

From Eye to Insight



THE OPTION MACHINE

Leica TCS SP8 – Confocal Platform





LEICA TCS SP8

"It is great if you can buy, with a small budget, a relatively standard microscope setup and if you need a special technique, you can upgrade it at a relatively low price."



Bram van de Broek
Netherlands Cancer Institute, NKI AvL
Amsterdam



Jose Xavier Neto
Laboratório Nacional de Biociências, Brasil

"I am very fond of the concept of modularity, because you don't have to buy entirely new equipment every time you need additional capability."



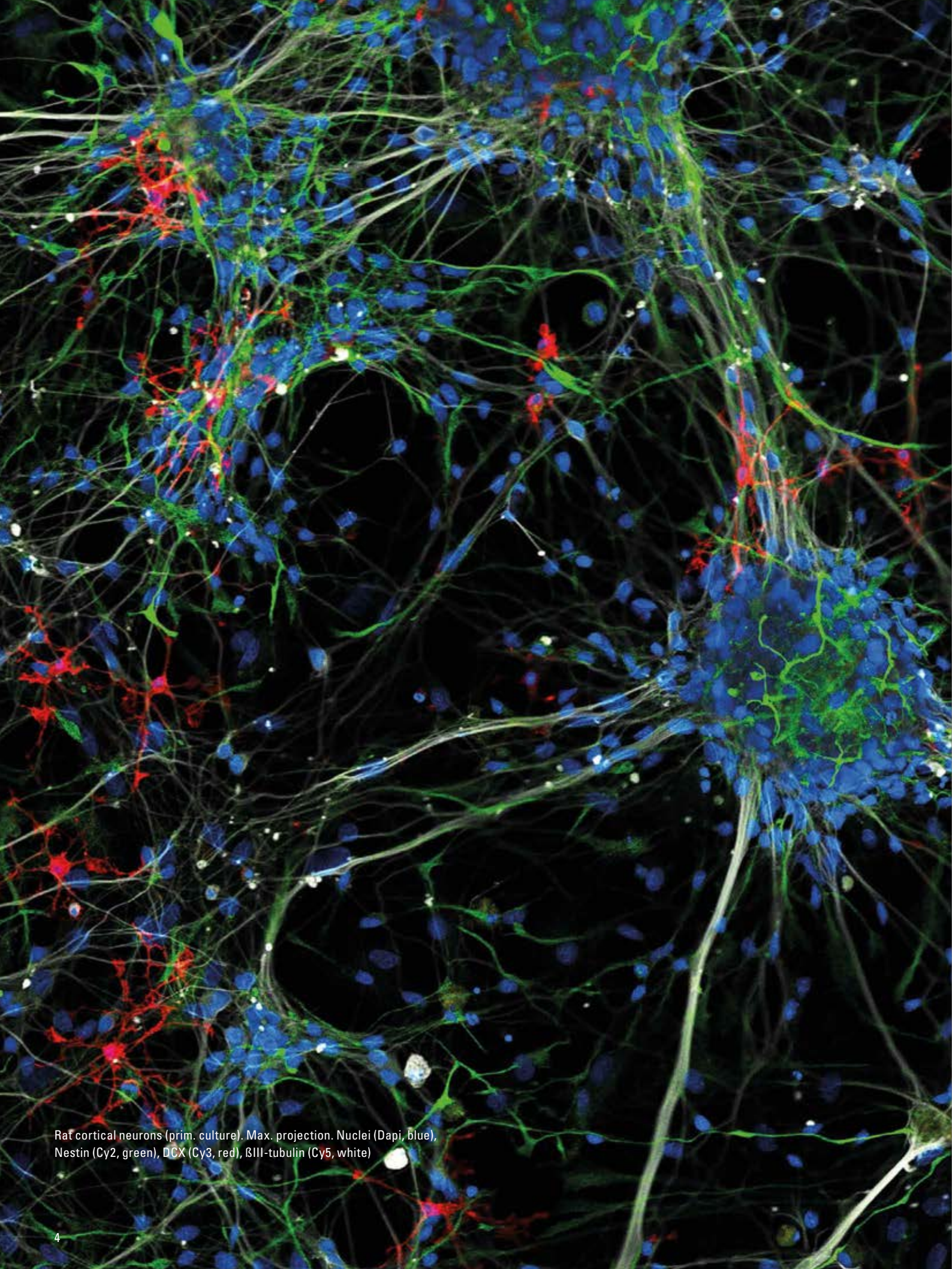
Astrid Schauss
CECAD Imaging Facility, University of Cologne, Germany

"When you start with a microscope configuration for your application, you don't know how the research will develop. Especially in imaging facilities, we need to be flexible. And this flexibility is what I really like about the system."

