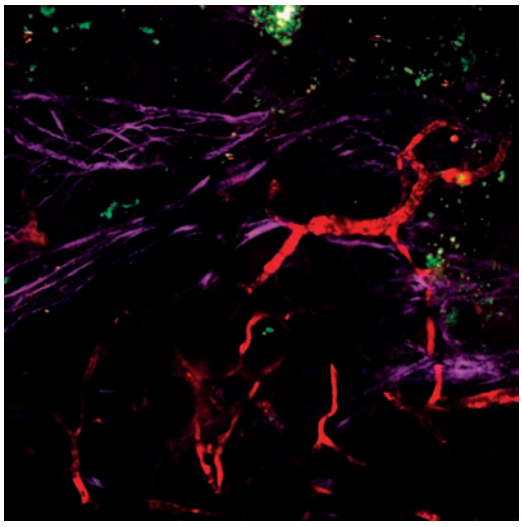
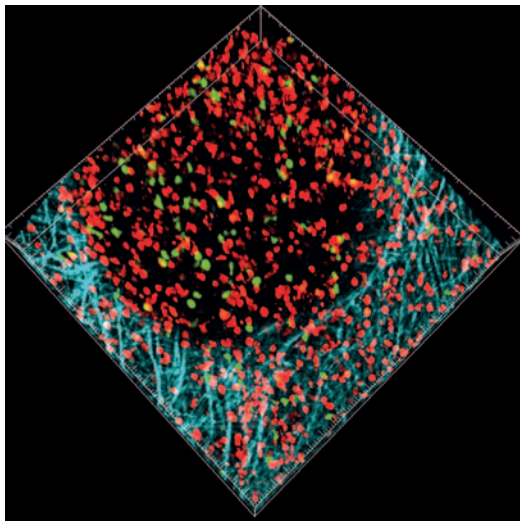
Leica TCS MP5

