

ABSTRACT

Mouse models are pivotal for translational research, enabling the detection of therapeutic-induced immune modifications within the tumor microenvironment (TME). We utilized a 40-marker panel targeting tumor and immune markers to investigate the spatial protein composition of the whole mouse embryo, mouse brain and mouse glioblastoma (GBM) tumor sections. Imaging Mass Cytometry™ (IMC™) technology was employed to capture the expression of 40 markers simultaneously while preserving tissue integrity and enabling a flexible workflow without autofluorescence artifacts.

Data analysis revealed distinct spatial patterns of tumor and immune markers across the GBM, identifying key features such as necrotic cores, regions of high immune infiltration, proliferating tumor cells and diverse populations of infiltrating immune cells. The wide detection range of expression provided by IMC technology enabled visualization and analysis of clusters exhibiting varying levels of key GBM markers, suggesting a probable dual origin of the GBM, coexistence of pro- and anti-tumorigenic immune responses and overall extreme heterogeneity that can only be visualized with high-plex antibody panels.

This approach demonstrates the capability of IMC technology to reveal the complex cellular and structural spatial landscape of the TME in GBM, providing valuable insights into tumor biology and potential therapeutic targets for patient stratification.

Methods and materials

We demonstrate the whole slide imaging (WSI) application using a 40-marker panel composed of the Maxpar™ OnDemand Mouse Immuno-Oncology IMC Panel Kit, the Maxpar Neuro Phenotyping IMC Panel Kit (Figure 1) and the Maxpar IMC Cell Segmentation Kit on mouse embryo, normal brain and GBM tissue. We performed imaging using two new features of the Hyperion™ XT1 Imaging System (Figure 2). Ultrafast Preview Mode was applied to rapidly screen entire tissue sections for marker expression signatures associated with tissue compartments and biological processes. This enabled biomarker-guided selection of areas in normal and tumor tissues that were imaged using region of interest (ROI)-based Cell Mode imaging and analyzed using single-cell analysis. In parallel, high-throughput Tissue Mode was applied to perform a detailed whole slide tissue scan of mouse embryo, normal brain and GBM tissues that were quantified using pixel-clustering analysis to unravel the composition of the TME.

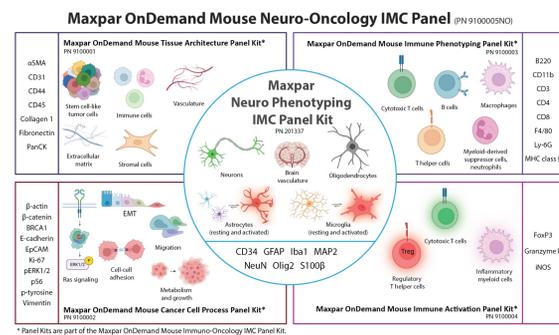


Figure 1. Maxpar OnDemand Mouse Neuro-Oncology IMC Panel Kit. This 35-marker modular panel was designed to study the TME of mouse neurological tissues. Markers in the kit offer comprehensive cellular and tissue phenotyping capabilities to understand the structure and function of mouse normal and diseased tissues.

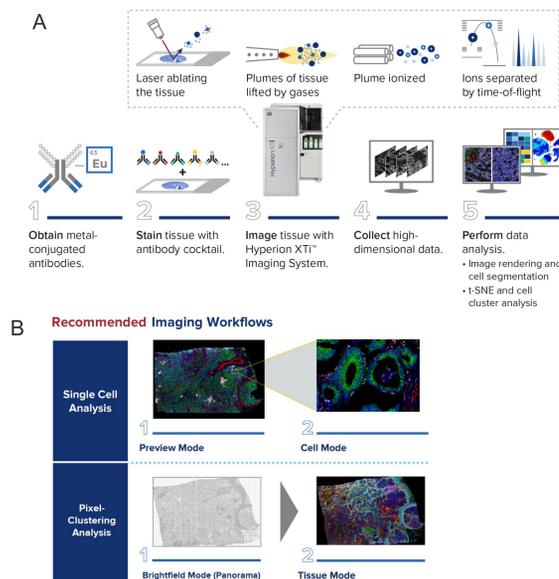


Figure 2. Imaging Mass Cytometry workflows.

(A) IMC technology offers a streamlined workflow that simplifies translational and clinical application of multiplexed tissue analysis. The five-step process, which consists of obtaining metal-conjugated antibodies, staining tissues with antibody cocktails, imaging tissues with the Hyperion XT1 Imaging System, and the collection and analysis of high-dimensional data, can be accomplished in as little as 72 hours (two slides with two 4 mm² ROIs each).

