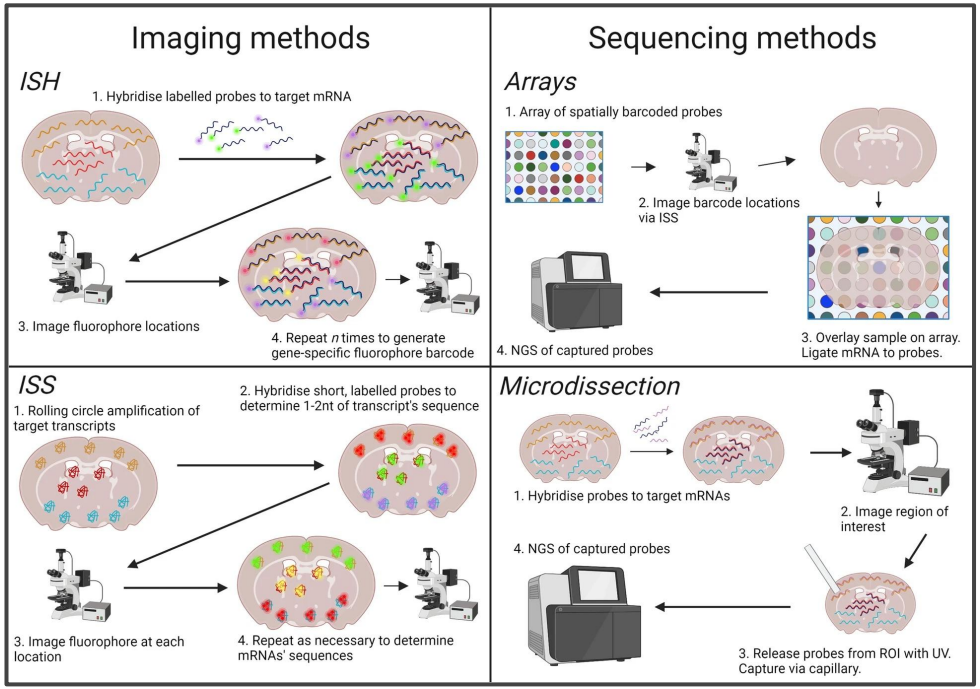
Identification of regionally variant genes

Orhan Hosten-Mittermaier

Spatial Transcriptomics Enables Profiling of Cells in Space

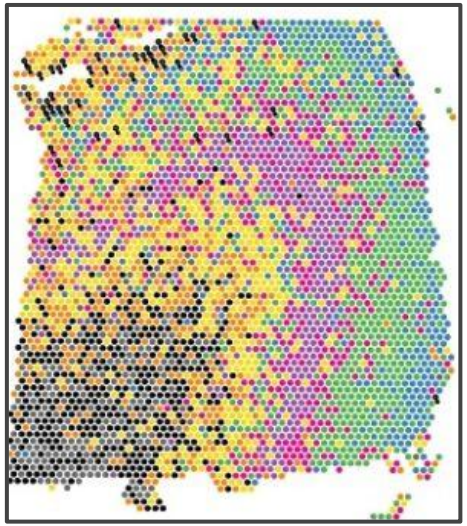
Goal: understand spatial relationships of mRNA expression



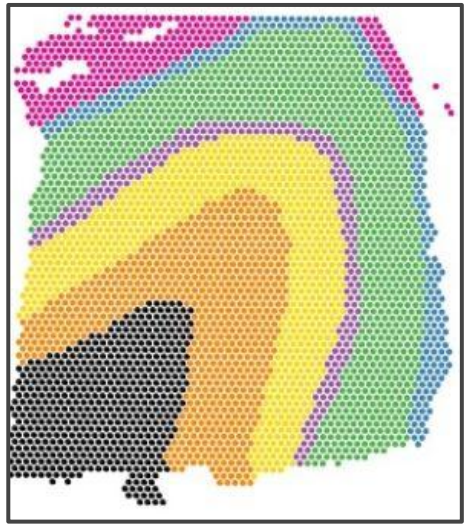
Williams, et. al.

Spatial Transcriptomics Enables Profiling of Cells in Space

Cell type clustering



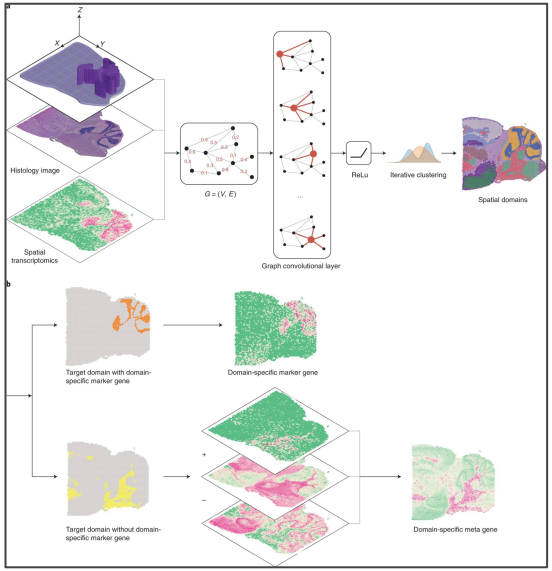
Regions in space



Maynard, et. al.

How are regions assigned?

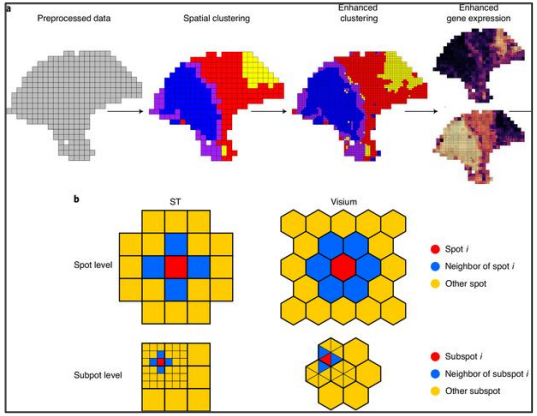
Neural Network



STAGATE, SpaGCN, GASTON

Hu, et.al.

