Miltenyi Biotec

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Same-section spatial multiomic analyses using MICS technology for investigating the dynamics of the tumor microenvironment

RNAsky Detection Probes

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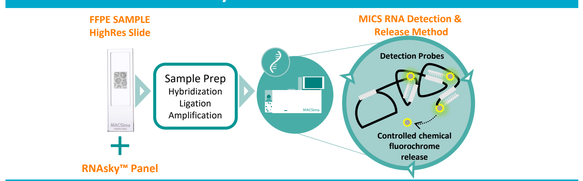
Abstract

The recent increase in image-based, spatially-resolved technologies enables researchers to profile the tumor microenvironment (TME) by capturing gene expression profiles within tissue sections. However, a significant limitation of these technologies is the lack of ability to resolve protein and RNA information in the same section, as well as conveniently analyze multimodal data sets. Here, we report a spatial RNA detection method, RNAsky, using Miltenyi Biotec's MACSima™ Platform as an automated, multiomic approach. Our method integrates spatial proteomics and transcriptomics data to provide in-depth profiling with single-cell resolution on the same tissue section. We demonstrate these capabilities by characterizing key immune-oncology markers across normal and diseased tissues. We investigated the impact of clustering using protein, RNA, or the combination to evaluate the contribution of different information modalities on TME spatial dynamics. This cutting-edge approach will enable the identification of valuable parameters and new cell types, furthering the discovery and development of predictive and prognostic biomarkers.

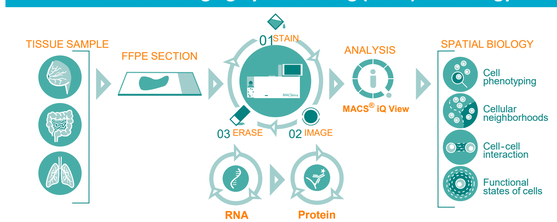
Introduction

Understanding the composition and architecture of cells within tissues is fundamental to studying tissue function, and more broadly, human biology. The complex dynamics of cellular functions and interactions between cell populations drive normal physiology, but dysregulation of these mechanisms cause diseases such as cancer. Here, we introduce RNAsky™, a targeted and amplified gene expression profiling assay designed for the MACSima platform. We combined this assay with multiplex protein imaging to generate multiomic datasets. We profiled colorectal and non-small cell lung cancer tissues using a targeted RNAsky Immuno-Oncology (I-O) Explore panel with additional customized gene targets and a selection of protein markers to look at the dynamics of the tumor microenvironment.

RNAsky™ molecular workflow



MACSima™ Imaging Cyclic Staining (MICS) technology

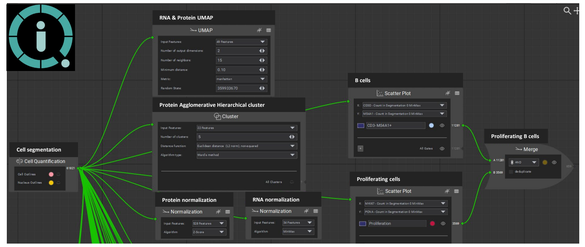


Methods and Materials

FFPE tissue sections were collected on HighRes glass slides, and samples were prepared following the RNAsky user guidelines. Multiplex transcriptomics and protein data acquired on the MACSima™ platform were combined with H&E staining collected on a single tissue section. The multiomic data were analyzed using Miltenyi image analysis software, MACS® iQ View.

In MACS® iQ View, transcripts were detected by an integrated algorithm, counts were normalized by a MinMax method, and gated for cell population analysis and transcript density maps. For hierarchical clustering analysis, Z-score normalized protein fluorescence intensity of each marker was used. The normalized protein intensity was combined with Log normalized RNA counts to generate UMAP plot.

MACS® iQ View Workflow Editor



Results/Discussion

1. Same section multiomics

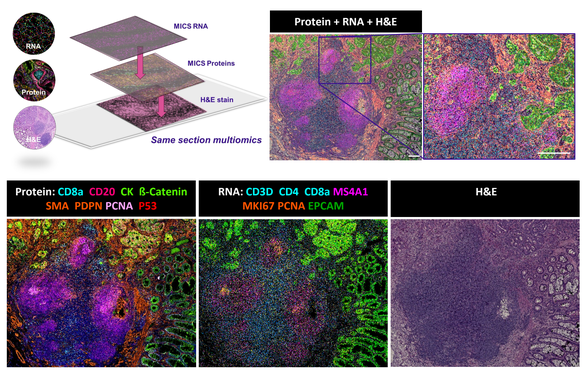


Fig 1. Miltenyi Biotec's multiomics technology combines quantitative gene expression profiles (mRNA) and protein signatures with classic H&E staining on the same section. In this example, a subset of the RNAsky I-O explorer panel and select proteins display a variety of immune, cancer, and proliferation markers. Scale bar = 200 µm.

