Exploring Spatial Transcriptomics

2020-05-28

Alma Andersson

https://github.com/almaan

SPATIAL research

https://www.spatialresearch.org

SciLifeLab

A brief Introduction

- Alma Andersson
- From : Utterbäck, Sweden
  - Population : 69
- Now : Stockholm, Sweden
  - Population : 1,605,030
- 2017-2018 : Delemotte Lab
  - Molecular Dynamics
  - Membrane proteins (Ion Channels)
- 2018-Current : Lundeberg Lab
  - Spatial Transcriptomics (ST)
  - Computational Method Development
- github : almaan
- Small disclaimer : first online teaching experience

Outline

- ~~Introduction~~
- Notation
- Background
- Data Processing
- Data Analysis (Overview)

- Break

- Questions
- Cont. Data Analysis (Overview)
- Exercises (Information)
- Questions

Notation

- Exercise session consists of 3 parts
- Symbols below used to indicate when material is included in one of these

- Material in Part 1

- Material in Part 2

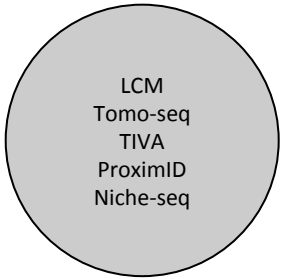
- Material in Part 3

Background

The spatial space | Overview of techniques

The spatial space | Overview of techniques

Microdissection

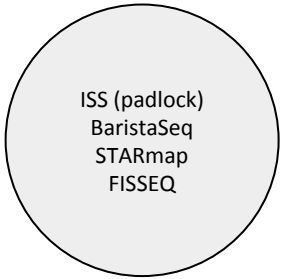


Isolate a region of interest, place isolate in separate well and sequence (either by bulk or single-cell methods).

“Brute Force” approach.

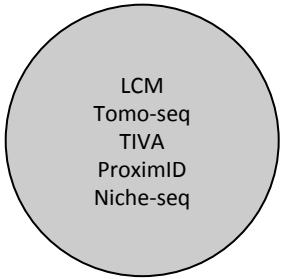
The spatial space | Overview of techniques

In situ sequencing



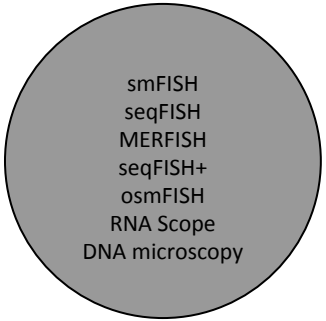
Sequence the transcripts in place. Offer sub-cellular resolution. Tend to rely on gene panels. Need "*a priori*" defined targets.

Microdissection



The spatial space | Overview of techniques

In situ hybridization



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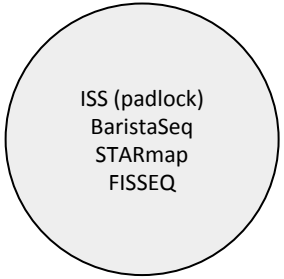

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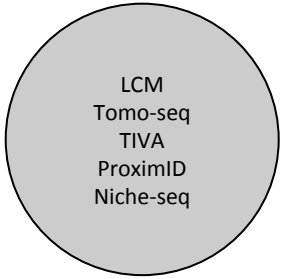
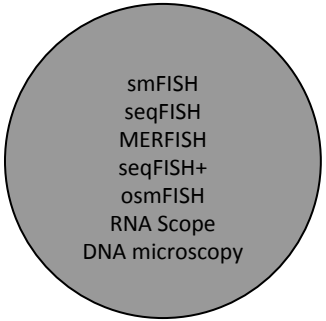


Labeled probes for specific targets, hybridize in place and visualized for spatial information.

The spatial space | Overview of techniques

In situ hybridization



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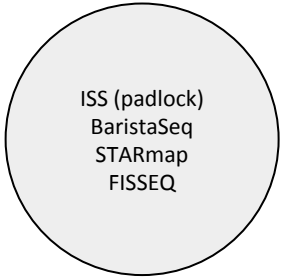

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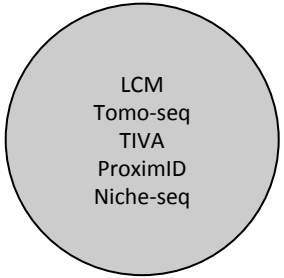
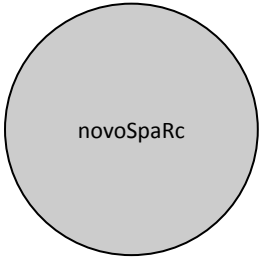


Infer and reconstruct spatial structure from non-spatial data (e.g., single cell).

In silico



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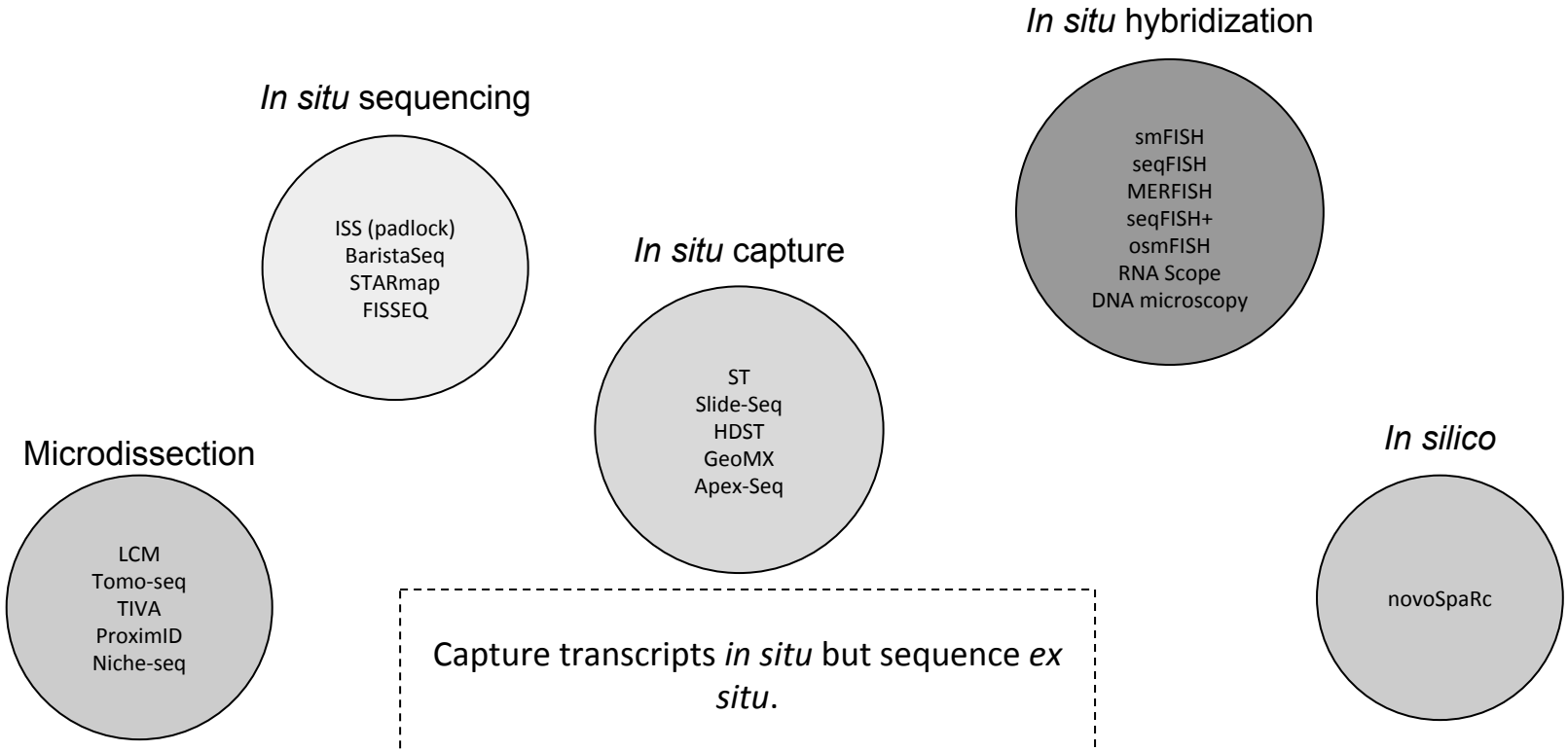

Credit to J.Bergenstråhle and M.Asp for categorization of techniques

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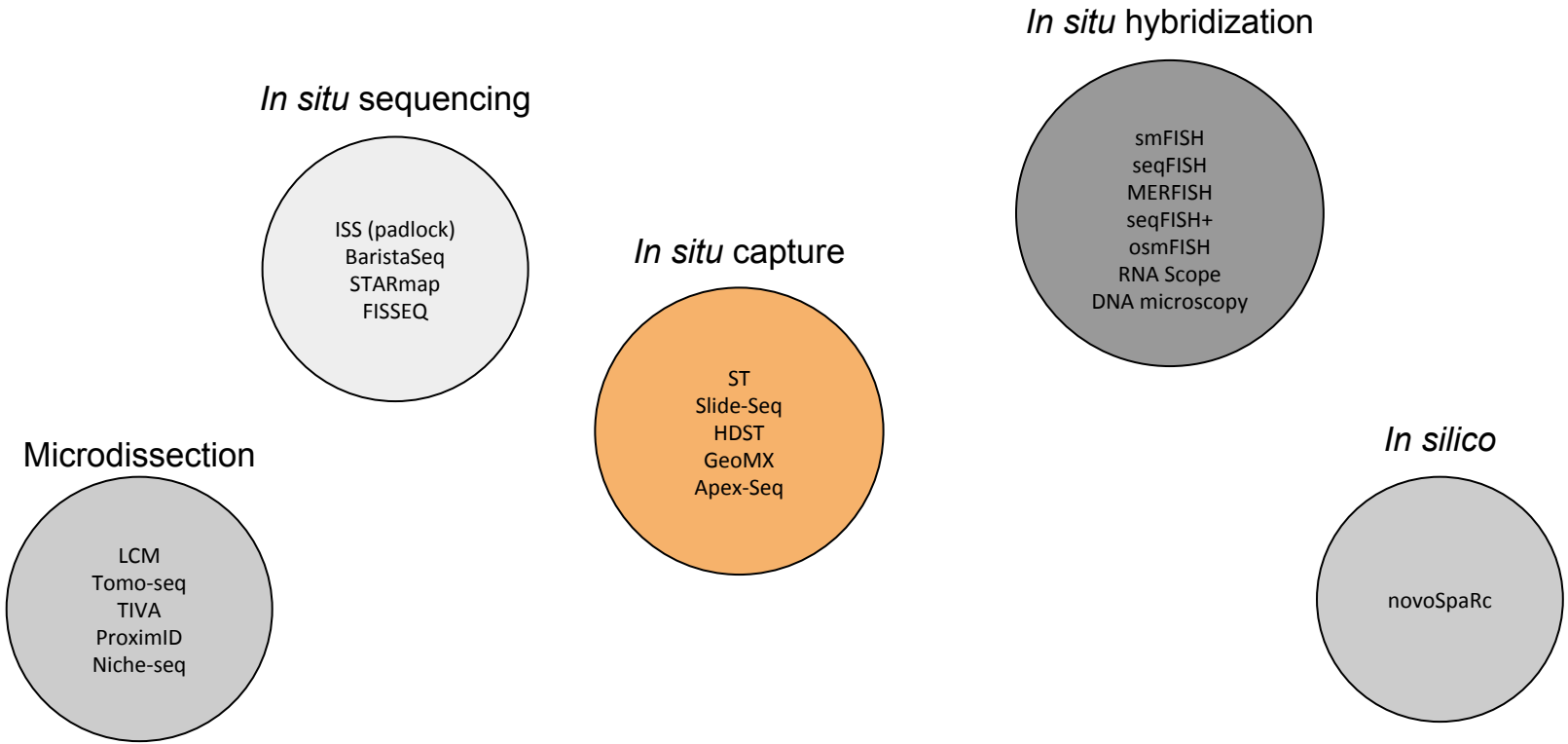

