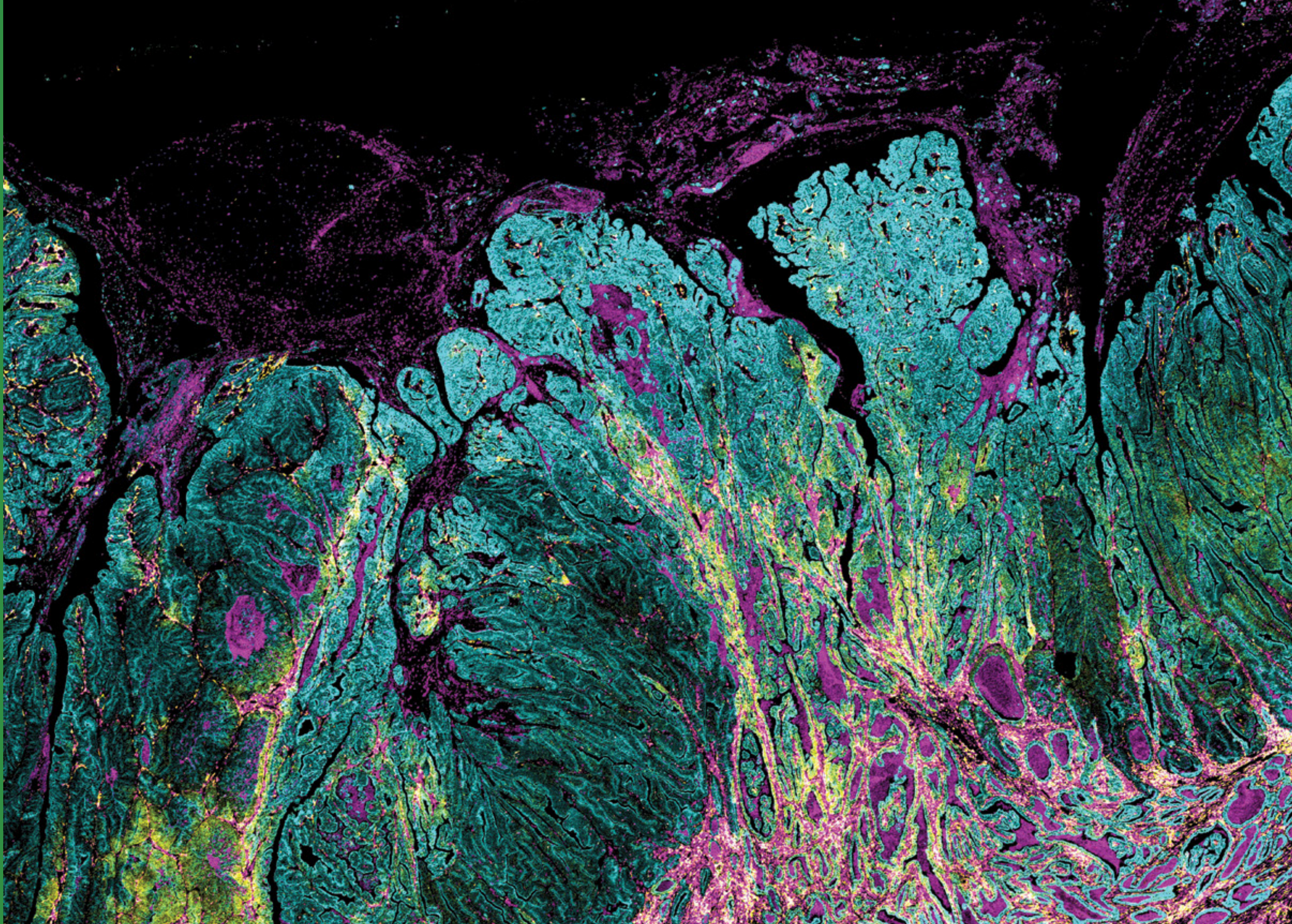
Image Lookbook

NEXT-GENERATION SPATIAL BIOLOGY

High-Throughput Multiplexed Imaging Mass Cytometry with Whole Slide Modes

Immuno-oncology

Take a deep dive into translational and clinical applications that explore new views of cellular composition within the tissue microenvironment



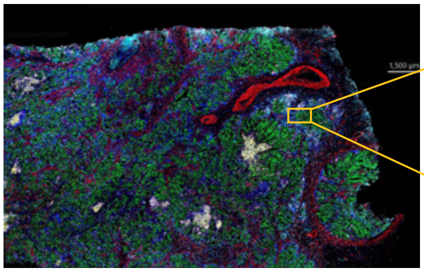
Imaging modes overview

Identifying cellular- and spatial-level composition of the tumor microenvironment (TME) is vital for interpretation of disease origin, progression, prognosis and treatment options. Whole slide imaging (WSI) modes and an integrated automated slide loader have been developed for Imaging Mass Cytometry™ (IMC™) platforms that **enable streamlined workflows using ultrafast Preview Mode and high-throughput Tissue Mode.**

Novel WSI modes with the Hyperion™ XTi Imaging System provide rapid and detailed assessment of tissue structure, identifying diverse cell types within functionally varied ecosystems of the TME. This lookbook showcases translational and clinical applications of multiplexed tissue analysis.

