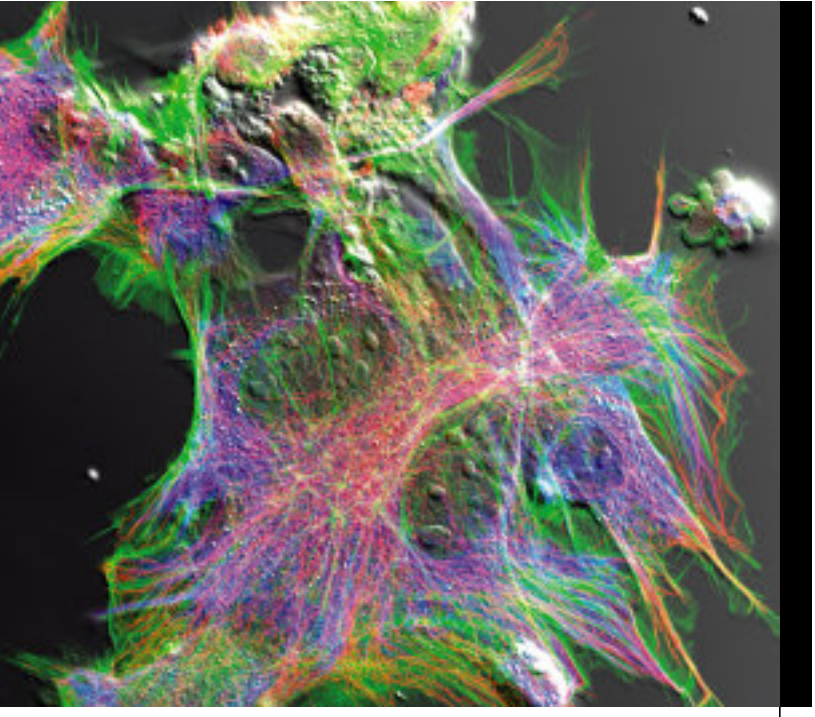


Spectral Detector Leica TCS SPE

Unique, Flexible and Powerful

Leica

MICROSYSTEMS

Evolving needs in reserach

Today, sensitive confocal systems allow detection of fluorescence signals even from a single fluorescent protein. At the same time, numerous antibody markers and DNA probes have been discovered and these can be labeled with multicolor fluorescent dyes.

The Leica TCS SPE detector concept

Many of the dyes and fluorescent proteins require specific fluorescence filter designs for optimal detection. As a consequence, almost each dye requires a specific combination of beam splitters and emission filters. And new dyes and fluorescence proteins are being developed every month. So what about new applications tomorrow?

