

LSM 7 *LIVE* and LSM 7 *DUO*
Your Vision Set in Motion



Localize, manipulate, visualize and analyze



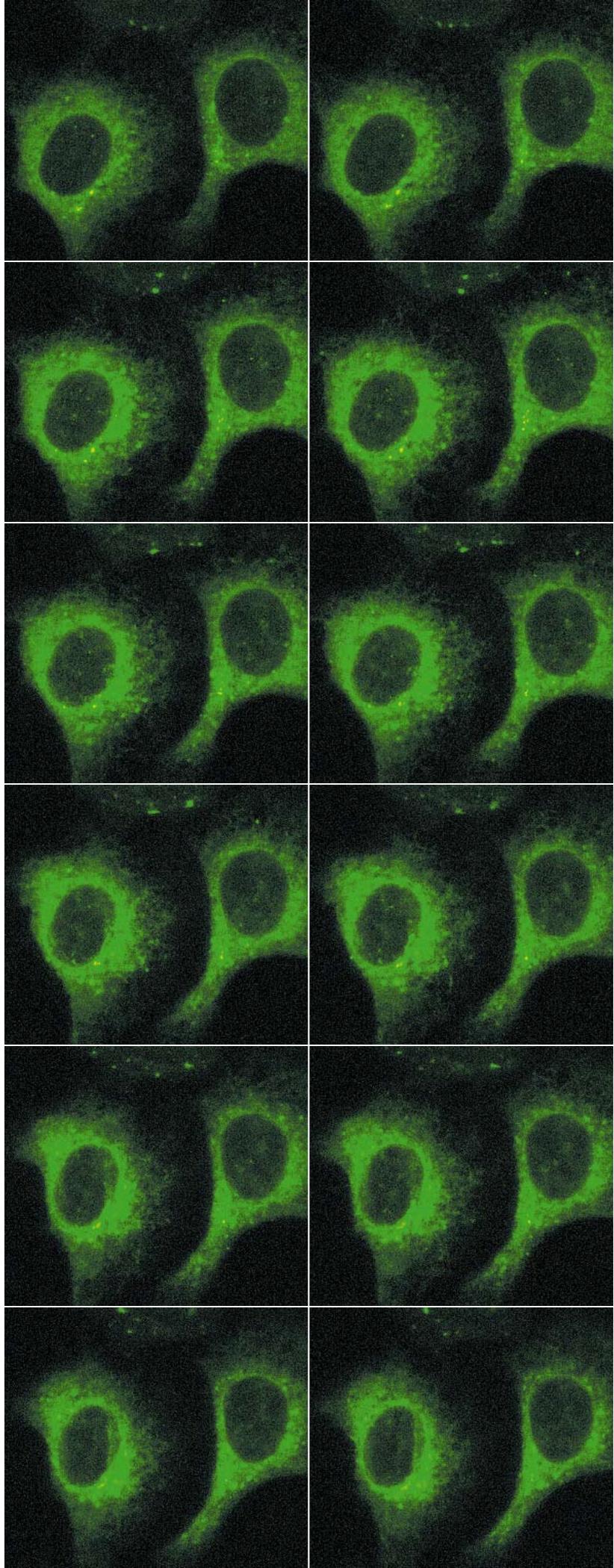
We make it visible.

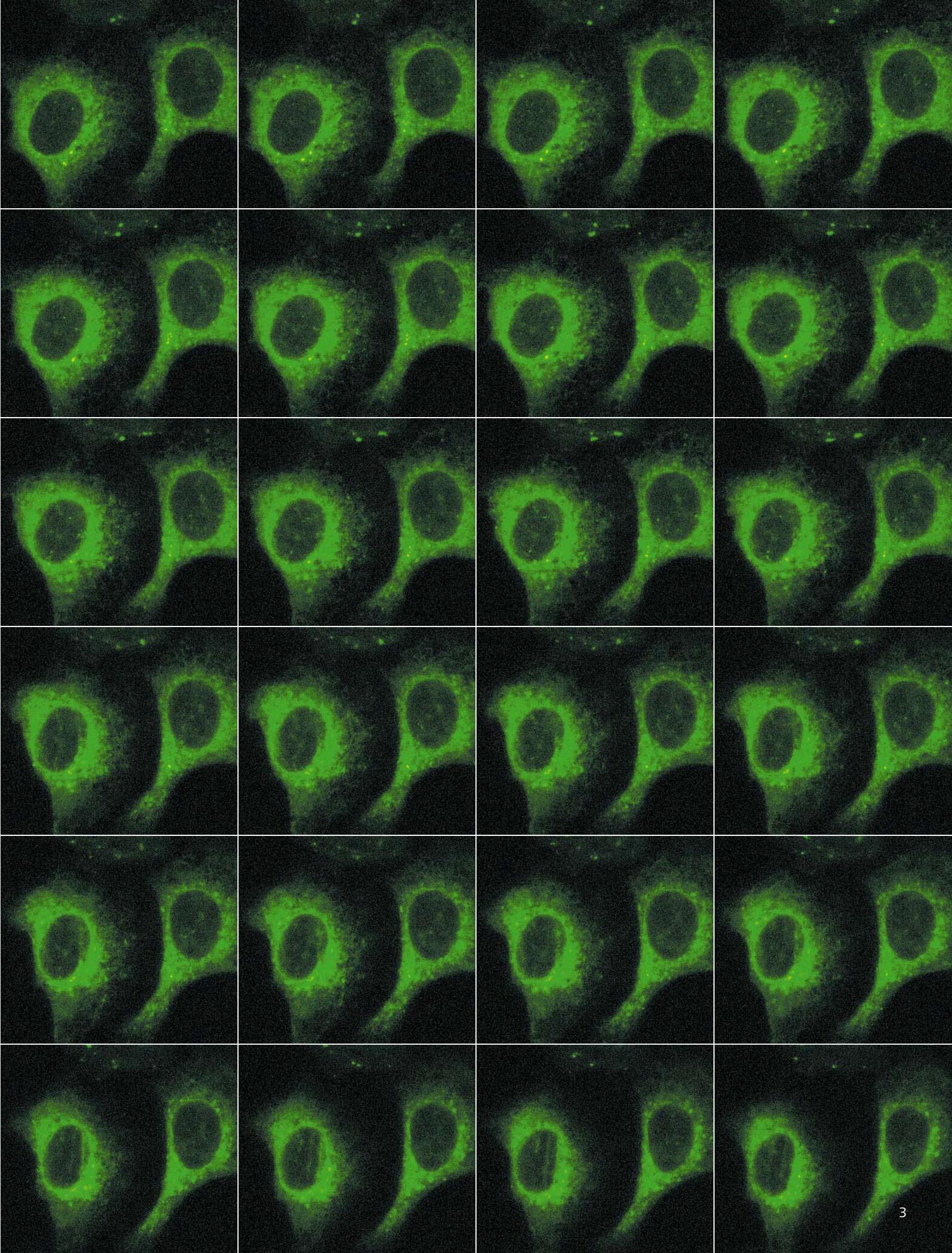
Content

LIVE Transmission	4
Access to living cells	8
Analyzing dynamic events with FRAP, FLIP und FRET	10
Follow up molecules with PA-GFP, DRONPA und KAEDE	12
Speed is the key in your physiological measurement	14
Deep insight by multiphoton excitation	16
Study motion in detail	17
Faster than in real time	18
Confocal microscopy without limits	20
Technical data	22
System overview	24
Excellent in combination	27

Title:
Motion trajectories of Shewanella oneidensis bacteria. Maximum intensity projections of 500 images of a XYZ time series with 44 fps.
Preparation: Dr. T. Teal, Dr. D. Newman, Biological Imaging Center, Caltech, Pasadena, USA

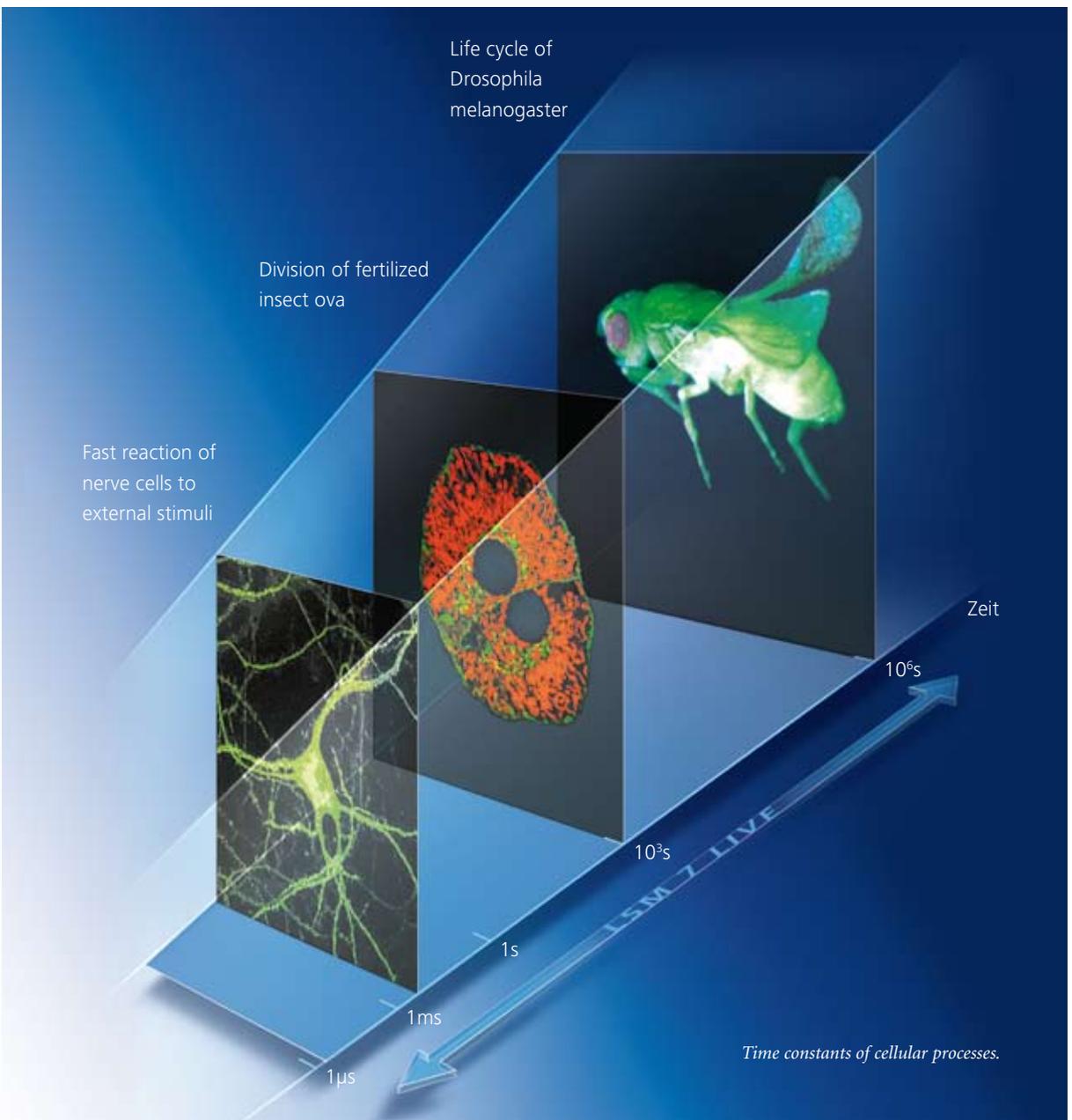
Page 3:
GalT-Y BFP Washout of cultivated cells, time series of intraplasmatic motion.
Preparation: Dr. Lippincott-Schwartz, NIH, Bethesda, USA



