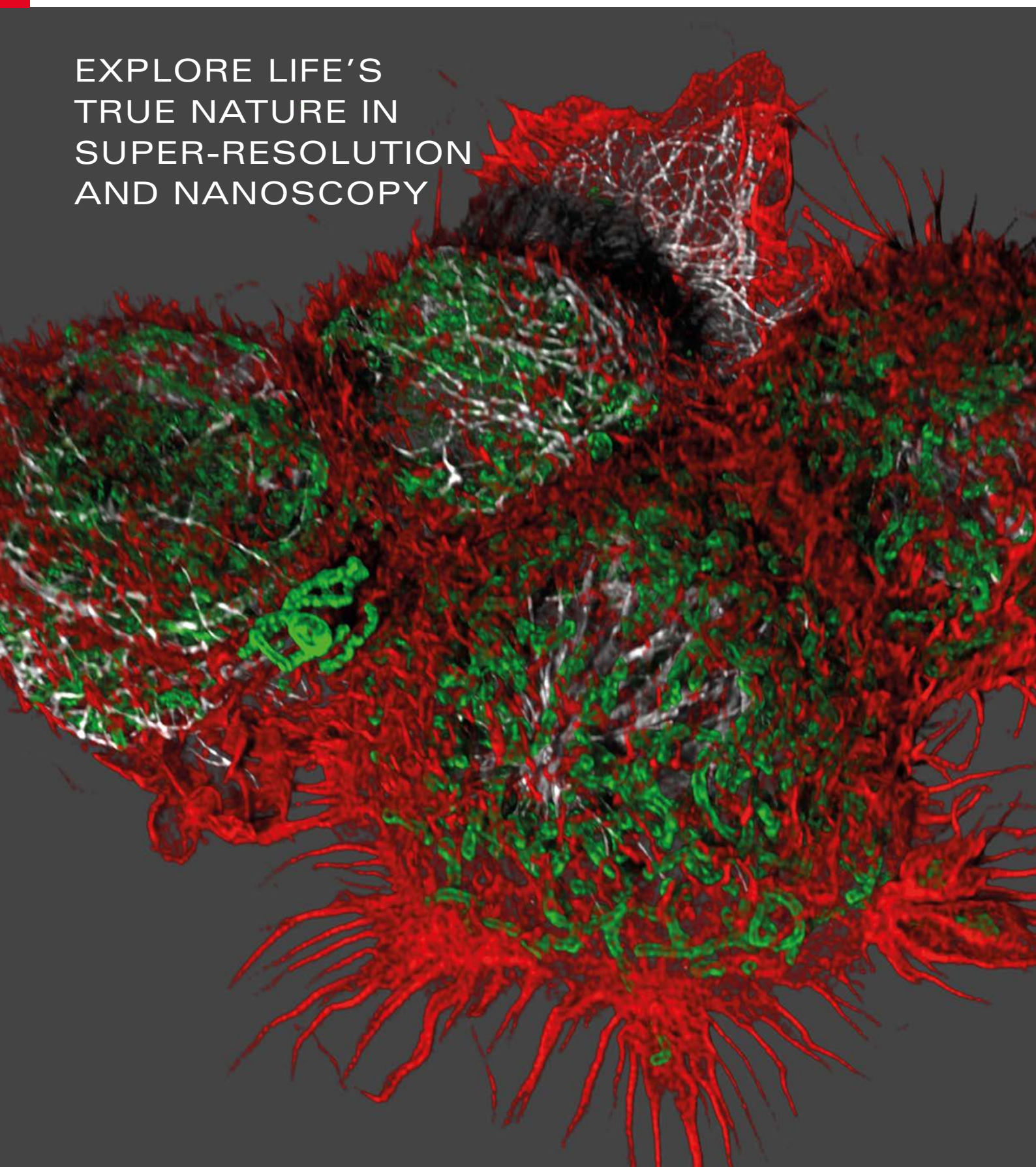


From Eye to Insight

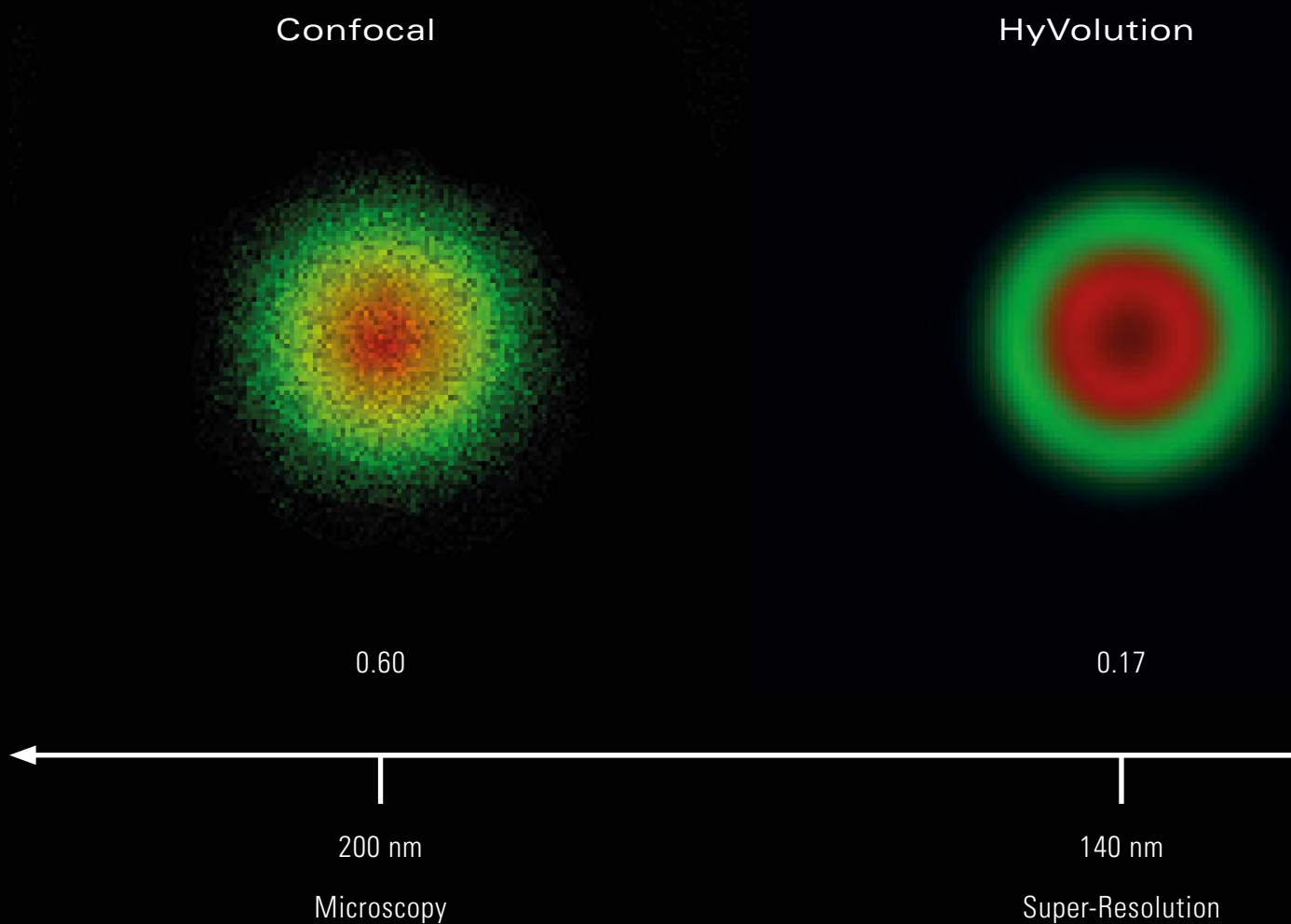


EXPLORE LIFE'S
TRUE NATURE IN
SUPER-RESOLUTION
AND NANOSCOPY



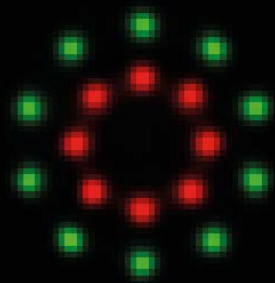
EXPLORE THE TRUE NATURE OF YOUR SPECIMENS

YOU DECIDE WHICH LEVEL OF DETAIL ANSWERS YOUR QUESTION



Simulation data, courtesy of Remko Dijkstra, Scientific Volume Imaging Deconvolution powered by Huygens – SVI
Cover Image: COS-7 cells. Sample: courtesy of Dr. Jana Doeber, Center of Microscopy and Image Analysis, University of Zurich, Switzerland.

STED



0.00

Pearson
Coefficient

30 nm

Nanoscopy

