Spatial domains

Tissue architecture and remodeling from spatial omics data

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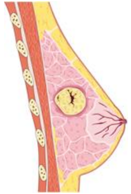
PI: Prof. Giovanni Ciriello

Outline

- What is a **spatial domain**?
- Types of spatial domain **methods**
- CellCharter
    - Downstream analysis: domain **shape**
    - Find the best **number** of domains
- Applications to:
    - Autoimmune diseases
    - Lung cancer

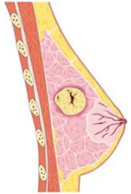
What is a spatial domain?

scRNA-seq



Spatial transcriptomics

scRNA-seq



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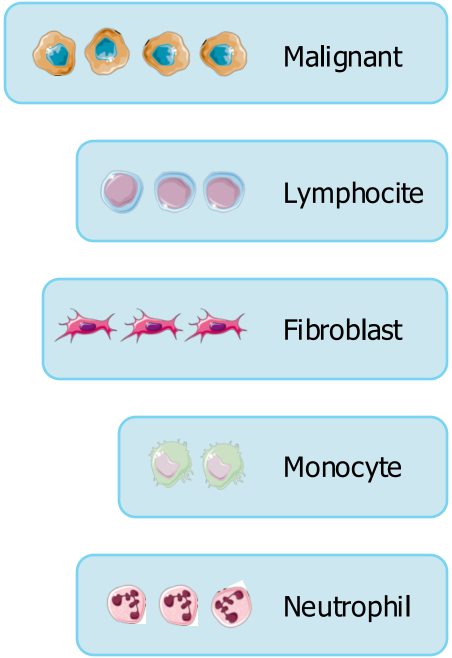


Malignant

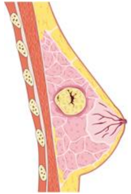
Lymphocite

Fibroblast

Monocyte

Neutrophil

scRNA-seq



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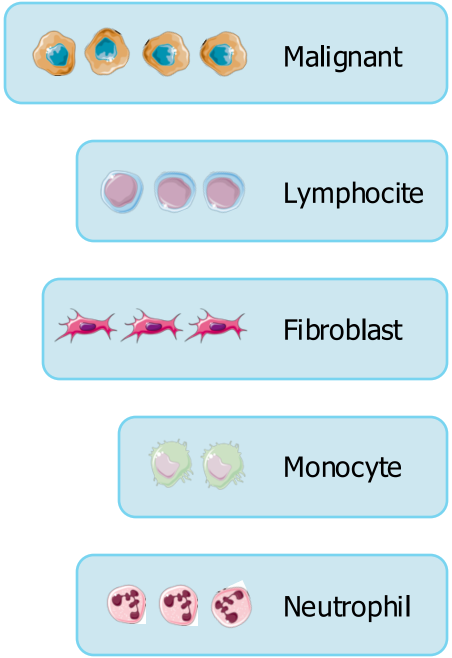

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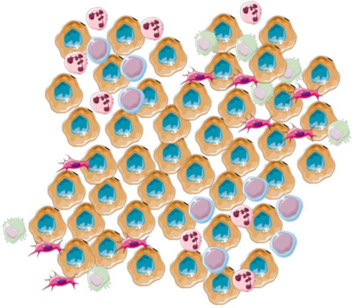

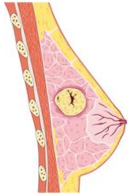

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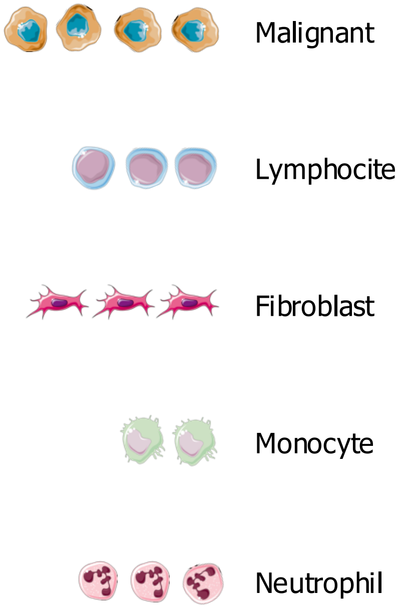

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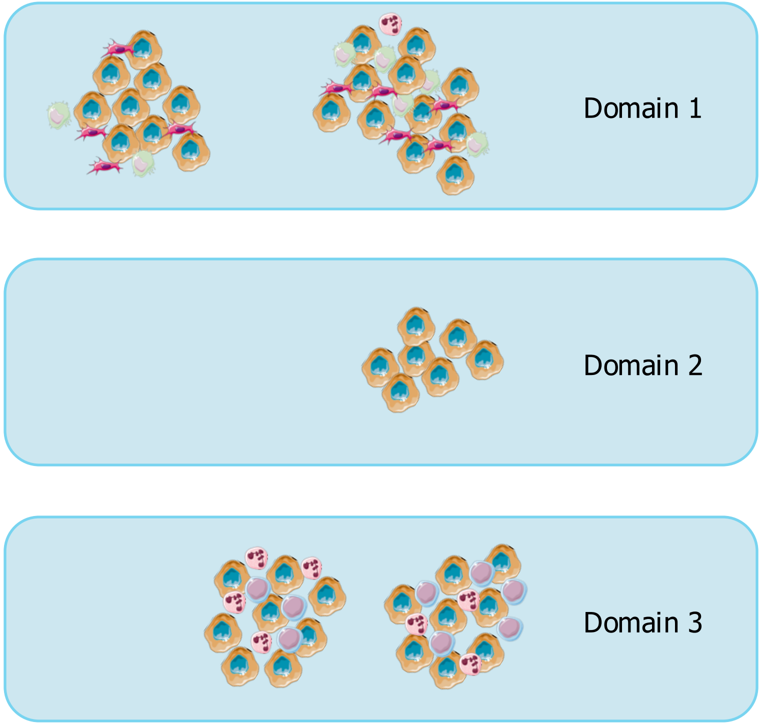
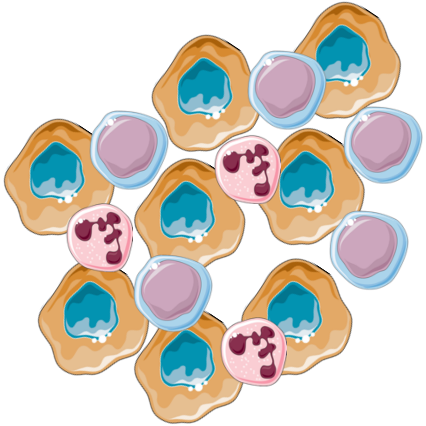


Spatial clustering principles

Cluster cells based on the gene expression of:



Spatial clustering principles

Cluster cells based on the gene expression of:

Cell

Spatial clustering principles

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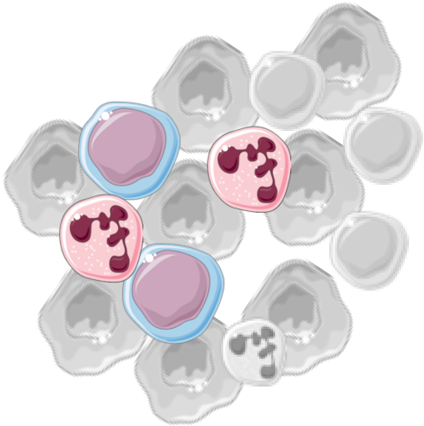

Cell
+
Immediate neighbors (niche)

Spatial clustering principles

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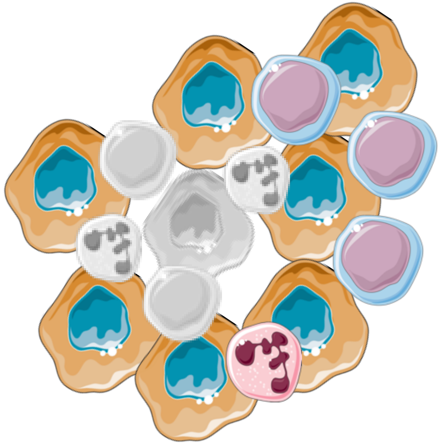
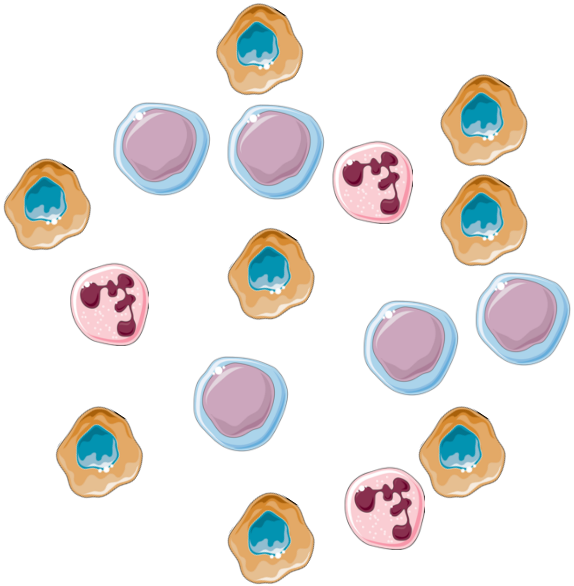


Cell
+
Immediate neighbors (niche)
+
Farther neighbors

Spatial domain identification approaches

Cell type proportions, Hidden Markov Random Fields and Graph Neural Networks

Approach 1: proportion of cell types



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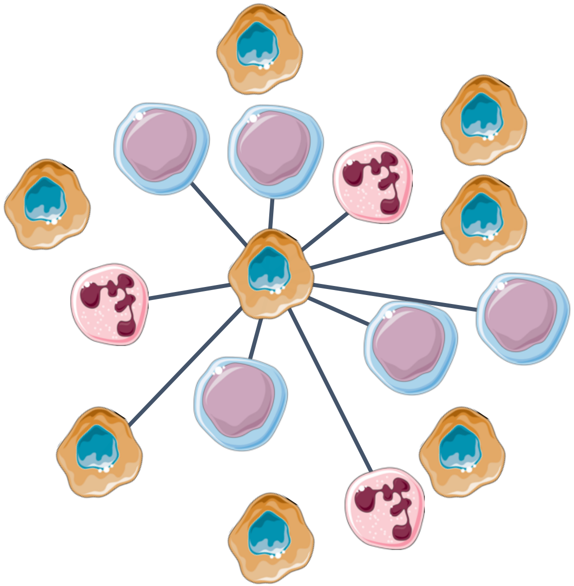

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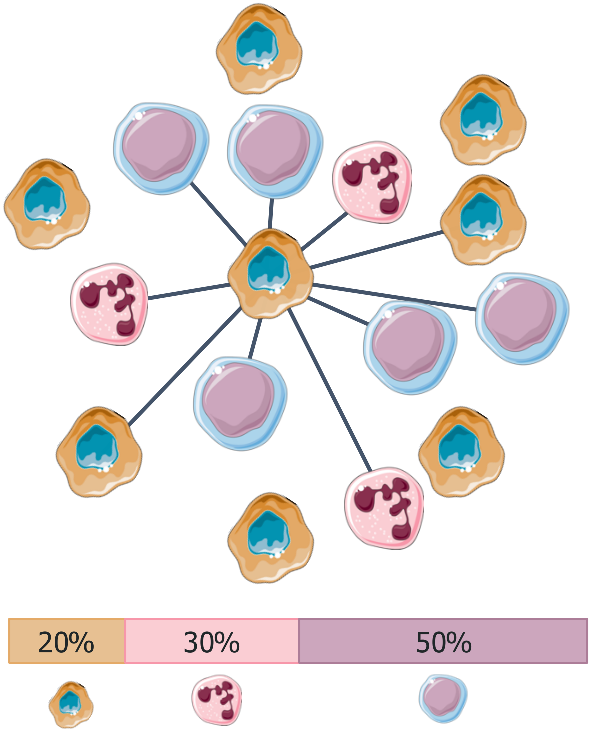

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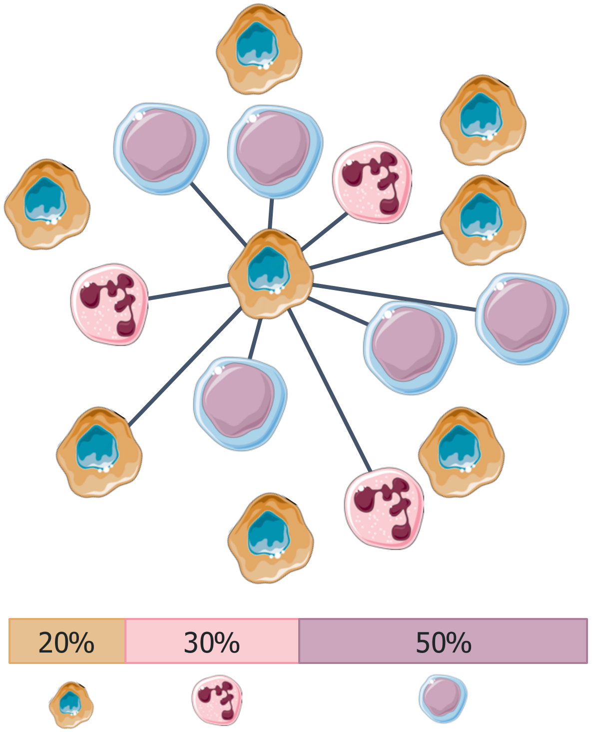
   for every cell
2. Cluster cells
   based on their proportions



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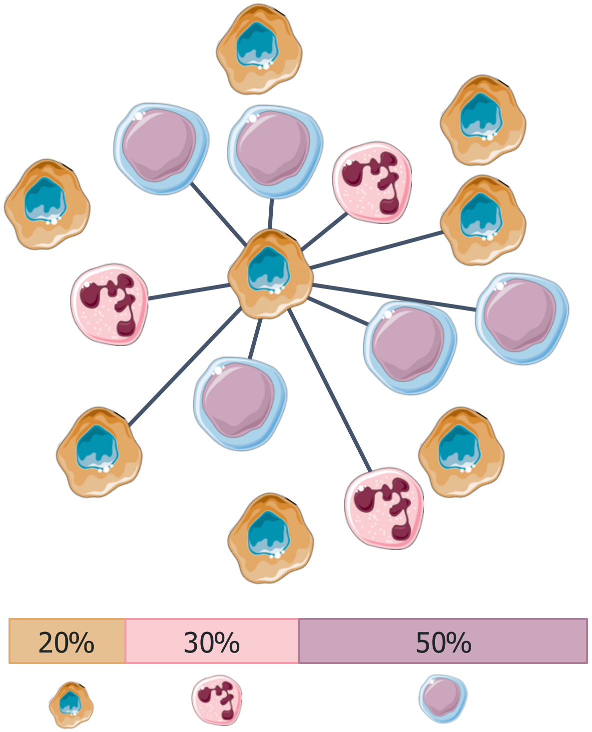

- Scalable
- Depends on manual annotations
  - *How detailed should the annotation be?*
  - *How to capture **variability** within cell types?*

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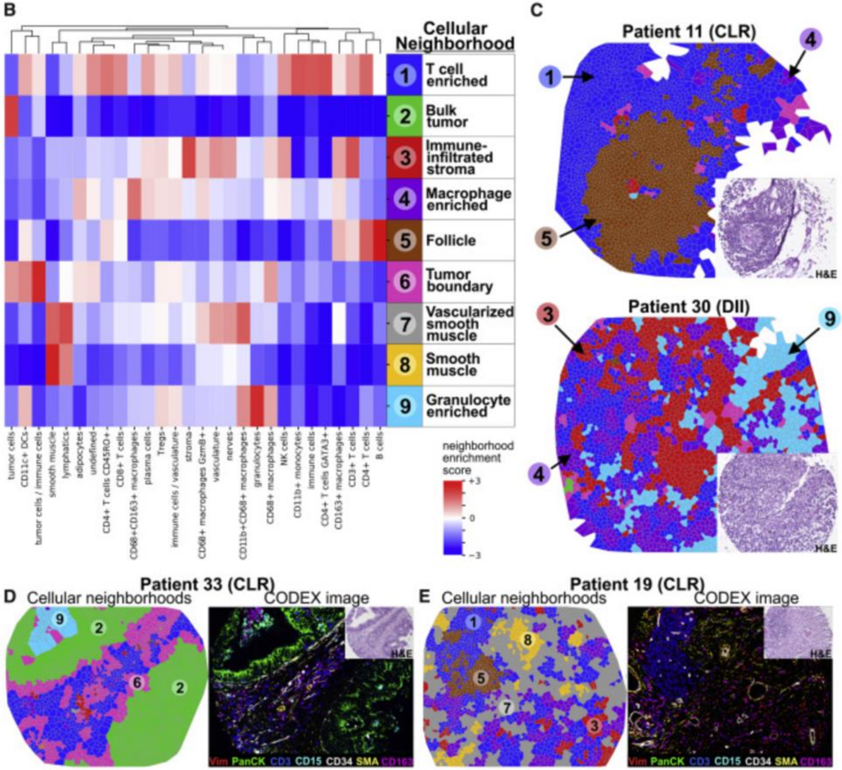

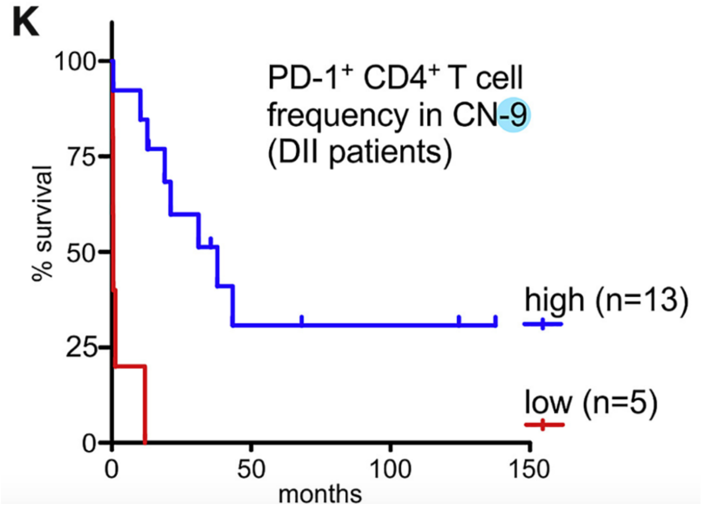

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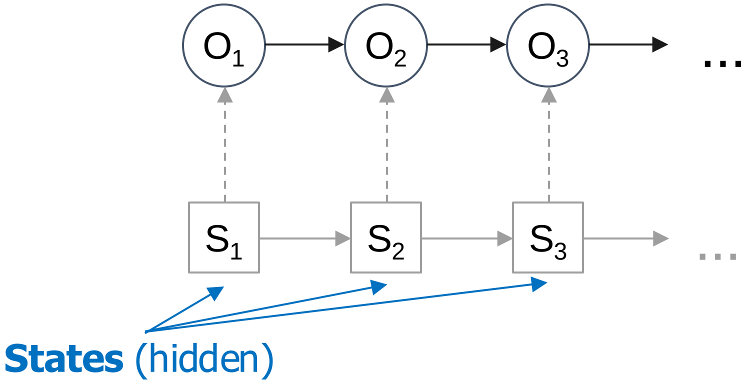
Approach 2: Hidden Markov Random Fields

Hidden Markov Model



Sequences → 1D graphs

Approach 2: Hidden Markov Random Fields

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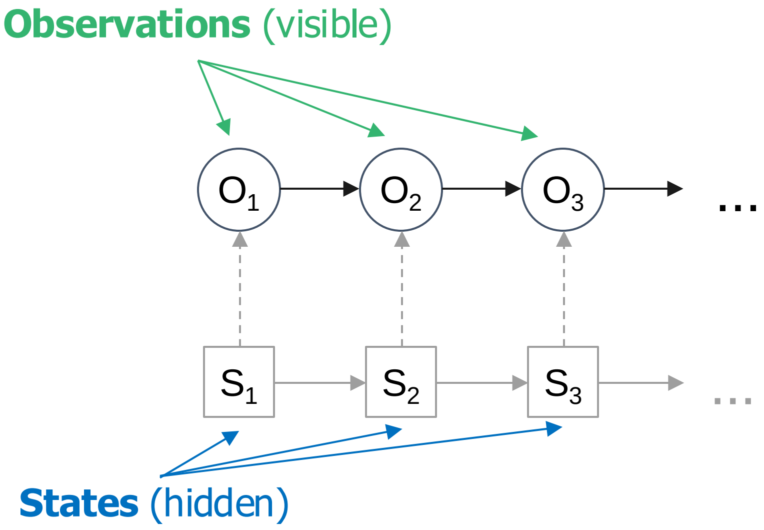

Sequences → 1D graphs

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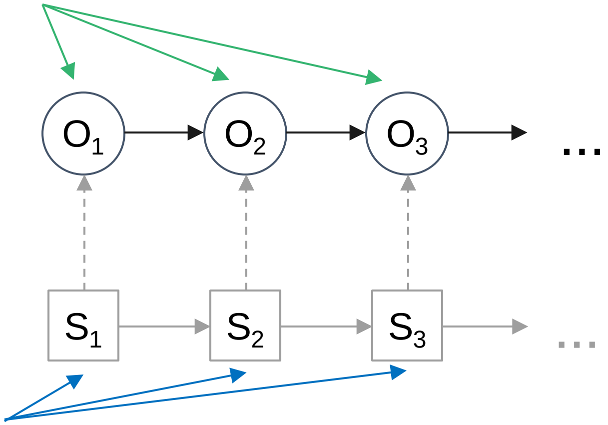
Hidden Markov Model

Histone marks levels

(high vs low)

Observations (visible)



Chromatin state

(active vs inactive)

States (hidden)

Genome sequence

Sequences → 1D graphs

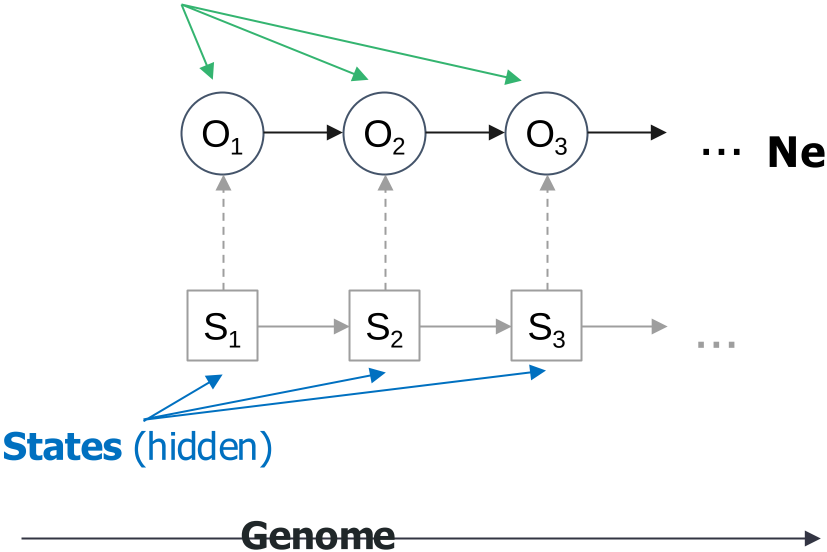
Approach 2: Hidden Markov Random Fields

Hidden Markov Model

Histone marks levels

(high vs low)

Observations (visible)



Neighboring observations (O)

are likely to have

similar states (S)

Chromatin state

(active vs inactive)

States (hidden)

Genome

sequence

Sequences → 1D graphs

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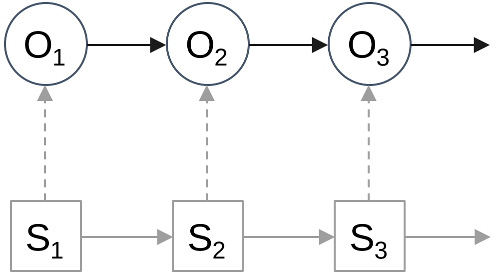

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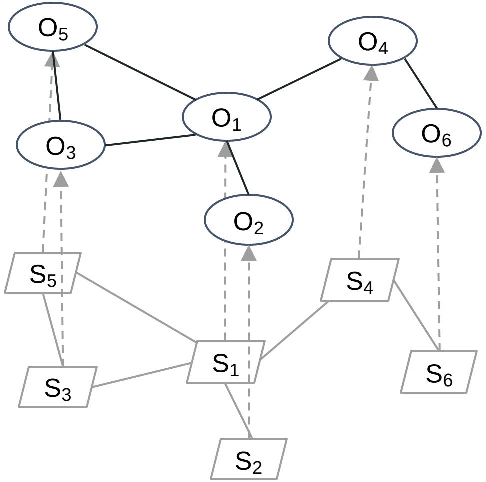

Arbitrary graphs

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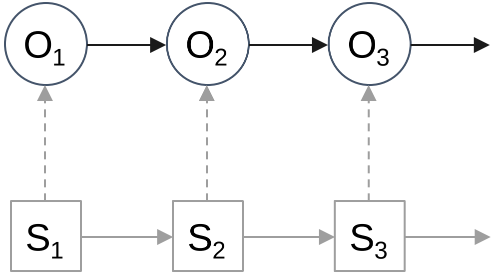
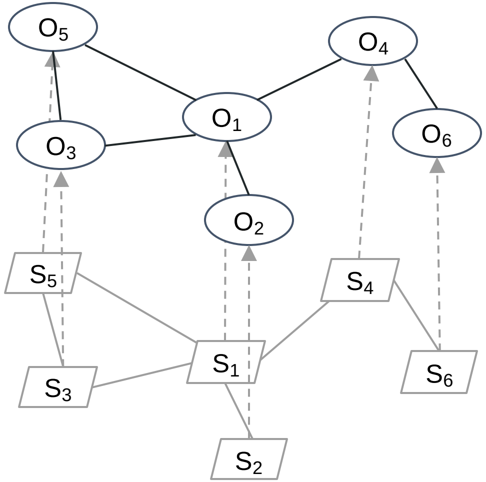


Sequences → 1D graphs

Hidden Markov Random Field

Gene expression



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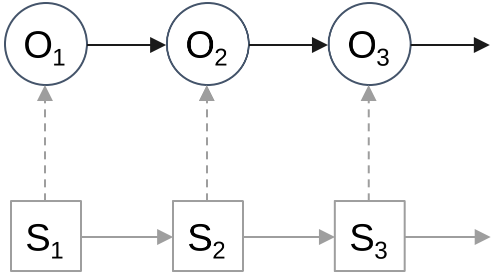

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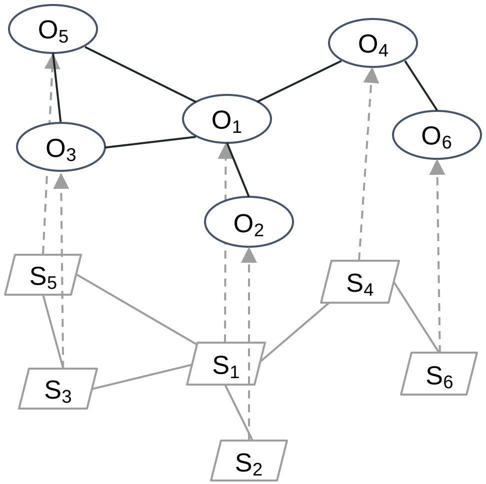

Spatial domain

Arbitrary graphs

- Medium scalability
- No annotation required
  Works directly on gene expression

