Spatial transcriptomics 1

- Why spatial transcriptomics?

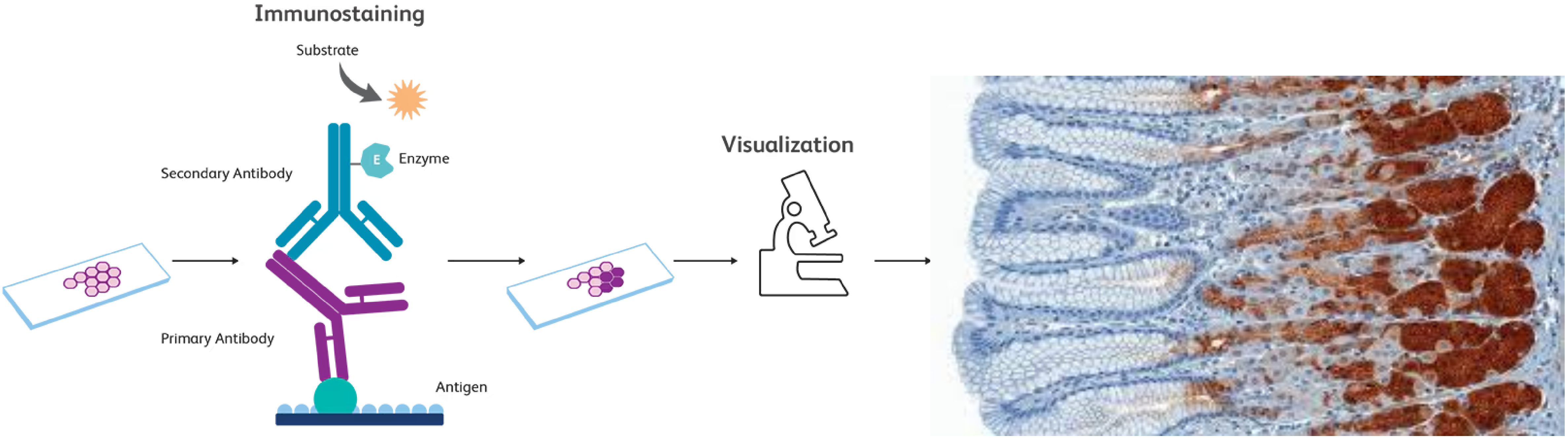
Bulk

Single Cell

Spatial

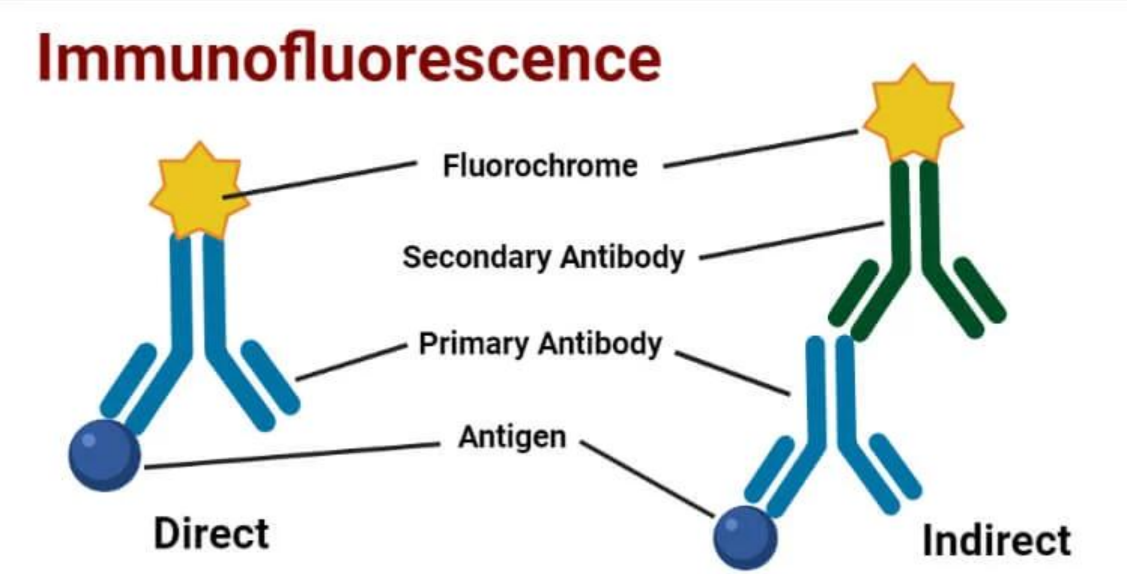
• Previous method

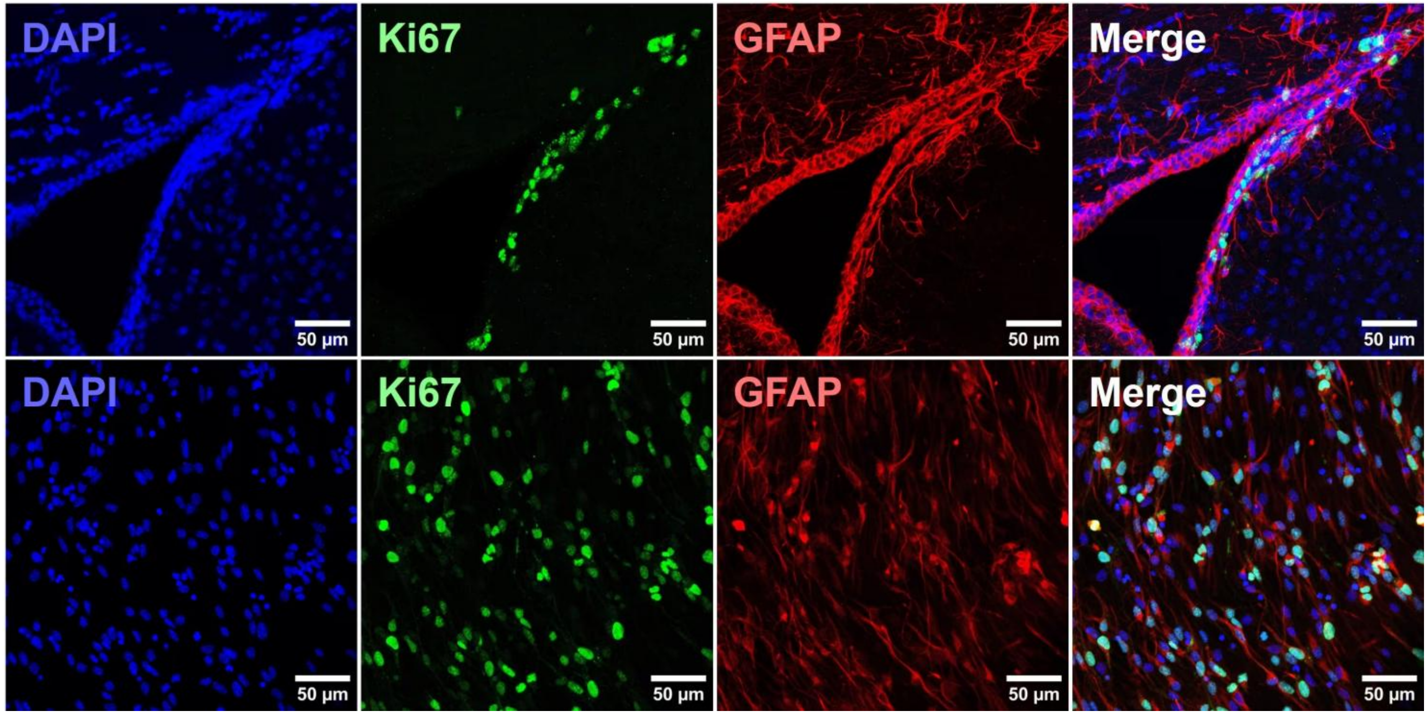
-Immunohistochemisty



- Previous method

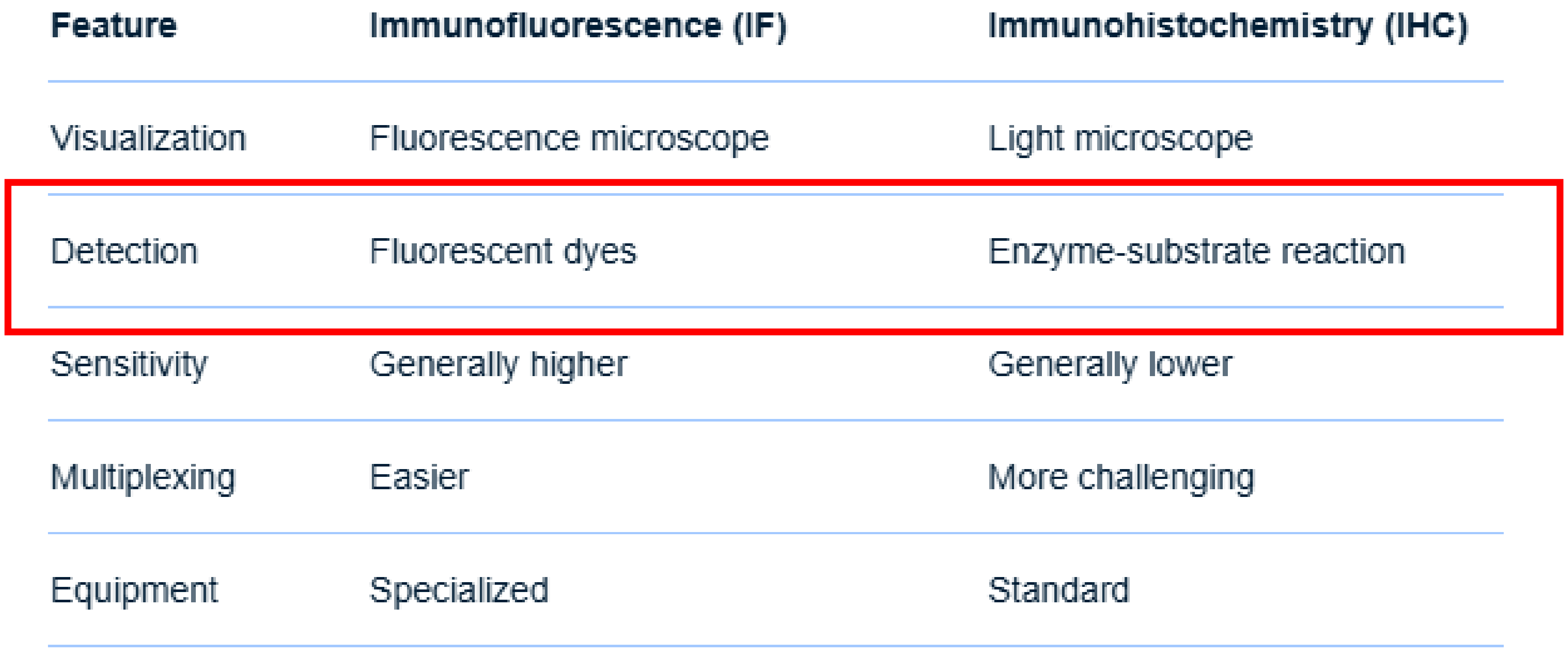
-ImmunoFluoresence





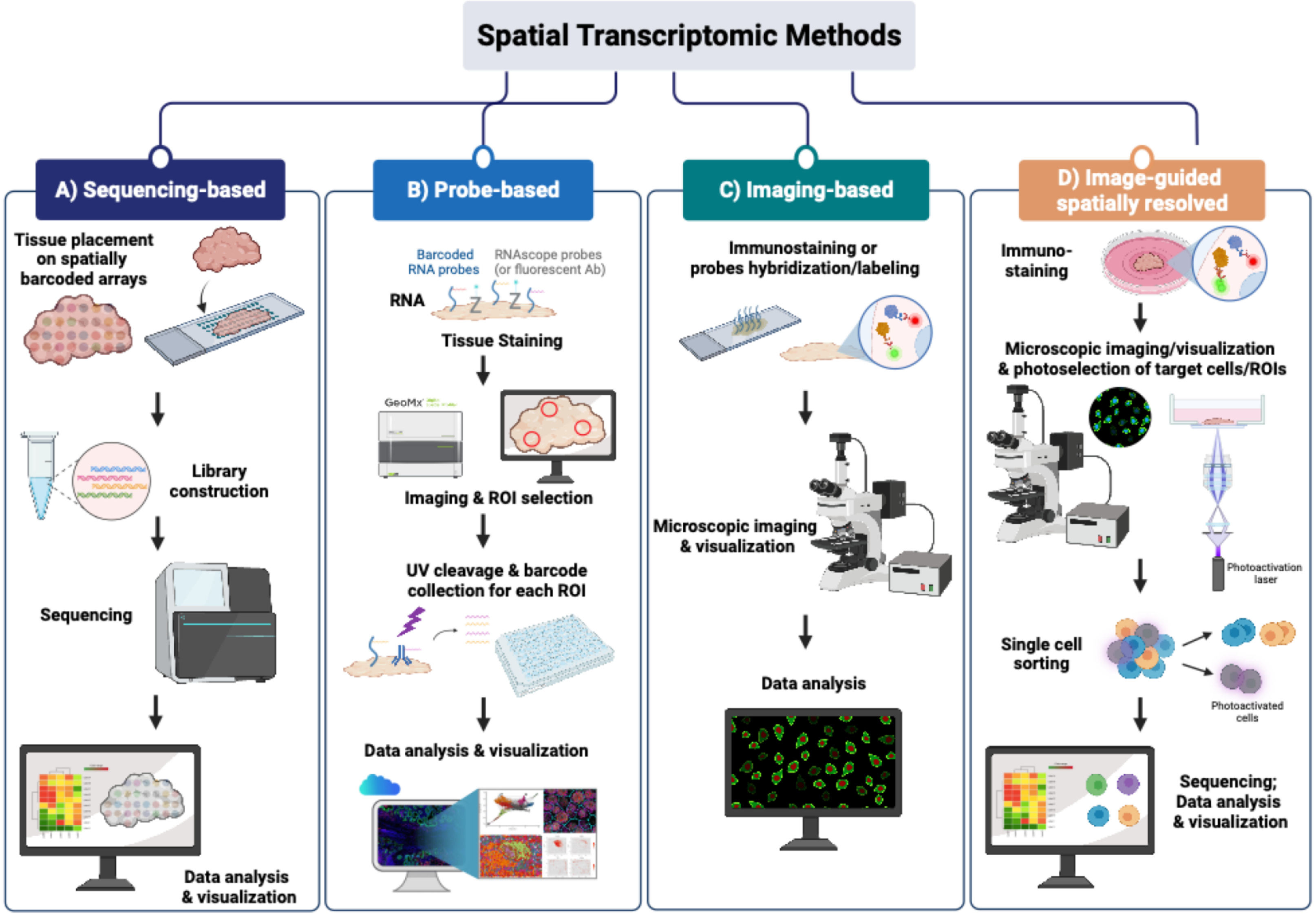
- Previous method

-IHC vs IF



Feature	**Immunofluorescence (IF)**	**Immunohistochemistry (IHC)**
Visualization	Fluorescence microscope	Light microscope
Detection	Fluorescent dyes	Enzyme-substrate reaction
Sensitivity	Generally higher	Generally lower
Multiplexing	Easier	More challenging
Equipment	Specialized	Standard

- Various techniques



Spatial Transcriptomic Methods

A) Sequencing-based

Tissue placement on spatially barcoded arrays

Library construction

Sequencing

Data analysis & visualization

B) Probe-based

Barcoded RNA probes

RNAscope probes (or fluorescent Ab)

