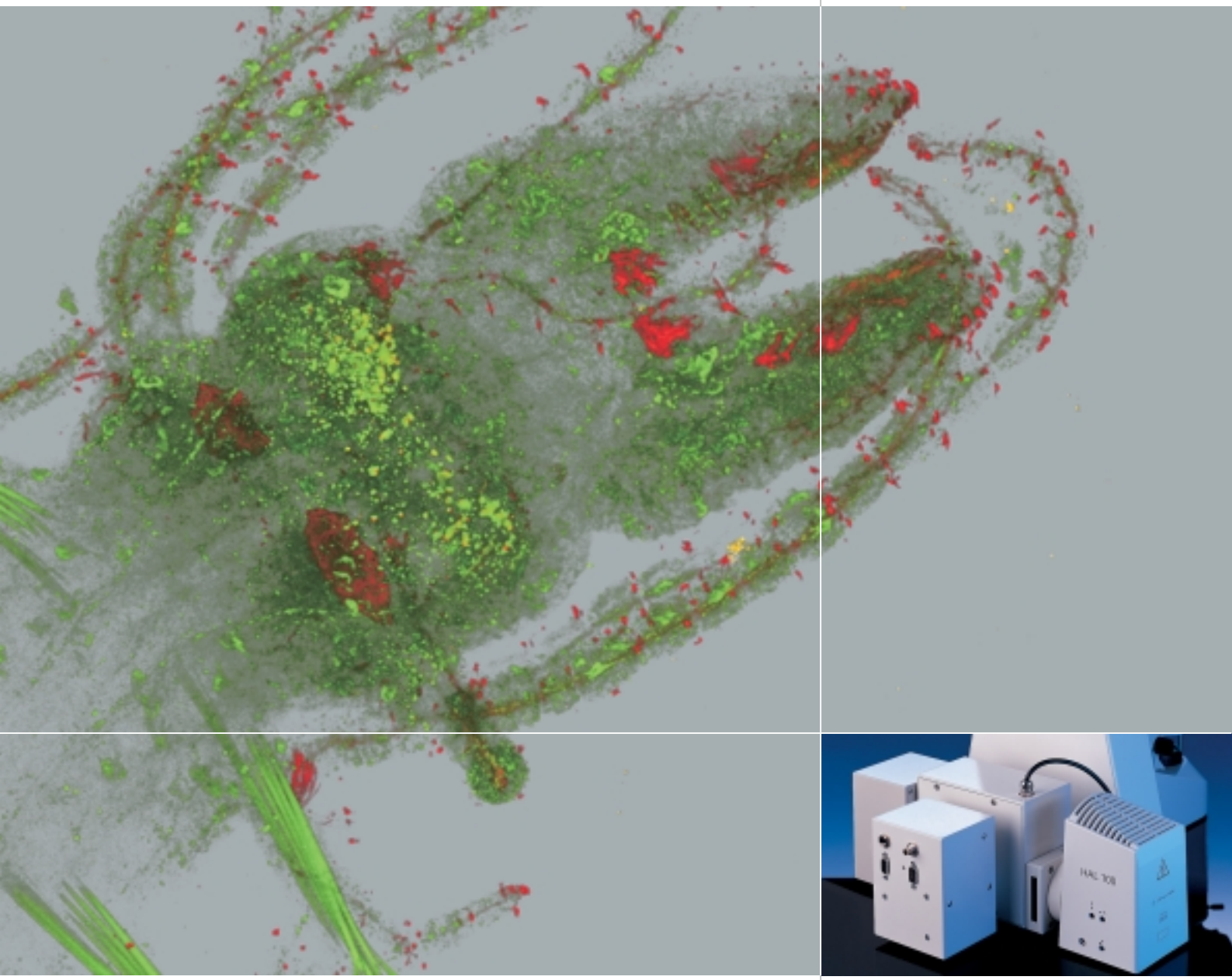
LSM 510 NLO and LSM 510 META NLO

Multiphoton Laser Scanning Microscopes



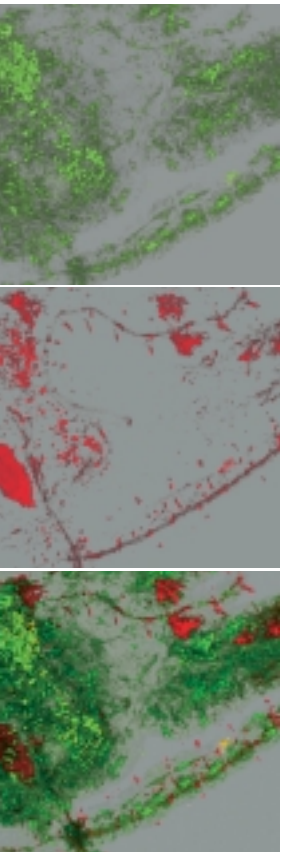
Deep Insights Carefully Gained

ZEISS

LSM 510 NLO and LSM 510 META NLO
Deep Insights Carefully Gained

In multiphoton microscopy, femtosecond lasers make it possible to create and detect fluorescence signals up to 500 µm deep within tissue. At the same time, the excitation of the fluorochrome by NIR radiation, which is limited to the focal spot, allows careful examination of living cells and tissue specimens. As many conventional fluorochromes used in multiphoton microscopy show very wide excitation spectra, a single wavelength can simultaneously excite a whole variety of these dyes in a specimen.

The system solutions offered by Carl Zeiss are superior in efficiency, sensitivity and flexibility.



Bristle-worm (Brania), labeled for tubulin (FITC, green) and serotonin (CY3, red). Both fluorochromes were excited simultaneously at 780 nm with a femtosecond laser. Specimen: Dr. R. Hessling, University of Osnabrück, Germany

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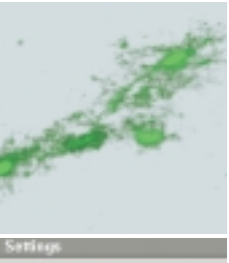
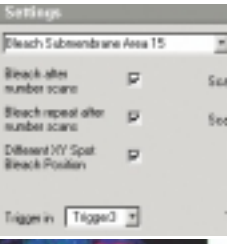
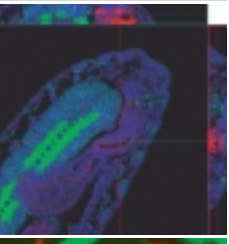
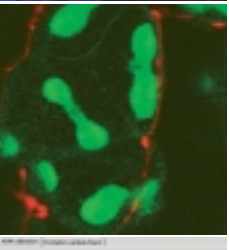
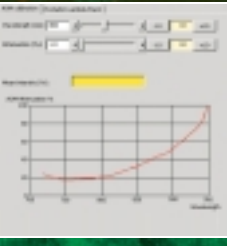
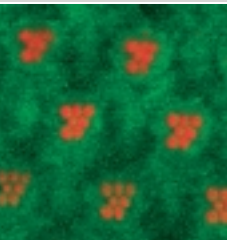


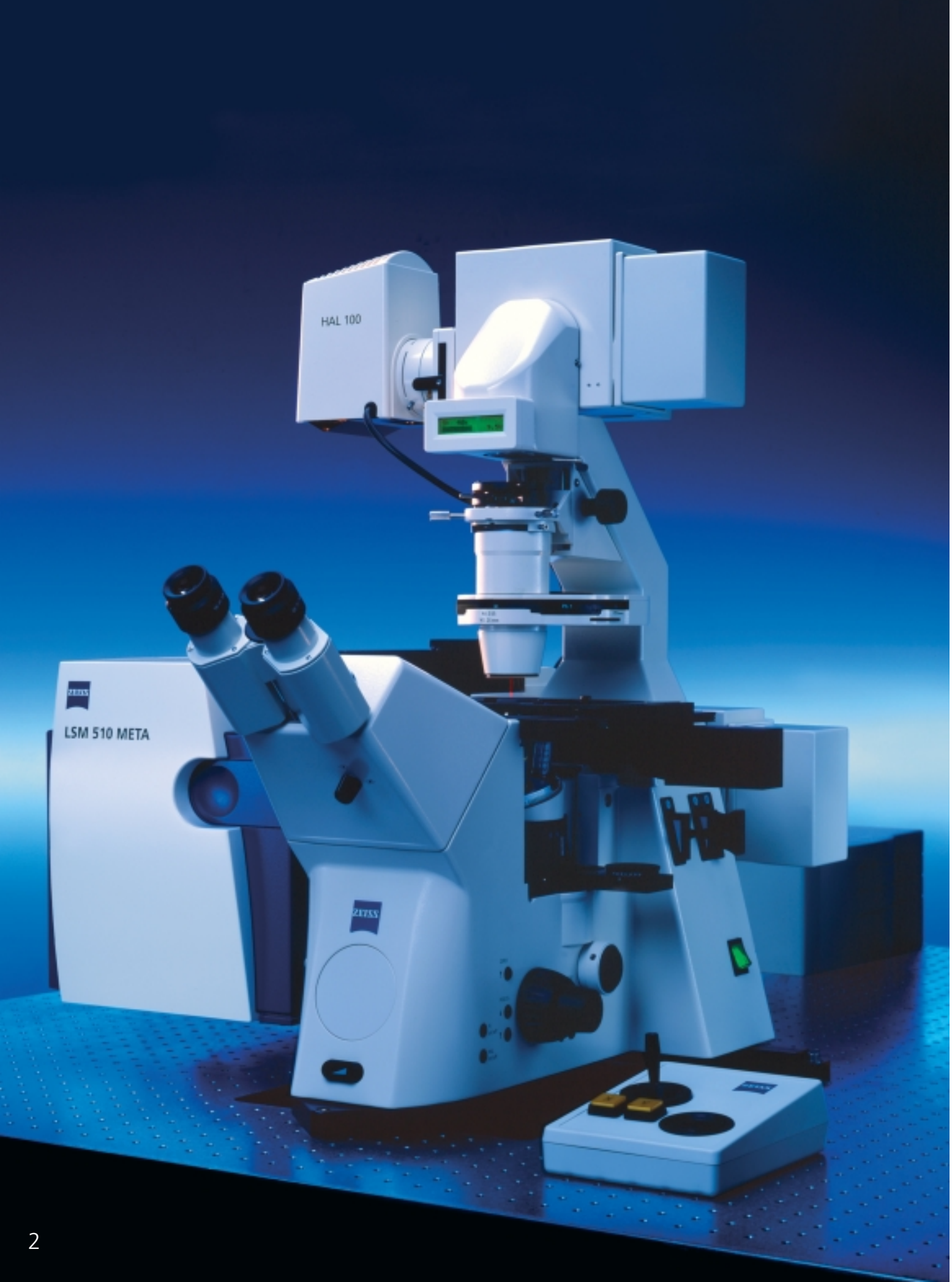











HAL 100
LSM 510 META

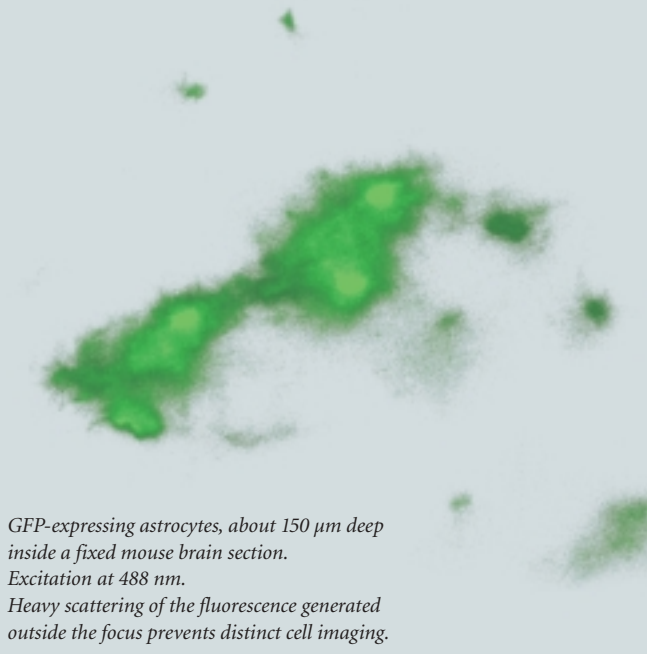

LSM 510
HAL 100

The Principle of Multiphoton Microscopy

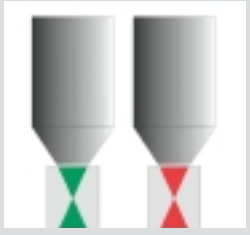
Excitation on the Spot

So far researchers attempting to generate fluorescence signals deep inside fluorescent tissue sections quickly came up against limitations. This was caused by the increase in absorption and scattering of the exciting and emitted light when focusing deeper into the tissue.

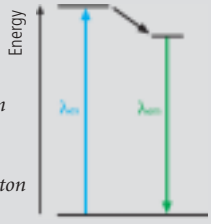
The LSM 510 NLO and LSM 510 META NLO conquer this drawback: they can image fluorescence signals emitted at depths of several hundreds of µm. This is mainly due to two properties: The bigger longer wavelength of the femtosecond laser used, and the restriction of fluorescence excitation to the focal spot.