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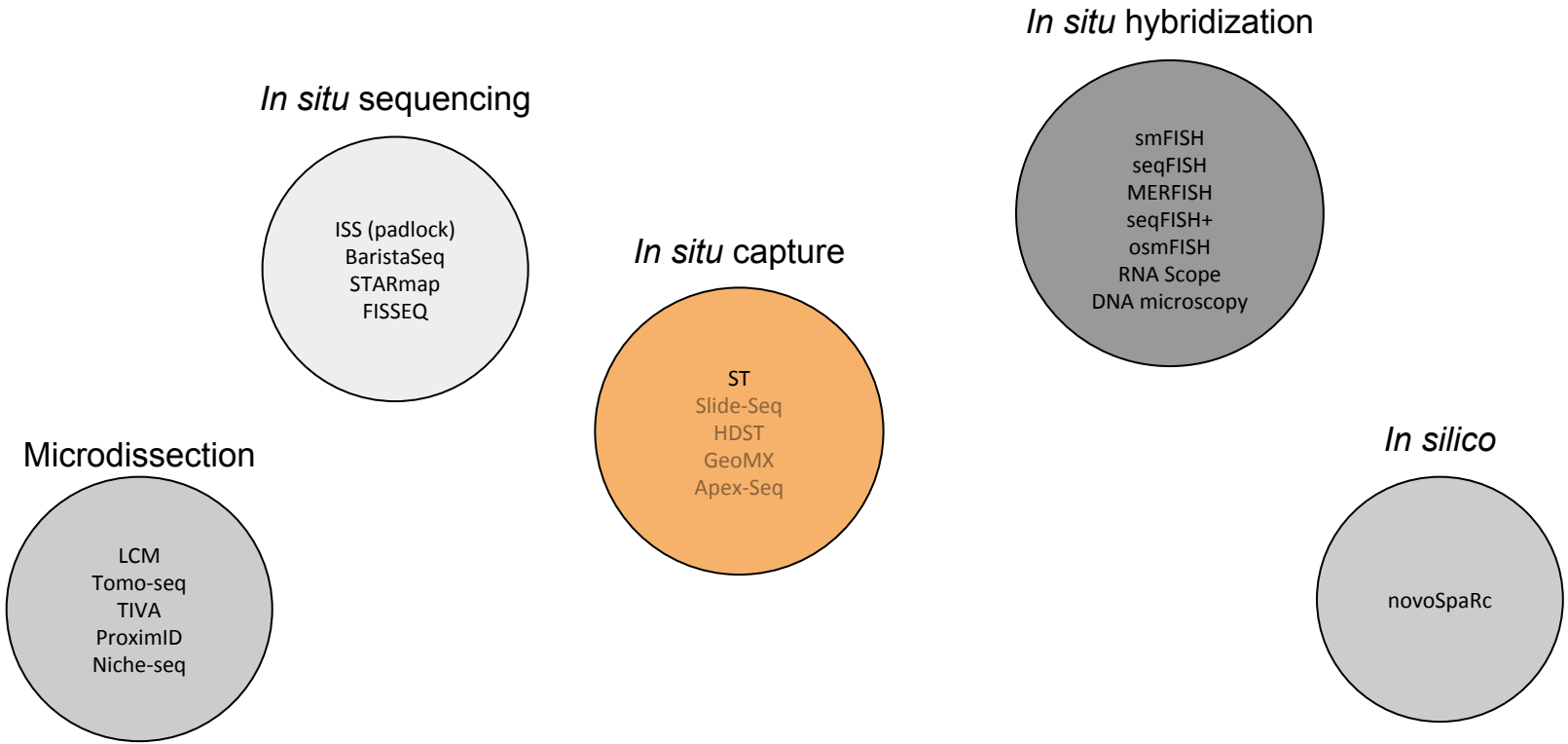

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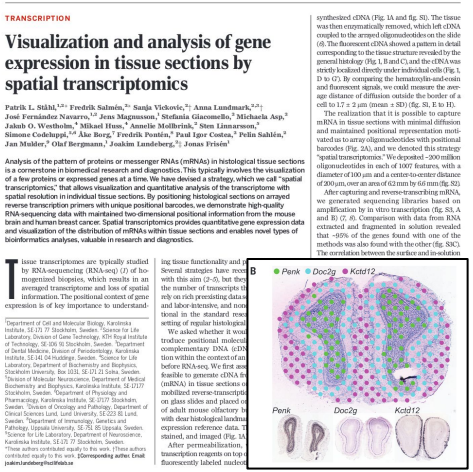
Spatial Transcriptomics (ST)

Mid 2016



TRANSCRIPTION

Visualization and analysis of gene expression in tissue sections by spatial transcriptomics

Patrik L. Ståhl,[1,2,*] Fredrik Salmén,[2,*] Sanja Vickovic,[2,*] Anna Lundmark,[2,*†] José Fernández Navarro,[1,2] Jens Magnusson,[2] Stefania Giacomello,[2] Michaela Asp,[2] Jakub O. Westholm,[3] Mikael Huss,[4] Annelie Mollbrink,[2] Sten Linnarsson,[3] Simone Codeluppi,[2,5] Åke Borg,[6] Fredrik Pontén,[4] Paul Igor Costea,[2] Pelin Sahlén,[2] Jan Mulder,[4] Olaf Bergmann,[1] Joakim Lundeberg,[2†] Jonas Frisén[1]

Analysis of the pattern of proteins or messenger RNAs (mRNAs) in histological tissue sections is a cornerstone in biomedical research and diagnostics. This typically involves the visualization of a few proteins or expressed genes at a time. We have devised a strategy, which we call "spatial transcriptomics," that allows visualization and quantitative analysis of the transcriptome with spatial resolution in individual tissue sections. By positioning histological sections on arrayed reverse transcription primers with unique positional barcodes, we demonstrate high-quality RNA-sequencing data with maintained two-dimensional positional information from the mouse brain and human breast cancer. Spatial transcriptomics provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections and enables novel types of bioinformatics analyses, valuable in research and diagnostics.

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DOI: 10.1126/science.aaf2403

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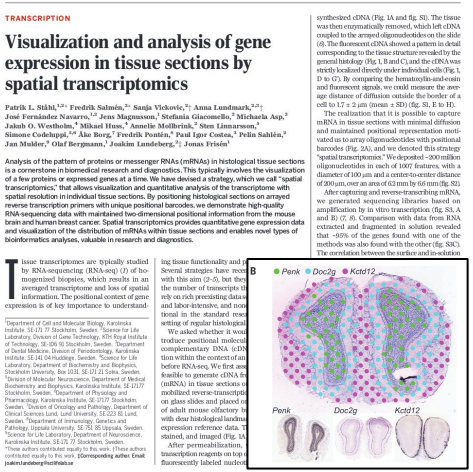

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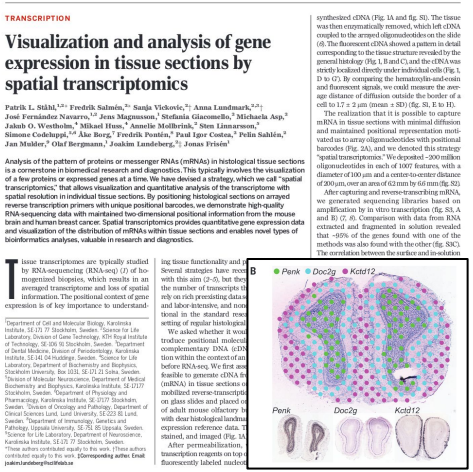

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Tissue transcriptomes are typically studied by RNA-sequencing (RNA-seq) (*1*) of homogenized biopsies, which results in an averaged transcriptome and loss of spatial information. The positional context of gene expression is of key importance to understand-

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*These authors contributed equally to this work. †These authors contributed equally to this work. ‡Corresponding author. Email: joakim.lundeberg@scilifelab.se

synthesized cDNA (Fig. 1A and fig. S1). The tissue was then enzymatically removed, which left cDNA coupled to the arrayed oligonucleotides on the slide (*6*). The fluorescent cDNA showed a pattern in detail corresponding to the tissue structure revealed by the general histology (Fig. 1, B and C), and the cDNA was strictly localized directly under individual cells (Fig. 1, D to G'). By comparing the hematoxylin-and-eosin and fluorescent signals, we could measure the average distance of diffusion outside the border of a cell to 1.7 ± 2 μm (mean ± SD) (fig. S1, E to H).

The realization that it is possible to capture mRNA in tissue sections with minimal diffusion and maintained positional representation motivated us to array oligonucleotides with positional barcodes (Fig. 2A), and we denoted this strategy "spatial transcriptomics." We deposited ~200 million oligonucleotides in each of 1007 features, with a diameter of 100 μm and a center-to-center distance of 200 μm, over an area of 62 mm by 65 mm (fig. S2).

After capturing and reverse-transcribing mRNA, we generated sequencing libraries based on amplification by in vitro transcription (fig. S3, A and B) (*7*, *8*). Comparison with data from RNA extracted and fragmented in solution revealed that ~95% of the genes found with one of the methods was also found with the other (fig. S3C). The correlation between the surface and in-solution

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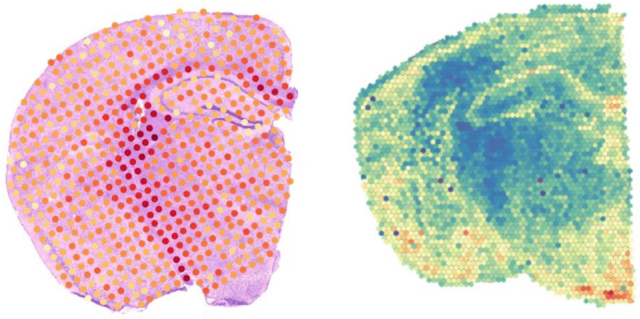

Launch of **Visium** Spatial Gene Expression Platform

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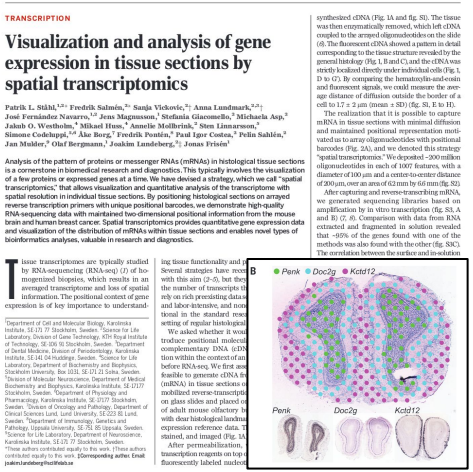
Spatial Transcriptomics (ST) → Visium

Mid 2016



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Several strategies have recei
with this aim (*2*–*5*), but thes
the number of transcripts th
rely on rich preexisting data s
and labor-intensive, and non
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setting of regular histological

We asked whether it woul
troduce positional molecul
complementary DNA (cDN
tion within the context of an
before RNA-seq. We first ass
feasible to generate cDNA fr
(mRNA) in tissue sections a
mobilized reverse-transcripti
on glass slides and placed o
of adult mouse olfactory b
with clear histological landma
expression reference data. T
stained, and imaged (Fig. 1A

After permeabilization,
transcription reagents on top
fluorescently labeled nucleot

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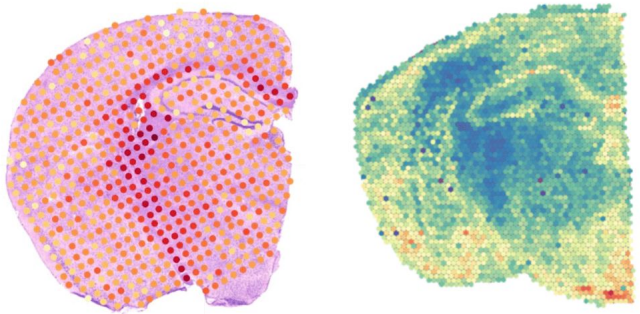
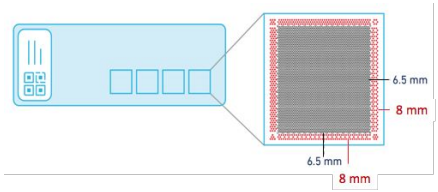
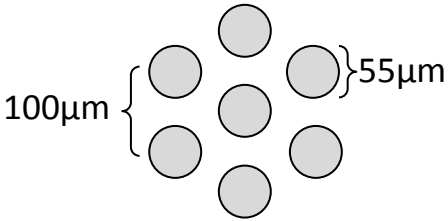


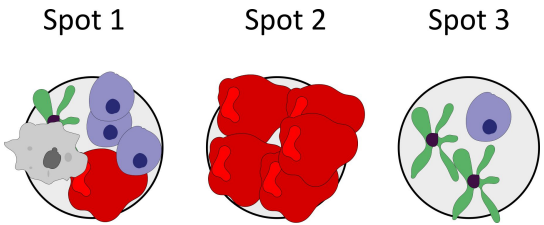
Launch of **Visium** Spatial Gene Expression Platform

