LSM 510 NLO and LSM 510 META NLO
Multiphoton Laser Scanning Microscopes

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.
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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.

Femtosecond lasers excite the dye only in the focal spot.

Energy diagram of fluorescence generation with single photon excitation.

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.

“...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 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.

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 online 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.
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 Axiocert 200M. 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 Achronplan 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; andSpot, 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  6 Scanning lens  12 Photomultiplier tubes
2 Motor-driven collimators  7, 8, 9 Secondary dichroic beamsplitters  13 META detector
3 Beam combiner  10 Pinholes  14 Neutral density filter
4 Main dichroic beamsplitter  11 Emission filters  15 Monitor diode
5 Scanning mirrors  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 collect the fluorescence signal before the scanning lens.
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.

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.
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.

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.

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

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.
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 µm³) is harnessed to provide an easy-to-handle tool.

*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.

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

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.

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 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 Phalloidin).*

*Excitation Fingerprinting clearly separates autofluorescence and emission signals.*

*Specimen: PD Dr. O. Baumann, University of Potsdam, Germany*
**LSM 510 NLO**

**Specification**

### Microscopes

<table>
<thead>
<tr>
<th>Models</th>
<th>Upright: Axioplan 2 imaging MOT, Axioskop 2 FS MOT; inverted: Axiovert 200 M BP (Base Port) or SP (Side Port)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z drive</td>
<td>DC motor with optoelectronic coding, smallest increment 25 or 50 nm; fast piezo focusing accessory</td>
</tr>
<tr>
<td>HRZ 200 (option)</td>
<td>High-precision galvanometric fine focusing stage, total lift 200 µm, smallest increment &lt;10 nm</td>
</tr>
<tr>
<td>XY stage (option)</td>
<td>Motor-driven XY scanning stage, with Mark &amp; Find (XY2) and Tile Scan functions, smallest increment 1 µm</td>
</tr>
<tr>
<td>Accessories</td>
<td>AxioCam digital microscope camera, incubation chambers, micromanipulators, etc.</td>
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</table>

### Scanning module

<table>
<thead>
<tr>
<th>Models</th>
<th>2 or 3 confocal channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanner</td>
<td>2 independent galvanometric scanning mirrors, DSP-controlled, for ultrafast line and vertical flyback</td>
</tr>
<tr>
<td>Scanning resolution</td>
<td>4 x 1 to 2048 x 2048 pixels, also for several channels, continuously adjustable</td>
</tr>
<tr>
<td>Scanning speed</td>
<td>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</td>
</tr>
<tr>
<td>Scanning zoom</td>
<td>0.7x to 40x, digital, variable in steps of 0.1</td>
</tr>
<tr>
<td>Scanning rotation</td>
<td>Free 360° rotation, variable in steps of 1 degree, free XY offset</td>
</tr>
<tr>
<td>Scanning field</td>
<td>Max. diagonal 18 mm in the intermediate image plane, homogeneous illumination</td>
</tr>
<tr>
<td>Pinholes</td>
<td>Up to 4 pinholes for each confocal channel, adjustable in size and position</td>
</tr>
<tr>
<td>Detection</td>
<td>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</td>
</tr>
<tr>
<td>Data depth</td>
<td>Selectable between 8 bit and 12 bit</td>
</tr>
</tbody>
</table>

### Laser module

<table>
<thead>
<tr>
<th>VIS laser module</th>
<th>Polarization-preserving single-mode fiber, temperature-stabilized VIS-AOTF for simultaneous intensity control of up to 6 visible-light laser lines, switching time &lt; 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)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiphoton module (option)</td>
<td>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</td>
</tr>
</tbody>
</table>

### Electronics module

<table>
<thead>
<tr>
<th>LSM 510 Control</th>
<th>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</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computer</td>
<td>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</td>
</tr>
</tbody>
</table>
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, XZ, XZt, Spot-t
On-line computation and presentation of ratio images.
Averaging and summation (line wise or frame wise, 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 fluorences; 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; 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**

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<td>Z drive</td>
<td>DC motor with optoelectronic coding, smallest increment 25 or 50 nm; fast piezo focusing accessory</td>
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<td>High-precision galvanometric fine focusing stage, total lift 200 µm, smallest increment &lt;10 nm</td>
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<td>Accessories (options)</td>
<td>AxioCam digital microscope camera, incubation chambers, micromanipulators, etc.</td>
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</table>

**Scanning module**

<table>
<thead>
<tr>
<th>Models</th>
<th>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</th>
</tr>
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<tr>
<td>Scanner</td>
<td>2 independent galvanometric scanning mirrors, DSP-controlled, for ultrafast line and vertical flyback</td>
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<td>4x1 to 2048x2048 pixels, also for several channels, continuously adjustable</td>
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<td>Scanning speed</td>
<td>13x2 speed stages; up to 5 frames/s with 512x512 pixels (up to 77 frames/s with 512x32 pixels); min. 0.38 ms for a line of 512 pixels</td>
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<td>Scanning zoom</td>
<td>0.7x to 40x, digital, variable in steps of 0.1</td>
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<tr>
<td>Scanning rotation</td>
<td>Free 360° rotation, variable in steps of 1 degree, free XY offset</td>
</tr>
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<td>Scanning field</td>
<td>Max. diagonal 18 mm in the intermediate image plane, homogeneous illumination</td>
</tr>
<tr>
<td>Pinholes</td>
<td>Pinholes for each epi-illumination channel (single-channel detector or META multichannel detector), individual adjustments of size and position, preadjusted</td>
</tr>
<tr>
<td>Detection</td>
<td>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</td>
</tr>
<tr>
<td>META detector</td>
<td>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</td>
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<td>Data depth</td>
<td>Selectable between 8 bit and 12 bit</td>
</tr>
</tbody>
</table>

