IMAGE LOOKBOOK

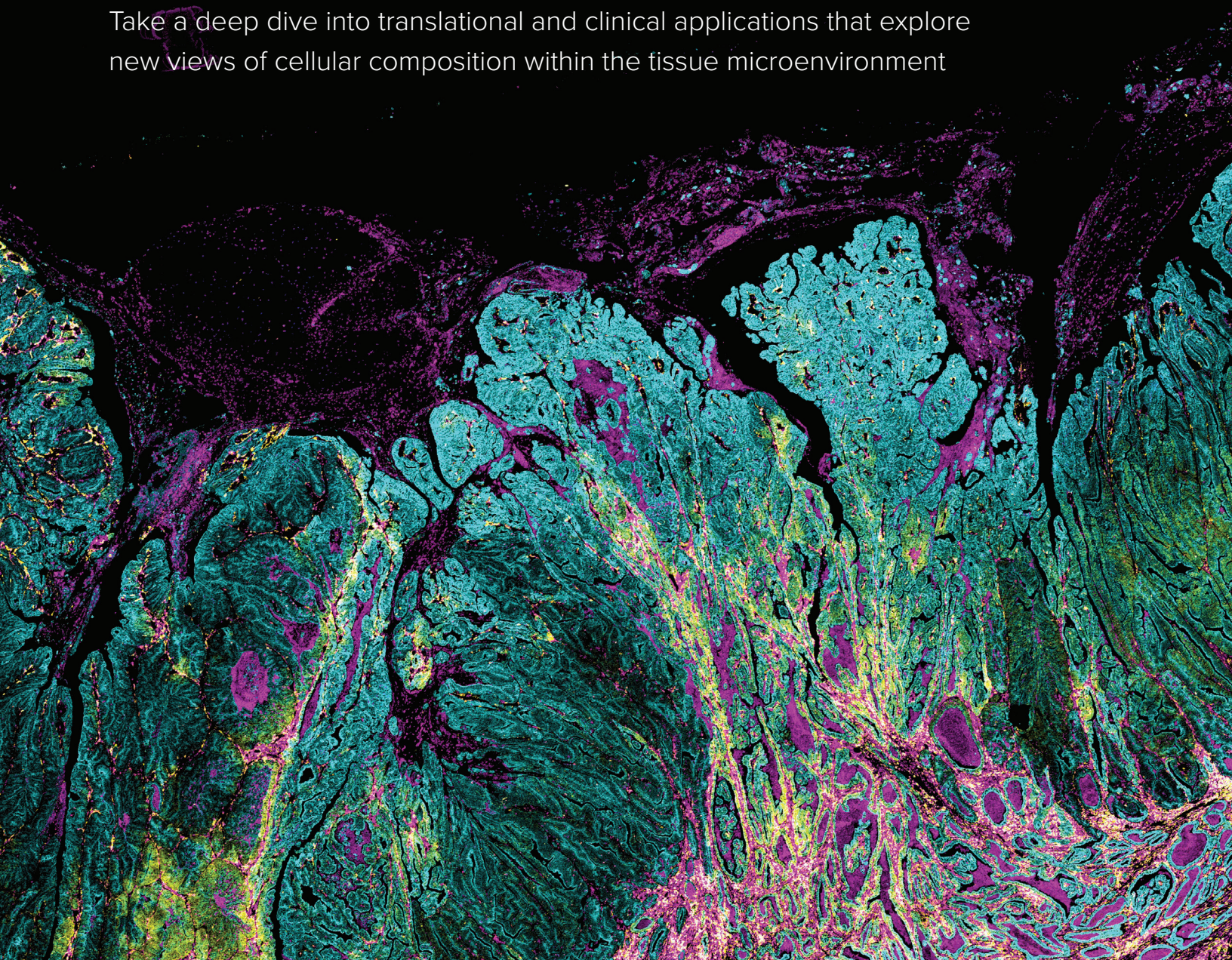
STANDARD BIOTOOLS™

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



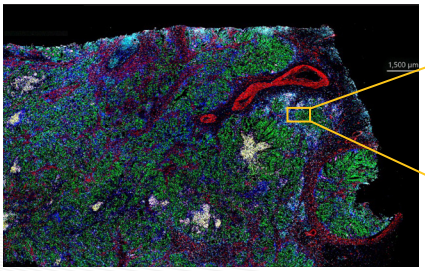
Introduction

Identifying cellular- and spatial-level composition of the tumor microenvironment (TME) is vital for interpretation of disease origin, progression, prognosis and treatment options. Two new whole slide imaging (WSI) modes and an integrated automated slide loader have been developed for Imaging Mass Cytometry™ (IMC™) 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 and cell features. This lookbook showcases translational and clinical applications of multiplexed tissue analysis.