Molecule interaction in fluorescence microscopy is often quantified by colocalization coefficients which are robust and easy to interpret. However, this analysis crucially depends on the resolution of the microscope.

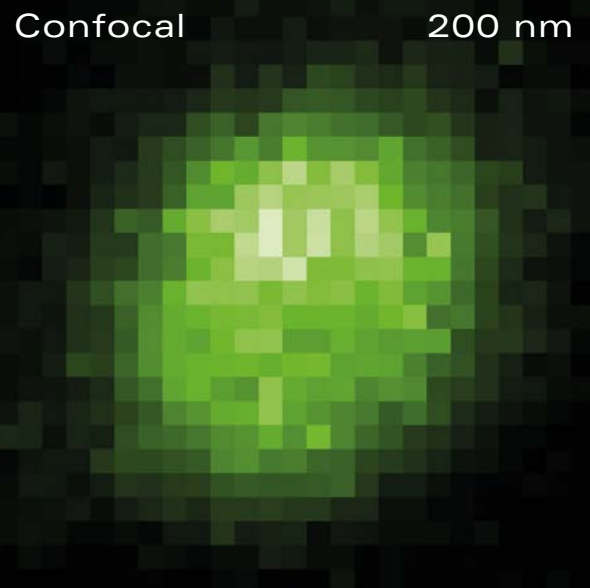
This data demonstrates the information gain realized by super-resolution and nanoscopy.

In the confocal microscope the structure appears as two colocalizing spheres, quantified with a Pearson correlation coefficient of 0.6, indicating significant correlation.

HyVolution yields super-resolution down to 140 nm by combining optimal confocal imaging with Huygens from SVI. HyVolution reveals that there are actually two concentric rings showing little colocalization.

