Lecture 13
Spatial transcriptomics and spatial domain detection

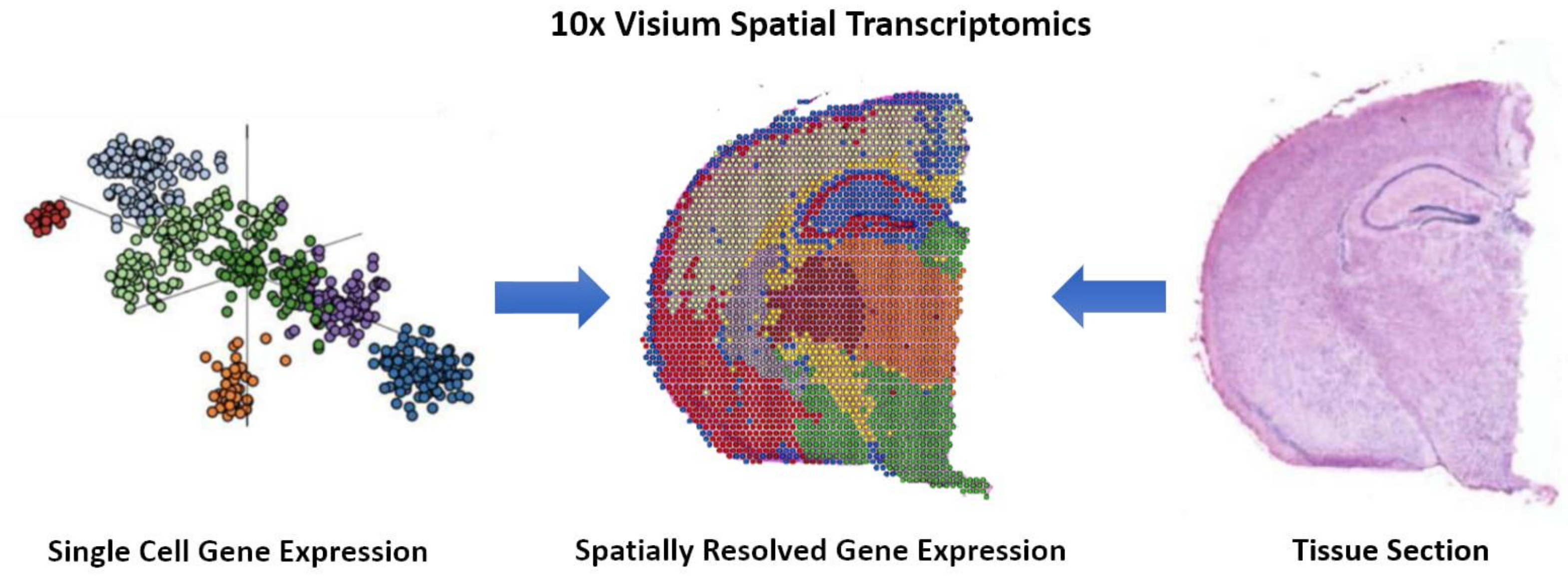
Outline

- Spatial transcriptomics
    - Histology
    - Image-based and sequencing-based technologies

- Spatial domain detection
    - Spatial statistics-based methods and GNN methods
    - Integration of multiple slices

What is spatial transcriptomics?

- Spatial transcriptomics measure both transcriptomics (gene expression levels across the whole genome) and spatial information
  - Many genes need to be properly regulated in space for the system to function
  - Understand spatial patterns of gene expressions


10x Visium Spatial Transcriptomics
Single Cell Gene Expression
Spatially Resolved Gene Expression
Tissue Section

Adapted from 10x Genomics

Histology

- Histology: spatial transcriptomics data often have an associated histology image
  - Microscopic anatomy of biological tissues
  - Staining provides colors:
    - H&E stain: stains the nuclei purplish-blue and cytoplasm and other tissues in various stains of pink
  - Can be used to diagnose cancer and other diseases

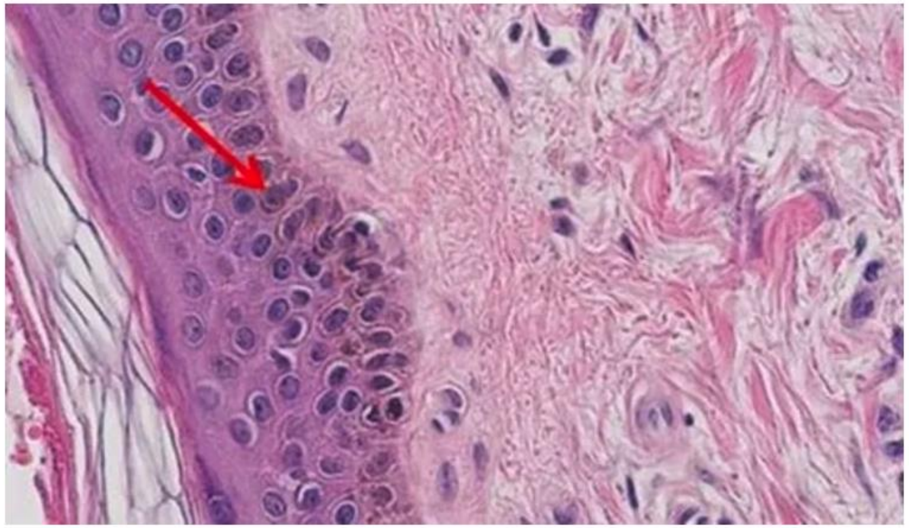
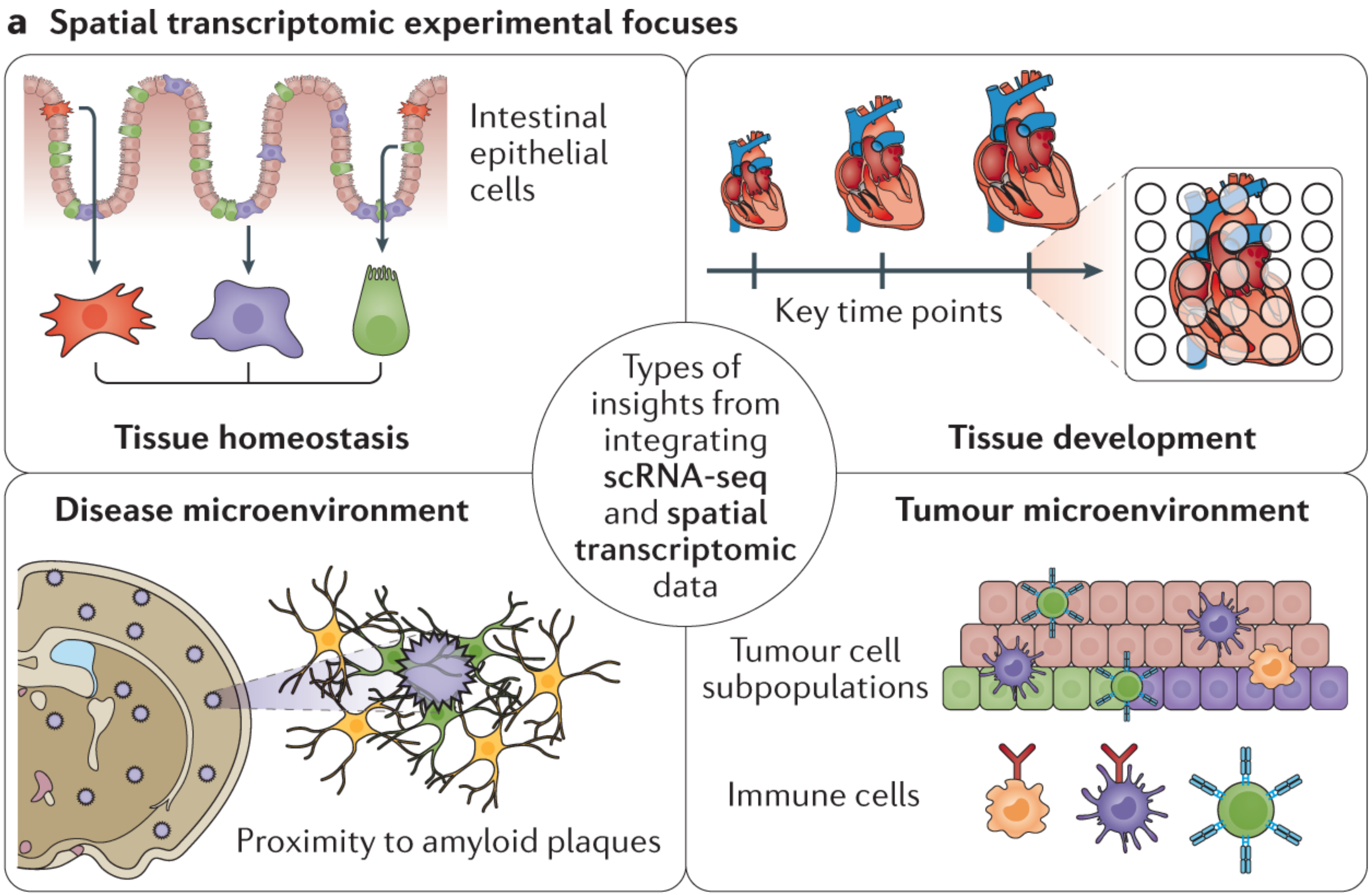


Fig 1: Skin H&E. Note the balanced coloration in this section of skin. The nuclei are stained purple, while the cytoplasmic components are pink.

https://www.leicabiosystems.com/us/knowledge-pathway/he-staining-overview-a-guide-to-best-practices/

Why spatial transcriptomics?

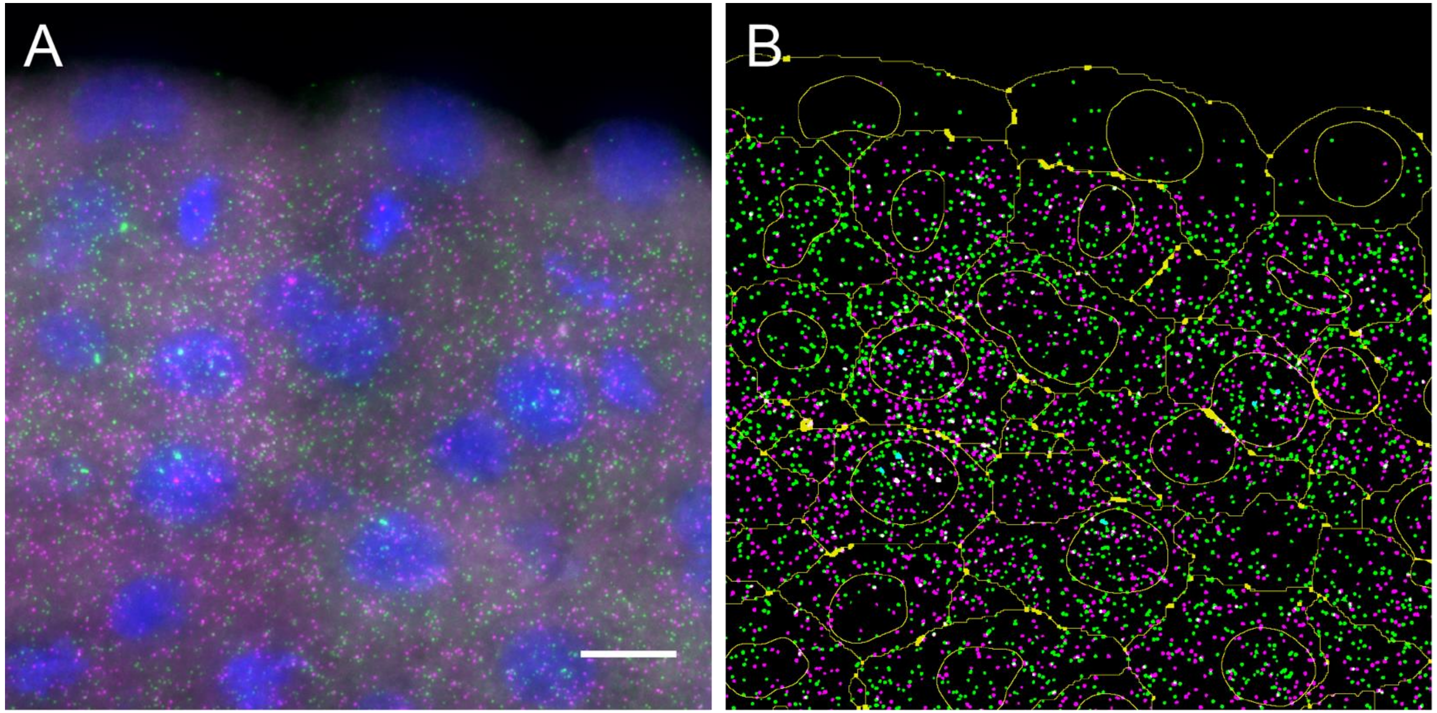


a Spatial transcriptomic experimental focuses
Intestinal epithelial cells
Tissue homeostasis
Key time points
Tissue development
Types of insights from integrating scRNA-seq and spatial transcriptomic data
Disease microenvironment
Proximity to amyloid plaques
Tumour microenvironment
Tumour cell subpopulations
Immune cells

