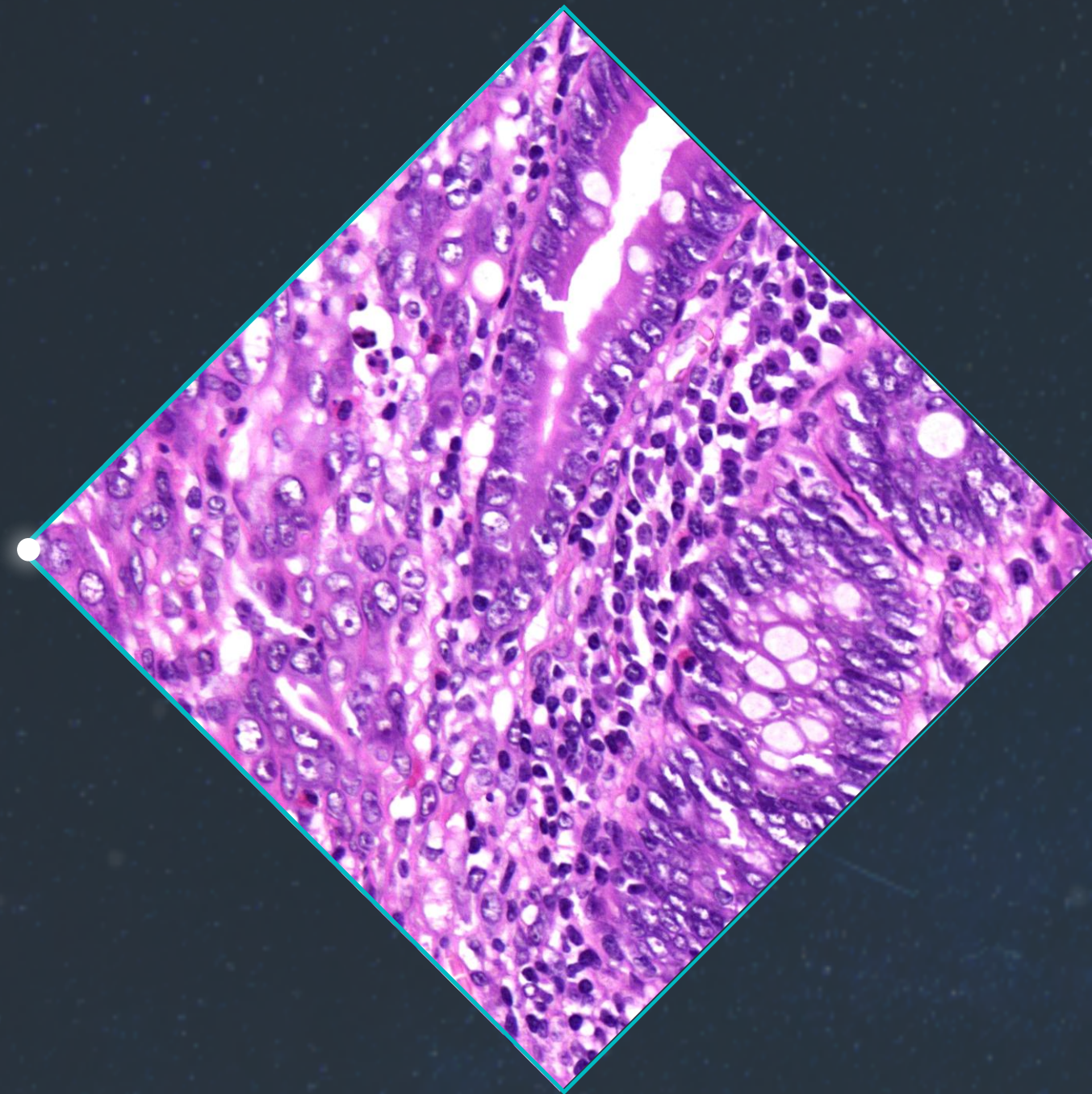


Spatial heterogeneity of immune response in colorectal cancer

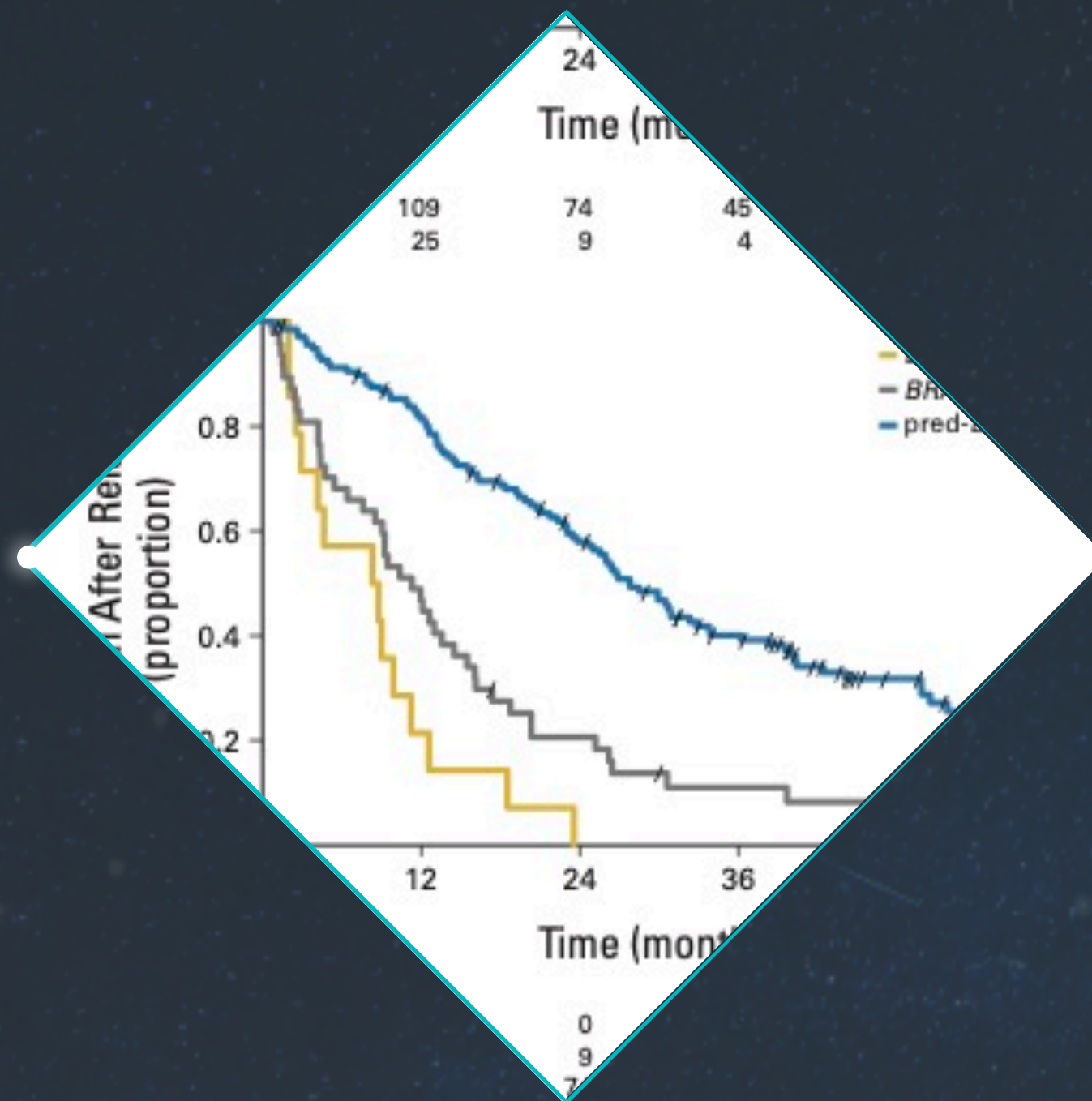


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Context



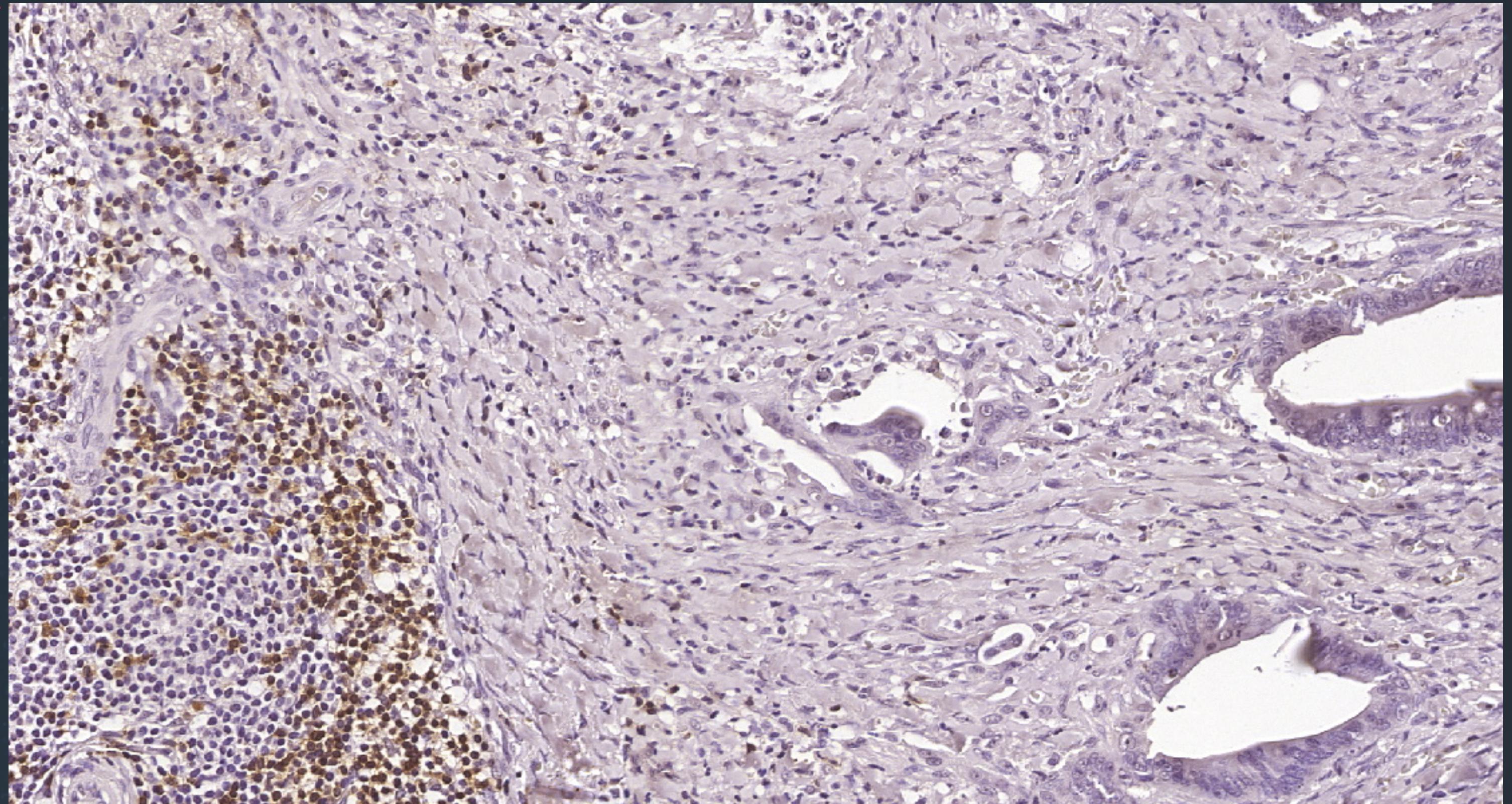
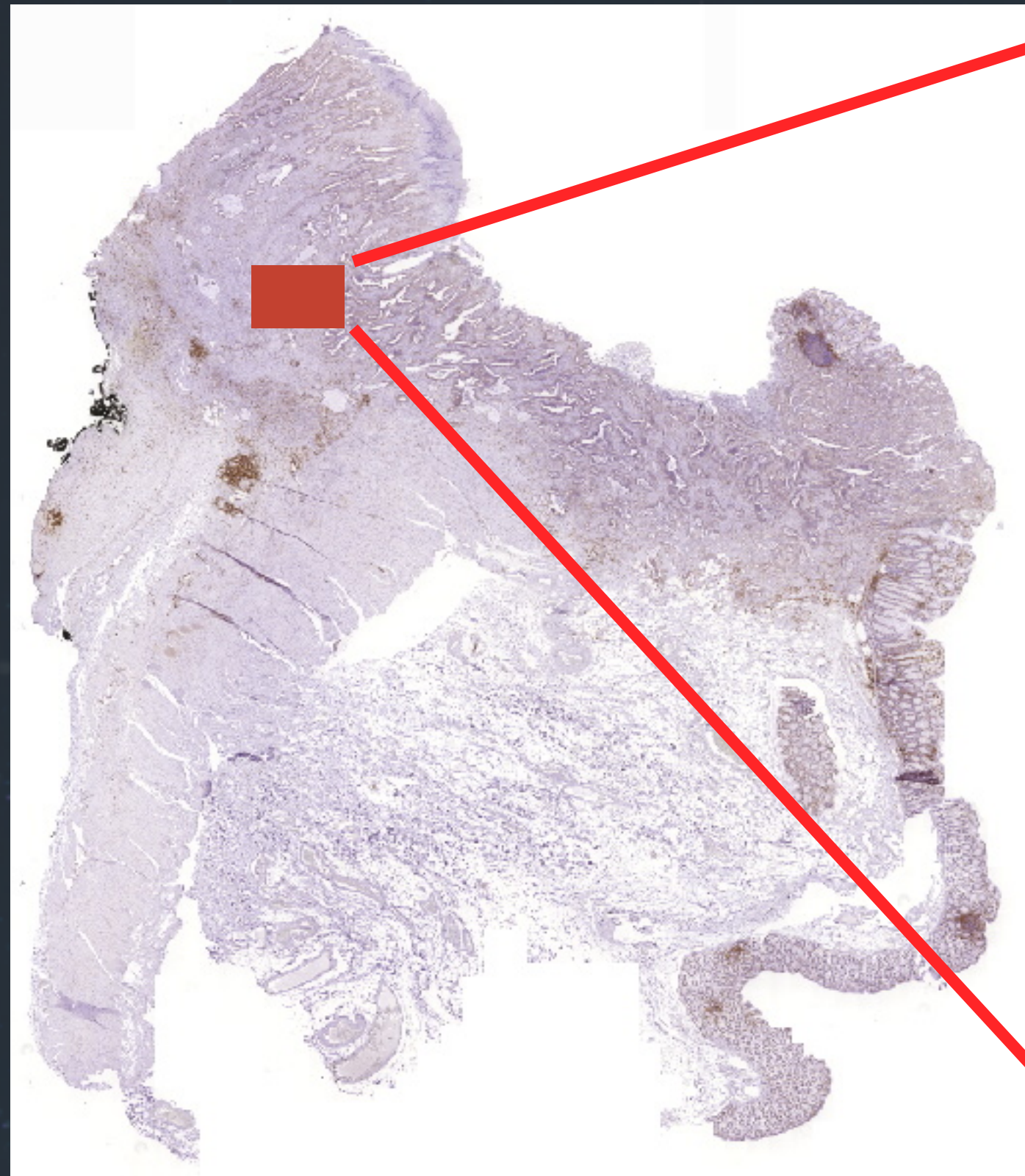
- tools for precision medicine and tumor heterogeneity (in colorectal cancer)
- multi-omics: genomics, transcriptomics, metagenomics, radiomics (computational pathology)
- inter-tumor heterogeneity: population stratification - molecular subtypes
- intra-tumor heterogeneity (ITH): morphological + molecular; **immune response**

ITH: immune response

Questions of interest:

- Is the distribution of T-cells uniform in (a region of) a section?
- Do the distributions of T-cells differs between sections corresponding to the same patient?
- Do the different types of T-cells have different distributions...?
- Are there interactions between T-cells populations?
- ...new associations with clinical covariates?

