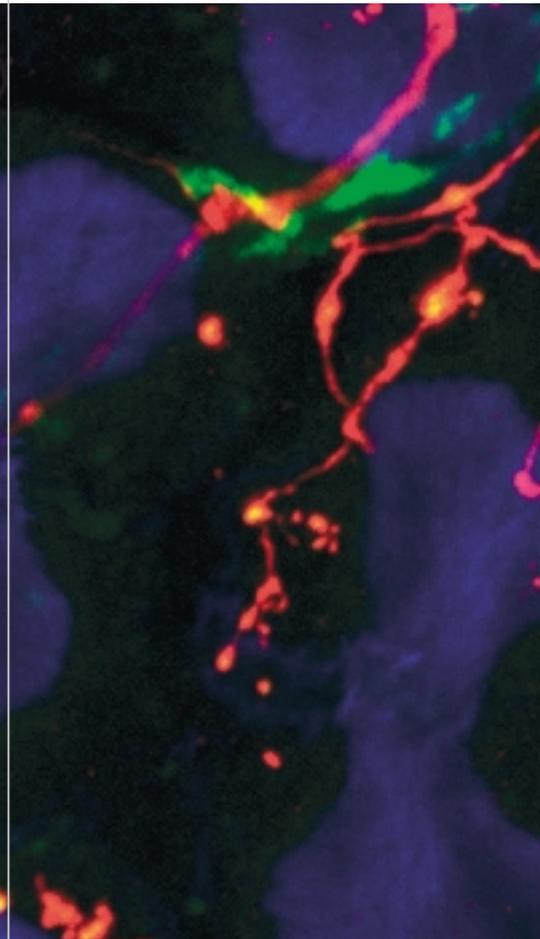
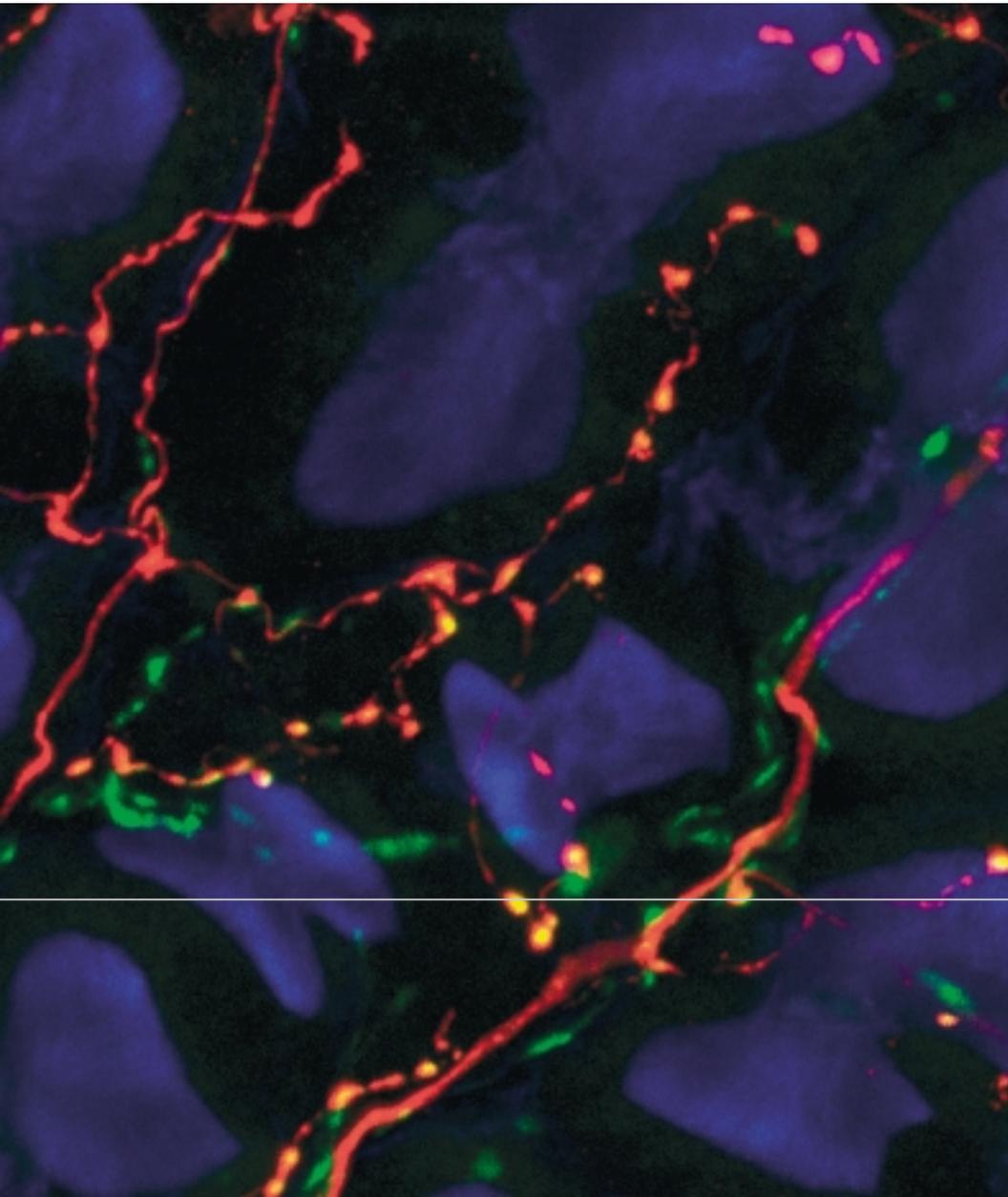


LSM 5 PASCAL

Laser Scanning Microscope

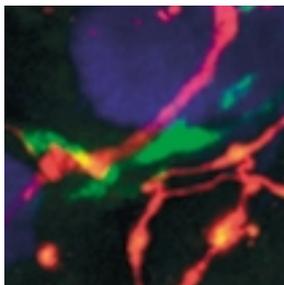
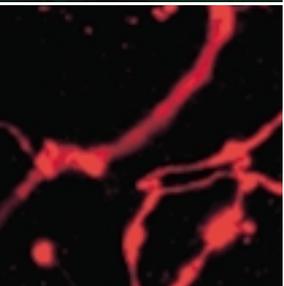
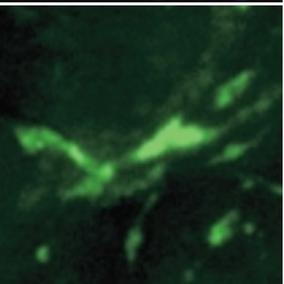
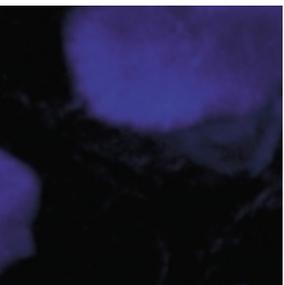


Cellular Functions Clearly Identified



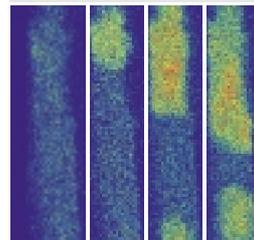
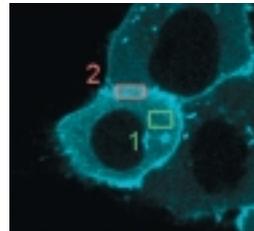
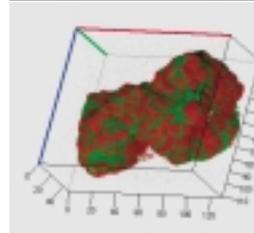
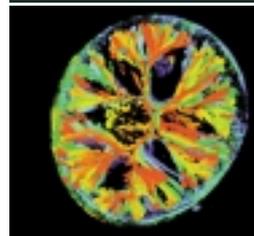
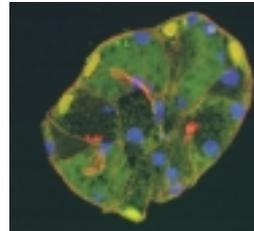
The Carl Zeiss LSM 5 PASCAL is a confocal laser scanning microscope for basic research in medicine and biology. Equipped with optimized, user-friendly hardware and software, this system delivers excellent two- and three-dimensional images, especially in fluorescence applications. It is ideal for the acquisition of time series in physiological experiments as well as for quantitative measurements.

With its cost-effective modular add-on capabilities, the LSM 5 PASCAL is the optimum solution both for an individual user and in a multi-user environment.



Isolated salivary gland of a cockroach. Actin filaments labeled with BODIPY-phalloidin (blue), serotonin with Cy3 (green), and synapsin with Cy5 (red). Specimen: Dr. O. Baumann, University of Potsdam, Germany

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LSM 5 PASCAL



Confocal Principle: A Crisp Image at Every Level

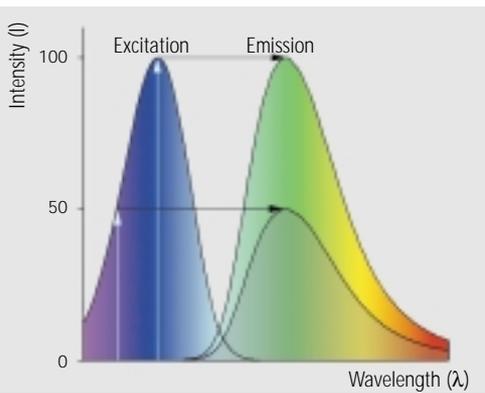
The LSM 5 PASCAL is a confocal microscope system that scans a specimen point by point and slice after slice. The picture elements (pixels) thus obtained assemble electronically into a three-dimensional stack of images. With different fluorescent emissions clearly separated, the images produced are rich in contrast and detail information.

Point-by-point
and line-by-line scanning

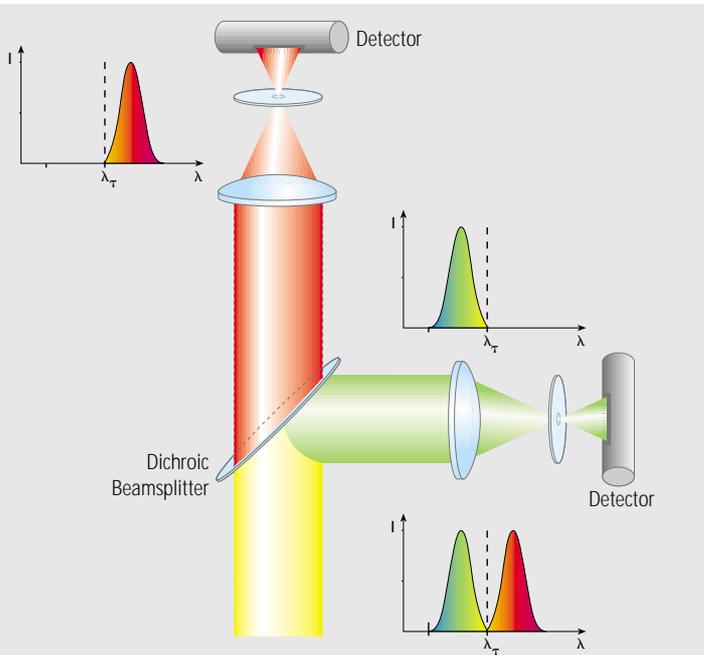
The special advantage of confocal laser scanning microscopy results from the use of a pinhole diaphragm located conjugate to the focal plane. The pinhole only admits light coming from the focal plane, while emissions from planes above or below it are rejected. Point-by-point and line-by-line scanning 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 indexed along the Z axis generates a series of optical sections (slices), which can then be combined into a three-dimensional data record.

Laser modules

For the LSM 5 PASCAL, lasers with several lines ranging from 405 to 633 nm are available. The lasers are coupled to the scanning module reliably and efficiently via separate optical fibers. Laser intensity can be adjusted continuously with a software-controlled mechano-optical tunable filter (MOTF).



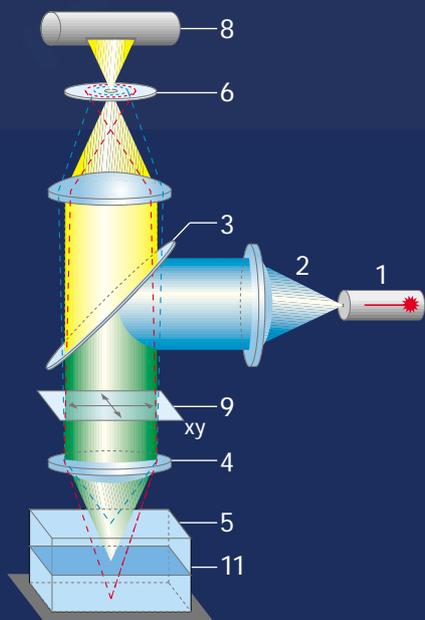
A fluorescent molecule can be excited by different wavelengths within its excitation spectrum. Accordingly, it emits different, characteristic emission spectra. The intensity of the emission signal is a function of the wavelength, intensity and efficiency of the excitation light.



