Statistical methods for spatial omics data

- Overview on the technologies (review)
- Finding spatially-variable genes
- Deconvoluting low-resolution (or aggregating high-resolution) spatial omics data
- Spatially-aware dimension reduction / clustering
- Cell-cell communication —> co-localization
- Classical spatial statistics
  - Point patterns: random, clustered, intensity/correlation
  - Lattice data: useful summaries / functions
  - Models with spatially correlated errors

From bulk to single-cell RNA-seq to imaging- & sequencing-based spatially resolved transcriptomics

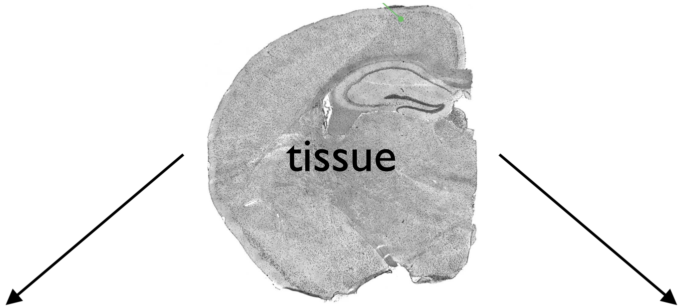
Background

Slide from
Helena Crowell

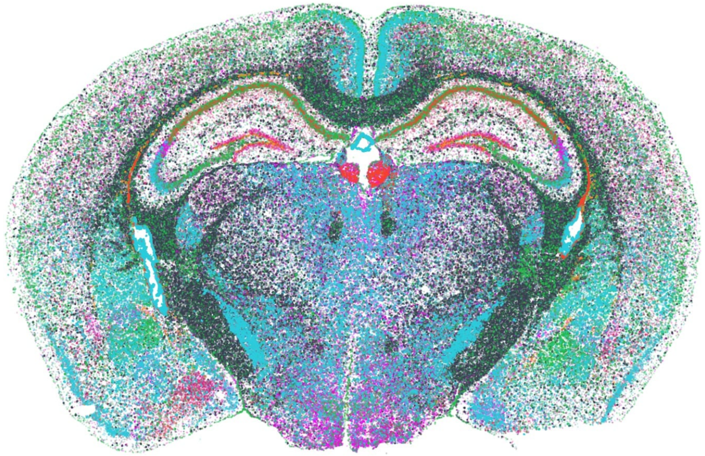
bulk

single-cell

spatial

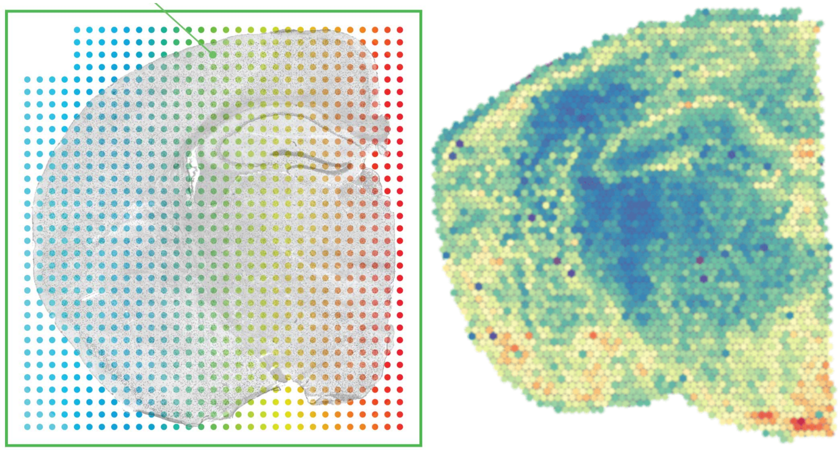


imaging-based



- molecule-level data
- targeted panel (100s of features; >2024: 1000s)
- single-cell resolution requires segmentation

sequencing-based



- spot-level data
- whole transcriptome (10,000s of features)
- single-cell resolutions requires aggregation or deconvolution

Technology choices: expression table + coordinates

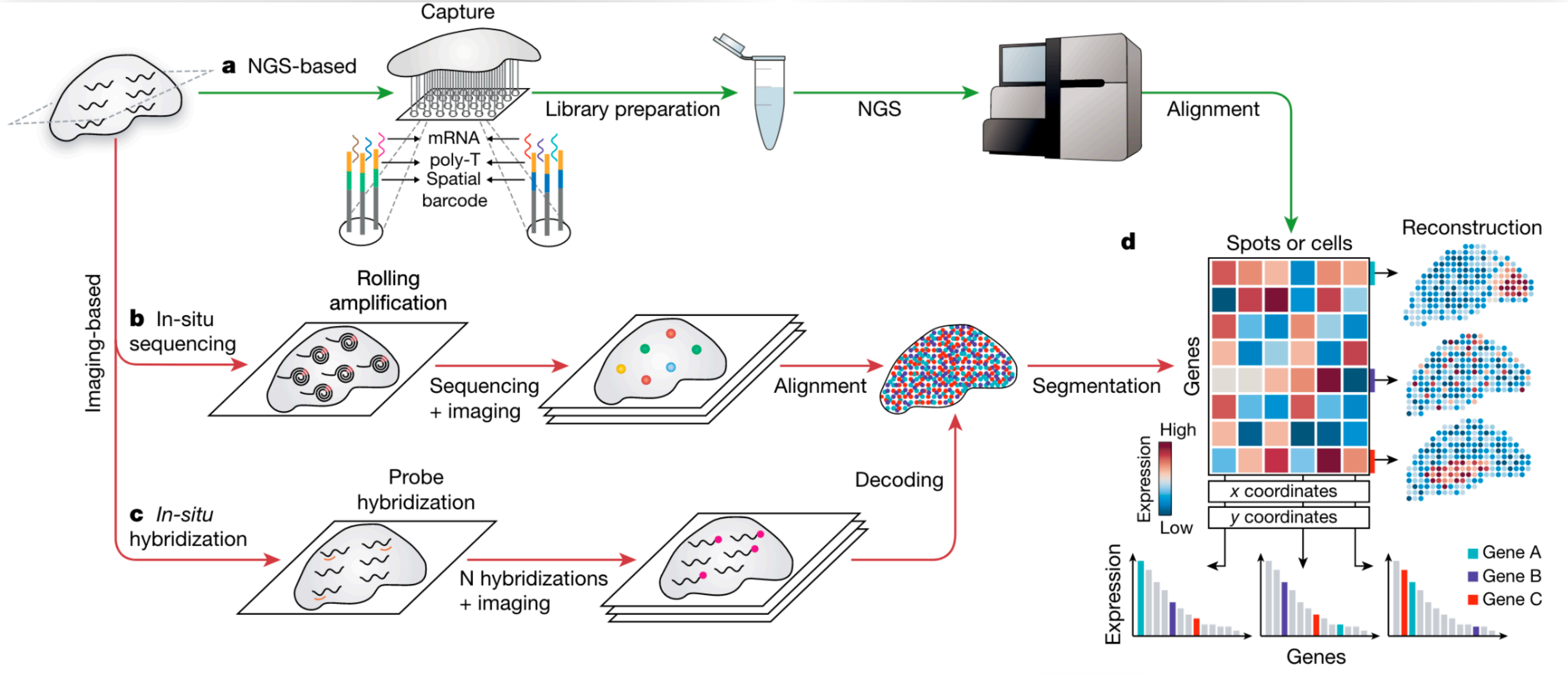


Fig. 1 | The technologies of spatial transcriptomics provide a gene-expression matrix. a, NGS-based spatial transcriptomic methods barcode transcripts according to their location in a lattice of spots. **b,** ISS approaches directly read out the transcript sequence within the tissue. **c,** ISH

methods detect ta
fluorescent probe
gene-expression m
genes and location

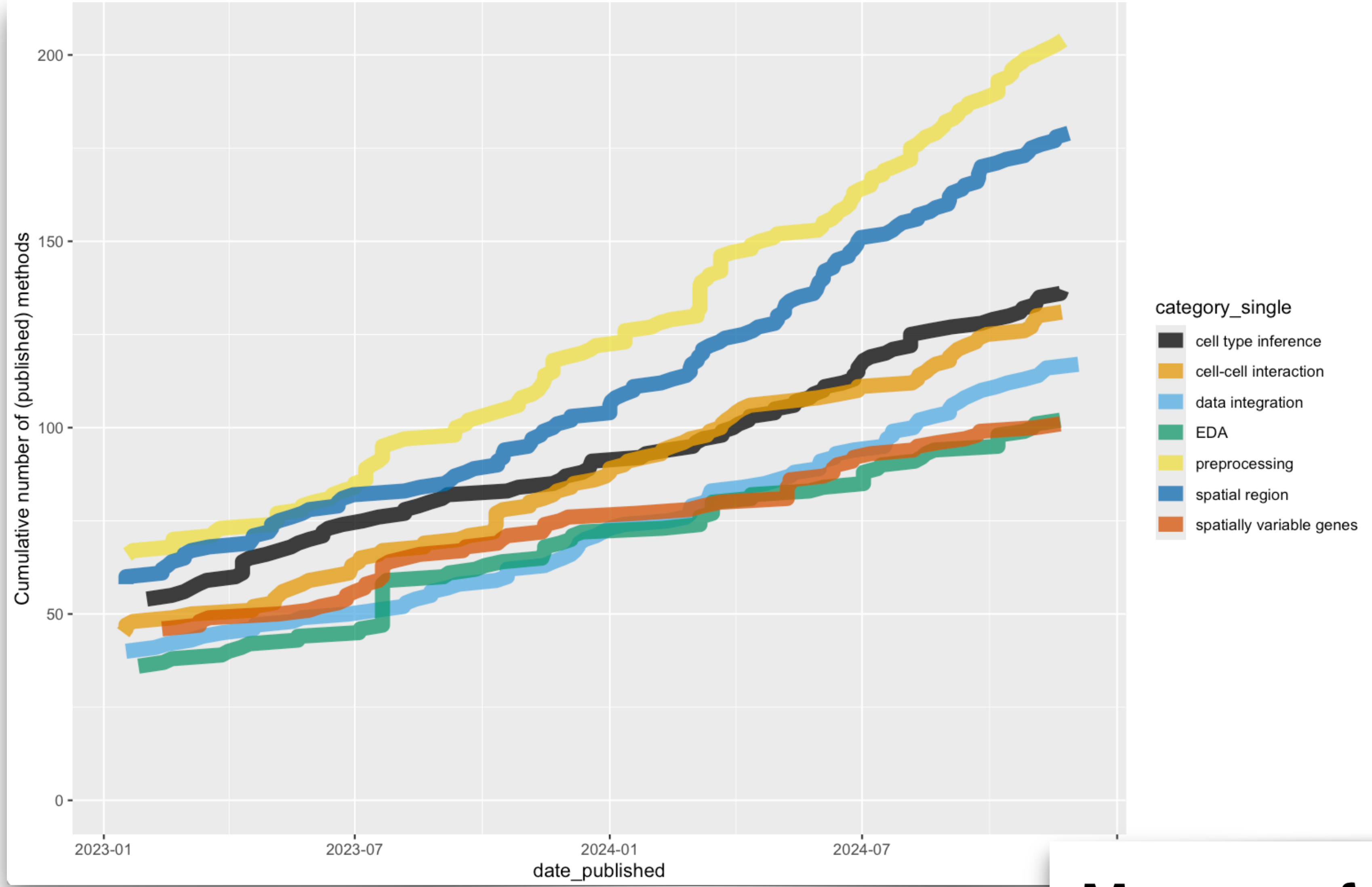
Review

Exploring tissue architecture using spatial transcriptomics

https://doi.org/10.1038/s41586-021-03634-9

Anjali Rao[1,3], Dalia Barkley[1,3], Gustavo S. França[1] & Itai Yanai[1,2]✉

(Spatial omics) computational method explosion —>



—> Google sheet

Museum of spatial transcriptomics

Lambda Moses[1] **and Lior Pachter**[1,2] ✉

Finding spatially-variable genes: SpatialDE

- SpatialDE: response = normal distribution with covariance with two components: i) based on distance b/w points - exponential decay; ii) constant non-spatial variance
- Null model: fit just the non-spatial variance (i.e., without sigma)
- Fit 2 models, likelihood ratio test

SpatialDE: identification of spatially variable genes

Valentine Svensson[1,2], Sarah A Teichmann[1,3] & Oliver Stegle[2,4]

SpatialDE model. SpatialDE models gene expression profiles $y = (y_1, \ldots, y_N)$ for a given gene across spatial coordinates $X = (x_1, \ldots, x_N)$, using a multivariate normal model of the form

$$P(y \mid \mu, \sigma_s^2, \delta, \Sigma) = N(y \mid \mu \cdot 1, \sigma_s^2 \cdot (\Sigma + \delta \cdot I)) \quad (1)$$

The fixed effect $\mu_g \cdot 1$ accounts for the mean expression level, and Σ denotes a spatial covariance matrix defined on the basis of the input coordinates of pairs of cells. SpatialDE uses the so-called squared exponential covariance function to define Σ:

$$\Sigma_{i,j} = k(x_i, x_j) = \exp\left(- \frac{|x_i - x_j|^2}{2 \cdot l^2} \right) \quad (2)$$

University of Zurich[UZH]

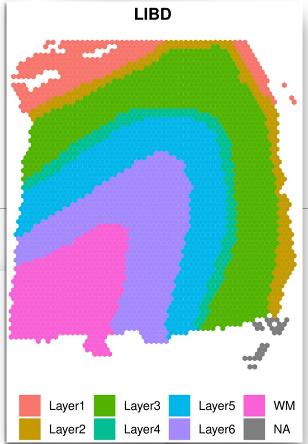
Statistical Bioinformatics // Department of Molecular Life Sciences

Article
https://doi.org/10.1038/s41467-023-39748-z

nnSVG for the scalable identification of spatially variable genes using nearest-neighbor Gaussian processes

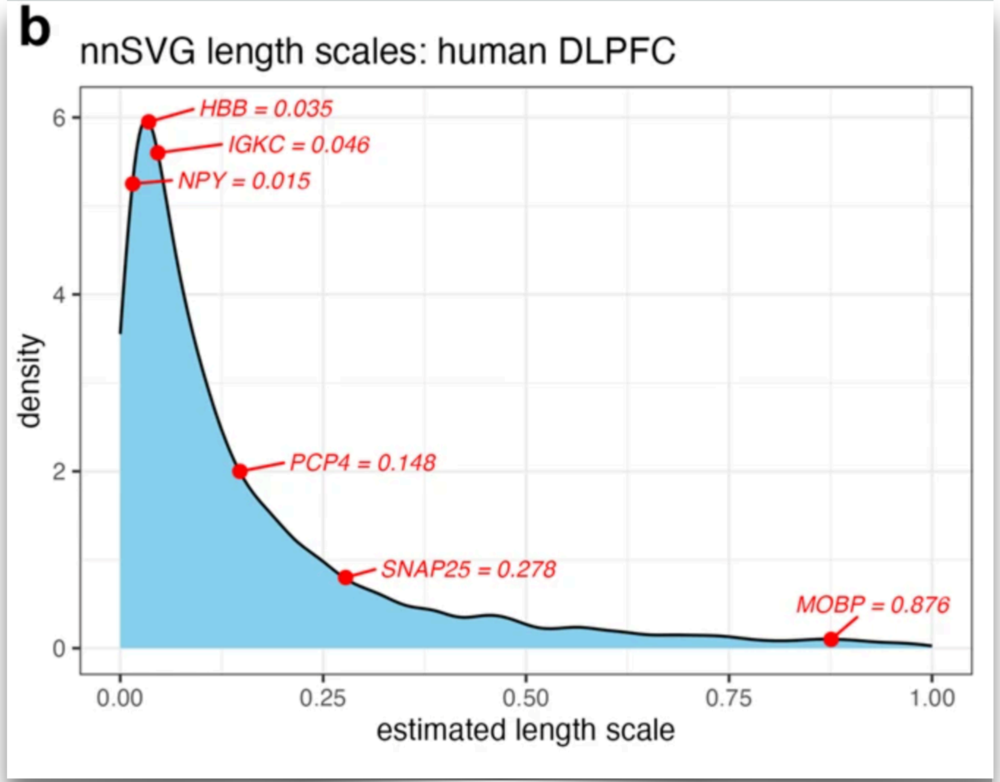
Received: 15 June 2022
Accepted: 23 June 2023

