

# An orientation in the spatial transcriptomics landscape

2021 :: Summer School Advanced Topics in Single Cell Analysis



<https://github.com/almaan>



<https://almaan.github.io>



@aalmaander

# Who am I?

- **Name :** Alma Andersson
- **Part of :** Lundeberg Lab (PhD Student)
- **Works with :** Computational Method Development
  - Mainly focus on spatial transcriptomics data
- **Background :**
  - Engineer by training
  - **Before:** Molecular Dynamics
  - **Now:** Spatial Transcriptomics
- **Work :**
  - Single cell and spatial transcriptomics data integration (*stereoscope*)
  - Model to find spatially variable genes (*sepal*)
  - Spatial characterization of HER2 breast cancer samples
  - Common coordinate frameworks for spatial data
- **Non-scientific Interests**
  - Trail/Ultrarunning, Hiking, Outdoor stuff



# My vision for today

## Introduction

- Broad overview of experimental spatial transcriptomics techniques
- A Recap on Visium
- Data character - what are we working with?

## Computational methods and frameworks

- Different flavors of currently available methods
- Example methods
- Extra focus on single cell mapping and integration
- squidpy : a framework for handling spatial data

## Observations from the wild

- General advice
- Example : A spatial survey of HER2-positive breast cancer
- Example : Spatial gene expression dynamics in the mouse liver

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### Slides ::

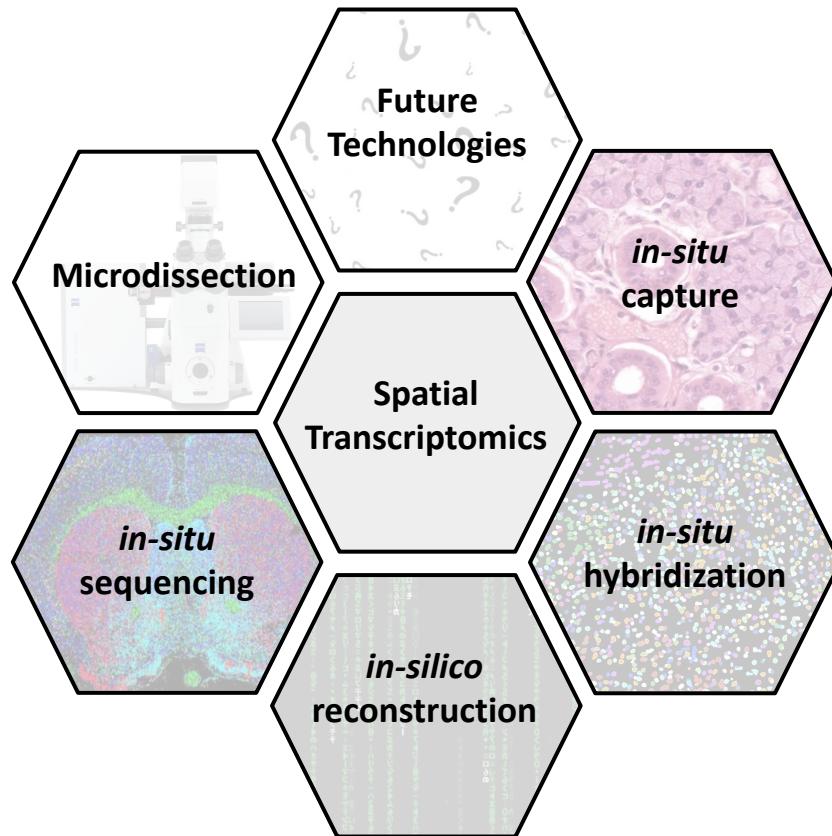
lectures/spatial\_transcriptomics.pdf

### Links ::

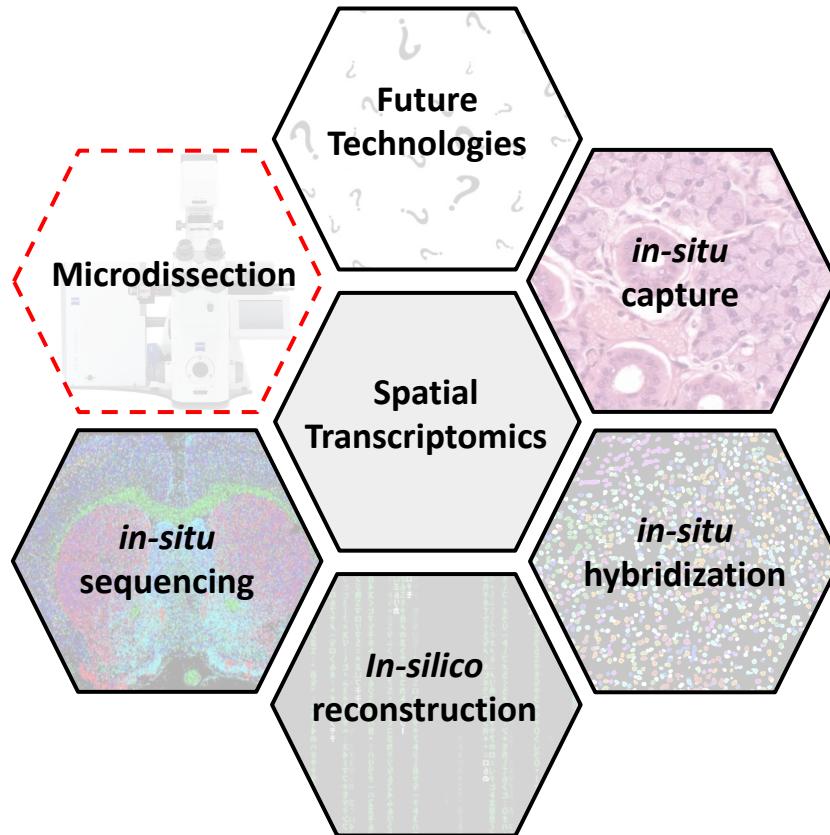
<https://almaan.github.io/extras/advsc-info/>



# Experimental techniques



# Experimental techniques



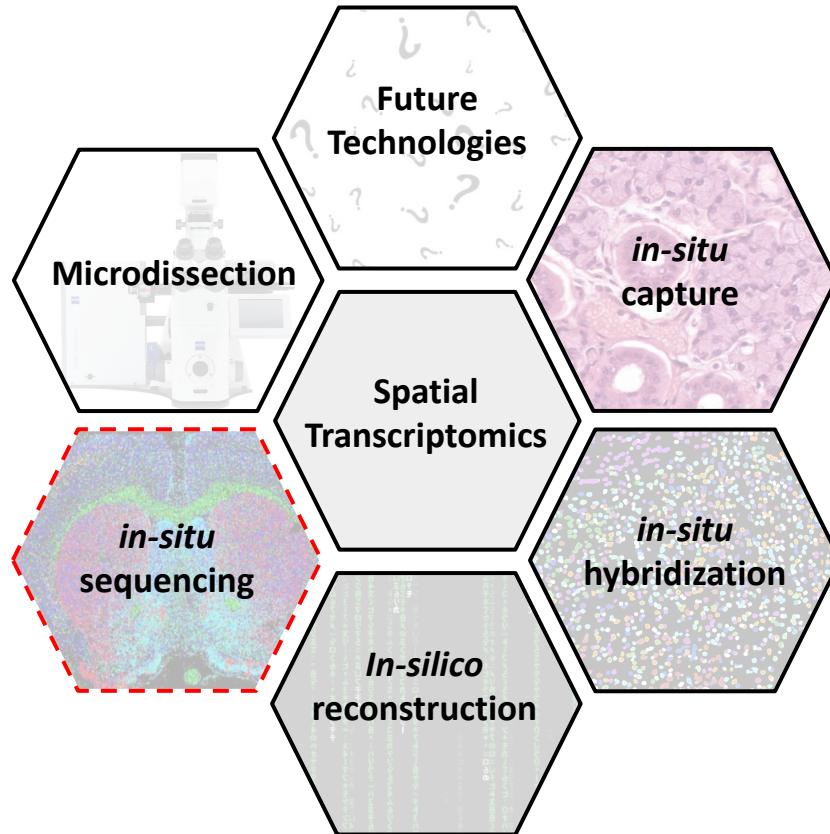
## Microdissection-based technologies

Isolate a region of interest, place isolate in separate well and sequence (either by bulk or single-cell methods).

A “Brute Force” approach.

**Examples :** LCM, Tomo-seq, TIVA, ProximID, Niche-seq

# Experimental techniques



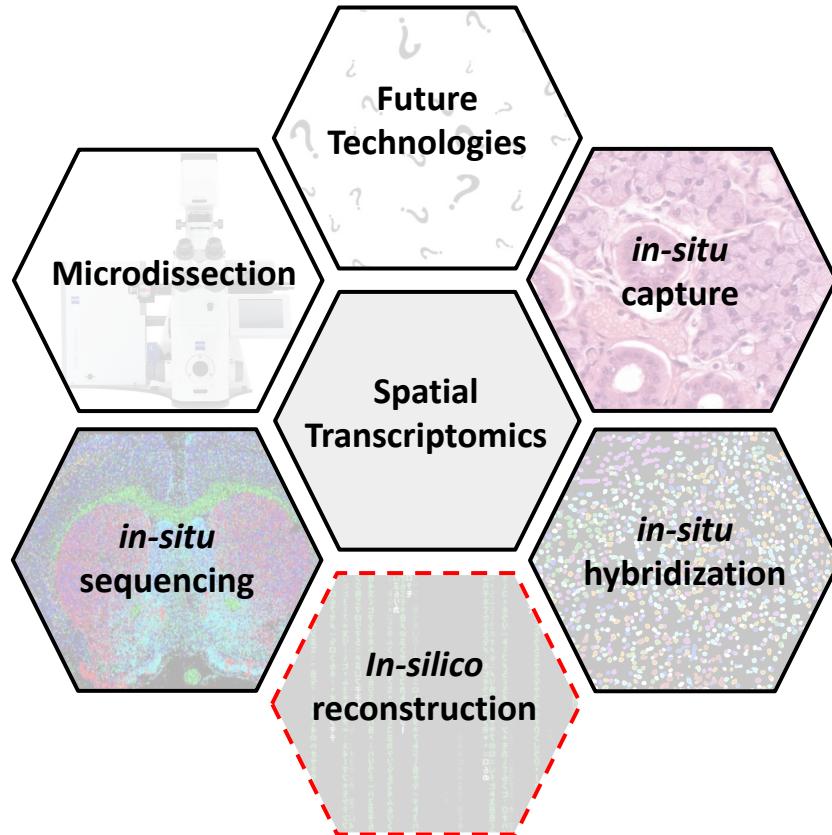
## *In-situ* sequencing based methods

Sequence the transcripts in place.

Offer sub-cellular resolution. Some relies on “*a priori*” defined targets, but not all.

**Examples :** ISS/Cartana (padlock probes), BaristaSeq, STARmap, FISSEQ

# Experimental techniques

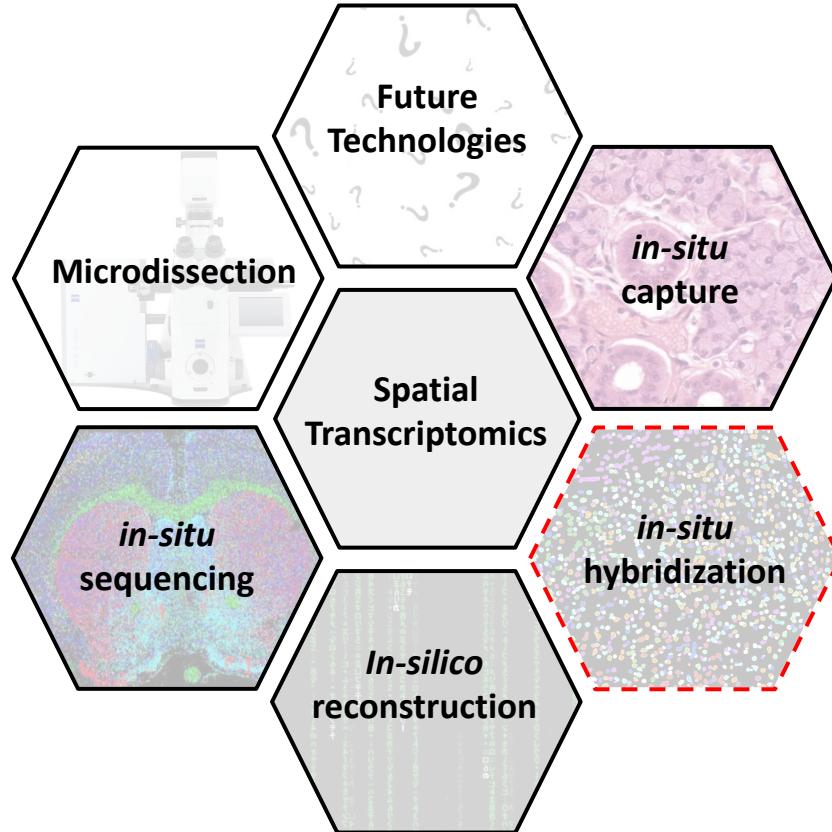


## *In-silico* reconstruction

Infer and reconstruct spatial structure from non-spatial data (e.g., single cell).

**Examples :** novoSpaRc, CSOmap, Seurat v3

# Experimental techniques



## *In-situ* hybridization based methods

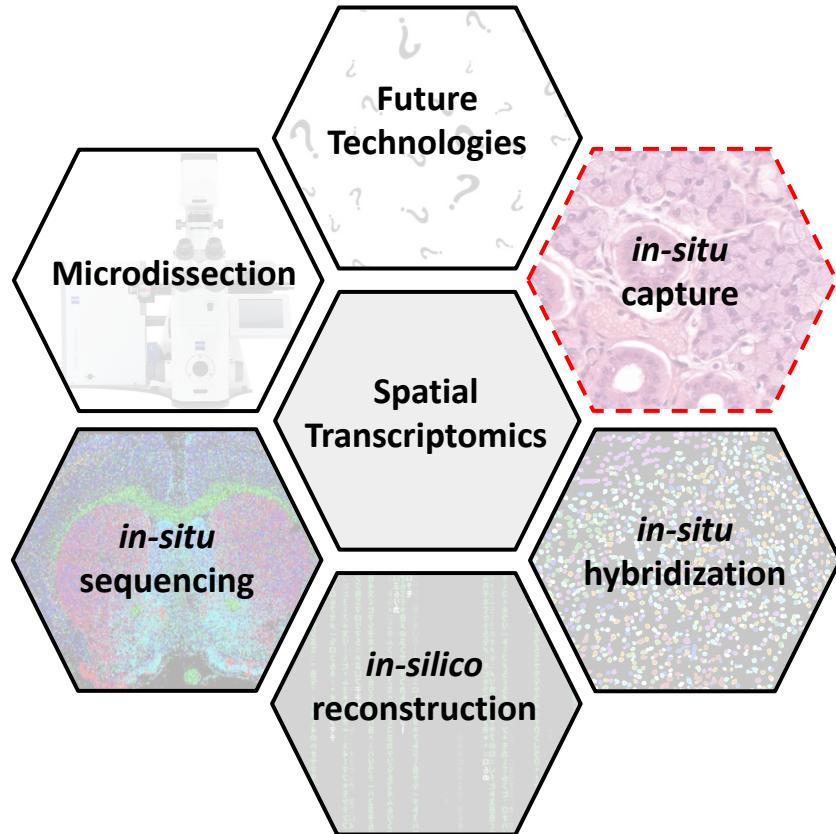
Labeled probes for specific targets, hybridize in place.

Requires “*a priori*” defined targets.

Expansion strategies and smart decoding scheme has helped to overcome spectral overlap.

**Examples :** smFISH, seqFISH, MERFISH, seqFISH+, osmFISH, RNA Scope, DNA microscopy

# Experimental techniques

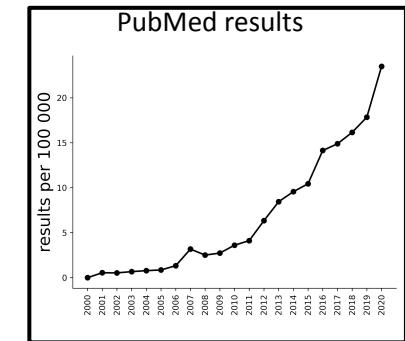
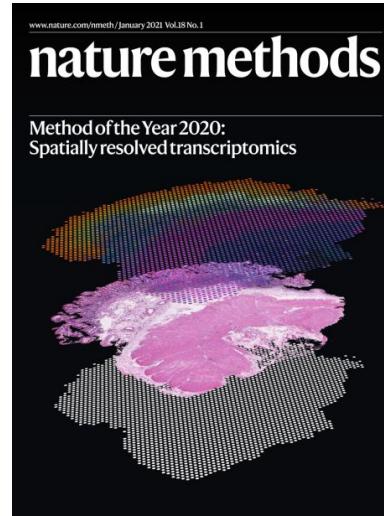
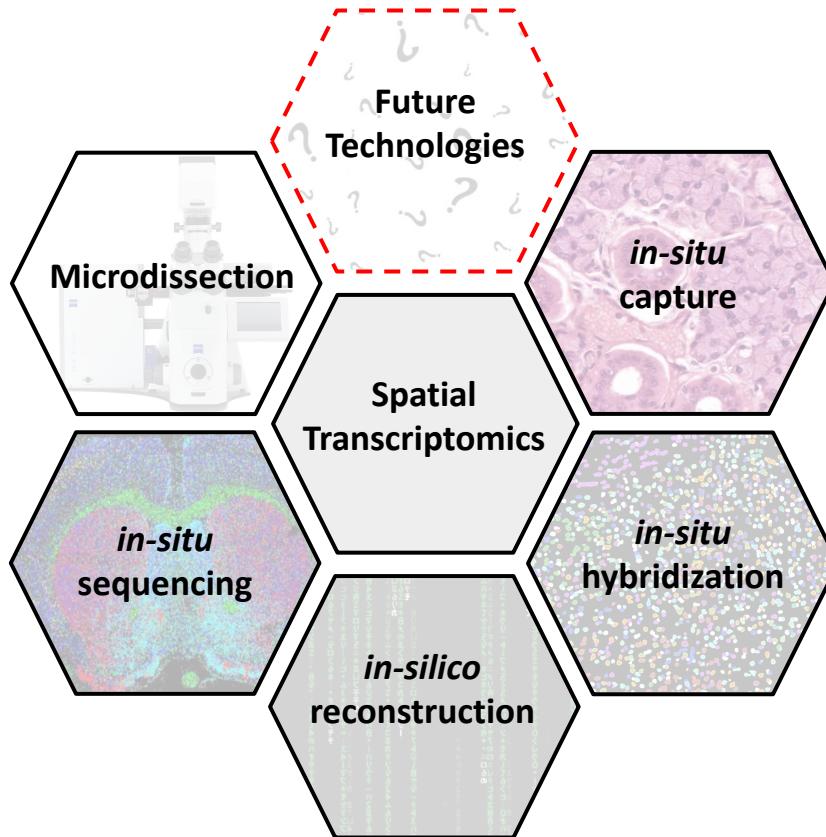


## *In-situ* capture based methods

Capture transcripts *in situ* but sequence *ex situ*.  
Usually less dependent on prior selection of targets.