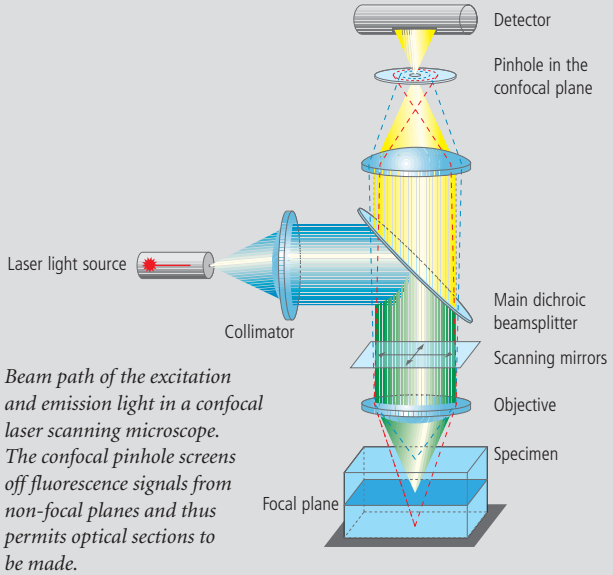
GFP-expressing astrocytes, about 150 µm deep inside a fixed mouse brain section.
Excitation at 488 nm.
Heavy scattering of the fluorescence generated outside the focus prevents distinct cell imaging.



Femtosecond lasers excite the dye only in the focal spot.



Energy diagram of fluorescence generation with single photon excitation.

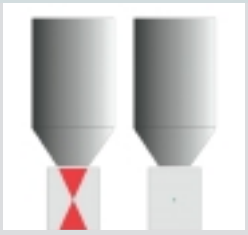


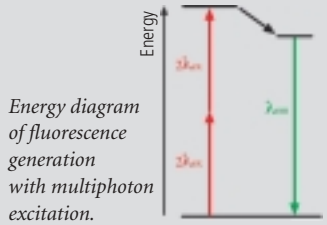
Beam path of the excitation and emission light in a confocal laser scanning microscope. The confocal pinhole screens off fluorescence signals from non-focal planes and thus permits optical sections to be made.

Prof. W. Denk,
Max Planck Institute of Medical Research,
Heidelberg, Germany

"To get high-resolution images from the depths of light-scattering tissue, there is no alternative to two-photon microscopy.
Half a millimeter below the surface of a brain section, the detection efficiency of a two-photon microscope (equipped with appropriate widefield detectors) is about 10,000 times better than that of a confocal microscope."

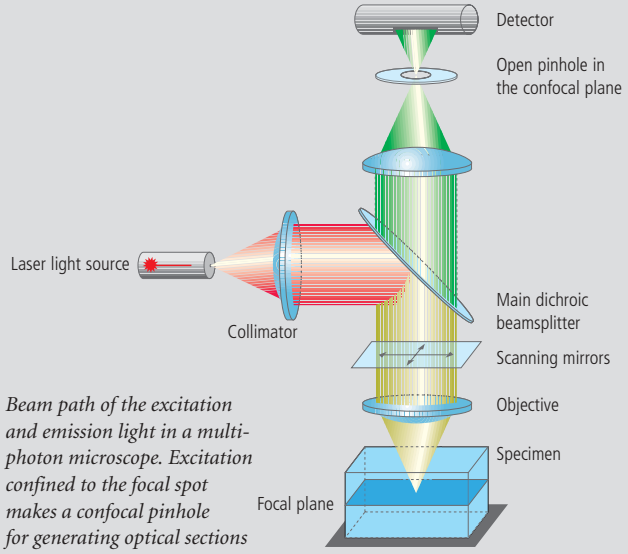
The same cells excited with a femtosecond laser at 890 nm. Excitation is confined to the focal spot. As a result, the fine cell processes are distinctly visible.





Energy diagram of fluorescence generation with multiphoton excitation.

Femtosecond lasers excite the fluorochrome only at the focus.



Beam path of the excitation and emission light in a multiphoton microscope. Excitation confined to the focal spot makes a confocal pinhole for generating optical sections superfluous.

The limits of confocal microscopy

The confocal microscope reaches its limits when it comes to imaging fluorescent structures in deeper layers of tissue. The deeper the target spot, the greater are the absorption and scattering of visible light. At a certain depth, the excitation intensity is no longer sufficient to create a fluorescence signal that provides a distinct image of the structure of interest.

Nonlinear optics (NLO): Excitation on the spot

A femtosecond laser used on the LSM 510 NLO or LSM 510 META NLO eliminates most of the restrictions of the confocal microscope. This laser emits light in the near infrared spectral range (between 700 and 1100 nm), in ultrashort pulses with a pulse energy of up to 170 kW and a repetition rate of 76 to 90 MHz. Spot-focused on the object of interest, the laser beam excites fluorescence if at least two photons are absorbed by a fluorochrome molecule within less than a femtosecond (10^{-15} seconds). This effect varies exponentially with the intensity of the excitation light. The probability of excitation is maximum at the objective focus, outside of which it drops extremely fast. Thus, the fluorescent light created by this nonlinear effect exclusively derives from the focal spot. Therefore, the entire light emitted can be utilized for imaging the signal.

System Components

Efficiency by Perfect Interaction

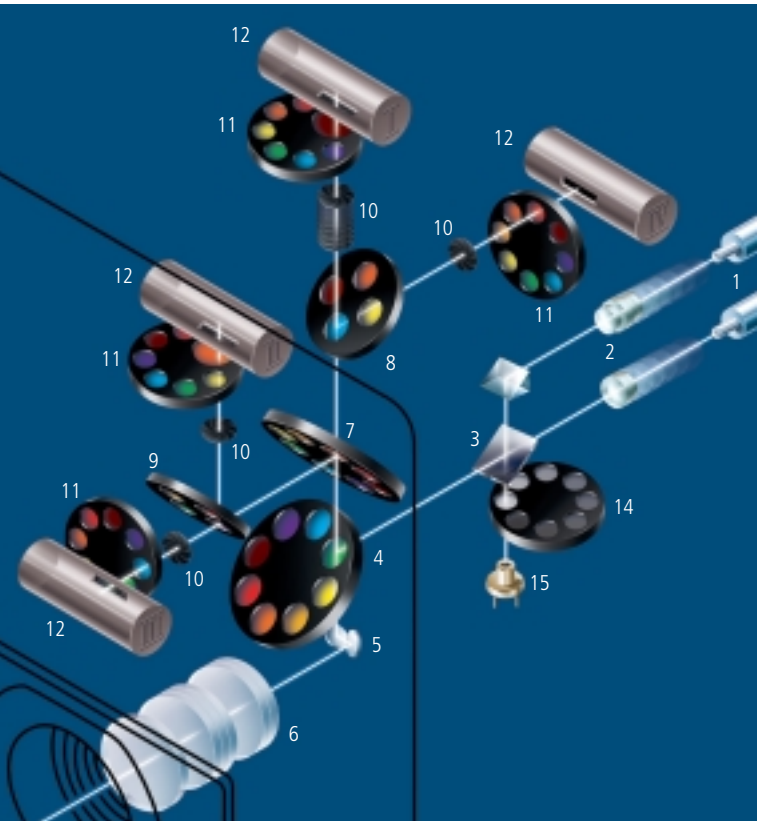
The LSM 510 NLO and LSM 510 META NLO microscopes implement multiphoton microscopy at its best. With their exactly matched components, they decisively expand your experimental capabilities.

Scanning module

The scanning module is the core of the LSM 510 NLO and LSM 510 META NLO.

It contains motor-driven collimators, scanning mirrors, individually adjustable and positionable pinholes, and highly sensitive detectors. All these components are arranged to ensure optimum specimen illumination and efficient collection of reflected or emitted light.

Beam path (schematic) in the LSM 510 NLO scanning module



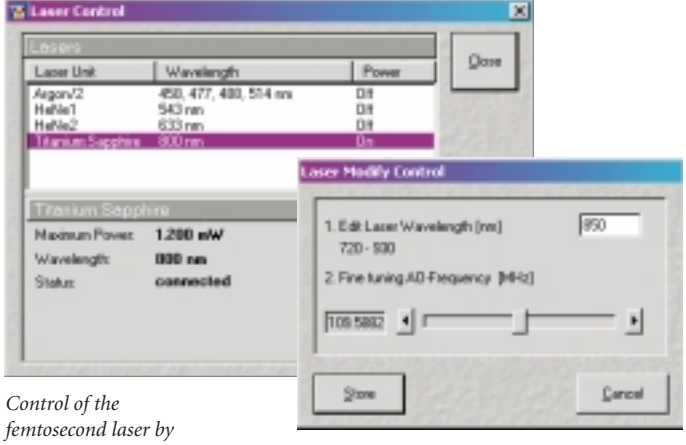
Detectors

1) Descanned: The LSM 510 NLO has up to four detectors with individual pinholes. The LSM 510 META NLO permits the emission signal to be split up according to spectral properties. With the innovative META detector you can identify fluorochromes by their spectral signature – either on-line or off-line.

2) Non-Descanned: Non-descanned detectors are an indispensable option for detecting heavily scattered fluorescence signals, for example in brain tissue. A motor-driven filter wheel allows you to detect two fluorescence signals simultaneously. You can use up to four detectors, two each in the reflected-light and transmitted-light beams.

Laser module

To excite fluorescent structures, the LSM 510 NLO and LSM 510 META NLO instruments provide laser lines in a range of 458 – 633 nm. The laser is attenuated by means of acousto-optical tunable filters (AOTF). The additional coupling of a femtosecond laser, either direct or via an optical fiber, turns the instrument into a multiphoton microscope. In the fiber-coupled configuration, optimum pulse width setting at the specimen is ensured by a grating dispersion compensator (GDC) and a post fiber compressor (PFC); laser attenuation is by an acousto-optical modulator.



Control of the femtosecond laser by the LSM software

Microscopes

The high-grade Zeiss research microscopes guarantee unsurpassed image quality and optical perfection. You can choose between Axioplan 2 imaging MOT, Axioskop 2 FS MOT and Axiovert 200 M. All are fully motor-driven, LSM-software-controlled, and equipped with ICS optics.

Objectives

Objectives especially designed for use with NIR light provide the best possible combination of resolving power, aperture and working distance. We highly recommend the IR Achroplan series.

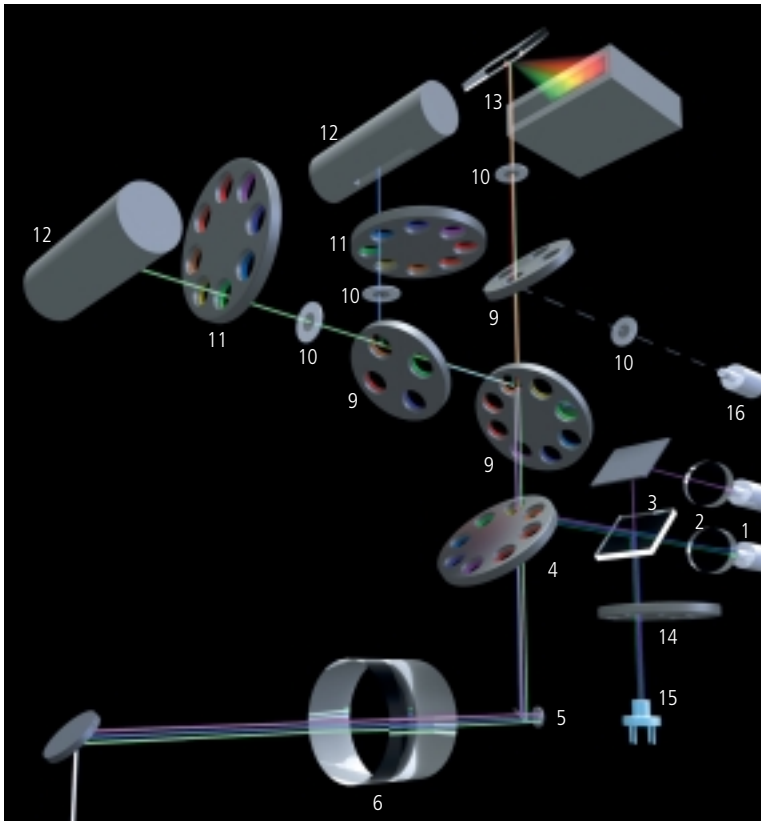
Control computer and software

With the PC featuring a powerful processor and the LSM software you can control all system components, including wavelength tuning for some types of directly-coupled femtosecond lasers, making them substantially easier to handle. In addition to the acquisition parameters, the software also saves the laser settings, so that the operating variables for a particular experiment can be reproduced in a later experiment with speed, ease and certainty.

Electronics module

The LSM 510 NLO and LSM 510 META NLO are controlled by digital signal processors (DSP). They bring about fast, flexible synchronization of scanners, AOTF, AOM and detectors. This enables such sophisticated functions as Multitracking; Spline Spot, and Step Scan; Region of Interest (ROI) Scan; and Spot, Z, and ROI Bleaching independent of the scan position. Moreover, this technology permits the implementation of new scanning functions through simple software upgrades.

