Miltenyi Biotec

Unbiased epitope analysis of postmortem multiple sclerosis brain tissue

Spatial biology with the MACSima™ Platform

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Background

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that leads to neurodegeneration, neuroinflammation, and demyelination. Patients with MS may suffer from visual, sensory, and motor impairments, as well as pain and cognitive deficits.[1] Myelin sheaths, which cover axonal projections of central neurons, are attacked by infiltrating immune cells. As a result, oligodendrocytes, including their protective myelin sheaths, become damaged and eventually die. This causes the typical formation of MS lesions that can be observed in the CNS of patients. Additionally, glial cells, such as microglia and astrocytes, turn into a reactive, inflammatory state and lead to areas of neuroinflammation and neurodegeneration.[2,3]

This complex disease includes the participation of many central and peripheral cell types. Peripheral immune cells such as T cells, macrophages, and B cells start infiltrating the brain of MS patients and attack the protective myelin sheaths. Centrally, astrocytes and microglia are responsible for the formation of neuroinflammation while even more cells are potentially involved in this response and are yet to be defined. Therefore, a thorough characterization of all involved cell types is needed for a better understanding of disease mechanisms. Ideally, this characterization includes information about the localization of cells, especially in the context of MS lesions. Cell and lesion characterization through conventional microscopy is often limited by the small number of cell markers that can be used simultaneously and is thereby limited in providing complete information.

Here, we used a spatial biology approach based on the MACSima Platform and its unique MICS (MACSima Imaging Cyclic Staining) technology to gain a deeper understanding of the cellular composition and organization in the brain of MS patients. MICS technology is based on an iterative cyclic staining process composed of three steps that are conducted in a fully automated manner by the MACSima System.

The process begins when a sample is stained with up to three different fluorochrome-conjugated antibodies, the images are then acquired with a widefield microscope, and to complete the cycle, the fluorescence signal is erased. This process is repeated automatically within the MACSima System for as many times as needed, allowing staining with an unlimited number of markers. To take advantage of the spatial biology technology and obtain a precise characterization of the cellular landscape in postmortem MS brain tissue, we stained 87 different markers including a variety of neural and immune cell markers in this study.

Methods and workflow

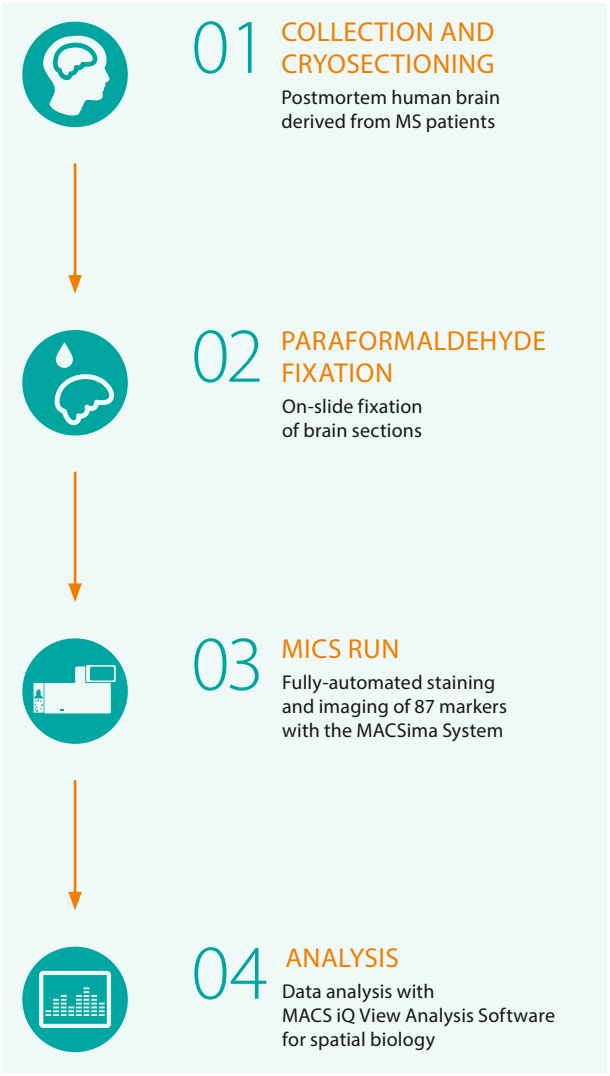

Figure 1: Experiment workflow.
Fresh-frozen brain samples were derived postmortem from human MS patients of the Netherlands Brain Bank. The frozen tissue samples were cryosectioned prior to the MICS run and the sections were fixed onto slides using a 4% paraformaldehyde solution. The region of interest (ROI) definition at the beginning of the workflow was performed based on hematoxilin/eosin stainings of sequential sections. After ROI definition, a MICS run with 87 different antibody conjugates was performed followed by data analysis with the MACS iQ View Analysis Software.

