Spatially Resolved Transcriptomics

Data modalities
&
Unsupervised Analysis

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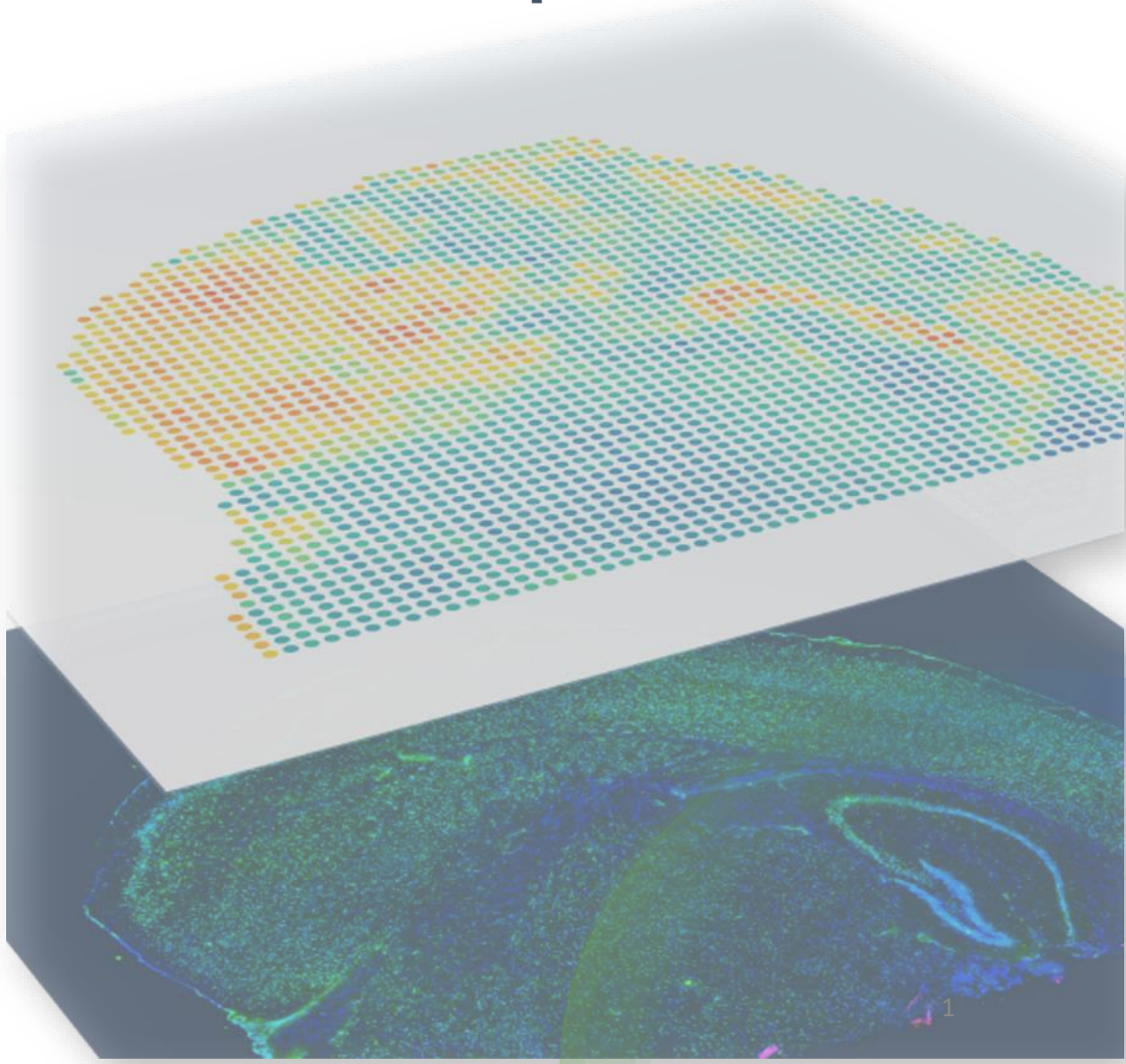
JOSEP CARRERAS LEUKAEMIA RESEARCH INSTITUTE
For a future without leukaemia



Guideline

- Data overview
- Pipeline overview
- Leverage new data modalities
- Spatially aware unsupervised analysis
    - Data normalization
    - Feature selection
    - Model based
  - Benchmarking and further specific results
- Latent representation from reference-based deconvolution
- Bonus: Deep Data Fusion

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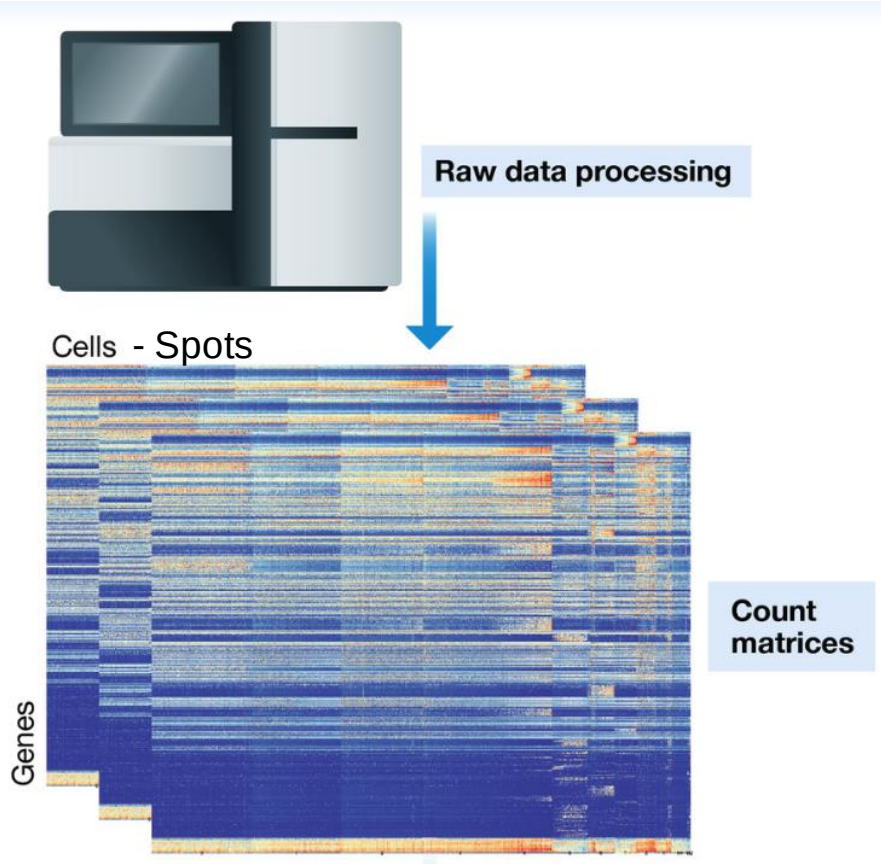
Data overview

Transcriptomic data

Count matrix



General notation for data science

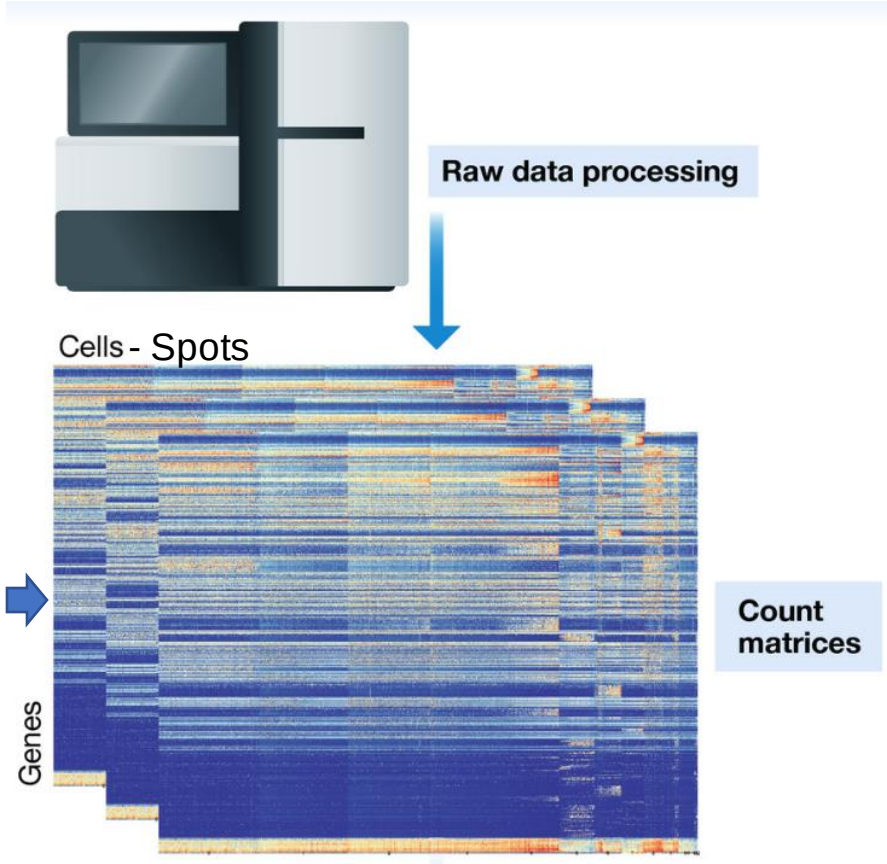
Cells / Spots → Samples

Genes → Features

Data overview

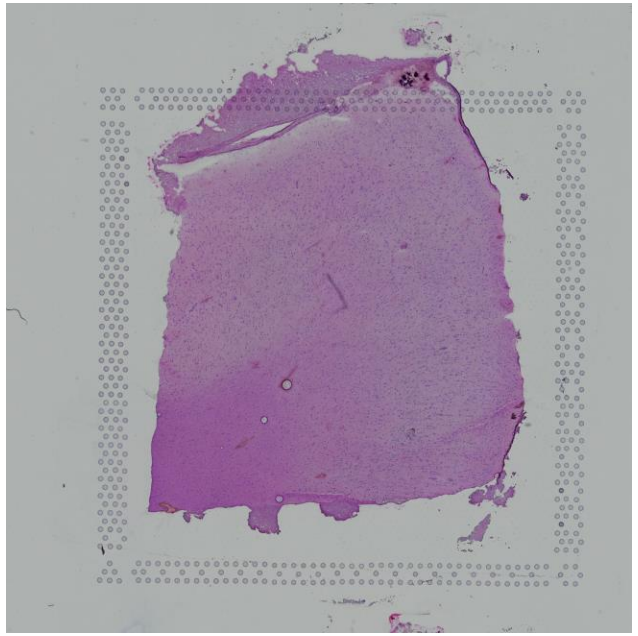
Transcriptomic data

Count matrix

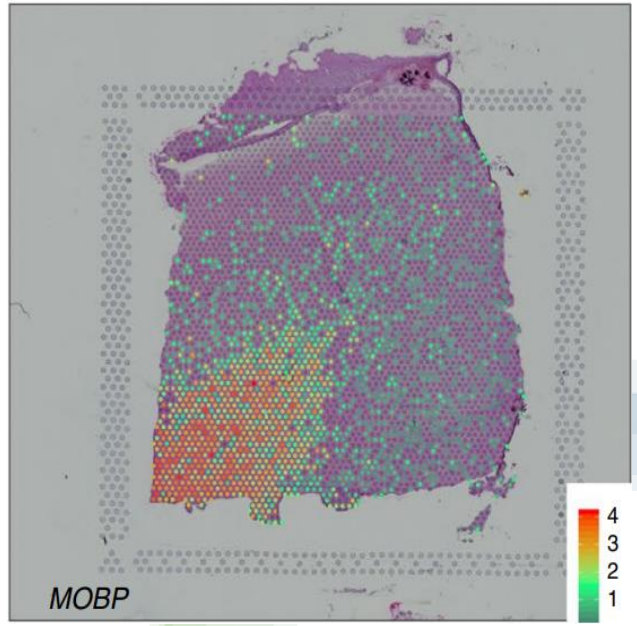


Spatial data

High resolution microscopy image



Spatial location



New data modalities!

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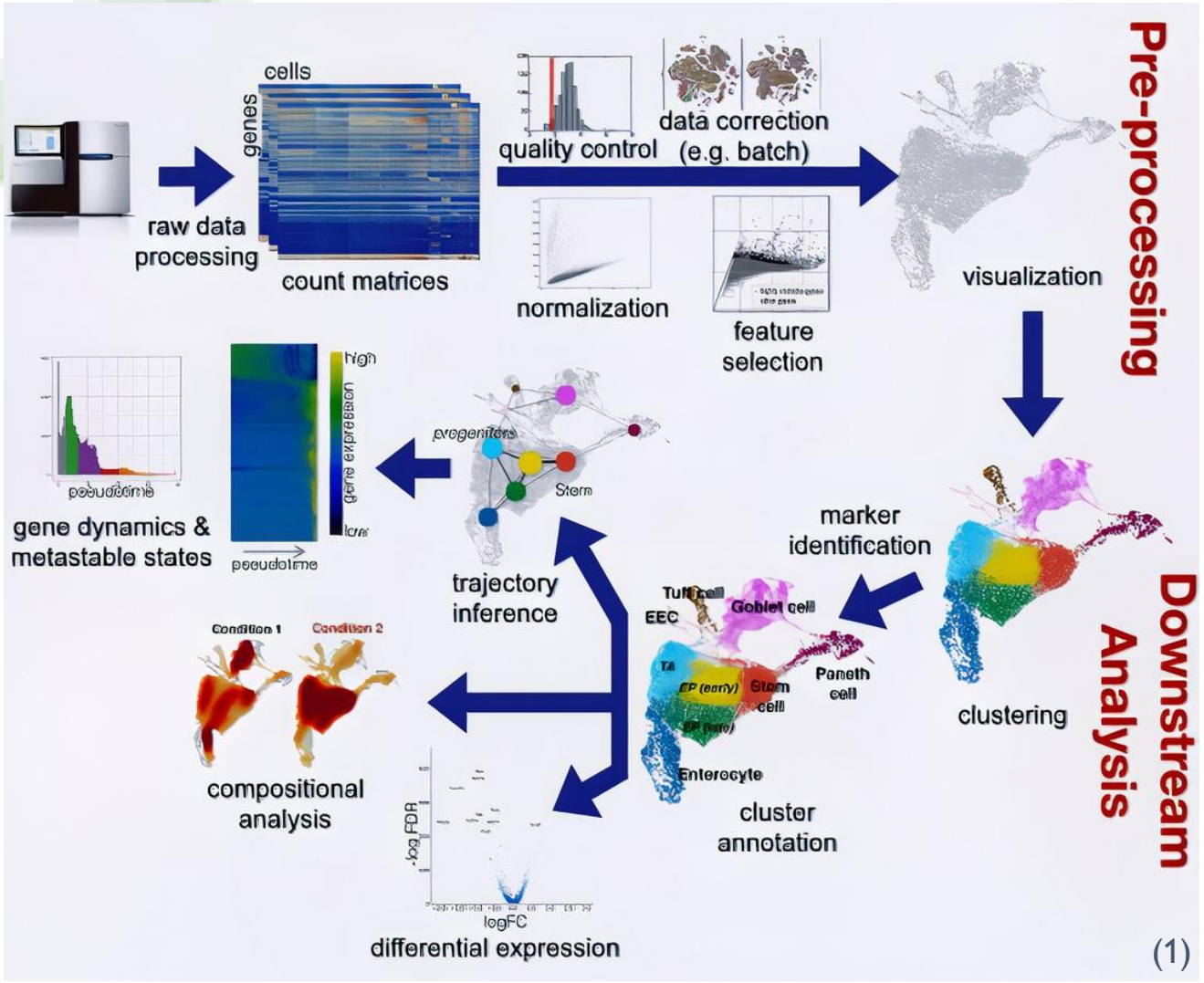
Pipeline overview

Unsupervised analysis



Standardized pipeline for scRNA-seq data

This pipeline can be applied on spatially resolved transcriptomics data to do a first exploration of the data.

Our aim shift from identifying and profiling:

Cell types → to → Tissue regions

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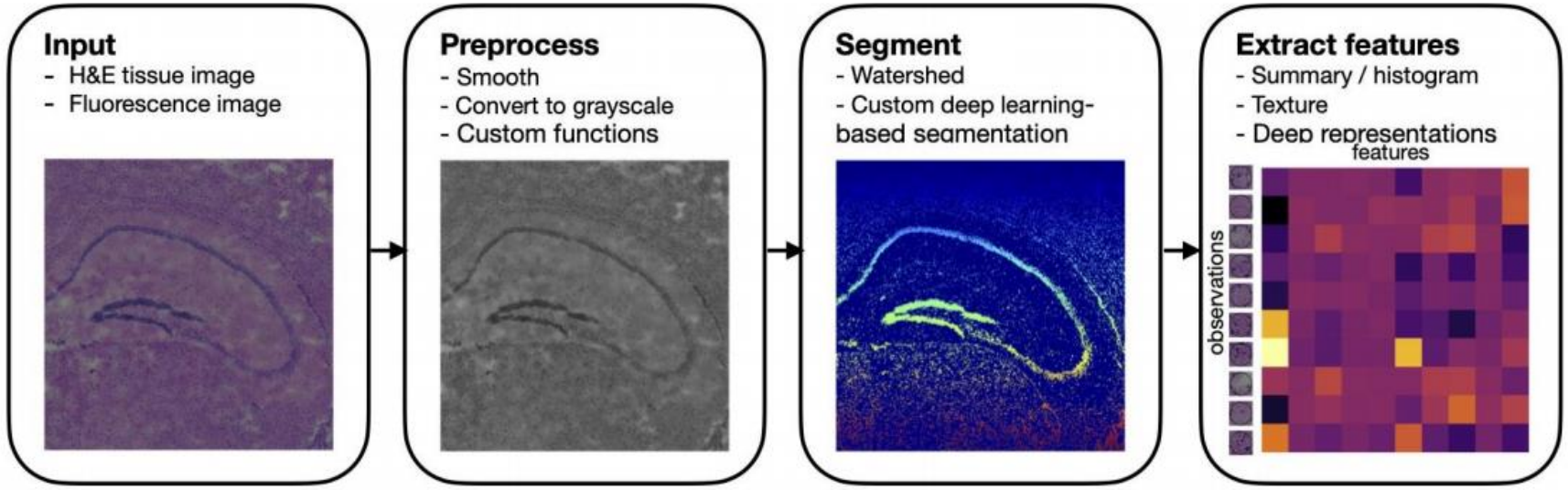
Leverage new data modalities

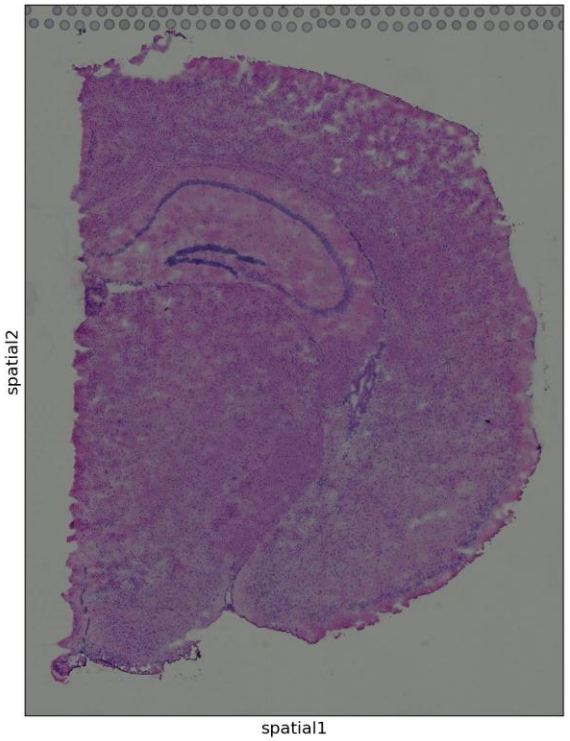
High resolution microscopy image

- Rich source of morphological information

- Useful for visualization and qualitative result assessment

- We can extract image features to complement gene information (transcriptomic features)

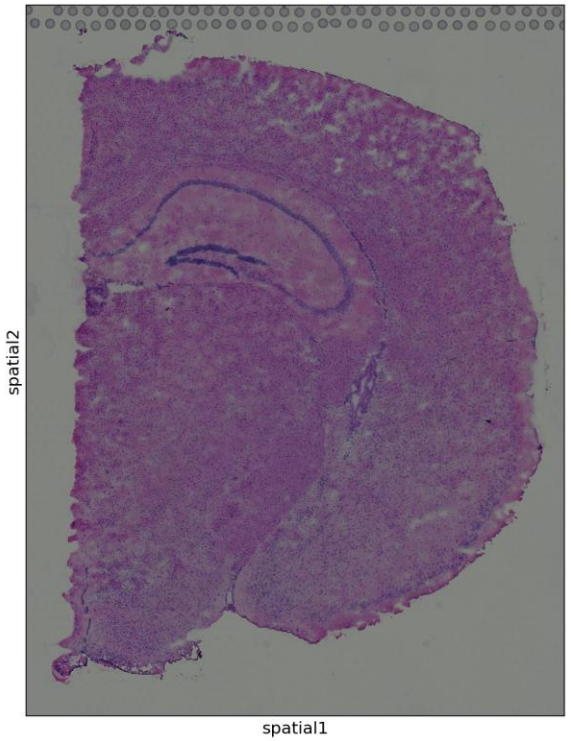




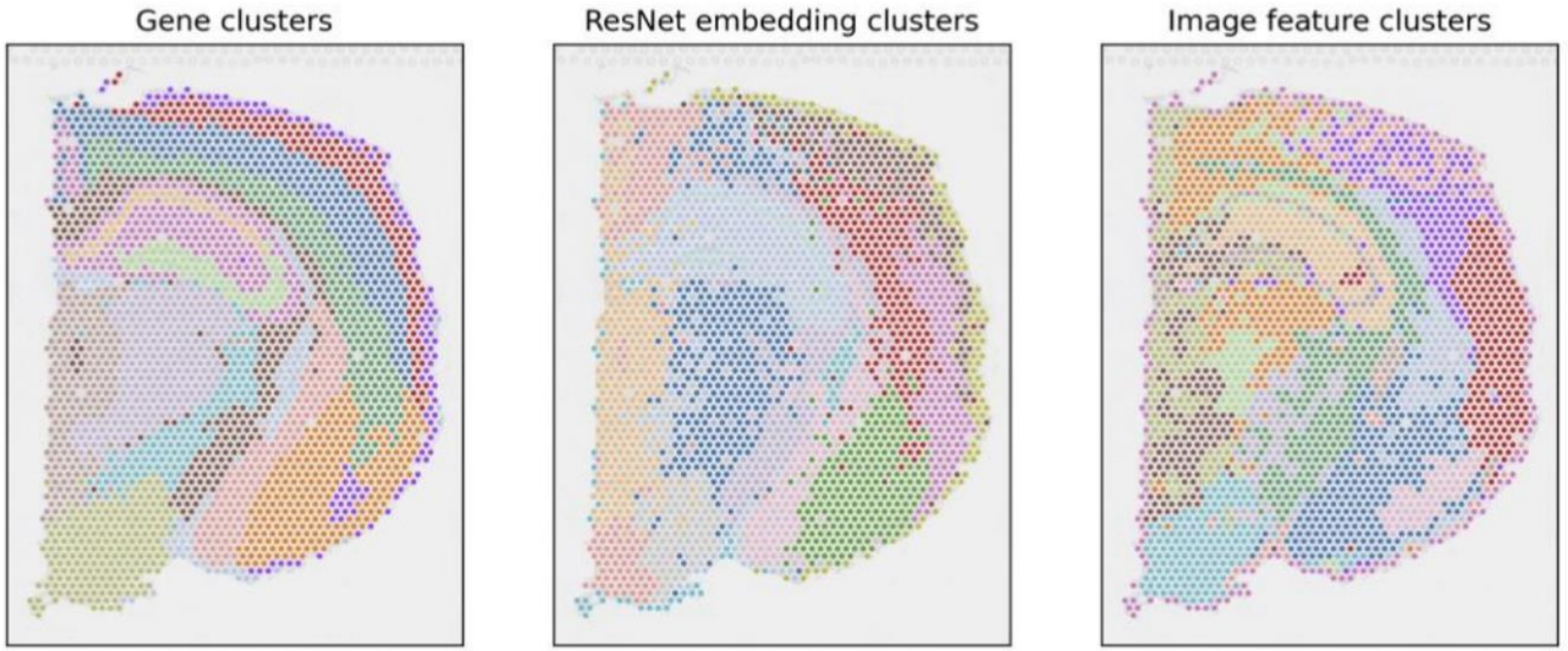
(2) squidpy

Leverage new data modalities

High resolution microscopy image



- **ResNet(DL model) embedding cluster:** Cluster labels obtained from a Deep learning model trained to predict Gene Cluster assignment.
- **Image features cluster:** Clustering based on the intensity mean, standard deviation and 0.1, 0.4 and 0.9 quantiles of the H&E stain at each spot location



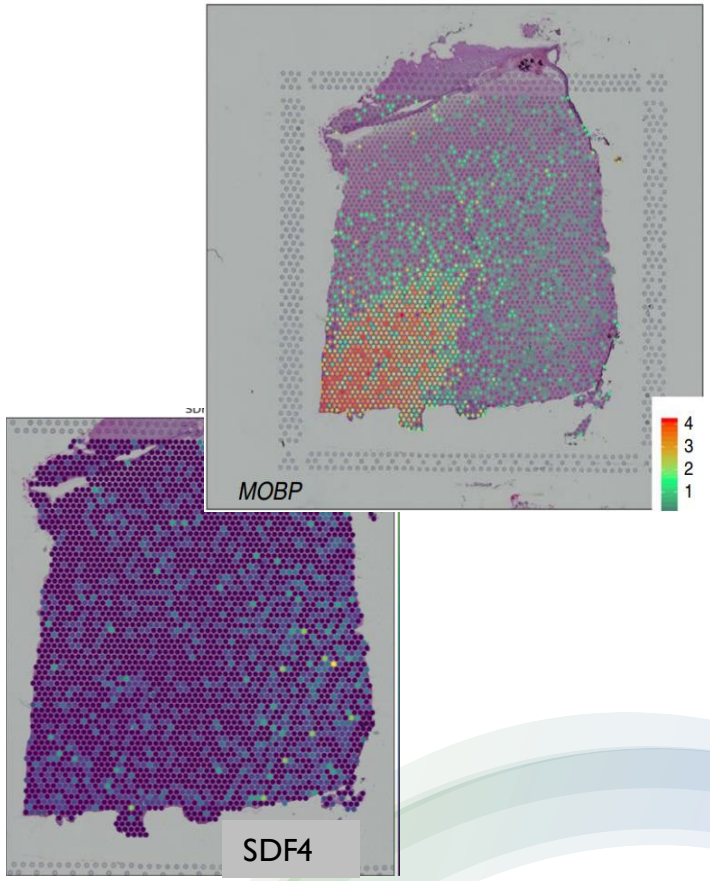
(2) squidpy

Leverage new data modalities

Spatial Location

- **Spatial Statistics** : This field studies entities by using topological, geographic or geometric properties. It offers statistical tests to score the spatial pattern shown by a gene (assess the spatial relevance of each of the features of our samples).

