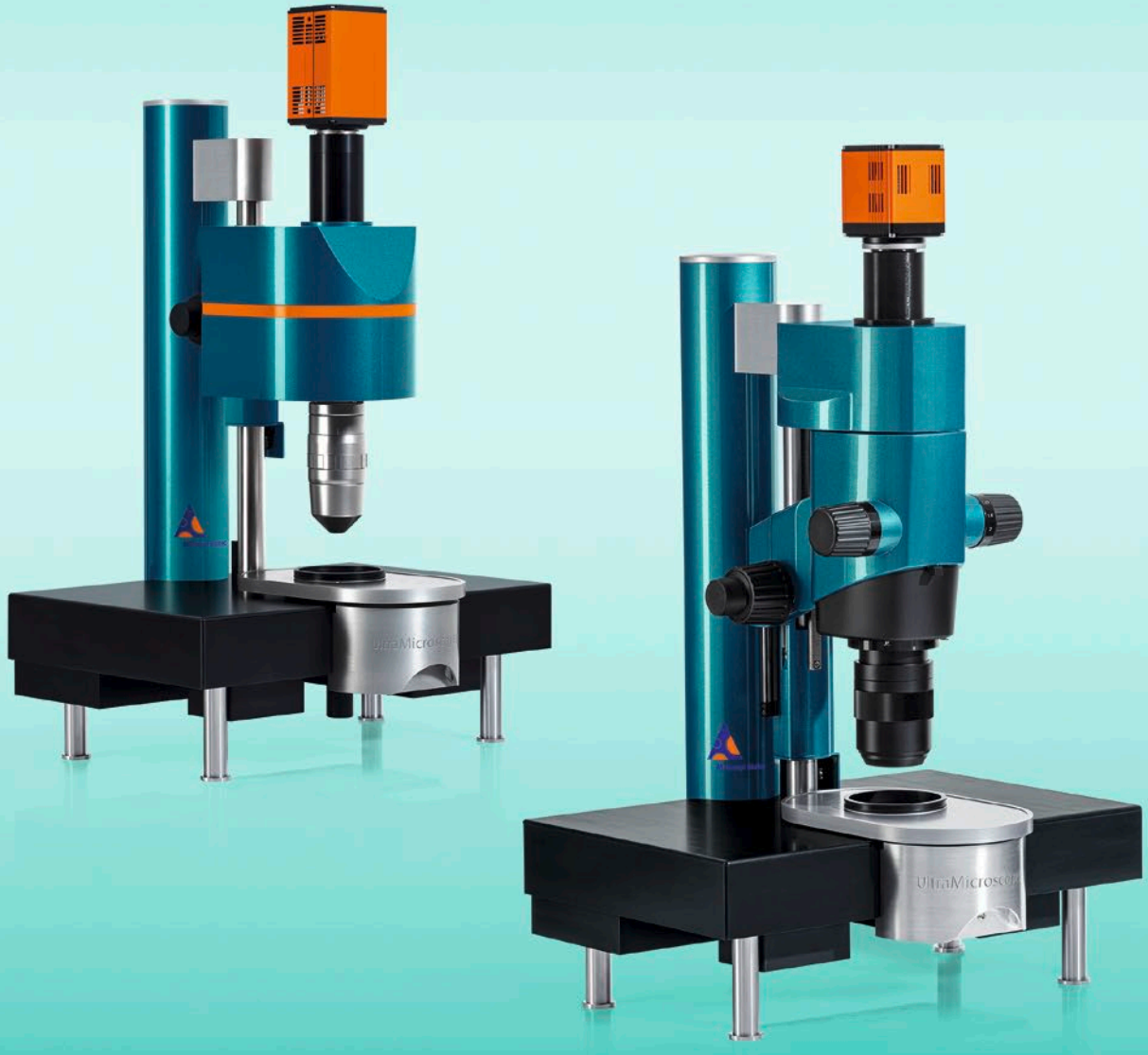
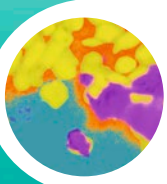
Miltenyi Biotec

The UltraMicroscope II

Fast 3D imaging and analysis of entire biological systems





UNDERSTAND
NATURE'S
COMPLEXITY

Revealing the architecture of nature

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

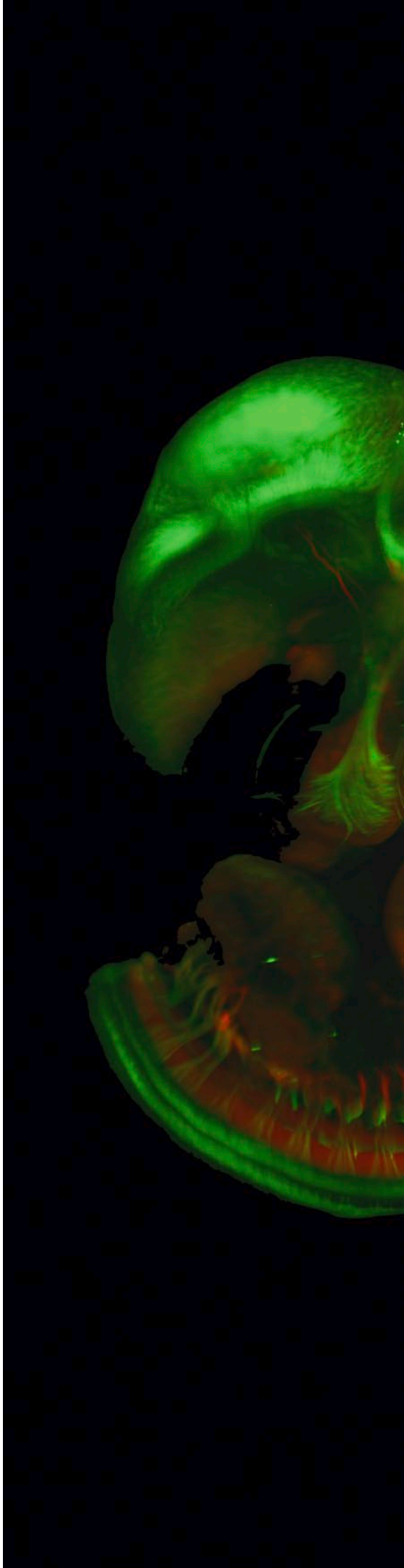
Adjust the six light sheets to optimally and homogeneously excite your sample and prevent light scattering, shadowing, refraction, and other artifacts.