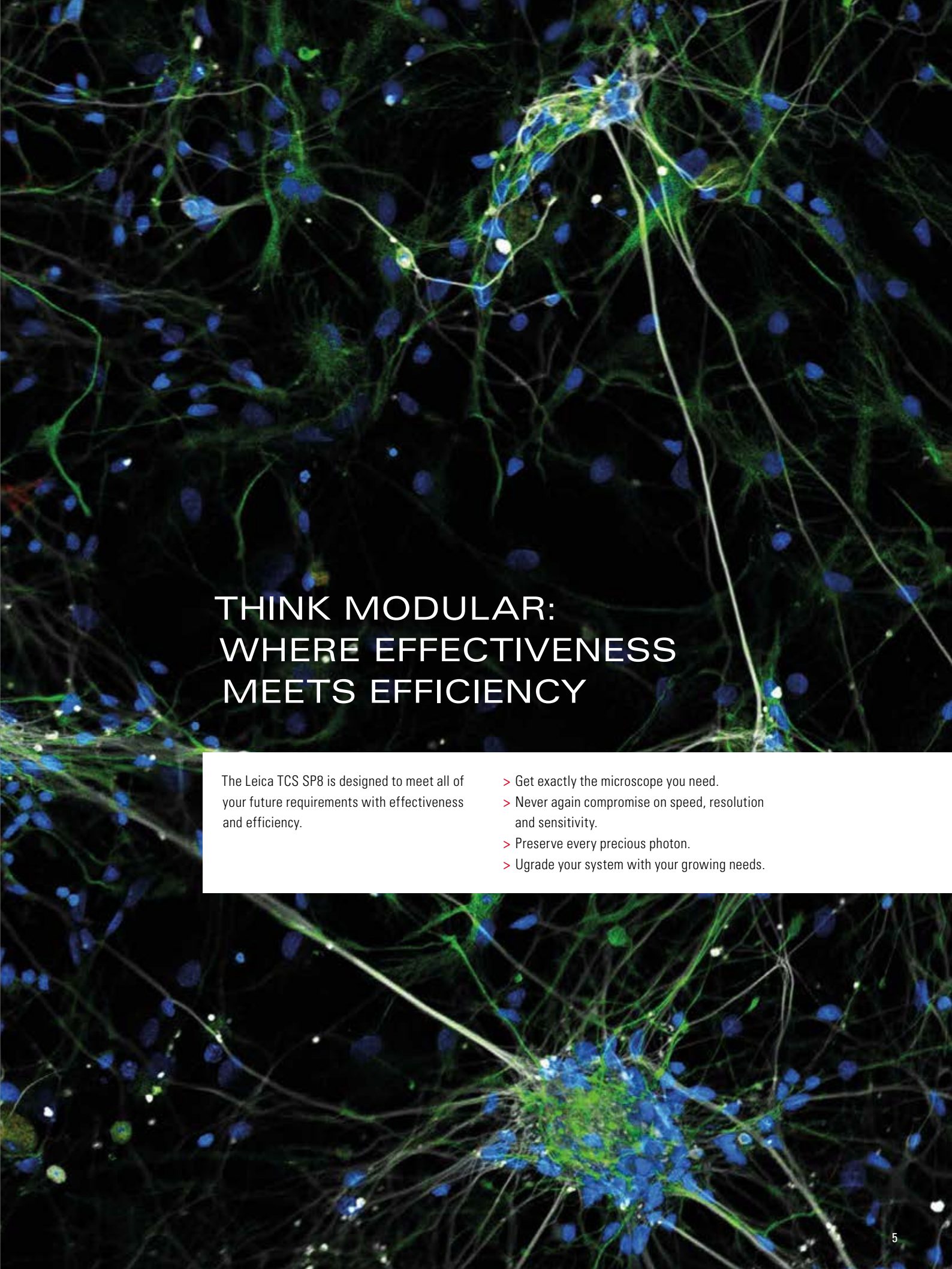


CURRENT AND FUTURE APPLICATIONS
BY YOUR PARTNERS IN SCIENCE





Rat cortical neurons (prim. culture). Max. projection. Nuclei (Dapi, blue), Nestin (Cy2, green), DCX (Cy3, red), β III-tubulin (Cy5, white)



THINK MODULAR: WHERE EFFECTIVENESS MEETS EFFICIENCY

The Leica TCS SP8 is designed to meet all of your future requirements with effectiveness and efficiency.

- > Get exactly the microscope you need.
- > Never again compromise on speed, resolution and sensitivity.
- > Preserve every precious photon.
- > Upgrade your system with your growing needs.

THE PLATFORM THAT IS READY FOR YOUR RESEARCH

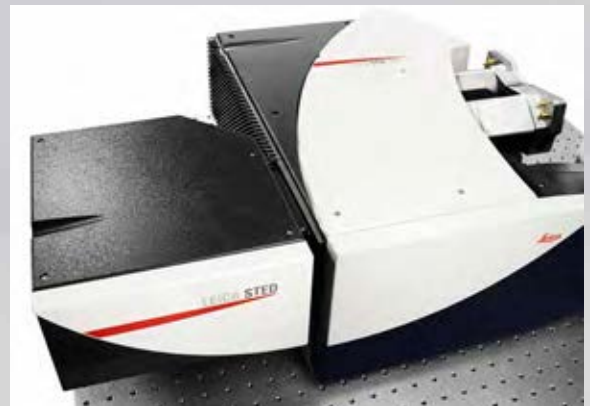
GET WHAT YOU NEED NOW AND UPGRADE WHENEVER NECESSARY.

Expand your possibilities

Do you know which direction your research will take in the future? From live cell to quantitative imaging, from super-sensitivity to super-resolution, from multiphoton to light sheet imaging – with the Leica TCS SP8 you can always expand your possibilities.

Design your future

We build on a modular concept: Tailor your microscope to your current needs and upgrade with additional functionalities at any time. Your investment in a Leica TCS SP8 will pay off – now and in the future.



Leica TCS SP8 STED 3X: Your next dimension in super-resolution



Leica TCS SP8 X: Spectral freedom with the white light laser



Leica TCS SP8 CARS: Label-free imaging



Leica TCS SP8 SMD: Precision in single molecule detection



Leica TCS SP8 DLS: The vertical turn in light sheet imaging



Leica TCS SP8 MP: Discover greater depths with multiphoton imaging



OPTIONS INSIDE

Mouse testis. Synaptonemal complex (Alexa 488, green), Nuclei (DAPI, blue), Protein X (Cy3, red).
Courtesy of Yu Katsuyama and Noriko Osumi, Tohoku University, Miyagi, Japan.

THE LAS X SOFTWARE PLATFORM CAN BE PERSONALIZED TO YOUR WORK AND ASSISTS YOU IN PLANNING, EXECUTING, AND ANALYZING YOUR EXPERIMENTS.

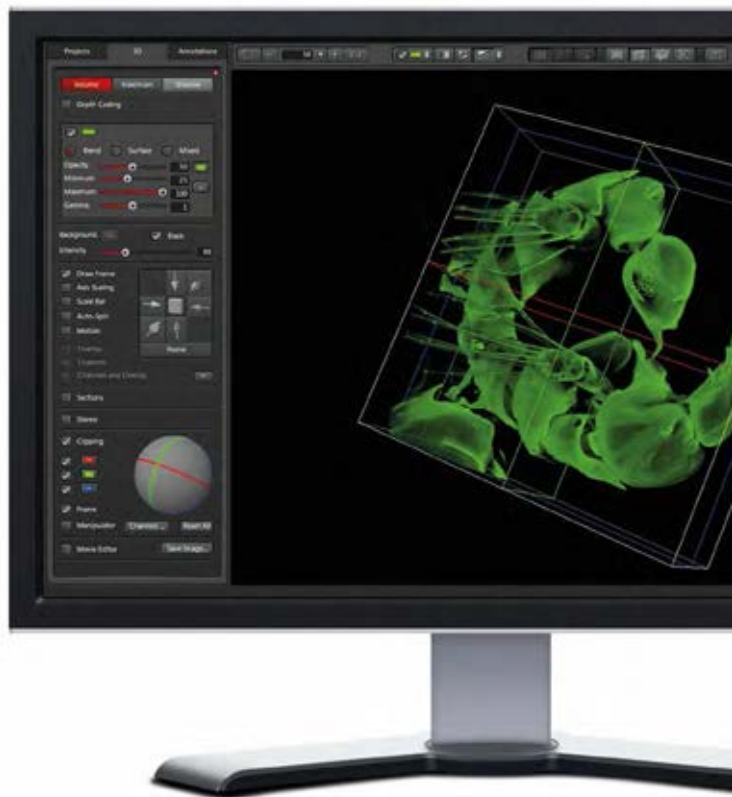
Explore and analyze

Spend your time on your research while the Leica Application Suite X (LAS X) attends to microscope control. Its workflow-oriented design and intuitive software wizards guide you step by step through image acquisition, processing, and analysis. Tailor LAS X to your needs with additional software packages like 3D Visualization and Analysis, which allow you to understand the topology of your 3D image and quantify various aspects of intracellular structures. The examination and analysis of your experiments become extremely easy using the Leica TCS SP8 and LAS X.

Start thinking big

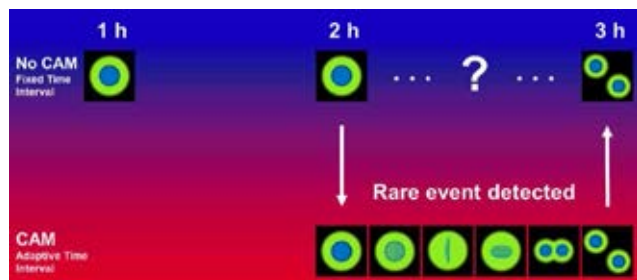
Breakthrough discoveries can happen when you are in the right place at the right time. Leica HCS A can speed up the process of discoveries through high content screening allowing you to standardize biological applications for rapid and reproducible results.

