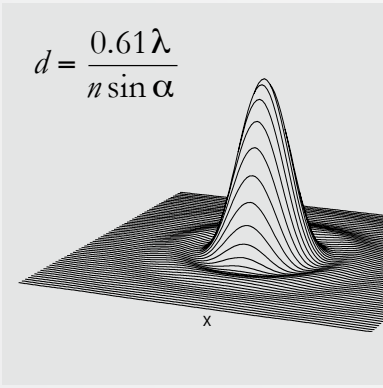
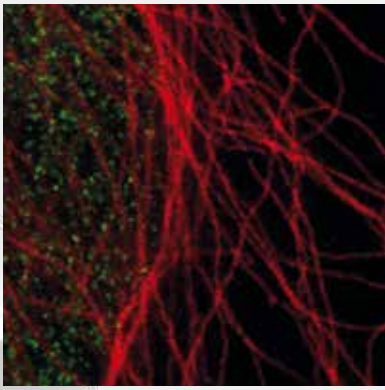
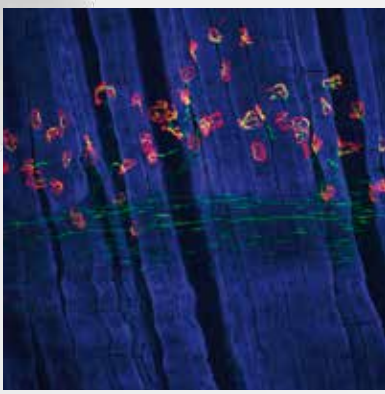
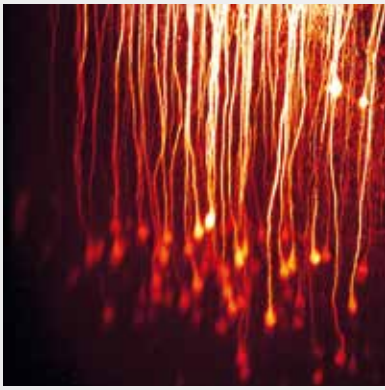
Living up to Life

Leica
MICROSYSTEMS

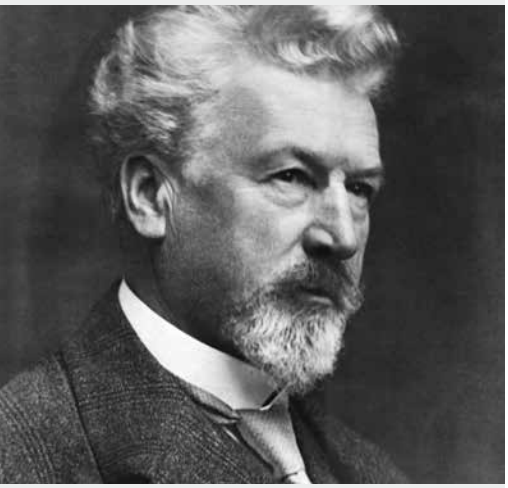




# Leica Objectives

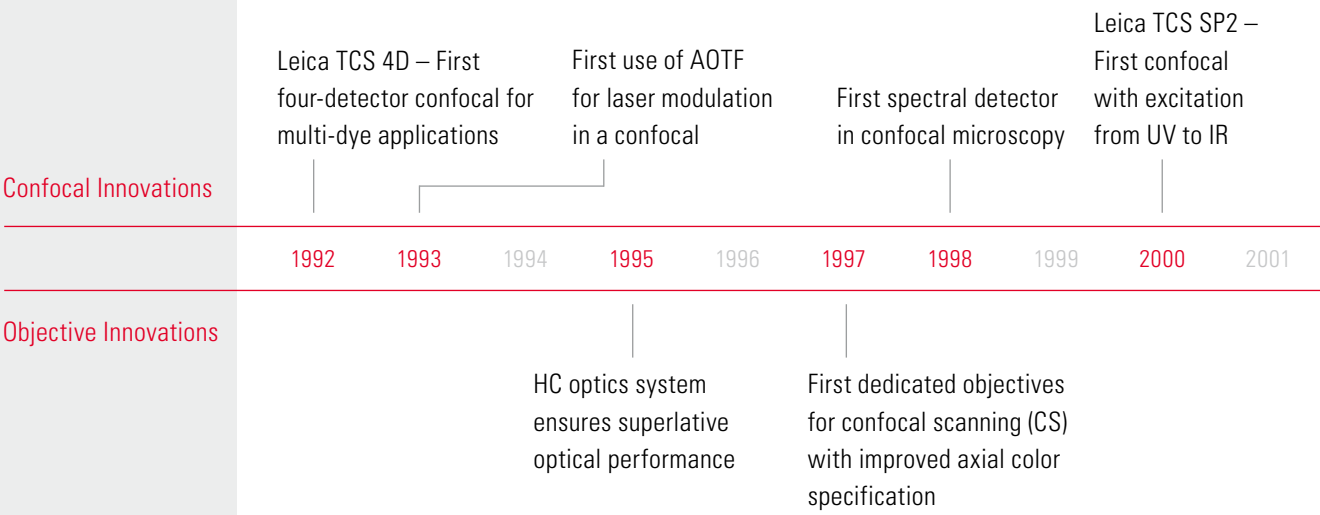
Superior Optics for Confocal and
Multiphoton Research Microscopy

# Leica Microsystems: Optics for Your Discoveries



Working "with the user, for the user" (Ernst Leitz I, 1843–1920) describes our drive to innovate and the success of Leica Microsystems for over 160 years.

We have designed and produced superior optics for a wide variety of applications in research, industry, and medicine for more than 160 years. Today, the innovation power of our optics designers and the experience and expertise of our precision optical engineers come together to provide microscopes with the best possible optics for spectral imaging. A sophisticated state-of-the-art production process yields objectives that deliver superlative image quality. We also help you to choose the best optics with optical characteristics that are tailored to your requirements.



2002 – AOBS – First reprogrammable beam splitter

2005 – Leica TCS SP5 – Broadband confocal with the first switchable tandem scanner

2008 – Leica TCS SP5 X – First white light laser confocal with tunable VIS excitation

2011 – Leica TCS SP5 MP with OPO, excitation up to 1300 nm

2012 – Leica TCS SP8 – New confocal platform, modular system

2014 – Leica TCS SP8 STED 3X – Fast and direct super-resolution

2002 – λ blue objectives optimized for 405 nm excitation

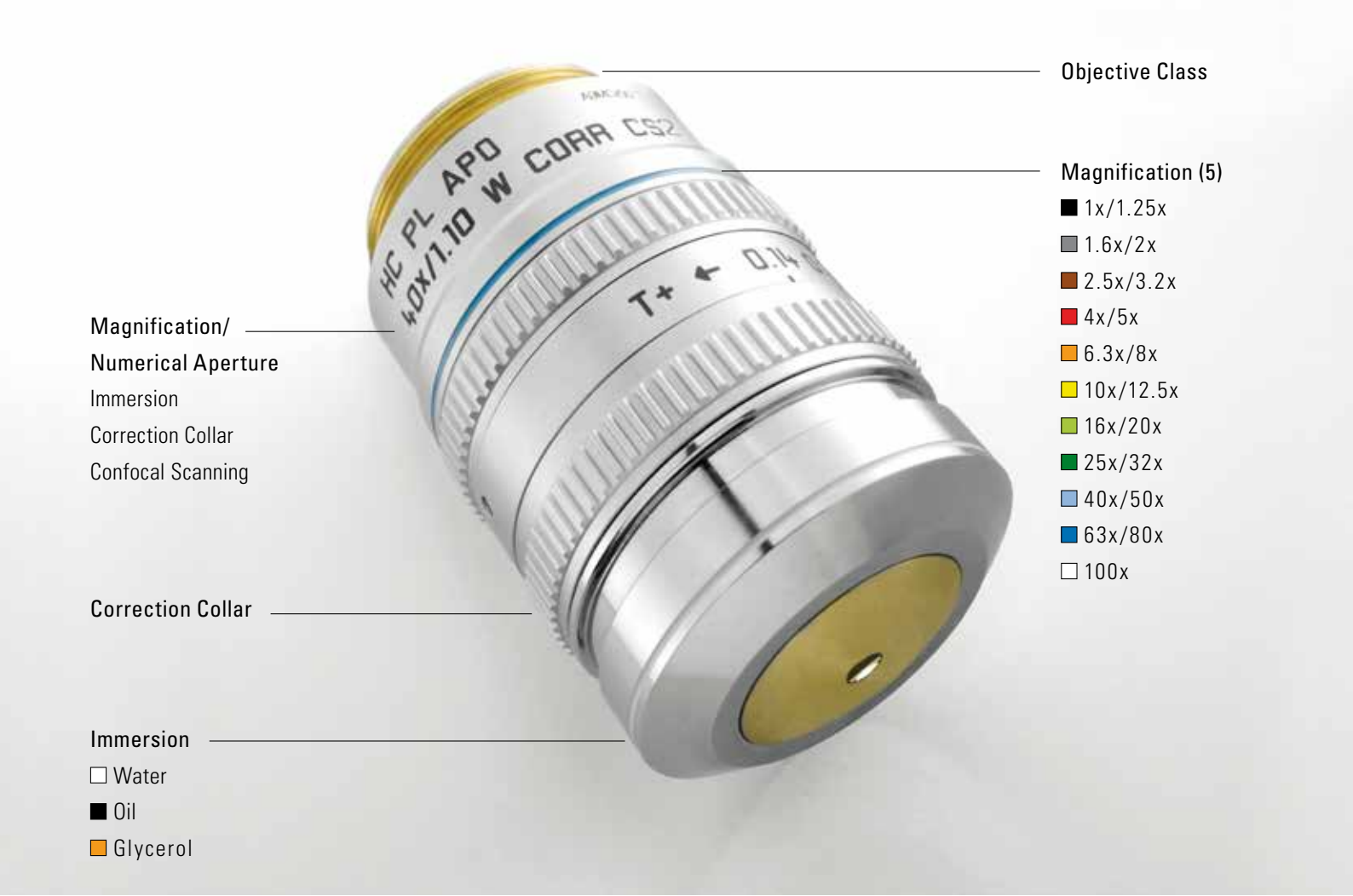
2011 – First motCORR objectives with motorized correction collar

