



Miltenyi Biotec

The LaVision BioTec UltraMicroscope II

Fast 3D imaging of entire biological systems



**UNDERSTAND
NATURE'S
COMPLEXITY**

Exploring the architecture of biology

Light sheet microscopy allows for fast imaging of large 3D biological samples, like whole rodent organs, embryos or larvae. Compared to other current fluorescence microscopy techniques only a single plane of a labeled sample is illuminated perpendicular to the direction of detection. The use of this decoupled optical pathways allows for fast, true volume, in-depth imaging with very low photo-damage and bleaching effects. Based on this technique, the UltraMicroscope II opens new avenues to study entire biological systems and processes in physiologically representative 3D samples.

Entire biological systems

Explore the cellular details while keeping the overview of the entire biological system in 3D.

Optimized illumination

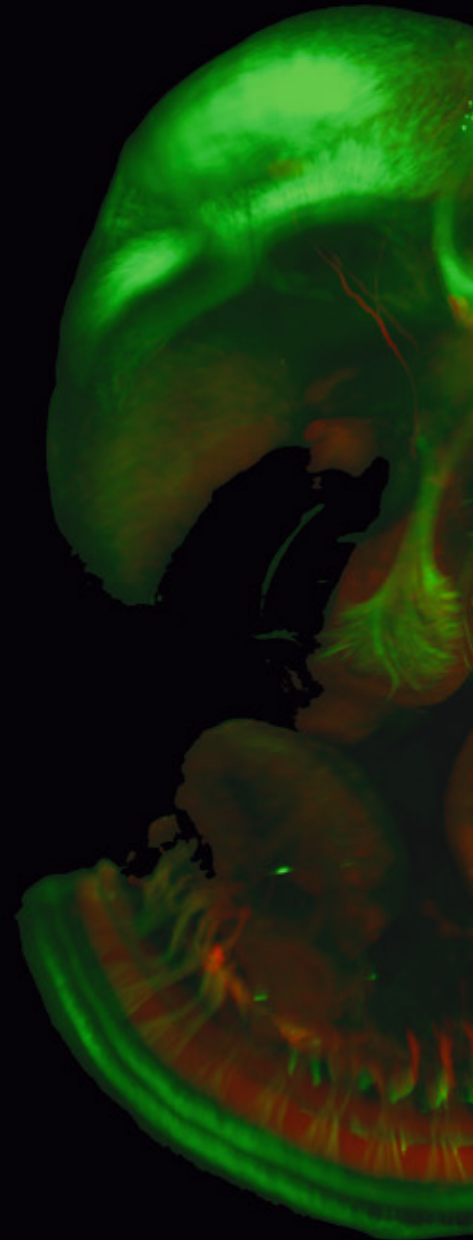
Take advantage from the most homogeneous fluorescence excitation to minimize artifacts like dark areas and stripes. Choose the ideal settings for your samples with the flexible light sheet technology.

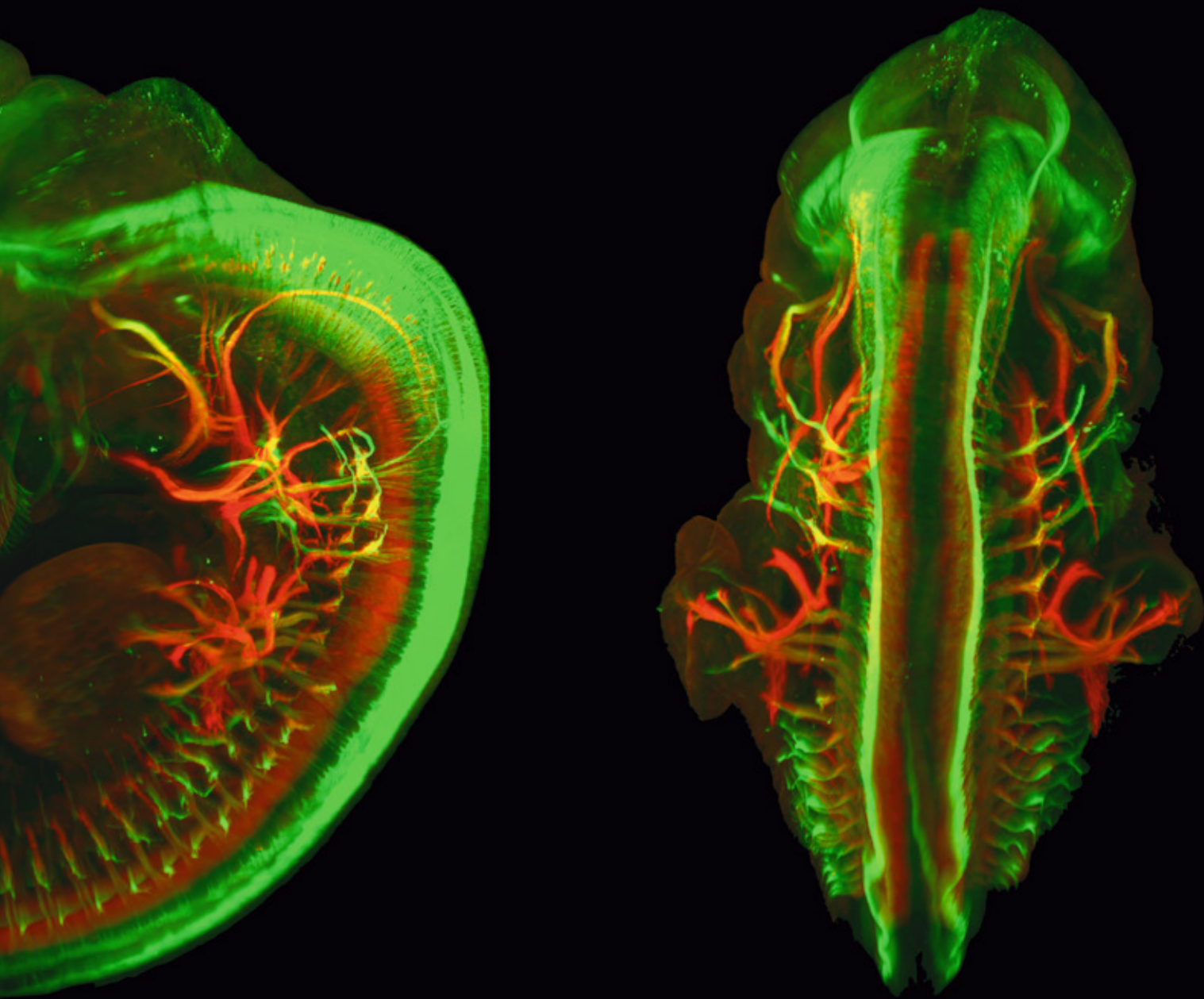
All clearing protocols

Apply all current clearing protocols and imaging solutions – from water to organic solvents.

