#8042 A complete pipeline for high-plex spatial proteomic profiling and analysis of neural cell phenotypes on a Spatial Molecular Imager and a Spatial Informatics Platform.

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Abstract

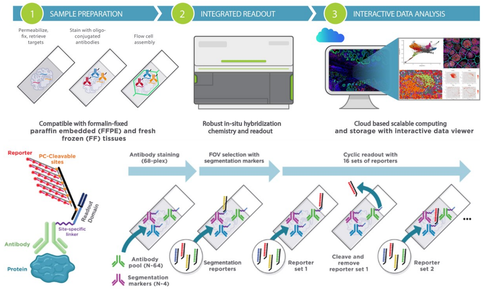
The brain is complex and heterogeneous where cell function and cell-to-cell communication are critical for rapid and accurate performance. The ability to explore protein-driven activities at high resolution within the spatial context of their immediate environment is critical to gain comprehensive pictures of brain development, activity, aging, disease or dysfunction, and inflammatory responses. Many existing approaches for high-plex single-cell spatial proteomics face issues around simplicity, speed, scalability, and big data analysis.

Here, we present an integrated workflow that addresses key concerns around high-plex proteomics. The CosMx™ Spatial Molecular Imager (SMI) and AtoMx™ Spatial Informatics Platform comprise an end-to-end workflow that efficiently handles highly multiplex protein analysis at plex sizes exceeding 68 targets. The CosMx protein assays use oligonucleotide-conjugated antibodies, that are detected using universal, multi-analyte CosMx readout reagents. The CosMx Mouse Neural Cell Typing and Alzheimer's Pathology panel is optimized to comprehensively profile neural cell lineages across the brain as well as the progression of Alzheimer's disease (AD). Furthermore, 80% of the antibodies making up both panels are cross-reactive with human tissue antigens. The AtoMx spatial informatics platform provides full analysis support, including whole-slide image viewer, and methods for performing built-in or fully customizable analyses for cell typing, ligand-receptor analysis, neighborhood analysis and spatial differential expression.

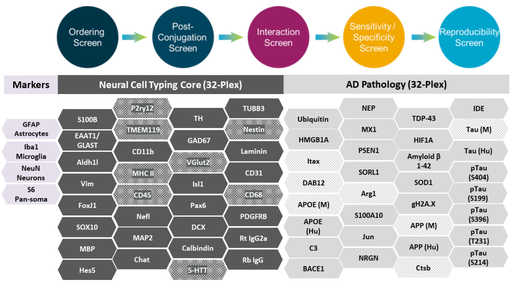
The CosMx protein assay reagents were validated on the FFPE adult mouse brain, mouse embryo, and Alzheimer's positive human brain. We used the CosMx Mouse Neural Cell Typing and Alzheimer's Pathology panel with the CosMx SMI to identify multiple neuronal subtypes, different reactive states of astrocytes and microglia, cell degeneration and proliferation. In a single-cell exploration of mitochondria, we noted distinct patterning of key immune targets based on their immediate microenvironment. Additionally, evaluated the co-expression patterns and activation states of microglia within 200 µm from the amyloid plaque and tau tangles.

The CosMx SMI is a high-plex spatial multi-omics platform that enables the detection of > 68 proteins at subcellular resolution. In combination with the high-plex CosMx Mouse Neural Cell Typing and Alzheimer's Pathology panel, we present a flexible and scalable informatics platform, a robust solution for comprehensive neural and disease phenotyping that captures the complexity of neuronal and glial cellular activity with full spatial context.

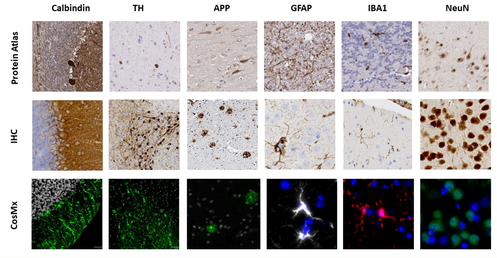
CosMx Spatial Molecular Imager (SMI) and AtoMx Spatial Informatics Platform



CosMx Mouse Neuroscience Protein Panel (64-Plex) Content



Specificity

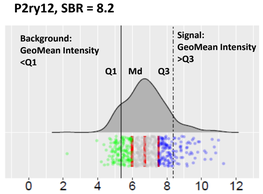


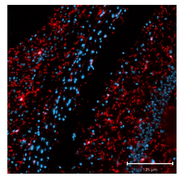
CosMx Mouse Neural Panel antibodies demonstrate comparable IHC and CosMx SMI staining patterns consistent with specific antigen targets

Sensitivity

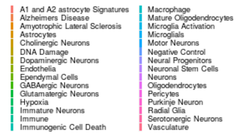
CosMx Mouse Neural Panel antibodies demonstrate on signal-to-background ratio (SBR) ≥ 2.5 on mouse or human FFPE tissue samples.

