Current Trends in Bioinformatics:

A Brief Introduction to Spatial Transcriptomics

ヴィラ･ジョ | Jo Villa, PhD
Researcher at KOTAI Biotechnologies, Inc.

November 17th, 2025

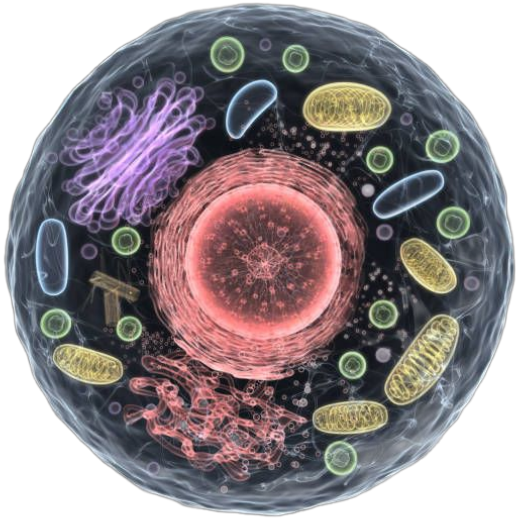
Overview of Today's Topics

- What is **Spatial Transcriptomics**?
  - How can it be used to **study various tissues**?
- What are some **differences between various methods**?
- How can we **analyze the data**?
- What are some of the **current challenges in the field**?
  - Challenge #1: Cell Segmentation
  - Challenge #2: Integration and Alignment of Multiple Samples

An Introduction to the Methods

A Comparative Analogy:

If **a cell** is a berry…

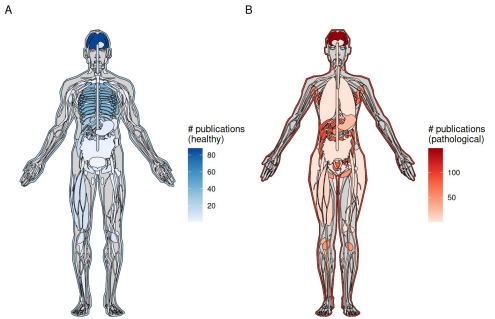


Then **bulk RNAseq** is the smoothie…

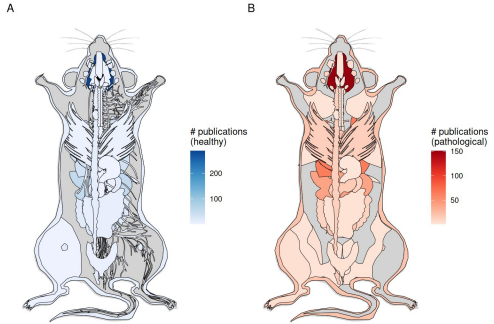
And **scRNAseq** is the fruit salad…

Spatial transcriptomics is the orchard

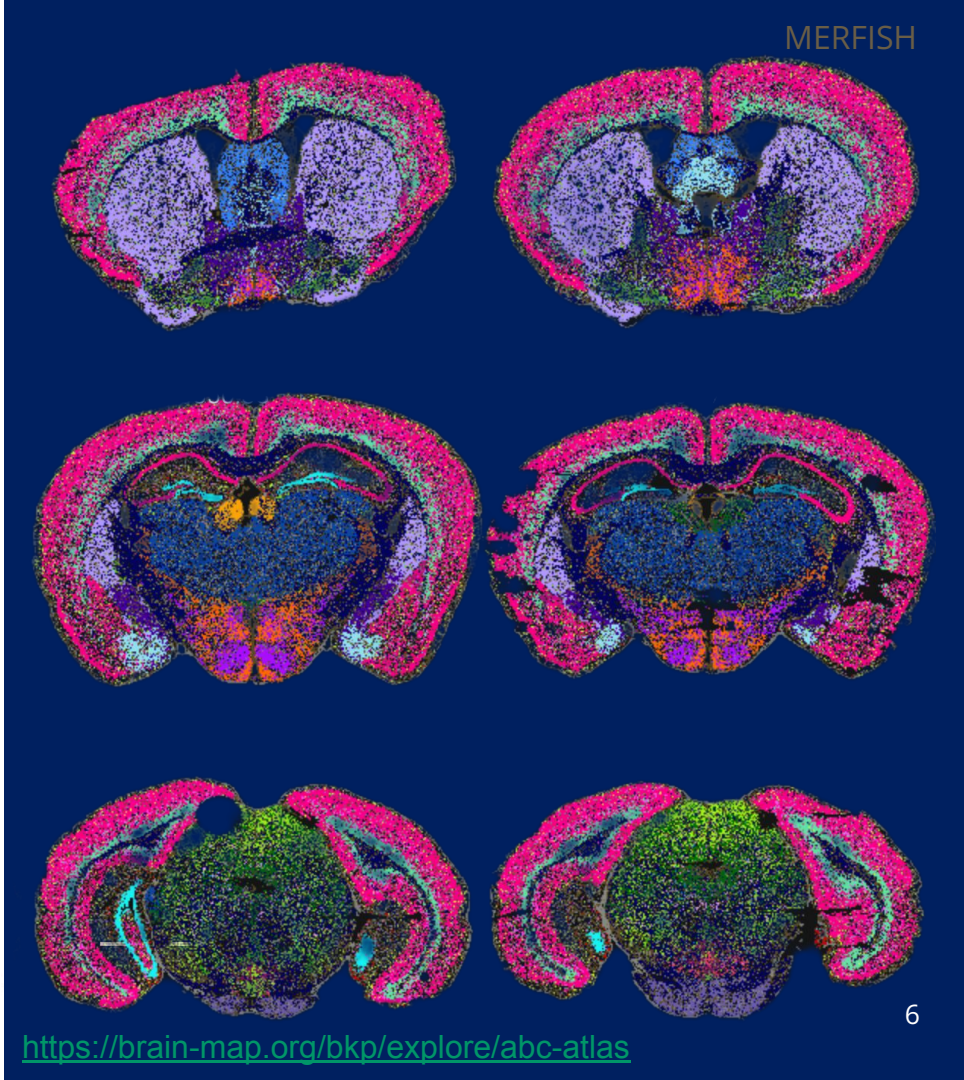
Applications Across Tissue Types



Most studies to date have been in human and mice tissues, with many brain and tumor studies



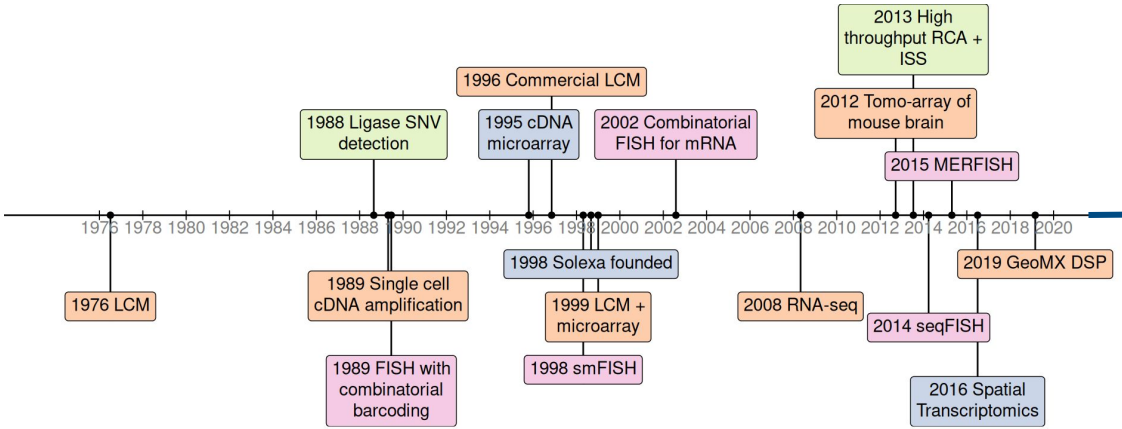
Museum of Spatial Transcriptomics (2025-10-18)



https://brain-map.org/bkp/explore/abc-atlas

Timeline of Spatial Transcriptomics Methods

The ability to examine RNA and protein expression in tissues isn't new (ex. in situ hybridization since 1960s), but the capabilities have exponentially increased in recent years



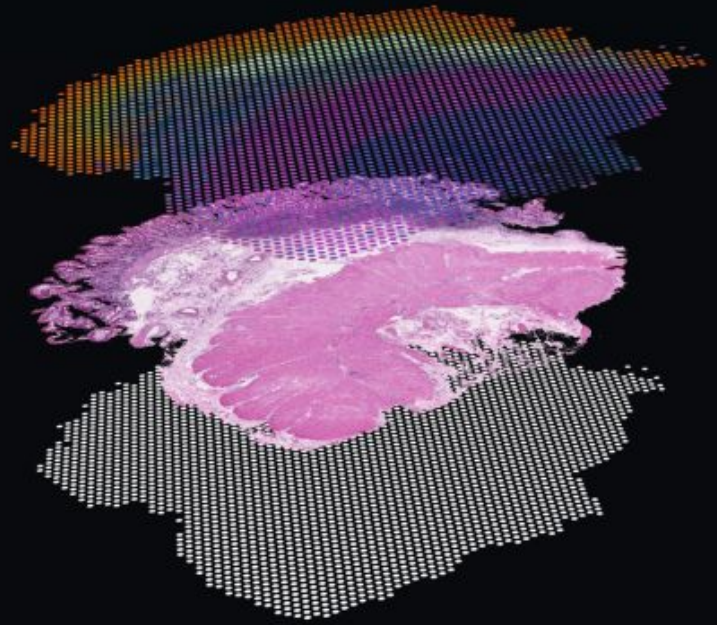
Modern usage: Increases in the number of genes and detection abilities

