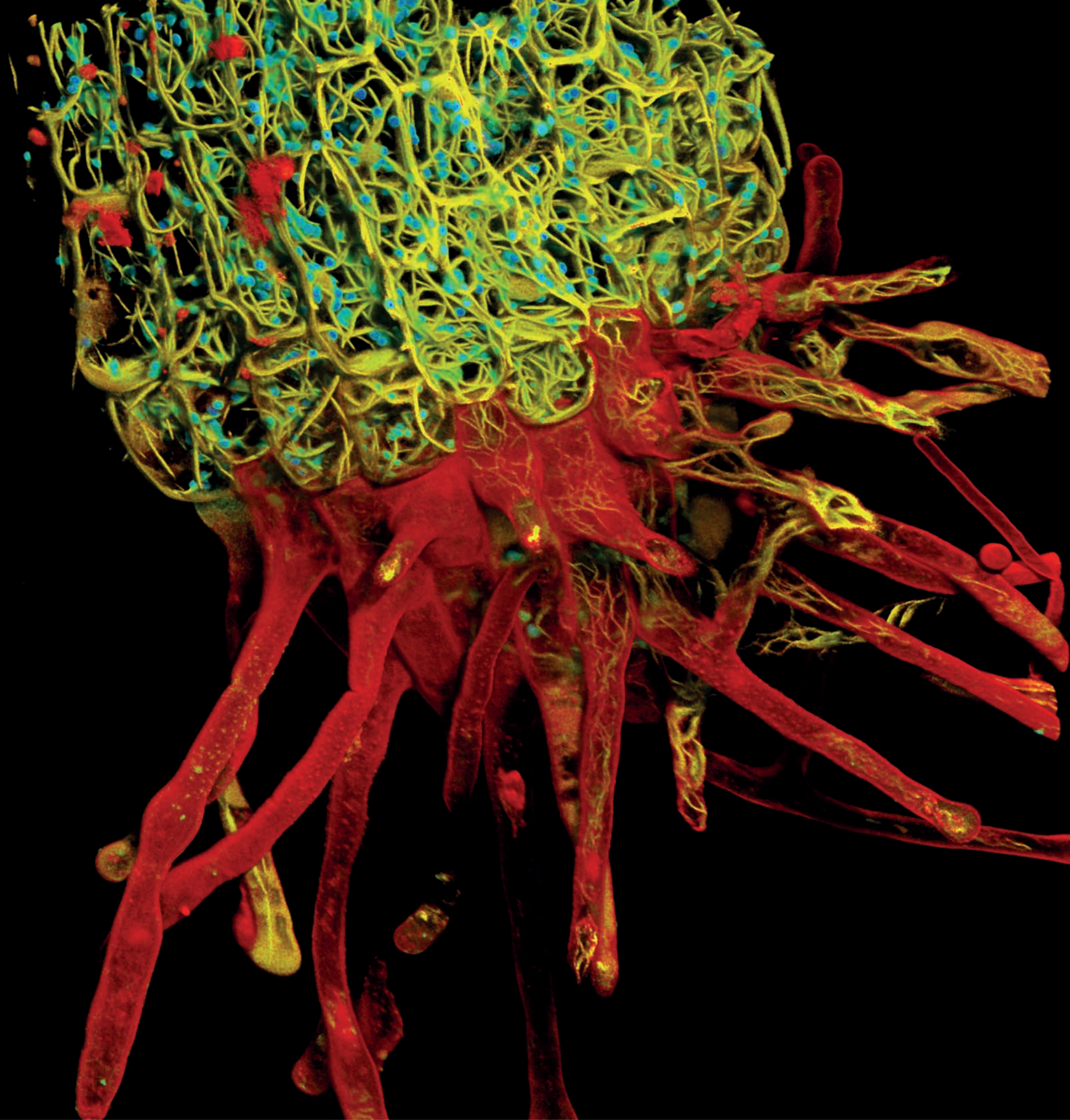
Leica

MICROSYSTEMS

STELLARIS

CONFOCAL RE-IMAGINED

GET CLOSER TO THE TRUTH



SEEING LIFE IN MORE DIMENSIONS

Nature is multi-dimensional. To truly comprehend its complexity, we need to look in multiple dimensions. This detailed multicolor image of *Arabadopsis thaliana* root junction was obtained with just one click and one detector. With conventional confocal microscopy, it would be black and white. With STELLARIS what we see is transformed by an extra dimension of lifetime information. Not only is the remarkable architectural diversity of the actin-containing root system revealed with more clarity, but also its relationship to the cell wall and chloroplasts—structures that would be difficult or impossible to distinguish using the intensity data alone. The result is not just a snapshot of nature's beauty, but an information-rich image that progresses our understanding of plant biology.

Root-hypocotyl junction of *Arabidopsis thaliana*. Image acquired with TauContrast. Actin: Lifeact-Venus (Era et al. Plant Cell Physiol., 2009); chloroplasts: endogenous fluorescence; membranes: Propidium iodide. Sample courtesy: Dr. Melanie Krebs, COS, University of Heidelberg.

GET CLOSER TO THE TRUTH

UNCOVERING TRUTH IS THE NATURE OF DISCOVERY

In microscopy, our mission is to empower you to drive progress in science. To help you get closer to the truth hidden in every sample, we have completely re-imagined confocal microscopy. Our new family of confocal microscopes – STELLARIS 5 and STELLARIS 8 – takes confocal imaging to a new level. Now you can see more, discover more, and do more than ever before, with STELLARIS.

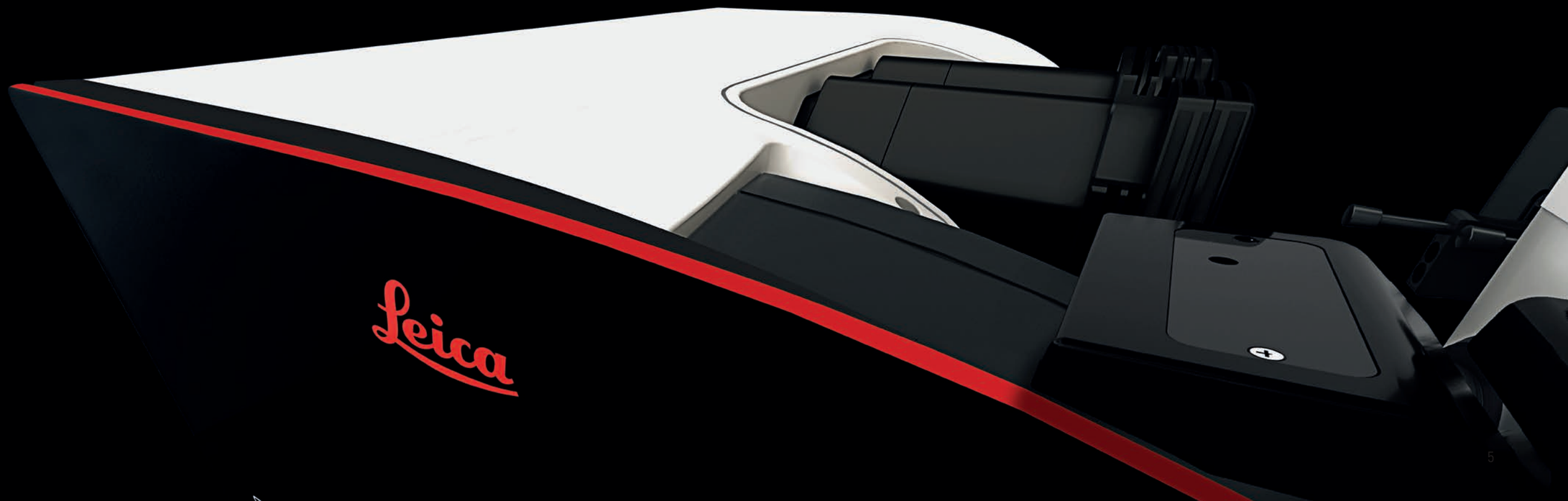
STELLARIS

CONFOCAL RE-IMAGINED

POWER to see more: Imagine having the power to observe more detail in every sample, to capture even the weakest signals, and to collect more accurate and reliable data across the spectrum.

POTENTIAL to discover more: Imagine the potential to make novel discoveries by adding an extra dimension to your experiments with fluorescence lifetime information.

PRODUCTIVITY to do more: Imagine the added productivity that comes with an easier setup and navigation, acquiring images from complex samples with just a few clicks.



POWER
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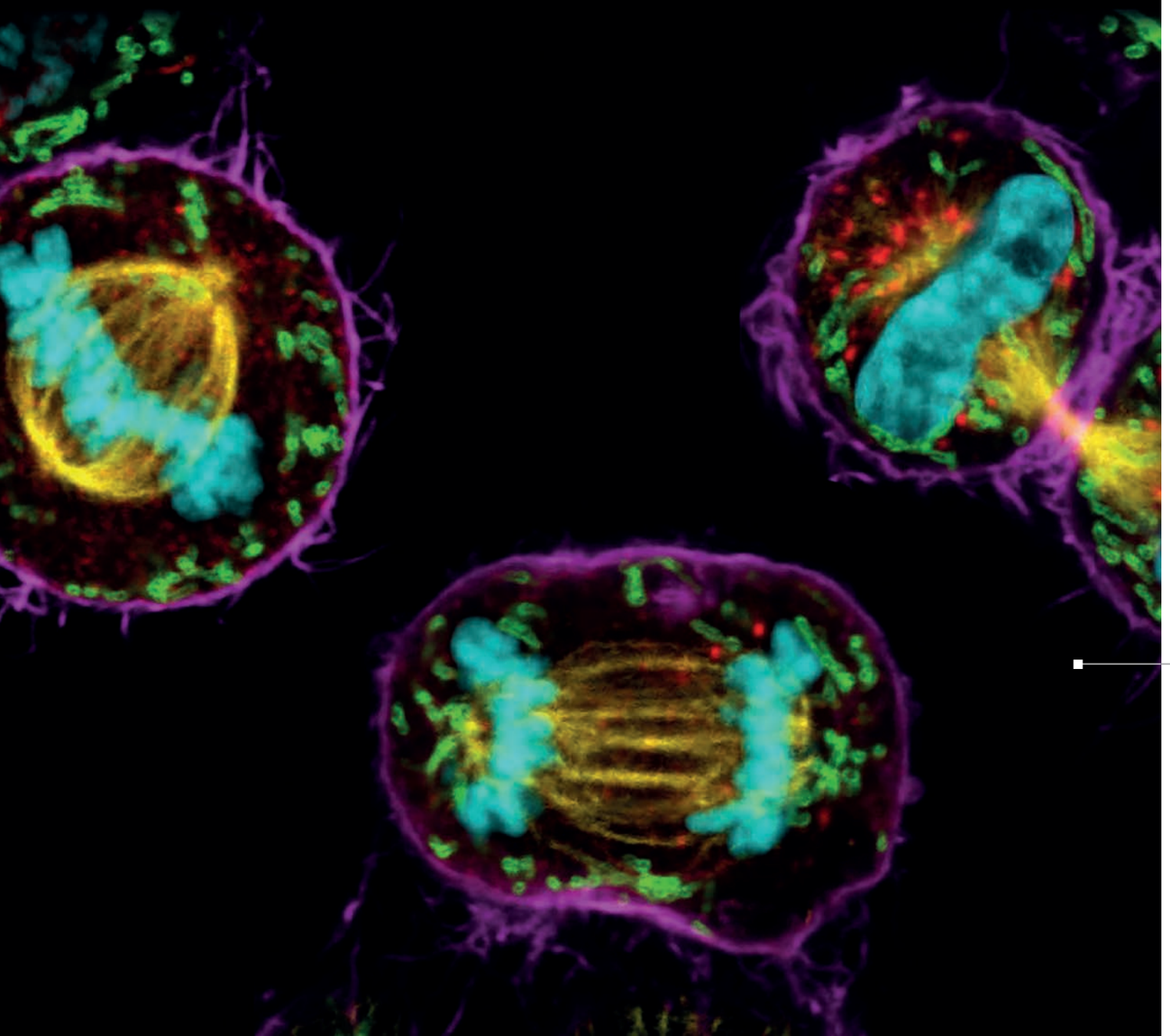
The perfect synergy between our newly designed family of Power HyD detectors, fully optimized beam path, and next-generation White Light Lasers delivers exceptional imaging performance. Your results are clearer, with greater detail derived from brighter signals, more contrast, and increased sensitivity – even when imaging low-abundance labels and events.