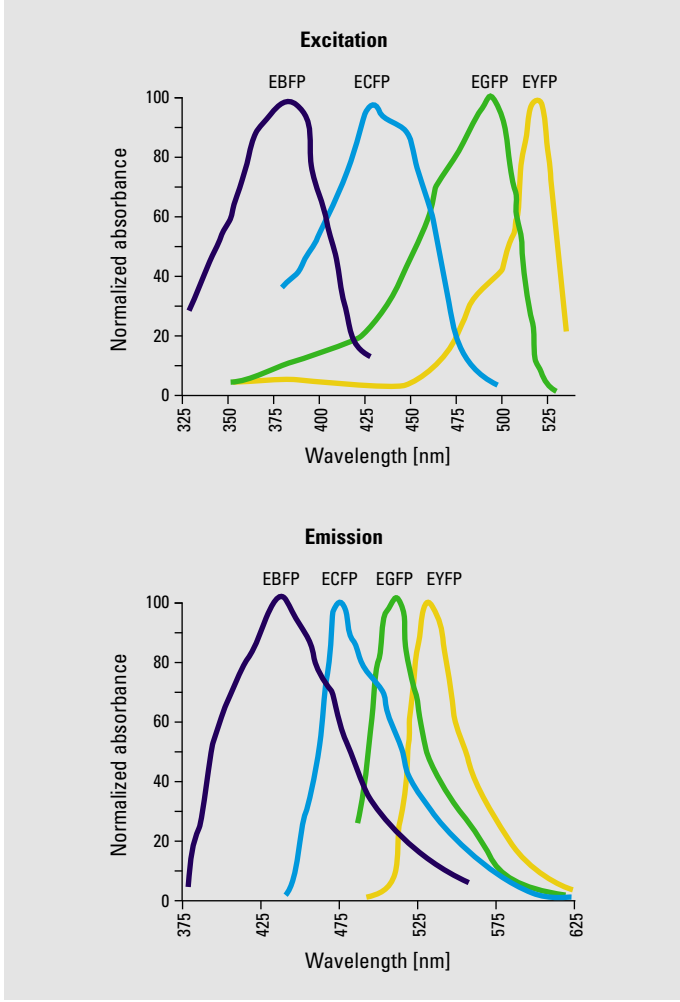
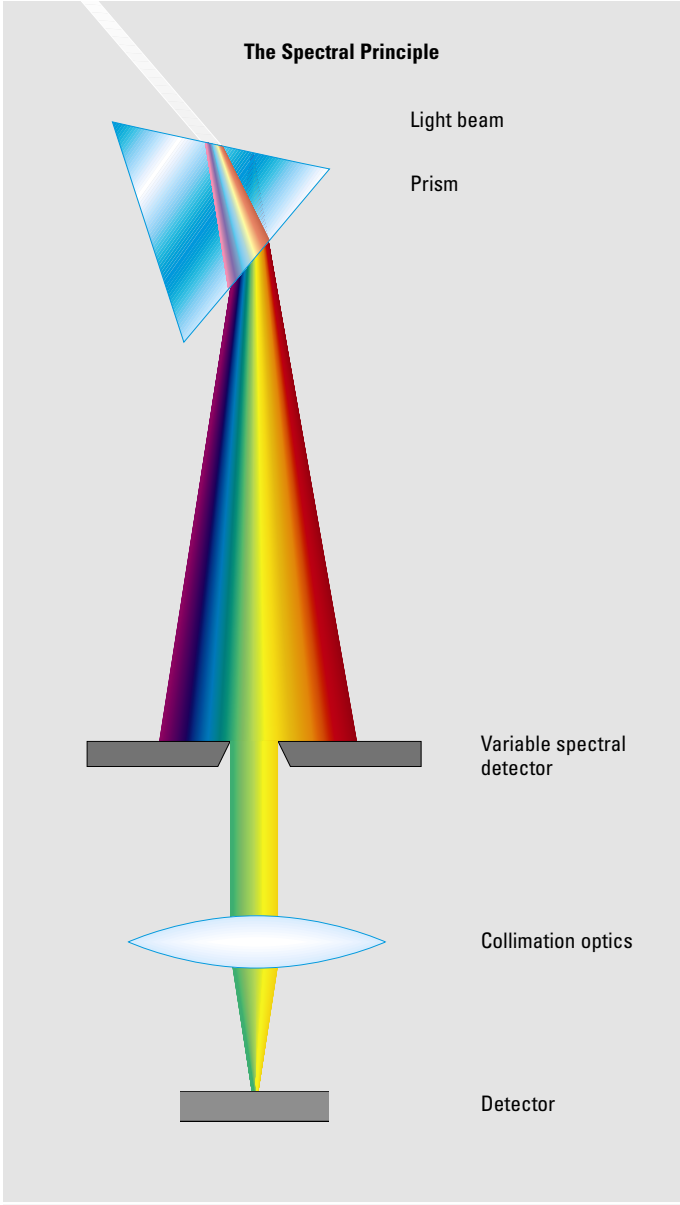


Fig. 1: Excitation and emission spectra for enhanced fluorescent proteins.
Data provided by G. Patterson & D. Piston, Vanderbilt University, Nashville TN, USA



