Ultrahigh-plex Spatial Phenotyping of the Glioma Tumor Landscape in IDH-1^{wt} and IDH-1^{R132H} Patient Tissues

Poster #97

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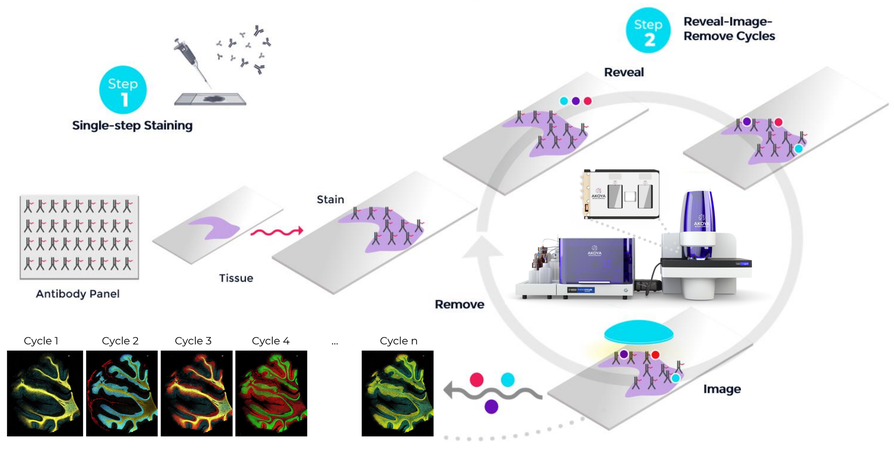
1. Introduction

Glioblastoma Multiforme (GBM), the most prevalent and aggressive brain tumor in adults, is characterized by high intra- and inter-tumoral heterogeneity. In addition, the highly immunosuppressive GBM tumor microenvironment (TME) leads to poor clinical outcomes with median survival of less than 15 months. The R132H mutation in the isocitrate dehydrogenase 1 gene (IDH-1^{R132H}) is the most important prognostic factor for the survival of glioma patients. IDH-1^{R132H} generates high levels of the 2-hydroxyglutarate – an oncometabolite that modulates cellular epigenetic programs and metabolic profiles, yet the mechanism(s) explaining the impact on the tumor immune microenvironment (TiME) remain unknown. In this study we designed an ultrahigh-plex antibody panel for **comparing FFPE human glioma tissues based on their IDH-1 status aiming to identify and characterize the effects of** IDH-1^{R132H} **on the GBM TiME**.

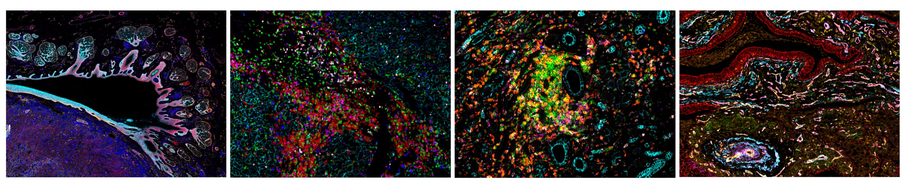
2. Rapid and Deep Spatial Phenotyping

The **Phenocycler®-Fusion 2.0** (PCF) workflow is compatible with a wide range of commercially available antibodies. Antibodies can also be customized via tagged to activated oligonucleotide barcodes that are complementary to existing antibody panels. Following antibodies are titrated and tested for appropriate target recognition, and then added to a panel. We routinely deploy panels with 100+ antibodies.

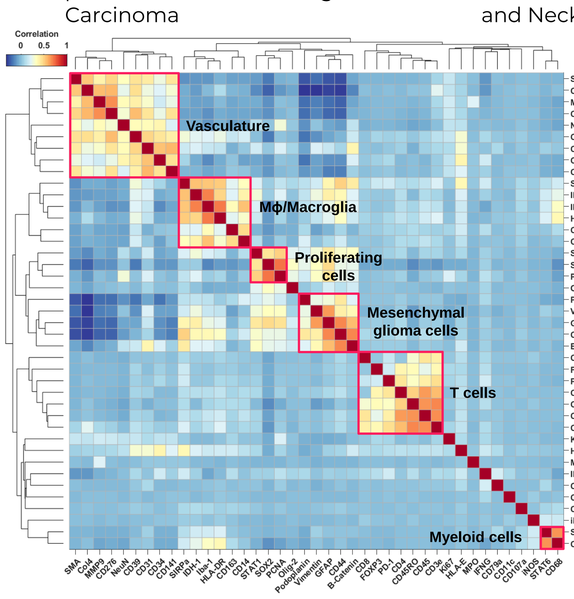
The **Phenocycler-Fusion** workflow consists of iterative cycles of labelling, imaging and removing fluorescent reporters. In each imaging cycle, three fluorescent reporters are assayed to their corresponding barcode-conjugated antibodies and imaged via standard fluorescent optics. Thereafter, the three reporters are removed, and a new cycle images additional reporters. The process is fully automated, and data are acquired **across whole slides at single-cell resolution**.

