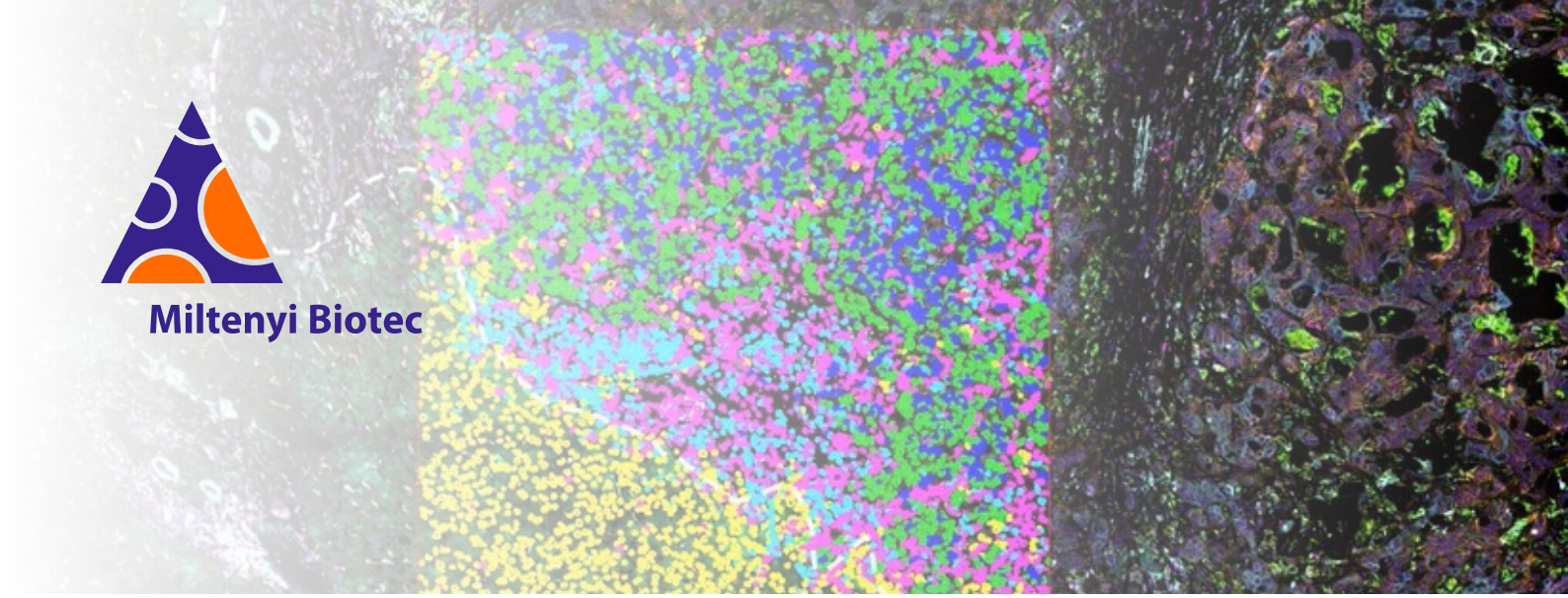

Miltenyi Biotec

Deciphering Cell–Cell Interactions in Liver Metastases

Dr. Marie Laviron

Advances in techniques such as single-cell transcriptomics have enabled researchers to better understand cellular functions. However, there is still a lack of knowledge about how cells are organized in their distinct microenvironmental niches, including the specific cell–cell interactions which determine cell identity within tissues. This application focus explores how spatial profiling with MACSima™ Imaging Cyclic Staining (MICS) technology can address this knowledge gap in liver metastases research.

Building a liver cell atlas with MICS

Understanding the liver environment in both healthy tissue and tumors requires the characterization of the signals defining the development, maintenance and function of each cell type. For instance, the resident macrophage population of the liver, Kupffer cells, are important for immune function. Their identity is programmed by the hepatic macrophage niche through signals delivered by neighboring cells, namely endothelial cells, stellate cells and hepatocytes (Figure 1).[1]

In 2022, a cell atlas of the liver was assembled, encompassing cell content, gene and protein expression and spatial arrangement for each cell type.[3] Cellular indexing of transcriptomes and epitopes by sequencing (CITE-Seq) was performed to gain information about both mRNA transcripts and protein expression, enabling the identification of population markers. Classical microscopy proved unsuitable for identifying the spatial distribution of these populations on such a large scale, due to the limited number of markers it can use. Instead, the MACSima Spatial Biology Platform was employed using MICS (MACSima Imaging Cyclic Staining) technology.