Museum of Spatial Transcriptomics (2025-10-18)

www.nature.com/nmeth / January 2021 Vol.18 No. 1

nature methods

Method of the Year 2020: Spatially resolved transcriptomics

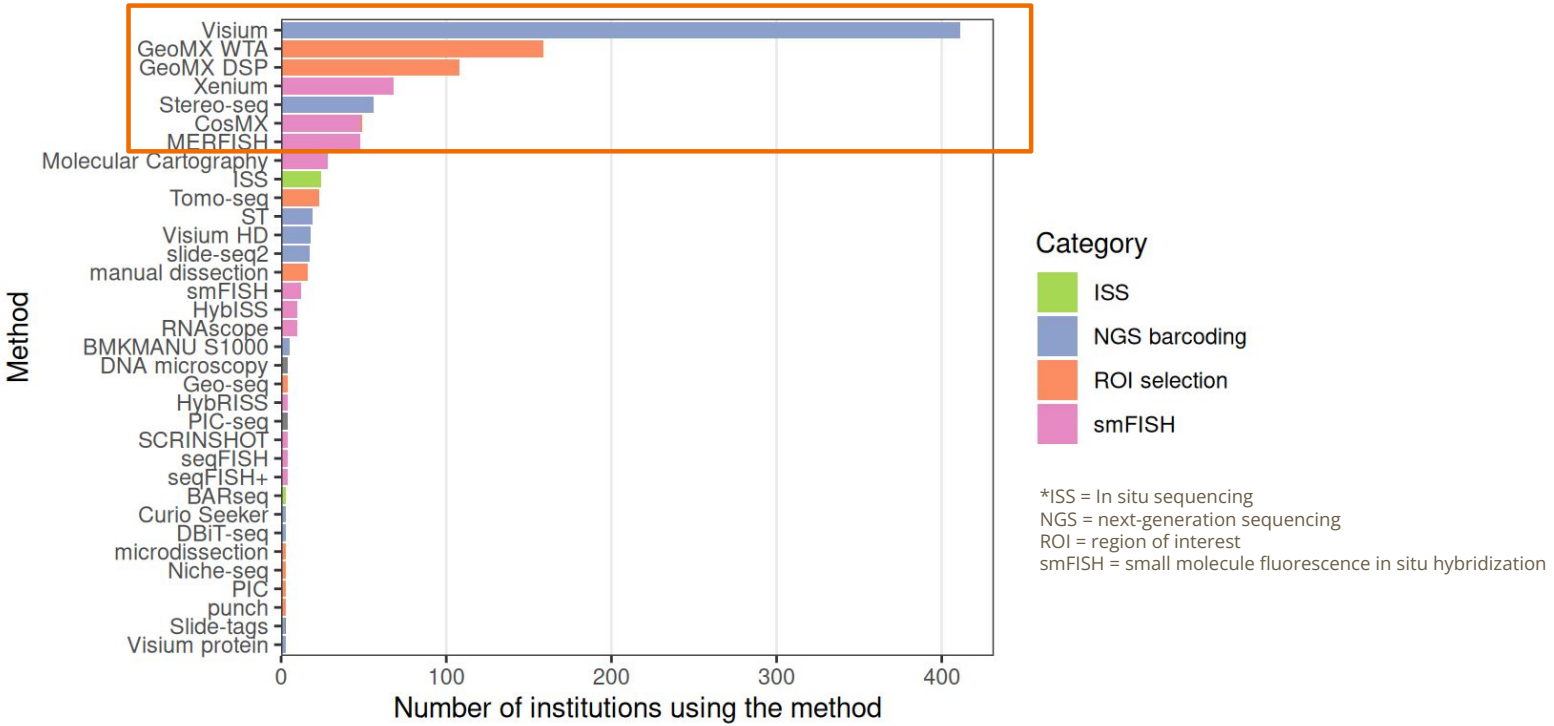


www.nature.com/nmeth / December 2024 Vol.21 No. 12

nature methods

Method of the Year 2024: spatial proteomics

Various Spatial Transcriptomics Methods Are Used

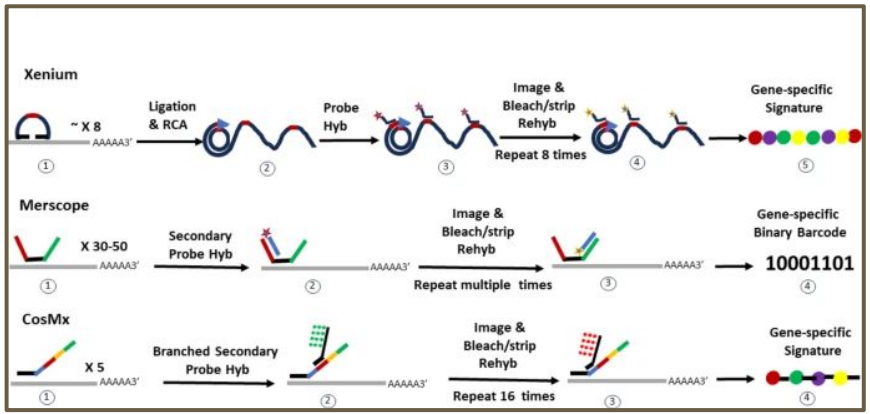


*ISS = In situ sequencing
NGS = next-generation sequencing
ROI = region of interest
smFISH = small molecule fluorescence in situ hybridization