RNA

Tissue Staining

Imaging & ROI selection

UV cleavage & barcode collection for each ROI

Data analysis & visualization

C) Imaging-based

Immunostaining or probes hybridization/labeling

Microscopic imaging & visualization

Data analysis

D) Image-guided spatially resolved

Immuno-staining

Microscopic imaging/visualization & photoselection of target cells/ROIs

Photoactivation laser

Single cell sorting

Photoactivated cells

Sequencing; Data analysis & visualization

E.g. 10X Genomics Visium

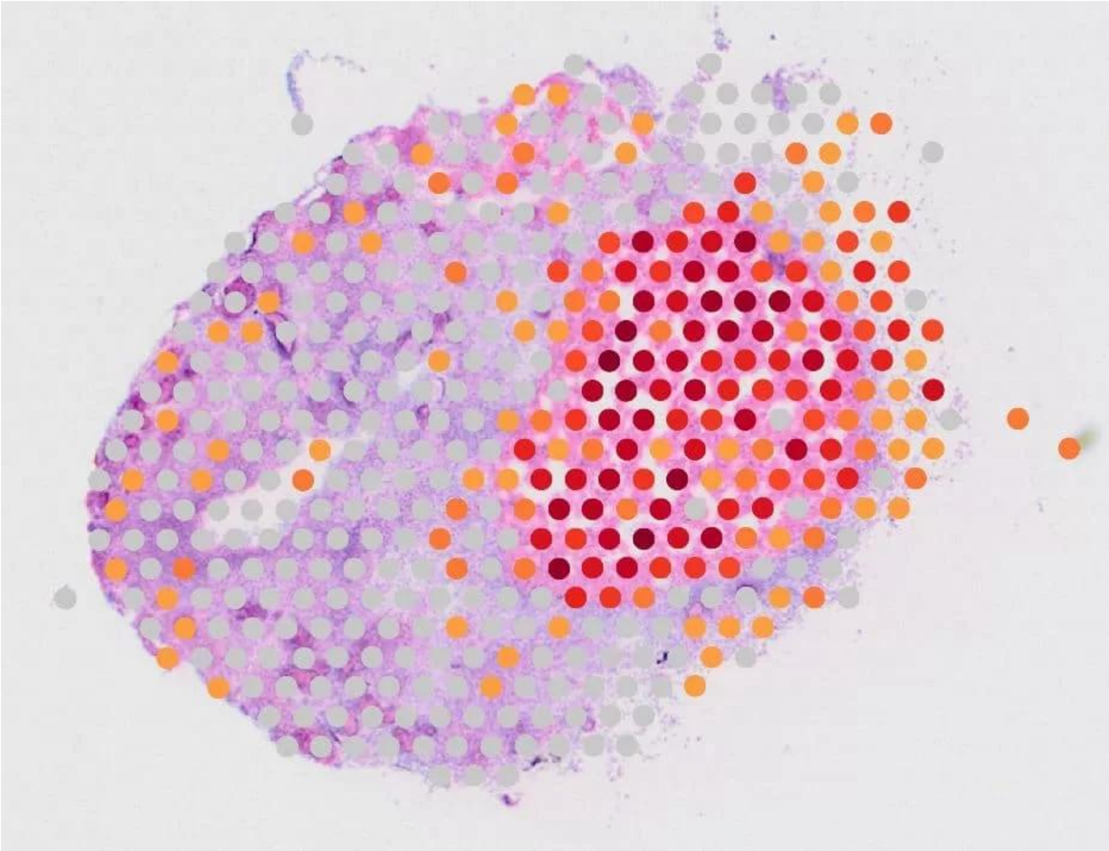
E.g. GeoMx™

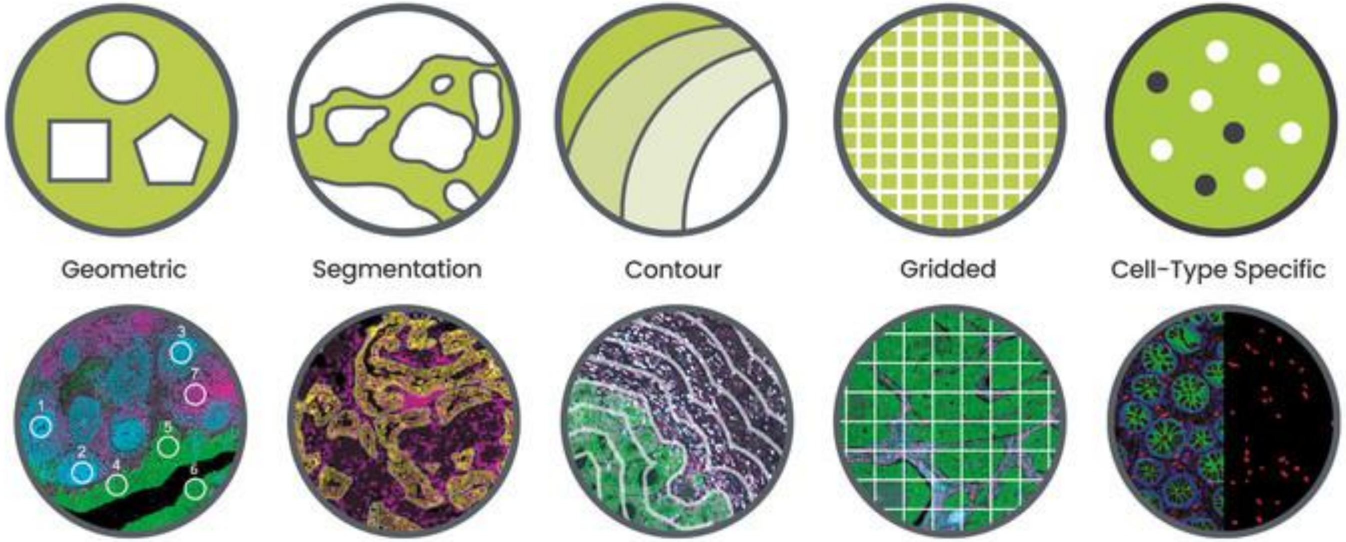
E.g. CosMx SMI

E.g. Spatially annotated FUNseq

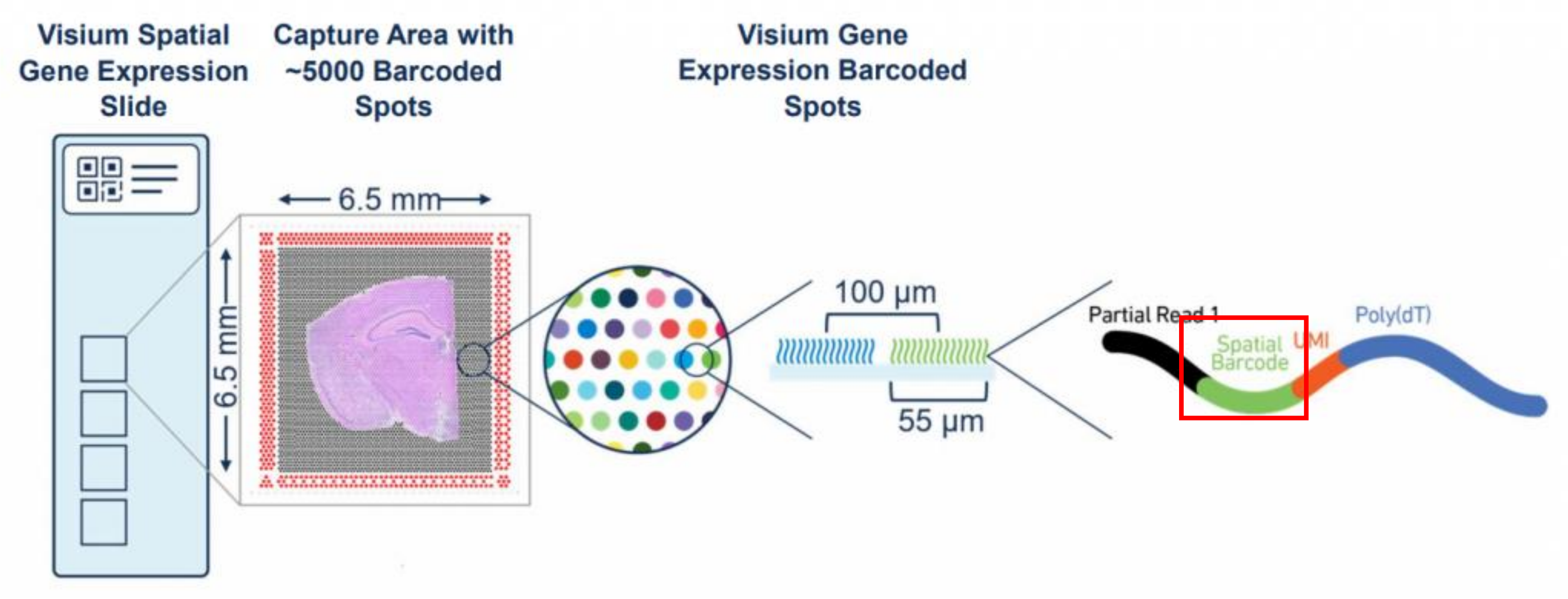
- Spot-based spatial transcriptomics

-Visium (10x), GeoMx (NanoString)



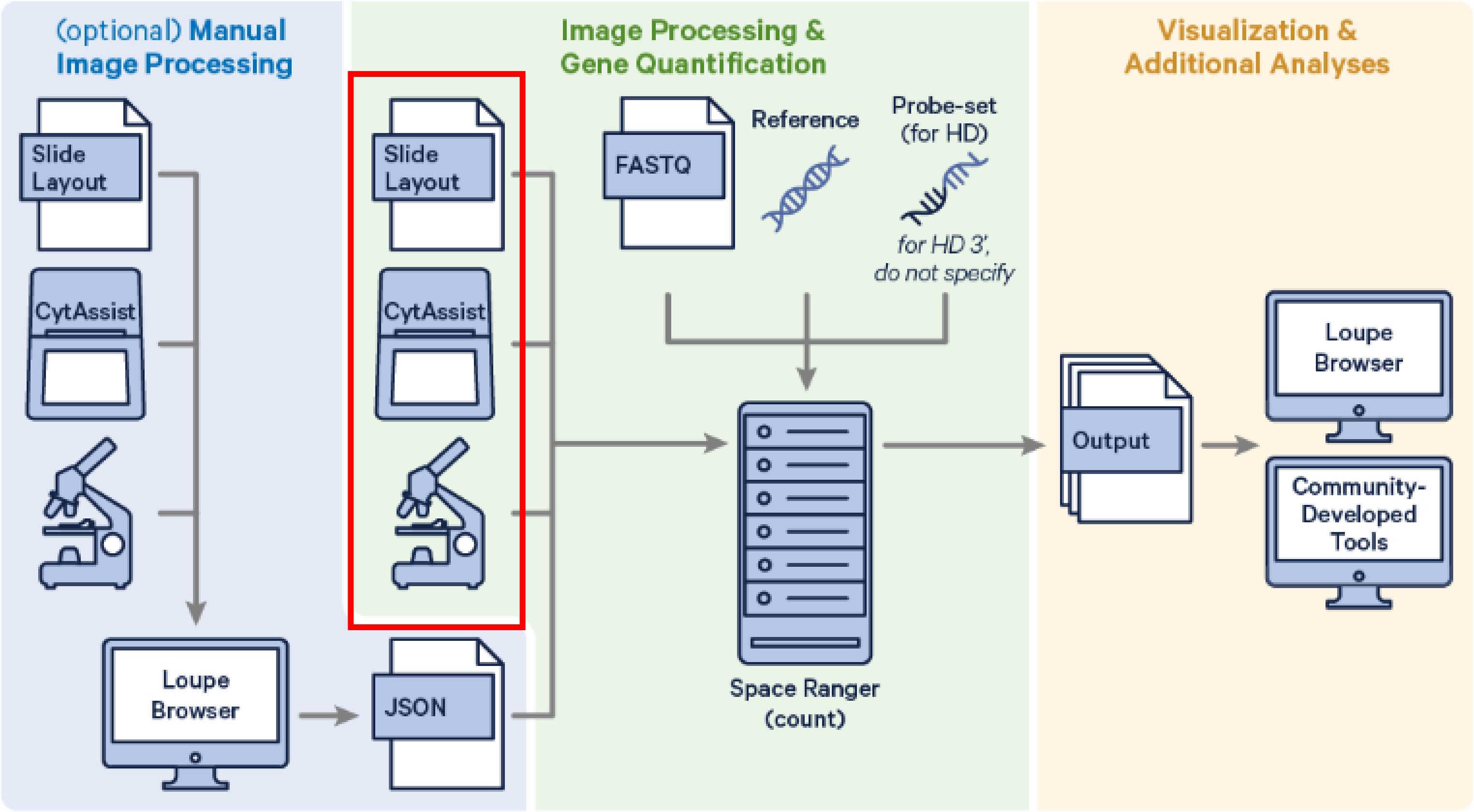


- Visium



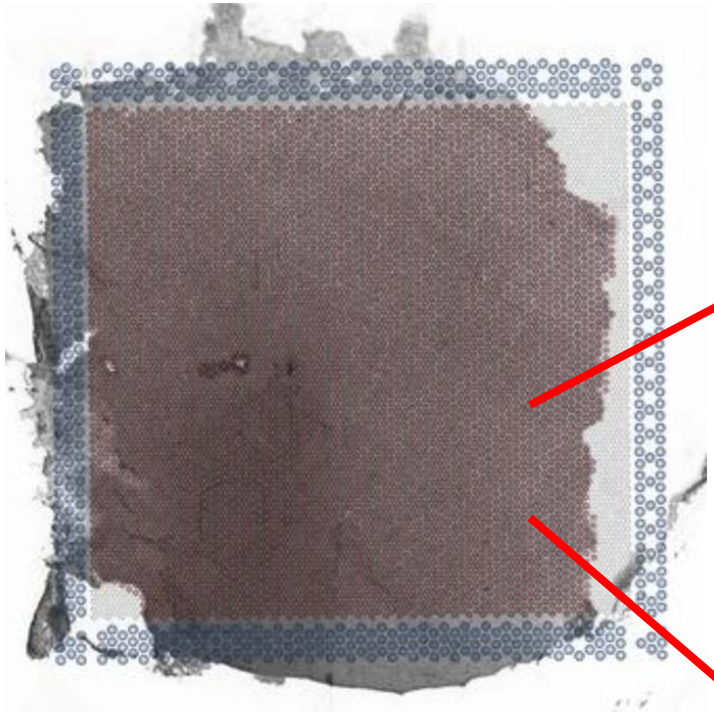
-Spatial barcode on each spot → instead of cell barcode, we could detect each spot (spatial location)
Center – center: 100 um

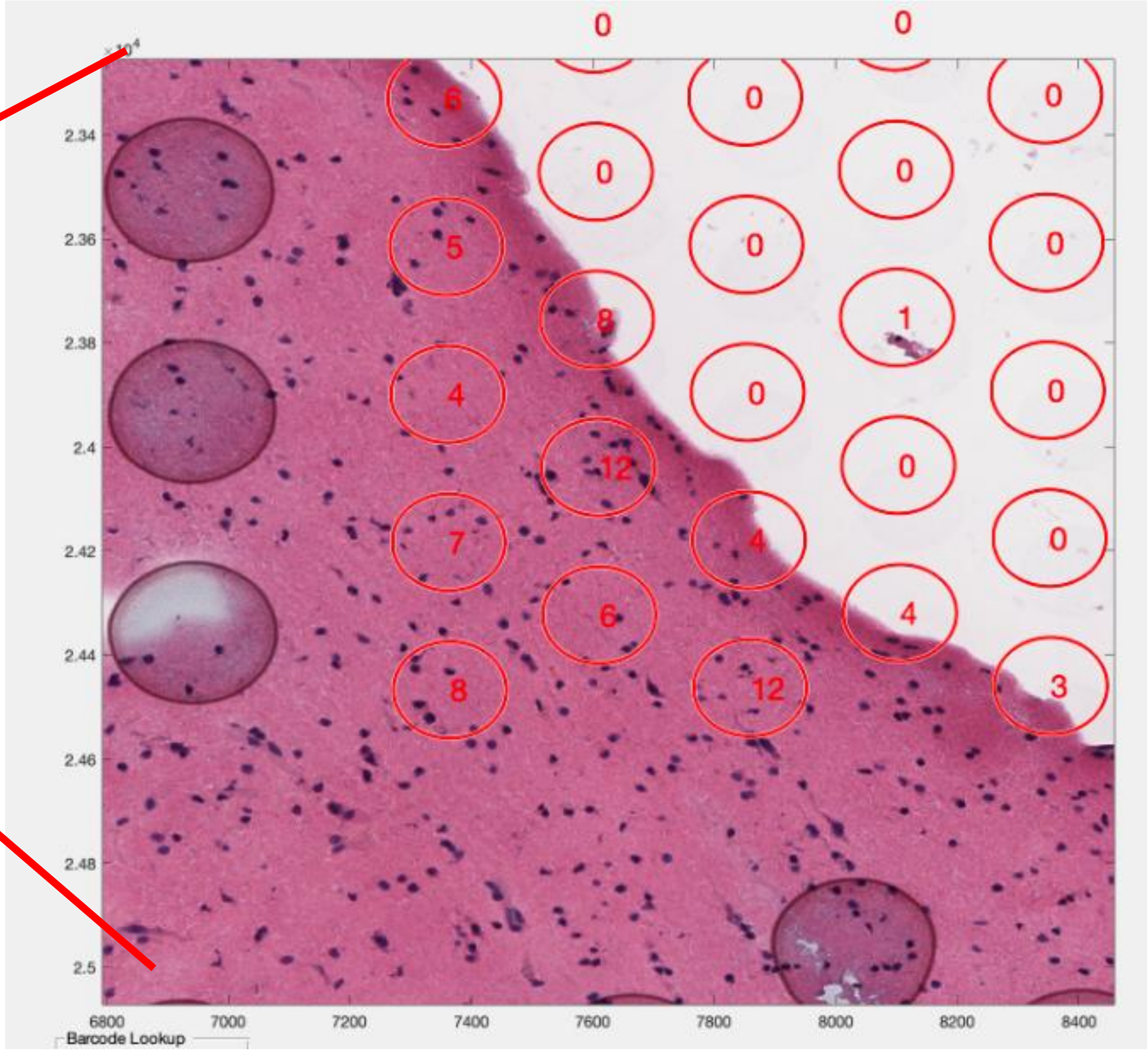
- Visium



-Spatial data require **spatial images** for processing
-spaceranger count
→ Count matrix (same as scRNA-seq) + spatial information

- Visium

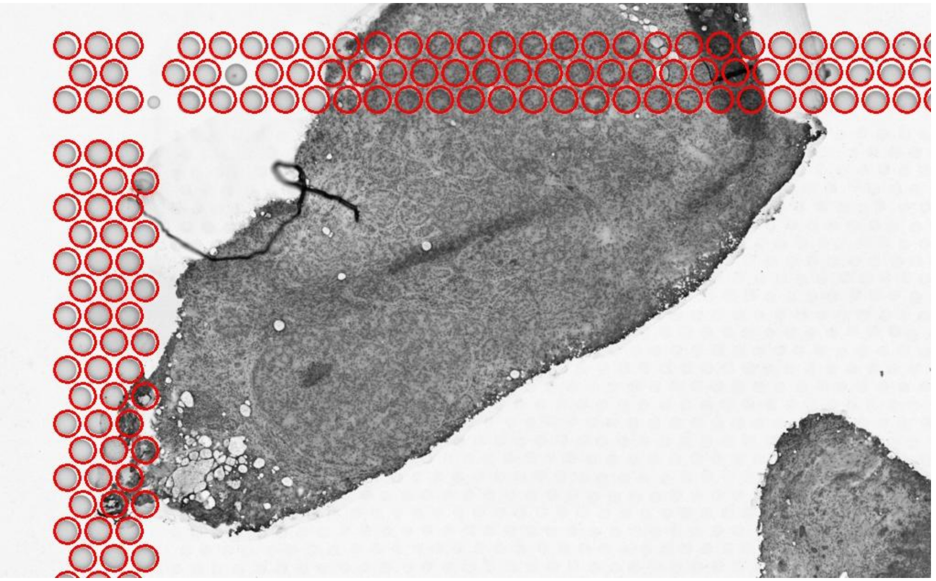




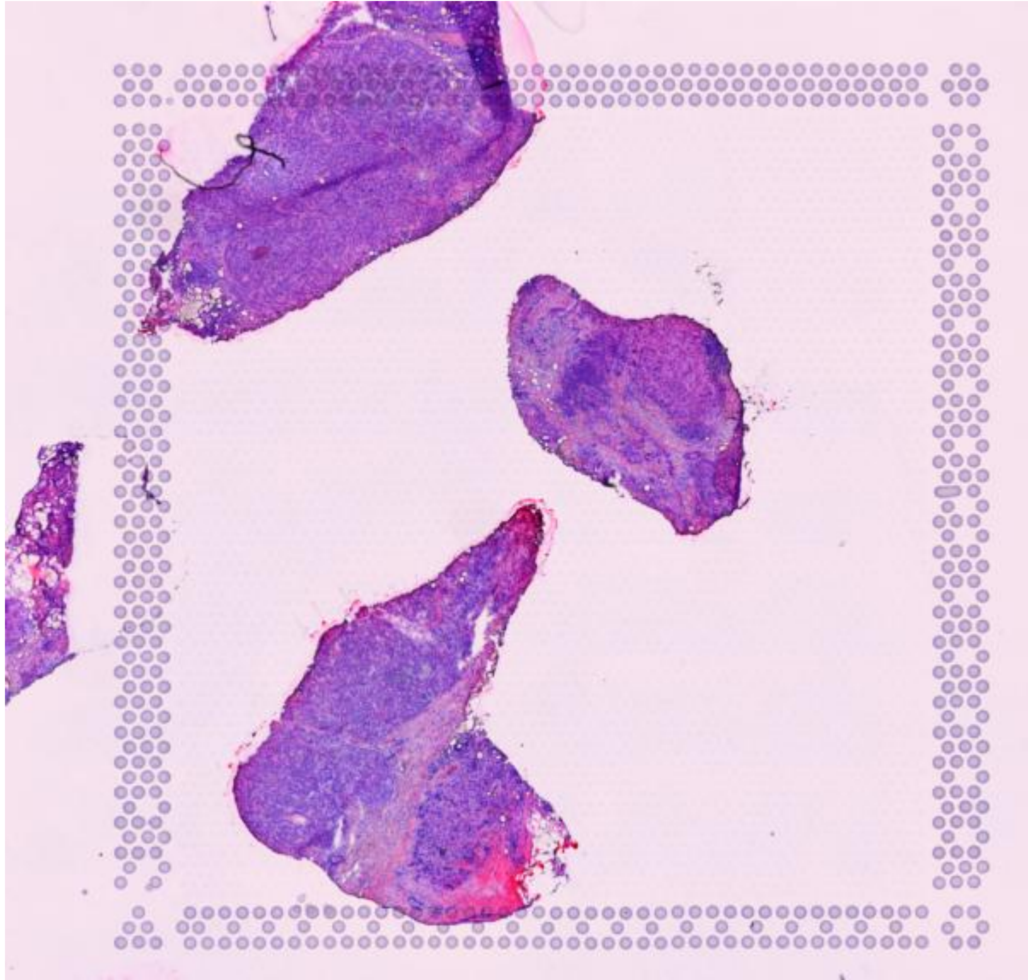
-Depends on the tissue size
-Usually, overlay with H&E results