MICS technology is based on fluorescence microscopy and uses the principle of cyclic staining with fluorochrome-conjugated antibodies to acquire data simultaneously ranging from 15 to hundreds of markers on a single sample (Figure 2).

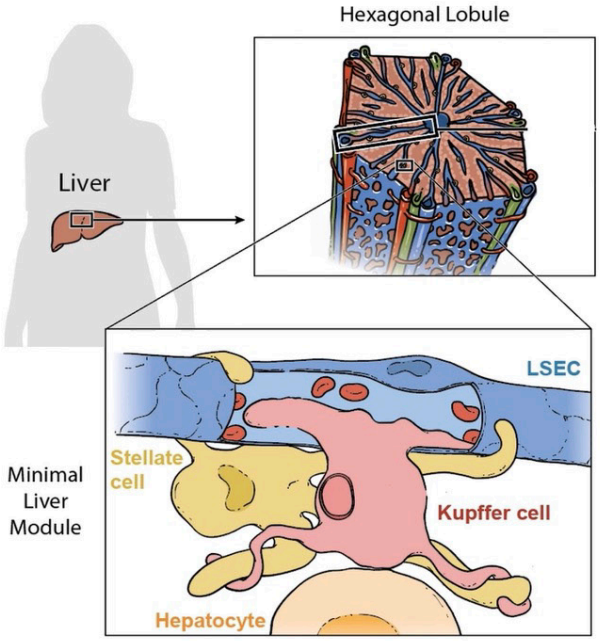

Figure 1. Liver cell types: liver sinusoidal endothelial cells (LSECs), Kupffer cells, stellate cells and hepatocytes. Credit: Adapted from Guilliams et al., 2020.[2]

Using a 100-plex protein panel, the different liver cell populations (immune and stromal) were spatially defined at single-cell resolution, visualizing their alignment to key liver structures such as the endothelium, the portal vein and the central vein. This wealth of information was key in defining the atlas at the spatial level and resolving the hepatic macrophage niche by characterizing cell–cell interactions.

Tip: When designing MICS experiments, it's a good idea to pre-test your antibodies using conventional epi-fluorescence and/or confocal microscopy. This will make panel design easier in the future.

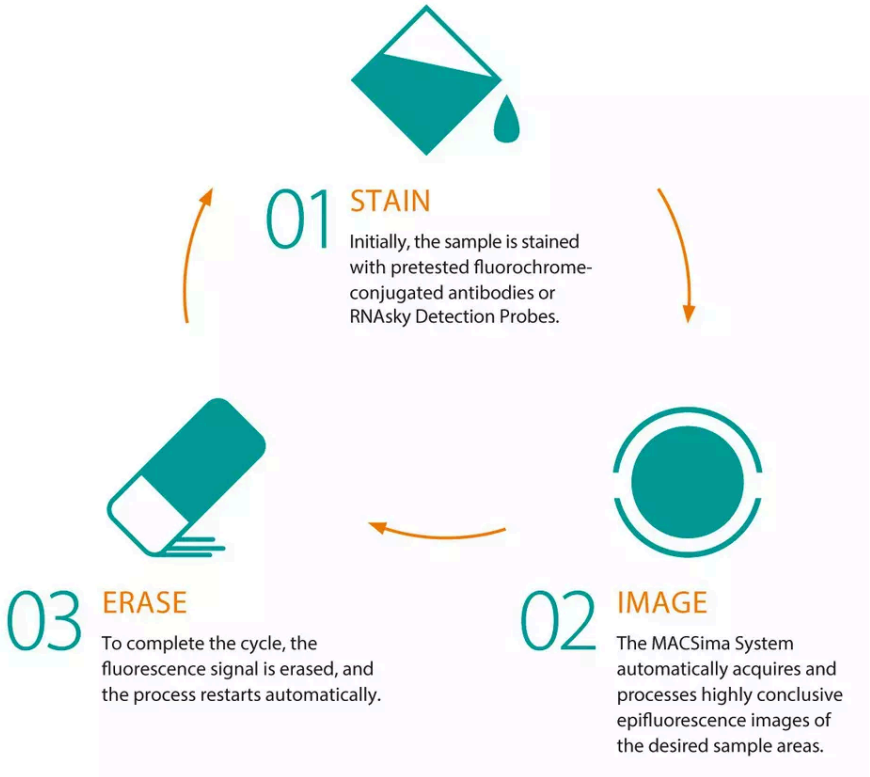

Figure 2. The principle of cyclic staining using MICS. Credit: Miltenyi Biotec.

