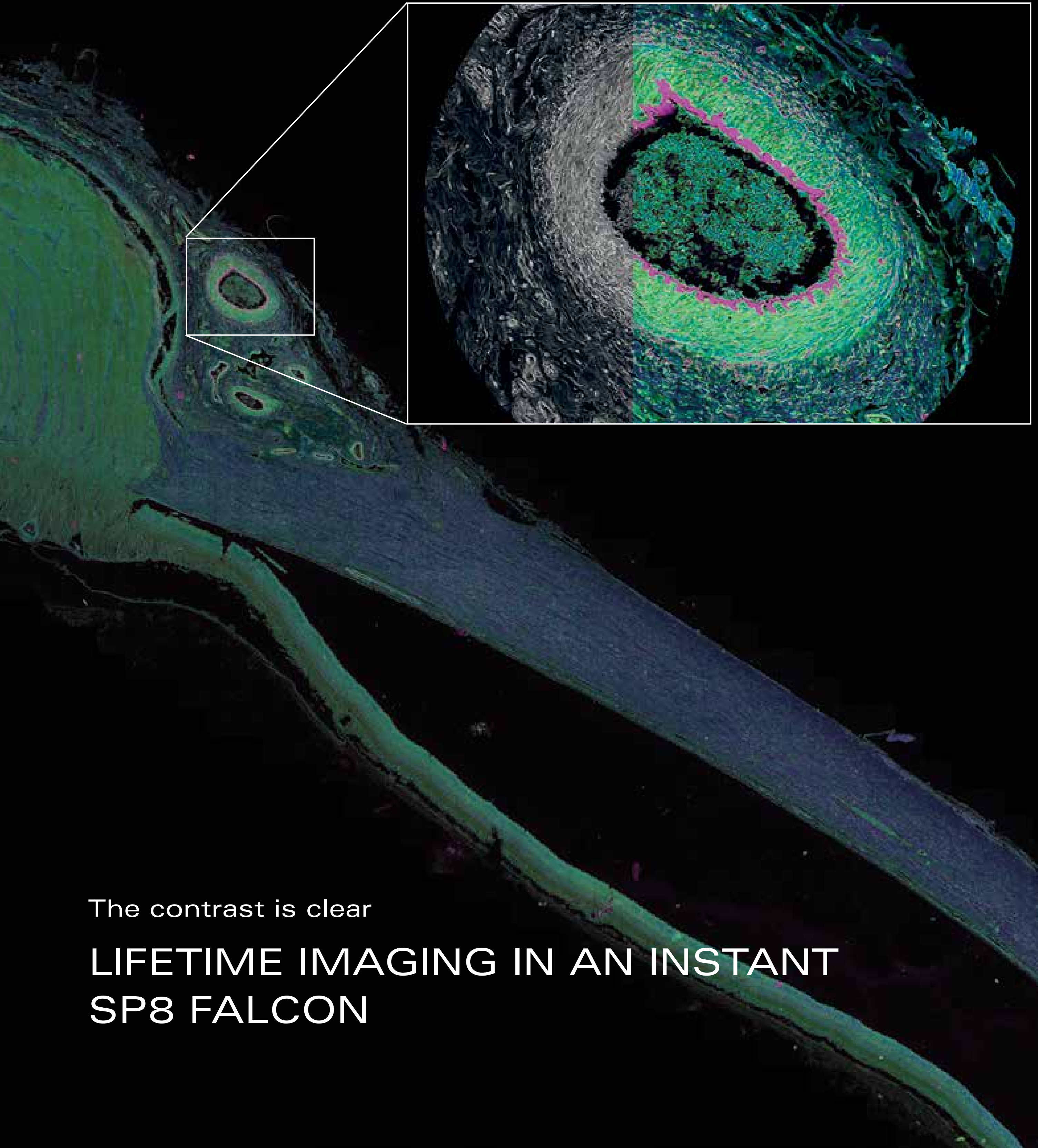
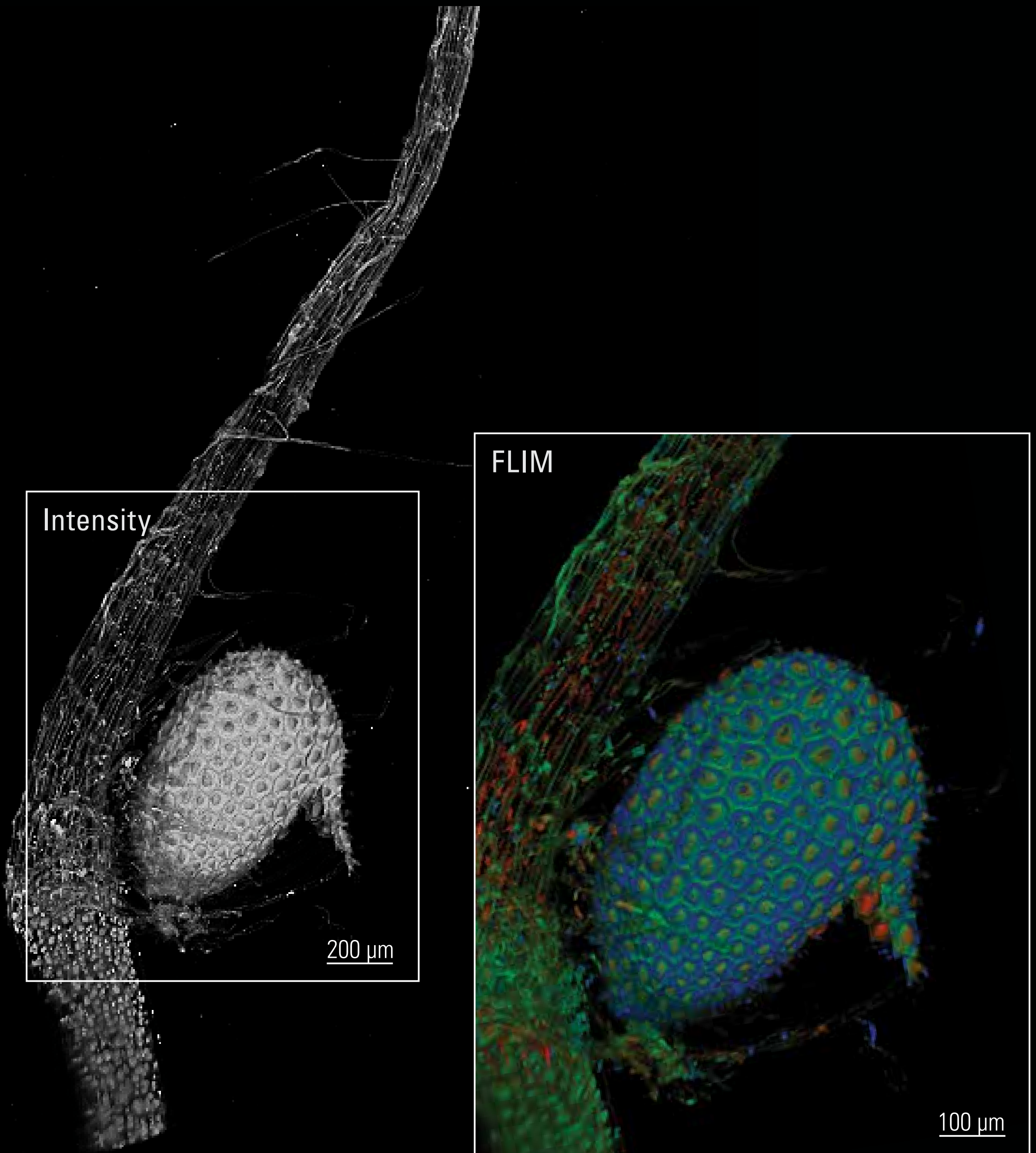
From Eye to Insight

Leica MICROSYSTEMS



The contrast is clear

LIFETIME IMAGING IN AN INSTANT SP8 FALCON



Arabidopsis seedling acquired with the SP8 DIVE FALCON. Detail with lifetime contrast. Z-stack 95 steps; 15 tiles.
Courtesy Melanie Krebs / Karin Schumacher, Ruprecht-Karls-Universität Heidelberg, Germany.

