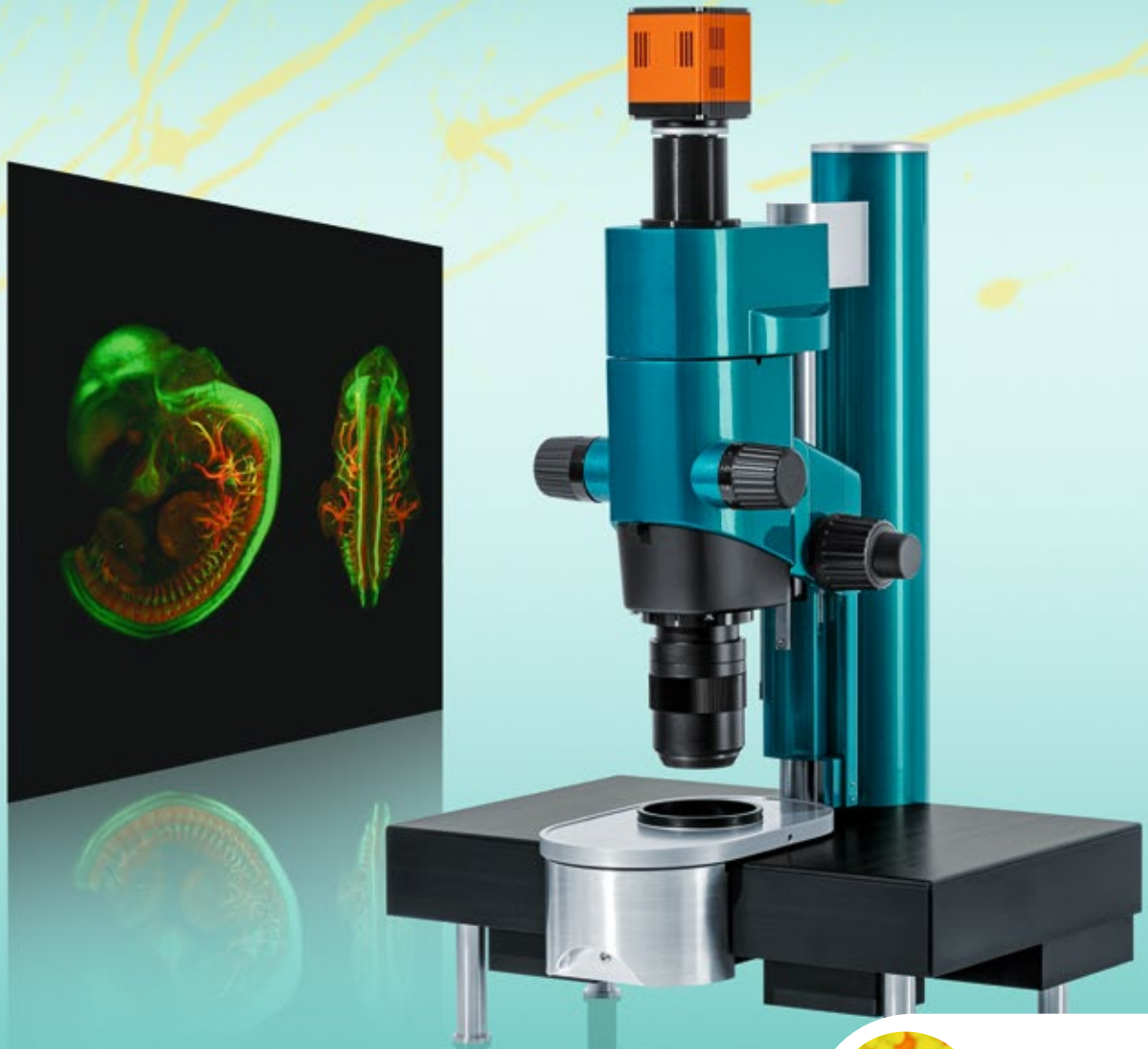
MACS

Miltenyi Biotec

The UltraMicroscope II

Fast 3D imaging and analysis of entire biological systems



UNDERSTAND
NATURE'S
COMPLEXITY

Revealing the architecture of biology

Fluorescent imaging of three-dimensional biological samples, like cell clusters, whole organs, embryos or larvae is challenging. Photobleaching and photodamage are common when full samples are illuminated although signals are captured only from the focal plane. The UltraMicroscope II eliminates these issues by concentrating six sheets of light exclusively on the focal plane. Moving the sample in the z-axis through the focal plane generates brilliant 3D images, and the thoughtful and intelligent engineering of the instrument makes visualizing biological processes in physiologically relevant contexts a staple methodology for any lab.

Visualize biological systems

Reveal process compartmentalization and time course of biological events in cellular detail without losing sight of the system as a whole.

Sharp, bright images

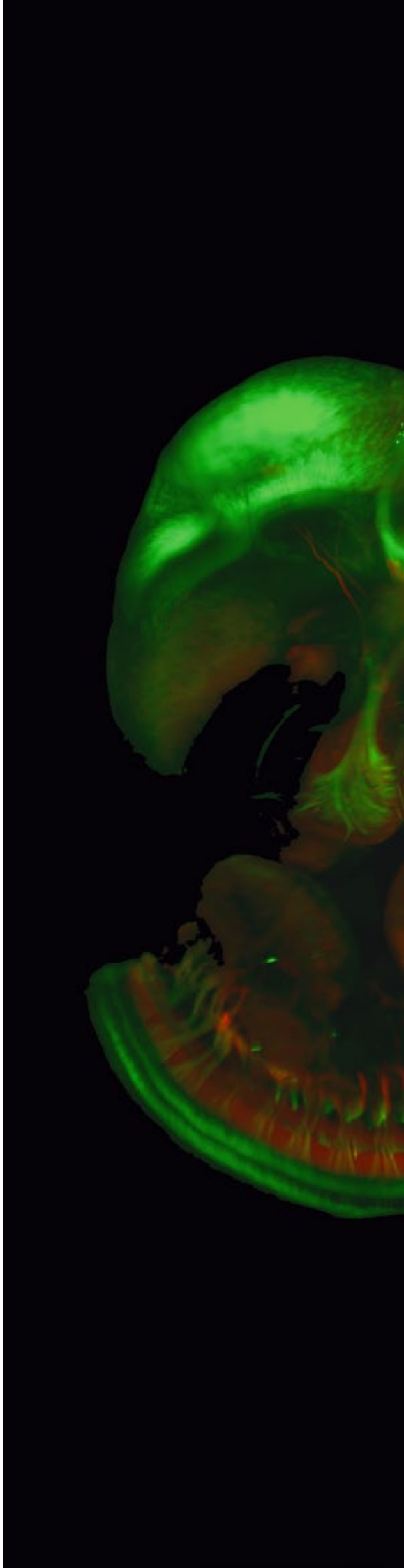
Adjust the six light sheets to optimally and homogenously excite your sample and prevent light scattering, shadowing, refraction, and other artifacts.