Results

In this project, the MACSima Platform was used to acquire images of 87 different markers from Miltenyi Biotec on postmortem human MS brain sections (fig. 2–4), allowing deep phenotyping of multiple cell populations within a spatial context. The visualization of vessels, astrocytes, oligodendrocytes, neurons, and microglia revealed detailed images of the MS lesions and surrounding tissue.

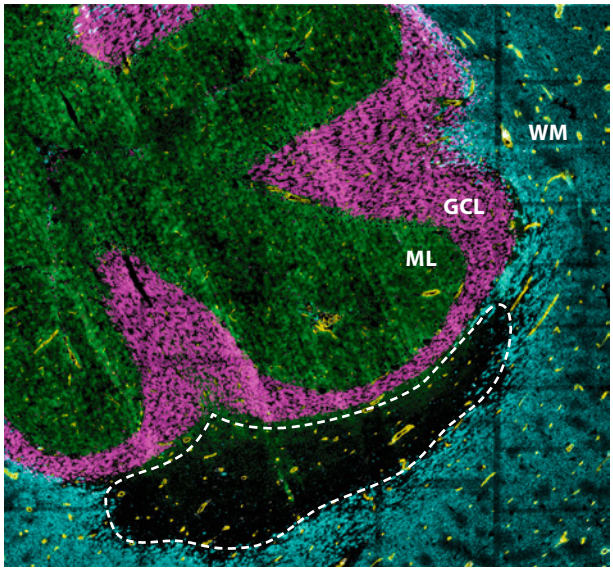

Figure 2: Overview of lesion site in the cerebellum. GLAST-positive Bergmann glia, located in the Purkinje cell layer and extending into the molecular layer, are visible in green. Neuronal cell bodies in the granular cell layer were stained with an Anti-NeuN–specific antibody (magenta). A myelin (MBP, cyan) lesion is visible as the unstained region in the white matter and outlined. T cells (CD8, grey) are sparsely scattered through the lesion site. Vessels were stained with an antibody specific for CD29 (yellow).
ML = molecular layer, **GCL** = granular cell layer, **WM** = white matter.

A set of typical neural cell markers was chosen out of all 87 antibodies to be presented in exemplary images (figs. 2–4). An overview image of the cerebellum clearly showed a MS lesion in the white matter with the typical lack of myelin signal (fig. 2). The white matter, mainly consisting of myelinated axons, is indicated by the myelin basic protein (MBP) positivity in cyan, whereas NeuN-positive neuronal cell bodies, depicted in magenta, are shown in high density in the granular cell layer of the cerebellum. GLAST-positive Bergmann glia, which are located in the Purkinje cell layer and extend their processes into the molecular cell layer, are shown in green. The unstained area below the granular cell layer indicates the MS lesion in this sample. CD29-positive vessels in the white matter are shown here in yellow.

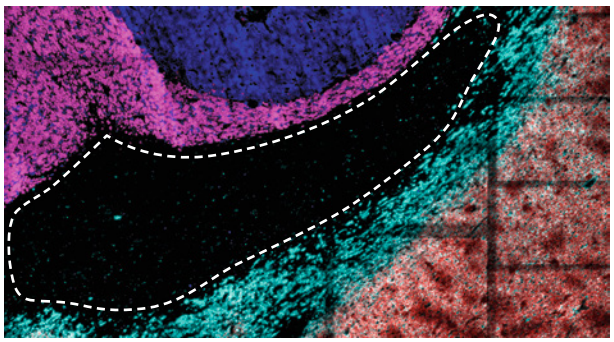

Figure 3: Myelin loss at lesion site. Loss of MBP (cyan) and PLP (red) staining indicates a demyelinated lesion. NeuN-positive neuronal cell bodies located in the granule cell layer are shown in magenta while synaptophysin-positive synaptic structures in the molecular cell layer are shown in blue.

A close-up of figure 2 shows complete lack of MBP staining (cyan) at the lesion site with interrupted staining surrounding the lesion (PLP, red; fig. 3). NeuN-positive neuronal cells are again depicted in magenta and synaptophysin-positive synaptic processes in blue.

