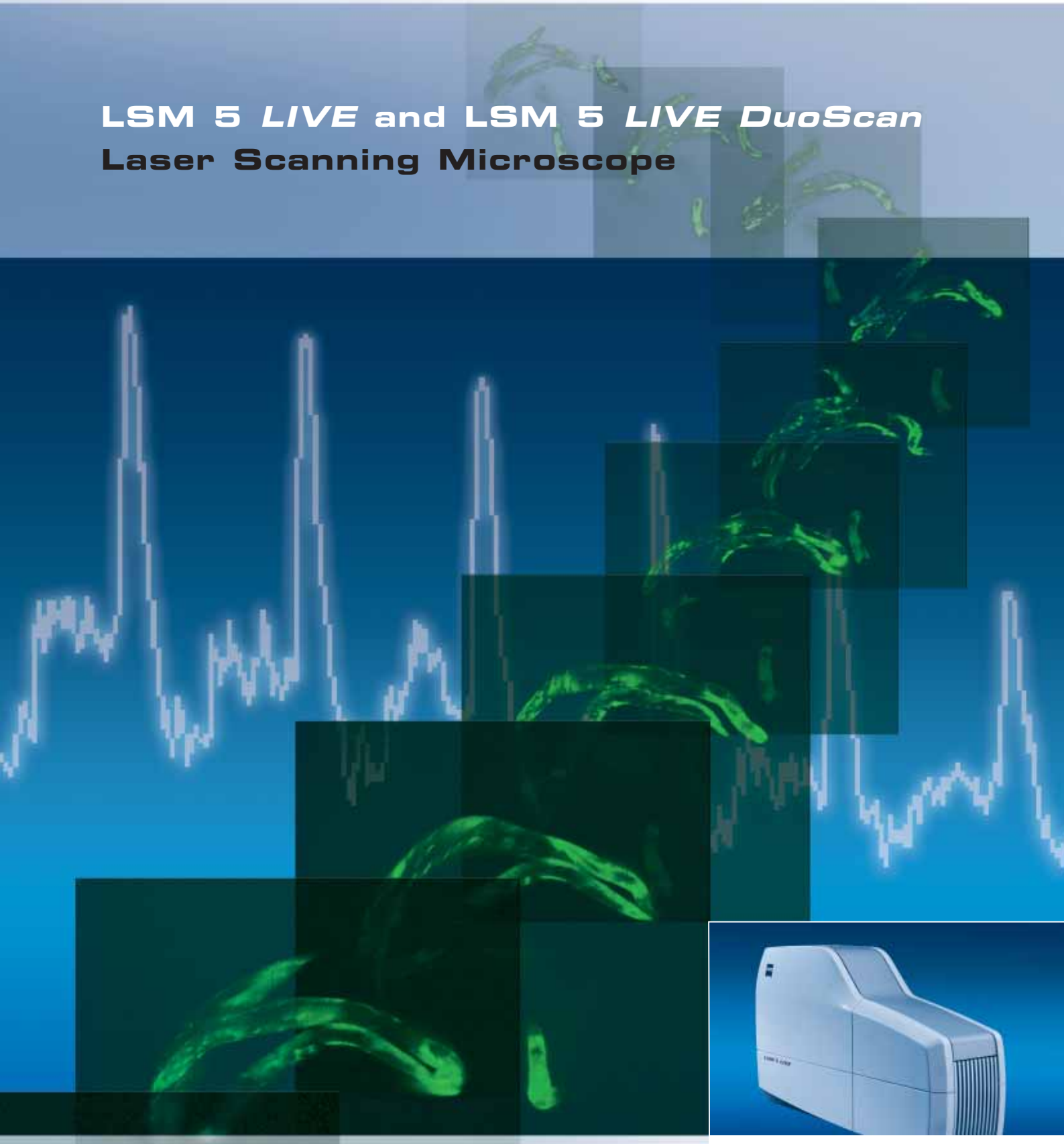
LSM 5 *LIVE* and LSM 5 *LIVE DuoScan*
Laser Scanning Microscope



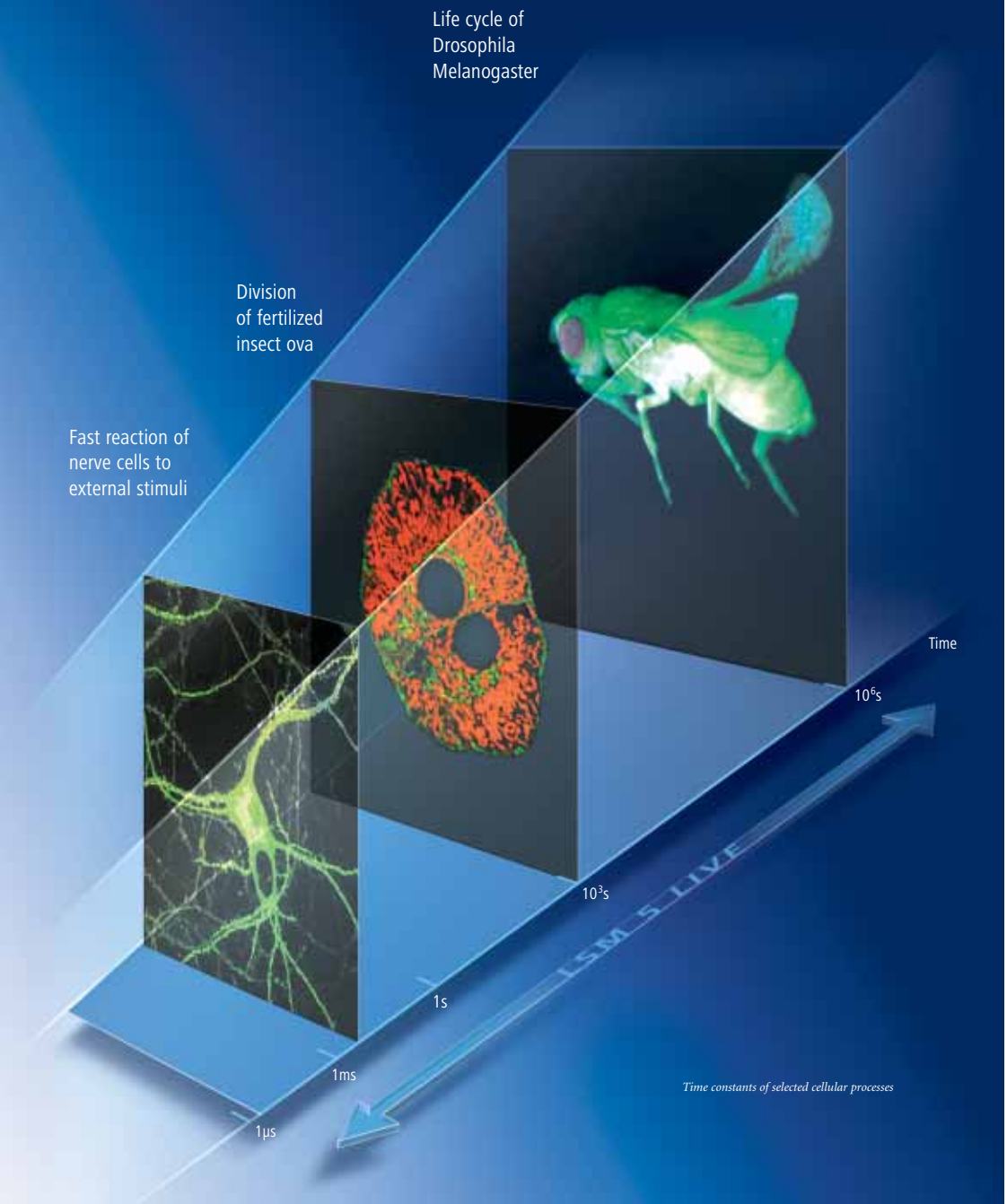
Vision Set in Motion

ZEISS

We make it visible.

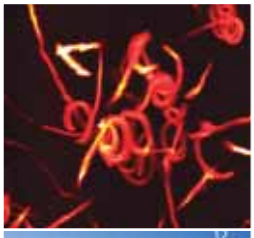
LIVE-Transmission

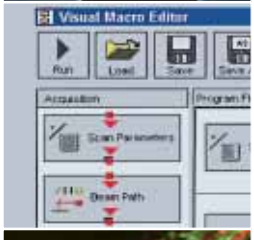
Fundamental processes in living cells can only be observed and understood when imaged live in motion. The ultimate movie about the living cell however was a dream so far in biological science. Now you can come close to this vision with the LSM 5 LIVE.