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

- 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



A Perfect Match Optics and System Components

Carl Zeiss will configure every LSM 5 PASCAL to suit the user's scope of applications. For that purpose, a great number of well-matched, high-quality system components are available. Additional flexibility is guaranteed by user-exchangeable beamsplitters and filters.

Scanning module and software

The scanning module includes collimators, the scanner, a freely positionable and adjustable pin-hole, and other components. Detection is by highly sensitive photomultipliers.

Scanner control, the symmetric design of the two scanning mirrors, and continuous laser attenuation permit the user to apply many different scanning strategies, such as the compilation of two- or three-dimensional images. Images can be recorded along a straight line a freely defined curve, or in a diffraction-limited spot. With XY images, frame sizes between 4 x 1 and 2,048 x 2,048 pixels are available. The two independent scanning mirrors allow the scanning field to be rotated to any angle between 0° and 360°. There are 26 different scanning speeds, with line frequencies ranging from 4 to 2,600 Hz.

LSM 5 PASCAL with Axiovert 200 M / SidePort: The 2D and 3D real-color images are rendered through a high-resolution graphics card.



Electronics module

The electronics module comprises the main components of scanner control and image acquisition, the digital signal processor (DSP), and a trigger interface for recording time series in physiological experiments. The trigger interface allows external devices (e.g., a micromanipulator) to be synchronized with the scanning process in time series experiments (trigger in/out).

Objectives

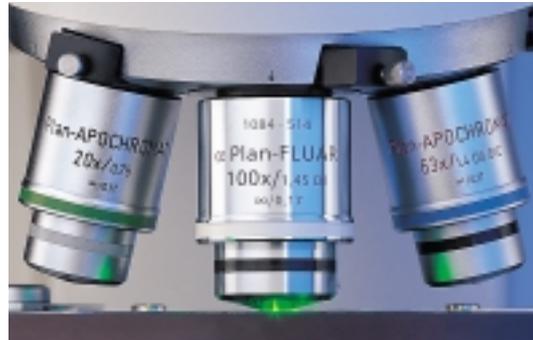
Carl Zeiss objectives are ideal for a wide diversity of applications. For every kind of experiment there is an objective providing just the right combination of resolving power, numerical aperture, working distance and aberration correction. For the confocal microscopy of samples immersed in aqueous media, Carl Zeiss specially designed the C-Apochromats, which ensure superior transmission and perfect image flattening from the UV to the IR region.

Microscopes

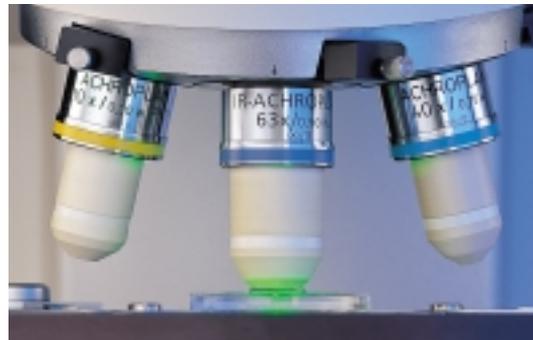
Depending on the user's application, several high-end research microscopes are available as platforms for the LSM 5 PASCAL system: Axioplan 2 imaging MOT, Axiovert 200M SidePort/BasePort, Axioskop 2 MOT, and Axioskop 2 FS MOT. All of them are equipped with ICS optics, which guarantee peerless image quality, flexibility, and optical perfection. The LSM software automatically senses the microscope settings and the objectives used, and accurately controls all system motions as well as image acquisition. The scanning module can be removed from one microscope and attached to another within a few minutes.



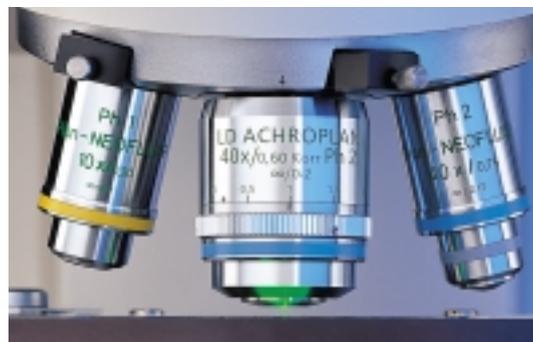
The C-Apochromats are designed for confocal laser scanning microscopy.



The α Plan-Fluar and the Plan-Apochromats combine the highest possible numerical aperture with best color correction.



The water dipping objectives of the Achromplan series are indispensable for applications in physiology.



The Plan-Neofluars are suitable for diverse applications. The LD objectives are available for long working distances.

The Piezo device for faster and precise objective focusing



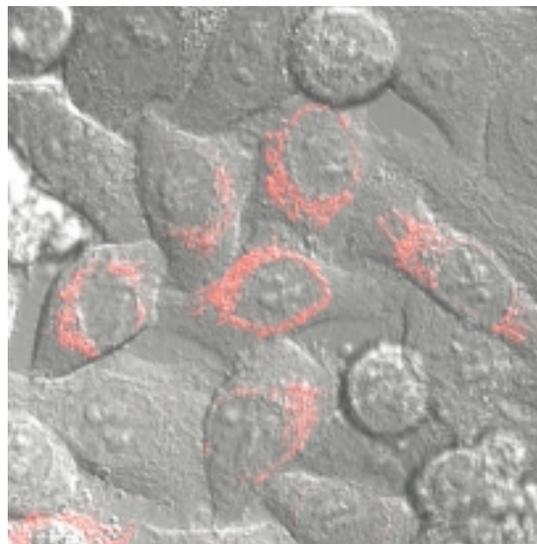
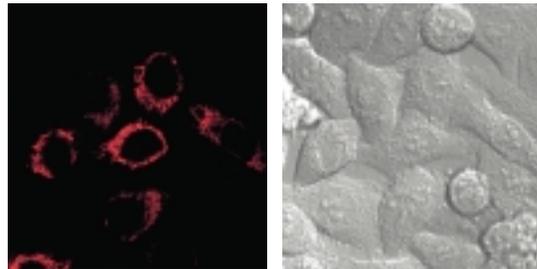
Single and Double Fluorescence: Configurations Generated and Saved in No Time

The success of a microscopical experiment is a matter of correct settings. For all parameters of the fully motor-driven LSM 5 PASCAL to be selected quickly and correctly, operation of its software is intuitive. The parameters of the experiment – from laser setting to image acquisition – are automatically saved and can be repeated whenever they are needed again. So rather than having to care too much about the microscope, the user can concentrate on his research.

Two simultaneous,
confocal detection channels

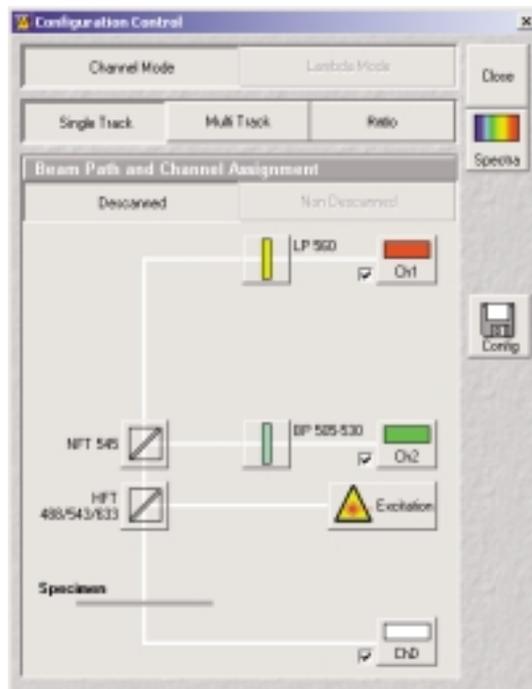


In the LSM 5 PASCAL, one or two channels are available for fluorescence and reflection measurements. In some experiments it is helpful to superimpose transmitted-light (e.g., DIC) and fluorescence images to "get the whole picture". For this purpose, a transmitted-light channel is provided. Each detector unit features a highly sensitive, low-noise photomultiplier. Pinhole adjustment in XY direction is effected through the software; the pinhole diameter can be controlled continuously, same as the intensity of the laser.



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

*Simple, clear, intuitive:
The software of the
LSM 5 PASCAL is
extremely user-friendly.*

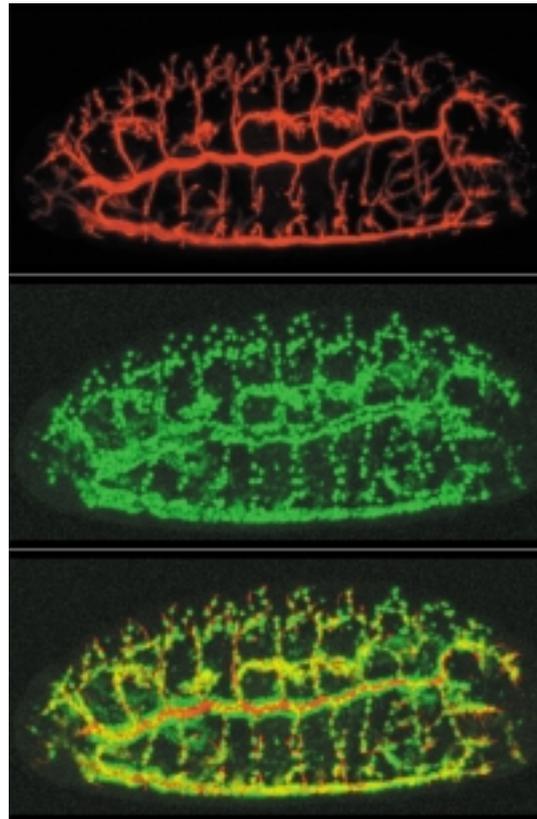




Easy to configure

Use the *Configuration Control* dialog window to select the main and secondary dichroic beamsplitters, the emission filters in the confocal beam path, and to define the detection parameters. As an alternative, the system falls back on settings made in earlier experiments. This tried-and-approved *ReUse* concept not only speeds up procedures in the laboratory but also exactly reproduces experimental conditions.

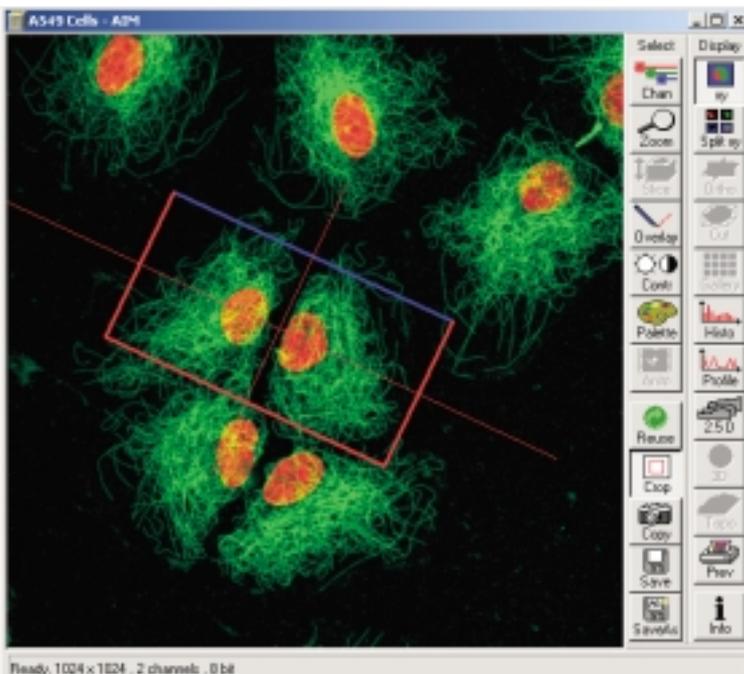
Use the *Scan Control* dialog window to define all scanning parameters, such as frame size (up to 2,048 x 2,048), scanning speed, data resolution, and scanning direction (uni- or bidirectional). Use the *Find* function to have the system find the optimum contrast and brightness settings within seconds – an advantage especially when you do not know the specific properties of a specimen.



The detection channels can be displayed individually or superimposed.