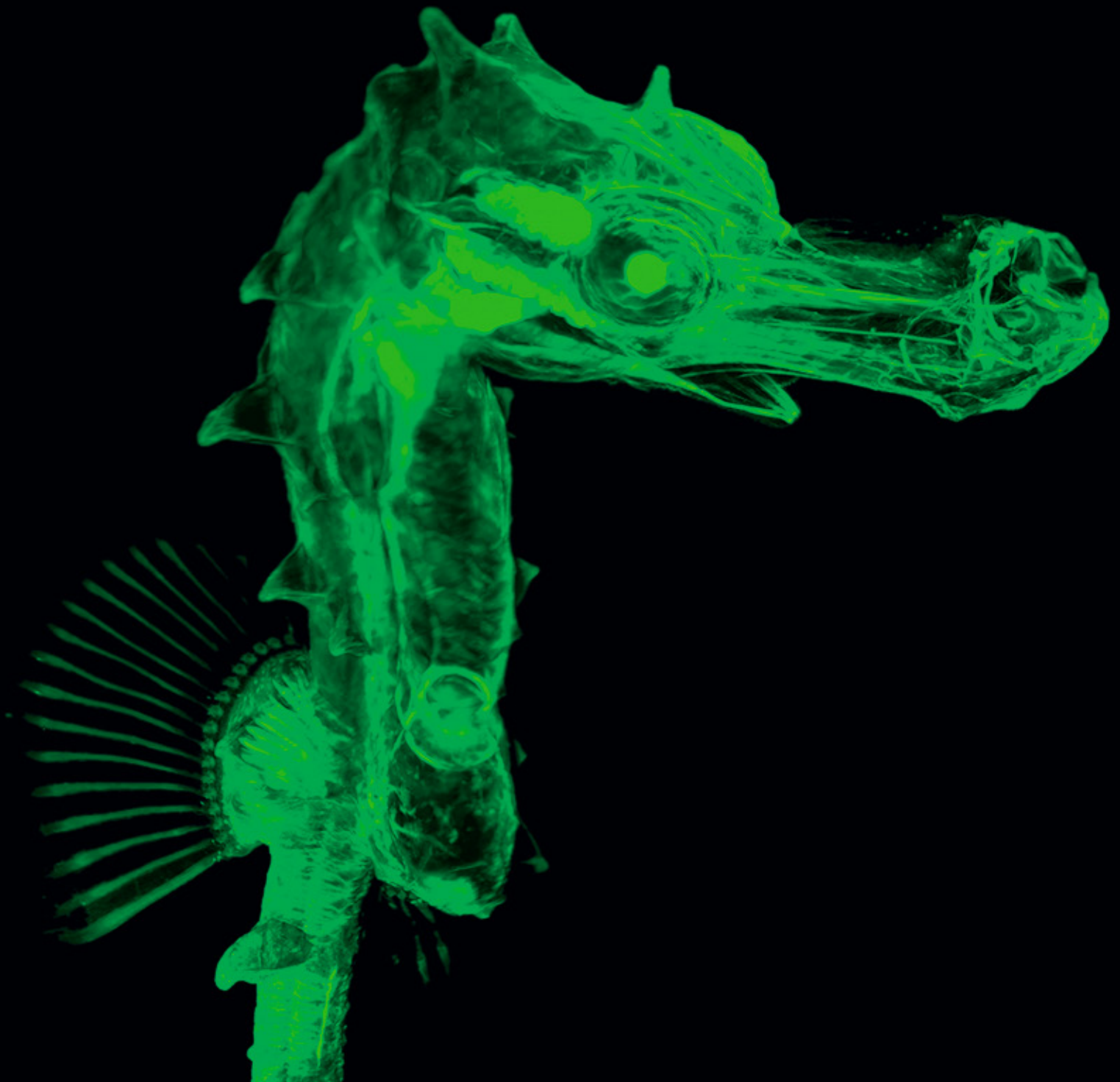
Flexible and easy

Short training time for new users and a low degree of staff support makes the UltraMicroscope II a perfect tool for multi-user environments like imaging facilities.





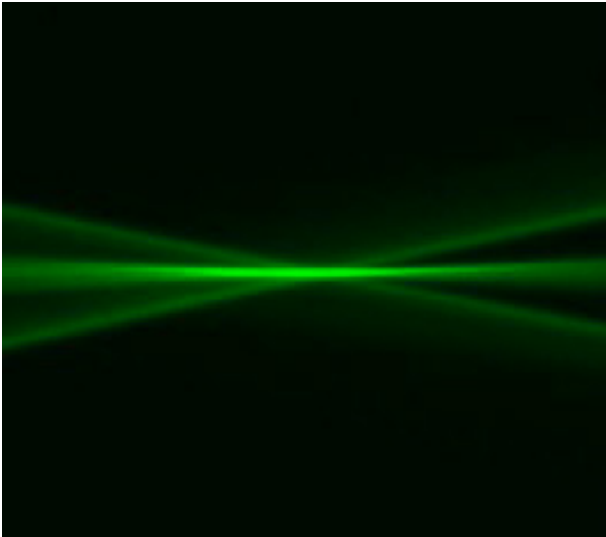
E12 mouse embryo labeled with anti-ChAT (red) and anti-TAG-1 (green) antibodies and cleared with 3DISCO. Chloé Dominici & Alain Chédotal, Institut de la Vision, Paris, France.



Large samples in 3D

The UltraMicroscope II serves to image a great variety of biological samples like whole rodent organs, embryos or larvae. With the vast sample chamber, the large field of view, and the long travel range it is designed to explore the cellular details in best resolution while keeping the overview of the complex 3D structures.

Optimized illumination



Flexible light sheet technology for optimal image quality

The light sheets of the UltraMicroscope II can easily be adjusted to suit different experimental requirements – simply choose the settings that perfectly match your sample via the software. High NA illumination is useful for imaging of small samples at a higher Z resolution. Low NA illumination supports a large field of view and is thus optimal for imaging of large samples. When images of high resolution across large areas are required, the dynamic horizontal focus is the best choice (fig. 2). The sample is kept in one place and the horizontal focus position of the light sheet is moved throughout the sample. Multiple images are acquired and then blended together to a final image just containing the best data.

Six light sheets for homogeneous illumination

The UltraMicroscope II comes with 2x3 light sheets that excite the sample from both sides under slightly different angles. This means the fluorescence excitation is most homogeneous and artifacts like dark areas and stripes are minimized (fig. 1).