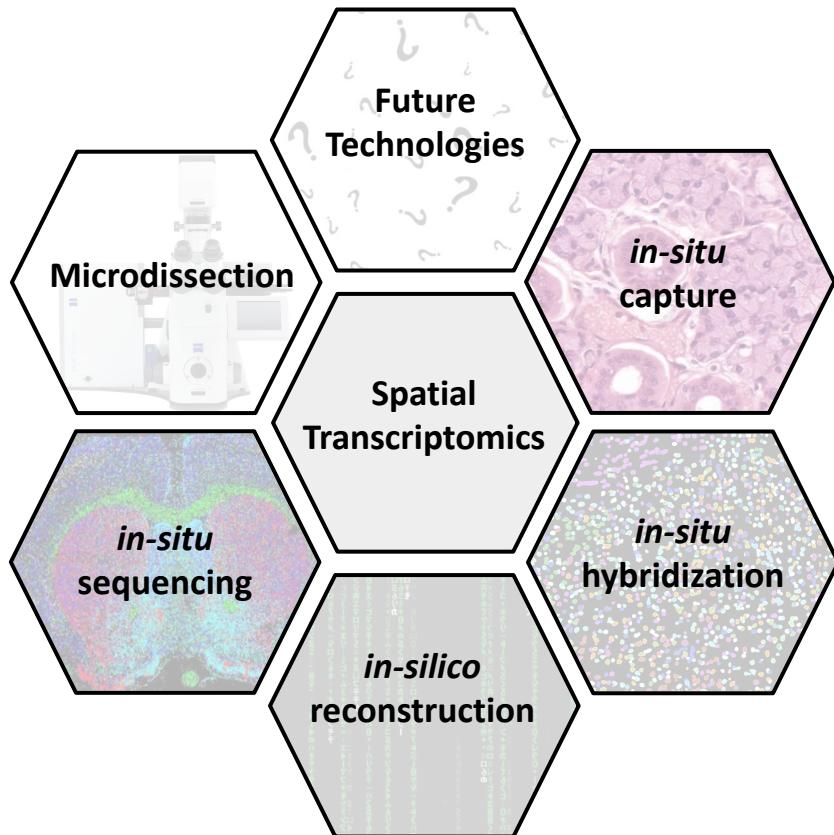
**Examples :** Visium, ST, Slide-Seq, HDST, GeoMX, Apex-Seq, Stereo-SEQ

# Experimental techniques



Search: Spatial Transcriptomics

# Experimental techniques



## Further Readings

***Spatially Resolved Transcriptomes—Next Generation Tools for Tissue Exploration***

**Authors :** Michaela Asp, Joseph Bergenstråhle, Joakim Lundeberg  
**Published :** 2020-05-04  
**DOI:** [10.1002/bies.201900221](https://doi.org/10.1002/bies.201900221)

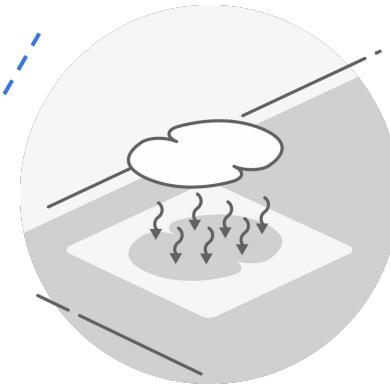
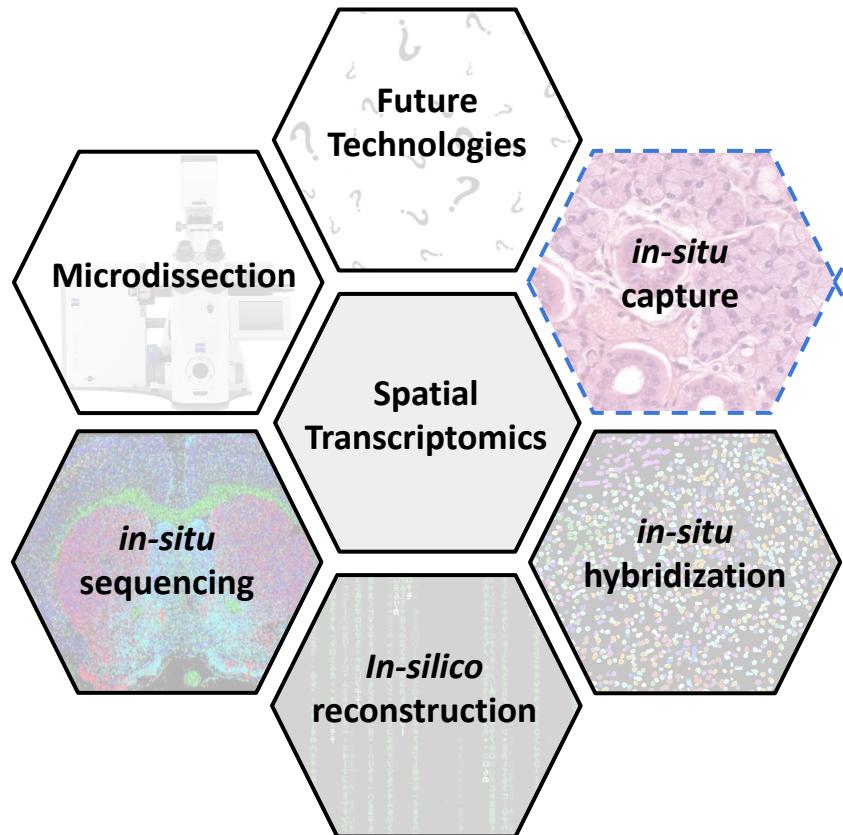
***Spatially resolved transcriptomics adds a new dimension to genomics***

**Authors :** Ludvig Larsson, Jonas Frisén & Joakim Lundeberg  
**Published :** 2021-01-06  
**DOI:** [10.1038/s41592-020-01038-7](https://doi.org/10.1038/s41592-020-01038-7)

***Museum of Spatial Transcriptomics***

**Authors :** Lambda Moses and Lior Pachter  
**Published :** 2021-05-12  
**Link:** [https://pachterlab.github.io/LP\\_2021/](https://pachterlab.github.io/LP_2021/)

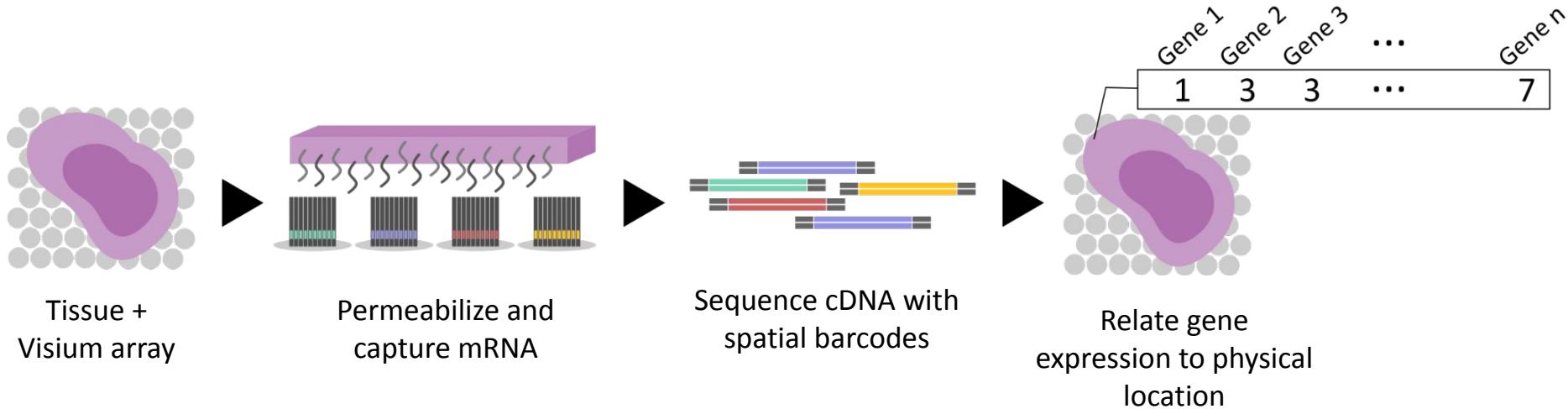
# Experimental techniques



**10X  
GENOMICS**

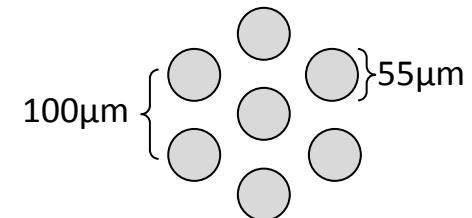
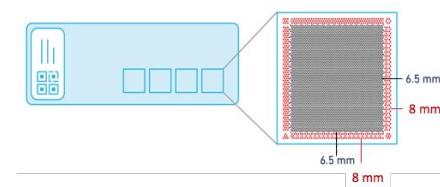
**Visium**

# Visium :: Recap

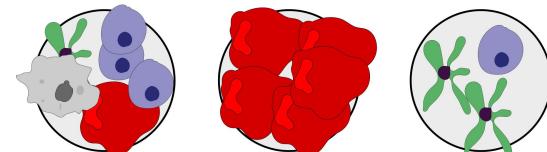


# Visium :: Recap

- Array based technique
- 6.5mm x 6.5mm area to put sample on
- 4992 spots arranged in hexagonal grid
- Array specs:
  - Spot diameter : 55 $\mu$ m
  - Center to center distance : 100  $\mu$ m
- Successor to Spatial Transcriptomics (ST)
- Data processing often includes :
  - Genome mapping and annotation
  - Spatial barcode demultiplexing
- Approx. 1-10 cells contribute to each spot
  - **NOTE** : Not single cell resolution!
- Data represented as [spot] x [gene] matrix
- You also get HE-image of the **same** tissue



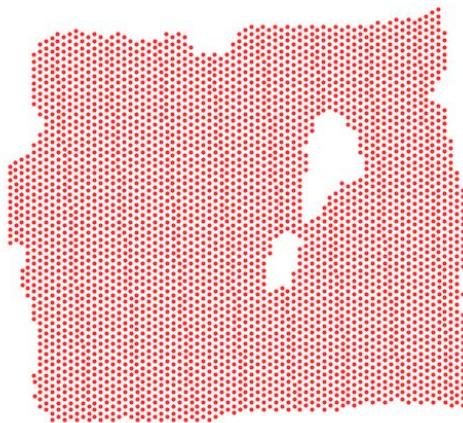
Spot 1      Spot 2      Spot 3



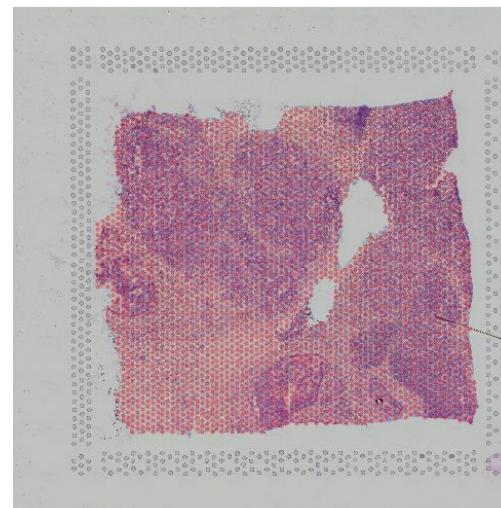
# Visium :: Recap

- Example with Human Breast cancer data
  - Public data : Available at 10x website