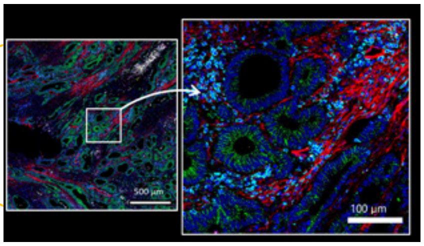
KEY TAKEAWAYS

- WSI modes provide a novel high-throughput workflow for multiplexed imaging of tumor samples
- Using a combination of imaging modes and ready-to-go high-plex panels on the same tissue section provides researchers with more flexibility to understand the extensive cellular heterogeneity of the TME
- Rapid imaging modes demonstrate key biological insights captured in spatial context that are relevant for identifying prognostic and diagnostic biomarkers for targeted therapies



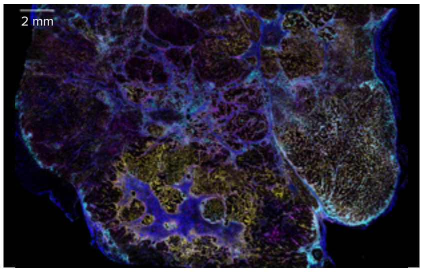
Preview Mode

Number of markers: 42
Acquisition time: 20 minutes
Sample: colon cancer (25 mm x 15 mm)



Cell Mode

Number of markers: 42
Acquisition time: 2 hours
Sample: colon cancer (2 mm x 2 mm)
Resolution: 1 µm



Tissue Mode

Number of markers: 42
Acquisition time: 5 hours and 50 minutes
Sample: breast cancer (24 mm x 16 mm)
Resolution: 5 µm

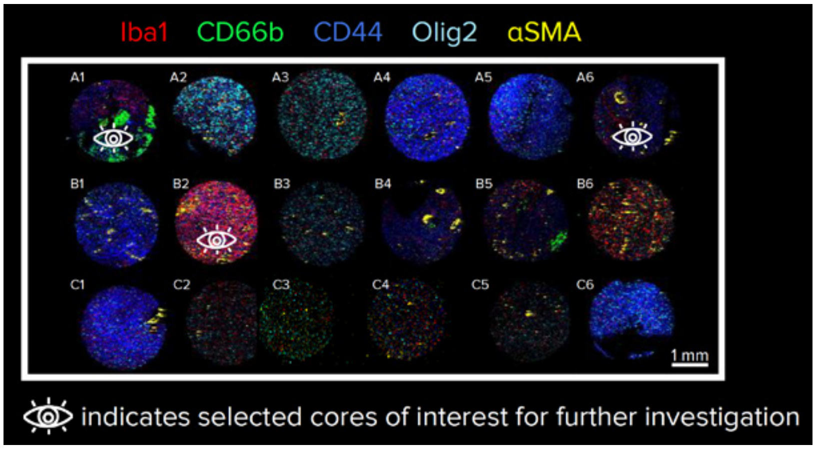
Imaging tissue microarrays

Rapid and high-throughput workflow unveils extensive cellular heterogeneity

Applying three rapid imaging modes to a tissue microarray (TMA) containing dozens of human glioma cores revealed the spatial distribution of over 40 distinct molecular markers.

