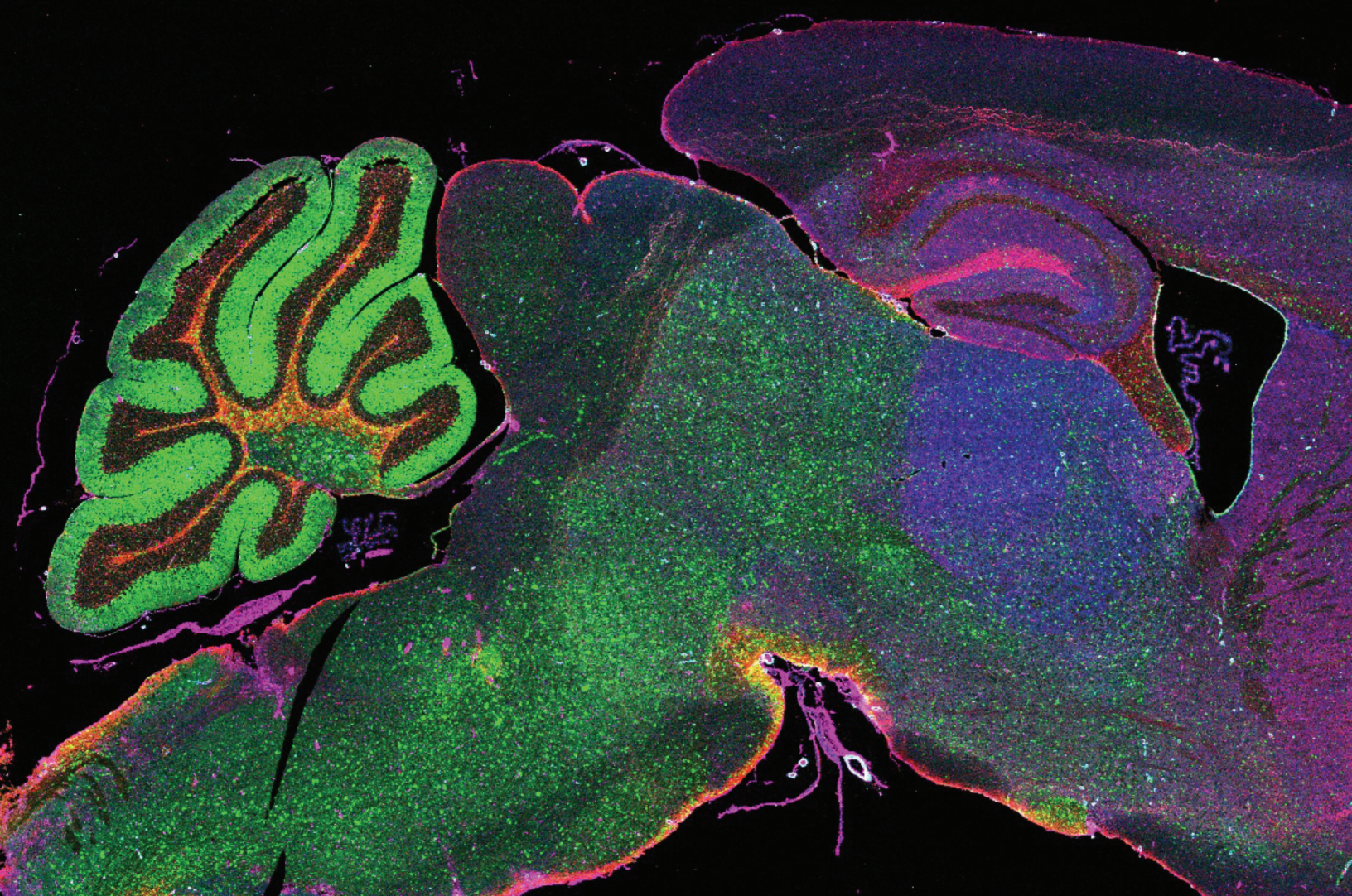
IMAGE LOOKBOOK

NEUROSCIENCE

Revealing Spatial Biomarkers in Neural Tissues with Multiplexed Imaging Mass Cytometry Technology

Explore new views of cellular and structural composition of neural tissues to unlock new understanding of spatial biology in clinical applications



Introduction

Understanding the cellular and spatial composition of tissues is crucial for interpreting neural disease origin, progression, prognosis and treatment options. Imaging Mass Cytometry™ (IMC™) is a spatial biology technology that can uncover novel phenotypes and identify therapeutic targets that may be relevant to developing biomarkers and future treatment strategies in neuro-oncology and neurodegenerative studies.