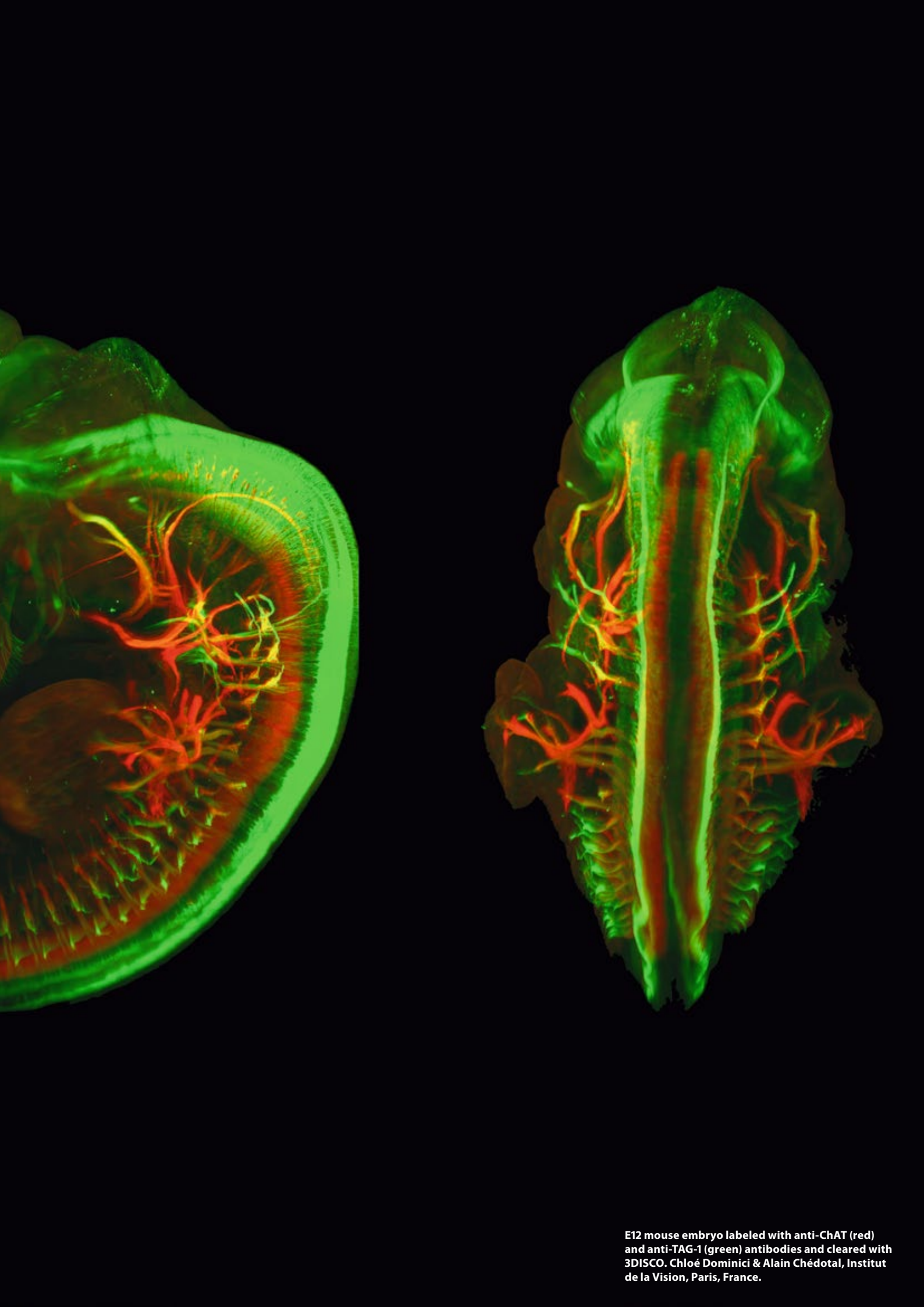
Flexible input

Use any common clearing protocol and any imaging solution – from water to organic solvents – for your visualization.

Smart, user-friendly features

With a quick-access sample chamber, easy-handling zoom body and modules for expanded applications, the UltraMicroscope II is designed for a multi-operator, high-use environment.





E12 mouse embryo labeled with anti-ChAT (red) and anti-TAG-1 (green) antibodies and cleared with 3DISCO. Chloé Dominici & Alain Chédotal, Institut de la Vision, Paris, France.

Capture cellular detail with 3D acuity

Location and timing are key features of biological processes. Spatial and temporal compartmentalization at the subcellular, tissue, and organismal level guarantee that the right biomolecules interact with one another to elicit a specific phenotype, and disruption can lead to dysfunction and disease. Visualizing this architecture in cellular detail but at a larger scale – cells, tissues, organisms – provides clues to molecular mechanisms and elucidates the physiological context in which these occur. In keeping with this bird's-eye perspective, the UltraMicroscope II has a large and open sample chamber, a wide field of view and a long objective travel range to accommodate those larger samples, like embryos, mouse organs, biopsies or 3D cell cultures.

Sea horse, autofluorescence.

Optimized illumination

Tailored illumination for profound images

Sharp, brilliant, and clear images offer a landscape of insights into the complex structure and processes of biological systems. To achieve this high-fidelity, the fluorescence excitation must be spot-on for each sample, regardless of nature, size or treatment.

Six light sheets evenly illuminate the sample

Structures of a sample illuminated from one side can block light, casting a shadow over areas behind them (fig. 1A). The UltraMicroscope II is equipped with two opposing sets of three light sheets, each positioned at a slightly different angle. All six light sheets converge on the focal plane either head-on, slightly above or slightly below to illuminate all areas of the sample and minimize artifacts like dark areas or stripes (fig. 1B).

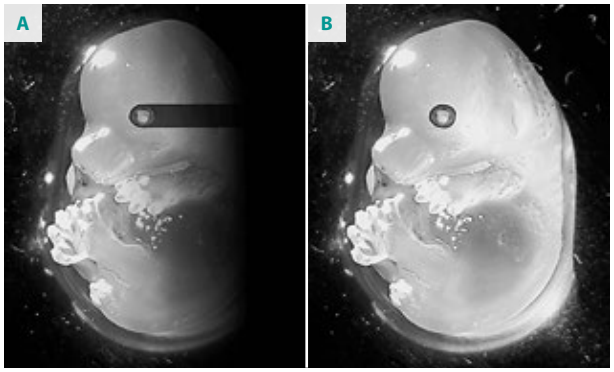


Figure 1: Unidirectional illumination (from the left) results in dark areas and stripes as parts of the sample are not exposed to the light sheet (A). The bidirectional, triple light sheet illumination of the UltraMicroscope II generates a clear, evenly illuminated image (B).

Adjustable light sheet parameters for the best image

No light sheet is perfectly planar but all approximate a plane over a given horizontal range. This is where the light sheet is thinnest and where detection in a light sheet microscope takes place. Controls in the UltraMicroscope II software allow you to make this planar section wider by decreasing the numerical aperture (NA) of the illumination. This enlarges the field of view to capture large samples that do not require high resolution in the z-axis. Boosting axial precision requires using thin light sheets, which are achieved by increasing illumination NA at the expense of decreasing the planar range for detection. The result is exceptionally high-precision images of smaller samples (fig. 2).
To visualize larger samples with the same z-resolution, the UltraMicroscope II uses dynamic horizontal focus. Samples remain stationary while the focus position moves, shifting the narrow range of detection horizontally to generate a series of snapshots that are blended into one high-quality image by the software.