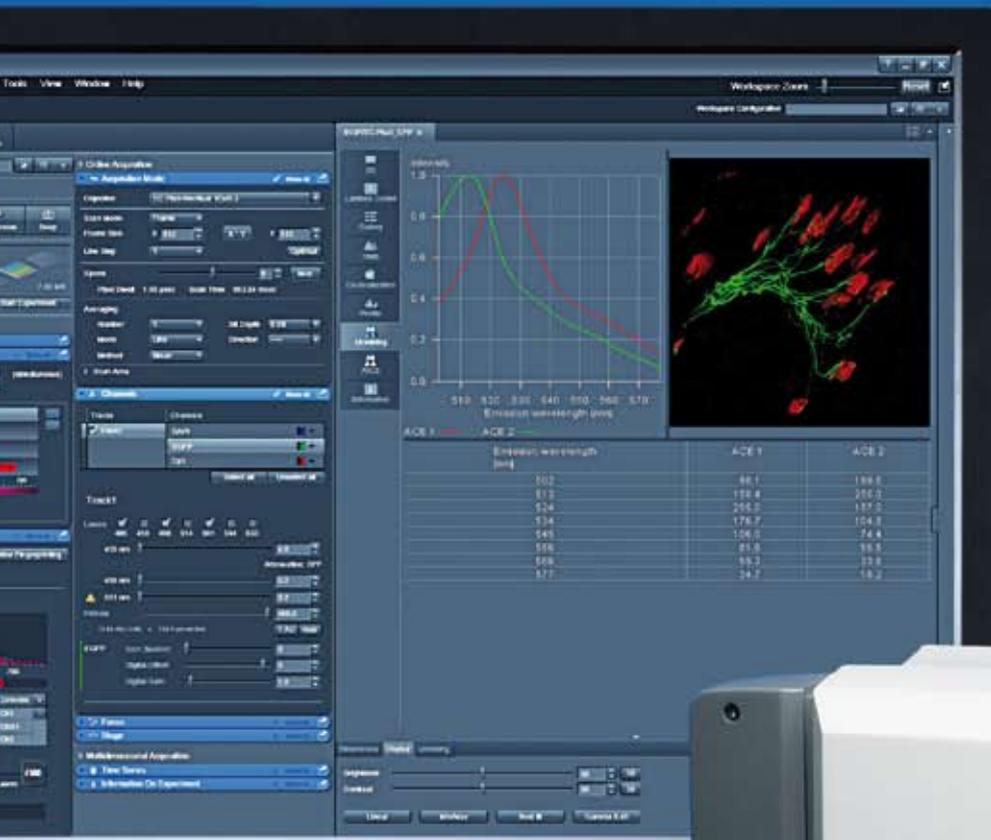


LIVE Transmission

Depict living cells live and in motion
to understand fundamental processes.



ZEN Software –
the interface to your applications.



The LSM 7 *DUO* unites LSM 7 *LIVE* and
LSM 710 at the Axio Observer.Z1



The LSM 7 LIVE with the manipulation module
LSM DuoScan at the Axio Observer.Z1



Access to Living Cells

The abundance of high-resolution multi-dimensional digital data requires new strategies in imaging, processing and visualizing image series.

The LSM 7 *LIVE* navigates and analyzes these data flows reliably and efficiently.

64-bit performance for your image data

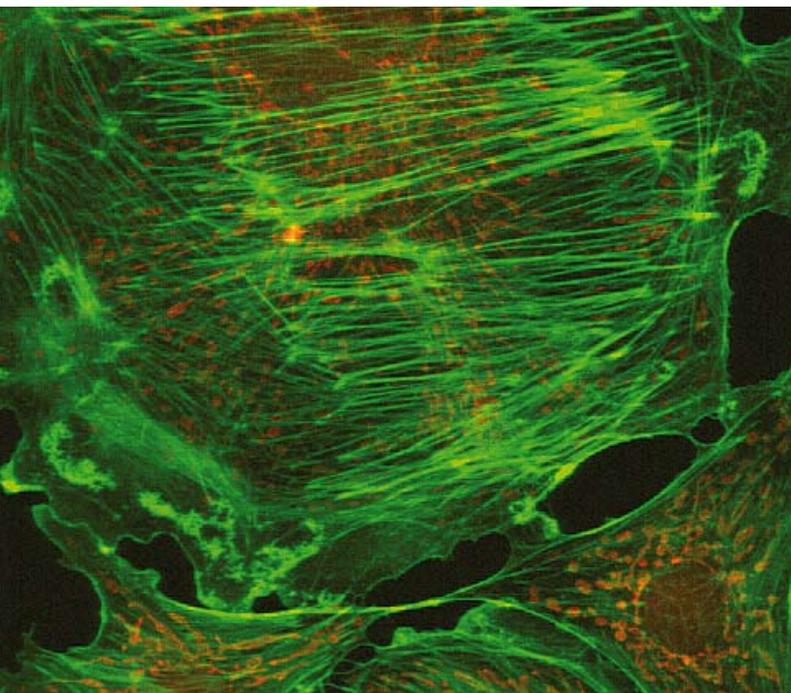
A thousand images of 512x512 pixels each in 10 seconds? Some 250 MB in just 10 seconds or a full CD-ROM of data in less than half a minute? Such outstanding performance is not difficult for the LSM 7 *LIVE*, thanks to novel real-time electronics paired with the new realtime computer system with Windows 64-bit. It handles these huge 4D data volumes with transmission rates of up to 100 MB per second.

Identifying objects from their paths

Process your time series, for example, with offline particle-tracking-software in AxioVision. This lets you capture the trajectories of your objects and draw valuable conclusions.

Precise play of laser light colors

The compact and long-lived solid-state laser puts even your thick or weakly fluorescent preparations in the right light, limiting tissue damage. The new fine-tuned laser coupling and beam formation of the LSM 7 *LIVE* set new standards for image quality with high-speed line scanners. Choose the excitation lines for your dyes from lasers with 405, 488, 532, 561 and 635 nm.



Cyto-skeleton and mitochondria of a cultivated cell. With its new fine-tuned laser coupling and beam formation, the LSM 7 LIVE delivers an artefact-free image, even from highly homogeneous preparations – an innovation in line scan systems.

The LSM 7 LIVE: High-performance, user-friendly ZEN Software with its 64-bit-operating system and scan head for improved image quality.



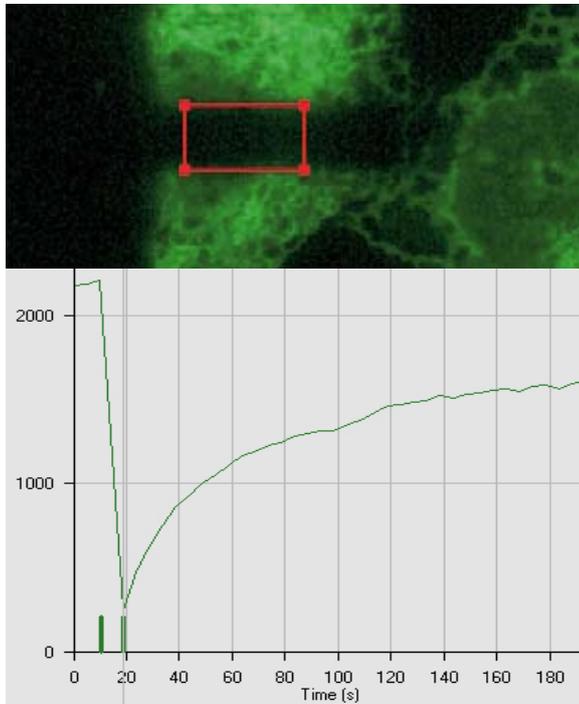
Automated imaging of ROIs

Define complex recurrent processes in time series. The new multilocation scan of ZEN 2009 Software automatically records your selected positions or a complete multiwell plate – with a correct Z-position even in extremely long series. This leaves you more time for complex tasks.

Multilocation scan for your time series.

Analyzing Dynamic Events with FRAP, FLIP and FRET

The LSM 7 LIVE DuoScan and LSM 7 DUO achieve enormous precision in investigating the kinetics of molecules. Perform your FRAP and FLIP experiments with direct laser bleaching in extremely quick time series.

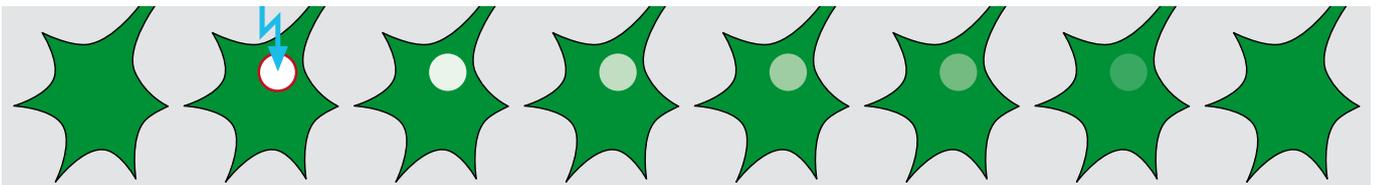


FRAP: Bleaching of a ROI and recovery in a GFP-marked CD3-cell with the LSM 7 DUO.

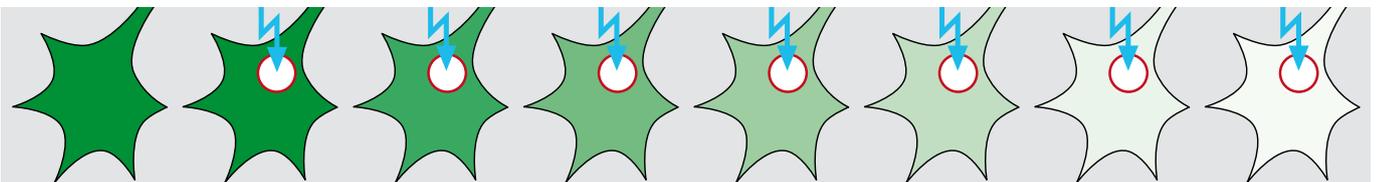
The LSM 7 LIVE depicts cells at high speed, but it can do a whole lot more. Two independent scanner groups, in combination as LSM 7 DUO or with the LSM DuoScan, bring you valuable flexibility in photobleaching. You can carry out quick FRAP experiments in freely definable ROIs with varying wavelengths – even simultaneously with quick recording in two channels.

