Swiss Institute of
Bioinformatics

Introduction to Spatial Transcriptomics Data Analysis

Multi-scale and multi-sample analysis

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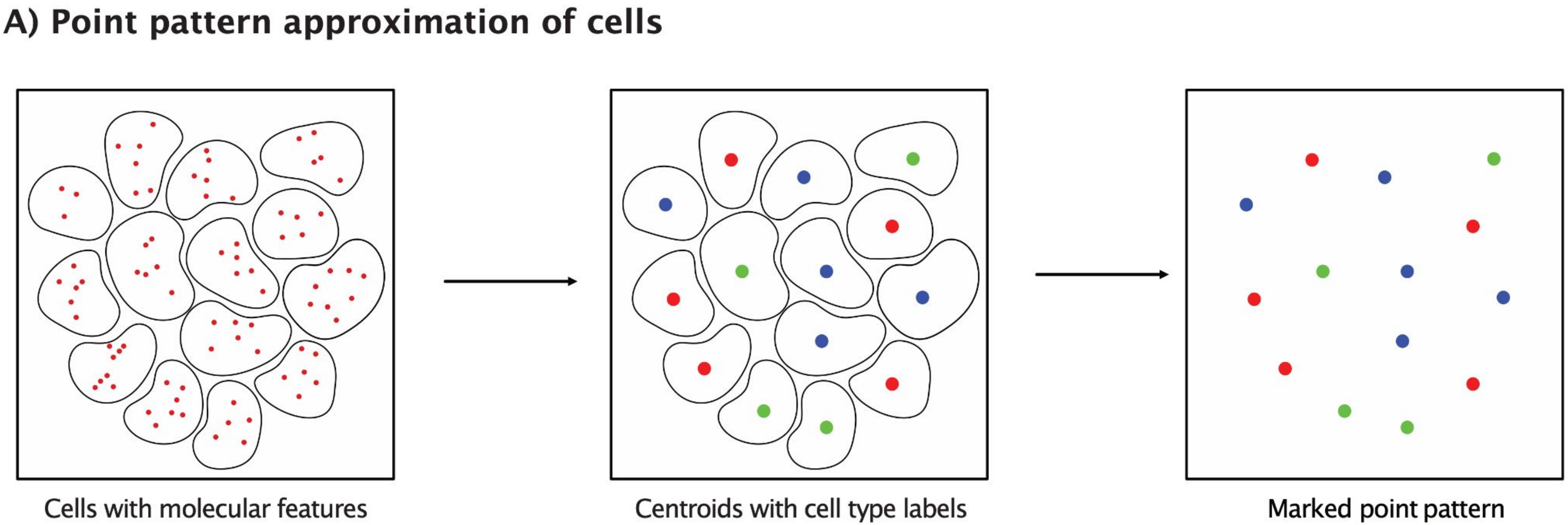
SWITZERLAND

Outline

- Lecture on downstream analysis of spatial omics data
  - i) Differential co-localisation analysis with `spatialFDA`
  - ii) Analysis of multicellular anatomical structures with `sosta`
  - iii) Differential spatial pattern analysis with `DEspace`

