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# Seeing more of the brain with high-plex multi-omics imaging at single-cell spatial resolution in a large contiguous tissue section

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## Abstract

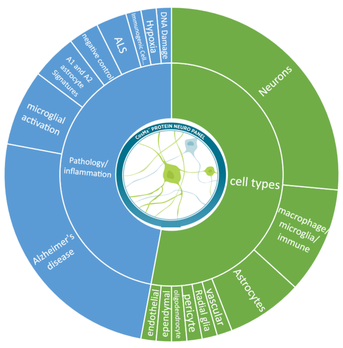
Neurophysiological and neurodegenerative processes occur over multiple spatial scales in the brain. While fine-scaled spatial molecular analyses of human brain function have vastly advanced in the last decade, these datasets often lack context of how microscopic features fit within the larger picture of macroscopic neuroanatomy. In the brain, critical cell functions and cell-to-cell communications take place over relatively large interstitial distances and far from the cell somas. The ability to explore protein and RNA-driven activities at high resolution, within the spatial context across a large environment and passing through multiple microenvironments, allows a comprehensive picture of brain biology to emerge and is applicable to open questions regarding brain development, activity, aging, and neurodegeneration.

Here we demonstrate a novel Larger Surface Area flow cell with > 1,600 mm2 of imageable area. Fluidic and imaging conditions of Spatial Molecular Imager (SMI) were optimized to deliver consistent performance. Combining the Larger Surface Area flow-cell imaging capabilities with the human-specific > 68-plex CosMx™ SMI Human Neuroscience protein panel (Neural Cell Profiling and Alzheimer's Pathology), we demonstrate spatial imaging of neural cell and neurodegenerative disease-specific targets (beta-amyloid and phospho-tau species), and key post-translational modifications. Furthermore, advanced neuronal segmentation algorithms allow for specific tracing and segmentation of single neurons, including axons, across hundreds of micrometers of space. Ultimately, the ability to image and evaluate 5-fold larger sections of human brain tissues with unparalleled spatial context and high-plex analyte contents allows the grander scale of disease anatomy and processes to be cataloged with exquisite precision and accuracy.

## Methods

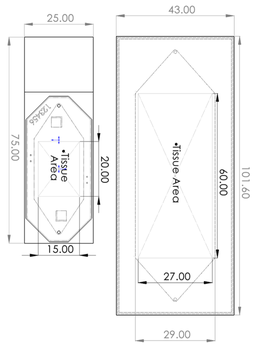
Taking advantage of oligonucleotide barcode-conjugated antibodies and large-format flow cell, the NanoString® CosMx™ Spatial Molecular Imager (SMI) platform allows high-plex profiling of protein expression in FFPE human brain section in spatial context, capturing the single-cell variance over large spatial dimension.

### CosMx 68-plex protein panel

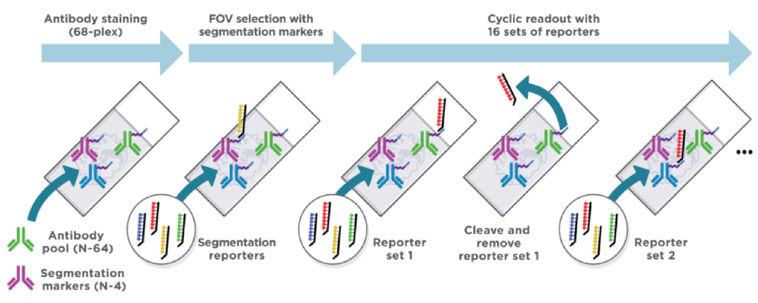



### Large format flow cell

- Imageable area > 1600 mm2, >5x increase from the imageable area of standard CosMx SMI flow cell.
- Fluidic system on the CosMx SMI 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



