Miltenyi Biotec

Spatial analysis of TILS in colon carcinoma

MACSima™ Platform

Background

Tumor-infiltrating leukocytes (TILs) are known to play crucial roles in tumor growth, cancer progression, and response to therapy[1]. Several studies have documented that the presence of activated and proliferating T cells within primary colorectal tumors is associated with improved survival[2]. Traditionally, the immunophenotyping of TILs in solid tumors has relied on resolving single cells by flow cytometry and immunohistochemical methods[1]. Flow-based methods can now record >30 channels per cell but lack the spatial information to distinguish infiltrated from non-infiltrated leukocytes. Immunofluorescence techniques provide spatial information but are typically limited to 4–6 channels[3].

Miltenyi Biotec's MACSima Imaging Cyclic Staining (MICS) technology (fig. 1) was developed to overcome these limitations and combine multiplexing with spatial context. MICS technology is based on an iterative cyclic staining process. This process is composed of three steps that are conducted in the MACSima System in a fully automated manner. First, a sample is stained with multiple fluorochrome-conjugated antibodies, produced and pre-tested by Miltenyi Biotec. This is followed by acquisition and subsequent processing of an image with a widefield microscope. To complete the cycle, the fluorescence signal is erased. This cycle is repeated automatically for as many times as needed, allowing a single sample to be stained with hundreds of markers for extensive spatial analysis. The MACSima System can analyze various kinds of fixed samples, ranging from tissue sections to adherent and suspension cells.

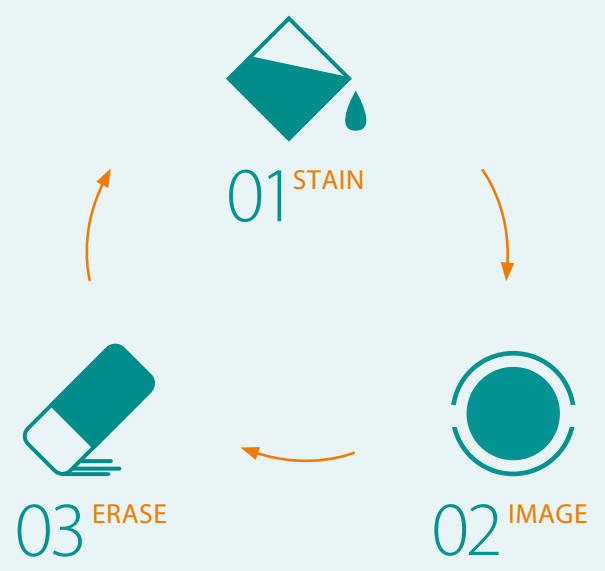

Figure 1: Schematic of the MICS principle. The MACSima System performs the three steps of the MICS technology in a fully automated manner: 1) The sample is stained, 2) imaged, and 3) the signal is erased. After a cycle is completed, the process automatically continues with the next cycle, enabling the analysis of hundreds of markers on a single sample.

Methods

Sample preparation

Fresh biopsies of colon carcinoma were "snap frozen" in freezing medium and sectioned with cryostat. The samples were fixed in 100% cold acetoneand stained with DAPI.

MACSima workflow

The antibody panel was defined and the antibody working solutions for staining was prepared in 96-well deep well plates. The prepared sample and antibody plates were loaded in the instrument and regions of interest (ROIs) were selected. The experiment was started and the MACSima System performed the entire process in an automated manner. Once a cycle was completed, the corresponding images were directly processed and saved in the common TIF format, which gave the user the option for on-the-fly data analysis while the experiment was still running.

