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# *Spatial multi-omics analysis targeting protein and RNA biomarkers on a single FFPE tissue section using an integrated staining and imaging workflow*

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## Background

Biomarker detection approaches capable of rapid identification, quantification, and spatial mapping of many cellular sub-types of the tumor environment in FFPE tissue sections are becoming increasingly valuable for investigating the highly complex biology of tumors and their respective microenvironments. Multiplexed immunofluorescence (mIF) enables simultaneous identification of multiple protein targets within their spatial context in FFPE tissues, thereby facilitating more in-depth analysis including cellular phenotyping. In parallel, spatial transcriptomic analysis can provide additional insights into cellular function. Multi-omics analyses combining protein and nucleic acid biomarkers on a single tissue section can provide a deeper understanding of the complex cellular interactions in the context of the tumor. In this study, we demonstrate the versatility of Ultivue InSituPlex technology (ISP) through an integrated workflow for co-detection of protein and RNA on a single tissue section.

## Methods

FFPE tumor tissue samples were processed for protein and/or RNA detection on a single slide with an integrated workflow using ISP (Refer to **Figure 1** for schema). Briefly, slides were stained for both protein and RNA targets using a Leica Biosystems BOND RX autostainer. Post-staining, the slides were imaged on ZEISS Axioscan.Z1 in five different fluorescent channels and evaluated using IndicaLabs HALO analysis software. In parallel, control serial sections were stained to perform comparative analyses.

## Results

Here we report an efficient and streamlined integrated workflow for co-detection of protein and RNA on a single section, powered by ISP technology. The protein and RNA targets were detected both sequentially (over different imaging rounds, **Figure 2**) with increased multiplexing capabilities, as well as concurrently (in the same imaging round, **Figure 3** ) for a higher throughput solution, thereby demonstrating the flexibility of the workflow. Comparative analyses between the different workflows confirmed minimal effect on staining performance of individual biomarkers (both protein and RNA, **Figures 4&5**).

## Conclusions

The gentle treatment of FFPE tissue sections through ISP technology preserves tissue integrity, and morphology enabling efficient detection of protein and RNA targets on a single section. The integrated workflows presented here demonstrate compatibility of the individual steps in the assay towards co-detection of protein and RNA targets with efficient sensitivity in terms of staining performance for individual biomarkers.

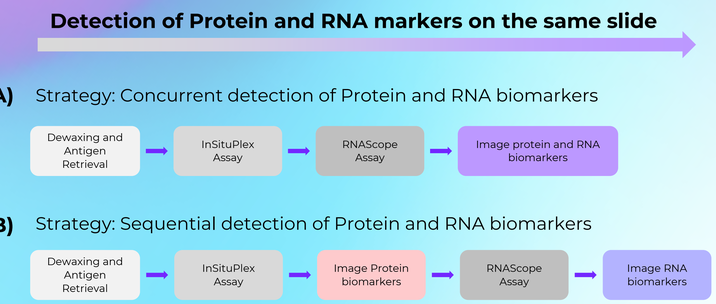


**Figure 1.** ***Proof-of-concept demonstration of integrated workflow combining InSituPlex (ISP) assay and RNAScope assays for codetection of protein and RNA biomarkers on a single slide.*** Schematic representation of representative integrated workflows for: **A)** concurrent detection or **B)** sequential detection, of protein and RNA targets on a single tissue section by combining ISP and RNAScope technologies.

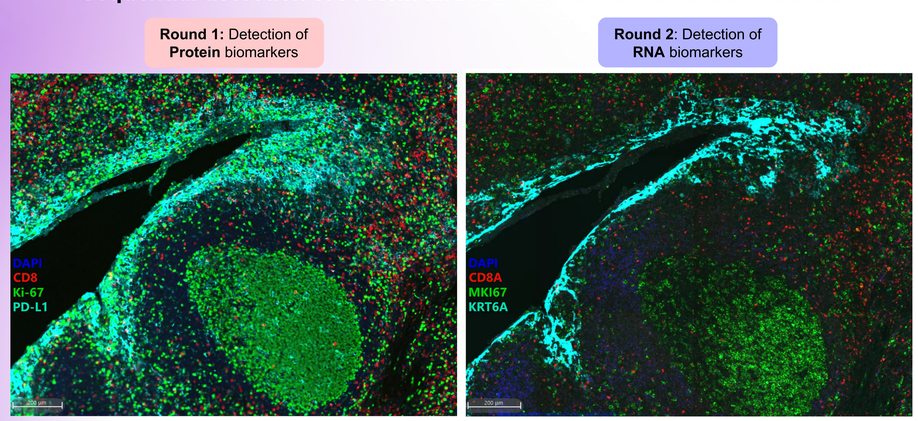


**Figure 2.** ***Sequential detection of Protein and RNA biomarkers on a single slide through integrated ISP-ISH workflow.*** Acquired images of a representative area on a tonsil tissue section highlighting detection of protein biomarkers (CD8, Ki-67, PD-L1) through InSituPlex assay (on the left), followed by detection of RNA biomarkers (CD8A, MKI67, KRT6A) through RNAScope assay (on the right).