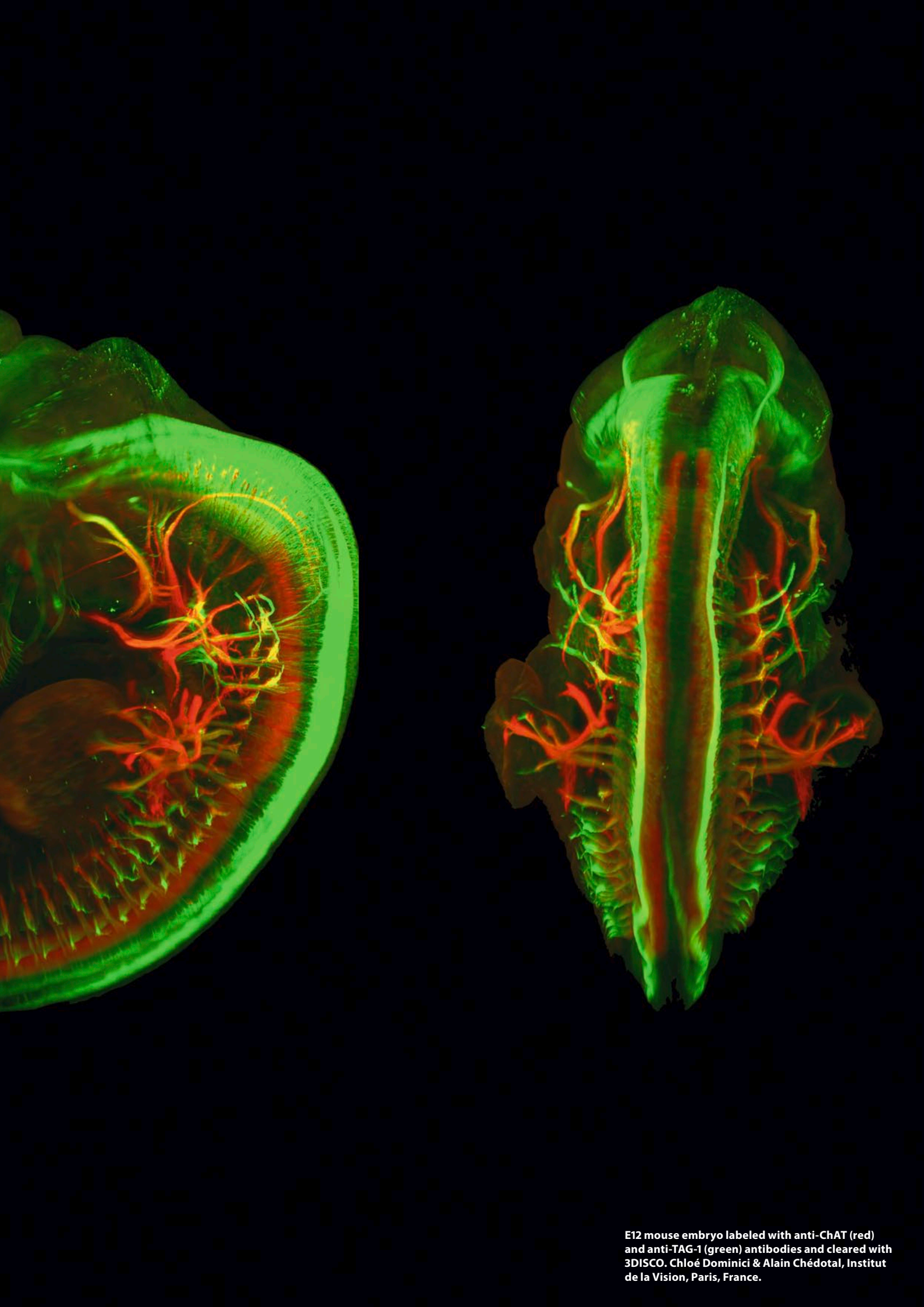
Flexible input

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 organism 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 within 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).

