Novel fully automated multiomics assay for profiling the immune cell landscape and activation states

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Introduction

The immune system plays a critical role in combating several types of malignancies. Immunotherapies including checkpoint blockade therapies have utilized the innate ability of the immune system to identify and eliminate malignant cells. It has also been established that the profile of tumor-infiltrating lymphocytes (TILs) can to some extent predict patient response and overall survival. Traditionally, standard techniques such as flow cytometry and immunofluorescence have enabled successful assessment of tumor immune profile but identifying information about their activation states and cytotoxic effects has been challenging. Spatial technologies that enable the simultaneous detection of cytokines and chemokines with immune cell markers, can provide information about the immune cell composition and enable a comprehensive understanding of mechanisms underlying immune recruitment, infiltration and exclusion.

Methods

The fully automated spatial multiomics protocol on the COMET™ enables RNA detection using the RNAscope™ HiPlex Pro assay combined with protein detection using sequential immunofluorescence (seqIF™), (PMID: 37813886) to integrate same-section sequential detection of up to 12 RNAs followed by up to 24 proteins. This workflow allows the user to detect any RNA and protein target of interest by utilizing the vast catalog menu of RNAscope probes or generate a custom design for RNA targets and the use of standard, non-conjugated primary antibodies for protein detection. Here, we have demonstrated the detection of cytokine RNA targets *IFNG*, *TNFA*, *TGFB, IL6, IL8, IL10 and* 1 control RNA, *PP1B* along with CD3,CD4,CD8, CD20, CD11c, CD68, αSMA, S100, PD-L1, Vimentin and FOXP3 proteins to study immune cell activation in the melanoma tumor microenvironment. In addition, we were also able to demonstrate T cell recruiting by visualizing chemokines such as *CXCL10, CXCL9 and CCL5* and spatially map tumor infiltrating T cells within tissue context and with subcellular resolution. For images captured, background was subtracted, and contrast was adjusted.

FIGURE 1. Fully automated same-section multiomics workflow on COMET

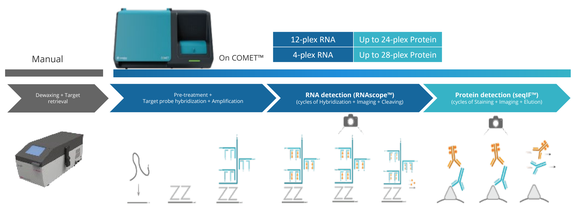


Figure 1: Schematic of the new automated workflow combining RNA and protein detection on COMET. A walk-away workflow that enables RNA detection with the RNAscope HiPlex Pro assay where all the steps after target retrieval are automated. This is followed by seqIF for protein detection.

FIGURE 2. Protease-free multiomic COMET workflow retains robust protein signal after RNAscope assay

Melanoma

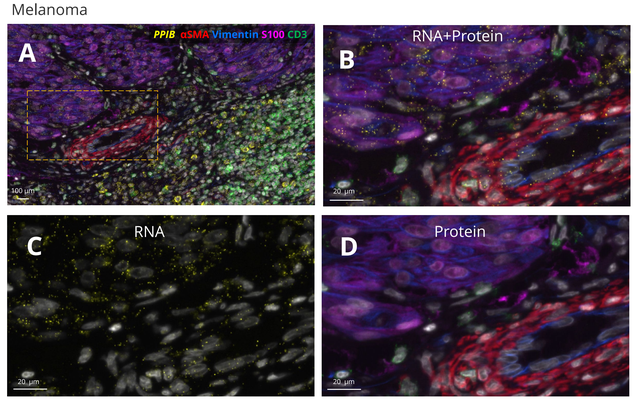
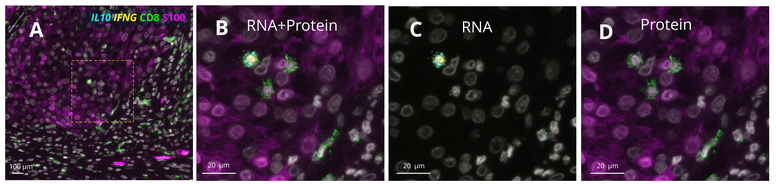


Figure 2: Sequential protein detection following RNAscope. A, The protease-free workflow on COMET enables successful detection of both protein and RNA signals as visualized with low (A) and high (B) magnification. C, RNA marker *PPIB* (yellow) was visualized with D, protein markers αSMA (red), Vimentin (blue), CD3 (green) and S100 (magenta). DAPI in gray.

