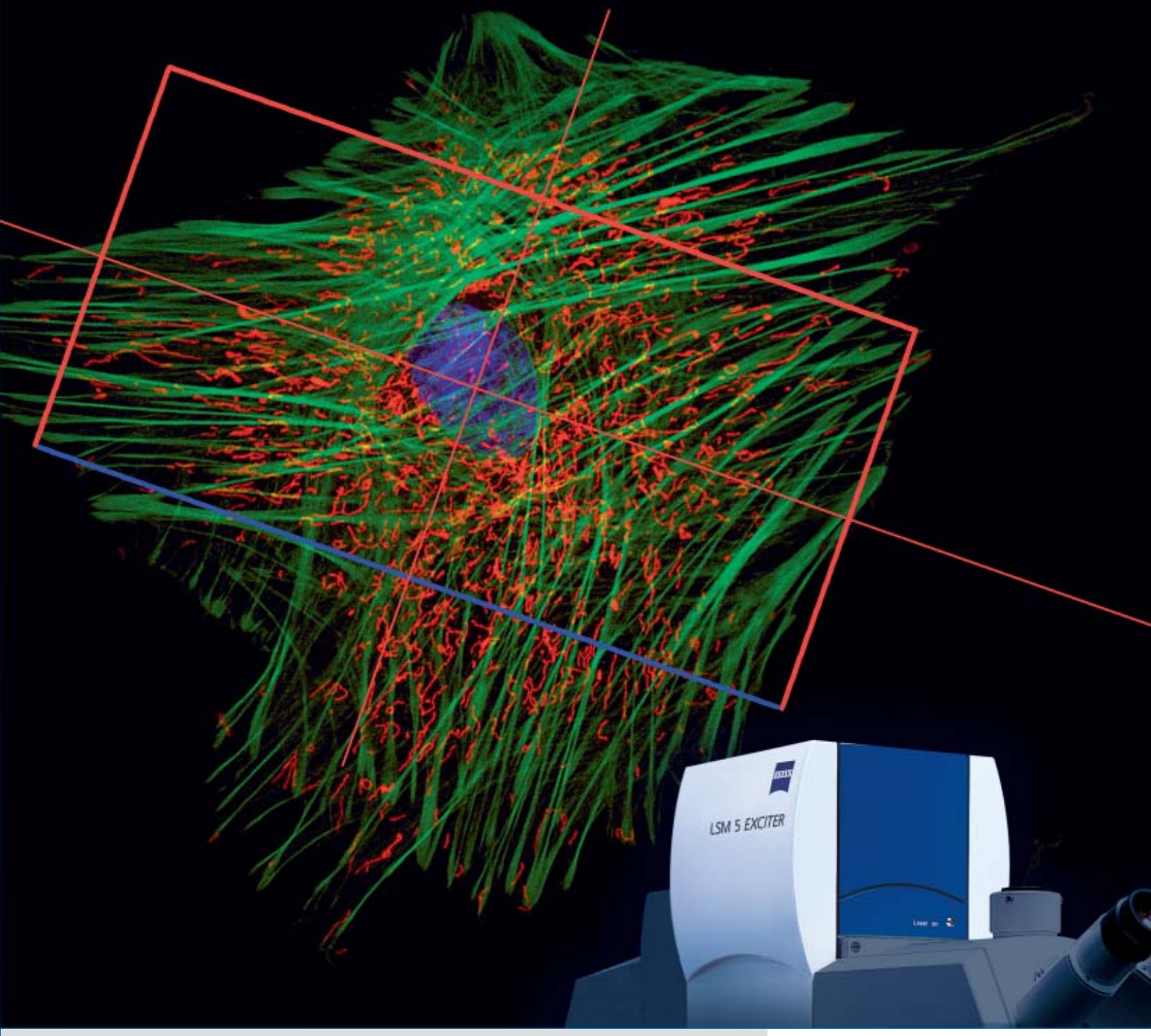
LSM 5 *EXCITER*

Laser Scanning Microscope

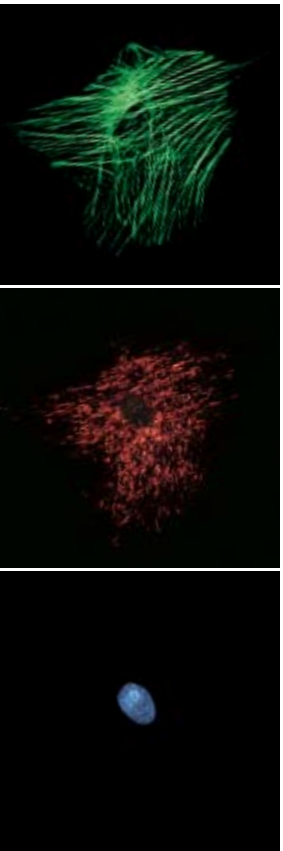


Tracking of Cellular Processes

ZEISS

We make it visible.

The LSM 5 *EXCITER* from Carl Zeiss is a confocal laser scanning microscope for fundamental research in medicine and biology. Equipped with optimized, user-friendly hardware and software, this system delivers excellent confocal images and image stacks, especially in fluorescence applications. It is optimally designed for the acquisition of time series for the analysis of molecule mobilities by bleaching or photoconversion.



Cultured rat ovary cells.
ß-Actin labelled with Alexa 488 - Phalloidin (green),
Mitochondria with Mitotracker - red (red),
Nucleus with DAPI (blue).

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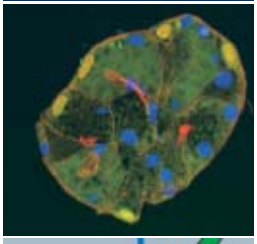
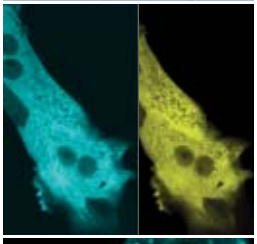
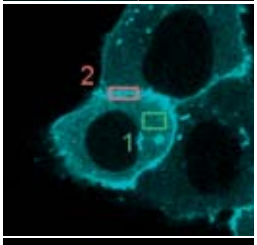
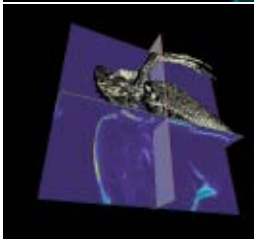
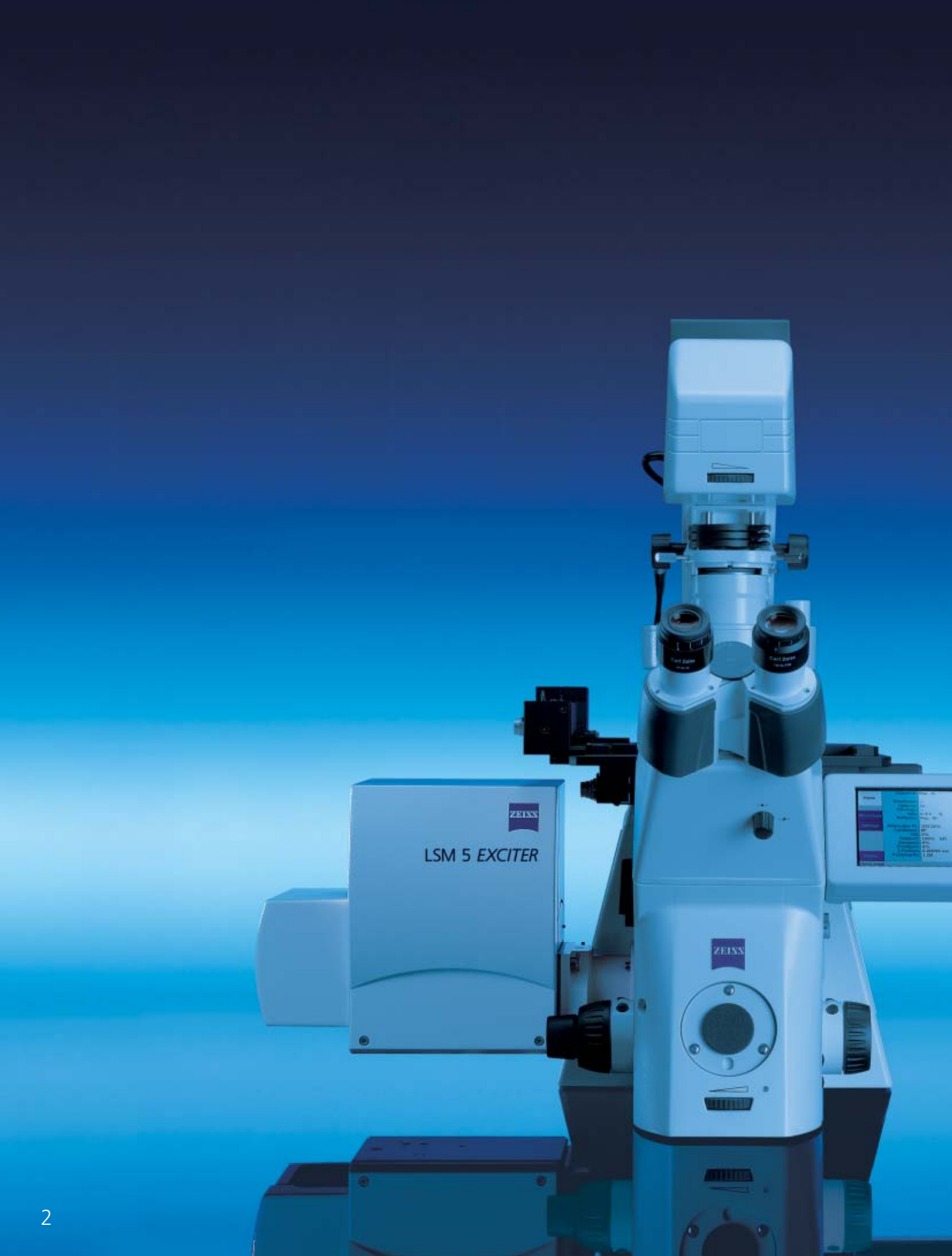









