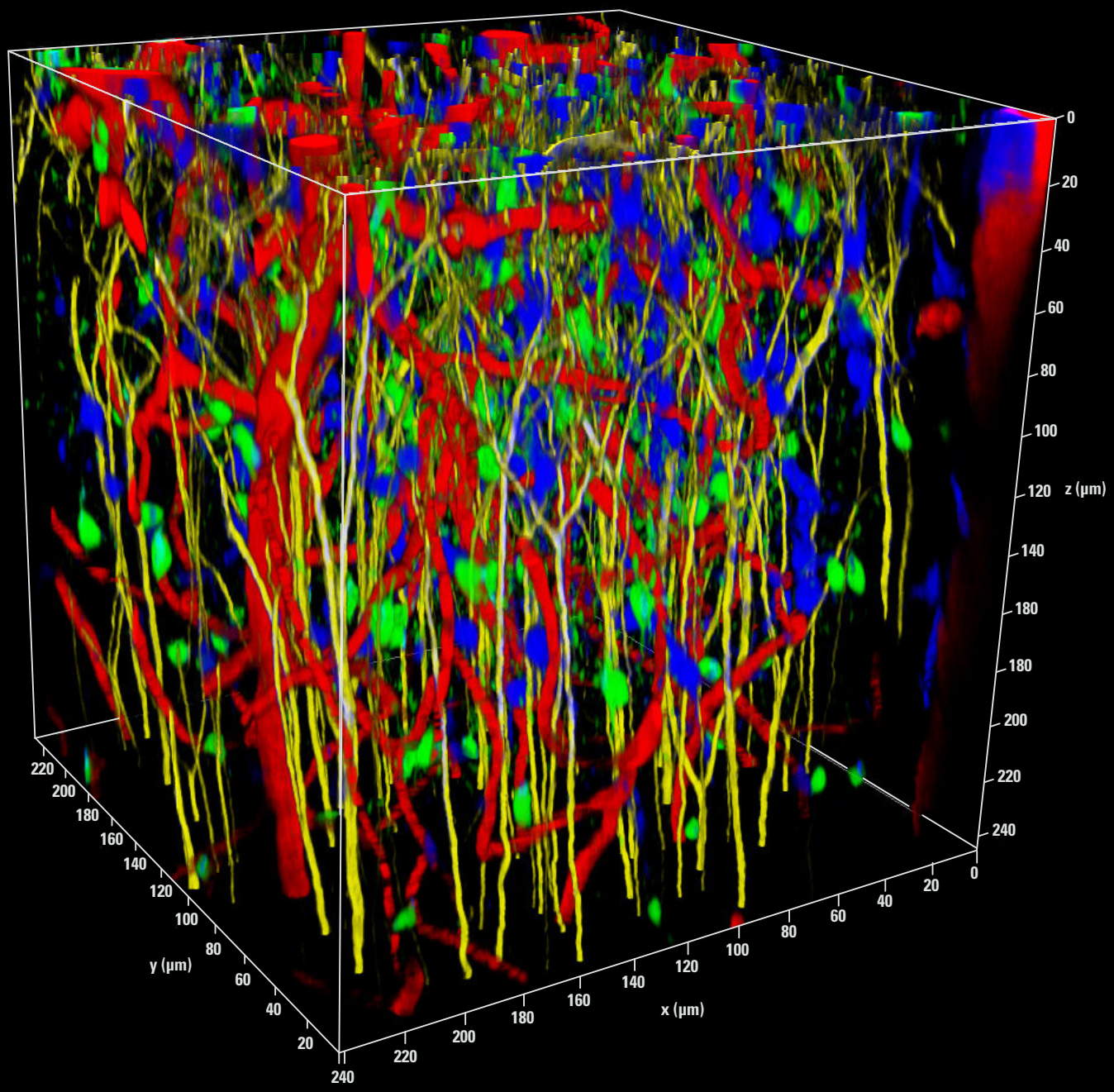
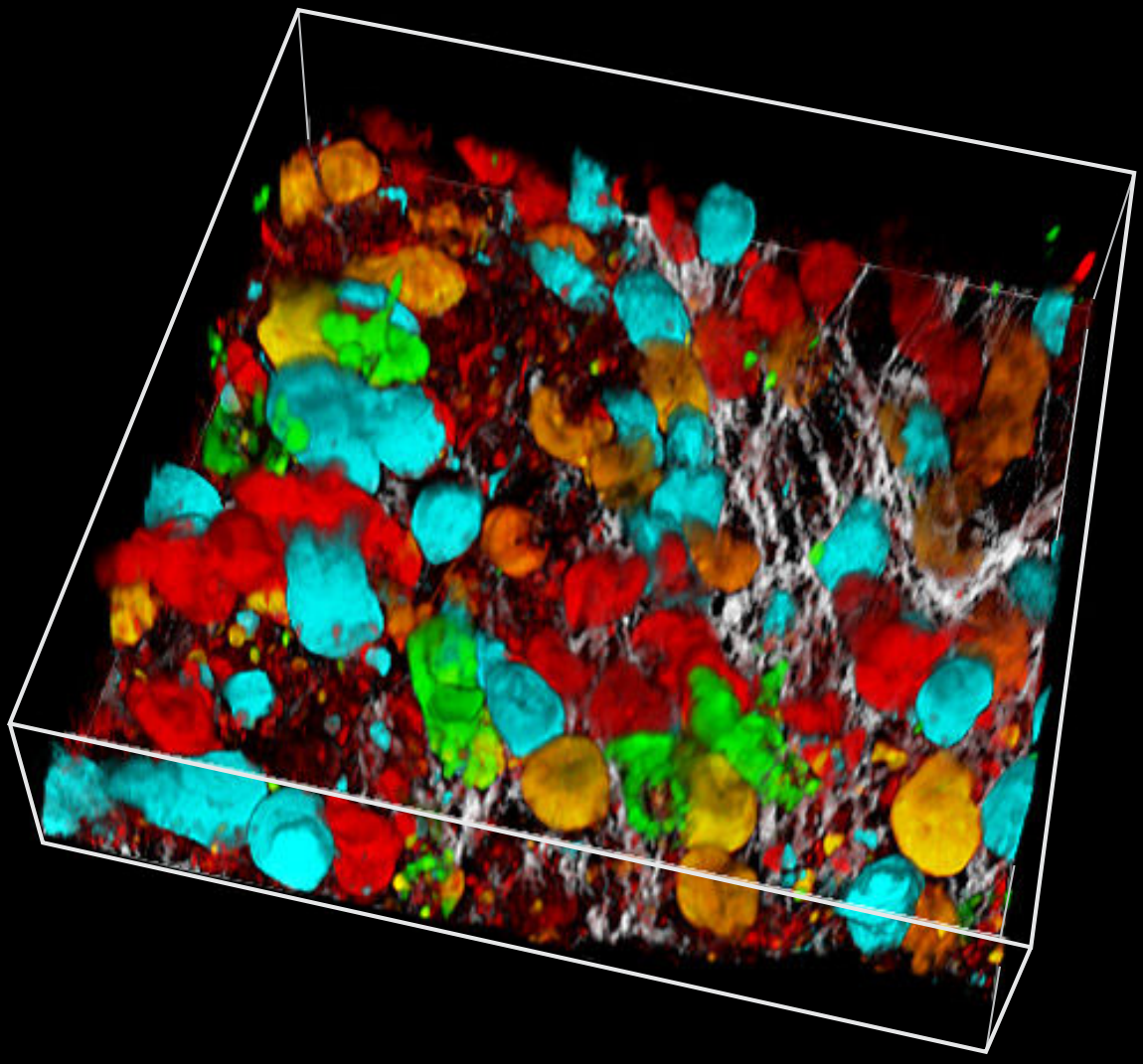
From Eye to Insight

Leica
MICROSYSTEMS

SP8 DIVE — DEEP IN VIVO EXPLORER

The world's first tunable deep imaging solution





Confetti mouse small intestines, fluorescently labeled and lineage traced from a multi-color tracer. The gray color codes for the SHG signal from Collagen. Lineage traced stem cells are shown in cyan (CFP), green (GFP), yellow (YFP) and red (RFP). Image size is ca. 700x700x150 µm³. Recorded with two-photon excitation, using the SP8 DIVE. Sample courtesy of J. van Rheenen, Netherlands Cancer Institute, Amsterdam (the Netherlands).

Title page: Mouse brain, transgenic and transiently labeled. Different types of nerve cells are shown in green (microglia, GFP), yellow (neurons, YFP), blue (astrocytes, sulforhodamine) and the blood system is shown in red (Alexa680-dextran). Sample courtesy of Kevin Keppler, Light Microscope Facility, DZNE Bonn (Germany).

SP8 DIVE
SPECTRAL FREEDOM FOR MULTICOLOR DEEP IN VIVO IMAGING

Spectral Freedom with 4Tune

Up to now, the possibilities to do multicolor deep in vivo experiments were limited by the choice of dichroic filters. The SP8 with the brand-new and unique 4Tune detector is the world's first fully spectrally tunable multiphoton microscope. Another degree of spectral freedom results from the newly implemented possibility to use up to three IR excitation lines.

Today's research focuses on complex biological processes such as human diseases. Complexity mainly results from the number of components involved: The need of labeling and imaging more than one or two components in a natural environment is evident.

