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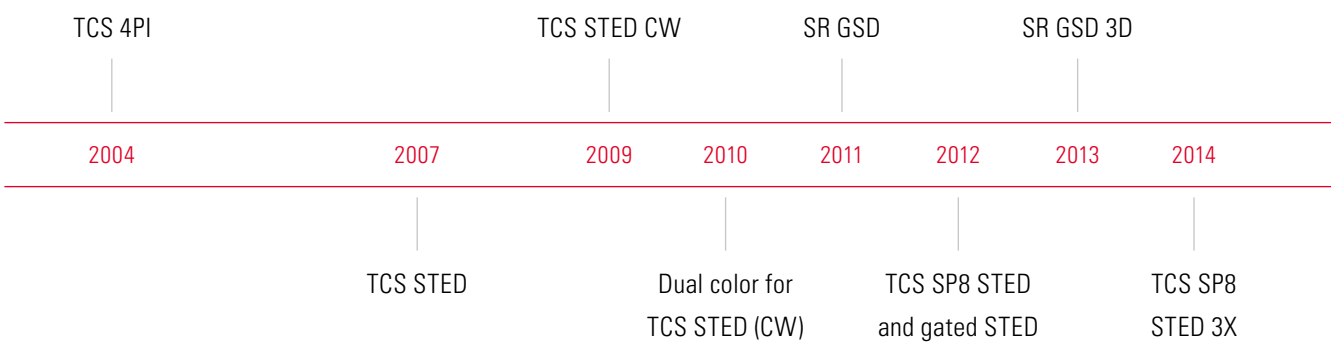
A Decade of Super-Resolution Innovation

Working with passion for the global research community: The TCS SP8 STED 3X marks one decade of innovative super-resolution technology by Leica Microsystems – providing quality data and efficient processes for your science.



TCS SP8 STED 3X

SR GSD 3D

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LASER RADIATION
VISIBLE AND INVISIBLE - CLASS 3B
AVOID DIRECT EXPOSURE TO BEAM
< 500mW 350-700nm
IEC 60825-1: 2007

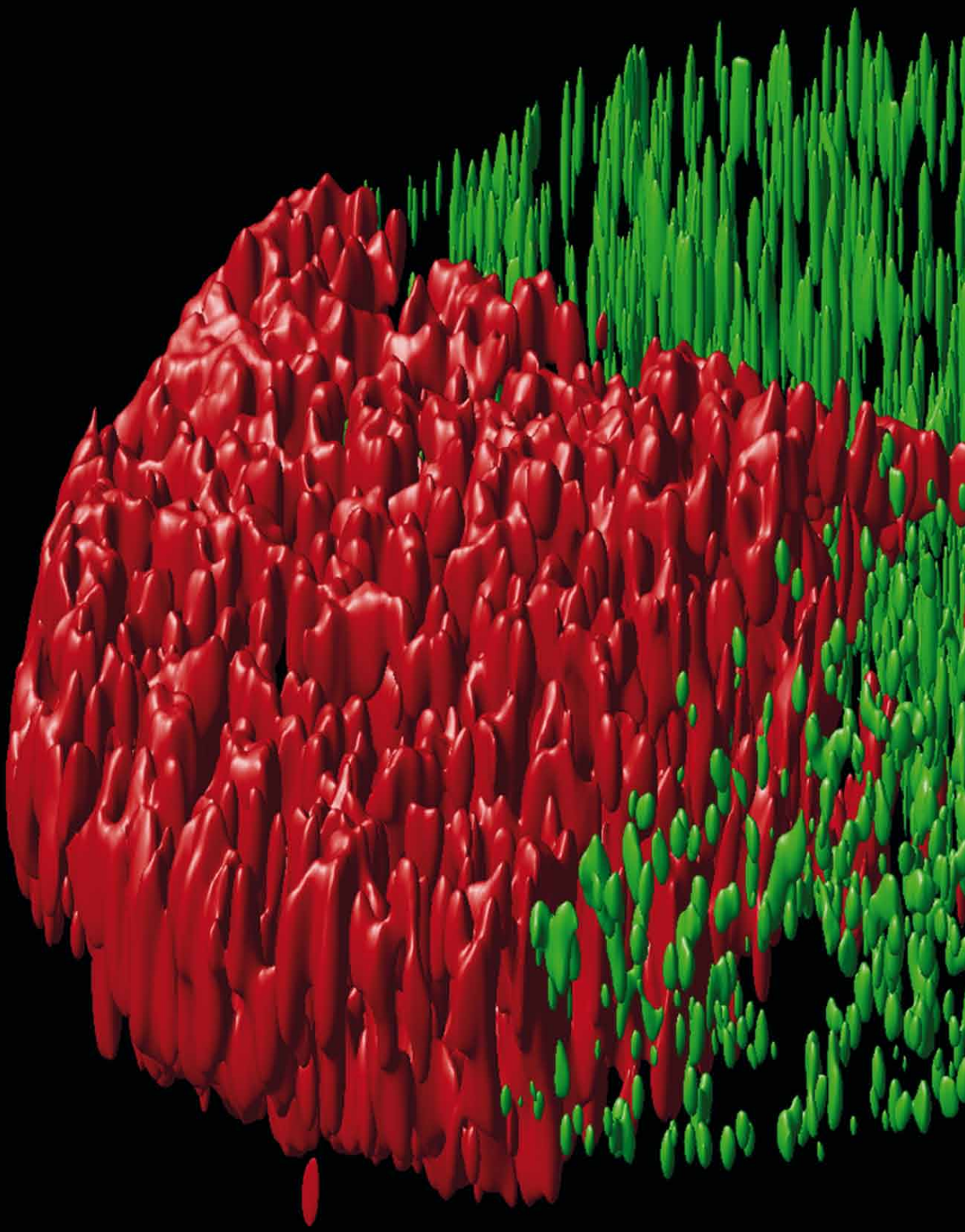
LASER RADIATION
VISIBLE AND INVISIBLE - CLASS 4
AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCAITERED RAOIATION
P< 4W 350-1600nm >801s
IEC 60825-1 : 2007

Living up to Life

Leica
MICROSYSTEMS

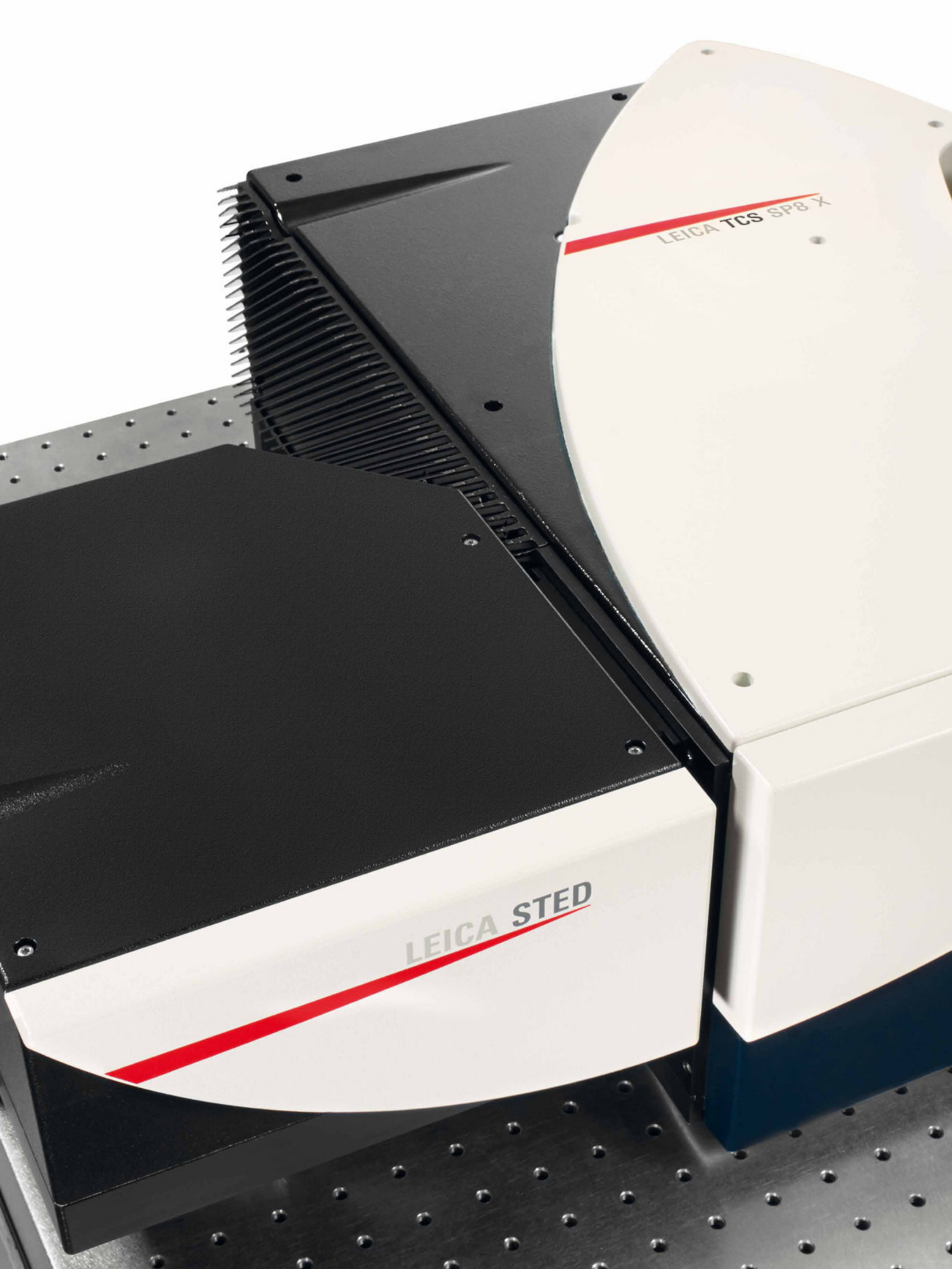
Leica TCS SP8 STED 3X

Your Next Dimension!



