10X Visium Spatial Transcriptomics Platform

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Content

Strengths of spatial transcriptomics

Spatial transcriptomics (ST) is an *in situ* capturing technique, which profile gene expression in RNA level, whilst preserving the spatial information of histological tissue sections.

Limit of other similar technologies:

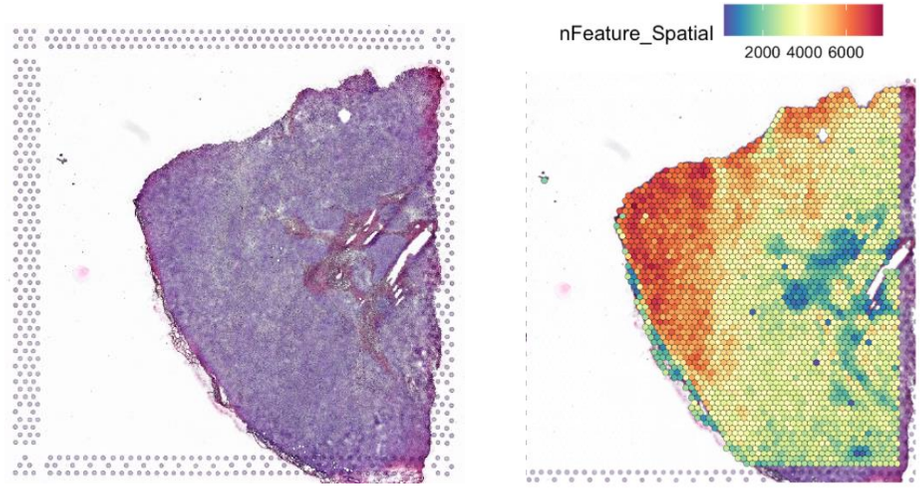
-- Bulk RNA-seq or single-cell RNA-seq techniques which result in loss of spatial information.

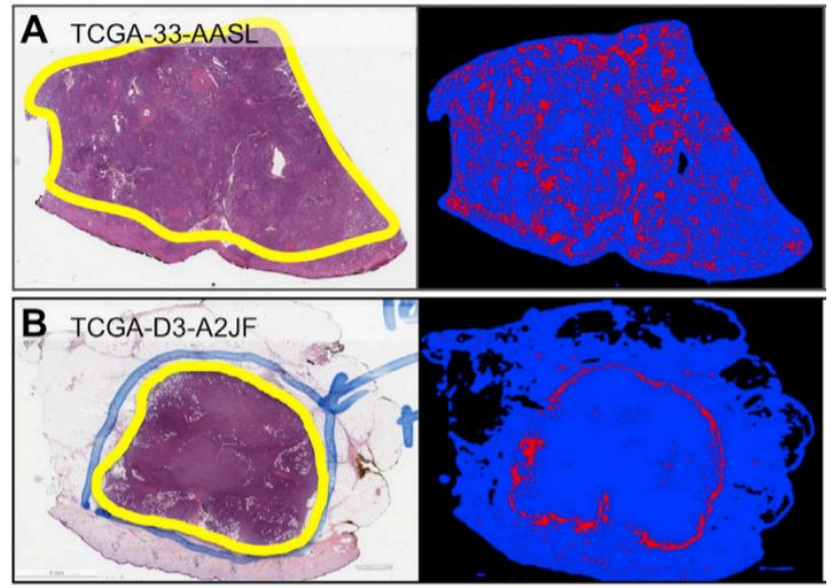
-- *in situ* hybridization technique which localize a single nucleic acid within a histologic section instead of whole transcriptome.

Strengths of ST:

- Maintaining the architecture of tissue
- Assigning expression data within histological H&E image
- Exploring molecular identities of diverse cells in the context of heterogeneous tissue

Spatial Transcriptomics





Lymphocytes infiltrated tumor
► Good prognosis

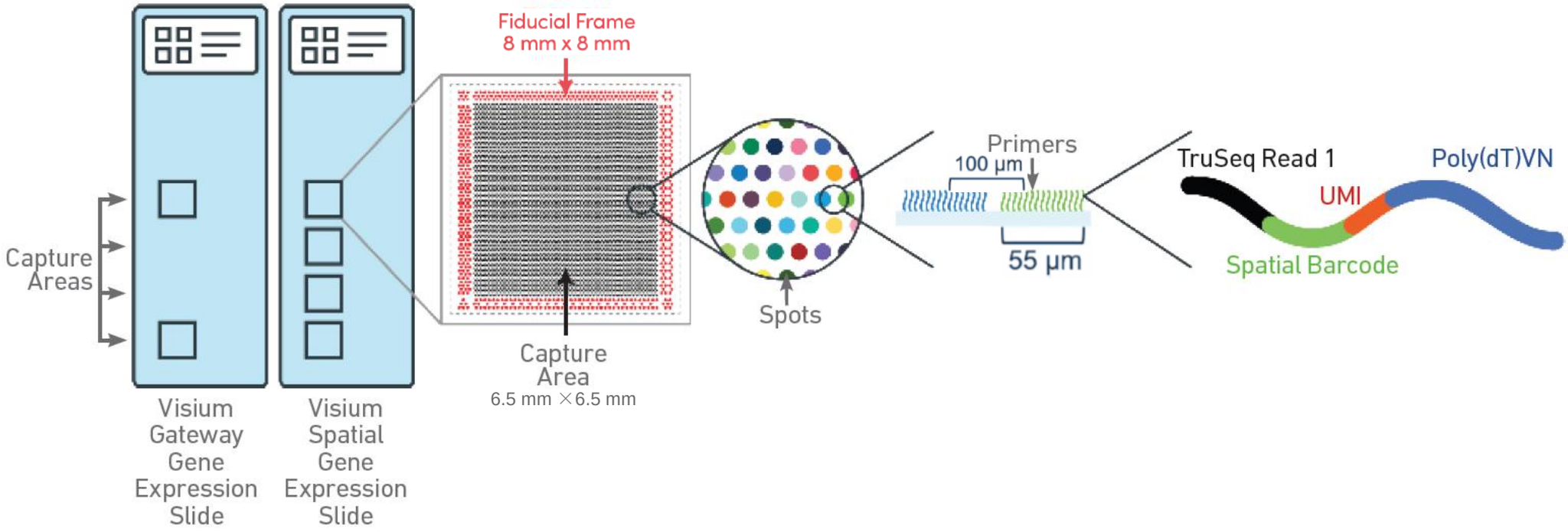
Lymphocytes stopped at tumor boundary
► Poor prognosis