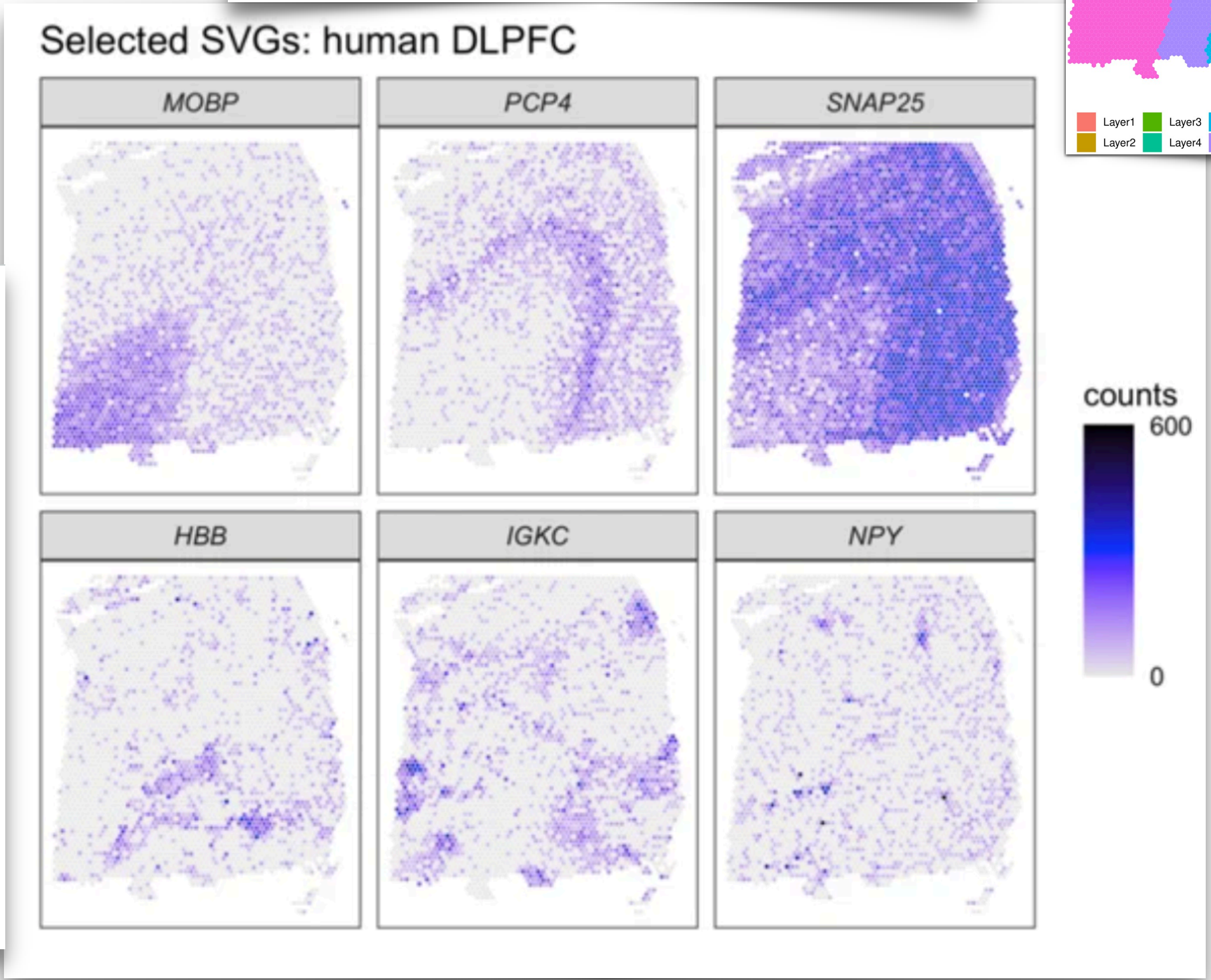
Lukas M. Weber[1], Arkajyoti Saha[2], Abhirup Datta[1], Kasper D. Hansen[1] & Stephanie C. Hicks[1]



Spatially variable genes —> scale

$$C_{ij}(\boldsymbol{\theta}) = \sigma^2 \exp\left(\frac{-||\mathbf{s_i} - \mathbf{s_j}||}{l}\right)$$





Alternatively, spatially variable features = DE between domains

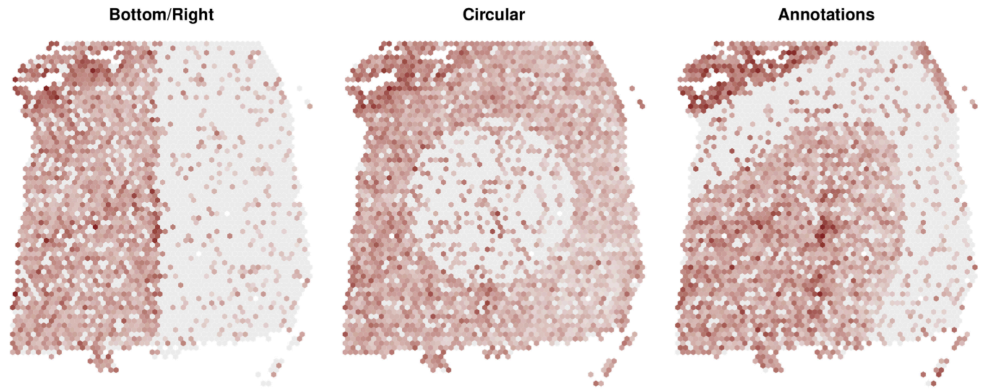
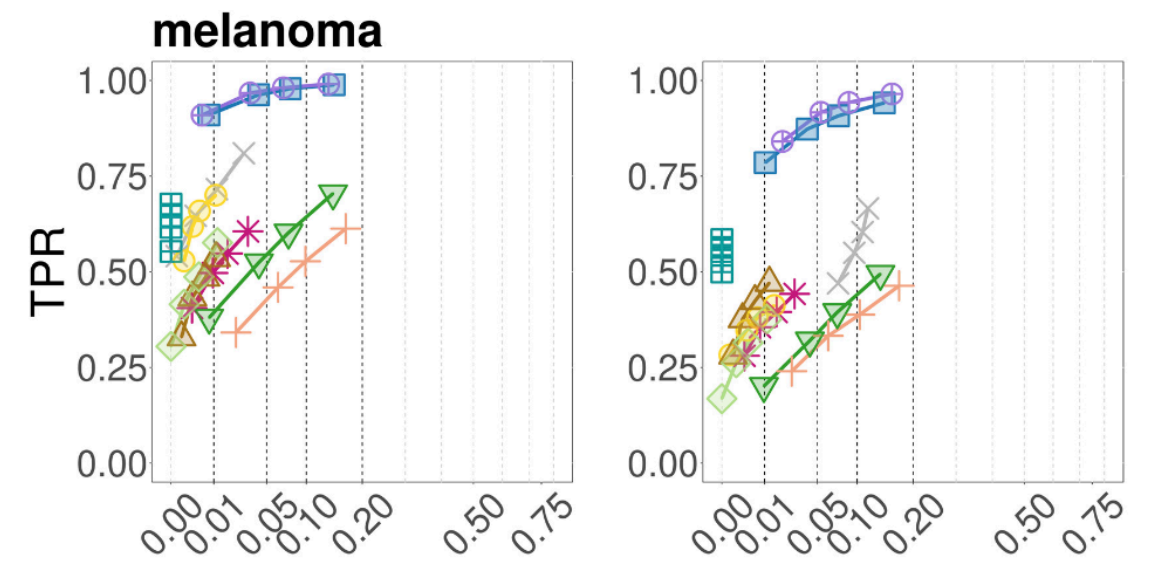
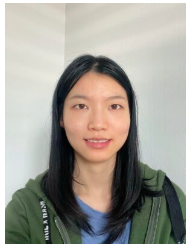


Figure 2. Three examples of simulated SVGs, from the *LIBD* data, following *bottom/right*, *circular*, and *annotations* patterns. Examples of SVGs from *mixture* and *inverted mixture* patterns are presented in Supplementary Fig. S1.



Simone Tiberi



Peiying Cai

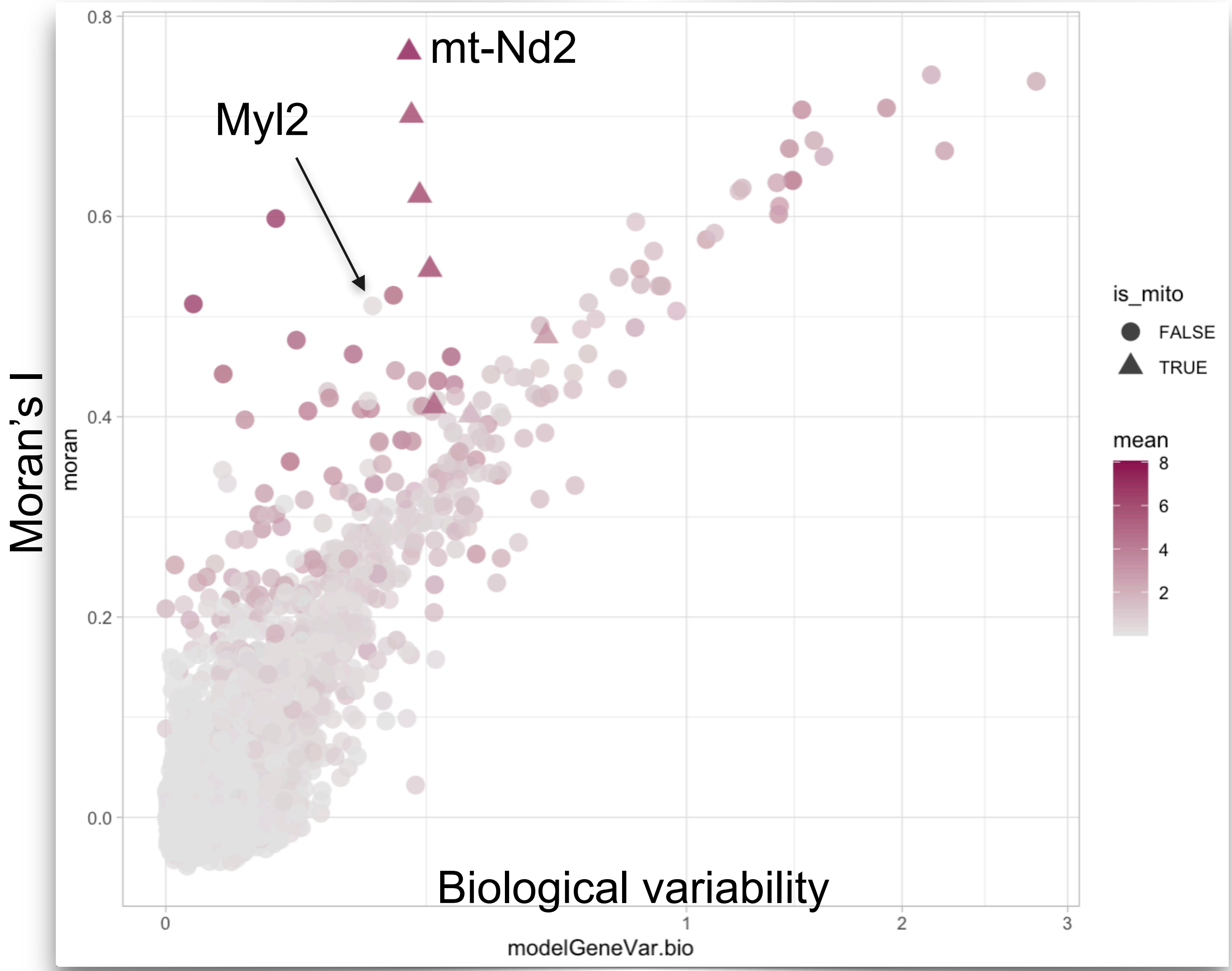
To find spatially variable genes (SVGs); spatial clustering + classical statistical method works quite well

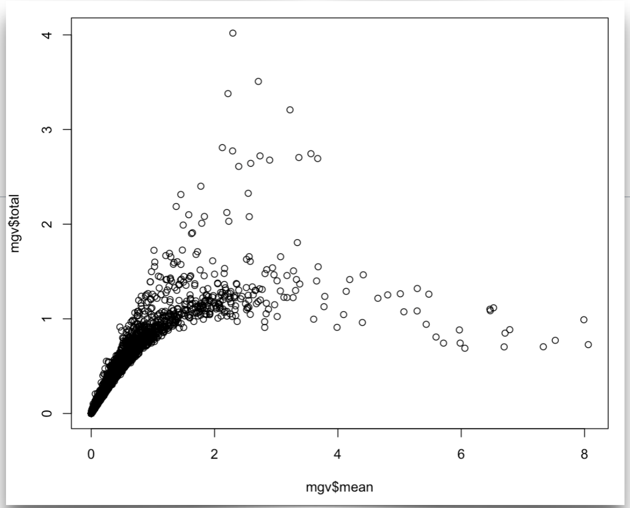
JOURNAL ARTICLE

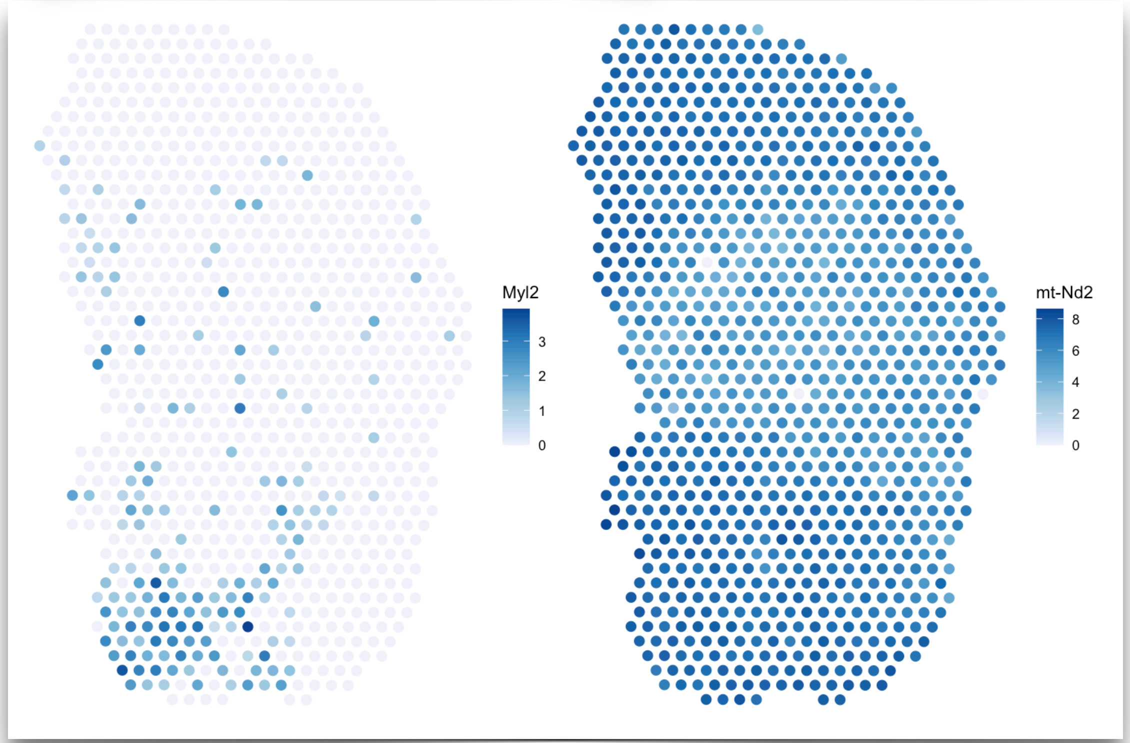
DESpace: spatially variable gene detection via differential expression testing of spatial clusters

