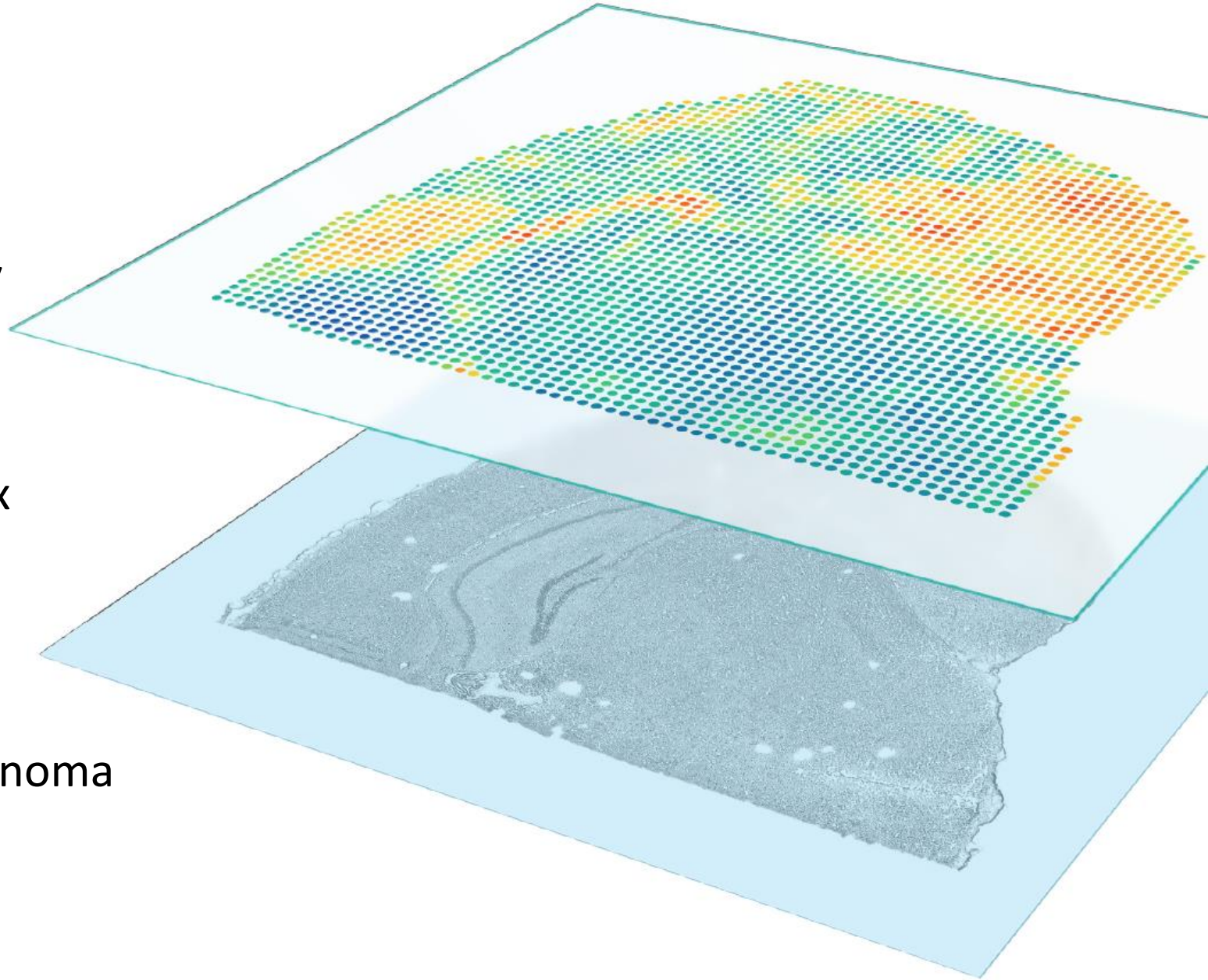# Spatial Clustering and Deconvolution

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Outline

- Visium spatial technology

- Spatial clustering
  - Method
  - Example: Prefrontal cortex

- Spatial deconvolution
  - Method
  - Example: Melanoma
 - Example: Merkel cell carcinoma