2011 – IRAPO objectives with color correction up to 1300 nm

2012 – Leica HC PL APO CS2 for full VIS application spectrum

2013 – HC PL APO STED WHITE with superior broadband color correction

2014 – First motCORR for objectives with 75 mm parfocal distance


Objective Class
Magnification (5)
1x/1.25x
1.6x/2x
2.5x/3.2x
4x/5x
6.3x/8x
10x/12.5x
16x/20x
25x/32x
40x/50x
63x/80x
100x
Magnification/
Numerical Aperture
Immersion
Correction Collar
Confocal Scanning
Correction Collar
Immersion
Water
Oil
Glycerol

# Choose the Best Objective for Your Needs

The name of an objective describes its specifications and target applications. This guide gives you an overview of the technical terms and abbreviations.

| | |
|---|---|
| PL/PLAN – Excellent field planarity (1) | Objective with a flattened field of view for the representation of thin specimens, crucial for confocal microscopy of thin objects. |
| APO/IRAPO/FLUOTAR – Wavelength range of correction (2) | Appropriate color correction is required for colocalization in multicolor specimens. In addition, high transmission of an objective for the excitation and emission wavelengths is necessary for maximum image brightness. |
| L – Long free working distance | Long distance between the objective front lens and the focus plane offers good access to the specimen for complex experimental setups and enables acquisition of large z-stacks in thick tissues. |
| Objective Magnification (3) | Factor by which the objective enlarges the object in the intermediate image plane. |
| Numerical Aperture | The numerical aperture determines the lateral and axial resolution as well as the image brightness. |
| Oil/W/Glyc/IMM – Type of immersion medium (4) | The ideal choice of immersion directly depends on the mounting medium, because focusing deep into the specimen is only possible with homogenous immersion, i.e. mounting medium and immersion having the same refractive index. |
| CORR/motCORR – Manual or motorized correction collar | Corrects for variations in coverglass thickness, temperature, and refractive index of the specimen. Motorized correction collar is remote-controlled for easy, precise adjustment. |
| CS/CS2 – Highest specifications for confocal scanning | Apochromatic objectives optimized for confocal scanning deliver the best color correction. |

Leica objectives comply with
(1) ISO19012-1,
(2) ISO19012-2,
(3) ISO8039,
(4) ISO8036,
(5) ISO8578.

# High Numerical Aperture for Best Resolution

Numerical aperture and wavelength directly influence the resolving power of a microscope. Resolution improves with higher numerical apertures and lower wavelengths.

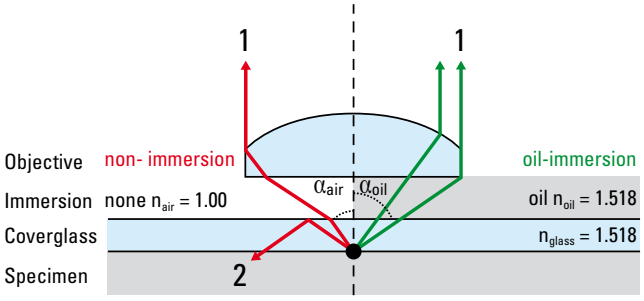
## NUMERICAL APERTURE

The numerical aperture (NA) of an objective is described by the sine of the half-angle $\alpha$ of the maximum cone of light that can enter or exit the lens multiplied by the refractive index $n$ of the immersion medium.

$$NA = n \sin \alpha$$

This also deterimines the maximum NA technically possible for different immersion media.

| | |
|---|---|
| Maximum angle | $\alpha_{max} \approx 72°$ |
| Dry immersion ($n_{air} = 1.0$) | $NA_{max} \approx 0.95$ |
| Oil ($n_{oil} = 1.518$) | $NA_{max} \approx 1.44$ |




1 Limiting ray
2 Total internal reflection

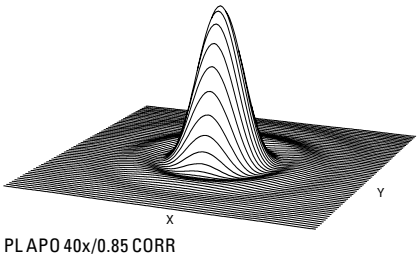
The numerical aperture of an objective depends on the half-angle of the aperture. For non-immersion objectives the maximum angle of light that can still be collected by the objective is smaller than for immersion objectives.

## POINT SPREAD FUNCTION

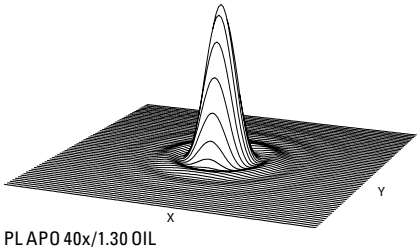
The point spread function (PSF) describes how an imaging system represents a point object in three dimensions. The PSF of a fluorescence microscope is dependent on the numerical aperture of the system and the wavelength.

In confocal microscopy, the pinhole diameter also has to be considered. If the pinhole diameter is set to 1 Airy Unit (AU) only the signal included in the Airy disk, i.e. the central maximum, is detected.

› High NA for best resolution in thin and well-defined specimen
› Low NA for larger depth of field for thick samples with lower requirements in resolution

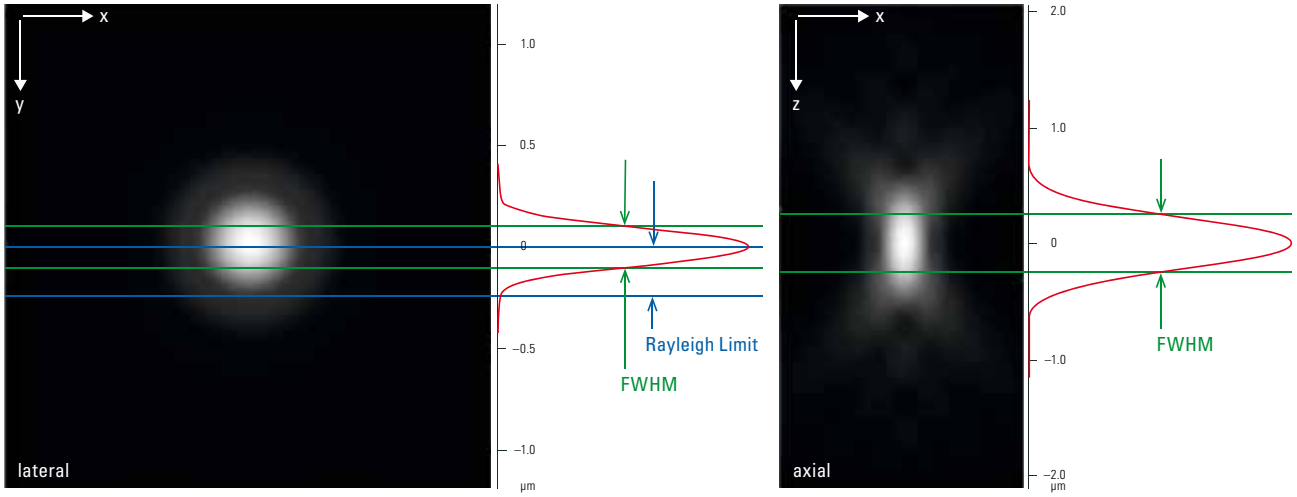



PL APO 40x/0.85 CORR




PL APO 40x/1.30 OIL

Calculated point spread functions for HCX PL APO 40x/0.85 CORR CS (no immersion) and HC PL APO 40x/1.30 Oil CS2. A larger NA results in a higher resolving power indicated by a smaller spot size and in higher intensities.




## LATERAL RESOLUTION

For a rough estimation of the resolving power of a fluorescence microscope in x and y, applying the Rayleigh criterion is usually sufficient. Here, the maximum of the Airy disk of one point overlaps with the first minimum of the Airy disk of the second point (left, shown in blue).

A more practical approach is to use the full width at half maximum (FWHM) of the PSF. This is also true for a confocal microscope with the pinhole equal to or larger than 1 Airy Unit (AU) (left, shown in green).

For pinholes significantly smaller than 1 AU, the FWHM decreases further. However, chosing a smaller pinhole diameter often reduces the signal-to-noise ratio, and any lateral resolution gain is lost in the noise.

› The lateral resolution linearly depends on the NA of the objective.

## AXIAL RESOLUTION AND OPTICAL SECTION THICKNESS

The volume of the PSF is not only restricted horizontally in the focus plane but also vertically along the optical axis of the microscope (z). The axial resolution of a microscope system is worse than its lateral resolution, approximately by a factor of two.

The major advantage of the confocal microscope is that only light originating from the focal plane is detected. Out-of-focus light is blocked by the detection pinhole. This drastically improves the effective axial resolution of a confocal microscope.

› The axial resolution depends on the square of the NA of the objective.
› For low NA objectives, the PSF becomes very elongated.
› For high NA objectives, the axial resolution is approximately twice the lateral resolution.

### Background: Estimating resolution and FWHM

Lateral resolution in xy according to the Rayleigh criterion:

$$d = \frac{0.61\lambda}{\mathrm{NA}}$$

Lateral FWHM of PSF for a fluorescent point object, with PH ≥ 1 AU

$$\mathrm{FWHM}_{\mathrm{lateral}} = \frac{0.51\lambda}{\mathrm{NA}}$$

Diffraction limited minimal axial FWHM from fluorescent specimens. The pinhole diameter is assumed to be zero.

