

LSM 5 Family

Objectives for Your Biomedical Applications

Whether your research subject is in cell biology, developmental biology, neurobiology or physiology, Zeiss offers you a wide range of objectives to fit the special properties of your specimen and the LSM. This brochure presents a selection of the best Zeiss objectives for LSM use, sorted by type of usage and correction properties. It is intended as a help for choosing the right objectives for your LSM, in order to guarantee the best possible image results.



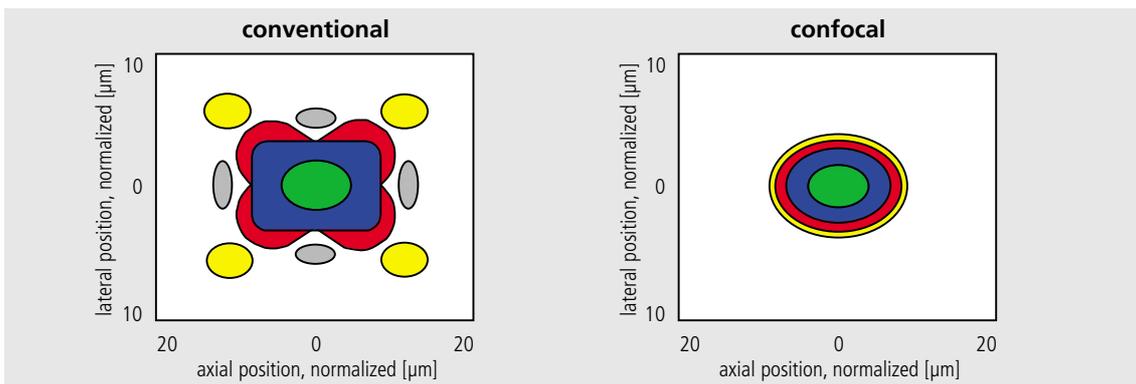
Confocal Laser Scanning Microscopy
Multiphoton Laser Scanning Microscopy
Fluorescence Correlation Spectroscopy



Why Special Objectives for Confocal Microscopy?

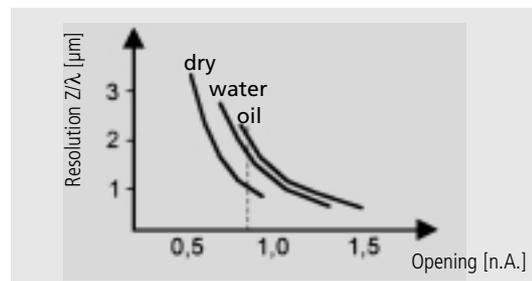
In confocal microscopy, the requirements for objective design and quality are much higher than in conventional light microscopy. Due to the ability to obtain optical sections in Z and to collect high resolution data of one point in the specimen at various wavelengths simultaneously, confocal microscope objectives need a perfect correction of longitudinal chromatic and spherical errors over the full wavelength range.

Maximum quality of point spread functions (PSF) in conventional (wide field) microscopy and in confocal microscopy. The almost ideal PSF in confocal microscopy is only available, of course, if perfectly corrected ("diffraction limited") objectives are used.



Specimen		matching objectives regarding coverslip, media correction and working distances			
Subject	optical property	dry	water immersion	oil immersion	water dipping
Cell Biology Microbiology	covered, thin	PI-Neofluar, Fluar, Plan LD	C-Apochromat	PI-Apochromat, Neofluar, Fluar	
Developmental Biology	covered, in dish etc.	Plan LD 40x & others	C-Apochromat	PI-Neofluar 25x & others	
Zoology, Botany Neurobiology	covered, thick	PI-Apochromat 20x & others	C-Apochromat	Achroplan 50x & others	
Physiology, Micromanipulation	uncovered	Fluar, Neofluar 2.5–10x range			Achroplan W

Half width of axial resolution in relationship to the numerical aperture of objective types. Surprisingly, the axial resolution at a given numerical aperture is highest in dry objectives, whereas the maximal possible resolution is achieved in oil immersion objectives. A good compromise of both properties is available in water immersion objectives.