Welcome To Your Next Dimension!

The new TCS SP8 STED 3X opens up new dimensions for your research: Discover the possibilities of super-resolution in real 3D.


LEICA TCS SP8 X
LEICA STED

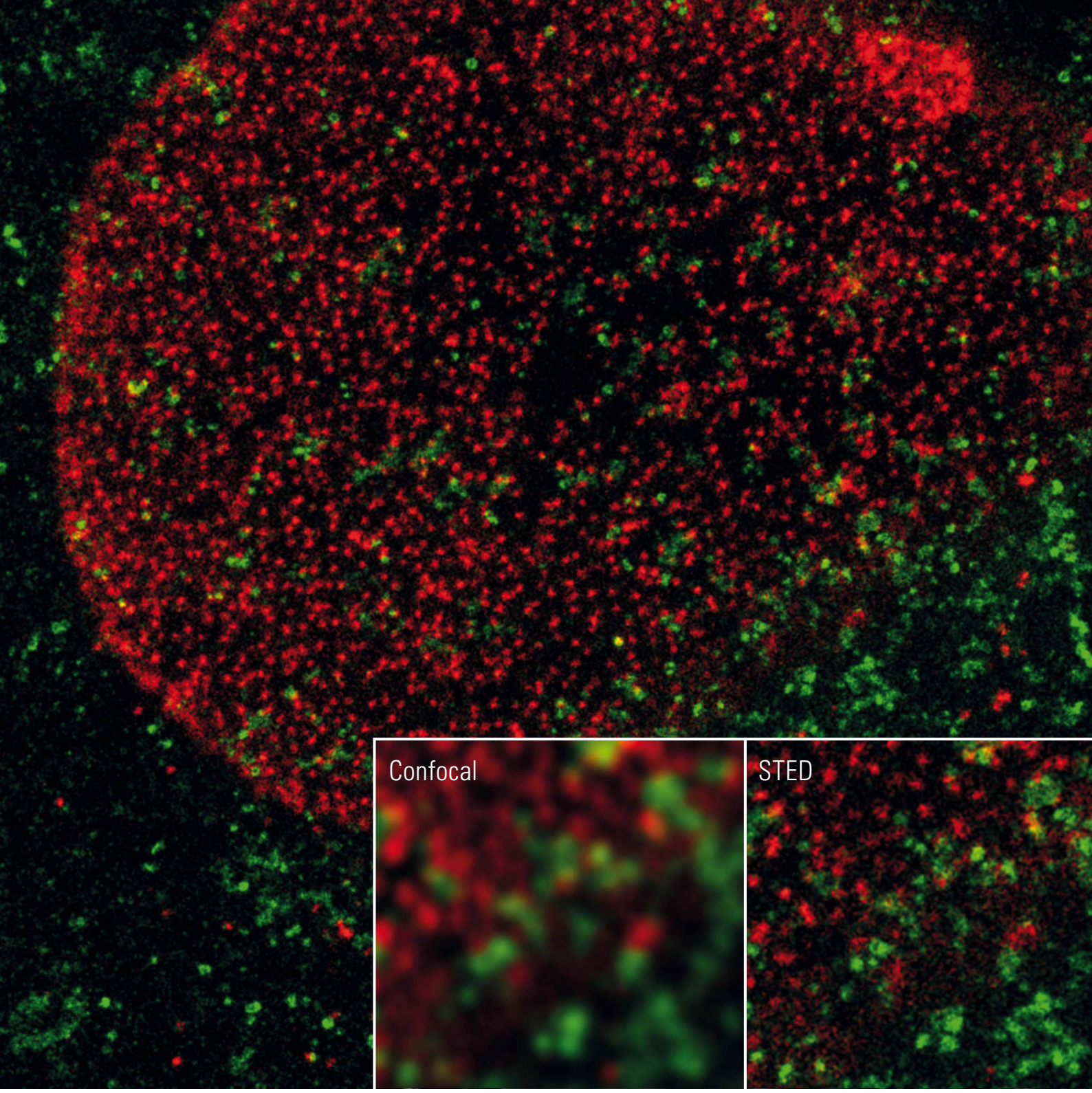
Pure Physics, More Efficiency

The new TCS SP8 STED 3X strengthens your science:

› Tunable and direct super-resolution in x, y and z reveal smallest details
› Multiple STED lines open up the full spectrum of visible light
› Gated detection improves resolution and increases live cell capabilities
› STED WHITE objective has optimal color correction for the full spectrum
› Auto beam alignment provides stability and reliability
› Modular concept based on the TCS SP8 allows you to upgrade at any time
› Smart STED Wizard intuitively controls your experiments
› Huygens deconvolution gets more from your raw data

CONNECT WITH US

Find more information online whenever you see that sign!


Confocal
STED


LEICA


STED WHITE

Here's Your Next Dimension!

STED microscopy by Leica Microsystems has revolutionized the study of subcellular architecture and cell dynamics at the nanoscale. The fully integrated STED (STimulated Emission Depletion) system meets the requirements of daily research and provides fast, intuitive, and purely optical access to structural details far beyond the diffraction limit. Gated STED substantially improves resolution to below 50 nm and increases live cell viability. Now, the next generation of STED, the TCS SP8 STED 3X, broadens the scope of super-resolution microscopy by offering the whole spectrum of visible light and opening the door to super-resolution in all dimensions.

LIFE HAPPENS IN 3D – NOW OBSERVE ITS DETAILS

STED microscopy easily reaches lateral resolutions below 50 nm. With the new TCS SP8 STED 3X you have the freedom of choice: Depending on your specimen and science, you can freely tune the effective focal spot to your needs and achieve super-resolution in x, y and z – online, fast and direct. Push the boundaries of your science with the smallest focal volume and the thinnest optical sections ever.

SEE THE FULL SPECTRUM OF LIFE

Many popular dyes and fluorescent proteins in the green spectrum are compatible with the 592 nm STED laser. Live cell imaging as well as dual color STED are routinely performed. However, with the new STED 3X module, multicolor applications become much easier since the TCS SP8 STED 3X opens up the full spectrum of visible light. Multiple STED laser lines at the instrument make a broad range of fluorophores accessible. This allows you to perform super-resolved co-localization studies while staying as close as possible to routine protocols, saving both time and money.

A PERFECT MATCH – CONFOCAL AND STED

STED microscopy is a super-resolution technology based on true confocal scanning. It is fully integrated into the Leica TCS SP8 platform, which gives you the greatest convenience and freedom ever. At the heart of Leica STED super-resolution you get the most versatile confocal microscope. You can purchase what you need now and develop the instrument with your future research. Upgrade from TCS SP8 or TCS SP8 STED to STED 3X at any time!

"STED 3X made a quantum leap by expanding into new dimensions – multicolor super-resolution imaging of the cell will surely revolutionize cell biology."

Dr. Yasushi Okada, RIKEN Quantitative Biology Center, Osaka, Japan

Immunofluorescence staining in HeLa cells:
red: NUP 153, green: Clathrin-TMR.
gSTED with 0% 3D STED

Pure Physics, More Reliability

STimulated Emission Depletion imaging reaches lateral resolution below 50 nm in true optical sections by downscaling the spot from where fluorescence is generated. STED is pure physics: What you see is what you get.