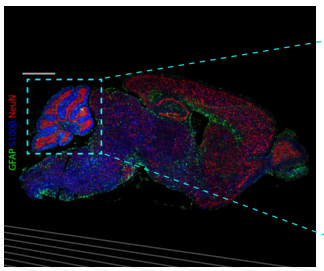
Unlike traditional cyclic fluorescent methods, IMC technology can uncover the spatial distribution of 40-plus distinct protein markers simultaneously without tissue degradation and autofluorescence artifacts usually observed in brain tissue. This lookbook showcases translational and clinical applications of multiplexed tissue analysis using IMC technology.

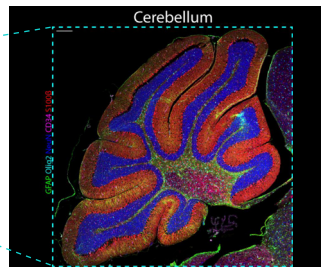
Imaging Mass Cytometry technology enables highly specific detection of **surface and intracellular targets** without interference from autofluorescent tissues.

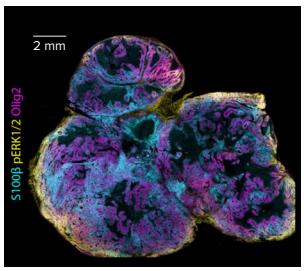
KEY TAKEAWAYS

- Using a combination of imaging modes and ready-to-go high-plex panels on the same tissue section provides researchers with more flexibility to resolve the distribution of neural and non-neural cell lineages.
- Rapid imaging modes reveal key biological insights within spatial context that are relevant for developing potential diagnostic and therapeutic applications.

NEURO-ONCOLOGY
PARKINSON'S DISEASE
ALZHEIMER'S DISEASE
MULTIPLE SCLEROSIS
PROTEINOPATHIES
SYNTAU MIXED PATHOLOGY







Ready-to-use panels

Fast high-parameter panel design by combining pre-optimized panels with other relevant IMC panels.

Preview Mode

Number of markers: 42
Acquisition time: 20 minutes
Sample: normal mouse brain (13 mm x 15 mm)

Cell Mode

Number of markers: 42
Acquisition time: 2 hours
Sample: normal mouse brain (2 mm x 2 mm)
Resolution: 1 µm

Tissue Mode

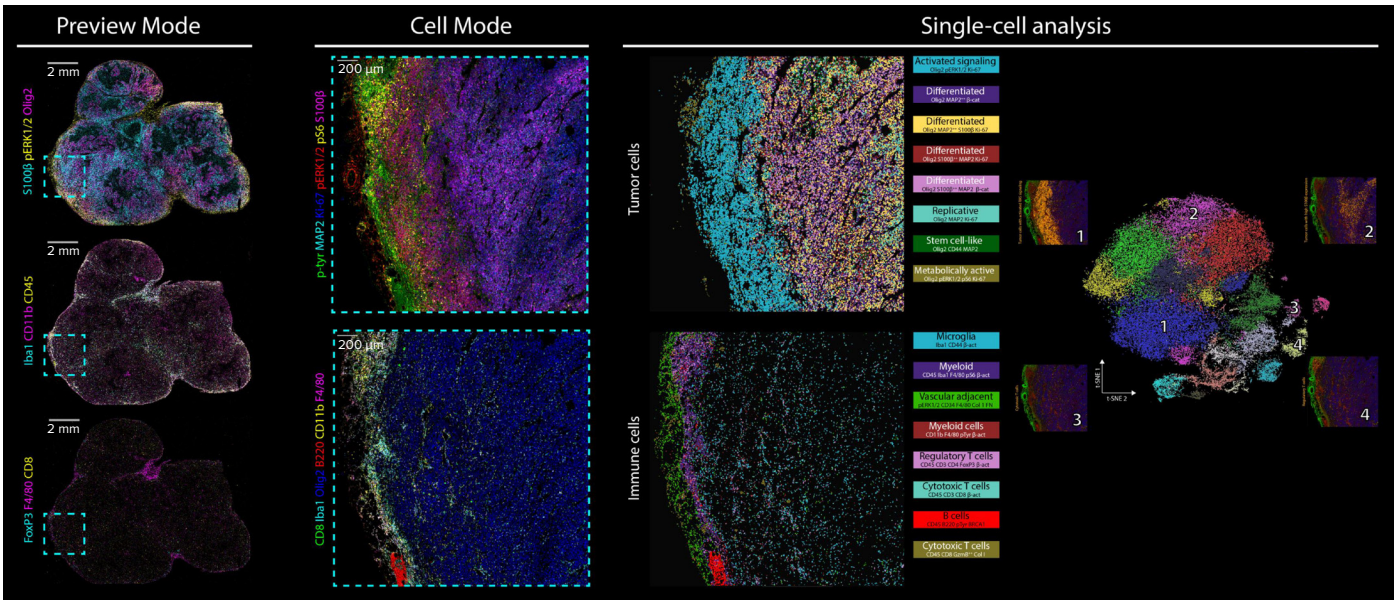
Number of markers: 42
Acquisition time:
5 hours and 50 minutes
Sample: mouse glioblastoma brain (24 mm x 16 mm)
Resolution: 5 µm