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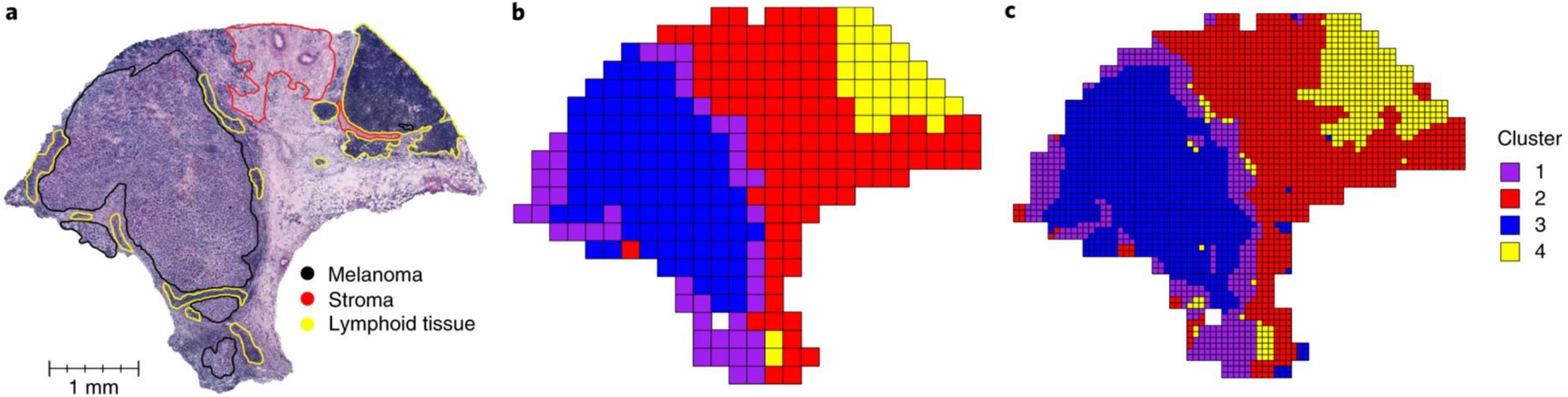

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Approach 3: Graph Neural Networks

Nodes with **similar neighborhoods** have **similar representations** (i.e. vectors)

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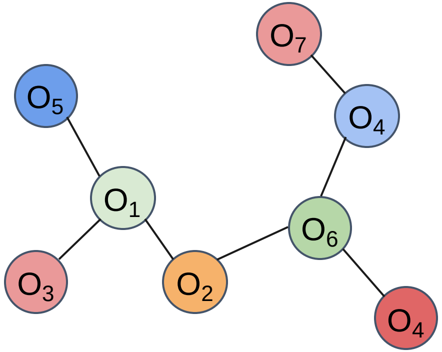
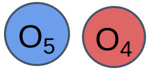
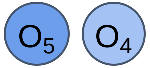




Different cell types → very different gene expression



Variability within cell type → slightly different gene expression

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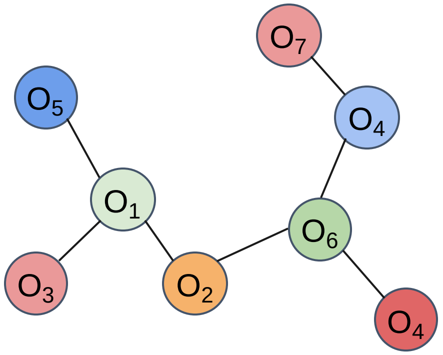

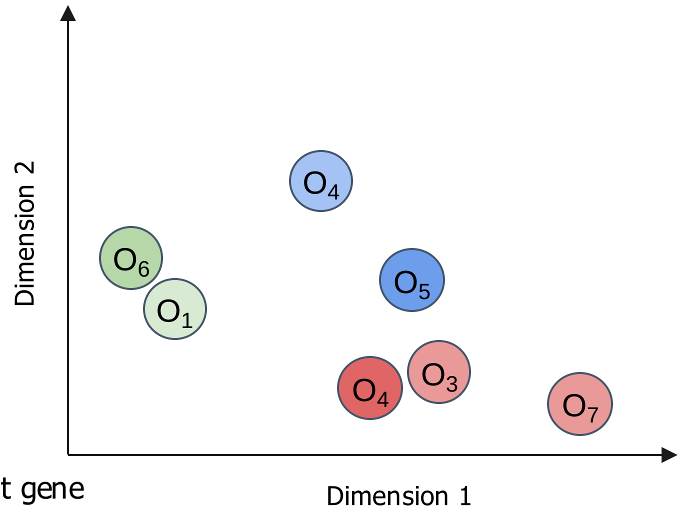

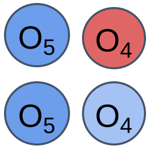

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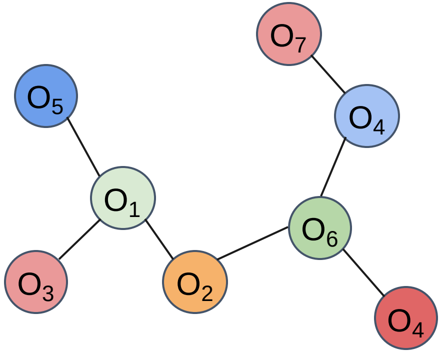
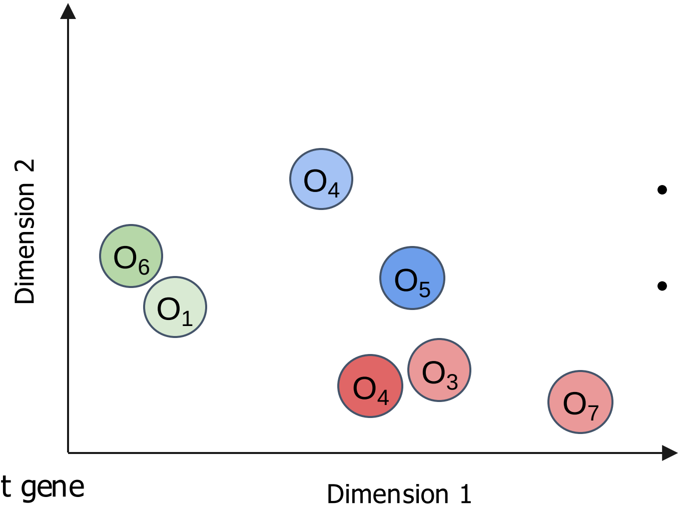
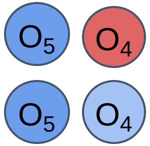




- Low/medium scalability
- No annotation required
  Works directly on gene expression



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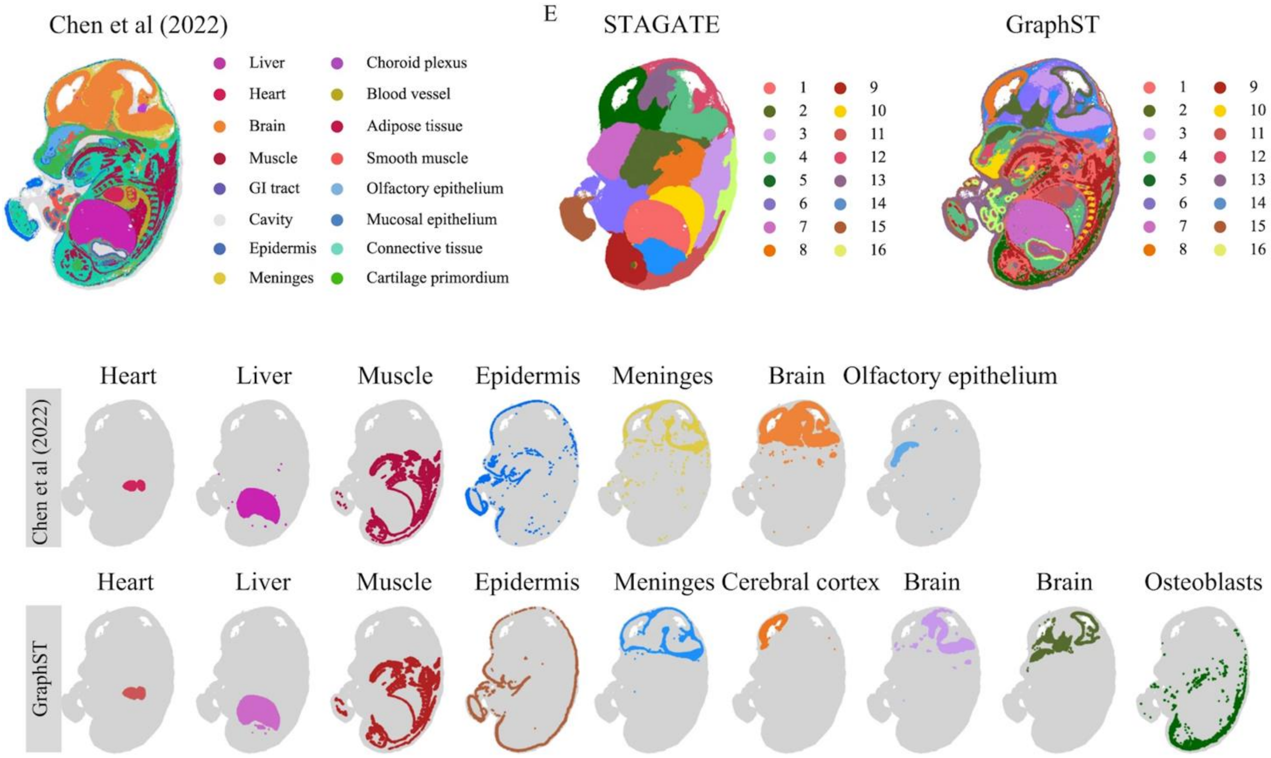

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Our approach: neighborhood aggregation

My PhD plan (2020) → Spatial domain identification with **Graph Neural Networks**

- Start with a simple baseline:
  - Acquire familiarity with the problem
  - Gradual increase of complexity in development

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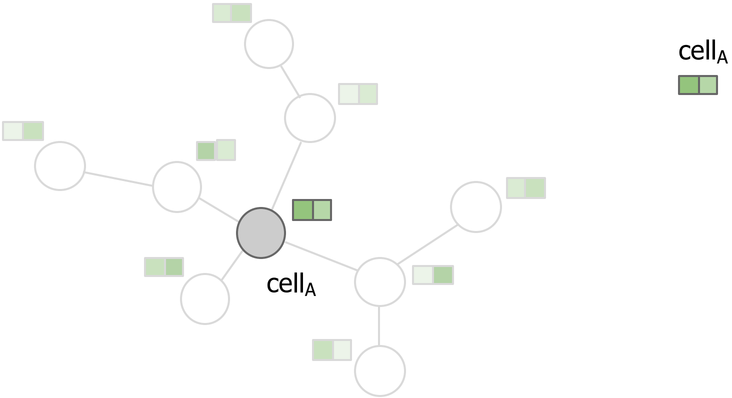

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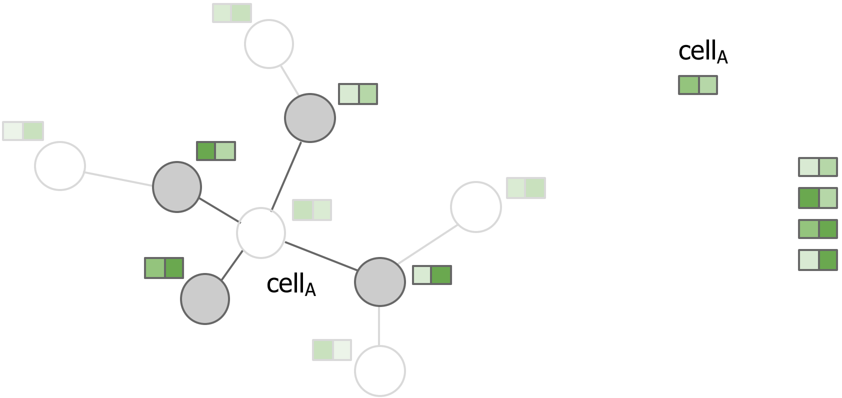

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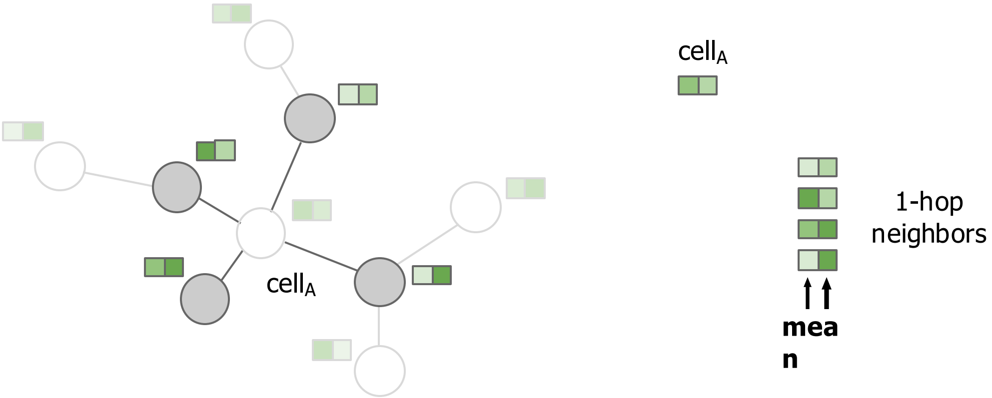

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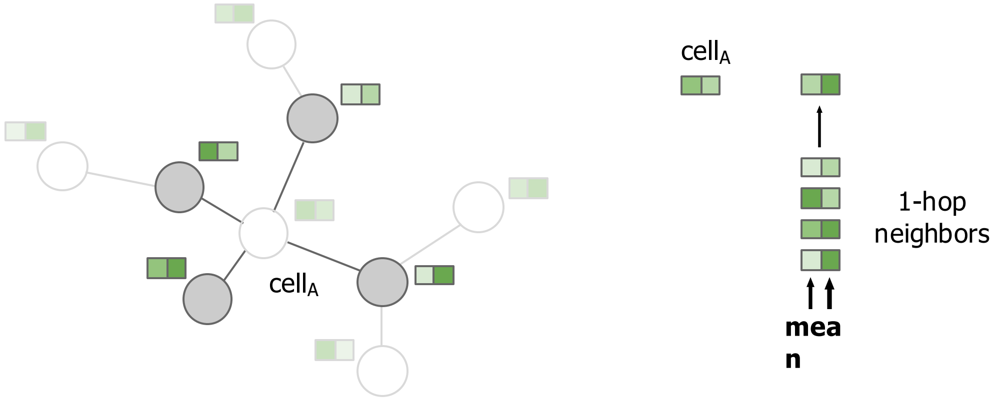

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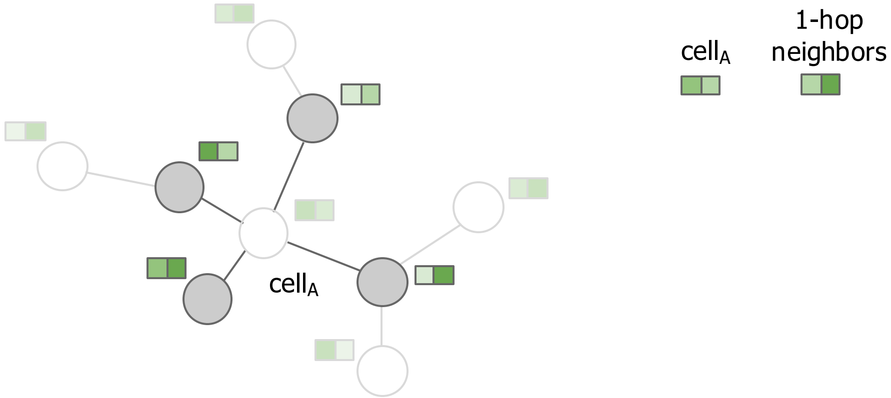

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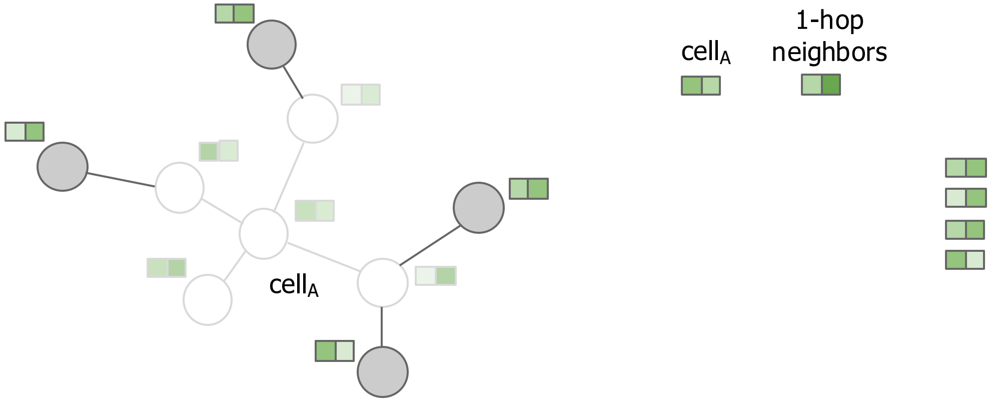

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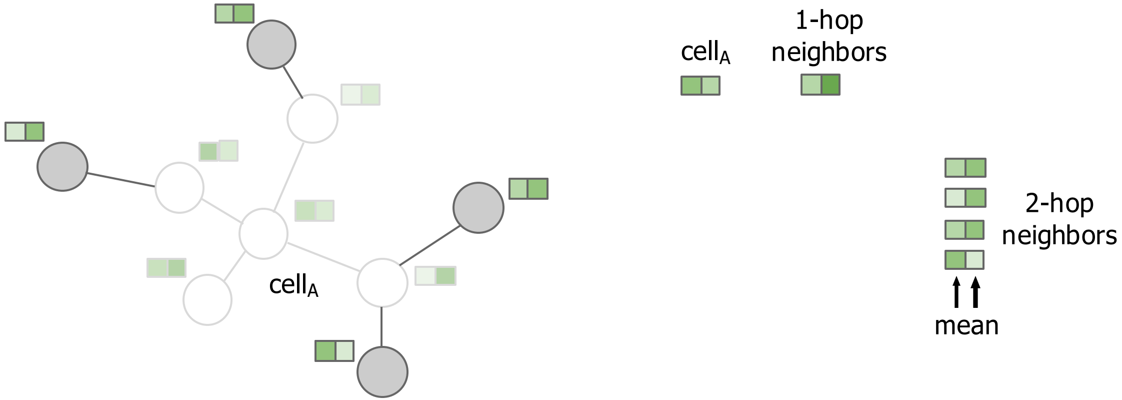

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

Our approach: neighborhood aggregation

My PhD plan (2020) → Spatial domain identification with **Graph Neural Networks**

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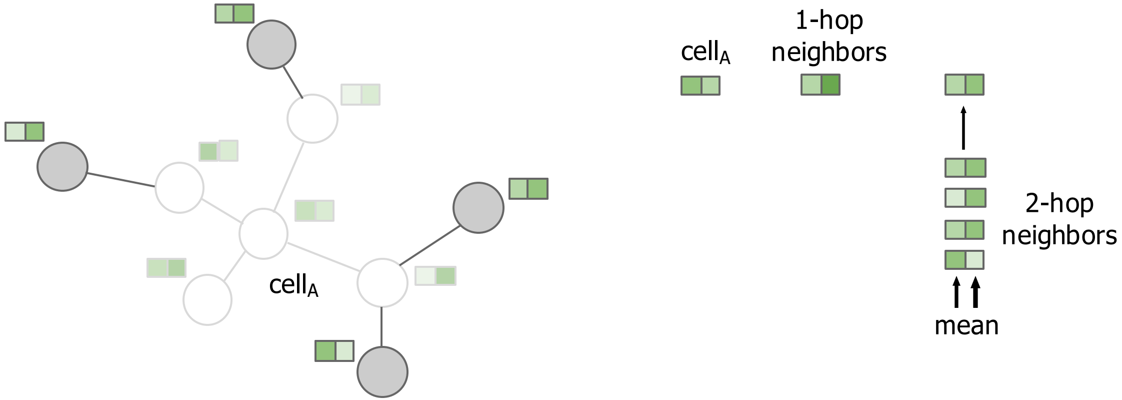

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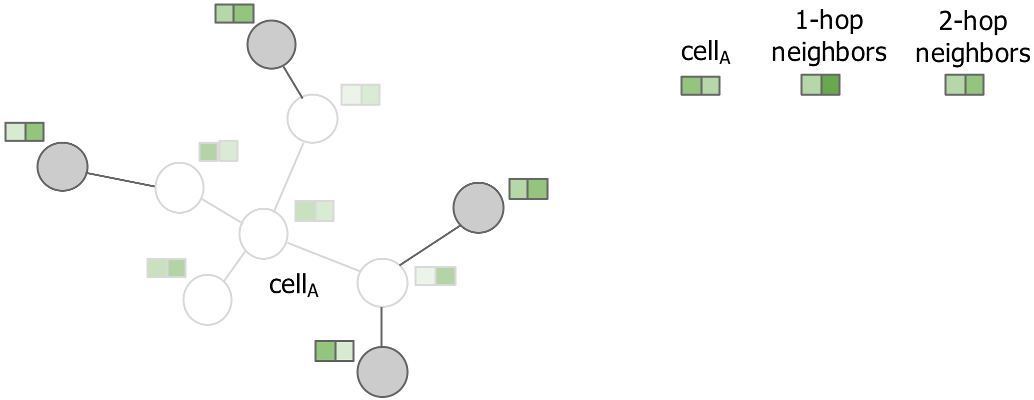

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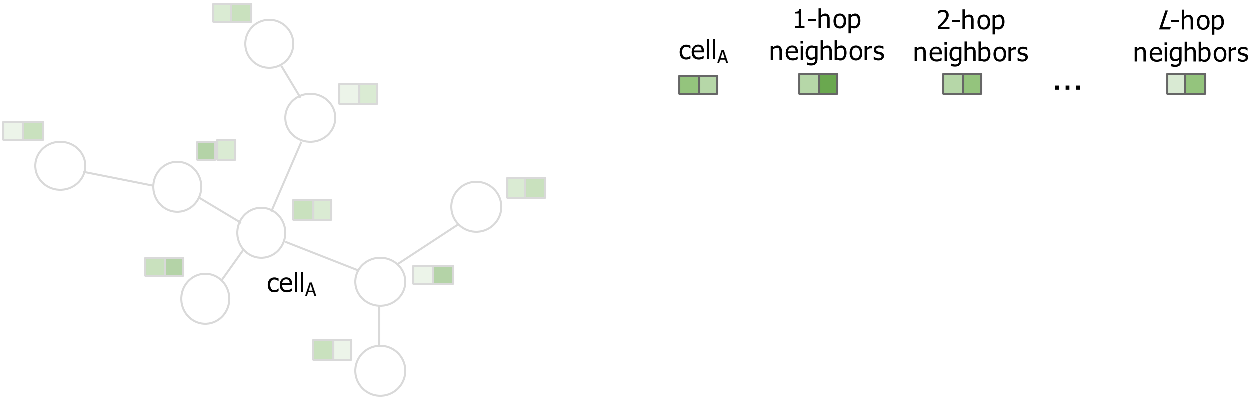

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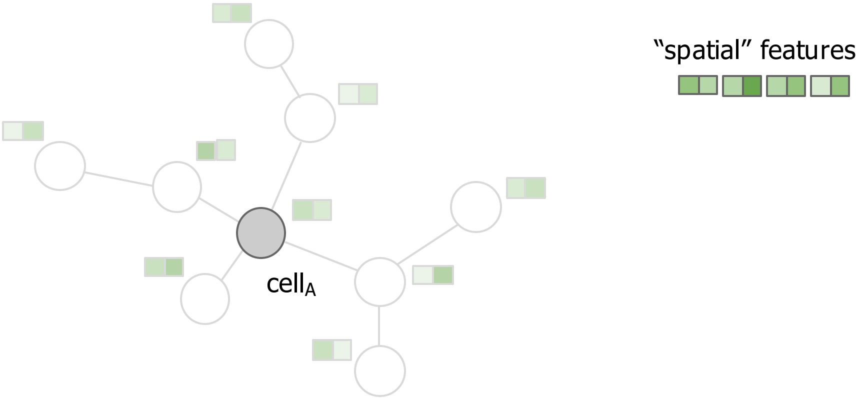

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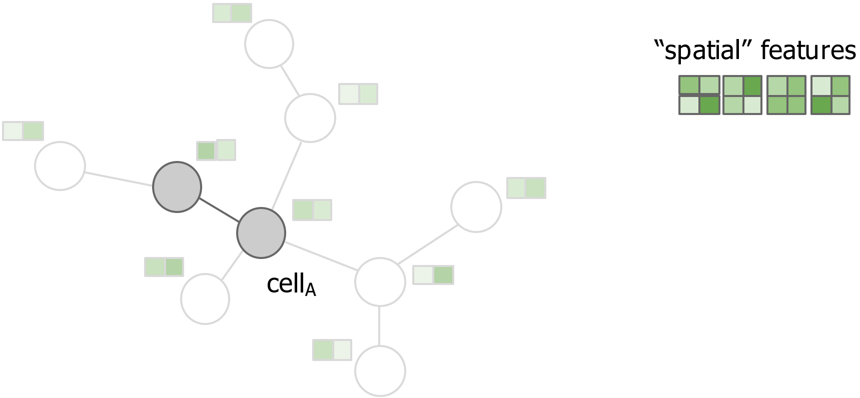

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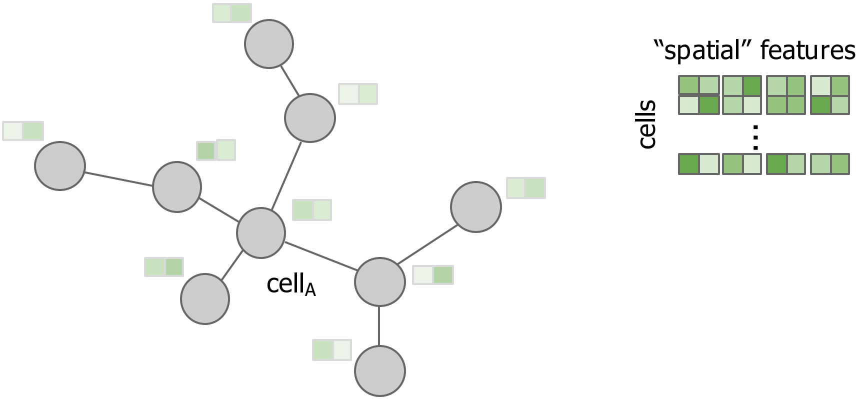

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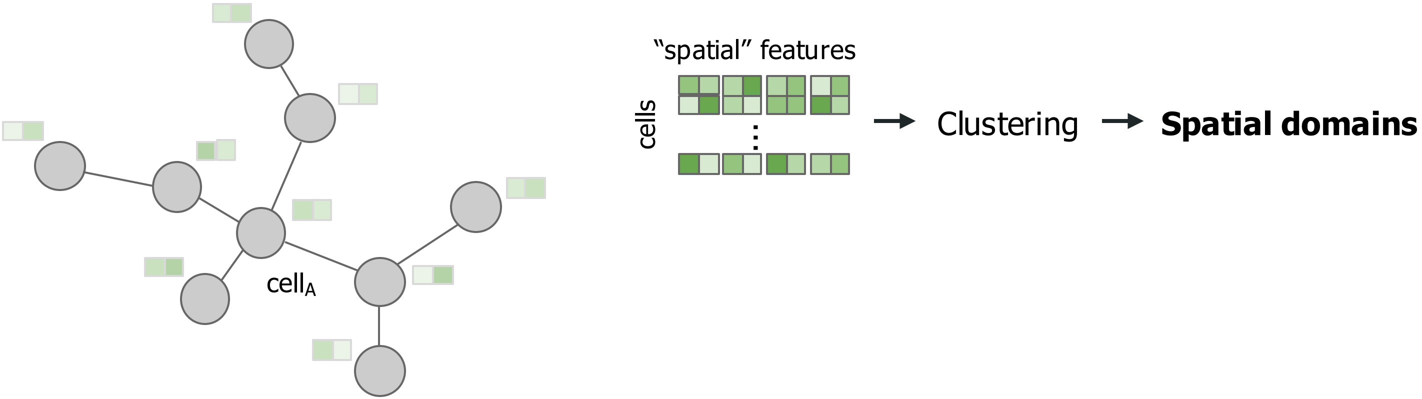