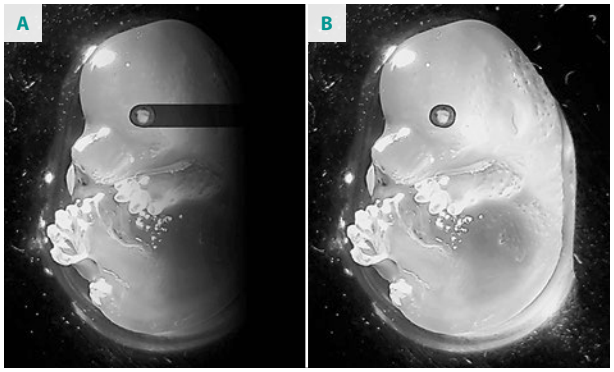


Figure 1: Unidirectional illumination (from the left) results in dark areas and stripes as parts of the sample are not exposed to the light sheet (A). The bidirectional, triple light sheet illumination of the UltraMicroscope II generates a clear, evenly illuminated image (B).

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 focusing. 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.

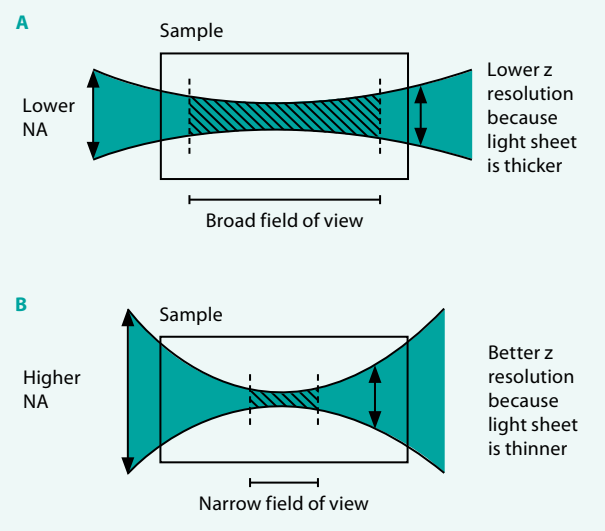


Figure 2: By adjusting the shape of the light sheets, illumination is tailored to sample size and imaging goals. A lower NA results in a broad field of view (A), and a higher NA results in a narrow field of view (B). While there is a tradeoff between the field of view, the thickness of the light sheet, 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 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 organic 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 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 unprecedented 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 compromising on 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.

Controls to optimize imaging results

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

The big picture in biology

A large, easy-to-access sample chamber can accommodate 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 magnification changer

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). They allow you to 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 chromatic 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 *in vivo* imaging. The *in vivo* sample cuvette is quickly mounted on the UltraMicroscope II and allows setting and maintaining of 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 removal for autoclaving.

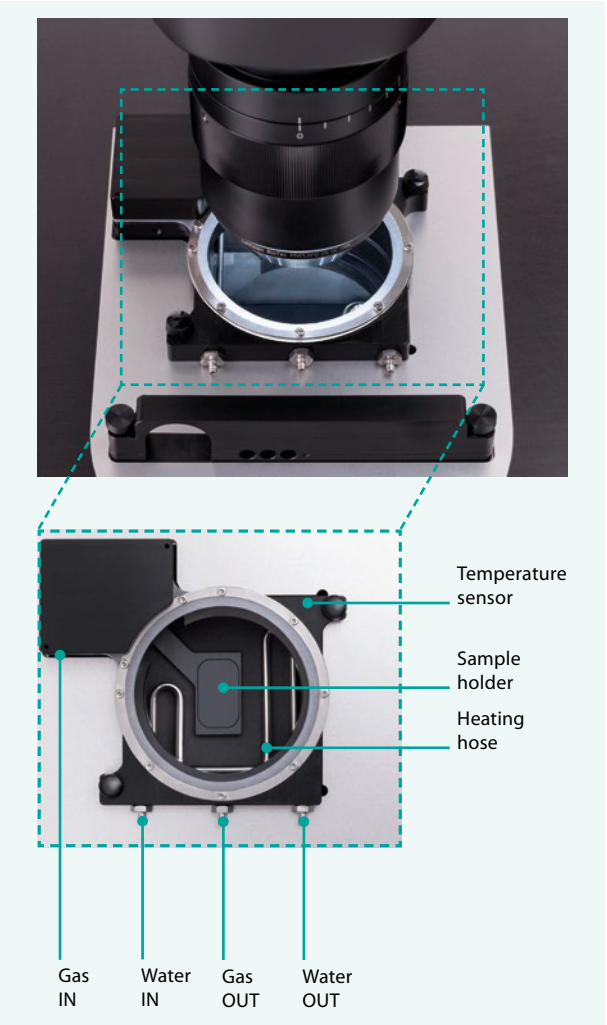


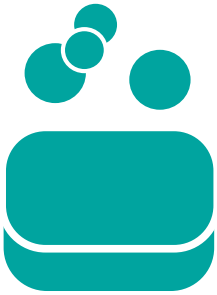
Figure 4: The *in vivo* sample chamber maintains constant temperature and CO_2/O_2 concentrations for functional and time-course imaging.

Three steps to a new view of biology

Visualizing the three-dimensional architecture of complex biological systems is convenient thanks to the UltraMicroscope II. To provide a complete, smooth, and hassle-free 3D imaging workflow, Miltenyi Biotec also offers solutions for sample staining and clearing. Antibodies specifically validated for 3D-immuno-fluorescence (IF) make time-consuming and costly validation processes obsolete. The MACS® Clearing Kit ensures fast and effective tissue clearing. And easy-to-follow protocols make this technology as easy as it gets, even if you are just about to start doing 3D imaging.