High sensitivity, broad spectral coverage and a wide dynamic range are essential to take full advantage of today's ever-expanding range of fluorescent probes, multi-label experiments and diverse applications. Our Power HyD detector family has been designed for high performance in terms of spectral coverage, sensitivity and dynamic range. Taking advantage of the latest innovations in detection technology, this powerful new family delivers uncompromising image quality across a wide range of conditions—from dim samples to fast dynamic acquisition. With their single-photon counting capabilities, Power HyD detectors also enable a unique set of fluorescence-lifetime-based imaging applications.

PUSH THE LIMITS OF YOUR RESEARCH WITH ADVANCED DETECTION CAPABILITIES

Introducing the new generation Power HyD detector family
To ensure you get the most out of your confocal applications, we have developed three types of detectors for STELLARIS. **Power HyD S detectors** have the highest dynamic range and the broadest spectral coverage of the family, making it an all-round detector for general confocal applications. **Power HyD R detectors** enable enhanced detection in the near infrared (NIR) range of the spectrum, getting the most out of the white light laser extended red spectral range on STELLARIS 8. **Power HYD X detectors** are specially optimized for fast lifetime imaging applications.

Power HyD S: a new breed of detector
Power HyD S detectors are the new core of STELLARIS. These silicon-based Multi-Pixel Photon Counter (MPPC) detectors use a multi-cell architecture and avalanche diodes to suppress dark noise and improve efficiency of photon collection, providing outstanding image quality. The ability to switch between analog detection and photon counting mode makes them exceptionally versatile for a wide range of applications. In photon counting mode, individual photons are resolved and counted with high fidelity, producing highly accurate and quantitative data. In analog mode, fluorescent signal is integrated over time, which produces crisp images with an exceptionally high dynamic range.

Uncovering hidden connections between cancer and mitosis
Unravelling the role of mitotic instability in a complex disease like cancer requires correlation of multiple biological markers in the same sample. This image faithfully captures individual COS-7 cells at three stages of mitosis: metaphase, late anaphase and telophase. The delicate interplay between chromosomes, spindle fibers, Golgi, mitochondria and actin cortex is revealed in exceptional detail. The power of STELLARIS to distinguish fluorescent labels that have closely overlapping spectra was essential for this 5-color experiment, taking multiplexing capability beyond ordinary limits.

Mitotic COS7 mitotic cells. Chromatin (cyan, mCherry), mitotic spindle (yellow, EGFP), Golgi (red, Atto647N), mitochondria (green, AF532), actin filaments (magenta, SiR700). Sample courtesy: Dr.Jana Döhner and Dr.sc.nat. Urs Ziegler, University of Zürich; cells expressing mCherry were a kind gift of Daniel Gehrlich. SiR was a kind gift of Spirochrome.

POWER
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New technology: introducing Power Counting, a novel photon counting approach to improve the precision of your results

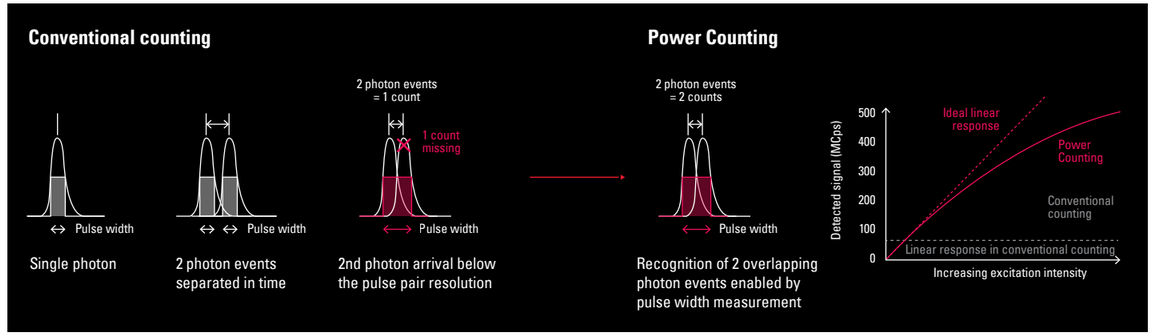
STELLARIS breaks new ground with Power Counting, its innovative photon counting approach. Traditional photon counting, based on thresholding of detector signals, is unable to distinguish a single photon from two or more photons arriving at the same time. This means valuable information is lost from your experiments. Power HyD detectors overcome this limitation by using accurate pulse width measurements to identify and count overlapping photons. As a result, more photons can be detected without saturating the detector. This significantly enhances the fidelity and dynamic range of confocal images and improves quantitative accuracy of your results. The entire Power HyD detector family is equipped with this new approach.

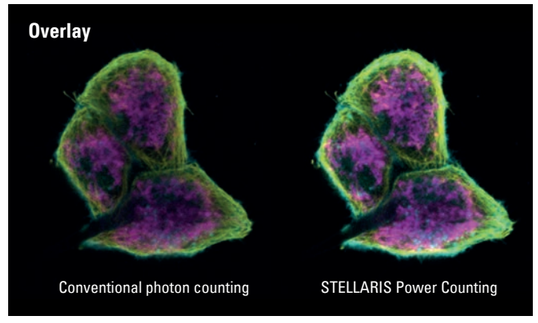
Power counting at work: more detail in every image

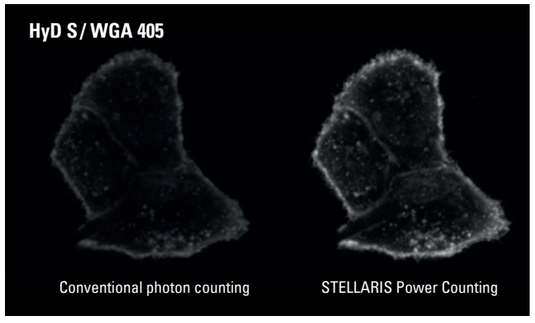
The dynamic range of the detector determines how many different levels of intensity you can distinguish within your images. A high dynamic range is essential to cope with the heterogeneous signal intensities and large fluctuations of target molecules common in biological samples. Our Power HyD S technology, in combination with Power Counting, delivers more than twice the dynamic range* of classical photon counting methods. This enhanced capacity to count photons improves image contrast, especially at high signal intensities where the likelihood of having overlapping photons is much greater.

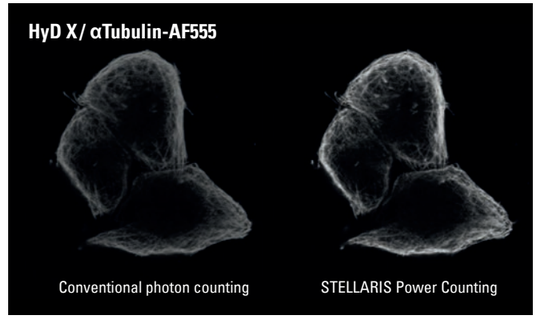
* Comparison of linear range of STELLARIS HyD S versus SP8 HyD in counting mode (CW)

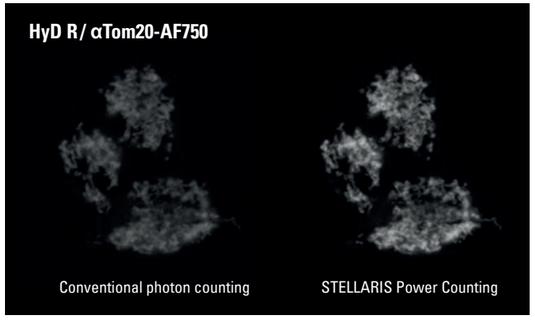
Traditional photon counting methods miss a count when two photons reach the detector in very close succession (frequency of occurrence above pulse pair resolution, grey bar). By accurately measuring and analyzing pulse widths, Power Counting resolves and correctly counts them as two individual photons. As a result, the dynamic range is higher, contrast between bright and dim features is more faithfully represented in the image, and quantitative accuracy is significantly improved.











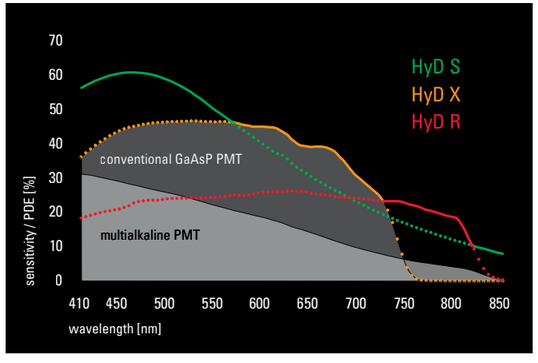
HeLa cells, fixed, imaged on STELLARIS 8, labelled with WGA-405 (HyD S), αTubulin-AF555 (HyDX) and αTom20-AF750 (HyD R).

Enhanced performance across the spectrum

Whatever your preferred fluorescent labels are, detector sensitivity can be critical to avoid photobleaching and to capture maximum signal, especially when working with low-abundance targets or events. For live-cell imaging, high sensitivity becomes even more critical to ensure physiological behaviors and functions are not altered or disrupted, thus obscuring the true nature of your sample.

With three detector types in the Power HyD family, STELLARIS is able to offer enhanced detection capability across the spectrum. The Power HyD S detectors core to STELLARIS 5 provide exceptionally high sensitivity in the blue-green range, with a Photon Detection Efficiency (PDE) of up to 56%—more than double that of conventional multi-alkali photomultiplier tube (PMT) detectors. This ultra-sensitive detection comes in the range where many commonly used live-cell probes such as CFP or GFP emit. Power HyD S detectors also provide good sensitivity into the red and even the near infrared spectral ranges, giving STELLARIS 5 the versatility to meet a wide range of application needs.

