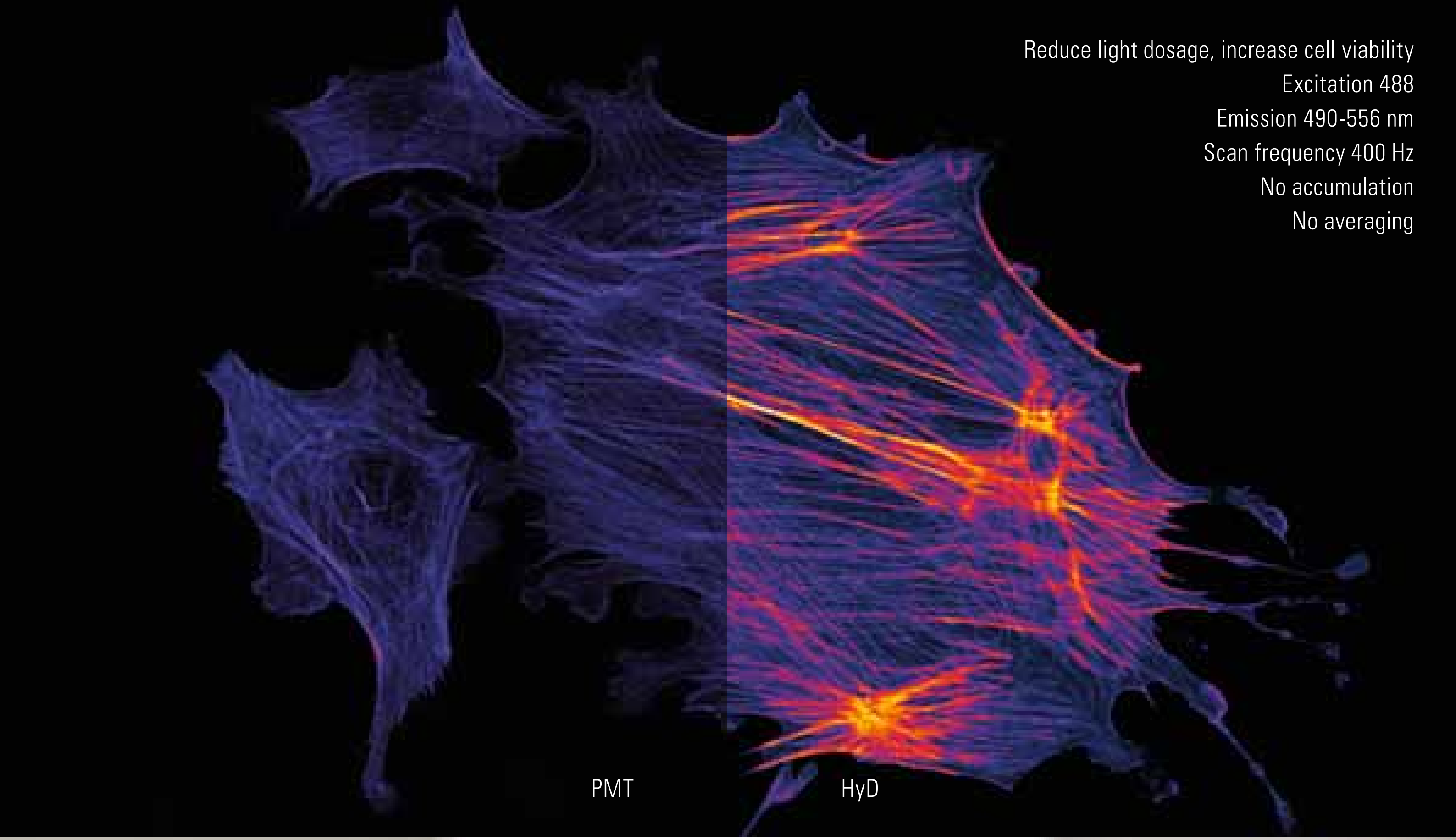
Living up to Life

Leica MICROSYSTEMS

Leica HyD for Confocal Imaging

All-Purpose Super-Sensitivity


Reduce light dosage, increase cell viability
Excitation 488
Emission 490-556 nm
Scan frequency 400 Hz
No accumulation
No averaging
PMT
HyD

Leica HyD – Your Road to Super-Sensitivity

Innovation is a driving force for discovery. The Leica HyD hybrid detector sets a new standard in super-sensitive imaging. There is no need to compromise: photon counting or imaging? Low-light or bright fluorescence? High speed or crisp images? With the Leica HyD you can do it all.

GO LIVE WITH HIGH DEFINITION

Imaging live cell dynamics presents a challenge for point scanning confocals. To scan fast enough to capture rapid cellular processes typicallly is a trade-off of sensitivity to gain speed of image capture. This results in lower signal level and higher noise. With our hybrid detector you go live with super-sensitivity, as it combines low noise with high signal levels. The Leica HyD produces sharp images that convey every detail in high fidelity.

A VIABLE SOLUTION

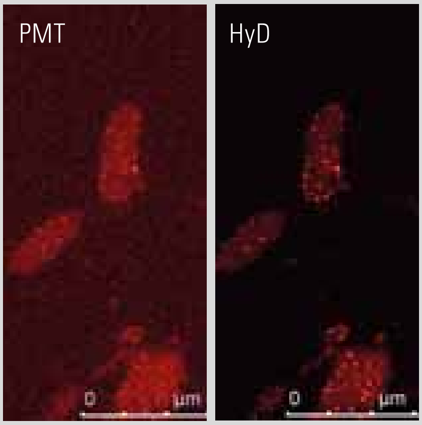
Live cells can suffer from inherent phototoxic effects as a result of imaging. While many of the underlying mechanisms are well understood, the effects of phototoxicity can be hard to pin down in the biological system being studied. High sensitivity directly allows for a reduction of light dosage delivered to the sample, and cell viability is improved by the reduction of free radicals. Even delicate organisms, such as yeast or worms, are accessible to hybrid detection – at full confocal resolution.

READY TO GROW

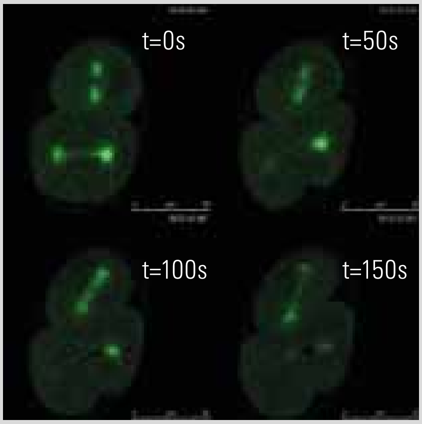
The Leica HyD can be integrated into any new or existing Leica TCS SP8 system. With its high quantum efficiency, low noise and large dynamic range, the Leica HyD is the most versatile detector in the Leica TCS SP8 confocal platform. It synergizes perfectly with our filter-free spectral detection system and the acousto-optical beam splitter (AOBS) in the gapless light detection with maximum photon efficiency. This makes the Leica TCS SP8 ideally suited for quantitative measurements and all-purpose imaging alike.

› Multi-spectral detection for diverse applications
› Superior sensitivity allows decreased light dosage
› Ideally suited for high-speed imaging
› Quantitative through single photon counting
› Descanned or non-descanned detection

Leica HyD Applications



Neuromuscular junction in *Drosophila melanogaster* labeled with Bruchpilot::mStrawberry. The background of the PMT image is blurred by residual noise amplified by the maximum projection, while the HyD image is devoid of noise.



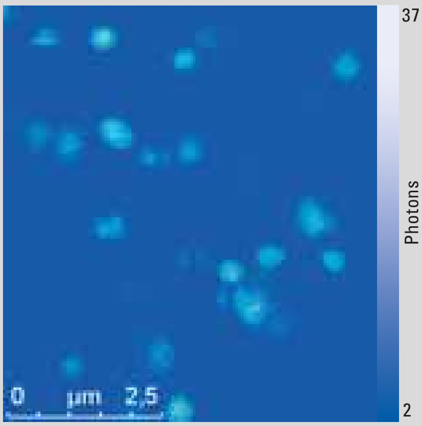
Three- to four-cell stage of *Caenorhabditis elegans* labeled by EGFP-tubulin.

LOW DARK NOISE IMPROVES Z-STACKS OF NEUROMUSCULAR JUNCTIONS

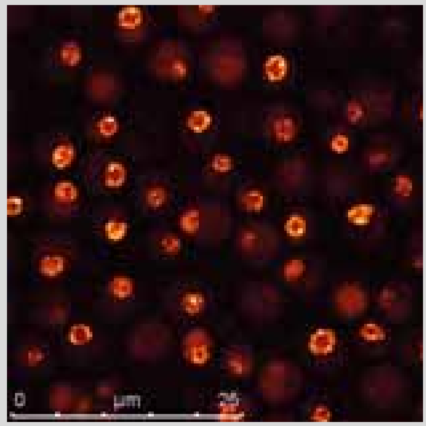
Sensitive live specimens need to be imaged under low-light conditions in high gain situations. Low noise can become the decisive advantage when it comes to recognizing weakly stained detail.

HIGH SPEED MAKES *C. ELEGANS* EMBRYOGENESIS ACCESSIBLE

Developmental processes intrinsically involve the time dimension. In order to unravel the spatio-temporal formation of structure one needs to find the right balance between acquisition speed and clarity. Along with Leica Microsystems' pioneering tandem scanner, the Leica HyD offers unprecedented image quality. The tandem scanner's resonant line frequency (8 or 12 kHz) leaves plenty of room for averaging or accumulation as needed while retaining a large field of view.



Polymer-embedded red fluorophores on a glass surface.



