LaVision BioTec

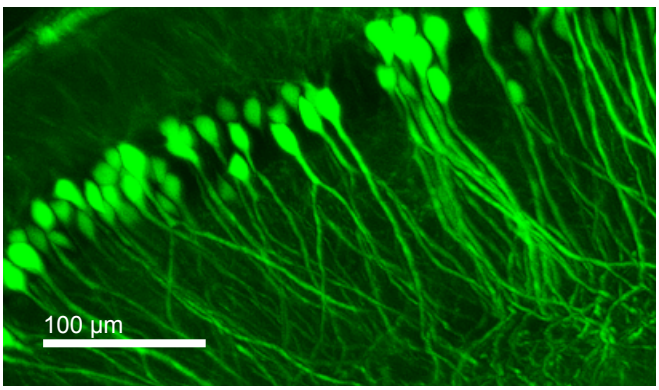
Light Sheet Microscopy
UltraMicroscope II

Bidirectional Triple Light Sheet Microscopy
UltraMicroscope II

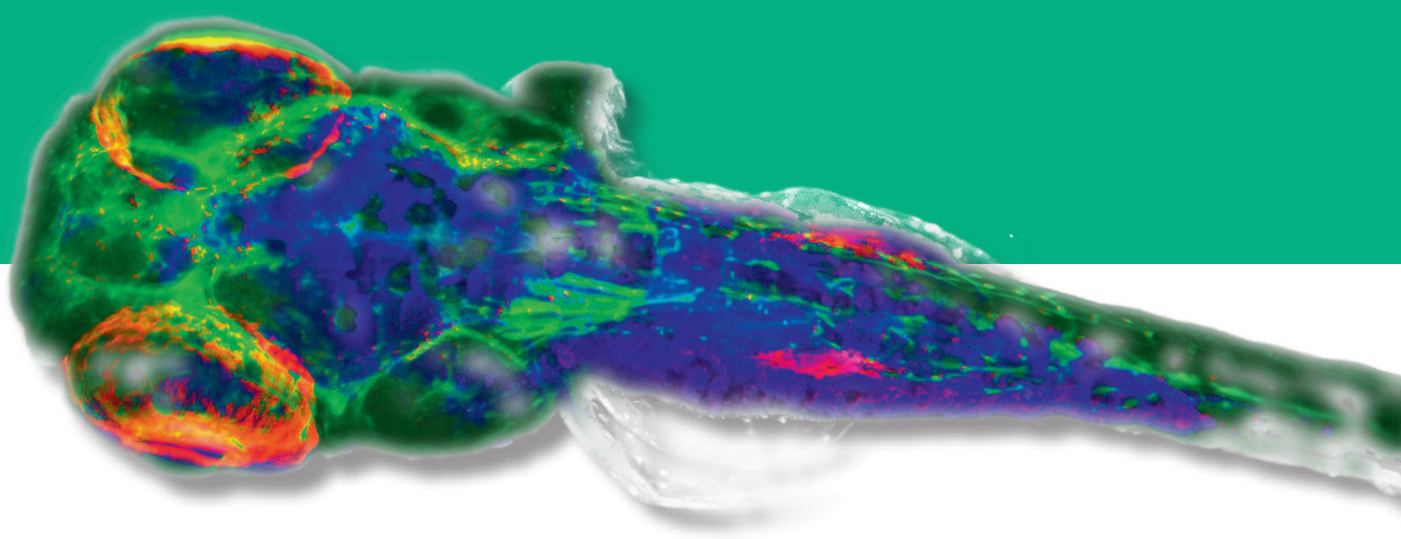
Six variable light sheets for perfect 3D fluorescence microscopy from macro view to cellular resolution in organic solvents and aqueous buffers

The bidirectional triple light sheet technology of the UltraMicroscope II generates 6 focused light sheets to excite samples from the side while the fluorescence light is detected by a sCMOS camera perpendicular to the illumination plane. Moving the sample through the light sheet generates a 3D image stack. Selective excitation of the focal plane reduces bleaching and photo toxicity significantly. The open setup allows the analysis of cleared samples in any clearing solution or in vivo data acquisition in aqueous media. The dynamic horizontal light sheet focus guarantees excellent Z-resolution covering the entire field of view.