Results

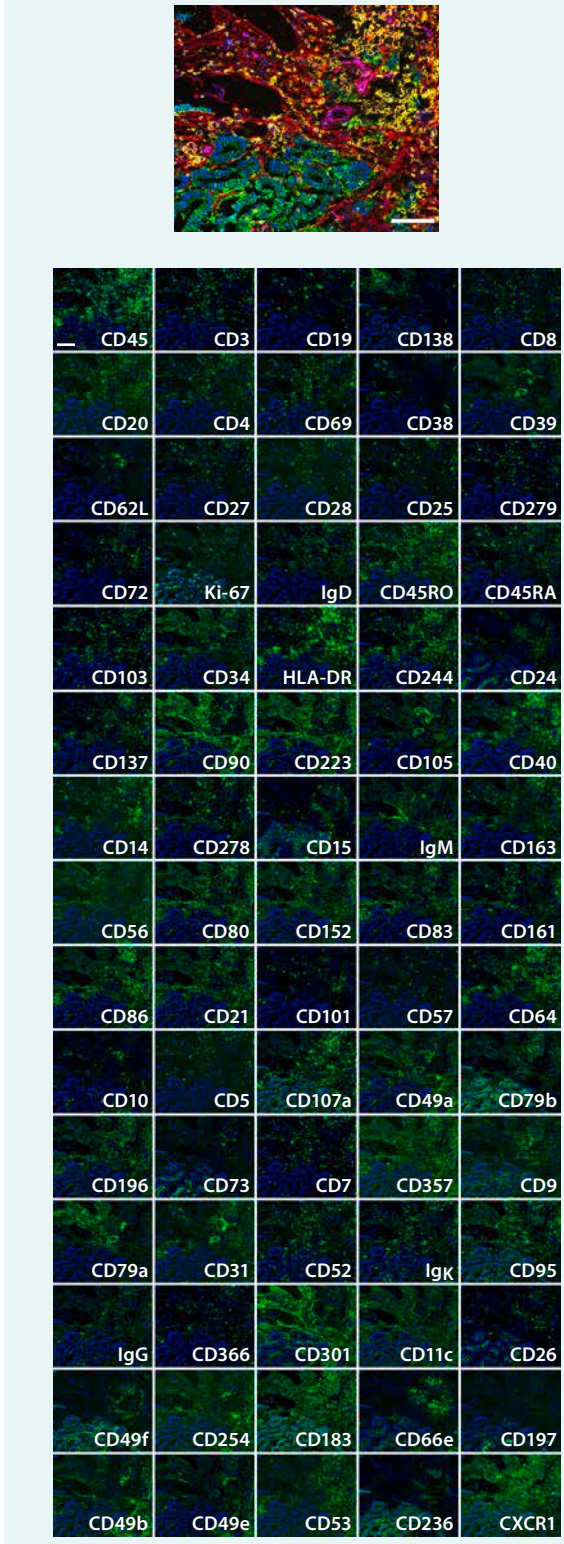

Figure 2: Colon carcinoma. Upper panel: Composite with four markers plus nuclear stain (DAPI – blue; CD45 – yellow; EPCAM – green; CD90 – red; CD31 – magenta). Lower panel: single-stain images of the 75 markers (green) acquired by a MACSima System counterstained with DAPI (blue). Scale bars: 100 µm.

In this experiment, the MACSima System acquired images of 75 different markers on a colon carcinoma section (fig. 2). The data were analyzed using our tailor-made MACS® iQ View Analysis Software. Integrated cell segmentation was performed to define the nuclei of each cell, as well as the cell contours. After the location and boundaries of each cell were defined, the cell-specific intensity profile of each marker could be extracted.

Based on the intensity profile of the cells, several distinct cell populations could be identified. Using this information, it was possible to identify the populations that could infiltrate the tumor region (fig.3).