*Projection of the tracheal system of a fruit fly (*Drosophila melanogaster*): Cell nuclei labeled with Cy2 (green), lumen with Cy3 (red).*

Specimen: Prof. S. Hayashi, National Institute of Genetics, Mishima, Japan



The Crop function allows a new scanning area to be selected and rotated with speed and ease. Cultured A 549 cells:

Alpha-tubulin labeled with FITC (green), cell nuclei with propidium iodide (red).

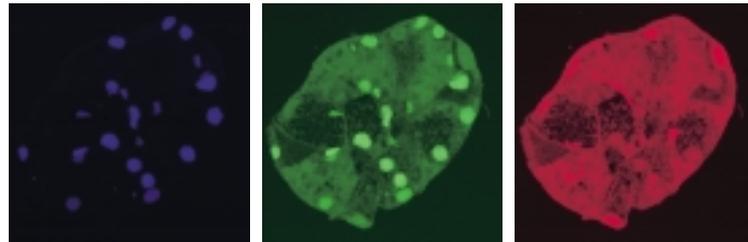
Specimen: Prof. H. Ayabe, Nagasaki University, Japan

Multifluorescence Images: Multitracking for Clear Separation without Crosstalk

The risk of inter-channel crosstalk increases with the number of dyes used in an experiment. This is a critical problem especially in colocalization studies. To avoid it, one would usually have to either acquire the images sequentially in a tedious step-by-step process, or select very narrow detection ranges. Framewise Multitracking is an elegant alternative with a high degree of certainty.

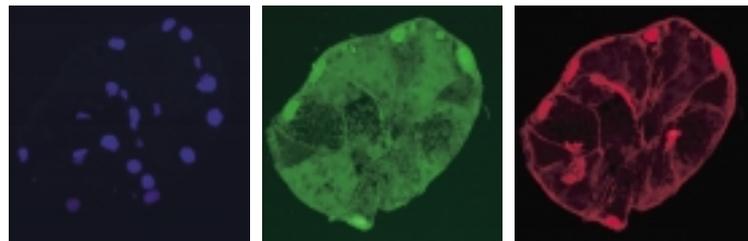
Unique capabilities

The LSM 5 PASCAL allows the user to define illuminating parameters and detection settings for the respective dye in individual tracks. A list of such tracks can be stored and used as a Multitrack for the automatic acquisition of multifluorescence images. Images from up to eight channels can thus be captured in a single run. The MOTF efficiently blanks and unblanks the laser lines between the tracks. Due to the selective excitation and detection of the dyes, signal crosstalk is prevented reliably.

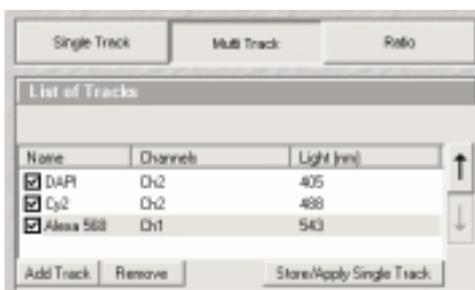


Isolated salivary gland of a cockroach. Cell nuclei are labeled with DAPI (blue), Na⁺/K⁺ ATPase with Cy2 (green), and F-actin with Alexa 568 phalloidin (red). Specimen: Dr. D. Malun, Free University of Berlin, Germany.

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).



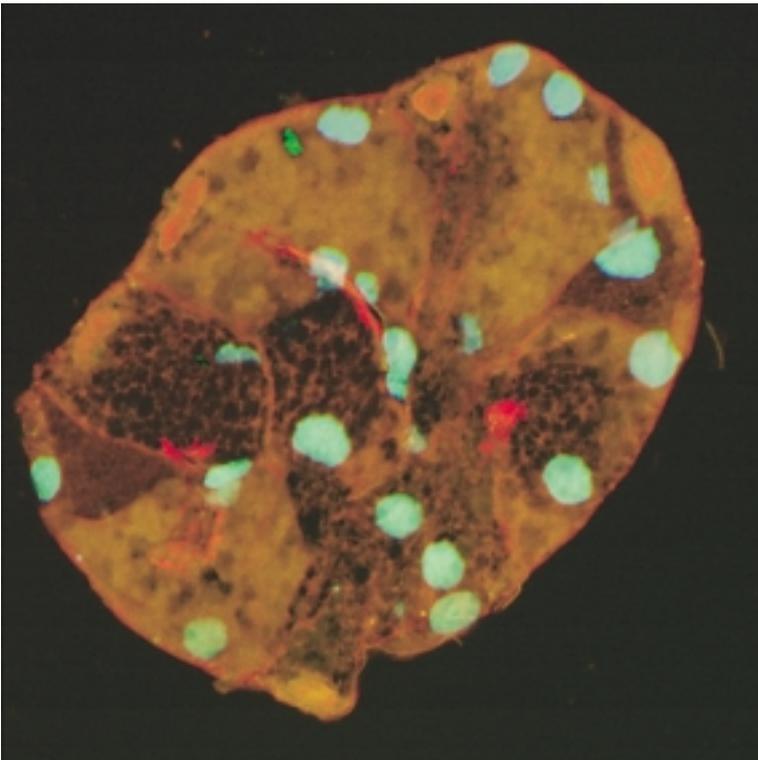
The same specimen recorded by Multitracking (with the same laser intensity). The channels are clearly separated without any crosstalk.



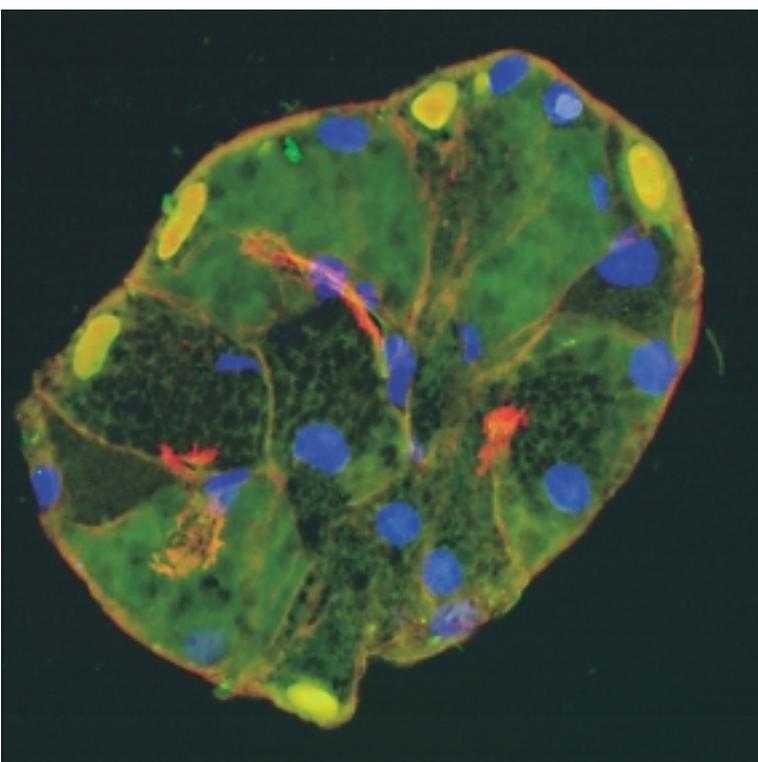
Setting up a Multitracking routine is easy as pie: Select the laser lines, main dichroic beamsplitters and emission filters for each channel in the Configuration Control dialog window. Once compiled, a configuration can be saved and activated later whenever needed.

Single Track

Multi Track



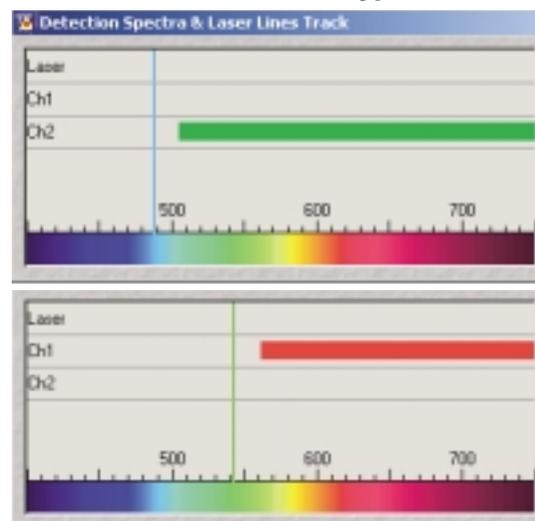
Superposition of the images obtained from three channels clearly illustrates the difference: Simultaneous recording results in massive signal crosstalk, which wrongly suggests a colocalization (orange and turquoise).



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

Multitracking is a benefit also in the case of very faint signals. Here, the researcher can now use long-pass emission filters and thus use the entire emission spectrum of the fluorochromes. The pinhole diameter can be set individually for each track to match the signal intensity. This allows optimum adjustment to different dye intensities. As an added advantage in critical colocalization studies it is possible to adapt optical slice thickness.

The Spectra function allows the selected laser lines and filters to be displayed in a clear overview. Example of a configuration for detecting Cy2 (green) and Alexa 568 (red) in a Multitrack with long-pass filters.

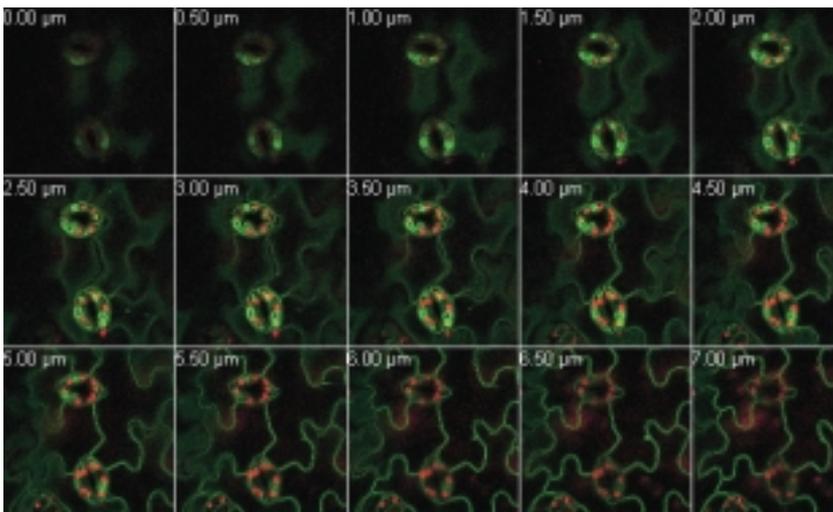


Acquisition of Image Stacks: Harnessing the Third Dimension

Confocal microscopy is distinguished from conventional microscopy mainly by its capability to analyze a specimen in three dimensions with enhanced depth of focus. With the LSM 5 PASCAL, a series of two-dimensional sections can be stacked on top of each other. Such image stacks allow the researcher to gain literally "deep" insights into interrelations that can hardly, if at all, be seen or verified in a two-dimensional representation.

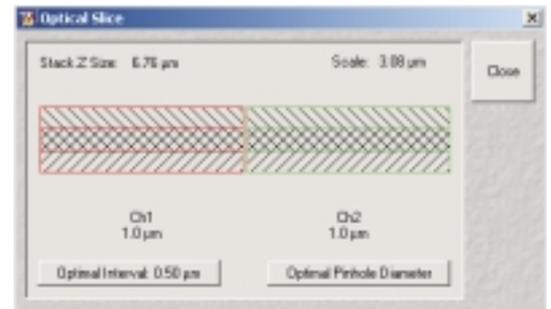
Slice by slice

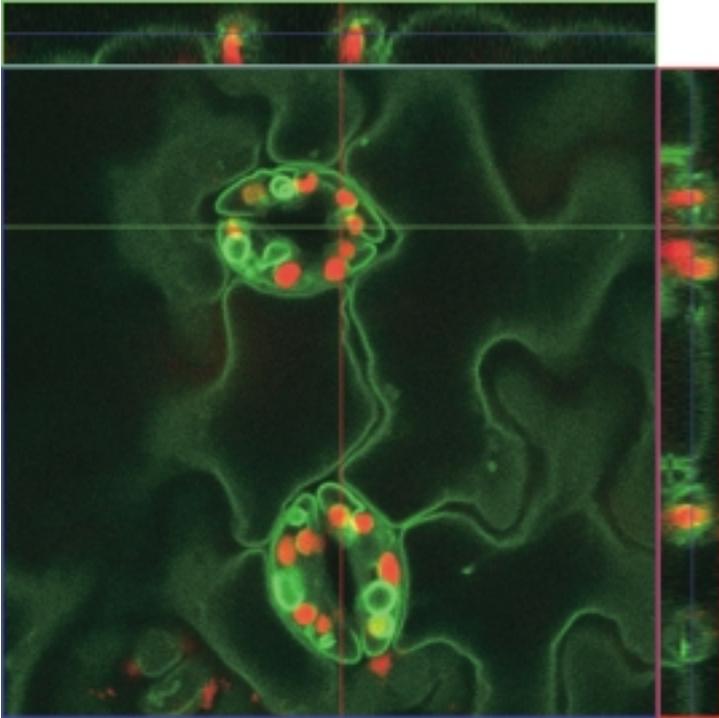
Thanks to the use of the confocal pinhole, which admits light from the focal plane only while rejecting emission from planes lying above or below it, the system produces a series of two-dimensional images (virtual sections) at different levels, which are free from scattered light. The thickness of the optical slices depends on the pinhole diameter and the wavelength of the laser light. Digitally stacked one above the other, these 2D images assemble into a 3D representation with high depth of focus. Thus the spatial structure of the specimen can be analyzed and supplies detailed information about complex interrelations.