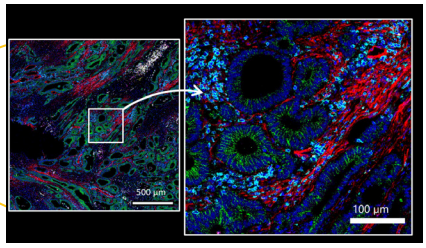
KEY TAKEAWAYS

- New imaging 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.



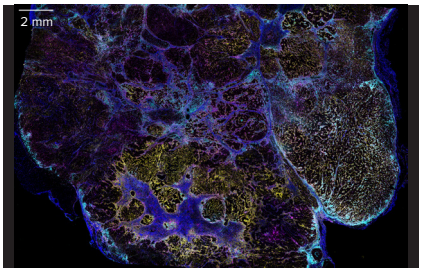
Preview Mode

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

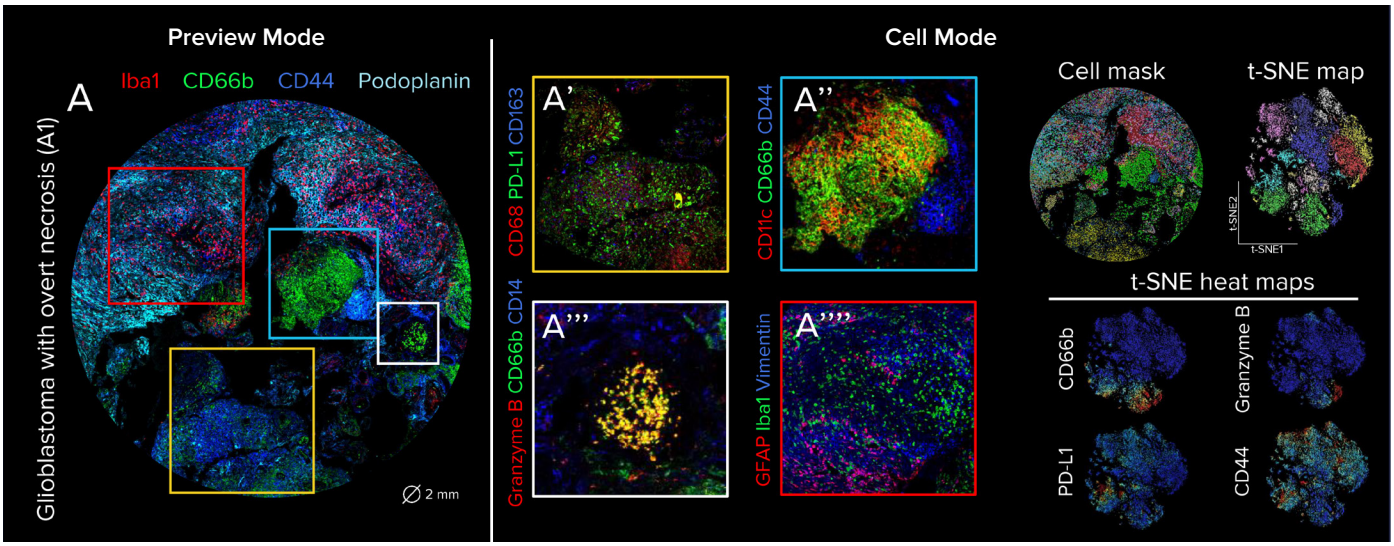
Imaging Modes Overview

View the study details

Rapid and high-throughout workflow unveils extensive cellular heterogeneity

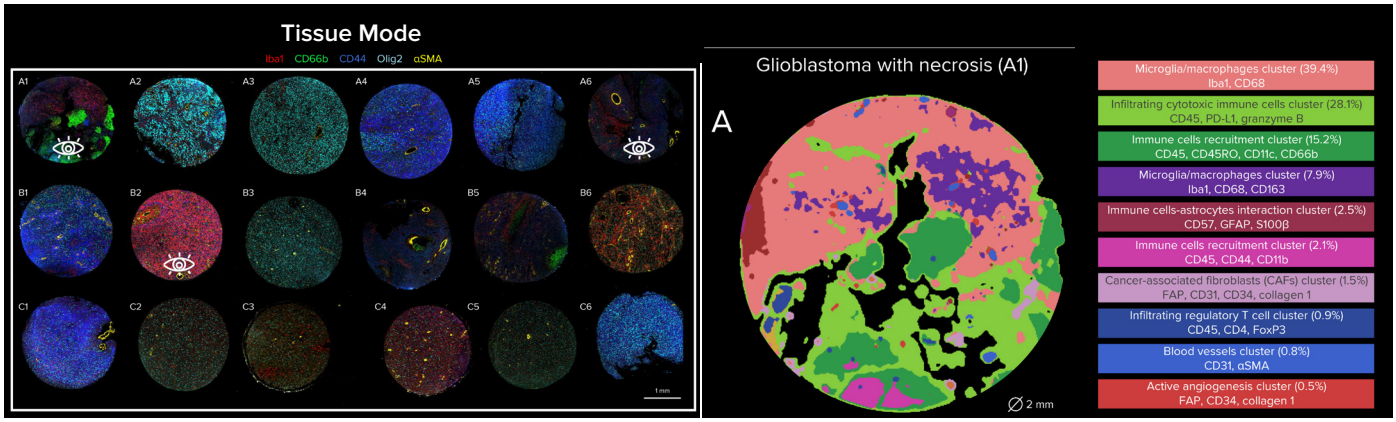
Applying three rapid imaging modes to a tissue microarray (TMA) containing dozens of human glioma cores identified the spatial distribution of over 40 distinct molecular markers.