Differential co-localisation analysis

Approximating cells as points



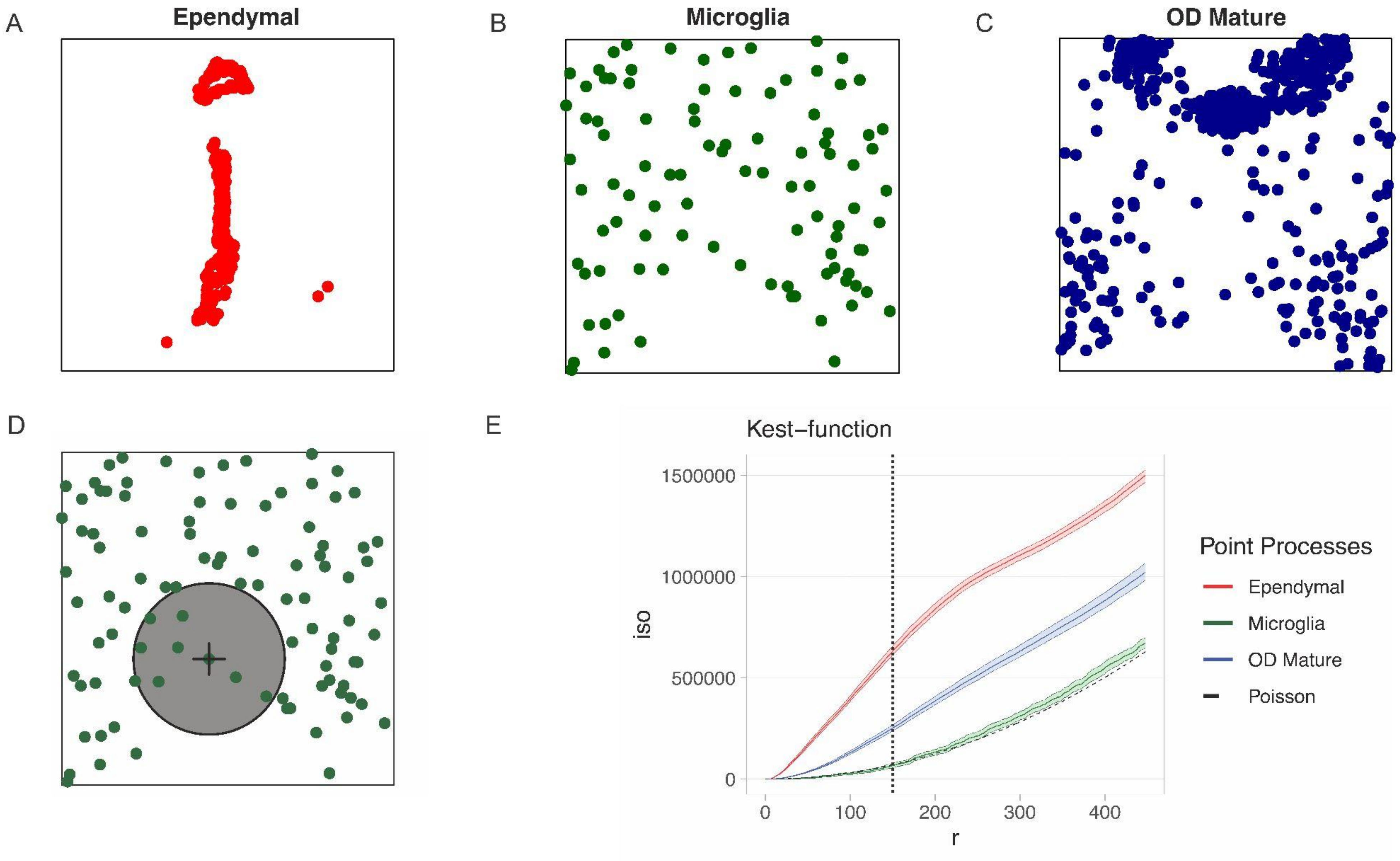
A) Point pattern approximation of cells

Cells with molecular features

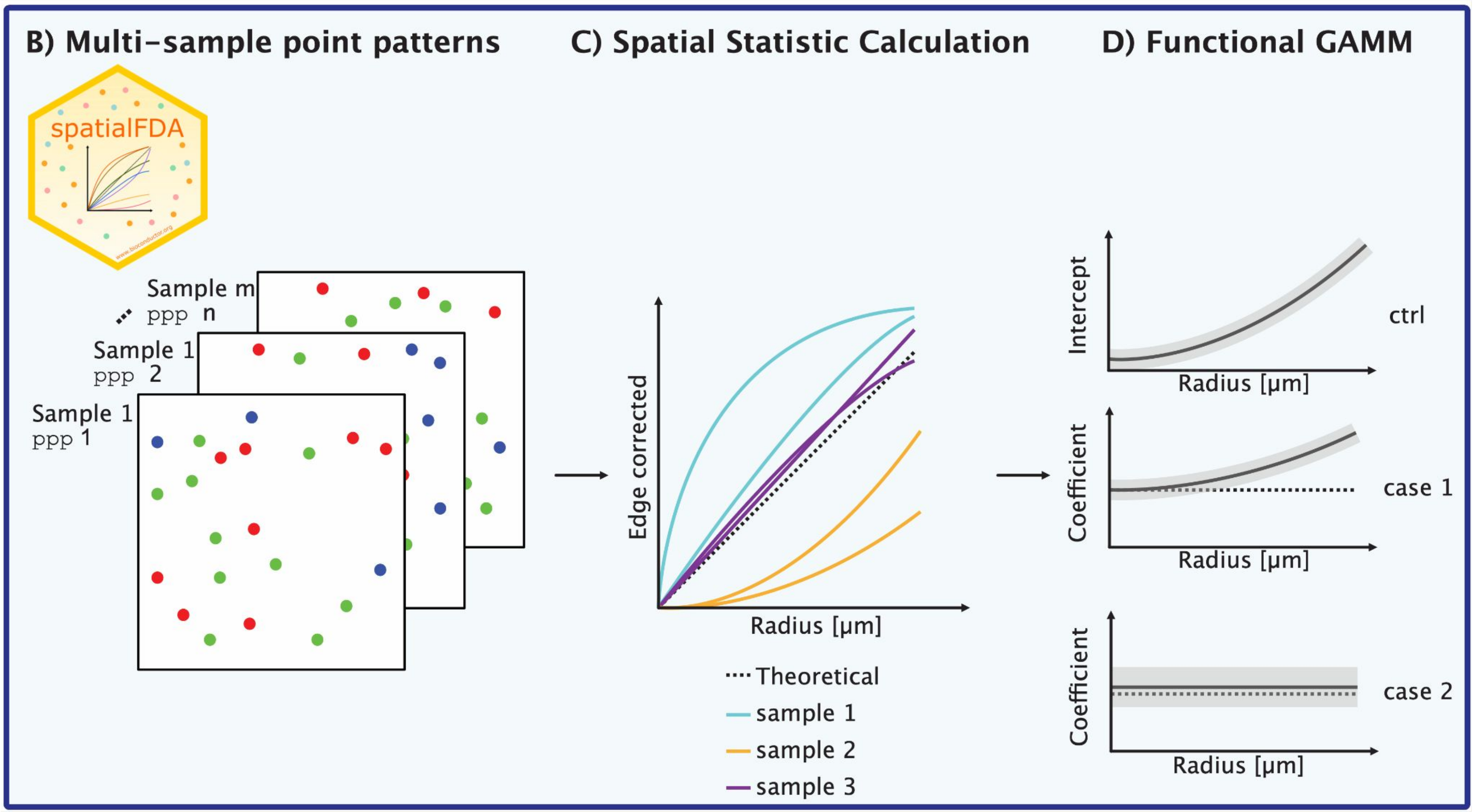
Centroids with cell type labels

Marked point pattern

Point pattern analysis summarises cell arrangements



`spatialFDA:` Differential co-localisation across scales



B) Multi-sample point patterns

C) Spatial Statistic Calculation

D) Functional GAMM

Generalised functional additive mixed models

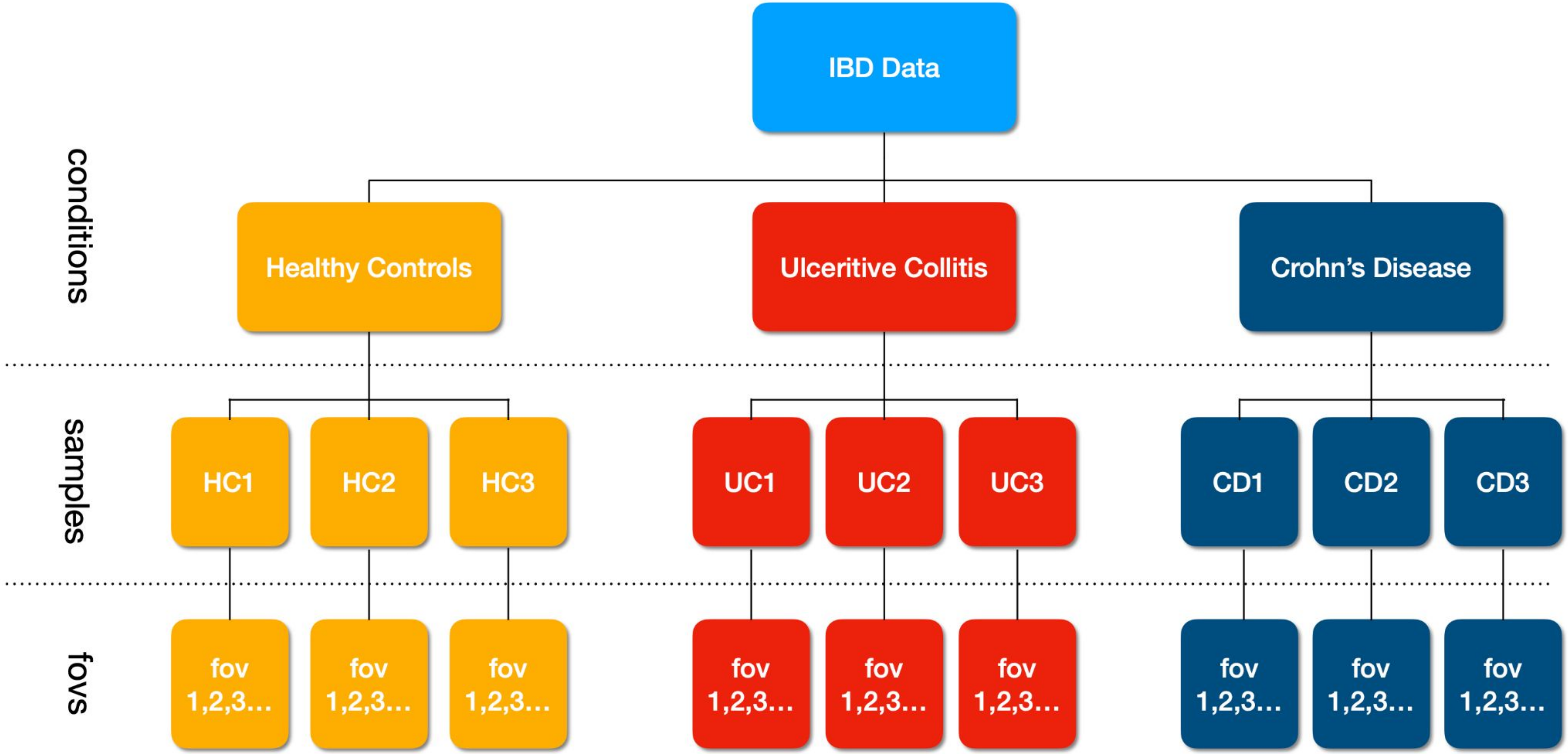
functional GAMM

$$\underbrace{\mathbb{E}[y_i(r)|X_i, g(i)]}_{functions} = \underbrace{g^{-1}}_{link}(\underbrace{\alpha(r)}_{intercept} + \underbrace{\beta_{0,g(i)}(r)}_{random\ \ intercept} + \underbrace{\sum_{j=1}^{J} f_j(X_{ji}, r))}_{predictors}$$