Use the LSM 7 LIVE for your FRAP, FLIP and FLAP experiments with double marking to compare the dynamic ratio between unbleached and bleached fluorescence markers.

In an FRAP experiment a defined region in a cell expressing a GFP-fusions protein is bleached by brief but intensive laser irradiation. The recovery of fluorescence is documented by time-lapse shots and measured.



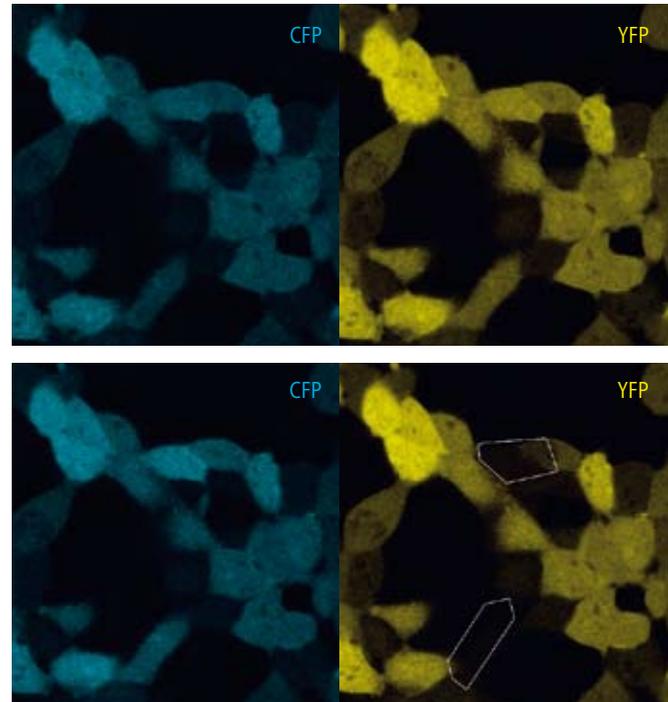
In a FLIP experiment the same region within a cell is bleached repeatedly and the fluorescence loss outside that region is measured.



In addition to the traditional bleach and recovery experiments used to analyze molecular kinetics and motility, the LSM 7 *LIVE* also lets you carry out molecular interaction studies and analysis of developmental events in a much more sophisticated manner.

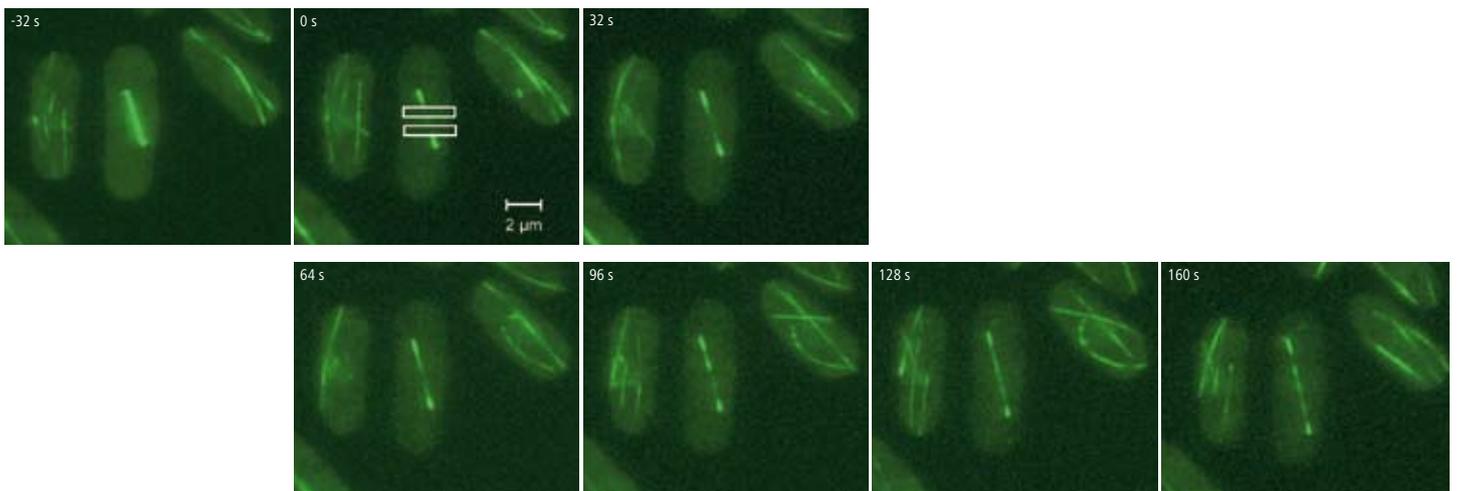
FRET is widely used to analyze the proximity and interaction of molecules. However, the LSM 7 *LIVE* is particularly suitable for conducting the reliable acceptor photobleaching method, a very secure FRET method.

In developmental studies, selective bleaching of structures can supply the answers to many localization and proliferation questions, something that pure staining alone cannot do.



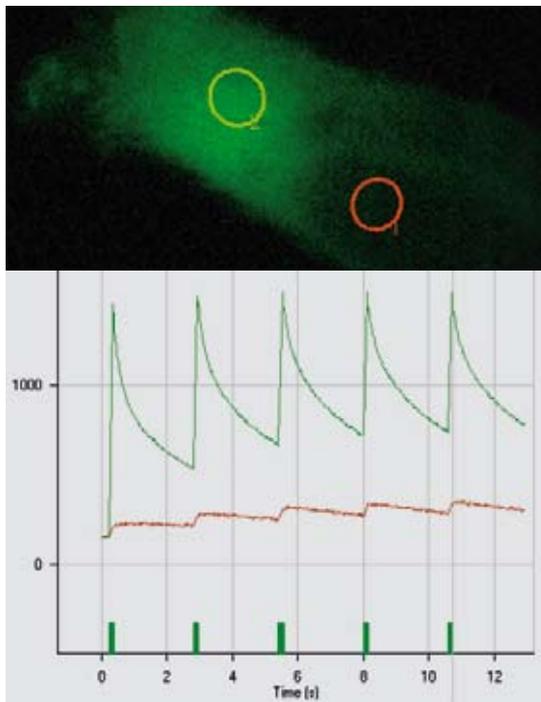
*Yeast cells expressing tubulin-GFP.
Mitotic spindle precisely bleached with LSM 7 DUO.
Preparation: Prof. M. Yoshida, Chemical Genetics Laboratory,
RIKEN, Wako, Japan*

*FRET analysis of CFP and YFP in cultivated cells, controlled
bleaching of the acceptor and increased donor signal.*



Follow Up Molecules with PA-GFP, DRONPA and KAEDE

Extend the scope of your biomedical research to carrying out experiments with flexible sample manipulation - for example, photoactivation and photoconversion – with excellent precision and resolution.

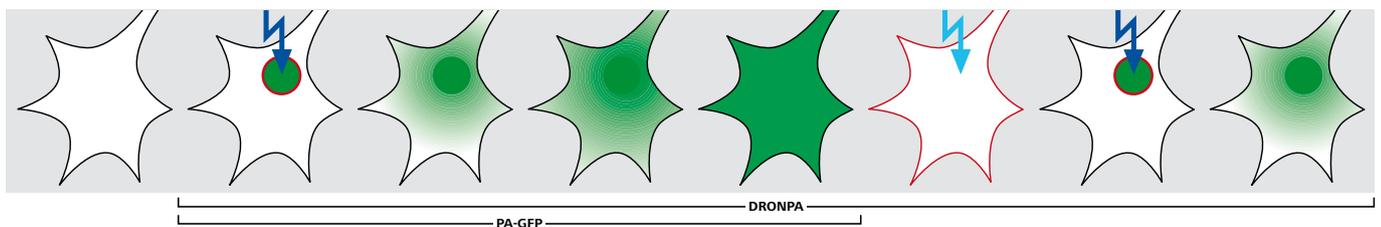


Cultivated cell transfected with DRONPA, activated multiple times with light impulses at 405 nm; fast imaging with excitation at 488 nm.

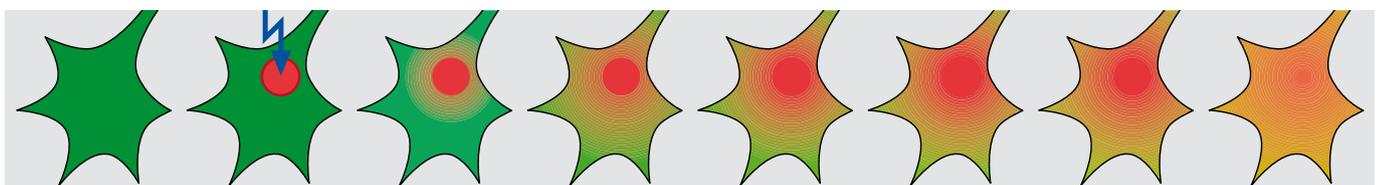
The recently developed fluorescent proteins PA-GFP, DRONPA and KAEDE support you in directly examining dynamic processes. With the two independent scanner groups of the combined systems, LSM 7 LIVE DuoScan and LSM 7 DUO, you can carry out your photoactivation and photoconversion experiments with all the high flexibility you need. Mark subpopulations of proteins, complete organelles or even whole cells in a tissue compound with PA-GFP, DRONPA or KAEDE, then activate them selectively or convert them by local irradiation with violet light. The high performance lasers of wavelengths 405, 488 and 561 nm are ideal for these experiments. They are used by both scan modules and can be monitored via a convenient ROI-Bleach user interface. With the LSM 7 DUO you can select from a variety of detection options: spectral via the QUASAR-Detector of the LSM 710 or at high speed via line scanning in the CCD detectors of the LSM 7 LIVE.

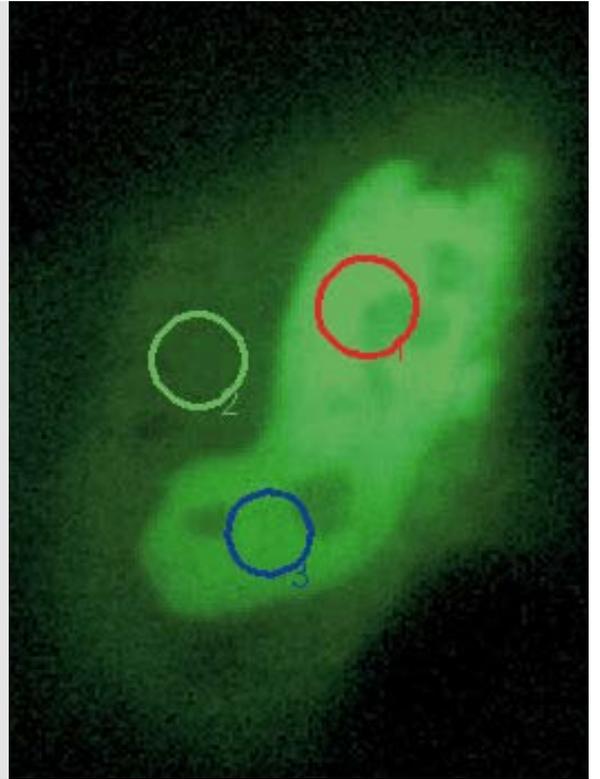
PA-GFP + DRONPA

The fluorescent protein DRONPA switches in optical excitation between a fluorescent and non-fluorescent state.