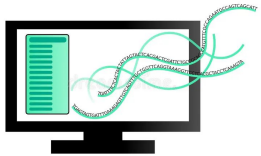
Museum of Spatial Transcriptomics (2025-10-18)

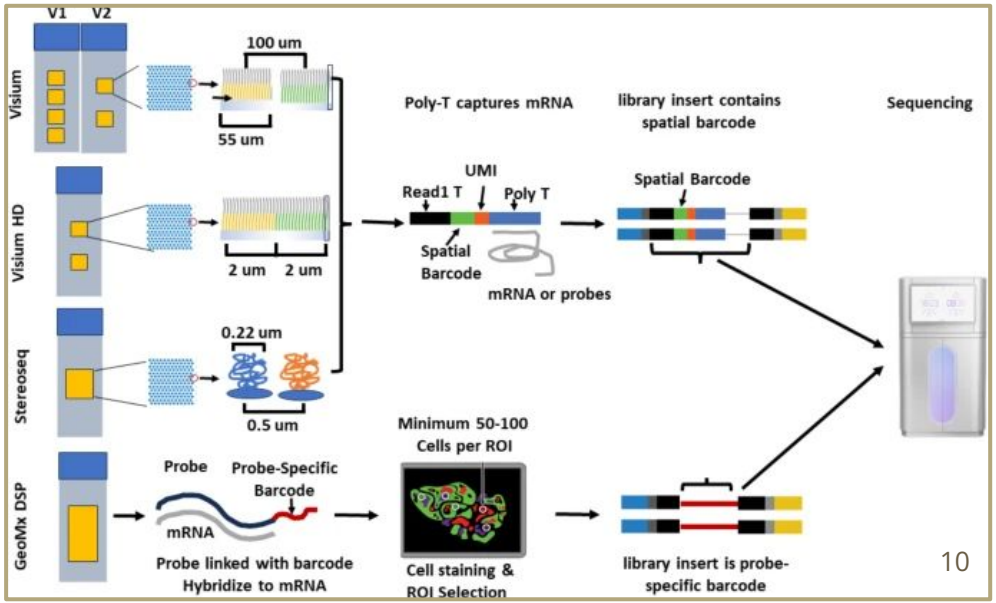
Classes of Spatial Transcriptomics Methods

Imaging-Based



Sequencing-Based



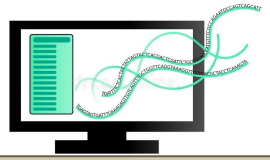


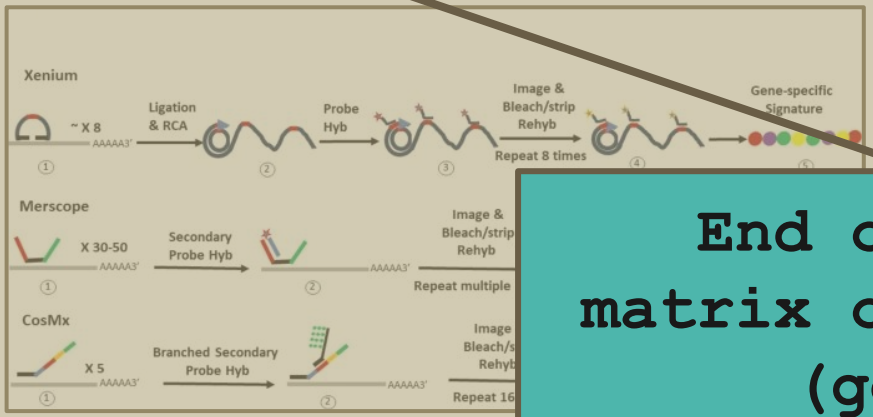
Lim, H.J., Wang, Y., Buzdin, A. *et al.* A practical guide for choosing an optimal spatial transcriptomics technology from seven major commercially available options. *BMC Genomics* 26, 47 (2025). https://doi.org/10.1186/s12864-025-11235-3

