Synergistic Spatial Profiling: Unifying Xenium Transcriptomics and Imaging Mass Cytometry Proteomics for Holistic Tissue Characterization

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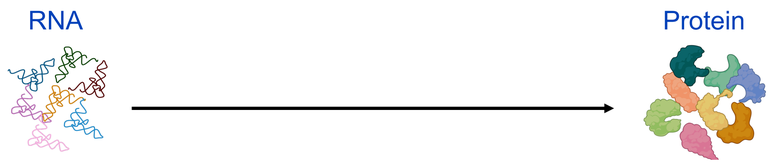
Introduction

Transcriptomics and proteomics provide synergistic information about the cell types, states and functions present in a tissue sample. Integrating spatial proteomics with transcriptomics allows for improved identification of functional protein networks and a deeper understanding of cellular organization. However, following up to several days of transcriptomic acquisition, many spatial proteomic methodologies that use sequential acquisition of data would further degrade the quality of the samples and eventual data. Imaging Mass Cytometry™ (IMC™) technology is a high-plex spatial biology imaging technique that enables deep characterization of the diversity and complexity of tissue microenvironments. IMC technology supports detailed assessment of cell phenotype and function using 40-plus metal-tagged antibodies simultaneously on a single slide without artifacts associated with fluorescence-based spectral overlap, tissue autofluorescence and multiple acquisition cycles. Here we showcase the application of IMC assays directly on slides following 10x Genomics Xenium acquisition. This novel approach enables simultaneous spatial transcriptomics and proteomics on the same tissue sample, thus delivering more comprehensive cellular signatures at a single-cell level. We demonstrate the utility of this new approach in liver cancer tissues.

Methods and materials

We performed spatial transcriptomics on hepatocellular carcinoma tissue using a Xenium V1 assay with a custom 400-marker panel. We then applied spatial proteomics using IMC technology on the same slide to detect proteins in single cells. We utilized the Human Immuno-Oncology IMC Panel, 31 Antibodies (PN 201509), which offers cell phenotyping of tumor and immune cell subtypes and their functional states. We also applied IMC technology on a separate serial section of the same tissue and compared performance.



Cellular transcript expression

- Captures the transcriptome at high spatial resolution, revealing gene activity across tissue architecture
- Reveals regulatory potential, cell identity and trajectory through RNA expression patterns
- Sensitive to early activation events, even before corresponding proteins are expressed

Cellular protein expression

- Directly measures functional molecules involved in signaling, structure and immune activity
- Reflects post-transcriptional regulation, protein stability and modifications not captured by RNA
- Correlates with therapeutic targets, such as surface markers, enzymes and immune checkpoints

Figure 1. Integration of spatial transcriptomics and proteomics amplifies the biological insights obtained from the same sample. Proteins reveal functional information beyond what RNA alone can provide. Since tissue sections run on a Xenium system remain largely intact after processing, the same slide can be used to obtain spatial proteomic insights on the Hyperion™ XTi Imaging System. Xenium data combined with Hyperion XTi data enables researchers to get a more complete picture of the biological processes in a tissue sample.

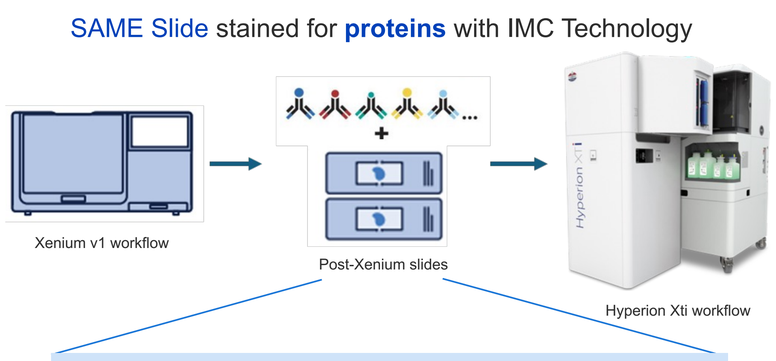


Figure 2. Combined workflow for spatial imaging of both RNA and protein on the same slide. The IMC staining protocol can be added onto the end of Xenium acquisition with an additional wash step prior to IMC staining. As a pause point, post-Xenium slides can be stored in PBS-Tween (0.05%) buffer for up to two days before proceeding with IMC staining workflow. Ideally, slides should be stained for IMC application immediately after the Xenium run. Slides must be kept hydrated between the Xenium workflow and IMC staining. Slides can be imaged immediately or stored long term for later acquisition.