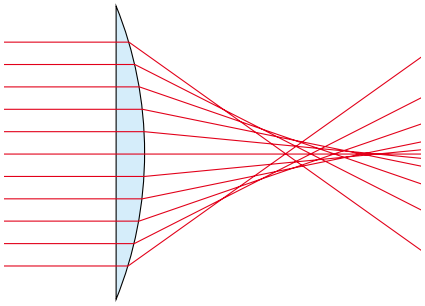
$$d_z \cong \frac{0.61\lambda_{\mathrm{exc}}}{n - \sqrt{n^2 - \mathrm{NA}^2}}$$

Optical section thickness dz in dependence of the pinhole diameter (PH) as it is implemented in Leica Application Suite Advanced Fluorescence (LAS AF) software.

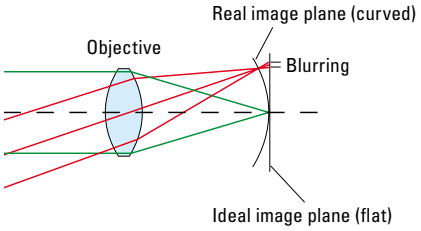
$$d_z\,(\mathrm{PH}) \cong \sqrt{\left(\frac{\lambda_{\mathrm{exc}} \cdot n}{\mathrm{NA}^2}\right)^2 + \left(\frac{n \cdot \sqrt{2} \cdot \mathrm{PH}}{\mathrm{NA}}\right)^2}$$

# Highly Corrected Optics for Better Images

Precise optical design and high manufacturing standards ensure that the imaging errors inherent in every optical system are reduced to a minimum.



Spherical Aberration




Field Curvature

## SPHERICAL ABERRATIONS

Spherical aberration is the dominant imaging error that needs to be corrected in high numerical aperture optics.
An objective with spherical aberrations has no well-defined focus.

Spherical aberrations blur the image across the whole field of view. The blurring cannot be compensated by refocusing. It already occurs in the center of field and remains constant over the whole field of view.

› Leica objectives are spherically corrected for at least the same wavelength range as the color correction.

## FIELD CURVATURE

Field curvature is a monochromatic aberration that causes the optimal focus position to vary with the image point position. It increases quadratically with the distance between the image point and the center of field. As a result, the image is increasingly blurred toward the edge of the field.

Objectives with high magnifications possess greater positive refractive power and therefore generally greater field curvature. Especially for confocal microscopy of thin specimens, field curvature should be corrected.

› PL – or PLAN – objectives are corrected for field curvature and show a flat field of view as well as minimized astigmatism
› Acquire sharp images over the whole field of view

### Background: Monochromatic Aberrations

Monochromatic aberrations already occur when using a single wavelength.
› Defocus: image is out of focus
› Spherical: no well-defined focus point
› Astigmatism: cross- or line-like deformation of image points
› Coma: comet-like distortion of a point
› Field curvature: curved image plane
› Image distortion: barrel or pincushion

## AXIAL COLOR

Axial color causes the optimal focus position to vary with the wavelength. This aberration occurs in the center of field and remains constant over the whole field of view.

In polychromatic applications this aberration causes a loss of contrast, colored fringes and a best focus that is not color-neutral.

Correction of axial color is critical in confocal microscopy, especially for time-sensitive multi-wavelength fluorescence applications when refocusing would take too long.

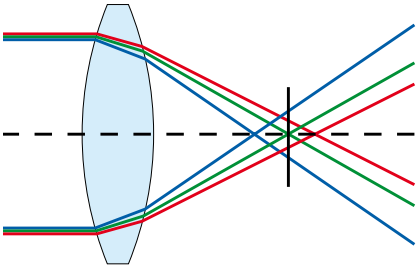
Axial color aberrations are caused by the natural dispersion of optical glasses. By chosing different glass types, this aberration can be eliminated.

## LATERAL COLOR

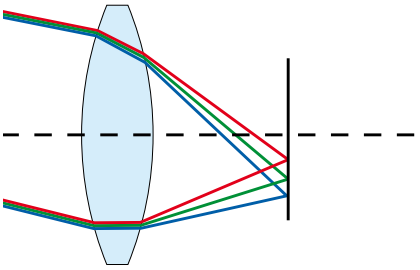
Lateral chromatic aberration causes the magnification to vary with the wavelength. It increases linearly with the distance between the image point and the center of field.

Image points near the edge of the field show a distinctive colored smearing for which the human eye is very sensitive. In quantitative applications, this makes overlay of images with different fluorophores difficult.

› The quality and wavelength range of axial and lateral color correction is indicated by the objective class.
› Chromatic correction matching the experimental setup guarantees optimal colocalization in xy and z.



Axial Color



Lateral Color

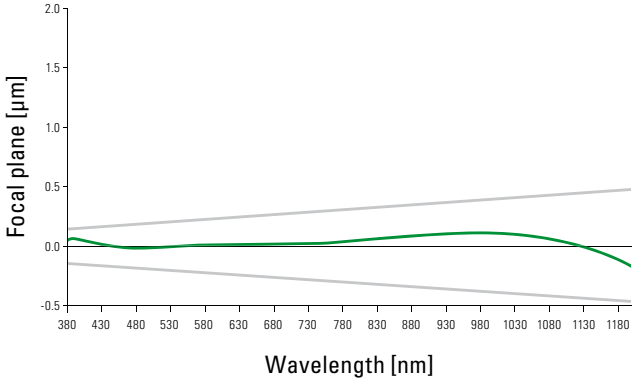
**Background: Chromatic Aberrations**

Chromatic aberrations appear in addition to monochromatic aberrations when using multiple wavelengths.

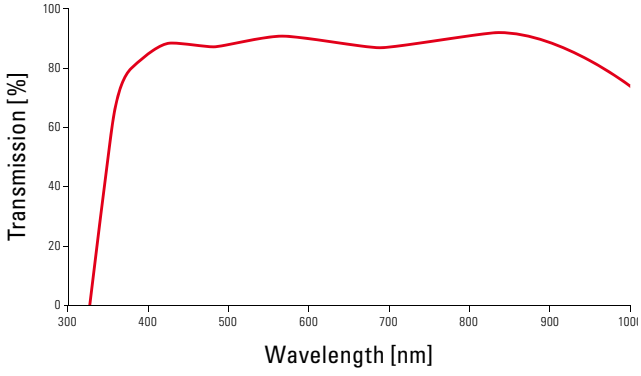
› Axial color: color-shift in z
› Lateral color: magnification dependent on color, color-shift in xy depends on position in image

# It's All About Color

Preparing your specimen for microscopy is only the first step to a stunning image. Choosing the best objective for your purpose should be done with as much care. Besides magnification and numerical aperture, the appropriate color correction is crucial.




Calculated axial chromatic aberration for HC PL APO 100x/1.40 OIL CS2. The position of the focus plane at different wavelengths shows almost no deviation over a wide range of wavelengths. Production tolerances can lead to small variations. The grey lines indicate the depth of of field.




Transmission curve for HC PL APO 40x/1.30 OIL. Production tolerances can lead to small variations.

## Apochromatic Color Correction

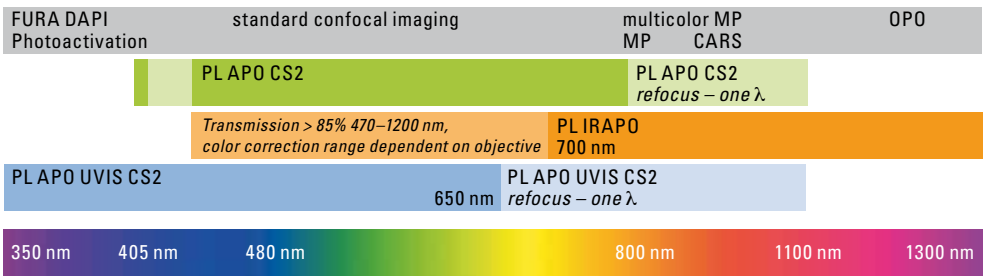
Apochromats allow the image resolution to reach the diffraction limit over a wide range of wavelengths. For fluorescence imaging, all excitation and detection wavelengths must be included to ensure that image resolution is as high as physically possible.

For optimal colocalization, superior color correction of apochromats guarantees a variation of the focus plane less or equal to half of the depth of field within a specified wavelength range.

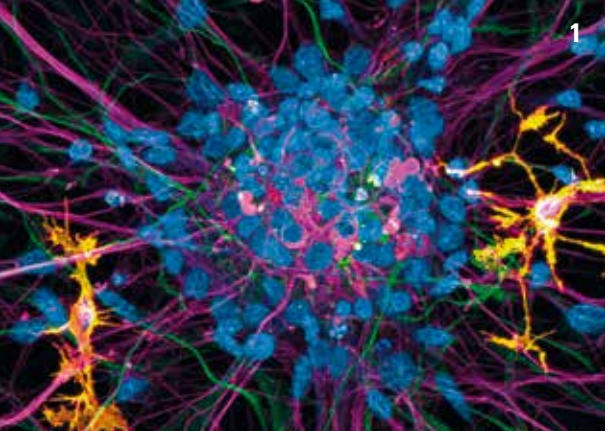
## Objective Transmission

The transmission of an objective is determined by the glass types it is manufactured from and the amount of losses due to reflections at the optical interfaces. The transmission is wavelength-dependent. As a general rule, transmission of short wavelengths is limited by glass properties, while transmission of long wavelengths is limited by the anti-reflective coatings.