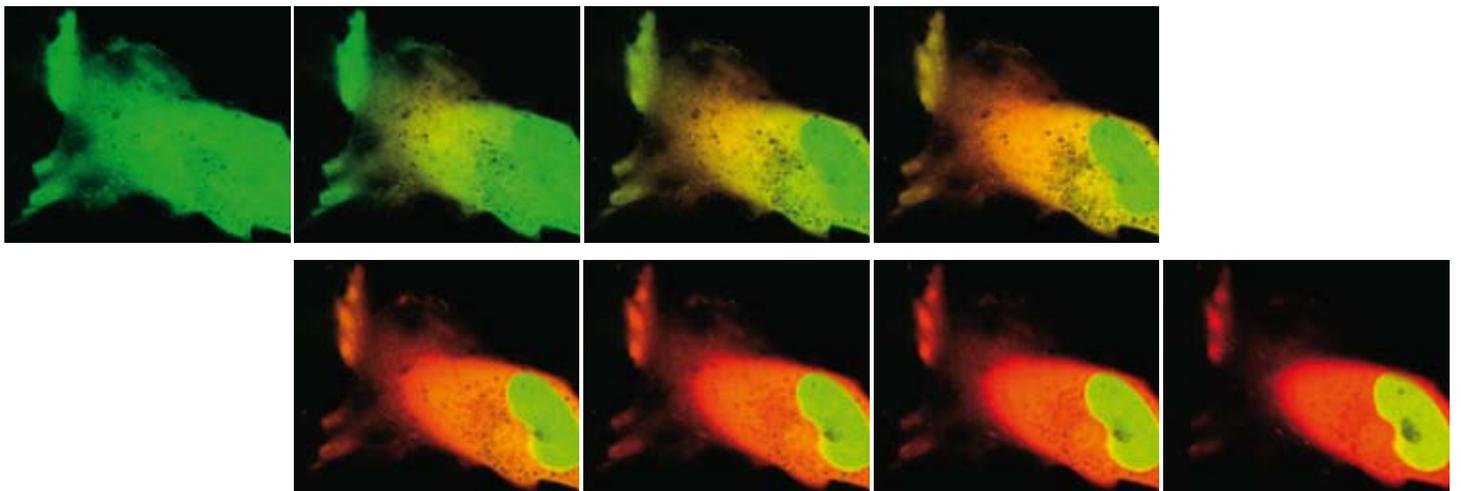
The fluorescent protein KAEDE switches from red to green when irradiated with UV light.





Cultivated cell transfected with PA-GFP in ROI 1 briefly activated with 405 nm light; analysis of molecule motion in ROI 2.

Photo conversion of a cell transfected with KAEDE. Irradiation at 405 nm in an exactly localized subcellular region converts the green fluorescence completely into red fluorescence.



Speed is the Key in Your Physiological Measurements

The LSM 7 *LIVE* is ideal for measurements that have to be adapted to the speed and change in color of ion indicators and stress-sensitive dyes.

The LSM *DuoScan* guarantees maximum precision while manipulating ROIs.

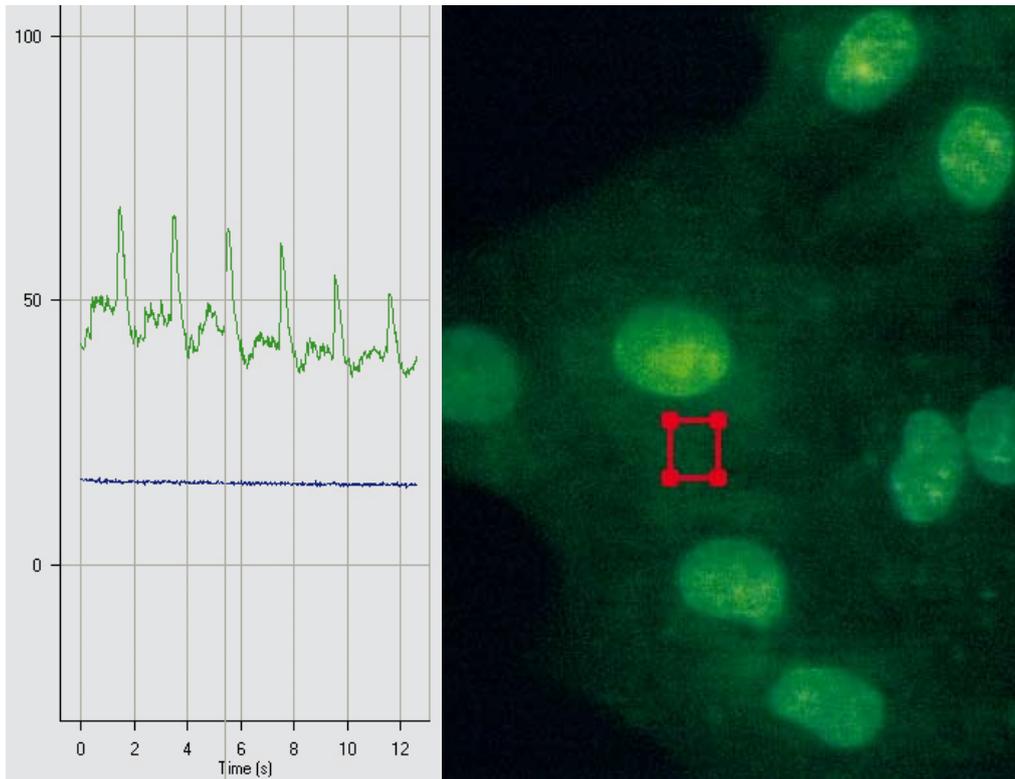
Image frequencies within the kilohertz range are achieved with the LSM 7 *LIVE* – for example, 1010 fps at 512x50 pixels. You reap the benefits from this exceptional speed in having a real confocal system with simultaneous two-channel imaging. Then, rounded off with a point scanning system, the LSM 7 *LIVE DuoScan* or LSM 7 *DUO* makes your system flexible enough for uncaging and sample stimulation - for example, with near UV light. Achromatic immersion objects are available for micromanipulation, like the plan Achromates 20x/1.0 W and 63x/1.0 W.

Presentation and evaluation of ion concentrations

- Online and offline ratio for ratiometric dyes
- Online and offline F/F0 for single-wave dyes
- Calibration for ratiometric and single-wave dyes
 - in situ and in vitro
 - including substrate compensation
 - after titration with various curve adaptations
 - according to Grynkiewicz
- Interactive scaling of image data series
- Interactive graphic presentation of measuring data from ROIs

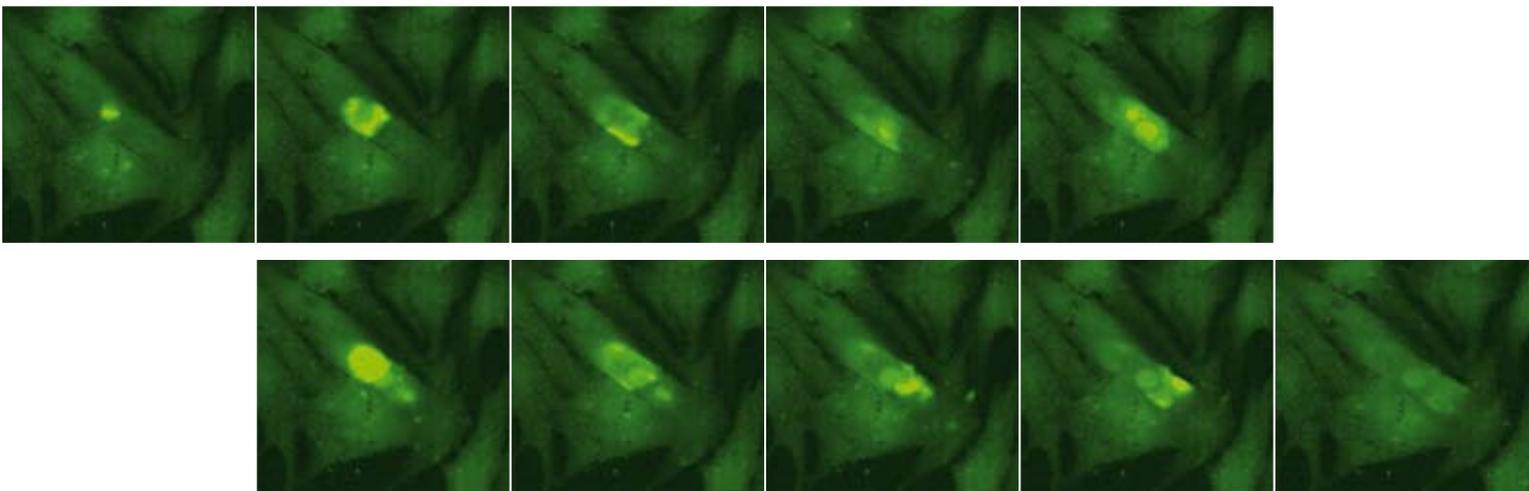
Easy evaluation of the absolute Ca²⁺ concentration using the calibrating function of the ZEN Software.

The screenshot shows the 'Calibration graph' window in ZEN Software. It includes a 'Load ...' and 'Save ...' button at the top right. Below are several dropdown menus: 'Background (Input 2)' (checked), 'Dye' (Single wavelength dye), 'Channel 1', 'Method' (Equation), and 'Calibration' (In Vitro). There are two spinners for 'Display minimum concentration' (0.10) and 'Display maximum concentration' (1.00). A mathematical formula is displayed:
$$\text{Concentration} = Kd * \frac{F - F_{min}}{F_{max} - F}$$
 Below the formula are input fields for 'Kd' (1.000), 'Fmin' (25.500), and 'Fmax' (255.000), each with a 'Select' button.