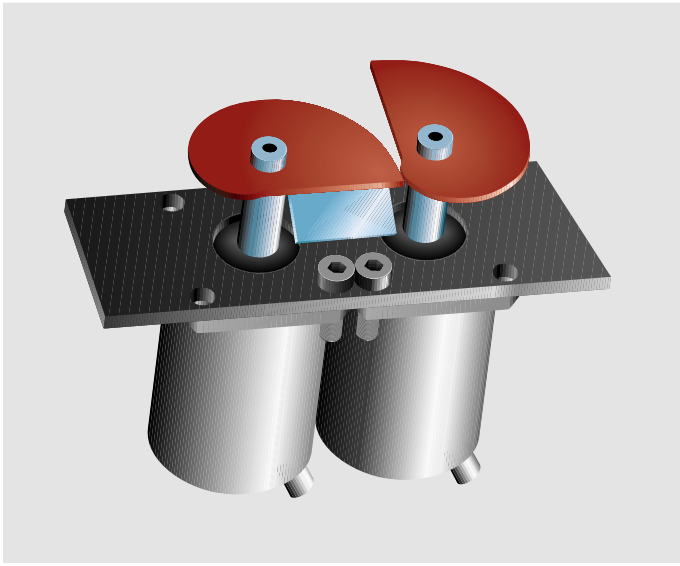


Fig. 2 (above): Principle of the Leica SP detector
Fig. 3 (below): High-speed variable wavelength selector (Leica patent)

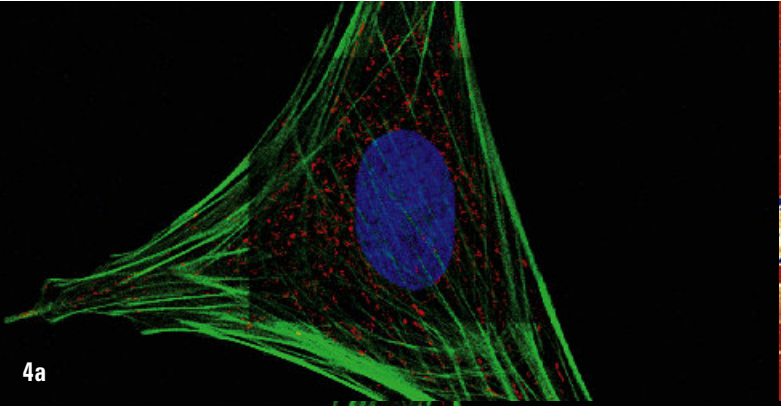
Design your own filter

Wouldn´t it be ideal to never have the need to change any filter again, to "design" the filter online in a confocal, whenever a new dye is available?

This is the idea leading to the development of the spectral detector in the Leica TCS SPE Confocal System!

Figure 3 shows a diagram of the concept of the Leica Spectral Detector: Emission light coming from the specimen is passing the confocal pinhole and is dispersed into its spectral colors by a prism. The desired spectral range is selected from the spectrum by two adjustable motorized blades – an ingeniously simple and effective concept!

This principle allows to "design" adjustable filters of any spectral characteristics (width and center wavelength) within the visible emission spectrum. It makes the system ready for new dyes and markers today and in the future, with an almost infinite "number of filters" built-in. Less photodamage, better sample protection.



