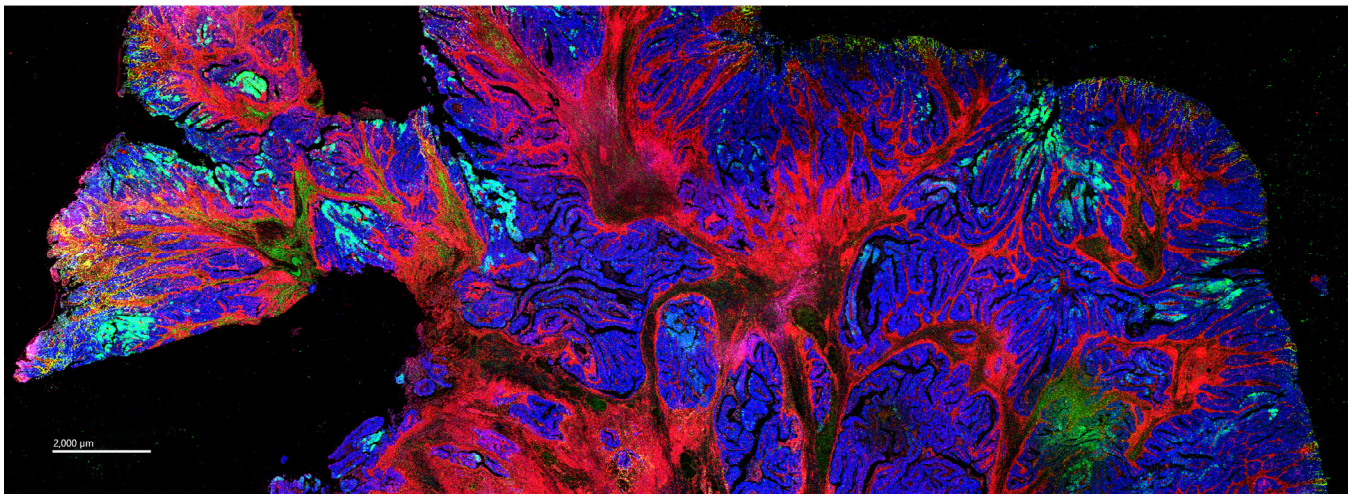
APPLICATION NOTE

Understanding Cell Signaling Dynamics in Cancer with Spatial Proteomics

Modular Imaging Mass Cytometry panels identify changes in signaling pathways across the tumor microenvironment

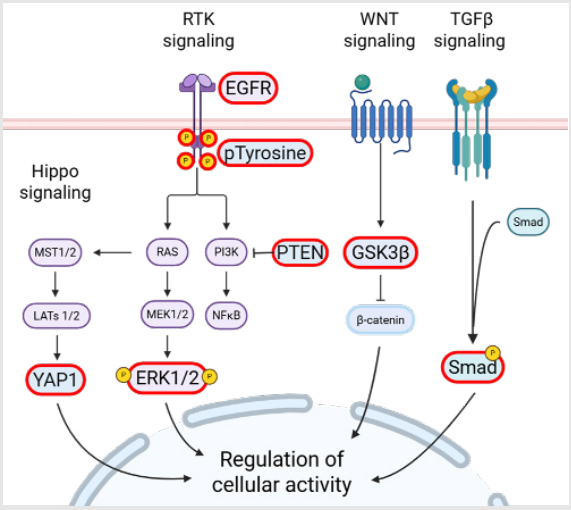


Introduction

The tumor microenvironment (TME) is a complex cellular ecosystem that can influence tumor development and treatment response. Cell signaling pathways in tumor cells may differ significantly from those of healthy cells, supporting uncontrolled proliferation and survival. Understanding activation states of these cells within the TME is essential for elucidating disease progression and advancing the development of immunotherapies.

This application note highlights how ready-to-use Imaging Mass Cytometry™ (IMC™) antibody panels simplify spatial profiling and the mapping of key signaling pathways within the TME, accelerating the investigation of processes that regulate tumor growth and cancer pathogenesis.

