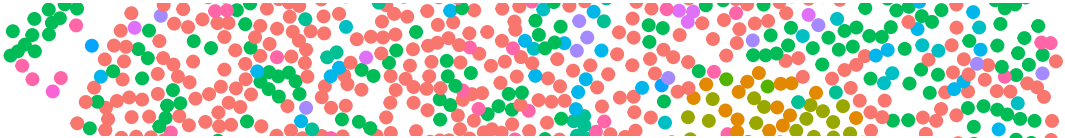
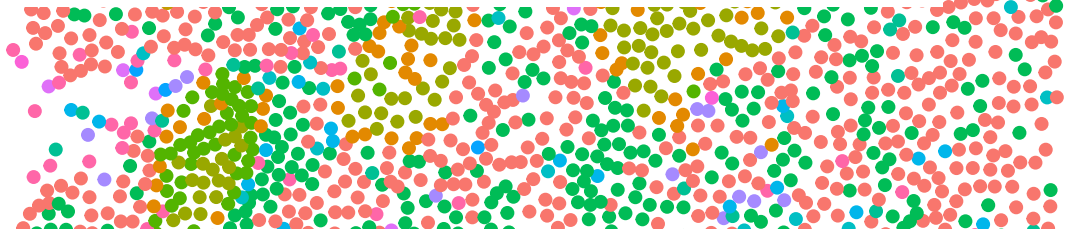


Sequencing-based Spatial Transcriptomics Data Analysis

Spatial Statistics for Differential Analysis

Martin Emons, Statistical Bioinformatics Group, UZH & SIB

December 10, 2025



Outline

- `pasta` - a resource for exploratory spatial analysis
- Lattice data analysis to compare spatial associatons on a grid
- Point pattern analysis for comparisons of stochastic processes
- Comparison of spatial statistics functions with `spatialFDA`

Cover picture data from [Damond et al., 2019]

Technological Overview

- High-throughput sequencing (HTS) of the transcriptome allows for high-throughput characterisation of cells
- Traditional HTS platforms work either on a homogenised sample (bulk) or on single cells (sc)
- These approaches come at the loss of spatial information
  - Tissue is either homogenised (bulk) or sorted by cells (sc)
- Recent advances allow for the characterisation of cells in their spatial environment

[Rao et al., 2021]

Imaging vs. HTS-based

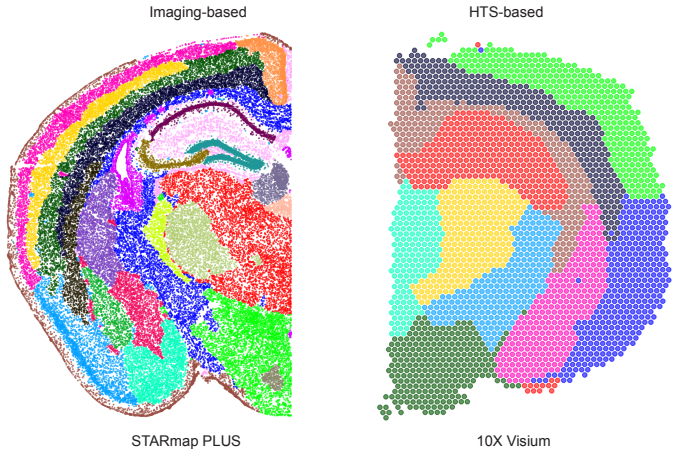


Figure: Data: [Shi et al., 2023, 10X, 2022], clustering: [Singhal et al., 2024]

Imaging vs. HTS-based

Imaging-based

Pros

- higher sensitivity
- better resolution
- adjustable area of detection

Cons

- lower number of features → targeted
- trade off area / acquisition time

→ hypothesis-testing

[Rao et al., 2021, Moffitt et al., 2022]

HTS-based

Pros

- higher number of features → untargeted

Cons

- lower sensitivity
- limited resolution (55 μm diameter Visium, 2 μm diameter Visium HD)
- standard area of detection (arrays)