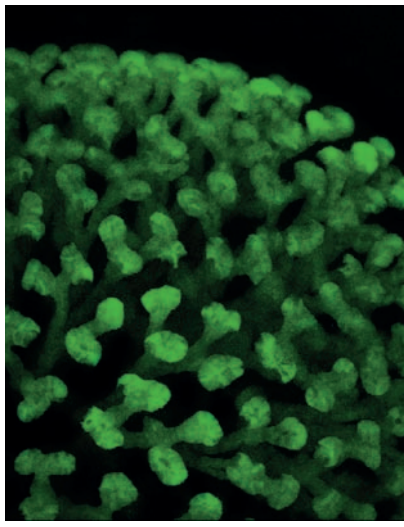
Optimized for Multiphoton Imaging

Living up to Life

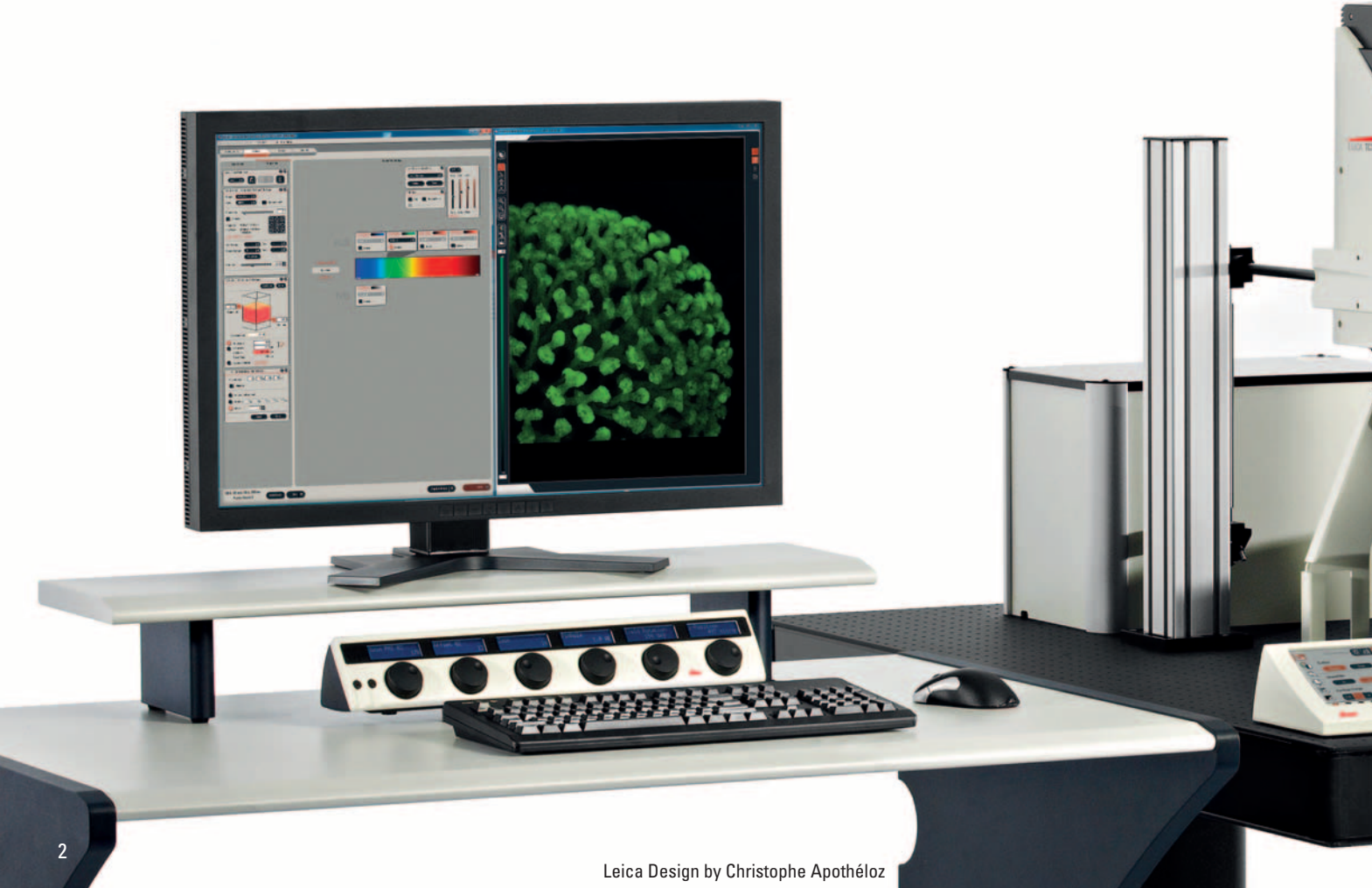
Leica
MICROSYSTEMS



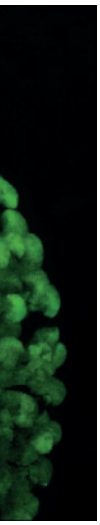


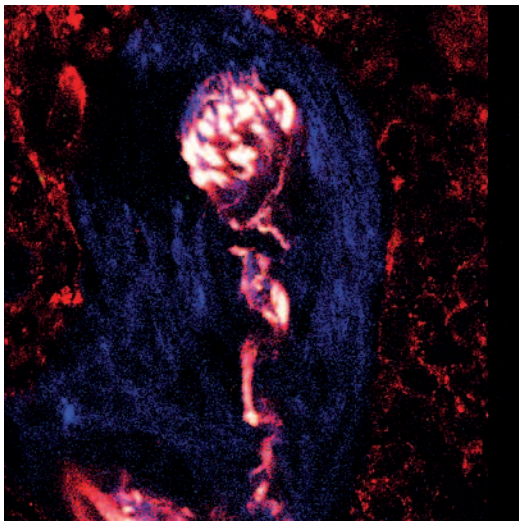


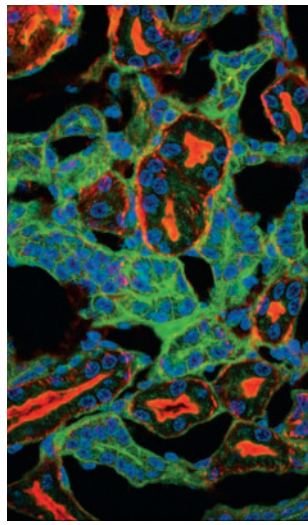
- Dedicated multiphoton platform – perfect for intravital imaging
- Follow fast cellular dynamics at video rate scan speed
- Combine up to seven channels for multimodal imaging
- Efficiently excite red fluorophores with up to 1300 nm
- Discover details from deeper tissue sections with prechirped femtosecond lasers



Leica Design by Christophe Apothéloz







Today, there is a major challenge for many scientists to monitor biological processes involved in neurological disorders, cancer and other fundamental diseases without interrupting the complex regulatory networks.

Researchers use thick tissue sections and whole animals to study the interactions between biomolecules and cells in their natural context.

Intravital imaging by multiphoton microscopy provides a detailed picture of cellular interactions and subcellular structures deep within tissues.

Multiphoton microscopy offers insights into the biology of the brain and other organs that are not accessible by standard fluorescence microscopy.