Fast screening of the entire slide combined with single-cell analysis

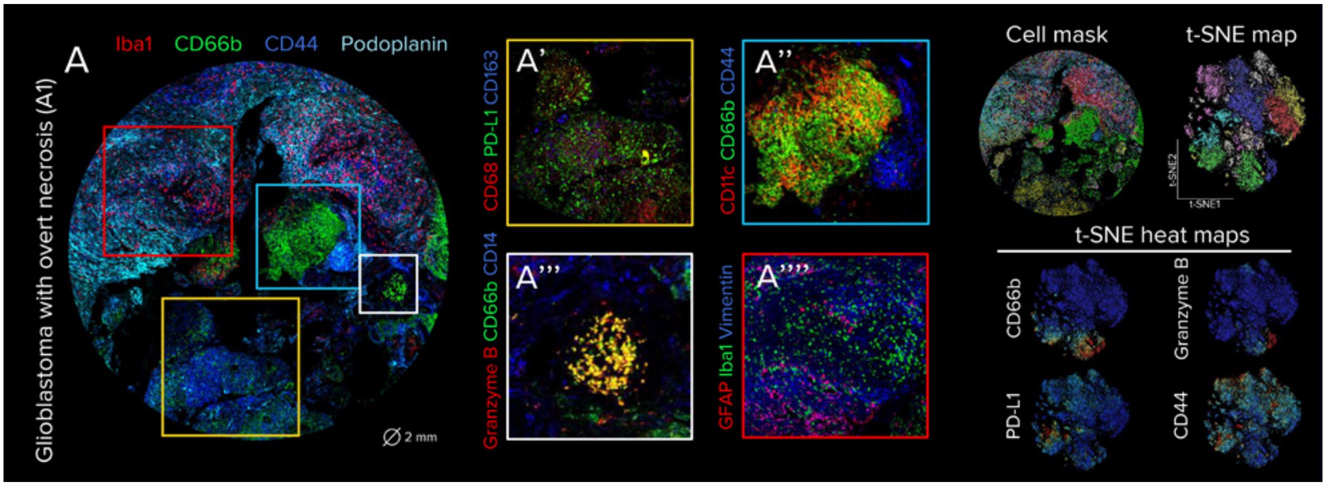
PREVIEW MODE



Preview Mode was applied to rapidly screen tumor cores for expression signatures associated with tumor immuno-oncology processes. This enabled biomarker-guided selection of areas in tumor tissue that were imaged at higher resolution and analyzed with single-cell analysis using **Cell Mode**.

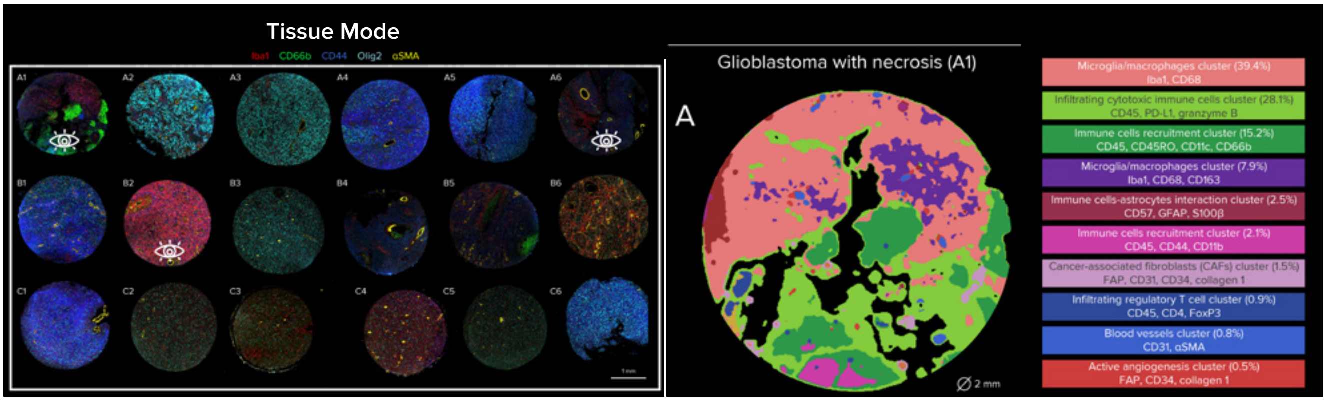
View the study details

CELL MODE



Tissue Mode facilitates identification of prominent features in all TMA cores

TISSUE MODE



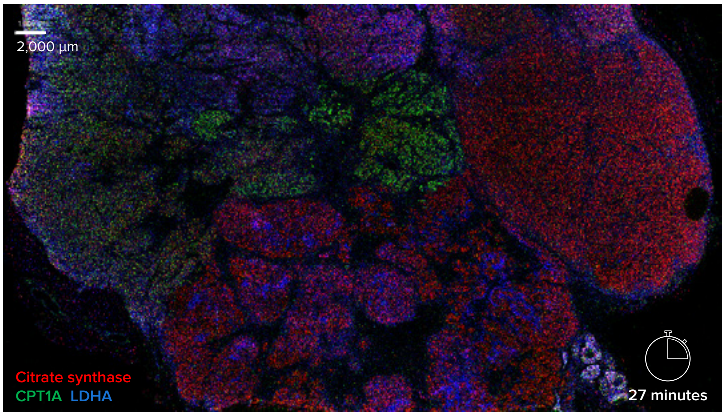
From larger samples to TMA cores, **Tissue Mode generates a high-quality scan of the entire tissue section in a matter of hours** with higher spot-size ablations, enabling entire tissue analysis using pixel-clustering methods. This is an especially high-throughput modality with TMAs, as eighteen 2 mm TMA cores can be **imaged in 1 hour and 35 minutes**.