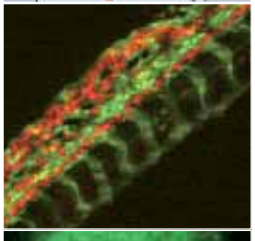
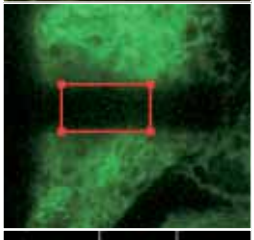
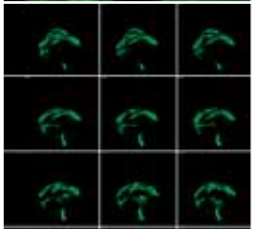


Time constants of selected cellular processes

Content





LSM 5 *LIVE* DuoScan

LSM 5 *LIVE*

Faster, more brilliant, more informative

The LSM 5 *LIVE* enables you to analyze the course of fundamental mechanisms in living cells – faster, more brilliant, more informative than ever before. Be it the visualization of movements, of metabolic events, developmental growth, or nerve signal propagation, the LSM 5 *LIVE* will bring your research to life.

Confocal High-Speed Camera

Motion Studies in Detail

Many cellular compartments as well as simple organisms show highly developed motility. By precise analysis of these motion patterns, the LSM 5 *LIVE* helps you to identify such structures, track their motion and better understand transport processes.

Confocal search for clues

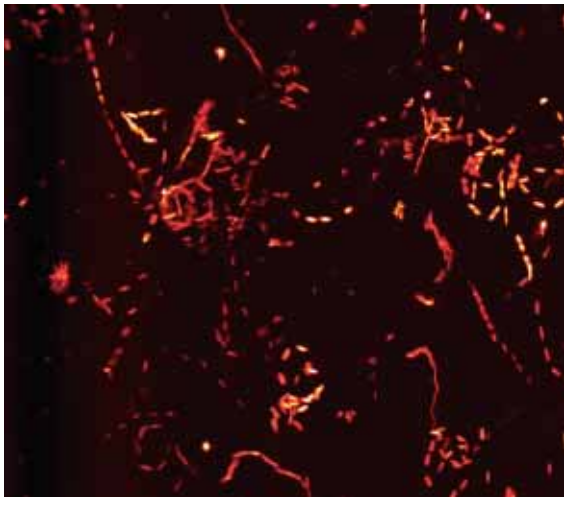
High image information content is always needed when tracking and analyzing transport processes in cells or organisms in high detail – for instance when a large number of very small structures are moving very rapidly.

Whether you are interested in erythrocytes in veins and arteries, bacteria or viruses penetrating the membrane of a host cell, mitochondria or dendritic spines on neurons: precise motion studies of these structures produce unique information about transport processes and turnover times of interacting partners.

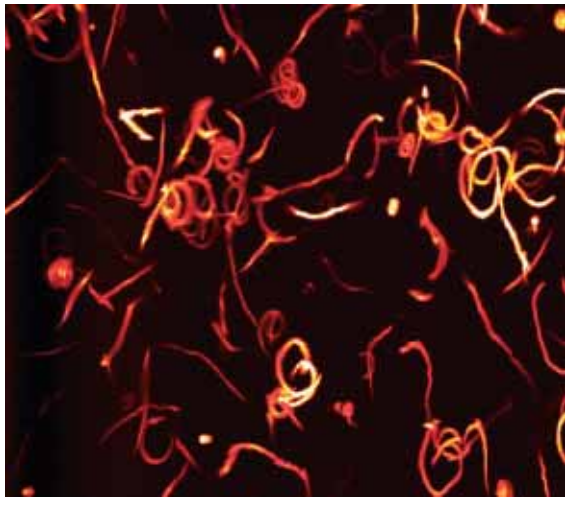
True to detail in space and time

With its high-resolution digital image series, in space and time, the LSM 5 *LIVE* allows you to gain entirely new insights into intercellular and intracellular interaction processes. It gives you constructive impetus for answering questions about cells and their ontogenesis. Single image formats up to 2.4 megapixels let you keep everything clearly in view. With the LSM 5 *LIVE* no important detail is lost. You recognize the motion trajectories clearly and distinctly – and it is always *LIVE* and confocal.

Motion trajectories of Shewanella oneidensis bacteria. Maximum intensity projections of XYZ time series. Specimen: Dr. T. Teal, Dr. D. Newman, Biological Imaging Center, Caltech Pasadena, USA



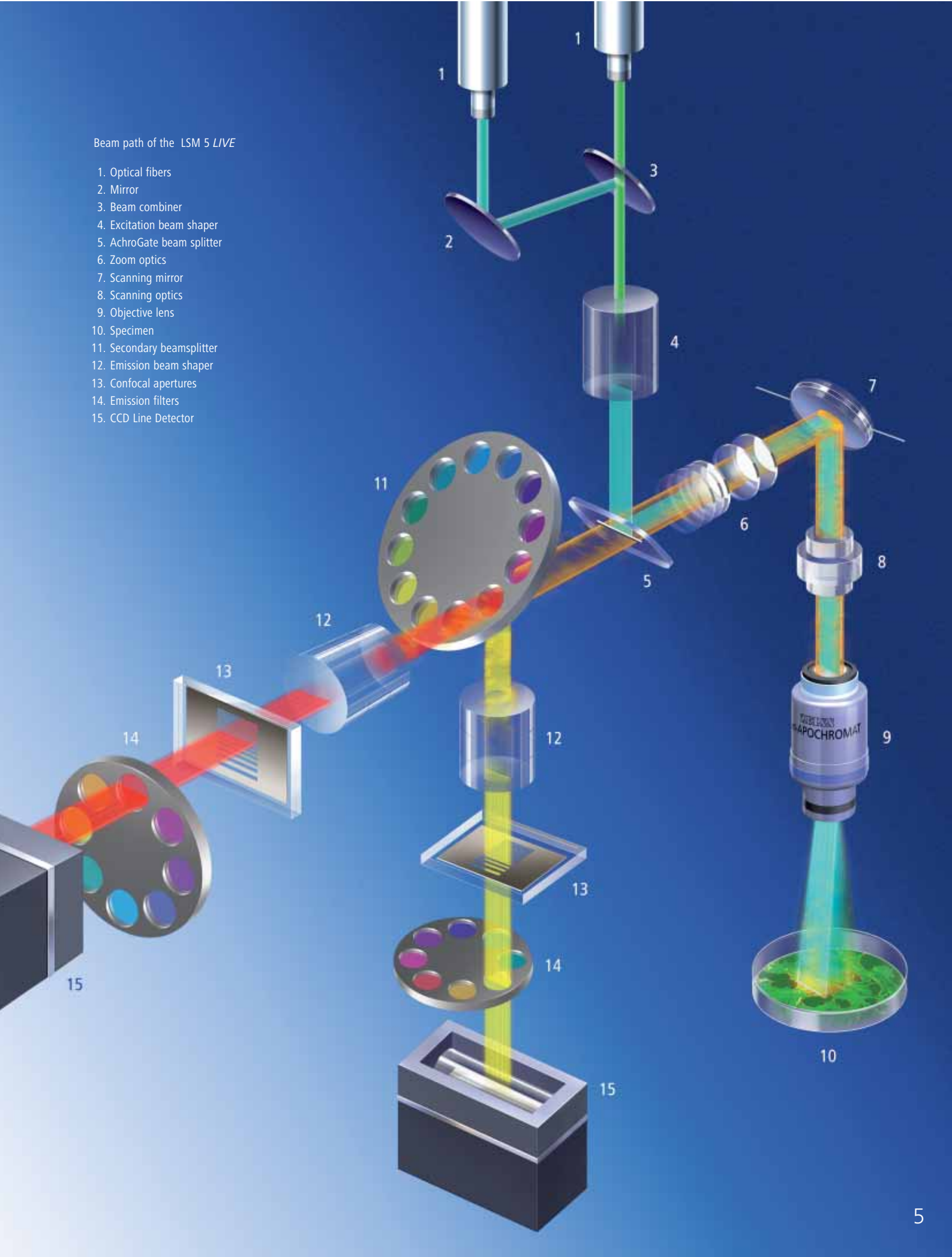
Projection of 50 images with resolution of 4.4 fps



Projection of 500 images with resolution of 44 fps

Beam path of the LSM 5 *LIVE*

1. Optical fibers
2. Mirror
3. Beam combiner
4. Excitation beam shaper
5. AchroGate beam splitter
6. Zoom optics
7. Scanning mirror
8. Scanning optics
9. Objective lens
10. Specimen
11. Secondary beamsplitter
12. Emission beam shaper
13. Confocal apertures
14. Emission filters
15. CCD Line Detector



Accessing Living Cells

Data Production in Realtime

A flood of high-resolution and multidimensional digital data calls for new strategies in data recording, management, compression and visualization.
The LSM 5 *LIVE* navigates and analyzes these streams for you reliably and effectively so that you can concentrate on your research.