Conclusions

Here, we demonstrate the complementary capabilities of a Xenium system 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 providing a comprehensive understanding of tissue biology.

- The IMC workflow can follow a spatial transcriptomic workflow on the same slide to deliver high-quality complementary data
- Seamless integration of Xenium and IMC technology is enabled by simplicity of the IMC workflow and existence of available data analysis solutions
- H&E staining can be incorporated between Xenium and IMC application for generating data for pathological review of tissue
- Cell phenotyping capabilities for tumor and immune components of the tissues are intact in post-Xenium slides imaged with IMC technology

Results

Imaging Mass Cytometry technology extracts valuable spatial proteomic data from the same tissue sample previously processed with Xenium-based spatial transcriptomics

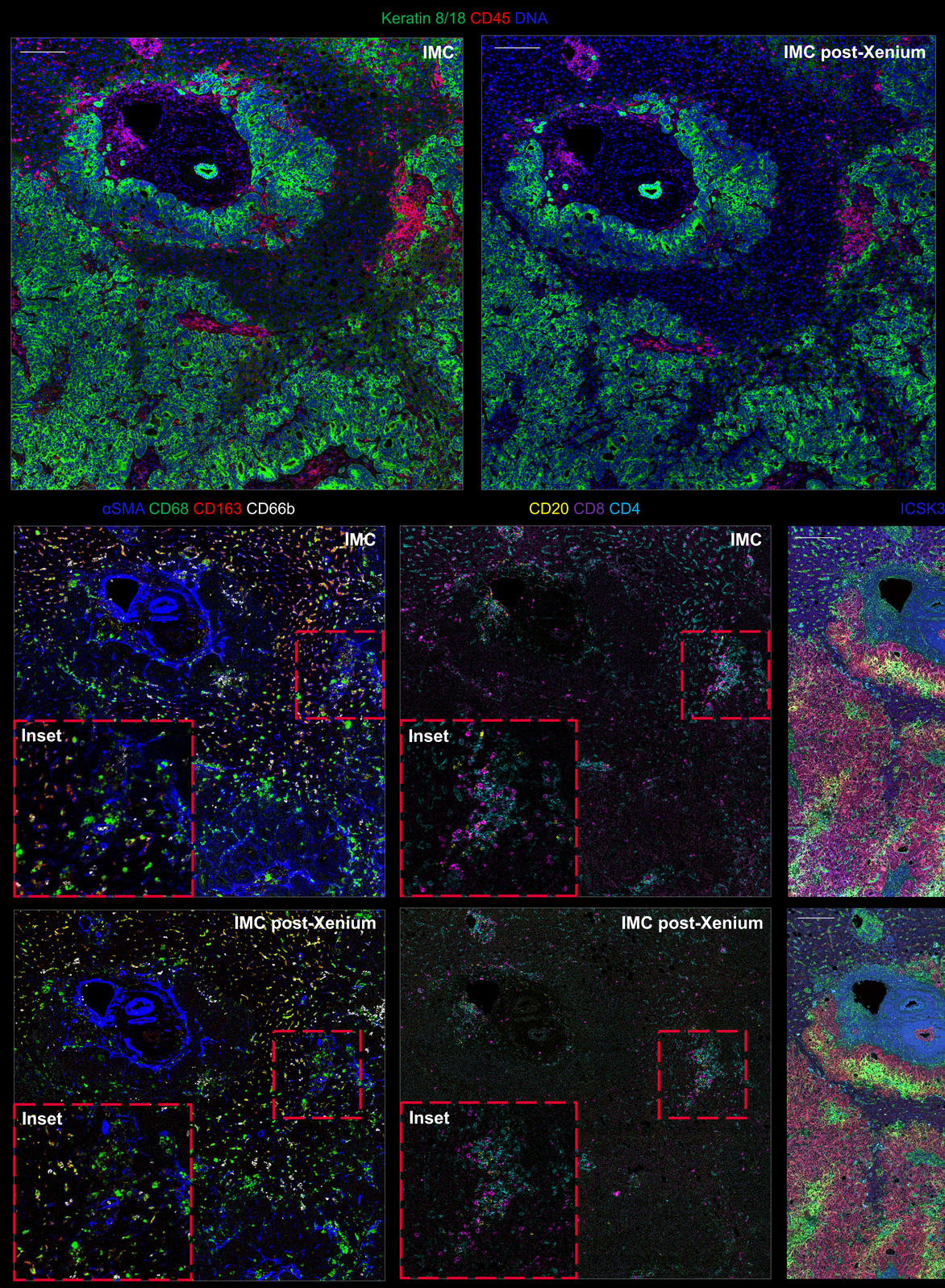


Figure 3. Imaging Mass Cytometry technology gives nearly identical results with or without prior Xenium acquisition. IMC alone and post-Xenium IMC generate an image of similar quality, demonstrating highly consistent tumor and immune cell phenotyping capabilities in human hepatocellular carcinoma. The keratin 8/18 transcript marks tumor cells and CD45 protein marks the location of immune cells. The Xenium assay used was a custom panel using the Xenium v1 workflow, although IMC technology can work with either 1K or 5K Xenium methodologies.

Figure 4. Imaging Mass Cytometry subtypes, myeloid and lymphoid immune cell subtypes in post-Xenium samples. IMC alone and post-Xenium IMC similarly detect localization of macrophages (M1 and M2), neutrophils (CD66b), B cells (CD20), cytotoxic T cells (CD8) and T helper cells (CD4) in specific locations around the tissue. Markers in the Maxpar™ IMC Cell Segmentation Kit were used to identically highlight cellular membranes of all cells in the tissue in both IMC alone and post-Xenium IMC tissue samples.

Integrating transcriptomic and proteomic data for enhanced cell phenotyping of critical immune cell populations

