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Integrated Spatial Proteomic Profiling with Same-Slide Orthogonal Validation Reveals Distinct Proteomic Signatures in Alzheimer's Disease

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Introduction

Beta amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) are hallmark pathological features of Alzheimer's disease (AD) and other neurodegenerative conditions. Even though these features have been reported 100+ years ago, they remain poorly understood. We developed a same-slide, multiplatform, multi-omic spatial profiling pipeline that enables comprehensive orthogonal study of transcriptomic and proteomic modalities in human FFPE brain sections. Using the GeoMx® Digital Spatial Profiler (DSP), we performed simultaneous whole-transcriptome and 643-plex protein profiling on FFPE human brain tissue to analyze molecular signatures in pathological hallmarks. We next profiled the same slides using CellScape™, a multiplex immunofluorescence platform with single-cell resolution and high dynamic range microscopy. CellScape data confirmed DSP-derived protein targets, resolved subcellular localizations, and identified features underrepresented in transcriptomic data, such as extracellular proteins and acellular plaque-core components. Our findings demonstrate the value of combining high-plex spatial profiling with same-slide single-cell validation and provide a generalizable framework for multi-omic analysis of complex human neuropathology.

Comprehensive Spatial Proteomics of Neurodegenerative Disease

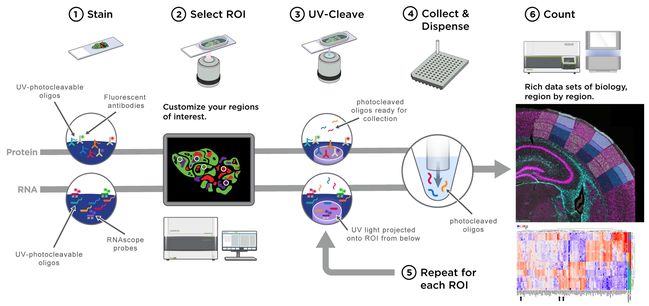

Figure 1. The GeoMx DSP uses antibodies coupled to photocleavable DNA barcodes that are read out with NGS sequencing, allowing for theoretically unlimited plex. Readouts can be attained with single-cell resolution.

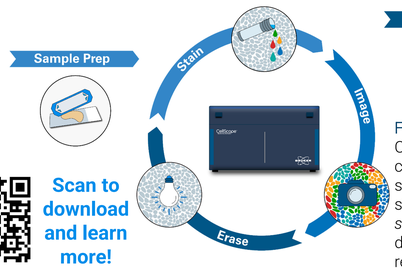

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Figure 2. The CellScape/EpicIF workflow consists of cycles of staining, imaging, and gentle signal removal to enable *in-situ* multiplexed biomarker detection at single-cell resolution.

Profiling AD pathology in the primary motor & visual cortex

Sample	AD with Neuritic Plaques	Control
Sex	M	F
Age at Death	80	56
PMI	8.3	6.3
Cognitive Status	Dementia	No dementia
Overall AD neuropathological Change	High	Not
BRAAK	6	1
CERAD	3	0
Thal	5	0
Overall CAA Score	0	0
Highest Lewy Body Disease	Not Identified	Not Identified

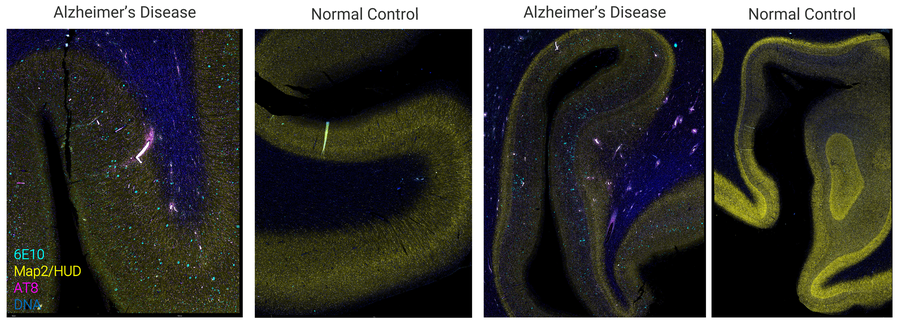
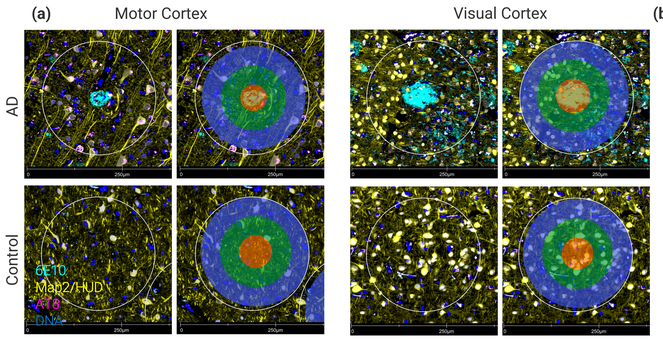
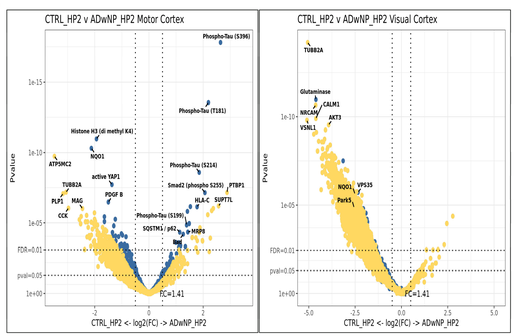


Figure 3. Sample information and region of interest selection strategy to profile Ab plaques in postmortem human brains. (a) Aβ plaques were identified using a 6E10 antibody (Aβ 1-16). Regions of interest (ROI) included a 100μm center ring (orange). Two additional concentric rings were placed around the center plaques (green and blue) in 100μm increments. A Map2/HuD combo labeled neurons (yellow); AT8 labeled NFTs (tau phosphorylated at Ser^{202}/Thr^{205}; magenta) and Syto to label nuclei (blue). (b) Volcano plots showing the top differentially expressed genes (yellow) and proteins (blue) in the plaque environment.