SP8 FALCON
REVEAL INTERACTION AND FUNCTION AT THE SPEED OF LIFE

The new SP8 FALCON (Fast Lifetime CONtrast) is the future of functional imaging. SP8 FALCON adds another dimension to confocal or multiphoton fluorescence imaging by harnessing the power of Fluorescence Lifetime Imaging (FLIM) to investigate cellular physiology and explore molecular dynamics in living cells.

SP8 FALCON is an integrated solution for FLIM and delivers benchmark results at confocal speed, opening the door to biosensing and tracking protein interactions. FALCON-FLIM mode can function as an additional dimension to all SP8 imaging modes, such as XYZ, XYZt, or XYλΛ.

With SP8 FALCON you can

> use biosensors to detect changes in metabolic state and microenvironment, do metabolic imaging using auto fluorescent molecules (e.g. NADH imaging with two-photon imaging)

> follow fast molecular interactions via FLIM-FRET (Förster Resonance Energy Transfer)

> apply lifetime contrast to separate multiple fluorophores with spectrally overlapping emission

Prof. Christian Eggeling

Provost Prof. Scott E. Fraser

Prof. Kees Jalink

Prof. Enrico Gratton

Feedback from Experts

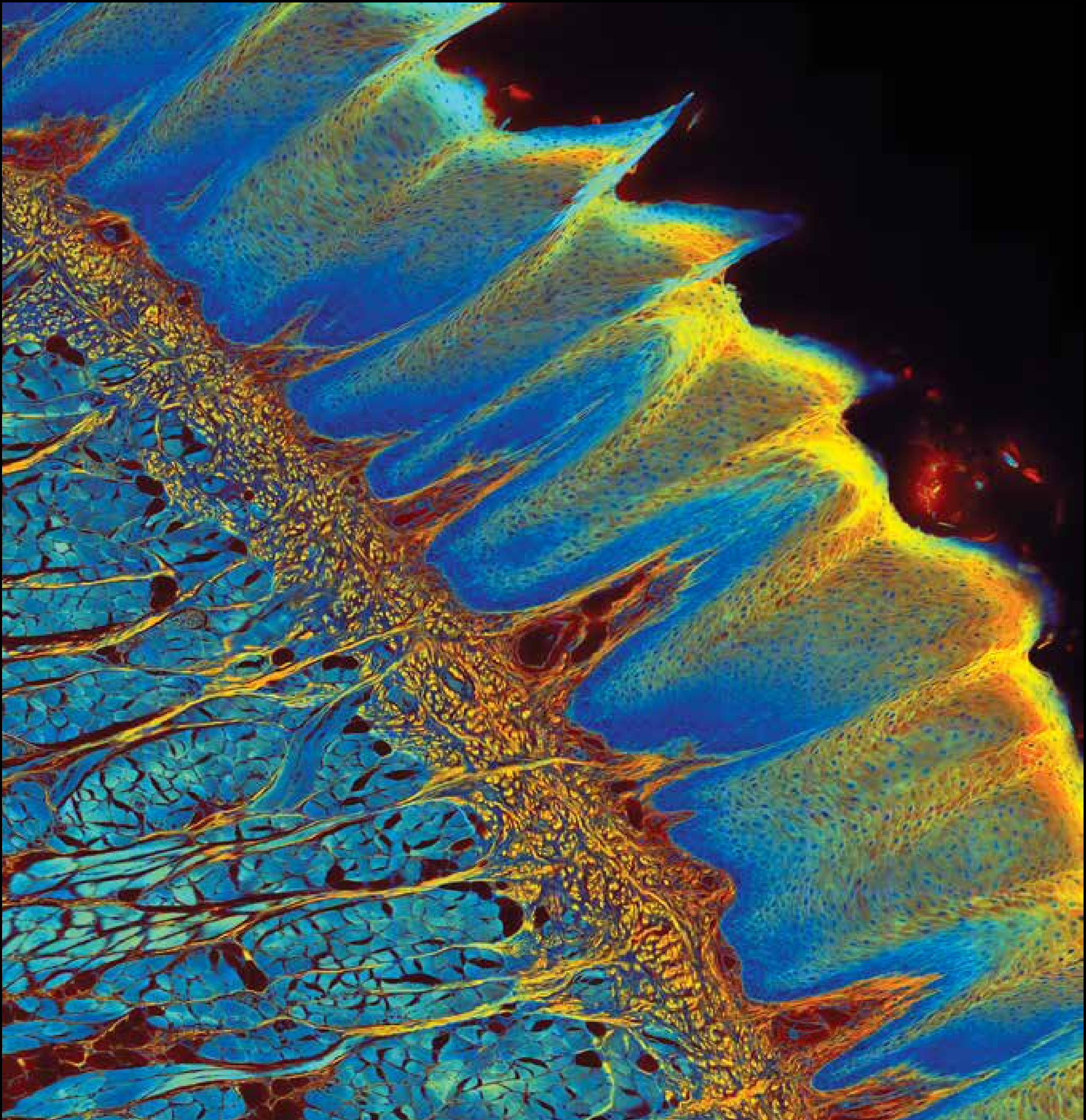
“We have scrutinized the Leica SP8 FALCON. It is every bit as accurate as dedicated TCSPC solutions, but with an intuitive interface and at turbo-speed.” „In my mind, this is a game changer for functional imaging.” **– Prof. Kees Jalink, Ph. D., Netherlands Cancer Institute, Amsterdam**

“The new Leica FLIM/ FCS solution will boost their applicability – fantastically fast, flexible and straightforward to use.” **– Prof Christian Eggeling, University of Oxford**

“Most FLIM instruments are attachments, this is a total change of perspective: having a truly integrated system – very powerful!” **– Prof. Enrico Gratton, UCI Samueli, University of California, Irvine**

“The Leica SP8 FALCON is the first commercial system that offers integrated confocal and lifetime imaging that I can imagine using in a core facility environment.” **– Provost Prof. Scott E. Fraser, Ph. D., University of Southern California, Los Angeles**

GAIN ADDITIONAL CONTRAST BY FLUORESCENCE LIFETIME IMAGING (FLIM)



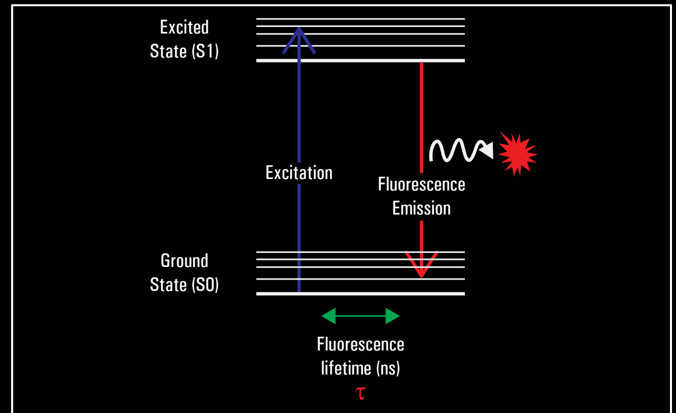
Transverse histological slice of a rabbit tongue (Filiform Papillae). 50 megapixels FLIM image (2326 µm x 1739 µm) acquired with SP8 FALCON and LAS X Navigator. Lifetime gives an additional contrast that allows the differentiation of different structures with histological staining.

Fluorescence imaging focus is on spectral contrast but fluorescence contains much more information about the fluorophore microenvironment. This information is encoded in the Fluorescence Lifetime (τ), that is the time the fluorophore spends in the excited state.