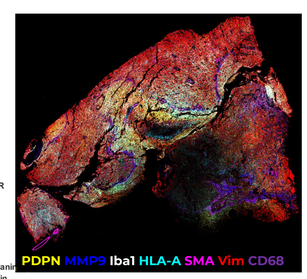


The Phenocycler-Fusion is a fast spatial biology solution that affords high parameter and high-throughput spatial readouts across whole slides with uncompromised single-cell optical resolution. **The images below show examples of high-plex Spatial Phenotyping in human FFPE tissues.**



Squamous Cell Carcinoma | Lung Cancer | Head and Neck Cancer | Renal Cell Carcinoma



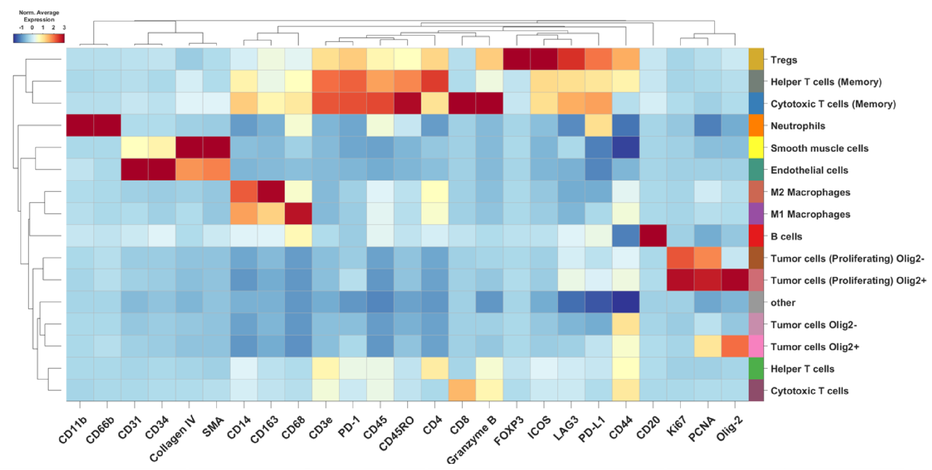


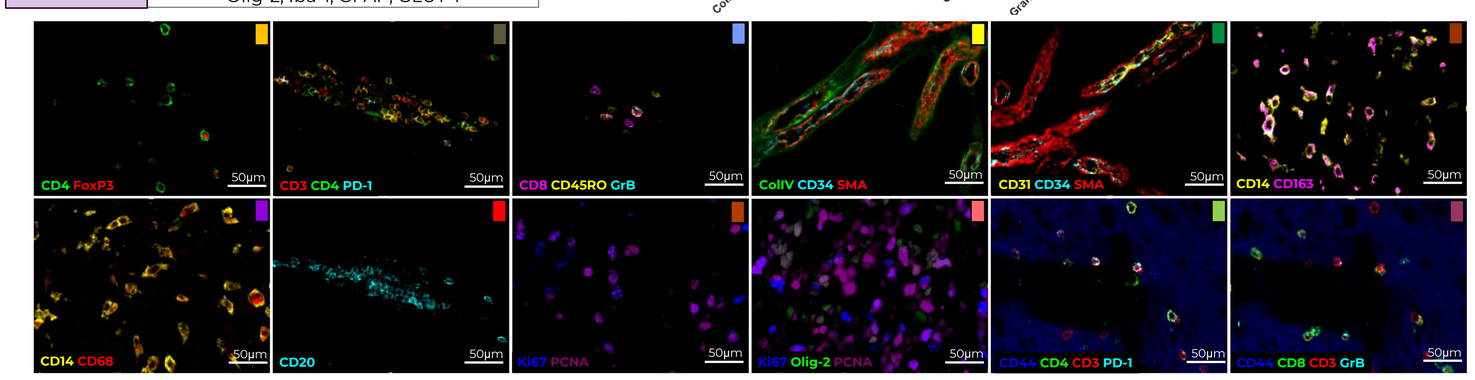
Whole slide human FFPE GBM tissue section at single-cell resolution; biomarkers as indicated. **Cross-correlation matrix** showing labeling of **41 markers**. Data show immune cell lineages and functional niches as well as vasculature/structural signatures