Saltz et al. Cell reports, May 2018

Slide-spot derived spatial transcriptomics

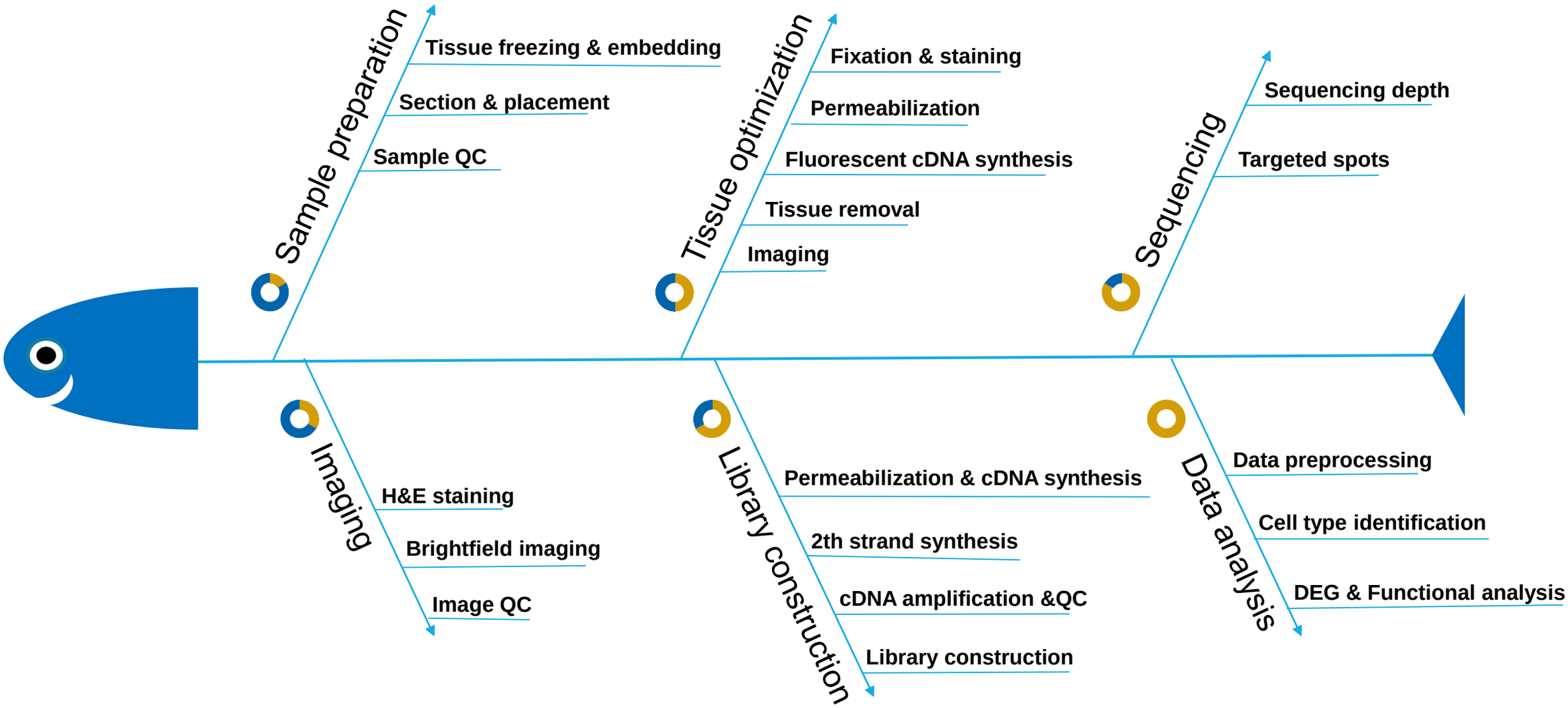
- **Whole transcriptome** in intact tissue sections by capturing and priming of poly-adenylated mRNA

- **High cell resolution**
  - 1-10 cells on average per spot depending on tissue
  - Spot size 55 µm diameter
  - ~5000 spots per capture area



From 10X genomics: Visium Spatial Gene Expression Reagent Kits User Guide • Rev D

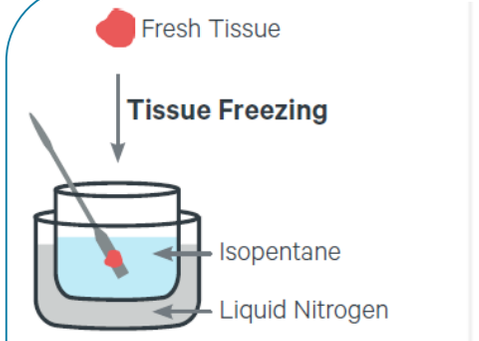
Workflow of spatial transcriptomics



Optimization of sample processing for reliable data

Sample preparation & imaging

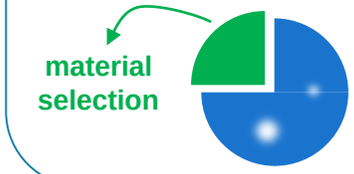
Freezing & Embedding

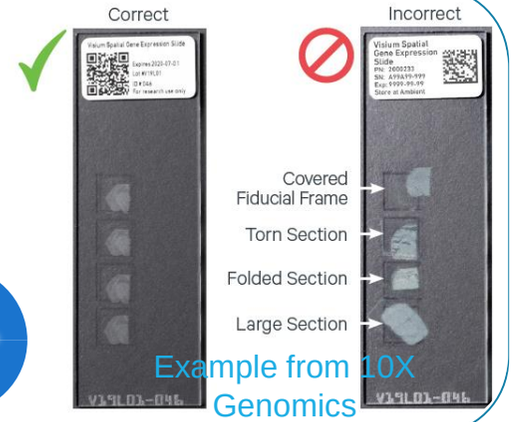


Example from 10X Genomics

Cryosectioning & Section Placement

- A tissue section of ≤6.5 x 6.5 mm
- Recommended section thickness for most tissue types is 10 µm.