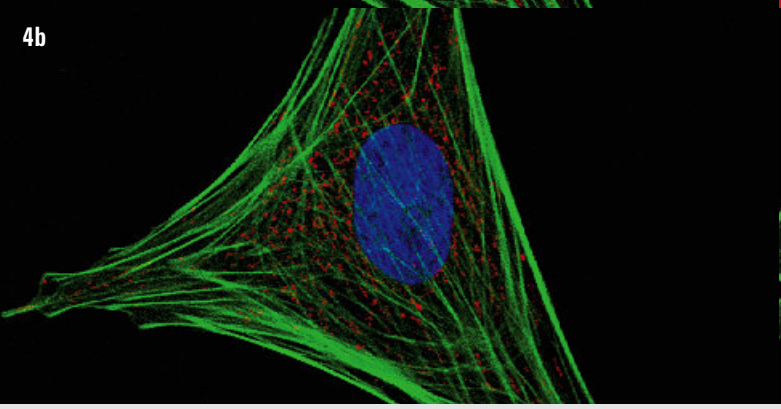
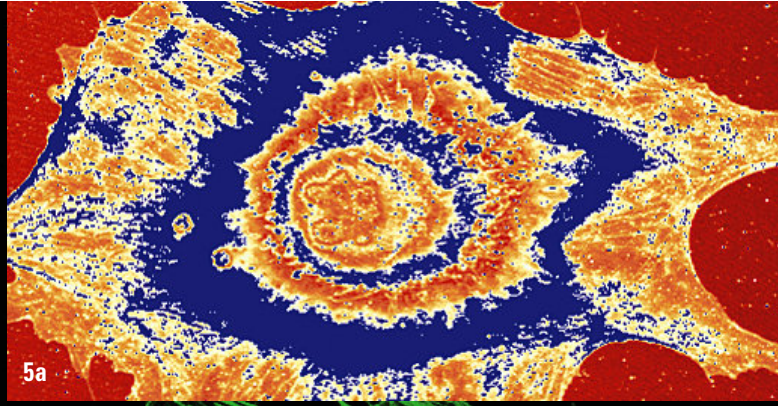


Fig. 4: Sample protection by high-sensitivity detection.
4a) High laser power and averaging is necessary in conventional confocal microscopes to generate noise-free images. Low illumination intensity and few averages will not damage this specimen when imaged with the SP detector. 4b) Low illumination intensity and few averages will not damage this specimen when imaged with the SP detector.



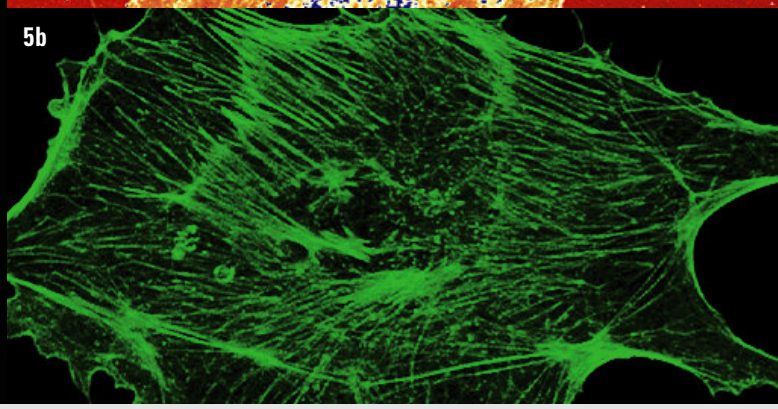
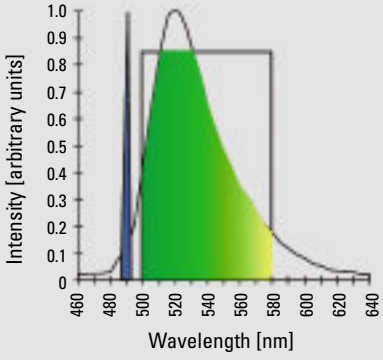


Fig. 5a) Spectral detector set to the excitation line.
Reflected light image with overflow.
5b) Spectral detector set only 5 nm off the excitation line.
A clean fluorescence image is obtained, due to the high selectivity of the SP detector outperforming conventional filters.

Less photodamage, better sample protection

Using this concept has additional advantages: direct, lossless coupling of a sensitive, performance-selected photomultiplier detector with high quantum efficiency is possible. As a result, the dose of excitation light can be reduced, sensitive specimens are better protected and exhibit less photodamage.

