

Combining Spatial Transcriptomics and Hyperion XTi Workflows for More Comprehensive Spatial Biology

Imaging Mass Cytometry enables proteome imaging of the same slide following spatial transcriptomic imaging

Integrating multi-omic imaging datasets significantly deepens biological understanding

The transcriptome and proteome each provide distinct layers of information that support the understanding of biological processes. While mRNA levels show the potential for protein production, protein levels indicate which proteins have been produced and are actionable targets. Due to post-transcriptional and post-translational regulation, protein concentrations often do not match mRNA levels. Combining transcriptomic and proteomic data provides a more comprehensive understanding of biological systems, aiding advancements in areas such as precision medicine.

A novel workflow combining a spatial transcriptomics platform, such as Xenium™ In Situ platform, and the

Hyperion™ XTi Imaging System enables the localization of both transcripts and proteins in the same cells, providing valuable insights into their regulation and function in tissue microenvironments (Figure 1).

With the flexibility of Imaging Mass Cytometry™ (IMC™) technology, the workflow allows any antibodies of interest to be added, or pre-configured and validated IMC panel sets to be used. This application note highlights how spatial transcriptomic and spatial proteomic workflows can easily be combined to enable more comprehensive data generation.

Spatial transcriptomic and spatial proteomic insights from the same sample

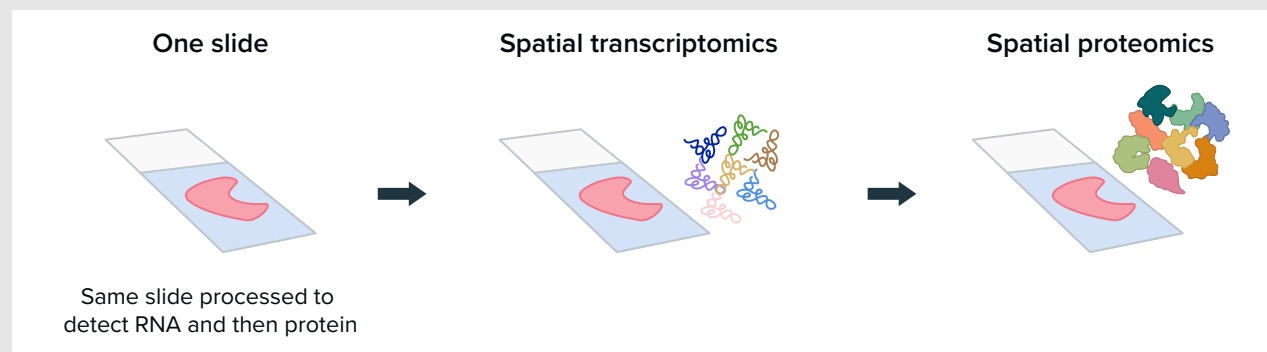


Figure 1. General workflow when integrating proteomic analysis following transcriptomic analysis. Proteins reveal functional information beyond what RNA alone can provide. Spatial transcriptomic data combined with Hyperion XTi data enables researchers to get a more complete picture than from their transcriptomic slides alone. Standard BioTools™ Services Labs are well-equipped to support this workflow.

IMC offers the flexibility to complement other spatial modalities using the same sample

IMC technology is a leading spatial proteomic solution that provides the highest dynamic range and throughput for protein detection on tissue samples (Figure 2).

Since some spatial transcriptomic platforms can preserve tissue integrity and protein epitopes, it is possible to perform IMC analysis on the same tissue section (Figure 3).

Cyclic immunofluorescence (CyclIF) workflows can be harsh on samples, causing challenges with post-RNA acquisition. In addition, assay development for CyclIF can be time-consuming and complicated given how precious post-RNA analyzed tissues can be. Unlike CyclIF, IMC technology stains and then acquires all markers simultaneously. This helps minimize tissue degradation prior to acquisition, greatly reduces assay development requirements and helps make this type of multi-omic workflow routine.