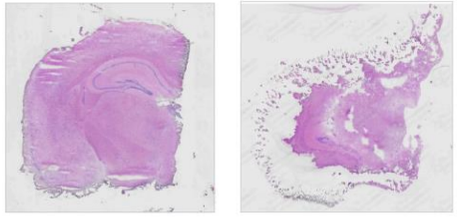




Example from 10X Genomics

Sample QC

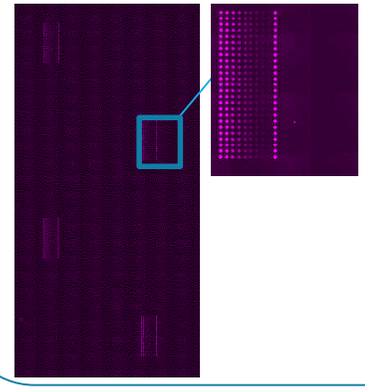
- **RNA quality**: RIN should be ≥ 7 and RNA quality assessment should be done before placing the tissue sections on Visium Spatial slides.
- **Tissue Sections** : pre-viewing by HE

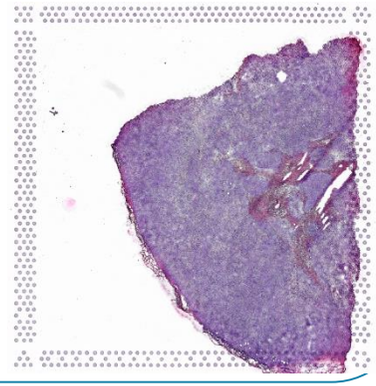


Example from 10X Genomics

Imaging

- Optimize imaging settings
- Brightfield imaging



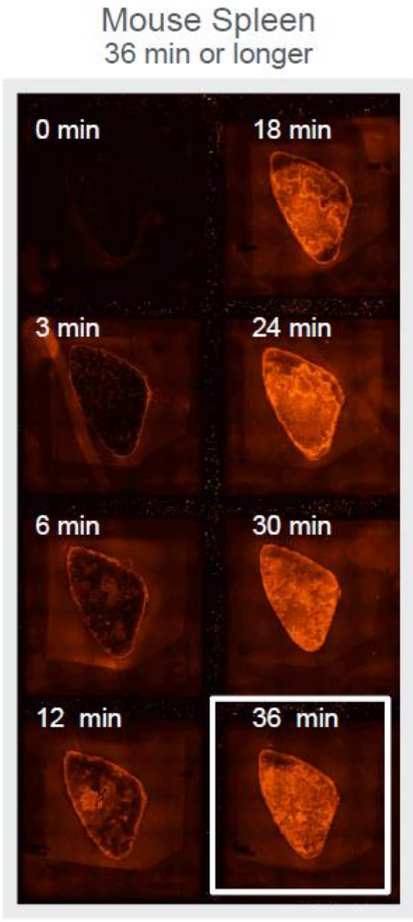


Optimization of sample processing for reliable data

Tissue optimization

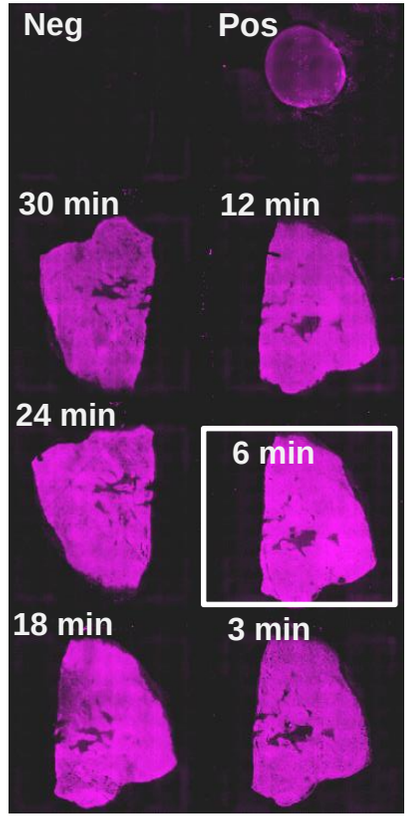
Customizing permeabilization conditions to boost the gene expression signal according to tissue type