*Fast Ca²⁺ transitions in Fluo-4 labelled heart muscle cells of a rat, imaged with 80 fps.
Preparation: Dr. W. J. Lederer and Dr. A. Ziman,
Medical Biotechnology Center,
Biotechnology Institute, University of Maryland,
Baltimore, USA*

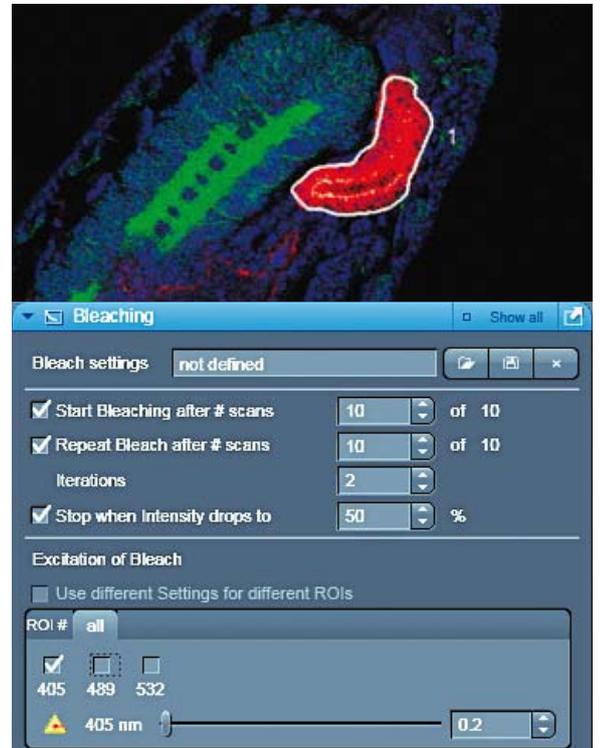
Manifold repeated Ca²⁺ increase in Fluo-4 marked heart muscle cells after stimulation.



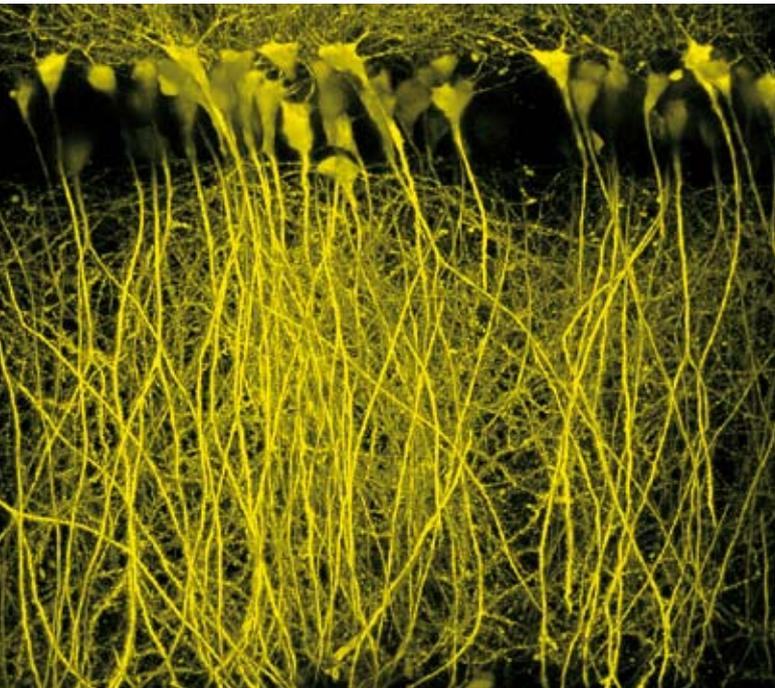
Deep Insight by Multiphoton Excitation

Multiphoton microscopy offers substantial progress in experiments with living cells by granting significant insight into living tissue, while simultaneously guaranteeing high laser performance and limiting tissue damage.

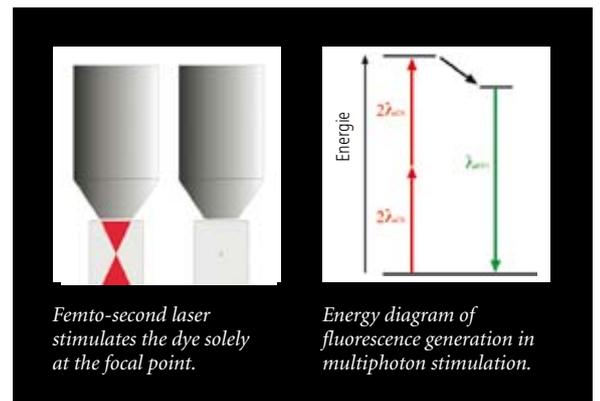
Photobleaching, photoactivating and uncaging, above all, with violet and ultraviolet laser – that is the outstanding advantage of the LSM 7 DUO. The multiphoton mode penetrates deep into the specimen. The high-speed line detection with the LSM 7 DUO NLO depicts living preparations at extremely high speed, simultaneously performing photomanipulation deep into the tissue with the multiphoton laser.



Ca-1 pyramid cells in the hippocampus of a mouse. The penetration depth of the multiphoton method enables highly detailed resolution. Preparation: M. Fuhrmann, Center for Neuropathology and Prion forschung, LMU Munich, Germany



Flexible manipulation: With the bleaching menu, manipulation timing and depth position can be set easily.

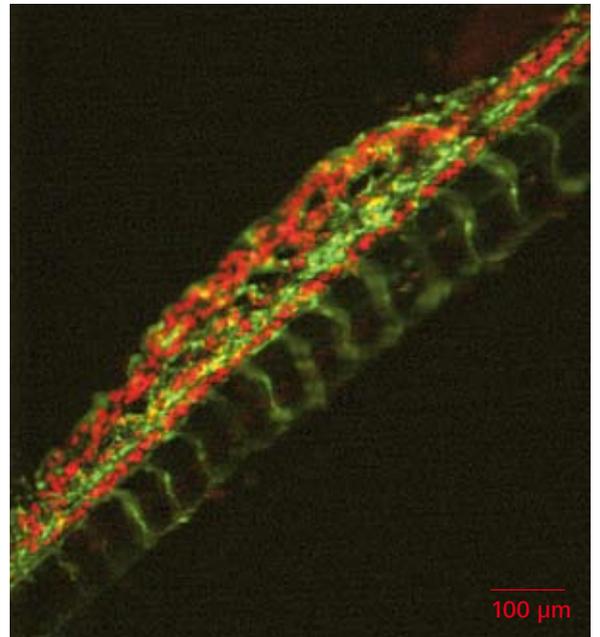
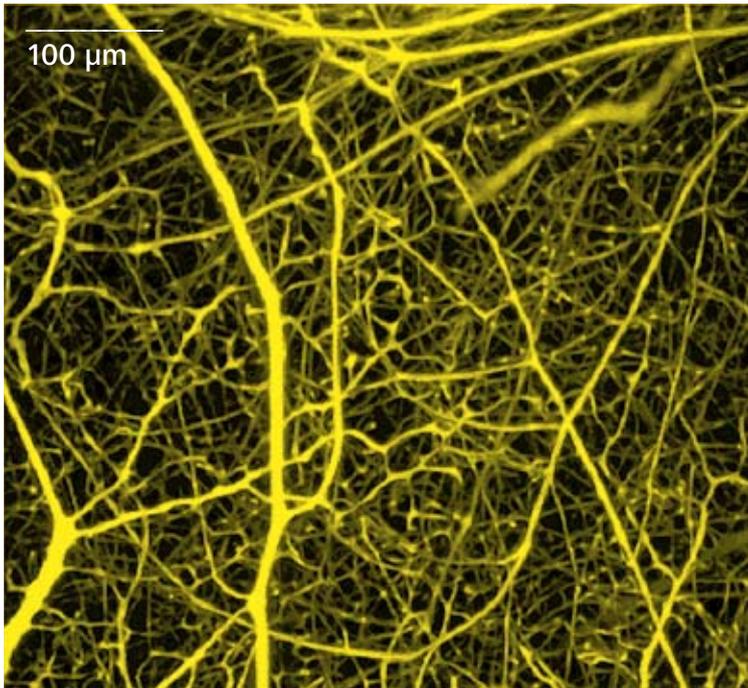


Study Motion in Detail

When complex cell processes are taking place at speeds higher than video frequencies, things become challenging. The revolutionary high-speed detection method implemented in the LSM 7 *LIVE* lets you follow these processes and analyze them in 3D and 4D.

Several innovations enable high-speed, ultra-quick parallel detection with the incomparable sensitivity of, for example, up to 1010 images per second and 512x50 pixels. With AchroGate, a trend-setting laser divider, you will detect emissions with an efficiency factor of 95 % without mechanical or electrical conversion. With the two integrated CCD line detectors (quantum efficiency of 75 % at 550 nm) and the clever double bandpass filters, it is possible to observe, for example, neuronal processes lasting only a few milliseconds with two or even more markers.

*High-resolution 3D imaging of blood vessels in the brain of a mouse visualized by the fluorochrome – labeled gelatine method.
Preparation: Dr. H. Hashimoto, Medical Faculty of the University Jikei; Dr. M. Kusakabe, Institute for Matrix Cell Research, Japan*



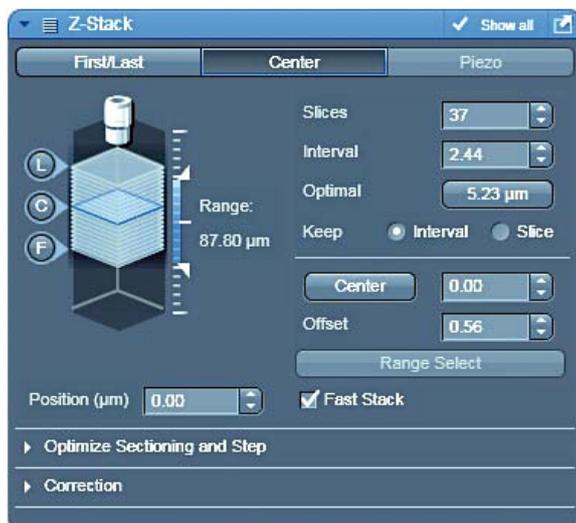
*Zebra fish embryo. Erythrocytes marked with dsRed (red) and endothelial cells with eGFP (green). Simultaneous imaging in two channels with 33 images per second.
Preparation: Dr. S. Hermanson and Dr. S. C. Ekker, University of Minnesota, USA*

