APPLICATION NOTE

Exploring Neurodegenerative Diseases with Imaging Mass Cytometry Systems

Interrogate various neurodegenerative diseases with the fastest, most comprehensive approach to assay design

Introduction

Neurodegenerative diseases (NDs) are chronic progressive conditions of the central nervous system (CNS) leading to physical and cognitive disability in humans across the globe. NDs affect approximately 15% of the worldwide population and patient numbers have been steadily climbing over the past decades[1].

In this application note, we demonstrate the spatial biology insights that Imaging Mass Cytometry™ (IMC™) platforms provide into NDs with a particular focus on Alzheimer's disease (AD) tissue. We showcase the comprehensive utilization of a specialized AD panel and demonstrate its effectiveness in exploring different populations of neurons that contribute to aggregate formation. The methodologies presented are equally applicable to other neurodegenerative tissues.

This application note outlines:

- Ready-to-use neurodegenerative panels compatible with human immuno-oncology IMC subpanels (Figure 1) provide swift high-parameter panel design with preselected antibodies
- Multiple imaging modes for IMC systems enable simultaneous detection of protein targets, from whole tissue sections down to single-cell resolution
- Researchers now have access to the fastest and most comprehensive workflow to interrogate various neurodegenerative diseases by combining high-parameter panels with various IMC imaging modes

Routine and scalable spatial profiling with ready-to-go IMC panels

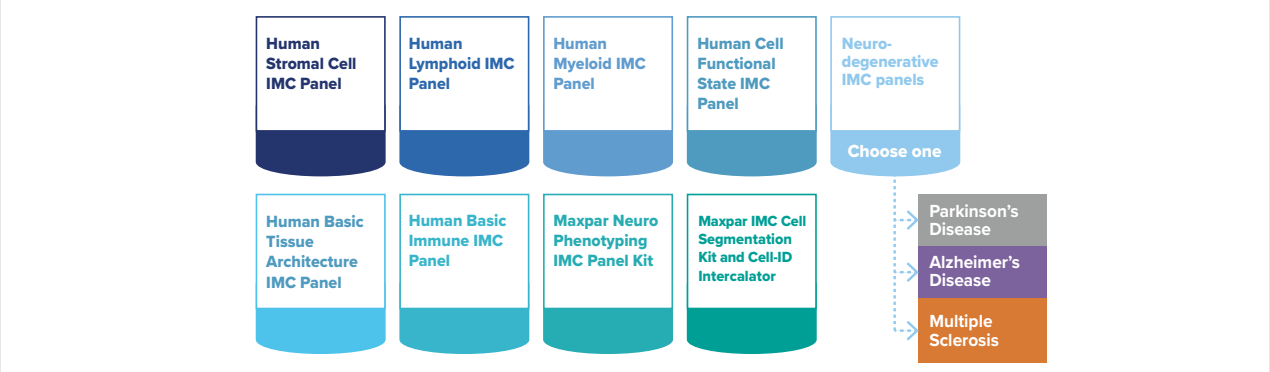

Figure 1. Three specialized panels are available to support neurodegenerative spatial biology studies. When combined with other relevant IMC panels tailored for neurophenotyping, tissue architecture and immune cell profiling, 3 different high-parameter 41-marker imaging panels for NDs can be easily assembled

Comprehensively visualize the pathological features of neurogenerative diseases

Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS) represent the 3 most prominent contributors to the global ND population. AD, PD and MS are currently incurable, with treatment focusing on palliative care and alleviating symptoms. Understanding the etiology of these diseases is a major challenge and requires deciphering the complex spatial biology processes that cause deterioration of neuronal tissue, such as formation of protein aggregates and tangles, neuroinflammation and demyelination. To fully address this complexity, it is important to clarify the underlying molecular mechanisms driving neurodegeneration and identify new targets and therapies.

Imaging Mass Cytometry (IMC) is a spatial biology technology that offers the ability to comprehensively visualize the pathological features of neurogenerative diseases. 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. IMC platforms offer various whole slide imaging (WSI) modes that range from visualization of an entire section to single-cell assessment, permitting in-depth exploration of tissue heterogeneity. WSI modes enable streamlined, comprehensive evaluation of human brain tissue using ready-to-use antibody panels specifically designed for neurological research.

Three specialized panels are available to support neurodegenerative spatial biology studies (Figure 2):

- The **Alzheimer's Disease IMC Panel, 3 Antibodies** identifies amyloid aggregates and Tau tangles
- The **Parkinson's Disease IMC Panel, 3 Antibodies** identifies Lewy bodies and Lewy neurites
- The **Multiple Sclerosis IMC Panel, 3 Antibodies** identifies the loss of major myelin components

When combined with other relevant IMC panels tailored for neurophenotyping, tissue architecture and immune cell profiling, 3 different high-parameter 41-marker imaging panels for NDs can be easily assembled (Figure 3). The modular panel-building format facilitates rapid and effortless design of high-parameter IMC panels tailored precisely to research objectives. IMC WSI provides a streamlined approach that expedites the discovery of valuable biological insights into each of these important neurodegenerative conditions.