FIGURE 3. Visualizing T cell sub-populations in melanoma tumor microenvironment

Cytotoxic T lymphocytes

Melanoma



Regulatory T lymphocytes

Melanoma

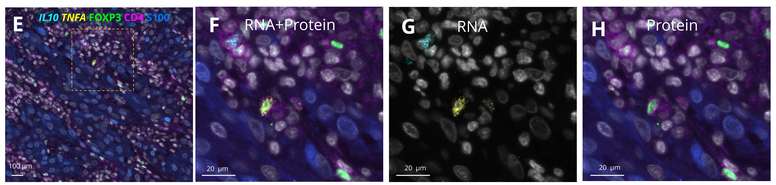
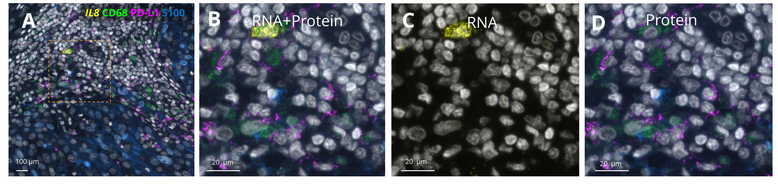


Figure 3: Simultaneous detection of RNA and protein markers enable immune cell phenotyping. A, Cytotoxic T cells were identified using CD8 protein (green) , *IFNG* (yellow) and *IL10* (cyan) cytokine RNAs . B, High magnification image shows a CD8/*IFNG*/*IL10* triple positive cell with C, cytokine RNAs and D, proteins, T cell marker CD8 and tumor/melanocyte marker S100. E, Regulatory T cells were identified using CD4 and FOXP3 proteins, *TNFA* (yellow) and *IL10 (*cyan) cytokine RNAs. F, High magnification image shows a CD4/*IL10* positive and CD4/FOXP3/*TNFA* positive cells with G, cytokine RNAs and H, T cell markers CD4, FOXP3 and tumor/melanocyte marker S100 proteins. DAPI in gray.

FIGURE 4. Cytokine expression reveals immunosuppressive signatures in multiple myeloid cells

Macrophages

Melanoma



Dendritic cells

Melanoma

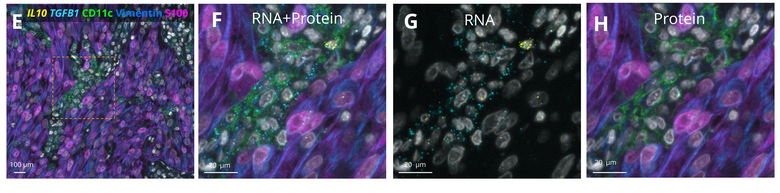


Figure 4: Macrophages and dendritic cells show an immunosuppressive gene signature. A, Macrophages were identified using CD68 protein (green) , PD-L1 protein (magenta) and *IL8* (green) cytokine RNAs . B, High magnification image shows a CD68/PD-L1/*IL8,* triple positive macrophage cell with C, *IL8* RNA and D, CD68, PD-L1 and tumor/melanocyte marker S100 proteins. E, Dendritic cells were identified using CD11c protein (green), *IL10* (yellow) and *TGFB1 (*cyan) cytokine RNAs. F, High magnification image shows a CD11c/*IL10*/*TGFB1* positive and CD11c/*TGFB1* positive cells with G, cytokine RNAs and H, CD11c and malignant melanocyte marker, vimentin proteins. DAPI in gray.