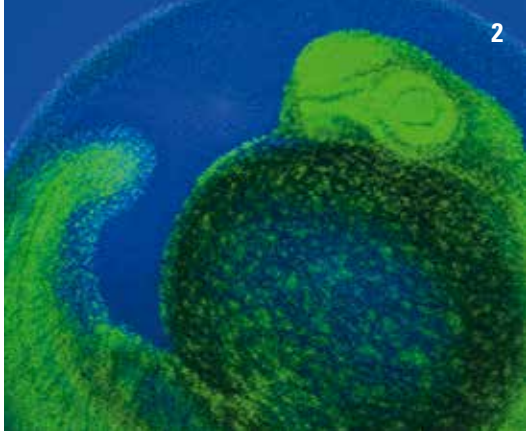
Leica Microsystems designs and manufactures complex anti-reflective coatings to increase transmission to the maximum. The Leica HC PL IRAPO objectives for example deliver superior broadband transmission that is higher than 85% from 470 to 1200 nm.



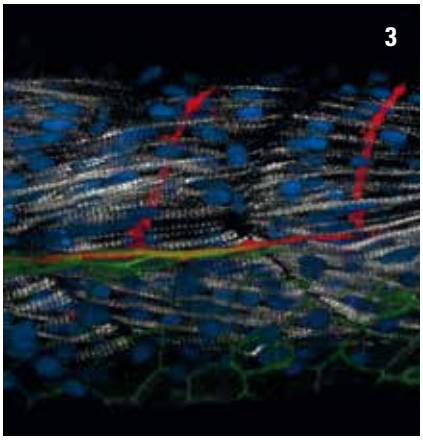

Wavelength ranges of color correction for objective classes recommended for confocal imaging.












## PL APO CS2

### Highest Specifications for Confocal Scanning

The apochromatic Leica CS2 objectives are optimized for confocal scanning (CS). Their color correction is outstanding over the whole field of view for precise colocalization of different fluorophores. In particular the lateral color has been further improved over the previous PL APO CS series.

The design of the CS2 objectives goes hand in hand with the innovative UV optics of the Leica TCS SP8, to give the most stable UV color correction.

Specialized objectives developed for 355 nm excitation or STED super-resolution microscopy are part of the CS2 objective class.

## PL IRAPO

### IR Color Correction for Multicolor Multiphoton Imaging and CARS

Leica PL IRAPO objectives are highly specified for improved multiphoton imaging.

The IR apochromats are color corrected from at least 700 nm up to 1300 nm to yield perfect overlap for multicolor multiphoton imaging and CARS (Coherent Anti-Stokes Raman Scattering).

Transmission is > 85 % from 470–1200 nm, maximizing the number of photons available for multiphoton excitation and emission detection. This results in brighter images from deeper tissue sections and reduced photodamage.

## PL FLUOTAR

### Objectives for Routine Fluorescence Imaging

The semi-apochromatic, universal PL FLUOTAR objectives feature good chromatic correction for visible wavelengths. This makes them well suited for standard fluorescence microscopy.

In confocal microscopy, these are an economic choice for overview images and imaging within a limited wavelength range.

# Matching Objective Immersion and Specimen

In addition to the objective itself, the refractive indices of all optical elements between the specimen and the front lens of the objective have a major influence on the image quality. Ideally, they should match the refractive index the objective has been designed for. This has to be kept in mind when choosing an objective and immersion medium for a certain application.

## OIL: STANDARD FOR FIXED SAMPLES

Immersion oil is designed to match the refractive index of standard crown glass ($n_e$=1.518). Oil immersion objectives are ideally suited for samples that are mounted in a medium that matches the refractive index of glass. This is the case for classically fixed specimens embedded in resin, Canada balm or glycerol-gelatine.

Oil immersion objectives can also be used when imaging close to the coverglass, i.e. less than a few micrometers deep. Further away from the coverglass, the image brightness and resolution will deteriorate quickly if the refractive indices are mismatched.

## GLYCEROL: THE OPTIMUM FOR MOUNTED SPECIMENS

Today, most fixed samples are mounted in Mowiol, Vectashield or similar mixtures based on glycerol. These media have refractive indices close to that of a 80/20 glycerol/water mixture ($n_e$=1.45).

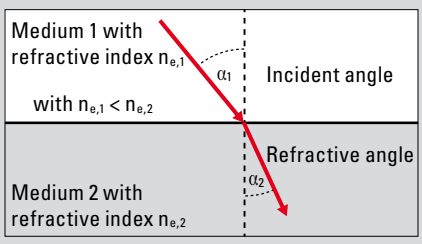
Leica glycerol objectives are an excellent choice for samples mounted in such media. They offer a correction collar to adjust the optics to varying refractive indices caused by changes of the composition of the mounting medium, or from variations in coverglass thickness or temperature.

## WATER: PERFECT MATCH FOR LIVE CELL IMAGING AND THICK SPECIMENS

Water immersion objectives are optimal for observing living cells in aqueous media. The refractive indices of the immersion medium and the specimen are a closer match than immersion oil, for example.

Water immersion objectives with high numerical apertures are very sensitive to refractive index variations. Therefore, each is equipped with a correction collar. It moves the central lens group to restore optimal image resolution and brightness.

Leica Microsystems offers water immersion lenses with motorized correction collars for accurate, remote-controlled adjustment of the correction collar (see page 17).




**Background: Refractive Index**

The refractive index (RI) describes the speed of light in a medium relative to the speed of light in a vacuum. The RI is dependent on temperature and wavelength. For the latter see the Background Box on the Abbe number.

For the generation of an image it is important that light changes direction when traveling from one medium to another if they have different RIs.

For an overview of RIs of common media please refer to the back page.

## IMM: GENERALISTS AND SPECIALISTS

Immersion objectives labelled with IMM are either for use with multiple immersion media like water, glycerol and oil or for specialized immersion media with refractive indices varying from the standard immersion media.

The generalists – Leica multi-immersion objectives:

› HC PL APO 10x/0.40 IMM CS

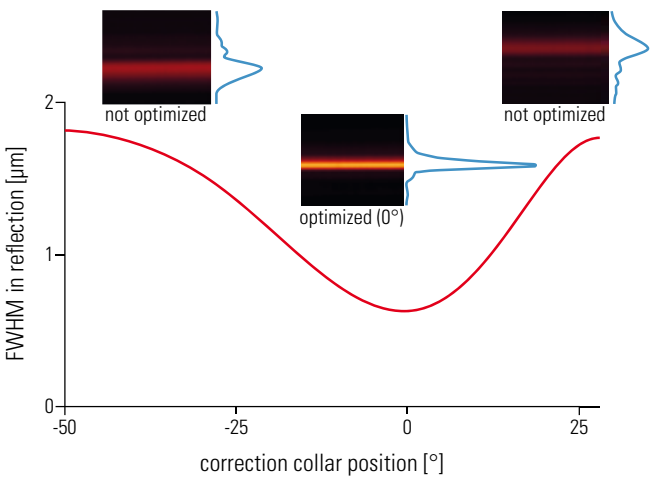
› HC PL APO 20x/0.75 IMM CS2

are for use with water, glycerol, and oil.

The specialists – special purpose immersion objectives:

› HC FLUOTAR L 25x/1.0 IMM motCORR VISIR for CLARITY-treated specimen ($n_e$=1.457)

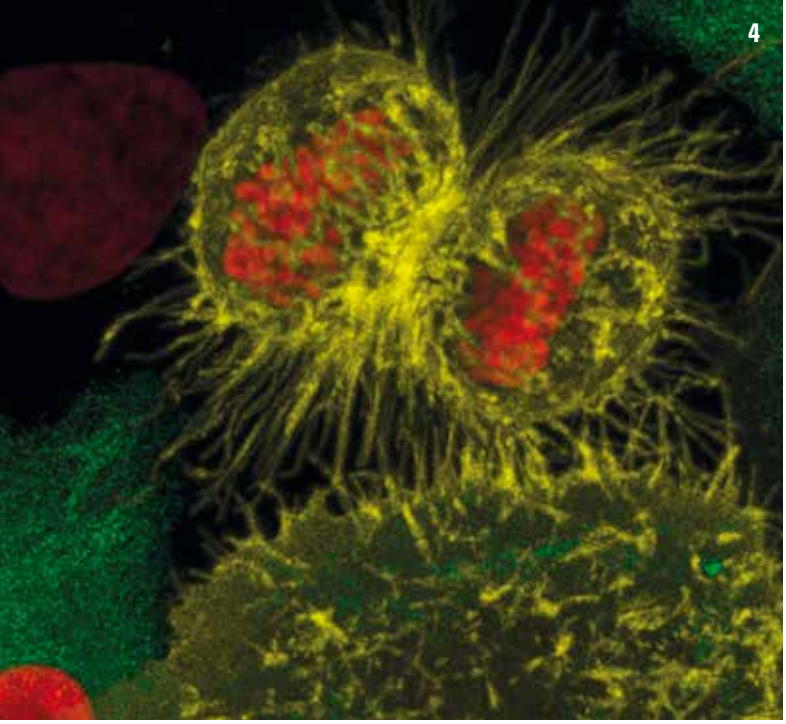
› HCX APO L 20x/0.95 IMM for BABB ($n_e$=1.563)




### Background: Abbe Number

For multicolor imaging, the dispersion of the immersion medium should not be neglected. It is a measure of the variation of the refractive index with wavelength and is described by the Abbe number.