Peiying Cai, Mark D Robinson, Simone Tiberi

Spatially variable versus highly variable







(More mathematical details on Moran's I below)

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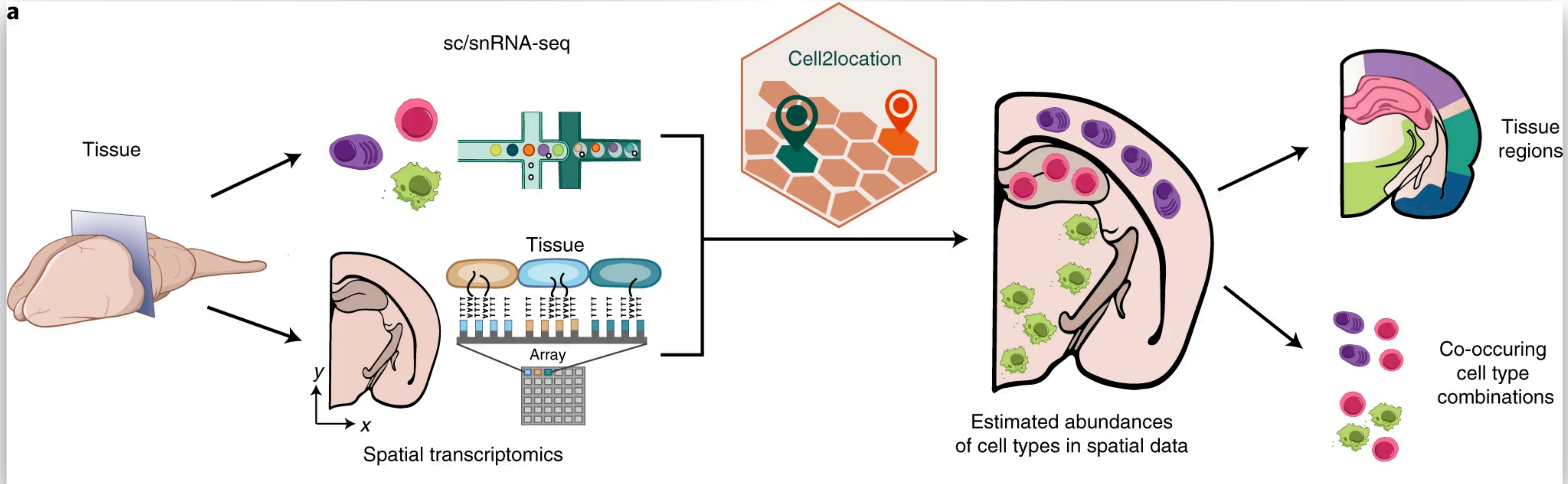
Deconvoluting low-resolution spatial omics (sequencing) data

- Cell2location: negative binomial regression for reference cell type signatures; decompose spot-level mRNA counts into reference cell types



Deconvoluting low-resolution spatial omics data

- Cell2location: negative binomial regression for reference cell type signatures; decompose spot-level mRNA counts into reference cell types

Cell2location model. Cell2location models the elements of the spatial expression count matrix $d_{s,g}$ as negative binomial distributed, given an unobserved gene expression level (rate) $\mu_{s,g}$ and gene- and batch-specific over-dispersion $\alpha_{e,g}$:

$$d_{s,g} \sim NB\left(\mu_{s,g}, \alpha_{e,g}\right).$$

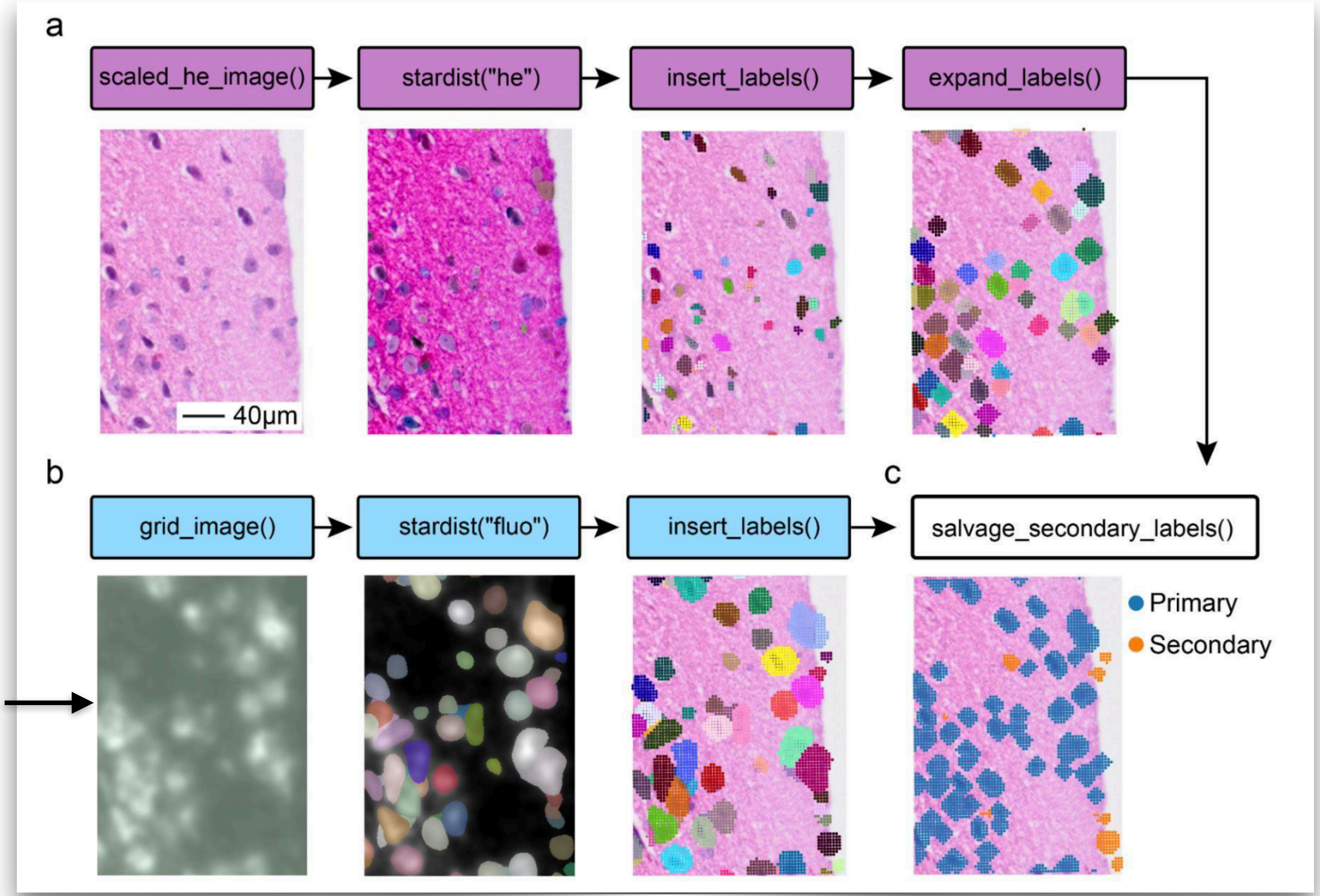
The expression rate of genes g at location s, $\mu_{s,g}$ in the mRNA count space is modeled as a linear function of reference cell types signatures $g_{f,g}$:

$$\mu_{s,g} = \left(\underbrace{m_g}_{\text{technology sensitivity}} \cdot \underbrace{\sum_f w_{s,f}\, g_{f,g}}_{\text{cell type contributions}} + \underbrace{s_{e,g}}_{\text{additive shift}} \right) \cdot \underbrace{y_s}_{\text{per-location sensitivity}}.$$

Aggregating high-resolution spatial omics (sequencing) data

- bin2cell: combines segmentation on H&E/IF and segmentation on gene expression counts

Image of counts per spot (smoothed)



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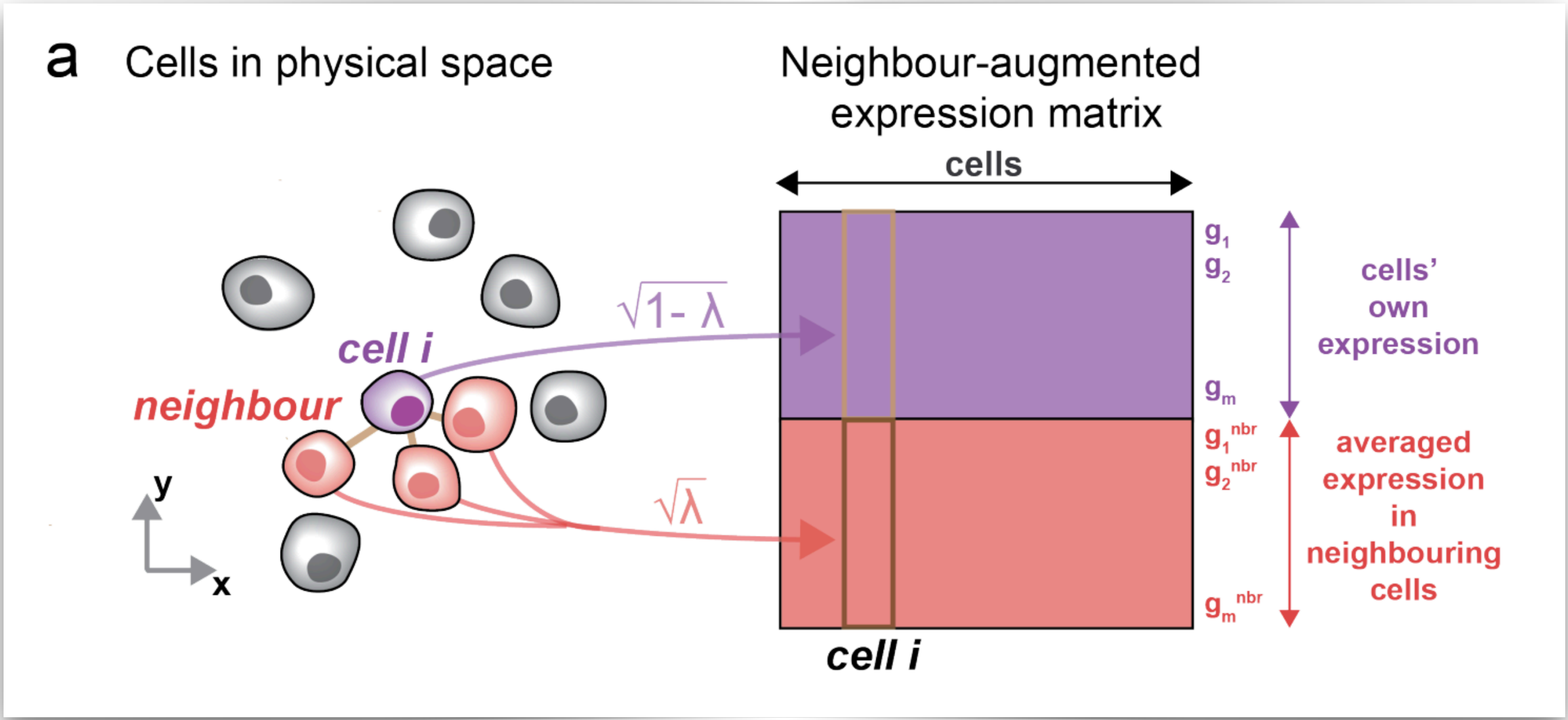
BANKSY unifies cell typing and tissue domain segmentation for scalable spatial omics data analysis

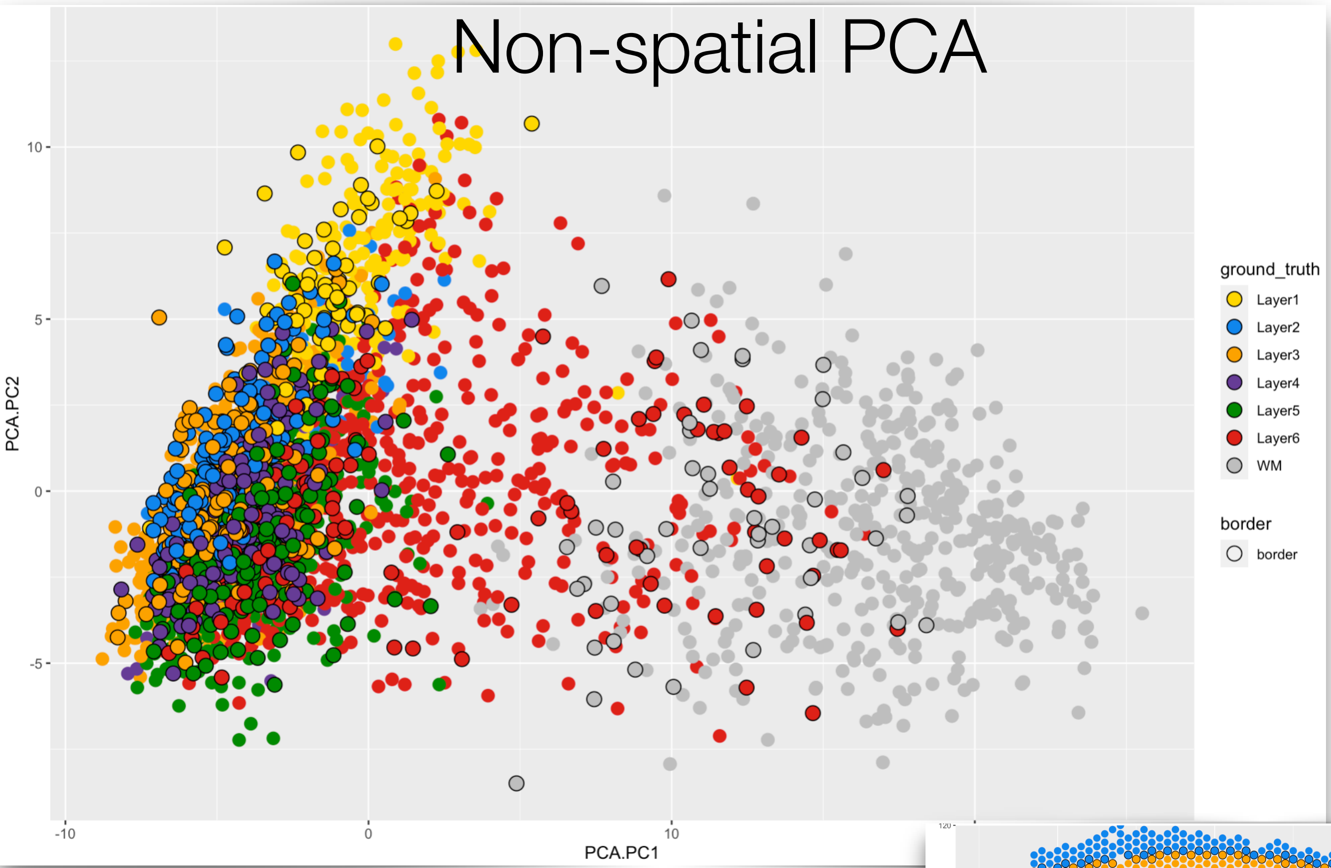
Received: 3 April 2023
Accepted: 16 January 2024
Published online: 27 February 2024