Confocal-Apochromat

Cell Biology
Microbiology

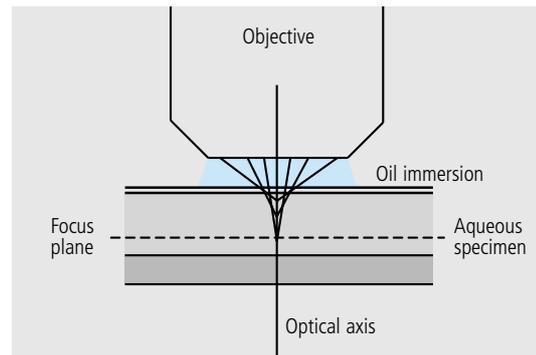
Developmental
Biology

Zoology, Botany
Neurobiology

The **C-Apochromat** objectives (C for confocal) have been developed to match the requirements of diffraction limited optics. The C-Apochromat objectives are the best offer for high performance confocal microscopy and combine high numerical apertures and perfect chromatic correction. The C-Apochromat series water immersion objectives are corrected for an extended range of six wavelengths instead of three or four in conventional Apochromats, starting at 360 nm (400 nm for the C-Apo 40 x) up to 700 nm, and therefore they work especially well for extended Z-scans in biological tissue and for spectral imaging with the META detector.

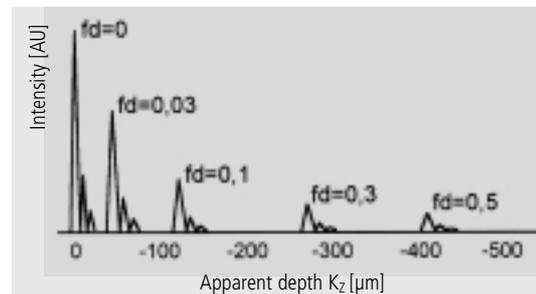
Using water immersion is the closest match to the refractive index of biological tissue and popular embedding media, and reduces spherical aberration even at very deep focus settings. Magnifications of C-Apochromats range from 10x overview to 40x and 63x with adjustable coverslip and temperature correction.

Objective	W.D.	Order #
C-Apochromat 10x/0.45 W	1.82	440638
C-Apochromat 40x/1.20 W Korr.	0.23	440052
C-Apochromat 63x/1.20 W Korr.	0.24	440668



Spherical aberration resulting from different refractive indices of sample and immersion media. This aberration is the greater, the deeper the focus goes into the sample.

Loss of axial resolution resulting from focussing an oil immersion objective into an aqueous sample (FD=focus distance). The deeper the focus goes into the sample, the smaller becomes the intensity maximum.



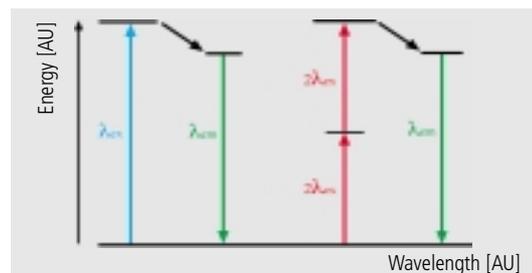
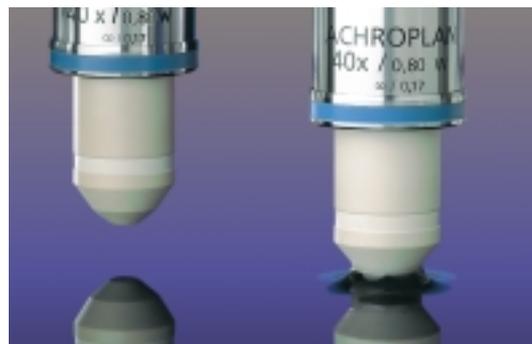
Confocal Achromplan

Developmental
Biology

Zoology, Botany
Neurobiology

For multiphoton applications and time lapse studies in living specimens, the C Achromplan NIR (near infrared ≤ 840 nm) is available. This super-achromatic objective made of special glasses with very high transmission matches the multiphoton excitation range above 800 – 850 nm with the resulting fluorescence emission wavelengths around 500 – 550 nm in chromatic correction terms, and offers an outstanding working distance even for deepest penetration of tissues. Thanks to the coverslip correction, living specimens can be immobilized and/or covered for long term experiments, and even inverted setups can be used.