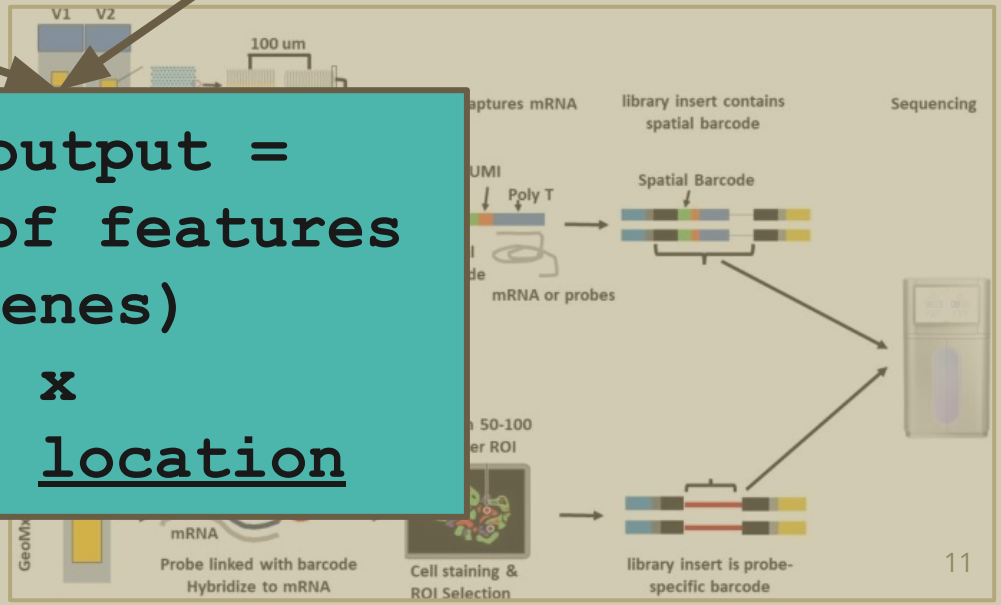
Classes of Spatial Transcriptomics Methods

Imaging-Based

Sequencing-Based







End output =
matrix of features (genes)
x
cell + location

Lim, H.J., Wang, Y., Buzdin, A. *et al.* A practical guide for choosing an optimal spatial transcriptomics technology from seven major commercially available options. *BMC Genomics* 26, 47 (2025). https://doi.org/10.1186/s12864-025-11235-3

Differences within Spatial Transcriptomics Methods

The many commercial and academic methods vary by:

- **Resolution** (“true” single-cell or single-cell level?)
- **Number** of genes detected (panel vs whole transcriptome)
- **Sensitivity** of detection
- **Size** of the imaging area
- **Experimental**: Cost, time, difficulty in sample preparation and processing
  - **Sample** considerations (fresh frozen vs FFPE tissue slices)

Benchmarking studies are still ongoing and companies continue to update their protocols and machines → ***Check the current benchmarking papers before designing your project!***