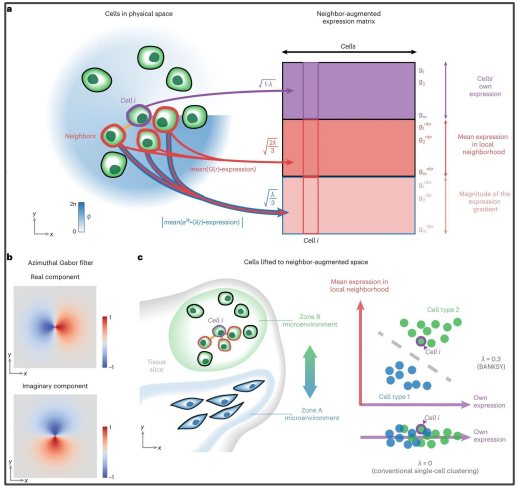
HMRF



BayesSpace, Giotto

Zhao, et. al.

Spatial Smoothing

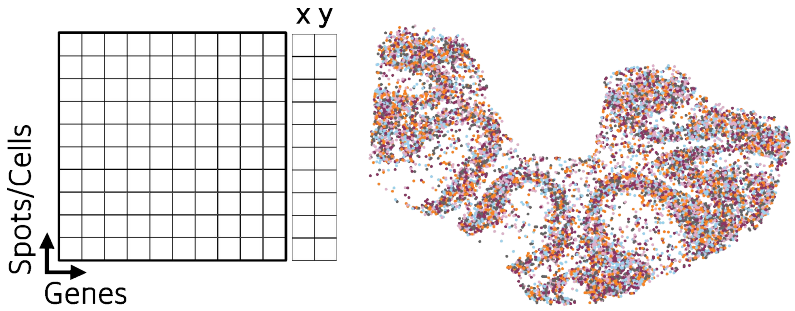


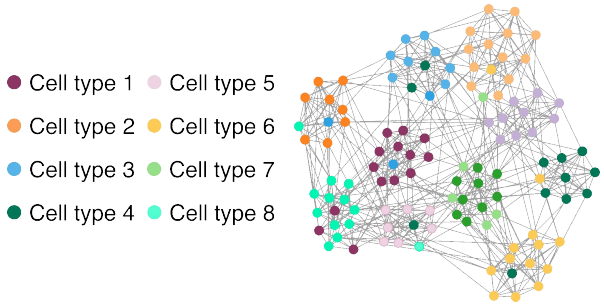
BANKSY, Concordex

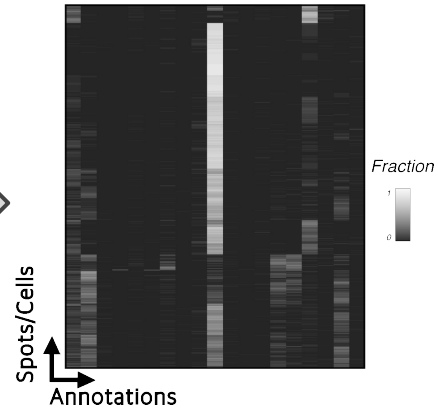
Singhal, et. al.

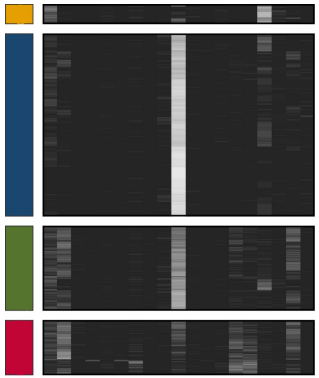
Concordex

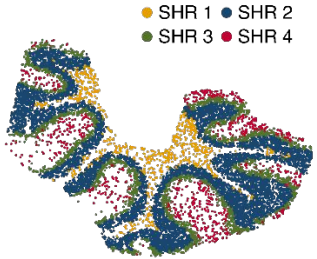
Input











1. kNN Graph

2. Neighborhood Consolidation Matrix

3. Region Clustering

Adapted from Jackson, et.al.

Guiding Research Questions

A. Are there region specific genes?
   a. genes expressed differentially across regions (“regional DE genes”)

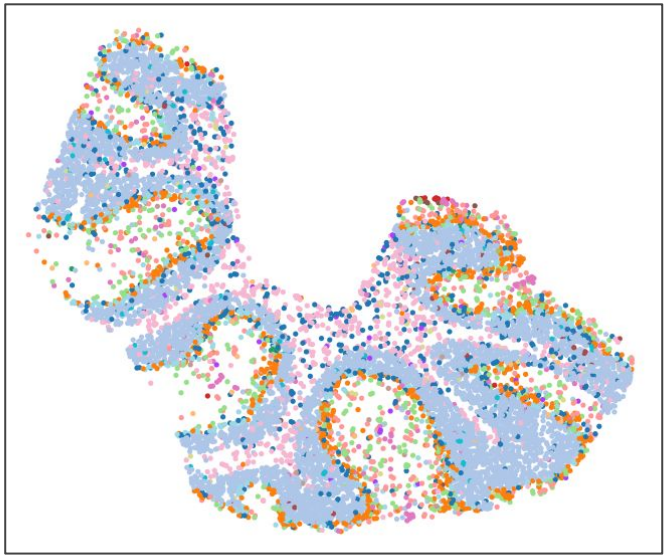
B. Are there region specific DE genes w/in a cell type?
   a. Genes expressed differentially in a cell type between regions (“cell type-regional DE genes”)

