LaVision BioTec

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.

Exciting the fluorescent sample perpendicular to the detection by a focused light sheet offers two major advantages:

1. **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.

2. **Second**, 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.

Dynamic Horizontal Focus

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.

Bidirectional Triple Light Sheet Microscopy

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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.
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.

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

Above from left to right: okolab® environmental control system, view into the in vivo cuvette, objective lens with dipping cap, lid of the in vivo cuvette

in vivo imaging

The in vivo setup for the UltraMicroscope II ensures constant environmental conditions within the sample cuvette considering the temperature and the CO₂/O₂ atmosphere. All settings are controlled by a touch-screen. The sample feedback mode measures the temperature at the sample with an additional temperature sensor. Thus, the temperature of the thermostat is automatically adjusted to the feedback of this sensor. The heating element and the sample holder can be easily unmounted for autoclaving. This in vivo imaging setup includes:

- in vivo sample cuvette
- Temperature control unit
- Active CO₂ controller
- Active humidity module
- Touch-screen control panel
- Feedback temperature sensor

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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
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 use this system as do oncologists verifying the efficiency of neovascularization inhibitors. 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 3DlSCO, iDISCO or CUBIC have been developed with the UltraMicroscope.

Applications and Literature

UltraMicroscope II

Sample Clearing

UltraMicroscope II

Sample Clearing articles


5) Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging. Suzuki EA, Tanaka K, Perrin D, Yukinaga H, Kuno A, Ueda HR; Nat Protoc. 2015 Nov


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 methanol 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 iDISCO 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 iDISCO.

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 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 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.

UltraMicroscope II

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## 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
- **Numerical aperture:** 0.5, 0.3
- **FOV diagonal (mm):** 1.7 – 17.6, 5.4
- **Total magnification (objective lens + zoom ratio):** 1.26x – 12.6x (w/o Zoom)
- **Working distance:** 4 mm, 6 mm, 10 mm, 6 mm
- **Refractive index matching:** 1.33 – 1.56
- **Chromatic detection:** Seven 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)**