The Maxpar Direct Immune Profiling Assay shows proven reproducibility across sites, essential for large multi-site studies

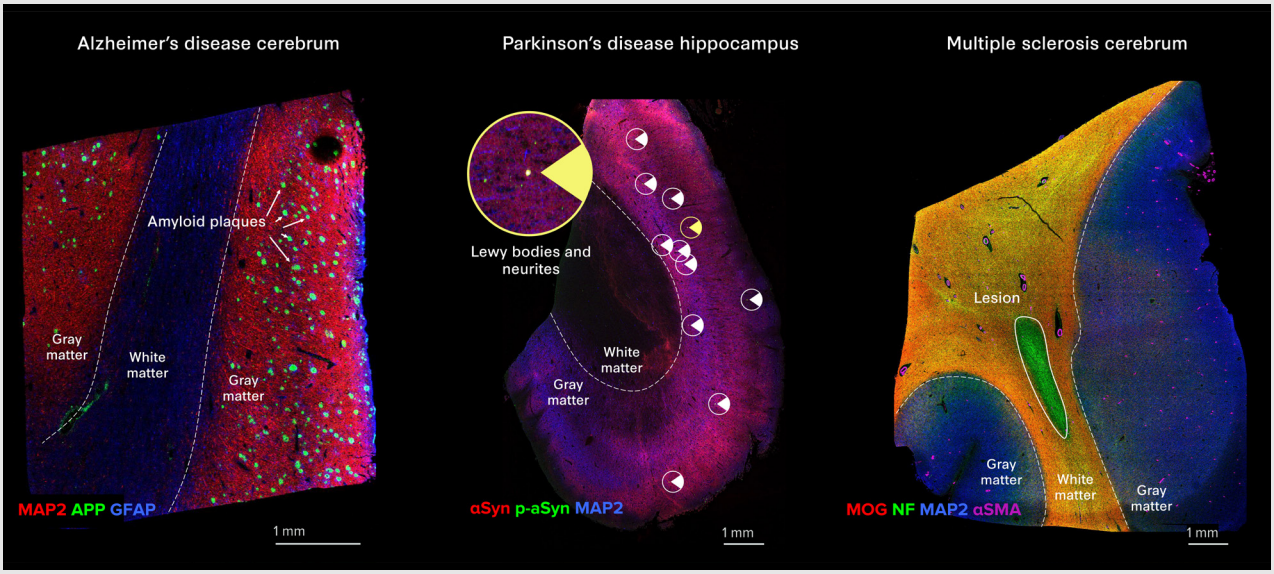

Figure 2. Main hallmark features of each neurodegenerative condition visualized in Tissue Mode: amyloid aggregates and tangles, Lewy bodies with Lewy neurites and multiple sclerosis lesions. Diseased tissues stained with applicable neurodegenerative panels (Alzheimer's Disease IMC Panel, Parkinson's Disease IMC Panel and Multiple Sclerosis IMC Panel) allow whole tissue visualization of the main protein contributors to disease pathology: amyloid precursor protein (APP) in amyloid plaques and Tau in tangles of Alzheimer's disease, phosphorylated αSynuclein (p-αSyn) in Lewy bodies and Lewy neurites of PD, and large areas of lost myelin (lesion) of MS.

Study design

Panel design

The 41-marker panel comprised of the following off-the-shelf modular subpanels and kits was assembled to investigate AD brain tissue (Figure 3):

- **Human immuno-oncology IMC subpanels, 26 antibodies** in total. Six human immuno-oncology IMC subpanels were used to interrogate structural, microglial and neuroinflammation components involved in the causality, development and progression of AD.
- **Maxpar™ Neuro Phenotyping IMC Panel Kit** for phenotyping of major cell types in the CNS, such as neurons, astrocytes, microglia, oligodendrocytes and endothelial cells. The Maxpar Neuro Phenotyping IMC Panel Kit is essential for accurately identifying all major cell types and their interactions with other cells when analyzing brain tissue.
- **Alzheimer's Disease IMC Panel** to locate major protein markers associated with each corresponding pathology, such as APP, Tau and phosphorylated Tau (pTau)
- **Maxpar IMC Cell Segmentation Kit (ICSK)** to facilitate an end-to-end workflow for single-cell data analysis
- **Cell-ID™ Intercalator-Ir (191Ir/193Ir)** for identification of nucleated cells
- **AD-relevant IMC catalog antibodies (αSyn, p-αSyn [S129])** to gain additional insights about αSyn participation in aggregate formation

See Appendix, Table 1 for detailed panel configuration and clone information.

A 41-marker assay built by combining ready-to-go modular panels characterizing phenotype, function and pathology of cells in a tissue sample