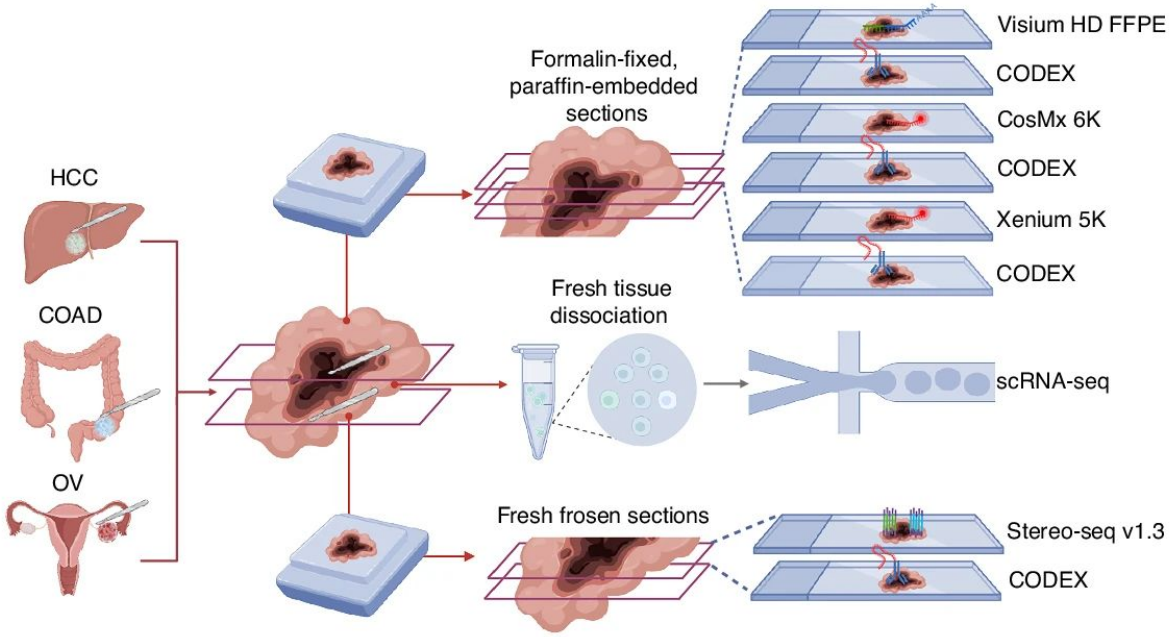
An example benchmarking study

Article | Open access | Published: 17 October 2025

Systematic benchmarking of high-throughput subcellular spatial transcriptomics platforms across human tumors

Pengfei Ren, Rui Zhang, Yunfeng Wang, Peng Zhang, Ce Luo, Suyan Wang, Xiaohong Li, Zongxu Zhang, Yanping Zhao, Yufeng He, Haorui Zhang, Yufeng Li, Zhidong Gao, Xiuping Zhang, Yahui Zhao, Zhihua Liu, Yuanguang Meng ✉, Zhe Zhang ✉ & Zexian Zeng ✉

Nature Communications **16**, Article number: 9232 (2025) | Cite this article

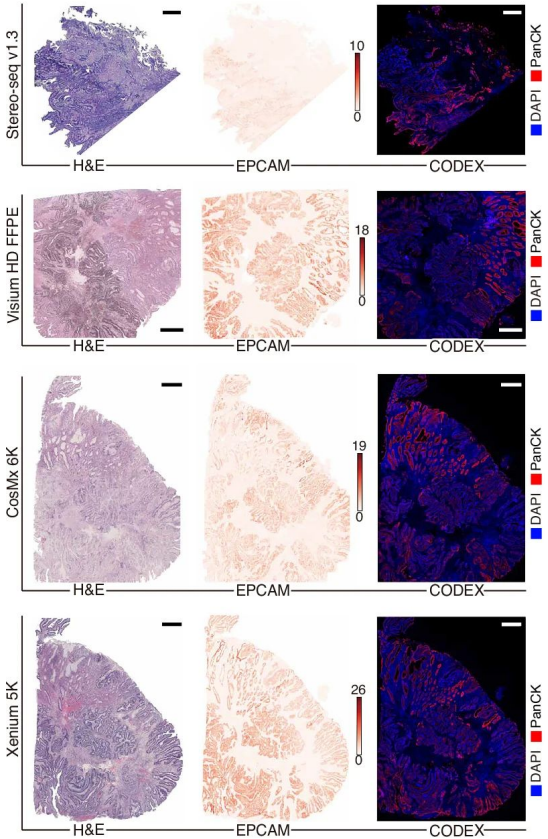


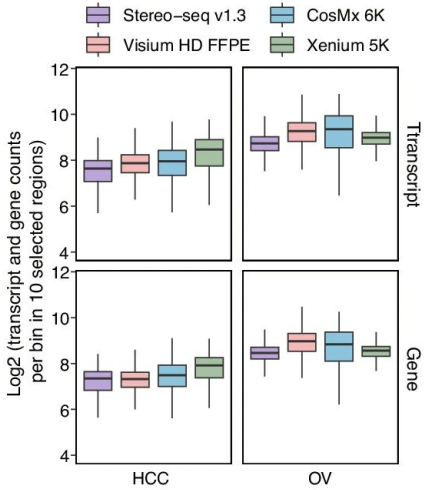
*HCC: Hepatocellular Carcinoma (liver cancer)
COAD: Colon adenocarcinoma (colon cancer)
OV: ovarian tumor

Imaging-based ST:
Xenium and CosMx
Sequencing-based ST:
VisiumHD and Stero-seq

"Ground Truth" Protein Imaging: CODEX (antibody-staining and imaging)
"Ground Truth" RNA Sequencing: scRNAseq

Benchmark: Gene Detection



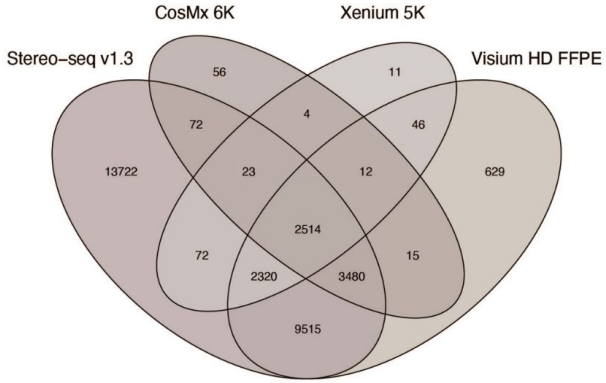


Article | Open access | Published: 17 October 2025

Systematic benchmarking of high-throughput subcellular spatial transcriptomics platforms across human tumors