Sample protection is further increased due to the fact, that the selectivity of the Leica SP detector is outperforming conventional filters. Selectivity means, that the fluorescence emission window can start already very close to the excitation line – as a result, wider detection windows can be used, and more fluorescence is recorded from the specimen.



With the SP detector, the Fluorescence detection window can be moved closer to the excitation line, thus maximizing efficiency of signal collection.

Fig. 6: Spectral Detection

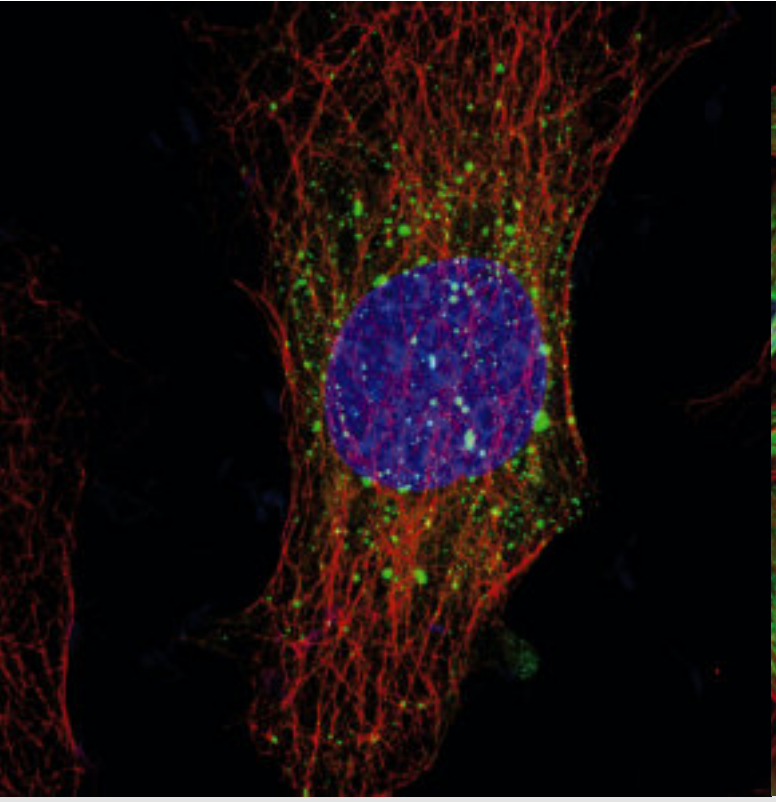


Fig. 7: COS 7 cells
Green: uncharacterized protein, GFP; Red: a-Tubulin, Cy3; Blue: Nuclei, DAPI
Courtesy of Prof. Wei Bian, Cell Research Center, Institute of Biochemistry and Cell Biology, SIBS, CAS, Shanghai, China