THE PRINCIPLE

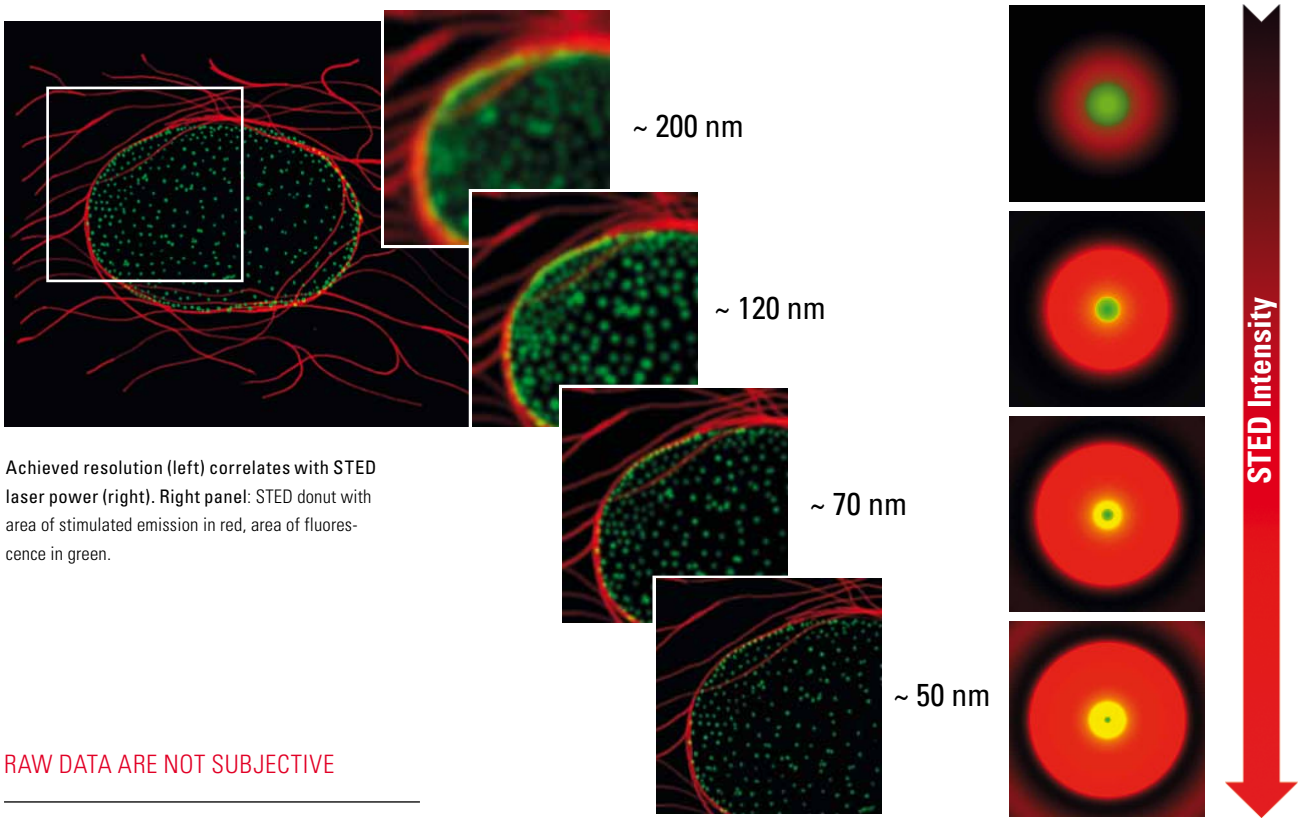
The basic principle of STED, which was first described by Stefan Hell in 1994[1], is simple. The effective focal spot scanning the specimen is reduced to an area smaller than the diffraction limit. To this end two laser foci are superimposed. A conventional excitation laser elevates the fluorophores to a higher energy level. In addition, a STED laser with a longer wavelength silences fluorophores at the periphery of the focal spot, returning molecules to the ground state by stimulated emission. Light from this process has the same wavelength as the STED laser and is easily filtered out. This prevents molecules outside the center from contributing to the built-up image. Both laser beams are focused through the objective and scan the specimen in perfect alignment.

A phase mask filter determines the area in the focus plane where STED light is dominant, e.g. resulting in a donut-like ring with zero energy at the center. In the absence of STED light, at the center of the STED donut or in classic confocal microscopy, the fluorophore returns to the ground state by spontaneous emission, creating conventional fluorescence. With increasing STED laser power the area where fluorescence originates – and consequently the effective focal spot – gets smaller. Resolution is now tunable!

SUPER-RESOLUTION – FAST AND DIRECT

STED technology provides fast and direct access to structural details at the nanoscale. Just one mouse click switches from confocal to super-resolution, even during live scans. Imaging parameters can be optimized within seconds. The resonant scanner together with the super-sensitivity of the HyD™ (Hybrid Detector) and the ability to freely adjust the observed area drives imaging speed to the extreme. Imagine being able to watch vesicles at their true size moving inside a living drosophila larva.

[1] Hell, S. W. & Wichmann, J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. Optics letters 19, 780-782 (1994).



Achieved resolution (left) correlates with STED laser power (right). Right panel: STED donut with area of stimulated emission in red, area of fluorescence in green.

RAW DATA ARE NOT SUBJECTIVE

Resolution improvement achieved by STED microscopy is purely optical and does not rely on mathematics. Of course, you can further process your images with deconvolution. STED allows you to directly compare the outcome with the raw data and makes your results more reliable. Don't waste time with artifacts!

Key Publications

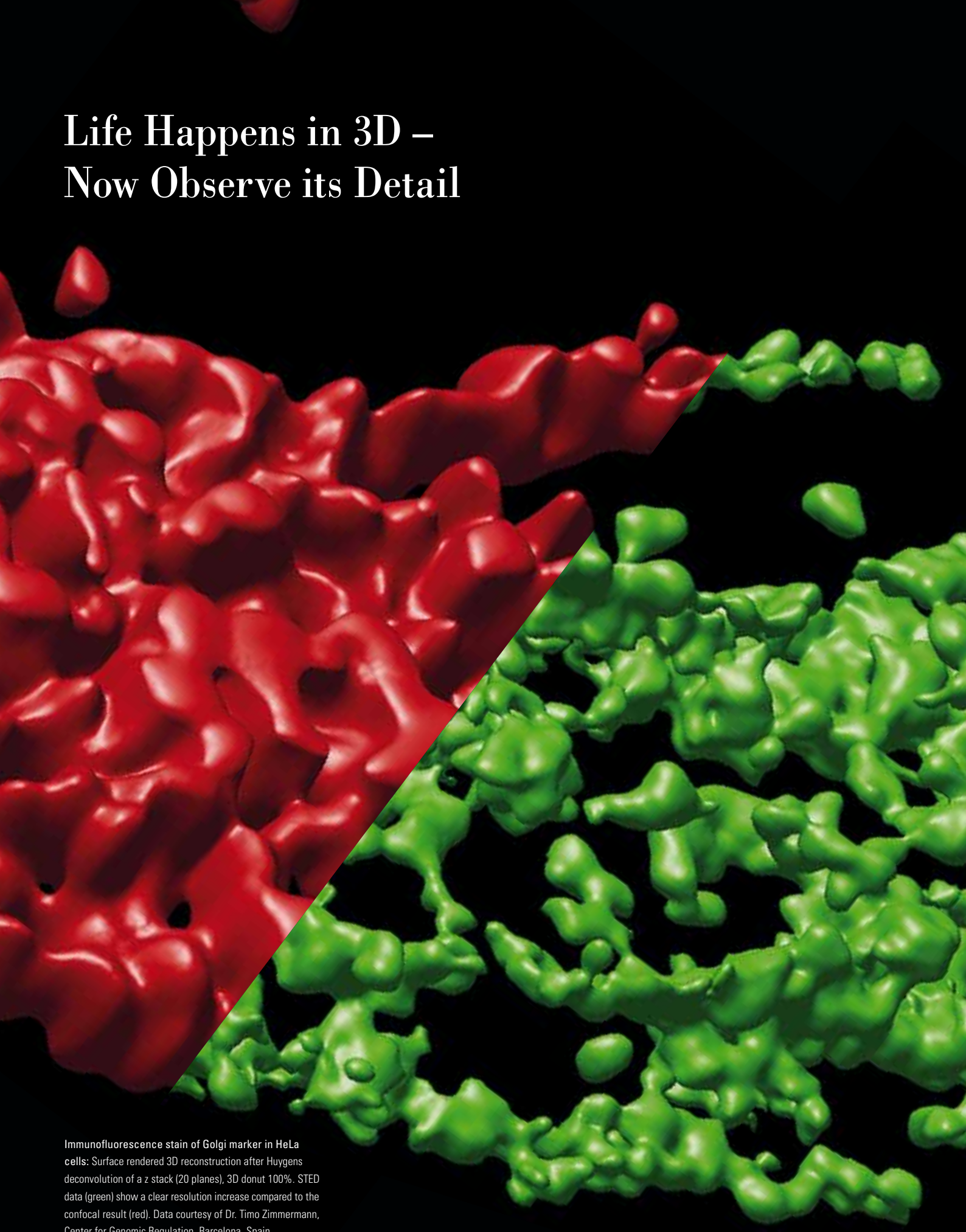
- Hell, S. W. & Wichmann, J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. Optics letters 19, 780-782 (1994).
- Willig, K. I., Harke, B., Medda, R. & Hell, S. W. STED microscopy with continuous wave beams. Nature methods 4, 915-918, doi:10.1038/nmeth1108 (2007).
- Vicidomini, G. et al. Sharper low-power STED nanoscopy by time gating. Nature methods 8, 571-573 (2011).

The resolution of STED can be approximated by a modified Abbe equation advanced by Stefan Hell in 1994. *n* is the refractive index; α is the half-angle of the maximum cone of light that can enter; λ is the STED wavelength. I is the intensity of the STED laser, and I_S a fluorophore, STED wavelength and detection gate specific parameter. Resolution is no longer limited but directly dependent on STED laser intensity.