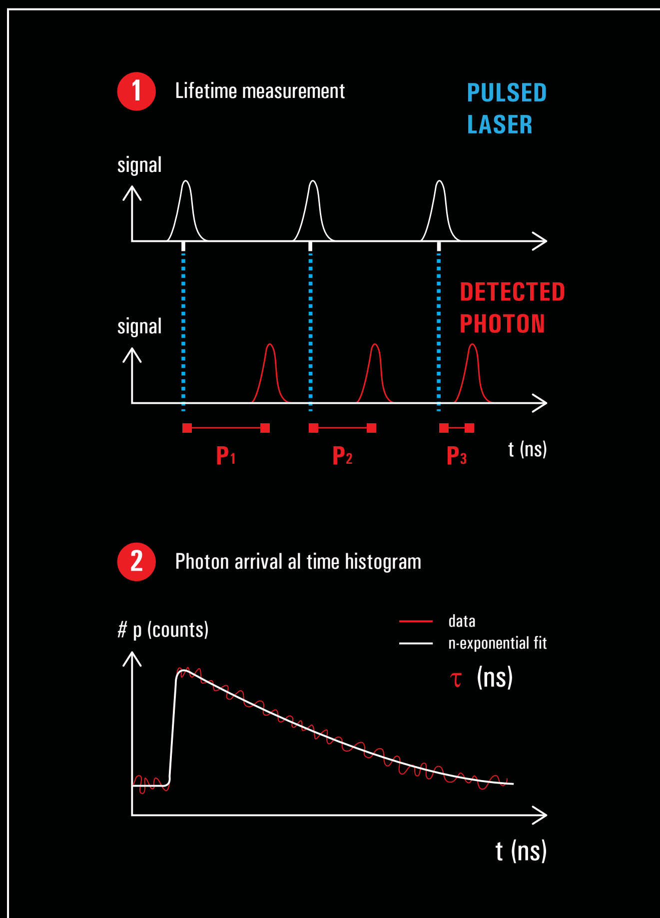
Fluorescence lifetime depends on

> Intrinsic dye proprieties
> Micro-environment
> Molecular binding

For these reasons Fluorescence Lifetime Imaging (FLIM) is a great tool for functional imaging.



How is lifetime measured?

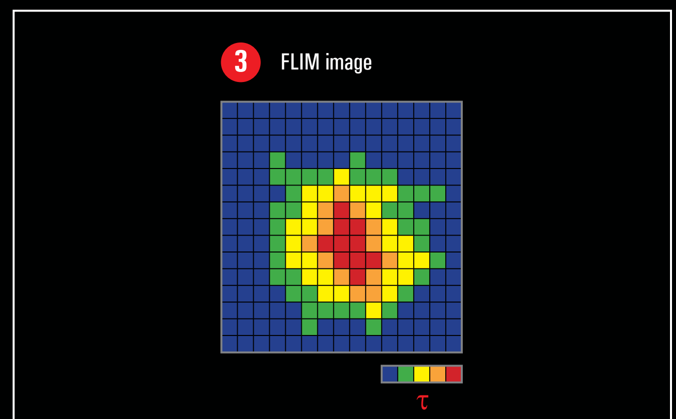


Fluorescence lifetime can be measured by Time Correlated Single Photon Counting (TCSPC). This method requires a pulsed excitation and it measures the time between laser pulse and photon detection (1).

The photon arrival time is reported in a histogram (2).

From the fit of the histogram with a (multi)exponential decay function it is possible to recover the lifetime pixel by pixel and generate a FLIM image (3).

Thanks to the very short system deadtime (1.5ns) the SP8 FALCON can measure 1 photon per pulse with each detector, allowing FLIM imaging at confocal speed.

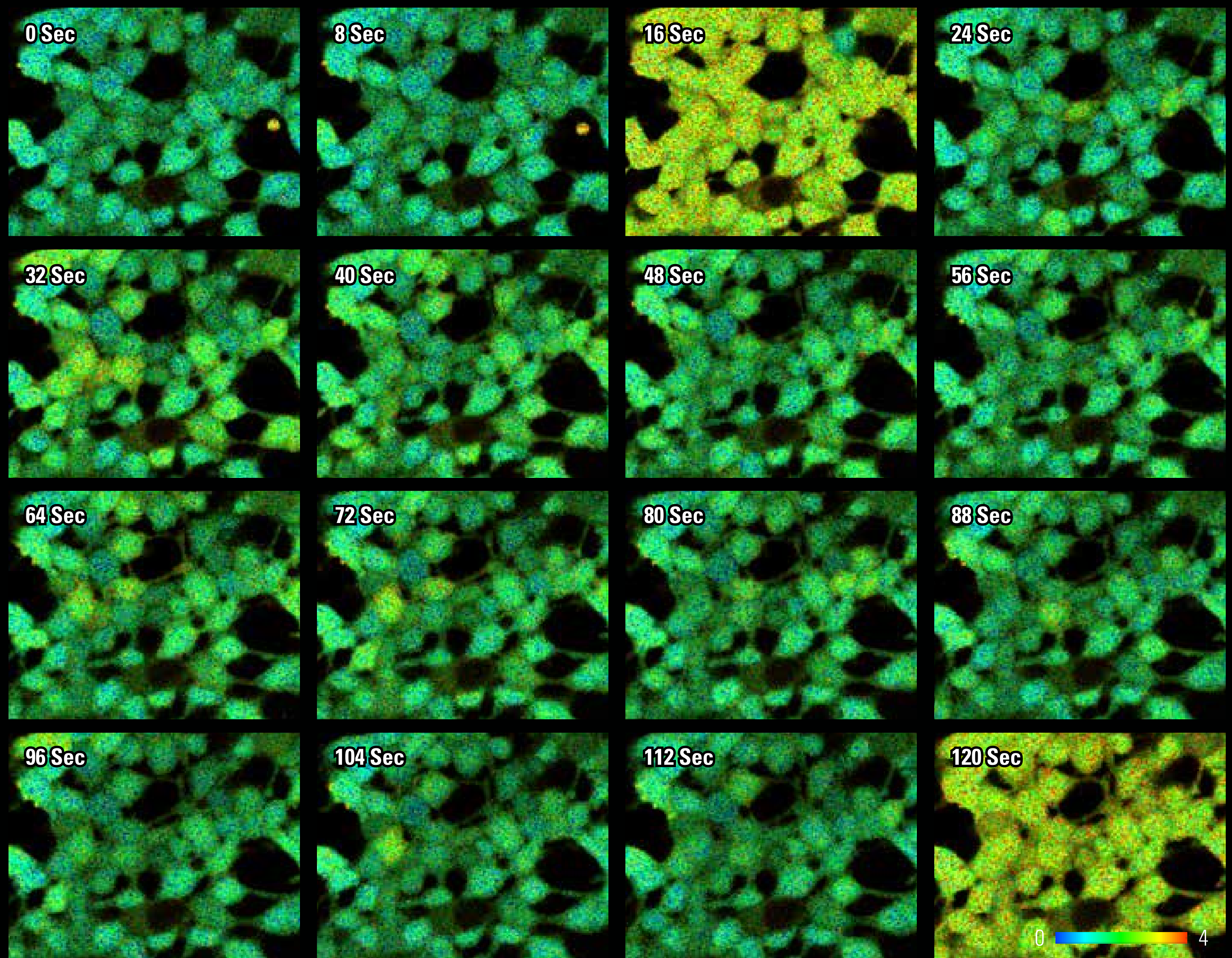


MONITOR SUBTLE AND FAST CHANGES WITH BIOSENSORS

With the SP8 FALCON, you access this information contained in the fluorescence lifetime, even for fast biological processes like calcium oscillations. This information complements the already powerful spectral imaging.

Application: Calcium signaling

Oregon Green (OG488) is a fluorophore sensitive to the calcium ion concentration. OG488 lifetime is increasing with calcium concentration, therefore it allows calcium waves in live cells to be followed.