  Can be used for:
  - Feature selection
  - Tissue "markers" exploration



- **Spatial Graphs :** Graphs are incredibly flexible tools. Spatial graphs encode spatial proximity. Can be used for a wide variety of purposes, in preprocessing and downstream analysis.

(2)

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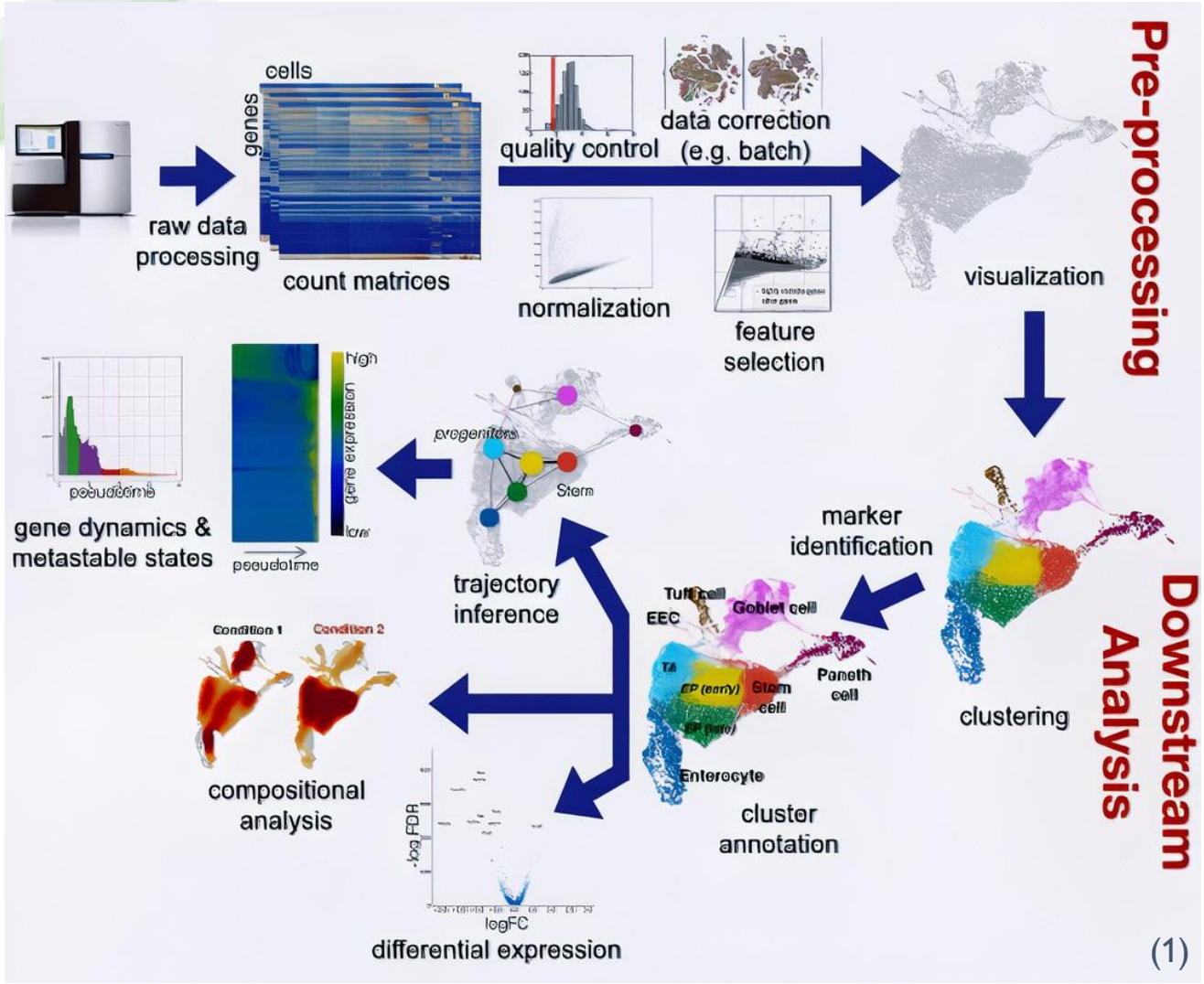
Spatially aware unsupervised analysis

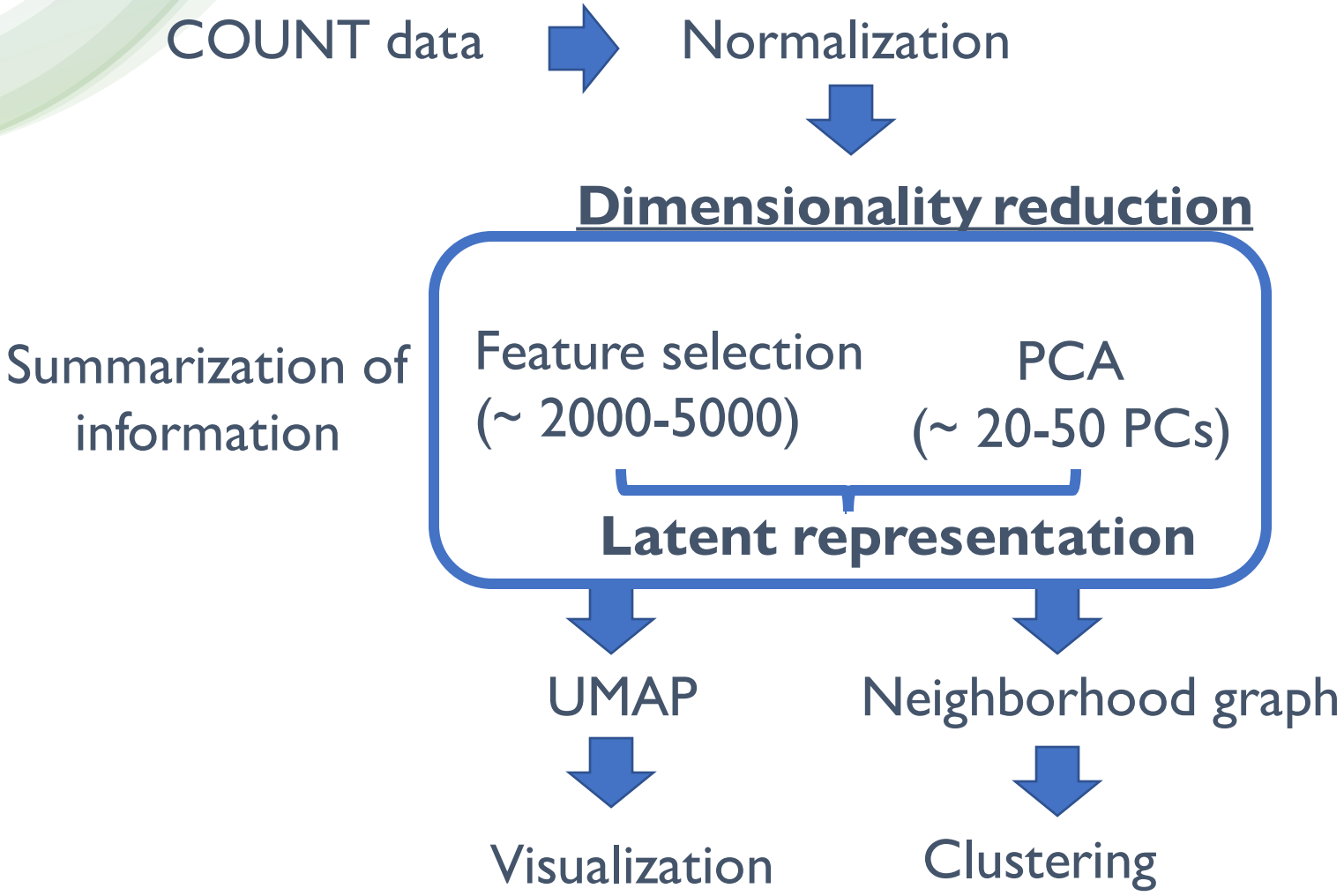


To properly dissect tissue heterogeneity and fully exploit the data:

An optimal integration of transcriptomics data and associated spatial information is essential

Let's take a careful look to the pipeline up to the clustering step

Spatially aware unsupervised analysis



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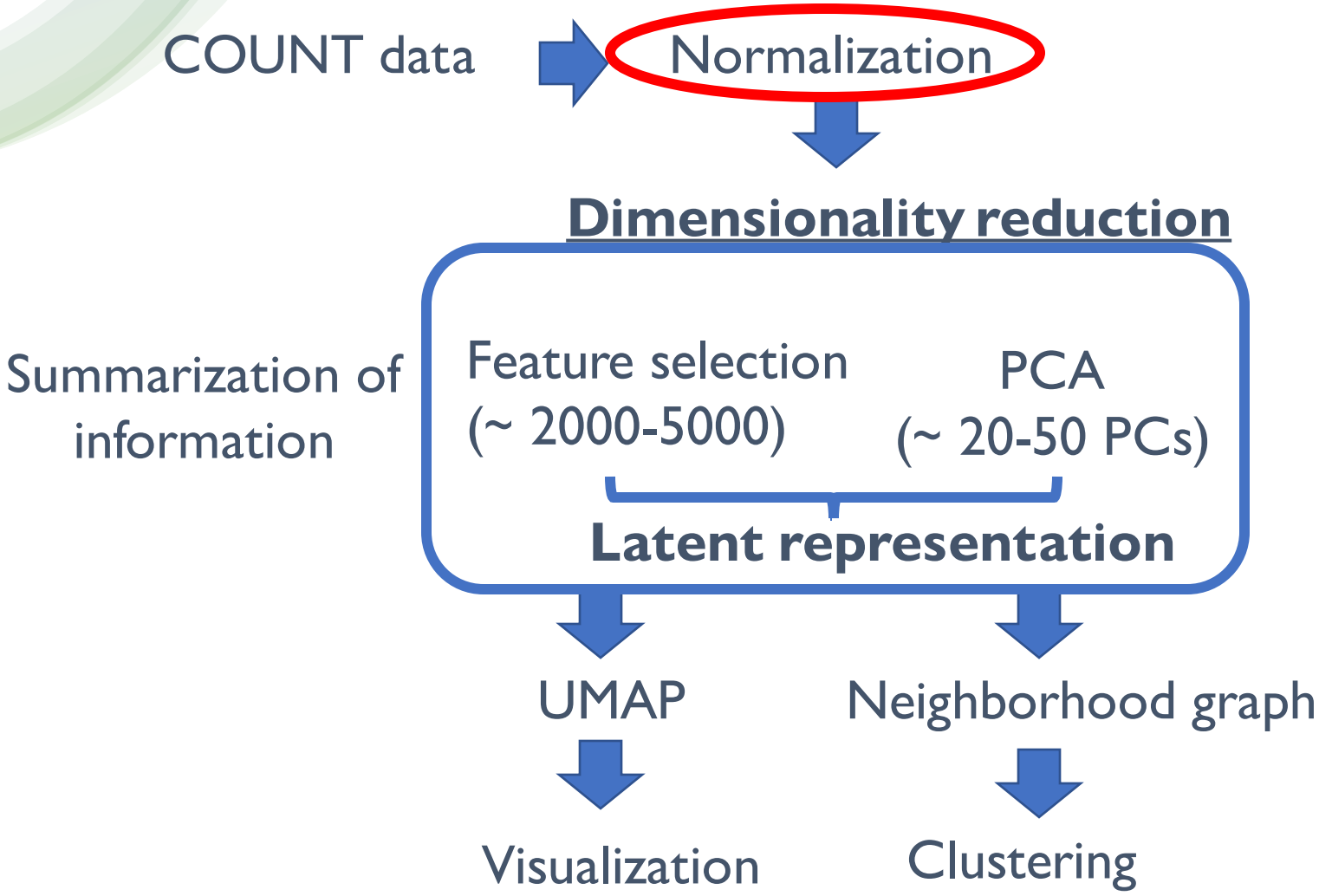

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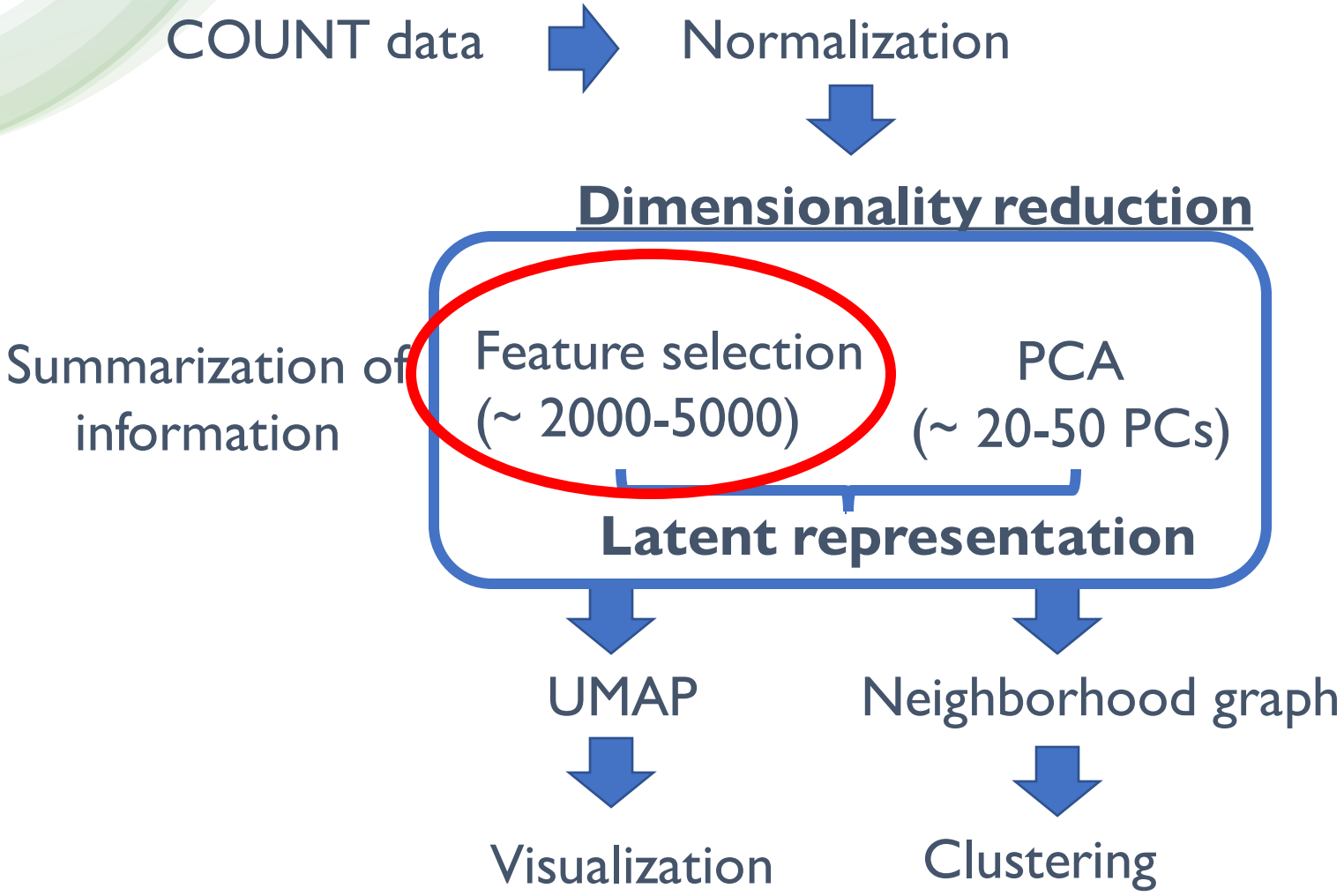

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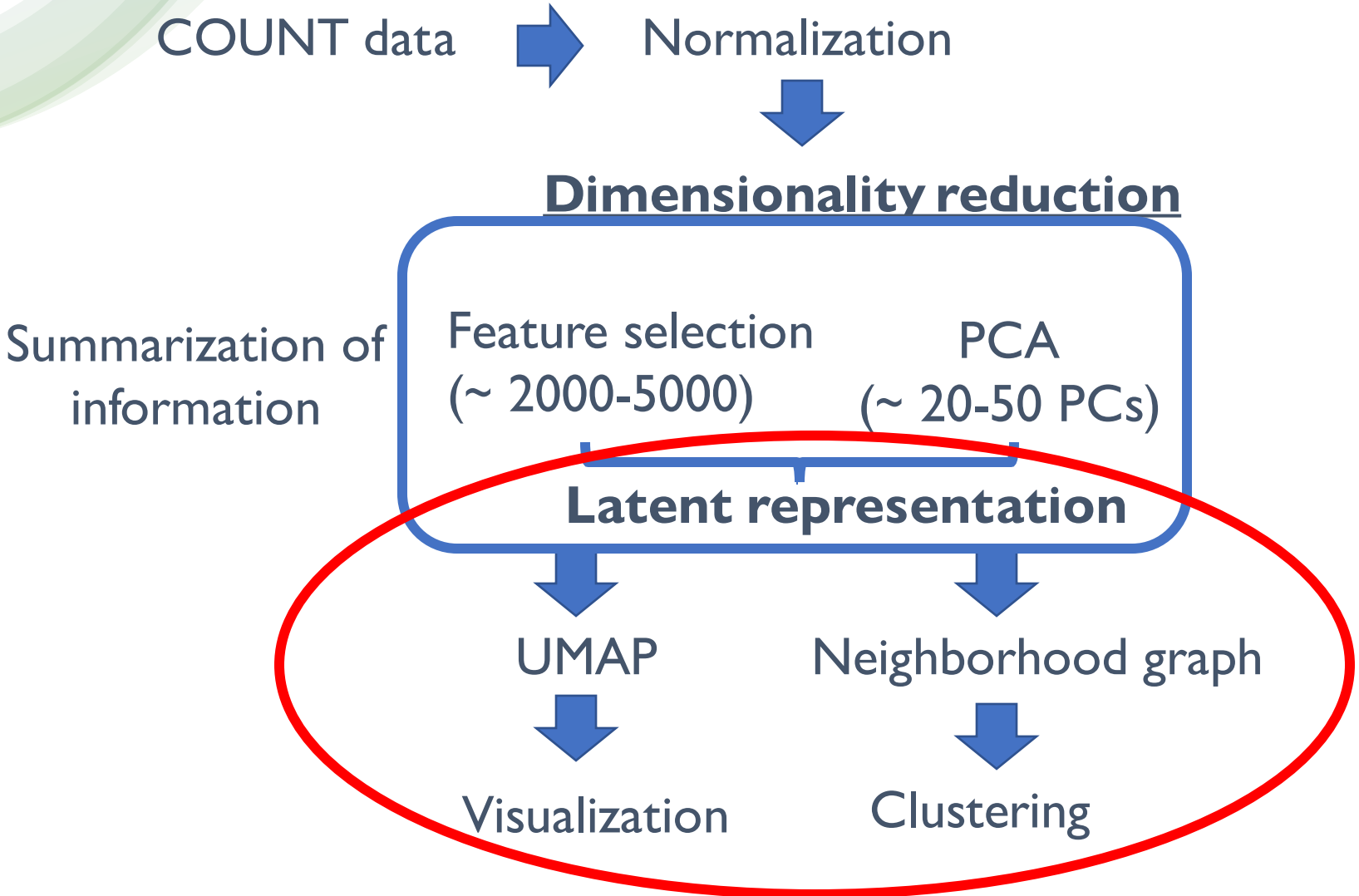