Moving towards nanoscopy with STED resolves the true structure, and clearly defines the position of individual dots. The STED image finally reveals that the structures in both channels are not colocalized at all.

WHICH WAY DO YOU CHOOSE
TO DISCOVER THE TRUTH?



Sample: Paramecium, courtesy of A. Aubusson-Fleury, CNRS I2BC, Gif sur Yvette, France

In design, form follows function. In nature,
function evolves into structure.

Leica super-resolution and nanoscopy systems
are designed to help you discover the truth.
You can now take the next evolutionary step
from microscopy to nanoscopy.

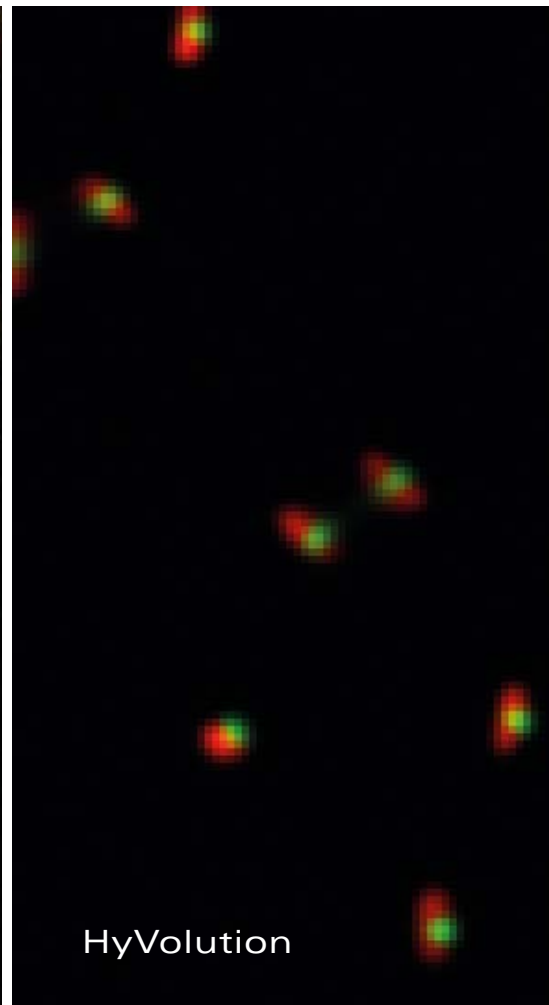
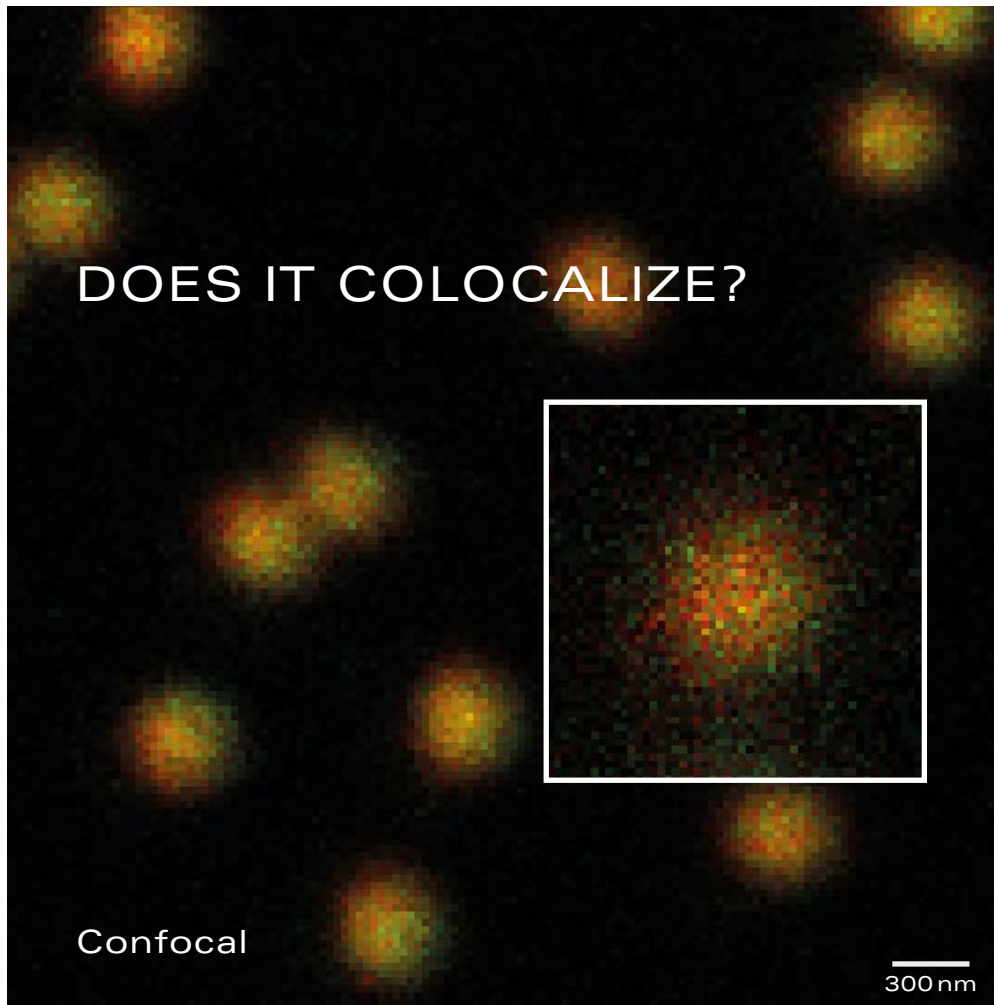
HyVolution