STELLARIS 8 brings added flexibility, allowing you to configure your system with any combination of Power HyD S, HyD R and HyD X detectors and enabling a wide range of applications.



Relative sensitivity (%PDE) of the Power HyD family of detectors compared to conventional PMT detectors.

DETECTION TECHNOLOGY IN FOCUS

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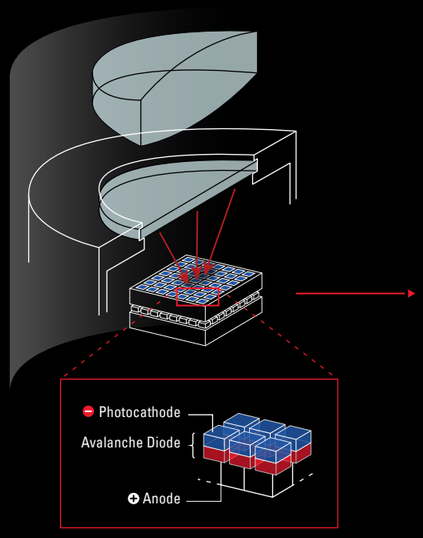
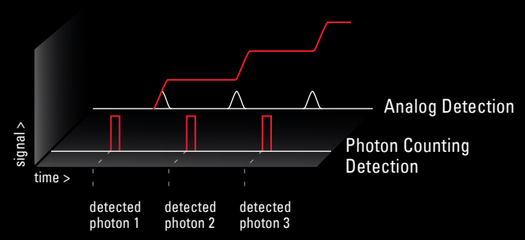
Overview of Power HyD detector family technology

Top: Power HyD S detectors deliver analog and photon counting detection. Bottom: Power HyD X and HyD R detectors are based on hybrid detector technology, allowing for ultra-low dark noise and high sensitivity.

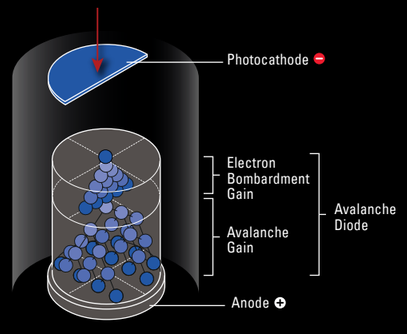
Power HyD S multi-cell architecture

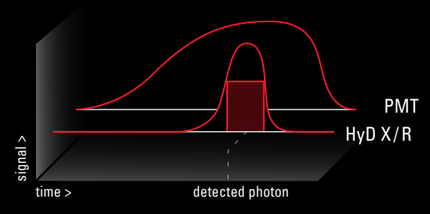


Processing of detector signals in the two available operating modes.



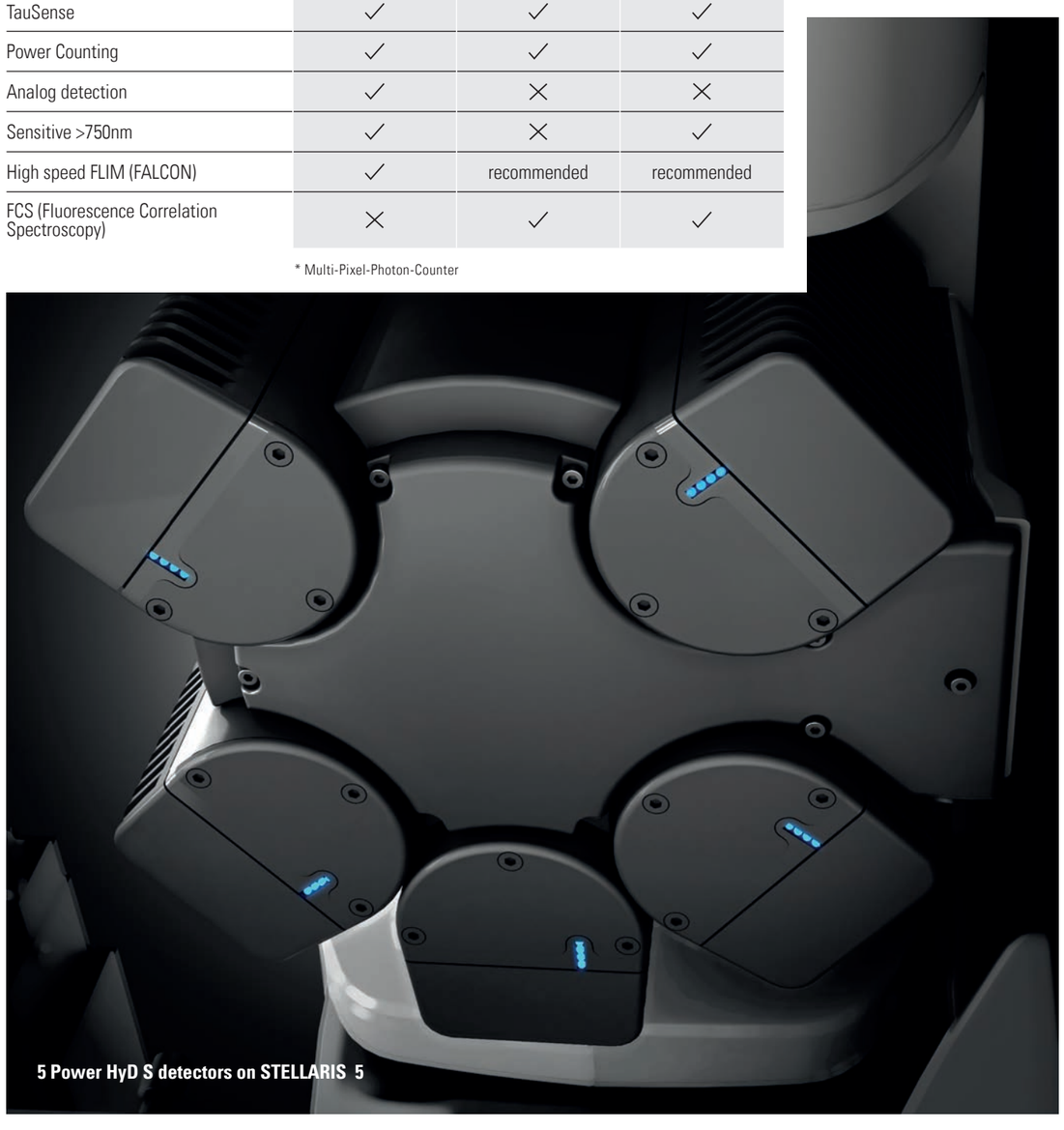
Hybrid detection principle in Power HyD X and HyD R detectors





Power HyD family at a glance	HyD S	HyD X	HyD R
Technology	Silicon MPPC*	GaAsP Hybrid	Extended red GaAsP Hybrid
TauSense	✓	✓	✓
Power Counting	✓	✓	✓
Analog detection	✓	✗	✗
Sensitive >750nm	✓	✗	✓
High speed FLIM (FALCON)	✓	recommended	recommended
FCS (Fluorescence Correlation Spectroscopy)	✗	✓	✓

* Multi-Pixel-Photon-Counter



5 Power HyD S detectors on STELLARIS 5

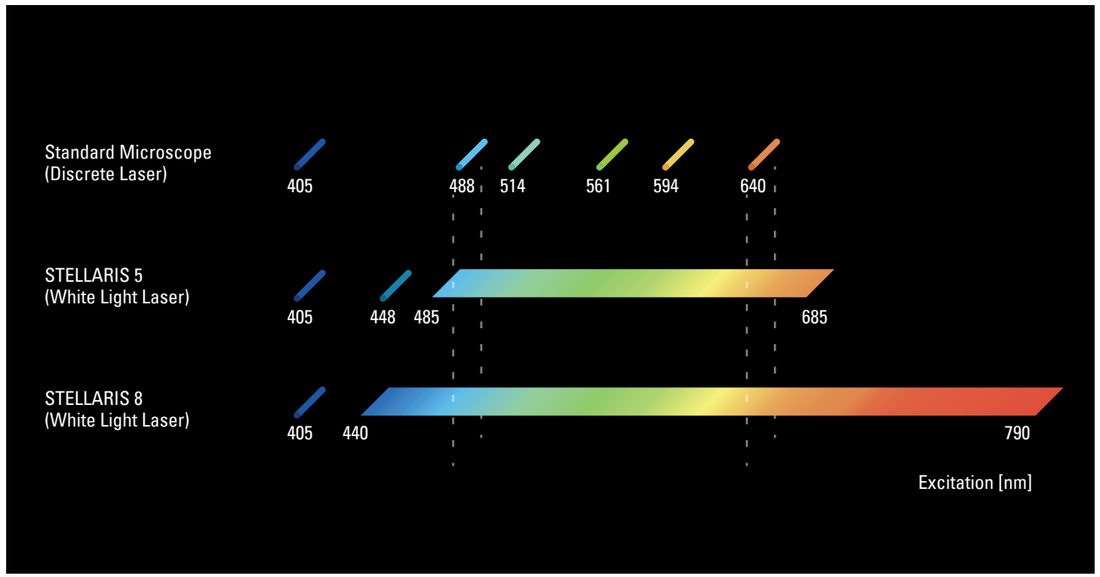
WHITE LIGHT LASER TECHNOLOGY IN FOCUS

Harnessing the rainbow: White Light Laser technology brings more color and more possibility into your experiments

Due to today's complex research questions, traditional confocal systems leave researchers wanting more: more choice in the fluorescent labels that can be used, more opportunities to multiplex, and more available wavelengths. With an ever-expanding toolbox of fluorescent probes, it is time for a microscope that can give you more flexibility to optimally excite your preferred labels and see more colors in a single image. Discrete lasers limit your flexibility when it comes to choosing fluorophores. The next generation White Light Laser (WLL) technology, coupled with the Power HyD detector family, allows you to optimally image virtually any fluorophore within the available spectral range and use up to 8 fully tunable laser lines simultaneously. This complete spectral freedom on STELLARIS makes it possible to work with more fluorescent labels and more fluorophore combinations than on any other confocal platform.

Use more of the spectrum, from blue to near infrared

The STELLARIS 5 WLL gives you a broad spectral range of 485-685 nm. In STELLARIS 8, the WLL range is extended even further going down to 440 nm giving you more excitation options in the blue region. The STELLARIS 8 WLL also features a significantly extended red emission, reaching out to 790 nm. In combination with the Power HyD R detector, this allows for imaging of fluorophores emitting all the way up to 850 nm. This enables you to add up to 3 additional red labels to your experiment and gives you the ability to work optimally with key NIR fluorophores such as AF750, AF790, CF 700, CF 750, and CF 770.



Compared to the limited selection of discrete laser lines available in standard confocal microscopy setups, the STELLARIS 5 and STELLARIS 8 WLLs vastly extend the spectral options. In addition, Discrete 405 and 448 nm lasers are also available as a complement to the WLL on STELLARIS.