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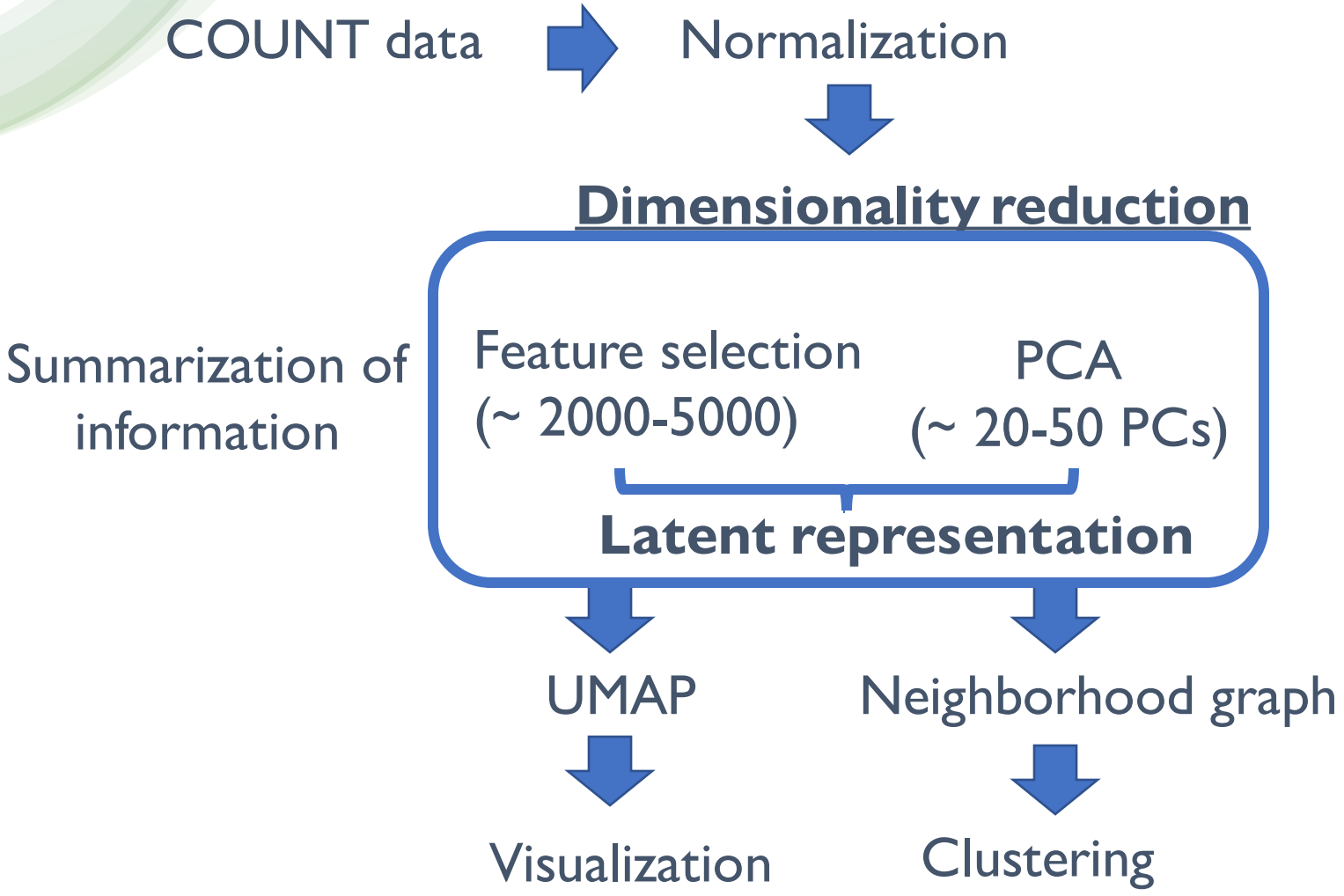

In terms of SUMMARIZATION:
We aim to compress spatial information in the latent representation of the data to **conduct a spatially aware clustering and downstream analysis**

For VISUALIZATION:
Alternatively to the UMAP, we can use SPATIAL COORDINATED to visualize CLUSTER ASSIGMENTS
We can **increase the spatial resolution of the transcriptomic information** with model-based approaches

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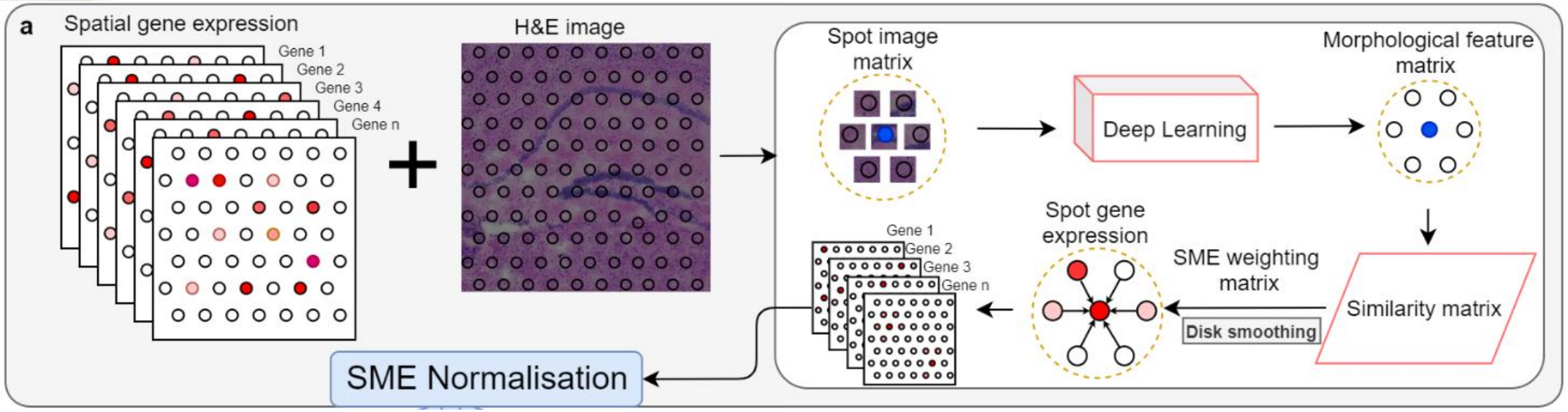
Spatially aware normalization

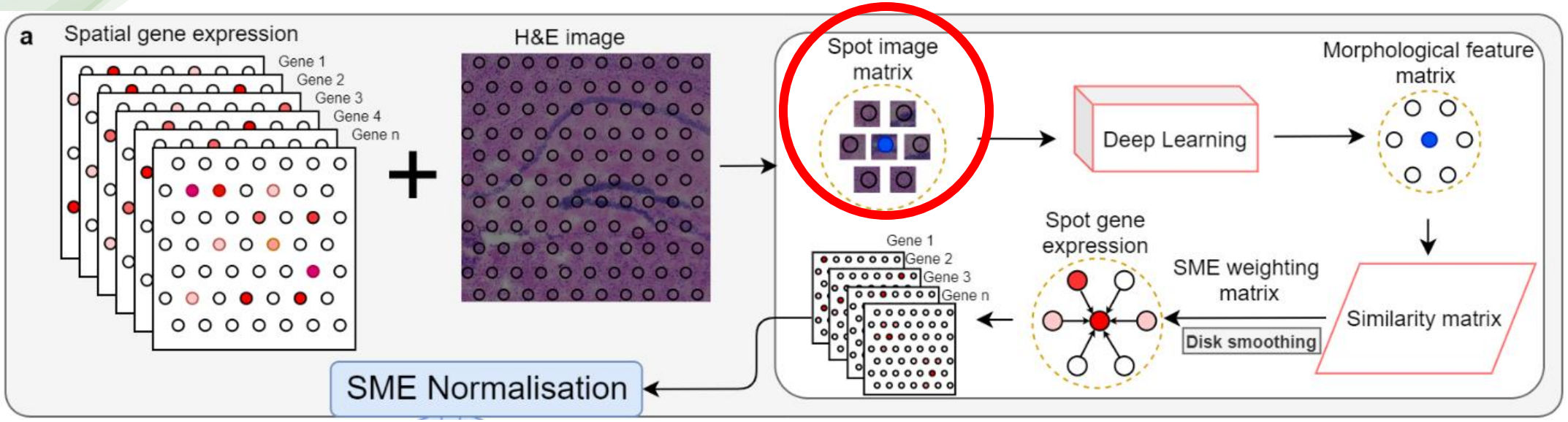
TOOL : **stLearn**[3] **Spatial Morphological gene Expression Normalization**



Spatially aware normalization

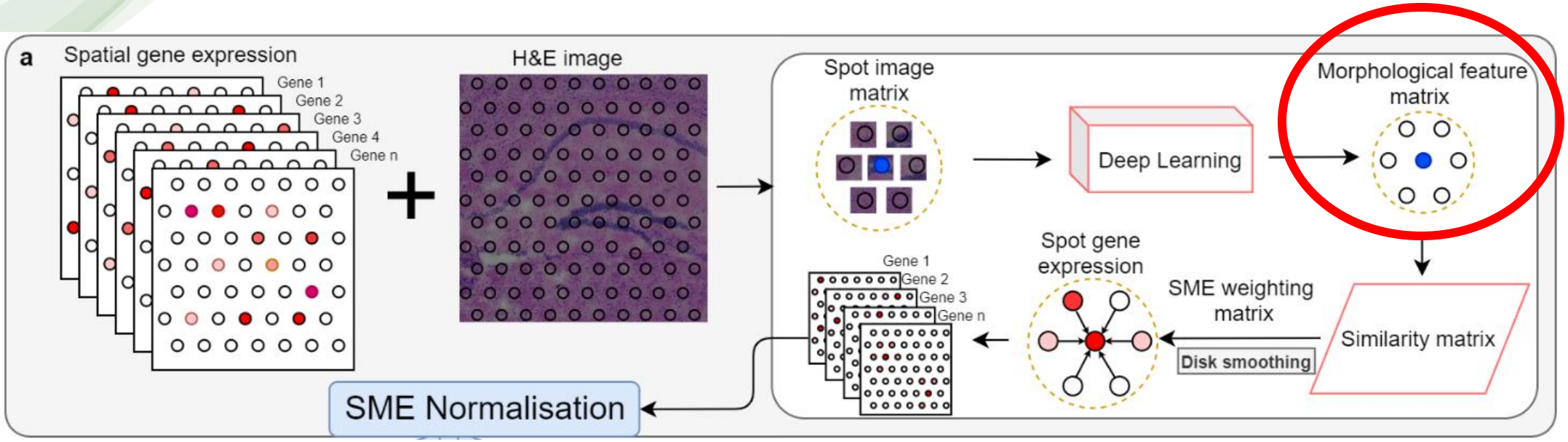
TOOL : **stLearn**[3]

Spatial Morphological gene Expression Normalization



Spatially aware normalization

TOOL : **stLearn**[3] DL models can convert an image into a 2048-dimensional vector. Then we can apply PCA to extract the first 50 PCs as latent features to represent the spot morphology (M)



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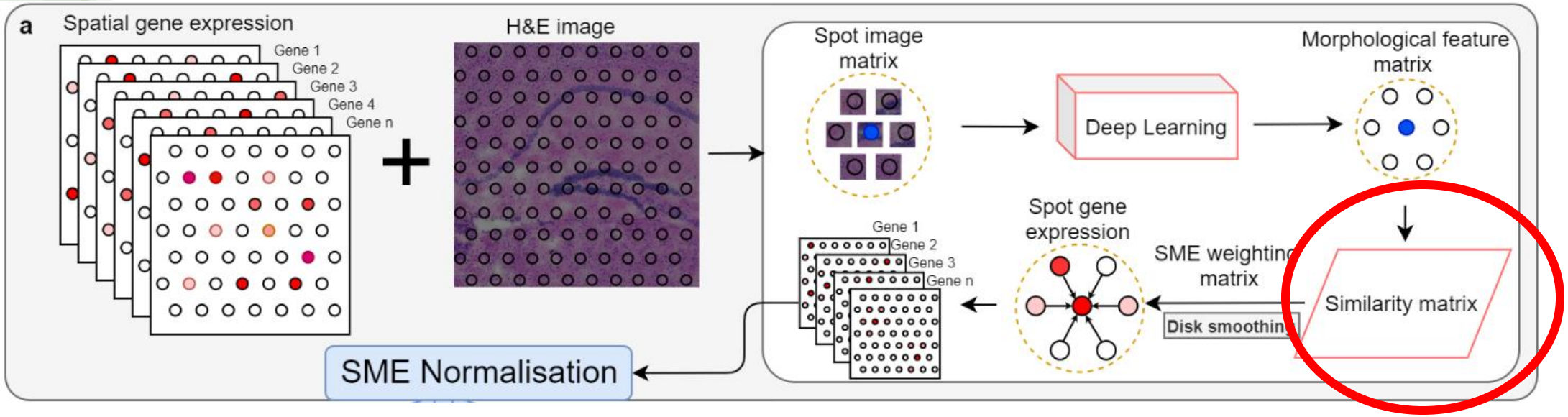
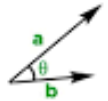


**Reminder

The Vector Dot Product



$$\mathbf{a} \cdot \mathbf{b} = |\mathbf{a}||\mathbf{b}| \cos\theta$$

$$\cos\theta = \frac{\mathbf{a} \cdot \mathbf{b}}{|\mathbf{a}||\mathbf{b}|}$$

Morphological distance
Similarity metric

$$MD(S_i, S_j) = MD_{ij} = \frac{M_i \cdot M_j}{\|M_i\| \|M_j\|}$$

if $PD_{ij} < r$

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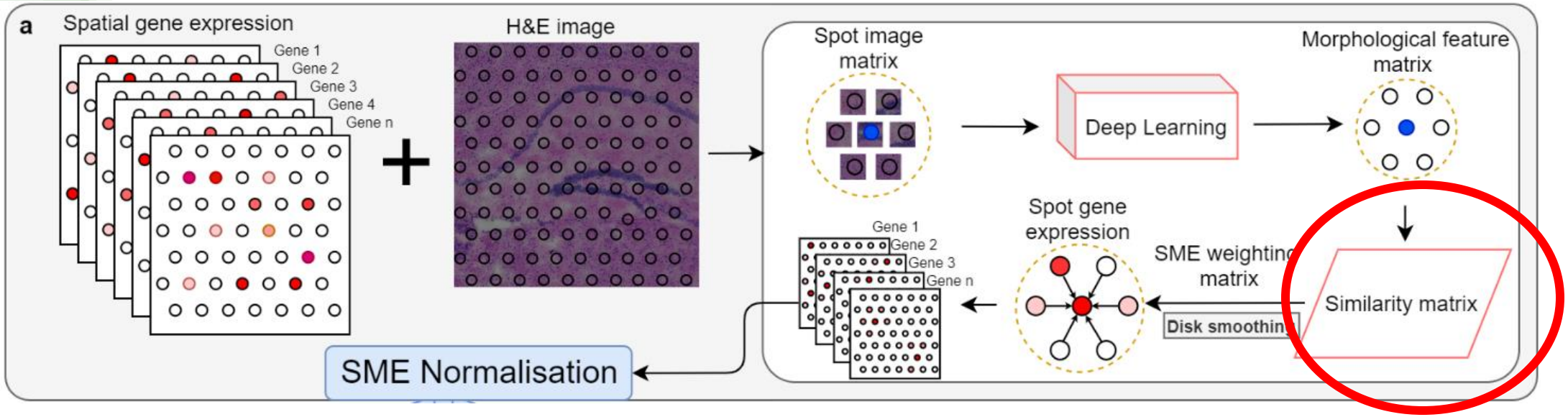

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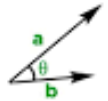

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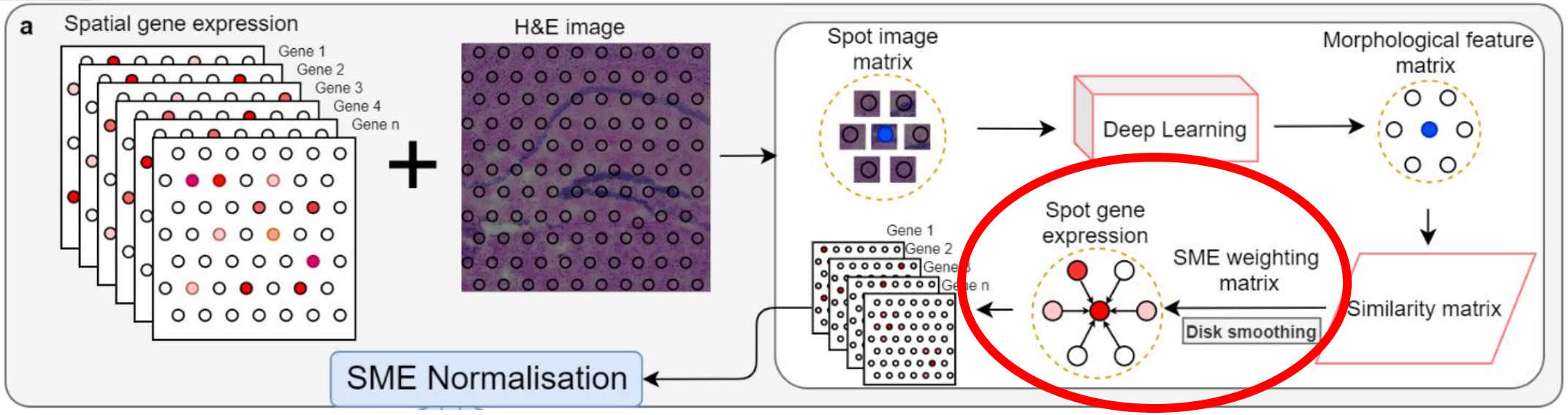

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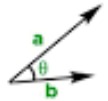

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Spatial Morphological gene Expression Normalizati

$$GE_i' = GE_i + \frac{\sum_{j=1}^{n} GE_j \cdot MD_{ij}}{n}$$

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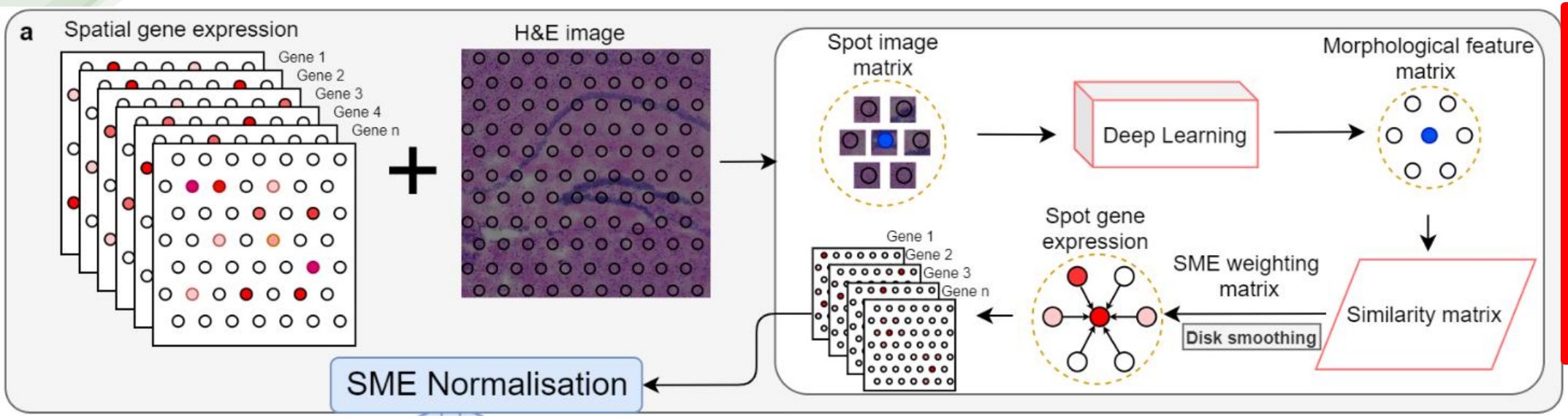

Alternatively, they propose to perform this normalization on the latent features (PCs / UMAP)

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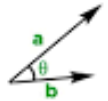

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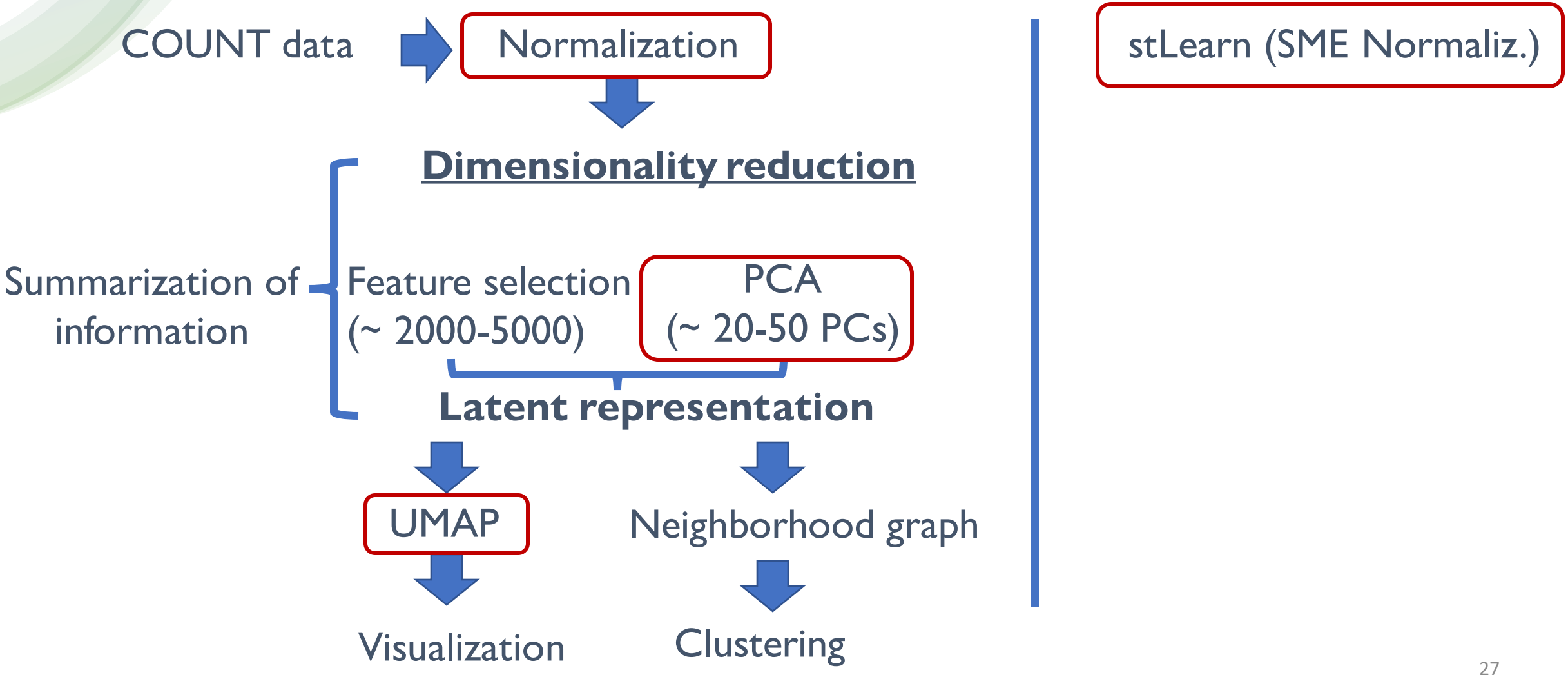
Spatially aware unsupervised analysis

Framed steps are tackled or replaced by the same-colored framed methods



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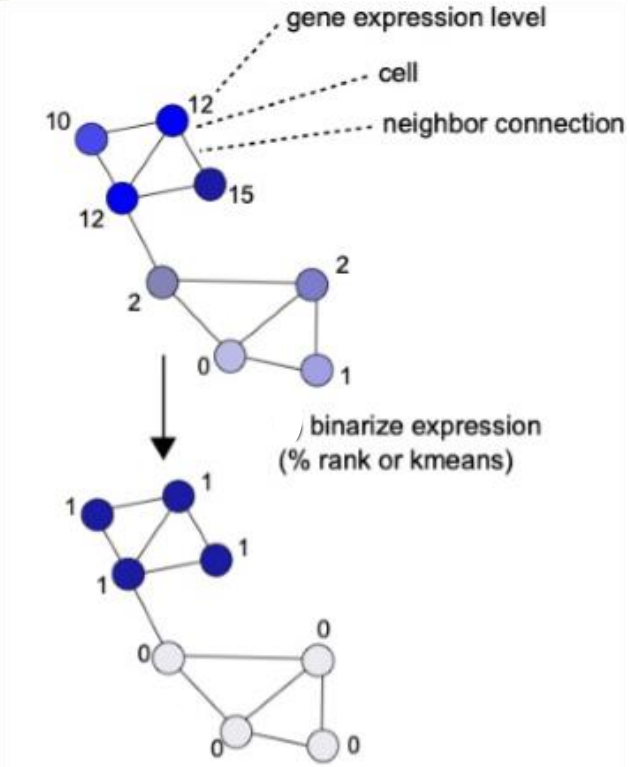
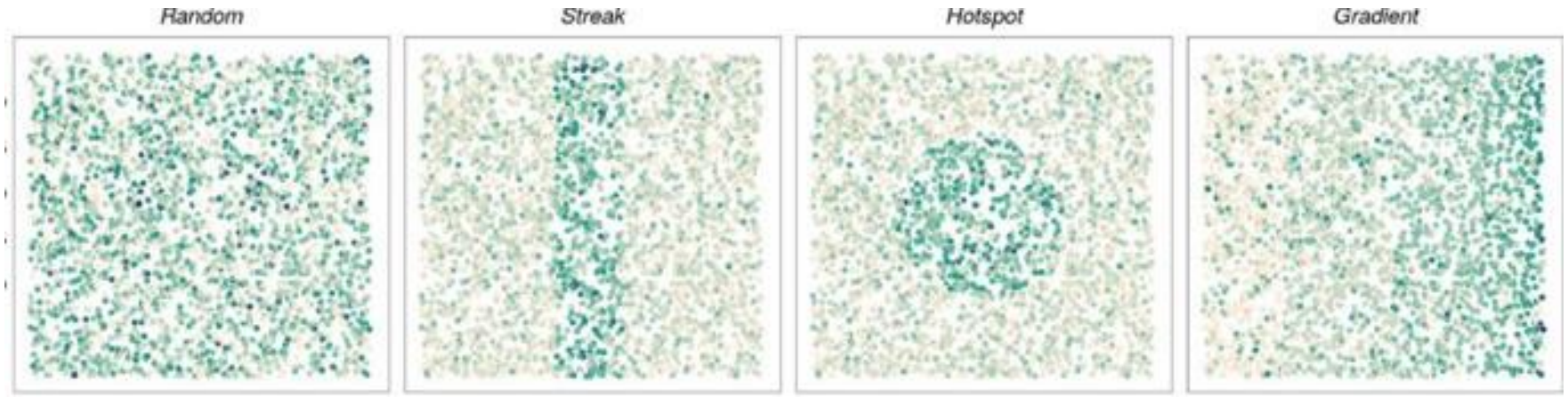
Spatially aware feature selection – Spatial Graphs