Beam path (schematic) in the LSM 510 META NLO scanning module



1 Optical fibers
2 Motor-driven collimators
3 Beam combiner
4 Main dichroic beamsplitter
5 Scanning mirrors
6 Scanning lens
7, 8, 9 Secondary dichroic beamsplitters
10 Pinholes
11 Emission filters
12 Photomultiplier tubes
13 META detector
14 Neutral density filter
15 Monitor diode
16 Fiber outcoupling

Femtosecond Laser and NDD

Exploring the Depth

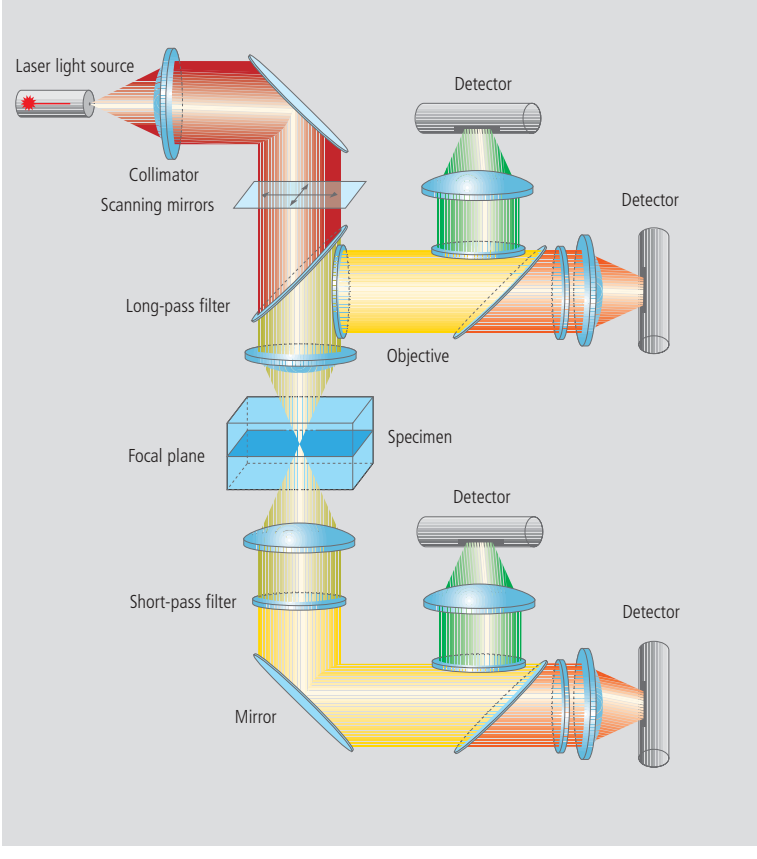
The structures and functions of biological systems are increasingly studied on close-to-life research models, including intact animals. High-resolution imaging techniques, however, usually have to rely on thin tissue sections, which yield limited information. Multiphoton microscopy adds a decisive tool to your experimental capabilities. Even in a living animal, fluorescence-labeled cellular structures at depths up to 500 µm can be precisely located and exactly imaged.

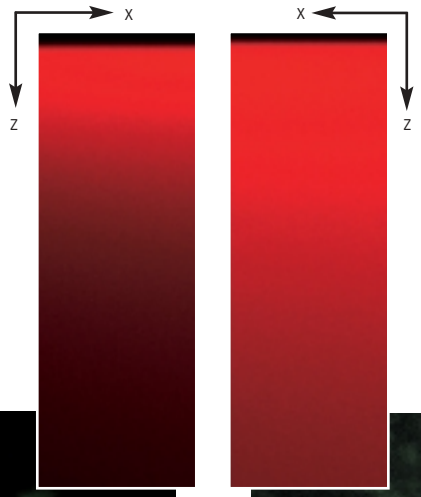
PD Dr. F. Kirchhoff,
Max Planck Institute of Experimental Medicine, Göttingen, Germany

"Analysis of highly branched processes of EGFP-positive astrocytes at high spatial and temporal resolution deep in acutely isolated tissue sections is an easy task with the Zeiss LSM 510 NLO.
The combination of the LSM scanhead, Axioskop 2 FS MOT, Coherent Ti:Sa laser and the straightforward software works from scratch. It is a faithful piece of equipment we rely on."

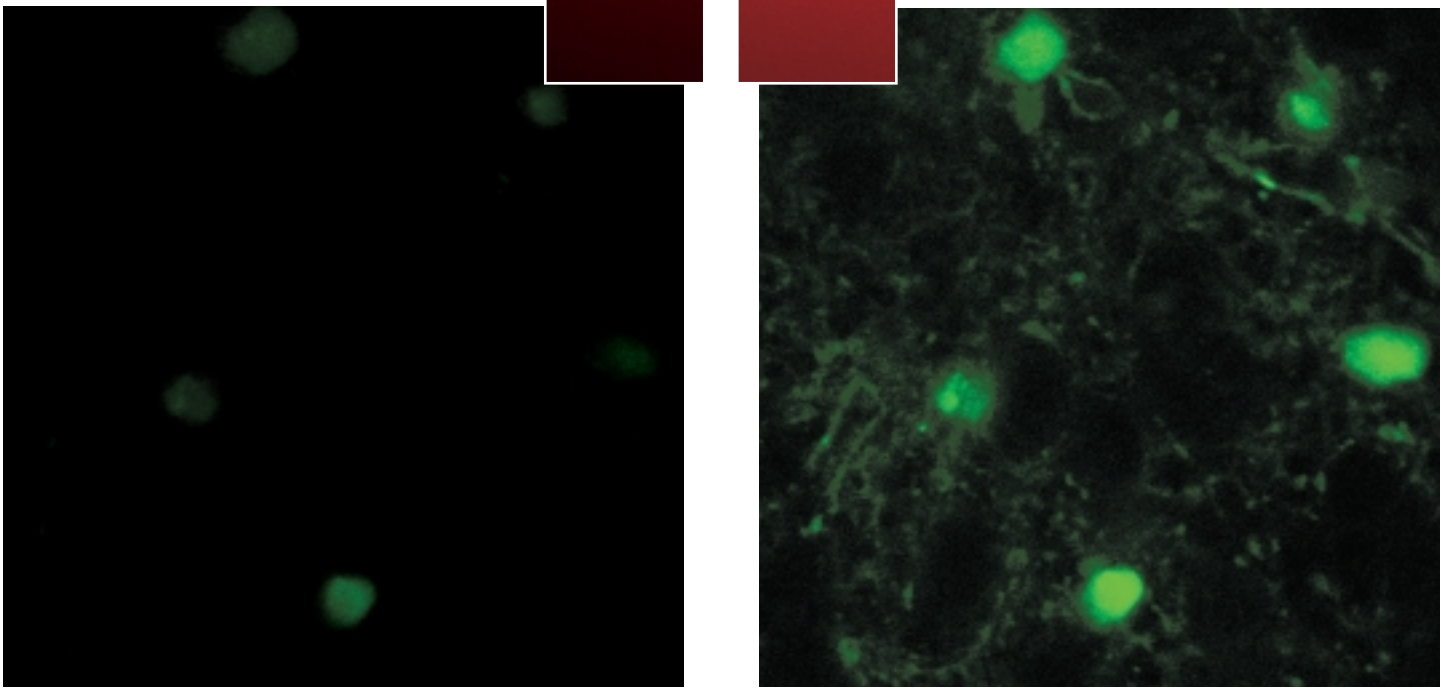
Non-descanned detectors (NDDs), arranged at the optimum microscope port, detect heavily scattered fluorescence signals from deep tissue layers.

Non-descanned detectors collect the fluorescence signal before the scanning lens.





Fluorescence signals generated by femtosecond lasers are precisely detected by non-descanned detectors at much greater depths compared to the detectors in the scanning module (left: descanned; right: non-descanned).



GFP-expressing astrocytes, 300 µm deep in a fixed brain section taken from a genetically engineered mouse, imaged with detectors in the scanning module (left) and with a non-descanned detector (right). Specimen: PD Dr. F. Kirchhoff, Max Planck Institute of Experimental Medicine, Göttingen, Germany.

The proper light source ...

The efficient generation of fluorescence signals by visible excitation light is drastically impaired in deeper tissue layers due to light absorption and scattering. The femtosecond laser, by comparison, efficiently excites fluorescent dyes even in regions several hundreds of µm below the surface.

... and the right detectors

Exciting fluorescence deep down in a tissue is one matter; detecting it is another. Where the fluorescent signal is emitted deep in tissue and subjected to heavy scattering, its registration calls for direct detectors located in front of the scanning lens and the confocal pinhole. Our systems provide up to four non-descanned detectors (NDDs) arranged in the reflected-light and transmitted-light beam paths of the microscope. This efficient method of detecting the emitted light has a higher signal-to-noise ratio and supplies more detail information.

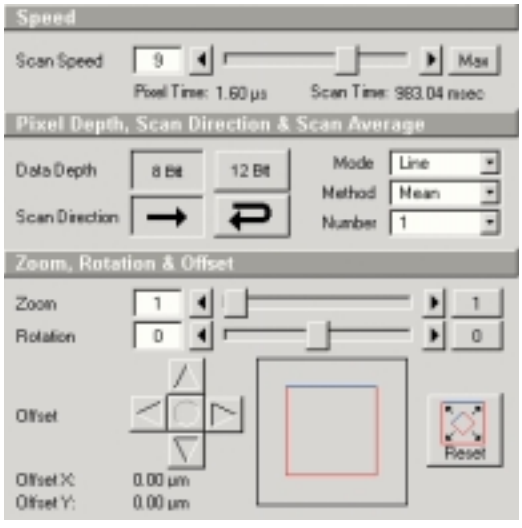
Signal Processor and Laser Attenuation

For Intelligent Scanning Strategies

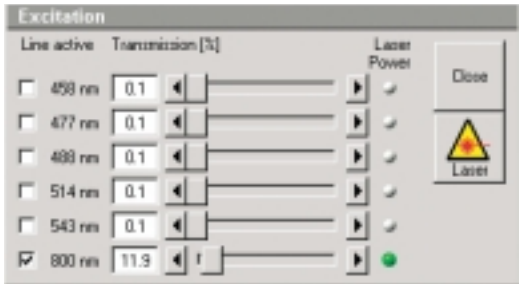
With their variety of scanning strategies, the LSM 510 NLO and LSM 510 META NLO are fundamental tools for many experimental approaches. Digital signal processors (DSPs) control the settings for optical manipulation and image acquisition, adapted to the respective experiment.

LSM software and DSPs control all system functions. Two independently controllable scanning mirrors enable such different scanning strategies as point illumination of different sites in the specimen or scanning along a freely defined line. All parameters for image acquisition like detector voltage and the attenuation of all lasers are exactly synchronized.

Some of the many scanning strategies provided are the definition of different laser settings depending on the focal plane in 3D imaging (Auto Z Brightness Correction), the limitation of specimen illumination and image acquisition to real regions of interest defined with single-pixel accuracy (RealROI), and bidirectional scanning (Dual Direction Scan, DDS). The fast and exact control of laser intensity is decisive also for a high level of specimen preservation. Outside the image acquisition times, the specimen is not subject to any light load.



Control of the scanning parameters by the LSM software.

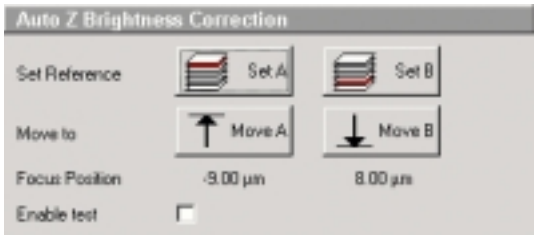


Laser intensity attenuation is set gradually in steps between 0.1 and 100.