Example from 10X Genomics



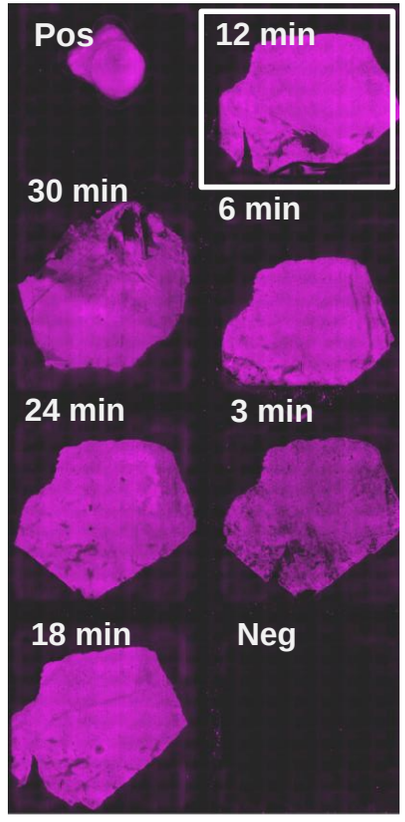
Tumor 1

6 min



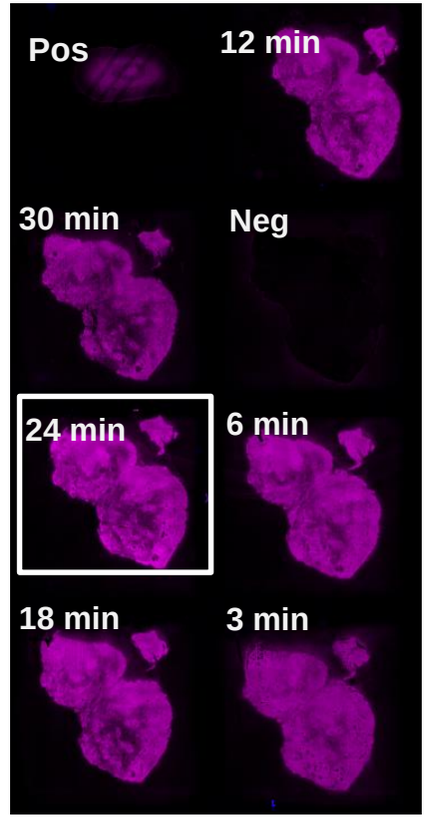
Tumor 2

12 min



Tumor 3

24 min



Optimization of sample processing for reliable data

cDNA library and sequencing

- Spatial transcriptomics library construction

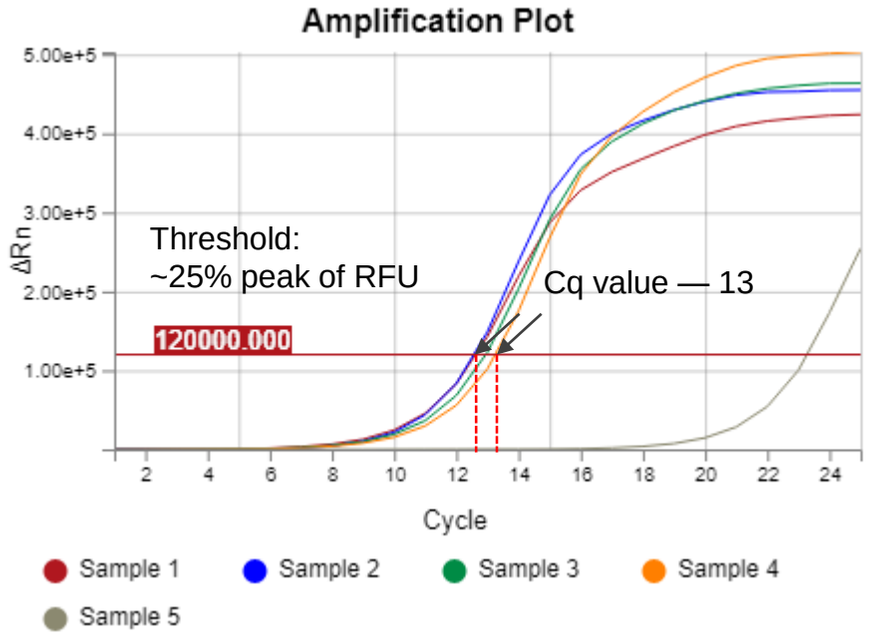
Step1: cDNA synthesis

Step2: 2th strand synthesis

Step3: cDNA amplification &QC

Step4: Library construction

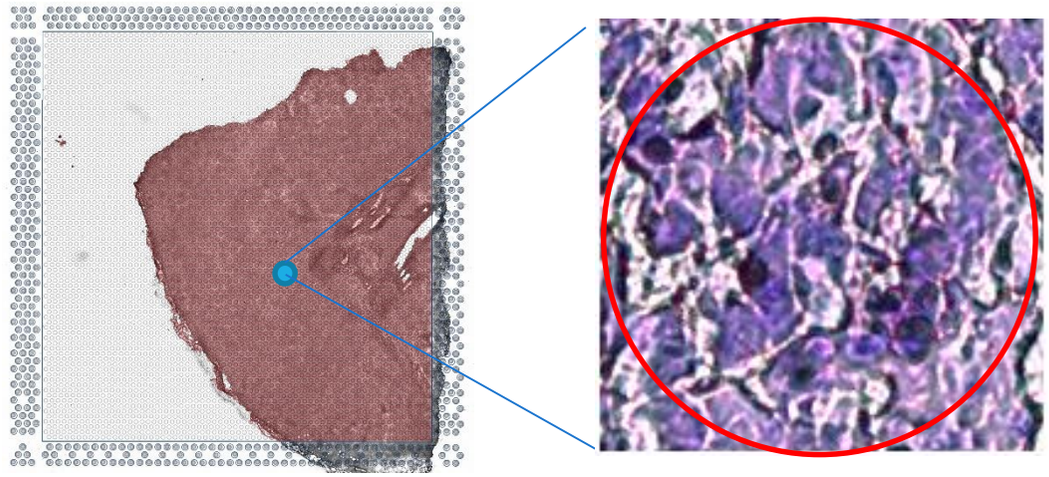
- cDNA is amplified based upon cycle number determined by qPCR
- Set threshold at 25% into the exponential phase to determine C(q) value



- Sequencing depth

Detected spots

Cell numbers/spot



Sequencing Depth/spot: Minimum 50,000 read pairs per covered spot

Total sequencing depth = covered spots × 50,000 read pairs/spot

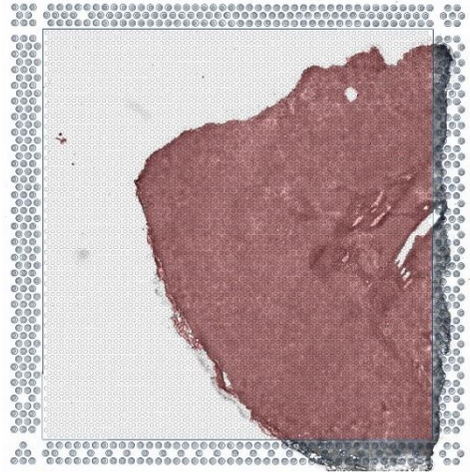
or estimated coverage area (%) ×5,000 (total spots)

