objectives – the APOCHROMATS*

## 1911

*Development of parfocal objectives which retain the focus position when the objective is changed*

## 1936

*Patenting of anti-reflection coatings on lens surfaces (T-coating)*

## 1876

*First oil-immersion objectives*

## 1904

*Discovery of fluorescence microscopy by Prof. August Köhler*

## 1934

*First test version of objectives for phase contrast*

## 1938

*Introduction of objectives with flatness of field – the Plan-ACHROMATS*



## **Considerations when selecting an objective:**

### **Magnification**

*Image scale of the objective in the real intermediate image plane*

### **Numerical aperture (NA)**

*Definition of the resolving power of an objective and the light intensity*

### **Free working distance**

*Distance between the front lens of an objective and the cover glass or specimen*

### **Flatness of field**

*Correction of field curvature to avoid blurred edges*

### **Color correction**

*Imaging of different colors of the light spectrum in one point*

### **Transmission**

*Light transmission of an objective for certain wavelengths*

### **Suitability for certain contrast techniques, e.g.**

*Brightfield*

*Darkfield*

*Phase contrast (Ph)*

*Differential Interference Contrast (DIC)*

*VAREL contrast*

*PlasDIC*

*Polarization (Pol)*

*Fluorescence*

### **1950**

*Introduction of objectives with specimen protection (objectives with spring system)*

### **1973**

*Introduction of infinity optics with the modular Axiomat microscope*

### **1982**

*Introduction of ICS optics*

### **1959**

*Development of first ULTRAFLUAR objectives with focus correction from ultraviolet through to the infrared range*

### **1975**

*Development of multi-immersion objectives Plan-NEOFLUAR (Imm. Corr.)*

### **2004**

*Introduction of ICS optics*

	A-Plan	LD A-Plan	ACHROPLAN	WACHROPLAN	FLUAR & ULTRAFUAR	EC Plan-NEOFLUAR	LD Plan-NEOFLUAR	LCI Plan-NEOFLUAR	LD LCI Plan-APOCHROMAT	Plan-APOCHROMAT	W Plan-APOCHROMAT	(LD) C-APOCHROMAT
Specimen with cover glass 0.17 mm ± 0.01	●	●	●	–	●	●	●	●	●	●	–	●
Specimen with cover glass 0.14 mm to 0.20 mm	up to NA 0.7	●	up to NA 0.7	–	up to NA 0.7	up to NA 0.7	●	●	●	up to NA 0.7	–	●
Specimen without cover glass	up to NA 0.3	up to NA 0.3	up to NA 0.3	●	up to NA 0.3	up to NA 0.3	●	up to NA 0.8	up to NA 0.8	up to NA 0.3	●	–
Culture plates with glass bottom 0.17 mm ± 0.01	●	●	●	–	●	●	●	●	●	●	–	●
Culture plate with glass bottom 0.14 mm to 0.20 mm	up to NA 0.7	●	up to NA 0.7	–	up to NA 0.7	up to NA 0.7	●	●	●	up to NA 0.7	–	●
Culture plate with plastic bottom	–	●	–	–	–	–	●	–	–	–	–	–
Open culture plate – objective is immersed in the culture medium	–	–	–	●	–	–	–	○ up to NA 0.8	○ up to NA 0.9	–	●	–
Multiwell culture plates with glass bottom 0.17 mm ± 0.01	●	●	●	–	●	●	●	●	●	●	–	●
Multiwell culture plates with glass bottom 0.14 mm to 0.20 mm	up to NA 0.7	●	up to NA 0.7	–	up to NA 0.7	up to NA 0.7	●	●	●	up to NA 0.7	–	●
Multiwell culture plates with plastic bottom	–	●	–	–	–	–	●	–	–	–	–	–
Field of view	23 mm		23 mm	23 mm	23 mm	25 mm	25 mm	25 mm	25 mm	25 mm	20 mm	25 mm
Flatness	**	**	**	**	*	****	****	****	****	****	****	****
Color correction	**	**	***	***	*	****	****	****	****	****	****	****
Working distance		very long		long			very long		long		long	
High transmission in UV	**	**	****	****	****	****	***	***	***	***	***	**
Transmission in IR	****	****	****	****	****	***	***	***	***	***	***	***
Correction for 37°C for Live Cell Imaging	–	–	–	–	–	–	–	yes	yes	–	–	yes
Classic stains e.g. HE	●	○	●	–	○	●	○	▲	●	●	–	●
Fluorescence	▲ VIS	▲ VIS	▲ VIS	●	●	●	●	●	●	●	●	●
3D Deconvolution	○	○	○	▲	▲	●	▲	●	●	●	●	●
ApoTome	–	–	–	–	○ VIS	●	○	●	●	●	○	●
Cell Observer	○	○	○	○	● UV	▲	○	●	●	●	○	●
TIRF	–	–	–	–	● *	–	–	–	–	● *	–	–
Laser Scanning Microscopy	–	–	○	▲	▲	●	–	●	●	●	●	●
FCS	–	–	–	–	–	–	–	–	–	–	–	● *

- Particularly well suited
- ▲ Well suited
- Possible, but not recommended
- Not possible
- \* Variant or special version



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## 1950

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## 1959

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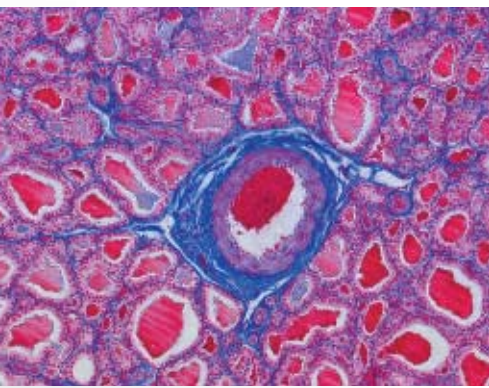
## 1975

*Development of multi-immersion objectives Plan-NEOFLUAR (Imm. Korr.)*

## 2004

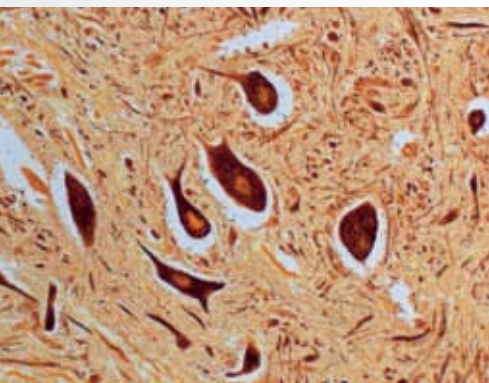
*Introduction of IC'S optics*

# Many Requirements Demand Many Objective Types. Each is in a Class of Its Own.



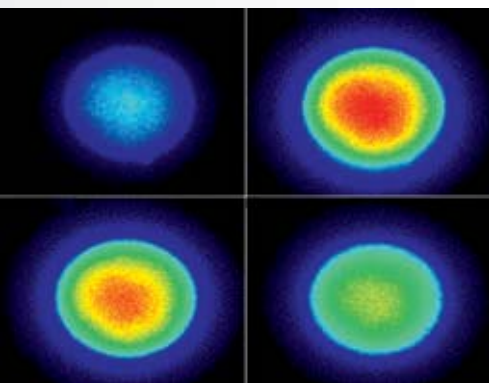
## **A-Plan - the A class**

A-Plan objectives from Carl Zeiss offer sound and reasonably priced entry into the world of microscopy. They are versatile in their use and deliver good optical quality.



