High-Plex Co-Detection of RNA and Protein to Explore Tumor-Immune Interactions Utilizing RNAscope With Imaging Mass Cytometry

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Introduction

The next breakthroughs in immuno-oncology will be driven by high-plex tools that decipher the spatial arrangement of different cell types within the tumor microenvironment (TME). Imaging Mass Cytometry™ (IMC™) is a proven tool for the study of complex cellular interactions in the TME. It utilizes CyTOF® technology for simultaneous assessment of 40-plus protein markers at subcellular resolution without spectral overlap or background autofluorescence, providing unprecedented insight into the organization and function of the TME. Despite this, some protein targets are challenging to include in IMC as they have very few or no commercial antibodies available. Moreover, although cellular identity can easily be deciphered through detection of protein targets, knowledge of the cell's transcriptome improves understanding of cellular function and activation state. Here we present a robust and reliable workflow that combines the highly sensitive and specific RNAscope™ technology for RNA detection with the multiplexing capability of IMC to visualize key RNA and protein markers in the same tumor samples. The RNAscope HiPlex Flex Assay v2 was combined with protein detection using IMC to evaluate expression of both RNA and protein targets in formalin-fixed, paraffin-embedded (FFPE) tumor tissue microarray (TMA).

Applications of RNA and protein co-detection

- Biological insights can be gained through multi-omic phenotyping of cells in standard FFPE tissue samples.
- Targets that were previously inaccessible via IMC due to a lack of commercial antibody availability can now be identified. This can assist investigation of chemokine milieus in melanoma, for example.
- Understanding of cellular function and activation state can be improved through identification of a cell's transcriptome. This can advance investigation of T cell abundance around chemokine-expressing cells in breast cancer stroma, for example.

Conclusions

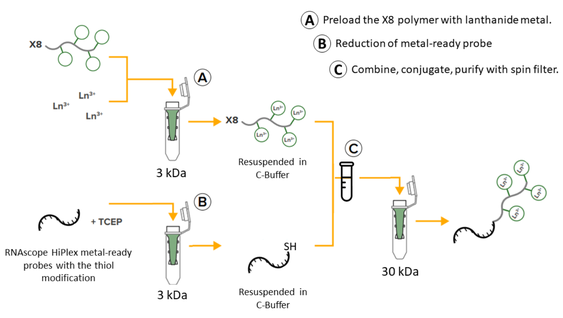
- Using RNA-protein co-detection with IMC, we identified that the pro-angiogenic factor VEGFA was present within the tumor compartment and was responsible for tumor growth (clusters 6 and 11).
- Pro-tumor cytokines TNFα and TGF-β appear to induce epithelial-to-mesenchymal transition of breast cancer cells (cluster 12), while anti-tumor chemokines CXCL9 and CXCL10 seem to be expressed by macrophages (cluster 14).
- Here we showcase a simple 3-step procedure for co-detection of RNA and protein targets within the same FFPE tumor samples.
- With our procedure, an investigator can visualize 40-plus markers, 12 of which can be used for RNA detection.

Methods

RNAscope HiPlex Flex protocol for Imaging Mass Cytometry

3-step procedure overview

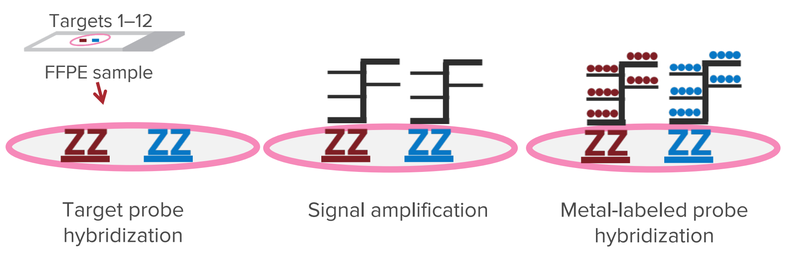
Step 1: Labeling detection oligonucleotides with metal tags for IMC



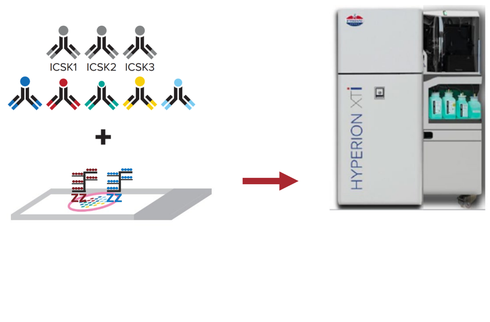
RNAscope HiPlex metal-ready probes with the thiol modification must be conjugated to metal tags (selected by the user/investigator).