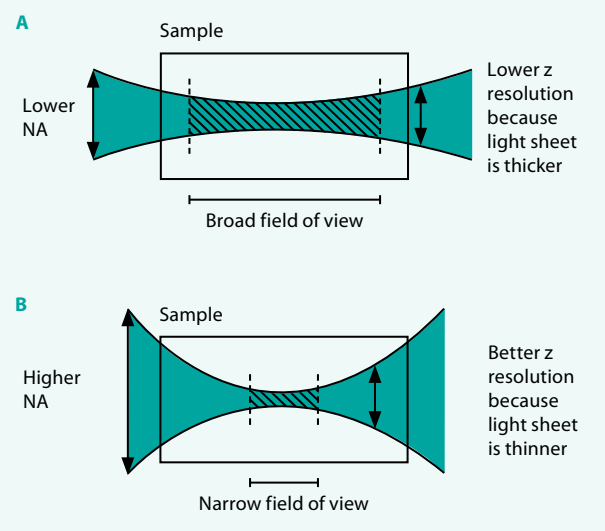


Figure 2: By adjusting the shape of the light sheets, illumination is tailored to sample size and imaging goals. A lower NA results in a broad field of view (A), and a higher NA results in a narrow field of view (B). While there is a tradeoff between the field of view, the thickness of the light sheet and the z-resolution, the UltraMicroscope II allows balancing these parameters to meet your specific requirements.

Compatible with all clearing protocols

Flexible and automatic adaptation to all clearing protocols

Deep tissue imaging often requires altering the optical properties of samples to render them transparent while keeping their structure intact. Most clearing agents and immersion media used today in clearing protocols have high refraction indices and cause specific dispersion. For optimal results, specifications of the objective lens used for imaging must match those parameters and accommodate a large free working distance to cover the full depth of the sample.

The UltraMicroscope II was designed to be compatible with all currently used clearing protocols, whether based on aqueous buffers or on organic solvents. The objectives, which come equipped with dipping caps, cover the full spectrum of differently cleared samples and immersion media to deliver high performance in water or solvent-based imaging solutions, with fixed or live samples. The system also features refractive index compensation which can be adjusted via the software to guarantee the perfect setup every time.

Flexible and easy

Designed for every sample and everyone

The development of the UltraMicroscope II revolutionized a technology that remained untapped for over a century. Every aspect of the design and engineering was built to address needs voiced by customers – "jump-right-in" usability, flawless operation, robust components, state-of-the-art optics. The instrument is now a powerful research tool that anyone can operate either in the lab or in high-use environments like imaging facilities.

Usability for the real world

Every detail of the UltraMicroscope II is a commitment to making your work easier. The sample chamber is large, and samples are easily inserted from the top without disassembly or removing imaging solution. Changing optical magnification by a factor of ten is done with the turn of a single knob of the Zoom Body Module, taking you from a full mouse organ to cells in the blink of an eye.

Intuitive and versatile

A positive, fluid and versatile user experience was a key driver in the software and instrument conception. New users can produce high-quality images after a short training and the intuitive operation ensures that they can work independently, with minimum support.

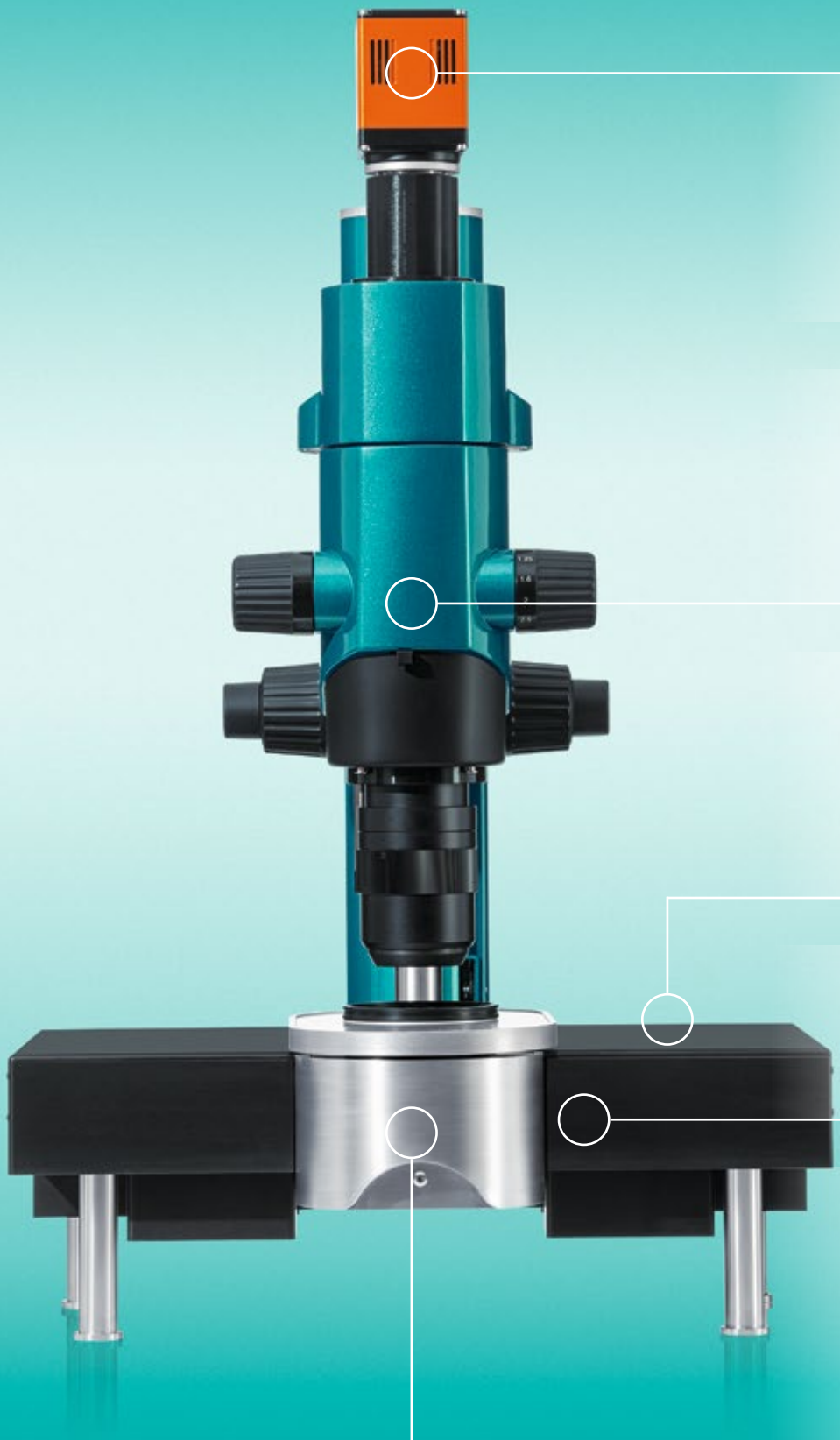
Software that eliminates guesswork

The software wizard is the sum of our decades-long experience in microscopy and guides you through the correct setup and an efficient data acquisition process to ensure the best results.