Fast and reliable orientation. The gallery view tells the researcher which structures are on which section level. A possible intensity drop of the fluorescence signal in case of large Z stacks can be compensated by automatic adjustment of the detector voltage. Guard cells, transfected with AtVAM3-GFP (green), and autofluorescence in chloroplasts (red). Specimen: Dr. M. Sato, Kyoto University, Japan

Indication of optical slice thicknesses (intervals) for various wavelengths. All relevant data are automatically converted to match the resultant slice thickness, displayed as a graph, and optimized at the touch of a button.

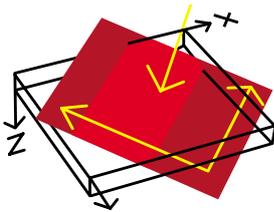




Orthogonal presentation of a stack of 15 images. Simultaneous view of XY, XZ and YZ planes.

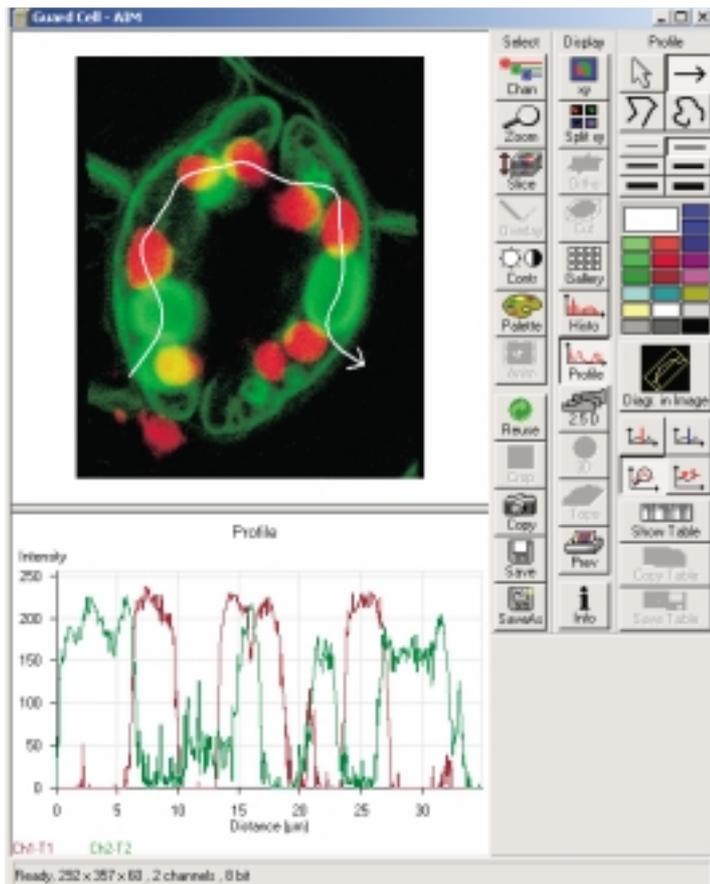
Display the section planes

Using the LSM 5 PASCAL, you can generate optical sections at different, self-defined planes. These virtual sections allow the specimen to be looked at from all sides and supply a pretty close idea of the spatial relations, even of complex or diffuse structures. At the same time, integrated measurement functions permit the user to establish all relevant quantities such as lengths, angles, circumferences and areas. The *Profile* function allows intensity measurements along freely defined curves. The data can be presented as a graph or in a structured table.



The Cut function allows virtual section planes to be illustrated. For example, structures extending into the depth at a slant can thus be visualized interactively.

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



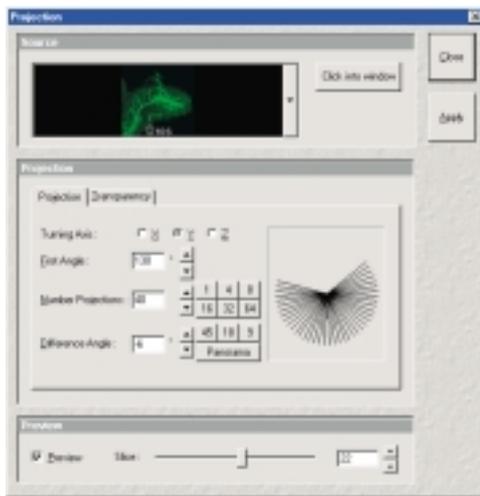
3D Visualization of Complex Structures: Overview and Details

Some structures become visible only if they are viewed from different angles. The powerful software of the LSM 5 PASCAL turns the stacks of confocal images into a three-dimensional virtual object that can be "handled" by the observer, i.e. turned and tilted to look at it from any projection angle.

Interesting Perspectives

The *Depth Code* function allows the depth information from an image stack to be visualized by coding with rainbow colors.

Transparent projections afford a view below the surface – you can see what happens inside the specimen. Stereo projections of 3D images for example provide exact information especially when you observe neuronal structures. To enhance the 3D effect, projections can also be animated.



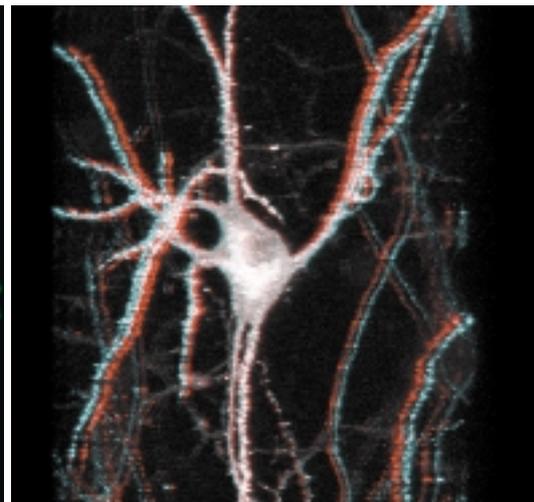
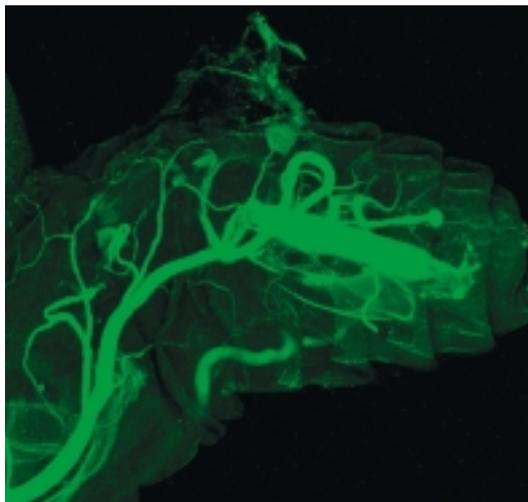
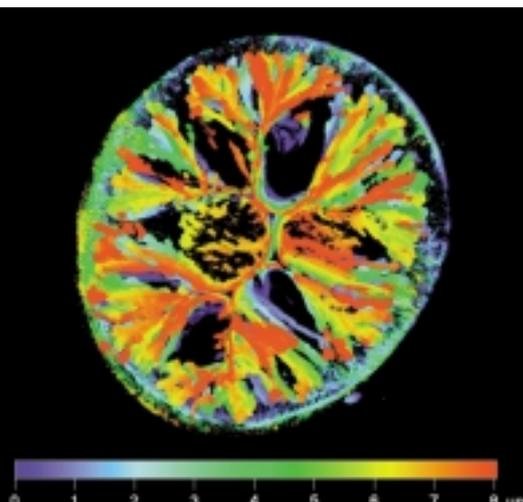
In the Projection dialog window, you can freely select the viewing angle, the number of projections and the transparency conditions. Use the Preview function for a fast preview of the result. The system allows you to interactively change all parameters; it will not compute the complete data record until the desired details are correct.



Depth coding compiled from 12 optical sections through the salivary gland of a fly (Calliphora), labeled with BODIPY-phalloidin. Specimen: Dr. B. Zimmermann, Potsdam University, Germany

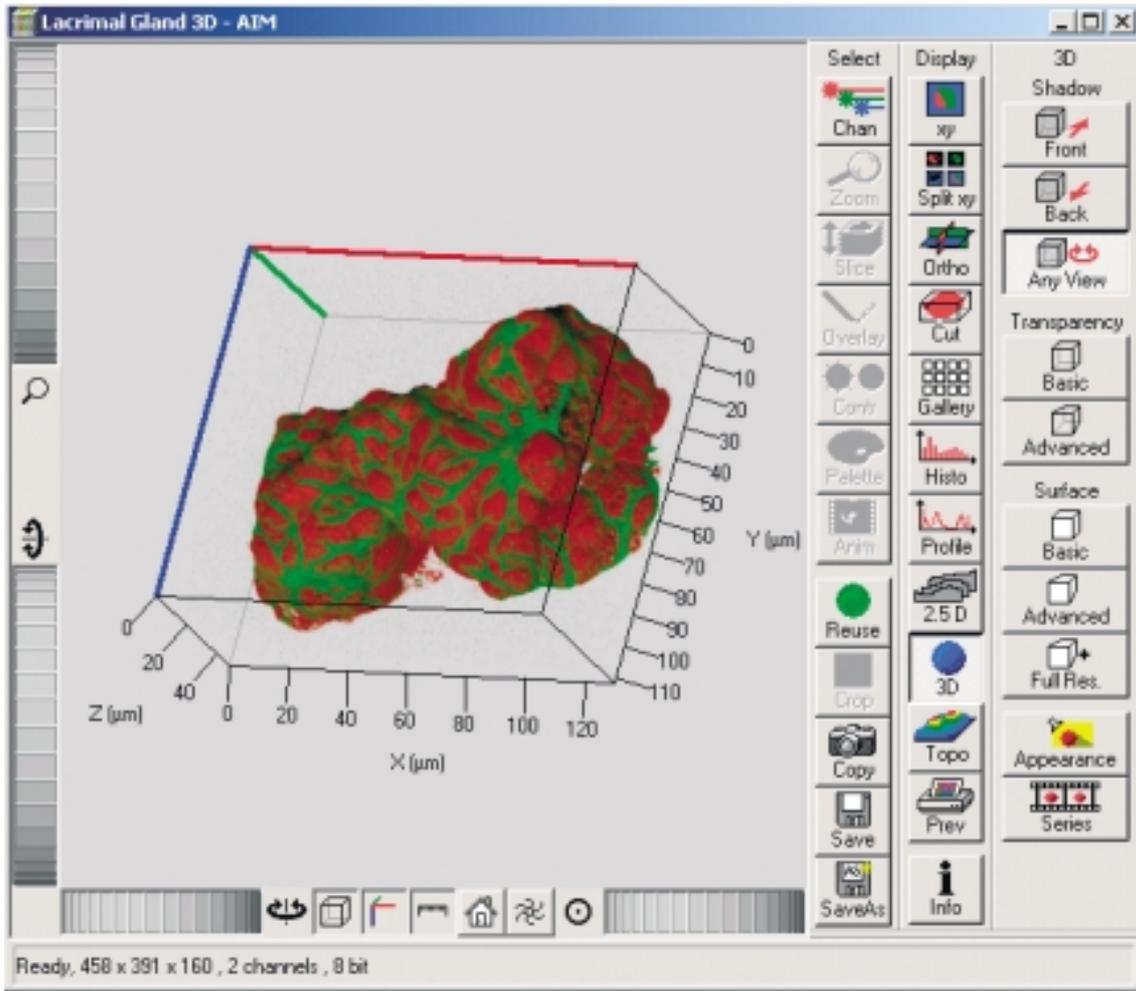
Projection compiled from 24 optical sections. Expression of GFP in a fruit fly (Drosophila). Specimen: Prof. K. Takeyasu, Kyoto University, Japan

Stereoprojection from 14 optical slices. Expression of GFP in a neuron. Specimen: Prof. S. Okabe, Tokyo University, Japan





3D reconstruction from 108 individual optical slices. Lachrymal gland. Actin filaments of myoepithelial cells labeled with BODIPY-FL phalloidin (green), cytoplasm and nuclei of acinar cells with ethidium homodimer-1 (red). Specimen: Prof. Y. Satoh, Iwate Medical University, Japan



Extended capabilities

The optional *Image VisArt* software package provides a broad range of functions for 3D and 4D visualizations of image stacks and time series, e.g., as shadow or transparency projection. *Image VisArt* not only provides additional information but also helps you to present and publish your findings in the most perfect way. Thanks to fast algorithms, such visualizations are generated at an

amazing speed. Handling this tool is extremely easy: To create an animation, simply specify the desired viewing angles. The system then automatically computes a "camera ride" (zooming) around and into the specimen. Many and varied parameters are available for setting the rendering properties for each function.