The workflow schematic outlines how the existing Maxpar® Antibody Labeling User Guide (PRD002) was adapted for conjugation of detection oligonucleotides. Here, the metal-ready probes were reduced with TCEP and mixed with metal-loaded X8 polymer. The final conjugated product was purified using a 30 kDa spin filter.

Step 2: Modified RNAscope HiPlex Flex Assay v2 with metal-labeled probes



Step 3: IMC antibody staining overnight



After following the RNAscope HiPlex Flex Assay workflow, FFPE tissues can be stained with metal-labeled antibodies overnight at 4 °C. Here, we utilized 4 Maxpar Human Immuno-Oncology IMC Panel Kits, available from Standard BioTools™, to stain/detect specific protein targets in FFPE tissues. Subsequently, the Hyperion XTi™ Imaging System was used to acquire IMC data.

Results

Co-detection of RNA and protein in human breast cancer

Check the quality of tissue using positive control probes.

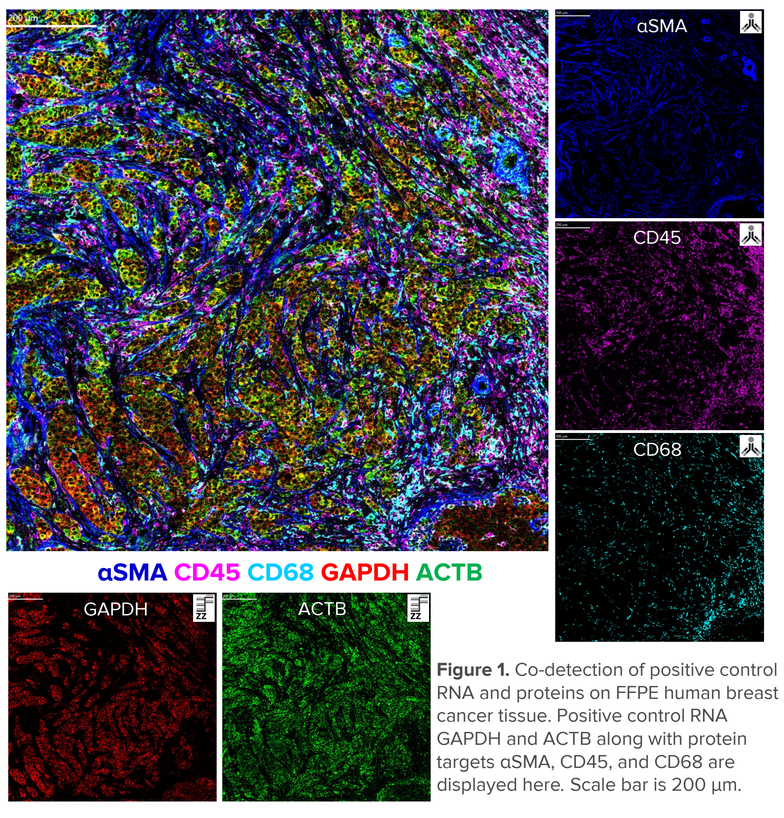


Figure 1. Co-detection of positive control RNA and proteins on FFPE human breast cancer tissue. Positive control RNA GAPDH and ACTB along with protein targets αSMA, CD45, and CD68 are displayed here. Scale bar is 200 µm.

Perform co-detection using target probes.

