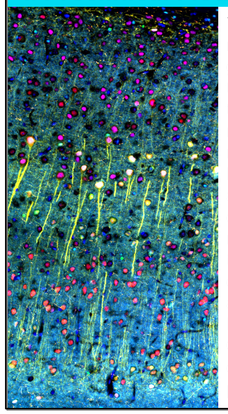
The Development and Application of an Ultrahigh-Plex Antibody Panel for Spatial Phenotyping of Mouse Brain Tissues in Homeostatic and Diseased States

Dmytro Klymyshyn[1], Aditya Pratapa[1], Lorcan Sherry[2], Matthew McFarlane[2], Nadezhda Nikulina[1], Tsz H.Tam[1], Gabriel Reines March[2], Roberta Mazzieri[3], Davide Moi[3], Riccardo Dolcetti[4], Tao Sun[5], Niyati Jhaveri[1*], Theo Mantamadiotis[4]

[1]Akoya Biosciences, Marlborough, MA; [2]OracleBio Ltd., Glasgow, United Kingdom; [3]Peter McCallum Cancer Centre, Parkville, Australia; [4]Department of Microbiology & Immunology, The University of Melbourne, Australia; [5]Cedar-Sinai Medical Center, Los Angeles, CA

*Corresponding Author – njhaveri@akoyabio.com

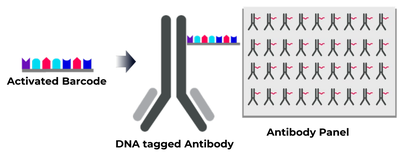
1. Preclinical Mouse Models for Neuroscience Research



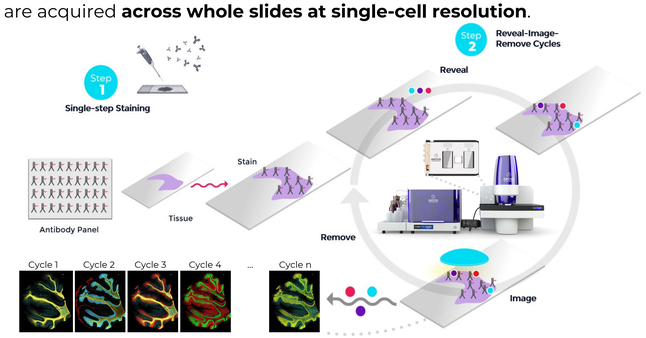
To develop new therapies, preclinical animal models are essential for analyzing the biology of glioblastoma (GBM), identifying new therapeutic targets, and evaluating the potential of new therapeutic strategies. While a variety of animal models are used to study GBM, the overwhelming majority of preclinical investigations involve mice. In this study we utilize a spatial phenotyping application that permits comprehensive characterization of key proteins in the microenvironment of healthy and GBM brain tissues. Our work encompasses the development of a custom antibody panel, an imaging workflow, as well as a novel bioinformatic analysis method. Deployment of this workflow on FFPE mouse GBM and normal tissues allowed us to study different cell populations, according to biomarker profiles and spatial distribution.

2. PhenoCycler-Fusion Workflow

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 attached 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.

3. Design and Validation of the FFPE Mouse Neuro Panel

Mature Neurons
NeuN
MAP2
Synaptophysin
Neurofilament
CTIP2
PSD95

Immature Neurons
Doublecortin

GABAergic Neurons
GAD67
Calbindin (CBDN)

Cholinergic Neurons
Choline Acetyltransferase

Serotonergic Neurons
Serotonin Transporter

Glutamatergic Neurons
GLUT-1

Dopaminergic Neurons
Tyrosine Hydroxylase

Astrocytes
GFAP
S100B

Structural
SMA
LamB1
CD31
Collagen
AQP4
Vimentin

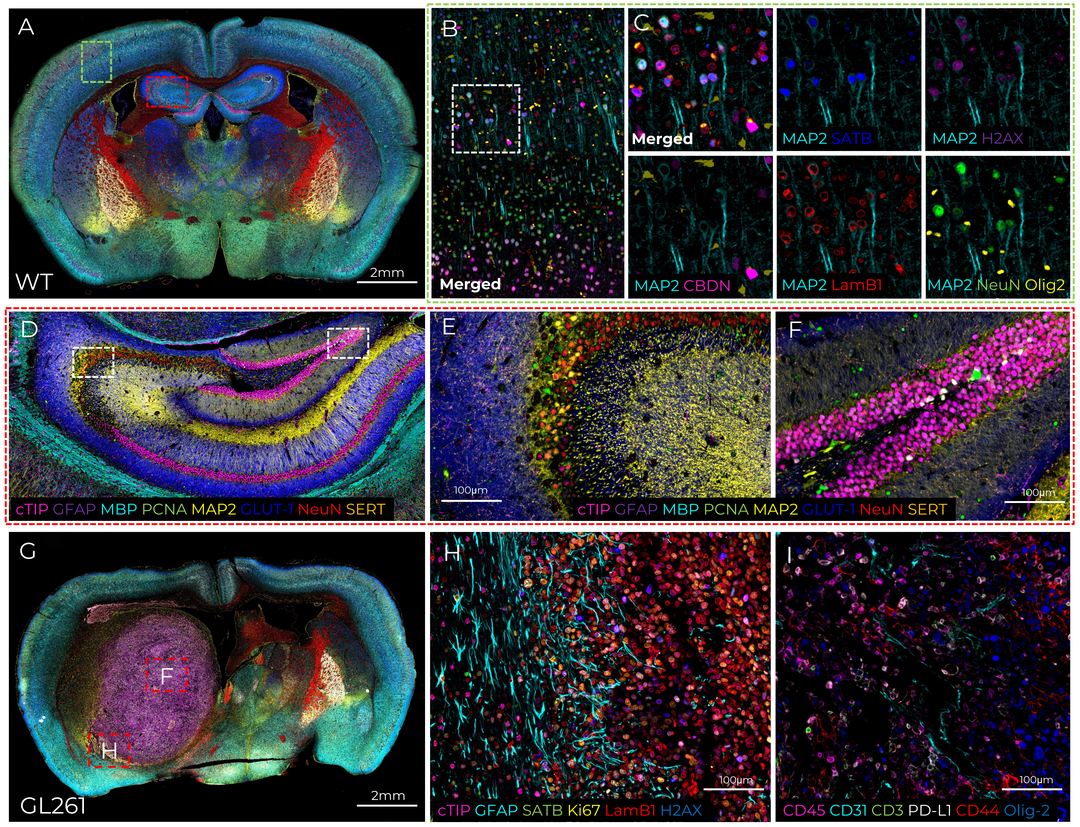
Microglia/Myeloid
Iba-1
TMEM119
F4/80
CD68
CD11b