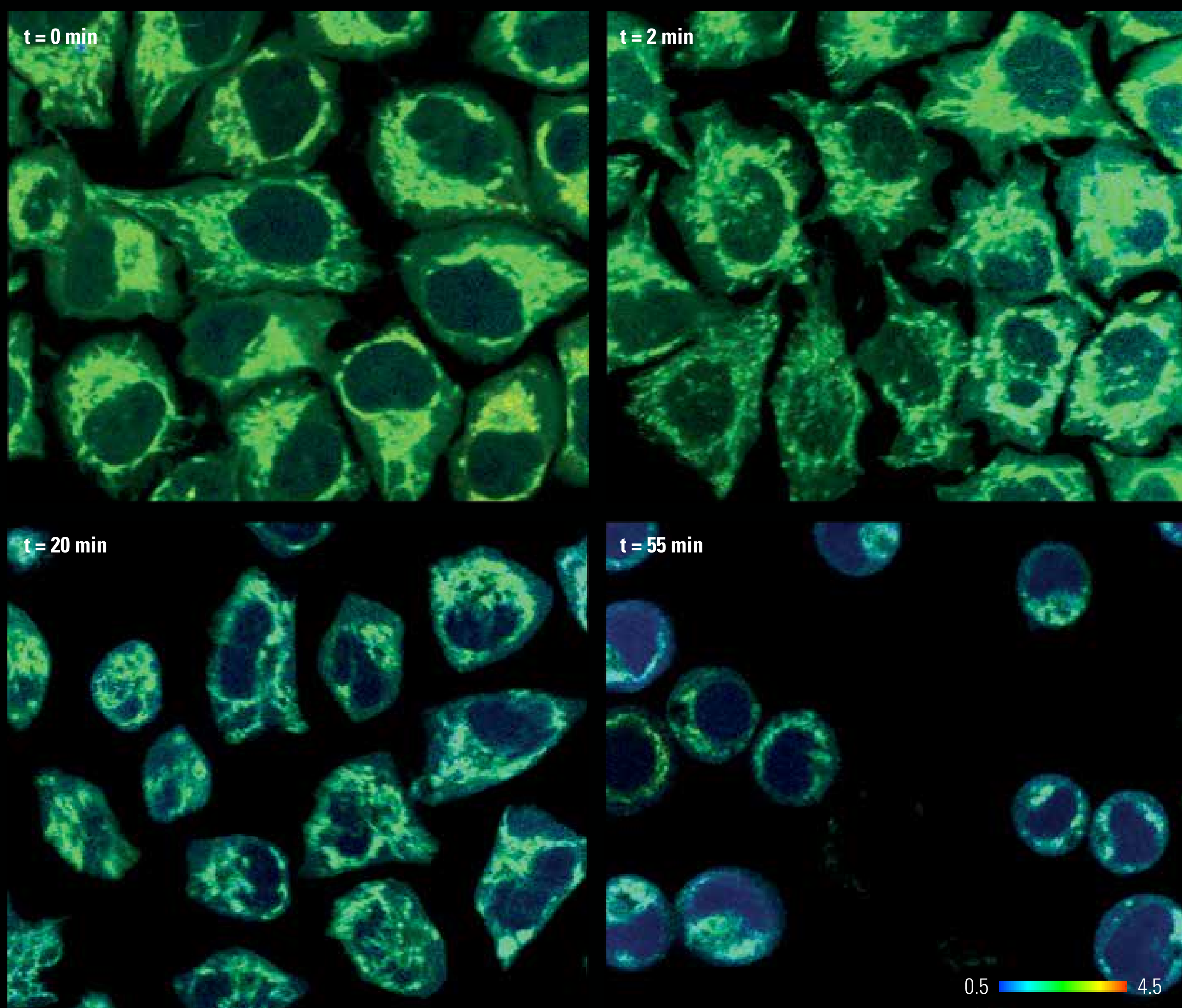


Calcium oscillations after stimulation with a thrombin activating peptide. The response in individual cells is recorded as a change in lifetime. Movie acquired at 4 fps. Image size: 256 x 256 pixels. Color bar scale (lifetime): ns. Courtesy Kees Jalink, Bram van den Broek, NKI Amsterdam.

MORE RELIABLE AND SENSITIVE METABOLIC IMAGING

Autofluorescence is an issue for conventional imaging. However, the SP8 FALCON turns it into valuable information. You can now transform autofluorescence into a reporter for metabolic status, cell differentiation, and cancer development.

Moreover, SP8 FALCON enables imaging contrast in living tissue, where fluorescence labeling is often unspecific or destroys physiological conditions.

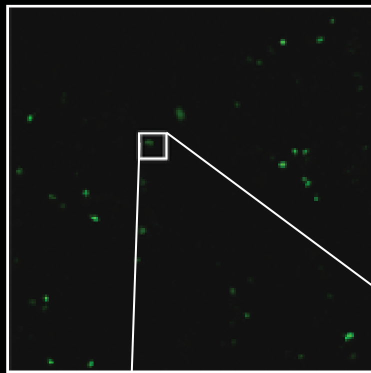


Autofluorescence in mammalian cells at non-physiological conditions (pH 8.5). The signal correlates with changes in the NAD/NADH endogenous pool. The development of oxidative stress reads out as a decrease of fluorescence lifetime over time. Original image size: 512 x 512 pixels. Color bar scale (lifetime): ns. Two-photon image acquisition performed with SP8 DIVE and SP8 FALCON.

FOLLOW MOLECULAR INTERACTIONS WITH FLIM-FRET

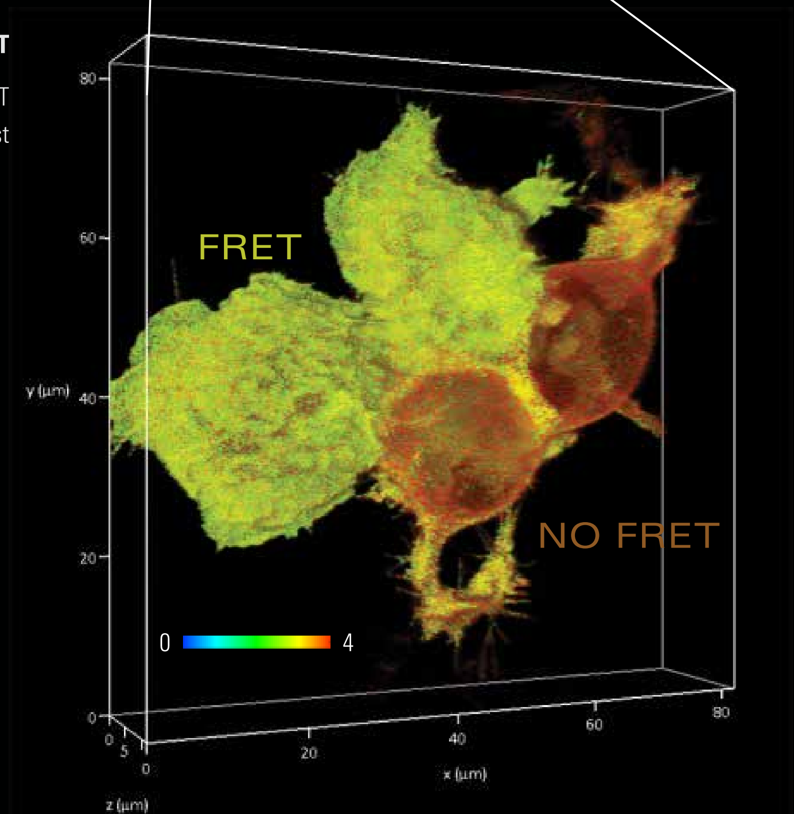
2D screen of 35 mm dish

Look for regions of interest by screening your sample using the LAS X Navigator software