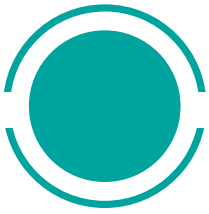
01 STAINING

Miltenyi Biotec's 3D-IF antibodies are specifically validated for whole-mount staining of large, cleared samples. For maximum reliability, ultimately producing conclusive results, these antibodies are functionally validated with the MACS Clearing Kit. Recombinantly engineered REAfinity™ Antibodies make for specific labeling and highly reproducible imaging data.



02 CLEARING

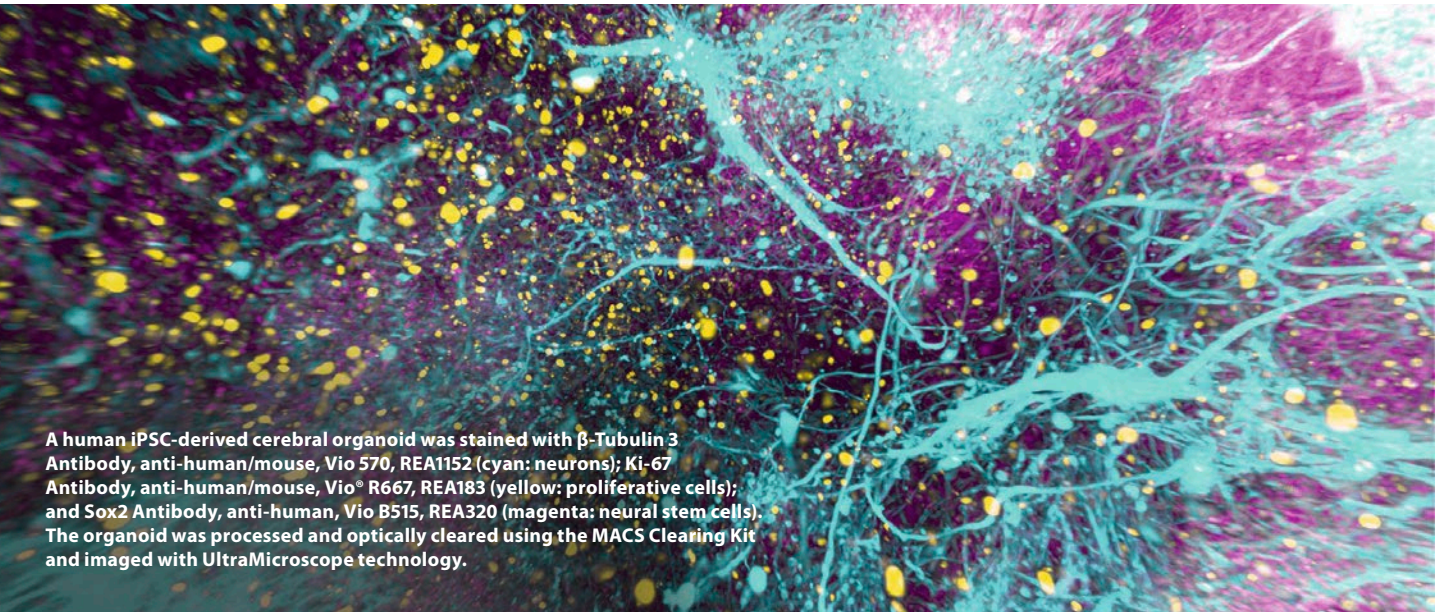
The MACS Clearing Kit provides a clearing process that is straightforward to use: fast, non-toxic, cost-effective, and easy. Clearing renders the optical properties of opaque organs transparent while keeping their structure intact. Following clearing, the sample is immersed in the non-toxic MACS Imaging Solution. Don't bother with toxic reagents in your 3D imaging workflow anymore.



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.

Antibodies validated for 3D imaging of cleared tissues



A human iPSC-derived cerebral organoid was stained with β-Tubulin 3 Antibody, anti-human/mouse, Vio 570, REA1152 (cyan: neurons); Ki-67 Antibody, anti-human/mouse, Vio® R667, REA183 (yellow: proliferative cells); and Sox2 Antibody, anti-human, Vio B515, REA320 (magenta: neural stem cells). The organoid was processed and optically cleared using the MACS Clearing Kit and imaged with UltraMicroscope technology.

REAfinity Recombinant Antibodies

ViO Dyes

LEARN MORE

Check out our portfolio of 3D-IF antibodies.

▸ **miltenyibiotec.com/3D-IF-antibodies**

Identifying appropriate antibodies to label structures of interest in large cleared samples is one of the most time-consuming steps in setting up the assays. Comprehensive screening and validation processes are needed to make sure that the antibodies give meaningful results. Miltenyi Biotec has already done this work for you: Recombinantly engineered REAfinity Antibodies are specifically validated and optimized for 3D-IF on tissues cleared with the MACS Clearing Kit.

- Validated and optimized for thorough whole-mount staining of large samples cleared with the MACS Clearing Kit
- Staining time decreased by 50% due to fluorochrome-conjugated primary antibodies
- Optimal signal-to-background ratios with primary antibodies conjugated to bright and photostable Vio® Dyes
- Recombinantly engineered for reproducible results and minimal background signals

Streamlined tissue 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 non-toxic organic solvent, providing the basis for the MACS Clearing Kit. This kit has been optimized for immunostaining with Miltenyi Biotec's 3D-IF antibodies for high-end imaging while completely avoiding toxic substances.