→ hypothesis-generating

`pasta` a resource for exploratory spatial analysis

- `pasta` is a webpage with several vignettes in R and Python to introduce and organise exploratory spatial analysis
- shows code examples for different technologies
- Assumptions depending on the data type are discussed and put into context

Technologies differ in their data modalities

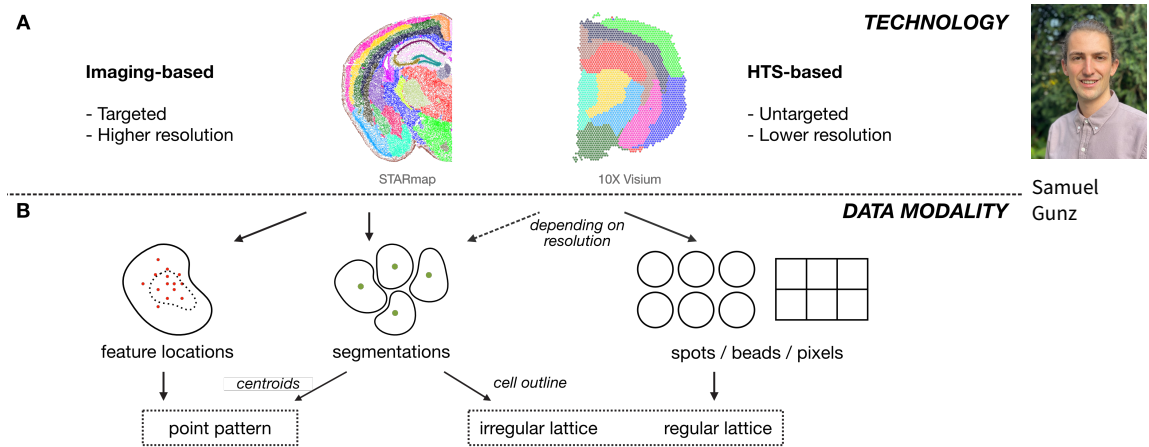


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Figure: [Emons et al., 2025, Rao et al., 2021], Data: [Shi et al., 2023, 10X, 2022]

Analysis options differ between technologies

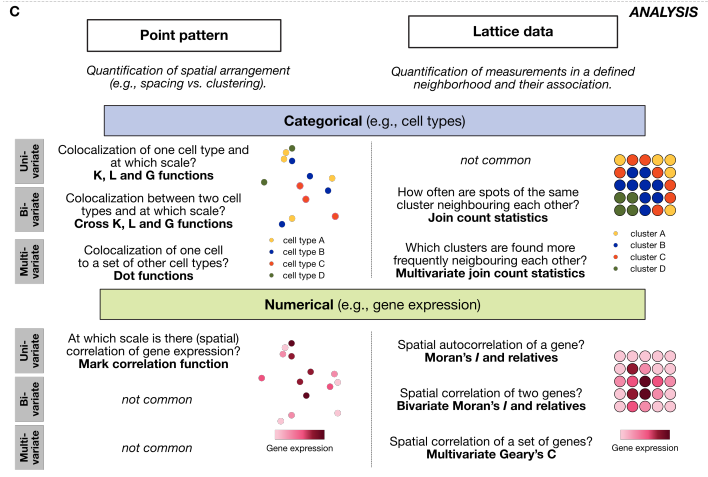

Figure: [Baddeley et al., 2015, Pebesma and Bivand, 2023, Emons et al., 2025]

