Highest plex spatial multiomics as a discovery tool for senescence cell biology in Alzheimer's disease

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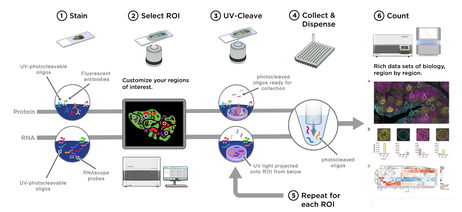
Abstract

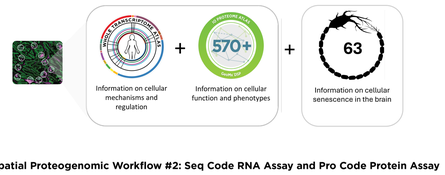
- Intraneuronal deposition of aberrant tau protein into neurofibrillary tangles (NFTs) closely correlates with neurodegeneration and cognitive decline. Pathogenic tau induces cell death in some neurons while other neurons evade apoptosis by entering cellular senescence, a complex stress response leading to a change in cell fate[1, 2]. We refer to senescent neurons as "neurescent" and are working to understand the role of tau in driving this cell fate.
- Removing senescent cells improves brain structure and function underscoring the importance of exploring senescent cells, their consequences across the brain and potential molecular targets for interventions[1-4].
- The advancement of spatially resolved, multiplex proteomic and transcriptomic technologies has revolutionized and redefined the approaches to complex biological questions pertaining to tissue heterogeneity, microenvironments, cellular interactions, cellular diversity, and therapeutic response in neurodegenerative diseases involving tau[5,6].
- Spatial transcriptomics has traditionally led the way in plex, multiple studies have demonstrated a poor correlation between RNA expression and protein abundance, owing to transcriptional and translational regulation, target turnover, and most critically, post-translational protein modifications. Therefore, a more holistic, ultra-high-plex, and high-throughput proteomic atlas approach becomes critical for the next phase of discovery biology.
- A Digital Spatial Profiler platform is uniquely suited to support high-plex proteomics, allowing for the simultaneous analyses of proteins from discrete regions of interest (ROIs) in FFPE tissue sections while preserving spatial context.
- Here, we use a barrier-breaking, highest plex spatial multiomics solution to explore the connection between tau neuropathology and senescence in human Alzheimer's disease. We also profiled human brains from patients with amyotrophic lateral sclerosis (ALS) and healthy controls to help identify disease-specific profiles.

GeoMx Digital Spatial Profiler

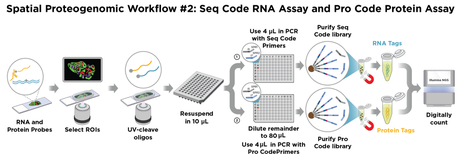


GeoMx Immuno Oncology Proteome Atlas is fully compatible with existing and well validated GeoMx Digital Spatial Profiler (GeoMx DSP) workflows. The GeoMx DSP utilizes antibodies coupled to photocleavable DNA barcodes readout with NGS sequencing, allowing for theoretically unlimited plex.

Highest Plex Spatial Multiomics



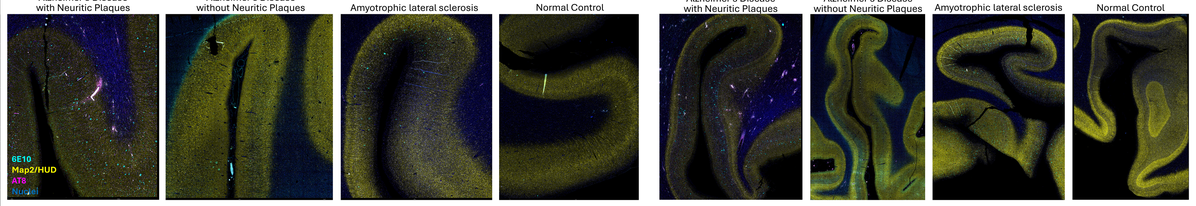
Spatial Proteogenomic Workflow #2: Seq Code RNA Assay and Pro Code Protein Assay



Here we introduce the Human Immuno-Oncology Proteome Atlas (IPA), a 570+ antibody-based proteomic discovery panel, compatible with immunohistochemistry on FFPE tissues with NGS readout, coupled with a 63 plex neuropathology custom antibody panel and a whole transcriptome RNA panel (WTA).