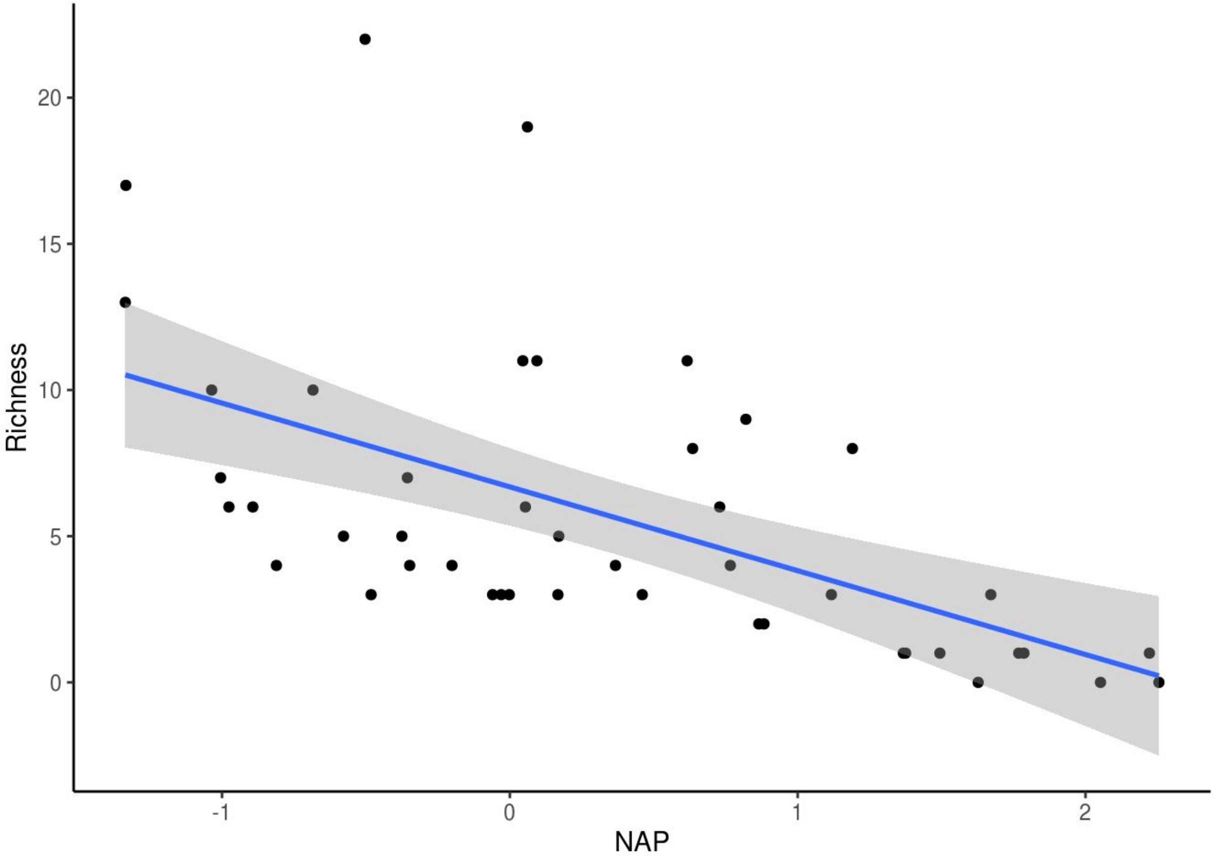
F-test of overall effect

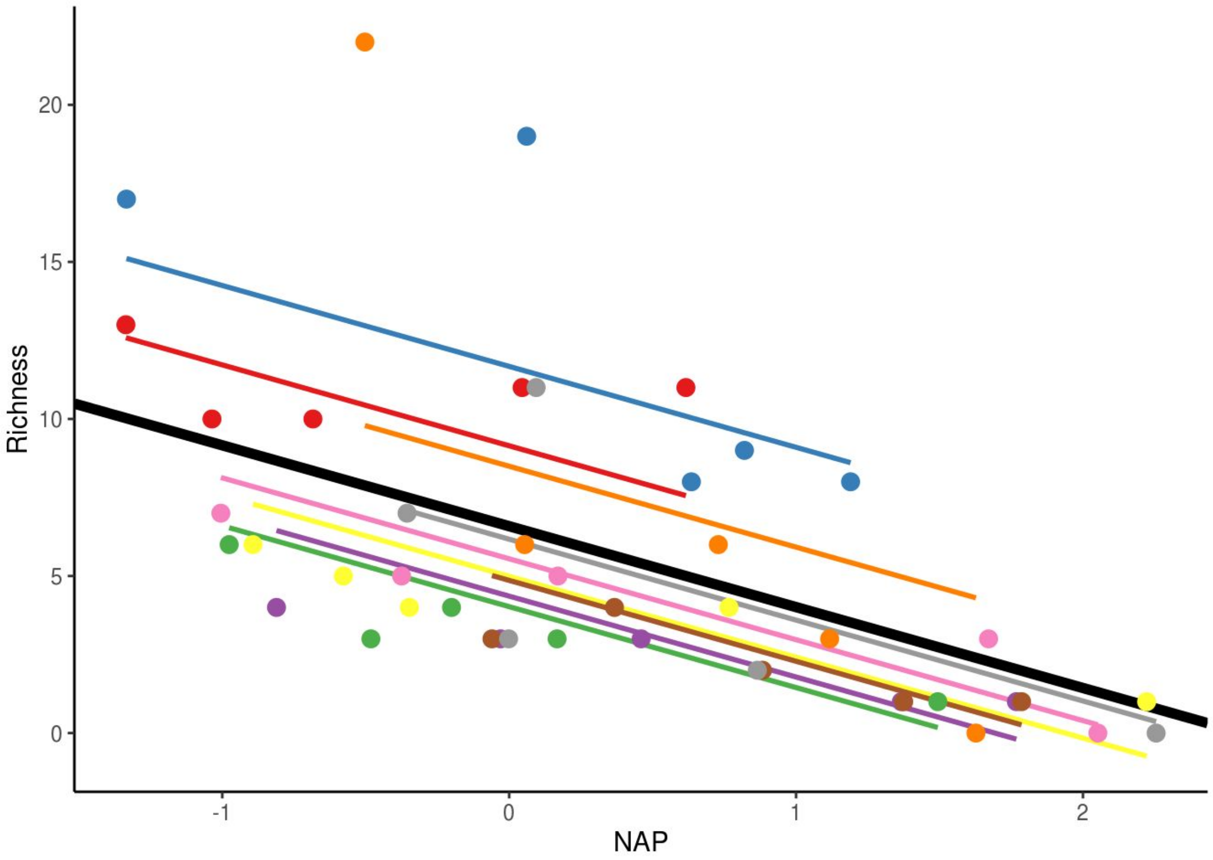
$$H_0 : \beta(r) = 0, \forall r \in [r_{min}, r_{max}]$$
$$H_1 : \exists r \in [r_{min}, r_{max}] s.t. \beta(r) \neq 0$$

Modern spatial datasets show nested covariances



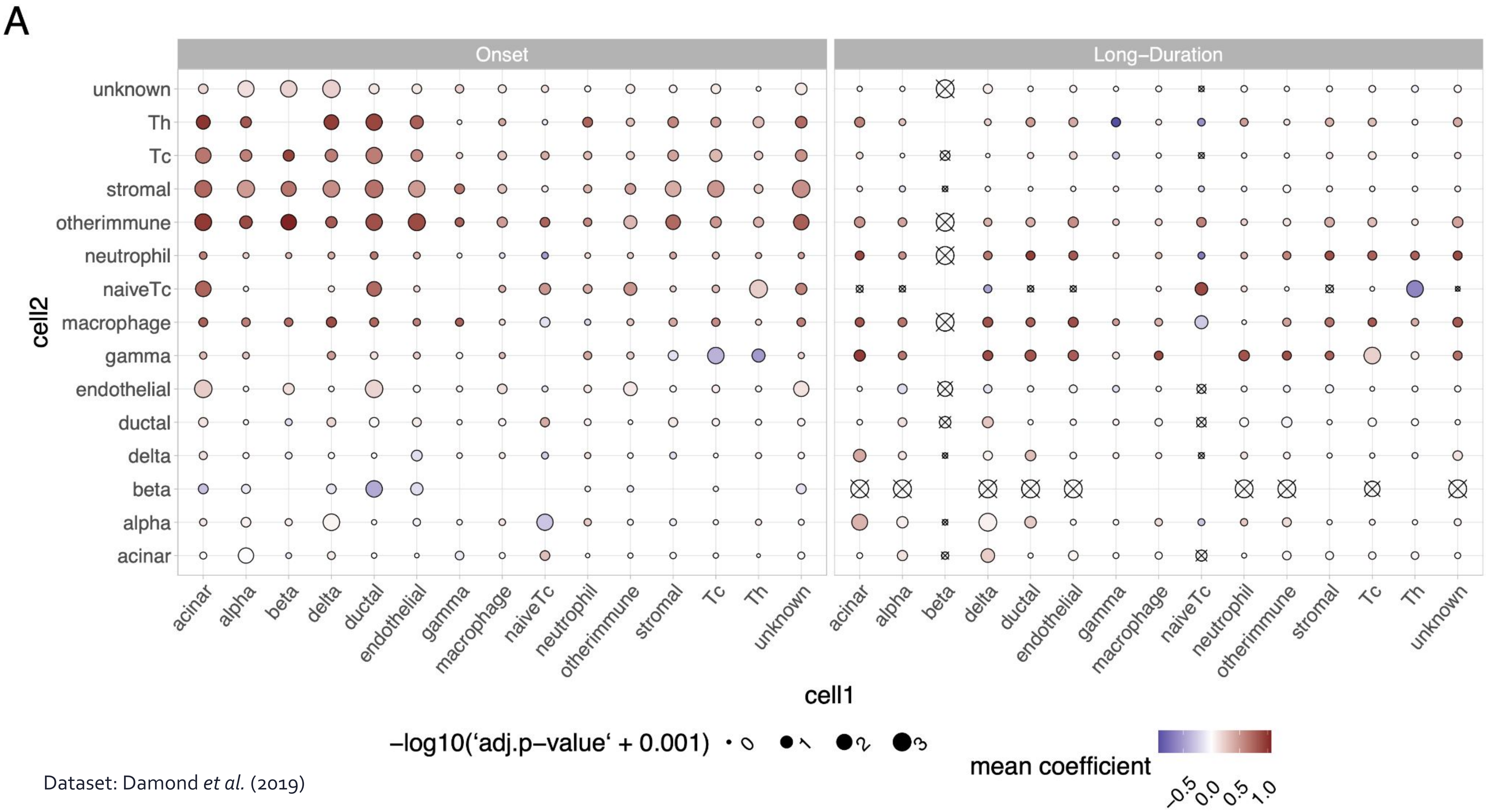
Random intercepts account for group effects