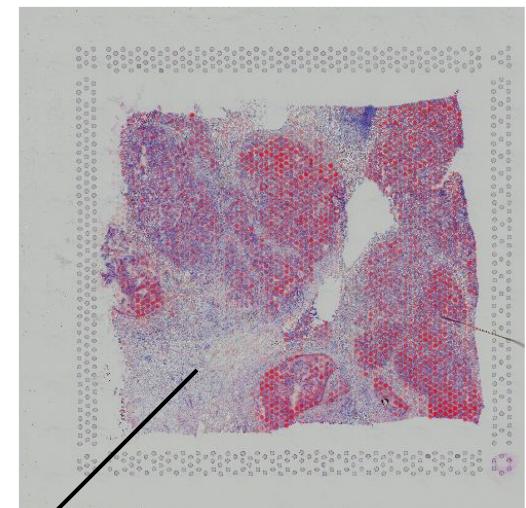
Spots Only



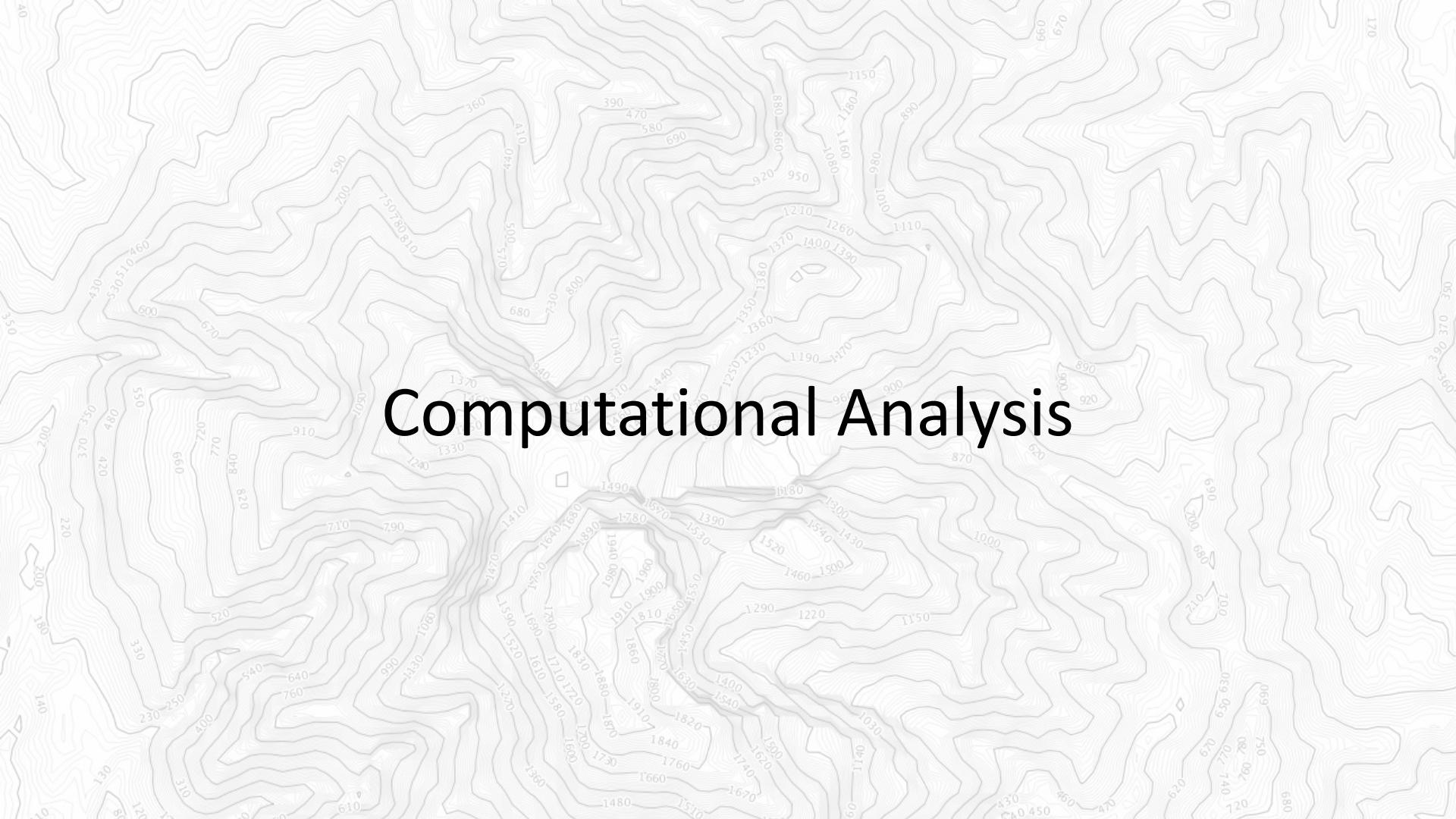
Spots + HE image



Spots + ERBB2 expression + HE image

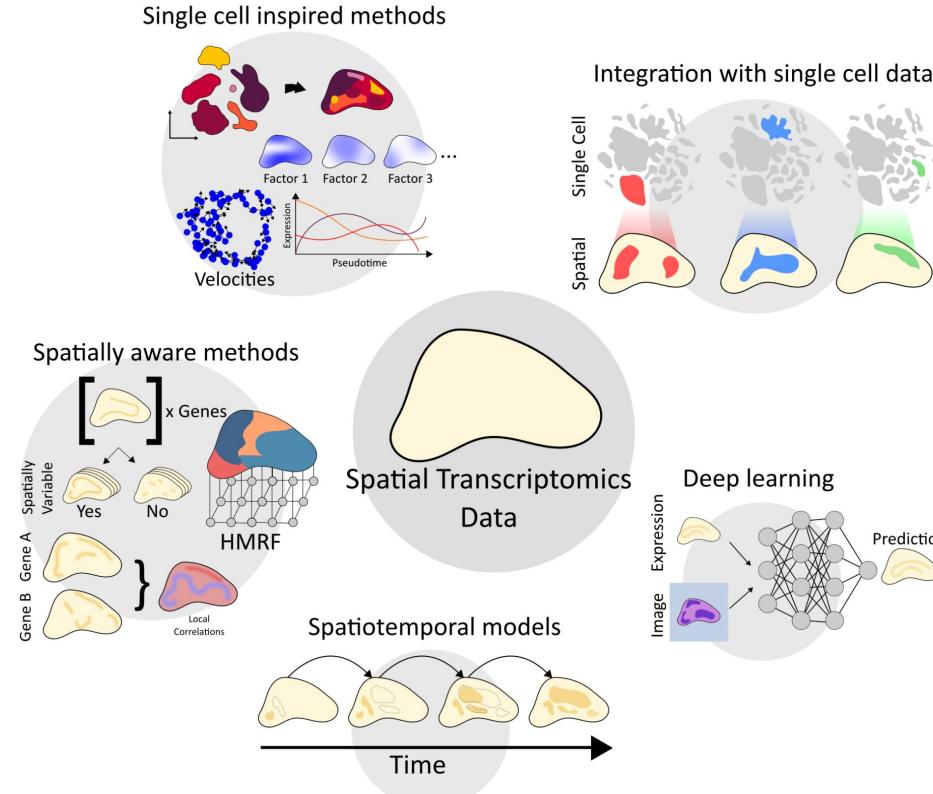


Facecolor intensity proportional  
to gene expression value



# Computational Analysis

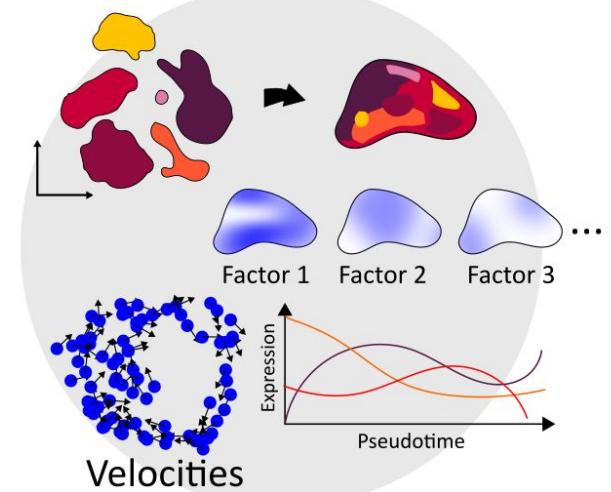
# A motley crew of diverse methods



# Single Cell Inspired methods

- **Basic idea :** apply existing methods and tools developed for single cell data.
- **Examples :**
  - Cluster spatial data, show clusters in space
  - Factor models for data decomposition
  - Trajectory Inference
- **Suites/Tools:**
  - Seurat : added support for spatial data
  - Scanpy : added support for spatial data
  - STUtility : built on Seurat tailored for spatial data
  - stLearn : built on scanpy tailored for spatial data
  - SpatialExperiment : (similar to SingleCellExperiment)
  - And many many more...

## Single cell inspired methods



# Clustering :: Human Breast Cancer Data

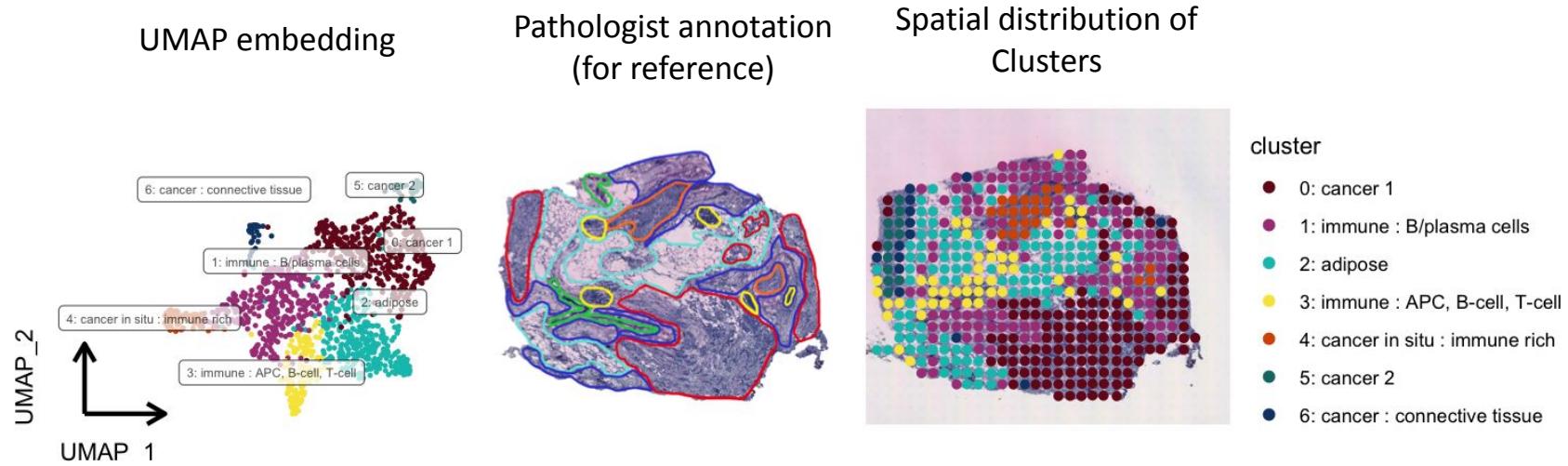
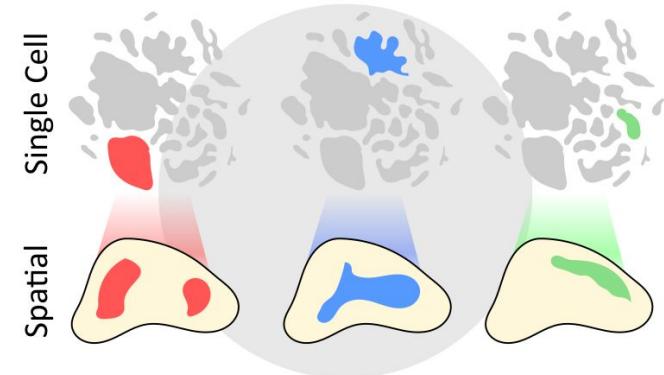


Figure from : *Spatial Deconvolution of HER2-positive Breast Tumors Reveals Novel Intercellular Relationships*, Andersson et al.

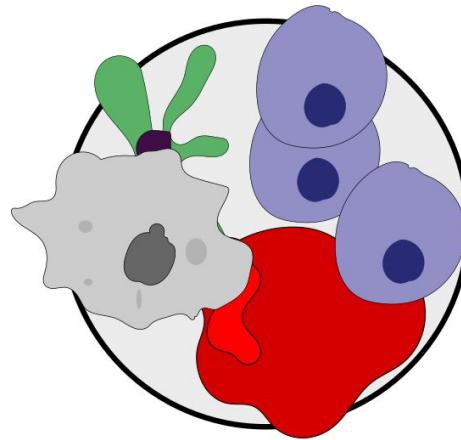
# Integration with single cell data

- **Basic idea :** use single cell data as a *reference* when working with spatial data.
- **Answers :** Where are cell types in SC data found in space?
- **But why?** Two main reasons :
  - **Efficient use of resources.** Leverage extensive annotation work done for single cell data.
  - Problem of **mixed contributions** (in Visium)

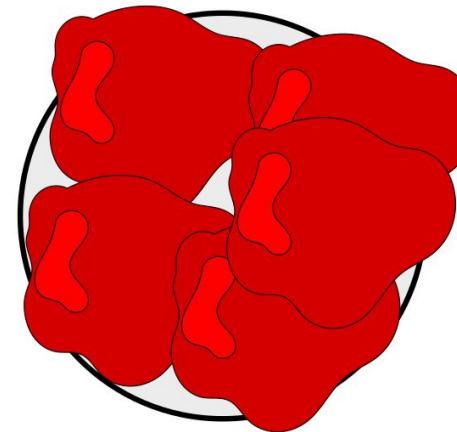
## Integration with single cell data



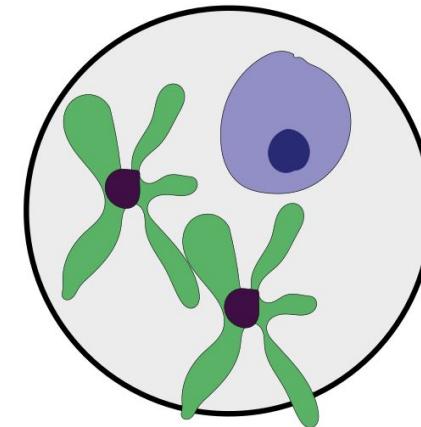
Spot 1



Spot 2



Spot 3



In several of the **capture based techniques** (e.g., Visium and Slide-seq), observed expression values are **contributions from multiple cells**. Not all necessarily of the same type.

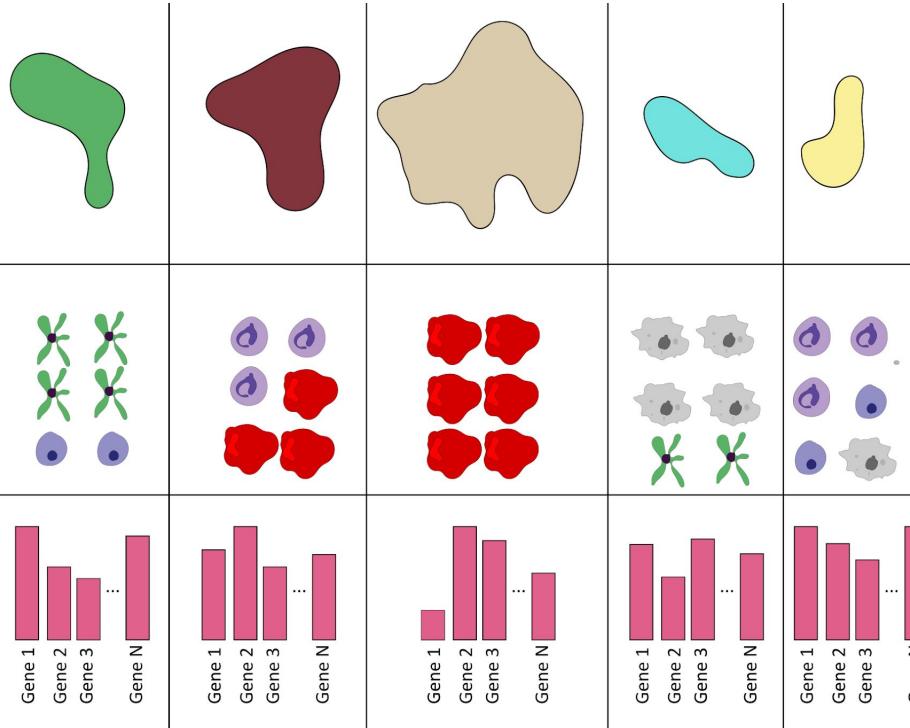
# Mixed contributions

16

Cluster

Population

Expression



- Clusters do not represent cell types
- Clusters are more an assembly of spots with **similar composition** of cell types.
- We have no idea what the cell type population looks like.  
**Only observe expression**

# Mixed contributions

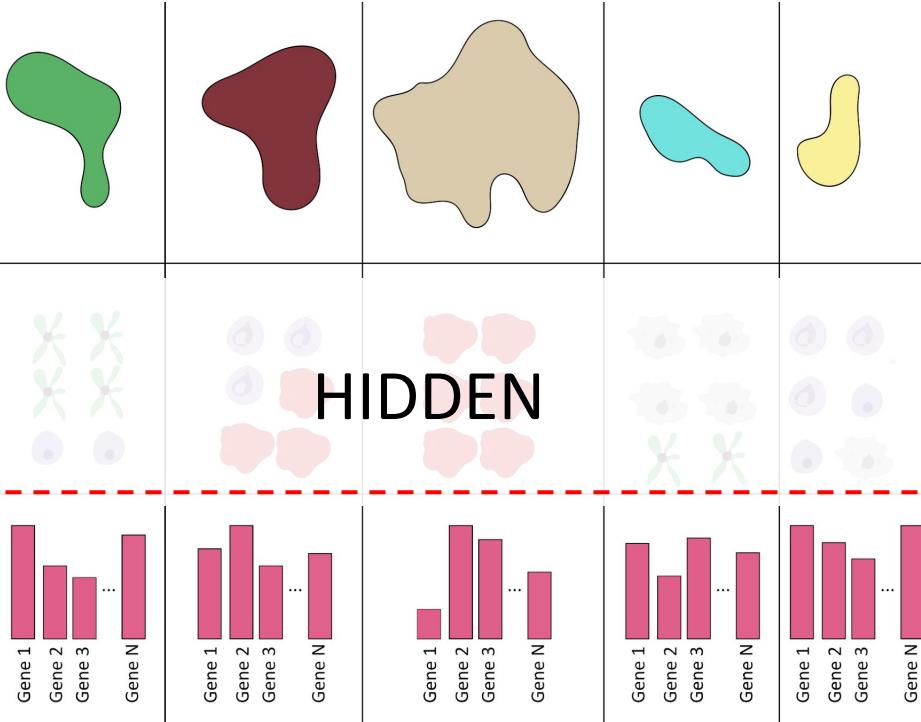
17

Cluster

Population

Expression

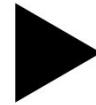
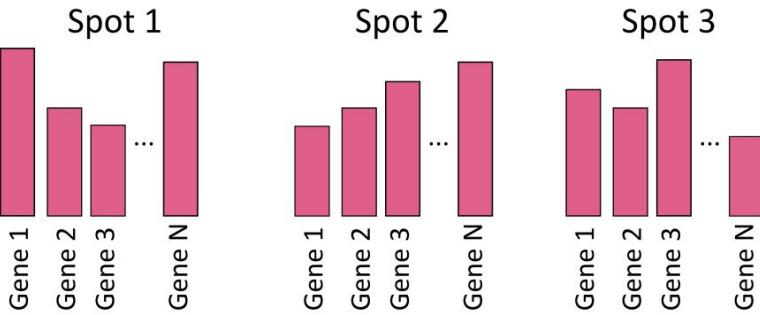
Observed



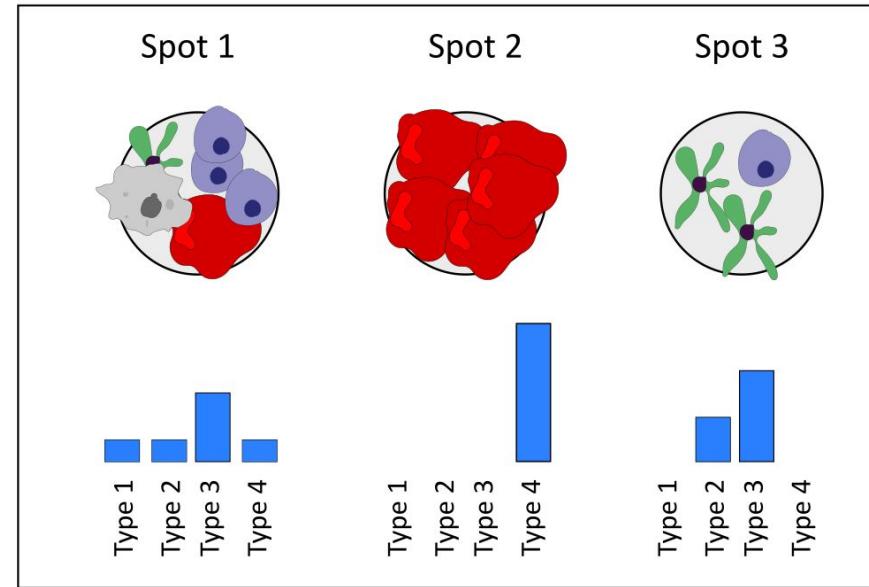
- Clusters do not represent cell types
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# Our objective : deconvolve expression data

From this

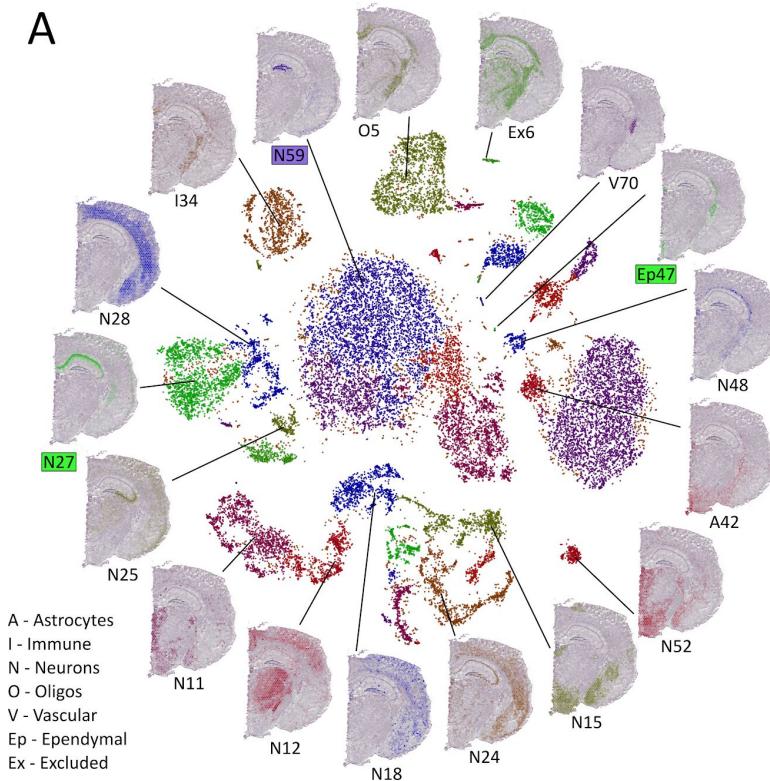


We want this



# Integration with single cell data

A



(generated with *stereoscope*)

- **Inner** : Single cell data from mouse brain, gt-SNE embedding. Colored by cluster.
- **Outer** : Visium data of mouse brain. Facecolor intensity indicates proportion value of cluster.