140 nm

STED

30 nm

30nm

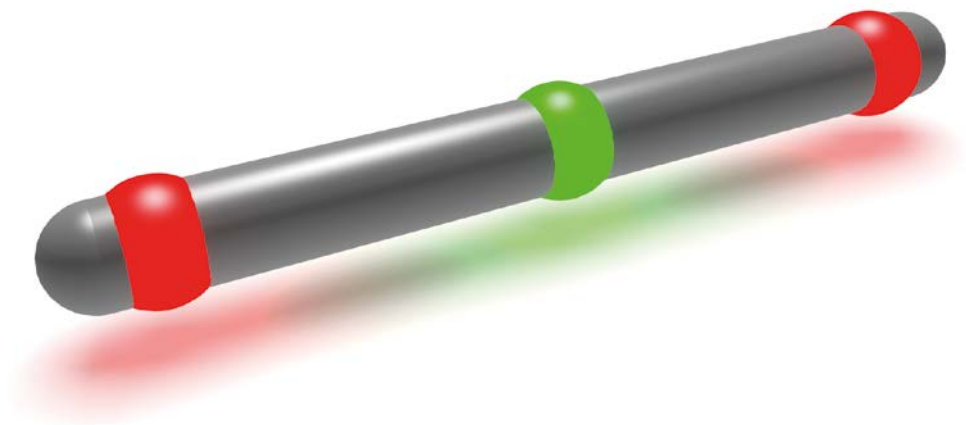


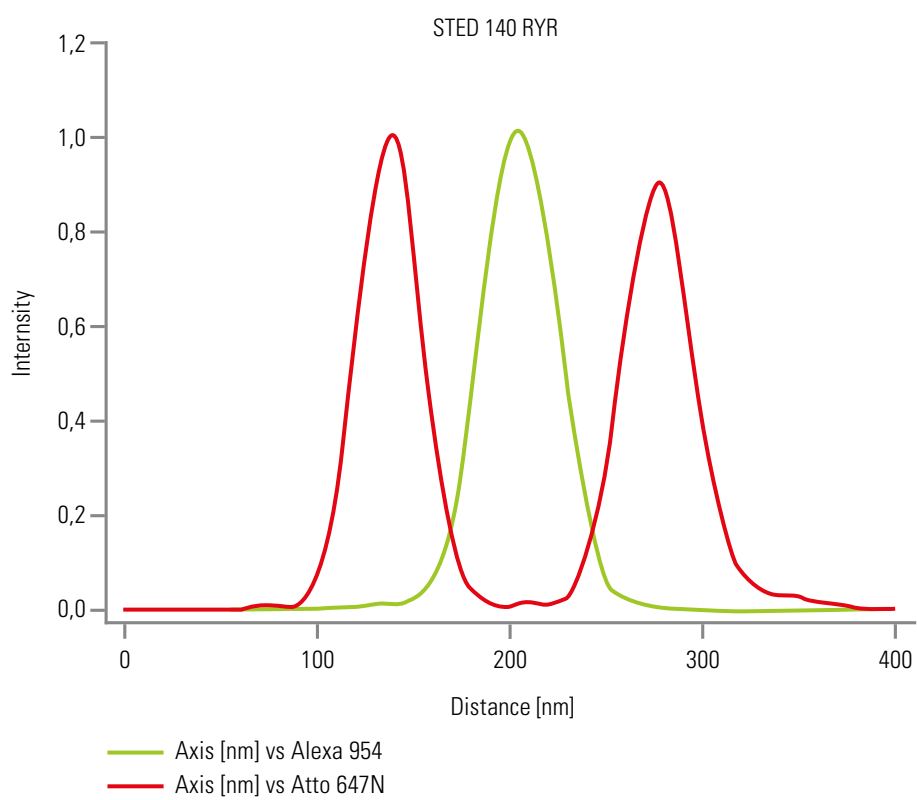
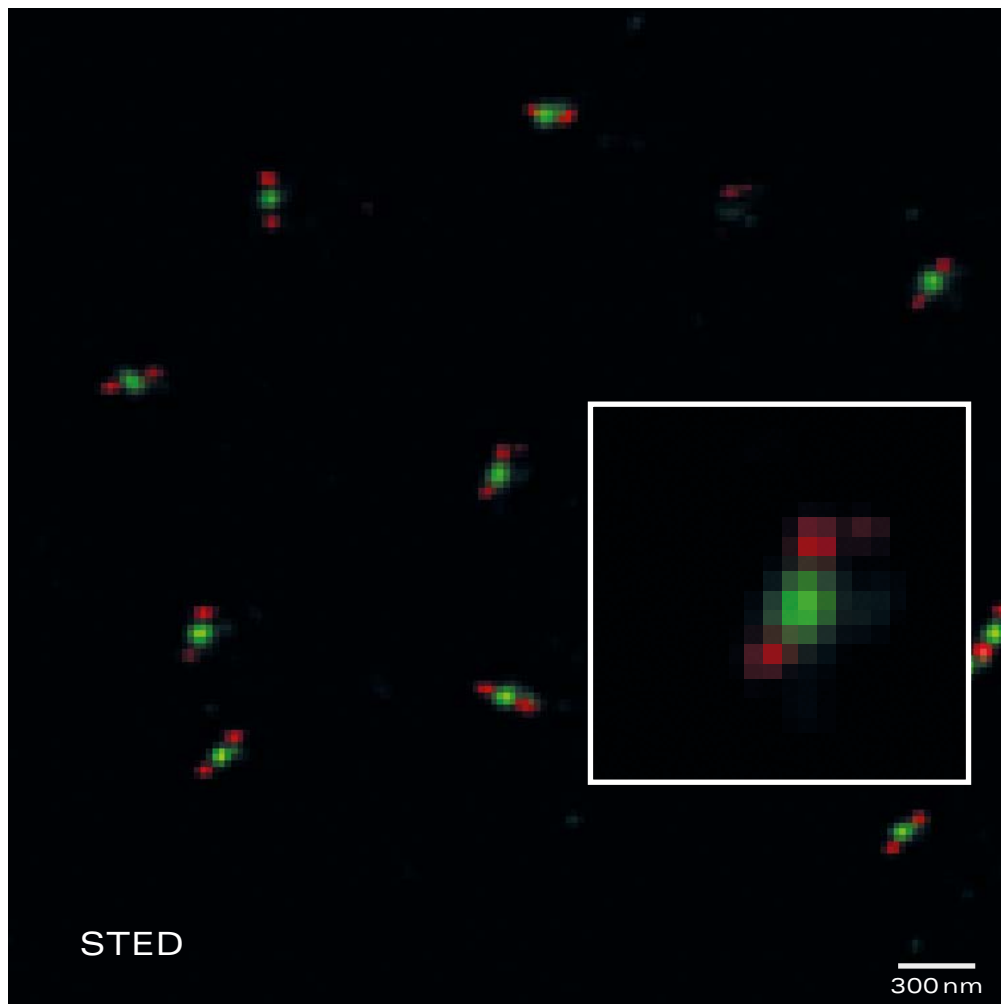
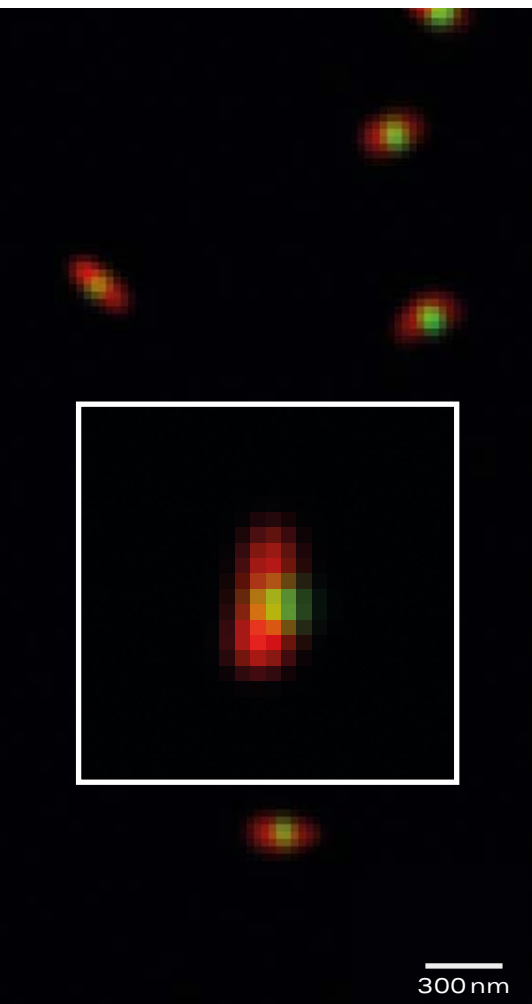
Nanorulers STED 140 RYR as imaged with confocal, HyVolution and STED. Judging from the confocal data it looks as if red and green structures are colocalized. Pearson's correlation coefficient as a measure for colocalization of 0.81 is very high. STED reveals the true nature of the structure (Pearson correlation coefficient of 0.14). Sample courtesy of GATTaQuant.