**Laser module**

<table>
<thead>
<tr>
<th>VIS laser module</th>
<th>Polarization-preserving single-mode fiber, temperature-stabilized VIS-AOTF for simultaneous intensity control of up to 6 visible-light laser lines, switching time &lt; 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)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiphoton module (option)</td>
<td>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</td>
</tr>
</tbody>
</table>

**Electronics module**

<table>
<thead>
<tr>
<th>LSM 510 Control</th>
<th>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</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computer</td>
<td>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</td>
</tr>
</tbody>
</table>
**Standard software**

<table>
<thead>
<tr>
<th>System configuration</th>
<th>Convenient control and configuration of all motorized microscope functions, of the laser and scanning modules; saving and restoration of application-specific configurations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ReUse function</td>
<td>Restoration of acquisition parameters with one mouse click.</td>
</tr>
<tr>
<td>Acquisition modes</td>
<td>Spot, Line, Spline, Frame, Z Stack, Lambda Stack, Time Series and combinations: XY, XYZ, XY1, XZ, XZ1, Spot-t, X, Y, Z, XYZ, XZ, XZ1, On-line computation and presentation of ratio images. Averaging and summation (linewise or framewise, configurable). Step scan (for higher frame rates, configurable).</td>
</tr>
<tr>
<td>Auto Z</td>
<td>On-line adaptation of Z stack acquisition parameters for uniform brightness distribution.</td>
</tr>
<tr>
<td>Crop</td>
<td>Convenient selection of scanning ranges (zoom, offset, rotation simultaneously).</td>
</tr>
<tr>
<td>RealROI Scan</td>
<td>Scanning of up to 99 ROIs (Regions of Interest) of any shape, with pixel-accurate laser blanking.</td>
</tr>
<tr>
<td>ROI Bleach</td>
<td>Localized photobleaching of up to 99 bleaching ROIs for applications such as FRAP (Fluorescence Recovery After Photobleaching) or uncaging.</td>
</tr>
<tr>
<td>Spline Scan</td>
<td>Scanning along a free-hand defined line.</td>
</tr>
<tr>
<td>Multitracking</td>
<td>Acquisition of multiple fluorences; minimized signal crosstalk (bleeding) by fast change of excitation lines.</td>
</tr>
<tr>
<td>Metatracking</td>
<td>Extension of multitracking by fast electronic change of detection channels, even with overlapping bandpasses, ensures optimum signal detection (only with META detection module).</td>
</tr>
<tr>
<td>Lambda Scan</td>
<td>Fast acquisition of image stacks with spectral information for every pixel (only with META detection module).</td>
</tr>
<tr>
<td>Emission Fingerprint</td>
<td>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).</td>
</tr>
<tr>
<td>Presentation</td>
<td>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.</td>
</tr>
<tr>
<td>Image analysis</td>
<td>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.</td>
</tr>
<tr>
<td>Image operations</td>
<td>Addition, subtraction, multiplication, division, ratio, shift, filters (low-pass, median, high-pass, etc.; user-definable).</td>
</tr>
<tr>
<td>Image archiving, export, import</td>
<td>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, JPEG, PSD, PCX, GIF, AVI, Quicktime …) for compatibility with all common image processing programs.</td>
</tr>
</tbody>
</table>

**Software Options**

<table>
<thead>
<tr>
<th>LSM Image VisArt</th>
<th>Fast 3D and 4D reconstruction and animation (silhouette projection, transparent projection, surface rendering).</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D Deconvolution</td>
<td>Image restoration based on computed point spread functions (Nearest Neighbor, Maximum Likelihood, Constraint Iterative).</td>
</tr>
<tr>
<td>Multiple Time Series</td>
<td>Assembled time series with change of application-specific configurations, autofocus and bleaching functions.</td>
</tr>
<tr>
<td>3D for LSM</td>
<td>3D reconstruction, thresholding, processing and measurement (surface area, volume, particle count).</td>
</tr>
<tr>
<td>Physiology</td>
<td>Analysis of time series, graphical mean-of-ROI analysis, on- and off-line display and calibration of ion concentrations.</td>
</tr>
<tr>
<td>Topography</td>
<td>Visualization of 3D surfaces (fast rendering modes) plus measurement functions (roughness, waviness, surface area, volume).</td>
</tr>
<tr>
<td>Macro Edit</td>
<td>Acquisition and editing of routines for the automation of scanning and analysis functions.</td>
</tr>
</tbody>
</table>

**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

- Laser module VIS
  - VIS AOTF (6 channels)
- NLO kit for fiber coupling
- NLO kit for direct coupling
- Ar-laser, 458, 477, 488, 514 nm, 30 mW
- HeNe laser, 543 nm, 1 mW
- HeNe laser, 633 nm, 5 mW

Options:
- VIS/NLO-Scan module LSM 510
- Upgrade kits LSM 510 to LSM 510 META
- Fluorescence Correlation Spectroscopy (FCS)
- Fluorescence Lifetime Imaging Microscopy (FLIM)

Actively vibration-absorbed system table for LSM 5
- Table surface 30" x 30" (76.2 cm x 76.2 cm)

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

Monitor 21" (50 cm)

Host computer

LCD TFT-Flatscreen 18"
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

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

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.

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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Subject to change.