C. How can we leverage spatial information to gain more insight?

The Dataset

Technology: Slide-seq V2

Tissue: Mouse Cerebellum

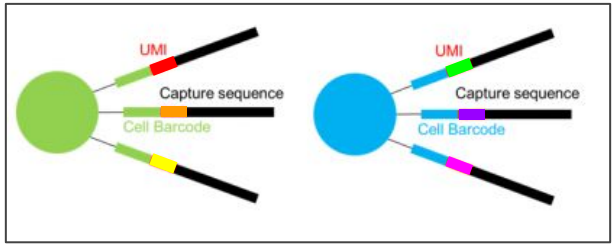


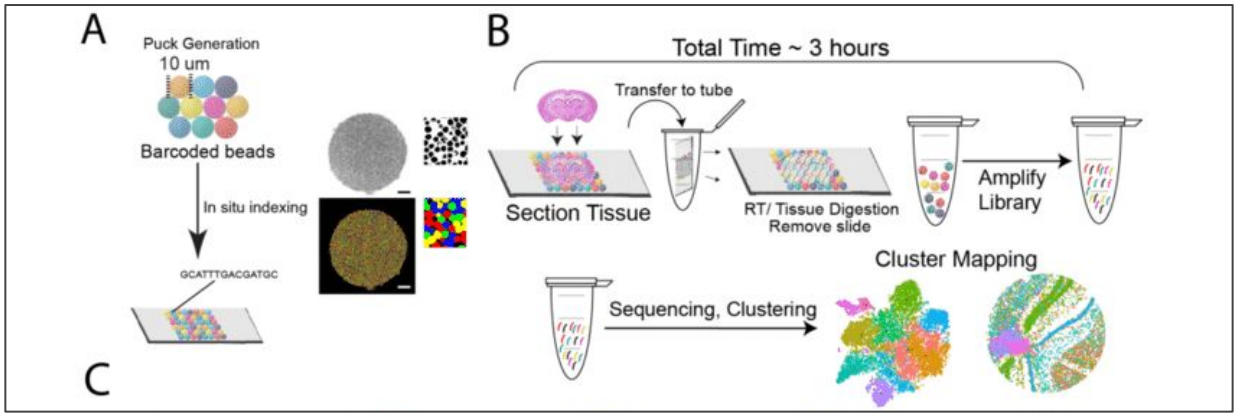
data from Cable, et. al.

The Dataset

Technology: Slide-seq V2

Tissue: Mouse Cerebellum





Rodriques, et. al.

The Dataset

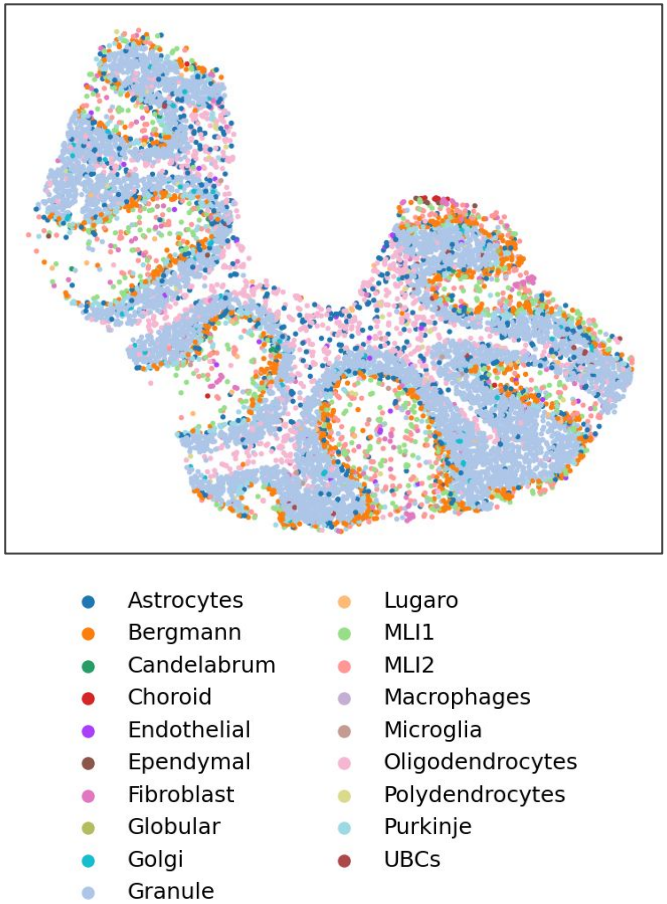
Technology: Slide-seq V2

Tissue: Mouse Cerebellum

cells captured: **9,985**

genes found: **23,096**

cell types identified: **19**



data from Cable, et. al.