Tailored for every application

The UltraMicroscope II can be fitted with either: the Zoom Body Module for easy handling in a multi-user environment or the Super Plan Module that can deliver unforeseen image resolution and quality. The Super Plan Module can be mounted directly to the focusing unit of existing UltraMicroscope II systems.

Zoom body configuration



Optimal sensitivity and speed

High-resolution camera with a large field of view without sacrificing frame rate or increasing read noise.

Fast and easy zoom

The Zoom Body Module supports fast and safe magnification changes with the simple turn of a knob. Ideal for multi-user environments.

Even and targeted illumination

Six light sheets illuminate your sample from both sides and at slightly different angles to create accurate and exact images.

The control to optimize

Adjust the settings of the light sheets to match your samples and your imaging goals.

The big picture in biology

A large, easy-access sample chamber accommodates rodent organs and tumors, mouse embryos, insect larvae, or biopsies from large animals.

Super Plan configuration

Unprecedented image quality

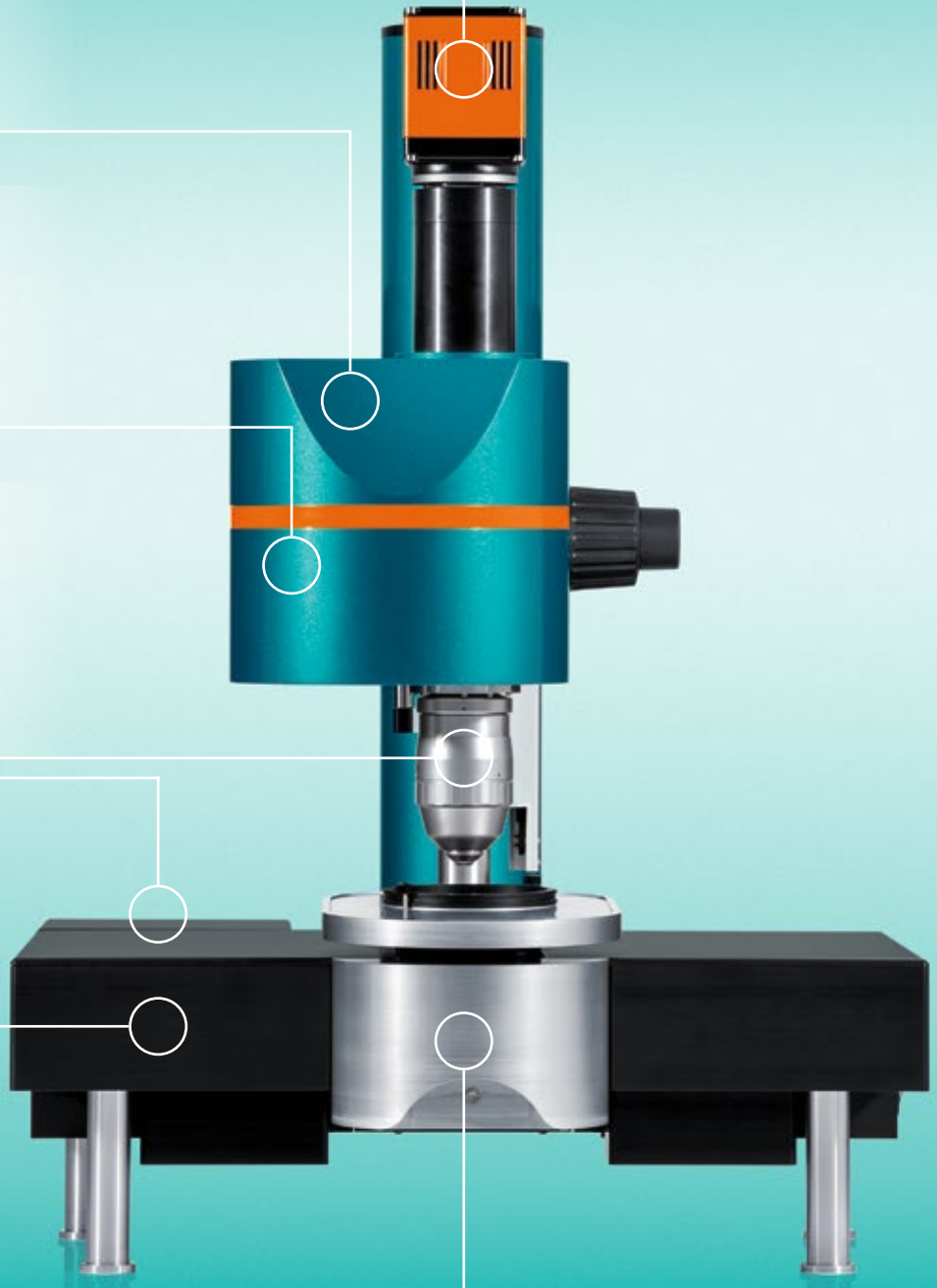
The large motorized tube lens guarantees high image quality free from aberrations.

Automated post-magnification

Easy and fast switching between four different post-magnification lenses (0.6×, 1×, 1.67×, and 2.5×).

Superior imaging capabilities

Especially developed for light sheet microscopy, this setup maximizes imaging performance with the MI Plan objective series.



Expandable to cover every application

Objectives designed for large-scale 3D imaging

The MI Plan series of objectives are apochromatic planar multi-immersion lenses developed specifically for the Super Plan Module of the UltraMicroscope II (fig. 3). Achieve optimal images regardless of sample size, clearing technique and immersion solution.

- Robust construction for repeated use in any immersion solution.
- Designed with optical characteristics to match common immersion media, such as high refractive indices from 1.33 (water) to 1.56 (organic solvents).
- Long working distances to accommodate large samples.
- Flat-field correction guarantees a flat focal plane matching the optical axis of the light sheets.
- Include chromatographic aberration correction.

Figure 3: The MI Plan objective lenses optimized for light sheet microscopy of large samples in a broad range of immersion solutions.

Controlled sample chamber for *in vivo* imaging

Keep your sample in a controlled environment for realistic *in vivo* imaging. The *in vivo* sample cuvette is quickly mounted on the UltraMicroscope II and allows setting and maintaining a constant immersion medium temperature and CO_2 / O_2 atmosphere between medium and objective (fig. 4).

- Intuitive control via touchscreen.
- Temperature feedback mode monitors and adjusts medium temperature as needed.
- Active CO_2 controller and humidity module.
- Easy dismount for autoclaving.

