TECHNICAL NOTE

Toward Integrated Spatial Biology with Same-Slide Omics: A Simple Workflow for Transcriptomic and Proteomic Imaging Enabled by the Hyperion XTi Imaging System

This technical note outlines:

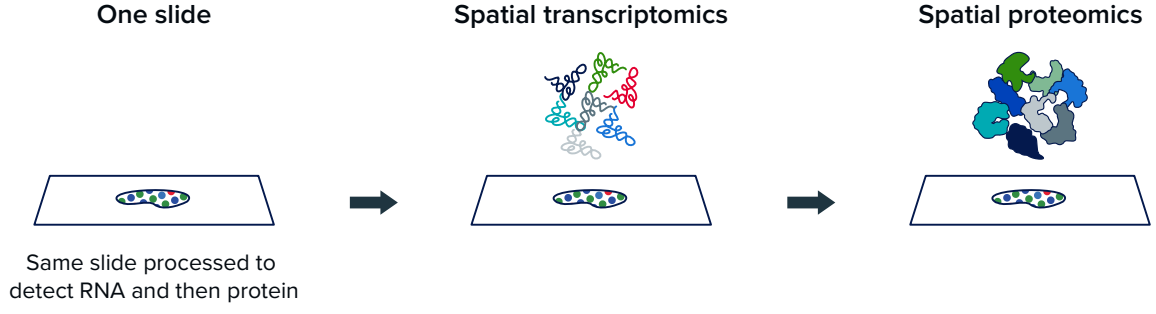
- A method for same-slide imaging of transcripts and proteins on individual tissue samples
- Best practices for combining RNA detection on a spatial transcriptomic platform and protein detection using the Hyperion™ XTi Imaging System for spatial proteomic analysis

Introduction

Imaging Mass Cytometry™ (IMC™) platforms are an ideal follow-up to spatial transcriptomic and tissue morphology analysis with an all-in-one staining and acquisition workflow, which preserves tissue integrity and enables a more accurate and comprehensive view of protein states. This streamlined approach, run on the Hyperion XTi Imaging System, minimizes tissue handling and degradation, ensuring higher-quality spatial proteomic data. Additionally, with access to over 300 catalog antibodies, IMC technology offers broad flexibility for multiplexed protein analysis across diverse research needs[1].

This technical note outlines steps that enable the combination of spatial transcriptomic and spatial proteomic workflows, along with best practices for maintaining high-quality data generation.

Spatial transcriptomic and spatial proteomic insights from the same sample



Overview of a combined spatial transcriptomic and Hyperion XTi workflow

Detection of RNA and proteins in the same cells enables a more complete picture than from transcriptomic data alone. Since some spatial transcriptomic platforms preserve tissue integrity and protein epitopes, it is possible to perform IMC analysis on the same tissue section. A novel workflow combining spatial transcriptomics and the Hyperion XTi Imaging System enables the localization of both transcripts and proteins on a single slide, providing valuable insights into their regulation and function in tissue microenvironments.

When moving from an RNA detection workflow to the Hyperion XTi workflow (Figure 1), wash the slides before proceeding to IMC FFPE staining. At this point, IMC staining can be initiated immediately using the Imaging Mass Cytometry Staining User Guide (FLDM- 01336), starting from the antigen retrieval step for FFPE samples. It is possible to pause the experiment if needed and store the slides in PBS-Tween-20 (0.05%) for up to 48 hours.

Refer to the IMC slide staining protocol[1] for continued processing of the slides post-RNA detection.