POWER
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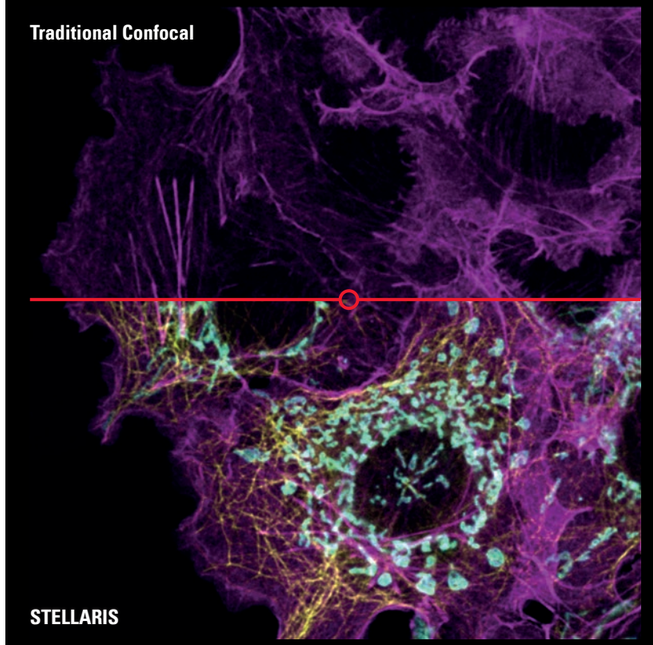
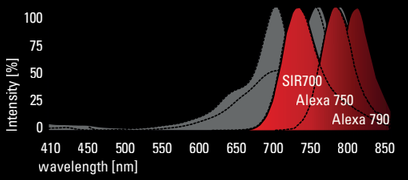
APPLICATIONS: THE POWER OF SEEING RED

Cytoskeletal and mitochondrial interactions revealed

Most conventional confocal systems are equipped with only one or two discrete laser lines in the red. In addition, conventional GaAsP detectors are not sensitive in the NIR range. Both of these factors severely limit the number of red dyes that can be imaged in a single experiment with traditional confocal imaging systems. In the experiment shown here, Cos-7 cells were labeled with red-excitable probes specific for actin, mitochondrial outer membrane, and tubulin. Traditional confocal microscopy with a GaAsP detector detects only the SiR-Actin (Top). Using the extended WLL on STELLARIS 8 together with the HyD R detector, all 3 fluorescent probes were optimally excited and are visible in the image (bottom). Imaging on STELLARIS not only made it possible to see the delicate interplay between mitochondria, actin and tubulin, but freed up the rest of the spectrum for additional fluorescent probes.





Top: shows Cos-7 cells labeled with SiR-Actin (657 – 740nm detection range), AF750-Tom20 (760 – 790nm), AF790-Tubulin (810 – 850nm). Above slider bar: seen by conventional GaAsP detectors. Below slider bar: seen by STELLARIS 8 equipped with a Power HyD R detector. Spectral traces (right) show excitation (gray) and emission (red) spectra of fluorophores used in the experiment. Sample courtesy: Dr.Jana Döhner and Dr.sc.nat. Urs Ziegler, University of Zürich.

WHITE LIGHT LASER TECHNOLOGY IN FOCUS

POWER
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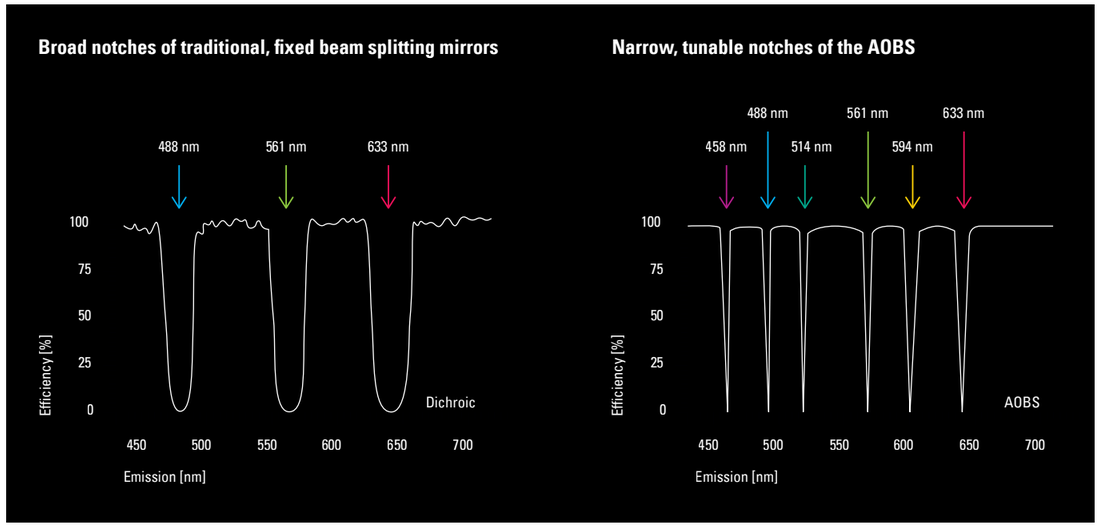
Acousto-optic technology gives you power to see more of the signal from your sample

Traditional beam splitting mirrors were designed with discrete lasers in mind, having high transmission for a fixed set of wavelengths. To fully utilize the next generation White Light Laser, STELLARIS is outfitted with an Acousto Optical Beam Splitter (AOBS). The AOBS connects the STELLARIS White Light Laser to the Power HyD detectors. Because the AOBS can be precisely tuned for efficient transmission of any wavelength that is produced by the White Light Laser, fluorescent labels in your sample are excited more efficiently. In addition, the AOBS excitation notches used to separate excitation light from emitted signal are much narrower compared to traditional mirrors. This means that less emission signal is prevented from reaching the detector. With more control and efficiency of both excitation and emission paths, this uniquely designed optical system provides the ideal solution for advanced multichannel confocal microscopy.



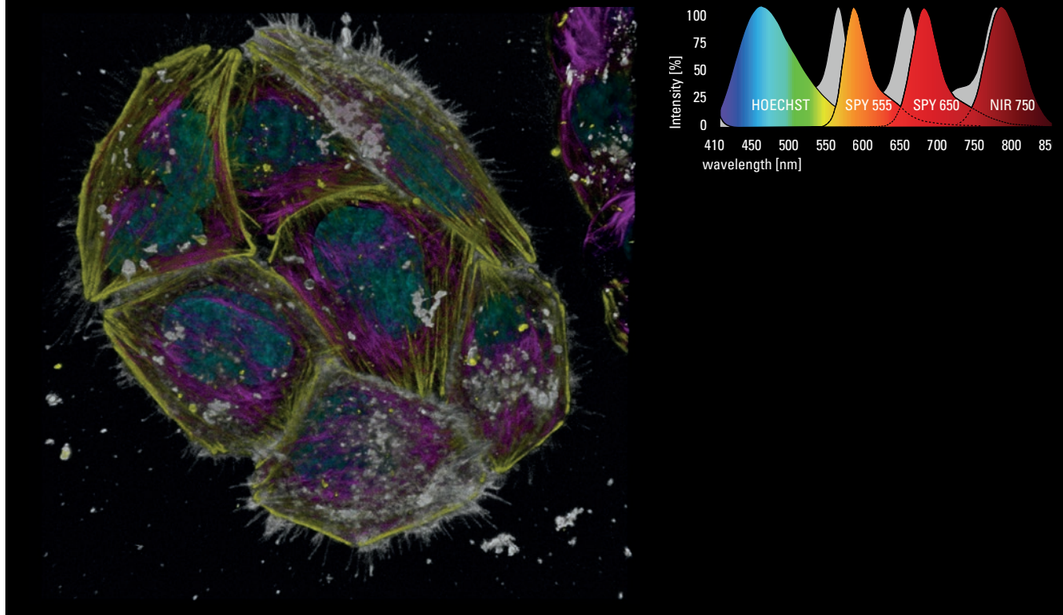
The synergistic design of STELLARIS allows for more gentle and efficient live-cell imaging

Lasers can bleach fluorescent labels, disrupt biological processes and burn delicate tissues. Live cell imaging experiments are all too often cut short, or undesirable trade-offs are made in the acquisition parameters, to avoid these damaging effects. STELLARIS uses the power of tunable acousto-optic technology and perfectly matched spectral detection to take full advantage of the white light laser and let more fluorescent signal through to the detectors. This means that the laser power can be turned right down to preserve your precious samples and enable you to image your samples for longer periods of time.

APPLICATIONS: LIVE CELL IMAGING

Getting the most from live-cell experiments: fewer exposures, more information

When using traditional confocal microscopy to image several different fluorescent labels in the same sample, sequential imaging of each color channel is often needed to avoid spectral bleed-through, which can degrade image quality. In the case of a kinetic experiment, that means you may miss rapid dynamic events due to the increased time it takes to acquire each time point. In addition, your sample remains on the stage for longer, making it more challenging to maintain cell health for the duration the experiment. The image below was captured from live HeLa cells labeled with 4 different fluorophores to identify nuclei, actin, tubulin and plasma membrane. With STELLARIS, it was possible to collect all 4 channels in a single pass, rather than having to image the cells 4 times in succession.



Simultaneous 4-color imaging with STELLARIS 8. Live HeLa cells have been labeled to identify nuclei (cyan, Hoechst), actin (yellow, SPY555), tublin (magenta, SPY650) and plasma membrane (gray, NIR750).

MORE POWER FROM YOUR CONFOCAL PLATFORM

With STELLARIS you get powerful features that are optimized to work in perfect harmony and bring you the best performance. The combination of the Power HyD detector family, the AOBS and the next-generation White Light Laser makes every photon count and gives you exciting new ways to design your experiments and generate new discoveries.

POTENTIAL
DISCOVER MORE

STELLARIS has re-imagined confocal microscopy by giving you an extra dimension of information in every experiment. With TauSense, we put revolutionary new lifetime-based tools at your fingertips.

With just a few clicks, you can remove unwanted signal to reveal more detail in your images, multiplex more labels in a single experiment, separate fluorophores that have overlapping spectra, and apply the power of lifetime-based information to explore localized functional and micro-environmental changes. With fluorescence lifetime-based information at your fingertips, STELLARIS opens up a world of new possibilities for your research.

The vast majority of imaging experiments measure fluorescence intensity to study targets of interest, but fluorescence has another property that is always present, but not always measured: **fluorescence lifetime.** STELLARIS TauSense technology gives you the potential to exploit fluorescence lifetime-based information to gain new functional insights, study more targets simultaneously, or simply improve signal-to-noise by removing unwanted signal.

TauSense is a revolutionary set of imaging tools fully integrated into STELLARIS: TauContrast, TauGating, TauScan and TauSeparation. Each of these tools takes advantage of fluorescence lifetime to bring unique benefits to your research.

