Whole Slide Imaging Modes for Imaging Mass Cytometry Quantitively Resolve Spatially Extensive Cellular Heterogeneity in Human Gliomas

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Abstract Poster
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Introduction

Gliomas present a complex form of brain cancer that is challenging to diagnose and treat, with a median survival of just over one year after diagnosis for primary glioblastoma (GBM). GBM can occur as multi-lesion, remote or diffuse tumors, and often contains a tumor microenvironment (TME) that is devoid of peripheral immune cells.

Additional hallmark features of GBM include necrosis, hemorrhage and pseudopalisades, making it a highly heterogeneous disease requiring further investigation. Identification of the cellular and spatial-level composition of the TME is vital for interpretation of GBM disease origin, progression, prognosis and treatment options. Specifically designed for high-throughput applications, a 40-slide loader for the Hyperion XTi™ Imaging System permits automated and continuous imaging of more than 40 large tissue samples (400 mm² per tissue) per week.

Methods and Materials

A 41-marker neuro-oncology Imaging Mass Cytometry™ (IMC™) antibody panel (Figure II) was used to determine the cellular and structural landscape of the brain TME. We applied the panel on a tissue microarray (TMA) containing dozens of human glioma cores and identified the spatial distribution of over 40 distinct molecular markers.

We performed imaging using two features of the Hyperion™ XTi Imaging System (Figure IA) that provide whole slide scanning capabilities. **Preview Mode** (Figure IB, top panel) 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 using single-cell analysis. In parallel, a high-throughput **Tissue Mode** (Figure IB, bottom panel) was applied to perform a detailed scan of the brain tumor TMA followed by pixel-clustering analysis to unravel the spatial composition of the TME.

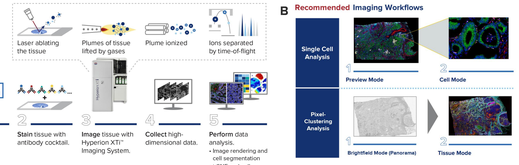

Figure I. Imaging Mass Cytometry workflows. (A) IMC offers a streamlined workflow that simplifies translational and clinical application of multiplexed tissue analysis. The five-step process, which consists of obtaining metal-conjugated antibodies, staining tissues with antibody cocktails, imaging tissues with Hyperion XTi and the collection and analysis of high-dimensional data, can be accomplished in as little as 72 hours for the whole slide. Additionally, the slide loader can accommodate two cassettes of 20 slides each (40 slides total) to greatly increase throughput. (B) The whole slide imaging modes for IMC offer a customized workflow for specific customer needs. Preview Mode offers a rapid scan of the sample and generates useful data for guiding region of interest (ROI) placement for Cell Mode acquisition for single-cell analysis application. Alternatively, Tissue Mode can be applied to generate a high-quality scan of entire tissue sections in a matter of hours with higher spot size ablations enabling entire tissue analysis using pixel-clustering methods. Both workflows offer unique advantages for specific research requirements.

Human Immuno-Oncology IMC Panel, 31 Antibodies (PN 201509)							Additional panels	
Human Tissue Architecture IMC Panel, 4 Antibodies	Human Stromal Cell IMC Panel, 4 Antibodies	Human Lymphoid IMC Panel, 4 Antibodies	Human Myeloid IMC Panel, 6 Antibodies	Human Cell Functional State IMC Panel, 5 Antibodies	Human Epithelial and Mesenchymal IMC Panel, 4 Antibodies	Human Basic Immune IMC Panel, 4 Antibodies	Maxpar® Neuro Phenotyping IMC Panel Kit	Maxpar IMC Cell Segmentation Kit and Cell-ID™ Intercalator-Ir
PN 201510	PN 201511	PN 201512	PN 201513	PN 201514	PN 201515	PN 201518	PN 201337	PN 201500 PN 201192B
Pan-cytokeratin CD31 Collagen 1 Fibronectin	FAP Podoplanin αSMA CD44	CD4 CD8 CD45RO CD57	CD66b HLA-DR CD163 CD14 CD11b CD11c	Granzyme B PD-L1 PD-1 FoxP3 Ki-67	E-cadherin β-catenin EpCAM Vimentin	CD45 CD3 CD20 CD68	Iba1 GFAP NeuN S100β MAP2 CD34 Olig-2	ICSK1 ICSK2 ICSK3 DNA1 DNA2

Figure II. The Human Immuno-Oncology IMC Panel. This 41-marker panel is designed to uncover relevant immuno-oncological processes in human tumors. The off-the-shelf modular structure of the panel offers excellent flexibility to customize IMC panels for application on translational and clinical samples. Metal assignments were carefully designated for each marker to extract the maximum performance from each individual antibody. The panel was optimized for FFPE tissues.

