APPLICATION NOTE

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

Imaging Mass Cytometry enables same-slide detection of RNA and proteins from individual tissue samples

Integrating multi-omic imaging datasets significantly deepens biological understanding

The transcriptome and proteome each offer distinct yet complementary insights into biological activity. RNA transcripts reflect gene expression status while proteins are the functional molecules of the cells and tissue. Due to post-transcriptional and post-translational regulation, RNA levels do not always correlate with protein abundance. While measuring protein alone can be more informative of both phenotype and function, combining RNA and protein detection in the same cells provides a broader view, capturing regulatory intent and functional outcome to reveal true cellular states and mechanisms.

A novel workflow combining a spatial transcriptomics platform, such as the 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).

The workflow can be customized by leveraging the inherent versatility in high-plex IMC™ panel design, allowing the inclusion of additional antibodies of interest, or use of pre-configured and validated IMC panel sets. This application note describes how spatial transcriptomic and spatial proteomic workflows can easily be combined to enable more comprehensive data generation.

Complementary insights from the same sample

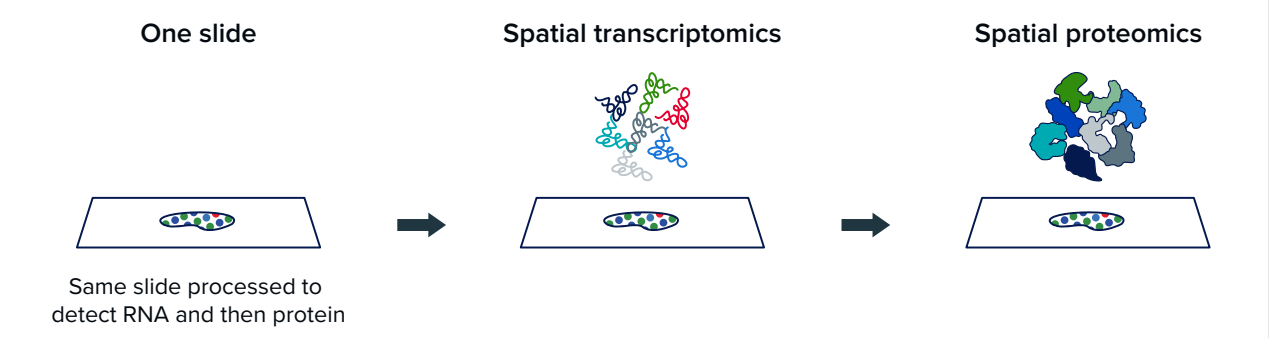

Figure 1. General workflow for same-slide detection of RNA followed by proteins. Spatial transcriptomic data combined with Hyperion XTi data enables researchers to get a more complete picture than from their transcriptomic slides alone. Standard BioTools™ Services Labs are well equipped to support this workflow.

Xenium is a registered trademark of 10x Genomics

IMC enables protein detection after other spatial modalities using the same sample

Imaging Mass Cytometry™ (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 (CycIF) workflows can be harsh on samples, causing challenges with post-RNA acquisition. In addition, assay development for CycIF can be time-consuming and complicated when working with precious post-RNA analyzed tissues. Unlike CycIF, IMC technology stains and then acquires all markers simultaneously, minimizing tissue degradation prior to acquisition, reducing assay development requirements and creating a routine multi-omic workflow.

IMC platforms capture the entire dynamic range of expression for biomarkers

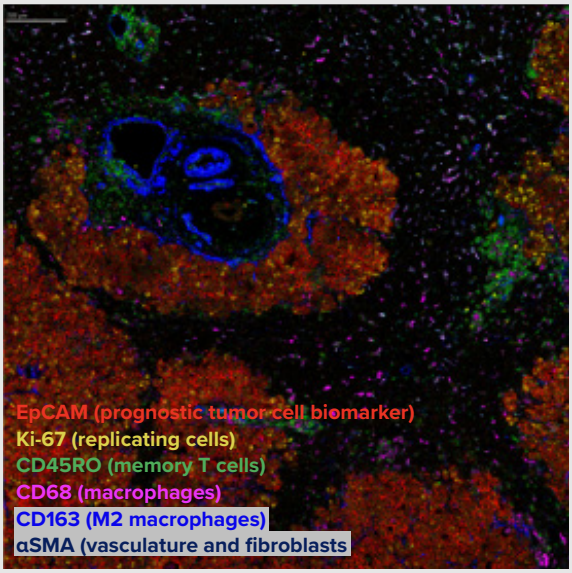

Figure 2. Spatial proteomic data can be simultaneously captured from 40-plus markers after spatial transcriptomic acquisition using the same slide. IMC platforms enable the comparison of spatial transcriptomic data with a choice of up to 45 spatial proteomic markers in the same section. Image of human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

Simply add spatial protein detection on the same slide following RNA imaging, with H&E staining option