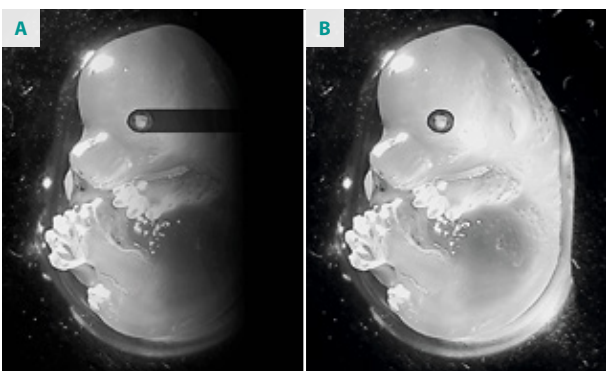


Figure 1: Illumination from the left side only (A). Bidirectional triple light sheet illumination (B).

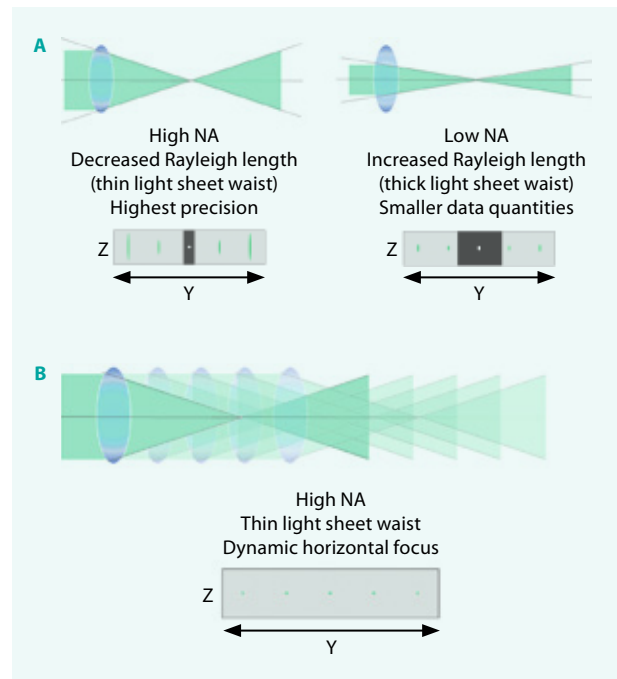


Figure 2: Trade-off between field of view, light sheet thickness and image quality (A). Dynamic horizontal focus (B).

All clearing protocols

Compatible with all current clearing solutions

Some samples like zebra fish are almost transparent by nature but the majority of samples are opaque counteracting all attempts to explore whole biological systems and processes. Tissue clearing methods modify the optical properties of large biological samples to render them transparent. Several methods have been published which can be divided into two large groups:

- Aqueous buffer-based protocols
- Organic solvent protocols

The compatibility of the UltraMicroscope II with organic solvents gives you access to some of the fastest and most effective clearing techniques. Did you know that clearing procedures like the DISCO series or CUBIC have been developed with the UltraMicroscope II?

Automatic adaption with the multi refractive index compensation

Running a system with different imaging solutions induces the necessity to correct for different refractive indices. With the refractive index compensation, the UltraMicroscope II can be adapted to clearing protocols. Utilized via the software interface the user chooses between current clearing techniques or water for *in vivo* imaging. This technology guarantees the perfect setting for every imaging solution – either water-based or solvent-based.

Name	Methodology	XFP	Clearing Time	RI	Publication
Solvent-based					
Methylsalicylat	Solvent	–	hours-days	1.55	Spalteholz, 1911
THF/DBE	Solvent	–	hours-days	1.56	Dotd <i>et al.</i> , 2012
3DISCO	Solvent	–	hours-days	1.56	Ertürk <i>et al.</i> , 2012
iDISCO	Solvent	–	hours-days	1.56	Renier <i>et al.</i> , 2014
iDISCO+	Solvent	–	hours-days	1.56	Renier <i>et al.</i> , 2016
uDISCO	Solvent	+	hours-days	1.55	Pan, Ertürk <i>et al.</i> , 2016
FluoClearBABB	Solvent	+	hours-days	1.55	Schwarz <i>et al.</i> , 2015
ECi	Solvent	+	hours-days	1.55	Klingberg <i>et al.</i> , 2016
PEGASOS	Solvent	+	hours-days	1.55	Jing <i>et al.</i> , 2018
MASH	Solvent	–	hours-days	1.56	Hildebrand <i>et al.</i> , preprint
vDISCO	Solvent	–	hours-days	1.56	Cai <i>et al.</i> 2019
Aqueous-based					
CLARITY	Hydrogel	+	2–6 weeks	1.45	Chung & Deisseroth, 2013
PACT	Hydrogel	+	1–2 weeks	1.38–1.48	Yang <i>et al.</i> , 2014
SWITCH	Hydrogel	–	1–4 weeks	1.47	Murray <i>et al.</i> , 2015
EDC-CLARITY	Hydrogel	+	2–4 weeks	1.45	Sylwestrak <i>et al.</i> , 2016
TDE	Immersion	–	days-weeks	1.52	Staudt <i>et al.</i> , 2007
ClearT2	Immersion	+	2–3 days	1.44	Kuwajima <i>et al.</i> , 2013
SeeDB2	Immersion	+	2 days	1.46/1.52	Ke <i>et al.</i> , 2016
CUBIC	Hyperhydration	+	1–2 weeks	1.38–1.48	Susaki <i>et al.</i> , 2014
ScaleS	Hyperhydration	+	some days	1.44	Hama <i>et al.</i> , 2015

Table 1: Overview on different clearing techniques classified by the methodology (XFP = Maintenance of fluorescent protein emission, RI = Refractive index).

Flexible and easy



Light sheet microscopy as simple as it can be

After 100 years of stagnancy, we brought light sheet microscopy to the next level. We listened to our customers and paid attention to their needs. Coming from a first concept, we developed an instrument that can be operated by anyone. We combine highest image quality with user friendliness to cover the request of today's demanding applications.

Easy operation

Short training time for new users and a low degree of staff support makes the UltraMicroscope II a perfect tool for imaging facilities and in a multi-user environment.

Comfortable sample swap

The big sample chamber allows an easy access and fast change of samples without dismantling the imaging chamber.

Zoom from overview to ROI

Changing the optical magnification by a factor of ten is done with our zoom body setup by turning a single knob. From largest overview of entire rodent organs to cellular resolution within a blink of the eye.

Intuitive software

Direct your imaging experiments easily towards best results with a supporting and intuitive software. The software wizard enables all required features for your data acquisition and takes care that the imaging process is set up correctly.

Zoom body setup

Optimal sensitivity and speed

Camera with a large field of view and high resolution without compromising read noise or frame rate.

Easy handling

The zoom body setup is ideal for multi-user environments. Simply set up the magnification just by turning the zoom knob.

