Maximizing spatial biology - A workflow combining 3D imaging with 2D multiparameter analysis of adult mouse brain

Poster ID: PSTR115.21

Kevin Bigott, Sara Rahmati, Luigi Prisco, Simon Merz, Gernot Ebel, Andreas Bosio, Melanie Jungblut
Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

Scan the QR code to download the poster (PDF):

Introduction

Spatial biology is an emerging field that studies spatial organization of cells and molecules within tissues to decipher complex biological processes. Current methods for two dimensional immunofluorescence microscopy only provide information from tissue sections, whereas three dimensional analysis utilizes immunophenotyping of intact organs, but only addresses a restricted set of markers. To maximize spatial analysis, we developed a workflow to combine 3D and 2D imaging of the same specimen. 3D analysis is first applied for visualization of a selected set of markers at single cell resolution in the whole organ. Subsequently, target structures within the sample are selected and sectioned for multi-parameter 2D analysis with hundreds of markers.

In summary, this workflow provides the link between two imaging technologies. The ability to obtain both, a comprehensive whole organ context and detailed information about cellular diversity in a spatial context from a single sample makes the procedure a valuable tool in spatial biology to maximize our understanding of the cellular and molecular architecture in whole organs.

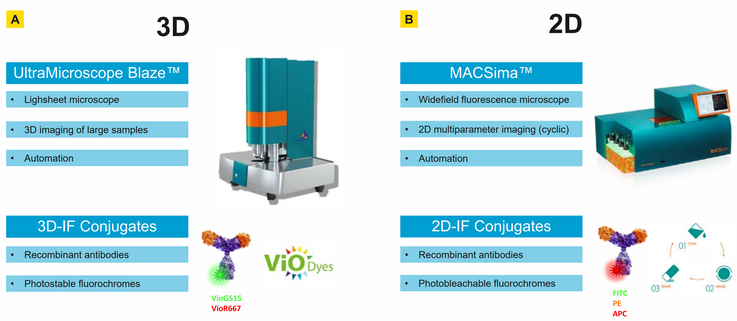
Methods

1 Imaging Technologies

For 3D imaging of large samples we utilized a fully automated light-sheet fluorescence microscope (UltraMicroscope Blaze™), a non-toxic organic solvent based clearing method (MACS® Clearing Kit) and recombinant REAfinity™ antibodies coupled to bright and stable Vio® dyes (Figure 1A).

For 2D analysis we applied our MACSima™ Imaging Cyclic Staining (MICS) procedure for cyclic immunofluorescence microscopy. This technology enables the analysis of tissue samples with hundreds of antibody-fluorochrome conjugates on a single section at single-cell resolution. Sections were imaged in a cyclic process that includes fluorescent staining with antibody-fluorochrome conjugates (fluorochromes: FITC = fluorescein isothiocyanate, PE = phycoerythrin, APC = allophycocyanin), image acquisition and erasure of the fluorescence signal (Figure 1B).

Figure 1



2 Combined workflow

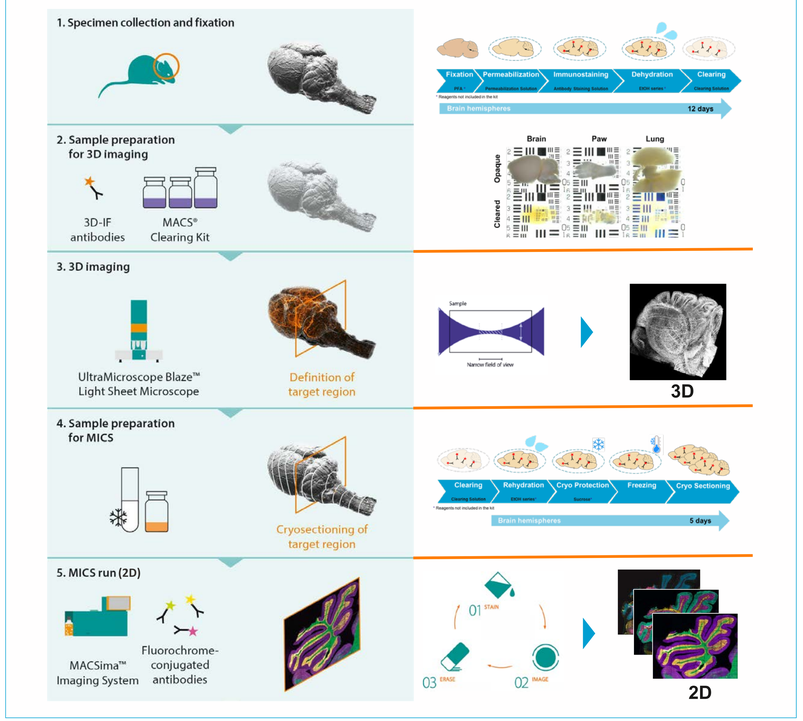
1. Specimen collection and fixation

2. Sample preparation for 3D imaging:

- Permeabilization
- Antibody staining
- Dehydration
- Tissue clearing

3. 3D imaging:
UltraMicroscope Blaze™ Light Sheet Microscope (Figure 1A) was used to obtain 3D images of optically cleared mouse brain hemispheres. For excitation, a 4 µm thin light sheet and Dynamic Horizontal Focusing were used to ensure optimal x-, y-, and z-resolution. For detection, a 12×/0.53 MI Plan immersion objective lens equipped with a dipping cap for organic solvents was utilized along with a 1.66× post magnifier lens, resulting in a total magnification of approximately 20×. A 7 × 7 tiles mosaic was acquired to cover the entire mouse cerebellum, with a z-step size of 4 µm and 20% overlap between tiles to allow stitching of the data into a single 3D image.