Conclusions

Whole slide imaging modes highlight the power of **Imaging Mass Cytometry** to simultaneously explore numerous **biological outputs** to provide **new perspectives** on the extensive **development, immune landscape and predictive markers of gliomas**. This enhanced understanding opens avenues for **new diagnostic and therapeutic options**.

Results

Preview Mode facilitates rapid whole slide screening and selection of TMA cores for single-cell analysis with Cell Mode

The Human Immuno-Oncology IMC Panel and Maxpar Neuro Phenotyping IMC Panel Kit uncover the extensive heterogeneity of glioma cores for subsequent selection of samples that are the most relevant for the research questions.

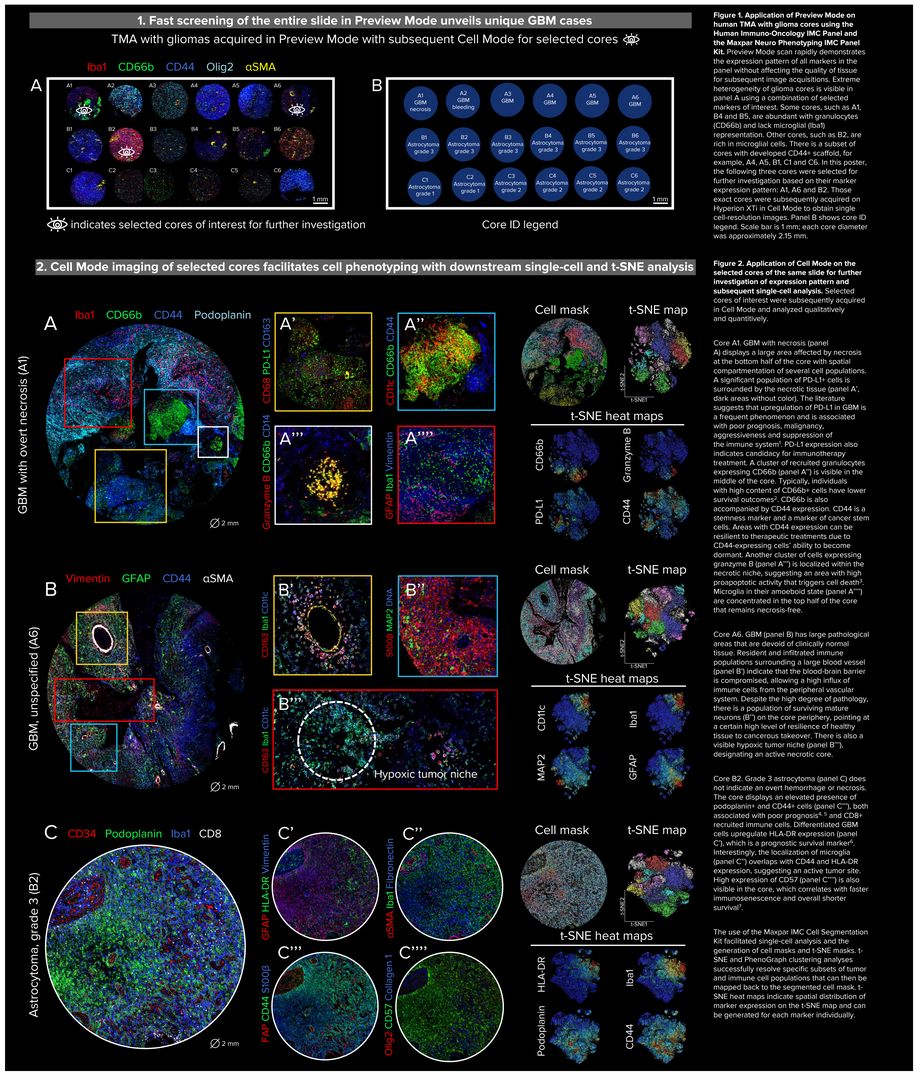

Figure 1. Application of Preview Mode on human TMA with glioma cores using the Human Immuno-Oncology IMC Panel and the Maxpar Neuro Phenotyping IMC Panel Kit. Preview Mode scan rapidly demonstrates the expression pattern of all markers in the panel without affecting the quality of tissue for subsequent image acquisitions. Extreme heterogeneity of glioma cores is visible in panel A using a combination of selected markers of interest. Some cores, such as A1, B4 and B5, are abundant with granulocytes (CD66b) and lack microglial (Iba1) representation. Other cores, such as B2, are rich in microglial cells. There is a subset of cores with developed CD44+ scaffold, for example, A4, A5, B1, C1 and C6. In this poster, the following three cores were selected for further investigation based on their marker expression pattern: A1, A6 and B2. Those exact cores were subsequently acquired on Hyperion XTi in Cell Mode to obtain single cell-resolution images. Panel B shows core ID legend. Scale bar is 1 mm; each core diameter was approximately 2.15 mm.