Human solid tumor

In this application, breast adenocarcinoma and colon adenocarcinoma tissue samples were stained with a 43-marker panel comprised of the high-plex Human Immuno-Oncology IMC Panel, 31 Antibodies and expanded targets of interest, and acquired with WSI modes.

Identifying varying levels of biomarker expression across breast cancer tissue

PREVIEW MODE



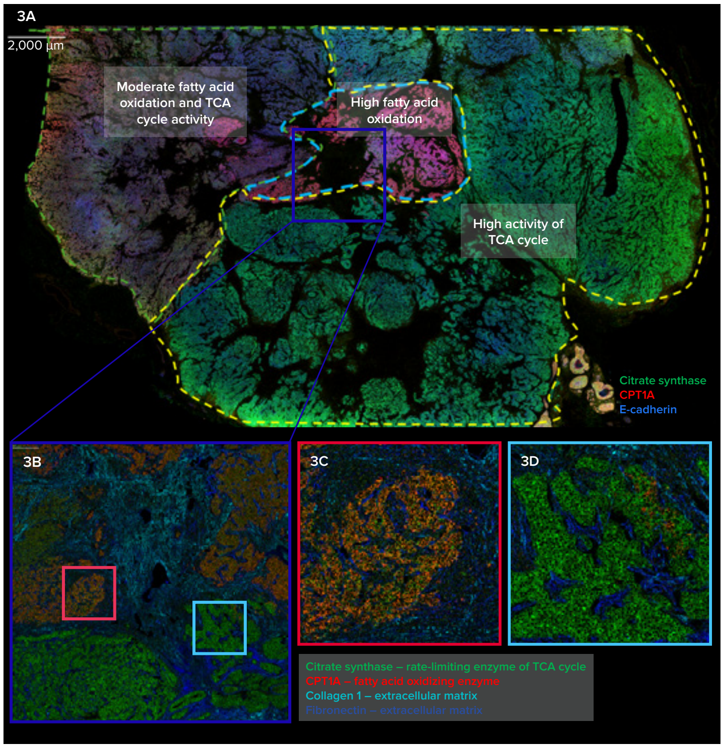
Tumor cells differentially utilize glucose and fatty acid oxidation to fuel growth in breast adenocarcinoma. A **Preview Mode** scan rapidly revealed the spatial organization and metabolic profile of cells across the entire breast cancer (invasive ductal carcinoma) tissue.

Citrate synthase is a rate-limiting enzyme of the TCA cycle; CPT1A is a fatty acid oxidizing enzyme; and LDHA catalyzes lactate production from pyruvate.

Revealing spatial organization of metabolically active tumor cells

TISSUE MODE

CELL MODE



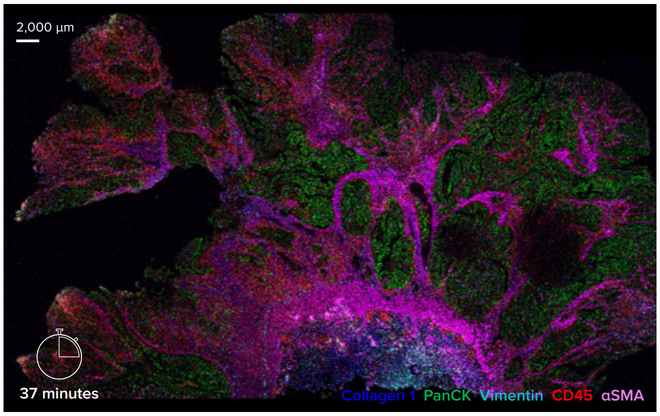
Using **Tissue Mode** (Image 3A), clear delineation of pathways can be visualized across the whole tissue, providing information on metabolic pathway activation and tumor growth patterns. Using **Cell Mode** (Image 3B, with inset regions shown in Images 3C and 3D), the differential utilization of energy sources can be spatially resolved on a single-cell level, highlighting the heterogeneity of tumor cells within the TME.