Figure 2



4. Sample preparation for MICS:

- Rehydration
- Cryoprotection
- Embedding and freezing

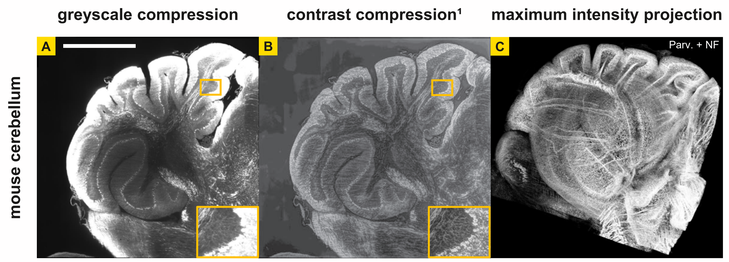
5. MICS run:
Multiplex analysis was performed using MICS Technology, which employs a three-step iterative cyclic staining process performed automatically by the MACSima™ Imaging System (Figure 1B). In this process, samples are stained with multiple fluorochrome-conjugated antibodies, an image is acquired using a widefield microscope and fluorescence signal is removed by photobleaching or fluorochrome release mechanisms. This cycle can be repeated, allowing for immunostaining of hundreds of markers on a single sample. For this feasibility, sequential staining of 12 fluorochome labeled antibodies was performed, together with 4',6-Diamidino-2-phenylindol (DAPI).

Results

1 3D analysis and post-processing

A 3D volume of the entire hemisphere was captured utilizing a UM Blaze™. Dataset is represented by a maximum intensity projection displayed in Figure 3C. The hemisphere was co-stained with Neurofilament-Vio R667 and Parvalbumin-Vio R667 (Figure 3A, B, C). This staining provided a structural overview of the brain in 3D at single cell resolution (Figure 3B, zoom), allowing for more accurate selection of the section for subsequent MICS, and also enabled the identification of the same biological structures within light sheet microscopy and MICS datasets. Denoising and contrast compression algorithms were used to further enhance the images (Figure 3B, C, Merz, Jansen *et al.*, 2021). Scale bar: 1000 µm.

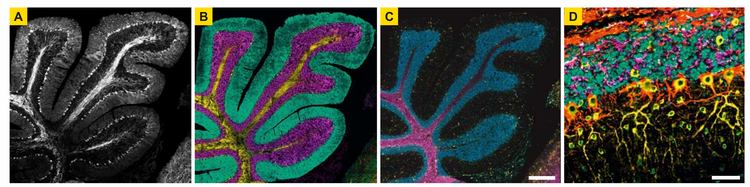
Figure 3



2 2D multi-parameer analysis

The selected section was successfully stained with 12 different fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated antibodies. Selected set of stainings are shown in Figure 4, indicating that the epitopes remained stable throughout the entire process of sample preparation for 3D imaging, 3D imaging itself, and sample preparation for MICS. Further, 3D-IF stainings (Neurofilament-Vio R667 and Parvalbumin-Vio R667) also remained stable and were still detectable with the MACSima™ Imaging Platform (Figure 4A), enabling selection of target regions. Neurofilament is shown in yellow, GLAST in cyan, DAPI in magenta (Figure 4B) and O4 in magenta, DAPI in blue, CD11b in yellow (Figure 4C). Enlarged view with neurofilament in red, β-tubulin III in yellow, VGLUT1 in magenta for visualization of neurons, and DAPI in cyan for visualization of nuclei (Figure 4D). Various cell types, such as neuronal cells, astrocytes and oligodendrocytes were detected with single-cell resolution (Figure 4D). Scale bars: 300 µm (Figure 4A, B, C) and 100 µm (Figure 4D).

Figure 4



Conclusion and Outlook

Spatial biology platforms typically utilize few thin tissue sections to analyze biological systems, such as organs. This approach poses a risk of missing critical contextual information that may exist beyond the chosen sections.

Here we describe a workflow that provides the link between two previously separated imaging technologies by combining 3D imaging of a whole tissue sample and 2D immunostaining/ imaging of a multitude of markers on a tissue section of the same sample. We demonstrated that during sample preparation as well as imaging, both the fluorochromes used for 3D imaging (Figure 4A) and the epitope were preserved for targeted 2D multiplex analysis (Figure 4B, C, D). The ability to obtain both, a whole organ analysis and detailed information about the cellular diversity from one sample in a 2D context makes this workflow a powerful tool to understand the localization and expression of specific proteins in complex tissues.

Future developments will focus on expanding this workflow to diverse tissues and/or applications and the correlation of resulting 3D and 2D datasets and extrapolation of 2D multiparameter expression profile into the 3D landscape.

References:
References 1. Merz, Simon *et al.* (2020). High-resolution 3-D imaging for precise staging in malignant melanoma.10.1101/2020.07.22.20159103.