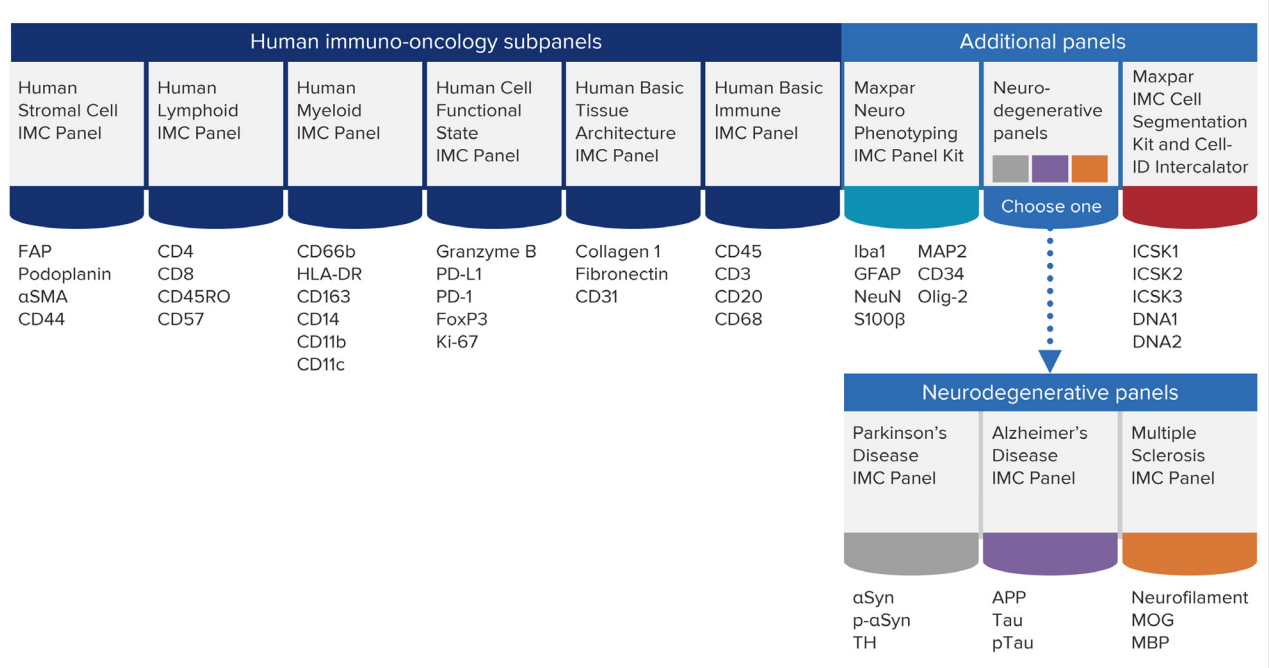

Figure 3. Combination of 3 ND IMC panels with other compatible panels and kits for comprehensive interrogation of each disease. The modular panel design provides flexibility, allowing researchers to create customized IMC panels for investigating most neurodegenerative conditions, including AD, PD and MS. When combined with other relevant IMC panels tailored for neurophenotyping, tissue architecture and immune cell profiling, 3 different high-parameter 41-marker imaging panels for NDs can be easily assembled. This enables visualization of the main cell types in the CNS and provide insights into the contribution of the immune system in NDs.

The full-configuration panel was applied on sections of human AD brain. The tissue slides were prepared and stained according to the Standard BioTools™ Imaging Mass Cytometry Staining Protocol for FFPE Sections (400322) using optimized antibody concentrations for Tissue Mode, Preview Mode and Cell Mode.

Imaging modes

Tissue slides were visualized with the Hyperion™ XTi Imaging System using all 3 imaging modes (Figure 3):

- **Preview Mode** enables quick visualization of all 41 markers within minutes across the whole tissue. This fast scan provides guidance for selecting regions of interest (ROIs) to be acquired on the same slide in Cell Mode.
- **Tissue Mode** is a complete WSI mode to visualize 41 markers, revealing the heterogeneity of the whole tissue
- **Cell Mode** facilitates acquisition of ROIs selected in Preview Mode at subcellular resolution for detailed characterization of individual cells and cell populations

One serial section of AD tissue utilized Preview Mode and Cell Mode, while another sequential section of AD tissue exclusively employed Tissue Mode (Figure 3).

Image analysis

Qualitative data analysis, high-parameter image rendering and single-channel image extractions were performed using MCD™ SmartViewer software. For AD tissue data obtained using Preview Mode and Cell Mode, quantitative single-cell analysis was performed using a 2-step data analysis pipeline: Cellpose was used for cell segmentation and PhenoGraph was used for clustering[2]. The data obtained using Tissue Mode was analyzed with a pixel-clustering analysis and neighborhood enrichment analysis approach using MCD SmartViewer software.

See Methods for additional experimental details regarding samples, staining, ablation and data analysis.

Recommended imaging workflows offer comprehensive and accelerated spatial analysis

