Multiomic profiling of healthy and diseased brains with high-plex single-cell spatial molecular imaging

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Introduction

Single cell transcriptomics and proteomics can provide complementary information about the form and function of neurons and glia throughout the brain. However, most high-plex spatial analyses to date have primarily utilized one of these two modalities to interrogate cell activity and cell-to-cell communication. Here, we simultaneously leveraged the detection of 68 proteins and over 6,000 RNA targets on the same FFPE human brain sections to perform extended segmentation of neural processes and integrated analyses of protein and RNA expression. The protein targets are well-suited for dissecting neurodegenerative disease pathology (e.g. amyloid beta variants). Moreover, they cover major neural cell types and enable robust cell typing, alongside 4,900 neuroscience-related genes.

CosMx™ SMI Multiomics Workflow

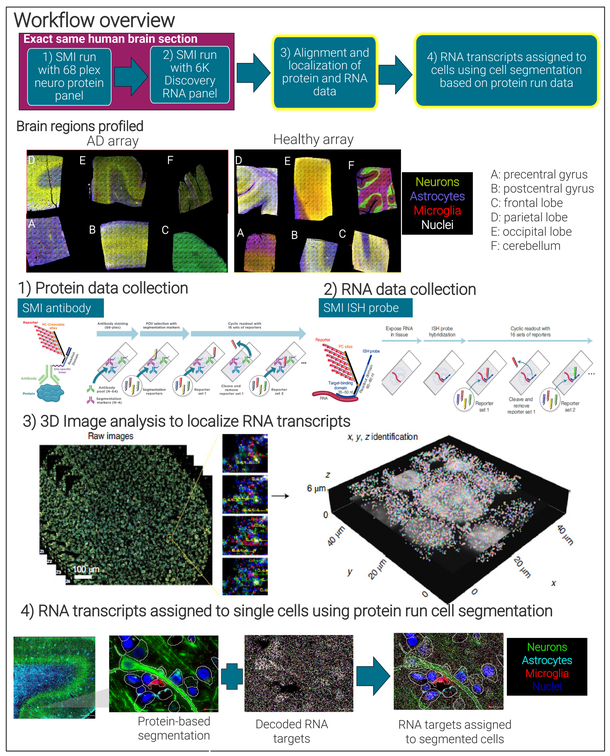

Fig 1. The SMI multiomics assay sequentially detects protein and RNA targets with oligonucleotide barcode-conjugated antibodies and barcoded RNA probes via several rounds of reporter binding and fluorescence imaging. Cells are segmented based on protein stains and decoded RNA targets are assigned to individual cells.

Protein augments segmentation

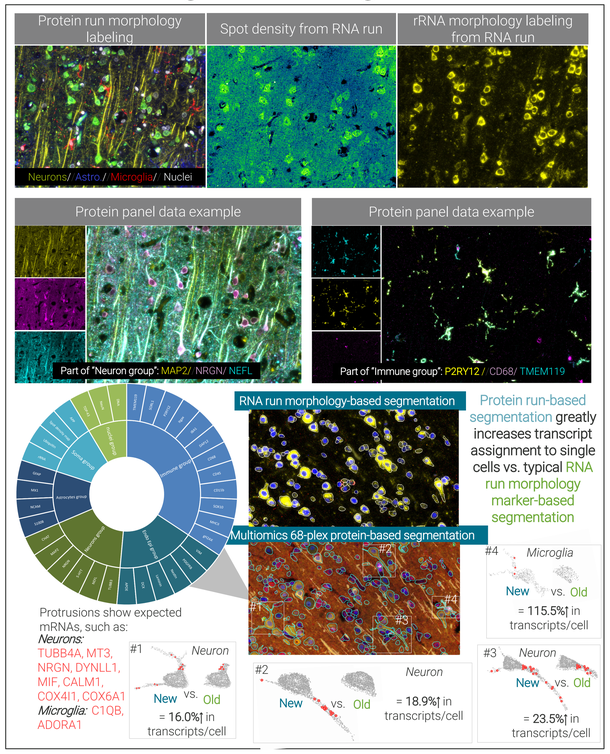

Fig 2. Example protein and RNA density images used for segmentation. Multiomics 68-plex protein-based segmentation captures neuronal and glial protrusions and associated transcripts.

RNA integrity is maintained through multiomics workflow

	Total cells		Total tissue area (mm2)		Cells per mm²	
	Healthy	AD	Healthy	AD	Healthy	AD
Cerebellum	21495	63385	5.1	10.1	4215	6276
Frontal lobe	11025	16836	4.7	14.1	2346	1194
Occipital lobe	22724	47195	8.2	20.9	2771	2258
Parietal lobe	16662	12713	6.2	7.1	2687	1791
Postcentral gyrus	7972	37300	4.1	18.6	1944	2005
Precentral gyrus	8580	26222	4.1	9.2	2093	2850

Data shown are post-QC

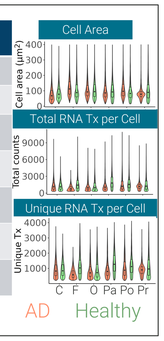

Fig 3. QC statistics about the six tissues processed through the SMI multiomics assay from a healthy and AD brain.