Objective	W.D.	Order #
C Achromplan NIR 40x/0.80 W	≥ 1.75	1080378 (on request)



Fluorescence excitation and resulting emission.
Left: excitation with 1 photon;
right: cumulated effect of 2 photons at the doubled wavelength.

Plan-Apochromat

Cell Biology,
Microbiology

Zoology, Botany
Neurobiology

The range of C-Apochromats can be complemented for conventional thin specimens with multiple fluorescence preferentially in the VIS range (420 – 670 nm) by the Plan-Apochromat dry and oil immersion objectives of highest numerical apertures and perfect image flatness. Our legendary Plan-Apochromat 63x/1.40 Oil now offers a comfortable 0.18 mm working distance. The Plan-Apochromat 20x/0.75 is the star of the dry lenses, with exceptional properties regarding fluorescence efficiency and free working distance, making it a capable lens also for multiphoton applications.



Objective	W.D.	Order #
Plan-Apochromat 20x/0.75	0.61	440649
Plan-Apochromat 63x/1.40 Oil DIC	0.18	1113108

Plan-Neofluar

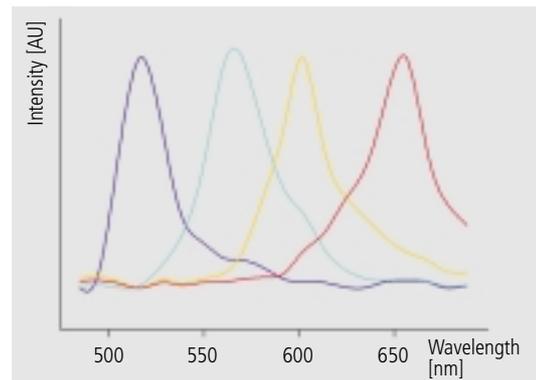
Cell Biology
Microbiology

Developmental
Biology

In addition to objectives dedicated to high end confocal microscopy, a universal objective solution is often required to match the properties of various specimens and protocols. The Plan-Neofluar objectives are perfectly designed for general fluorescence microscopy. The universal Plan-Neofluar dry and oil immersion objectives are corrected for an extended range from 435 nm up to 670 nm, sufficient even for 4-color fluorescence, with a correction level matching or even surpassing yesterday's finite system Apochromats.

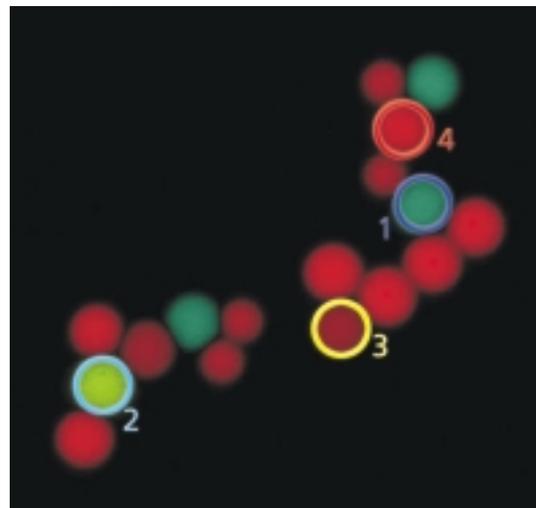
Transmission even starts in the UV range at 365 nm. As a truly versatile solution, various types are available including the multi-immersion Plan Neofluar 25x/0.80 Imm Korr., which can be used with water, glycerine and oil to match any refractive media index.