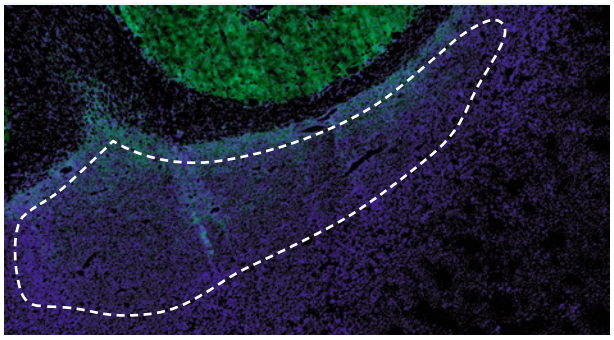

Figure 4: Astrogliosis at lesion site. Astrocyte staining with GLAST (green) for homeostatic Bergmann glia cells in the molecular layer and GFAP (violet) for reactive astrocytes in the white matter.

When looking specifically at astrocytes (fig. 4), a different staining pattern of the astrocyte-specific markers GLAST (green) and GFAP (violet) becomes visible. Reactive astrocytes that are characterized by high expression of GFAP, but are GLAST negative, are concentrated in the lesion site, indicating reactive gliosis in this area.

Analysis

The data was analyzed using MACS iQ View Analysis Software.

Usually, the first step in analyzing and quantifying spatial biology data in detail is the segmentation of the region of interest. For cell segmentation, the intuitive MACS iQ View Analysis Software offers an integrated tool that automatically segments the cells into nuclei and cytoplasm using proven and fast algorithms. However, neurons have complex and elongated shapes that are often present in several layers. Hence, super pixel segmentation was used instead of cell segmentation algorithms. In a super pixel analysis, the image is divided into a grid and an identity is assigned to every square/super pixel. From this, all squares are linked to specific values which can then be plotted, thus all features from every super pixel can be easily extracted.

To identify myelinated and demyelinated areas within the sample, a marker-based analysis was performed. For analysis, the molecular and granular cell layers were excluded due to no expression of myelin (black area). PLP is a known marker for myelinated structures and was plotted against beta-tubulin III which is commonly expressed in most neuronal cells. The plot shows two major peaks; the top consists of cells that express a high level of PLP and thus are myelinated, while the lower shows cells with a low PLP expression. By gating the cells into three different gates (high peak, intermediate cells, and low peak), the selected cells of the plot are equally highlighted within the sample image. This then allows for the spatial information of cells expressing high, moderate, and low PLP to be directly extracted.

By this means, the sample can be easily analyzed in a spatial and quantitative way. Low expressing PLP cells mark the lesion area (fig. 5B, magenta), cells surrounding the lesion area express moderate PLP (fig. 5B, yellow), and cells that show a high level of PLP depict the healthy, myelinated tissue (fig. 5B, cyan).

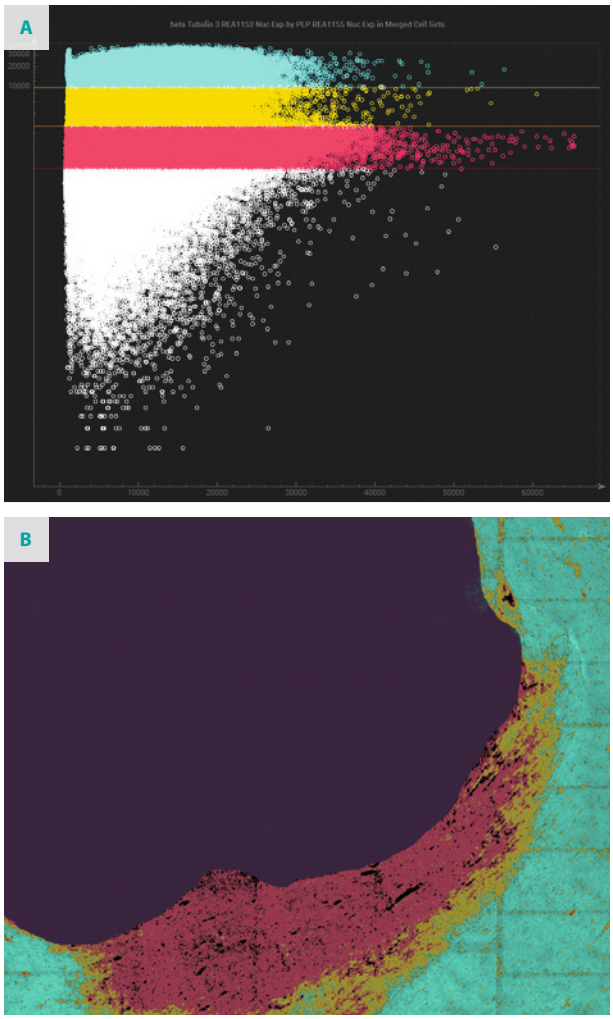

Figure 5: (A) Plot of PLP against beta-tubulin III. All white matter cells were plotted. The x-axis shows the expression level of beta-tubulin III while the y-axis shows the expression levels of PLP – a known marker for myelinated structures. Cells were gated into three regions – cells expressing high PLP (cyan), cells expressing a moderate level of PLP (yellow), and cells expressing low levels of PLP (magenta). **(B) Sample image showing the three gated areas of (A).** Cells expressing a high level of PLP show the healthy region of the tissue (cyan), cells that express a moderate level of PLP depict an intermediate area (yellow), and cells expressing a low level of PLP illustrate the lesion area (magenta). Cells from the black area were excluded from the analysis.