ZEISS
LSM 5 EXCITER
ZEISS

ZEN

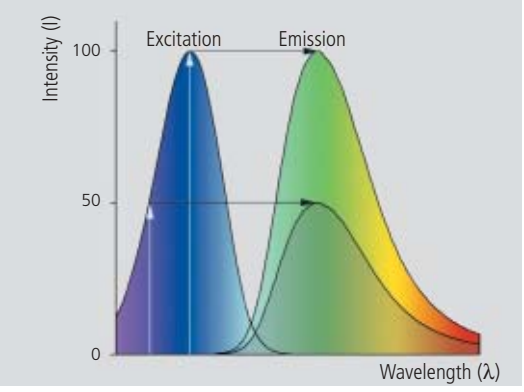
Efficient Navigation

With the new software ZEN we have made it possible to concentrate completely on the essentials. Discover a new era in laser scanning microscopy.

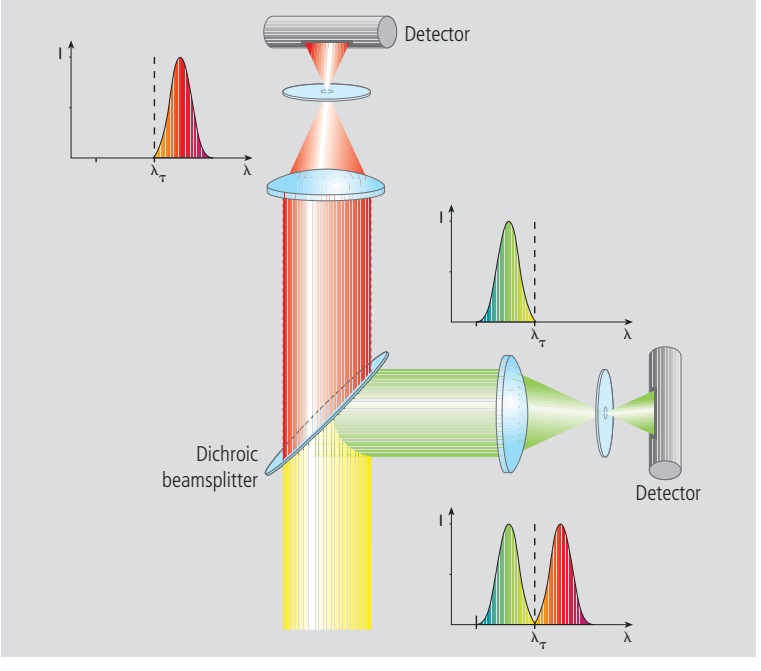
Experience Confocality

Fascinating Contrast

The LSM 5 *EXCITER* is a confocal microscope system that scans a specimen with laser light, point by point and line by line, in order to acquire an optical section. Many optical sections collected from different Z planes form a 3D image stack. Confocal image stacks acquired with fluorescent light provide information on selectively labelled functional regions of cells, tissues and organisms.



A fluorochrome can be excited by different wavelengths within its excitation spectrum. Light is then emitted with a characteristic emission spectra. The emission intensity is a function of the radiation intensity and excitation efficiency of the light.



In case of a multiple-stained specimen, the various emission signals are separated by high-quality dichroic filters, which can be selected and changed to suit the application.

Confocal ...

The special advantage of confocal laser scanning microscopy results from the use of a pinhole diaphragm located conjugate to the focal plane. The pinhole only admits light coming from the focal plane, while emissions from planes above or below it are rejected.

... laser ...

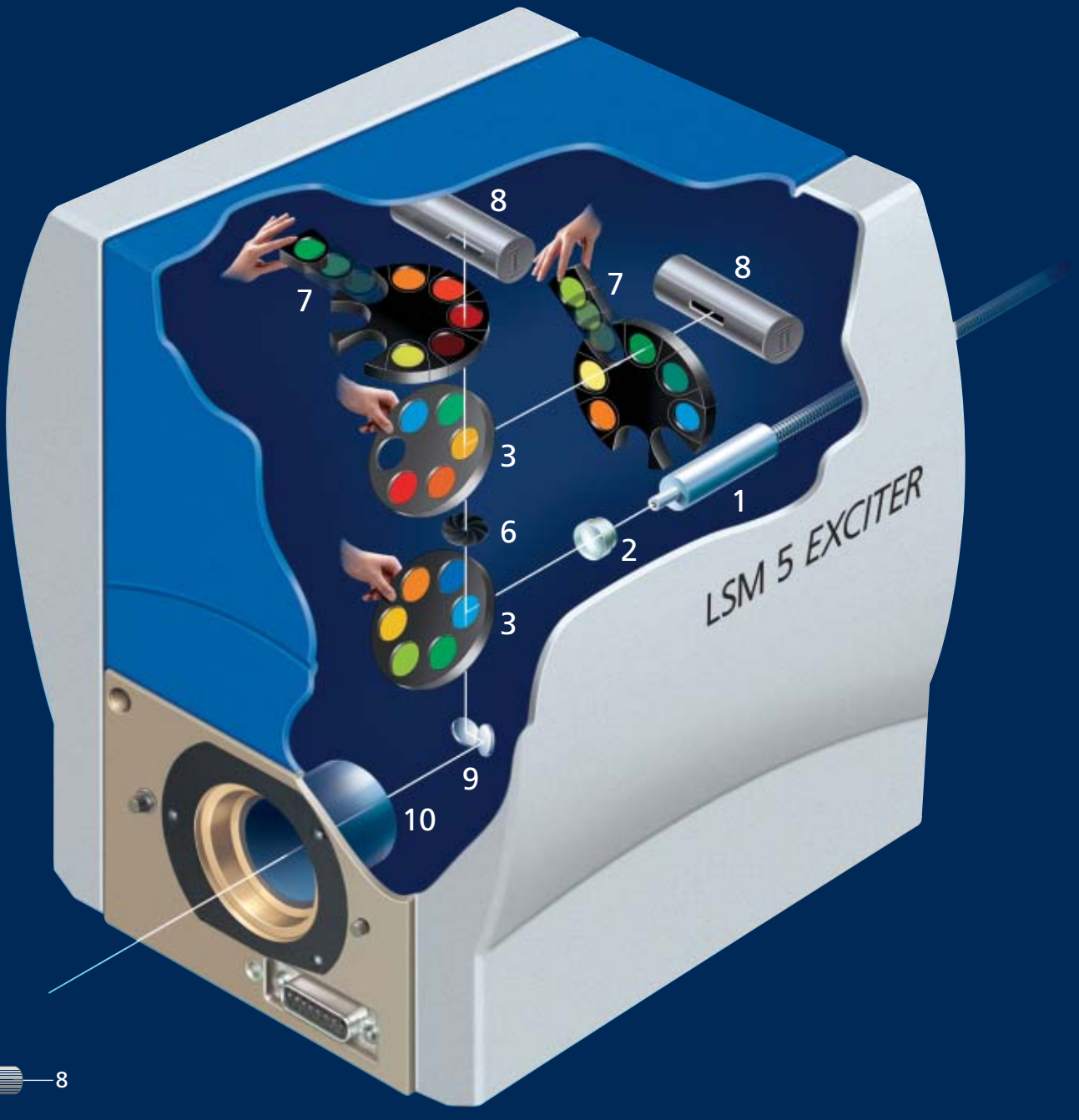
For the LSM 5 *EXCITER*, lasers with several lines ranging from 405 to 633 nm are available for excitation. The lasers are coupled to the scanning module reliably and efficiently via separate optical fibers. Laser intensities can be adjusted with a software-controlled acousto-optical tunable filter (AOTF).

... scanning

Point-by-point and line-by-line scanning of the specimen with a focused laser beam produces two-dimensional images free from scattered light. Scanning at different levels as the laser focus is shifted along the Z axis generates a series of optical sections (slices), which can then be combined into a three-dimensional image stack.

1 *Fiber (from laser source)*
2 *Collimator*
3 *Main dichroic beamsplitter**
4 *Objective**
5 *Specimen*
6 *Pinhole*
7 *Emission filter**
8 *Detector*
9 *Scanning mirror*
10 *Scanning optics*
11 *Focal plane*

**user-exchangeable components*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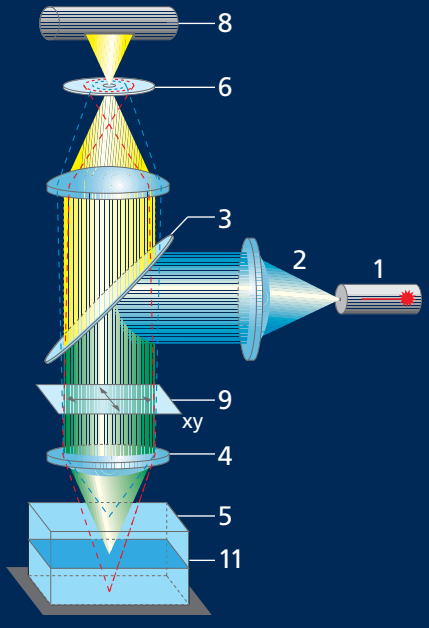
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Components Matched for Efficient Operation

A Perfect Team

Carl Zeiss configures every LSM 5 *EXCITER* to suit the user's scope of applications. For that purpose, a great number of well-matched, high-quality system components are available.