TOOL : **Giotto** (4) (Binary Spatial extract / BinSpect)



- Binarize the gene expression value (0/1)
- Assess the spatial pattern by checking whether a gene is usually expressed in neighboring cells



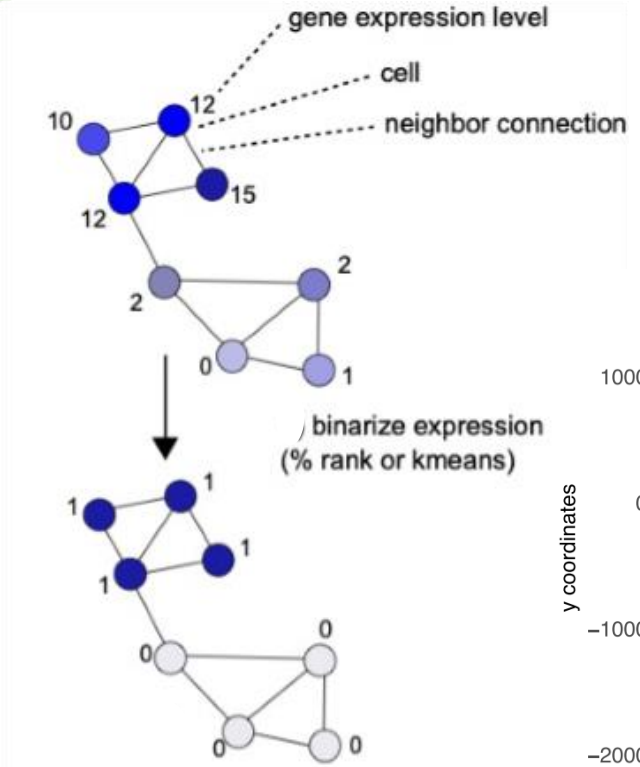
Does not exhibit more potential than Spatial Statistics approaches but is COMPUTATIONALLY MORE EFFICIENT

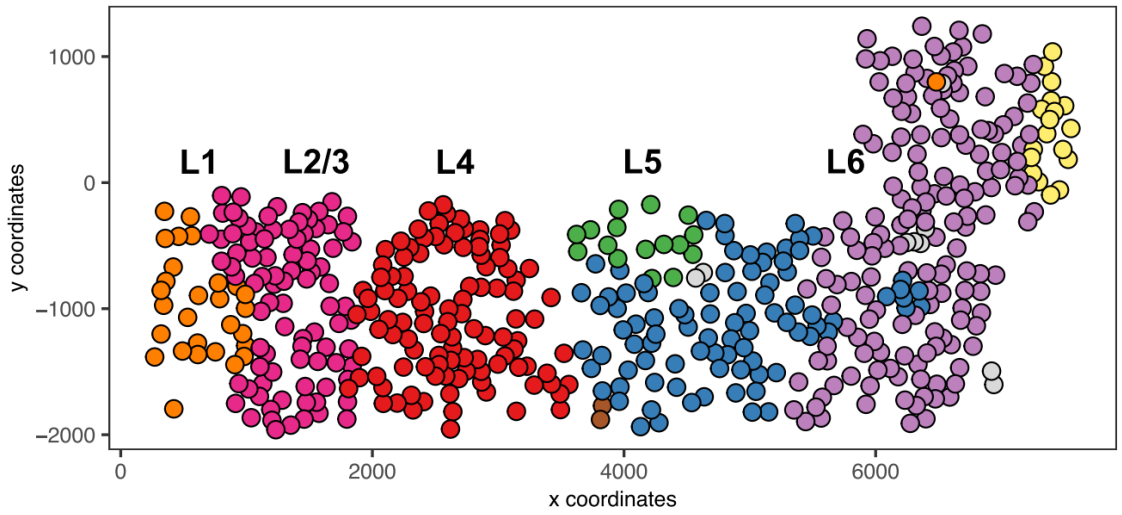
Spatially aware feature selection – Spatial Graphs

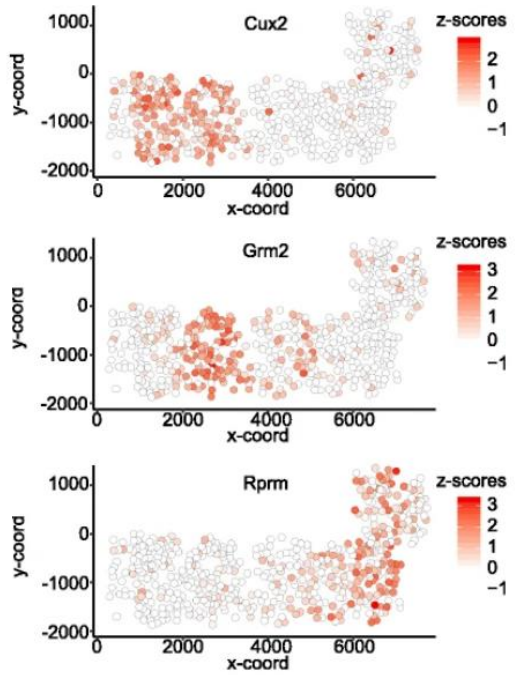
TOOL : **Giotto** [4] (Binary Spatial extract / BinSpect)

- Binarize the gene expression value (0/1)
- Assess the spatial pattern by checking whether a gene is usually expressed in neighboring cells

Suitable for cases such as: seqFISH+ mouse somatosensory cortex (underlying layered structure)







Spatially aware feature selection – Spatial Statistics

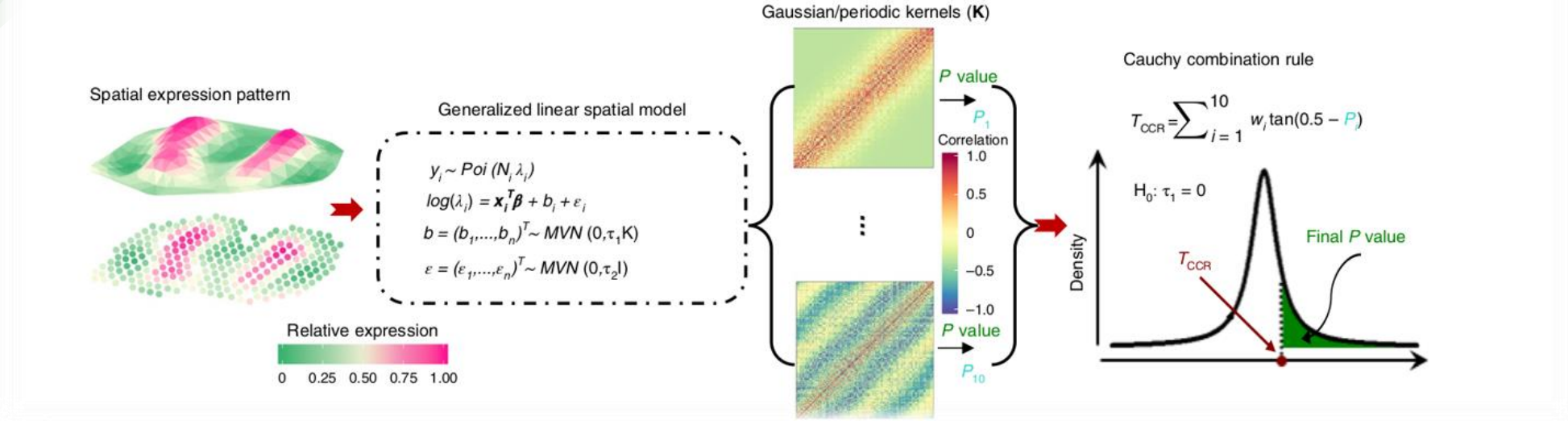
TOOL : **SPARK**[5] (Spatial pattern recognition via kernels)

PREVIOUS APPROACHES		CHALLENGES
SpatialDE[2]	Poor control of type **I** errors* →	Statistical
Based on efficient linear mixed models		
Trendseek[3]	Not Scalable →	Computational
Expensive permutation strategies with non-parametric test statistics		

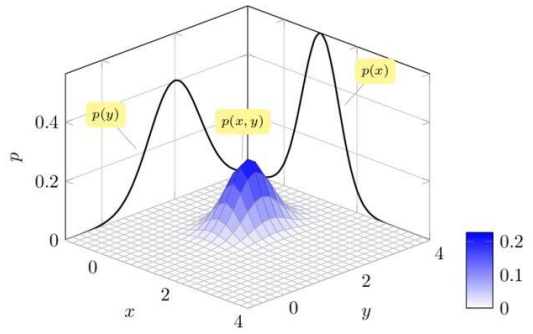
Type **I errors lead to concluding that purely random results are statistically significant*

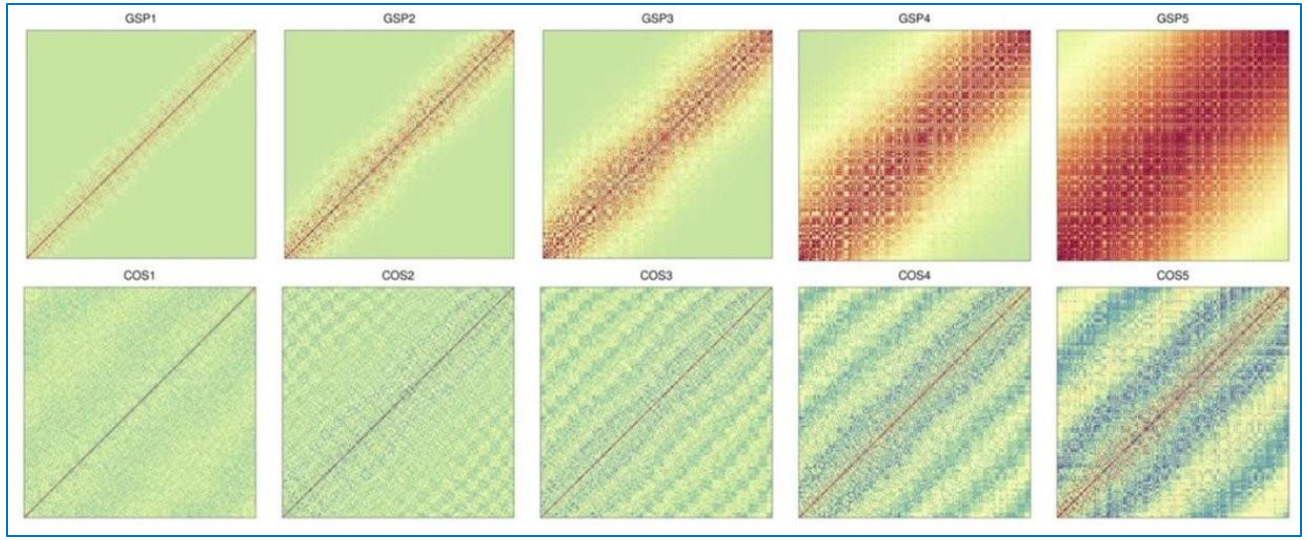
Spatially aware feature selection – Spatial Statistics

TOOL : **SPARK** (5) (Spatial pattern recognition via kernels)



MVN ~ Multivariate normal distribution





Kernels

Gaussian

Periodic

Spatially aware feature selection – Spatial Statistics

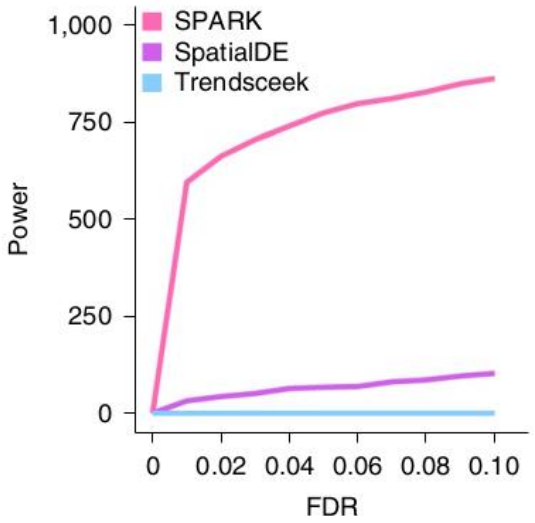
TOOL : **SPARK** (5) (Spatial pattern recognition via kernels)

Olfactory bulb

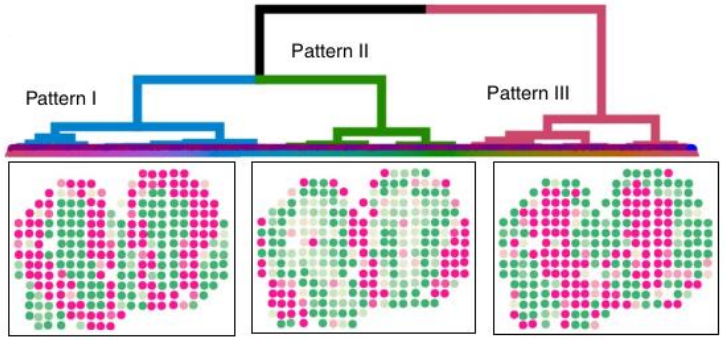
Number of detected genes displaying spatial patterns:

SPARK: 772

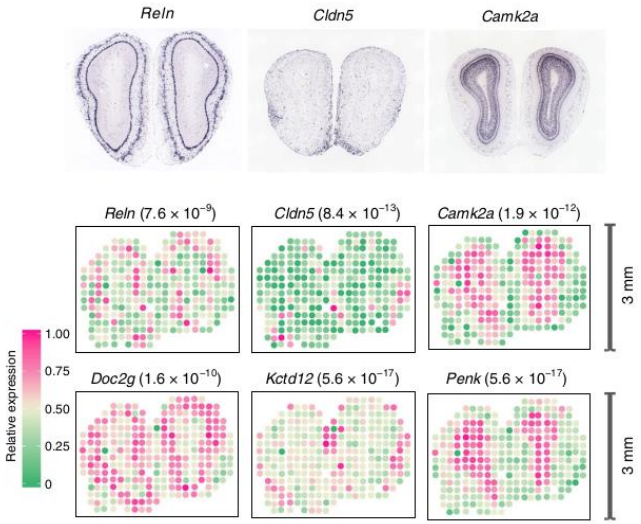
SpatialDE: 67 (62 overlaps)



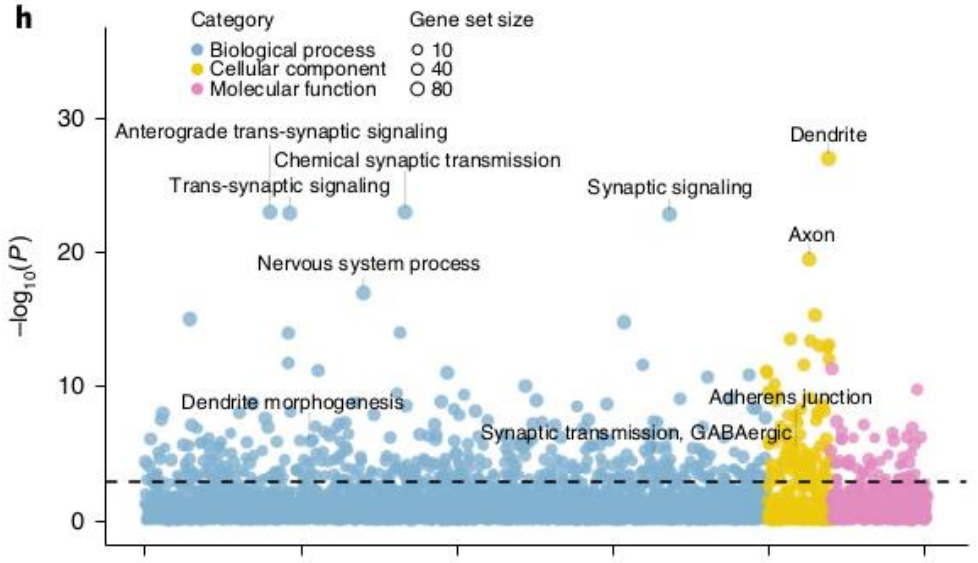
Clustering



Markers



Ontology enrichment analysis



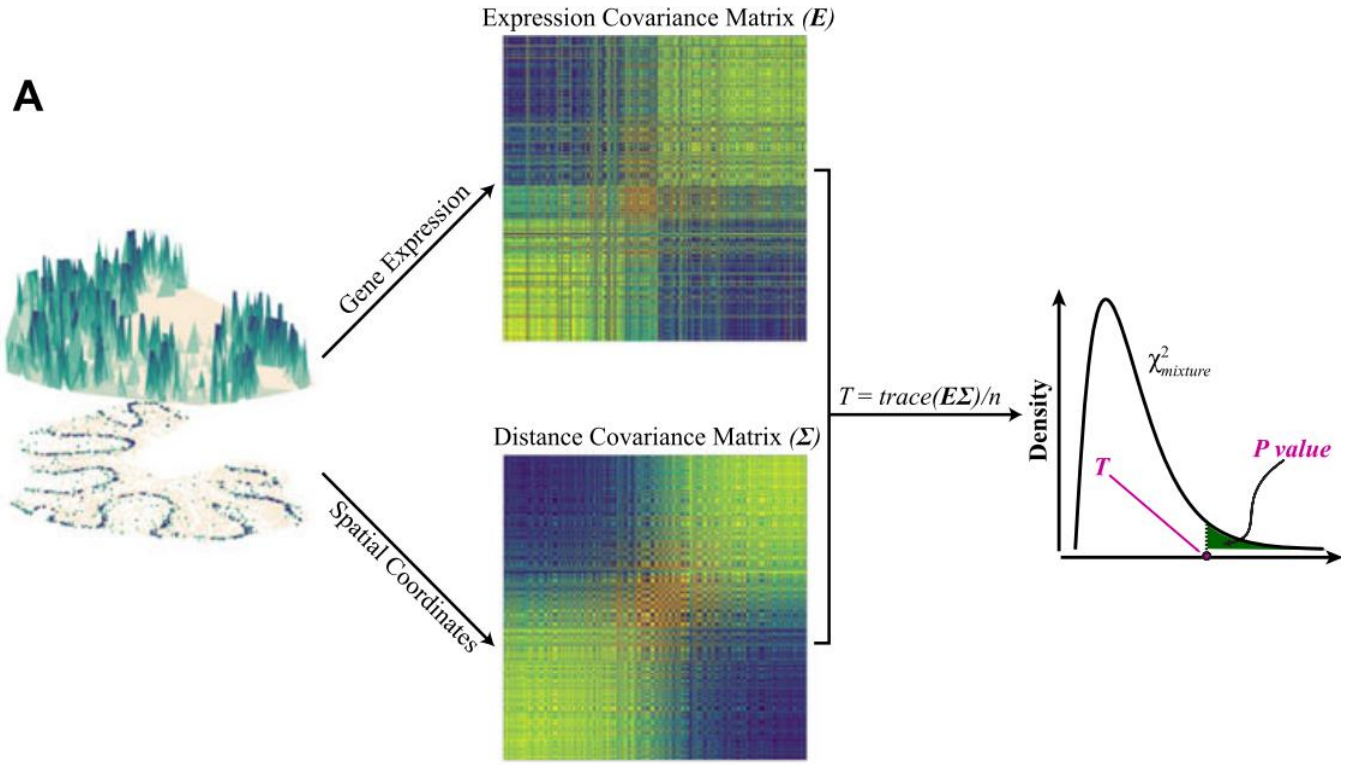
- SYNAPTIC ORGANIZATION
- BULB DEVELOPMENT

Spatially aware feature selection – Spatial Statistics

TOOL : **SPARK-X**[6] (Non-parametric version)

More suitable than SPARK for **sparse, large-scale data**

Scalable for dataset with more than ~10.000 of genes measured on ~10.000 spots.

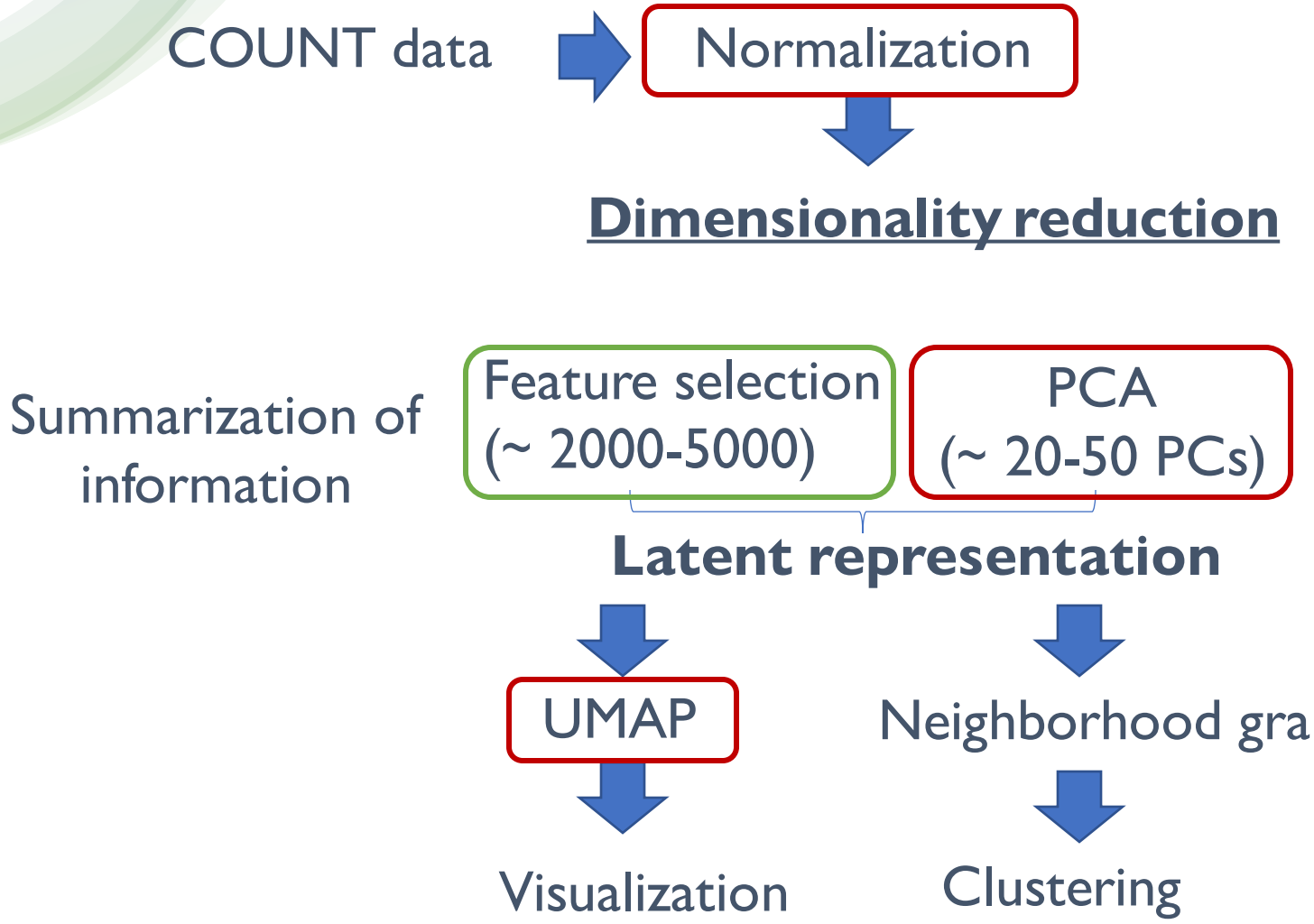


Intuition: if y (gene expression) is independent of S (spatial coordinates), then the spatial distance between two locations ***i*** and ***j*** would also be independent of the gene expression difference between the two locations

2

Spatially aware unsupervised analysis

Framed steps are tackled or replaced by the same-colored framed methods



stLearn (SME Normaliz.)