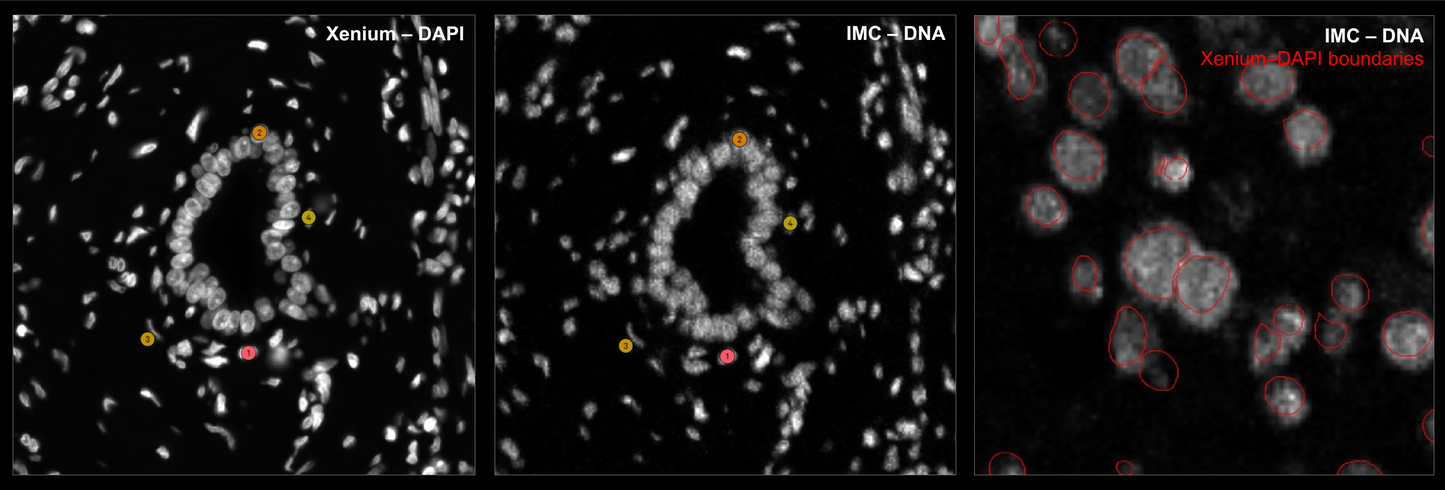


Figure 5. Excellent co-registration of transcriptomic and proteomic images using Xenium Explorer. Xenium Explorer software permits import of IMC data through user-friendly co-registration algorithm included in the software, which incorporates location of DAPI-stained nuclei from transcriptomic data and DNA-stained nuclei from IMC data. IMC data, extracted as an OME-TIFF file format, is imported into Xenium Explorer and the location of 50–100 cells matching on both datasets is manually registered by the user. This results in accurate cellular co-registration for both datasets, providing combined RNA and protein cellular phenotypes.

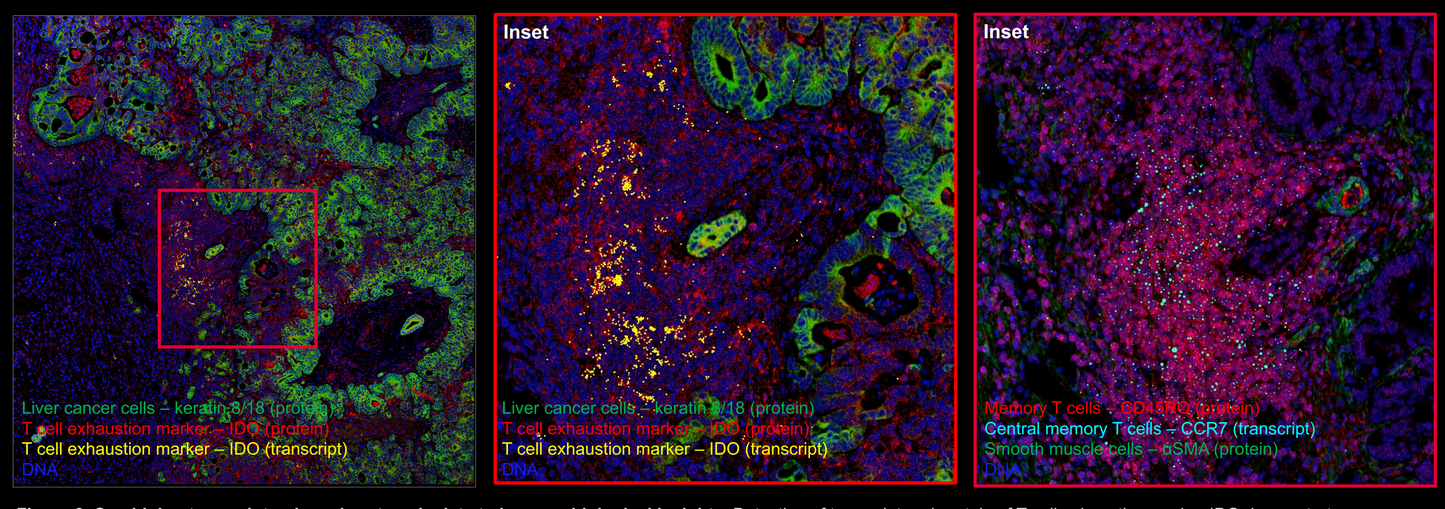


Figure 6. Combining transcriptomic and proteomic data to improve biological insights. Detection of transcript and protein of T cell exhaustion marker IDO demonstrate discrepancies in spatial localization. Merging expression of CCR7 transcript with memory T cell marker CD45RO spatially distinguishes central memory T cells.

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