41-marker IMC panel for spatially resolved profiling of metabolic activation

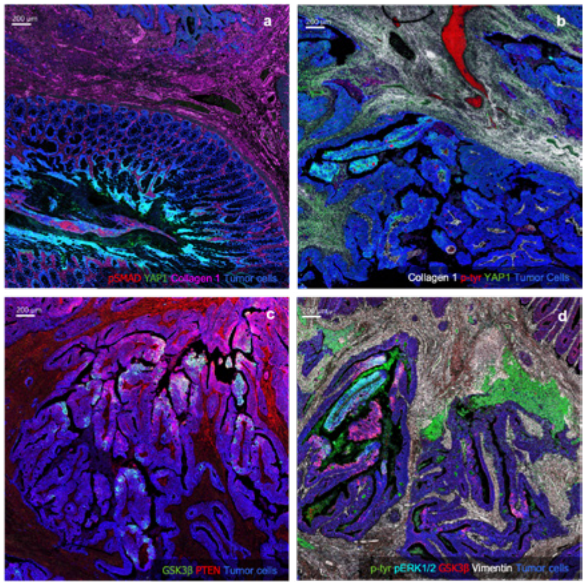
Human Immuno-Oncology IMC Panel, 31 Antibodies (PN 201509)							**Human Cell Metabolism IMC Panel, 7 Antibodies** PN 201521	**Maxpar™ IMC Cell Segmentation Kit** PN 201500
Cell Functional State PN 201514	Stromal Cell PN 201511	Basic Immune PN 201518	Lymphoid PN 201512	Myeloid PN 201513	Tissue Architecture PN 201510	Epithelial and Mesenchymal PN 201515		

Changes in signaling activity in different regions of the TME in colon adenocarcinoma

PREVIEW MODE



CELL MODE

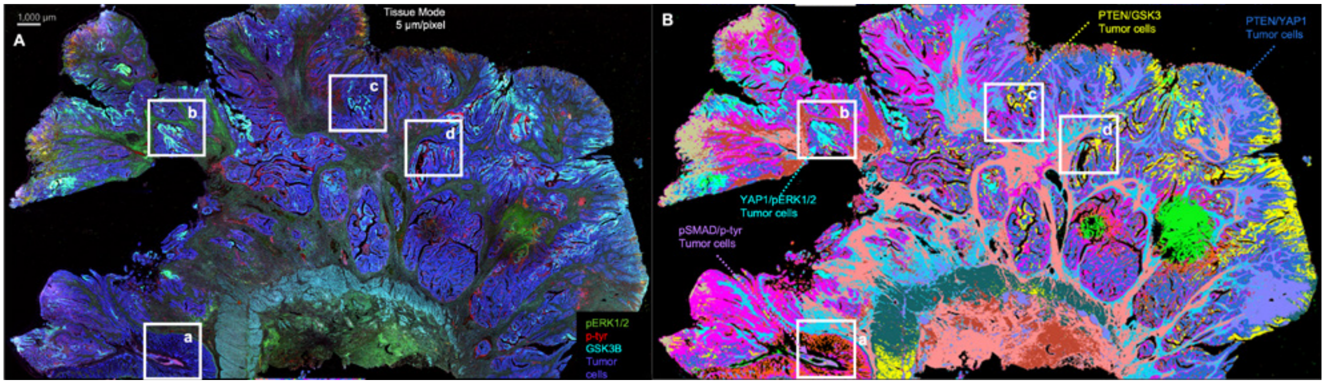


A quick tissue scan using **Preview Mode** guides single-cell analysis of selected regions of interest (ROIs).

Cell Mode 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; (C) Wnt (GSK3β) signaling activity is activated in different tumor regions; and (D) Wnt signaling is seen in some tumor regions while other tumor cells use MAPK (pERK1/2) signaling.

Mapping differential expression across signaling mechanisms

TISSUE MODE



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 performed using MCD™ SmartViewer shows differential activation of signaling cascades in colon adenocarcinoma cells.

View the
study details

41-marker IMC panel enabling spatially resolved profiling of cell signaling pathways

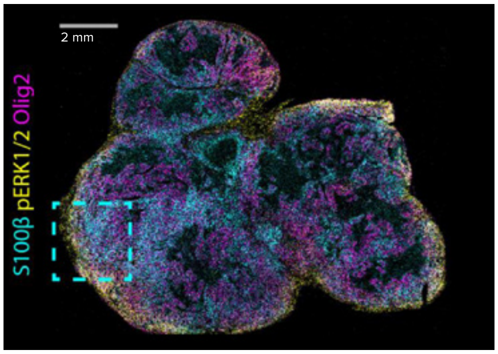
Human Immuno-Oncology IMC Panel, 31 Antibodies (PN 201509)							**Human Cell Signaling IMC Panel, 7 Antibodies** PN 201522	**Maxpar IMC Cell Segmentation Kit** PN 201500
Cell Functional State PN 201514	Stromal Cell PN 201511	Basic Immune PN 201518	Lymphoid PN 201512	Myeloid PN 201513	Tissue Architecture PN 201510	Epithelial and Mesenchymal PN 201515		