Maneuvering gigabytes

1000 images of 512 x 512 pixels in 10 seconds? That means 250 MByte in 10 seconds, more than a CD-ROM full of data every half a minute. Not an unusual amount of data with the LSM 5 *LIVE*. Thanks to new realtime electronics and a realtime computer system, the LSM 5 *LIVE* can efficiently process these huge 4D data quantities (XYZt) with data rates up to 100 MByte a second.

Identifying objects from their paths

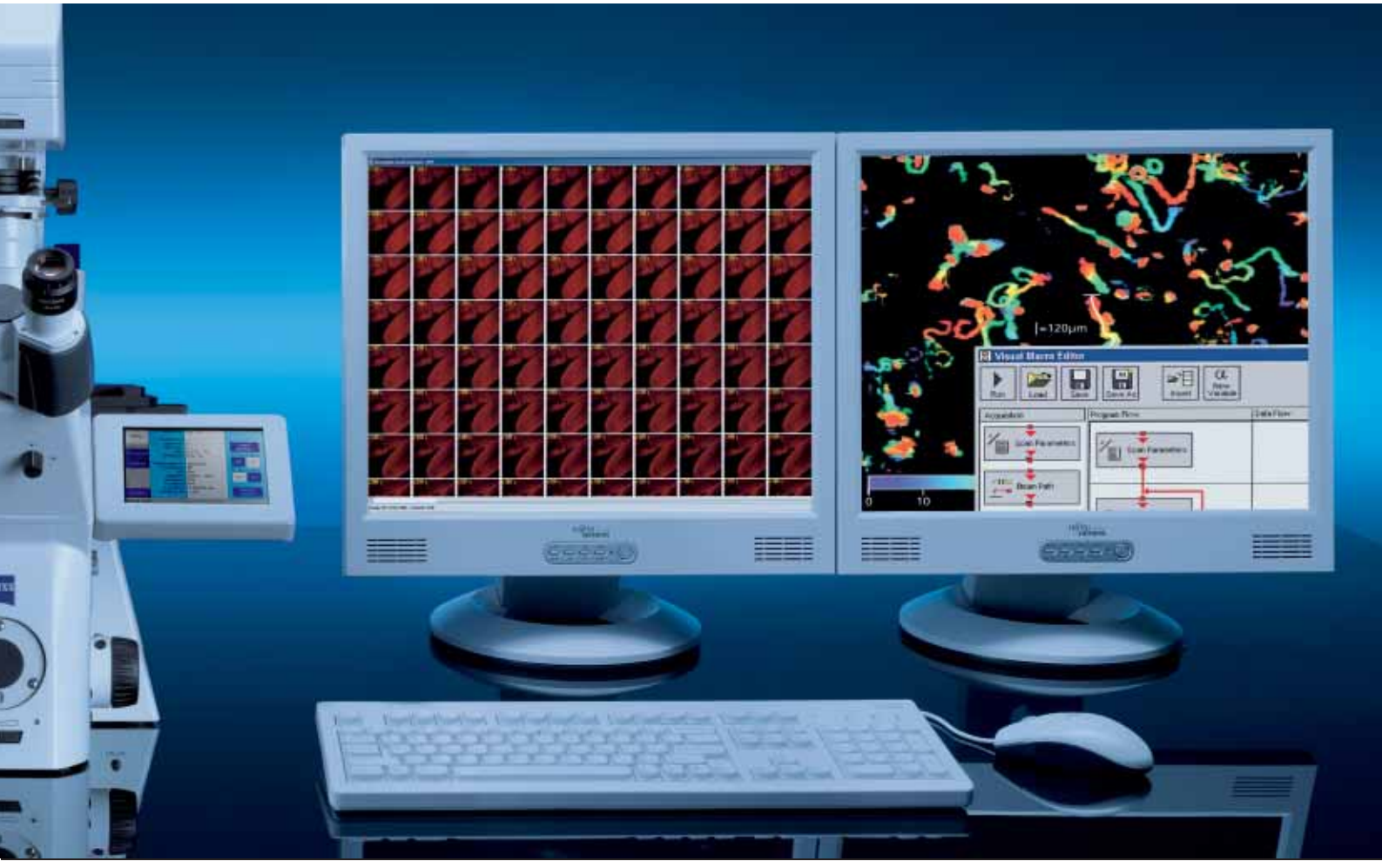
Time series acquired with the LSM 5 *LIVE* are processed e.g. by professional offline particle tracking software. With such software, motion trajectories of all objects of interest may be investigated reliably and quantitatively.

Simple duplication of proven procedures

The new Visual Macro Editor of the LSM 5 software enables you to automate complex and repetitive work procedures – by a few mouse clicks or drag & drop, without in-depth programming skills. This optimizes your time, enabling you to focus on more sophisticated tasks.

Application oriented objectives allow you to create a variety of optimized constellations for resolution, signal intensity, contrast, homogeneity and working distance.

LSM 5 LIVE at Axio Observer.Z



New high-performance objectives

C-Apochromat, LD C-Apochromat
for confocal perfection with correction into NIR wavelengths.

LD LCI Plan-Apochromat, LCI Plan-Neofluar
or sophisticated requirements in life cell imaging.

C-Plan-Apochromat, EC Plan-Neofluar
for greater contrast on fixed specimens under glass

W Achroplan, W Plan-Apochromat
for VIS-IR applications in physiology

Precise laser light play

Compact and long-lived solid-state lasers also put thick or weakly fluorescent specimens in the right light, limiting tissue damage. You can concentrate on the emission of your specimen: the disturbing side effects of conventional gas lasers like heat or sound emissions are a topic of the past. Choose up to 4 lines from the range of 405, 440, 488, 532, 561 and 635 nm lasers.

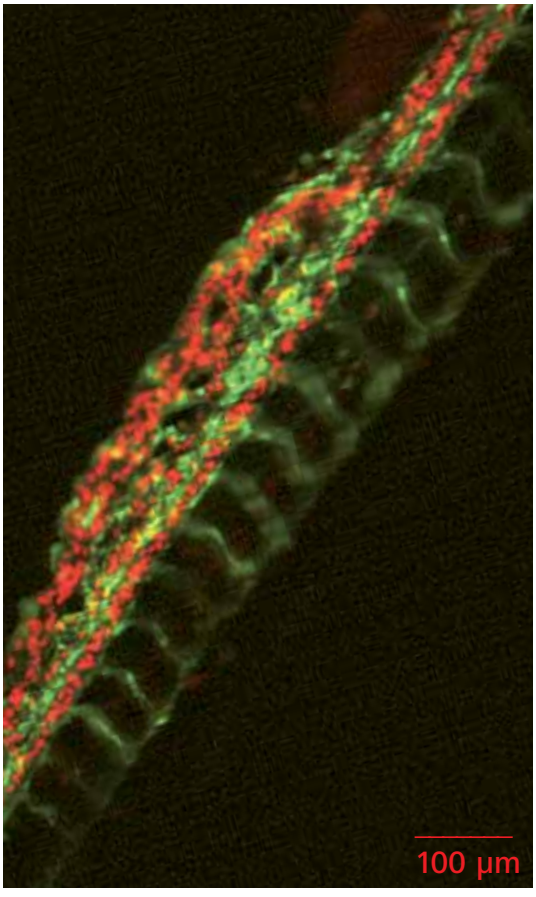
Detailed Motion Studies

Careful Imaging at Highest Speeds

Complex cellular processes can be difficult to capture because they often happen at higher speeds than real-time video rate acquisition. But the revolutionary high-speed detection technology in the LSM 5 *LIVE* enables you to track and analyse them in 3 or 4 dimensions.