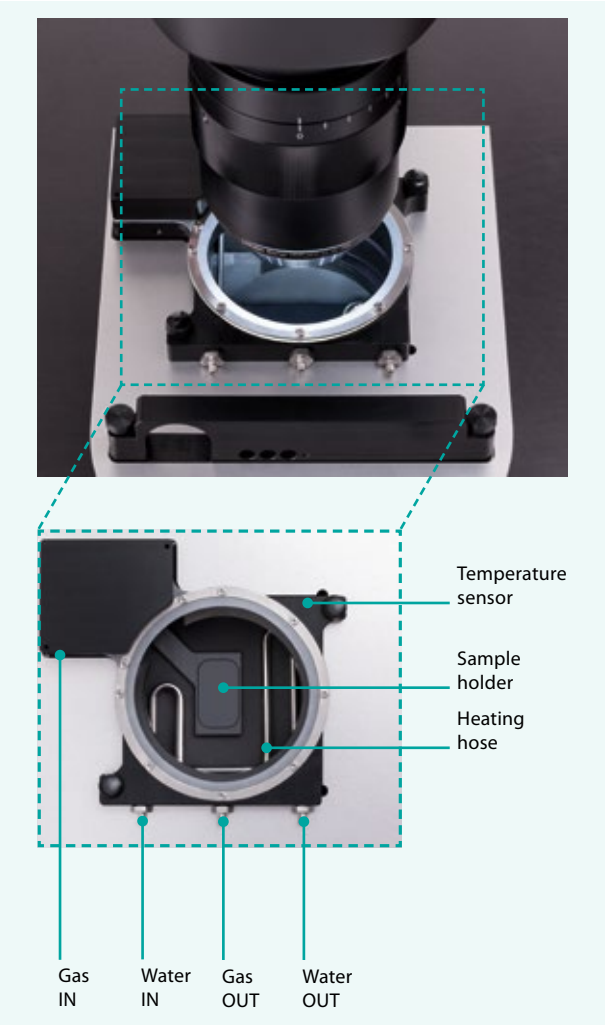


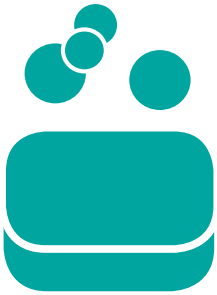
Figure 4: The *in vivo* sample chamber maintains a constant temperature and CO_2/O_2 concentrations for functional and time-course imaging.

Three steps to a new view of biology

With its powerful optics, smart engineering, and user-minded operation, the UltraMicroscope II reveals a new perspective on organisms, how they are built and how they function. High-speed imaging of fragile and cleared specimens allows capturing fast biological processes and obtaining realistic three-dimensional renditions of complex biological systems. Appropriate sample preparation is essential in this workflow. Miltenyi Biotec offers a complete portfolio of validated antibodies and antibody-fluorochrome conjugates as well as strategies for sample clearing. Get started knowing you are using the right tools to get the best results.

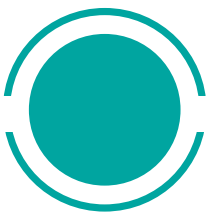
01 STAINING

Structural and functional biomolecules are (immuno-) labeled with specific fluorescent dyes, proteins, or conjugated antibodies.



02 CLEARING

Tissue-clearing methods using organic solvents or aqueous buffers render large biological samples transparent while maintaining their internal three-dimensional structure.



03 IMAGING

A single z-section of the stained sample is excited by six focused light sheets and the resulting fluorescence is recorded. The sample is moved through the focal plane, exciting fluorophores at each layer and creating 3D image stacks while keeping photodamage and bleaching to a minimum.

Colorful opportunities to see more than meets the eye

Unique antibodies for unmatched staining

Explore our portfolio of more than one thousand REAfinity™ Recombinant Antibodies for your imaging applications.

- Extensive portfolio for imaging all biological samples.
- Recombinant nature ensures high experimental reproducibility.
- Unique antibody design eliminates background signals.
- Directly conjugated primary antibodies achieve perfect balance between signal strength and sensitivity.

Partner with us to test REAfinity Antibodies for your application! For further information, contact your local Miltenyi Biotec representative.

REAfinity Recombinant Antibodies

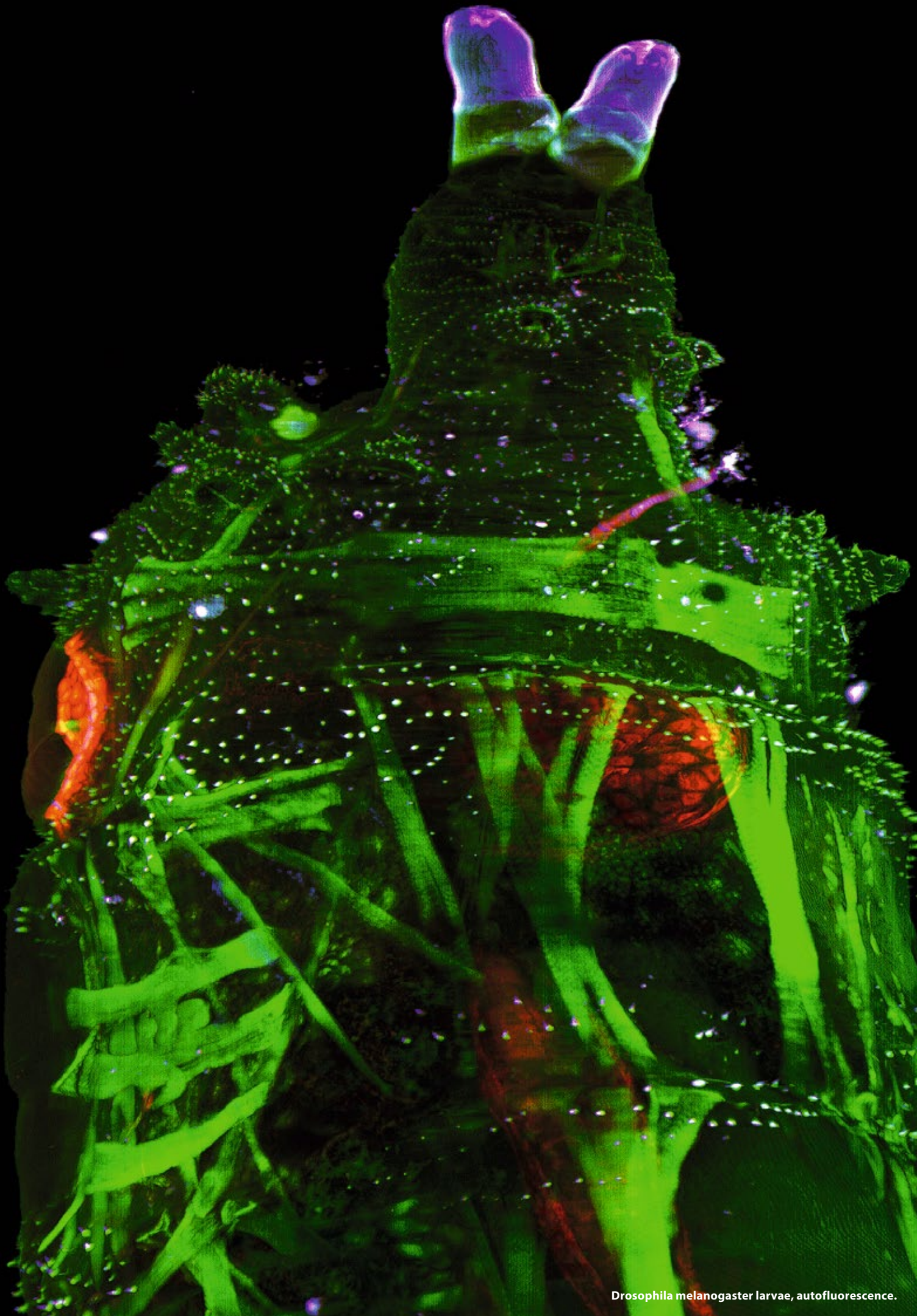
Streamlined sample clearing to get started immediately

Current protocols for tissue clearing involve laborious steps that often use toxic reagents to speed up the clearing process. We have established an easy and fast method to clear large tissue samples using a nontoxic organic solvent. This protocol has been optimized for various tissues, including bone, preserving endogenous fluorescence and antibody labeling for high-end imaging while completely avoiding toxic substances.