3D FLIM-FRET

Acquire and analyze 3D FLIM-FRET data from your sample's region of interest



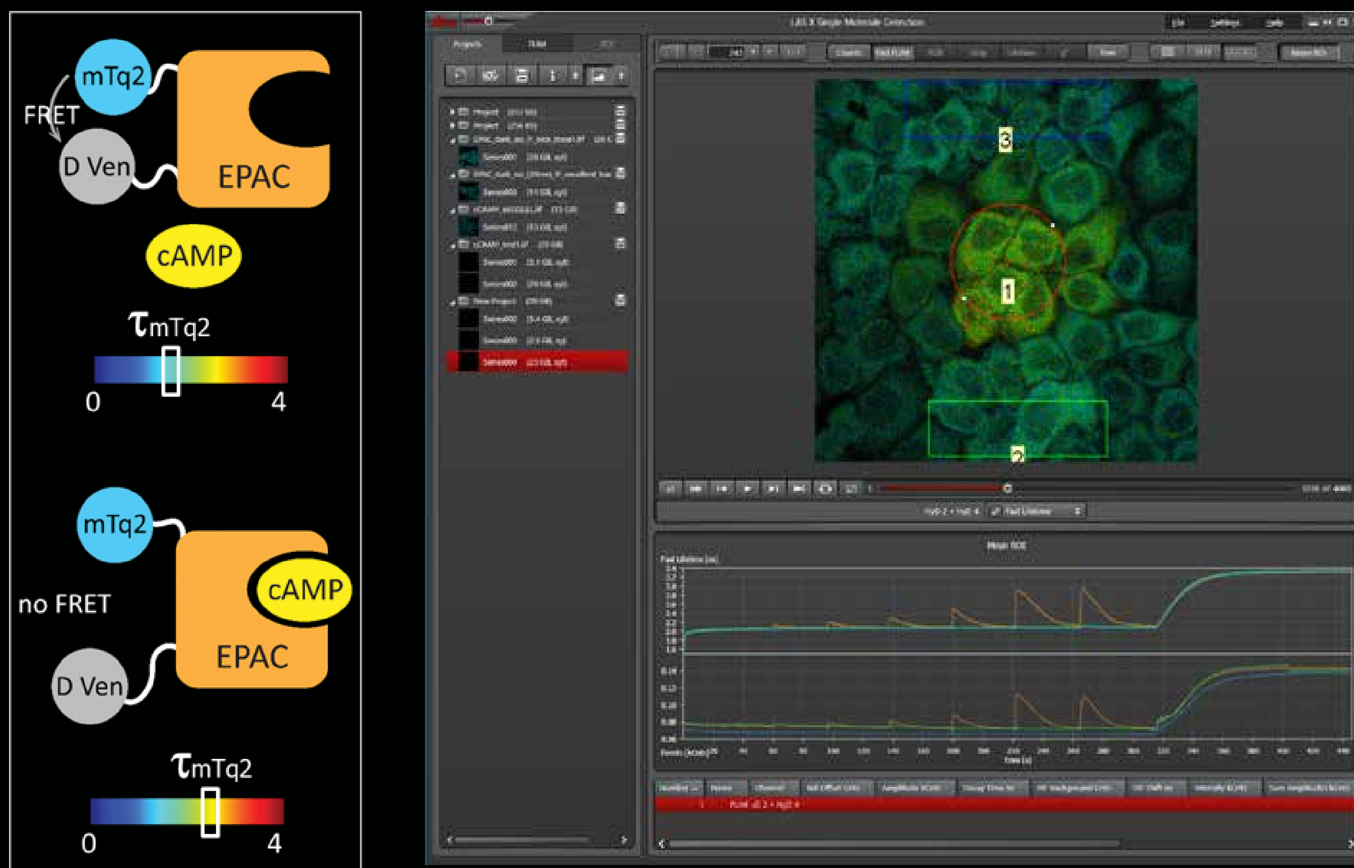
PH domain interactions at the plasma membrane. FLIM-FRET with ECFP/EYFP. Color bar scale (lifetime): ns. Courtesy Kees Jalink, Bram van den Broek, NKI Amsterdam.

The SP8 FALCON sets a new speed standard for FLIM instruments. It enables FRET in highly dynamic cellular events. Modern research investigates how molecules interact and/or change conformation during biological processes.

FLIM-FRET is considered the gold standard for measuring interactions, as it is reproducible, easily quantifiable, and independent of concentration and bleaching.

Application: FRET biosensors cAMP signaling

EPAC mT2-dVenus is a FRET sensor for cAMP. In the absence of cAMP, the sensor is fretting (donor is quenched, it has a shorter lifetime). When cAMP is bound to the sensor there is no FRET, therefore the lifetime of the donor is longer. With the SP8 FALCON, we can follow the activity of this FRET sensor.



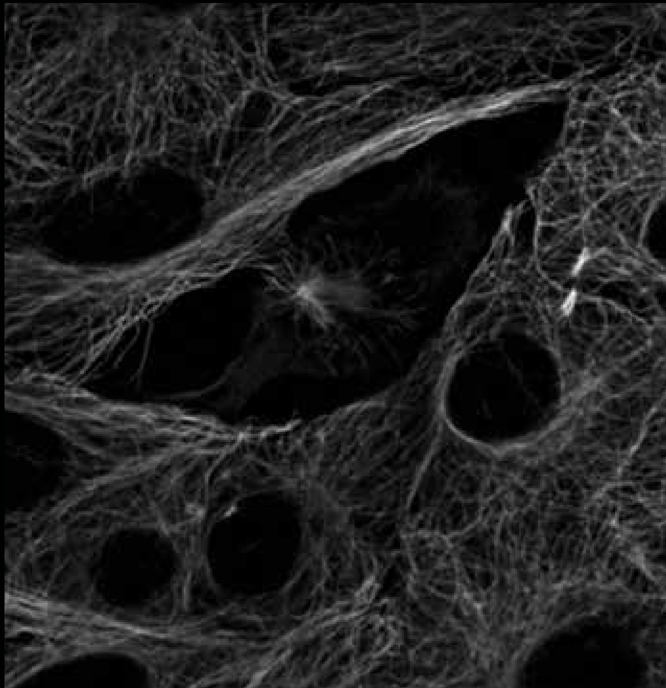
Caged cAMP in HeLa cells expressing EPAC mT2-dVenus FRET sensor. EPAC response to UV-mediated cAMP uncaging (central area). Movie recorded at 4 fps. Image size: 256 x256 pixels. Color bar scale (lifetime): ns. Courtesy Kees Jalink, Bram van den Broek, NKI Amsterdam.

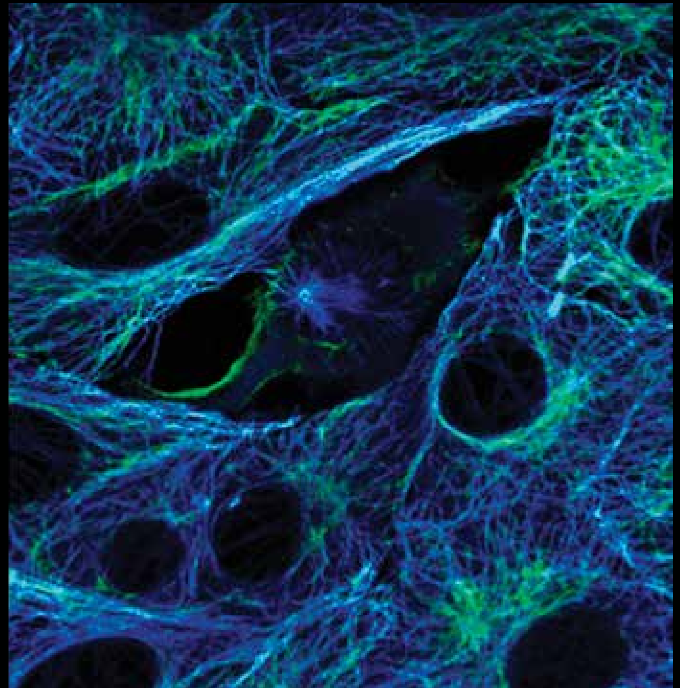
FLUOROPHORE SEPARATION BEYOND THE SPECTRAL OPTIONS

Fluorescence labeling is the standard way to differentiate intracellular structures. Spectral separation is very powerful, but sometimes limited when the emission spectra are too close.

With SP8 FALCON, you can overcome this limit by using the additional fluorescence lifetime dimension. This parameter unlocks the potential for separating multiple fluorescent probes.

You can enjoy even more freedom by combining options of the SP8 platform. Options include the white light laser excitation source, acousto-optical beam splitter and multichannel spectral detection.

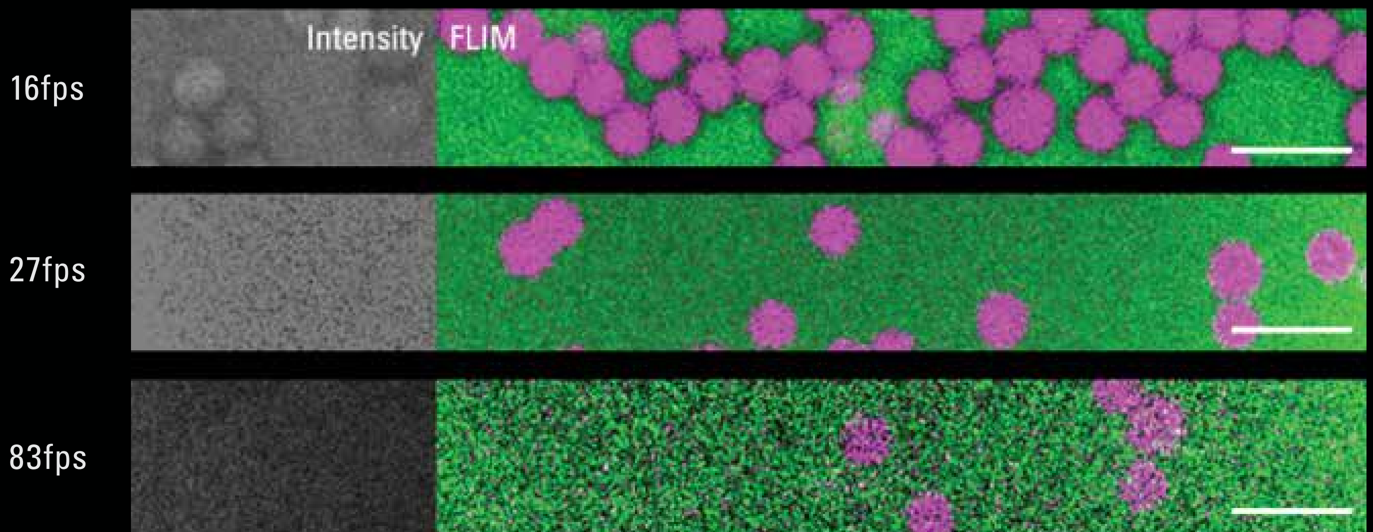




Cytoskeleton structures distinguished by lifetime contrast. Vimentin immunolabeled with Alexa Fluor 555 (green), and tubulin immunolabeled with Alexa Fluor 546 (blue). The fluorophores are spectrally very similar, but they are separated using fluorescence lifetime information. Image size: 512 x 512 pixels.