The Abbe number of a medium should match the objective design, otherwise chromatic aberrations will occur. Let us assume that two different immersion oils with Abbe numbers that lie at opposite ends of what is specified by the ISO norm are used with the HC PL APO 100x/1.40 OIL CS2 objective. Then, the resulting axial color between 405 nm and 544 nm is around 300 nm. This is significant when considering the axial resolution of a confocal microscope. Leica CS and CS2 oil immersion objectives are designed for Leica immersion oil type F.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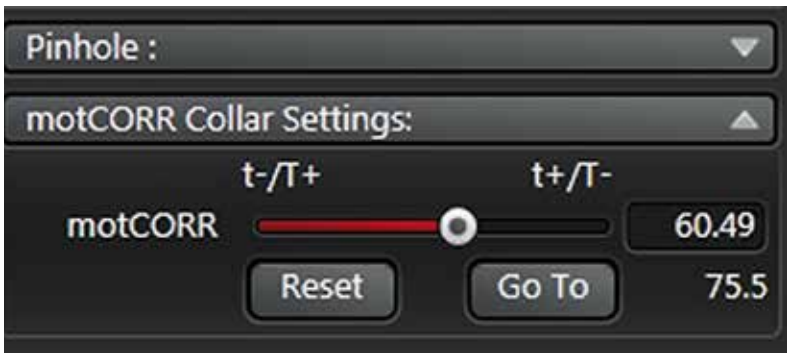
Leica


Pinhole :
motCORR Collar Settings:
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motCORR
60.49
Reset
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75.5


Pinhole
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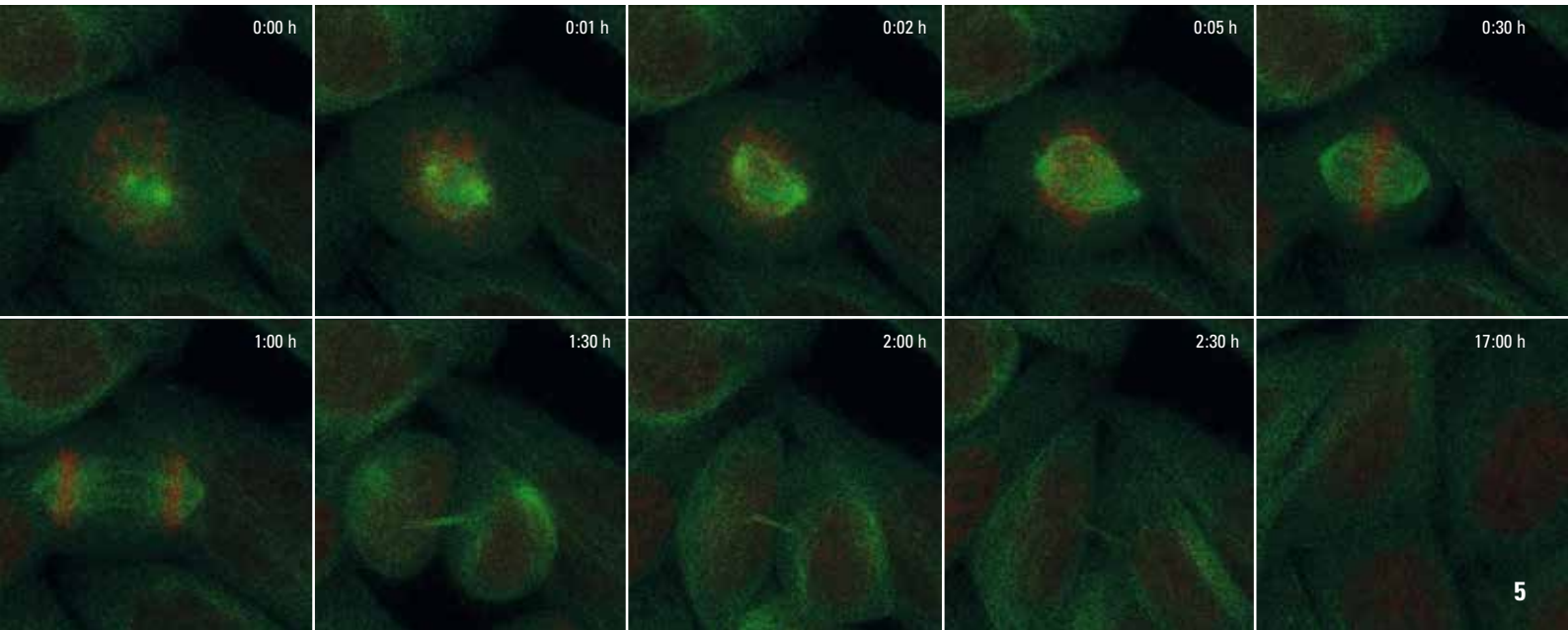
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5

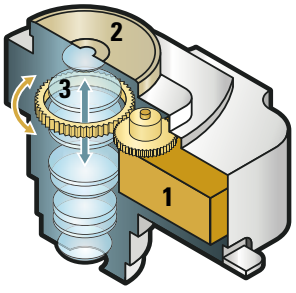
# Designed for Live Cell Imaging

The refractive index of living cells and their surrounding culture medium is close to the refractive index of water. Therefore, the best choice for live cell imaging is a water immersion objective. With the unique motorized correction collar, water immersion micro dispenser, and adaptive focus control you can easily acquire high-resolution images during long time-lapse experiments.

## MOTORIZED CORRECTION COLLAR FOR BEST OPTICAL PERFORMANCE

The optical performance of high NA water immersion objectives is highly sensitive to refractive index mismatches. Precise adjustment of the correction collar ensures that optimal resolution, signal intensity and penetration depth is restored.

The motorized correction collar of the Leica motCORR™ objectives simplifies the workflow by quickly adjusting the objective lenses to varying coverglass thickness, changes in temperature, and specimen inhomogeneities.




1 Motor
2 Water cap
3 Lens group to correct for changes in refractive index or coverglass thickness

## AUTOMATIC SUPPLY OF WATER IMMERSION DURING EXPERIMENTS

Water immersion has one drawback: water quickly evaporates. Especially at 37 °C and during screening or Mark & Find experiments the immersion film can be disrupted.

The Water Immersion Micro Dispenser overcomes these problems by adding immersion automatically during a running experiment.

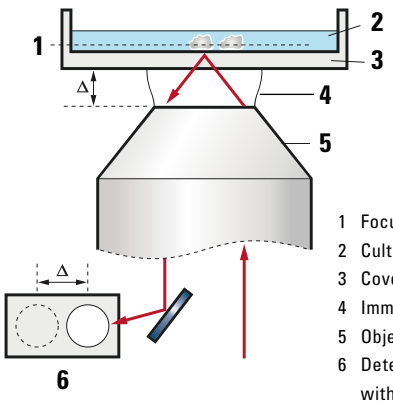
› Software-controlled water pump, no interaction required
› Steady water supply for long-term experiments at 37 °C
› Cap design prevents disruption of water film during stage movement

## KEEP YOUR FOCUS WITH ADAPTIVE FOCUS CONTROL

The Leica DMI6000 with Adaptive Focus Control (AFC) for live cell applications ensures that the specimen is actively kept in focus even under demanding environmental conditions.

The fully automated AFC is the ultimate tool for long-term time-lapse recordings in combination with multi-positioning, z-stacking and multifluorescence experiments.

› AFC is compatible with a large selection of objectives




1 Focus position
2 Culture medium
3 Cover glass
4 Immersion medium
5 Objective
6 Detection sensor with light spot

# A Clear View Deep in Tissues

Imaging depth in biological tissues is limited by light scattering caused by refractive index mismatches within the specimen. There are two approaches to overcome this limitation. For multiphoton excitation, longer wavelengths up to 1300 nm are used to reduce scattering. Additionally, novel optical clearing methods use organic solvents to reduce scattering and maximize imaging depth in intact tissue. Clearing and multiphoton imaging can be combined.

## COLOR CORRECTION FOR MULTIPHOTON IMAGING

Multiphoton excitation requires wavelength about twice as long as for standard fluorescence imaging, i.e. in a range of 680-1300 nm. Emission of the fluorophores remains in the visible range (450-650 nm). Therefore, both the PL IRAPO and the FLUOTAR VISIR objectives show excellent transmission in the visible and infrared.

FLUOTAR VISIR objectives can be used for confocal imaging as well as multiphoton imaging with a broadband color correction. The IRAPO objectives are designed for multiphoton imaging, with excellent color correction up to 1300 nm for perfect overlap in multicolor multiphoton imaging and CARS (Coherent Anti-Stokes Raman Scattering).

