# #33527 High-plex single-cell spatial transcriptomic analysis of the mouse brain for massive-scale hypothesis generation

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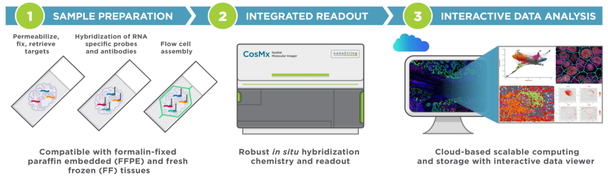
## Abstract

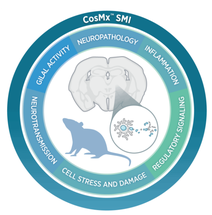
Omics datasets have long been used for hypothesis generation – mass, unbiased discovery of correlations deserving further investigation. Within the brain, neuronal function is heavily dependent on cell-cell interactions, not just on isolated cell states. When applied to the well-researched mouse model, single-cell spatial transcriptomics (SCST) datasets, which record both location and gene expression profiles for potentially millions of cells, would appear to be particularly promising veins to mine for novel mammalian neurobiology. However, exploratory systems biology analyses are seldom applied in SCST, perhaps because most studies have used panels dedicated to cell-type mapping, with plexity in the low hundreds. Here we demonstrate the utility of a high-dimension systems biology approach to SCST analysis, using the 1,000-plex Mouse Neuroscience Panel with the CosMx™ Spatial Molecular Imager (SMI). This panel covers robust neural and glial cell typing, neurodegeneration, neurodevelopment, and key aspects of cell state and signaling. We generated a dataset of over 170,000 cells from three coronal sections of a healthy, eight-week-old male C57BL/6 mouse, detecting an average of more than 1,200 transcripts per cell across all three samples. Leveraging the 1,000 genes on the panel, we categorized each cell into 50 cell types with distinct expression patterns and anatomical locations comprising excitatory neurons, inhibitory neurons, glia, and vascular populations. Then, we used spatial clustering analysis to partition the tissue into 16 niches with distinct cell-type compositions and calculated the enrichment of > 700 curated gene sets across all the cells in the sample. For cell types with at least 50 cells in four or more niches, we compared pathway activity across niches, testing > 12,000 hypotheses and identifying > 750 pathway-cell type combinations with highly concentrated activity (Gini score > 0.3). Additionally, we identified novel modules of co-expressed genes that show spatial correlations beyond what would be expected due to cell type distribution alone. As demonstrated from these complementary approaches in a healthy brain, high-plex single-cell spatial transcriptomics can support massive-scale hypothesis generation and new insights inaccessible to previous technologies.



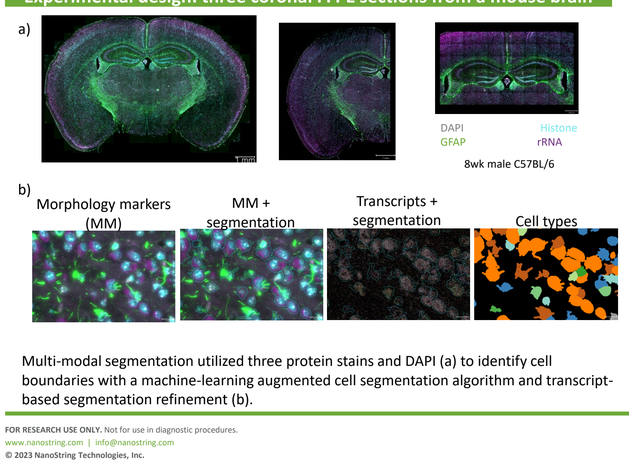
## CosMx Spatial Molecular Imager




**CosMx SMI is the first high-plex, *in situ* spatial multiomics platform for formalin-fixed paraffin-embedded (FFPE) and fresh frozen (FF) tissue samples with single-cell and subcellular resolution**.
CosMx SMI is an integrated system with mature cyclic fluorescent in situ hybridization (FISH) chemistry, high-resolution imaging readout, and interactive data analysis and visualization software. With 1000 highly curated targets, the CosMx Mouse Neuroscience Panel is designed to provide robust cell typing, cell-cell interaction analysis, and more in mouse brain and other neuronal tissues.