Data analysis

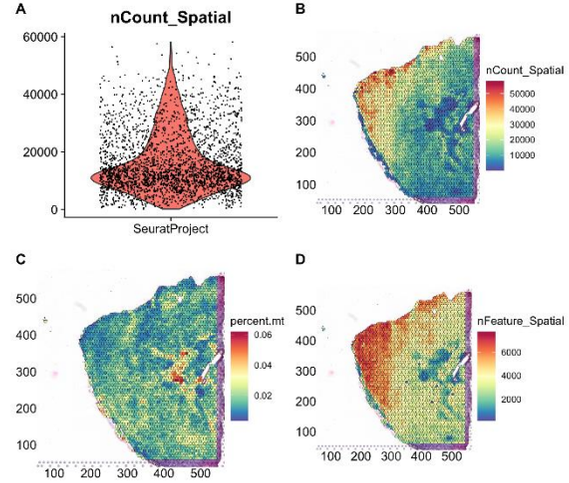
Data preprocessing

In data pre-processing, multiple QC metrics to ensure high-quality gene-spot data.

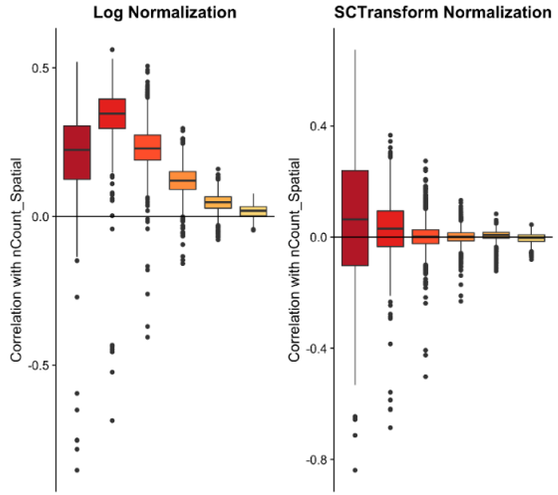
Aligned fiducials



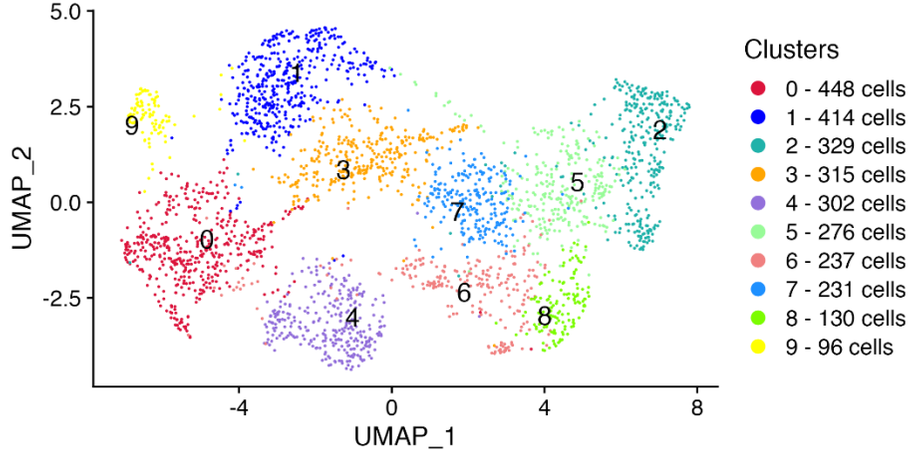
QC metrics



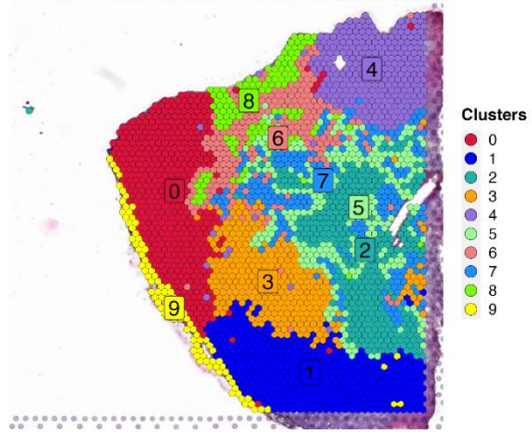
Normalization methods comparison



Dimensionality reduction & clustering



Assigning clusters to spatial

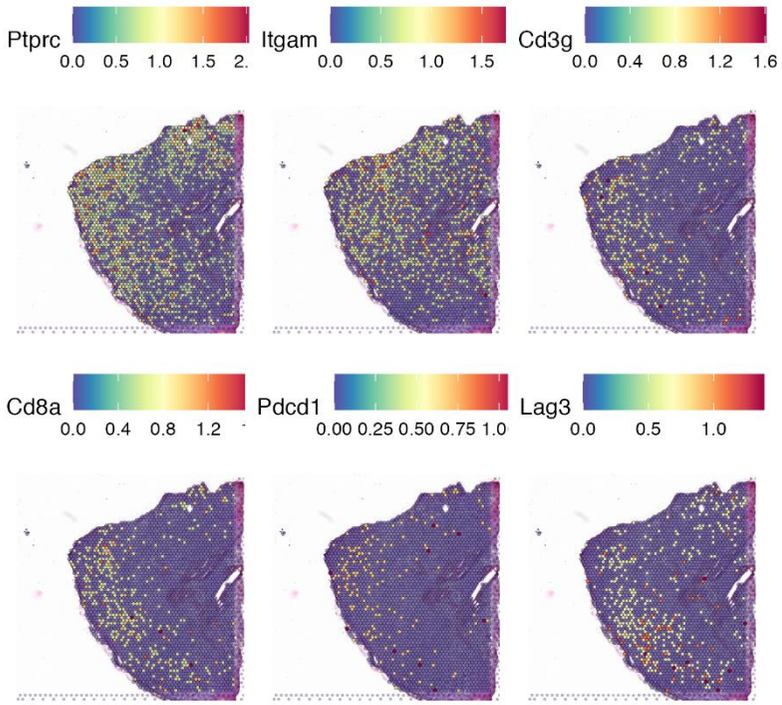


Data analysis

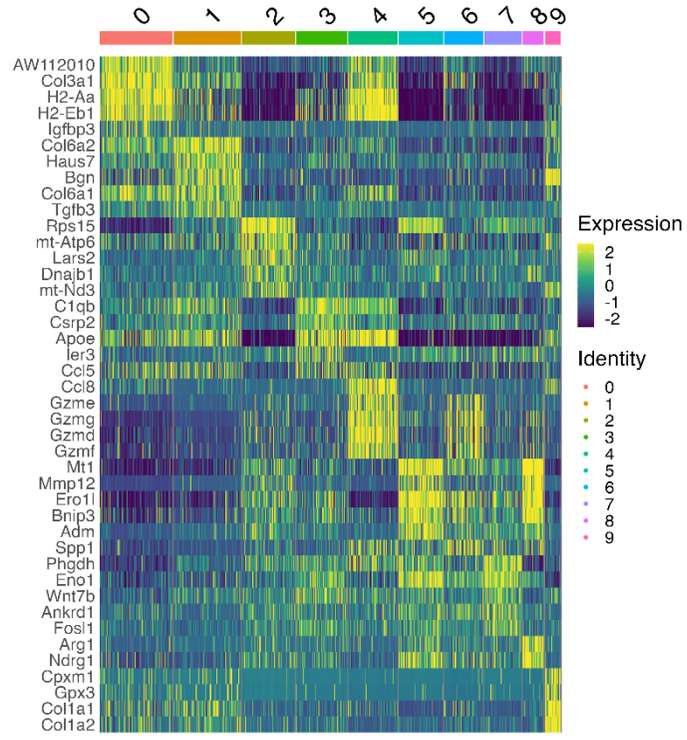
Cell components architecture

- Cell components analysis cross-validate by multiple approaches.

