



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

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A robust and reliable workflow combines RNAscope™ technology with the multiplexing capability of Imaging Mass Cytometry™ (IMC™) to **visualize key RNA and protein markers in the same samples**. Detection of protein targets and knowledge of the cell's transcriptome improves understanding of cellular function and activation state.

KEY TAKEAWAYS

- A three-step procedure enables the co-detection of RNA and protein targets within the same formalin-fixed, paraffin-embedded (FFPE) tumor samples.
- With this workflow, 40-plus markers can be visualized, 12 of which can be used for RNA detection.
- Biological insights into activation, proliferation and transcriptional activity can be gained through multi-omic profiling of cells in standard FFPE samples.

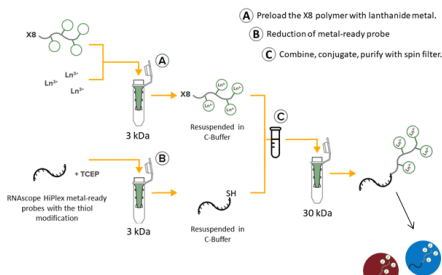
Background

Characterizing the tissue microenvironment has become a crucial step in understanding disease progression and response to immunotherapies. Combining spatial phenotyping with knowledge of the cell's transcriptome improves understanding of cellular function and activation state.

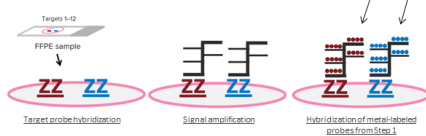
The RNAscope HiPlex Flex Assay v2 was combined with protein detection using IMC to evaluate expression of both RNA and protein targets in FFPE tumor tissue microarray.

Study Design

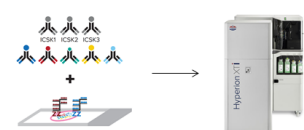
Step 1 – Labeling of Detection Oligonucleotides



Step 2 – Modified RNAscope HiPlex Flex Assay v2

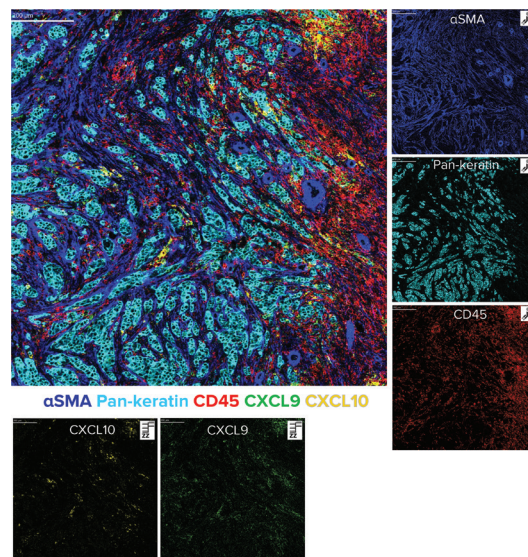


Step 3 – IMC Antibody Staining Overnight



Results

- Staining with RNAscope HiPlex 12 Positive Control Probes identifies 12 housekeeping genes, helping to confirm RNA quality in FFPE tissue.
- The pro-angiogenic factor VEGFA was present within the tumor compartment and was responsible for tumor growth in human breast cancer tissue.
- Pro-tumor cytokines TNF α and TGF- β appear to induce epithelial-to-mesenchymal transition of breast cancer cells, while anti-tumor chemokines CXCL9 and CXCL10 seem to be expressed by macrophages.



Co-detection of target RNA and proteins on FFPE human breast cancer tissue. RNA targets CXCL9 and CXCL10 along with protein targets α SMA, pan-keratin, and CD45 are displayed. Scale bar is 200 μ m.

CO-DETECTION

PROTOCOL

Co-Detection of RNA and Protein

Day	Step	Time
Step 1 – Labeling of Detection Oligonucleotides		
Day 1	Reduction of metal-ready probe	60 min
	Preload X8 polymer with lanthanide metal.	60 min
	Combine, conjugate, and purify with spin filter.	180 min
Step 2 – Modified RNAscope HiPlex Flex Assay v2		
Day 2	Prepare FFPE tissue sections.	
	Bake slides.	60 min
	Deparaffinize FFPE sections.	20 min
	Prepare pretreatment materials.	30 min
	Perform target retrieval.	15–30 min
	Create a barrier.	15 min
	<i>Optional stopping point</i>	
Apply RNAscope Protease III.	15–30 min	
	Proceed immediately to HiPlex Assay.	240 min
Step 3 – IMC Antibody Staining Overnight		
Day 2	Prepare metal-labeled antibody cocktail.	45 min
	Apply antibody cocktail to FFPE sections overnight.	10 min
Day 3	Slide washing and DNA intercalator stain	45 min
	Dry the slide, then proceed to IMC ablation.	30 min

Table 1. An estimated timeline of the workflow

Ordering Information | Standard BioTools™

Product Name	Product Number
Maxpar® Human Immuno-Oncology IMC™ Panel Kit	201508
Maxpar IMC Cell Segmentation Kit	201500
Maxpar X8 Antibody Labeling Kit	Multiple available

Ordering Information | Advanced Cell Diagnostics

Product Name	Product Number
RNAscope HiPlex 12 Positive Control Probes – Hs	324311
RNAscope HiPlex metal-ready probes T1-T12	322710
RNAscope HiPlex Flex Reagent Kit for IMC users	322725

For a full list of required consumables, reagents and equipment, refer to the **Co-Detection of RNA and Protein to Explore Tumor-Immune Interactions Utilizing RNAscope With Imaging Mass Cytometry Technical Note (FLDM-01182)**.



Scan the QR code to see the full digital version of the poster.

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