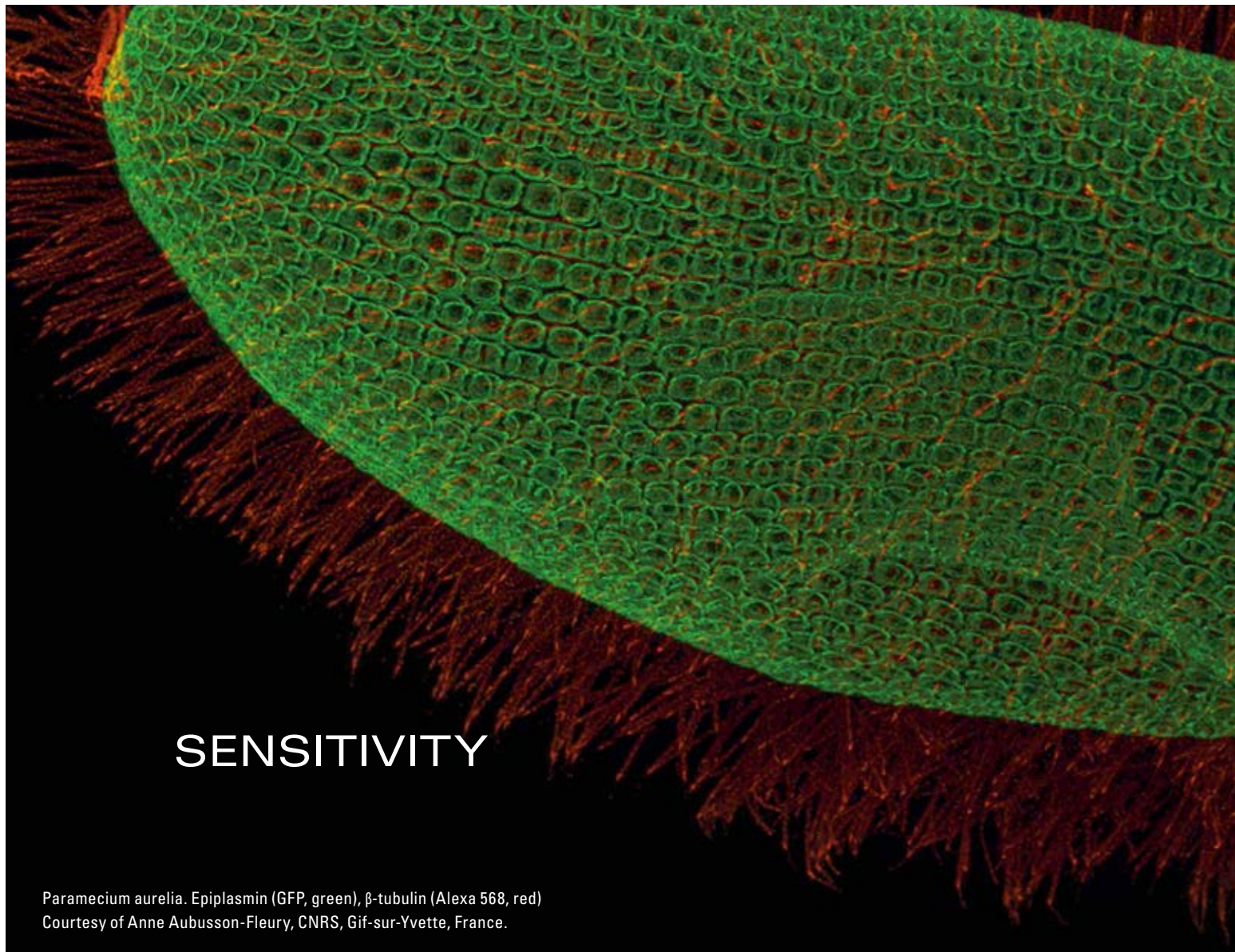
Computer Aided Microscopy (CAM) allows the detection of rare events using external image analysis software such as MatLab or the open-source ImageJ or CellProfiler software packages. Data is continuously streamed to external storage, and image analysis takes place simultaneously with data acquisition. Leica HCS A can respond to feedback from the analysis software, which simplifies large screening campaigns.



Without CAM (top): Important events may be missed.

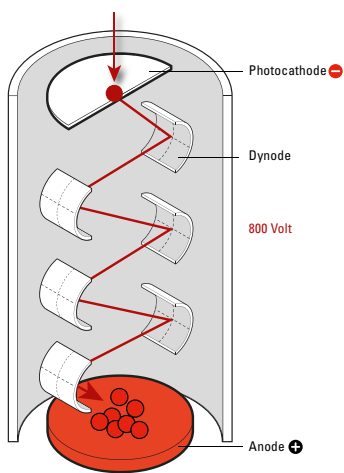
With CAM (bottom): Time resolution is temporarily increased as needed. Process is studied in more detail and with better yield.



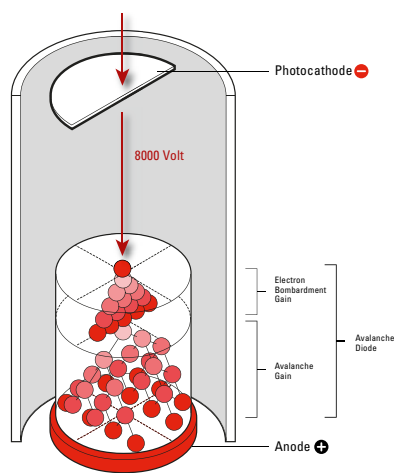


SENSITIVITY

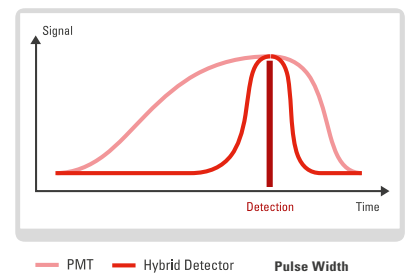
Paramecium aurelia. Epiplasm (GFP, green), β -tubulin (Alexa 568, red)
 Courtesy of Anne Aubusson-Fleury, CNRS, Gif-sur-Yvette, France.

