#651 Ultra high-plex single-cell spatial multi-omic imaging of human brain sections

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Introduction

High-plex spatial imaging of the complex cell shapes in the brain is an unsolved problem. Brain cells often have intricate projections that can extend far from their cell body and escape segmentation by existing algorithms. As a result, roughly 50% of transcripts are left unassigned to cells when brain imaging. Thus, improving brain cell segmentation is essential for a better understanding of neural cell functions.
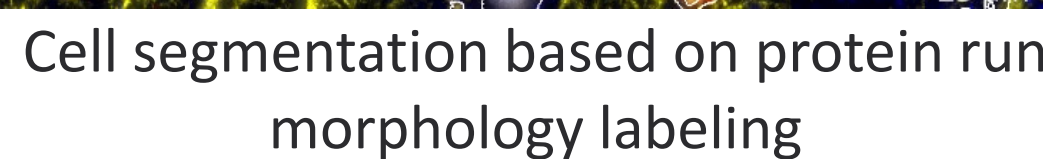
The Spatial Molecular Imager (SMI) addresses this problem by enabling high-plex detection of both proteins and RNAs on the same slide to improve brain cell segmentation and assignment of transcripts to single cells. In this multi-omic system, 68 proteins and over 6,000 transcripts covered by the Human 6K Discovery Panel are targeted on the exact same FFPE human brain section
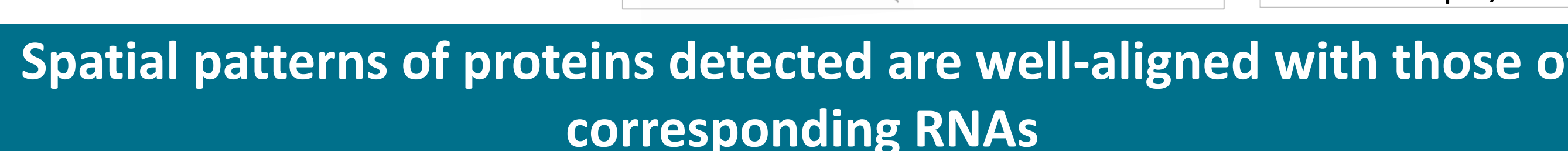
68 plex human protein panel coverage

CosMx 6K Discovery RNA panel: coverage of nearly all biology

>4900 gene targets related to neuroscience + >80 relevant pathways

Overview of the CosMx™ SMI multi-omic chemistry workflow
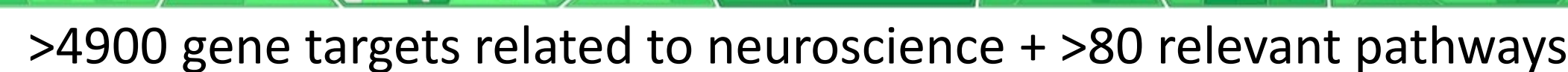
Multi-omic workflow overview

Exact same human brain section

SMI run with 68 plex neuro protein panel #1 → SMI run with 6K Discovery RNA panel #2 → Alignment of protein and RNA data → RNA transcripts assigned to cells using cell segmentation based on protein run data #3

Brain regions profiled

- Parietal lobe
- Postcentral gyrus
- Precentral gyrus
- Cerebellum
- Occipital lobe
- Alzheimer's disease tissue array
- Healthy tissue- Frontal lobe

Neurons/ Astrocytes/ Microglia/ Nuclei

#1- Protein data collection

Sample preparation, flow cell assembly and target detection

#2- RNA data collection

Sample preparation, flow cell assembly and target detection

#3- RNA transcripts assigned to single cells using protein run cell segmentation

Occipital lobe

Neurons (MAP2)/ Astrocytes (GFAP)/ Microglia (IBA1)/ Nuclei (DAPI)

175 µm

Cell segmentation based on protein run morphology labeling | Decoded RNA targets + protein cell segmentation

High-plex protein data enables unprecedented neural cell segmentation and assignment of transcripts to cells

Cell segmentation based on protein run morphology captures neuron and glial processes in addition to cell bodies and associated transcripts

Neurons (MAP2)/ Astrocytes (GFAP)/ Microglia (IBA1)/ Nuclei (DAPI)

Parietal lobe

50 µm

Protein cell segmentation | Decoded RNA targets + protein cell segmentation

Cell segmentation based on protein run morphology marker labeling captures astrocytic protrusions and associated transcripts