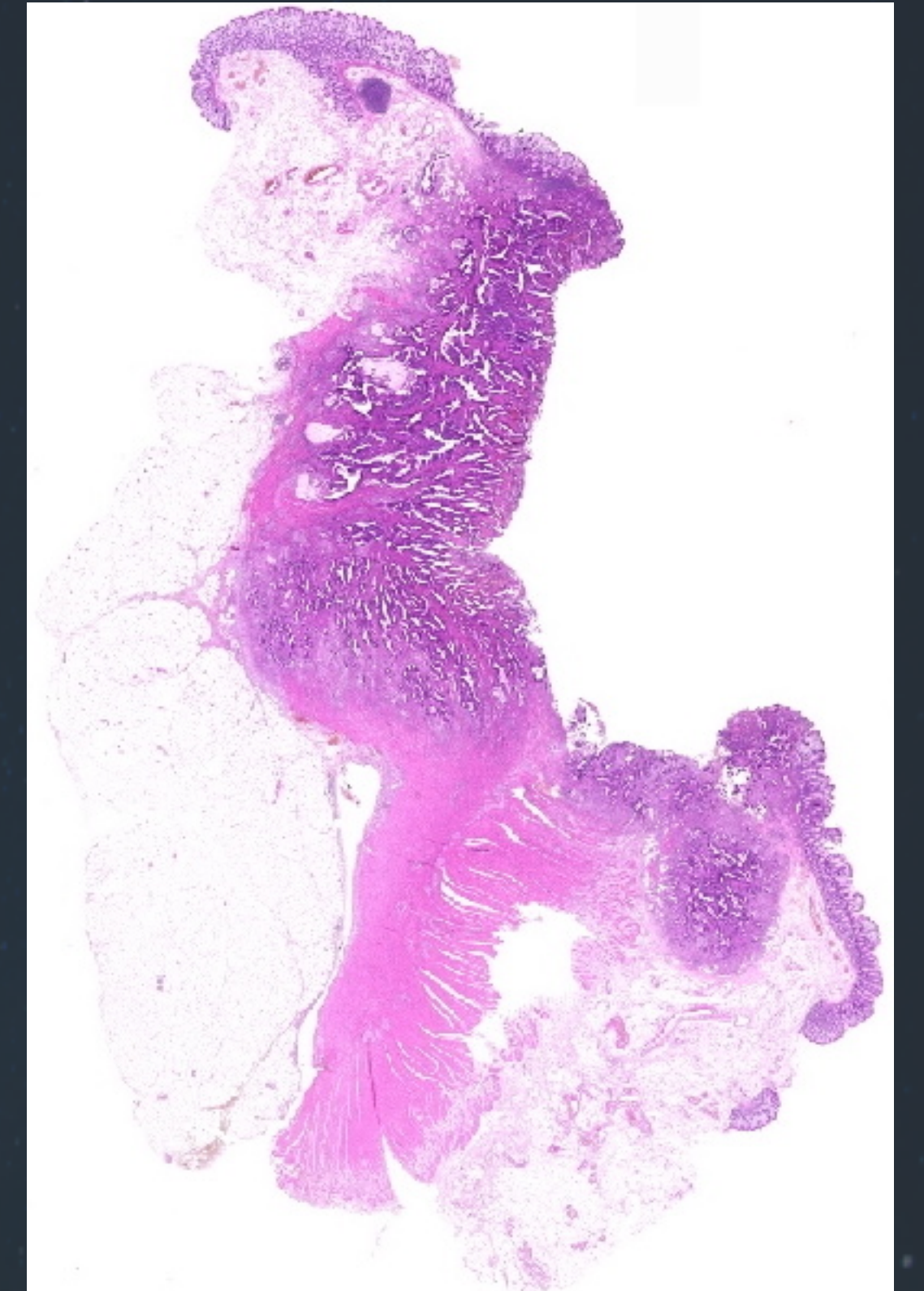
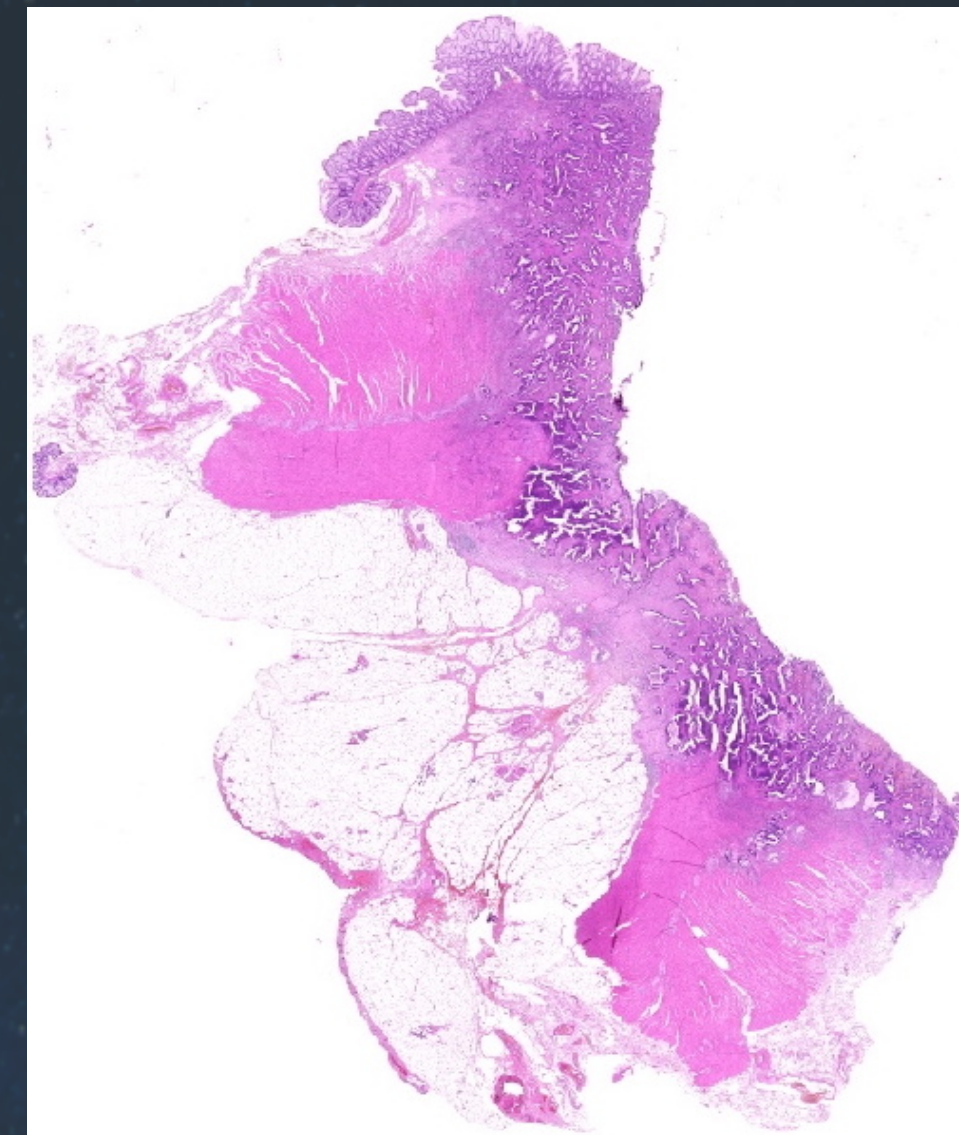
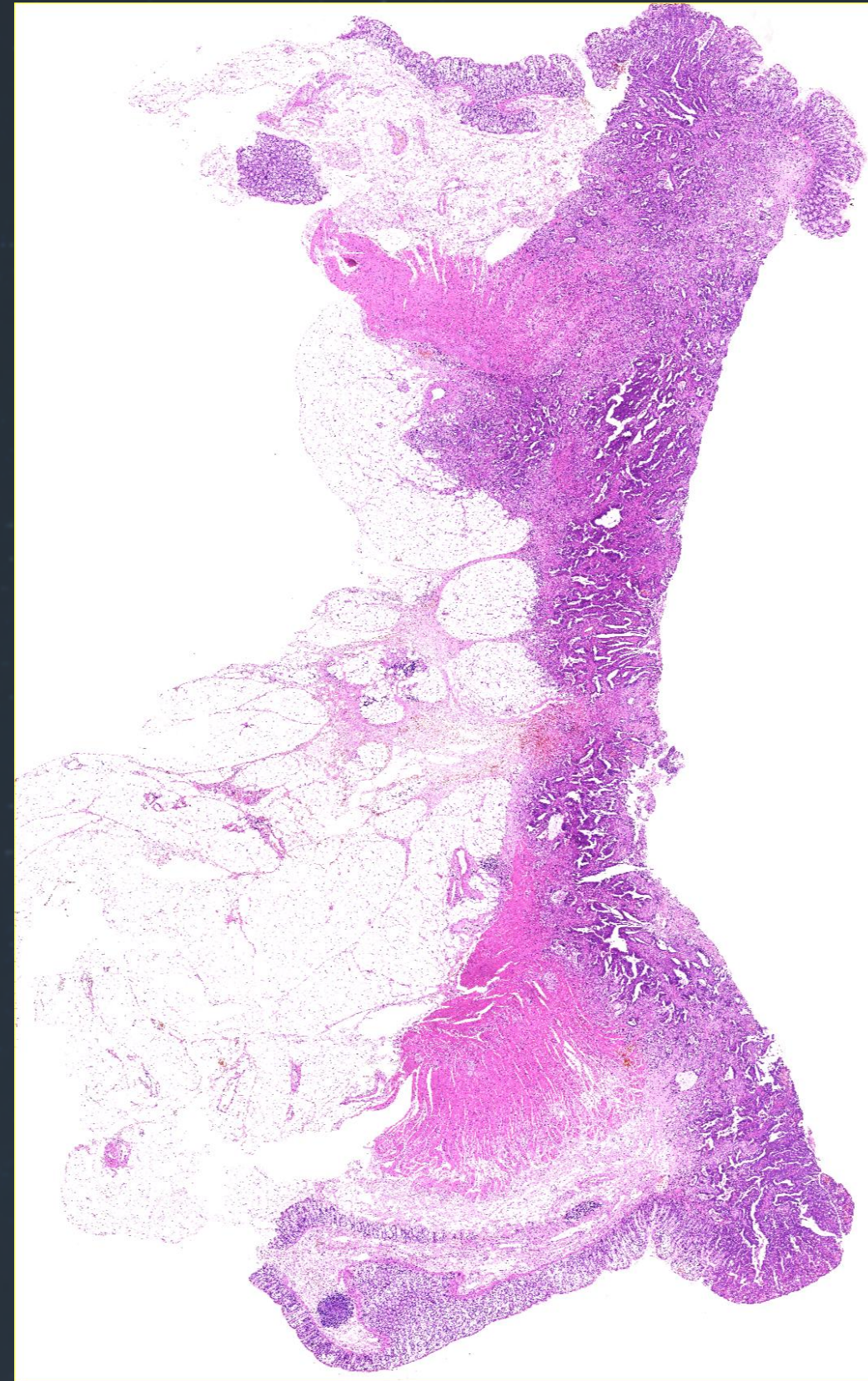
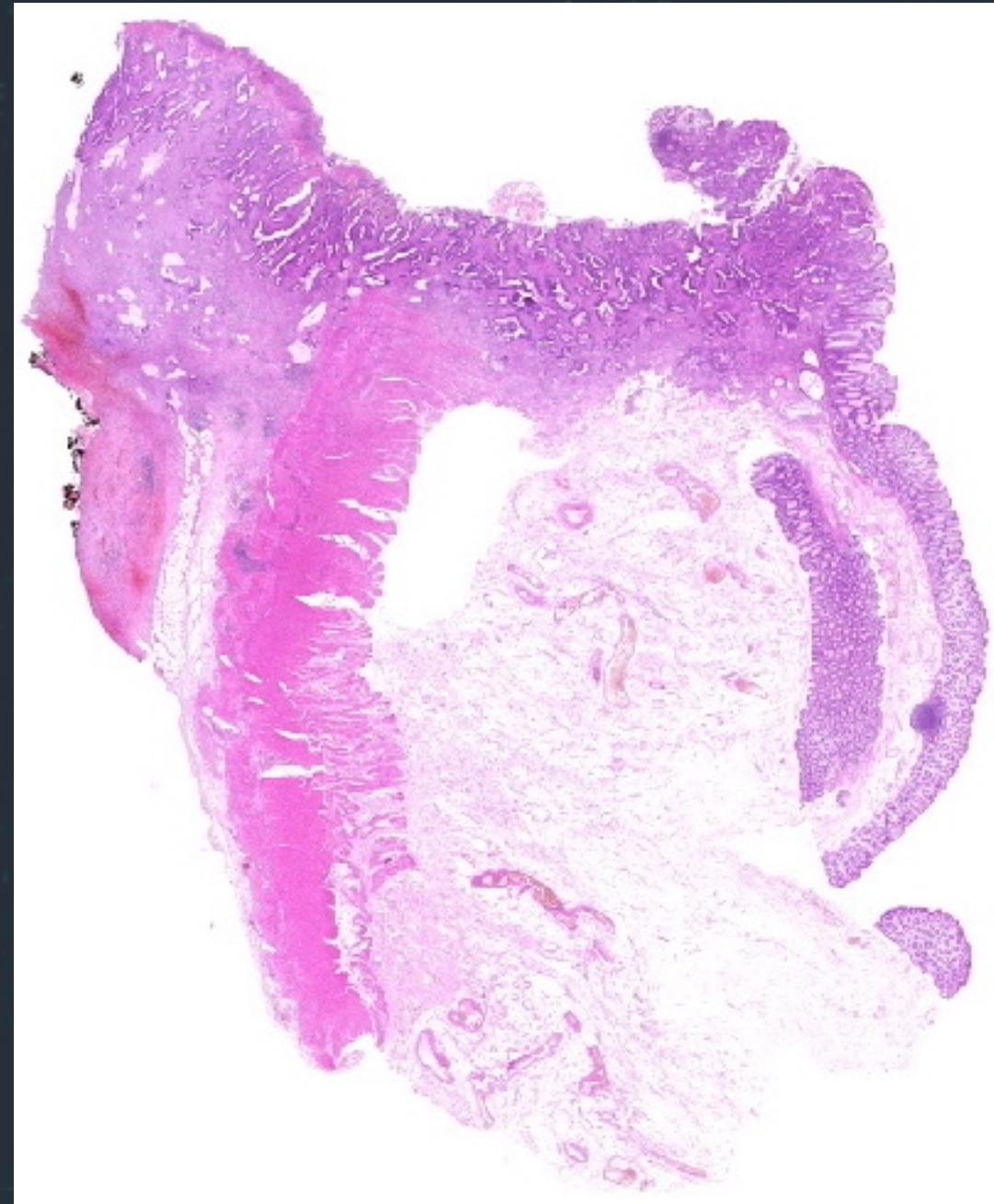
ITH: immune response