Neuro-oncology

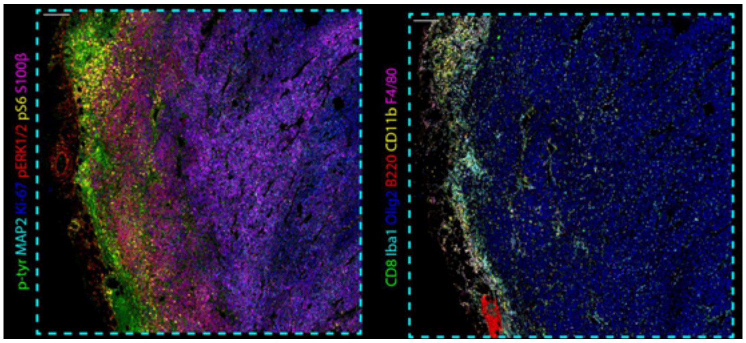
In a study of mouse glioblastoma (GBM) tissue, spatial complexity is revealed in heterogeneous brain tissue without the challenges of tissue degradation and autofluorescence artifacts.

Mouse neuro-oncology panel detects tumor cell and immune cell infiltration in GBM

PREVIEW MODE

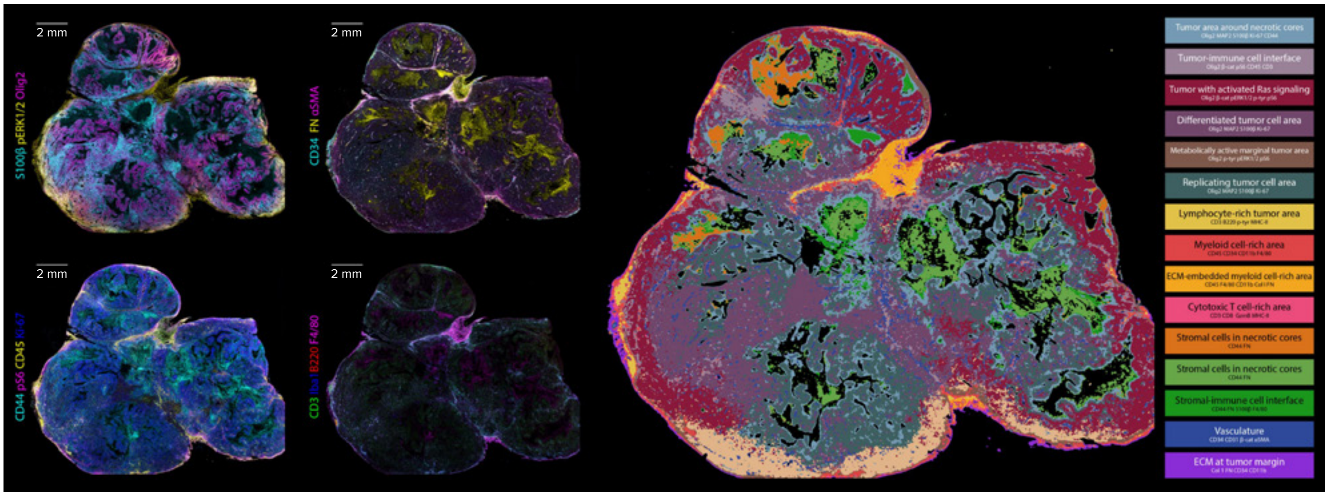


CELL MODE



Pixel-clustering analysis reveals highly specialized tumor, immune and stromal tissue compartments

TISSUE MODE



Tissue Mode imaging demonstrates the tumor and immune cell heterogeneity of mouse GBM tissue and the spatial positioning of tissue compartments. Metabolically active tumor cells were detected at the periphery of the tumor. Immune cells were detected in high concentration at the tumor margin and in necrotic cores. Unsupervised pixel-clustering analysis with hierarchical clustering quantitatively segregates highly specialized subcompartments and detects areas containing subsets of differentiated tumor cells, immune hot and cold areas, stromal compartments, vasculature and extracellular matrix.