## **ACHROPLAN - the solid performers**

Solid and reliable: the objectives of the ACHROPLAN class stand out through their excellent flatness of field. They are a highly recommendable solution for image documentation in pathology.



## **FLUAR - the photon collectors**

The objectives of the FLUAR series are manufactured from special optical glasses. High numerical apertures, good contrast and very high transmission for the entire visible spectrum to the near UV result in great optical performance. The objectives of choice for making the weakest fluorescence signals visible.



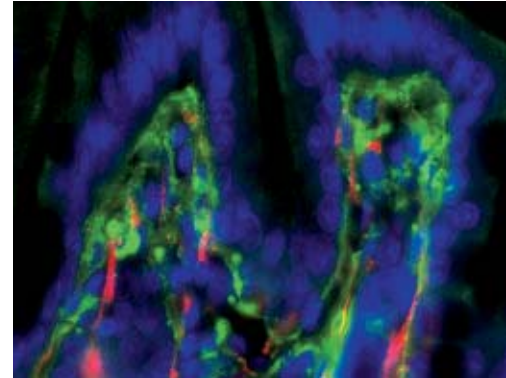


# Overview



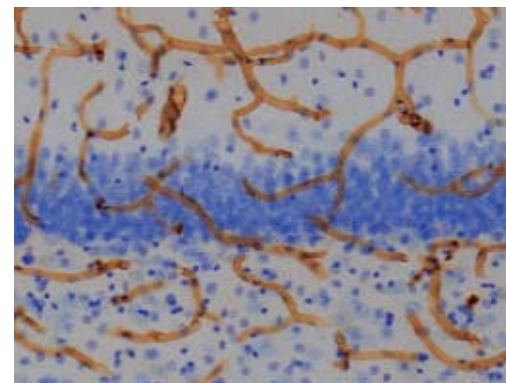
## **EC Plan-NEOFLUAR - the all-round performers**

Where flexibility and multiple imaging methods are required, the EC Plan-NEOFLUAR objectives are often selected. The optimized IC<sup>2</sup>S optics makes it possible to achieve contrast-rich imaging with excellent homogeneity and high resolution. From transmission to the near UV, outstanding flatness of field and achromatic correction, to high numerical apertures, the EC Plan-NEOFLUAR class meets the high demands of applications using brightfield, darkfield, phase contrast, DIC, polarization and fluorescence.



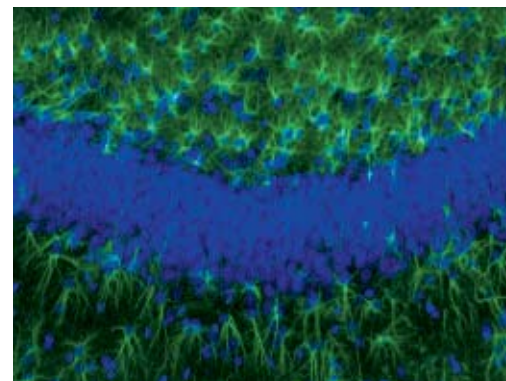
## **Plan-APOCHROMAT - the precision performers**

With the best color correction and highest numerical apertures, Plan-APOCHROMAT objectives deliver brilliant images in brightfield, DIC and fluorescence techniques. Their outstanding point spread function and extreme chromatic correction are particularly impressive. High resolution and excellent image sharpness make even the finest details and color nuances visible.



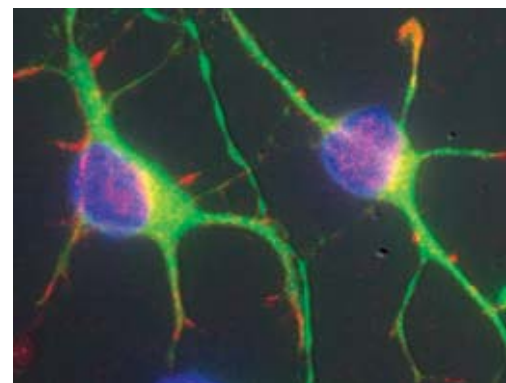
## **C-APOCHROMAT - the top performers**

These high-performance objectives are able to compensate optically for different refractive indices and layer thicknesses of the mounting medium by means of a correction collar. They are perfectly suited to extremely demanding applications in research of living organisms and immersion specimens. For brilliant images in all applications and 3D techniques such as confocal Laser Scanning Microscopy, ApoTome and 3D Deconvolution.

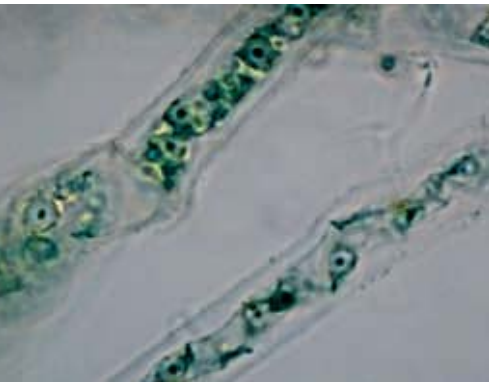


## **LCI - the immersion specialists**

LCI stands for Live Cell Imaging. The high-performance objectives of this class have been specifically developed for complex applications involving living cells and tissues. They are calculated for temperature intervals of 23° C to 37° C. Spherical aberrations caused by deviating cover slip thicknesses, different temperatures or refractive indices are ideally compensated by use of correction collar. Therefore, more visible details and reliable results for your scientific analyses are possible.

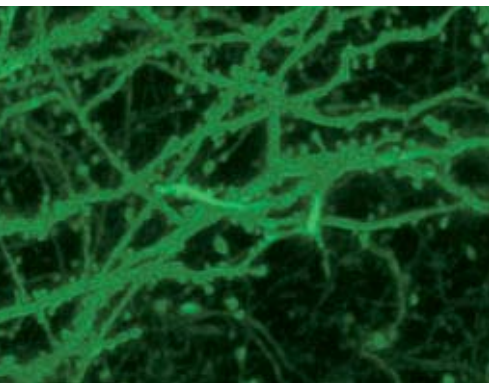


# Objectives for special applications



## **LD - the flexible performers**

Special applications require special objectives. Long distance objectives are used if, for example, you need to focus deep into a thick specimen or through the plastic bottom of cell culture plates. LD objectives are as varied as the tasks they perform: with LD A-Plan and LD Plan-NEOFLUAR objectives for inverted microscopes it is possible to examine cells in plastic culture plates and specimens under a standard cover glass. LD variants of the LCI and C-APOCHROMAT series have been developed for applications involving living cells: using a correction collar, deviations in cover glass thicknesses, for example, can be compensated optically.