The heart of the system

The scanning module includes collimators, the scanner, and a freely positionable and adjustable pinhole. Detection is by highly sensitive photomultipliers. Advanced scanning control allows the precise positioning of the two scanning mirrors, and continuous laser attenuation to permit the user to apply many different scanning strategies. Fluorescence signals can for example, be detected along a straight line or a freely defined curve, or at a diffraction-limited spot. Pixel resolutions of confocal images can be freely selected between 4 x 1 and 2,048 x 2,048 pixels. The two independent scanning mirrors allow the scanning field to be rotated to any angle between 0° and 360°. The scanning speed can be precisely varied in 26 steps, with line frequencies ranging from 4 to 2,600 Hz.

LSM 5 EXCITER with Axio Observer, Axioskop 2 FS MOT and Axio Imager

The intelligent control center

The electronics module houses the main component of scanner control and image acquisition. A trigger interface allows synchronization of external devices (TriggerOut), e.g., a micromanipulator, as well as "remote-controlled" triggering of confocal image acquisition (TriggerIn) by external devices such as a breath sensor.

The stable basis

Depending on the user's application, several high-end research microscopes are available as platforms for the LSM 5 *EXCITER* system: Axio Imager, Axio Observer and Axioskop 2 FS MOT. All of them are equipped with IC^{2}S optics, which guarantee unrivalled image quality, brilliant contrast, and perfect color correction. The LSM software automatically detects all objectives in use, reproduces saved microscope settings at the touch of a button, and accurately controls the system's image acquisition process.

The keen eyes

High-grade objectives from Carl Zeiss allow you to set just the right combination of resolving power, speed, working distance, the refractive index of the immersion liquid, etc., as required for your application. C-Apochromat objectives provide the ultimate in confocal microscopy: diffraction-limited resolution and perfect chromatic correction from the UV to the NIR range. The flare-reduced EC Plan-Neofluar objectives deliver enhanced contrast, and the slender LCI Objectives provide extra space for convenient manipulation and temperature control of live cell specimens.

C-Apochromats: Optimized for confocal microscopy.

EC Plan-Neofluars: High contrast for general use.

Plan-Apochromats and Fluars: High-aperture photon collectors.

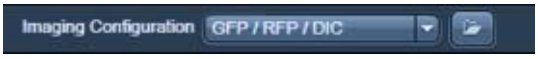
Achroplans: Water-immersed objectives for electrophysiological specimens.

LCI Plan-Neofluars: With temperature compensation for living specimens.

Configuration with just a Mouse Click

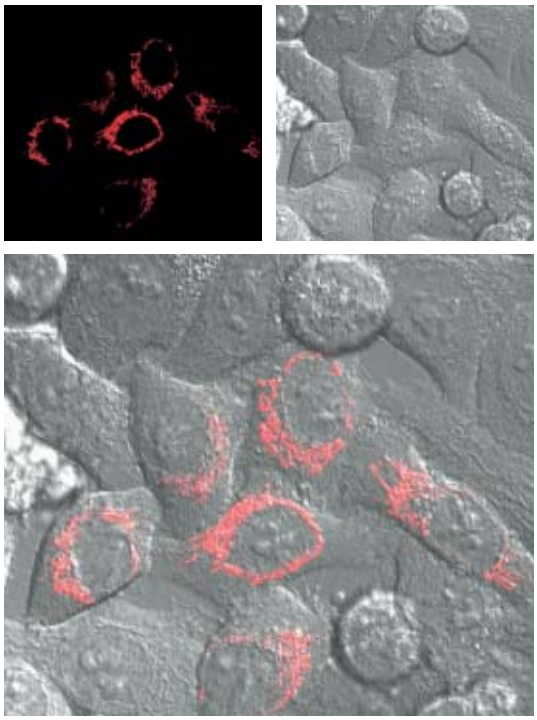
Proper Settings

The success of a microscopic experiment is a matter of correct settings. All parameters of the fully motor-driven LSM 5 *EXCITER* can be selected quickly and correctly, via its intuitive software ZEN.
The parameters of the experiment – from laser setting to image acquisition – can be precisely restored whenever they are needed again. So rather than having to care too much about the microscope, you can fully concentrate on your research.

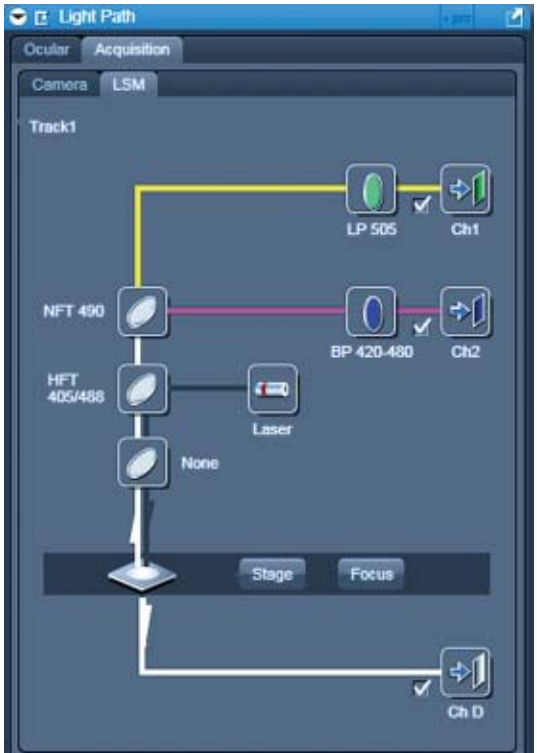


(2+1) detection channels up to 4 dyes simoultaneously!

In the LSM 5 *EXCITER*, one or two channels are available for fluorescence and reflection measurements, each featuring a highly sensitive, low-noise photomultiplier. XY adjustment of the pinhole is effected through a software-controlled motor; the pinhole diameter can be controlled continuously, as can the intensity of the laser. In some experiments it is helpful to superimpose transmitted-light and fluorescence images to "get the whole picture". Especially with differential interference contrast (DIC), the optional transmitted-light channel supplies important information about the topology of your specimen. New double-bandpass emission filters allow the detection of up to 4 dyes using fast simultaneous imaging.



Cultured cells. Fluorescence image superimposed with differential interference contrast in the transmitted-light channel. HeLa cells, mitochondria labeled with DsRed. Specimen: Prof. S. Yamamoto, Hamamatsu Medical University, Japan

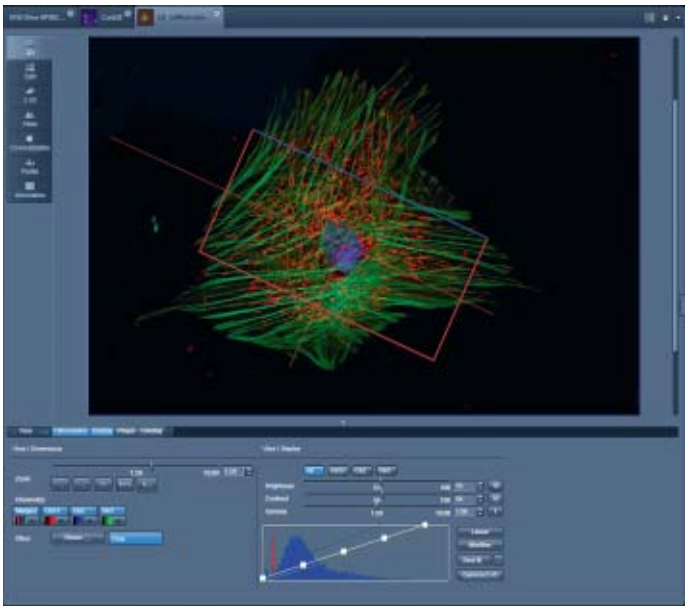


Clear and easy to use: The software of the LSM 5 EXCITER.

