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Integrated Spatial Multiomics Pipeline For Visualizing Protein Interactions In The Tumor Microenvironment

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Introduction

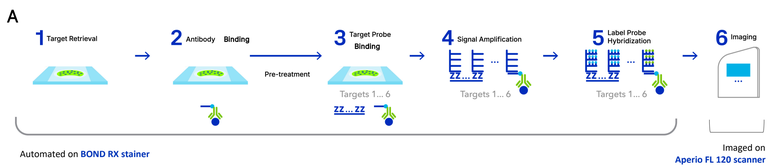
Protein-protein interaction is one of the many mechanisms where individual cells communicate with nearby cells or extracellular matrix to modulate the tissue environment. Visualizing these interactions using a spatial platform can validate known interactions implicated in disease pathology within relevant spatial domains, especially in cancer. We developed the ProximityScope™ assay that can be used with the RNAscope™ Multiomic LS assay to detect protein interactions and their impact on molecular pathways by simultaneously visualizing proteins and RNA on a single tissue section.

Here, we demonstrate a complete end-to-end workflow by staining FFPE human tonsil tissue samples to detect PD1-PDL1 proximity along with immune cell markers such as *PDCD1 RNA*, *CD68* RNA, CD4 protein, CD8 protein and PanCK protein. Images were acquired using the Aperio FL 120 by Leica Biosystems. Using the HALO® image analysis software from Indica Labs, we performed RNA and protein quantification as well as cell phenotyping. PD-1/PD-L1 proximity was successfully visualized between immune cells and tumor cells.

T cells were identified by either CD8 or CD4 protein detection, macrophages were identified with *CD68* RNA, while tumor cells were identified by PanCK protein staining. Interactions/proximity appear as punctate dots or dot clusters between two cells or on the cell surface.

This study demonstrates the importance of having an end-to-end spatial solution for staining, imaging, and quantification of target protein interactions to evaluate protein function. The ProximityScope assay can be used to study a broad range of protein interactions to evaluate signaling pathways and gain biological insights.

Method



C

RNA	Protein	Interaction
PDCD1, *CD68*	CD8, CD4, PanCK	PD1-PDL1

Figure 1: RNAscope Multiomic LS assay and ProximityScope workflow schematic. A, The Multiomic LS assay supports detection of RNA, protein targets and protein-interactions with TSA-based chemistry. The workflow run on the BOND RX research stainer involves antibody incubation followed by protease-free target retrieval , probe binding, signal amplification and detection. Images were scanned on the Aperio FL 120 scanner at 40x magnification. HALO® data analysis was performed on selected regions in the scan. **B**, Schematic for detection of PD1/PDL1 interaction using oligonucleotide-conjugated primary antibodies targeting PD1 and PDL1. **C**, List of target markers used on tonsil tissues.

Result

PD1-PDL1 proximity was visualized between immune cell and tonsillar epithelial cells in combination with RNA and protein markers