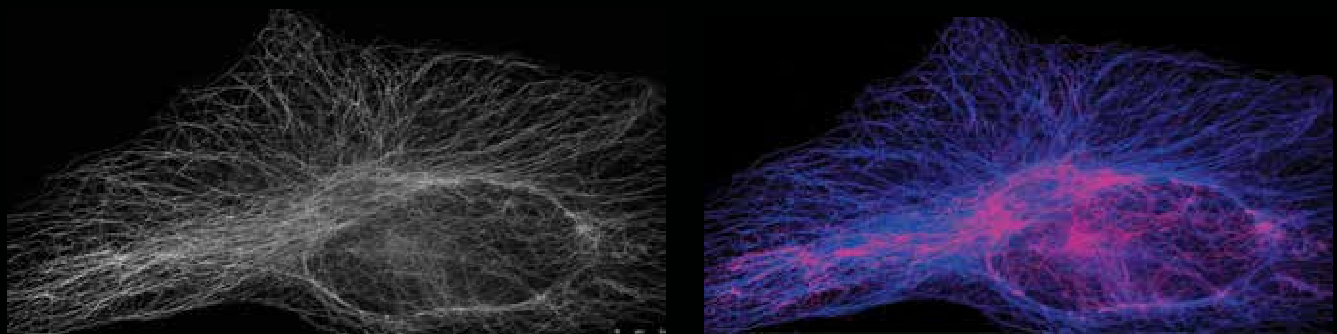
Separate fluorophores at video rates with lifetime contrast

SP8 FALCON acquisition speed allows FLIM imaging at video rate and faster using the resonant scanning technology.



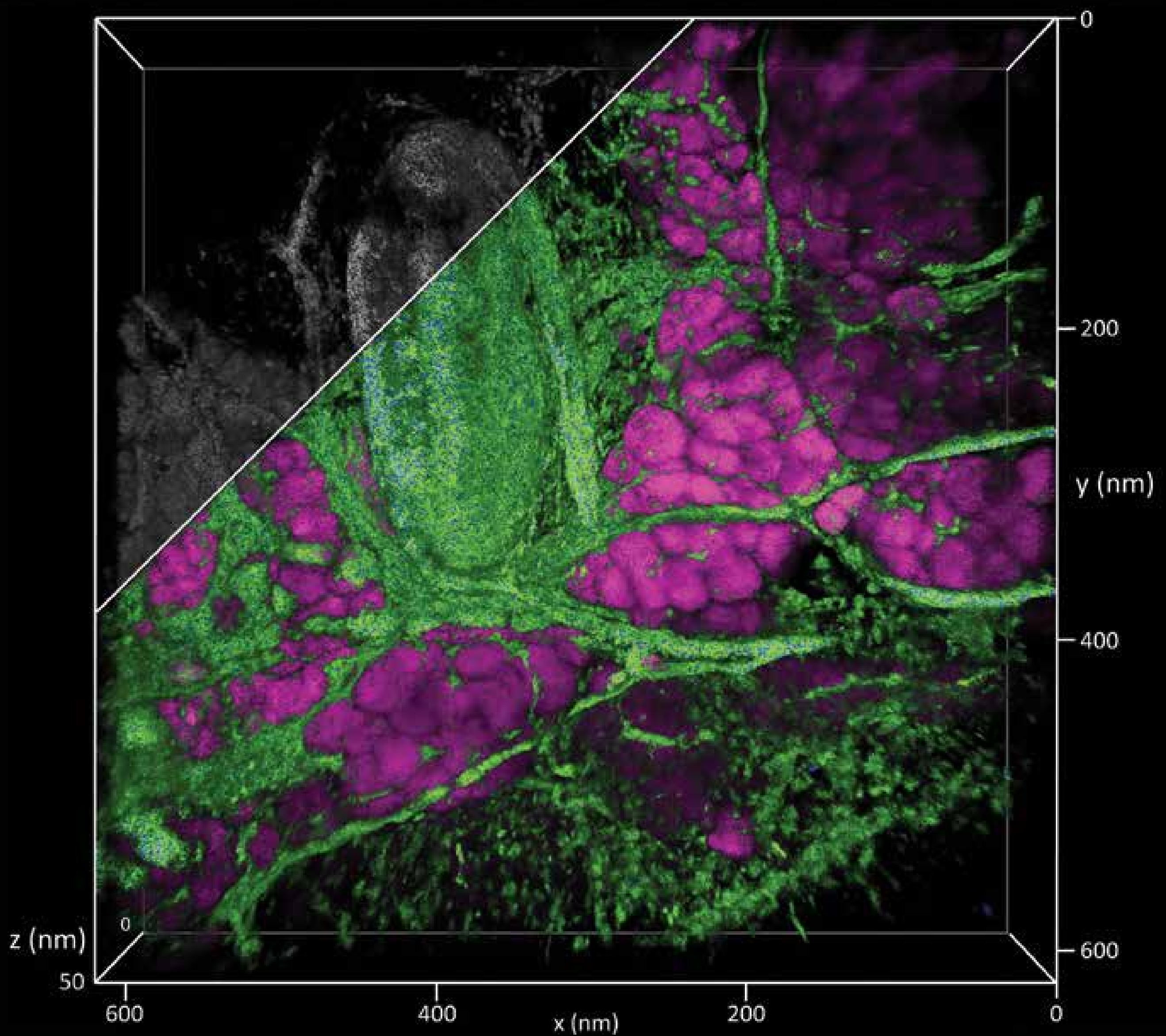
Fluospheres 4 µm (nanoparticle, magenta) in AF555 (green). These dyes are separable by their lifetime: the Fluospheres nanoparticles show an average lifetime of 2 ns, while AF555 lifetime is 0.5 ns. Scale bar: 10µm. Image size: 512x64 pixels.

Enhance Nanoscopy with dye separation by FLIM: STED FLIM



Single STED laser line and a single HyD SMD detector. We separate and super-resolve Atto647N-vimentin (magenta) and STAR635P-tubulin (blue) filaments, two fluorophores with very similar emission spectra (gray, intensity) but, different lifetimes.

ALL-IN-ONE MULTIMODAL IMAGING



Simultaneous 3D spectral (gray) and FLIM (color) multiphoton imaging reveals contrast encoded in fluorescence lifetime. The lipid droplets (magenta) are clearly identified. Stack acquisition and rendering using SP8 DIVE FALCON and LAS X software.