→ Clustering → **Spatial domains**

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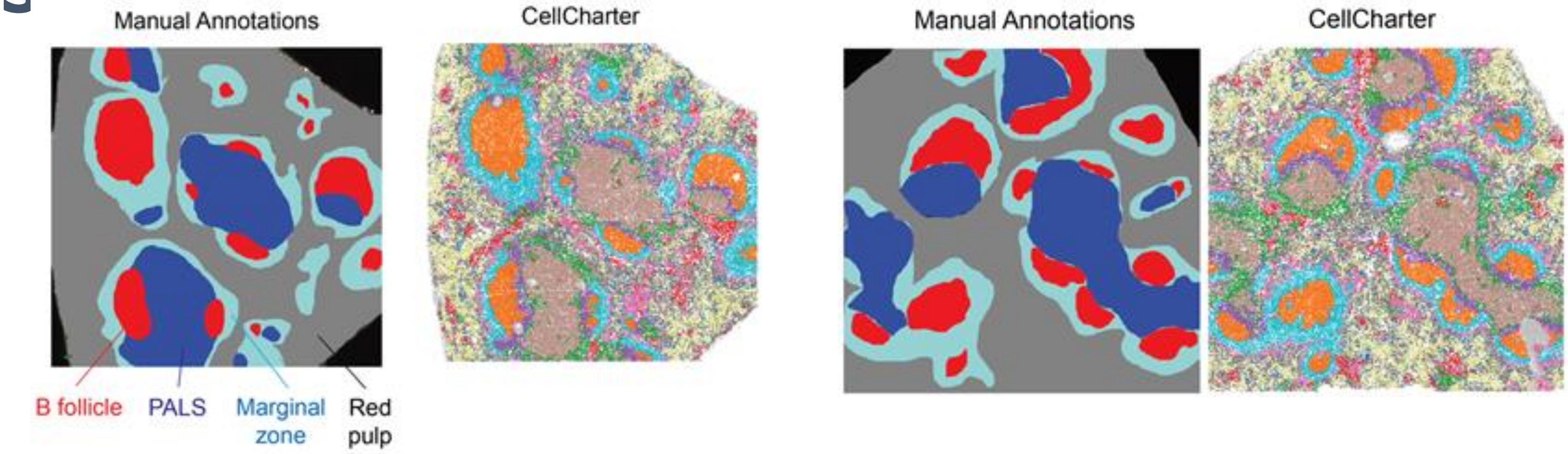
First results on spatial proteomics mouse spleen



Strong match with the anatomical areas mouse spleens

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

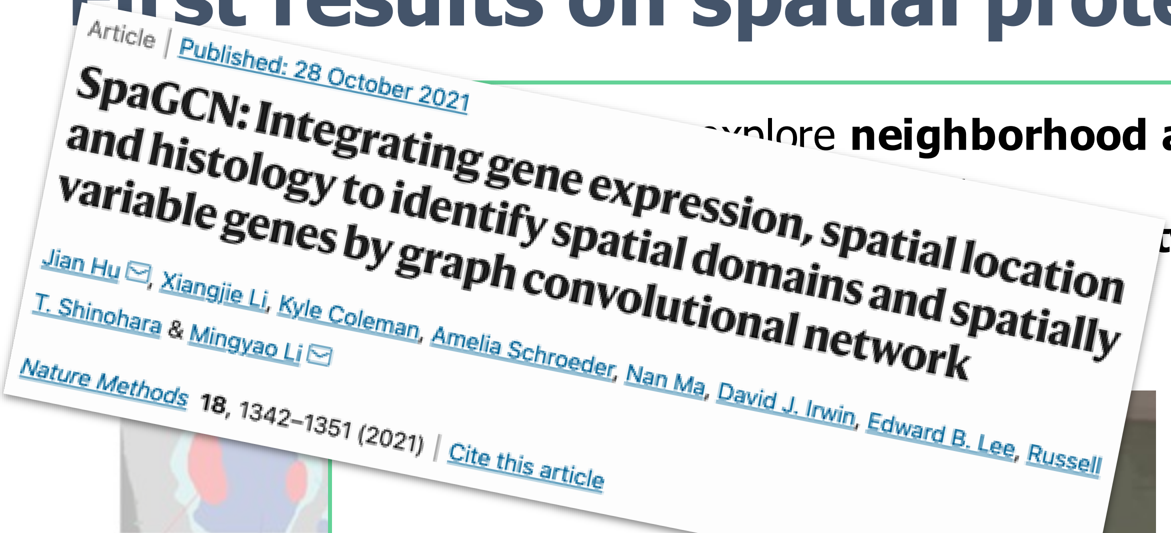
First results on spatial proteomics mouse spleen

Continue to explore **neighborhood aggregation**
or
Move to develop a **Graph Neural Network** method?

First results on spatial proteomics mouse

xplore **neighborhood aggregation**

work method?



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SpaGCN: Integrating gene expression, spatial location and histology to identify spatial domains and spatially variable genes by graph convolutional network

Jian Hu, Xiangjie Li, Kyle Coleman, Amelia Schroeder, Nan Ma, David J. Irwin, Edward B. Lee, Russell T. Shinohara & Mingyao Li

Nature Methods **18**, 1342–1351 (2021) | Cite this article

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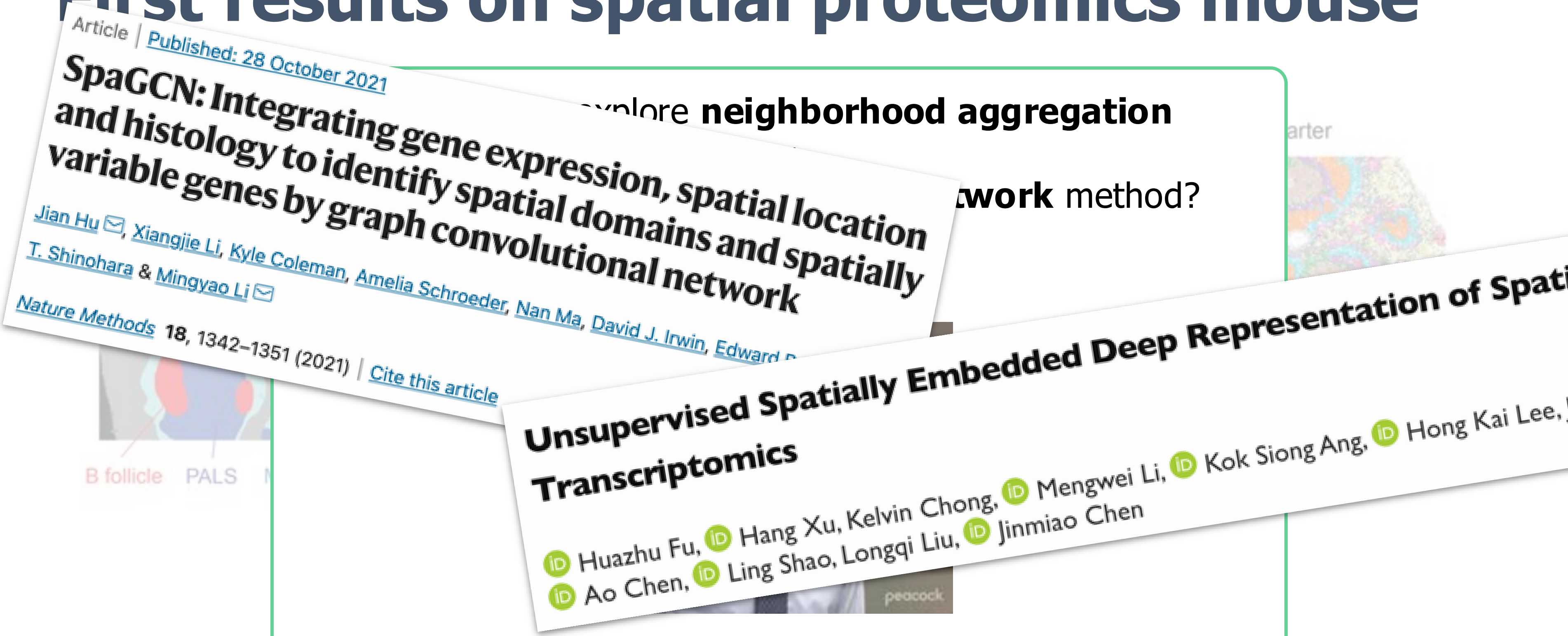

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**Unsupervised Spatially Embedded Deep Representation of Spati
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Huazhu Fu, Hang Xu, Kelvin Chong, Mengwei Li, Kok Siong Ang, Hong Kai Lee,
Ao Chen, Ling Shao, Longqi Liu, Jinmiao Chen

First results on spatial proteomics mouse

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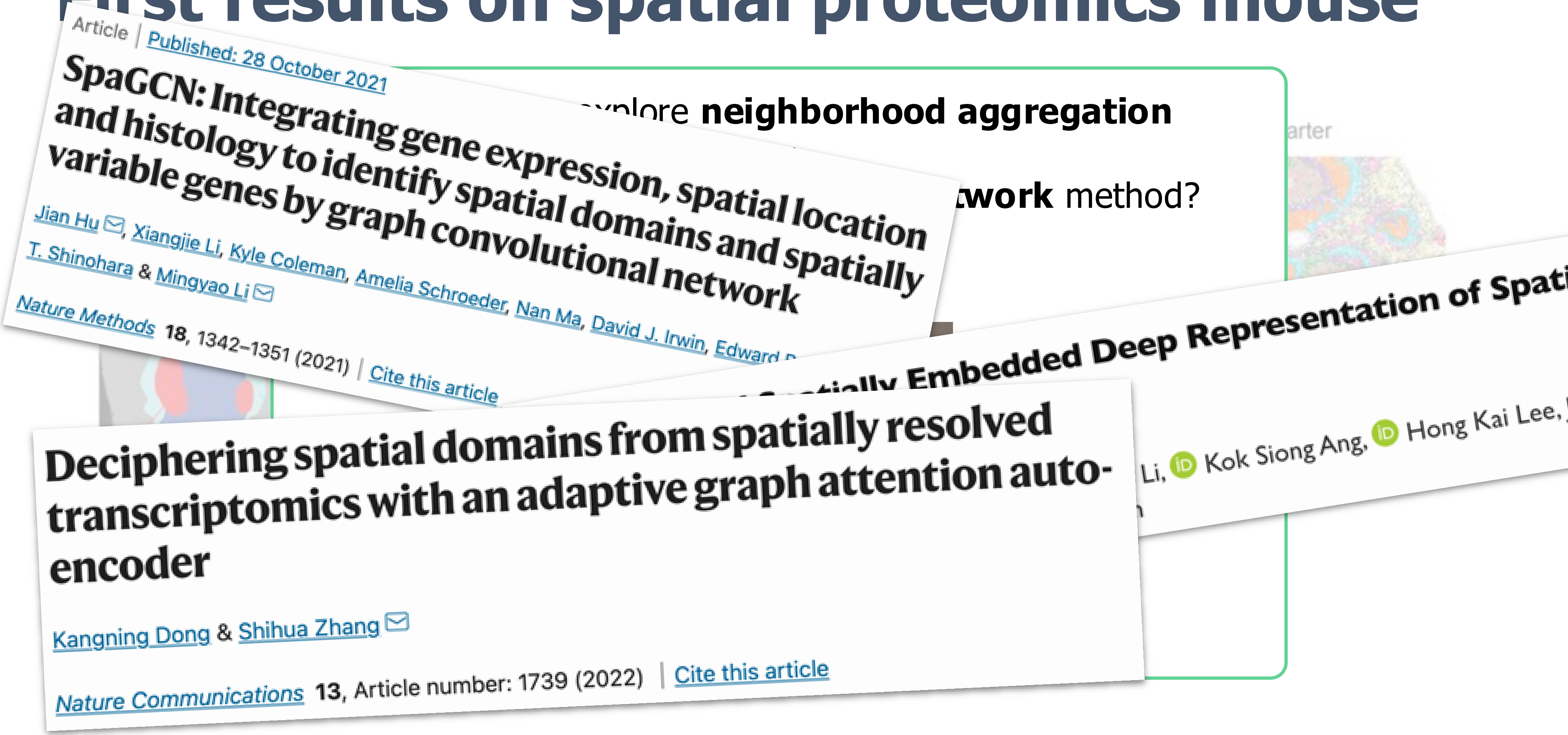

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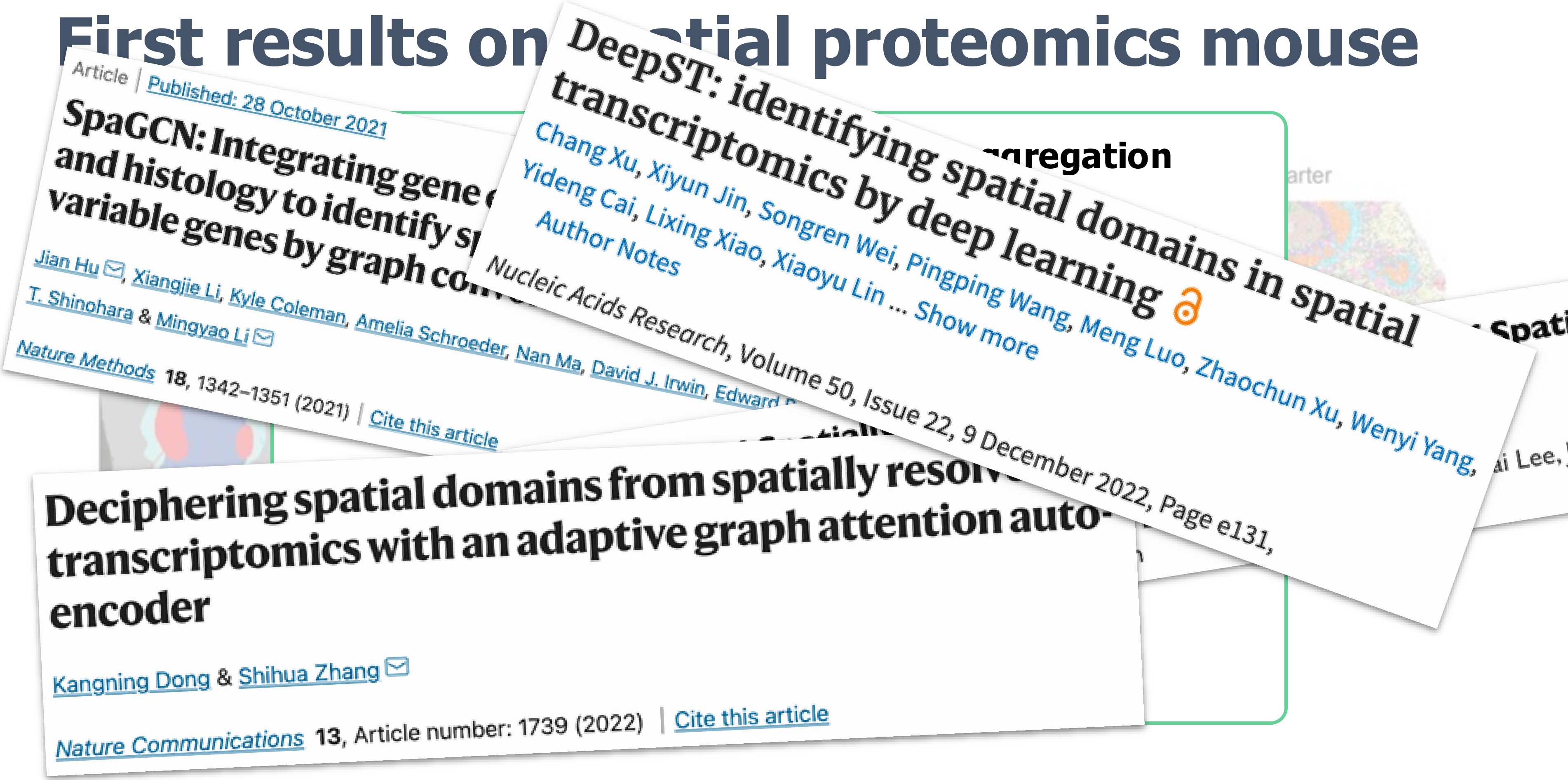

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DeepST: identifying spatial domains in spatial transcriptomics by deep learning

Chang Xu, Xiyun Jin, Songren Wei, Pingping Wang, Meng Luo, Zhaochun Xu, Wenyi Yang,
Yideng Cai, Lixing Xiao, Xiaoyu Lin ... Show more
Author Notes

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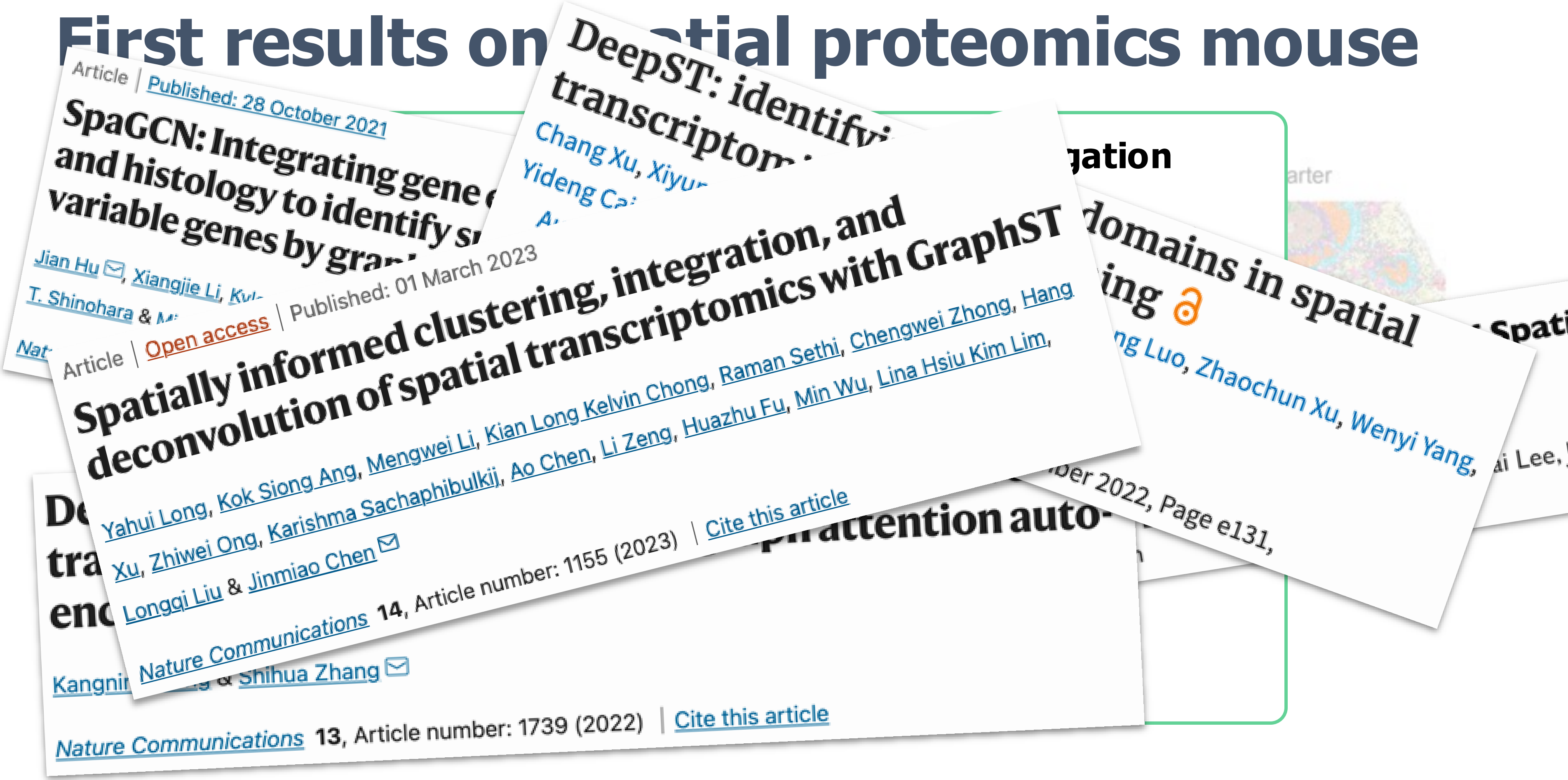

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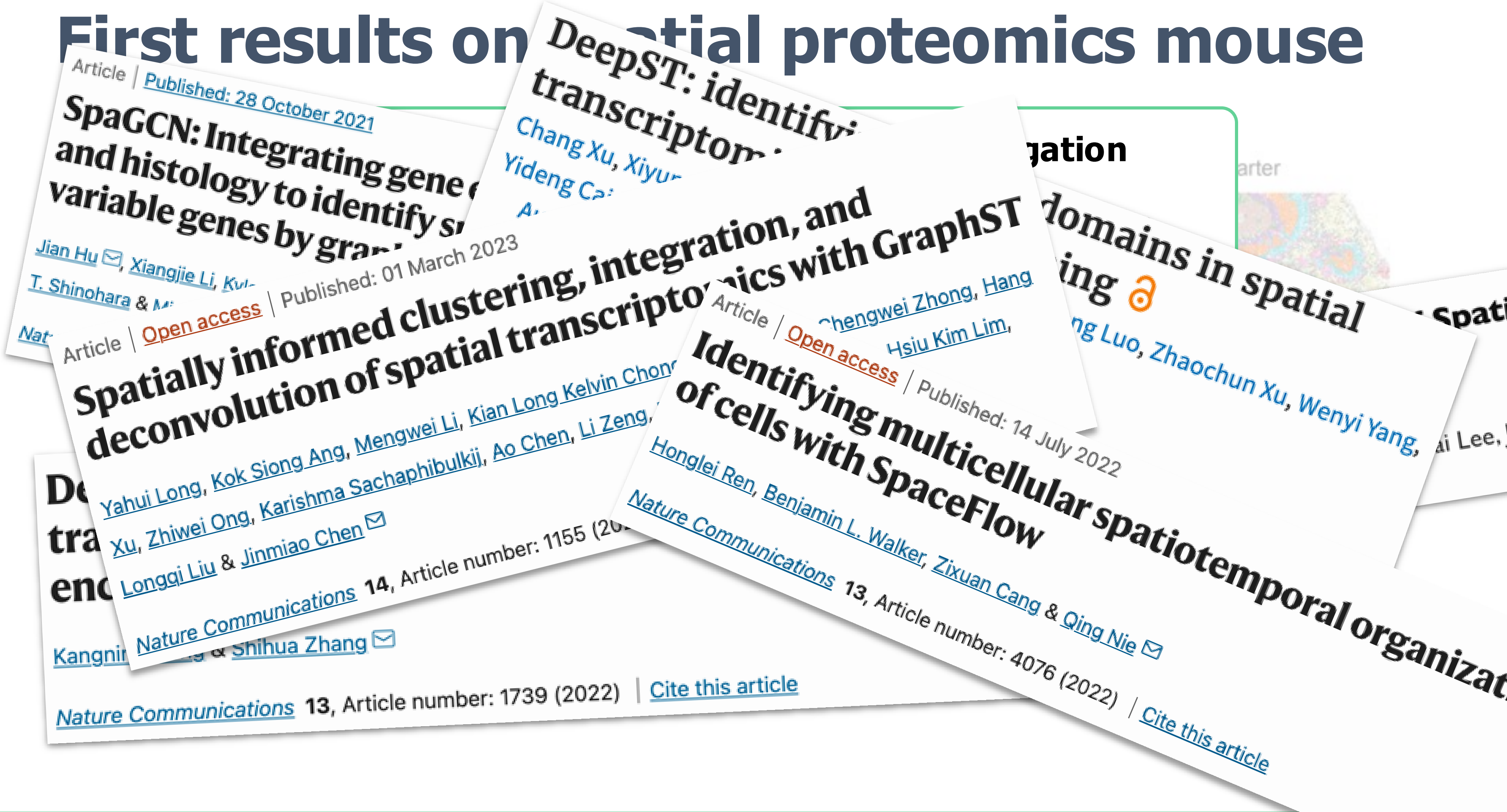

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Article | Open access | Published: 14 July 2022

Identifying multicellular spatiotemporal organizat
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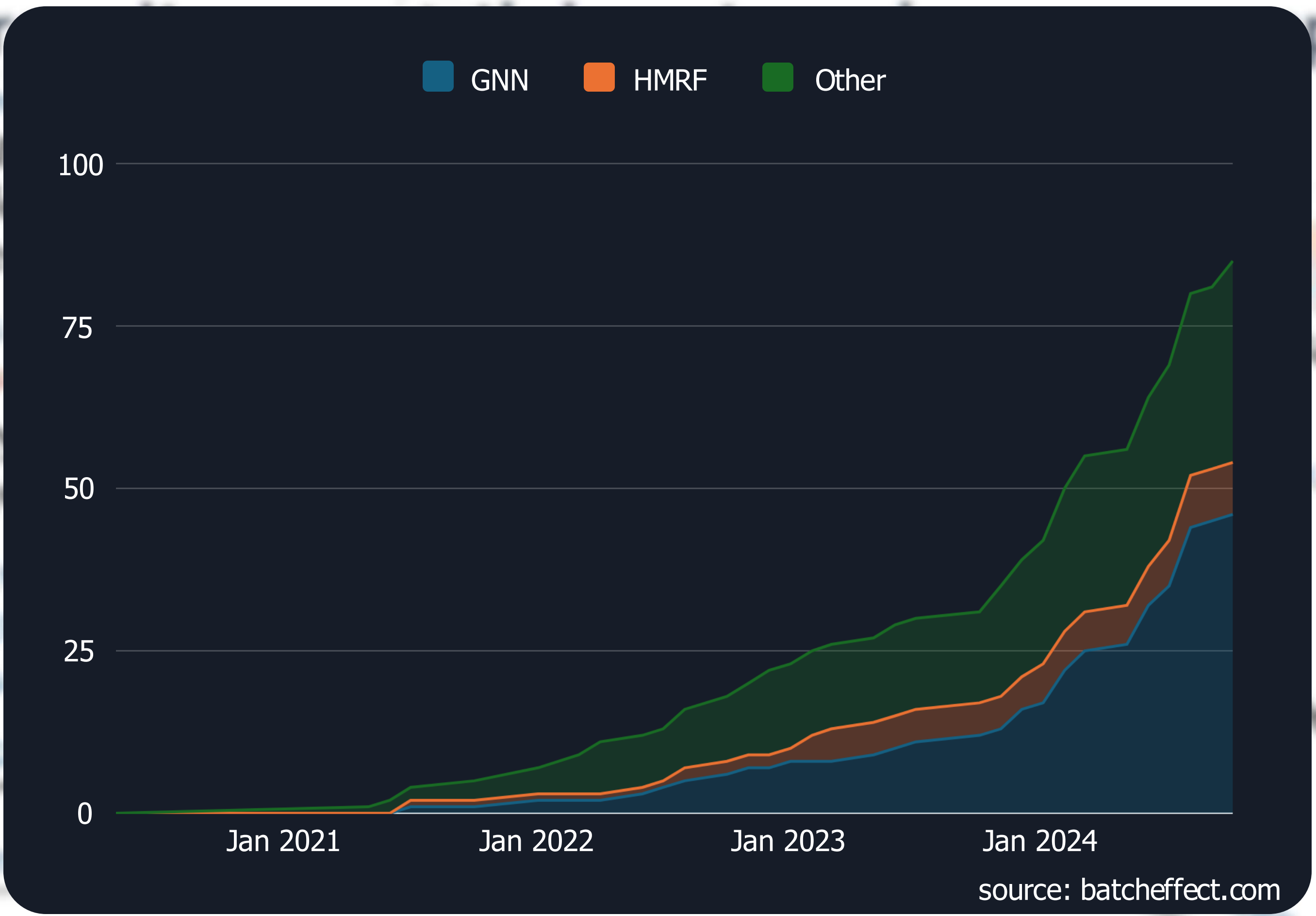
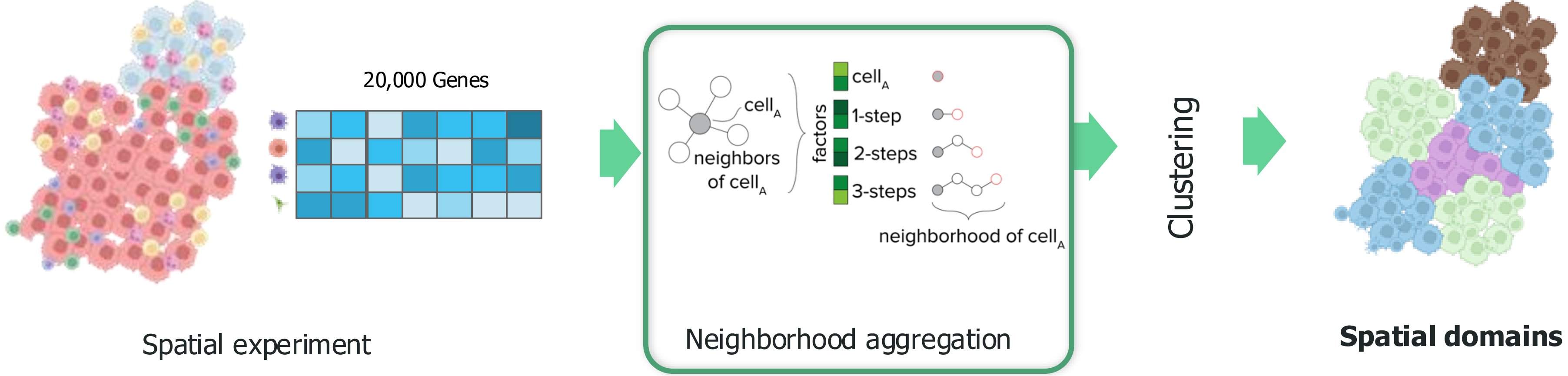
Honglei Ren, Benjamin L. Walker, Zixuan Cang & Qing Nie