Homogeneous illumination

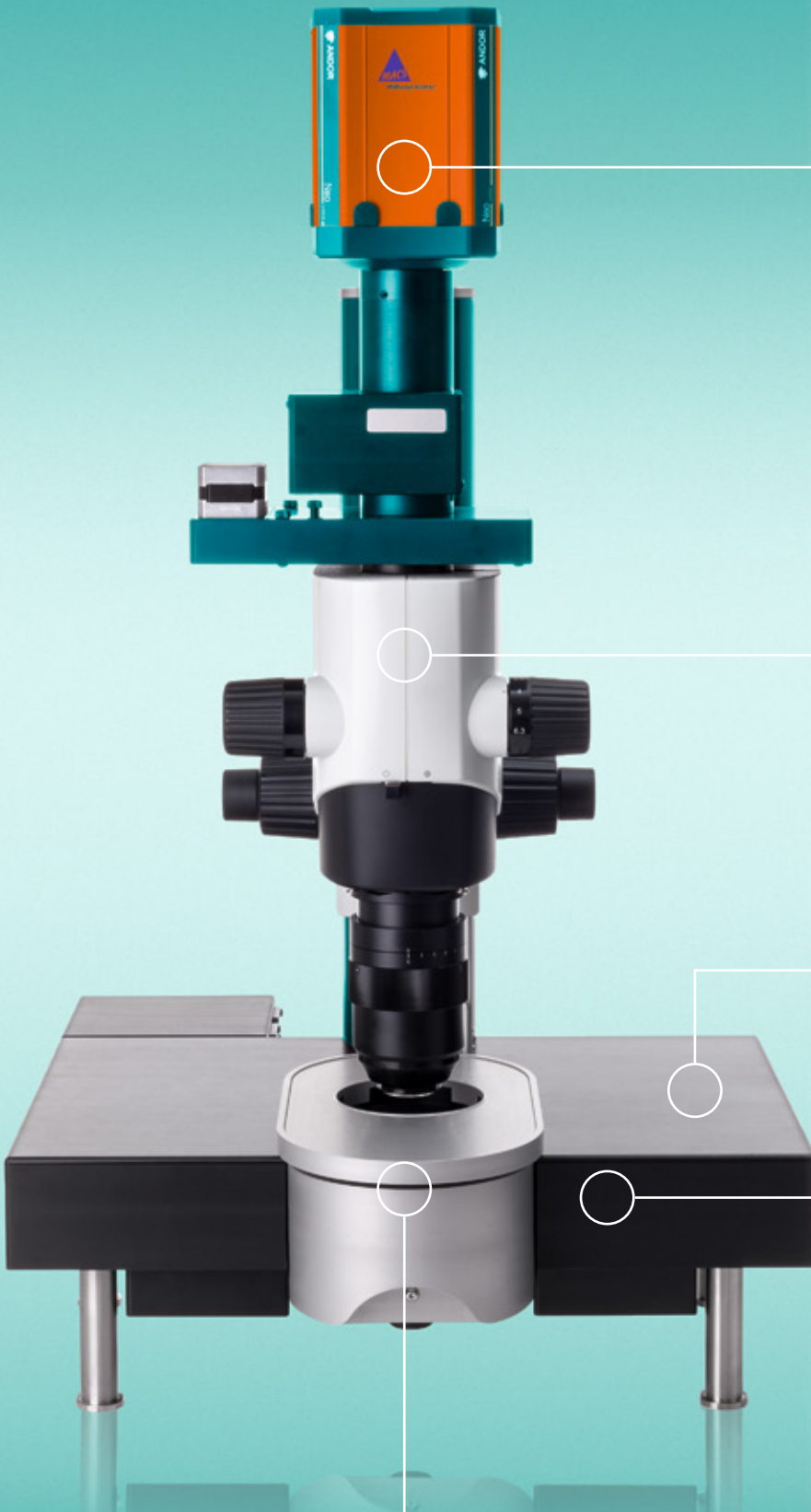
Illuminate your samples from two sides under slightly different angles with up to six light sheets.

Adjustable light sheets

Chose settings that perfectly match your samples.

Large samples

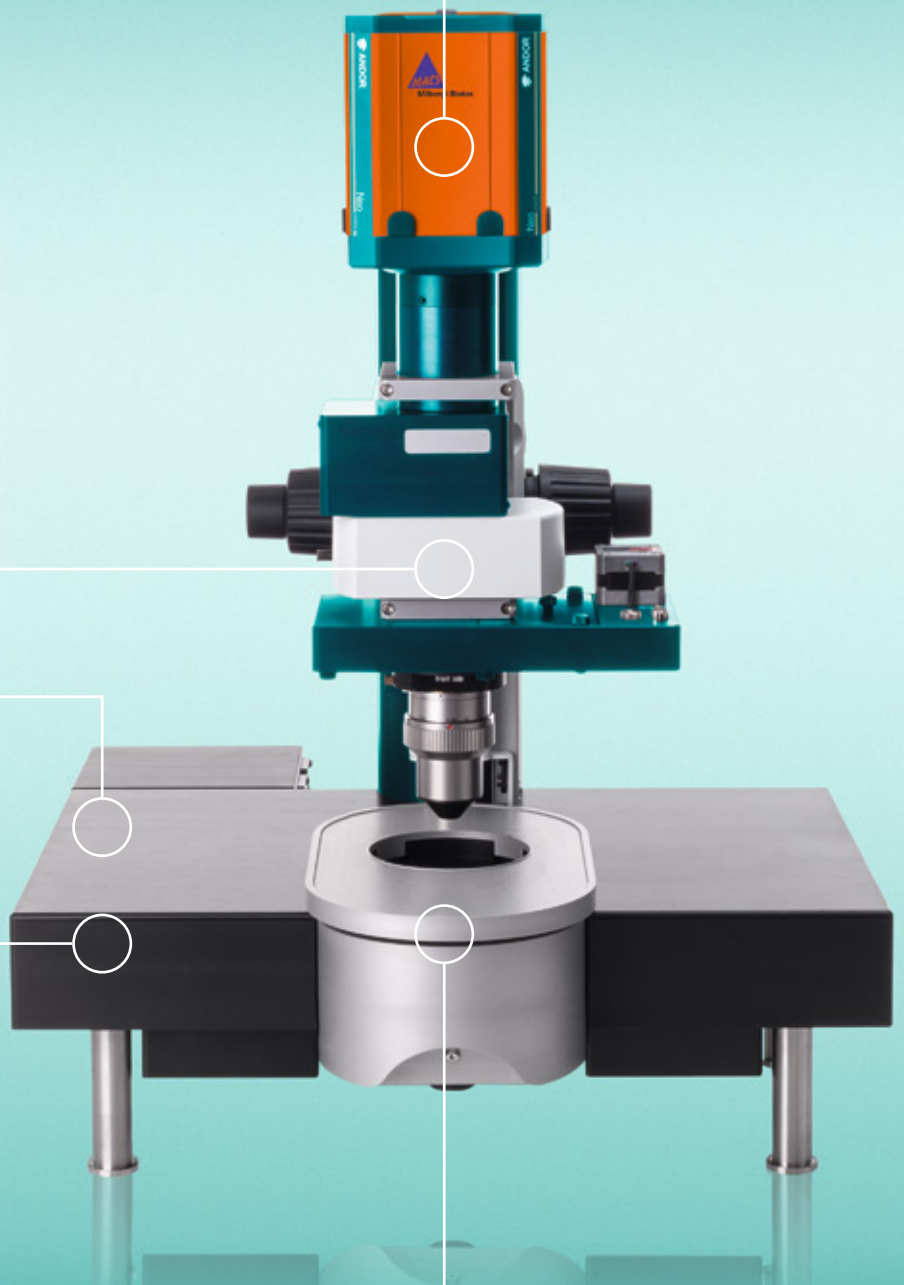
Examine entire rodent organs, rodent tumors, mouse embryos or biopsies from large animals.