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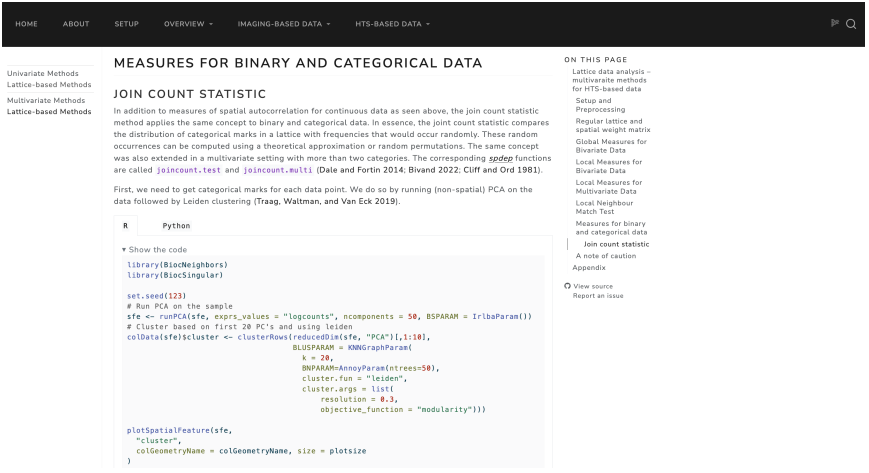
Vignettes in R and Python for analyses



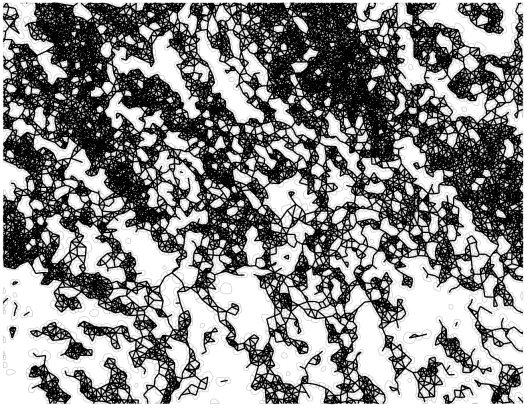
available under `robinsonlabuzh.github.io/pasta`

Spatial association measures per neighbourhood

$$\sum_i \sum_j f(x_i, x_j) w_{ij}$$

- Generally, lattice data analysis metrics are a double sum over all locations
- Neighbourhood is defined in a weight matrix w_{ij}.
- Typical choices are contiguity-, k-NN or distance based neighbourhoods.
- For a given neighbourhood, a measure of spatial association $f(x_i, x_j)$ is calculated
- More details in [Moses et al., 2023] and [Emons et al., 2025].

Weight matrix construction depends on the biological question



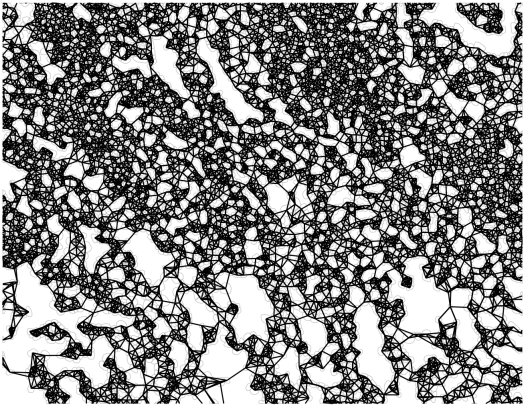


Figure: NSCLC CosMx Data from [He et al., 2022]