## MOTORIZED CORRECTION COLLAR FOR DEEP TISSUE IMAGING

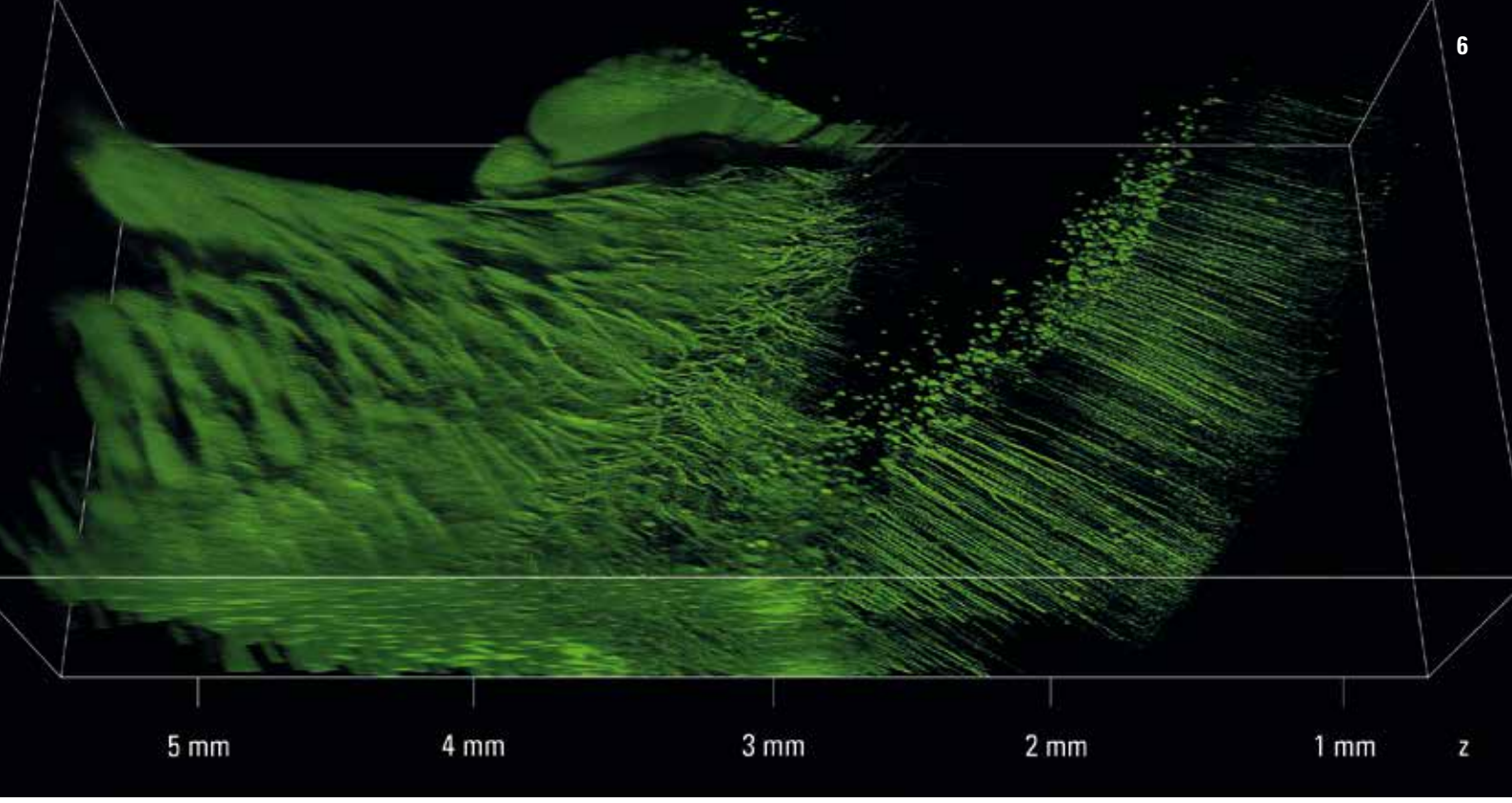
We have designed objectives ideally suited for deep tissue imaging, combining excellent color correction with our motorized correction collar. Adjustment of the correction collar to the refractive index of the specimen allows imaging of deeper in tissues with increased brightness and better contrast.

The Leica motCORR™ is easily remote-controlled by the control panel and by LAS AF (Leica Application Suite Advanced Fluorescence) microscope software. This ensures that the specimen remains undisturbed during movement of the correction collar.

## MAXIMUM ACCESS AND ISOLATION FOR ELECTROPHYSIOLOGY

High numerical aperture dipping objectives with inert ceramic fronts and large access angles are optimal for sensitive electrophysiology experiments. Leica Microsystems offers a complete range of dipping lenses and high resolution objectives to match your needs.

The Leica HC FLUOTAR L 25x/0.95 W VISIR objective provides maximum clearance around the specimen with an access angle of 41° and a free working distance of 2.5 mm. It can be used with confocal microscopes. A version for use with coverglass is available, too.

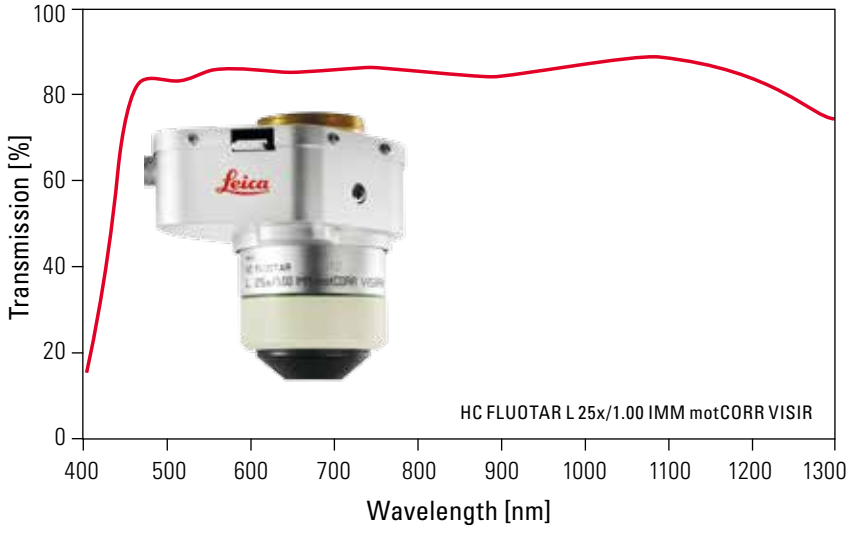



## SPECIALIZED OBJECTIVES FOR CLEARED TISSUE SAMPLES

The Leica objective HC FLUOTAR L 25x/1.00 IMM (ne=1.457) motCORR VISIR has been designed for the latest tissue clearing techniques in laser scanning microscopy. The result: maximum imaging depth and highest resolution.

The objective matches the refractive index of CLARITY-treated specimen. It is equipped with a motorized correction collar to adjust for remaining refractive index variations.

With a free working distance of 6 mm, whole organ imaging is possible. The broad range VISIR correction is ideal for use with single photon and two-photon excitation.




**Background: Long Free Working Distance Objectives (L)**

Free working distance is a decisive measure for many applications.
For example it is important in the following cases:

› Low magnifications: accessibility of specimen
› Medium magnifications: safety while focusing
› Water immersion objectives: focusing deep into the specimen

A large free working distance can only be achieved through special arrangements of the refractive powers inside the objective and so is contrary to other requirements.

The free working distances of a number of objectives can be found on pages 22/23.

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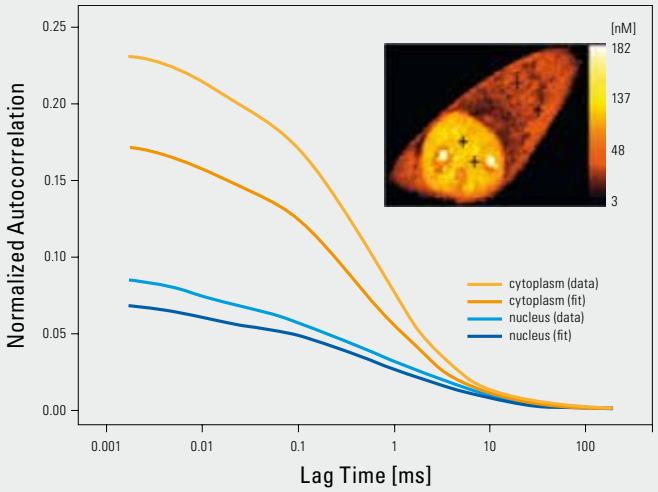
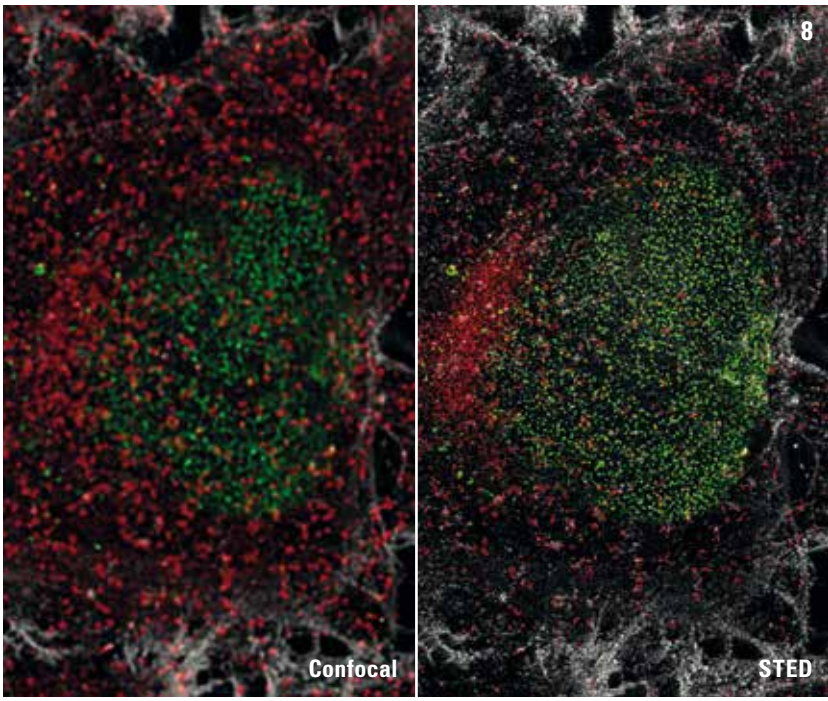
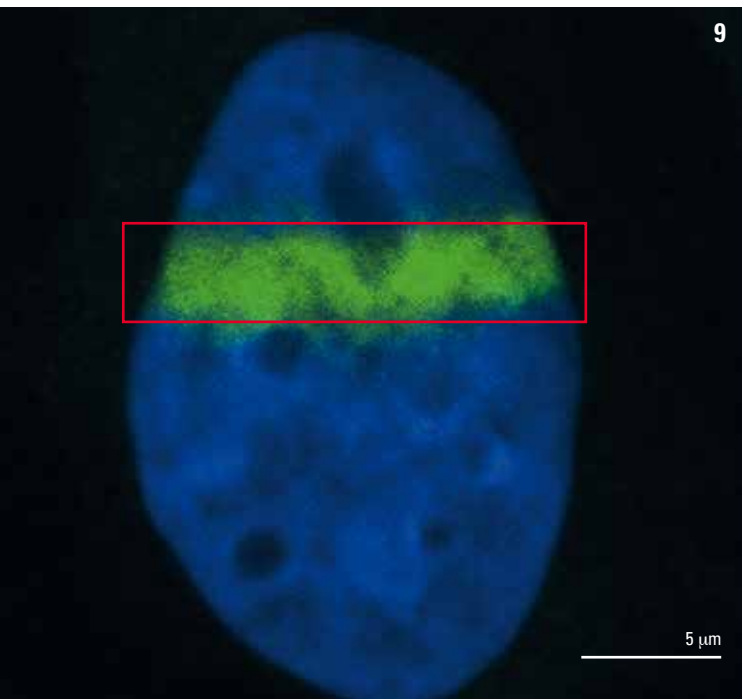

Normalized Autocorrelation
0.25
0.20
0.15
0.10
0.05
0.00
0.001
0.01
0.1
1
10
100
Lag Time [ms]
[nM]
182
137
48
3
cytoplasm (data)
cytoplasm (fit)
nucleus (data)
nucleus (fit)


Leica


OIL STED WHITE


Confocal
STED

8


5 µm

9


PL APO
W CORR UVIS CS2

# High-end Optics for Demanding Applications

For research involving highly specialized techniques or challenging specimens, specifically optimized objectives are often needed. Leica Microsystems offers a wide choice of application-specific objectives.