Objective	W.D.	Order #
Plan-Neofluar 10x/0.30	5.60	440330
Plan-Neofluar 40x/0.75	0.47	440350-9902
Plan-Neofluar 25x/0.80 Imm. DIC	0.21	440542
Plan-Neofluar 40x/1.30 Oil DIC	0.20	1056602 (1022818)



Spectral signature of a fluorescence emission. Four different fluorochromes alone can be matched into the VIS range of 450–670 nm.

Lambda- (wavelength-) coded view of multi-color fluorescence beads.



Fluar

Cell Biology
Microbiology

Developmental
Biology

To offer ideal prerequisites for physiological measurements, photon collecting, FISH analysis and even TIRF microscopy, the Fluar series of dry and oil immersion objectives are available as the fluorescence specialists in the Zeiss objective range. Extended transmission range from true UV 340 nm to 670 nm, extreme high transmission values for weak fluorescence and special high numerical apertures are the hallmarks of the Fluars, as can be seen e.g. in the outstanding α Plan-Fluar 100x/1.45 Oil. The Fluar 2.5x/0.12 offers a numerical aperture typical for lenses of twice the magnification, making it a perfect LSM "macroscopy" lens with inbuilt "zoom" capacity.



Objective	W.D.	Order #
Fluar 2.5x/0.12	6.40	1052092
F-Fluar 40x/1.30 Oil	0.10	440258
α Plan-Fluar 100x/1.45 Oil	0.10	1084514

Achroplan W/LD

Developmental
Biology

Physiology,
Micromanipulation

For developmental and physiological cell observations with LSMs on fixed stage microscopes, the Zeiss Achroplan W objectives are available. They combine long working distances in water with special inert coatings and good fluorescence transmission in a correction range from 400 to 850 nm. The water immersion 40x and 63x objectives for physiology are also available in versions corrected for the IR range up to 1100 nm. Hence they are ideally suitable for IR-microscopy without visible light and for multiphoton excitation.

To offer a perfect solution for inverted microscopes, the Achroplan 40x/0.60 LD objective provides excellent fluorescence transmission even in combination with thick culture dishes. The Achroplan 50x Oil offers a truly outstanding working distance of 0.3 mm with oil immersion and is another transmission "star" in the Zeiss range.



Objective	W.D.	Order #
Achroplan 10x/0.30 W Ph 1	3.10	440039
Achroplan 40x/0.80 W (option: piezo & IR version)	3.60	440090 9901 (440095 IR)
Achroplan 63x/0.95 W (option: IR version)	2.20	1036023 (1036025 IR)
LD Achroplan 40x/0.60 Korr.	1.80	440864
Achroplan 50x/0.90 Oil	0.30	440057

Accessories for Confocal Objectives

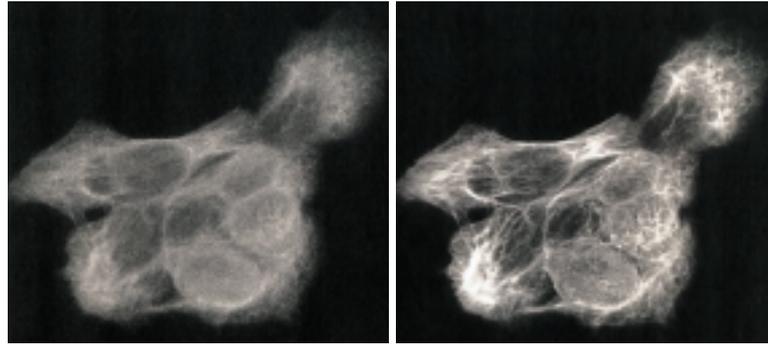
Developmental
Biology

Zoology, Botany
Neurobiology

To match even advanced requirements in confocal imaging, Zeiss offers a very unique range of accessories to complement the LSM dedicated range of objectives.

The immersion medium *Immersol W* with a refractive index of $n_e = 1.33$ exactly matches the C-Apochromat and Plan-Neofluar multiimmersion range of objectives for demanding applications. While offering an ultimate solution for the effective reduction of spherical aberrations, it still maintains the physical advantages of immersion oils regarding evaporation and run-off behavior. Chromatic properties and temperature stability are nearly perfect, and handling even in inverted configurations is as easy as you ever wanted it to be.