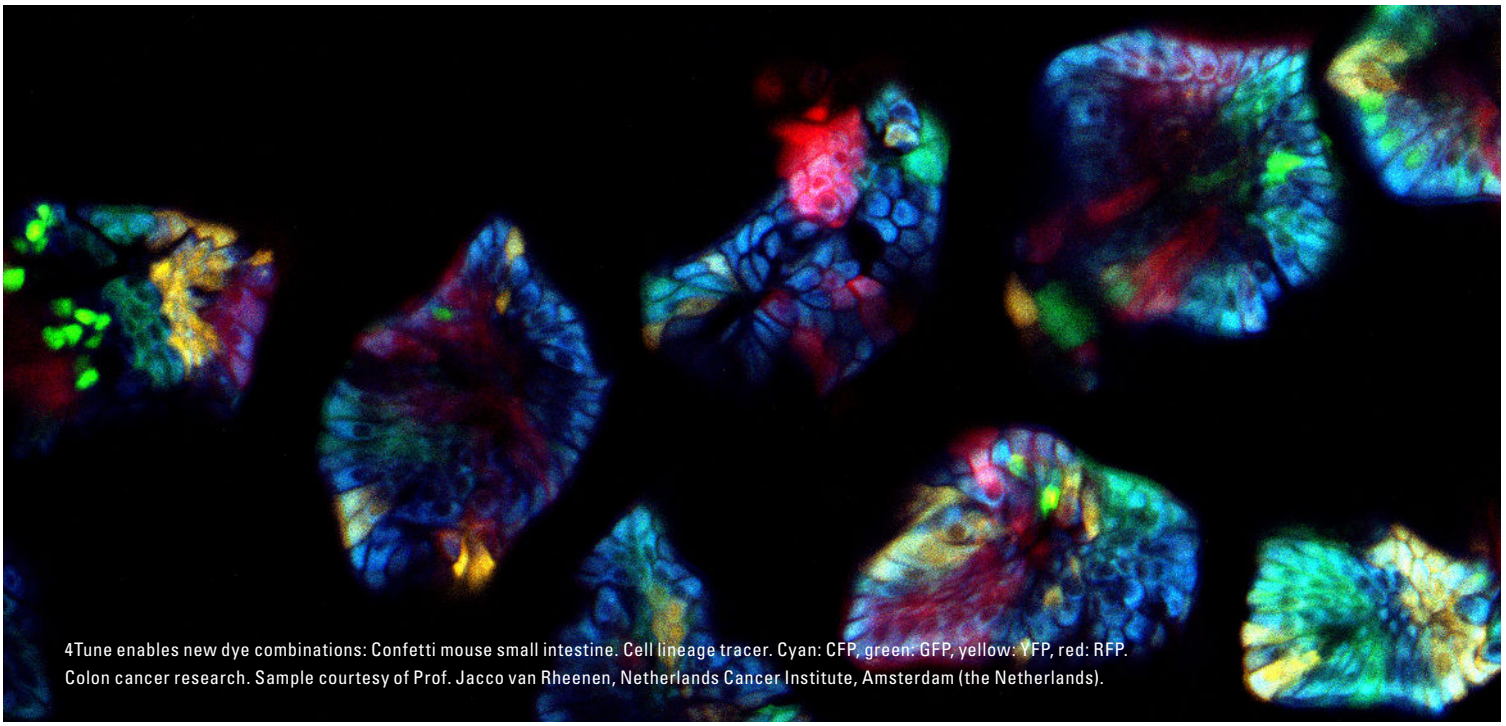
The SP8 DIVE offers you unlimited flexibility to reveal the interactions of multiple structures by imaging in whole animal model organisms.

Deep Insights with the Vario Beam Expander

Most times, the information you need is hidden deep within the scattering tissue. The longer infrared wavelengths used by multiphoton microscopy allow you to penetrate several hundreds of micrometers into the tissue and uncover details from inside a living model organism with low phototoxicity. Even deeper insights come with the SP 8 DIVE: The SP8 DIVE features lasers up to 1300 nm and the new Vario Beam Expander (VBE) that allows tuning for best depth penetration and for best resolution.

"The conventional dichroics are never optimal to distinguish all fluorophores, but with the spectral detectors, this is now possible and much easier, since we can really optimize for each flurophore the wavelengths you want to detect."

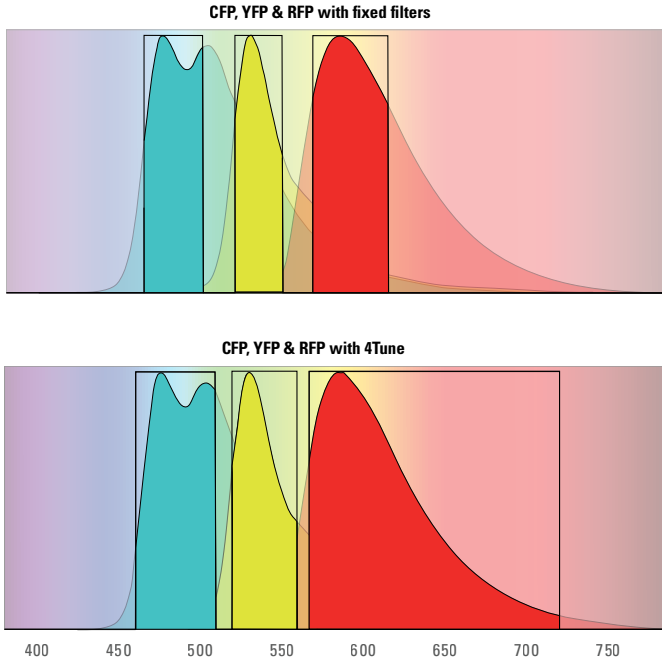
Prof. Dr. Jacco van Rheenen. Netherlands Cancer Institute, Amsterdam (the Netherlands).



4Tune enables new dye combinations: Confetti mouse small intestine. Cell lineage tracer. Cyan: CFP, green: GFP, yellow: YFP, red: RFP. Colon cancer research. Sample courtesy of Prof. Jacco van Rheenen, Netherlands Cancer Institute, Amsterdam (the Netherlands).

ENJOY SPECTRAL FREEDOM

Tune your detection and collect more fluorescence with 4Tune



4Tune increases detection efficiency. Comparison CFP, YFP, RFP filter based detection vs. 4Tune. Improve signal collection for CFP by 35 %, for YFP 8 % and RFP 98 %.

Adapt to any transgenic marker

The evolution of new fluorescent transgenic markers in combination with genetic tools like the CRISPR/Cas system speed up many research areas and challenges traditional multiphoton imaging technologies.

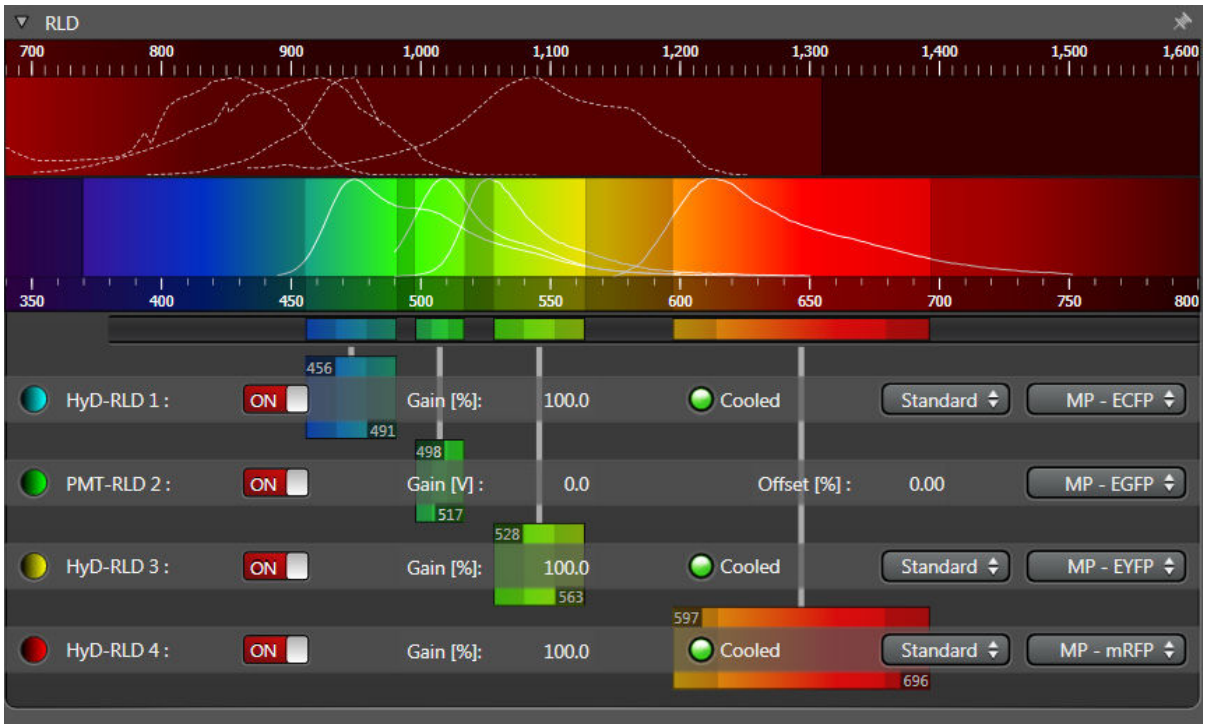
The SP8 DIVE keeps pace with developments of fluorophores and can easily adapt to any upcoming or existing markers with just a few mouse clicks.

Our patent-pending spectral detector on the non-descanned site, 4Tune, allows you to define up to four detection bands simultaneously anywhere in the emission spectrum. 4Tune allows you to adapt to any combination of fluorophores and separate strongly overlapping spectra.

Twice as much fluorescence

Depending on the dye combination used, the 4Tune detector enables you to capture twice as much of the fluorescent signal compared to commercial filter sets. This translates to improved penetration depth, faster scanning speed or less phototoxicity for deep in vivo imaging.

No need to design or exchange filter cubes, detection windows can be easily adjusted live and on the fly!



The intuitive 4Tune user interface of the Leica LAS X software. Up to four channels can be defined and imaged simultaneously – unlimited in the sequential mode.

