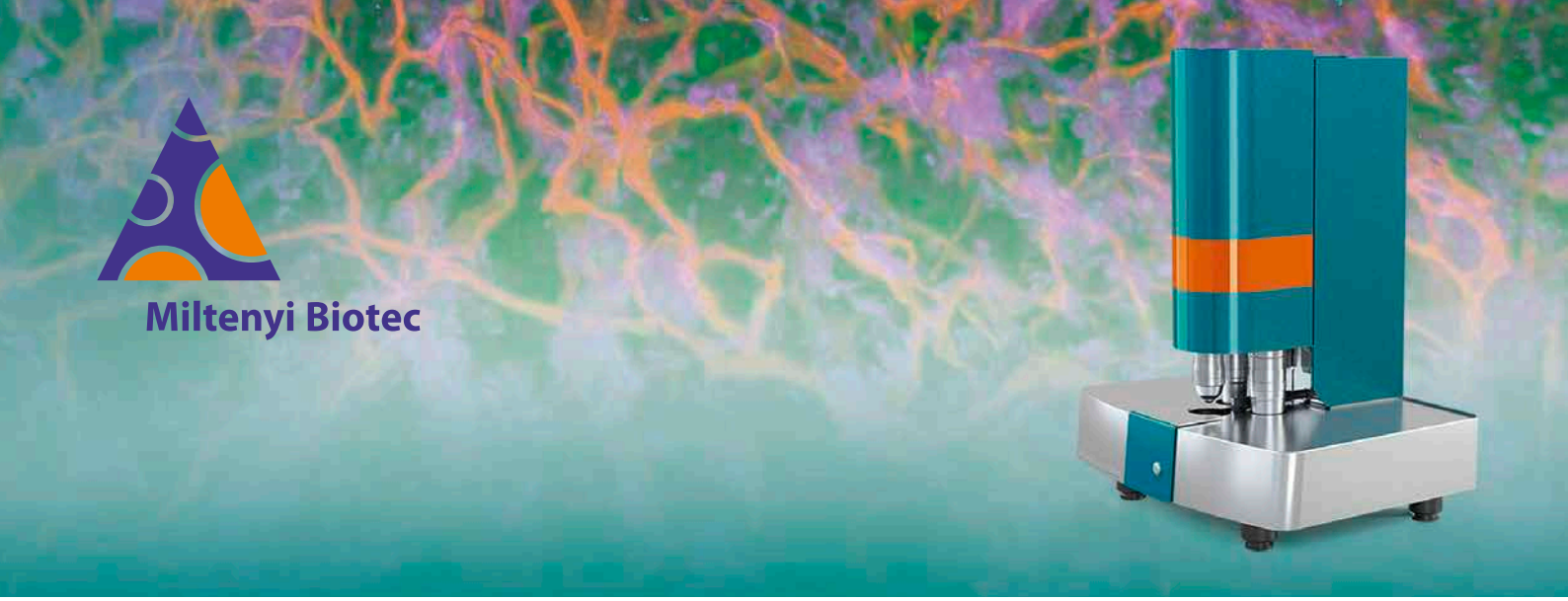

Miltenyi Biotec

3D light sheet imaging workflow

From sample preparation to data optimization

A complete workflow solution for large-scale 3D fluorescence microscopy

Acquiring meaningful 3D-immunofluorescence (3D-IF) microscopy data from large samples such as whole mouse brain, hemispheres or brain organoids, whole rodent organs, or even human organs is a four-step process. Miltenyi Biotec offers a complete workflow solution that allows you to analyze your intact sample in its entirety and dive deep into the details.

VIDEO

See how easy it is to create detailed 3D images with our workflow solution:

▸ **miltenyibiotec.com/UM-Blaze-workflow-video**

LEARN MORE

Explore our complete workflow solution online.

▸ **miltenyibiotec.com/3D-light-sheet-imaging-workflow**

01 STAINING

The sample is stained with validated fluorochrome-conjugated antibodies. Thorough epitope labeling within the entire 3D structure and high signal-to-noise ratios are key for reliable analysis.

02 TISSUE CLEARING

The tissue is rendered transparent using the non-toxic MACS® Clearing Kit to allow excitation of fluorochromes deep inside the sample and efficient detection of fluorescence.