COLOCALIZATION DONE RIGHT

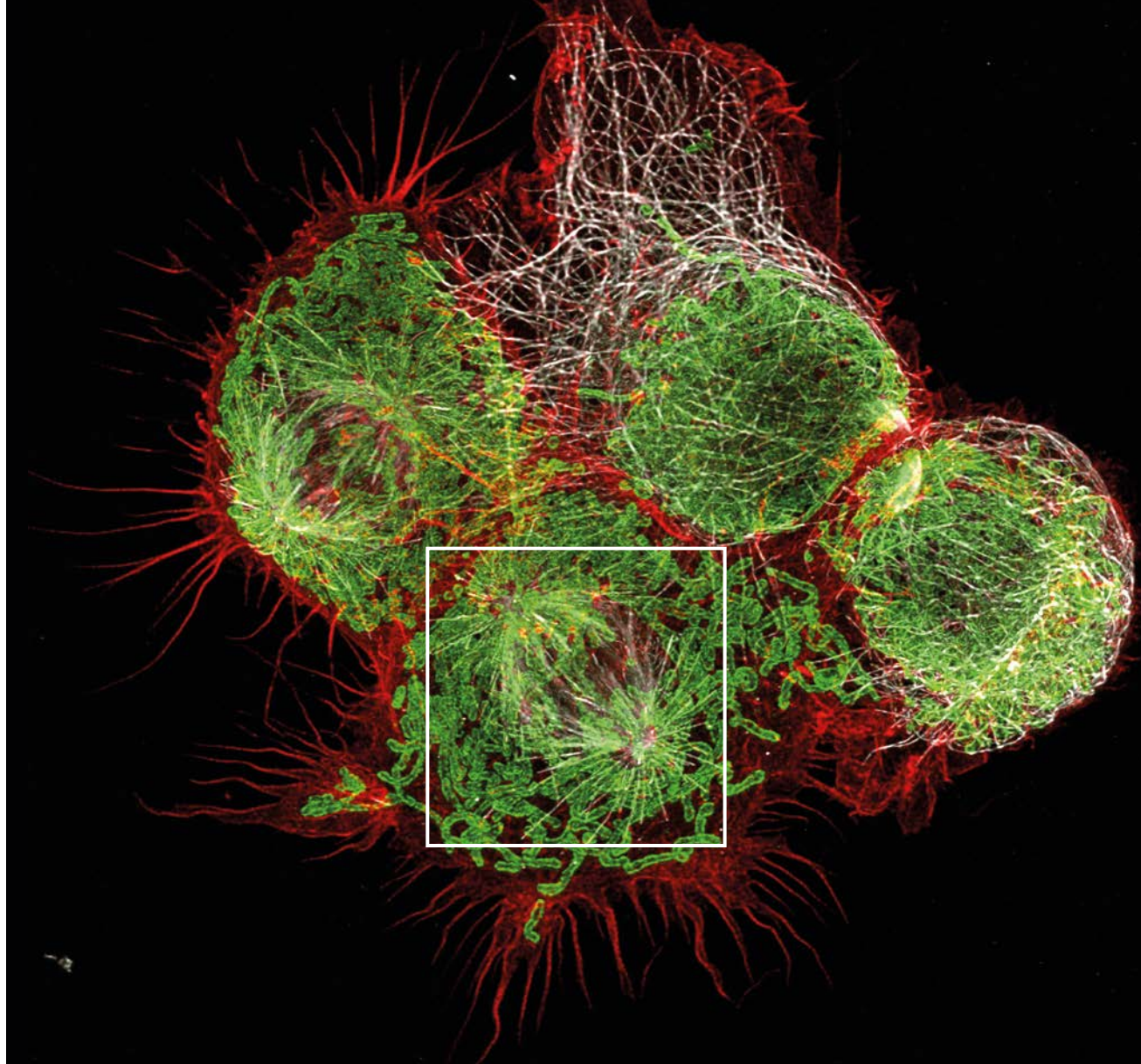
When studying molecular interactions in microscopy colocalization analysis is an indispensable tool due to its straightforward approach and robustness. The key to success is to pin down the volumes occupied by the molecules of interest correctly. That's why resolution matters.

Only super-resolution and nanoscopy represent colocalization precisely.





HYVOLUTION – ADDING COLOR TO SUPER-RESOLUTION



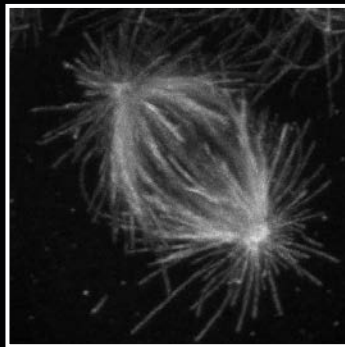
WOULD YOU LIKE SOME MORE?

Unlike super-resolution systems that use a single external detection channel HyVolution integrates seamlessly with all confocal imaging modalities. Therefore, you can record all five channels simultaneously – without the need for sequential scanning, which would only slow down the acquisition speed. With HyVolution you can quantify colocalization of multiple molecules correctly at full speed.

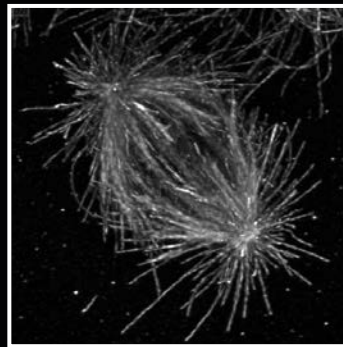
DEEPER INSIGHTS WITH HYVOLUTION

In conjunction with multiphoton excitation HyVolution reveals detail even at deep depths. There is an option to correct sample-induced spherical aberrations and reconstruct image information in deep image planes. Every multiphoton acquisition benefits from the resolution boost afforded by HyVolution – as deep as 800 μm .

Confocal



HyVolution



COS-7 cells. Sample: courtesy of Dr. Jana Doeber, Center of Microscopy and Image Analysis, University of Zurich, Switzerland.

DON'T SPEND YOUR TIME WAITING

Today's GPUs take HyVolution to a new level in terms of speed. HyVolution fully supports CUDA by NVIDIA, which leverages the highly parallelized computation on GPU. This way you can benefit from the underlying constrained iterative approach. This is the physically correct way to bring to light the information contained in your images – in an instant.

PARTNERING WITH THE BEST

HyVolution helps you drive your TCS SP8 confocal system towards super-resolution without compromise. Seamless integration with industry-leading Huygens by SVI means you can rely on more than 20 years of excellence in image restoration at the push of one button. With the photon budget afforded by Leica hybrid detectors (HyD) you have more freedom to use HyVolution to get the most out of your images.

STED – THE NEW STANDARD

MICROSCOPY BECOMES NANOSCOPY

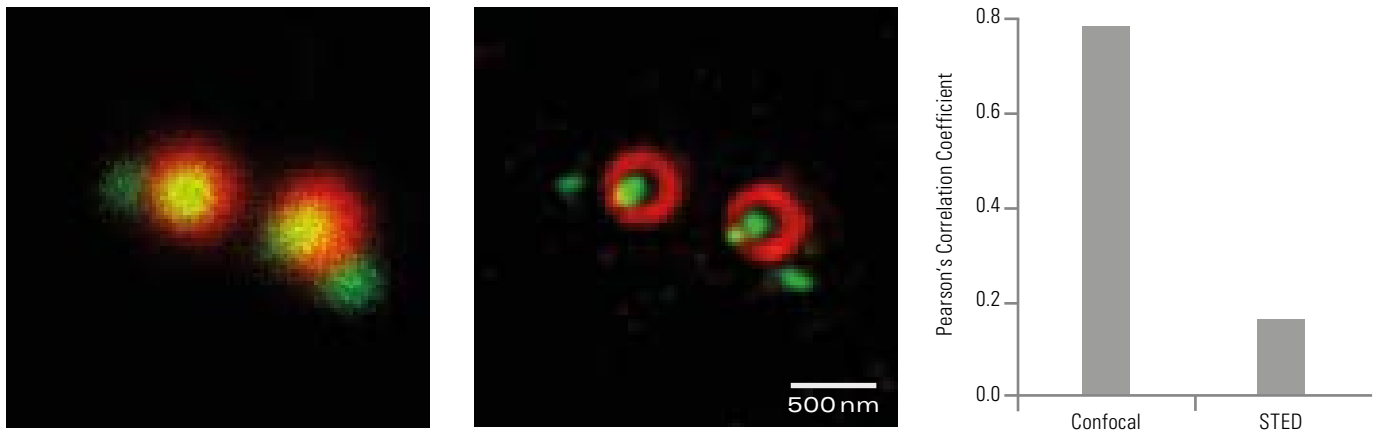
Microscopy beyond the diffraction limit has revolutionized the study of subcellular architecture and dynamics at the nanoscale and is on its way to becoming the new gold standard in fluorescence imaging (Nobel Prize for Chemistry 2014).