Pengfei Ren, Rui Zhang, Yunfeng Wang, Peng Zhang, Ce Luo, Suyan Wang, Xiaohong Li, Zongxu Zhang, Yanping Zhao, Yufeng He, Haorui Zhang, Yufeng Li, Zhidong Gao, Xiuping Zhang, Yahui Zhao, Zhihua Liu, Yuanguang Meng, Zhe Zhang & Zexian Zeng

Nature Communications **16**, Article number: 9232 (2025) | Cite this article



Gene detection and sensitivity varies across platforms
→ panel vs whole-transcriptome
→ probe vs transcript capture

Benchmark: Cell Typing

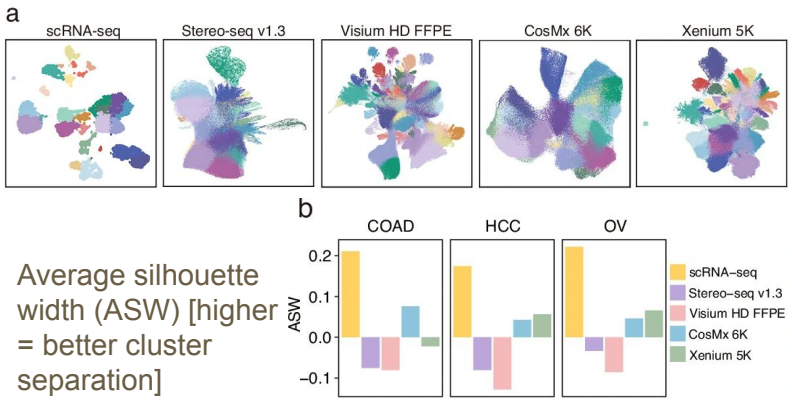
Article | Open access | Published: 17 October 2025

Systematic benchmarking of high-throughput subcellular spatial transcriptomics platforms across human tumors

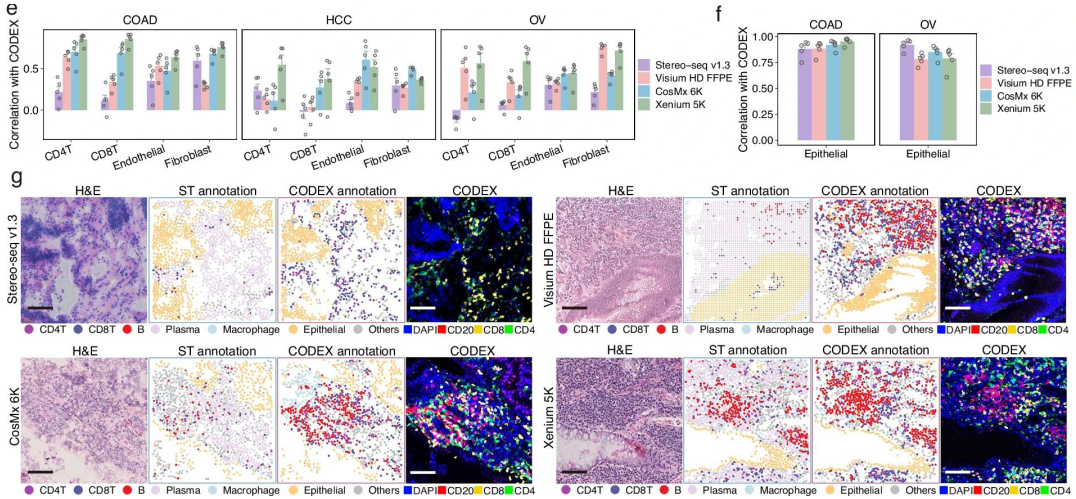
Pengfei Ren, Rui Zhang, Yunfeng Wang, Peng Zhang, Ce Luo, Suyan Wang, Xiaohong Li, Zongxu Zhang, Yanping Zhao, Yufeng He, Haorui Zhang, Yufeng Li, Zhidong Gao, Xiuping Zhang, Yahui Zhao, Zhihua Liu, Yuanguang Meng ✉, Zhe Zhang ✉ & Zexian Zeng ✉

Nature Communications **16**, Article number: 9232 (2025) | Cite this article

Detection effects the clustering…



Average silhouette width (ASW) [higher = better cluster separation]



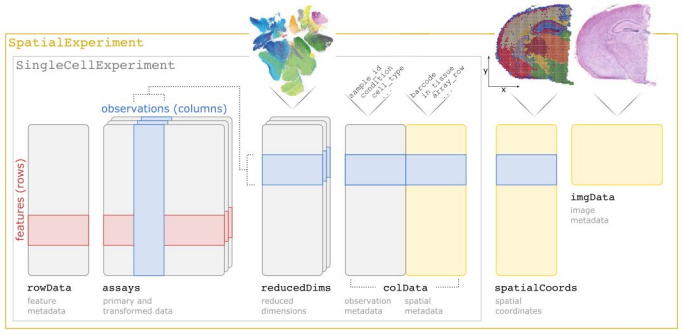
…and cell typing!