Nature Communications 13, Article number: 4076 (2022) | Cite this article



source: batcheffect.com

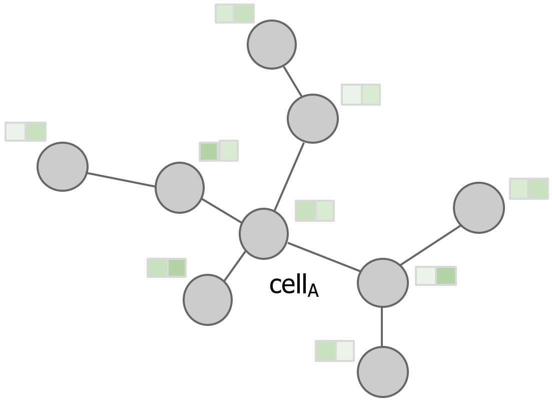
Our approach: neighborhood aggregation



Spatial experiment

Neighborhood aggregation

Spatial domains



→ Clustering → **Spatial domains**

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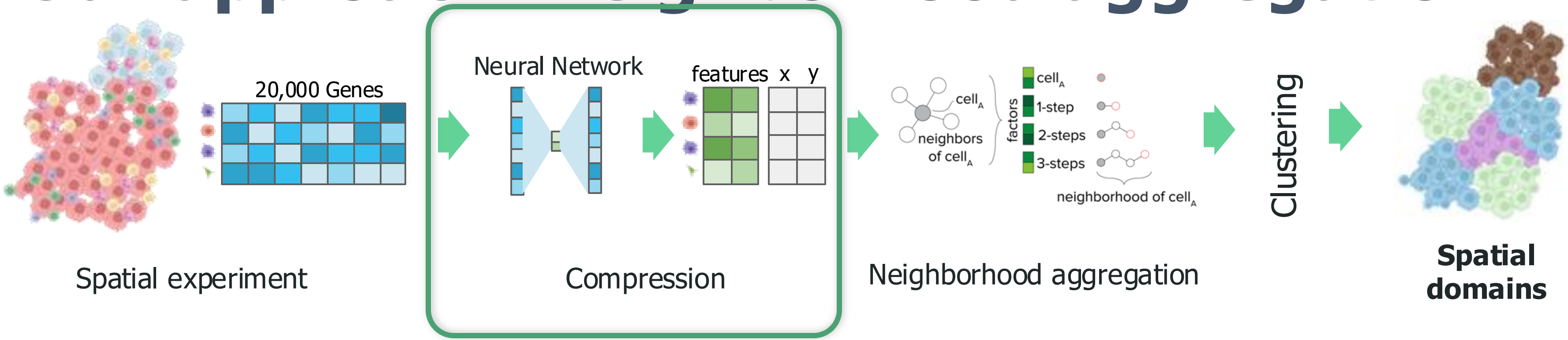

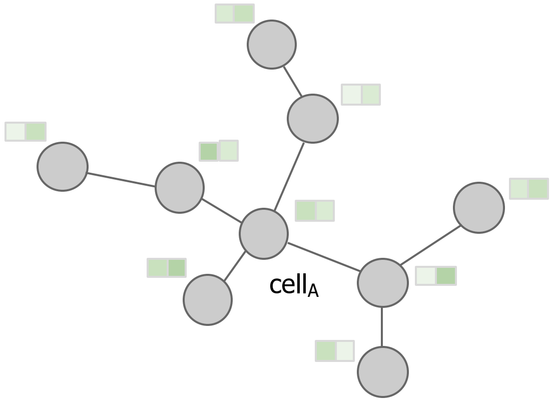

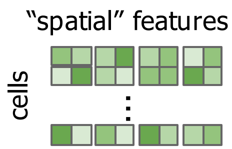

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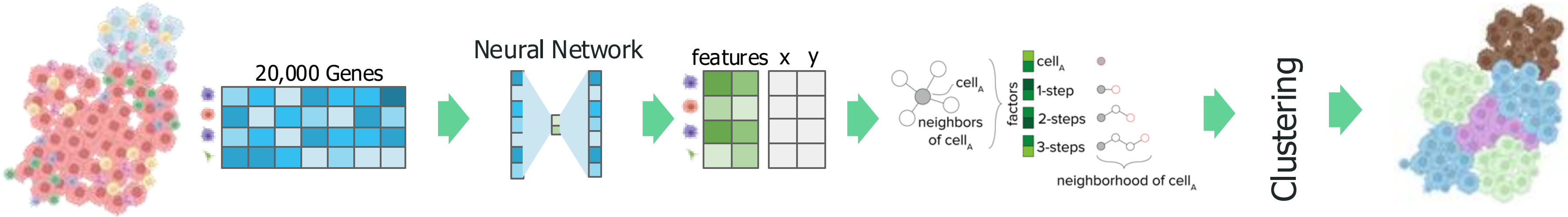

Spatial experiment

Compression

Neighborhood aggregation

Spatial domains

Scalable

+8M cells in 14 mins

No annotation required

Works directly on gene expression

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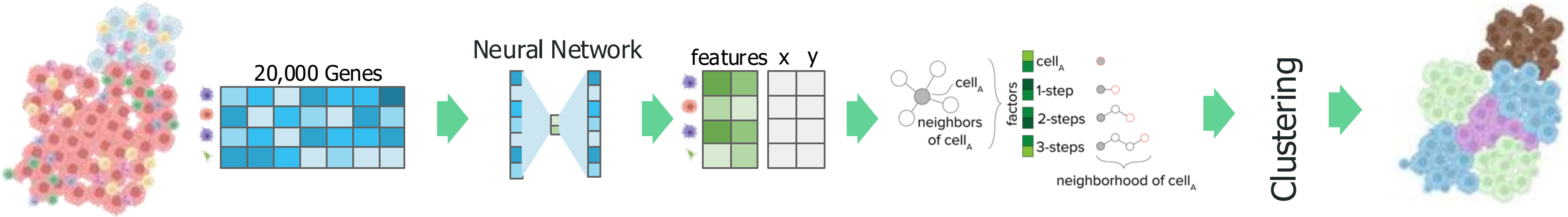

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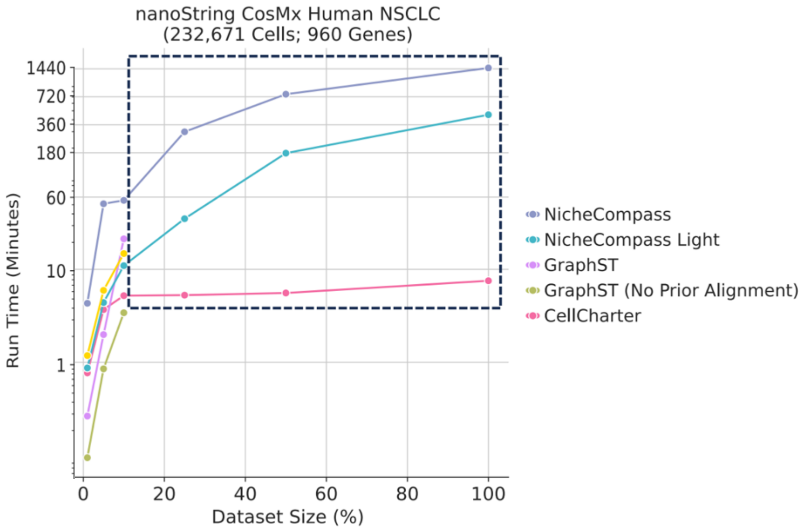

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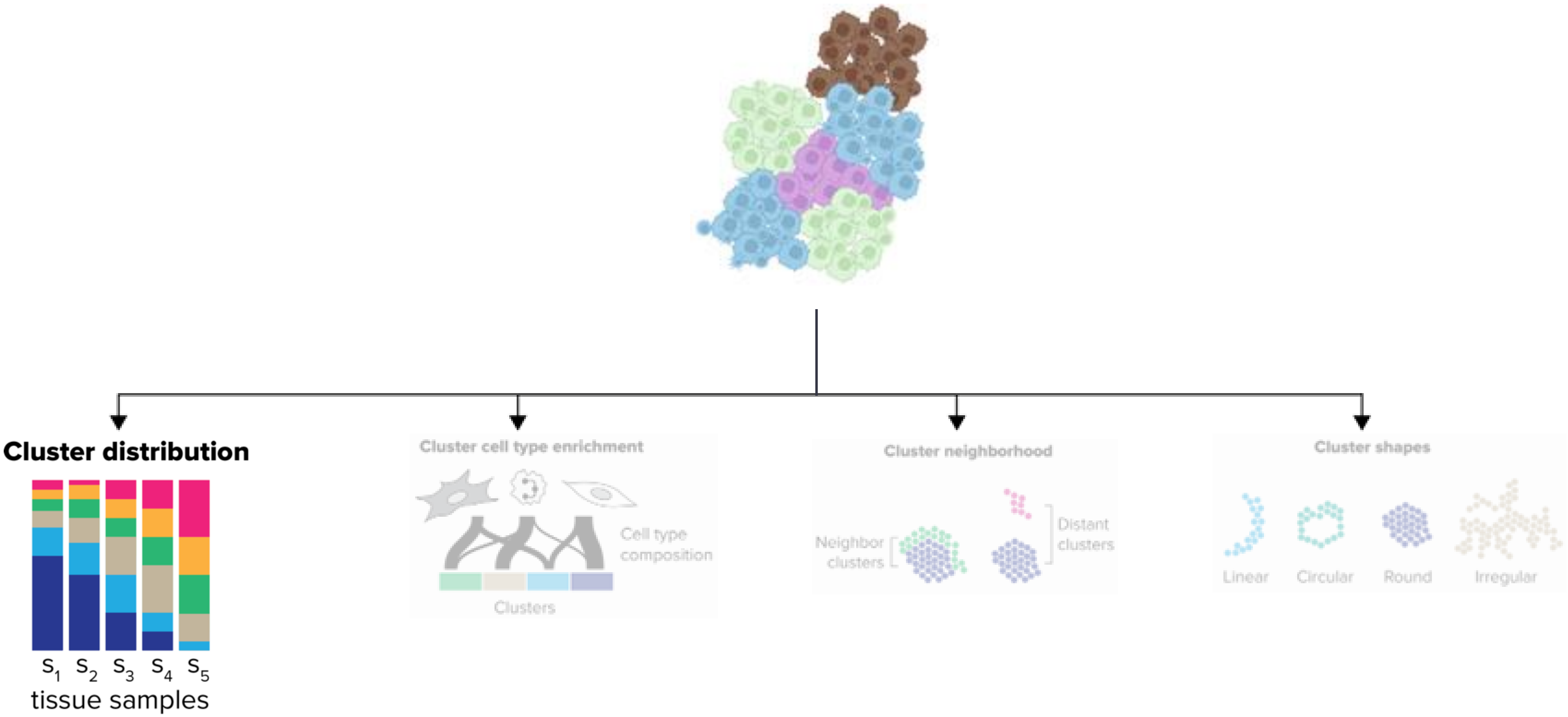

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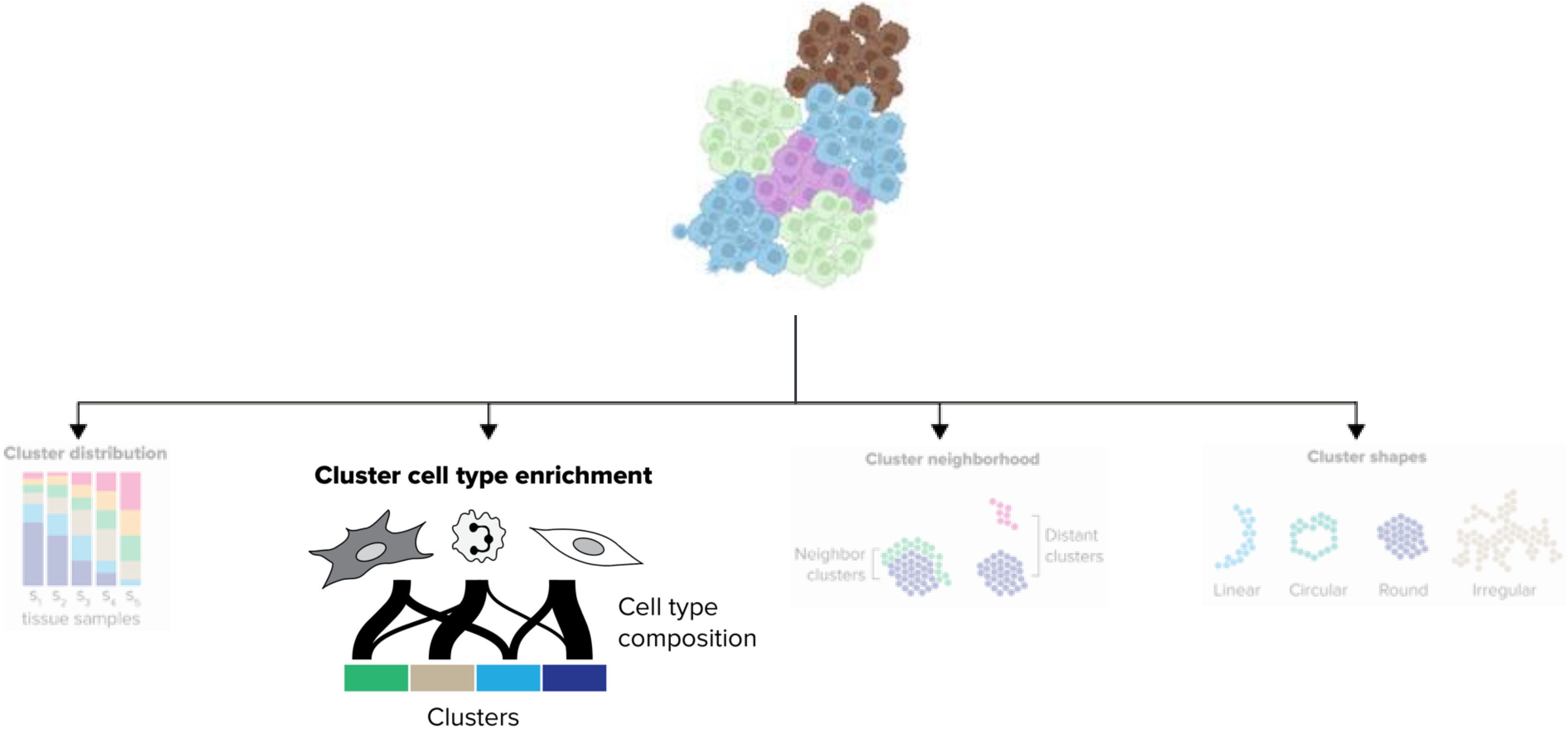

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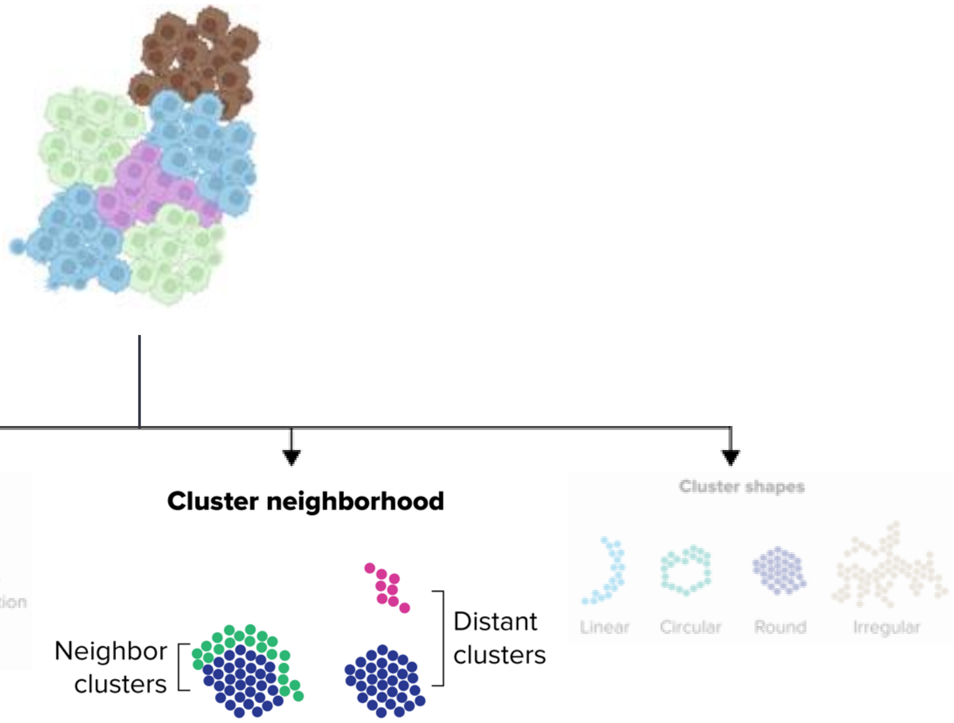

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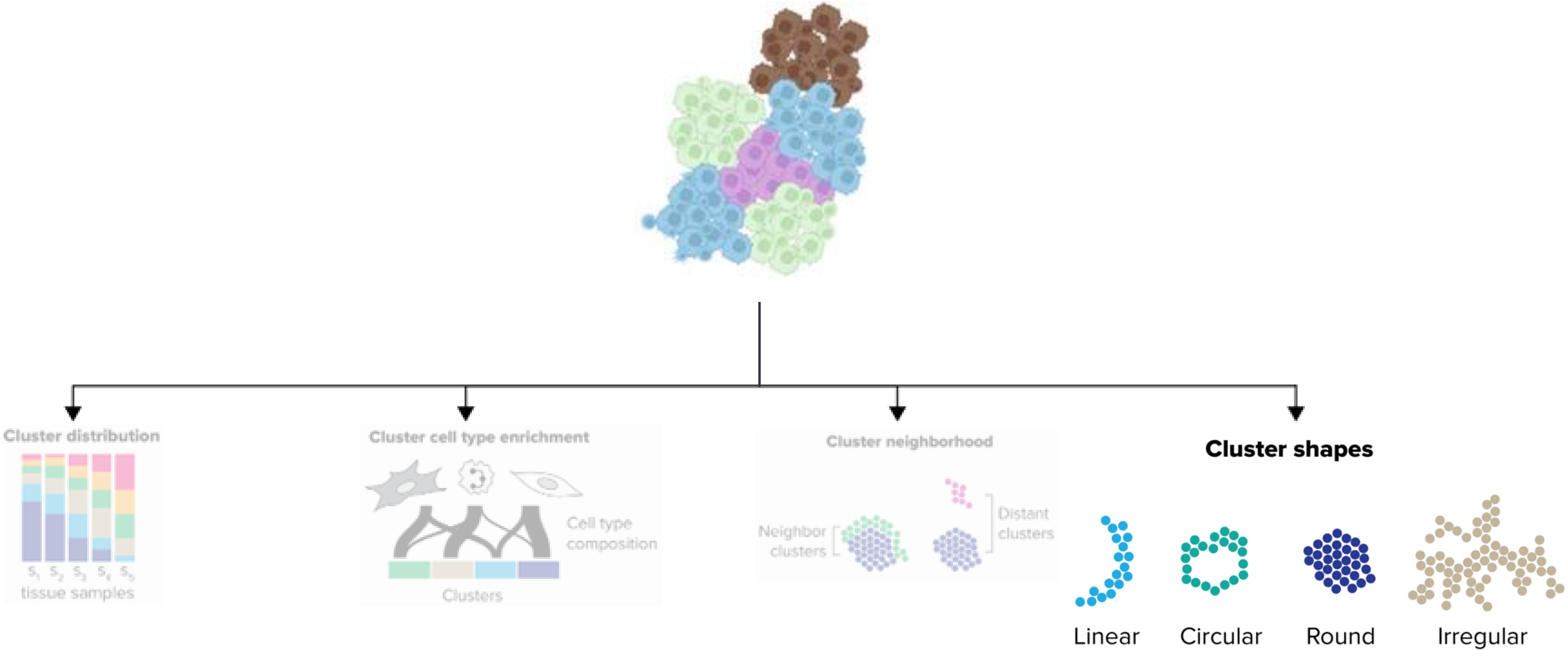

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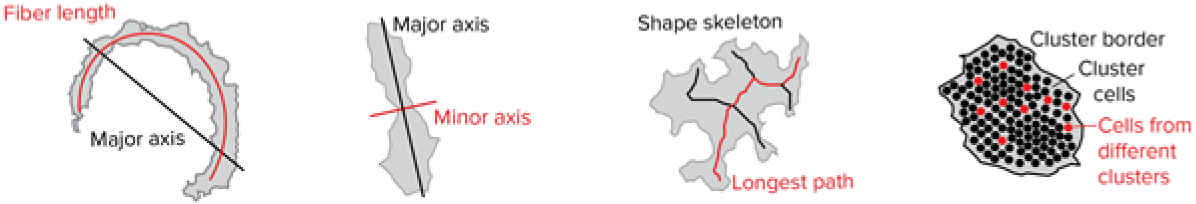
Domain shape analysis

Shape measures



$$\text{Curl} = 1 - \frac{\text{Major Axis}}{\text{Fiber Length}}$$

$$\text{Elongation} = 1 - \frac{\text{Minor Axis}}{\text{Major Axis}}$$

$$\text{Linearity} = \frac{\text{Longest path}}{\text{Shape skeleton}}$$

$$\text{Purity} = \frac{\text{\# cluster cells}^*}{\text{\# cells}^*}$$

(* within the cluster border)

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

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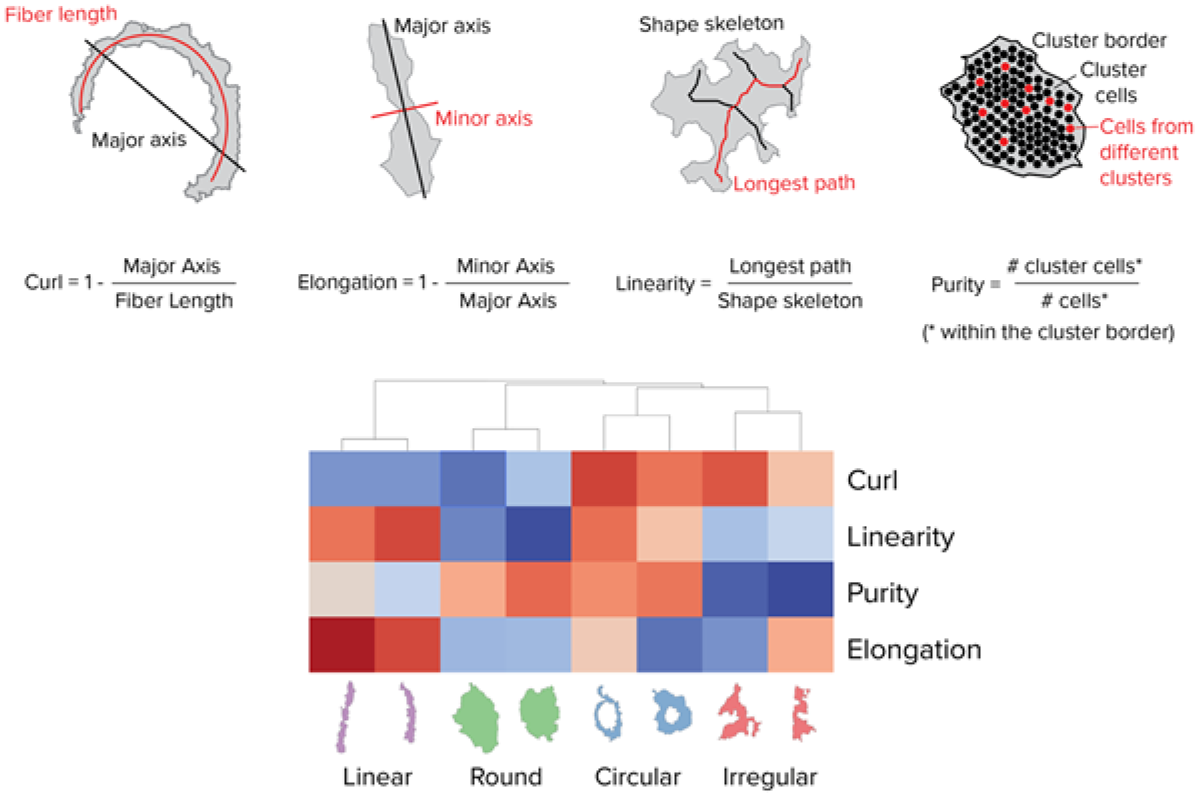

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(* within the cluster border)

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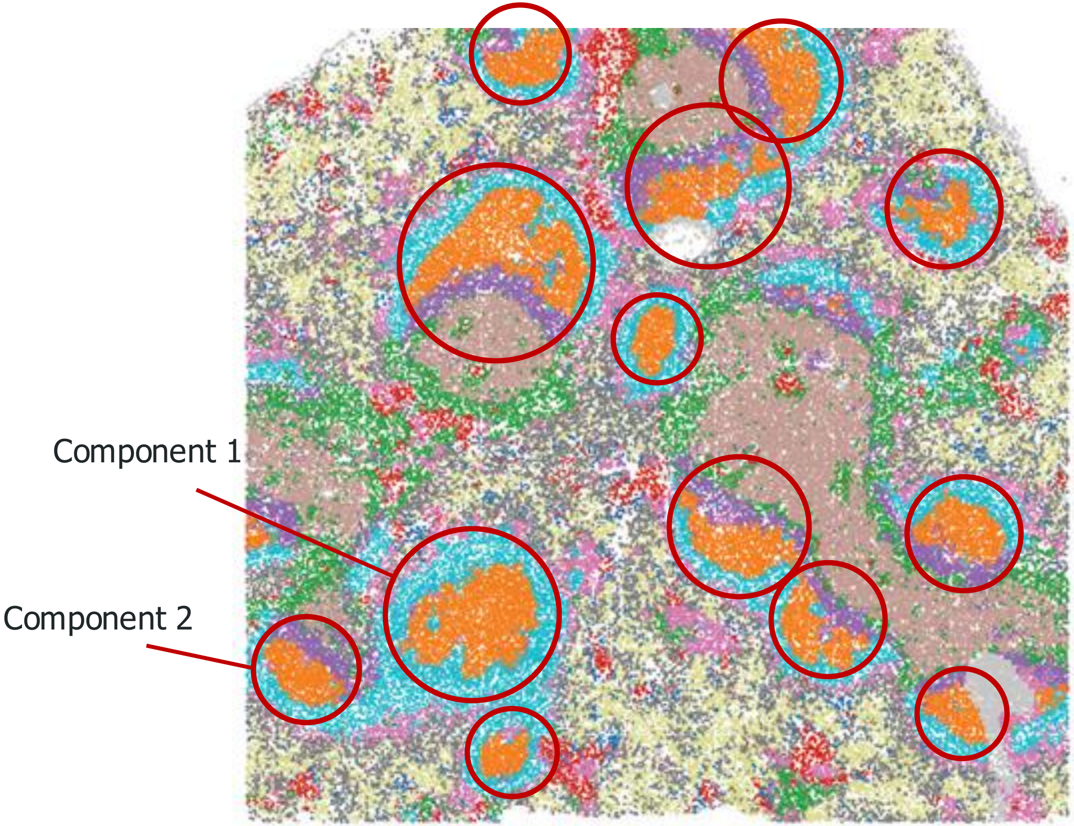
Shape measures