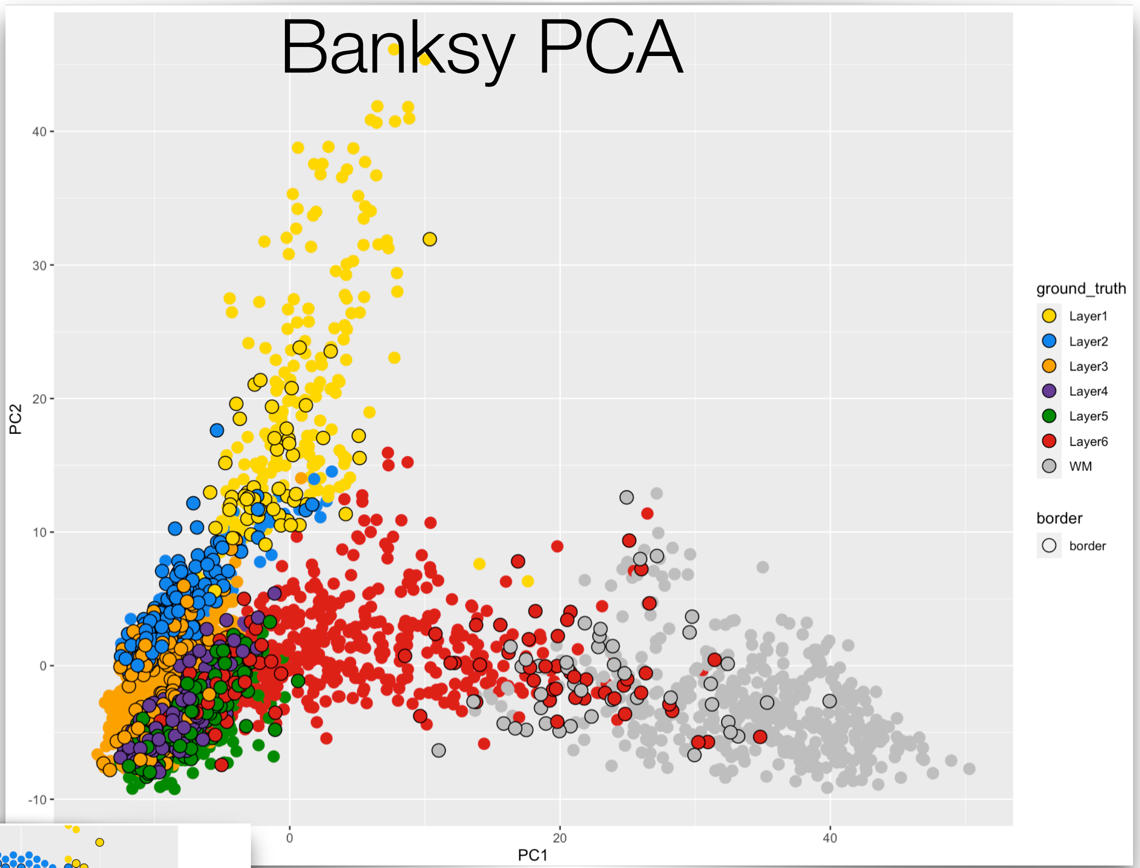
Vipul Singhal[1,13], Nigel Chou[1,13], Joseph Lee[2], Yifei Yue[3], Jinyue Liu[1], Wan Kee Chock[1], Li Lin[4], Yun-Ching Chang[5], Erica Mei Ling Teo[5], Jonathan Aow[1], Hwee Kuan Lee[4,6,7,8,9,10], Kok Hao Chen[1] & Shyam Prabhakar[1,11,12]

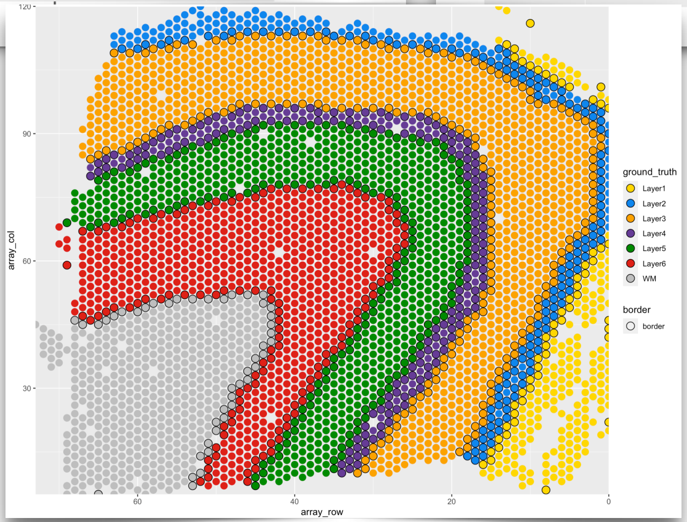
Spatial clustering / domain detection (BANKSY)

—> combine transcription and spatial information









Sample 151673

Article
https://doi.org/10.1038/s41467-022-34879-1

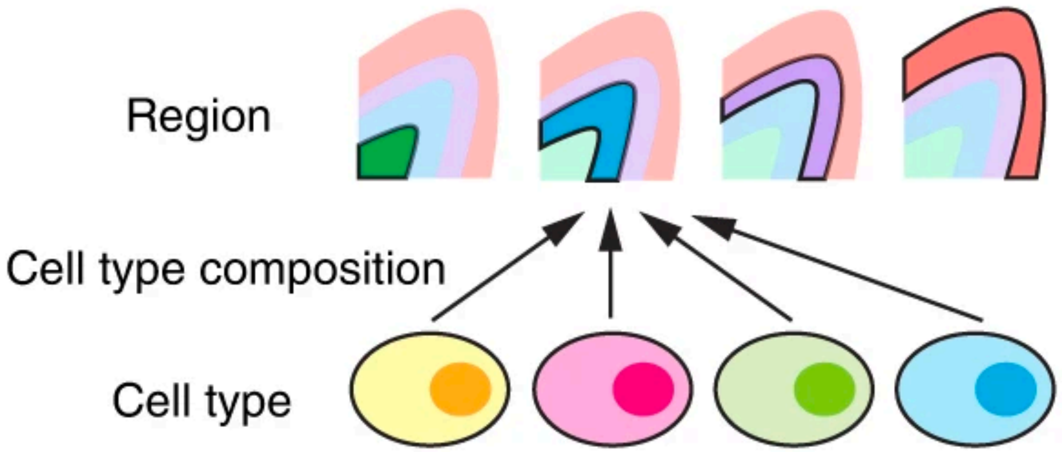
Spatially aware dimension reduction for spatial transcriptomics

Received: 10 March 2022

Lulu Shang [1,2] & Xiang Zhou [1,2]

Spatial domain detection ~ spatially homogeneous regions ~ spatial niches
—> needs a definition!

b.

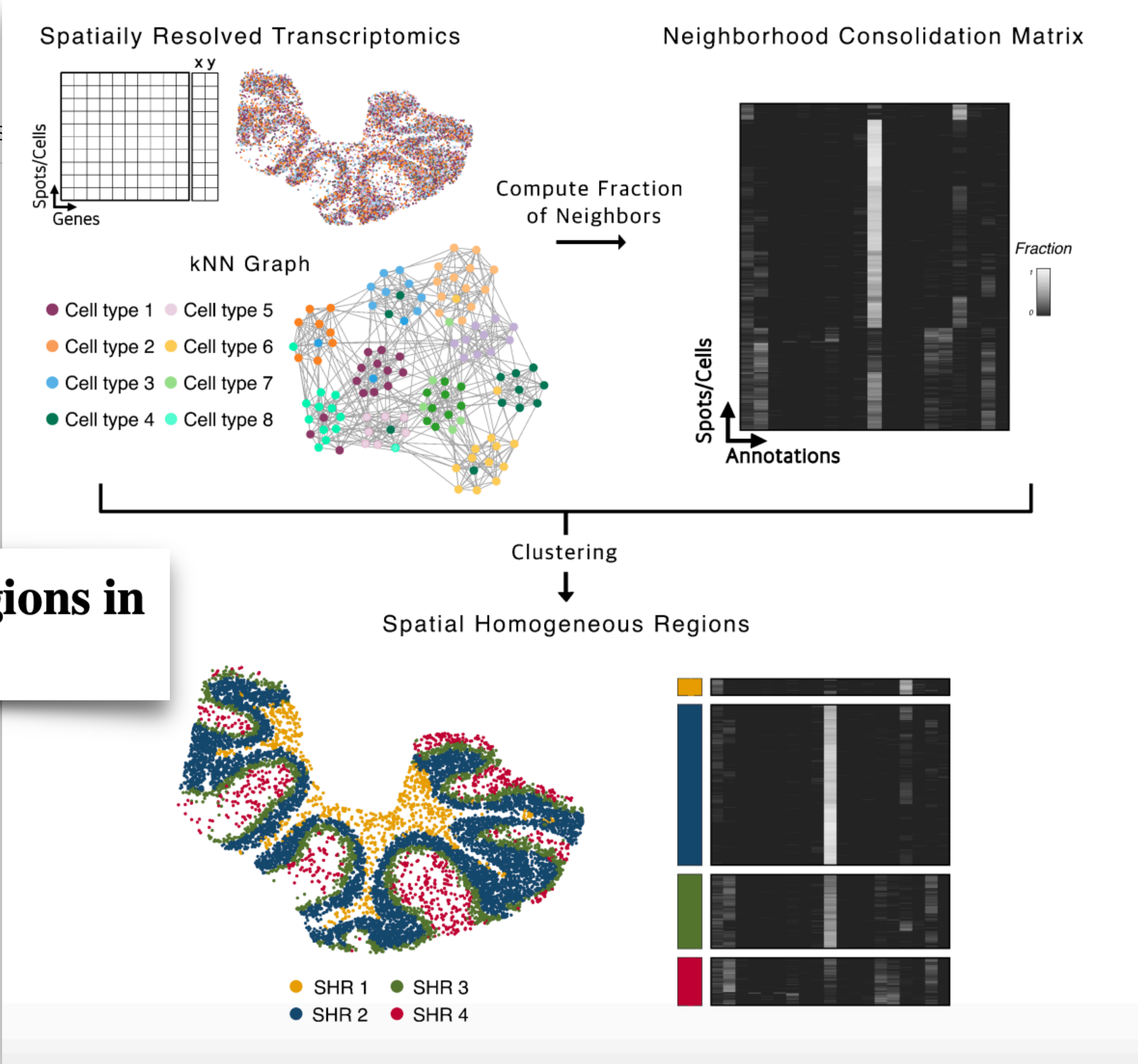


Scenario 1	70%	10%	10%	10%
Scenario 2	45%	45%	5%	5%
Scenario 3	60%	30%	5%	5%
Scenario 4	35%	30%	30%	5%

https://www.nature.com/articles/s41467-022-34879-1

Spatial domain detection ~ spatially homogeneous regions

Identification of spatial homogeneous regions in tissues with concordex



https://www.biorxiv.org/content/10.1101/2023.06.28.546949v2

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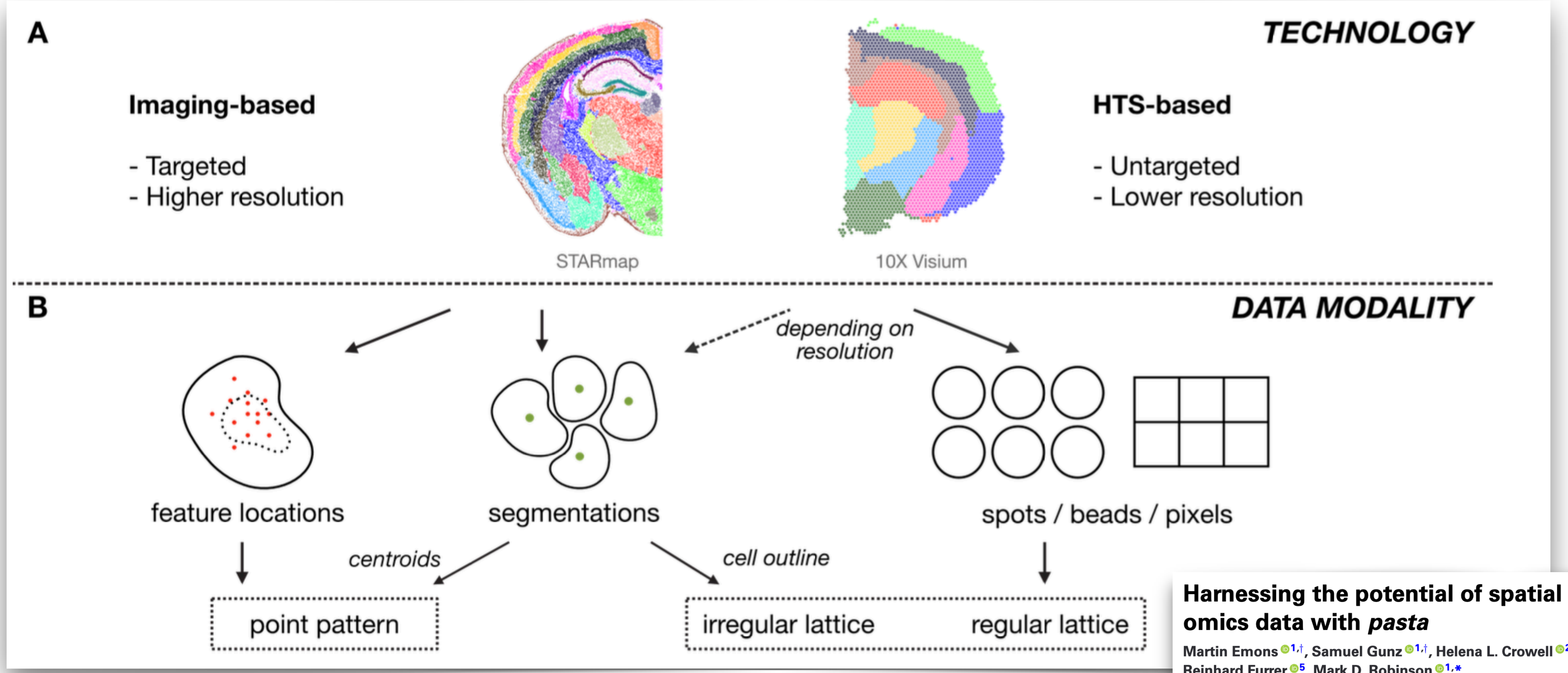
`pasta`: Data representations determine spatial statistics options