Growth patterns of liver metastasis are associated with prognosis

Many primary cancers (most commonly, colorectal cancer) metastasize to the liver, further emphasizing the need to understand cell–cell interactions and how they are altered in the metastatic liver environment.[4] Colon cancer-derived liver metastases are difficult to detect early, and it has been shown that they impact both liver immunity and systemic immunity. This makes them highly challenging to treat and very unresponsive to immunotherapy.

Two distinct growth patterns are found in patients: desmoplastic and replacement (Figure 3). The desmoplastic pattern is characterized by a dense fibrotic frame, which is hypothesized to be either fibroblasts, or possibly dead hepatocytes, that are crushed together – separating the tumor tissue from the healthy tissue. In comparison, the replacement pattern mimics the liver architecture more, with less defined tumor tissue and fewer immune infiltrates.

The replacement growth pattern correlates with a worse prognosis than the desmoplastic one, so understanding what drives the formation of a certain pattern and uncovering the components involved in the structure of both growth patterns is key to advancing treatment.[5]

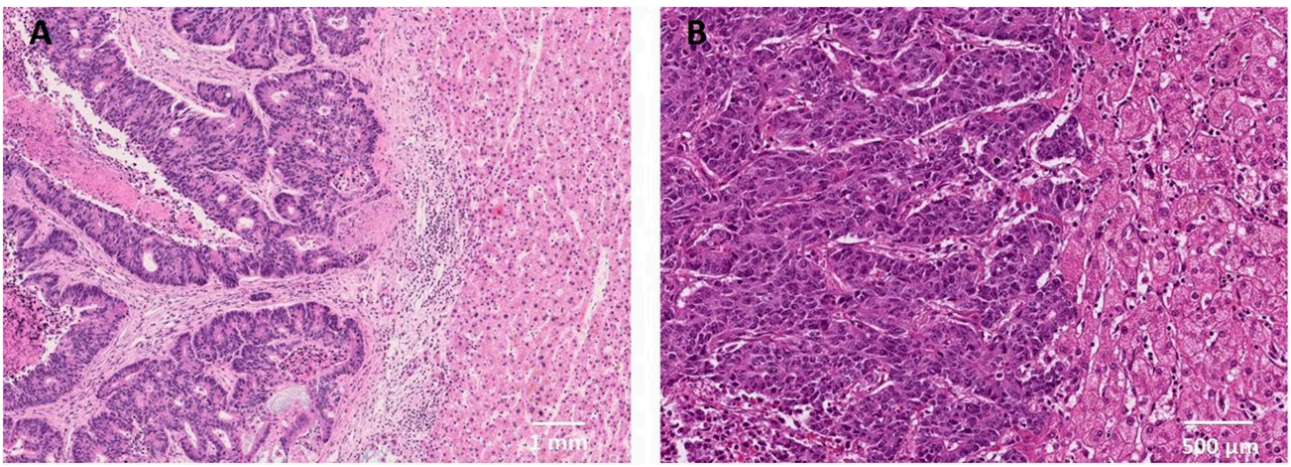

Figure 3. Histopathological growth patterns in liver metastases. (A) Desmoplastic pattern. (B) Replacement pattern. Credit: Bohlok et al., 2023.[5]

A hypothesis for the hepatic macrophage niche in tumor development

Confocal imaging was used to study the macrophage niche upon metastatic development (Figure 4A). Kupffer cells were only found in the healthy tissue and at the border of the metastatic nodule and very rarely in the metastatic nodule, due to signals that exclude them from infiltrating the tumor (Figure 4B). However, a population of monocyte-derived macrophages infiltrated the tumor, becoming tumor-associated macrophages (TAMs).

Stellate cells, the resident fibroblasts of the liver parenchyma, were also analyzed alongside a stain for general fibroblasts (Figure 4C). In the healthy tissue, stellate cells were present in the parenchyma as expected. However, fibroblasts recruited or derived from other structures were present within the metastatic nodule. At the border between the metastasis and the healthy tissue, the presence of both signals indicated that the fibroblasts derived from stellate cells had acquired a new morphology upon contact with cancer cells to support tumor growth. This activated phenotype is distinct from the stellate cells found in healthy tissue.