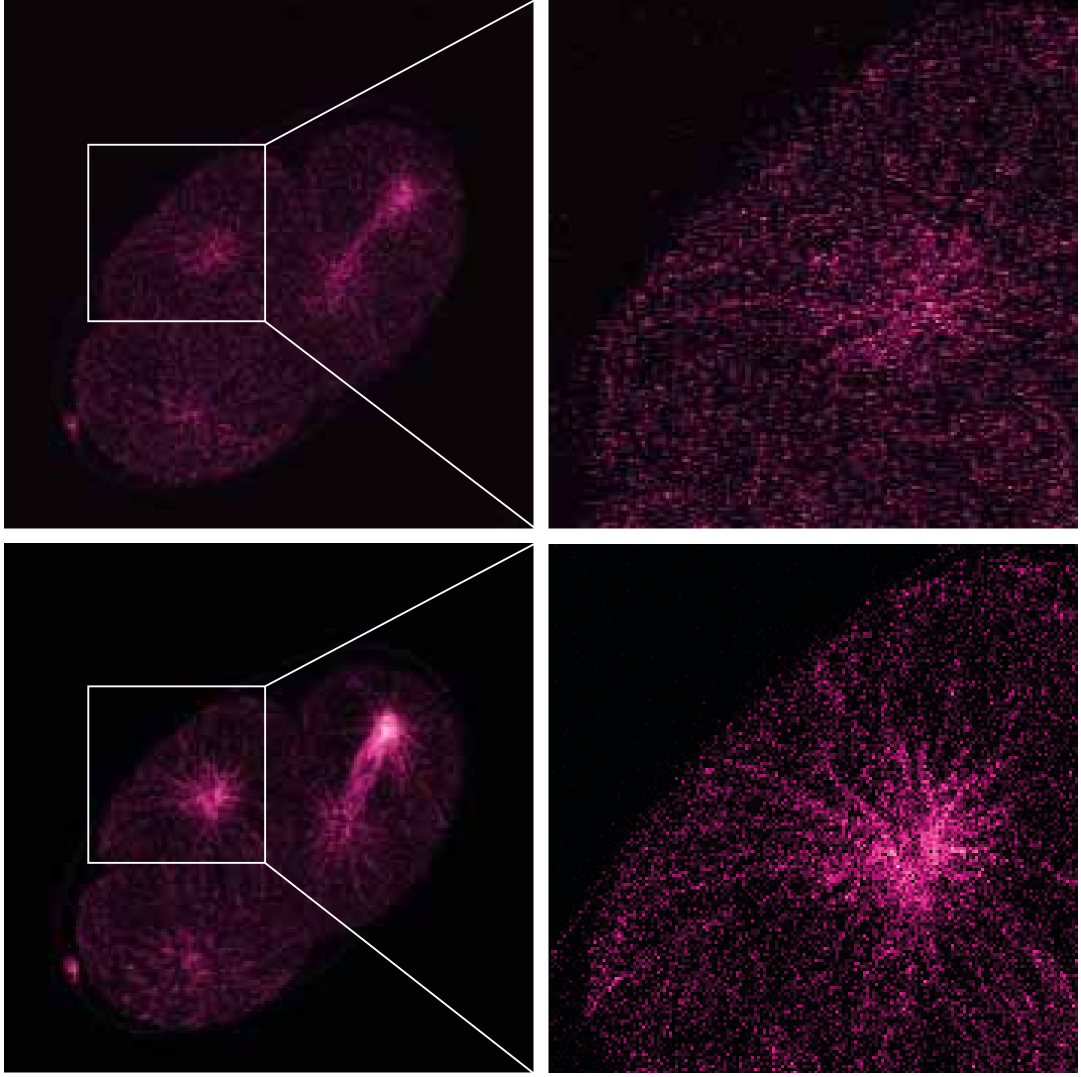
Live yeast cells double-labeled with EGFP at both the nuclear envelope and the telomere.

HIGH SENSITIVITY FOR SINGLE MOLECULE

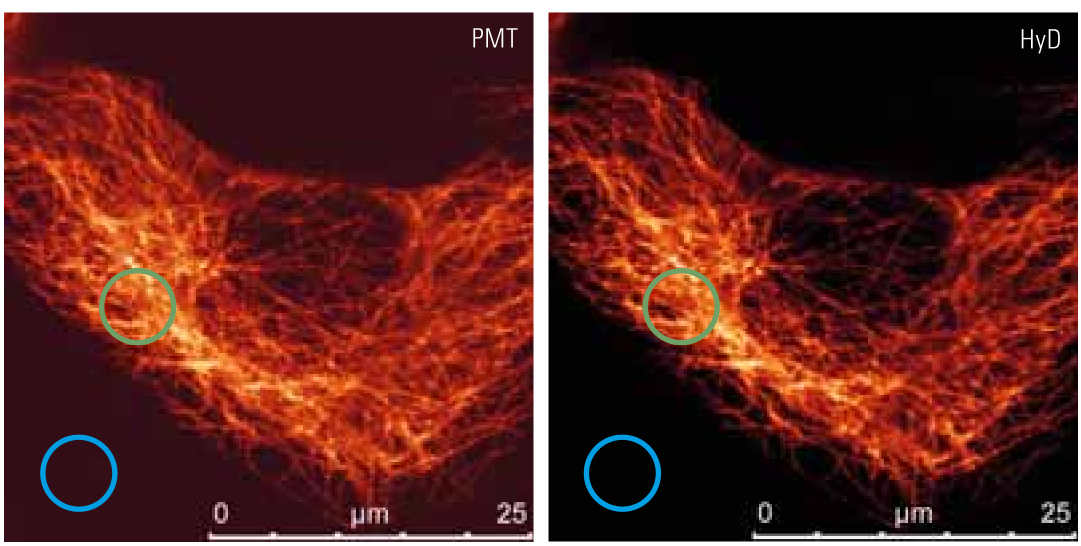
Fixed single molecules represent the ultimate imaging frontier. When measuring such weak signals close to a reflective surface, sensitivity, dark noise and the efficiency of the beam splitting system are stretched to their limits. Note the half-moon shapes and horizontal lines in the image above, which show that molecules are blinking on and off. This reveals the single molecule nature of the diffraction-limited spots.

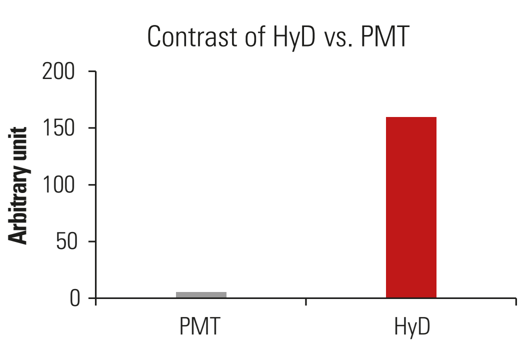
INCREASED CELL VIABILITY PERMITS YEAST IMAGING WITH A CONFOCAL POINT SCANNER

High sensitivity directly translates into reduced light delivered to the specimen and less bleaching. The Leica HyD detects even delicate systems such as yeast at full confocal resolution.



C. elegans embryos labeled with EGFP-tubulin imaged using either PMT (top row) or HyD (bottom row) with identical instrument parameters.
The insets (right column) reveal how the Leica HyD captures the delicate microtubules radiating from the aster. Courtesy of Prof. Pierre Gönczy, EPFL, Lausanne, Switzerland.





Leica HyD strongly improves contrast in comparison to PMTs.
Sample: Tubulin
Excitation: Argon laser 488 nm, 0.2% total power
Imaging: 16x accumulation
Detection: 500-620 nm
PMT Voltage: 800 V
HyD gain: 100%

The contrast ratio was plotted as the ratio of mean intensity in the darkest region (blue) and the brightest region (green circle).

Hybrid Detection Technology for High Fidelity

With its unparalleled contrast the Leica HyD delivers publication-ready images without post-processing. All imaging tasks benefit from the Leica HyD's low dark noise, superior sensitivity and large dynamic range.

BROADEN YOUR SCOPE

The Leica HyD is designed to provide years of high performance. Due to its hybrid photo-detector design, the photo-cathode and downstream amplifying elements remain sensitive over time. Techniques borrowed from silicon chip manufacturing and a simplified geometry combine to produce perfectly smooth internal surfaces that are very robust. This long-term stability ensures brilliant images for many years to come.

SUPERIOR SIGNAL-TO-NOISE

Low dark noise is necessary for maximum signal efficiency, especially when photons are accumulated for more information. Otherwise, noise will pile up in the background of the image. With the latest HyD generation, a built-in Peltier cooling improves the superior signal-to-noise ratio to help render the finest details from any specimen – even tricky ones, such as highly scattering tissue slices. By reducing dark noise, the Leica HyD automatically improves image contrast. You obtain more information content, and the images are immediately publication-ready without any need for image processing.

STANDARD QUANTITATIVE IMAGING

The number of emitted photons directly correlates with the concentration of fluorophores within sample ROI, and single photon counting is the most accurate approach to image quantification. Biochemical information becomes accessible through single photon counting and in situ spectroscopy. Unlike PMTs, which intrinsically have a longer flight time for photoelectrons, the Leica HyD generates ultra-short pulses. In combination with rapid sampling at a 40 MHz rate, this allows precise photon counting with everyday samples. Quantitative imaging is now the standard for your research.

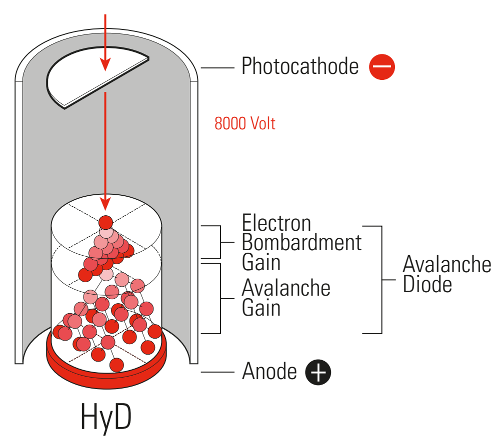
› Signal efficiency for maximum information content
› Low dark noise for high fidelity
› Rendition of minute details
› Publication-ready images out of the box

Hybrid Detector Technology – The Best of Two Worlds

Photodetectors translate light into electric signal, which makes them a critical part of the recording process. Leica HyDs combine the best characteristics of the classic PMT and the highly sensitive avalanche photodiodes (APDs). This results in super-sensitivity and large dynamic range combined with rapid detection speed and low dark noise, making them an ideal detector for all samples.