The Dataset

Technology: Slide-seq V2

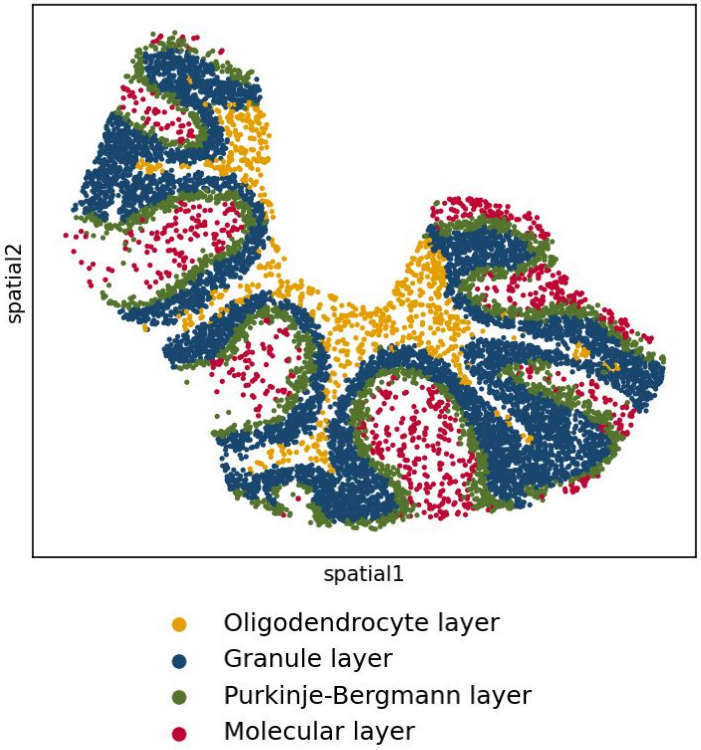
Tissue: Mouse Cerebellum

cells captured: **9,985**

genes found: **23,096**

cell types identified: **19**

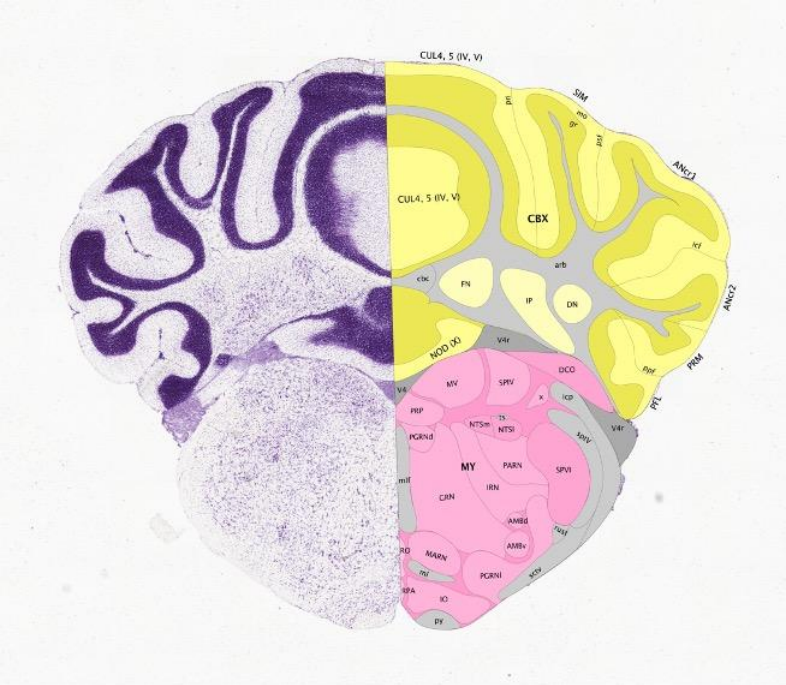
Region assignments by concordex

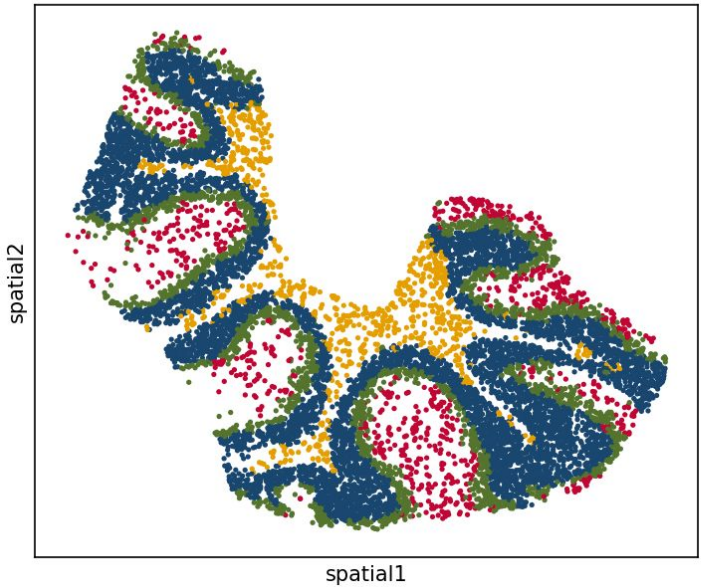


data from Cable, et. al.

The Dataset

Assigned regions correspond to functional regions

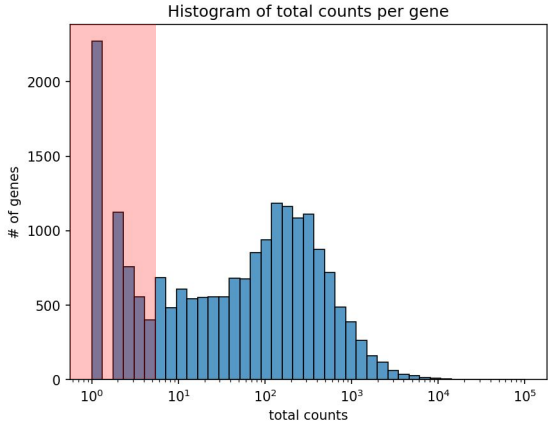


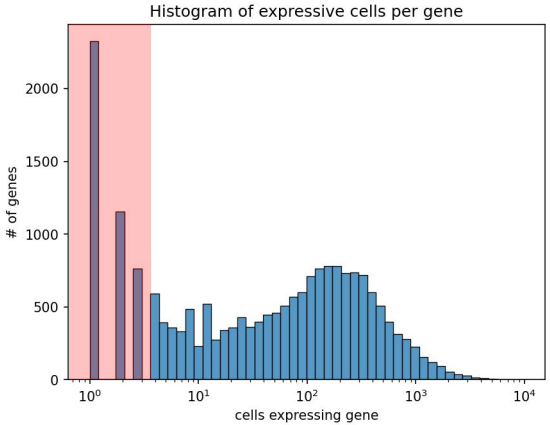


data from Cable, et. al.

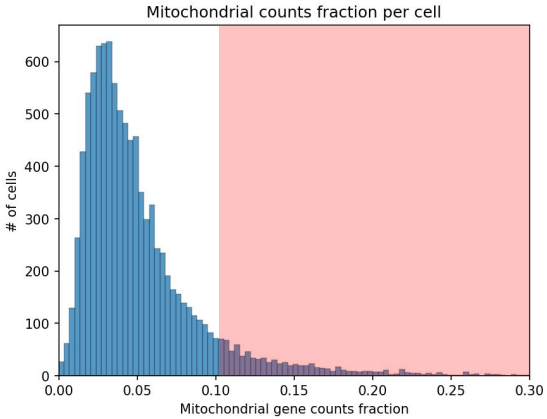
Pre-processing

Filtering:
- total counts per gene > 50
- cells expressing gene > 25
- mitochondrial gene expression fraction < 0.1