EXPLORE A NEW DIMENSION OF INFORMATION WITH THE UNIQUE TAUSENSE TECHNOLOGY

TauContrast
Gain immediate access to informative functional and physiological parameters such as pH, temperature and ion concentration changes. In addition to capturing fluorescence intensity information, each pixel contains details about photon average arrival times (a function of fluorescence lifetime), which can change depending on shifts in micro-environment. Using TauContrast, such changes can be mapped and visualized in the image. For example, differences in the pH values of intracellular vesicles (arrows) are not apparent by fluorescence intensity alone. With TauContrast, these differences are revealed because the lifetime of the chosen fluorophore varies depending on the local pH.

TauGating
TauGating can be used to remove unwanted signal based on the photon arrival times. For example, the intensity images of basal membrane in live cells often contain a reflection contribution that can mask the signal from your label of interest. Because reflected photons have a much shorter arrival time compared to the fluorescence signal, they can be distinguished and removed by TauGating.

TauScan and TauSeparation
TauScan and TauSeparation make use of fluorescence lifetime-based information to distinguish fluorophores that could not be separated with spectral tools. By exploiting differences in photon arrival times, signals from LifeAct-GFP and MitoTracker Green (magenta) are clearly separated, even though the two fluorophores have significant spectral overlap. This expands the potential number and combinations of fluorescent labels you can use in a single experiment.

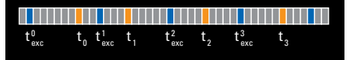
Gaining valuable insights from your sample
Cells and tissues are fluorescent by nature. This endogenous fluorescence (autofluorescence) is often seen as a problem to be overcome in confocal microscopy, because it can interfere with the spectrum of specific fluorescent markers. But instead of getting rid of it, what if we could use autofluorescence as an additional informative parameter in imaging experiments? With the lifetime-based TauContrast functionality on STELLARIS, fluorescence signals can be discriminated by their average photon arrival times, as in this image of *Arabidopsis thaliana* leaf tissue, which shows actin (LifeAct-Venus) in red and chloroplast autofluorescence in blue-green. Here, the arrangement of actin fibers, particularly around a leaf pore (stomatum), can be visualized nicely in combination with the distribution and size of chloroplasts. The blue-green color is the result of varying photon average arrival times associated with differences in the fluorescence lifetime. Since the fluorescence lifetime of chloroplasts is known to be influenced by the local environment, this color variation provides additional insight into physiological conditions—without the need for an additional label. Crucially, STELLARIS gathered all this information from a single detector, leaving the remaining detectors free for additional labels.

Arabidopsis thaliana leaf with stomatum. Red: Actin, LifeAct-Venus (Era et al. Plant Cell Physiol., 2009). Blue-green: chloroplasts, endogenous fluorescence. Image acquired with TauContrast. Sample courtesy: Dr. Melanie Krebs, COS, University of Heidelberg.

TauContrast

Average Arrival Time* (AAT)

*in a pixel

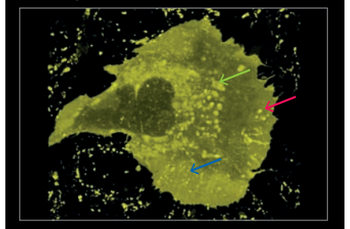


$AT_i = t_i - t^i_{exc}$ $\rangle$ $AAT = \frac{\sum_{i=1}^{N} AT_i}{N}$

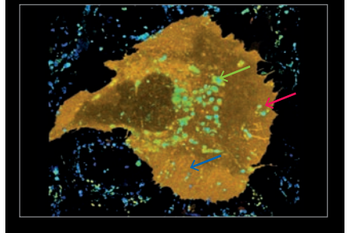
ATi = Arrival time photon i

N = number of photons detected during the pixel dwell time

Intensity



TauContrast

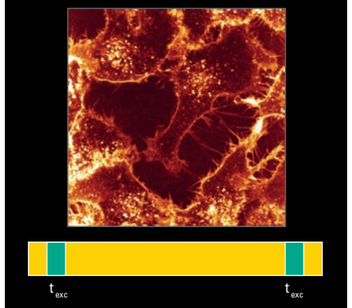


TauGating

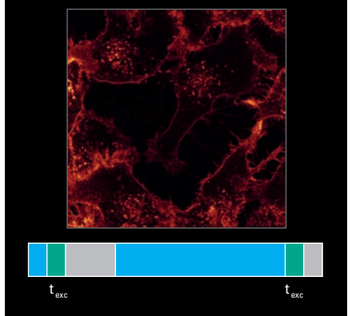
Gated Arrival Time

Photons arriving outside the selected time gates are not counted

Intensity



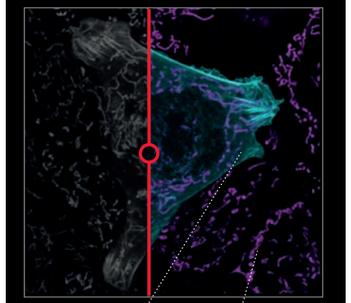
TauGating

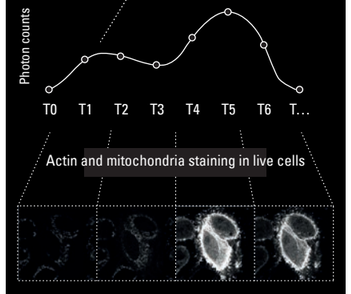


TauScan and TauSeparation

Gate based multi-exponential components distribution

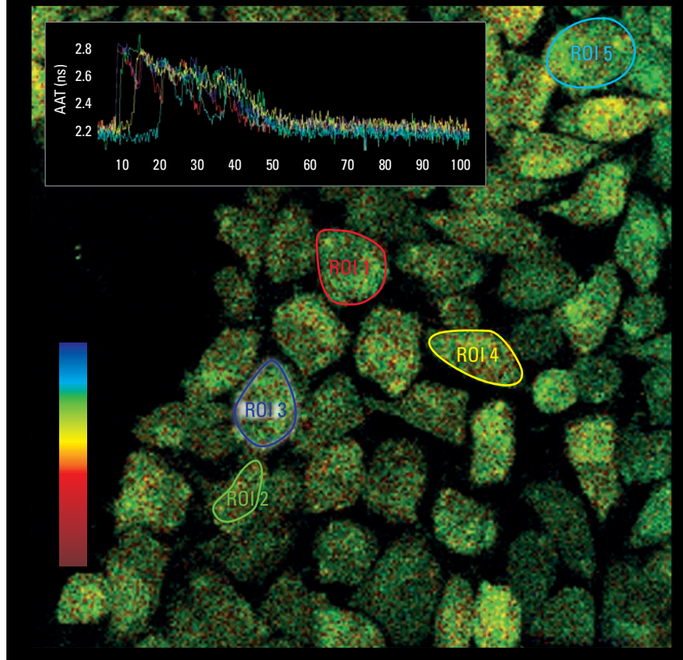
Intensity TauSeparation





TauContrast. The contrast in each pixel is given by the average arrival times (AAT) of the photons detected during the pixel dwell time. Images show a mammalian cell labeled with near infrared membrane stain. Arrows indicate vesicles with differing pH values (red higher, blue lower, green intermediate). Intensity image: punctate vesicles show higher intensity than the surrounding cytoplasm. TauContrast image: look-up table (LUT) of the color overlay corresponds to AAT (0-1 ns); changes in vesicular pH during internalization are more apparent than in the intensity image. Scale bar, 20 µm. **TauGating** enables splitting photons arriving at different times. Live HeLa cells stained with WGA CF680 (top). Reflection removed by TauGating (bottom). **TauScan and TauSeparation.** TauScan and TauSeparation of mammalian cells expressing LifeAct-GFP (manufactured by ibidi GmbH) and labeled with a green mitochondrial stain. Schematic shows the distribution of lifetime components. Scale bars, 10 µm.

APPLICATIONS: UNLOCKING THE POTENTIAL OF FLUORESCENCE LIFETIME BASED INFORMATION



Tackling calcium signaling with TauSense, for more robust results

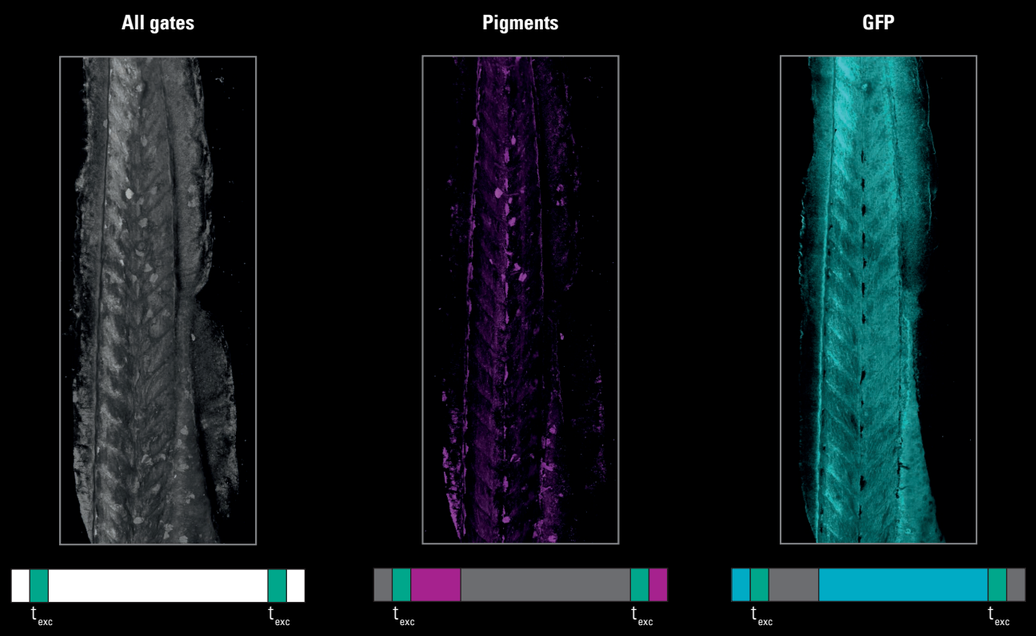
Calcium signaling plays a central role in many essential biological processes, including metabolic regulation, muscle contraction, and neurotransmission. Robust methods for monitoring dynamic spatial and temporal changes in free calcium are, therefore, essential to unravel mechanisms of calcium regulation. While calcium imaging has become increasingly routine for investigation of calcium signaling mechanisms, subtle changes in the fluorescence intensity of calcium indicators like Oregon Green 488 BAPTA can be difficult to measure reliably at the individual cell level using its intensity changes. With TauContrast, STELLARIS delivers more robust results by taking advantage of calcium concentration-dependent changes in the lifetime of Oregon Green 488 BAPTA. In this time-series experiment, TauContrast clearly reveals distinct variations in intracellular calcium concentration.

Calcium waves after mechanical stimulation of mammalian cells loaded with Oregon Green 488 BAPTA. The response in individual cells is recorded as a change in TauContrast. Traces (inset) show time series for individual regions of interest (line color corresponding to circled ROI) acquired at 4.5 frames per second. Image size: 256 x 256 pixels. Rainbow LUT (TauContrast): 0-4 ns.