- Bidirectional triple light sheet microscopy
- Imaging of living animals or cleared samples
- Imaging in all kinds of clearing solutions or water
- Dynamic horizontal light sheet focus
- Allows samples up to 10 x 10 x 10 mm^3
- Variable magnification from 1.26x to 12.6x
- Easily accessible sample chamber



Mouse hippocampus, Thy-1 GFP,
courtesy of Bianca Schmid,
Max Planck Institute for Psychiatry, Munich, Germany

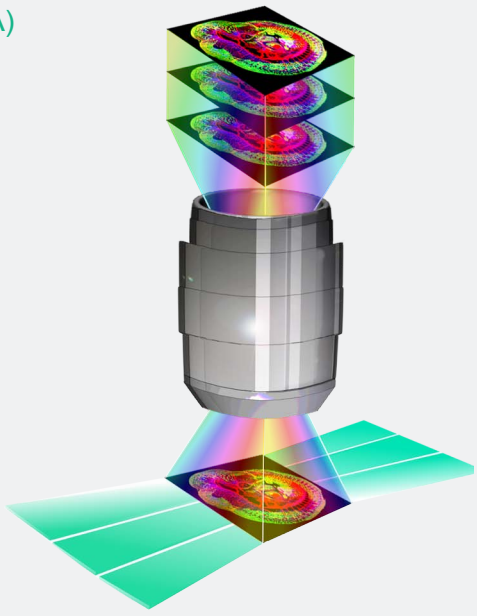


Zebra Fish
in vivo imaging, GFP & autofluorescence

Dynamic Horizontal Focus (DHF)

Custom-made optics form two triple light sheets that are focused into the sample. Z-resolution and contrast is best within the light sheet focus. To optimize this, the focus diameter and the focus length [Rayleigh length] can be adapted to the imaging conditions and will be optimized by software. Then, the UltraMicroscope II provides the Dynamic Horizontal Focus that shifts the focus through the sample while imaging. In combination with advanced software algorithms, the UltraMicroscope II delivers a pin sharp 3D image of the sample.

A)



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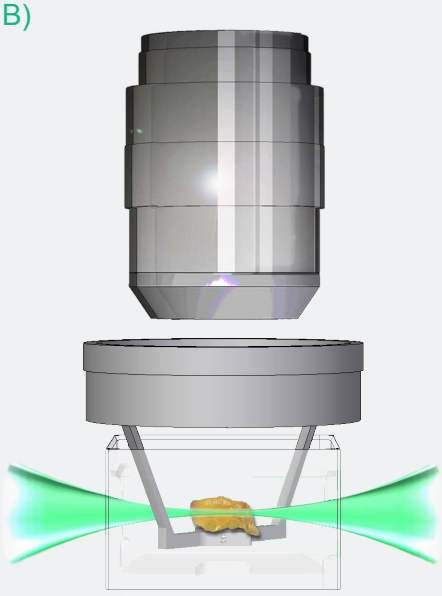


A) Bidirectional fluorescence excitation with up to six light sheets perpendicular to the detection.

B) Dynamic horizontal focus fluorescence excitation of the sample positioned by up to six focused light sheets (travel range 1 cm x 1 cm x 1 cm).

Bidirectional Triple Light Sheet Microscopy (BTLSM)

Exciting the fluorescent sample perpendicular to the detection by a focused light sheet offers two major advantages:
First, it allows 3D microscopy utilizing a wide field microscope. A camera-based wide field microscope delivers much higher frame rates than any laser scanning microscope.

Second, the combination of wide field microscopy and sheet excitation reduces bleaching and photo toxicity significantly. LaVision BioTec's UltraMicroscope II comes with up to six light sheets that excite the sample from different angles. This means the fluorescence excitation is most homogenous and artifacts like dark areas and stripes are minimized.

Bidirectional Triple Light Sheet Microscopy
UltraMicroscope II

Multi Refractive Index Compensation (RIC)

Several different clearing procedures for fixed tissue have been developed and utilized. Most clearing reagents differ from protocol to protocol and so does the refractive index. Running a system with different clearing or imaging solutions induces the necessity to correct for different refractive indices. The refractive index compensation of the UltraMicroscope II is utilized via the software interface. The user chooses between current clearing techniques such as CLARITY* or Benzyl Ether. Water can also be selected for in vivo imaging. This technology guarantees the perfect setting for every imaging solution. The UltraMicroscope II is the only light sheet microscope handling organic clearing solutions as well as aqueous buffers.

Variable Light Sheet Parameter

The UltraMicroscope II is the flexible solution for a variety of applications and diverse samples. The UltraMicroscope II has an adjustable light sheet that allows the user to set width, NA and Rayleigh length of the focused light sheets. This unique feature helps to meet the demand for the highest flexibility. The user can choose the perfect matching settings for any kind of sample via the software. In fully automated mode, the software selects the settings. Together with the multi refractive index compensation and the chromatic correction from 400 nm to 800 nm, the UltraMicroscope II can be adapted to different samples and clearing protocols.

