Uncover laminar organization of the developing human neocortex using 6,000-plex RNA spatial imaging

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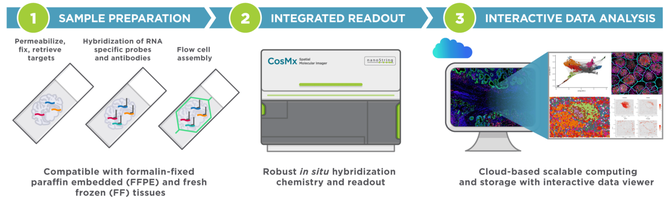
Abstract

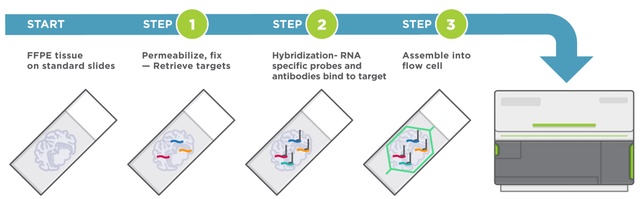
The human cerebral cortex is composed of billions of morphologically and functionally distinct neurons. These neurons are produced in an organized fashion during development. The neocortex is a 6-layered laminar structure, with a precise anatomical organization ensuring proper function. Each layer is comprised of distinct populations of neurons distinguished by differences in size, shape, connectivity, and gene expression. The complex cognitive functions of the adult neocortex depend on the precise emergence of these properties during development. Elucidating the molecular mechanisms that regulate human neocortical development has been a challenge for many years. Development of the neocortex requires an orchestration of a series of processes including the appropriate generation, migration, positioning of the neurons, acquisition of layer-specific transcriptional hallmarks, and formation of precise axonal projections and networks. Over the past years, fate-mapping, genome-wide analysis, and transcriptome profiling has been used to characterize this neocortical cellular diversity. In our pilot study, we apply the 6,000-plex NanoString's CosMx™ SMI, a spatial molecular imaging platform to detect RNA and protein markers in situ at single-cell and subcellular resolution. The pre-defined content from the 6,000-plex panel has comprehensive coverage of genes and pathways. The assay utilizes standard IHC-grade antibodies or in situ RNA hybridization probes that are covalently linked to small (~20 nm) high information content single-molecule imaging barcodes. The platform's ability to perform high-plex multi-omic imaging with sub-cellular resolution allows the visualization and quantification of targeted RNA and proteins directly from tissue samples. Using second trimester primary brain samples, focusing on the neurogenic and gliogenic periods, we visualize known and novel patterns of RNA transcripts across cell types. We identify transcripts involved in cell-cell interactions, synaptogenesis and cortical layering spanning cortical laminae. Using this platform reiterates fundamental differences between neurogenic and gliogenic radial glia, excitatory neurons, and other cell types, highlighting differences in local transcription between the two significant time periods, across the germinal zones and cortical plate.

CosMx Spatial Molecular Imager(SMI) was used for single-cell spatial profiling

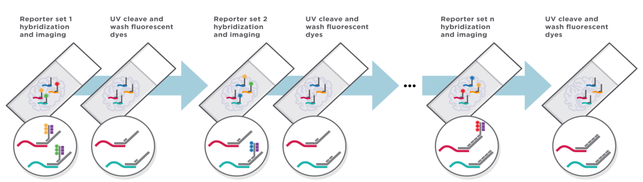
CosMx SMI delivers a comprehensive package which includes validated reagents, instrument, and data analysis software for seamless sample-to-result.



CosMx assay enables efficient single-cell spatial transcriptome profiling in intact FFPE tissue with automatable sample preparation.

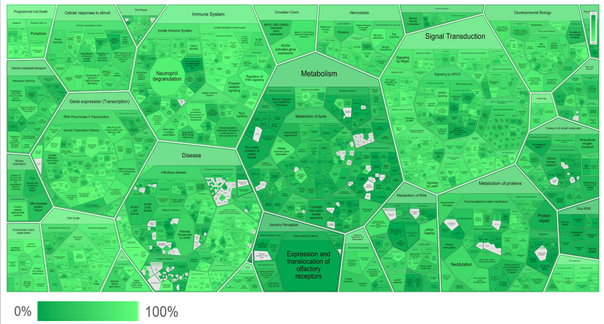


Automated Cyclic Chemistry for in situ detection of transcripts.