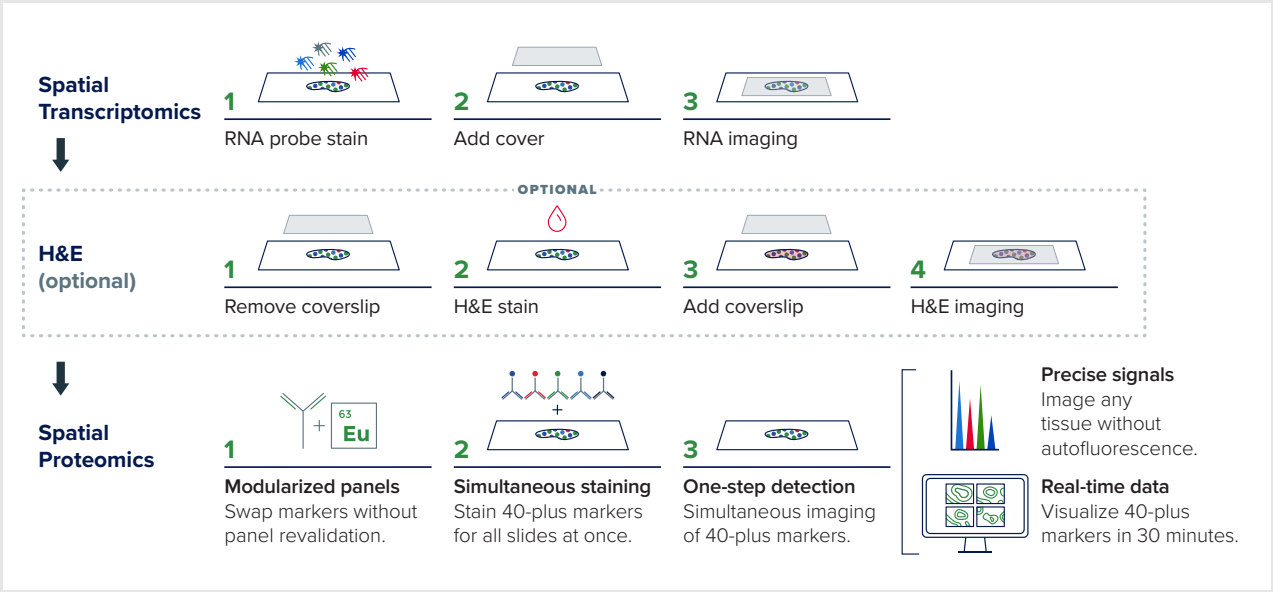

Figure 3. IMC workflows can easily follow transcriptomic imaging and/or an H&E workflow. The Hyperion XTi workflow provides the ability to add essential complementary proteomic insights to previously acquired spatial transcriptomic data. H&E staining can be incorporated between transcriptomic and Hyperion workflows, if desired.