Neuro-Oncology

View the study details

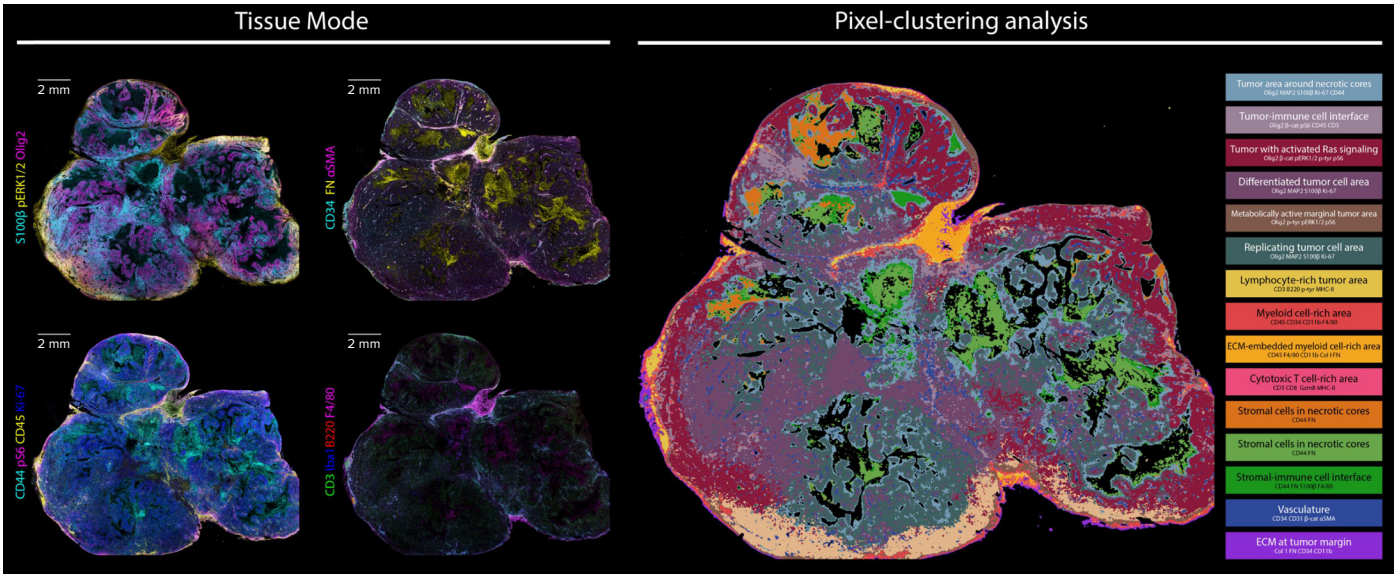
In a study of mouse embryo, normal brain and glioblastoma (GBM) tissue, a 43-marker neuro-oncology panel composed of the Maxpar™ OnDemand Mouse Immuno-Oncology IMC Panel Kit and the Maxpar Neuro Phenotyping IMC Panel Kit revealed the spatial distribution of over 40 distinct molecular markers.