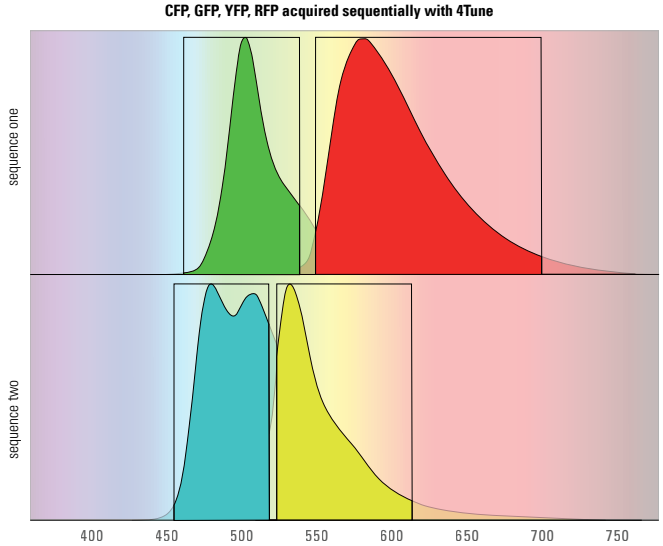
DIVE WITH EASE

Experimental set-up made easy with Leica LAS X

Optimize your settings live and with minimal effort

The intuitive 4Tune user interface lets you optimize the emission settings of multiple fluorophores live and on the fly. Due to its clear and user-friendly design, operation is easy and requires minimal training.

If more than four markers are investigated or overlapping spectra require sequential imaging, 4Tune detection bands can be defined for multiple sequences and tuned automatically – new experiments suddenly become feasible!



4 channel sequential setting for the confetti construct. Imaging sequentially, CFP and YFP as well as GFP and RFP can be well separated. Sequence 1 uses 900 and 1000 nm for excitation, sequence 2 uses 860 and 960 nm for excitation.

Image sequentially and omit crosstalk

Transgenic markers like CFP, GFP, YFP and RFP can only be separated by excitation with dedicated excitation wavelengths, however, using fixed filters for emission detection cuts off most of the emission light. In most cases, this impairs their separation and as a consequence their efficacy in tracing multiple targets in the same experiment. A clever combination of balanced excitation and tuned 4 Tune detection enables you to separate strongly overlapping fluorophores without the need of mathematical restauration.

LASER RADIATION
VISIBLE AND INVISIBLE- CLASS 3B
AVOID DIRECT EXPOSURE TO BEAM
P < 500 mW 350- 700nm
IEC 60825-1: 2014
LASER RADIATION
VISIBLE AND INVISIBLE- CLASS 4
AVOID EYE OR SKIN EXPOSURE TO
DIRECT OR SCATTERED RADIATION
Paverage < 4 W 350- 1600nm >40fs
IEC 60825-1: 2014
Leica
Newport
SG Breadboard

4 REASONS FOR 4TUNE

Flexible Deployment of Fluorescent Markers

4Tune, the heart of the SP8 DIVE, is a unique spectral non-descanned detection unit that enables you to define your detection band freely between 380 and 800 nm with variable bandwidth. The detection can be adjusted live by simple drag and drop.

Efficient Photon Collection

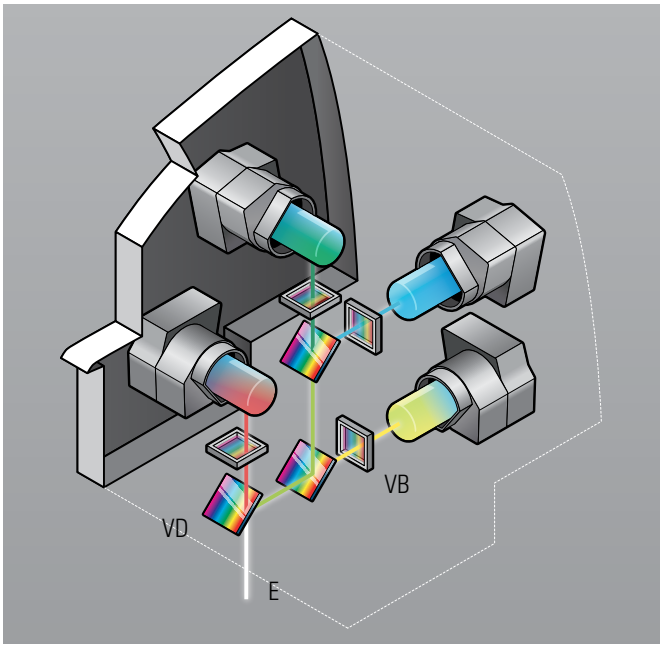
4Tune, located at the non-descanned site, enables multicolor deep in vivo imaging in 1 mm depth and beyond. In contrast, confocal detection strongly limits penetration depth in multiphoton mode. Equip your 4 Tune detection with HyD detection and tune the detection bandwidth optimally for each fluorophore to achieve most efficient photon collection.

Ready for FLIM

4Tune can be equipped with 2 to 4 detection units, which are freely configurable with the proven super-sensitive HyD hybrid detectors, photomultipliers or a combination of both. HyD detectors are prepared for fluorescence lifetime imaging (FLIM). Upgrade anytime to your FLIM imaging system with the SP8 FALCON.

New Aera for Sequential imaging

4Tune allows you to configure multiple sequences with different detection windows. Without the need of exchanging filter cubes in between – an unlimited number of channels can be defined, independent of the number of detectors available!



How 4Tune works:
4Tune spectral non-descanning detection: emission (E) from the sample is spatially separated into sub-spectra by a set of continuously variable dichroics (VD). The final signal fractions can be fine-tuned by continuously variable band-passes (VB) before being picked up by sensors.

Technical specification

- 380 – 800 nm detection range
- 10 nm minimal bandwidth
- 1 nm stepsize
- Live adjustment of detection range
- 2 – 4 channels, HyD or PMT

Hybrid detection for highest sensitivity:
The 4Tune detector can be equipped with four HyD hybrid detectors – the standard in super-senstive imaging.
Lower excitation power ensures less specimen damage, while the HyDs' superior signal-to-noise ratio reveals more detail from deeper tissue sections.

DIVE ENABLES NEW RESEARCH

Lambda Square Scanning

Fully explore the photonic landscape of your sample by carrying out an excitation-emission scan using 4Tune detection. Emission spectra are captured exploiting the tunable 4Tune bands, excitation spectra acquisition becomes easy using the newly introduced constant power mode.

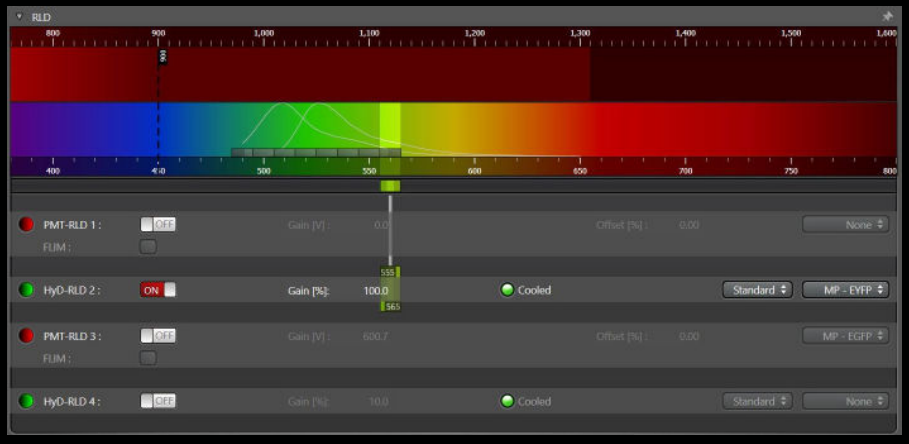
You can investigate the autofluorescence of your sample or hunt for new fluorescent markers. With only a few mouse-clicks you receive a two-dimensional plot of excitation versus emission for each pixel of your image – a spectral fingerprint. You can either use this to adjust imaging parameters or to characterize your sample.

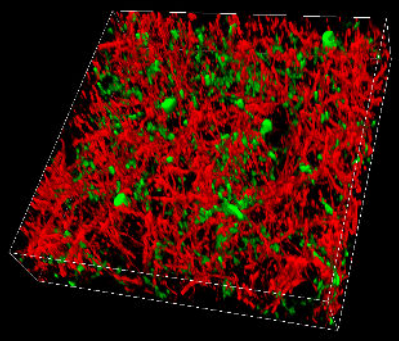
Constant power is maintained by adjusting the AOM accordingly to the absolute output-power of the laser as indicated by the greyed range.

Spectral and Channel Dye Separation

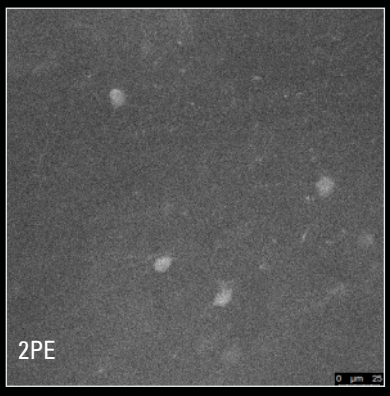
The SP8 DIVE supports the separation of stronger overlapping spectra by spectral or channel unmixing. SP8 DIVE therefore provides the best basis for successful separation of fluorescence spectra.

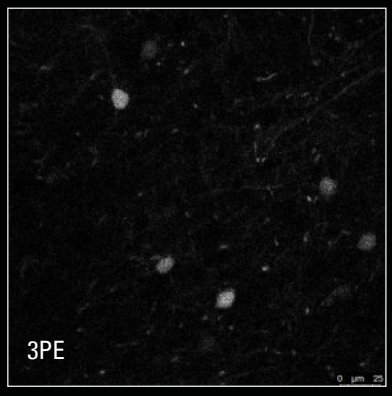
The workflow is simple: Up to four simultaneous channels can be balanced out individually for signal intensity and captured in one shot. With channel dye separation this is then split into the expected number of signals. With an emission lambda scan, spectral dye separation can be used to gain even better separation if required.