Profiling Alzheimer's disease pathologies across the primary motor cortex and visual cortex

Sample	AD without Neuritic Plaques	AD with Neuritic Plaques	ALS	Control
Sex	M	M	F	F
Age at Death	73	80	72	56
PMI	7.4	8.3	9.8	6.3
Cognitive Status	Dementia	Dementia	No dementia	No dementia
Overall AD neuropathological Change	Low	High	Low	Not
BRAAK	1	6	1	1
CERAD	0	3	0	0
Thal	1	5	3	0
Overall CAA Score	0	0	0	0
Highest Lewy Body Disease	Neocortical (Diffuse)	Not identified	Not identified	Not identified



Ultra-high-plex multiomics reveals distinct microenvironments immediately surrounding plaques specific to disease and brain region

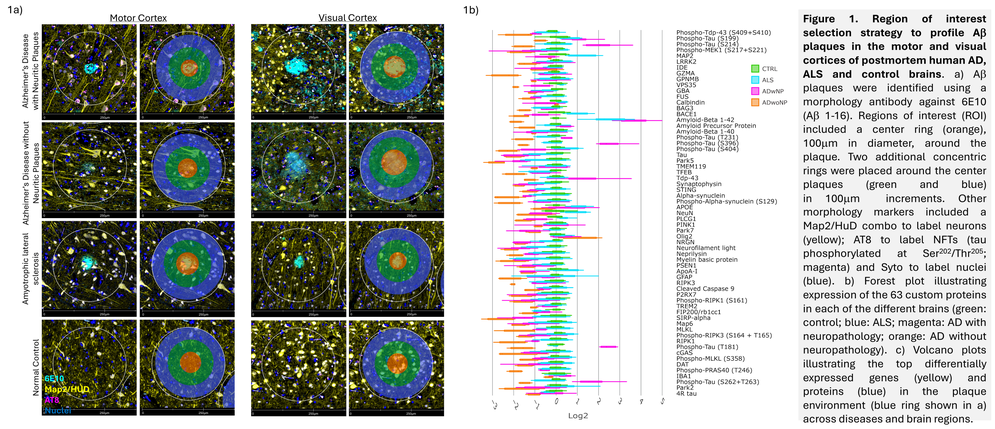
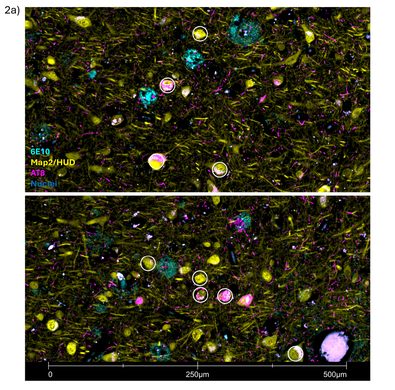
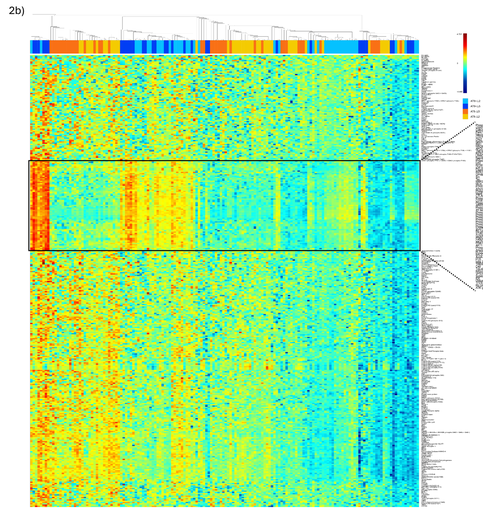


Figure 1. Region of interest selection strategy to profile Aβ plaques in the motor and visual cortices of postmortem human AD, ALS and control brains. a) Aβ plaques were identified using a morphology antibody against 6E10 (Aβ 1-16). Regions of interest (ROI) included a center ring (orange), 100µm in diameter, around the plaque. Two additional concentric rings were placed around the center plaques (green and blue) in 100µm increments. Other morphology markers included a Map2/HuD combo to label neurons (yellow); AT8 to label NFTs (tau phosphorylated at Ser^{202}/Thr^{205}; magenta) and Syto to label nuclei (blue). b) Forest plot illustrating expression of the 63 custom proteins in each of the different brains (green: control; blue: ALS; magenta: AD with neuropathology; orange: AD without neuropathology). c) Volcano plots illustrating the top differentially expressed genes (yellow) and proteins (blue) in the plaque environment (blue ring shown in a) across diseases and brain regions.