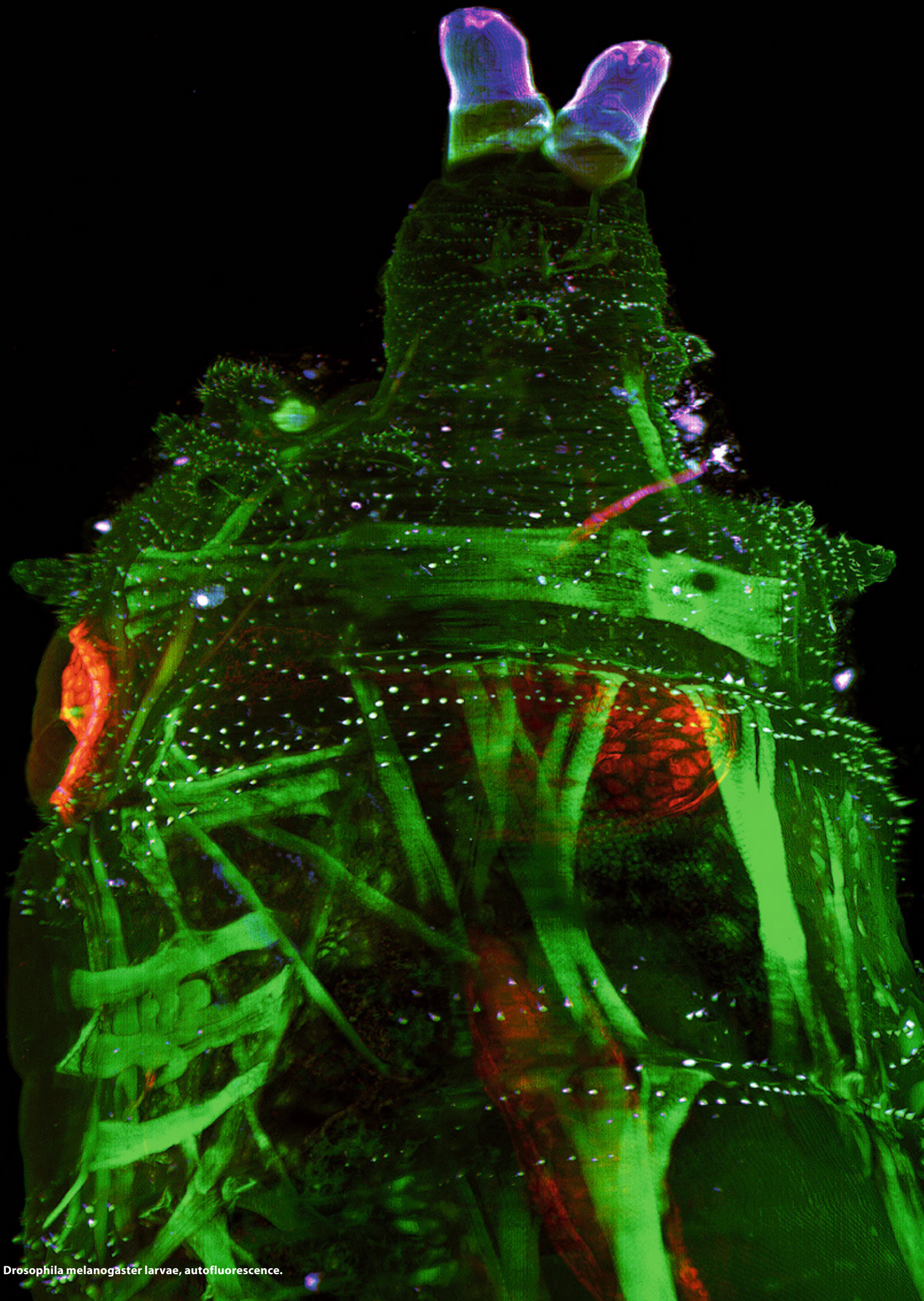


Infinity corrected optics setup

Superior imaging capabilities

The infinity corrected optics setup allows the implementation of all infinity corrected objective lenses like those of the MI PLAN series, specially developed for light sheet microscopy and covering all imaging solutions.



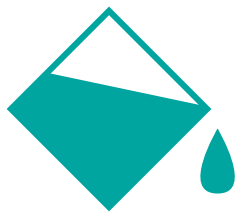


Drosophila melanogaster larvae, autofluorescence.

Light sheet microscopy in three steps

The UltraMicroscope II enables high-speed imaging of fragile specimens, fast biological processes, and large cleared samples. Achieving realistic three-dimensional images cannot depend alone on the imaging system. Large samples require a special preparation in which

they are rendered transparent to prevent light scattering within the sample. Miltenyi Biotec covers the whole workflow by offering a complete solution from validated antibodies and antibody-fluorochrome conjugates including a broad range of reagents.



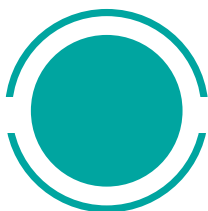
01 STAINING

Proteins, structures, and biological processes are (immuno) labeled with fluorescent dyes, proteins or conjugated antibodies.



02 CLEARING

Tissue clearing methods based on organic solvents or aqueous buffers render large biological samples transparent while keeping the internal three-dimensional structure.



03 IMAGING

The fluorescent sample is excited perpendicular to the detection pathway by a focused light sheet. 3D image stacks are generated by moving the sample through the light sheet. As only the actually observed section is illuminated, photodamage and fluorophore bleaching are kept at a minimum.