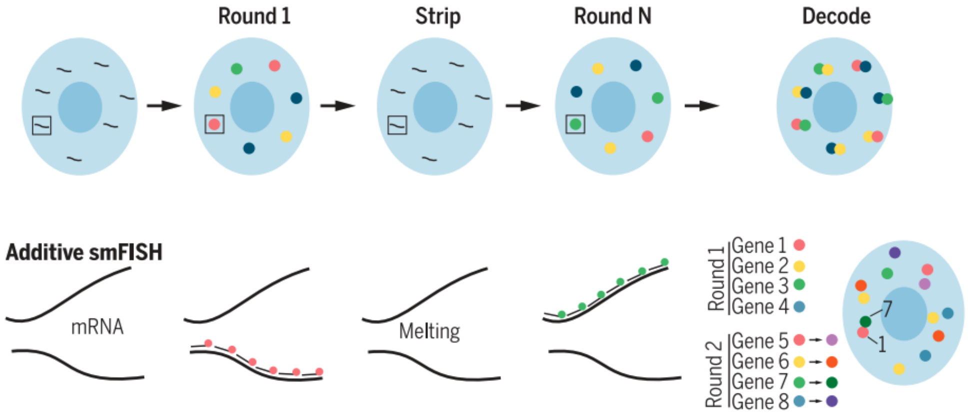
(Longo et. al., Nature Reviews Genetics 2021)

RNA-Fluorescence in situ hybridization (FISH)

- FISH is a technique using fluorescently labeled probe to detect specific DNA/RNA sequence
  - Keep the location of the cells but can only detected a limited number of genes



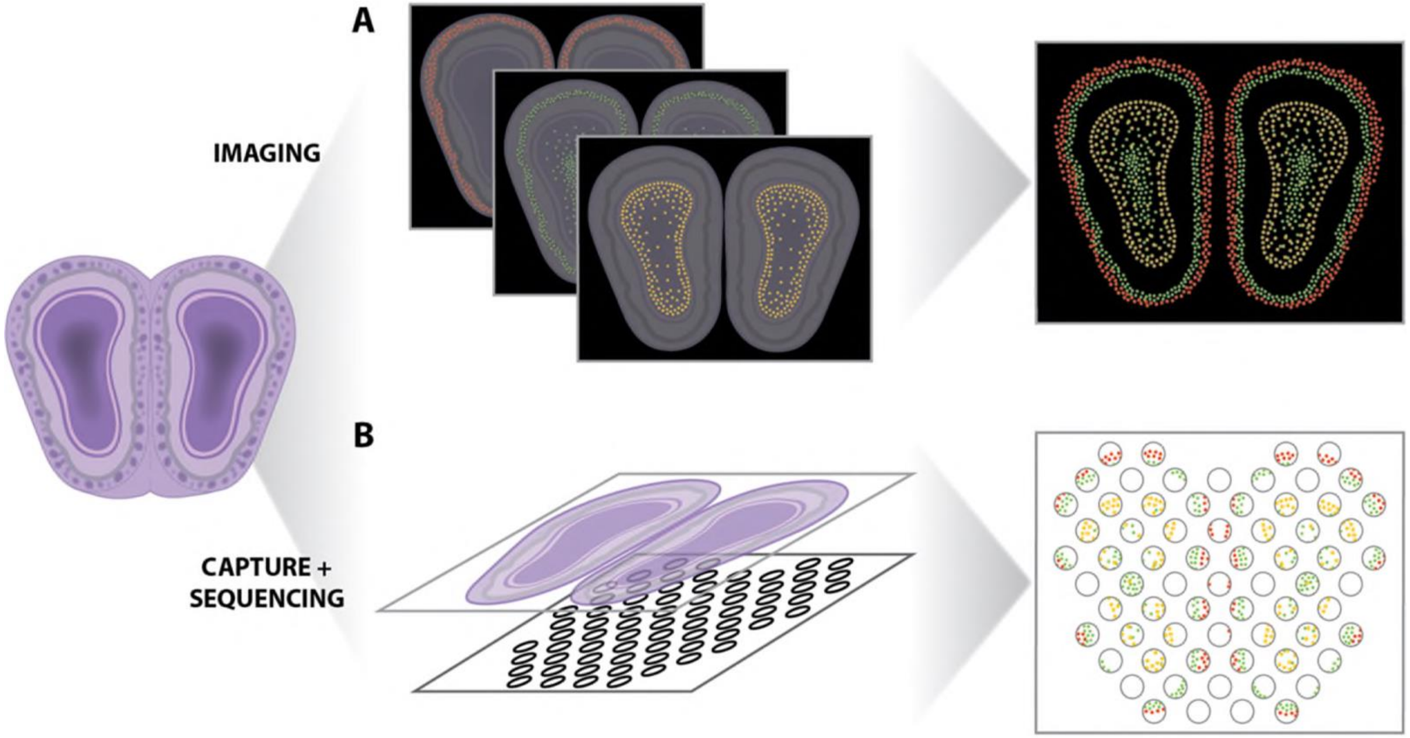
Transcripts detected by smFISH. (**A**) Original smFISH image. Blue: nuclei, magenta: smFISH for *apoeb*, green: smFISH for *aldob*. (**B**) Results of the smFISH analysis pipeline when applied to the image shown in (A). Yellow: outlines of cells and nuclei, magenta: detected *apoeb* transcripts, white: detected transcription foci for *apoeb*, green: detected *aldob* transcripts, cyan: detected transcription foci for *aldob*. Scale bar: 10 µm.



https://thenode.biologists.com/fishing-fish-2/resources/

Two types of spatial transcriptomics technologies

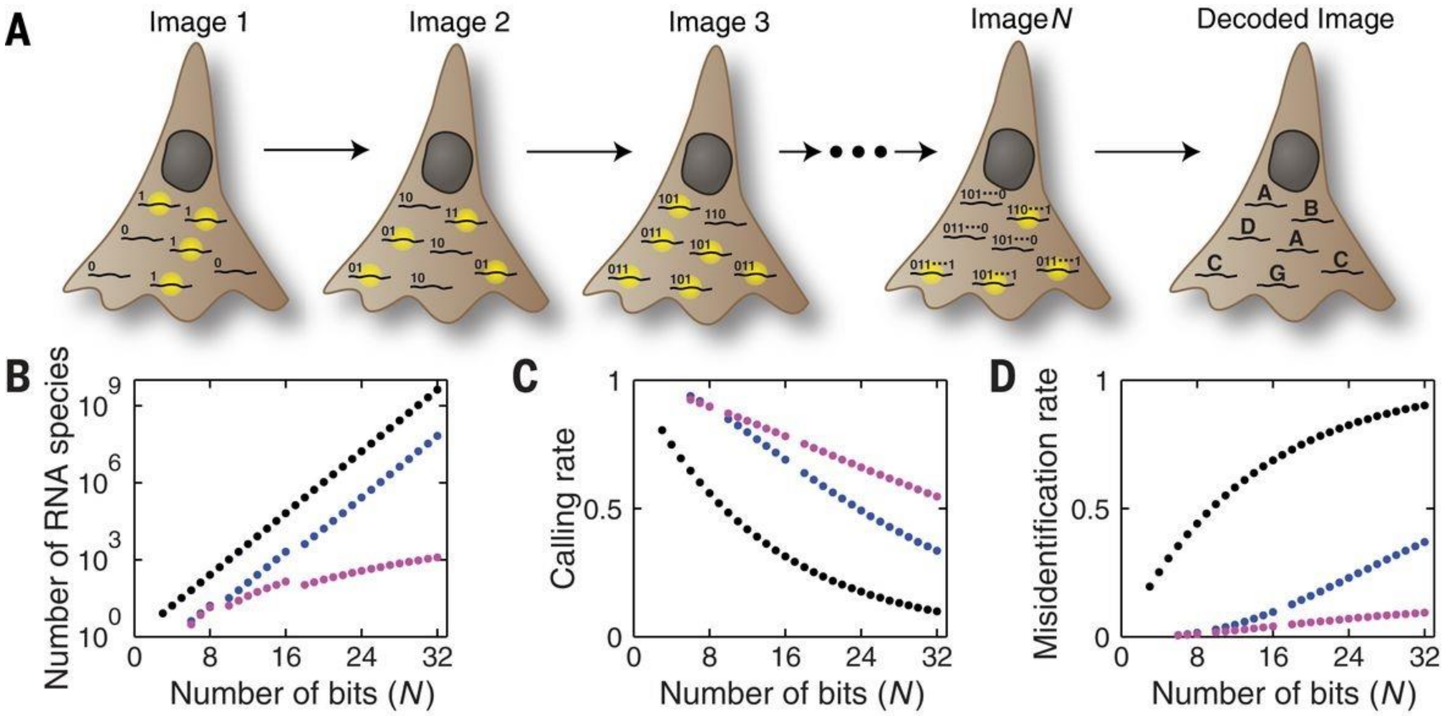
- Sequencing based spatial transcriptomics
  - Use scRNA-seq techniques to measure transcriptomics profiles for each spatial spot
- Image-based spatial transcriptomics
  - Use FISH techniques, increase the number of genes detected to a few hundreds



(Atta and Fan, Nature Comm, 2021)

MERFISH (Chen et. al., Science 2015)

- Multiplex error-robust FISH that can measure 100-1000 genes
- smFISH: K round→ measure K gene
- Combinatorial barcoding of the genes: K round → measure $2^K - 1$ genes at most
  - Problem: calling rate also has an exponential decay (black dots)
    - Assume 1 -> 0 error p_1, 0 -> 1 error p_2, the code has m 1s, recall rate will be $(1-p_1)^m(1-p_2)^{K-m}$

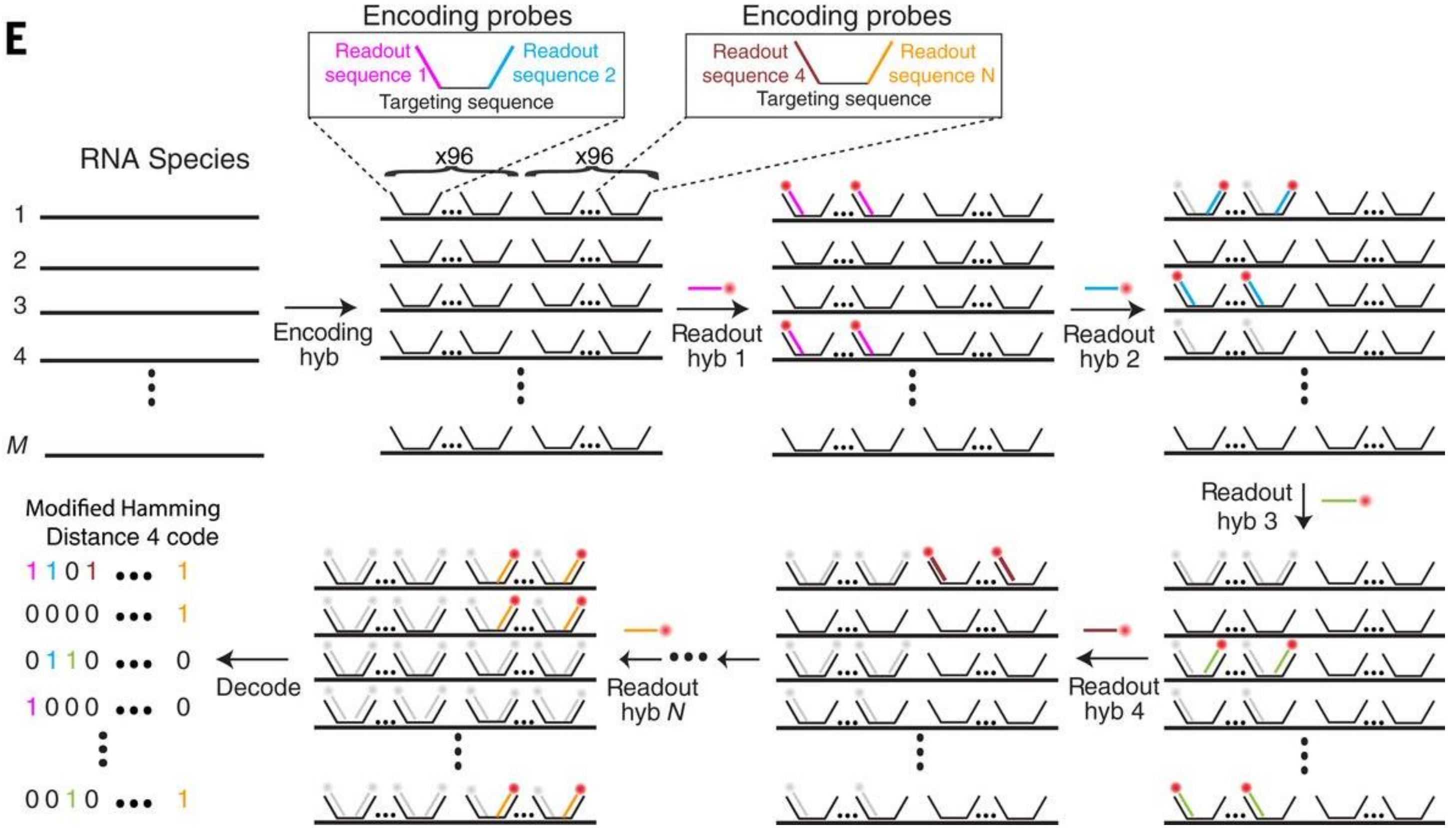


Black: simple encoding
Blue: HD at least 4
Purple: HD at least 4 + exactly 4 1s

MERFISH (Chen et. al., Science 2015)

