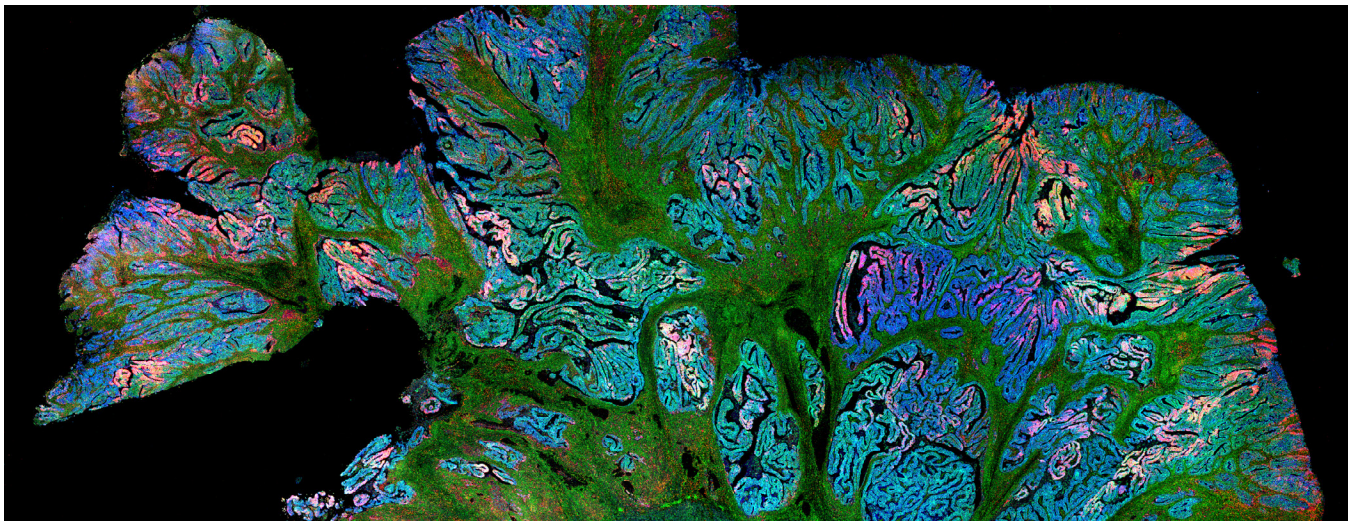
APPLICATION NOTE

Unraveling Metabolic Reprogramming in Cancer with Spatial Proteomics

Modular Imaging Mass Cytometry panels identify cell metabolic states across the tumor microenvironment

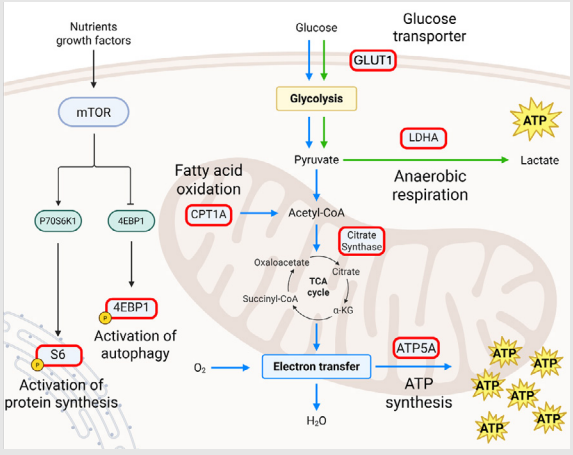


Introduction

The tumor microenvironment (TME) is a metabolically and functionally diverse ecosystem that can influence tumor development and treatment response. Monitoring cell metabolism and revealing the spatial positioning of tumor and immune cells can shed light on distinct alterations in metabolic activity and cellular energy production. 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 metabolic profiling and the accurate mapping of rate-limiting enzymes and activation markers, offering valuable targets for drug development and clinical research.

The Human Cell Metabolism IMC Panel detects metabolic pathway proteins that play critical roles in tumor growth and therapeutic resistance



One IMC assay can incorporate multiple panels encompassing phenotype, function and metabolic activity across immune cells and cancer cells in the TME