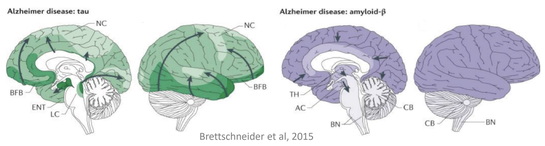

### Sample preparation, flow cell assembly and target detection




## High-plex Proteomics Profiles of Large Area Alzheimer's Disease Tissue

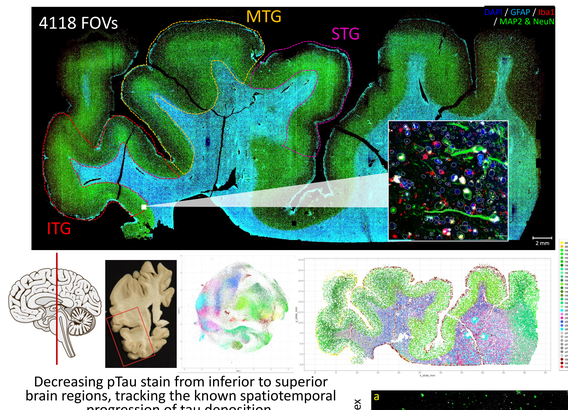
Alzheimer's disease (AD) is characterized by the spatiotemporal progressive deposition of pathologic peptides amyloid beta (Aβ) and hyper-phosphorylated tau (pTau). The burden of pTau, in the form of neurofibrillary degeneration, corresponds strongly with cognitive impairment and progresses generally from medial temporal lobe structures to neocortex. The middle temporal and superior temporal gyri (MTG and STG, respectively) represent a critical transition zone in disease progression, separating pTau accumulation associated with aging and pre-clinical AD from advanced stages of dementia-associated pTau pathology.

### *Spreading of pathology in AD*

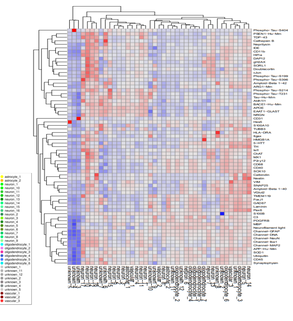



The novel Large Surface Area flow cell of the CosMx SMI prototype has > 1600 mm2 of imaging area, which is a > 5-fold increase of standard CosMx SMI flow cell, and thus allows the study of pTau pathology within spatial context of both immediate and far-ranging neuroanatomy. Combining the large-format flow cell with a 68-plex CosMx SMI Human Neuroscience 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.

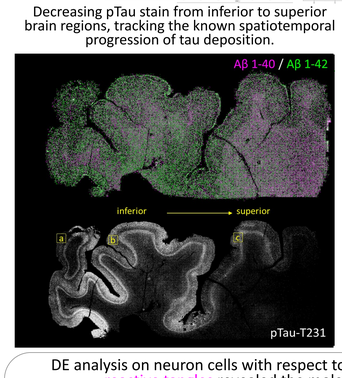
### *Brain section #1 from AD patient at Braak stage VI*

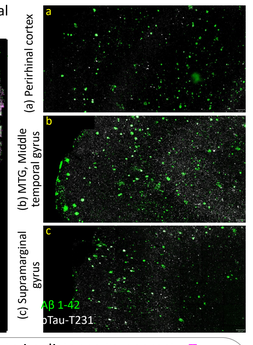



Unsupervised clustering of protein profiles identified 16 clusters within 5.15 million cells across ~1100 mm² scanned area.

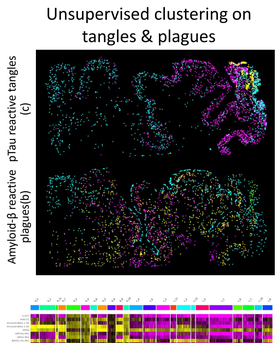


Decreasing pTau stain from inferior to superior brain regions, tracking the known spatiotemporal progression of tau deposition.

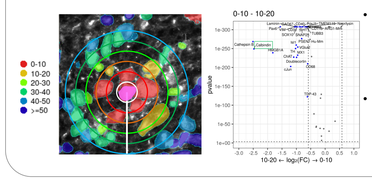


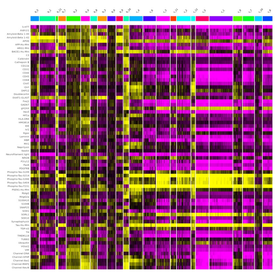

Unsupervised clustering on tangles & plaques




DE analysis on neuron cells with respect to its distance to nearest pTau-reactive tangles revealed the molecular response within microenvironment of neurofibrillary tangles.