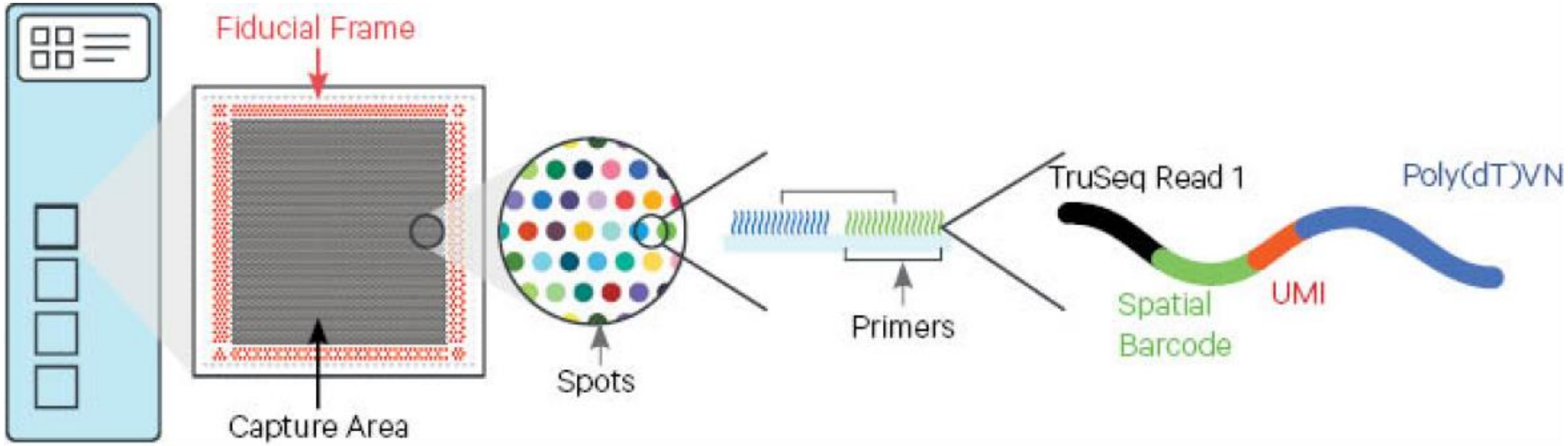
Solution: error-robust coding

- Encode each gene so that the barcode Hamming distance is at least 4
- Each gene barcode has exactly 4 1s to increase recall rate (as $p_1 > p_2$)

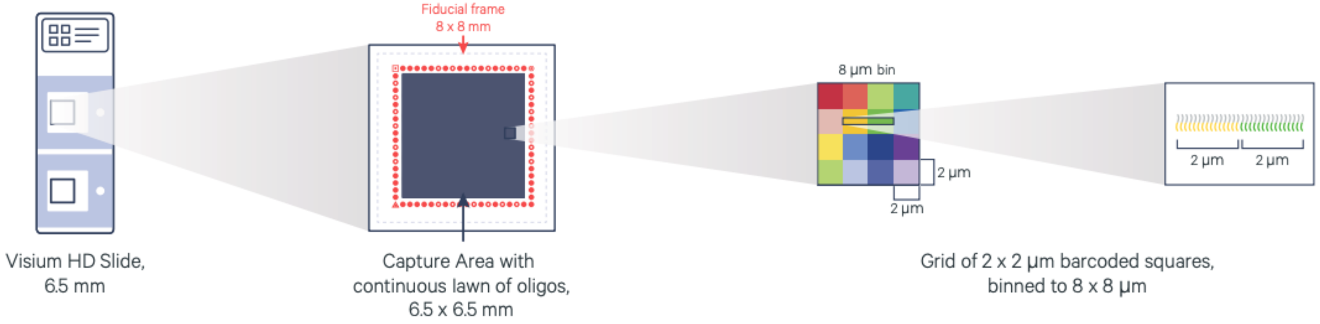


10X Visium

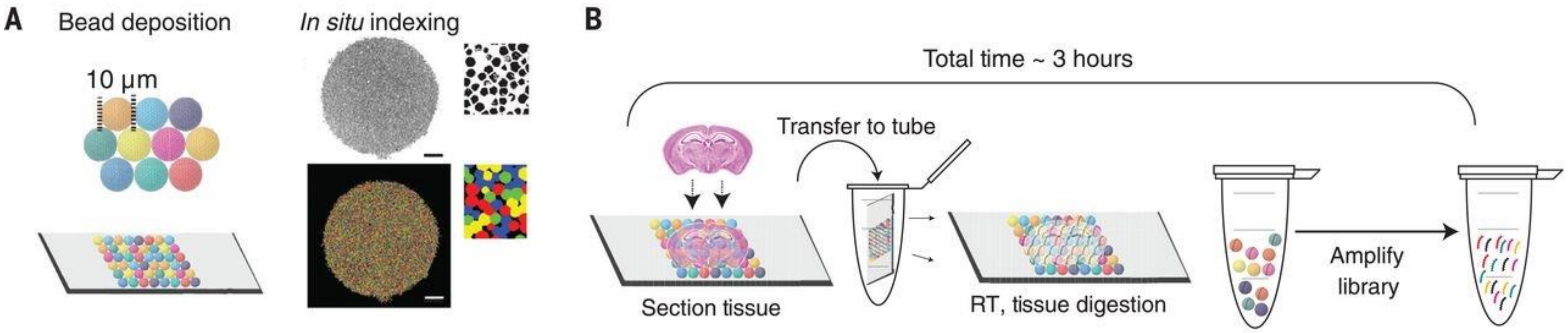
- Resolution: 55 µm spot (Stahl et. al., Science 2016)
- Typical human cell dimension: 10-15 µm in diameters, depend on the cell type



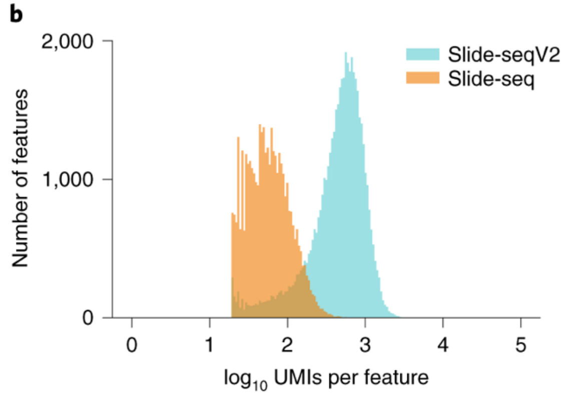
- Visium HD: 3 µm resolution, binned to 8 * 8 µm bins as a starting point
  - Much more expensive

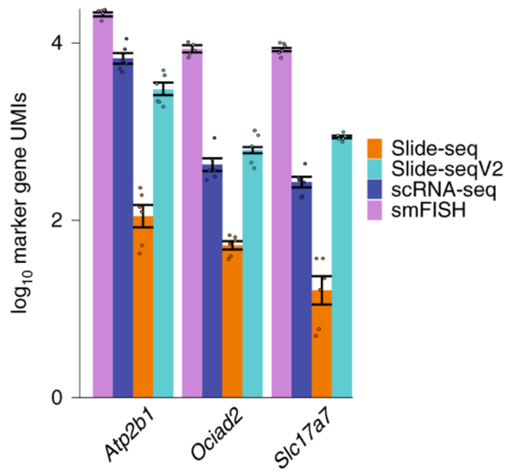


Slide-seq (Rodrigques et. al. Science 2019) & Slide-seqV2 (Stickels et. al., Nature Biotech 2021)

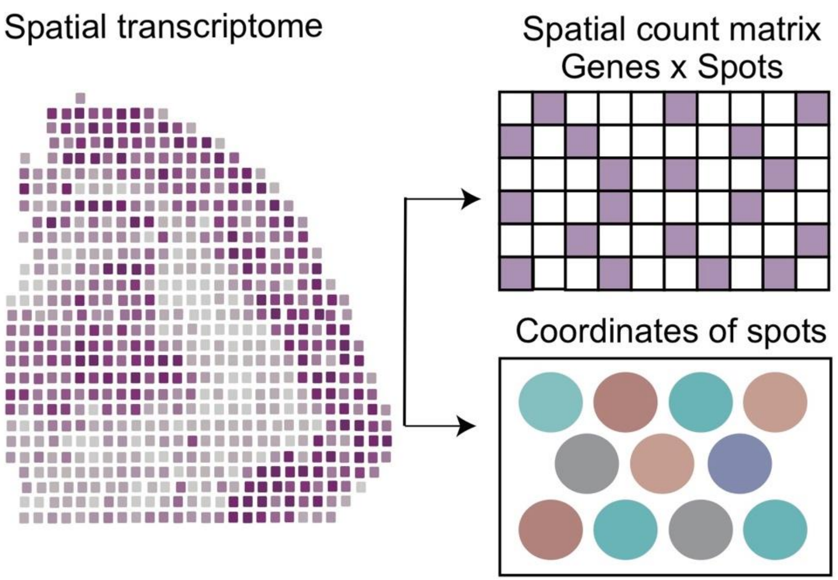


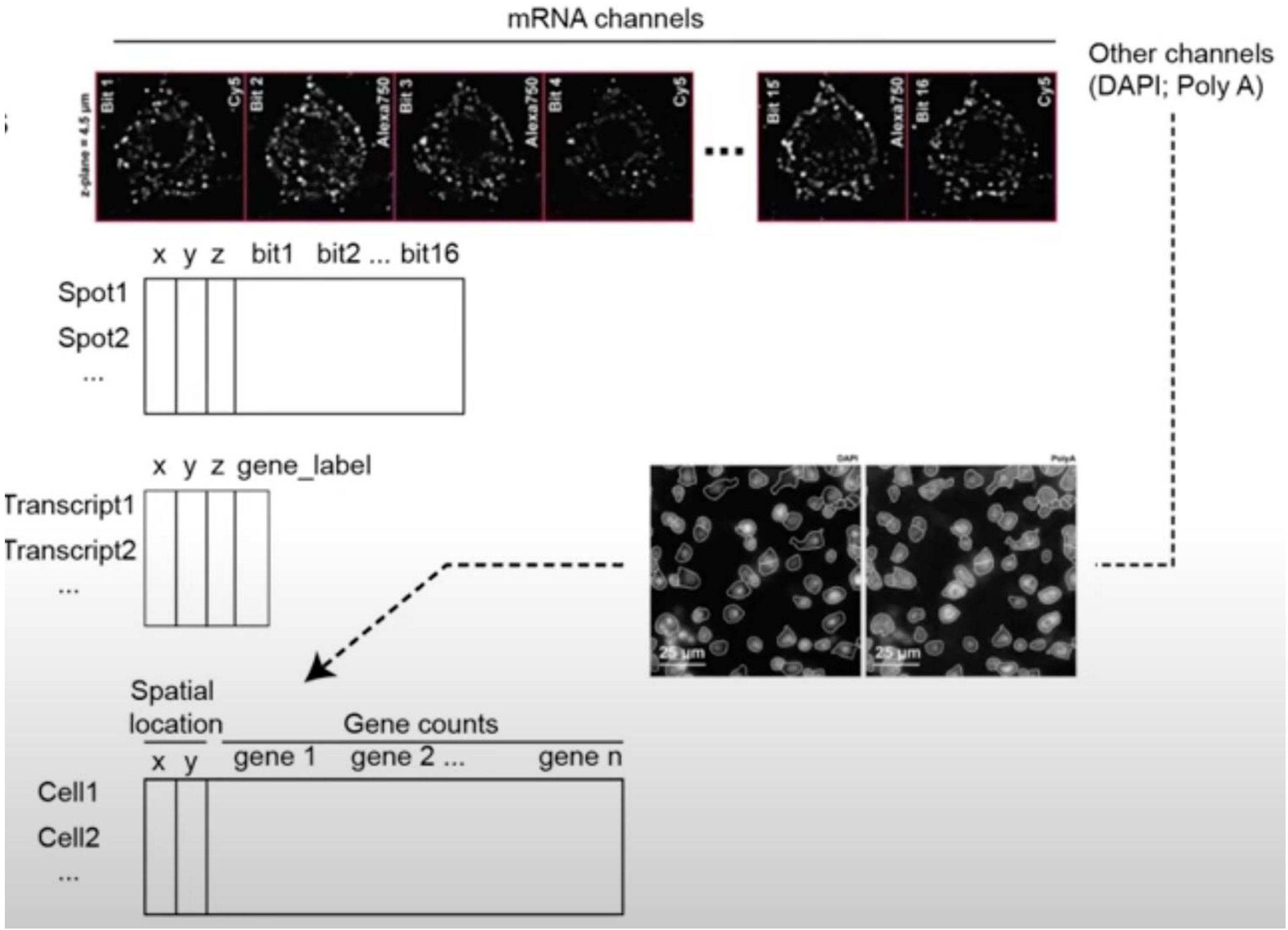
- Slide-seqV2 keeps the 10 µm resolution but has much higher mRNA capture efficiency