GFP (green) and YFP (red) expressed in the cortex of a live mouse can be separated effortlessly by capturing either two channels simultaneously or conducting a spectral emission scan and applying channel unmixing in LAS X. Sample courtesy of Kevin Keppler, Light Microscope Facility, DZNE, Bonn (Germany).





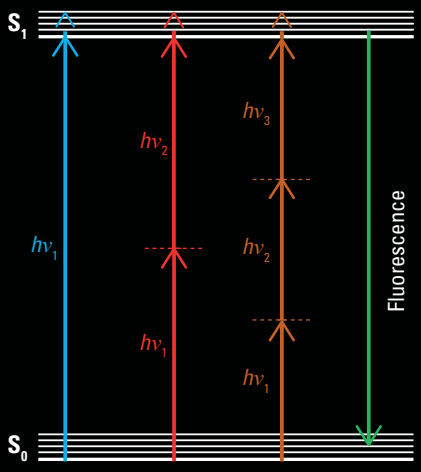
Comparison of 2PE with 920 nm (left) and 3PE with 1300 nm (right), 800 µm deep in the live mouse brain cortex. Thy1-eYFP cortical neurons, IRAPO 25x1.0 W motCorr, both images autoscaled from darkest to brightest pixel.

Sample courtesy of Kevin Keppler, Light Microscope Facility, DZNE, Bonn (Germany).

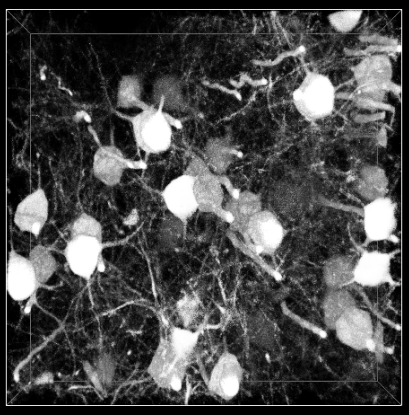
Three photon excitation

The new SP8 DIVE can be equipped with laser sources that allow excitation up to 1300 nm. This is the ideal wavelength for three-photon excitation (3PE) of GFP, YFP or other markers within the same spectral range such as GCaMP or Alexa488.

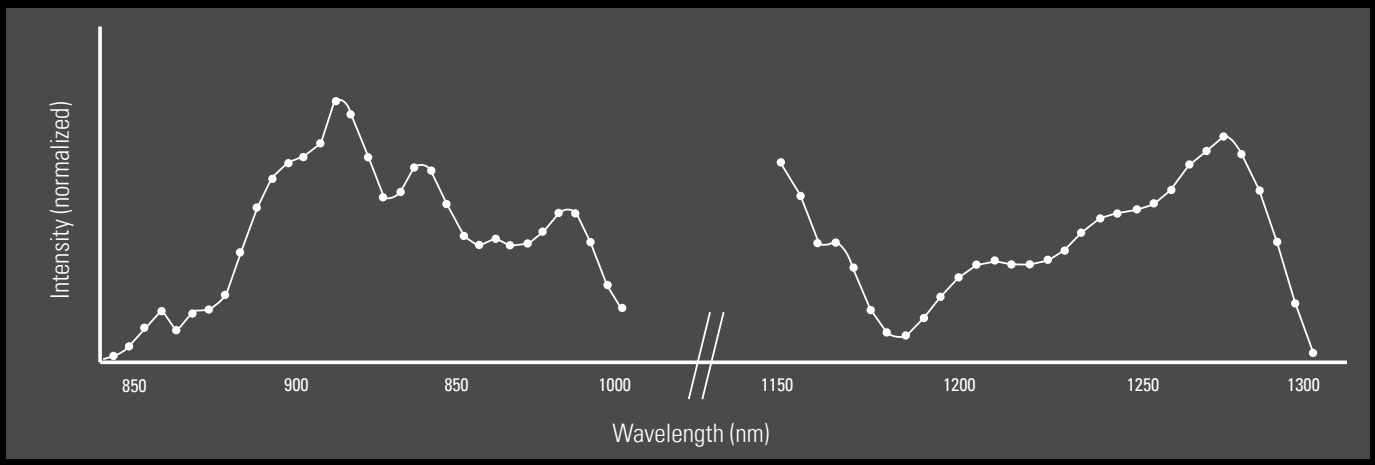
3PE even exceeds 2PE in terms of penetration depth and contrast as longer wavelengths are less prone to scattering and therefore penetrate even deeper. Plus, they trigger less autofluorescence of the tissue, and the background is substantially lower – even as deep as 800 µm in highly scattering brain tissue.



Simplified principles of one-, two- and three-photon excitation.



Live mouse brain cortex. Thy1-eYFP cortical neurons,imaged in 450 µm depth with 1300 nm excitation. Sample courtesy of Kevin Keppler, Light Microscope Facility, DZNE, Bonn (Germany).



Excitation spectra of GFP labeled neurons showing the 2PE peak at 900 nm and the 3PE peak at 1280 nm. Spectra captured with SP 8 DIVE using the constant power mode.

LABEL — FREE IMAGING WITH DIVE

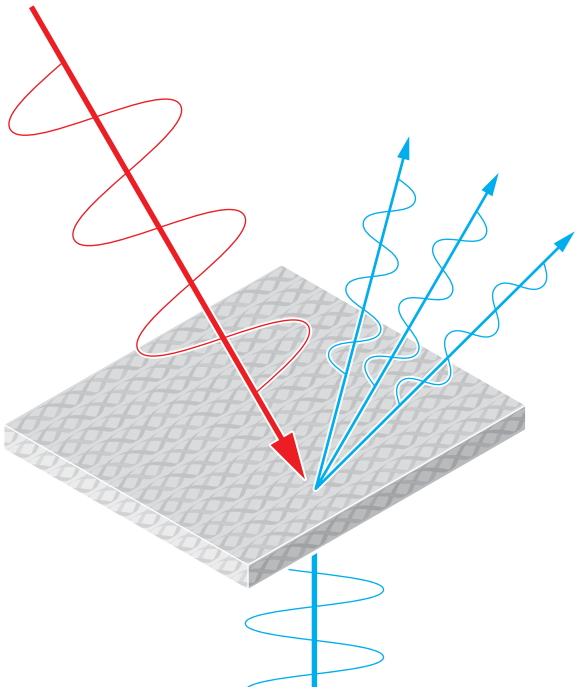
Second and third harmonics on the fly

Second harmonic generation (SHG) has emerged as a powerful label-free imaging modality to visualize fibrillar collagen in diverse tissues.

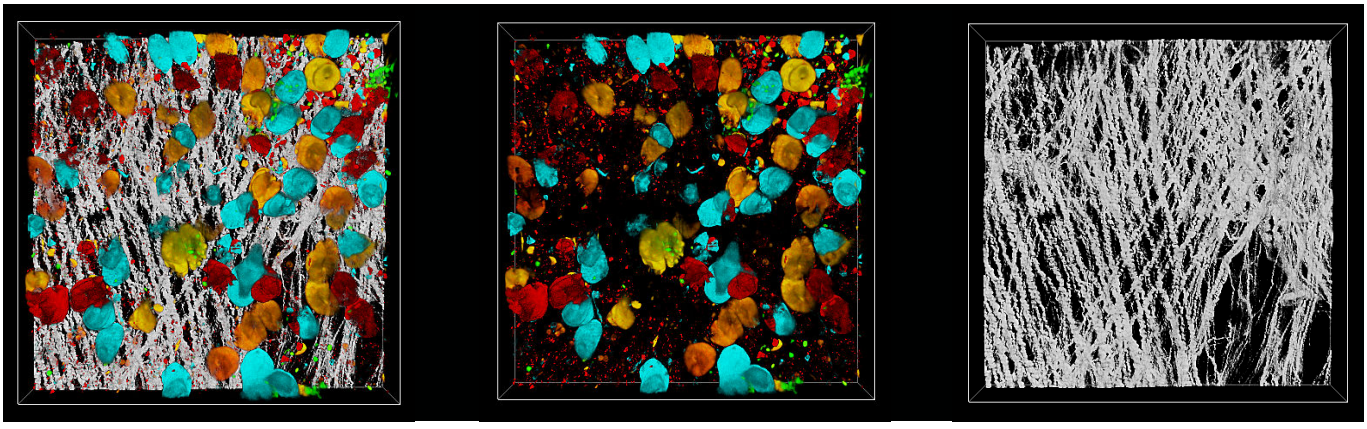
SHG signals occur from large non-centrosymmetric structures with a periodic alignment. These structures double the frequency of the infrared pulsed excitation light used for multiphoton imaging. In conclusion, no labels need to be incorporated and since the light is not absorbed but scattered, photodamage does not occur.

The information from this channel can be used in various ways: Either by studying the change of collagen caused by a disease or the use as an orientation landmark within your tissue. Since SHG does not require labeling, it can be picked up at any time at half of the wavelength that is used.

The SP8 DIVE with its tunable 4Tune detector enables you to select a narrow detection bandwidth at exactly double the frequency of any excitation wavelength you use to visualize unlabeled structures like collagen, myosin or microtubules in addition to your fluorophores. As a consequence, spectral detection with 4Tune allows you to add important structural information to your experiment – at any time.



Principle of SHG formation: The incoming light (red) frequency is doubled (blue) which translates to emission with half of the incoming wavelength.



Images of small intestine of Lgr5CreERT2-GFP confetti mice. Gray: SHG signal (collagen 1) which is used as an orientation landmark, green: Lgr5+ stem cells. Red, cyan and yellow, traced cells that are progeny of the Lgr5 stem cells. Sample courtesy of Prof. Jacco van Rheenen, Netherlands Cancer Institute, Amsterdam (the Netherlands).