Visium Platform

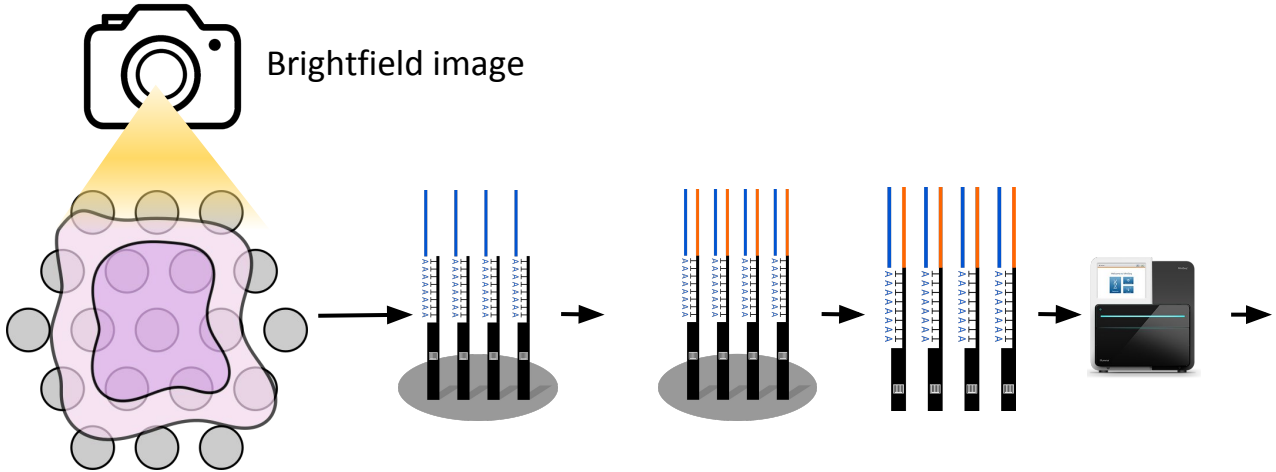
- Array based technique
- 6.5mm x 6.5mm area to put sample on
- 4992 spots arranged in hexgonal grid
- Spot specs:
  - Spot diameter : 55µm
  - Center to center distance : 100 µm
- Each spot has millions of capture probes
  - spatial barcode
  - polyT sequence
  - captures polyadenylated mRNA
  - Full transcritptome(-ish)
- ~ 1-10 cells contribute to each spot
  - **NOTE** : Not single cell resolution!







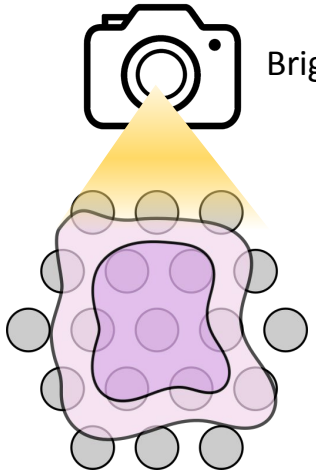
The experimental workflow (in a nutshell)



spot (barcode)	gene_1	...	gene_{G-1}	gene_G
AAACATACAACCAC-1	5	...	0	9
AAACATTGAGCTAC-1	3	...	1	3
⋮	4	⋱	0	0
AAACATTGATCAGC-1	0	...	8	7

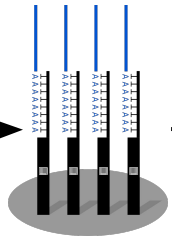
Count Matrix

The experimental workflow (in a nutshell)

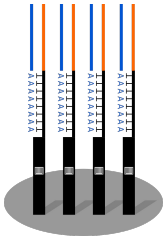


Brightfield image

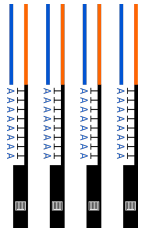
Tissue +
Visium-array



mRNA
capture



cDNA
synthesis



Cleave
Probes

Sequence +
preprocess

Where the fun starts!

spot (barcode)	gene_1	...	gene_{G-1}	gene_G
AAACATACAACCAC-1	5	...	0	9
AAACATTGAGCTAC-1	3	...	1	3
⋮	4	⋱	0	0
AAACATTGATCAGC-1	0	...	8	7

Count Matrix

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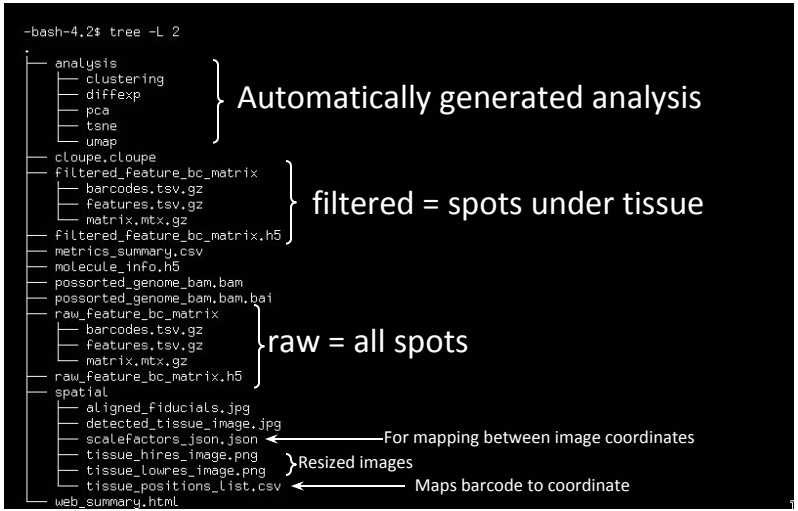
Data Processing

After sequencing (brief)

- spaceranger mkfastq | BCL files to FASTQ
- spaceranger count |tissue detection/alignment, UMI counting

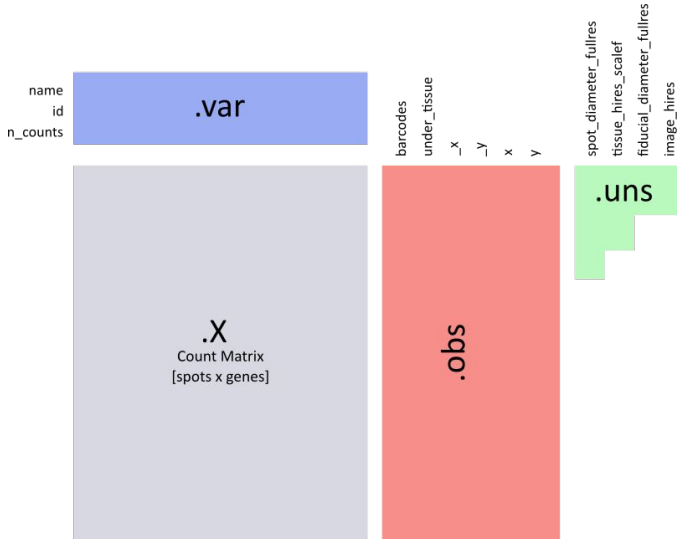


Example of spaceranger count output

Processed data

P1

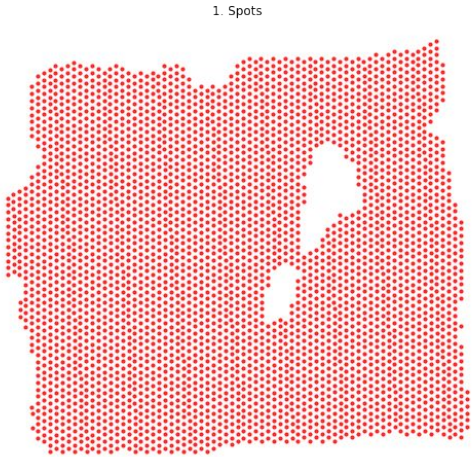
- Either use .mtx files or .h5 files to assemble a data object to work with
  - No standardized format
- Personal preference : convert to .h5ad file (will be using in exercises)
  - scanpy/anndata teams working on - soon to release - their own (similar) format



An initial assessment

P1

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



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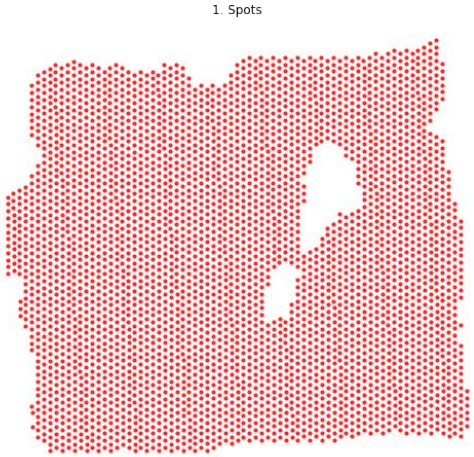

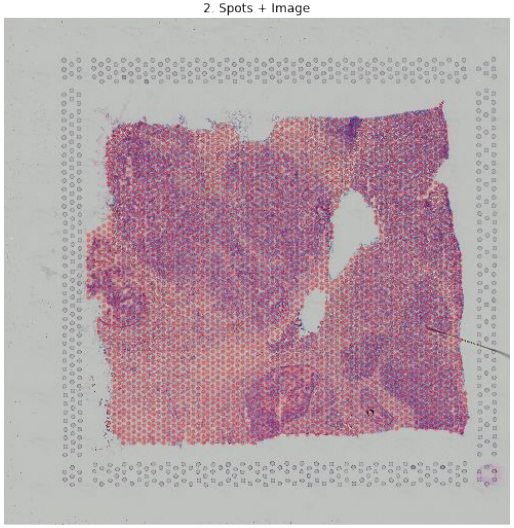

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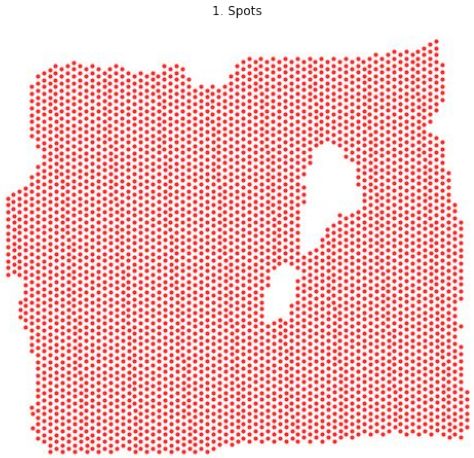

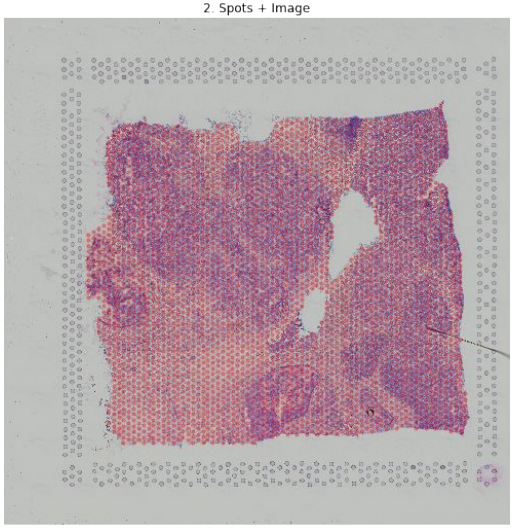

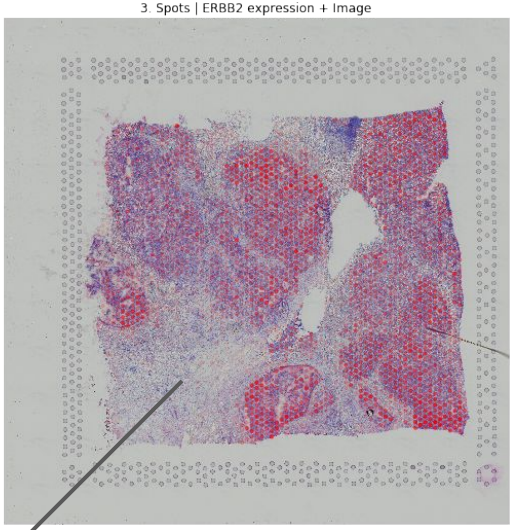

Facecolor intensity proportional to gene expression value

Visualizing high dimensional spatial data

(Tips)

- We visualized one feature/gene (*ERBB2*)
- How do we handle multiple non-mutually exclusive features?

Visualizing high dimensional spatial data

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- One idea :
  - Embed gene expression data in 3 dimensional space (e.g. using UMAP)
  - Do affine transformation to unit cube
  - Consider values as RGB values (or other colorspace) and color spots accordingly

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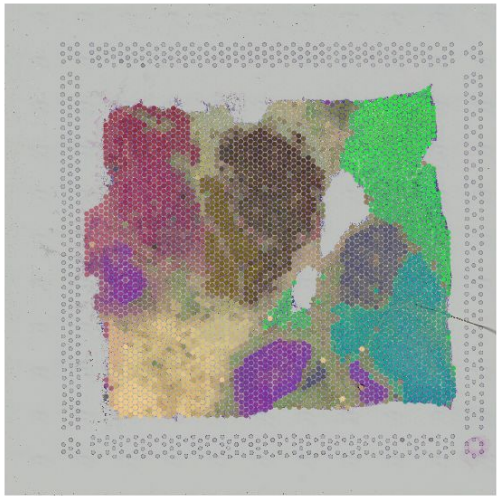

Regions with similar colors have similar gene expression.