- Visium

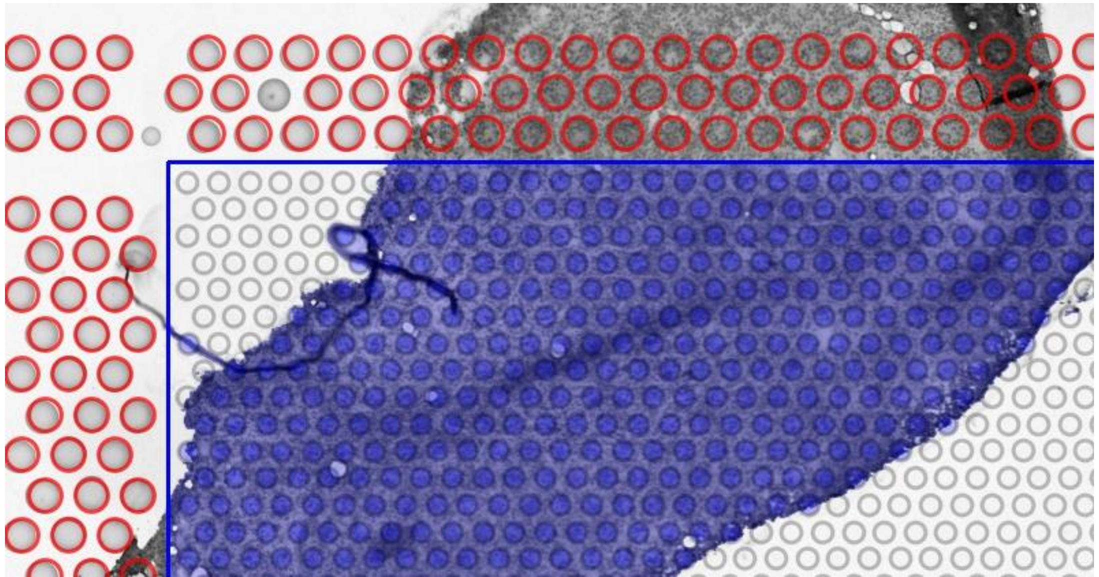
- Aligned_fiducial: bullet point of the imaging focusing → boundary of the sample



- Tissue_image: H&E staining (high or low resolution)

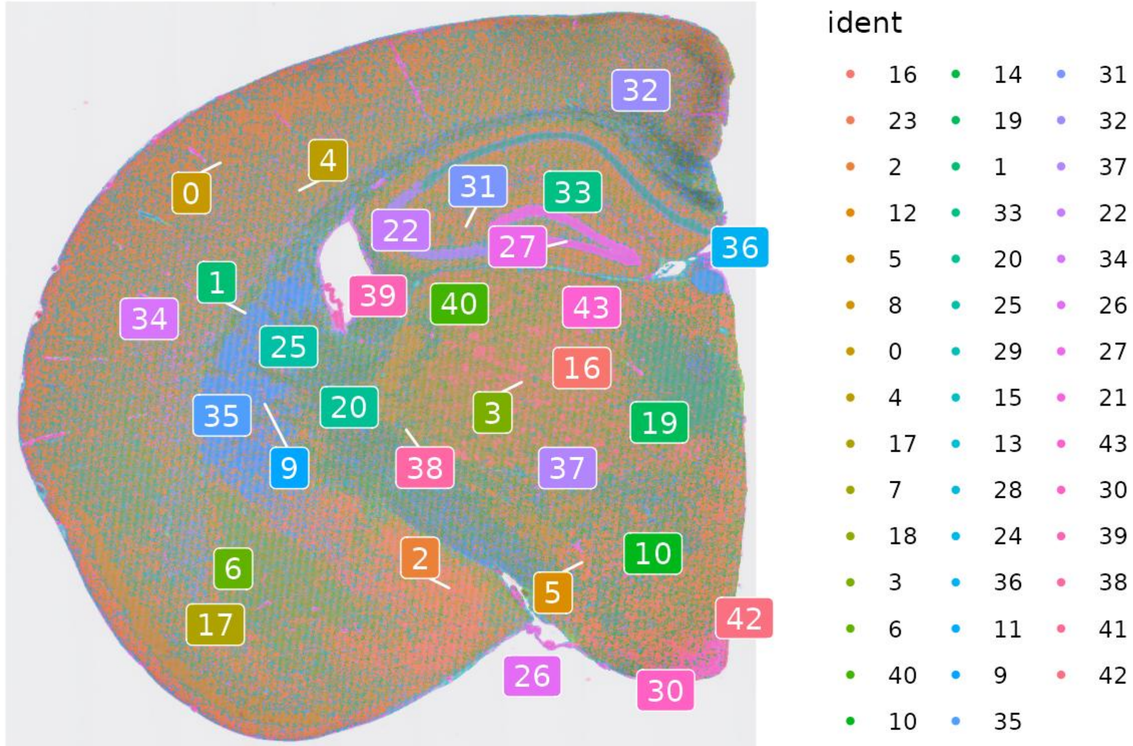


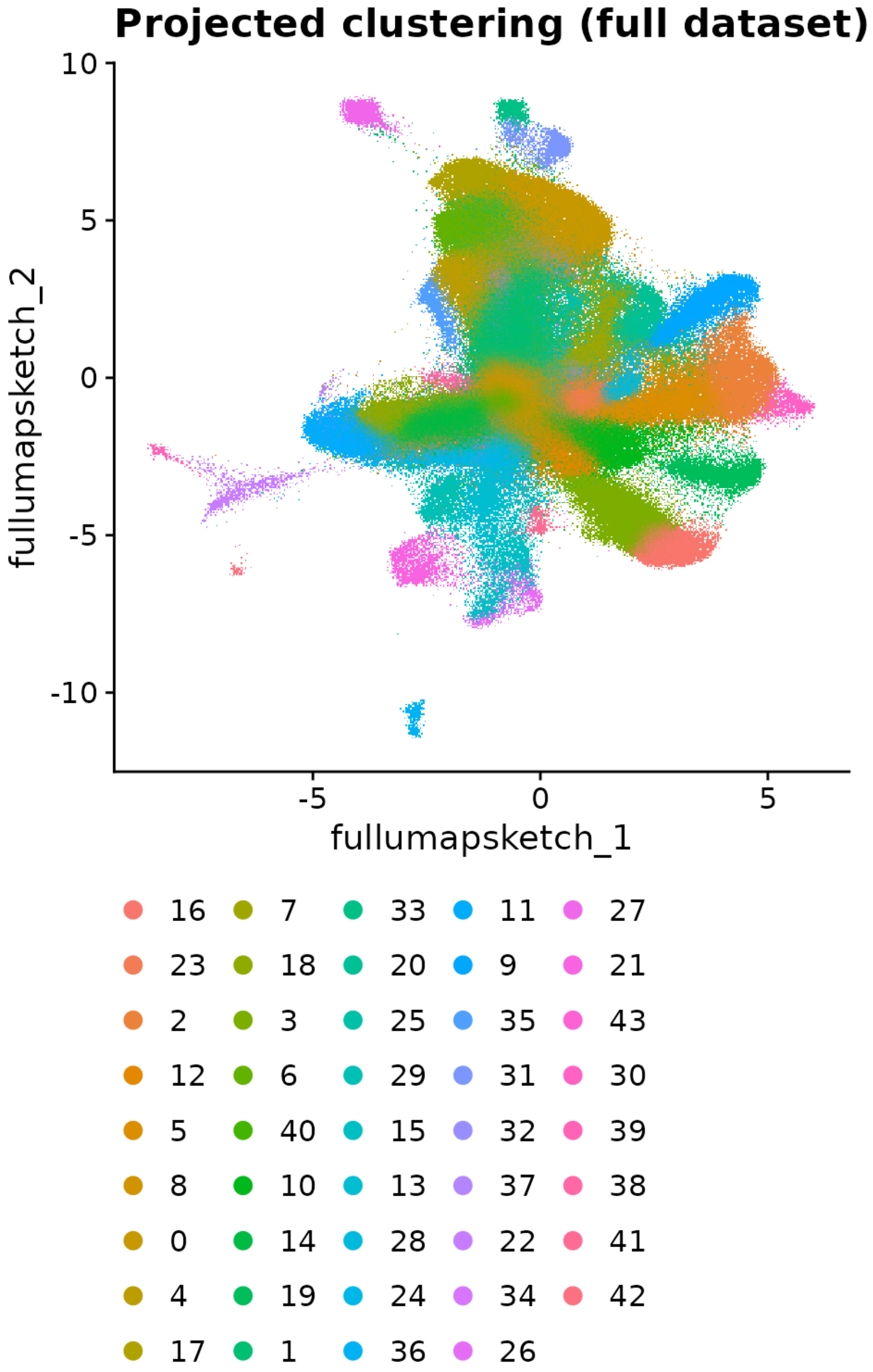
- Detected_tissue_image → detected spots



- # Visium (Data processing)

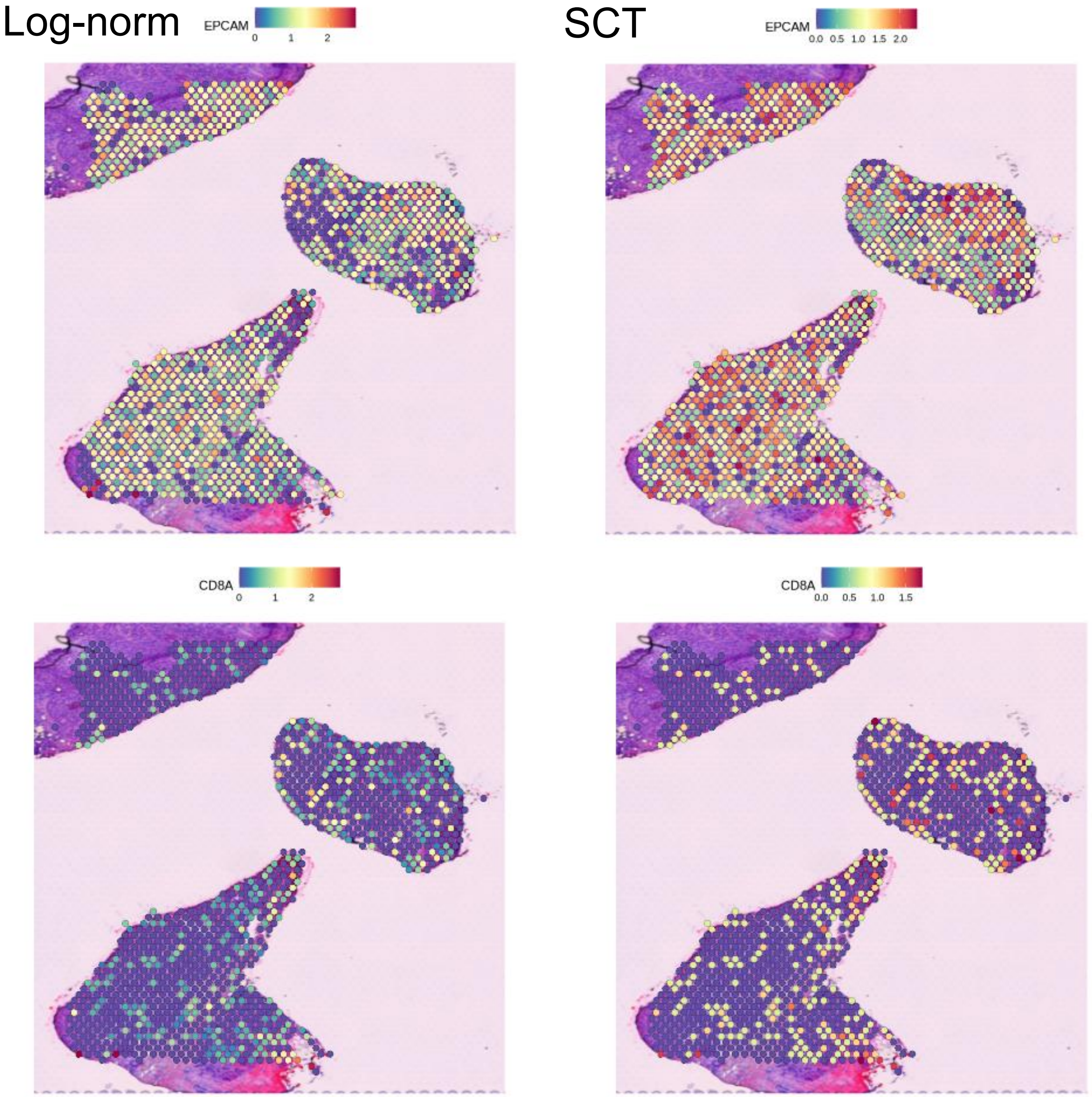
-Normalization (log-norm) or SCTransform
-FindVariableFeatures
-ScaleData
-PCA, neighbor graph, clustering, UMAP …





- Visium (Data processing)

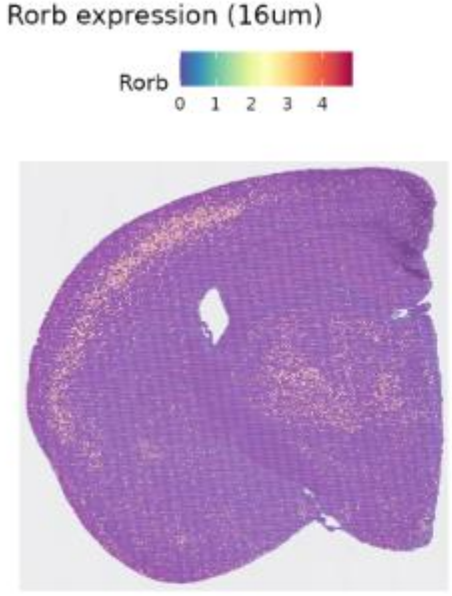
Log norm vs SCTransform
→ SCTransform make low signal → high (higher sensitivity)

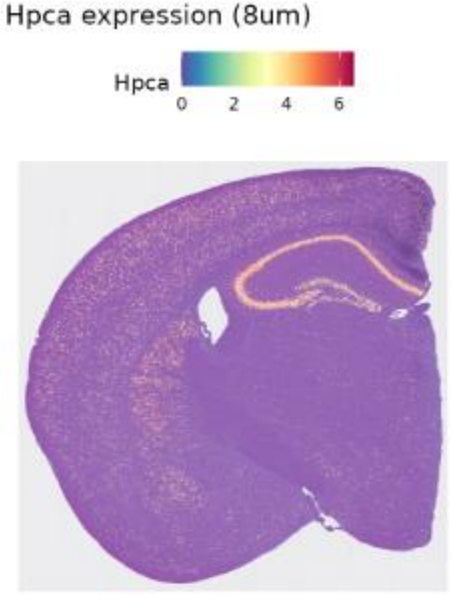


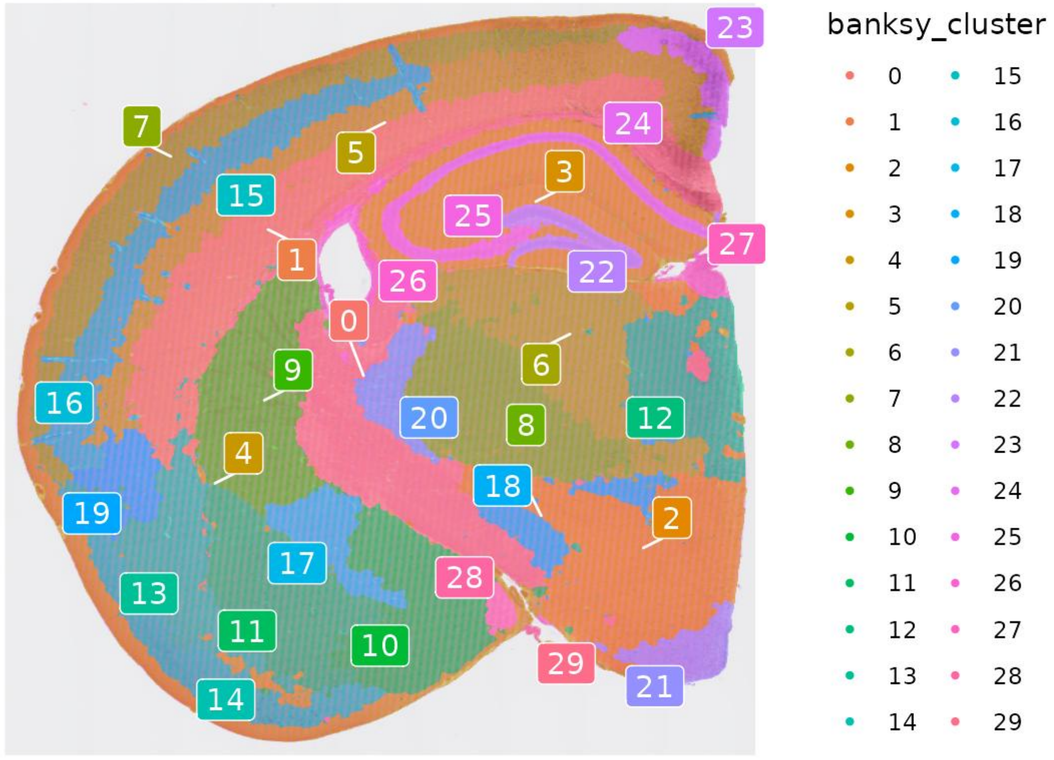
- Visium (Data processing)