Faster than in Real Time

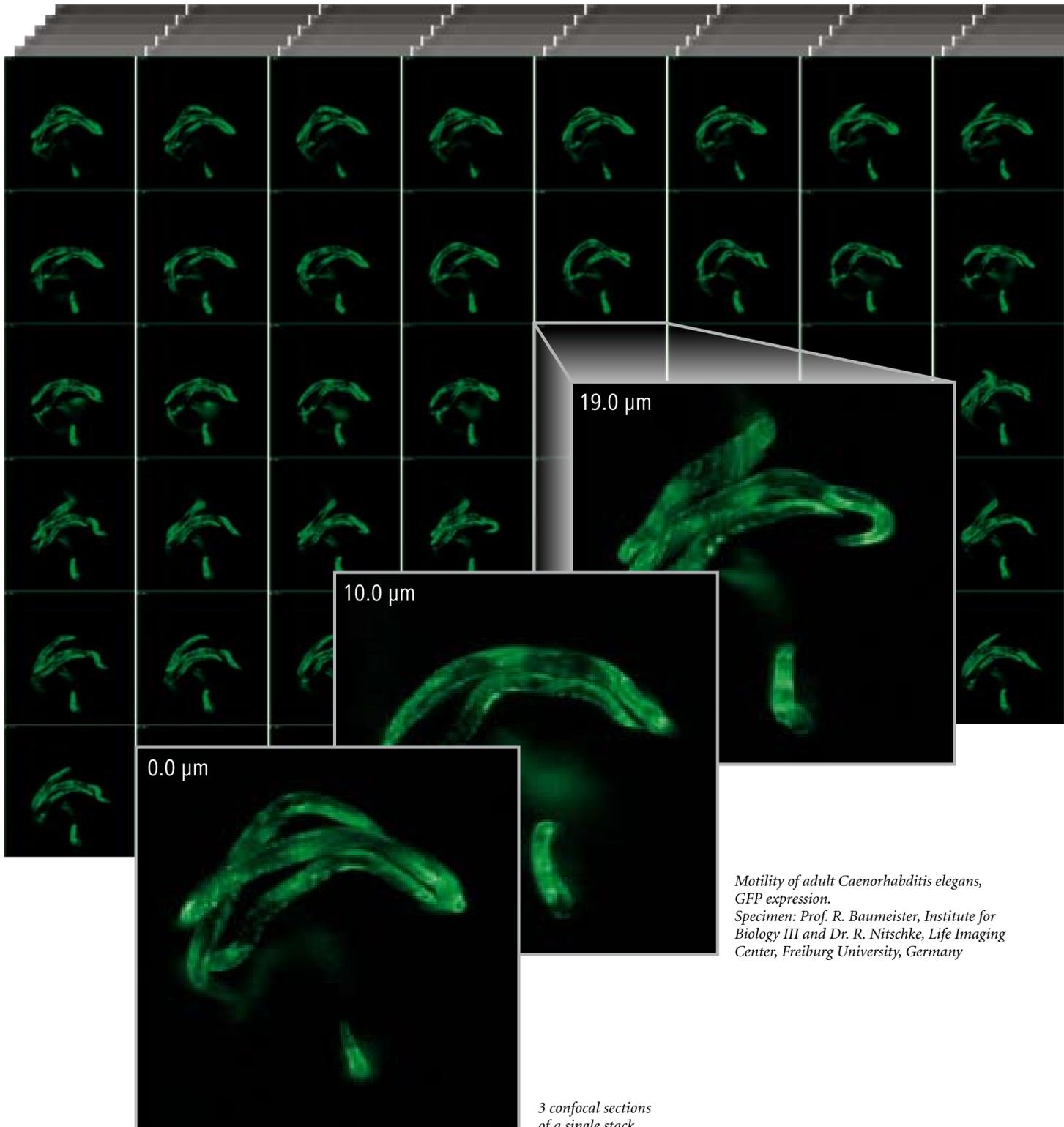
Imaging the developmental processes of organisms undergoing rapid changes is a challenge – they are taking place deep inside the organism itself so you have to carry out your development investigations at high speed in 4D – that is, you need true confocality.

The LSM 7 *LIVE* offers you the confocal imaging precision you need for your 4D development studies. The confocal apertures are set according to requirements and lens. Optical imaging is performed at high speed with excellent 3D resolution over time in the 4th dimension. With the latest piezo focusing features you can process Z-image stacks of up to 70 optical cuts per second and extend the focusing range up to 250 μm , making it ideal for imaging living objects. The objectives have been developed especially for your Live-Cell Imaging – that is, the ZEISS LCI Plan-Neofluare or the LD C-Apochromate. A generous working distance with water or glycerin immersion gives you sufficient scope.

*Fast Z image acquisition
with piezo focusing drive.*



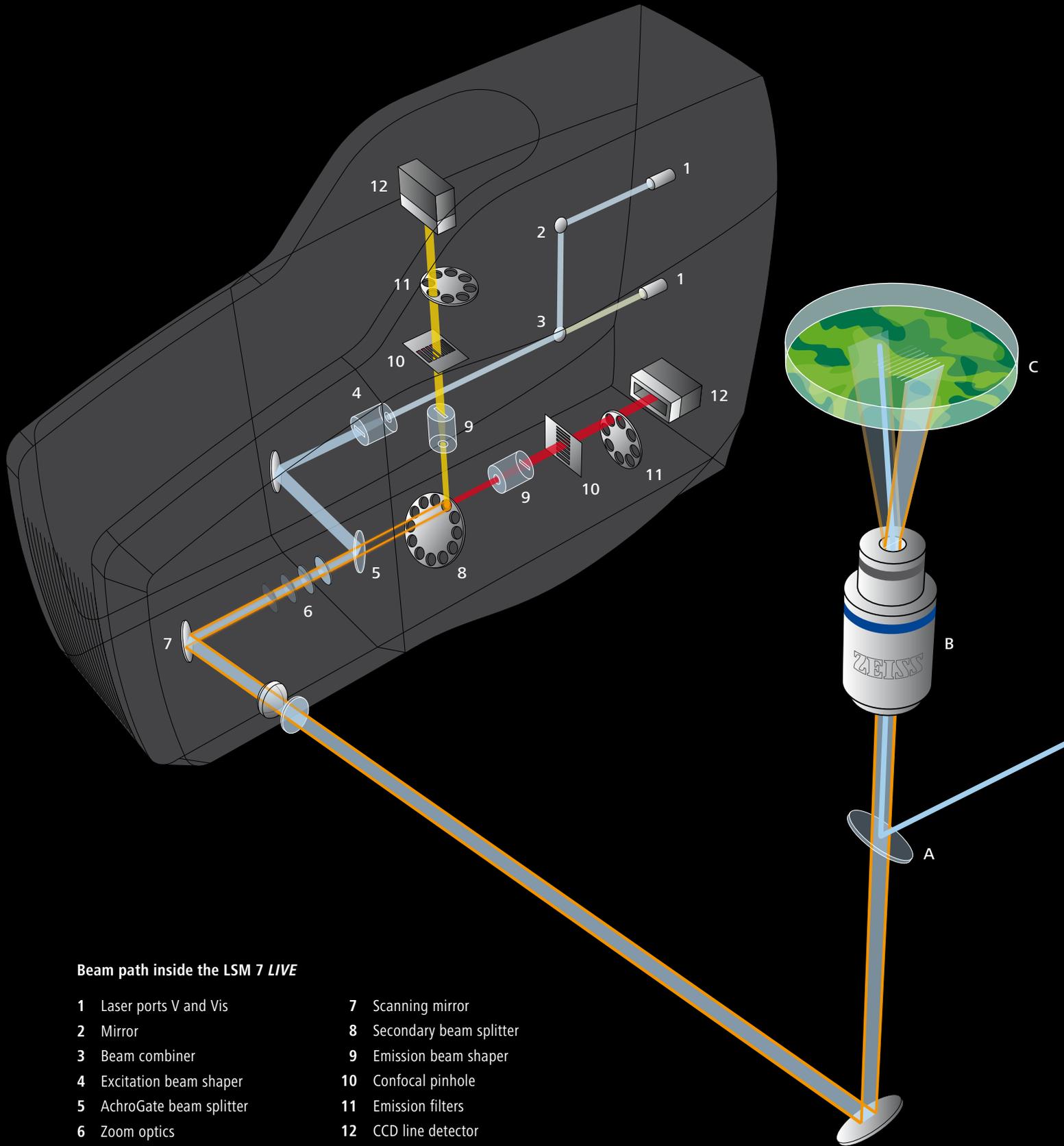
Gallery of projections of a XYZt time series, recorded at 40 frames per second or in 1.23 sec per single stack, total duration of the experiment: 54 s.



Motility of adult *Caenorhabditis elegans*, GFP expression.
Specimen: Prof. R. Baumeister, Institute for Biology III and Dr. R. Nitschke, Life Imaging Center, Freiburg University, Germany

3 confocal sections of a single stack.

Confocal Microscopy Without Limits

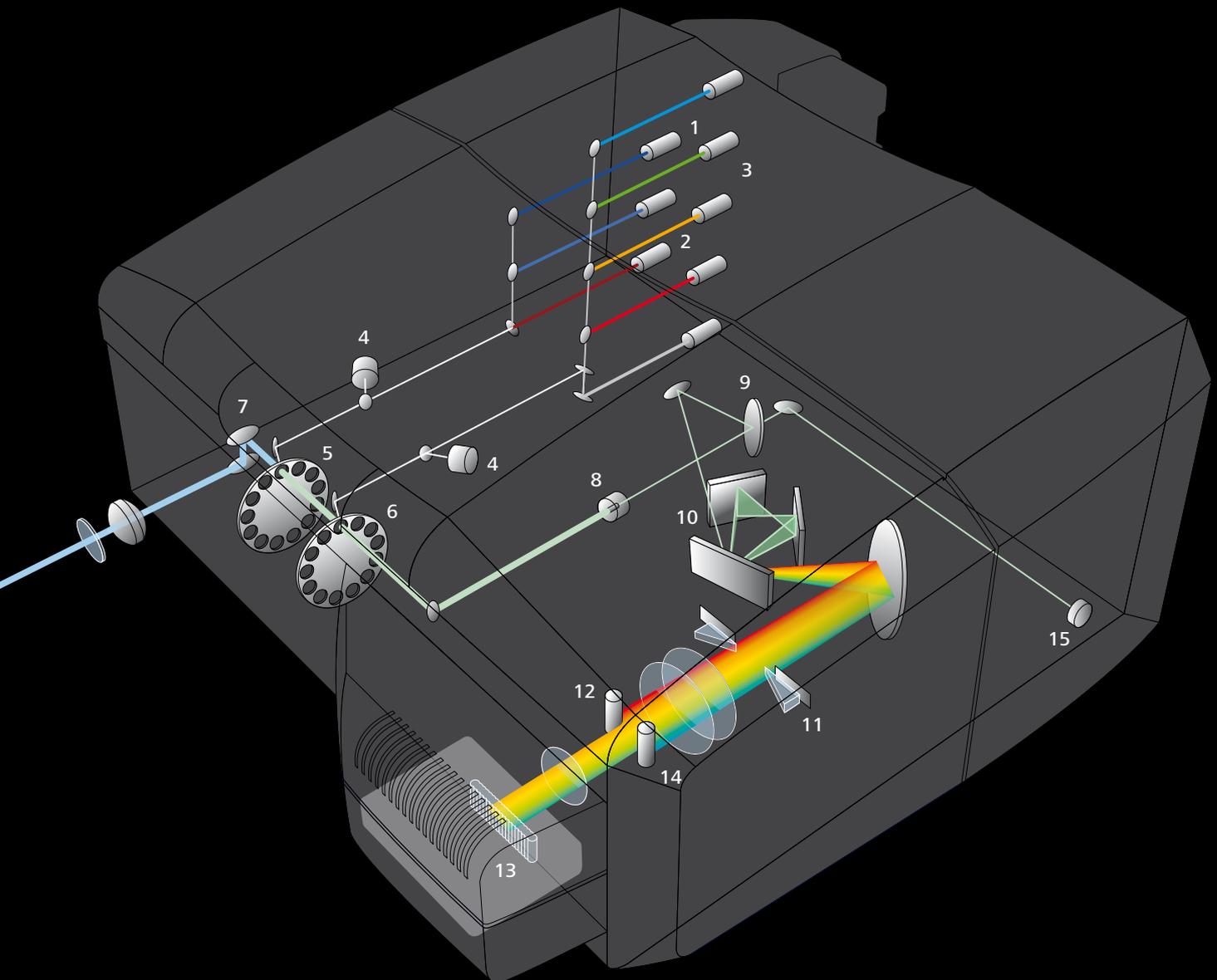


Beam path inside the LSM 7 LIVE

- | | |
|---------------------------------|---------------------------|
| 1 Laser ports V and Vis | 7 Scanning mirror |
| 2 Mirror | 8 Secondary beam splitter |
| 3 Beam combiner | 9 Emission beam shaper |
| 4 Excitation beam shaper | 10 Confocal pinhole |
| 5 Achromatic Gate beam splitter | 11 Emission filters |
| 6 Zoom optics | 12 CCD line detector |