Oligodendrocytes
Olig-2
MBP

Neural Stem-Progenitor
SOX2

Immune
CD8
CD11c
CD44
Ki67
CD206/MRC1
CD3
PD-L1
CD20
FoxP3
CD56
CD45

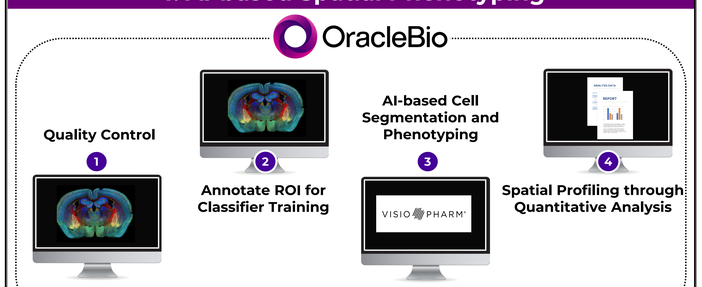
Neuropathology
H2AX
pTau (S396)
PCNA
A-Beta



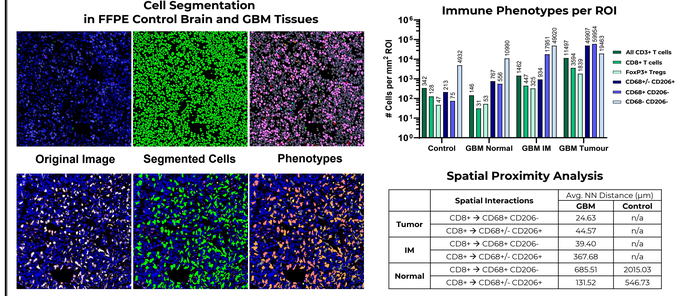
A. Multiplexed imaging of the healthy mouse brain. B. Magnified view of the green dotted box region of the mouse cortex. Images on the right show the overlay (C) and the single-channel images of region B. D-F Magnified views of the red dotted box region of the mouse hippocampus. G. Multiplexed imaging of the GBM mouse brain and magnified views of the GL261 tumor (H-I).

4. AI-based Spatial Phenotyping

OracleBio



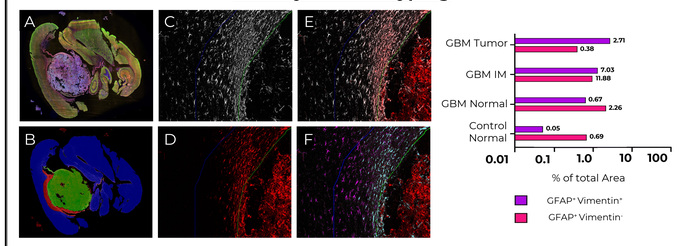
Immune Phenotyping



Spatial Proximity Analysis

	Spatial Interactions	Avg. NN Distance (µm) GBM	Control
Tumor	CD8+ → CD68+ CD206-	24.63	n/a
	CD8+ → CD68+/- CD206+	44.57	n/a
IM	CD8+ → CD68+ CD206-	39.40	n/a
	CD8+ → CD68+/- CD206+	367.68	n/a
Normal	CD8+ → CD68+ CD206-	685.51	2015.03
	CD8+ → CD68+/- CD206+	131.52	546.73

Astrocyte Phenotyping



(A) Whole brain section from GBM Tumor Model, (B) ROI classification showing Normal Brain (blue), GBM Tumour (green) and Invasive Margin (red), magnified region relates to following images; (C) GFAP staining (white); (D) Vimentin staining (red); (E) GFAP and vimentin staining; (F) image analysis overlay showing detection of single GFAP+ areas (pink) and dual GFAP+ Vimentin+ areas (cyan) within ROI.

5. Development of a Mouse FFPE Neuro Panel for Single-Cell Spatial Analysis

In this study we developed a comprehensive Spatial Biology workflow aimed at uncovering the biology of GBM TiME in FFPE mouse tissues. Our work encompasses the development of a custom antibody panel, an imaging workflow, and a novel bioinformatic analysis method. Deployment of this workflow on the mouse FFPE tissues allowed us to study different cell populations, according to biomarker profiles and spatial distribution. Our workflow will allow large-scale unsupervised analyses of the GBM TiME that is needed to better characterize this disease.