

3D imaging with the UltraMicroscope Blaze™

Preparation of rodent gastrointestinal tract for optimal 3D light sheet microscopy

Background

Three-dimensional (3D) imaging of tissues and organs provides valuable *in situ* and spatial information that traditional two-dimensional (2D)-imaging techniques cannot offer.[1] However, many tissues and organs do not retain their 3D structure once removed from a living subject.[2] For example, the small intestine – whose turgidity and structural integrity are maintained by the presence of fluid and food waste under normal physiological conditions – tends to collapse during sample preparation for imaging or analysis.[3]

Here, we present a simple and cost-effective method that uses agarose stabilization to preserve the 3D structure of mouse intestinal fragments during 3D imaging with the UltraMicroscope Blaze. This approach is compatible with established immunostaining, tissue dehydration, and tissue clearing techniques, as it does not cause autofluorescence or other imaging artifacts.[2,4] Using this method, we visualized both the structural organization of the ileum vasculature via CD31 staining of endothelial cells – also known as platelet endothelial cell adhesion molecule-1 (PECAM-1) – and the network of gut-associated lymphoid tissue (GALT) through CD45 staining of immune cell aggregates.



Figure 1: The 3D structure of the ileum fragment was recovered when the agarose solidified inside the ileum.

Materials and methods

Ileum filling

Mouse ileum samples from an 8-week-old male fixed in 4% paraformaldehyde (PFA) and washed with phosphate-buffered saline (PBS), were cut into 4 cm fragments and placed in a glass Petri dish containing PBS. For each ileum fragment, one end was sealed using a trimmed square knot tied with a 30 cm string of nylon monofilament suture thread, positioned 5 mm from the end of the tissue.

Cooled, viscous 1.5% agarose was carefully injected into the ileum fragment using an 18G blunt needle until it reached the sutured end. The needle was then slowly withdrawn, while 100 µL of agarose was simultaneously and gradually dispensed along the length of the ileum fragment.

Using forceps to clamp the open end, the fragment was transferred to a glass Petri dish containing ice-cold PBS and placed on top of a cooling block for 30 seconds to quickly solidify the agarose. The open end of the ileum fragment was then closed using the same knotting procedure as for the first end (fig. 1).

The ileum fragments, filled with solidified agarose, were stored in PBS in a MACSwell™ 24 Imaging Plate at 4 °C prior to immunostaining and clearing.

Note that the ileum fragments can be stored at 4 °C for one to two weeks.

Immunostaining

The suture-sealed, agarose-stabilized mouse ileum fragments, as described above, were immunostained and cleared following the procedure detailed in the Miltenyi Biotec protocol "Immunostaining and clearing of mouse intestine and lung for 3D imaging analysis."



Figure 2: The cleared ileum fragment retained a 3D structure.

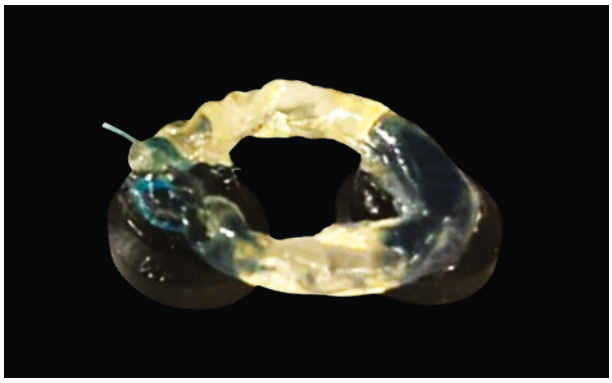


Figure 3: The ileum fragment was attached to the sample pedestals.

The proprietary reagents described here are part of the MACS® Clearing Kit, which includes Permeabilization Solution, 10× Antibody Staining Solution, and Clearing Solution.

The agarose-stabilized ileum fragments were permeabilized by incubation in 5 mL of Permeabilization Solution with slow, continuous agitation for 24 hours at room temperature.

Following permeabilization, the ileum fragments were immunostained with CD31 and CD45 antibodies in Antibody Staining Solution at 37 °C with gentle shaking for 7 days. To remove unbound antibodies, the samples were washed twice with Antibody Staining Solution at room temperature for 4 hours each. A final overnight wash was then performed under the same conditions. After washing, the ileum fragments were dehydrated at room temperature with slow, continuous agitation (as in the permeabilization process), using increasing concentrations of ethanol (EtOH) diluted with 2% Tween® 20, as previously described.[2]

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Follow our detailed clearing and staining protocol for optimal 3D imaging!