Data Analysis

Before the analysis | Filtering, Normalization, Batch correction, etc.

- No magic recipe to give
  - How to process your data is very much dependent on the samples and objective
  - Much can be learnt from analysis of single cell data
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  - Spots based on expression levels (total gene expression at spot > thrs) †
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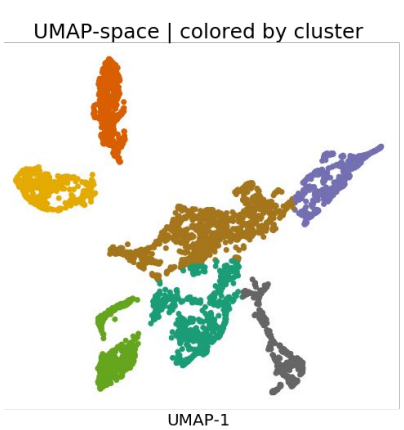
  - Spots based on expression levels (total gene expression at spot > thrs) †
  - Ribosomal (RP) and mitochondrial genes (MT) tend to exhibit spurious expression patterns. Exclusion of these is common
- Normalization / batch correction :
  - Recommend to account for spot “library size“ - varying cell density
  - Include slide/array as covariate (sometimes big variation is observed)
  - Popular tools for batch correction : sctransform and Harmony

† Only necessary if you expect “defunct” spots under the tissue

Example : Basic Analysis

P1

- Cluster the spots based on gene expression
  - Normalize → $PCA_{n=20}$ → UMAP → GMM
- Backmap clusters onto tissue
- Use HE-image as reference
  - Sanity check - does it make sense?
  - Valuable resource
- Next, annotate clusters
  - Find genes associated with cluster
  - Functional enrichment analysis



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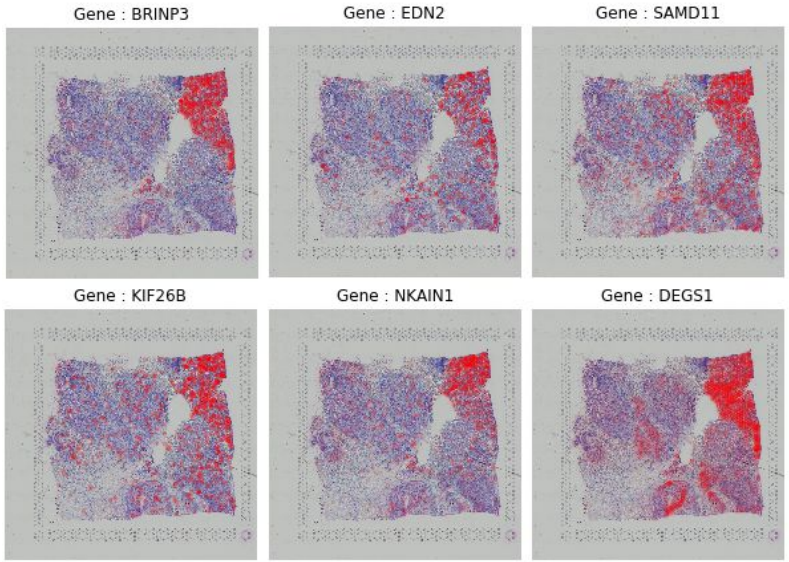
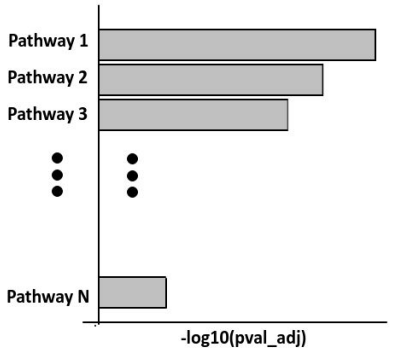
Example : Basic Analysis

Clustered data



DE analysys | Here cluster 1 (orange) vs. all

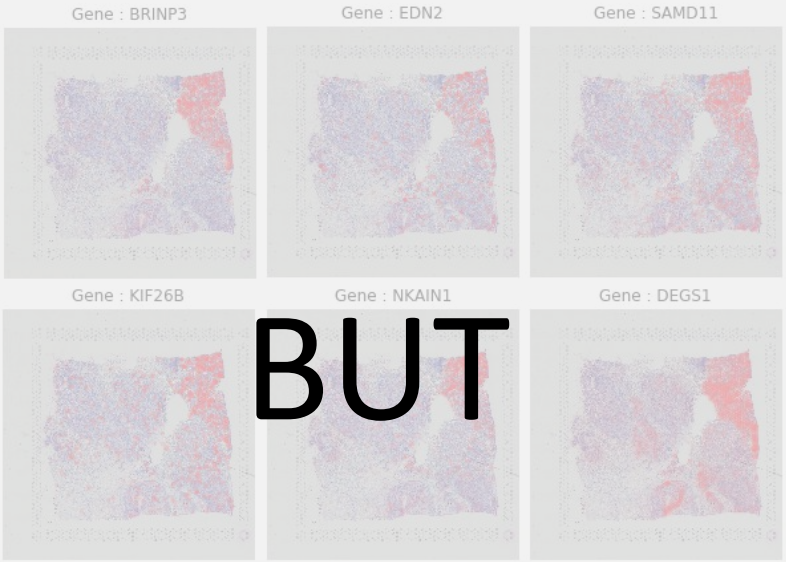


Functional Enrichment Analysis

All helpful in annotation of clusters

Example : Basic Analysis

Clustered data



BUT

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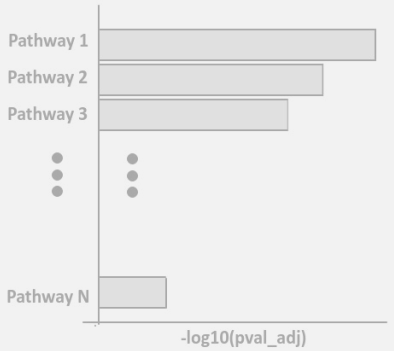

Functional Enrichment Analysis

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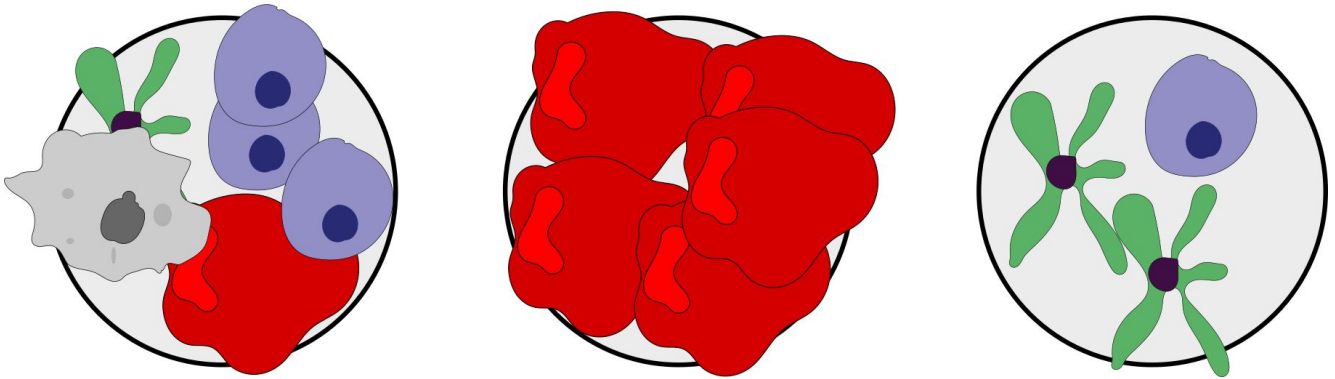
Example : Basic Analysis

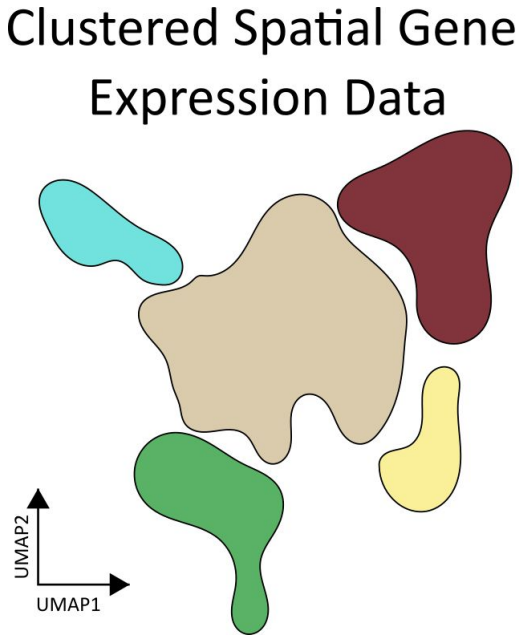
Remember :

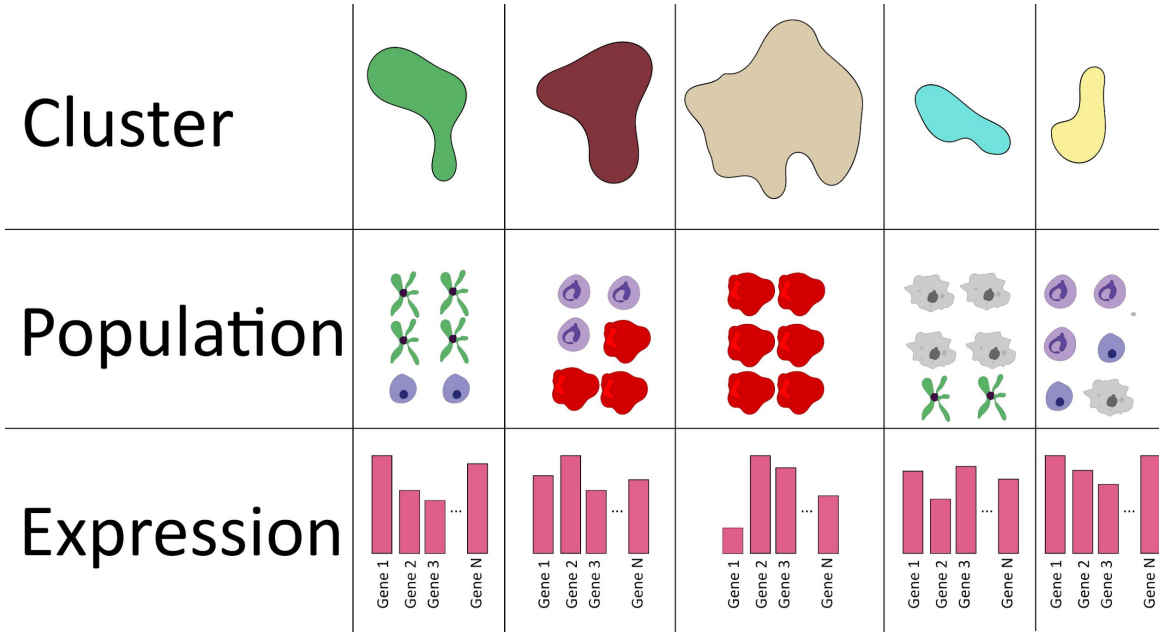
"each spot is a mixture of multiple cells,
i.e., one spot may contain multiple cell types"

Spot 1 Spot 2 Spot 3



Cluster ≠ Cell type

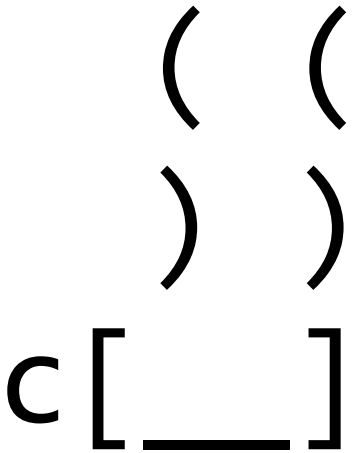




So where are my cell types?

Break

- 10 min Break
- During :
  - Read through questions (for me)
- After :
  - Discuss (some) questions when everyone is back
  - Data Analysis Cont.
  - Information about the exercises
  - Wrap up and questions



Questions

So where are my cell types?

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- Marker genes?

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- Marker genes? Easy and straightforward, but
  - Requires knowledge of marker genes (not always true)
  - Risk for overlap among marker genes
  - How do we interpret expression values?
  - Lowly expressed markers genes may not always be observed

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- Alternative solution - Integrate single cell (SC) and spatial data!