Data from spatial transcriptomics





https://qcb.ucla.edu/collaboratory/workshops/w31-spatial-transcriptomics/

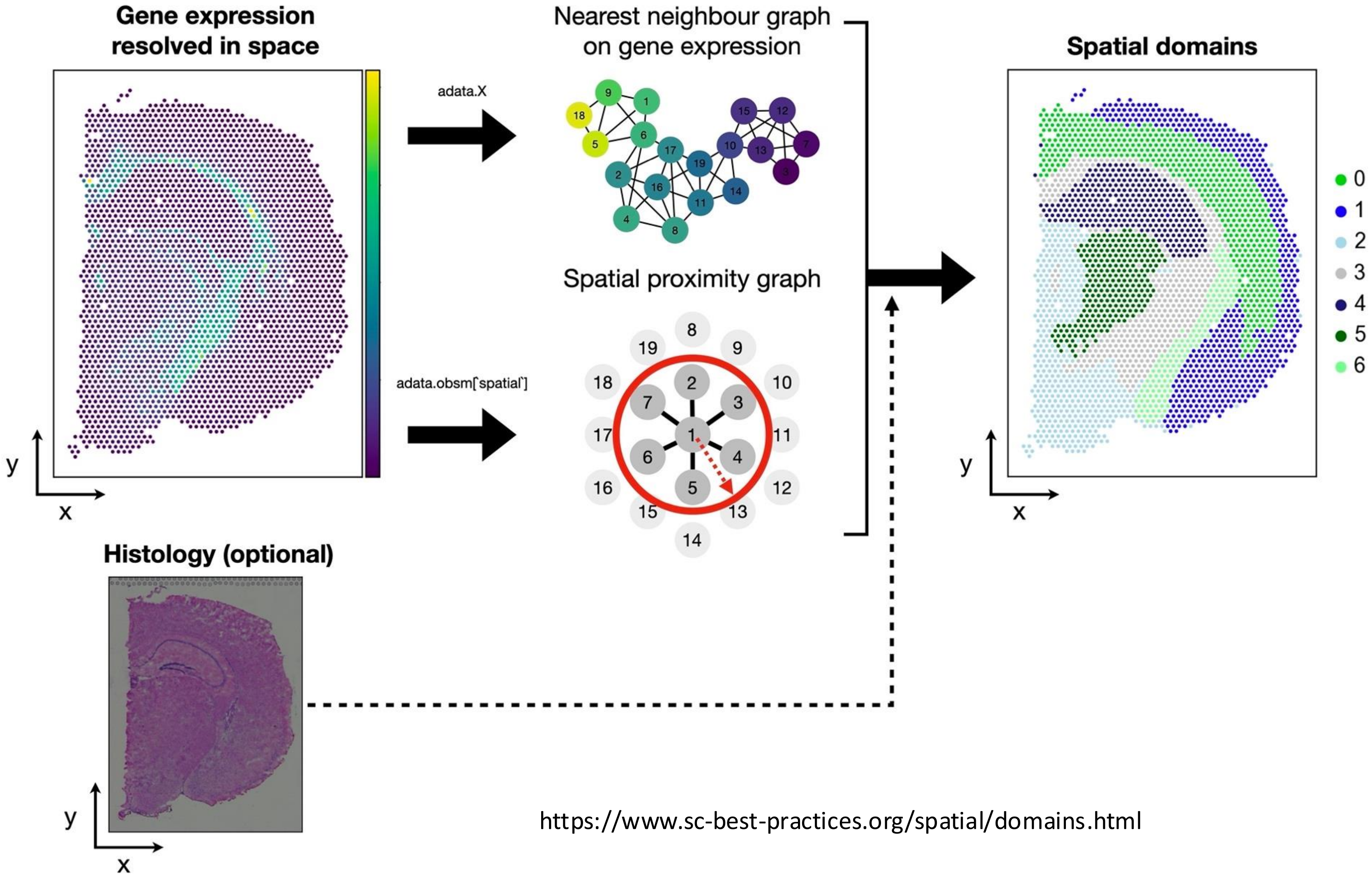
Common types of downstream analyses:

- Spatial domain detection
- Deconvolution and cell type annotation
- Imputation with external data
- Finding spatially variable genes
- Understand cell-cell interactions

- Detecting cell boundaries can be a challenge
  (Prabhakaran, Bioinformatics advances, 2022)

Spatial domain detection

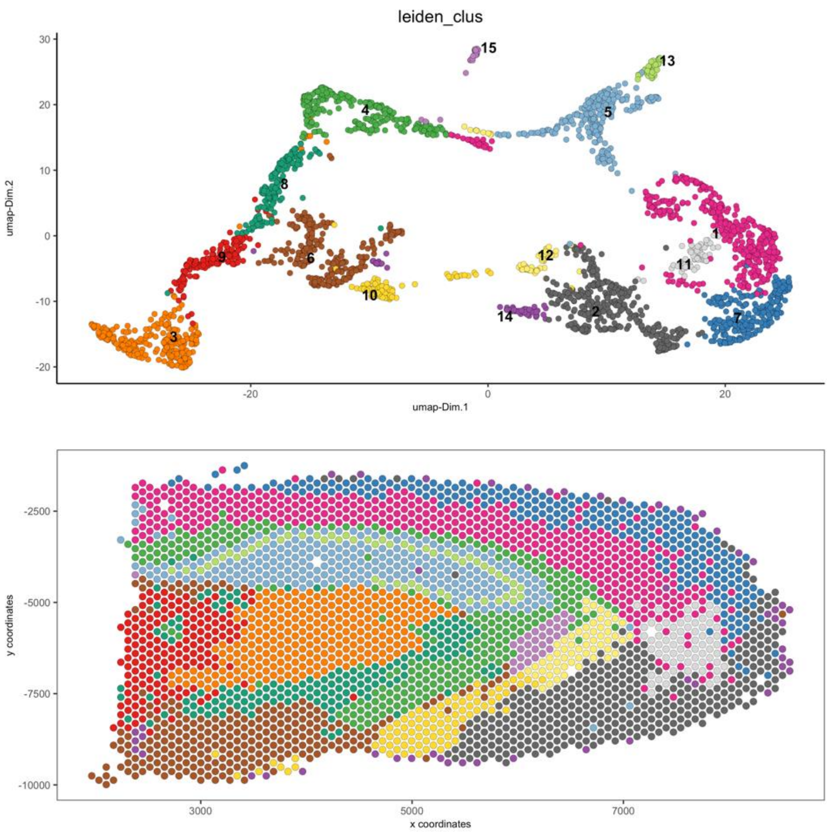
- How to perform clustering of the cells/spots taking spatial coordinates into consideration?



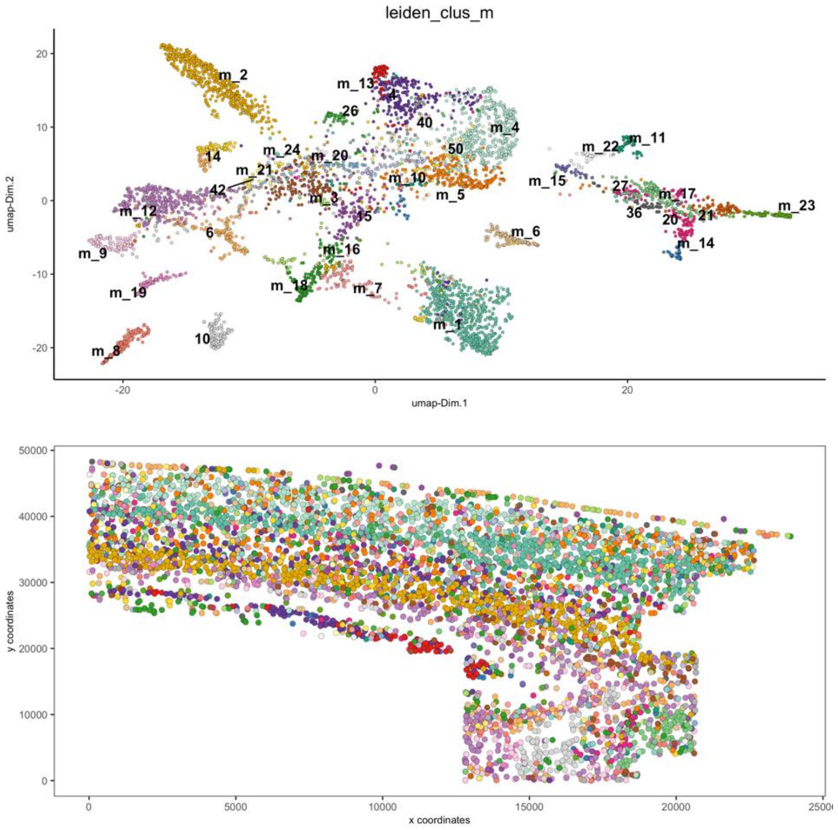
https://www.sc-best-practices.org/spatial/domains.html

Giotto (Dries et. al., Genome Biology, 2021)

- Spatial domain detection in Giotto uses hidden-Markov random field (HMRF)
- Clustering without using spatial information seems not too bad



Visium Brain data



osmFISH mouse SS cortex data

Giotto (Dries et. al., Genome Biology, 2021)

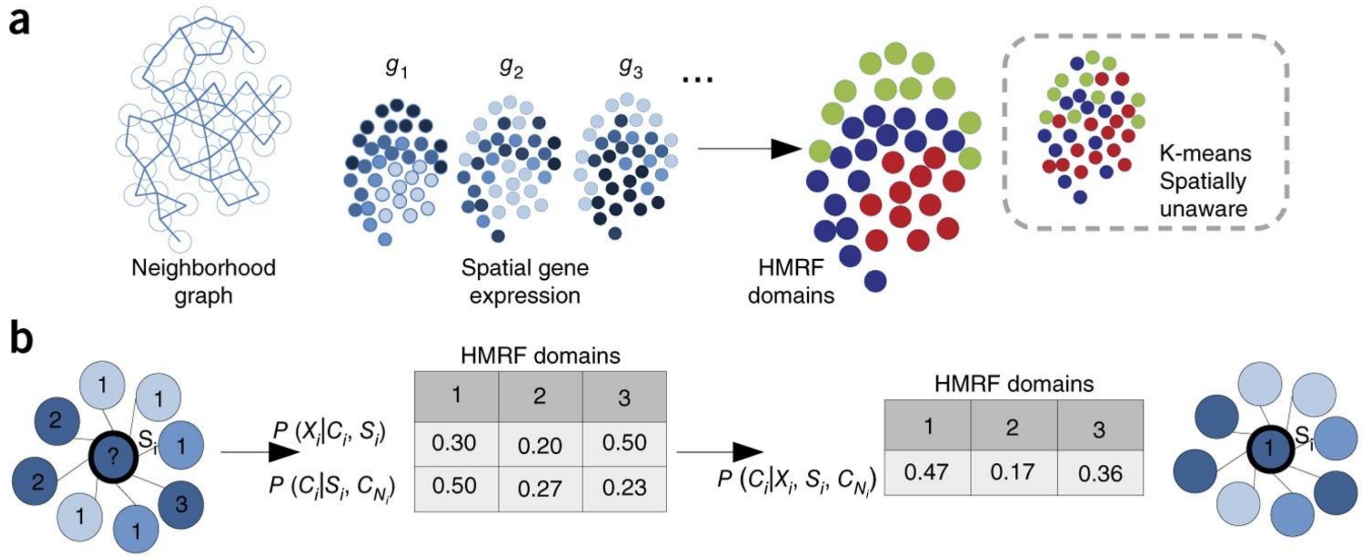
- hidden-Markov random field (HMRF) -> two-dimensional hidden Markov model
- Key assumptions (Zhu et. al., Nature Biotech, 2018):
  - For a spot/cell i, gene expressions given the hidden state c_i are independent across i

$$p(y \mid x, \theta) = \prod_{i \in \mathcal{S}} \mathcal{N}(y_i | x_i = k, \mu_k, \Sigma_k)$$