The Human Cell Signaling IMC Panel highlights key proteins that regulate activation states of cells in the TME



One IMC assay can incorporate multiple panels encompassing phenotype, function and differential activation of signaling pathways in a spatial context across immune and cancer cells

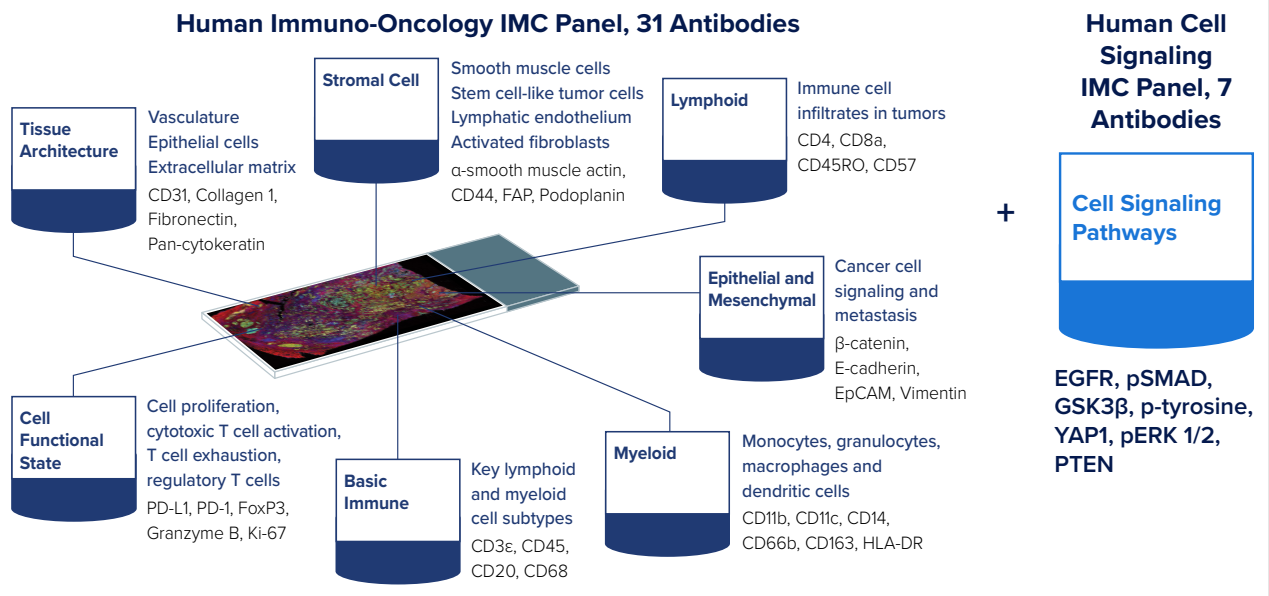


Figure 1. Mix-and-match IMC panels provide broad immuno-oncology coverage with options for more disease- or application-focused targets. The Human Immuno-Oncology IMC Panel is designed to explore immuno-oncological processes in human tumors. It includes 31 pathologist-verified antibodies in the base panel and is optimized for FFPE tissues. When expanded with the Human Cell Signaling IMC Panel, it enables the detection of immune cell subtypes, tumor characteristics and microenvironment components, and the presence of cancer-associated fibroblasts in a comprehensive, high-plex approach.

Modular IMC panels enable spatially resolved profiling of cell signaling pathways

Signaling pathways can be differentially activated in spatially distinct tumor cell populations. Visualizing this heterogeneity offers insights into how and where a tumor may be vulnerable to immune or therapeutic targeting. Key signaling pathways in the TME can be mapped using one IMC assay combining cell phenotype, function and signaling markers (Figure 1).

The Human Cell Signaling IMC Panel, 7 Antibodies (PN 201522) contains essential markers for the identification and study of major signaling pathways in human cells. This panel includes seven pathologist-verified antibodies – EGFR, pSMAD, GSK3β, p-tyrosine, YAP1, pERK1/2 and PTEN – that can be stained and acquired simultaneously, enabling the assessment of EGFR, TGF-β, Wnt and Hippo signaling, and MAPK and PI3K/Akt pathways.