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



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**.

Tissue Mode facilitates identification of prominent features in all TMA cores



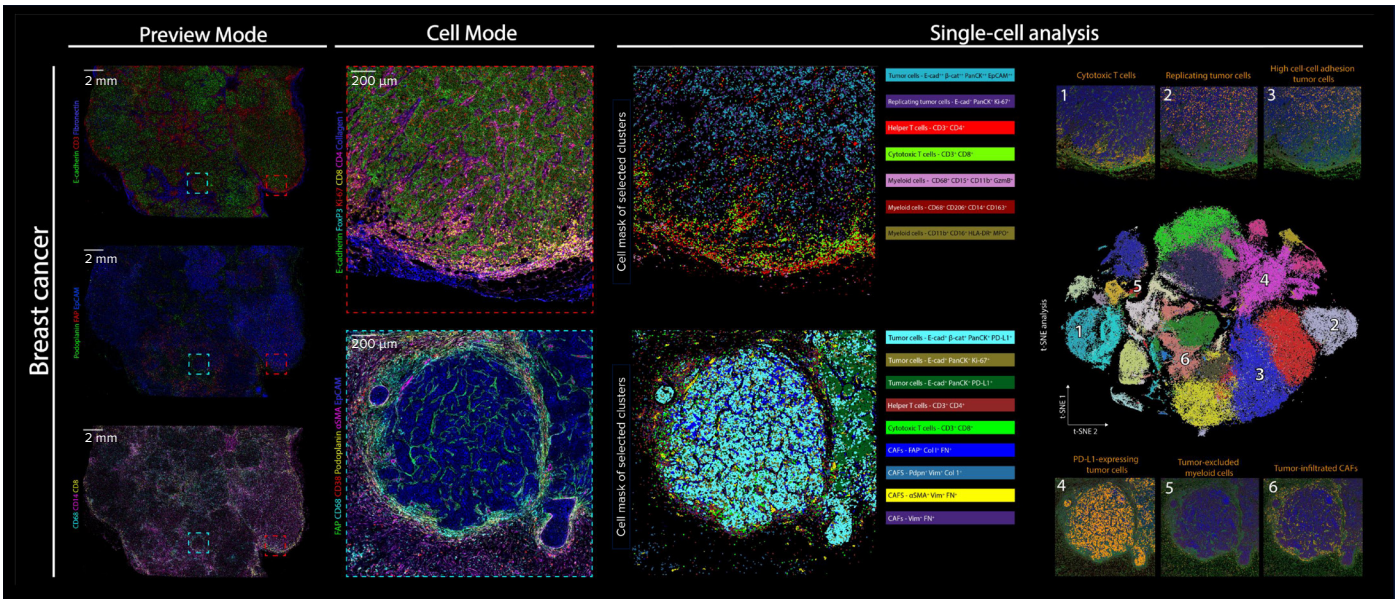
From larger samples to TMA cores, **Tissue Mode generates a high-quality scan of the entire tissue section in a matter of hours** with higher spot-size ablations enabling entire tissue analysis using pixel-clustering methods. This is an especially high-throughput modality with TMAs, as 18 2 mm TMA cores can be **imaged in 1 hour and 35 minutes**. In the figure above, Tissue Mode visualizes tissue compartments and indicates high heterogeneity of human glioma cores. Cores of interest are selected for subsequent pixel-clustering analysis.

Human Solid Tumor

In this application, breast cancer and colon cancer tissue samples were stained with a 41-marker panel comprised of the high-plex Human Immuno-Oncology IMC Panel, 31 Antibodies and expanded targets of interest, and acquired with WSI modes.