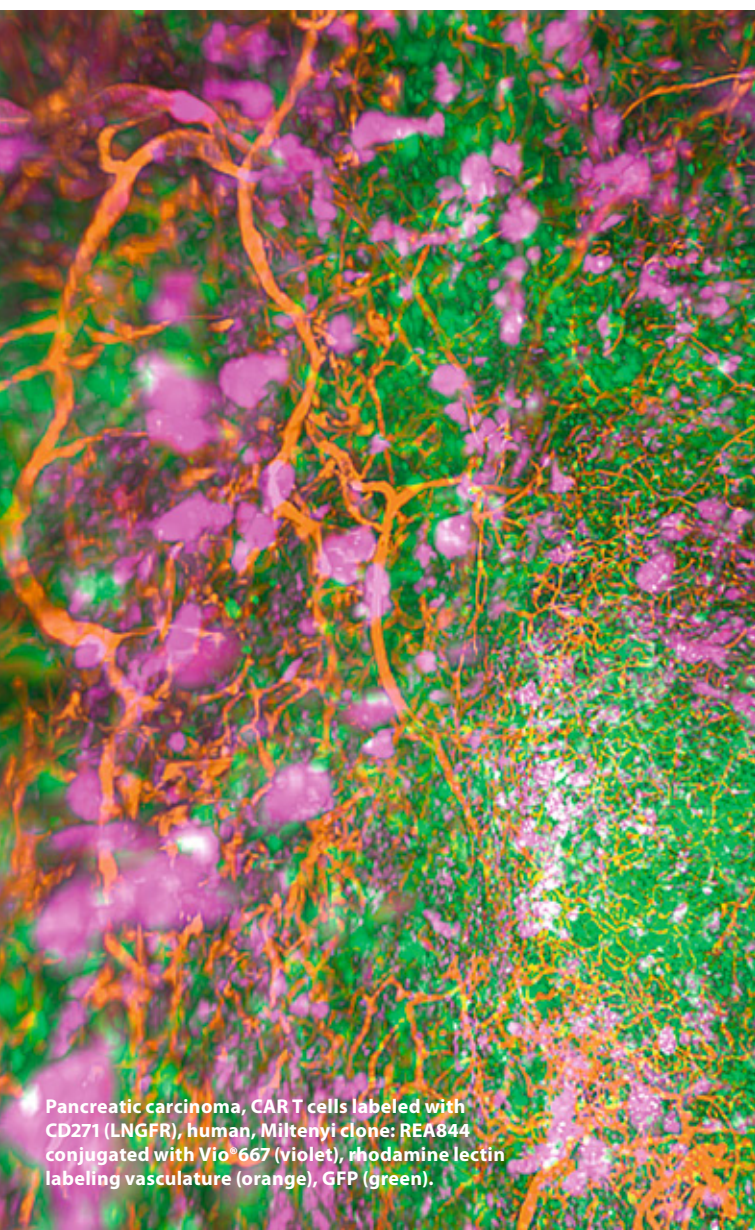
Staining and clearing solutions

Sample staining

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

The recombinant nature of these antibodies ensures high experimental reproducibility. Their unique design to show no background signal and availability as directly conjugated primary antibodies provides a perfect balance between signal strength and sensitivity for imaging all biological samples.

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



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

Sample clearing

Current protocols for tissue clearing involve laborious steps using often toxic reagents in order 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.

This protocol has been optimized to clear various tissues including bone while it completely avoids the use of toxic substances. At the same time, it preserves the endogenous fluorescence and antibody labeling for high-end imaging.

- New clearing process that is non-toxic, cost-effective, and easy to use for untrained personnel
- Simple protocol with one fast clearing step that allows for optimal clearing results and preservation of endogenous fluorescence
- Efficient clearing process for whole organ clearing, including whole brain and tumor tissues

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

The complete package for every application

Objective lenses for optimized image quality

The apochromatic planar multi-immersion objective lenses of the MI PLAN series were specially developed for the infinity corrected optics setup of the UltraMicroscope II. They are not only resistant against common media used for tissue clearing but also tailored to their optical characteristics covering refractive indices from 1.33 (water) to 1.57 (organic solvents). The long working distances are ideally suited for large cleared samples. The flat-field-correction guarantees a flat focal plane matching the optical axis of a light sheet.



Figure 3: 12× NA 0.53 MI PLAN objective lens.

In vivo imaging

The UltraMicroscope II can be equipped with an incubation cuvette to ensure optimal environmental conditions. The temperature of the immersion medium as well as the atmosphere (CO_2/O_2) between medium and objective lens can be regulated using an environmental control system. All settings are controlled by a touchscreen. The heating element and the sample holder can be easily unmounted for autoclaving.

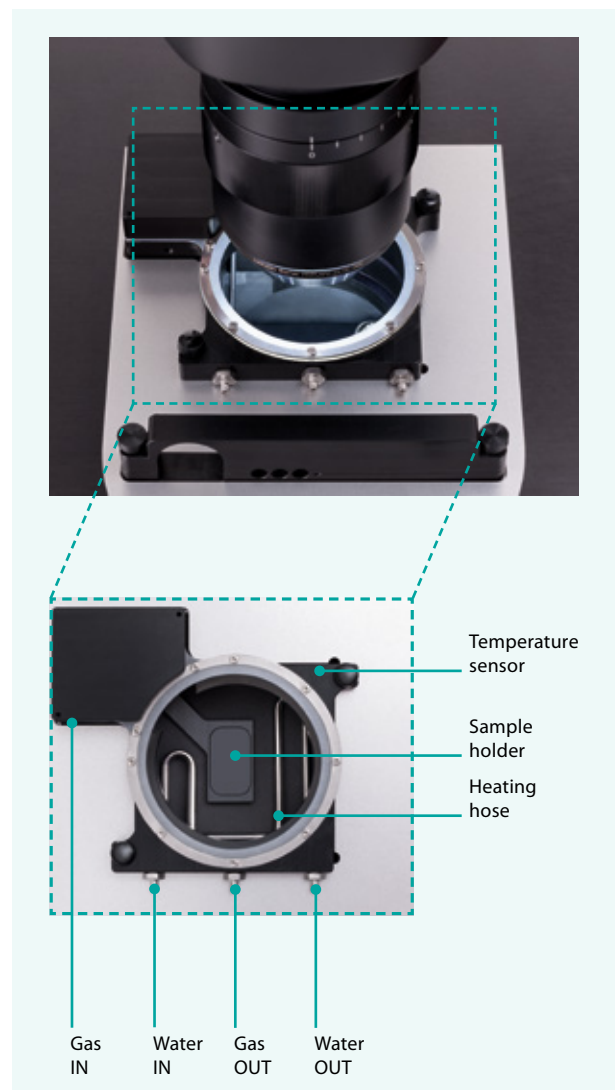


Figure 4: In vivo imaging setup of the UltraMicroscope II.