-SpatialFeaturePlot

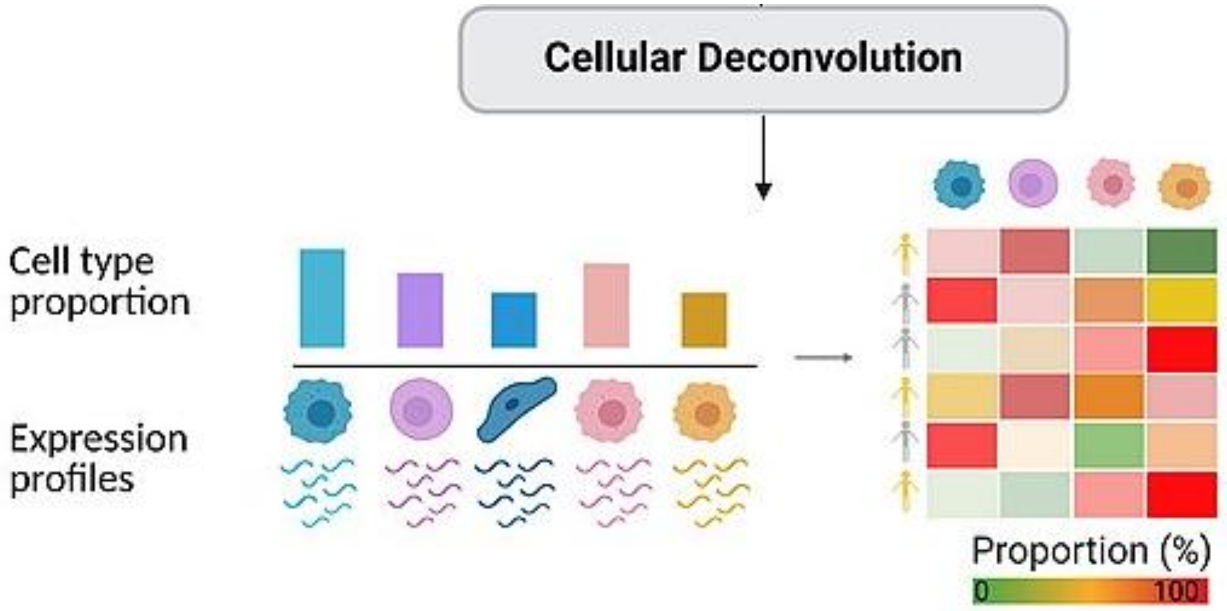
-Spatialcluster


Rorb expression (16um)


Hpca expression (8um)



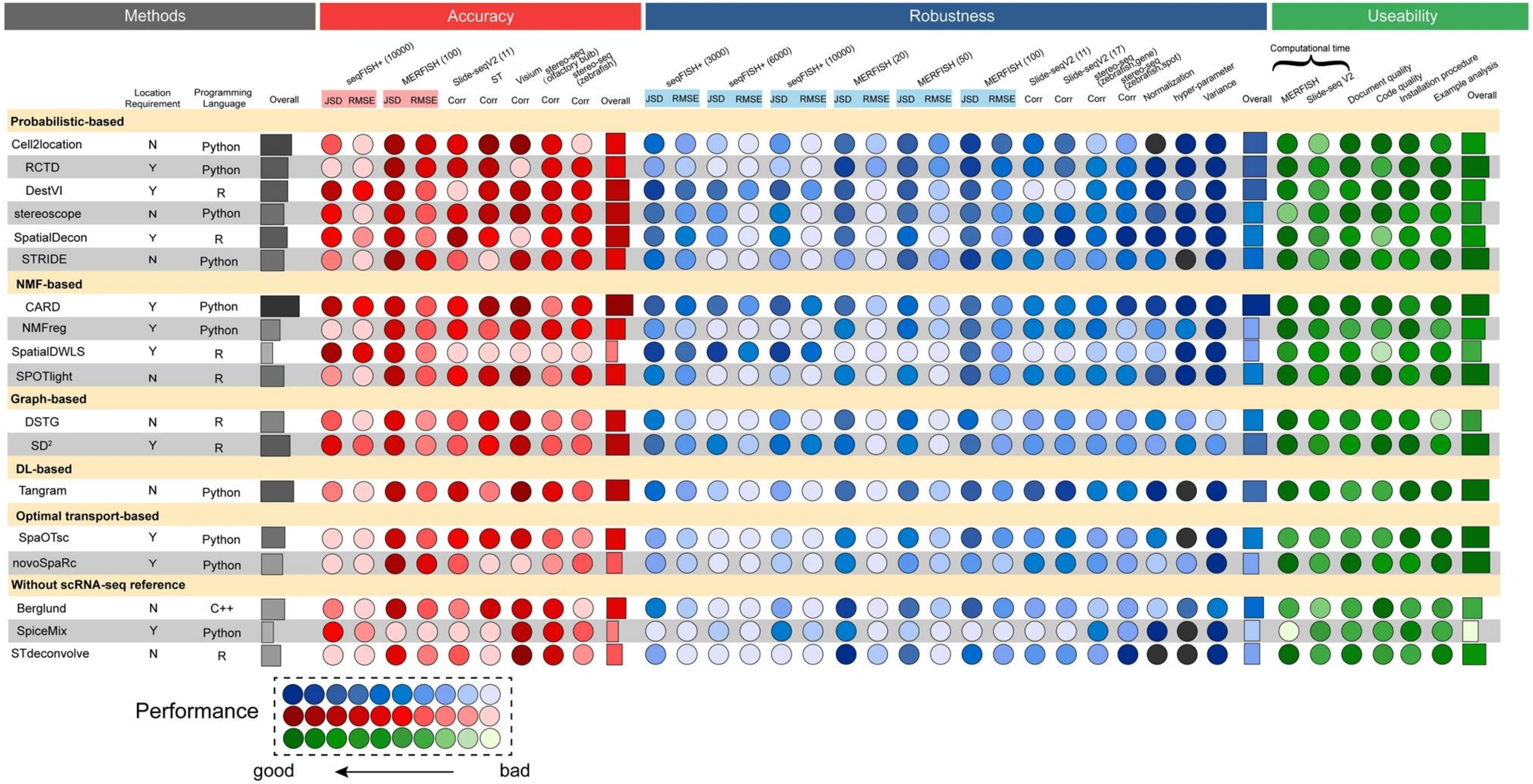
- Visium (Deconvolution)



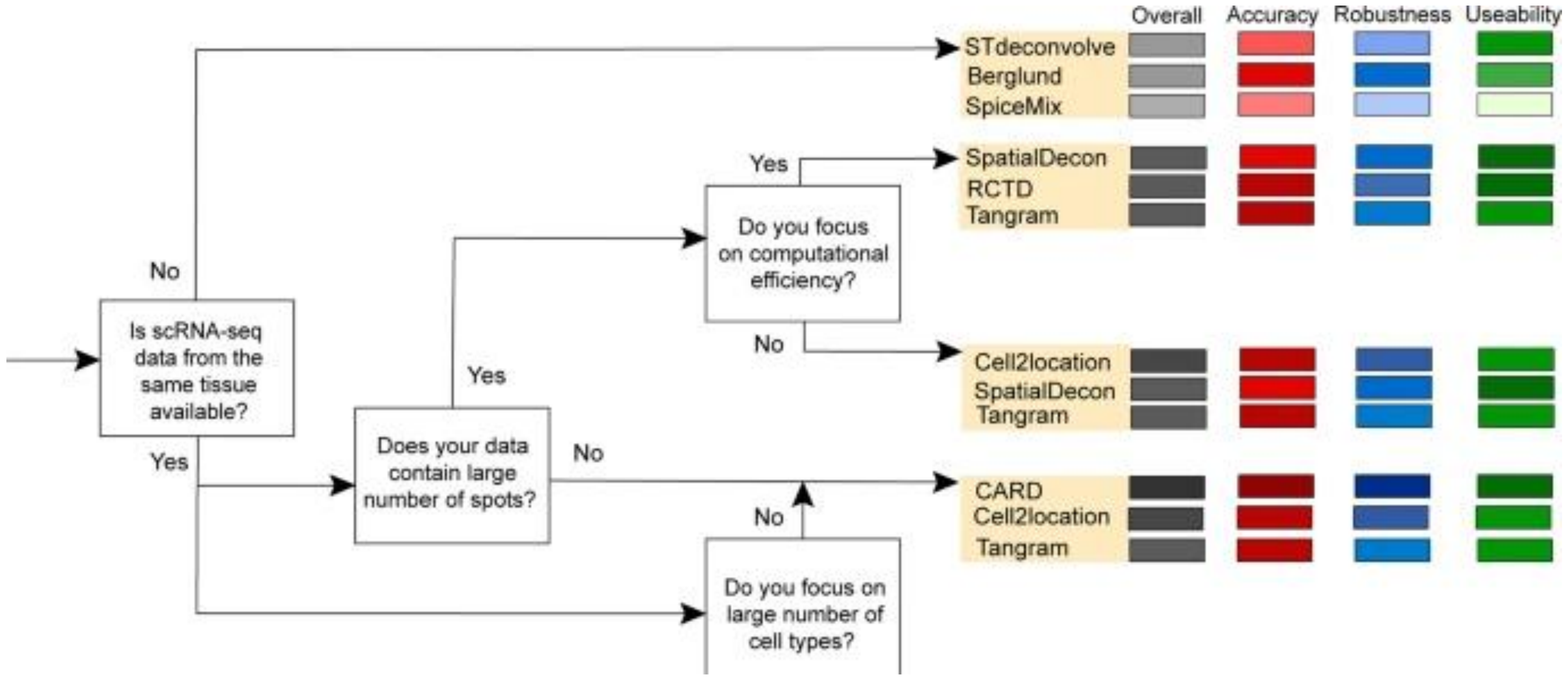
Spatial data for each spot
→ Aggregate of a few cells
→ Deconvolute the signals into each cell type

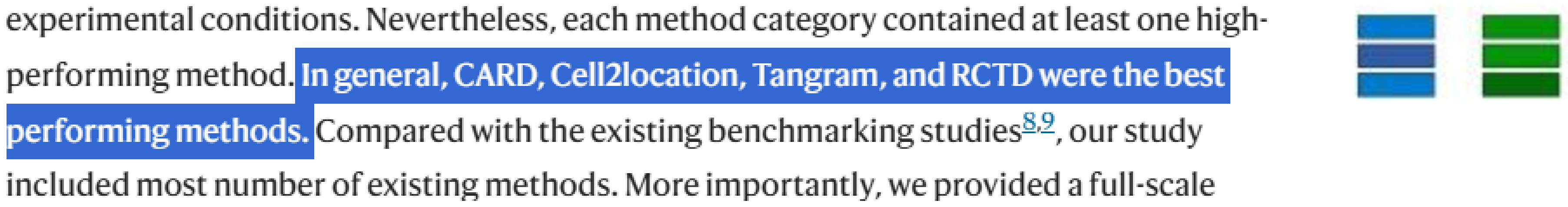
- Visium (Deconvolution)

A comprehensive benchmarking with practical guidelines for cellular deconvolution of spatial transcriptomics



- Visium (Deconvolution)




experimental conditions. Nevertheless, each method category contained at least one high-performing method. In general, CARD, Cell2location, Tangram, and RCTD were the best performing methods. Compared with the existing benchmarking studies[8,9], our study included most number of existing methods. More importantly, we provided a full-scale

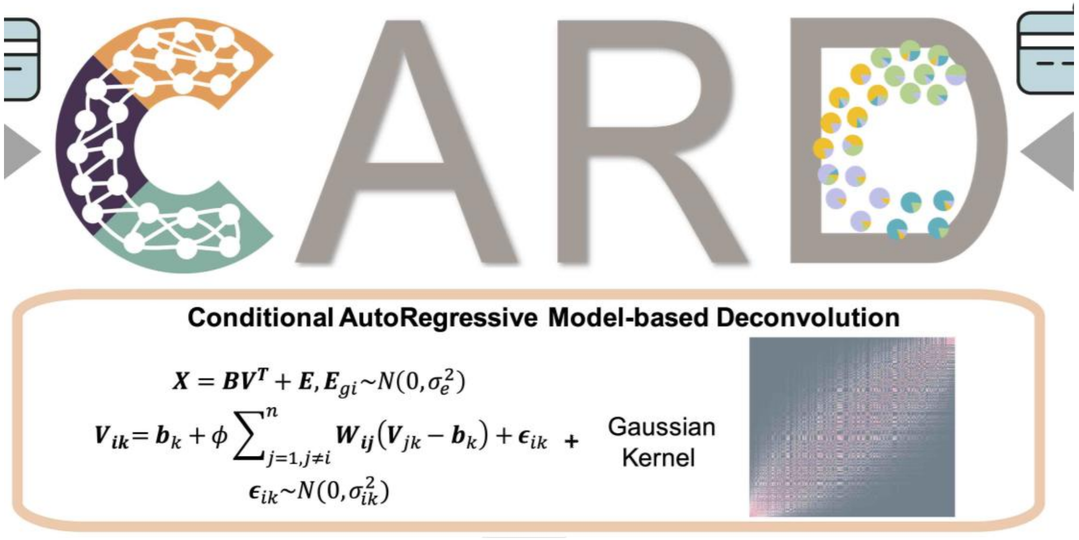
• Visium (Deconvolution)

-scRNA-seq based
→ generate reference cell type specific signature matrix

Article | Published: 02 May 2022

Spatially informed cell-type deconvolution for spatial transcriptomics

Ying Ma & Xiang Zhou



scRNA: K celltype * G informative gene
B: mean exp per celltype
V: N(spot) ~ celltype (proportion)
X: spatial data, E: error (gaussian)

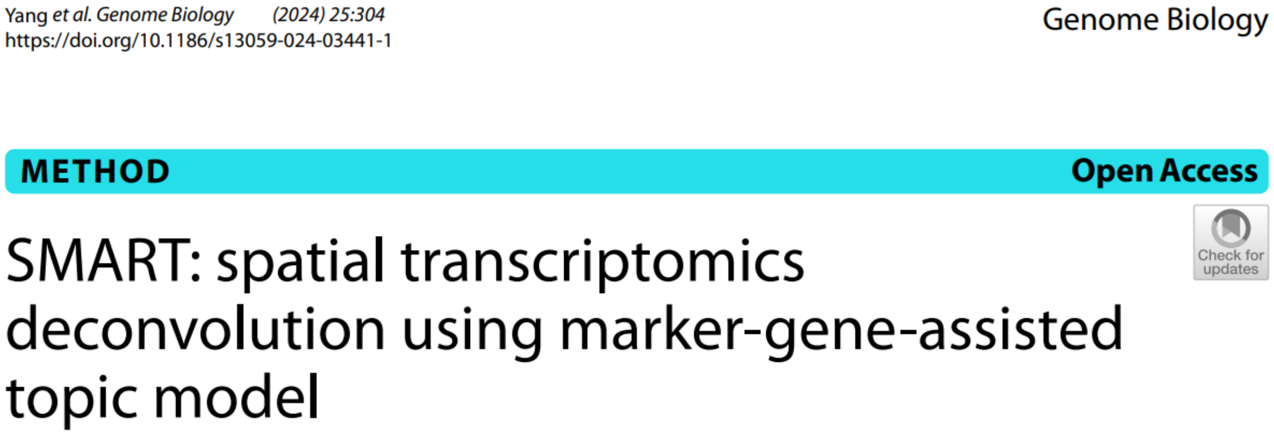
$$X = BV^T + E$$

X: real gene expression (scRNA-seq)

$$V_{ik} = b_k + \phi \sum_{j=1, j \neq i}^{n} W_{ij} \left(V_{jk} - b_k \right) + \epsilon_{ik};$$

V: overall cell-composition +
autocorrelation (high autocorrelation → similar cell composition)

- Visium (Deconvolution)



Yang *et al. Genome Biology* (2024) 25:304
https://doi.org/10.1186/s13059-024-03441-1