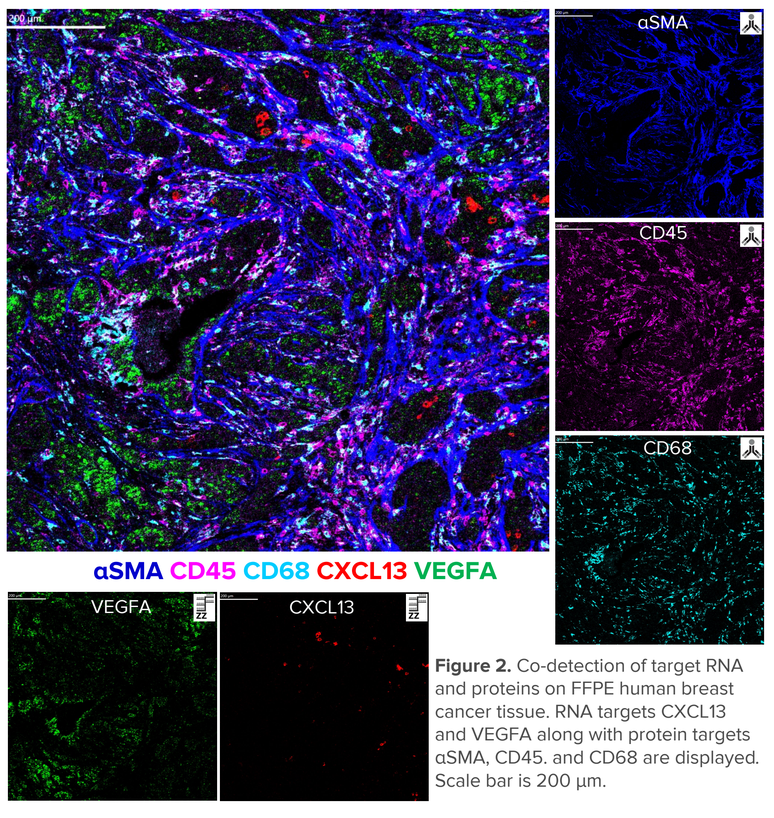


Figure 2. Co-detection of target RNA and proteins on FFPE human breast cancer tissue. RNA targets CXCL13 and VEGFA along with protein targets αSMA, CD45. and CD68 are displayed. Scale bar is 200 µm.

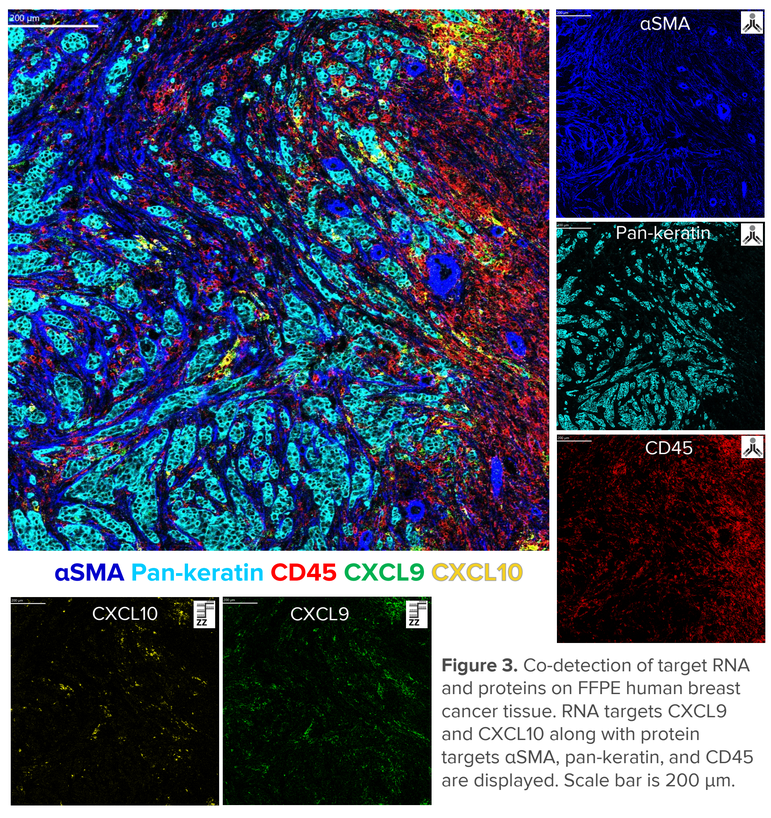


Figure 3. Co-detection of target RNA and proteins on FFPE human breast cancer tissue. RNA targets CXCL9 and CXCL10 along with protein targets αSMA, pan-keratin, and CD45 are displayed. Scale bar is 200 µm.