Spatially aware feature selection

Guideline

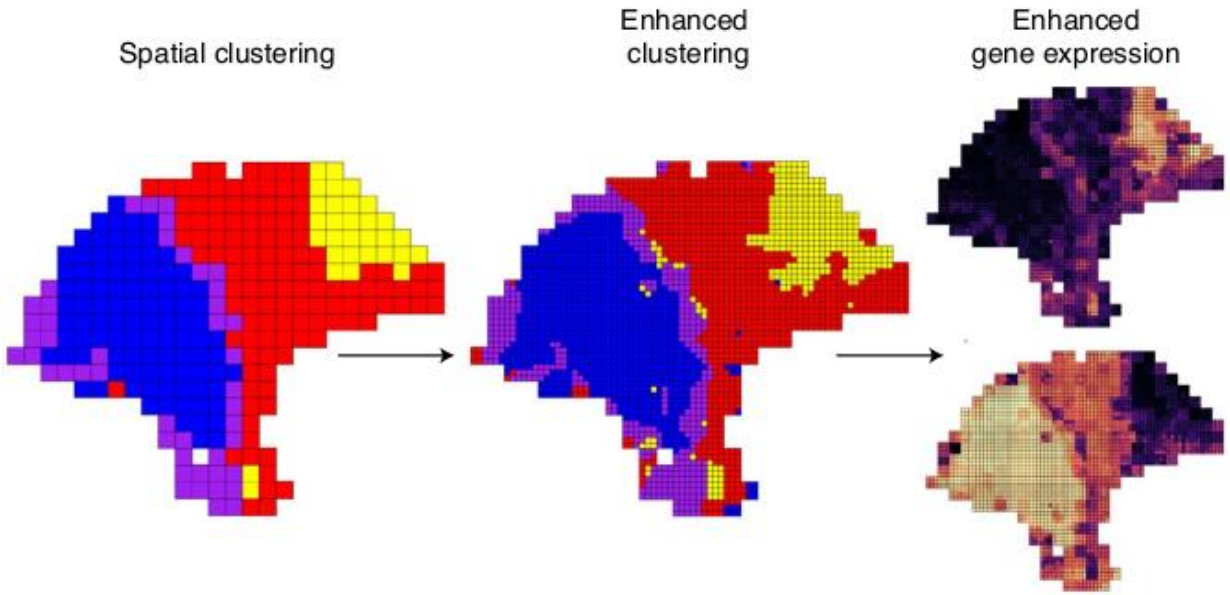
- Data overview
- Pipeline overview
- Leverage new data modalities
- Spatially aware unsupervised analysis
    - Data normalization
    - Feature selection
    - **Model based**
  - Benchmarking and further specific results
- Latent representation from reference-based deconvolution
- Bonus: Deep Data Fusion

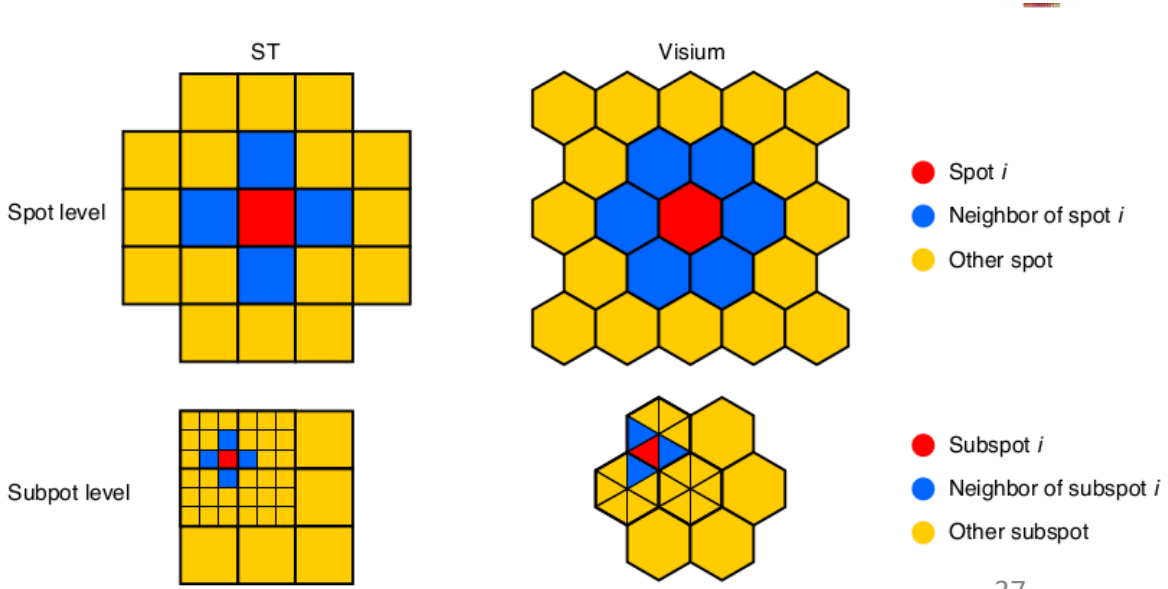
Model based approaches

TOOL : **BayesSpace**(6) Provides the SPOT CLUSTER ASSIGMENTS + SUB-SPOT RESOLUTION

Fully Bayesian Model with Markov Random Field. Inspired on widely used computer vision models for denoising and segmentizing images in a statistical / probabilistic manner

- ***Preprocessing*** : **Normalization + Log-Transformation + top HVG + top PCs (~ 15)**.
- Performance ***relies on empirical knowledge*** for the selection of HVG, PCs, nº of Clusters
- BayesSpace performs iterative clustering, ***CONSTRAINING*** spots to join neighboring clusters (spatial awareness)



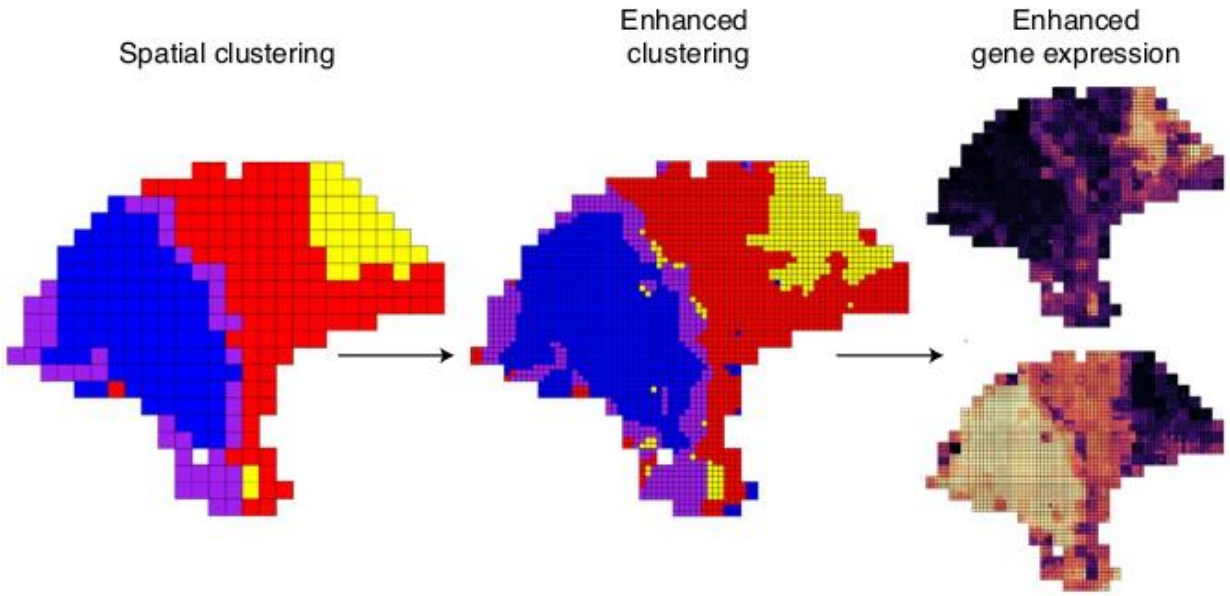


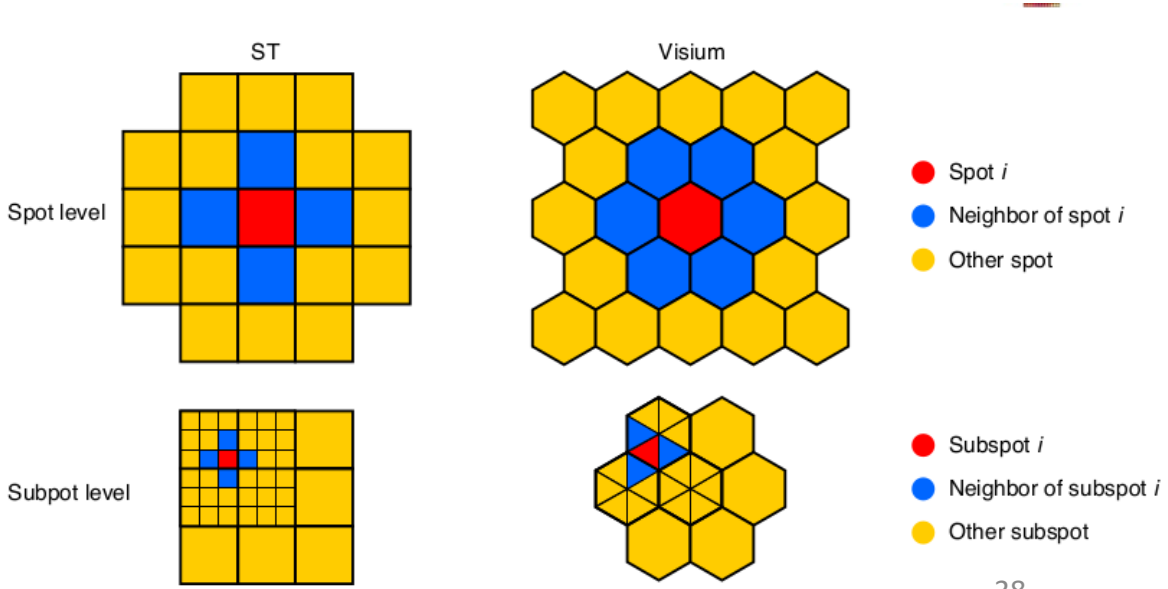
Model based approaches

TOOL : **BayesSpace**(6) Provides the SPOT CLUSTER ASSIGMENTS + SUBS-POT RESOLUTION

if the model **only works with the PCs** of the data:

HOW CAN WE GET SUBSPOT RESOLUTION EXPRESSION MAPS ?





Model based approaches

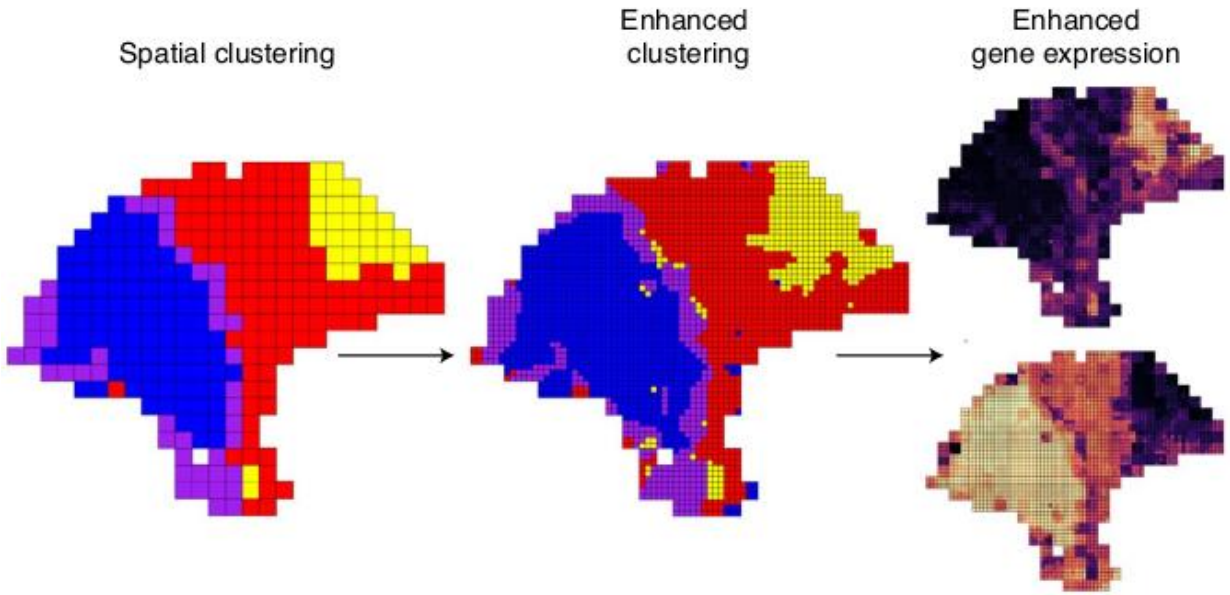
TOOL : **BayesSpace**(6) Provides the SPOT CLUSTER ASSIGMENTS + SUB-SPOT RESOLUTION

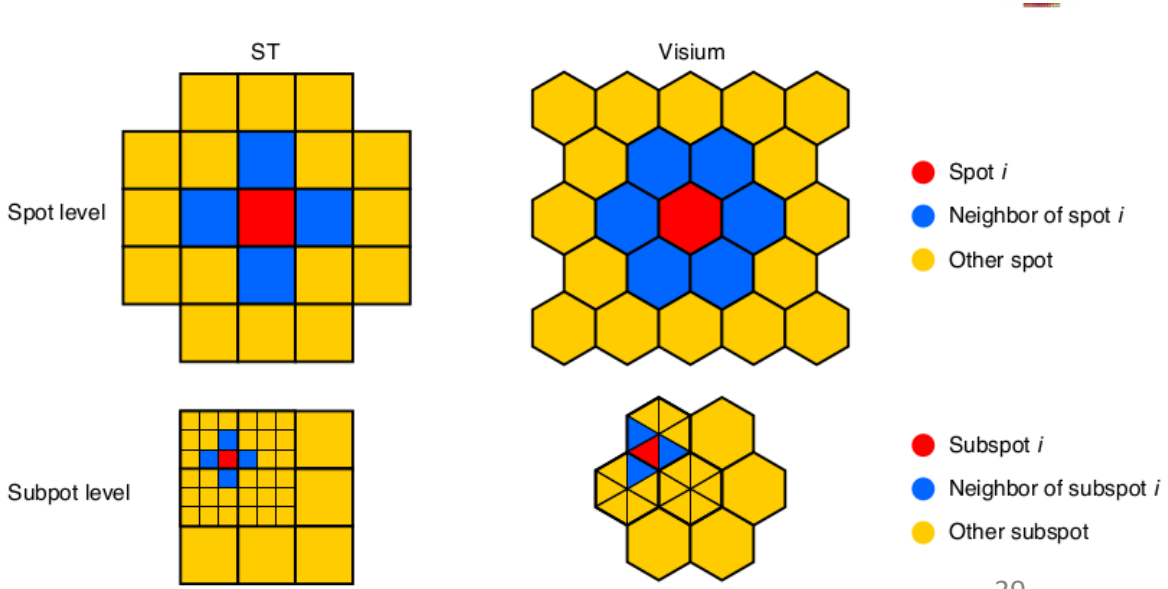
if the model **only works with the PCs** of the data:

HOW CAN WE GET SUBSPOT RESOLUTION EXPRESSION MAPS ?

Need an additional step ➡ Train a model to predict gene expression from PCs on original data

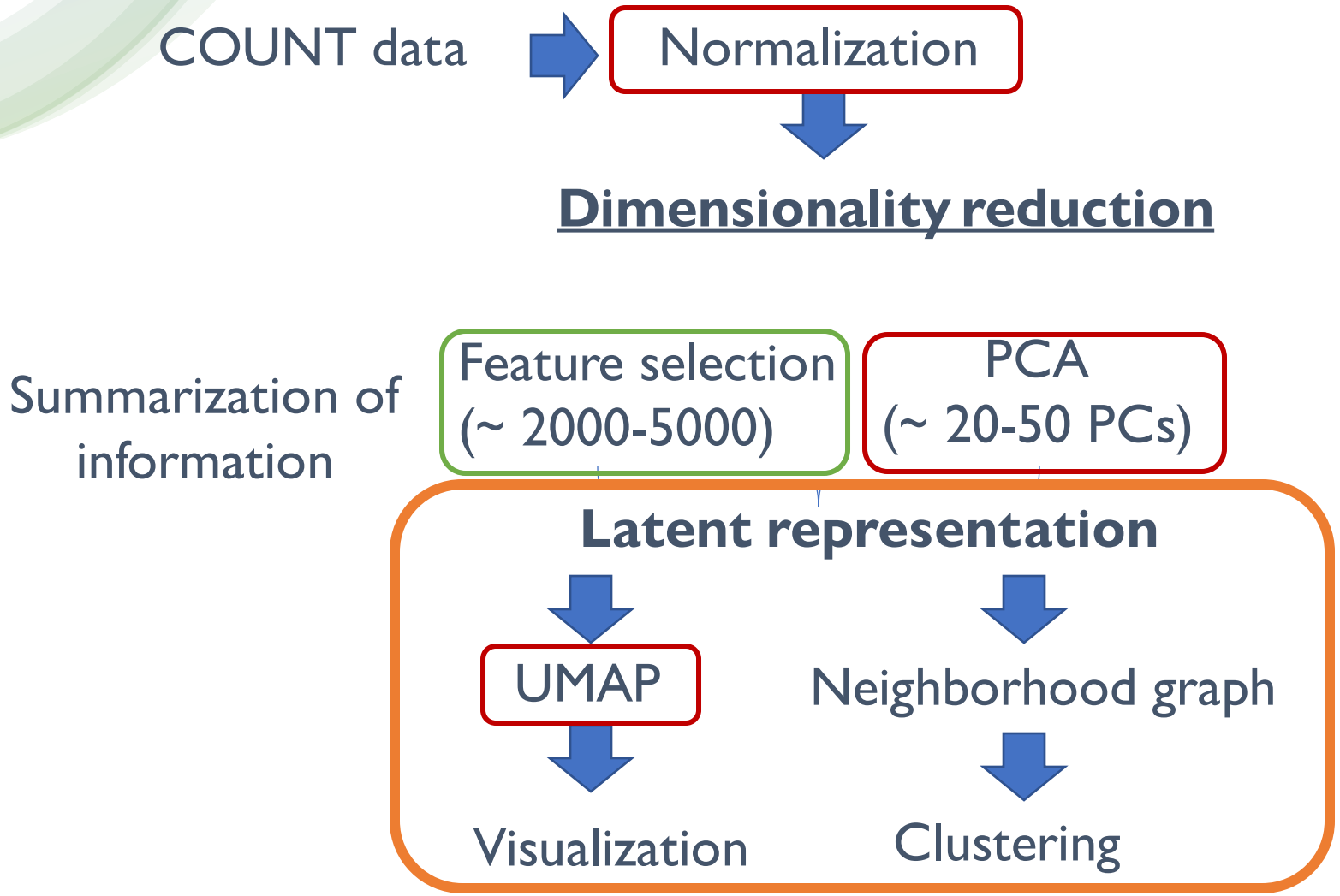
Use this model on the sub-spot PCs values to get Enhanced gene expression maps





Spatially aware unsupervised analysis

Framed steps are tackled or replaced by the same-colored framed methods



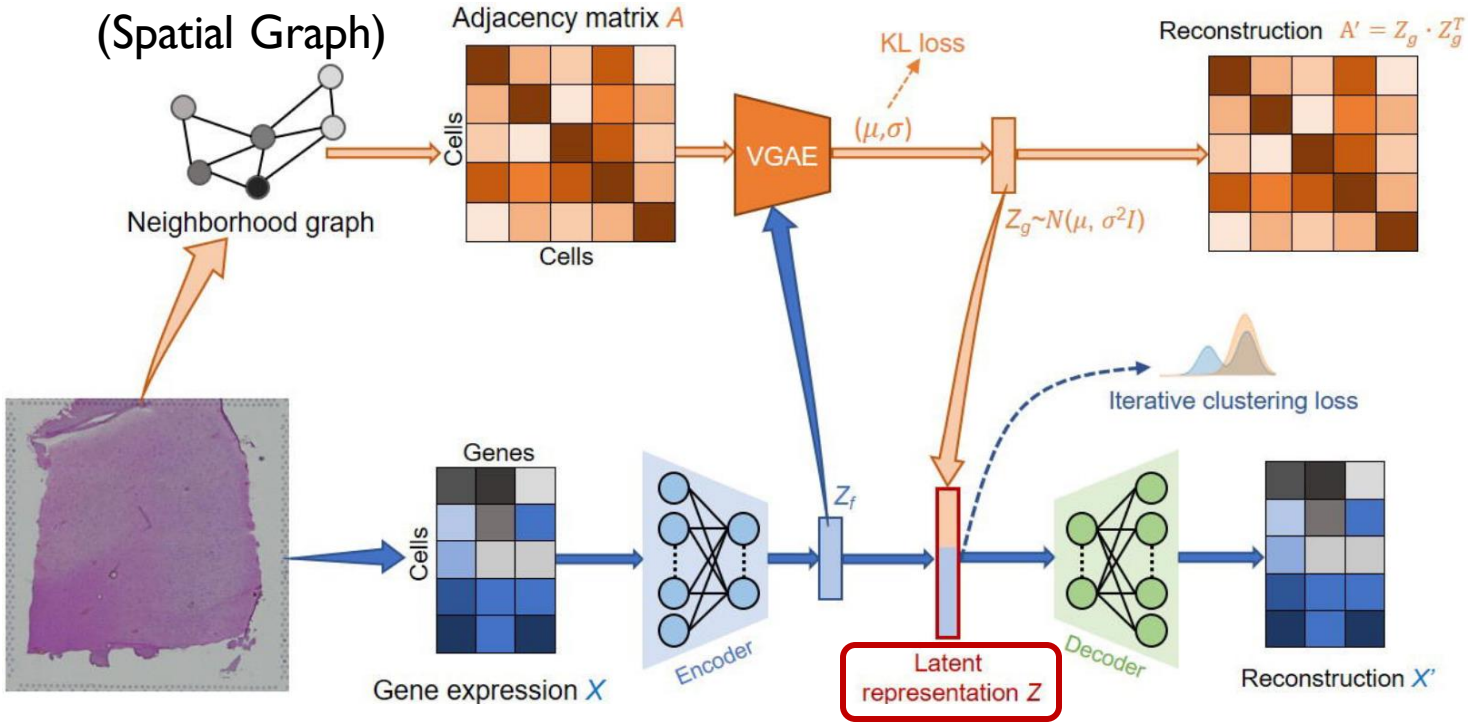
stLearn (SME Normaliz.)