Visium spatial technology

- Spatial RNA sequencing provides high-throughput gene expression profiling while preserving the morphological context of the tissue
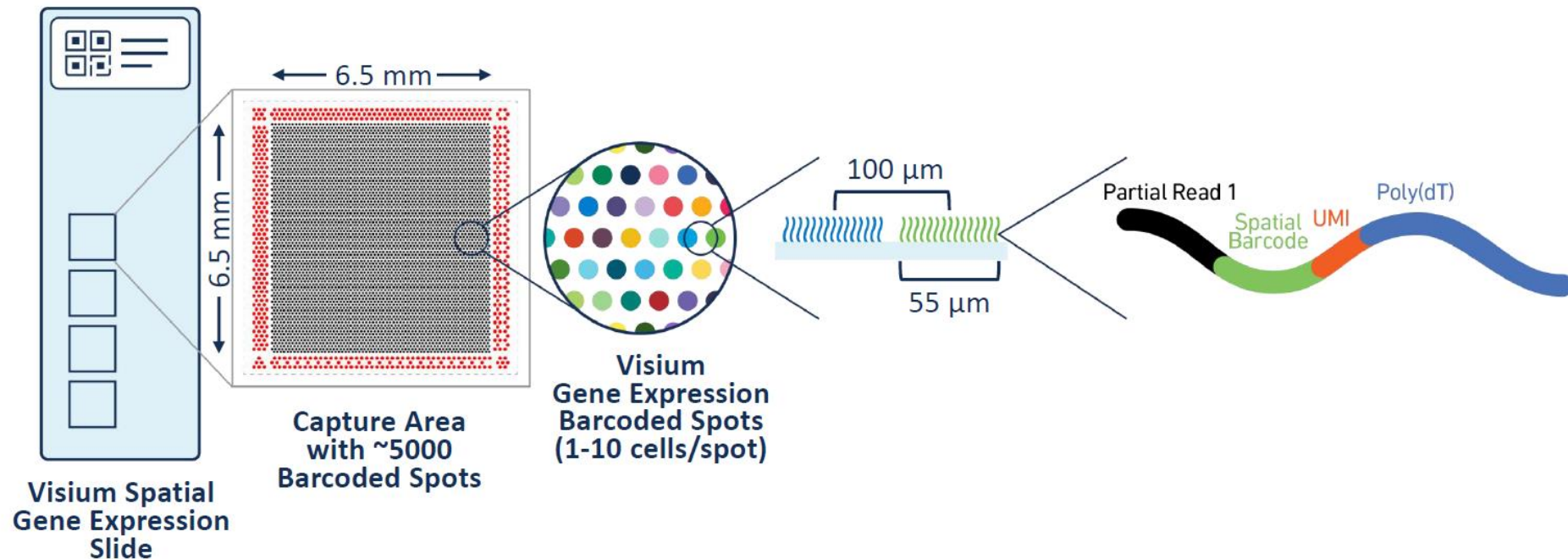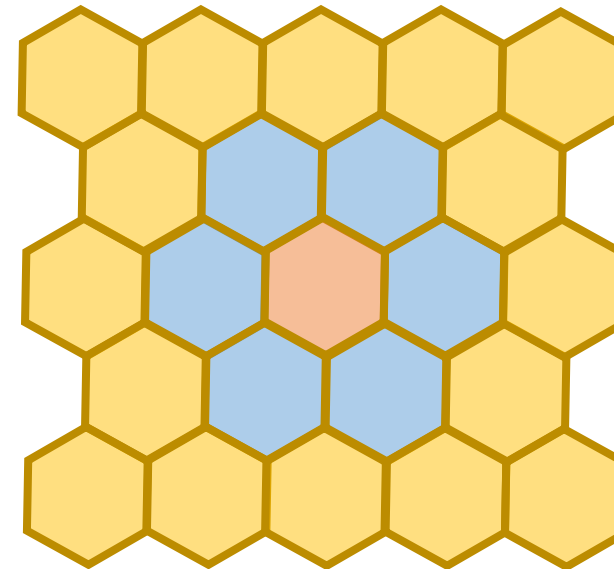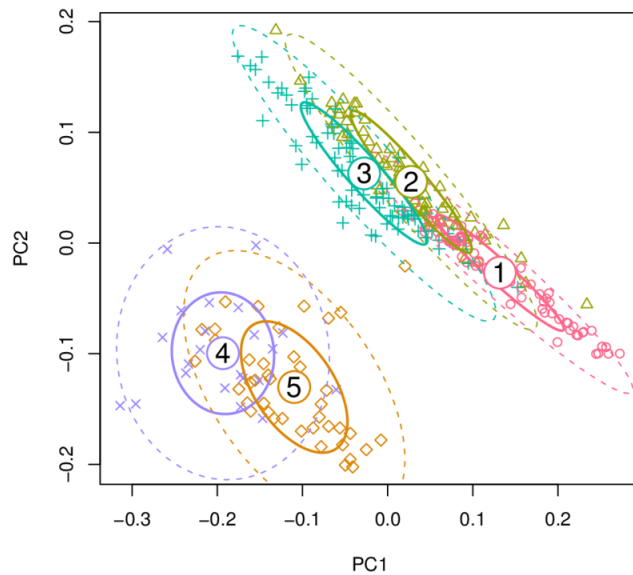
Spatial clustering

- Accurate clustering is an important step that allows downstream analyses such as cell type or tissue annotation and differential expression to provide unbiased biological insights
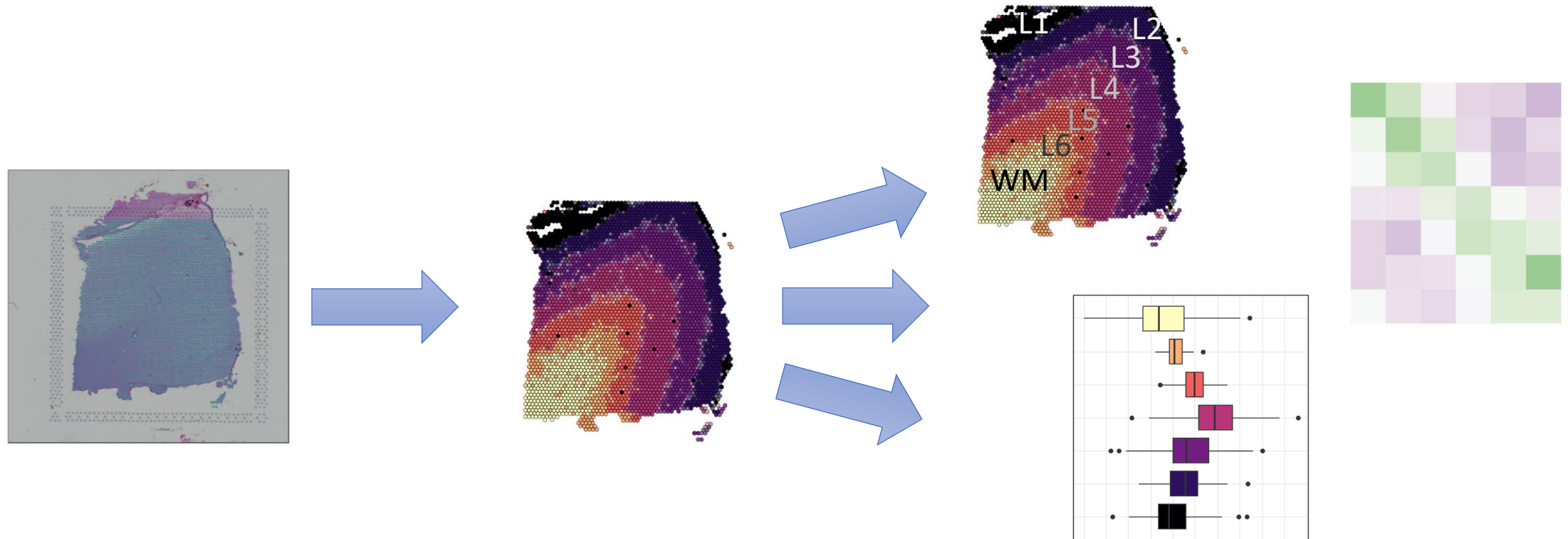
Method