## THE BEST SPHERICAL AND CHROMATIC CORRECTION FOR FCS

Reliable, reproducible fluorescence correlation spectroscopy (FCS) data requires a confocal volume that closely resembles the mathematical model used in the FCS analysis. Leica PL APO optics feature excellent spherical and color correction for FCS and FCCS (Fluorescence Cross-Correlation Spectroscopy).

As the specimen is usually in an aqueous environment, water immersion objectives are ideal. A high numerical aperture ensures a small confocal volume for FCS. However, this requires correction collar optimization for each specimen to adjust for variations in coverglass thickness and temperature. Our motorized correction collar simplifies this adjustment.

## STED WHITE – ENJOY THE FULL SPECTRUM

Super-resolution microscopy places the highest demands on objective design. Especially multicolor super-resolution can separate very small structures from each other, provided that the optics are highly color-corrected.

The Leica HC PL APO 100x/1.40 OIL STED WHITE objective enables you to perform STED microscopy in the full spectrum of visible light in all three dimensions. Its chromatic correction and transmission are optimally designed for 3D STED.

## UVIS: A SHIFT TO THE BLUE FOR 355 NM EXCITATION

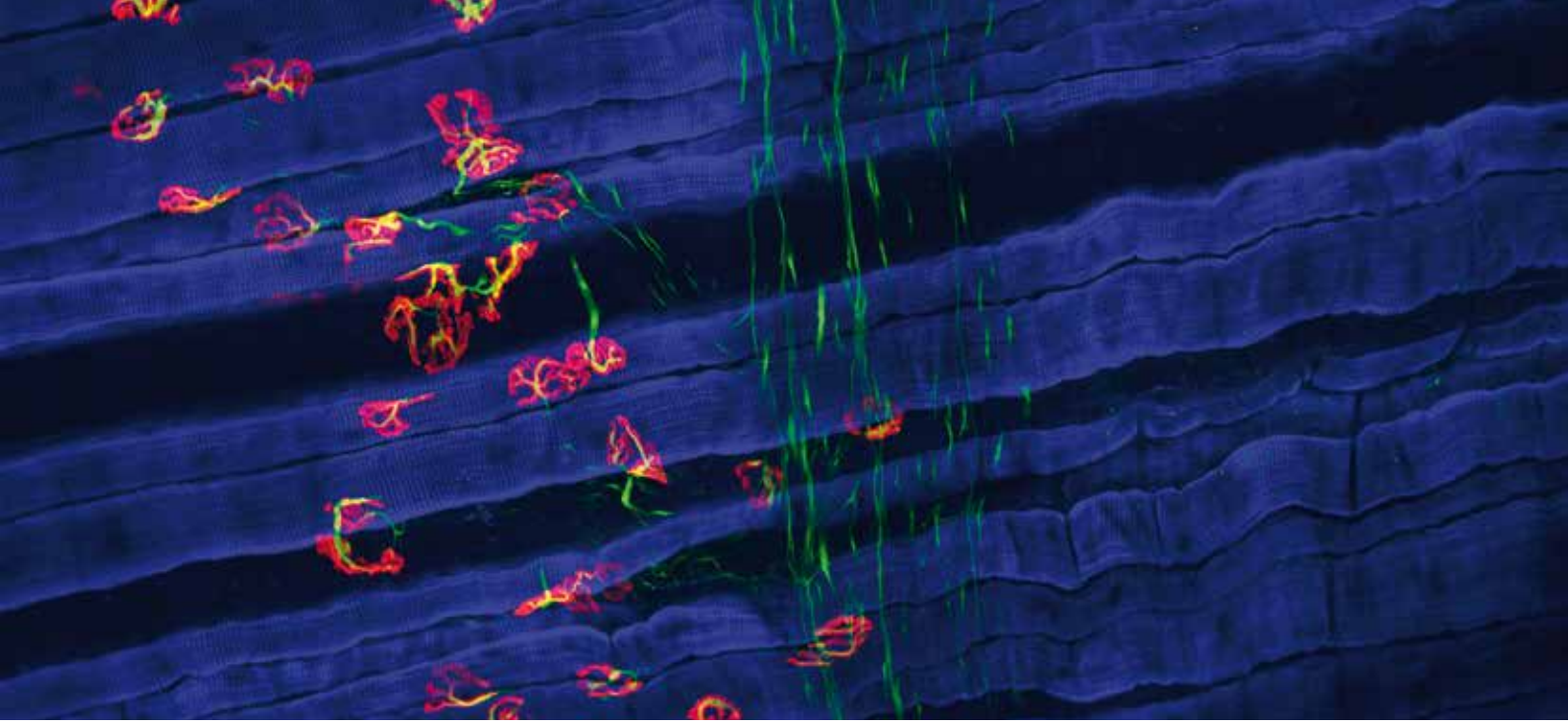
The Leica HC PL APO 63x/1.20 W CORR UVIS CS2 is an ultra-broadband objective dedicated for excitation with 355 nm.

It provides excellent color correction from 345-730 nm. This makes it the ideal objective for DNA lesions, photoactivation, uncaging, and physiological experiments using $Ca^{2}$+-single line and ratio imaging, monitoring gene expression or autofluorescence.

# Acknowledgements

Title Page:
Top: Dual color STED image. Green: histone 3. Red: microtubules. Both visualized in HeLa cells with Chromeo 505 and BD HorizonV500, respectively. Leica Microsystems
Middle: Mouse diaphragm. Green: nerve fiber, Alexa 488. Red: synapses, Rhodamin. Blue: muscle fiber, myosin. DODT contrast. Sample: courtesy of Ulrike Mersdorf, Max Planck Institute for Medical Research, Heidelberg, Germany.
Bottom: Adult Thy1-EYFP H line mouse, in vivo (cranial window). Excitatory pyramidal neurons in Layer 5 partly express EYFP. Courtesy of Dr. Masahiro Fukuda and Prof. Haruo Kasai, Center for Disease Biology and Integrative Medicine, Faculty of Medicine, The University of Tokyo, Tokyo, Japan.

[1] Rat primary culture labeled with DAPI, NG2-Cy3 and β3-Tubulin-Cy5. Leica Microsystems
[2] Zebrafish (Danio rerio), Neurogenin-GFP. H2A. Courtesy of J. Legradi, Dr. U. Liebel, KIT Karlsruhe Institute of Technology, Germany.
[3] Zebrafish embryo: Lateral Line (GFP, red), neurons (DsRed, red), muscles (SHG, grey), nuclei (BFP, blue). Courtesy of Lionel Newton and Darren Gilmour, EMBL, Heidelberg, Germany.
[4] HeLa cells expressing three different fluorescent proteins: GFP-tubulin (green) Ex 476 nm, Em 485-509 nm, YFP-GPI-filipodia (yellow) Ex 514 nm, Em 517-556 nm, mCherry-H2B-nucleus (red) Ex 561 nm, Em 571-671 nm. Three channels, simultaneously recorded. Courtesy of Jutta Bulkescher, ALMF, EMBL, Heidelberg, Germany.
[5] HeLa cells expressing tubulin-EGFP (green) and H2B-mCherry (red). Time series acquired at 1 min intervals over 17 hours on a Leica TCS SP8. Courtesy of Jutta Bulkescher, ALMF, EMBL, Heidelberg, Germany.
[6] Thy1-YFP adult mouse brain treated with CLARITY. Confocal imaging with excitation at 514 nm. Courtesy of Karl Deisseroth and Raju Tomer, Stanford University, Palo Alto, CA, USA.
[7] Concentration mapping of TIF1a-GFP in HeLa cells. ROIs for FCS measurements (red and blue graphs) shown as black crosses. Courtesy of Dr. Matthias Weiss, Dr. Jedrzej Szymanski and Nina Malchus, DKFZ, Heidelberg.
[8] Triple immunostaining in HeLa cells. Green: NUP153-Alexa 532. Red: Clathrin-TMR. White: Actin-Alexa 488. HeLa cells, fixed in methanol, 660 nm gated STED. Huygens deconvolved xy images, Leica Microsystems.
[9] Immunostaining in HeLa cells shows increased level of cyclobutane pyrimidine dimers (CPDs) (green) after locally induced DNA lesions by UVA laser (355 nm) (see region of interest in red). Nuclei were visualized by DAPI staining (blue). Image acquisition, cell irradiation and immunofluorescence was performed by Petra Sehnalová and Soňa Legartová from the Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno.



## LOOKING FOR A SPECIFIC OBJECTIVE?

Detailed information for all objectives is available in the objective finder, including transmission curves and dimensions for each objective.

CONNECT WITH US

www.leica-microsystems.com/products/microscope-objectives

## LEARN MORE ABOUT THE LEICA TCS SP8

More information about the Leica TCS SP8 platform, its applications, technology, and software are provided on the Leica TCS SP8 product page.

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www.leica-microsystems.com/sp8

## HOW CAN WE HELP YOU?

No matter if you have a demo request, questions about your existing Leica system or any other topic, contact us via our website.