For each spatial domain

1. Split it into different **components**

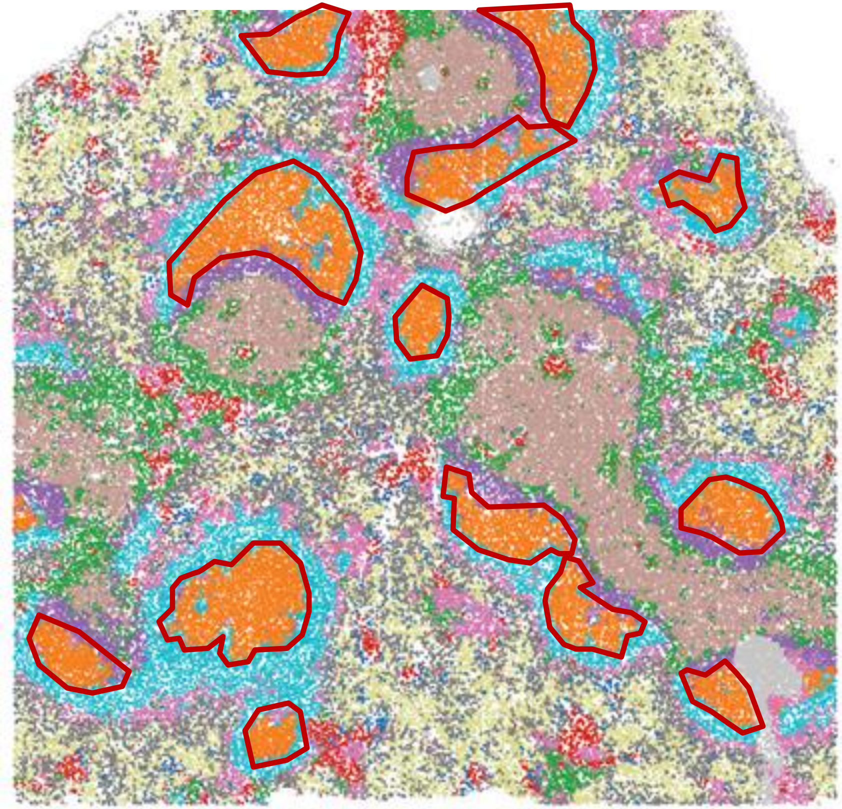


Shape measures

For each spatial domain

1. Split it into different **components**

2. Automatically draw the **boundary** around each components
   - Using alpha shapes



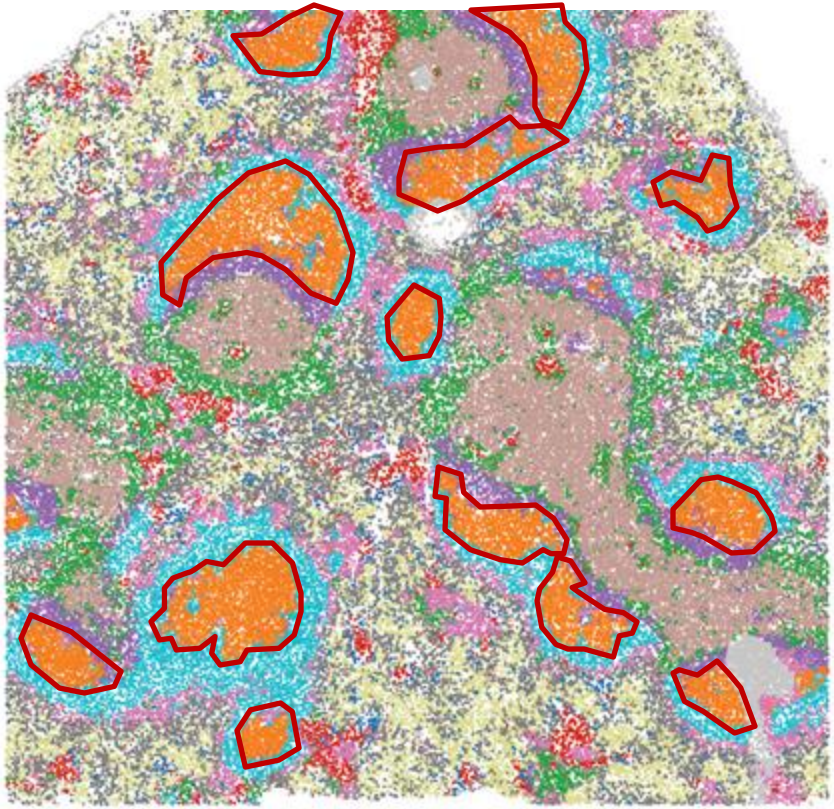
Shape measures

For each spatial domain

1. Split it into different **components**

2. Automatically draw the **boundary** around each components
   - Using alpha shapes

3. Compute **shapes metrics** for each component

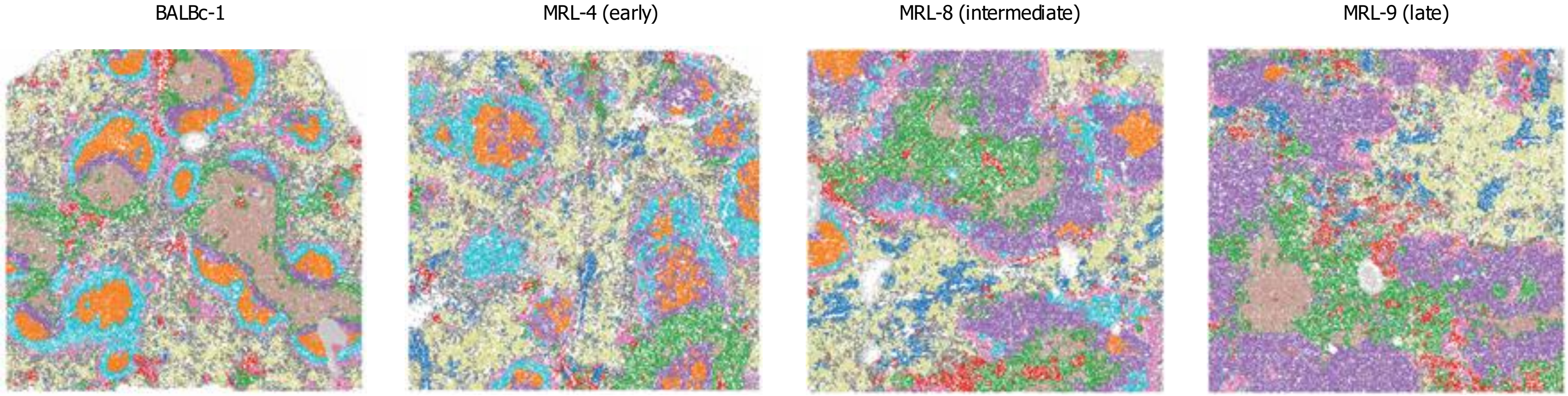


Domain shape changes in mouse lupus

Spatial proteomics with 30 markers applied to 9 samples of **mouse spleen** in 2 conditions:

- **3 Normal** (BALBc)
- **6 Sytemic lupus** (MRL): autoimmune disease

Goltsev et al., 2018



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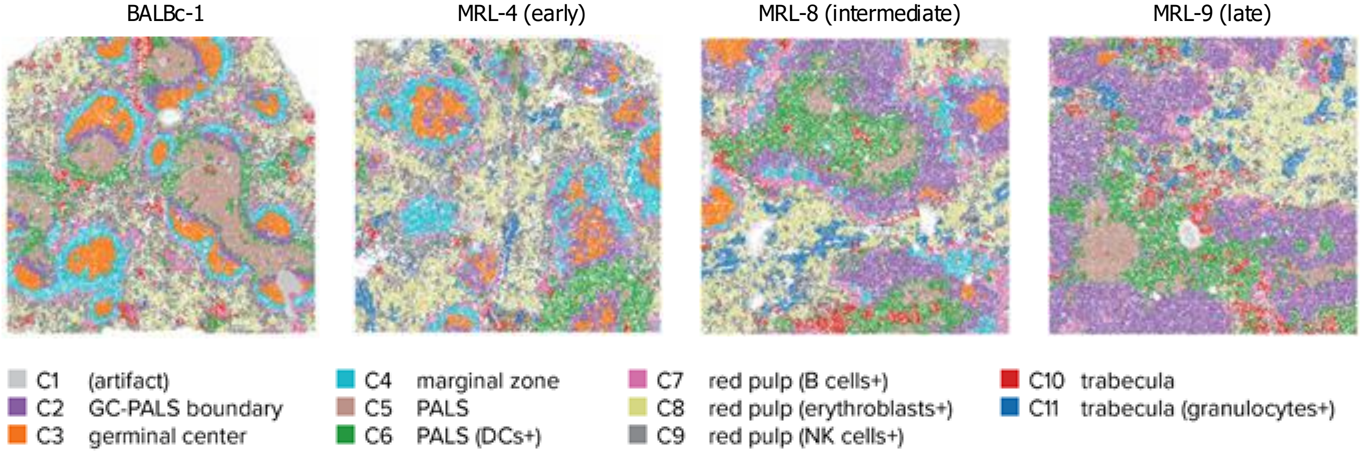
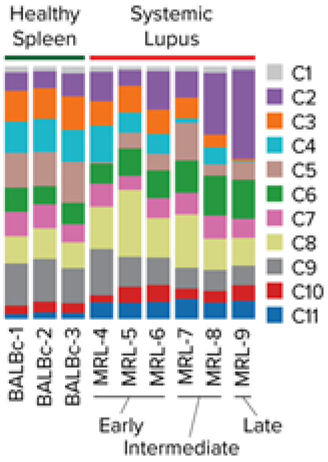


From normal to lupus

- No new spatial domain



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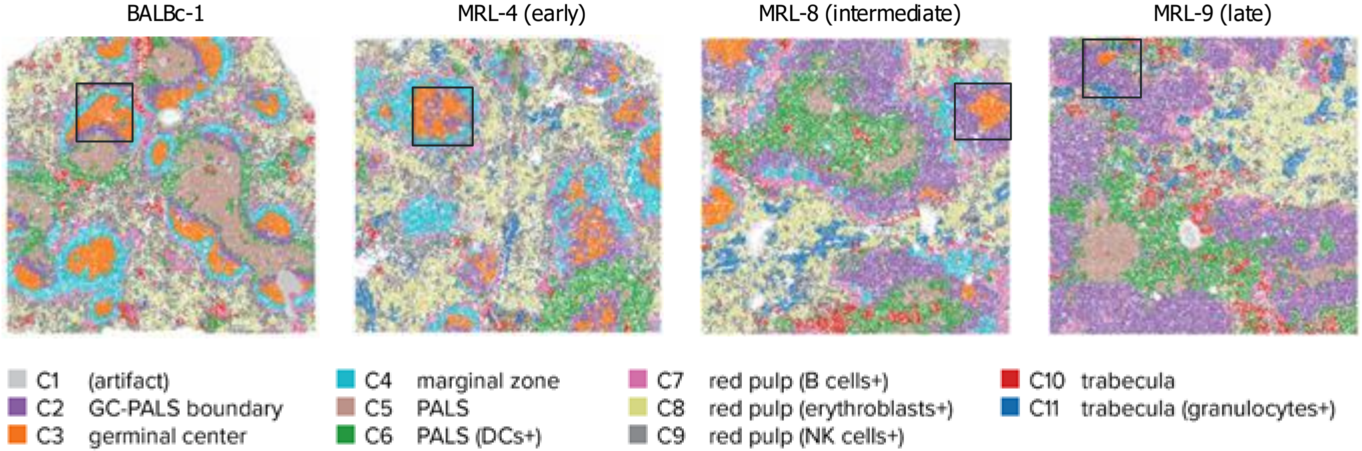
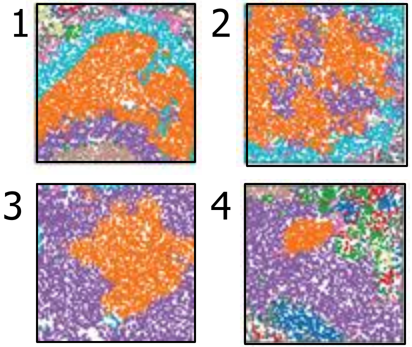
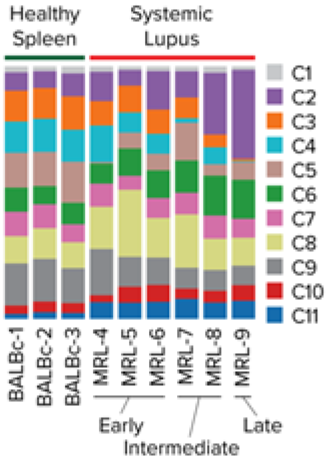




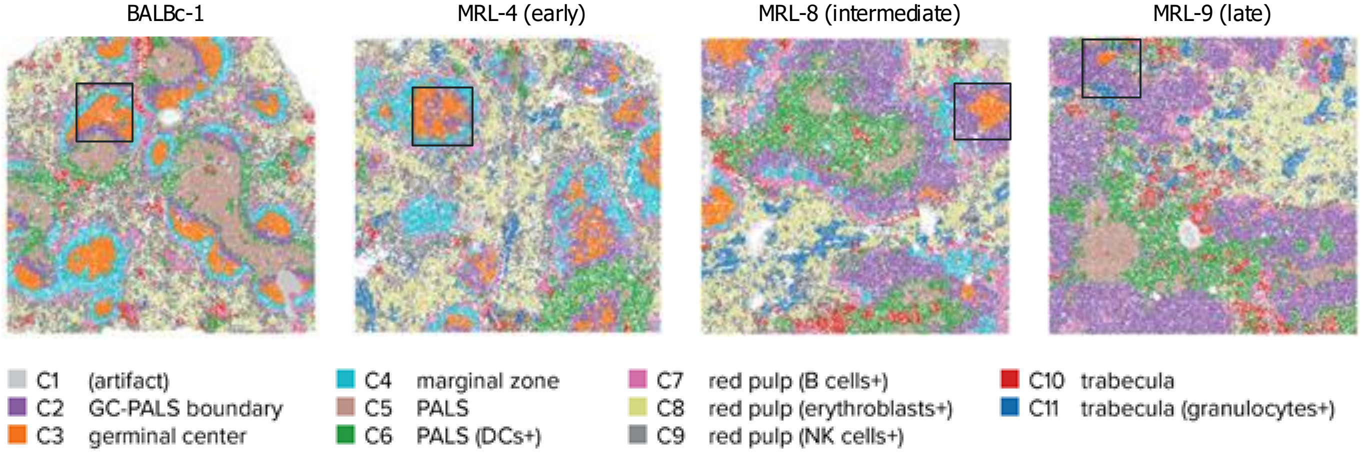


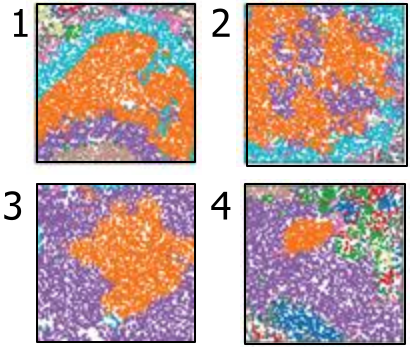
From normal to lupus

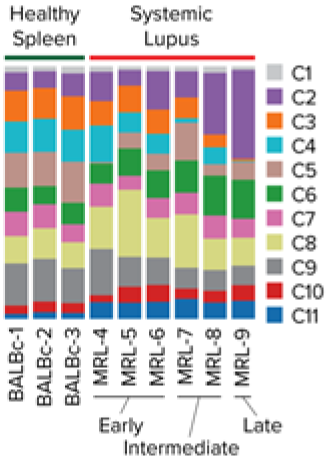
- No new spatial domain
- **C2 - GC-PALS** expands and infiltrates **C3 - germinal center**

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

Domain shape changes in mouse lupus



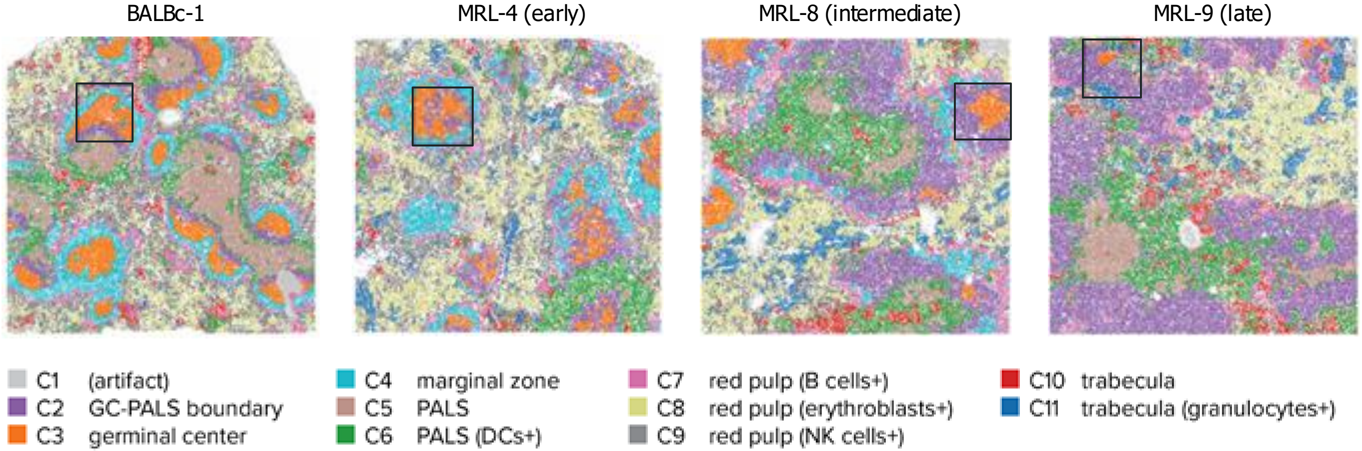


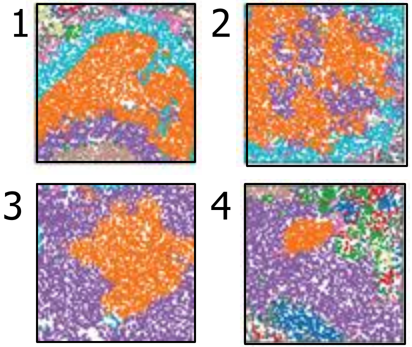


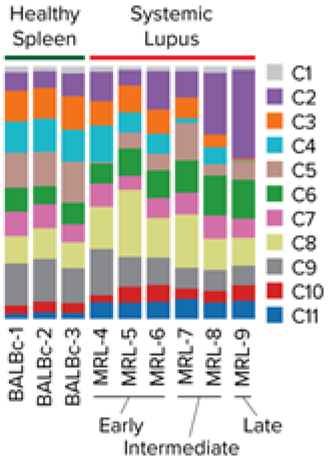
From normal to lupus

- No new spatial domain
- **C2 - GC-PALS** expands and infiltrates **C3 - germinal center**
- **C3 - germinal center** acquires a more **irregular** shape

Domain shape changes in mouse lupus



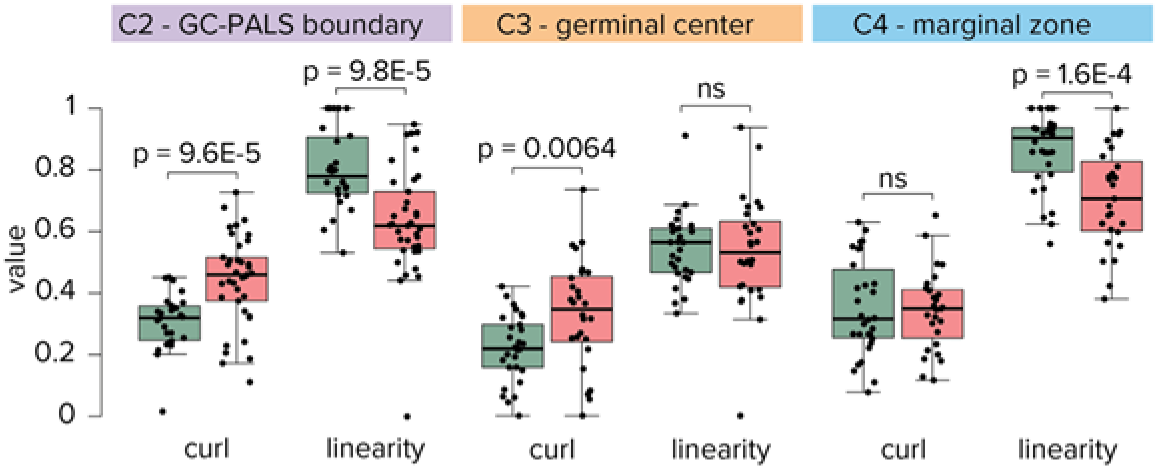


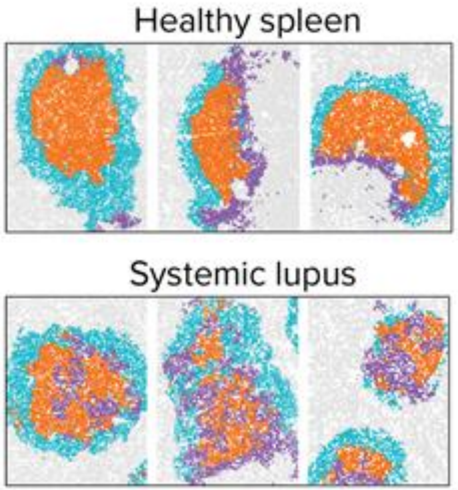


From normal to lupus

- No new spatial domain
- **C2 - GC-PALS** expands and infiltrates **C3 - germinal center**
- **C3 - germinal center** acquires a more **irregular** shape
- **C2 - GC-PALS** and **C4 - marginal zone** lose their **linear**/**circular** shape

Shape analysis





- **C3 - germinal center acquires a more irregular shape**
- **C2 - GC-PALS and C4 - marginal zone lose their linear/curved shape**

How to find the “best” number of clusters?

There may not be a “best” one

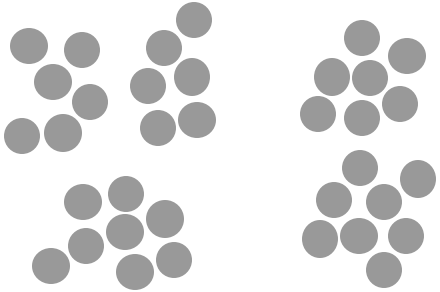
Finding K

- There may not be an unequivocally **best** number of clusters K
- Tissues and samples are often organized into hierarchies

Cancer

Individual tumors

Tumor subpopulations



Tumor cells

- All levels may be valid clusterings

Find one or more good candidates within a range (K_{min}, K_{max})

Finding K

- There may not be an unequivocally **best** number of clusters K
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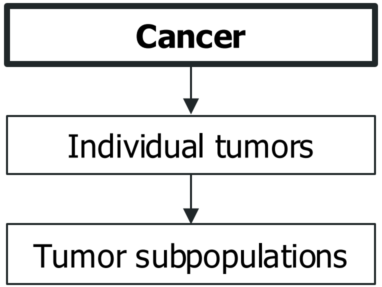
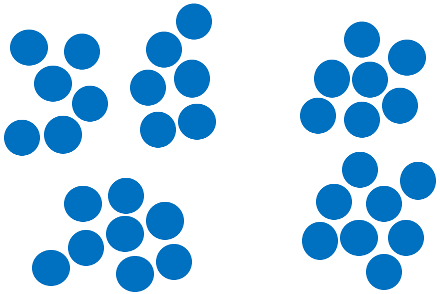


Cancer

Individual tumors

Tumor subpopulations



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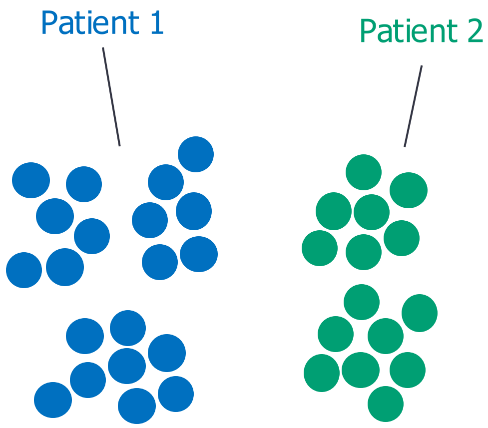
Cancer
↓
Individual tumors
↓
Tumor subpopulations



Tumor cells

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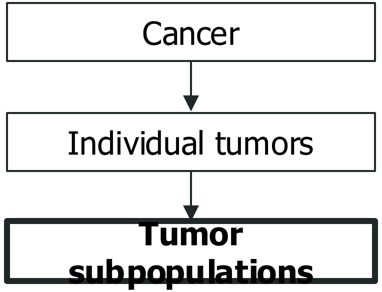
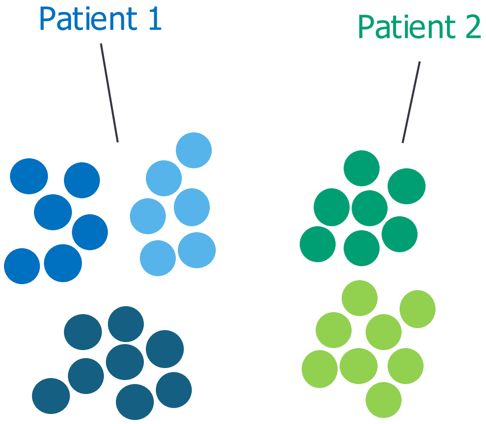

Cancer

Individual tumors

Tumor subpopulations



Tumor cells

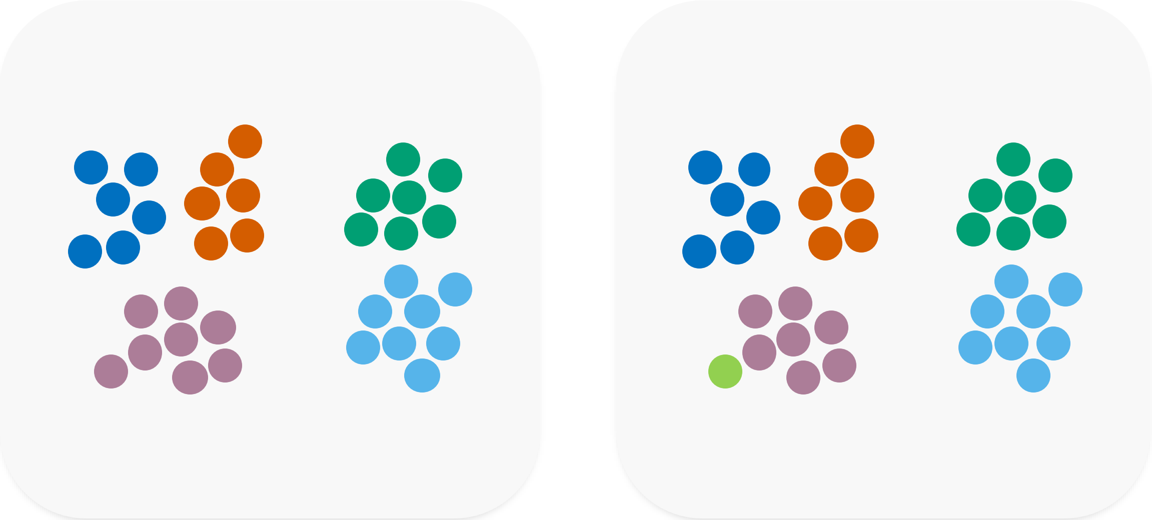
- All levels may be valid clusterings

Find one or more good candidates within a range (K_{min}, K_{max})

