Disentangling the relationship between pathology and cell-specific vulnerability using large format highly multiplexed in situ single-cell profiling of human brain

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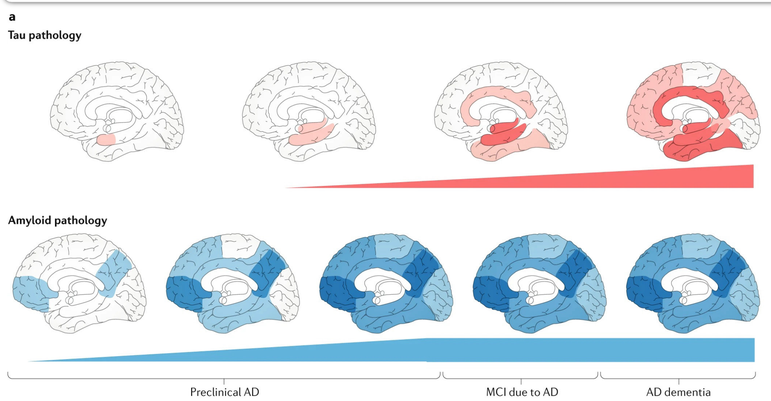
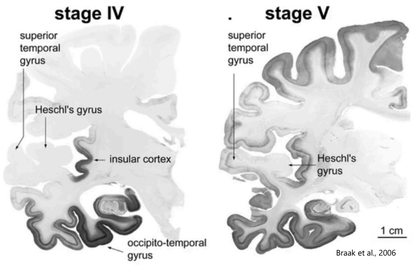
BACKGROUND





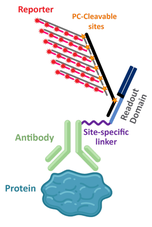
Braak et al., 2006

Transition zones in AD

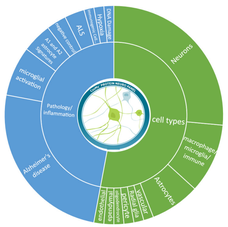
- Allocortical to neocortical progression
  - Entorhinal cortex
  - Hippocampus
  - Temporal cortex (inferior → middle → superior temporal gyri)
  - Frontal cortex

METHODS

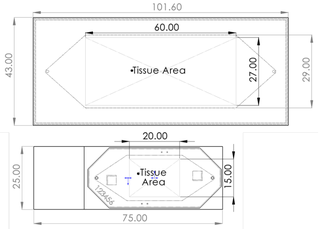
SMI antibody



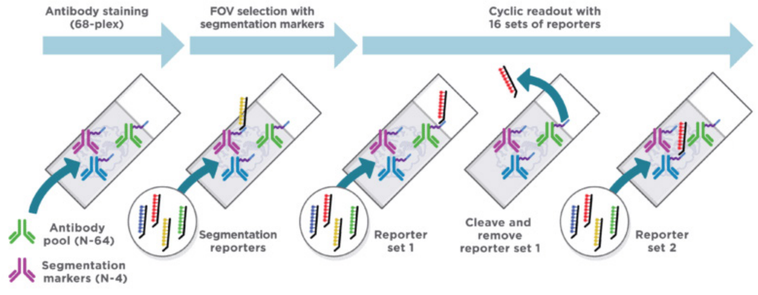
68-plex protein panel



Large format flow cell

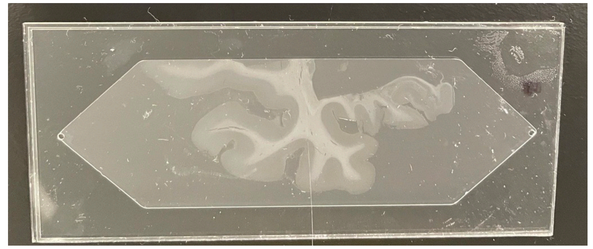


Sample preparation, flow cell assembly and target detection



LARGE SURFACE AREA FLOW CELL

The CosMx™ SMI Spatial platform efficiently handles highly multiplexed protein analysis on FFPE tissues. Here we demonstrate a novel Large Surface Area flow cell with > 1600 mm² of imaging area, a > 5-fold increase. Combining the Large Surface Area flow cell imaging capabilities with a human specific > 68 plex CosMx™ Neural Cell Profiling and Alzheimer's Pathology Protein Panel, we demonstrate spatial imaging of brain cell typing (GFAP, Iba1, NeuN), disease-specific targets (APP, Aβs, pTau), and key post translational modifications (pTau at multiple sites), tracking the spread of AD pathology across brain structures.