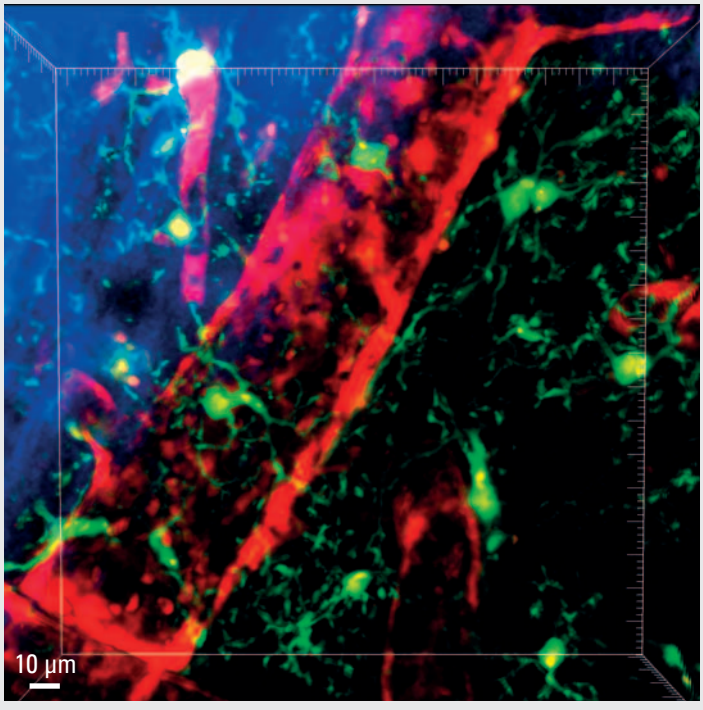
Leica TCS MP5

Optimized for Multiphoton Imaging

The Leica TCS MP5 is dedicated to the needs of multiphoton imaging. Optimal transmission up to 1300 nm and an integrated OPO (optical parametric oscillator) solution allow you to reach even deeper into the sample.

Follow fast cellular dynamics in living animals and rapidly acquire large z-stacks by high-speed image acquisition for groundbreaking results – with the Leica TCS MP5.

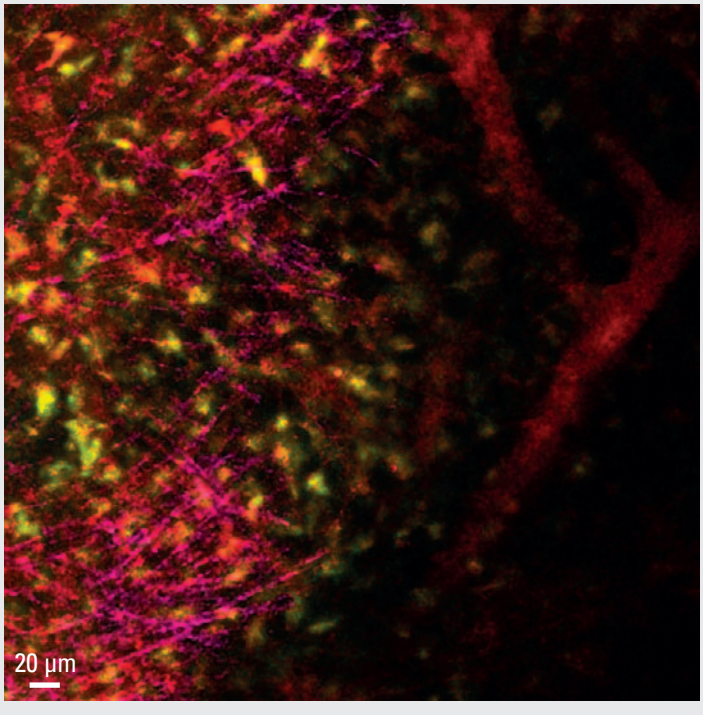
Intravital Multiphoton Applications



Multicolor Imaging of the Brain

Cellular connections are crucial to the nervous system. Disruption of this complex cellular network results in an artificial representation of brain function. To view these connections, neuroscientists use thick brain slices, spinal preparations or whole animals for imaging the nervous system. By intravital multiphoton microscopy they reach further into the intact brain and make substantial progress in the neurosciences.

Imaging of live mouse brain through thinned skull. Representative 50 µm z-stack acquired in less than 30 seconds extracted from a time lapse. Microglia (green) are shown in relation to blood vessels (red) residing in the brain parenchyma. Some microglia have their processes wrapped around blood vessels. Excitation at 910 nm.