  - The hidden state c_i depends on hidden states of spatially nearby points (Potts model)

$$P(x; \beta) = \frac{1}{Z_\beta} \exp\{-U(x)\} \qquad U(x) = \sum_{i,i' \in \mathcal{N}_i} \beta[1 - \delta(x_i, x_i')]$$

  - Assign spatial domains / clusters based on the posterior probability of the hidden states



HMRF domains

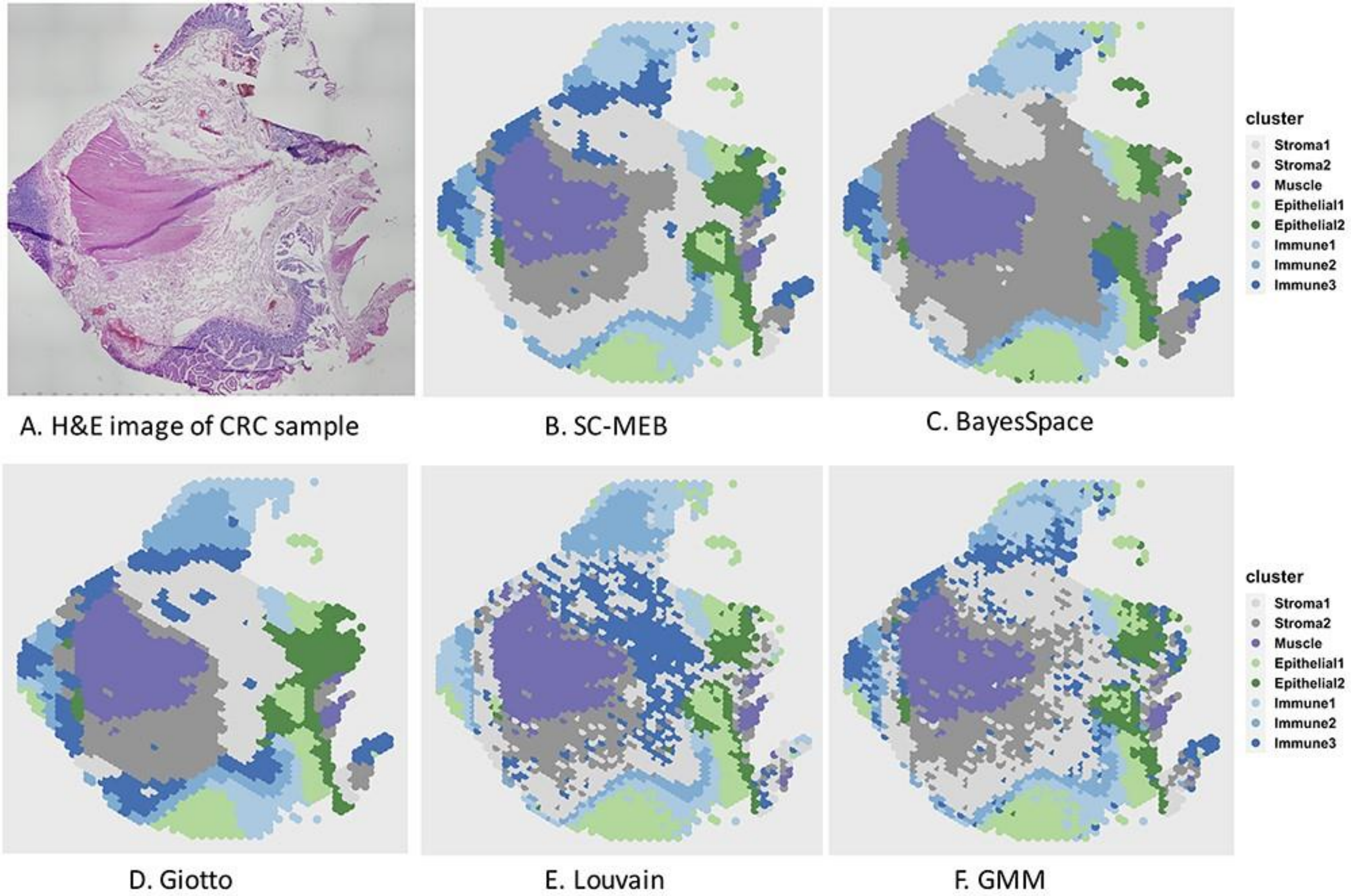
1	2	3
0.30	0.20	0.50
0.50	0.27	0.23

HMRF domains

1	2	3
0.47	0.17	0.36

Giotto (Dries et. al., Genome Biology, 2021)

- Performance of HMRF models (SC-MEB, Yang et. al. Briefings in Bioinformatics 2021)



A. H&E image of CRC sample

B. SC-MEB

C. BayesSpace

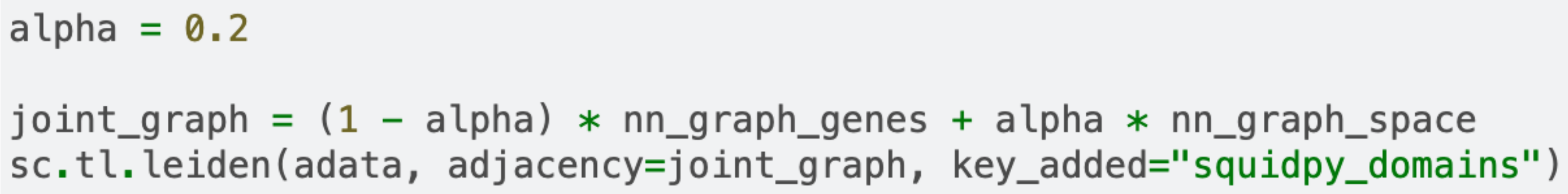
D. Giotto

E. Louvain

F. GMM

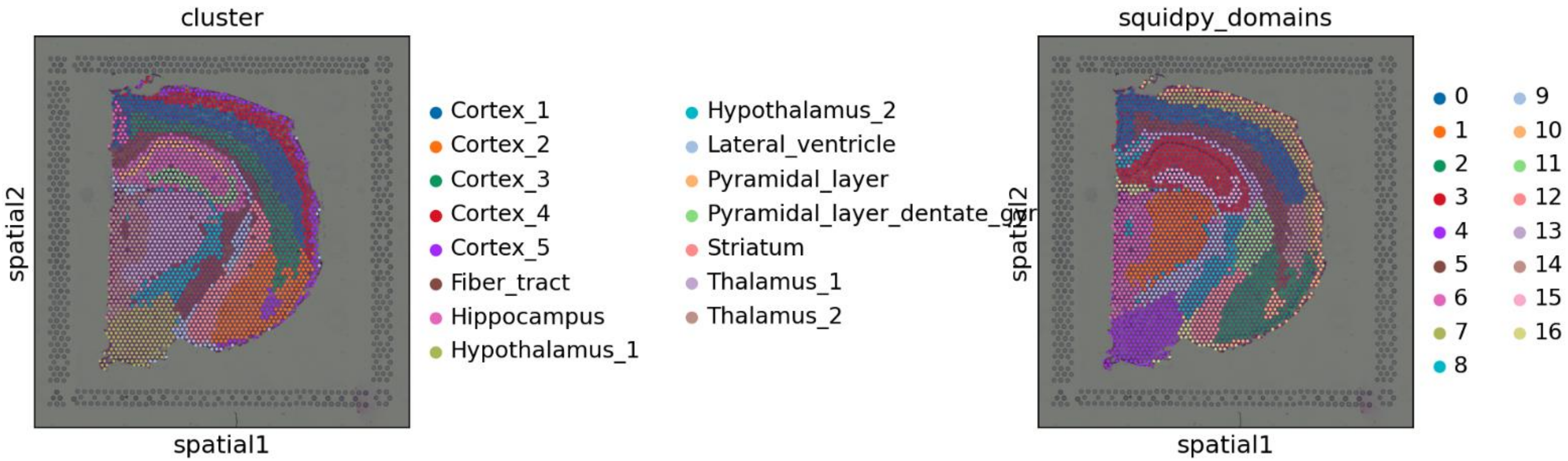
A simple weighted graph method

- Illustrated using Squidpy (Palla et. al., Nature Methods 2022): https://www.sc-best-practices.org/spatial/domains.html#id555
- Idea: spatial smoothing in clustering
  - Compute cell-cell connectivity graph using both graphs:
    - Nearest neighbor graph based on gene expression PCA
    - Nearest neighbor graph based on spatial coordinates
    - Weighted average to create a new graph for clustering



```
alpha = 0.2

joint_graph = (1 - alpha) * nn_graph_genes + alpha * nn_graph_space
sc.tl.leiden(adata, adjacency=joint_graph, key_added="squidpy_domains")
```



SpaGCN (Hu et. al., Nature Methods, 2021)

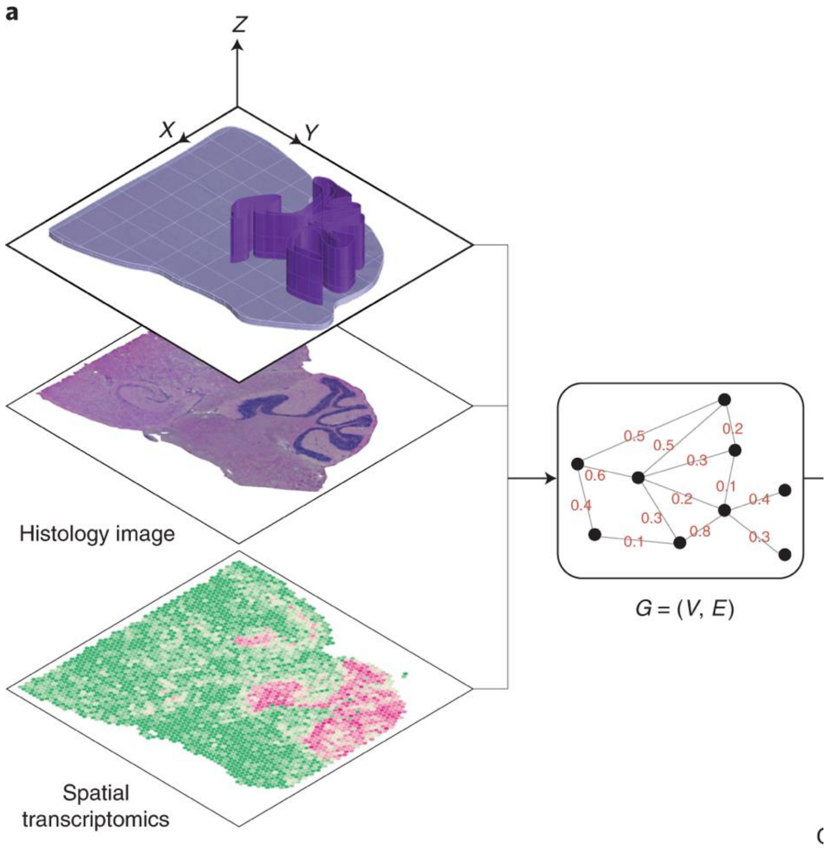
- Use both histology image and spatial locations to build connectivity graph between two spots
  - Convert histology RGB values to a single value and treat it as a 3rd dimension when calculating cell-cell distances

Higher weights given to channel with larger variances

$$z_v = \frac{r_v \times V_r + g_v \times V_g + b_v \times V_b}{V_r + V_g + V_b}$$

$$z_v^* = \frac{z_v - \mu_z}{\sigma_z} \times \max\left(\sigma_x, \sigma_y\right) \times s$$

$$d\left(u, v\right) = \sqrt{\left(x_u - x_v\right)^2 + \left(y_u - y_v\right)^2 + \left(z_u^* - z_v^*\right)^2}$$