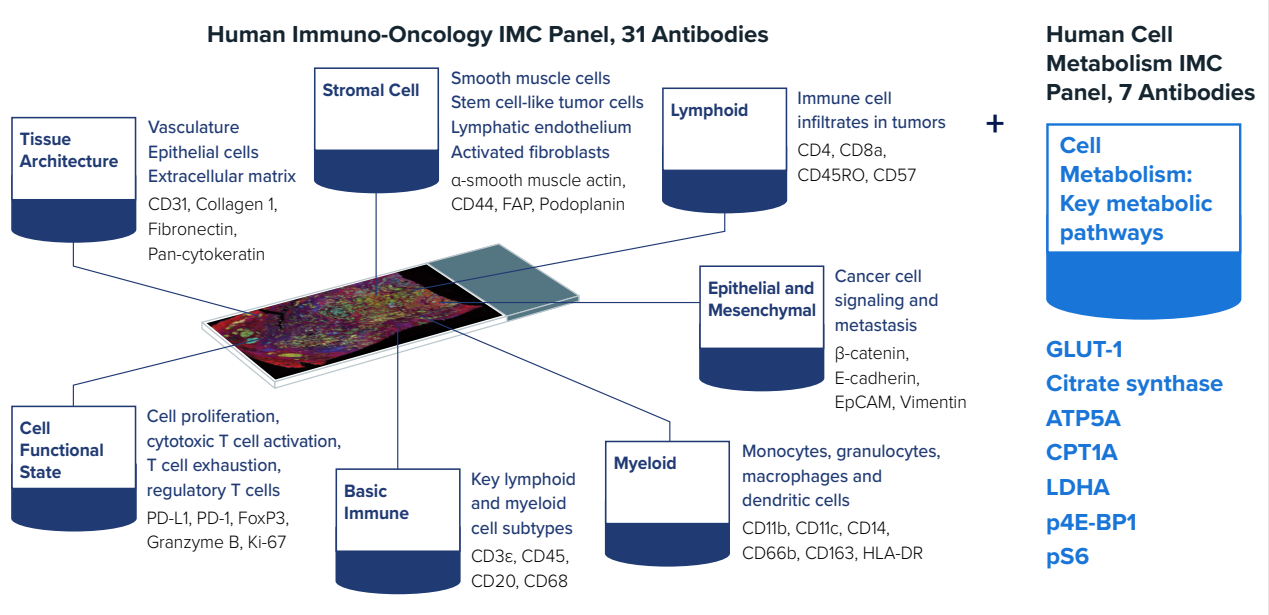


Figure 1. Mix-and-match IMC panels provide broad immuno-oncology coverage with options for more disease- and application-focused targets. The Human Immuno-Oncology IMC Panel is designed to explore immuno-oncological processes in human tumors. It includes 31 pathologist-verified antibodies in the base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

Modular IMC panels enable spatially resolved profiling of metabolic activation across tissue

Metabolic 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 metabolic pathways in the TME can be mapped using one IMC assay combining cell phenotype, function and metabolism markers (Figure 1).

The Human Cell Metabolism IMC Panel (PN 201521) provides markers for the identification and study of six metabolic pathways: glucose metabolism, fatty acid oxidation, lactate metabolism, the tricarboxylic acid (TCA) cycle, oxidative phosphorylation and mTOR signaling. This panel includes seven pathologist-verified antibodies – GLUT1, citrate synthase, ATP5A, CPT1A, LDHA, p4E-BP1 and pS6 – that can be stained and acquired simultaneously.

Thorough investigation of processes that regulate energy production can reveal metabolic dysfunction linked to cardiovascular diseases, cancer, neurological conditions and age-related diseases.

The following IMC images reveal significant insights into the spatial organization and metabolic profile of cells across cancer tissues (Figures 2 and 3). To visualize immune and tumor cells and assess the activation status of these cells on the Hyperion™ XTi Imaging System, Tissue Mode was used to image the whole tissue section for component analysis and feature identification. The Hyperion XTi Imaging System offers 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 Metabolism IMC Panel reveal differential utilization of glucose and fatty acid oxidation to fuel growth in breast adenocarcinoma