Endothelial cell staining was also performed to identify subsets specific to the metastasis (Figure 4D). The leading hypothesis was that endothelial cells could also be used to support metastatic nodule development, in the same way that stellate cells are co-opted by cancer cells.

Currently, there is no reliable marker to distinguish between potentially co-opted blood vessels in healthy tissue and newly formed blood vessels. However, these observations suggest that metastatic growth interrupts the formation of the distinct macrophage niche. For example, as stellate cells are co-opted by cancer cells, they could be responsible for delivering new signals to Kupffer cells at the tumor border.

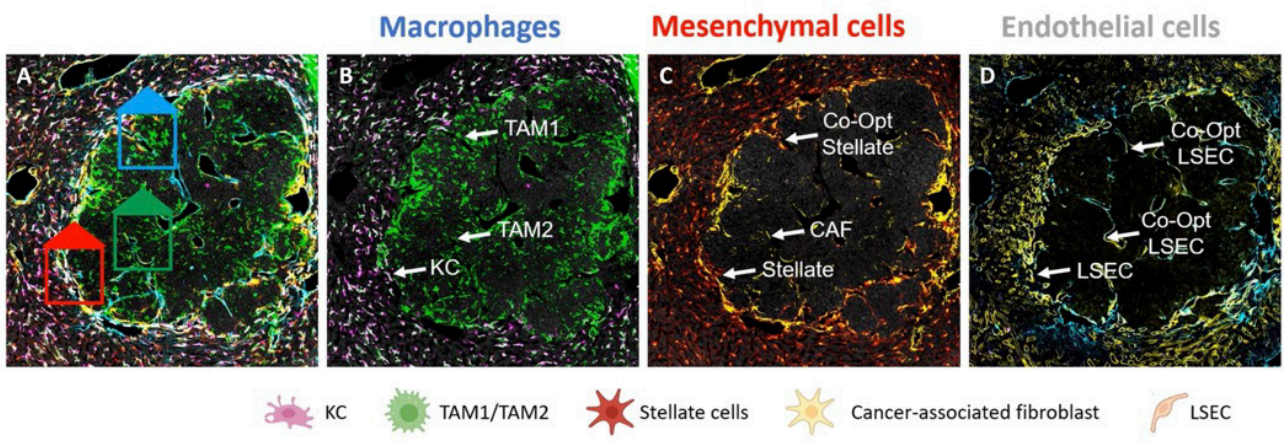

Figure 4. Confocal imaging of a metastatic nodule from a mouse liver, circled by a capsule of fibroblasts and surrounded by the healthy liver. (A) Macrophages, mesenchymal cells and liver sinusoidal endothelial cells (LSECs) overlayed. In red: normal Kupffer cells, stellate cells and LSECs that are bordering cancer cells. In blue: population of TAMs interacting with co-opted stellate cells and normal LSECs. In green: TAMs interacting with fibroblasts that are not derived from stellate cells and co-opted LSECs. (B) Kupffer cells (pink) and tumor-associated macrophages (green). (C) Stellate cells (red) and cancer-associated fibroblasts (yellow). (D) LSECs. Credit: Marie Laviron in collaboration with Peter Vermeulen.

Translating the hypothesis to the human liver

Since these observations were made in the mouse liver, it was unknown whether the hypothesis surrounding the modification of the macrophage niche was translatable to humans. To address this, MICS was performed on paraffin-embedded human metastatic liver samples, using a 200-plex tumor microenvironment panel to determine the different liver cells in the metastasis.

Data analysis was performed by using MACS iQ View Software for spatial biology. First, cells were segmented in the region of interest that encompasses both tumor and healthy tissues. Then, dimensional reduction and cell clustering were performed for a few populations to see if a pattern could be determined. The results reinforced the previous observations made about the spatial distributions of the immune subsets. Moreover, they also showed the infiltration of T cells and tumor-associated macrophage cells in the tumor (Figure 5), indicating a possible correlation between the distribution of macrophage populations and T cells.

To further investigate this possibility, correlation matrices were generated to determine the co-localization between the CD8 T cells and Kupffer cells or TAMs. Overall, the correlation between Kupffer cells and CD8 T cells was not significant. However, the clusters at the border of both populations could indicate a different activation status of Kupffer cells in that region, potentially revealing an interaction between both cell types.