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- Marker genes? Easy and straightforward, but
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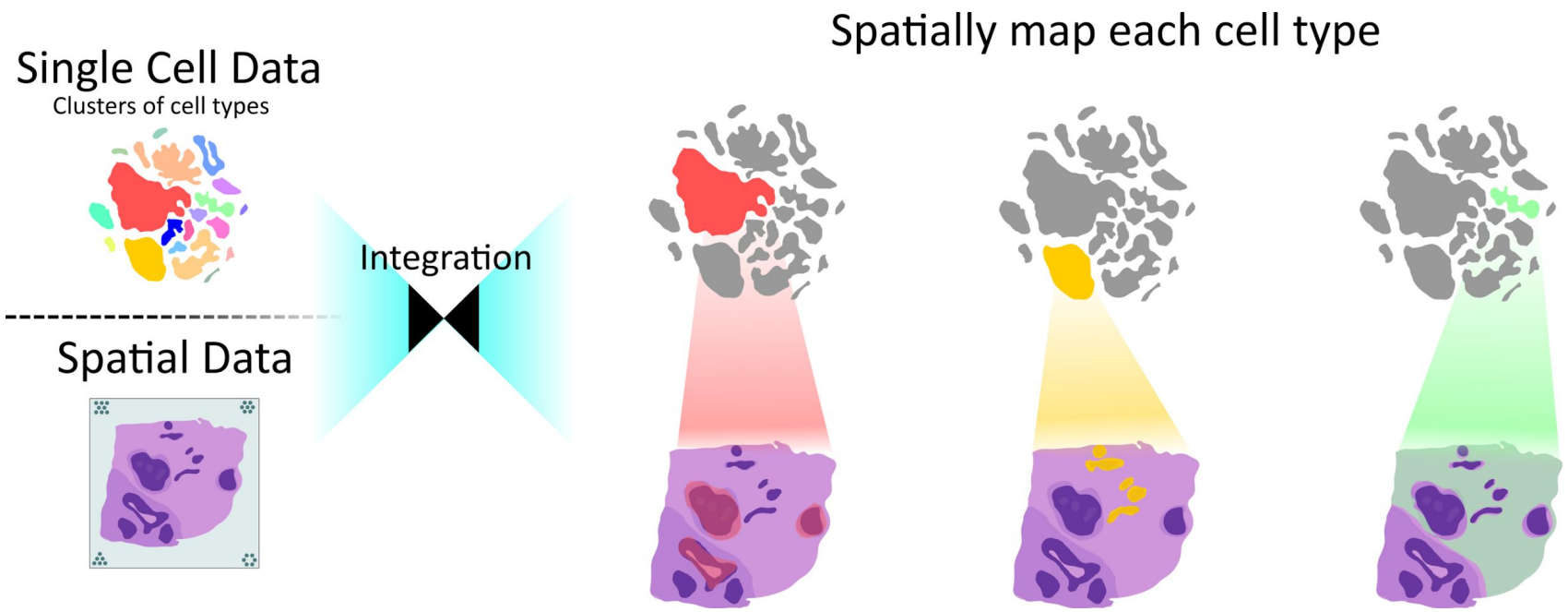
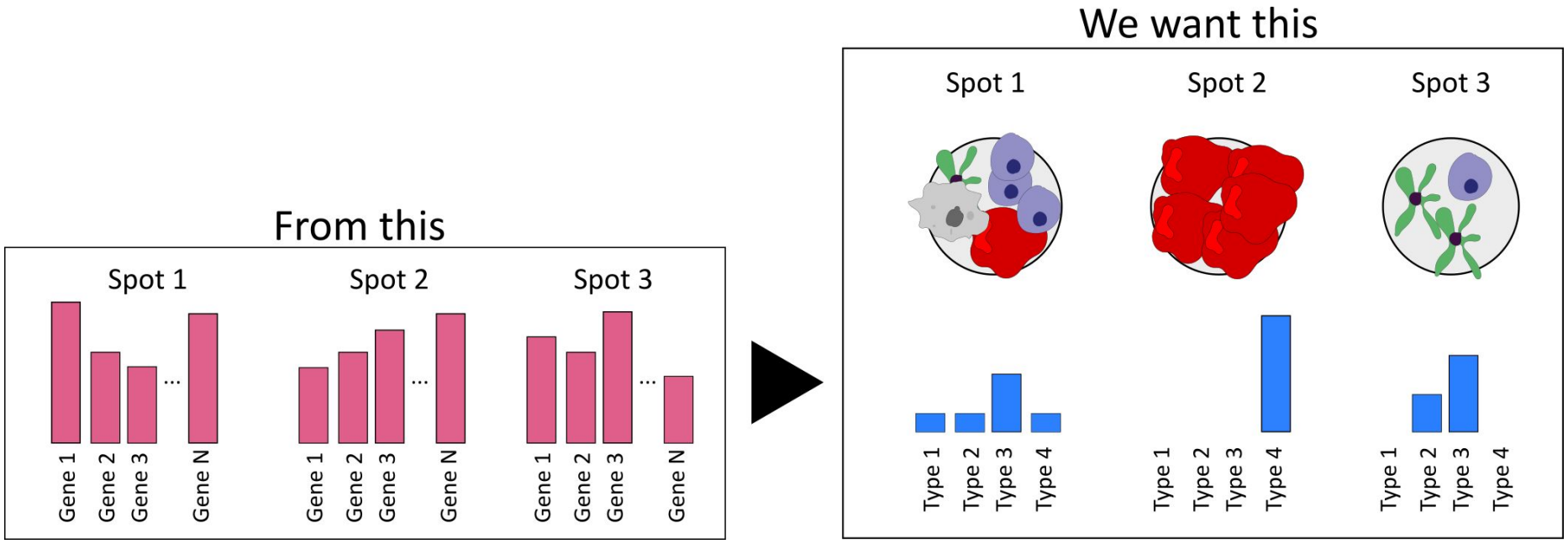
  - Risk for overlap among marker genes
  - How do we interpret expression values?
  - Lowly expressed markers genes may not always be observed
- Alternative solution - Integrate single cell (SC) and spatial data!
  - Extract information of cell types from SC data and apply to spatial data
  - **Big challenge : deconvolution required (on Visium data)**
  - Multiple approaches have been proposed, e.g., :
    - MIA by Tirosh et al.
    - Seurat's spatial module

Integration of Single Cell and Spatial Data



Our objective : deconvolve expression data



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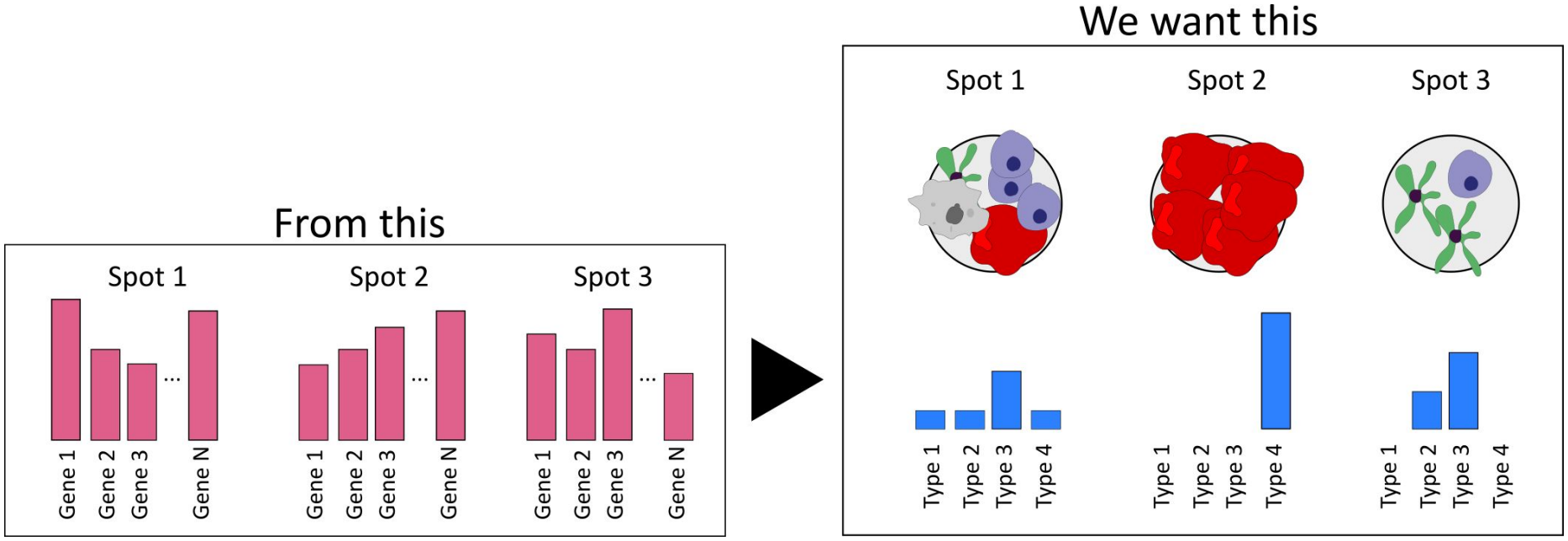
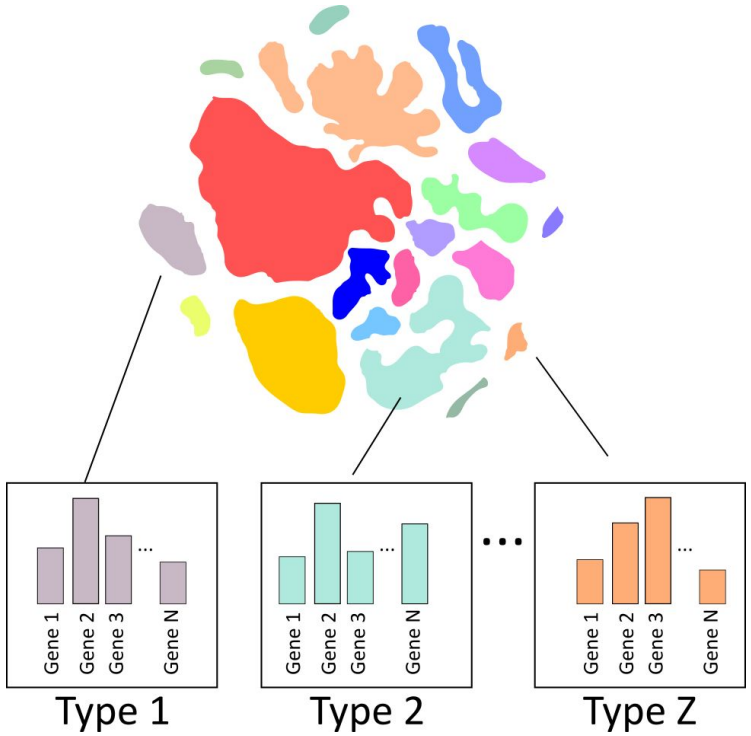