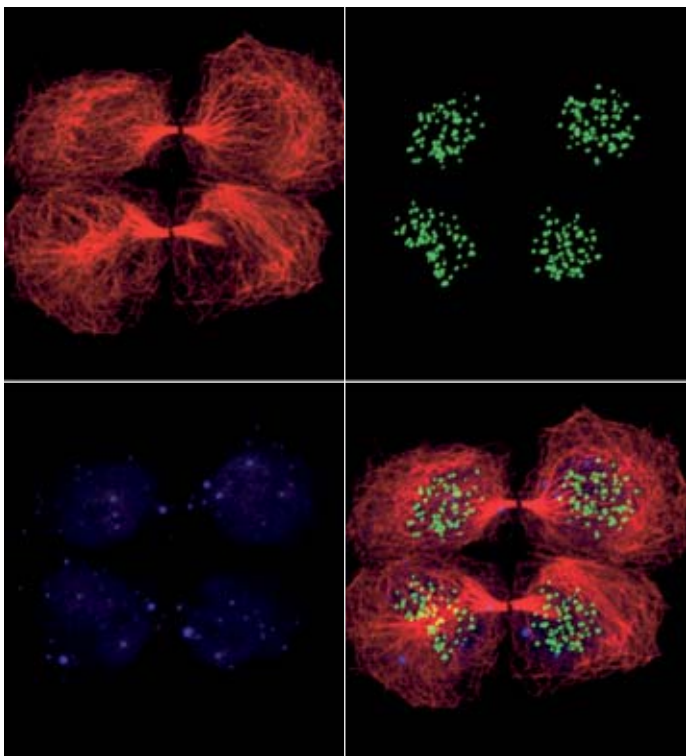
Complex functionality under control

Use the Light Path tool to select the main and secondary dichroic beamsplitters and emission filters in the confocal beam path, and to optimize the detection parameters. Alternatively, the system falls back on settings made in earlier experiments: the tried-and-approved *ReUse* function not only speeds up procedures in the lab but also exactly reproduces experimental conditions.

Use the Aquisition Mode tool to define all scanning parameters, such as frame size (up to 2,048 x 2,048 pixels), scanning speed, data resolution, and scanning direction (uni- or bidirectional). Use the *Find* function to have the system find the optimum contrast and brightness settings within seconds of clicking the mouse.



The Crop function allows a new scanning area to be selected and rotated with speed and ease.
Cultured ovary cells:
ß-actin labeled with Alexa 488 - phalloidin (green), mitochondria with Mitotracker (red),
Nucleus with DAPI (blue).



The images can be displayed individually or superimposed.
Cell division of HEp-2 cells.
Microtubules labeled with rhodamine (red), centromeres with FITC (green),
PML nuclear bodies with Alexa 633 (blue).
Specimen: S. Weidtkamp-Peters, Fritz-Lipmann-Institute Jena, Germany

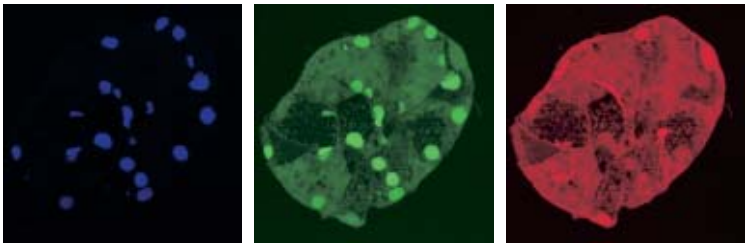
Sequential Imaging for Clear Signal Separation

No Crosstalk

Sequential Imaging is an elegant and reliable solution which efficiently prevents interchannel crosstalk between several fluorochromes. Colocalization studies, in particular, benefit from the increase in reliability gained from Sequential Imaging.

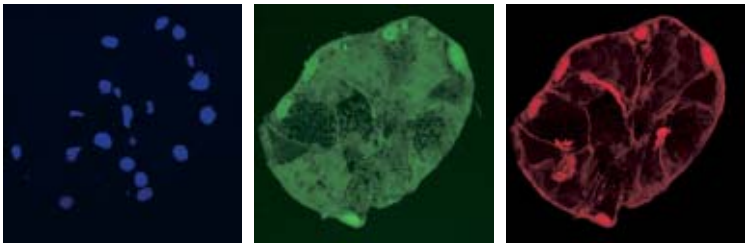
Unique capabilities

The LSM 5 *EXCITER* allows the user to optimize excitation parameters and detection settings for a single dye signal, and to define and save it as a track. A list of such tracks for independently optimized dye signals can then be run automatically as an Experiment in Channel Mode. Unblanking and blanking of the laser lines between the tracks is accomplished by acoustooptical devices. Up to eight fluorescent signals can thus be imaged sequentially in a single run, section by section or line by line. Due to the selective excitation and detection of the dyes, signal crosstalk is reliably prevented.

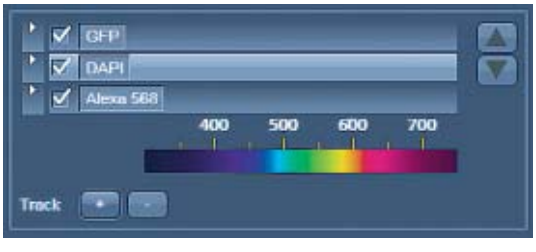


Isolated salivary gland of a cockroach. Cell nuclei are labeled with DAPI (blue), Na^+/K^+ ATPase with Cy2 (green), and F-actin with Alexa 568 phalloidin (red).
Specimen: Dr. D. Malun, Free University of Berlin, Germany

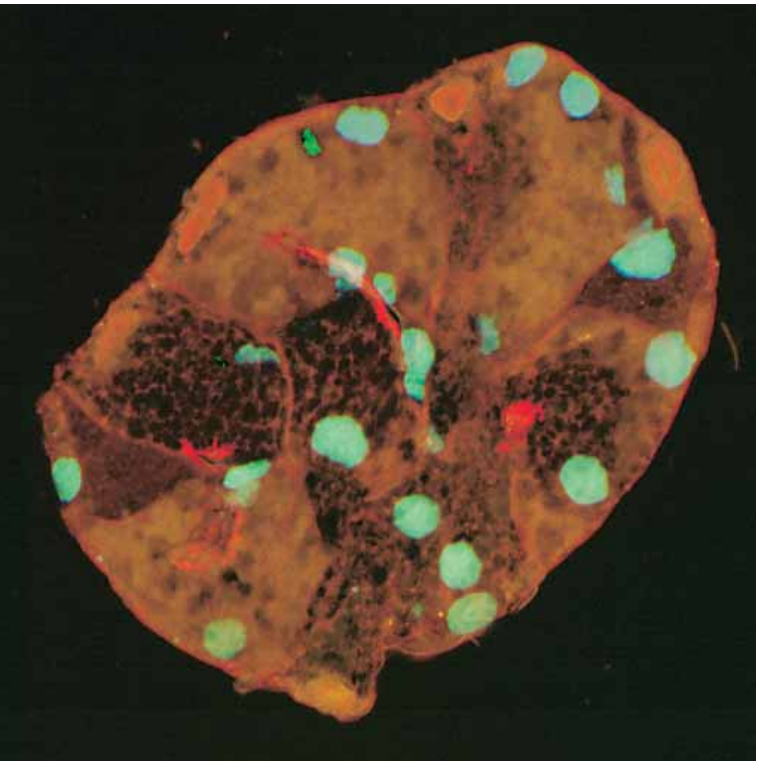
The simultaneous recording of either DAPI and Cy2, or Cy2 and Alexa 568 shows strong bleedthrough of the DAPI signal into the Cy2 channel (middle), or of the Cy2 signal into the Alexa 568 channel (right).



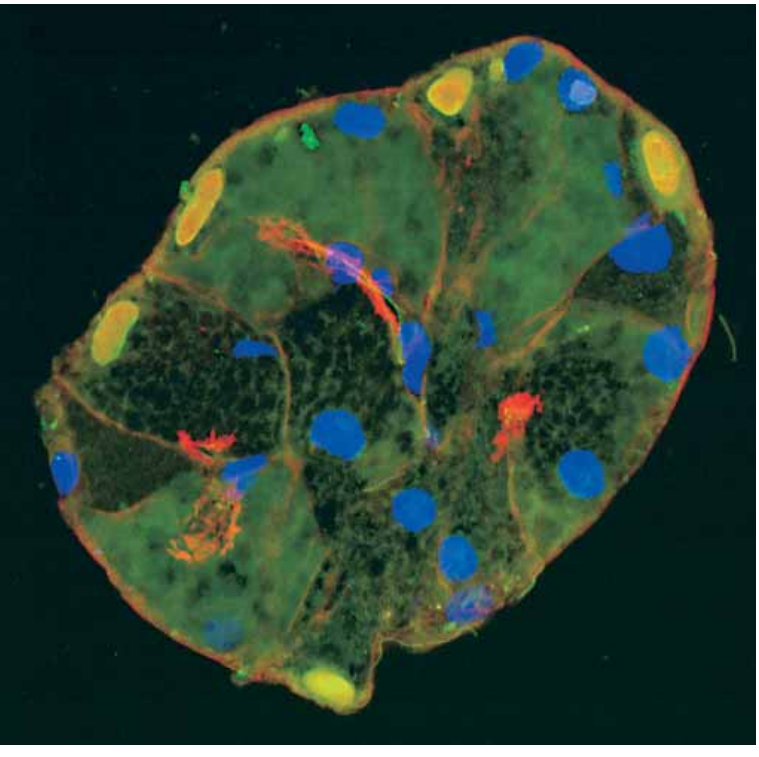
The same specimen recorded by Sequential Imaging (with the same laser intensity).
The channels are clearly separated.



Sequential Imaging is as easy as pie: Select the laser lines, main dichroic beamsplitters and emission filters for each channel in the Light Path tool. Once compiled, a list of tracks can be saved and activated later whenever needed.



Superposition of the images obtained from three channels clearly illustrates the difference: Simultaneous recording results in massive signal crosstalk, which wrongly suggests a colocalization (shown in orange and turquoise).