- **Nontoxic, cost-effective, and easy:** a clearing process that anyone can perform.
- **Simple:** one fast clearing step that optimally clears samples and preserves endogenous fluorescence.
- **Efficient:** clears whole organs, including whole brain and tumor tissues.

A ready-to-use kit will be available soon, featuring a protocol and components for antibody labeling and sample clearing. For more information please contact your local Miltenyi Biotec representative.

Figure 5: Cleared mouse testicles.



Drosophila melanogaster larvae, autofluorescence.

Limitless applications

Pancreatic carcinoma, CAR T cells labeled with CD271 (LNGFR), human, Miltenyi clone: REA844 conjugated with Vio®667 (violet), rhodamine lectin labeling vasculature (orange), GFP (green).

Oncology

The complexity of cancer tumors often hinders examining and understanding internal regulatory mechanisms and avenues of therapy delivery. Light sheet microscopy has already contributed to overcoming some of these challenges.

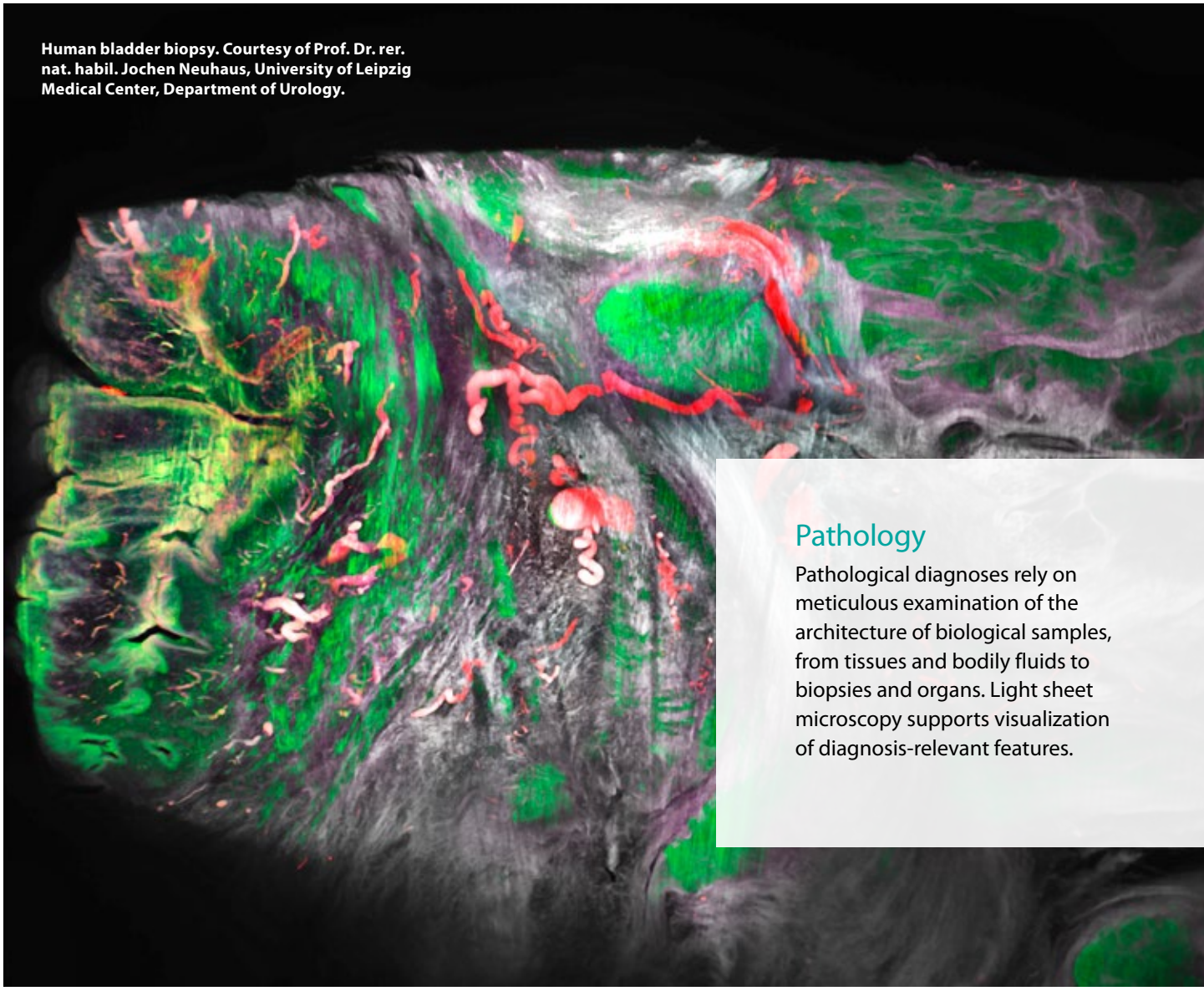
Locally renewing resident synovial macrophages provide a protective barrier for the joint.
Culemann, S. *et al.* (2019) Nature 572: 670–675.

Glioblastoma multiforme restructures the topological connectivity of cerebrovascular networks.
Hahn, A. *et al.* (2019) Scientific Reports 9, 11757.

Deep learning reveals cancer metastasis and therapeutic antibody targeting in whole body.
Pan, C. *et al.* (2019) bioRxiv: https://doi.org/10.1101/541862

Correlated MRI and Ultramicroscopy (MR-UM) of brain tumors reveals vast heterogeneity of tumor infiltration and neoangiogenesis in preclinical models and human disease.
Breckwoldt, M.O. *et al.* (2019) Front. Neurosci. 12, 1004.

Tumor uptake of anti-CD20 fabs depends on tumor perfusion.
Mendler, C.T. *et al.* (2016) J. Nuc. Med. 57: 1971–1977.



Human bladder biopsy. Courtesy of Prof. Dr. rer. nat. habil. Jochen Neuhaus, University of Leipzig Medical Center, Department of Urology.