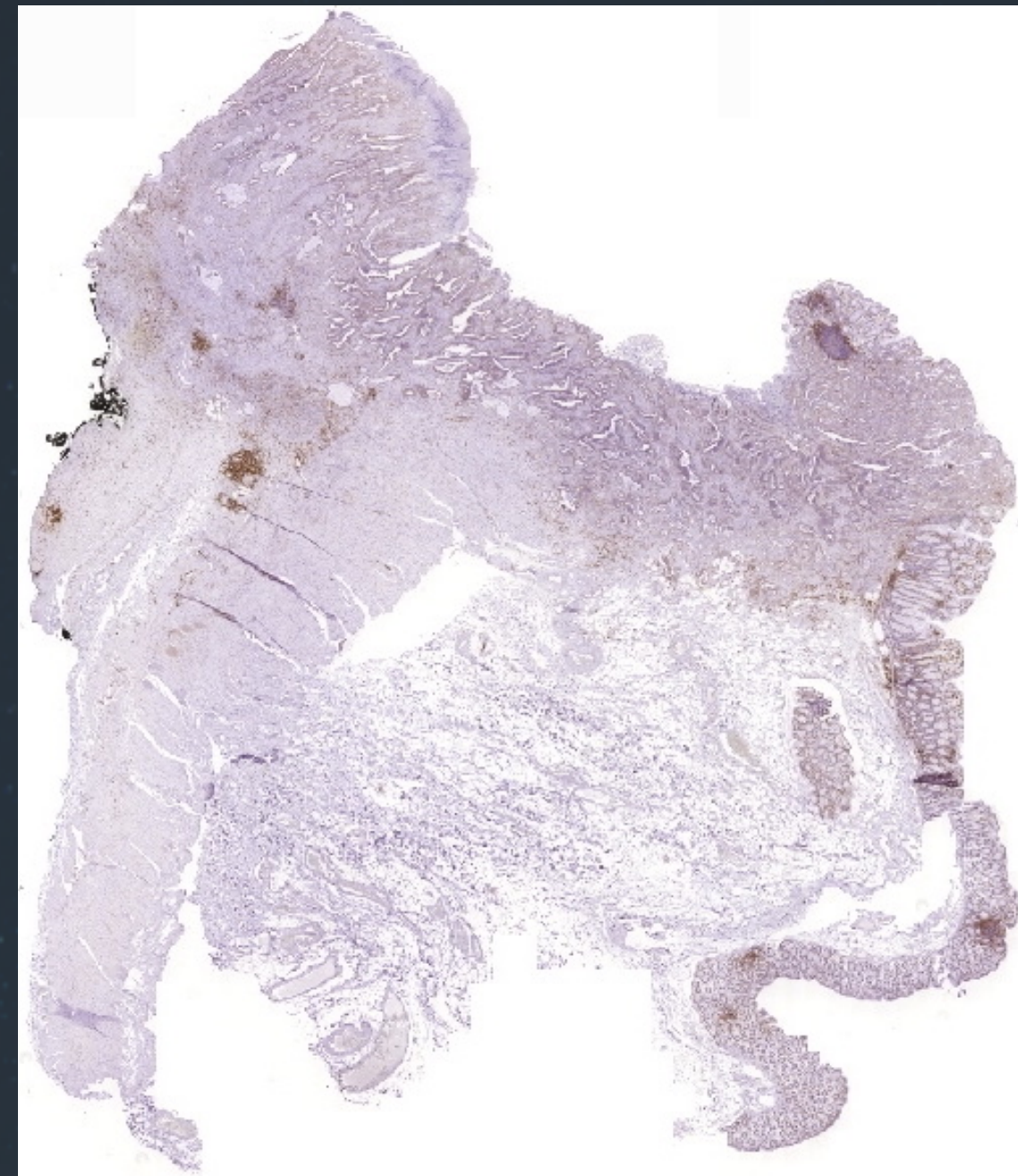
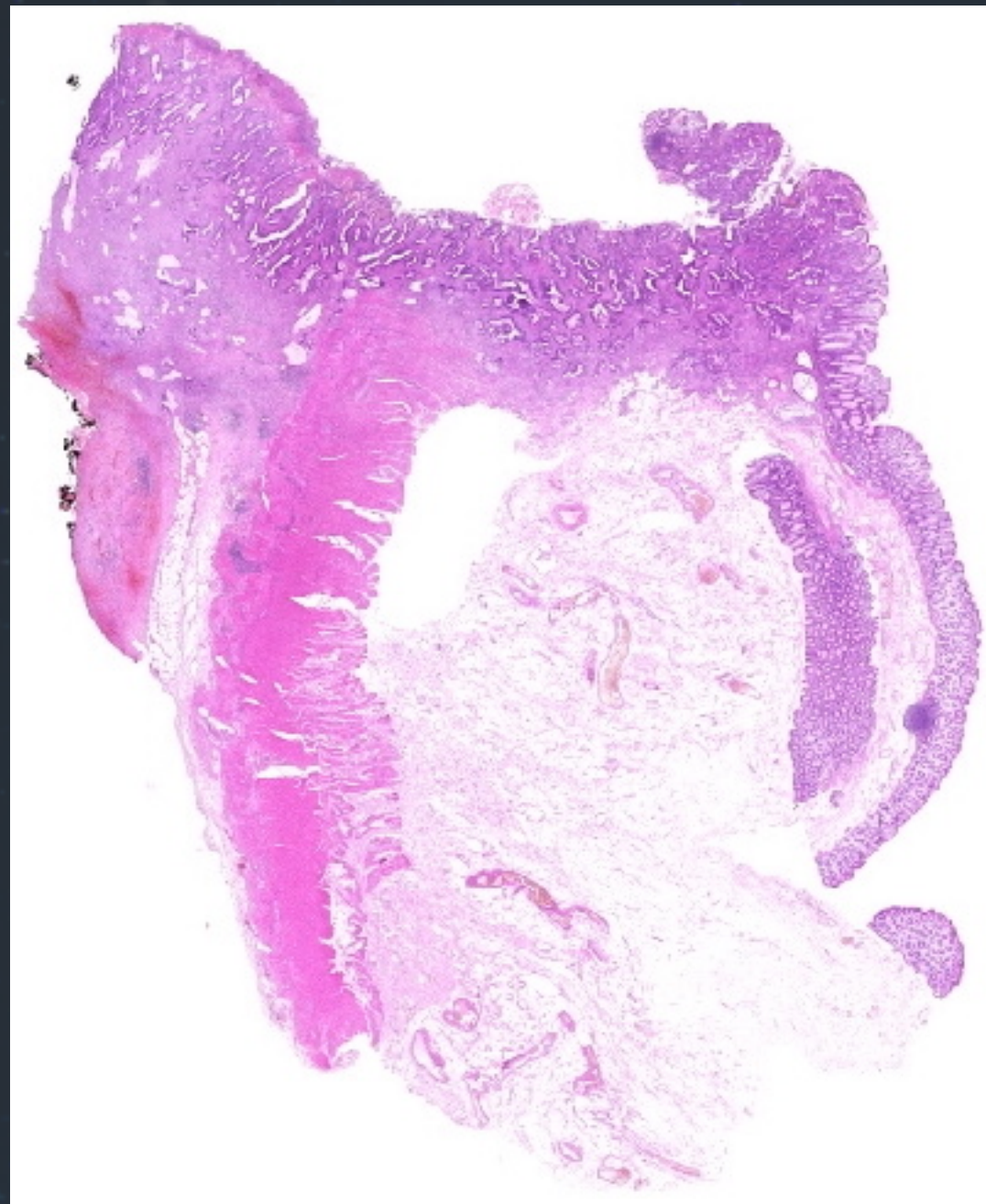
IH: immune response - experimental setup

- 24 CRC patients (stage II,III)
- 4 tissue blocks per patient
- H&E + 7 IHC sections per block





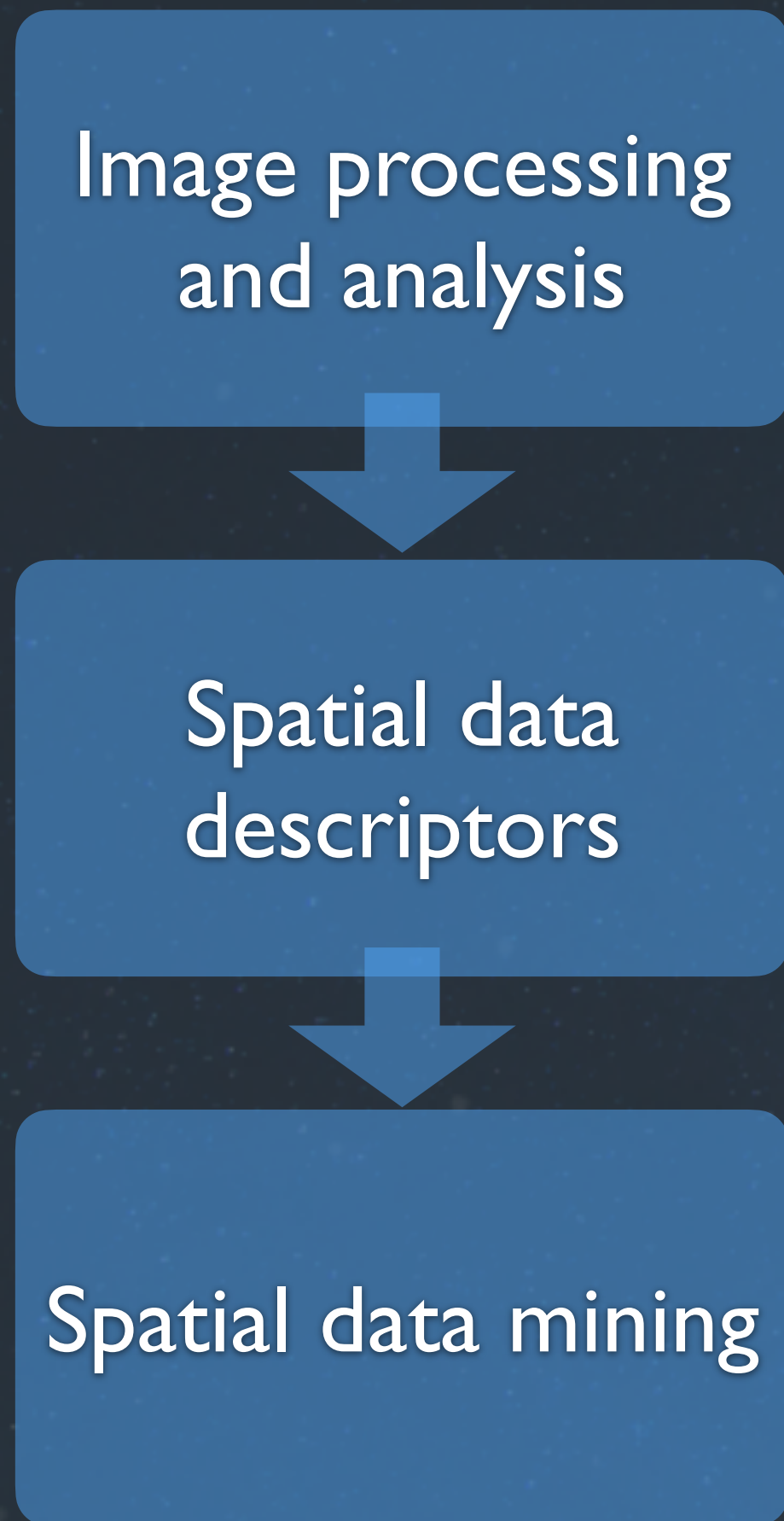
4 different blocks



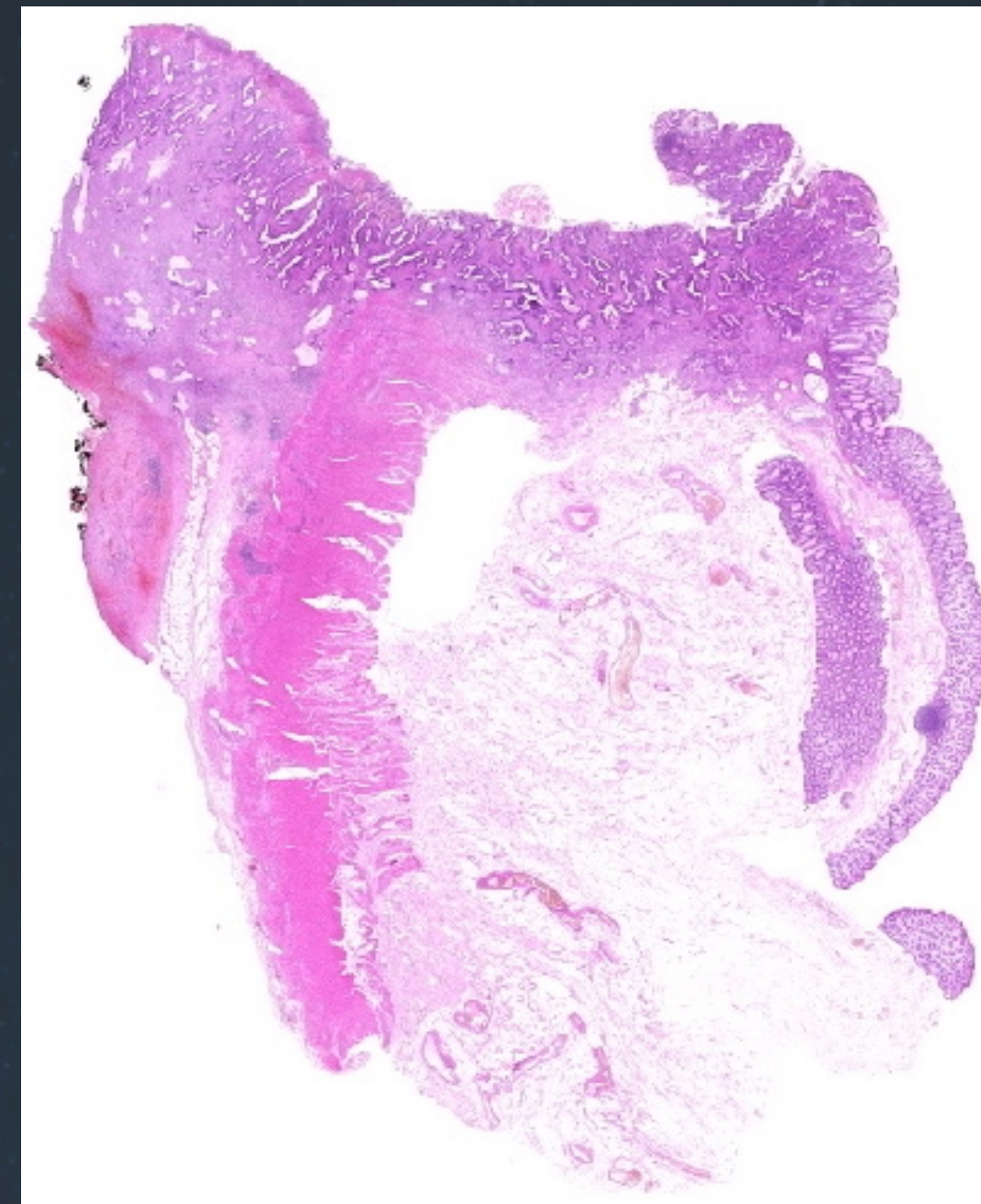
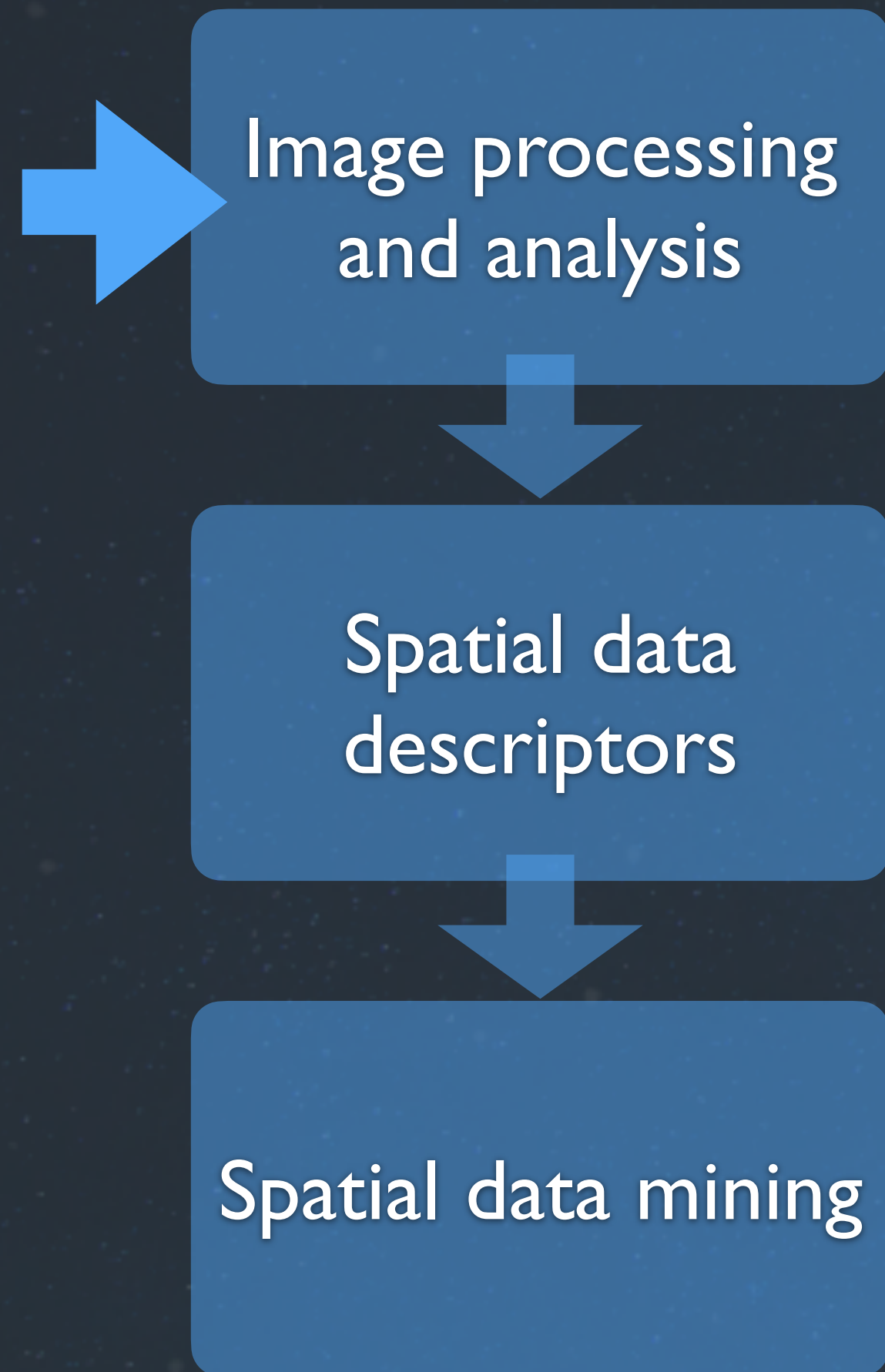
- CD3: T-cell membrane protein; presence: better prognosis
- CD45RO: memory T-cell membrane protein; presence: better survival
- CD68: cell membrane protein, associated with macrophages; presence: worsen survival
- CD8: cytotoxic T-cells membrane protein; presence: better survival
- FOXP3: transcription factor for Treg; associated with better survival
- PD1: immune checkpoint; surface protein of T-cells
- PD-L1: immune checkpoints; surface protein of *tumor cells*; interaction with PD1 inhibits T-cell response, allowing tumor to evade the immune system