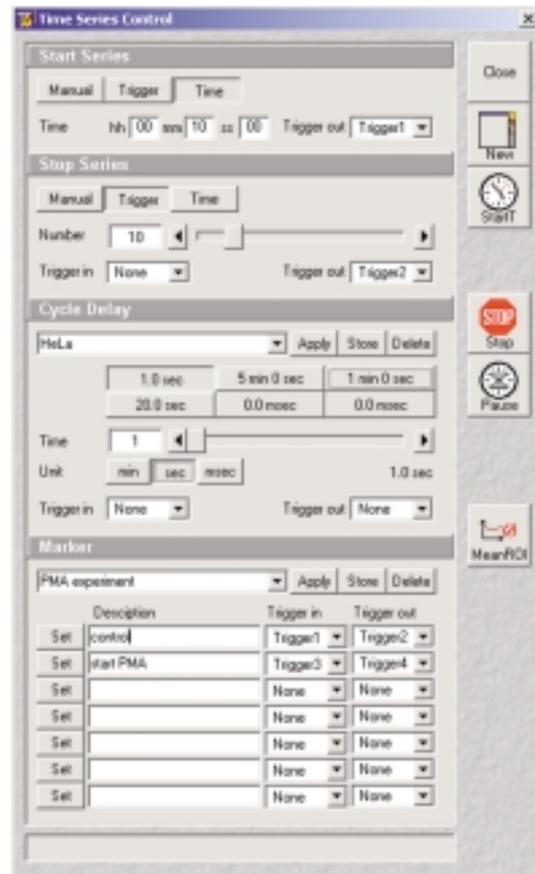
Physiology: More than just Time Series

Physiological experiments are a field in which the LSM 5 PASCAL can show its strengths. This image acquisition and analysis tool is, as a whole, ideally suited to investigate intracellular ion concentrations, transport mechanisms in organelles, and other dynamic processes in live cells and tissues.

Whether the subject of study is a fast process or a long-time change: with interval times between 0.1 ms and 10 h, the system can handle the whole spectrum of applications. The absolutely linear scanner movements ensure dependable quantitative measurements. All parameters set can be changed on-line during image acquisition.

Outgoing trigger signals provide control of external equipment, e.g., to start some process at a specified point in time. Incoming trigger signals can be used to have external signals or processes control confocal image acquisition. This is a definite requirement for synchronization with electrophysiological experiments.

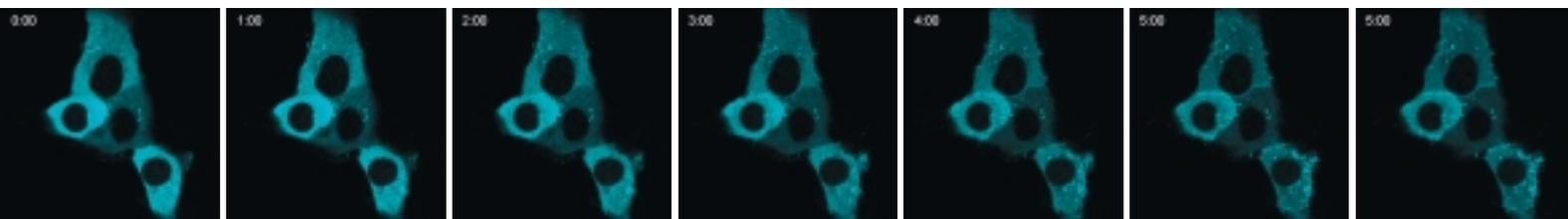
In addition, the *Multiple Time Series* module can be used to create complex time series experiments. This allows you to automatically switch between complete configurations, e.g., capture of an XY image in one configuration (e.g. GFP), and recording of a Z stack in another.

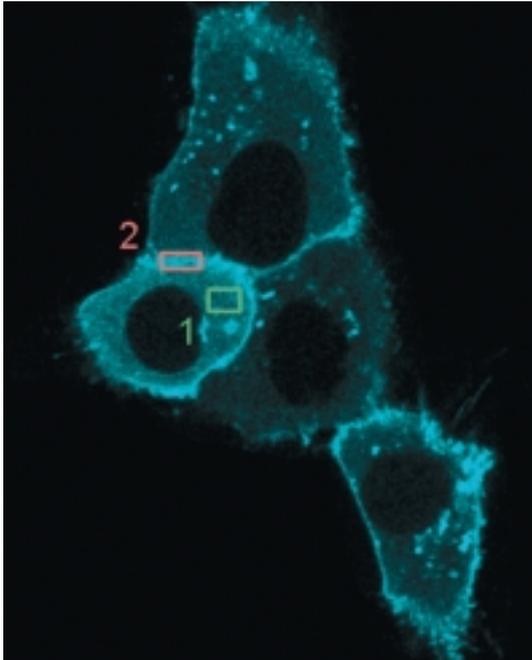


In the Time Series Control dialog window you can set all parameters for data acquisition such as the number and duration of cycles, triggers, or markers for substance addition.

Investigating protein movements

Time series of an experiment in HeLa cells, transfected with PKC-GFP. At the time $t=1$ min, the cells were stimulated with PMA. Specimen: Dr. S. Yamamoto, Hamamatsu Medical University, Japan

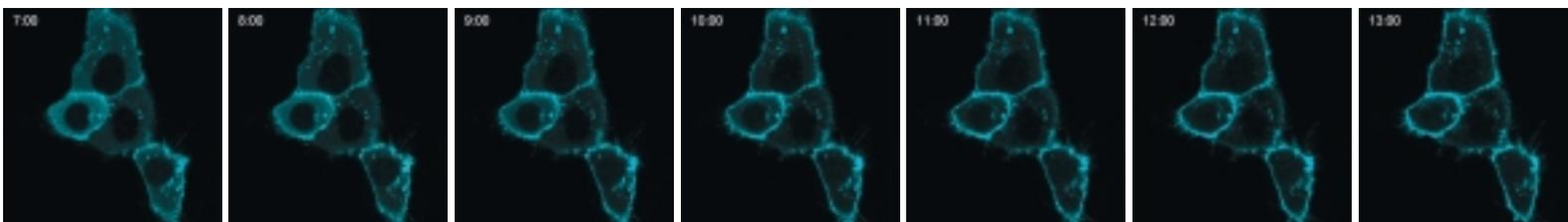
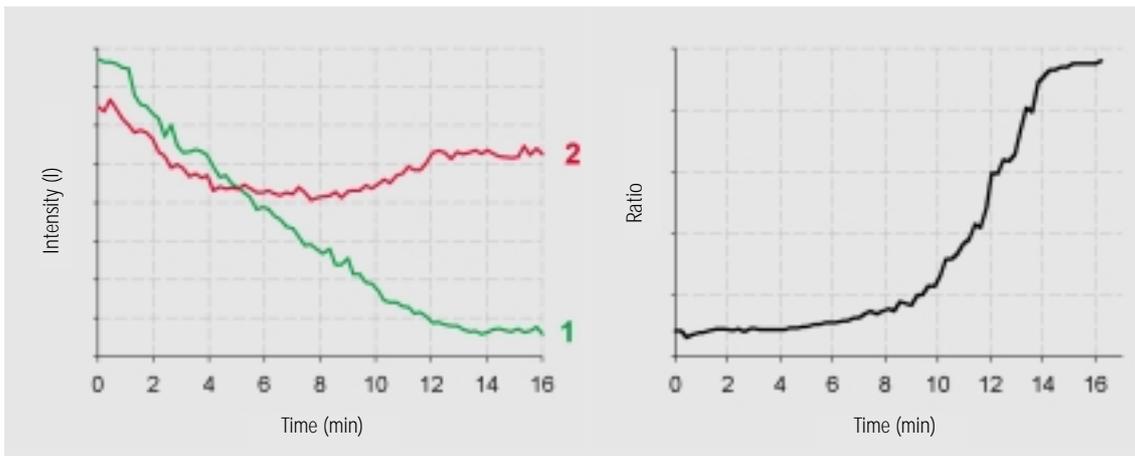




With freely defined regions of interest (ROIs) you can investigate precisely those structures of a specimen you are interested in. During image acquisition, either the series of images or the intensity curves inside the ROIs or both can be displayed. In ratiometric measurements, rather than waiting until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

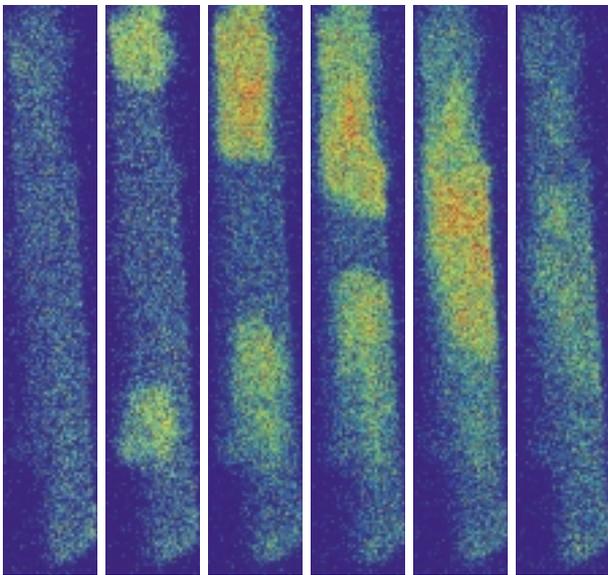
*Selection of regions of interest (ROIs) within the specimen.
ROI 1: Cytosol
ROI 2: Cell membrane*

The intensities and the ratio of the two ROIs marked above, plotted in corresponding colors.



Physiology: Observe Fast Processes with Certainty and Efficiency

The extremely fast scanning modes of the LSM 5 PASCAL, in combination with scanning fields or lines exactly matched to the specimen, allow time resolutions in the order of milliseconds. Z line scanning permits scans in the third dimension along a straight line or spline curve positioned anywhere within the specimen, thus optically cutting the specimen into vertical slices. In combination with the HRZ 200 fine-focusing stage or the piezo objective focusing device attached to the system, you can, for example, study morphological and physiological changes with superior Z and time resolution and at a rapid speed.