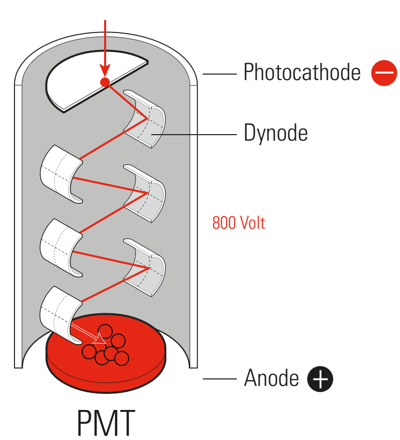
HYBRID DETECTOR TECHNOLOGY

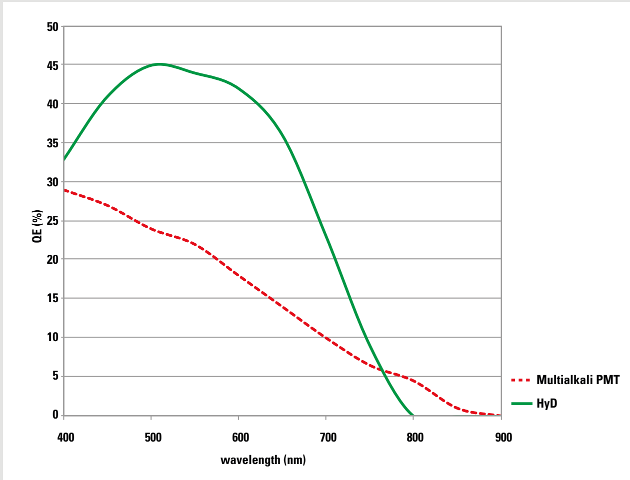
Leica HyD photodetectors combine functional elements used in PMTs and APDs. At the photocathode, the arriving photon is converted into an accelerated electron (photoelectric effect). This primary electron generated at the GaAsP cathode is accelerated in a vacuum by a high electrical field followed by electron bombardment and an avalanche element. Thus a high gain is achieved. The HyD's photon detection is very efficient as there is virtually no loss of electrons. The dark noise level is very low, which gives an efficient recovery of the signal. The avalanche element allows immediate response and a very sharp electrical pulse. Photon counting is possible even at high intensities.

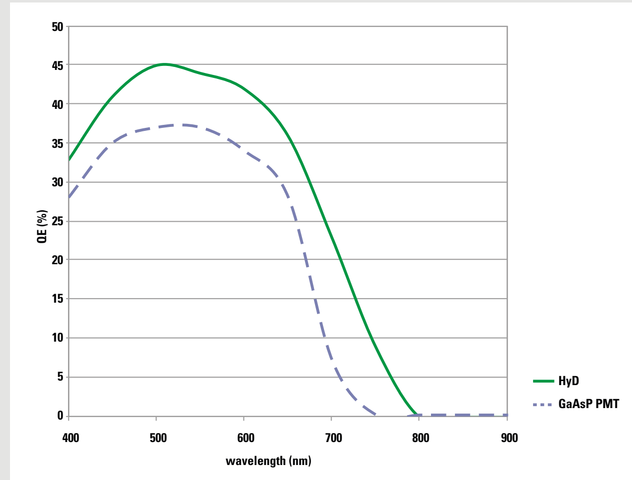


TRADITIONAL PMT

Classic photomultiplier tubes (PMTs) have a large dynamic range and reasonable noise levels, but are limited in sensitivity and the speed of their response. Avalanche photodiodes are highly sensitive, but suffer from low dynamic range and long dark states. At the photocathode of the PMT the primary electron is subsequently amplified in the multiplier tube by a cascade of increasingly positively charged dynodes. Amplification is achieved with each dynode stage. PMTs have several limitations. Due to the dynode design, a substantial amount of electrons is lost for detection. Each step also amplifies noise. Due to the time it takes for flight dispersion, photon pulses are broad and not recognized as individual signals. Therefore, photon counting is possible only at low intensities.



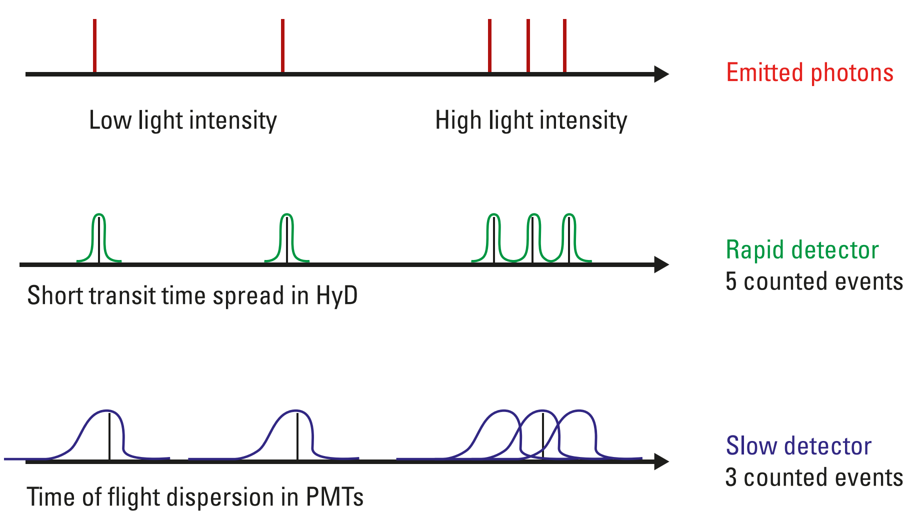




LEICA HYD'S QUANTUM EFFICIENCY IS SUPERIOR TO STANDARD PMTS AND GaAsP PMTS

Quantum efficiency is a measure for a detector's capability to translate photons into electrons. With a typical quantum efficiency of 45% at 500 nm, the hybrid detector is two to three times more sensitive than a standard PMT. This new level of sensitivity improves low-light applications where traditional PMT-based confocals would fail, such as imaging of yeast or C. elegans.

Traditional PMTs using a GaAsP (Gallium-Arsenide-Phosphide) photocathode are susceptible to damage by overexposure. The Leica HyD's design avoids this shortcoming, making the detector both highly sensitive and versatile with a large dynamic range. In terms of quantum efficiency the Leica HyD even supersedes standard GaAsP PMTs by delivering outstanding sensitivity along with durability..



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From Single Photons to Whole Organisms

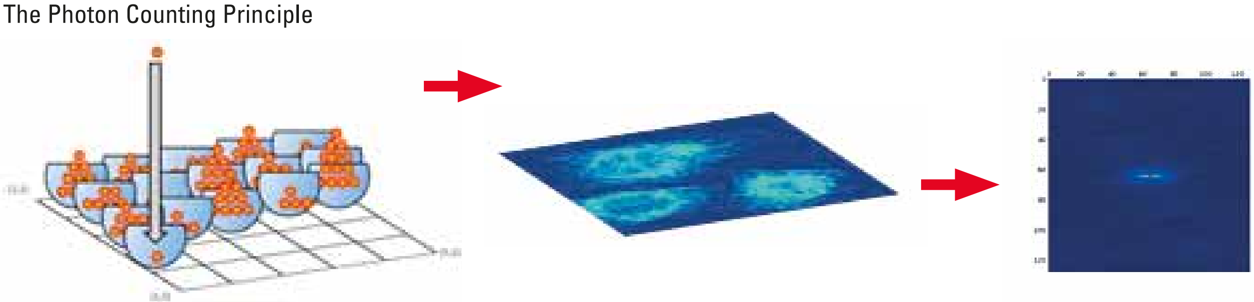
Photon counting enhances the dynamic range of an imaging system and opens the door to a new level of quantitative imaging. Enhanced dynamic range is achieved by preventing the dynamic overflow of single pixels or regions. Quantitative imaging benefits from photon counting as researchers can study photophysical processes and apparent concentrations of labeled molecules. The light emitted by a specific fluorophore is directly proportional to its concentration in the specimen. Photon counting provides for quantitative measurements such as ratio imaging, FRET, and image correlation.

HOW PHOTON COUNTING WORKS

Photon counting thresholds electrical pulses and treats them as binary events (photon or no photon). The read-out of photons is done sequentially. The arrival time of a photon pulse in relation to a scanner's internal clock signal determines which pixel, line and frame the photons are assigned to. In a scanning device such as a confocal microscope this information is available as default. The photons assigned to individual pixels are displayed as a color-coded image, which represents a spatial map of signal intensities.

Due to the nature of photon counting, there exists a direct link between pixel intensity and the number of molecules. Knowing the molecular brightness (photons per molecule per unit time) means that the photon counting image can be used as a concentration map for monitoring biochemical reactions and molecular stoichiometries.

