Rapid Antibody Drug Conjugate Markers and RNA Biomarkers in Solid Tumors Using a Hybrid Ultivue InSituPlex and Protease-Free RNAscope Workflow

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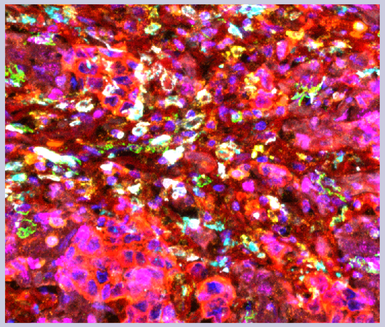

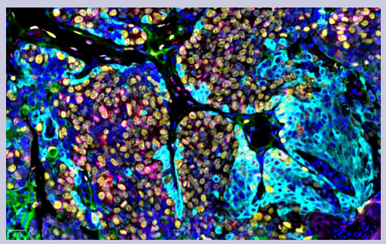

Conclusion: This innovative, combined Ultivue InSituPlex and protease-free ACD ISH assay provides a powerful, single-slide platform for spatially resolved multi-omic analysis. It overcomes the technical limitations of traditional sequential staining, offering a high-throughput, high-fidelity tool that can be rapidly deployed for complex biomarker validation, detailed tumor microenvironment characterization, and improved patient stratification strategies in translational oncology and clinical trials.

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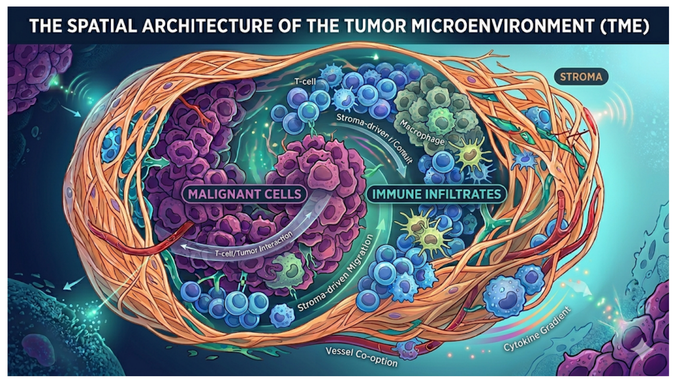
INTRODUCTION

The spatial architecture of the tumor microenvironment (TME)—defined by the interplay between malignant cells, immune infiltrates, and stroma—is a primary determinant of therapeutic response and patient prognosis. However, capturing this complexity with high fidelity remains a technical challenge:

- The Limitation: Traditional spatial profiling often relies on serial sections, which compromises precise cellular co-localization and structural alignment.
- The Barrier: Harsh processing steps frequently degrade RNA/protein integrity, limiting multi-omic insights from a single sample.
- The Need: We address the urgent requirement for single-slide, high-throughput methods capable of co-detecting protein and RNA with high fidelity and exact spatial registration.

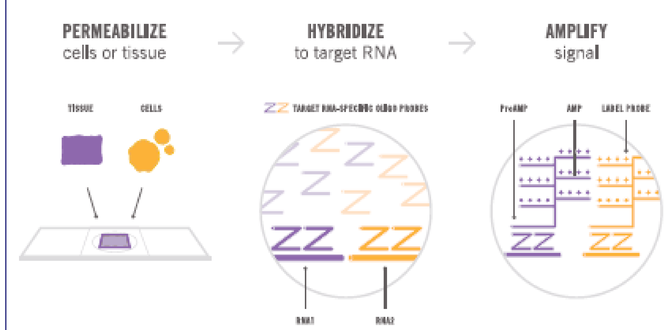


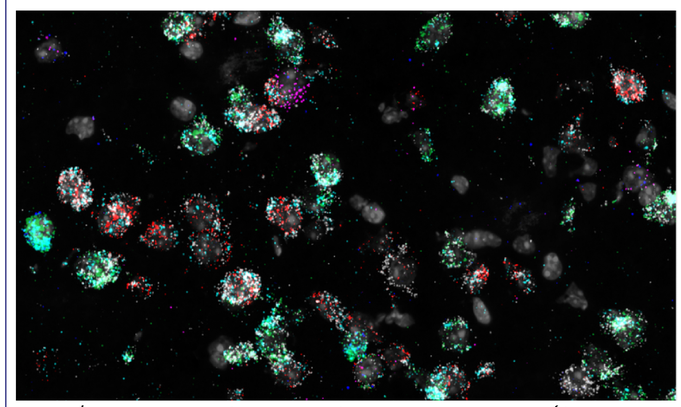
METHODS AND MATERIAL

We developed a streamlined, single-slide hybrid workflow integrating Ultivue InSituPlex (protein) and ACD RNAscope™ (RNA) technologies:

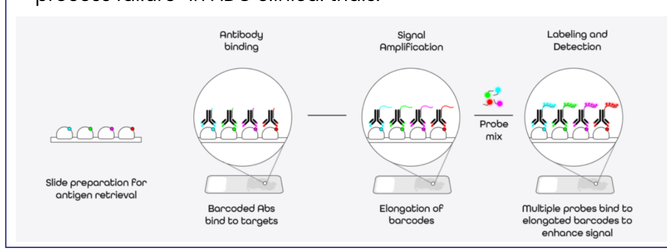
- Integrated Workflow: To preserve target integrity, we utilized ACD's protease-free ISH capability. This critical modification prevents the degradation of cell surface epitopes, ensuring robust downstream antigenicity for protein detection
- Panel Composition: The multiplex assay included antibody drug conjugate markers (e.g., HER2) via InSituPlex, co-detected with relevant transcriptional targets (e.g., mRNA) via ISH.
- Validation Suite: Applicability was demonstrated across multiple FFPE prepared cancer tissues.

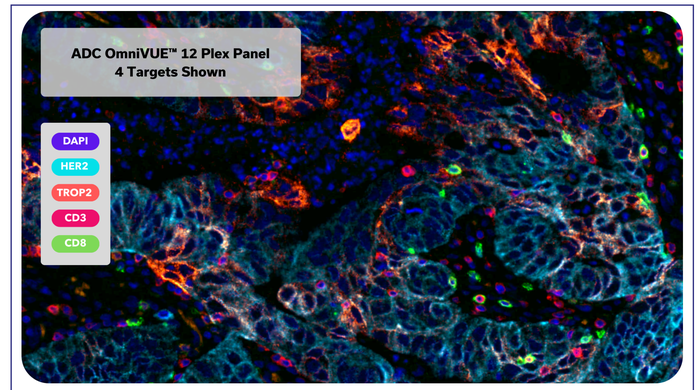
Figure 1. ACD based In-situ hybridization with protease free methodology

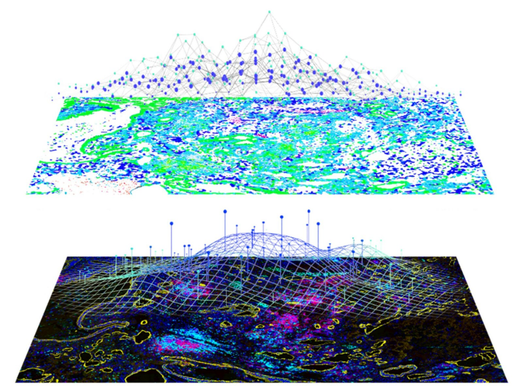




- TOP1 / TOP2A mRNA: If you are using a Dxd-based ADC (like Enhertu), running ISH for the TOP1 gene can show you if the cell has the machinery required for the drug to work. If a cell has low TOP1 expression, the ADC might bind, but it won't kill.
- CXCL10 / IFNG (Interferon Gamma): These ISH markers show an inflamed, "hot" tumor microenvironment. If the ADC is killing cells and releasing "danger signals," these mRNA levels will spike in the surrounding stroma, even in cells that the IHC showed were "negative" for the drug target.
- ABCB1 (MDR1) mRNA: This is the "vacuum cleaner" of the cell. If a cell has high ABCB1 expression via ISH, it is likely pumping the ADC payload out as fast as it comes in. This is a classic reason for "process failure" in ADC clinical trials.







Computerized alignment of DAPI from RNA image to Protein Image.

CONCLUSION

We successfully achieved simultaneous, high-resolution co-localization of up to 4 protein markers and 3 RNA transcripts within a single tissue section across all three heterogeneous cancer types. The protease-free pre-treatment maintained optimal tissue and epitope morphology, enabling exceptional signal-to-noise ratios for both protein and RNA channels. Critically, this combined multi-omic readout allowed for the precise spatial phenotyping of cell populations (e.g., CD8+ T cells co-expressing specific cytokine mRNA) and the assessment of spatial proximity between cells defined by combined protein/transcriptional signatures. The assay can be expanded up to 12 protein markers via the same method described and adding a flourophore quenching step in between staining rounds.