7 different immuno-stains

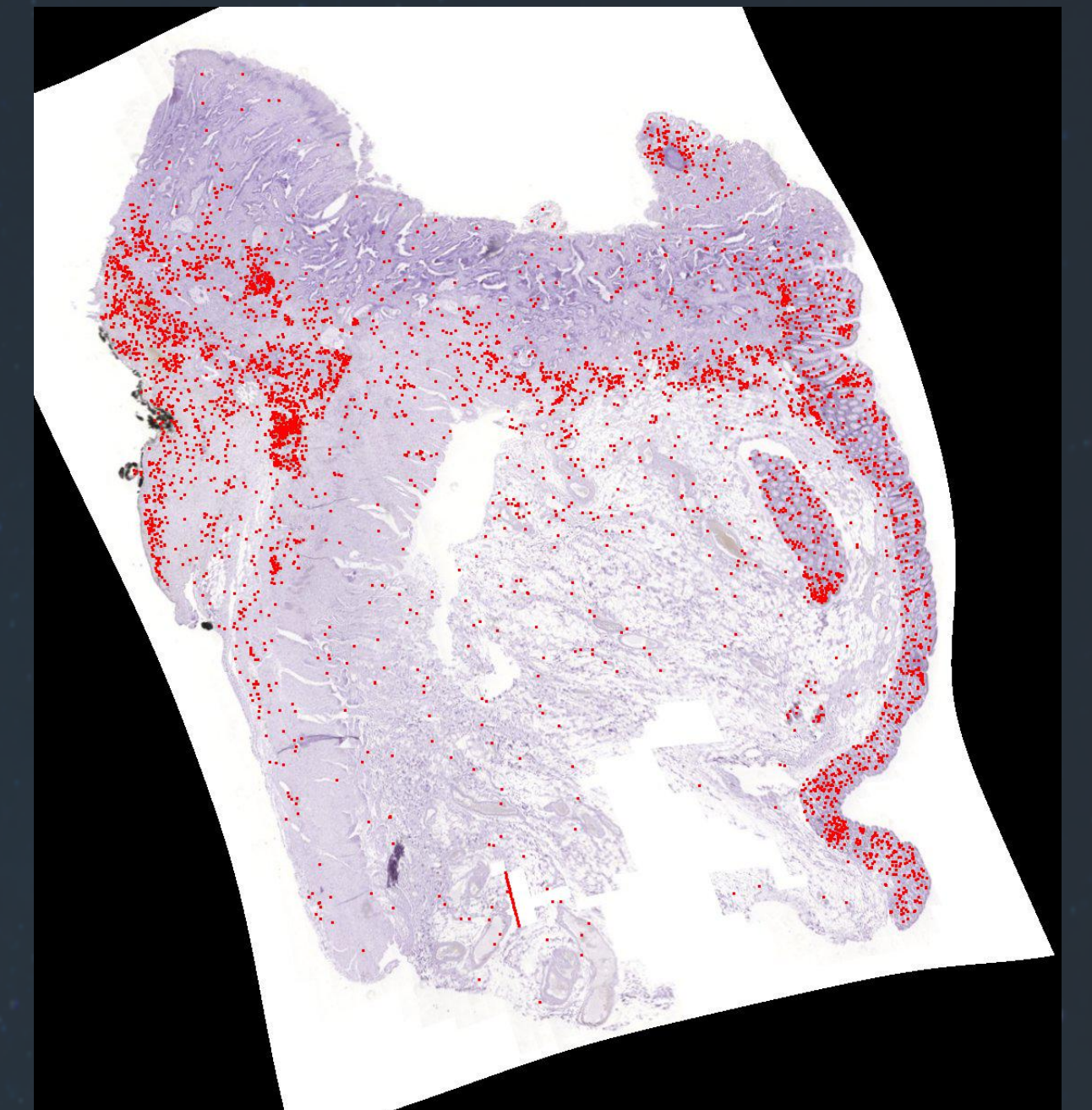
Approach



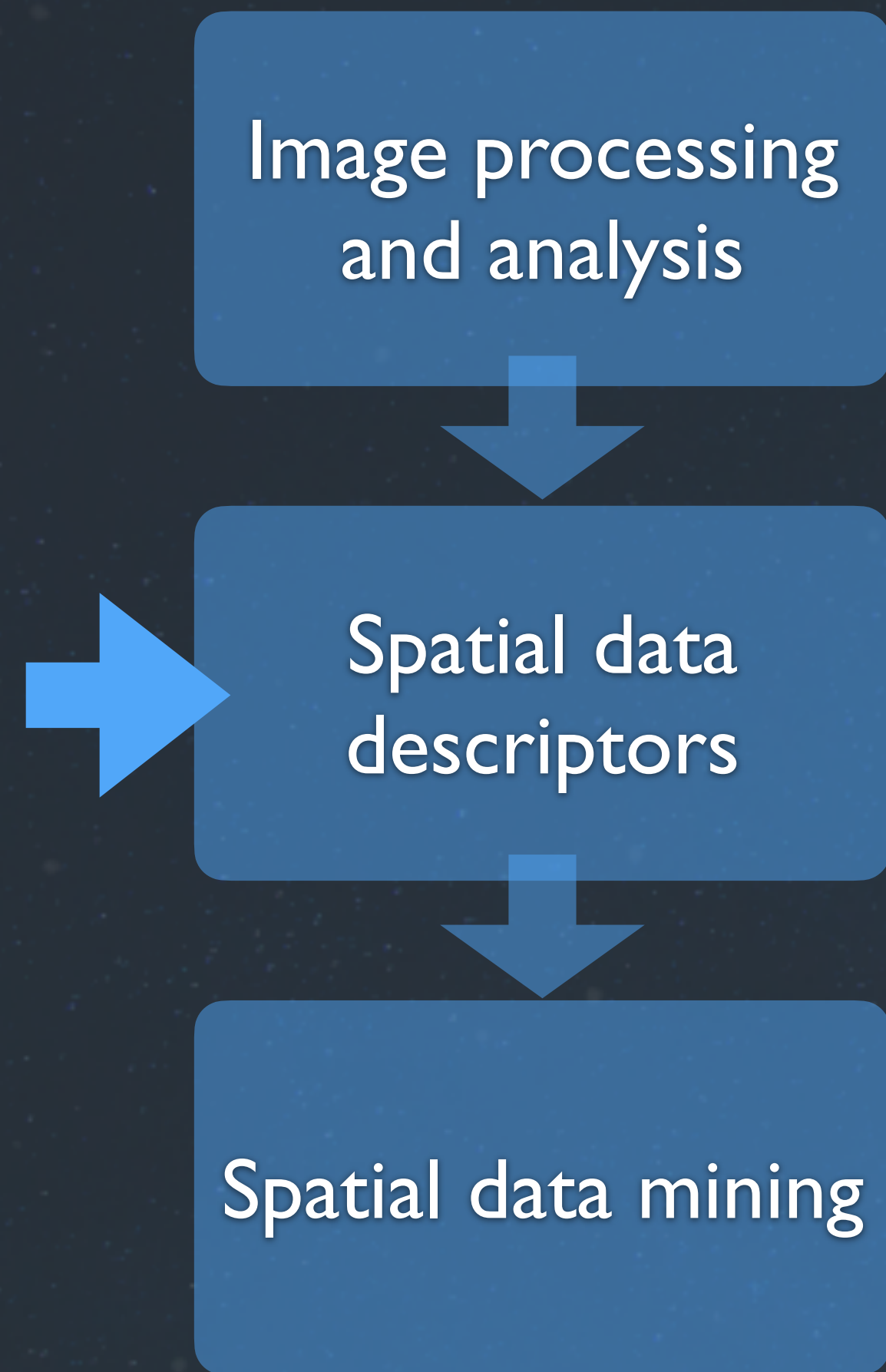
- detection of stain-positive cells (density counting)
- IHC image registration on the H&E image (elastic registration)
- mapping of the pathologist annotations



H&E



CD8



- use standardized Bessag's L functions to describe the point processes

$$L(r) - r = \sqrt{\frac{K(r)}{\pi}} - r,$$

where we use for Ripley's K function the estimator:

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

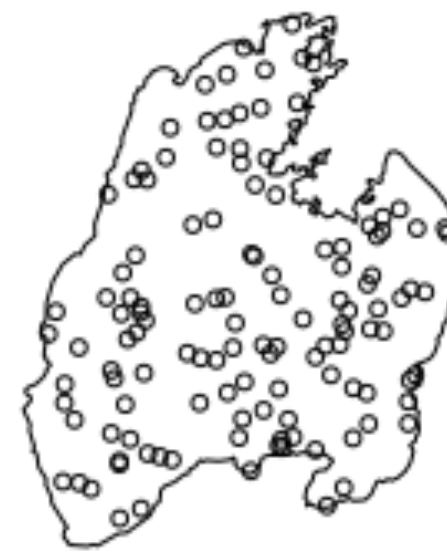
- use marked point processes: each event's position s_i is marked with a label ξ_i corresponding to the stain

Image processing
and analysis

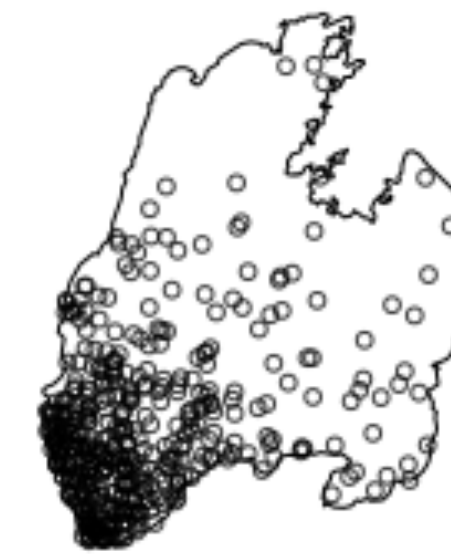
Spatial data
descriptors

Spatial data mining

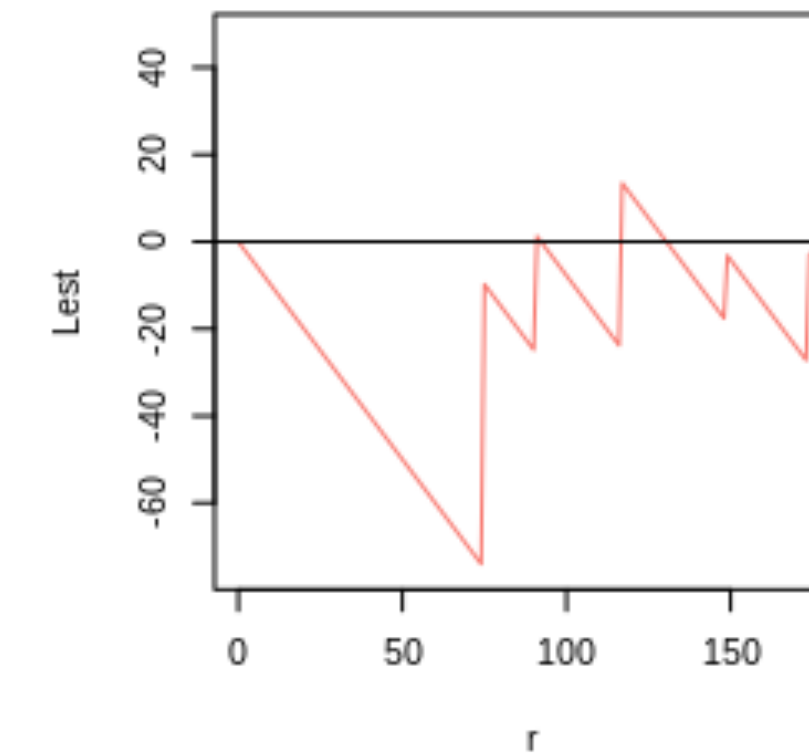
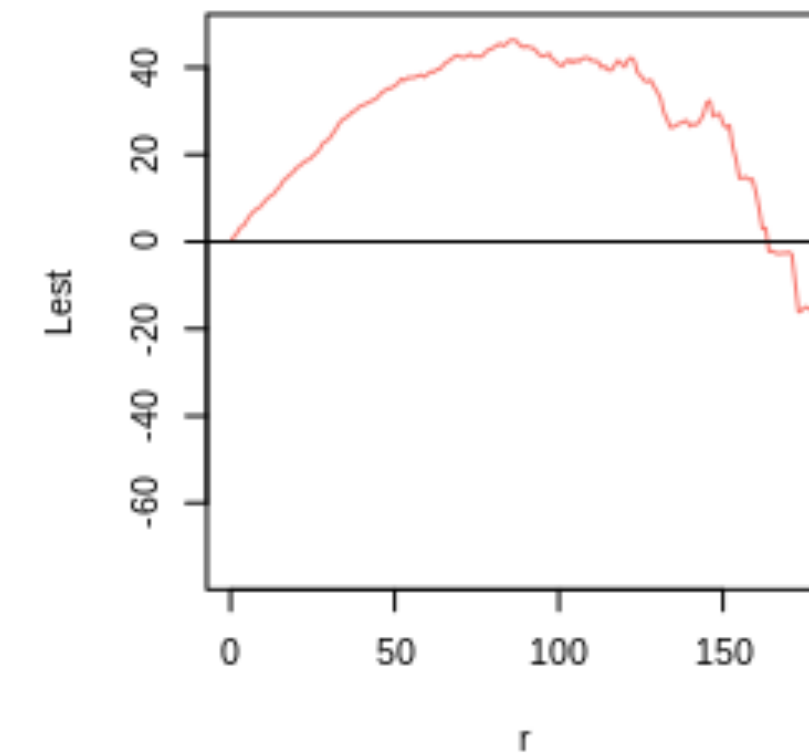
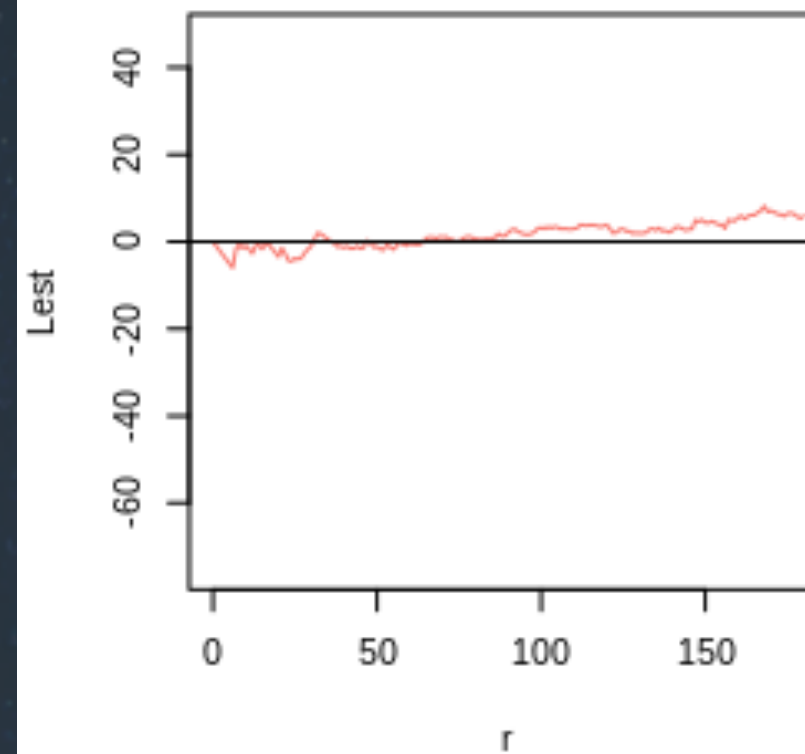
CSR