Samuel

Martin

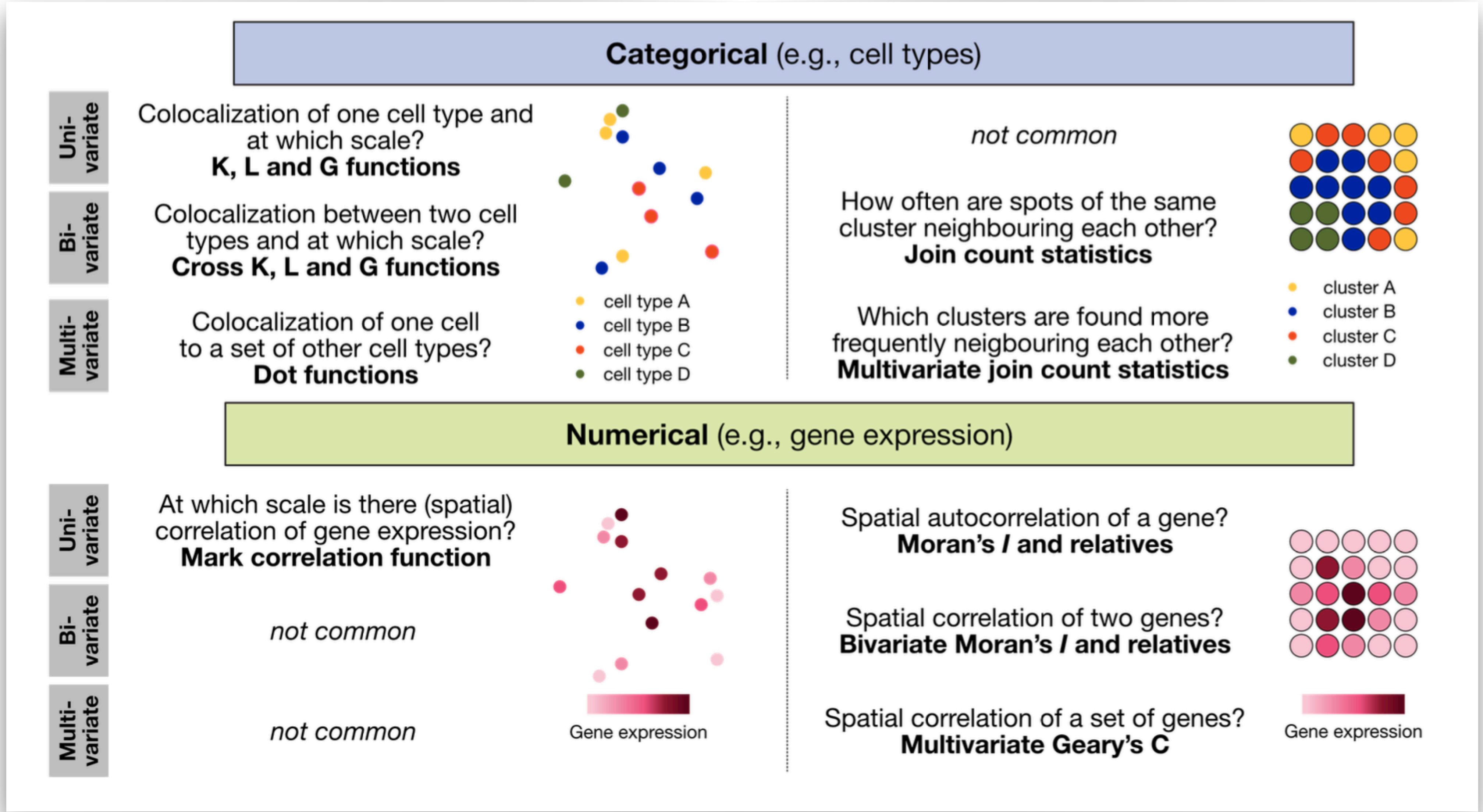
Harnessing the potential of spatial statistics for spatial omics data with *pasta*

Martin Emons[1,†], Samuel Gunz[1,†], Helena L. Crowell[2], Izaskun Mallona[1], Malte Kuehl[3,4], Reinhard Furrer[5], Mark D. Robinson[1,*]

[1]Department of Molecular Life Sciences and SIB Swiss Institute of Bioinformatics, University of Zurich, 8057 Zurich, Switzerland
[2]Centro Nacional de Análisis Genómico (CNAG), 08028 Barcelona, Spain
[3]Department of Clinical Medicine, Aarhus University, 8200 Aarhus N, Denmark
[4]Department of Pathology, Aarhus University Hospital, 8200 Aarhus N, Denmark
[5]Department of Mathematical Modeling and Machine Learning, University of Zurich, 8057 Zurich, Switzerland

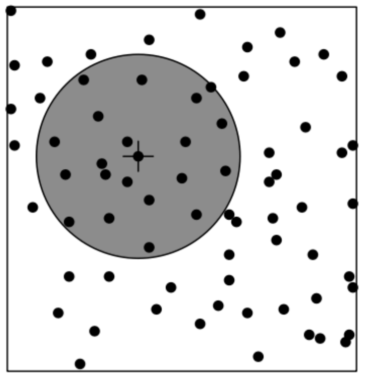
[*]To whom correspondence should be addressed. Email: mark.robinson@mls.uzh.ch
[†]The first two authors should be regarded as Joint First Authors.

pasta: Data representations determine spatial statistics options



Samuel

Martin



Correlation for **point patterns**

- Ripley's K function
- mathematical definition:

$$K(r) = \frac{1}{\lambda}\,\mathbb{E}\left[\text{number of } r\text{-neighbours of u} \;\middle|\; \mathbf{X} \text{ has a point at location } u\right]$$

$$t(u,r,\mathbf{x}) = \sum_{j=1}^{n(\mathbf{x})} \mathbf{1}\{0 < \|u - x_j\| \leq r\}$$

Definition 7.1. *If* $\mathbf{X}$ *is a stationary point process, with intensity* $\lambda > 0$*, then for any* $r \geq 0$

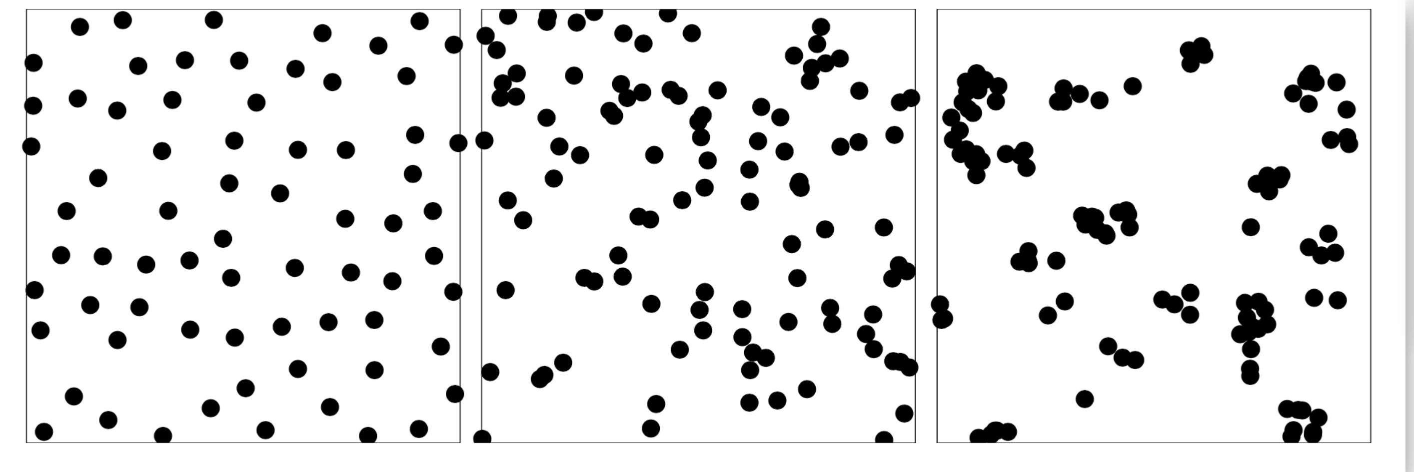
$$K(r) = \frac{1}{\lambda}\mathbb{E}\left[t(u,r,\mathbf{X}) \;\middle|\; u \in \mathbf{X}\right] \qquad (7.6)$$

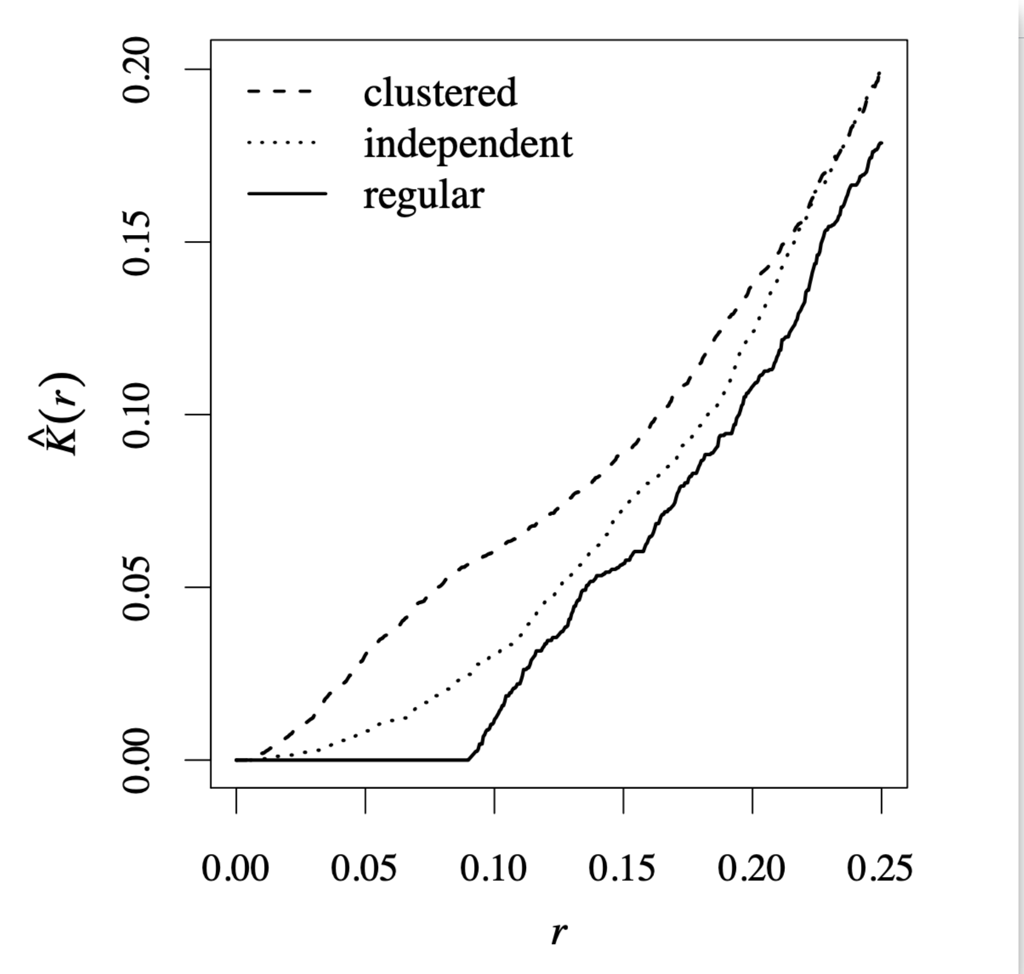
does not depend on the location u, and is called the K-function of $\mathbf{X}$.

Correlation for **point patterns**

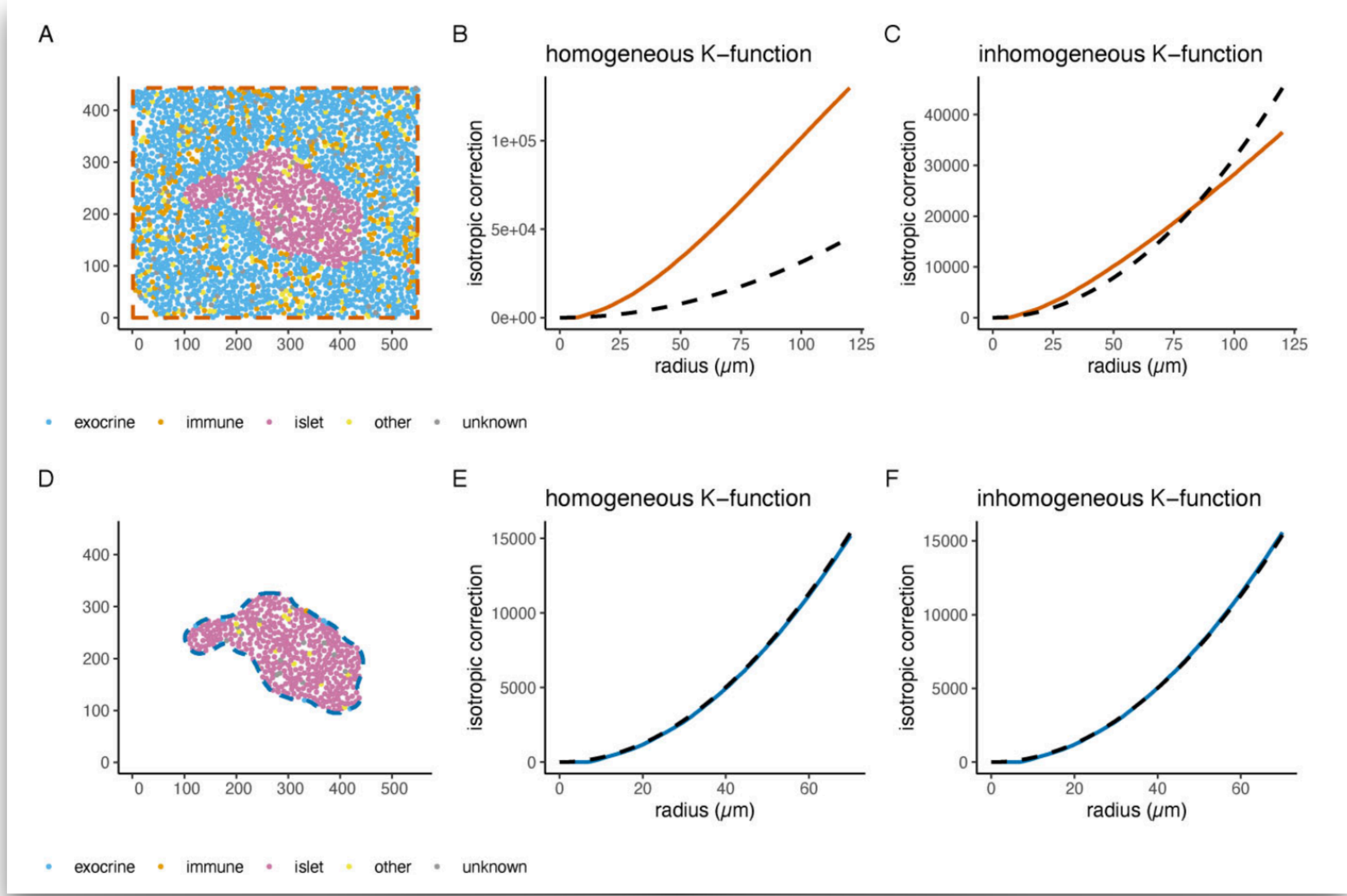
- Ripley's K function
- words definition: *the empirical K-function K(r) is the cumulative average number of data points lying within a distance r of a typical data point*

regular independent clustered





`pasta`: the 'gotcha' of spatial statistics — is it clustering or intensity?