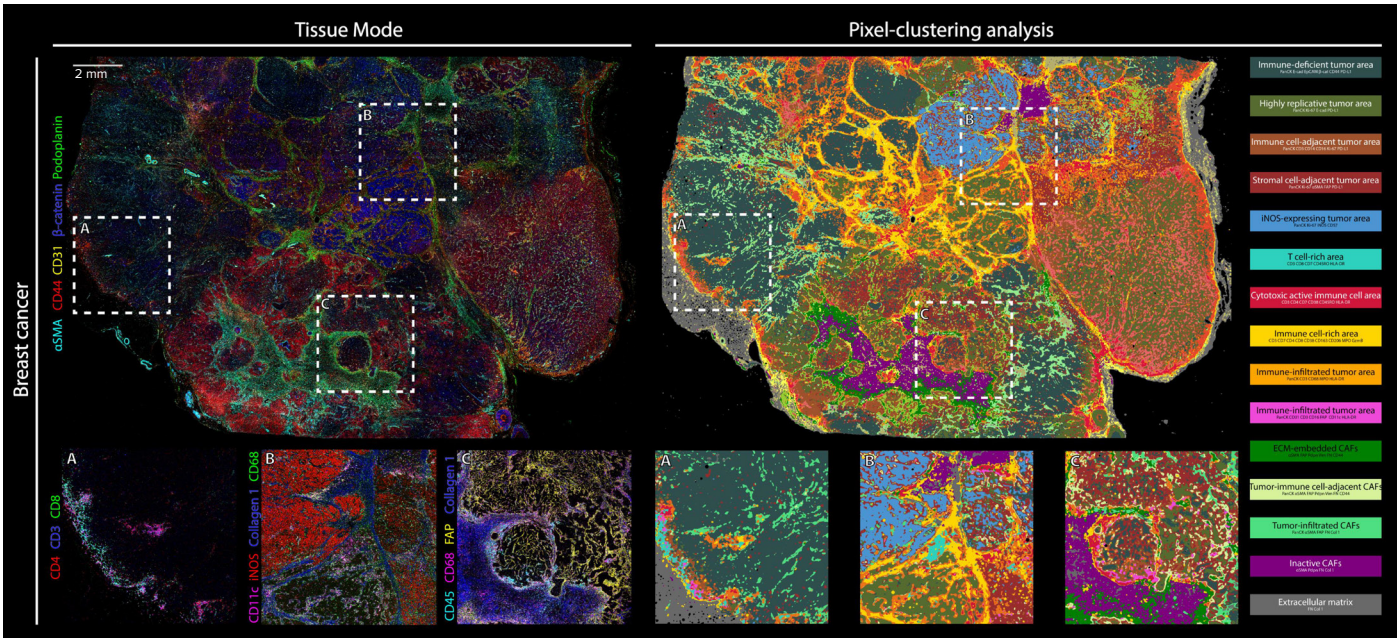
View the study details

Identifying regions of immune activity and infiltration in tumor tissue



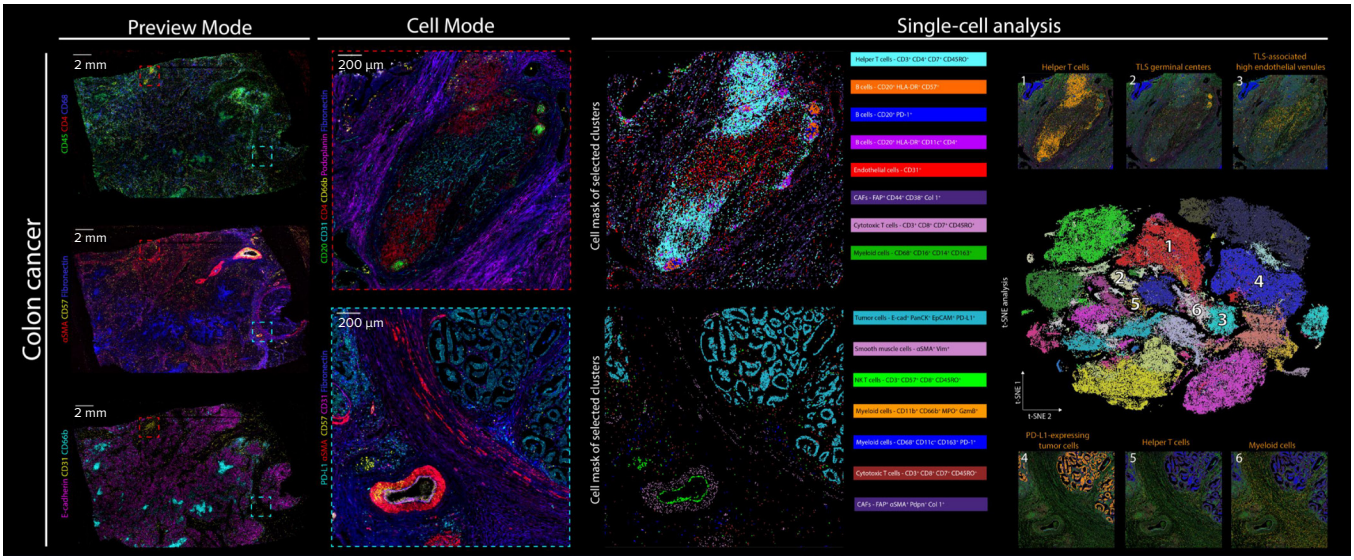
Multiple types of lymphoid and myeloid immune cells were detected at the tumor margin (red inset). A tumor structure with low immune penetration was detected surrounding the necrotic tissue (cyan inset). Single-cell analysis quantitatively delineated cell phenotype of PD-L1-expressing tumor and immune cell populations. The detection of subpopulations of tumor and immune cells defines the highly heterogeneous nature of the breast cancer sample.