Applications

The potential applications of light sheet microscopy are limitless. The UltraMicroscope II features applications ranging from *in vivo* imaging to cleared samples

independent of the size of the sample and their labeling.

Mouse tumor, xenograft vessel staining,
Feuchtinger, Helmholtz Zentrum Munich, Germany.

Oncology

Verification of the efficiency of neovascularization inhibitors.

Apoptosis imaging for monitoring DR5 antibody accumulation and pharmacodynamics in brain tumors noninvasively.

Weber, T.G., Osl, F., Renner, A., Pöschinger, T., Galbán, S., Rehemtulla, A., Scheuer, W.
Cancer Res. (2014) 74: 1913–1923.

Tumor uptake of anti-CD20 fabs depends on tumor perfusion.

Mendler, C.T., Feuchtinger, A., Heid, I., Aichler, M., D'Alessandria, C., Pirsig, S., Blechert, B., Wester, H.J., Braren, R., Walch, A., Skerra, A., Schwaiger, A.
J. Nuc. Med. (2016) 57: 1971–1977.

Deep learning reveals cancer metastasis and therapeutic antibody targeting in whole body.

Pan, C., Schoppe, O., Parra-Damas, A., Cai, R., Todorov,

M.I., Gondi, G., Neubeck, v.B., Ghasemi, A., Reimer, M.A., Coronel, J., Garvalov, B.K., Menze, B., Zeidler, R., Erturk, A.

bioRxiv (2019). <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., Bode, J., Sahm, F., Krüwel, T., Solecki, G., Hahn, A., Wirthschaft, P., Berghoff, A.S., Haas, M., Venkataramani, V., Deimling, v.A., Wick, W., Herold-Mende, C., Heiland, S., Platten, M., Bendzus, M., Kurz, F.T., Winkler, F., Tews, B.

Front. Neurosci. (2019) 12: 1004.

Mouse testicles, Cd31, Simon Merz, Institute for Experimental Immunology and Imaging, Essen, Germany.



Pathology

Imaging of different developmental stages of animal models for phenotyping or characterization.

CUBIC pathology: three-dimensional imaging for pathological diagnosis.

Nojima, S., Susaki, E.A., Yoshida, K., Takemoto, H., Tsujimura, N., Iijima, S., Takachi, K., Nakahara, Y., Tahara, S., Ohshima, K., Kurashige, M., Hori, Y., Wada, N., Ikeda, J.I., Kumanogoh, A., Morii, E., Ueda, H.R. *Sci. Rep.* (2017) 7: 9269.

VIPAR, a quantitative approach to 3D histopathology applied to lymphatic malformations.

Hägerling, R., Drees, D., Scherzinger, A., Dierkes, C., Martin-Almedina, S., Butz, S., Gordon, K., Schäfers, M., Hinrichs, K., Ostergaard, P., Vestweber, D., Goerge, T., Mansour, S., Jiang, X., Mortimer, P.S., Kiefer, F. *JCI Insight.* (2017) 2: e93424.

Scalable cytoarchitectonic characterization of large intact human neocortex samples.

Hildebrand, S., Schueth, A., Herrler, A., Galuske, R., Roebroek, A. *bioRxiv* (2018). <https://doi.org/10.1101/274985>

Immunolabeling of cleared human pancreata provides insights into three-dimensional pancreatic anatomy and pathology.

Noë, M., Rezaee, N., Asrani, K., Skaro, M., Groot, V.P., Wu, P-H., Olson, M.T., Hong, S-M., Kim, S.J., Weiss, M.J., Wolfgang, C.L., Makary, M.A., He, J., Cameron, J.L., Wirtz, D., Roberts, N.J., Offerhaus, G.J.A., Brosens, L.A.A., Wood, L.D., Hruban, R.H. *Am. J. Path.* (2018) 188:1530–1535.

Mouse cortex

Neuroscience

Regeneration potential of neurons and axonal path finding.

GABAergic inhibition in dual-transmission cholinergic and GABAergic striatal interneurons is abolished in Parkinson disease.

Lozovaya, N., Eftekhari, S., Cloarec, R., Gouty-Colomer, L.A., Dufour, A., Riffault, B., Billon-Grand, M., Pons-Bennaceur, A., Oumar, N., Burnashev, N., Ben-Ari, Y., Hammond C.
Nat. Commun. (2018) 9: 1422.

Three-dimensional study of Alzheimer's disease hallmarks using the iDISCO clearing method.

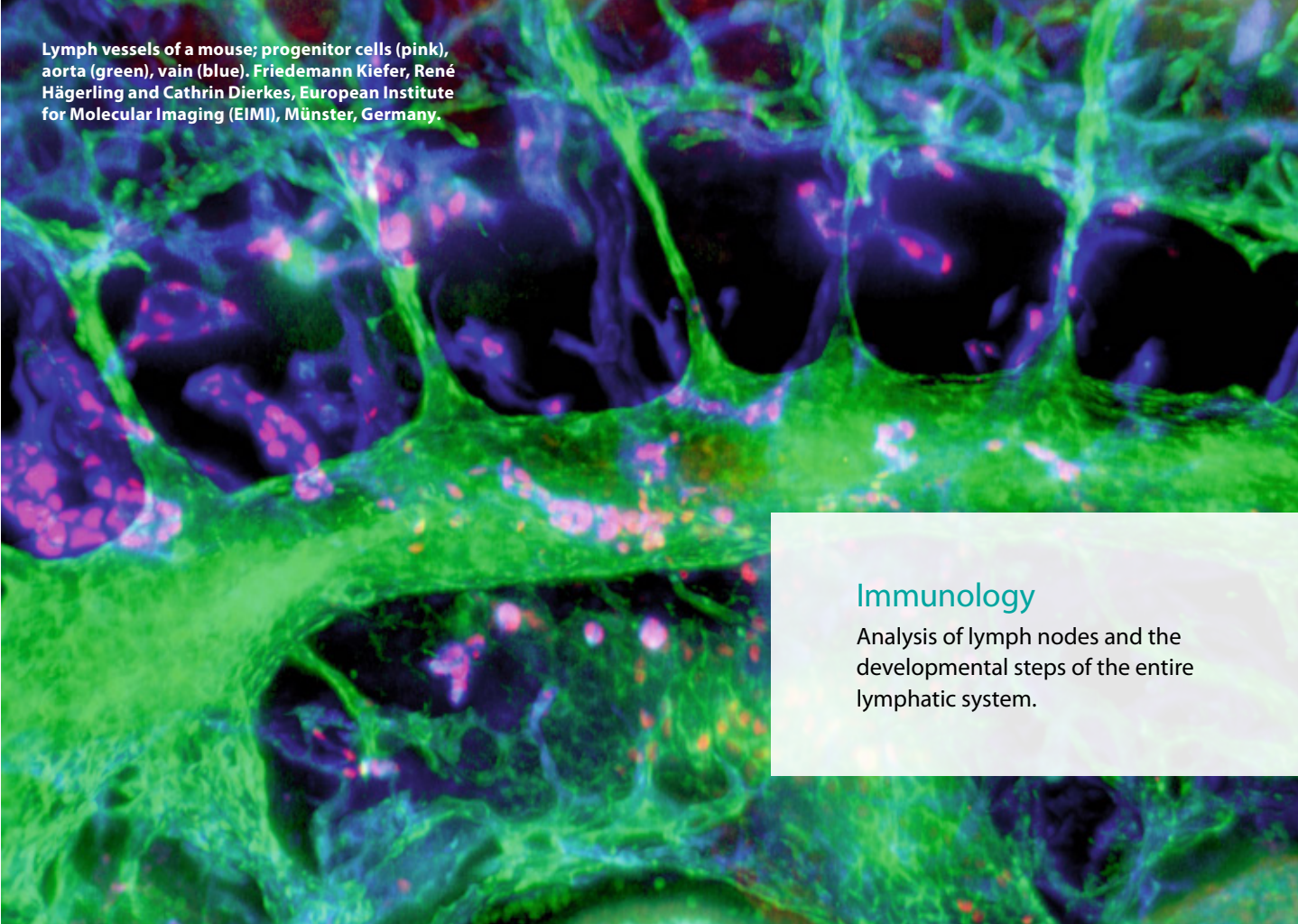
Liebmann, T., Renier, N., Bettayeb, K., Greengard, P., Tessier-Lavigne, M., Flajolet, M.
Cell Rep. (2016) 6: 1138–1152.