(B) The novel WSI modes for IMC systems offer a customized workflow for specific imaging applications. The novel Preview Mode offers a rapid scan of the sample and generates useful data for guiding ROI placement for Cell Mode acquisition for single-cell analysis application. Tissue Mode can be applied to generate 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 analysis. Combining these new workflows with the Hyperion XT1 Slide Loader streamlines IMC application and makes it a useful resource for high-throughput clinical and translational studies.

Results

The Mouse Neuro-Oncology IMC Panel detects spatial distribution of individual cells in mouse tissues using Preview and Cell Mode workflows.

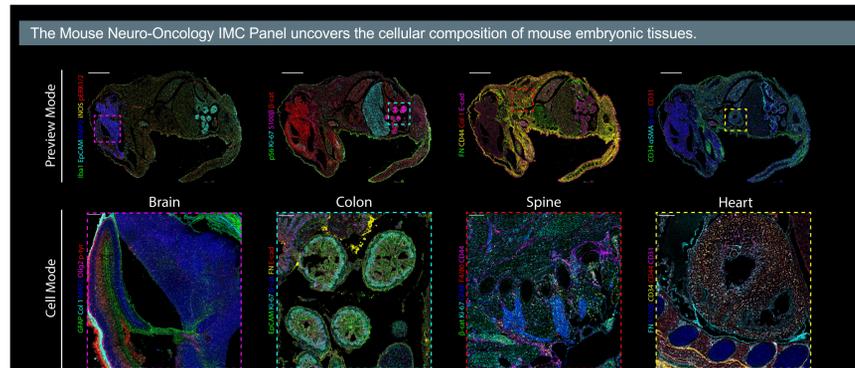


Figure 3. Rapid identification of tissue composition with intact spatial resolution in E18.5 embryo using the Mouse Neuro-Oncology IMC Panel. A Preview Mode scan rapidly demonstrates the expression pattern of all markers in the panel without affecting the quality of the subsequent Cell Mode scan. Using Preview Mode data, we identified relevant ROIs in the brain, colon, spine and heart to evaluate TME composition. In the developing brain, we noted the cerebral cortex layers composed of brain-specific cell populations. In the developing colon, structured epithelium expressing cell-cell adhesion proteins can be observed. In the developing spine, various cell populations including macrophages, stem cells and replicating cells are observed around the cervical vertebrae. In the developing heart, a highly structured and dense network of vasculature is detected. This tool provides streamlined assessment of abnormalities in mouse models of developmental diseases. Scale bar is 2 mm in Preview Mode and 200 µm in Cell Mode.

The Mouse Neuro-Oncology IMC Panel classifies the cellular composition of mouse normal brain tissues.

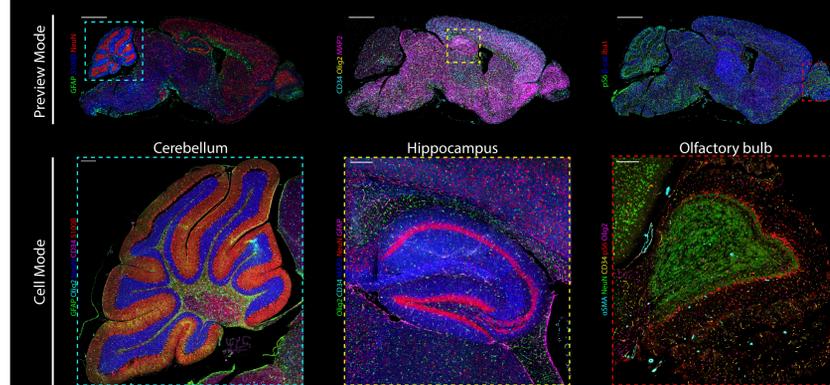


Figure 4. Generating spatial maps of the normal mouse brain with the Mouse Neuro Phenotyping Panel. A Preview Mode scan rapidly identified spatial positioning of brain-specific compartments. Using Preview Mode data, we identified relevant ROIs in the mouse brain to evaluate TME composition using Cell Mode imaging. In the cerebellum, we detected highly structured tissue morphology with specific cellular compartments such as the cortex, individual lobules and neuronal cell bodies. In the hippocampus, we detected the typical horseshoe-shaped structure with structured spatial cellular distribution. In the optic lobe, various cell populations including oligodendrocytes, neurons, metabolically active cells and vasculature are highlighted. Detection of the relative spatial and highly specialized cellular structures of the brain is crucial for understanding its normal or diseased state. Scale bar is 1.5 mm in Preview Mode and 200 µm in Cell Mode.

The Mouse Neuro-Oncology IMC Panel detects tumor cell activity and immune cell infiltration in mouse GBM.