In case an extremely fine Z-focus capacity is required without moving the specimen, the Zeiss piezo focus attachment, especially for physiological applications, adds ultrafine objective focusing to every microscope stand. A special short-built parfocal version of the Achroplan 40x/0.80 W is available.



Resulting image quality in an aqueous sample in case of the use of an immersion medium with incorrect refractive index (unsharp) and correct refractive index (sharp).

Immersol W matches the handling properties of conventional oil and the water-like refractive index of thick biological specimens.



Piezo device for faster and more precise objective focusing

Imaging Methods and Objective Types

General VIS	Inverted, Time lapse	Physiol.	NLO-IR/ 2-Phot.	UV	Objective Type / Magnification / n. A.	W.D.	Order #
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Dry							
●	●	●		●	Fluar 2.5x/0.12	6.40	1052092
●		●			Plan-Neofluar 10x/0.30	5.60	440330
			●	● (405)	Plan-Apochromat 20x/0.75	0.61	440649
	●				LD Achromplan 40x/0.60 Korr.	1.80	440864
●				● (2D)	Plan-Neofluar 40x/0.75	0.47	440350 9902

Water Immersion with coverslip							
●	●		●	●	C-Apochromat 10x/0.45 W	1.82	440638
	●		● (850)		C Achromplan NIR 40x/0.80 W*	≥1.75	1080378*
●			● (850)	● (405)	C-Apochromat 40x/1.20 W Korr.	0.23	440052
●				●	C-Apochromat 63x/1.20 W Korr.	0.24	440668

Oil Immersion							
●	● (thin)				Plan-Neofluar 25x/0.80 Imm. DIC	0.21	440542
●			●		Plan-Neofluar 40x/1.30 Oil DIC	0.20	1056602 (1022818)
				●	F-Fluar 40x/1.30 Oil	0.10	440258
●			● (NDD)		Achromplan 50x/0.90 Oil	0.30	440057
●				● (405)	Plan-Apochromat 63x/1.40 Oil DIC	0.18	1113108
●	● (TIRF)				α Plan-Fluar 100x/1.45 Oil	0.10	1084514

Water dipping without coverslips							
		●			Achromplan 10x/0.30 W Ph 1	3.10	440039
		●	● (850)	● (2D)	Achromplan 40x/0.80 W (opt. piezo version*)	3.60	440090 9901
		●	● (NDD)		Achromplan IR 40x/0.80 W	3.60	440095
		●	● (850)	● (2D)	Achromplan 63x/0.95 W	2.20	1036023
		●	● (NDD)		Achromplan IR 63x/0.90 W	2.20	1036025

405: allows UV imaging with 405 nm laser; not recommended with 365 nm excitation

* available on request

2D: limited confocal 3D-reconstruction capability (elongated PSF in Z) in the UV range

thin: recommended for time lapse imaging of specimens up to 150 μm thickness

TIRF: allows Total Internal Reflection Fluorescence (see light microscope equipment)

850: NLO imaging with excitation up to 850 nm, not recommended above 900 nm

NDD: recommended for non-descanned detection (chromatic focus difference VIS-IR)

Markings on Objectives

Designation of Objective

Objective class
plus special properties, e.g.
LD for long working distance

Magnification / Numerical Aperture

plus special properties:
- immersion liquid: Oil/W(ater)/Glyc(erin)
- adjustable cover slip correction

Tube Length / Coverslip Thickness (mm)