▸ **miltenyibiotec.com/MouseGITract-staining-clearing-protocol**

Ileum clearing

After immunostaining, the dehydrated ileum fragments were cleared by incubation in 5 mL of Clearing Solution with slow, continuous agitation for 6 hours at room temperature, protected from light. An example of the cleared tissue is shown in Figure 2.

The cleared ileum fragments were then mounted onto two sample pedestals (fig. 3) using clear silicone waterproof sealant, stored in MACS Imaging Solution at room temperature, and protected from light until imaging.

Imaging

Acquisition settings

The pedestals supporting the cleared ileum fragments were clipped onto the sample holder (fig. 4A), which was then inserted into the imaging chamber of the UltraMicroscope Blaze (fig. 4B).

To optimize the imaging procedure, the chamber was filled with MACS Imaging Solution. The fluorescence channels used were 488 nm for background signal (autofluorescence), 561 nm for the CD31 marker (Vio® G570), and 640 nm for the CD45 marker (Vio R667).

For all channels, a 5.5 µm light sheet with a 40% width and a numerical aperture of 0.21 was applied. A Z-stack was acquired at 4× magnification using the objective lens, with a Z-step size of 2.7 µm. Image processing was performed using the software Imaris v10.2.0.

After acquisition, the raw UltraMicroscope Blaze images (OME-TIFF format) were converted to Imaris files (OME-IMS format) using the Imaris File Converter v10.2.0, and subsequently stitched using the Imaris Stitcher v10.2.0.

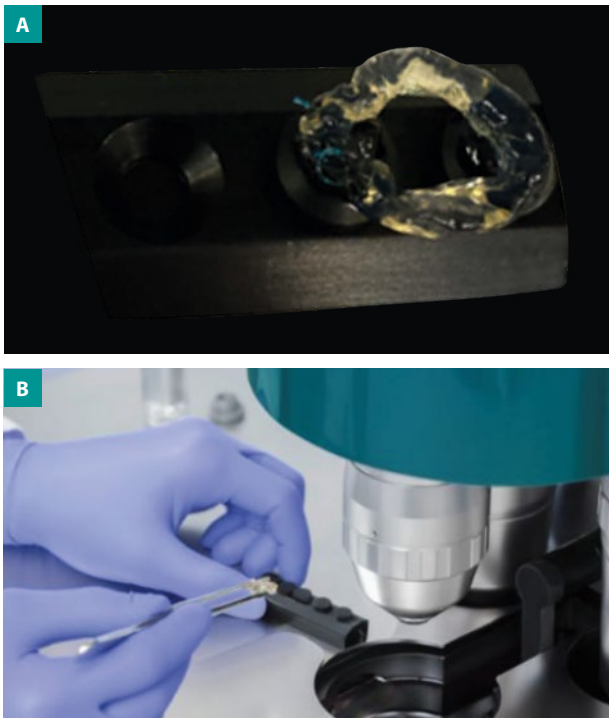


Figure 4: The pedestals were clipped onto the sample holder (A), ready for imaging with the UltraMicroscope Blaze (B).