IMC workflows can easily follow spatial transcriptomic imaging

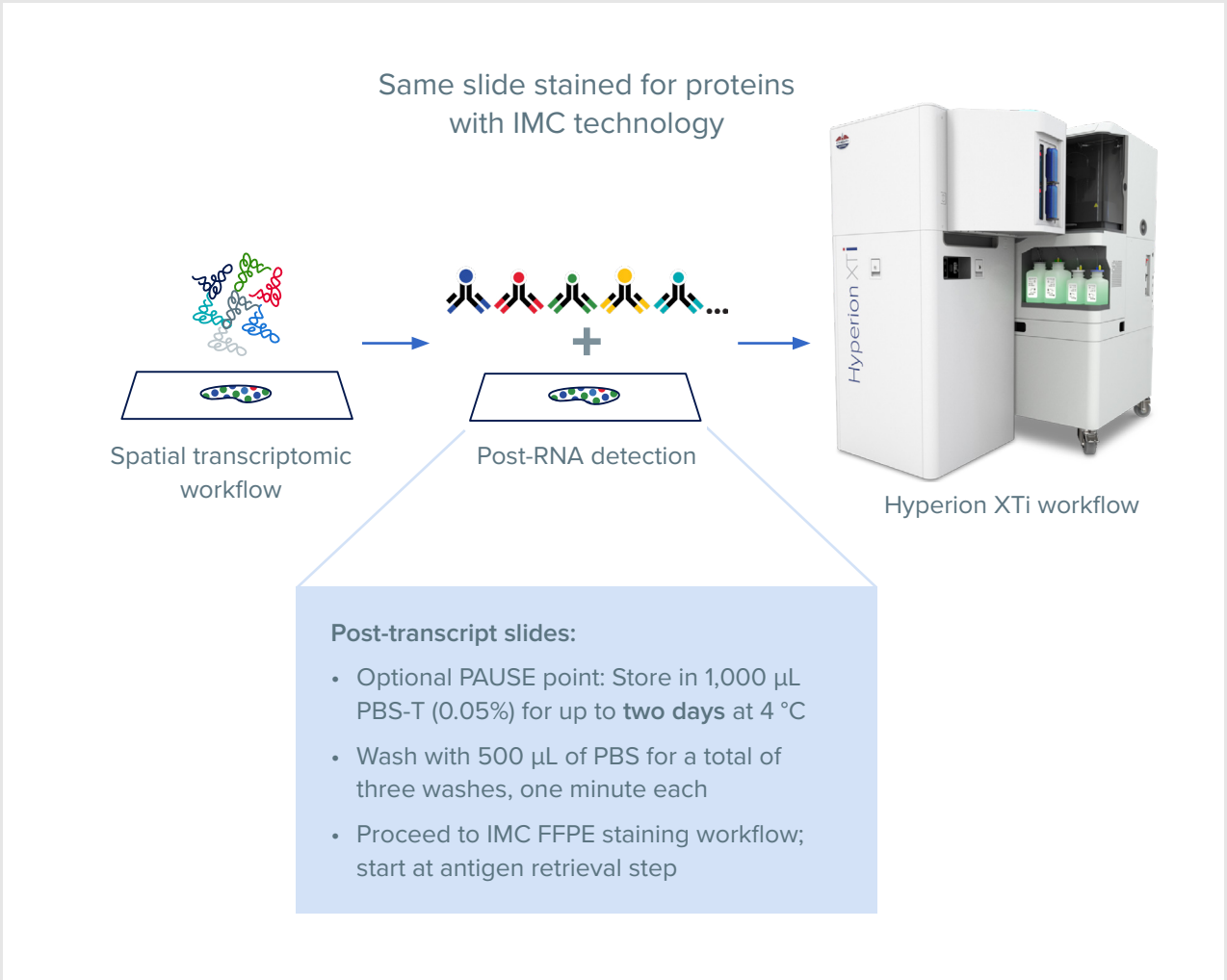


Figure 1. Combined workflow for spatial imaging of both RNA and protein on the same slide. The IMC staining protocol can be added onto the end of transcript acquisition with an additional wash step prior to IMC staining. Slides can be imaged immediately or stored for later acquisition.

High-quality same-slide omics using IMC

In a comparison of staining performance between post-transcript detection images and control images from IMC technology alone, similar high-quality protein staining can be observed (Figure 2). Proteins were accurately detected on both slide sets, along with expected tumor and immune cell markers, indicating that cell phenotyping capabilities were intact and the spatial location of structures could be assessed in the post-transcriptomic IMC workflow.