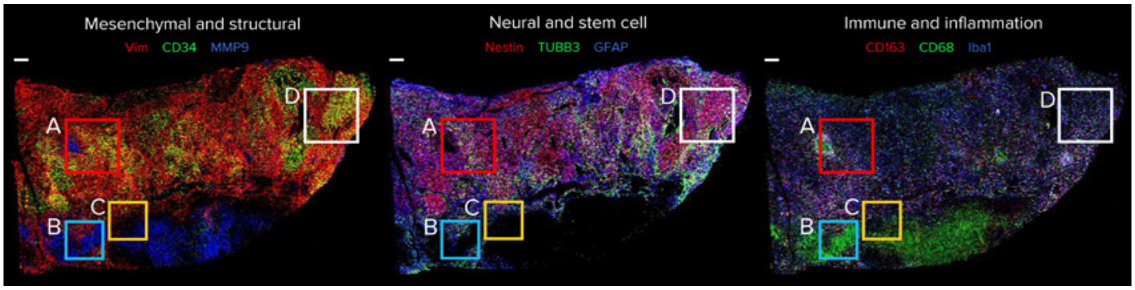
40-marker IMC panel for the study of the TME in mouse neurological tissues

Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle (PN 9100005NO)					**Maxpar IMC Cell Segmentation Kit** PN 201500
Maxpar OnDemand Mouse Tissue Architecture IMC Panel Kit PN 9100001	Maxpar OnDemand Mouse Cancer Cell Process IMC Panel Kit PN 9100002	Maxpar OnDemand Mouse Immune Phenotyping IMC Panel Kit PN 9100003	Maxpar OnDemand Mouse Immune Activation IMC Panel Kit PN 9100004	Maxpar Neuro Phenotyping IMC Panel Kit PN 201337	

Applying imaging modes to a human whole GBM tumor tissue section provided new perspectives on GBM's neuronal and mesenchymal origins.

PREVIEW MODE

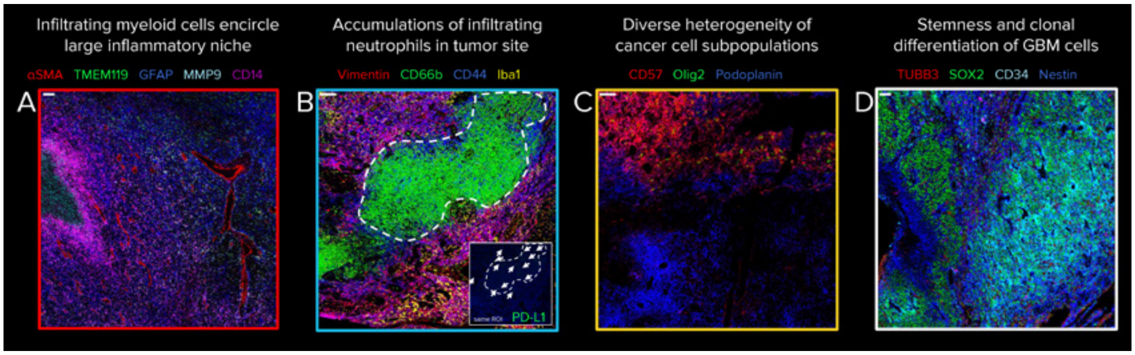
Unveiling distinct patterns of marker distribution in whole GBM tissue



A **Preview Mode** scan rapidly reveals the expression pattern of all markers in the panel and underscores the complex interplay between tumor cells, the extracellular matrix, immune activity and other processes.

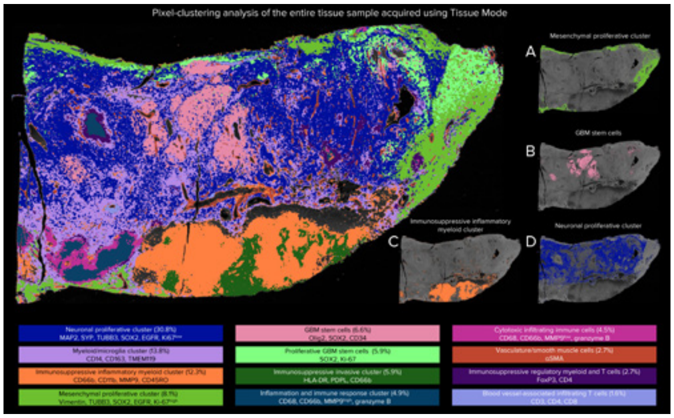
CELL MODE

In-depth investigation identifies localized niches with aggregated cell populations



Detailed visualization of some GBM niches in **Cell Mode** indicates potential active epithelial to mesenchymal transition and demonstrates the diversity of cancer cells expressing various stem cell-like markers, highlighting the increased ability of GBM to self-renew.