Long-term Investigation of Tumor Metastasis

Cancer is one of the most life threatening diseases. Intravital imaging provides oncologists with dynamic information on tumor development and metastasis in an intact organism.

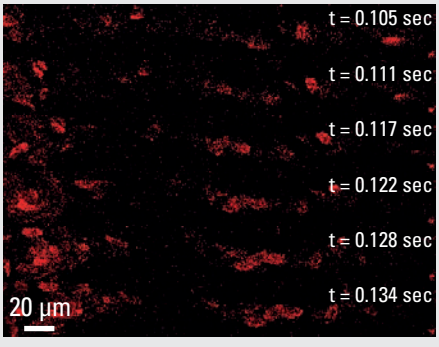
Novel techniques for long-term intravital multiphoton imaging deep within soft tissues have substantially facilitated this research. Implanted imaging windows allow for repeated observation of tumor metastases and blood vessel formation over several days.

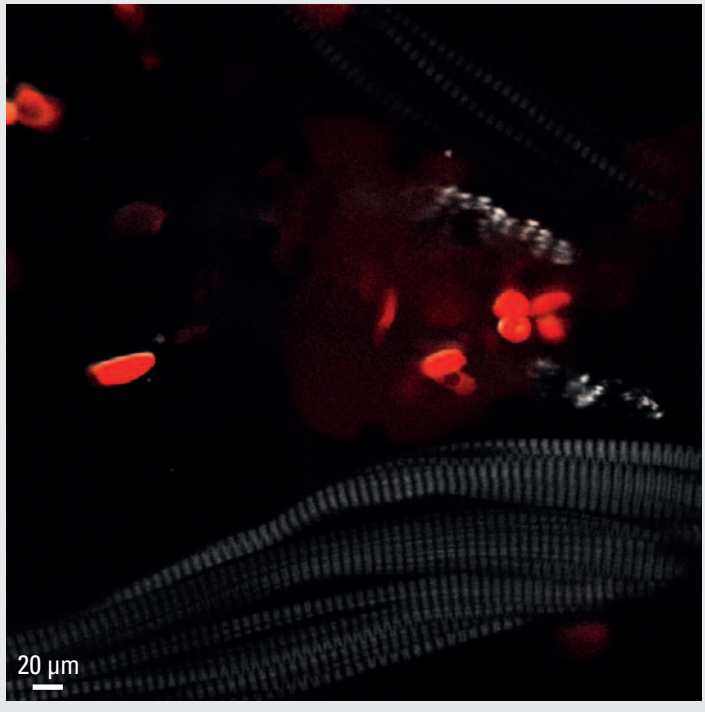
Mouse spleen with Texas Red 70 kD labeled blood vessels (red), imaging depth 75 µm. Texas Red was excited at 1150 nm with OPO. Simultaneous excitation with 800 nm resulted in second harmonic generation signal (SHG, purple) of type I collagen and autofluorescence of single cells (green).

Ultra-rapid Imaging of Embryonic Blood Flows

During embryonic development biological fluid flows play an important role. To investigate how cells interpret physical information from their environment, high-speed intravital multiphoton imaging is used to study fluid flows with minimal photodamage to the developing organism.

Blood cells labeled with DsRed (red). 167 frames/second at 512 x 64 pixels with Resonant Scanner. Multiphoton excitation at 1100 nm with OPO.

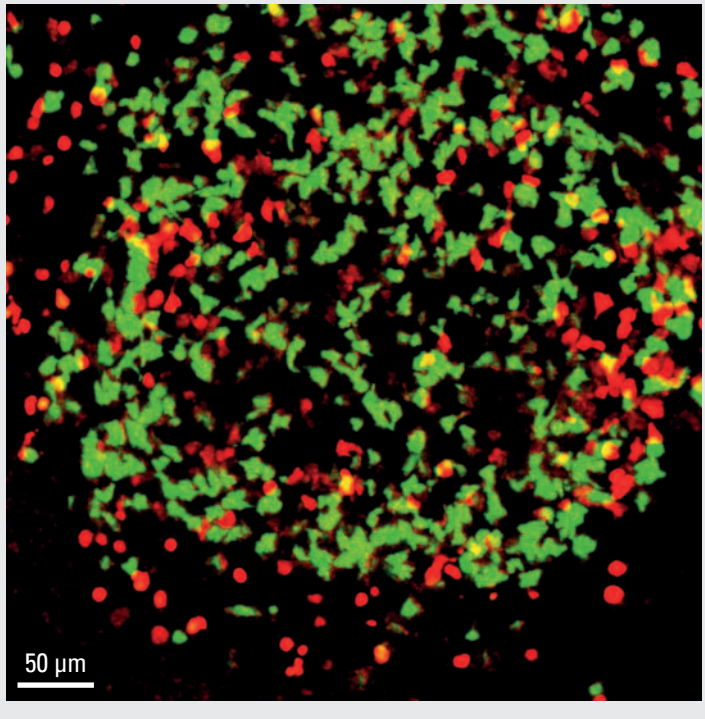




Zebrafish embryonic heart, 100 µm deep. Blood cells labeled with DsRed (red), SHG of muscle (gray)

