# Fully automated, novel protease-free workflow for co-detection of protein-protein interaction, individual proteins and mRNA using RNAscope Multiomic LS assay

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## INTRODUCTION

Background Protein-protein interaction (PPI) is one of the many mechanisms where individual cells understand and modulate the surrounding environment through communication with nearby cells or extracellular matrix. In many diseases including cancer, some PPIs have been identified to adversely impact immune response. One well-known example is the interaction between programmed cell death ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1). Tumor cells utilize PD-1/PD-L1 interaction to evade immune cell activities. While many immunotherapies targeting PD-1/PD-L1 blockade have been approved by FDA, there is a critical need for biomarkers that are more predictive of clinical outcomes than PD-L1 immunohistochemistry. Direct detection of PD-1/PD-L1 interactions in patient tissues in the context of a multiomic readout is likely to have better correlation to the therapeutic effect of checkpoint inhibitors than PD-L1 test alone and pinpoint specific tumor-immune cell interactions from accidental proximity.

We have developed a fully automated workflow that can visualize PPI with multiomic context of tumor-immune microenvironment (TIME) on a single FFPE tissue section. We observed PD-1/PD-L1 interaction, individual proteins, and mRNA at high spatial resolution in tumor tissues using new workflow enabled by high sensitivity and specificity of RNAscope™ technology.

Methods Oligonucleotide-conjugated anti-PD-1 and anti-PD-L1 primary antibodies or secondary antibodies were prepared to integrate the detection of PD-1/PD-L1 interaction or protein-protein proximity into RNAscope Multiomic LS assay. The protease-free workflow is fully automated and performed on BOND Rx instrument. New 6-plex workflow allows imaging of one PPI and any combination of up to 5 protein and mRNA targets of interest with tyramide signal amplification.

Results On human normal and cancer tissues, we show microenvironment surrounding the PD-1/PD-L1 interaction signals using oligonucleotide-conjugated primary anti-PD-1 and anti-PD-L1 antibodies, cell phenotyping protein markers for immune and tumor cells (CD4, CD8, CD68, and PanCK), as well as mRNA markers for secreted proteins such as chemokines, cytokines, or enzymes (*CXCL9*, *CXCL10*, *IFNG* or *GZMK*). The PPI signals appear as a group of punctate dots enabling semi-quantitative analysis. This assay is also capable of visualizing other protein-protein interaction or proximity using oligonucleotide-conjugated secondary antibodies.

Conclusion New protease-free RNAscope™ multiomics workflow is a powerful technique to resolve protein-protein proximity including PPI in the context of TIME. Spatial multiomic analyses of PPI will expand our knowledge of tumor immune evasion strategies and potentially offer new patient stratification strategy for checkpoint inhibitor immunotherapies.

## RESULTS

### 1. RNAscope Multiomic LS assay visualizes tumor-immune microenvironment surrounding PD-1/PD-L1 interaction on human cancer tissues

A. Immune response regulation by interferon gamma: Hodgkin's Lymphoma

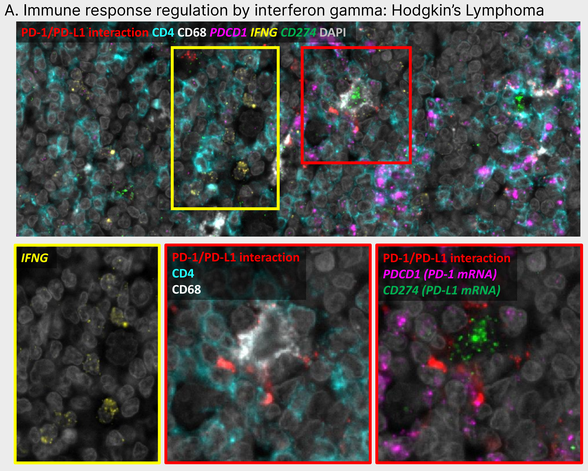


**Figure 1.** VIsualization of 6 markers including PD-1/PD-L1 interaction (PPI), 3 mRNAs targets (*IFNG*, *PDCD1*, and *CD274*), and 2 immune cell protein markers (CD4 and CD68) in Hodgkin's Lymphoma.