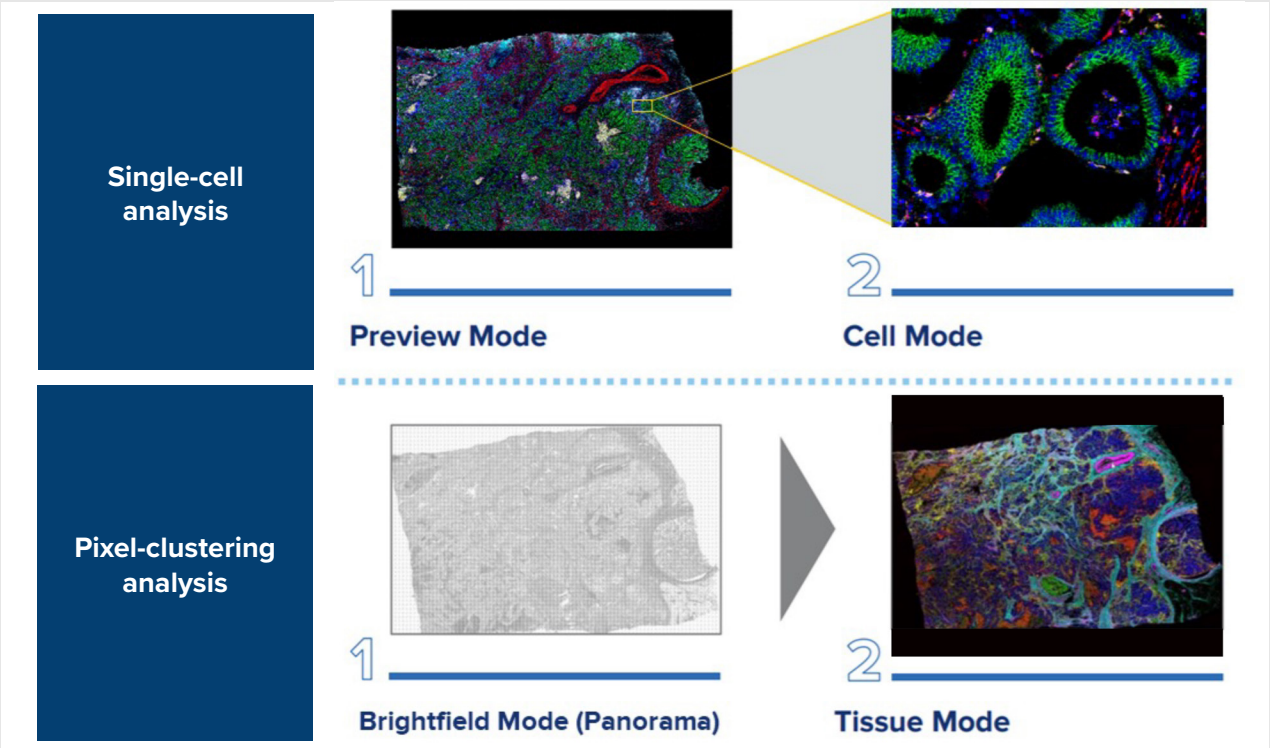

Figure 4. WSI modes for IMC platforms offer versatile workflows to accelerate quantitative spatial biology discoveries. Preview Mode rapidly scans the sample and generates useful data for guiding ROI placement used in Cell Mode single-cell analysis. Tissue Mode generates a high-quality scan of the entire tissue section in a matter of hours with higher spot-size ablations enabling pixel-clustering analysis of the whole tissue. Combining these new workflows with the Hyperion XTi Slide Loader streamlines IMC application and makes it a useful resource for high-throughput clinical and translational studies.

Results

Whole slide Preview Mode imaging enables the spatial localization of protein aggregates in AD tissue

Multiple brain sections from a 73-year-old male donor diagnosed with AD were commercially obtained. The tissue sections were stained with a 41-marker assembly containing 3 markers from the Alzheimer's Disease IMC Panel, then analyzed using the Hyperion XTi Imaging System.

Tissue slides were first imaged using Preview Mode, with visualization of the whole post-central gyrus tissue measuring 3.9 mm x 5.2 mm completed in less than 5 min. One of the most notable benefits of visualizing brain tissue in Preview Mode is the ease of marker-guided identification of specialized brain regions such as gray and white matter. For example, the utilization of MAP2 or NeuN markers visibly defines the gray matter area (Figure 4A).

Localization of all 3 markers from the Alzheimer's Disease IMC Panel (APP, total Tau protein, and phosphorylated [S202/T205] Tau protein) is also clearly visible in the tissue within the MAP2+ gray matter of AD brain (Figure 4A).

The striking abundance of insoluble plaques containing APP and pTau (Figure 4A, left and middle images) suggests an advanced stage of the disease, possibly leading to irreversible cognitive decline[3].

These large plaques scattered around the gray matter served as landmarks for selecting ROIs for visualization in Cell Mode using the same tissue section. By utilizing the same tissue slide for imaging using both Preview Mode and Cell Mode, we eliminated the need for additional stained slides, thereby conserving valuable patient samples.

IMC systems allow for qualitative and quantitative analysis to reveal cell-specific neurodegenerative pathology