Some tumor zones enriched for CD8 T cells were shown to colocalize with TAMs. Interestingly, the tumor-associated macrophages in close vicinity to CD8 T cells expressed a higher level of HLA-DR compared to the ones that are not in contact, suggesting that some macrophage subsets might be more involved in antigen presentation. Depending on how they are distributed in the tumor, this could give information about the growth pattern and the correlating immune activation in those different zones.

To investigate further, the organization and distribution of fibroblasts in patients with desmoplastic and replacement growth patterns were compared (Figure 6). In the central region of the tumor, the architecture was quite similar:

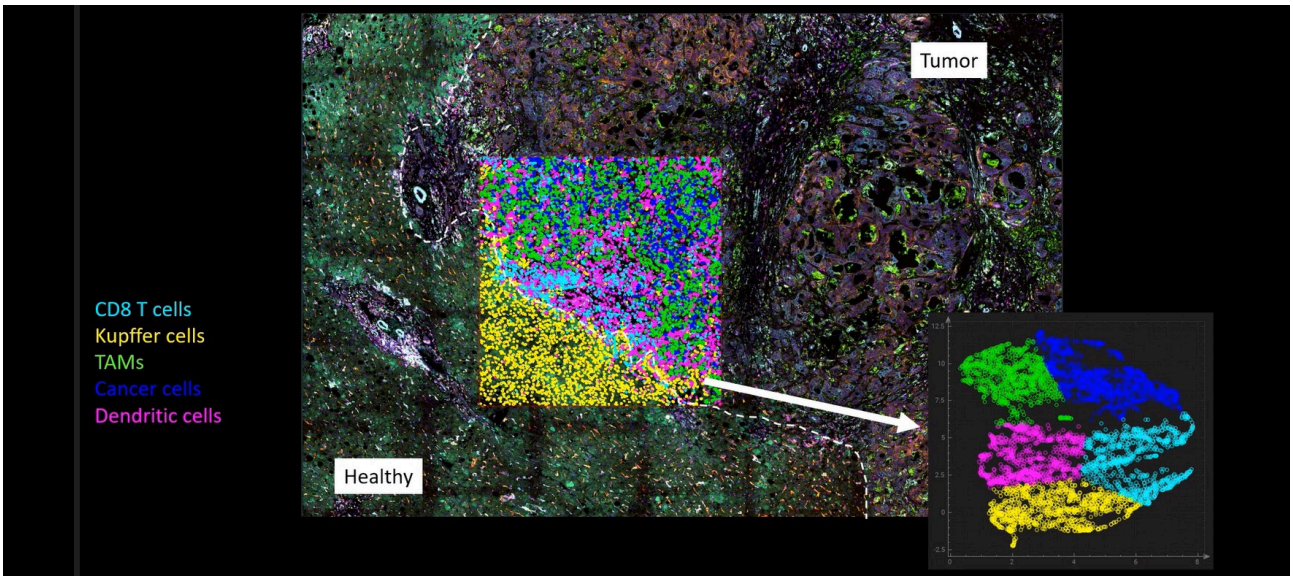

Figure 5. High-plex image of a metastatic nodule from a human liver taken by the MACSima platform and analyzed with MACS iQ View to create a UMAP. Credit: Marie Laviron in collaboration with Peter Vermeulen.