*Isoproterenol-induced calcium changes in insulated myocardium cells of a guinea pig, visualized with Fluo-4; frame rate about 20 frames/sec.
Specimen: Prof. M. Endo, Yamagata University, Japan*

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

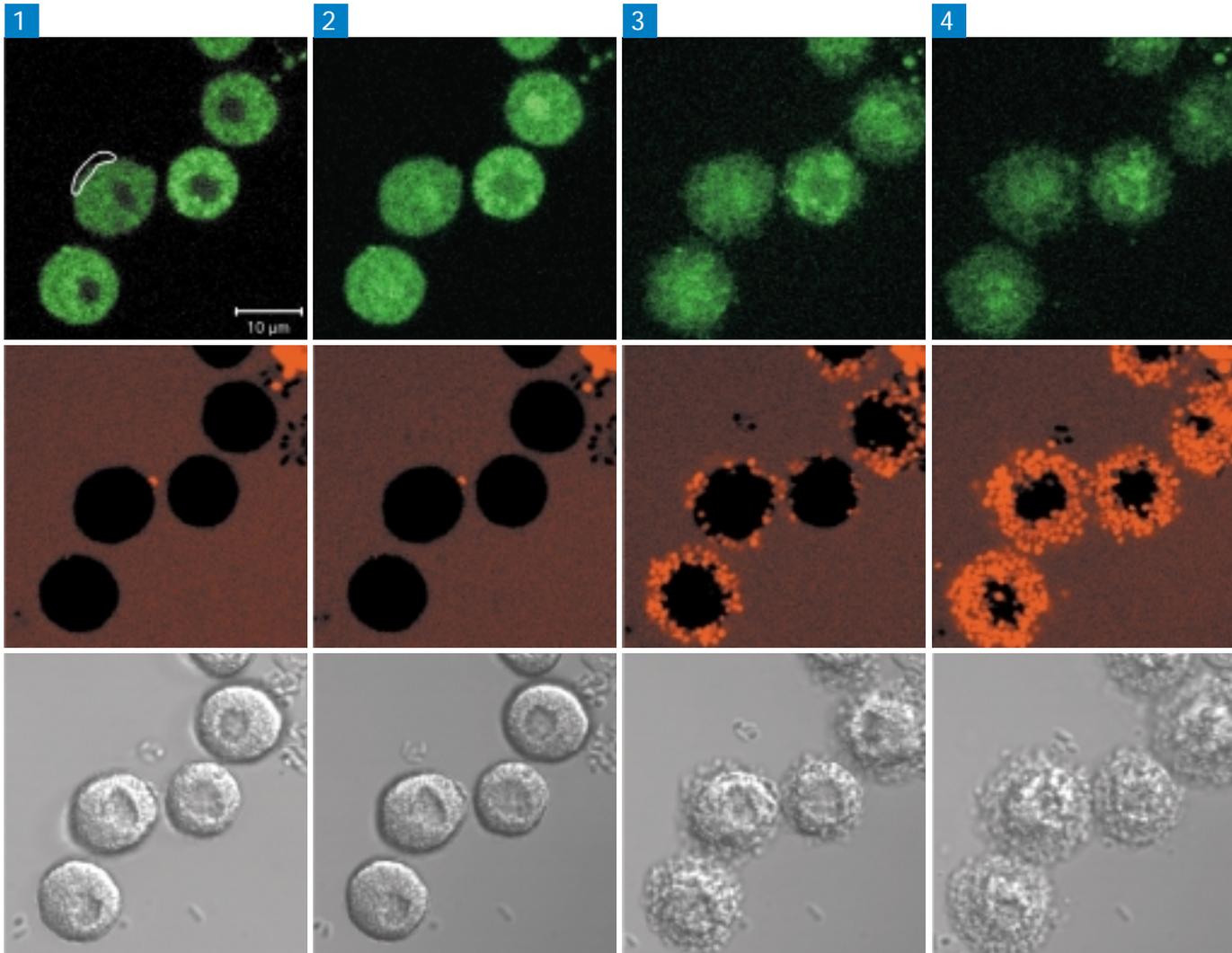
Visualization and analysis of ion concentrations:



On-line and off-line ratio for ratiometric dyes
On-line and off-line F/F_0 for single-wavelength dyes
Calibration for single-wavelength and ratiometric dyes <ul style="list-style-type: none"> • in situ and in vitro • inclusive of background correction • after titration with various curve fittings • according to Grynkiewicz
Interactive scaling of the image data series
Interactive graphic representation of the data measured in ROIs

On-line ratio computations according to stored and preselected formulae with parameters defined by the user allow the data to be presented right during acquisition. Various modes are available for the calibration of dyes for concentration analysis and display. With these features, the LSM 5 PASCAL is able to cope with the specific fluorescence properties of any dye.





Calcium release and exocytosis

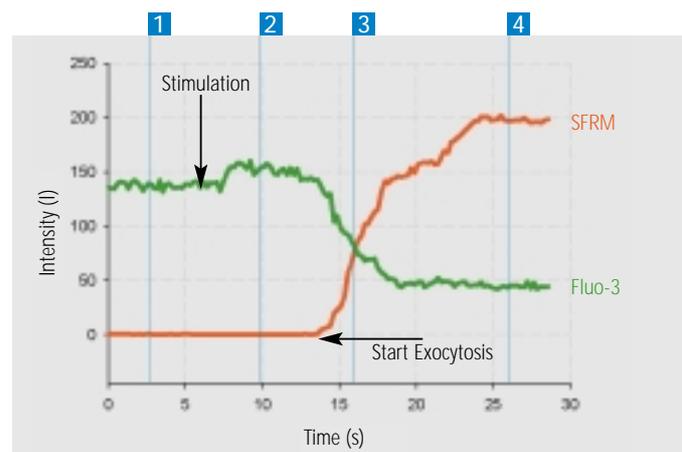
Simultaneous detection of Fluo-3 for the measurement of cytosolic calcium (green, top row) and sulphorhodamine-B (SFRM) for labeling exocytosed vesicles (red, middle row) in mast cells.

The high time resolution (6 frames/sec) was achieved by acquisition in the Dual Direction Scanning (DDS) mode.

Stimulation of the cells with Compound 48/80 causes an initial increase in intracellular calcium concentration, which then leads to exocytosis.

The differential interference contrast images (gray, bottom row) distinctly show the morphological changes after stimulation.

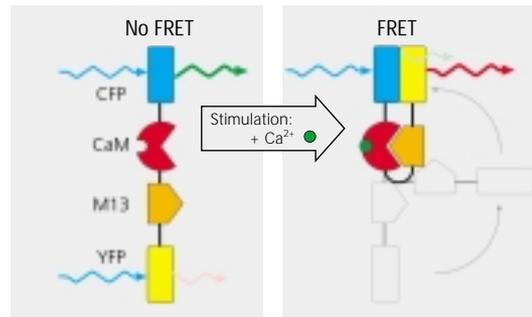
Specimen: Prof. Y. Satoh, Iwate Medical University, Japan



Intensity curves of Fluo-3 and SFRM in the region of interest shown above. The numbers above the curves correspond to the points in time indicated in the images above.

FRET: Visualize Molecular Interactions

FRET (Fluorescence Resonance Energy Transfer) is an analytical method for investigating and quantifying protein-protein interactions, in which the proteins of interest are labeled with fluorescent dyes. The emission wavelength of one dye (the donor) overlaps the excitation wavelength of the other (the acceptor). With the two spaced sufficiently close (<10 nm), the donor transfers its energy to the acceptor without any emission, whereas the acceptor emits light that can be detected.

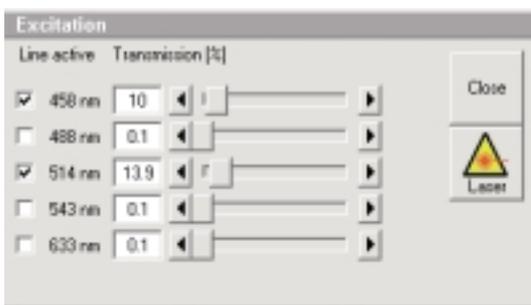


■ CaM: Calmodulin
■ M13: Calmodulin binding site

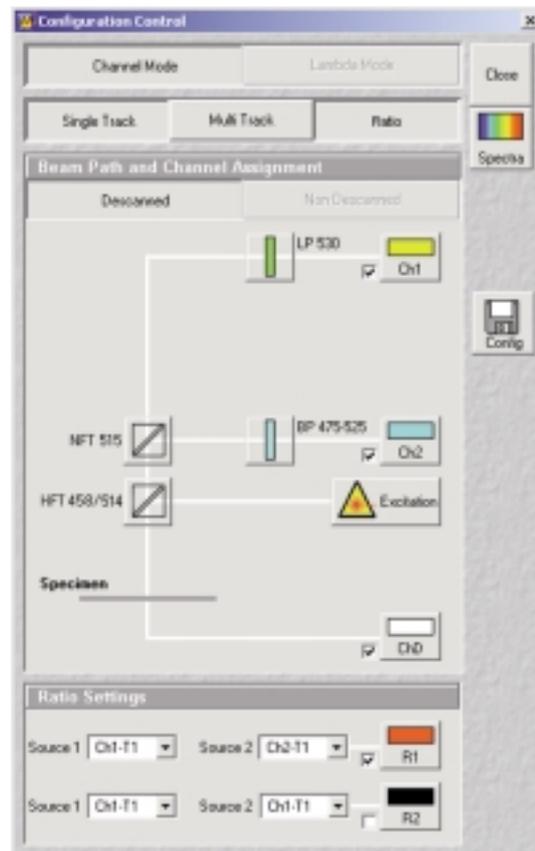
*The experiment:
Calcium imaging with the FRET indicator Yellow Cameleon 2.*

The LSM 5 PASCAL with its many hardware and software functions is an ideal environment for FRET studies. These functions include not only the broad selection of laser lines for optimum dye excitation but also the specially matched dichro mirrors and emission filters for best possible detection.

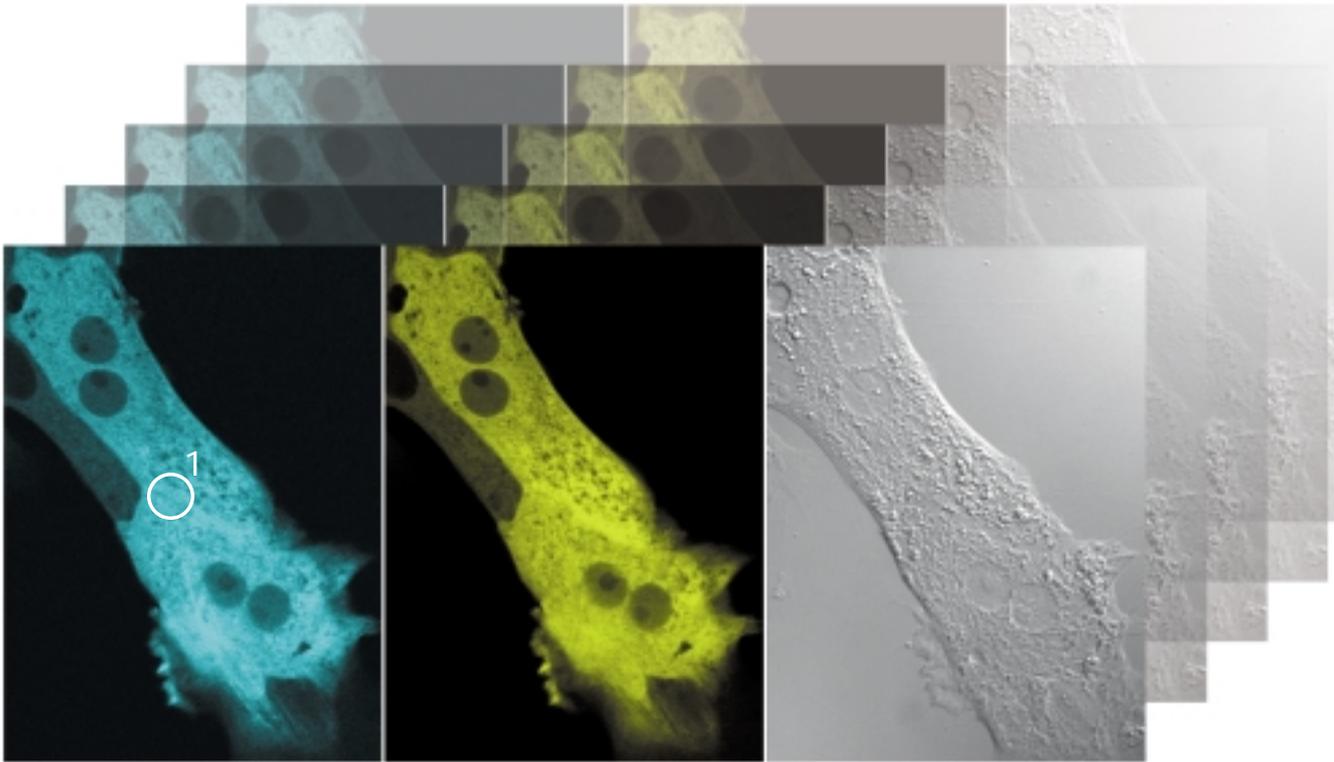
Through separately defined channels, the user can keep track of ratio measurements on the screen in real time.