- Non-toxic, cost-effective, and easy: A clearing method that anyone can perform.
- Fast and efficient: One rapid clearing step that optimally clears samples and preserves tissue morphology.
- Versatile: Clears various whole organs, including mouse brain and tumor tissue.
- Sharp images: Non-toxic MACS Imaging Solution, matching the refractive index of the cleared tissue, allows aberration-free image acquisition.

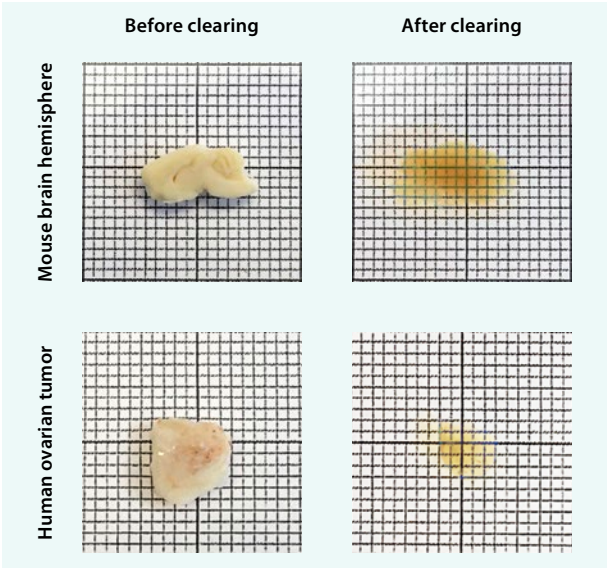
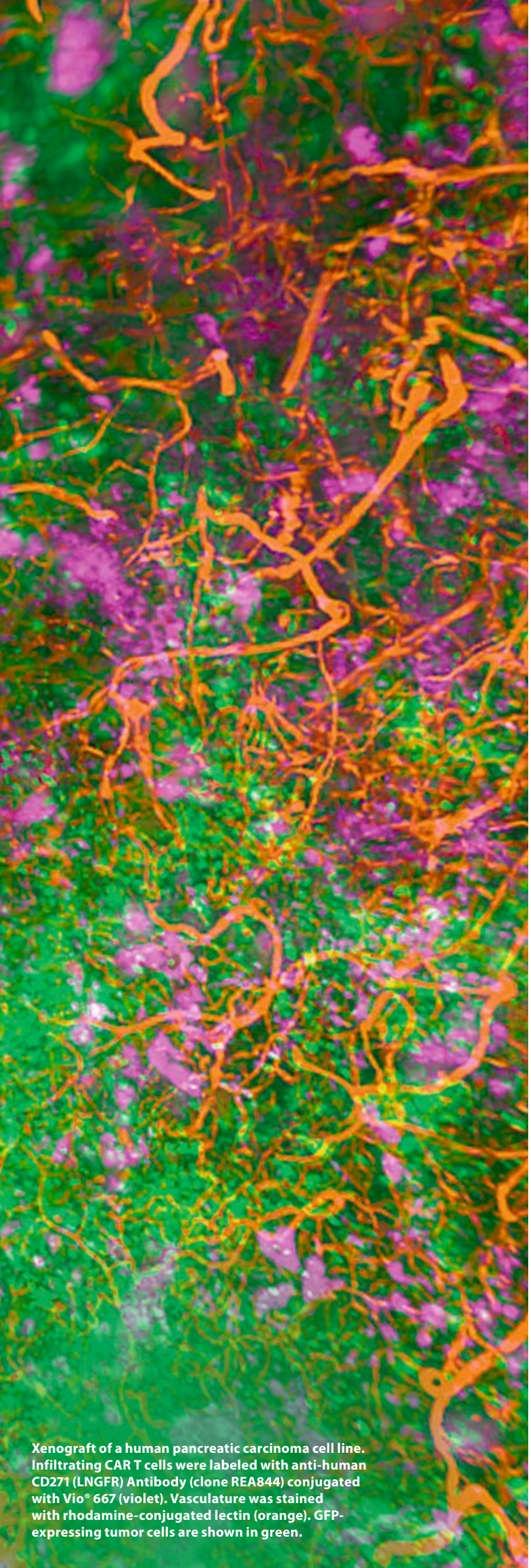


Figure 5: The MACS Clearing Kit enables effective clearing of a mouse brain hemisphere or human ovarian tumor within only six hours.

LEARN MORE

Browse our protocols to get started right away. You will find dedicated protocols for efficient clearing of samples like mouse brain hemispheres, human-derived xenograft tumors, and organoids.

▸ **miltenyibiotec.com/tissue-clearing-protocols**



Xenograft of a human pancreatic carcinoma cell line. Infiltrating CAR T cells were labeled with anti-human CD271 (LNGFR) Antibody (clone REA844) conjugated with Vio® 667 (violet). Vasculature was stained with rhodamine-conjugated lectin (orange). GFP-expressing tumor cells are shown in green.

Visualize and quantify CAR T cells in large solid tumors

UltraMicroscope technology has many applications in immuno-oncology, such as

- visualization of single disseminated cancer cells in whole animal models,
- drug target identification for cancer treatments in a whole mouse body,
- section-free 3D histological analysis.

3D microscopy and deep learning reveal the heterogeneity of crown-like structure microenvironments in intact adipose tissue.
Geng, J. *et al.* (2021) Sci. Adv. 7: eabe2480.