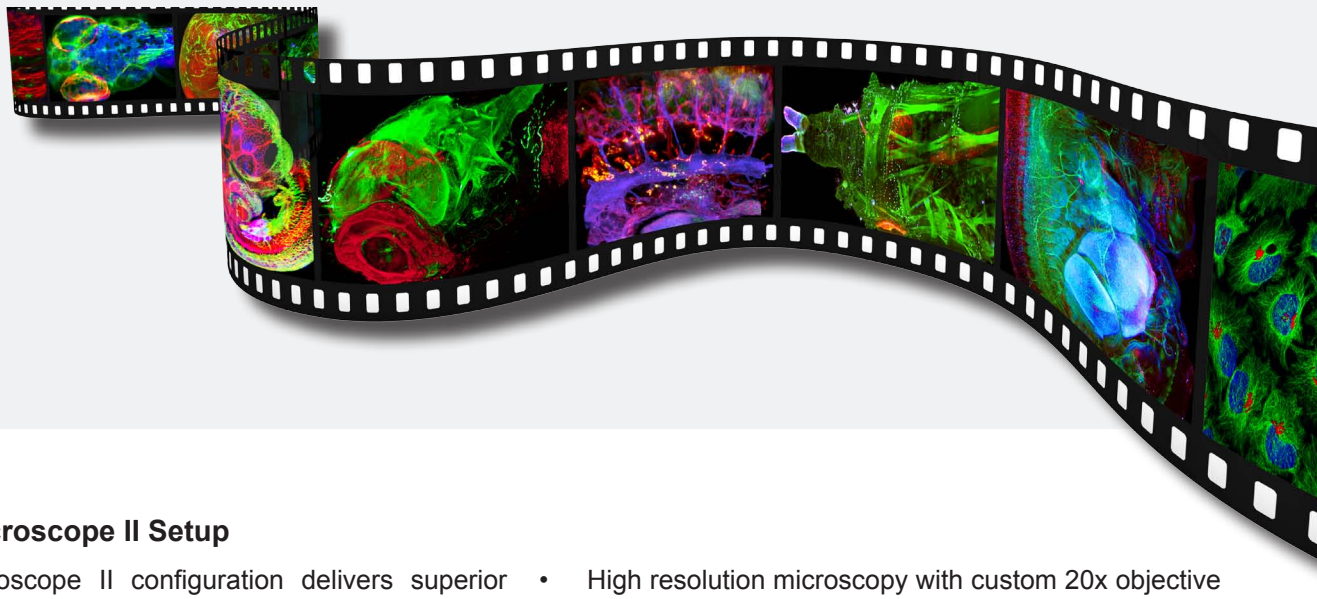
Users speak about the UltraMicroscope II:

"LaVision BioTec's UltraMicroscope has revolutionized the way we analyze vascular development. It allows us to proceed to a new and much deeper level of structural and mechanistic understanding."

Professor Friedemann Kiefer
Max Planck Institute for Molecular Biomedicine
Münster, Germany

"By giving access to three dimensions, the technology developed by LaVision BioTec in their UltraMicroscope has transformed the way we study and understand the organization of brain connections."

Alain Chédotal, Ph.D
Institut de la Vision
Paris, France



The UltraMicroscope II Setup

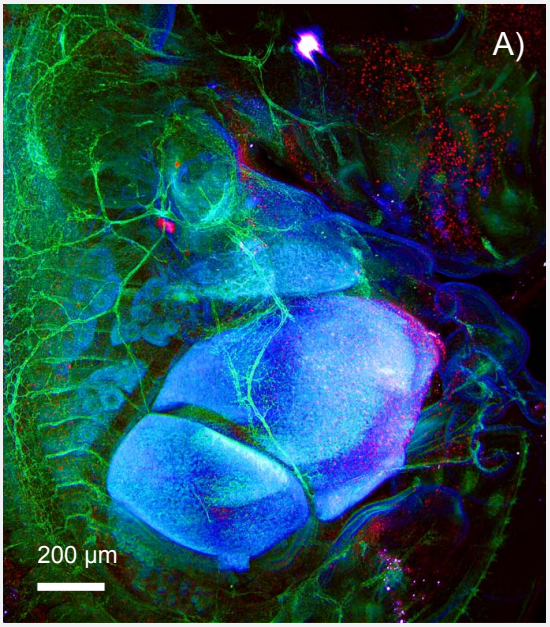
The UltraMicroscope II configuration delivers superior imaging capabilities and user friendliness. Just choose the camera, objective lens and laser light source to adapt the UltraMicroscope II to your application:

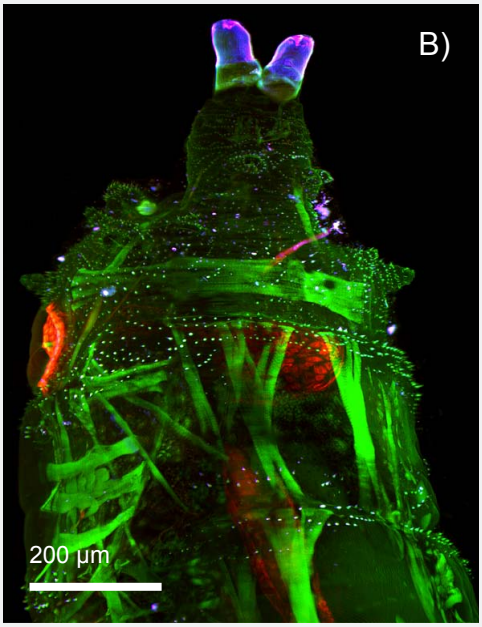
- sCMOS camera technology for high quality images
- Double triple sheet excitation allows a consistent illumination
- High resolution microscopy with custom 20x objective lens
- Low magnification lenses for macro imaging
- Laser module with up to five laser diodes or supercontinuum white light laser for maximum flexibility

Applications and Sample Clearing
UltraMicroscope II

Applications

The UltraMicroscope II serves diverse applications. They share the fact that imaging only a minor part of the sample is not sufficient and distorting artifacts introduced by sectioning have to be excluded. Researchers who need artifact free data from overview to a specific region of interest with cellular resolution implement this technology into their projects. Neuroscientists focusing on the regeneration potential of neurons and the axonal path finding[1] use this system as do oncologists verifying the efficiency of neovascularization inhibitors[2,3]. In the field of immunology lymph nodes and the developmental steps of entire lymphatic system are analyzed. The different developmental stages of animal models can be imaged for phenotyping or characterization of pathologies. The image acquisition in vivo is also possible as is the imaging of samples prepared by any clearing procedure. Tissue with endogenous fluorescent proteins like GFP or stained with labelled antibodies can be analyzed fast and easily with this setup. Clearing procedures like 3DISCO[5] or CUBIC[1] have been developed with the UltraMicroscope.





A) Mouse embryo E12, whole mount, triple staining; courtesy of Serge van de Pavert, VU Amsterdam

B) Drosophila melanogaster larvae, autofluorescence; LaVision BioTec

Right side : mouse embryo E12 tag1 ChAT ; courtesy of Chloé Dominici and Alain Chédotal, Institut de la Vision, Paris, France

UltraMicroscope articles

1) Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis
Susaki EA, Tainaka K, Perrin, Kishino F, Tawara T, Watanabe TM, Yokoyama C, Onoe H, Eguchi M, Yamaguchi S, Abe T, Kiyonari H, Shimizu Y, Miyawaki A, Yokota H, Ueda HR;
Cell. 2014 Apr

2) Apoptosis Imaging for Monitoring DR5 Antibody Accumulation and Pharmacodynamics in Brain Tumors Noninvasively
Weber TG, Osl F, Renner A, Pöschinger T, Galbán S, Rehemtulla A, Scheuer W;
Cancer Res. 2014 Apr

3) Multispectral fluorescence ultramicroscopy: three-dimensional visualization and automatic quantification of tumor morphology, drug penetration, and antiangiogenic treatment response
Dobosz M, Ntziachristos V, Scheuer W, Strobel S;
Neoplasia. 2014 Jan

4) Three-dimensional evaluation of retinal ganglion cell axon regeneration and pathfinding in whole mouse tissue after injury
Luo X, Salgueiro Y, Beckerman SR, Lemmon VP, Tsoulfas P, Park KK;
Experimental Neurology. 2013 Mar

5) Three-dimensional imaging of solvent-cleared organs using 3DISCO
Ertürk A, Becker K, Jährling N, Mauch CP, Hojer CD, Egen JG, Hellal F, Bradke F, Sheng M, Dodt HU;
Nature Protocols . 2012 Oct

Sample Clearing

Imaging large samples even into the depth of the tissue needs certain procedures to reduce the opacity. The tissue has to be virtually transparent. Some samples like Zebra Fish are mostly transparent by nature but the majority of samples are opaque. This counteracts all attempts to image the sample in total. Nowadays, two main principles of creating translucent samples have been established. In the case of organic solvent clearing, the principle of operation is matching the different refractive indices. On the other hand, the sample may be cleared by using aqueous buffers which have a certain depolymerizing effect on structures like lipid chains.