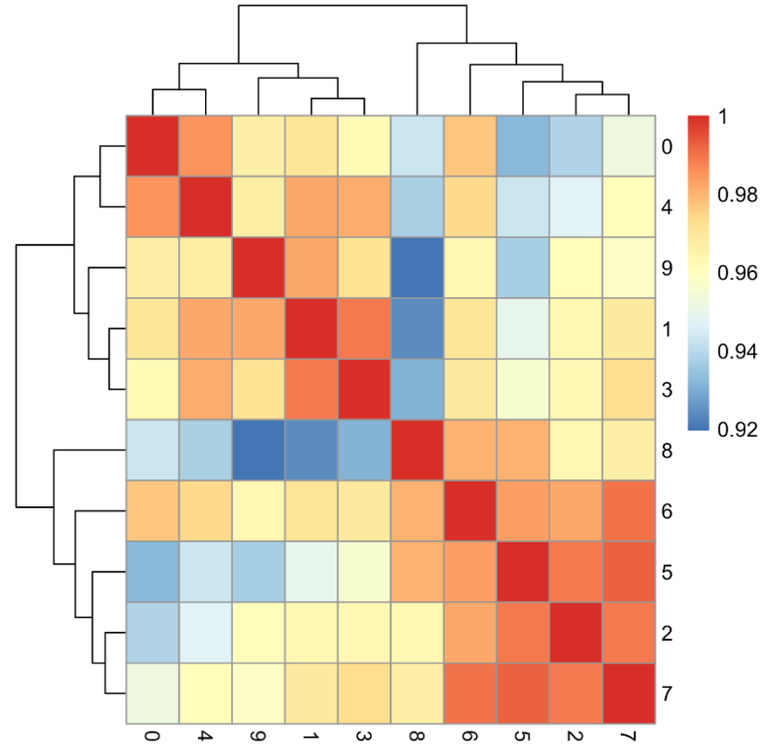
Visualization of canonical markers



Top5 differential expressed genes in each cluster



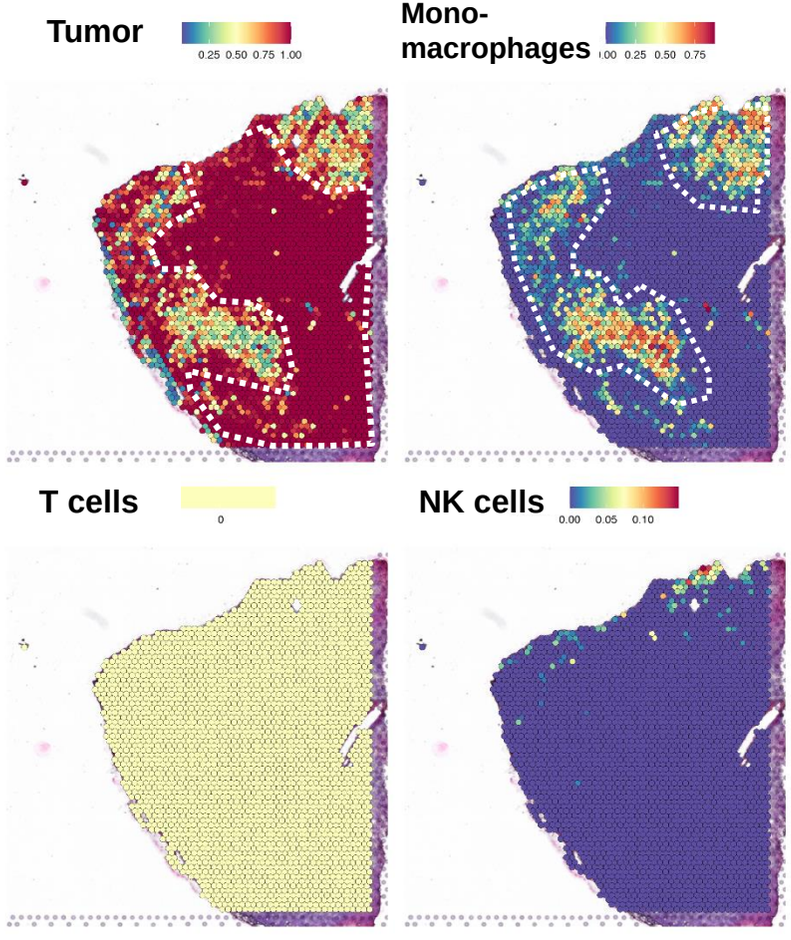
Pearson correlation among clusters