- Fit a parametric model to the observed data (principal components)
  - Using MCMC, iteratively estimate the center of each cluster, variance within clusters, and cluster label of each spot
  - Spatially smooth cluster labels by encouraging neighboring spots to belong to the same cluster

Dorsolateral prefrontal cortex (Maynard et al., 2020)

- Manual annotation by expert based on cytoarchitecture and selected marker genes
 - Considered ground truth
  - Labor-intensive
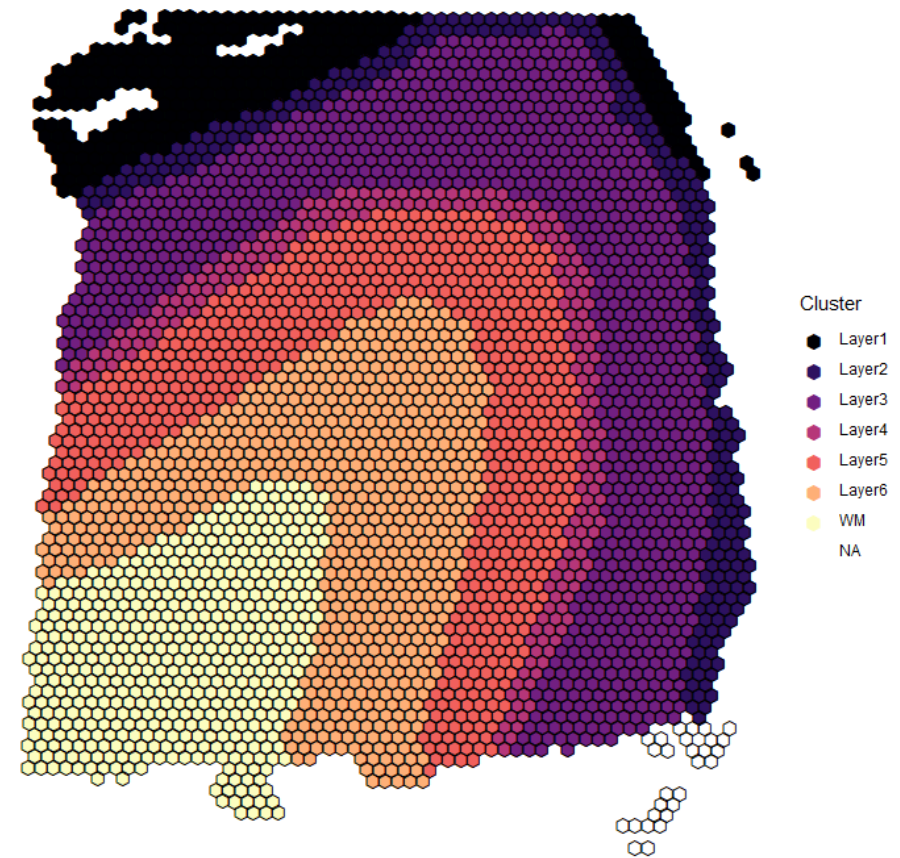
Non-spatial clustering

- Shared nearest neighbor clustering on top 50 principal components (PCs) generated from highly variable genes (HVGs) + spatial coordinates

- Set to return 8 clusters to improve clustering performance

- Adjusted Rand Index (ARI) = 0.27
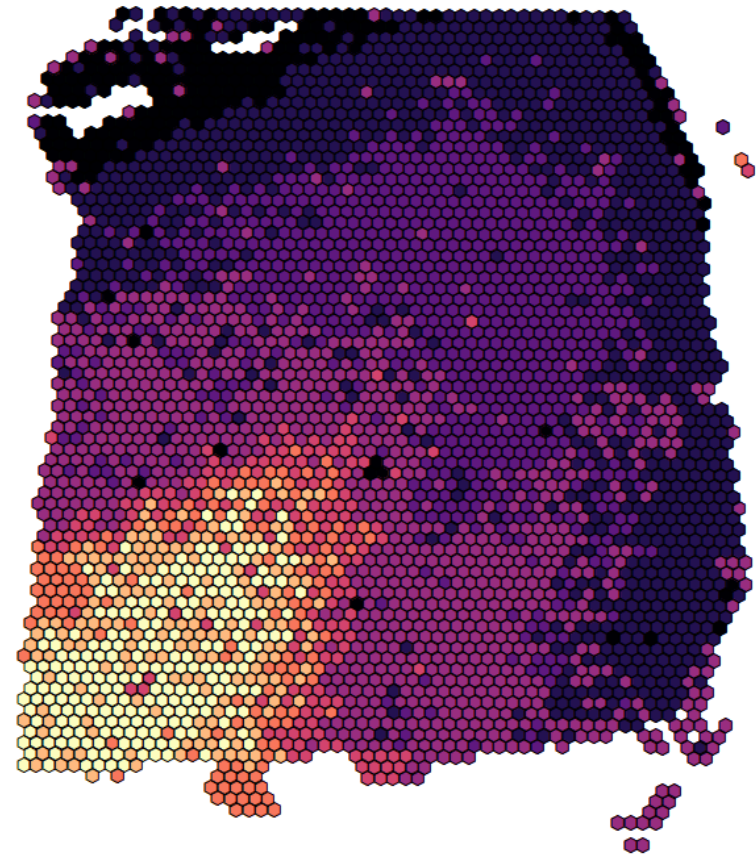
Spatial clustering