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



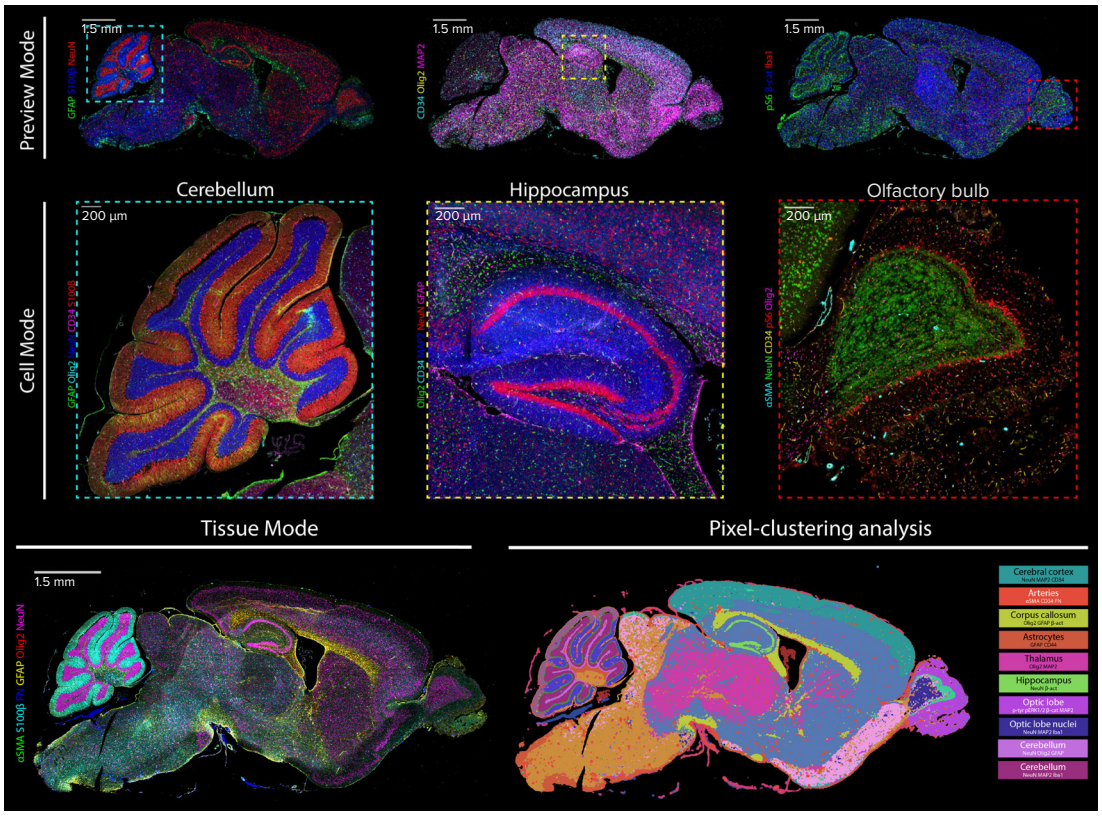
Preview Mode scan rapidly identified areas with high tumor and immune cell activity, which was used to identify relevant regions of interest for detailed Cell Mode investigation. Multiplex Cell Mode images using tumor- (top) and immune- (bottom) specific markers demonstrate the heterogeneity of the TME.

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



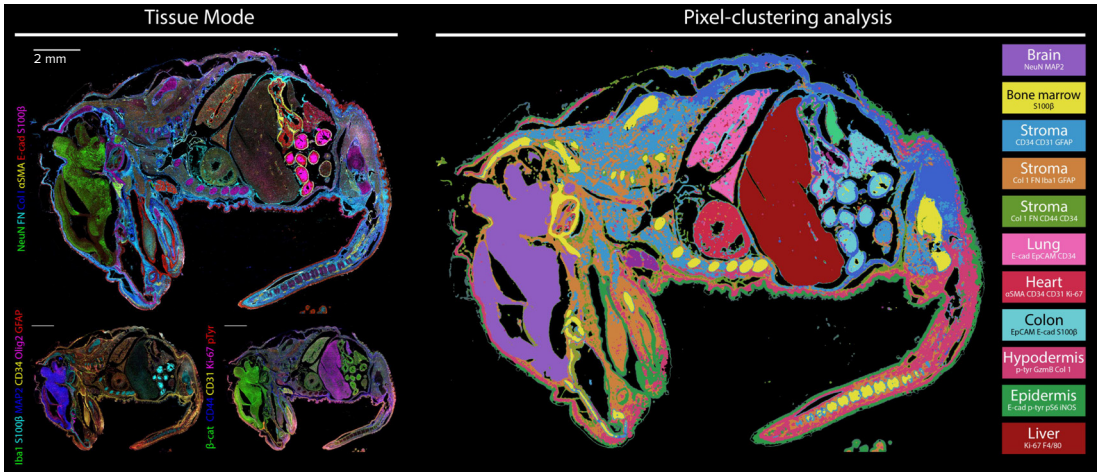
Tissue Mode imaging demonstrates the tumor and immune cell heterogeneity of mouse glioblastoma tissue. Metabolically active tumor cells were detected at the periphery of tumor. Vascularization was observed across the tumor in non-necrotic areas. Immune cells were detected in high concentration at the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