STED: NANOSCOPY IN 3D

The fully integrated STED (STimulated Emission Depletion) systems by Leica Microsystems meet the requirements of daily research, providing fast, intuitive, and purely optical access to structural details far beyond the diffraction limit. Resolution down to 30 nm becomes tunable in x,y and z. Life happens in 3D – now observe its details.

COLOCALIZATION PRECISION REDEFINED

Molecular interaction occurs on the nanoscale, so it is intrinsically difficult to discover with traditional confocal imaging. STED opens the door to the nanoscale, thus providing new insights into molecular interactions. Colocalization, a pre-requisite for the existence of molecular interactions, can be quantified reliably.



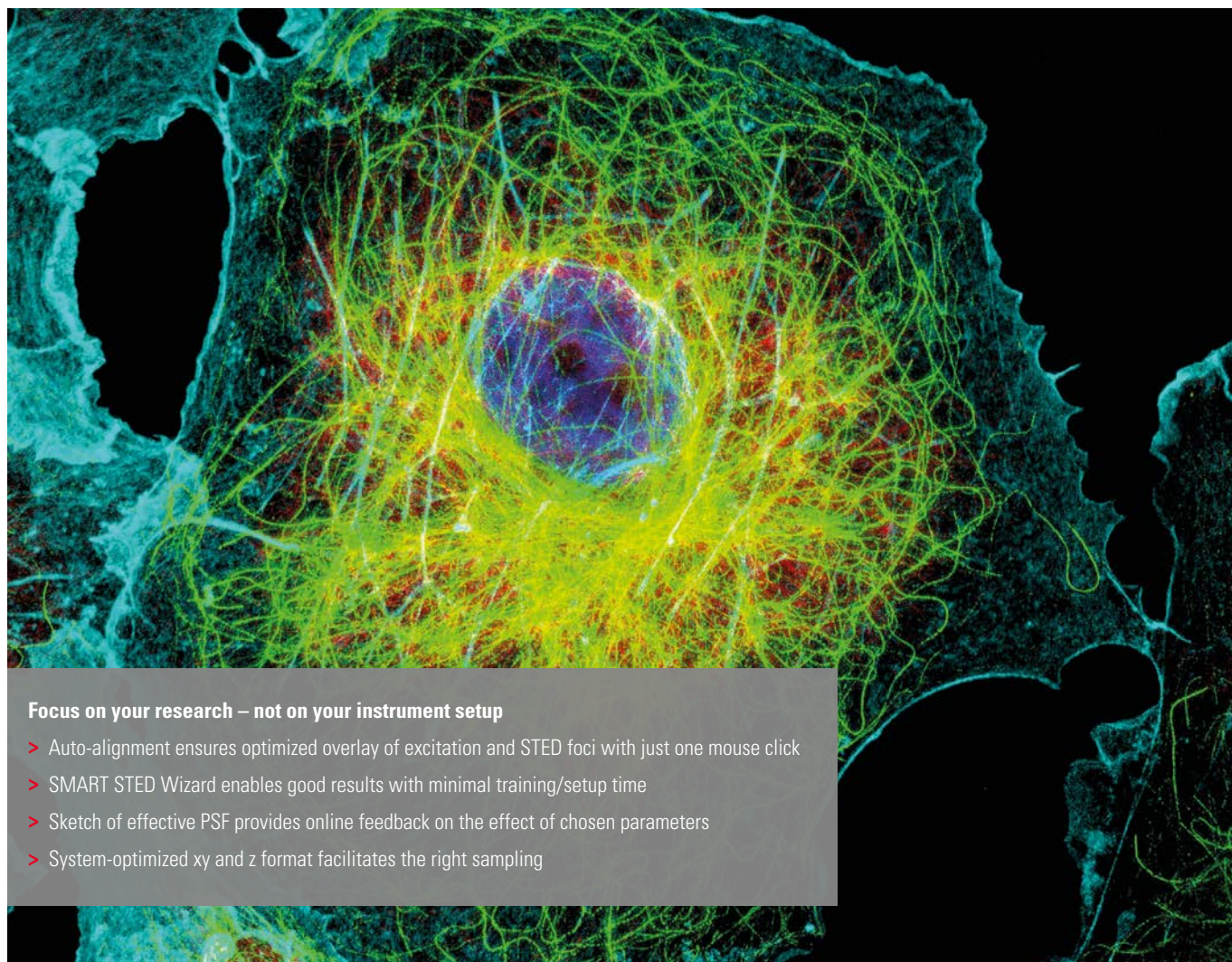
Centrioles in U2OS cells visualized by indirect immunofluorescence. Colocalization of Centrin3 – Alexa Fluor 594 (green) and Cep152 – Alexa Fluor 647 (red) in confocal (left) and deconvolved STED image (right). Due to the diffraction-limited resolution one would judge the two structures to be strongly colocalized based on the confocal data. A look at these structures with STED resolution tells a completely different story: The Pearson correlation coefficient drops dramatically from 0.78 to 0.14, indicating hardly any colocalization of these structures. Further, the Cep152 ring structure – not seen at all in the confocal images – becomes clearly visible. Sample courtesy of Ella Fung, CRUK/MRC Oxford Institute for Radiation Oncology, UK. The anti-Cep152 antibody was kindly provided by E. Nigg, Biozentrum, University of Basel, Switzerland (Sonne KF et. al. J Cell Science 2013).

GETTING STARTED WITH STED

With the new Leica TCS SP8 STED ONE 592 and STED ONE 660 your entry into nanoscopy has never been easier. Built on the basis of the highly versatile confocal platform Leica TCS SP8, every Leica STED system covers the full range of fluorescence imaging. Spectral freedom in excitation and detection make a Leica STED instrument a tool of maximal flexibility not only for nanoscopy.

A STED INSTRUMENT FOR EVERY APPLICATION

STED ONE 592 is ideally suited to life cell imaging with fluorescent proteins GFP and YFP. For colocalization studies, STED ONE 660 offers great flexibility for a wide range of standard fluorophores. The STED 3X with its dedicated Leica exclusive 775 nm STED laser achieves the best signal-to-noise ratio and resolution. Alternatively, you can choose all three STED lasers in a STED 3X system facilitating STED over the full visible spectrum.