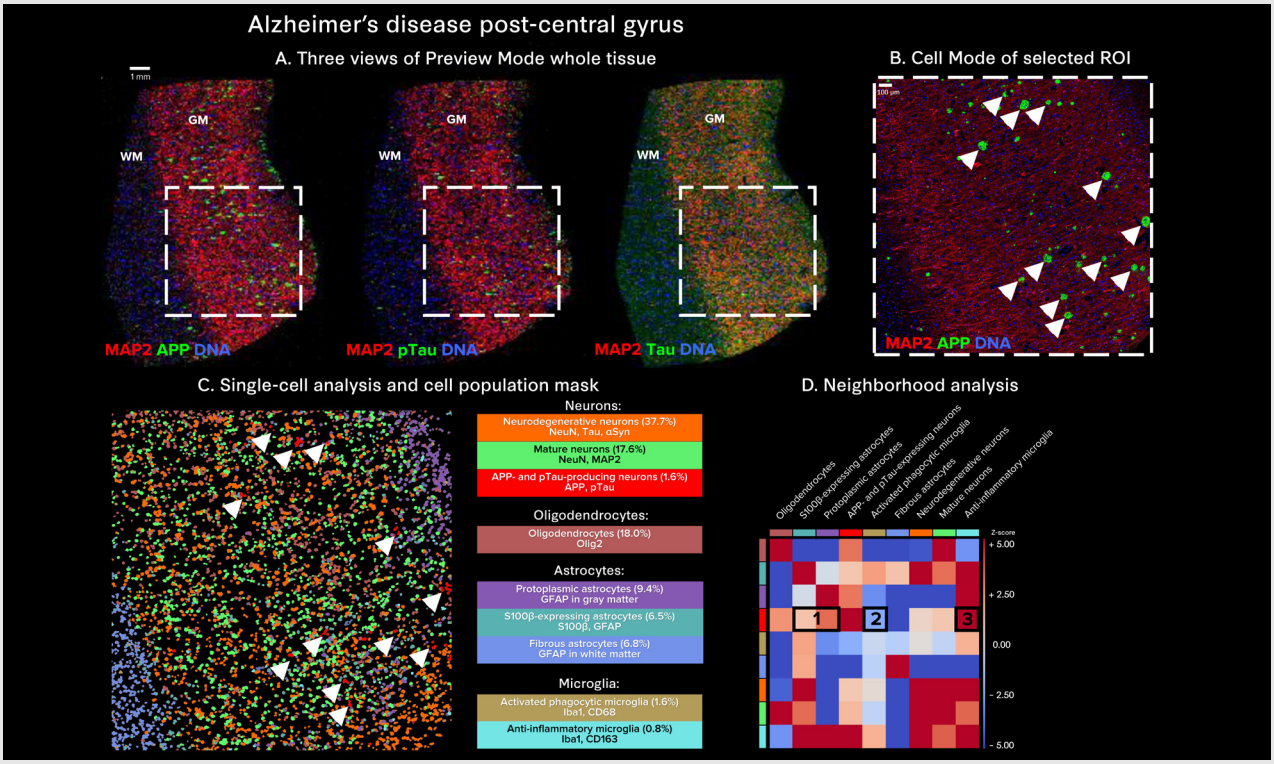

Figure 5. Qualitative and quantitative assessment of AD tissue using IMC Preview Mode and Cell Mode with subsequent single-cell and neighborhood enrichment analysis uncovers cell populations associated with aggregate-producing neurons. Preview Mode facilitates marker-guided ROI selection for subsequent visualization in Cell Mode. Cell Mode ROI was used to conduct single-cell analysis. (A) Three views of a single ROI. (B) Locations of the same aggregates with the most abundant presence of APP- and pTau-producing neurons are marked with arrowheads in a Cell Mode image and (C) cell population mask. Cell populations were identified using ICSK and Cell-ID Intercalator-Ir staining. Population names were assigned based on marker expression. (D) Subsequent quantitative neighborhood analysis demonstrates a heat map with an enrichment score (Z score) that indicates enrichment or depletion of the spatial proximity between all identified clusters. WM – white matter, GM – gray matter

One region with high concentration of aggregates with both APP and pTau was selected to further investigate the tissue in Cell Mode (Figure 4B) using single-cell analysis (Figure 4C).

Single-cell analysis of high-resolution Cell Mode images reveals diverse composition of cell populations within the aggregate vicinity

High-resolution Cell Mode visualization of 1 ROI measuring 2.1 mm x 2.1 mm was acquired at 800 Hz and completed in 1 hr 43 min. Three functionally distinct populations of both neurons and astrocytes, 2 populations of microglia and 1 population of oligodendrocytes were identified through single-cell analysis of the Cell Mode ROI. Most segmented nucleated elements were neurons, accounting for 56.9% of all cells, with roughly every 7 out of 10 being neurodegenerative neurons producing elevated levels of Tau and αSyn. Another population of neurons, the smallest at 1.5% of all cells, was observed to produce high levels of APP and pTau (Figure 4C). Interestingly, these neurons tended to gravitate toward the periphery of large aggregates (Figure 4B and 4C, arrowheads), suggesting their involvement in the process of actively producing toxic amyloid species that contribute to aggregate formation[4]. Inhibiting this smallest population of "rogue" neurons producing high levels of APP and pTau to mitigate or slow the secretion of toxic amyloid species could potentially have a significant clinical impact.

These aggregate-forming neurons were associated with populations of protoplasmic astrocytes and astrocytes expressing elevated levels of S100β as indicated by the neighborhood enrichment analysis heat map (Figure 4D, Box 1). S100β is an important inflammatory regulator of astrocytes by inducing their phenotype change to become reactive[5, 6]. Direct inhibition of S100β has been shown to have a neuroprotective effect in some populations of neurons and reduce neuronal loss in mouse AD models[7].

Neighborhood analysis also suggests a close spatial proximity of aggregate-forming neurons with microglia that exhibit anti-inflammatory properties (Iba1+, CD163hi), while activated phagocytic microglia (Iba1+, CD68hi) appear to be located at a **distance** (Figure 4D, Boxes 2 and 3). Dissociation of phagocytic microglia from amyloid plaques may indicate microglial exhaustion due to prolonged exposure to aggregates, resulting in defective autophagy[8]. As microglia play a critical role in mediating the phagocytosis of aggregates, successful efforts were made to reverse microglial phagocytic deficits by treating microglia with anti-inflammatory agents[9].

Overall, the data presented highlights the power of Preview Mode to rapidly locate desirable areas of the tissue for subcellular resolution imaging via Cell Mode on the same slide. Furthermore, combining this workflow with single-cell analysis effectively identifies diverse cellular organization and cellular interactions in the AD brain.

While single-cell analysis provides valuable insights, nuclei-based segmentation of brain tissue is not sufficient to draw a complete picture of the disease. Additional complex analysis of neuronal, astrocytic and microglial extensions that are not associated with DNA, especially of ramified phenotypes, is required to comprehensively understand the cellular population landscape of the diseased brain. Moreover, single-cell analysis is limited in capturing extracellular aggregates because they are devoid of membrane and nucleus markers. Therefore, pixel-clustering analysis offers a complementary perspective, allowing for the examination of non-nucleated extracellular aggregates at a tissue level.