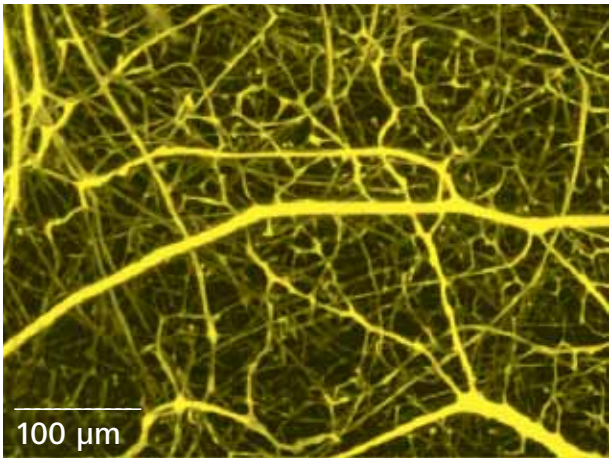
Several innovative achievements allow ultra-fast parallel detection with unparalleled sensitivity (e.g. 1010 fps at 512 x 50 pixel). A groundbreaking AchroGate beam splitter gives you 95% efficiency in emission detection without the need for any mechanical or electrical switching. This means you can track neuronal processes lasting just a few microseconds with the high quantum efficiency (≥75% at 550 nm) of the two internal line detectors.

Even with the cost efficient one-channel LSM 5 *LIVE* version, two dyes can be observed very fast due to the use of an AOTF for lag-free laser switching and double bandpass filters for quick detection of the most popular dye combinations.



Zebrafish embryo. Erythrocytes (dsRed : red) and endothelial cells (eGFP : green). Two channels captured simultaneously at 33 frames/second.
Specimen: Dr. S. Hermanson and Dr. S. C. Ekker, University of Minnesota, USA

High resolution 3-D image of blood vessels in the mouse brain visualized by the fluorochrome – labeled gelatine method.
Specimen: Dr. H. Hashimoto, Jikei Univ. School Med., Dr. M. Kusakabe, Matrix Cell Res. Inc., Tokyo, Japan

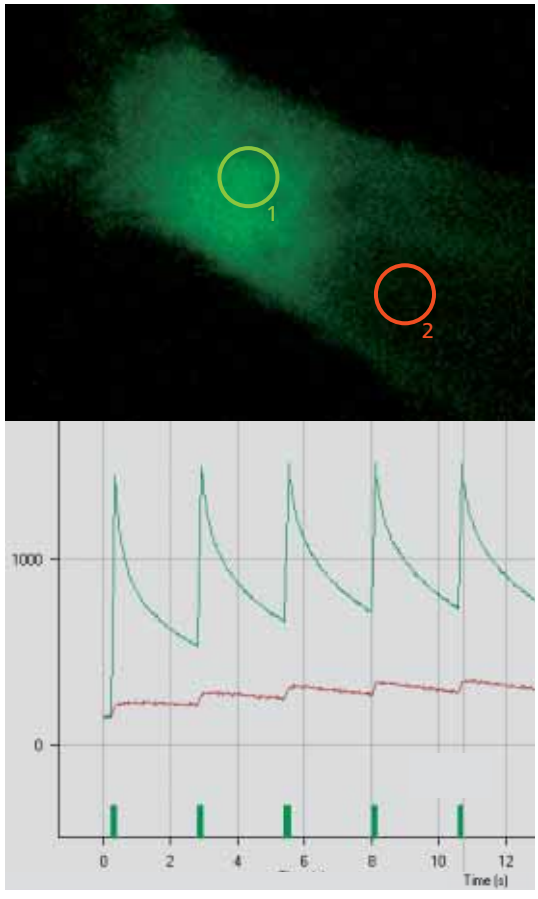


PA-GFP, Dronpa and Kaede

Selective Activation of Fluorescent Proteins with Violet Light

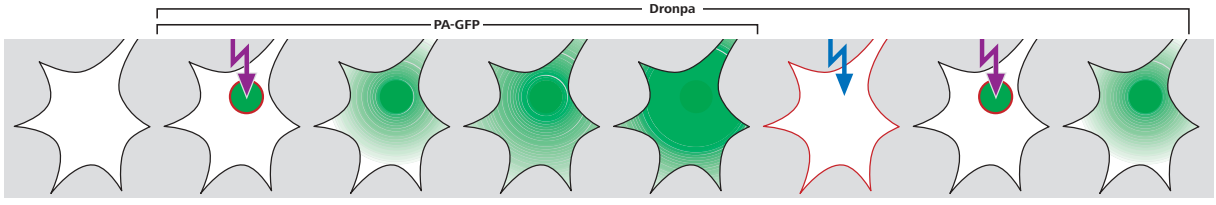
You can push back the frontiers of biomedical research by means of flexible sample manipulation experiments, such as photo-activation and -conversion, conducted with great recision and at high resolution time scales.

Recently developed fluorescent proteins enable you to study dynamic processes directly – PA-GFP, Dronpa and Kaede. The two independent scanner groups of the universal ZEISS confocal system LSM 5 *LIVE DuoScan* give you a great deal of flexibility for such photoactivation and photoconversion experiments.

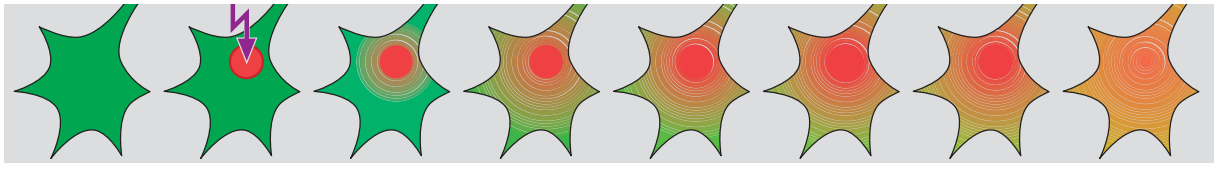


Dronpa transfected cultured cell, repeatedly activated by pulses of 405 nm light, imaged fast with 488 nm excitation.

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



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



Physiological Measurements

Comprehensive Acquisition and Analysis Options

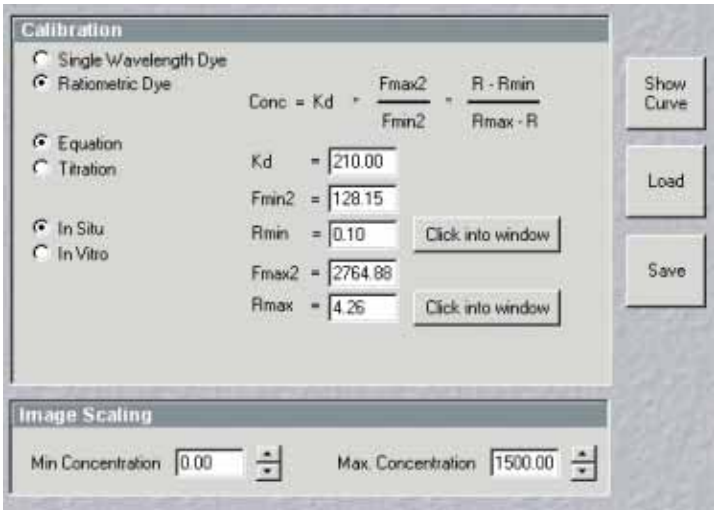
The LSM 5 LIVE is the ideal workstation for obtaining measurements that are perfectly matched to the biological time scale as well as the spectral properties of ion indicators and voltage sensitive dyes. In addition, the ROI manipulation capability of the LSM DuoScan point scanners ensures excellent precision for uncaging experiments.

The LSM 5 *LIVE*'s ultra-fast image acquisition capability makes it the ideal tool for observing dynamic events, even at kilohertz resolution (e.g. 1010 fps at 512x50 pixel). Even more important, this speed is delivered in a true confocal system with simultaneous two-channel acquisition. Complemented by a point scanner, the LSM 5 *LIVE DuoScan* gives you the flexibility for uncaging and sample stimulation, e.g. with UV light (351+364 nm). Apochromatic dipping objectives such as the Plan Apochromat 20x/1.0 W or 63x/1.0 W are also available for micro-manipulation.