Same-section validation of GeoMx data on CellScape

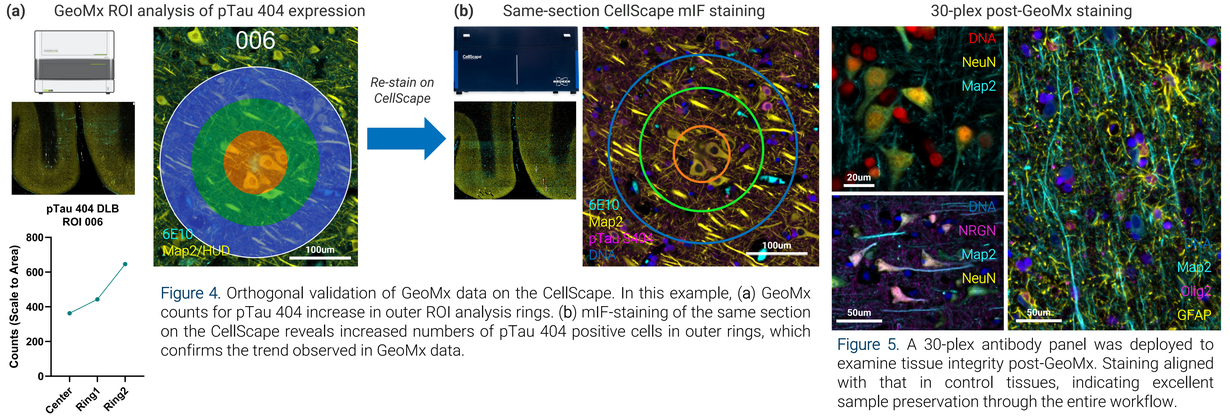


Figure 4. Orthogonal validation of GeoMx data on the CellScape. In this example, (a) GeoMx counts for pTau 404 increase in outer ROI analysis rings. (b) mIF-staining of the same section on the CellScape reveals increased numbers of pTau 404 positive cells in outer rings, which confirms the trend observed in GeoMx data.

Figure 5. A 30-plex antibody panel was deployed to examine tissue integrity post-GeoMx. Staining aligned with that in control tissues, indicating excellent sample preservation through the entire workflow.

Comprehensive Spatial Proteomics from CellScape to GeoMx

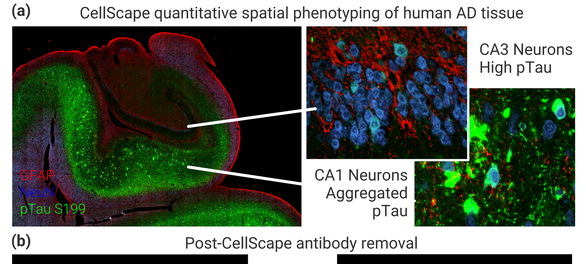


Figure 6. (a) CellScape quantitative spatial phenotyping reveals a large range of pTAU expression in human AD brain tissue. To further evaluate proteomic signatures of differing pTAU expression, we first removed CellScape antibodies by performing two rounds of antigen retrieval with a proprietary buffer (b) and then interrogated the same tissue on GeoMX (see Fig. 7).

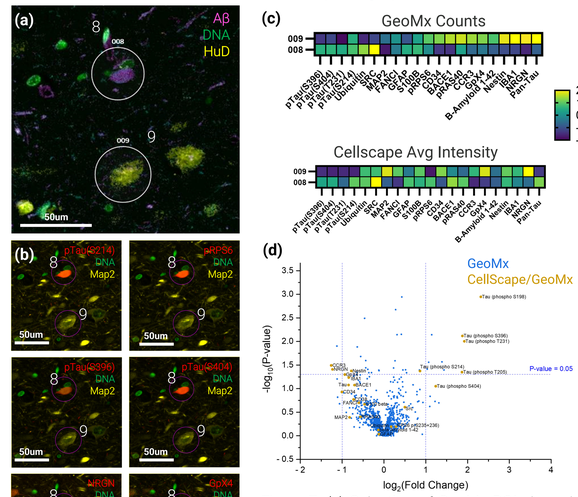


Figure 7. (a) Selection of GeoMx ROIs based on CellScape data (b). As shown, cells differ in their degree of pTau staining & the combinatorial staining with antibodies. (c) Heatmaps indicate good concordance between CellScape intensity measurements and read counts from the post-CellScape GeoMx assay; they are also consistent with IF data in (b). (d) Differential expression analysis was then performed to gain a deeper understanding of the proteomic signatures in select cells, demonstrating that it is possible to use this workflow to achieve deep-single cell proteomic profiling.

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