These processes are fundamental regulators of cell proliferation, differentiation, survival and tumorigenesis, making the panel a powerful tool for studying cancer biology, developmental signaling and targeted therapeutics. For example, Wnt signaling plays an essential role in cell proliferation and differentiation, so aberrant activation is a key contributor to tumorigenesis.

The following IMC images reveal significant insights into the spatial organization and signaling activity of cells across cancer tissues (Figures 2 and 3). To visualize immune and tumor cells and assess the activation status of immune cells on the Hyperion™ XTi Imaging System, Tissue Mode was used to image the whole tissue section for component analysis and feature identification. Hyperion XTi Imaging Systems offer scalable acquisition and a high dynamic range, generating high-quality data without fluorescence-based limitations such as spectral overlap and autofluorescence.

Tissue Mode images using the Human Cell Signaling IMC Panel reveal differential expression across signaling mechanisms in colon adenocarcinoma

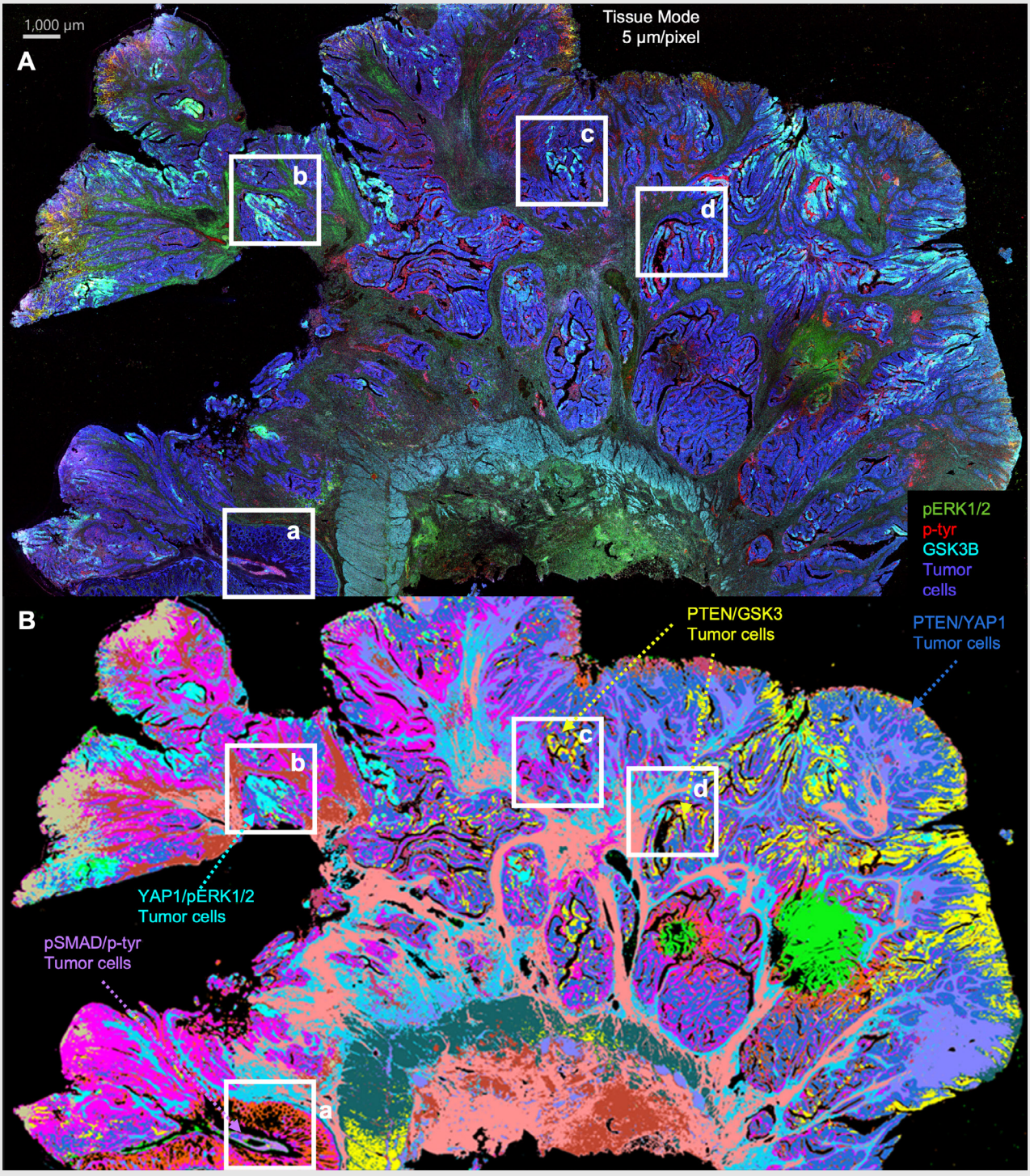


Figure 2. IMC images generated on the Hyperion XTi Imaging System show the heterogeneity in signaling mechanisms among tumor cells. Tissue Mode (A) imaging of a whole slide tumor section, combined with pixel-clustering analysis (B), provides a spatially resolved quantitative assessment of specific tumor and immune components within the TME. Pixel-based clustering of Tissue Mode (5 µm resolution) performed using MCD™ SmartViewer shows differential activation of signaling cascades in colon adenocarcinoma cells. Boxes (a, b, c, d) highlight regions of interest selected for Cell Mode (1 µm resolution) analysis shown in Figure 3.

Cell Mode images show clear changes in signaling activity in different regions of the TME in colon adenocarcinoma