Weight matrix construction will influence results

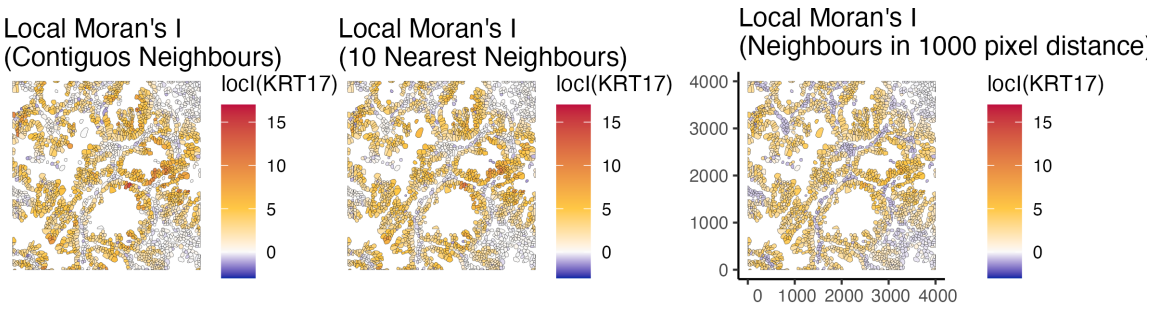


Figure: NSCLC CosMx Data from [He et al., 2022]

Spatial autocorrelation of gene expression

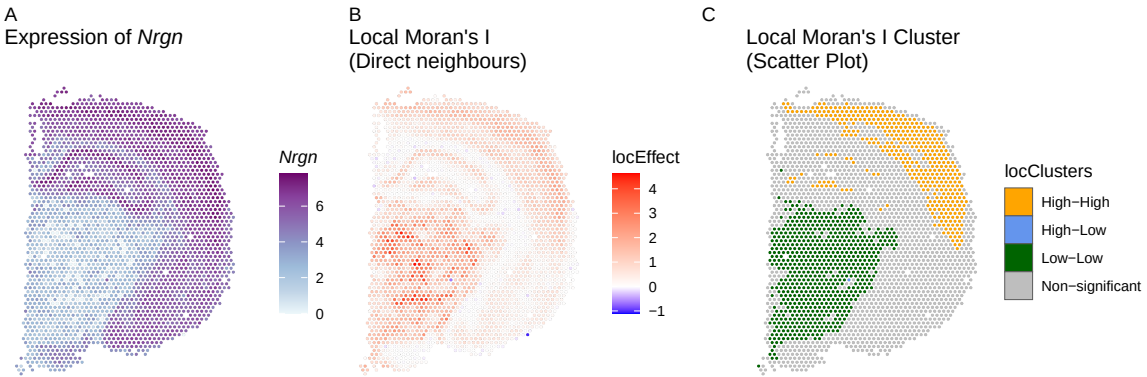


Figure: Mouse coronal brain 10x Visium data from [10X, 2022]

Lattice data analysis reveals triple positive receptor regions

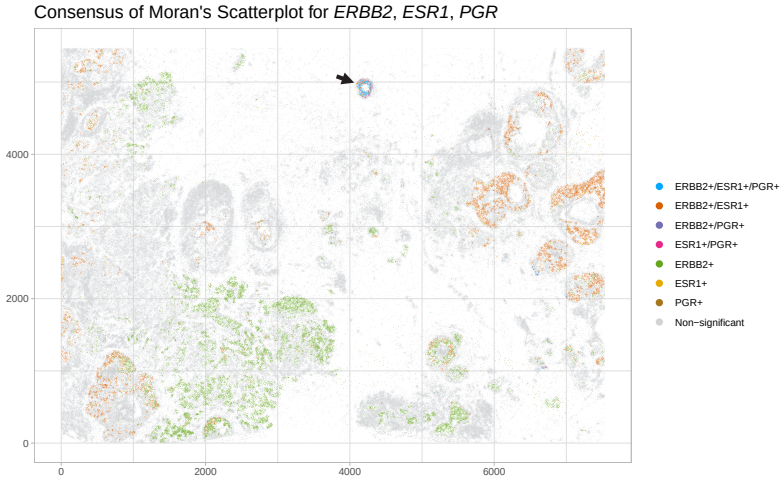


Figure: Breast cancer data from 10x Xenium [Janesick et al., 2023, Emons et al., 2025]