With Sequential Imaging, the signals from the various channels are clearly separated, and optimum signal yield is guaranteed.

Increased efficiency

Sequential Imaging is a distinct benefit especially when signals are very faint. Here, you can now use longpass emission filters to obtain the entire emission spectrum of the dyes. The pinhole diameter can be set individually for each track to help balance the signal intensity for the respective dye type or dye concentration. This allows either optimum balance between greatly differing dye intensities, or to perfectly match the optical slice thicknesses in critical colocalization studies.

The Imaging Setup Tool displays the detection bands and the selected laser lines for a clear overview of the experiment. Example of a configuration for detecting DAPI (blue), GFP (green) and Alexa 568 (red).

3D Colocalization Quantified

Perfect Coincidence

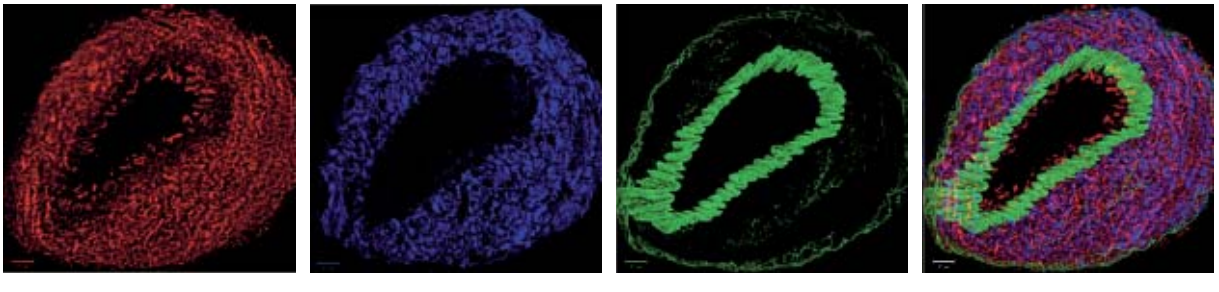
With the LSM 5 *EXCITER*, quantitative colocalization analyses can be made with unprecedented reliability and precision. Image presentation, scatter plot and data table are interactively linked to the region-of-interest (ROI) and thresholding tools.

The colocalization of labeled cell structures is often considered a first indicator of a potential functional interaction. Through Sequential Imaging with the LSM 5 *EXCITER*, genuine colocalization can readily be distinguished from emission channel crosstalk. Therefore, a region of interest can be selected immediately in the scatter plot, whereupon the system immediately indicates the occurrence of these colocalized fluorophores in the image. In the same way, the data table is interlinked with the scatter plot and the image. There is no more intuitive and precise way of analyzing your data.

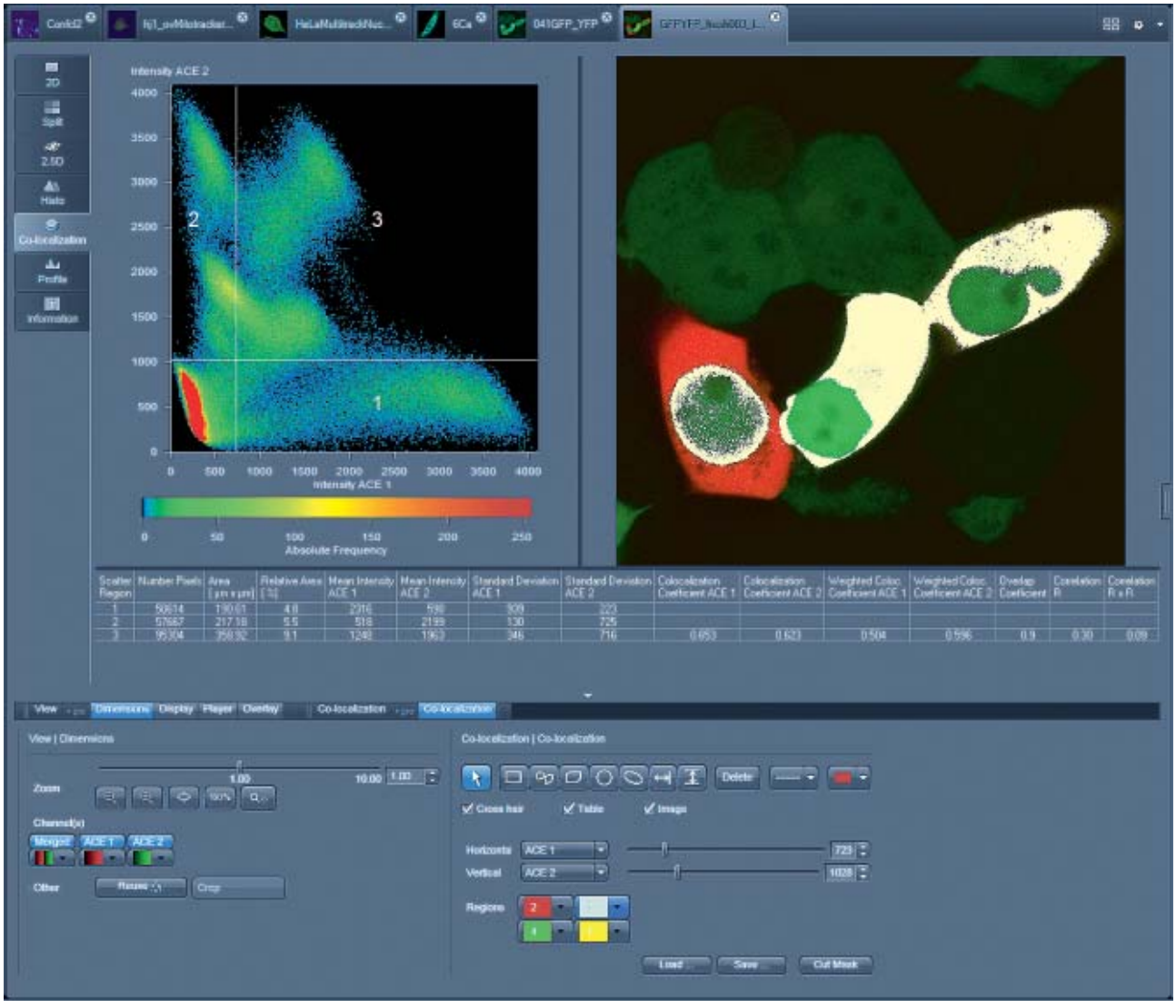
Visualization and analysis of colocalization experiments

- Interactive linking of images, scatter plots and data tables
- Interactive or automatic threshold determination
- Results of the colocalization analysis superimposed on image channels
- Quantitative colocalization analysis for up to 99 ROIs, including
  – area and mean gray level intensity
  – degree of colocalization
  – coefficient of colocalization
  – Pearson's correlation coefficient
  – Manders overlap coefficient
- Export of analysis results

Artery of a rat's lung.
Endothelin B labeled with Alexa 555 (red),
α-actin (SMA) with Alexa 647 (blue),
autofluorescent elastic fibers (green).
Specimen: L. Villeneuve,
Heart Montreal Institute Research Center,
Montreal, Quebec, Canada

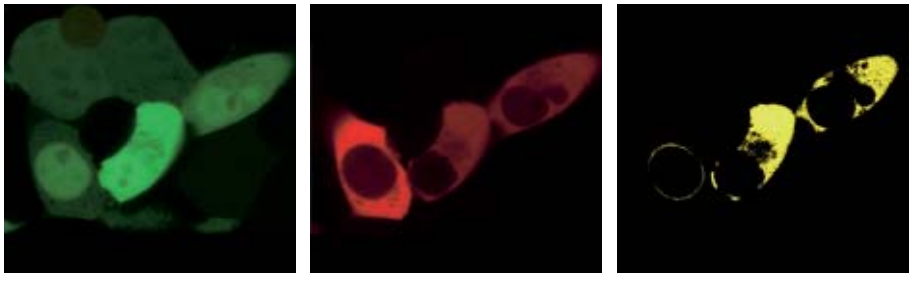


First-rate tools properly applied: Image presentation, scatter plots and data table are interactively linked with the ROI and thresholding tools.



Analysis of the colocalized area in a double tagged HEK 293 cell (green GFP, red YFP).

Coloc view with scattergram and quantitative parameters. Presentation of single-tagged (red and green) and colocalized image areas (white).



Extracted colocalized areas, according to regions 1, 2 and 3 in the scattergram.

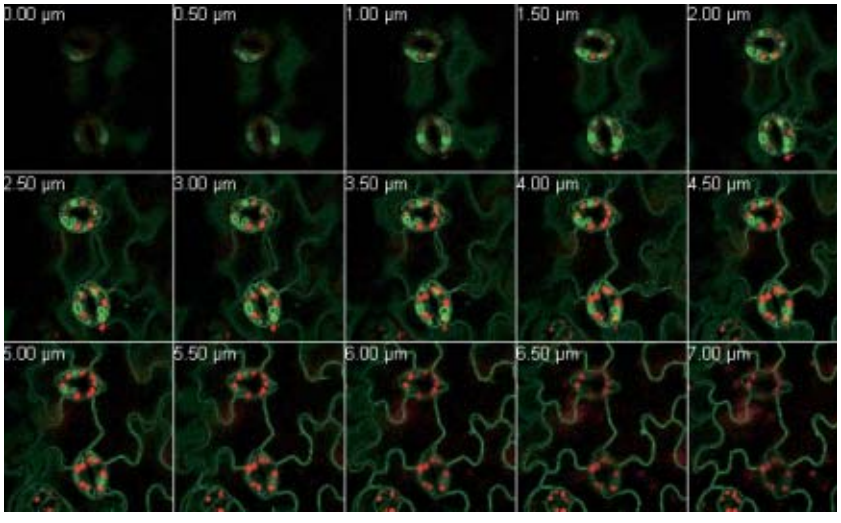
Analyzing 3D Image Stacks to µm Accuracy

Governed Space

Confocal microscopy is distinguished from conventional microscopy mainly by its capability to precisely analyze a specimen in three dimensions. From 3D image stacks, the researcher can gain new insights into the complex structures and interrelations on the cellular level. The LSM 5 *EXCITER* can turn such image stacks into projections of infinite depth of field, even from thick tissue sections.