Spatially aware feature selection

BayesSpace

Model based approaches

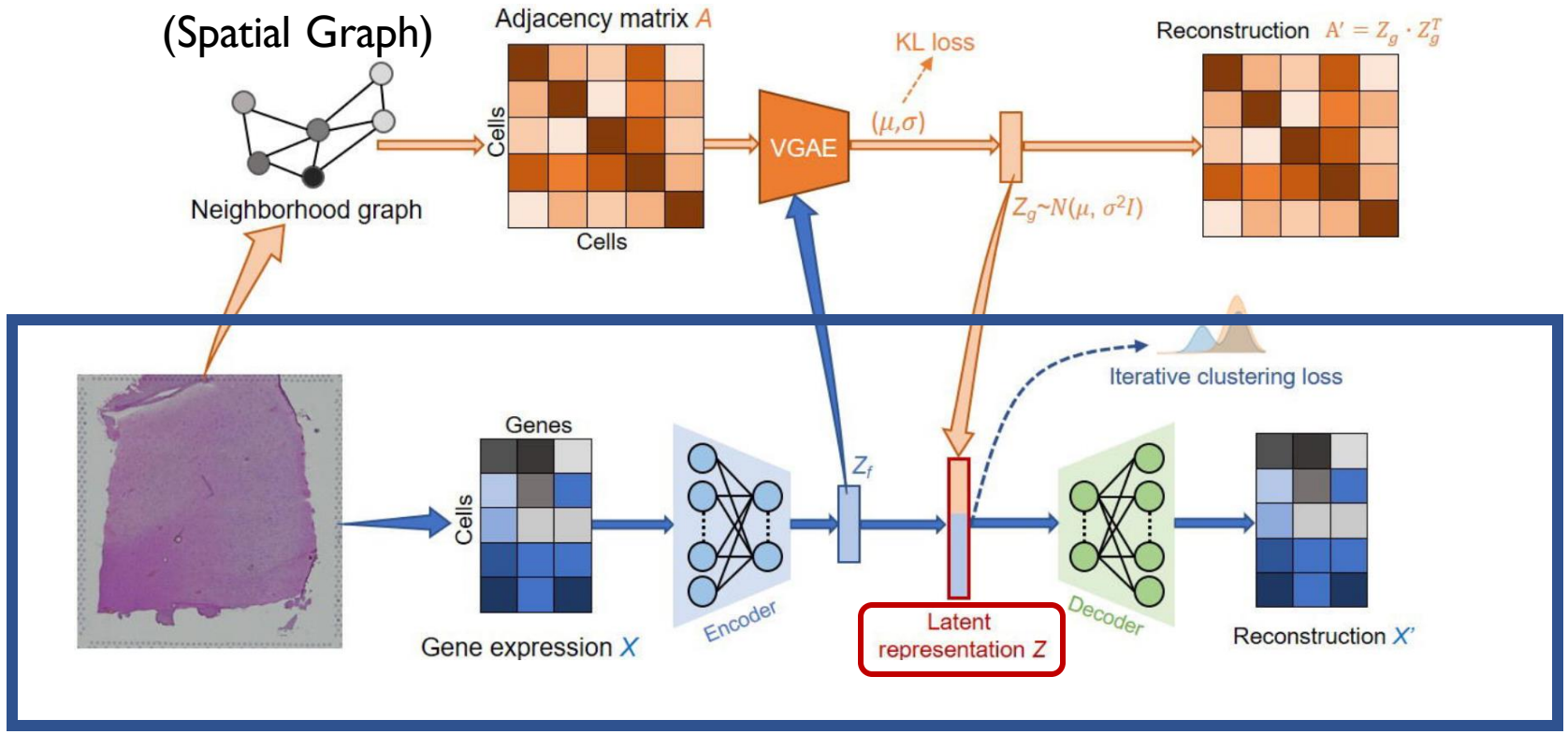
TOOL : Spatially Embedded Dimensionality Reduction (**SEDR**)[7] Provides a Refined Latent Representation



Model based approaches

TOOL : Spatially Embedded Dimensionality Reduction (SEDR)[7] Provides a Refined Latent Representation

Autoencoder

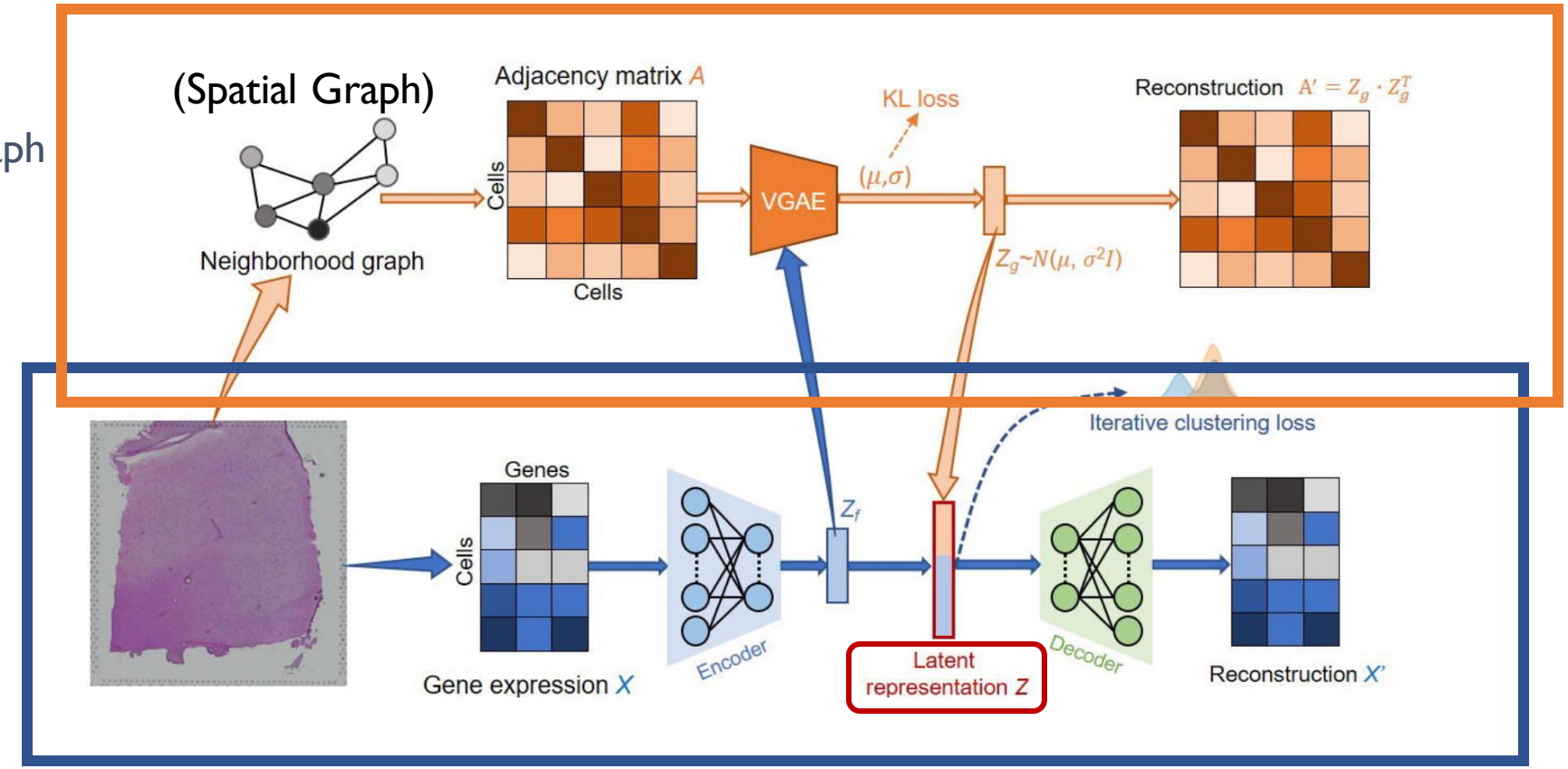


Model based approaches

TOOL : Spatially Embedded Dimensionality Reduction (SEDR)[7] Provides a Refined Latent Representation

Variational graph autoencoder

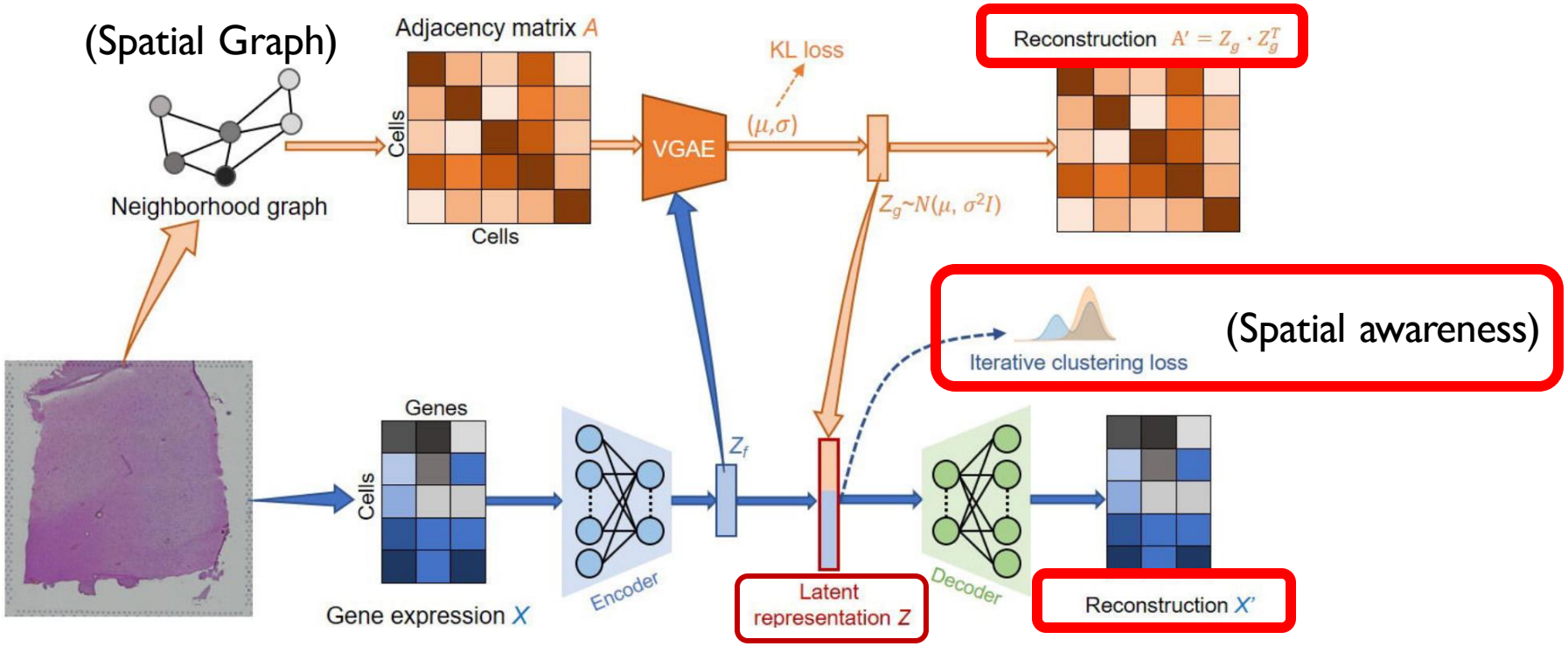
Autoencoder



Model based approaches

TOOL : Spatially Embedded Dimensionality Reduction (SEDR)[7]

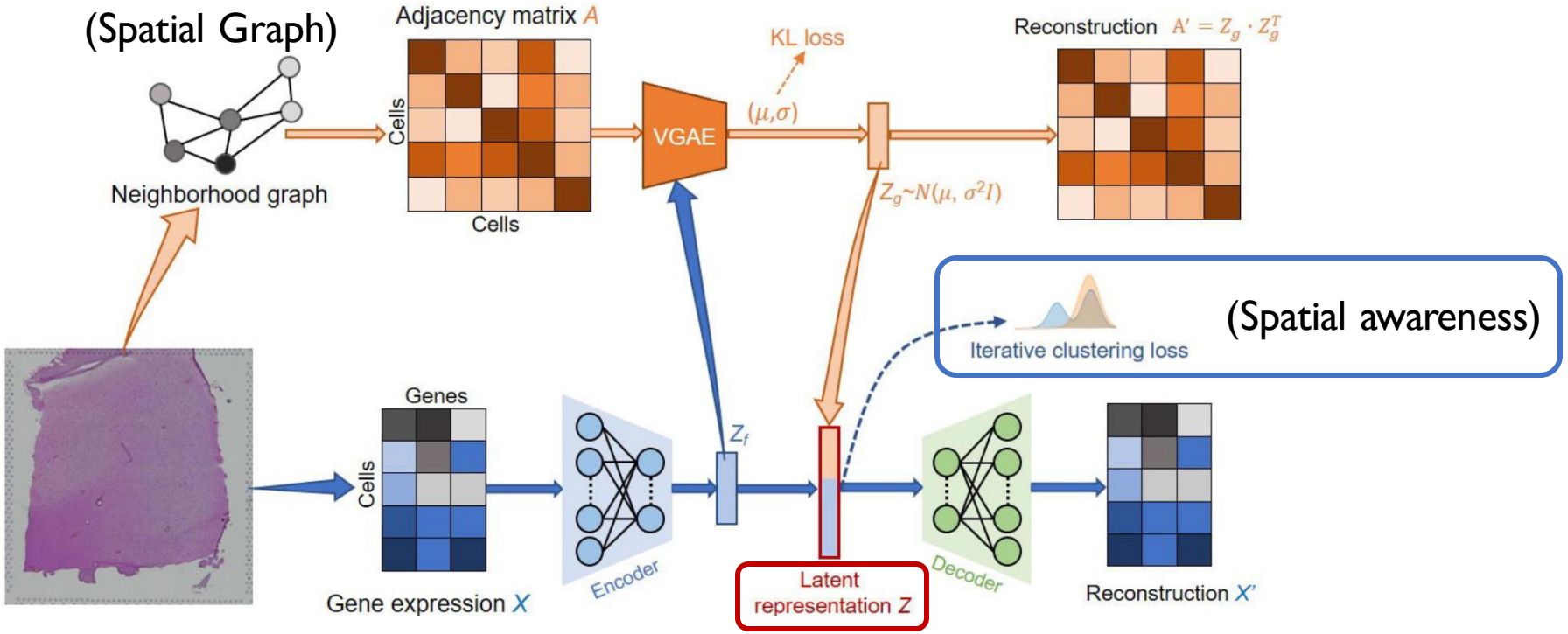
Provides a Refined Latent Representation



Model based approaches

TOOL : Spatially Embedded Dimensionality Reduction (**SEDR**)[7] Provides a Refined Latent Representation

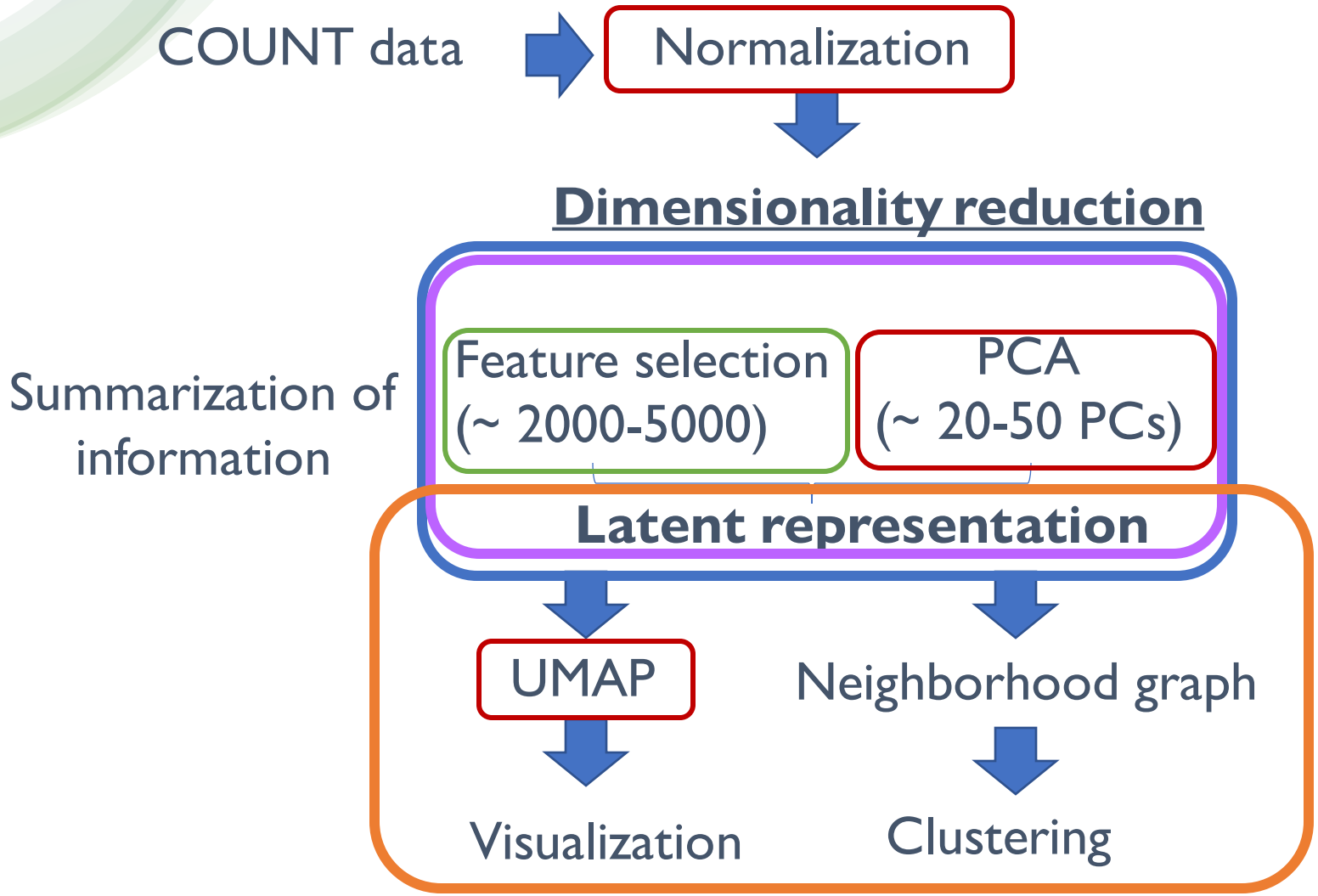
Preprocessing :
Normalize, log-norm. and PCs computation.
Authors recommendation:
~ 300 PCs as input



Latent Representation Z is composed by : - Encoded features of the gene expression +
- Spatial embedded features outputted by the Variational Graph Autoencoder (Spatial Graph)
(encoded features)

Spatially aware unsupervised analysis

Framed steps are tackled or replaced by the same-colored framed methods



stLearn (SME Normaliz.)

Spatially aware feature selection

BayesSpace

SEDR

Guideline

- Data overview
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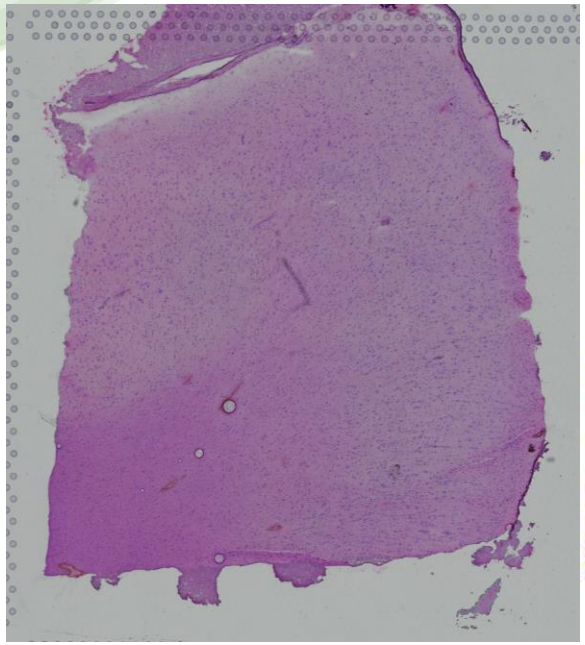
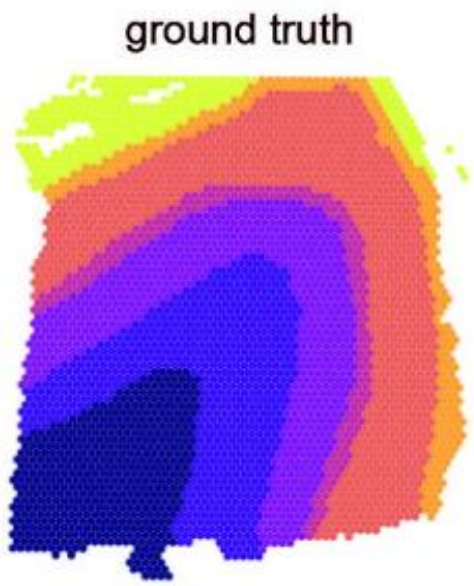
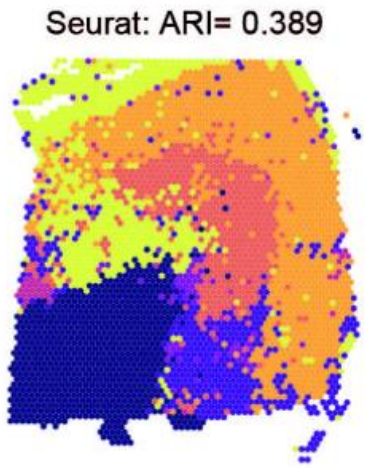
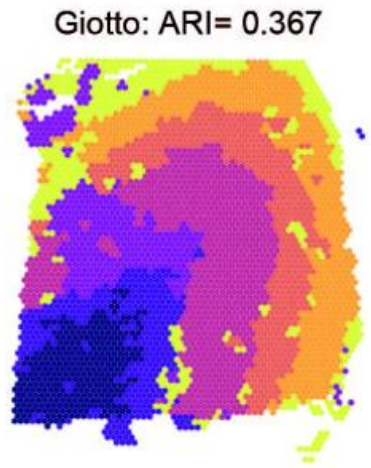
Benchmarking results - DLPFC