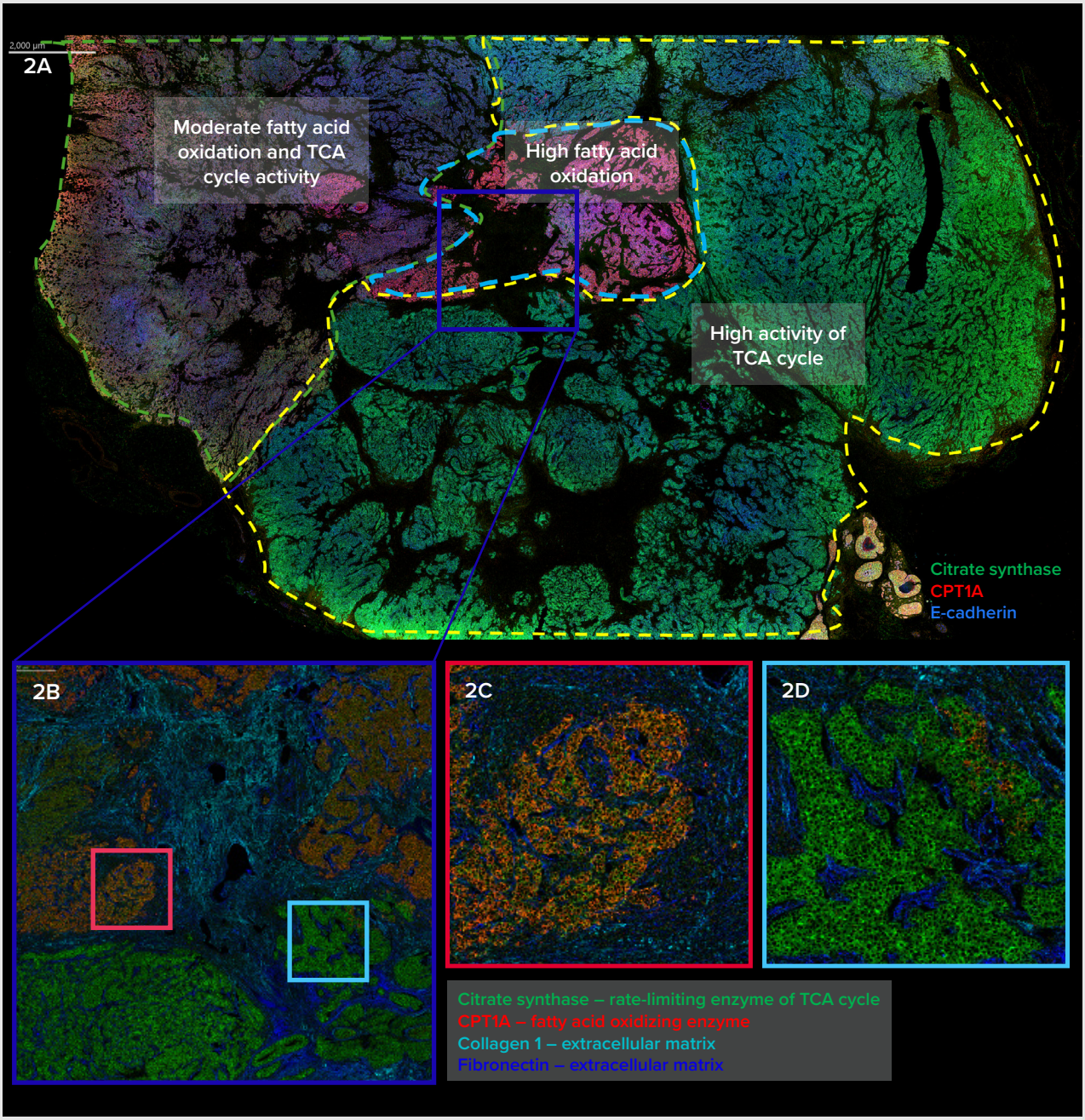


Figure 2. IMC images generated on the Hyperion XTi Imaging System show the spatial organization of metabolically active tumor cells in breast adenocarcinoma. Using Tissue Mode (Figure 2A), clear delineation of pathways can be visualized across the whole tissue, providing information on metabolic pathway activation and tumor growth patterns. Using Cell Mode (Figure 2B, with inset regions shown in Figures 2C and 2D), the differential utilization of energy sources can be spatially resolved on a single-cell level, highlighting the heterogeneity of tumor cells within the TME. Images shown are only subsets of the total 43-marker assay, in which each marker is an individual quantitative assay that can be visualized in useful combinations. Here, five markers are shown for easy identification of each marker.

Cell Mode image and single-cell analysis show that immune cells preferentially infiltrate tumor areas with fatty acid oxidizing activity