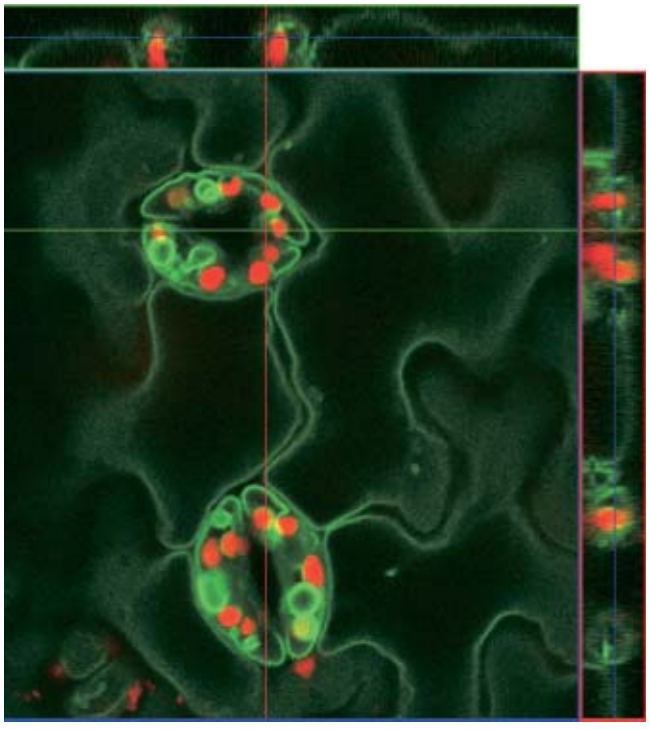
Slice by slice

Thanks to the use of the confocal pinhole, which only admits light from the focal plane while rejecting emission from planes lying above or below it, the system produces a confocal "optical section", which is free from out-of-focus scattered light. The thickness of the slice depends on the pinhole diameter and the wavelength of the laser light. Digitally stacked one above the other, many of such sections made at equidistant intervals along the Z axis assemble into a 3D representation of quasi-infinite depth of field. This stack of images is the basis for an analysis of the specimen's spatial structure.



Guard cells, transfected with AtVAM3-GFP (green), and autofluorescence in chloroplasts (red). The gallery view of the image stack shows the in-focus information in different Z positions. Specimen: Dr. M. Sato, Kyoto University, Japan

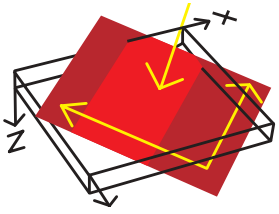
Indication of optical slice thicknesses (intervals) for various wavelengths. All relevant parameters are automatically used to calculate the resultant slice thickness, displayed as a graph, and equalized at the click of a button.



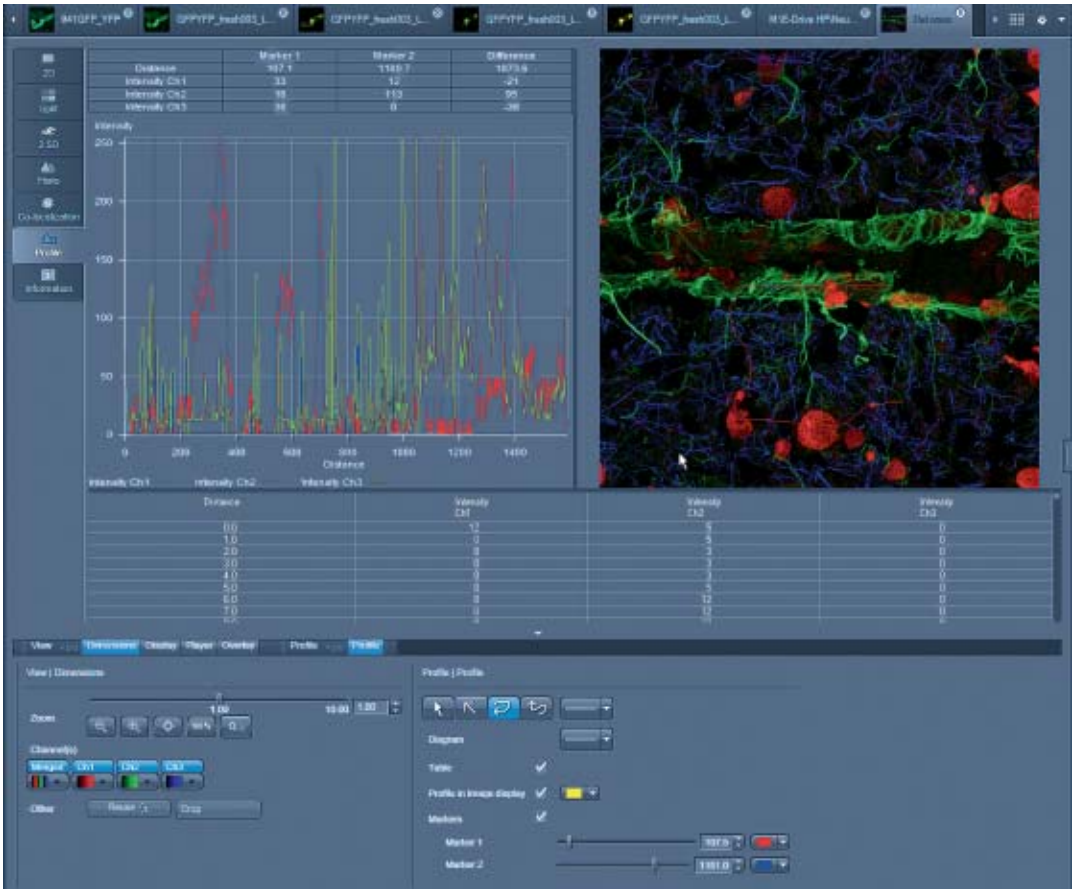
Orthogonal presentation of a stack of 15 images. Simultaneous view of XY, XZ and YZ planes.

Rendering the section planes

Using the LSM 5 *EXCITER*, you can generate virtual optical sections of any orientation inside the image stack. These virtual sections show you the spatial arrangements inside the specimen, even with structures that are only faintly fluorescent. Integrated measurement functions furnish geometric measurements such as length, angles, circumference or area, as well as densitometric parameters. The *Profile* function measures signal intensities along freely defined curves. The gray level data are presented in a clearly arranged table.



The Cut function allows virtual section planes of any orientation to be visualized.



Intensity measurement of the various channels along a freely defined curve by means of the Profile function.

FRAP

Dynamic Processes Observed with Single-Pixel Accuracy

Using acousto-optical attenuation (AOTF) of the laser beam, and the FRAP tool of the LSM software, you can conveniently study the distribution dynamics of biomolecules. The LSM 5 *EXCITER* delivers quantitative results within a few minutes.

In addition to the mere imaging of cellular structures, it is also possible to irreversibly quench the fluorescence of many dyes or fusion proteins from defined regions. Non-bleached dye molecules from the surrounding regions will diffuse into this bleached region, therefore causing a signal increase within the bleached region.

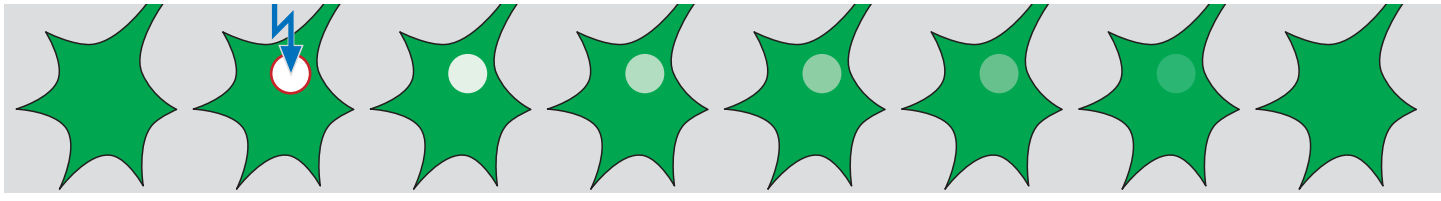
Fast, precise light flashes

The AOTF technology allows the laser output to be maximized within microseconds to efficiently bleach a region defined with single-pixel accuracy. Therefore, the LSM 5 *EXCITER* is capable of running many photobleaching techniques such as **FRAP** (**F**luorescence **R**ecovery **A**fter **P**hotobleaching) or **FLIP** (**F**luorescence **L**oss **I**n **P**hotobleaching), which have become established as standard experiments in biomedical analysis.