Figure 2. Application of Cell Mode on the selected cores of the same slide for further investigation of expression pattern and subsequent single-cell analysis. Selected cores of interest were subsequently acquired in Cell Mode and analyzed qualitatively and quantitively.

Core A1, GBM with necrosis (panel A) displays a large area affected by necrosis at the bottom half of the core with spatial compartmentation of several cell populations. A significant population of PD-L1+ cells is surrounded by the necrotic tissue (panel A', dark areas without color). The literature suggests that upregulation of PD-L1 in GBM is a frequent phenomenon and is associated with poor prognosis, malignancy, aggressiveness and suppression of the immune system[3]. PD-L1 expression also indicates candidacy for immunotherapy treatment. A cluster of recruited granulocytes expressing CD66b (panel A'') is visible in the middle of the core. Typically, individuals with high content of CD66b+ cells have lower survival outcomes[4]. CD66b is also accompanied by CD44 expression. CD44 is a stemness marker and a marker of cancer stem cells. Areas with CD44 expression can be resilient to therapeutic treatments due to CD44-expressing cells' ability to become dormant. Another cluster of cells expressing granzyme B (panel A''') is localized within the necrotic niche, suggesting an area with high proapoptotic activity that triggers cell death[5]. Microglia in their amoeboid state (panel A'''') are concentrated in the top half of the core that remains necrosis-free.

Core A6, GBM (panel B) has large pathological areas that are devoid of clinically normal tissue. Resident and infiltrated immune populations surrounding a large blood vessel (panel B') indicate that the blood-brain barrier is compromised, allowing a high influx of immune cells from the peripheral vascular system. Despite the high degree of pathology, there is a population of surviving mature neurons (B'') on the core periphery, pointing at a certain high level of resilience of healthy tissue to cancerous takeover. There is also a visible hypoxic tumor niche (panel B'''), designating an active necrotic core.

Core B2, Grade 3 astrocytoma (panel C) does not indicate an overt hemorrhage or necrosis. The core displays an elevated presence of podoplanin+ and CD44+ cells (panel C''), both associated with poor prognosis[4, 5] and CD8+ recruited immune cells. Differentiated GBM cells upregulate HLA-DR expression (panel C'), which is a prognostic survival marker[6]. Interestingly, the localization of microglia (panel C''') overlaps with CD44 and HLA-DR expression, suggesting an active tumor site. High expression of CD57 (panel C'''') is also visible in the core, which correlates with faster immunosenescence and overall shorter survival[7].

The use of the Maxpar IMC Cell Segmentation Kit facilitated single-cell analysis and the generation of cell masks and t-SNE masks. t-SNE and PhenoGraph clustering analyses successfully resolve specific subsets of tumor and immune cell populations that can then be mapped back to the segmented cell mask. t-SNE heat maps indicate spatial distribution of marker expression on the t-SNE map and can be generated for each marker individually.

Tissue Mode highlights tissue compartments and supports subsequent pixel-clustering analysis

Tissue Mode visualizes tissue compartments and indicates high heterogeneity of human glioma cores.
Cores of interest are selected for subsequent pixel-clustering analysis.