## **W - for immersion**

Physiological applications involving living cells and/or tissues often require water objectives which can be dipped directly into the culture medium. W objectives have a conical tip made from special inert plastic. They are often, but not only used in combination with a fixed-stage microscope. These objectives are distinguished through their outstanding optical performance with good flatness of field and high transmission for perfect results in physiology.





# Color Coding of Objectives

## Labeling of the objective

Objective class, special designations are used for this, e.g.

LD for Long Working Distance

## Magnification/numerical aperture

plus additional details on

- immersion medium (Oil/W/Glyc)
- adjustable cover glass correction (Korr.)
- contrast method

## Tube length/cover glass thickness (mm)

ICS optics:  $\infty$

Infinity Color Corrected System

standard cover glass: 0.17

without cover glass: 0

insensitive: -

## Mechanical correction collar for

- cover glass thickness correction
- different immersion
- different temperature
- adjusting an iris diaphragm

## Color of writing

Contrast method

Standard 

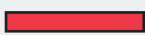
Pol/DIC 

Ph 0 1 2 3 

## Color coding of magnification


1.0/1.25 

2.5 

4/5 

6.3 

10 

16/20/25/32 

40/50 

63 


100/150 

## Immersion fluid

Oil 

Water 

Glycerin 

Oil / Water / Glycerin 

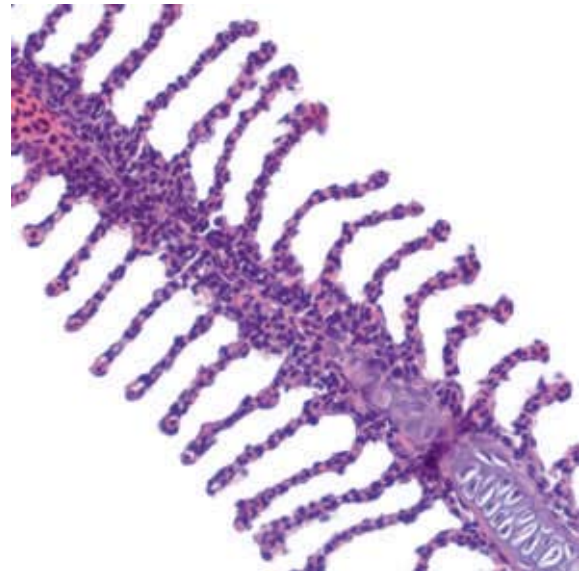


## Every Objective Meets Just One Demand: Perfection Down to the Last Detail



### **A-Plan: good entry-level product with excellent performance**

From laboratory and routine microscopy through to the research class, A-Plan objectives are the right entry-level choice. They are suitable for brightfield and phase contrast and deliver good contrast. A-Plan objectives can also be used in fluorescence applications with excitation wavelengths in the visible spectral range.



- Field of view: 23 mm
- Flatness: ★
- Color correction: ★

Entry-level objectives for laboratory, routine and research microscopes



### **LD A-Plan: versatile in inverse microscopy**

This entry-level line for inverted microscopy is economical, flexible and rich in contrast. These objectives have a particularly long working distance that makes it possible to carry out observations through thicker cell culture vessels. They are corrected for the use of cover glasses and vessel bottom thicknesses of up to 2 mm. From brightfield, phase contrast, VAREL to Hoffman Modulation Contrast and PlasDIC, these objectives can be used in a wide range of contrast methods for unstained cells and tissue. Suitable for excitation wavelengths within the visible spectral range, just like the A-Plan objectives these can also be used in fluorescence microscopy. A high-performance entry-level series for sophisticated microscopy.



- Field of view: 23 mm
- Flatness: ★★
- Color correction: ★★

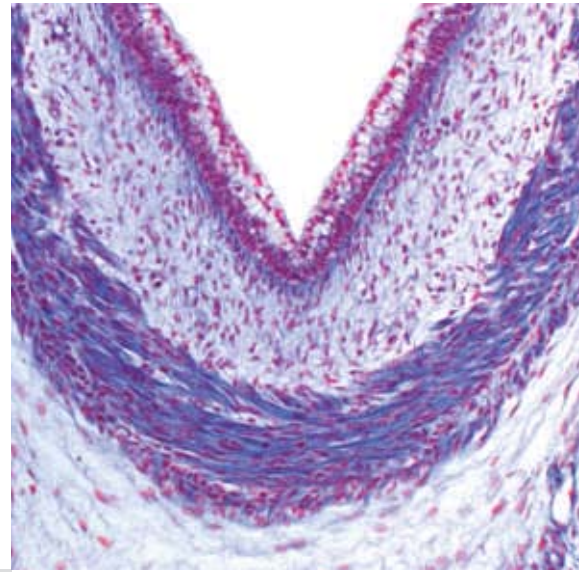
Objectives with long working distance for inverted microscopes; attachable cover glass cap for thinner cover glasses measuring 0.17 to 0.6 mm

# ACHROPLAN



## **ACHROPLAN: versatility like no other**

The ACHROPLAN objectives have good flatness of field and color correction and are well-suited for microphotography. In accordance with the wide range of applications, ACHROPLAN objectives are available in various versions. The possible contrast techniques that can be used are brightfield and phase contrast in transmitted light and fluorescence with excitation in the visible range.



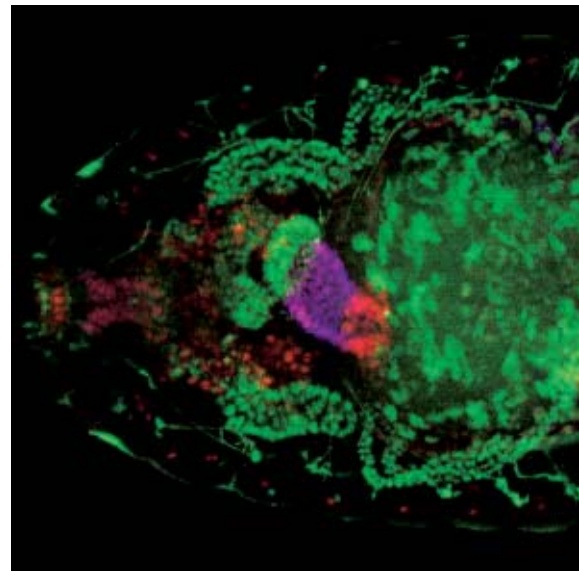
- Field of view: 23 mm
- Flatness: ★★
- Color correction: ★★★

Objectives in many variations for diverse applications



## **W ACHROPLAN: dive to physiological depths**

The water objectives of the ACHROPLAN series are primarily used in connection with an upright fixed-stage microscope in the area of electrophysiology. Such set-ups make it possible to dip into a medium using the immersion objective and to examine a specimen from above. Here, microscopic techniques are combined with physiological methods. Typical areas of use include the patch-clamp technique and intracellular recording in electrophysiology, intravital microscopy as well as the examination of microcirculation and of thick specimens when working with vital brain sections. Thanks to the slender tip and the long working distance, electrodes and microinjection capillaries can be brought to the specimen without any problems. Objectives belonging to the W ACHROPLAN class are impressively flexible. All contrast techniques are possible, including fluorescence and infrared DIC. The optical performance and exceedingly high transmission are particularly outstanding features for visibly more information in physiological applications.