03 3D IMAGING

The UltraMicroscope Blaze™ detects the fluorescent signals, capturing a detailed 3D image stack that offers both a complete sample overview and subcellular insights.

04 IMAGE PROCESSING

The image stacks are processed using MACS iQ View – 3D Large Volume, a software package specifically designed to optimize UltraMicroscope Blaze data with ease.

Staining

Staining with antibodies validated for 3D-IF imaging of cleared tissues

Identifying appropriate antibodies to label structures of interest in large cleared samples is one of the most time-consuming steps in designing the experiment. 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 for 3D-IF on tissues cleared with the MACS Clearing Kit.

- Validated for whole-mount staining of large samples cleared with the MACS Clearing Kit.
- Compatible with other methods like iDISCO+.
- 50% faster staining with fluorochrome-conjugated primary antibodies.
- Superior tissue penetration.
- Optimal signal-to-background ratios with bright and photostable Vio® Dyes.
- Recombinantly engineered for reproducible results and minimal background signals.

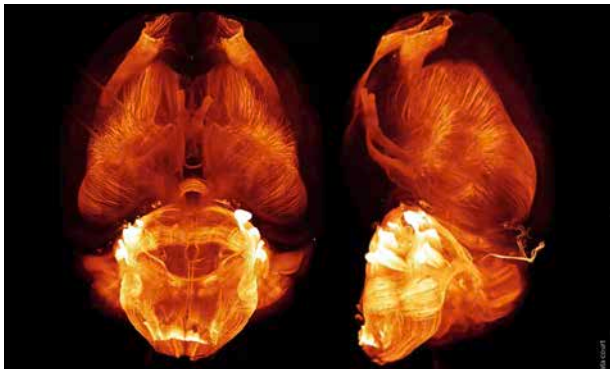

Figure 2: Mouse brain stained with Neurofilament Antibody (anti-human/mouse, Vio R667, REAfinity) and cleared with iDISCO+. Data courtesy of Gubra, a CRO and Biotech company, Denmark.

LEARN MORE

Check out our portfolio of 3D-IF antibodies.

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

Tissue clearing

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.

- Non-toxic and user-friendly clearing method.
- Fast and efficient clearing in just one step while preserving tissue morphology.
- Easy-to-follow protocols for pigmented and non-pigmented tissues and for optimal preservation of endogenous protein signals.
- Non-toxic MACS Imaging Solution, perfectly matching the refractive index of cleared tissue for stunningly sharp images.

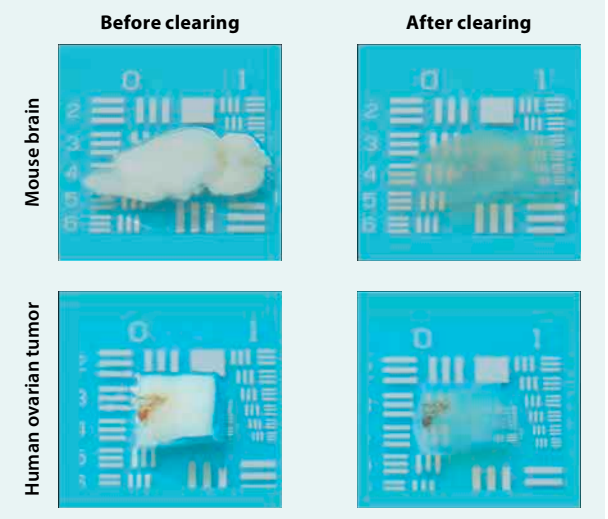

Figure 3: The MACS Clearing Kit enables effective clearing of tissues.

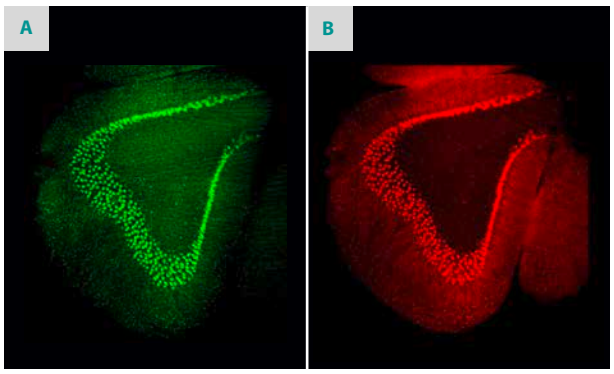

Figure 4: Endogenous GFP signal vs. staining with GFP Antibody. GAD67-EGFP mouse brain cleared with the MACS Clearing Kit using the dedicated protocol. Preserved endogenous GFP signal is shown in green (A) and staining with GFP Antibody (Vio R667) is shown in red (B).