- Compute cell-cell similarity matrix A
  - Edge weights

$$w\left(u, v\right) = \exp\left(-\frac{d(u, v)^2}{2l^2}\right)$$

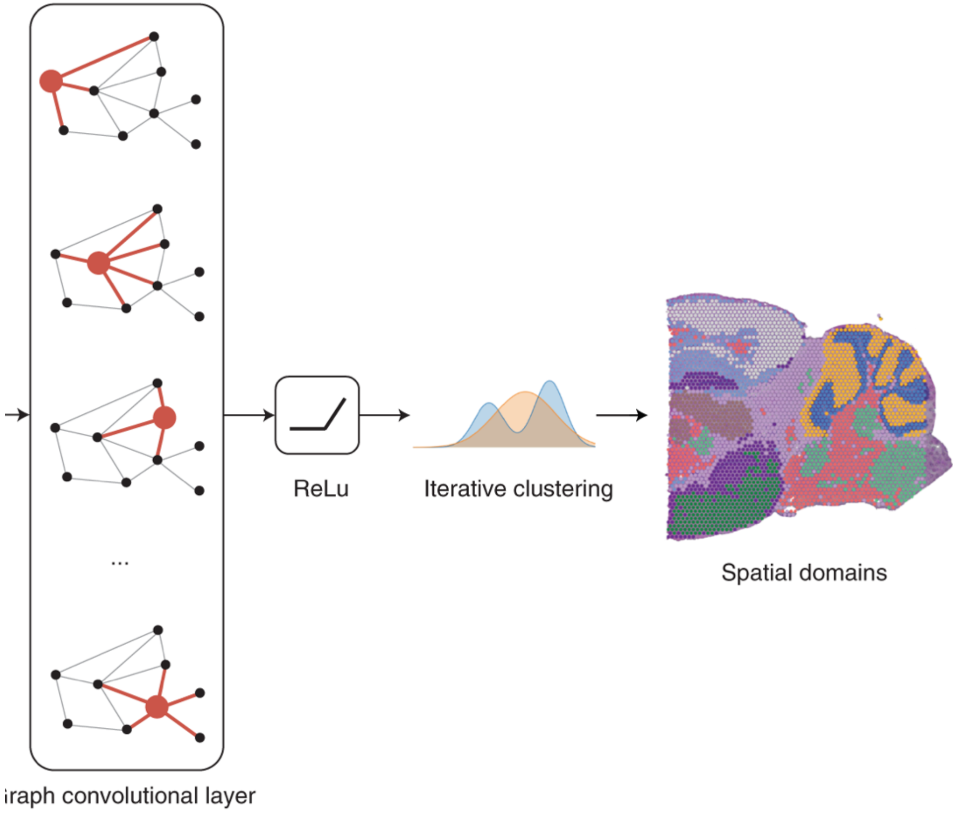
SpaGCN (Hu et. al., Nature Methods, 2021)

- Use graph convolutional layer to perform smoothing
    - Use the top PCs as input X
    - Graph convolutional later:

$$f\left(X,\,A\right)=\delta\left(AXB\right)$$

- Loss function: measuring the clustering performance
    - Perform Louvain clustering on based on the output of the graph convolutional layer
    - Calculate "assignment probability" assuming t-distributions

$$q_{ij}=\frac{\left(1+h_i-\mu_j^2\right)^{-1}}{\sum_{j'=1}^{K}\left(1+h_i-\mu_{j'}^2\right)^{-1}}$$



- Minimize the loss to encourage q_{ij} to be close to 0 or 1

$$L=\mathrm{KL}(P||Q)=\sum_{i=1}^{N}\sum_{j=1}^{K}p_{ij}\log\frac{p_{ij}}{q_{ij}}$$

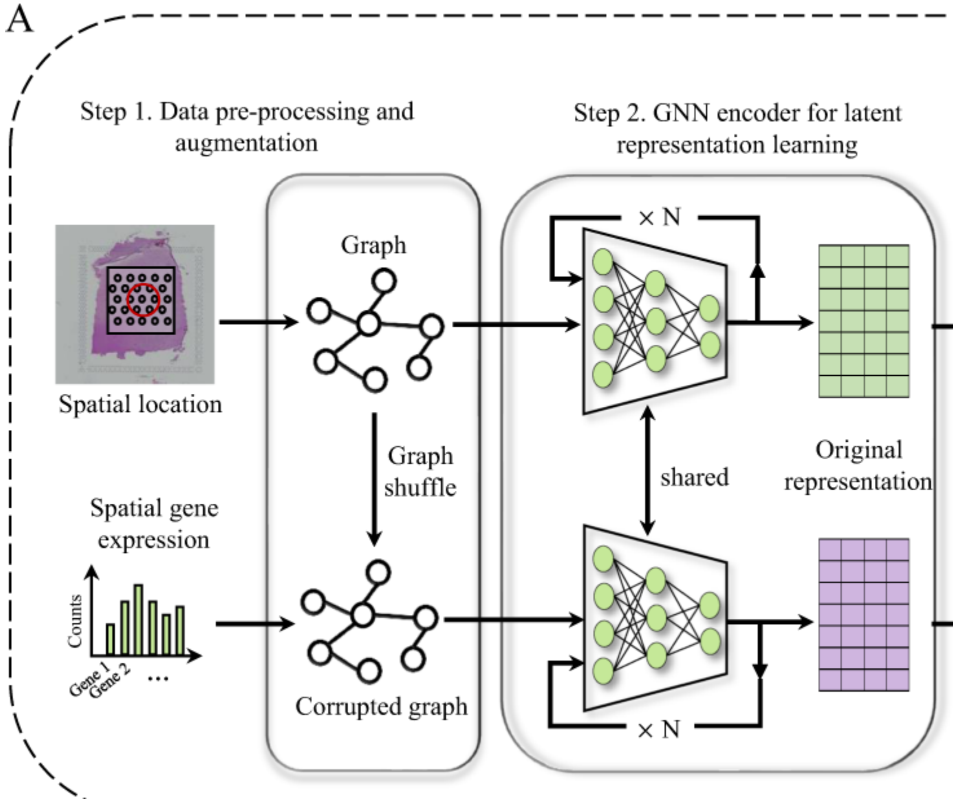
$$p_{ij}=\frac{q_{ij}^2/\sum_{i=1}^{N}q_{ij}}{\sum_{j'=1}^{K}\left(q_{ij'}^2/\sum_{i=1}^{N}q_{ij'}\right)}$$

GraphST (Long et. al. Nature Comm, 2023)

- Main idea: GNN + self-supervised contrastive learning
  - Build KNN graph using spatial locations and obtain adjacency matrix A
  - Use graph convolutional network to build the encoder

$$Z_s^l = \sigma\left(\widetilde{A} Z_s^{l-1} W_e^{l-1} + b_e^{l-1}\right)$$

$$\widetilde{A} = D^{-\frac{1}{2}} A D^{-\frac{1}{2}}$$



  - Data augmentation
    - Permute spot locations to create a new dataset
    - Keep the original graph unchanged
  - Minimize self-reconstruction loss

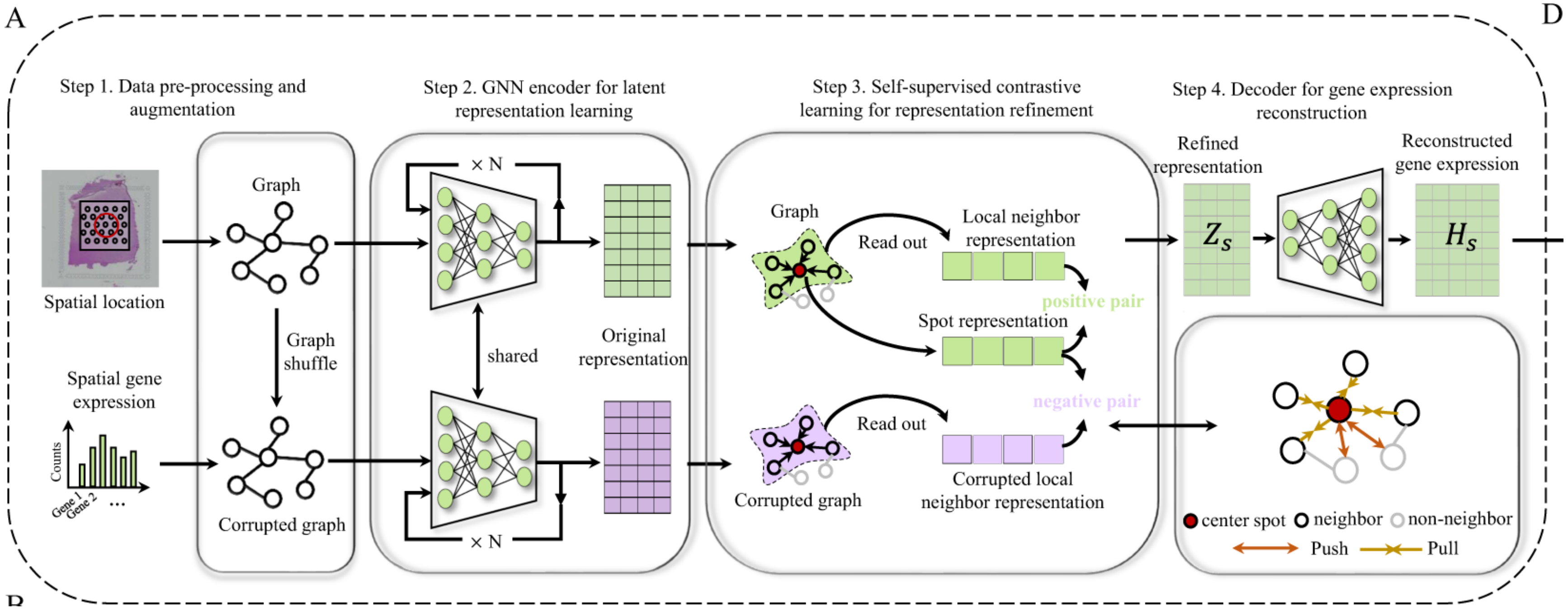
$$\mathscr{L}_{recon} = \sum_{i=1}^{N_{spot}} ||x_i - h_i||_F^2$$

  - Final loss (see next page)

$$\mathscr{L} = \lambda_1 \mathscr{L}_{recon} + \lambda_2 \left(\mathscr{L}_{SCL} + \mathscr{L}_{SCL\_corrupt}\right)$$

GraphST (Long et. al. Nature Comm, 2023)

- Contrastive learning:
  - Motivated by Deep Graph Infomax (Velickovic et. al., 2019, ICLR poster)
  - Make positive pairs more similar to each other and contrast negative pairs
    - Local context vector: some average of a cell's immediate neighbors
    - Positive pairs: the true expression a cell and its local context vector
    - Negative pairs: the corrupted (permuted) expression and its local context vector

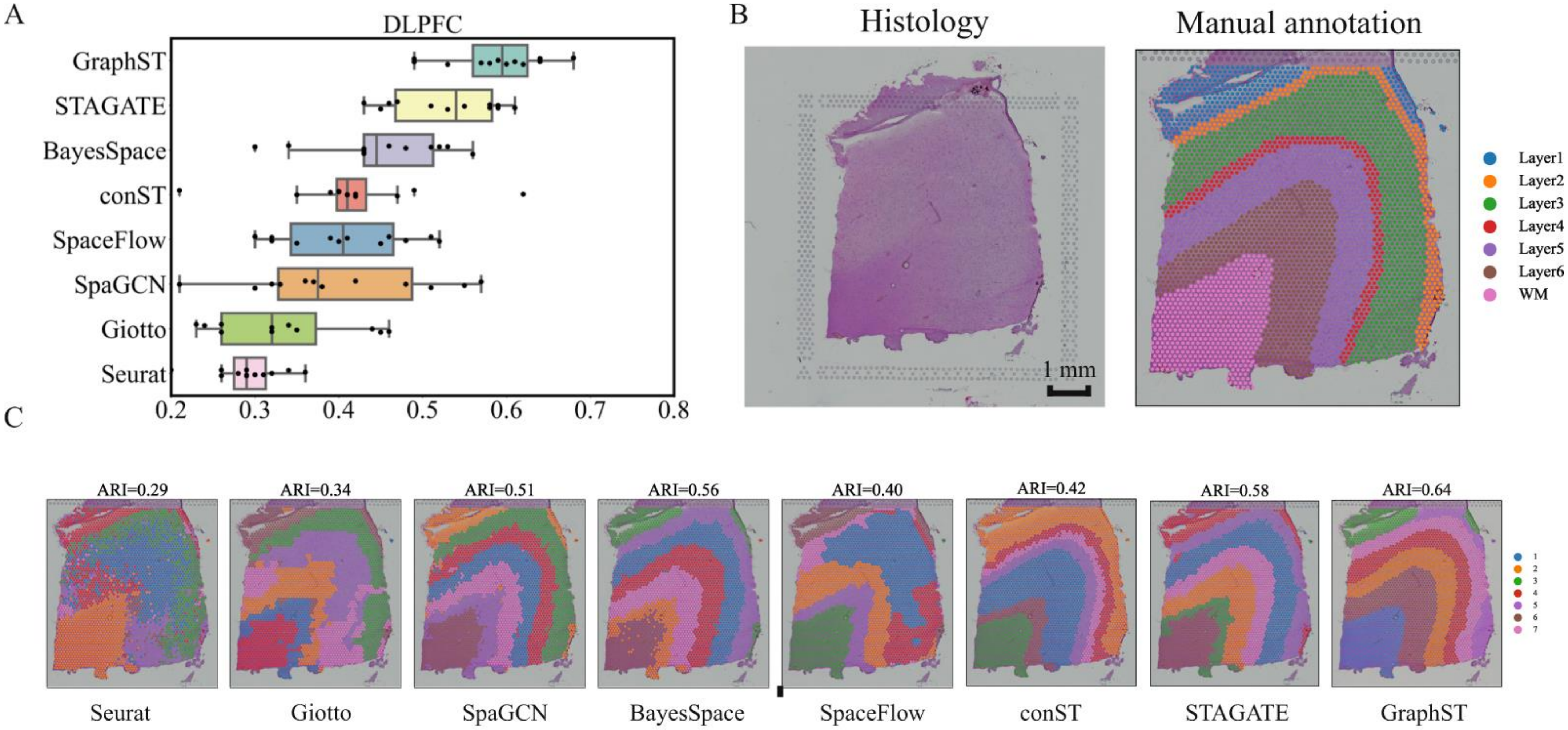

A
D
Step 1. Data pre-processing and augmentation
Spatial location
Graph
Graph shuffle
Spatial gene expression
Counts
Gene 1
Gene 2
...
Corrupted graph
Step 2. GNN encoder for latent representation learning
× N
shared
Original representation
× N
Step 3. Self-supervised contrastive learning for representation refinement
Graph
Read out
Local neighbor representation
positive pair
Spot representation
negative pair
Read out
Corrupted graph
Corrupted local neighbor representation
Step 4. Decoder for gene expression reconstruction
Refined representation
Z_s
Reconstructed gene expression
H_s
center spot
neighbor
non-neighbor
Push
Pull
B