Suggested approach : *Model-based Probabilistic Inference*

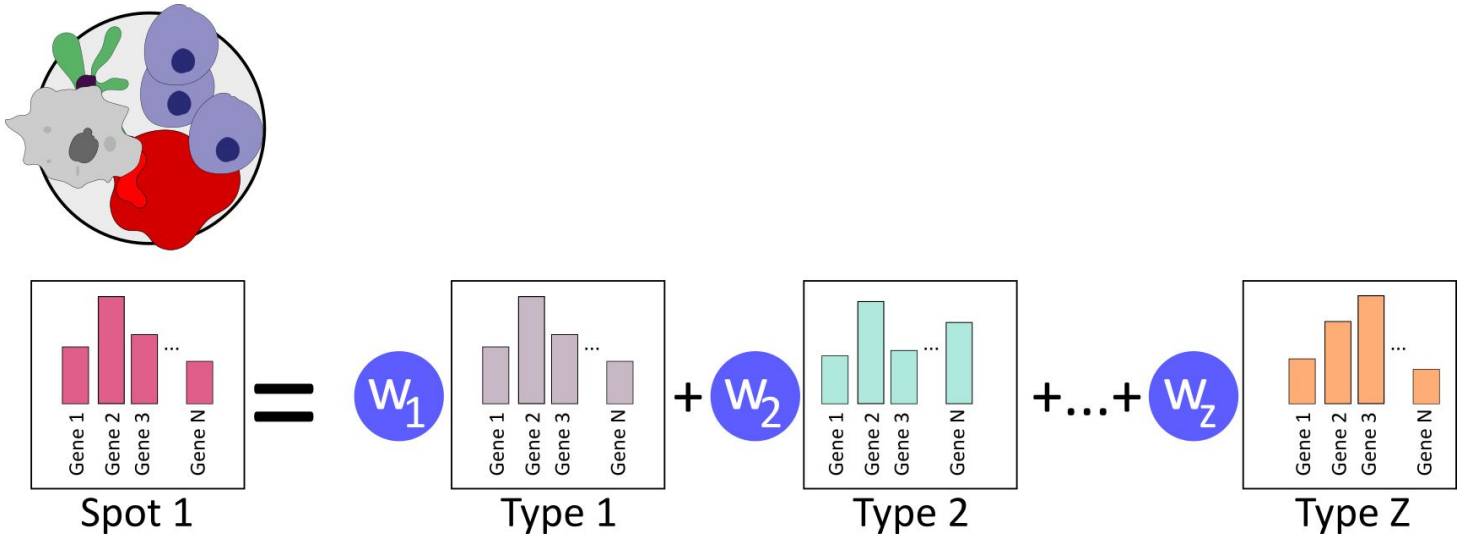
It’s as easy as 1-2-3

1. Infer cell type expression parameters from SC data



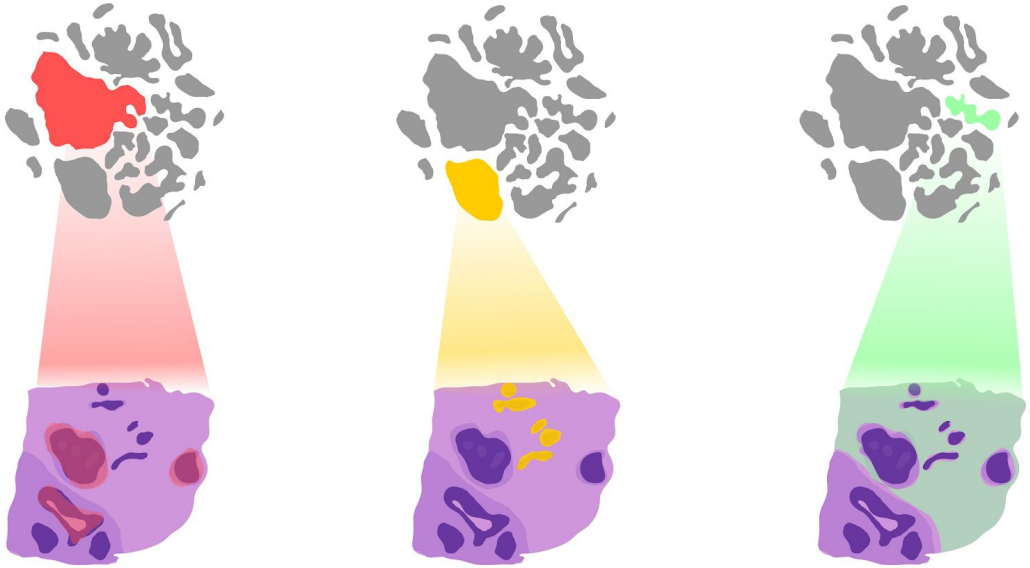
It’s as easy as 1-2-3

2. Use inferred parameters to find optimal combination of cell types in spot



It’s as easy as 1-2-3

3. Map cell type proportions back onto the tissue



Slightly more complex than adding bar graphs..

- Probabilistic Model

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- Probabilistic Model

Observed counts for gene g at spot s

Scaling factor

Gene Bias

$$x_{gs} = \sum_{c \in C_s} x_{gsc}, \qquad x_{gsc} \sim NB(\beta_g \alpha_s r_{gz_c}, p_g)$$

Cell type specific Rate

Success probability

= Learn from Single Cell Data

$$y_{gc} \sim \mathcal{NB}(s_c r_{gz_c}, p_g)$$

Slightly more complex than adding bar graphs..

- Probabilistic Model

$$x_{gs} \sim NB(\alpha_s \sum_{c \in C_s} \beta_g r_{gz_c}, p_g)$$

Change Index of summation

$$x_{gs} \sim NB(\alpha_s \sum_{z \in Z} \beta_g n_{sz} r_{gz}, p_g)$$

Number of cells from type z at spot s

Slightly more complex than adding bar graphs..

- Probabilistic Model

$$x_{gs} \sim NB(\alpha_s \sum_{z \in Z} \beta_g n_{sz} r_{gz}, p_g)$$

join scaling factor and cell counts

$$v_{sz} = \alpha_s n_{sz}$$

$$x_{gs} \sim NB(\sum_{z \in Z} \beta_g v_{sz} r_{gz}, p_g)$$

Slightly more complex than adding bar graphs..

- Probabilistic Model

$$w_{sz} = \frac{v_{sz}}{\sum_{z \in Z} v_{sz}} = \frac{\alpha_s n_{sz}}{\alpha_s \sum_{z \in Z} n_{sz}} = \frac{n_{sz}}{\sum_{z \in Z} n_{sz}}$$

Slightly more complex than adding bar graphs..

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Number of cells from cell type z at spot s

Total number of cells at spot s

Slightly more complex than adding bar graphs..

- Probabilistic Model

Number of cells from cell type z at spot s

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Proportion of cell type “z” at spot “s”

Total number of cells at spot s

Slightly more complex than adding bar graphs..

- Probabilistic Model
  - Use MLE estimate to find unadjusted proportions (V = [v_{sz}])
  - Minimize : $$l(\mathbf{V}, \beta) = -\log[L(\mathbf{V}, \beta|\mathbf{r}, \mathbf{p}, \mathbf{X})]$$
  - Stochastic optimization (PyTorch)

Likelihood function

Slightly more complex than adding bar graphs..

- Probabilistic Model
  - Assumes single cell and spatial data are both NB distributed
- Tool : *stereoscope*
- Output : [spot] x [cell_type] matrix
  - Elements are proportion of cell belonging to the given cell type at each spot

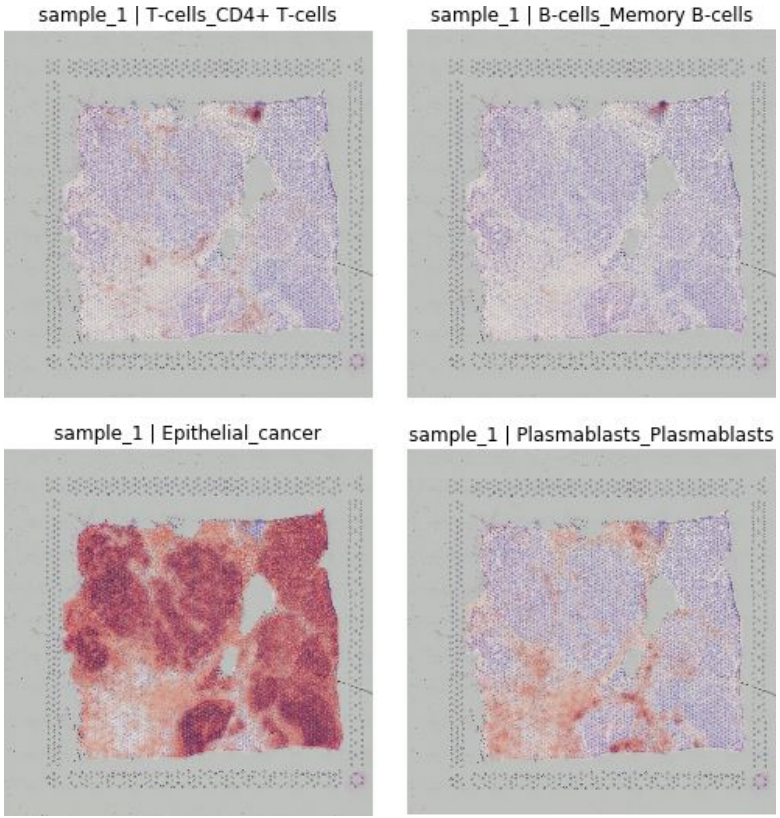
https://github.com/almaan/stereoscope

#shameless self-advertising

SciLifeLab

Applying it to our breast cancer data

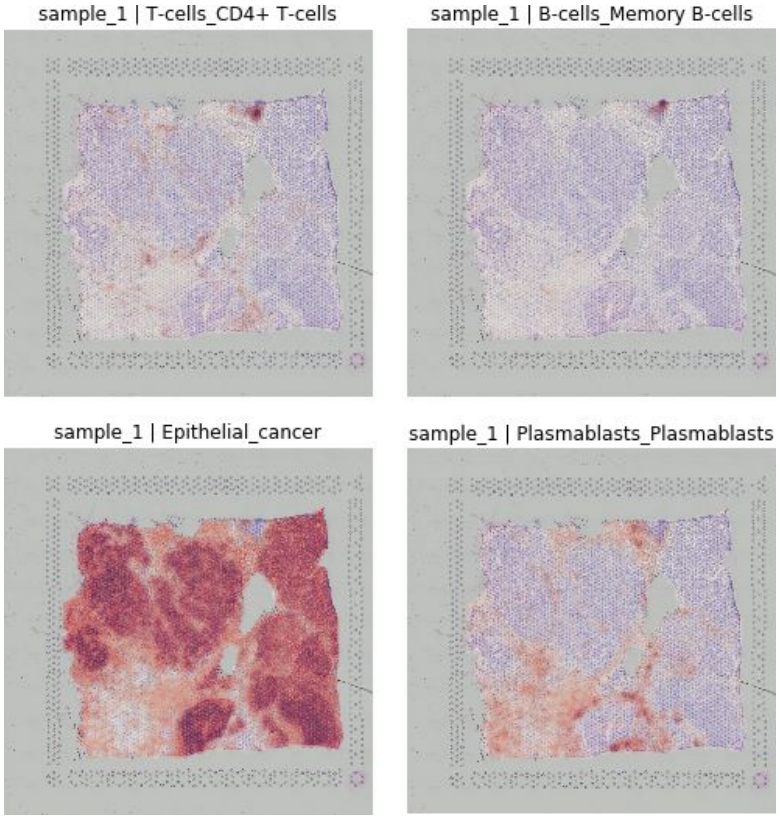
Proportion estimates



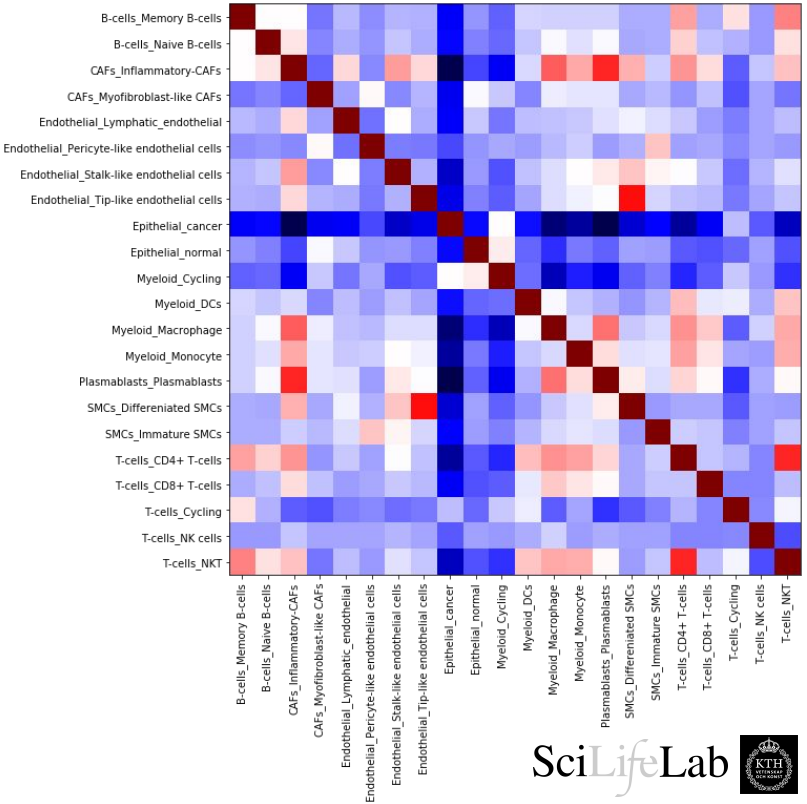
Applying it to our breast cancer data

P2

Proportion estimates



Cell type co-localization



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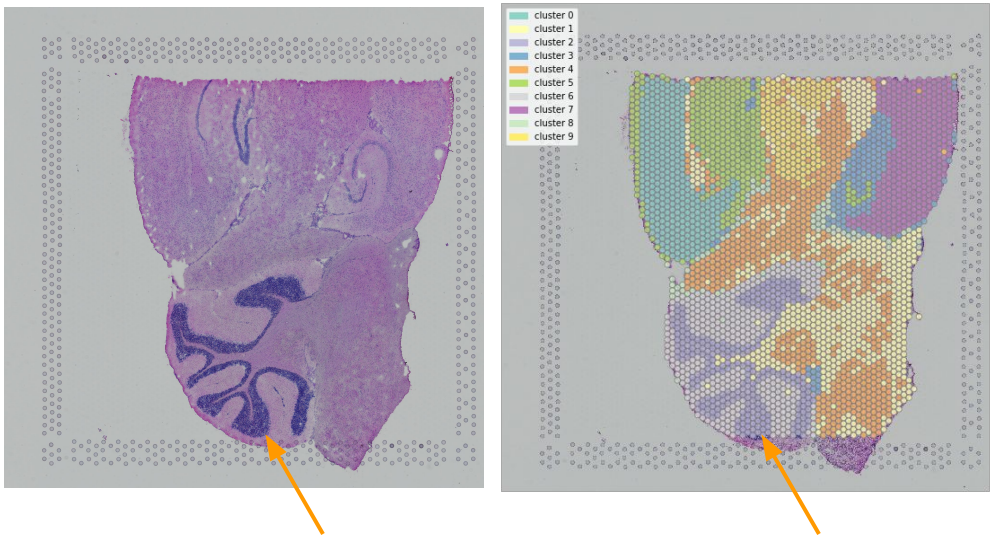
Summary : Integration of single cell and spatial data

- Leverages strengths from respective technique
    - Spatial resolution of well defined cell types
- Can be used as basis in subsequent analyses
    - Patterns of cell type co-localization
- Solution until experimental techniques reach single cell res.
- Atlases are exciting!

