Exploring Spatial Transcriptomics

2020-05-28

Alma Andersson

https://github.com/almaan

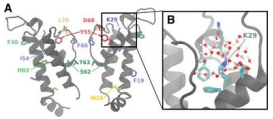
SPATIAL research

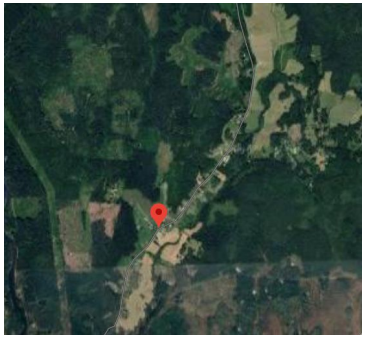
https://www.spatialresearch.org

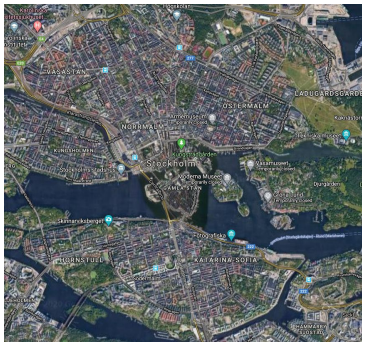
SciLifeLab

A brief Introduction

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







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Outline

- ~~Introduction~~
- Notation
- Background
- Data Processing
- Data Analysis (Overview)
  - Basic Analysis (Clustering and Visualization)
- Break
  - Questions
- Data Analysis
  - Mapping of cell types
  - More “spatially” focused
- Exercises (Info)
- 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

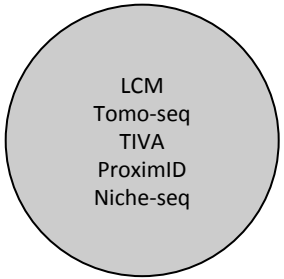
- Material in Part 3

Background

What is currently out there?

The spatial space | Overview of techniques

Microdissection-based



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

ISS (padlock)
BaristaSeq
STARmap
FISSEQ

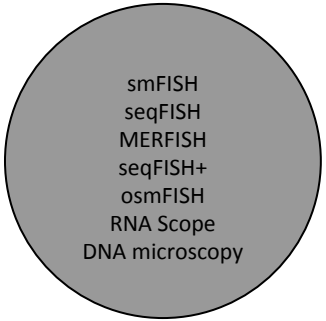
Sequence the transcripts in place. Offer sub-cellular resolution. Some relies on "*a priori*" defined targets, but not all.

Microdissection-based

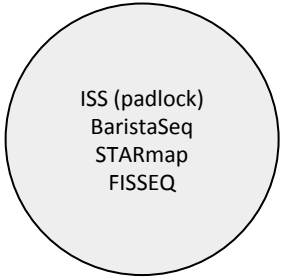
LCM
Tomo-seq
TIVA
ProximID
Niche-seq

The spatial space | Overview of techniques

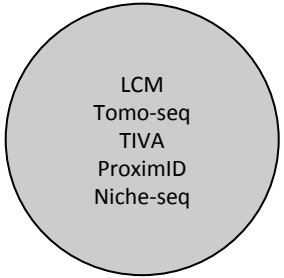
In situ hybridization



In situ sequencing



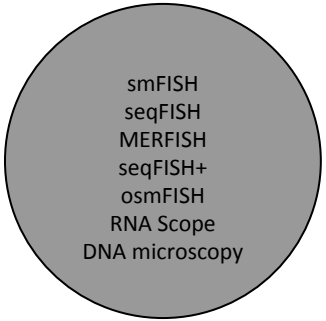
Microdissection-based



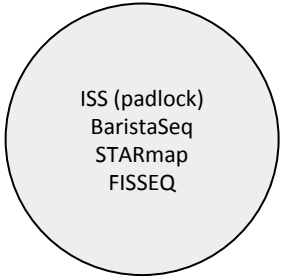
Labeled probes for specific targets, hybridize in place. Requires "*a priori*" defined targets. Expansion strategies and decoding scheme has helped to overcome spectral overlap.

The spatial space | Overview of techniques

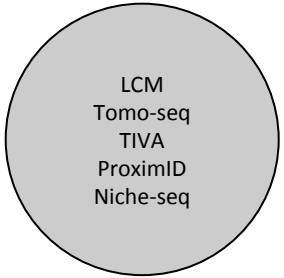
In situ hybridization



In situ sequencing

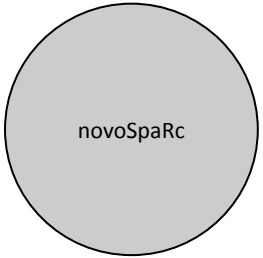


Microdissection-based



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

In silico



The spatial space | Overview of techniques

In situ hybridization

smFISH
seqFISH
MERFISH
seqFISH+
osmFISH
RNA Scope
DNA microscopy

In situ sequencing

ISS (padlock)
BaristaSeq
STARmap
FISSEQ

In situ capture

ST
Slide-Seq
HDST
GeoMX
Apex-Seq

Microdissection-based

LCM
Tomo-seq
TIVA
ProximID
Niche-seq

In silico

novoSpaRc

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

The spatial space | Overview of techniques

In situ hybridization

smFISH
seqFISH
[illegible]
[illegible]
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[illegible]
[illegible]copy

In situ sequencing

ISS (pad
Barista
STARm
FISSE

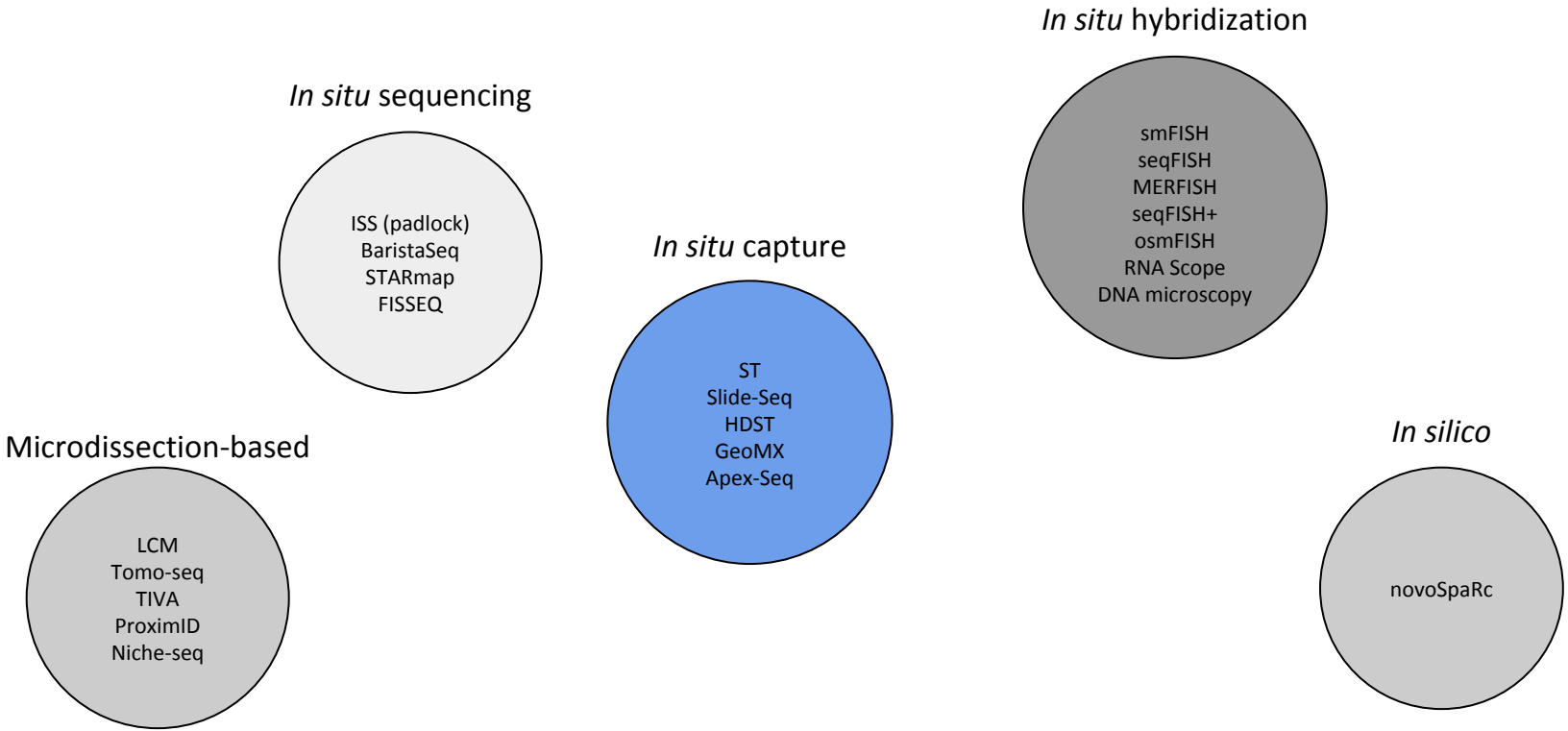
Microdissection-based

LCM
Tomo-seq
TIVA
ProximID
Niche-seq

In silico

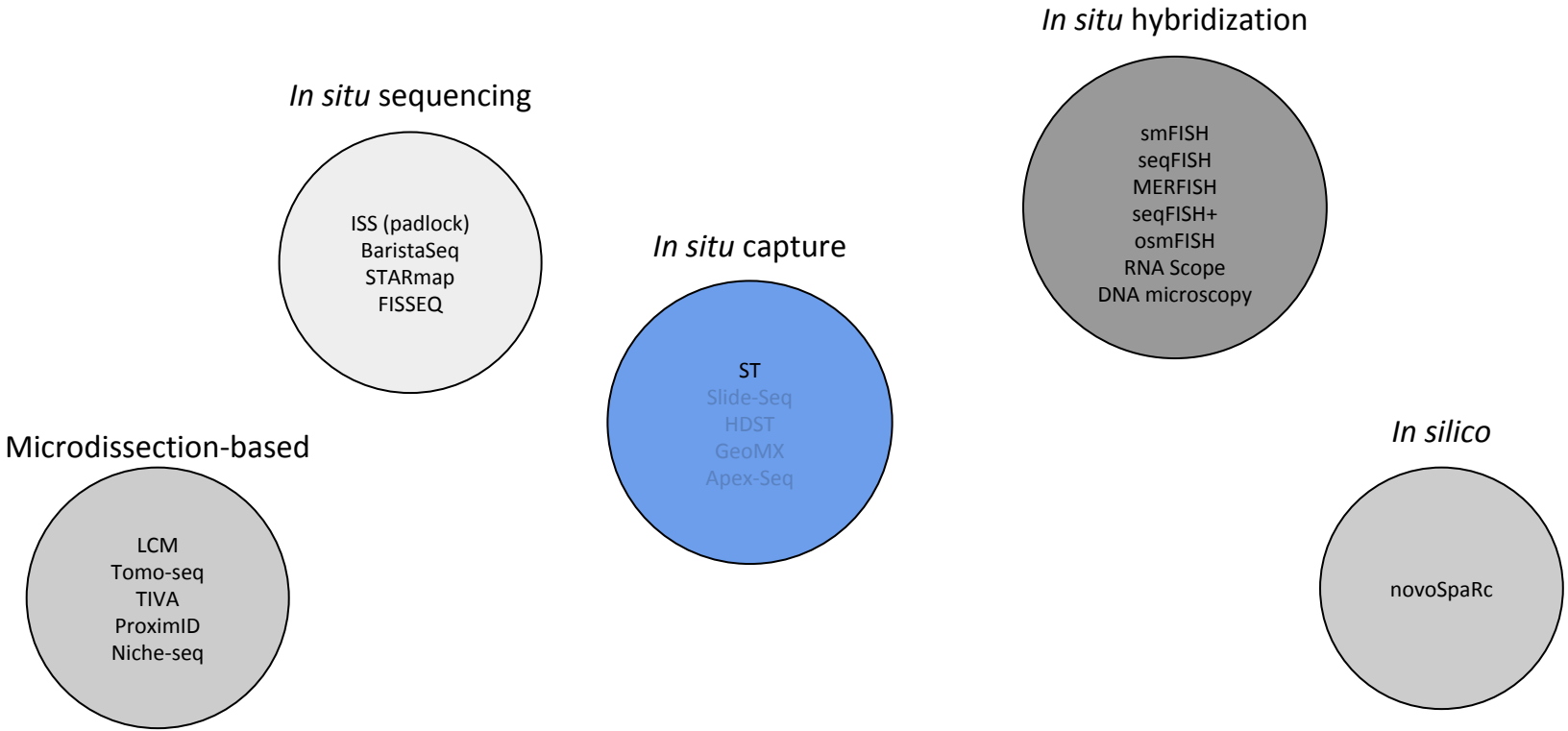
novoSpaRc

The spatial space | Overview of techniques



From : https://doi.org/10.1002/bies.201900221

The spatial space | Overview of techniques



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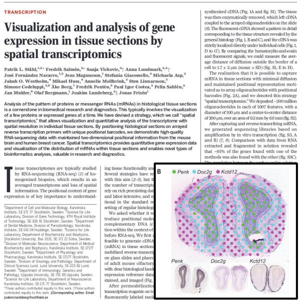
Spatial Transcriptomics (ST)