Pixel-clustering analysis of Tissue Mode images complements single-cell analysis by providing additional biological insights

A slide with a sequential section was visualized in Tissue Mode (Figure 5A). Visualization of the whole tissue section of post-central gyrus measuring 3.9 mm x 5.2 mm was completed in 27 min. Subsequent pixel-clustering analysis using MCD SmartViewer software (Figure 5B) performed unsupervised clustering, automatically grouping pixels with similar characteristics by analyzing the intensity and distribution of 41 markers in each pixel. Pixel-clustering analysis unveiled numerous distinct morphology clusters, such as gray matter-associated and white matter-associated microglia, 3 populations of neurons, astrocytes and oligodendrocytes, and vasculature, alongside the identification of 2 functional amyloid aggregate clusters.

A cluster of neurodegenerative neurons expressing higher levels of Tau and αSyn encompassed a substantial portion of the whole tissue area (41.8% of total analyzed pixels). This is consistent with the observations from single-cell analysis implying a high degree of pathology, such as cellular dysfunction and disease progression.

Aggregates of a relatively small size exhibited an exclusive enrichment of APP, whereas most of the larger aggregates demonstrated a spatial organization characterized by the central concentration of APP surrounded by a halo of Tau protein. 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[10].

Overall, these findings demonstrate the power of combining Tissue Mode imaging with pixel-clustering analysis to clearly unveil the heterogeneity of non-nucleated extracellular aggregates and nucleated cellular clusters in neurodegenerative conditions.

IMC Tissue Mode combined with pixel-clustering analysis captures AD-specific protein expression

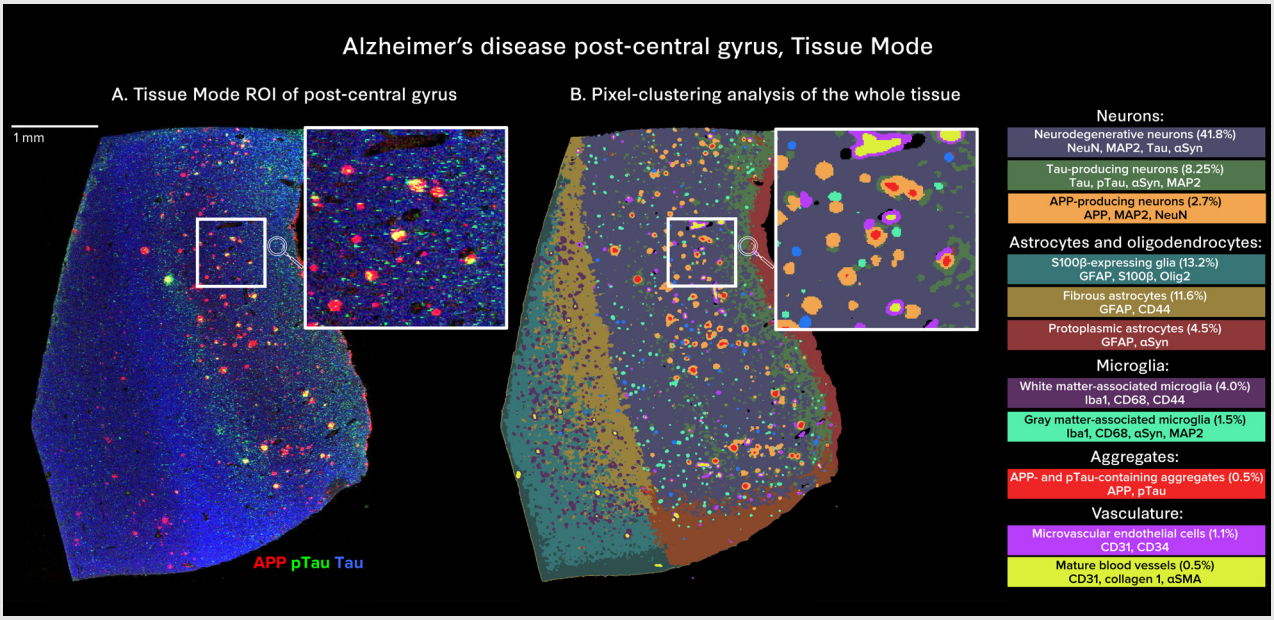

Figure 6. Visualization of Alzheimer's disease tissue using IMC Tissue Mode with subsequent pixel-clustering analysis reveals distinct aggregate populations. (A) Tissue Mode imaging successfully captures the expression pattern of APP, pTau and Tau in AD brain tissue. (B) Subsequent pixel-clustering analysis captures different types of cell clusters and aggregates based on the marker expression in each pixel.

Conclusions

IMC technology captures key biological insights and provides a comprehensive multimodal approach to understanding the complex pathology of neurodegenerative conditions by utilizing ready-to-use antibody panels, synergistic WSI modes and complementary data analysis pipelines.

Preview Mode allows for rapid and simultaneous 40-plus-marker visualization across the entire tissue section, facilitating the identification of ROIs for use in Cell Mode analysis. Cell Mode and subsequent downstream single-cell analysis provide detailed insights at the highest resolution, uncovering distinct neuronal populations and their protein expression profiles, crucial for understanding disease mechanisms. Additionally, Tissue Mode enables comprehensive visualization of all markers, revealing the heterogeneity of aggregate distribution across the whole tissue.