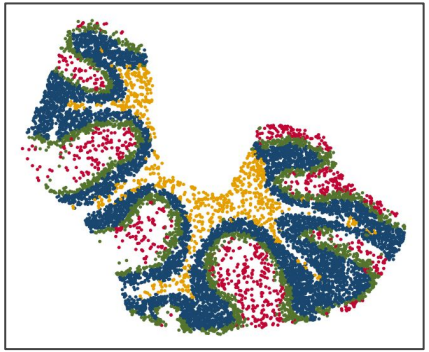


Genes: **23,096 → 10,293**
Cells: **9,985 → 9,051**

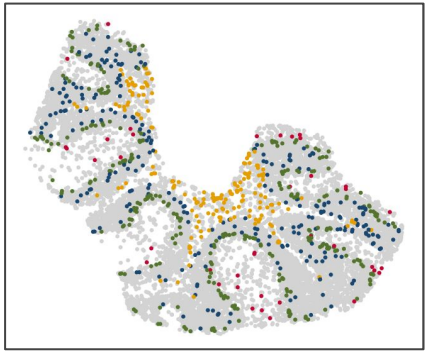


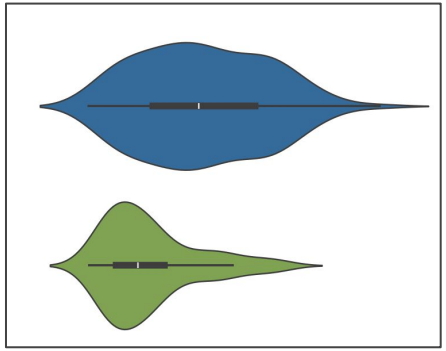
Normalization:
- By total counts across each cell
- log1p

Identifying DE genes



- Oligodendrocyte layer
- Granule layer
- Purkinje-Bergmann layer
- Molecular layer





Gene	Cell Type	Regions	p-value
…	…	…	…
…	…	…	…
…	…	…	…
…	…	…	…

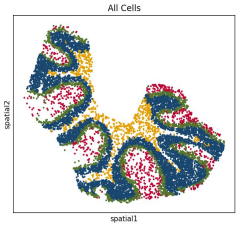
1. Isolate a cell type

2. Compare expression

3. Tabulate results

204 significant comparisons
124 unique genes

Interpretation of significant results



- Oligodendrocyte layer
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- Purkinje-Bergmann layer
- Molecular layer

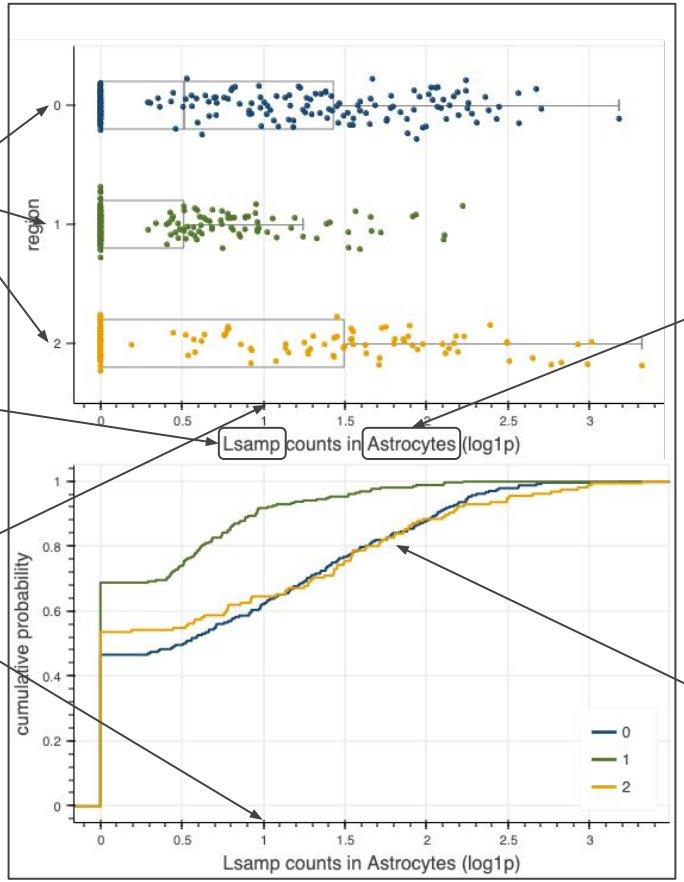
Regions included in plot if part of a pairwise stat-sig relationship