Cluster stability

Find one or more good candidates within a range (K_{min}, K_{max})

Best K = highest stability when changing K



K=5 **K=6** …

Cluster stability

Find one or more good candidates within a range (K_{min}, K_{max})

Best K = highest stability when changing K

Cluster stability

Find one or more good candidates within a range (K_{min}, K_{max})

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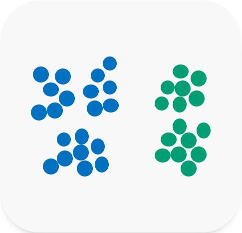
Run 1



number of clusters

Cluster stability

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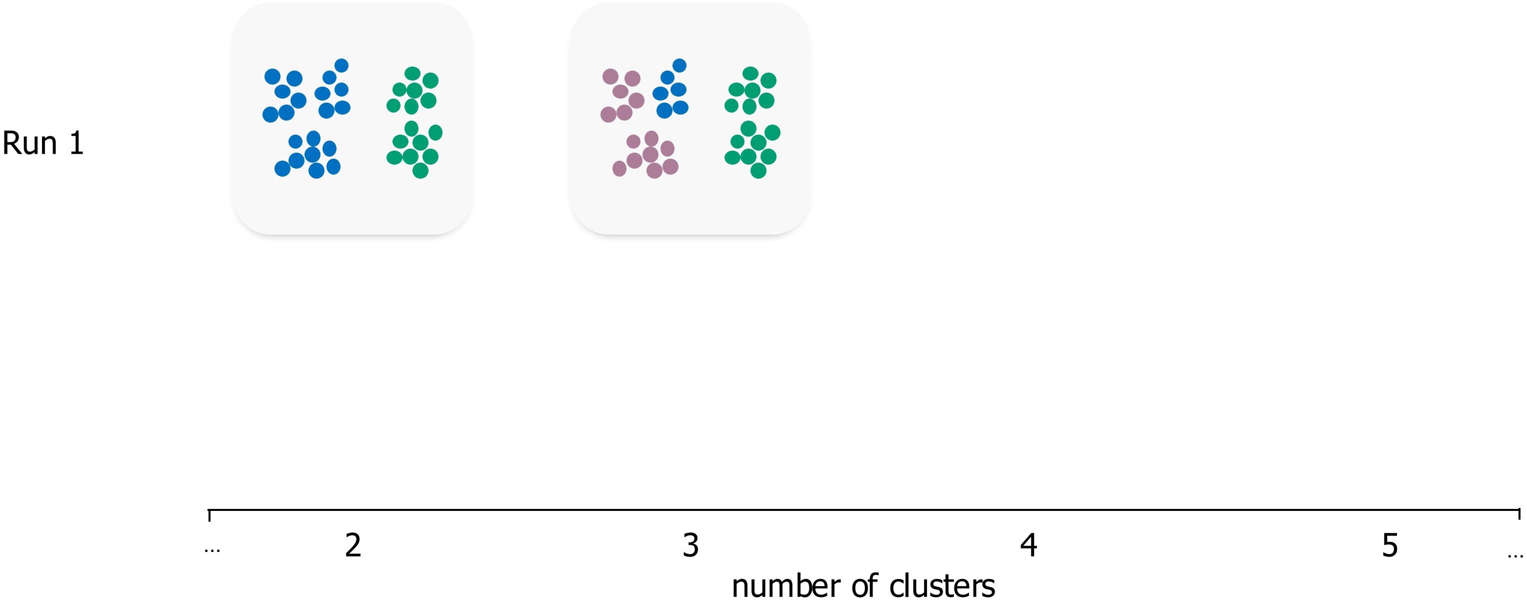

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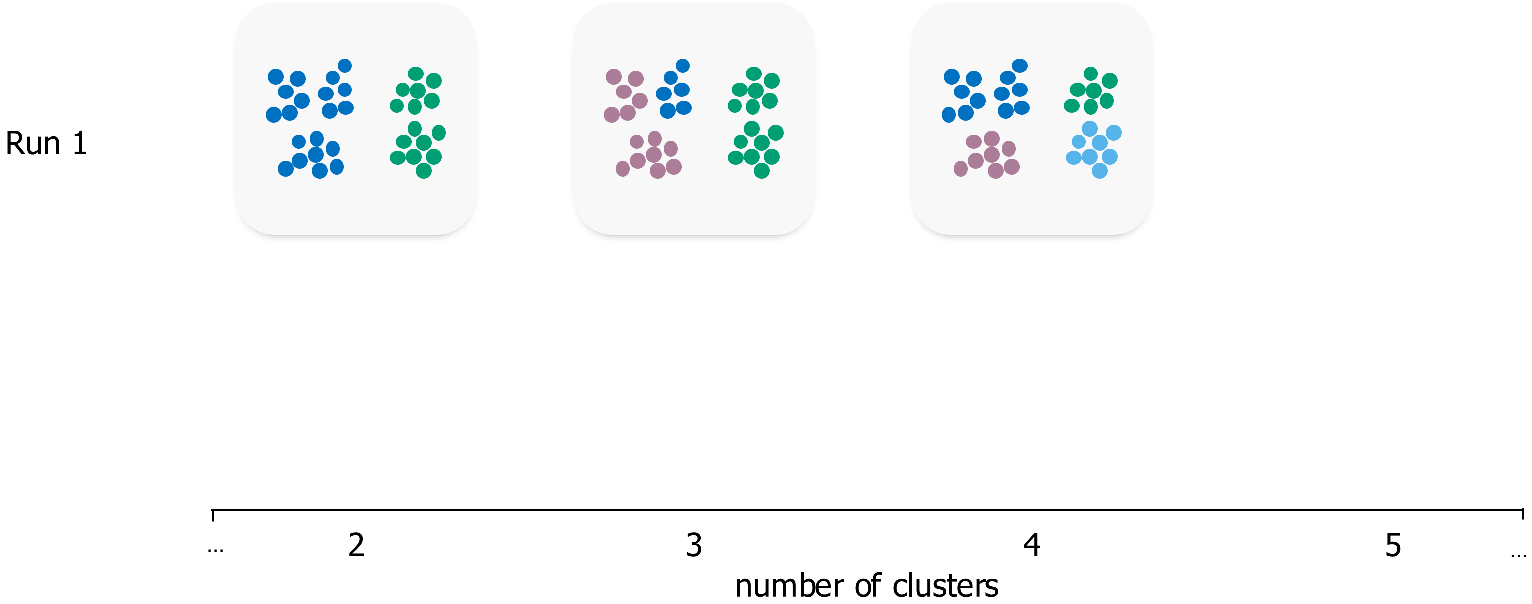

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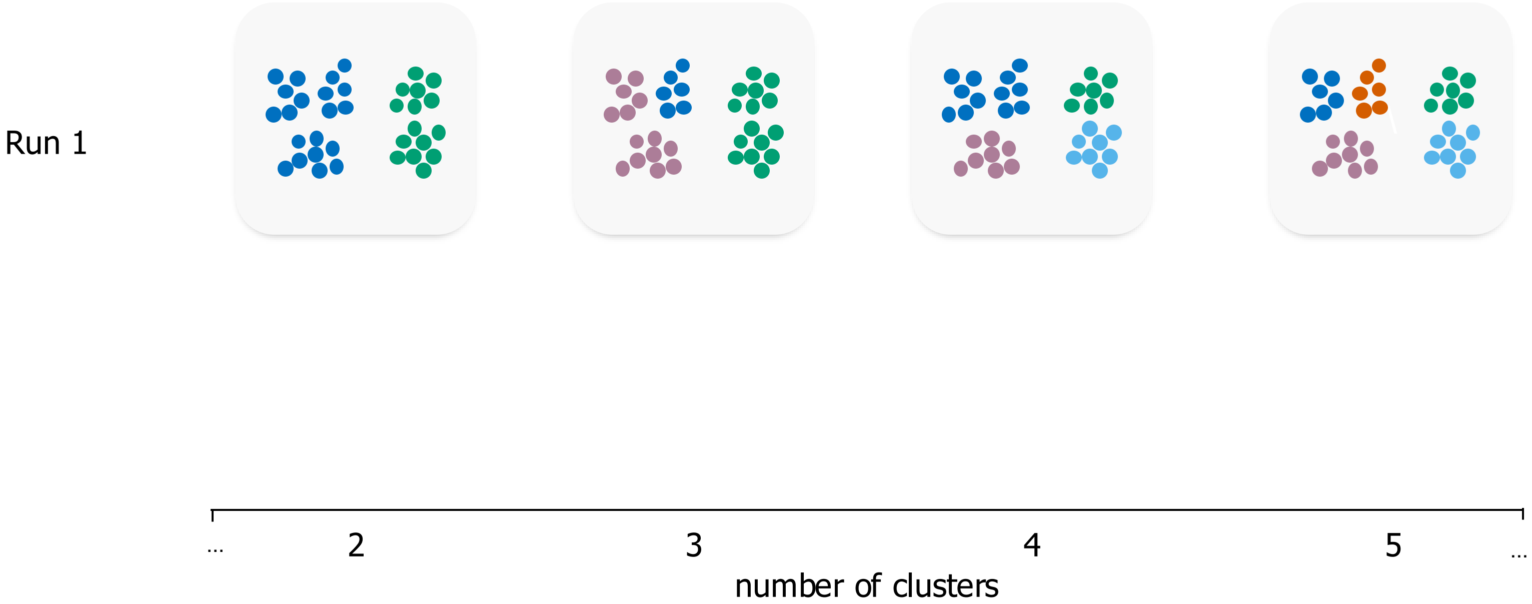

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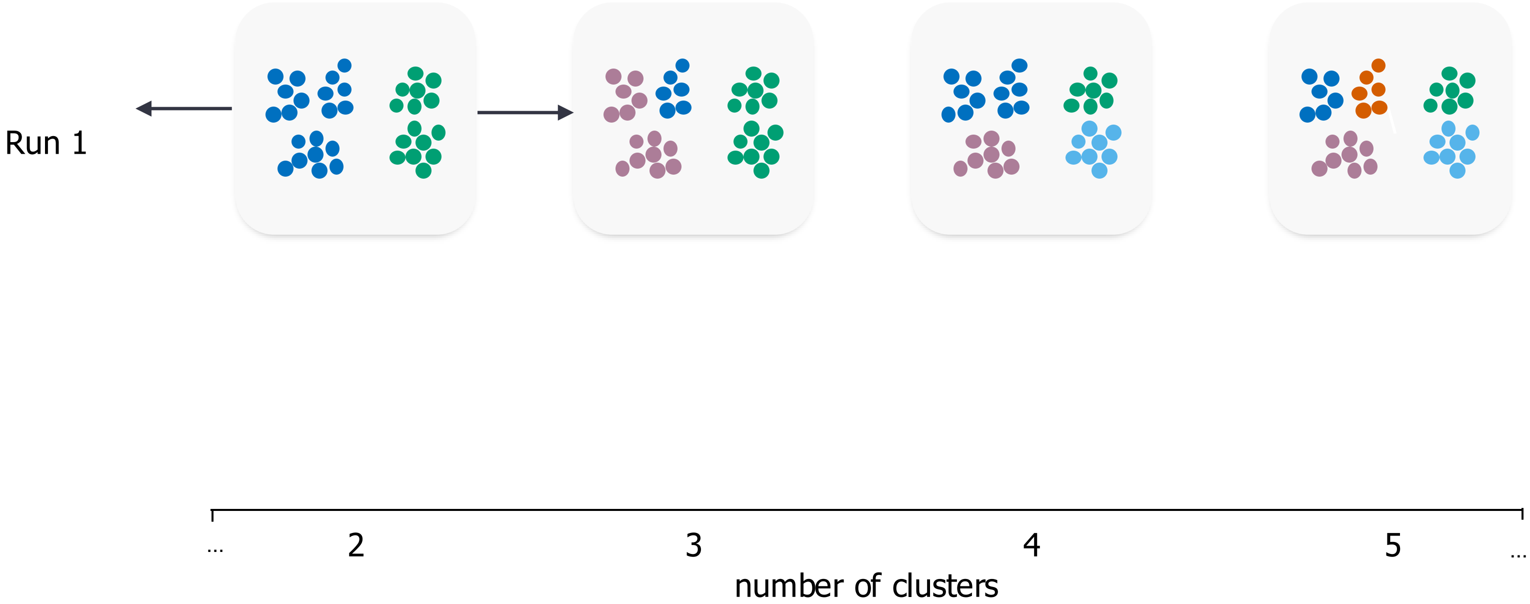

Run 1

… 2 3 4 5 …

number of clusters

Cluster stability

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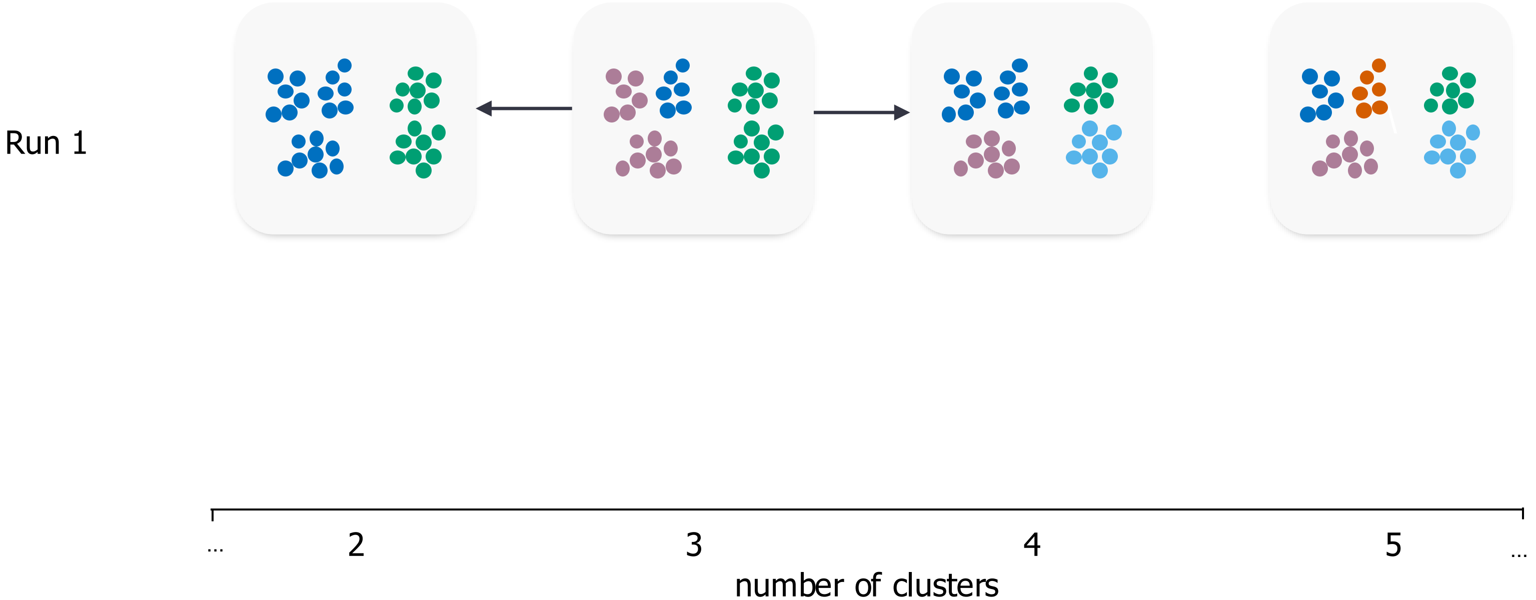

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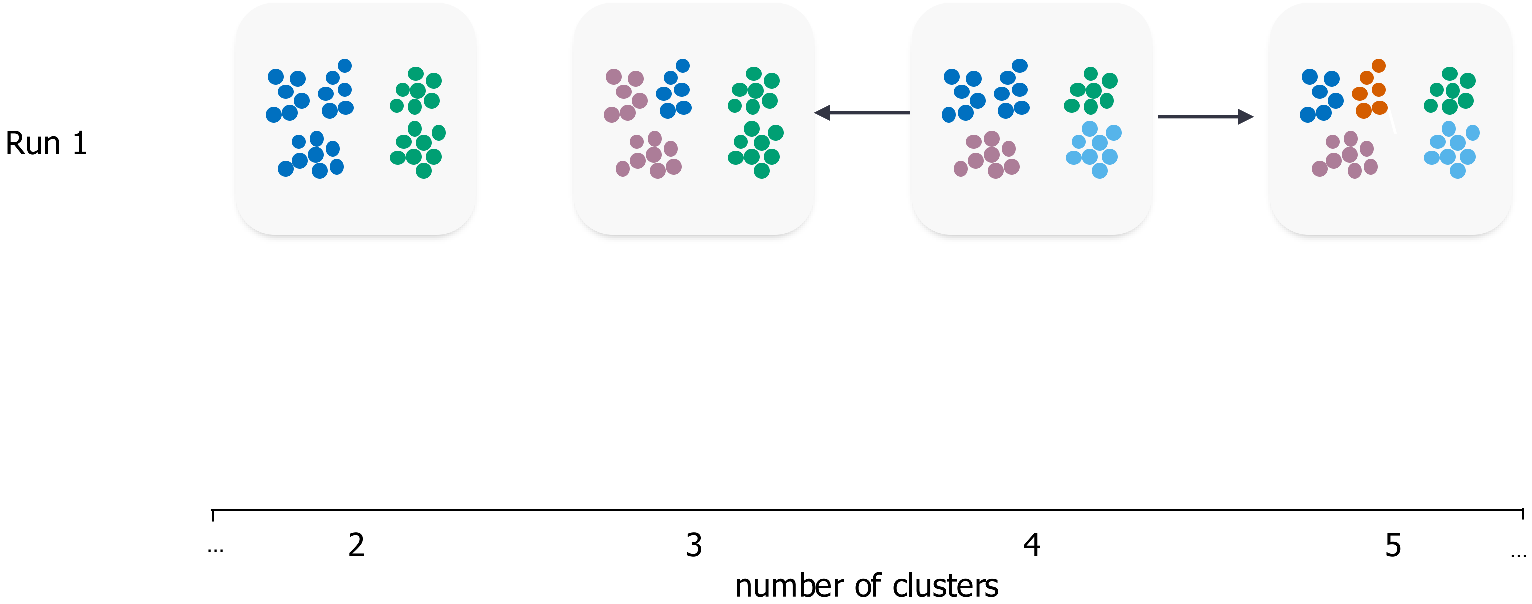

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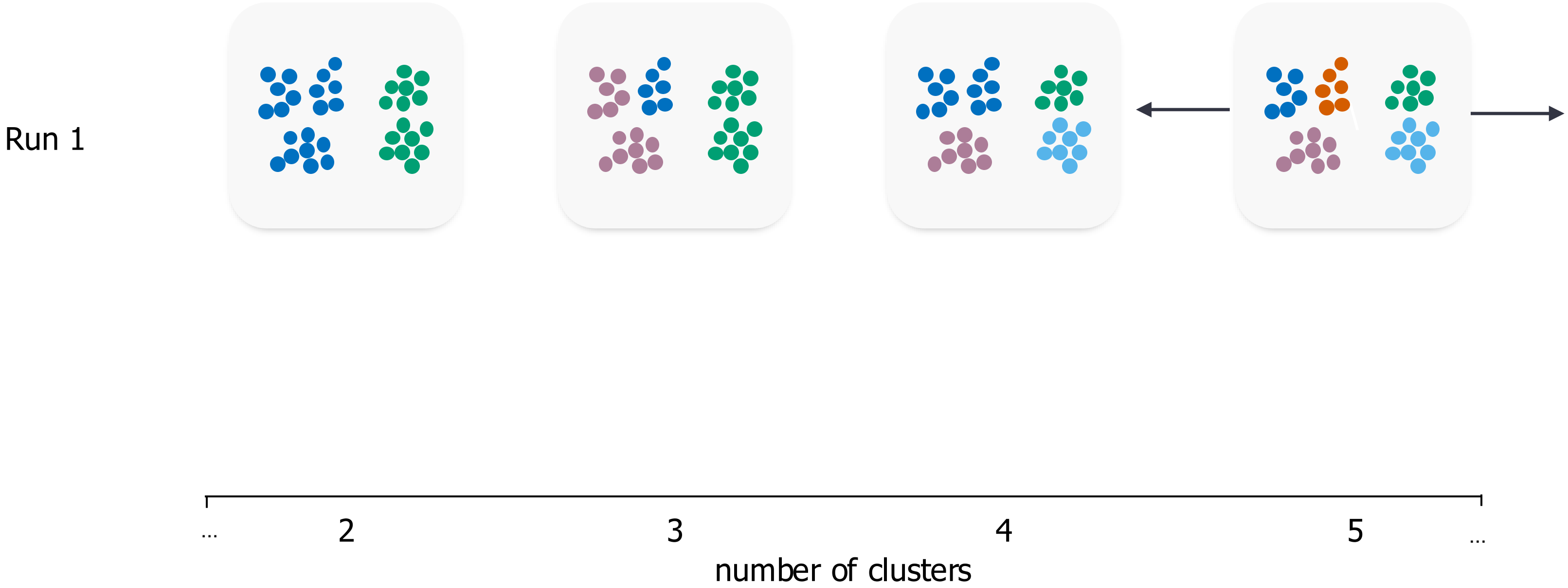

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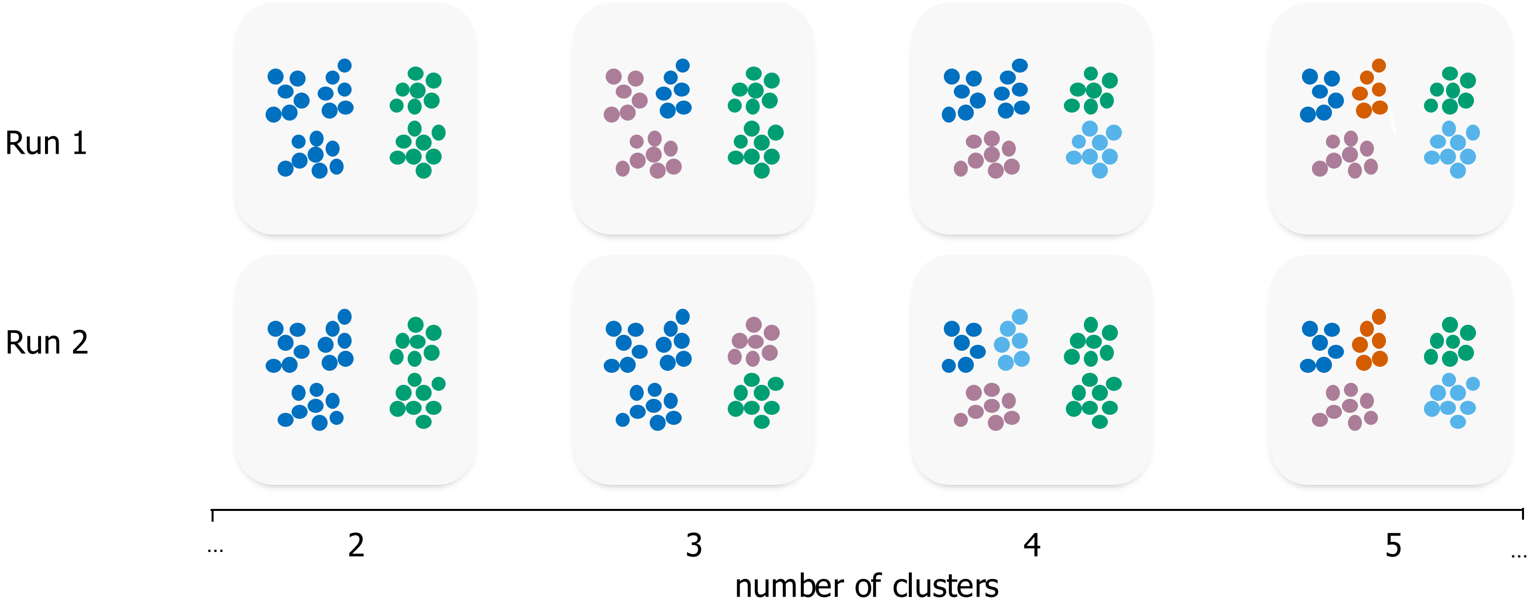
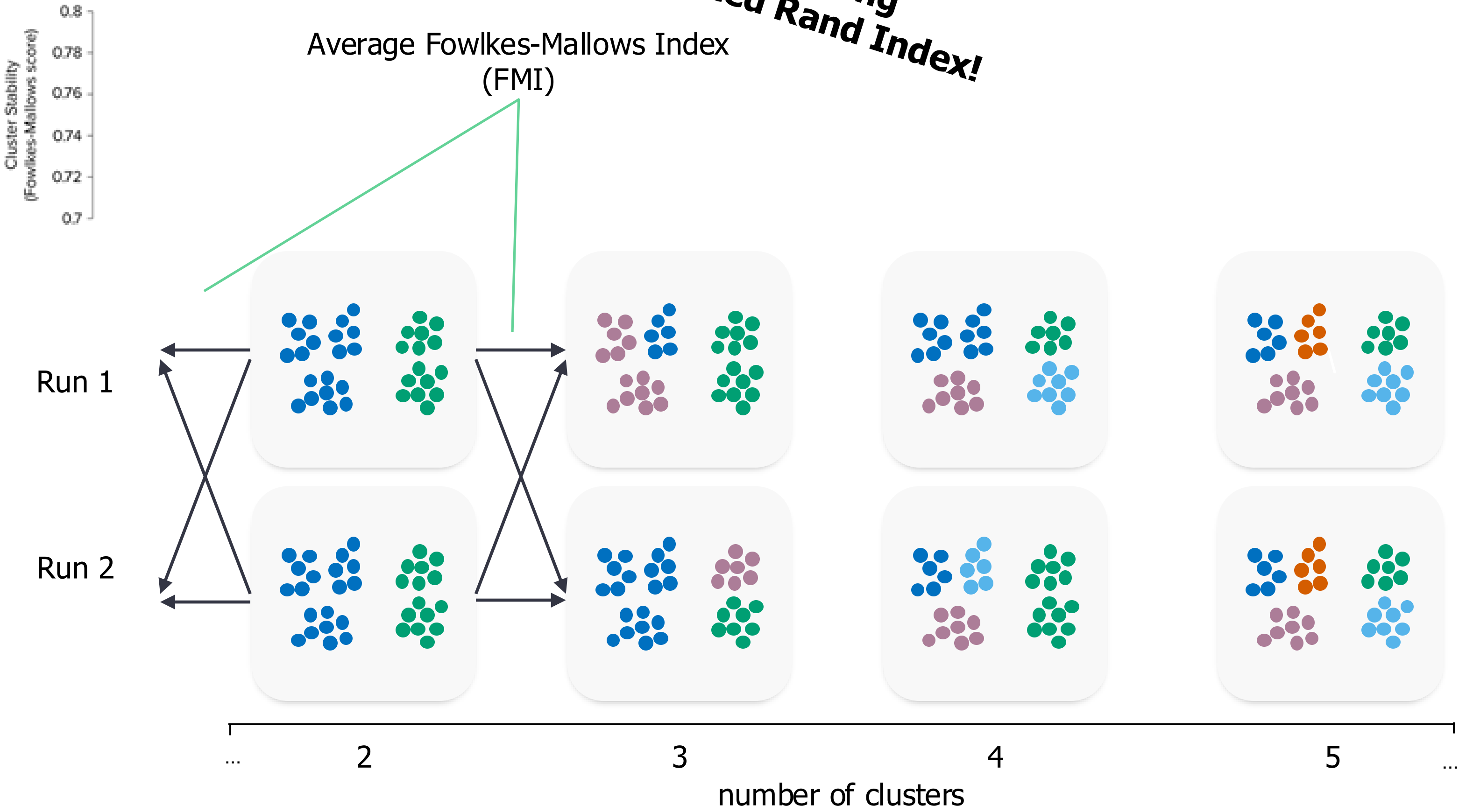


Cluster stability

Now using Adjusted Rand Index!