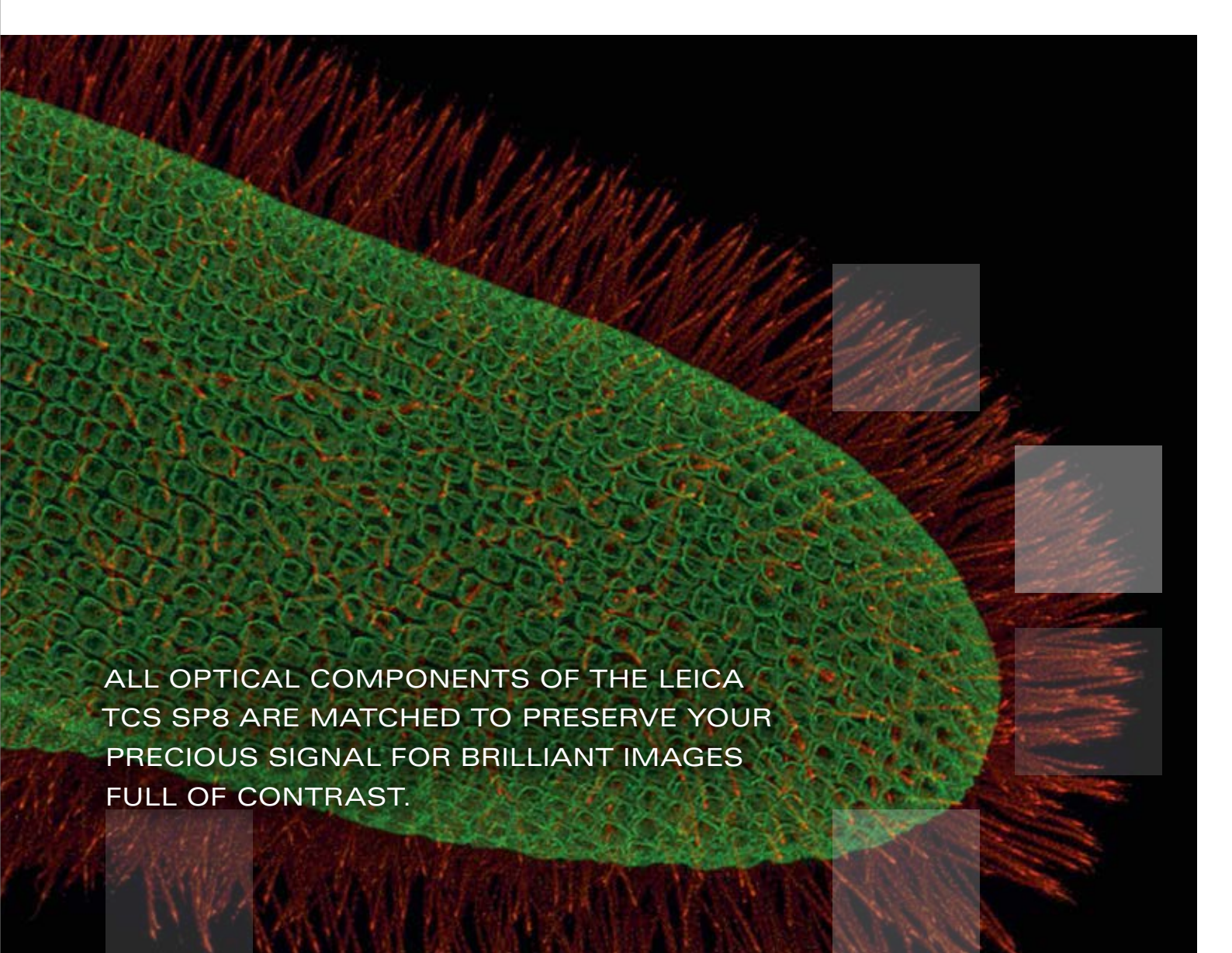


Principle of photomultiplier tube, which generates broad electrical pulses.



Principle of hybrid detector, which results in sharp electrical pulses.





ALL OPTICAL COMPONENTS OF THE LEICA TCS SP8 ARE MATCHED TO PRESERVE YOUR PRECIOUS SIGNAL FOR BRILLIANT IMAGES FULL OF CONTRAST.

More details

With the synergies of the multiband spectral detector, acousto-optical beam splitter (AOBS), and super-sensitive Leica HyD hybrid detectors you get a confocal microscope with maximum photon efficiency and gapless spectral detection. The high signal-to-noise ratio of the Leica HyDs helps to render the finest details of any specimen. The superior sensitivity of the Leica TCS SP8 directly translates into less laser power and improved viability of live specimens.

Effective quantification

Due to their rapid pulse response, Leica HyDs are ideal for photon counting and can give you direct insights into molecular stoichiometries. With Leica HyDs you get information from your images that is more reliable than any other detector fully integrated into a confocal microscope.

Even more sensitivity

The Leica TCS SP8 platform offers advanced possibilities for gentle imaging of sensitive specimens. Multiphoton (MP) imaging reduces out-of-focus excitation and increases the viability of your samples. Light sheet imaging offered by the Leica TCS SP8 DLS is the most gentle imaging technique, as the specimen is illuminated in only a single plane at a time, which reduces phototoxic effects dramatically. The combination of low illumination and high-speed acquisition allows you to follow developing organisms over time.

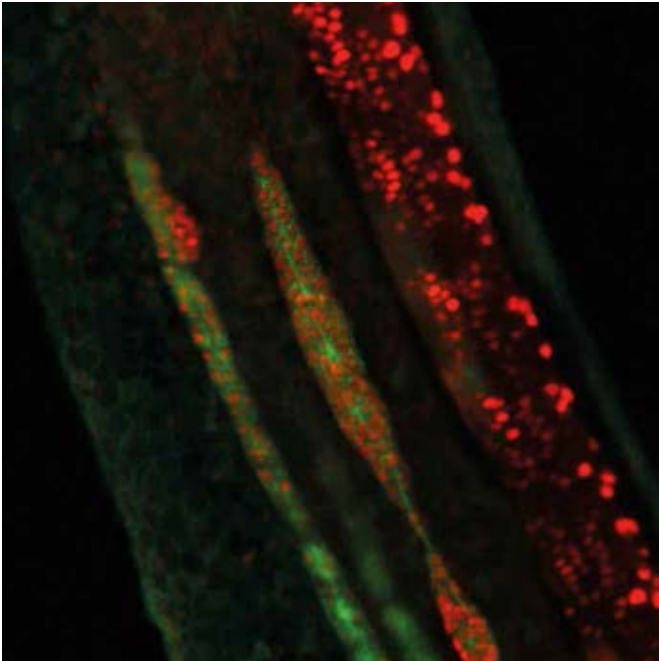
LIVE CELL IMAGING

Reliable results

With a Leica TCS SP8, imaging of living specimens can have many facets. It gives you the freedom to conveniently perform any advanced live imaging method with reliable results. You can expect highest image fidelity and specimen viability from its light efficient detection and high speed scanning system.

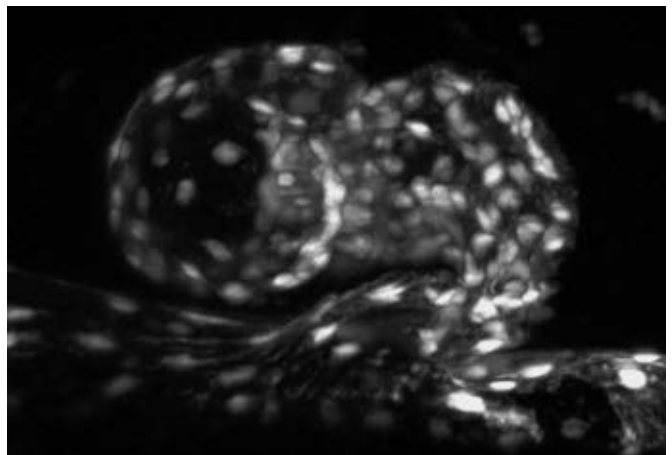
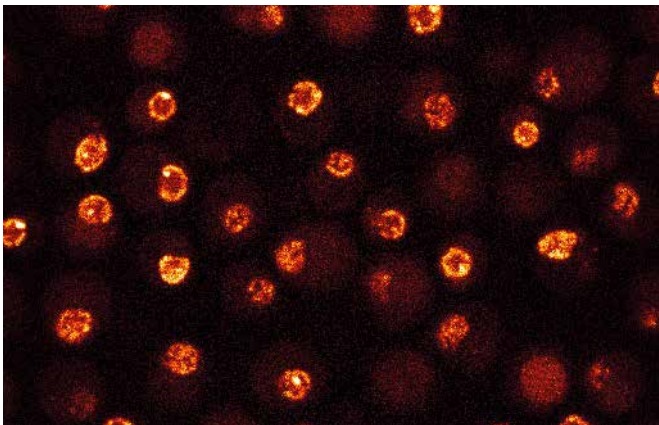
Bigger and deeper

Thinking bigger and deeper the Leica TCS SP8 MP accommodates live organisms. At the opposite end of the scale you can study nanoscopic detail in live cells using STED super-resolution. Or follow embryonic development using gentle light sheet illumination with digital light sheet imaging – all options on the same system if you like. But no matter which way you choose to look at it – a Leica TCS SP8 is always optimized for speed.