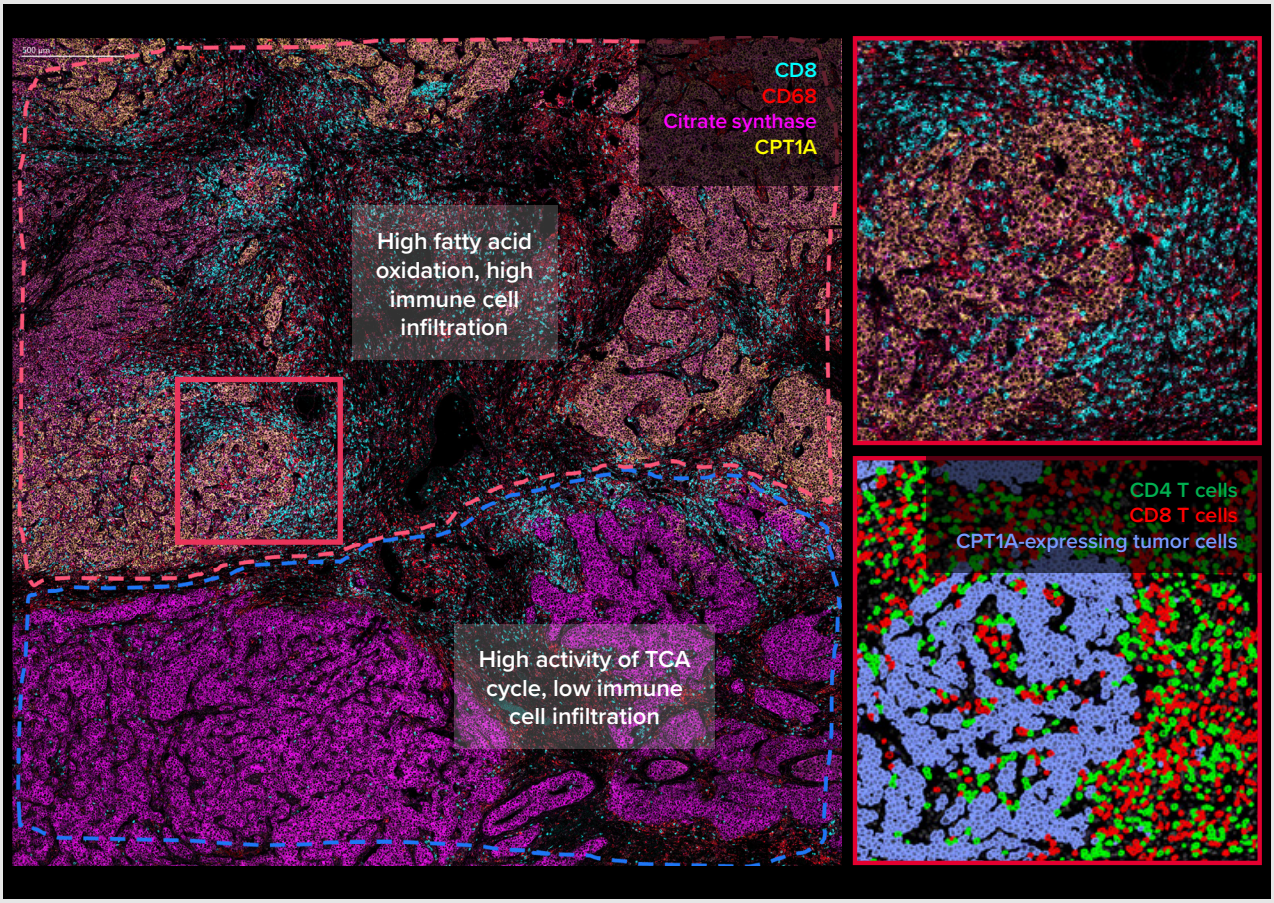


Figure 3. Single-cell analysis demonstrates the utility of the Human Cell Metabolism IMC Panel by revealing distinct metabolic zones and selective immune cell infiltration into specific zones. Two distinct tumor cell populations with varying expression of fatty acid oxidizing enzyme CPT1A display differential infiltration of cytotoxic T cells and macrophages. The inset image (top) shows a high abundance of immune cells detected adjacent to a high CPT1A-expressing tumor cell population. The inset image (bottom, supervised clustering using QuPath) enables quantitative single-cell analysis highlighting the close spatial proximity of cytotoxic and T helper cells with the high CPT1A-expressing cell population.

Single-cell analysis using IMC distinguishes tumor immune microenvironments associated with metabolic activity

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 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 metabolic activity.

Conclusion

Modular IMC panels enable comprehensive spatial and metabolic profiling, revealing the interconnected roles these pathways play in promoting tumor survival and resistance to therapies. Their application uncovers metabolic heterogeneity 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 Metabolism IMC Panel, with seven pathologist-verified antibodies targeting GLUT1, citrate synthase, ATP5A, CPT1A, LDHA, p4E-BP1 and pS6, 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 Metabolism IMC Panel enhances studies of cancer, autoimmune diseases, age-related conditions and more – demonstrated here in breast 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: mTOR signaling and oxidative phosphorylation in colon adenocarcinoma, acquired on the Hyperion XTi Imaging System. IMC markers shown: pS6 (red), ATP5A (green), E-cadherin (blue)

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