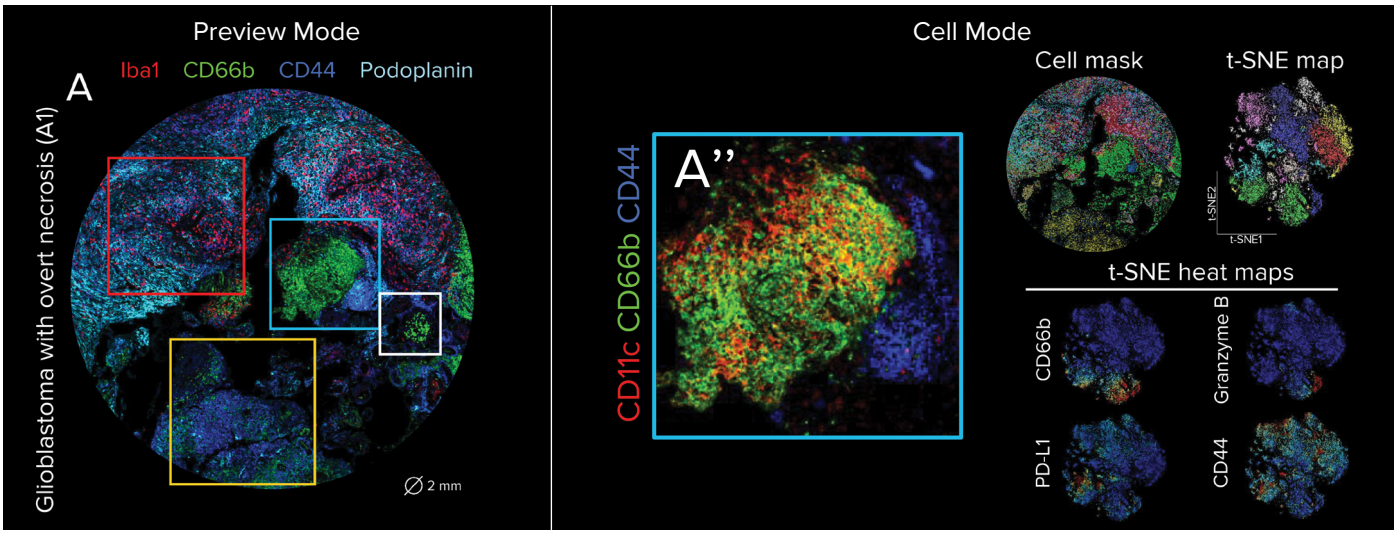
A 40-marker panel was designed to study the TME of mouse neurological tissues.

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	

View the study details

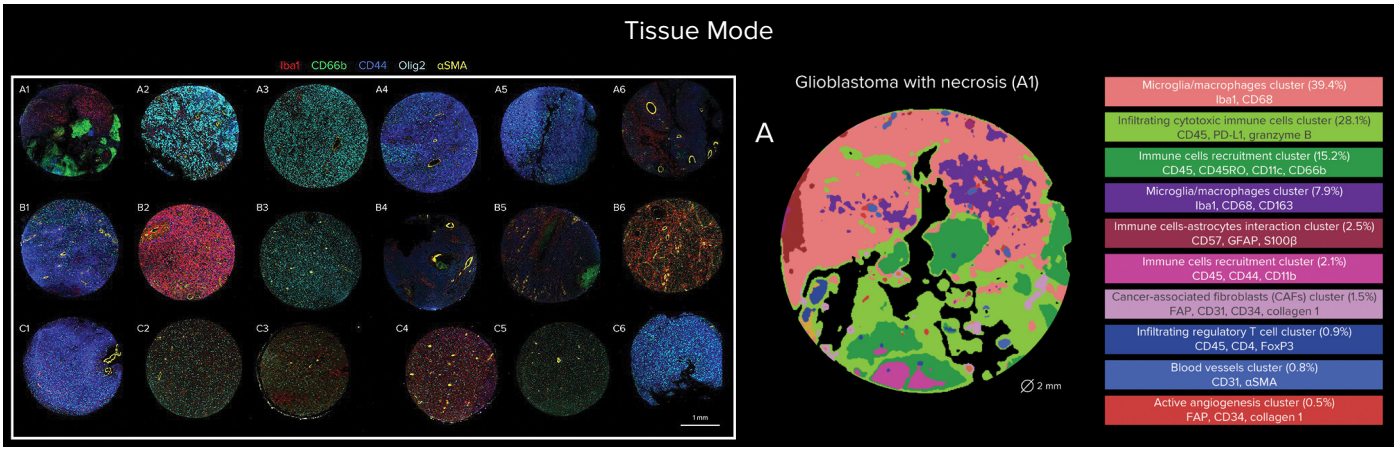
Fast screening of the entire slide combined with single-cell analysis