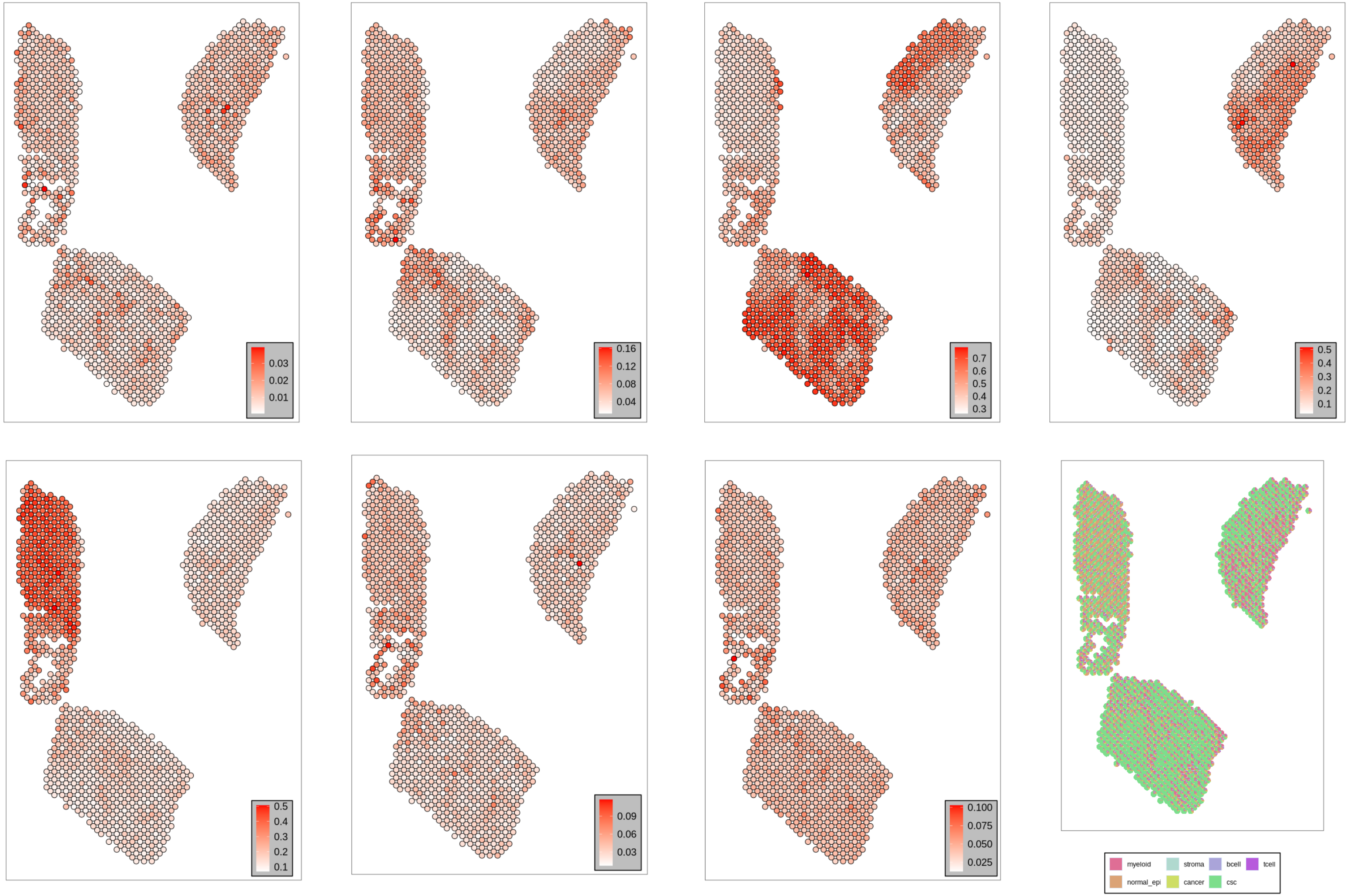
Genome Biology

METHOD **Open Access**

SMART: spatial transcriptomics deconvolution using marker-gene-assisted topic model

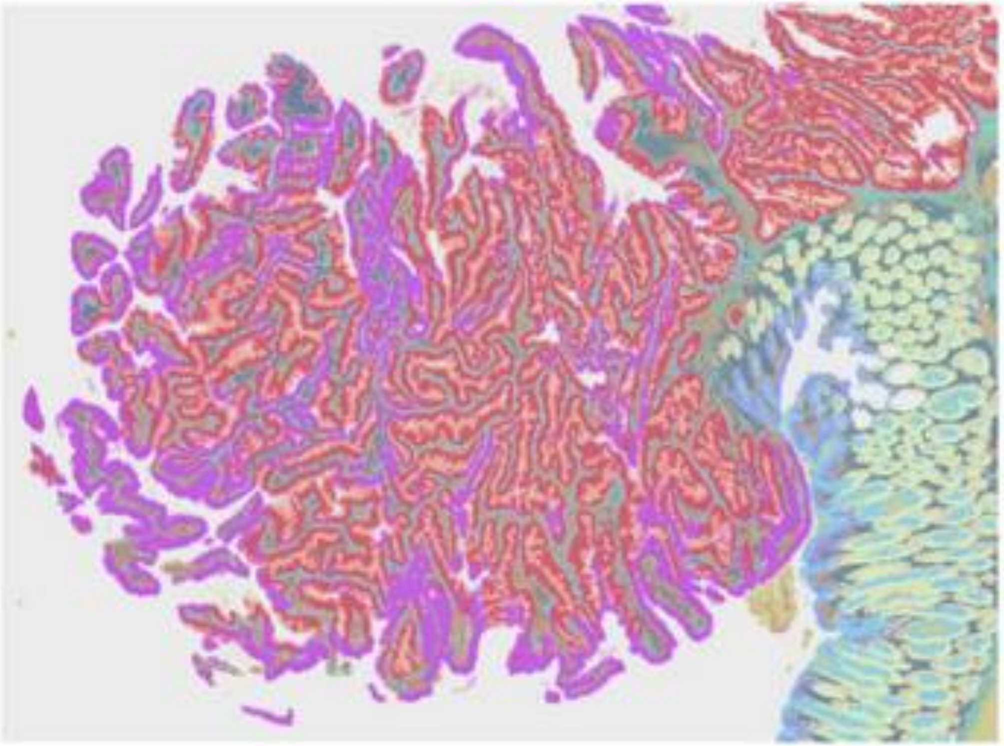
MARker-gene-assisted Topic model (SMART)
-scRNA-seq based → batch problem (ex: scRNA-seq & spatial data is not paired sample)
→ Marker-based (celltype-specifc marker gene list)

- Visium (Deconvolution)



- Visium

Visium

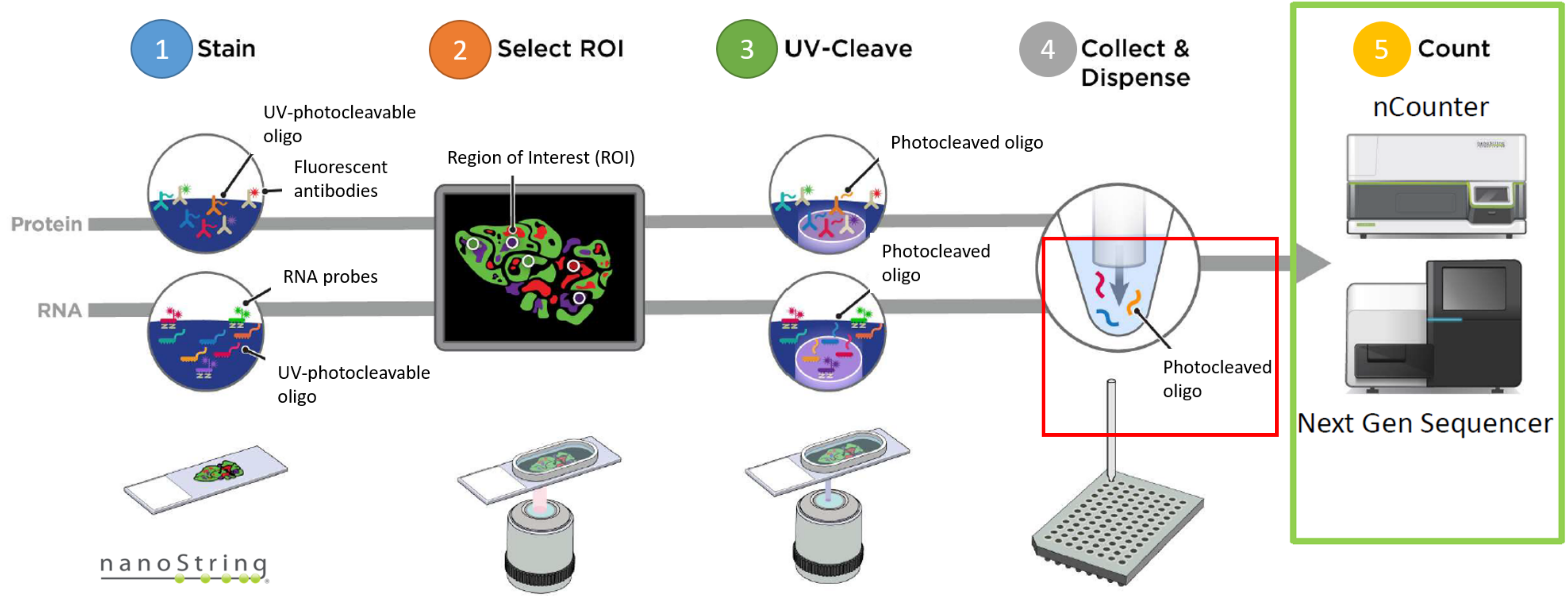


Visium HD

-Visium: 55um spot → multiple cells
-Visium HD: 2um → subcellular

- GeoMx

GeoMx DSP with nCounter or Next Gen Sequencer Workflow



1 Stain

2 Select ROI

3 UV-Cleave

4 Collect & Dispense

5 Count

UV-photocleavable oligo

Fluorescent antibodies

Protein

RNA

RNA probes

UV-photocleavable oligo

Region of Interest (ROI)

Photocleaved oligo

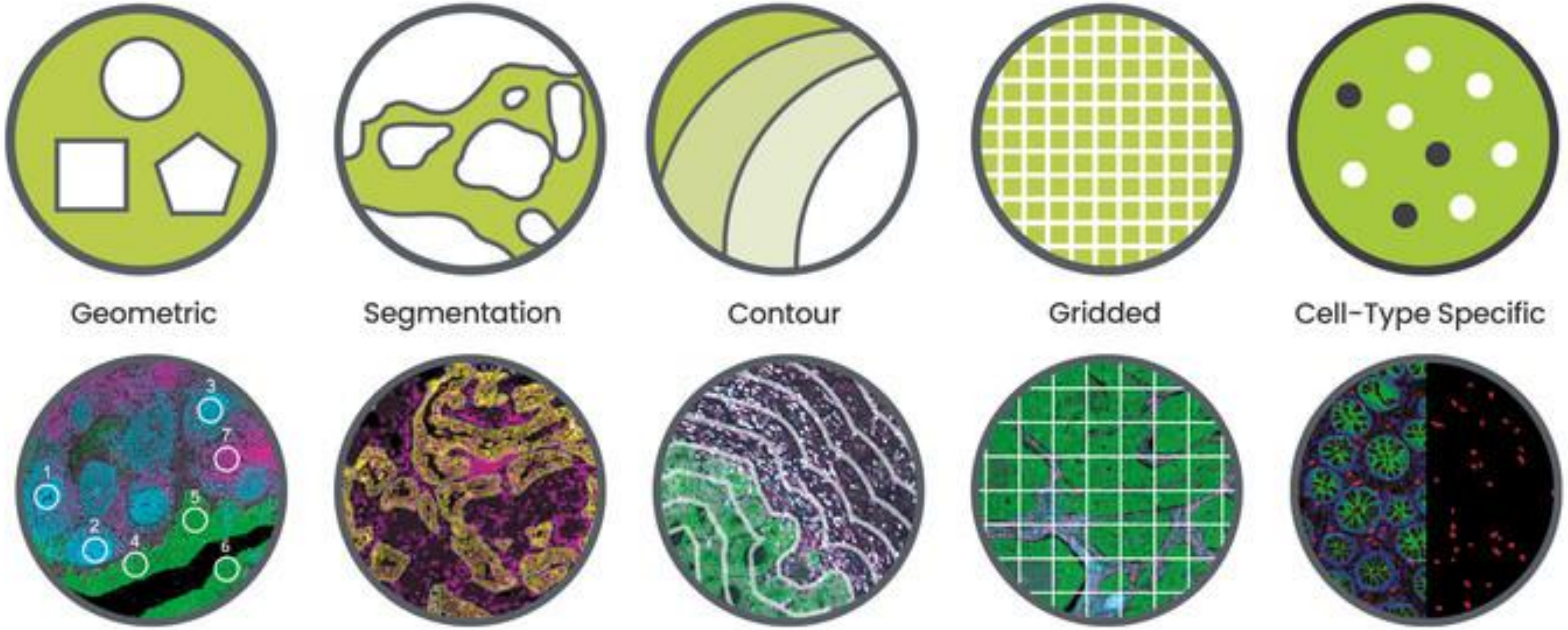
Photocleaved oligo

Photocleaved oligo

nCounter

Next Gen Sequencer

- GeoMx



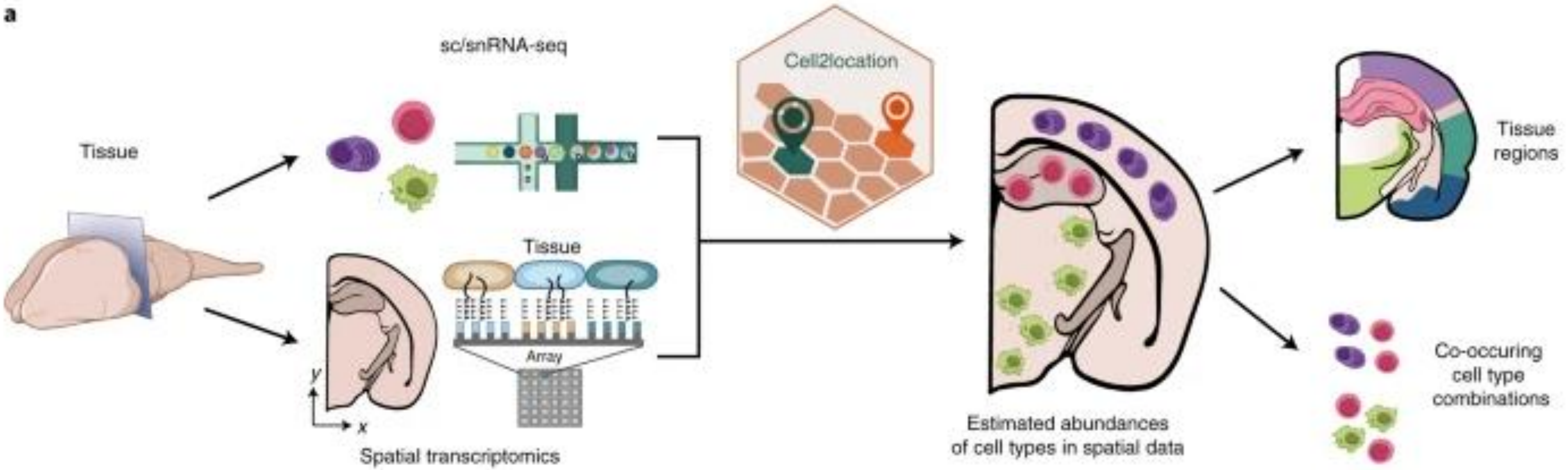
- Region of Interest (ROI)
-10~600um: typically, 20~300 cells

- GeoMx (deconvolution)

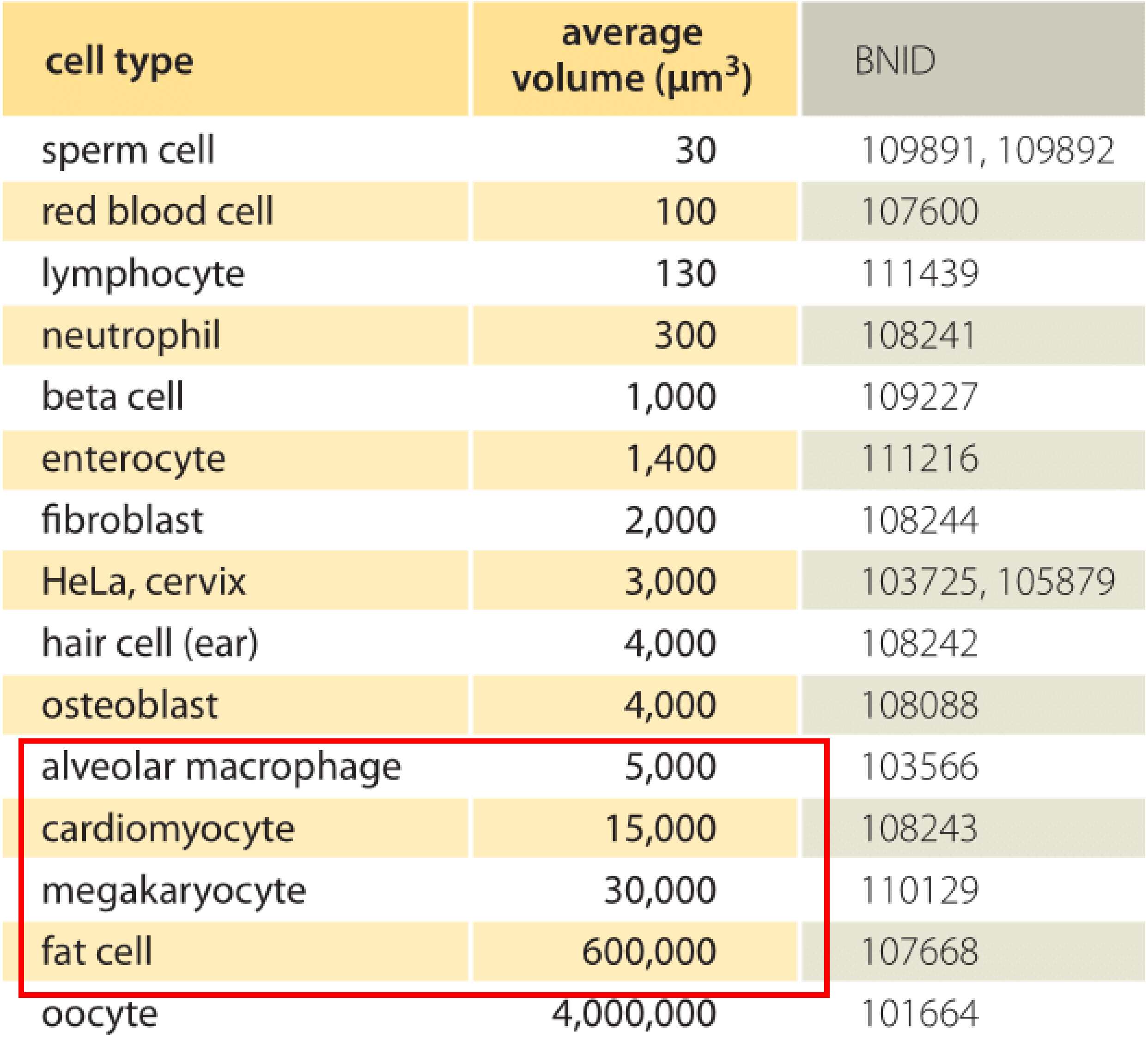
-Cell2location

-scRNA-seq based (reference cell type specific signature matrix)

