Ultivue
Profiling Cancer Biology

Strategies for Spatial Multiomics: Co-detection of Protein and RNA biomarkers on a single FFPE tissue section powered by InSituPlex® Technology

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Ultivue E-Book

Introduction

Biomarker detection approaches capable of rapid identification, quantification, and spatial mapping of the tumor environment within FFPE tissue sections is becoming increasingly valuable for understanding the highly complex biology of tumors. For researchers, this information helps to gain a more complete understanding of mechanisms of action and cellular phenotypes that may be promoting or hindering response to therapies. In the clinical space, this information can vastly improve the accuracy of immuno-oncology prognosis and accelerate the discovery of patient-focused immunotherapy targets.

Multiplex immunofluorescence (mIF) staining enables the detection of multiple protein targets within their spatial context in a single tissue sample. This in turn enables the identification of diverse cellular sub-types, shedding light on the phenotypic microenvironment of the tumor. Although many mIF staining methods exist, most require long assay development time, provide limited information on small regions of heterogenous tissue or require specialized instrumentation and long imaging times. In contrast, the Ultivue® InSituPlex® (ISP) technology enables rapid, pre-optimized staining of multiple targets simultaneously in a single FFPE tissue section. Ultivue's flexible portfolio of ISP-based kits, including FixVUE™ panels (formerly called UltiMapper kits), include ready-to-use marker panels with automated or manual staining protocols for use with a variety of commercially available imaging systems.

In the field of spatial biology, phenotypic and transcriptomic analyses are evolving in parallel. To investigate complex cellular interactions, in-depth multi-omics analyses often require detecting several protein and RNA targets on a single tissue section. Co-detection of protein and RNA can also be valuable for contextualizing the tissue microenvironment by visualizing specific secretory proteins like cytokines. Additionally, co-detection of protein and RNA enables correlative analyses of different markers based on protein and RNA expression levels. Further, detecting RNAs for specific biomarkers can serve as a proxy for detecting proteins using antibodies when antibody use is unfeasible (e.g., when antibodies are not available, have sub-optimal specificity, or have longer production timelines). Because FixVUE™ reagents efficiently preserve the tissue microenvironment throughout the entire assay workflow, ISP assays can be integrated with other spatial detection assays for combined protein and RNA detection.

We demonstrate the compatibility of ISP assays for protein detection with a commercially available RNAScope® assay (Advanced Cell Diagnostics, Inc.) for RNA detection. This eBook workflow highlights the value of an integrated ISP-in situ hybridization (ISH) workflow for co-detection of protein and RNA targets on a single FFPE 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. For a detailed step by step protocol, please contact us at ultivue.com/contact

Figure 1

DETECTION OF PROTEIN AND RNA MARKERS ON THE SAME SLIDE

a. Strategy: Concurrent detection of Protein and RNA biomarkers

b. Strategy: Sequential detection of Protein and RNA biomarkers

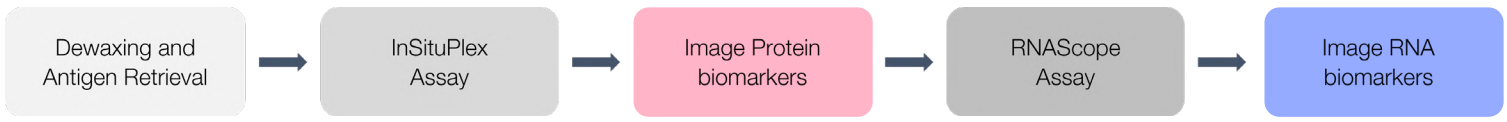

Figure 1. Proof-of-concept demonstration of integrated workflow combining InSituPlex (ISP) assay and RNAScope assays for codetection of protein and RNA biomarkers on a single slide. Schematic representation of representative integrated workflows for: A) concurrent detection or B) sequential detection,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

Results

The use of ISP technology effectively preserves the tissue microenvironment for potential downstream applications on the same tissue section. This feature enables the development of integrated workflows by combining ISP with complementary technologies for simultaneous detection of different classes of targets (e.g. protein, RNA, etc.) on the same tissue section. This study demonstrates the compatiblity of ISP technology with RNAScope technology for co-detection of protein and RNA targets on the same tissue section through two representative flexible workflows: a) either concurrently in a single imaging round, or b) sequentially over two or more imaging rounds. Importantly, the gentle signal removal and reapplication steps with ISP technology preserve the endogenous RNAs in the tissue sections for subsequent targeting with the RNAScope assay.