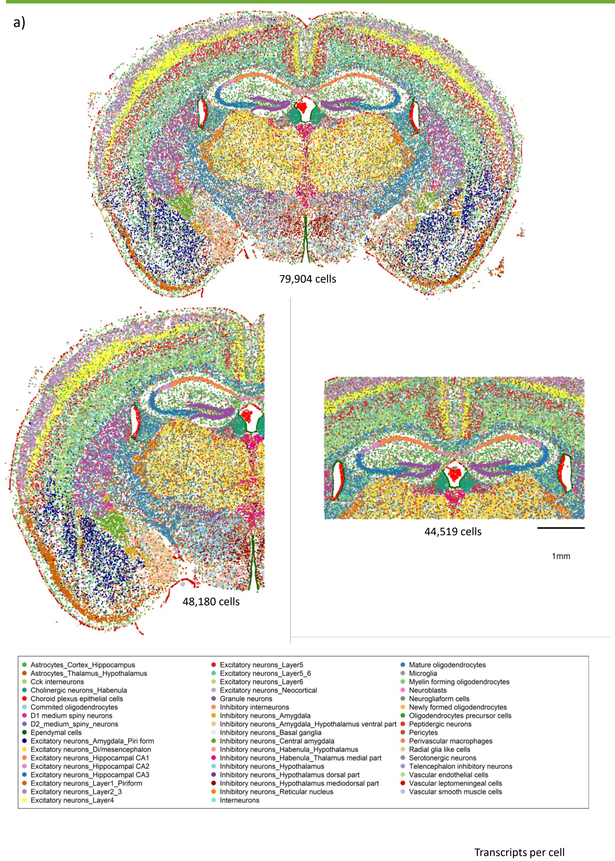


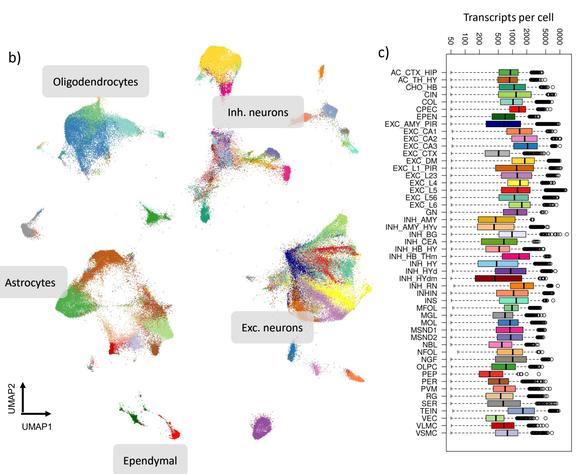

## Experimental design: three coronal FFPE sections from a mouse brain




Multi-modal segmentation utilized three protein stains and DAPI (a) to identify cell boundaries with a machine-learning augmented cell segmentation algorithm and transcript-based segmentation refinement (b).

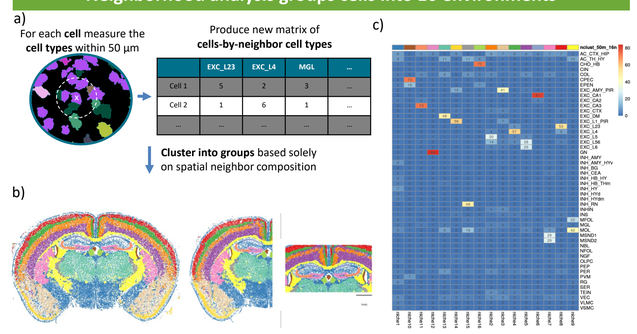
## 50 cell types were identified across three samples



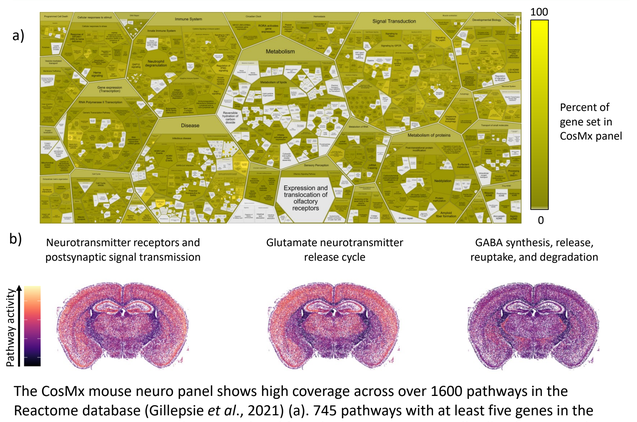

Over 170,000 cells annotated as 50 distinctly localized cell types using a negative binomial model and a reference single cell RNA-seq dataset (Zeisel *et al*, 2018) as implemented in the InSituType package (Danaher *et al*, 2022; panel a). Two dimensional UMAP projection separates major classes of cells (b). All cell types show high numbers of transcripts detected, with per cell averages ranging from 488 to 2334 (c).