Figure 2 from "Single-cell and spatial transcriptomics enables probabilistic inference of cell type topography", Andersson et al.



# Integration with single cell data

## Marker gene based

Extract marker genes (MG) for each cell type from SC data

Compute enrichment score for each set of MGs in spatial locations

Normalize to make scores sum to 1

**Ex:** Moncada et al.

## Anchor based

Find anchors between modalities (MNNs). Create correction vector based on differences in expression.

Use correction vectors to remove platform effects.  
Integrated data sets.

Transfer labels of single cells to spatial data points.

**Ex:** Seurat

## Probabilistic Modelling

Assume gene expression follows certain statistical distributions.

Joint model for SC and spatial data. Learn cell type parameters from SC data, use to deconvolve spatial data (when mixed).

Correct for eventual platform differences

**Ex:** stereoscope, RCTD, cell2location

## Optimization based

Find spatial location where each cell is most likely to reside.

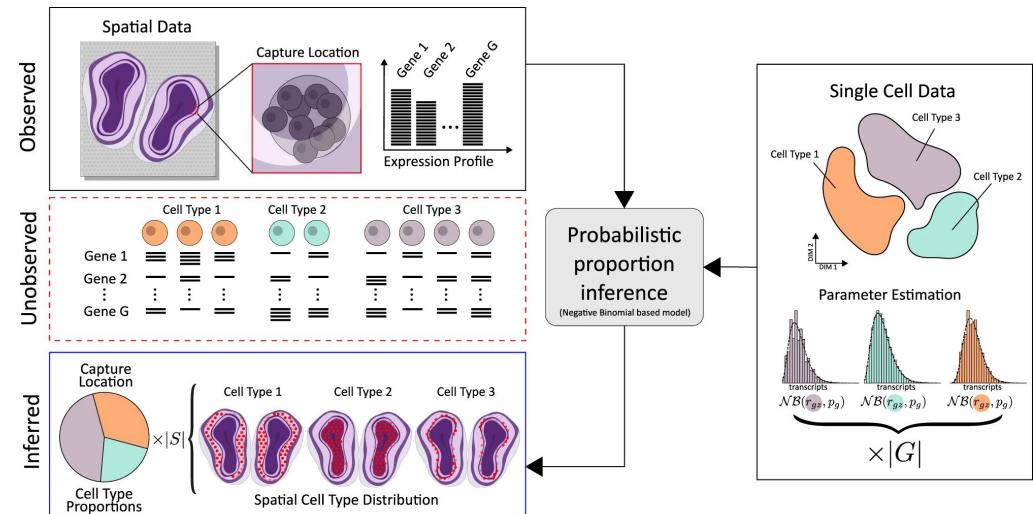
Tries to simultaneously optimize terms such as:

- Cell density
- UMI distribution across genes within spots
- gene distribution across spots

**Ex:** Tangram

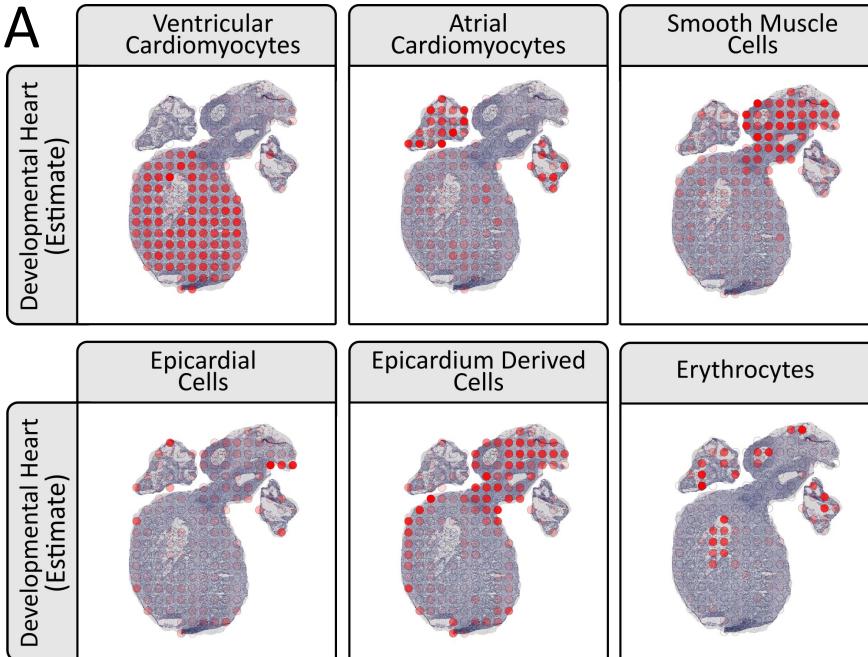
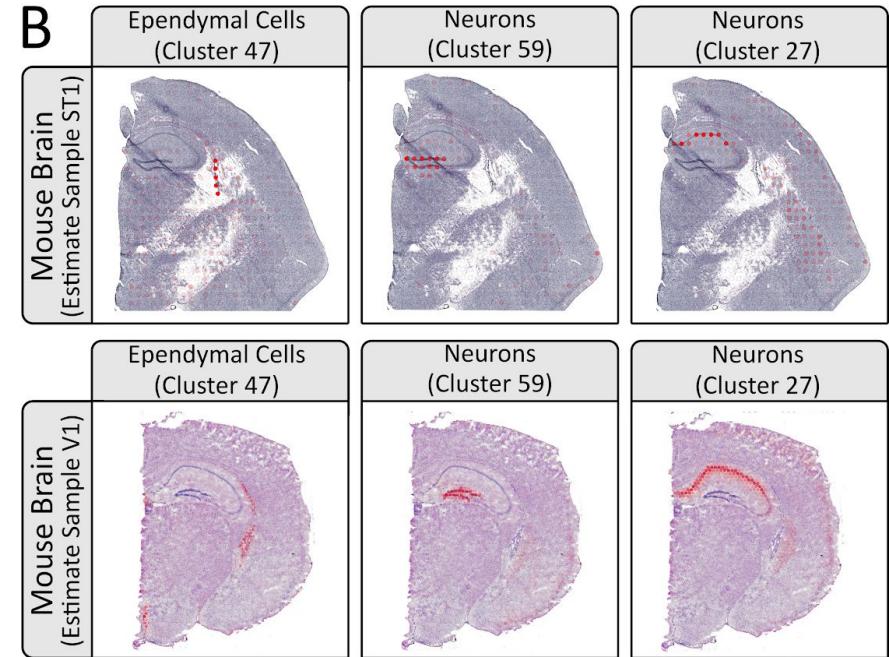
# Integration with single cell data :: *stereoscope*

- Probabilistic, models single cell and spatial transcriptomics data with **negative binomial** distribution
- Two-step process:
  1. Learn parameters from sc-data
  2. Infer proportions in spatial data
- Parameters from single cell data can be reused, cut computational time in half.
- Accounts for missing cell types by including a “dummy cell types”
- *“Single-cell and spatial transcriptomics enables probabilistic inference of cell type topography”*, Communications Biology, Andersson et al.

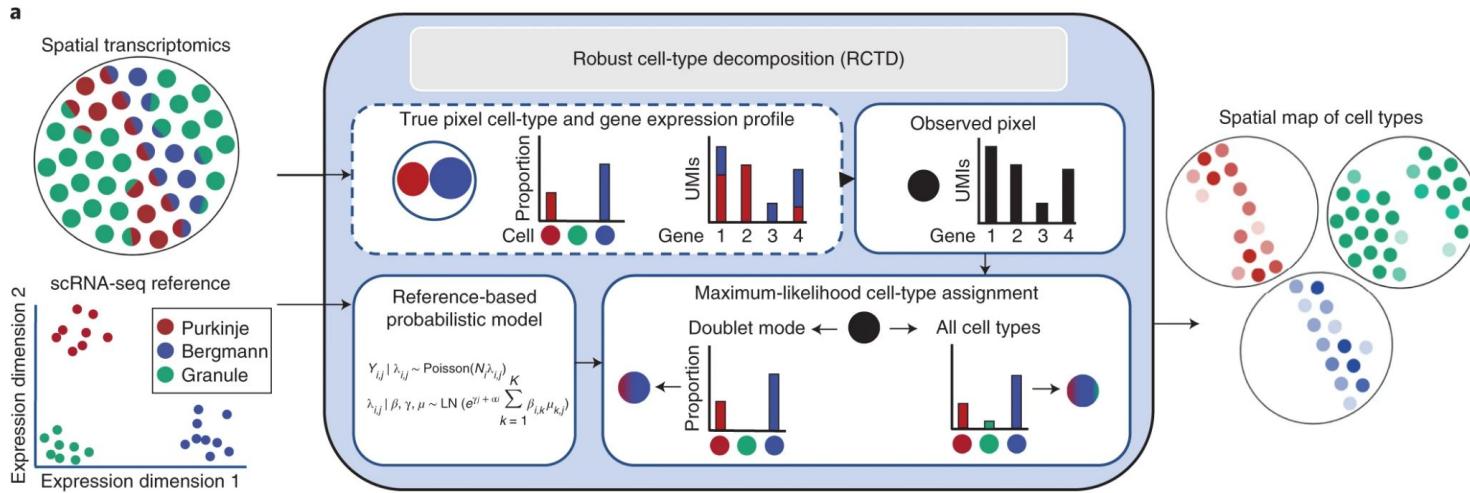


<https://github.com/almaan/stereoscope>

# Integration with single cell data :: *stereoscope*

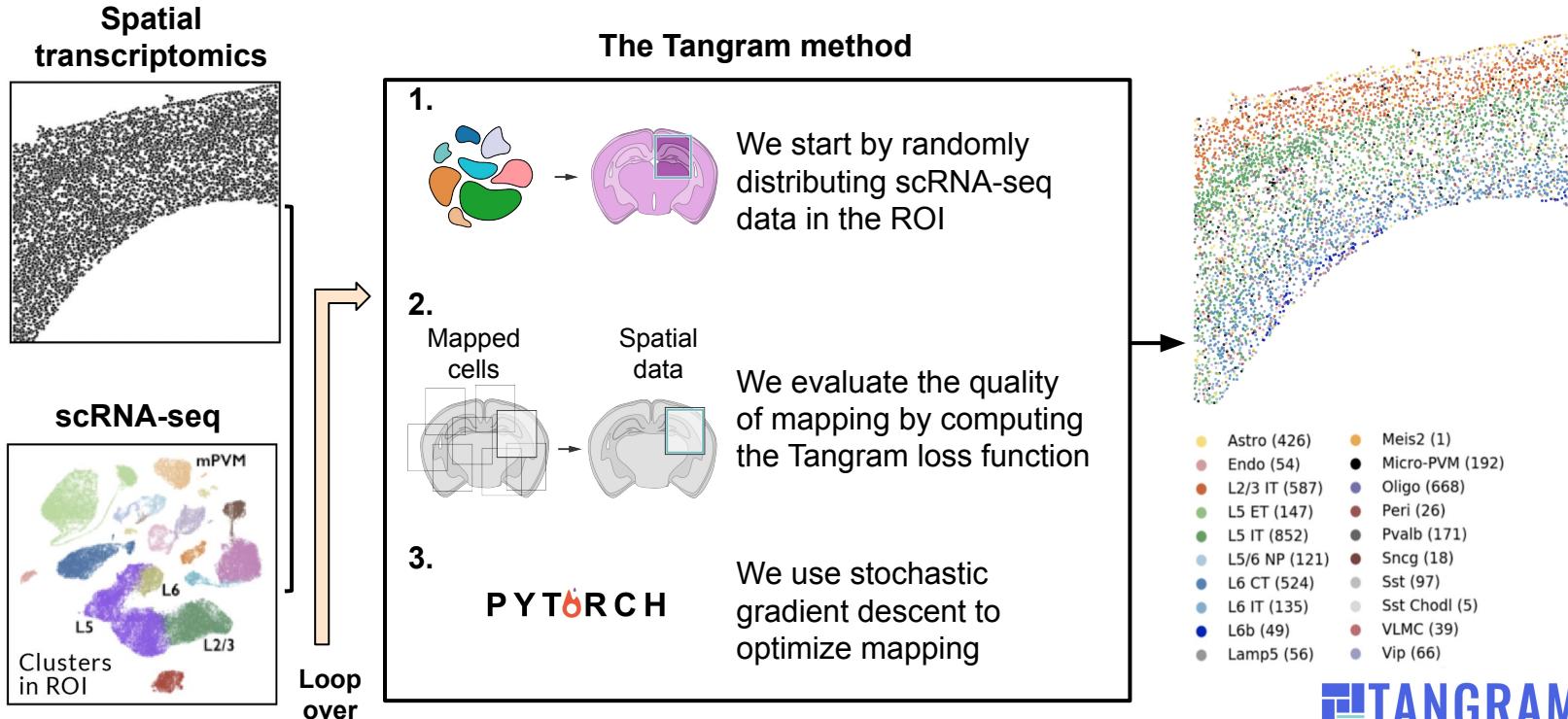
**A****B**Developmental heart : DOI: [10.1016/j.cell.2019.11.025](https://doi.org/10.1016/j.cell.2019.11.025)Mouse Brain : 10X Genomics website + [mousebrain.org](http://mousebrain.org)

# Integration with single cell data :: RCTD



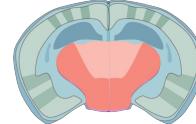
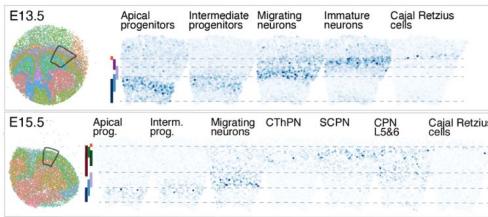
- Probabilistic model for **inferring cell types in spatial transcriptomics data**, supervised with a labeled single-cell RNA-seq reference.
- Infers **platform effects** (or technical differences across sequencing platforms) in order to correct for differences between the single-cell reference and the spatial target dataset.
- RCTD uses maximum likelihood estimation to **identify cell types present on each spatial transcriptomics spot, in addition to estimating cell type proportions**.
- Robust decomposition of cell type mixtures in spatial transcriptomics, Nat. Biotech, Cable et al.

# Integration with single cell data :: Tangram



# Integration with single cell data :: Tangram

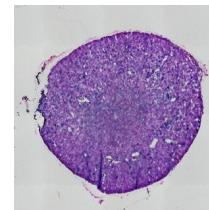
## Spatial maps of cell types in developmental mouse brain



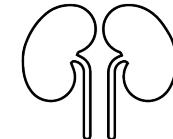
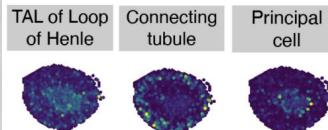
with Paola Arlotta lab  
(*Nature* 2021 *in press*)

## Assessing cross-species conservation in kidney

### Mouse kidney histology

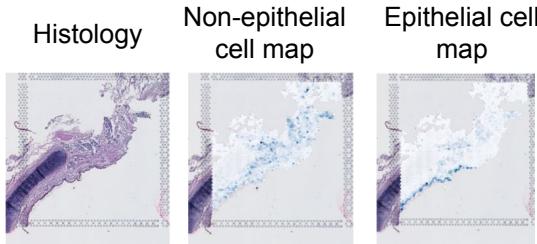


### Cell type maps in kidney



with Aviv Regev lab  
(*Nature Methods* 2021 *in press*)

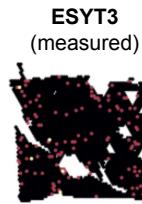
## Localization of epithelial cell types in human lung



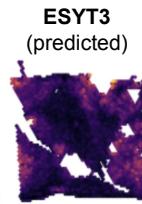
with Jay Rajagopal lab  
(*in preparation*)

## Correction of gene expression in colorectal cancer

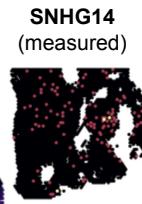
ESYT3  
(measured)



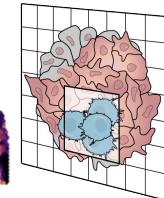
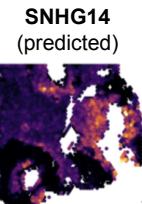
ESYT3  
(predicted)



SNHG14  
(measured)

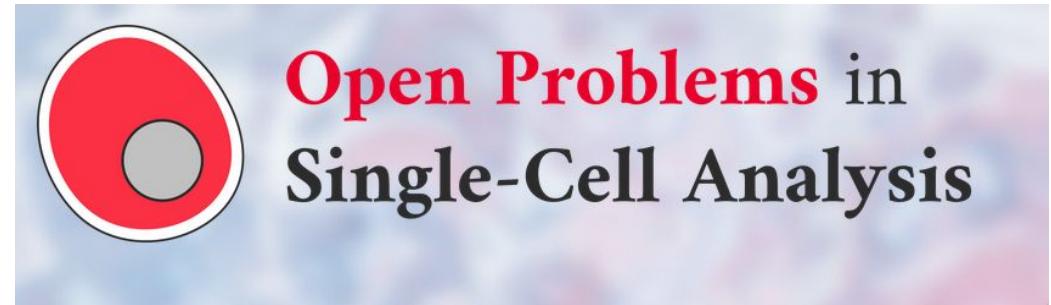


SNHG14  
(predicted)

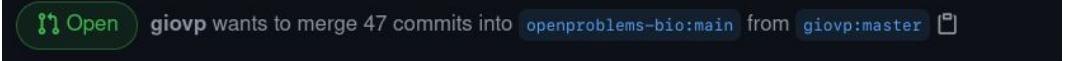


with Fred De Sauvage

- Initiative to formalize problems in single cell (and spatial) analysis. Includes *proper* definition.
- Provide datasets for unbiased evaluation of data, and define metrics to be used.
- Build framework for said evaluation.
- Allows you to make informed choice.
- <https://openproblems.bio/>