Auto Z Brightness Correction

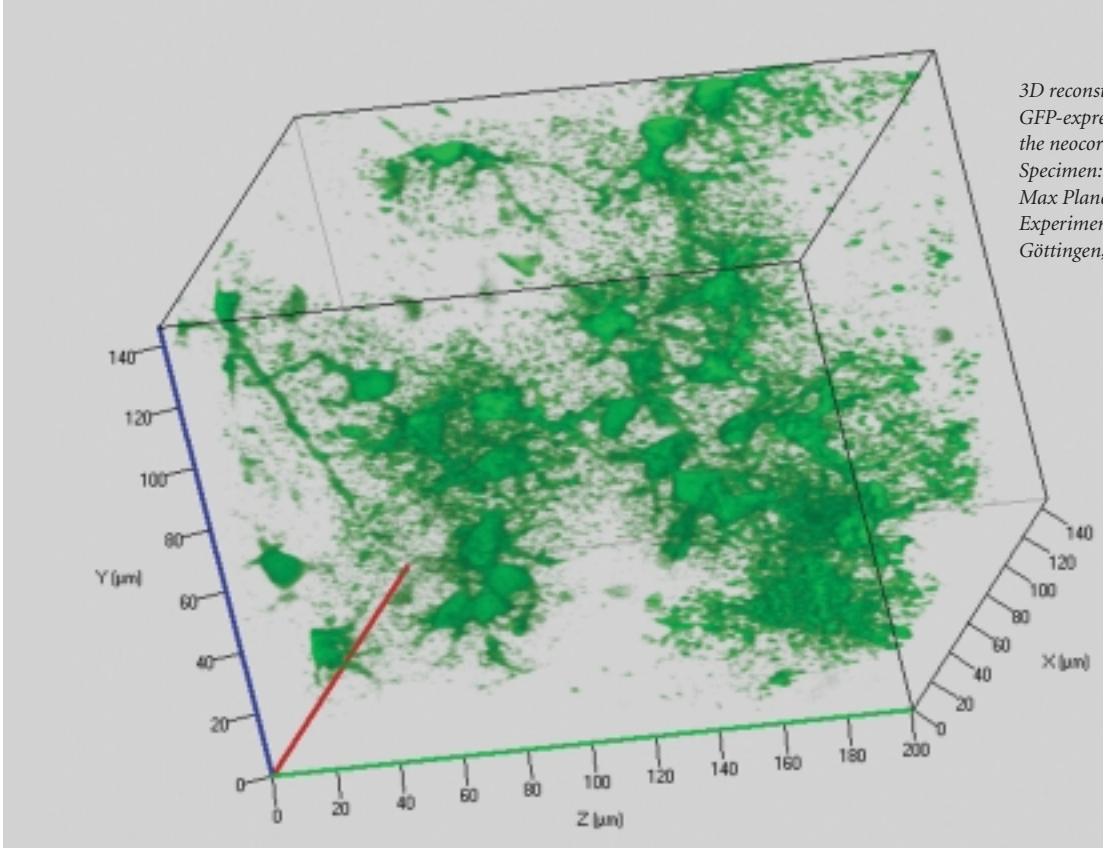
Constant Image Quality over a Wide Depth Range

Complete three-dimensional reconstructions of fluorescent structures deliver valuable information about the architecture of cells and tissues.



Once defined, the settings of laser intensity, detector voltage and offset for the beginning and end of the Z stack are saved.

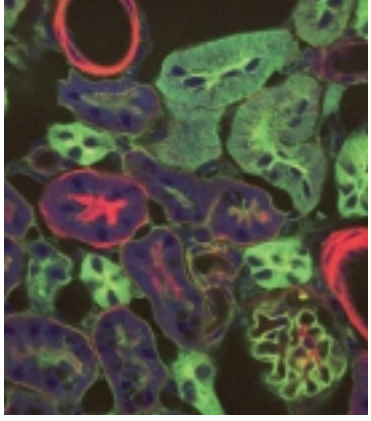
In the recording of extended Z stacks, the fluorescence signal gets fainter as the focus is moved to greater depth. To compensate the loss, conventional techniques require the detector voltage and laser intensity settings for the various optical sections to be adapted manually. The Auto Z Brightness Control function of the LSM software does away with manual adjustment. You only need to define the settings for the first and the last optical section, and you will get a stack of images with uniformly intense fluorescence signals. 3D reconstructions of such stacks supply highly informative spatial representations of cells or tissues.



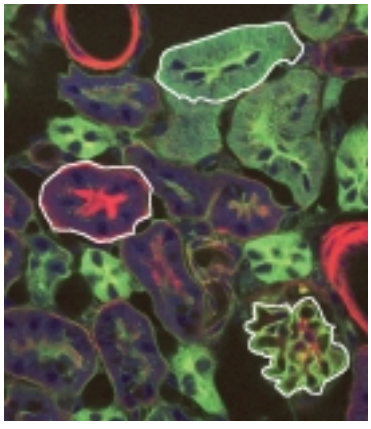
3D reconstruction of GFP-expressing astrocytes in the neocortex of a mouse. Specimen: Dr. J. Hirrlinger, Max Planck Institute of Experimental Medicine, Göttingen, Germany.

Region of Interest (ROI)

Arbitrarily Defined Areas

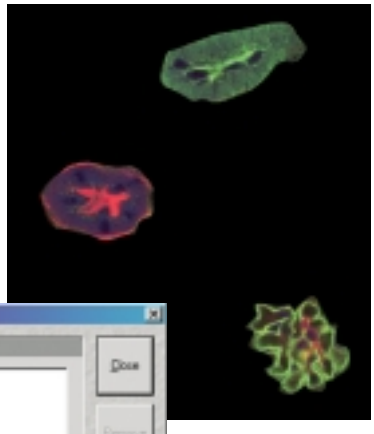


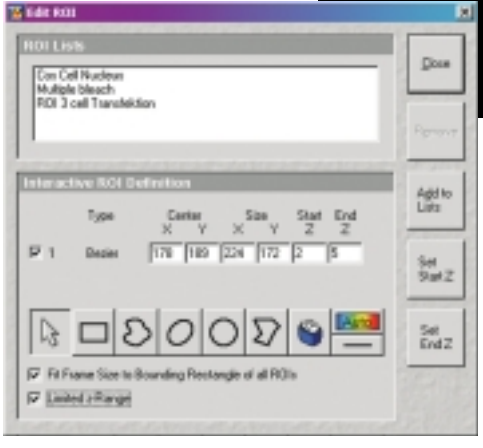
Manipulations with living cells such as the bleaching of fluorescent proteins or the photochemical uncaging of biologically active substances have to be done in precisely defined areas to create reproducible data. Such areas, termed ROIs, are defined in the LSM software with single-pixel accuracy.



You can define a number of ROIs of varied sizes and with any outlines. Select one or several of them and the laser light will only irradiate the areas selected. This flexible technique supports many experimental tasks such as Fluorescence Recovery after Photobleaching (FRAP), Fluorescence Loss in Photobleaching (FLIP), photochemical uncaging of substances, or photoactivation. Moreover, the MultiTimeSeries module, combined with a motor-driven XY stage, allows the completely automatic irradiation of individual regions with varied laser lines and light intensities. ROIs can be defined differently for different scanning configurations. In long-time experiments, the Auto Focus function keeps the region to be examined in the focal plane.

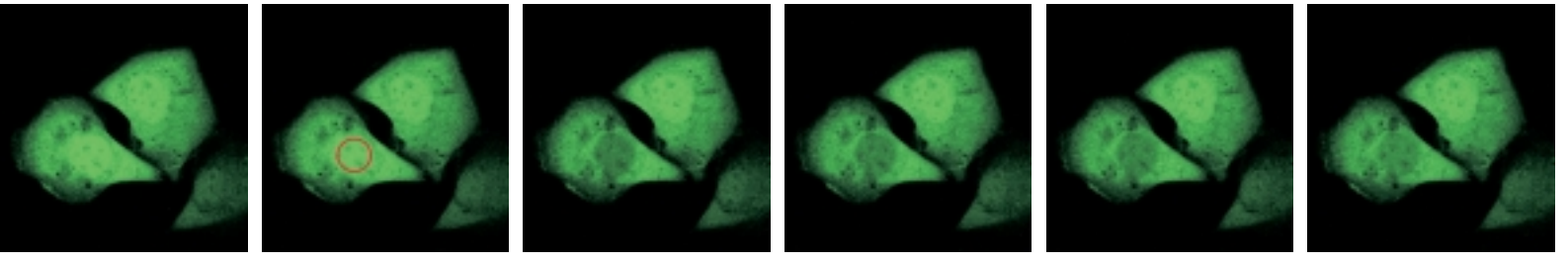
If a Region of Interest (ROI) is defined in the specimen image, only this region will then be illuminated. Signal detection from several ROIs is effected by a single image acquisition action.





ROIs are defined in the image by means of drawing tools or by entering their exact positions, and saved.

Bleaching the nucleus region of GFP-expressing COS 7 cells. The area to be irradiated is defined with an ROI. Specimen: A. Böhmer, Friedrich Schiller University of Jena, Germany.

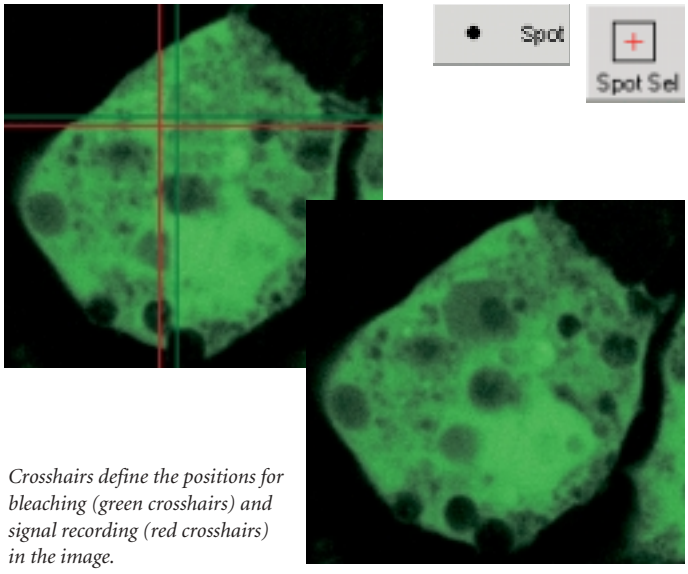


Spot Scan and Spot Bleach

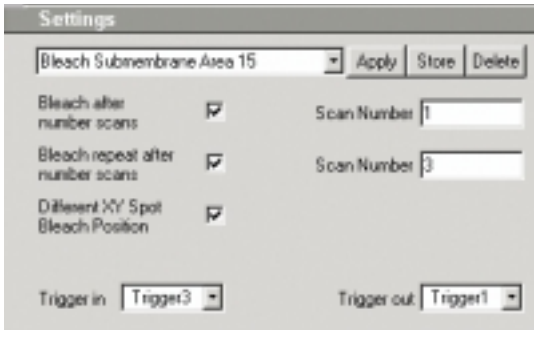
Detection of the Smallest Possible Area

To capture fast processes taking place in small regions of a cell, the changing fluorescence signal must be imaged with a high temporal resolution. For that purpose, the Spot Scan and Spot Bleach functions reduce the detection area to the smallest possible size.

In the Spot Scan and Spot Bleach modes, the scanning mirrors are parked in a defined position, so that only a spot in the specimen is illuminated. The fluorescent signals at the focal spot are recorded at intervals of a few microseconds. If you want to combine specimen imaging with bleaching, you may select differing positions in the specimen. In this way you can track, for example, the bleaching of the fluorescent label or the photolytic uncaging of a chemically modified messenger substance in the immediate vicinity with the appropriate temporal resolution.

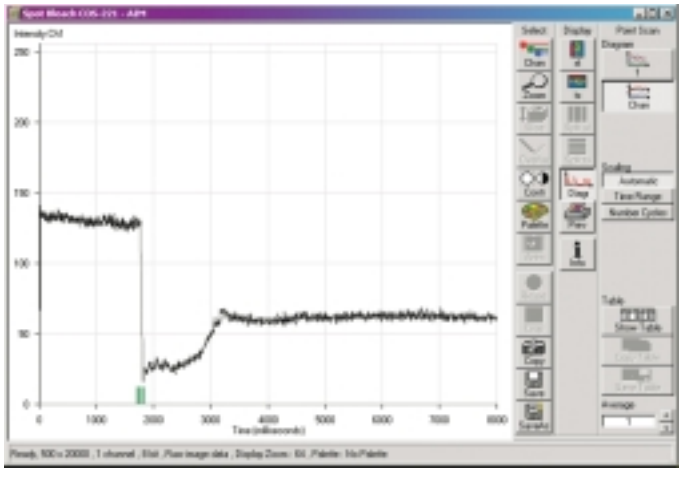


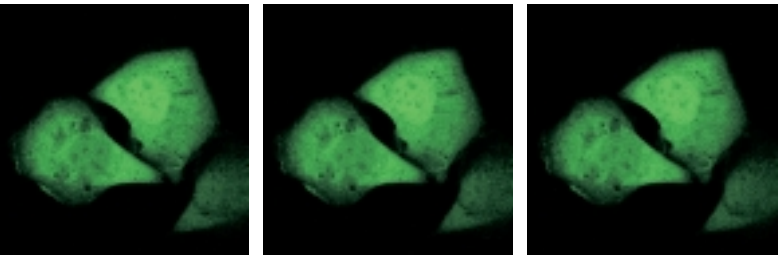
Crosshairs define the positions for bleaching (green crosshairs) and signal recording (red crosshairs) in the image.



In the Spot mode, you can repeatedly bleach a position different from that defined for signal recording.

The intensity graph informs about the effect of bleaching on the fluorescent protein in the position of signal detection.





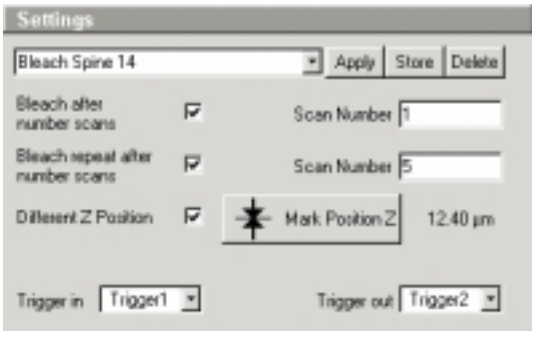
Z Bleach
Optimum Use of Focal Excitation

The femtosecond laser excites fluorochromes exclusively in the focal plane. With the Z Bleach function of the LSM software, this property can be optimally utilized for different experimental approaches. You can define different planes for the optical manipulation of specimens and the detection of the emission signals.