- Spatial clustering on top 9 principal components (PCs) generated from highly variable genes (HVGs)

- Set to return 7 clusters to match prior knowledge of prefrontal cortex cytoarchitecture

- ARI = 0.47
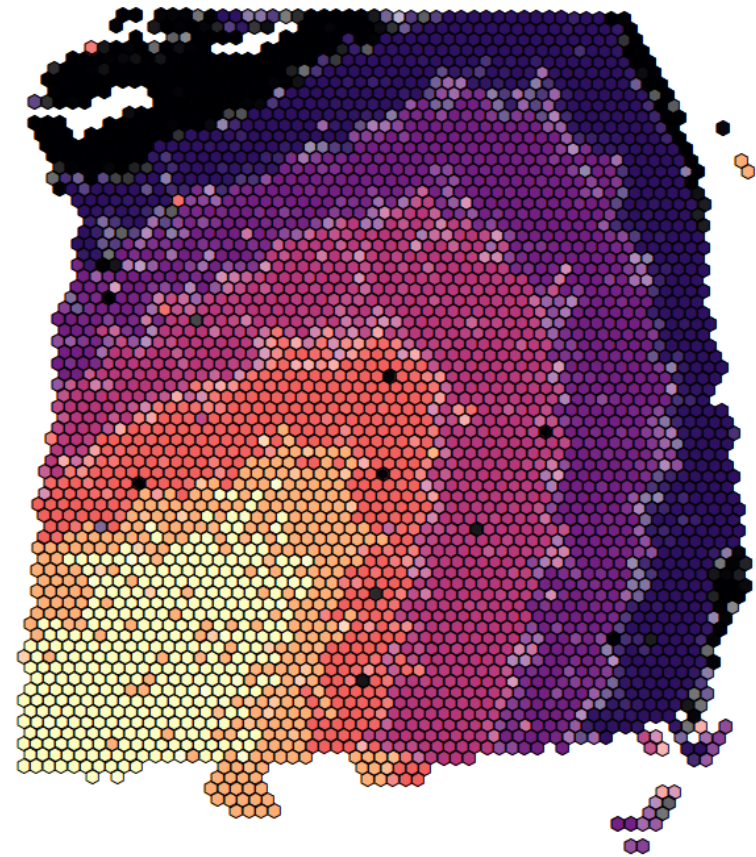
Spatial deconvolution

- Visium is not a single cell technology

- Deconvolution can:
  - Increase the resolution of the spatial map
  - Allow for better understanding of tissues composed of heterogenous mixtures of cells (e.g. tumor microenvironment)
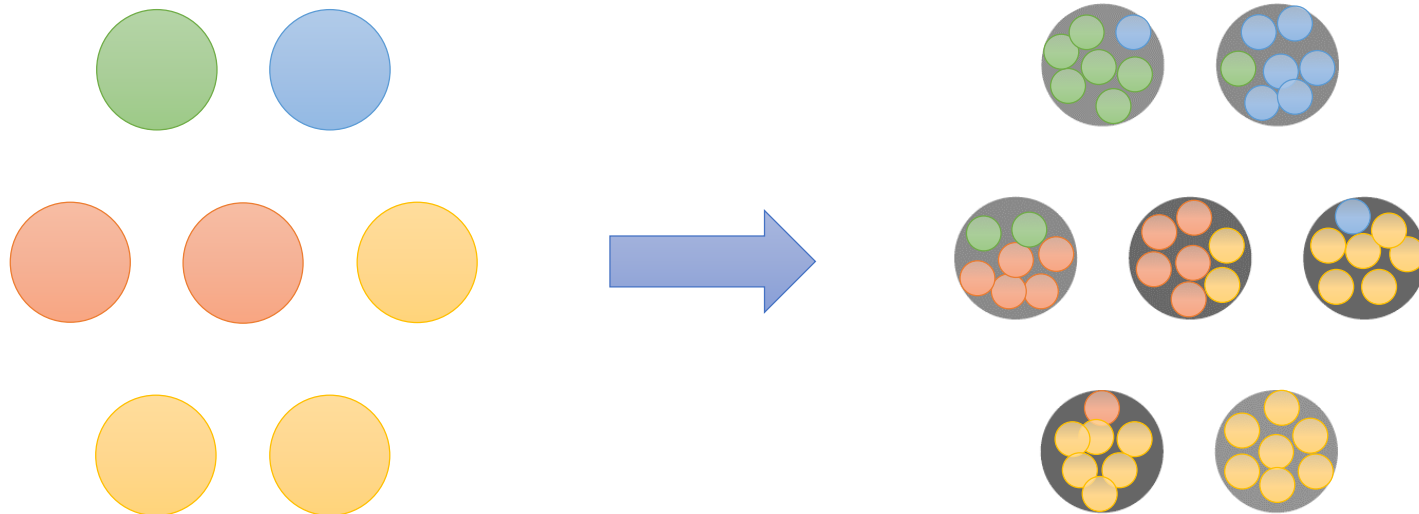
Method