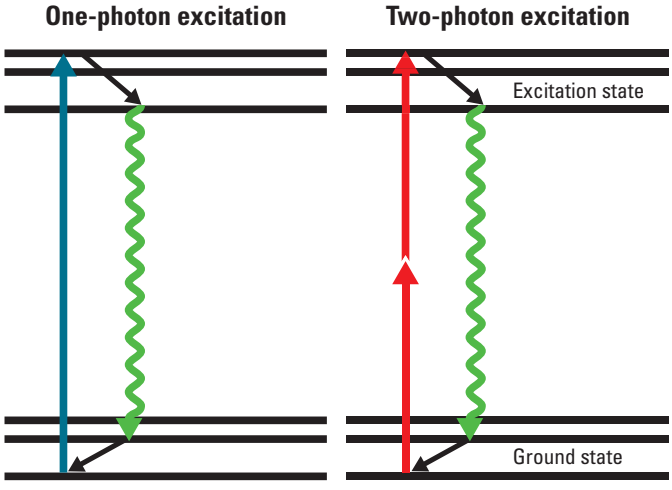
Dynamics of Anti-viral Response in Spleen

Our immune system constantly defends us against attacks by viruses and other microorganisms. If it fails to respond quickly, microorganisms can cause fatal diseases. Immunologists use video rate intravital multiphoton microscopy to follow infections and the response of the immune system in real time deep within tissues.

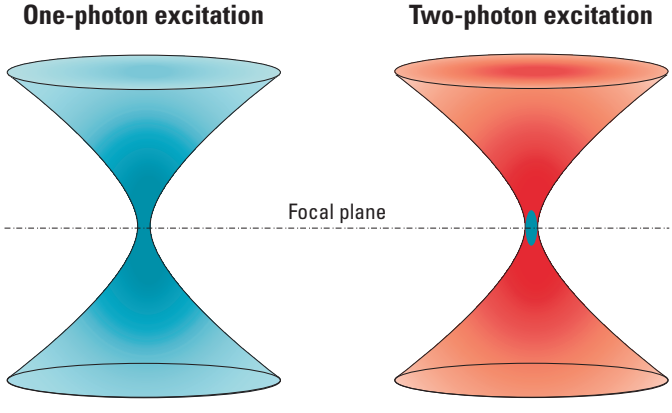


3D reconstruction of a 50 µm z-stack from a time lapse captured in the spleen 7 days following infection with lymphocytic choriomeningitis virus (LCMV). Anti-viral CD8-CFP (red) and CD4-YFP (green) T cells excited with 910 nm. Spectral unmixing was performed using Leica LAS AF software.

Principle of Multiphoton Microscopy



Energy levels of a fluorophore in one-photon (left) and two-photon (right) excitation. Colors correspond to the absorbed and emitted wavelengths.



The probability of excitation in one-photon excitation (left) linearly depends on the amount of photons from the light source. In two-photon excitation (right) it is proportional to the square of the intensity of the light source and limited to the vicinity of the focal plane.

Visualize deeper tissue sections

One of the main purposes for using multiphoton microscopy in biomedical research is the ability to make observations deep within thick tissue sections. The red-shifted excitation light used for multiphoton imaging is less prone to scattering in biological tissues and can therefore reach deeper into samples.

For multiphoton excitation, a fluorescent molecule has to absorb multiple photons almost simultaneously. When two photons are absorbed, we speak of two-photon excitation. Three-photon excitation is less common but sometimes used to improve resolution. For many fluorophores, the wavelength for two-photon excitation is roughly twice the wavelength for single-photon excitation while the emission spectrum usually remains the same.

Reduced phototoxicity and bleaching by localized excitation

The high photon density necessary for multiphoton excitation is generated by pulsed IR lasers with high peak power. While peak powers of several hundred kW are reached, average power is still low enough to avoid damage to the sample. The probability of two photons reaching the fluorophore almost simultaneously is only sufficiently high in the vicinity of the focus and drops exponentially outside of the focus. This reduces photobleaching and phototoxicity outside of the focal plane.