[new task] Spatial decomposition #309



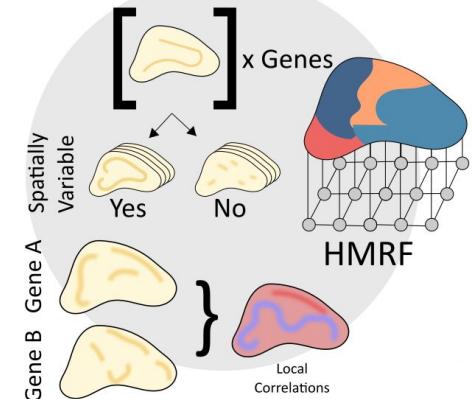
# Spatially aware methods

**Basic Idea :** Attempts to include knowledge of spatial structure in the analysis, not only to visualize results.

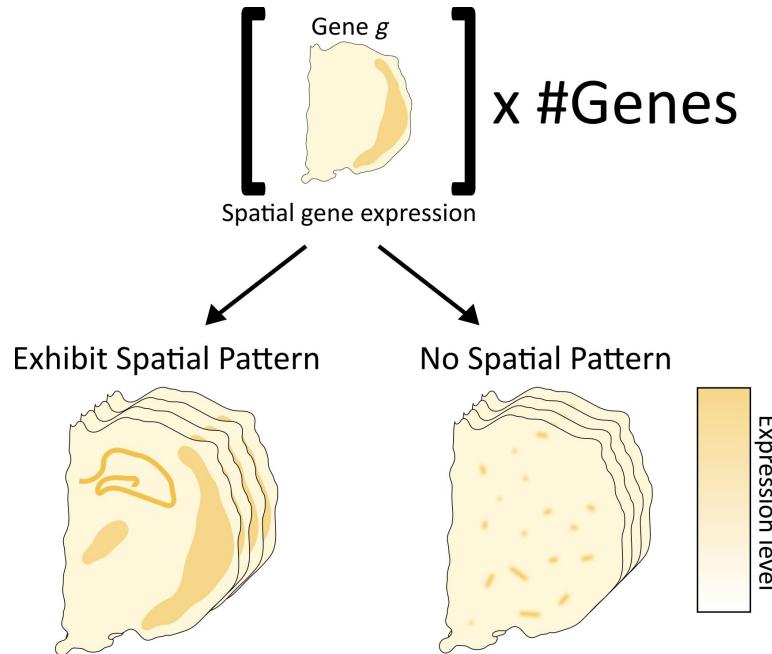
**Designed for tasks like :**

- Identifying *spatially* variable genes and features
  - Why not just select highly variable genes ([more later](#))
- Finding spatially coherent expression domains
- Leverage spatial proximity to increase robustness of inference (e.g., CNA inference)
- Find *local* correlations between features

Spatially aware methods

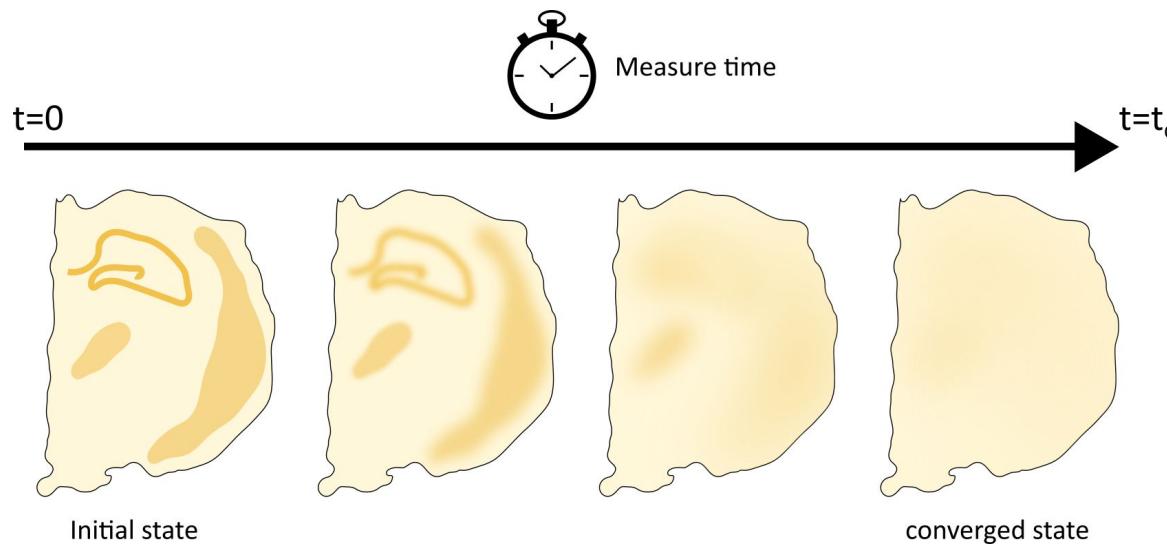


# Spatially Variable Genes



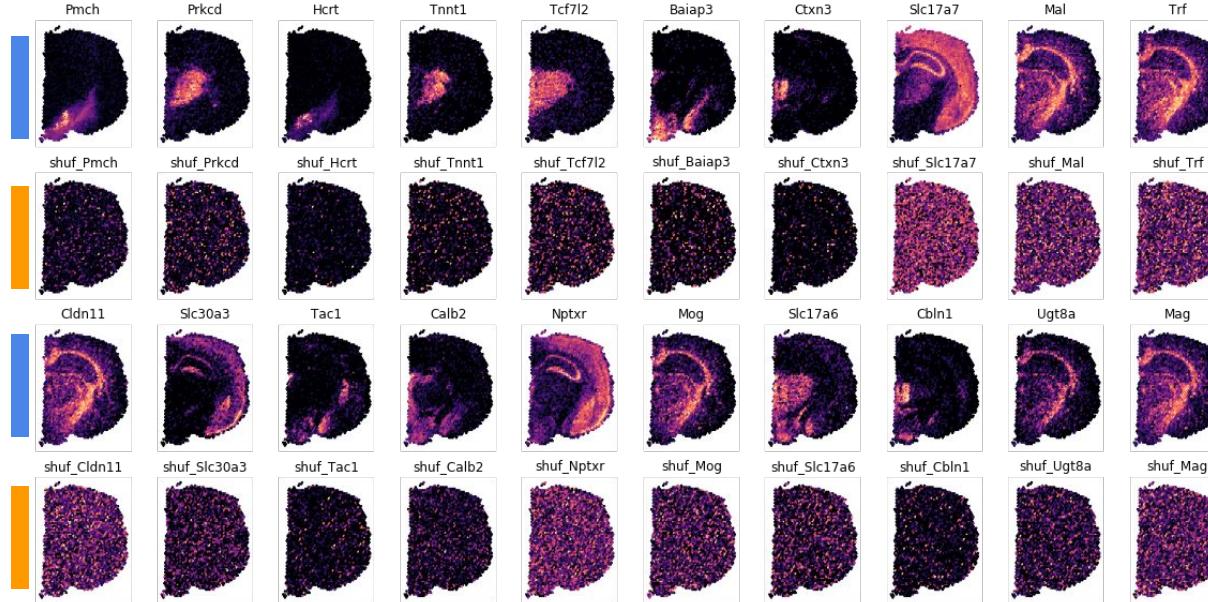
- Sort expression profiles into spatially variable or not.
- SpatialDE, SVCA and SPARK use probabilistic models
- Leverage *Gaussian Processes* to model data
- Essentially, test whether a “spatial” term in the covariance function significantly increase model’s ability to explain data

# Spatially Variable Genes



- *sepal* is not probabilistic
- Uses finite differences to simulate diffusion of transcripts.
- Measures time until converges
- Ranks genes by the time it takes to converge.
- **Key Idea** : The longer the time, the more structured the initial state.

# Spatially Variable Genes



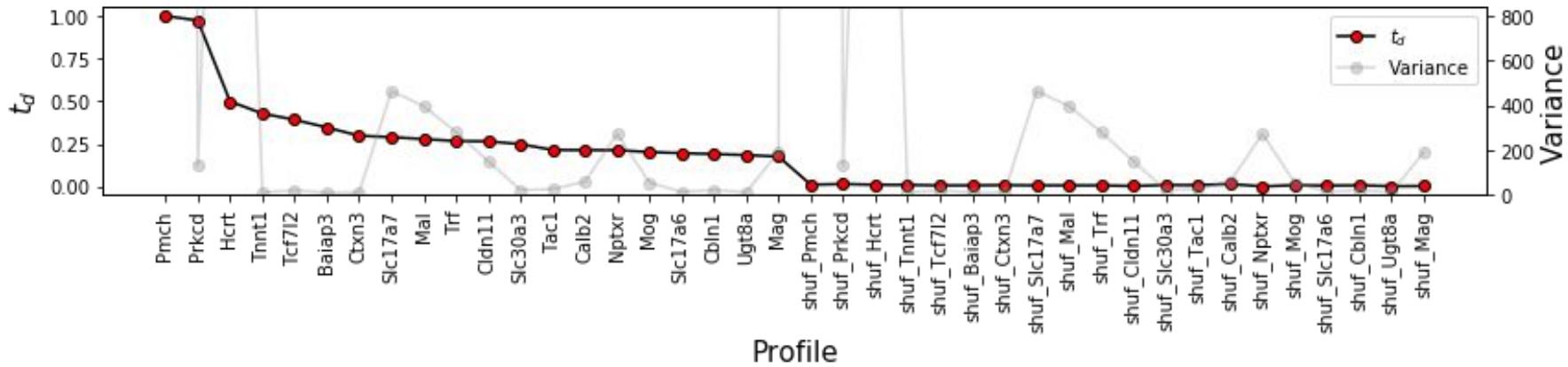
- 20 Expression profiles from mouse brain
- Shuffle spots to get random expression profiles. Has “shuf” prefix.

Observed Profiles

Shuffled Profiles

# Spatially Variable Genes

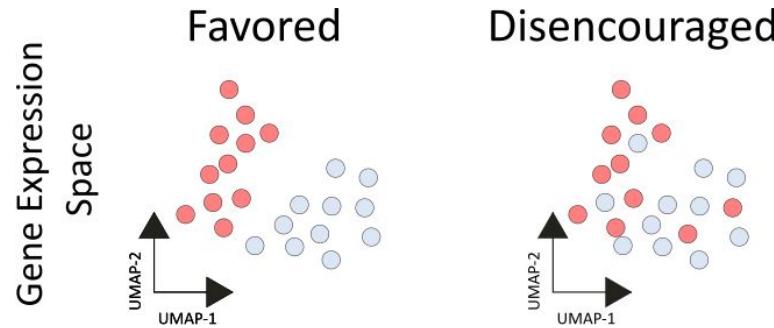
33



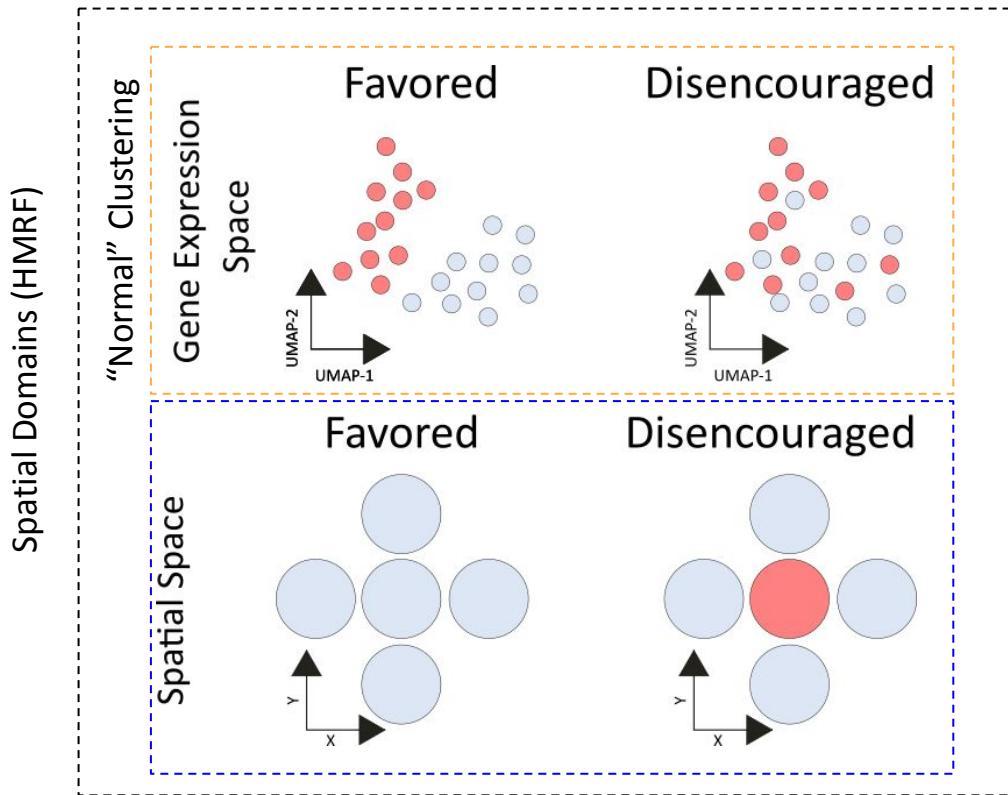
- Variance or dispersion metrics render exactly the same value (gray) for shuffled and non-shuffled profiles
- *sepal's* ranks real profiles higher than shuffle ones (spatial structure considered)
- Similar results obtained for other methods as well (SpatialDE, SPARK, etc)

# Spatials domain patterns

- Normal clustering mainly focus on gene expression



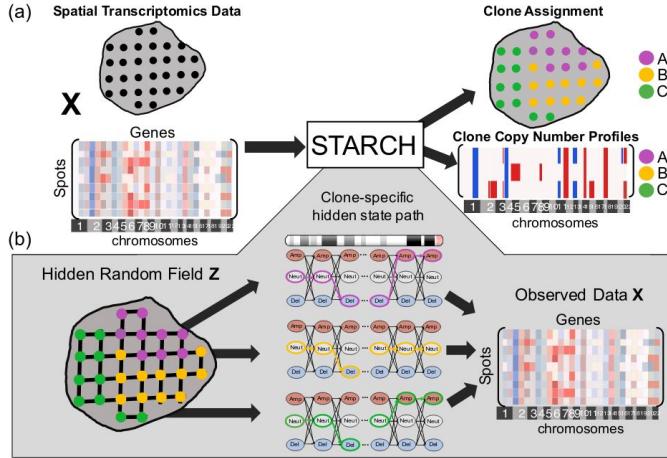
# Spatial domain patterns



- Normal clustering mainly focus on gene expression
- Leverage spatial information to find spatially coherent clusters (domains)
- Common to use HMRF (Hidden Markov Random Field)
- Construct a graph based on spatial proximity
- Probability of node (spot) belonging to a specific domain depends on:
  - Agreement with domain expression profile
  - Coherence with neighbors

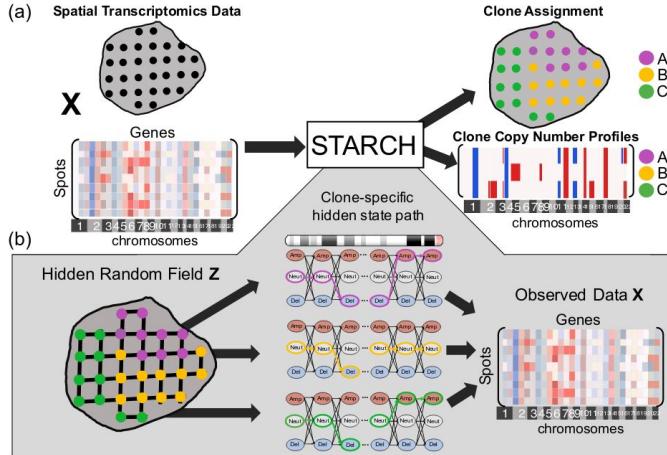
Example : Identification of spatially associated subpopulations by combining scRNAseq and sequential fluorescence *in situ* hybridization data", Zhu et al.

# Spatially aware methods :: STARCH and scHOT



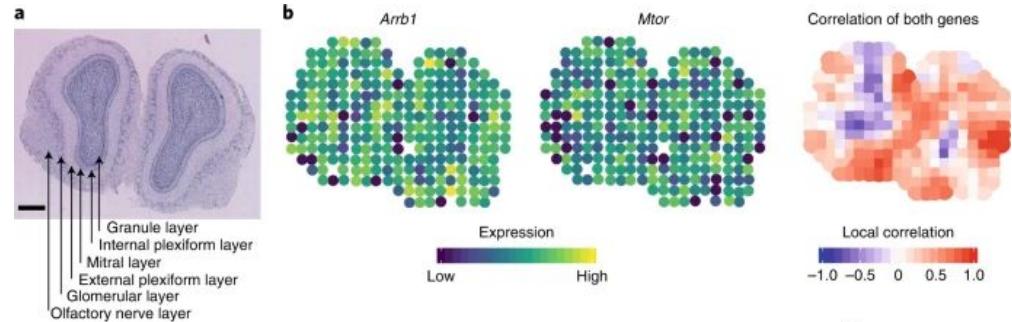
- **Name :** STARCH
- Infer Copy Number Aberrations (CNA) from spatial transcriptomics data
- Increase robustness of inference by aggregating data in same domains (similar profiles)
- Also uses Hidden Markov Random Fields (HMRF)
- “*STARCH: Copy number and clone inference from spatial transcriptomics data*”, Elyanow et al.