- Similar to clustering method, except now the unit of analysis is not a spot but instead a "cell"
  - Using MCMC, iteratively estimate the center of each cluster, variance within clusters, and cluster label of each "cell" while accounting for the spatial structure
  - In each iteration, jitter the features of each "cell" while keeping the sum over all "cells" within a spot fixed
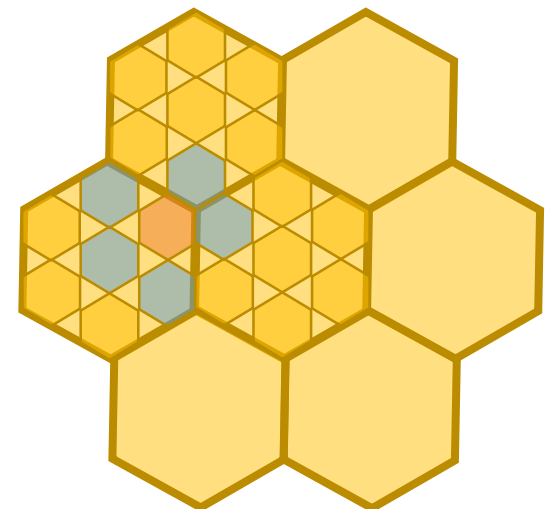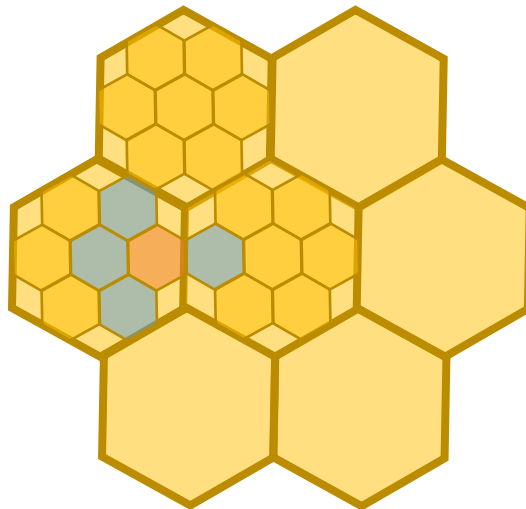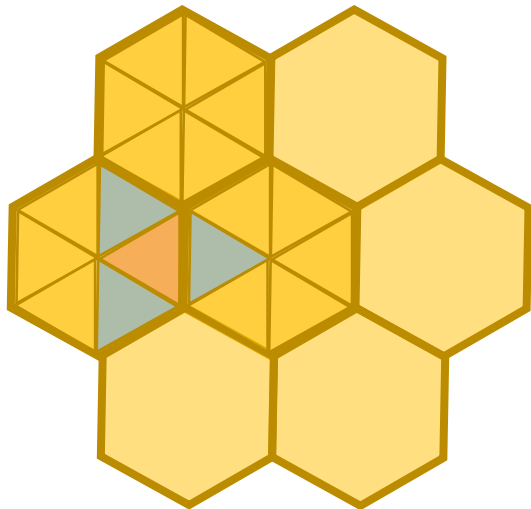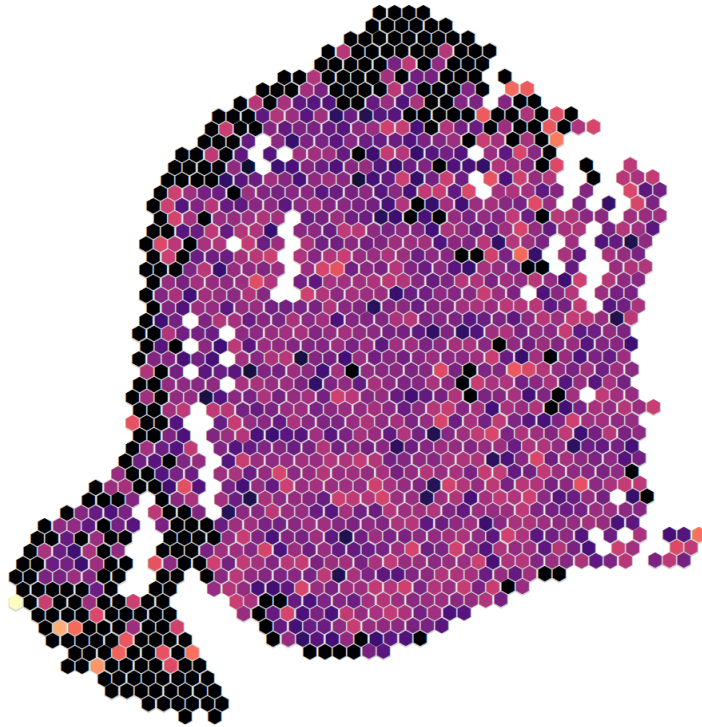
Melanoma (Thrane et al., 2018)