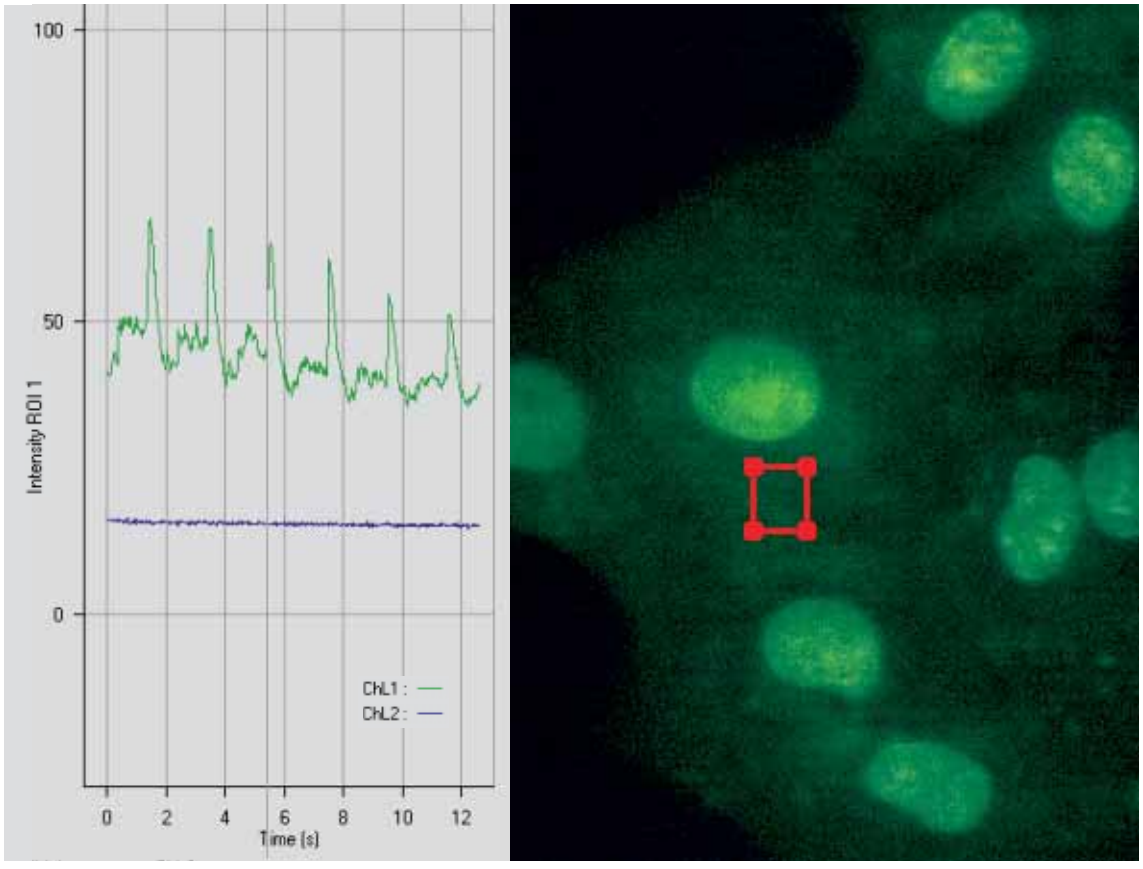
Display and Analysis of Ion Concentrations

- Online and offline ratio for ratiometric dyes
- Online and offline F/F_0 for single-wavelength dyes
- Calibration for single-wavelength and ratiometric dyes
  – *in situ* and *in vitro*
  – including background correction
  – after titration with various curve fittings
  – according to Grynkiewicz
- Interactive scaling of image data series
- Interactive graphic display of the measured data from ROIs

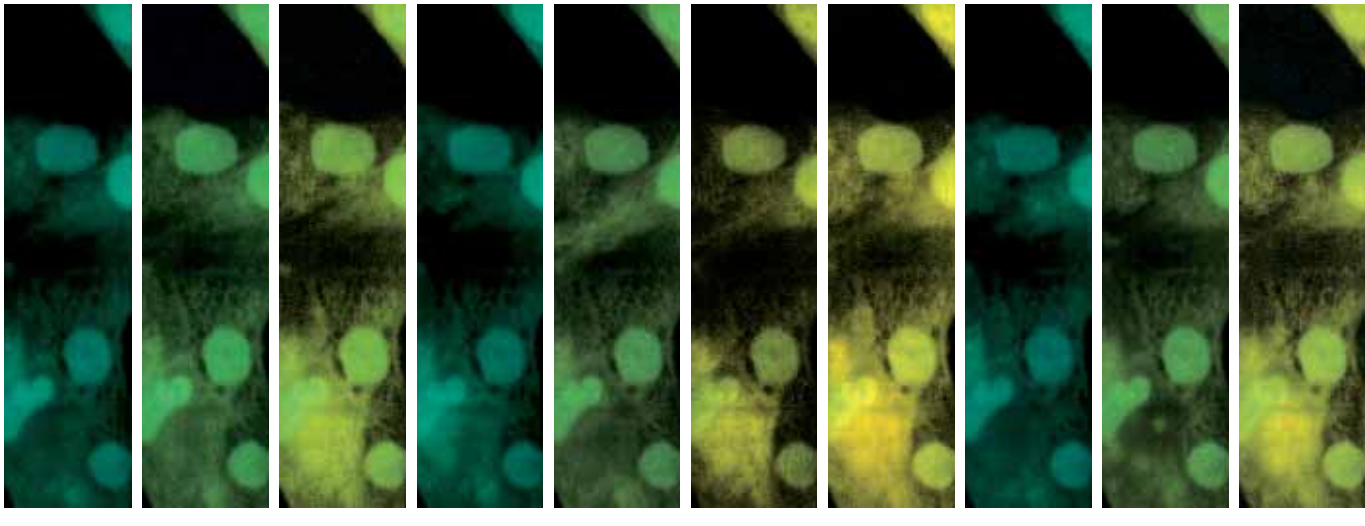
The absolute Ca^{2+} concentration can easily be analyzed with the calibration tool in the ZEISS LSM software.



Fast Ca^{2+} transients in Fluo-4 loaded rat cardiac myocytes, imaged at 80 fps.
Specimen: Dr. W. J. Lederer and Dr. A. Ziman, Medical Biotechnology Center, Biotechnology Institute, Universtity of Maryland, Baltimore, USA



Repetitive Ca^{2+} increase in Fluo-4 loaded heart muscle cells after stimulation.

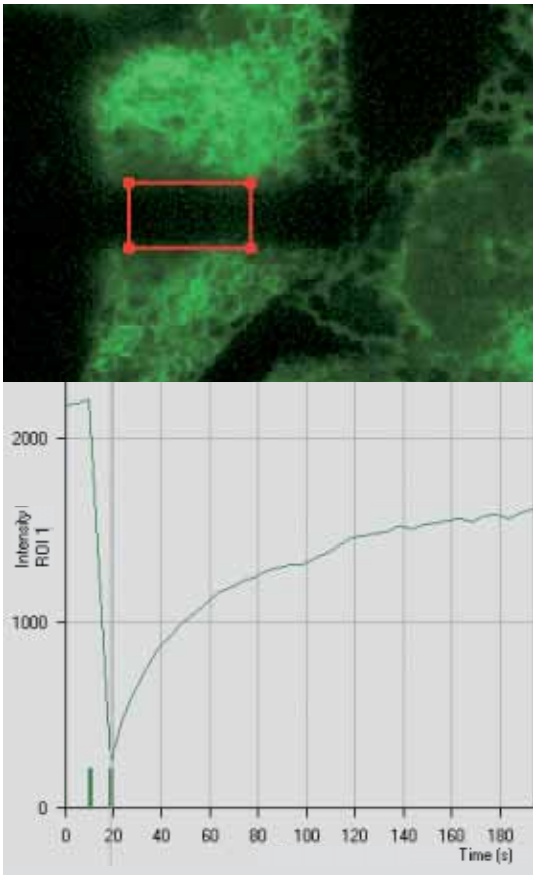


FRAP, FLIP and FRET

Tracking Down Biological Molecules

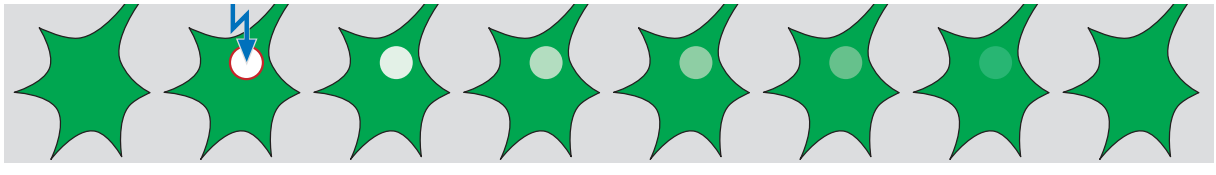
Photo-bleaching experiments such as FRAP and FLIP provide you with a flexible tool for molecular kinetics studies. The LSM 5 *LIVE DuoScan* allows you to carry out such experiments with great precision and at high-resolution time scales.