Single cell ultra-high-plex proteomics demonstrates distinct tau tangle profiles within cortical layers in the primary motor cortex of human Alzheimer's disease





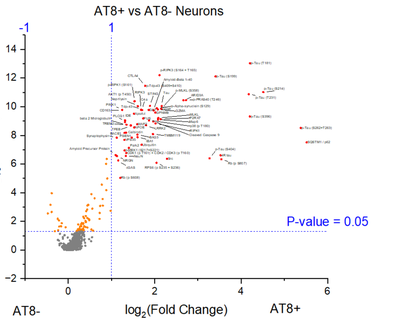


Figure 2. Differential protein expression between neurons with and without neurofibrillary tangles (NFTs) in the primary motor cortex of AD with neuritic plaques. a) Region of interest (ROI) selection strategy included 25µm circles around neurons positive for AT8 (NFTs, pTau(Ser^{202}/Thr^{205}; magenta) and neighboring neurons without phospho-tau (Map2/Hud only, yellow); n=192 neurons. b) Heatmap illustrating expression levels of all 643 proteins (rows) in each of the 192 ROIs (columns). c) Volcano plot illustrating the differentially expressed proteins between neurons with (AT8+) and without (AT8-) neurofibrillary tangles.

Orr Custom 63-Plex

Phospho-Tdp-43 (S409+S410)	RIPK1	GZMA	Phospho-RIPK3 (S164 + T165)
DAT	cGAS	IDE	Park7
4R tau	Phospho-Tau (S262+T263)	LRRK2	PINK1
Phospho-Tau (T181)	IBA1	MAP2	PLCG1
Phospho-MLKL (S358)	Phospho-PRAS40 (T246)	Phospho-MEK1 (S217+S221)	PSEN1
Map6	ApoA-I	Phospho-Tau (S214)	NeuN
FIP200/rb1cc1	APOE	Phospho-Tau (S199)	Phospho-Alpha-synuclein (S129)
P2RX7	Amyloid-Beta 1-40	Phospho-Tau (T231)	Alpha-synuclein
Cleaved Caspase 9	Amyloid Precursor Protein	Phospho-Tau (S396)	STING
Park2	Amyloid-Beta 1-42	Phospho-Tau (S404)	Synaptophysin
RIPK3	BACE1	Tau	Tdp-43
Phospho-RIPK1 (S161)	BAG3	Myelin basic protein	TFEB
TREM2	Calbindin	Neprilysin	TMEM119
SIRP-alpha	FUS	Neurofilament light	Park5
MLKL	GFAP	NRGN	VPS35
GBA	GPNMB	Olig2	

Summary

GeoMx DSP Platform Capabilities:

- Single neuron resolution of multiple analytes across entire FFPE tissue sections.
- Spatially-resolved multiomic data including whole transcriptome + 643-plex proteins with or without post-translational modifications.

Preliminary Findings and On-Going Analyses:

- Neuropathologies (NFTs and Aβ plaques) display molecular signatures that differ across diseases (AD with or without neuritic plaques, ALS and control), between cortical regions, and between cortical layers within the same region).
- Whole transcriptome analyses reveals spatially resolved, co-localized gene modules in the plaque environment that differ across diseases and brain regions.
- Evaluating RNA and protein expression in single neurons and across pathologies suggests low concordance; analyses are ongoing.
- Combining IPA with the neuro panel is providing insights into neurescence at an unprecedented molecular detail.

References

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Funding (to MEO): Department of Veterans Affairs I01BX005717, NIA R01AG068293, Cure Alzheimer's Fund, AFAR/Hevolution, Rainwater Charitable Foundation).