Revealing complex compartmentalization and tissue heterogeneity



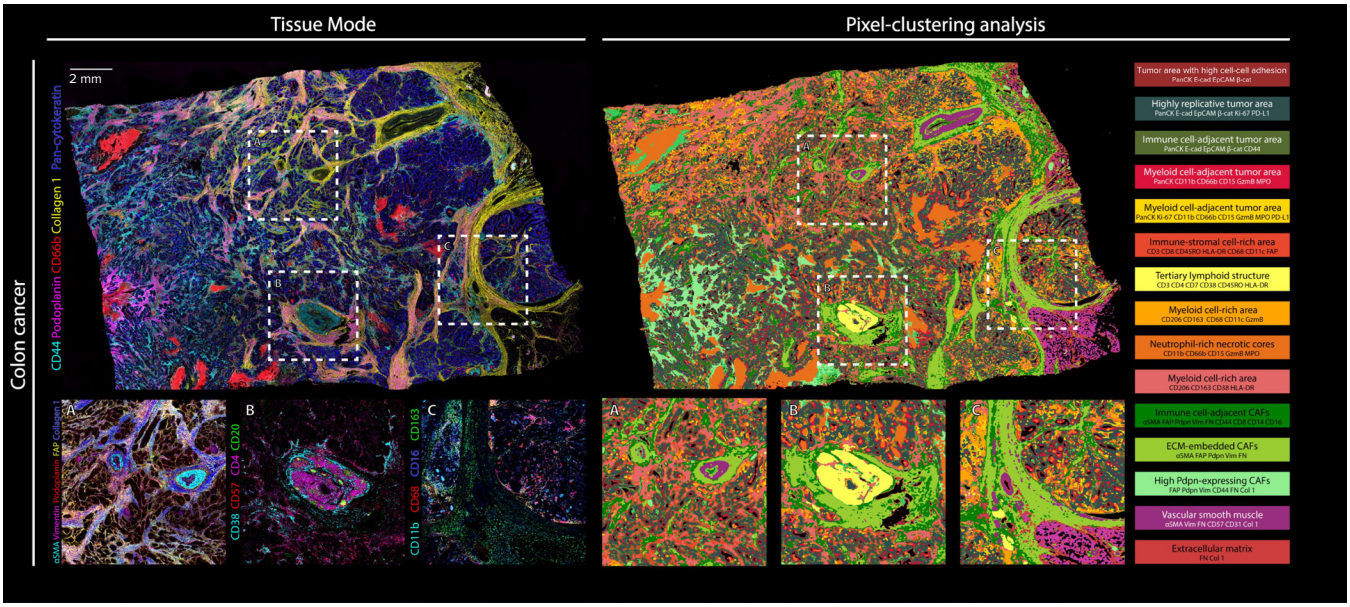
Striking heterogeneity of breast cancer tissue can be detected with specialized tumor structures across the tissue. Lymphocyte localization is observed at the tumor margins (A, inset). The presence of specialized tumor cells expressing iNOS (B, inset) and tissue compartments containing interactive niches of cancer-associated fibroblasts (CAFs) and immune cells (C, inset) are detected. Pixel-clustering analysis highlighted several tumor areas such as immune-deficient, highly replicative, immune cell-adjacent and stromal cell-adjacent tumor regions.

Detection of tertiary lymphoid structures and PD-L1-expressing tumor cells in the colon



Preview Mode highlighted several tumor-specific structures such as tertiary lymphoid structures (TLSs) and high vascular and immune cell-density landmarks. Subsequent single-cell analysis demonstrated immune evasive tumor cell populations, CAFs and myeloid and lymphoid immune cell types.

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



Immune-stromal interactive niches, myeloid cells, neutrophil-rich necrotic cores and TLSs can be visualized. Striking heterogeneity of CAFs and a large TLS are detected. Myeloid cells are observed within the tumor and stromal compartments. Analysis identified multiple tumor areas with high expression of cell-cell adhesion markers, replication markers and immune cell-adjacent areas.

A 41-marker panel comprised of pathologist-verified antibodies permits detection of various immuno-oncological processes in human tumors.