- negative binomial regression



Cell size



cell type	average volume (µm³)	BNID
sperm cell	30	109891, 109892
red blood cell	100	107600
lymphocyte	130	111439
neutrophil	300	108241
beta cell	1,000	109227
enterocyte	1,400	111216
fibroblast	2,000	108244
HeLa, cervix	3,000	103725, 105879
hair cell (ear)	4,000	108242
osteoblast	4,000	108088
alveolar macrophage	5,000	103566
cardiomyocyte	15,000	108243
megakaryocyte	30,000	110129
fat cell	600,000	107668
oocyte	4,000,000	101664

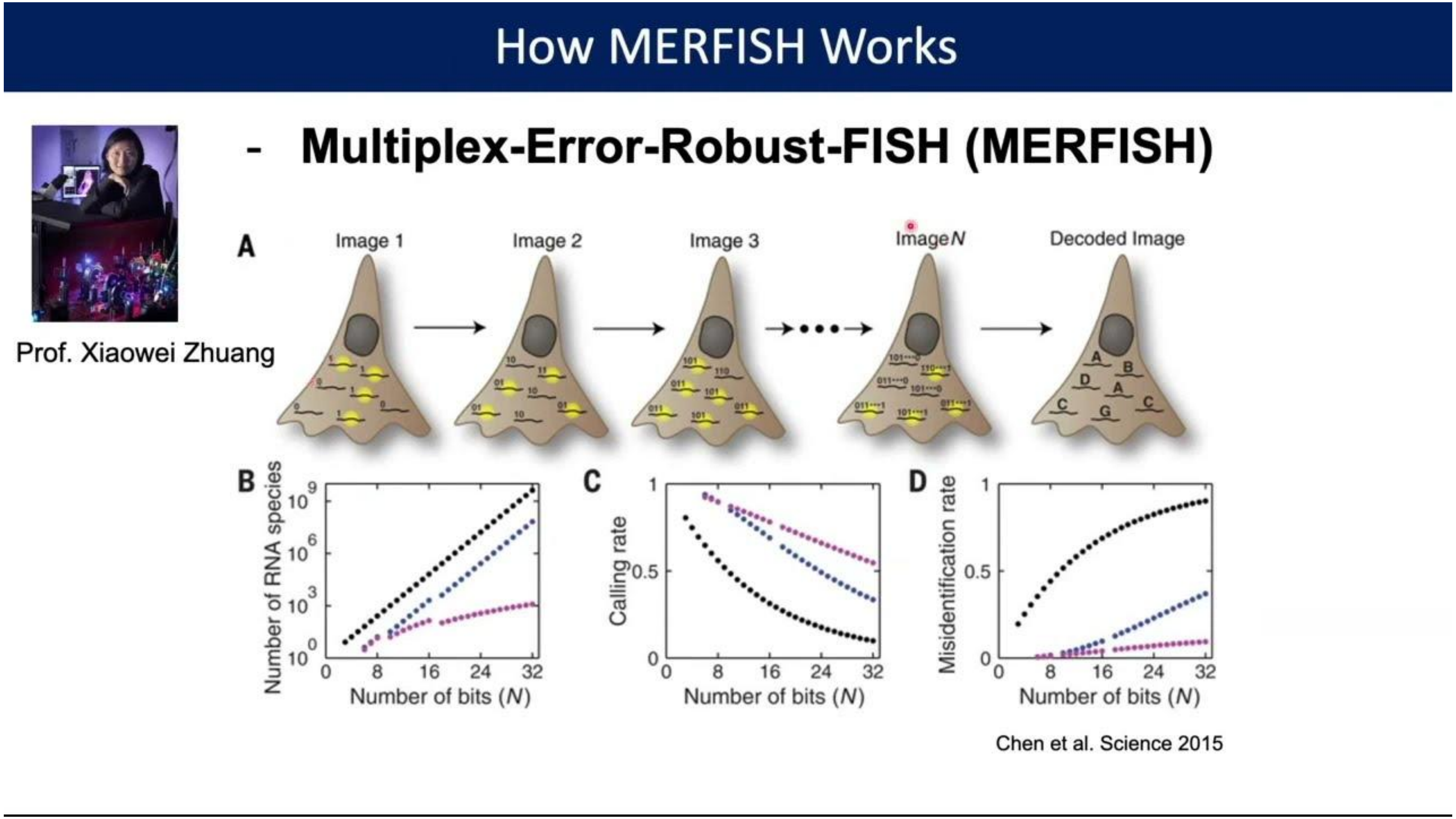
-Lymphocyte: diameter: ~10um
→ Cell size is important for spatial transcriptomics
→ Spot size is fixed
→ Variability between different spots for cellular content

Macrophage: too big!

-Visium: ~20 cells
Center-to-center: 100um

-GeoMx: 20~300 cells
→only spatial profiling
→ No cellular analysis

- Probe-based spatial transcriptomics



How MERFISH Works

- Multiplex-Error-Robust-FISH (MERFISH)

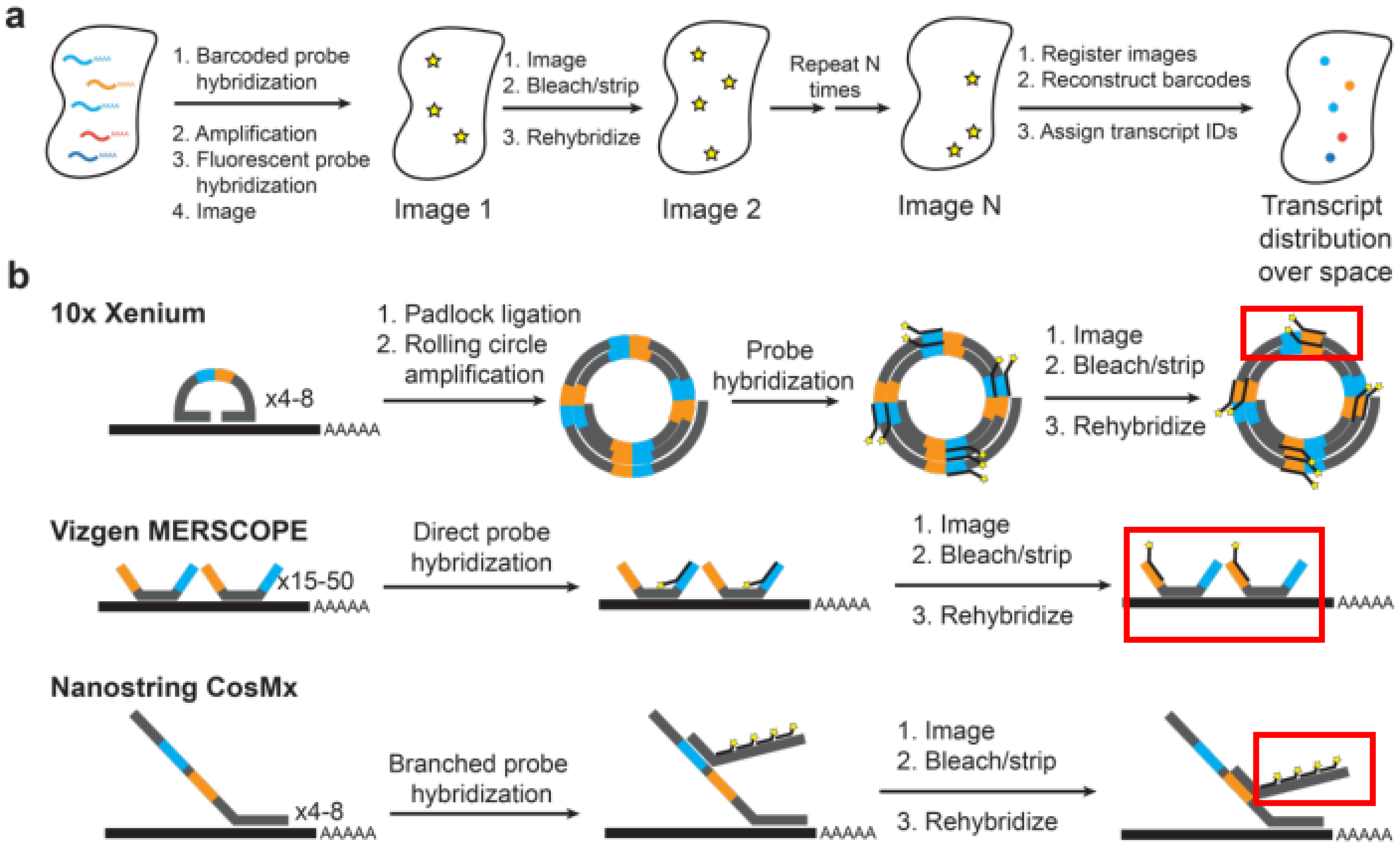
Prof. Xiaowei Zhuang

Chen et al. Science 2015

- MERFISH, , MERSCOPE, COSMX, XENIUM

→ Subcellular resolution → cellular analysis is possible

Probe-based spatial transcriptomics



Imaging strategy
→ Different amplification method

Xenium: Rolling amplification

Merscope: Direct probe amplification

Cosmx: Branched probe amplification

cf) typically: FFPE blocks → may lead to poor RNA integrity

• CosMx

nature biotechnology

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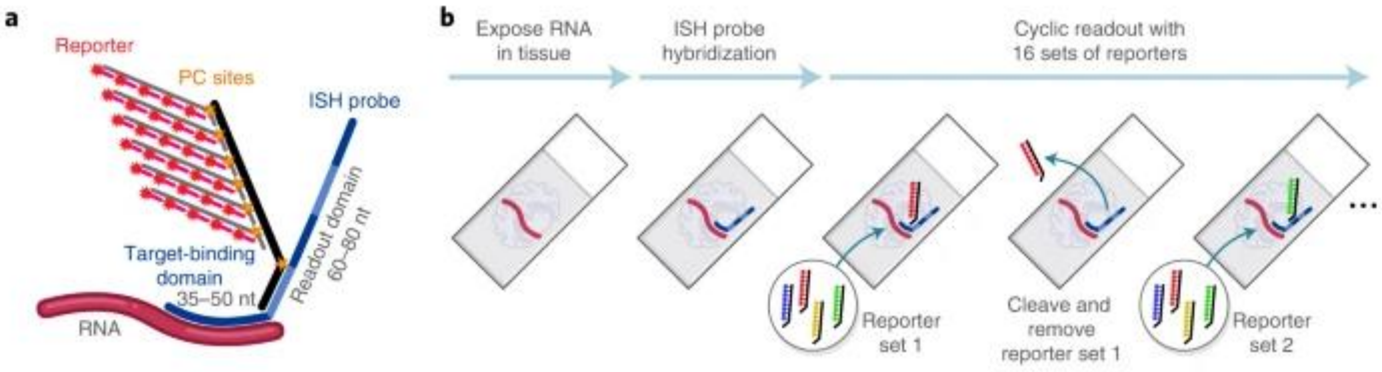
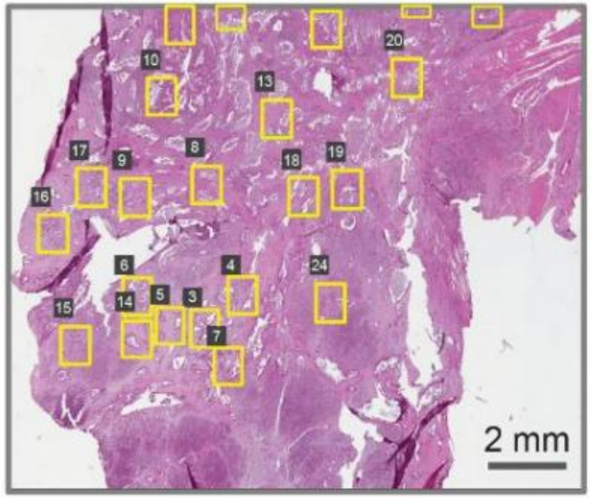
Article | Published: 06 October 2022

High-plex imaging of RNA and proteins at subcellular resolution in fixed tissue by spatial molecular imaging



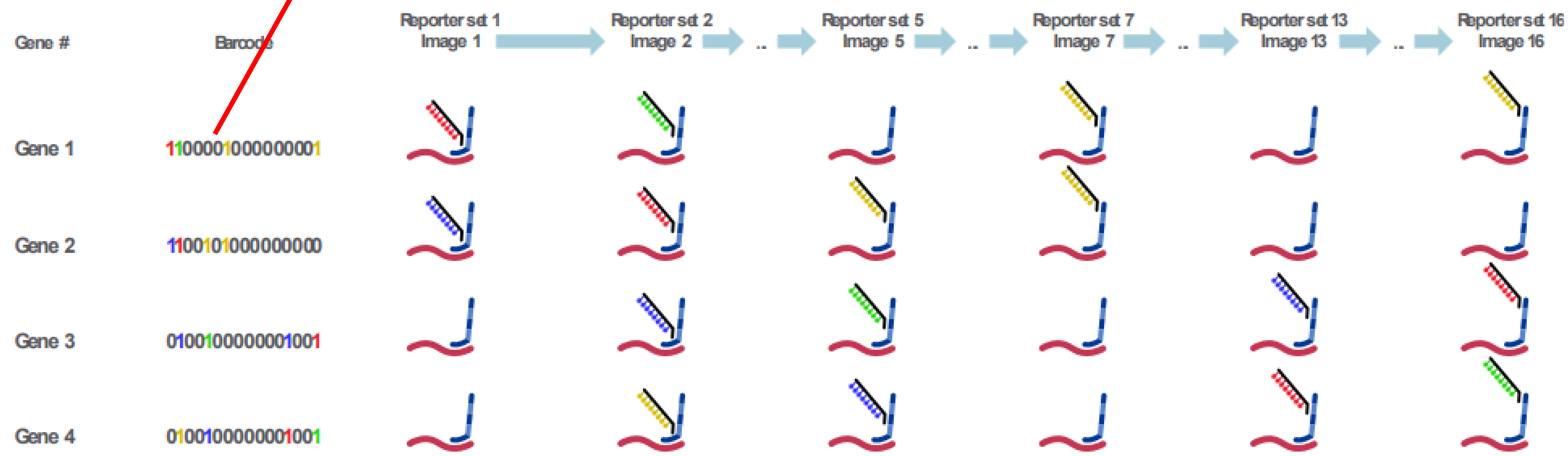
*Technical procedure
-Wide field laser
-Stage moving system → each FOV imaging (Field of view)
-Objective (water → trash)



- CosMx

*RNA barcode
-4 reporter + “off” (16round → 16-serial barcode) → UV fluorescence

-Hamming distance (barcode similarity) > 4 (HD4) → 1210 barcode



CosMx

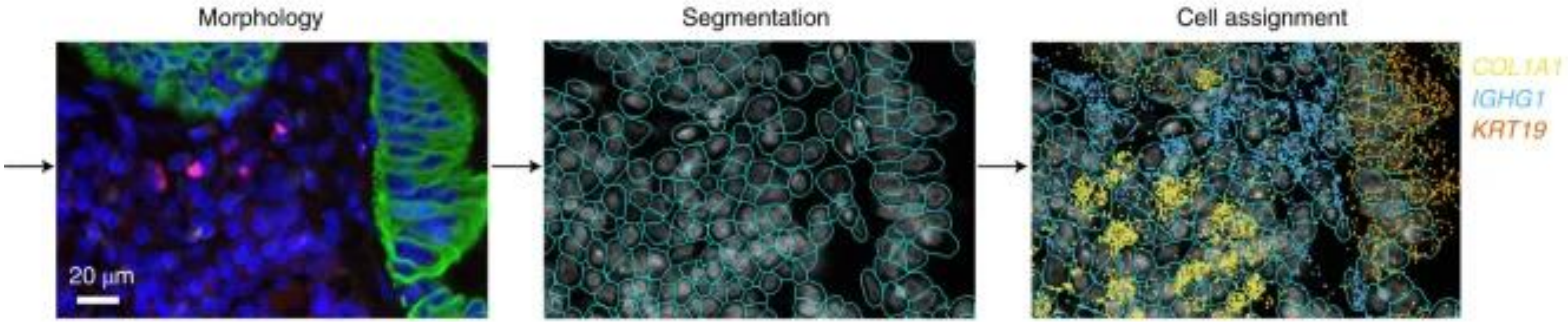
*Image reconstruction

1) Image stacking (z-direction) → 2D Laplacian of Gaussian filter (increase optical resolution)→ fluorescnece background correction

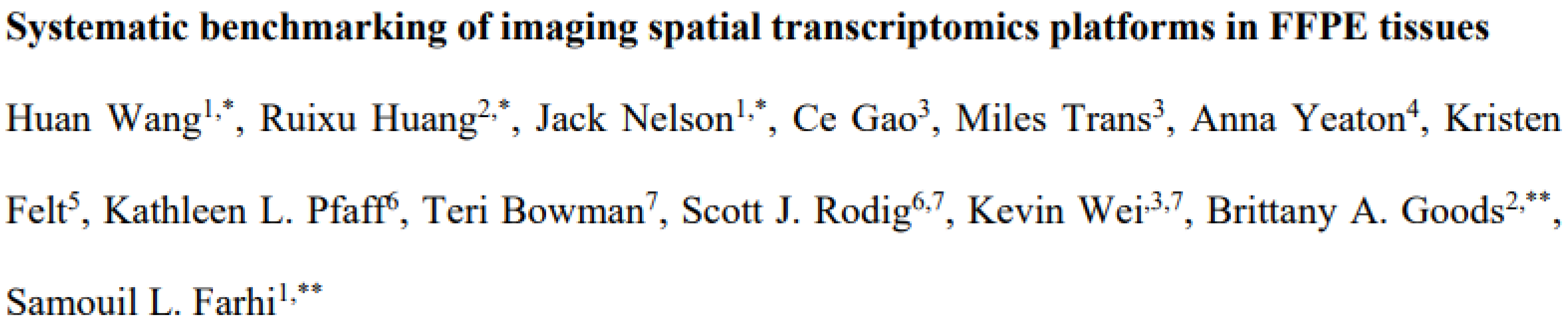
2) Gene assignment: integrate the signals within 0.5 pixel (90nm)