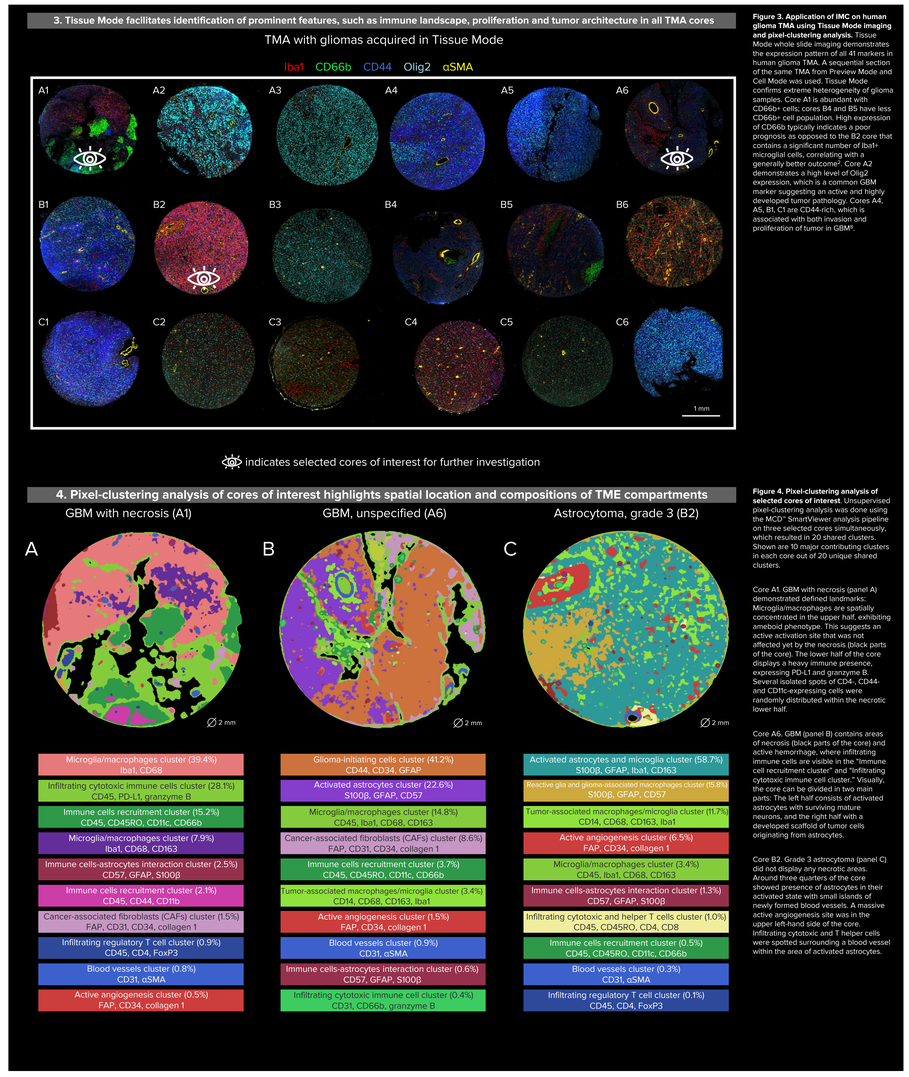

Figure 3. Application of IMC on human glioma TMA using Tissue Mode imaging and pixel-clustering analysis. Tissue Mode whole slide imaging demonstrates the expression pattern of all 41 markers in human glioma TMA. A sequential section of the same TMA from Preview Mode and Cell Mode was used. Tissue Mode confirms extreme heterogeneity of glioma samples. Core A1 is abundant with CD66b+ cells; cores B4 and B5 have less CD66b+ cell population. High expression of CD66b typically indicates a poor prognosis as opposed to the B2 core that contains a significant number of Iba1+ microglial cells, correlating with a generally better outcome[2]. Core A2 demonstrates a high level of Olig2 expression, which is a common GBM marker suggesting an active and highly developed tumor pathology. Cores A4, A5, B1, C1 are CD44-rich, which is associated with both invasion and proliferation of tumor in GBM[8].

Figure 4. Pixel-clustering analysis of selected cores of interest. Unsupervised pixel-clustering analysis was done using the MCD™ SmartViewer analysis pipeline on three selected cores simultaneously, which resulted in 20 shared clusters. Shown are 10 major contributing clusters in each core out of 20 unique shared clusters.

Core A1, GBM with necrosis (panel A) demonstrated defined landmarks: Microglia/macrophages are spatially concentrated in the upper half, exhibiting amebоid phenotype. This suggests an active activation site that was not affected yet by the necrosis (black parts of the core). The lower half of the core displays a heavy immune presence, expressing PD-L1 and granzyme B. Several isolated spots of CD4+, CD44- and CD11c-expressing cells were randomly distributed within the necrotic lower half.

Core A6, GBM (panel B) contains areas of necrosis (black parts of the core) and active hemorrhage, where infiltrating immune cells are visible in the "Immune cell recruitment cluster" and "Infiltrating cytotoxic immune cell cluster." Visually, the core can be divided in two main parts: The left half consists of activated astrocytes with surviving mature neurons, and the right half with a developed scaffold of tumor cells originating from astrocytes.

Core B2, Grade 3 astrocytoma (panel C) did not display any necrotic areas. Around three quarters of the core showed presence of astrocytes in their activated state with small islands of newly formed blood vessels. A massive active angiogenesis site was in the upper left-hand side of the core. Infiltrating cytotoxic and T helper cells were spotted surrounding a blood vessel within the area of activated astrocytes.

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