Protein and transcript detection may be concordant or discordant

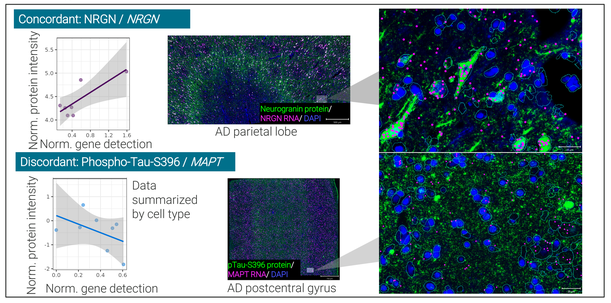

Fig 4. Levels of some, but not all, RNA transcripts are predictive of corresponding proteins within cell types. The neuronal protein NRGN correlates well with *NRGN* transcript detection whereas the *MAPT* transcript does not predict cellular levels of the secreted, phosphorylated protein pTau-S396.

Protein staining is leveraged for RNA cell typing

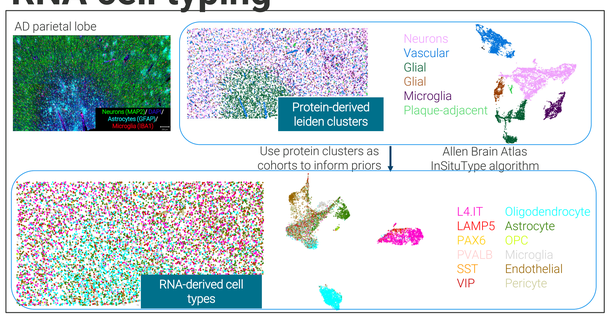

Fig 5. Cells were clustered using the Leiden algorithm based on protein intensity. For finer annotation, particularly of neuron types, RNA counts were used in the InSituType algorithm to define cell types, leveraging the protein clusters to inform model priors.

Proteins and transcripts define co-localized expression modules

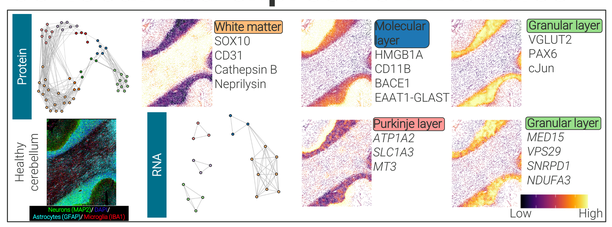

Fig 6. Modules of co-detected proteins (top) or transcripts (bottom) highlight brain structures with the package InSituCor.

Gene expression differs by protein-defined neighborhoods

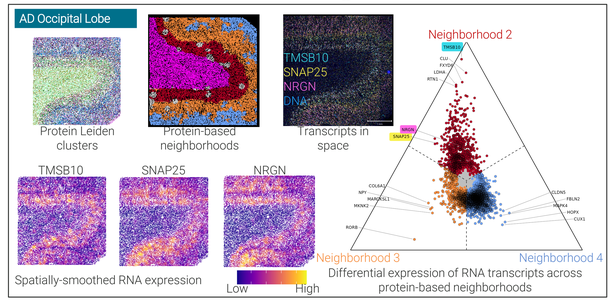

Fig 7. Protein defined neighborhoods of similar cellular composition highlight cortical layers of the occipital lobe as well as amyloid beta plaques (grey). Differential expression on neurons across the cortical layer neighborhoods identified 2035 spatially-regulated RNA transcripts.

References

Bakken *et al*. Nature (2021). [Allen Brain Atlas]
Danaher *et al*. BioRxiv (2022). [InSituType]
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Hao *et al*. Nat Biotech (2023). [Seurat v5]
Hodge *et al*. Nature (2019). [Allen Brain Atlas]
Vasconcelos *et al*. BioRxiv (2024). [smiDE]

Open source packages are highlighted in blue in citations above.

Conclusions

- Leveraging 68 neurobiology-related proteins enabled unparalleled cell segmentation
- Across 12 tissue sections of healthy and diseased brain a median of 1132 RNA transcripts per cell were detected after protein detection
- Protein and RNA were correlated for some, but not all, corresponding pairs.
- Over 6000 RNA transcripts contributed to cell typing informed by protein staining
- RNA and protein together identified hundreds of genes differentially expressed across cortical layers and differentially localizing modules of co-expressed proteins

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