Pathology

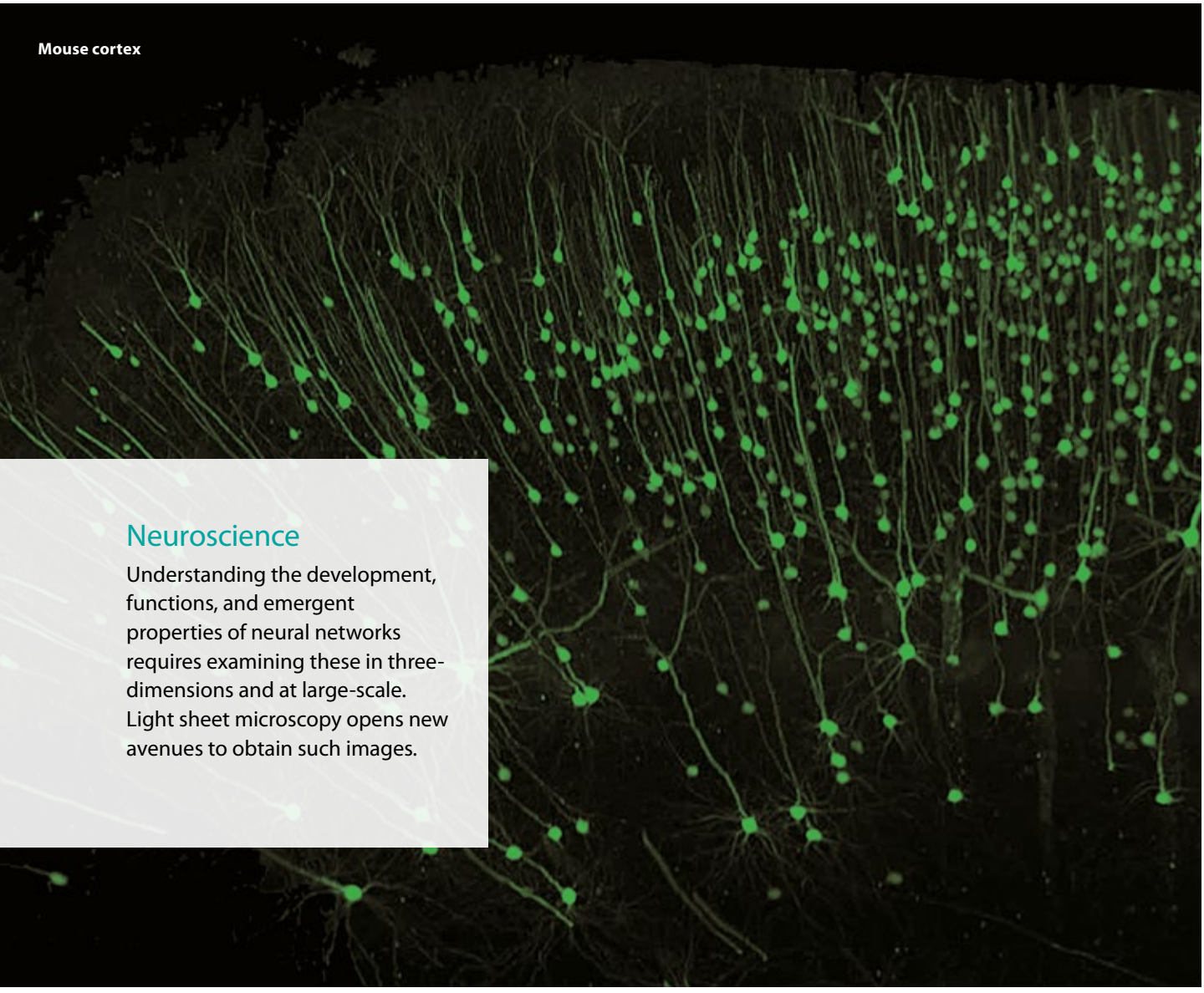
Pathological diagnoses rely on meticulous examination of the architecture of biological samples, from tissues and bodily fluids to biopsies and organs. Light sheet microscopy supports visualization of diagnosis-relevant features.

Contemporaneous 3D characterization of acute and chronic myocardial I/R injury and response.
Merz, S.F. *et al.* (2019) Nat. Commun. 10: 2312.

Scalable cytoarchitectonic characterization of large intact human neocortex samples.
Hildebrand, S. *et al.* (2018) bioRxiv: https://doi.org/10.1101/274985

Immunolabeling of cleared human pancreata provides insights into three-dimensional pancreatic anatomy and pathology.
Noë, M. *et al.* (2018) Am. J. Path. 188: 1530–1535.

VIPAR, a quantitative approach to 3D histopathology applied to lymphatic malformations.
Hägerling, R. *et al.* (2017) JCI Insight 2: e93424.



Neuroscience

Understanding the development, functions, and emergent properties of neural networks requires examining these in three-dimensions and at large-scale. Light sheet microscopy opens new avenues to obtain such images.

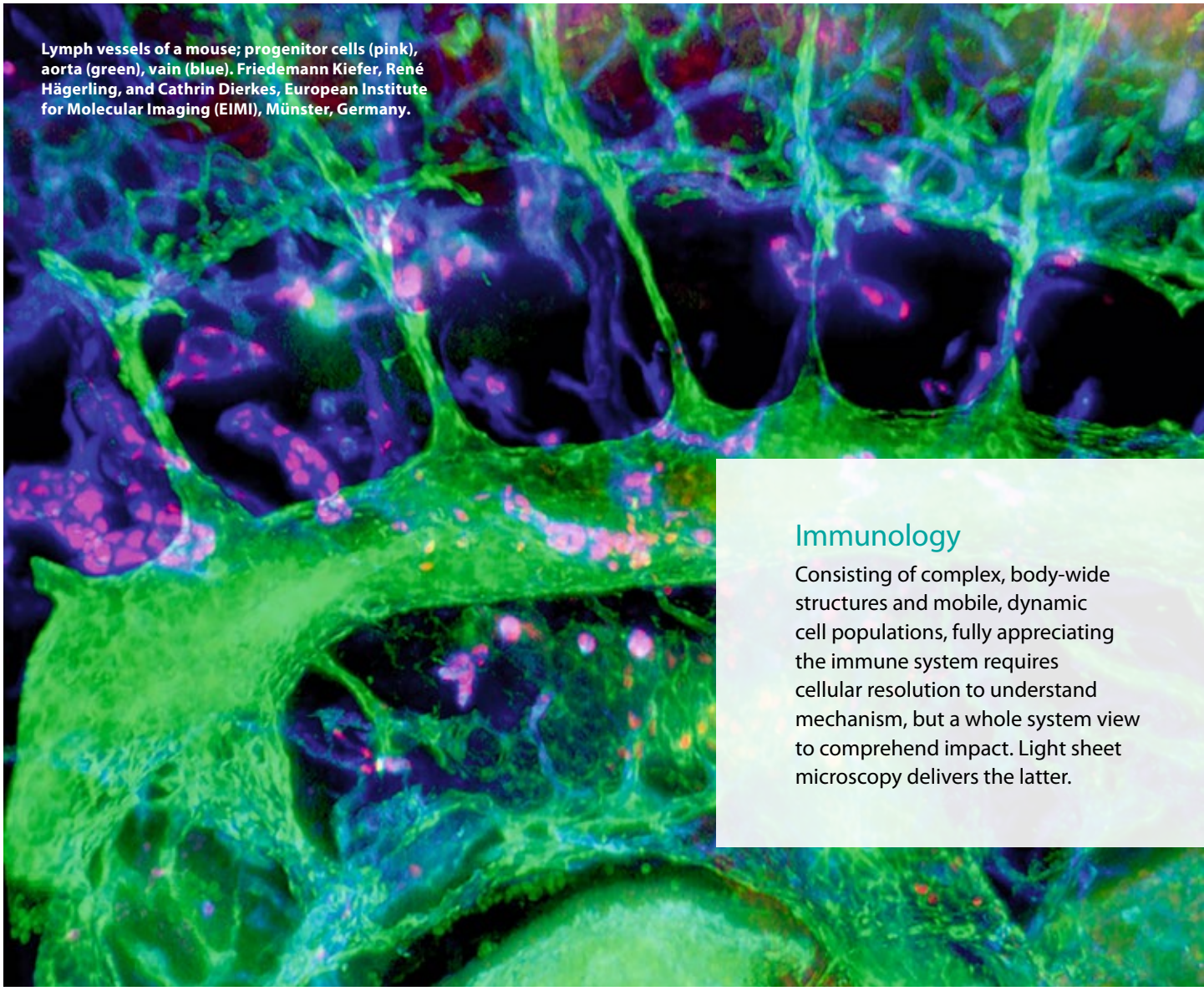
Circuit asymmetries underlie functional lateralization in the mouse auditory cortex.
Levy R.B. *et al.* (2019) Nat. Commun. 10 : 2783.