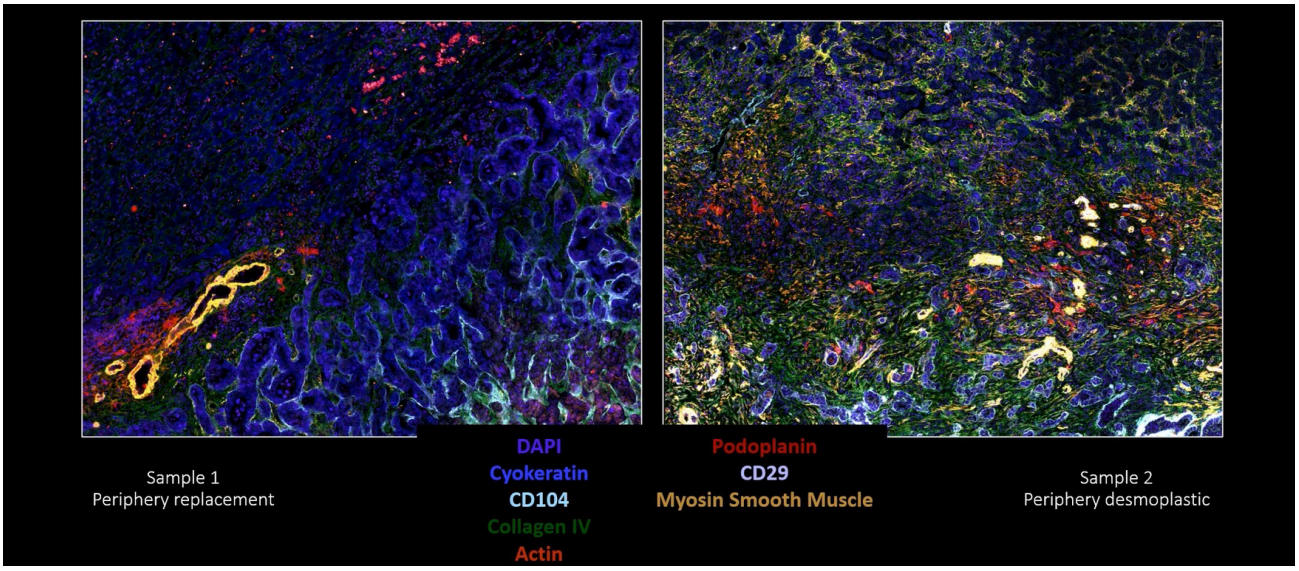

Figure 6. High-plex images taken by the MACSima Platform of human liver metastases samples from patients exhibiting replacement pattern (left) and desmoplastic pattern (right). Credit: Marie Laviron in collaboration with Peter Vermeulen.

fibroblasts and collagen deposition were widespread. However, at the periphery, the desmoplastic pattern showed significantly more collagen deposition and myosin accumulation (suggesting more fibroblast activation and accumulation). Thus, it was proposed that, in the desmoplastic pattern, a fibrous capsule could isolate the tumor and prevent it from further damaging healthy tissue. Whereas in the replacement pattern, cancer cells are more likely to invade the hepatocytes, as the boundaries are more ambiguous.

Optimizing automated cell analysis for growth pattern determination

The ability to screen samples with 200 markers at once is a powerful tool. Yet, to avoid human bias, it should be combined with automated analysis to determine the common features between patients exhibiting the same growth pattern. However, automated analysis of protein alone has difficulty achieving accurate cell segmentation in highly heterogeneous tissue where cells closely interact with each other. By also imaging mRNAs, users can overcome this problem and improve the identification of origin cells for activation markers, such as cytokines. Integrating the RNAsky assay with the MACSima Platform enables automated detection of both RNA and protein in the same section, thereby expanding the platform's capabilities to include spatial RNA detection and analysis within the MACSima spatial biology workflow (Figure 7 and 8).