Technologies differ in their data modalities

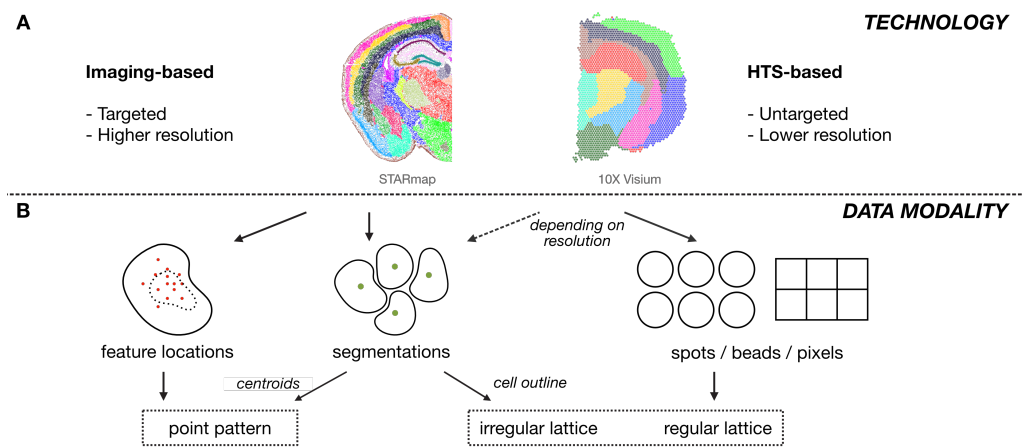


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Figure: [Emons et al., 2025, Rao et al., 2021], Data: [Shi et al., 2023, 10X, 2022]

Point pattern analysis compute curves across radii r

$$\hat{K}(r) = \frac{|W|}{n(n-1)} \sum_{i=1}^{n} \sum_{j=1, j \neq i}^{n} \{d_{ij} \leq r\} e_{ij}(r)$$

- Compute a measure of spatial assocation in a r-neighbourhood $\rightarrow$ distance-based neighbourhood
- Calculate the measure of spatial assocation across all radii r.
- Normalises for differences in field-of-view ($|W|$) and number of points (n)
- Corrects for edge-effects at the image boundary $e_{ij}(r)$

Edge effects are differences in association due to the FOV border

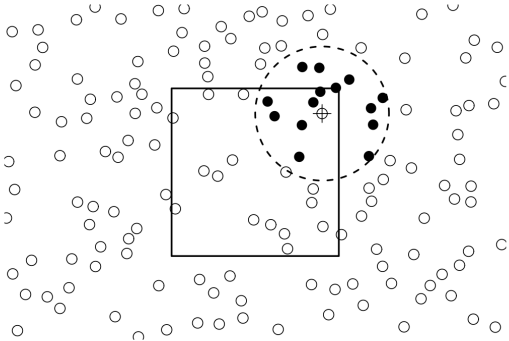


Figure 7.12. *Counting, without edge effects, the number of neighbours of each wildflower within a sampling frame (black rectangle) in a field of wildflowers.*

Figure: Taken from [Baddeley et al., 2015]