CONNECT WITH US

www.leica-microsystems.com/contact-support

| Order No | Objective Name | FWD (mm) | Immersion | Coverglass | Correction Collar | Color Correction VIS | High Transmission VIS |
|---|---|---|---|---|---|---|---|
| 15506224 | HCX PL Fluotar 5x/0.15 | 13.70 | Dry | ♦ | – | O | ●● |
| 15506505 | HCX PL Fluotar 10x/0.30 | 11.00 | Dry | ♦ | – | O | ●● |
| 15506507 | HCX PL Fluotar 10x/0.30 PH1 | 11.00 | Dry | ♦ | – | O | ●● |
| 15506142 | HCX APO L 10x/0.30 W U-V-I | 3.60 | Water | ♦ | – | ● | ●● |
| 15506285 | HCX PL APO 10x/0.40 CS | 2.20 | Dry | 0.17 | – | ●● | ● |
| 15506293 | HCX PL APO 10x/0.40 IMM CS | 0.36 | IMM | ♦ | – | ●● | ● |
| 15506503 | HC PL Fluotar 20x/0.50 | 1.15 | Dry | 0.17 | – | O | ●● |
| 15506506 | HC PL Fluotar 20x/0.50 PH2 | 1.15 | Dry | 0.17 | – | O | ●● |
| 15506147 | HCX APO L 20x/0.50 W U-V-I | 3.50 | Water | ♦ | – | ● | ●● |
| 15506517 | HC PL APO 20x/0.75 CS2 | 0.62 | Dry | 0.17 | – | ●● | ●● |
| 15506343 | HC PL APO 20x/0.75 IMM CORR CS2 | 0.68 | IMM | ♦ | CORR | ●● | ●● |
| 15506344 | HC PL IRAPO 20x/0.75 W | 0.67 | Water | ♦ | – | O | ●● |
| 15507701 | HCX APO L 20x/1.00 W | 2.00 | Water | 0 | – | ● | ●● |
| 15507702 | HCX APO L 20x/0.95 IMM | 1.95 | $n_e$=1.563 | 0 | – | ● | ●● |
| 15506374 | HC FLUOTAR L 25x/0.95 W VISIR | 2.50 | Water | 0 | – | O | ●● |
| 15506375 | HC FLUOTAR L 25x/0.95 W 0.17 VISIR | 2.40 | Water | 0.17 | – | O | ●● |
| 15507704 | HC IRAPO L 25x/1.0 W motCORR | 2.60 | Water | ♦ | motCORR | – | ●● |
| 15507703 | HC FLUOTAR L 25x/1.0 IMM motCORR VISIR | 6.00 | $n_e$=1.457 | ♦ | motCORR | O | ●● |
| 15506295 | HCX PL APO 40x/0.85 CORR CS | 0.21 | Dry | 0.11–0.23 | w | ●● | O |
| 15506155 | HCX APO L 40x/0.80 W U-V-I | 3.30 | Water | 0 | – | O | ● |
| 15506357 | HC PL APO 40x/1.10 W CORR CS2 | 0.65 | Water | 0.14–0.18 | CORR | ●● | ●● |
| 15506360 | HC PL APO 40x/1.10 W motCORR CS2 | 0.65 | Water | 0.14–0.18 | motCORR | ●● | ●● |
| 15506352 | HC PL IRAPO 40x/1.10 W CORR | 0.65 | Water | 0.14–0.18 | CORR | – | ●● |
| 15506358 | HC PL APO 40x/1.30 Oil CS2 | 0.24 | Oil | 0.17 | – | ●● | ●● |
| 15506359 | HC PL APO 40x/1.30 Oil PH3 CS2 | 0.24 | Oil | 0.17 | – | ●● | ●● |
| 15506362 | HCX APO L 63x/0.90 W U-V-I CS2 | 2.20 | Water | 0 | – | O | ● |
| 15506346 | HC PL APO 63x/1.20 W CORR CS2 | 0.30 | Water | 0.14–0.18 | CORR | ●● | ●● |
| 15506361 | HC PL APO 63x/1.20 W motCORR CS2 | 0.30 | Water | 0.14–0.18 | motCORR | ●● | ●● |
| 15506356 | HC PL APO 63x/1.20 W CORR CS2 0/D | 0.30 | Water | 0 | CORR | ●● | ●● |
| 15506355 | HC PL APO 63x/1.20 W CORR UVIS CS2 | 0.22 | Water | 0.14–0.19 | CORR | ●● | ●● |
| 15506353 | HC PL APO 63x/1.30 Glyc CORR CS2 | 0.30 | Glycerol | 0.14–0.19 | CORR | ●● | ●● |
| 15506350 | HC PL APO 63x/1.40 Oil CS2 | 0.14 | Oil | 0.17 | – | ●● | ●● |
| 15506351 | HC PL APO 63x/1.40 Oil PH3 CS2 | 0.14 | Oil | 0.17 | – | ●● | ●● |
| 15506372 | HC PL APO 100x/1.40 Oil CS2 | 0.13 | Oil | 0.17 | – | ●● | ●● |
| 15506325 | HCX PL APO 100x/1.44 Oil CORR CS | 0.10 | Oil | 0.10–0.22 | CORR | ●● | O |
| 15506378 | HC PL APO 100x/1.40 Oil STED WHITE | 0.13 | Oil | 0.17 | – | ●●* | ●● |

♦ For use with and without coverglass
– Not available

●● Superior performance, highly recommended
● Excellent performance
O Good performance

* STED WHITE objective especially designed for use with STED and STED 3X. Axial color shift of objective <100 nm in VIS

| Color Correction UV / 405 nm on TCS SP8 | High Transmission UV | Color Correction >800 nm | High Transmission IR | Recommended for FCS | Compatible with | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Electro-physiology | Adaptive Focus Control | Water Immersion Micro Dispenser | Phase Contrast |
| – | ●● | – | ○ | | | | | |
| – | ●● | – | – | | | yes | | |
| – | ●● | – | – | | | yes | | yes |
| – | ●● | ○ | ● | | yes | | | |
| – | ● | – | ○ | | | yes | | |
| – | ○ | – | – | | | | | |
| – | ● | – | – | | | yes | | |
| – | ● | – | – | | | yes | | yes |
| – | ● | ○ | ● | | yes | | | |
| ● | ● | – | ○ | | | yes | | |
| ●● | ● | ○ | ● | | | yes | yes | |
| – | – | ●● | ●● | | | | | |
| – | ● | – | ● | | yes | | | |
| – | ● | – | ● | | | | | |
| – | ● | ● | ●● | | yes | | | |
| – | ● | ● | ●● | | | yes | yes | |
| – | – | ●● | ●● | | yes | | | |
| – | – | ● | ●● | | | | | |
| – | ○ | – | – | | | yes | | |
| – | ○ | ○ | ○ | | yes | | | |
| ● | ● | – | ● | ● | | yes | yes | |
| ● | ● | – | ● | ● | | yes | yes | |
| – | – | ●● | ●● | | | | yes | |
| ● | ●● | – | ● | | | Yes | | |
| ● | ●● | – | ● | | | yes | | yes |
| ● | ● | ○ | ○ | | yes | | | |
| ● | ● | – | ● | ●● | | yes | yes | |
| ● | ● | – | ● | ●● | | yes | yes | |
| ● | ● | – | ● | | | | yes | |
| ●● | ● | – | ● | | | yes | yes | |
| ● | ● | – | ● | | | yes | | |
| ● | ● | – | ● | | | yes | | |
| ● | ● | – | ● | | | yes | | yes |
| ●● | ● | ○ | ● | | | yes | | |
| – | – | – | – | | | yes | | |
| ●● | ● | ○ | ● | | | yes | | |

Subject to change without prior notice.

www.leica-microsystems.com

# Useful Equations and Numbers

### Rayleigh Criterion

$$d = \frac{0.61\lambda}{n \sin \alpha}$$

Distance of two point sources at which the maximum of the second PSF overlaps with the first minimum of the first PSF.
Details see page 7.

### Full Width Half Maximum

$$\mathrm{FWHM}_{\mathrm{lateral}} = \frac{0.51\lambda}{n \sin \alpha}$$

Lateral FWHM of PSF for a fluorescent point object, with PH ≥ 1 AU.

### STED Resolution

$$\Delta x \sim \frac{\lambda}{n \sin \alpha \sqrt{1 + \frac{I}{I_s}}}$$

| Mountant | Manufacturer | RI |
|---|---|---|
| Fluoromount-G™ | Southern Biotech Assoc. Inc. | 1.40 |
| ProLong®/ProLong® Gold | Molecular Probes | 1.46 after curing |
| VECTASHIELD® | Vector Laboratories | 1.44 |
| VECTASHIELD® Hard+Set™ | Vector Laboratories | 1.46 after hardening |
| Mowiol® | Kuraray Europe GmbH | 1.41–1.49 |
| TDE/Water | – | 1.33–1.52 |

| Immersion Medium | RI |
|---|---|
| Air | 1.000 |
| Water | 1.333 |
| Immersion Type G at 23°C (Glycerol/Water) | 1.450 |
| Glycerol 100% | 1.474 |
| Immersion Type F (Oil) | 1.518 |

How sensitive an objective is to varying coverglass thickness depends on the immersion medium and the numerical aperture (NA). For objectives designed for use with coverglass (0.17 mm), the following table can be referenced as a general rule.

| Immersion Medium | With or Without Coverglass | Coverglass No 1.5 (0.16 – 0.19 mm) | Coverglass No 1.5H (0.17 mm ± 0.005 mm) |
|---|---|---|---|
| Air | NA <0.30 | NA <0.70 | NA >0.70 |
| Water | NA <0.60 | NA <0.90 | NA >0.90 |
| Immersion Type G | NA <0.80 | NA <1.10 | NA >1.10 |
| Immersion Type F | NA <0.90 | NA <1.30 | NA >1.30 |