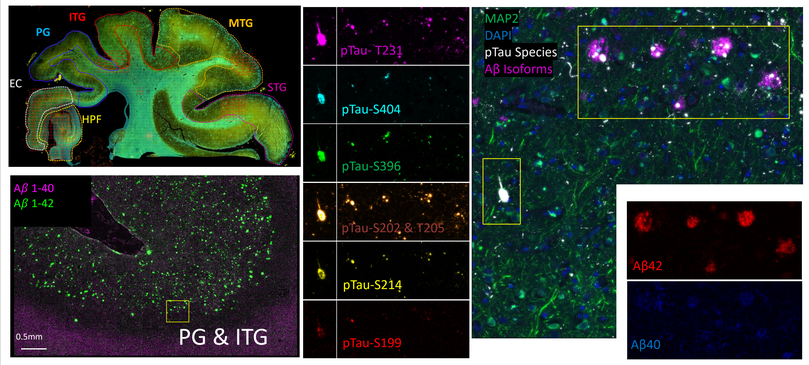


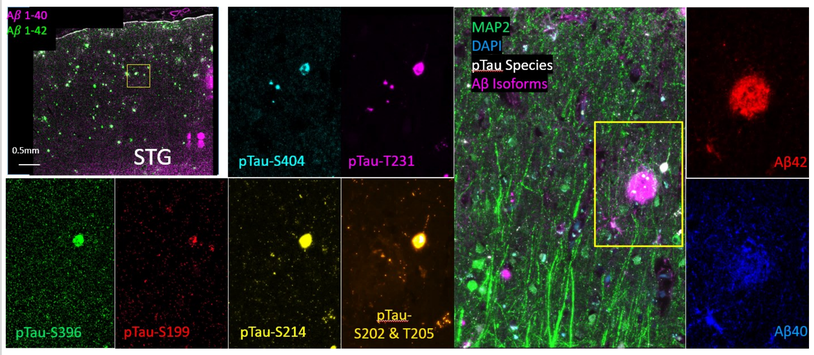

- Cortical neurons within 0-10 um distance to pTau+ tangles showed reduced expression of Calbindin protein compared to neurons within 10~20 um distance or further.
- Similar behavior has been reported for basal forebrain cholinergic neurons which lose expression of calbindin-$D_{28k}$ protein during tangle formation in Alzheimer's disease [6].



## Heterogeneity of plague & tangle composition across brain regions

### *Brain section #2 from AD patient at Braak stage V*




## Conclusions

The large format CoxMx SMI technology provides the unique opportunity to study vulnerable cells continuously across multiple brain structures to understand the earliest neurodegenerative changes in disease. Using this approach, we have begun to shed light on unanswered questions such as how pTau species, comorbid protein-autopathies (such as TDP-43), and immune cells differentially impact vulnerable cell types and change dynamically change with disease progression.

By applying the 68-plex protein assay to AD tissue we observed:

- pTau pattern that tracks known spatiotemporal progression of tau
- Intriguing heterogeneity in plaque composition
- Differential subcellular location of pTau species

Critically, by sampling larger brain areas spanning multiple disease-associated regions from a single donor, we can begin to uncover the cellular and protein-level underlying key transition points along each stage of disease progression.

## References

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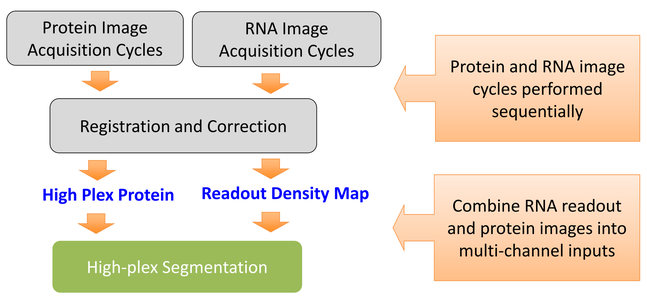
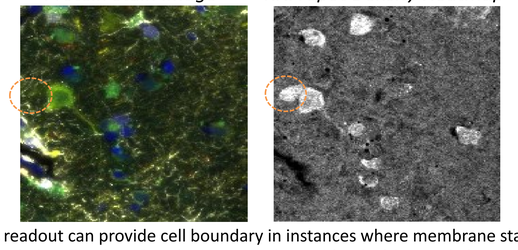
[6] Ahmadian SS, Rezvanian A, Peterson M, et al. Loss of calbindin-D28K is associated with the full range of tangle pathology within basal forebrain cholinergic neurons in Alzheimer's disease. *Neurobiol Aging*. 2015;36(12):3163-3170. doi:10.1016/j.neurobiolaging.2015.09.001