While the comparison demonstrates high similarity across images, some differences in staining outcomes were observed, such as lower signal intensities in the post-transcript IMC slides. It is important to note that IMC staining following transcript detection may result in slightly weaker signals compared with control staining, due to the effect of the protease treatment on the tissue proteins that is part of many RNA imaging protocols.

Specifically, not all protein epitopes remain intact after a transcriptomic slide staining protocol, which can result in some antibody clones such as CD44 (IM4) not performing after transcript processing, as anticipated. These differences are not technology-related but are likely due to the impact to the actual epitopes during initial RNA staining and processing. This impact could influence staining of certain antibody clones, thus performance is not guaranteed for all antibodies in the post-transcript IMC workflow.

Other factors that may impact this workflow include the amount of time the slides are stored between workflows and tissue type and quality.

Best practices for combining spatial transcriptomic and Hyperion XTi workflows

Slide storage and handling

- Post-transcript detection slides can be immediately used or stored in PBS-Tween (0.05%) at 4 ˚C for up to 48 hours before staining
- Ideally, slides should be stained for IMC application immediately after the initial RNA detection run
- Slides must be kept hydrated between the spatial transcriptomic workflow and IMC staining

IMC workflow following transcript detection

- After RNA staining and acquisition, wash then proceed with IMC staining
- Titrate antibody concentrations using serial sections for optimal signal
- Note that antibody clones known to be compatible with protease-based epitope retrieval are recommended for use in this protocol
- Control slides can be prepared following the IMC staining protocol, and processing performed concurrently with post-transcript detection slides to reduce potential variability between the experimental and control samples
- Tissue quality can impact results; it is important to confirm tissue quality prior to running slides according to other spatial transcriptomic protocols
- It is recommended that slides be stained by IMC technology within two days after RNA processing to avoid any compromise in performance
- Staining reagents and conditions mentioned in this technical note can be found in the IMC staining protocol

For additional information, refer to the Imaging Mass Cytometry Staining User Guide and the Combining Spatial Transcriptomics and Hyperion XTi Workflows for More Comprehensive Spatial Biology Application Note.

Consistent high-plex protein detection by IMC after a spatial transcriptomic protocol