Organic Solvent Clearing Protocols

When performing organic solvent clearing, the water has to be removed in the first step by incubating the sample in increasing concentrations of ethanol or another dehydrating solution. After this step, the refractive index of water (1.33) is virtually no longer present. Within a second step, the remaining refractive indices are matched by ether incubation as in case of the 3DISCO[5] clearing. The organic solvent clearing leads to very transparent samples and is perfectly suited for dense tissue like tumors, adult tissue or highly myelinated brain. The majority of immuno-histochemical staining is well conserved. To preserve the fluorescence of proteins like GFP, the pH has to be adjusted. The UltraMicroscope II can be used for all current organic solvent clearing procedures including BABB and 3DISCO[5].

Water-Based Clearing Protocols

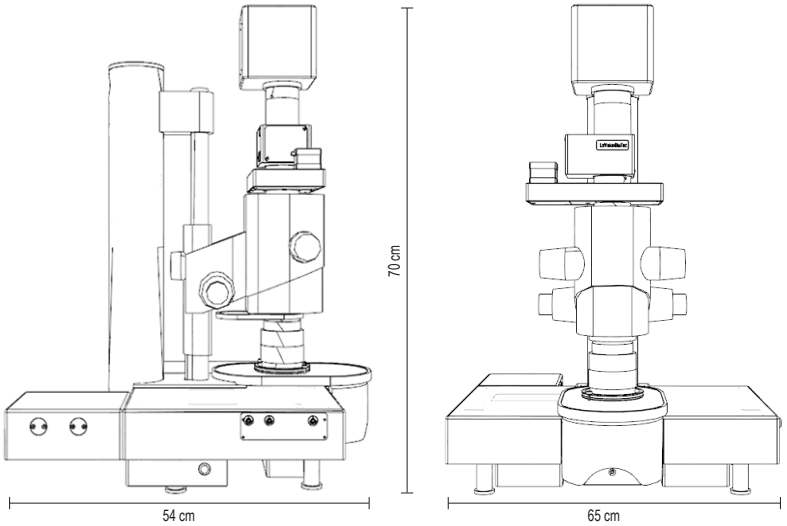
The most common operating principle of water-based clearing protocols is by depolymerization. By dividing large structures like lipid chains into small micelles of different sizes, the opacity is remarkably reduced. As depolymerizing reagent aqueous buffers can contain urea as it is used for CUBIC[1] clearing. A SDS buffer and an advanced electrophoresis protocol are used for CLARITY* clearing. The clearing protocols differ in complexity and in the degree of translucency which can be achieved. By depolymerization, the entire structure of a sample can be debilitated while the fluorescence of proteins like GFP is well preserved.

Both organic solvent-based clearing and water-based clearing methods are powerful tools for successful sample preparation. The variety of clearing protocols shows that clearing procedures have to be optimized for the sample of interest. The UltraMicroscope II is capable to handle all current clearing solutions.

** CLARITY for mapping the nervous system. Chung K, Deisseroth K; Nature Methods, 2013 June*

Specifications
UltraMicroscope II

Sheet optics	Illumination	Uni- & bidirectional		
	Number of light sheets	3 – 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 for 2x objective lens	Zoom	Mono zoom		
	Zoom ratio	0.63x – 6.3x (1:10)		
Detection optics	Objective lenses	2x	4x	20x
	Numerical aperture	0.5	0.3	0.35
	FOV diagonal (mm)	1.7 - 17.6	5.4	1.1
	Total magnification (objective lens + zoom ratio)	1.26x – 12.6x	(w/o Zoom)	(w/o Zoom)
	Working distance	4 mm, 6 mm, 10 mm	6 mm	2.5 mm
	Refractive index matching	1.33 – 1.56		
	Chromatic detection	Eight filters		
	Chromatic correction	Dynamic 400 nm – 850 nm		
Detector	Type	sCMOS		
	Pixel	2560 x 2160		
	Pixel size	6.5 µm x 6.5 µm		
	Maximum frame rate	100 fps @ full frame		
	Read noise	1 e-		
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		
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		
Dimensions	54 cm x 70 cm x 65 cm (W x H x D)			
Weight	47 kg (w/o controller and laser)			



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