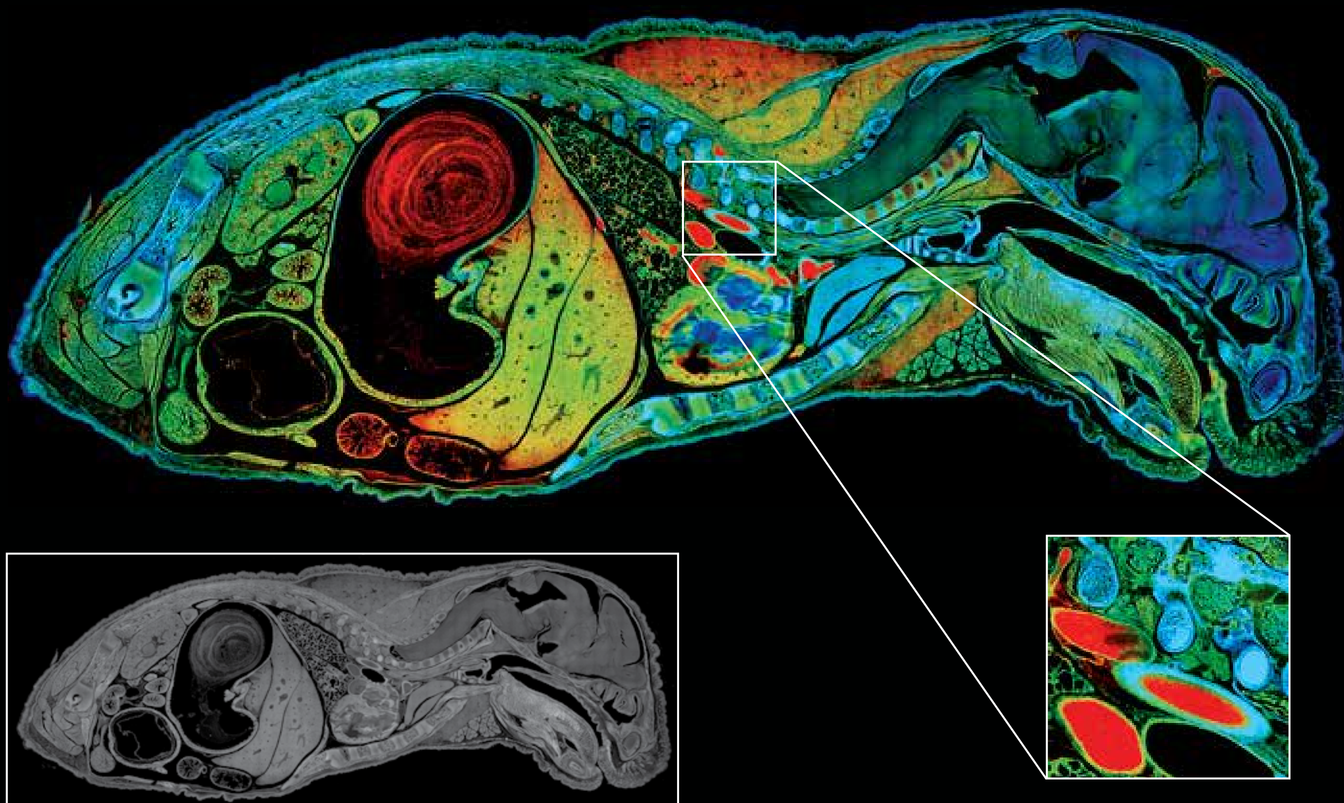
Combining FLIM with other modalities was never as easy as with the SP8 FALCON. Until now, researchers had to cope with complex wiring and cumbersome file transfer tasks. With SP8 FALCON, you can integrate lifetime information into your standard confocal workflow.

SP8 FALCON is fully integrated in the LAS X acquisition and analysis software. It can record FLIM with four spectral channels simultaneously and up to 10 channels sequentially.

SP8 FALCON enables lifetime contrast with any imaging workflow, such as:

- 3D stacks
- Time lapse
- Lambda scan
- Mosaic/ tile scanning
- Combinations of the above and more...

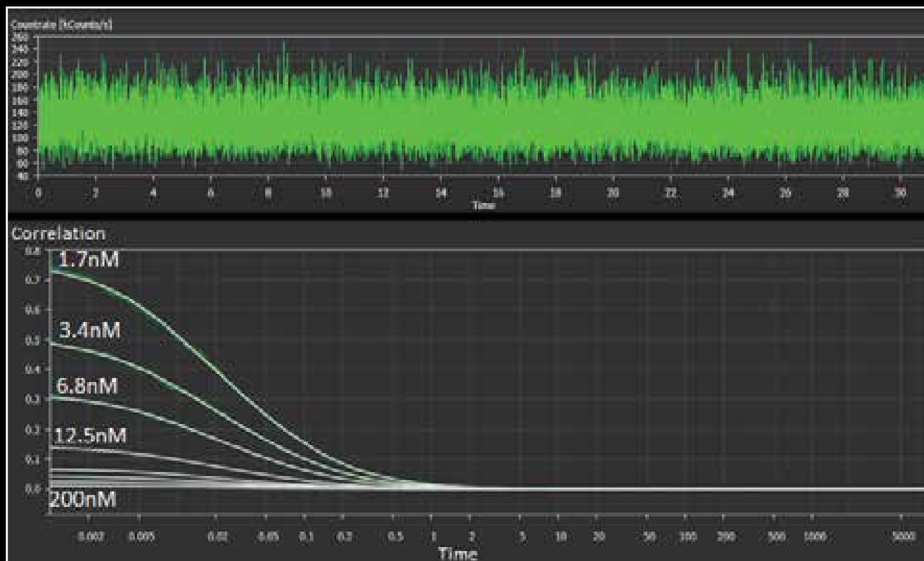
With the new LAS X Navigator, you can expand your viewing area up to 10,000 times. You save precious time identifying regions of interest while exploring your samples in a whole new way.

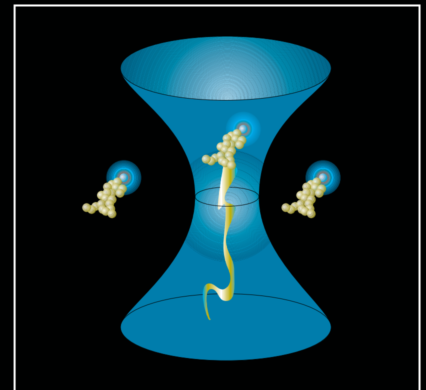


Straightforward acquisition of complex samples. High resolution mosaic image of mouse embryo with 722 tiles containing 190 Megapixels. FLIM data fitted with four characteristic fluorescence lifetimes (color coded). Acquisition: 1:23 h. Analysis: 1:00 h

FOLLOW MOLECULAR DYNAMICS WITH SP8 FCS

Fluorescence Correlation Spectroscopy (FCS) is a quantitative technique for determining particle concentrations, diffusion coefficients, viscosity, molecular mass, binding constants, and photo-physical properties.

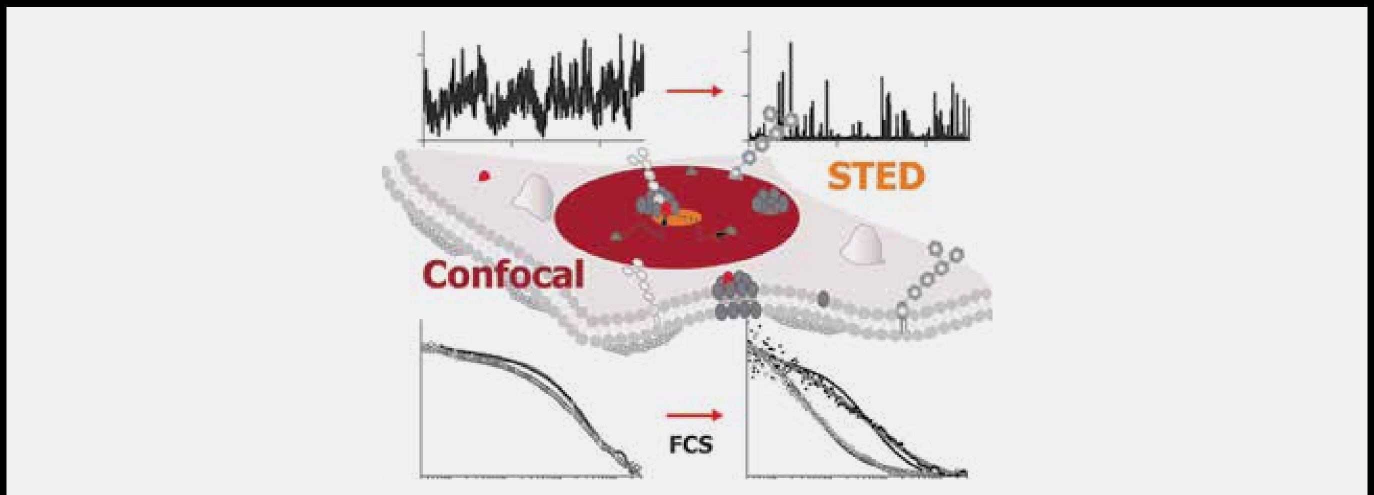




Intensity traces and autocorrelation curves for different concentration of Atto 488. The amplitude decreases with the concentration.