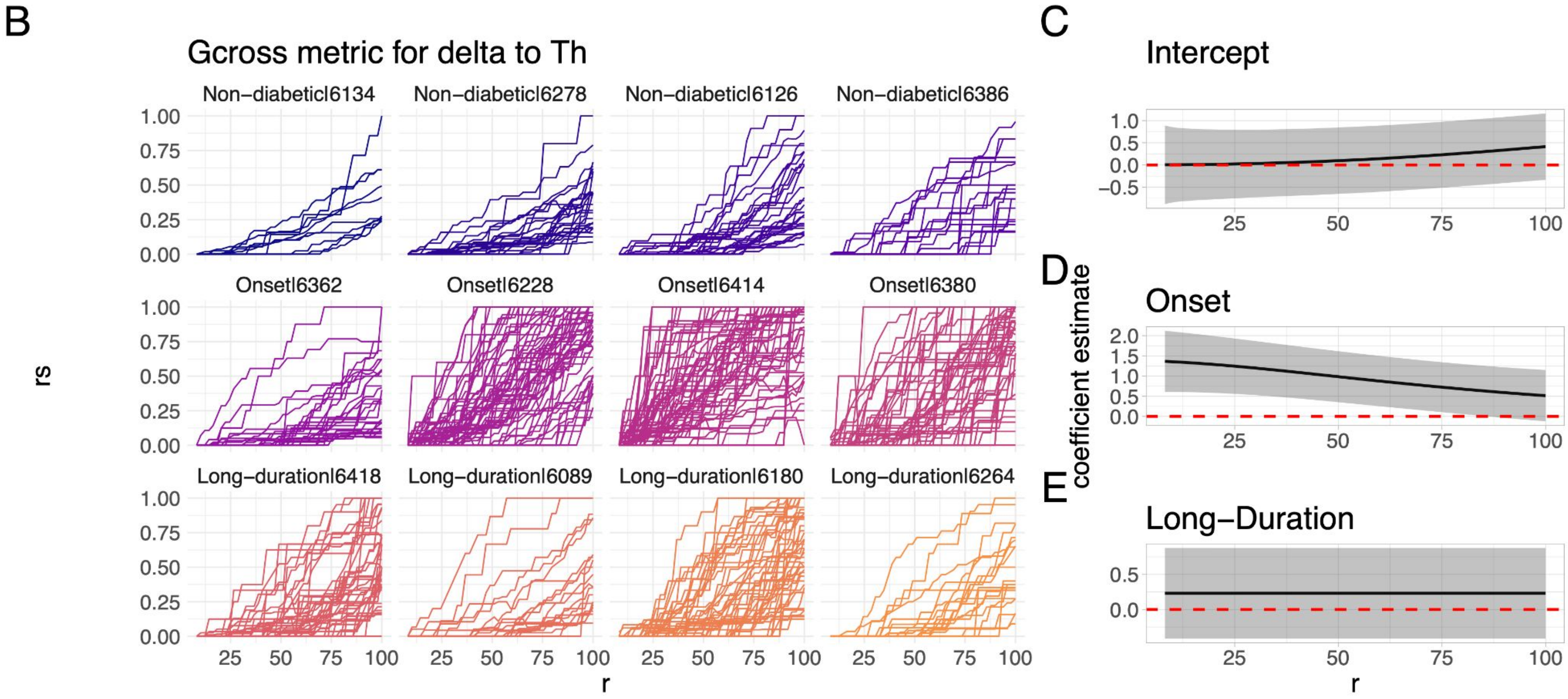
adapted from [Santangelo, 2018], licensed as CC BY 4.0

Overall *F*-test results of pairwise cell type combinations



Dataset: Damond *et al.* (2019)

fGAMM shows scale-effect of delta-Th interaction



Dataset: Damond *et al.* (2019)

Summary: i) Differential co-localisation

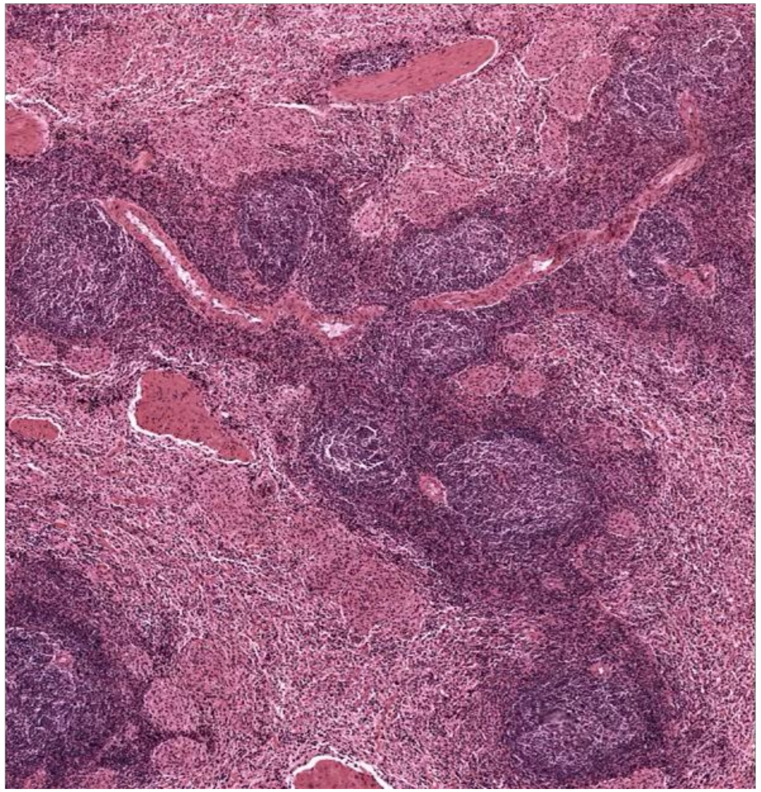
- We approximate cells as points in space by their centroid
- The cell type is added as a mark → marked point pattern
- `spatialFDA` is a tool for differential co-localisation analysis
- mixed effects models can account for nested covariances
- The spatial scale of differential co-localisation is maintained in `spatialFDA`
- `spatialFDA` is available on R/Bioconductor

Analysis of multicellular anatomical structures

Biological function is often reflected in morphology and location

Ileum, villi and crypt

Spleen, red and white pulp



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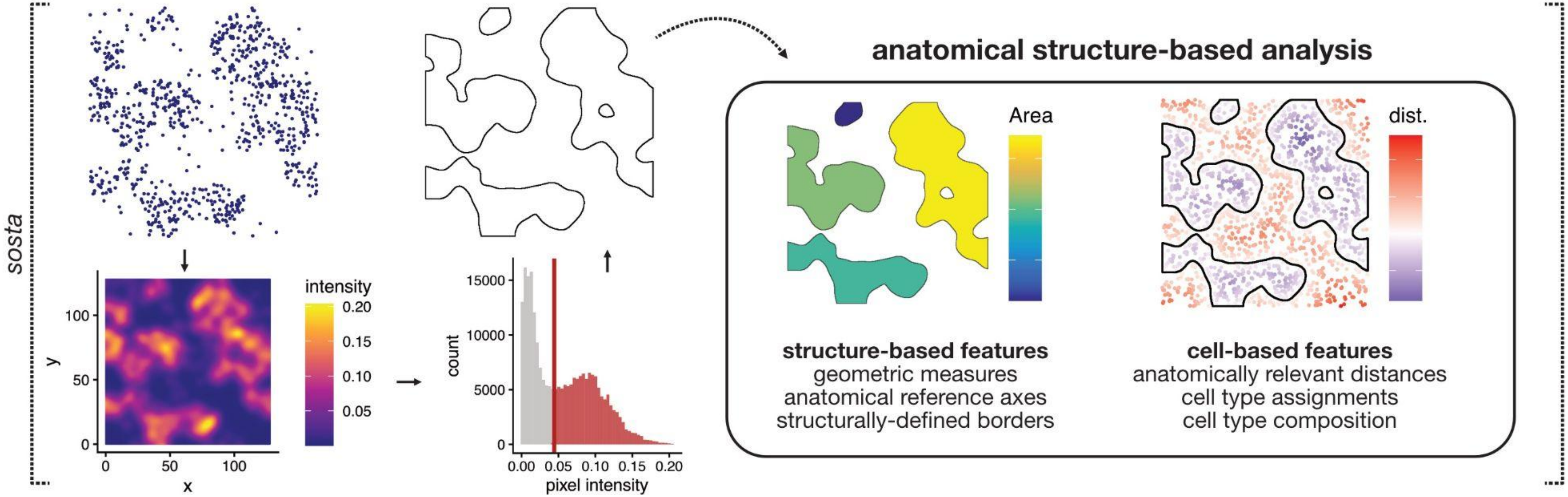
Virginia University - Digital Histology; Pakura, Bigbee *et al.*; https://digitalhistology.org/; (CC BY-NC-SA 4.0)