Gene

Aligned axes

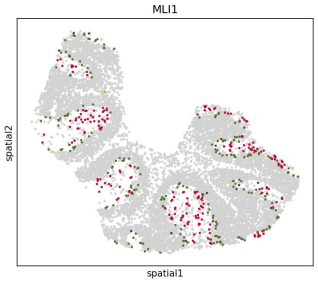
Cell type

Region colors are consistent

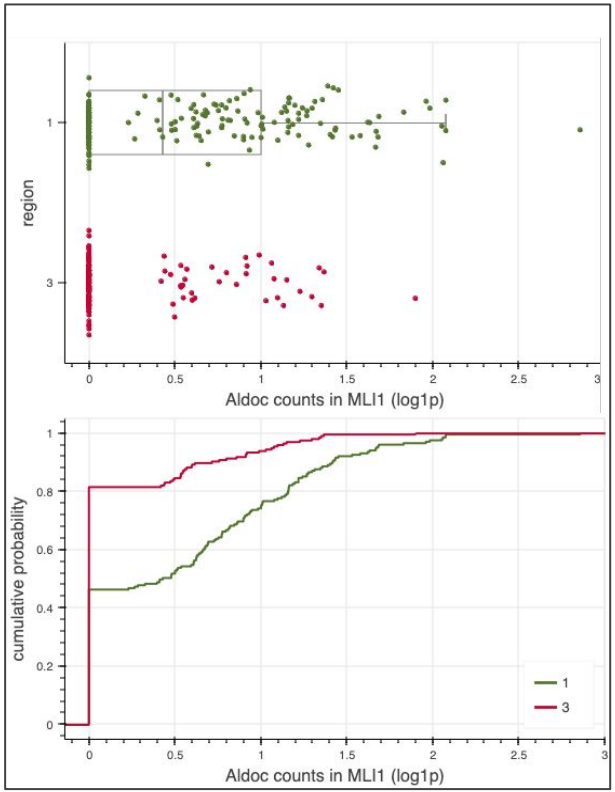


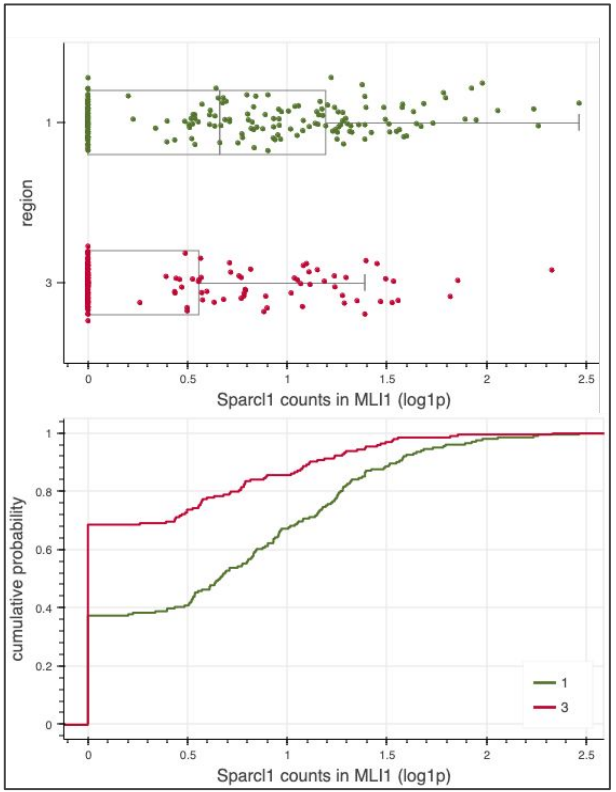
MLI1s are more active in Purkinje-Bergmann layer

- Oligodendrocyte layer
- Granule layer
- Purkinje-Bergmann layer
- Molecular layer