Furthermore, single-cell and pixel-based analyses complement each other effectively. Single-cell analysis elucidates the heterogeneity of nucleated elements associated with the aggregates, while pixel-based analysis offers a broader perspective by capturing different types of aggregates at a tissue level. The combination of these approaches allows for a comprehensive understanding of cellular dynamics. Researchers can now gain a more holistic understanding of neurodegeneration to discover new potential therapeutic targets and interventions.

Besides the Alzheimer's Disease IMC Panel, the Parkinson's Disease IMC Panel and the Multiple Sclerosis IMC Panel effectively reveal Lewy bodies with Lewy neurites and lesions in PD and MS, respectively, facilitating a comprehensive high-parameter sample investigation with WSI modes and downstream analysis.

Tips for success

- For best results, use freshly cut FFPE tissue samples when possible
- Perform a 3-point titration and include positive control tissue for all antibodies when optimizing their working concentrations on tissue sections. Recommended dilution ranges for each antibody can be found in the technical datasheet.
- Increase maximum threshold values in MCD SmartViewer software to better visualize small Lewy bodies and Lewy neurites in PD tissue
- After staining, samples should be stored at room temperature in slide holders inside a sealed bag in a non-humid environment
- Customers should reach out to their local Field Applications Scientist (FAS) for ordering and product support. To be connected to a FAS, contact **technical support**.

Methods

Staining

Slide preparation and staining were conducted according to the Imaging Mass Cytometry Staining Protocol for FFPE Sections.

Imaging

Imaging was performed using the Hyperion XTi Imaging System with CyTOF™ Software v9.0. Before ablation, instrument tuning was performed using a tuning slide. Ablation frequency was 800 Hz with optimized laser power for all imaging modes.

Cell segmentation and analysis

Qualitative data analysis, high-parameter image rendering and single channel image extractions were performed using MCD SmartViewer software. For AD tissue data obtained using Preview Mode and Cell Mode, quantitative single-cell analysis was performed using a 2-step data analysis pipeline: Cellpose was used for cell segmentation and PhenoGraph was used for clustering. The data obtained using Tissue Mode was analyzed with a pixel-clustering analysis and neighborhood enrichment analysis approach using MCD SmartViewer software.

Panel compatibility

Neurodegenerative IMC panels are compatible with the following products:

Product Name	Part Number
Human Stromal Cell IMC Panel, 4 Antibodies	201511
Human Lymphoid IMC Panel, 4 Antibodies	201512
Human Myeloid IMC Panel, 6 Antibodies	201513
Human Cell Functional State IMC Panel, 5 Antibodies	201514
Human Basic Tissue Architecture IMC Panel, 3 Antibodies	201517
Human Basic Immune IMC Panel, 4 Antibodies	201518
Maxpar Neuro Phenotyping IMC Panel Kit	201337
Maxpar IMC Cell Segmentation Kit	201500
Cell-ID Intercalator-Ir – 125 µM	201192A

Appendix

Core panel

Panel or Product Name	Marker	Clone	Metal	Concentration (µg/mL)		Part Number
				Tissue Mode – Working	Preview Mode and Cell Mode – Working	
Human Basic Tissue Architecture IMC Panel	Collagen 1	Polyclonal	89Y	1	5	201517
	Fibronectin	EPR23110-46	171Yb	0.2	1	
	CD31	EPR3094	151Eu	1	5	
Human Stromal Cell IMC Panel	FAP	E1V9V	161Dy	0.5	2.5	201511
	Podoplanin	D2-40	164Dy	0.5	2.5	
	αSMA	1A4	209Bi	0.125	0.5	
	CD44	IM7	153Eu	1	5	
Human Basic Immune IMC Panel	CD45	D9M8I	152Sm	0.25	2.5	201518
	CD3ε	D7A6E	170Er	1	5	
	CD20	H1	115In	1	5	
	CD68	KP1	159Tb	0.25	1	
Human Lymphoid IMC Panel	CD4	EPR6855	156Gd	1	5	201512
	CD8a	C8/1448	162Dy	0.5	2.5	
	CD45RO	UCHL1	173Yb	0.2	1	
	CD57	NK/804	163Dy	0.5	2.5	
Human Myeloid IMC Panel	CD66b	BLR111H	160Gd	0.25	1.25	201513
	HLA-DR	LN3	174Yb	0.2	1	
	CD163	EDHu-1	147Sm	1	5	
	CD14	EPR3653	175Lu	0.5	2.5	
	CD11b	EPR1344	144Nd	0.25	1.25	
	CD11c	D3V1E	154Sm	0.5	2.5	
Human Cell Functional State IMC Panel	Granzyme B	EPR20129-217	176Yb	0.125	1	201514
	PD-L1	73-10	166Er	5	10	
	PD-1	D4W2J	165Ho	5	10	
	FoxP3	PCH101	155Gd	5	10	
	Ki-67	B56	150Nd	0.5	2.5	
Maxpar Neuro Phenotyping IMC Panel Kit	GFAP	GA5	143Nd	0.125	0.25	201337
	NeuN	EPR12763	145Nd	2.5	5	
	S100β	EP1576Y	146Nd	0.125	0.25	
	Olig2	EPR2673	168Er	2.5	5	
	Iba1	EPR16588	142Nd	0.63	1.25	
	CD34	EP373Y	167Er	2.5	5.0	
	MAP2	EPR19691	148Nd	0.25	0.42	
Cell-ID Intercalator-Ir	DNA1		191Ir			201192A
	DNA2		193Ir			
Maxpar IMC Cell Segmentation Kit	ICSK1		195Pt		2.5	201500
	ICSK2		196Pt		2.5	
	ICSK3		198Pt		5	