FIGURES 5. Detecting pro-inflammatory cytokine secreting B cells in melanoma tumor tissue

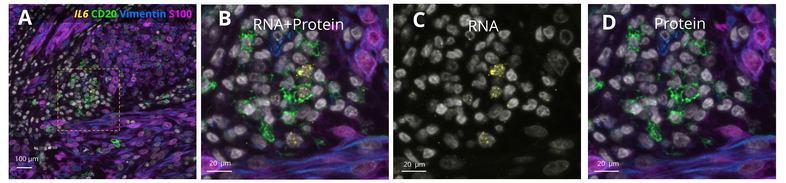
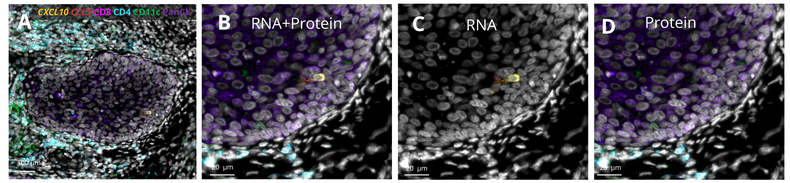


Figure 5: Pro-inflammatory B cells in melanoma tumor. A-B, B cells were identified with CD20 protein along with *IL6* cytokine RNA surrounded by malignant melanocytes marked by Vimentin (purple) and tumor/melanocyte marker S100 (magenta), C, cytokine RNA markers, D, cell marker proteins. DAPI in gray.

FIGURES 6. The new multiomic assay demonstrates immune cell recruitment in the presence of chemokines within the tumor

Infiltrating T cells and dendritic cells at the tumor-stroma interface

Head and Neck cancer



T cells and Dendritic cells

Head and Neck cancer

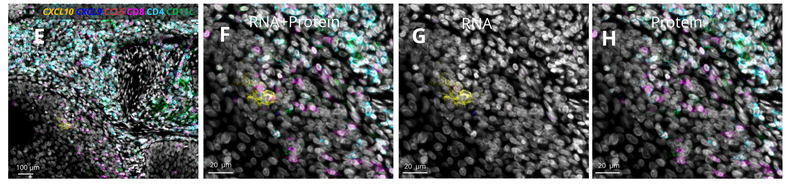


Figure 6: T cell and dendritic cell recruitment at the tumor-stroma interface. A, T cells and dendritic cells were identified using CD8 protein (magenta), CD4 (cyan) and CD11c (green) protein, respectively , tumor cells were detected using PanCK along with chemokine marker RNAs *CXCL10* (yellow) and *CCL5* (red). B, High magnification image highlighting PanCK/*CXCL10*/*CCL5* positive tumor cell with immune cells infiltrating in the surrounding, C, cytokine RNA markers, D, protein cell markers. E, T cells and dendritic cells were identified using CD8 protein (magenta), CD4 (cyan) and CD11c (green) protein, respectively, along with chemokine marker RNAs *CXCL10* (yellow), *CXCL9* (blue) and *CCL5* (red), F, High magnification image highlighting cluster of CD4/*CXCL10* positive, CD8/*CXCL10* positive, CD8/*CCL5* positive T cells and CD11c/*CXCL9* positive dendritic cells, G, cytokine RNA markers, H, protein cell markers. DAPI in gray.

Summary

- The HiPlex Pro assay on COMET was able to detect key cytokine markers within the tumor-immune landscape in melanoma tissue.
- The single-cell resolution of the HiPlex Pro workflow on COMET enables robust immune cell profiling to identify sub-types implicated in disease initiation and progression.
- The multiomics workflow was successfully able to demonstrate the detection of chemokines involved in T cell and dendritic cell recruitment which is crucial for activation of the anti-tumor adaptive immune response.

Conclusions

The use of RNAscope HiPlex Pro on COMET along with seqIF allows true multiomic analysis with simultaneous visualization of RNA and protein targets for immune cell profiling. Detecting cytokine and chemokine expression provides vital information about immune cell activation and recruitment, thereby increasing our understanding of phenomenon such as immune exclusion which is key in establishing predictive signatures for immunotherapy response.

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