$$\mathscr{L}_{SCL} = -\frac{1}{2N_{spot}}\left(\sum_{i=1}^{N_{spot}}\left(\mathbb{E}_{(X,A)}\left[\log\Phi\left(z_i,\, g_i\right)\right] + \mathbb{E}_{(X',A')}\left[\log\left(1-\Phi\left(z'_i,\, g_i\right)\right)\right]\right)\right)$$

Define a similar loss for the corrupted graph

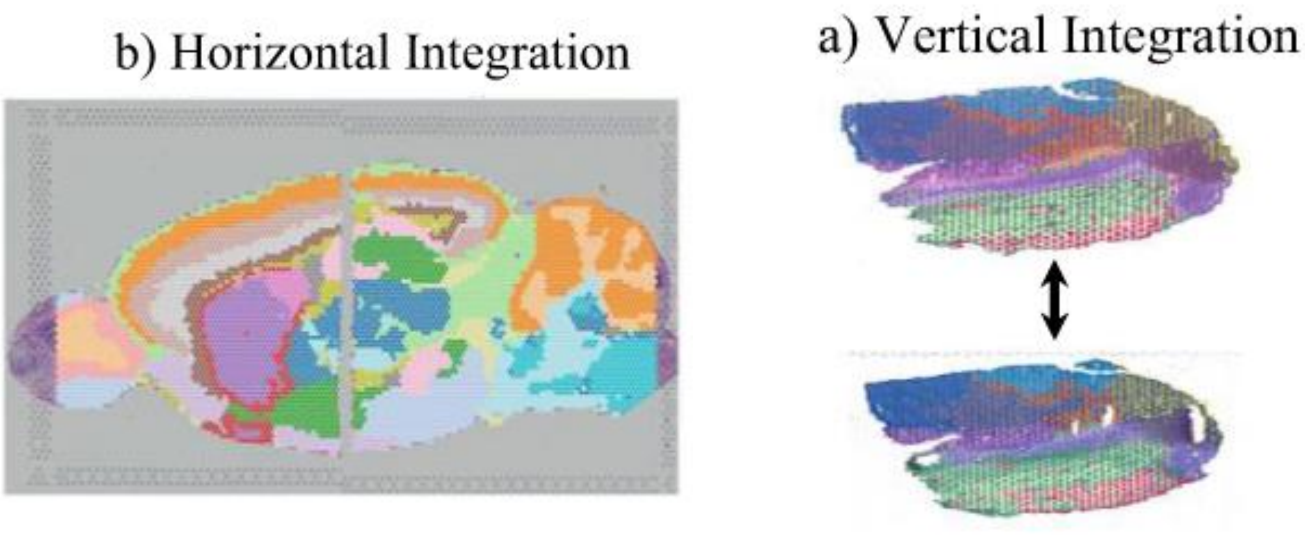
- Perform standard clustering on reconstructed gene expression matrix + surrounding refinement

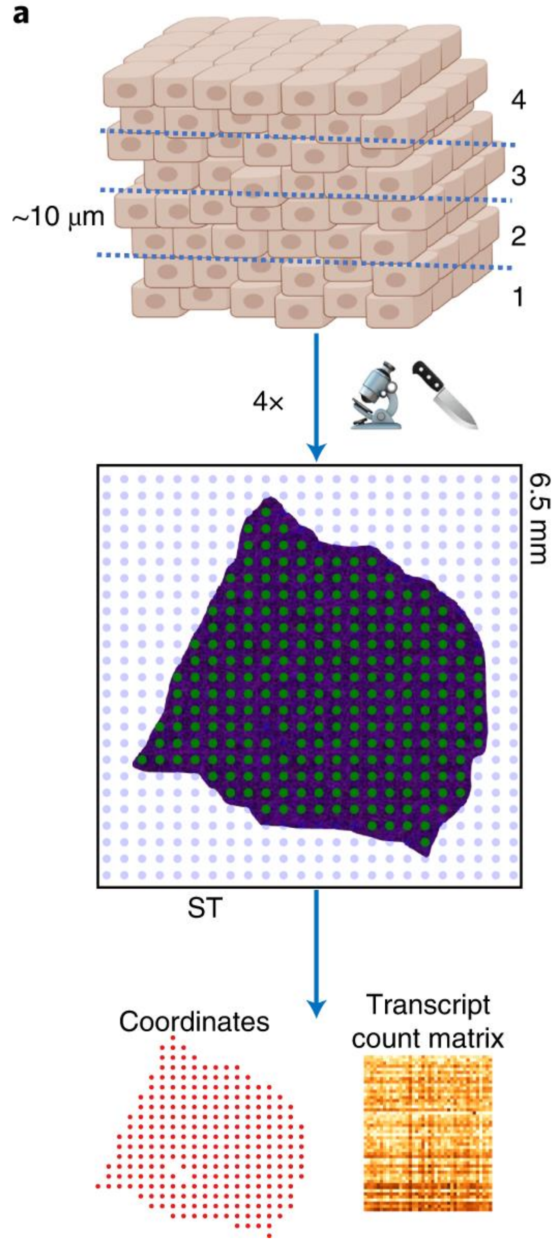
GraphST (Long et. al. Nature Comm, 2023)



Integration of spatial transcriptomics data

- Tissue sample can be dissected into multiple sections
- Serial tissue slices can be used to infer 3D information





- Extract 3D spatial domains

- Challenge: placement and orientation of the tissue on the array can be arbitrary

PASTE (Zeira et. al., Nature Methods 2022)

- Focus: vertical integration using optimal transport
- Pairwise alignment of ST slices
  - Convert spatial coordinate matrix to spatial distance matrix between any two spots on the same slice D
  - Define alignment matrix $\Pi = [\pi_{ij}] \in \mathbb{R}_{+}^{n \times n'}$
  - Minimize the transport cost

$$F(\Pi\,;\, X, D, X', D', c, \alpha) = (1-\alpha) \sum_{i,j} c(x_{\cdot i}, x'_{\cdot j}) \pi_{ij} + \alpha \sum_{i,j,k,l} \left(d_{ik} - d'_{jl}\right)^2 \pi_{ij} \pi_{kl}$$

  - Computational cost using fused Gromov–Wasserstein optimal transport: $O(n^2 n' + nn'^2)$

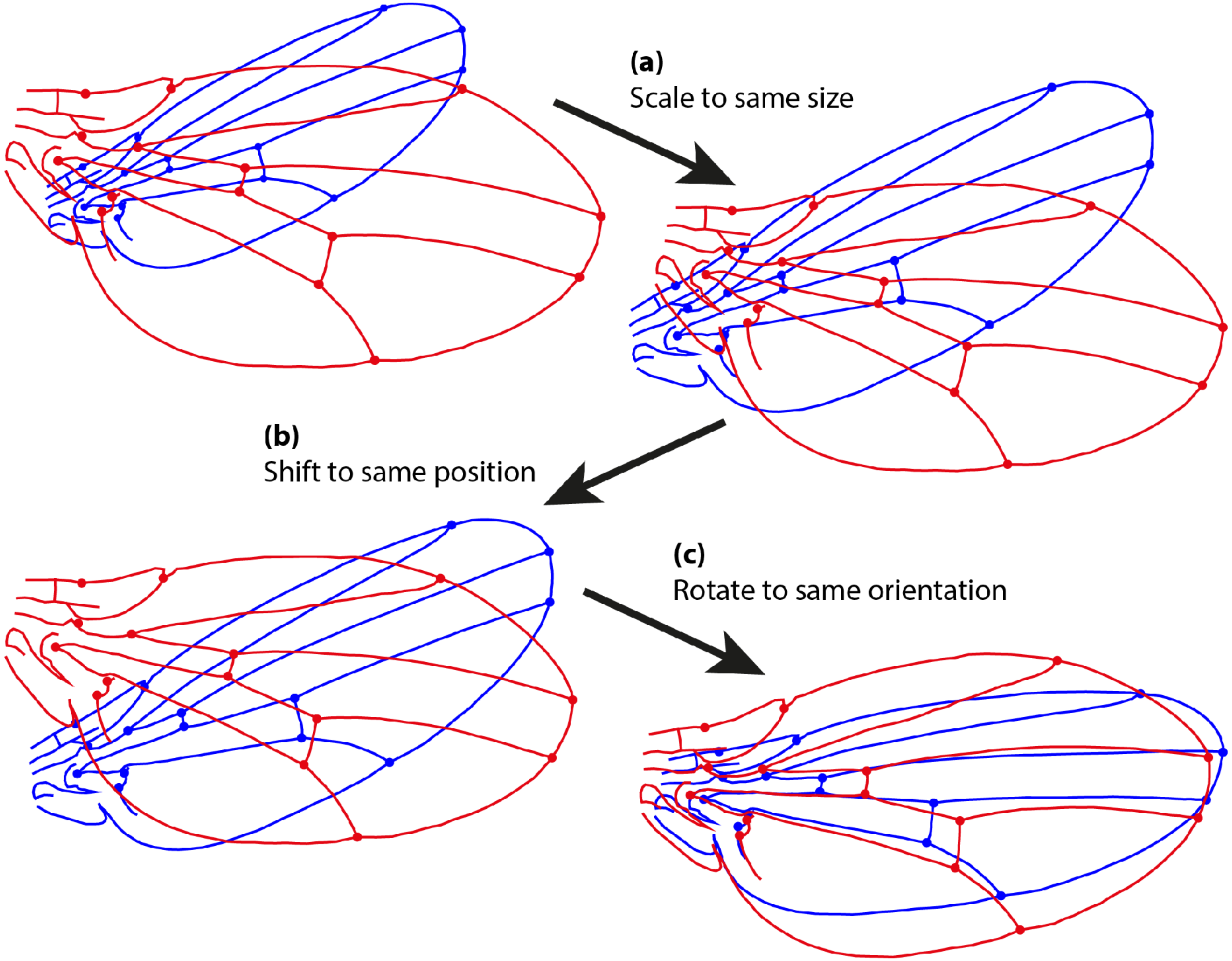
- Reconstruct stacked 3D spatial representation
  - Obtain pairwise alignment matrix between adjacent slices
  - Estimate a rotation matrix and translation vector for each adjacent pair of slices (generalized weighted Procrustes problem)

$$\hat{R}, \hat{v} = \min_{\substack{R \in \mathbb{R}^{2\times 2}, v \in \mathbb{R}^2 \\ R^T R = I}} \sum_{i,j} \pi_{ij}^{(k)} \left|\left| z_{\cdot i}^{(k)} - R z_{\cdot j}^{(k+1)} - v \right|\right|^2$$