Analysis and cell identification using histoCAT

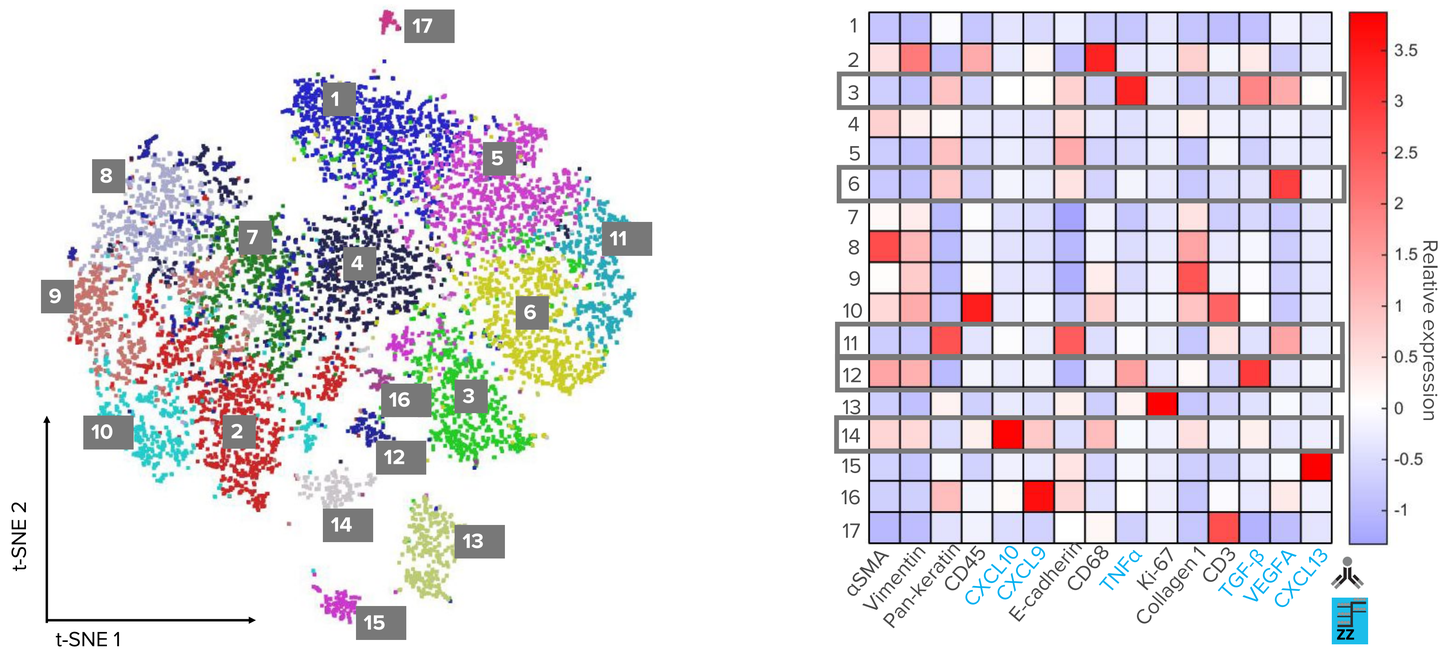


Figure 4. Heat map (right) of marker expression identified by PhenoGraph clustering` (left) on histoCAT™ using segmented cells (n=9,751). PhenoGraph clusters are numbered (1–17) on the Y-axis and marker names are indicated on the X-axis, where protein targets are in black type, RNA targets in blue.

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Link to technical note on the RNAscope protocol

Link to web page