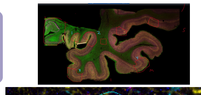
- Fluidic system on the CosMx™ prototype was modified to enable uniform reagent exchange through the flow cell with minimal use of expensive reagents
- The system was able to achieve uniform reagent exchange with a reagent volume of only 250µL, despite the enormous imaging area of the flow cell

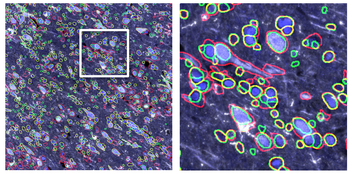
68-PLEX PROTEIN PANEL

Cell types and pathological peptides each have their own distinct roles in the pathophysiological progression of AD. Glia, such as astrocytes (GFAP) and microglia (IBA1), increase their abundance and aggregate in areas of high pathological peptide burden, whereas neurons (MAP2), particularly excitatory neurons, appear to decrease in relative abundance with increasing pathological stage. The NanoString CosMx high plex protein panel enables the assessment of cell subtypes as well as intracellular (such as pTau) and extracellular (such as Aβ) pathological peptides. Further, the panel captures a range of phosphorylation states for key AD proteins like pTau.

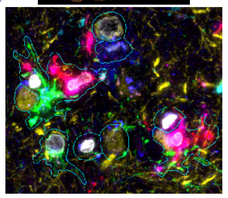
CELL TYPING AND SEGMENTATION

High-plex protein images → Spatial colocalization protein groups → Machine-learning cell segmentation → Combining outputs over multiple groups → Final cell segmentation

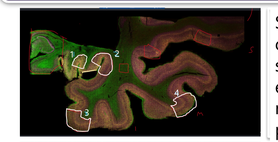




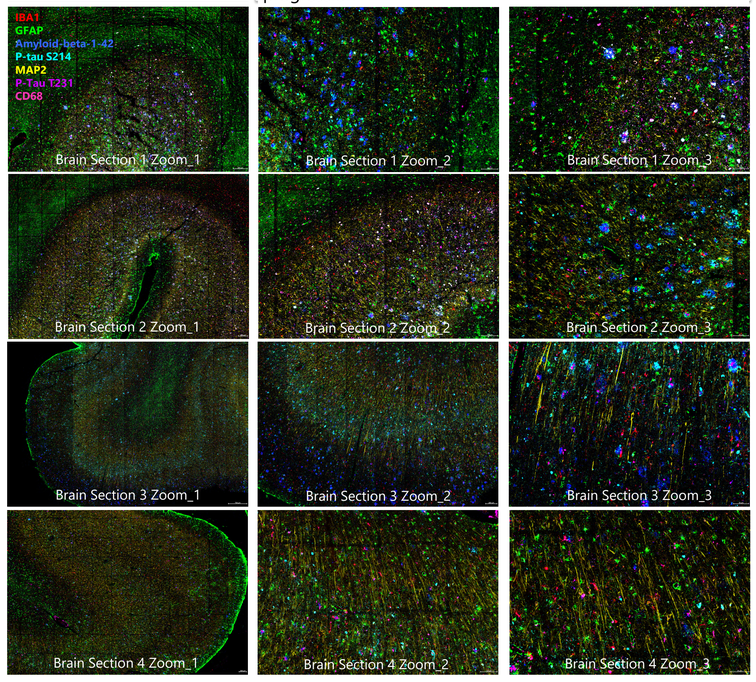
- Green outline - Previous segmentation pipeline
- Red outline - New pipeline using high-plex protein images
- Blue shading – DAPI
- Gray shading – Max projection of high-plex protein images



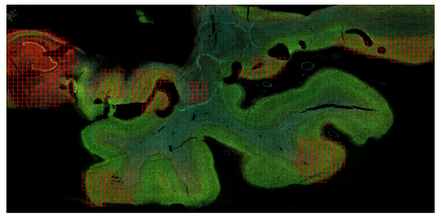
AD SPATIAL PROGRESSION



Several key brain regions were selected to capture the continuous progression of pathological peptides within a single brain donor. Regions 1 and 2 represent the regions earliest affected by tau pathology, while regions 3 and 4 represent cortical regions affected later in disease progression.



FUTURE DIRECTIONS



FOVs (highlighted in red) selected for profiling on the NanoString 6K RNA panel that is currently being run on the same tissue section.

Three core components are essential to disentangle the relationship between pathology and cell-specific vulnerability: spatial context, pathological protein distribution, and highly resolved cell type labels. This dataset provides a detailed yet large-scale protein landscape. Combining this dataset with NanoString's 6K RNA panel will unlock the full potential of the technology and complete this goal.

REFERENCES

[1] He, S., Bhatt, R,. Brown, C., et al. (2022). High-plex imaging of RNA and proteins at subcellular resolution in fixed tissue by spatial molecular imaging. Nature Biotechnology. 40(12):1794-1806. doi:10.1038/s41587-022-01483-z

[2] Stringer, C., Wang, T., Michaelos, M., & Pachitariu, M. (2021). Cellpose: a generalist algorithm for cellular segmentation. Nature Methods, 18(1), 100-106.