Point pattern analysis summarises cellular arrangements

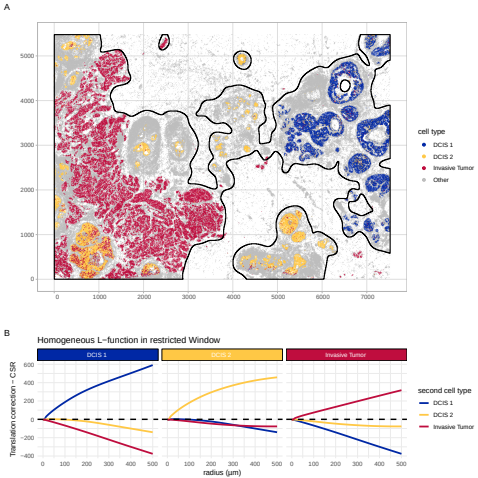


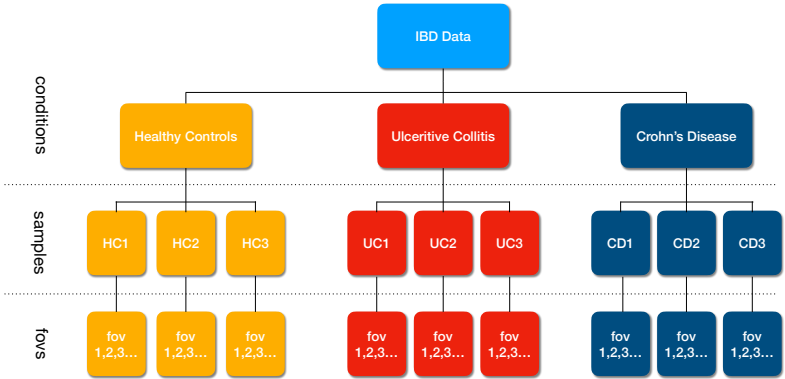
Figure: Breast cancer data from 10x Xenium [Janesick et al., 2023, Emons et al., 2025]

Conclusions `pasta`: Highlights the usability of spatial statistics in R and Python

- vignettes introduce and contextualise known concepts from spatial statistics and their usage for Omics data.
- point pattern analysis is shown in R with the package `spatialFDA`
- lattice datta analysis is shown in R with `Voyager` and in Python with `Pysal`
- paper available for further detail [Emons et al., 2025]

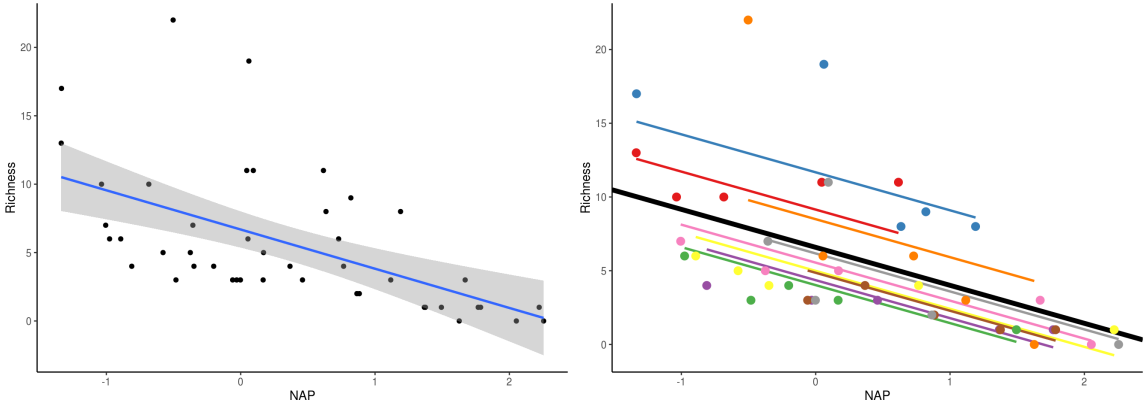
Modern datasets have complex nested variance structures

- imaging-based spatial transcriptomics (CosMX)
- compares three conditions - Healthy, Crohn's Disease, Ulceritive Collitis
- per condition three samples
- per sample $\sim$ 20 FOVs



[Garrido-Trigo et al., 2023]

Mixed-effects to account for nested covariance



Taken from [Santangelo, 2018], licensed as `CC BY 4.0`

`spatialFDA` set up

- Open question in the field is how to efficiently and sensitively compare different co-localisation of cells across samples and/or conditions
- There are only a few options $\rightarrow$ often limited to scalar comparisons [Canete et al., 2022, Hawinkel et al., 2025] or without random effect estimation [Seal et al., 2024]
- aim: build a framework to compare spatial statistics functions over their entire domain r.