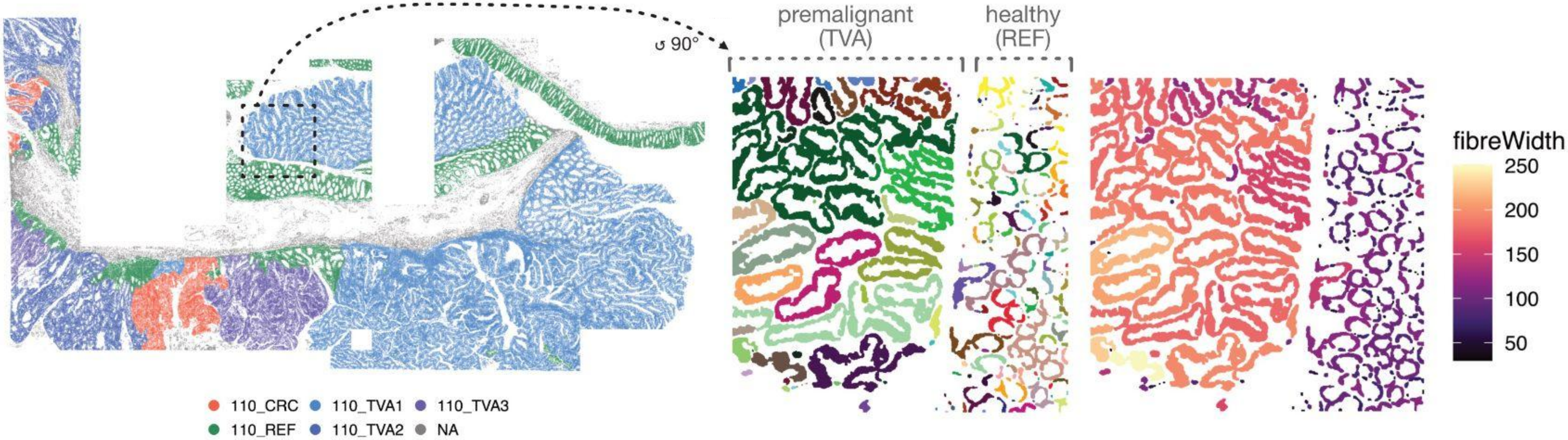
Analysis of anatomical structures using *sosta*



Gunz *et al.* (2025)

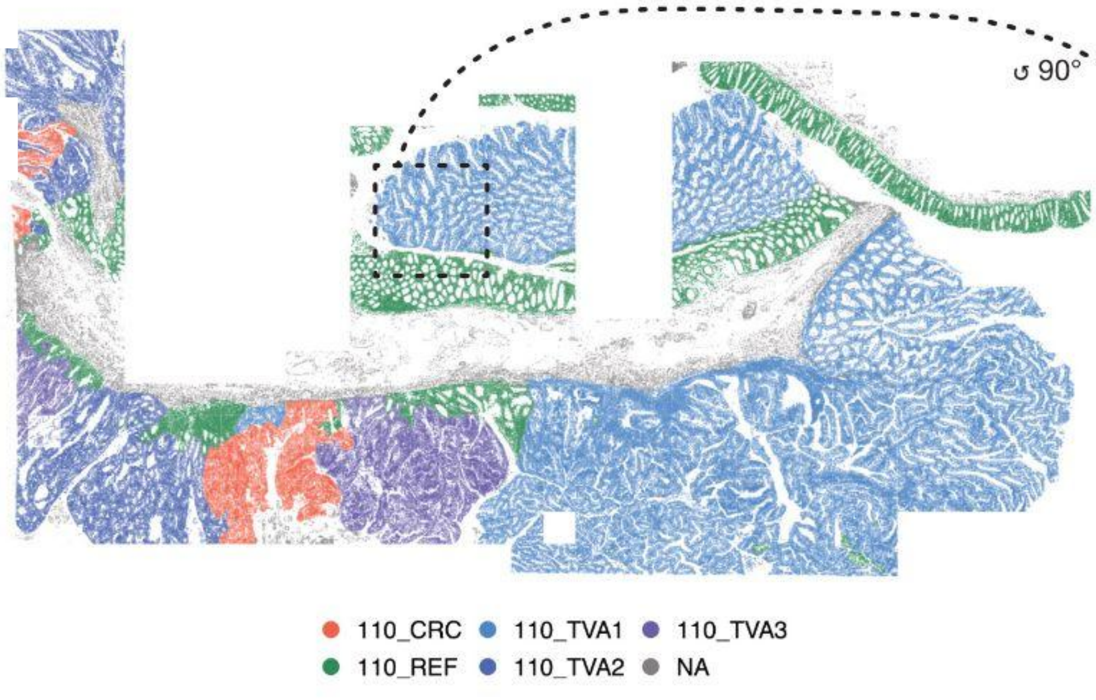
Point pattern intensity based segmentation

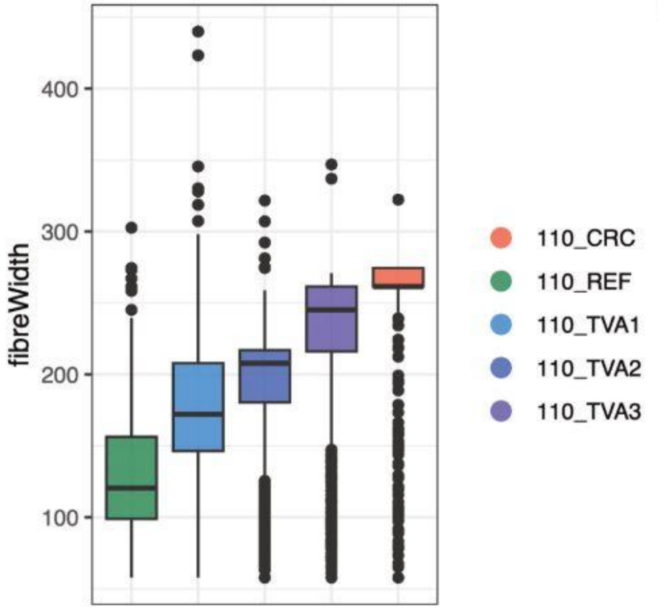
Structure based analysis: Geometric features



Dataset: Crowell *et al.* (2025)

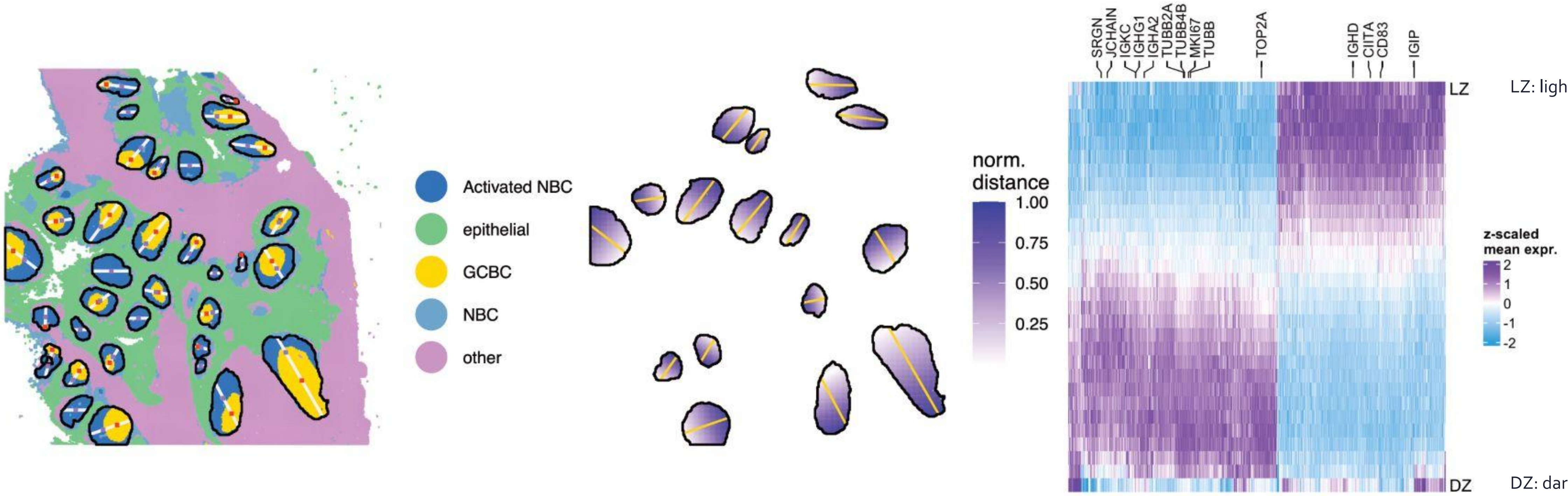
Structure based analysis: Geometric features





- Fibre width increases along the pathological progression: healthy (HC) to tubulovillous adenoma (TVA) to colorectal cancer (CRC)
- Reflects gradual morphological changes related to increase in crypt thickness.