APPLICATIONS: UNLOCKING THE POTENTIAL OF FLUORESCENCE

Removing unwanted contributions to your signal with TauGating

Fluorescence lifetime-based information gives you unprecedented control over the information that is accumulated in your image. Now you can easily remove unwanted signals that could be masking lower intensity fluorophores. For example, the intensity image of a zebrafish (all gates, left image) is comprised of signals from both endogenous pigments and genetically encoded GFP. TauGating can be applied to show pigment only (magenta, middle image) or GFP only (right image).

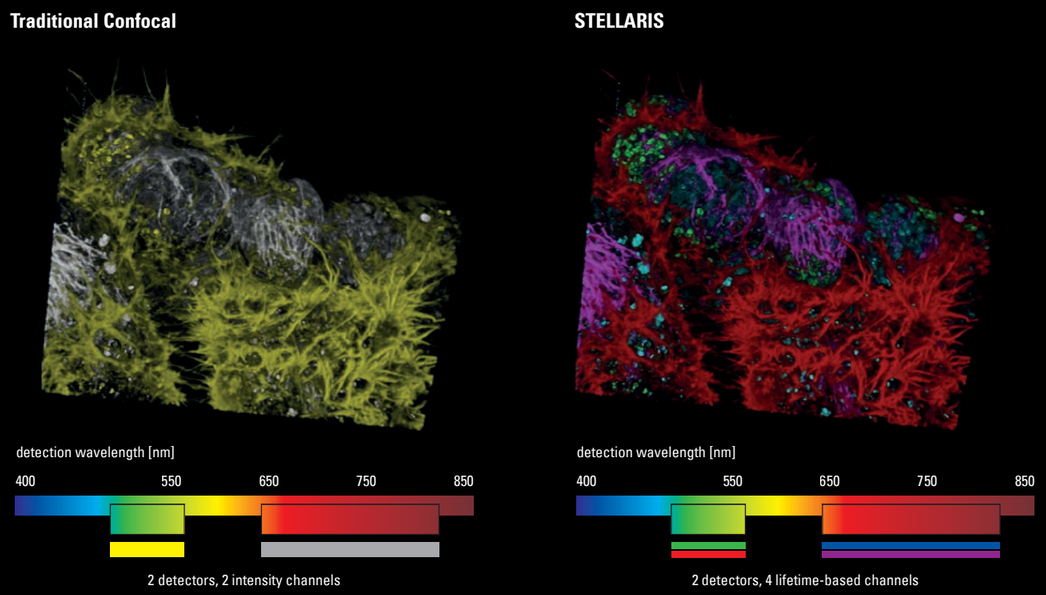


Zebrafish (labelled with the transgenic construct 4xGTIIC:d2GFP) exhibiting native pigments. GFP fluorescence provides a read-out of Yap1/Taz-Tead activity (Link & Miesfeld 2014, Mech. Dev) and is used here to visualize the striated muscle of the trunk at 55 hpf. Using TauGating, the signal of interest (cyan, long arrival times) is singled-out from endogenous pigment contributions (magenta, short arrival times). Scale bar: 200 µm. Sample courtesy: Julien Vermot, IGBMC, Strasbourg.

LIFETIME-BASED INFORMATION

Acquire usable images from overlapping fluorophores with lifetime-based information

In the past it was difficult, if not impossible, to distinguish two fluorophore species that had closely overlapping spectra. In the lab, this meant researchers were severely restricted in the number and combinations of fluorescent probes they could combine in a single experiment. TauSense imaging toolbox can remove these restrictions by exploiting fluorescence lifetime-based information, allowing you to unmix even fully overlapping fluorophores. Because more than one label can be distinguished in a single channel with lifetime information, this effectively expands the number of targets of interest that can be studied simultaneously. Where traditional confocal microscopy shows you two colors, STELLARIS adds lifetime information to give you the possibility to measure four.



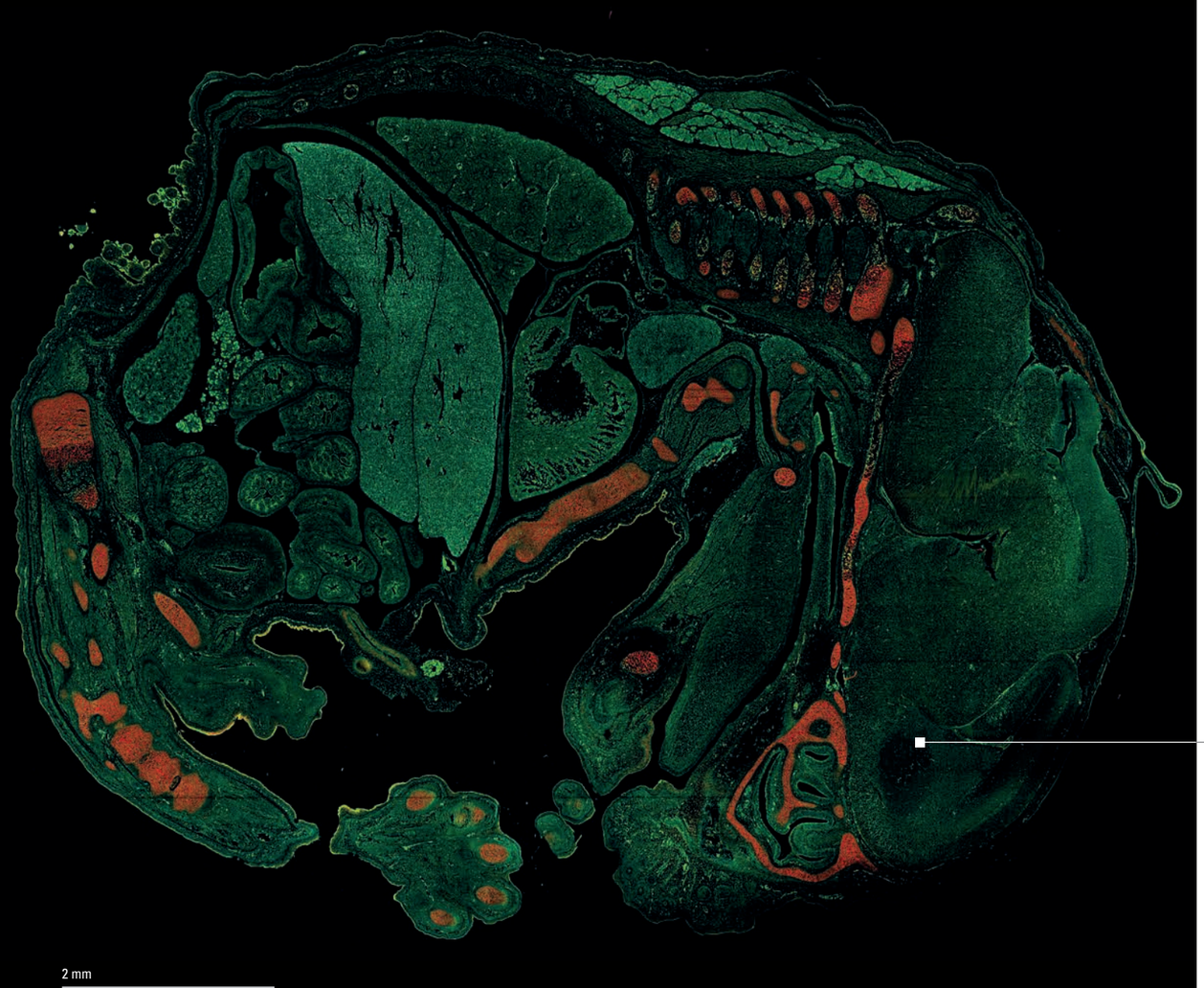
NE-115 cells imaged with two detectors. **Left:** without TauSeparation, fluorophores with spectral overlap detected in the same image channel cannot be distinguished: LifeAct m-NeonGreen (yellow), MitoTracker Green (yellow), NUC Red (gray), and SiR-tubulin (gray). **Right:** the four fluorophores have been separated using TauSeparation: LifeAct-mNeonGreen (red), MitoTracker Green (green), Nuc Red (cyan), and SiR-tubulin (magenta). Sample courtesy: Max Heydasch, University of Bern and Spirochrome.

MORE POTENTIAL AT YOUR FINGERTIPS

With fluorescence lifetime information at your fingertips, you can instantly add an extra dimension to your experiments. The entire suite of TauSense tools is available with the click of a button, and is fully integrated into the STELLARIS platform. This frees you to imagine cutting-edge experiments and unlocks new potential to explore your samples in more detail than ever before.

PRODUCTIVITY
DO MORE

Today's imaging experiments are more demanding than ever, requiring the capture of thousands of images and the ability to monitor rapid dynamic changes in context and real time. STELLARIS takes advanced new technologies for confocal imaging and packages them in a way that is simple, adaptable, and scalable. With a re-imagined software interface that seamlessly integrates cutting edge imaging tools, you can break free from traditional equipment and technology limitations to increase your productivity like never before. STELLARIS gives you the freedom to perform the experiments you've always dreamed of doing.



FAST AND EASY SETUP OF CONFOCAL MULTICOLOR EXPERIMENTS WITH IMAGECOMPASS

Too often, software gets in the way of your confocal microscopy experiments, complicating the setup process and wasting valuable time. STELLARIS redefines the image acquisition workflow with ImageCompass, its smart user interface. Drag-and-drop tools make it easy to match each fluorophore to the appropriate detector and set up complex multicolor experiments correctly— every time. ImageCompass automatically adjusts the excitation and detection parameters for each channel to enable optimal results. With its intuitive interface, ImageCompass readily adapts the system configuration to your unique sample requirements and chosen fluorophore combination. The display lets you see your entire experiment configuration at a glance, so that you can achieve an optimal setup quickly, and easily maintain control over your experiment during image acquisition—all without a steep learning curve. With less time spent on training and setup, you'll have more time to focus on your experiments and gain the productivity to do more.

ImageCompass smart user interface. Set up of a 6-color experiment.

See the bigger picture in high resolution

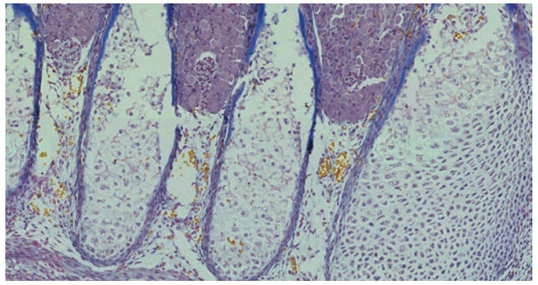
Advances in confocal techniques allow you to see the smallest structural details of your samples. However, the deeper we delve into our sample preparations, the more we risk missing out on the "bigger picture" and how these small-scale interactions affect the overall structure and physiology. The LAS X Navigator lets you zoom in and out of your sample as seamlessly as if you were looking for places to visit on your smartphone. Here we see the whole picture of a mouse embryo with all the fine details preserved in high resolution. Thanks to the integration with ImageCompass, you can quickly assess and optimize the imaging parameters across the entire sample. Once the image has been captured, you can then quickly zoom in on regions of interest without losing perspective to truly understand the nature of your sample.

Whole mouse embryo labelled with Heidenhain's Azan stain.