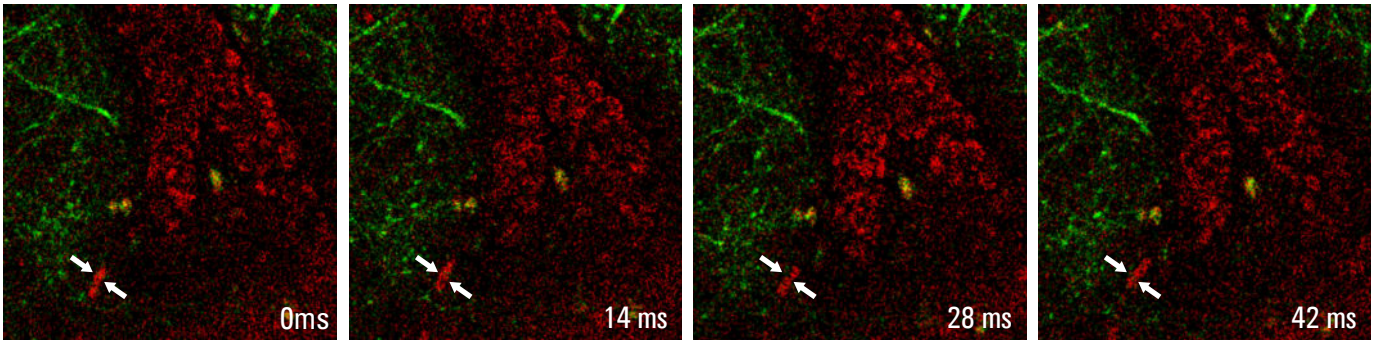
Like SHG, third harmonic generation (THG) is a label-free imaging method, however it occurs at exactly one third of the incoming pulsed laser light wavelength. By equipping the SP8 DIVE with a laser source that provides wavelengths up to 1300 nm, THG can be detected in the visible range at 430 nm.

With 4Tune detection, you can easily adapt the detection window to the tripled frequency signal. Without the need of a dedicated filter, for example live blood flow tracking is possible with just a few mouse clicks.

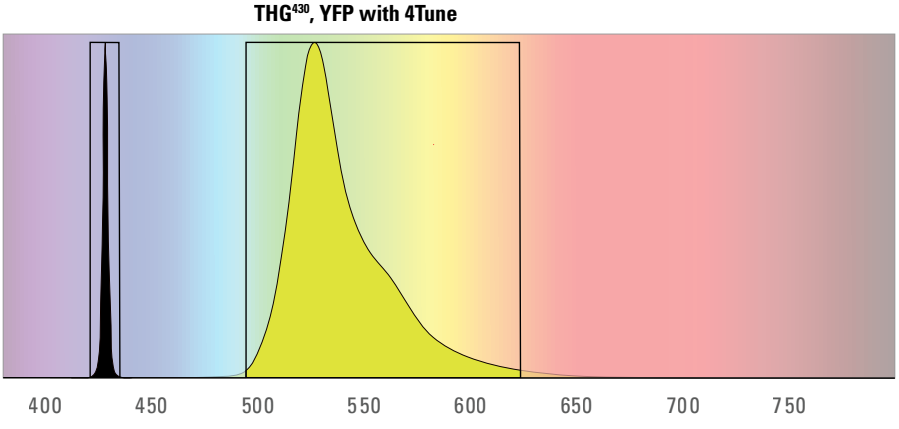
Combine THG with Ultra-fast imaging

Blood flow happens at very fast time scales. To enable live blood flow tracking, the optional 12 kHz resonant scanner with frame rates up to 428 fps, is the ideal tool. In combination with the ultrasensitive HyD detection even weak signals can be picked up.

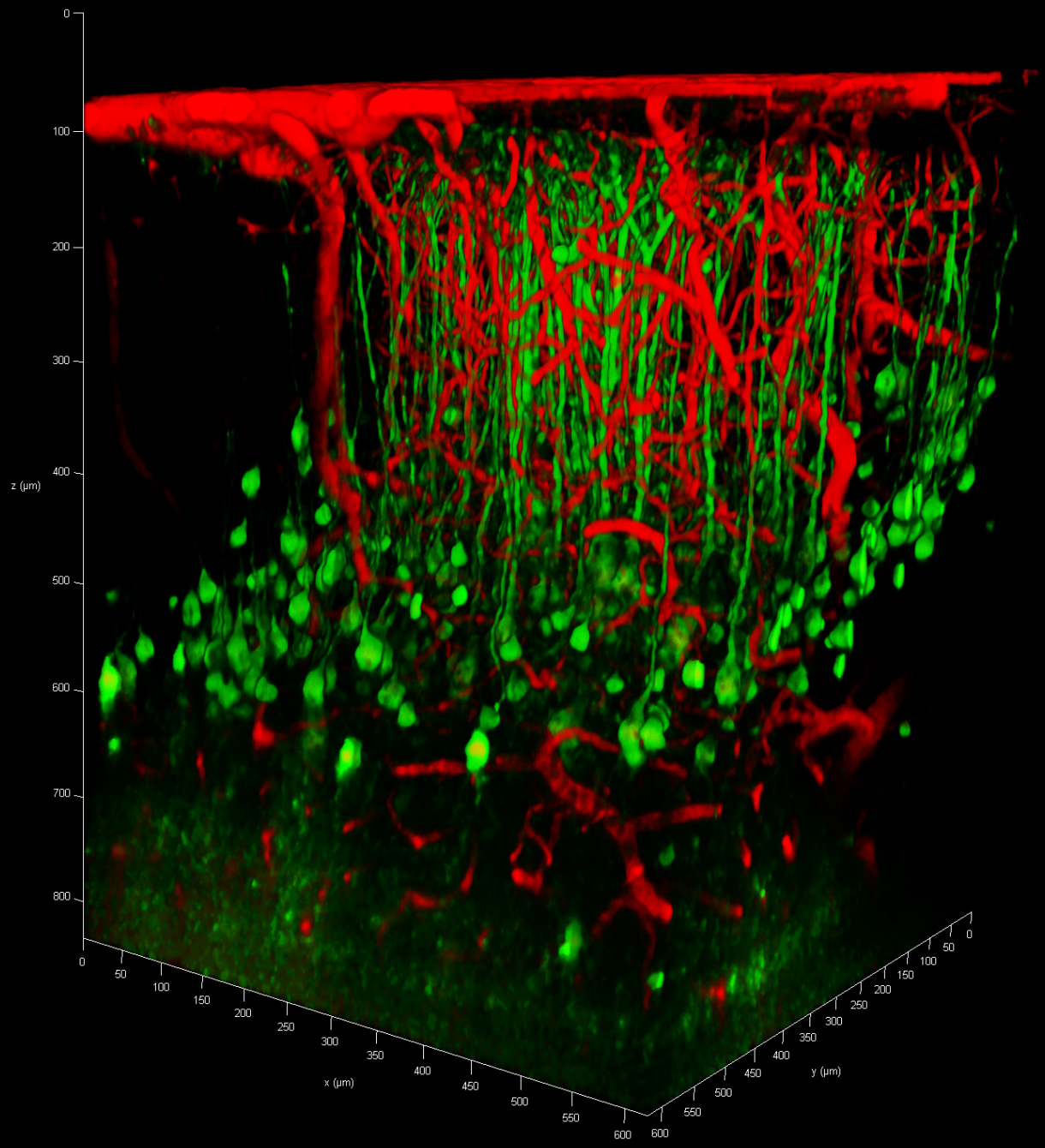
Since THG also occurs at refractive index mismatches, it gives the same information as phase contrast microscopy but due to local confinement of the focal spot, the phase contrast information is even obtained in three dimensions.



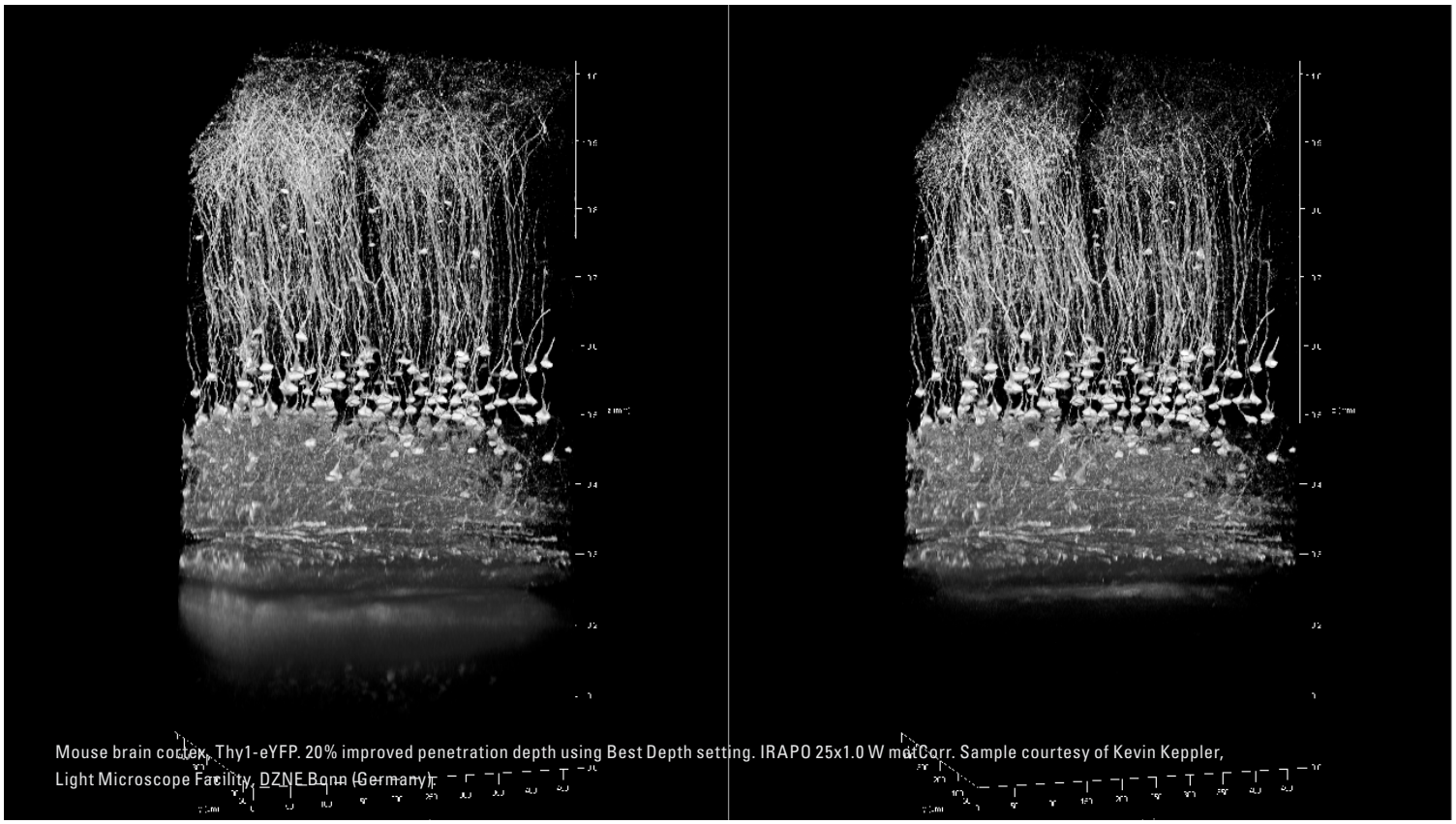
Mouse brain cortex. Green: Thy1-eYFP cortical neurons, red: THG blood cells, 1300 nm excitation, IRAPO 25x1.0 W motCorr; 70 fps, 256x256 px, Sample courtesy of Kevin Keppler, Light Microscope Facility, DZNE, Bonn (Germany).