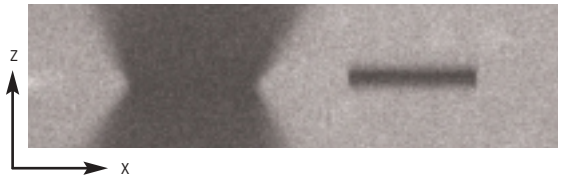
Fluorescence Recovery after Photobleaching (FRAP) is a method used in the investigation of transport processes between cells or cell compartments. For highly informative results, it is important to limit the area to be manipulated not only in the X and Y but also in the Z direction, as this avoids the unintentional bleaching of non-defined areas. In experiments that track the loss of fluorescence in unbleached areas (FLIP), the LSM software makes it possible to select different planes for the bleaching action and for fluorescence change tracking.

Within a time series, experimental routine procedures can be set up in a very quick and easy way and then run automatically. As a result you obtain image series that supply quantitative data immediately.

The method also permits targeted illumination of individual cells in a tissue in order to label them by the uncaging of a fluorochrome or by photoactivation of PA-GFP. The activation can be repeated time and again after a defined number of images is acquired.

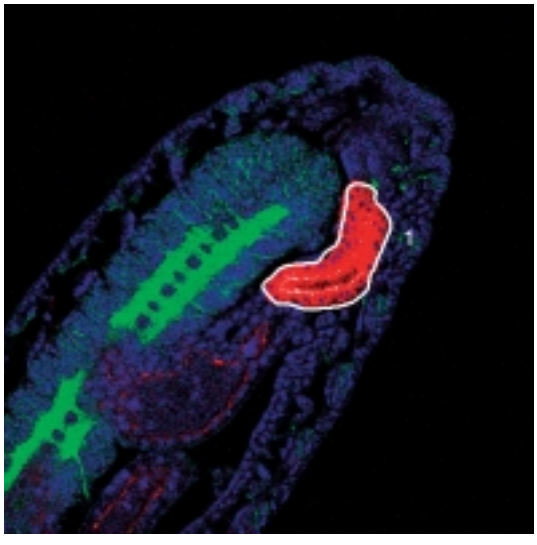


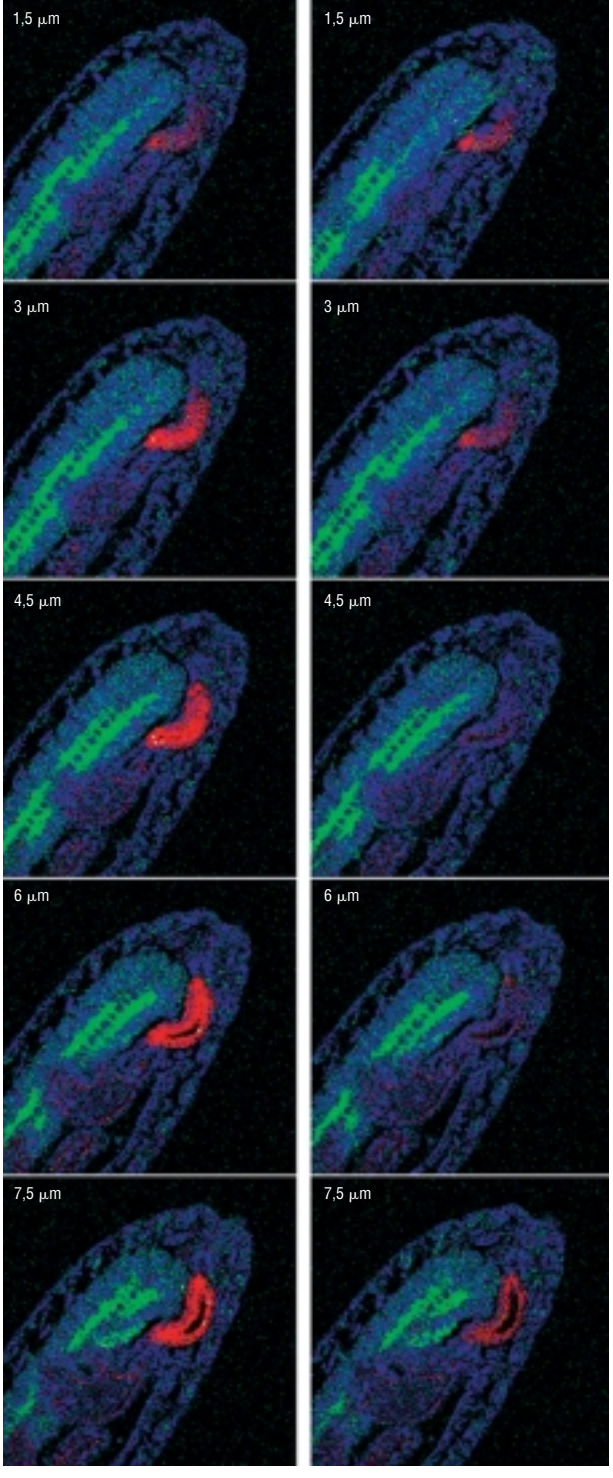
The predefined Z plane is moved into focus during bleaching.



Axial extension of the bleached area in confocal scanning microscopy (left) and multiphoton microscopy (right).

Definition of a ROI for bleaching.





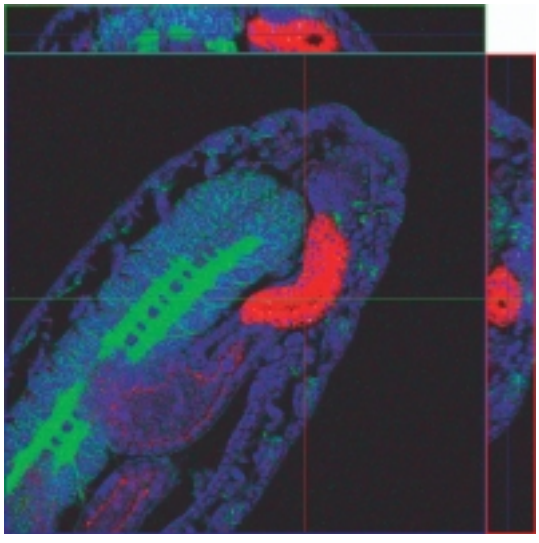
Drosophila embryo labeled for glial cells (CY3, red), neurons (FITC, green), and nuclei (DAPI, blue). Excitation with 780 nm, detection with META detector in the Online Fingerprinting mode. After the experiment, the labeling of the glial cells has bleached within a confined axial range.

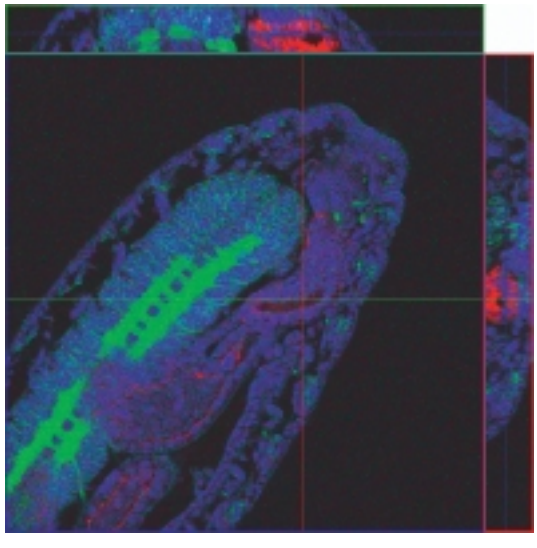
Prof. Y. Oshima,
Kyushu University, Fukuoka, Japan

"Our LSM 510 NLO is very useful in morphological analysis of deeper portions of GFP-expressing C. elegans worms especially in that ROI (Region Of Interest) and Time Lapse programs enable us to perform FRAP and FLIP by which we can get in vivo molecular data that we cannot get with other methods."

This brings about the complete activation or uncaging, respectively, within a cell, as fluorochromes not irradiated will gradually diffuse into the focal plane.

For the user of an LSM 510 NLO or LSM 510 META NLO instrument, the property of the femtosecond laser to excite a fluorochrome only within a volume of less than a femtoliter (1 μm^3) is harnessed to provide an easy-to-handle tool.





In the orthogonal view, the Z extension of the bleached region can be determined.

With motor-driven collimators, the excitation planes of near infrared and visible light can be precisely matched.

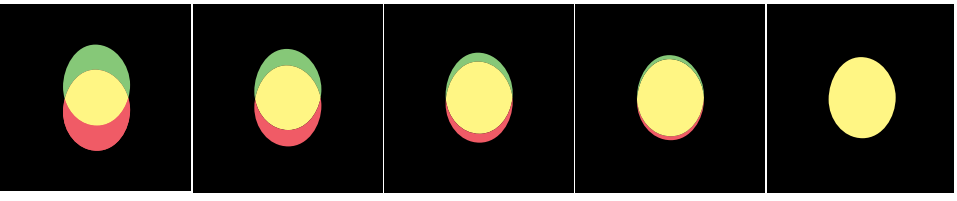
Visible Light and Near Infrared

A Clever Combination

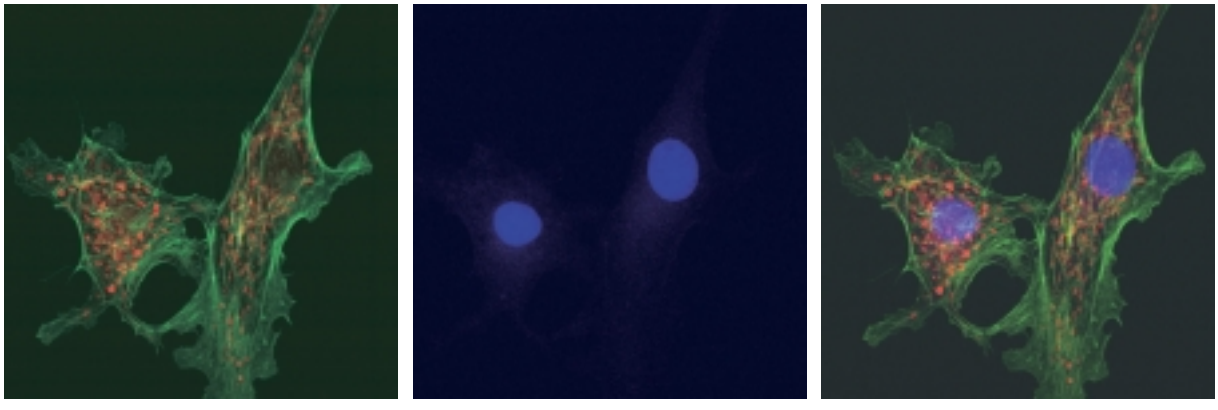
The mere availability of lasers emitting visible and infrared light is one thing; making the best possible use of these light sources is another. For the latter purpose, the LSM 510 NLO and LSM 510 META NLO instruments feature motor-driven collimators, which precisely match the excitation planes for different wavelengths.

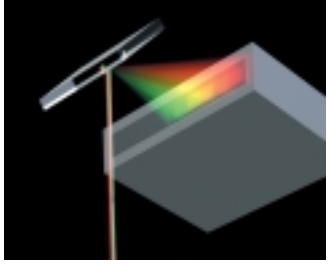
Computer-controlled, servomotor-driven collimators ensure that the excitation with visible and infrared light takes place in precisely the same specimen plane, even if the excitation wavelengths are far apart. Collimator settings are optimized for each objective and saved together with the image data. A desired setting can be activated any time with the ReUse function. The high output power of the femtosecond laser – one watt plus – is suitable, e.g., for the targeted damaging of cell processes. Thanks to the collimators, you can subsequently record the fluorescence signal of the manipulated structures with any desired excitation laser. This technique also enables you to choose from an exceptionally broad range of fluorochromes. For example, you can use DAPI to label a cell nucleus and excite it with the femtosecond laser.

Establishing the optimum collimator position by means of a fluorescent bead.
The collimator for the near infrared excitation range is shifted step by step to make the visible light and near infrared excitation planes coincide.



Simultaneous use of femtosecond and visible light lasers.
In a triple-stained specimen, two fluorescence signals are exited by visible light (green and red), and the third one by infrared light (blue).





The META detector separates mixed fluorescence signals and positively assigns the light emissions to the fluorochromes from which they originate. The method (patent pending) supplies images of substantially higher information content.

META Detection

Fast, Positive Separation of Fluorescence Signals

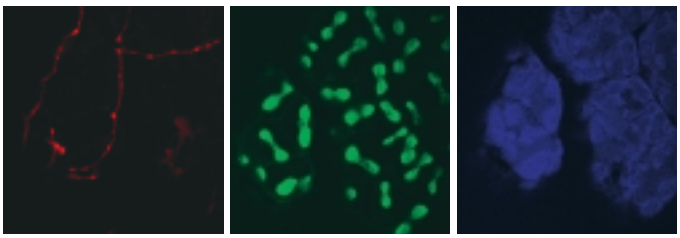
A femtosecond laser often excites more than one of the fluorochromes applied; the emitted mix may even contain autofluorescence. The LSM 510 META NLO will identify marker dyes and autofluorescence by their spectral properties. The method of Emission Fingerprinting clearly separates the fluorescent components from each other.