The concurrent detection workflow is a fully automated assay, where the protein targets in a tissue section are initially stained with ISP technology, followed by RNA target detection using RNAScope technology. The final steps involve adding ISP probes for protein targets before imaging both protein and RNA targets (up to 4 targets) concurrently in a single imaging round (Figure 1A).

The sequential detection workflow enables detection of more than four targets. In the first round, a tissue section is stained with ISP technology and imaged to detect multiple protein targets. The ISP probes are then removed, and the tissue section is processed using RNAScope technology, and multiple RNA targets are visualized in the subsequent imaging round. (Figure 1B). This workflow allows detection of more than four targets without spectral unmixing by combining the two assays.

Figure 2

CONCURRENT DETECTION OF PROTEIN AND RNA BIOMARKERS ON THE SAME SLIDE

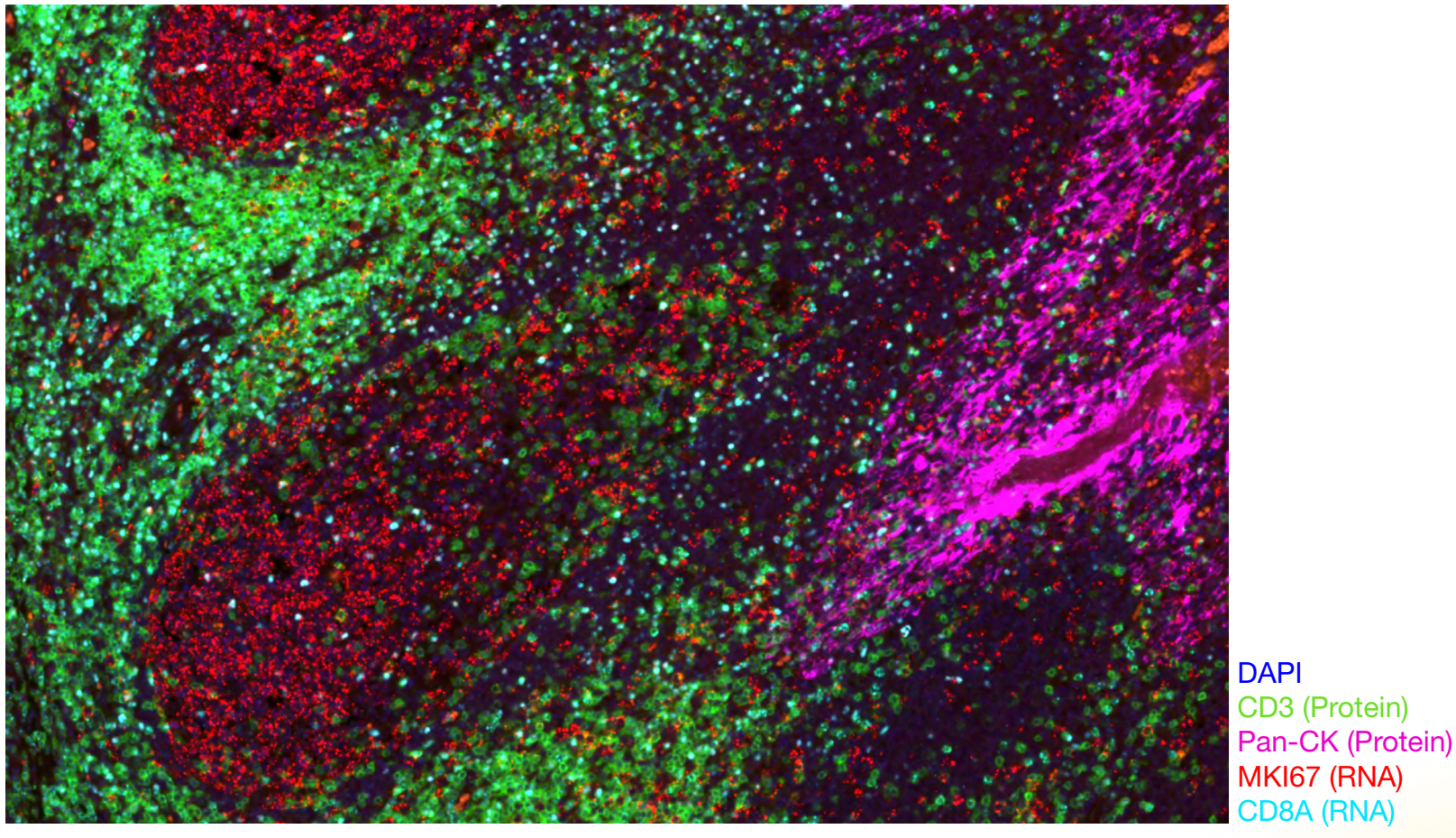

Figure 2. Concurrent detection of Protein and RNA biomarkers on a single slide through integrated ISP-ISH workflow. Acquired image of a representative area on a tonsil tissue section highlighting biomarkers detected concurrently in a single imaging round, after processing the section through the ISP/BOND RX 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 2 shows a representative image of an FFPE tonsil tissue section subjected to the integrated ISP-ISH workflow for concurrent detection of protein and RNA targets. Samples were stained using an automated assay protocol on the BOND RX autostainer, wherein the tissue section was first processed with ISP technology to detect CD3 and pan-cytokeratin (pan-CK) protein targets, followed by RNAScope assay with detection probes corresponding to Ki67 (MKI67) and CD8 (CD8A). The RNAScope assay is initiated with a protease treatment step to remove any RNA-binding proteins for better detection of RNA targets on FFPE tissue sections, however, the protease treatment can significantly affect the ISP staining performance by digesting the antibodies used for detecting protein targets. To reduce any effect of the protease step included in RNAScope protocol on the subsequent detection of protein targets using the ISP protocol, a cross-fixation step was included in between ISP and RNAScope protocols in the workflow. The integrated protocol concluded with addition of ISP probes for visualizing the protein targets. This workflow demonstrates the integration of ISP and RNAScope technologies for concurrent detection of protein and RNA targets in a single imaging round.