Structure based analysis: Gene expression



LZ: light zone

DZ: dark zone

Dataset: 10x (2025)

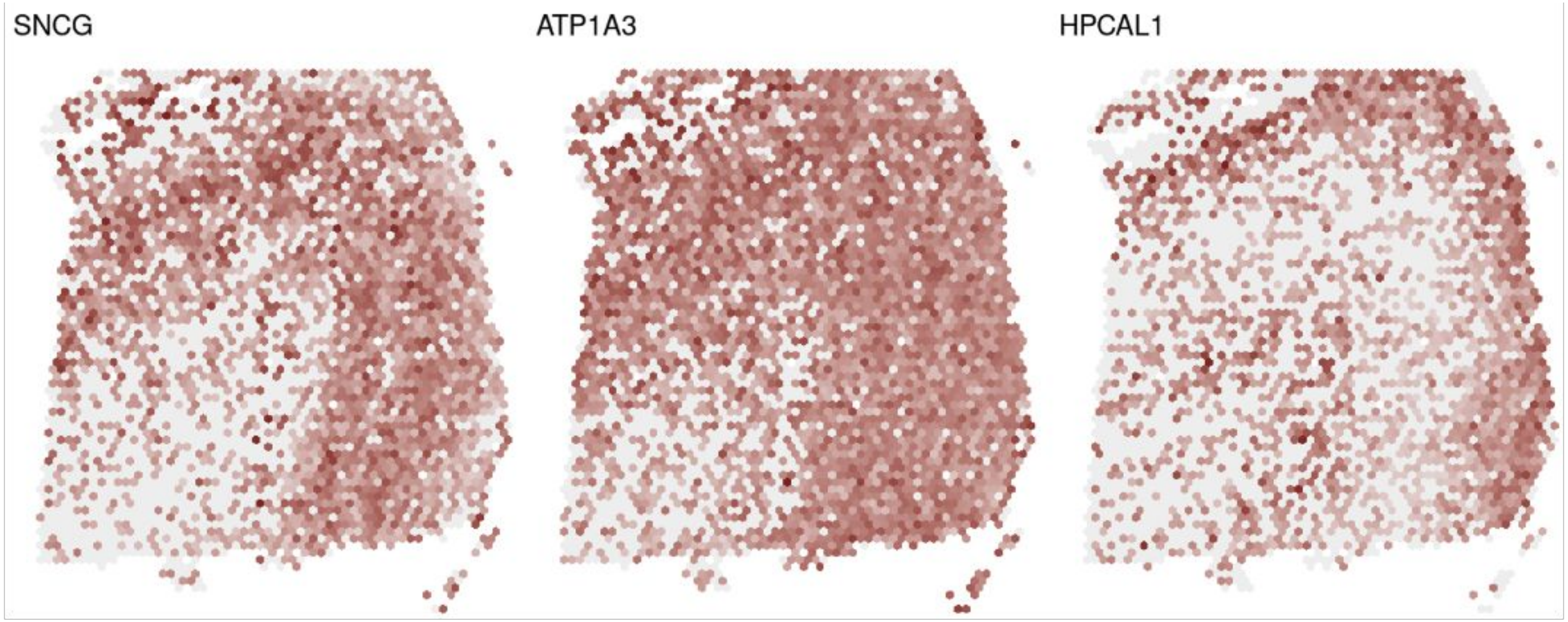
- Germinal centers are well defined anatomical structures with clear orientation
- Structure-based gene expression analysis to recover DZ / LZ markers

Summary: ii) Analysis of multicellular anatomical structures

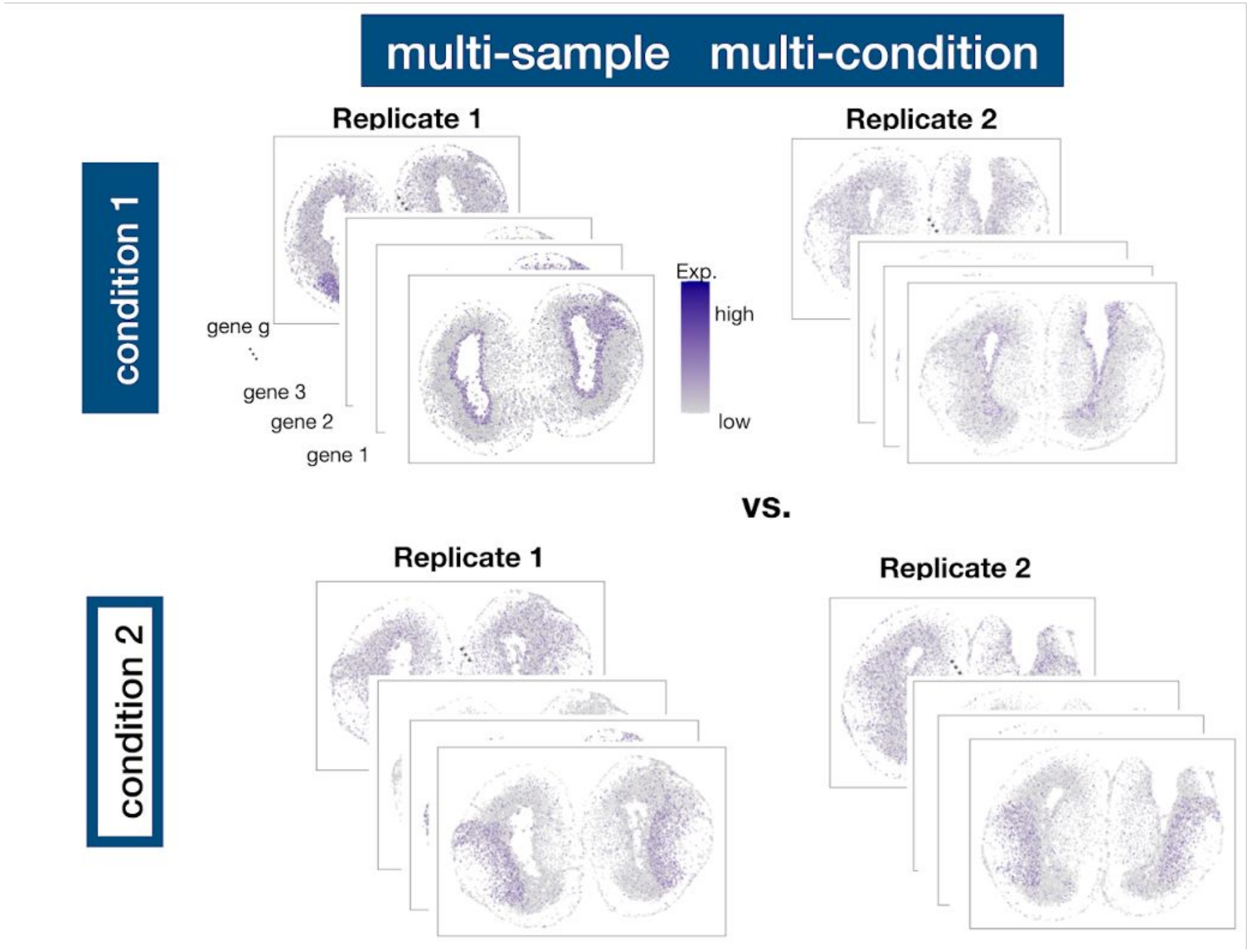
- Analysis of anatomical structures in spatial omics data inspired by “form reflects function”
- Geometrical features can reflect disease progression
- Anatomical information can be used as a reference system for spatial gene expression analysis
- Common challenge: what are relevant anatomical structures and how to identify them
- `sosta` is an R/Bioconductor package for analysis of anatomical structures in spatial omics data