- Field of view: 23 mm
- Flatness: ★★
- Color correction: ★★★

Objectives for immersing directly into cell culture media, specifically for physiological applications

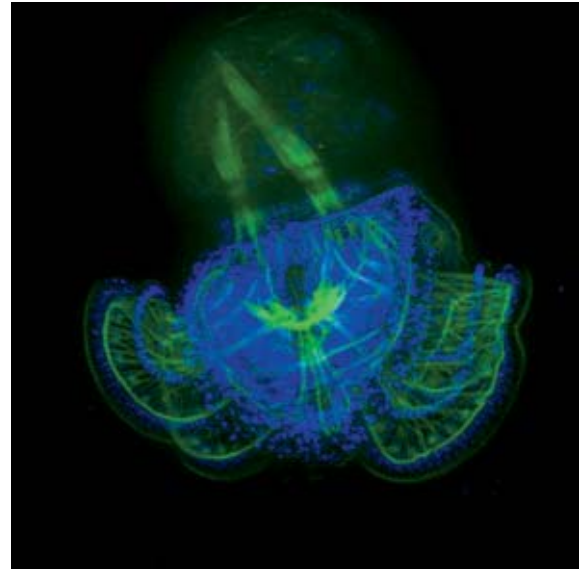


# FLUAR



## **FLUAR: detects very weak fluorescence signals**

The FLUAR objectives stand for maximum light transmission and photon collection. Manufactured from special glass, these objectives have been developed specifically for qualitative and quantitative analyses of ion modifications and for demanding fluorescence applications. Good flatness of field up to 23 mm, high numerical apertures and very high transmission from a wavelength of 340 nm – making even the weakest signals clearly visible.



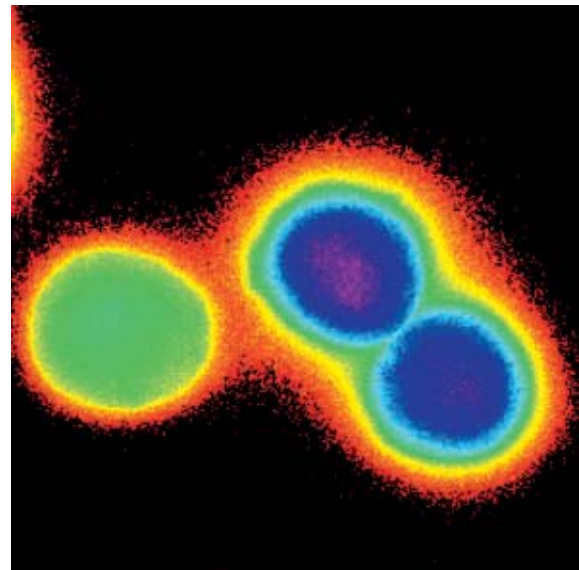
- Field of view: 23 mm
- Flatness: ★
- Color correction: ★

Objectives with high numerical apertures and extremely high transmission properties from 340 nm



## **ULTRAFLUAR: ultra-effective in UV light**

ULTRAFLUAR for ultraviolet light – with ULTRAFLUAR objectives, it is possible to carry out applications using fluorescence excitation in the UV wavelength range. Only quartz glasses are used in their manufacture. These objectives demonstrate outstanding transmission from 240 nm to the infrared range. Consequently, they cover the widest spectral range and have good flatness of field up to 20 mm. With these objectives you will always obtain a reliable result, even in applications with excitation light in the UV range.



- Field of view: 20 mm
- Flatness: ★
- Color correction: ★

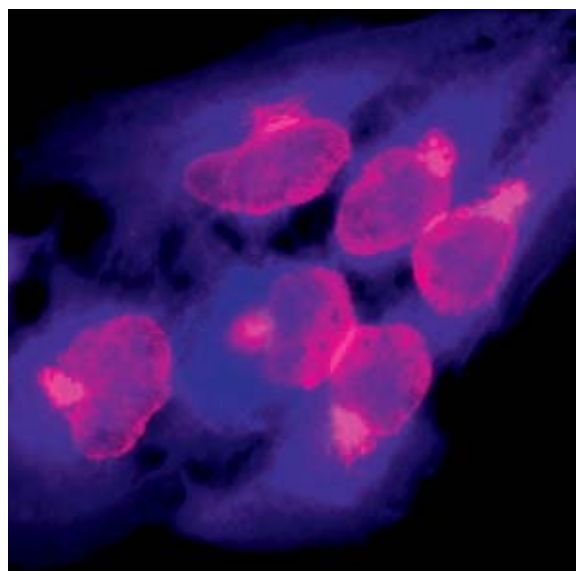
Objective for fluorescence excitation in the UV range from 240 nm

# EC Plan-NEOFLUAR



## EC Plan-NEOFLUAR: excellent contrast

EC stands for Enhanced Contrast. In combination with the chromatic correction and high resolving power, these universal objectives deliver brilliant images that are rich in contrast, while retaining excellent flatness of field. Glass with low intrinsic fluorescence is used in their manufacture, which, in addition to their high transmission from the near UV range, virtually predestines the EC Plan-NEOFLUAR class for fluorescence applications. Special objectives in this class include EC Plan-NEOFLUAR Antiflex for reflection contrast and EC Plan-NEOFLUAR Pol for polarization.



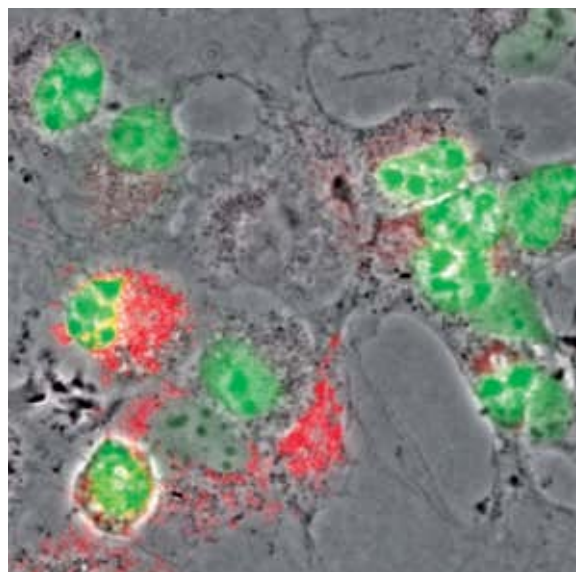
- Field of view: 25 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Universal objectives with excellent properties for fluorescence microscopy



## LD Plan-NEOFLUAR: go the distance

LD Plan-NEOFLUAR objectives with an extra long working distance are objectives designed for cell culture. These objectives are used on the inverted research platforms such as Axiovert and Axio Observer. With a correction collar, the objectives can be adapted seamlessly to various optical conditions, e.g. the use of conventional cover glasses or plastic culture plates in the 0 to 1.5 mm range. Due to the outstanding fluorescence properties of all EC Plan-NEOFLUAR objectives, the LD variants are also ideally suited for fluorescence microscopy. In addition, all current contrast techniques in transmitted light, such as brightfield, phase contrast, DIC and PlasDIC, are also possible. For brilliant, high-contrast and meaningful images even at long working distances.