Non-descanned detection (NDD) for optimal light collection

In multiphoton microscopy all emitted photons are a result of excitation in the focal plane. This results in optical sectioning without the need for a detection pinhole. Thus, it is possible to improve the efficiency of light collection by placing the detectors as close as possible to the source of emission.

Multicolor Deep Tissue Imaging at High Speed

Leica TCS MP5 is a dedicated multiphoton microscope that gives you the choice between the high speed of a resonant scanner for following rapid processes and slower scan speeds for morphological studies. This multiphoton system acquires multiple colors and a transmitted light contrast image simultaneously.

Full range of scan speeds in one system
The Tandem Scanner combines two scanners in the Leica TCS MP5. The resonant scanner allows users to record fast dynamics and acquire detailed z-stacks faster by high-speed scanning. Switch to slower scan speeds of the conventional scanner for optimal spatial resolution.

For both scanning methods you can rotate the scan field and use panning in x and y to optimally position the imaging area in relation to the sample.

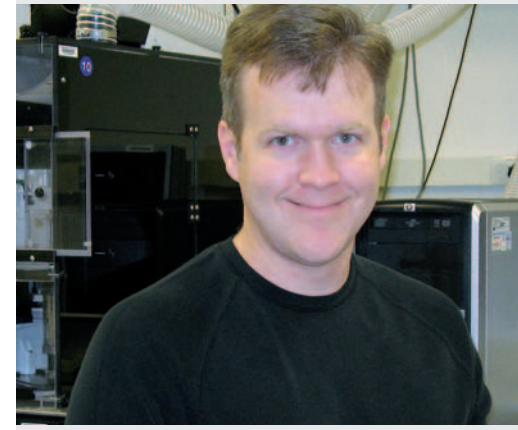
Detect up to seven channels simultaneously
For multicolor imaging you can use up to four non-descanned detectors with large photo sensor areas in either reflected or transmitted light position. The short coupling of the detectors ensures high photon collection efficency.

On Leica multiphoton systems it is easy to exchange and customize filter set for the NDDs. Signals with overlapping emission spectra are easily separated with the Dye Separation algorithm in LAS AF (Leica Application Suite Advanced Fluorescence).

Brightfield images (DIC, Dodt) can be acquired simultaneously with TLD (transmitted light detection) and RLD (reflected light detection) signals from the NDDs*. Dodt contrast on the Leica DM6000 CFS fixed stage microscope gives you optimal light collection efficiency for brightfield and fluorescent signals without additional prisms in the light path.

* not available on Leica DMI6000

- Freely configurable dedicated multiphoton microscope
- Small footprint
- True single point Tandem Scanner – conventional and resonant
- Frame rates up to 250 frames/s
- Up to 7 external detectors
- Simultaneous acquisition of RLD, TLD and Dodt contrast/brightfield



“The Tandem Scanner adds impressive speed to our multiphoton microscope. Combining high-speed scanning with the Leica TCS MP5 offers a real advantage for intravital microscopy.”

Dr. Dorian McGavern
NIH/NINDS, Bethesda, MD, USA

Exciting a Broader Range of Fluorophores

- Optimal transmission up to 1300 nm
- Fully integrated OPO solution
- Femtosecond and precompensated IR lasers with pulse widths < 70 fs
- Rapid modulation by EOM

"With the OPO we have access to a much larger selection of fluorophores for multiphoton imaging. And the integration of the OPO in Leica's multiphoton microscopes makes setup and operation really easy."

Dr. Jacco van Rheenen
Hubrecht Institute
Utrecht, The Netherlands

You want to see as deeply into your sample as possible. Leica Microsystems offers you the integration of the most advanced pulsed IR laser sources for multiphoton imaging. With pulses of less than 70 femtoseconds or wavelengths up to 1300 nm, the system is perfectly prepared for your most challenging samples.

Expand into the red for multicolor applications
Excitation up to 1300 nm with an optical parametric oscillator (OPO) allows you to explore the full range of fluorescent proteins and dyes.

With Leica's OPO solution, simultaneous multiphoton excitation of green and red fluorophores is possible. The longer wavelengths of the OPO can reach deeper into tissue and are less harmful to samples.

Leica Microsystems offers an OPO solution that is fully integrated into the software interface of LAS AF for easy tuning and setup of laser wavelengths. High transmission, up to 1300 nm, of the Leica TCS MP5 scan optics brings all the power from the OPO to the sample.