Identification and characterization of a non-conventional CD45 negative perivascular macrophage population within the mouse brain.
Siret, C. *et al.* (2021) Research Square: preprint. DOI: 10.21203/rs.3.rs-479980/v1

Cellular and molecular probing of intact human organs.
Zhao, S. *et al.* (2020) Cell 180, 1–17.

Deep learning reveals cancer metastasis and therapeutic antibody targeting in the entire body.
Pan, C. *et al.* (2019) Cell 179: 1661–1676.e19.

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.

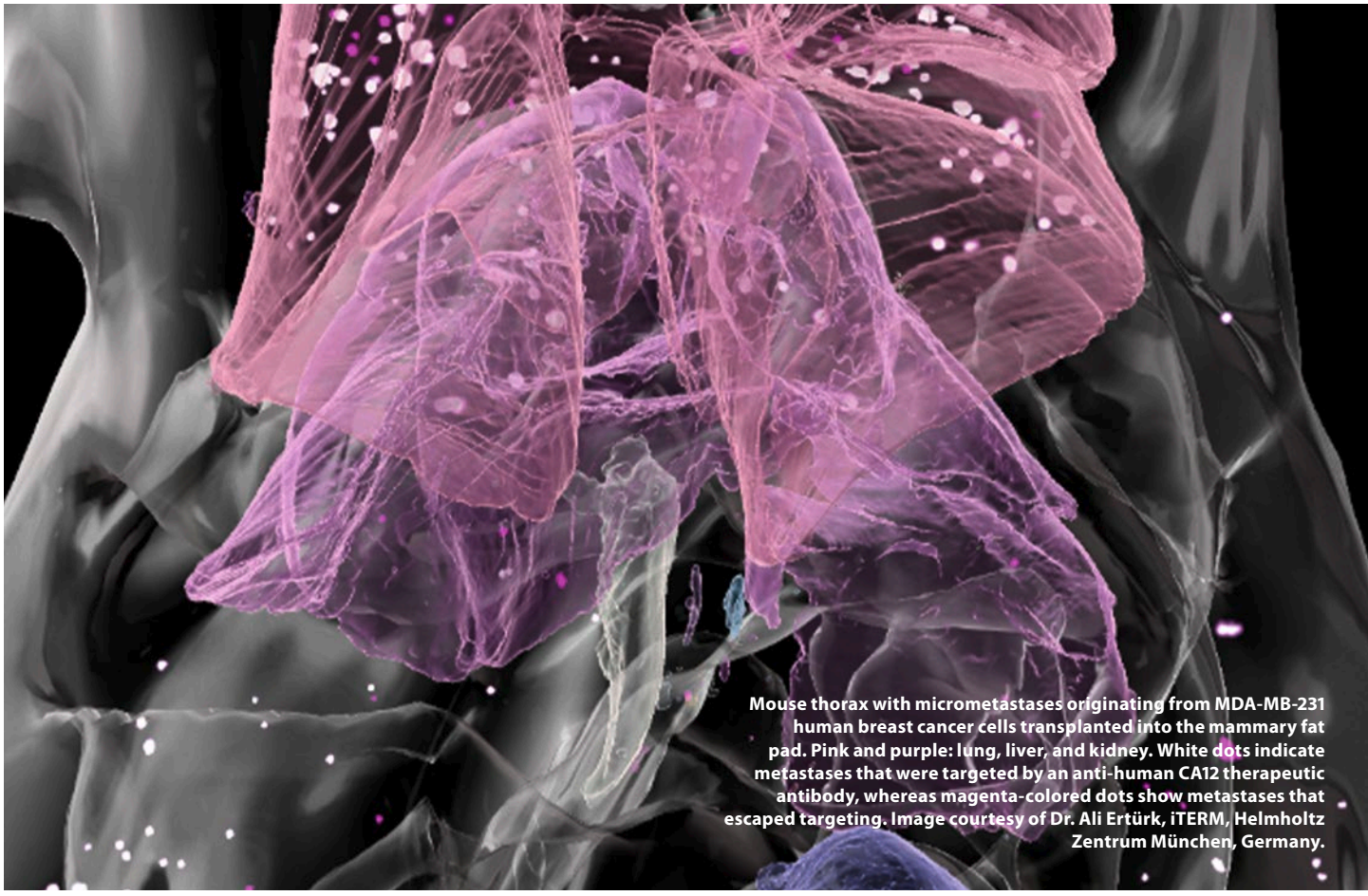
Correlated MRI and Ultramicroscopy (MR-UM) of brain tumors reveals vast heterogeneity of tumor infiltration and neoangiogenesis in preclinical models and human disease.
Breckwoldt, M.O. *et al.* (2019) Front. Neurosci. 12, 1004.

VIDEO

Watch our 3D rendering of a pancreatic carcinoma xenograft.

▸ **miltenyibiotec.com/pancreas-carcinoma-video**

Visualize micro-metastasis in an entire mouse body



Mouse thorax with micrometastases originating from MDA-MB-231 human breast cancer cells transplanted into the mammary fat pad. Pink and purple: lung, liver, and kidney. White dots indicate metastases that were targeted by an anti-human CA12 therapeutic antibody, whereas magenta-colored dots show metastases that escaped targeting. Image courtesy of Dr. Ali Ertürk, iTERM, Helmholtz Zentrum München, Germany.

LEARN MORE

Visit our webpage to learn how you can visualize single tumor cells in a whole mouse body.