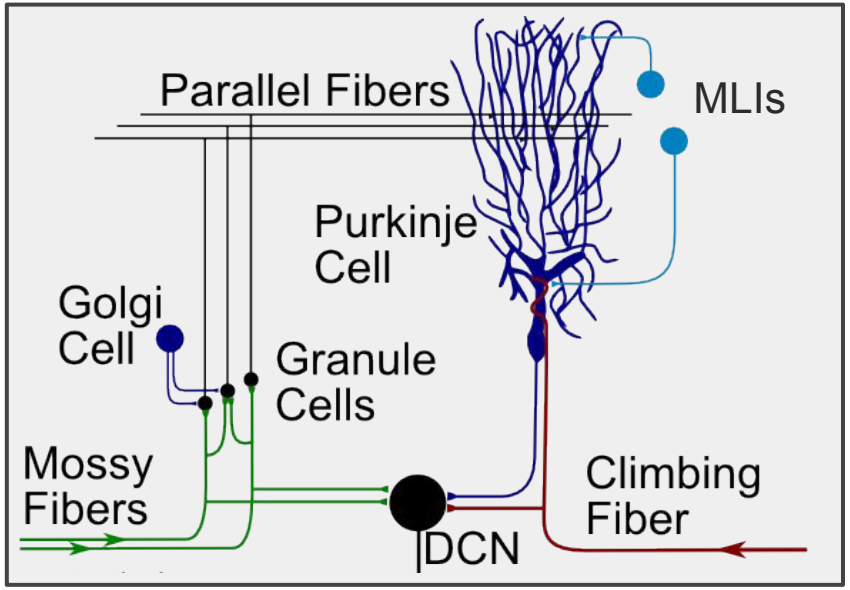


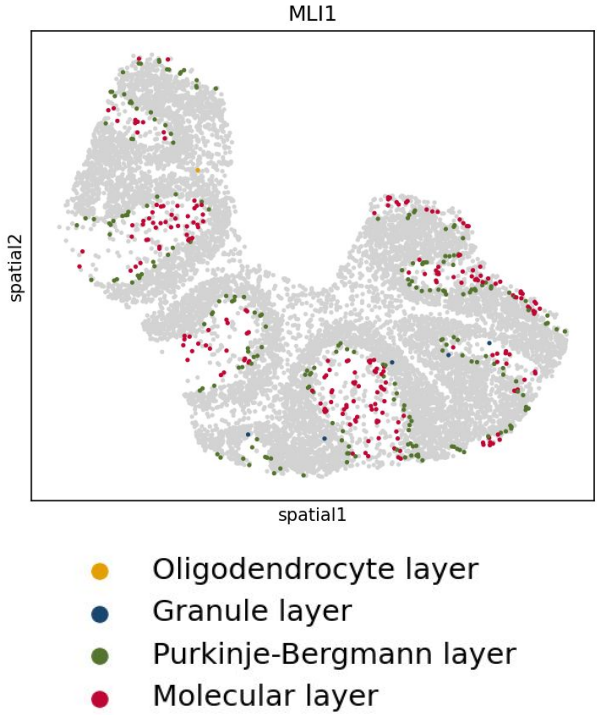
Cell type: MLI1





MLI1s are more active in Purkinje-Bergmann layer

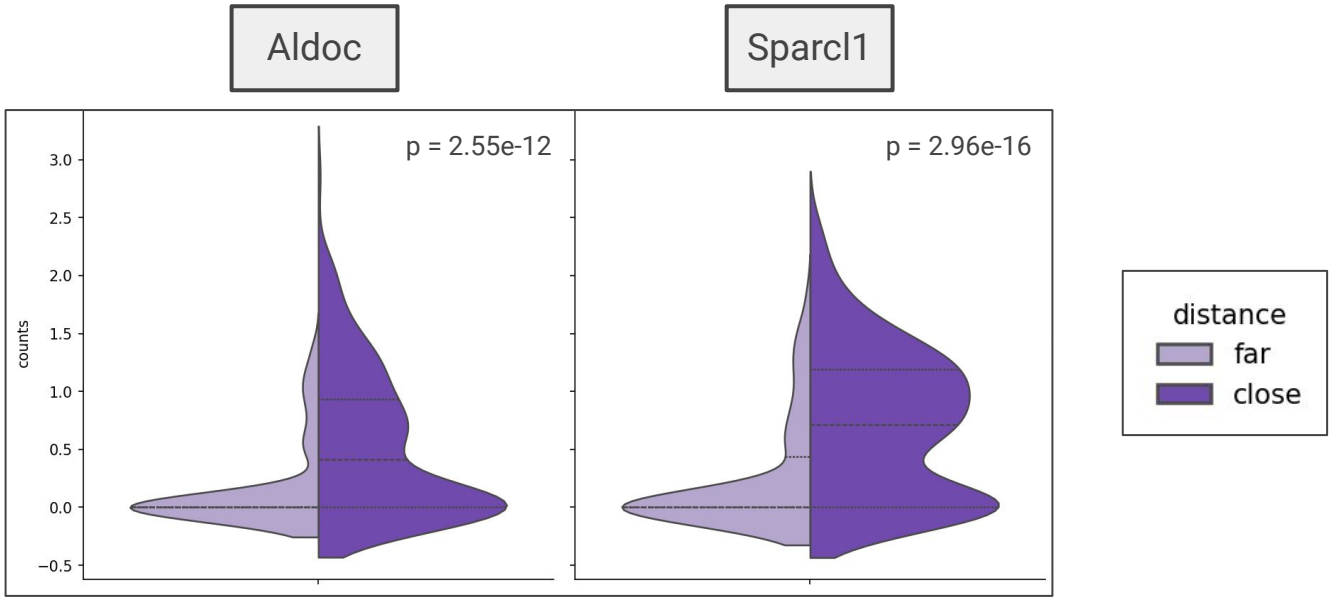




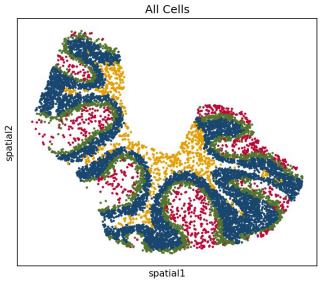
Schlerf, et. al.

MLI1 activity levels depend on proximity to granules

Cell type: MLI1

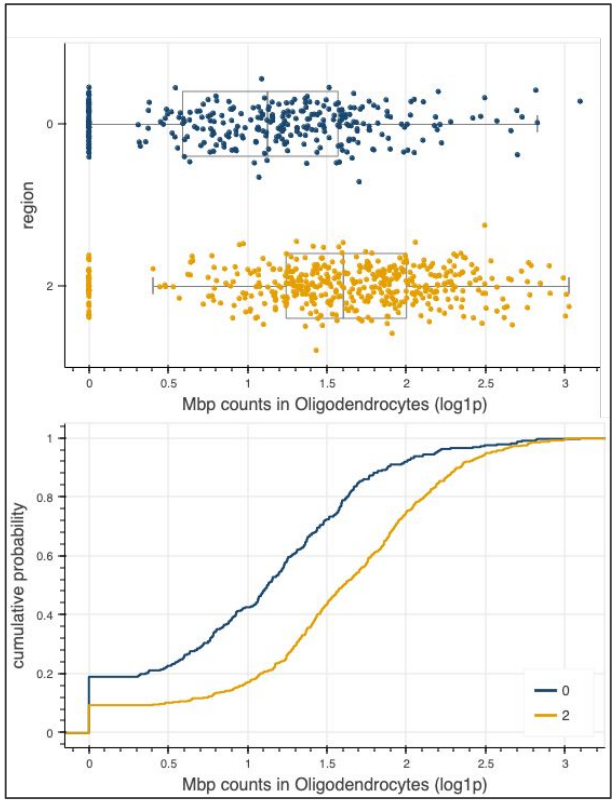


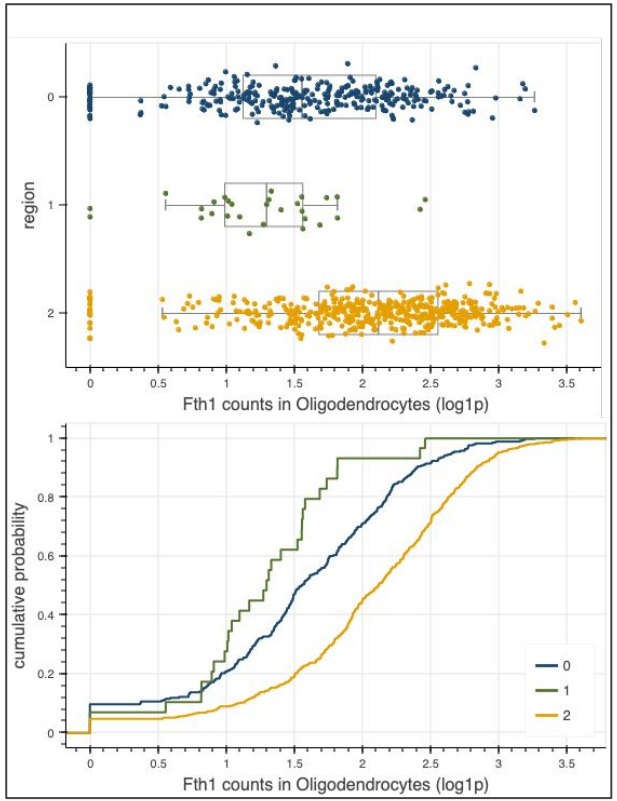
The oligodendrocyte layer has a regionally upregulated myelination function



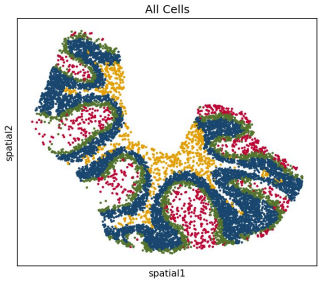
- Oligodendrocyte layer
- Granule layer
- Purkinje-Bergmann layer
- Molecular layer