- ● Melanoma
- ● Stroma
- ● Lymphoid tissue
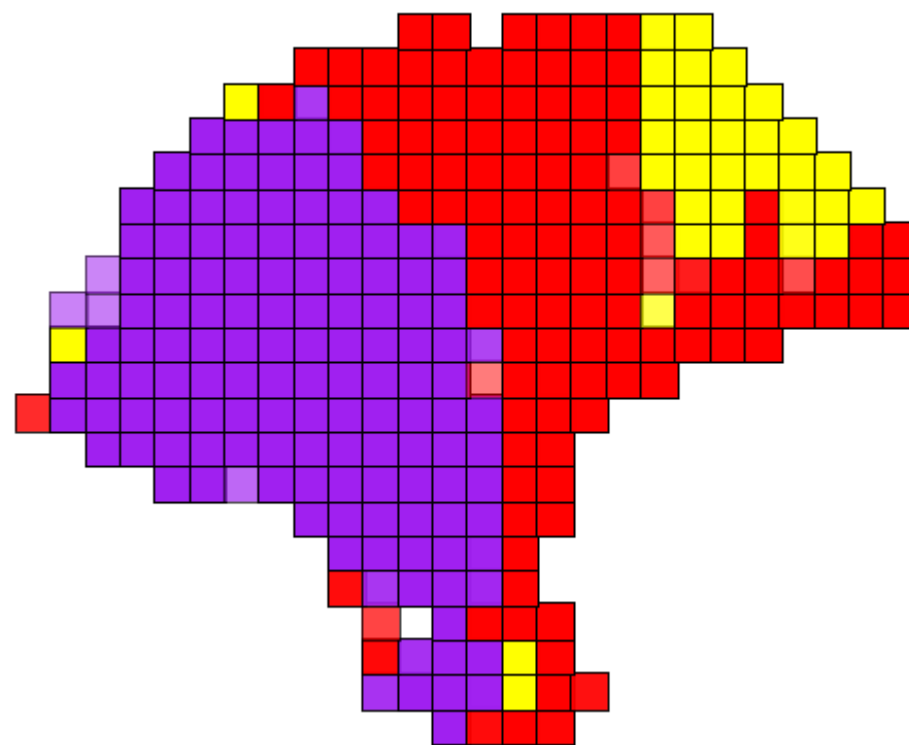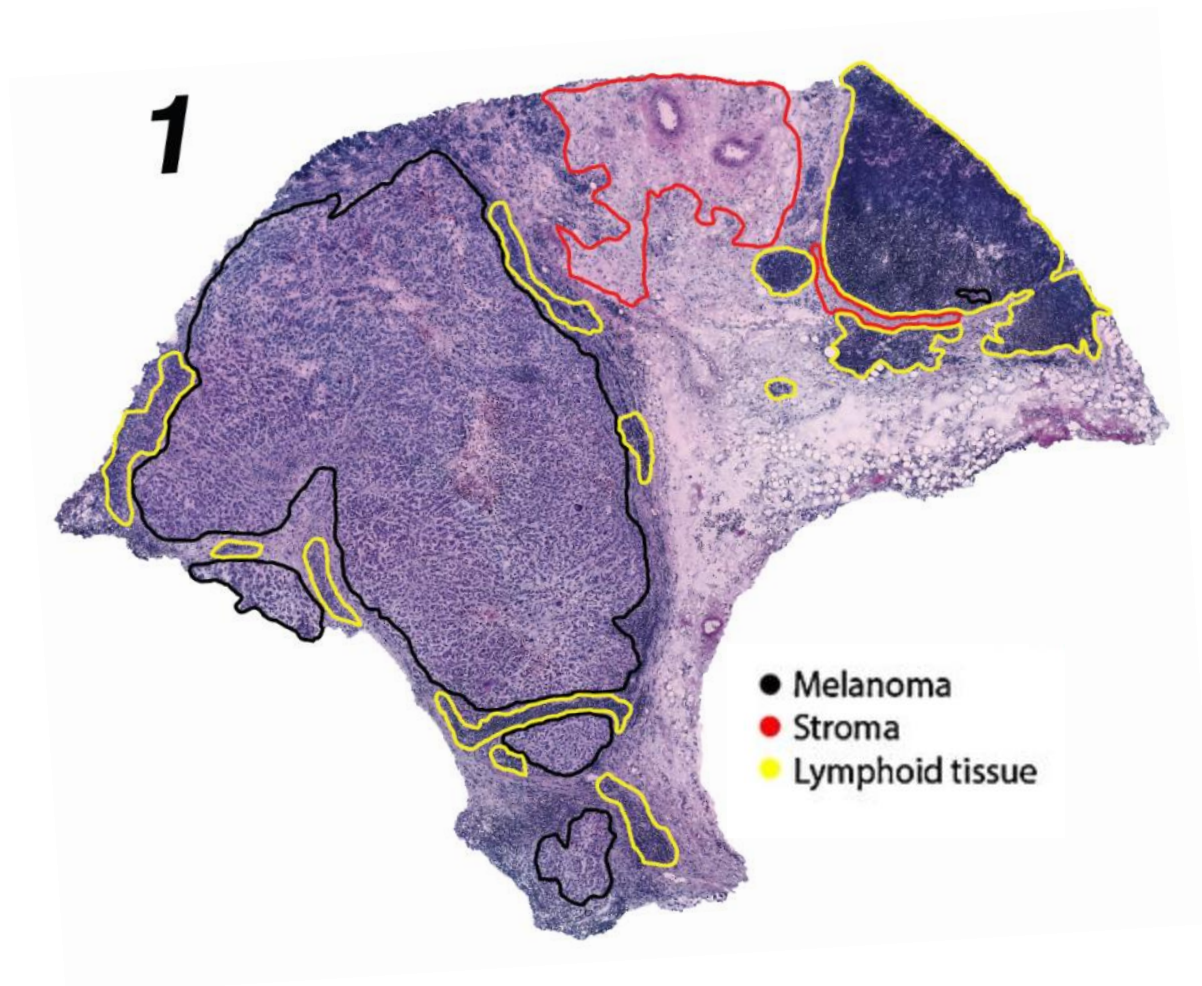
Deconvolution reveals heterogeneity in the tumor immune microenvironment