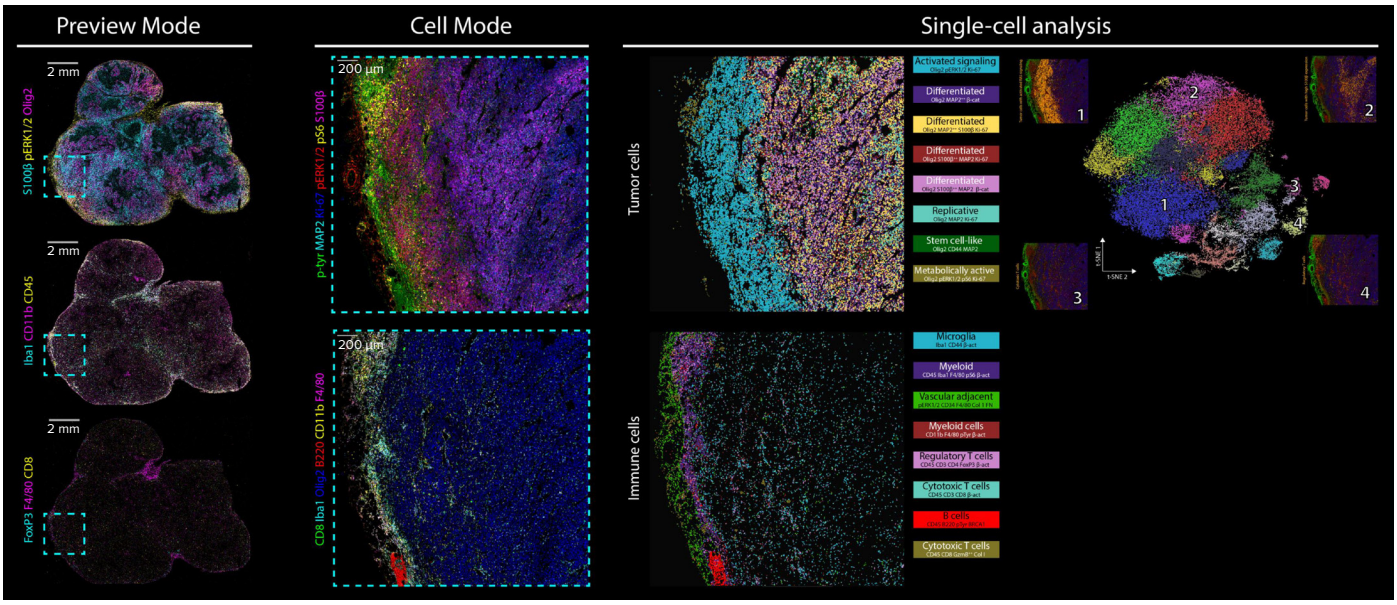
Human Immuno-Oncology IMC Panel, 31 Antibodies (PN 201509)							Human Immune Cell Expansion IMC Panel, 7 Antibodies PN 201516	Maxpar® IMC Cell Segmentation Kit PN 201500
Cell Functional State PN 201514	Stromal Cell PN 201511	Basic Immune PN 201518	Lymphoid PN 201512	Myeloid PN 201513	Tissue Architecture PN 201510	Epithelial and Mesenchymal PN 201515		

Neuro-Oncology

View the study details

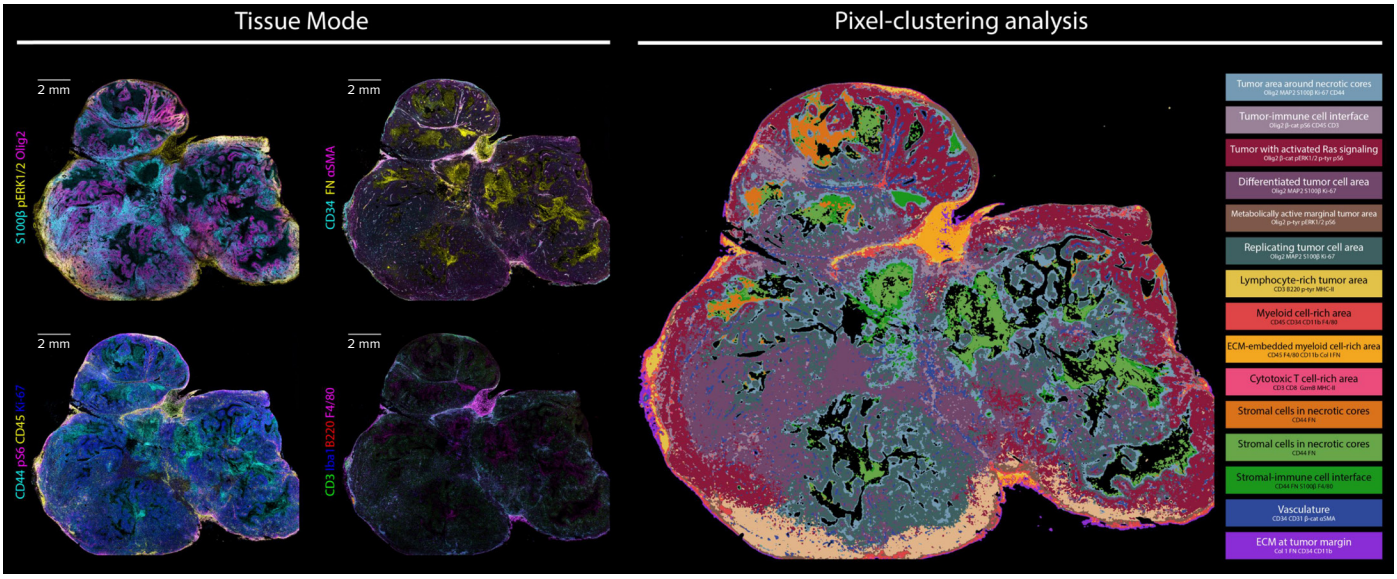
In a study of mouse embryo, normal brain and glioblastoma (GBM) tissue, a 44-marker neuro-oncology panel composed of the Maxpar OnDemand™ Mouse Immuno-Oncology IMC Panel Kit and the Maxpar Neuro Phenotyping IMC Panel Kit revealed the spatial distribution of over 40 distinct molecular markers.