Precompensation for maximum power
Precompensation of the dispersive effects of glass ensures that the narrow pulse width of ultrashort light pulses is maintained. The maximum peak power is delivered at the sample for brightest images.

Modulation of laser power with minimal pulse broadening
In addition to a polarizing filter wheel, a continuously adjustable electro-optical modulator (EOM) attenuates the laser power. The EOM is highly efficient over the complete excitation range and causes minimal pulse broadening.

The rapid switching time of less than a microsecond is optimal for fast ROI (region of interest) scans.

Reaching a New Dimension in Intravital Imaging

The Leica DM6000 CFS provides the best mechanical and electronic stability for sensitive experiments. The new motorized 2-position objective changer allows smooth and vibration-free switching and dipping of objectives – all controlled remotely.

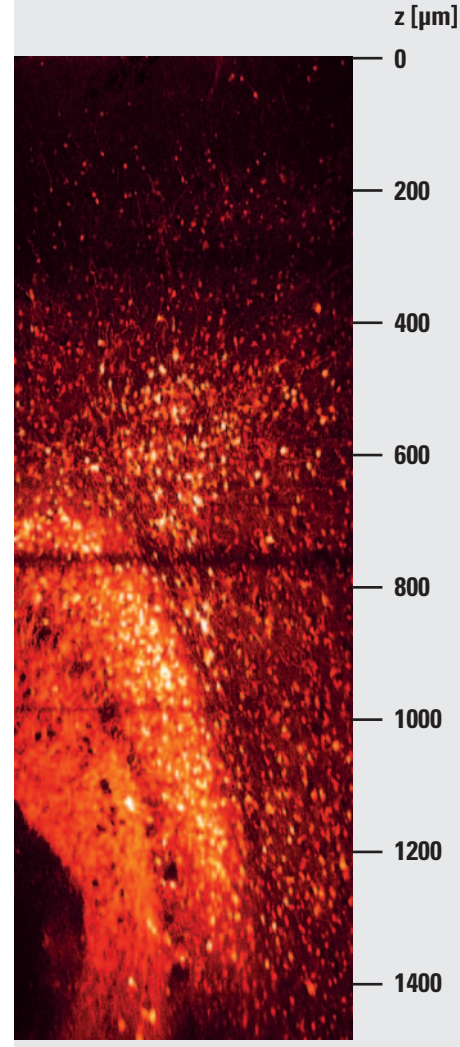
Dipping objectives for high resolution and optimal access
The Leica HCX IRAPO L 25x/0.95 W objective provides maximum clearance around the specimen with an access angle of 41° and a free working distance of 2.5 mm. A complete range of dipping lenses and high resolution objectives is available to match your needs.

Deeper tissue imaging with BABB and specialized objective
Reach even deeper into tissues prepared with BABB (Benzyl Alcohol Benzyl Benzoate, 1:2) with the Leica HCX APO L20×/0.95 IMM objective. The optics perfectly match the refractive index of BABB for optimal performance.

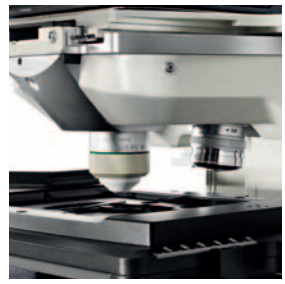
CCD camera simplifies positioning of manipulators
Changing the magnification in CCD camera mode without actually changing the objective allows for perfect positioning of pipettes without interrupting the setup. Alternating CCD camera and NDD detection can be combined in time lapse experiments.

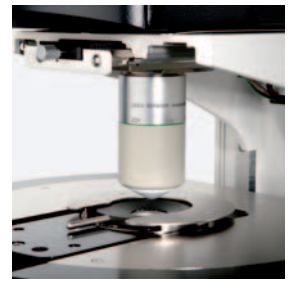
Online data evaluation gives faster results
Physiological studies often require data on morphological as well as electrophysiological responses. LAS AF Electrophysiology correlates voltage recordings with the fluorescence intensity data to simplify data evaluation.

- Leica DM6000 CFS
- Remote control of microscope
- Dedicated dipping objectives
- Specialized 20x/0.95 IMM objective
- Combine CCD camera and NDD
- Correlation of optical and electrical data
- LAS AF Electrophysiology



YFP expressed in mouse olfactory bulb prepared with BABB.