Choice of 1 neurodegenerative panel

				Concentration (µg/mL)		
IMC Panel	Marker	Clone	Metal	Tissue Mode	Cell Mode	Part Number
Parkinson's Disease IMC Panel (9100006)	αSyn	E4U2F	141Pr	1.67	3.33	91H052141
	p-αSyn [S129]	EP1536Y	169Tm	1.25	2.5	91H053169
	TH	E2L6M	172Yb	1.25	2.5	91H054172
Alzheimer's Disease IMC Panel (9100007)	APP	4G8	141Pr	1.25	2.5	91H055141
	Tau	D1M9X	149Sm	0.25	0.63	91H056149
	pTau [S202/T205]	AT8	172Yb	0.31	0.63	91H057172
Multiple Sclerosis IMC Panel (9100008)	NF	SMI 312	141Pr	2.5	5.0	91H060141
	MBP	MBP101	149Sm	0.083	0.167	91H058149
	MOG	EP4281	158Gd	0.83	1.25	91H059158

Additional single antibodies used

				Concentration (µg/mL)		
IMC Product	Marker	Clone	Metal	Tissue Mode	Cell Mode	Part Number
Single antibodies for Alzheimer's Disease IMC Panel	αSyn	E4U2F	158Gd	0.63	1.25	91H052158
	p-αSyn [S129]	EP1536Y	169Tm	1.25	2.5	91H053169

Table 1. Antibodies used in this application note

Ordering Information

Standard BioTools Products		Part Number
Cell-ID Intercalator-Ir – 500 µM		201192B
Human Immune Cell Expansion IMC Panel, 7 Antibodies		201516
Human Immuno-Oncology IMC Panel, 31 Antibodies		201509
Maxpar IMC Cell Segmentation Kit		201500
Maxpar PBS		201058
Maxpar Water		201069
Reagents from Other Suppliers		**Part Number**
Commercial Alcohols	Ethyl Alcohol Anhydrous, USP	P006EAAN
Agilent	Dako Target Retrieval Solution, pH 9 (x10)	S236784-2
Sigma-Aldrich	Bovine Serum Albumin solution, 10% in DPBS	A1595
	m-Xylene ReagentPlus, 99%	185566-1L
Thermo Fisher Scientific	Triton X-100 Surfact-Amps Detergent Solution	85111

References

1. Van Schependom, J. and D'haeseleer, M. "Advances in neurodegenerative diseases." *Journal of Clinical Medicine* 12 (2023): 1,709.
2. Stringer, C. et al. "Cellpose: a generalist algorithm for cellular segmentation." *Nature Methods* 18 (2021): 100–106.
3. Nelson, P.T. et al. "Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship." *Journal of Neuropathology & Experimental Neurology* 68 (2009): 1–14.
4. Ashraf, G.M. et al. "Protein misfolding and aggregation in Alzheimer's disease and type 2 diabetes mellitus." *CNS & Neurological Disorders – Drug Targets* 13 (2014): 1,280–1,293.
5. Rodríguez, J.J. et al. "Astrocyte S100β expression and selective differentiation to GFAP and GS in the entorhinal cortex during ageing in the 3xTg-Alzheimer's disease mouse model." *Acta Histochemica* 126 (2024): 152131.
6. Monterey, M.D. et al. "The many faces of astrocytes in Alzheimer's disease." *Frontiers in Neurology* 12 (2021): 619626.
7. Cirillo, C. et al. "S100B inhibitor pentamidine attenuates reactive gliosis and reduces neuronal loss in a mouse model of Alzheimer's disease." *BioMed Research International* (2015): 508342.
8. Pomilio, C. et al. "Microglial autophagy is impaired by prolonged exposure to β-amyloid peptides: evidence from experimental models and Alzheimer's disease patients." *GeroScience* 42 (2020): 613–632.
9. Mandrekar-Colucci, S. and Landreth, G.E. "Microglia and inflammation in Alzheimer's disease." *CNS & Neurological Disorders – Drug Targets* 9 (2010): 156–167.
10. Moussaud, S. et al. "Alpha-synuclein and tau: teammates in neurodegeneration?" *Molecular Neurodegeneration* 9 (2014): 43.

Learn more at **standardbio.com**

Unleashing tools to accelerate breakthroughs in human health™

CORPORATE HEADQUARTERS
2 Tower Place, Suite 2000
South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US and Canada
Fax: 650 871 7152
standardbio.com

SALES
North America | +1 650 266 6000 | info-us@standardbio.com
Europe/Middle East/Africa/Russia | +33 1 60 92 42 40 | info-europe@standardbio.com
Latin America | +1 650 266 6000 | info-latinamerica@standardbio.com
Japan | +81 3 3662 2150 | info-japan@standardbio.com
China (excluding Hong Kong/Macau) | +86 21 3255 8368 | info-china@standardbio.com
All other Asia-Pacific countries/India/Australia | +1 650 266 6000 | info-asia@standardbio.com

FLDM-01349 Rev 2 062025
Exploring Neurodegenerative Diseases with Imaging Mass Cytometry App Note