Example Analysis : Expression as a function of distance

- Say we cluster spatial data and find an interesting domain (e.g. cluster 2)



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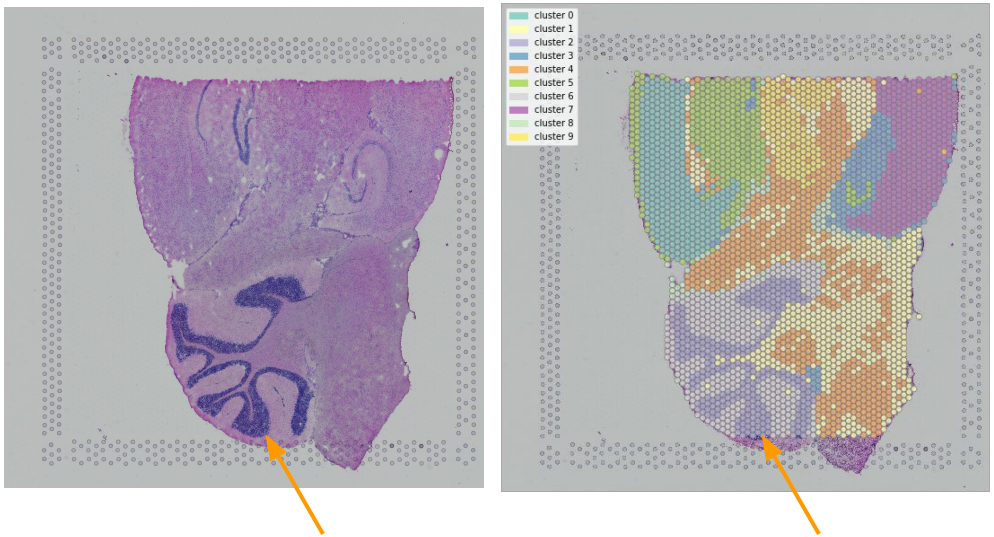
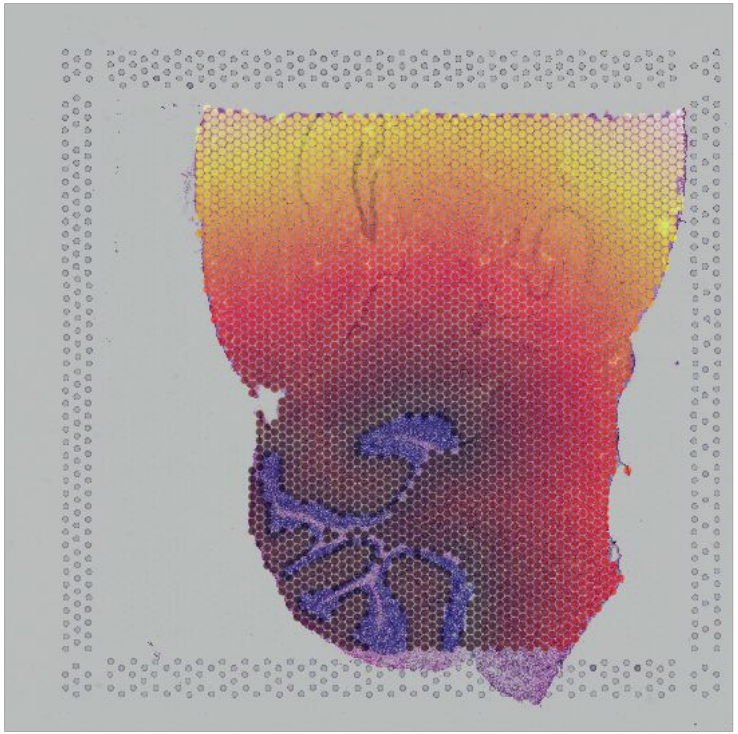
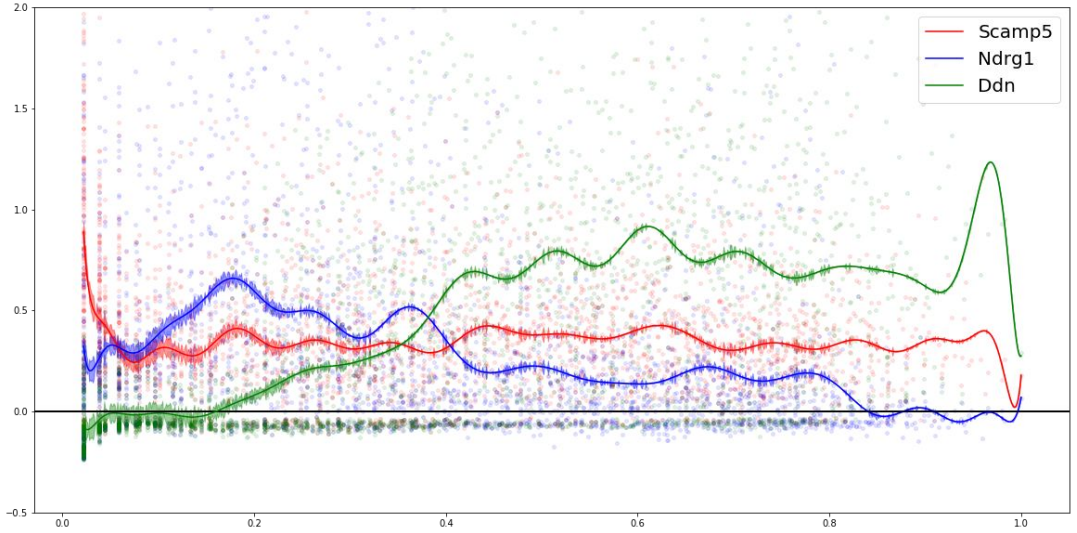


- We may then ask how gene expression changes with the distance to this cluster

Example Analysis : Expression as a function of distance

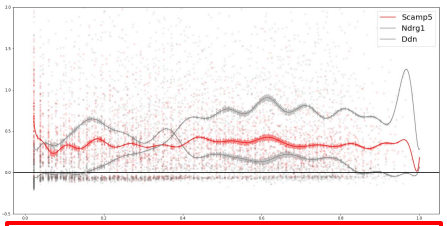


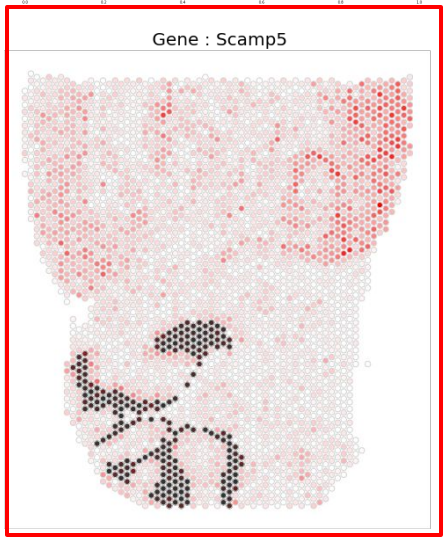
Spots colored by distance do cluster 2

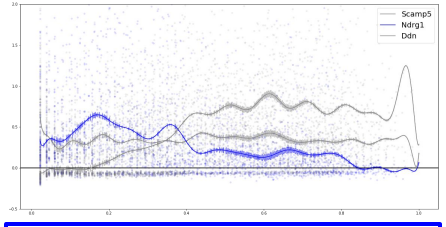


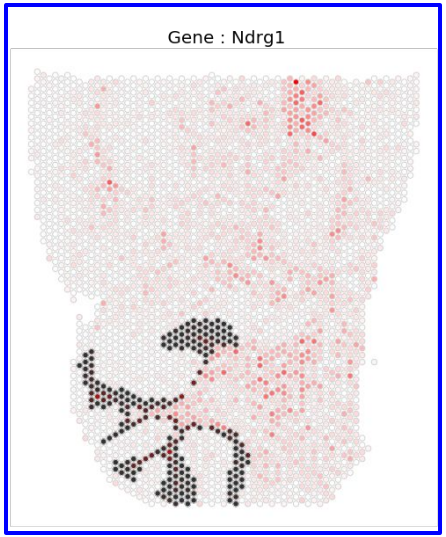
Gene expression as a function of the distance to cluster 2

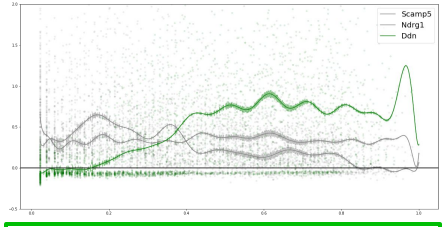
Example Analysis : Expression as a function of distance

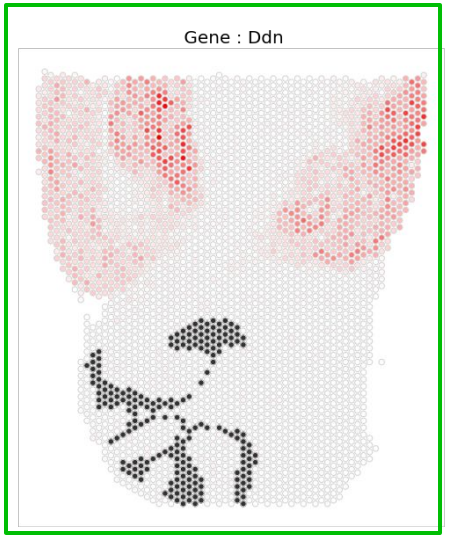












Example Analysis : Expression as a function of distance

- Can also ask : "*within which distance (d_g) from cluster 2 is ε % of all transcripts from gene g contained?*"

$$\varepsilon = 100 \times \frac{\int_0^{d_g} f(x)dx}{\int_0^1 f(x)dx}$$

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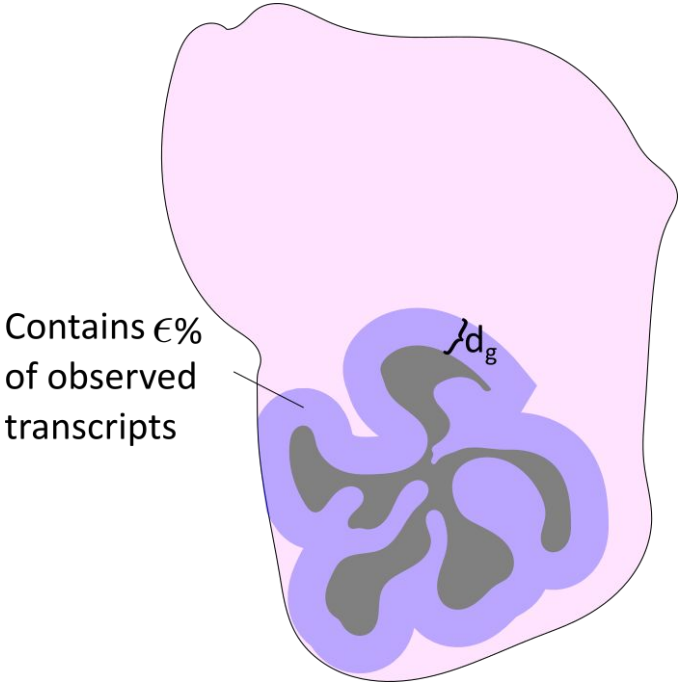