Long-term, multicolor observations: 17 hours of zebrafish lateral line development. Zebrafish 36hpf, CldnB:lynGFP / Cxcr4B:nuclearRFP. Sample courtesy of Darren Gilmour, EMBL Heidelberg, Germany.

Live yeast cells double-labeled with EGFP at both the nuclear envelope and the telomere.



High-speed imaging: Beating zebrafish heart, transgenic line flik: EGFP. Post-acquisition alignment after xyzt recording. Recording speed: 120 frames/second. Courtesy of Emily Steed, Vermot Lab, IGBMC Strasbourg, France.



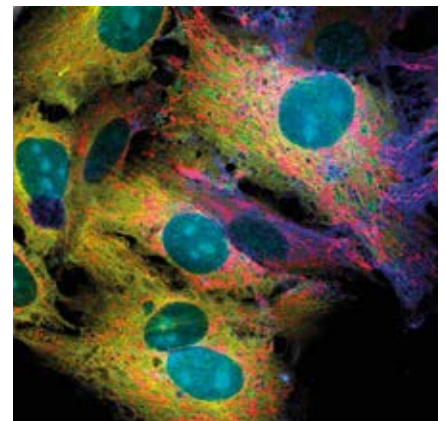
NIH3T3 cells transduced with five individual fluorescent protein (FP) vectors. FPs: Cerulean, EGFP, Venus, tdTomato and mCherry. Each FP was visible only in the cells transduced with the corresponding vector. AOBS fast line sequential scan with five excitations and five emission bands. No unmixing. Image courtesy of Daniela Malide, NIH Bethesda, MD USA

MULTICOLOR IMAGING

Spectral freedom

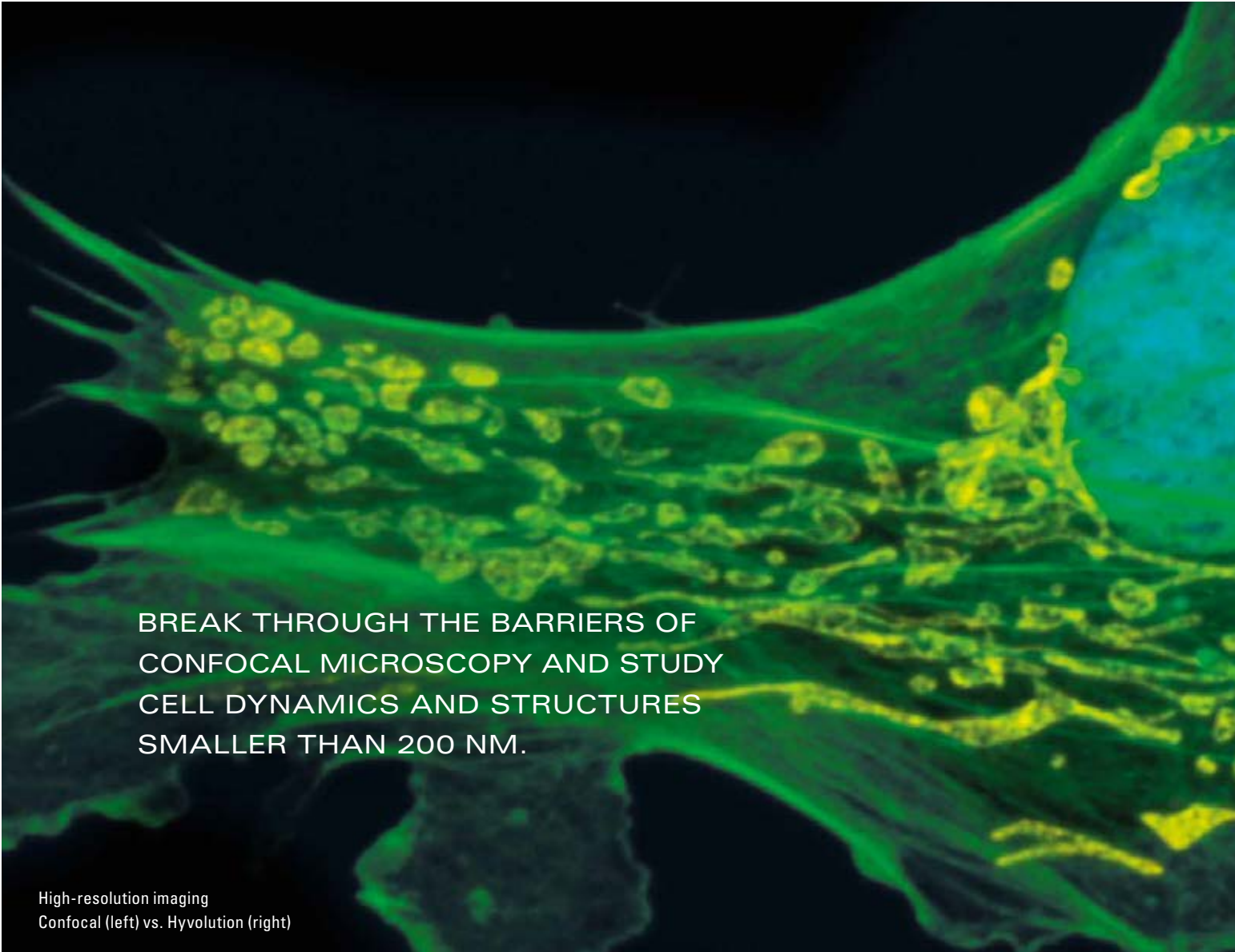
Stop worrying about challenging multicolor experiments. Get the spectral freedom you need to image any kind of dye combination. Easily explore cell connectomics using Brainbow fluorescent proteins with the Leica TCS SP8¹.

With the filter-free spectral detection system of the Leica TCS SP8, you can simultaneously record up to 5 different dye emissions coming from your specimen without any loss. The AOBS, a uniquely flexible beam splitter, makes it possible to acquire spectrally close fluorophores like GFP and YFP in a detection window that is more narrow than on any other system. This speeds up the acquisition because you need less multi-tracking. The white light laser, a laser source that covers the full spectrum of visible light, gives you the most flexibility for your multicolor experiments.



PAE cell line stably expressing five fluorescent subcellular markers (FPs) with ex and em. Image courtesy of Philipp Berger, Paul Scherrer Inst. Villigen, CH.