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



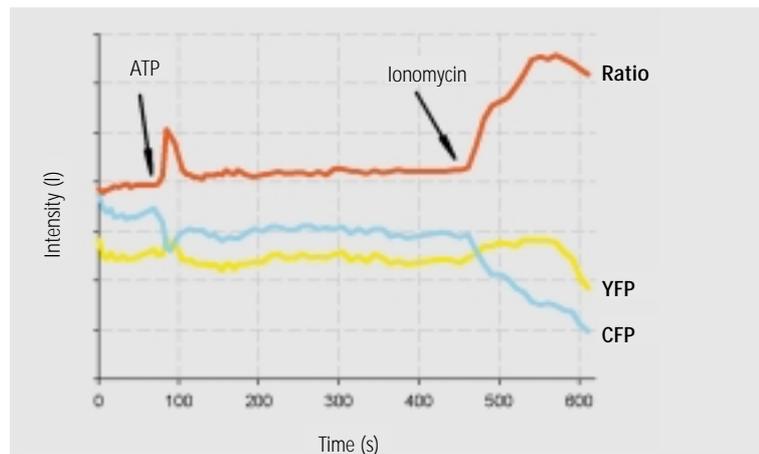
Time series of the CFP and YFP fluorescences of Yellow Cameleon 2, and differential interference contrast (in hepatocytes) after stimulation with ATP and Ionomycin. Specimen: Prof. T. Kawanishi, National Institute of Health Science, Tokyo, Japan



The time series function, optionally combined with Multitracking, allows complete FRET experiment runs to be configured conveniently and reproducibly. An elegant and well-established way of detecting FRET is known as acceptor bleaching, in which the operator uses the zoom function of the LSM 5 PASCAL to select a certain region within the specimen, and eliminates acceptor fluorescence with high laser intensity.

Since some time ago the FRET method has been established also as a sensor system for investigating enzyme activities, ion concentrations and interactions between second messengers in cells. One of the FRET sensors used to detect changes in intracellular calcium concentration is Yellow Cameleon 2.

The intensities of the CFP and YFP fluorescences and the YFP/CFP signal ratio in the ROIs marked above (left image).



Quantitative Colocalization: Looking for the Needle in the Haystack

With the LSM 5 PASCAL, quantitative colocalization analyses reach an unprecedented level of reliability and precision. Image presentation, scatter plot and data table are interactively linked to the ROI and thresholding tools.

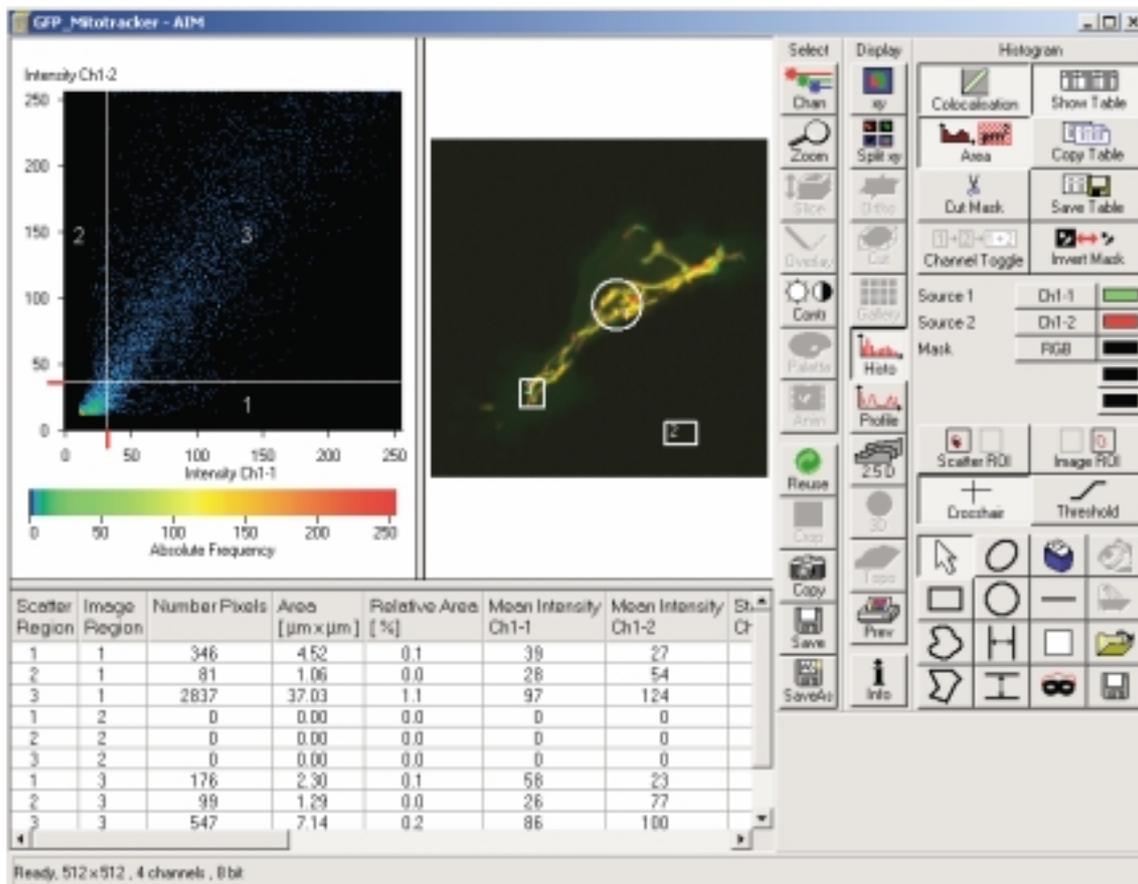
For example, the user selects a region in the scatter plot, whereupon the system immediately indicates the occurrence of this colocalization in the image. In the same way, the data table is interlinked with the scatter plot and the image. There is hardly any more intuitive and precise way of analyzing the 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 pixel intensity
 - degree of colocalization
 - coefficient of colocalization
 - Pearson's correlation coefficient
 - Manders overlap coefficient
- Export of analysis results

First-rate tools properly applied: Image presentation, scatter plots and data table are interactively linked with the ROI and thresholding tools. Expression of GFP-hMsrA (green), and mitochondria labeled with Mitotracker (red). Specimen: Prof. S. H. Heinemann, University of Jena, Germany



LSM Image Database: Faster Archiving and Retrieval



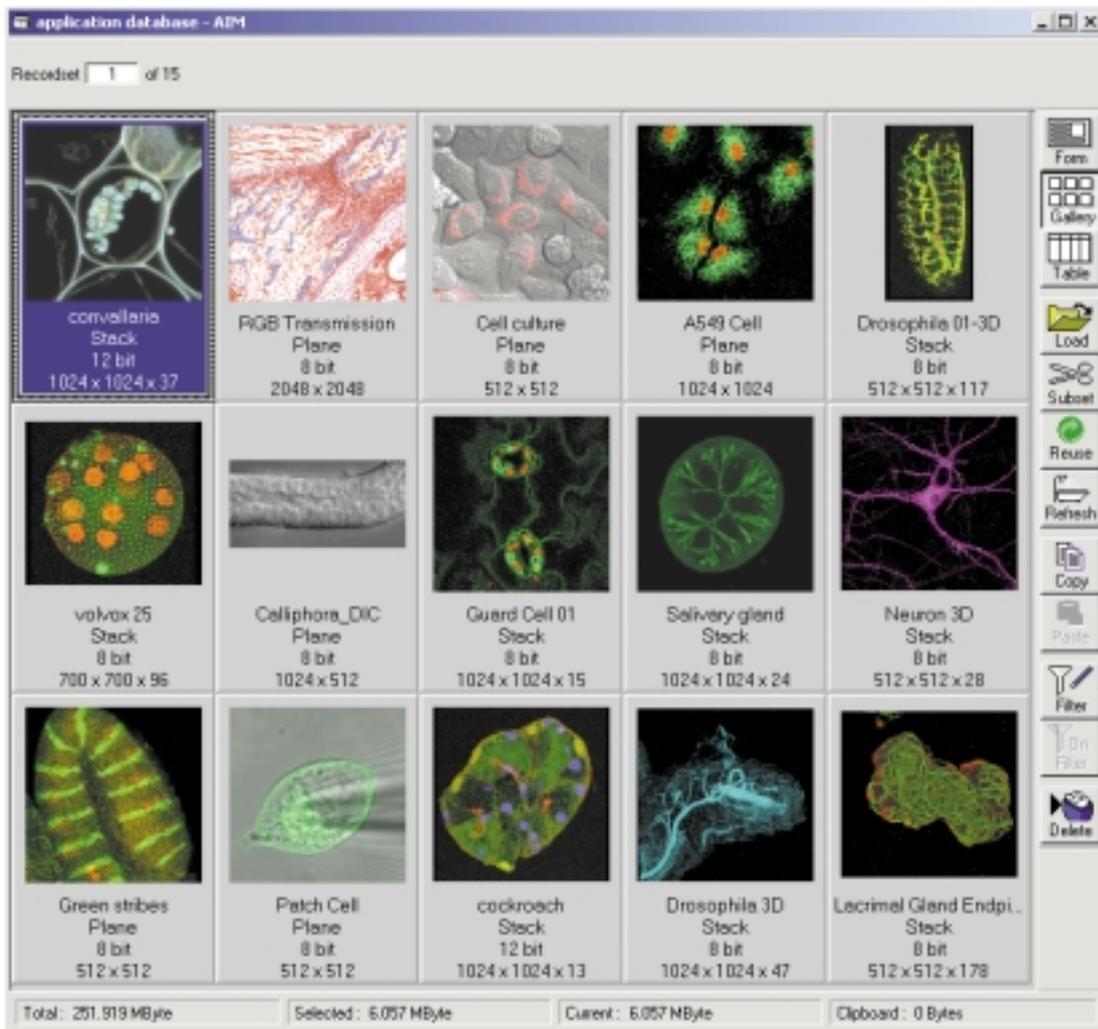
Easy overview and accessibility have been major goals in designing the archiving system for the LSM 5 PASCAL. The database stores all data, such as the laser lines used, pinhole diameter, scanning mode, objective, etc. The proven ReUse concept allows these settings to be retrieved with a mouse click. Different experiments can thus be reproduced under identical conditions at will, which saves time and guarantees reliability.

To suit individual requirements, users can freely select from different presentation capabilities.

A great number of image file formats are provided for exporting the image documents into other programs. This also applies to measured data and tables.

As a genuine multi-user system, the LSM 5 PASCAL allows the saving of user-specific configurations. Thus, all users in the lab can quickly avail themselves of a system optimized to suit their individual applications.

LSM database: The gallery presentation gives a clear overview. Each image represents the entire acquisition method. Special filter functions permit convenient database searches.



Specification

LSM 5 PASCAL System Components

Microscopes

Models	Upright: Axioplan 2 imaging MOT, Axioskop 2 MOT, Axioskop 2 FS MOT; inverted: Axiovert 200 M BP (BasePort) or SP (SidePort)
Z drive	DC motor with optoelectronic coding; smallest increment 25 or 50 nm; fast piezo focusing accessory
HRZ 200 (option)	High-precision galvanometric fine-focusing stage; focusing range 200 μm , smallest increment <10 nm
XY stage (option)	Motorized XY scanning stage with Mark & Find (XYZ) and Tile Scan (mosaic scan) functions; smallest increment 1 μm
Accessories	High-resolution digital AxioCam microscope camera; integration of incubation chambers, micromanipulators, etc.