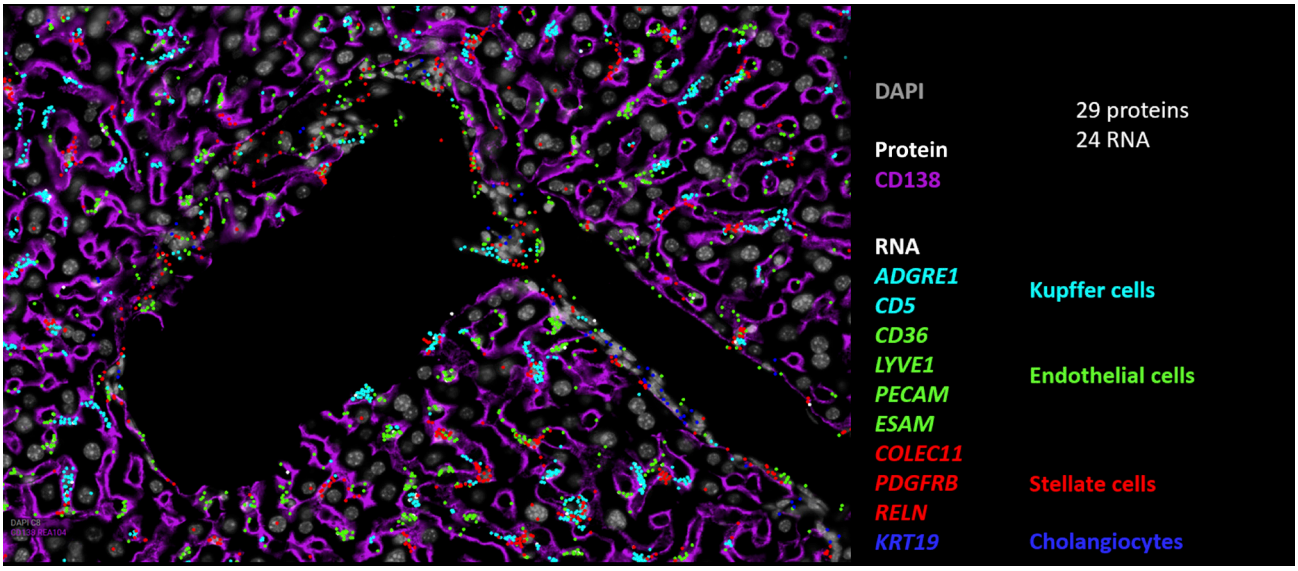

Figure 7. Same-section multiomics (RNA and protein) of a mouse liver FFPE sample. High-plex images were taken with the MACSima Platform using 29 antibodies and 24 RNAsky detection probes. Credit: Marie Laviron in collaboration with Peter Vermeulen.

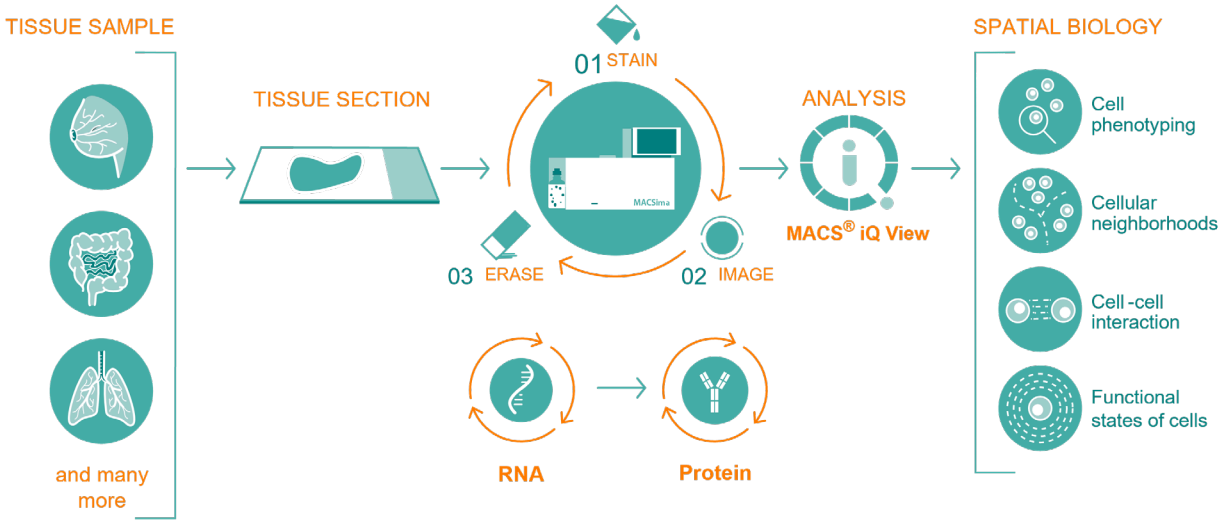

Figure 8. Multiomics workflow using MICS. Credit: Miltenyi Biotec.

The future lies in spatial multiomics

Combining information from RNA and protein can help resolve single-cell spatial organization and provide more information about the activation state of cells. This technology is already being trialed on human samples to better characterize the two liver metastases growth patterns. The specific activation profile of fibroblasts and macrophages – which is predicted to be key in determining growth pattern – is a focus of the ongoing research effort to improve prognosis and determine the best treatment for patients.

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Dr. Marie Laviron is a postdoctoral researcher in the laboratory of Professor Martin Guilliams at the Flanders Institute for Biotechnology (VIB) UGent Centre for Inflammation Research in Belgium, where her work focuses on the cells and interactions driving the immune response in the metastatic liver. She obtained her PhD in 2021 at Sorbonne University in Paris, where she studied the modulation of macrophage niches during tumor development and in response to chemotherapy.