Although the LSM 5 *LIVE* is the ideal system for fast cell imaging, this unique multi-purpose workstation can actually do significantly more. Two independent scanner groups in the LSM 5 *LIVE DuoScan* give you a great deal of flexibility in photo-bleaching so you can carry out fast FRAP experiments in freely definable ROIs at a variety of wavelengths – even with fast parallel two-channel image acquisition. The LSM 5 *LIVE DuoScan* not only makes FRAP and FLIP possible but also two-label FLAP, which compares the dynamic ratio of an unbleached and bleached label.

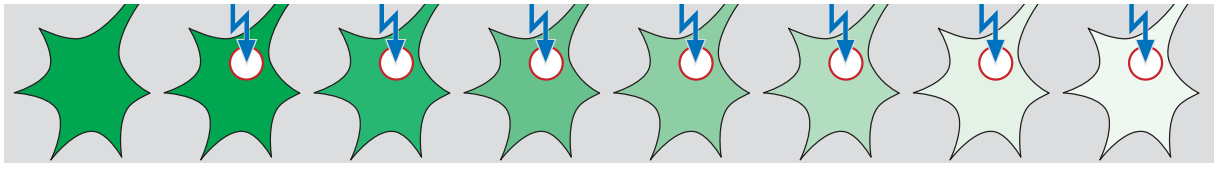


FRAP ROI-bleaching and recovery of a GFP-labelled CD3 cell with a LSM 5 LIVE. Specimen: D. W. Hailey, Dr. J. Lippincott-Schwartz, NICHD, NIH, Bethesda, USA

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

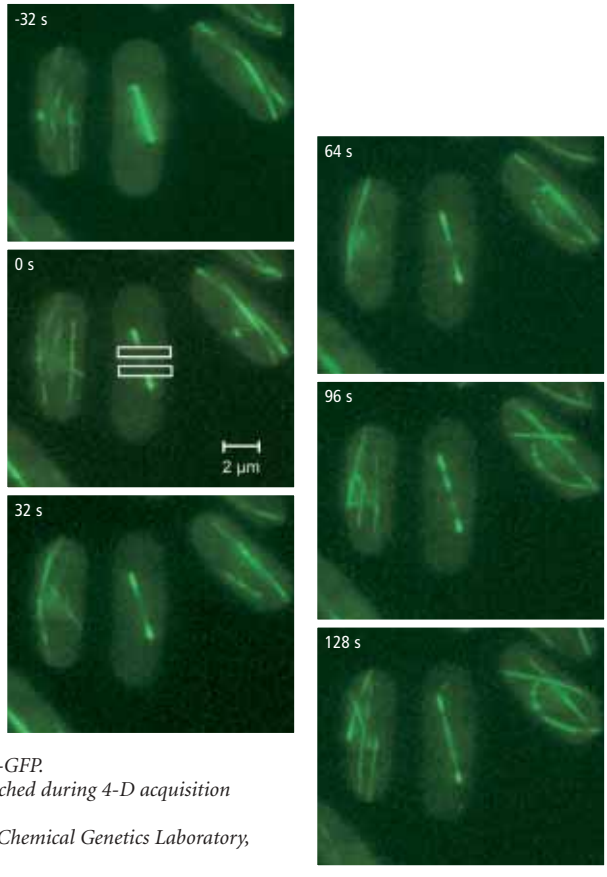


In a FLIP experiment, the same region within a cell is bleached repeatedly, and the loss in fluorescence outside that region is measured.



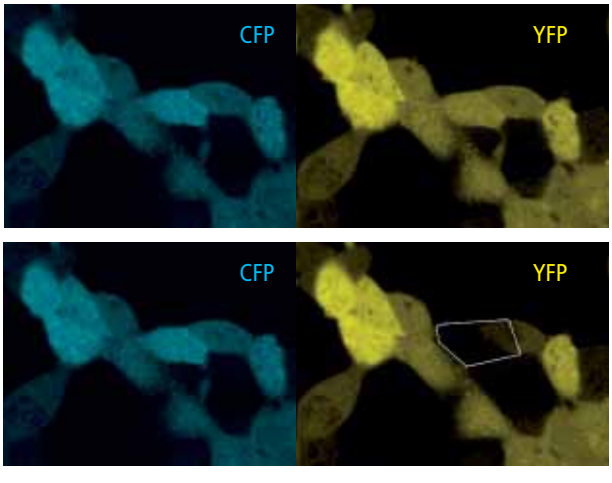
In addition to the traditional bleach and recovery experiments to analyze molecular kinetics and motility, the LSM 5 *LIVE DuoScan* also enables molecular interaction studies and analysis of developmental events to be carried out in a much more sophisticated manner.

FRET is widely used to analyze the proximity and interaction of molecules. While several FRET methods are available, the LSM 5 *LIVE DuoScan* is particularly suitable for easily conducting the reliable acceptor photo bleaching method. In developmental studies, selective bleaching of structures can supply the answers to many localization and proliferation questions, which pure staining alone cannot.



Yeast cells expressing tubulin-GFP.
Mitotic spindle precisely bleached during 4-D acquisition with the LSM 5.
Specimen: Prof. M. Yoshida, Chemical Genetics Laboratory, Riken Institute, Wako, Japan

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

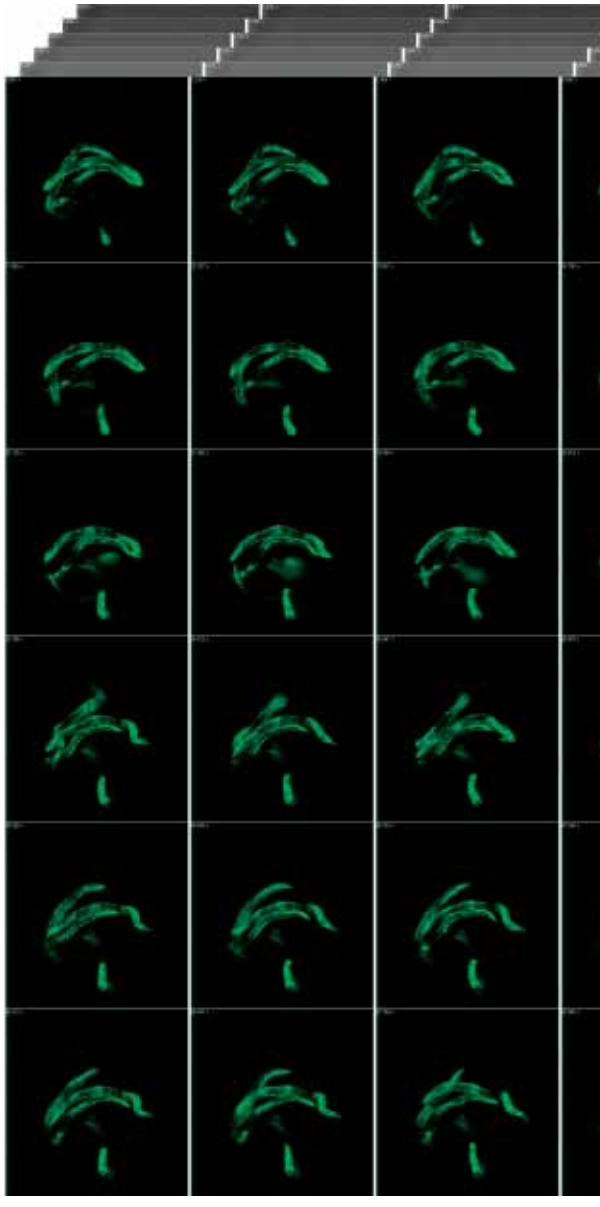


Faster than Real Time

Development in 4 Dimensions

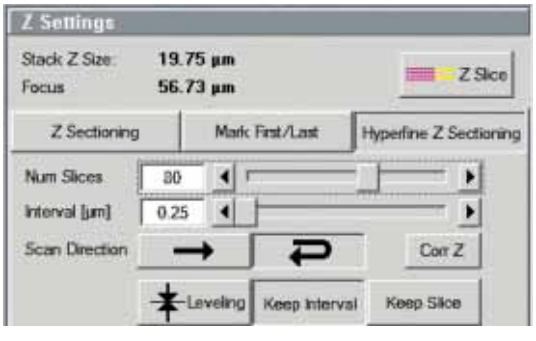
Examining living cells often requires a tissue context or imaging of an entire animal. However, imaging the developmental processes of organisms showing rapid changes is a challenge. 4-D developmental studies have to be done at high speed and require true confocality, even with low-magnification lenses.