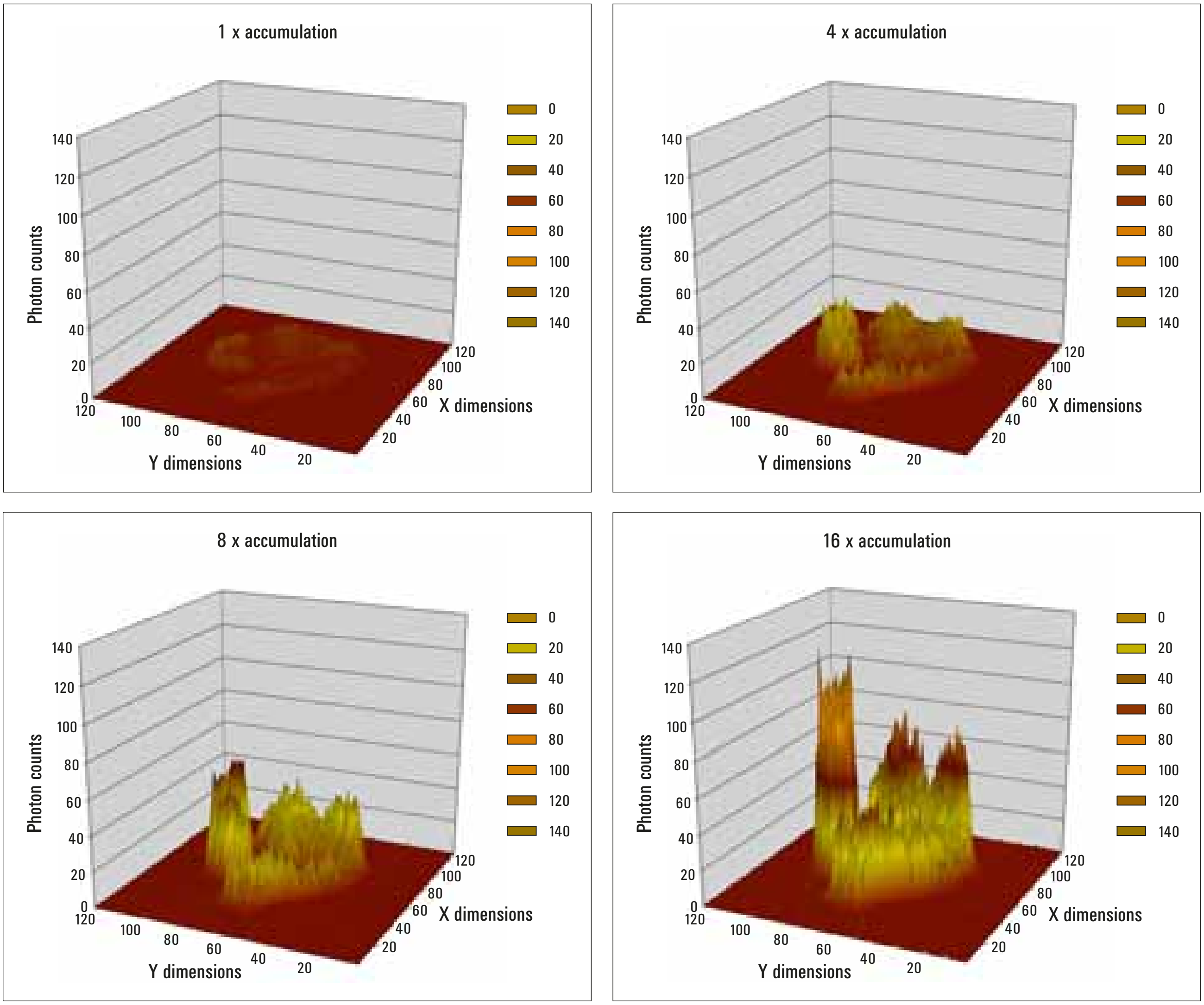
The Photon Counting Principle



Single photon counting during acquisition – filling up pixels with photons

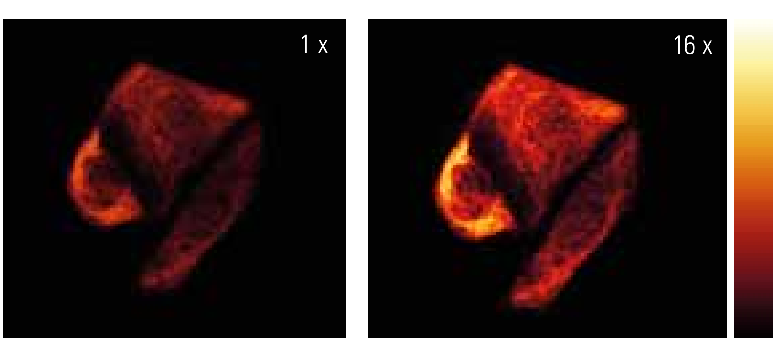
Color-coded image scaled in photon counts

Statistical analysis

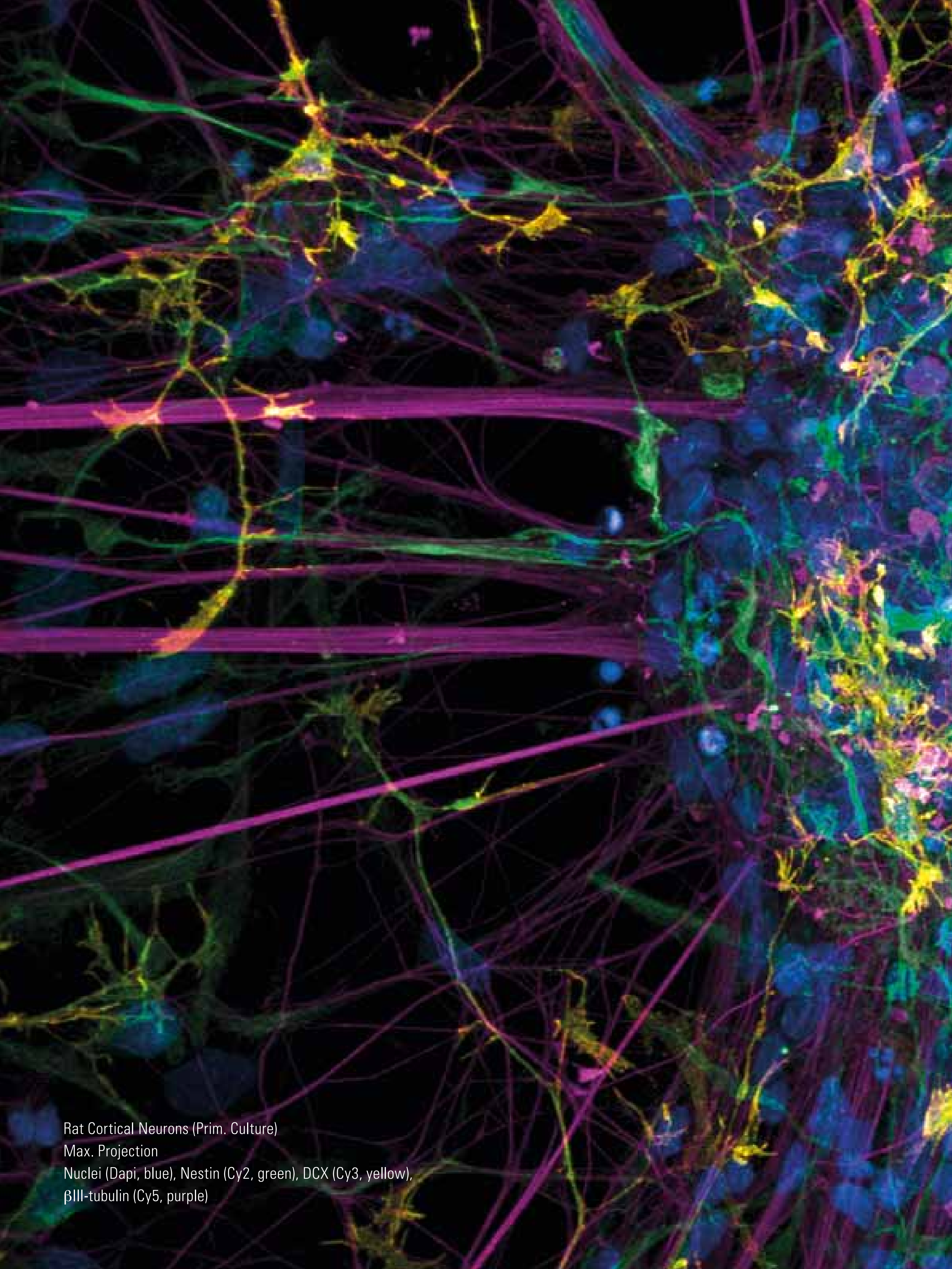


MAXIMUM DYNAMIC RESOLUTION BY PHOTON COUNTING

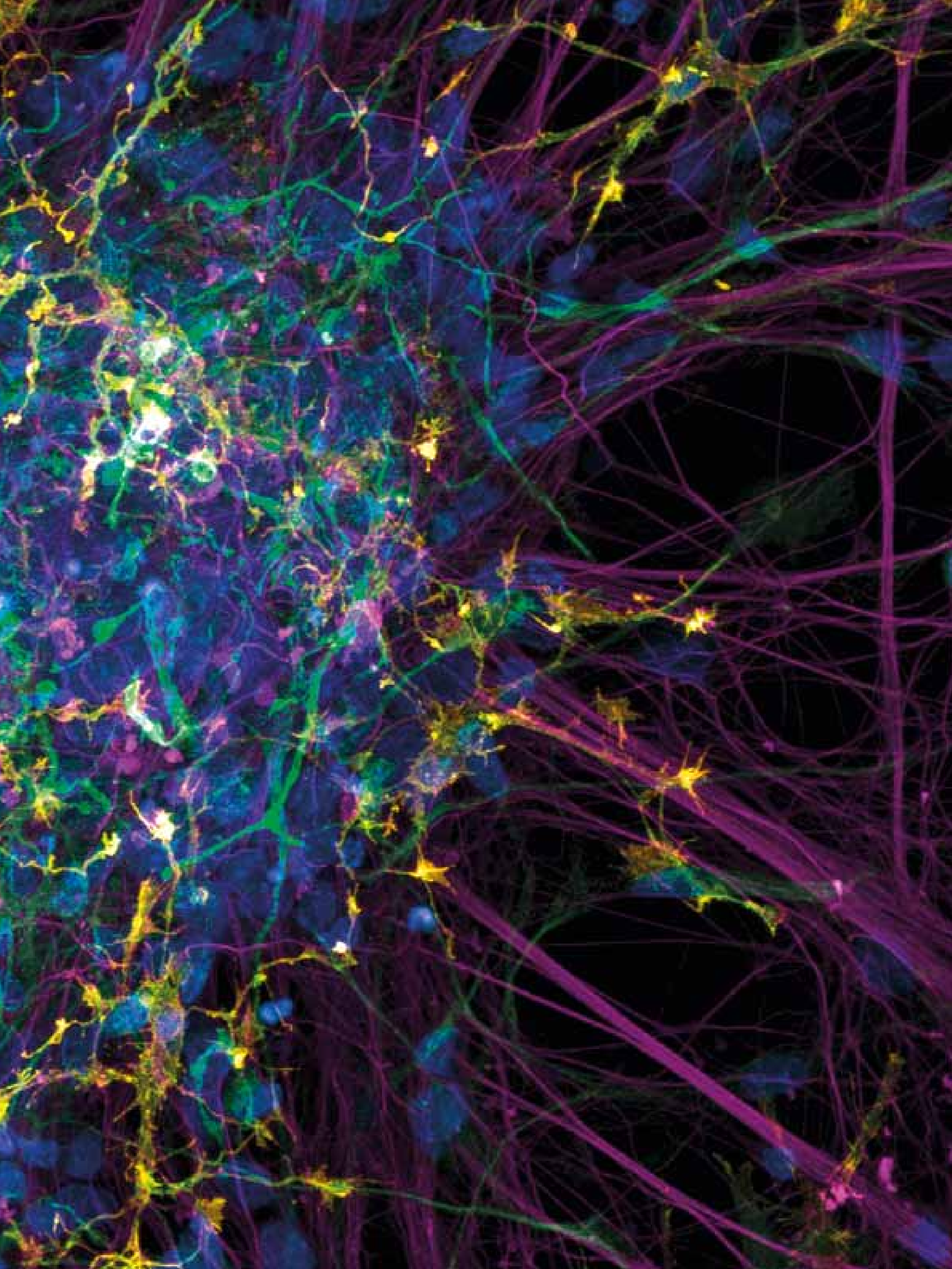
Due to the very low background of our hybrid detectors, photon counting allows as much information to be accumulated as needed for any statistical analysis. In photon counting each pixel behaves like a bucket, which can be filled with photons. The longer one counts, the more photons are collected. The higher bit-depth modes available, 12 bit and 16 bit, represent very large buckets: in 12 bit mode, one can fill 4096, in 16 bit 65356 photons into one pixel. Thus, an enormous dynamic range with very low statistical per-pixel variance is available. The photon numbers are displayed via a look-up-table (LUT) on the screen. In this case the colors have a physical equivalent: photons.