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,[2,3†] José Fernández Navarro,[1,2] Jens Magnusson,[1] Stefania Giacomello,[2] Michaela Asp,[2] Jakub O. Westholm,[4] Mikael Huss,[4] Annelie Mollbrink,[2] Sten Linnarsson,[5] Simone Codeluppi,[5,6] Åke Borg,[7] Fredrik Pontén,[8] Paul Igor Costea,[2] Pelin Sahlén,[2] Jan Mulder,[9] 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.

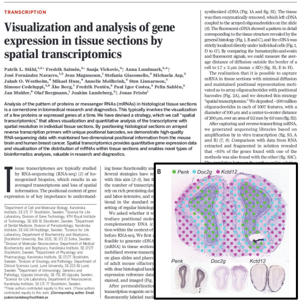
Science Publication
Ståhl et.al
DOI: 10.1126/science.aaf2403

"Spatially barcoded arrays"

Technique/Method Name : *Spatial Transcriptomics*

Spatial Transcriptomics (ST)

Mid 2016



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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-

[1]Department of Cell and Molecular Biology, Karolinska Institute, SE-171 77 Stockholm, Sweden. [2]Science for Life Laboratory, Division of Gene Technology, KTH Royal Institute of Technology, SE-106 91 Stockholm, Sweden. [3]Department of Dental Medicine, Division of Periodontology, Karolinska Institute, SE-141 04 Huddinge, Sweden. [4]Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Box 1031, SE-171 21 Solna, Sweden. [5]Division of Molecular Neuroscience, Department of Medical Biochemistry and Biophysics, Karolinska Institute, SE-17177 Stockholm, Sweden. [6]Department of Physiology and Pharmacology, Karolinska Institute, SE-17177 Stockholm, Sweden. [7]Division of Oncology and Pathology, Department of Clinical Sciences Lund, Lund University, SE-223 81 Lund, Sweden. [8]Department of Immunology, Genetics and Pathology, Uppsala University, SE-751 85 Uppsala, Sweden. [9]Science for Life Laboratory, Department of Neuroscience, Karolinska Institute, SE-171 77 Stockholm, Sweden.
*These authors contributed equally to this work. †These authors contributed equally to this work. ‡Corresponding author. Email: joakim.lundeberg@scilifelab.se

ing tissue functionality an
Several strategies have re
with this aim (2–5), but t
the number of transcripts
rely on rich preexisting dat
and labor-intensive, and n
tional in the standard re
setting of regular histologi
We asked whether it w
troduce positional mole
complementary DNA (c
tion within the context of
before RNA-seq. We first a
feasible to generate cDNA
(mRNA) in tissue section
mobilized reverse-transcri
on glass slides and placed
of adult mouse olfactory
with clear histological land
expression reference data
stained, and imaged (Fig.
After permeabilizatio
transcription reagents on to
fluorescently labeled nucl

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 6.2 mm by 6.6 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).

Science Publication
Ståhl et.al
DOI: 10.1126/science.aaf2403

Late 2018

10x GENOMICS®

(acquisition)

SciLifeLab KTH

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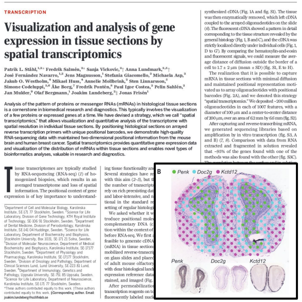

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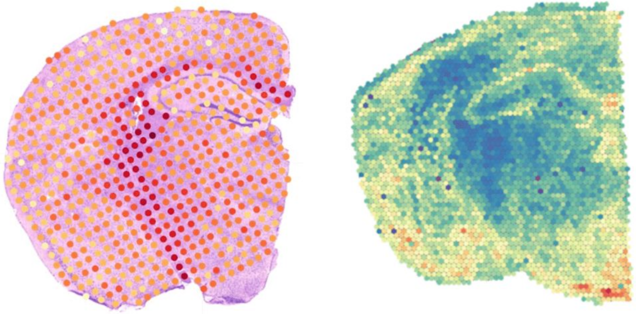
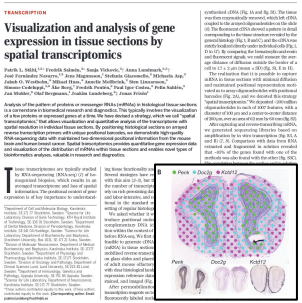
Late 2019



Launch of **Visium** Spatial Gene Expression Platform

~~Spatial Transcriptomics (ST)~~ Visium

Mid 2016



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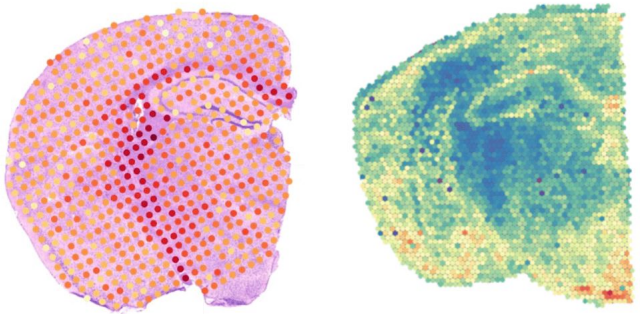
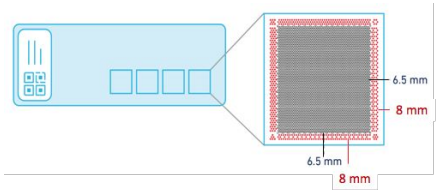
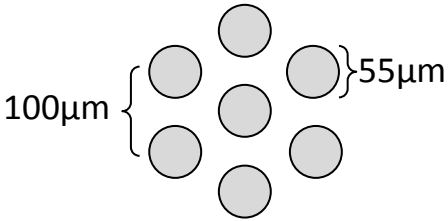


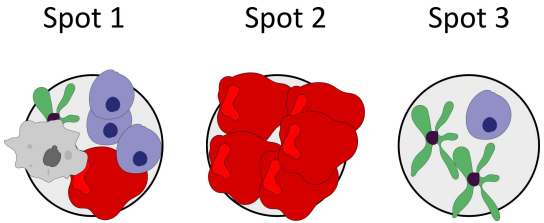
Launch of **Visium** Spatial Gene Expression Platform

Visium Platform

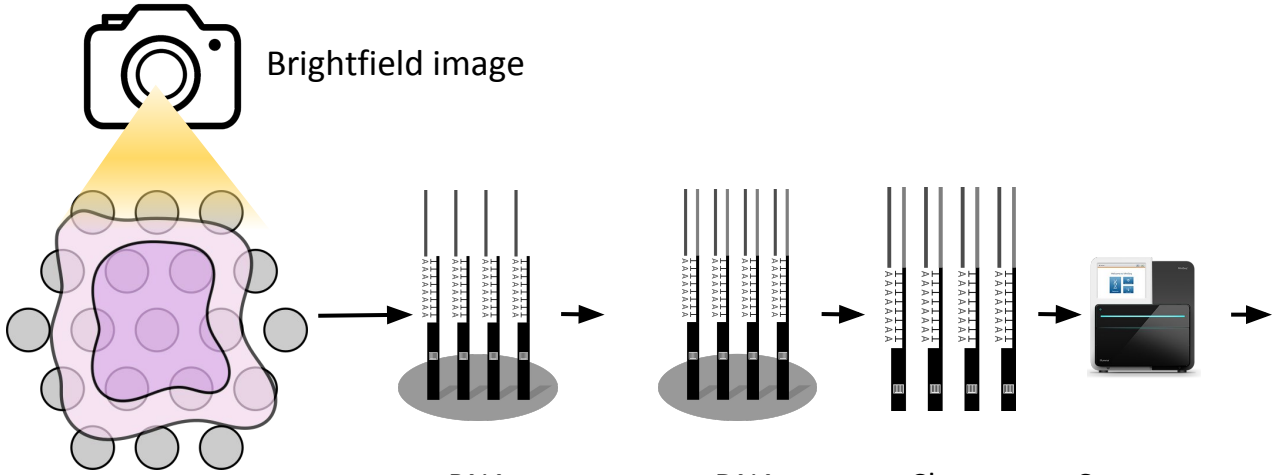
- Array based technique
- 6.5mm x 6.5mm area to put sample on
- 4992 spots arranged in hexagonal grid
- Spot specs:
  - Spot diameter : 55µm
  - Center to center distance : 100 µm
- Each spot has millions of capture probes
  - spatial barcode
  - polyT sequence
  - captures polyadenylated mRNA
  - Full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







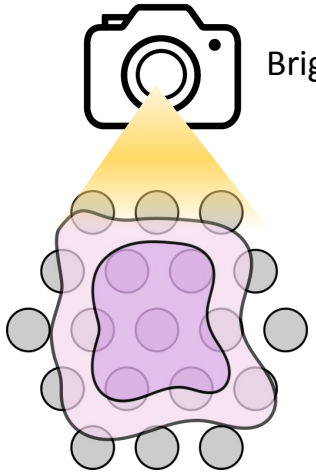
The experimental workflow (in a nutshell)



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

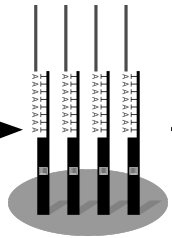
Count Matrix

The experimental workflow (in a nutshell)

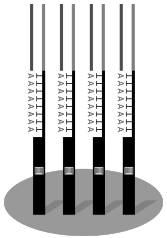


Brightfield image

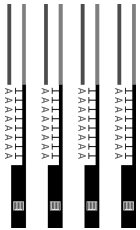
Tissue +
Visium-array



mRNA
capture



cDNA
synthesis



Cleave
Probes

Sequence +
preprocess

Where the fun starts!