3) cell segmentation: DAPI (nucleus), PANCK (epithelial cell), CD3, CD298

+ Cellpose



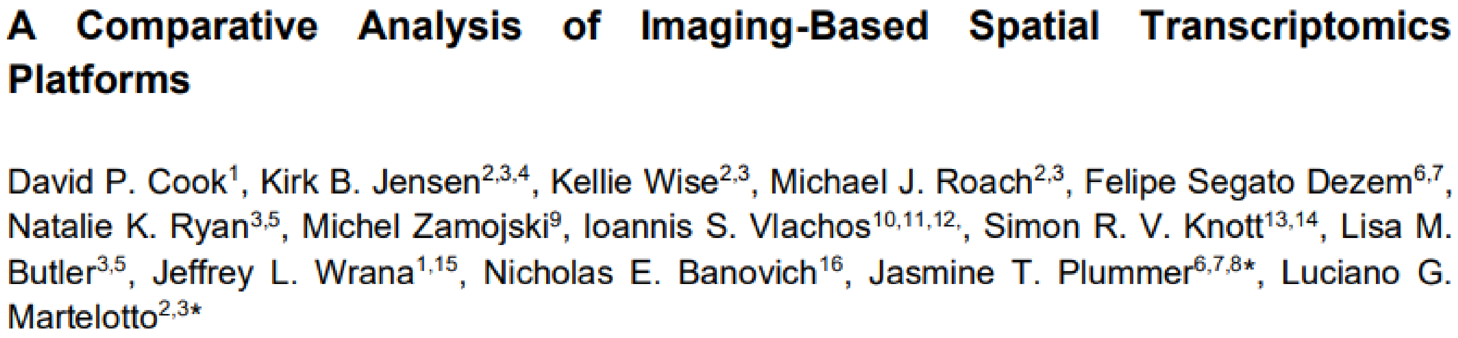
Benchmarking of image-based spatial transcriptomics



Systematic benchmarking of imaging spatial transcriptomics platforms in FFPE tissues

Huan Wang[1,*], Ruixu Huang[2,*], Jack Nelson[1,*], Ce Gao[3], Miles Trans[3], Anna Yeaton[4], Kristen Felt[5], Kathleen L. Pfaff[6], Teri Bowman[7], Scott J. Rodig[6,7], Kevin Wei[3,7], Brittany A. Goods[2,**], Samouil L. Farhi[1,**]

Broad Institute, BWH
bioRxiv: 20231208



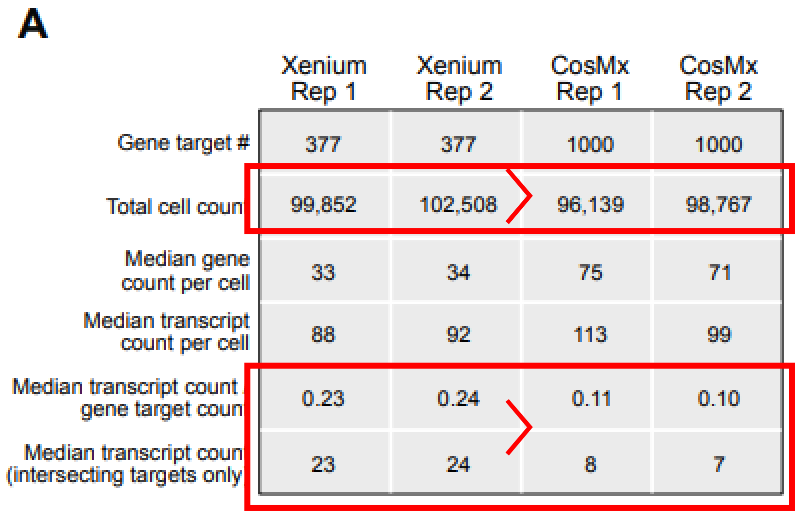
A Comparative Analysis of Imaging-Based Spatial Transcriptomics Platforms

David P. Cook[1], Kirk B. Jensen[2,3,4], Kellie Wise[2,3], Michael J. Roach[2,3], Felipe Segato Dezem[6,7], Natalie K. Ryan[3,5], Michel Zamojski[9], Ioannis S. Vlachos[10,11,12,], Simon R. V. Knott[13,14], Lisa M. Butler[3,5], Jeffrey L. Wrana[1,15], Nicholas E. Banovich[16], Jasmine T. Plummer[6,7,8*], Luciano G. Martelotto[2,3*]

South Australia
bioRxiv: 20231214

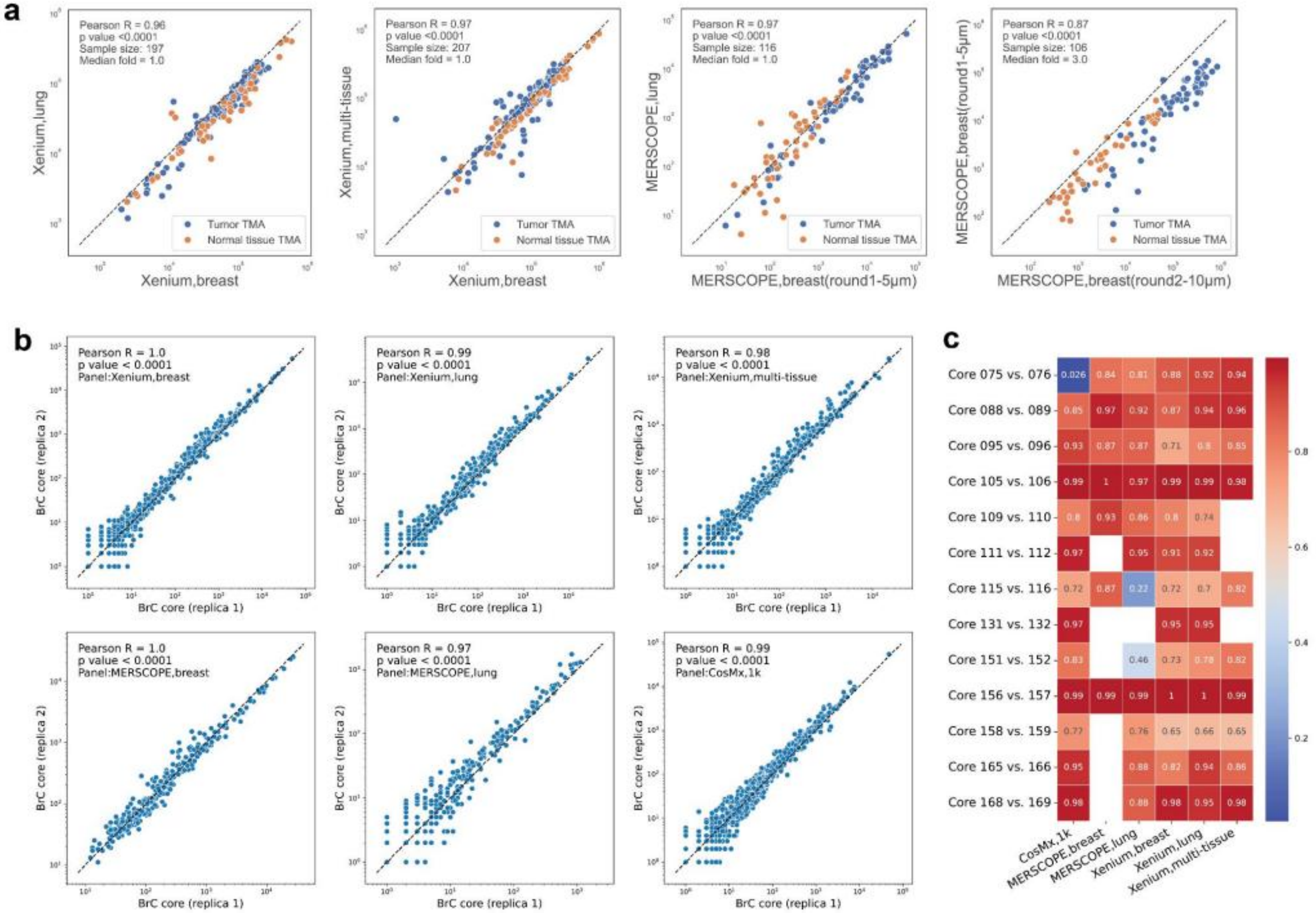
- Benchmarking of image-based spatial transcriptomics

Total transcript: Xenium > Cosmx > Merscope
Number of cells: Xenium > Merscope > Cosmx



	Xenium Rep 1	Xenium Rep 2	CosMx Rep 1	CosMx Rep 2
Gene target #	377	377	1000	1000
Total cell coun	99,852	102,508	96,139	98,767
Median gene count per cell	33	34	75	71
Median transcript count per cell	88	92	113	99
Median transcript count gene target coun	0.23	0.24	0.11	0.10
Median transcript coun (intersecting targets only	23	24	8	7

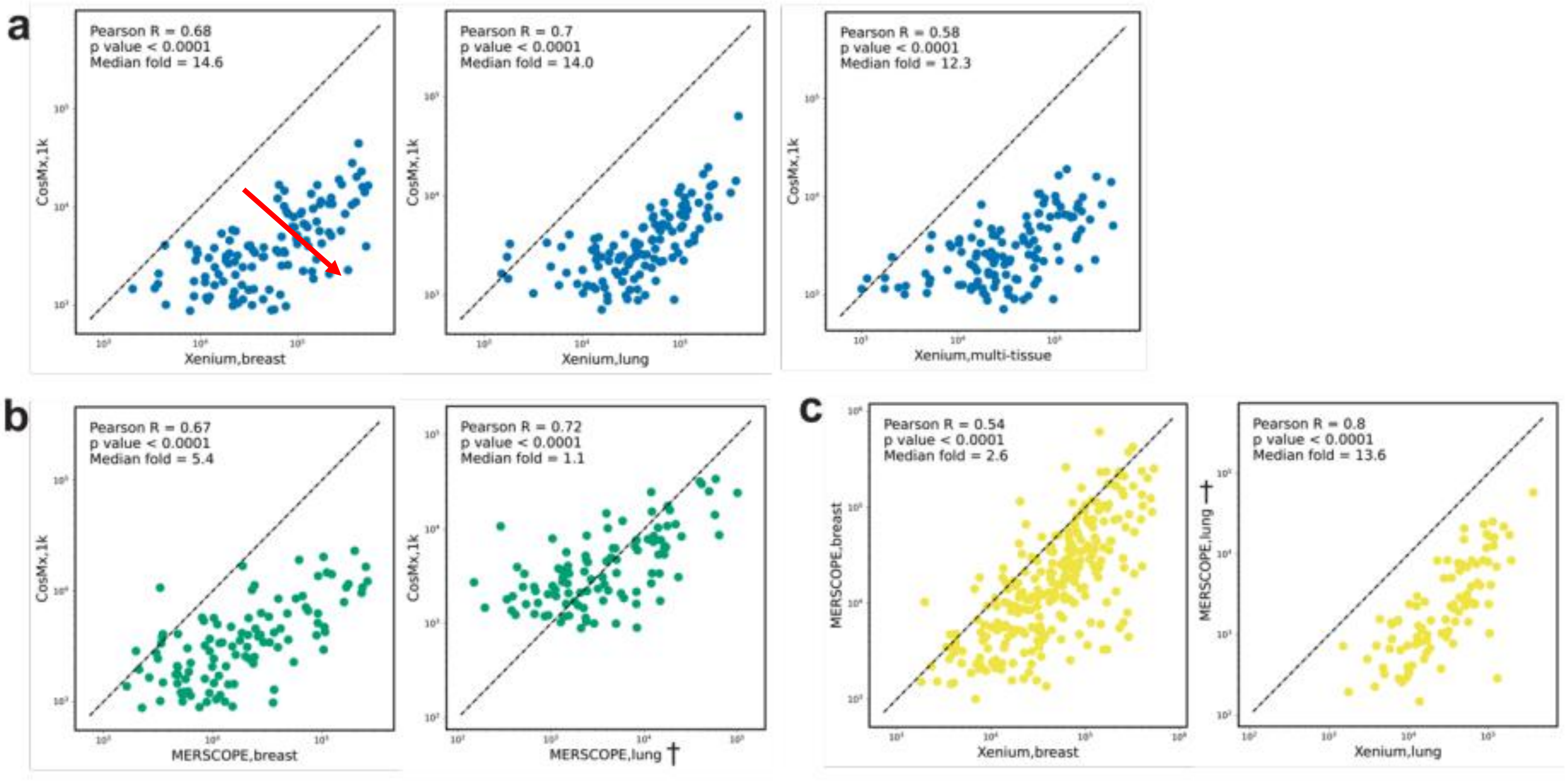
- Benchmarking of image-based spatial transcriptomics



	CosMx,1k	MERSCOPE,breast	MERSCOPE,lung	Xenium,breast	Xenium,lung	Xenium,multi-tissue
Core 075 vs. 076	0.026	0.84	0.81	0.88	0.92	0.94
Core 088 vs. 089	0.85	0.97	0.92	0.87	0.94	0.96
Core 095 vs. 096	0.93	0.87	0.87	0.71	0.8	0.85
Core 105 vs. 106	0.99	1	0.97	0.99	0.99	0.98
Core 109 vs. 110	0.8	0.93	0.86	0.8	0.74	
Core 111 vs. 112	0.97		0.95	0.91	0.92	
Core 115 vs. 116	0.72	0.87	0.22	0.72	0.7	0.82
Core 131 vs. 132	0.97			0.95	0.95	
Core 151 vs. 152	0.83		0.46	0.73	0.78	0.82
Core 156 vs. 157	0.99	0.99	0.99	1	1	0.99
Core 158 vs. 159	0.77		0.76	0.65	0.66	0.65
Core 165 vs. 166	0.95		0.88	0.82	0.94	0.86
Core 168 vs. 169	0.98		0.88	0.98	0.95	0.98

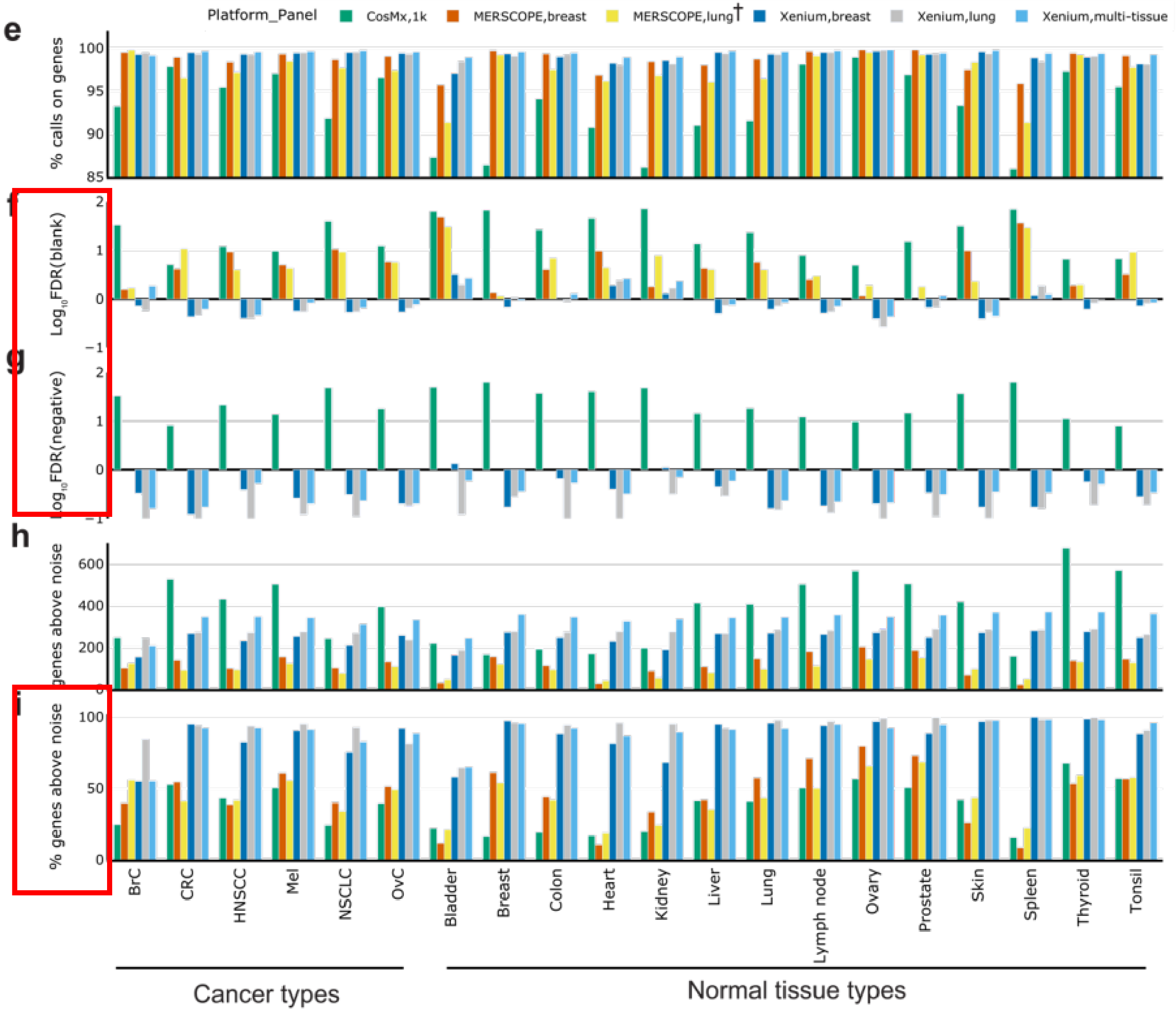
Total transcript / gene ← pseudo-bulk from each core
Intra-platform reproducibility (same patient, different core) → very good

