Leica MICROSYSTEMS

The Power HyD family of detectors for confocal microscopy

We introduce the Power HyD family of detectors from Leica Microsystems, designed for high performance in terms of spectral coverage, sensitivity and dynamic range in confocal laser scanning microscopy. These advances offer unprecedented flexibility in multi-color imaging throughout the visible and near-infrared spectrum and result in excellent image quality in conditions ranging from dim samples to fast dynamic acquisitions. The underlying high-speed photon counting concept provides the basis for a unique set of fluorescence-lifetime-based imaging applications.

The ability to observe cellular structures and probe biomolecular functions with fluorescence microscopy is one of the most powerful experimental assets in the life sciences. The study of molecular function typically requires innovative approaches to probing the interactions among partners in the cellular context[1] with unprecedented detail[2] and in closer-to-physiological conditions[3,4]. The development of increasingly sophisticated labeling strategies[5,6] and the continuing emergence of new genetically encoded fluorescent probes[7,8] enable the study of almost all known cellular processes even at low expression levels. The plethora of fluorophores available today provides a wealth of opportunities for researchers but also poses a technological challenge. Indeed, it would be desirable that any microscope could cope well with all current and future probes, delivering high-resolution spatial and dynamic information. Laser scanning confocal microscopy is a widely accepted imaging technique when it comes to matching these requirements, and the choice of suitable detectors is crucial for realizing the full potential of this approach[9]. Modern detectors must ensure maximum sensitivity across a broad spectral range and be capable of detecting low photon numbers.

We have developed a new family of detectors to get the most out of confocal technology. The Power HyD detector family comprises three types of detectors—Power HyD S, Power HyD X and Power HyD R—featuring a combination of strengths that fulfill these demands. The implementation of the Power HyD family enables sensitive detection of fluorescence from 410 nm to 850 nm, covering the entire visible spectrum and extending the detection capabilities into the near infrared (NIR). These detectors, combined with a light path optimized for the extended spectral range and laser excitation lines from 355 nm to 790 nm, seek to maximize the flexibility of a confocal system for multicolor imaging applications. As an example, we selected five different fluorophores with emission maxima from the blue to the NIR (Fig. 1, top). We used them to target five key components of a mammalian cell: the plasma membrane, DNA, mitochondria, microtubules and actin (Fig. 1, bottom) and performed confocal imaging with a combination of all members of the Power HyD family. The resulting images show the five labeled structures with excellent quality, with bright signal and low background in all channels.

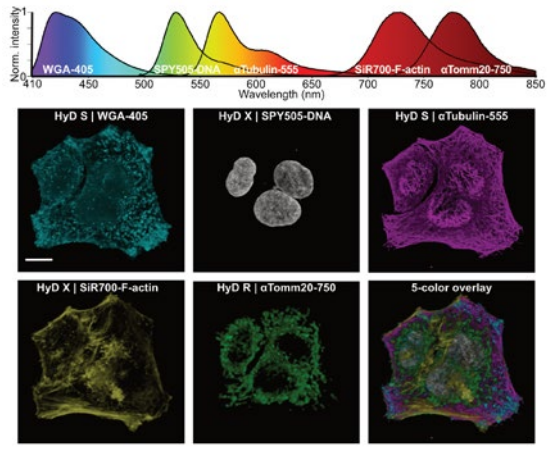

Figure 1 | The Power HyD detector family enables flexible multi-color imaging across the visible to near infrared range. Mammalian cells stained for five key cellular components. Emission spectra of the fluorophores used, and five-color image of cellular structures with detector types, fluorophores and targets indicated. Scale bar, 10 µm

The Power HyD family is built on two main architectures. The Power HyD X and Power HyD R are based on hybrid detector technology[10] (Fig. 2a) whereby a photocathode front plate (gallium arsenide phosphide for HyD X and extended-red gallium arsenide phosphide for HyD R) is coupled to an avalanche diode. This architecture not