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.



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.

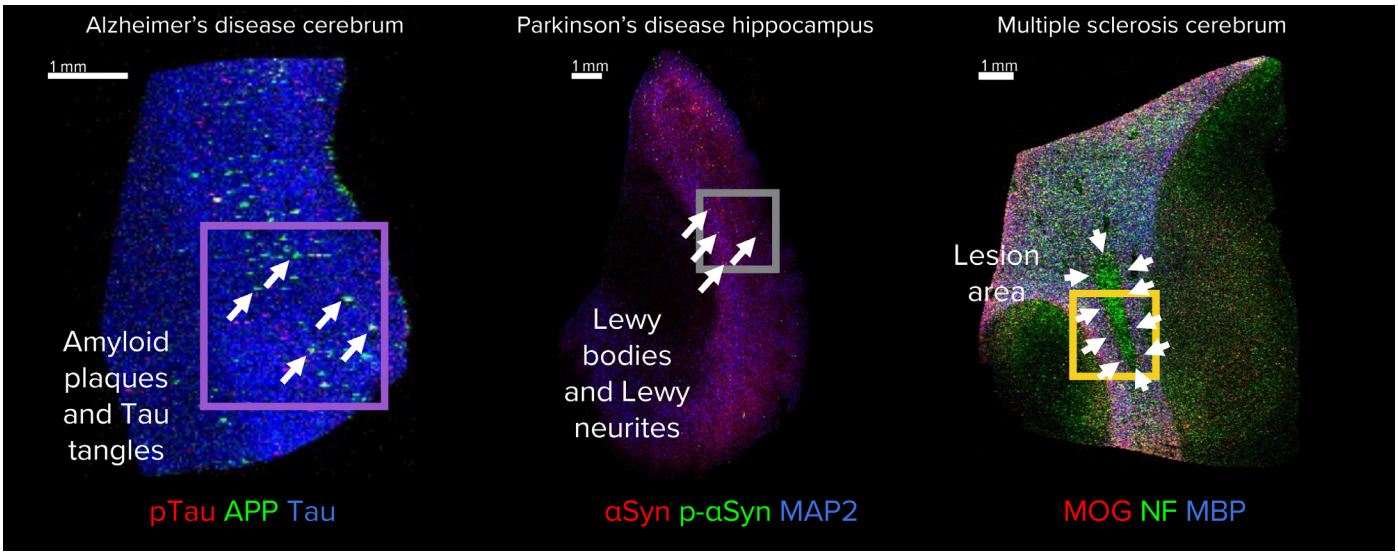
18 2 mm TMA cores can be imaged in **1 hour and 35 minutes**