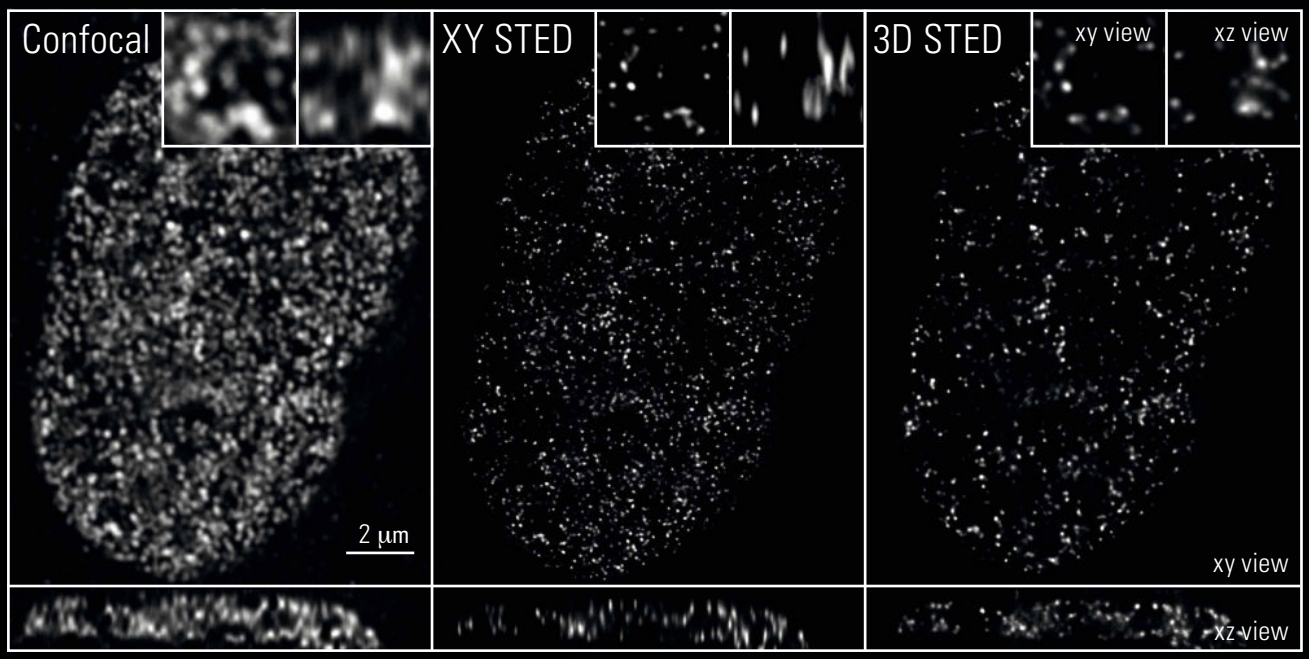
$$\Delta x = \frac{\lambda}{2n\sin\alpha\sqrt{1+\frac{I}{I_S}}}$$

The resolution of STED – the principle

Life Happens in 3D – Now Observe its Detail

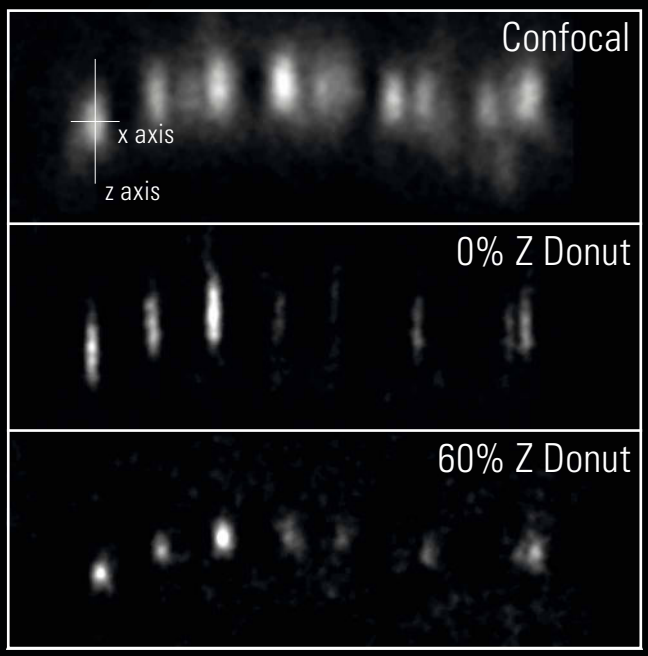


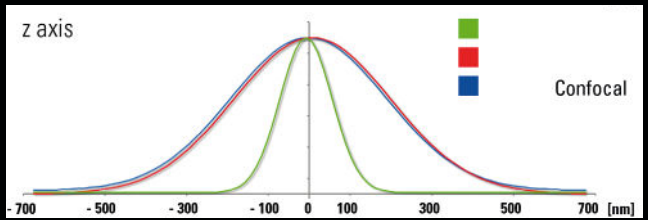
Immunofluorescence stain of Golgi marker in HeLa cells: Surface rendered 3D reconstruction after Huygens deconvolution of a z stack (20 planes), 3D donut 100%. STED data (green) show a clear resolution increase compared to the confocal result (red). Data courtesy of Dr. Timo Zimmermann, Center for Genomic Regulation, Barcelona, Spain.



Histone H3-Alexa 568 in HeLa cells: The highest lateral resolution increase is achieved by the vortex donut, maximal resolution increase in x, y and z by 3D STED. Note the loss of structures of objects that are not in the super-resolved focus plane when using 3D STED. 3D STED also achieves an increase of resolution in the xy dimension.

XZ axis plot of Histone H3-Alexa 568 in HeLa cells.
0% z donut allocates all STED light to the vortex donut resulting in maximal resolution increase in x and y, but not in z compared to confocal microscopy. A small focal volume is achieved by using 60 % of STED light in the z and 40% in the vortex donut.





Push the Boundaries!

Organisms, tissues and cells are three dimensional. To investigate cellular processes you want to consider all directions. The new TCS SP8 STED 3X allows you to extend the boundaries of super-resolution in all dimensions.

DISCOVER MORE DETAILS IN X, Y AND Z

With TCS SP8 STED 3X, two STED light paths generate different STED patterns (see figure below). For best resolution in x and y the light is allocated to the STED pathway, which creates the established STED donut by a vortex phase mask. The resulting effective focal volume, the point spread function (PSF), is rodshaped (see figure at the bottom right). A second light path with a different phase mask forms a z donut, yielding resolution increase mainly in z but also in x and y. Ultra-thin optical sections reveal previously unseen details irrespective of the orientation of the specimen.

ENGINEER YOUR PSF TO YOUR SCIENCE

TCS SP8 STED 3X gives you the possibility to match the resolution of your microscope in all dimensions to your scientific question and specimen. It's not an "either/or" decision between the classic STED path with best resolution in xy or the novel z donut. A variable allocator allows you to freely distribute the light to both paths. You can choose between the best lateral resolution, best vertical resolution or anything in between to get optimal results. The smallest focal volume ever as well as spherical isotropic PSF are adjustable. The new three-dimensional STED is fully integrated into LAS AF (Leica Application Suite, Advanced Fluorescence) software and intuitively controlled. A sketch of the effective PSF gives live feedback on how instrument parameters influence the obtained resolution. Super-resolution imaging has never been more flexible. Tailor your effective PSF to your needs – online during a live scan.

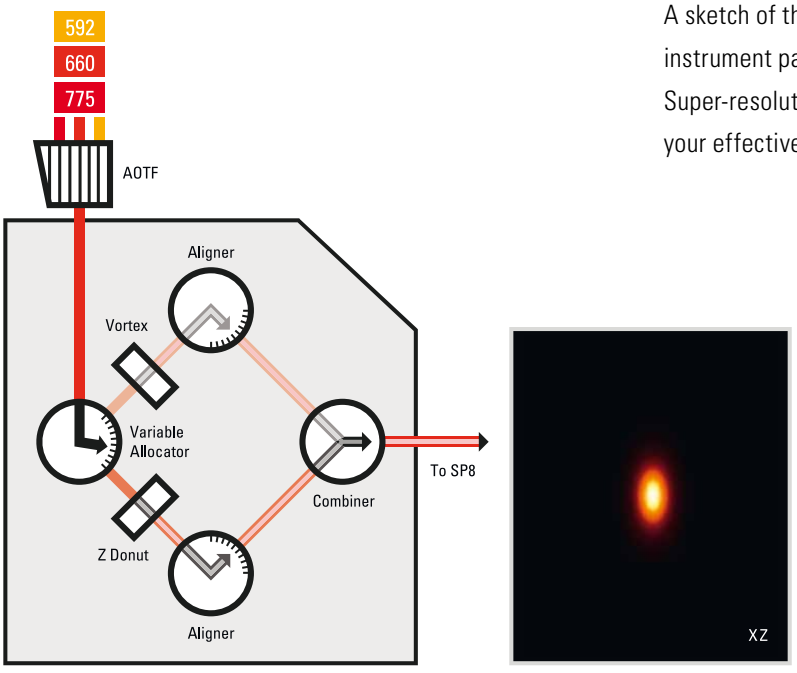
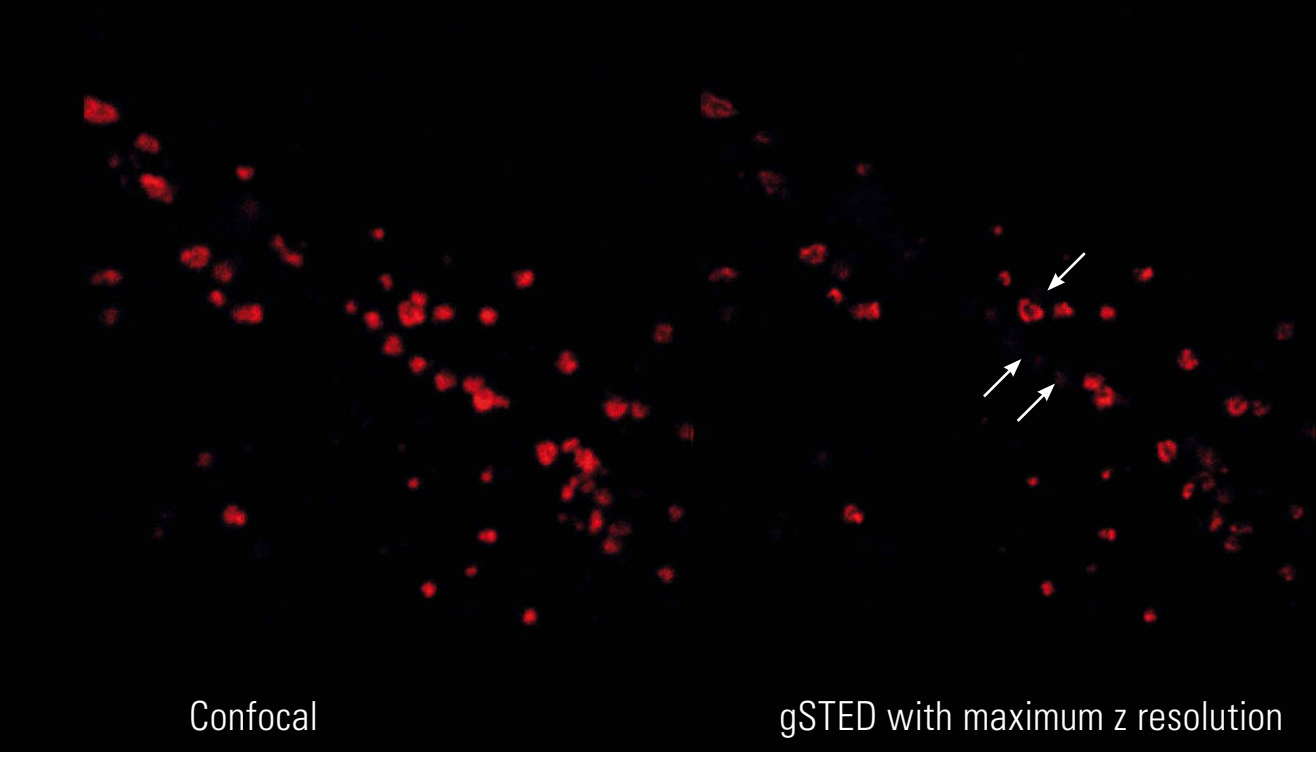