## Robust Cell Segmentation Pipeline using High-plex multi-omics Images

### (I) Preprocess Protein & RNA images for multi-omics assays.

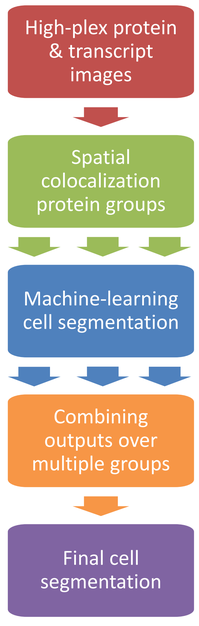



*Protein Stain image* *RNA Spot Density Heatmap*

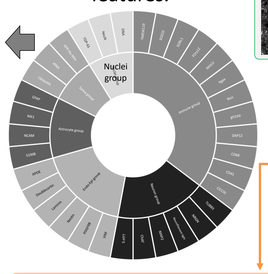


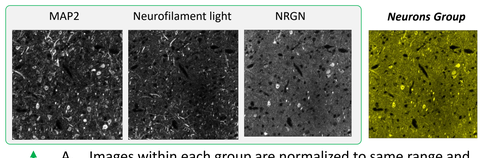
RNA readout can provide cell boundary in instances where membrane staining is missing. The Spot Density Heatmap is combined with protein images in Soma group for downstream segmentation.

### (II) Perform high-plex segmentation leveraging protein colocalization & ML models.

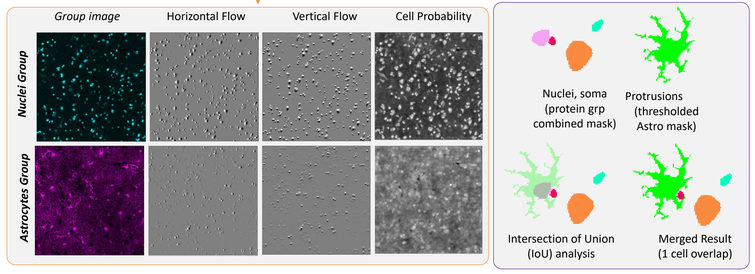



**Spatial colocalization groups** characterized by shared morphology features.



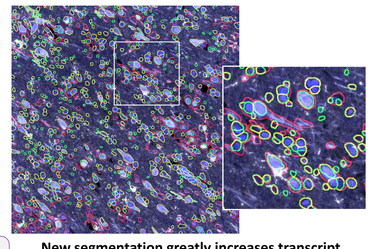




A. Images within each group are normalized to same range and then combined into one via maximum intensity projection.
B. Combined image of each group is fed into ML segmentation model (e.g. Cellpose).
C. The ML outputs (flows & cell probability) of each group are combined to create the combined flow & cell probability, which are then converted into cell masks using gradient tracking.
D. Protrusions are segmented using Moment auto thresholding and then combined with protein group cell masks using intersection of union analysis.

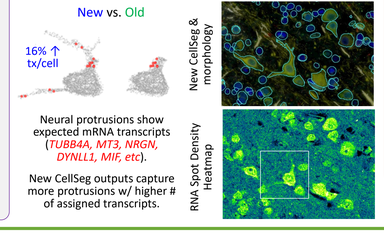



### Improved cell segmentation outcomes

- 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



**New segmentation greatly increases transcript assignment to single cells.**




Neural protrusions show expected mRNA transcripts (*TUBB4A, MT3, NRGN, DYNLL1, MIF, etc*).

New CellSeg outputs capture more protrusions w/ higher # of assigned transcripts.

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