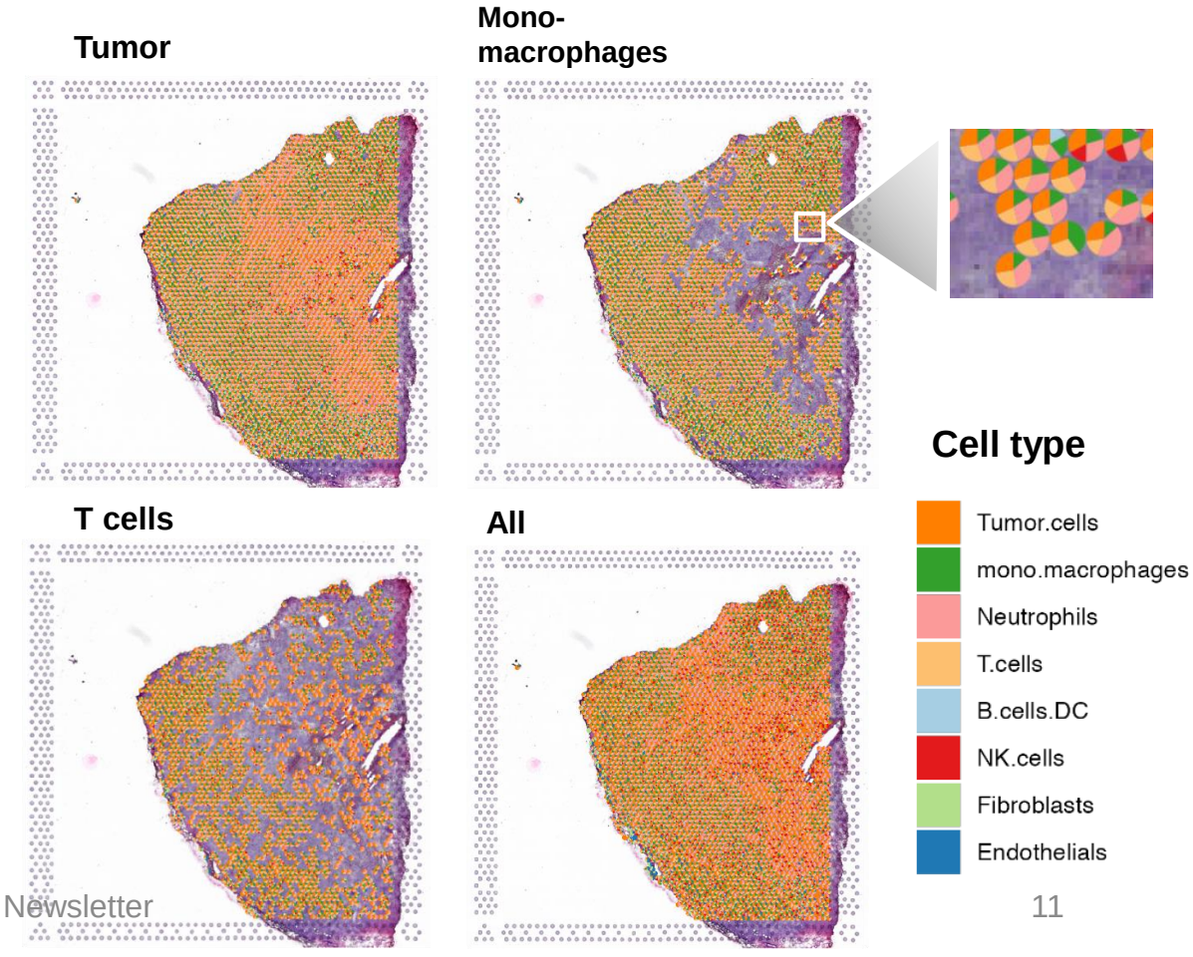
Data analysis

Cell components architecture

- Cell components identification by integrating spatial transcriptomics and scRNA-Seq data.