6,000 plex RNA panel + Custom Spike-in was used for in situ transcripts detection

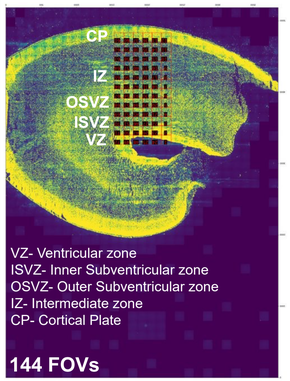
This high-plex RNA panel provides broadest coverage available on biological areas with special emphasis on oncology, immunology, and neuroscience.



Results

Fresh frozen coronal sections from primary developing human prefrontal cortices (PFC) were used from GW18 and GW24. These ages correspond to neurogenic and gliogenic time windows.

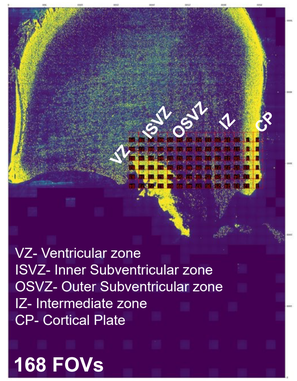
GW18



VZ- Ventricular zone
ISVZ- Inner Subventricular zone
OSVZ- Outer Subventricular zone
IZ- Intermediate zone
CP- Cortical Plate

144 FOVs

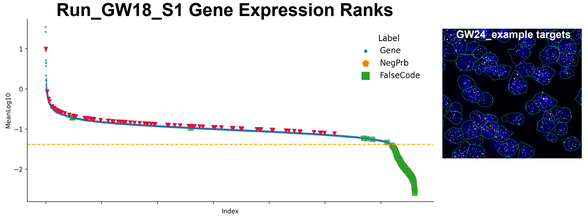
GW24



VZ- Ventricular zone
ISVZ- Inner Subventricular zone
OSVZ- Outer Subventricular zone
IZ- Intermediate zone
CP- Cortical Plate

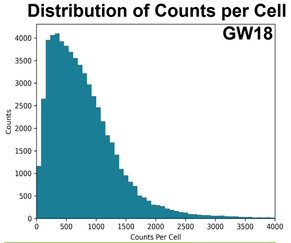
168 FOVs

Run_GW18_S1 Gene Expression Ranks

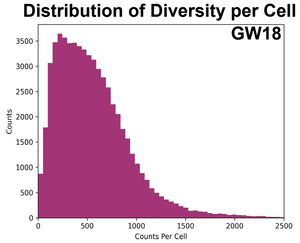


6150 core genes and all 66 isoforms detected above LOD

Distribution of Counts per Cell



Distribution of Diversity per Cell



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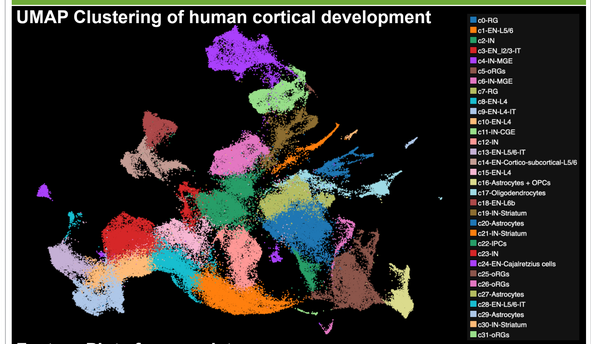
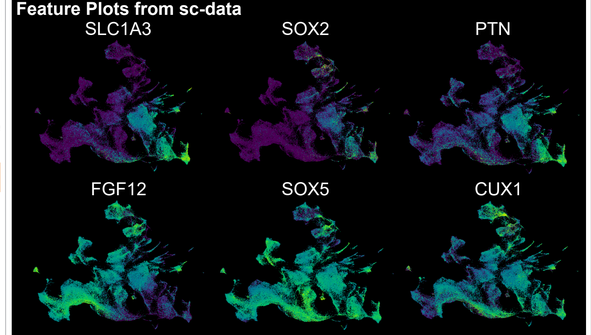
CosMx SMI validates the single-cell gene expression patterns