Detection scheme for the above experiment.
The narrow detection window for THG at exactly 1/3 of the exciting wavelength can be defined on the fly – no dedicated filter needed! Using 3PE for YFP, both signals can be captured well separated in a single sequence experiment – ideal for fast blood flow tracking!



Mouse brain cortex., Thy1-eYFP, Tx Red. IRAPO 25x1.0 W motCorr.
Sample courtesy of Kevin Keppler, Light Microscope Facility, DZNE Bonn (Germany).

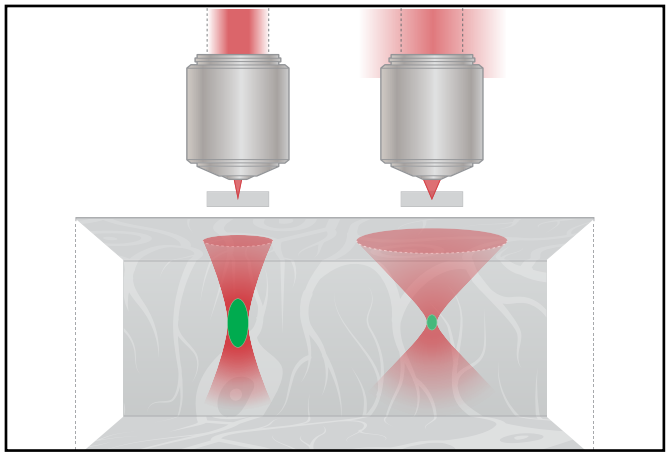


Mouse brain cortex. Thy1-eYFP. 20% improved penetration depth using Best Depth setting. IRAPO 25x1.0 W motCorr. Sample courtesy of Kevin Keppler, Light Microscope Facility, DZNE Bonn (Germany).

EXPLORE NEW DIMENSIONS IN DEPTH

The thicker the sample, the more scattering occurs and the more information is lost from deeper layers. This is particularly challenging when you want to image in dense tissue of living model organisms. The novel and unique Vario Beam Expander (VBE) gives you the choice between maximum imaging depth or maximum resolution by simply moving a slider in the LAS X software.

In addition, the new excitation beam path perfectly harmonizes with the SP8 DIVE and optimized coatings for best transmission improve excitation efficiency in highly scattering tissue.



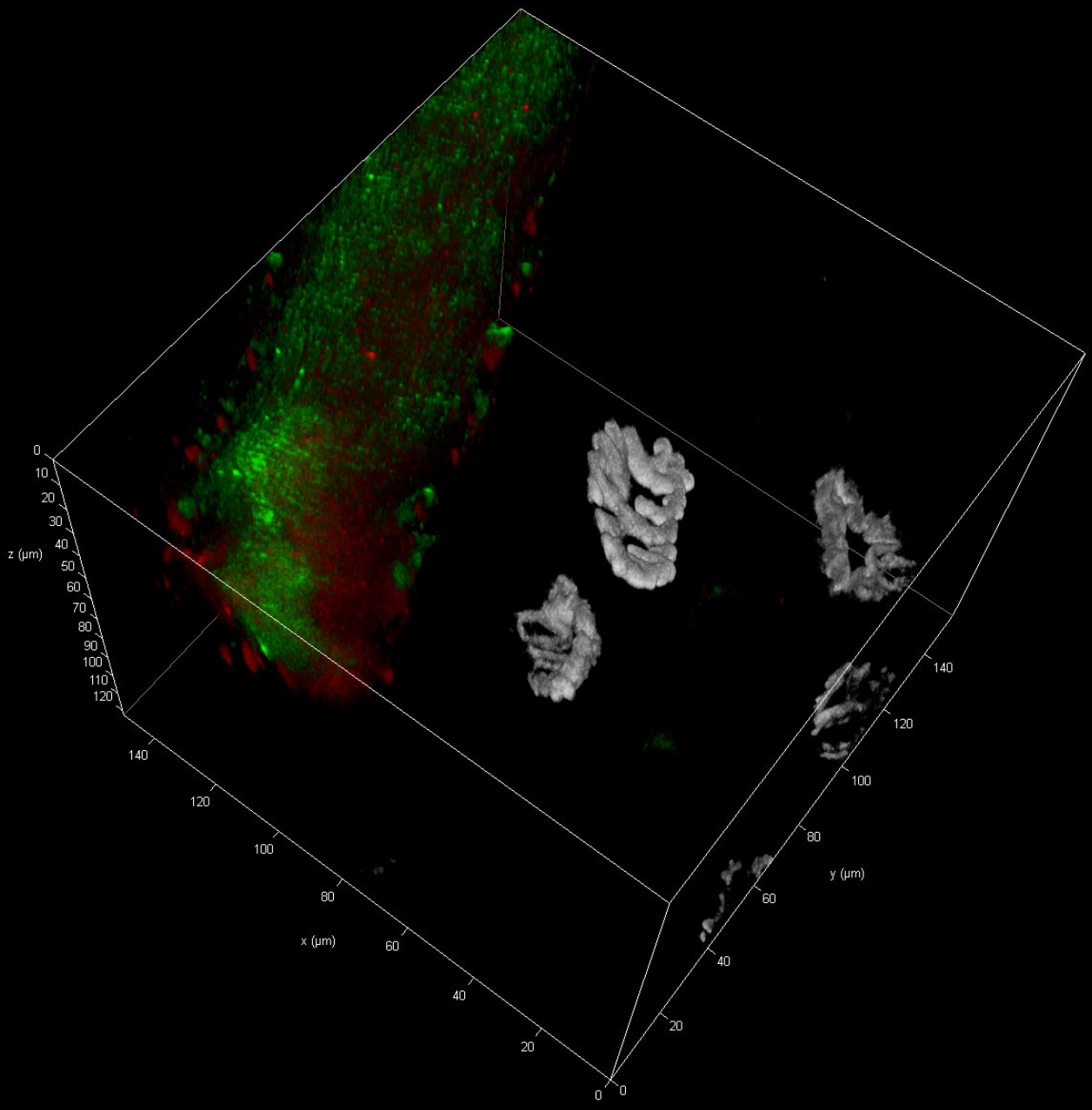
The Vario Beam Expander:
Tune for penetration depth (left) and optical resolution (right).

DIVE deeper with the Vario Beam Expander

The VBE adapts the input of your excitation laser by tuning the diameter of the beam.

- By widening the beam you obtain homogeneous illumination of the back focal plane of your objective lens. In combination with a high numerical aperture, this results in maximal resolution.
- Narrowing the beam causes higher energy input in the focal volume. This reduces the effective numerical aperture of the objective lens which results in a better penetration of the sample and therefore deeper imaging.

The VBE allows independent adjustment of up to three individual IR wavelengths simultaneously. With the VBE, the SP8 DIVE adapts to your experimental setup.



Mouse tibia: Striated muscle. Morphological analysis of neuromuscular junction development.
Green: GFP – Rab 5 (GTPase, endosomes)
Red: mCherry – Bif 1
Grey: AF647- Alpha bungarotoxin (NMJ)
Sample courtesy of Prof. Rudolf, University of Applied Science, Mannheim (Germany).

DEEP IN VIVO COLOCALIZATION

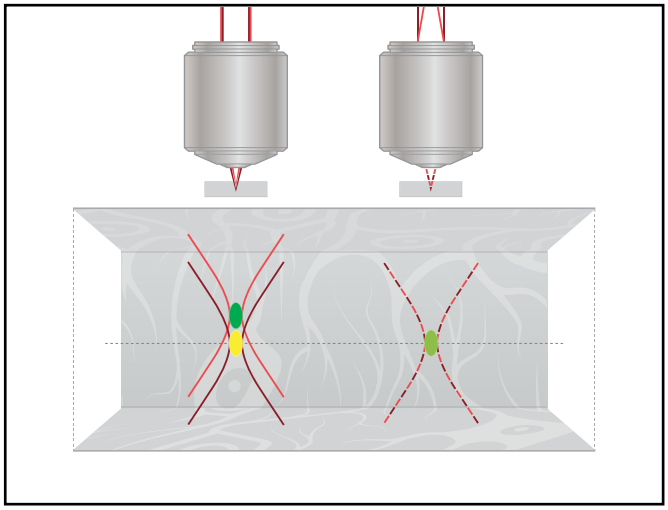
Multicolor excitation

Excite multiple transgenic markers in a single experiment with perfect color separation. Or even carry out localized high-precision photomanipulation and simultaneous imaging in a diffraction limited volume. The SP8 DIVE can be equipped with up to three excitation lines simultaneously, balanced by acousto-optical modulation. Since lasers tunable up to 1300 nm can be integrated, you can even use red and far red dyes for multiphoton experiments. Less scattering enables deeper penetration and results in bright images full of detail even from deep layers.