Scanning module

Scanner	Two independent galvanometric scanning mirrors for rotation, zoom, offset
Scanning resolution	4 x 1 to 2048 x 2048 pixels, user-definable
Scanning speed	13 x 2 speeds; line frequencies from 4 to 2600 lines/sec, 5 fps with 512 x 512 pixels (max. 77 fps with 512 x 32 pixels)
Scanning zoom	0.7x to 40x, variable in steps of 0.1
Scanning rotation	Free rotation through 360°, variable in steps of 1°
Scanning field	Field diagonal 18 mm (max.) in the intermediate image plane, homogeneous field illumination
Pinhole	1 confocal pinhole, continuously variable diameter, preadjusted
Detection	1 or 2 confocal channels (R/FL), 1 optional external transmitted-light channel (DIC-capable); each channel equipped with high-sensitivity PMT detectors
Data depth	Selectable between 8 and 12 bit

Laser Modules

Lasers	Ar laser (458, 488, 514 nm) 25 mW; ArKr laser (488, 568 nm) 30 mW; HeNe laser (543 nm) 1 mW; HeNe laser (633 nm) 5 mW; Diode laser (405 nm) 25 mW (end-of-lifetime specifications)
Fiber optics	Polarization-preserving single-mode fibers
Attenuation	Individual and variable intensity setting of all laser lines by means of MOTF

Electronics Module

LSM 5 Control	Control of microscope, laser modules, scanning module and other (accessory) components, using a high-performance digital signal processor (DSP)
Computer	High-end PC with ample RAM and hard disk storage capacity; with ergonomic high-resolution monitor or TFT flat panel display; many accessories. Windows 2000/NT 4.0 operating system with multi-user capability



Standard Software

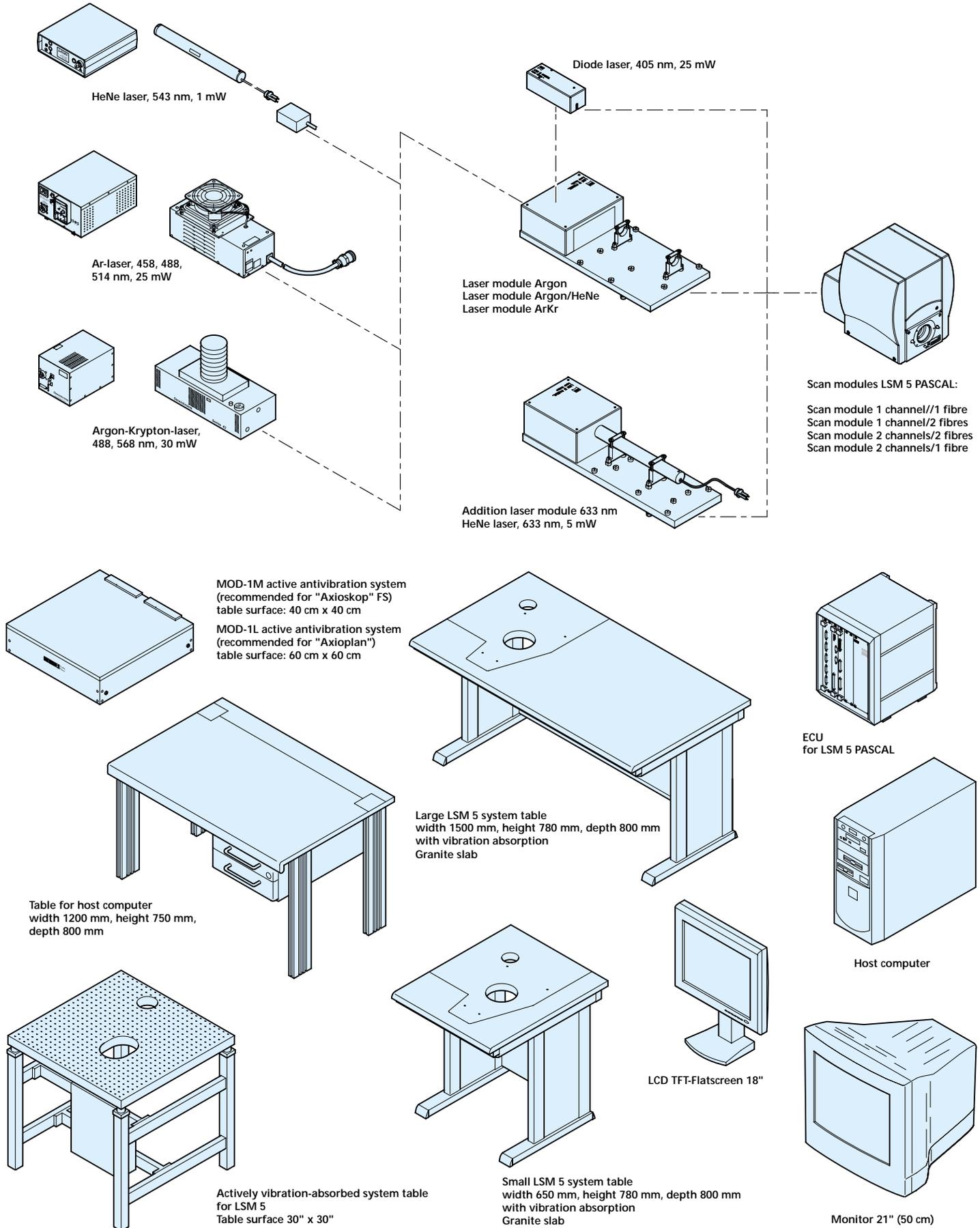
System configuration	Convenient control and configuration of all motor-driven microscope functions, the laser modules and the scanning module; saving and retrieval of application-specific configurations
ReUse function	Restoration of acquisition parameters per mouse click
Acquisition modes	Spot, line/spline, frame, Z stack, time series and combinations: xy, xyz, xyt, xyzt, xz, xt, xzt, spot-t. On-line computation and presentation of ratio images; averaging and summation (linewise or framewise, configurable); Step Scan (for higher frame rates, configurable)
Crop function	Convenient selection of scanning ranges (simultaneous zoom, offset and rotation)
ROI scan	Scanning of up to 99 regions of interest (ROIs) of any shape, and blanking of the lasers
Spline scan	Scanning along a freehand defined line
Multitracking	Signal crosstalk in multifuorescence acquisition minimized by fast framewise change between excitation lines
Image processing	Image processing options for any kind of mathematical procedures, such as crosstalk correction by selective channel subtraction
Visualization	Orthogonal view (xy, xz, yz in one view), cut view (3D section made under a freely definable spatial angle), 2.5D view for time series of line scans, projections (stereo, maximum, transparent) for single frames and series (animations), depth coding (pseudo-color presentation of height information), brightness and contrast variation, off-line interpolation for Z stacks, selection and modification of color lookup tables (LUTs), drawing functions for documentation
Analysis, measurement	Advanced tools for colocalization and scatter plot analysis with individual parameters and options, profile measurement on straight lines and curves of any shape, measurement of lengths, angles, areas, intensities, and many other capabilities
Image operations	Addition, subtraction, multiplication, division, ratioing, shift, filters (low pass, median, high pass, etc., or user-definable)
Data archiving, export, import	LSM image database with comfortable functions for managing images together with the associated acquisition parameters; Muprint function for creating assembled image-plus-data views; more than 20 file formats (TIF, BMP, JPG, PSD, PCX, GIF, AVI, Quicktime, ...) for compatibility with all common image processing programs

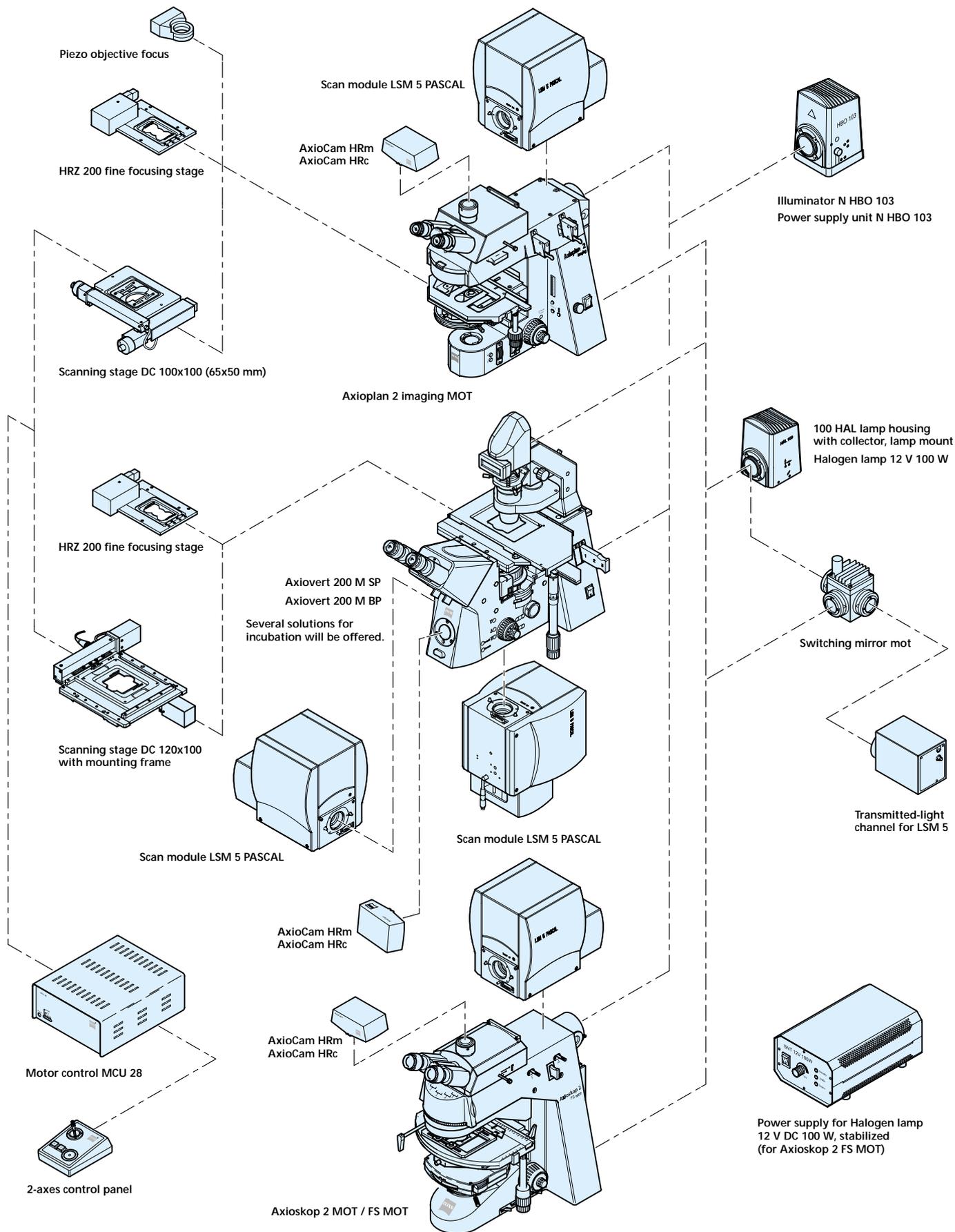
Software Options

LSM Image VisArt	Fast 3D and 4D reconstruction and animation (various modes: shadow projection, transparency projection, surface rendering)
3D Deconvolution	Image restoration based on computed point spread functions (modes: nearest neighbor, maximum likelihood, constraint iterative)
Multiple Time Series	Complex time series with varied application-specific configurations
3D for LSM	3D presentation and 3D measurement of volume data records
Physiology	Comprehensive software for the analysis of time series, graphic mean-of-ROI analyses, on-line and off-line calibration of ion concentrations
Topography Package	Visualization of 3D surfaces (fast rendering modes), plus many measurement functions (roughness, surface areas, volumes)

Image Browser Free software package for the display, editing, sorting, printing and export/import of LSM 5 images

System Overview LSM 5 PASCAL





Functions
of the LSM 5 PASCAL
from Carl Zeiss

Multitracking

Scanning mode available with the LSM 5, generates multi-fluorescence images without crosstalk of emission signals, by means of fast switching between excitations, and quasi-simultaneous detection.

Spline Scan

Scanning along a freehand-defined line for recording fast (physiological) processes, e.g. along neurons.

Spot Scan

Scanning mode in which the signal intensity at a confocal point can be tracked with extremely high temporal resolution.

Step Scan

Fast overview scan in which intermediate lines are added by interpolation.

Tile Scan

Records an overview image consisting of a number of tiled partial images for the recording of larger objects with improved resolution.

Glossary

CFP	Cyan Fluorescent Protein
DDS	Dual Direction Scan
DIC	Differential Interference Contrast
DSP	Digital Signal Processor
FRET	Fluorescence Resonance Energy Transfer
GFP	Green Fluorescent Protein
MOTF	Mechano-optical Tunable Filter
PMA	Phorbol 12-Myristate 13-Acetate
ROI	Region of Interest
SFRM	Sulforhodamine-B
YFP	Yellow Fluorescent Protein

Highlights of Laser Scanning Microscopy

1982

The first Laser Scanning Microscope from Carl Zeiss.
The prototype of the LSM 44 series is now on display in the Deutsches Museum in Munich.

1988

The LSM 10 – a confocal system with two fluorescence channels.

1991

The LSM 310 combines confocal laser scanning microscopy with state-of-the-art computer technology.

1992

The LSM 410 is the first inverted microscope of the LSM family.

1997

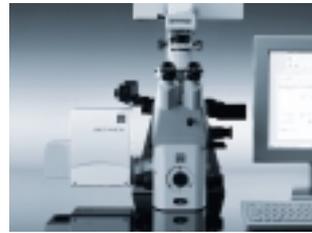
The LSM 510 – the first system of the LSM 5 family and a major breakthrough in confocal imaging and analysis.

1998

The LSM 510 NLO is ready for multiphoton microscopy.

1999

The LSM 5 PASCAL – the personal confocal microscope.





Extensive Customer Support

It is part of our tradition to ensure that our customers can use our products efficiently. To that end, Carl Zeiss Jena renders a range of after-sale services. Consultants and technicians especially responsible for your region will help you to make the best possible use of your LSM 5 PASCAL in your research.

Every system installation is followed by intensive operator training, and users are offered in-depth courses and workshops on practice-oriented aspects of laser scanning microscopy.



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