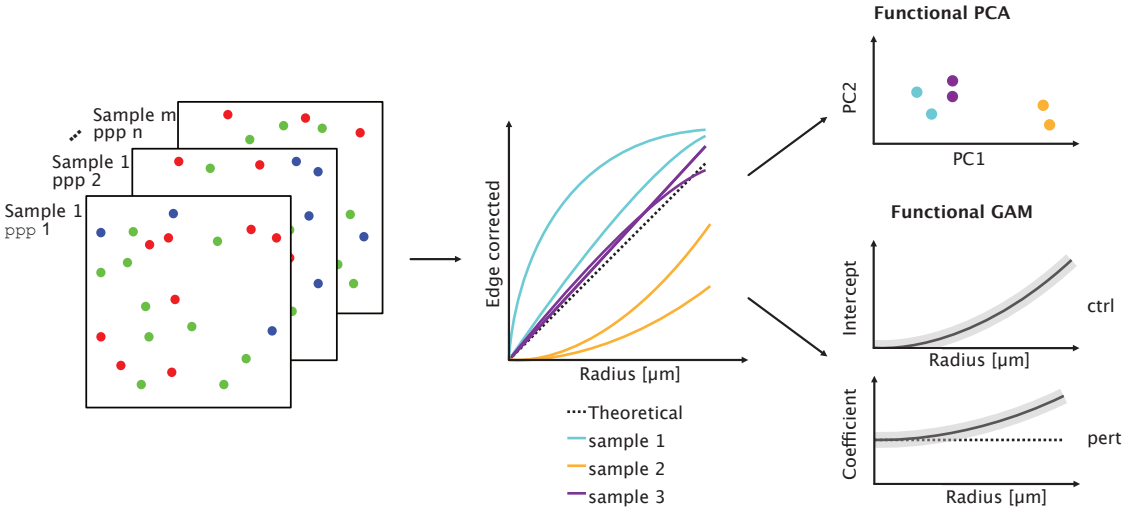
State of the art I

package	metric	full distribution	testing
spicyR	AUC of L function	no	linear (mixed effects) model
smoppix	AUC of G function	no	linear mixed effects model
SpaceANOVA	pair correlation function	yes	functional ANOVA

areas of improvement:

- improved functional testing
- systematic evaluation and validation of approaches
- larger flexibility
- software for simple installation and integration into existing analysis workflows → `Bioconductor` package

`spatialFDA`: Differential co-localisation across scales



Functional additive mixed model

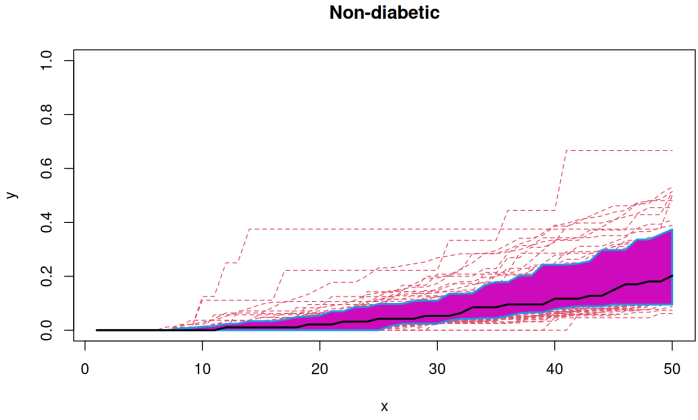
— Compare not a summary statistic between conditions but the function over the domain r
$\rightarrow$ Functional data analysis (FDA)

— Additive mixed model with a functional response

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

[Scheipl et al., 2015, Scheipl et al., 2016]