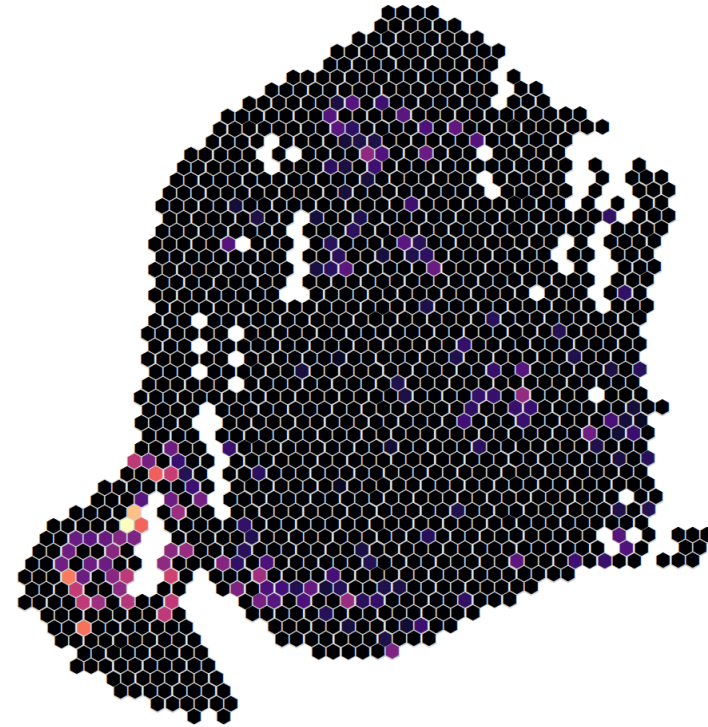- ● Melanoma
- ● Stroma
- ● Lymphoid tissue
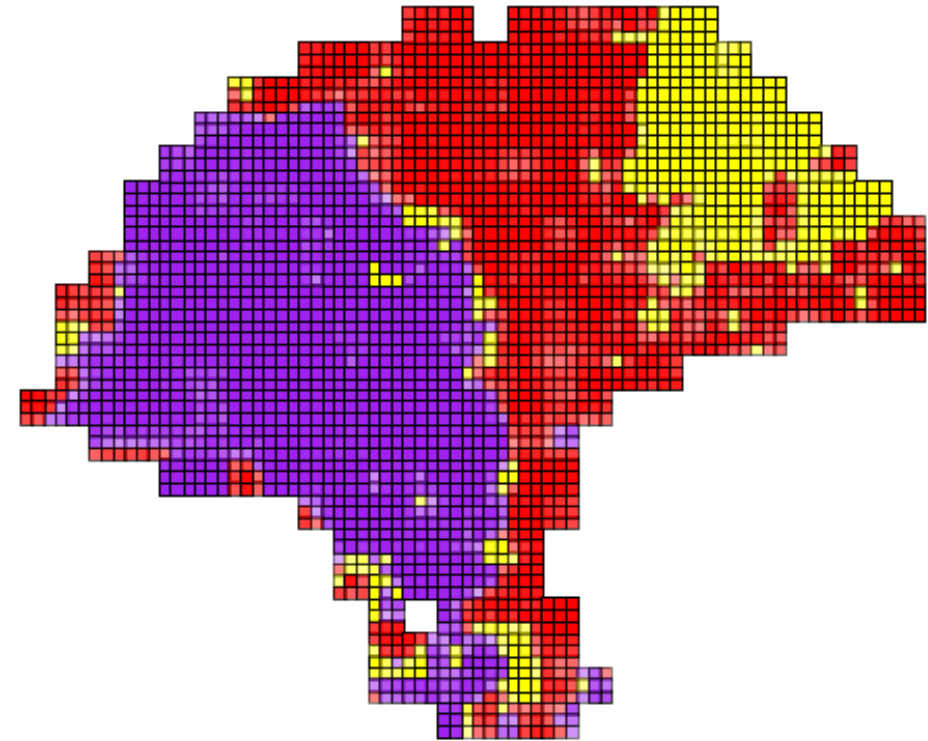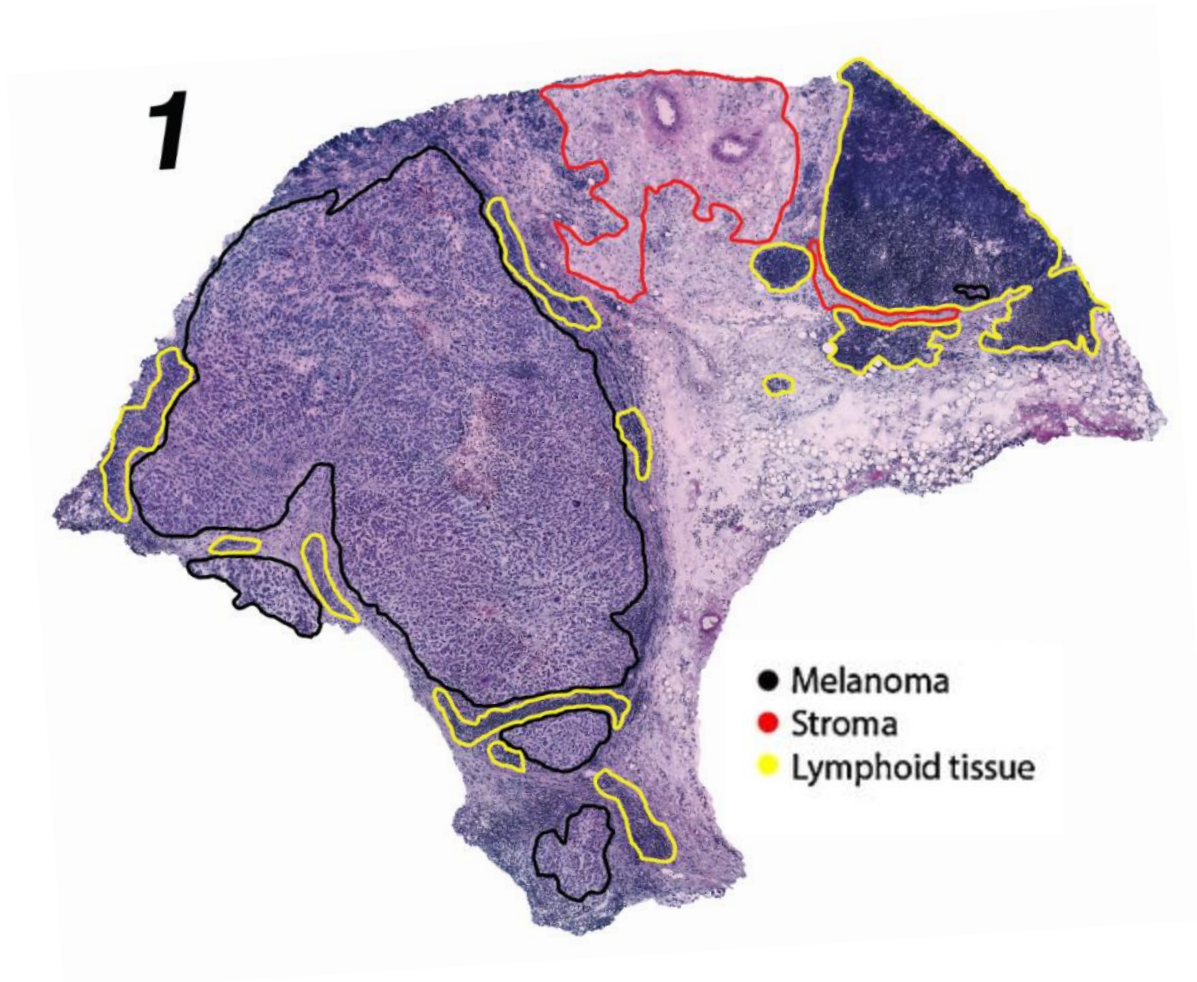
Merkle cell carcinoma (MCC)