Figure 3

EFFECT OF ISP ASSAY ON RNASCOPE ASSAY PERFORMANCE

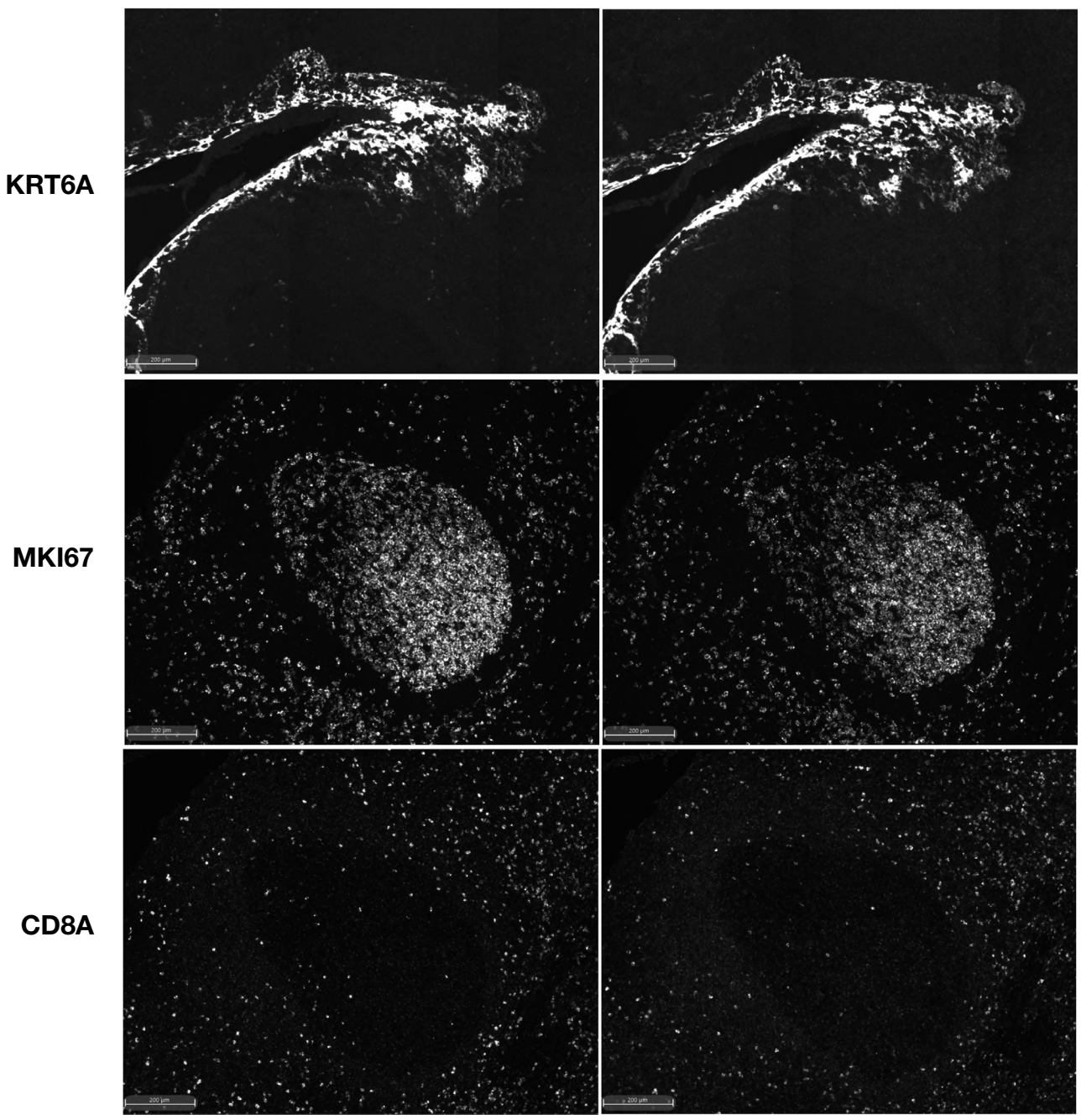

Figure 3. Evaluation of the effect of InSituPlex assay workflow on staining performance of RNAScope assay. Acquired images highlighting RNA biomarkers (KRT6A, MKI67, CD8A) on serial sections of tonsil tissue subjected to either: (left) RNA biomarker detection through RNAScope assay only, or (right) Integrated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

To establish an integrated workflow combining both ISP and RNAScope technologies, it is essential to evaluate the compatibility of the two technologies for minimal interference in staining performance. To this end, the staining of multiple RNA targets in a tissue section subjected to integrated ISP-ISH workflow was compared to serial sections processed with RNAScope assay only (Figure 3). Since the integrated workflow includes processing of tissue sections with ISP followed by RNAScope, it is essential to evaluate the effect of the preceding ISP protocol on RNA detection with the RNAScope protocol. The results showed no qualitative difference in staining of multiple RNA targets (cytokeratin (KRT6A), CD8 (CD8A) and Ki67 (MKI67)) between serial sections subjected to either the RNAScope assay or the ISP-ISH integrated workflow.