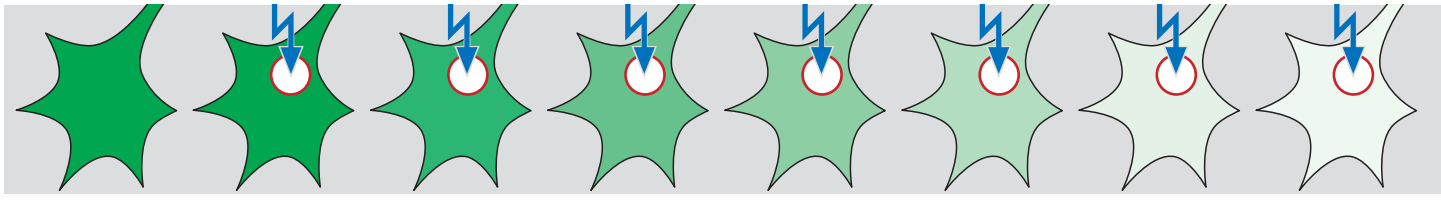
The FRAP tool

The LSM 5 *EXCITER* readily supports other designs of bleaching experiments as well. Various kinetic models (single- and bi-exponential fits) are provided for the reliable determination of regeneration times and mobile or immobile molecule fractions – all with simple menu prompts.

FRAP
In a FRAP experiment, a defined region in a cell expressing, e.g., a GFP fusion protein is bleached by brief but intense laser irradiation.
The recovery of fluorescence is documented by time-lapse images and measured.



FLIP
In a FLIP experiment, the same region within a cell is bleached repeatedly, and the loss in fluorescence outside that region is measured.



The Use of Photoconvertible Fluorescent Proteins

Telling Changes

The fluorescence of organelles or tissue cells expressing PA-GFP, Kaede or Dronpa can be selectively activated, deactivated or spectrally changed by local irradiation with violet light. With the proper light, the LSM 5 *EXCITER* visualizes your photoactivable proteins by fluorescence.

Single-molecule activation

Compared to bleaching experiments, photoactivation has the advantage that individual molecules can be activated selectively and their movements analyzed directly. With fast AOTF laser switching and the 405 nm laser diode of the LSM 5 *EXCITER*, you have everything you need to directly follow the journey of a protein inside a living cell.

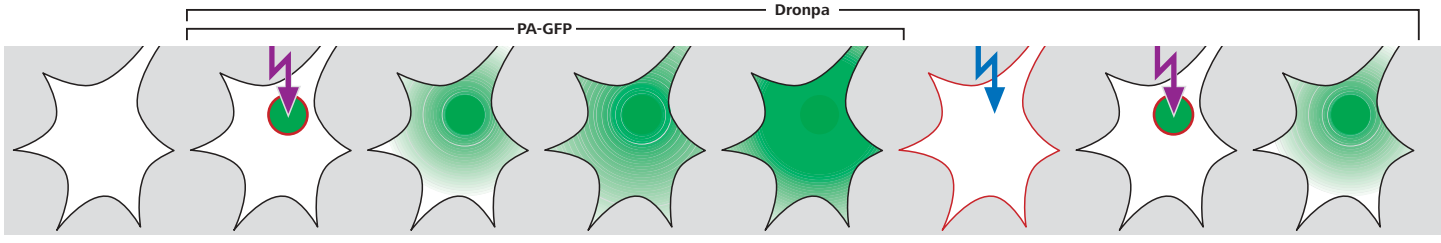
Tracking the green-to-red ratio

The *Time Series* functionality and the unmixing of spectrally overlapping emission signals allow you to observe the changing ratio of photoconverted (red) to non-converted (green) Kaede. Labeled molecules taking part in transport processes in cells can thus be precisely identified and localized at any time.

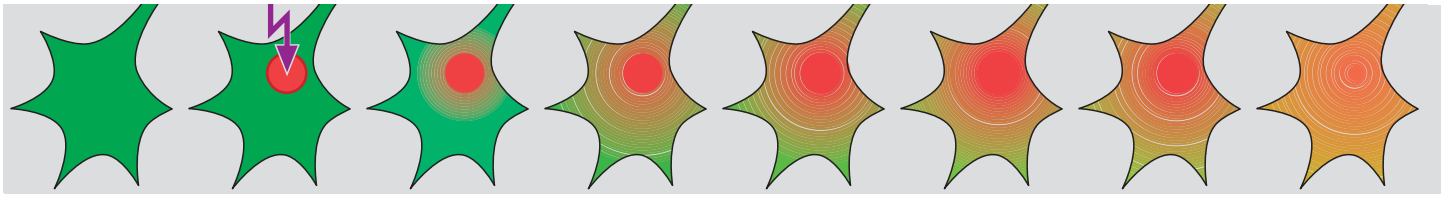
The blinker protein

The photoswitchable protein Dronpa can be "turned on" with a dose of 405 nm light, and "turned off" again with 488 nm, repeatedly. Using the Visual Macro Editor of the LSM software, you can thus repeat experiments with the same cell several times. This facilitates optimization of the ambient conditions (including stimulants), and it increases the certainty of your assessment of the result.

PA-GFP + Dronpa
Dronpa is a fluorescent protein which can be optically stimulated to switch between a fluorescent and a non-fluorescent state.



Kaede is a fluorescent protein which changes from green to red color when irradiated with ultraviolet light.



FRET

Visualize Molecular Interactions

FRET (Fluorescence Resonance Energy Transfer) is a radiationless energy transfer between two fluorophores located close to each other. Its intensity change can be harnessed for the investigation of intramolecular protein-protein interactions, enzyme activities, ion concentrations, and interactions between messenger substances in cells.

Proper excitation

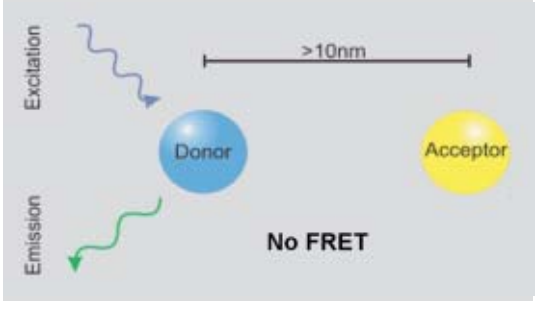
With its wide range of suitable laser lines, dichroic beamsplitters and emission filters, the LSM 5 *EXCITER* is optimally suited to excite selected dye combinations – known as FRET fluorophore pairs – such as CFP/YFP, GFP/mRFP or GFP/Rhodamine.

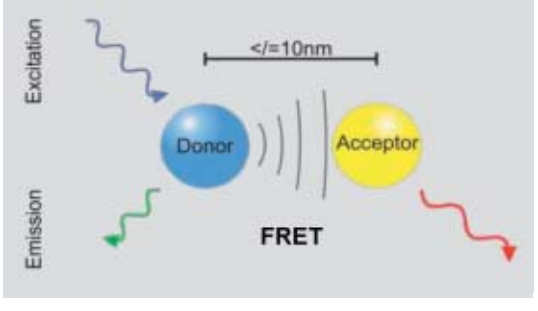
FRET-sensitive emission

Regions of arbitrary shape can be selected precisely thanks to fast, pixel-accurate switching of the laser intensity by the AOTF. With the linewise or frame-wise Sequential Imaging capability of the LSM 5 *EXCITER*, donor, acceptor and FRET signal portions and their overlaps can be clearly identified.

The intensive bleaching program

Alternatively, the FRET effect can be detected elegantly and quickly by the bleaching of the acceptor fluorophore. An increase in donor intensity in a Region of Interest (ROI) immediately after bleaching can be unambiguously proven by means of the *Time Series* function of the LSM 5 *EXCITER*.