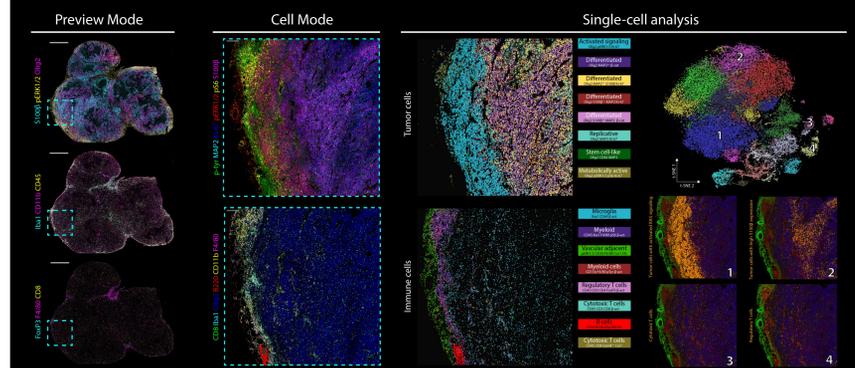


Figure 5. Application of IMC technology on mouse GBM using the Mouse Neuro-Oncology IMC Panel. A Preview Mode scan rapidly identified areas with high tumor and immune cell activity, which was used to identify relevant ROIs for detailed Cell Mode investigation. Rendered multiplexed Cell Mode images using tumor- (top) and immune- (bottom) specific markers demonstrate the heterogeneity of the TME. Single-cell analysis through Cellpose and histoCAT enabled detection of tumor and immune cell populations on both tissue samples, as shown in the segmented cell mask. t-SNE and PhenoGraph clustering analysis successfully resolved specific subsets of tumor and immune cell populations, which could then be mapped back to the segmented cell mask for each tissue. Determining the spatial relationship between cell populations is relevant to identify prognostic and diagnostic biomarkers for targeted therapies. Scale bar is 2 mm in Preview Mode and 200 µm in Cell Mode.

Conclusion

The Mouse Neuro-Oncology IMC Panel highlights the power of Imaging Mass Cytometry technology to simultaneously explore dozens of relevant tissue features to better understand the mouse glioblastoma microenvironment.

Moreover, IMC platforms offer pre-validated panels for human and mouse tissue samples (FFPE/frozen), ensuring broad applicability across oncology, immunology and neuroscience studies.

Whole sample imaging using Tissue Mode highlights critical immune, stromal and tumor structures in mouse tissues.

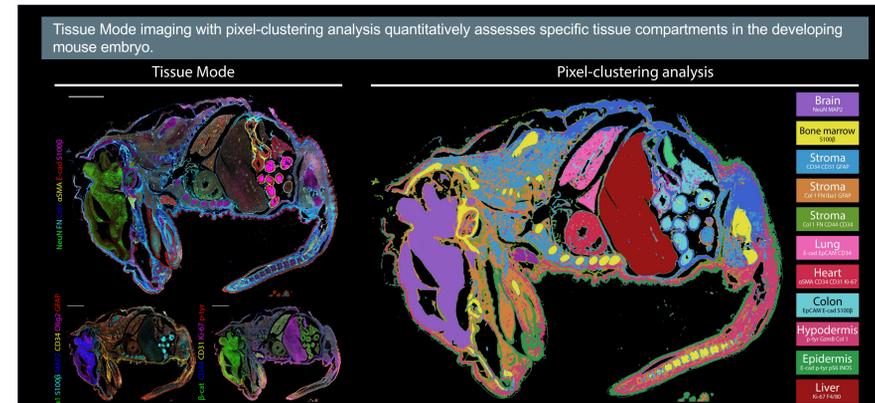


Figure 6. Rapid assessment of whole mouse E18.5 embryo tissue structure and composition using the Mouse Neuro-Oncology IMC Panel. Tissue Mode imaging enables detection of tissue-specific compartments in the mouse embryo by employing the Mouse Neuro-Oncology IMC Panel. The tissue scan was performed in hours. Expression of neuronal-specific markers was observed in the developing brain as well as along the spinal column. Several stromal compartments were identified with combinatorial expression of several stromal markers. The epidermis and hypodermis are visualized and differentiated by the expression of specific epithelial and stromal markers. Organ-specific tissue compartments were also highlighted. In the lungs, specific bronchiolar and alveolar tissue compartments can be observed. In the heart, the expression of vasculature-specific markers and cell replication markers is detected. In the liver, high expression of cell replication markers and the presence of Kupffer cells are observed. In the colon, epithelial tissue and surrounding smooth muscle are highlighted. Unsupervised pixel-clustering analysis along with hierarchical clustering using the MCD SmartViewer analysis pipeline highlighted successfully and quantitatively segregated highly specialized subcompartments in the developing mouse embryo. Scale bar is 2 mm.

Tissue Mode imaging with pixel-clustering analysis detects the spatial location of specialized tissue substructures in the mouse brain.