- Field of view: 23 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Objectives with long working distances for inverted research microscopy, very well-suited to fluorescence applications

# Plan-APOCHROMAT

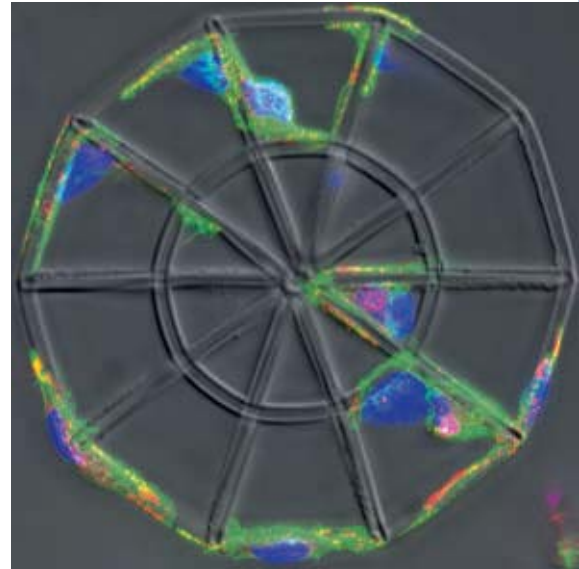


## Plan-APOCHROMAT: protects sensitive samples

Plan-APOCHROMAT objectives demonstrate top-class optical performance. They make it possible to see structures at the boundary of what is visible. Their outstanding performance features include: excellent correction, extremely high apertures and maximum resolution, color purity, contrast and flatness of field. All this combines to produce brilliant, needle-sharp images for observation, digital documentation and, in particular, fluorescence applications. The i Plan-APOCHROMAT of the 63x objective has been developed specifically for Live Cell Imaging – for optimal focus stability for time-lapse experiments.

- Field of view: 25 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Objectives with optimum correction of flatness of field and color; suitable for Digital Imaging



*Image courtesy of Martin Bastmeyer und Franziska Klein, University of Karlsruhe, Germany*

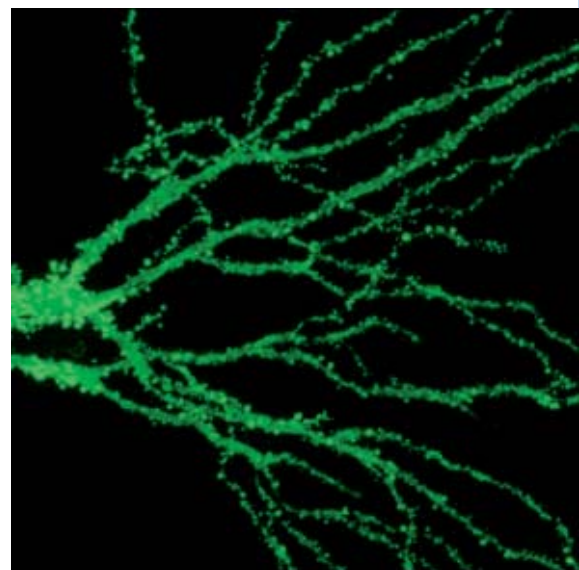


## W Plan-APOCHROMAT: apochromatically correct

The immersion variant of the Plan-APOCHROMAT series – an addition to the water objectives of the ACHROPLAN class – has been specifically designed for electrophysiology. W Plan-APOCHROMAT objectives have apochromatic correction from visible light to the near infrared (VIS - IR) and are intended for use without a cover glass. Typical transmission values are greater than 80% from 450 nm to 1,000 nm and greater than 50% at 365 nm. These are also ideal prerequisites for use in 2-photon microscopy. The front of this slender objective is made of a special inert plastic that was originally developed for food technology.

- Field of view: 20 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Apochromatically corrected immersion objectives for applications in physiology





## Resolution

The resolution of an optical system is generally defined as the smallest distance between two object structures at which these objects are still imaged separately or perceived as being separate. Due to the wave nature of light and the diffraction associated with this, the resolution of an objective is limited. This limit is theoretical, i.e. even a theoretically ideal objective without any imaging errors has a finite resolution.

Resolution can be calculated according to the famous formula introduced by Ernst Abbe and represents a measure of the image sharpness of a light microscope:

$$d = \frac{\lambda}{2n \sin \alpha}$$

$\lambda$  = wavelength of the light used (effective wavelength of white light: 550 nm)

$n$  = refractive index of the optical medium between the front lens and cover glass (air = 1; H<sub>2</sub>O = 1.33; immersion oil = 1.518)

$\alpha$  = half the opening angle of the objective used

It becomes apparent from Abbe's formula that resolution is determined by the wavelength of the light used ( $\lambda$ ), as well as by the product of the refractive index ( $n$ ) of the medium between the cover glass and front lens and the sine of half the opening angle ( $\alpha$ ) of the objective used. Due to the central significance of this interrelationship for imaging in microscopy, Abbe introduced the concept of numerical aperture.

## Numerical aperture

Microscopic images are generated through the interaction (interference) of the light diffracted at the sample with the uninfluenced light that penetrates the sample. The interference of these light components leads to an intermediate image that already contains all the image information. This intermediate image is magnified in the microscope by the eyepiece. This is, therefore, also referred to as two-stage imaging process.

The larger the opening angle of an objective, the more (diffracted) light can be gathered from the sample and the higher the resolution of the resulting image.

This fundamental correlation was identified for the first time by Ernst Abbe at Carl Zeiss in 1872. He introduced the concept of the numerical aperture (NA) of an objective. This is defined as the product of the refractive index between the cover glass and front lens of the objective and the sine of half the opening angle of the objective:

$$NA = n \cdot \sin(\alpha)$$

The numerical aperture is a measure of the size of the cone of light captured by the objective, taking the immersion medium used into consideration.

Resolution values are given in the table below for a number of typical objectives with different apertures. In practice, however, these calculated resolutions are only achieved as long as the imaging system does not show any imaging errors, i.e. if the imaging is diffraction-limited.

Resolution table using green light with  
 $\lambda = 0.550 \mu\text{m}$ :

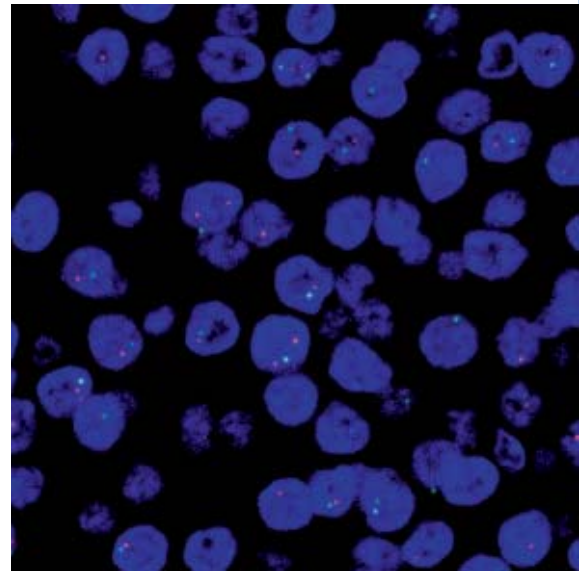
Magnification	/	NA	Resolution ( $\mu\text{m}$ )
10x	/	0.30	1.10
40x	/	0.75	0.45
63x	/	1.40 Oil	0.24
100x	/	1.30 Oil	0.26

# C-APOCHROMAT



## **C-APOCHROMAT: top performance in 3D**

The high-quality and extremely powerful objectives of the C-APOCHROMAT class are perfect for studying living cells. They are optimally corrected in terms of flatness and color and meet even the highest requirements in 3D microscopy using confocal LSM, ApoTome Structure Illumination or 3D Deconvolution. The specimens used here are often in a watery medium that has a similar refractive index to water. With the C-APOCHROMAT objectives it is possible to compensate for spherical aberrations, which frequently occur if the refractive indices of the immersion and mounting medium are different, by means of a correction collar. Furthermore, the correction collar also allows very small deviations in the thickness of the cover glass and different temperatures to be compensated for. Optimum performance parameters provide the best possible results.