Photon counting allows as much information to be accumulated as needed for statistical analysis.



Rat Cortical Neurons (Prim. Culture)
Max. Projection
Nuclei (Dapi, blue), Nestin (Cy2, green), DCX (Cy3, yellow),
βIII-tubulin (Cy5, purple)



Sensitivity by Design

Photons emitted by the sample need to be preserved so they can contribute to your brilliant imagery. The Leica TCS SP8 provides high photon efficiency and gapless spectral detection using the innovation synergy of hybrid detectors, multiband spectral detector and acousto-optical beam splitter.

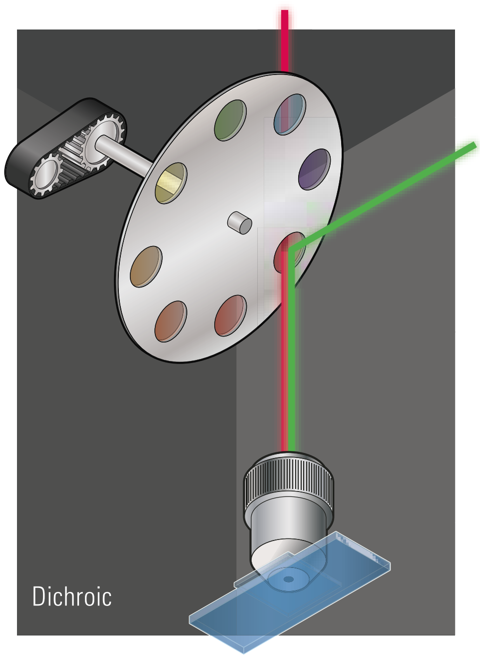
SPECTRAL MULTIBAND SP DETECTOR

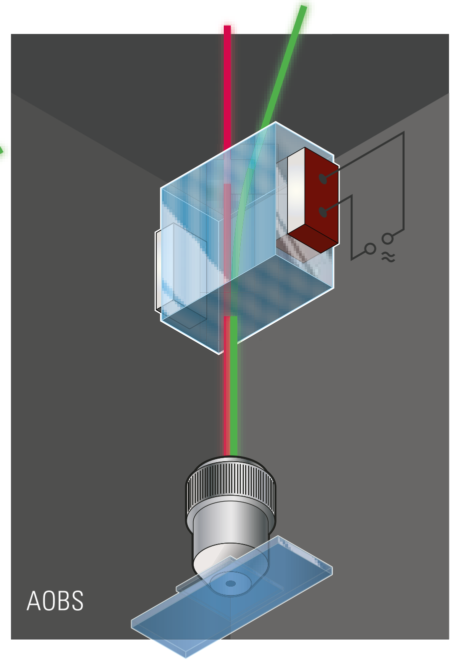
The Leica HyD spectral detectors seamlessly integrate into the Leica SP detector module. This design offers simultaneous detection of variable gapless emission bands. The SP detector resembles a multiband spectrophotometer, based on a prism and mirror sliders. This patented design using a prism represents the most efficient dispersion concept without reducing intensity by a grating-based design, and therefore eliminates the need for a recycling loop.

Unlike array-based spectral detection designs, the Leica SP detector allows a customized balance between the highest sensitivity and highest dynamic range. Discrete detectors permit individual gain settings for each detector, rather than being forced to use the same gain for all array elements.

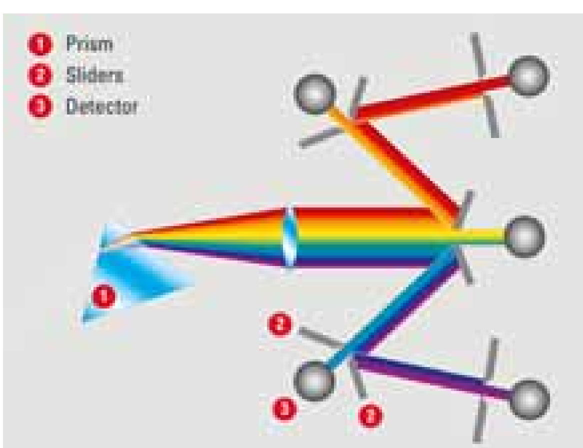
YOUR CHOICE: DICHROIC BEAM SPLITTER OR AOBS

Leica Microsystems offers two beam splitter technologies: the acousto-optical beam splitter (AOBS) and low incident angle dichroics (LIAchroics). The AOBS is an active optical crystal that is completely transparent and offers the highest photon efficiency of any beam splitting device. Unlike filter wheels, it switches within microseconds by simply changing the radiofrequency of the acoustic wave coupled into the crystal. LIAchroics are Leica Microsystems' high-efficiency beam splitters for customized performance to produce improved image contrast when compared to standard dichroics.



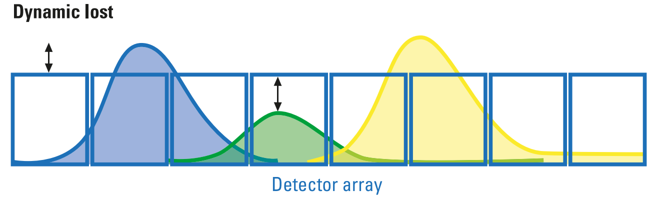


Acousto-Optical Beam Splitter.
This device replaces all conceivable dichroic and multichroic mirrors and wheel- or slider-based arrangements or combinations of these. The AOBS is a single programmable optical element for visible range laser scanning microscopy.

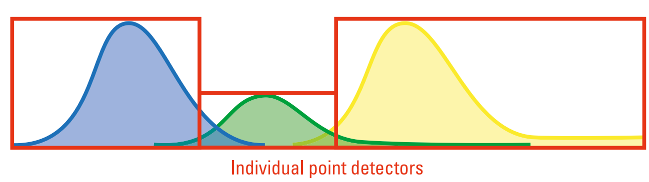


Prism Dispersion and Spectral Detection. Emitted light passes through a prism that breaks the light up into its spectral constituents. Specific wavelengths can be separated from the spectrum for detection. A narrow band of wavelengths can be selected by the insertion of a mechanical slit (sliding mirrors). The rest of the spectrum is directed by the highly reflective mirrors to the subsequent detectors. A cascade of mechanical slits built from highly reflective sliding mirrors permits recording of up to five channels simultaneously without losses.

Dynamic lost



Adaptive dynamic range



Adaptive dynamic range of the SP detector. The SP detection design using individual point detectors avoids two inherent drawbacks of multianode arrays: Loss of dynamics and spectral gaps. Individual point detectors adapt dynamically to the changing needs of a diverse range of samples.

Dynamic Range is Key

Sensitive detection systems, such as avalanche photon diodes or classical GaAsP photomultipliers or arrays have, by their nature, a low dynamic range and cannot convert high light intensities into signals. This characteristic limits them to very dedicated applications. In contrast, the high dynamic range of the spectral Leica HyD makes your system highly flexible for every application.

HAVE THE CONFOCAL SYSTEM ADAPT TO YOUR SAMPLE

For optimal imaging of specimens with varying brightness, the hybrid detectors offer a large dynamic range that enables imaging bright and dim structures simultaneously.

The combination of up to five channels comprised of PMTs and HyDs, each with an adjustable gain, maximizes the overall dynamic range of the confocal system. This effectively eliminates the need to record time-consuming exposure series and hence reduces the photon dosage delivered to the sample. Moreover, the workflow remains straightforward, because no additional software tools are needed to create images with a large dynamic range.

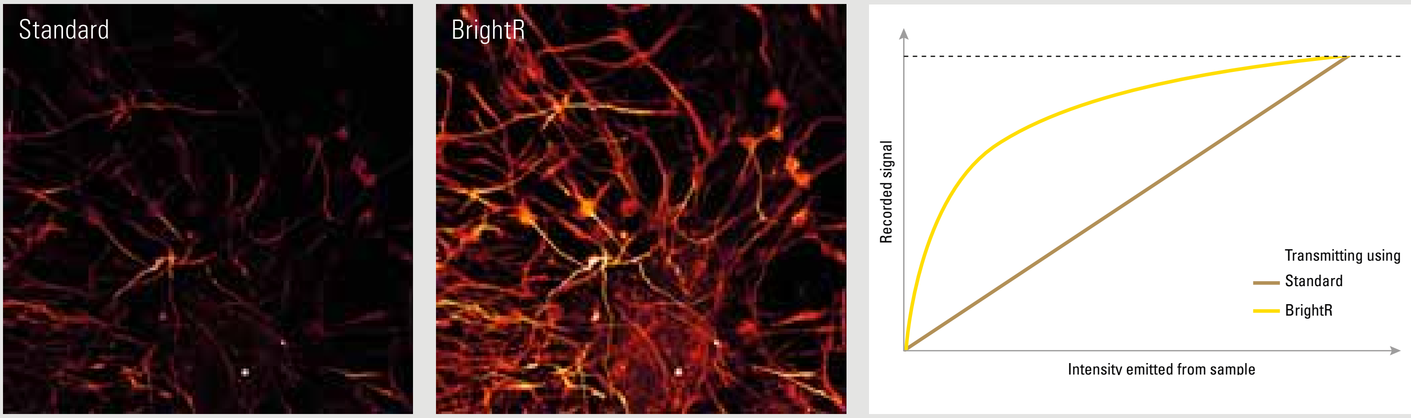
FOUR WAYS TO MAXIMIZE DYNAMIC RANGE

› Use 12 bit or 16 bit dynamic resolution
› Use BrightR mode for large dynamic range
› Use photon counting
› Combine PMTs with Leica HyD in one system