IMC platforms capture the entire dynamic range of expression for biomarkers

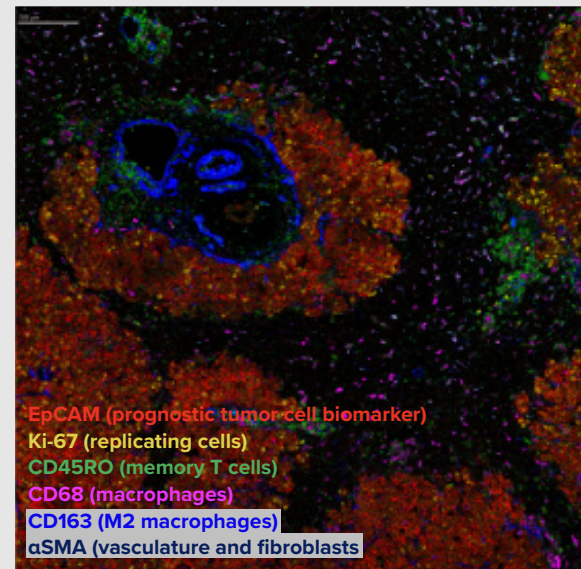


Figure 2. Spatial proteomic data can be simultaneously captured from 40-plus markers after spatial transcriptomic acquisition using the same slide. IMC platforms enable the comparison of spatial transcriptomic data with a choice of up to 45 spatial proteomic markers in the same section. Image of human hepatocellular carcinoma tissue.

Simple integration of Hyperion XTi protein imaging on the same slide following RNA imaging, with the option of H&E staining

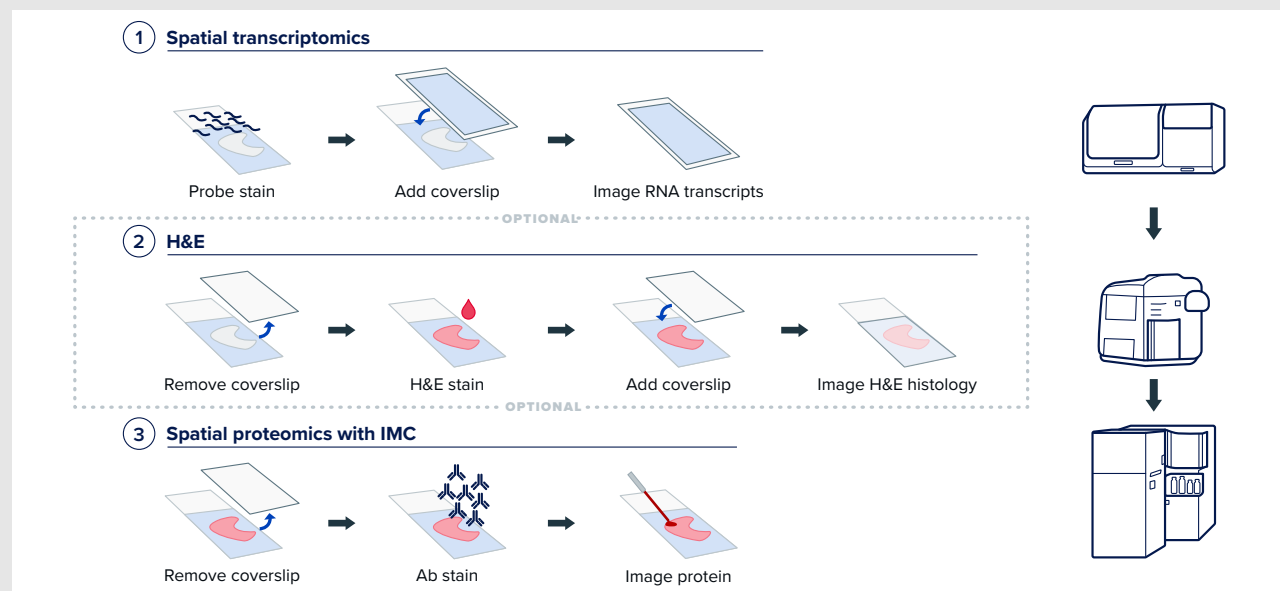


Figure 3. IMC workflows can easily follow transcriptomic imaging and/or an H&E workflow. The Hyperion XTi workflow directly follows transcriptomic acquisition on the same slide for a critical additional layer of spatial proteomic data. H&E staining can be incorporated between transcriptomic and Hyperion workflows, if desired.