B. Immune cell recruiting chemokines (Non-small cell lung cancer)

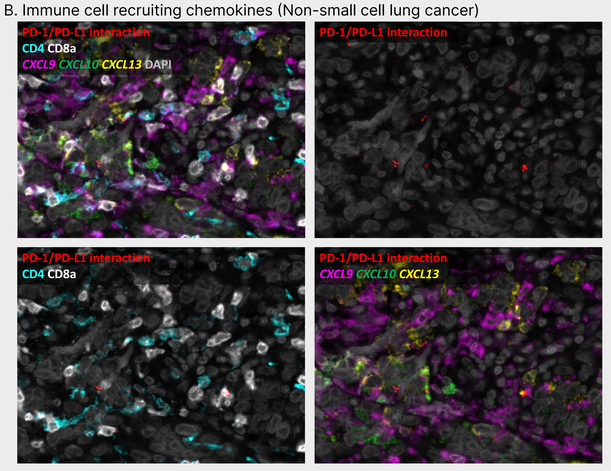


**Figure 2.** Visualization of 6 markers: PD-1/PD-L1 (PPI), 3 immune-cell-recruiting chemokine mRNA targets (*CXCL9*, *CXCL10*, and *CXCL13*), and 2 T lymphocyte protein markers (CD4 and CD8a) in non-small cell lung cancer.

### 2. Multiomic co-detection of protein-protein proximity/interaction using oligonucleotide-conjugated secondary antibodies

A. Cytotoxic T cell activation by MHC class 1

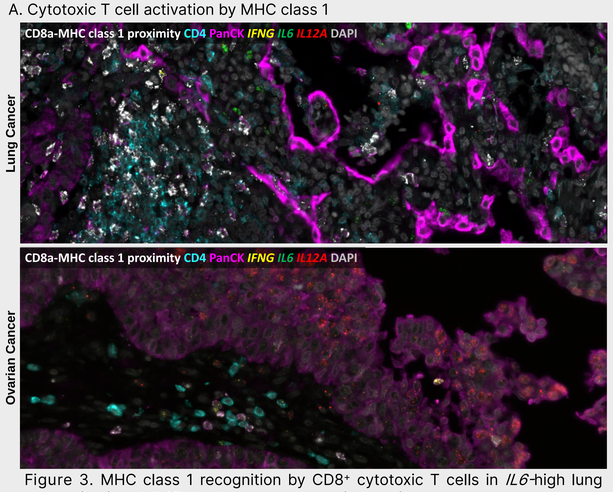


Figure 3. MHC class 1 recognition by CD8⁺ cytotoxic T cells in *IL6*-high lung cancer (top) and *IL12A*-high ovarian cancer (bottom).

B. EGFR-HER2 heterodimer

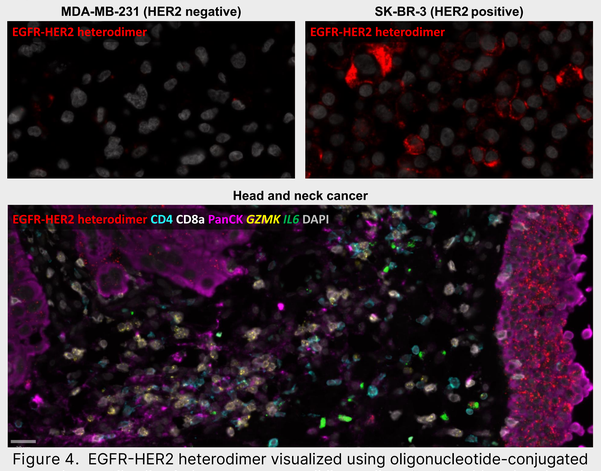


Figure 4. EGFR-HER2 heterodimer visualized using oligonucleotide-conjugated secondary antibodies in breast cancer cell line pellets (top) and head and neck cancer (bottom).

## METHOD


**Figure 5. RNAscope Multiomic LS Assay workflow.** FFPE sections are first pretreated, followed by application of conjugated antibodies and target RNA specific probes. RNA transcripts and protein-protein proximity/interaction appear as punctate dots.

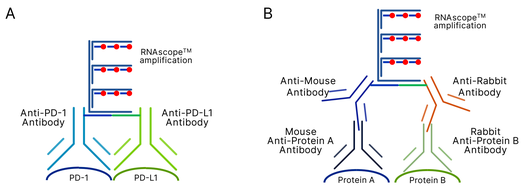


**Figure 6. Antibodies for protein-protein interaction/proximity detection.** (A) PD-1/PD-L1 interaction detected using oligonucleotide-conjugated primary antibodies targeting PD-1 and PD-L1. (B) Protein A-B proximity detected using oligonucleotide-conjugated secondary antibodies targeting mouse and rabbit antibodies.

## CONCLUSIONS

- This fully-automated assay on BOND Rx enables simultaneous detection of 1 protein-protein proximity/interaction and up to 5 targets of mRNA and individual protein on a single FFPE tissue section.
- PD-1/PD-L1 interaction is visualized at high resolution with oligonucleotide-conjugated primary antibodies using RNAscope™ Multiomic LS assay on human cancer tissues.
- Oligonucleotide-conjugated secondary antibodies provide flexibility to visualizing various protein-protein proximity of interest.
- This technology can provide meaningful insights into cancer immunotherapies targeting protein-protein interaction, and spatial progression of disease pathology.

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