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



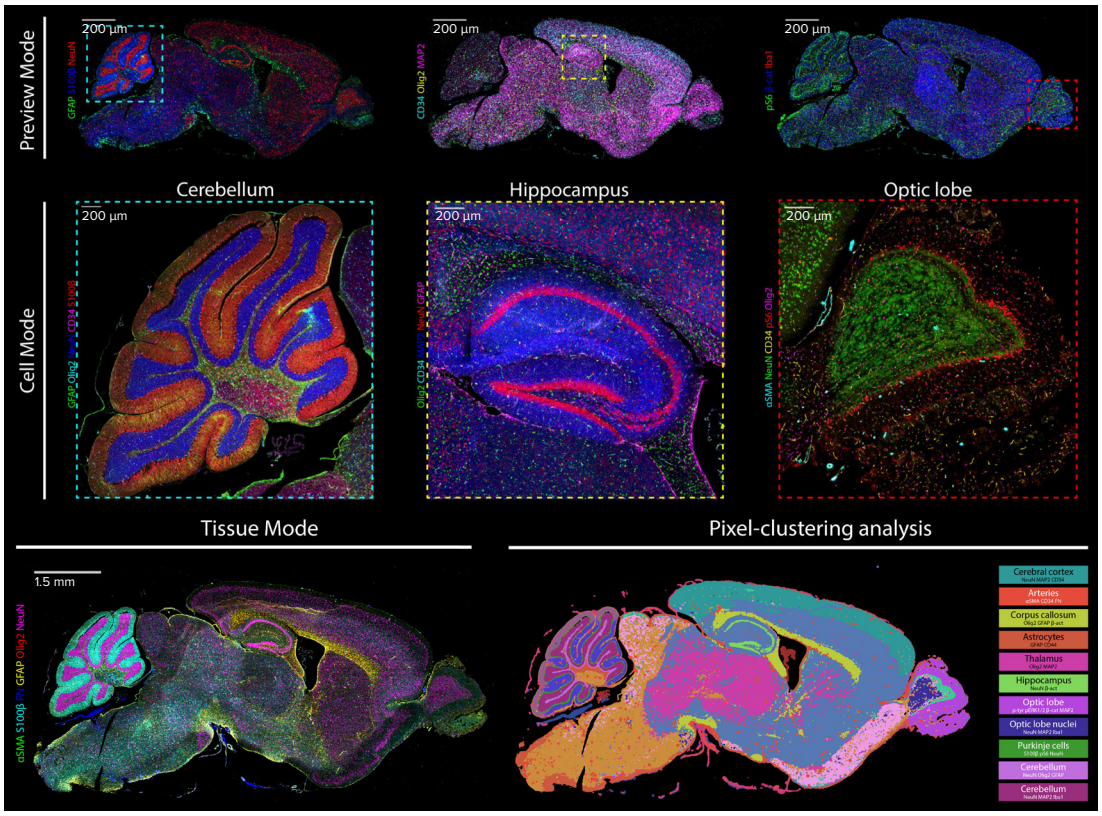
Preview Mode scan rapidly identified areas with high tumor and immune cell activity, which was used to identify relevant regions of interest for detailed Cell Mode investigation. Multiplex Cell Mode images using tumor- (top) and immune- (bottom) specific markers demonstrate the heterogeneity of the TME.