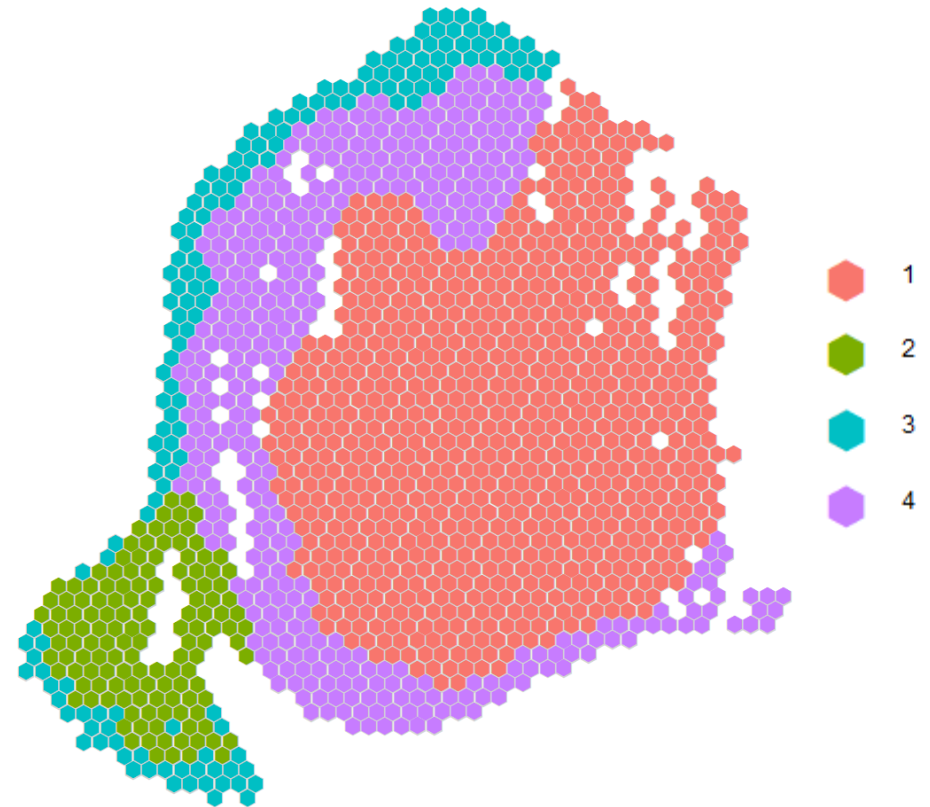**CHGA (cancer marker) expression**

CD3 (T cell marker) expression

Spatial clustering

- Clusters 1 and 4 from spatial clustering correspond to the tumor
- Cluster 2 has the highest immune expression

Spatial deconvolution

- Most of the immune cells (green) are at the periphery of the tumor

- However, there is some evidence of immune infiltration inside the tumor