In the next step, heatmaps of all three regions (healthy (cyan), intermediate (yellow), and lesion (magenta)) were created to identify and compare the expression level of all markers in each corresponding region (fig. 6). This is an easy and effective method to identify new biomarkers or drug targets and to gain valuable insights of the composition of demyelinated (lesion) vs. myelinated (healthy) tissue.

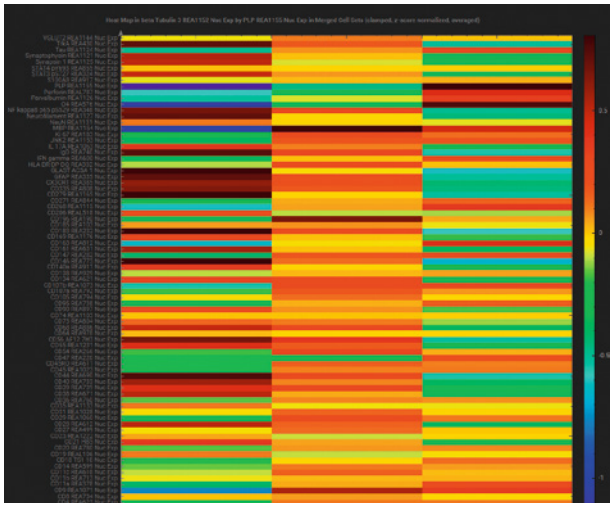

Figure 6: Heatmap of healthy, intermediate, and lesioned tissue areas. The x-axis shows the three different regions (healthy, intermediate and lesion areas), while the y-axis depicts the 87 markers that were analyzed. Dark violet/blue represents low marker expression while bright/red colors represent high marker expression.

Conclusions

Spatial biology is a new and evolving field in research that gives scientists the opportunity to image and visualize hundreds of markers in one single sample. Especially for rare and precious samples, like postmortem human brain samples, the staining of a high number of markers in one sample is of high importance. Here, we show that with the easy-to-use and fully automated MACSima System, combined with the powerful MACS iQ View Analysis Software, the expression of a multitude of markers can be analyzed in postmortem MS brain tissue in a detailed and precise way.

Using MICS technology and the MACSima System together with recombinant pretested REAfinity™ Antibodies provides clear signals to characterize the main neural cell types that surround MS lesions or are involved in lesion formation. In addition, many other markers can be detected for specific cell types that could help identify new biomarkers or drug targets for MS research.

By demonstrating different strategies for the analysis of MICS data, focusing on an unbiased epitope analysis, different areas of sample tissue were visualized and differentiated into healthy, intermediate, and lesion areas. Furthermore, heat maps of the three regions show expression levels of all stained markers.

These analysis strategies allow deep phenotyping that helped create a comprehensive cell map of brain lesions in MS patients and could lead to the discovery of new biomarkers and drug targets. Furthermore, such spatial biology strategies have extended the understanding of cell organization and interaction which could lead to the improved development of personalized and more efficient therapies.

Highlights:

- High-quality and single-cell staining of postmortem brain tissue with 87 antibody conjugates.
- Unbiased and qualitative analysis allows the identification of healthy and lesion areas in a spatial context.
- Quantitative analysis of marker expression by, for example, heatmaps, can lead to the discovery of new biomarkers and drug targets.

References

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Acknowledgements

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Appendix

Marker	Cell type	Clone	Product no.
MBP Antibody, anti-human/ mouse, REAfinity	Myelin/ oligodendrocytes	REA1154	130-120-341
PLP	Myelin/ oligodendrocytes	REA1155	130-120-273
GLAST	Astrocytes	ACSA-1	130-095-822
GFAP	Reactive astrocytes	REA335	130-118-489
NeuN	Neuronal cell bodies	REA1131	130-119-492
Synaptophysin	Neuronal cell bodies	REA1121	130-119-348
CD29	Endothelial cells	REA1060	130-118-122
CD8	T cells	REA734	130-110-681
Beta Tubulin 3	Neuronal cells	REA1152	130-120-265

Table 1: Selected markers (9 out of 87) that were used for MICS of MS brain samples.

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