Diagram of the available light paths in the STED 3X module.
Light can be freely allocated to both paths.
Left: sketch of STED 3X extension.
Right: resulting effective PSF.



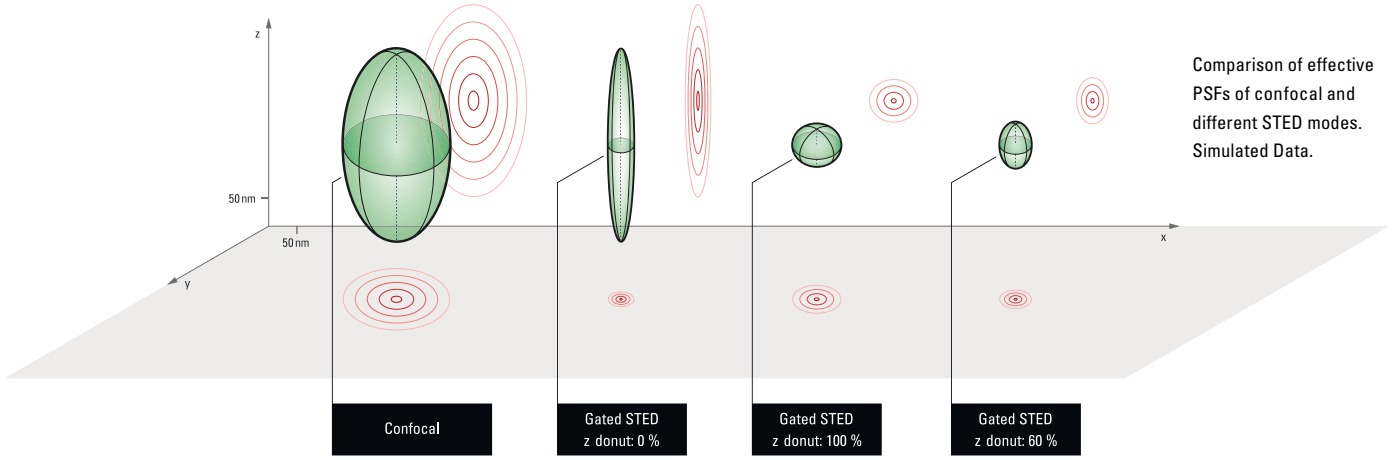
Immunostaining of endosomal marker (Lamp M) in HeLa cells. Note the loss of structures out of super-resolved focal plane (white arrows) in the middle compared to confocal images. Courtesy of Shem Johnson, University of Geneva, Switzerland.

Confocal

gSTED with maximum z resolution

PUSH THE LIMITS EVEN FURTHER

The powerful Huygens STED deconvolution package, exclusively supplied with every TCS SP8 STED 3X system, helps to improve your data. Huygens decreases noise levels dramatically and also enhances resolution in x, y and z. After deconvolution of STED data, even smaller details are resolved in all dimensions. As you can compare the outcome directly with your raw data, you avoid being misled by image processing artifacts. Leica and SVI (Scientific Volume Imaging) have not only enabled Huygens to handle 3D STED data. The newly developed LAS AF ↔ Huygens data exchange also facilitates time-saving interaction of the two software packages. One mouse click sends acquired data to Huygens, where you can directly start deconvolution. And deconvolved images are just as easily sent back to LAS AF for data storage, quantification or advanced visualization.



Comparison of effective PSFs of confocal and different STED modes. Simulated Data.

See the Full Spectrum of Life

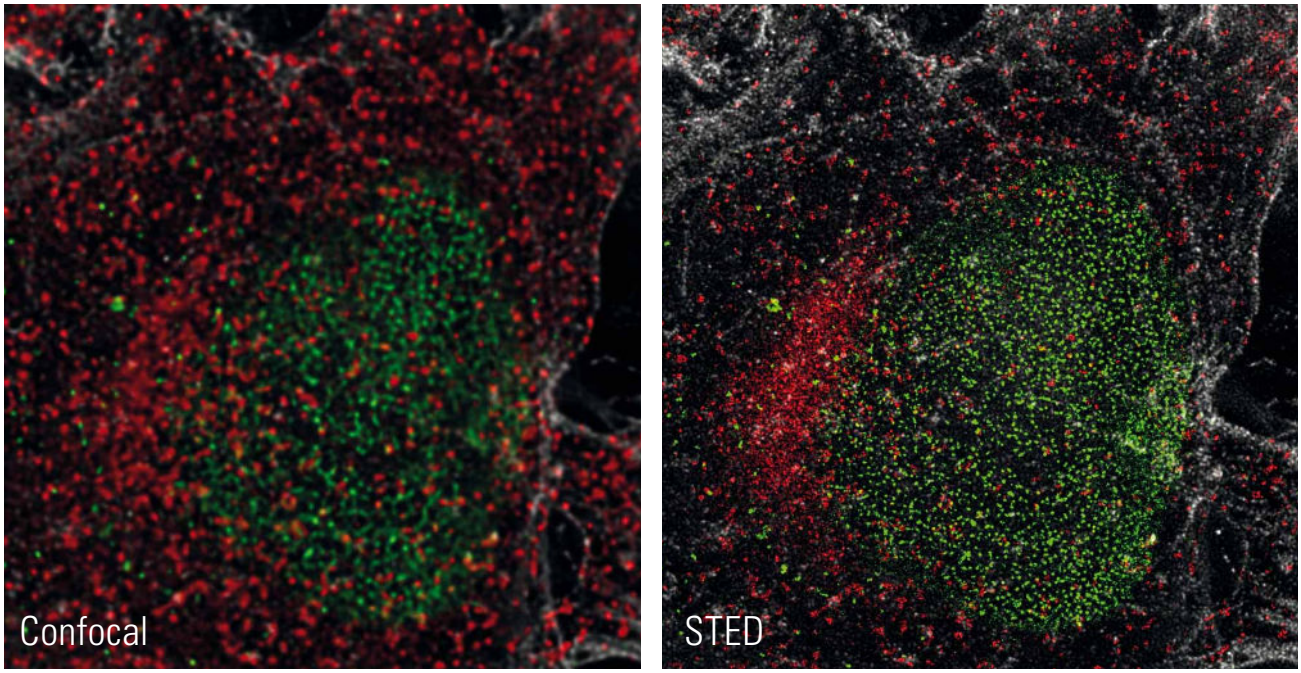
Seeing subcellular structures in nanoscopic detail opens a new world to scientists. Multicolor applications give access to detailed information about the interrelationships of various structures. The new STED 3X module offers multiple STED laser lines at different wavelengths in one instrument. Super-resolved colocalization studies have never been so easy.