Differential spatial pattern analysis

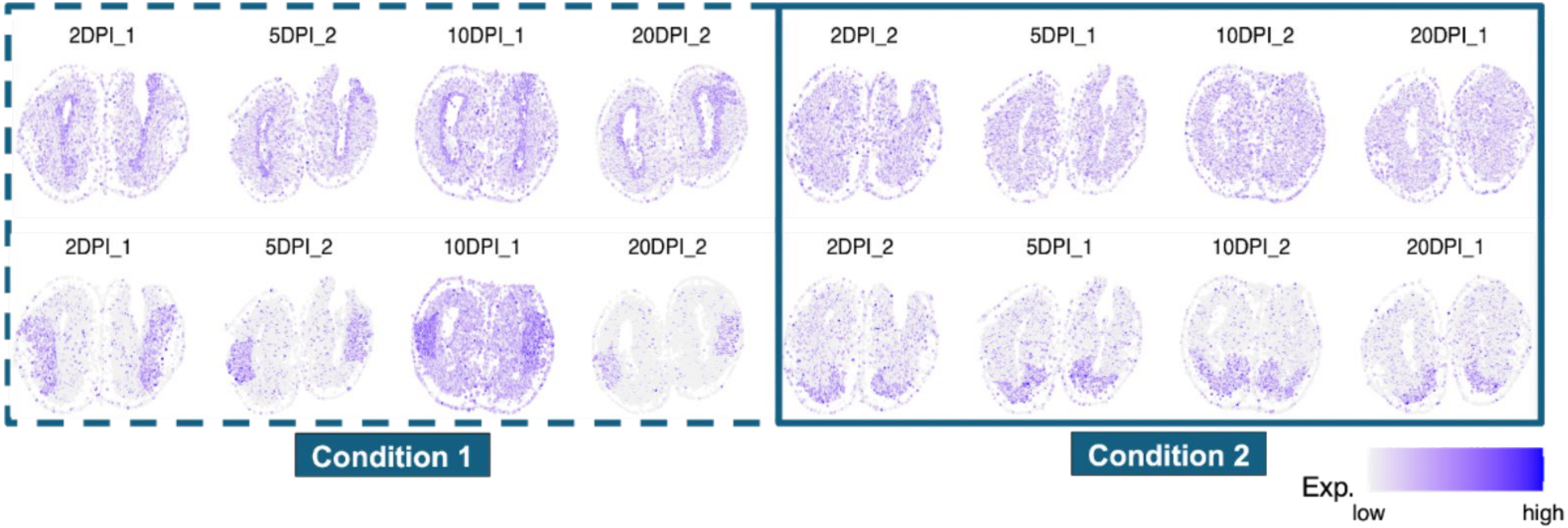
Recap: spatially variable genes



From one tissue section to multiple samples and conditions



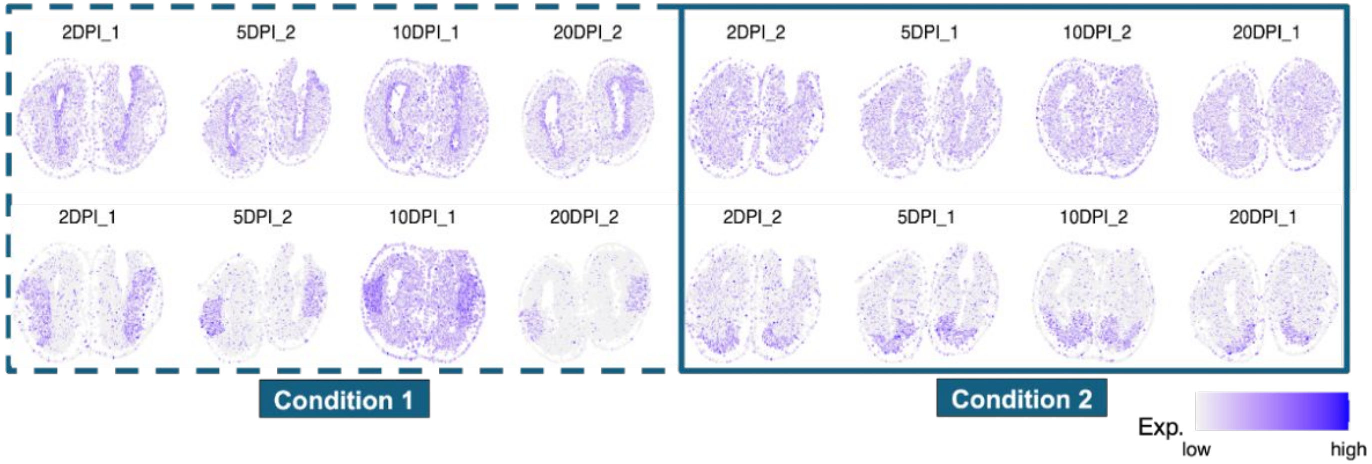
Differential spatial patterns (DSP)



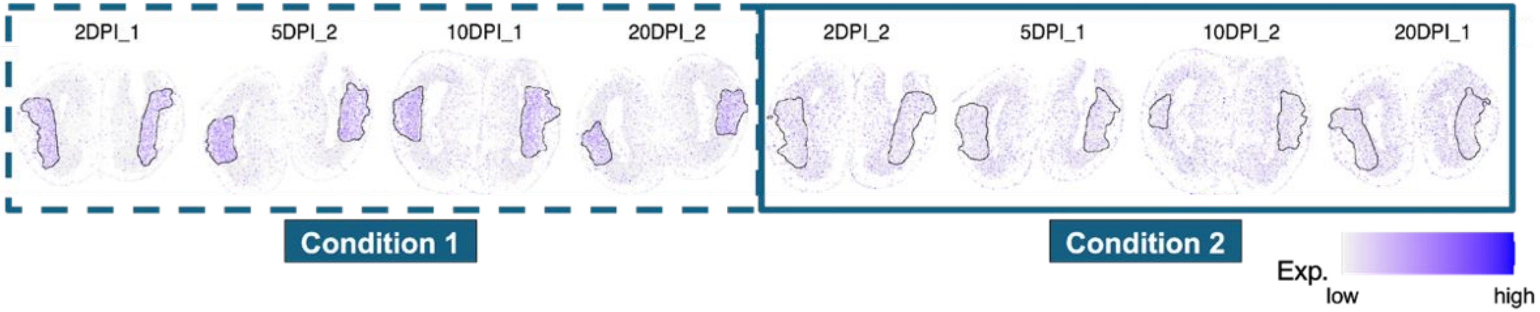
DSP genes are those whose spatial expression patterns change across groups, such as different treatment conditions or time phases.

DESpace:

1. Global test: detect DSP genes.



2. Individual cluster test: identify the spatial clusters where gene abundance varies across conditions

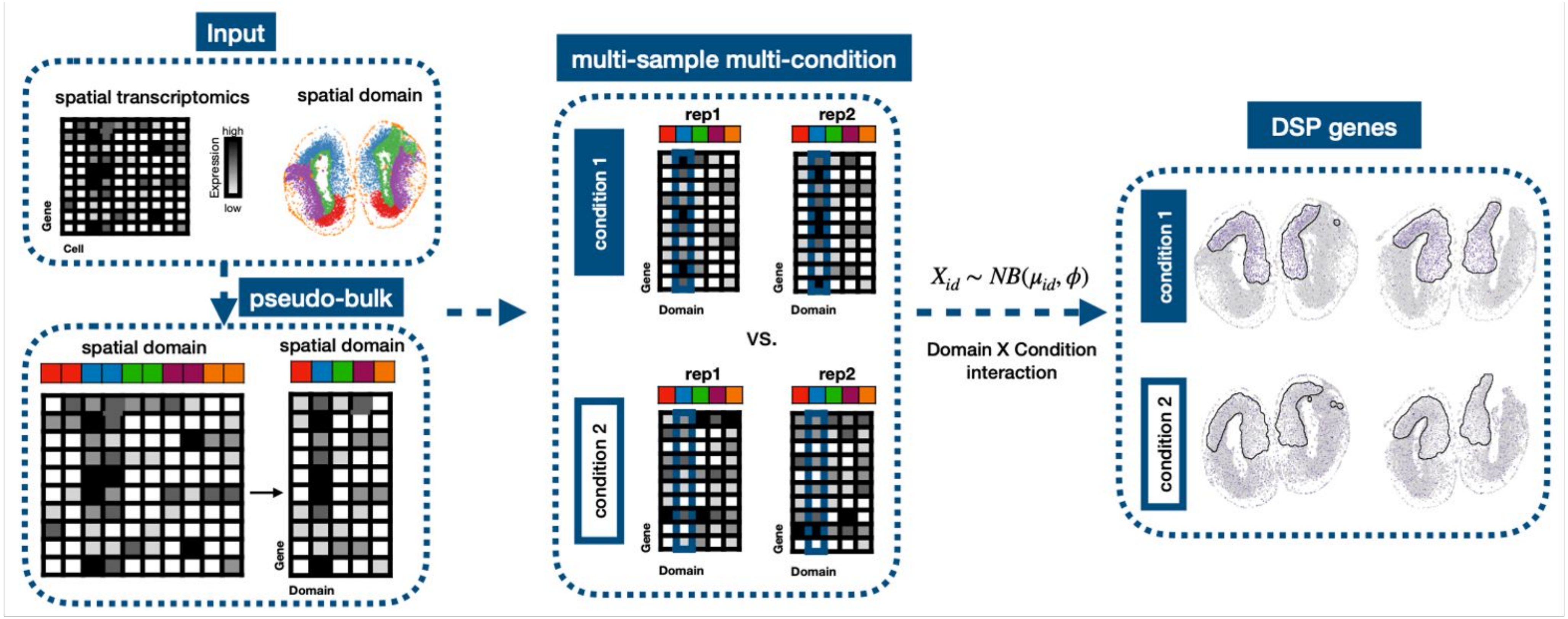


Obtaining shared domains: BANKSY + Harmony

Harmony correction on Banksy PCA embedding

1. Compute BANKSY representation per sample
2. PCA on BANKSY representation
3. Use Harmony to correct sample/batch effects in PCA space
4. Run UMAP/clustering on Harmony-corrected embedding