GABAergic inhibition in dual-transmission cholinergic and GABAergic striatal interneurons is abolished in Parkinson disease.
Lozovaya, N. *et al.* (2018) Nat. Commun. 9: 1422.

Three-dimensional study of Alzheimer's disease hallmarks using the iDISCO clearing method.
Liebmann, T. *et al.* (2016) Cell Rep. 6: 1138–1152.

Mapping of brain activity by automated volume analysis of immediate early genes.
Renier, N. *et al.* (2016) Cell 165: 1789–1802.

Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis.
Susaki, E.A. *et al.* (2014) Cell 157: 726–739.



Lymph vessels of a mouse; progenitor cells (pink), aorta (green), vain (blue). Friedemann Kiefer, René Hägerling, and Cathrin Dierkes, European Institute for Molecular Imaging (EIMI), Münster, Germany.

Immunology

Consisting of complex, body-wide structures and mobile, dynamic cell populations, fully appreciating the immune system requires cellular resolution to understand mechanism, but a whole system view to comprehend impact. Light sheet microscopy delivers the latter.

Panicle-Shaped Sympathetic Architecture in the Spleen Parenchyma Modulates Antibacterial Innate Immunity.
Ding, X. *et al.* (2019) Cell Reports 27 (13): 3799-3807.e3

Compartmentalized gut lymph node drainage dictates adaptive immune responses.
Esterházy, D. *et al.* (2019) Nature 569: 126–130.

A network of trans-cortical capillaries as mainstay for blood circulation in long bones.
Grüneboom, A. *et al.* (2019) Nature Metabolism 1: 236–250.

Matrix stiffness controls lymphatic vessel formation through regulation of a GATA2-dependent transcriptional program.
Frye, M. *et al.* (2018) Nat. Commun. 9: 1511.

Technical specifications

UltraMicroscope II specifications				
Sheet optics				
Illumination	Uni- and bidirectional			
Number of light sheets	1–6			
Thickness	4 µm–24 µm			
Width	1 mm–20 mm			
Numerical aperture	0.0135–0.135			
Focus positioning	Dynamic			
Refractive index matching	1.33–1.56			
Detection optics	**Zoom body configuration**	**Super Plan configuration**		
Objective lenses	2×	1.1×	4×	12×
Numerical aperture	0.5	0.1	0.35	0.53
FOV diagonal (5.5 Megapixel camera)	1.7 mm–17.6 mm	≥33 mm	≥9.1 mm	≥3 mm
Zoom | post-magnification	0.63× – 6.3× (manual)	0.6×, 1×, 1.67×, and 2.5× (automated)		
Total magnification	1.26×–12.6×	0.66×–2.75×	2.4×–10×	7.2×–30×
Working distance	5.6 mm (corrected), 10 mm	≤17 mm	≤16 mm	≤10.9 mm
Refractive index matching	1.33–1.56			
Chromatic detection	Seven filters			
Chromatic correction	Dynamic 400 nm–850 nm			
Imaging chamber				
Imaging solution	Aqueous buffers and organic solvents			
Sample travel range (X, Y, Z)	1 cm, 1 cm, 1 cm			
Sample size	µm range to cm range			
Chamber size	72 mm × 74 mm × 35 mm			
Light source				
Laser module	Max. 5 laser lines (405, 488, 561, 639, 785 nm)*, 50-100 mW per diode			
Supercontinuum laser	Emission 460 nm–800 nm, 1 mW/nm–3 mW/nm			
General information	**Zoom body configuration**	**Super Plan configuration**		
Dimensions (w × h × d)	54 cm × 82 cm × 65 cm	54 cm × 73 cm × 65 cm		
Weight	47 kg (w/o controller and laser)			

*Other laser lines available upon request.

Camera specifications				
Detector	**5.5 Megapixel sCMOS camera**		**4.2 Megapixel sCMOS camera**	
Sensor type	Front-illuminated scientific CMOS		Front-illuminated scientific CMOS	
Active pixels (w × h)	2560×2160 (5.5 Megapixel)		2048×2048 (4.2 Megapixel)	
Pixel size	6.5 µm × 6.5 µm		6.5 µm × 6.5 µm	
Sensor size	16.6 mm × 14.0 mm; 21.8 mm diagonal		13.3 mm × 13.3 mm; 18.8 mm diagonal	
Pixel well depth	30,000 e^-		30,000 e^-	
Pixel readout rate	200 MHz (100 MHz×2 sensor halves) 560 MHz (280 MHz×2 sensor halves)		Slow read 216 MHz (108 MHz×2 sensor halves) Fast read 540 MHz (270 MHz×2 sensor halves)	
Readout modes	Rolling shutter and true global shutter (snapshot)		Rolling shutter and global clear	
Read noise [median] rolling shutter	200 MHz: 0.9 e^- [1.2 rms]	560 MHz: 1.2 e^- [1.6 rms]	216 MHz: 0.90 e^- [1.1 rms]	540 MHz: 1.10 e^- [1.3 rms]
Read noise [median] global shutter	200 MHz: 2.3 e^- [2.5 rms]	560 MHz: 2.4 e^- [2.6 rms]	-	-
Max readout rate	560 MHz		540 MHz	
Max frame rates	Camera link: 100 fps; USB 3.0: 53 fps		Camera link: 100 fps; USB 3.0: 53 fps	
Maximum quantum efficiency	60%		82%	

Miltenyi Biotec and LaVision BioTec – joined forces to empower knowledge creation from image analysis

A pioneer in the market for commercial light sheet microscopes, LaVision BioTec GmbH has been driving innovation for high-end microscopy in oncology, pathology, neuroscience and immunology since 2000 and is now part of the Miltenyi Biotec family.

As experts in cell analysis, detection reagents, microscopy, image capture and processing, our goal is to offer fully scalable solutions for structural and functional analyses of biological samples at all levels – from organ and tumors, to tissue and further down to single cells. This way, our customers gain a more comprehensive view of cellular processes and disease mechanisms that leads to novel insights and breakthroughs.

▸ miltenyibiotec.com

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