Tissue clearing protocols

Browse our protocols to get started right away. You will find dedicated protocols for efficient clearing of:

- Mouse brain
- Endogenous protein signal preservation
- (Blood-rich) tumors
- Mouse liver, kidney, and spleen
- Mouse embryo
- Mouse lymph node
- Cerebral organoids
- Mouse lung and intestine.

LEARN MORE

Find the perfect tissue clearing protocol for your sample and quickstart your research.

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

3D Imaging

Light sheet microscopy with the UltraMicroscope Blaze for large-scale 3D-IF imaging

The UltraMicroscope Blaze provides comprehensive 3D visualization of entire samples, enabling detailed analysis down to the subcellular level. With its revolutionary LightSpeed Mode and advanced image processing software, it sets new benchmarks for fast and high-quality 3D imaging. This versatile tool is ideal for imaging a wide range of samples, from organoids to entire mouse models or human kidneys, and finds applications in neuroscience, immuno-oncology, developmental biology, and beyond.

- Effortless operation with automated objective exchange, autofocus, sample release, chromatic correction, and a batch measurement mode for imaging multiple samples.
- Blazing speed for high-resolution imaging. Capture an entire mouse brain in just 3 minutes.
- Intuitive design for researchers of all levels. No expertise required.
- Compatible with any clearing media, from water to organic solvents, with dedicated objective caps.
- Unprecedented image quality through six light sheets for uniform illumination, high numerical aperture (NA) objectives, and long working distance immersion objectives.

LEARN MORE

Browse our website for more information on the UltraMicroscope Blaze.

▸ **miltenyibiotec.com/blaze**

Image processing

Powerful image processing with MACS iQ View – 3D Large Volume Software

MACS iQ View – 3D Large Volume offers a comprehensive and user-friendly solution for processing images captured with the UltraMicroscope Blaze. A unique feature of the software is the integration of various processing algorithms (3D Crop, Destripe, Denoise, Deconvolution, Stitching, and Contrast Compression) into a unified workflow.

- Seamless integration: Direct processing of UltraMicroscope Blaze data from the acquisition workstation, eliminating data transfer, saving time, and effort.
- Streamlined processing workflow: Automated image processing by simply selecting and arranging processing modules in the workflow.
- Effortless stitching with a single click: Automatic assembly of 3D mosaic stacks, eliminating the need for manual processing.
- Deconvolution tailored to the specific optical characteristics of your UltraMicroscope Blaze.
- Batch processing: Queuing and initiation of various workflows across multiple datasets. This feature proves especially valuable when dealing with extensive datasets and imaging multiple samples using the UltraMicroscope Blaze, particularly in the LightSpeed Mode, ensuring a smooth processing workflow.
- Side-by-side view of the original and processed data to assess the results. This feature helps users experiment with different settings and combinations of algorithms to find the best fit for their data.

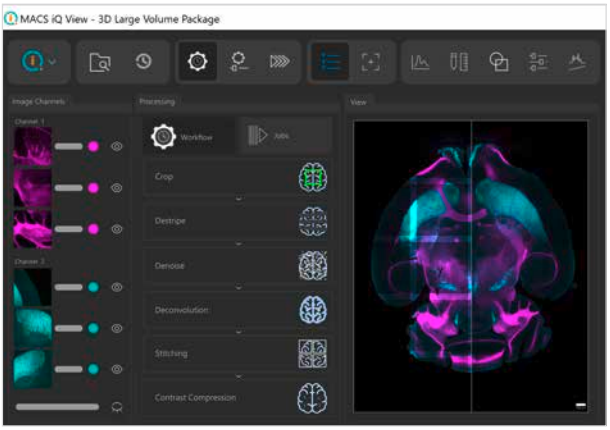

Figure 5: Processing of images in a straightforward and efficient manner with MACS iQ View – 3D Large Volume Software.

LEARN MORE

Learn more about MACS iQ View – 3D Large Volume:
▸ **miltenyibiotec.com/UltraMicroscope-image-processing**

Miltenyi Biotec

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