¹ Malide D, Metais JY, Dunbar CE. Blood (2012)120:e105-16.



**BREAK THROUGH THE BARRIERS OF
CONFOCAL MICROSCOPY AND STUDY
CELL DYNAMICS AND STRUCTURES
SMALLER THAN 200 NM.**

High-resolution imaging
Confocal (left) vs. Hyvolution (right)

Pushing the limits

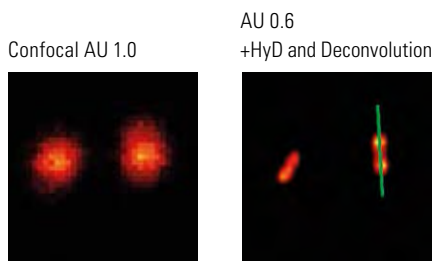
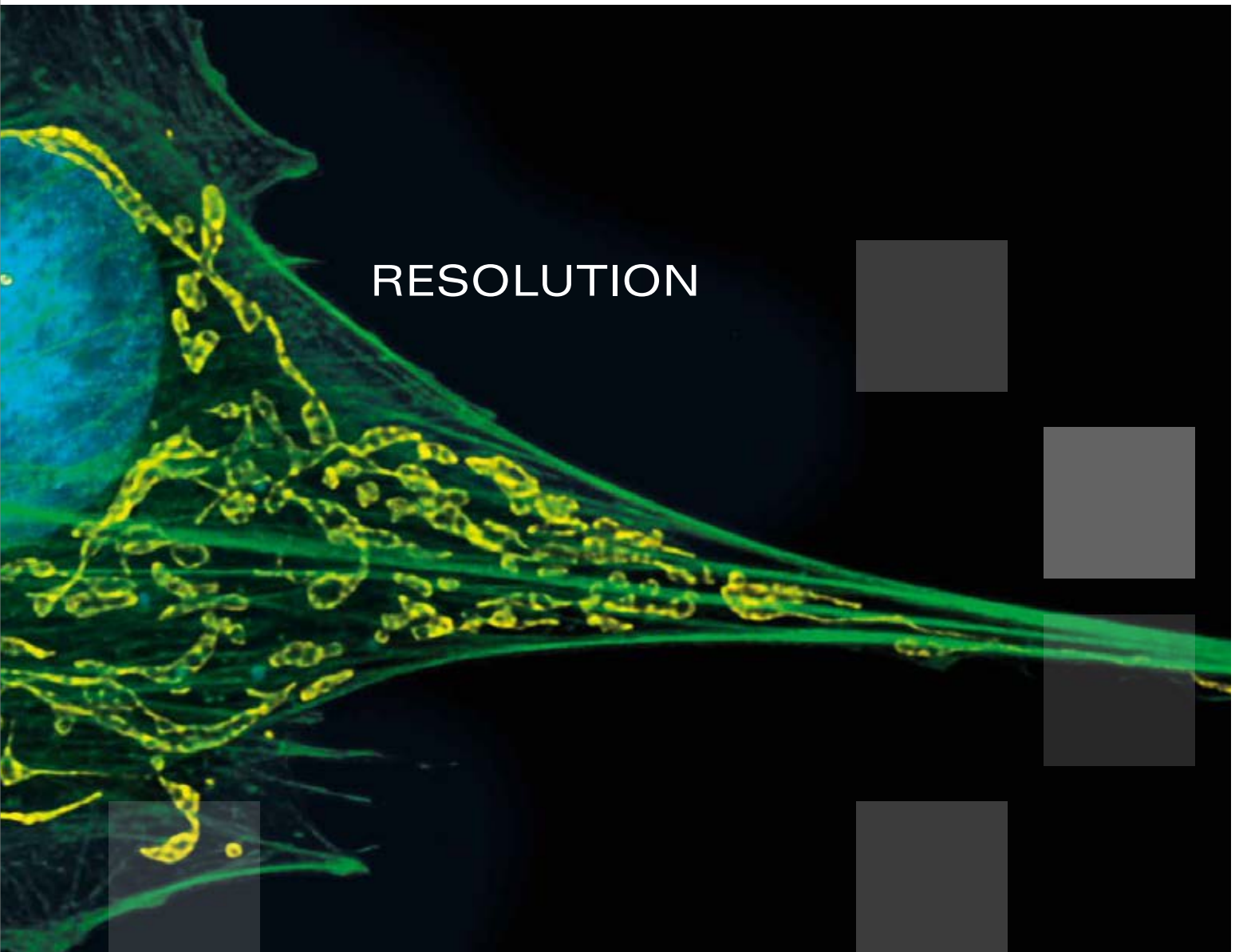
Pushing your confocal to its limits requires highly sensitive detection with low noise. The Leica TCS SP8 plus HyVolution combines super-sensitive HyD detectors, which reliably collect weak signals, with industry-leading Huygens deconvolution by SVI. This combination achieves resolution of 140 nm in a case study with DNA origamis and delivers crisp multicolor images, which convey every detail at high fidelity.

Resolve at nanoscale

The Leica TCS SP8 STED 3X provides super-resolution to resolve details down to 30 nm. It is fully integrated into the Leica TCS SP8 confocal platform and gives fast, intuitive, and purely optical access to structural details far beyond the diffraction limit – fast enough even for live cell imaging. The system offers the freedom to optimize resolution in all spatial dimensions as well as to perform multicolor colocalization studies over the whole spectrum of visible light. With a Leica exclusive STED deconvolution package, you get more from your images.

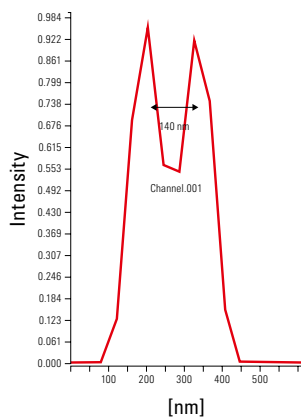
How to maximize resolution

- > Use super-sensitive HyD detectors
- > Get the most out of your data using Huygens deconvolution
- > Get sub-30 nm super-resolution with Leica TCS SP8 STED 3X

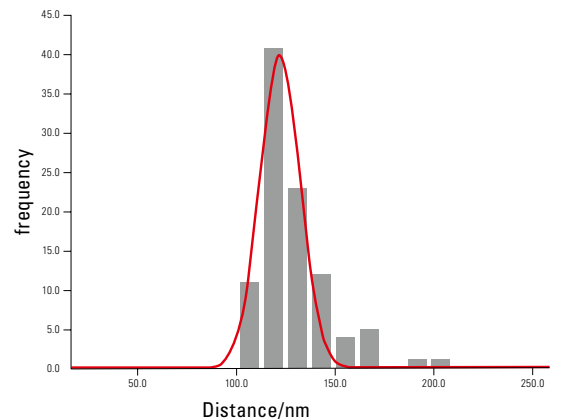


A

B



C



D

High-resolution imaging with super-sensitive HyD detectors and Huygens deconvolution can resolve 140 nm in a case study with DNA origamis. Specimen: single molecule DNA-origamis with 140 nm defined spacing. (A) Confocal image. (B) Hyvolution (C) Distance measured in B (green line). (D) Distribution of measured distances in nanometers.