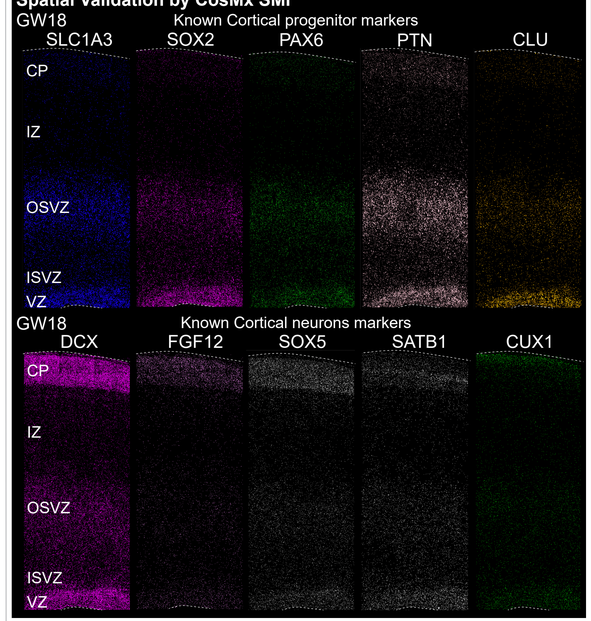
UMAP Clustering of human cortical development



Feature Plots from sc-data

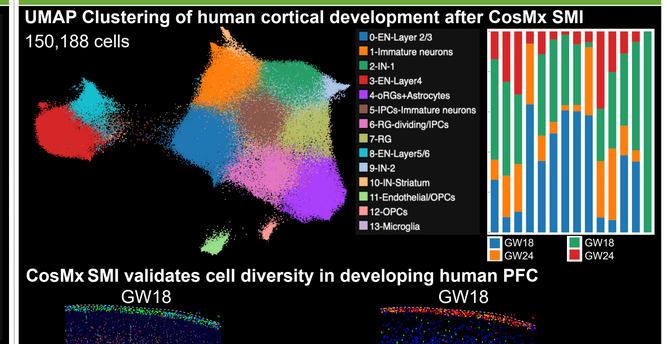


Spatial Validation by CosMx SMI

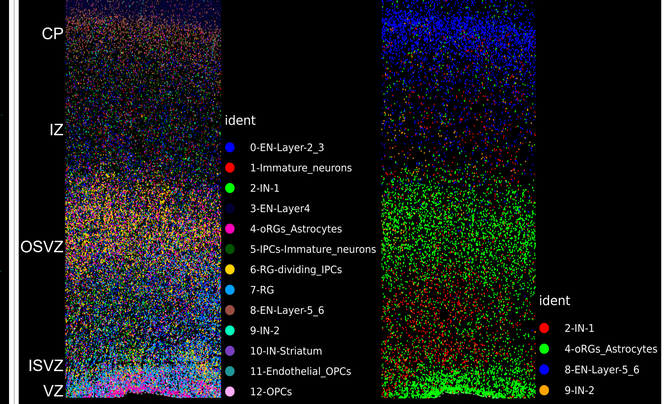


CosMx SMI validates cell diversity in developing human PFC

UMAP Clustering of human cortical development after CosMx SMI

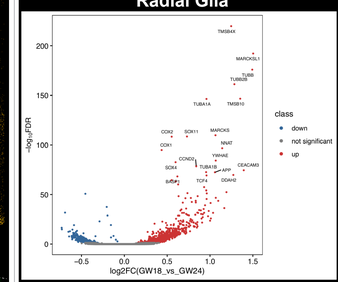


CosMx SMI validates cell diversity in developing human PFC

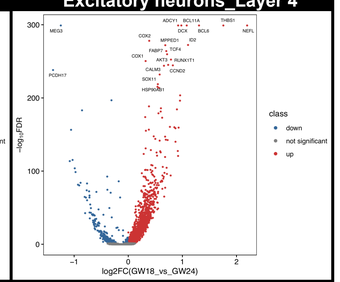


CosMx SMI identifies temporal gene expression changes in PFC

Radial Glia



Excitatory neurons_Layer 4



Spatial feature plots for example DEGs