Samuel

Martin

K-functions here: clustering / intensity of pink cells (islets).

https://en.wikipedia.org/wiki/Moran%27s_I
https://en.wikipedia.org/wiki/Geary%27s_C

Spatial autocorrelation: Global Moran's I

- Global measure of auto-correlation (correlation to signal nearby in space); assume homogeneity!
- Alternative: Geary's C

$$I = \frac{1}{\sum_{ij} w_{ij}} \frac{\sum_{ij} w_{ij}(X_i - \bar{X})(X_j - \bar{X})}{N^{-1} \sum_i (X_i - \bar{X})^2}$$

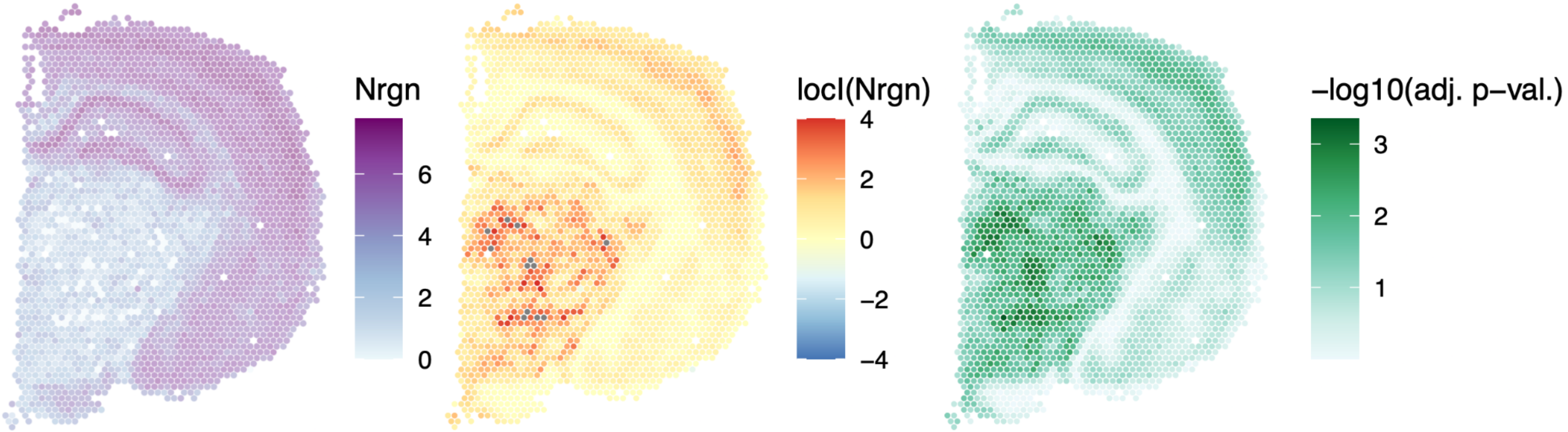
$$C = \frac{(N-1)\sum_i \sum_j w_{ij}(x_i - x_j)^2}{2W \sum_i (x_i - \bar{x})^2}$$

https://en.wikipedia.org/wiki/Moran%27s_I
https://en.wikipedia.org/wiki/Geary%27s_C

Spatial autocorrelation: Local Moran's I

– Local measure of auto-correlation (correlation to signal nearby in space)

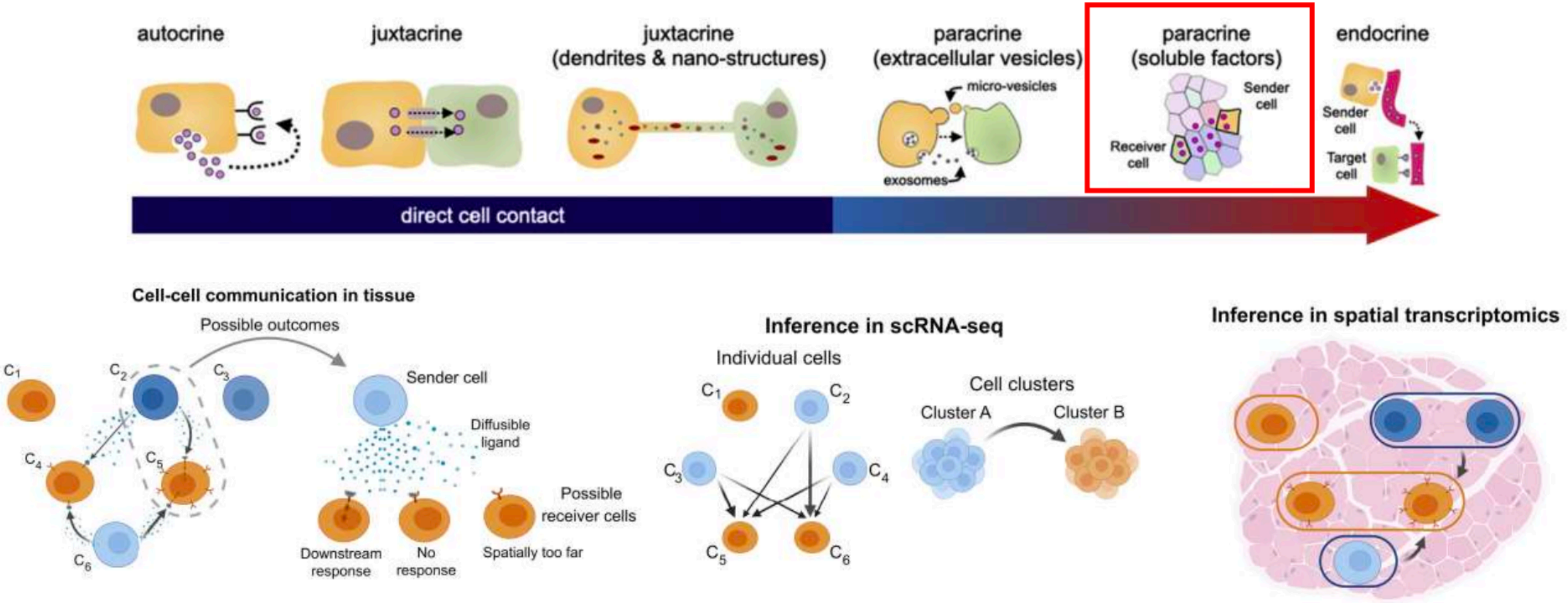
$$I_i = \frac{x_i - \bar{x}}{\sum_{k=1}^{n}(x_k - \bar{x})^2/(n-1)} \sum_{j=1}^{n} w_{ij}(x_j - \bar{x})$$



Cell-cell communication (CCC)

Slide from
Yvan Saeys

Various modes of CCC exist

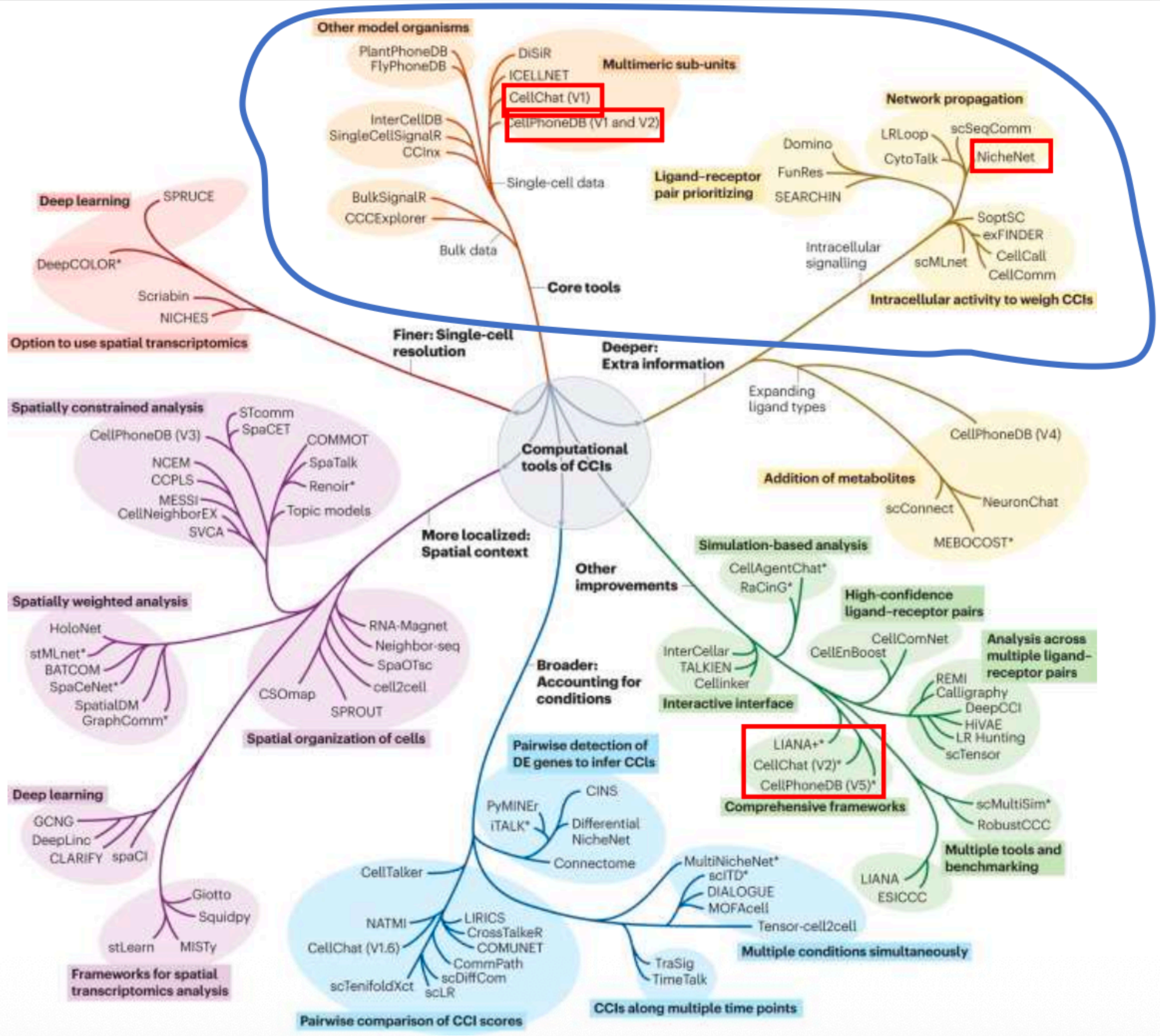


Luthria G, et al. Cell-Cell Communication Networks in Tissue: Toward Quantitatively Linking Structure with Function. Curr Opin Syst Biol. 2021 Sep;27:100341
Almet AA, Cang Z, Jin S, Nie Q. The landscape of cell-cell communication through single-cell transcriptomics. Curr Opin Syst Biol. 2021 Jun;26:12-23

University of Zurich[UZH]

Cell-cell communication (CCC)

Slide from Yvan Saeys



Armingol, E., Baghdassarian, H. M. & Lewis, N. E. The diversification of methods for studying cell–cell interactions and communication. *Nat. Rev. Genet.* (2024)

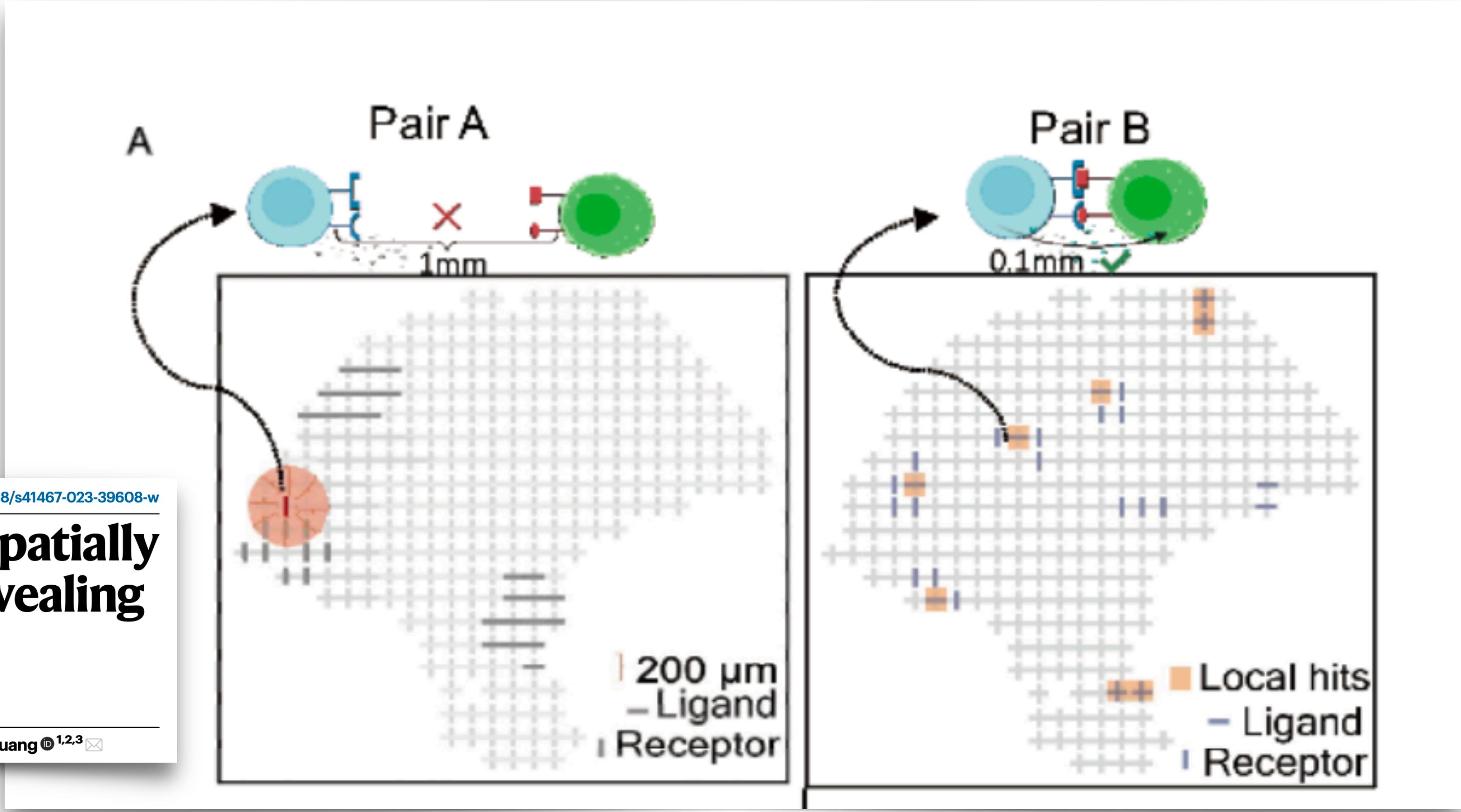
Core tools:

- CellPhoneDB
- CellChat
- NicheNet
- Liana(+)

$$\text{Global Moran's } R = \frac{\sum_i \sum_j w_{ij}(x_i - \bar{x})(y_j - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2}\sqrt{\sum_i (y_i - \bar{y})^2}},$$

Cell-cell communication

– SpatialDM: Global Moran's R, which is a bivariate version of Moran's I



Article

https://doi.org/10.1038/s41467-023-39608-w

SpatialDM for rapid identification of spatially co-expressed ligand–receptor and revealing cell–cell communication patterns

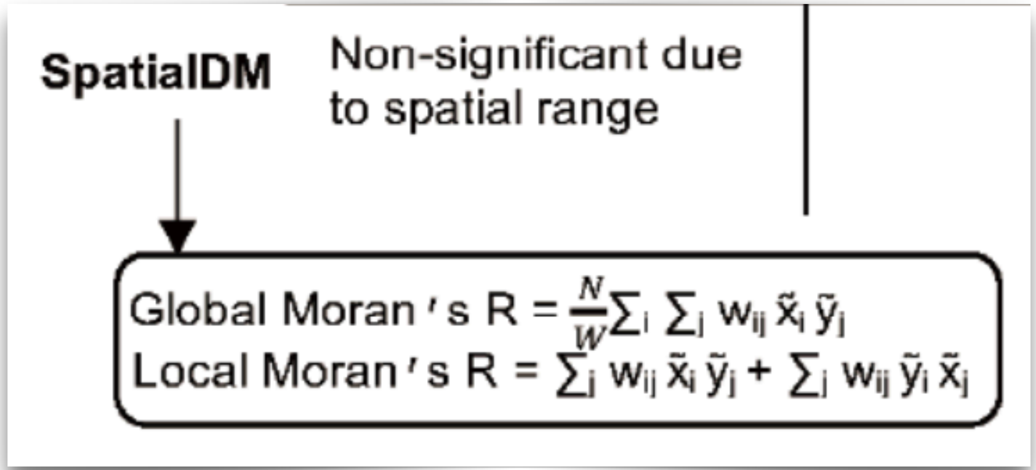
Received: 28 September 2022

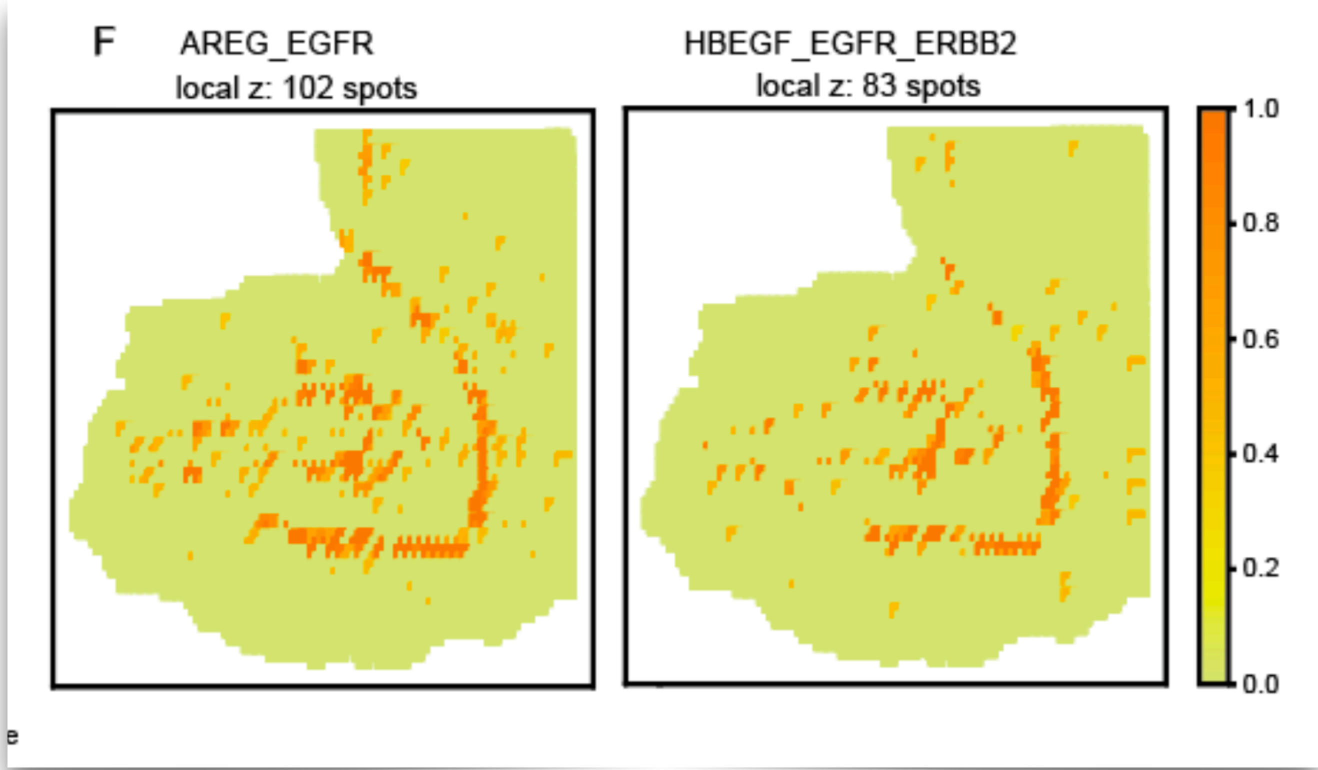
Zhuoxuan Li[1], Tianjie Wang[2], Pentao Liu[1,3] & Yuanhua Huang[1,2,3]

$$\text{Global Moran's } R = \frac{\sum_i \sum_j w_{ij}(x_i - \bar{x})(y_j - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2}\sqrt{\sum_i (y_i - \bar{y})^2}},$$