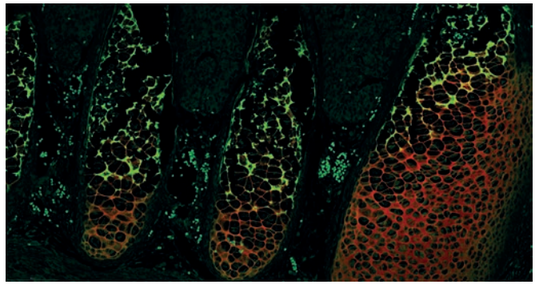
NAVIGATION SOFTWARE IN FOCUS

LAS X Navigator – a GPS for your experiment

Intuitive sample navigation tools are essential to assure optimal setup of complex multicolor experiments and enable efficient exploration of large specimens. The LAS X Navigator is like a GPS for your experiment, helping you to quickly switch from searching image-by-image to seeing a full overview of your sample. With easy access to both the high-level overview and the minute details, the LAS X Navigator ensures that you always have a clear path to high-quality data.



Detailed view (single tile) from the 201-tile stitched image of a mouse embryo (shown on page X) labelled with Heidenhain azan trichrome stain. Transmitted light detector (TLD) imaging was used to create an RGB overlay through excitation with 488nm, 561nm and 638nm wavelengths.



TauContrast image of the same field of view as above, maximum projection. Both the TLD and the TauContrast images were acquired with a 10x objective, and in each case a single tile is shown from the larger stitched image comprised of 201 tiles.

"The LAS X Navigator achieves something very difficult in a complex environment, namely providing maximum resolution while also giving you the high-level overview. It makes navigating your sample as easy as using a map on your smartphone.

Once you've used the LAS X Navigator, you can appreciate that without it you were essentially flying blind – wasting a lot of time and potentially missing important information in your sample. Now that has changed. STELLARIS delivers something truly special by giving you an efficient way to systematically explore every detail."

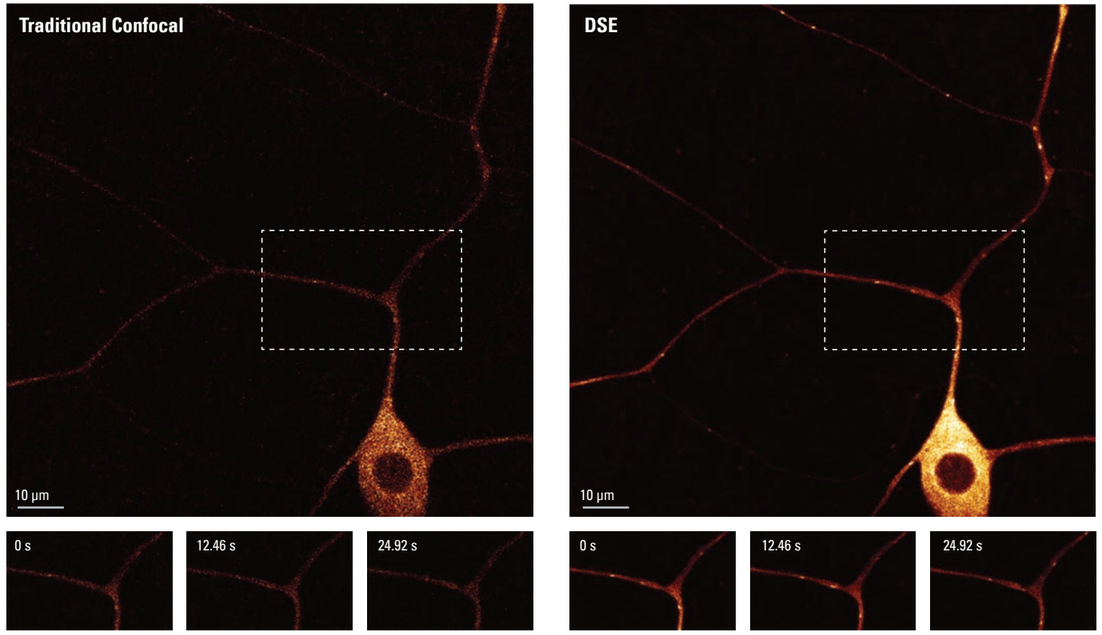
Urs Ziegler, Dr. sc. nat. Head of Facility, Center for Microscopy and Image Analysis, University of Zürich

LIVE CELL IMAGING IN FOCUS

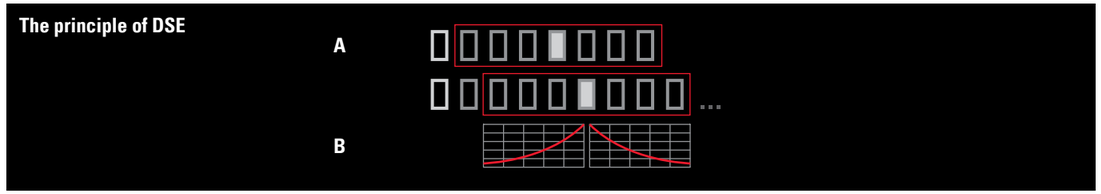
PRODUCTIVITY
DO MORE

When your samples move fast, so does STELLARIS

Capturing dynamic processes inside living cells is difficult using traditional confocal microscopy, often requiring trade-offs that reduce the quality of the data that can be collected. With STELLARIS Dynamic Signal Enhancement (DSE), you can capture biological events in detail while the structures and objects in your sample move, develop, and change. DSE acts like a rolling average, using information from neighboring frames to remove noise while preserving a high temporal resolution. This powerful digital tool can be applied in real-time or post acquisition, giving you more flexibility in how you set up your experiments.



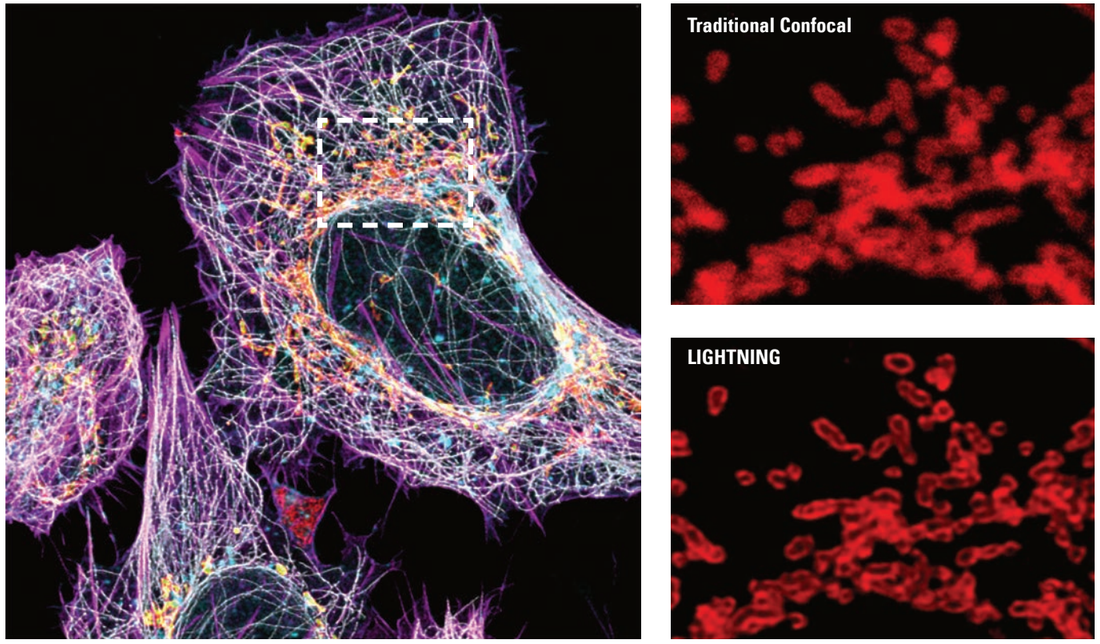
DSE (Dynamic Signal Enhancement) enhances faintest dynamic features to improve clarity and quantifiability. Live drosophila larva expressing EB1-GFP in neuronal cells imaged with resonant scanner on STELLARIS. The 3D time lapse was acquired at 20fps, or 4 volumes per second. EB1-GFP comets along the neurites are hardly visible in the raw data (left). After DSE processing (right), comets are distinct and can be easily followed over time. Sample courtesy: Bu Shufeng and Dr. Yu Fengwei, Temasek Life Sciences Laboratory, Singapore.



A: two consecutive time points showing how neighboring frames (red box) are used on a rolling basis for improving the SNR of the central frame (filled box)
B: additional parameter allows to define how neighboring frames are weighted, less weight means less influence, and less motion blurring.

Discover at the speed of LIGHTNING

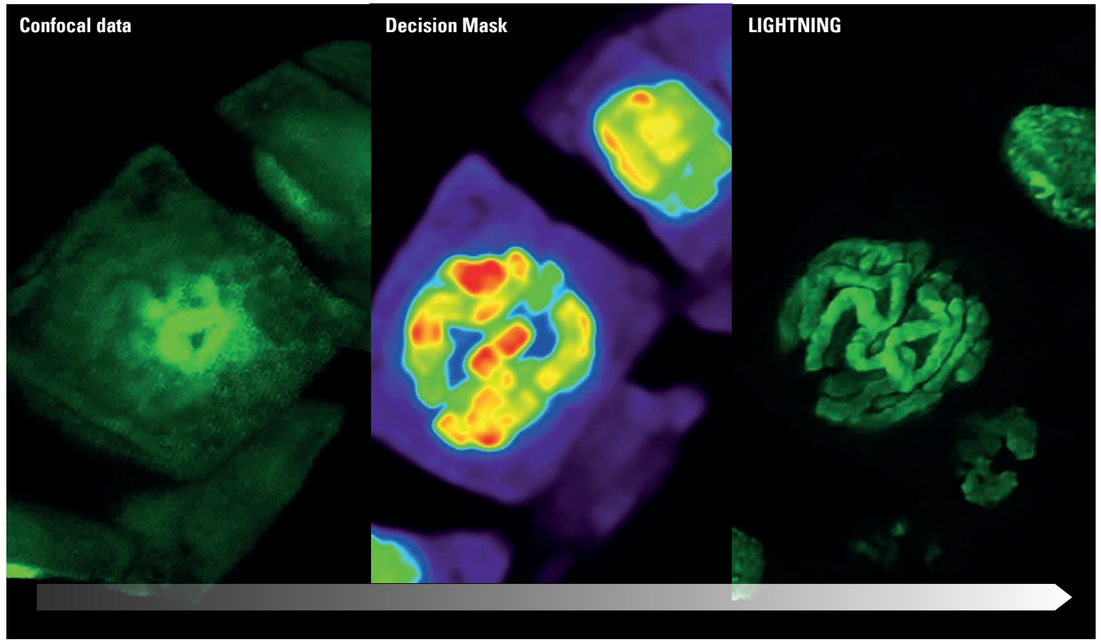
Confocal images obtained with STELLARIS contain more information than you see on the first glance. The LIGHTNING detection concept helps to uncover this hidden information from your specimen. With a single click and fully automatically, LIGHTNING extends the resolution of STELLARIS into super-resolution territory: resolve the fine structures and details, as small as 120 nm lateral resolution, which are elsewise simply not visible. Thanks to its deep integration and optimized GPU processing, LIGHTNING allows for the (near) real-time acquisition of super-resolution images with up to five color channels and a large field of view. Due to this flexibility, LIGHTNING works for any type of specimen and experiment.