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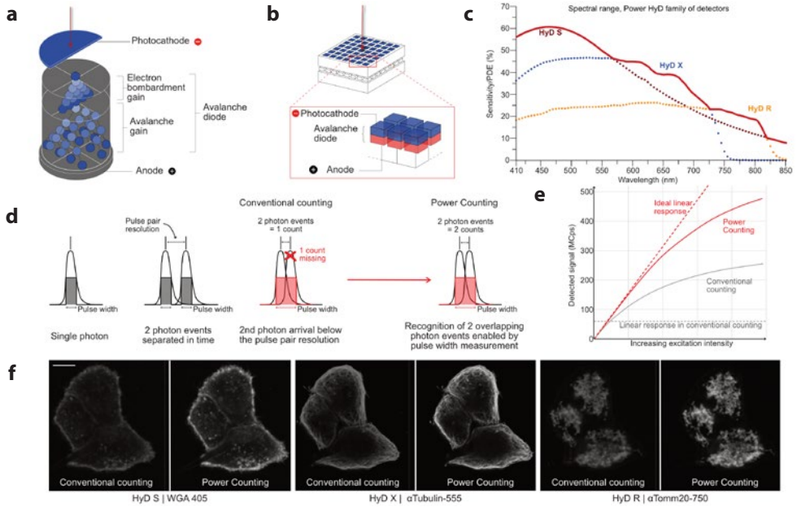

Figure 2 | Technical overview of the Power HyD family. a,b, Schematic of Power HyD X or R hybrid detectors and Power HyD S, highlighting its multi-pixel photon counter architecture. **c**, The Power HyD family delivers high photon detection efficiency across the spectrum. Solid red: PDE envelope of the Power HyD family; dotted red, blue, orange: PDE curves of HyD S, X and R, respectively. **d**, All Power HyD detectors provide added dynamic range and quantifiability through Power Counting technology. Conventional photon counting misses a second photon event occurring within the pulse pair resolution time; Power Counting correctly accounts for the second photon by using the pulse width information. **e**, Power Counting signal (red solid curve) as a function of excitation intensity shows enhanced dynamic range compared to conventional counting (gray curve). MCps, million counts per second. **f**, Images of cellular structures recorded without or with Power Counting. Power Counting delivers brighter, crisper images and more detail. Scale bar, 10 µm.

only enables single-photon sensitivity but also ensures optimal electronic amplification of the detected photon signals. The readout for these detectors is fully digital and photon counting based[10]. The Power HyD S is based on silicon multi-pixel photon counter (MPPC) technology[11] (Fig. 2b). Here, the fluorescence photons are spread onto a detection area comprising an array of independent detector subunits, each consisting of a silicon avalanche photodiode, which are all wired in parallel and read out over a common anode. Harnessing recent advances in MPPC technology and a unique engineering concept, this detector achieves an image quality comparable to that of Power HyD X and R (Fig. 1). An efficient three-stage cooling scheme suppresses dark noise, while an optimized optomechanical design ensures maximal photon collection in the MPPC architecture. In addition, Power HyD S efficiently operates in photon-counting or in analog mode, thereby providing the highest dynamic range in the Power HyD family. The combination of these strengths positions Power HyD S as the all-around detector for general confocal applications across a broad spectral range.

We can characterize the detection sensitivity of the Power HyD family first by looking at the photon detection efficiency (PDE). The PDE describes the probability of converting an incident photon into a detected signal and thus is the preferred metric for accounting for sensitivity independent of detector architecture. (The relation between PDE and quantum efficiency (QE) is PDE = QE × FF × PA. QE describes the probability of converting incident photons into electrons in the photocathode. The fill factor FF denotes the ratio of active detector area to total detector area and PA the probability of triggering an avalanche. Since FF ≤ 1 and PA ≤ 1, the corresponding QE will always yield a larger value than its associated PDE.) The PDE response across the visible–NIR spectrum (Fig. 2c) agrees with the observed performance of the Power HyD family in multicolor applications (Fig. 1). Power HyD S delivers excellent sensitivity in the blue–green spectral range, key for some of the most widespread live-cell fluorescence protein tags, while providing a broad overall detection range extending out to 850 nm. Power HyD X is particularly well suited for the red–orange region, and Power HyD R is especially tailored for the NIR region.

The purpose of an imaging experiment often goes beyond obtaining insightful details and requires quantifying the fluorescence signal[1,12]. To deliver a robust and accurate quantification, maximum fidelity between the signal in the image and the photons reaching the photocathode is key. In this respect, how faithfully the emitted photons are recorded depends not only on the sensitivity of the photocathode, but also on the temporal response of the detector itself and the attached readout circuitry. All photon-counting detectors experience a dead time following the detection of the first photon, during which the detector is unable to detect a second photon. To understand the importance of this effect, we can examine what would happen with a detector having a very high PDE—for example, 70%—and a long dead time—for example, 15 ns. When one photon hits the photocathode, the detector would record it as detected signal 70% of the time, as indicated by the nominal PDE. However, the detector would be ready to detect the next photon only after the dead time, which means it would remain blind for 15 ns, during which all emitted photons are lost. Therefore, the detector would have a limited effective PDE, despite a high nominal PDE. By contrast, HyD X, HyD R and HyD S have a dead time below 1.5 ns (detector and system electronics).