Mapping of brain activity by automated volume analysis of immediate early genes.

Renier, N., Adams, E.L., Kirst, C., Wu, Z., Azevedo, R., Kohl, J., Autry, A.E., Kadiri, L., Umadevi Venkataraju, K., Zhou, Y., Wang, V.X., Tang, C.Y., Olsen, O., Dulac, C., Osten, P., Tessier-Lavigne, M.
Cell (2016) 165: 1789–1802.

Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis.

Susaki, E.A., Tainaka, K., Perrin Kishino, F., Tawara, T., Watanabe, T.M., Yokoyama, C., Onoe, H., Eguchi, M., Yamaguchi, S., Abe, T., Kiyonari, H., Shimizu, Y., Miyawaki, A., Yokota, H., Ueda, H.R.
Cell (2014) 157: 726–739.



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

Immunology

Analysis of lymph nodes and the developmental steps of the entire lymphatic system.

A novel multistep mechanism for initial lymphangiogenesis in mouse embryos based on ultramicroscopy.

Hägerling, R., Pollmann, C., Andreas, M., Schmidt, C., Nurmi, H., Adams, R.H., Alitalo, K., Andresen, V., Schulte-Merker, S., Kiefer, F.
EMBO J. (2013) 32: 629–644.

A network of trans-cortical capillaries as mainstay for blood circulation in long bones.

Grüneboom, A., Hawwari, I., Weidner, D., Culemann, S., Müller, S., Henneberg, S., Brenzel, A., Merz, S., Bornemann, L., Zec, K., Wuelling, M., Kling, L., Hasenberg, M., Voortmann, S., Lang, S., Baum, W., Ohs, A., Kraff, O., Quick, H.H., Jäger, M., Landgraaber, S.,

Dudda, M., Danuser, R., Stein, J.V., Rohde, M., Gelse, K., Garbe, A.I., Adamczyk, A., Westendorf, A.M., Hoffmann, D., Christiansen, S., Engel, D.R., Vortkamp, A., Krönke, G., Herrmann, M., Kamradt, T., Schett, G., Hasenberg, A., Gunzer, M.
Nature Metabolism (2019) 1: 236–250.

Matrix stiffness controls lymphatic vessel formation through regulation of a GATA2-dependent transcriptional program.

Frye, M., Taddei, A., Dierkes, C., Martinez-Corral, I., Fielden, M., Ortsäter, H., Kazenwadel, J., Calado, D.P., Ostergaard, P., Salminen, M., He, L., Harvey, N.L., Kiefer, F., Mäkinen, T.
Nat. Commun. (2018) 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

Zoom microscope

Zoom	Mono zoom
Zoom ratio	0.63 \times –6.3 \times (1:10)

Zoom body setup

Detection optics

	Zoom body setup	Infinity corrected optics setup		
Objective lenses	2 \times	1.1 \times	4 \times	12 \times
Numerical aperture	0.5	0.1	0.3	0.53
FOV diagonal (5.5 Megapixel camera)	1.7 mm–17.6 mm	19.7 mm	5.4 mm	1.8 mm
Total magnification (objective lens + zoom ratio)	1.26 \times –12.6 \times	(w/o zoom)	(w/o zoom)	(w/o zoom)
Working distance	5.6 mm (corrected), 10 mm	16 mm, 17 mm	5.6 mm	8.5 mm, 10 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 \times 74 mm \times 35 mm

Light source

Laser module	Max. 5 laser lines, 50 mW–100 mW per diode
Supercontinuum laser	Emission 460 nm–800 nm, 1 mW/nm–3 mW/nm

General information

	Zoom body setup	Infinity corrected optics setup
Dimensions (w \times h \times d)	54 cm \times 82 cm \times 65 cm	54 cm \times 73 cm \times 65 cm
Weight	47 kg (w/o controller and laser)	

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 joining forces

To complement expertise in the area of cell analysis

The high-end microscopy specialist LaVision BioTec GmbH is now part of the Miltenyi Biotec family.

By combining our expertise in cell analysis and innovative detection reagents with LaVision BioTec's imaging know-how, we will develop pioneering analytical tools for biomedical research. Our aim is to offer fully scalable workflow solutions to our customers, allowing the analysis at all levels, starting at an organ or full-tumor level, going a step down to the tissue level and even further into detail on a single-cell level. This allows a much more comprehensive view on disease mechanisms and cellular treatments.

Located in Bielefeld, Germany, LaVision BioTec is a leading global specialist for advanced light sheet and multiphoton microscopy solutions for life science applications. Since its foundation in 2000, LaVision BioTec has developed innovative products that successfully address the high-end microscopy market for applications in oncology, pathology, neuroscience, and immunology. With its UltraMicroscopes, LaVision BioTec launched the first commercial light sheet microscopes, allowing imaging of large cleared tissue samples.



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