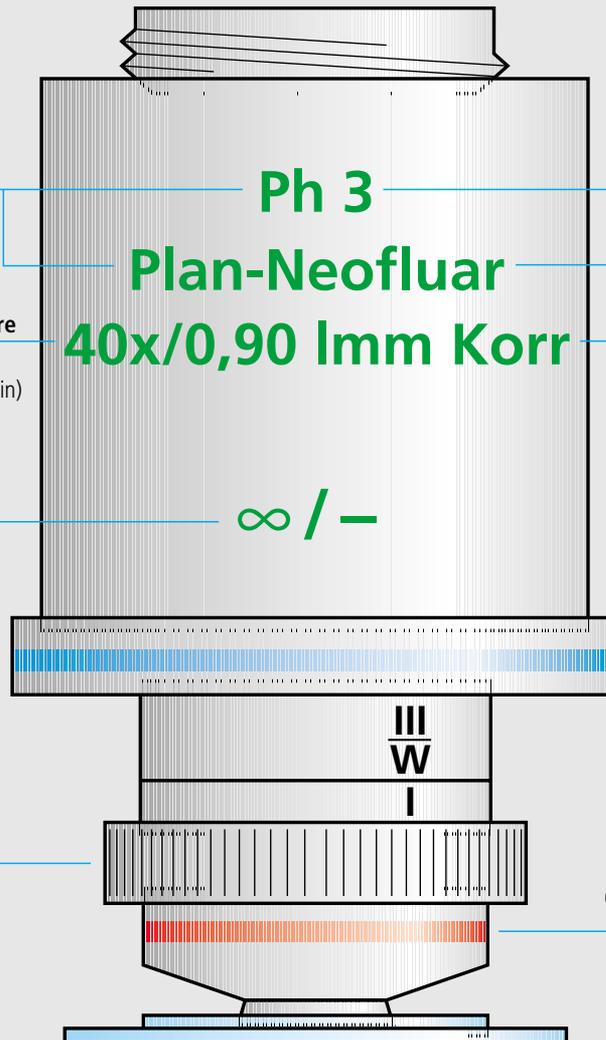
ICS-optics: ∞
Infinity Color corrected System

Standard cover slip: 0.17
Without cover slip: 0

Universal: —

Mechanical Function Ring

- Cover slip correction
- Correction for different immersion liquids or
- Aperture iris



Lettering Color

Contrast method

Standard	Black
Pol/DIC	Red
Ph 1.2.3	Green

Magnification Color Code

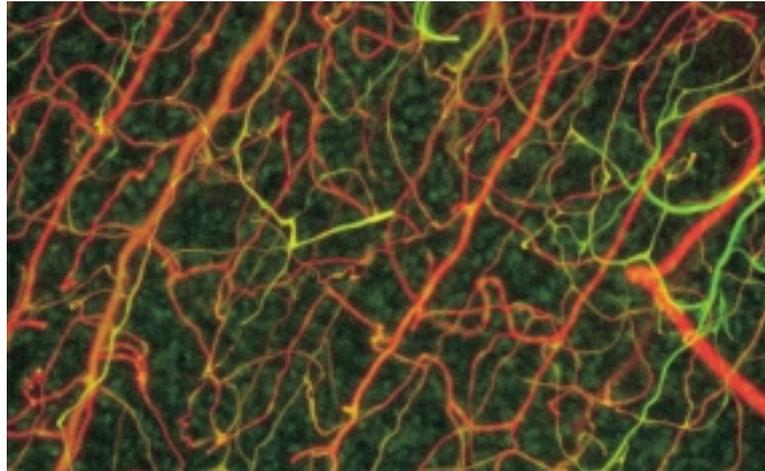
1.0/1.25	Black
2.5	Gold
4/5	Red
6.3	Orange
10	Yellow
16/20/25/32	Green
40/50	Blue
63	Dark Blue
100/150	White

Immersion Liquid

Oil	Black
Water	White
Glycerin	Gold
Oil/Water/Glycerin	Red

Selected Objectives for Laser Scanning Microscopes

Zeiss offers a large range of ICS-optics especially suiting the needs of confocal microscopy. The objectives selected for this brochure offer some of the best optical correction and transmission properties on the market. Low longitudinal chromatic aberration, low chromatic magnification difference, corrected spherical aberration and transmission values of 85 – 90 % in the main wavelength range (70 % at the borders given) combined with excellent image flatness create the best possible results. All this in combination with comfortable handling properties – that's Zeiss, the inventor of modern optics.



Blood vessels in brain tissue

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