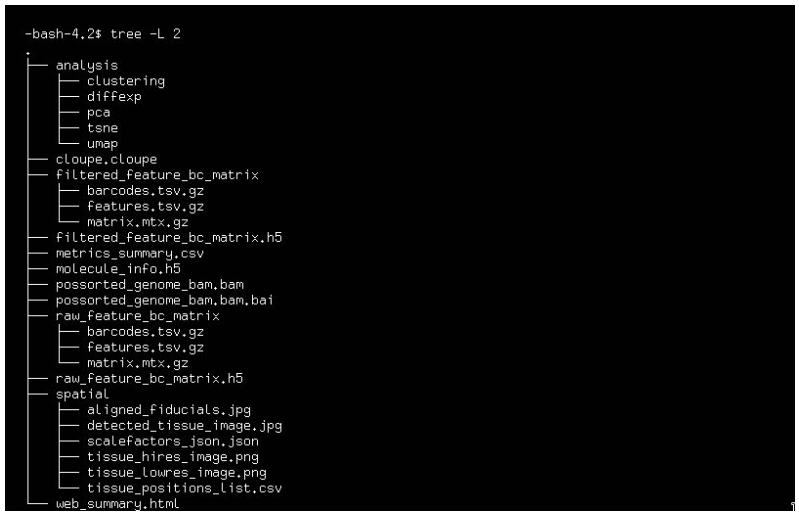
spot (barcode)	gene_1	...	gene_{G-1}	gene_G
AAACATACAACCAC-1	5	...	0	9
AAACATTGAGCTAC-1	3	...	1	3
⋮	4	⋱	0	0
AAACATTGATCAGC-1	0	...	8	7

Count Matrix

Data Processing

After sequencing (brief)

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



count data provided as *.mtx* and *.h5*

- *raw* : all spots
- *filtered* : spots under tissue

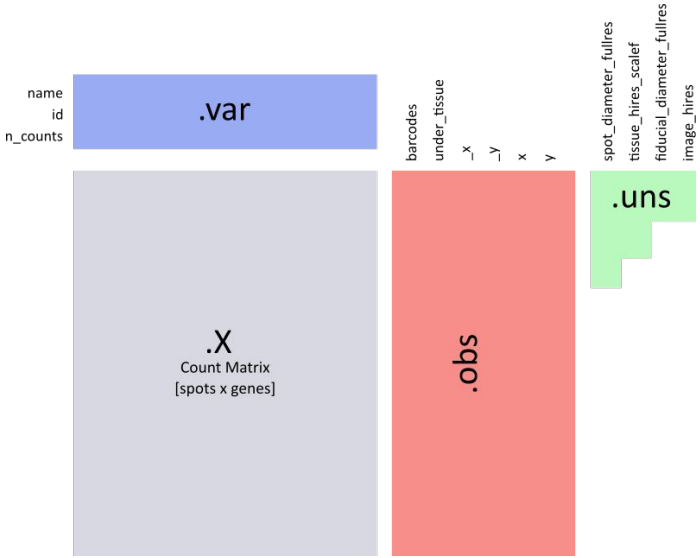
Example of spaceranger count output

https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/what-is-space-ranger

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



In short:

.var - holds gene identifiers
.obs - spot identifiers and coordinates
.uns - image and scaling factors

https://github.com/almaan/space2h5ad/

An initial assessment

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

An initial assessment

P1

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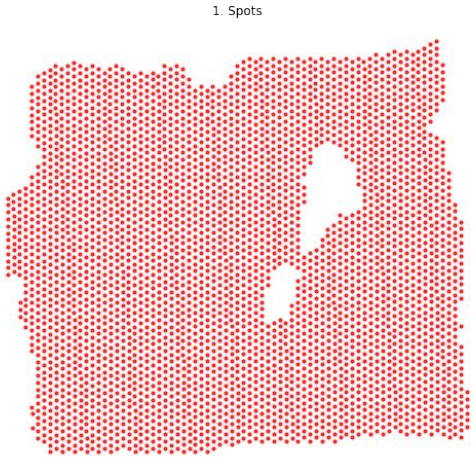
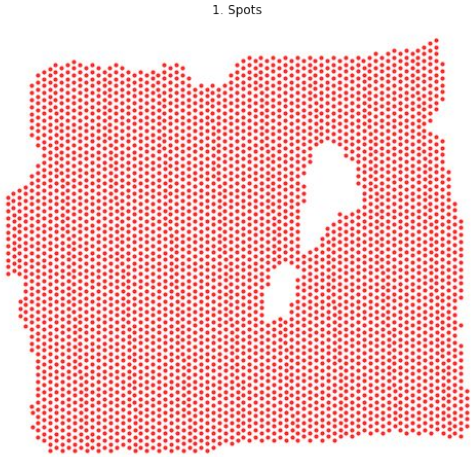
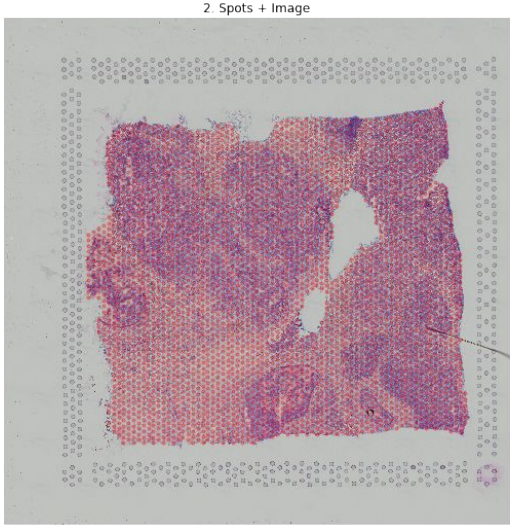


An initial assessment

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





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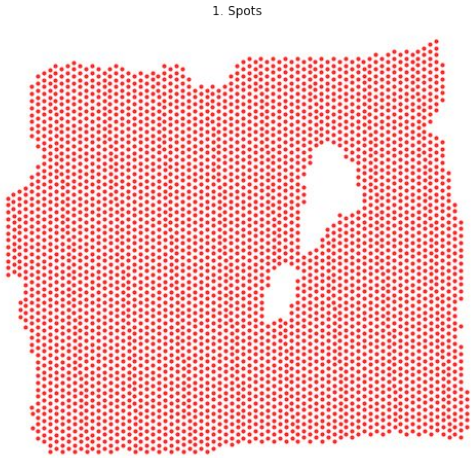
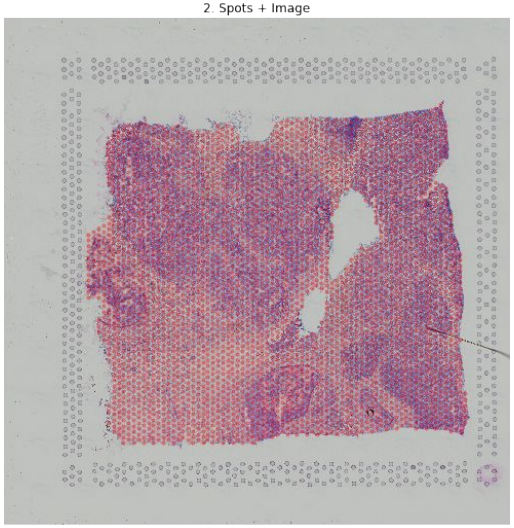
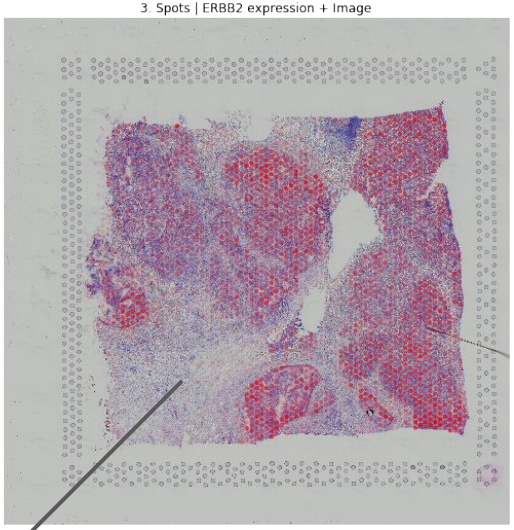
- Example with Human Breast cancer data
  - Public data : Available at 10x website





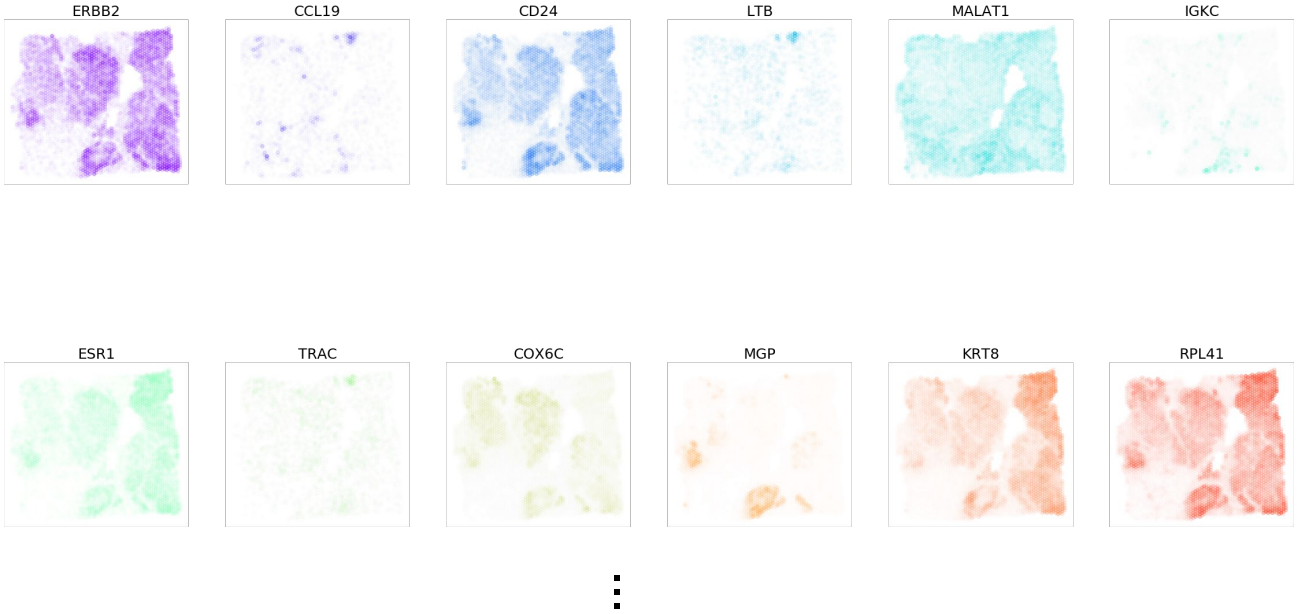


Facecolor intensity proportional to gene expression value

Visualizing high dimensional spatial data

(Tips)

- We visualized one feature/gene (*ERBB2*)
- **Q:** How do we “visualize” ~20000 features?



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All in one image

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  - Embed gene expression data in 3 dimensional space (e.g. using UMAP)
  - Do affine transformation to unit cube
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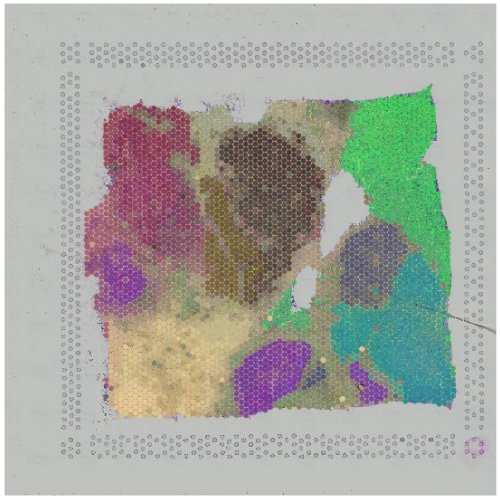

Regions with similar colors have similar gene expression.