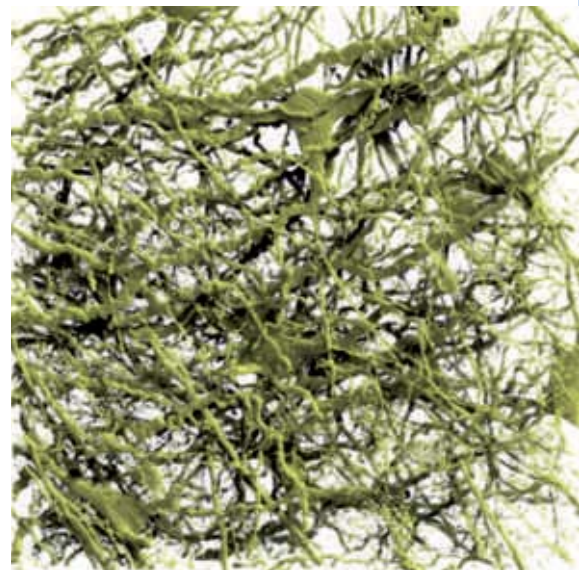
- Field of view: 25 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Water immersion objectives with very high numerical apertures for 3D microscopy; optimum correction and high transmission



## **LD C-APOCHROMAT: developed for multiphoton microscopy**

The LD C-APOCHROMAT objective has a long working distance while retaining a high numerical aperture of 1.1. This expert technology is particularly suited to confocal multiphoton microscopy in which extremely high penetration depths are achieved through the use of infrared excitation light. However, it is also possible to achieve excellent results with this objective using other 3D techniques such as ApoTome Structured Illumination and 3D Deconvolution.



- Field of view: 25 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Objective with long working distance optimized for multiphoton microscopy

## Imaging properties

Glass lenses that are used in a light microscope fundamentally show imaging errors. These imaging errors can be reduced to a practically insignificant level by optical design measures.

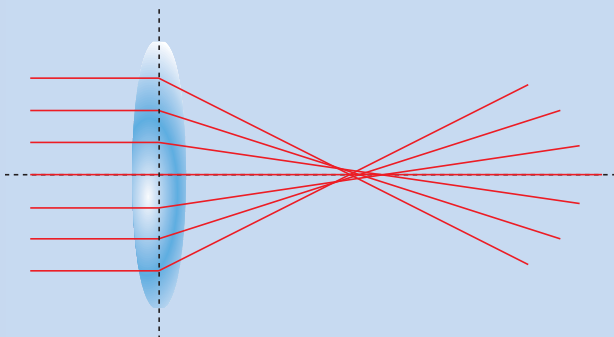
In general, imaging errors (aberrations) are understood to mean deviations from the ideal diffraction-limited imaging.

Aberrations may affect microscopic images in different ways, e.g. through reduced contrast, poor resolution or geometric distortions. Two types of aberrations well known in microscopic systems are spherical and color aberrations.

## Spherical aberrations

Spherical aberrations, also known as aperture errors, can significantly impair the imaging quality of a microscope objective.

Spherical aberrations occur because the focal distance of an individual lens depends on the distance of the incident light beams from the center of the lens. The focal distance of the lens is shorter for light beams at the edge than for beams near to the optical axis. Consequently, there is no single focal point but a focal line along the optical axis. This leads to a reduction in imaging contrast and sharpness (see diagram). High-magnification dry objectives with high numerical apertures are particularly sensitive to this type of image error.



In principle, it is possible to correct this error in the objective. This correction can be carried out on a fixed or an adjustable basis, as in the case of objectives with a correction collar.

Spherical aberrations are not only influenced by the optical properties of an objective but also by the properties of the cover glass and mounting medium. That is why, in the case of high-magnification objectives with high apertures, the cover glass is viewed as a component of the optical system and a standard thickness of 0.17 mm is required.

In practice, there are mainly two factors that considerably intensify spherical aberration:

1. The difference in the refractive indices of the immersion medium and mounting medium. The more the refractive index of the immersion medium deviates from the refractive index of the mounting medium, the more marked the spherical aberration. An objective that, for example, is calculated for oil immersion ( $n = 1.52$ ) therefore demonstrates a considerable spherical aberration if the specimen structures are imaged in a watery solution ( $n = 1.33$ ).
2. The distance between the cover glass and the specimen structure to be examined. In general, spherical aberration intensifies as the sample depth increases. Ideally, therefore, the specimen should be positioned directly under the cover glass.

In the case of objectives that do not have a correction collar, attention should therefore be paid to observing the recommended cover glass thickness and adjusting the refractive index of the mounting medium to the refractive index of the objective immersion. Otherwise, the result is an image with poor contrast and bad resolution.

In the case of objectives that do have a correction collar it is possible to compensate for deviating values in the cover glass thickness and differences in the refractive indices of the objective immersion and mounting medium. In addition, the increasing spherical aberration that occurs when imaging deep-lying structures in thick samples can also be corrected.

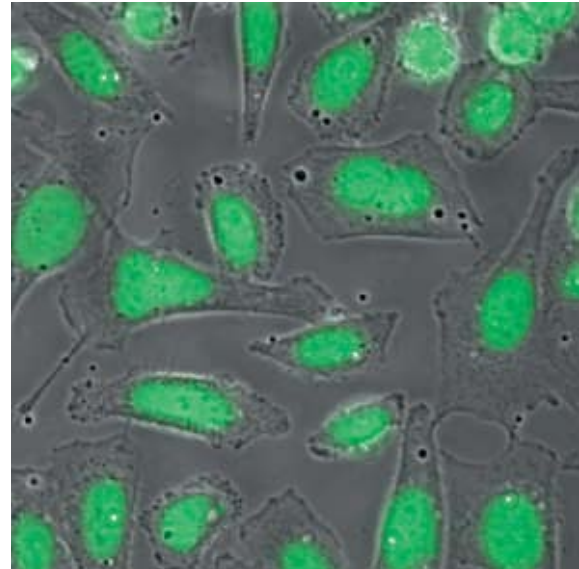


# Live Cell Imaging



## LCI Plan-NEOFLUAR: flexible with immersion

Whether it's with water, glycerin or oil – with their high numerical apertures and, therefore, optimal resolution, the LCI Plan-NEOFLUAR objectives are used for Live Cell Imaging (LCI) – with and without cover glass. The objectives of this series can always be flexibly adapted to the refractive index of the culture or mounting medium. This allows the ideal matching of the immersion fluid to the specimen's embedding media, thus eliminating spherical aberration caused by refractive index mismatch. In addition, the LCI Plan-NEOFLUAR range also allows living cells to be observed in physiological conditions of 37° C – under optimum optical conditions. With just one correction collar, immersion, cover glass thickness and temperature can all be set appropriately for brilliant insights into the dynamic processes of living organisms. The version i LCI Plan-NEOFLUAR with isolation collar is especially suited for incubation.