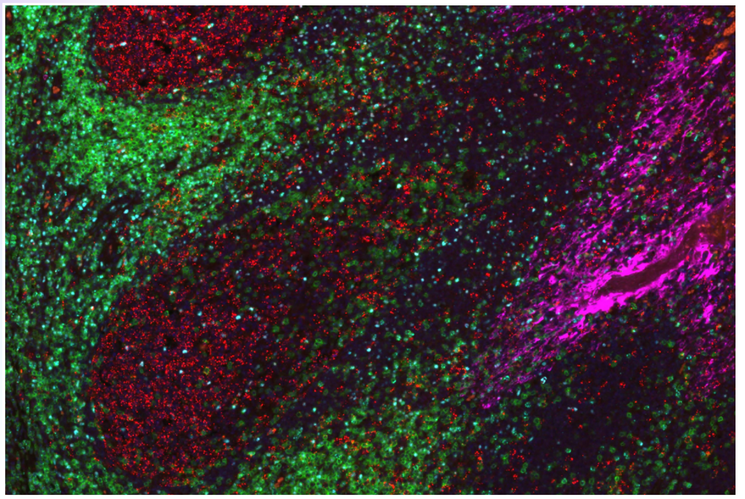


**Figure 3.** ***Concurrent detection of Protein and RNA biomarkers on a single slide through integrated ISP-ISH workflow.*** Acquired image of a representative area on a tonsil tissue section highlighting biomarkers detected concurrently in a single imaging round, after processing the section through the ISP/BONDRX automated assay protocol followed by RNAScope LS automated assay protocol. Antigen sites corresponding to biomarkers CD3 (green) and pan-cytokeratin (magenta) were detected using InSituPlex technology, while RNAs corresponding to biomarkers Ki67 (MKI67 - red) and CD8 (CD8A - cyan) were detected using the RNAScope assay..

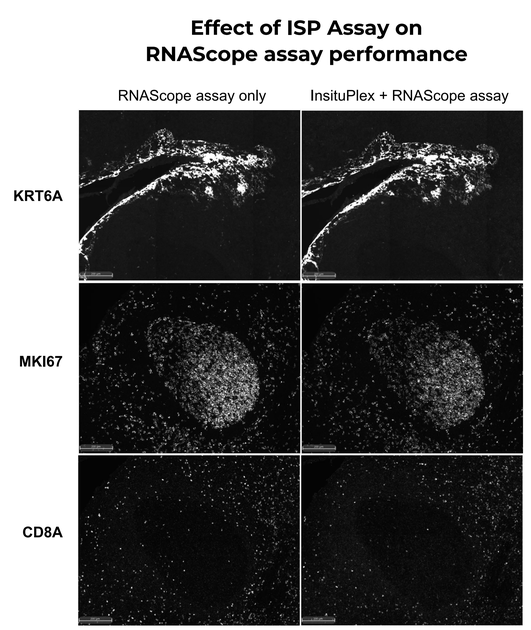


**Figure 3.** ***Evaluation of the effect of InSituPlex assay workflow on staining performance of RNAScope assay.*** Acquired images highlighting RNA biomarkers (KRT6A, MKI67, CD8A) on serial sections of tonsil tissue subjected to either: **(left)** RNA biomarker detection through RNAScope assay only, or **(right)** Integrated ISP-ISH workflow: protein biomarker detection through InSituPlex assay, followed by RNA biomarker detection through RNAScope assay (RNA data shown).

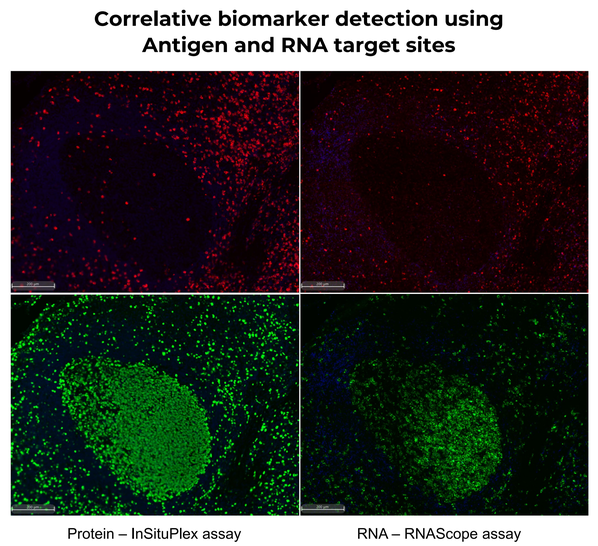


**Figure 5.** ***Correlative detection of biomarkers via Protein and RNA target sites using dual ISP-ISH assay.*** Correlative images highlighting protein and RNA detection on the same region of the tissue, corresponding to CD8 (top) and Ki-67 (bottom).