Data 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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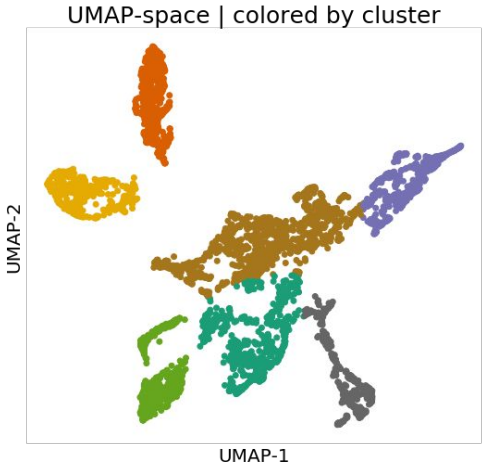
  - Ribosomal and mitochondrial genes tend to exhibit spurious expression patterns.
- Normalization / batch correction :
  - Recommend to account for spot “library size“ - varying cell density
  - Include slide/array as covariate (sometimes big variation is observed)
  - Tools that have performed well : *sctransform* and *Harmony*

† Not always necessary

Example : Basic Analysis

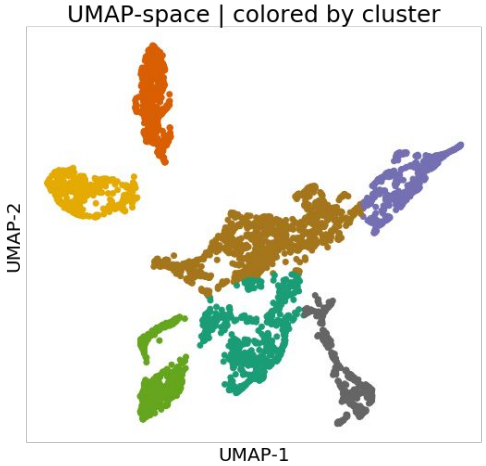
- Cluster the spots based on gene expression

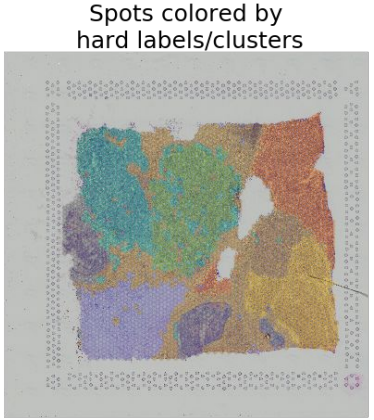


Example : Basic Analysis

P1

- Cluster the spots based on gene expression
- Backmap clusters onto tissue
- Use HE-image as reference
  - Sanity check - does it make sense?
  - Valuable information resource
- Next, what do these clusters represent?





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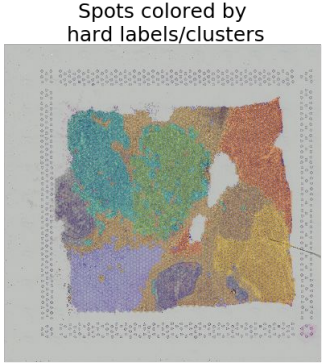
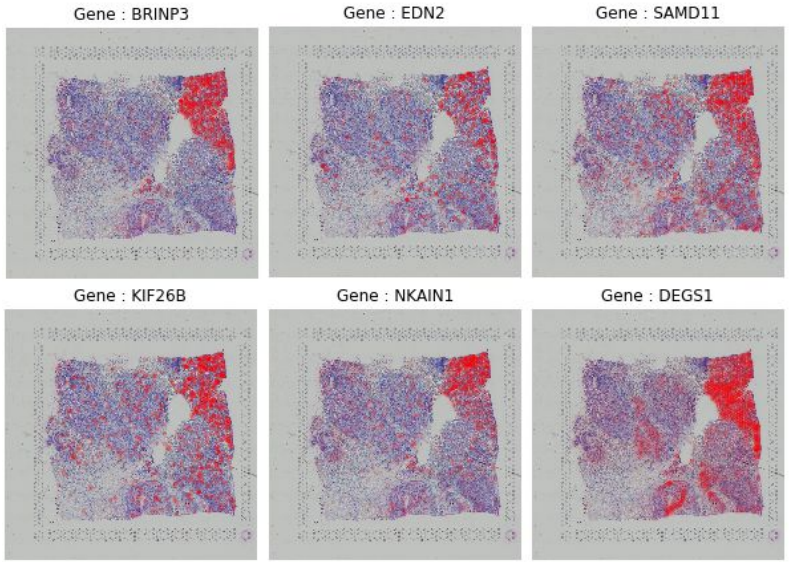
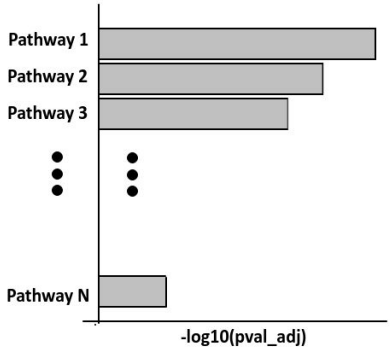


Clustered data



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



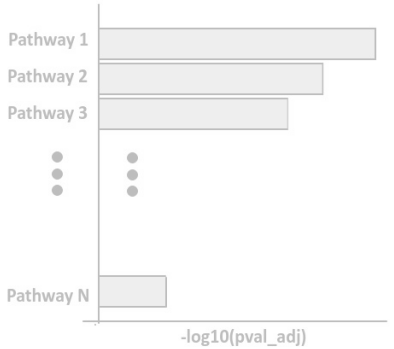
Functional Enrichment Analysis

Example : Basic Analysis

Clustered data

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

BUT



Functional Enrichment Analysis

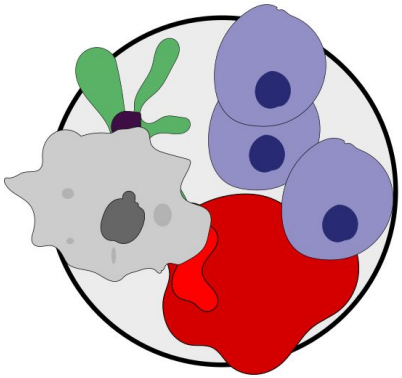
All helpful in annotation of clusters

Example : Basic Analysis

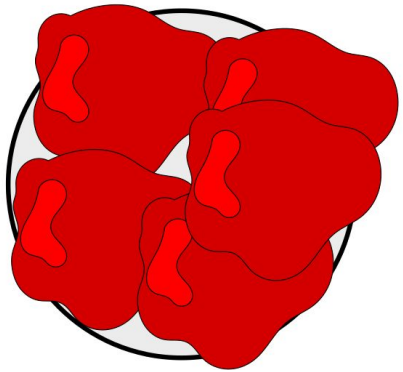
Remember :