3. Ultrahigh-Plex Spatial Phenotyping of Human FFPE GBM Reveals High Degree of Cellular Heterogeneity

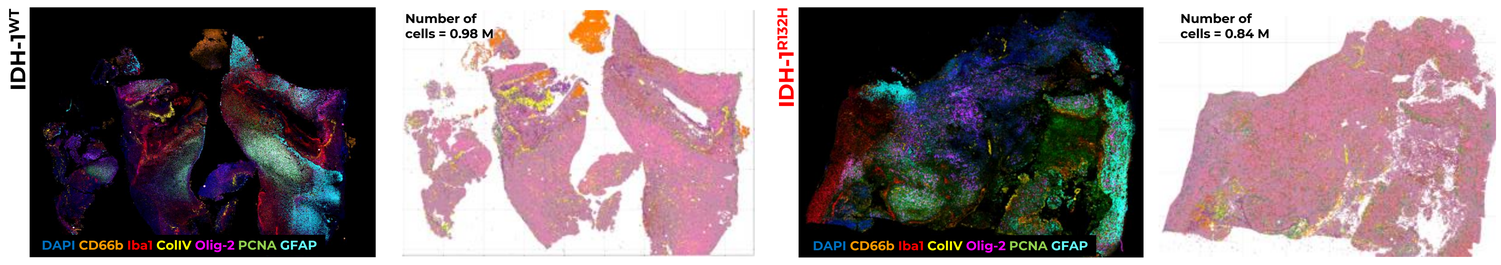
PhenoCode™ Discovery Panels

Immune Profiling Core	CD8, CD44, HLA-DR, CD4, CD45, Ki67, CD45RO, CD14, CD20, HLA-A, CD68, CD3e, CD11b, CD163, CD57		
Immune Activation & Proliferation	PD-1, PD-L1, IDO1, PCNA, HLA-E, IFNG		
Lymphocyte Profiling	FOXP3, CD79, CD21, GR B	***Tissue Architecture***	Vimentin, Collagen IV, CD31, SMA, CD34
Custom Module	DNMT1, CD11b, S100A4, C1Qa, CD66b, CD138, MPO, CXCR3, Histone H3(K9), MMP9, PHH3, BAX, Histone H3 (K27), ASCT2, GLUT1, G6PD, HIF1A, pNRF2, ATPA5, CS, LDHA, HK1, CytC, SDHA, CPT1A, Olig-2, Iba-1, GFAP, GLUT-1		