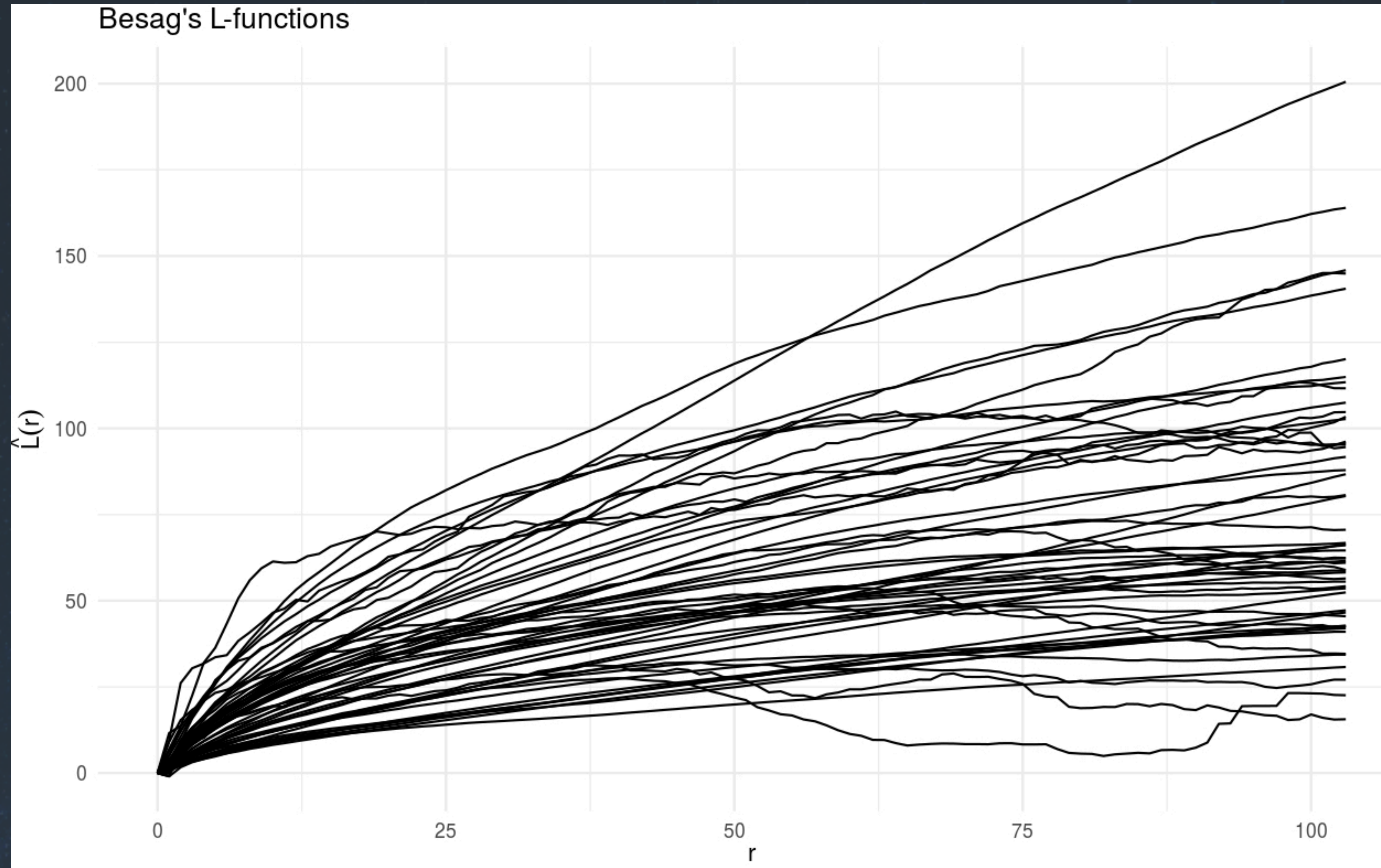
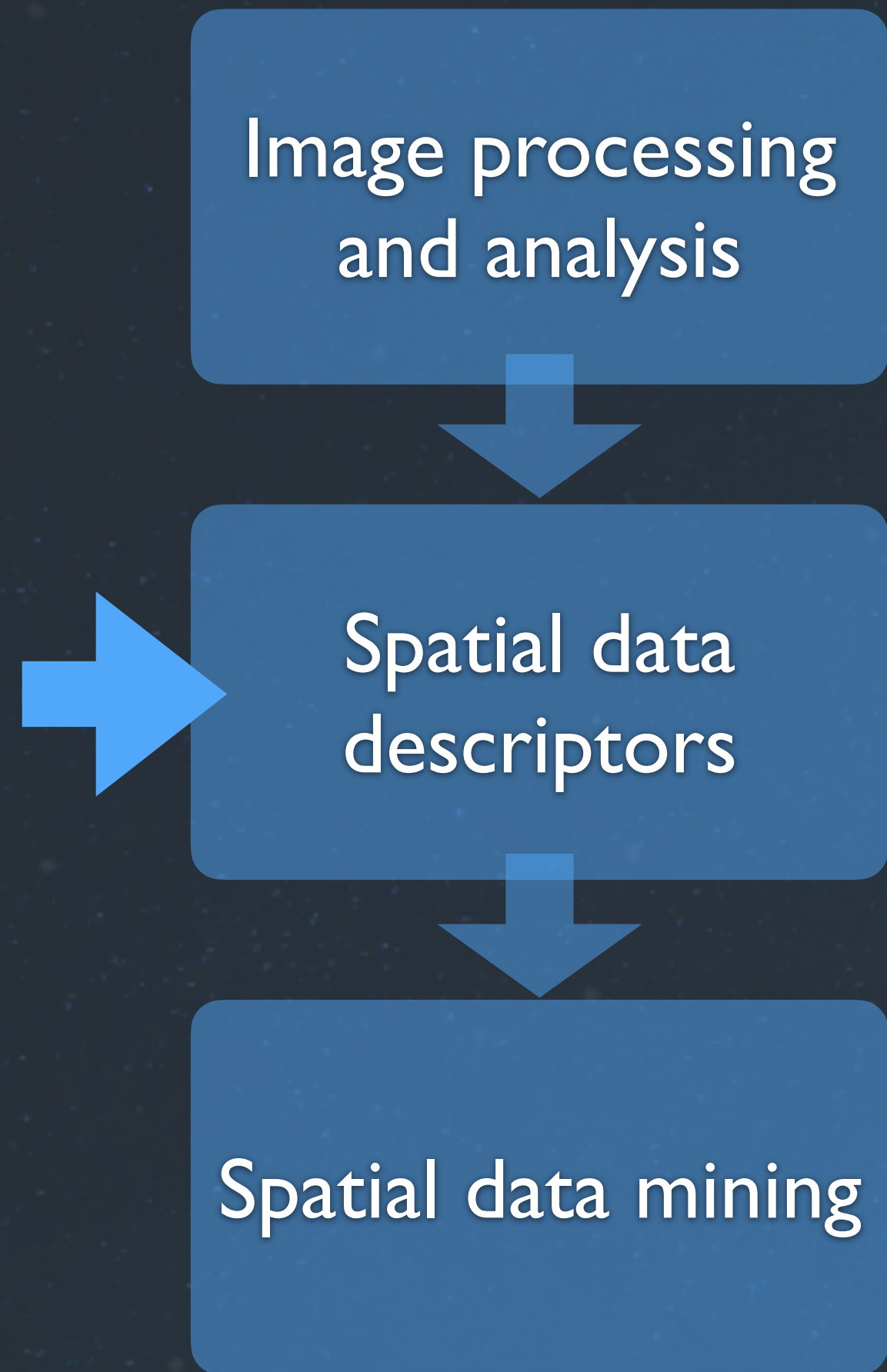


Clustering



Regularity





- use B-splines to obtain a continuous smooth version of L 's
- use functional PCA followed by VARIMAX
- hierarchical clustering (in FPCA space)