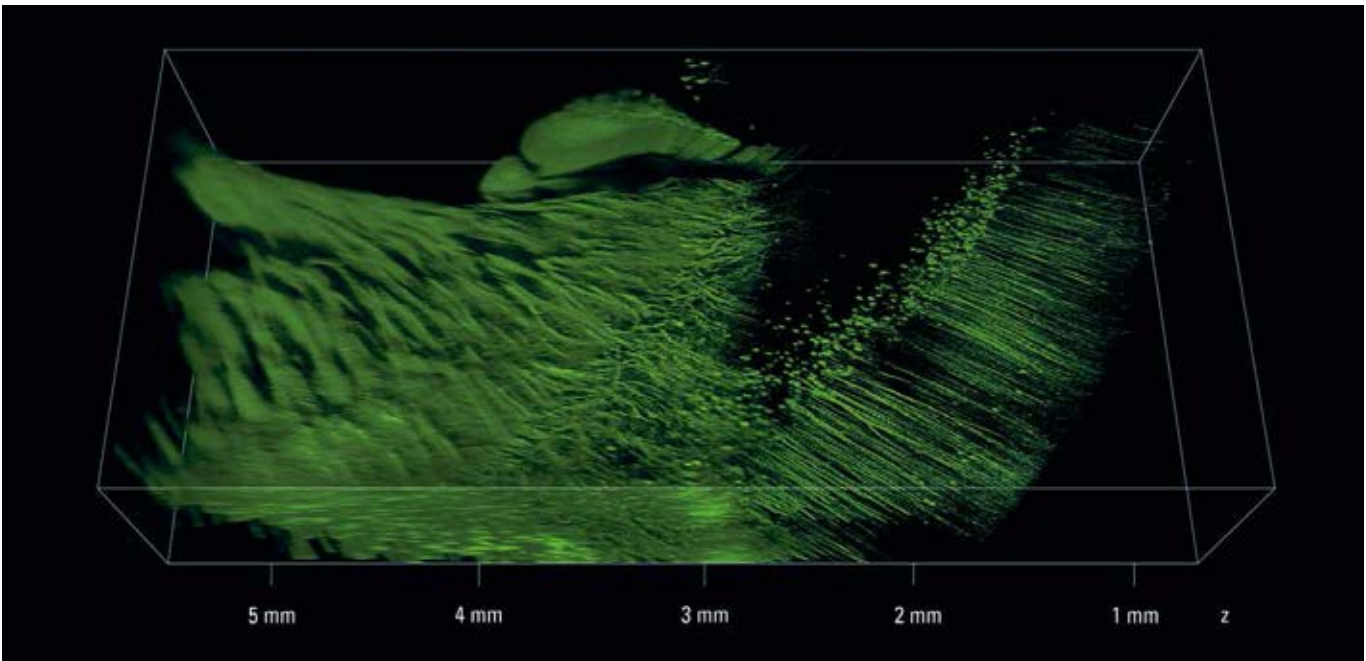
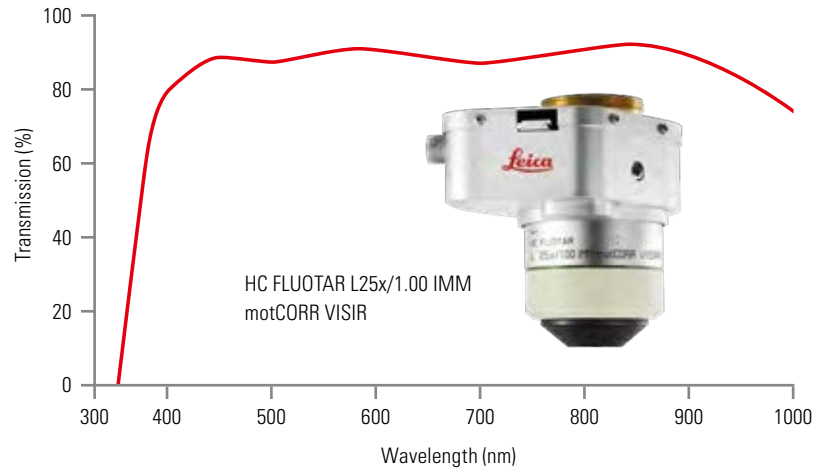
TISSUE CLEARING

Maximum depth, highest resolution

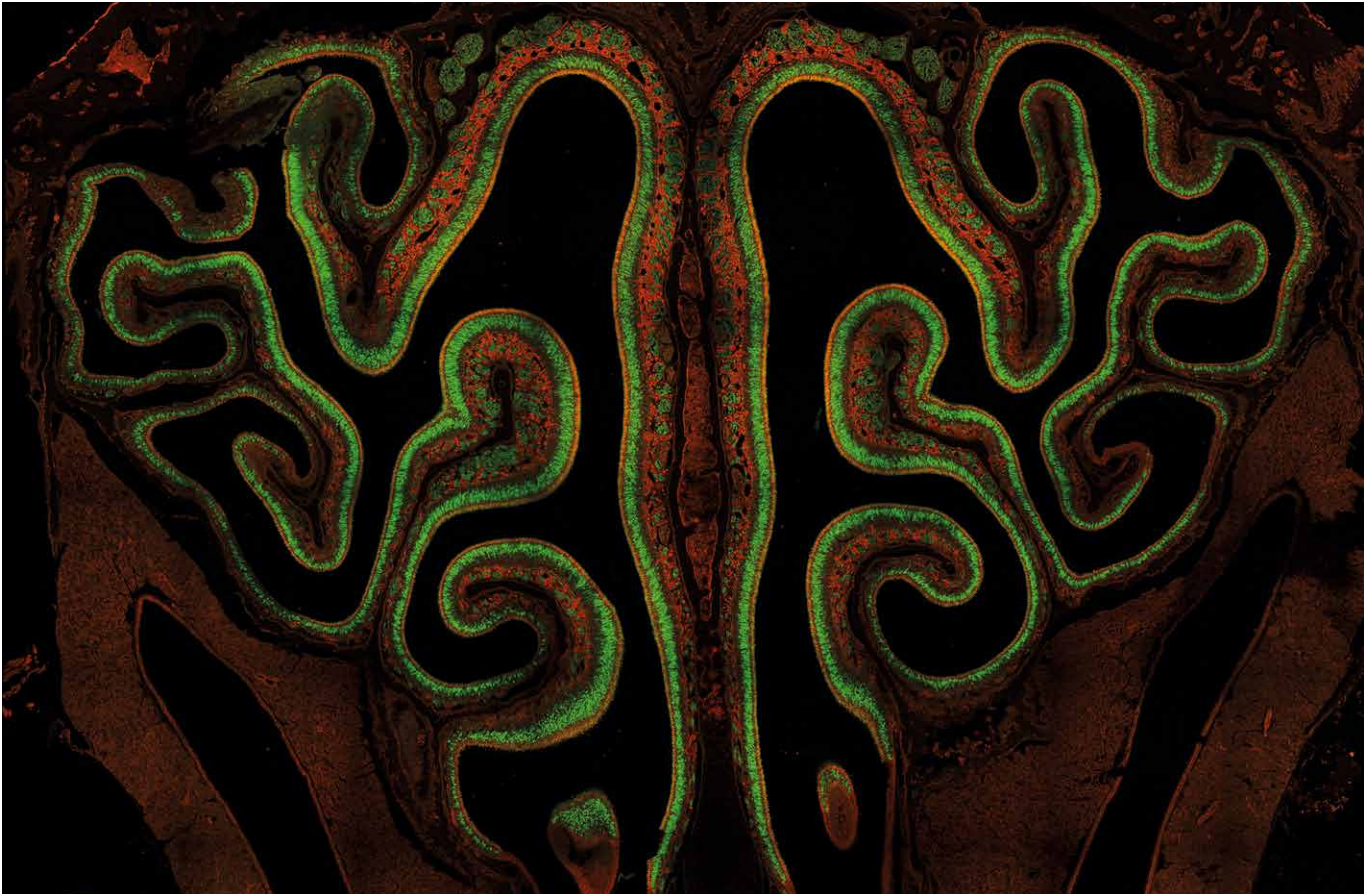
Transparent tissues help us to identify spatial arrangements and connectivity of cells and tissues. This is particularly helpful for identifying neuronal circuits. Clearing techniques provide maximum imaging depth and highest resolution when combined with the appropriate optics.