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

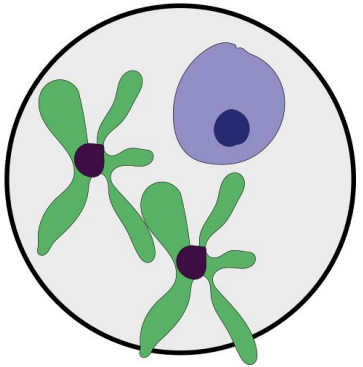
Spot 1



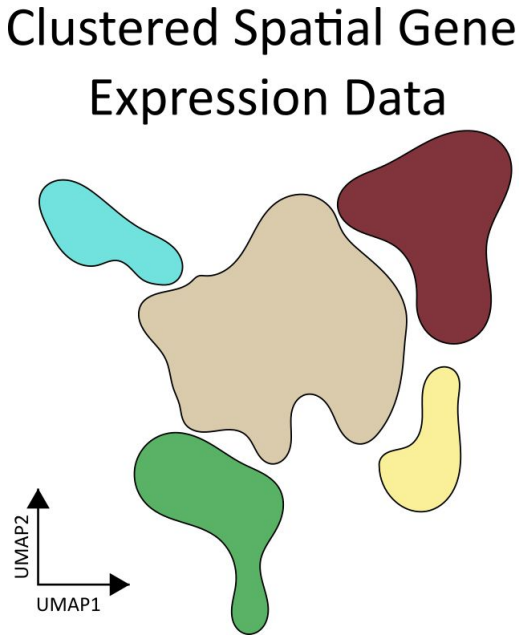
Spot 2

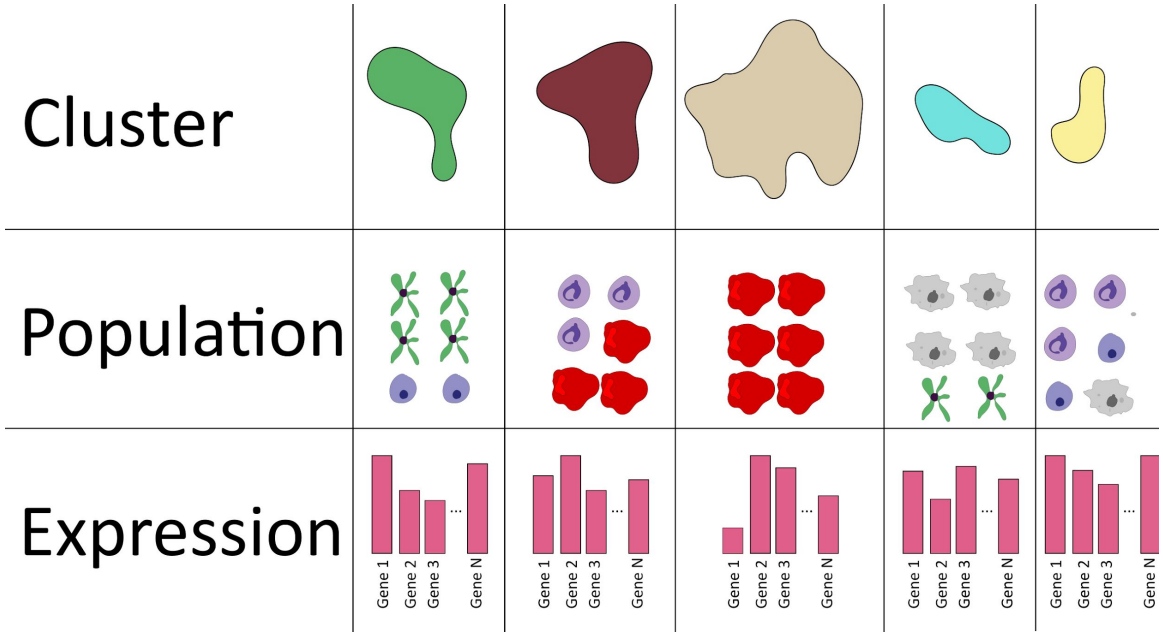


Spot 3



Cluster ≠ Cell type

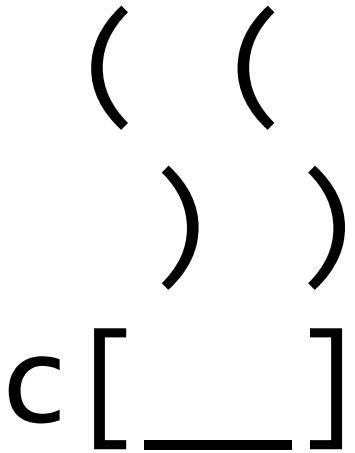




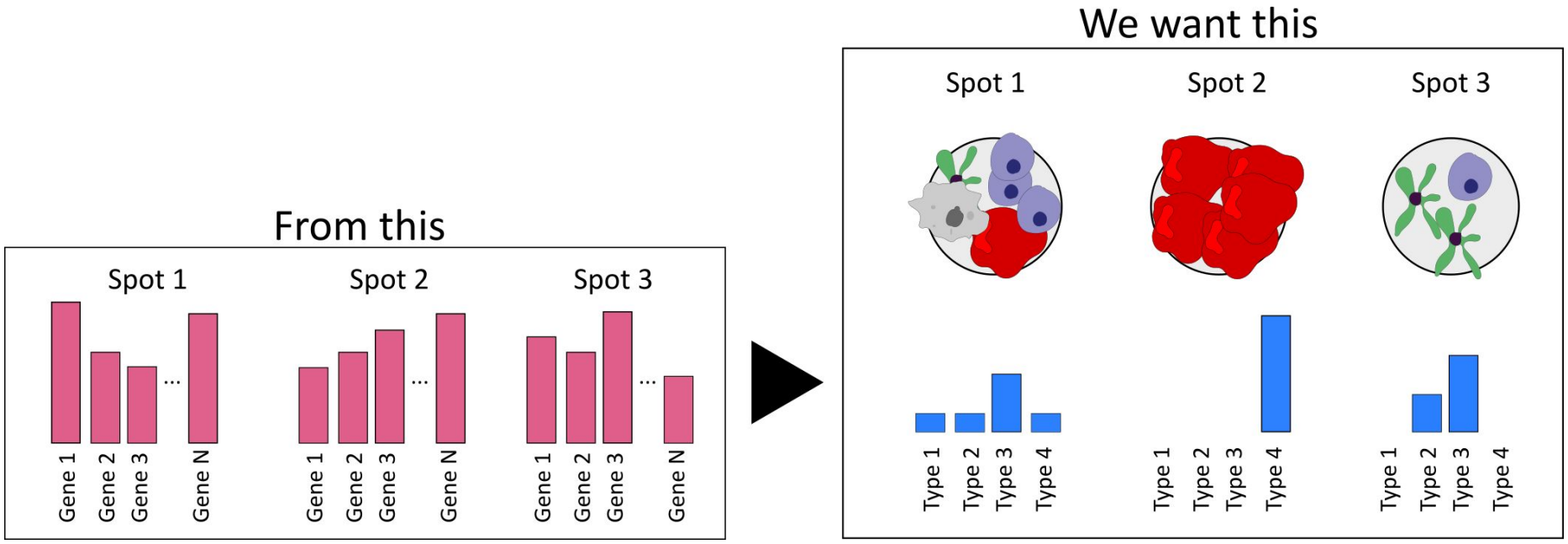
So where are my cell types?

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



Our objective : deconvolve expression data



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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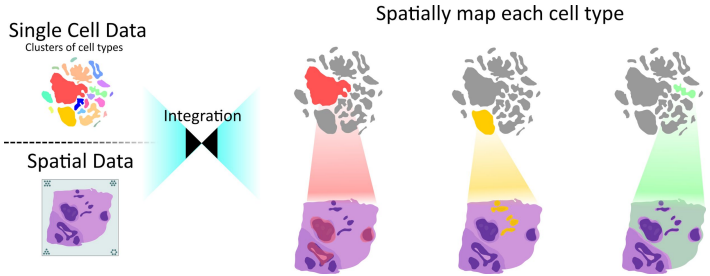
- Alternative solution - Integrate single cell (SC) and spatial data!
  - Extract information of cell types from SC data and apply to spatial data



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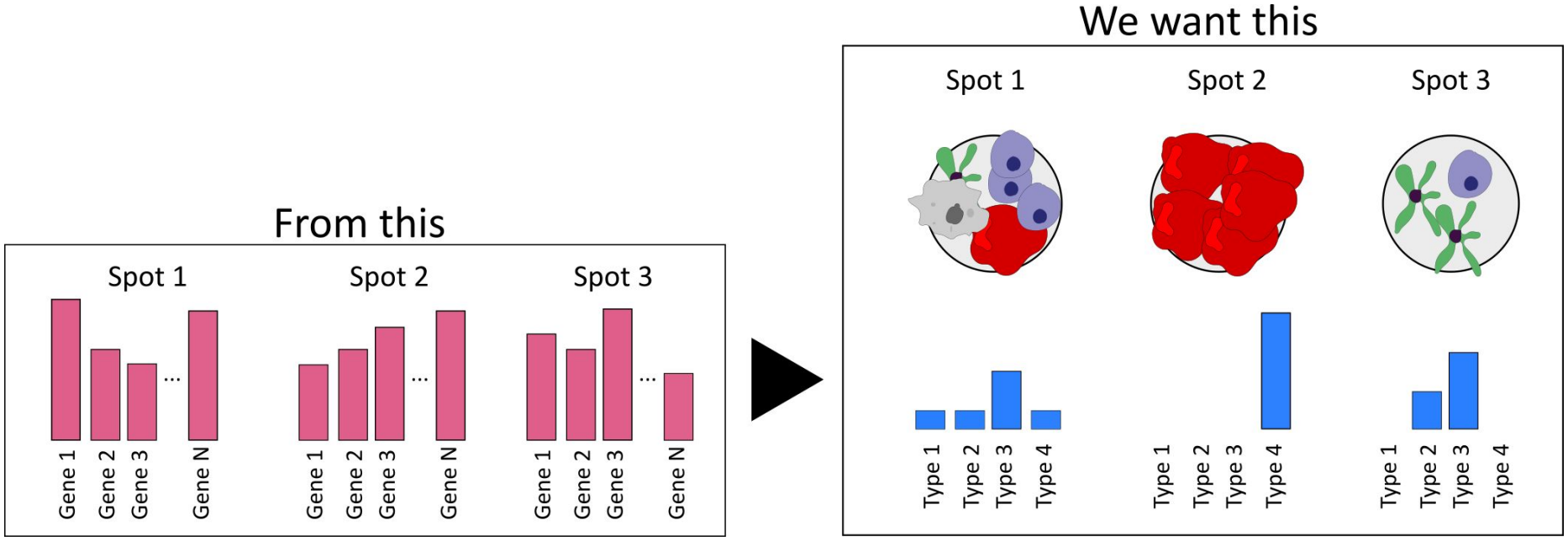
- Alternative solution - Integrate single cell (SC) and spatial data!
  - Extract information of cell types from SC data and apply to spatial data
  - **Big challenge : deconvolution required (on Visium data)**
    - How “much” of each cell type at each spot

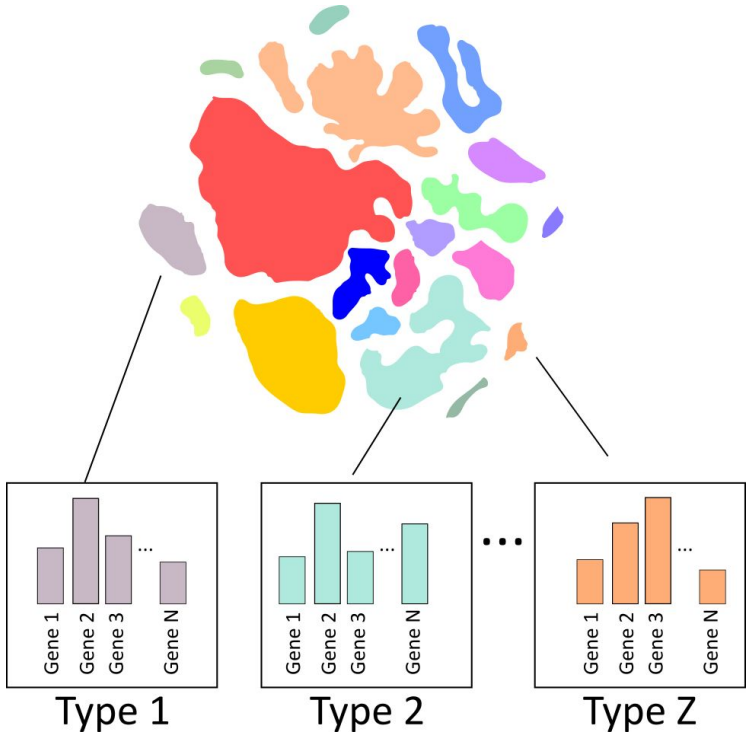
Our objective : deconvolve expression data



Suggested approach : *Model-based Probabilistic Inference*

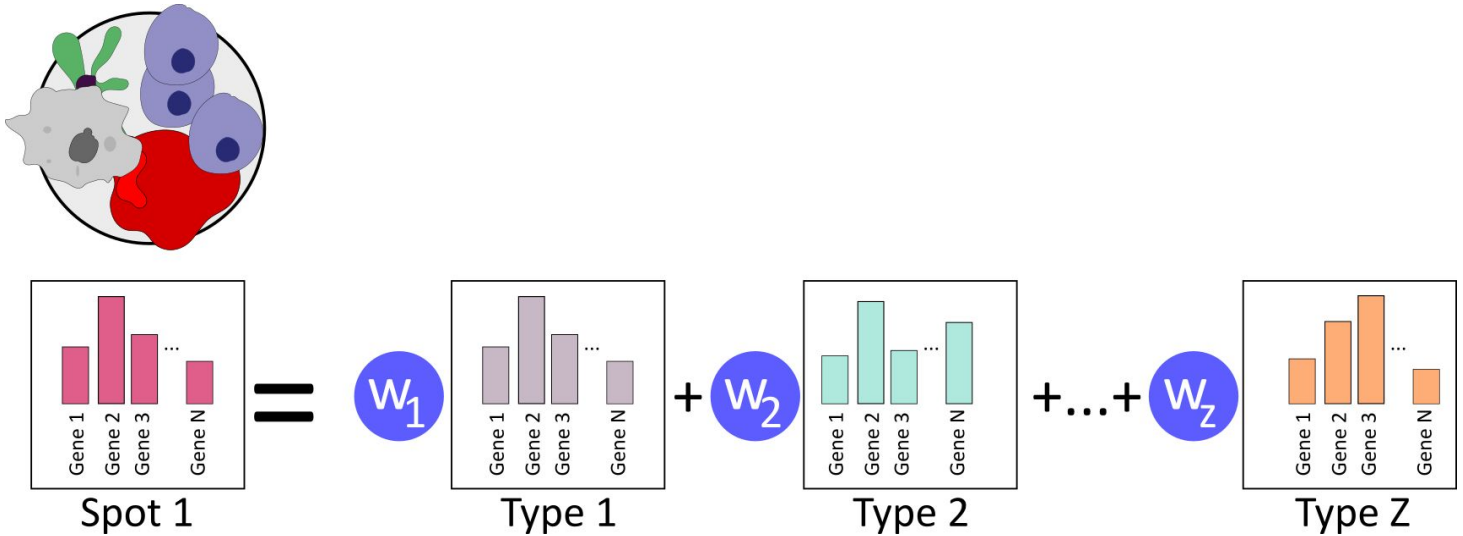
It’s as easy as 1-2-3

1. Infer cell type expression parameters from SC data



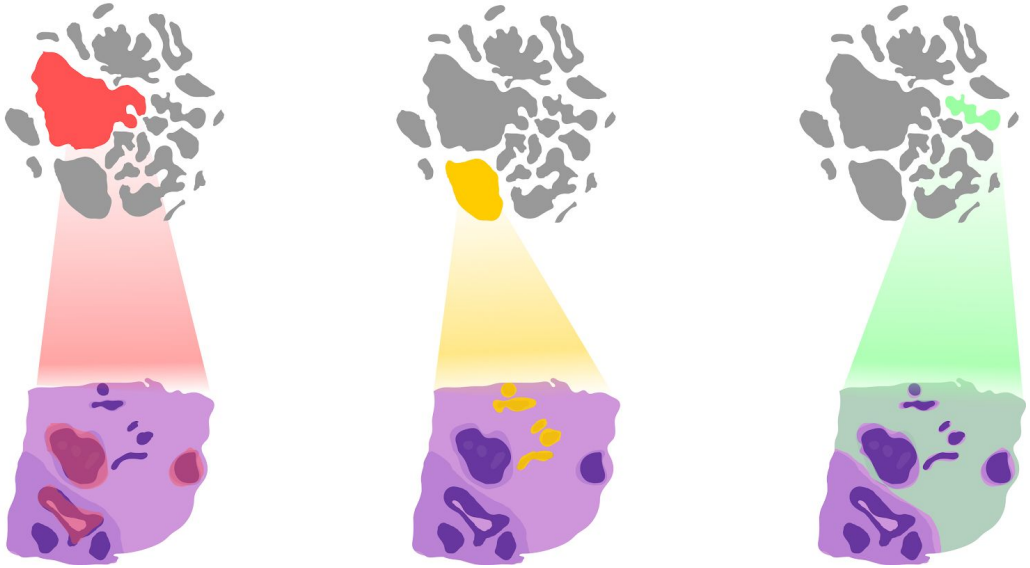
It’s as easy as 1-2-3

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



The machinery behind it

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Single Cell

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

Expression of gene “g” in cell “c”.