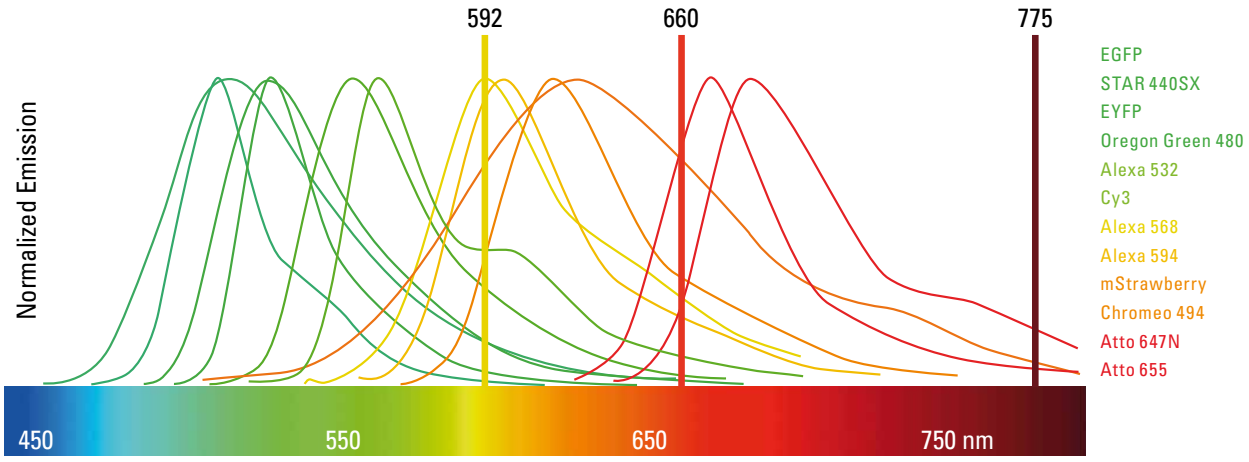
TUNABLE SPECTRAL SUPER-RESOLUTION – THE FREEDOM TO CHOOSE

Super-resolution and standard labeling strategies are not mutually exclusive. A broad variety of popular dyes and fluorescent proteins in the green spectrum of light can be used for STED with the proven STED laser at 592 nm. With two additional STED laser lines at the new TCS SP8 STED 3X – one continuous wave laser at 660 nm and a pulsed laser at 775 nm – Leica opens up the whole spectrum of visible light and gives you access to even more applicable fluorophores.

The Leica White Light Laser, the AOBS™ (acousto optical beam splitter) and the tunable spectral detector synergistically enable you to image any kind of fluorophore combination and give you the highest flexibility for your multicolor super-resolution experiment.

Triple immunostaining in HeLa cells: Three colors are achieved with one STED line. Green: NUP 153-Alexa 532, red: Clathrin-TMR, white: Actin- Alexa 488. 660 nm gated STED.





	592 GATED/CW	660 GATED/CW	775 PULSED
Strength	GFP/YFP	Multicolor	Most established spectral range
Colocalization studies	+	++	+
Photostability	+	++	++
Live cell	++	+	(+)

COLOCALIZATION BEYOND LIMITS

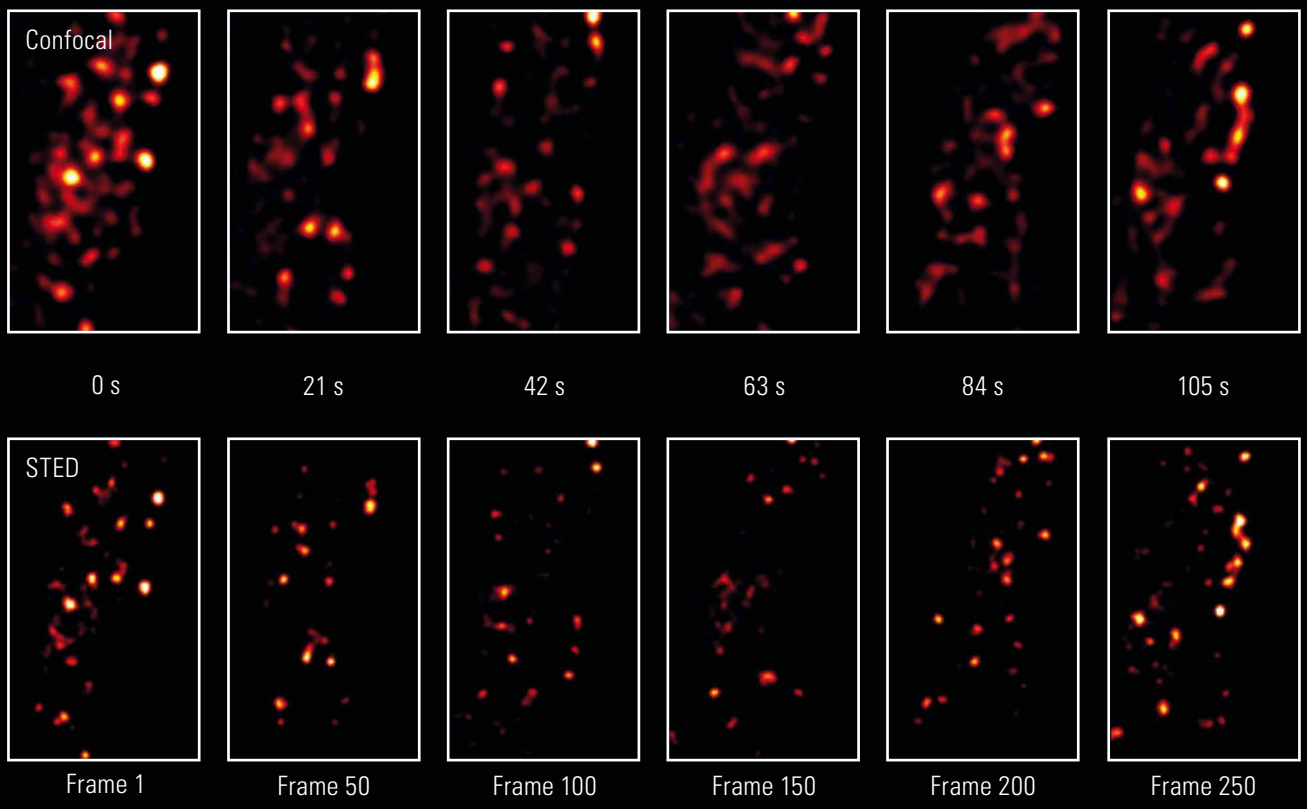
In order to investigate spatial relationship between different structures it is essential to investigate them in one experiment with different labels. Two-color applications are routinely performed with the 592 nm STED laser using the appropriate dye pair. With the additional STED lasers of TCS SP8 STED 3X, you not only have a larger selection of fluorophores to choose from, you can even have more than two colors in one experiment. Discover protein interactions and colocalization beyond the limits. More colors make the difference!

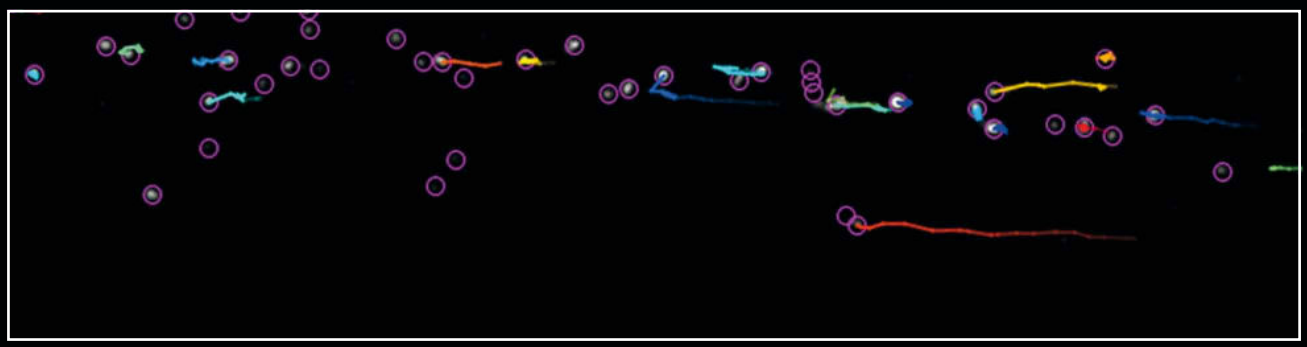
STED WHITE – ENJOY THE FULL SPECTRUM

Objectives are the eyes of every microscope and critical for determining the resolving power of a confocal system. An objective's transmission and color correction influence excitation and detection efficiencies. Based on the excellent Leica CS2 objectives, Leica Microsystems has designed a new objective with optimal chromatic correction and transmission for TCS SP8 STED 3X. The Leica HC PL APO 100x/1.40 OIL STED WHITE enables you to perform STED microscopy in the full spectrum of visible light.

"Adding the third dimension and an additional STED line to STED imaging, STED 3X allows us to see things that were impossible to see before."

Dr. Timo Zimmermann,
Center for Genomic Regulation,
Barcelona, Spain.





Times series: ANF GFP labeled dense core vesicles moving along axons ca. 10 µm deep inside an intact anaesthetized drosophila larva. A confocal and STED image were recorded every 0.45 seconds.
Upper panel: Confocal.
Middle panel: 20% STED light with gate start at 1 ns.
Lower panel: Particle tracking performed by TrackMate (FiJi). Sample Courtesy of Prof. Stephan Sigrist, FU Berlin, Germany.