Occipital lobe

Neurons/ Astrocyte/ Microglia/ Nuclei/ Astrocyte gene markers

Protein cell segmentation | Decoded RNA targets + protein cell segmentation

New cell segmentation framework increases transcript assignment to single cells vs. typical RNA run morphology marker-based segmentation

New framework leverages:

Protein run morphology labeling | Spot density from RNA Run | rRNA morphology labeling from RNA run

Neurons/ Astrocytes/ Microglia/ Nuclei

Protein panel data example

Part of "Neuron group": MAP2/ NRGN/ NEFL

Protein panel data example

Part of "Immune group": P2RY12/ CD68/ TMEM119

RNA run morphology-based "old" segmentation

"New" cell segmentation framework output

Neural protrusions also show expected mRNAs, such as:

Neurons: TUBB4A, MT3, NRGN, DYNLL1, MIF, CALM1, COX4I1, COX6A1

Microglia: C1QB, ADORA1

#1 Neuron — New vs. Old = 16.0%↑ in transcripts/cell

#2 Neuron — New vs. Old = 18.9%↑ in transcripts/cell

#3 Neuron — New vs. Old = 23.5%↑ in transcripts/cell

#4 Microglia — New vs. Old = 115.5%↑ in transcripts/cell

Spatial patterns of proteins detected are well-aligned with those of corresponding RNAs

#1 Neurofilament light protein/ NEFL RNA/ Nuclei

#2 Calbindin protein/ CALB1 RNA/ Nuclei

Parietal lobe | Cerebellum

Neurons/ Astrocytes/ Microglia/ Nuclei

#3 Neurogranin protein/ NRGN RNA

High-plex protein data reveals major cell types in the brain, and same slide ultra high-plex RNA data enables refined astrocyte cell typing

Protein data-based unsupervised cell typing

Protein markers | RNA markers

Healthy tissue- Frontal lobe

Protein data-based cell typing + RNA data identifies astrocyte subtypes consistent with distinct populations of astrocytes found in white and gray matter

Cell Type: Astrocyte 1/ Astrocyte 2/ Other

High-plex protein data reveals major cell types in the brain, and same-slide ultra high-plex RNA data enables refined neuron cell typing

Protein data-based unsupervised cell typing

Protein markers | RNA markers

Alzheimer's disease tissue- Occipital lobe

Protein data-based cell typing + RNA data identifies finer neuronal subtypes corresponding to distinct cortical layers

Unsupervised neighborhood analysis identifies tissue architecture and allows studies of cell behavior with respect to neighborhood environment

Cluster IDs:
1. Vasculature
2. Oligodendrocyte 1
3. Neuron 1
4. Neuron 2
5. Immune-related 1
6. Astrocyte 1
7. Oligodendrocyte 2
8. Immune-related 2
9. Neuron 3
10. Microglia 1
11. Astrocyte 2
12. Astrocyte Cxcl3

Cell Type: L2-L3 IT / L3-L5 / L4 IT / L4-L5 / L5-6 CT / L6 IT Car3 / L6b / Other

Multi-omic workflow enables assessment of transcriptional changes near hallmarks of Alzheimer's disease pathology

Amyloid Beta 1-40 | Amyloid Beta 1-42

Parietal lobe

Protein cell segmentation | Decoded RNA targets + protein cell segmentation + Alzheimer's pathology

Neurons/ Astrocytes/ Microglia/ Nuclei/ Phospho-Tau species (neurofibrillary tangles)/ Amyloid-Beta (plaques)

Summary & Conclusions

By combining high-plex neuro protein and RNA assays, we gain extraordinary access to the spatial dynamics of multimodal interactions. Specifically, the high-plex SMI multi-omic assay:

- Covers 68 proteins focused on neural cell typing and neural pathology/ inflammation.
- Profiles >4,900 neuroscience-related genes that cover areas ranging from basic neurobiological signaling mechanisms to neurodegenerative disease pathology on the exact same slide.
- Enables unparalleled segmentation of extended cell processes for neurons and glia.
- Rescues many of the 50% unassigned orphan RNA transcripts and quantitates the sub-cellular spatially localized expression in the human brain, an area of extensive biological activity.
- Enables refined cell typing that takes advantage of both protein and RNA data.
- Opens numerous avenues for biological inquiry by combining cell typing with neighborhood analyses.

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