Multiple labeling ...

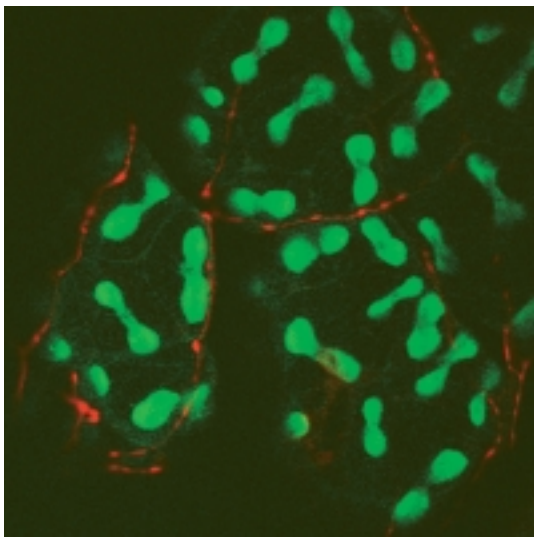
Many of the dyes used in conventional fluorescence microscopy are also suitable for multiphoton microscopy. As many excitation spectra overlap considerably in the near infrared range, it often happens that several fluorochromes are excited simultaneously. The same applies to autofluorescent substances. The phenomenon makes it difficult to separate labeled structures with filters.

... clearly separated

The META detector overcomes this handicap in a technically elegant and plausible manner: A highly efficient optical grating projects the entire fluorescence spectrum onto the 32 channels of the META detector. Thus, the complete spectral signature is recorded for every pixel of the scanned image and subsequently used for digital separation into the signal components.



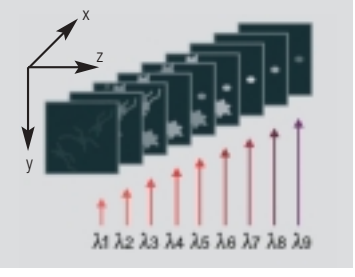
The Emission Fingerprinting function detects autofluorescence separately which can then be filtered out or used for additional information. Salivary gland of a cockroach (P. americana) labeled for dopamine (CY3, red) and actin (Oregon Green Phalloidine, green), autofluorescence (blue). Specimen: PD Dr. O. Baumann, University of Potsdam, Germany.



Excitation Fingerprinting

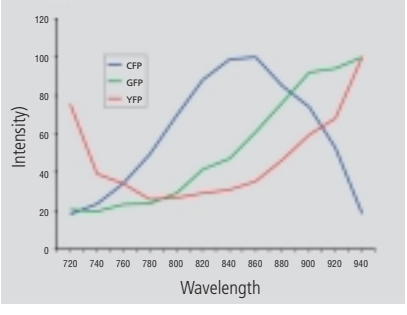
The Expert Way of Using Excitation Spectra

With the femtosecond laser, many conventional fluorochromes are excitable over a wavelength range of more than 100 nm. The LSM 510 NLO and LSM 510 META NLO microscopes will determine the optimum excitation wavelength quickly and reliably. Moreover, Excitation Fingerprinting uses excitation spectra to positively identify fluorescence signals.



The images of an excitation lambda stack are excited with different wavelengths.

Excitation spectra show signal intensities as a function of excitation wavelength.



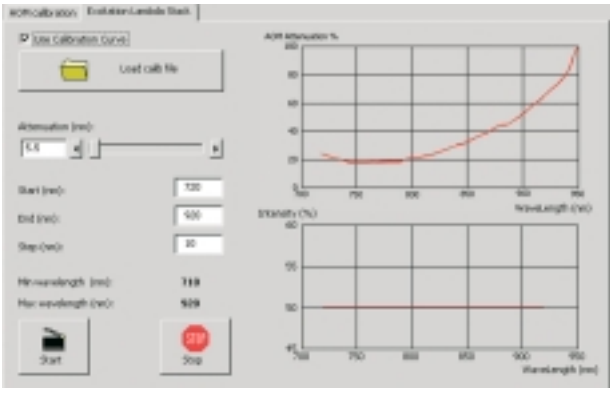
Record excitation spectra ...

Finding the optimum excitation wavelength for a fluorochrome used to be a time-consuming job. With the LSM 510 NLO and LSM 510 META NLO and a software-controlled femtosecond laser, excitation spectra tailored to a particular specimen can be recorded quickly and efficiently. Image acquisition at varied wavelengths supplies what is called a lambda stack, from which excitation spectra are obtained.

Real spectra are produced with a uniform laser intensity in the specimen plane, which is implemented by an appropriate setting of the AOM. Calibration curves for the AOM are established by an automatic procedure using a power meter, and saved. The normalized calibration curves are then used in the recording of lambda stacks.

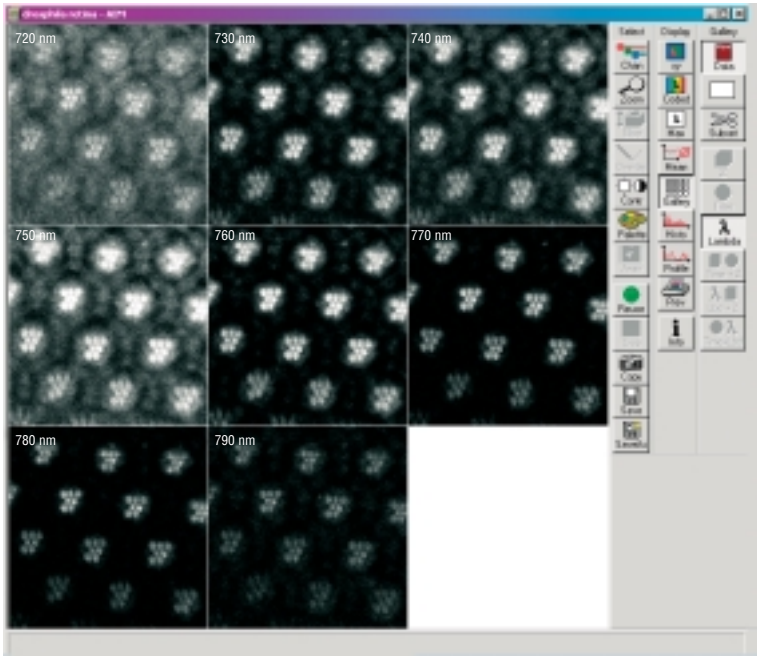
Calibration of the AOM is done with the Excitation Fingerprinting macro. The result is a constant intensity level of the laser light in the specimen plane, irrespective of wavelength.

The macro is also used to produce excitation lambda stacks. The user defines the excitation wavelength range, the spacing between the various wavelengths used, and the appropriate AOM calibration curve.

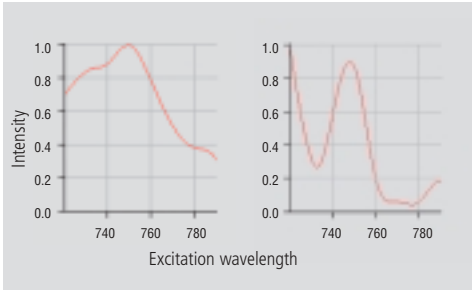


... and use them for Excitation Fingerprinting

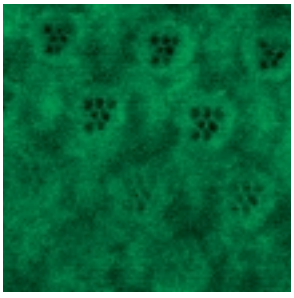
The excitation spectra not only greatly facilitate the definition of the optimum excitation wavelength of one or several fluorochromes, but are also helpful in distinguishing between fluorescence signals with overlapping emission spectra. The Linear Unmixing function of the LSM software uses excitation spectra to reliably compute the shares of the fluorochromes in the specimen. The Automatic Component Extraction function (ACE) can be used for the identification of fluorescent dyes with but short topographic overlaps. Non-descanned detectors are recommended to detect fluorescence signals deep in heavily scattering specimens. If overlapping, these signals, too, can be exactly separated.

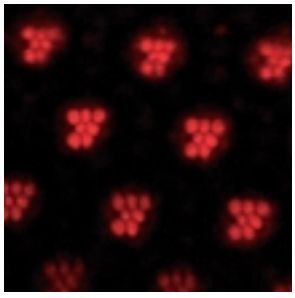


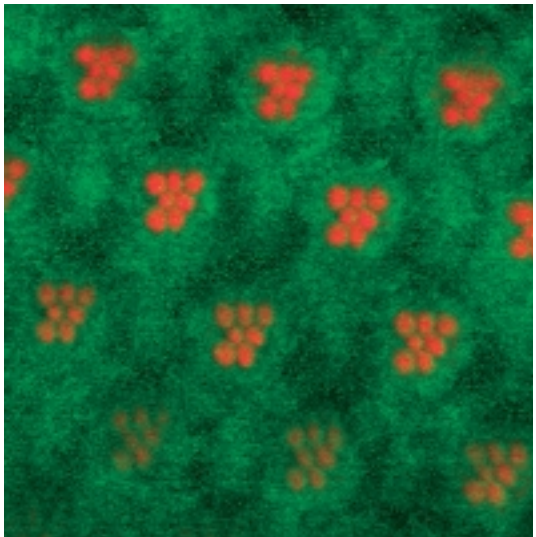
Excitation Fingerprinting separates widely overlapping emission signals using their excitation spectra.



Drosophila retina, labeled for actin (Alexa Fluor 568 Phalloidine). Excitation Fingerprinting clearly separates autofluorescence and emission signals.
Specimen: PD Dr. O. Baumann, University of Potsdam, Germany







LSM 510 NLO

Specification

Microscopes

Models	Upright: Axioplan 2 imaging MOT, Axioskop 2 FS MOT; inverted: Axiovert 200 M BP (Base Port) or SP (Side Port)
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, total lift 200 µm, smallest increment <10 nm
XY stage (option)	Motor-driven XY scanning stage, with Mark & Find (XYZ) and Tile Scan functions, smallest increment 1 µm
Accessories	AxioCam digital microscope camera, incubation chambers, micromanipulators, etc.

Scanning module

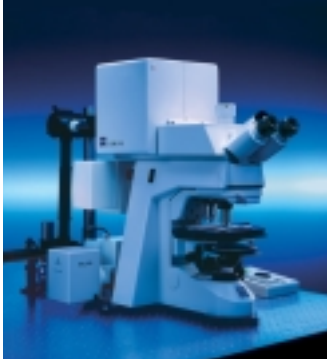
Models	2 or 3 confocal channels
Scanner	2 independent galvanometric scanning mirrors, DSP-controlled, for ultrafast line and vertical flyback
Scanning resolution	4 x 1 to 2048 x 2048 pixels, also for several channels, continuously adjustable
Scanning speed	13 x 2 speed stages; up to 5 frames/s with 512 x 512 pixels (up to 77 frames/s with 512 x 32 pixels); min. 0.38 ms for a line of 512 pixels
Scanning zoom	0.7x to 40x, digital, variable in steps of 0.1
Scanning rotation	Free 360° rotation, variable in steps of 1 degree, free XY offset
Scanning field	Max. diagonal 18 mm in the intermediate image plane, homogeneous illumination
Pinholes	Up to 4 pinholes for each confocal channel, adjustable in size and position
Detection	Up to 4 confocal channels for fluorescence/reflectance with short, efficient beam paths, each with a high-sensitivity PMT detector. Options: Transmitted-light channel with PMT Monitor diode for measuring the excitation intensity Non-descanned detectors (NDD) for multiphoton microscopy – up to 4 detectors for reflected- and transmitted-light fluorescence; max. 2 channels each in the reflected- and transmitted-light beam paths
Data depth	Selectable between 8 bit and 12 bit

Laser module

VIS laser module	Polarization-preserving single-mode fiber, temperature-stabilized VIS-AOTF for simultaneous intensity control of up to 6 visible-light laser lines, switching time < 5 µs; AOTF reprogramming via the LSM software. Ar laser (458, 477, 488, 514 nm), 30 mW; HeNe laser (543 nm), 1 mW; HeNe laser (633 nm), 5 mW (end-of-lifetime specification)
Multiphoton module (option)	Direct or fiber coupling of pulsed Ti:Sa lasers into the scanning module; various makes are supported. Grating Dispersion Compensator (GDC) and Post Fiber Compressor (PFC) for optimum pulse shaping. Fast change of laser intensity by means of AOM. Objectives optimized for use in the NIR range