# Spatially aware methods :: STARCH and scHOT



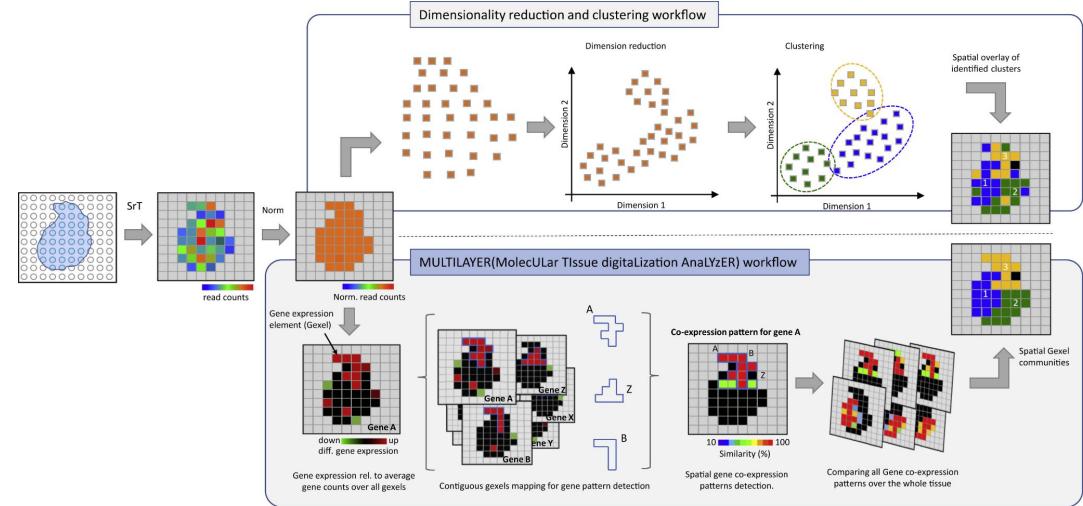
- **Name : STARCH**
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- Increase robustness of inference by aggregating data in same domains (similar profiles)
- Also uses Hidden Markov Random Fields (HMRF)
- “*STARCH: Copy number and clone inference from spatial transcriptomics data*”, Elyanow et al.

- **Name : scHOT**
- Computes (spatially) weighted correlations to find local correlations.
- “*Investigating higher-order interactions in single-cell data with scHOT*”, Ghazanfar et al.



# Spatially aware methods :: MULTILAYER

- Inspired by digital image processing.  
Introduces terminology of “gexels”.
- Looks at relative gene expression of each gexel compared to the rest. Find locally up and down-regulated genes.
- Uses agglomerative clustering to find contiguous patterns that share similar structures (co-expression modules)
- Extracts communities (clusters) from co-expression modules by using Louvain clustering.



# Spatiotemporal Modeling :: Splotch

- Hierarchical generative probabilistic model for analyzing Spatial Transcriptomics data
- Uses Zero Inflated Poisson (ZIP) regression model to account for:
  - Tissue region context
  - Local components
  - Spot effects
- Also aligns sections
- Can identify genes that changes over both space and time

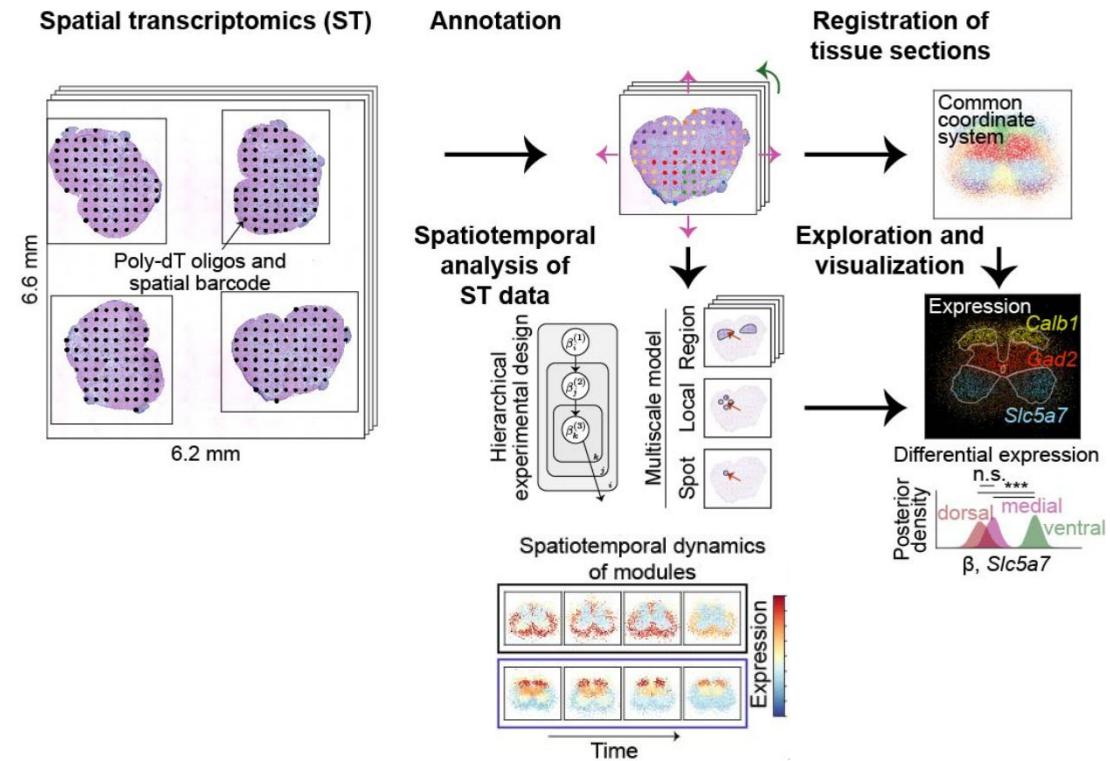
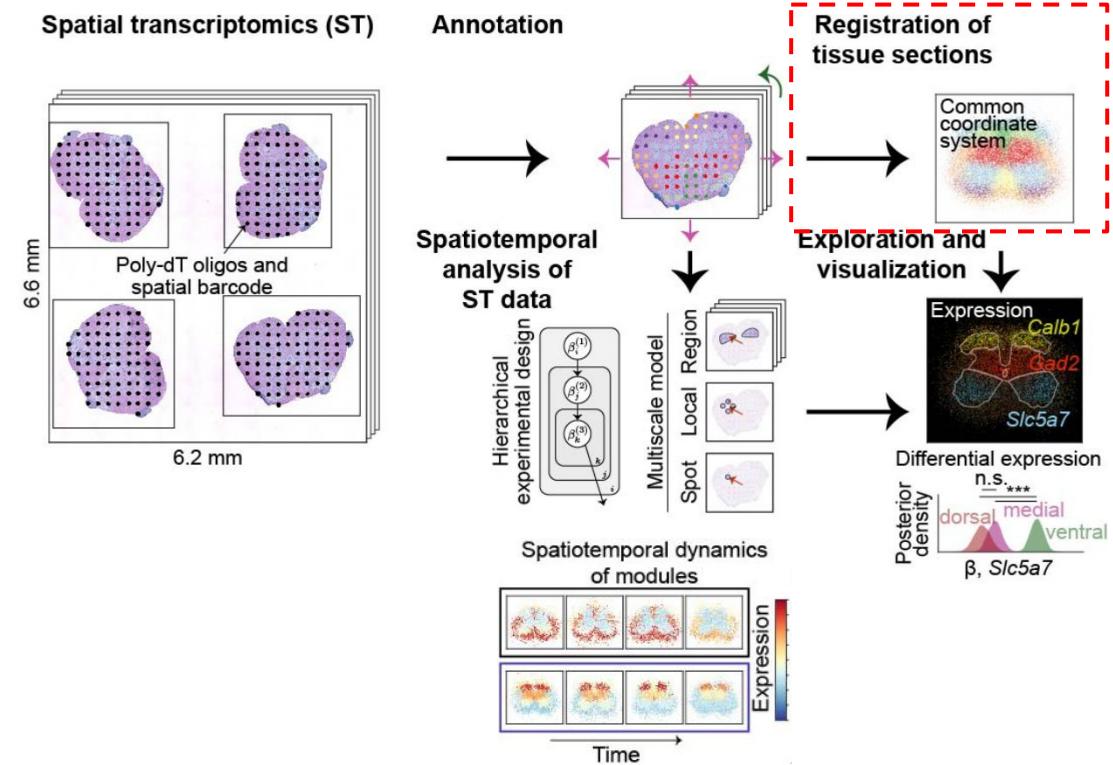


Image adaptation from : Splotch: Robust estimation of aligned spatial temporal gene expression data, T Äijö et al. (Supplementary Figure 1,2)

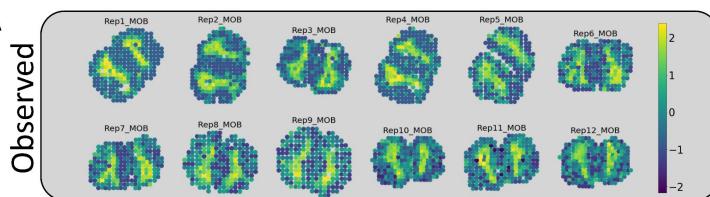
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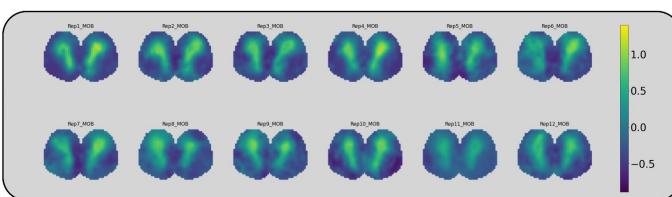
# Sneak Peek :: *eggplant*

A

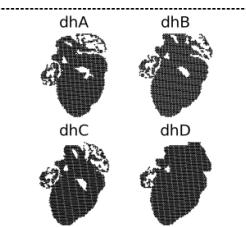


- New method (*eggplant*) that maps features (gene expression, cell type proportions) to a *common coordinate framework (CCF)*
  - Allows user to define a reference and then transfer feature values to it
  - Enables spatiotemporal modeling and facilitates construction of atlases

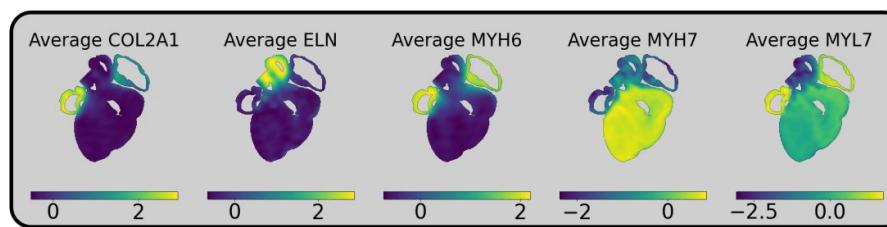
Mapped



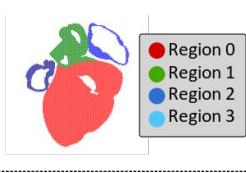
### Observed



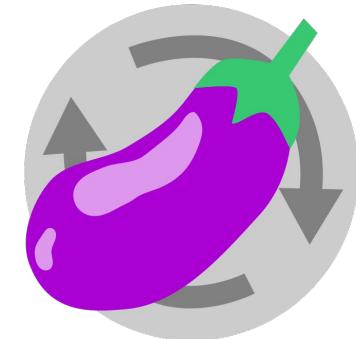
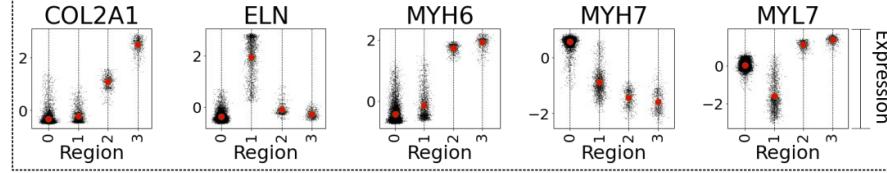
Transferred



Reference



Enrichment



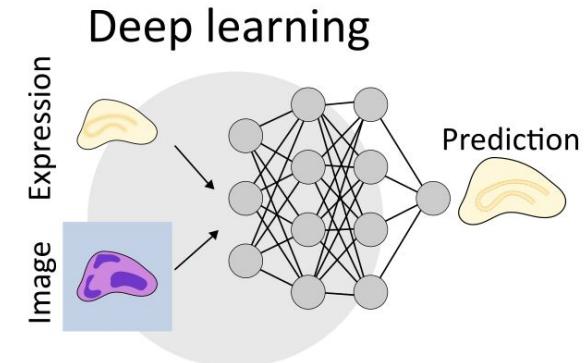
# Deep Learning

**Basic Idea :** Apply deep learning to spatial data (very broad)

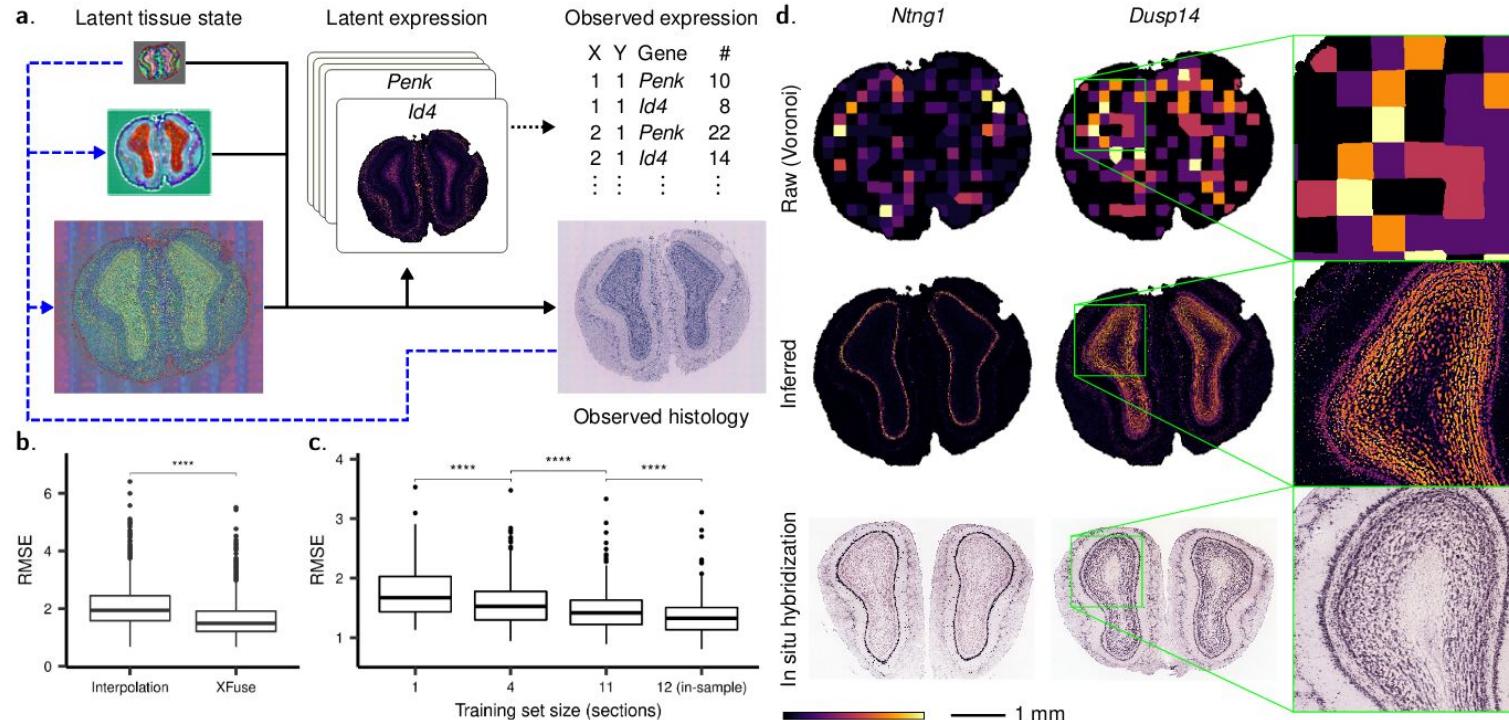
**Fairly nascent :** Relatively few examples. Limited amount of high quality available data.

**Current examples :**

- **XFuse** : “superresolution” (pixel) of gene expression by learning joint representation of image and expression data.
- **stPlus** : Uses scRNA-seq data and autoencoders to enhance spatial transcriptomics data
- **SpaGCN** : simultaneous domain and SVG detection using graph convolution layers
- **RESEPT** : Uses graph convolutional network to embed spatial data in RGB space, then uses a CNN to segment data into spatially coherent tissue domains



# Deep Learning :: XFuse

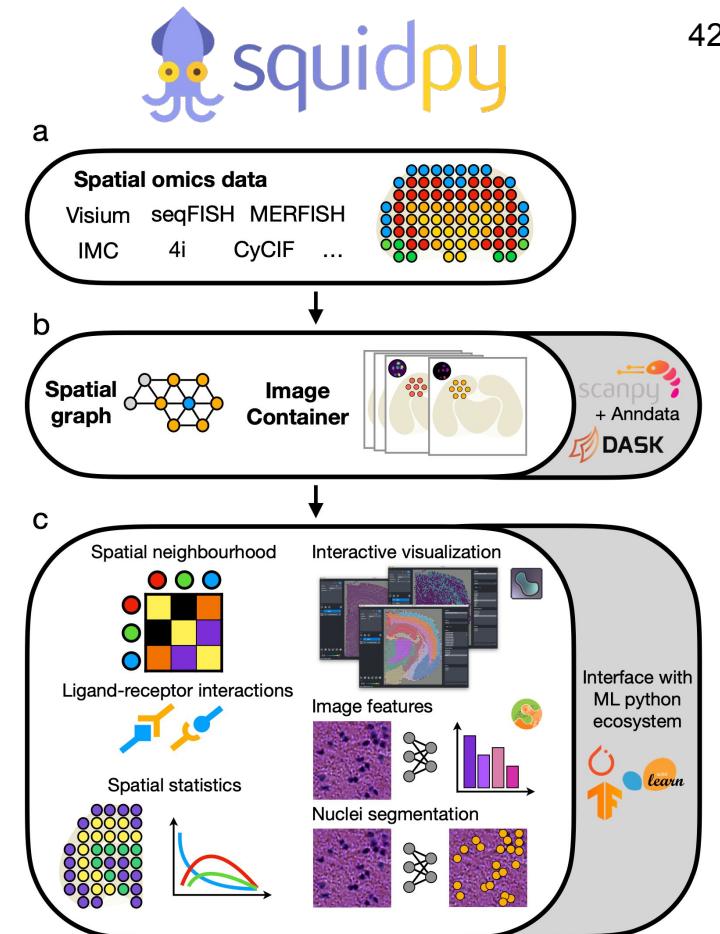


From : “Super-resolved spatial transcriptomics by deep data fusion”, Bergenstråhlé et al. (in press *Nature Biotechnology*)