The LSM 5 *LIVE* offers this true confocal imaging precision you need for 4-D developmental studies. Optical images with outstanding 3-D resolution are acquired ultra-fast in the 4th dimension over time. Modern piezo focus accessories help to speed up Z acquisition for up to 70 sections/sec, and expand the travel range to 250 µm – ideal for living specimens with modern live cell objectives like the ZEISS LCI Plan Neofluars or LD C-Apochromats.

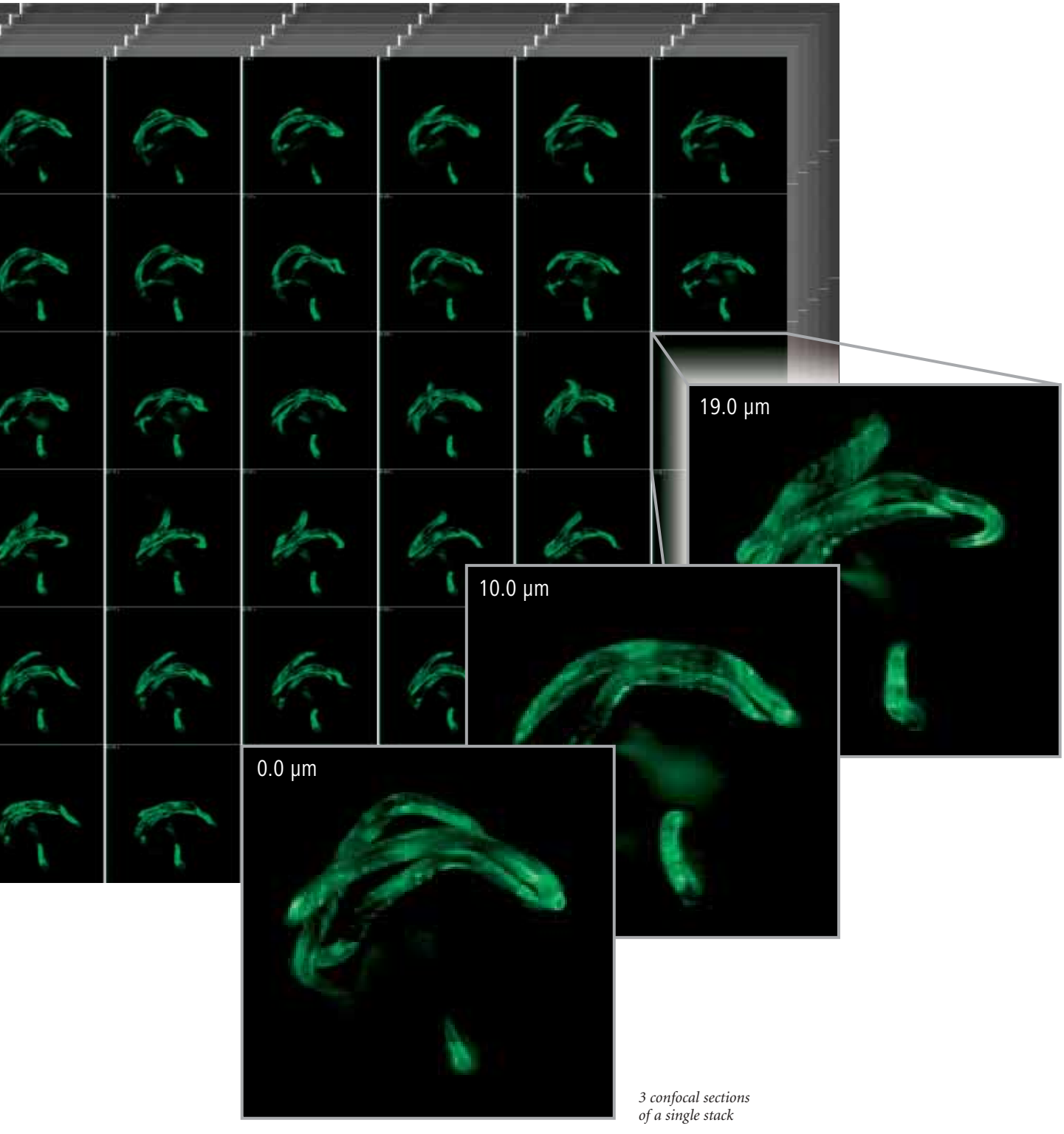


Motility of adult Caenorhabditis elegans,
GFP expression,
Specimen: Prof. R. Baumeister, Institute for Biology III and Dr. R. Nitschke, Life Imaging Center, Freiburg University, Germany

Fast Z sectioning with piezo focussing drives



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



3 confocal sections of a single stack

Specifications

LSM 5 *LIVE* and LSM 5 *LIVE DuoScan*

Microscopes

Models	Upright: Axio Imager.Z1, Axioskop 2 FS MOT. 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 Modules LSM 5 *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 1536x1536 pixels, also for several channels, continuously variable
Scanning speed	Variable up to 120 frames/s with 512x512 pixels; faster modes with smaller frames (e.g. 505 frames/s with 512x100 pixels, 1010 frames/s with 512x50 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 5 *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, alternatively 440 nm, 16 mW; 488nm laser diode, 100 mW; diode-pumped solid-state laser 532nm, 75 mW; laser diode 561 nm, 40 mW; laser diode 635nm, 35 mW

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 512x512 pixels (max. 77 regions/s with 512x32 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 X/Y offset
Scan field	18 mm field diagonal (max.) in the intermediate image plane, homogeneous field illumination

Laser Modules LSM *DuoScan*

Variable beam splitting	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 or 532nm laser lines
UV Laser Module	Polarization-preserving single-mode fiber, temperature-stabilized UV-AOTF for simultaneous intensity control of two ultraviolet laser lines, switching time < 5 µs; Ar laser (351, 364 nm), 80 mW

Electronics Module

LSM 5 *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 19" (4:3) or 24" (16:10), many accessories; Windows XP 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 19" (4:3) or 24" (16:10), many accessories; Windows XP multi-user operating system