Localization of transcripts and proteins in the same cells

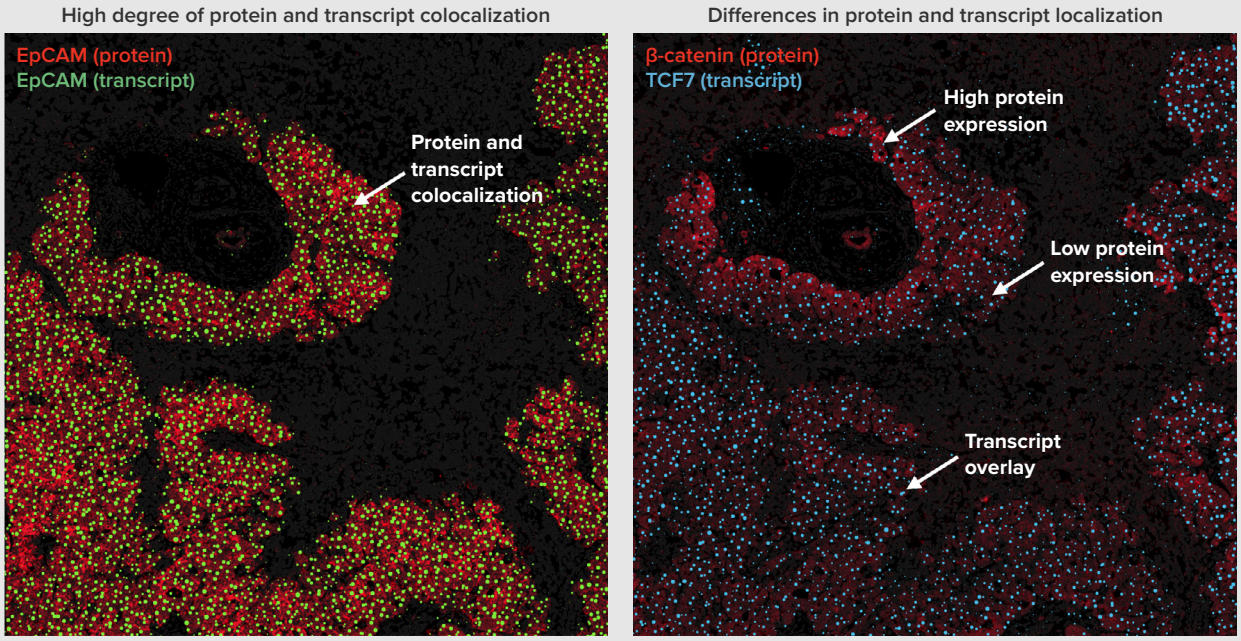

Figure 4. Spatial proteomic data from IMC systems can be overlaid on spatial transcriptomic data. Expression of both transcript and protein for EpCAM and β-catenin/TCF7 (β-catenin transcript) is detected in tumor cells. Transcript expression level for EpCAM corresponds closely to protein signal, whereas 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 options available for merging spatial transcriptomics and spatial proteomics images using standard image files.

RNA and protein data provides more detailed information that RNA 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

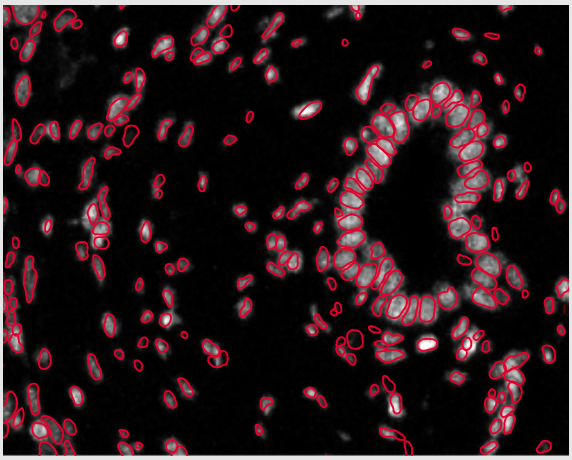

Figure 5. Image co-registration of transcriptomic data and IMC data along with cell segmentation performed by third-party software.

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 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 clarifies cellular phenotype and function

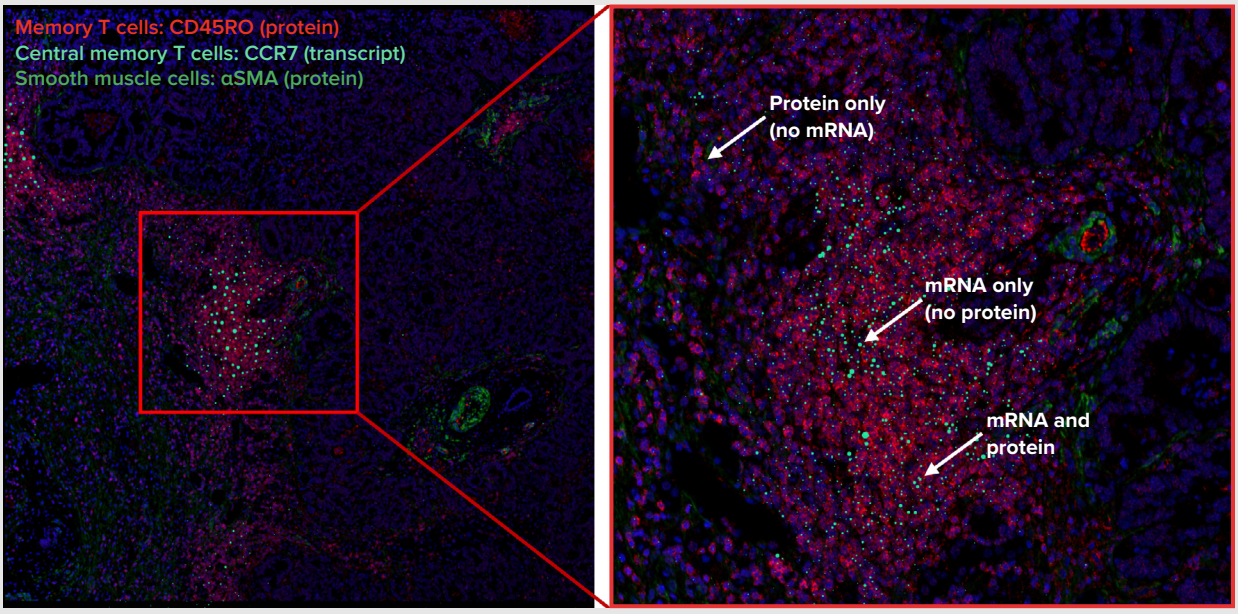

Figure 6. Visualizing transcripts and protein from the same tissue section offers complementary information. Image shows localization of CCR7 transcript transposed on CD45RO protein to further categorize the central memory T cell phenotype. There are cells that contain protein only (and no mRNA), mRNA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

Conclusion

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 demonstrate the complementary capabilities of spatial transcriptomic platforms for transcript detection followed by use of the Hyperion XTi for protein detection 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

The Standard BioTools Services Labs provide fast turnaround time for projects, a simple process for sample shipping and the ability to easily tailor antibody selection. **Contact us** for more information about IMC services.

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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