▸ **miltenyibiotec.com/Blaze-oncology**

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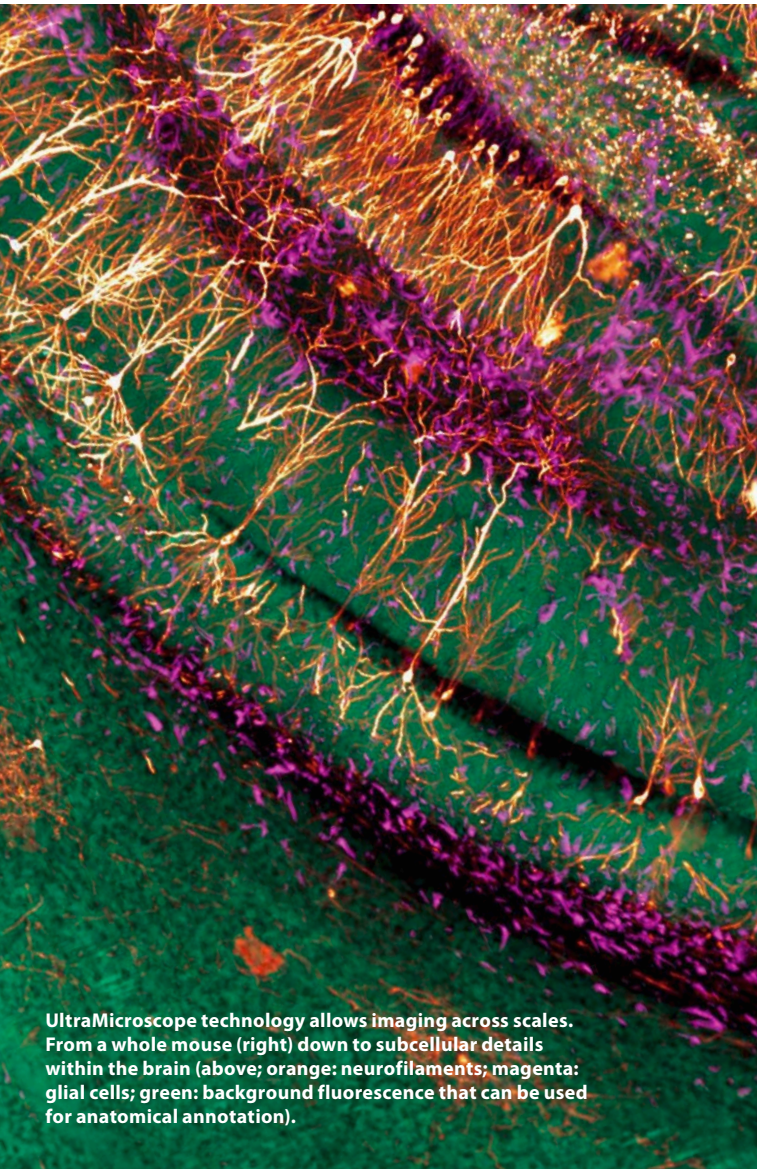
Download a list of selected references.

▸ **miltenyibiotec.com/UM-cancer-references**

3D neuroimaging across scales – from a whole mouse to single neurons

Understand the complex orchestration of neural circuits with whole-brain imaging at subcellular resolution. UltraMicroscope technology offers many applications in neuroscience, such as

- system-level identification of neuronal circuits in whole brains at subcellular resolution,
- 3D study of the pathology of Alzheimer's and Parkinson's diseases in whole brains in unprecedented detail,
- holistic visualization of affected areas in the central and peripheral nervous system after stroke and traumatic brain injury.



UltraMicroscope technology allows imaging across scales. From a whole mouse (right) down to subcellular details within the brain (above; orange: neurofilaments; magenta: glial cells; green: background fluorescence that can be used for anatomical annotation).

Microglia facilitate repair of demyelinated lesions via post-squalene sterol synthesis.
Berghoff, S.A. *et al.* (2021) Nat. Neurosci. 24: 47–60.

Ventral arkypallidal neurons inhibit accumbal firing to promote reward consumption.
Vachez, Y.M. *et al.* (2021) Nat. Neurosci. 24: 379–390.

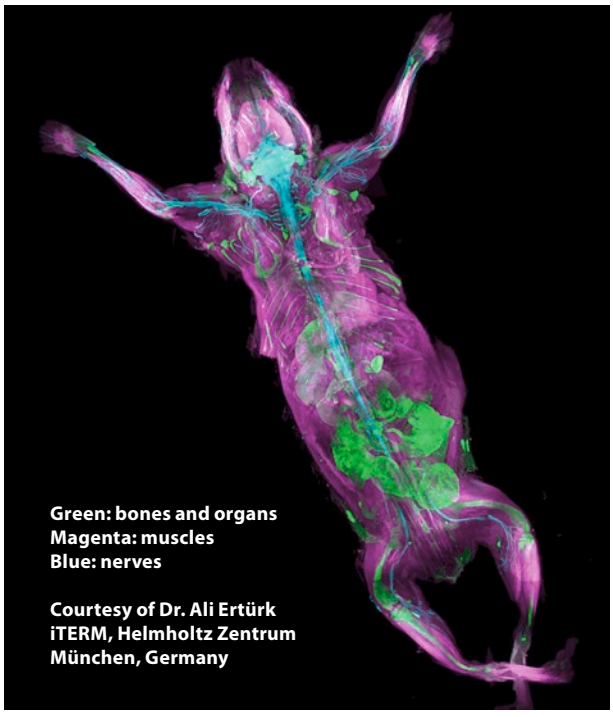
Mapping the fine-scale organization and plasticity of the brain vasculature.
Kirst, C. *et al.* (2020) Cell 180, 780–795.e25.