FCS can be also combined with FLIM to enable Fluorescence Lifetime Correlation Spectroscopy (FLCS) experiments. FLCS is used to study the interaction of molecules which are not spectrally separable, and to eliminate background contributions.

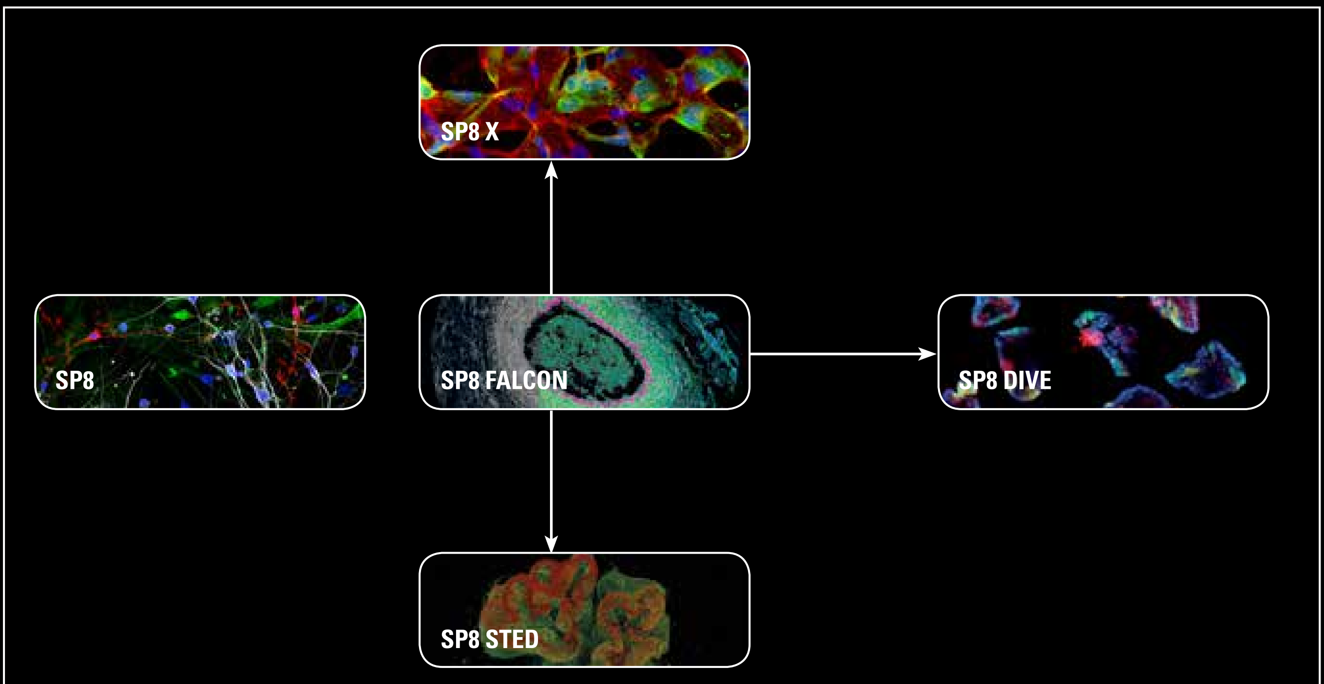
STED effectively decreases the observation volume. For this reason, STED FCS provides molecular information even at high concentrations (> 100 nM) where confocal FCS is limited.



THE RESULTS YOU NEED – WITH ONE CLICK

The LAS X software enables FLIM to be done with one click, making it as easy and routine as spectral imaging thanks to the same software philosophy.

FLIM is another contrast channel that you can add to your existing imaging or more specialized workflow.



Leica
MICROSYSTEMS

MULTIDIMENSIONAL IMAGING IN ALL DIRECTIONS – NO LIMITS

Following the direction of your research – now and in the future.

Histological section from a blood vessel of a cat eye.
Acquisition and visualization using SP8 FALCON and LAS X Software.

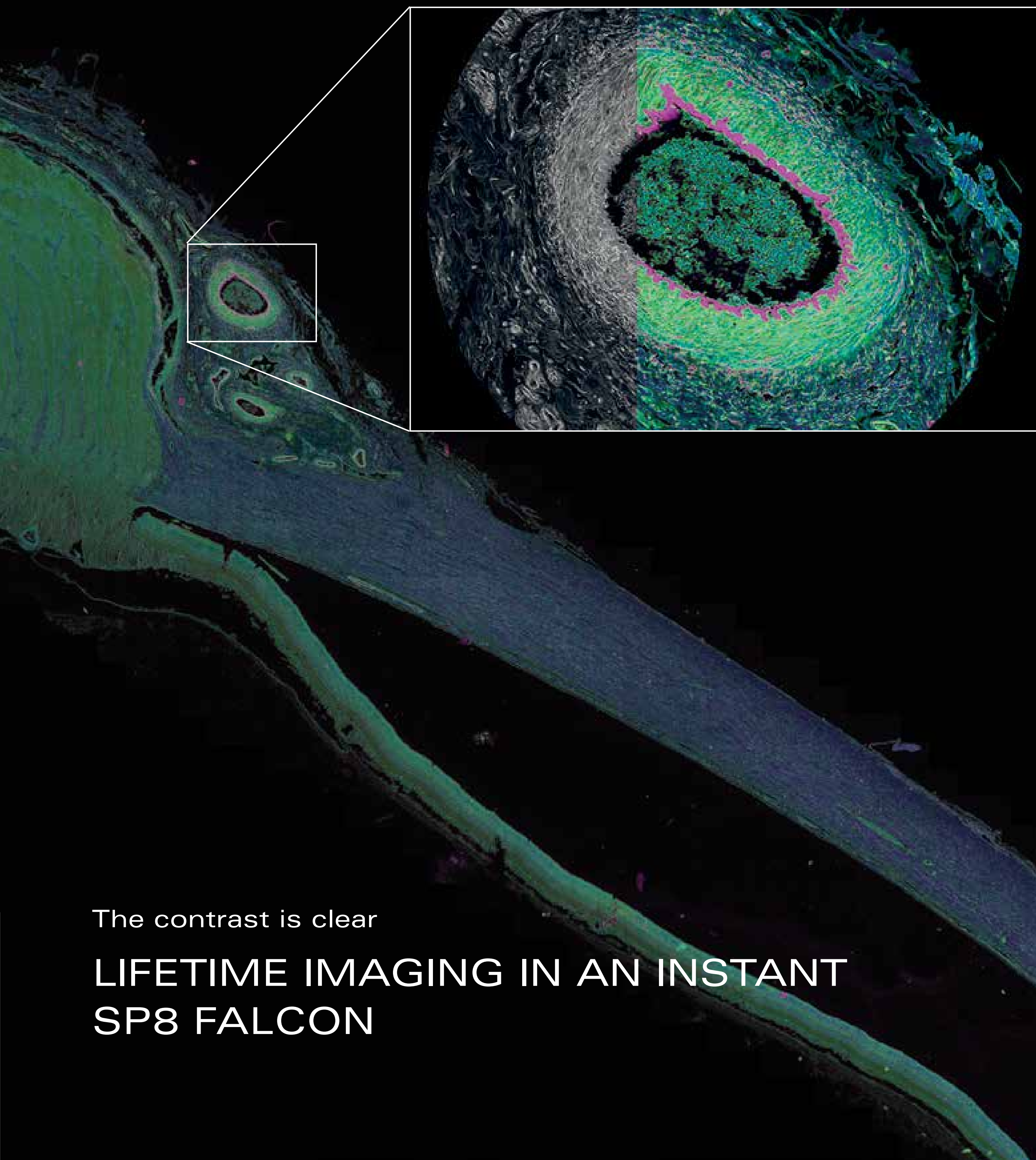
CONNECT WITH US!

Leica Microsystems CMS GmbH | Am Friedensplatz 3 | D-68165 Mannheim (Germany)
Tel. +49 (0) 621 7028-0 | F +49 (0) 621 7028-1028

www.leica-microsystems.com/falcon

From Eye to Insight

Leica
MICROSYSTEMS



The contrast is clear

LIFETIME IMAGING IN AN INSTANT
SP8 FALCON