Electronics module

LSM 510 Control	Control of the microscope, the VIS and NLO laser modules, the scanning module and further accessories. Monitoring of data acquisition and synchronization by a Digital Signal Processor (DSP). Data exchange between DSP and computer via ultra-wide SCSI port
Computer	High-end PC with generous working memory and hard disk storage capacity; 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 motorized microscope functions, of the laser and scanning modules; saving and restoration of application-specific configurations
ReUse function	Restoration of acquisition parameters with one 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)
Auto Z	On-line adaptation of Z stack acquisition parameters for uniform brightness distribution
Crop	Convenient selection of scanning ranges (zoom, offset, rotation simultaneously)
RealROI Scan	Scanning of up to 99 ROIs (Regions of Interest) of any shape, with pixel-accurate laser blanking
ROI Bleach	Localized photobleaching of up to 99 bleaching ROIs for applications such as FRAP (Fluorescence Recovery After Photobleaching) or uncaging
Spline Scan	Scanning along a free-hand defined line
Multitracking	Acquisition of multiple fluorescences; minimized signal crosstalk (bleeding) by fast change of excitation lines
Presentation	Orthogonal view (XY, XZ, YZ in a single presentation), 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 adjustments; off-line interpolation for Z stacks, selection and modification of color lookup tables (LUT), drawing functions for documentation
Image analysis	Modern tools for colocalization and histogram analysis with diverse parameters and options, profile measurement of straight lines and curves of any shape, measurement of lengths, angles, areas, intensities, etc.
Image operations	Addition, subtraction, multiplication, division, ratio, shift, filters (low-pass, median, high-pass, etc.; user-definable)
Image archiving, export, import	LSM image database with convenient functions for managing images together with their acquisition parameters; multiprint function for creating assembled image and 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 (silhouette projection, transparent projection, surface rendering)
3D Deconvolution	Image restoration based on computed point spread functions (Nearest Neighbor, Maximum Likelihood, Constraint Iterative)
Multiple Time Series	Assembled time series with change of application-specific configurations, autofocus and bleaching functions
3D for LSM	3D reconstruction, thresholding, processing and measurement (surface area, volume, particle count)
Physiology	Analysis of time series, graphical mean-of-ROI analysis, on- and off-line display and calibration of ion concentrations
Topography	Visualization of 3D surfaces (fast rendering modes) plus measurement functions (roughness, waviness, surface area, volume)
Macro Edit	Acquisition and editing of routines for the automation of scanning and analysis functions

Image Browser

Free software	Presentation, editing, archiving, printing and export/import of LSM 5 images

LSM 510 META NLO

Specification

Microscopes

Models	Upright: Axioplan 2 imaging MOT, Axioskop 2 FS MOT Inverted: Axiovert 200 M BP (Base Port) or SP (Side Port)
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, total lift 200 µm, smallest increment < 10 nm
XY stage (option)	Motor-driven XY scanning stage, with Mark & Find (XYZ) and Tile Scan functions, smallest increment 1 µm
Accessories (options)	AxioCam digital microscope camera, incubation chambers, micromanipulators, etc.

Scanning module

Models	META scanning module with 2 single-channel detectors and 1 polychromatic multichannel detector (each genuinely confocal with selected, high-sensitivity PMTs), prepared for lasers from VIS to NIR
Scanner	2 independent galvanometric scanning mirrors, DSP-controlled, for ultrafast line and vertical flyback
Scanning resolution	4 x 1 to 2048 x 2048 pixels, also for several channels, continuously adjustable
Scanning speed	13 x 2 speed stages; up to 5 frames/s with 512 x 512 pixels (up to 77 frames/s with 512 x 32 pixels); min. 0.38 ms for a line of 512 pixels
Scanning zoom	0.7x to 40x, digital, variable in steps of 0.1
Scanning rotation	Free 360° rotation, variable in steps of 1 degree, free XY offset
Scanning field	Max. diagonal 18 mm in the intermediate image plane, homogeneous illumination
Pinholes	Pinholes for each epi-illumination channel (single-channel detector or META multichannel detector), individual adjustments of size and position, preadjusted
Detection	Standard: 3 confocal epi-illumination channels simultaneously (META detector + 2 single-channel detectors), each with a high-sensitivity PMT detector. Options: Transmitted-light channel with PMT Monitor diode for measuring the excitation intensity Non-descanned detectors (NDD) for multiphoton microscopy – up to 4 detectors for reflected- and transmitted-light fluorescence; max. 2 channels each in the reflected- and transmitted-light beam paths
META detector	Polychromatic 32-channel detector for fast acquisition of lambda stacks and metatracking; simultaneous acquisition in up to 8 channels; also in combination with time series
Data depth	Selectable between 8 bit and 12 bit

Laser module

VIS laser module	Polarization-preserving single-mode fiber, temperature-stabilized VIS-AOTF for simultaneous intensity control of up to 6 visible-light laser lines, switching time < 5 µs; AOTF reprogramming via the LSM software. Ar laser (458, 477, 488, 514 nm), 30 mW; HeNe laser (543 nm), 1 mW; HeNe laser (633 nm), 5 mW (end-of-lifetime specification)
Multiphoton module (option)	Direct or fiber coupling of pulsed Ti:Sa lasers into the scanning module; various makes are supported. Grating Dispersion Compensator (GDC) and Post Fiber Compressor (PFC) for optimum pulse shaping. Fast change of laser intensity by means of AOM. Objectives optimized for use in the NIR range

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LSM 510 Control	Control of the microscope, the VIS and NLO laser modules, the scanning module and further accessories. Monitoring of data acquisition and synchronization by a Digital Signal Processor (DSP). Data exchange between DSP and computer via ultra-wide SCSI port
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ROI Bleach	Localized photobleaching of up to 99 bleaching ROIs for applications such as FRAP (Fluorescence Recovery After Photobleaching) or uncaging
Spline Scan	Scanning along a free-hand defined line
Multitracking	Acquisition of multiple fluorescences; minimized signal crosstalk (bleeding) by fast change of excitation lines
Metatracking	Extension of multitracking by fast electronic change of detection channels, even with overlapping bandpasses, ensures optimum signal detection (only with META detection module)
Lambda Scan	Fast acquisition of image stacks with spectral information for every pixel (only with META detection module)
Emission Fingerprinting	Technique for generating crosstalk-free multiple-fluorescence images with simultaneous excitation, online or offline unmixing, automatic or interactive definition of reference spectra (only with META detection module)
Presentation	Orthogonal view (XY, XZ, YZ in a single presentation), 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 adjustments. Off-line interpolation for Z stacks. Selection and modification of color lookup tables (LUT). Drawing functions for documentation
Image analysis	Modern tools for colocalization and histogram analysis with diverse parameters and options, profile measurement of straight lines and curves of any shape, measurement of lengths, angles, areas, intensities, etc.
Image operations	Addition, subtraction, multiplication, division, ratio, shift, filters (low-pass, median, high-pass, etc.; user-definable)
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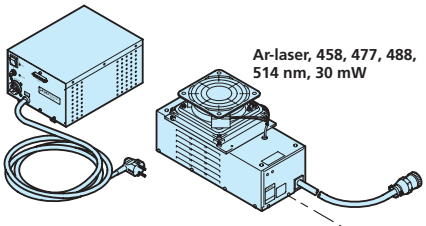
Macro Edit	Acquisition and editing of routines for the automation of scanning and analysis functions

Image Browser

Free software	Presentation, editing, archiving, printing and export/import of LSM 5 images

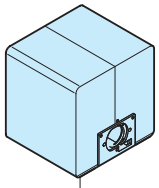
LSM 510 NLO and LSM 510 META NLO System Overview



Ar-laser, 458, 477, 488,
514 nm, 30 mW

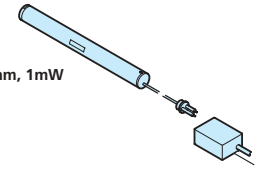
NLO kit for fiber coupling

VIS/NLO-Scan module LSM 510

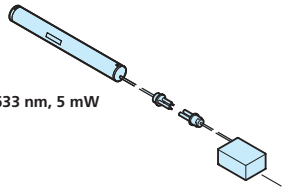


Upgrade kits LSM 510 to
LSM 510 META

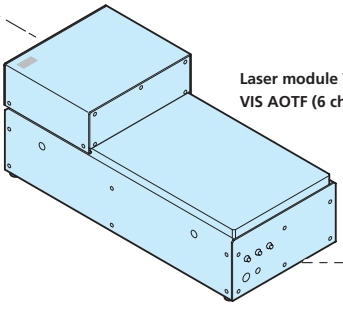
NLO kit for direct coupling



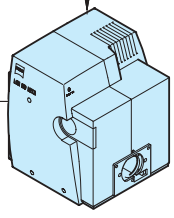
HeNe laser, 543 nm, 1mW



HeNe laser, 633 nm, 5 mW



Laser module VIS
VIS AOTF (6 channels)

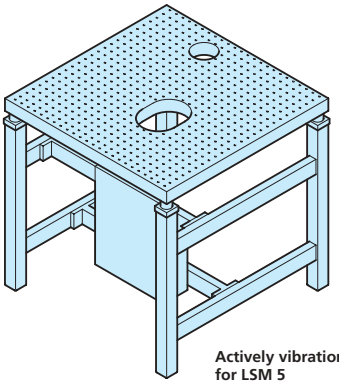


VIS/NLO Scan module LSM 510 META

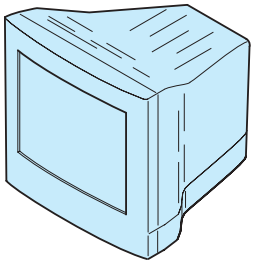
Fiber decoupling, channel 4

Options:
-Fluorescence Correlation
Spectroscopy (FCS)

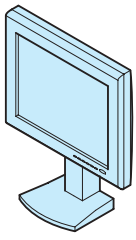
-Fluorescence Lifetime
Imaging Microscopy (FLIM)



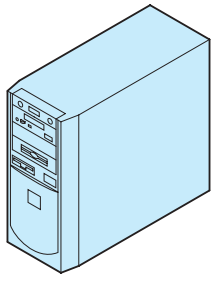
Actively vibration-absorbed system table
for LSM 5
Table surface 30" x 30"



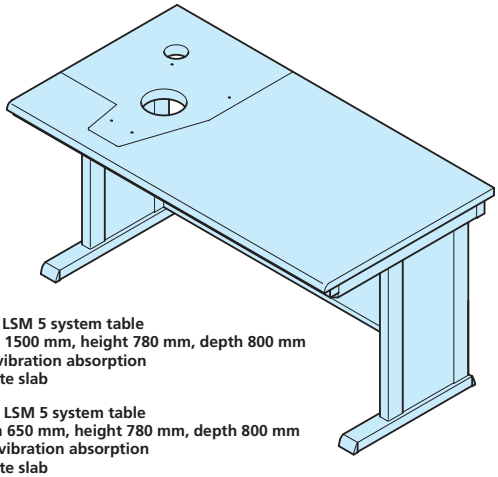
Monitor 21" (50 cm)



LCD TFT-Flatscreen 18"