Left: maximum projection of 5 color U2OS fixed cells, overlay shows LIGHTNING data set labelled with AF488 (Tubulin, grey), SPY555 (Actin, magenta), MitoTrackerRed (Lumen of Mitochondria, green), Atto 647N (TOM 20, Mitochondria, red), CF770 WGA (Membranes, cyan); **Right:** detail of Tom20 labeling raw (top) and with LIGHTNING (bottom).

Fast across scales in time and space

Unlike traditional methods which use a global set of parameters for the entire image, LIGHTNING is an adaptive process and calculates an appropriate set of parameters for each voxel to uncover details with high fidelity using a Decision Mask. The LIGHTNING decision mask applies the best fitting restoration strategy for each specific volume element. Additionally, using the resonant scanner in combination with DSE allows you to reduce the effective light dosage to protect your specimen for long imaging times. This enables you to adapt the parameters to your sample's dynamics to get the best image resolution with live analysis.



LIGHTNING enhances resolution capability using adaptive methods to deconvolve image data obtained from samples presented in a wide array of formats and applications.

PRODUCTIVITY TO MEET YOUR DEMANDS

With STELLARIS, you know that every time you sit at the microscope you will be able to get more done. The software tools ImageCompass and LAS X Navigator give you full control of your experiments allowing you to truly break free of traditional equipment limitations and imagine new ways to see your samples. The DSE and LIGHTNING technologies enable you to capture your samples with high temporal and spatial resolution, either on the fly or after the images have been acquired.

SELECT THE BEST STELLARIS FOR YOUR RESEARCH

STELLARIS 5

STELLARIS 5 delivers the very best of our platform. It is a completely re-imagined core system that sets a new standard for confocal microscopy. It is the only confocal system with an integrated WLL, combined with our proprietary Acousto-Optical Beam Splitter (AOBS) and new Power HyD S detectors.

Together with the new and unique TauSense technology, STELLARIS 5 sets a new standard for the quality of images and quantity of information generated. This perfected imaging performance is easily attainable thanks to the smart user interface, ImageCompass, which guides you through your experiment set up and acquisition in an easy and intuitive manner.

STELLARIS 8

STELLARIS 8 is a forward-looking system, offering all the benefits of the STELLARIS 5 core system plus added functionality thanks to an extended spectrum WLL and specialized detector options of the Power HyD detector family. This allows you to expand the range of confocal applications for your research.

STELLARIS 8 can be combined with all Leica Microsystems modalities, including STED (STimulated Emission Depletion), DIVE (Deep In Vivo Explorer), FALCON (FAst Lifetime CONtrast), DLS (Digital LightSheet) and CRS (Coherent Raman Scattering). The new features of STELLARIS 8 maximize the potential of these modalities and give you the power and potential to set new standards for research.

Detectors	STELLARIS 5	STELLARIS 8
Power HyD S	standard	standard
Power HyD X	X	✓
Power HyD R	X	✓
White Light Laser	✓	standard
Features		
TauSense	✓	standard
ImageCompass	standard	standard
LIGHTNING	standard	standard
Dynamic Signal Enhancement	standard	standard
Navigator	standard	standard
Modalities		
STED	X	✓
DIVE	X	✓
FALCON	X	✓
DLS	✓	✓
CRS	X	✓

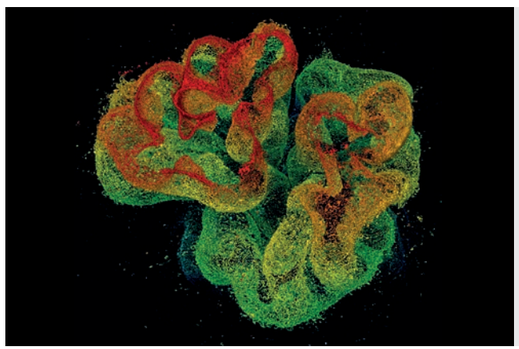
X = feature not available ✓ = feature available as option

Applications		
Gentle confocal live cell imaging	++	++
Integrated super resolution imaging in real time	++	++
Monitor lifetime-based changes	+	++
Spectral or lifetime-based species separation to accommodate difficult dye combinations	+	++
Flexibility for multicolor confocal experiments	++	++
2D and 3D multicolor STED Super-Resolution to study molecular relationships and structure well beyond the diffraction limit	n.a.	++
High-speed lightsheet imaging for live and cleared samples	++	++
Fluorescent Lifetime Microscopy (FLIM) to quantitativly study molecular interactions	n.a.	++
Support of stage applications in multiwells, slide scans, and multi-position experiments	++	++
Information of molecular dynamics (FCS)	n.a.	++
Multiphoton deep tissue In Vivo multicolor imaging	n.a.	++
FRET and FRAP	++	++
Complex experimental set-up (Live Data Mode)	++	++
Superresolution	++	++

+ Good fit ++ Excellent fit … to your application.

EXPAND YOUR RESEARCH

With our modular concept, you can tailor your confocal microscope to your current needs and upgrade with additional functionalities at any time.

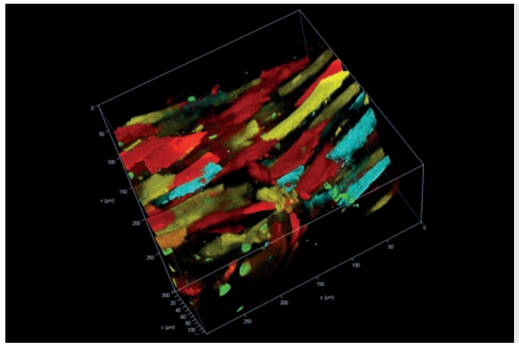


STED – Optical Super-Resolution

STED (STimulated Emission Depletion) delivers powerful and cutting-edge multicolor, deep, and live-cell nanoscopy, for 2D and 3D applications. STED enables you to characterize details in your sample and unveil molecular relationships at the nanoscale level.

Get detailed information here:
https://go.leica-ms.com/STED

STED for kidney disease research: 3D STED 775 deep nanoscopy of glomerulus in cleared kidney tissue immunostained for nephrin. Scale bar: 10 µm, z-depth color coded. Sample courtesy: David Unnersjö Jess, KTH, Stockholm, Sweden.

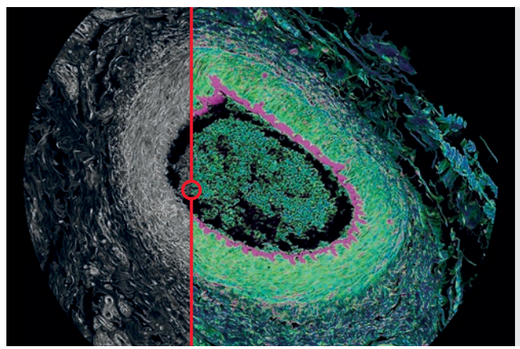


DIVE – Deep In Vivo Explorer

DIVE (Deep In Vivo Explorer) is the first multiphoton microscope with spectrally tunable detection. Equipped with 4Tune, a tunable, non-descanned detection unit, DIVE offers you unlimited flexibility and enables you to develop new multicolor deep in vivo experiments.

Get detailed information here:
https://go.leica-ms.com/DIVE

Small intestines of a confetti mouse, fluorescently labeled and lineage traced from a multi-color tracer. 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 STELLARIS 8 DIVE. Sample courtesy of Prof. Dr. J. van Rheenen, Netherlands Cancer Institute, Amsterdam (the Netherlands).

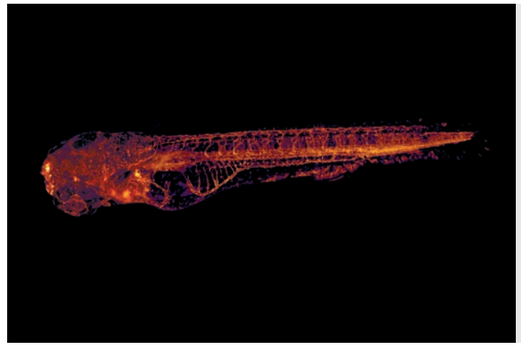


FALCON – FAst Lifetime CONtrast

Lifetime imaging in an instant. STELLARIS 8 FALCON (FAst Lifetime CONtrast) is the future of functional imaging. Harness the power of fluorescence lifetime to investigate cellular physiology and explore dynamics in living cells.

Get detailed information here:
https://go.leica-ms.com/FALCON

Histological section from cat eye. Simultaneous spectral (grey) and FLIM (color) confocal imaging reveals contrast by lifetime. Acquisition and visualization using STELLARIS 8 FALCON and LAS X software.

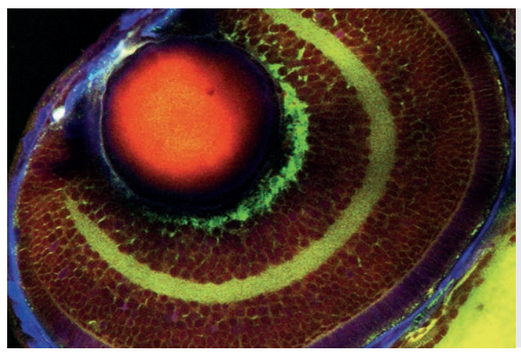


DLS – Digital LightSheet

With DLS (Digital LightSheet) you can benefit from two imaging systems in one: a full confocal and an easy-to-use light-sheet microscope with single plane illumination, making your research more versatile.

Get detailed information here:
https://go.leica-ms.com/DLS

Stitched image of a 4 day old Zebrafish embryo with labeled endothelial cells.
Images courtesy of Dr. Elvire Guiot, Imaging Center of the IGBMC, Illkirch-Graffenstaden, France.

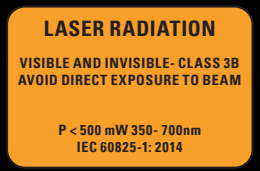


CRS – Label Free Imaging

The CRS (Coherent Raman Scattering) technology exploits image contrast arising from characteristic vibrational states of different molecules within a specimen. As no labeling is required, the specimen remains almost unaffected from preparation and imaging.

Get detailed information here:
https://go.leica-ms.com/CRS

Label-free Vibrational Imaging of Cellular Structures in an Intact Zebrafish. Sample provided by Dr. Julien Vermot, Institute of Genetics and Molecular and Cellular Biology (IGBMC) Strasbourg, France, and Elena Remacha Motta, Leica MS and IGBMC. Objective: HC PL IRAPO 20x/0.75 W



Leica Microsystems CMS GmbH | Am Friedensplatz 3 | 68165 Mannheim, Germany
Tel. +49 621 70280 | F +49 621 70281028

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