Standard Software

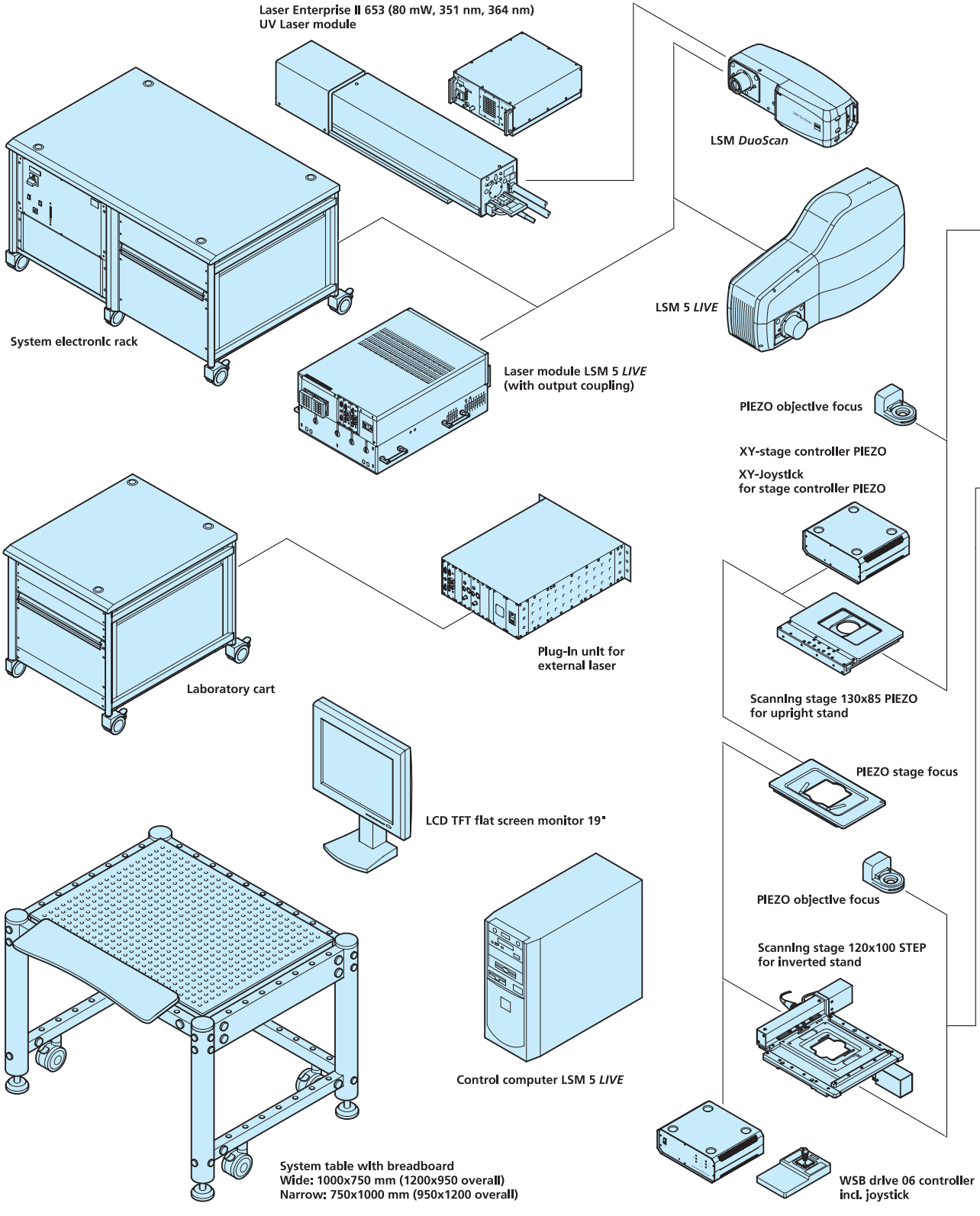
System configuration	Convenient control and configuration of all motor-driven microscope functions and of the laser and scanning modules; saving and restoration of application-specific configurations
ReUse function	Restoration of acquisition parameters with a mouse click
Acquisition modes	Line, Frame, Z-stack, time-lapse series and combinations: xy, xyz, xyt, xyzt, xz, xt, xzt; 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; Multiprint 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

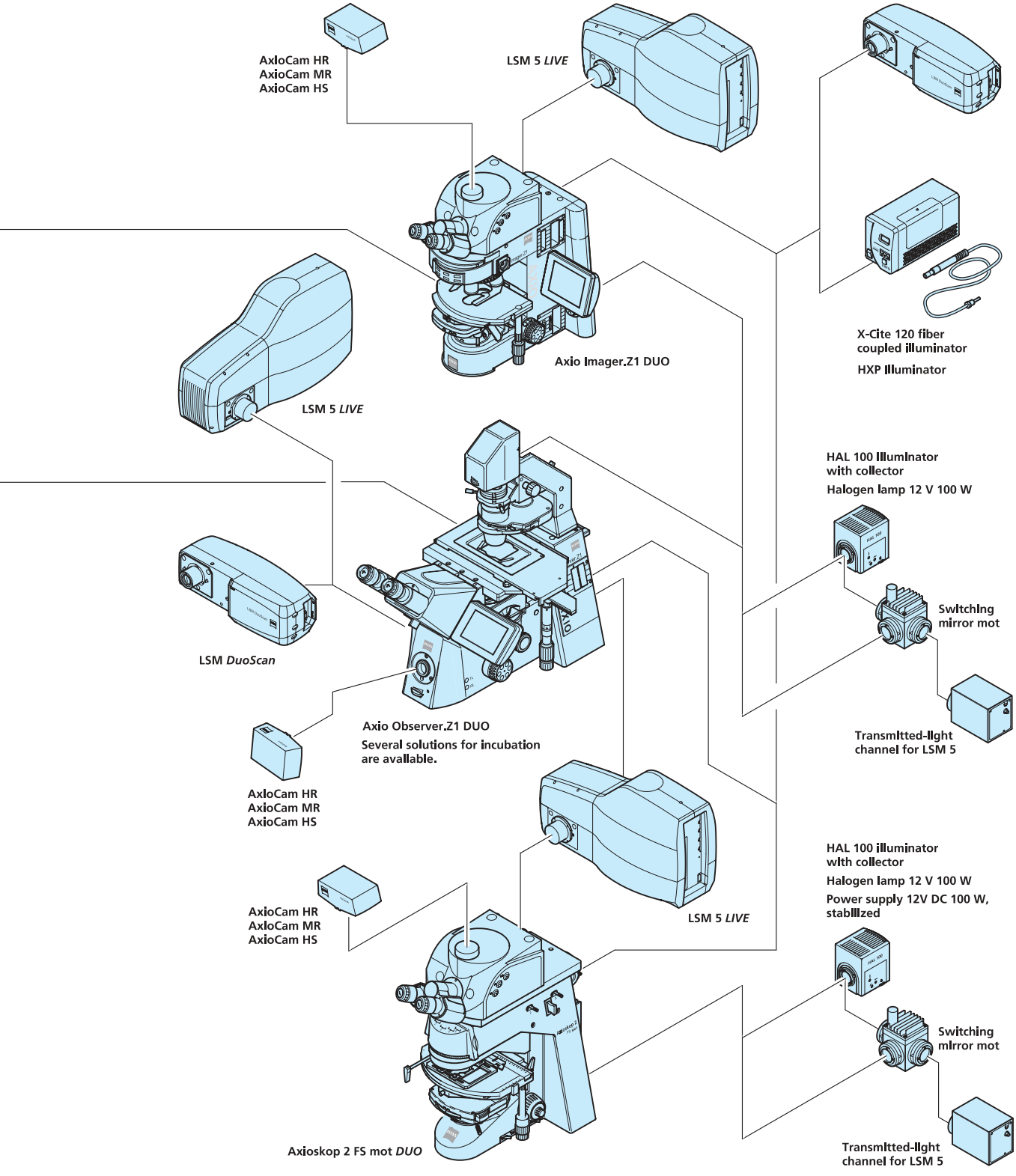
Software Options for all Systems

Image VisArt plus	Fast 3D and 4D reconstruction and animation (various modes: Shadow projection, transparency projection, surface rendering)
Multiple Time Series	Multiple time series with varied application configurations, autofocus and bleaching 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
VBA Macro Editor	Recording and editing of routines for the automation of scanning and analysis functions
Visual Macro Editor	Graphical compilation of routines for scanning and analysis functions
3D for LSM	3D visualization and 3D measurement of volume data records

System Overview

LSM 5 *LIVE* and LSM 5 *LIVE DuoScan*




AxioCam HR
AxioCam MR
AxioCam HS
LSM 5 *LIVE*
X-Cite 120 fiber coupled illuminator
HXP Illuminator
Axio Imager.Z1 DUO
LSM 5 *LIVE*
HAL 100 Illumination with collector
Halogen lamp 12 V 100 W
LSM *DuoScan*
Switching mirror mot
Transmitted-light channel for LSM 5
Axio Observer.Z1 DUO
Several solutions for incubation are available.
AxioCam HR
AxioCam MR
AxioCam HS
HAL 100 illuminator with collector
Halogen lamp 12 V 100 W
Power supply 12V DC 100 W, stabilized
LSM 5 *LIVE*
AxioCam HR
AxioCam MR
AxioCam HS
Switching mirror mot
Transmitted-light channel for LSM 5
Axioskop 2 FS mot *DUO*

Optical perfection, creative foresight and a sure feeling for the technical challenges in the life sciences: the basis for superior microscopy concepts from Carl Zeiss. We have given a name to our focus on the key method in research into life: FluoresScience.

LSM 5 *LIVE*
US Patents: 6848825, 6888148
6947127, 6037583
6462345, 6486458
6941247

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45-0067 e/11.06