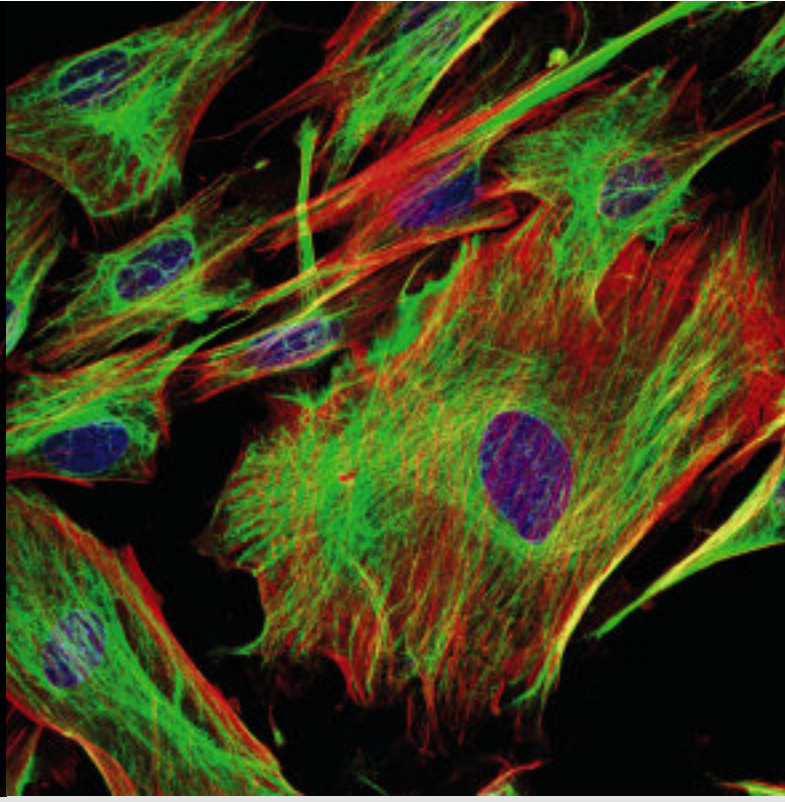


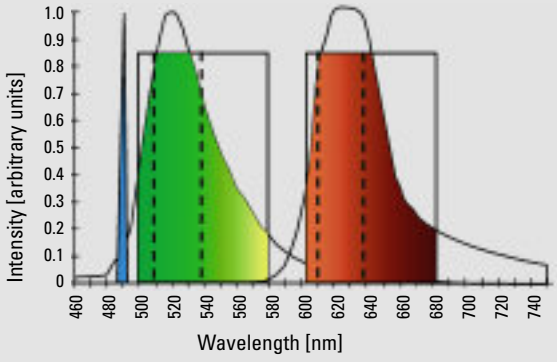
Fig. 8: Mouse fibroblasts
Green: F-Actin, FITC; Red: Tubulin, Cy5; Blue: Nuclei, DAPI
Courtesy of Dr. Günter Giese, Max Planck Institute for Medical Research, Heidelberg, Germany

Less crosstalk, better dye separation

This high selectivity also allows to efficiently eliminate crosstalk in multi-labelled specimens by excluding the areas of overlap in the sequential image acquisition process.

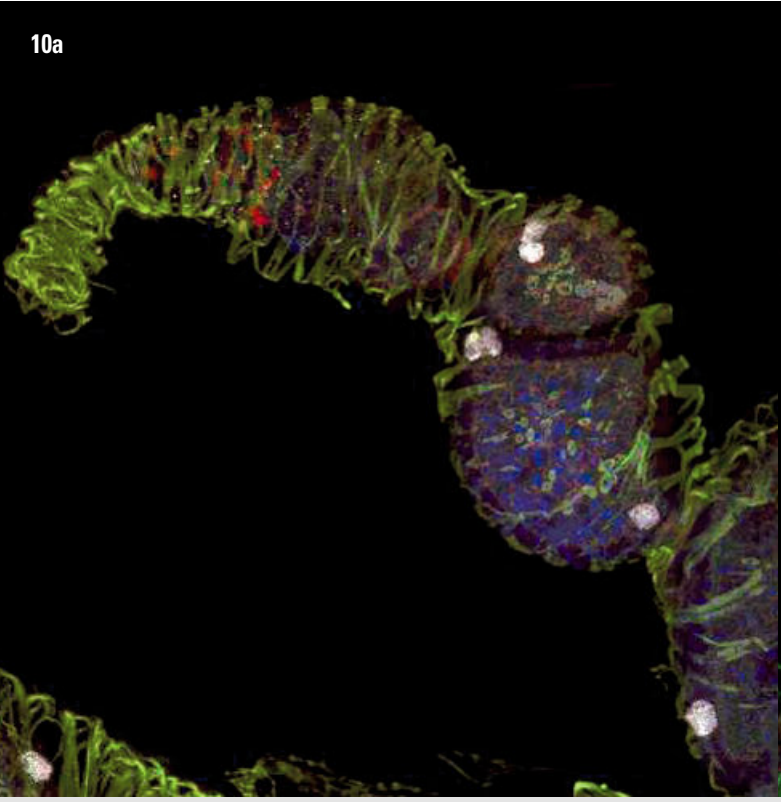
As a result, highly specific, well separated images of multi-labelled specimens are obtained with the Leica SP detector – up to 8 labels in a specimen in one fully automated sequence!