Spatial transcriptomic and spatial proteomic insights from the same sample

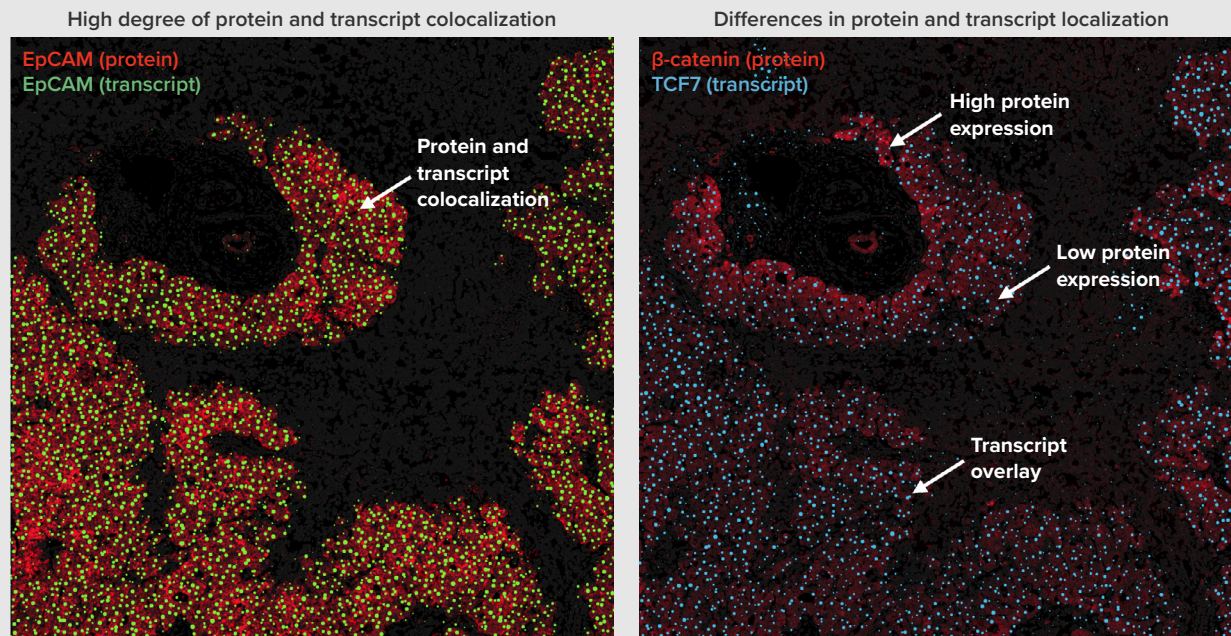


Figure 4. Spatial proteomic data by IMC platforms can be overlaid on spatial transcriptomic data captured by a leading spatial transcriptomic platform. Expression of both transcript and protein for EpCAM and β -catenin/TCF7 (β -catenin transcript) is detected in tumor cells. Transcript expression level for EpCAM corresponds closely to protein signal, whereas protein expression for β -catenin does not correlate well with the TCF7 transcript level. The images above were generated by importing protein images into third-party software. There are several other options available for merging spatial transcriptomics and spatial proteomics images using standard image files.

Overlay of IMC data onto transcriptomic data provides a deeper understanding of tissue biology than from spatial transcriptomics alone

The addition of protein data to transcript information on the same slide provides complementary insights about the locations where transcripts are present, and can reveal differences in transcript and protein patterns (Figure 4). Excellent alignment and co-registration of markers can be observed, which enables a comparison of same-cell data from different markers captured by IMC technology to transcripts captured by spatial transcriptomics.

As can be seen using third-party software following co-registration of IMC protein images, accurate alignment can be confirmed (Figure 5). Cell segmentation from IMC DNA staining is shown by red outlines and displayed onto spatial transcriptomic DAPI images. Because the images are from the same section, the alignment is excellent.

Cell segmentation of aligned RNA and protein images

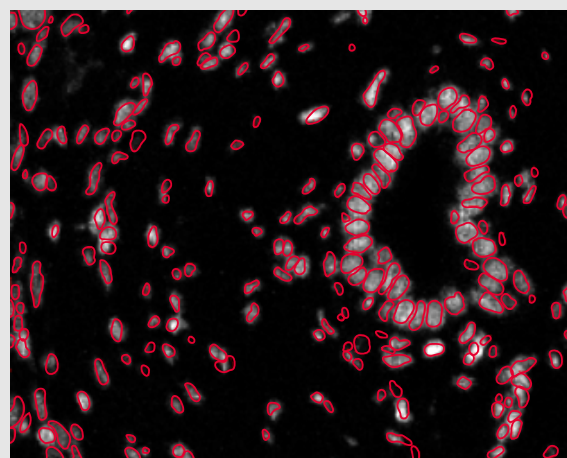


Figure 5. Image co-registration of transcriptomic data and IMC data along with cell segmentation performed by third-party software.