Figure 4

SEQUENTIAL DETECTION OF PROTEIN AND RNA BIOMARKERS ON THE SAME SLIDE

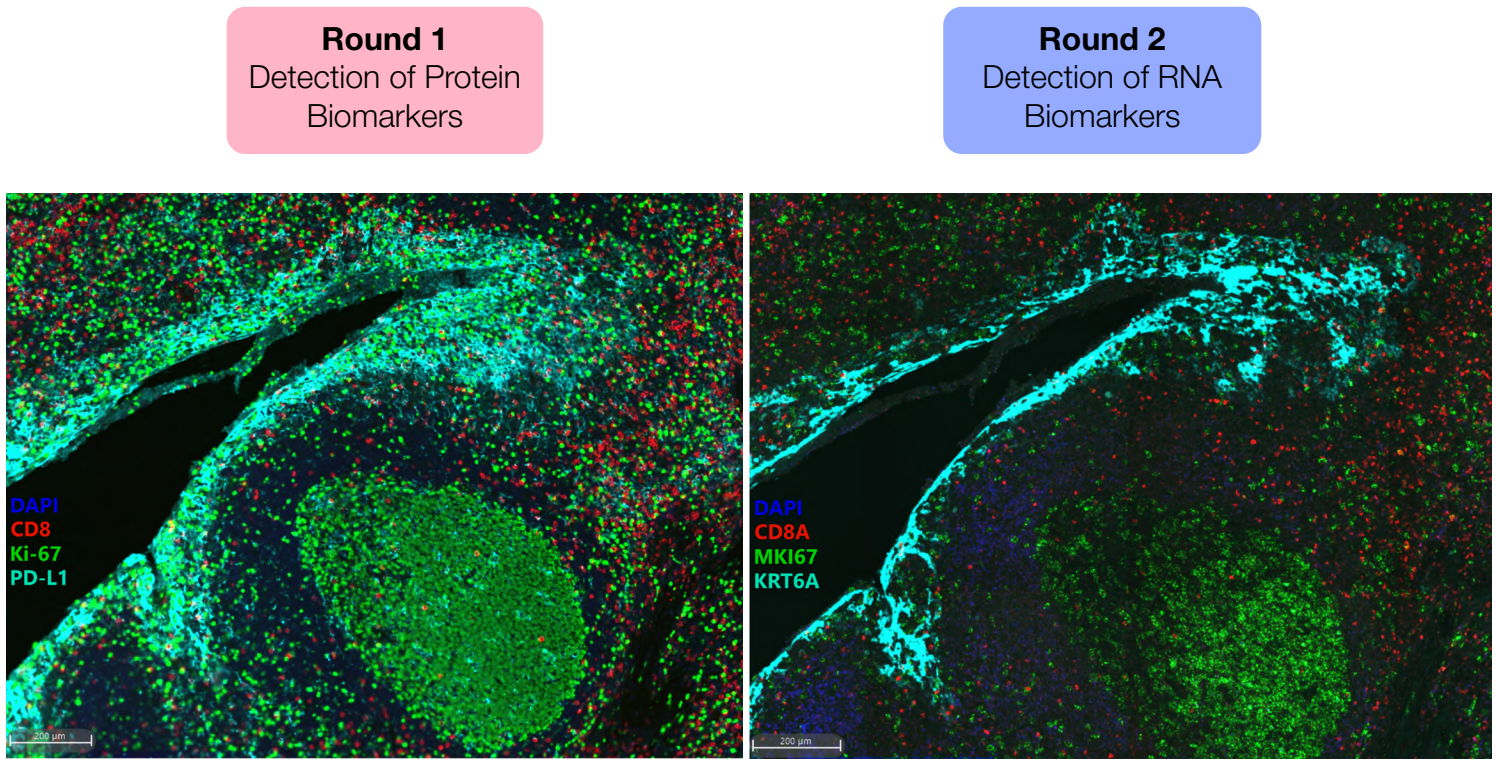

Figure 4. Sequential detection of Protein and RNA biomarkers on a single slide through integrated ISP-ISH workflow. Acquired images of a representative area on a tonsil tissue section highlighting detection of protein biomarkers (CD8, Ki-67,PD-L1) through InSituPlex assay (on the left), followed by detection of RNA biomarkers (CD8A, MKI67, KRT6A) through RNAScope assay (on the right).

The applicability of this integrated workflow can be extended towards high-multiplexed detection of protein and RNA targets (>4 targets) on the same tissue section via a sequential detection approach. Using this approach, up to four protein targets can be detected on a single tissue section in a single imaging round using ISP technology. The signal corresponding to the protein targets is then gently removed before detecting multiple RNA targets on the same tissue section using RNAScope technology in a subsequent imaging round.
Figure 4 highlights a proof-of-concept demonstration of the integrated workflow for sequential detection of protein and RNA targets on a single tissue section. Here, three protein targets (CD8, Ki67 and PD-L1) in an FFPE tonsil tissue section were initially detected with ISP technology in the first imaging round. Subsequently, three RNA targets (CD8A, MKI67 and KRT6A) were detected on the same FFPE tissue section using RNAScope technology in the second imaging round.