Average Fowlkes-Mallows Index
(FMI)

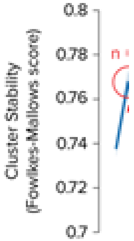
Run 1

Run 2

... 2 3 4 5 ...

number of clusters

Cluster stability



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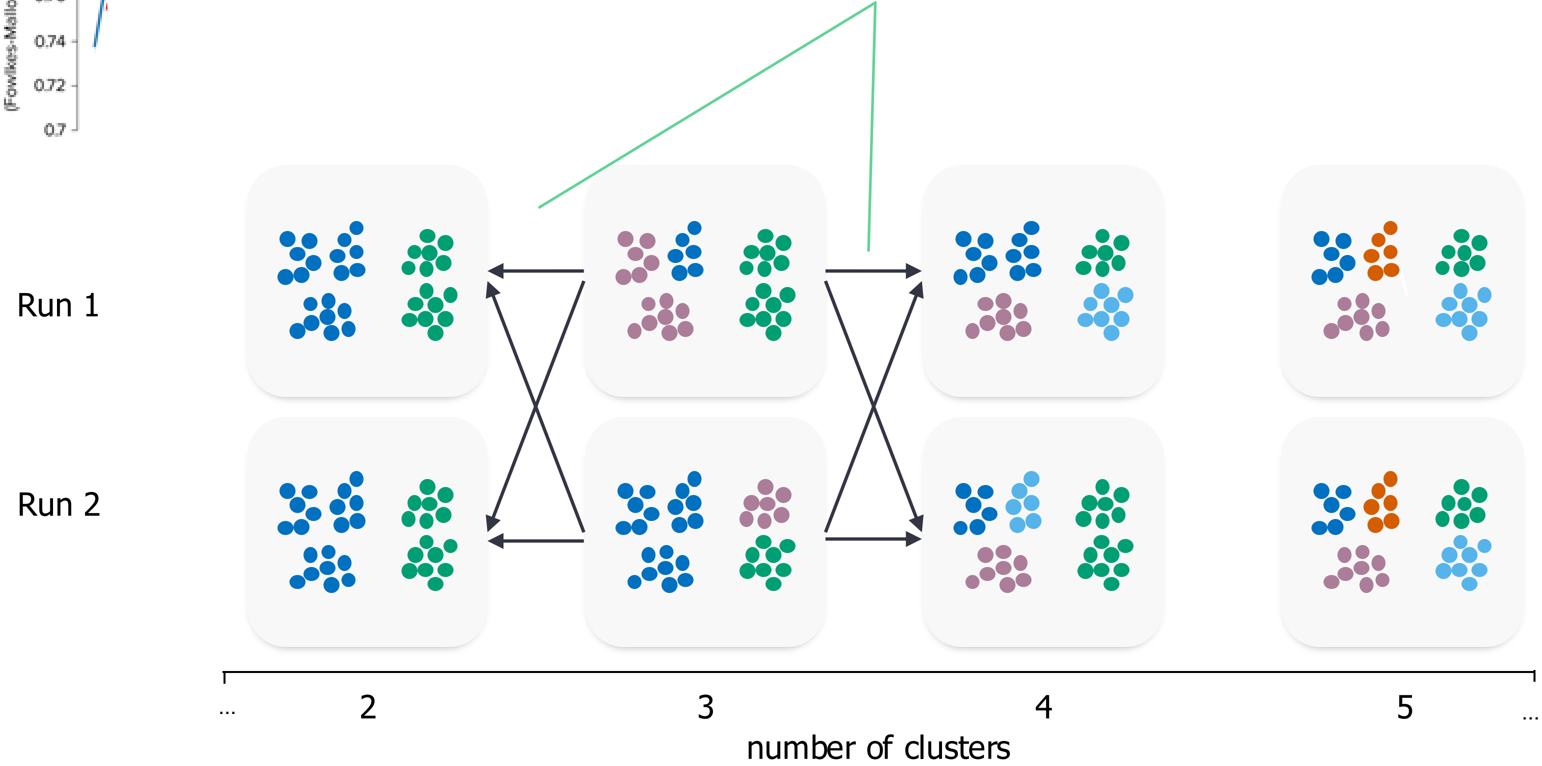


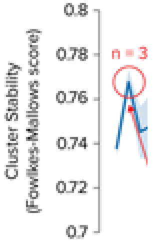
Run 1

Run 2

… 2 3 4 5 …

number of clusters

Cluster stability



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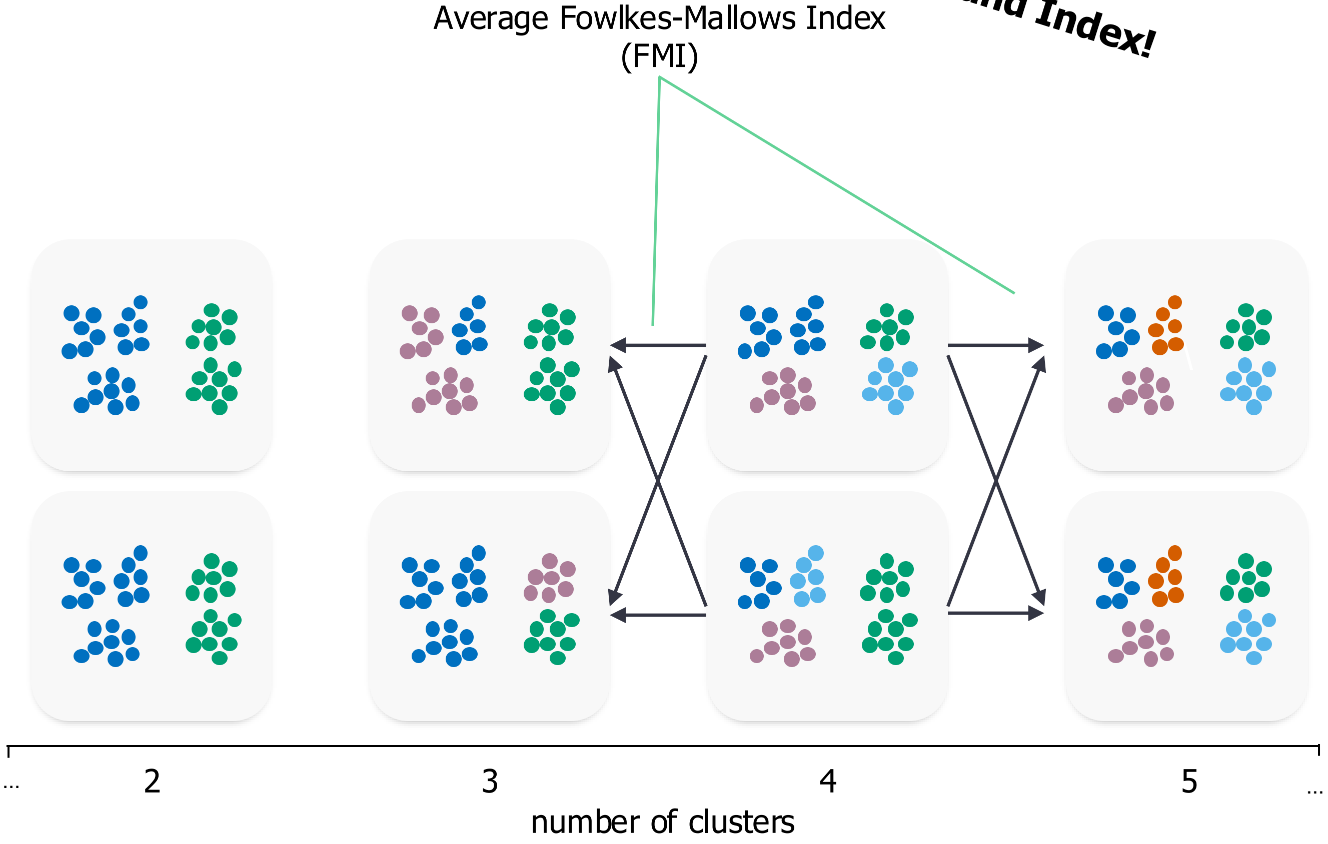
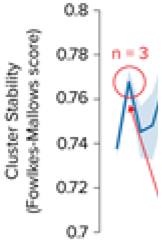

Average Fowlkes-Mallows Index (FMI)

Cluster stability

Now using Adjusted Rand Index!



Average Fowlkes-Mallows Index
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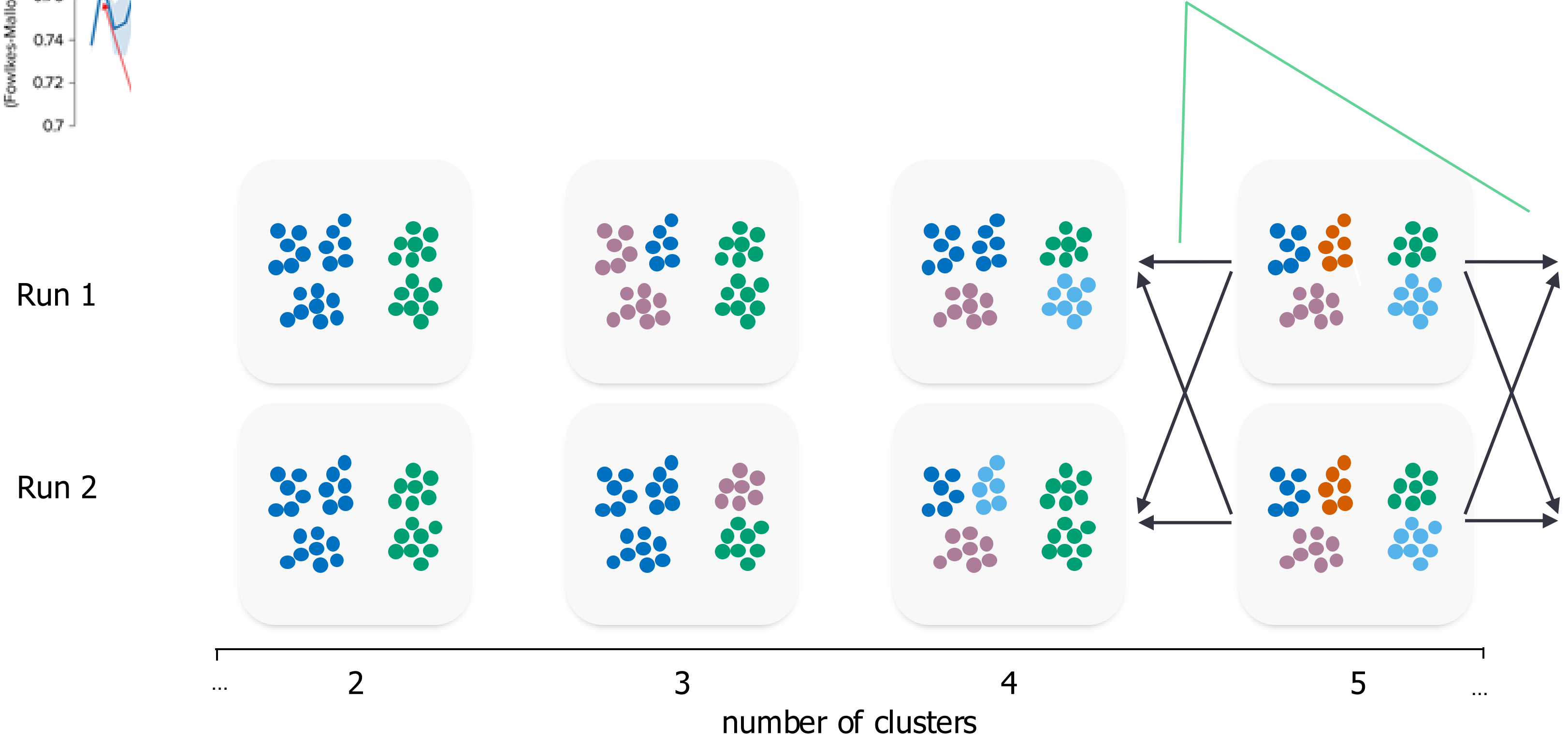
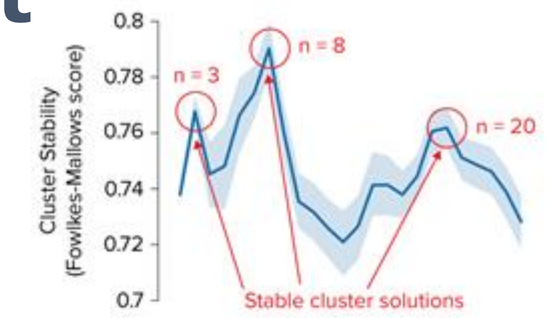
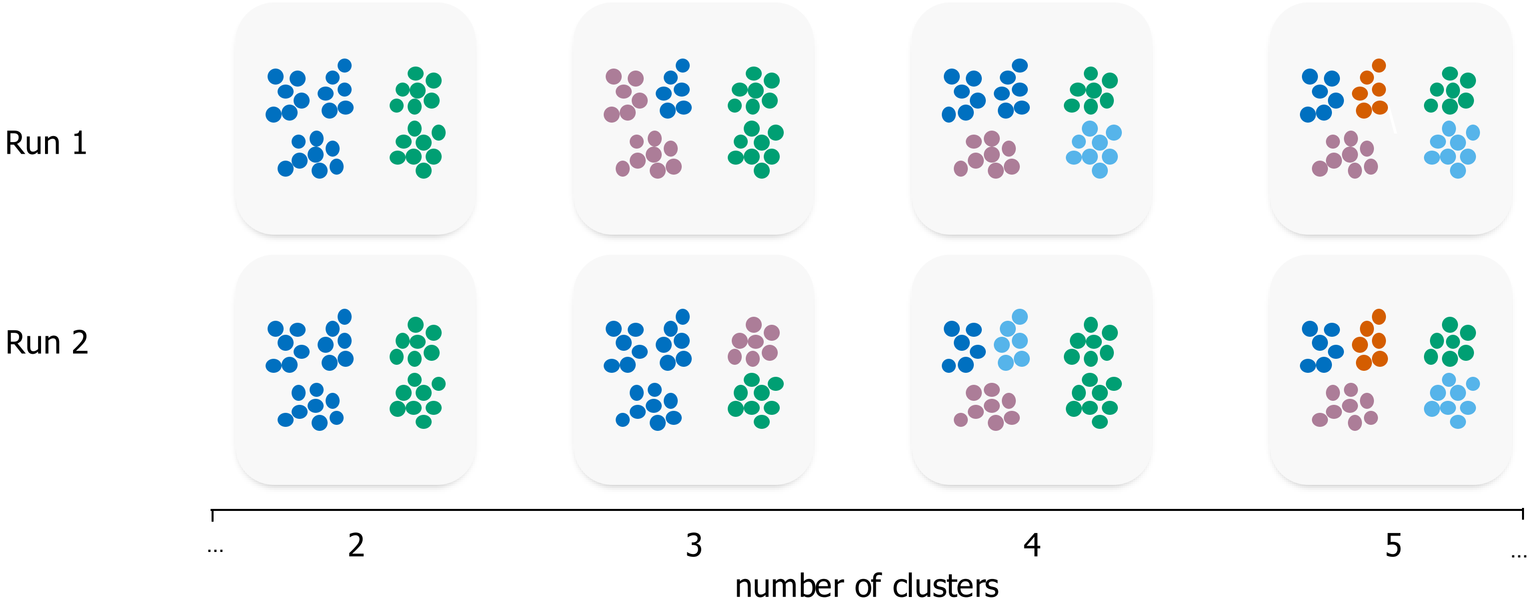


Cluster stability





Run 1

Run 2

… 2 3 4 5 …

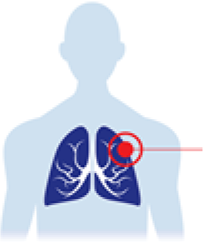
number of clusters

Spatial cell niches in Non-Small Cell Lung Cancer

Using CellCharter to decipher intra-tumor heterogeneity

Spatial domains in Non-Small Cell Lung Cancer

He et al., 2021

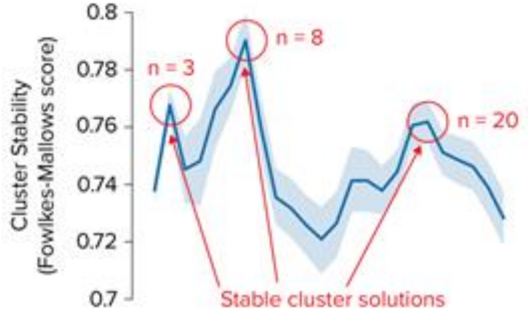


Lung cancer

n = 5 patients
n = 8 regions

Spatial transcriptomics
(Nanostring CosMx)

750,000 cells / 960 genes



Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

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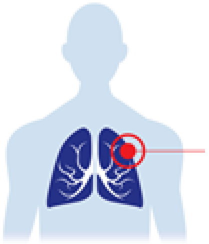

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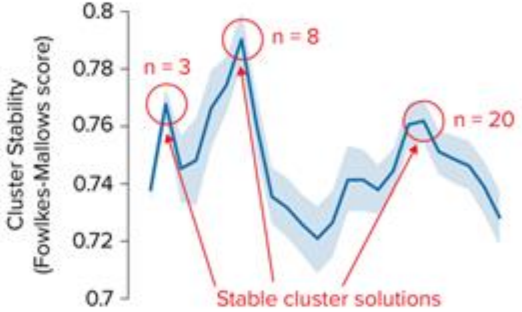
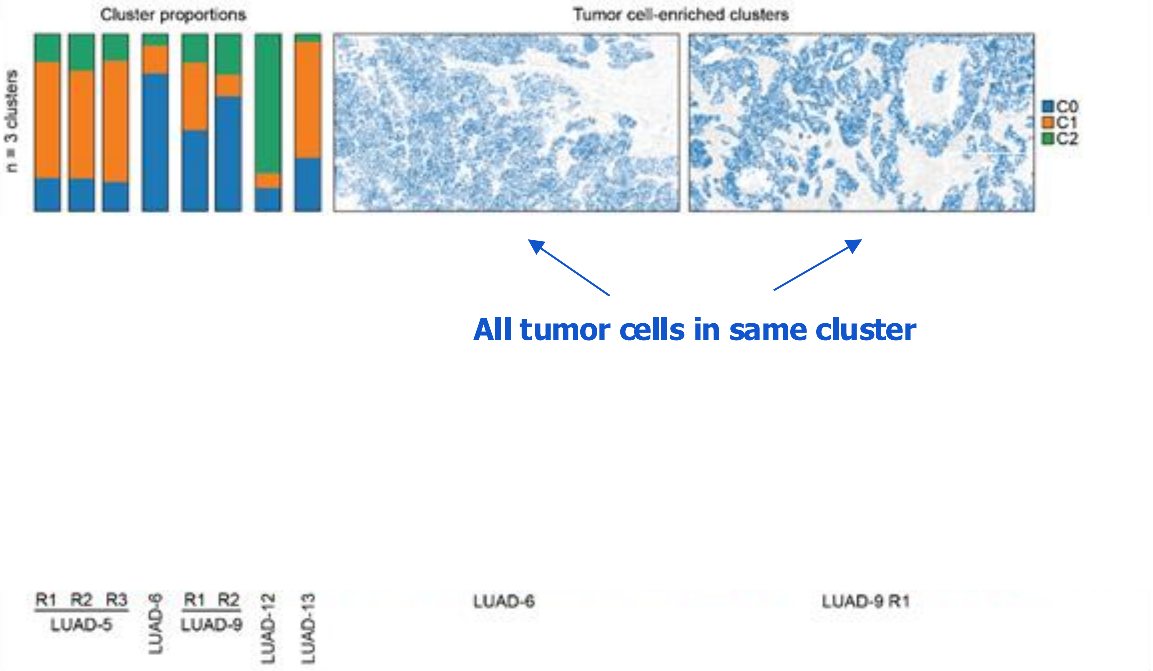


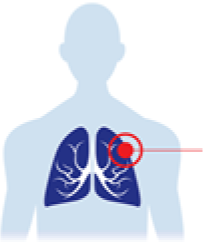


All tumor cells in same cluster

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

Spatial domains in Non-Small Cell Lung Cancer

He et al., 2021



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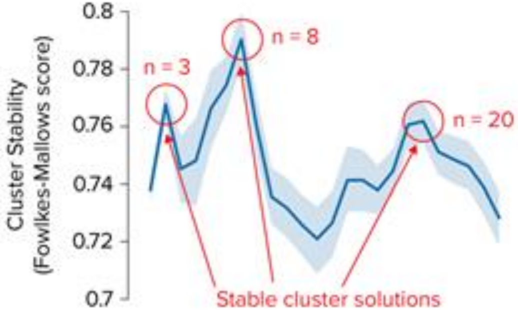
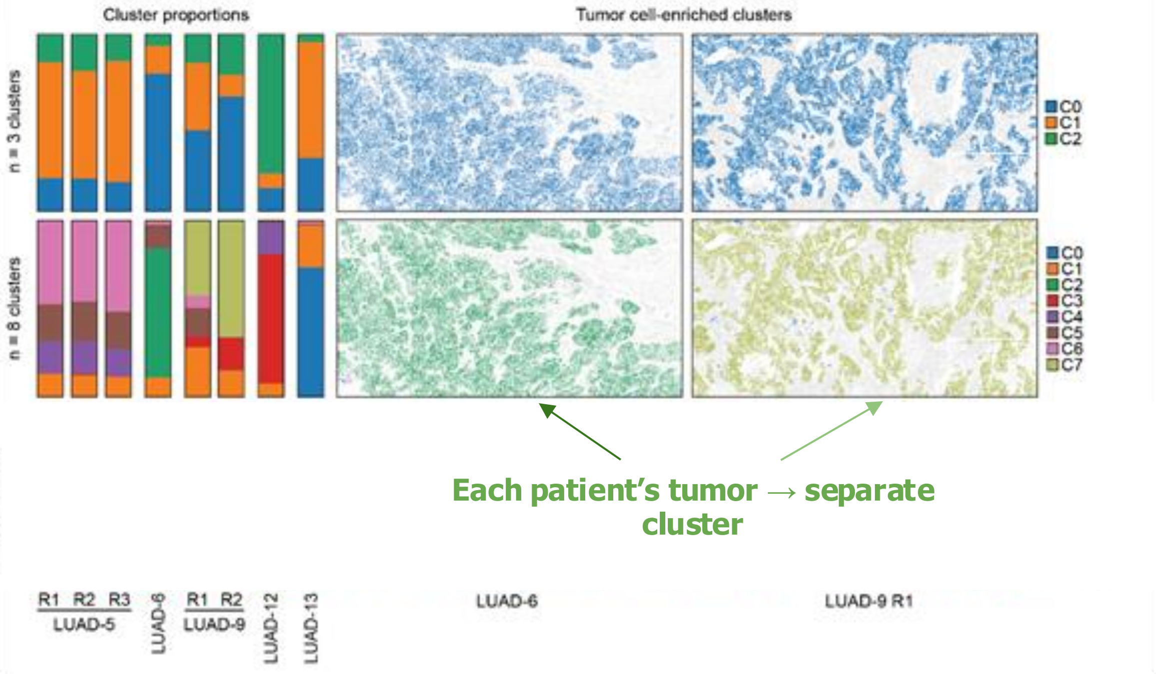


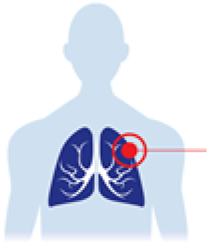


Each patient’s tumor → separate cluster

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

Spatial domains in Non-Small Cell Lung Cancer

He et al., 2021



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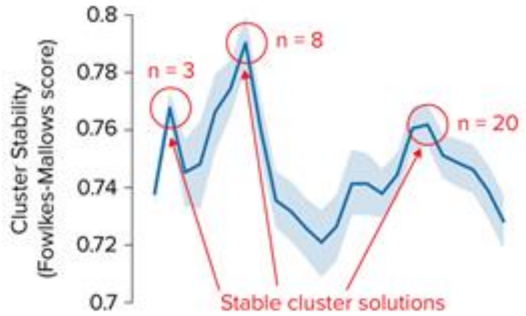
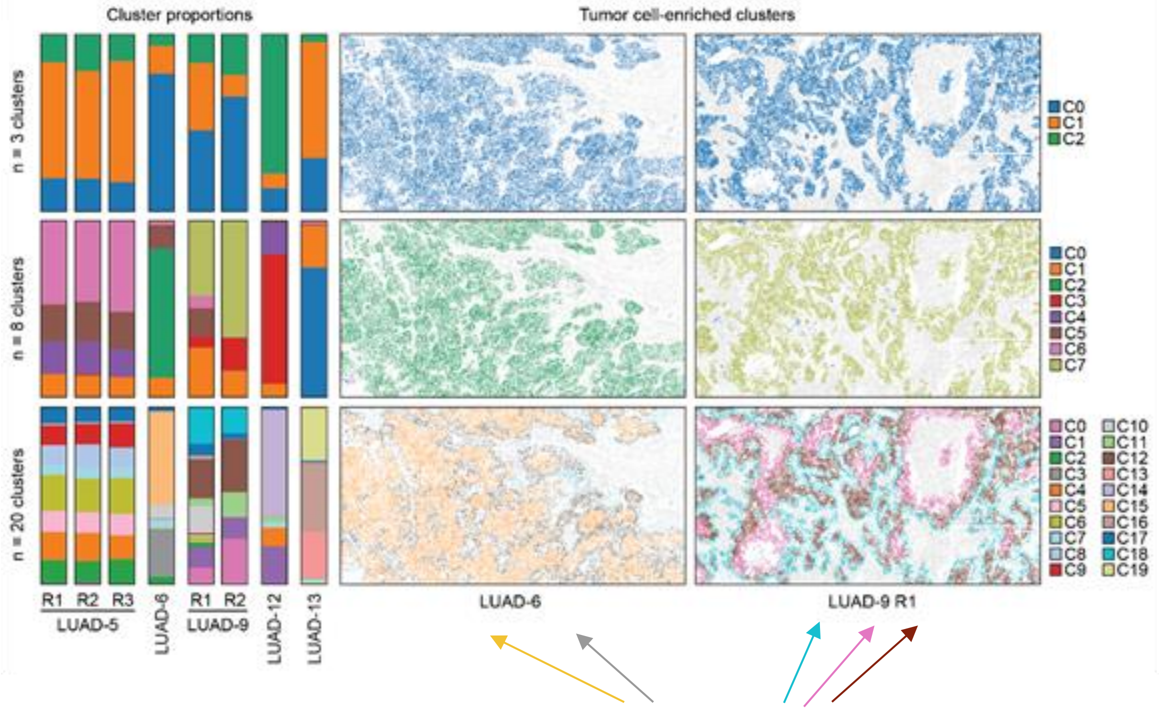


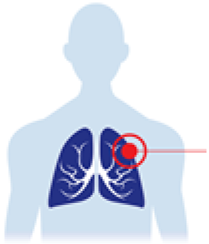


Within each patient: tumor cell states → separate clusters

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

Spatial domains in Non-Small Cell Lung Cancer

He et al., 2021



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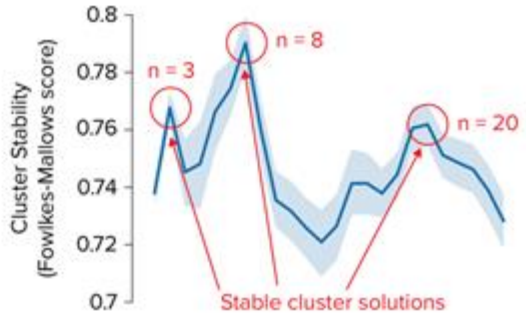