“*c*” is of cell type “z”

The machinery behind it

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Spatial

$$x_{gsc} \sim NB(\beta_g \alpha_s r_{gz_c}, p_g)$$

Expression of gene “*g*” at spot “*s*” from cell “*c*”.

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The machinery behind it

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Spatial

Gene Bias

Scaling factor

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The machinery behind it

Hidden values

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

This is what we observe,
elements in count matrix

The machinery behind it

Hidden values

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This is what we observe, elements in count matrix

Sum of NB-distributed variables with shared second parameter (p_g) is also NB-distributed

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

The machinery behind it

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

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”

The machinery behind it

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

join scaling factor (α_s) and cell counts (n_{sz}).
unadjusted proportions

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

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

The machinery behind it

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

Use MLE to get estimates of cell type parameters

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

Given the cell type parameters, use MLE to get values of gene scaling factors (β_g) and unadjusted proportions (v_{sz})

The machinery behind it

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

The machinery behind it

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

Number of cells from cell type "*z*" at spot "*s*"

Total number of cells at spot "*s*"

The machinery behind it

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

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

The machinery behind it

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

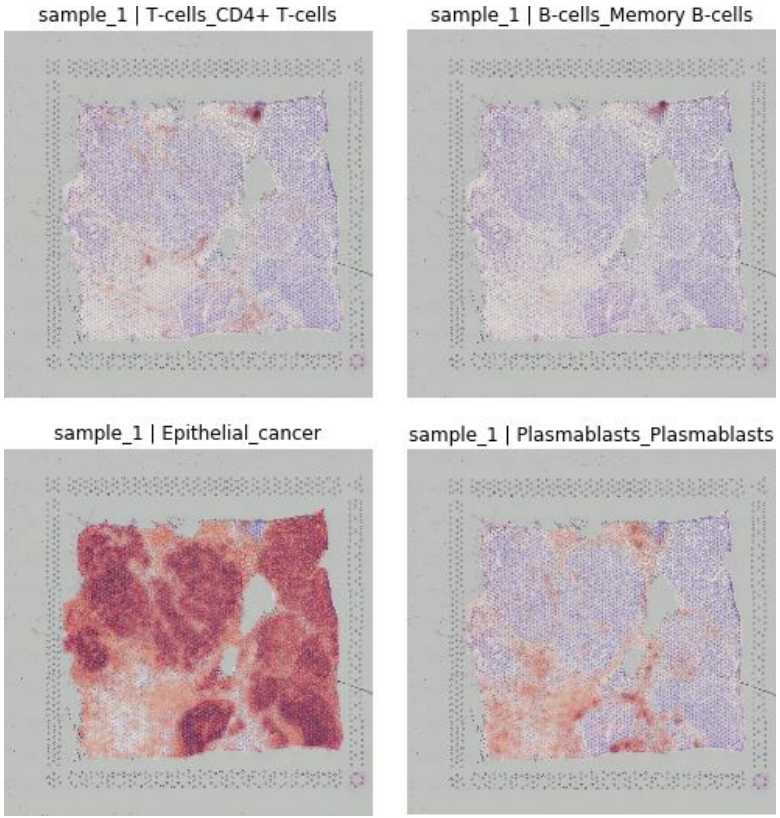
https://github.com/almaan/stereoscope

#shameless self-advertising

SciLifeLab

Applying it to our breast cancer data

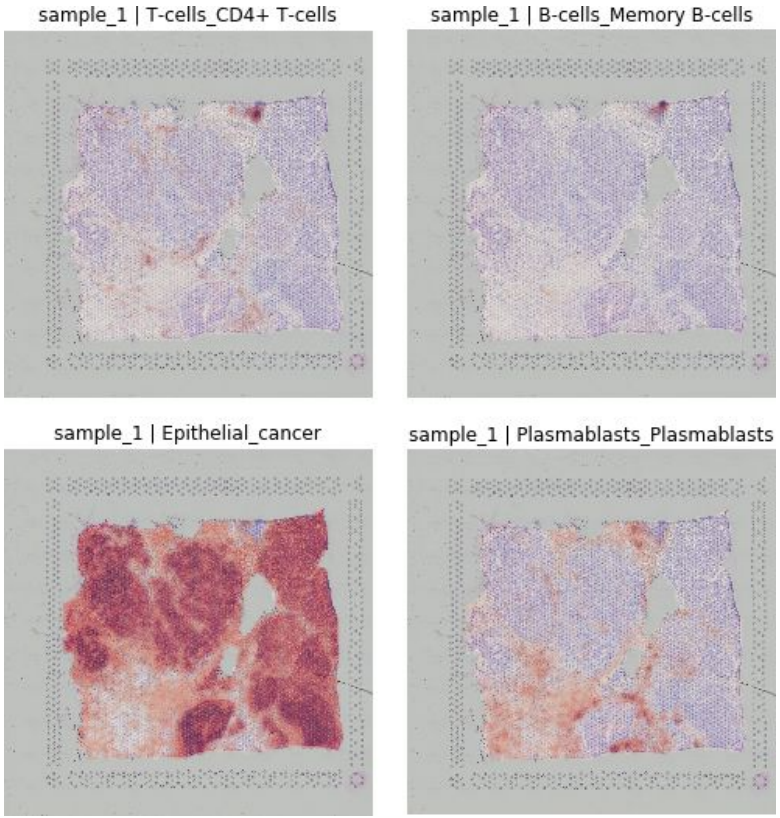
Proportion estimates



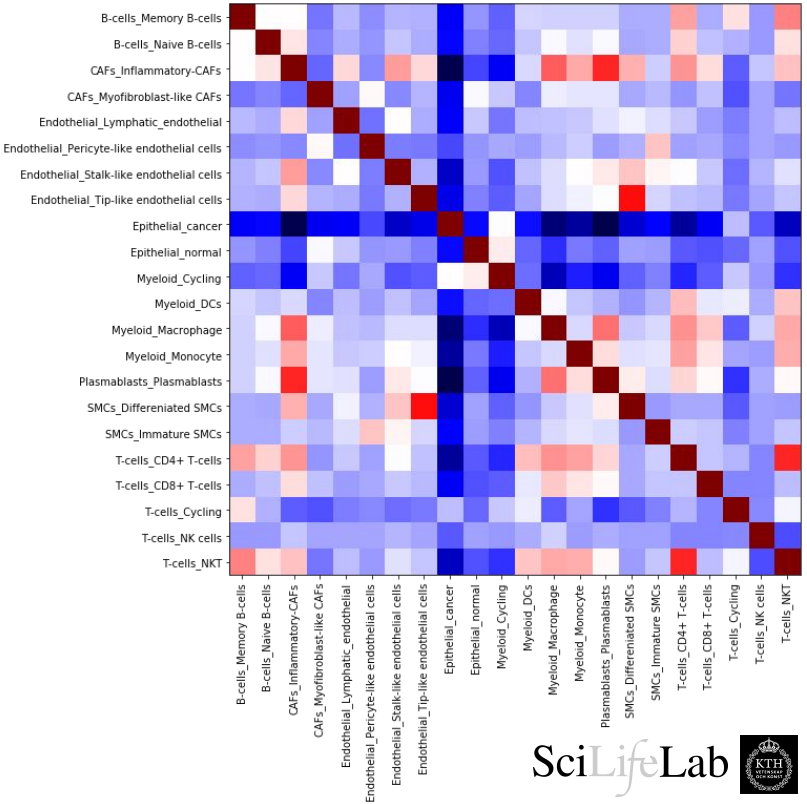
Applying it to our breast cancer data

Single cell data from Swarbrick lab, Australia

Proportion estimates



Cell type co-localization

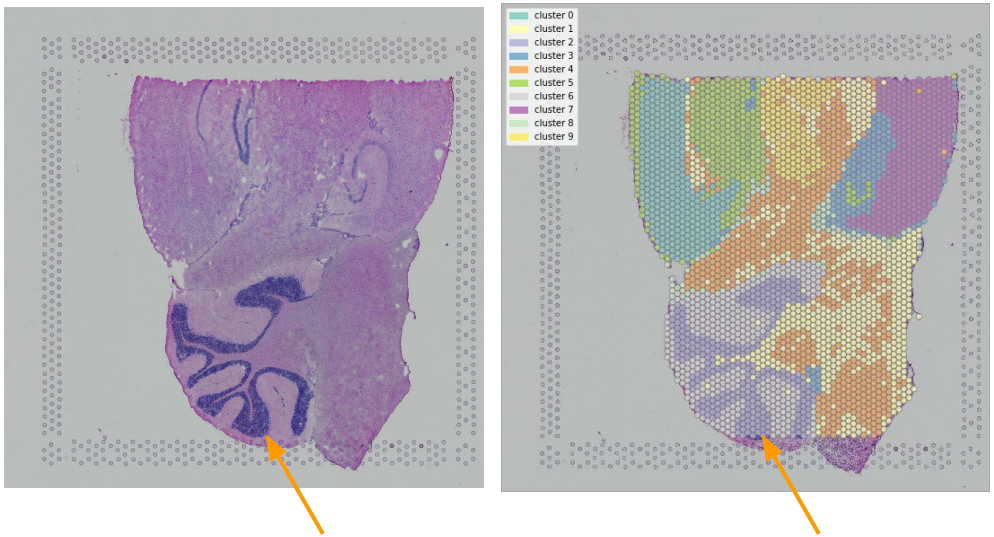


Summary : Integration of single cell and spatial data

- 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
- Until experimental techniques reach single cell resolution
- 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)