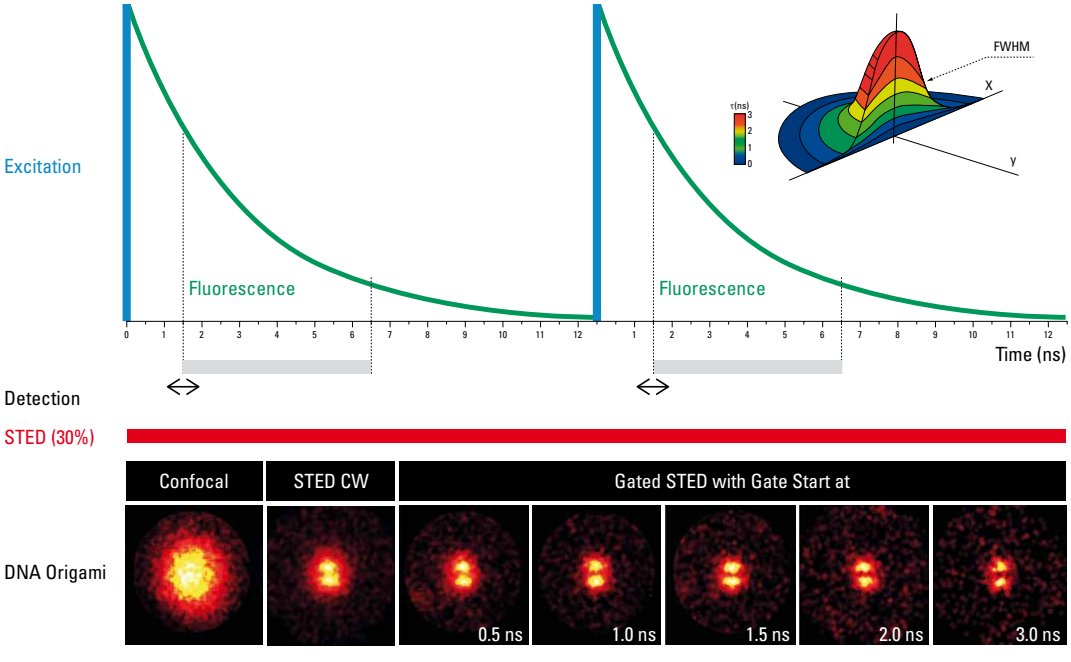
Enter Your Gateway to Live Cell Super-Resolution

Gated STED substantially extends the functionality of the proven STED CW, giving you the option of higher resolution or lower laser power. More images are obtained and smaller details are revealed.

SHARPER IMAGES AT LOWER POWER

With STED CW imaging, resolution is dependent on the lifetime of the fluorophore. The STED laser silences fluorophores with long lifetimes more efficiently than short-living ones. An instantly fluorescing molecule cannot be turned off by stimulated emission, as there is no time for the photons of the STED CW laser to return it to the ground state. The longer a fluorophore stays in the excited state on average, the higher the chance for stimulated emission and therefore the better the resolution. Thereby, the lifetimes of excited electrons are not evenly distributed in the effective STED CW excitation spot. Long-living states are found in the center, whereas short-living states are located at the periphery where the STED laser is active. Observing only the long-lived states further shrinks the effective spot. Smaller details can be observed without the need to apply more STED light, which increases the viability of living cells.

Leica's HyD™ detectors together with the White Light Laser as a pulsed excitation source offer the possibility to detect only in a certain time gate after the excitation pulse. Starting the detection 0.5 ns later already increases resolution significantly. By shifting the time gate away from the excitation pulse a resolution far below 50 nm can be reached. With the same laser power, gated STED achieves a more than 50% higher resolution than STED CW. Alternatively, gated STED uses less laser power for the same resolution, thus preserving photostability and increasing live cell capability. Super-resolved recordings of GFP-labeled vesicles moving inside a living drosophila larva over hundreds of frames become a reality.



Resolution increase by gated STED: Upper right corner: lifetime distribution of fluorophores within an effective STED CW PSF. Short-living states (blue/green) are not contributing to the according time gated image.

Smart STED Workflow:

1: Adjust the desired effective PSF with the STED and 3D slider.

2: Balance signal-to-noise and number of images with the dosage slider.

3: Define the area of interest during a confocal live scan and adjust the excitation accordingly.

4: Collect your super-resolved data by capturing an image or starting a series.

"The ability to do STED in 3D will bring our research to the next level – right on spot of the researcher's needs."

Dr. Christian Eggeling,
University of Oxford, UK.

Less Time for Set-up, More Time for Research

With increasing speed of research and more and more projects to handle, you do not want to waste time with elaborate system care. The TCS SP8 STED 3X attends to the alignment of laser beams and offers maximum convenience for setting up and controlling your experiments by implemented software tools.

AUTO-ALIGNMENT IS ONE MOUSE CLICK AWAY

Accurate spatial overlay of foci generated by the excitation laser and the STED laser is crucial for optimal results. TCS SP8 STED 3X ensures this using a software-controlled integrated alignment route, which automatically adjusts the lasers. The entire calibration routine takes place inside the scanner chassis without illuminating your specimen. Auto-alignment is activated by a single mouse click and completed within a few minutes at most. You don't need to change the specimen or worry about instrument settings. Save time for imaging and immediately continue generating reliable data.

KNOW YOUR RESOLUTION

STED microscopy is the fast and direct way to super-resolution. Application-specific modules integrated into LAS AF (Leica Application Suite, Advanced Fluorescence) makes your work convenient. A sketch of the estimated effective PSF gives you direct and online visual feedback on the effects of your chosen technical parameters on the achieved focal volume. Fit your focus to your science.

SMART STED

Leica has implemented the Smart STED Wizard into LAS AF as an additional innovation. It starts from your needs rather than defining technical parameters. The intuitive workflow allows you to operate the instrument with three simple sliders. Assisted by the sketch of the effective PSF you define the general level of resolution increase and the amount of super-resolution in 3D. With a third slider you adjust between signal-to-noise and the number of achievable images for your application. For the optimal outcome the wizard controls all necessary settings like STED laser intensity, pixel size, z step size, the pinhole, gate settings and averaging. Focus on your science rather than on system setup!

Software features that facilitate your experiments:

› Auto alignment of laser beams
› Smart STED wizard and online sketch of estimated PSF
› System optimized xy format and number of z slices
› Huygens STED Deconvolution Package included
› LAS AF ↔ Huygens Data exchange

A Platform that Grows with Your Research

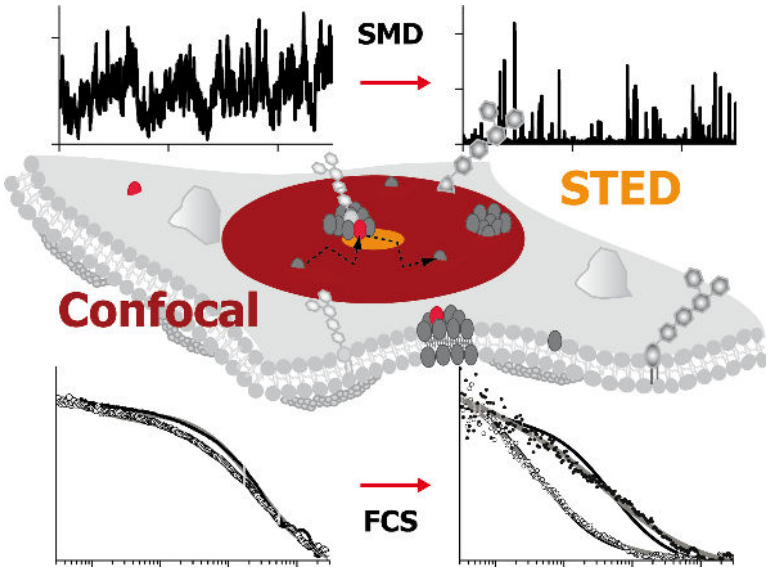
Life sciences are continuously changing, and it may be difficult to say which direction your research will take in the future. The modular concept of the TCS SP8 and TCS SP8 STED 3X offers you maximum flexibility in choosing your options. No matter where you start, you can configure additional functionality as your requirements evolve. Your investment in a TCS SP8 will pay off – now and in the future.

LEICA INNOVATIONS – PERFECT SYNERGIES FOR HIGHER PERFORMANCE

STED 3X microscopy is fully integrated into the TCS SP8 platform. With TCS SP8 STED 3X you always get a high-end confocal at the heart of the system. Superior optics combined with our multispectral HyD™ detectors and the Leica White Light Laser increase sensitivity and contrast while reducing laser power. This results in images of super-resolved real optical sections, even from weakly stained specimens. Resonant scanning technology combined with gated STED provides best results in super-resolved live cell imaging. The 12 kHz resonant scanner is able to record up to 420 fps at 512x16 format for super-resolution at maximum speed. Single molecule detection (SMD) with STED-FCS (fluorescent correlation spectroscopy) is also feasible. Leica innovations work together. Benefit from their synergies.

FOR EVERY IMAGING APPLICATION LEICA HAS A CONFIGURATION TO MATCH

The modular TCS SP8 STED 3X allows you to enter the confocal super-resolution world at any level. One STED laser or super-resolution over the full spectrum? Best resolution in xy or 3D? Acquire the system you need now and upgrade later. Your next project may have additional requirements: More colors, more resolution, more flexibility. You can purchase additional STED laser lines, the 3D STED functionality or gated STED whenever you need them and on almost all TCS SP8 configurations. 50 nm lateral resolution is already available on the TCS SP8 with a compact supply unit. Upgrade to TCS SP8 STED 3X – at any time!



STED-FCS: Small observation volumes created by STED allow the recording of single molecule based fluctuations. FCS data can be acquired at much higher concentrations than using diffraction-limited confocal microscopy.