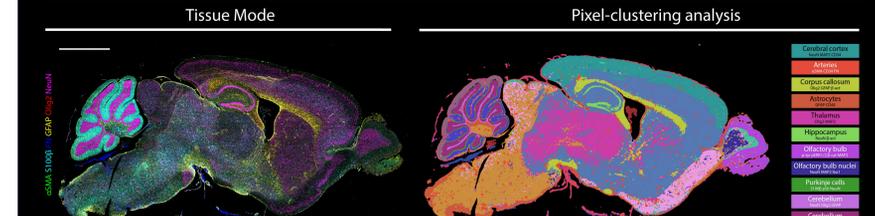


Figure 7. Detecting highly compartmentalized tissue structures in whole normal mouse brain using the Mouse Neuro Phenotyping Panel. Tissue Mode imaging enables detection of tissue-specific compartments in the mouse sagittal brain tissue section using the Mouse Neuro Phenotyping Panel. The cellular composition of brain-specific compartments such as the cerebral cortex, cerebellum, thalamus, optic lobe, midbrain and corpus callosum, which are enriched with neurons, astrocytes and oligodendrocytes, are detected in multiplexed images. Unsupervised pixel-clustering analysis along with hierarchical clustering using the MCD SmartViewer analysis pipeline highlighted successfully and quantitatively segregated highly specialized subcompartments in mouse brain. In the cerebellum and optical lobe, several cortical layers can be observed expressing a distinct combination of markers. Corpus callosum and hippocampus, thalamus, and midbrain and hindbrain structures can be readily observed as well. Scale bar is 1.5 mm.

Tissue Mode imaging with pixel-clustering analysis quantitatively resolves the spatial distribution of tumor and immune-enriched structures in mouse GBM.

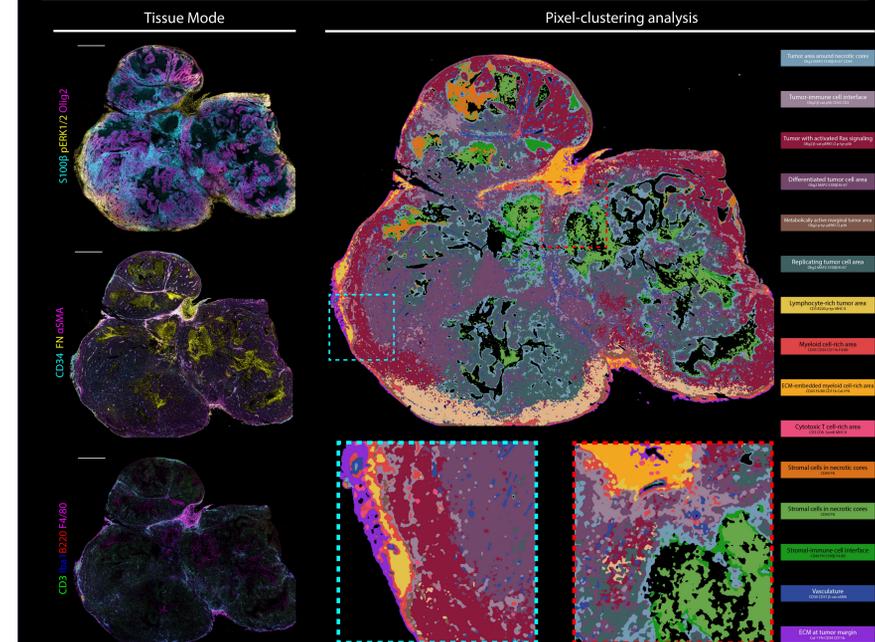


Figure 8. Visualizing the entire mouse GBM immuno-oncological landscape using the Mouse Neuro-Oncology IMC Panel. Tissue Mode imaging demonstrates the tumor and immune cell heterogeneity of mouse GBM tissue and the spatial positioning of tissue compartments. The activation of the Ras signaling pathway can be observed at the tumor margins. Expression of tumor cell activation marker S100β can be observed in specific tumor compartments. The presence of fibroblasts in fibronectin-enriched necrotic cores was detected. Metabolically active tumor cells were detected at the periphery of the tumor and cell replication markers were observed in virtually all tumor cells. A high degree of vasculature was observed across the tumor in non-necrotic areas. Lymphoid and myeloid immune cells were detected in high concentrations at the tumor margin and in necrotic cores. Unsupervised pixel-clustering analysis with hierarchical clustering using MCD SmartViewer successfully and quantitatively segregated highly specialized subcompartments in mouse GBM and detected various tumor areas containing subsets of differentiated tumor cells, immune hot and cold areas, stromal compartments, vasculature and the extracellular matrix. Such detailed spatial maps of tissue are critical for identifying expression signatures and tissue landmarks, which can help researchers and clinicians perform rapid screening of disease-modifying drugs in preclinical settings. Scale bar is 2 mm.