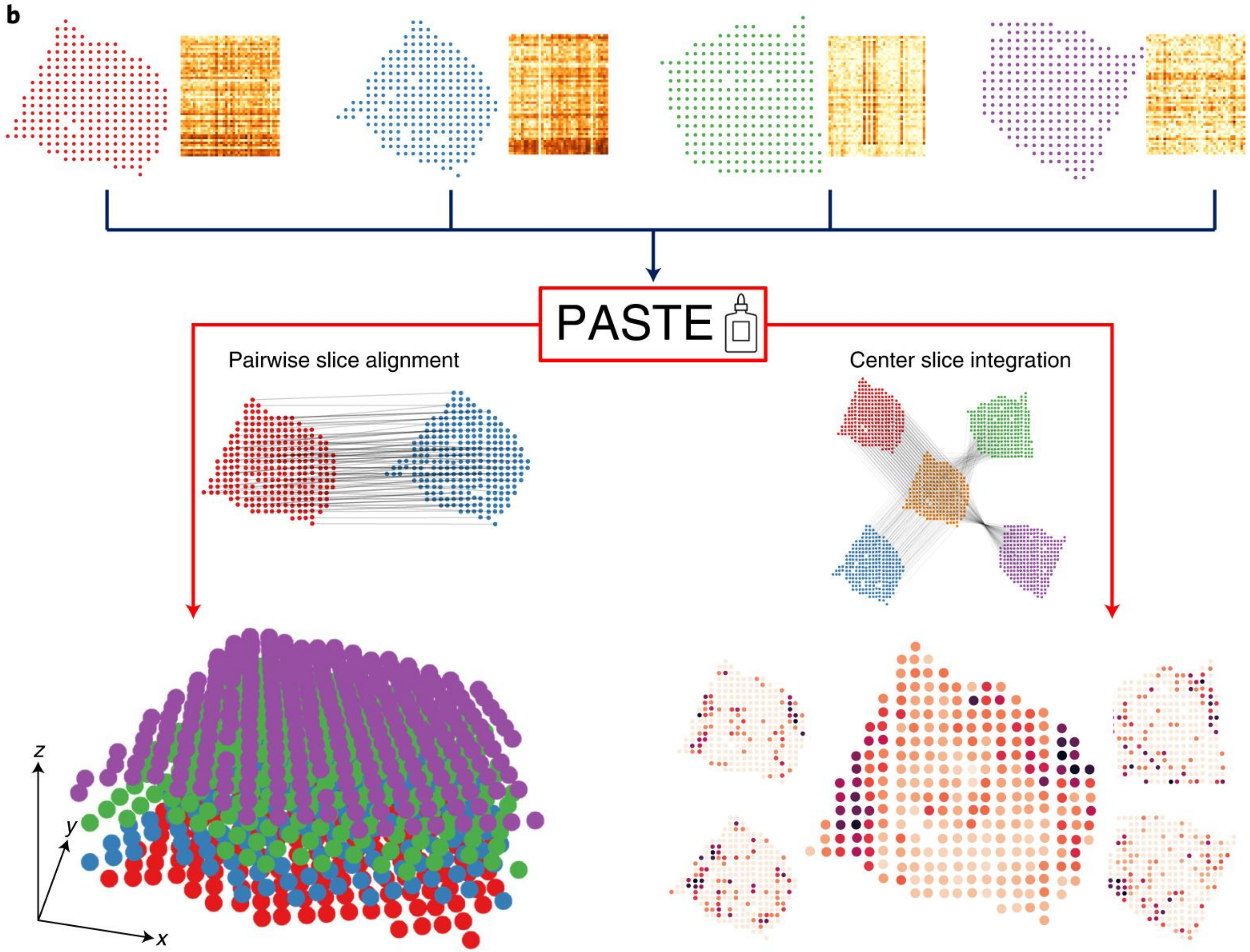
- Construct a center slice to represent all slices if the slices are similar to each other

PASTE (Zeira et. al., Nature Methods 2022)

- Procrustes analysis (from Wikipedia)



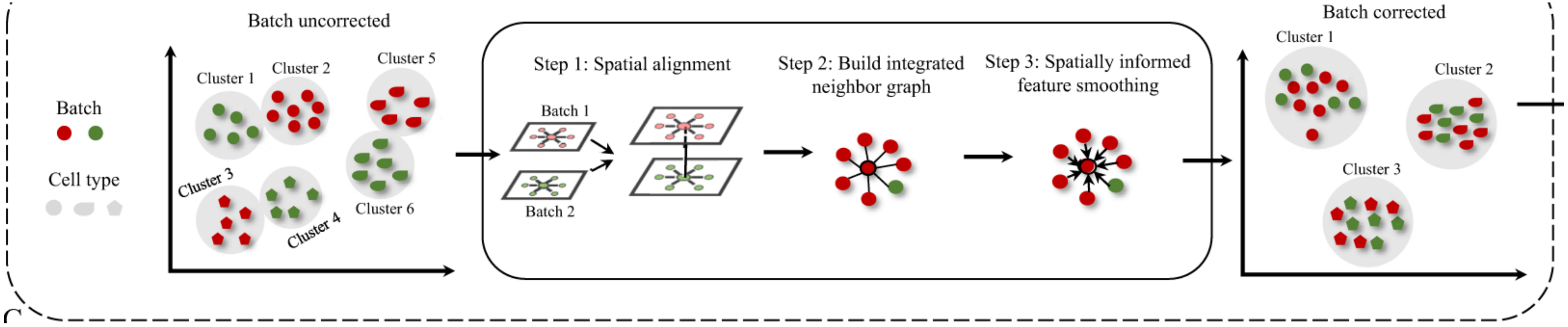
PASTE (Zeira et. al., Nature Methods 2022)



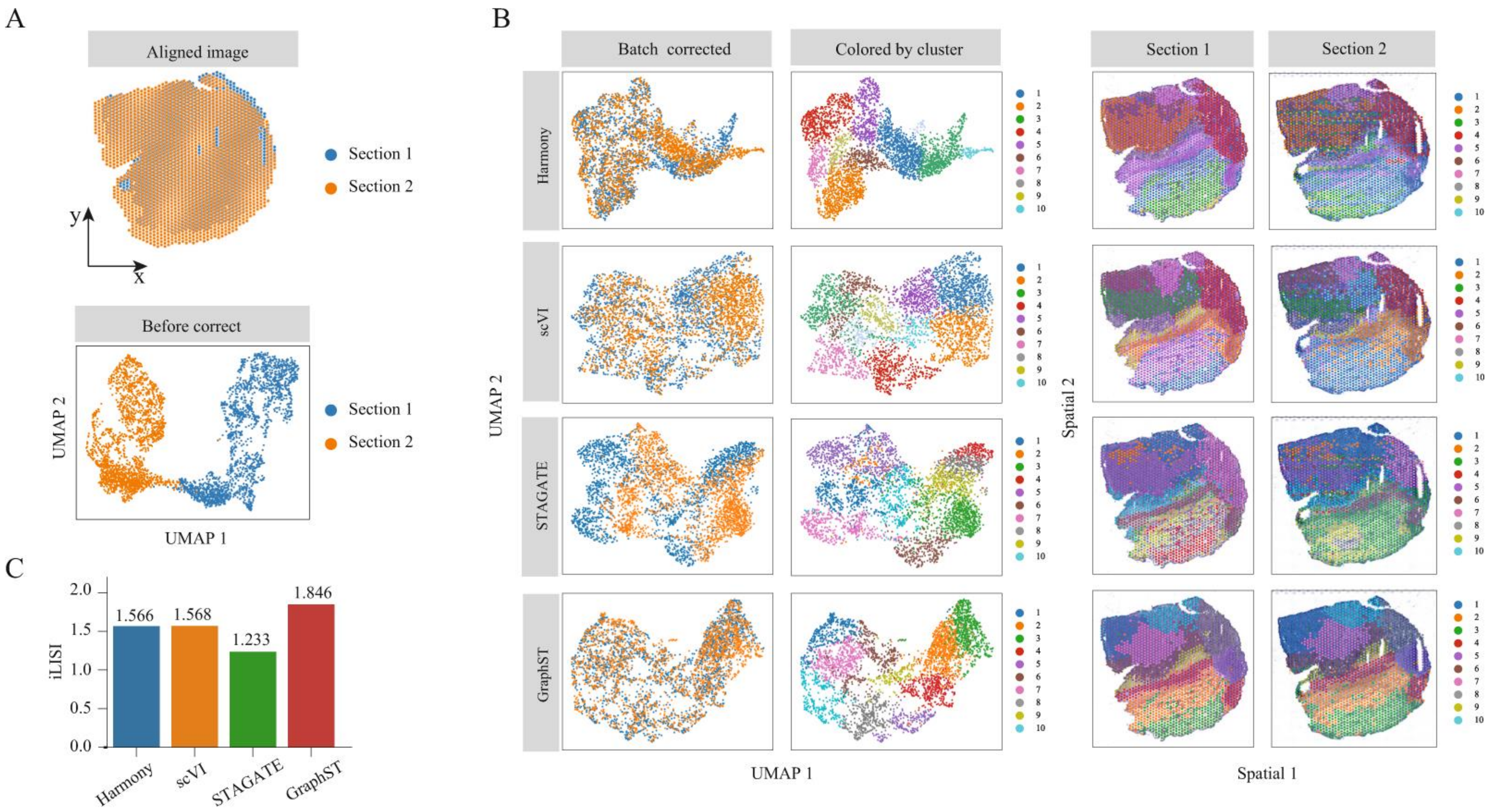
Integrative domain detection using GraphST

- Align the spatial locations
  - Horizontal integration (not sure how they did it …)
    - Align the two histological image to ensure slices are adjacent in space
  - Vertical integration
    - Use PASTE to align the coordinates using the histological images

- Joint neighborhood construction
  - Construct neighborhood graph including both intra-slice and inter-slice adjacent spots
- Train all slices together given the joint graph using the GraphST algorithm
  - Implicitly removes batch effects



Integrative domain detection using GraphST



Related papers

- Longo, S. K., Guo, M. G., Ji, A. L., & Khavari, P. A. (2021). Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. *Nature Reviews Genetics*, *22*(10), 627-644.
- Atta, L., & Fan, J. (2021). Computational challenges and opportunities in spatially resolved transcriptomic data analysis. *Nature Communications*, *12*(1), 5283.
- Chen, K. H., Boettiger, A. N., Moffitt, J. R., Wang, S., & Zhuang, X. (2015). Spatially resolved, highly multiplexed RNA profiling in single cells. *Science*, *348*(6233), aaa6090.
- Ståhl, P. L., Salmén, F., Vickovic, S., Lundmark, A., Navarro, J. F., Magnusson, J., ... & Frisén, J. (2016). Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science*, *353*(6294), 78-82.
- Rodriques, S. G., Stickels, R. R., Goeva, A., Martin, C. A., Murray, E., Vanderburg, C. R., ... & Macosko, E. Z. (2019). Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. *Science*, *363*(6434), 1463-1467.
- Stickels, R. R., Murray, E., Kumar, P., Li, J., Marshall, J. L., Di Bella, D. J., ... & Chen, F. (2021). Highly sensitive spatial transcriptomics at near-cellular resolution with Slide-seqV2. *Nature biotechnology*, *39*(3), 313-319.
- Prabhakaran, S. (2022). Sparcle: assigning transcripts to cells in multiplexed images. *Bioinformatics Advances*, *2*(1), vbac048.
- Dries, R., Zhu, Q., Dong, R., Eng, C. H. L., Li, H., Liu, K., ... & Yuan, G. C. (2021). Giotto: a toolbox for integrative analysis and visualization of spatial expression data. *Genome biology*, *22*, 1-31.
- Zhu, Q., Shah, S., Dries, R., Cai, L., & Yuan, G. C. (2018). Identification of spatially associated subpopulations by combining scRNAseq and sequential fluorescence in situ hybridization data. *Nature biotechnology*, *36*(12), 1183-1190.
- Yang, Y., Shi, X., Liu, W., Zhou, Q., Chan Lau, M., Chun Tatt Lim, J., ... & Liu, J. (2022). SC-MEB: spatial clustering with hidden Markov random field using empirical Bayes. *Briefings in bioinformatics*, *23*(1), bbab466.
- Palla, G., Spitzer, H., Klein, M., Fischer, D., Schaar, A. C., Kuemmerle, L. B., ... & Theis, F. J. (2022). Squidpy: a scalable framework for spatial omics analysis. *Nature methods*, *19*(2), 171-178.
- Hu, J., Li, X., Coleman, K., Schroeder, A., Ma, N., Irwin, D. J., ... & Li, M. (2021). SpaGCN: Integrating gene expression, spatial location and histology to identify spatial domains and spatially variable genes by graph convolutional network. *Nature methods*, *18*(11), 1342-1351.
- Long, Y., Ang, K. S., Li, M., Chong, K. L. K., Sethi, R., Zhong, C., ... & Chen, J. (2023). Spatially informed clustering, integration, and deconvolution of spatial transcriptomics with GraphST. *Nature Communications*, *14*(1), 1155.
- Zeira, R., Land, M., Strzalkowski, A., & Raphael, B. J. (2022). Alignment and integration of spatial transcriptomics data. *Nature Methods*, *19*(5), 567-575.