Data from 10X Genomics website

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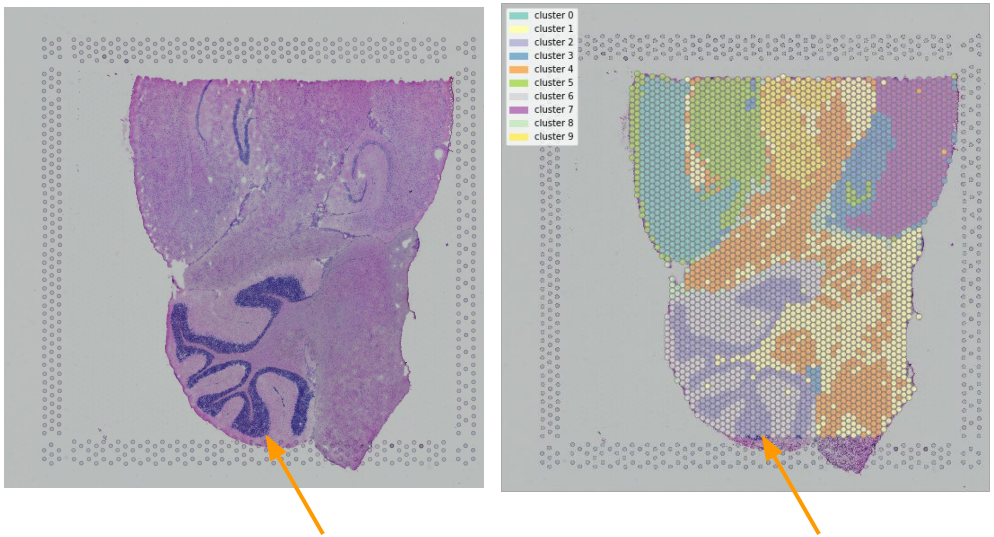
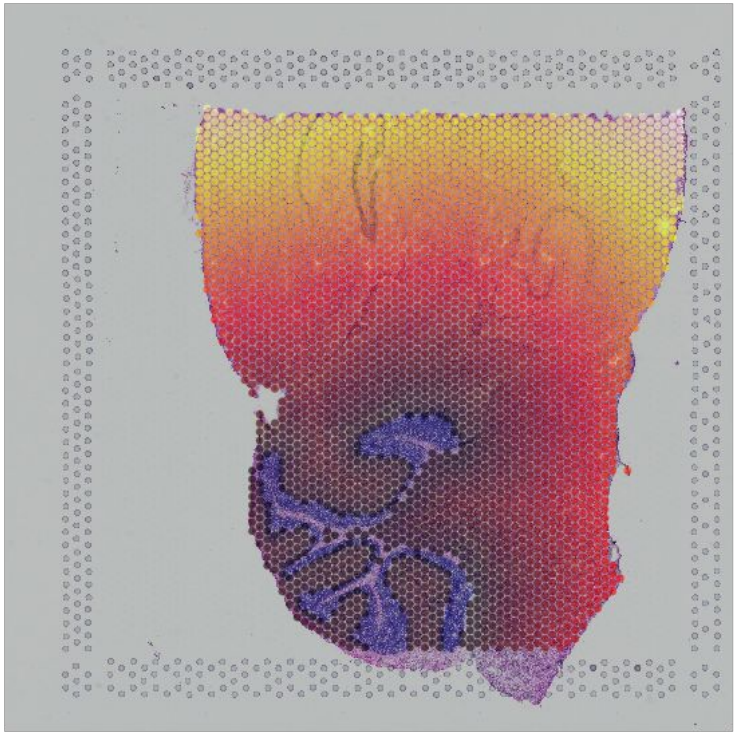


- We may then ask how gene expression changes with the distance to this cluster

Data from 10X Genomics website

Example Analysis : Expression as a function of distance



Spots colored by distance do cluster 2

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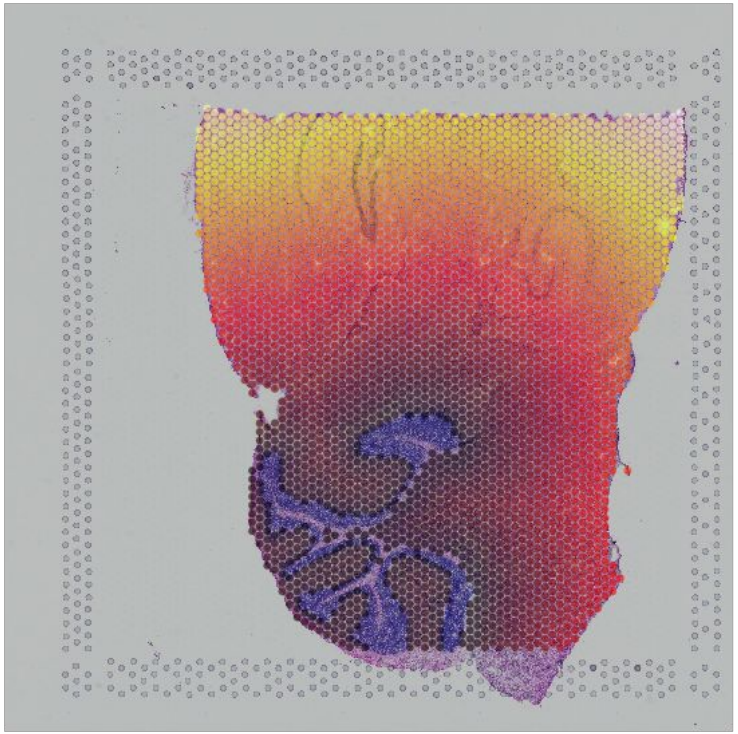

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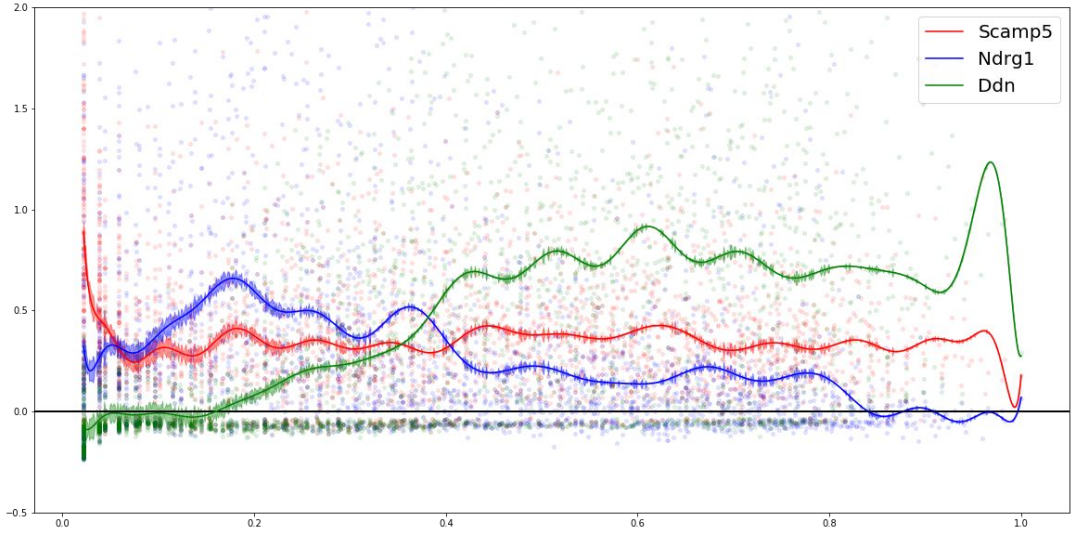
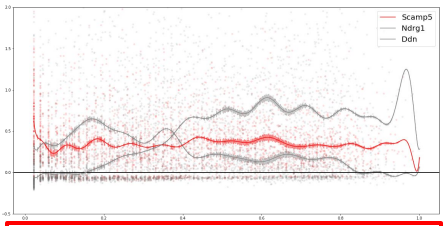
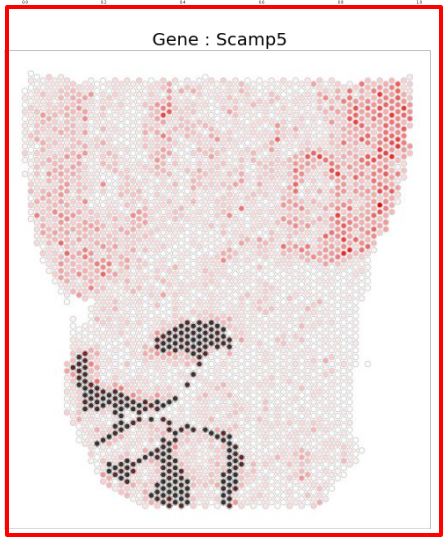
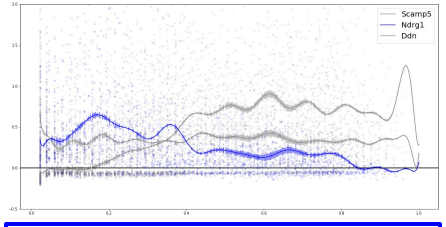


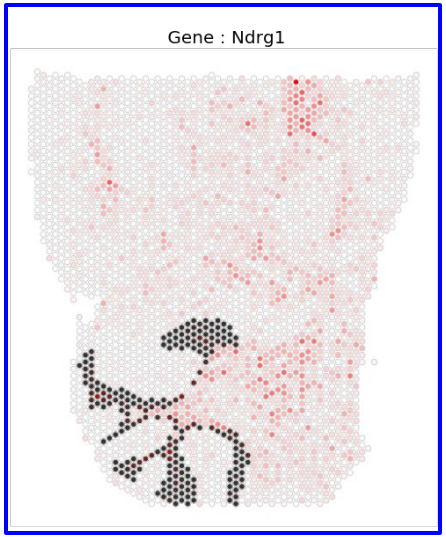
Gene expression as a function of the distance to cluster 2

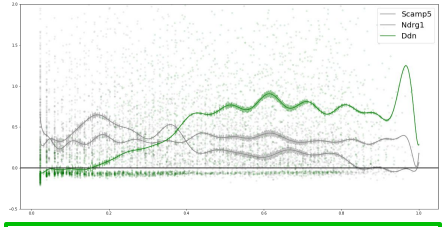
Example Analysis : Expression as a function of distance

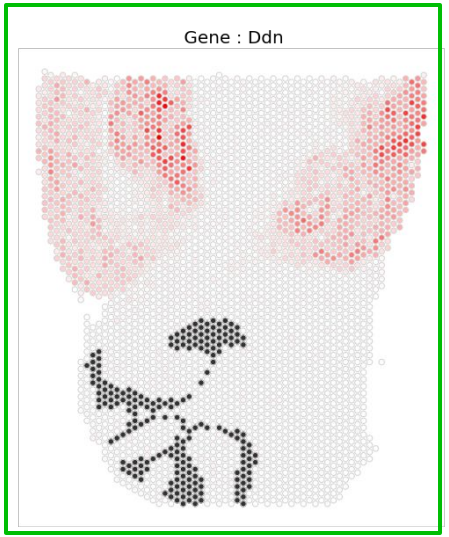








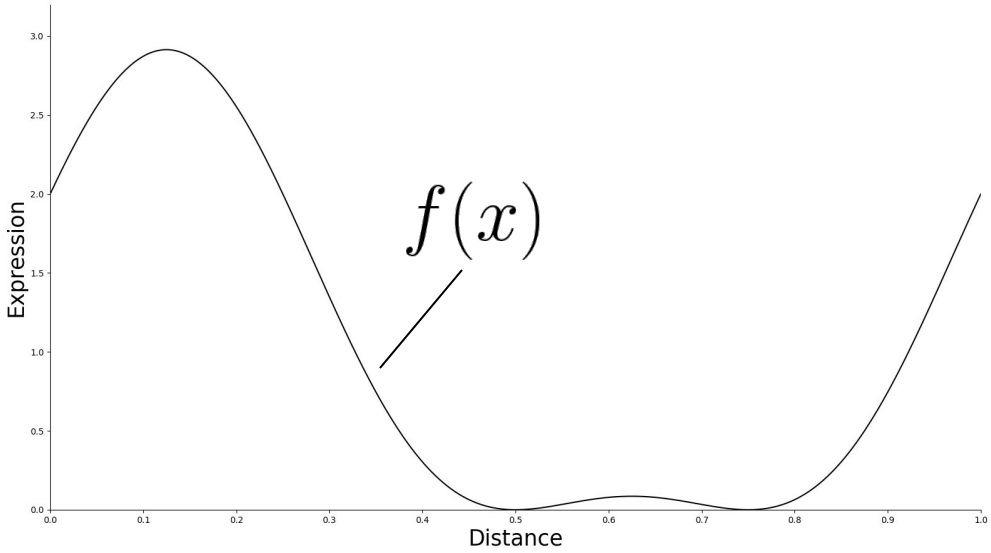




Example Analysis : Expression as a function of distance

P3

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



Equivalent to solving :