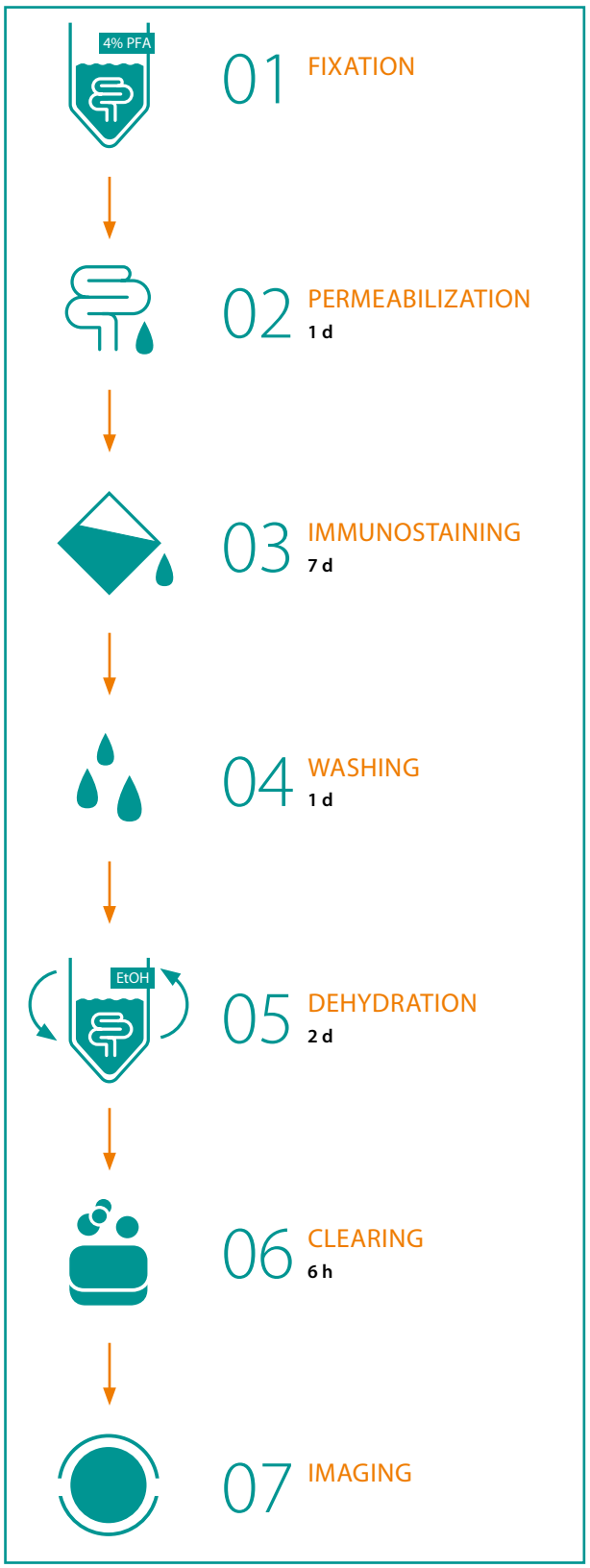


Figure 5: Workflow timeline from tissue fixation to imaging.



Figure 6: 3D image of immunostained, agarose-stabilized mouse ileum acquired with the UltraMicroscope Blaze.

VIDEO

Watch the 3D video data by scanning the QR code or visiting the URL below.

▸ **bit.ly/MouseGITract**

Results and conclusion

The agarose stabilization method proved to be a highly effective and practical solution for preserving and visualizing the intricate architecture of delicate intestinal tissues. Specifically, it enabled clear 3D imaging of both the vascular endothelium (CD31 Antibody, anti-mouse, Vio G570, REAfinity® [REA784]) and immune cell aggregates (CD45 Antibody, anti-mouse, Vio R667, REAfinity [REA737]), including the gut-associated lymphoid tissue (GALT) within the mouse ileum (fig. 5). This innovative approach opens new possibilities for spatial biology research and the discovery of novel therapeutic targets.

When integrated into a streamlined imaging pipeline (fig. 5) – including immunostaining, tissue clearing with the MACS Clearing Kit, and high-resolution imaging on the UltraMicroscope Blaze – this method delivers consistent, artifact-free results while preserving native tissue morphology.

Key advantages of this workflow include:

- **Robust and cost-effective tissue stabilization:** preserves spatial integrity for large-scale 3D imaging studies.
- **Seamless compatibility with REAfinity Antibodies:** ensures optimal signal fidelity and reproducibility across experiments.
- **Versatility across tissue types:** while optimized for mouse ileum, this protocol is readily adaptable to other soft or structurally fragile tissues, expanding its utility across diverse research areas.
- **Scalability and automation:** the UltraMicroscope Blaze system's large field of view and automated acquisition capabilities support high-throughput imaging without compromising resolution or detail.

Together, this comprehensive imaging workflow empowers researchers to explore the complex vascular and immune networks within the mouse ileum with unmatched clarity. By preserving both the structural integrity and molecular signals of biological specimens, it significantly advances the capabilities of spatial biology, and sets a new standard for 3D tissue imaging in biomedical research.

References

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2. Vieites-Prado, A. and Renier, N. (2021) Development. 148(18):dev199369. doi: 10.1242/dev.199369
3. Williams, J.M. *et al.* (2016) Curr. Protoc. Mouse Biol. 6(2):148-168. doi: 10.1002/cpmo.2
4. Gleave, J.A. *et al.* (2013) PLoS One. 8(8):e72039. doi: 10.1371/journal.pone.0072039

Product	Order no.
Reagents	
MACS Clearing Kit	130-126-719
MACS Imaging Solution	130-126-335
MACSwell 24 Imaging Plates	130-124-677
Antibodies	
CD31 Antibody, anti-mouse, Vio G570, REAfinity	130-133-792
CD45 Antibody, anti-mouse, Vio R667, REAfinity	130-128-737
Instrument	
UltraMicroscope Blaze	

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