Figure 5

CORRELATIVE BIOMARKER DETECTION USING ANTIGEN AND RNA TARGET SITES

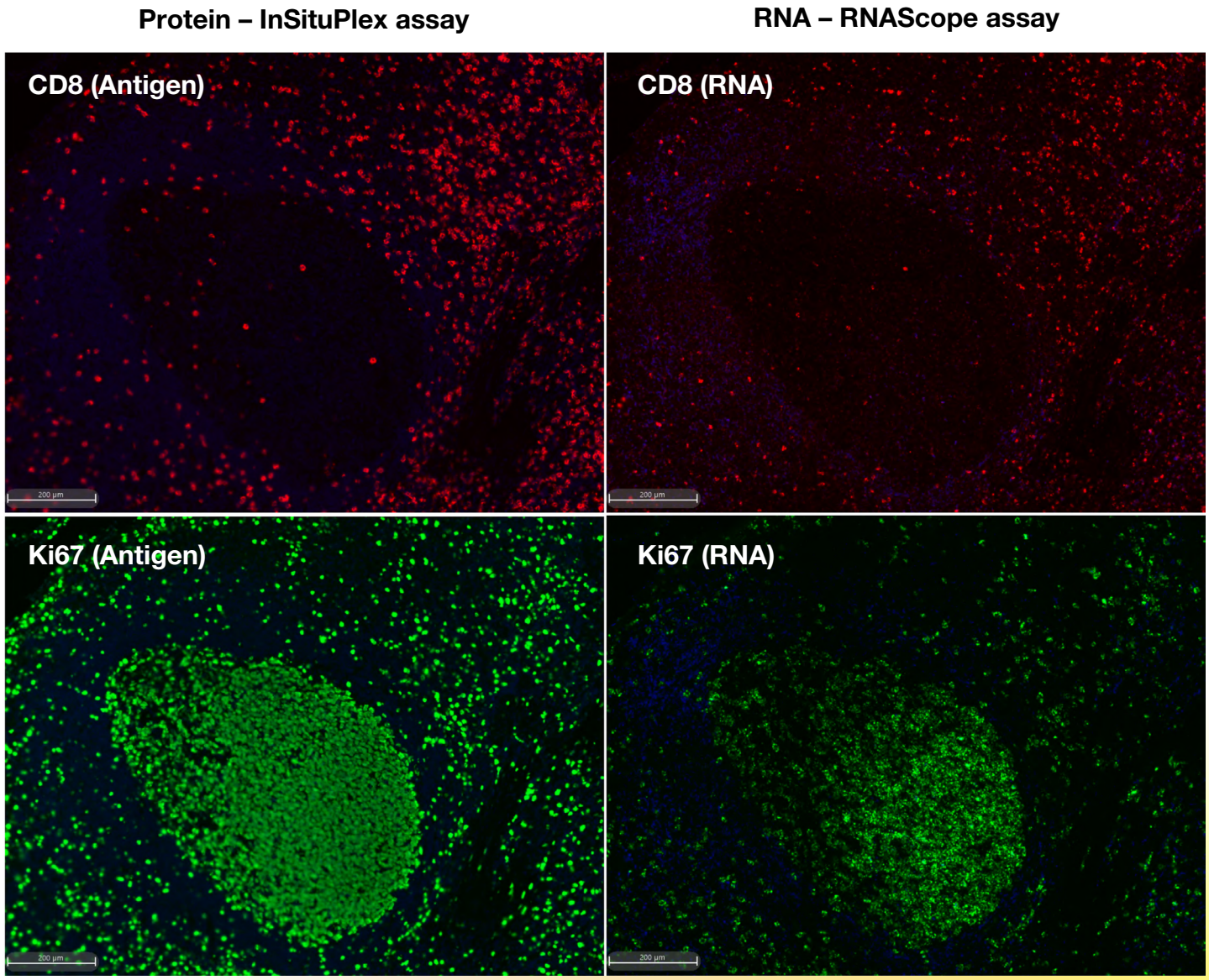

Figure 5. Correlative detection of biomarkers via Protein and RNA target sites using dual ISP-ISH assay. Correlative images highlighting protein and RNA detection on the same region of the tissue, corresponding to CD8 (top) and Ki-67 (bottom).

To qualitatively evaluate the integrated ISP-ISH workflow, an FFPE tonsil tissue section was subjected to the sequential ISP-ISH workflow, where two biomarkers (CD8 and Ki67) were detected via ISP and RNAScope assays consecutively. Figure 5 shows a representative area of the tissue section over two different imaging rounds, detecting CD8 and Ki67 via ISP assay on Imaging Round 1 (left) and CD8A and MKI67 RNA via RNAScope assay on Imaging Round 2 (right). A high degree of correlation between protein and RNA detection was observed qualitatively for both biomarkers, highlighting the efficacy of the integrated ISP-ISH workflow.

Conclusion

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.

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