Challenges in inference on functions

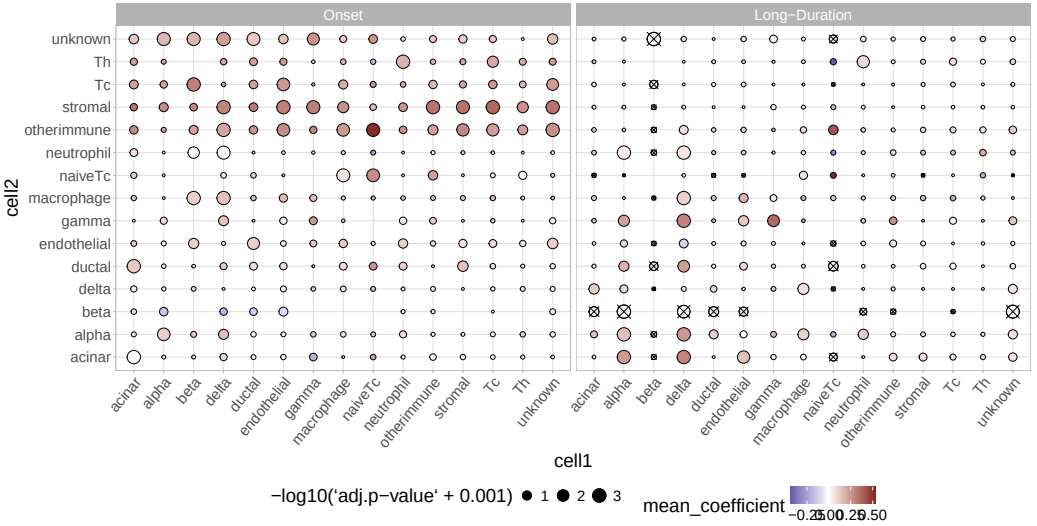


— Heteroscedasticity: Data-transformations and sandwich corrections

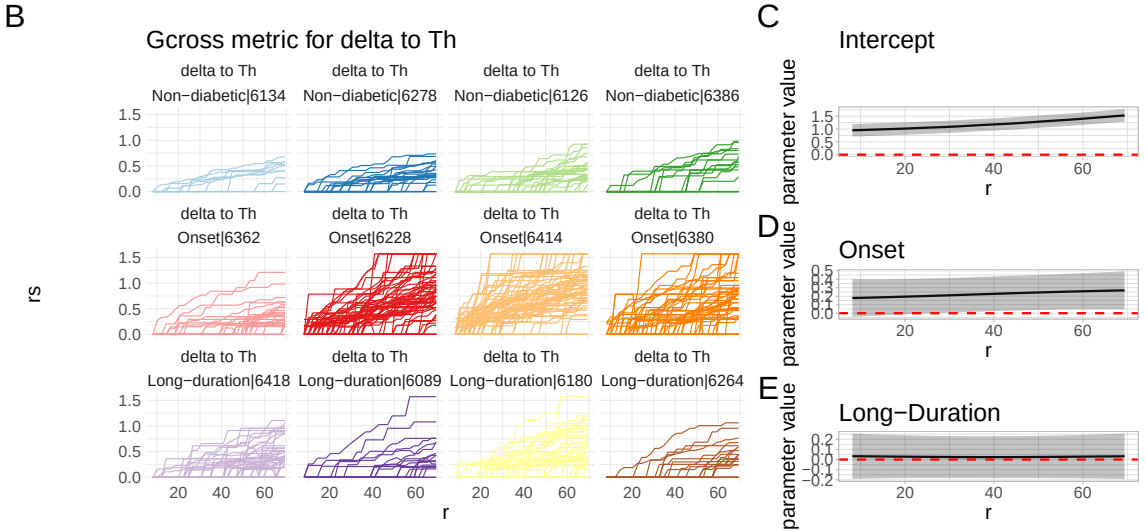
— Autocorrelation: AR(1) error model - residuals are correlated

Imaging mass cytometry dataset of type-I diabetes [Damond et al., 2019].

Pairwise differential co-localisation in type-I diabetes



Individual functional tests provide scale-effects



Conclusions

— Lattice data analysis compares relative locations of numeric marks on a grid → observation-based view

— Point pattern analysis is for inference of the stochastic process underlying the pattern → event-based view

— `spatialFDA` compares spatial statistics functions across spatial scales

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