Neurodegenerative Disease

View the study details

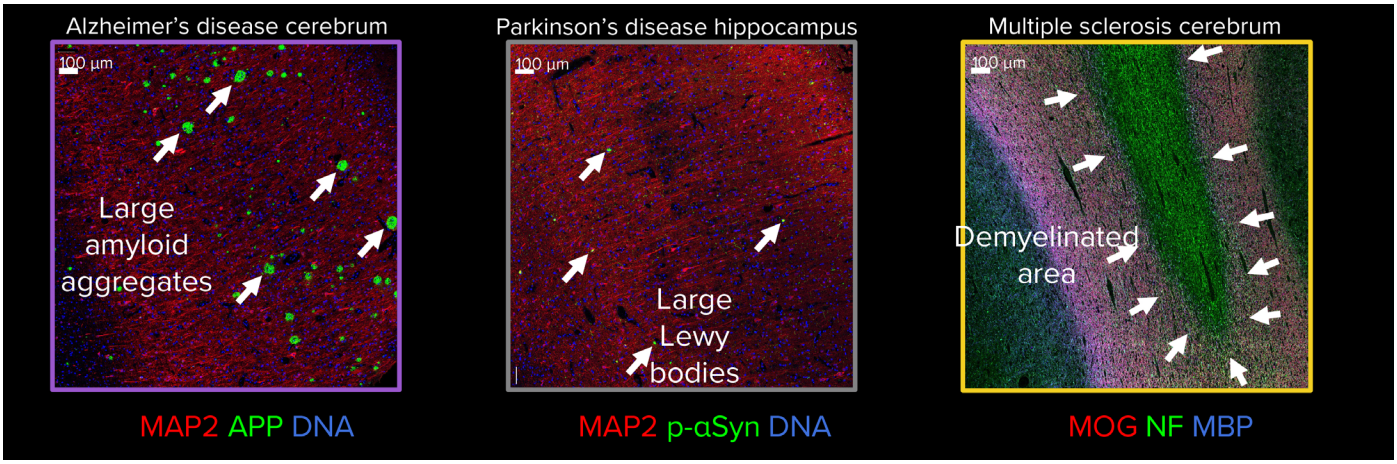
In this application, diseased brain tissues samples were stained with modular subpanels including one of three neurodegenerative subpanels, each specific to a disease type: Parkinson's disease (PD), Alzheimer's disease (AD) or multiple sclerosis (MS).

Identifying regions of main protein contributors to disease pathology



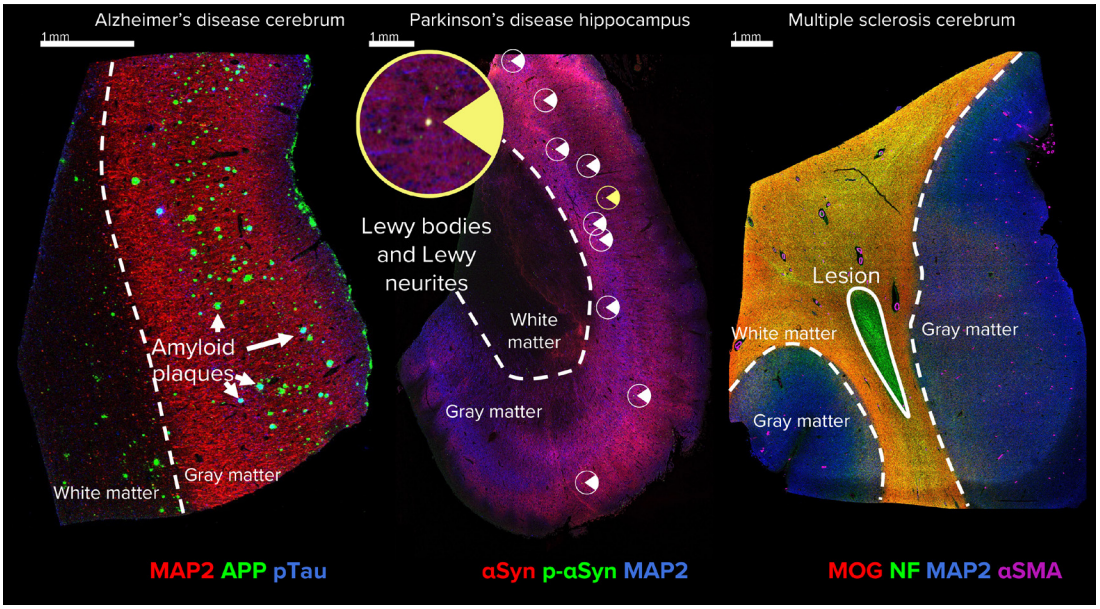
Preview Mode in combination with neurodegenerative panels allow whole tissue visualization of the main protein contributors to disease pathology: amyloid precursor protein (APP) in amyloid plaques and Tau in tangles of AD (left panel); p-αSynuclein (p-αSyn) in Lewy bodies and Lewy neurites of PD (middle panel); and large areas of lost myelin in a lesion of MS (right panel).