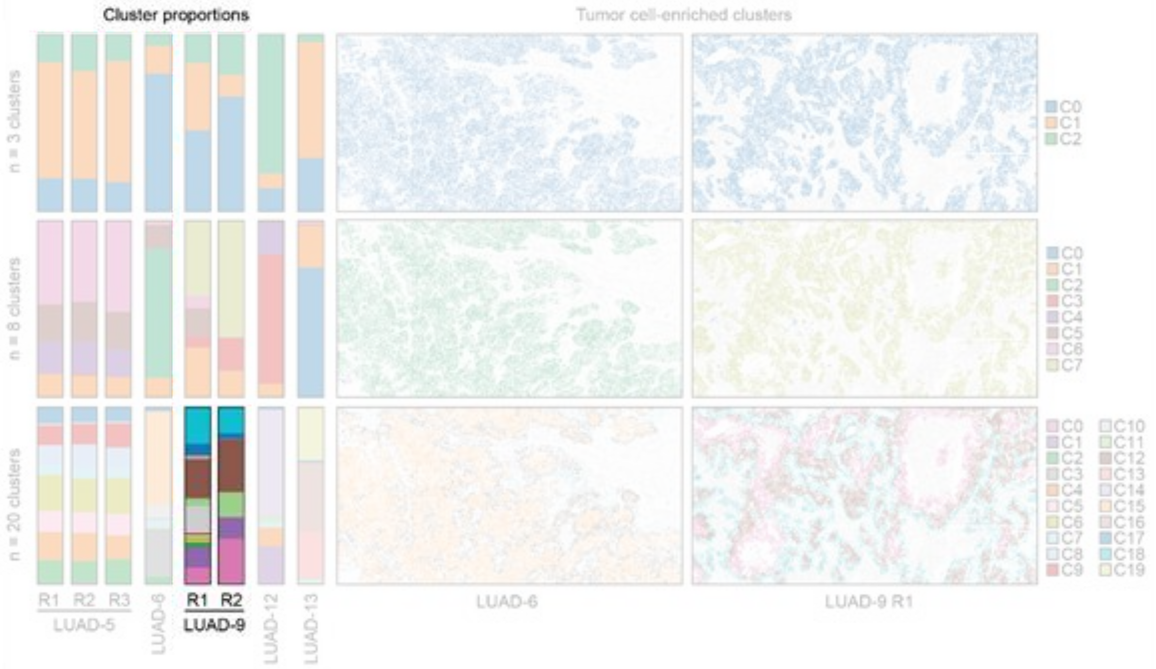

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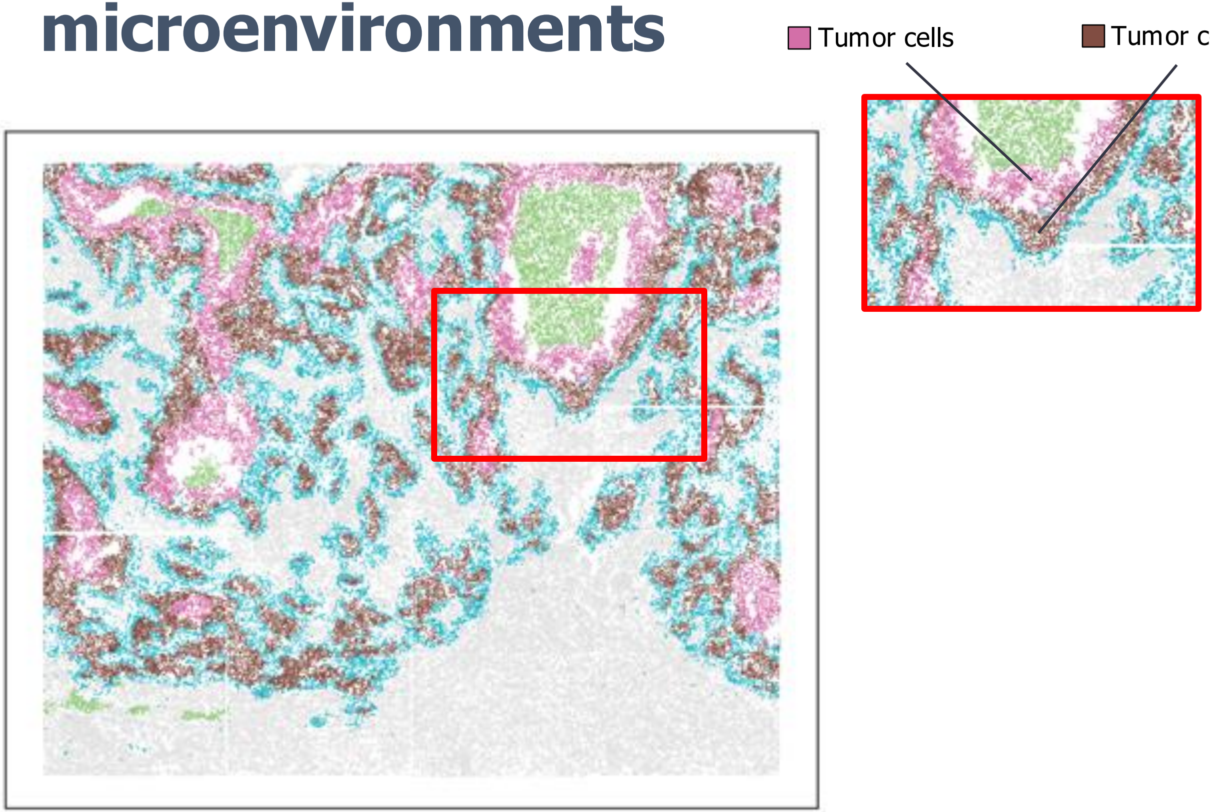
Two tumor subpopulations with distinct microenvironments



Lung 9 - Replicate 1

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

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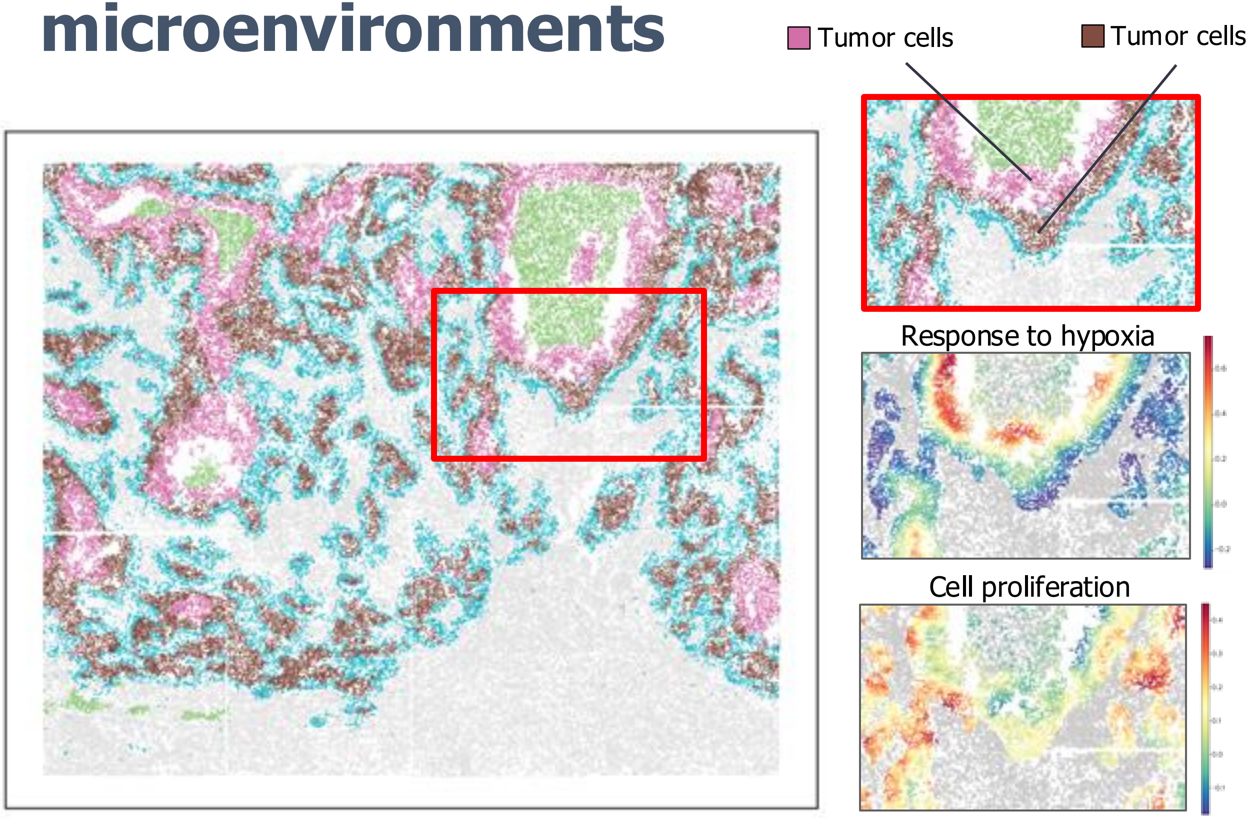

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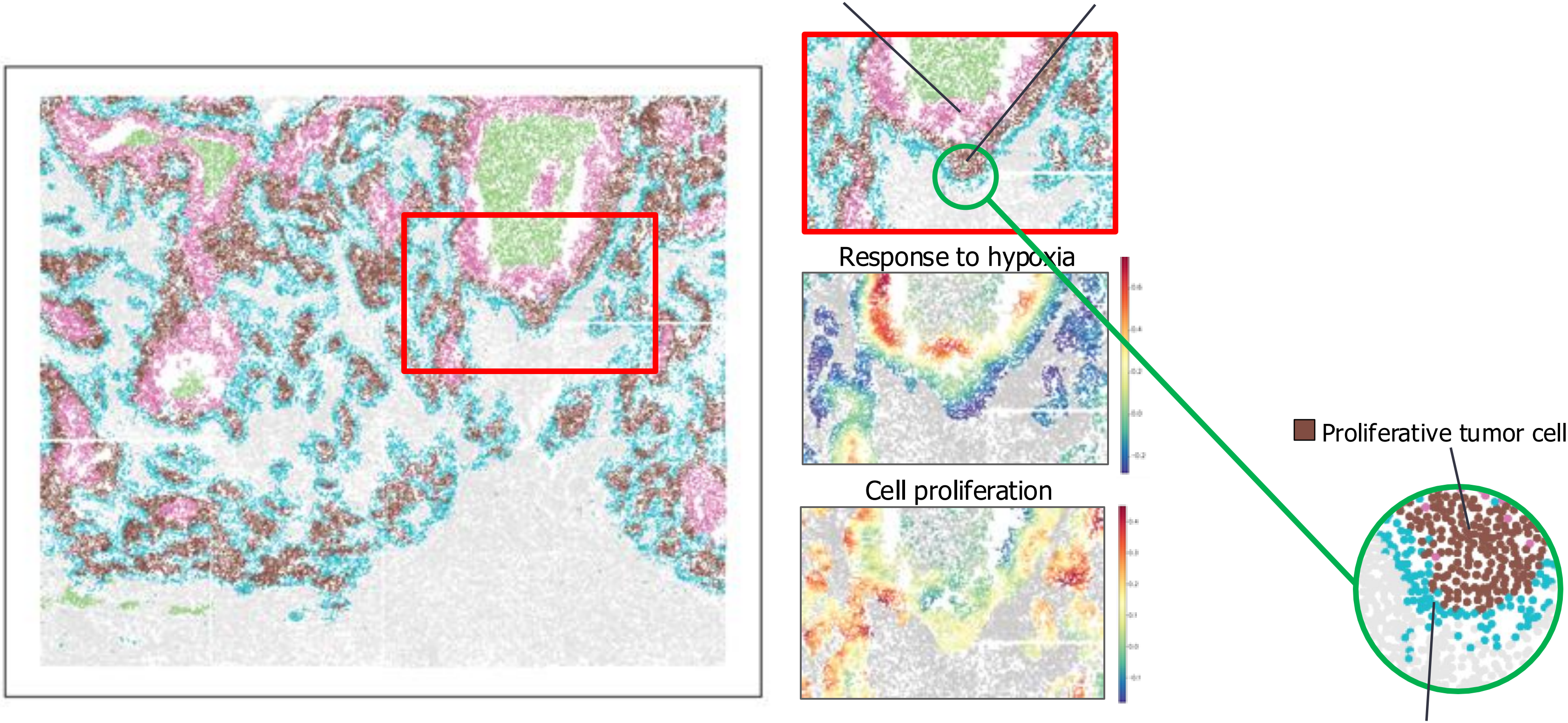

🟪 Tumor cells

🟫 Tumor cells

Response to hypoxia

Cell proliferation

🟫 Proliferative tumor cells

Lung 9 - Replicate 1

🟦 Tumor cells + CD4 memory T cells

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

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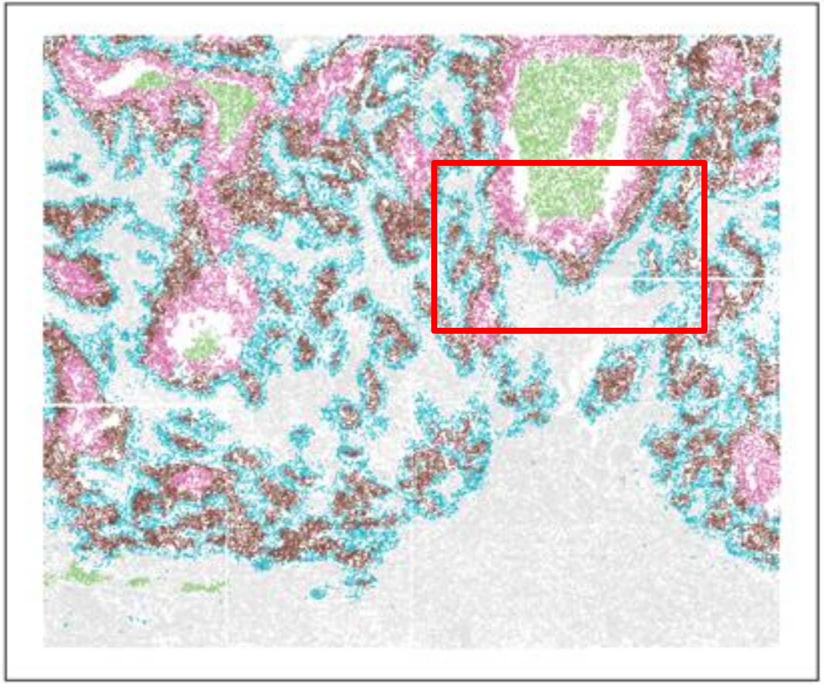

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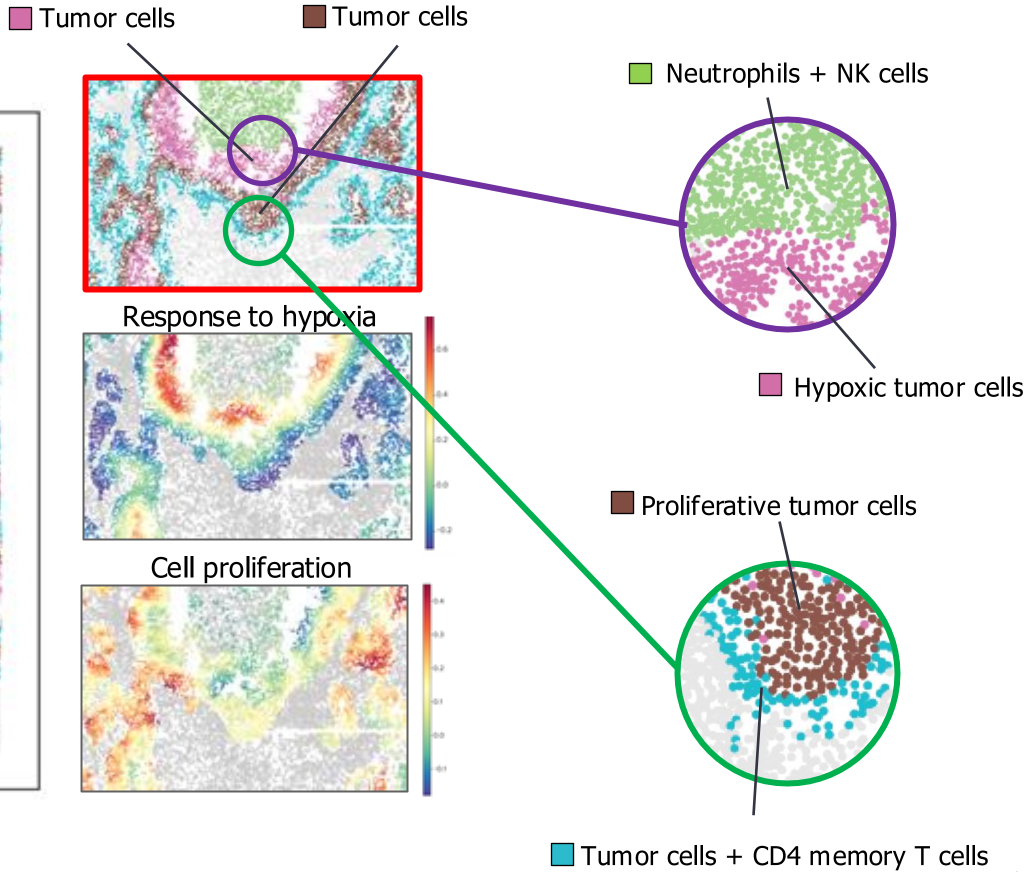

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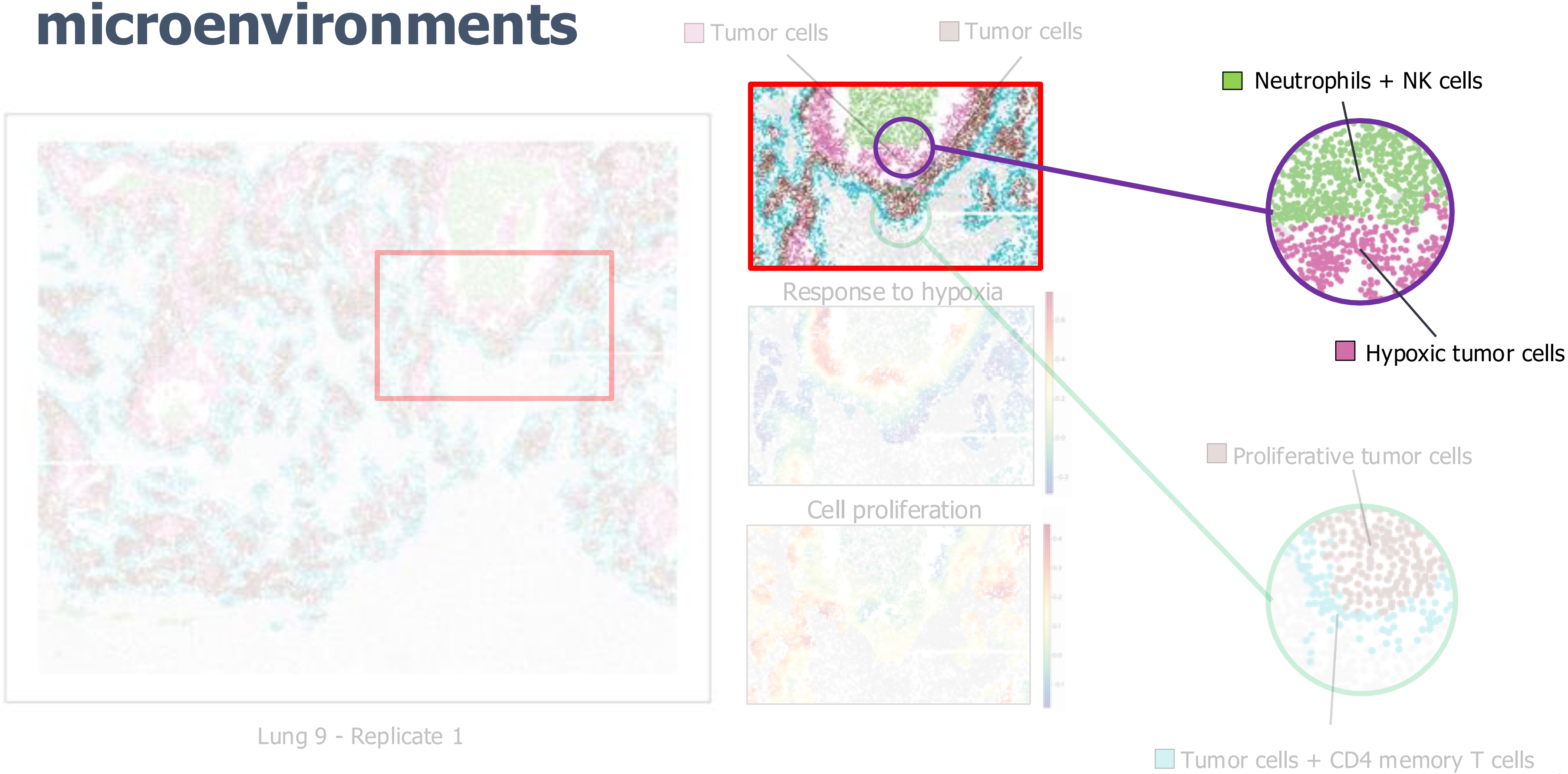


□ Tumor cells

□ Tumor cells

🟩 Neutrophils + NK cells

🟪 Hypoxic tumor cells

Response to hypoxia

Cell proliferation

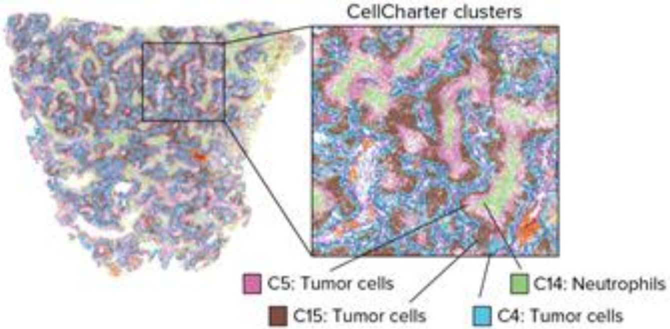
□ Proliferative tumor cells

□ Tumor cells + CD4 memory T cells

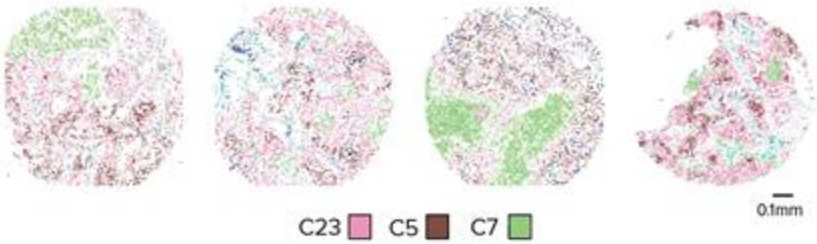
Lung 9 - Replicate 1

Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

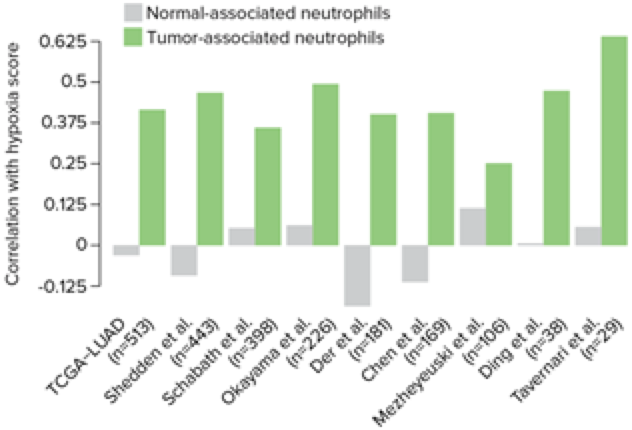
Neutrophil-hypoxia confirmed in multiple datasets



MERFISH dataset - Human Lung Cancer Patient 1 - ~350,000 cells



416 lung cancers - Imaging Mass Cytometry (IMC) - 35 protein markers

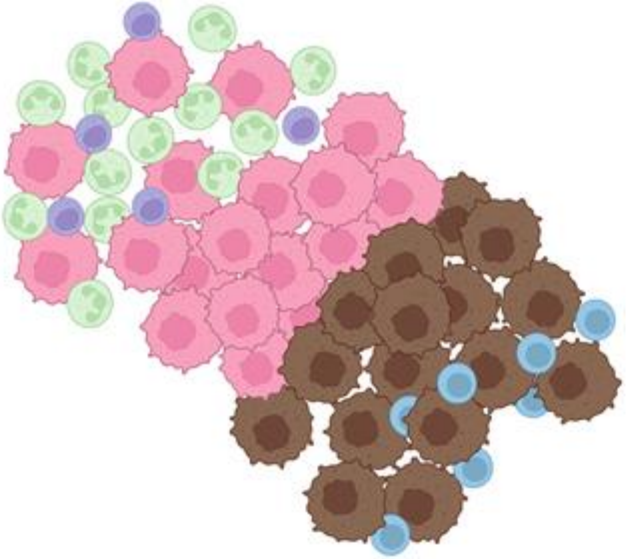


Varrone, et al. "CellCharter reveals spatial cell niches associated with tissue remodeling and cell plasticity." *Nature Genetics* (2024)

Intra-tumor heterogeneity in NSCLC

Tumor hypoxic state

Tumor proliferative state



Neutrophil + NK cells
tumor microenvironment

CD4 memory T cells
tumor microenvironment

Heterogeneous

tumor cells

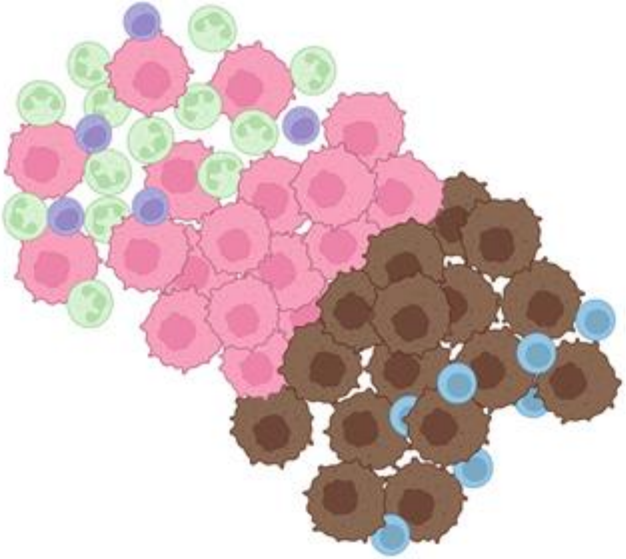
Heterogeneous

tumor microenvironment

Intra-tumor heterogeneity in NSCLC

Tumor hypoxic state

Tumor proliferative state



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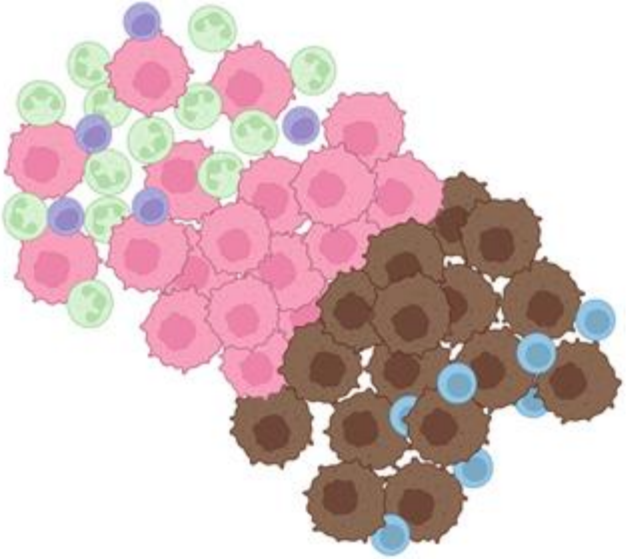
Heterogeneous

tumor microenvironment

Intra-tumor heterogeneity in NSCLC

Tumor hypoxic state ●

Tumor proliferative state ●



● ● Neutrophil + NK cells
tumor microenvironment

● CD4 memory T cells
tumor microenvironment

Heterogeneous
tumor cells

Heterogeneous
tumor
microenvironment

Conclusions

- 3 most common spatial domain approaches
    - Cell type proportion in neighborhood
    - Hidden Markov Random Field
    - Graph Neural Networks
- CellCharter
    - Find best number of domains
    - Shape analysis
- **Spatial domains** characterize **tissue architecture**
  Disease → tissue architecture remodeling