TISSUE MODE



Pixel-clustering analysis highlights spatial location and composition of TME compartments in GBM

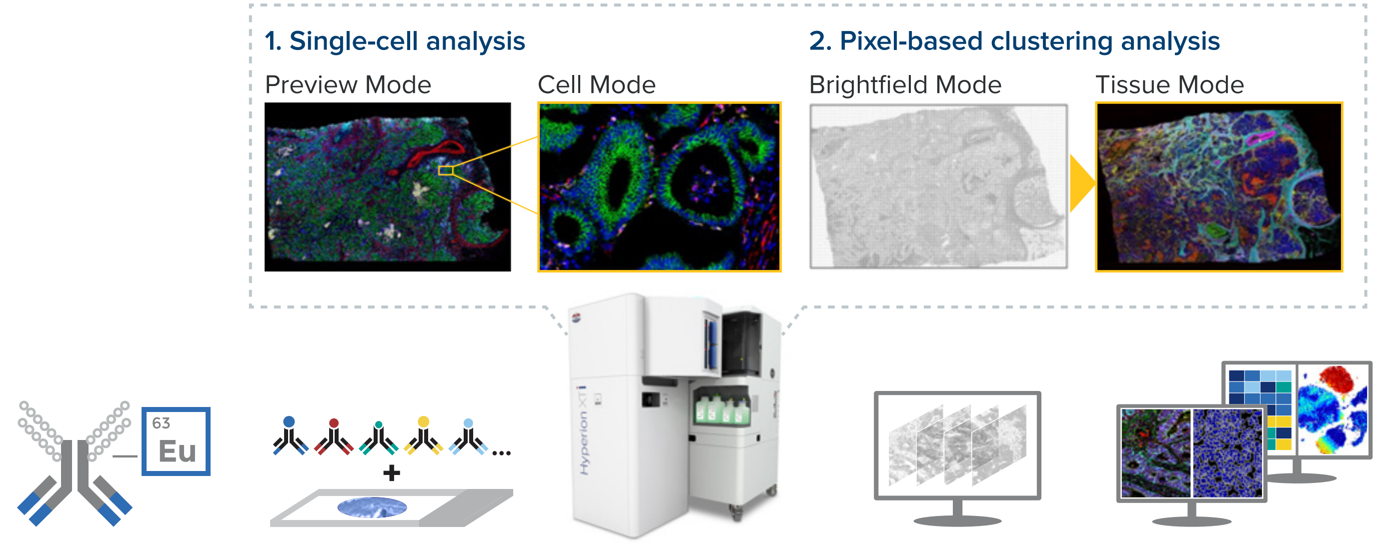
Tissue Mode confirms extreme intratumor heterogeneity. Unsupervised pixel-clustering analysis was performed using the MCD SmartViewer analysis pipeline on the entire GBM tissue, which resulted in identification of 12 distinct clusters based on their marker expression patterns.

View the
study details

41-marker IMC panel targeting neuronal-specific markers in human gliomas

Human Immuno-Oncology IMC Panel, 31 Antibodies (PN 201509)					**Glioblastoma IMC Panel, 5 Antibodies** PN 9100011	**Human Neuro Expansion IMC Panel, 3 Antibodies** PN 9100012	**Maxpar Neuro Phenotyping IMC Panel Kit, 7 Antibodies** PN 201337	**Maxpar IMC Cell Segmentation Kit** PN 201500
Stromal Cell PN 201511	Lymphoid PN 201512	Myeloid PN 201513	Cell Functional State PN 201514	Basic Immune PN 201518				

Protocol



1. Single-cell analysis

Preview Mode

Cell Mode

2. Pixel-based clustering analysis

Brightfield Mode

Tissue Mode

1 **Obtain** metal-conjugated antibodies.

2 **Stain** tissue with antibody cocktail.

3 **Image** tissue with Hyperion XTi Imaging System.

4 **Collect** high-dimensional data.

5 **Perform** data analysis.

- Single-cell analysis
- Pixel-clustering analysis

References

Pfister, T.D. et al. "Spatial Profiling of the Tumor Microenvironment Using Imaging Mass Cytometry: Unraveling Metabolic Reprogramming and Cell Signaling Dynamics in Cancer." Presented at FOCIS 2025 Annual Meeting.

Raza, Q. et al. "Novel whole slide imaging modes for Imaging Mass Cytometry reveal cellular and structural composition of mouse glioblastoma." *Cancer Research* 84 (2024): 1,450–1,450.

Raza, Q. et al. "Next generation of spatial biology: High-throughput multiplexed Imaging Mass Cytometry with whole slide modes." *Cancer Research* 84 (2024): 3,800–3,800.

Zabinyakov, N. et al. "Novel whole slide imaging modes for Imaging Mass Cytometry unveil extensive cellular heterogeneity in human gliomas." *Cancer Research* 84 (2024): 5,501–5,501.

Zabinyakov, N. et al. "Reveal the heterogeneity of glioblastoma and other tumors using high-plex Imaging Mass Cytometry empowered by whole slide imaging modes." Presented at 2024 Society for Neuroscience Conference.

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