## Neighborhood analysis groups cells into 16 environments



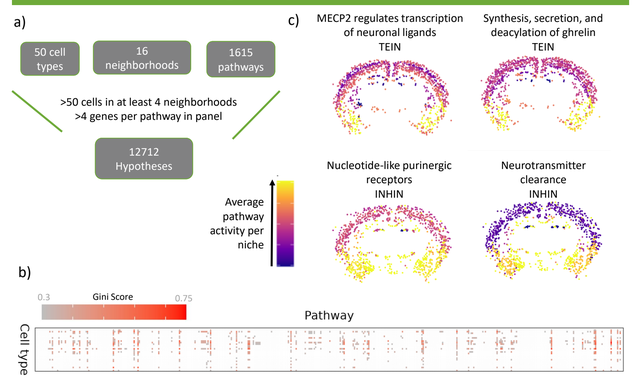

Sixteen neighborhoods represent areas with similar cell type distribution (a) and localize to similar anatomical regions across sections (b). Neighborhood cell type composition (c ).

## Activity scores for > 700 curated gene sets form spatial patterns



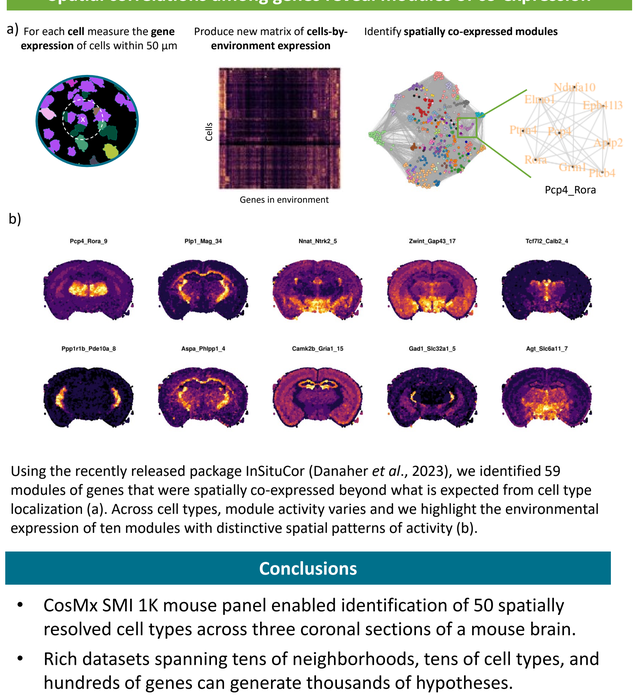

The CosMx mouse neuro panel shows high coverage across over 1600 pathways in the Reactome database (Gillespie *et al*., 2021) (a). 745 pathways with at least five genes in the CosMx mouse neuro panel were analyzed for relative activity across all cells. Shown are three example pathways in one sample with high activity in neurons broadly (neurotransmitter receptors), the cortex and thalamus (glutamate release), or the striatum (GABA synthesis).

## Per cell activity scores give rise to > 12,000 hypotheses across niches




We generated >12,000 sufficiently powered spatial hypotheses to consider (a), from which we identified 769 pathways with Gini scores over 0.3 when comparing in a single cell type across niches (b). As one example, telencephalon inhibitory neurons exhibit high variability in the activity of the pathway “MECP2 regulates transcription of neuronal ligands” (gini = 0.42), with highest activity in niche 3. This niche localizes to the amygdala, where MECP2 expression is reported to reduce anxiety behaviors (Adachi *et al*, 2009; panel c ).

## Spatial correlations among genes reveal modules of co-expression




Using the recently released package InSituCor (Danaher *et al*., 2023), we identified 59 modules of genes that were spatially co-expressed beyond what is expected from cell type localization (a). Across cell types, module activity varies and we highlight the environmental expression of ten modules with distinctive spatial patterns of activity (b).

## Conclusions

- CosMx SMI 1K mouse panel enabled identification of 50 spatially resolved cell types across three coronal sections of a mouse brain.
- Rich datasets spanning tens of neighborhoods, tens of cell types, and hundreds of genes can generate thousands of hypotheses.
- Utilizing neighborhoods to capture spatial variability, we identified > 700 gene set / cell type combinations that differed significantly in activity level by brain region.
- We further identified 59 modules of genes *de novo* showing non-trivial spatial correlation including sets of genes highly co-localized in the striatum, thalamus, or hippocampus.

## References

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