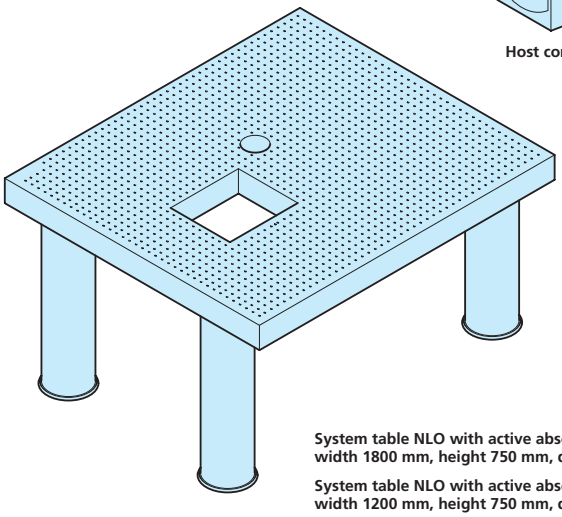


Host computer



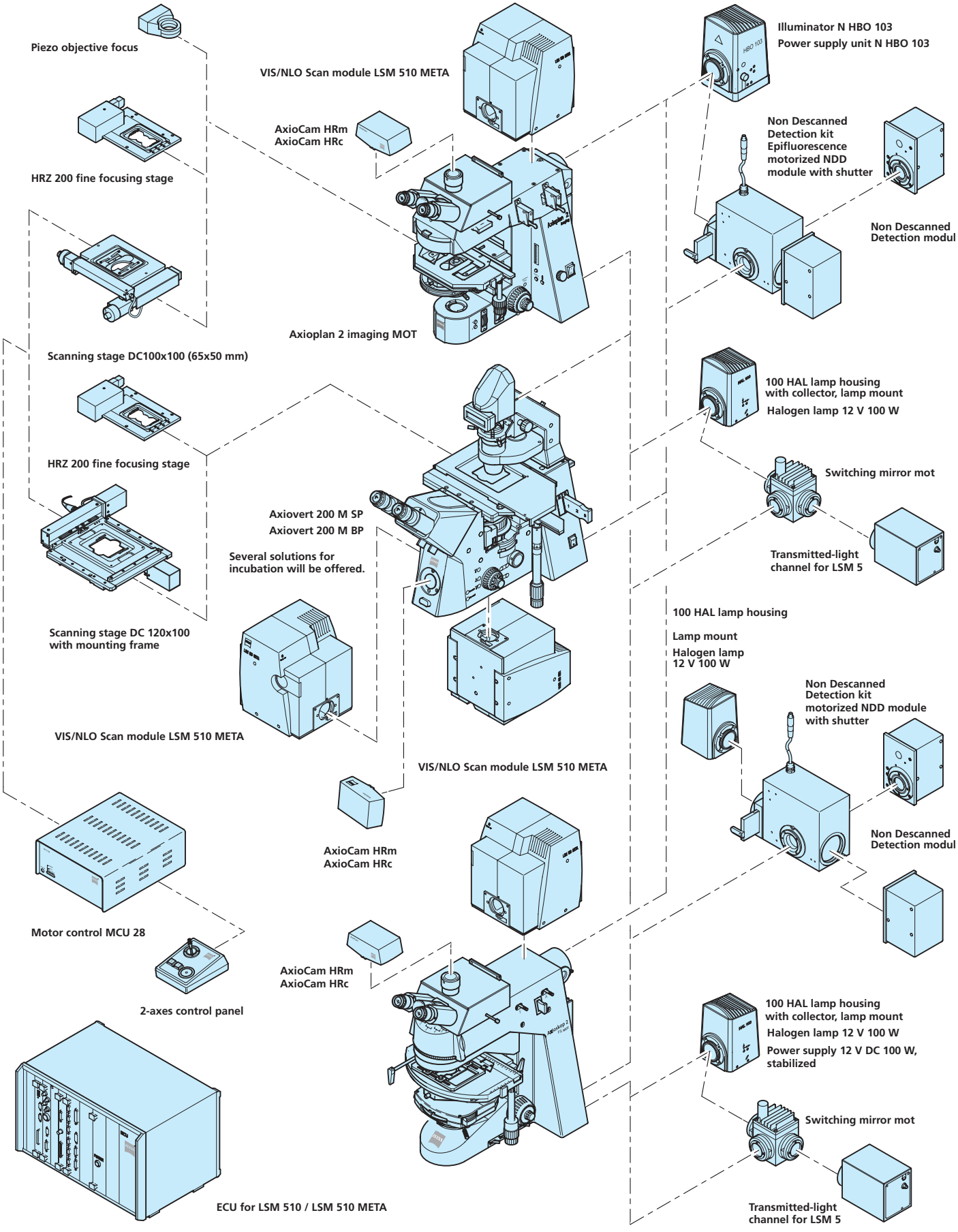
Large LSM 5 system table
width 1500 mm, height 780 mm, depth 800 mm
with vibration absorption
Granite slab

Small LSM 5 system table
width 650 mm, height 780 mm, depth 800 mm
with vibration absorption
Granite slab



System table NLO with active absorption
width 1800 mm, height 750 mm, depth 1400 mm

System table NLO with active absorption
width 1200 mm, height 750 mm, depth 1400 mm


Piezo objective focus
HRZ 200 fine focusing stage
Scanning stage DC100x100 (65x50 mm)
HRZ 200 fine focusing stage
Scanning stage DC 120x100
with mounting frame
Motor control MCU 28
2-axes control panel
ECU for LSM 510 / LSM 510 META
VIS/NLO Scan module LSM 510 META
AxioCam HRm
AxioCam HRc
Axioplan 2 imaging MOT
Illuminator N HBO 103
Power supply unit N HBO 103
Non Descanned
Detection kit
Epifluorescence
motorized NDD
module with shutter
Non Descanned
Detection modul
100 HAL lamp housing
with collector, lamp mount
Halogen lamp 12 V 100 W
Switching mirror mot
Transmitted-light
channel for LSM 5
Axiovert 200 M SP
Axiovert 200 M BP
Several solutions for
incubation will be offered.
VIS/NLO Scan module LSM 510 META
AxioCam HRm
AxioCam HRc
100 HAL lamp housing
Lamp mount
Halogen lamp
12 V 100 W
Non Descanned
Detection kit
motorized NDD module
with shutter
Non Descanned
Detection modul
VIS/NLO Scan module LSM 510 META
AxioCam HRm
AxioCam HRc
100 HAL lamp housing
with collector, lamp mount
Halogen lamp 12 V 100 W
Power supply 12 V DC 100 W,
stabilized
Switching mirror mot
Transmitted-light
channel for LSM 5

LSM 510 NLO and LSM 510 META NLO

Functionality at a Glance

Automatic Component Extraction
Statistical procedure for identifying individual fluorochrome spectra in a lambda stack.

Auto Z Brightness Correction
Procedure for compensating signal loss in deep tissue layers in Z stack acquisition, to obtain signals of equal intensity from the different planes.

Emission Fingerprinting* (patent pending)
Method of the LSM 510 META NLO for the acquisition, analysis and separation of emission signals in multiple fluorescence images; also applicable to widely overlapping spectra.

Excitation Fingerprinting (patent pending)
Method for the acquisition, analysis and separation of emission signals from multiple-fluorescence-labeled specimens by means of excitation spectra.

Lambda Stack
Image stack with information in the X, Y and λ dimensions; combinable with Z and/or time series; for the determination of spectral signatures anywhere in the specimen.

Linear Unmixing
Mathematical procedure for the spectral deconvolution of multiple emission signals.

Metatracking*
Scanning mode in the LSM 510 META NLO, similar to Multitracking, but supplemented by fast switching between detection settings.

Multitracking
Scanning mode in all models of the LSM 5 family, creates multiple fluorescence images without emission signal crosstalk, thanks to fast switching between excitation and quasi-simultaneous detection.

RealROI Scan
Scanning mode in which freely defined areas of the specimen are excited and imaged; maximum specimen preservation thanks to exact blanking of the laser lines outside the selected areas.

ROI Bleaching
Defined bleaching of several freely defined specimen areas, used, e.g., in FRAP or uncaging experiments.

Spline Scan
Scanning along a freely defined line for recording fast (physiological) changes, e.g. along neuron processes.

Spot Scan
Scanning mode in which the signal intensity in a confocal spot is tracked with extremely high temporal resolution.

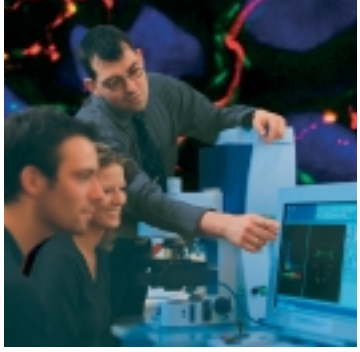
Spot Bleach
Bleaching mode used in time series, in which a defined specimen spot is bleached and the signal recorded at a different spot with extremely high temporal resolution.

Step Scan
Fast overview scan, in which intermediate lines are interpolated.

Tile Scan
Acquisition of the image of a large object as a mosaic of partial images, to achieve better resolution.

Z Bleach
Bleaching mode in which the plane of bleaching is defined independent of the image acquisition plane.

* only for LSM 510 META NLO



Carl Zeiss Consulting

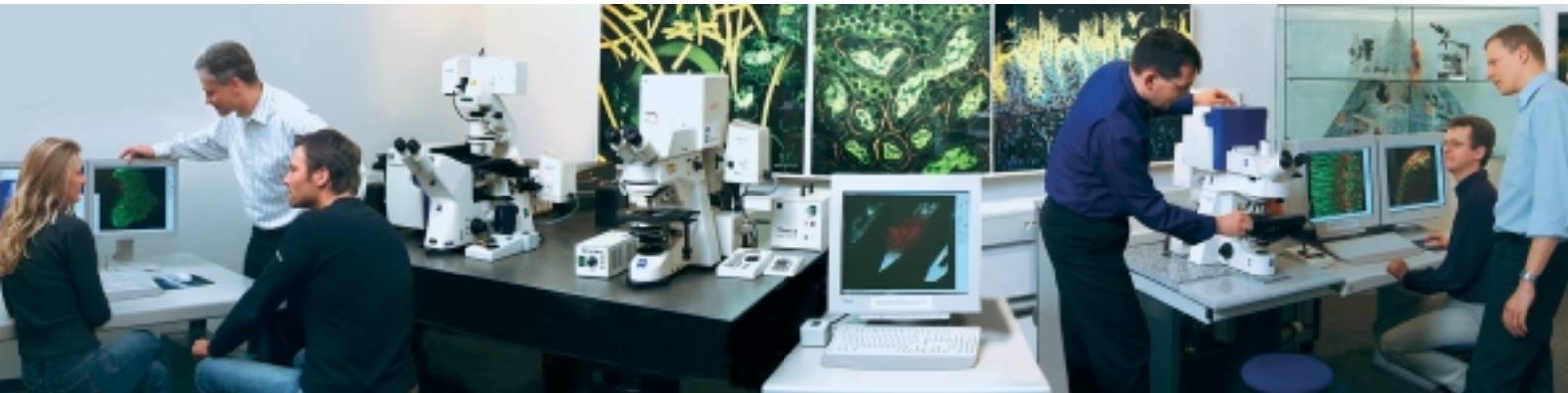
With their know-how of products and markets, our team will assist you in selecting the system that meets your specific requirements, including components of other makes.
After every system installation, our regional specialists will intensively train your operators.
Consult us about latest applications to continuously extend the use of the LSM 510 NLO or the LSM 510 META NLO in your research.

Carl Zeiss Training Courses

To you and your staff, Carl Zeiss offers comprehensive training courses about every aspect of confocal microscopy. Our seminars, whether entry-level or advanced, are always practice-related. In local workshops you will learn everything about current applications and new technical features introduced by Carl Zeiss, as well as gain hands-on experience in how to use the systems most efficiently.

Carl Zeiss Service

Dependable, competent Zeiss staff will install and maintain your system.
The new detection module can readily be retrofitted to any LSM 510 NLO system installed, to make up a fully functional LSM 510 META NLO.
The existing optical, mechanical and electronic interfaces permit further configuration for methods such as the measurement of molecule interactions by means of FCS (fluorescence correlation spectroscopy). New scanning and analysis methods can be made available quickly and easily through software upgrades.

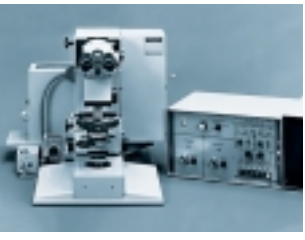


Glossary

AOM	Acousto Optical Modulator
AOTF	Acousto Optical Tunable Filter
DDS	Dual Direction Scan
DSP	Digital Signal Processor
FLIP	Fluorescence Loss in Photobleaching
FRAP	Fluorescence Recovery after Photobleaching
GDC	Grating Dispersion Compensator
GFP	Green Fluorescent Protein
ICS	Infinity Color Corrected System
MOT	Motor-driven Microscope Model
NDD	Non-Descanned Detector
NLO	Nonlinear Optics
NIR	Near Infrared
PFC	Post Fiber Compressor
PMT	Photo Multiplier Tube
ROI	Region of Interest

The History of Multiphoton Microscopy

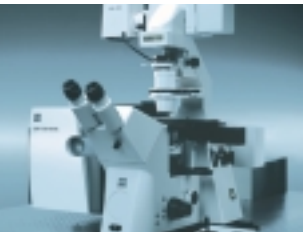
1931
Maria Goeppert-Mayer (Nobel prize winner in physics, 1963) describes two-photon processes in her doctoral thesis *Über Elementarakte mit zwei Quantensprüngen* (On Elementary Acts Involving Two Quantum Leaps) (Göttingen): Ann.Phys. 9:273-294.



1982
The first Carl Zeiss Laser Scanning Microscope. The prototype of the series is now an exhibit at the German Museum, Munich.

1970–1990
Several publications on experiments with two-photon processes in biological specimens
(Lesclaux R, Vsevolodov NN, Berns MW).

1990
First publication on two-photon laser scanning microscopy: Denk W, Strickler JH, Webb WW, Science 1990, 248 (4951):73-6.

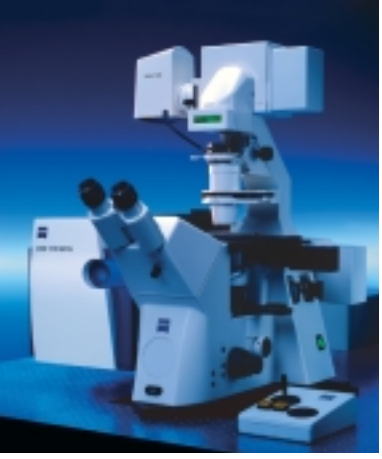


1991
The LSM 310 combines confocal laser scanning microscopy with leading-edge PC technology.

1997
First system of the LSM 510 series.

1998
Model LSM 510 NLO, prepared for multiphoton microscopy.

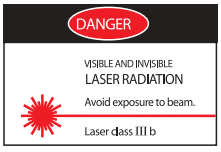
2001
LSM 510 META NLO, most advanced technology plus convenient operation.



LSM 510 META NLO and LSM 510 NLO for Multiphoton Microscopy

The integration of laser control into the LSM software adds convenient operation to an efficient technology.
An instrument combination suitable for many applications in biomedical research.

Carl Zeiss Jena GmbH
TÜV CERT
DIN EN ISO 9001
DIN EN 46001
Certified Quality Management System



US Patents: 6178041, 6521899, 6462345, 6486458, 6167173, 6278555, 6269206, 5995281, 6037583, 6563632, 6403332, 6631226

German Patents: 19919091C2, 19758744C2, 19758745C2, 19758746C2, 19758748C2, 19702753C2, 19829981C2, 19827139C2, 19702752C2, 19827140C2, 19915137C2

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