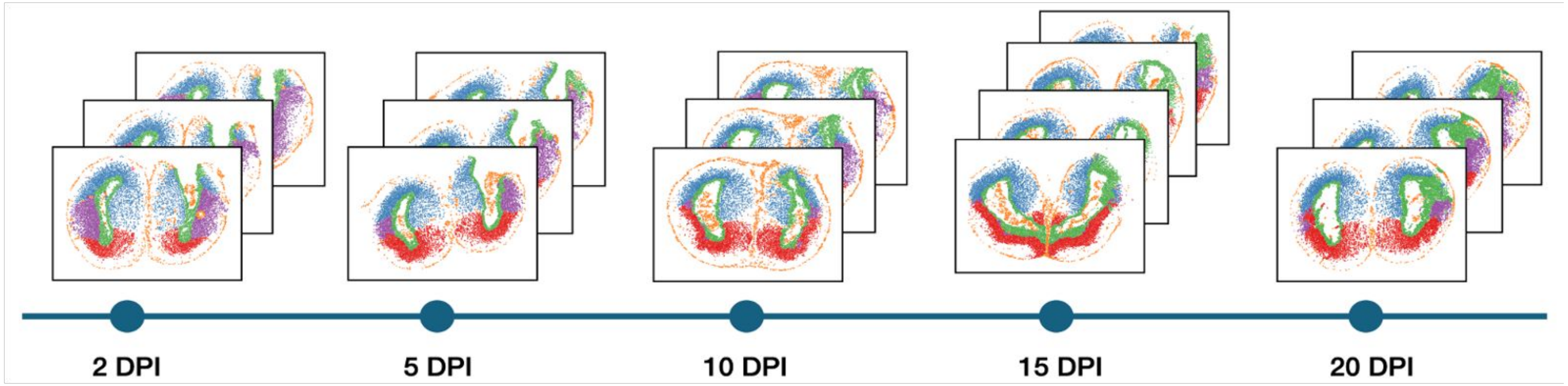
Methodological details



$$\begin{aligned} X_{id} &\sim NB(\mu_{id}, \phi), \\ \log(\mu_{id}) &= \log(M_{id}) + \beta_d + \beta_{c_i} + \beta_{c_i d}, \\ &\quad \text{for } i = 1, \ldots, N,\ d = 1, \ldots, D, \\ &\quad \text{and } c_i = 1, \ldots, N_c, \end{aligned}$$

Biological example

- ARTISTA (Stereo-seq) dataset captures axolotl brain regeneration at single-cell resolution
- 16 samples from 5 time points, i.e., days post-injury (DPI)
- 5 consistent spatial clusters

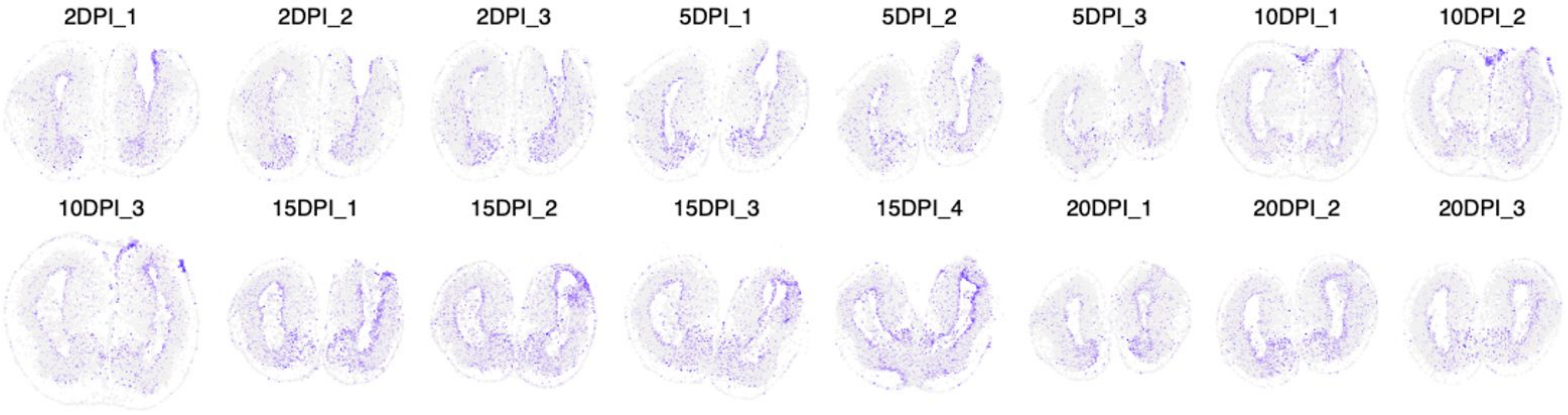


Wei, Xiaoyu, et al. "Single-cell Stereo-seq reveals induced progenitor cells involved in axolotl brain regeneration." Science 377.6610 (2022): eabp9444.

Biological example

DSP gene example

TNC: a glycoprotein in adult neurogenic niches, involved in tissue repair and regeneration



Summary: iii) Differential spatial pattern

- SVGs capture expression patterns that vary across tissue structure within sample.
- DSP analysis asks whether spatial expression patterns change across conditions.

References

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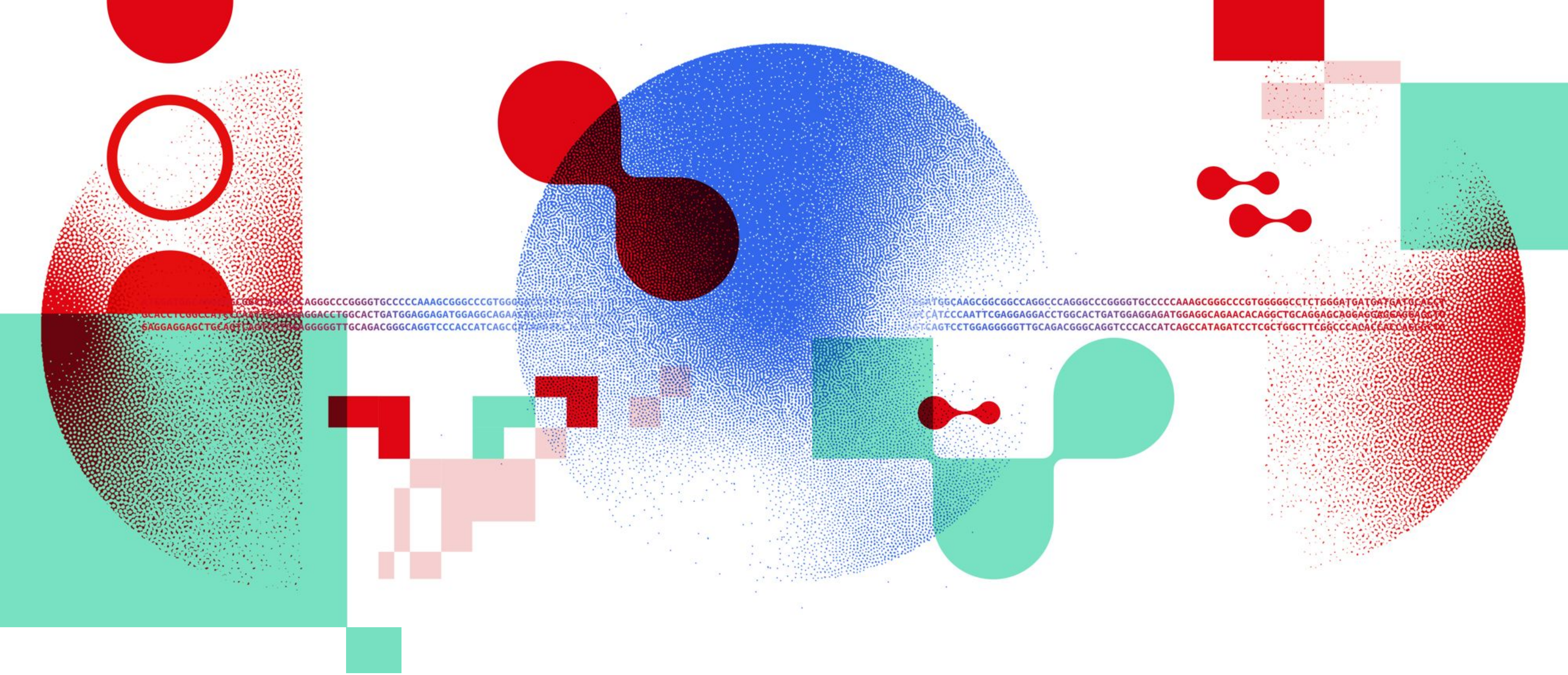

Thank you

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