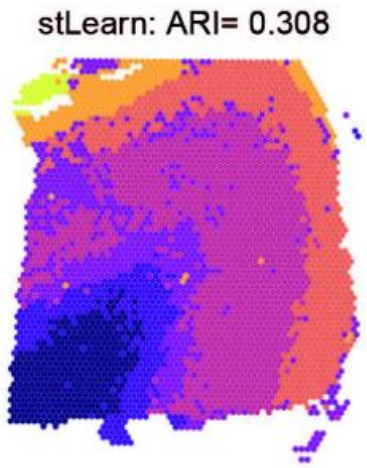
Anatomical structure detection

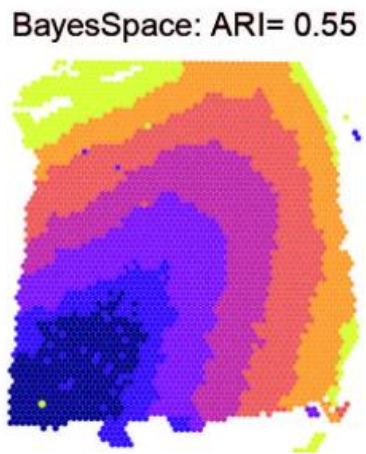


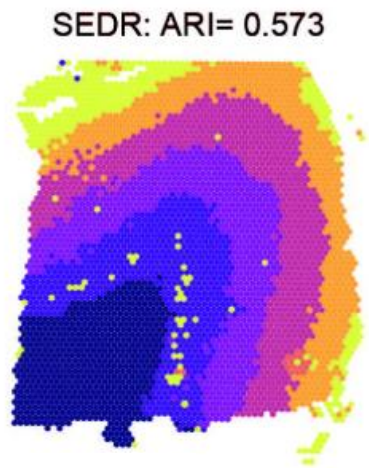












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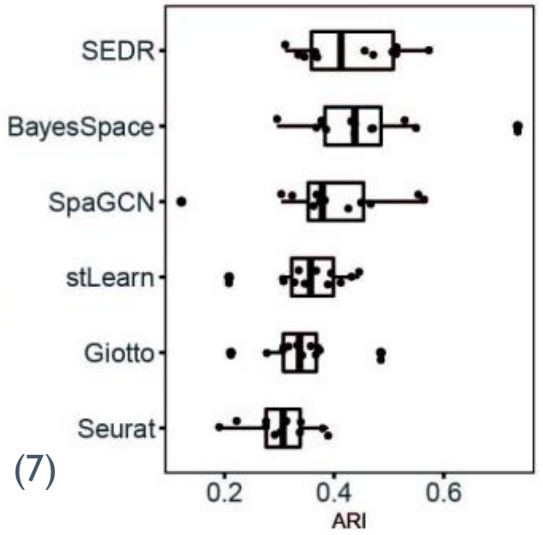
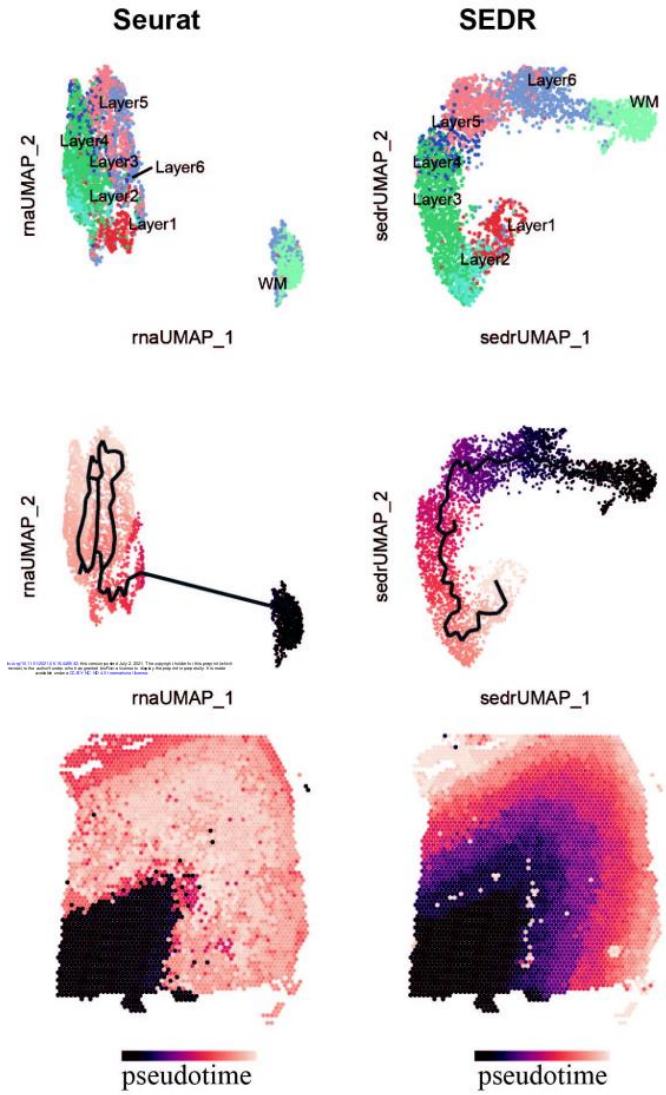


Benchmarking results - DLPFC

SEDR - Trajectory Inference

Monocle3
Tool for pseudotime estimation



SEDR results reflected the correct "inside-out" developmental ordening of cortical layers

**In tumoral samples pseudo-time can show the tumor progression

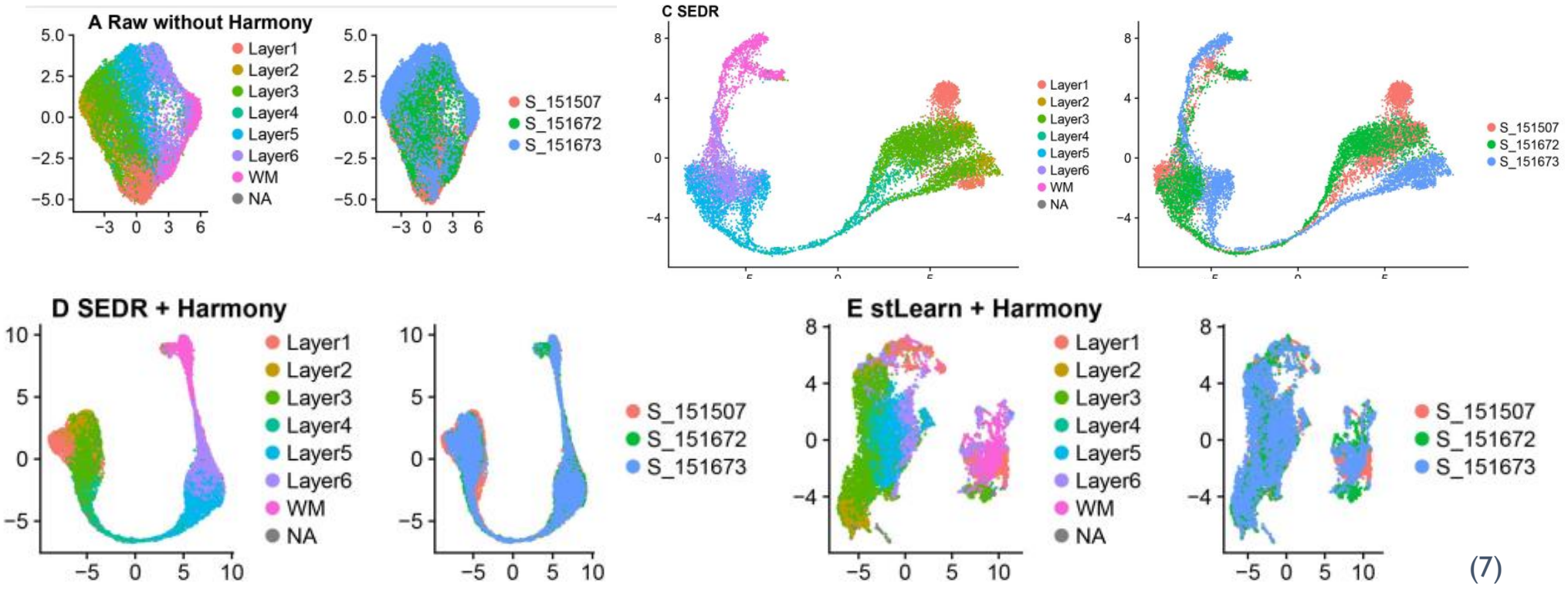
(7)

Benchmarking results - DLPFC

SEDR - Trajectory Inference & Batch effect

Harmony

Batch effect removal tool that aligns the samples information in the PC space



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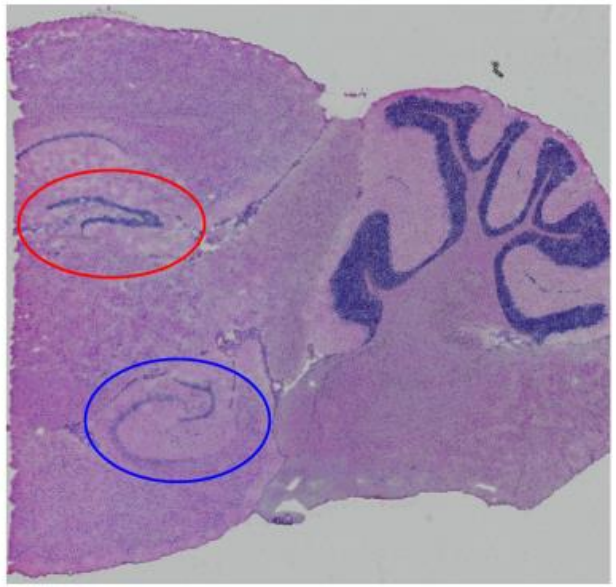
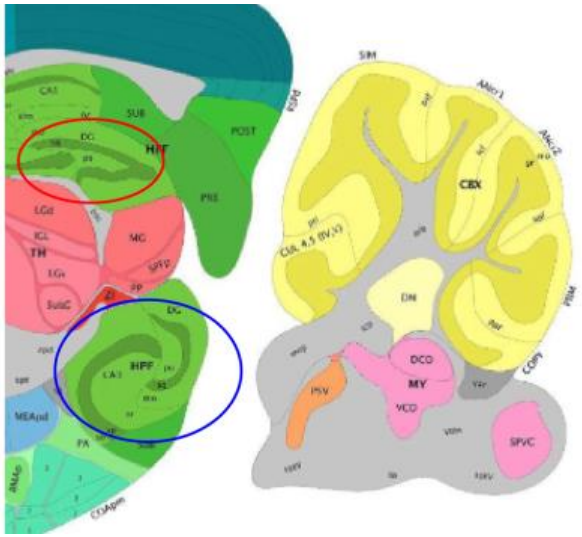
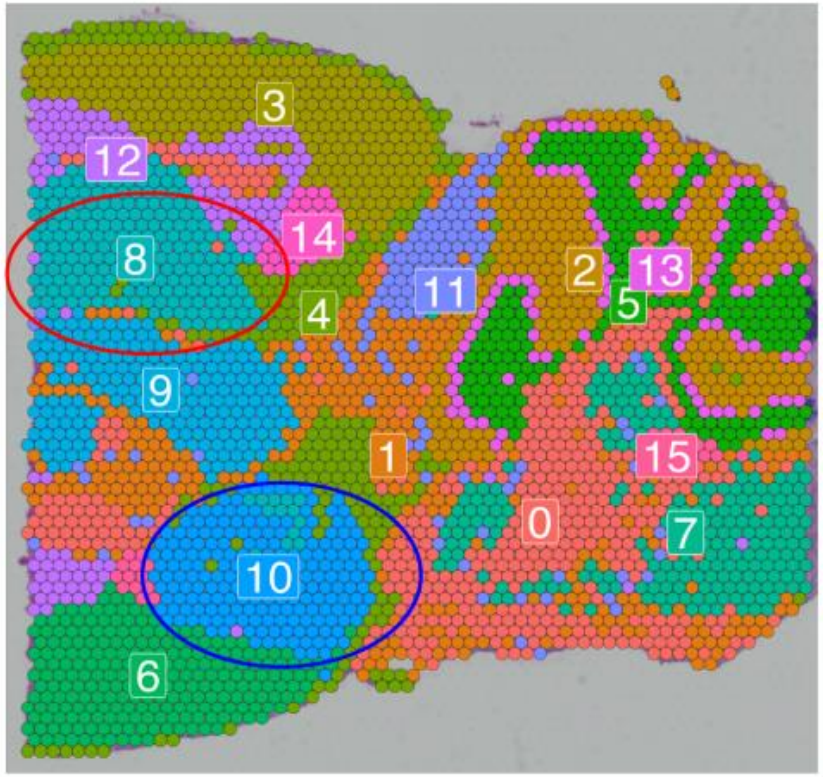
Benchmarking results – Saggital Posterior



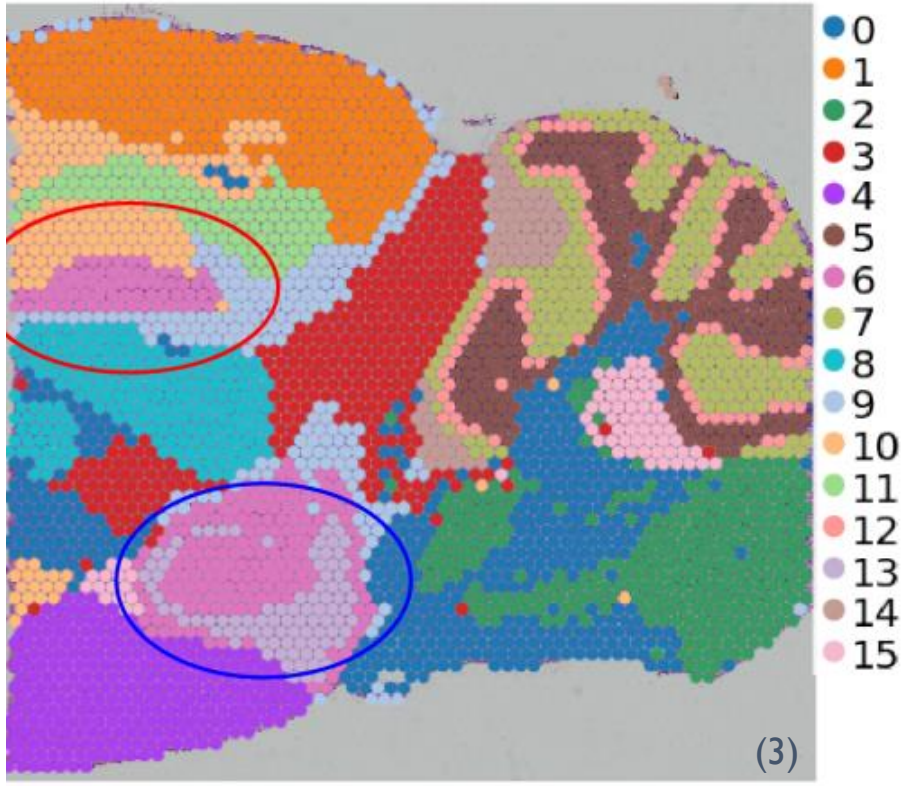


Complex anatomical regions detection – Dentate gyrus

Seurat / Scanpy

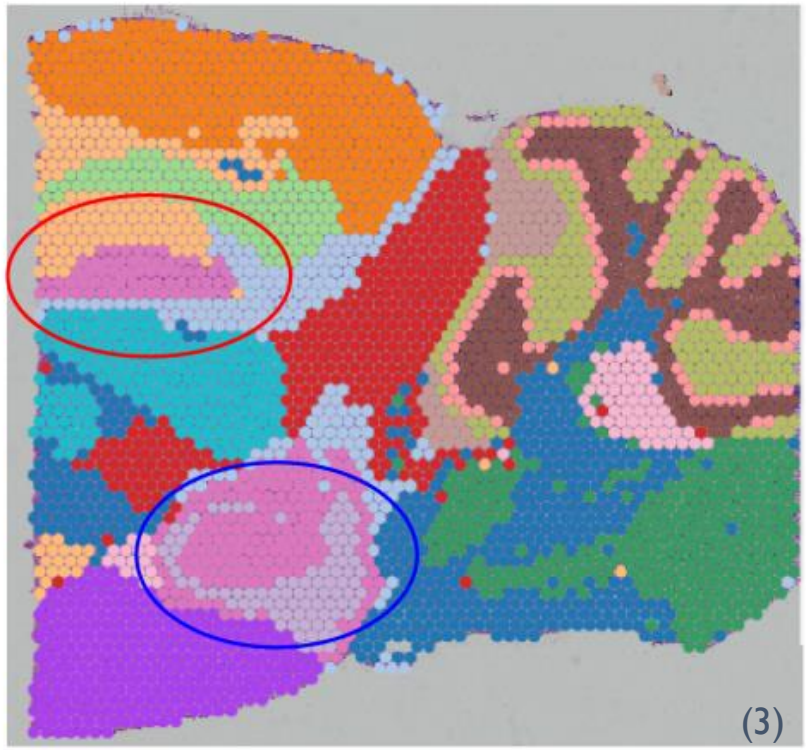


stLearn

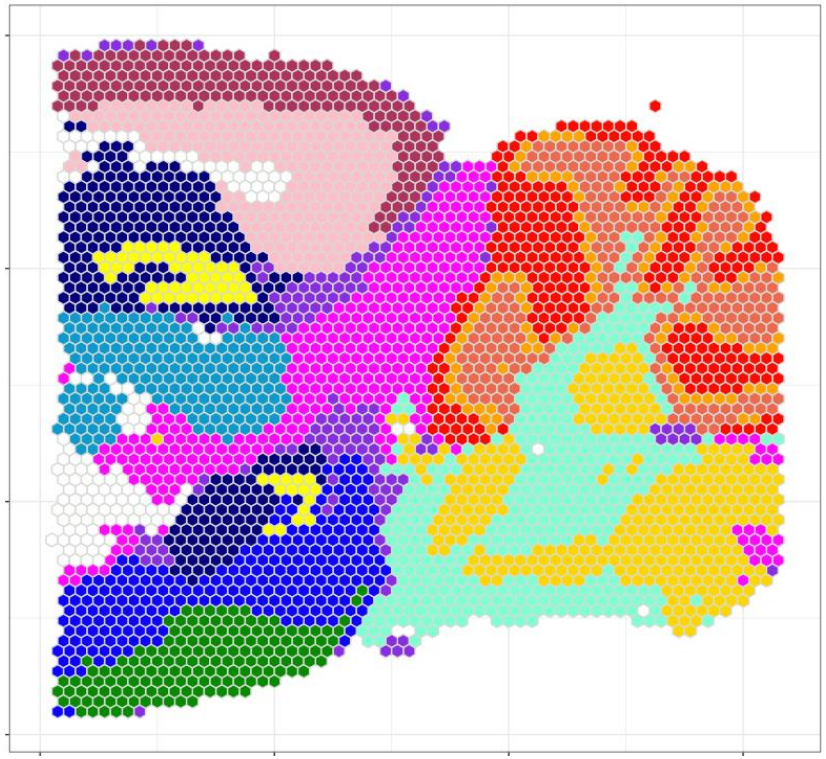


Benchmarking results – Saggital Posterior

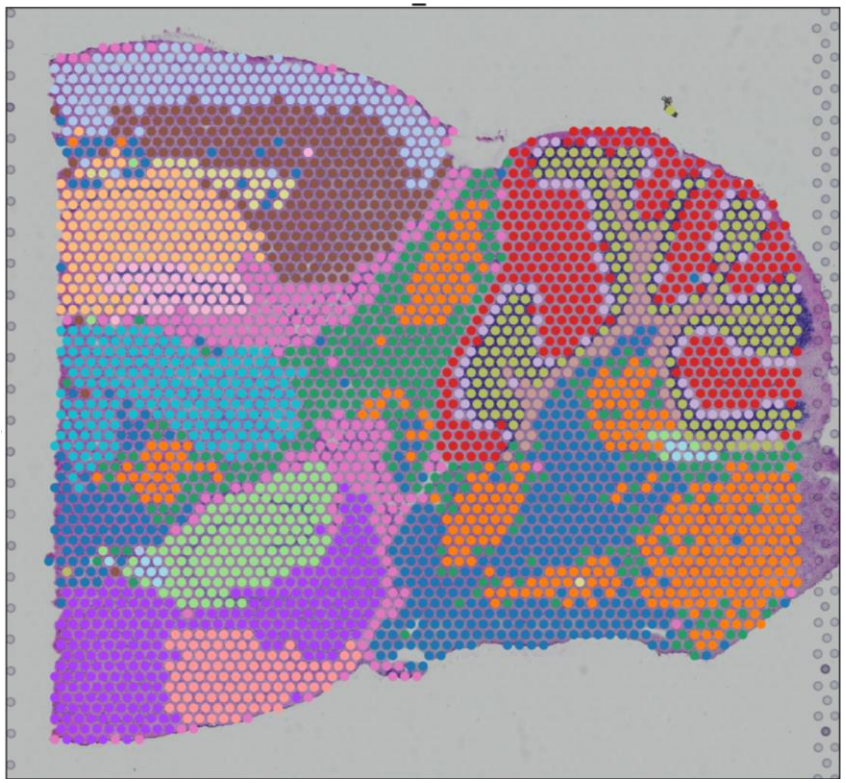
stLearn



BayesSpace

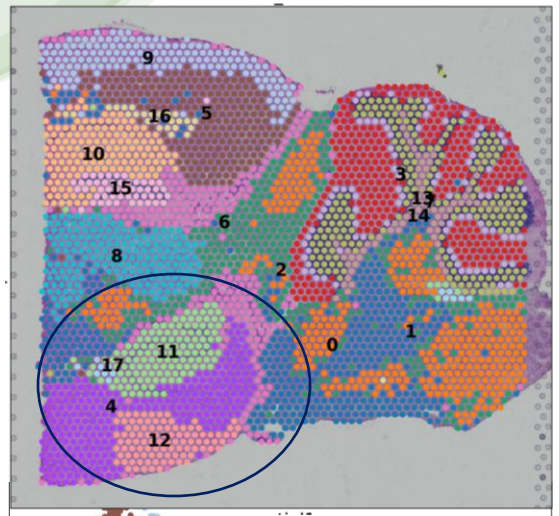


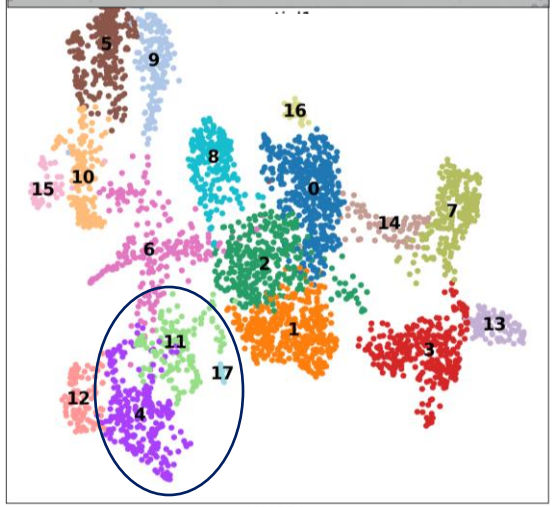
SEDR



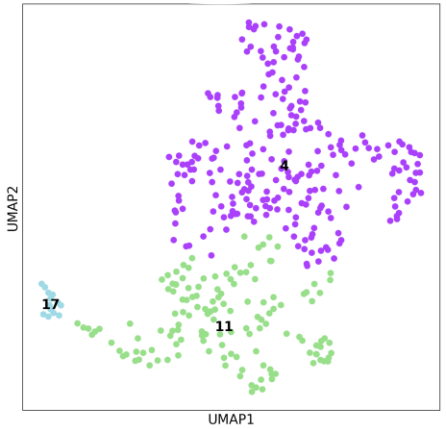
Specific results – SEDR

Allows for user-supervised re-clustering

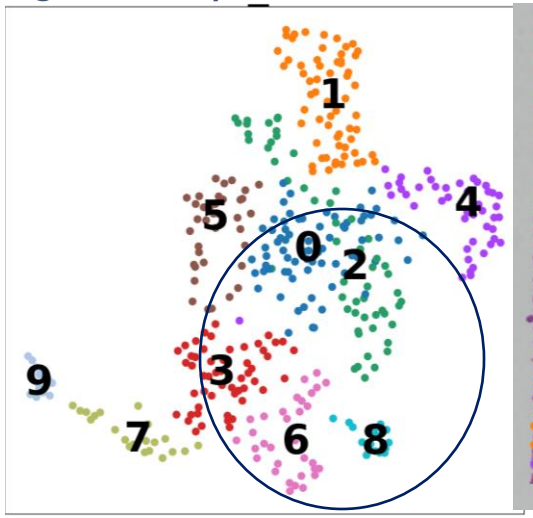


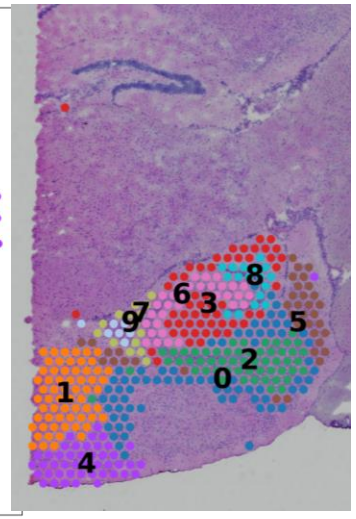


1.- Isolate clusters of interest (4,11,17)