Cell type:
Oligodendrocytes

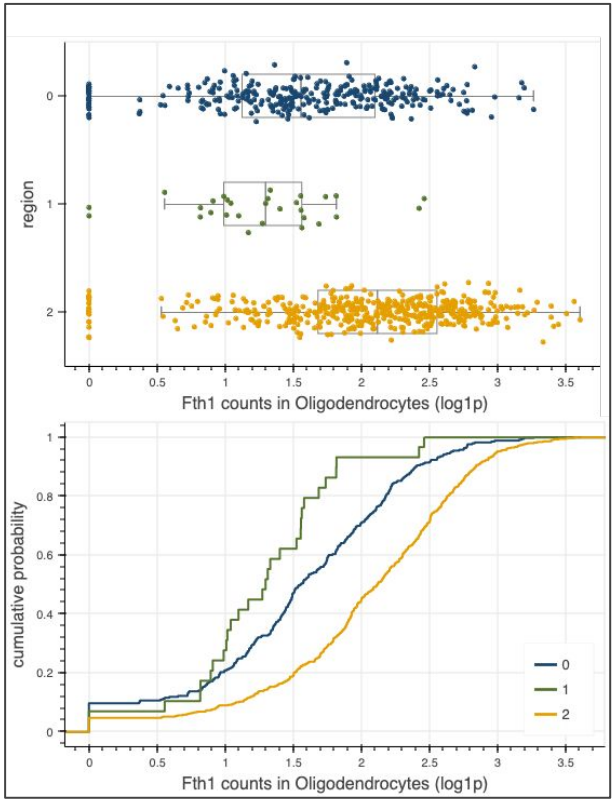


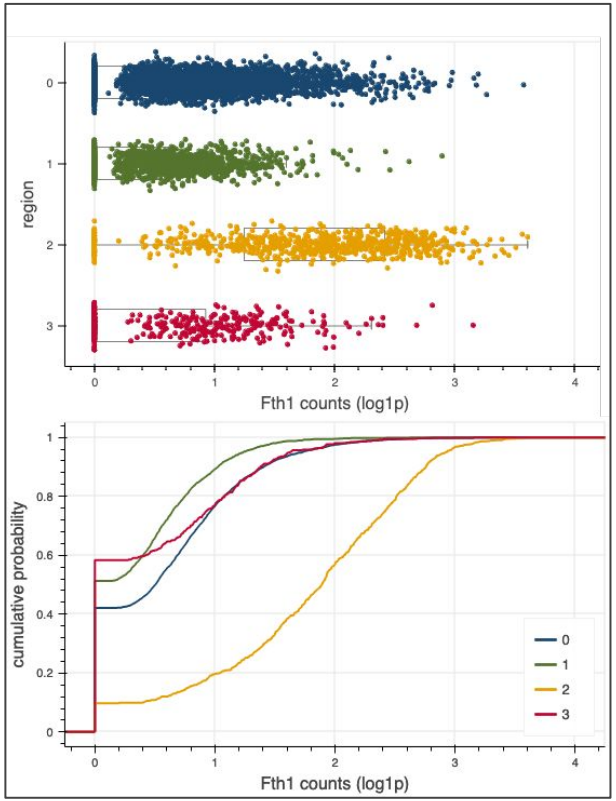


Iron storage regulation may be important for all cells in the oligodendrocyte layer

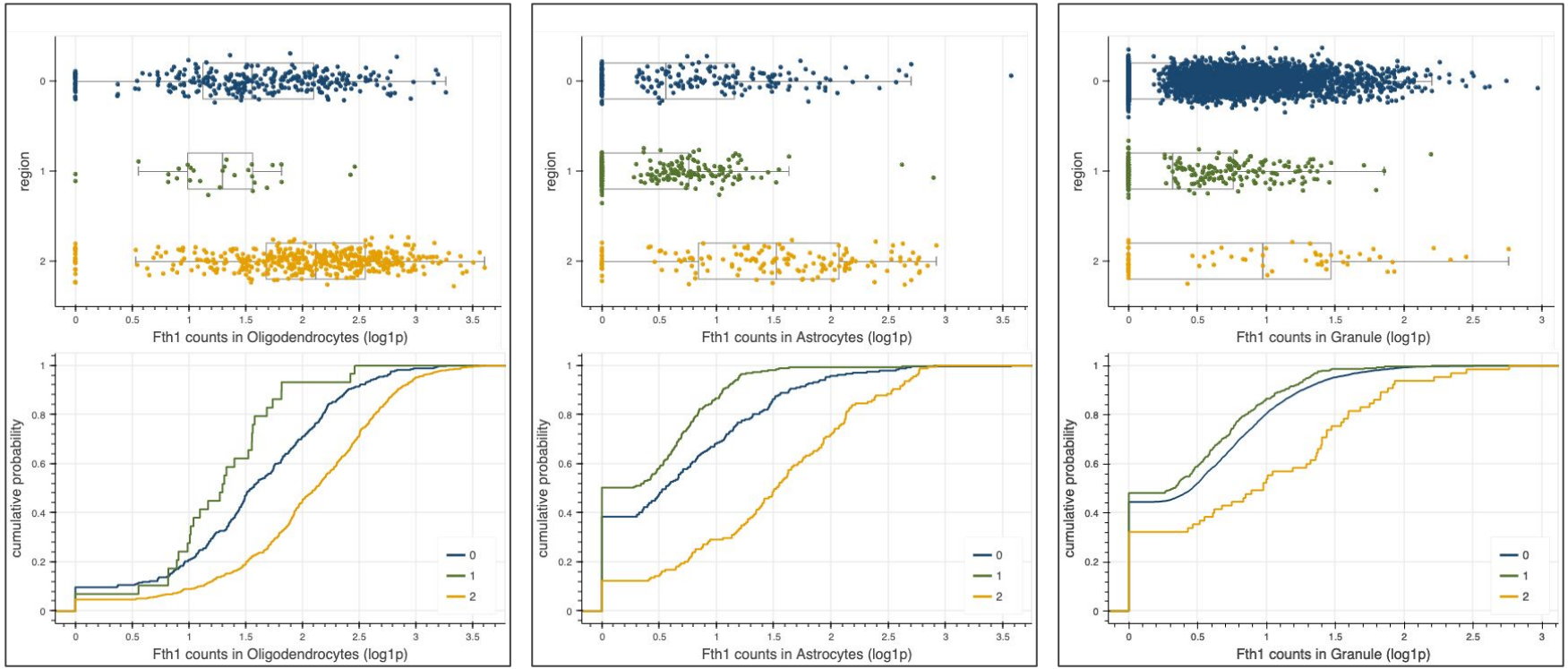


Cell type:
Oligodendrocytes





Iron storage regulation may be important for all cells in the oligodendrocyte layer



Conclusions

A. Regional differences between cells of the same type suggest differences in cell state

B. Examining regional gene expression can be used to gain further insight into region-specific functionality

C. Cell type clustering alone is insufficient to detect regional DE genes shared by multiple cell types

Dr. Lior Pachter

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Thank you :-)