Highly specific, crosstalk-free images are obtained with the Leica SP detector.



In multi-labelled specimens, spectral areas of "crosstalk" can be simply excluded from the acquisition process, resulting in clear separation of the dyes.

Fig. 9: Sequential Scan



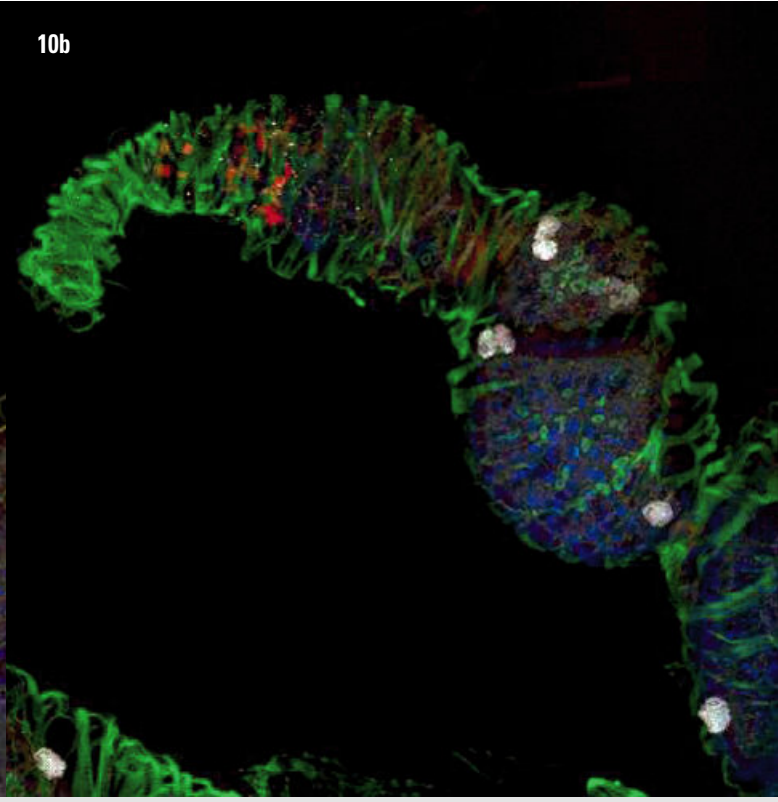


Fig. 10: Drosophila, Alexa 488, Alexa 543. Alexa 594. Toto 3
Fig. 10a) Raw image overlay with residual crosstalk due to overlapping

Fig. 10b) Residual crosstalk is eliminated with dye finder emissions
Courtesy: Dr. Ralf Pflanz, Max Planck Institute for Biophysiological Chemistry, Göttingen, Germany

Discriminating overlapping stainings

Stainings with completely overlapping spectra cannot be effectively separated by proper detector settings. This also applies to background autofluorescence, with specific markers superimposed.

Here, spectral data (either from single detection channels or from recorded emission spectra) serve as a base for eliminating crosstalk. By means of the "Dye Finder" software, even this residual crosstalk is eliminated and clear color separation of four stainings is obtained (see Fig. 10)