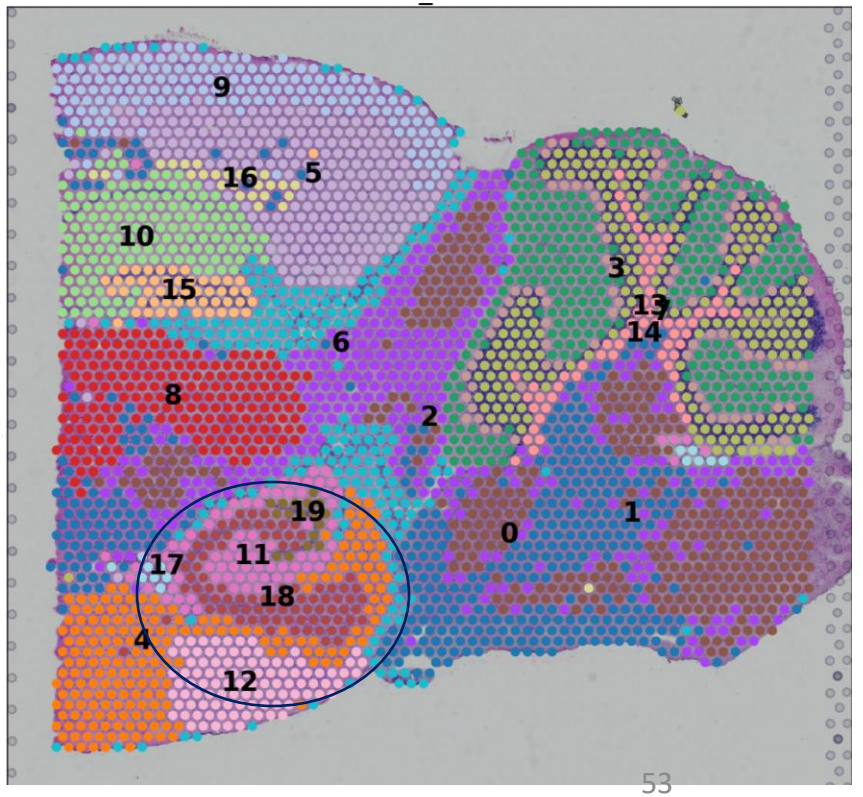


2.- Compute *finer granularity clusters*





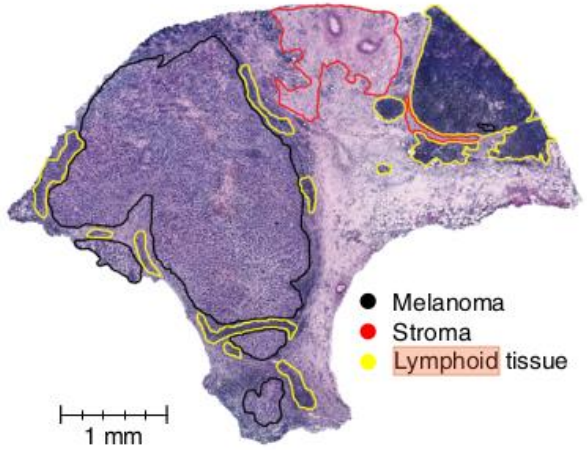
3.- Select finer granularity clusters of interest and merge.
2, 6 ➡ 18 ; *8* ➡ 19

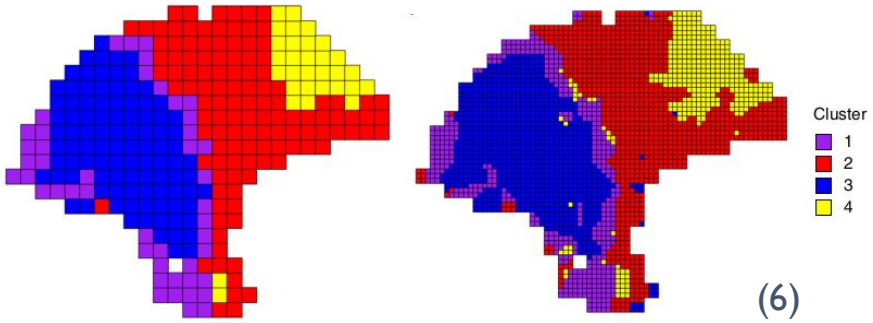


Specific results – BayesSpace

Melanoma sample

Tumor-proximal lymphoid tissue





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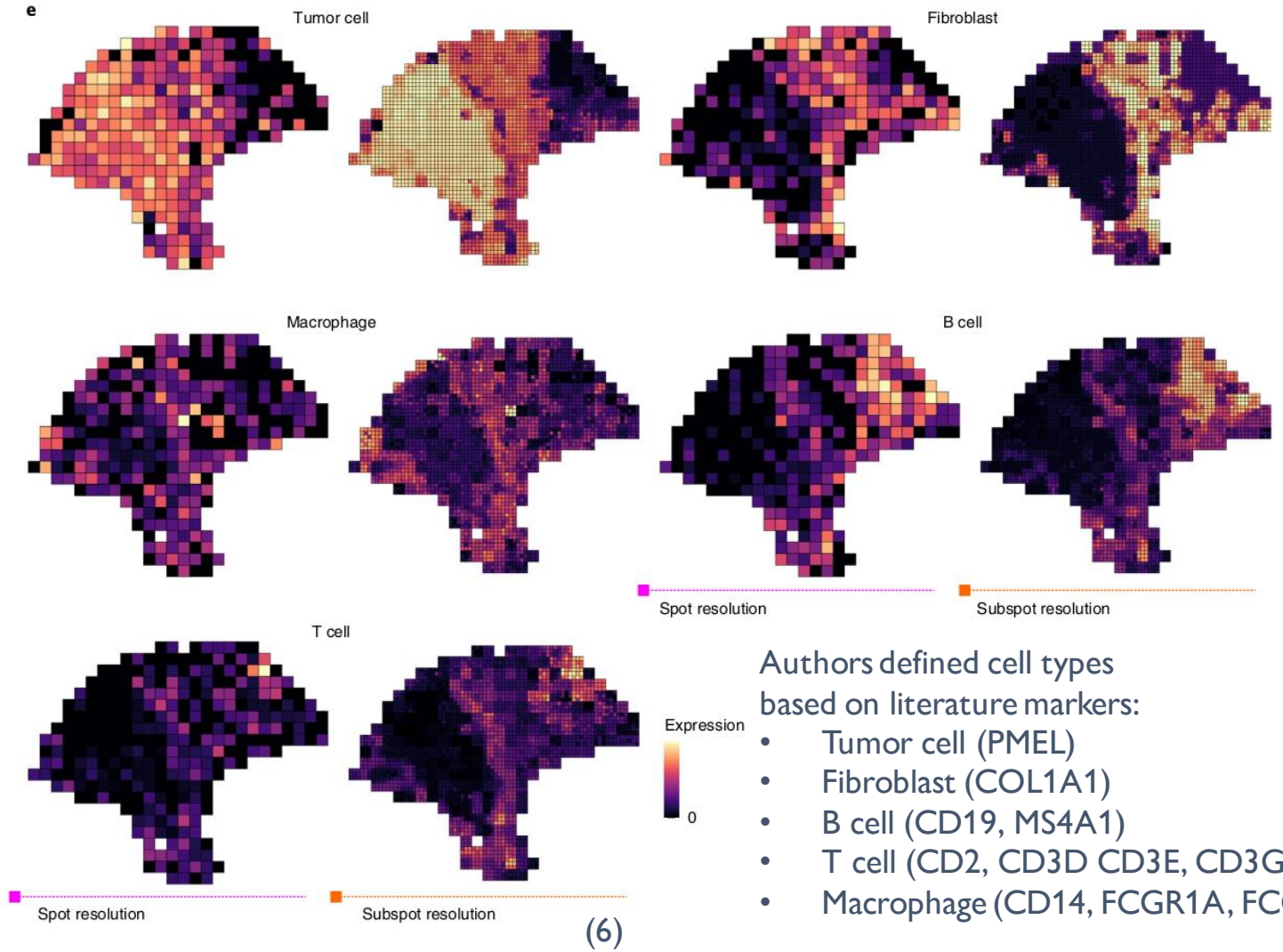

(6)

Authors defined cell types based on literature markers:

- Tumor cell (PMEL)
- Fibroblast (COL1A1)
- B cell (CD19, MS4A1)
- T cell (CD2, CD3D CD3E, CD3G, CD7)
- Macrophage (CD14, FCGR1A, FCGR1B)

Guideline

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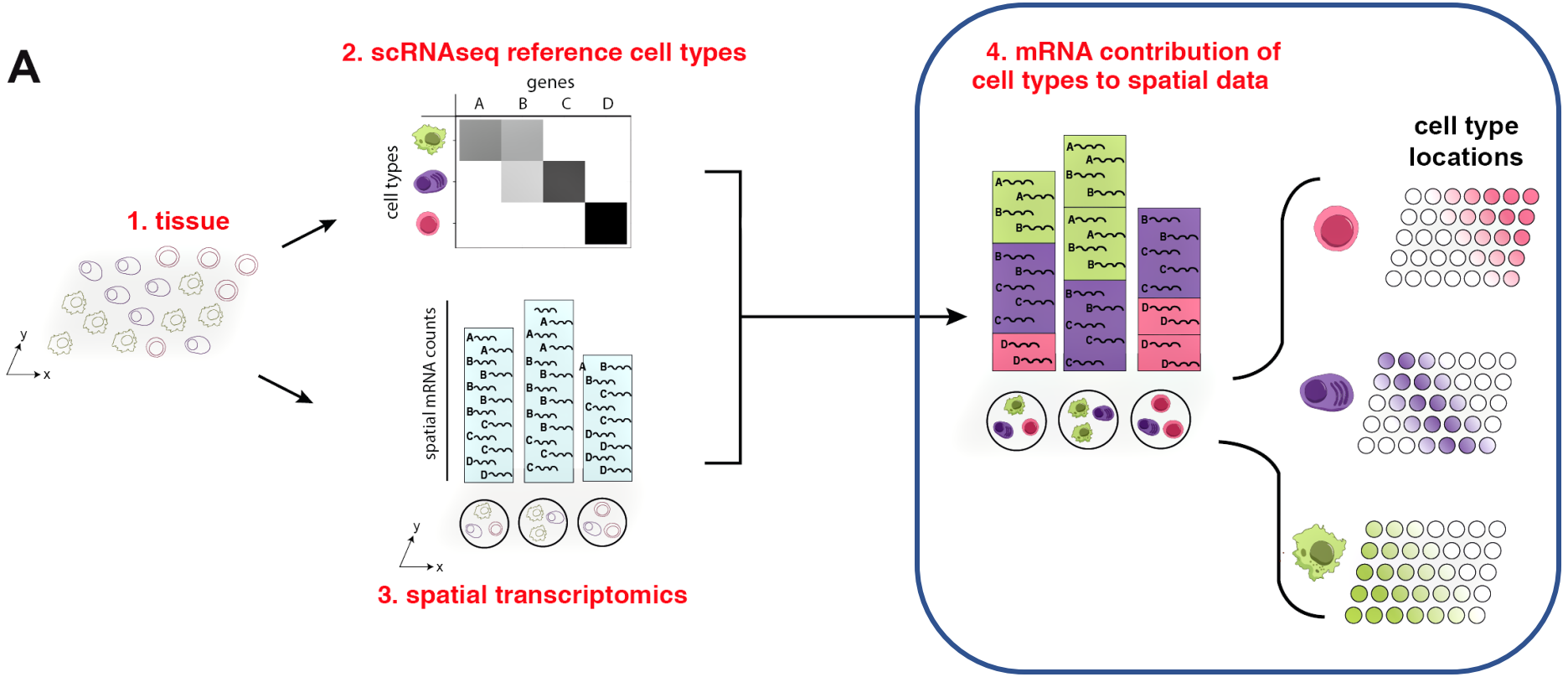
- **Latent representation from reference-based deconvo**
- Bonus: Deep Data Fusion

Latent representation from reference-based deconvolution

ST technologies not always provide single cell resolution

Is common to perform spot deconvolution:



Here we obtain a spot representation based on the cell type proportion

Latent representation of the data

Guideline

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- Pipeline overview
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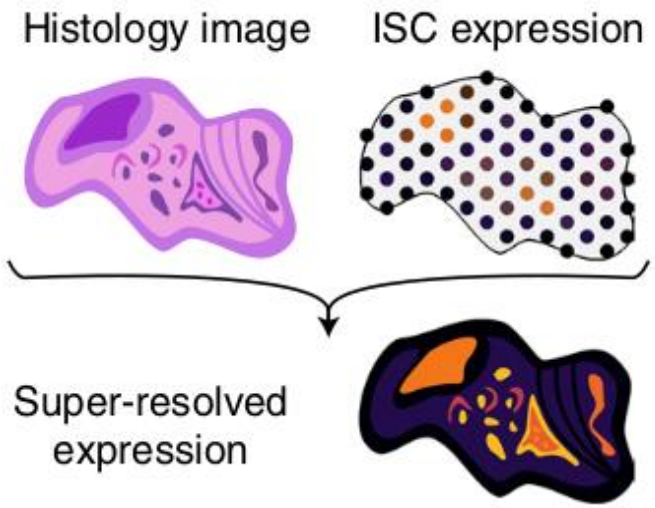
- **Bonus: Deep Data Fusion**

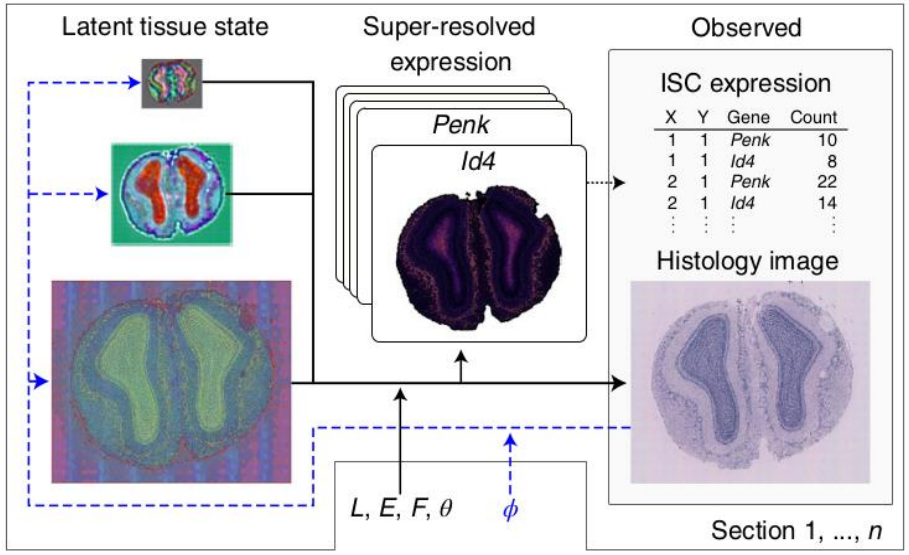
Deep Data Fusion

- Deep generative model that merge ideas from computer vision and generative statistical modeling

Provides SUPER-RESOLVED gene expression maps



Model scheme



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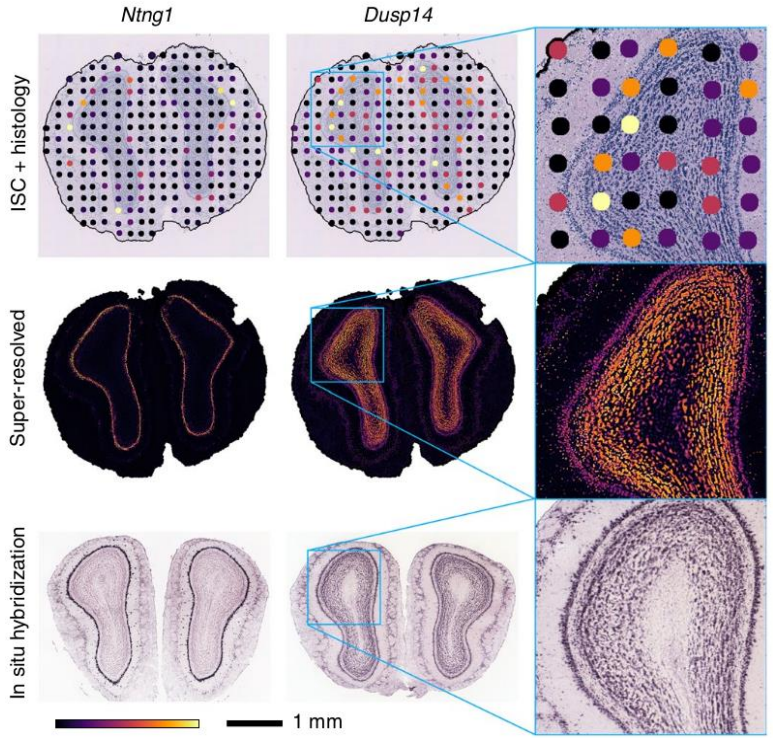
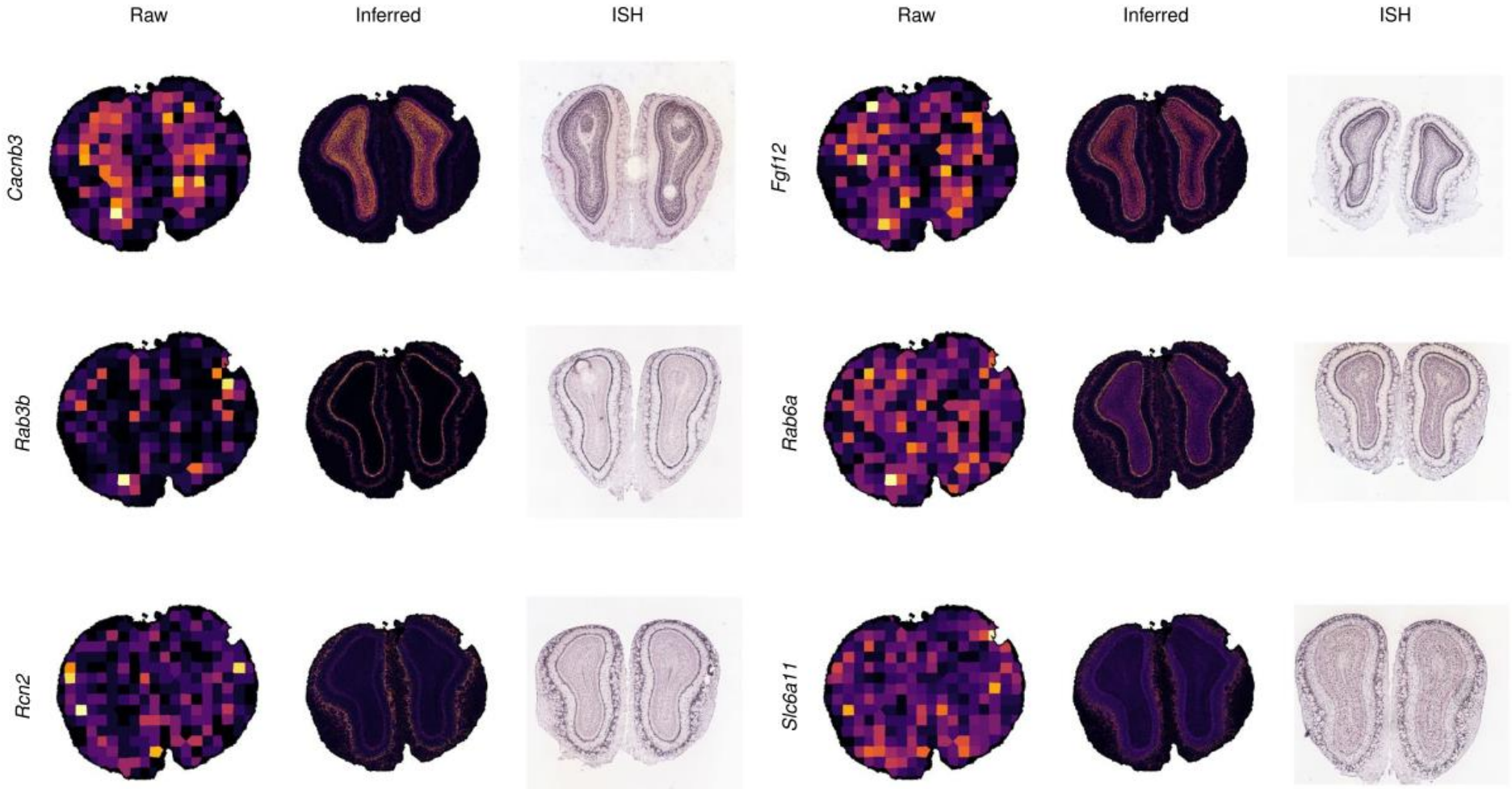


Spatial approaches

Model based approaches

TOOL : **Deep Data Fusion** Provides SUPER-RESOLVED gene expression maps



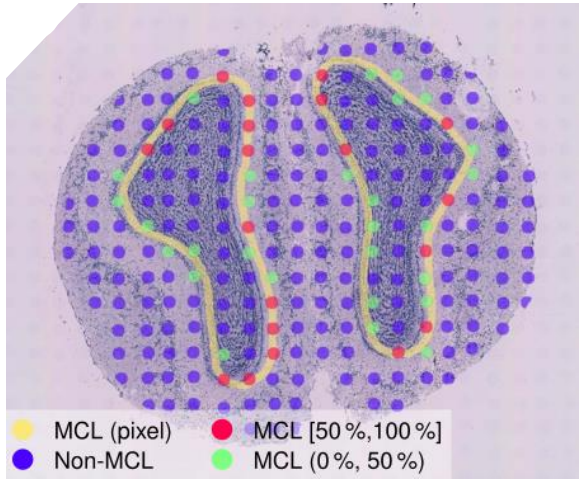
(8)

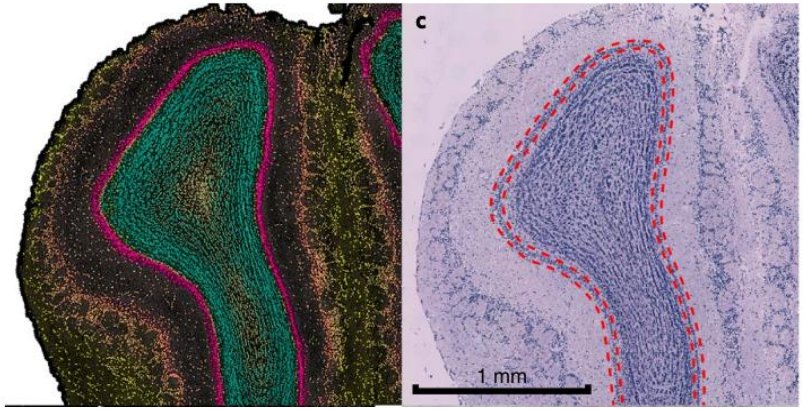
Spatial approaches

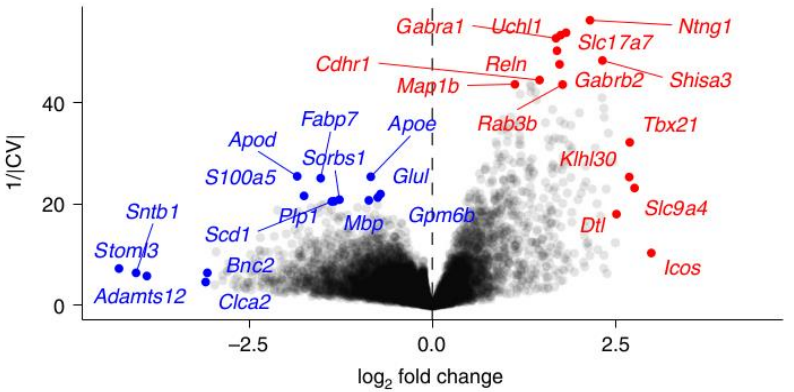
Model based approaches

TOOL : **Deep Data Fusion** Provides SUPER-RESOLVED gene expression maps

- Instead of clustering assigment we have a latent tissue state
- We can still run differential expression analysis: we select measurement locations overlapping with an annotation region (eg. Mitral Cell Layers) . Then, we log-normalize the data and compute differentially expressed genes using the FindMarkers (Seurat) function







(8)

METHODS SUMMARY

	INPUT	OUTPUT
stLearn	RAW ST + Histological Image	Spatial Morphological Gene Expression Normalization
SPARK(-X) // GIOTTO	RAW ST DATA	Spatially Variable Genes
BayesSpace	(~15) Top PCs from HVG	Cluster labels + Sub-spot resolution
SERD	(~300) Top PCs from ALL GENES	Spatially Embedded Latent Representation
Spot deconvolution	RAW ST DATA + Annotated sc-RNAseq reference	Cell type proportion (alternative latent representation)
Deep data fusion	RAW ST + Histological Image	Super-resolved gene expression maps

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THE END

Thanks for your attention!

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