Cell-cell communication

- SpatialDM: Global Moran's R, which is a bivariate version of Moran's I

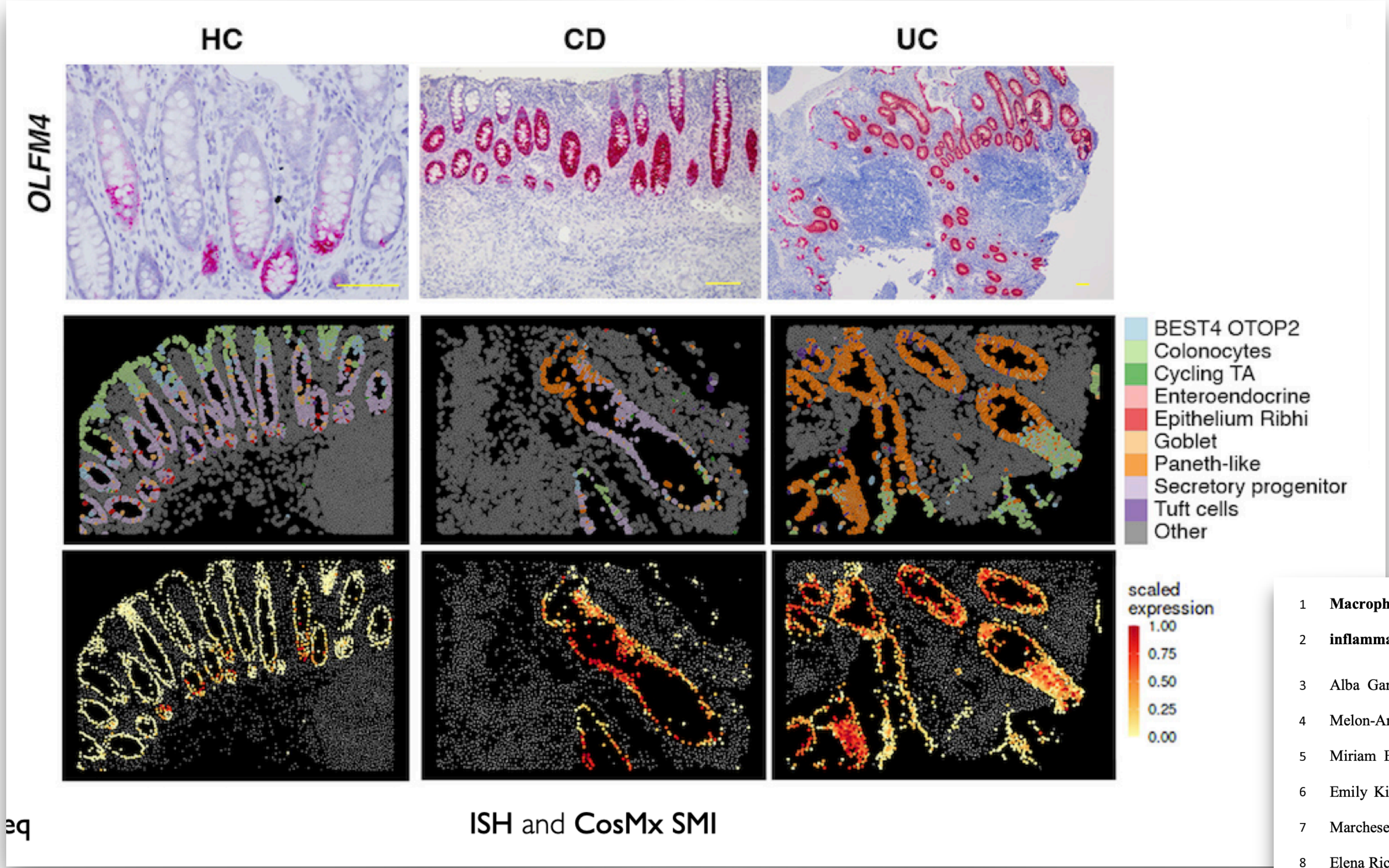




Research

- `spatialFDA`: Flexible modeling of point pattern summaries —> Martin
- `DESpace2`: DE beyond markers/SVGs: “differential spatial patterns” —> Peiying
- `sosta`: “Spatial structure”-focused analyses —> Samuel
- OSTA: Orchestrating spatial transcriptomics analysis with Bioconductor
- SpaceHack: using consensus clustering to consolidate domain detection

Tissue “structures” are often visible



- healthy control (HC)
- Crohn's disease (CD)
- ulcerative colitis (UC)

Macrophage and neutrophil heterogeneity at single-cell spatial resolution in inflammatory bowel disease

Alba Garrido-Trigo[1,2], Ana M. Corraliza[1,2], Marisol Veny[1,2], Isabella Dotti[1,2], Elisa Melon-Ardanaz[1,2], Aina Rill[3], Helena L. Crowell[4], Ángel Corbí[5], Victoria Gudiño[1,2], Miriam Esteller[1,2], Iris Álvarez-Teubel[1,2], Daniel Aguilar[1,2], M Carme Masamunt[1,2], Emily Killingbeck[6], Youngmi Kim[6], Michael Leon[6], Sudha Visvanathan[7], Domenica Marchese[8], Ginevra Caratù[8], Albert Martin-Cardona[2,9], Maria Esteve[2,9], Julian Panés,[1,2] Elena Ricart[1,2], Elisabetta Mereu[3,*], Holger Heyn[8,10,*], Azucena Salas[1,2]

Research

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- `DESpace2`: DE beyond markers/SVGs: “differential spatial patterns” —> Peiying
- `sosta`: “Spatial structure”-focused analyses —> Samuel
- **OSTA: Orchestrating spatial transcriptomics analysis with Bioconductor**
- SpaceHack: using consensus clustering to consolidate domain detection

Orchestrating Spatial Transcriptomics Analysis with Bioconductor

- https://bioconductor.org/books/OSTA

Orchestrating Spatial Transcriptomics Analysis with Bioconductor

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Book is available. Preprint on bioRxiv.
(Successor of the OSCA book: https://bioconductor.org/books/OSCA/)

Research

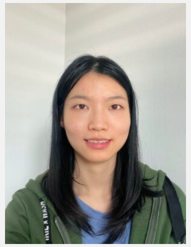
- `spatialFDA`: Flexible modeling of point pattern summaries —> Martin
- `DESpace2`: DE beyond markers/SVGs: “differential spatial patterns” —> Peiying
- `sosta`: “Spatial structure”-focused analyses —> Samuel
- OSTA: Orchestrating spatial transcriptomics analysis with Bioconductor
- **SpaceHack: using consensus clustering to consolidate domain detection**

Meta-benchmark

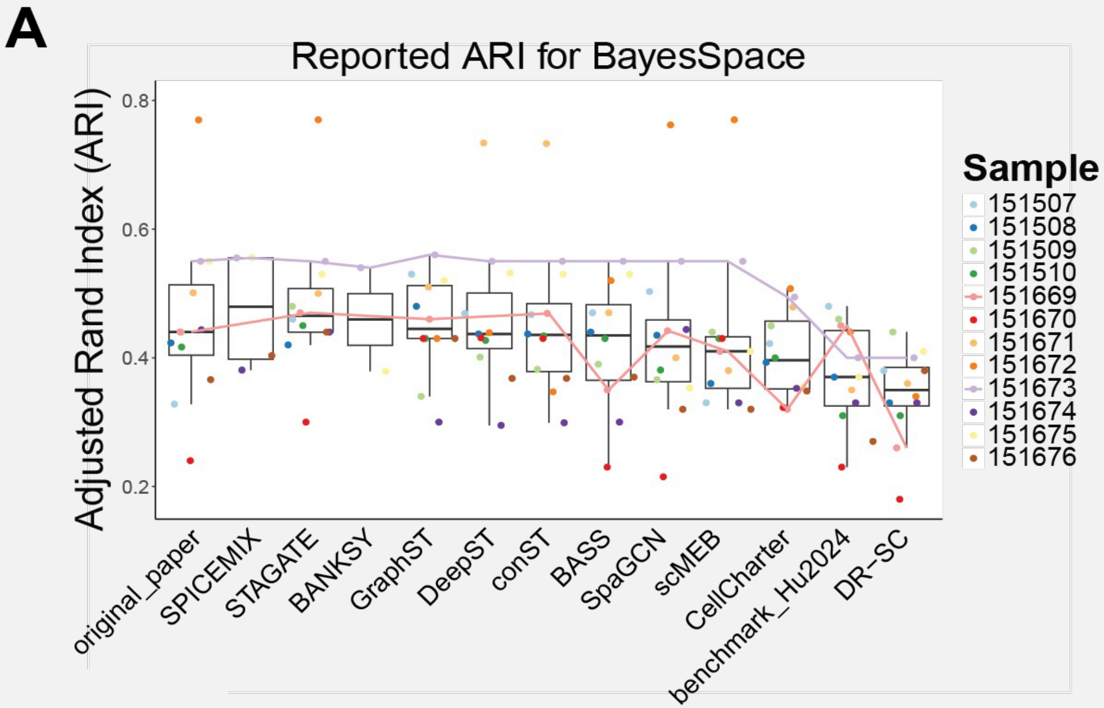
Reported method performances are inconsistent across studies

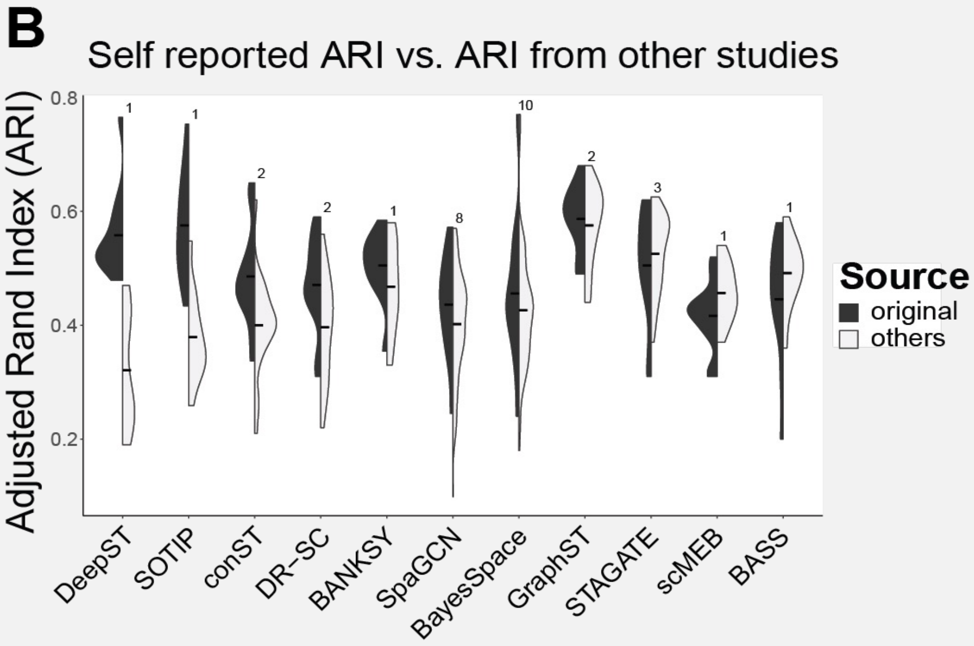
Beyond benchmarking: an expert-guided consensus approach to spatially aware clustering

Jieran Sun[1†], Kirti Biharie[2,3†], Peiying Cai[4†], Niklas Müller-Bötticher[5†], Paul Kiessling[6†], Meghan A. Turner[7†], Søren H. Dam[8,9†], Florian Heyl[10,11†], Sarusan Kathirchelvan[4], Martin Emons[4], Samuel Gunz[4], Sven Twardziok[5], Amin El-Heliebi[12], Martin Zacharias[13], SpaceHack 2.0 participants, Roland Eils[5], Marcel Reinders[3], Raphael Gottardo[1], Christoph Kuppe[6], Brian Long[7*], Ahmed Mahfouz[2,3*], Mark D. Robinson[4*], Naveed Ishaque[5*]



Peiying Cai





Ensemble clustering


Beyond benchmarking: an expert-guided consensus approach to spatially aware clustering