Image processing
and analysis

Spatial data
descriptors

Spatial data mining

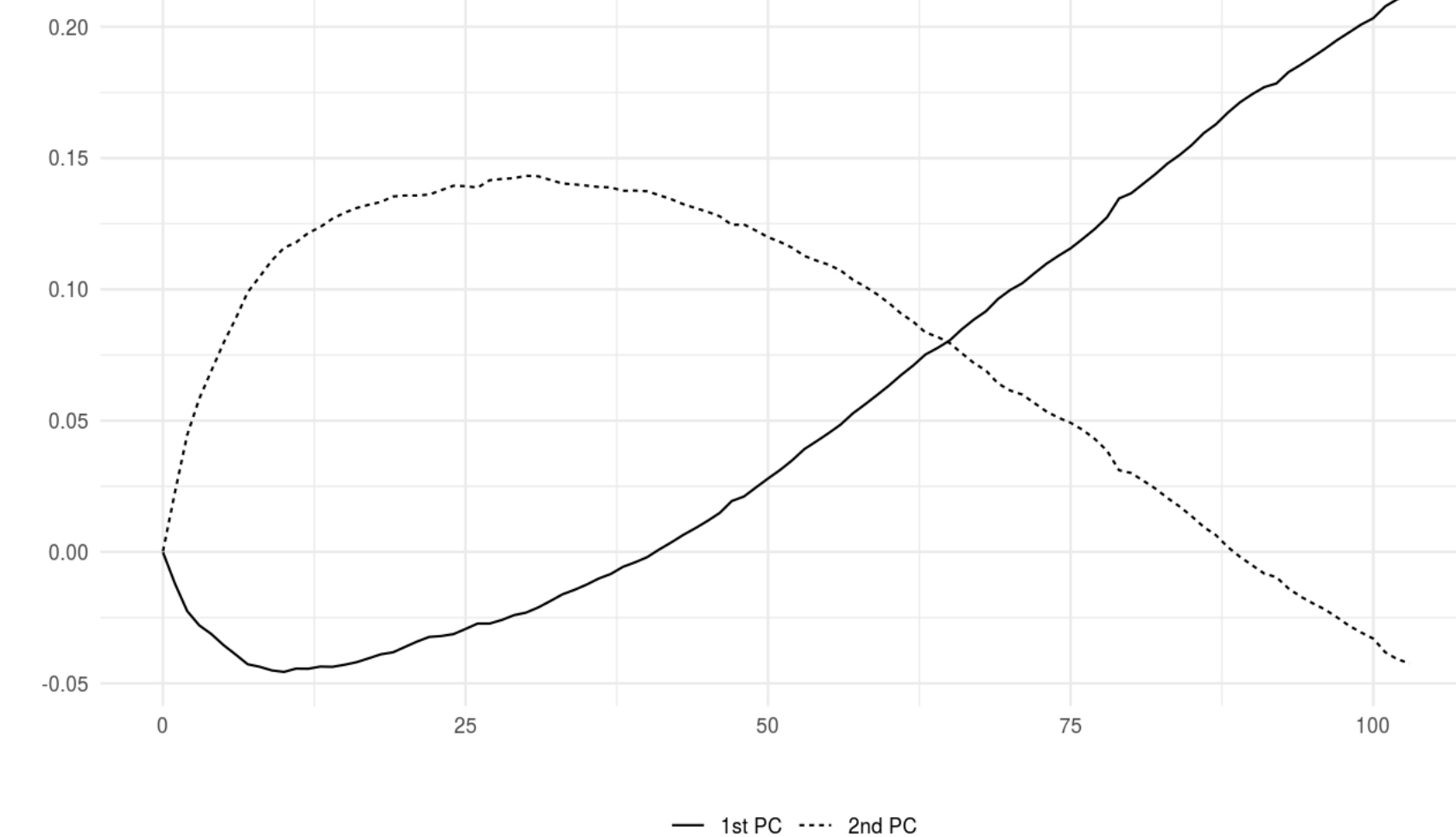
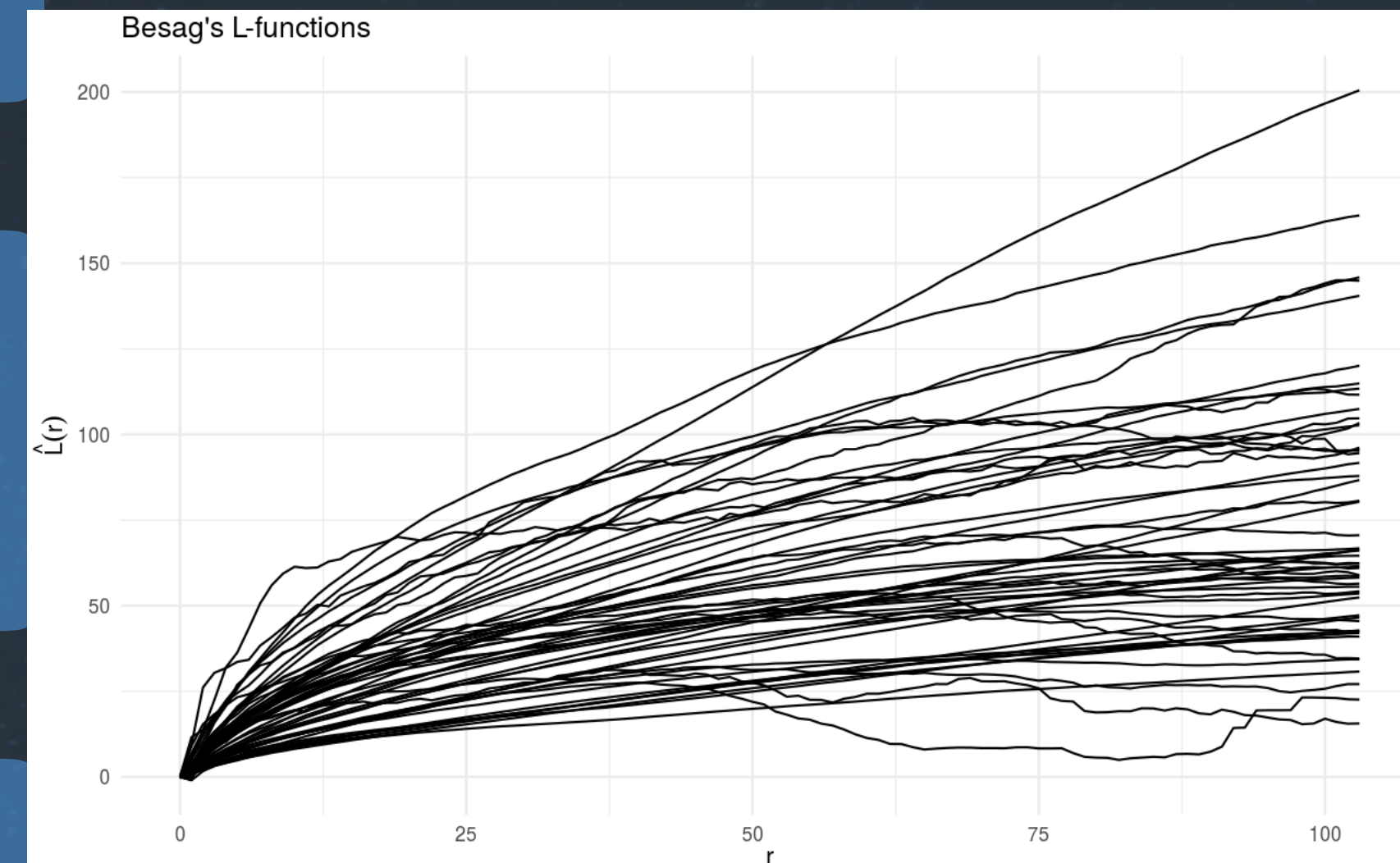
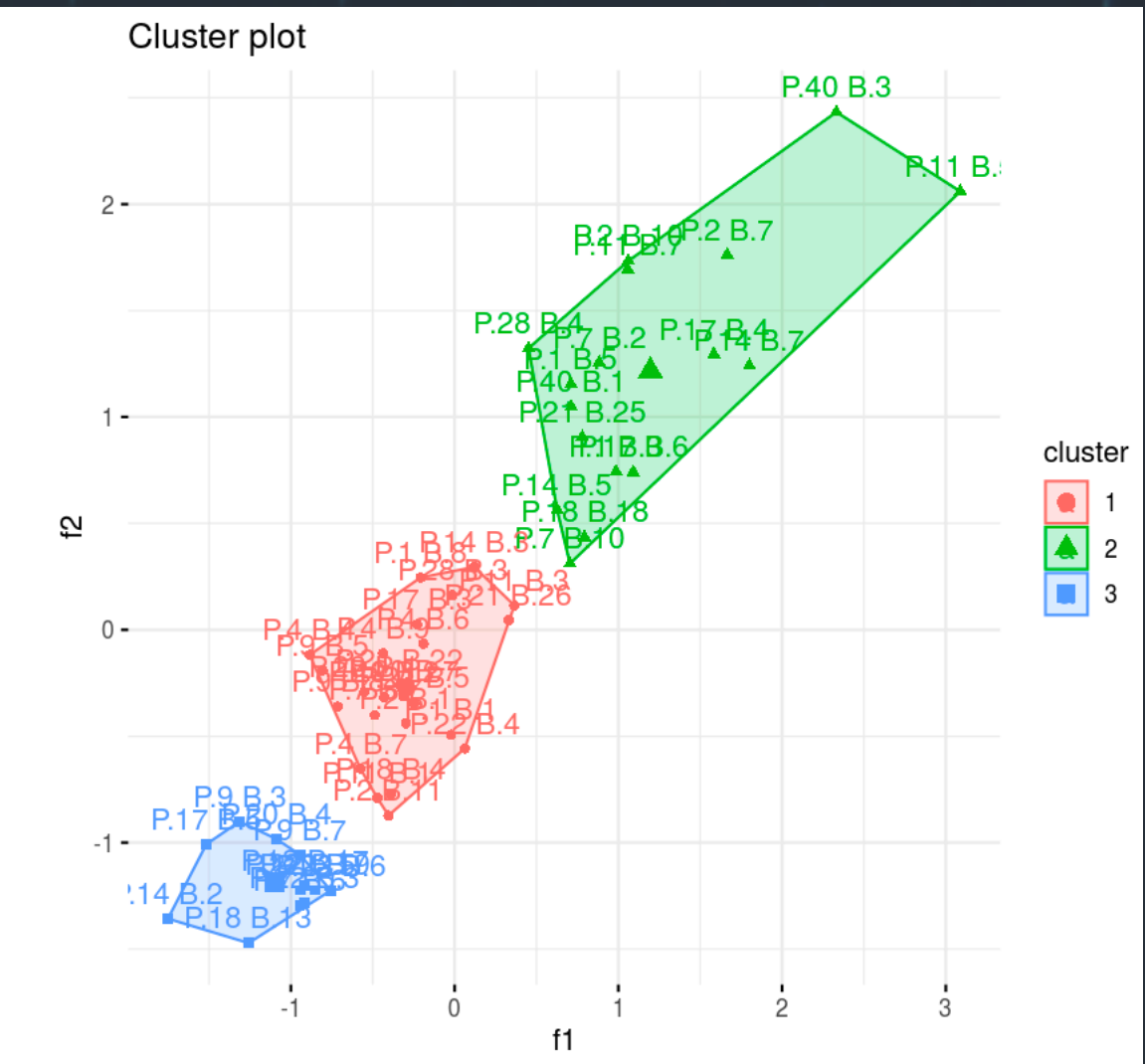
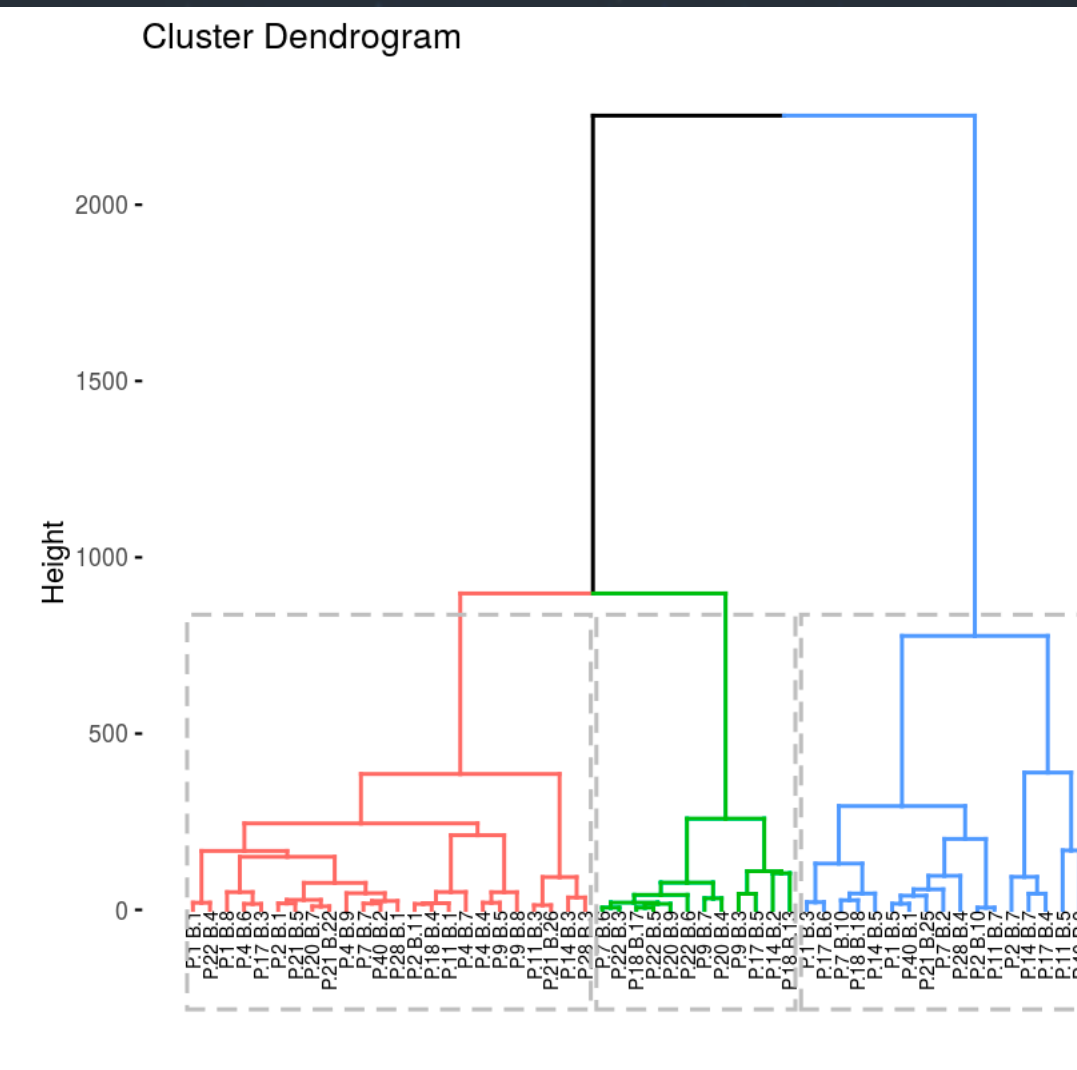
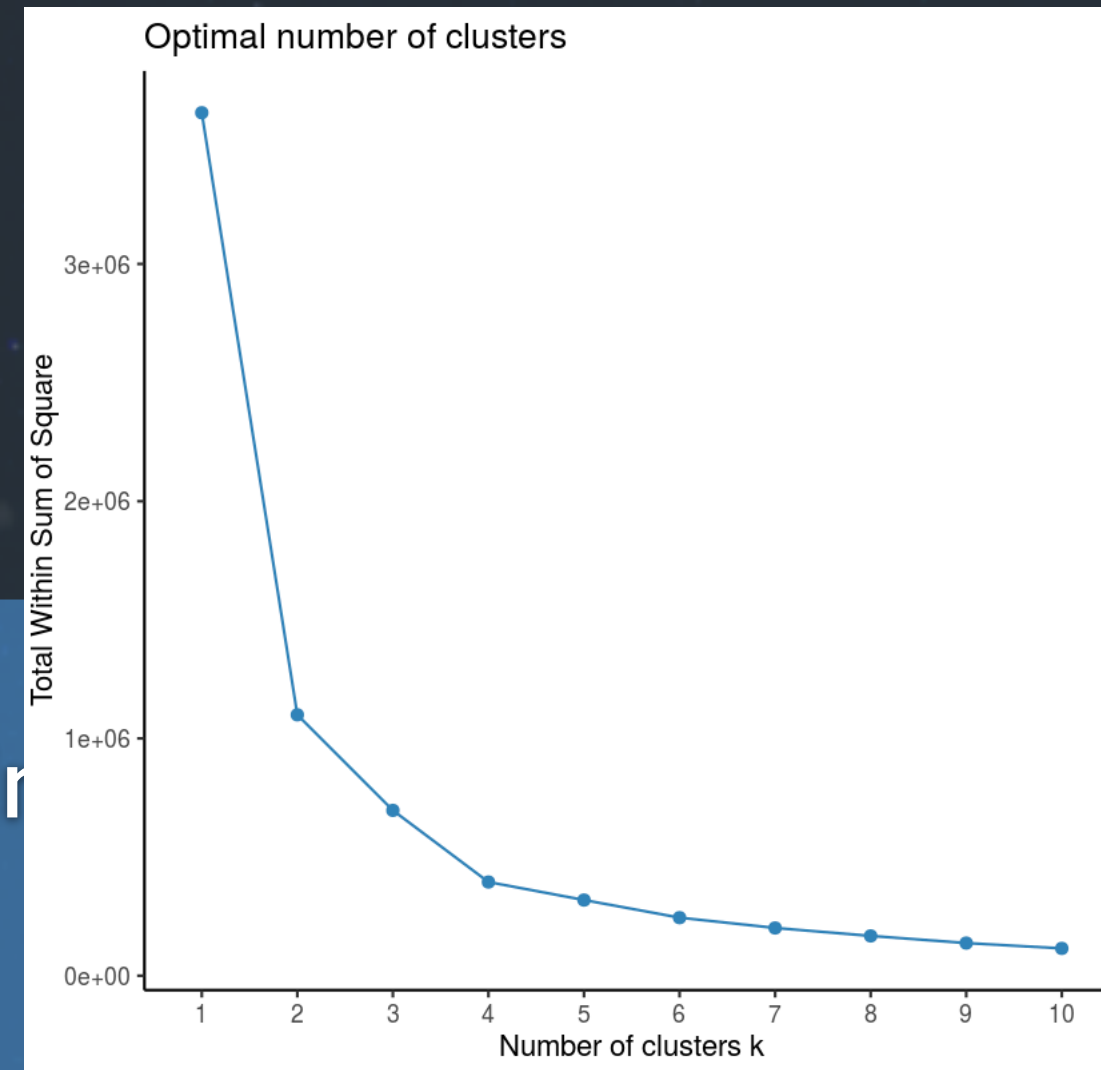
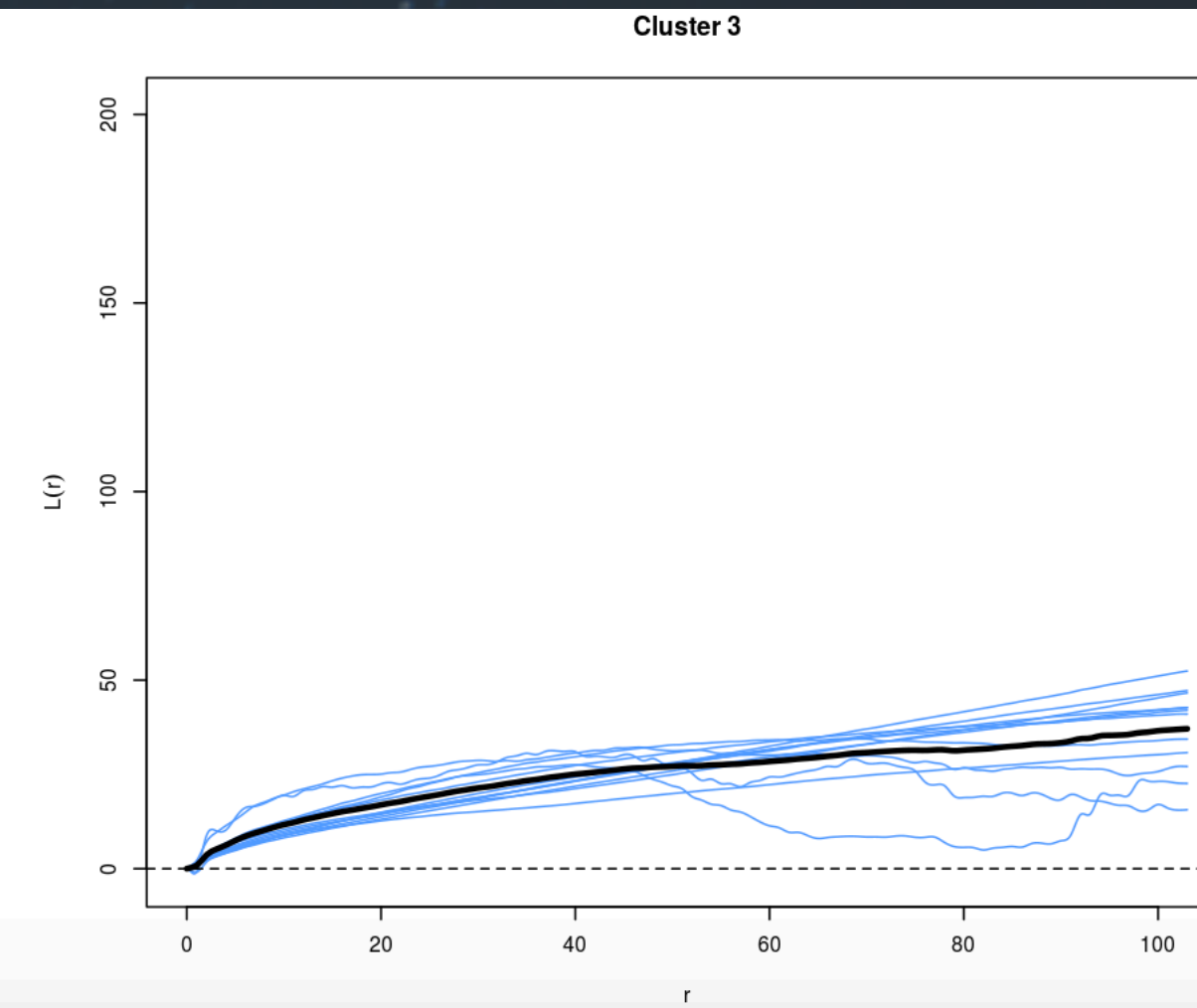
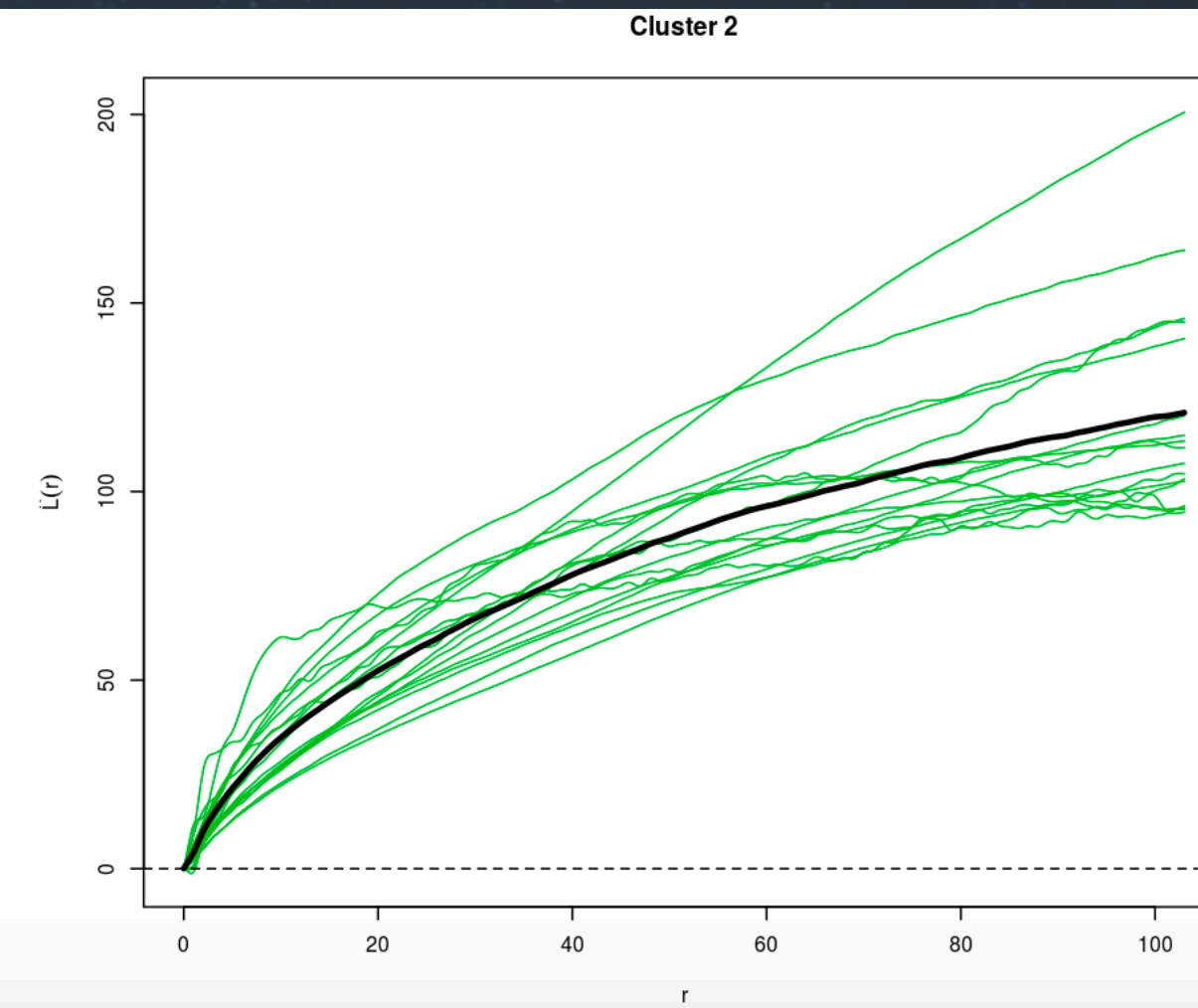
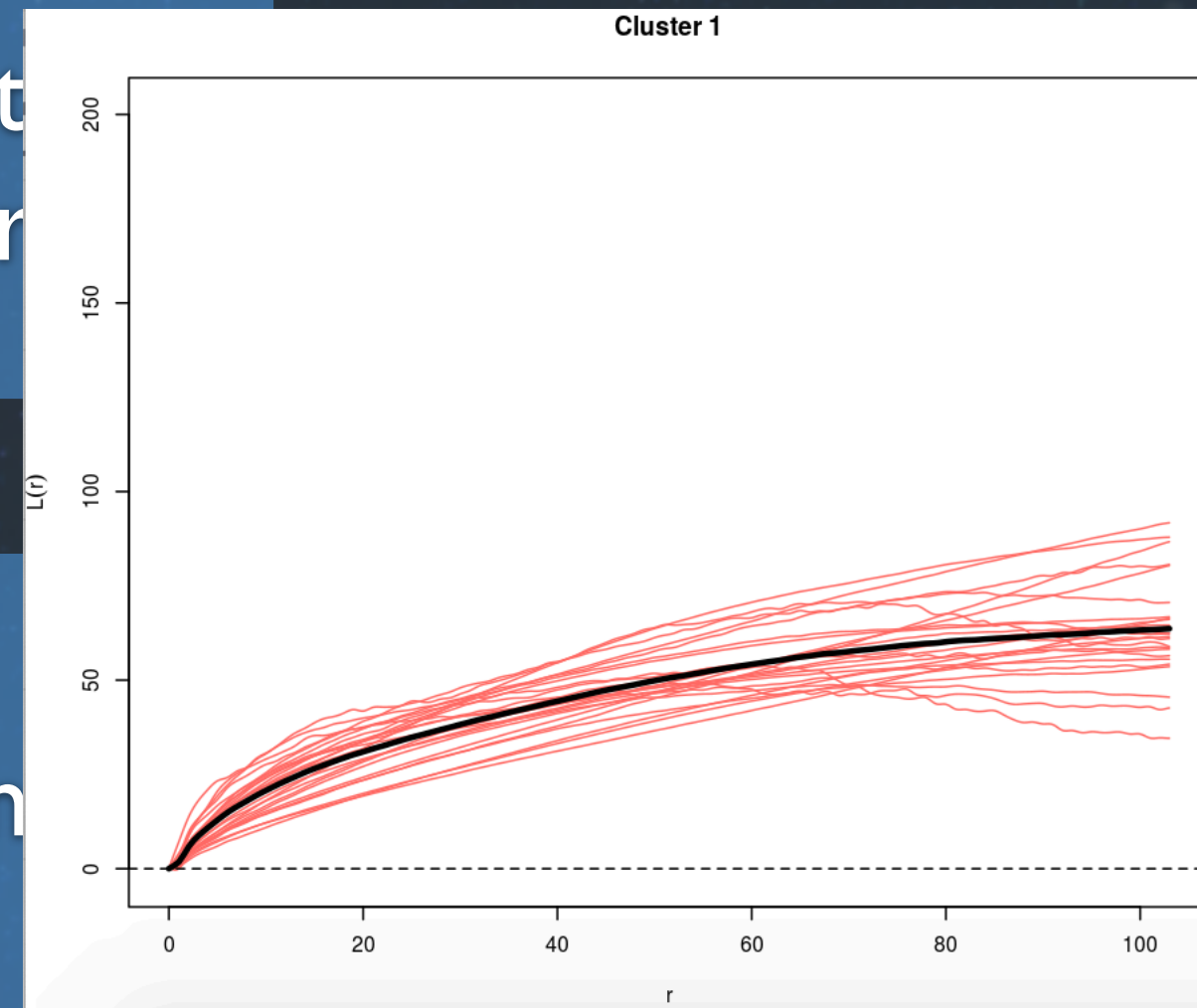