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

w.r.t. d_g

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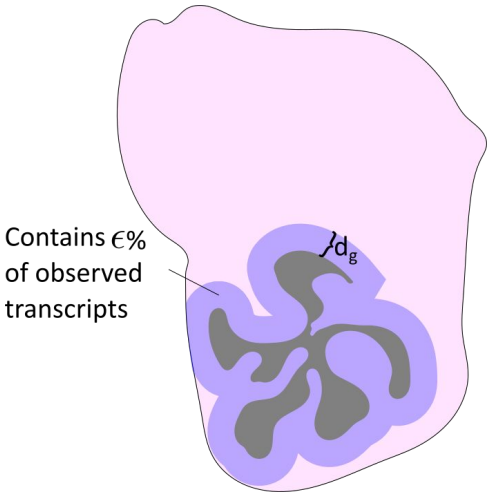

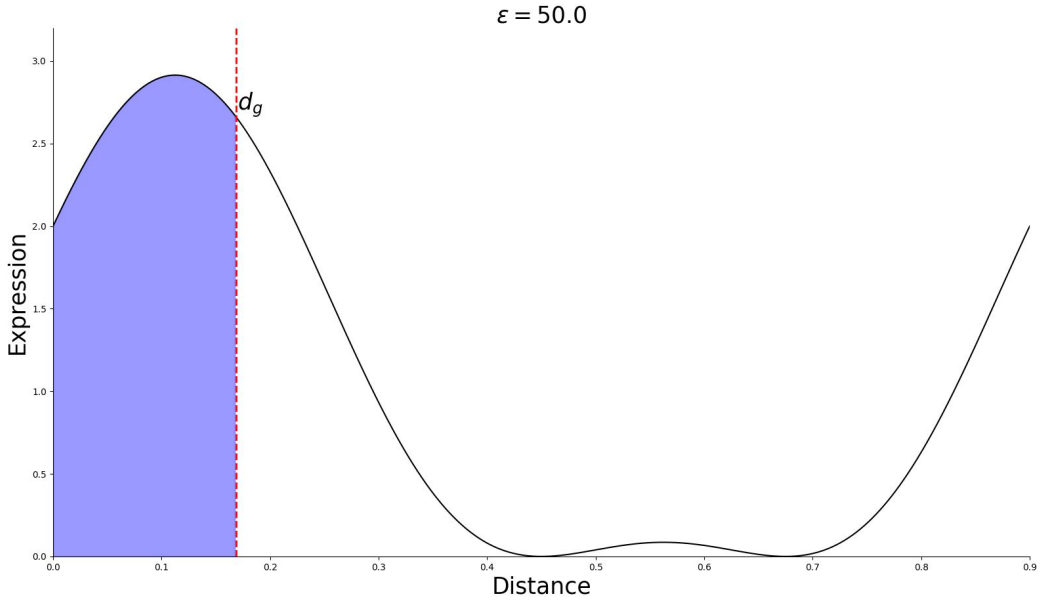

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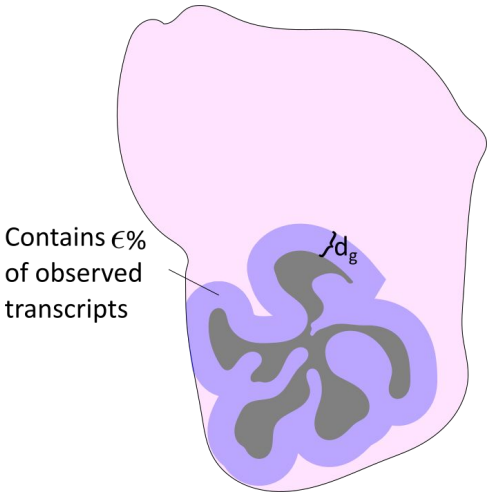

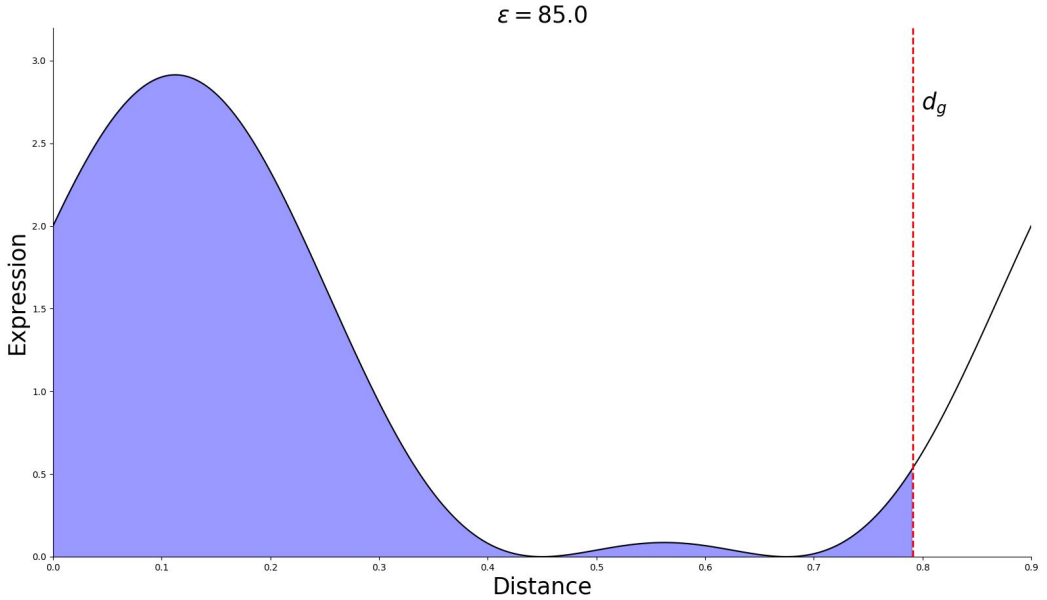

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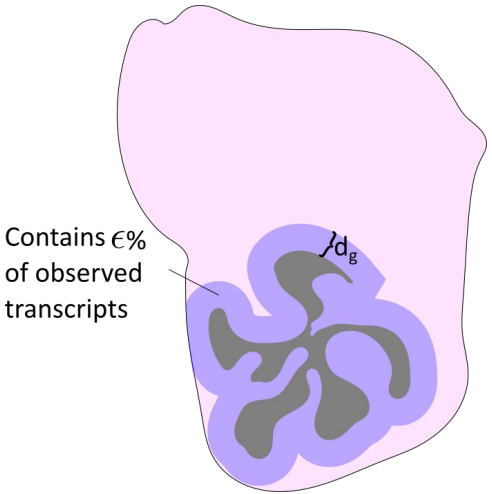

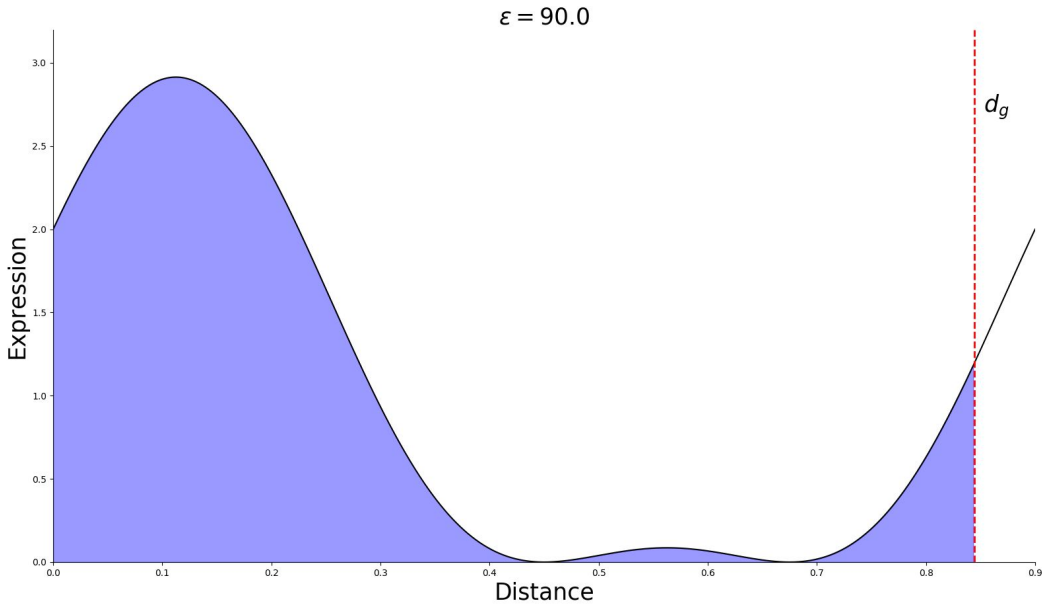

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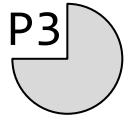

- Alternatively : "*which genes has ε % within the shortest distance (d_g) from cluster 2?*"

Example Analysis : Expression as a function of distance

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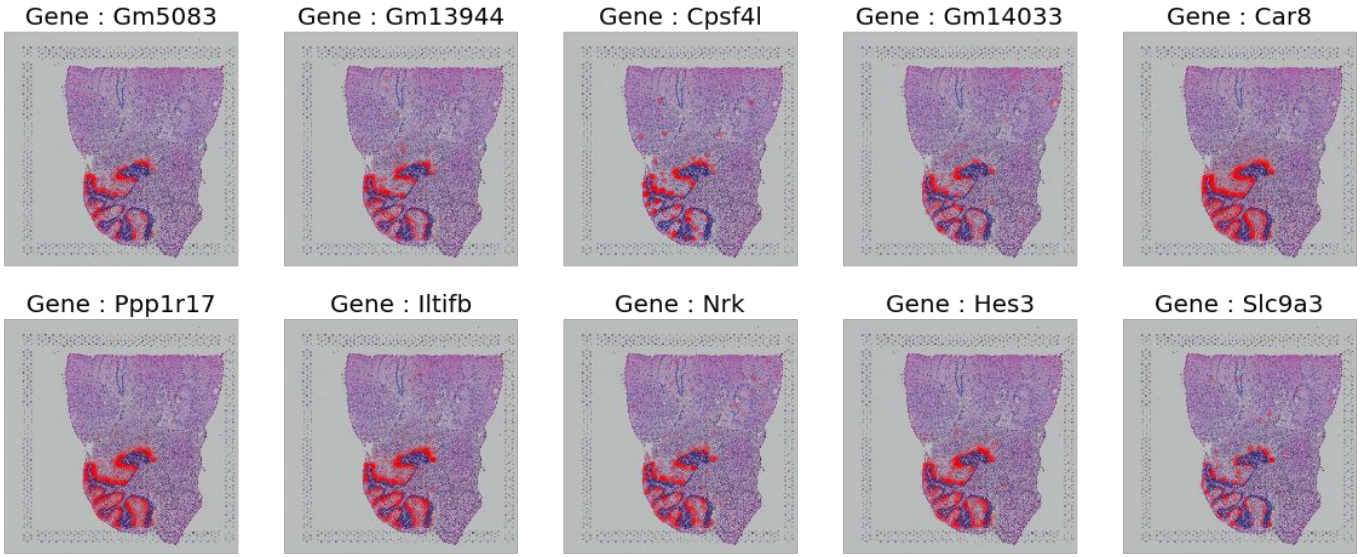

Exercises

Exercise Session

- Aims:
  - Getting familiar with spatial data
  - Overview - concept focused
- Written to be “independent” of lectures
  - Might experience some redundancy
- 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!

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Questions