Beam path inside the LSM 710

- | | |
|--|---|
| 1 V/Flex PTC laser ports
(405, 440, In Tune; ps+cw) | 8 Master pinhole |
| 2 IR PTC laser port
(tunable Ti:Sa) | 9 Splitter for external channels |
| 3 Vis PTC laser ports & Vis AOTF | 10 Spectral separation and recycling loop |
| 4 Monitoring diodes | 11 Spectral beam guides |
| 5 InVis TwinGate beam splitter
(upgradeable) | 12 QUASAR PMT spectral channel # 1 |
| 6 Vis TwinGate beam splitter
(user exchangeable) | 13 QUASAR PMT spectral channels # 2–33 (or # 2) |
| 7 Scan mirrors (FOV 20, 6k×6k) | 14 QUASAR PMT spectral channel # 34 (or # 3) |
| | 15 Ext. channels (# 4+5: APDs, FLIM, FCS etc.) |



- A Beam splitter *DUO*
- B Objective lens
- C Specimen

LSM 7 LIVE DuoScan and LSM 7 DUO Technical Data

Microscopes	
Models	Upright: Axio Imager.Z2, Axio Examiner.Z1; Inverted: Axio Observer.Z1 RP (Rear Port) or SP (Side Port)
Z drive	DC motor with optoelectronic coding, smallest increment 25 or 50 nm
Fine focusing	Accessory piezoelectric drive acting on stage or objective; total travel approx. 250 µm, smallest increment < 10 nm
XY stage (option)	Motor-driven XY scanning stage with Mark&Find (xyz) and Tile Scan (Mosaic Scan) functions; smallest increment 1 µm
Accessories	AxioCam Digital Microscope Camera, incubation chambers, micromanipulators, etc.

Scanning Module LSM 7 LIVE	
Models	Choice of one or two genuinely confocal channels
Scanner	One galvanometric scanning mirror for ultrafast image scanning; optional second scanning mirror for positioning the zoom region
Scan resolution	Up to 1536 x 1536 pixels, also for several channels, continuously variable
Scanning speed	Variable up to 120 frames/s with 512 x 512 pixels; faster modes with smaller frames (e.g. 505 frames/s with 512 x 100 pixels, 1010 frames/s with 512 x 50 pixels); ultrafast line scan mode with >60,000 lines/s
Scan zoom	0.5x to 2.0x, digital, free XY offset (depending on configuration)
Scan field	Maximum field diagonal 18 mm in the intermediate image plane, homogeneous illumination
Pinholes	Individually variable confocal pinholes for each detection channel
Detection	Up to two confocal channels for fluorescence, equipped with highly sensitive Detectors (QE 70% or better), Bright-field transmitted-light mode possible
Data depth	Selectable: 8 bits or 12 bits

Laser Module LSM 7 LIVE	
VIS Laser Module	Polarization-preserving single-mode fiber, temperature-stabilized VIS-AOTF for simultaneous intensity control; switching time < 5 µs
Lasers	All lasers of maintenance-free diode or solid-state type without significant heat dissipation. 405nm laser diode, 50 mW; 488nm laser diode, 100 mW; diode-pumped solid-state laser 532 nm, 75 mW; laser diode 561 nm, 40 mW; laser diode 635nm, 30 mW
Variable beam splitting (LSM DuoScan)	Additional outlet from existing LIVE Laser Module with polarization-preserving single-mode fiber; splitting proportion between the outlets freely variable through the software; for 405, 488, 532 or 561 nm laser lines

Scanning Module LSM DuoScan	
Scanner	Two independent galvanometric scanning mirrors, real-time controlled, with ultrashort line and frame flyback
Scanning speed	13 x 2 speed stages; up to 5 regions/s with 512 x 512 pixels (max. 77 regions/s with 512 x 32 pixels), 0.38 ms for a line of 512 pixels
Scan zoom	0.7x to 40x, digitally variable in steps of 0.1
Scan rotation	Free 360° rotation in steps of 1°, free XY offset
Scan field	18 mm field diagonal (max.) in the intermediate image plane, homogeneous field illumination

Electronics Module

LSM 7 LIVE Control	Controls the microscope, the laser modules, the scanning module and other accessories. Controls and synchronizes data acquisition through real-time computer; data exchange with user PC through Gigabit Ethernet Interface
Computer I	Standard PC with main and hard disk memory space appropriate to practical requirements; ergonomic high-resolution flat-panel displays of 30" (16:10), many accessories; Windows 32 or 64 bit multi-user operating system
Computer II	High-end PC with abundant main memory space and ultrafast RAID 0 hard disk system; ergonomic high-resolution flat-panel displays of 30" (16:10), many accessories; Windows 32 or 64 bit multi-user operating system