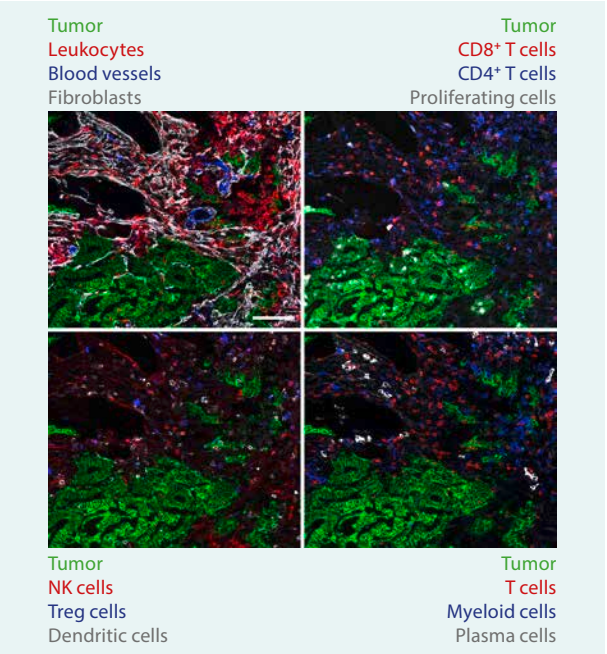

Figure 3: Identification of different cell populations in the colon carcinoma sample. Scale bar: 100 µm.

The identification of different cell populations in combination with the spatial analysis showed that some leukocytes were able to enter the tumor area. To further distinguish the infiltrating from the non-infiltrating cell population, we took a closer look at their respective expression profiles (fig. 4). Based on our results, seven markers were selected for further analysis after comparing the markers that were differently expressed in infiltrating vs. non-infiltrating leukocytes.

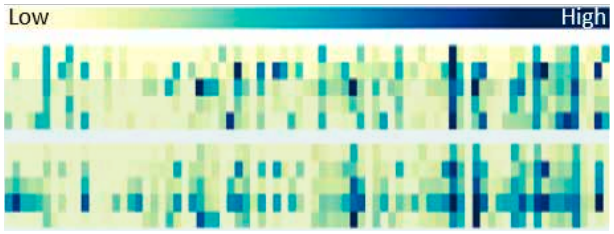

Figure 4: Expression profiles of infiltrating (upper panel) and non-infiltrating (lower panel) leukocytes. Marker names not disclosed because this data is part of an ongoing research project.

The chosen markers, as well as additional T-cell markers, were visualized in a close-up of the tumor region (fig. 5). We could observe that most TILs were $CD8^+$ T cells, and that Marker1, Marker3, and Marker4 were most promising targets for further analysis in the characterization of TILs in this colon carcinoma sample.

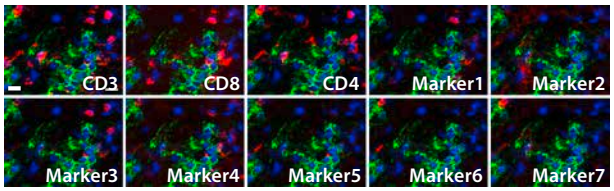

Figure 5: TIL characterization with different markers shown in red. Tumor cells are displayed in green and nuclei are displayed in blue. Some marker names are not disclosed because this data is part of an ongoing research project. Scale bar: 20 µm.

Conclusions

- The MACSima Platform preserves the spatial context of cells and markers within tissue samples. This spatial information is crucial for pathologists to comprehend the distribution and organization of cells within tissues, offering insights into disease pathology and progression.
- The MACSima Platform enables the simultaneous analysis of hundreds of markers (up to 75 in this study) on a single tissue sample. This comprehensive analysis facilitates the identification and profiling of various TIL subsets, providing a more detailed view of the immune response within the tumor.
- The MACSima Platform, as a workflow solution, assists pathologists in discovering new diagnostic or prognostic indicators and analyzing tissue architecture to gain a deeper understanding of disease mechanisms.
- The MACSima Platform provides a complete workflow solution that includes a wide range of validated antibodies, a fully automated imaging system, and advanced software for image analysis. This integrated approach simplifies the entire research process, from experimental design and sample preparation to data acquisition and analysis, ensuring a seamless and efficient workflow for acquiring and analyzing spatial data.

References

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3. Bendall, S. C. *et al.*(2011). Science. 6030:687–696. doi:10.1126/science.1198704

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