Image processing
and analysis

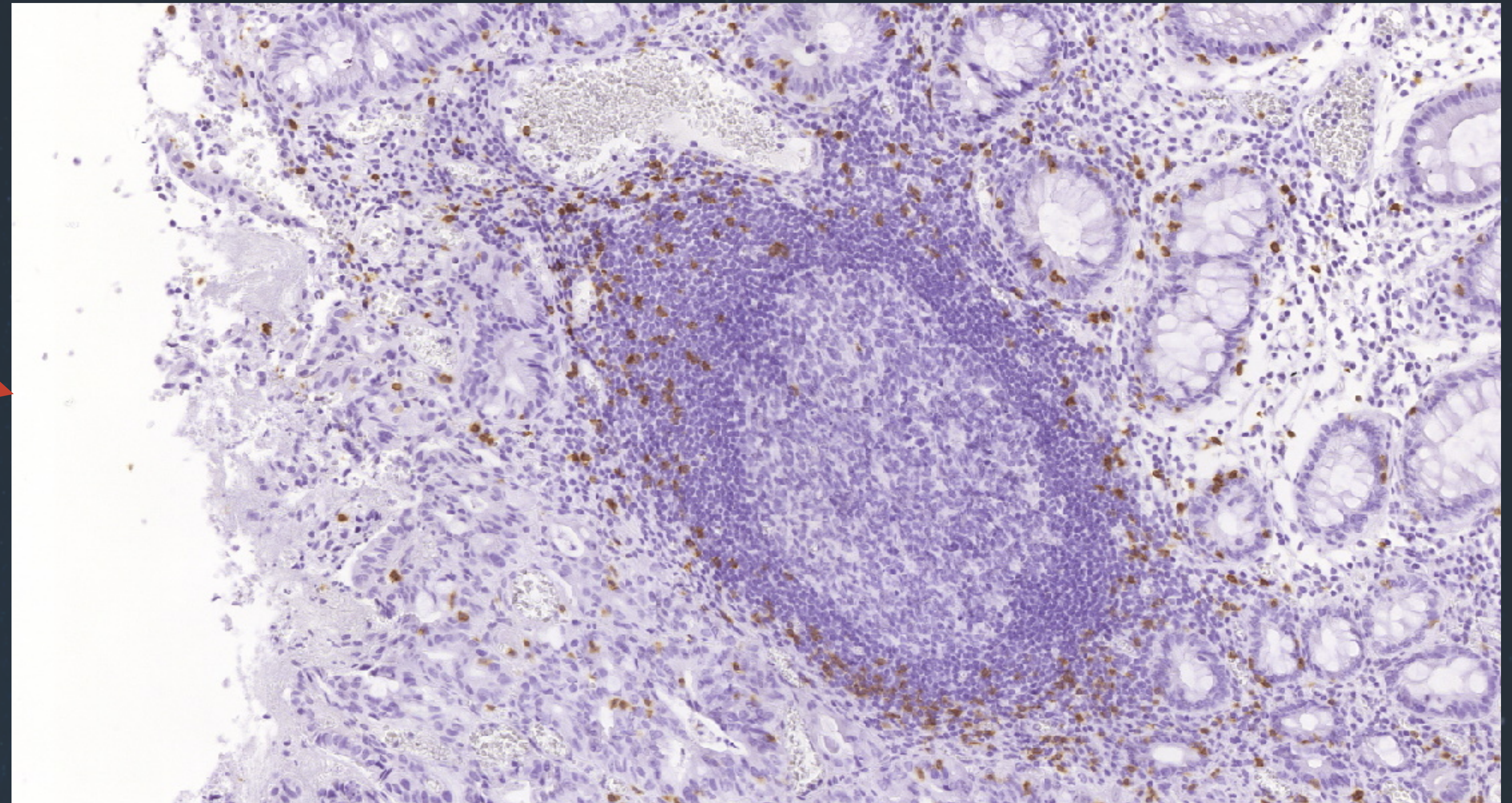
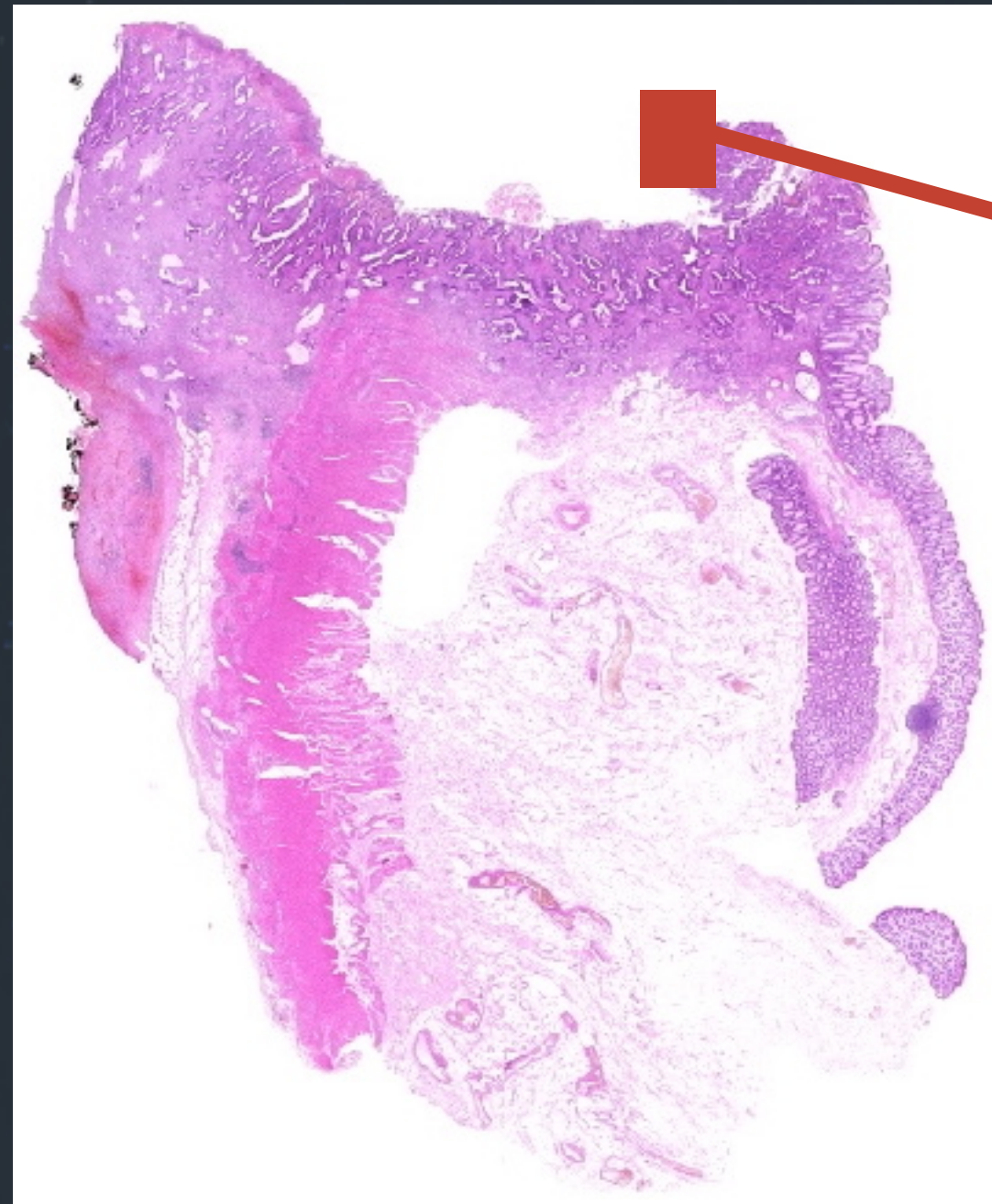