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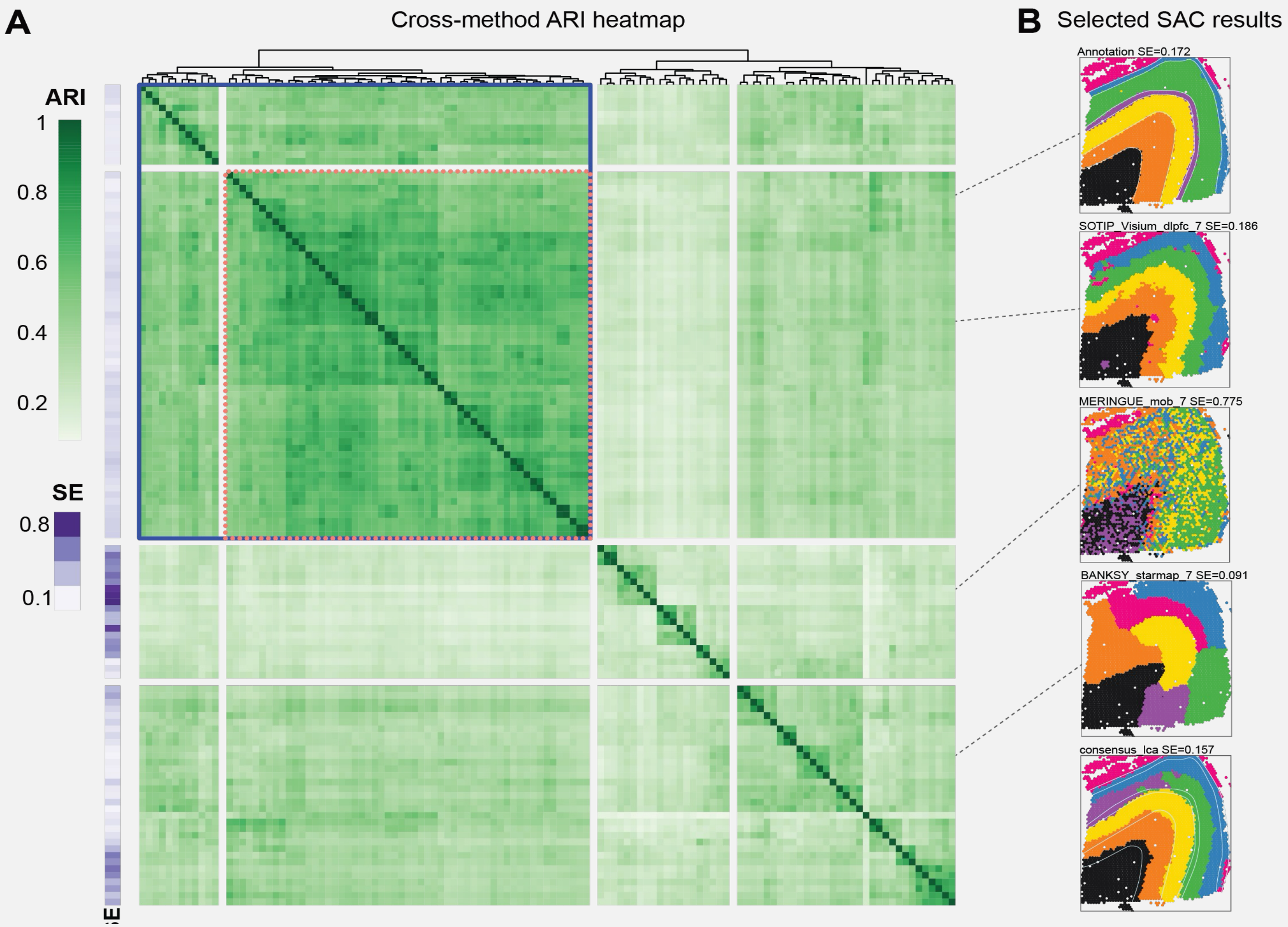
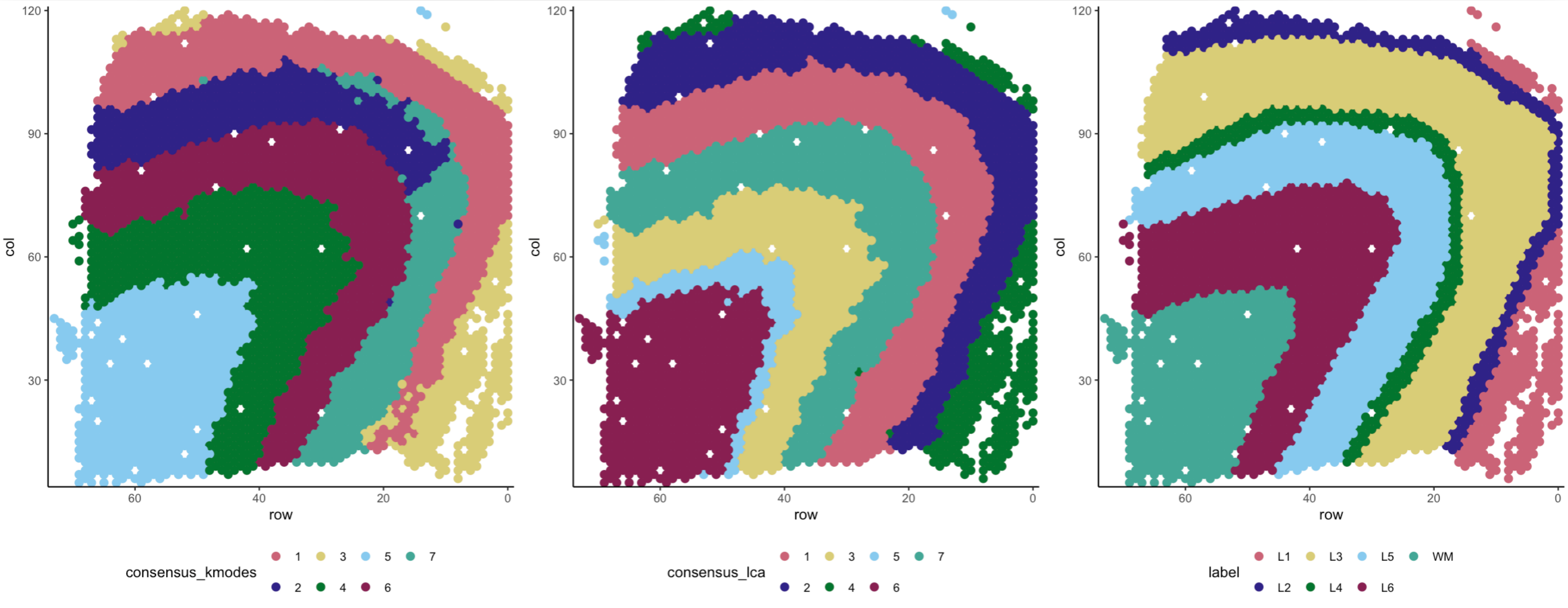
Methods are often more similar to each other than to the ground truth.

Smoothness Entropy (Low = smooth)



Consensuses

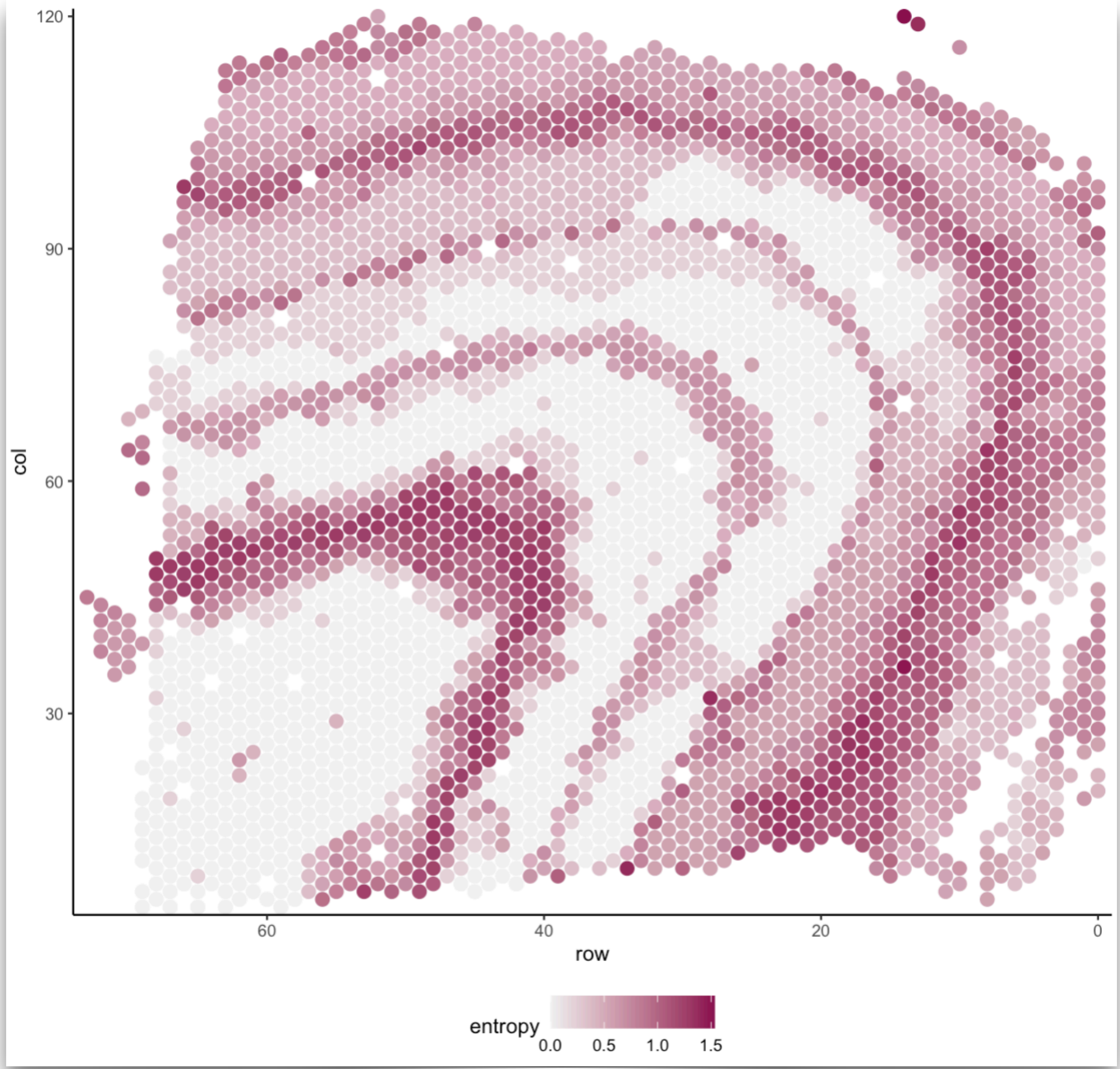
"ground truth"



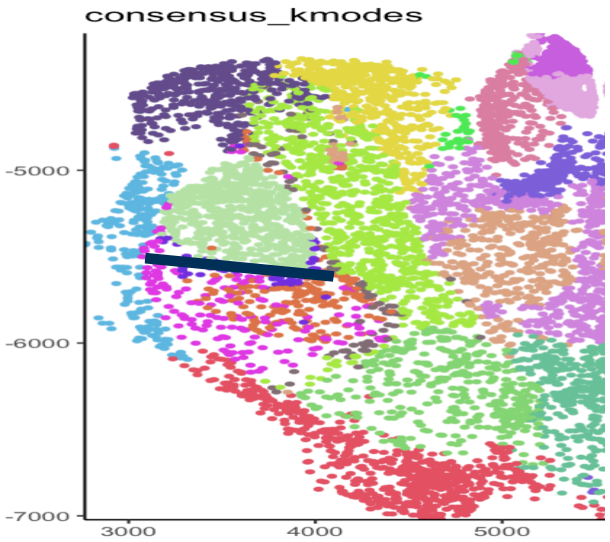
Entropy #2: Understanding spot-level uncertainty (across methods)

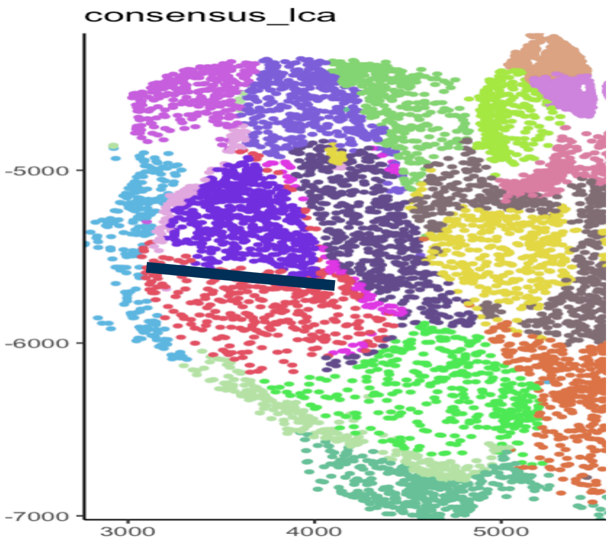
Entropy in the sense of <u>how stable across algorithms</u>

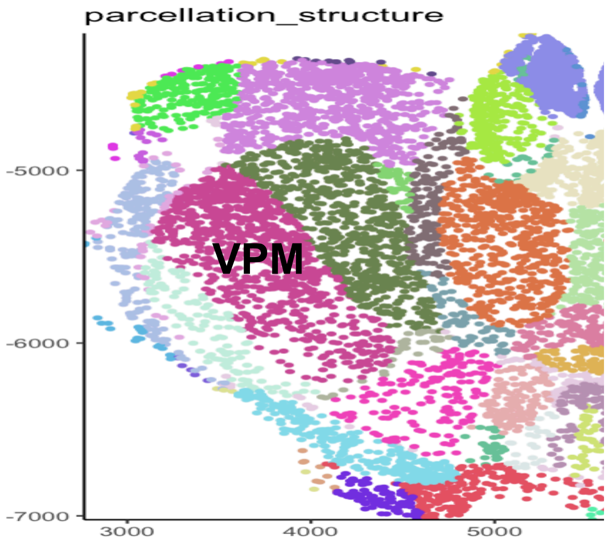
(align the spot-wise cluster labels across methods, entropy across label proportions)



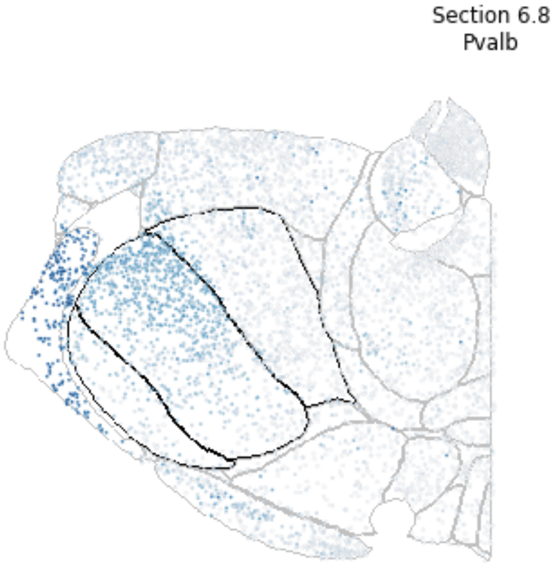
VPM

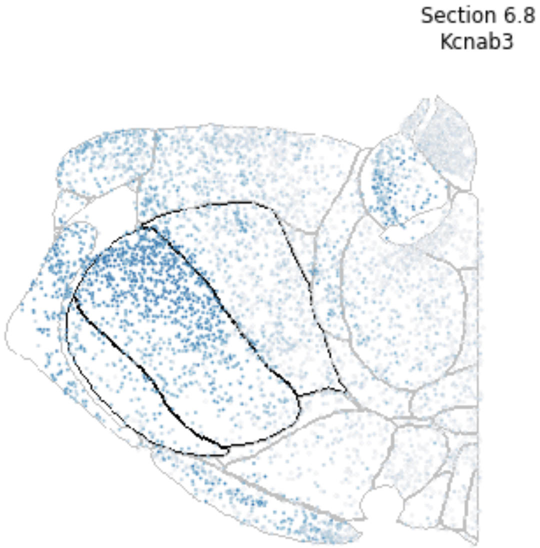


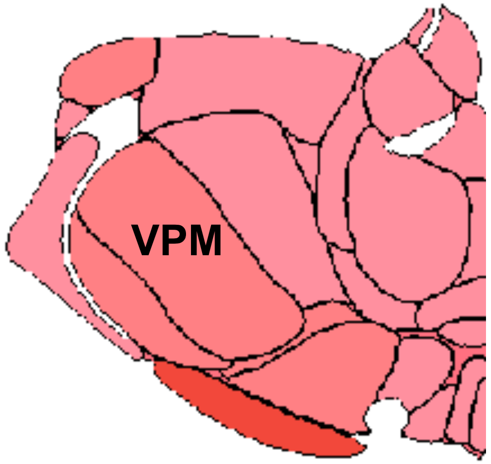




Slide from
Meghan Turner







Concluding remarks

- You are collecting/analyzing spatial data: what **spatial features** do you want to quantify?
- A few places where (classical) spatial statistics might be useful; data determines what you can do: point patterns versus lattice
- Tools available for various task: Functional data analysis (Martin), multi-cellular structure-based analyses (Samuel),