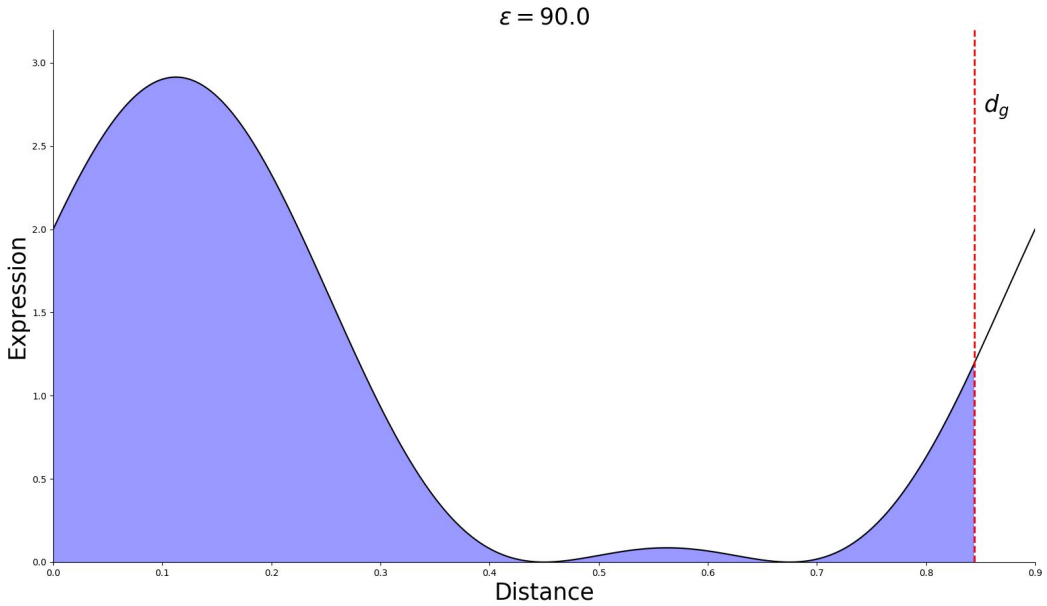

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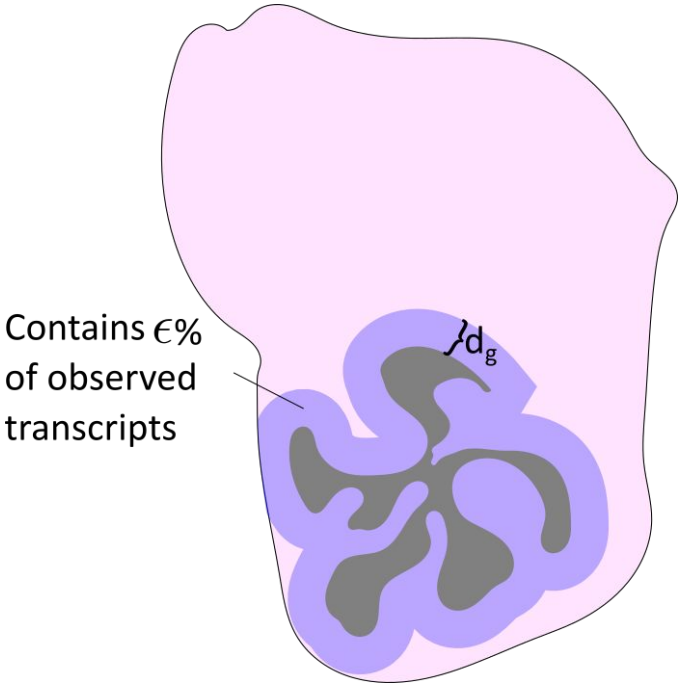

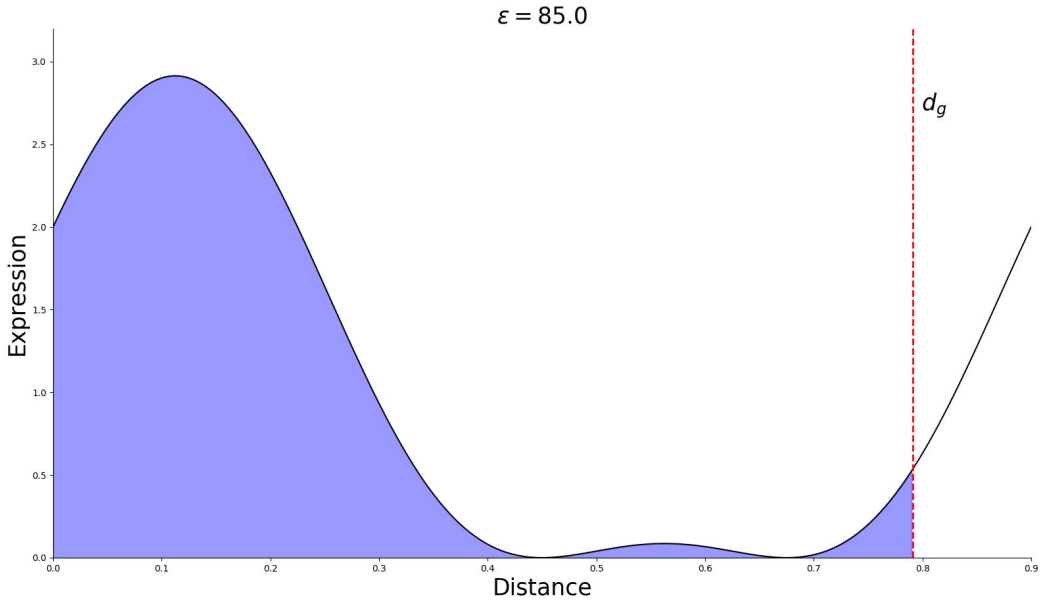

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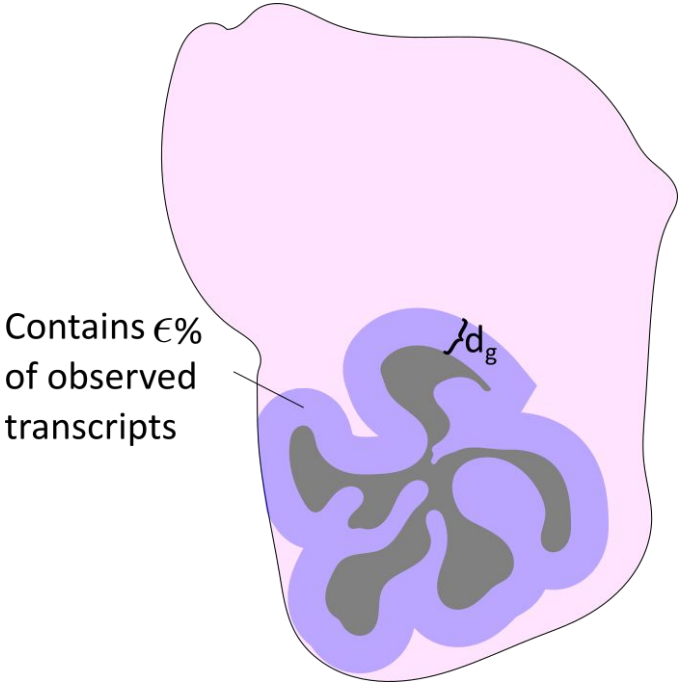

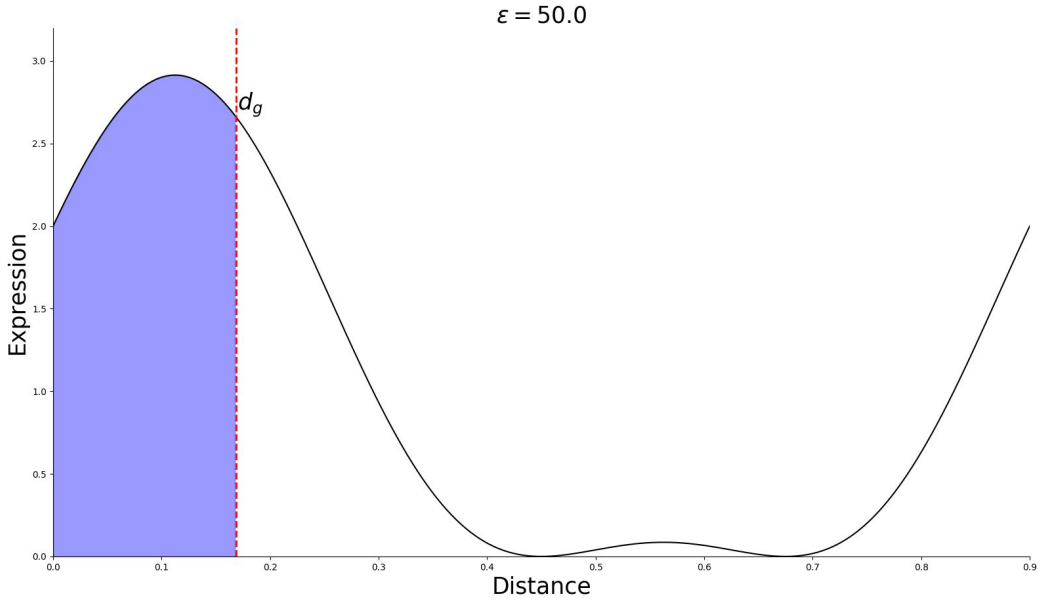

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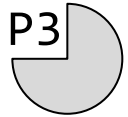

- Alternatively : "*which genes has ε % within the shortest distance (d_g) from cluster 2?*"

Example Analysis : Expression as a function of distance

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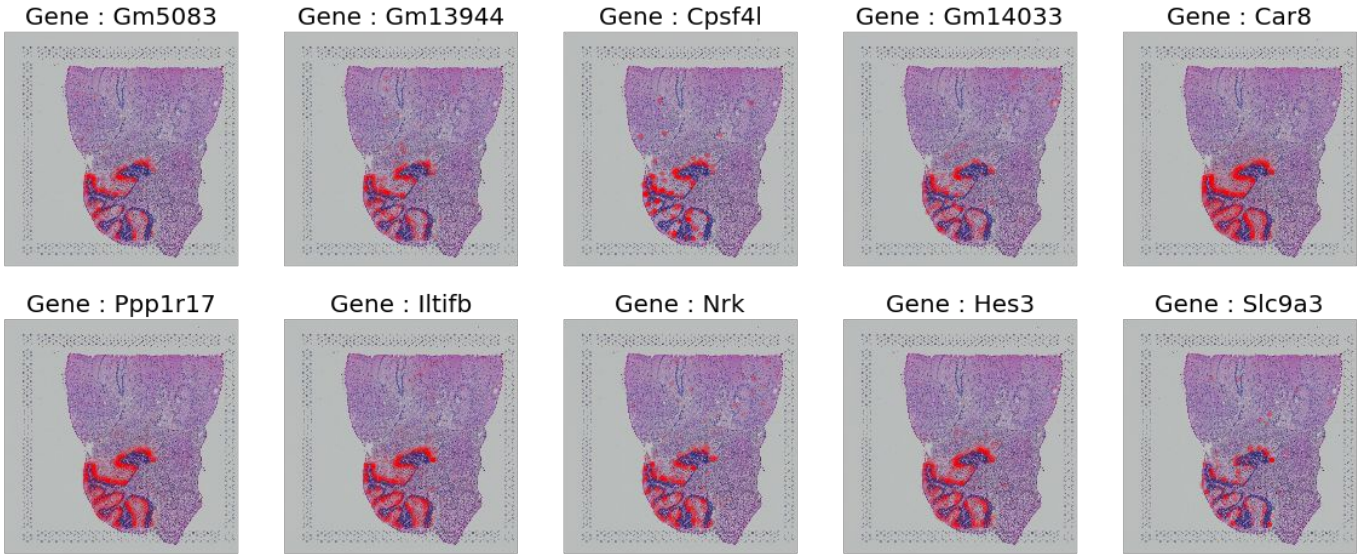

Exercises

Exercise Session

- Aims:
  - Getting familiar with spatial data
  - Overview - concept focused
- Written to be “independent” of lectures
  - Might experience some redundancy
  - but… *repetitio est mater studiorum*
- Three Parts
  - Part 1 - “*Getting Comfy with Spatial Data*”
    - Orienting, Inspecting and visualizing spatial data
    - Basic analysis workflow
  - Part 2 - “*Integrating Single Cell and Spatial RNA-Seq Data*”
    - Working with mapped data
    - Downstream analysis
  - Part 3 - “*Digging deeper into spatial analysis*”
    - Spatial gene set enrichment
    - Expression as a function of distance

Thank you for the attention

Questions