Flexible Solutions for High-Quality Results

Configure a Leica TCS MP5 for your applications:	Neuroscience	Electrophysiology	Cancer Research	Developmental Biology	Immunology	Cell Biology
Perfect resolution and ultrafast scanning – combine both with the Tandem Scanner						
Resonant Scanner Video rate scanning at up to 29 fps (512 x 512 pixels)	●●	●●	●	●●	●●	●
Conventional Scanner High optical resolution with up to 8192 x 8192 pixels and an exceptionally large field of view of 22 mm	●	●	●●	●	●	●●
Wide range of excitation sources						
Leica OPO Solution Excitation up to 1300 nm for deeper tissue imaging in multiple colors	●●	●●	●●	●●	●	●
Microscope stands to fit your needs						
Leica DM6000 CFS Fixed stage stand for intravital imaging	●●	●●	●	●	●	
Leica DMI6000 Fully automated inverted stand	●		●●	●	●	●●
Software with a clearly structured workflow lets you concentrate on your work						
LAS AF Live Data Mode Set up sophisticated time series to follow the most complex processes		●	●	●●	●●	●
LAS AF 3D Reconstruction Visualize x, y, z images	●●	●●	●	●●	●	
LAS AF Electrophysiology Software tool for synchronized correlation of optical and electrophysiological data	●	●●				
High resolution objectives combined with optimal free working distances (FWD)						
HCX APO L 20x/1.00 W, FWD 2.0 mm HCX APO L 20x/0.95 IMM, FWD 1.95 mm HCX IRAPO L 25x/0.95 W, FWD 2.5 mm	●●	●●		●	●●	

●● highly recommended

● recommended

Acknowledgements

We gratefully acknowledge the following scientists for providing images, data, samples and valuable support:

Page2/3 (from left to right)
Mouse mammary gland. Blood vessels labeled with 70 kD-Texas Red (red), SHG of type I collagen (purple), autofluorescence of single cells (green).
Courtesy of Evelyne Beerling, Dr. Jacco van Rheenen, Hubrecht Institute, Utrecht, The Netherlands

Lymph node. CD8-CFP (red) and CD4-GFP (green) T cells. SHG of collagen (cyan). Representative z-stack of 3D time lapse series.
Courtesy of Dr. Bernd Zinselmeyer and Dr. Dorian McGavern, NIH/NINDS, Bethesda, MD, U.S.A.

Embryonic day 16 kidney from a HoxB7-eGFP mouse.
HoxB7 labels the ureteric bud.
Courtesy of Prof. Deborah Hyink, Mount Sinai School of Medicine, New York, NY, U.S.A.

Lymph node. Alexa 488 (green), PE (red), SHG (blue).
Courtesy of Dr. Pierre Bourdoncle, Institut Cochin, Paris, France

Mouse kidney section. Wheat germ agglutinin-Alexa 488 (green), phalloidin-Alexa 568 (red) and DAPI (blue).
Courtesy of Dr. Jacco van Rheenen, Hubrecht Institute, Utrecht, The Netherlands

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Top: Microglia labeled with GFP (green), 655 nm quantum dots injected in brain vasculature (red), SHG of skull bone (blue). Representative z-stack of 3D time lapse series.
Courtesy of Dr. Debasis Nayak, Dr. Bernd Zinselmeyer and Dr. Dorian McGavern, NIH/NINDS, Bethesda, MD, U.S.A.

Bottom: Mouse kidney. Blood vessels labeled with 70 kD-Texas Red (red), SHG of Type I collagen (purple), autofluorescence of single cells (green).
Courtesy of Evelyne Beerling, Dr. Jacco van Rheenen, Hubrecht Institute, Utrecht, The Netherlands

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Top: Zebrafish embryo, blood cells labeled with DsRed.
Courtesy of Dr. Julien Vermot, IGBMC, Strasbourg-Illkirch, France

Bottom: Spleen. Anti-viral CD8-CFP (red) and CD4-GFP (green) T cells. Representative z-stack of 3D time lapse series.
Courtesy of Dr. Bernd Zinselmeyer and Dr. Dorian McGavern, NIH/NINDS, Bethesda, MD, U.S.A.

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Mouse olfactory bulb labeled with venusYFP. Sample cleared with BABB.
Courtesy of Jan Thomas Herb, Dr. Andreas Schäfer, Annemarie Scherbarth, Dr. Günter Giese and Prof. Winfried Denk, MPI for Medical Research, Heidelberg, Germany

“With the user, for the user”
Leica Microsystems

Leica Microsystems operates globally in four divisions, where we rank with the market leaders.

• Life Science Division

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems’ customers at the leading edge of science.

• Industry Division

The Leica Microsystems Industry Division’s focus is to support customers’ pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

• Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

• Medical Division

The Leica Microsystems Medical Division’s focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life**.

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