Nevertheless, even with such short dead times in the Power HyD family, it is still possible to miss some photon counts, since there is a small probability that more than one photon reaches the detector simultaneously. To further minimize the effects of dead time on the dynamic range and fidelity of photon detection, we explored how to take advantage of the time-resolved capabilities of our detection architecture[13]. As a result, we introduced Power Counting, an improved signal processing concept for identifying and counting photons that otherwise would be missed due to apparent temporal overlap (Fig. 2d). This novel photon counting approach significantly improves the precision of the recorded signals and extends the linear dynamic range, as shown with the comparison of the response curves for Power Counting versus conventional photon counting (Fig. 2e). Here, the improvement of signal stems from the microscope's ability to account for as many of the detected photons as possible, and not from an intrinsic improvement of detector sensitivity. The increased brightness and dynamic range achieved with Power Counting result in a higher level of detail than in images acquired with conventional counting, which are dimmer and have a restricted dynamic range (Fig. 2f).

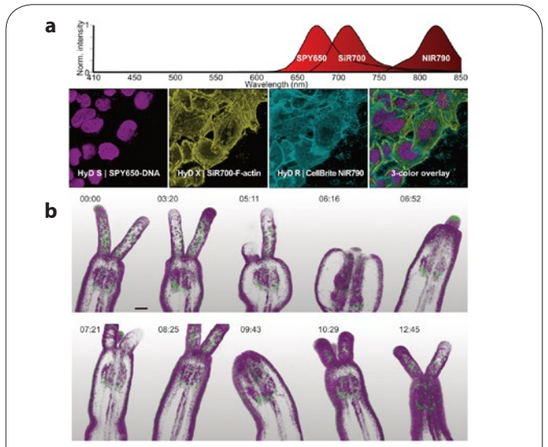

Figure 3 | Application examples. a, Emission spectra of SPY650-DNA, SiR700 actin and an NIR790 membrane stain (top). Corresponding live imaging of HeLa cells (bottom). Scale bar, 10 µm. **b**, Fast 3D in vivo imaging of *Nematostella vectensis* (Cnidaria) showing endogenous signal for cilia, nematosomes and clusters of freely circulating cnidocytes (green) and dextran red fluorescence (magenta). Endogenous signals and fluorescence separated by TauGating[14] on HyD S, 340 volumes acquired in 12 min 45 s. Scale bar, 50 µm. Sample courtesy of Anniek Stokkerman and Aissam Ikmi, EMBL Heidelberg.

The combination of the technological advances presented here translates into performance enhancements for a range of applications (Fig. 3). As a first example, we focused on classical three-channel confocal imaging, typically done with fluorophores emitting in blue (DAPI), green (GFP or FITC), and red (mCherry or Cy3.5). To reveal the new possibilities of the Power HyD family, we show live cell imaging with three fluorophores in the far red and NIR spectral range (Fig. 3a). The images of a HeLa cell labeled with SPY650-DNA, SiR700-F-actin and a NIR 790 membrane stain show the potential for enhanced multiplexing with more fluorophores. As a second example, we used the Power HyD family together with pulsed laser excitation sources to take advantage of the new TauSense lifetime-based tools for confocal imaging[13]. We imaged *Nematostella vectensis*, aiming to capture the fast dynamics of its morphological changes in 3D under gentle imaging conditions over 12.45 min. The sample was labeled with a red fluorophore–dextran conjugate. Using a single Power HyD S detector and TauGating[13], we generated images in two channels, one capturing the endogenous nematosomes and another capturing the dextran signal (Fig. 3b). A 3D-rendered movie can be accessed at http://www.leica-microsystems.com/Fast-3D-Nematostella.

In summary, technological advances in confocal laser scanning microscopy provide a match to the growing imaging needs in life science research. Leica Microsystems' concept for confocal microscopy is based on the deep integration of a single spectrally configurable pulsed excitation source (supercontinuum white-light laser technology) with a matching detection unit comprising multiple spectrally configurable detectors. In this context, the Power HyD family of photon counting detectors provides enhanced sensitivity over an extended spectral range, facilitates quantification, and provides the basis for an expanding set of fluorescence-lifetime-based imaging tools (for example, TauSense[13], FALCON[14] and τ-STED). Indeed, all fluorophores are welcome.

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