- Benchmarking of image-based spatial transcriptomics



Xenium is always better

- Benchmarking of image-based spatial transcriptomics

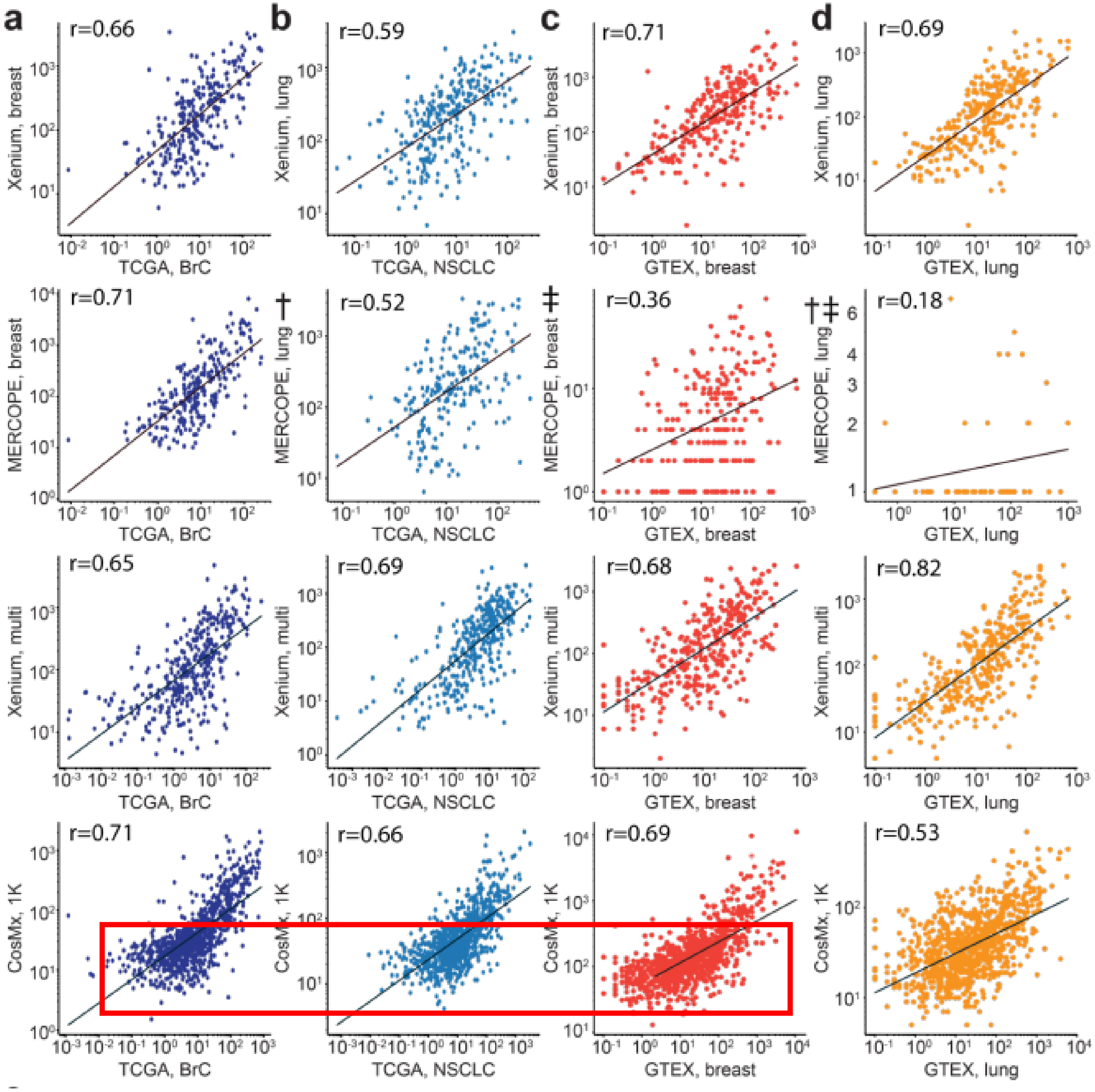


- Probe specificity: negative probe (experimental probe: does not present in human) (cf: Merscope: X) & negative barcode (computational barcode: algorithmically)

- On target / total
- above noise (2 S.D. > Avg)

→ Xenium is the best

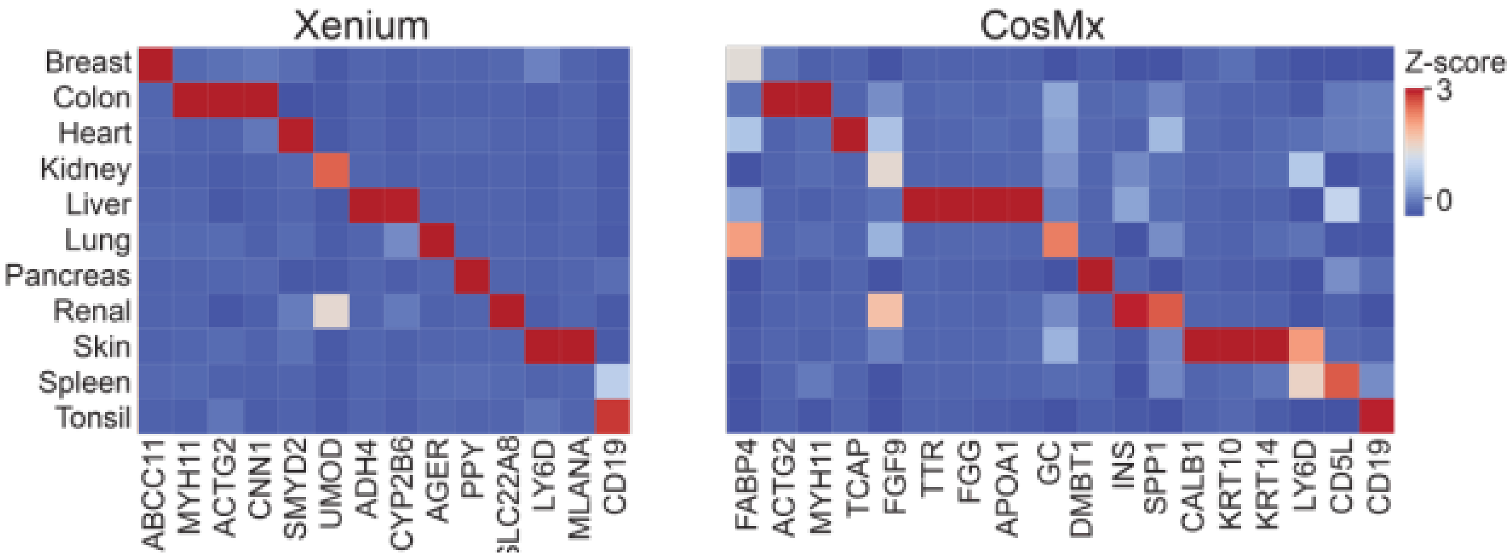
- Benchmarking of image-based spatial transcriptomics

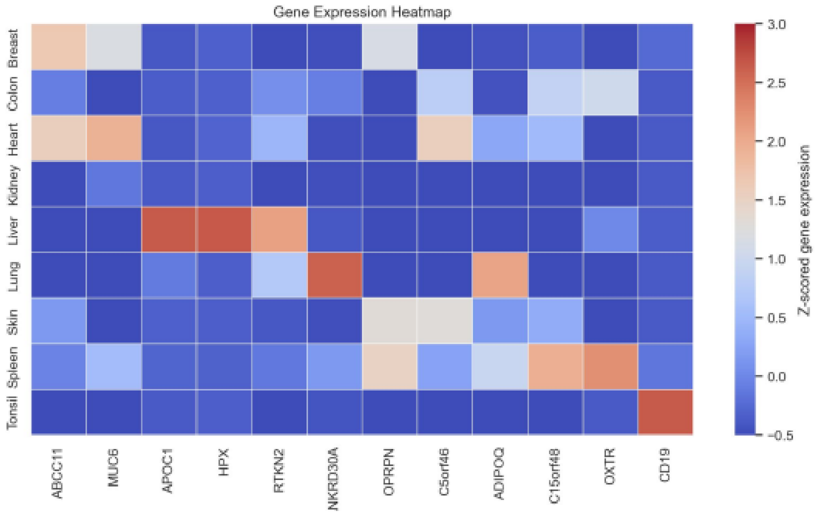


-Specificity assessment by TCGA and GTEX: gene-gene correlation

→ Cosmx: low exp: skewed
→ (low expression → non-specific binding high)

- Benchmarking of image-based spatial transcriptomics





*Specificity assessment by TCGA and GTEX

- Tissue-specific marker (> 20 fold btw other tissues)

→ Xenium > Cosmx

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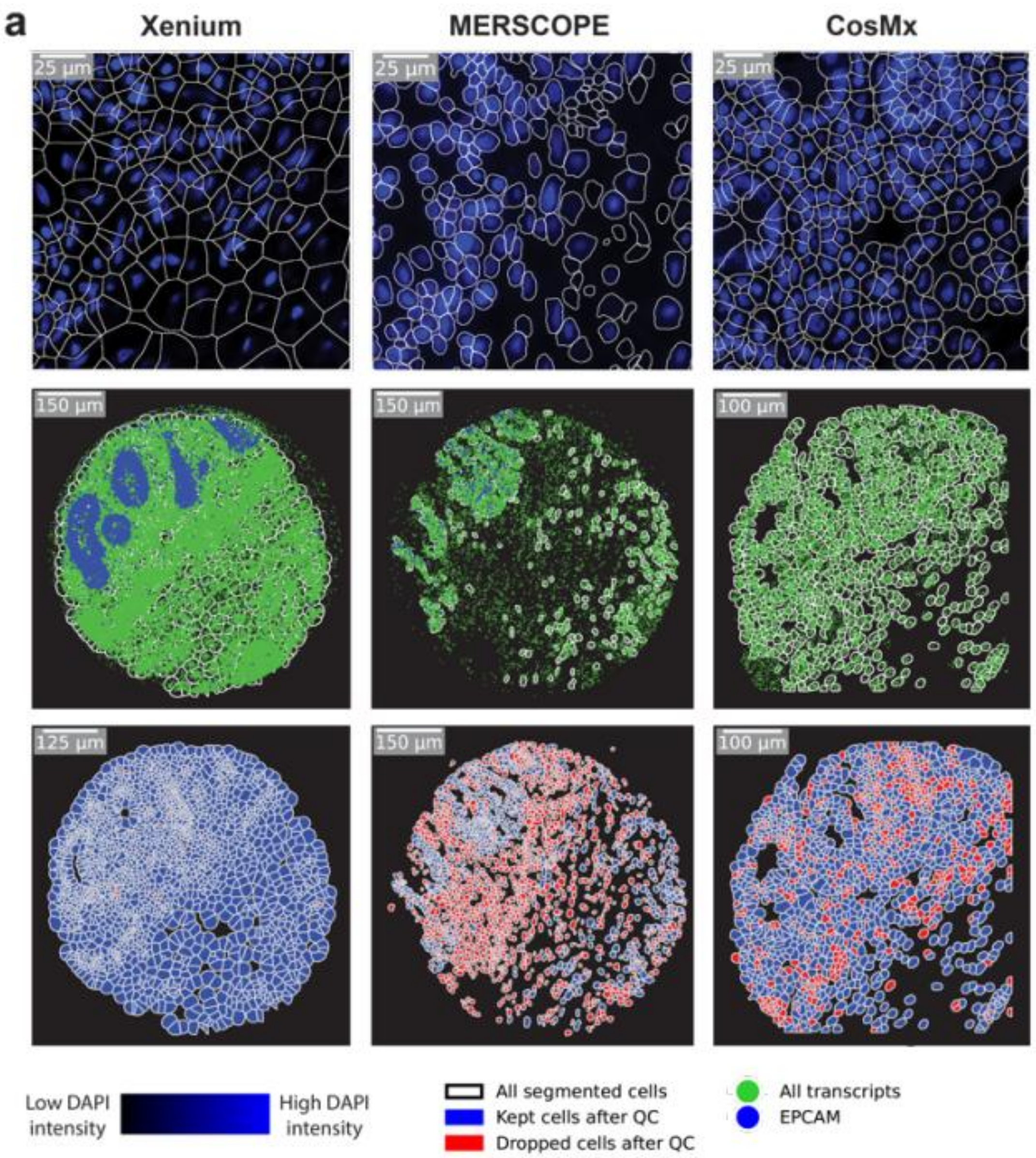
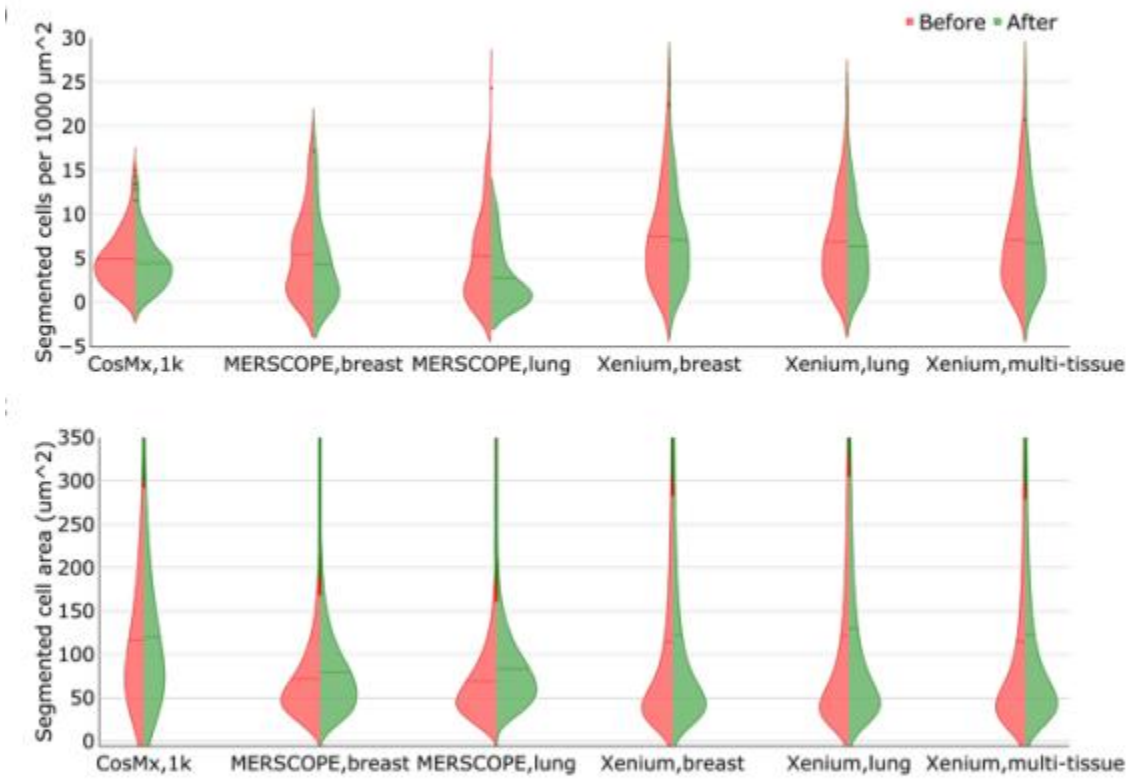


Xenium: large boundary
(though less dropped cells after QC)



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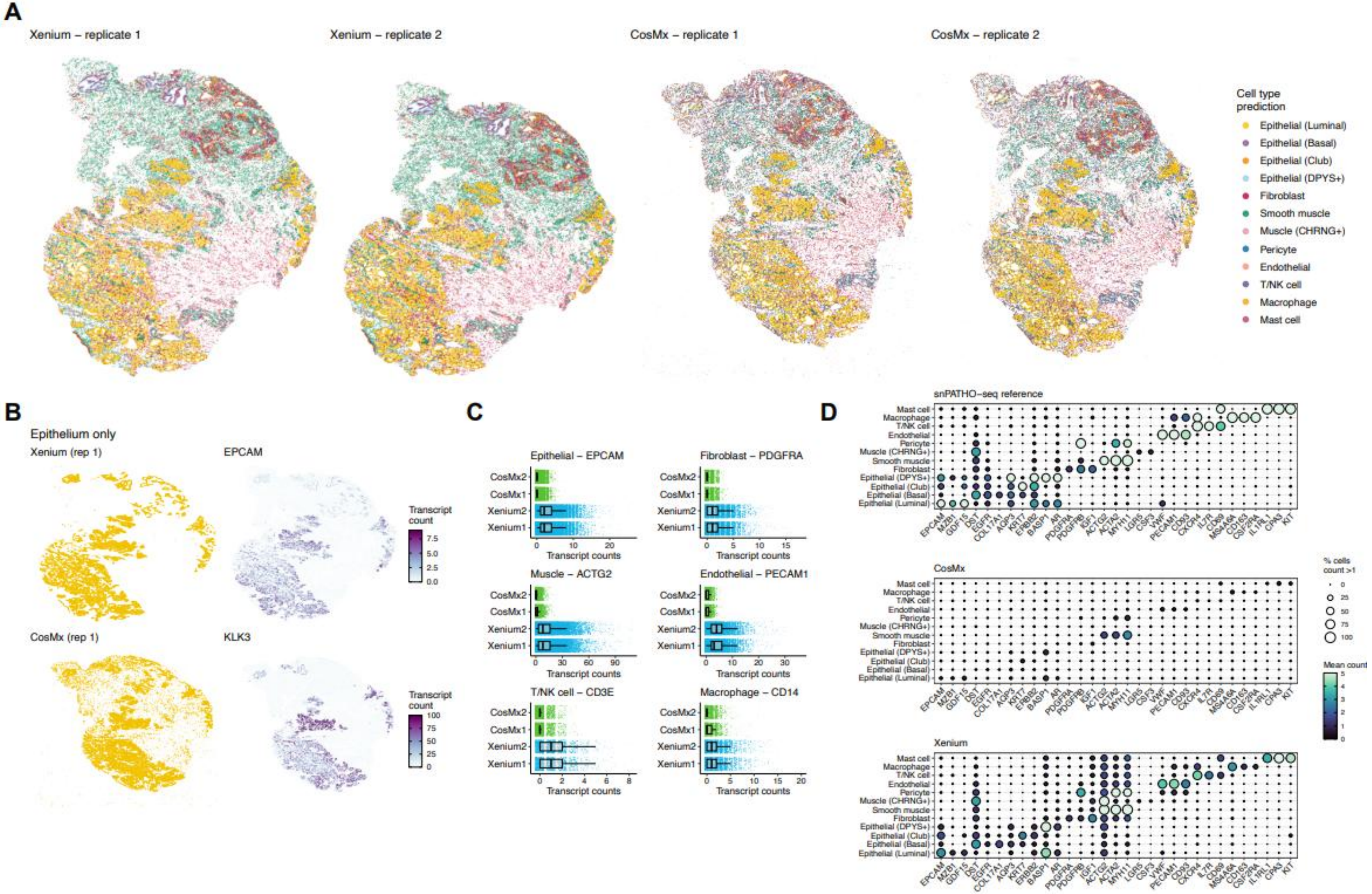

Marker gene: Xenium > Cosmx (Sensitivity)
Marker plot: Xenium is similar to snRNA-seq