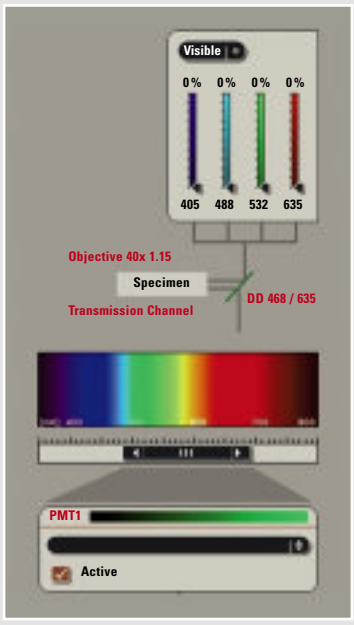


Fig. 11

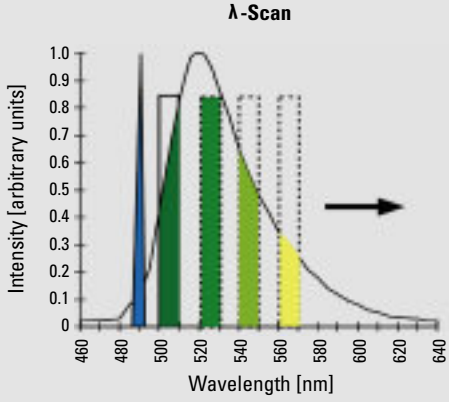
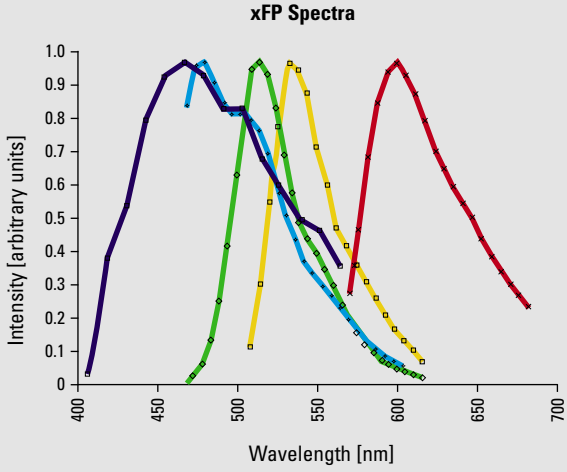


Fig. 12



Emission spectra of various fluorescence proteins in real sample environment.

Fig. 13

Ergonomic Control

Ergonomic software control is as important as superior hardware. With a slider the user can design intuitively the required filter properties (wavelength band and center wavelength) of the detector online and one can directly see the effect during scanning of the sample. This allows very quick adaptation and optimization to any sample condition, important for best protection of sensitive specimens. The optimized settings can be saved and re-called for future use. In the Leica TCS SPE, up to 8 different settings can be defined in a sequential "multiplexing" mode which are then applied in a single automated run to image up to 8 labels in a specimen.

Characterization of Emission Spectra

As a further important feature, the Leica SP detector allows recording of emission spectra. Emission spectral curves are important to characterize the dyes or fluorescent proteins in their actual environment, which often is different compared to theoretical curves. An example is given in Fig. 13, showing the emission spectra of various fluorescent proteins.

Major advantages over other spectral solutions

Compared to other spectral solutions on the market, the Leica SP design has major advantages: by far highest efficiency is achieved due to a highly transparent prism as dispersion element and close coupling of selected photomultiplier detector – a crucial point for best sample protection in fluorescence microscopy. In addition, sharp spectral selectivity for excellent dye separation and an excellent spectral resolution of 5 nm are outstanding performance characteristics of the Leica SP detector.

Other spectral solutions use gratings as dispersion elements which typically have an efficiency of only 40-50 % across the visible wavelength range vs 96 % in the Leica SP detector prism – resulting in doubled sensitivity!

Summary:

- The Leica SP detector is ready for new dyes and physiological conditions, allows to "Design your own filter"
- It provides better image quality than filter-based systems, especially for weak emissions, resulting in best sample protection
- Online interaction and intuitive control helps for quick optimization of imaging conditions
- The sequential image acquisition strategy optimises separation of fluorescent probes
- Characterisation of fluorescence emission spectra is standard
- Spectral unmixing separates even widely overlapping dyes