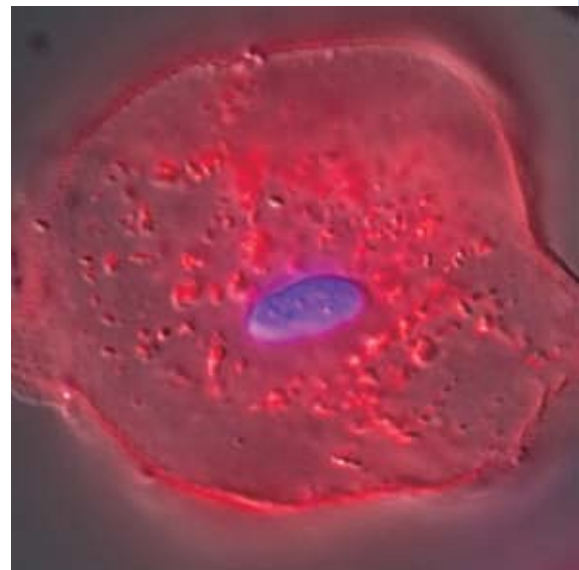
- Field of view: 25 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Flexible objective for Live Cell Imaging with and without cover glass for oil, glycerin or water immersion



## LD LCI Plan-APOCHROMAT: multi-tasking for Live Cell Imaging

This objective has been developed to meet the very highest requirements in Live Cell Imaging. Besides the performance features of all LCI Objectives already mentioned, LD LCI Plan-APOCHROMAT offers an extremely long working distance for this objective class of 0.57 mm. This working distance makes it possible to focus through thick specimens, e.g. through a brain section or a whole mount embryo. Color correction and flatness of field are identical to the Plan-APOCHROMAT series and represent the maximum standard of performance – despite the long working distance. Maximum quality for maximum reliability in scientific analysis.



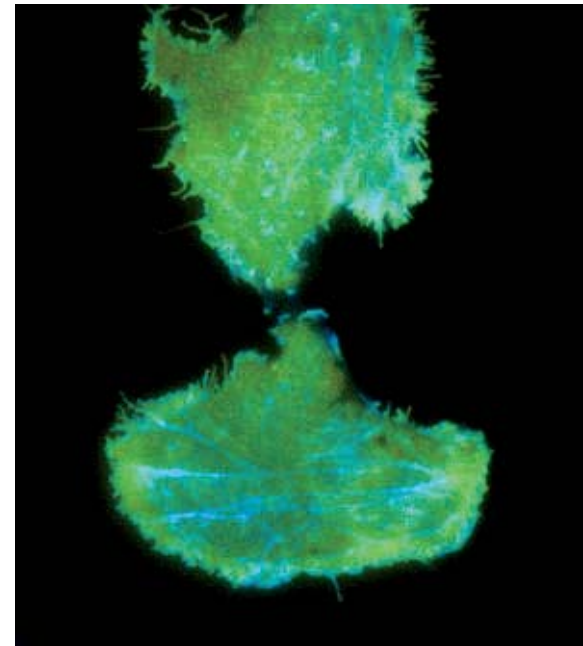
- Field of view: 25 mm
- Flatness: ★★★★★
- Color correction: ★★★★★

Flexible multi-immersion objective with long working distance for Live Cell Imaging



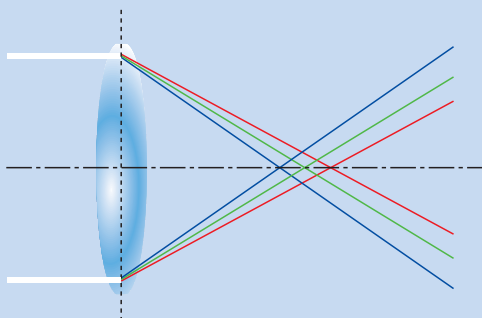
**α Plan-FLUAR 100x and  
α Plan-APOCHROMAT 100x:  
make cell membranes visible**

The special fluorescence technique TIRF (Total Internal Reflection Fluorescence) calls for objectives with particular properties. In this method fluorescence molecules are excited in a thin layer directly at the surface of the cover glass. Molecular mechanisms around the cell membrane, e.g. transport processes, are made visible – at layer thicknesses below 200 nm. In addition to high contrast and high resolution, an appropriate objective must also have a high numerical aperture of at least 1.45. Carl Zeiss has developed both α Plan-FLUAR 100x with a numerical aperture of 1.45 and α Plan-APOCHROMAT 100x with a numerical aperture of 1.46 for TIRF. Both objectives have excellent transmission properties from 340 nm and, with their extremely high numerical aperture, are also ideally suited to conventional fluorescence techniques with maximum resolution.



## Chromatic aberration

Single lenses have different focal distances for different wavelengths, i.e. different colors of light. This phenomenon is known as dispersion. Chromatic aberration shows itself in the form of narrow reddish or greenish color fringes around specimen structures.



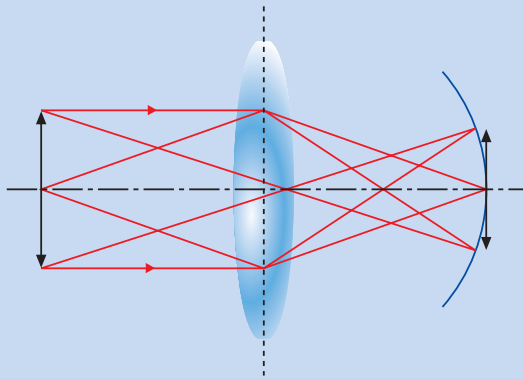
This color error can be almost completely rectified by making an appropriate choice of types of glass with various dispersion values. Chromatic aberration has

practical significance because increasing the aperture of an objective improves the sharpness of the imaged object but also magnifies color errors of the optical system. High resolution microscope objectives therefore place extremely high demands on the elimination of color errors. Depending on the degree of correction, a distinction is made between, in order of increasing color error elimination, ACHROMATS, fluorite objectives and APOCHROMATS.

At Carl Zeiss, APOCHROMATS are fully color-corrected for up to 7 wavelengths from UV through to IR. APOCHROMAT objectives are virtually free of any traces of color fringes. They were calculated for the first time at Carl Zeiss by Ernst Abbe in 1886. The correction of chromatic aberration is determined through the choice of the type of objective used and can scarcely be influenced in practice. Simply using the wrong immersion medium (e.g. anisol rather than immersion oil) leads to color fringes becoming more perceptible.