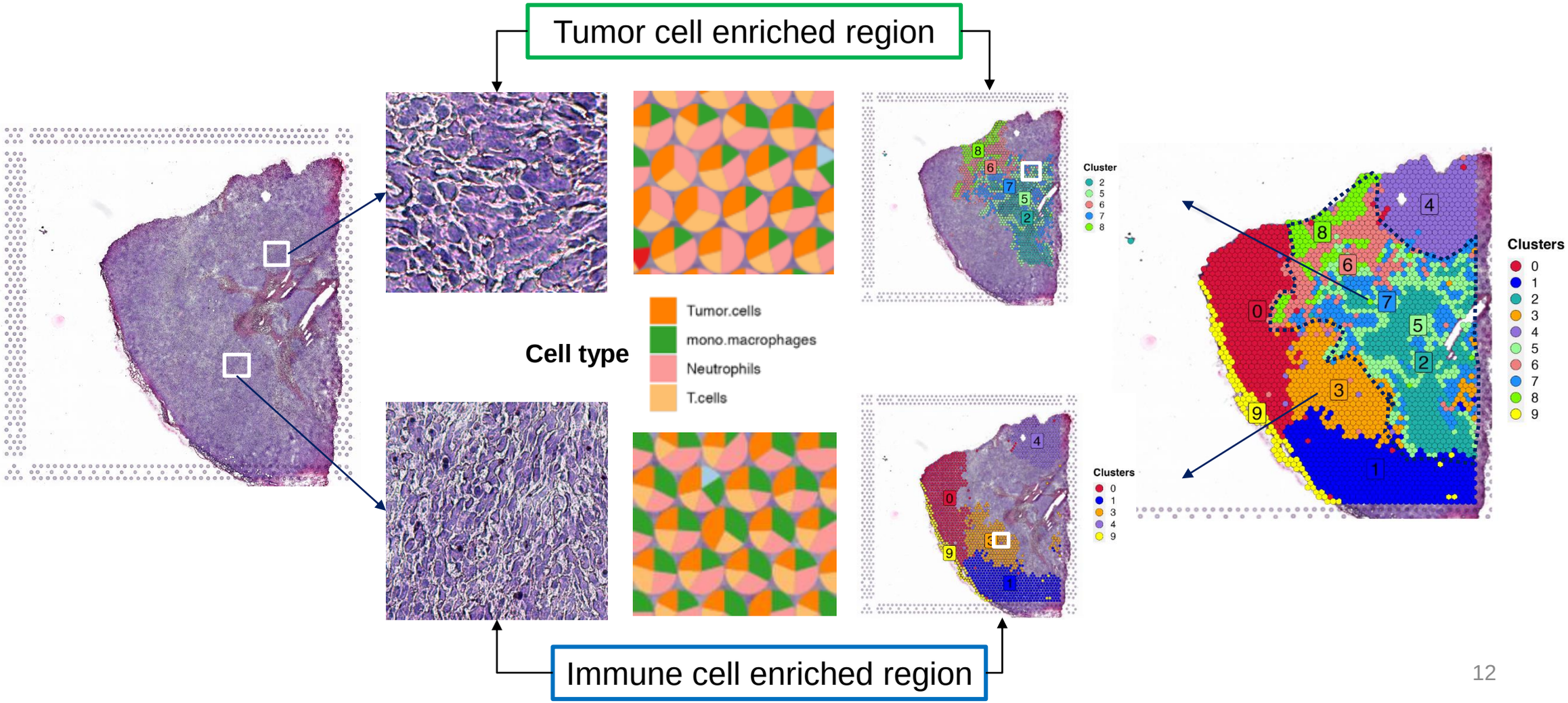
- Cell component identification by deconvolution method based on an NMFreg model.



Data analysis

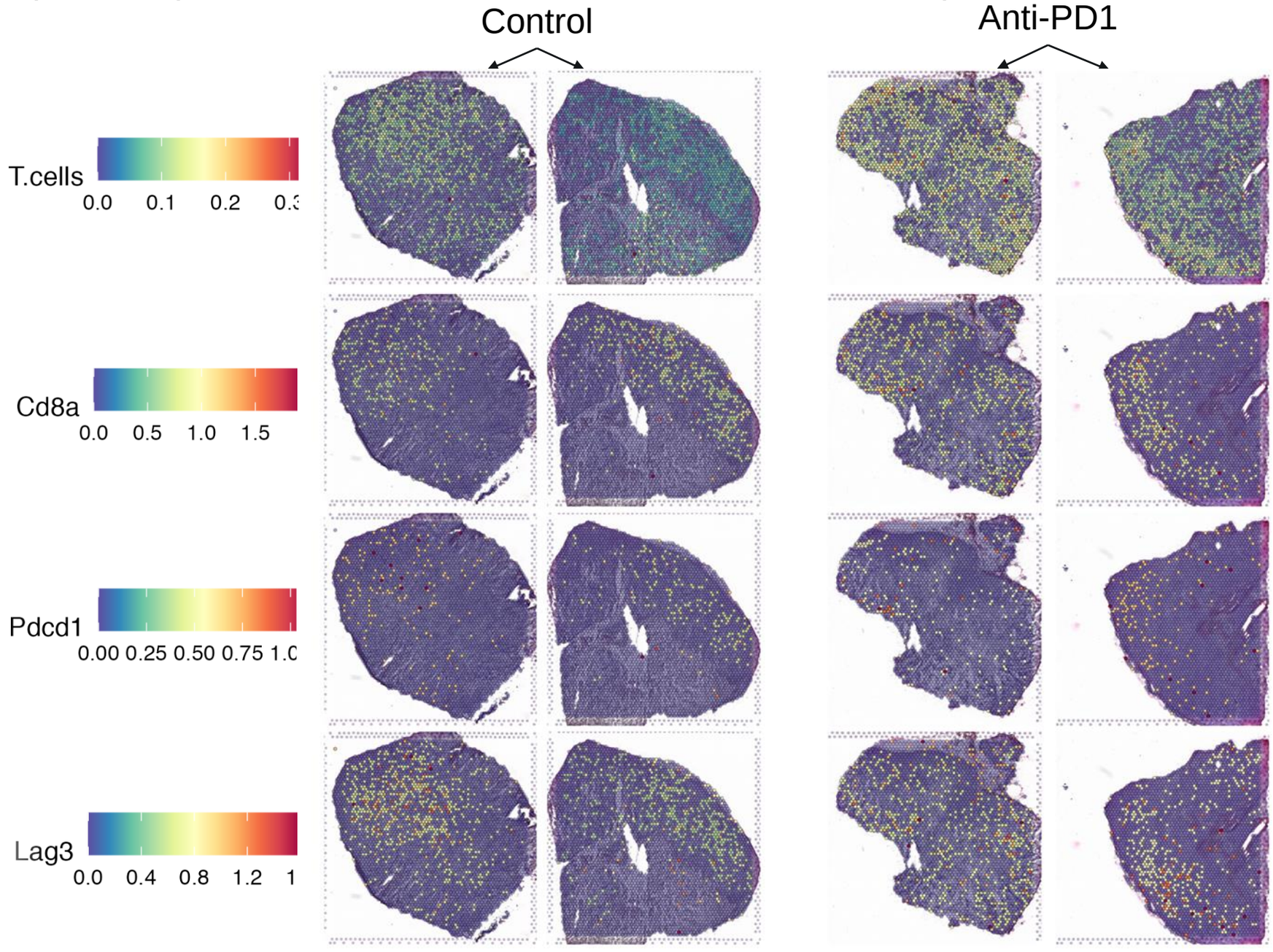
Cell components architecture

- Cell components analysis shows consistent results between pathological morphology analysis and by spatial transcriptomics.



Case Study

Gene expression profiles modification after anti-PD-1 treatment in spatial scale



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