Spatial proteomics combined with spatial transcriptomics offers a unique look into cell function and interactions

The images in Figure 6 show detection of CCR7 (cysteine-cysteine chemokine receptor 7) transcript and CD45RO protein to capture the presence of central memory T cells (TCMs) in an immune-rich tumor sample. Since TCMs are long-lived T cells that reside in lymphoid tissues, patrol for pathogens and can differentiate into effector memory T cells upon encountering their specific antigen, they play a crucial role in long-term immunity.

CCR7 is primarily expressed on naive and central memory T cells; this receptor directs cellular homing and recirculation of these cells within tertiary organs that form in response to chronic inflammation or immune activation. CCR7 is important for T cell and dendritic cell migration to the T cell zone in these organs. Detection of CD45RO protein can provide clues about the behavior and location of TCMs, in addition to revealing regions in which there is differing transcriptional activity – either only transcript, only protein or both together.

Combining spatial transcriptomics and spatial proteomics improves the understanding of cellular phenotype and function

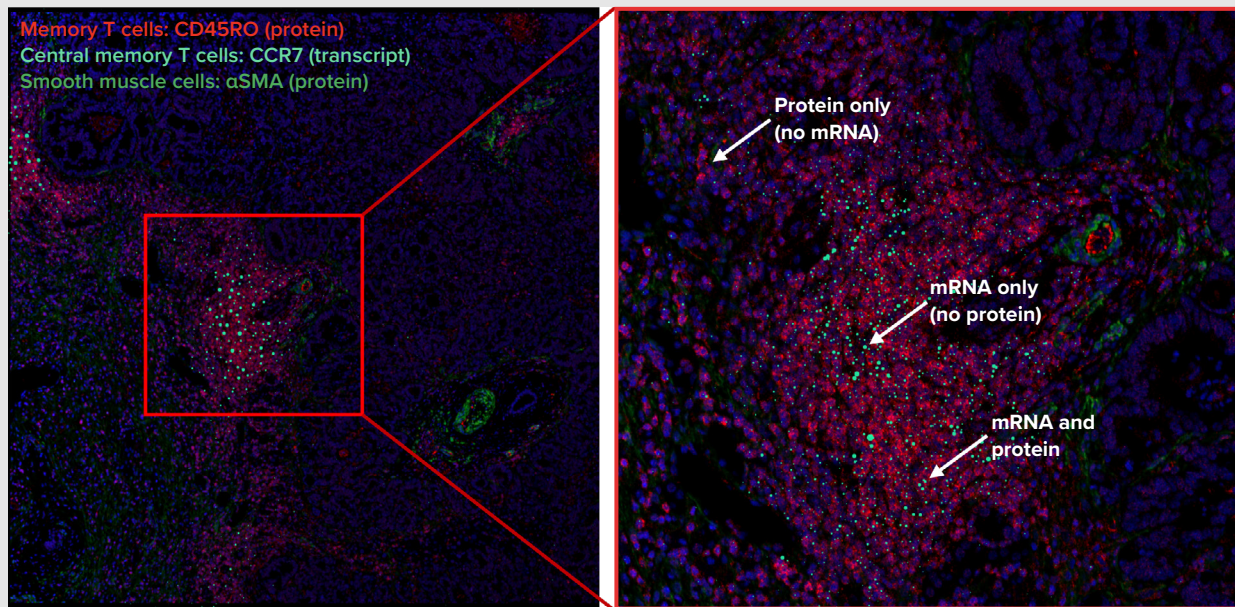


Figure 6. Combining transcripts and protein from the same tissue section offers complementary information. Image shows localization of CCR7 transcript transposed on CD45RO protein to further categorize the central memory T cell phenotype. There are cells that contain protein only (and no mRNA), mRNA only (and no protein) and some with both. αSMA is shown to highlight the tissue architecture.

Conclusion

It is now possible to collect transcriptomic and proteomic information from the same slide. Here, we demonstrate the complementary capabilities of a leading spatial transcriptomic platform for transcript detection followed by use of the Hyperion XTi Imaging System for protein detection on the same slide, enabling high-quality data to be more directly assessed and provide a better understanding of tissues.

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

Interested in taking advantage of IMC services for your project? [Contact us](#). The Standard BioTools Services Labs provide a fast turnaround time for projects, a simple process for sending samples and the ability to easily tailor antibody selection.

Request help with this approach from a Standard BioTools Field Applications Specialist [here](#).

References

1. Su, E.Y. et al. "Direct comparison of mass cytometry and single-cell RNA sequencing of human peripheral blood mononuclear cells." *Scientific Data* 11 (2024): 559.

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