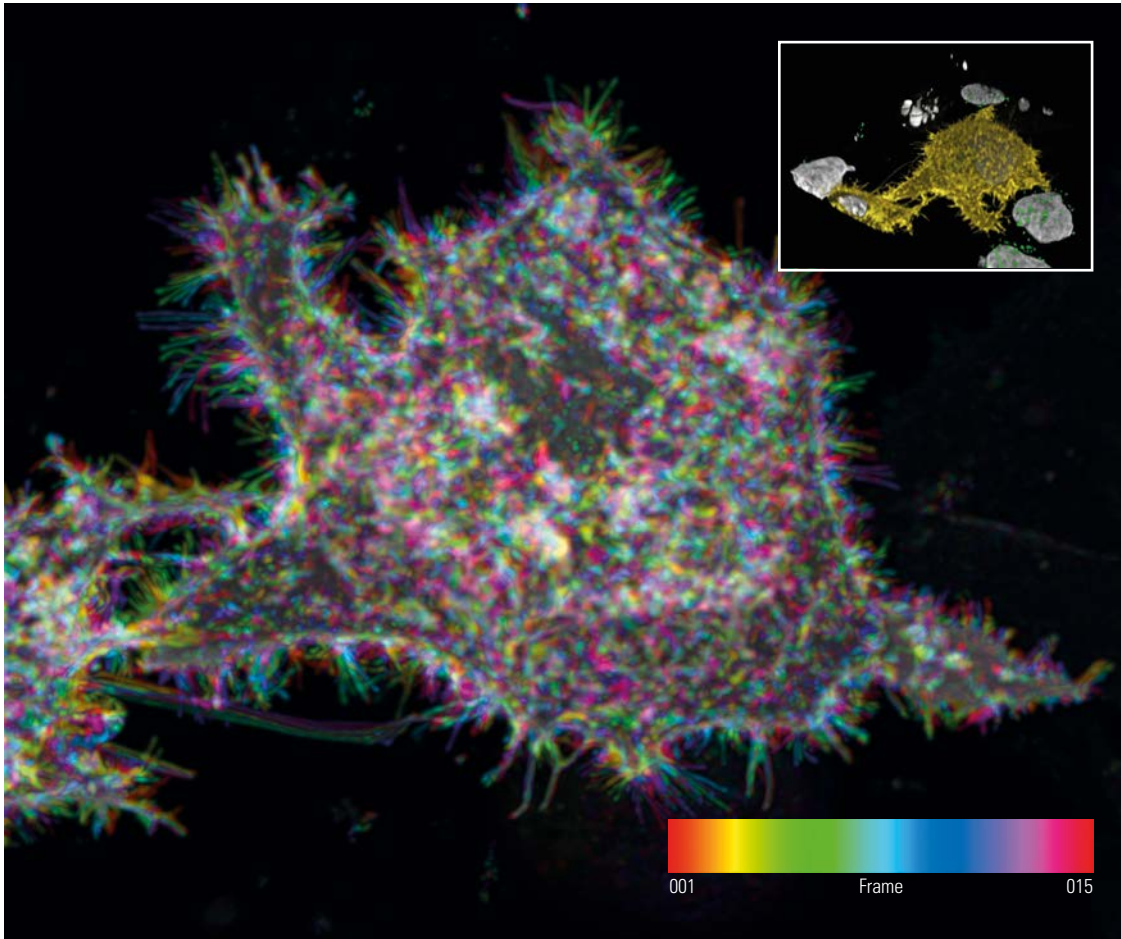
Focus on your research – not on your instrument setup

- > Auto-alignment ensures optimized overlay of excitation and STED foci with just one mouse click
- > SMART STED Wizard enables good results with minimal training/setup time
- > Sketch of effective PSF provides online feedback on the effect of chosen parameters
- > System-optimized xy and z format facilitates the right sampling

Multicolor nanoscopy: 3 STED + 1 confocal channel. STED: Vimentin-Alexa 647 (red), alpha tubulin Alexa 594 (green), phalloidin Alexa 488 (cyan). Confocal: DAPI (blue).
Courtesy of Eugene Katrukha, Utrecht University, The Netherlands.

SUPER-RESOLUTION GOING LIVE

FROM STATIC TO DYNAMIC WITH HYVOLUTION



View the live image:



Time-encoded representation of GPI-YFP expressed in HeLa Kyoto cells. Areas with low motility appear white whereas colored areas are indicative of high motility. The morphodynamics of the cell membrane were recorded in the context of cell nuclei and Golgi apparatus labeled with mCherry and GFP, respectively (see inset). 101 Nyquist sampled z slices were recorded to capture the entire cell volume. Three channels were recorded with an 8 kHz tandem scanner. The maximum frame rate of the 8 kHz tandem scanner at 512x512 pixels is 28 fps. Sample courtesy of Sabine Reither, EMBL Heidelberg, Germany.

Cell morphodynamics at super-resolution

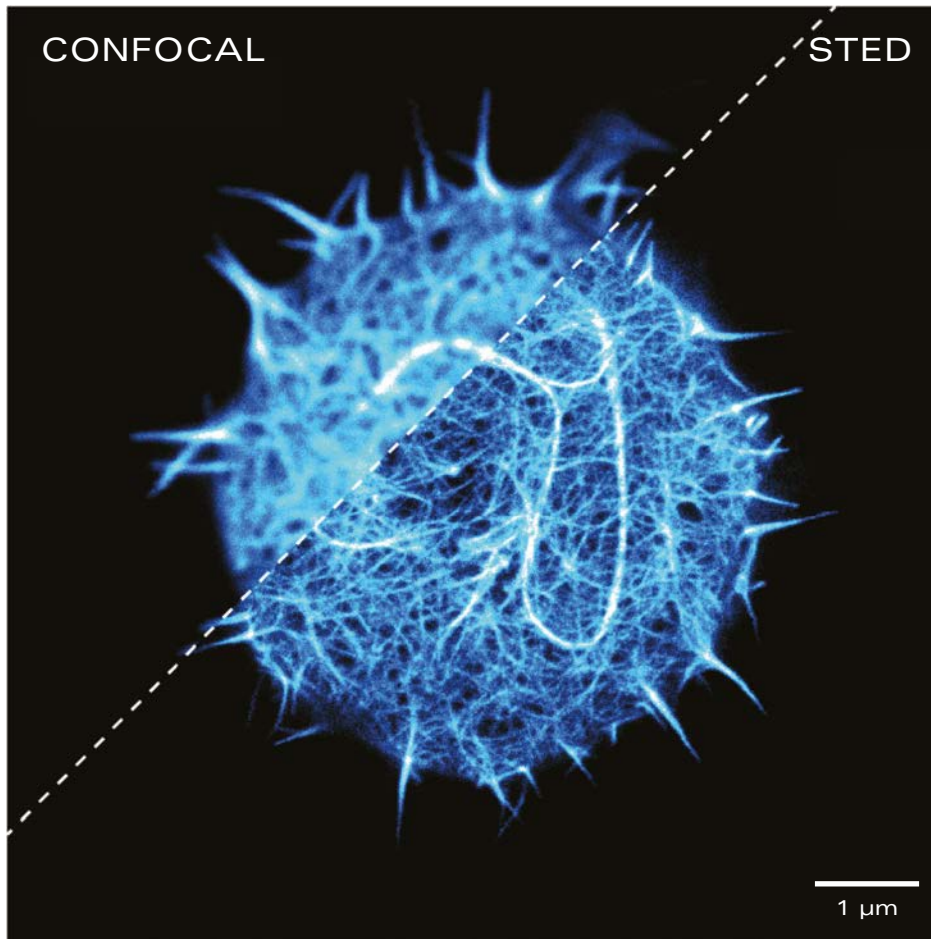
Utilizing the speed advantage of HyVolution over other super-resolution techniques allows you to acquire complete multicolor volumes of live cells – temporally resolved. This way you can capture the spatio-temporal dynamics in full detail – and are ready to study cell migration and cell morphology at super-resolution.