## Field curvature

The effect of field curvature means that a flat structure is imaged on a curved surface.



This image error can be completely rectified by making a suitable choice of the lens curvatures in the objective. Objectives with a flattened field of view contain the word 'Plan' in their name. Objectives with a completely flat field of view were invented at Carl Zeiss in 1938 by Hans Boegehold. Depending on the color correction, the following Plan objectives are available: Plan-ACHROMAT, Plan-Fluorite and Plan-APOCHROMAT.

In practice, field curvature is particularly disruptive in the case of flat specimens such as blood smears or histological sections. Modern objectives of the Plan-NEOFLUAR and Plan-APOCHROMAT class are fully flattened up to a field of view of at least 25 mm.

## Cover glasses and mounting media

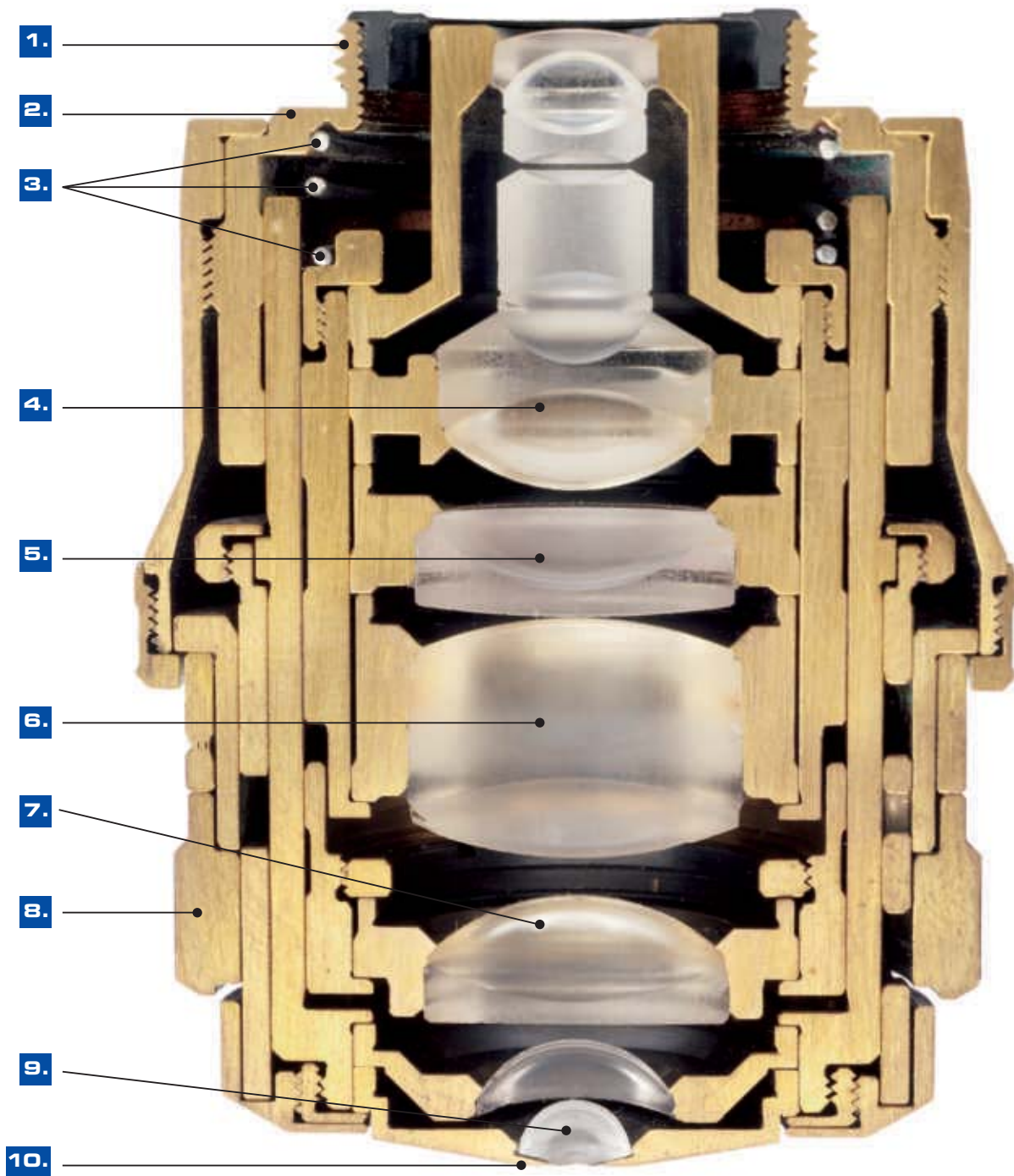
Cover glasses have a decisive influence on the imaging quality of a microscope as they form an optical component of the objective. Many objectives are calculated for a cover glass thickness of 0.17 mm exactly. For special purposes, e.g. smears, there are also objectives for uncovered objects (cover glass thickness = 0). If, however, the thickness of the cover glasses used deviates from the calculated value, the result is a clearly perceptible deterioration of the image caused by spherical aberration. It should also be taken into consideration that the thickness of the mounting medium also has an impact on the effective cover glass thickness. In practice, a deviating effective cover glass thickness becomes noticeable above a numerical aperture of 0.35. Above a numerical aperture of 0.7, even extremely small deviations ( $\pm 0.01$  mm) from the specified cover glass thickness have a significant effect on the image. For this reason, many high-aperture objectives are equipped with a correction collar.

In practice, please set the correction collar as follows:

- Set the correction collar on the objective to 0.17 mm/to a marking that corresponds with this value.
- Use a position on the specimen with small structures and as high contrast as possible. Focus this using the fine focusing control.
- Carefully turn the correction collar in one direction and observe the change in imaging quality – pay particular attention to the contrast of the image. As a rule, the image sharpness is lost during this process. This should be readjusted through continuous refocusing using the fine focusing control.

If the imaging becomes worse, turn the correction collar back slightly in the opposite direction and optimize the image until the structures are imaged sharply with exceptional contrast.





#### Cross section of an objective

- 1 Objective thread
- 2 Stop face of the objective
- 3 Spring system for the specimen-protection mechanism
- 4 - 7. Lens groups for the correction of image errors
- 8. Correction collar for adapting to deviating cover glass thicknesses or temperatures
- 9. Front lens system
- 10. Front lens holder

# Where to Find More Detailed Information

Choosing the right objective depends on a number of different factors and users may find themselves faced with unexpected issues. You will find everything that you want to know about each individual objective – down to the last detail – in the comprehensive Carl Zeiss objective database. From field of view, flatness of field, color correction and transmission properties to technical details and dimensions – it's all here. Naturally, you can also make selections on the basis of search criteria such as magnification, numerical aperture, contrast technique, etc.

In the objective class database you will find the most up-to-date information and the ideal solution for you: the best Carl Zeiss objective for your application.

**www.zeiss.de/objectives**

In addition to the right objective, clean microscope optics are prerequisite for perfect images. For more information see the website below.

**www.zeiss.com/cleanmicroscope**

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