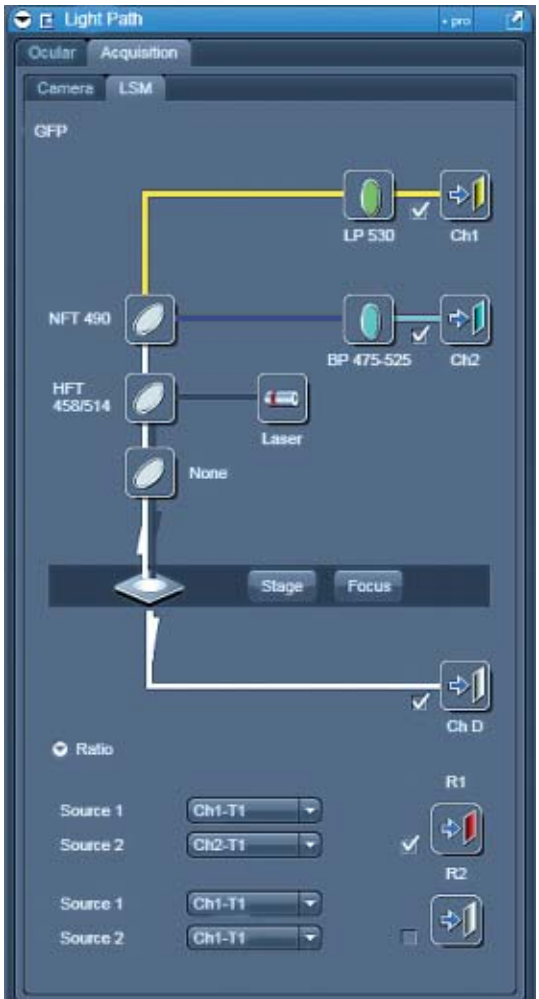




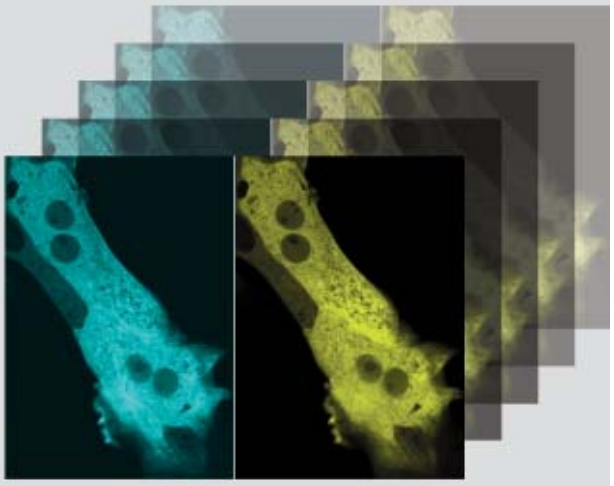
Example beam path configuration for the simultaneous acquisition of CFP and YFP for FRET studies. Continuous variation of laser intensities with AOTF. One or two ratio channels can be defined in addition and the result displayed during the measurement.

With the *Visual Macro Editor* of the LSM 5 *EXCITER* complete experiments for unmistakable detection of the FRET signal can be configured in a convenient and reproducible way. If needed, also in combination with Sequential Imaging.

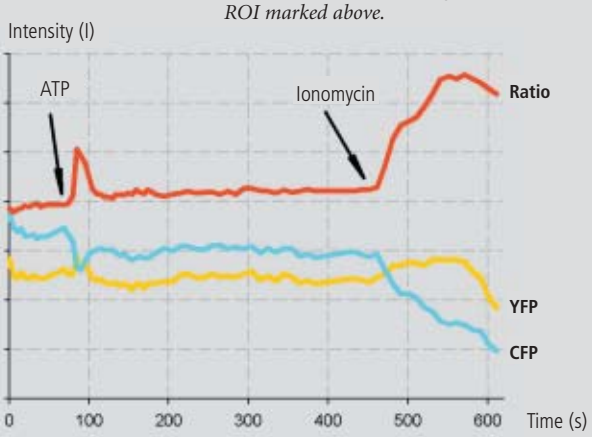
A number of bio-sensors have been designed recently that permit ion concentrations to be changed via a variation of FRET intensity. One of these bio-sensors used to detect changes in intracellular calcium concentration is Yellow Cameleon 2.

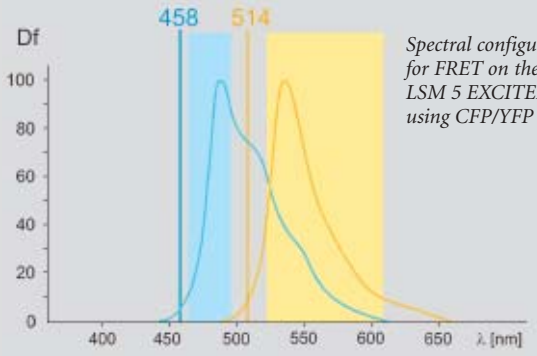


Time series of the CFP and YFP fluorescences of Yellow Cameleon 2 (in hepatocytes) after stimulation with ATP and ionomycin.
Specimen: Prof. T. Kawanishi, National Institute of Health Science, Tokyo, Japan



The intensities of the CFP and YFP fluorescences and the YFP/CFP signal ratio in the ROI marked above.





Spectral configuration for FRET on the LSM 5 EXCITER using CFP/YFP

Close Monitoring of Physiological Processes

Fast Action

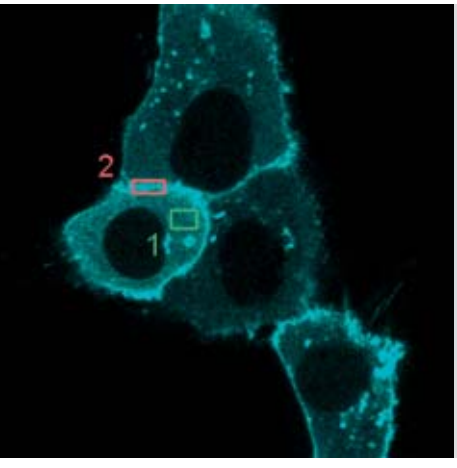
Physiological experiments are another field in which the LSM 5 *EXCITER* can show its strengths. Its image acquisition and processing functions are ideally suited to the investigation of fast processes in live cells and tissues.

Capture processes with correct timing

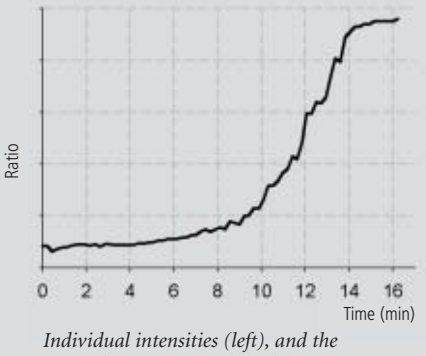
Whether you study a fast process or a long-time change: the LSM 5 *EXCITER* covers a wide range of biologically relevant time intervals between 0.1 ms and 10 hours. Highly frequent, linear scanner movements ensure reliable image data. All parameters can be optimized on-line during image acquisition.

Release pulses on time

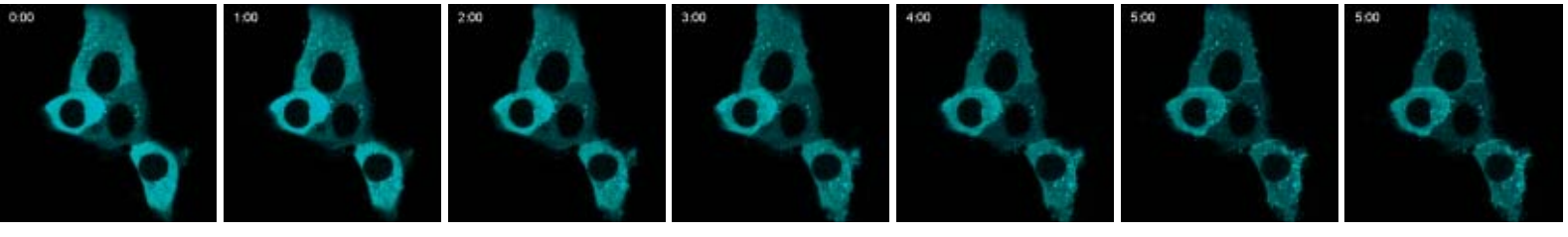
Outgoing trigger signals provide precise control of external equipment, e.g., microinjectors. Incoming trigger signals can be used, e.g., for synchronization with electrophysiological experiments in order to start confocal image acquisition.

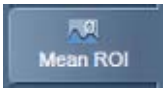


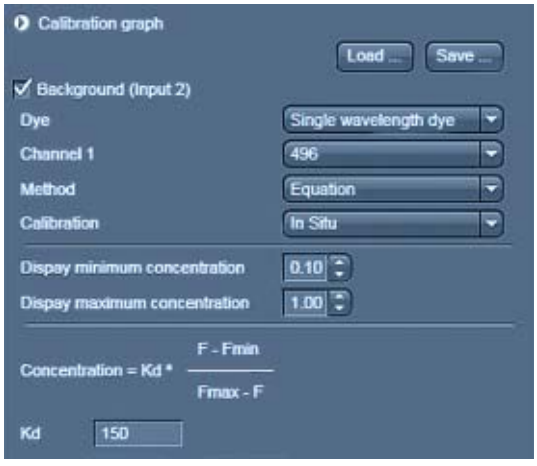
Selection of Regions of Interest (ROIs) within a specimen.
ROI1: Cytosol
ROI2: Cell membrane



Individual intensities (left), and the ratio of intensities (right) of the two ROIs boxed in the picture (far left). Colors are assigned accordingly.







Dialog window for the interactive calibration of ion-sensitive dyes.

Results available at any time

With freely defined regions of interest (ROIs) you can investigate precisely those structures of a specimen you are looking for. During image acquisition, either the series of images or the intensity curves inside the ROIs or both can be displayed. In ratiometric measurements, rather than waiting until the end of the time series, you can keep track of the results in a separate channel in real time.

On-line ratio calculations allow the data to be presented live during image acquisition. The system utilizes preset calculation formulas with user-defined parameters. Various calibration routines and display modes are available for calibrating fluorophores to be used in concentration analysis. Equipped with these process features, the LSM 5 *EXCITER* can do justice to any fluorescent dye.

Vary experimental setups from time to time

The optional *Visual Macro Editor* can be used to create complex time series experiments. This allows you to automatically switch between complete configurations, e.g., capture of an XY image in one configuration (GFP), and recording of a Z stack in another.

Investigation of protein movements

Time series experiment in HeLa cells transfected with PKC-GFP. Stimulation of the cells with PMA at the time t =1 min causes redistribution of PKC from the cytosol to the cell membrane.
Specimen: Dr. S. Yamamoto, Hamamatsu Medical University, Japan