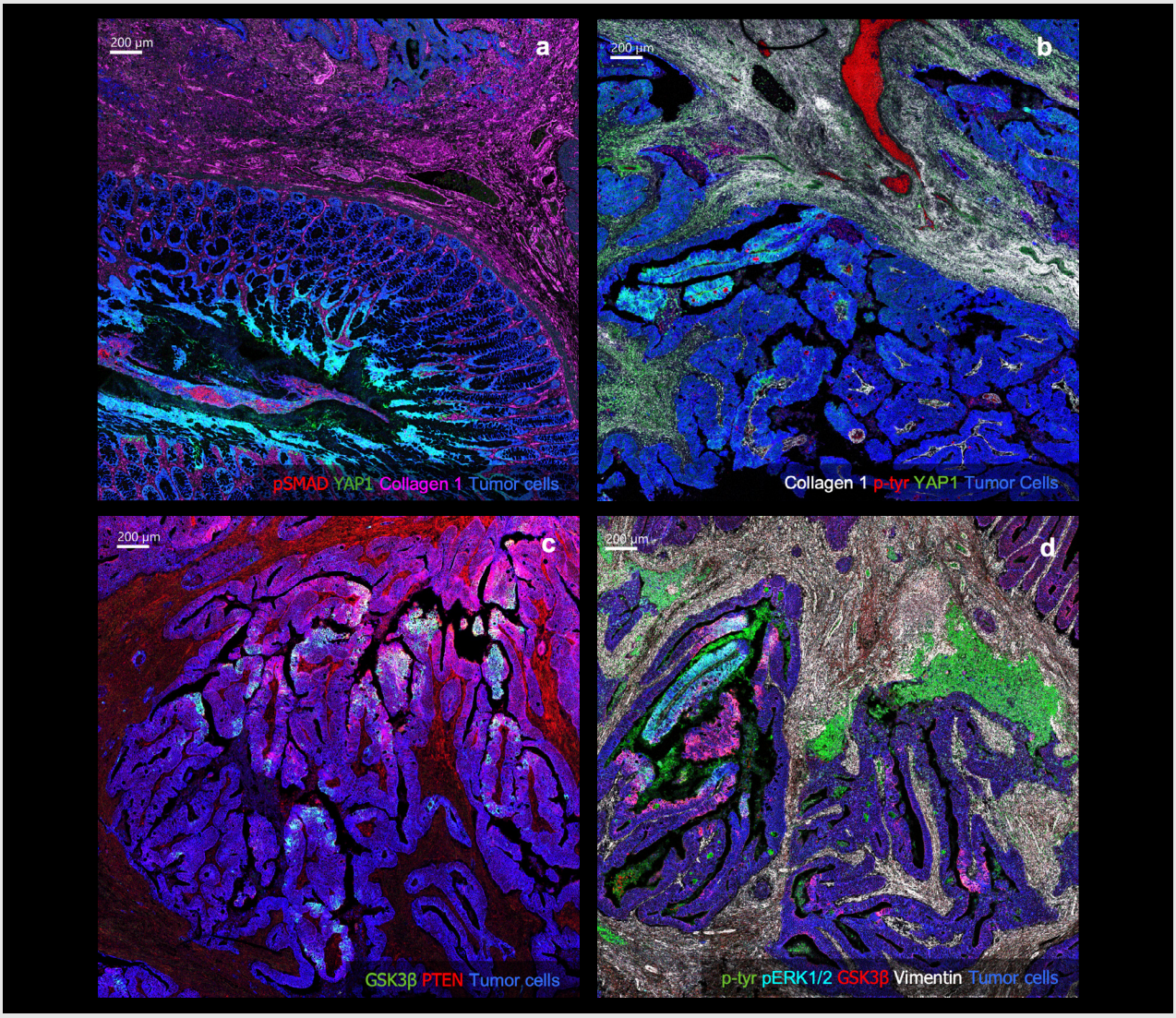


Figure 3. Single-cell analysis demonstrates the utility of the Human Cell Signaling IMC Panel to investigate spatial changes in specific cell activation states. The Human Cell Signaling IMC Panel shows clear distinction of regions where (a) areas of Hippo signaling (YAP1) in the tumor are surrounded by tumor areas with TGF-β (pSMAD) signaling; (b) Hippo signaling (YAP1) is observed in isolated tumor areas, some of which express p-tyrosine. p-tyr is also observed in the extracellular matrix (collagen 1); (c) Wnt (GSK3β) signaling activity is activated in different tumor regions. In some areas, it is co-expressed with PTEN, which is observed in both tumor and stromal cells; (d) Wnt signaling is seen in some tumor regions while other tumor cells use MAPK (pERK1/2) signaling. Elevated receptor tyrosine kinase (p-tyr) signaling localized to stroma (vimentin) is also observed.

Single cell analysis using IMC further details signaling activity across a tumor

The approach described in Figure 2 was further enhanced by a quick tissue scan using Preview Mode (20-minute whole slide acquisition) to guide single-cell analysis of selected regions of interest (ROIs) in serial tissue sections that were then acquired at 1 µm resolution using Cell Mode.

With the ability to spatially distinguish activation patterns down to each single cell, the Hyperion XTi Imaging System and targeted IMC panels provide insights on actionable phenotypic and functional targets to clarify mechanisms underlying signaling dynamics and identify patient response.

Conclusion

Modular IMC panels enable comprehensive spatial profiling, revealing the interconnected roles signaling pathways play in promoting tumor survival and resistance to therapies. Their application uncovers cell signaling dynamics in the TME, crucial for designing future prognostic assessments and supporting the development of effective, personalized cancer therapies.

These panels can be combined to quickly and easily create a single high-parameter assay. The Human Cell Signaling IMC Panel, with seven pathologist-verified antibodies targeting EGFR, pSMAD, GSK3β, p-tyrosine, YAP1, pERK1/2 and PTEN, is one of several targeted panels available for in-depth tissue analysis. See how the different panels work together here.

This application note illustrates how expanding spatial biology assays with the Human Cell Signaling IMC Panel enhances studies of cancer, autoimmune diseases and more – demonstrated here in colon adenocarcinoma.

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

Request help with this approach from a Standard BioTools Field Applications Scientist here.

Cover image: MAPK signaling and immune cell infiltration in colon adenocarcinoma, acquired on the Hyperion XTi Imaging System. IMC markers shown: CD45 (red), pERK1/2 (green), tumor cells (blue)

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