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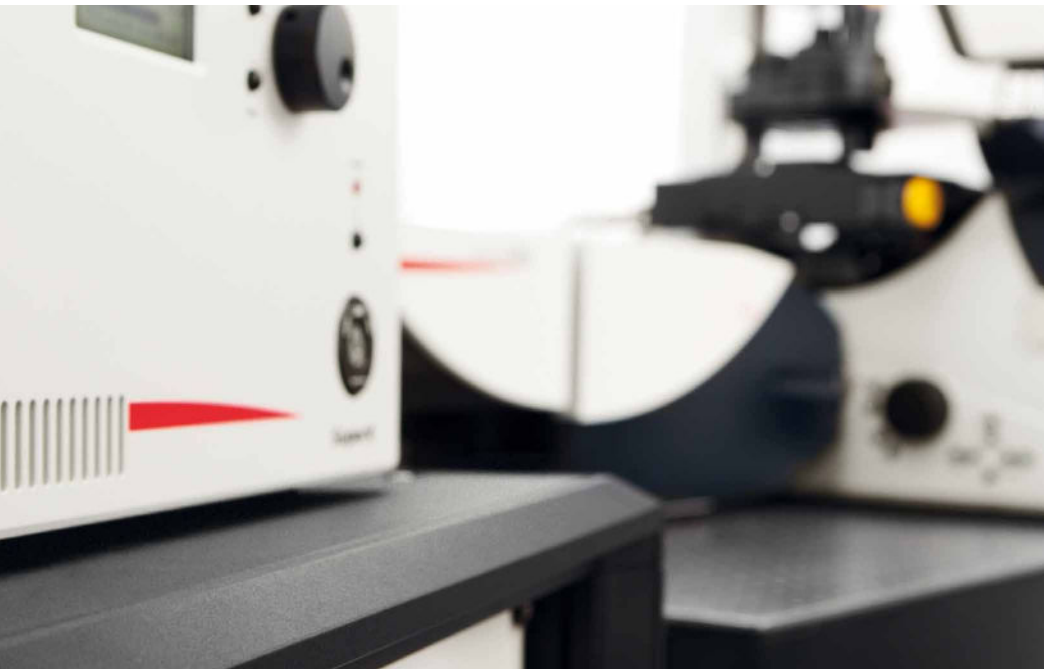
www.leica-microsystems.com/pdf/sted-application



All the Information You Want

Are you curious to delve into the world of microscopy? Do you need experienced advice? Or do you just want to know more about the TCS SP8 STED 3X? Get in touch with Leica Microsystems – connect with us on our online platforms!

LEICA SCIENCE LAB: LEARN. SHARE. CONTRIBUTE.

The knowledge portal of Leica Microsystems offers scientific research and teaching material on the many subjects of microscopy. The platform is designed to support beginners, experienced practitioners and scientists alike in their everyday work and experiments. Explore interactive tutorials and application notes, understand the basics of microscopy and study high-end technologies. Stay informed about about interesting meetings and by attending free webinars.

More than 350 authors from all over the world have contributetd to Leica Science Lab and there will be more. You are very welcome to join this community and share your expertise!

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Science Lab
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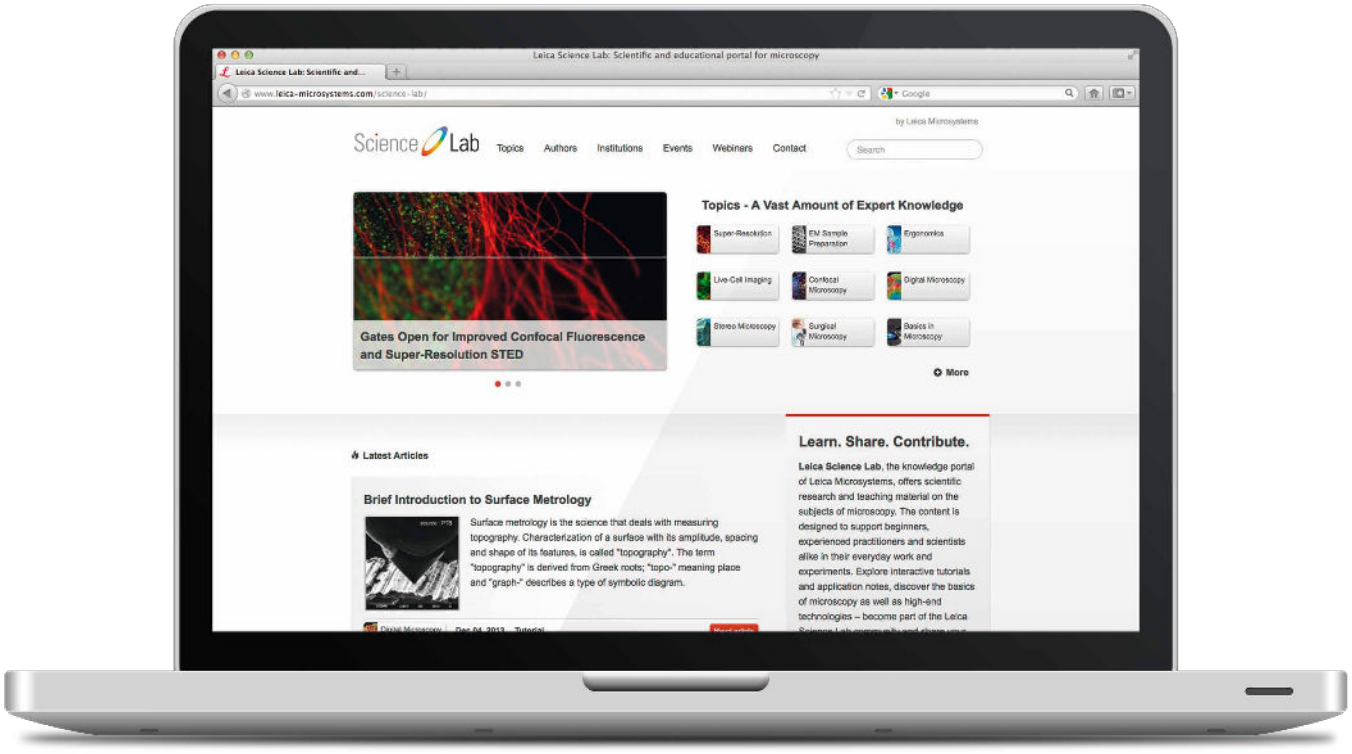

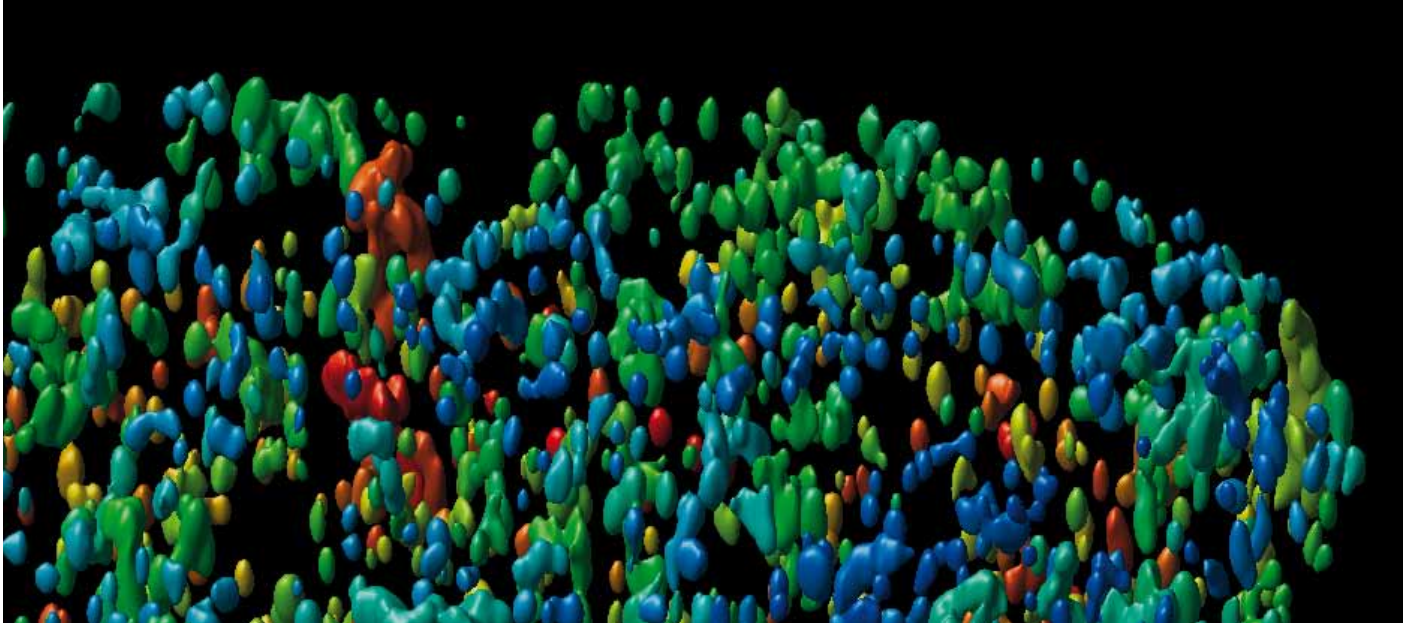

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