Reveal unexplored secrets

With the dedicated objectives for CLARITY and BABB treated specimens specifically designed by Leica Microsystems, you can reveal previously unexplored secrets. A motorized correction collar improves the resolution in deep tissue sections. Image whole organs at maximum imaging depth and resolution, and discover perfect CLARITY with single or multiphoton excitation.



Thy1-YFP adult mouse brain treated with CLARITY. Confocal imaging with excitation at 514 nm. Courtesy of K. Deisseroth and R. Tomer, Stanford University, Palo Alto, CA, USA.



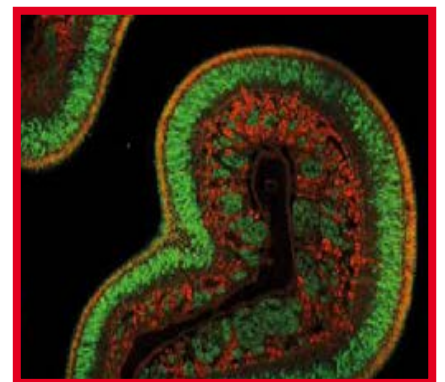
Large field – whole organism. Corona section of mouse nasal cavity.

STITCHING

From whole organisms to the highest level of detail

The Leica TCS SP8 offers the largest field of view in point scanning by using an X2Y scan mirror architecture. You can take images of whole animals or plants, often in one shot.

Mosaicking (stitching) is available for even larger specimens. Save time on stitching by covering the same area with a smaller number of images. Due to the homogenous field illumination provided by our scanner, your images are devoid of stitching artefacts. At the same time, every detail is preserved with high confocal resolution.



Coronal section of mouse nasal cavity. Mosaic compiled from 48 single images acquired using a high NA objective lens. (B) Close up from (A) indicating that high resolution is preserved at the whole field of view.

CREATING THE OPTION MACHINE

AN INTERVIEW WITH BILL HAY, SENIOR PROJECT MANAGER OF THE SP8 DEVELOPMENT TEAM.

Why was the SP8 built on a platform principle?

That was an answer to the customer requirements, of course. If customers want to start with a confocal, but can't afford the tricked-out system with all the bells and whistles, they can add new features year by year, just as their budget allows and science requires. When purchasing an SP8, our customers can look into the future and ask themselves what they are going to use it for and what they may need to add to it. We made an 'option' machine, so that the researcher has more options for the future.

So is it basically an expression of Ernst Leitz's principle "with the user, for the user"?

It does sum up very well what we're doing. If we don't do it for the user, there is no benefit to any of the things we do. If we're not making a product that people need, we're not helping to advance research and science. Therefore, we work with our customers to understand the science they are doing and how they're doing it. Then we can incorporate certain features and technologies and anticipate some of the needs that will arise from the development of science. We learn from the user, so we help him again in return – it's a symbiotic relationship.

How did you resolve the conflicting requirements of a confocal microscope, such as sensitivity, speed and resolution?

First of all, efficiency is an area where we won't compromise. Efficiency leads to sensitivity, so you don't lose light. That's the core principle of the SP8: efficient collection of light and

brightness. We improved optical coatings for the SP8 wherever possible with newer manufacturing processes that help us to increase the optical efficiency. In the end the SP8 is designed to gather photons.

But the challenge is to accommodate all three: sensitivity, speed, and resolution. And where the requirements really conflict, you have to make sure that you have the flexibility to serve all customers across the spectrum. If they need the higher speed, at some point they have to accept compromise in resolution. But we have to be able to offer the highest resolution for the people who don't need speed. You have to make sure you deliver the optimum combination of all three.

What is the key to the SP8's success?

One of the major keys to its success, I would say, is the flexibility of the different options that we offer. But the SP8 also comes with an intelligent user interface, so that customers aren't overwhelmed by all the features and complexity. In the end, they shouldn't even notice that we enable them to concentrate on their experiments, while we take care of controlling the system. In addition, we have a wide variety of tools and innovations – e.g., the white light laser, STED, and gating function – that are combined in one system. And not to be forgotten, our optics, efficiency, and image quality are second to none. It is extremely important to the user that they have the best possible image quality.

What does it mean to be a developer at Leica?

It's never boring. We work in a field that is developing incredibly quickly. And with all the innovation that's taking place, it's a constant challenge to keep up and to keep ahead. The SP8 is certainly not the end of the road. We are always looking to improve on the things we already do, and to implement new features and methods that we don't have yet. You never know what new developments might come along. One of the biggest challenges is to make a system that accommodates new developments without having to change the platform. The light sheet module is a prime example of that. But that's not the only thing that makes the work interesting. A whole team of gifted individuals comes together with a lot of brilliant ideas and innovations. This is the real basis for the SP8. I think that we have great people here with innovative ideas and a dedication to translate a vision into a real system for the user. We get to play with a machine like this and work every day on challenging new ideas. After all, it's a pretty damn cool machine!

William C. Hay holds a Bachelor in Physics from Davidson College and a Master in Electrical Engineering from Duke University in North Carolina. He joined Leica Microsystems in 1989 in the semiconductor applications department and switched to R&D project management in 1996. He was the project manager for the development of three generations of confocal microscopes: Leica TCS SP2, Leica TCS SP5 and Leica TCS SP8.



PLATFORM	MODULE	LIVE IMAGING	DEEP TISSUE	RESOLUTION BEYOND DIFFRACTION	QUANTITATIVE IMAGING
Leica TCS SP8	TCS SP8 + HyD	✓	✓	✓	✓
	TCS SP8 DLS	✓			
	TCS SP8 CARS	✓			
	TCS SP8 HCS A	✓			✓
	TCS SP8 MP	✓	✓		
	TCS SP8 STED	✓		✓	
	TCS SP8 X	✓			✓
	TCS SP8 SMD	✓			✓

ONE PLATFORM, 8 MICROSCOPES, COUNTLESS OPTIONS

NO MATTER WHAT YOU WANT
TO IMAGE WE HAVE AN SP8
TO MATCH YOUR NEEDS.



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 SCATTERED RADIATION
P< 4W 350-1600nm >80fs
IEC 60825-1: 2007