The colors of life – truly simultaneously

Whereas other solutions for confocal super-resolution work with external modules, HyVolution uses the SP detector at the heart of every Leica confocal instrument. This way HyVolution integrates fully with your existing workflow while it leverages the full speed of simultaneous multicolor detection – up to 40 fps with 5 independent colors. You can record live specimens at full speed without temporal aliasing artifacts incurred by sequential recording.

NANOSCOPY GOING LIVE

DEEPER LIVE CELL STED IMAGING WITH GLYCEROL IMMERSION



Living T cell in suspension. 3D reconstruction of confocal and STED stack. Maximum projection. Courtesy of Marco Fritsche, Mathias Clausen and Christian Eggeling, MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK.

STED WHITE – don't compromise on optics

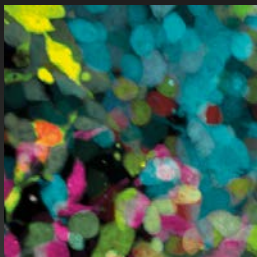
When it comes to resolving very small details, it's crucial to start with the best optics. The outstanding chromatic correction of the two STED WHITE objective lenses ensures optimal overlay of excitation and STED PSF in z all over the visible spectral range. The white light laser can be used as excitation source for STED at 592/660/775 and makes adjustment of prefocusing obsolete. Enjoy the freedom to choose from more fluorophores than ever for STED nanoscopy.

New STED objective for live cell nanoscopy

Standard fixed samples are easily imaged with STED oil objective lenses, which deliver highest NA and resolution. For more complex experiments, the STED WHITE Glycerin objective lens HC PL APO 93X/1.30 GLYC motCORR creates new opportunities. The motorized correction collar allows precise and swift adjustment of the optical lenses to varying coverglass thickness, changes in temperature and specimen inhomogeneity. Shed light on details deep inside your specimen with 3D STED, not only at room temperature, but also at 37 °C.

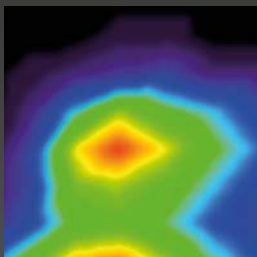
THE BEST TEAM WINS

IN A LEICA TCS SP8 ALL COMPONENTS ARE MATCHED TO PRESERVE YOUR PRECIOUS SIGNAL FOR MAXIMAL PERFORMANCE IN SUPER-RESOLUTION AND NANOSCOPY.



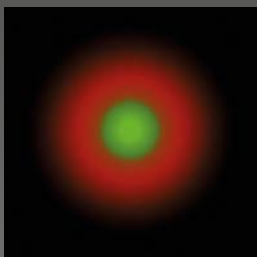
Noise-free performance

Leica HyDs are ideal for super-resolution and nanoscopy, because noise is not recorded. HyDs provide raw images with practically no background. Unlike any other implementations of GaAsP or hybrid detectors, Leica's HyD uses single photon counting with bright signals – higher sensitivity than any other confocal microscope. This opens up new possibilities – be it with STED or HyVolution. With Leica HyDs you get more reliable information than with any other detector fully integrated into a confocal microscope.



Spectral freedom in live cell super-resolution and nanoscopy

Stop worrying about challenging multicolor experiments. Get the spectral freedom you need to image any kind of dye combination. With the filter-free spectral detection system of the Leica TCS SP8, you can multiplex up to 5 independent channels for maximal speed using live specimens. The filter-free design of the SP detector is a perfect match for the HyD's superior signal-to-noise ratio. Every image taken with the TCS SP8 and HyD is more brilliant than any other GaAsP photomultiplier can deliver. Critical colocalization experiments in live cell applications benefit from Leica HyDs.



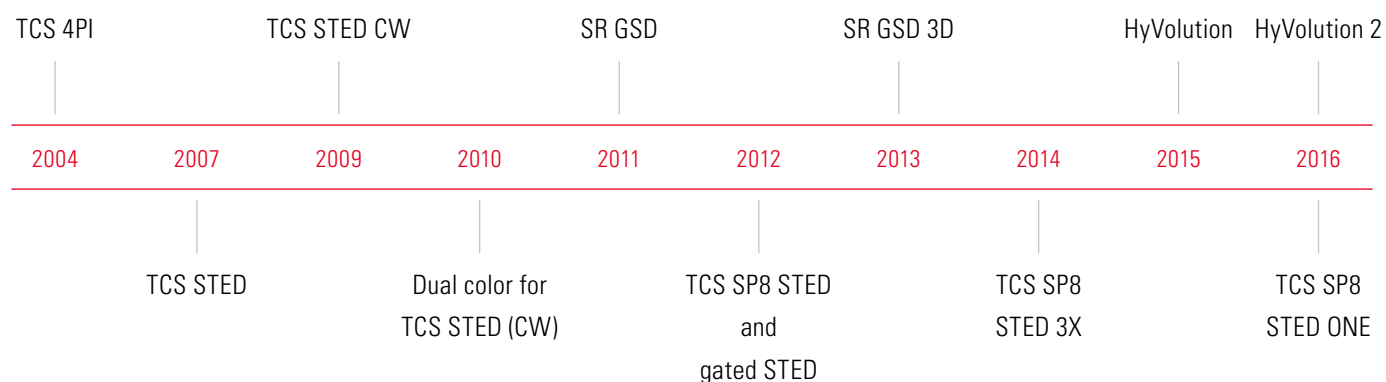
A gateway to nanoscopy

Being a pulsed supercontinuum source, the white light laser is synchronized with the HyD's gating input. This LightGate technology produces crisp, contrast-rich images and affords an additional resolution boost with STED. Thus free of detrimental background you can get down to 30 nm resolution.



DRIVEN BY YOUR RESEARCH

TWELVE YEARS OF INNOVATION TOWARDS NANOSCOPY



CONNECT
WITH US!



Leica Microsystems CMS GmbH

Am Friedensplatz 3 · 68165 Mannheim, Germany · T +49 621 70280 · F +49 621 70281028

www.leica-microsystems.com/products/super-resolution-microscopes