2. Transcript density map analysis

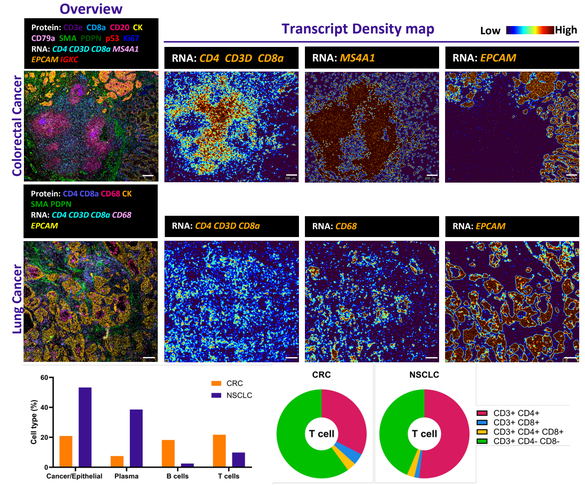
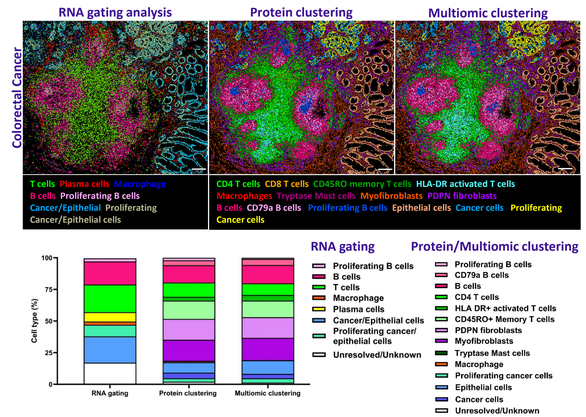


Fig 2. Identification of spatial transcript expression trends using density map analysis. Simultaneous multiplex imaging of protein and RNA targets shows complexity of the TME in colorectal and non-small cell lung cancer (overview). Gated RNA transcripts were represented by a density map to identify spatial expression patterns, where warmer colors (red, orange, yellow) represent a greater concentration of puncta. Based on expression levels of RNA transcripts, four different cell types were quantified in each cancer. For the T cell population, characteristic markers of T cell genes, *CD3D*, *CD4* and *CD8a*, were combined and represented in a density map. For B cells, epithelial cells, and macrophage, *MS4A1*, *EPCAM*, and *CD68* were used respectively. Cell type distributions were visualized as percentages of the total cells in the bar graph, and distribution of T cell subpopulations were shown in the pie chart. Scale bar = 200 µm.

3. Multiomic clustering analysis



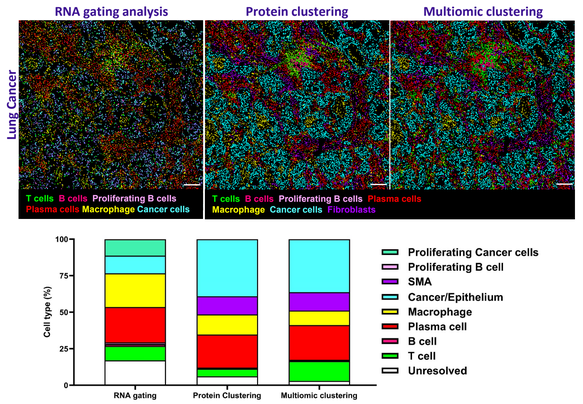


Fig. 3. Multiomic clustering analyses by combining spatial information of RNA and protein expression. For RNA gating analysis, transcript data was analyzed with a stringent gating strategy based on known gene relationships. An agglomerative hierarchical method was used for both protein and multiomic data clustering, using protein immunostaining intensity and RNA counts. Gated and clustered cell types are annotated in the images. Cell population abundance are represented in the stacked bar graph. Scale bar = 200 µm.

4. UMAP Visualization

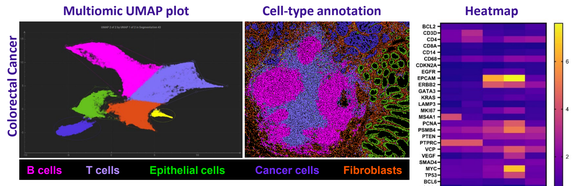


Fig 4. UMAP visualization of the main cell clusters in colorectal cancer. A UMAP plot was generated based on a combined dataset of 27 RNA targets and 22 protein markers. Using our gating strategy, all cells were classified into five different cell populations in the UMAP, and individual cells were mapped onto locations in the colorectal cancer to represent spatial organization. The gene expression profile of each cell population was shown in a heatmap. Scale bar = 200 µm.

Conclusions and Outlook

The MACSima™ Imaging Cyclic Staining technology provides the capability to image RNA transcripts and protein markers in a single tissue section, enabling spatial profiling of cell phenotypes. The resulting multiomic data allow for a deep dive into the spatial architecture and mechanisms of tumor progression, while providing the spatial context within which these events occur. In depth, single-cell resolution analyses on the same tissue section facilitate the visualization of spatial architecture and identification of key immune-oncology markers across normal and diseased tissues.