# ■ ■ ■ Computational suites :: squidpy

*“One framework to rule them all, one framework to find them...”*

- Similar philosophy as scanpy, uses same kind of API, built on AnnData objects
- Tailored towards spatial data with support for multiple different experimental platforms (not only Visium)
- Easy to construct spatial graphs and perform graph operations
- Has great interface with ML ecosystems such as PyTorch, TensorFlow and sklearn
- Simplified my life a lot and something I tend to use now in method development
- (Also has sepal integrated into the suit)



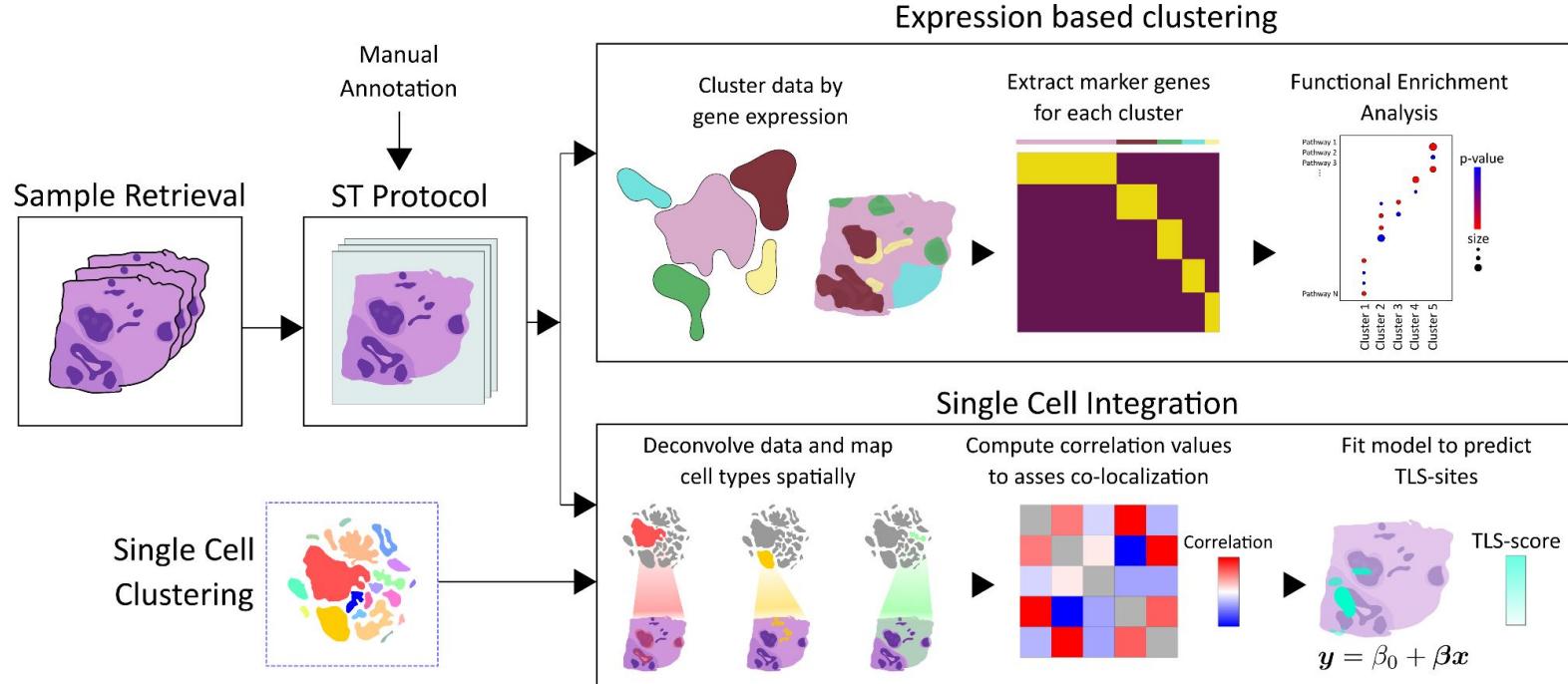


# Observations from the wild

# General advice

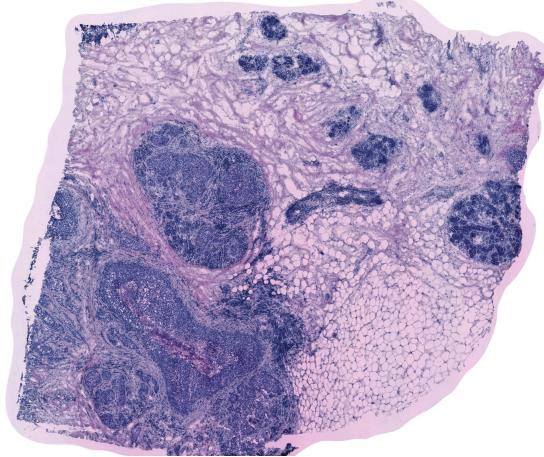
- **Batch effects** between sections are usually observed, try to account for this. Single cell methods have worked great so far.
- **Cell density** is often not homogeneous across tissue. Good idea to normalize based on the library size to account for this.
- Keep in mind that expression profiles are **mixtures**, often it makes more sense to analyze them accordingly; looking at **factor** contributions rather than hard cluster identities.
- Single cell mapping is often **improved by use of HVG** genes or curated lists
- **Trajectory inference is tricky**, no method that I'm aware of accounts for the fact that several temporal states might be present at each observation. Incorporation of spatial information has been done fairly heuristically so far.
- Filtering **ribosomal, mitochondrial and Hb-genes** usually have a positive effect on the result. They usually constitute irrelevant sources of variation. However, keep them if relevant!
- Use the image to visualize and inspect your data, one of the best quality checks there is. Always ask yourself: "does this make sense"?

# A spatial survey of HER2-positive breast cancer

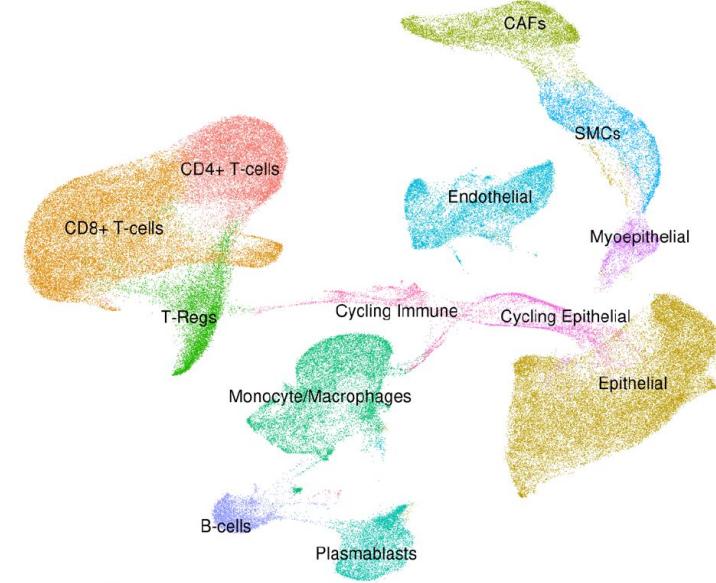


*"Spatial deconvolution of HER2-positive Breast cancer delineates tumor-associated cell type interactions"*, Andersson et al. (in press Nature Communications)

# Human HER2-positive breast cancer



**Spatial Data**  
Human Breast Cancer  
HER2-positive



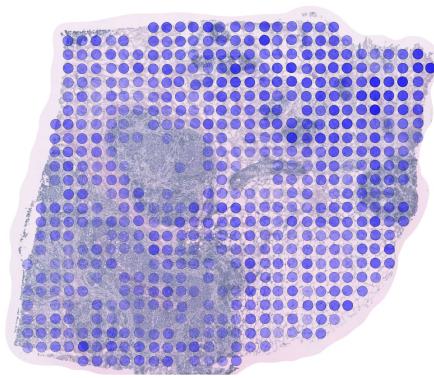
**SC Data**  
Human Breast Cancer  
Multiple types (incl. HER2)



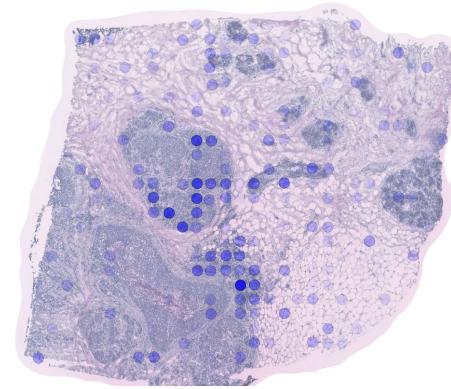
## Proportion estimated overlaid on tissue

46

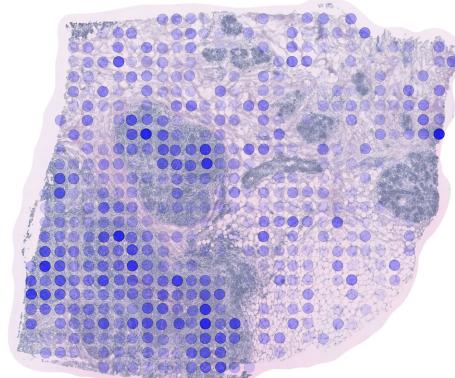
## Plasma Cells



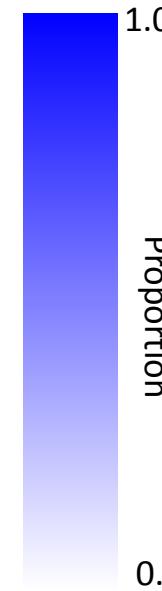
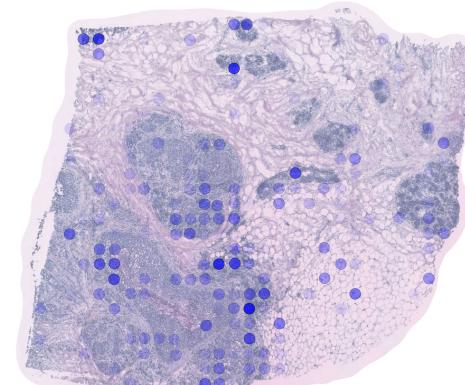
## CD4+ T-cells



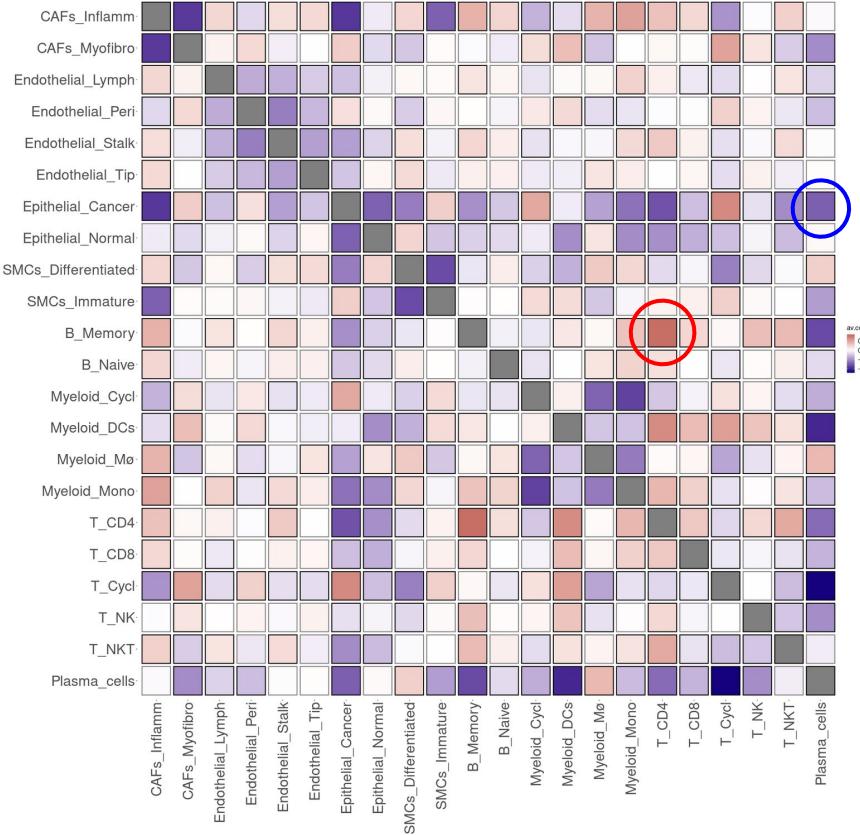
# Epithelial Cancer



## Memory B-cells

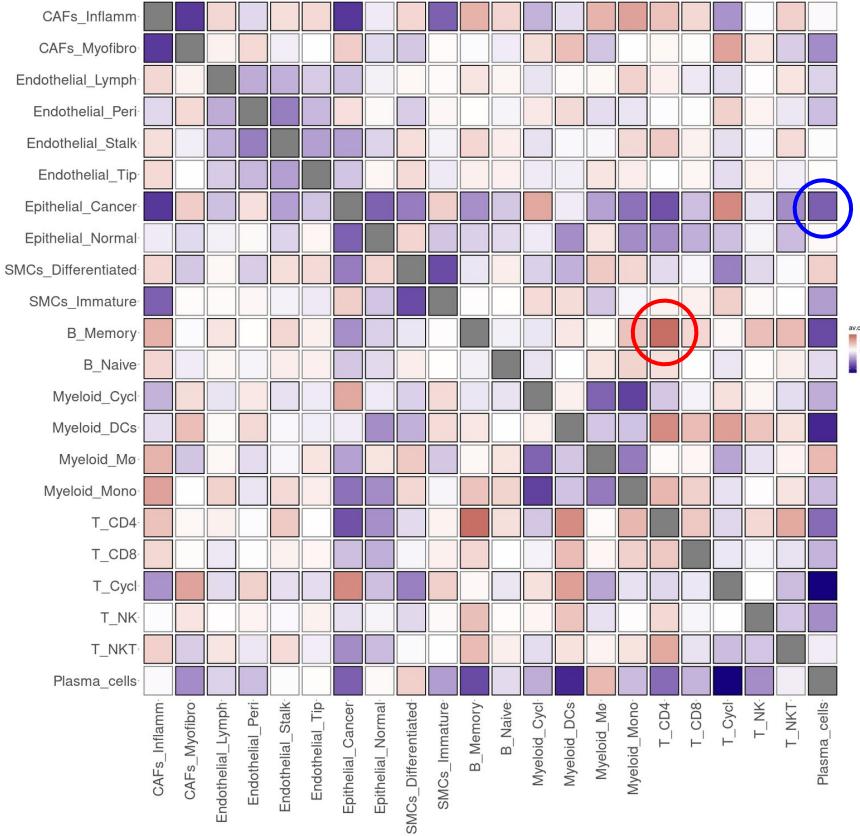


# Cell type co-localization



- Find cell types with similar spatial distributions
- Confirms previous observations
  - Plasma cells and Epithelial Cancer anticorrelate
  - Memory B-cells and CD4+ T-cells co-localize

# Cell type co-localization



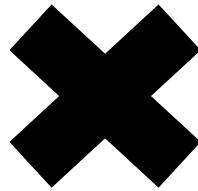
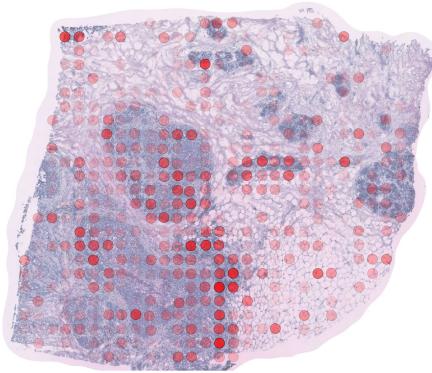
- Find cell types with similar spatial distributions
- Confirms previous observations

● Plasma cells and Epithelial Cancer  
anticorrelate

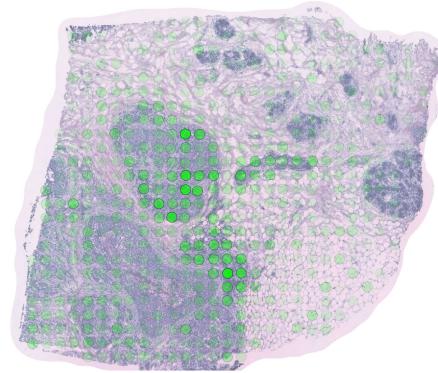
Memory B-cells and CD4+ T-cells co-localize

- Characterized by high presence of **B** and **T**-cells
- Interesting for several reasons
  - Partially dictates degree of TILs (Tumor Infiltrating Lymphocytes)
  - Implications in tumor treatment and outcome
- **Question :** Can we locate TLSs in our samples?

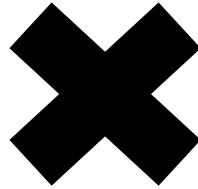
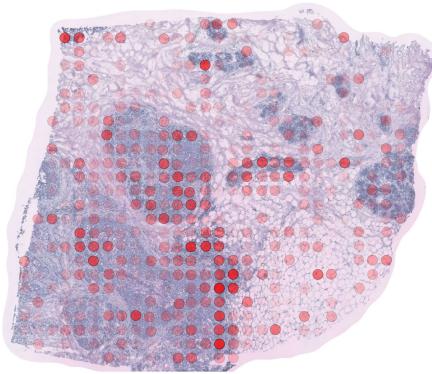
B-cells



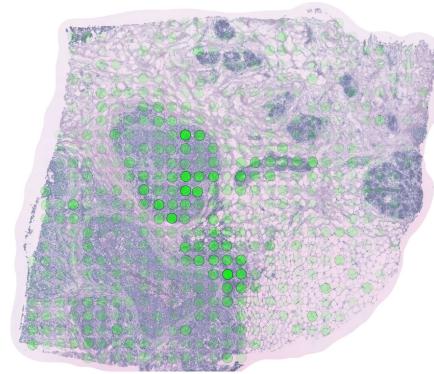
T-cells



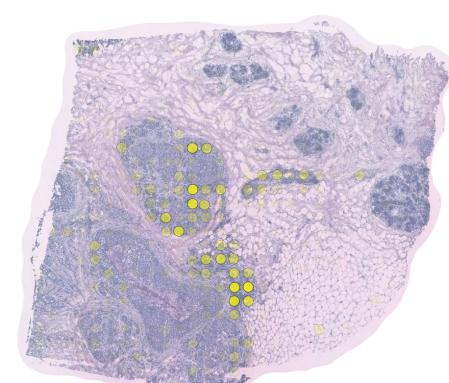
B-cells



T-cells



TLS

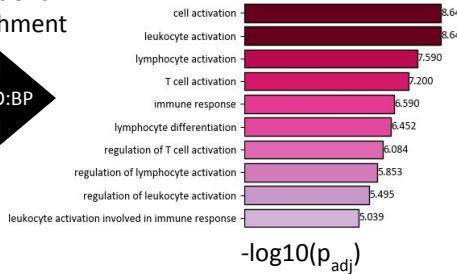


# Characterizing the expression landscape of TLSs

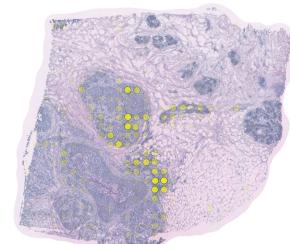
$$\beta_0 + \beta_1[\text{Gene 1}] + \dots + \beta_N[\text{Gene N}] =$$

Set of  
TLS-related genes:  
*CXCL13*  
*LTB*  
*CXCR5*  
...