FROM PHOTON COUNTING TO IMAGING WITH DETECTOR

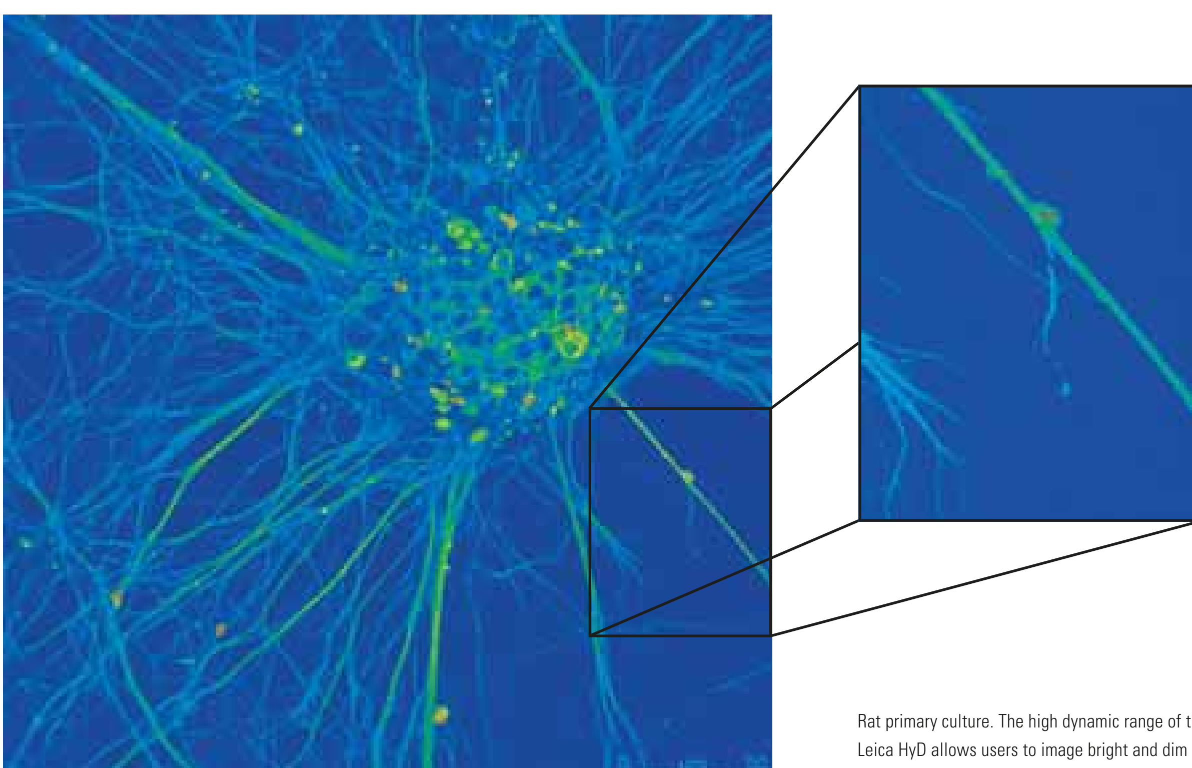
Sampling rate in a photon counting system is strongly linked to the signal-to-noise ratio. A conventional photon counting system with low sampling rate, e.g. 15 MHz, can detect only a small number of photons, which is associated with a relatively high noise level. At higher count rates, the signal saturates and is no longer quantitative. Integrating the signal often gives good results at higher count rates above 40 MHz. However, typical dyes in biological specimens emit at rates between 15 and 40 MHz.

With its fast sampling, the Leica HyD can detect higher photon rates with low noise, resulting in better images than PMTs or APDs. In photon counting mode, the HyD is linear up to 60 MHz. A calibration is used in standard mode to ensure linearity up to 300 MHz. The Leica HyD covers the full frequency range in one detector from photon counting to imaging. The complete information is contained in one image. This means high flexibility for your confocal experiments and less artifacts from data processing.



BRIGHTNESS REINFORCEMENT FOR HIGHLY DYNAMIC SAMPLES

In some biological specimens, fluorescent labels are not distributed homogenously. Certain structures are very bright while others only show very low label concentrations. This results in a highly dynamic distribution of light intensity. Such samples are intrinsically difficult to record, because either the bright parts of the image get overexposed or the dim parts are underexposed. The innovative BrightR addresses this imaging task. It amplifies dim structures more than bright ones. An image with an extended dynamic range results, capturing both very bright structures and intricate detail in the same image and making exposure series and post processing obsolete.



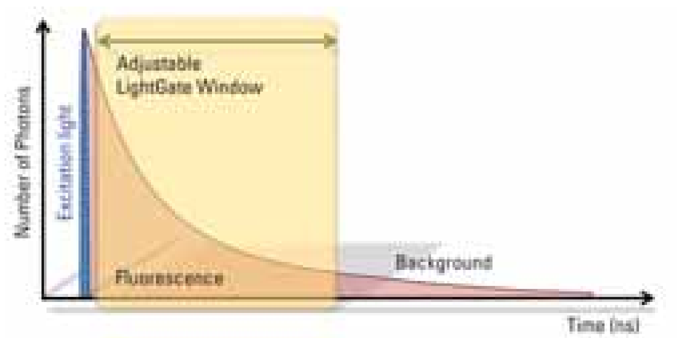
Rat primary culture. The high dynamic range of the Leica HyD allows users to image bright and dim structures simultanesoulsy.

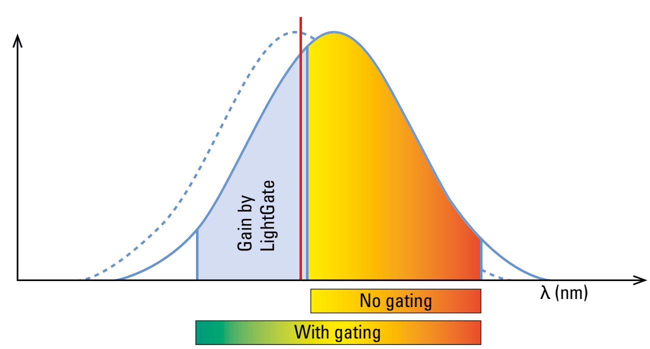
The LightGate – Filter Free Removal of Unwanted Signal

In conventional microscopy, reflected and backscattered light is removed by spectral blocking filters. Leica HyD detectors together with the White Light Laser as a pulsed excitation source allow the restriction of detection to a certain time gate after the detection pulse. This removes unwanted signal in an ingeniously simple way.

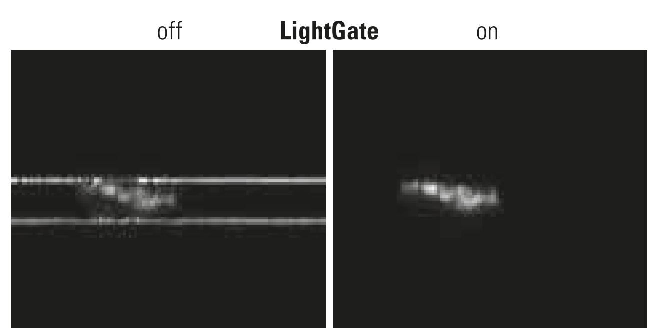
LIGHTGATE FOR MAXIMUM IMAGE CONTRAST

LightGate utilizes the time decay of the fluorescence signal. A light pulse from the White Light Laser (WLL) excites the fluorophores and triggers the start point for time measurement. Only signals arriving at the hybrid detector in a flexible and adjustable time window are collected. Unwanted signal from autofluorescence, backscattered light at the begin of the fluorescent decay, or detector noise at the end of decay can be removed. This way, the highest image contrast can be obtained – even from weakly stained samples.





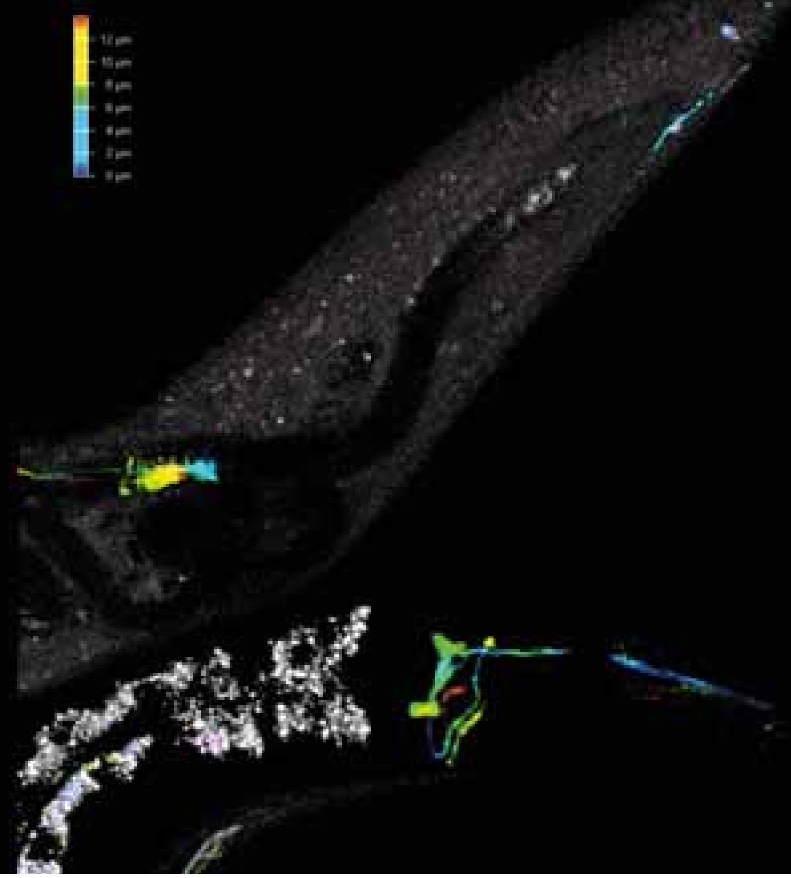
LightGate allows direct detection underneath the laser line to collect more data.