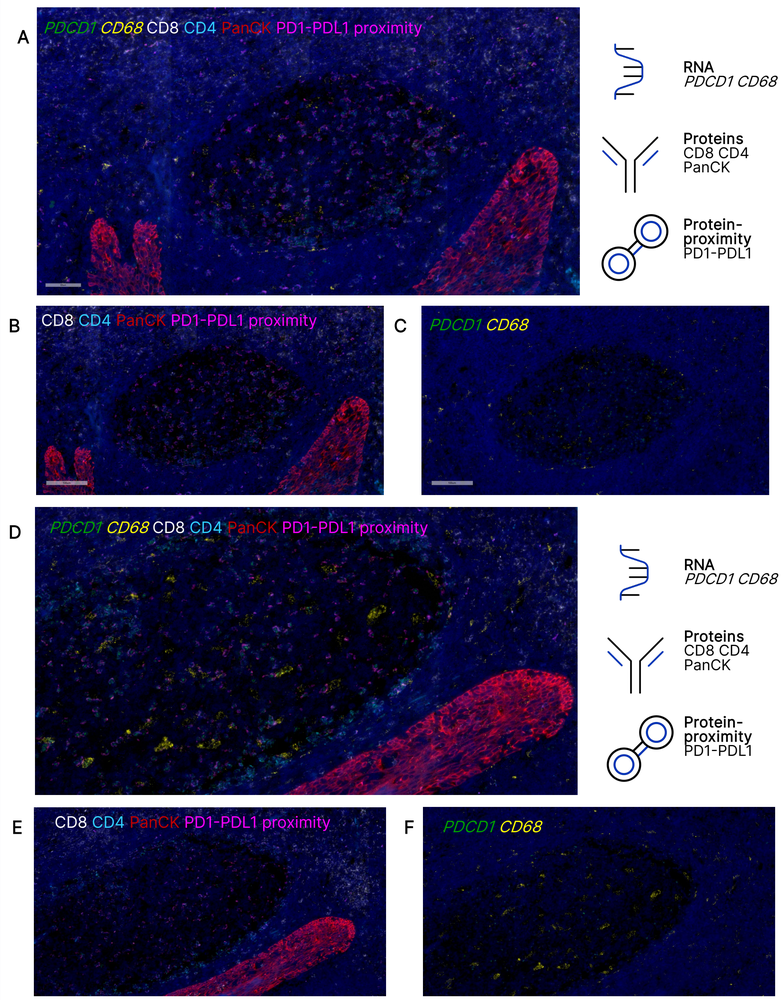


Figure 2: RNAscope Multiomic LS assay with ProximityScope to identify protein proximity in tonsil tissue. A and **D**, PD1-PDL1 protein proximity signal (pink) visualized with RNA markers *PDCD1* (green) and *CD68* (yellow). **B** and **E**, Protein signal for CD8 (white) and CD4 (cyan) visualized in and around the germinal center, PanCK signal visualized specifically in epithelial cells. **C** and **F**, *CD68* and *PDCD1* RNA signal visualized in and around the germinal center.

HALO AI based tissue classification enabled region specific quantification of PD1-PDL1 proximity

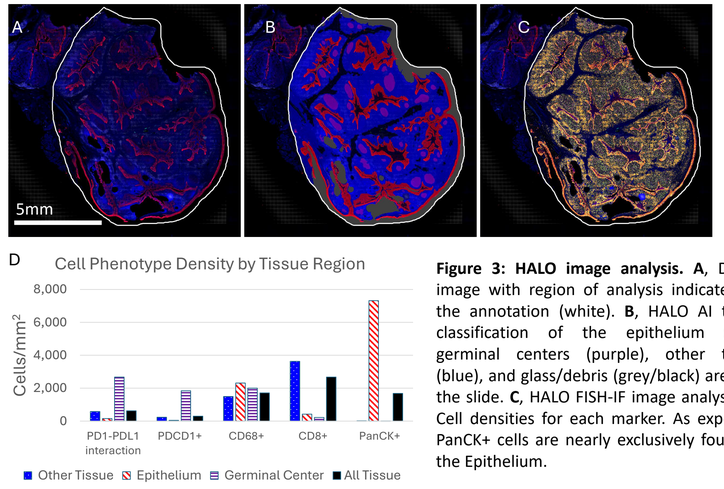


Figure 3: HALO image analysis. A, Digital image with region of analysis indicated by the annotation (white). **B**, HALO AI tissue classification of the epithelium (red), germinal centers (purple), other tissue (blue), and glass/debris (grey/black) areas of the slide. **C**, HALO FISH-IF image analysis. **D**, Cell densities for each marker. As expected PanCK+ cells are nearly exclusively found in the Epithelium.

PDCD1 RNA expression correlates with PD1-PDL1 proximity signal in the germinal centers

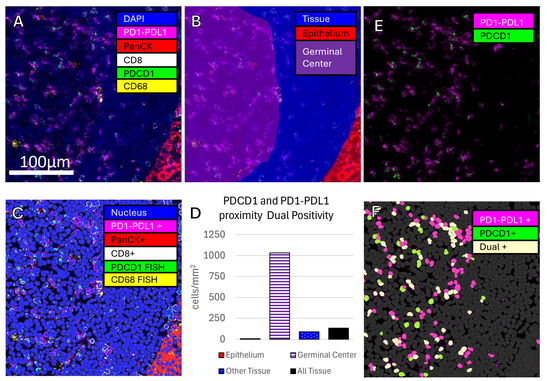


Figure 4: HALO FISH-IF image analysis results. A, 20X region highlighting an area containing the germinal center, other tissue, and epithelium. **B**, HALO AI tissue classification of the epithelium (red), germinal centers (purple), and additional tissue (blue) areas of the slide. **C**, HALO image analysis leveraging custom AI. For this assay, the proximity signal marker was analyzed as a protein marker due to the staining pattern for PD1-PDL1.

D, A higher density of PD1-PDL1 interaction and *PDCD1* dual positive cells are found in the germinal center tissue compartment (1,033 cells/mm²). **E** and **F**, 20X region of interest displaying PD1-PDL1 and *PDCD1* FISH staining and simplified HALO overlay. In the germinal center tissue, 39% of ProximityScope positive cells are dual positive for PDCD1.

Conclusion

The ProximityScope assay can be used to visualize protein-interactions such as PD1-PDL1 that provide functional insights to better assess tissue biology.

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