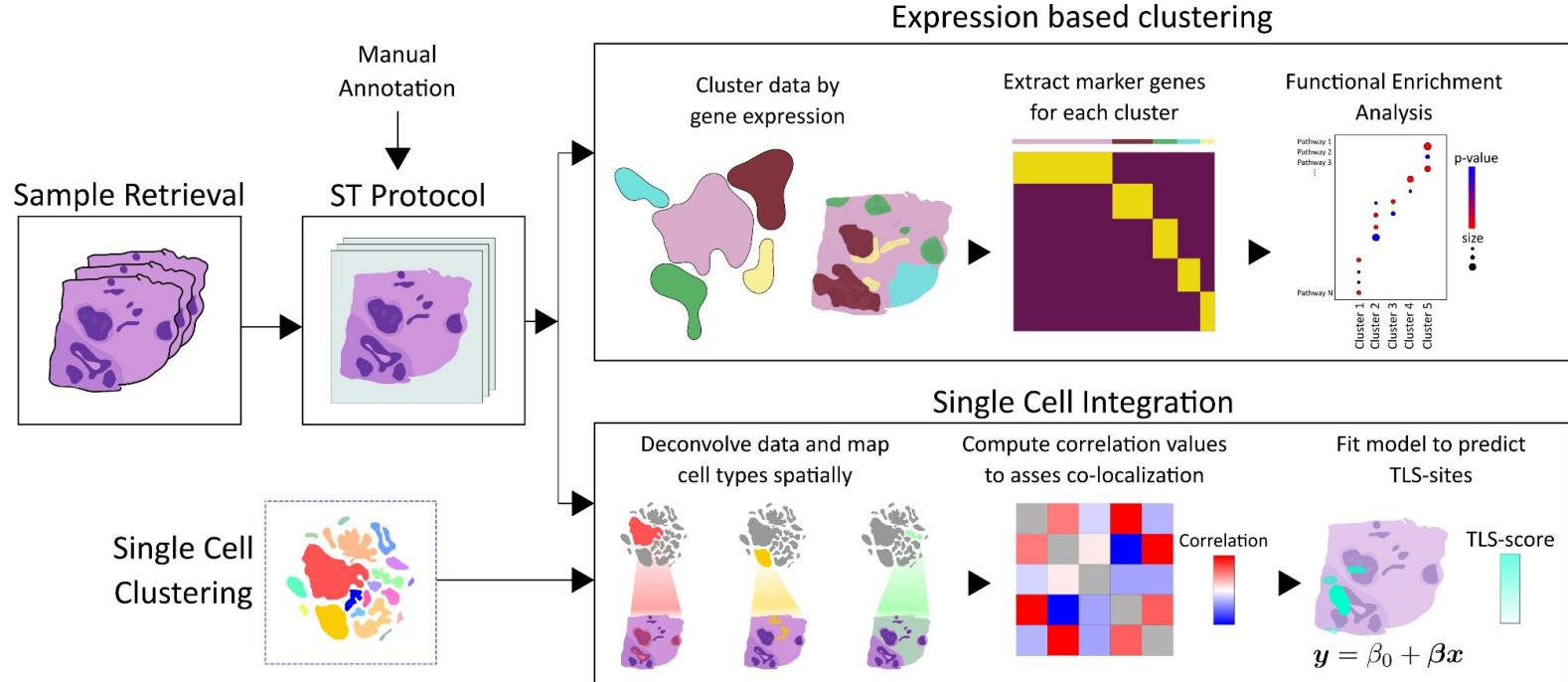
Functional  
Enrichment



TLS-score

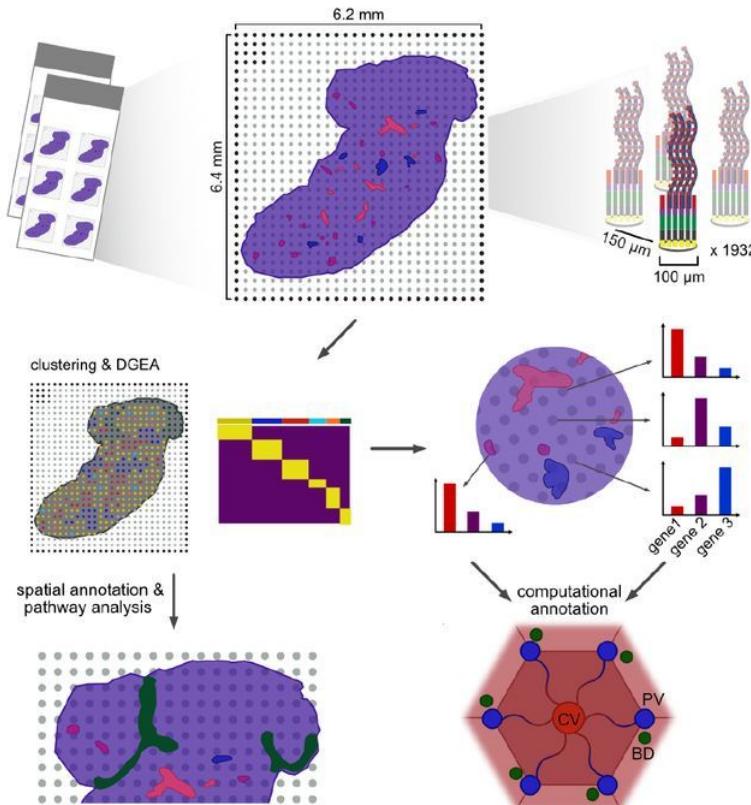


# A spatial survey of HER2-positive breast cancer



*"Spatial deconvolution of HER2-positive Breast cancer delineates tumor-associated cell type interactions"*, Andersson et al. (in press Nature Communications)

# Spatial gene expression dynamics in the mouse liver

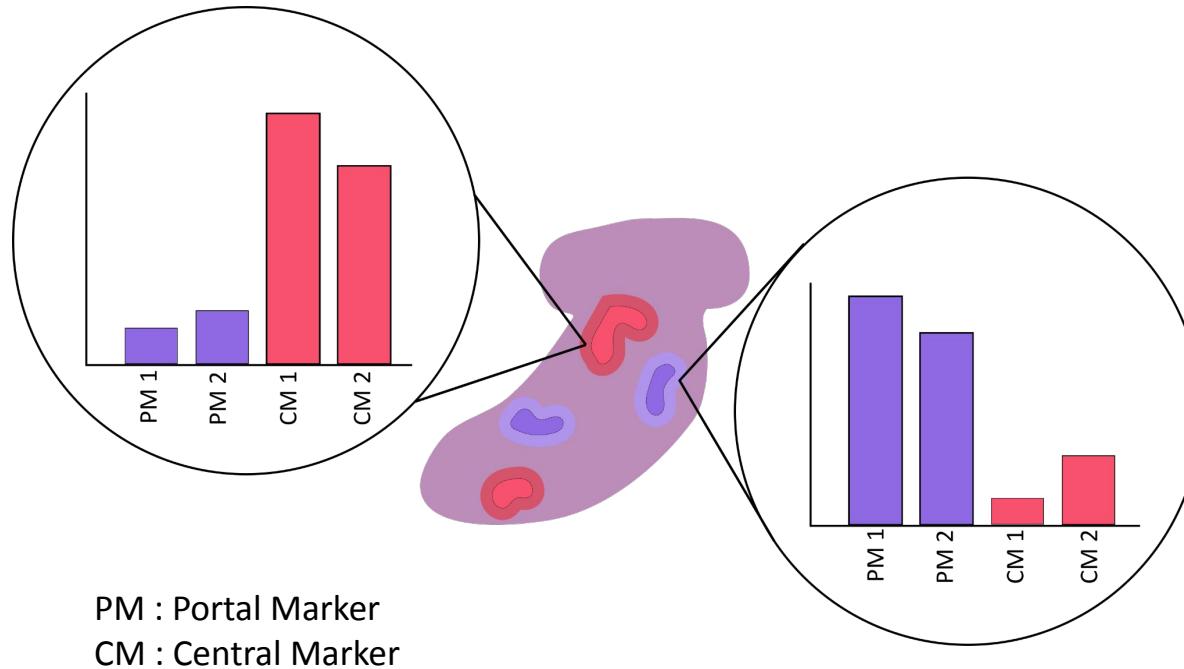


*“Spatial Transcriptomics to define transcriptional patterns of zonation and structural components in the liver”*, Hildebrandt and Andersson et al.

bioRxiv <https://doi.org/10.1101/2021.01.11.426100>

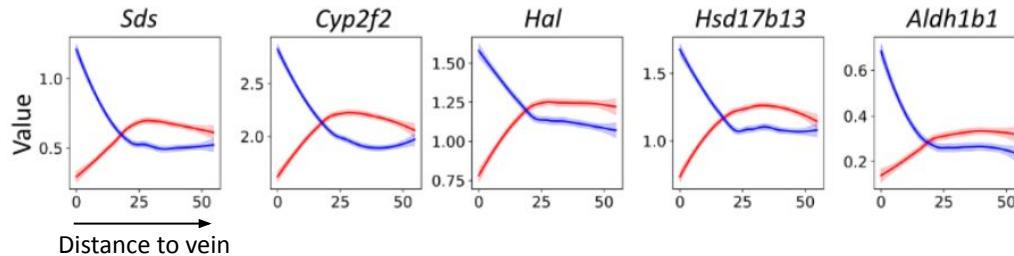


# Spatial gene expression dynamics in the mouse liver



- **Portal** and **central** veins have certain marker genes associated with them
- **Key concept :** Marker gene expression is dependent on distance to the veins

# Expression as function of distance

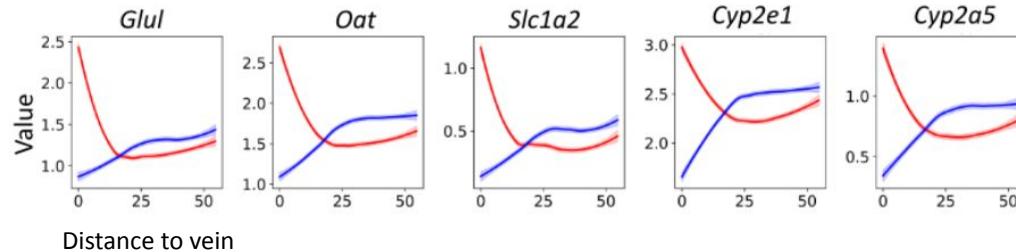


Blue curves : expression as a function of distance to **portal** veins

Red curves : expression as a function of distance to **central** veins

- Model gene expression as a **function of the distance** to respective vein

# Expression as function of distance



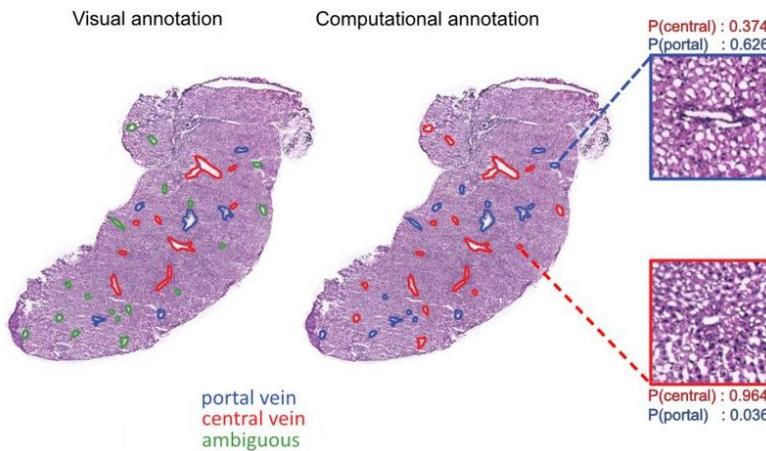
- Model gene expression as a ***function of the distance*** to respective vein

Distance to vein

Blue curves : expression as a function of distance to **portal** veins

Red curves : expression as a function of distance to **central** veins

# Expression as function of distance



- **Objective :** Unsupervised classification of vein types
- **Implementation :**
  - Construct neighborhood expression profile (NEP).
  - Train logistic classifier on NEPs from (expert annotated) veins
  - Predict vein type based on NEP for un-annotated veins. Gives probabilistic assignment.

# Summary

- Tons of spatial techniques
  - I'm very fond of Visium, but you should always pick whatever is best for you!
- Ever increasing repertoire of computational methods!
  - Be **careful** when transferring single cell methods, make sure the methods' assumptions are valid
  - A lot of tools out there, but sometimes beneficial to construct **custom solutions**
- **Don't just treat spatial data as a different form of single data**, it has much more to offer

# Acknowledgements



Spatial research group : [www.spatialresearch.org](http://www.spatialresearch.org)

Organizers : NBIS and SIB

External slide contributions : Tommaso Biancalani and Dylan Cable

# Thank **you** for the attention!



<https://github.com/almaan>

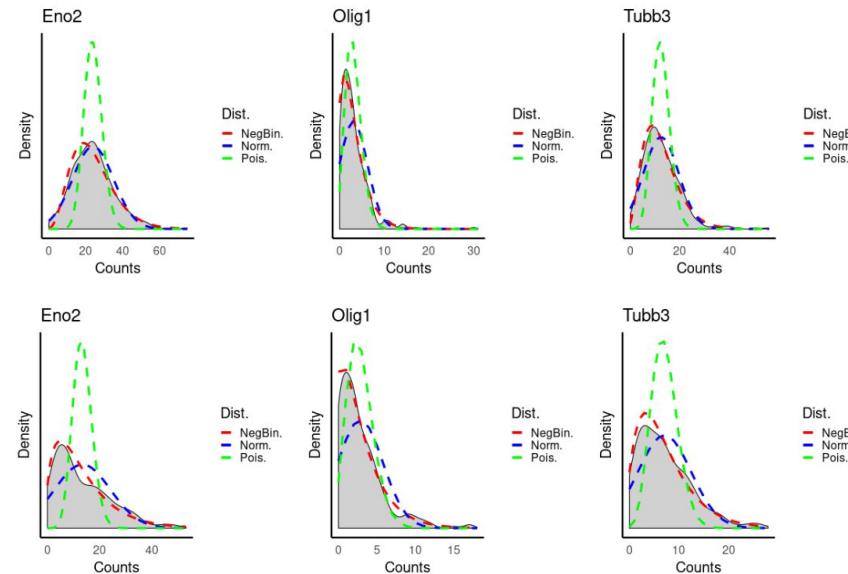
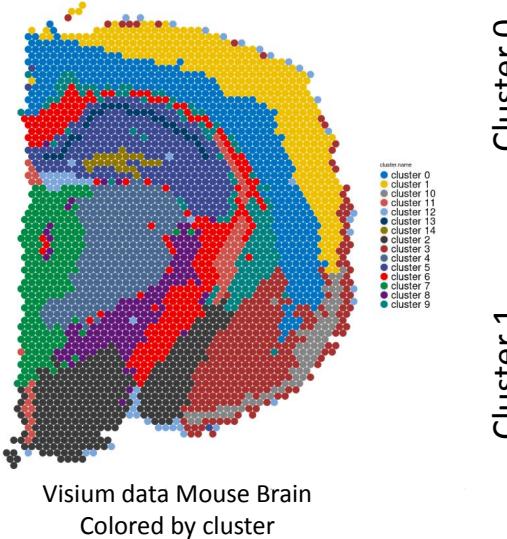


<https://almaan.github.io>



@aalmaander

- Single cell data usually modelled as overdispersed Poisson distribution (Negative Binomial). Basis for several analysis methods (Normalization, DE, etc.)
  - Applicable to ST/Visium data as well



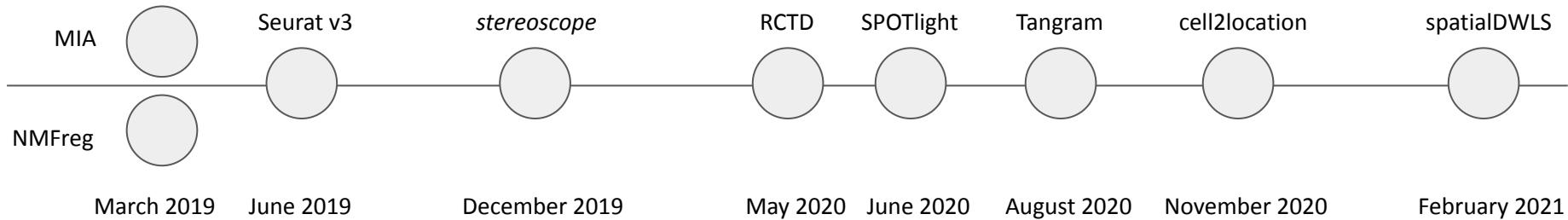
Similar trends for all clusters and genes.

Supports NB distribution, also when corrected for increased parameter number compared to Poisson).

# Integration with single cell data

21

A non-comprehensive overview based on bioRxiv releases



# Integration with single cell data

21

A non-comprehensive overview based on bioRxiv releases