LightGate completely removes cover glass reflection. HeLa cells, nucleus stained with Chromeo 505. Excitation: 510 nm (within detection range). Detection: 495-540 nm

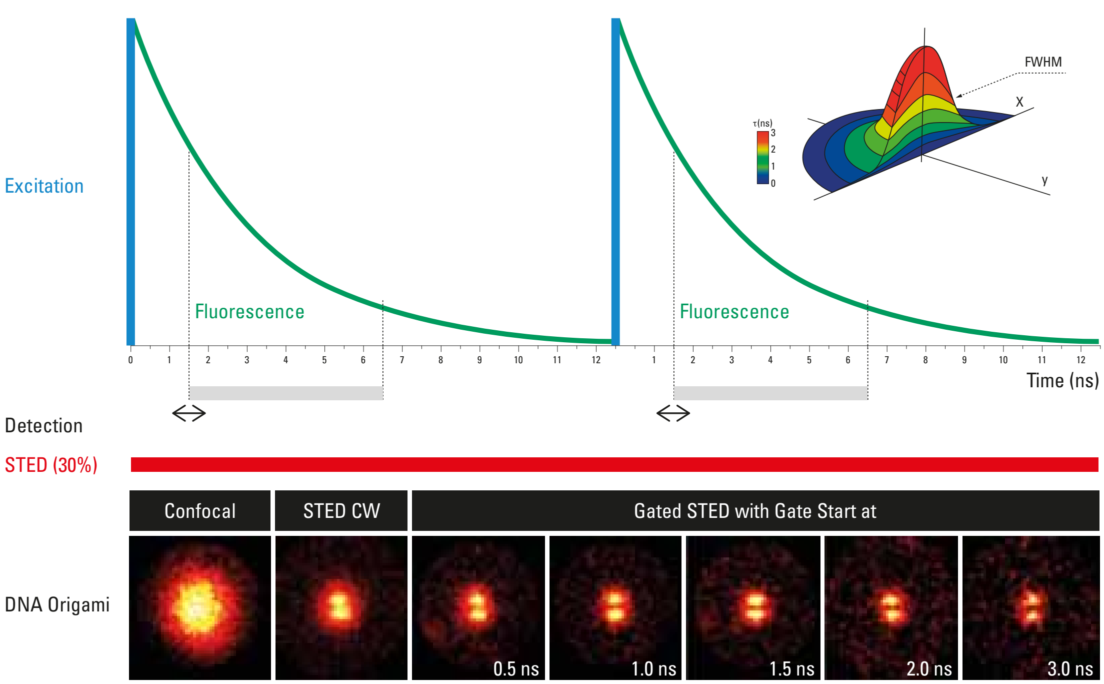
Separation of dyes or autofluorescence with spectral overlap, but different life times.
C. elegans, expression of myr-GFP at cell membranes of ciliated sensory neurons
Excitation: 486 nm
Detection: 496-621 nm

Overlay of two images:
Grey: Autofluorescence, LightGate off
Color-coded: GFP, LightGate on (1.5-7.6 ns)
Courtesy of A. North and A. Singhal, Rockefeller University, NY, USA



THE RATIONALE FOR USING LIGHTGATE

- Removal of instantly reflected laser light
- Detection underneath the laser line allows the collection of more signal
- Separation of dyes or autofluorescence with spectral overlap but different life times
- Gated STED achieves even higher resolution



Resolution increase by gated STED. Only long-lived (red/yellow) states contribute to the image. Short-lived states (blue/green) are filtered out by the LightGate.

Superior Sensitivity for Deep Tissue Imaging

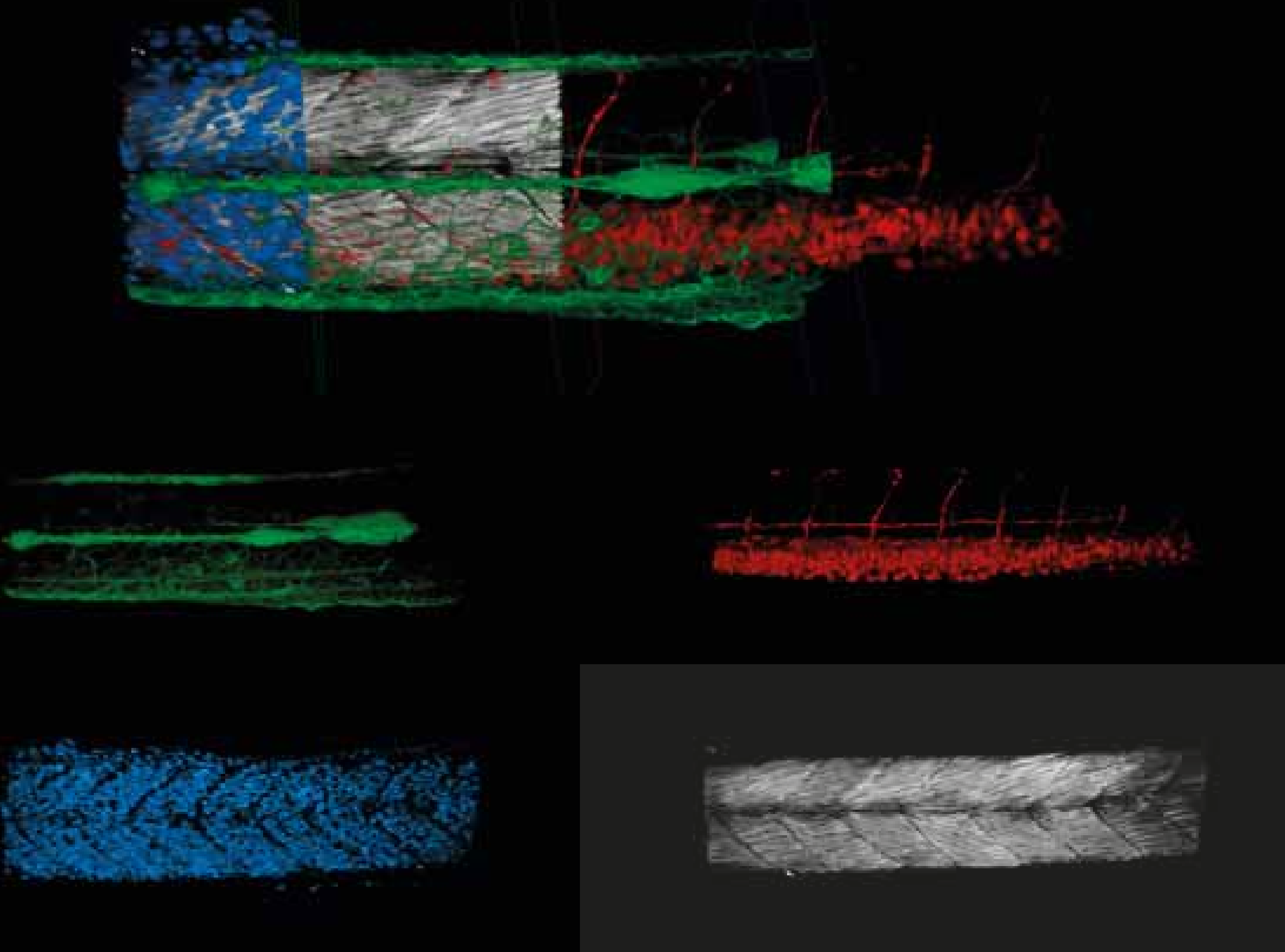
Multiphoton microscopy has special requirements for signal detection, as the emitted light is coming from deep tissue sections and backscattered from surrounding structures. Super-sensitive Leica HyD non-descanned detectors record the faintest structures from deep tissue sections. The new QUAD Module allows up to four super-sensitive HyDs in RLD (reflected light detection) position to collect all your precious photons from multiple stained samples.

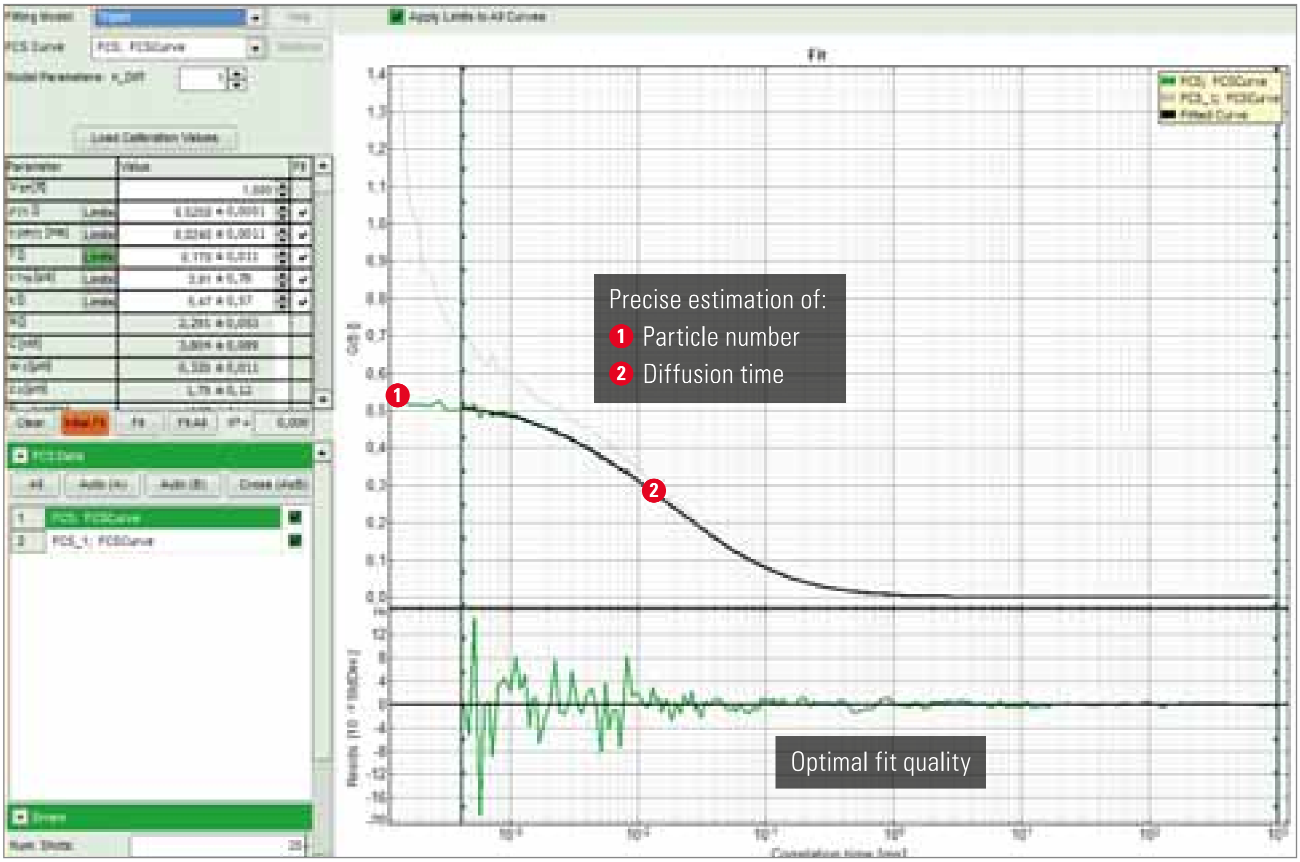
LOWER EXCITATION – BRIGHTER IMAGES

Multiphoton excitation can only take place in the focal plane, which makes the pinhole in the detection light path dispensable. To improve the efficiency of light collection, the detectors are placed as close as possible to the source of emission (non-descanned detection, NDD). A hybrid detector in RLD position gives you superior sensitivity for brighter images. Lower excitation power is needed which ensures less specimen damage, while a better signal-to-noise ratio shows more details from deeper tissue sections.