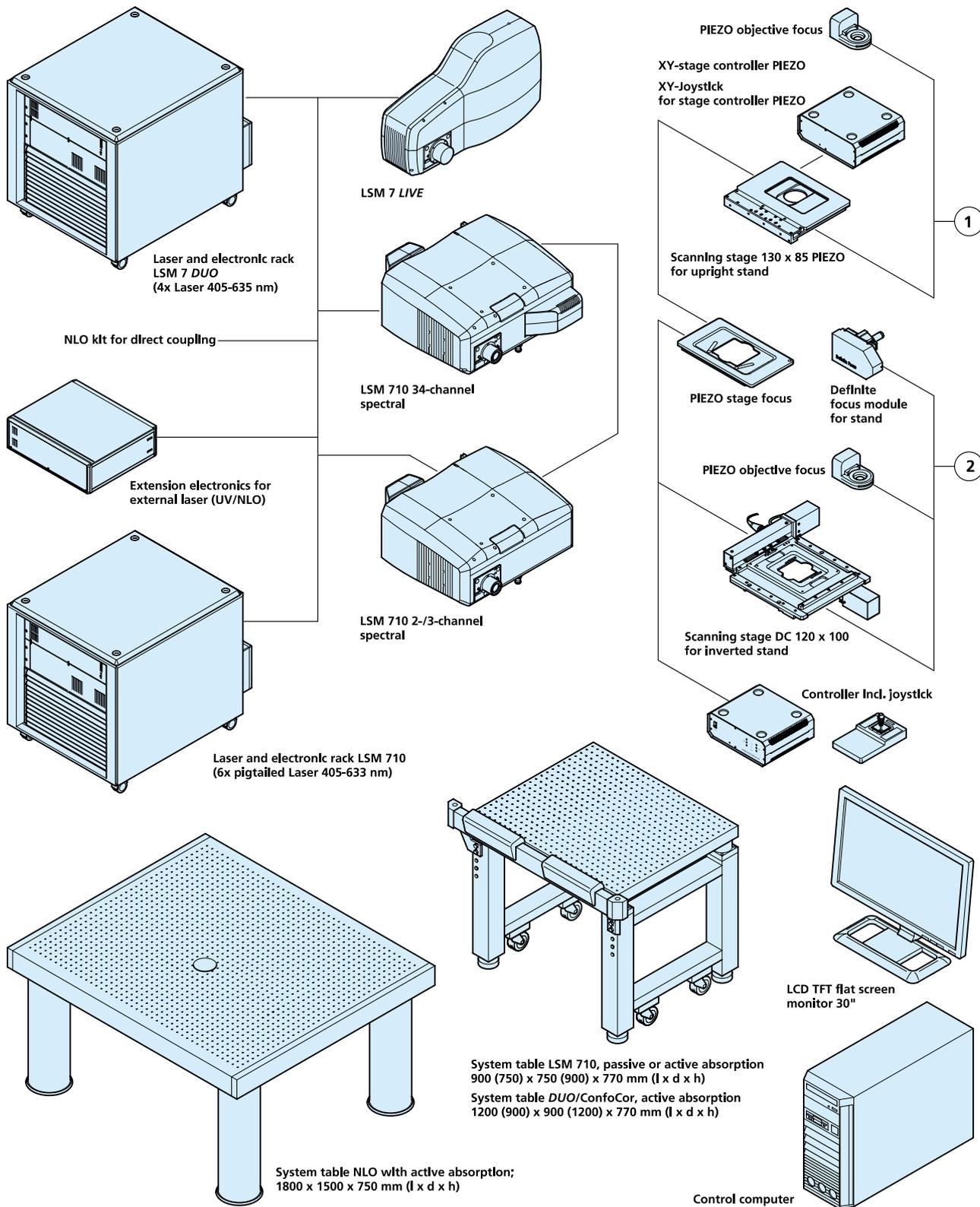
Standard Software ZEN

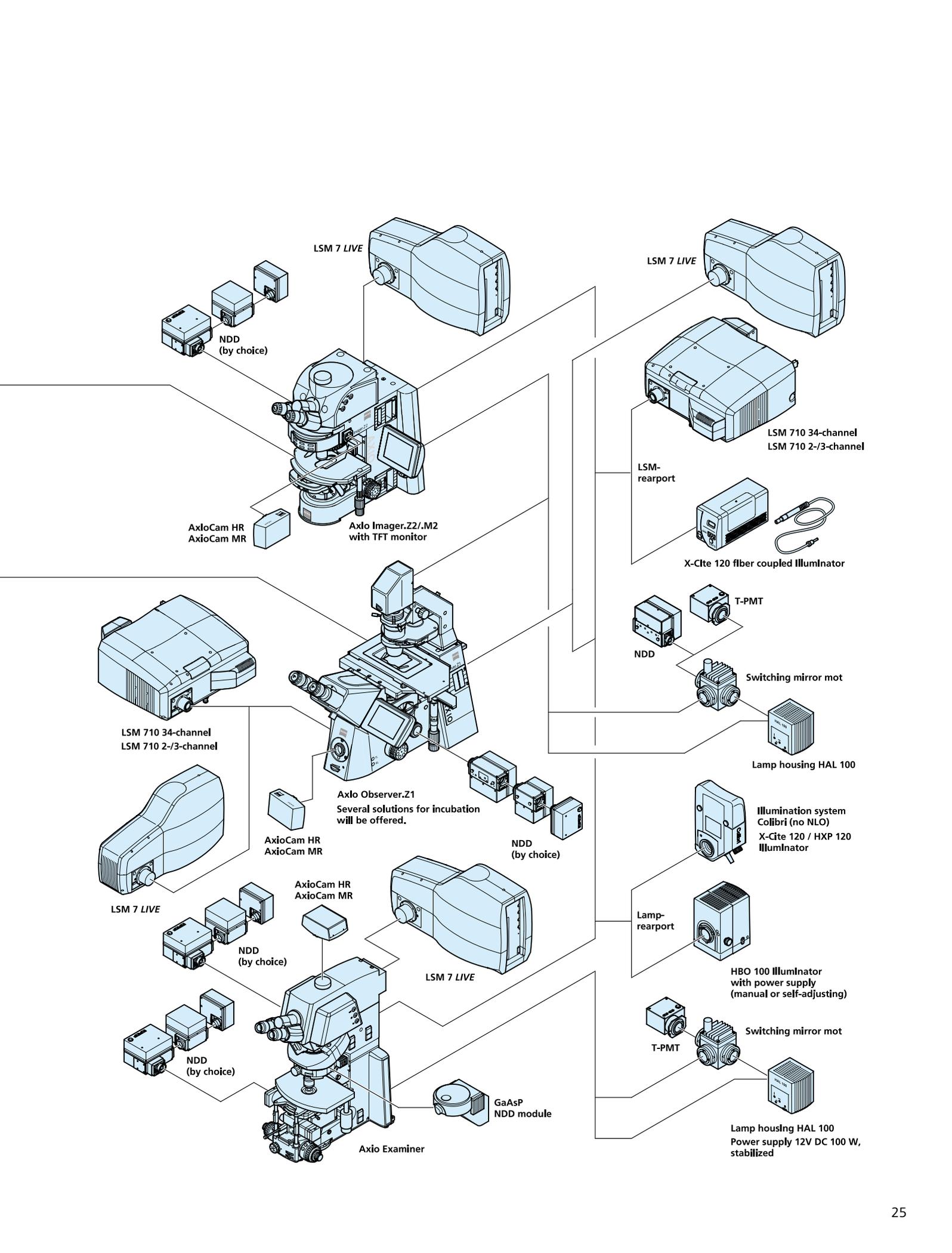
System configuration	Convenient control and configuration of all motor-driven microscope functions and of the laser and scanning modules; saving and restoration of application-specific configurations with a mouse click
System self test	Automatic adjustment with Kollimatic and System Maintenance Tool
Acquisition modes	Line, Frame, Z-stack, time series and combinations: xy, xyz, xyt, xyzt, xz, xt, xzt, multilocation scan with autofocus; on-line computation and visualization of ratio images. Averaging and summation.
Auto-Z function	On-line adaptation of acquisition parameters for Z-stacks for uniform brightness distribution
Zoom Crop function	Convenient selection of scanning areas (Zoom, Crop, Offset)
ROI Bleach	Localized photobleaching in up to 99 bleaching ROIs for such applications as FRAP (Fluorescence Recovery After Photobleaching) or Uncaging; up to 99 ROIs (Regions of Interest) of any shape, and laser blanking with single-pixel accuracy
Multitracking	Acquisition of multiple fluorescence signals by fast change of the excitation lines
Visualization	Orthogonal view (xy, xz, yz in one display), cut view (3D section at freely definable solid angles), 2.5D view for time-lapse series of line scans, projections (stereo, maximum, transparency projection) for single images and series (animations), depth coding (false-color view of height information). Brightness and contrast adjustment; off-line interpolation for Z-stacks, selection and modification of color look-up tables (LUTs), drawing functions for documentation
Image analysis	Modern tools for colocalization and histogram analysis with various parameters and options, profile measurement along straight lines and curves of any kind, 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 the images and the associated acquisition parameters; Multipart function for compiling 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
Image Browser	Free software package for visualization, processing, sorting, printing and export/import of LSM 5 images

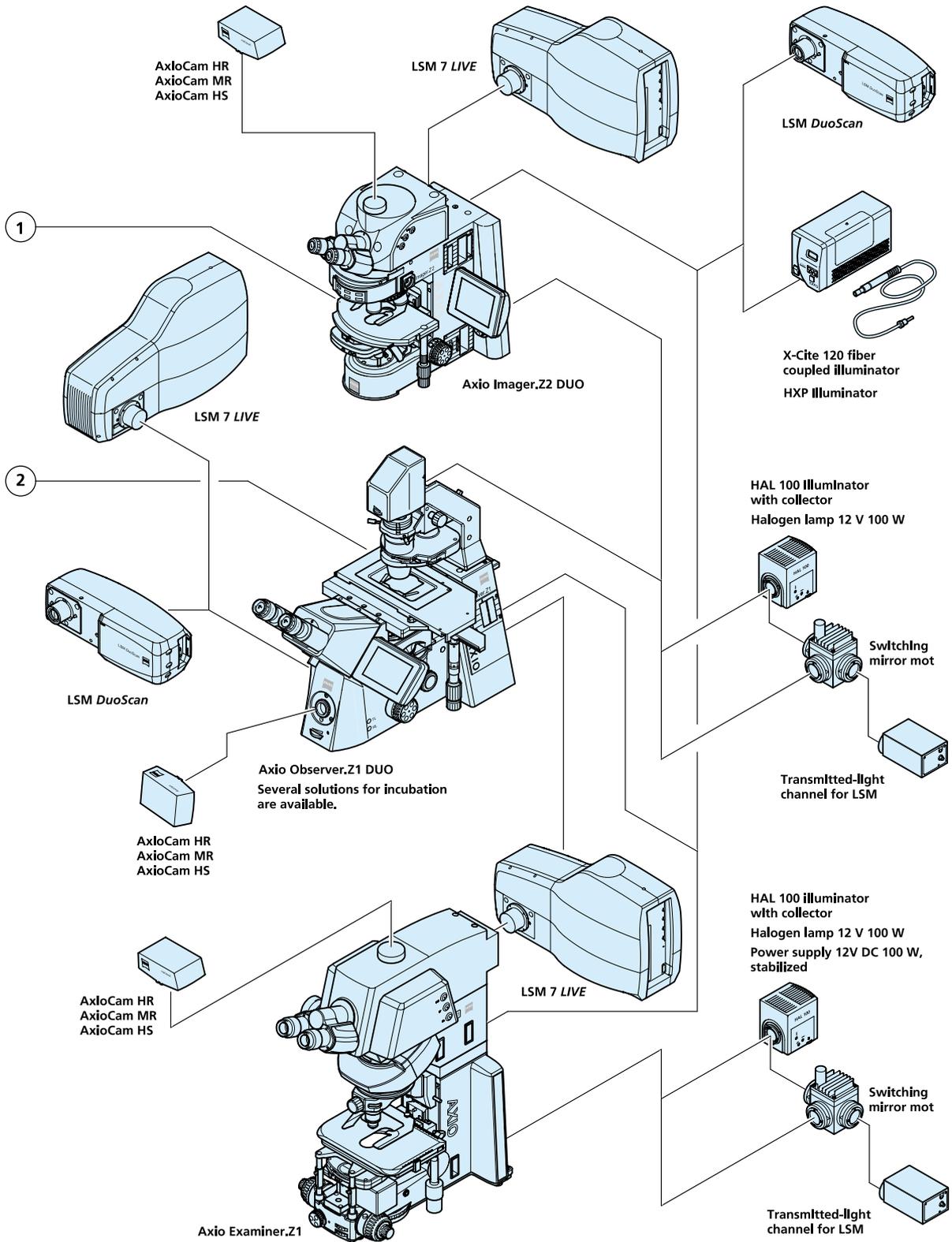
Software Options for all Systems

Image VisArt plus	Fast 3D and 4D reconstruction and animation (various modes: Shadow projection, transparency projection, surface rendering)
3D Deconvolution	Image restoration based on calculated point spread functions
Physiology	Comprehensive analysis software for time-lapse series, graphical Mean-of-ROI analyses, on-line and off-line calibration of ion concentrations
FRET plus	Analysis of experiments with the Sensitized Emission or Acceptor Photobleaching methods
FRAP	User guiding for, and analysis of FRAP and FLIP experiments, with calculation of the quantitative parameters
Visual Macro Editor	Graphical compilation of routines for scanning and analysis functions
VBA Macro Editor	Recording and editing of routines for the automation of scanning and analysis functions
Multiple Time Series	Multiple time series with varied application configurations, autofocus and bleaching functions

LSM 7 LIVE DuoScan and LSM 7 DUO System Overview







Excellent in Combination

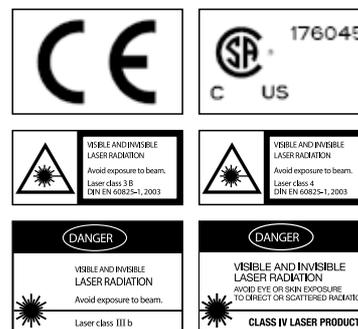
When carrying out a wide variety of experiments, you can combine the LSM 7 *LIVE*, LSM 7 *LIVE DuoScan* and LSM 7 *DUO* with the matching equipment.

There is nothing this system cannot do: spectral imaging, high-speed imaging of living cells and laser manipulation for FRAP, FLIP, photo activation or uncaging.

	Detection options and manipulation modes		
	CCD line with double bandpasses	CCD line with PMTs spectral	CCD line, PMTs spectral and NDDs
LSM 7 LIVE	1-/2-channel, stripe bleach	—	—
LSM 7 LIVE DuoScan	1-/2-channel, ROI manipulation	—	—
LSM 7 DUO	—	1-/2+34-channel, ROI manipulation	—
LSM 7 DUO NLO	—	—	1-/2+34+9-channel, ROI manipulation, multiphoton laser

Patents:
www.zeiss.de/micro-patents

Literature:
www.zeiss.de/lsm





Optical perfection, creative vision and a reliable feeling for technical challenges in bio-sciences. Those are the ingredients for brilliant microscopy concepts made by Carl Zeiss.

Carl Zeiss Microscopy GmbH
07745 Jena, Germany
BioSciences
microscopy@zeiss.com
www.zeiss.com/microscopy