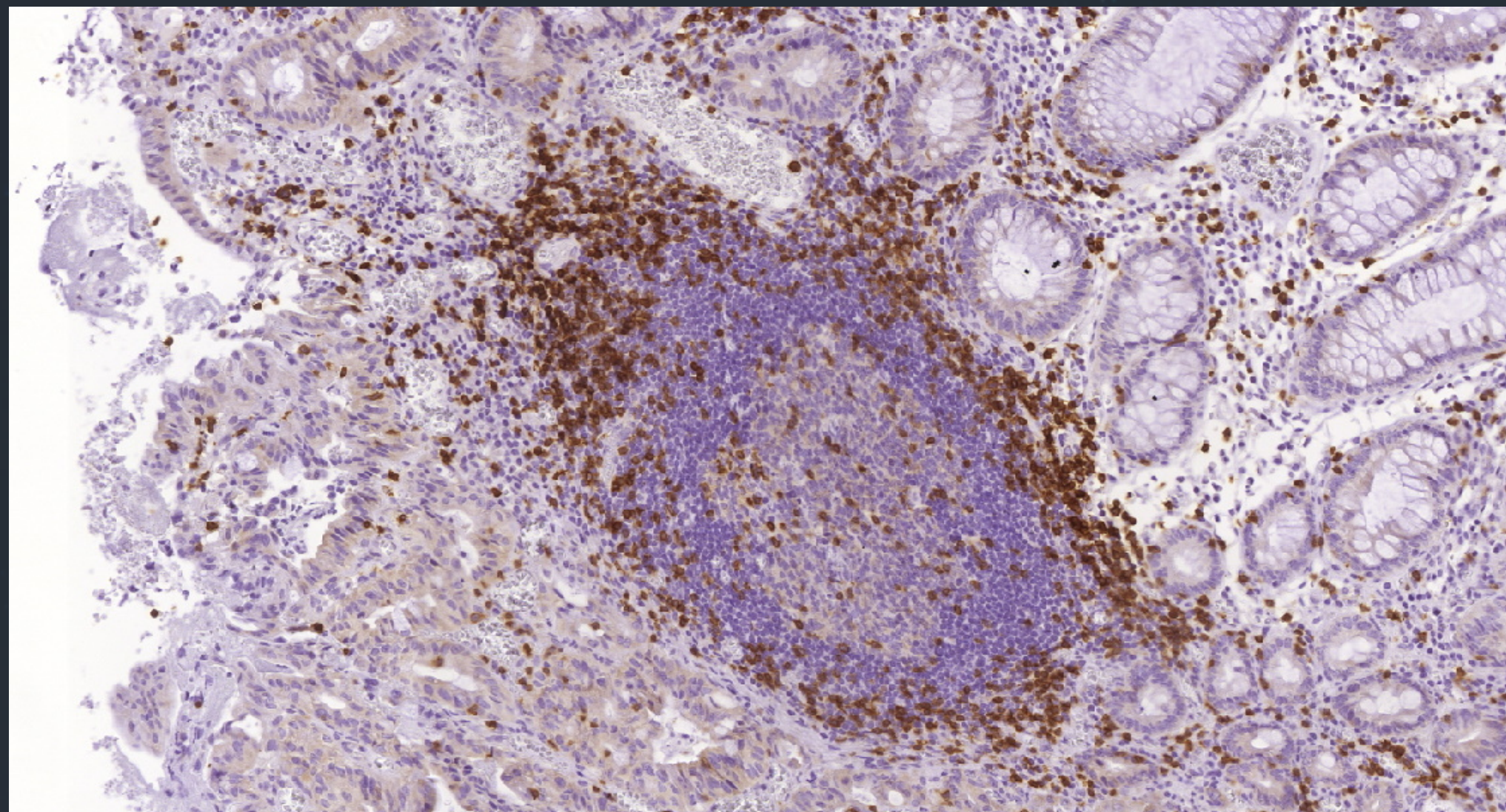
Spatial data
descriptor



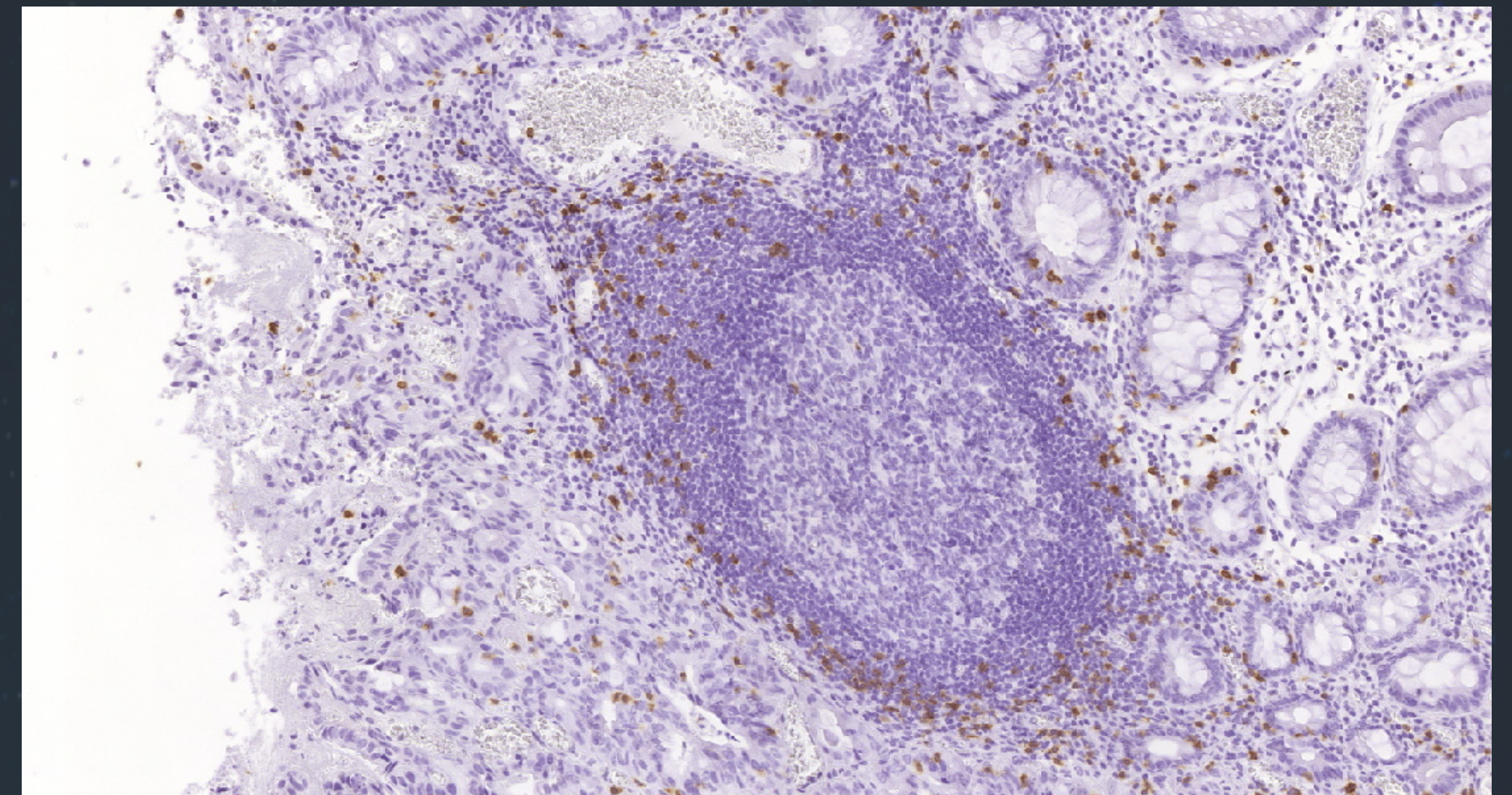
Spatial data m

- caveat: short-range interactions vs tertiary lymphoid structures

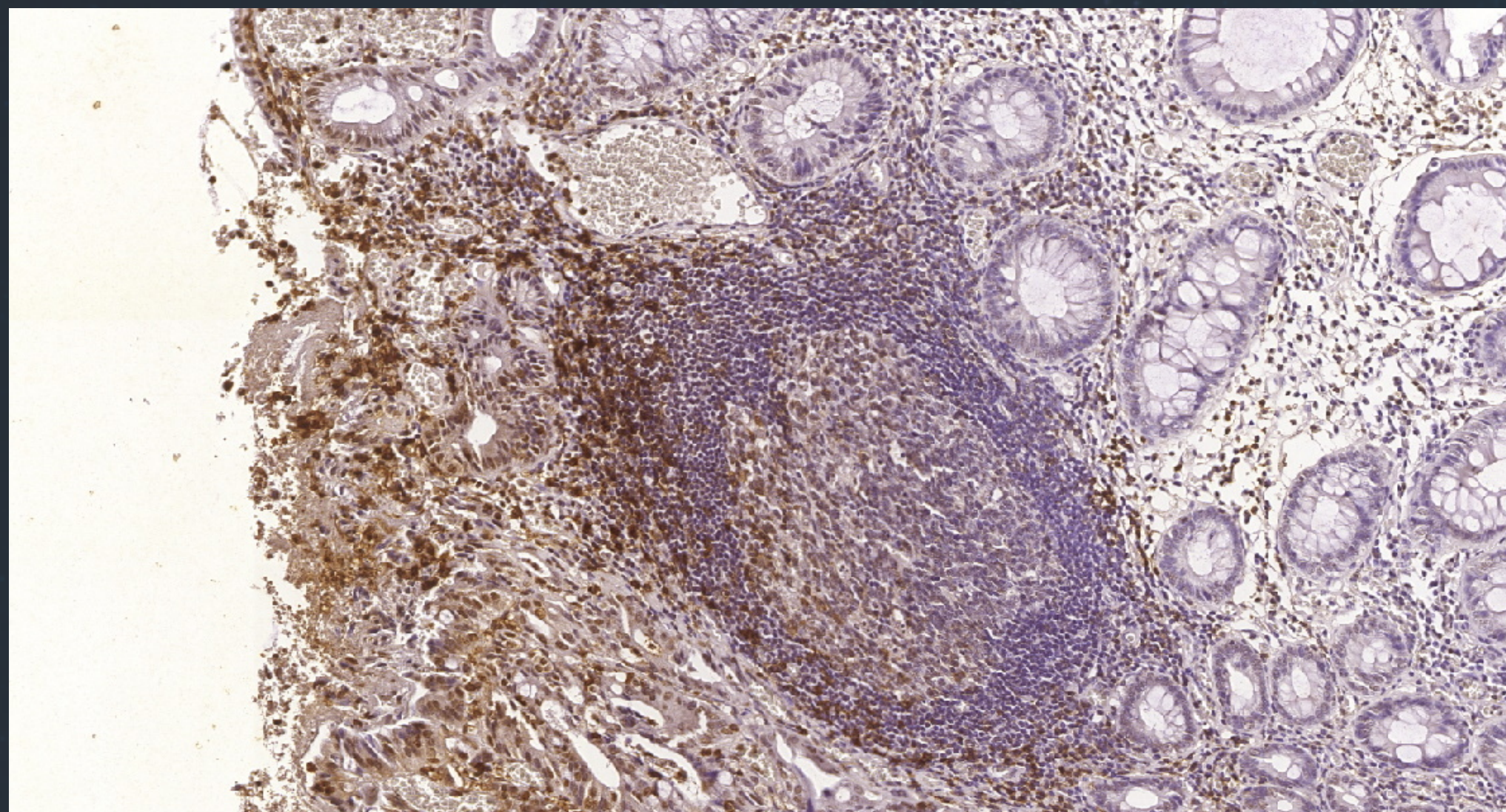




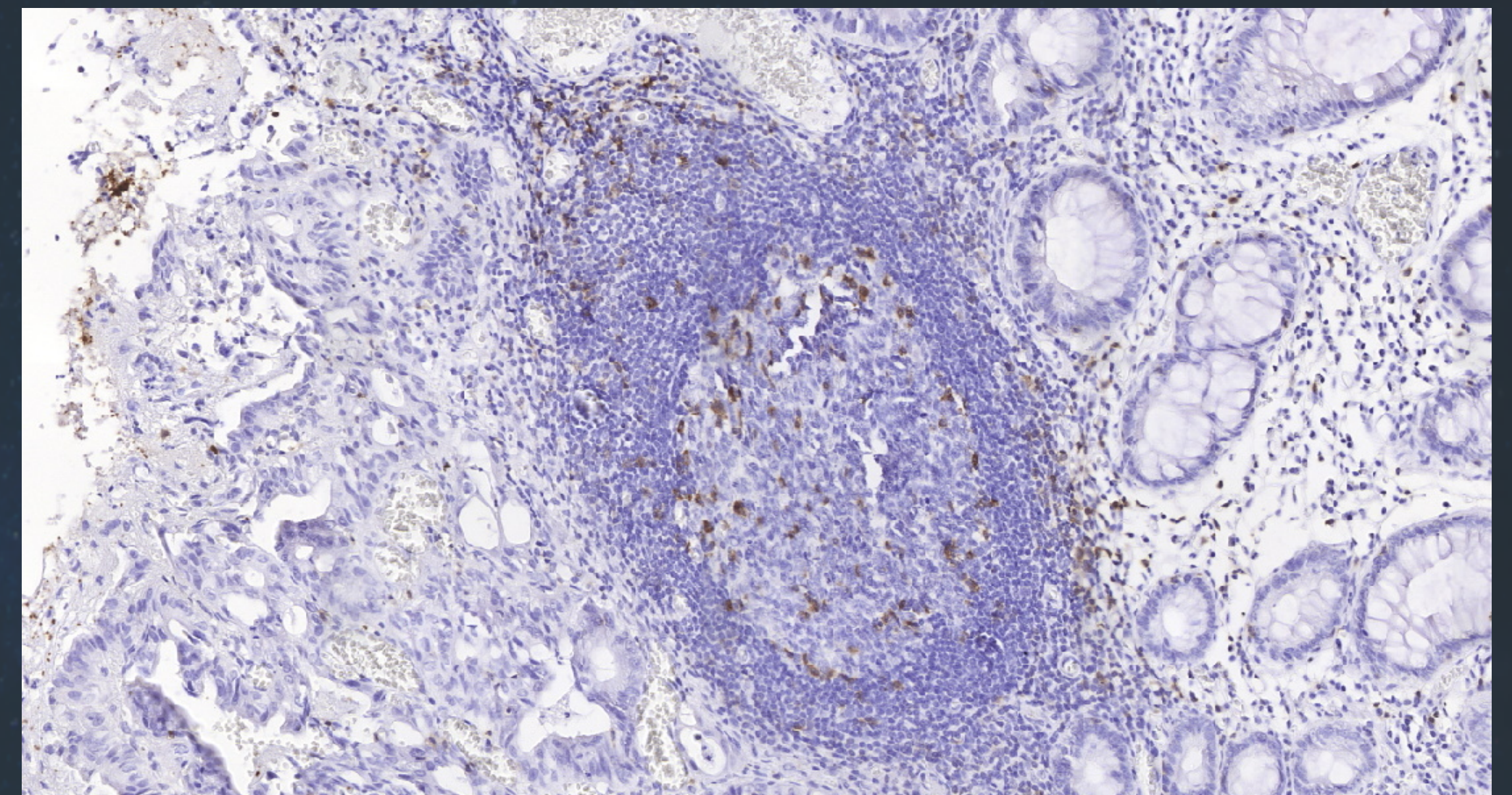
CD3



CD8



FOXP3



PD1

Other issues

- resolution: from scanned image (e.g. 20x, 0.25 mpp) to working resolution (e.g. 0.325x)
- artifacts: staining quality, tissue tearing, etc.
- grid deformation under image registration
- staining specificity: e.g. PD-L1+ cells must be tumoral cells to be of interest

Conclusions

- in our data, there are, in general, 3-4 patterns of spatial distributions of T-cells (per T-cell type)
- lymphoid tertiary structures, while important biologically, bias the analysis
- the immune reaction is heterogeneous across tumor
- the spatial statistics framework helps exploring the tumor-host interaction
- need for biological interpretation

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