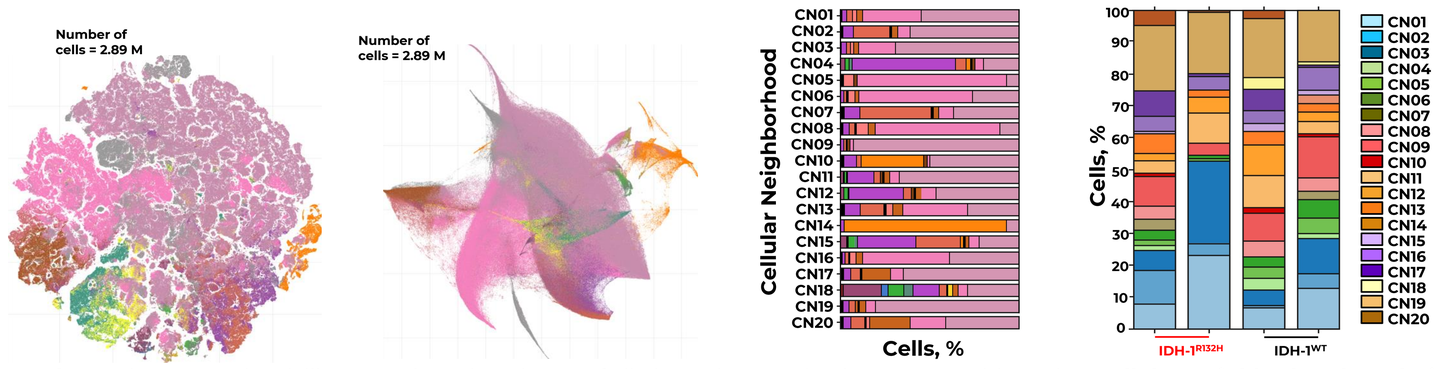




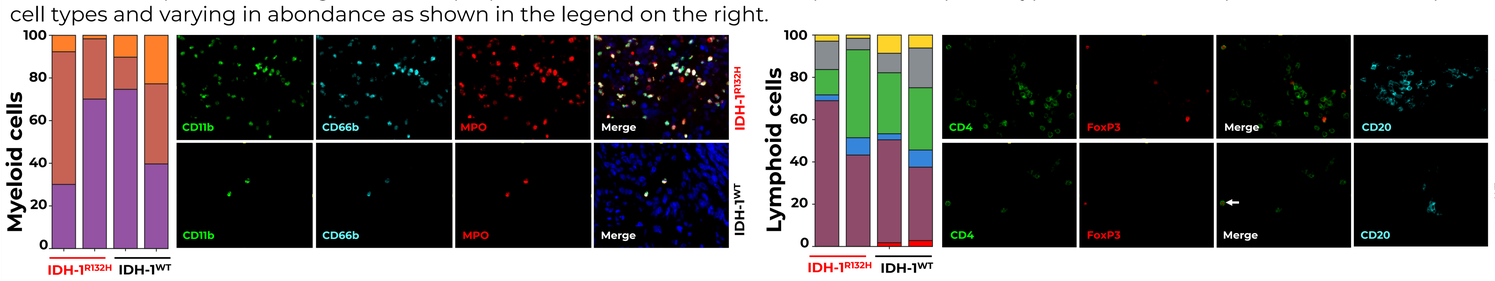
Deep analysis of 59 proteins comprising immune, tumor, metabolism, vascular & neuronal landscapes of the human FFPE GBM tissues. The antibody panel (top left) includes commercially available antibodies and antibodies customized via conjugation. **Single Cell Spatial Phenotyping** of ~2.89 M cells in a single specimen (top right). Unsupervised clustering (Leiden) yielded a minimum of **16 cell phenotypes**, including multiple immune and myeloid cell types. The heatmap shows a curated clustering dendrogram with all cell types summarized and annotated. Certain **cell types** are shown. The colored boxes within each image corresponds to the cell phenotypes in the heatmap.



Whole slide human FFPE GBM tissue sections at single-cell resolution and **Spatial Phenotyping Maps** of the entire IDH-1^{WT} (left) and GBM IDH-1^{R132H} (right) sections represent the overall organization of the different cell types relative to each other; biomarkers as indicated



A **tSNE Plot** and a **UMAP** (n=4) illustrate the abundance of distinct phenotypic clusters sorted by color. **Cellular Neighborhood Analysis** reveals 20 unique cellular neighborhoods (CN). Colors in the bar chart correspond to cell phenotypes in the heatmap. CNs enriched in specific cell types and varying in abondance as shown in the legend on the right.



IDH-1^{R132H} correlates with **fewer infiltrating Tregs** and **larger number of CD20+ B cells** when compared with IDH-1^{WT} gliomas. IDH-1^{R132H} GBMs, which are less aggressive compared with their IDH-1^{WT} counterparts, have lower neutrophil infiltration. Colors in the bar chart correspond to cell phenotypes in the heatmap.

4. Uncovering the Heterogeneity in GBM Microenvironment via Spatial Phenotyping

This study encompasses the development of a custom antibody panel, an imaging workflow, as well as a novel bioinformatic analysis method. Deployment of this workflow on GBM IDH-1^{R132H} and GBM IDH-1^{wt} tissues allowed us to identify clearly district immune cell profiles: (i) reduced numbers of Neutrophils in IDH-1^{R132H} compared with IDH-1^{wt} gliomas; (ii) fewer infiltration of Tregs and; (iii) larger number of CD20+ B cells in IDH-1^{R132H} when compared with IDH-1^{WT} gliomas. The research on IDH-mutant gliomas and the immunosuppressive mechanisms in the glioma microenvironment will help develop immunotherapy drugs and design new immunotherapies will help guide the development of new immunotherapies and identify potential glioma targets to kill tumor cells more effectively.

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