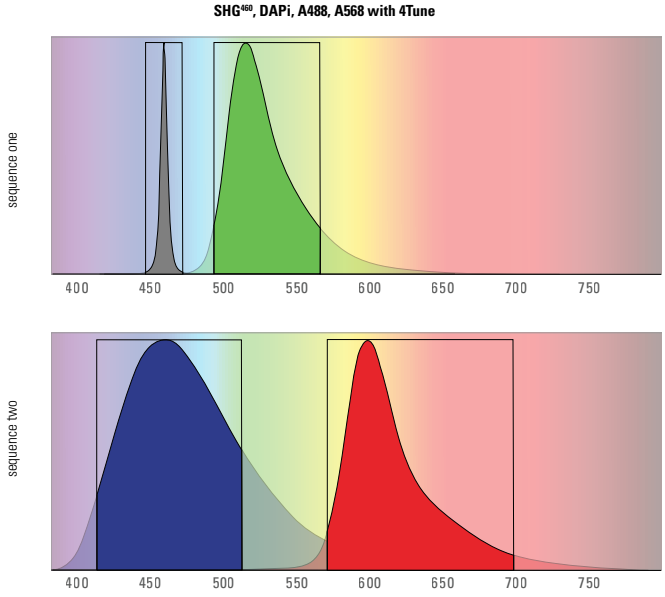
TuneIR – Tune for full color correction

Reproduce your results as often as you need to. TuneIR ensures lateral and axial overlay of multiple IR beams. Lateral alignment can be restored with just one mouse click.

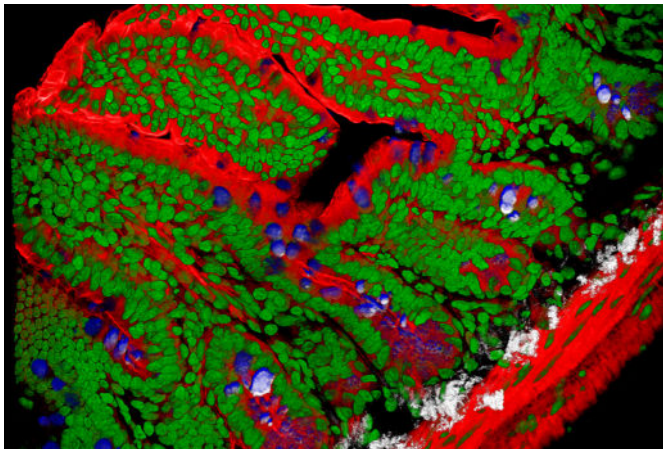
Even objective lenses without color correction along the whole spectrum can now be easily used in multicolor experiments. Prefocusing via the Vario Beam Expander corrects the axial color shift for different IR wavelengths. It is possible to excite the same focal plane using up to three different IR-lines.



Axial color correction with TuneIR: Axial color shift between different IR wavelength can easily be corrected for by prefocusing. Up to three IR lines can be matched.



Sequential setting of the four detection windows used for the image acquisition of the image on the right.



Mouse intestine, mucus of goblet cells, WGA (blue), Nuclei, SYTOX Green (green), Alexa568-phalloidin (red), SHG (white) Sample: Molecular Probes FluoCells #4 Mouse Intestine.

DEEP INSIGHTS RESULT FROM THE SYNERGY OF PARTS

Leica objective lenses for best optical performance

Efficient sample protection with IRAPO Objectives

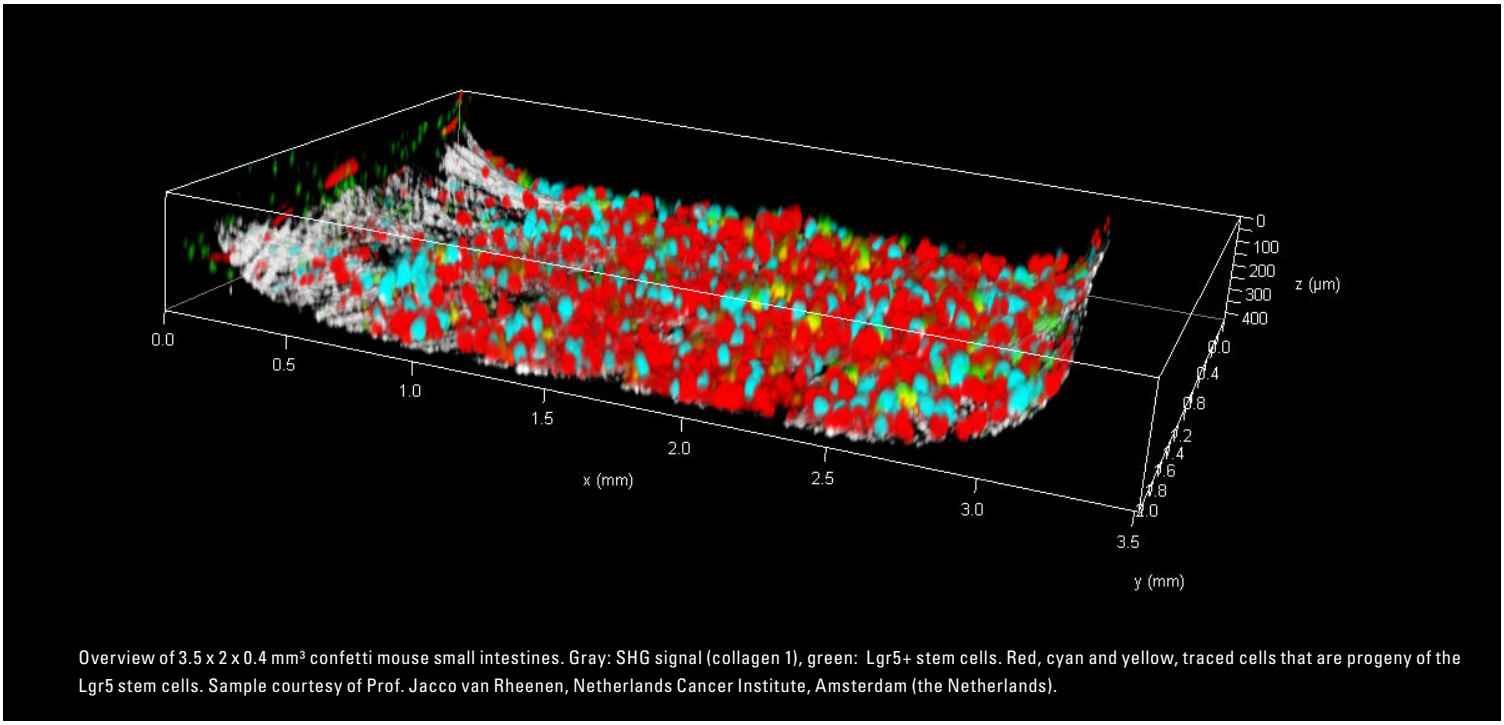
Multiphoton microscopy requires special objectives to ensure optimal color correction and transmission. Designed for distinct colocalization on multicolor multiphoton images, the dedicated IRAPO objectives reduce the need for high laser power and help to protect your precious sample from photodamage.

Precision at all depths: The motorized correction collar of the Leica motCORR objectives is able to compensate for refractive index mismatches and specimen inhomogeneity using real-time optical adjustment to restore optimal imaging in x, y and z dimensions.

Tissue clearing for deep insights

Our family of dedicated objective lenses for bright, high-resolution images from deep within tissues includes:

- **FLUOTAR L 16x/0.8 IMM motCORR VISIR**, FWD 8 mm, n_e~1.33 – 1.46, coverslip 0 – 0.17 mm (lens for in vivo and cleared samples)
- **FLUOTAR L 16x/0.6 IMM CORR VISIR**, FWD 2.5 mm, n_e~1.33 – 1.52, coverslip 0-0.17 mm (lens for in vivo and cleared samples)
- **HCX APO L 20x/0.95 IMM**, FWD 1.95 mm, n_e~1.563 (e.g. BABB)
- **HC FLUOTAR L 25x/1.00 IMM motCORR VISIR**, FWD 6 mm, n_e~1.457 (e.g. CLARITY).

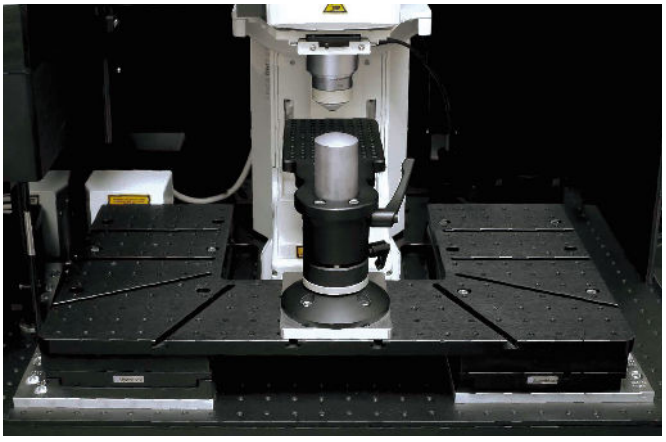


Overview of 3.5 x 2 x 0.4 mm³ confetti mouse small intestines. Gray: SHG signal (collagen 1), green: Lgr5+ stem cells. Red, cyan and yellow, traced cells that are progeny of the Lgr5 stem cells. Sample courtesy of Prof. Jacco van Rheenen, Netherlands Cancer Institute, Amsterdam (the Netherlands).