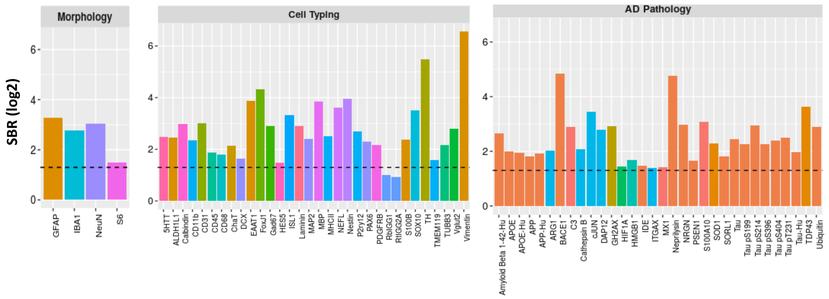
P2ry12, SBR = 8.2





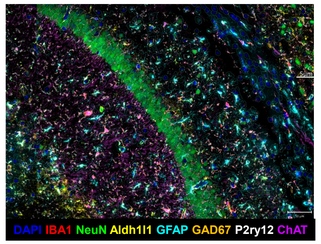
$$SBR = \frac{GeoMean\ Intensity > Q3}{GeoMean\ Intensity < Q1}$$

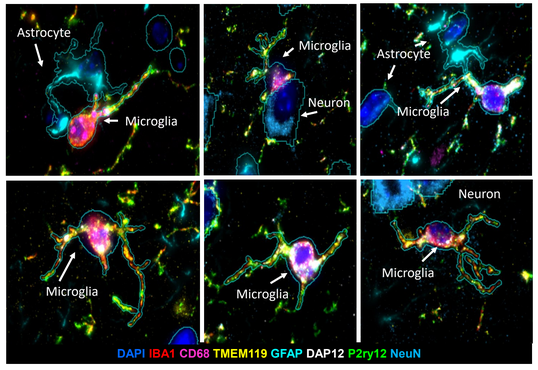




Advanced Segmentation Critical for Neural Single Cell Spatial Proteomics

Specifically identify multiple neural cell types in mouse hippocampus and capture distinct morphologies





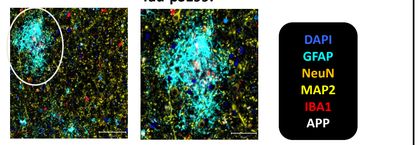
Conclusion

- End-to-end workflow from sample preparation to data analysis
- Enables high-plex proteomics up to 68 proteins from a single FFPE slide with spatial context at single-cell resolution
- Protein assay uses validated antibodies conjugated with oligonucleotides, detected via microfluidic-based cyclical immunofluorescence imaging process

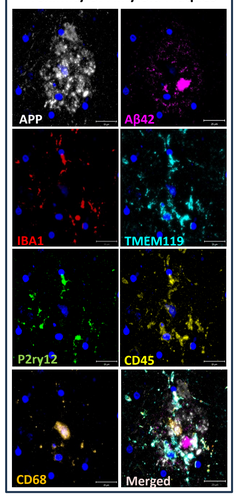
Neural Single Cell Spatial Proteomics on Human Alzheimer's Diseased Tissue

Evaluate the pathology of co-expression patterns of phosphorylated Taus and the activation states of microglia in proximity of amyloid βeta plaques

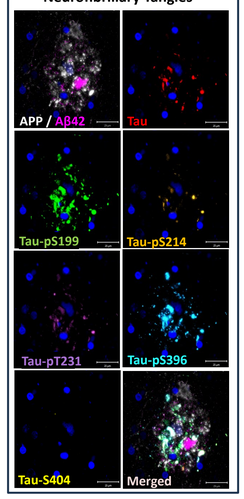
FOV 63: Postcentral gyrus enriched with activated microglia and Tau-pS199.



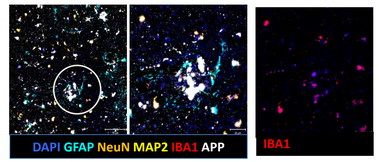
Activated Microglia in Proximity of Amyloid Plaques



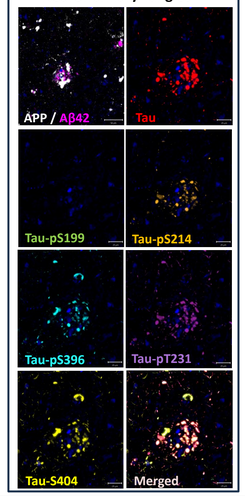
Amyloid Plaques and Neurofibrillary Tangles



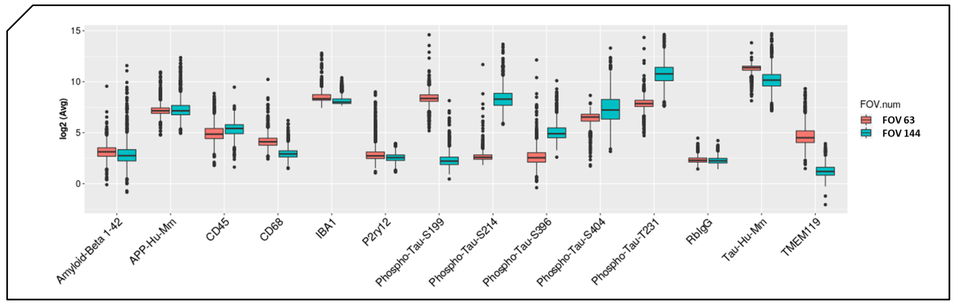
FOV 144: Frontal lobe enriched with ameboid microglia and Tau-pS404.



Amyloid Plaques and Neurofibrillary Tangles



Differential expression of microglial markers and phosphorylated Taus across an FOV-elaborate signal of individual cells across an FOV



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3. Uhlén M et al., 2015. Tissue-based map of the human proteome. Science.

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