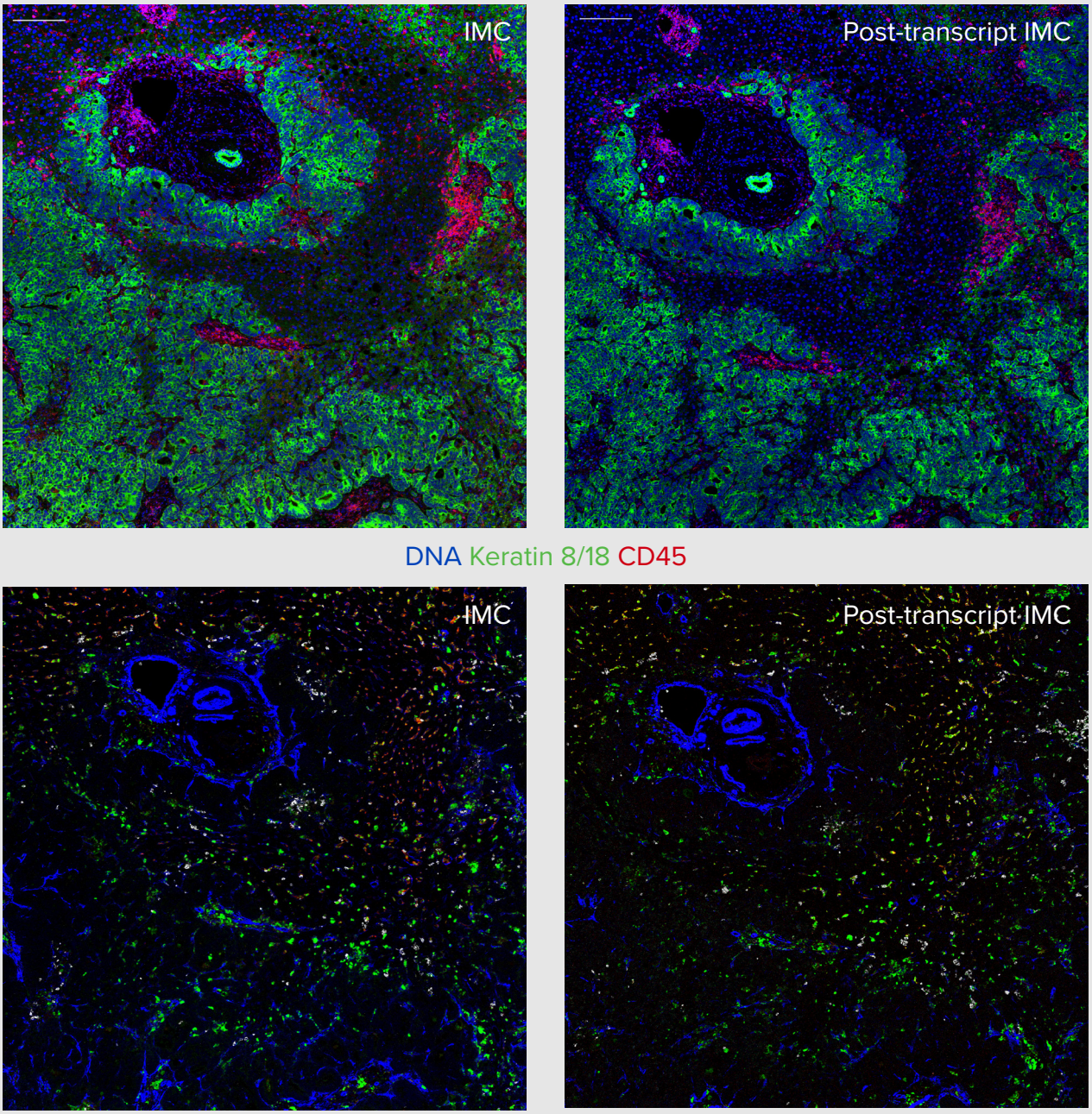


Figure 2. Low- and high-abundance proteins were detected by IMC systems regardless of processing approach. IMC processing alone and post-transcript IMC processing generate the same-quality image, demonstrating highly consistent and reproducible data. For post-transcript slides, a common spatial transcriptomic assay was used with a custom panel using the system's associated workflow. For all slides, human hepatocellular carcinoma tissue was stained with a customized IMC panel based on the Human Immuno-Oncology IMC Panel with the Human T Cell Exhaustion IMC Panel.

Conclusion

Leveraging spatial transcriptomics and the Hyperion XTi Imaging System, it is now possible to collect transcriptomic and proteomic information from the same slide. Here, we provide guidance and best practices for successful use of these complementary approaches on individual tissue samples.

Uniting the power of spatial transcriptomics and proteomics:

- ✓ Bridges the gap between gene expression and functional protein output, revealing the full molecular phenotype of cells *in situ*
- ✓ Improves cell type and state resolution, especially when transcriptomic and proteomic markers differ in specificity or abundance
- ✓ Uncovers post-transcriptional regulation, such as differing mRNA and protein levels, due to translational control or protein stability
- ✓ Enhances spatial context, allowing more accurate mapping of cell-to-cell interactions, signaling pathways and tissue architecture
- ✓ Strengthens biomarker discovery and therapeutic targeting, integrating upstream (RNA) and downstream (protein) molecular data

References

1. Combining Spatial Transcriptomics and Hyperion XTi Workflows for More Comprehensive Spatial Biology Application Note **(LAB-00067)**

2. Imaging Mass Cytometry Staining User Guide **(FLDM-01336)**

Interested in taking advantage of IMC services for your project? **Contact us**.

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