DIVE IN AND SEE MORE

Always maintain the overview: LAS X Navigator

Switch from searching image by image to seeing the full overview of your samples. Like a GPS for your disease model, LAS X Navigator software ensures that you always have a clear roadmap to the data you need. Create fast overviews of your area of interest and identify the important details instantly. Then set up high resolution image acquisition automatically.



The SP8 DIVE equipped with a Scientifica scanning stage. The variable sample holder can host different sizes of dishes and slides as well as small animals.

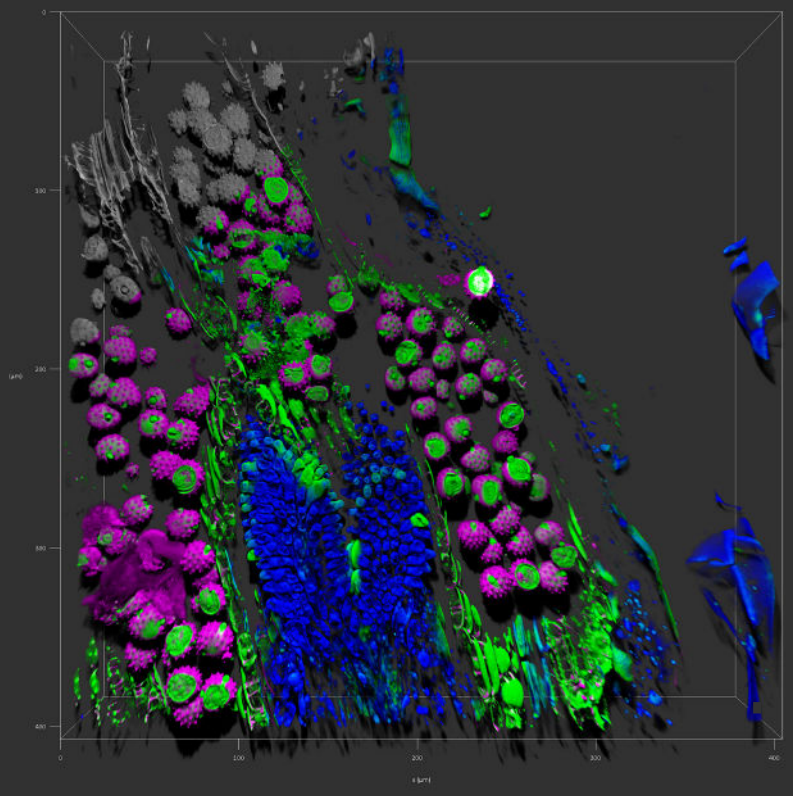
- Generate live overviews – fast!
- Create spiral scans to search in the vicinity of your current location
- Display images in sample carrier templates for quick orientation
- Correlate any magnification, camera, detector, and contrasting method in the same workspace
- Define an unlimited number of regions and positions for high resolution scans or multiwell projects
- Zoom swiftly in and out of your sample
- Move to any stage location by mouse click.

IDEAL COMPANION TO DIVE: SP8 FALCON

FAst Lifetime CONtrast for Functional Imaging
Understanding the function of molecules is key to find the origin of diseases. Fluorescence lifetime imaging (FLIM) is a very powerful tool because fluorescence lifetime is very sensitive to changes in molecular environment. At the same time it is independent of fluorophore concentration and photobleaching. FLIM provides contrast orthogonal to spectrum that multiplies the readout of molecular species. The new SP8 FALCON delivers lifetime and spectral information simultaneously and provides multimodal imaging faster than ever.

Video-rate FLIM for molecular interaction and biosensing
Unveil molecular interactions via FLIM-FRET. Measure metabolic activity, detect subtle pH changes in cells and tissue. Identify multiple species based on lifetime contrast. SP8 FALCON opens the door to multidimensional imaging with full temporal, spectral and spatial flexibility. SP8 FALCON works 10x faster than classical TCSPC, enabling video-rate timelapse imaging, 3D scanning, and mosaic scanning.

1-Click philosophy to focus on your science
SP8 FALCON is built on fast electronics and sensitive Leica HyD detectors. Fast algorithms and synergies with the SP8 platform and LAS X software make complex experiments easy. Retrieve the information contained in lifetime by combining SP8 FALCON with SP8 DIVE for deep multiphoton imaging, SP8 STED for nanoscopy resolution, and LAS X Navigator to reach every corner of large samples.



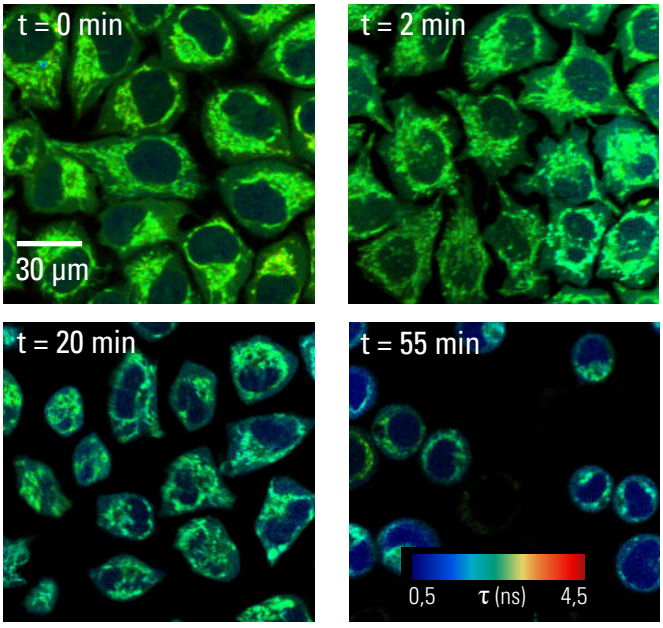
Daisy pollen, unstained sample. Simultaneous 3D spectral (gray) and FLIM (color) confocal imaging reveals contrast encoded in fluorescence lifetime. Stack dimensions: 1423 x 1423 pixels, 102 planes. The time to result was 25 min (acquisition + FLIM analysis + 3D visualization). Experiment performed using the SP8 FALCON and LAS X software including the new LAS X Navigator tool.

REVEAL THE HIDDEN CONTRAST AT THE SPEED OF LIFE WITH SP8 FALCON

Investigate metabolic state with SP8 DIVE and SP8 FALCON

The autofluorescence from tissue and cells contains metabolic information in conditions close to native. The species that contribute to autofluorescence are amino acids, nicotinamide and flavin adenine dinucleotides (NADH and FAD, respectively), and fluorescent pigments. NADH and its phosphate derivative NADPH are responsible for cell metabolism, signaling, and oxidative stress control.

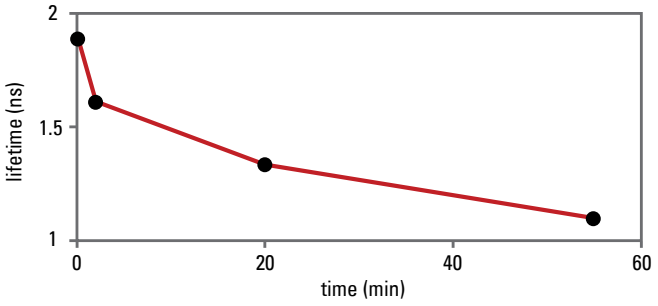
Due to its strong dependence on microenvironment, the fluorescence lifetime of NAD(P)H is a robust biosensor of cellular metabolic state. However, 1P excitation generates cell photodamage (365nm) and it is not suitable. 2P excitation with SP8 DIVE combined with SP8 FALCON becomes the tool of choice to address the modification of cellular redox state with high spatial and temporal resolution.



Reasons to believe

SP8 DIVE is ideal for NADH metabolic imaging, as 1P excitation would cause significant cell photodamage.

SP8 FALCON adds to SP8 DIVE the lifetime dimension and enables getting the experiment done, from acquisition to fit to anaylsis and visualization.



Autofluorescence in mammalian cells at non-physiological conditions (pH 8.5). The signal correlates with changes in the NAD/NADH endogenous pool. The development of oxidative stress reads out as decrease of fluorescence lifetime over time in the images (top panels) and average fluorescence lifetime values (right panel). Images size (top): 512 x 512 pixels. Color bar scale: ns.

"The Leica FALCON is the first commercial system that offers integrated confocal and lifetime imaging that I can imagine using in a core facility environment.

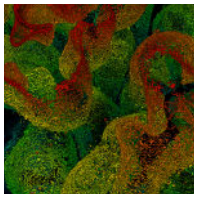
Its combination of ease of use with performance more typical of dedicated FLIM systems makes it a system of choice."

Prof. Scott Fraser, University of Southern California, Los Angeles (USA)

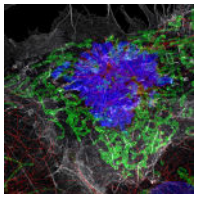
Leica
MICROSYSTEMS

SP8: FROM CONFOCAL TO MULTIMODAL

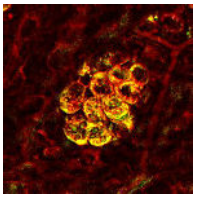
SP8 – the open Leica platform that covers your research



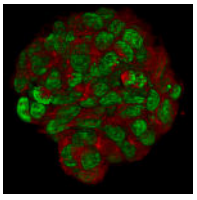
Nanoscopy
(STED)



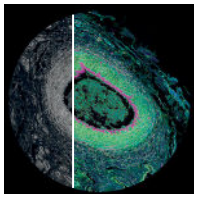
Super-resolution
(LIGHTNING)



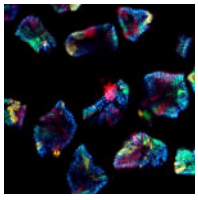
Vibrational Imaging
(CARS, SRS)



Light Sheet
Imaging (DLS)



Lifetime Imaging
(FALCON)



Multiphoton
(DIVE)

DIVE DEEP
WITH US!

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