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.

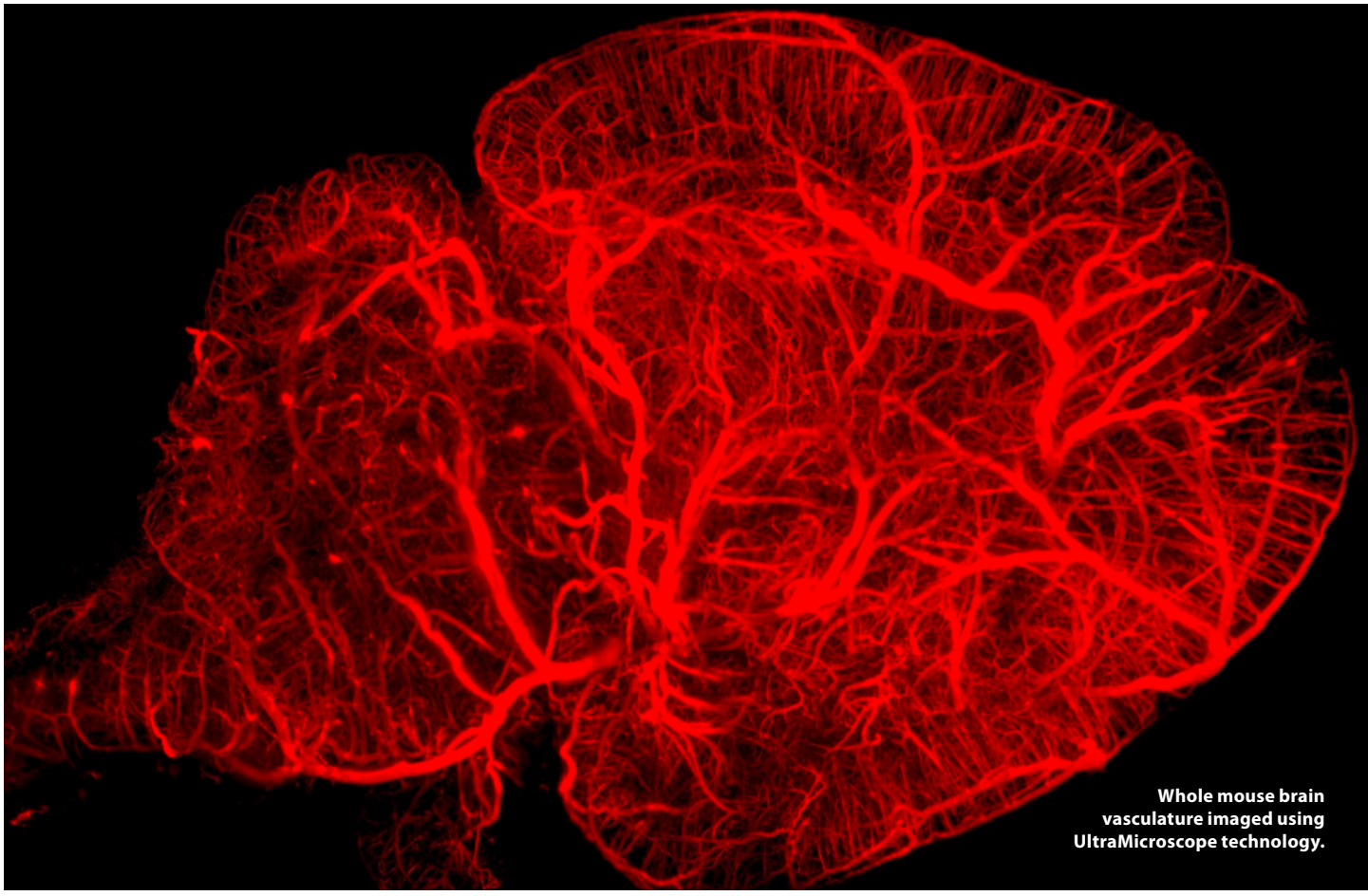
Mapping of brain activity by automated volume analysis of immediate early genes.
Renier, N. *et al.* (2016) Cell 165: 1789–1802.



Green: bones and organs
Magenta: muscles
Blue: nerves

Courtesy of Dr. Ali Ertürk
iTERM, Helmholtz Zentrum
München, Germany

Visualize an entire brain at subcellular resolution



Whole mouse brain vasculature imaged using UltraMicroscope technology.

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Download a list of selected references.

▸ miltenyibiotec.com/UM-neuroscience-references

Technical specifications

UltraMicroscope II specifications				
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			
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			
Camera specifications				
Detector	4.2 Megapixel sCMOS camera		5.5 Megapixel sCMOS camera	
Active pixels (w×h)	2048×2048		2560×2160	
Pixel size	6.5 µm × 6.5 µm		6.5 µm × 6.5 µm	
Sensor size	13.3 mm × 13.3 mm; 18.8 mm diagonal		16.6 mm × 14 mm; 21.8 mm diagonal	
Readout noise	0.8 med e^-		1 med e^-	
Maximal frame rates	100 fps		100 fps	
Maximum quantum efficiency	82%		60%	
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			
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.

▸ miltenyibiotec.com

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