Revealing heterogeneity of protein distribution at subcellular resolution



Data from Cell Mode acquisition was used to conduct single-cell analysis. Locations of the same aggregates with the most abundant presence of APP and pTau, Lewy bodies and area of demyelination are marked with arrowheads in a Cell Mode image.

Detection of pathological tissue compartments in the neurodegenerative brain



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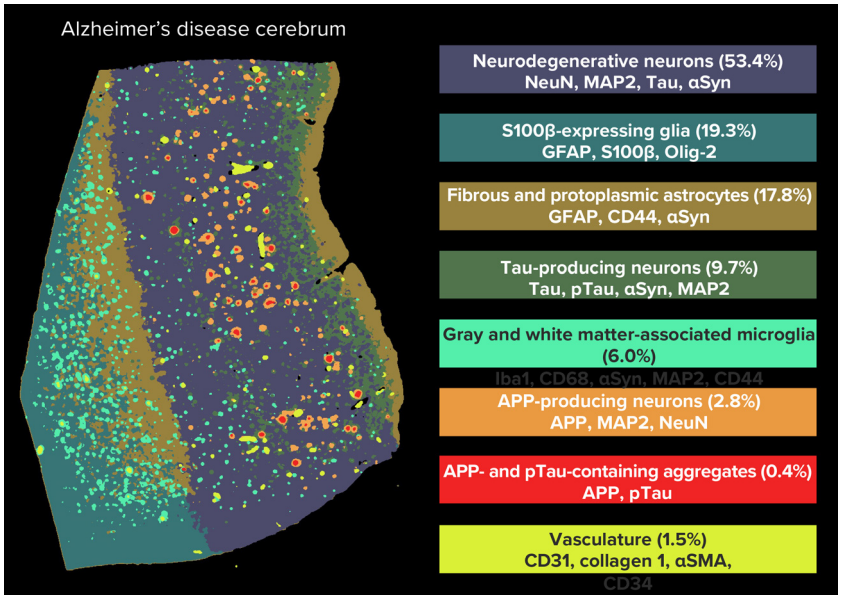
Pixel-clustering analysis reveals extracellular aggregates and distinct morphology clusters



In AD, pixel-clustering analysis unveiled eight distinct morphology clusters, such as gray matter-associated and white matter-associated microglia (combined in one cluster); three distinct populations of neurons, fibrous and protoplasmic astrocytes (combined in one cluster); oligodendrocytes; and vasculature, alongside the identification of two functional amyloid aggregate clusters.

The arrangement of APP and Tau hints at a potential aggregate stabilization and overall synergy among those proteins, alongside αSyn, all known to be prone to misfolding in the diseased brain.

A 41-marker panel, comprised of disease-specific neurodegenerative subpanels, was designed to study diseased brain tissue.

<table>
<tr><th colspan="6">Human Immuno-Oncology IMC Panel, 3 1 Antibodies (PN 201509)</th><th rowspan="2">Maxpar Neuro Phenotyping 7 Antibodies
PN 201337</th><th>Parkinson's Disease
PN 9100006</th><th rowspan="2">Maxpar IMC Cell Segmentation Kit
PN 201500</th></tr>
<tr><td>Cell Functional State
PN 201514</td><td>Stromal Cell
PN 201511</td><td>Basic Immune
PN 201518</td><td>Lymphoid
PN 201512</td><td>Myeloid
PN 201513</td><td>Basic Tissue Architecture
PN 201517</td><td>Alzheimer's Disease
PN 9100007</td></tr>
<tr><td colspan="6"></td><td></td><td>Multiple Sclerosis
PN 9100008</td><td></td></tr>
</table>

Protocol

1 **Section** tissue or tissue microarray onto a slide.

2 **Stain** tissue with one 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
Maxpar Neuro Phenotyping IMC Panel Kit	201337
Maxpar OnDemand Mouse Neuro-Oncology IMC Bundle	9100005NO
Parkinson's Disease IMC Panel, 3 Antibodies	9100006
Alzheimer's Disease IMC Panel, 3 Antibodies	9100007
Multiple Sclerosis IMC Panel, 3 Antibodies	9100008
Maxpar IMC Cell Segmentation Kit	201500
Cell-ID™ Intercalator-Ir	201192B

SBI Services Lab

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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.

Zabinyakov, N. et al. "Imaging Mass Cytometry spatially resolves immune activity in neurodegenerative brain pathology." *Journal for Neuroscience* (2024).

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