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



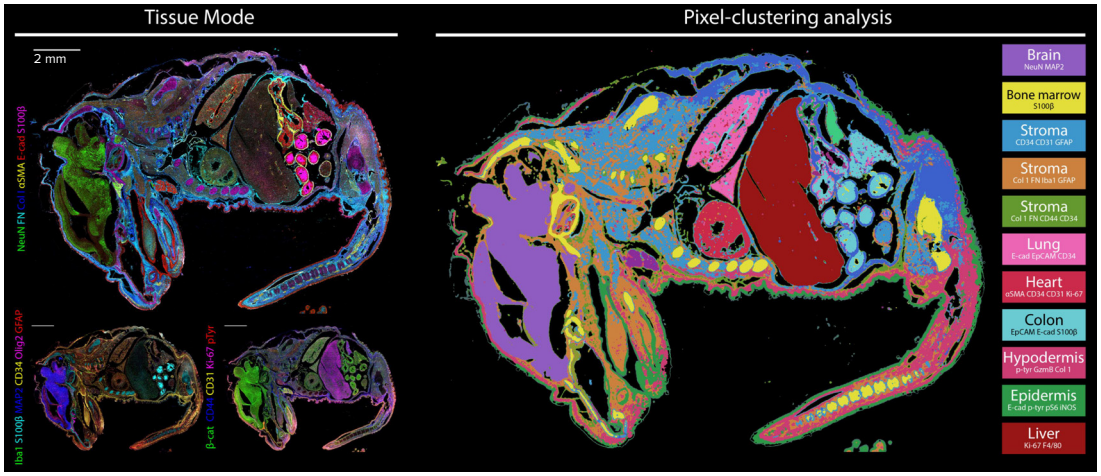
Tissue Mode imaging demonstrates the tumor and immune cell heterogeneity of mouse glioblastoma tissue. Metabolically active tumor cells were detected at the periphery of tumor. Vascularization was observed across the tumor in non-necrotic areas. Immune cells were detected in high concentration at the 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.

Generating spatial maps of specialized tissue substructures in the mouse brain



Preview Mode scan rapidly identified spatial positioning of brain-specific compartments. In the cerebellum, tissue morphology with specific cellular compartments such as the cortex, individual lobules and neuronal cell bodies is visualized. The hippocampus demonstrates structured spatial cellular distribution. In the optic lobe, various cell populations including oligodendrocytes, neurons, metabolically active cells and vasculature are highlighted.

Quantitative assessment of specific tissue compartments in the developing mouse embryo

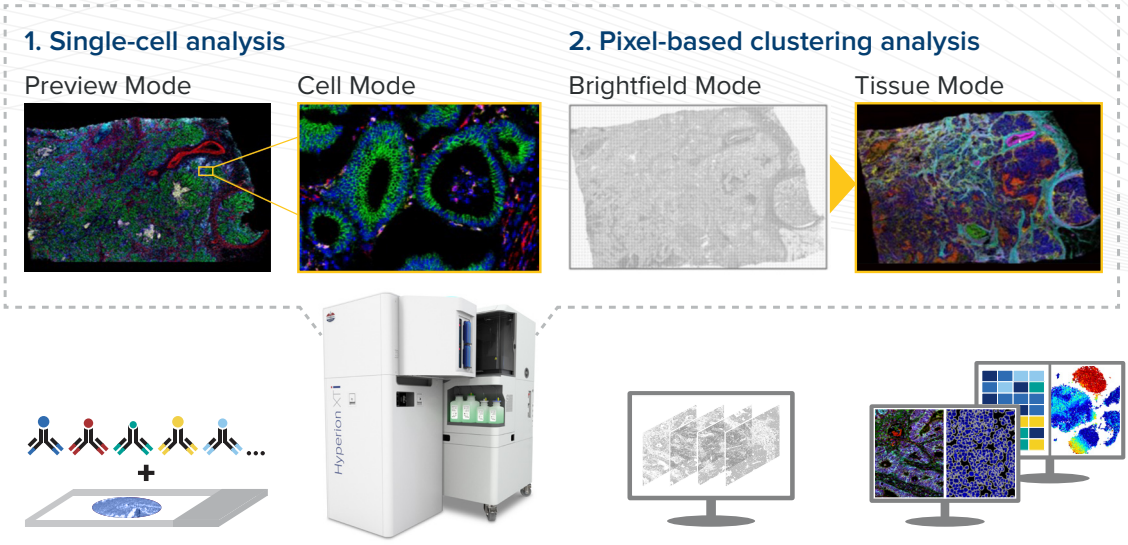


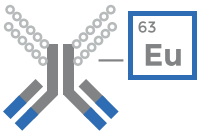
Tissue Mode imaging was performed in hours to assess whole mouse E18.5 embryo tissue structure and composition. Expression of neuronal specific markers was observed in the developing brain and spinal column. Organ-specific tissue compartments were also highlighted. Unsupervised pixel-clustering analysis along with hierarchical clustering quantitatively segregates highly specialized subcompartments in the developing mouse embryo.

A 40-marker panel was designed to study the TME of 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	

Protocol





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

Ordering information for referenced panels

Product	Part Number
Human Immuno-Oncology IMC Panel, 31 Antibodies	201509
Human Immune Cell Expansion IMC Panel, 7 Antibodies	201516
Maxpar Neuro Phenotyping IMC Panel Kit	201337
Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle	9100005NO
Maxpar IMC Cell Segmentation Kit	201500
Cell-ID™ Intercalator-Ir	201192B

References

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.

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