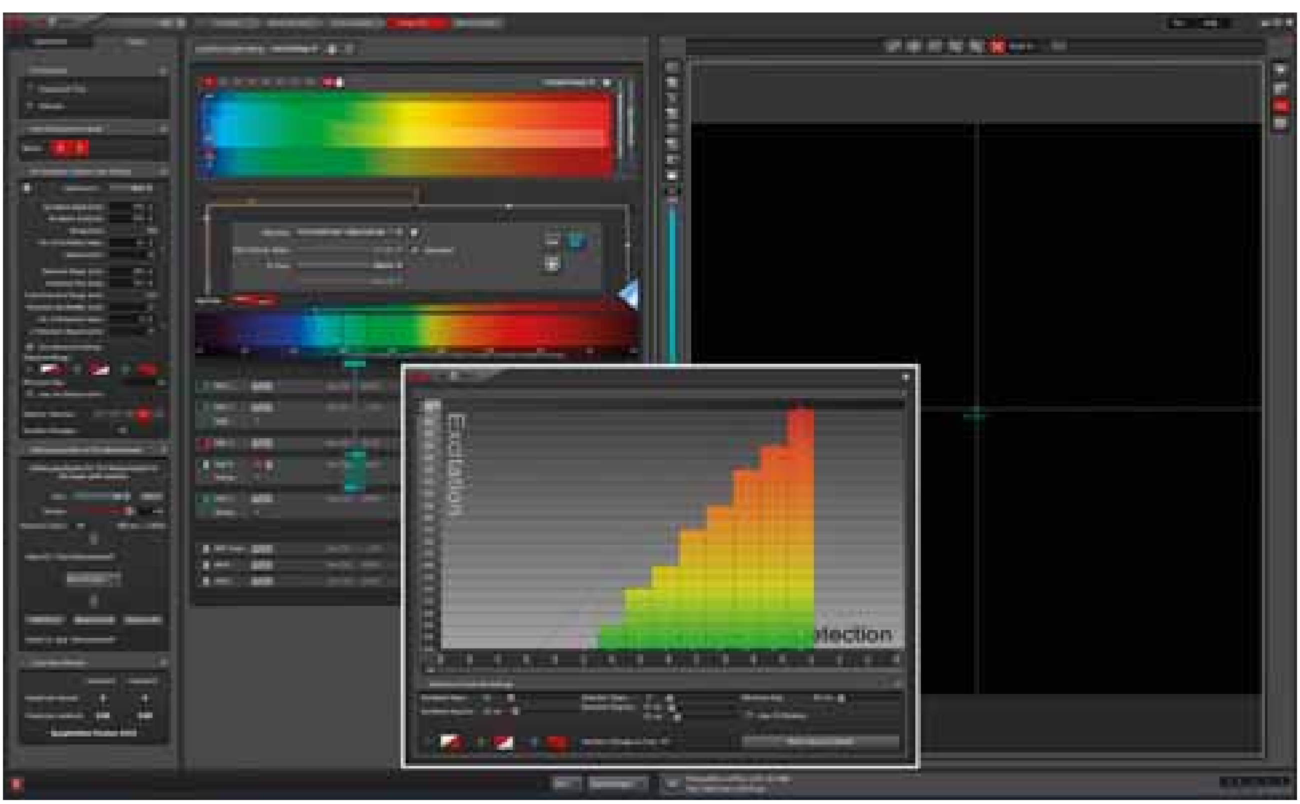
The new QUAD module couples up to four hybrid detectors in RLD position, which gives you the highest flexibility for multicolor deep tissue experiments. Due to its modular set-up you can choose as much super-sensitivity as you need for fast, easy non-descanned multicolor acquisition.

Fast and easy NDD image acquisistion. Zebrafish embryo: lateral Line (GFP, green), neurons (DsRed, red), muscles (SHG, grey), nuclei (BFP, blue). Courtesy of Lionel Newton, EMBL Heidelberg (Gilmour lab).





Autocorrelation of free dye moving in water, detected with HyD SMD (green) and an APD (gray). APDs show an afterpulsing at short time ranges overlaying information about dye triplet states. Sample: Alexa 488, Ex. 488 nm, Det. 500-550 nm



Identification of optimal FCS excitation and detection settings using λ-FCS stacks

The Optimal Detector for Single Molecule Detection

Quantitative characterization of biological phenomena is increasingly important in life science research. Analytical sensitivity is a prerequisite for reliable detection of single molecules. Its low dark noise and high quantum efficiency combined with the directly coupled, filter-free SP detector make the Leica HyD SMD the universal detector for all SMD applications and advanced imaging tasks alike.

THE UNIVERSAL SMD DETECTOR

Single molecule detection (SMD) is here used as an umbrella term for quantitative measurement methods like FLIM (fluorescence lifetime imaging), FCS (fluorescence correlation spectroscopy) or combined methods like FLCS (fluorescence lifetime correlation spectroscopy). For these methods, a sensitive and fast detection system is crucial.

Leica Microsystems has developed a special hybrid detector with superior characteristics for all SMD methods which is integrated in the SP detection systems. An active cooling system comprised of built-in Peltier cooling and additional external cooling reduces the specified dark noise of the Leica HyD SMD, resulting in highest SMD data quality.

RELIABLE MEASUREMENT OF CONCENTRATION

As the Leica HyD SMD has virtually no detector afterpulsing, precise diffusion data for small molecules can be acquired. There is no need for afterpulsing removal by cross-correlation. The positioning of the detectors in the confocal detection system allows FCS data acquisition with up to 4 detectors simultaneously. The spectral detection module gives you the greatest convenience in handling spectral FCS experiments: Less photobleaching due to optimization of the detection range results in less artifacts in your FCS curve. The crosstalk in cross-correlation experiments can be minimized. With the Leica HyD SMD you can rely on your FCS data.

TRUE FLIM DATA

Due to the descanned position of the SMD detector, all the various excitation sources like UV lasers, visible light laser or the white light laser offered by Leica Microsystems are also available for FLIM measurements. The system adapts to the emission of the used dyes. The direct coupling also leads to high photon count numbers.

With the superior signal-to-noise ratio of the HyD SMD, trusted FLIM data are obtained even from weak signals. The short instrument response time and the absence of dynode pulses give access to dyes with short lifetimes, which makes the interpretation of your FLIM-FRET data more reliable.

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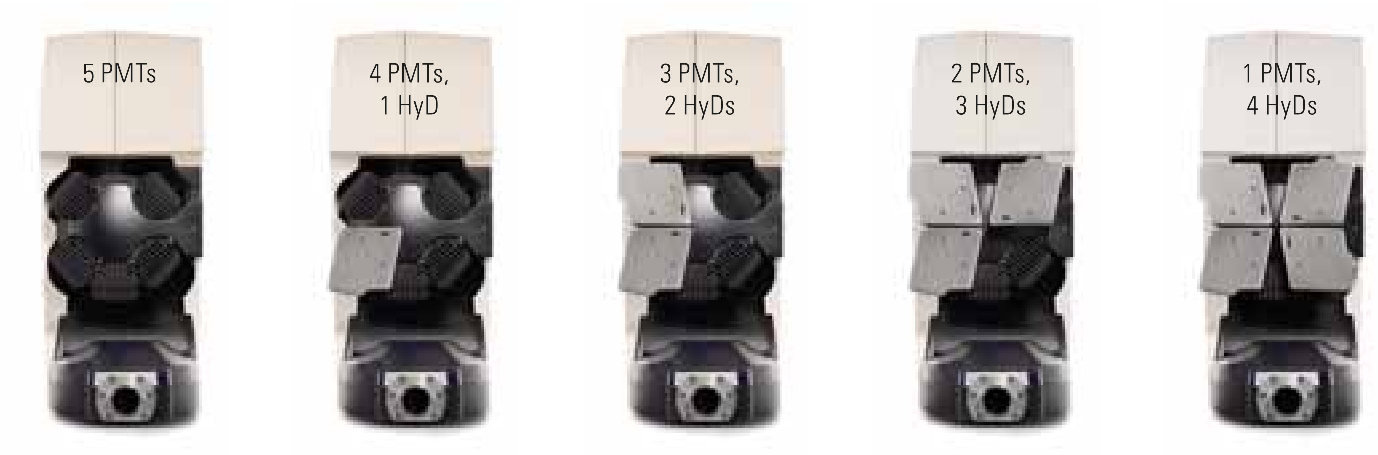

CHOOSE AS MUCH SUPER-SENSITIVITY AS YOU NEED

The Leica HyD was designed with upgradeability in mind. You can equip your confocal with up to four Leica HyDs right away for parallel detection of up to four multispectral super-sensitive channels, or you can start with one HyD and increase this number later as the lab's requirements grow. It is even possible to retrofit existing Leica TCS SP8 systems. Invest in tomorrow's technology today.

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