Analysis Methods

Tools for Spatial Transcriptomics Analysis

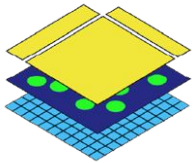
R

Seurat: https://satijalab.org/seurat/



SpatialExperiment (Bioconductor): https://lmweber.org/OSTA/

Python



SpatialData: https://spatialdata.scverse.org

SpatialData

squidpy

GUI

[Each commercial method has their own analysis tools]

Use whatever tool you (and your colleagues) are most comfortable working with!

“Standard” Method Workflow

Looks a lot like scRNAseq methods…

Steps:

1) Quality Check (#genes, #counts per cell/region)
2) Normalization [and Integration]
3) Dimensional Reduction and Clustering
4) Cell typing
5) DEG + further analysis

“Standard” Method Workflow

Looks a lot like scRNAseq methods…
…but with spatial coordinates

Steps:

1) Cell Segmentation or Region Selection
2) Quality Check (#genes, #counts per cell/region)
3) Normalization [and Integration]
4) Dimensional Reduction and Clustering
5) Cell typing
6) Spatial Clustering/Regions
7) DEG + further analysis

Challenges within the “Standard” Method Workflow

1) Cell segmentation
2) Integration, alignment, and spatial clustering of multiple slices

Steps:

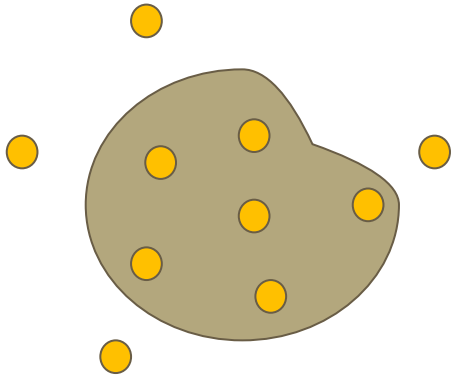
1) Cell Segmentation or Region Selection
2) Quality Check (#genes, #counts per cell/region)
3) Normalization [and Integration]
4) Dimensional Reduction and Clustering
5) Cell typing
6) Spatial Clustering/Regions
7) DEG + further analysis

Challenges #1: How to Best Segment Cells?

The dilemma of cell segmentation

Where is the cell?

Imaging-based
(ex 10X Xenium)



Sequencing-based
(ex. 10X Visium)

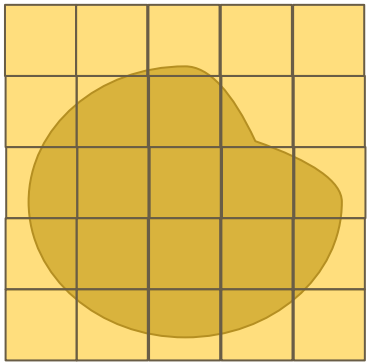


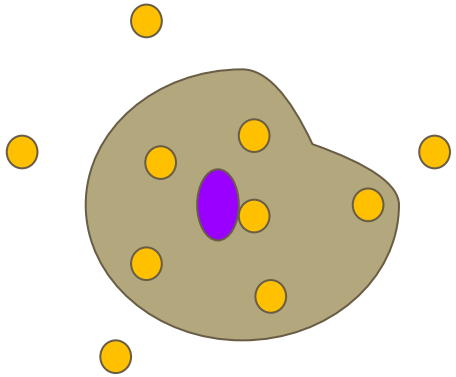
*diagram definitely not to scale

Most methods start with finding the nuclei

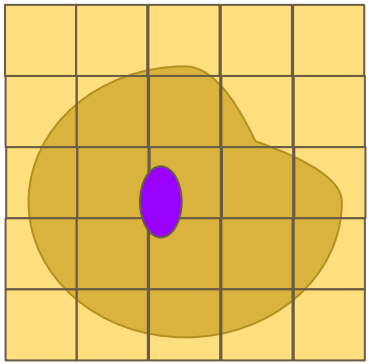
*Example methods: Mesmer, **STARDist**, Cellpose

Where is the nuclei?

Imaging-based
(ex 10X Xenium)



Sequencing-based
(ex. 10X Visium)



Nuclear staining

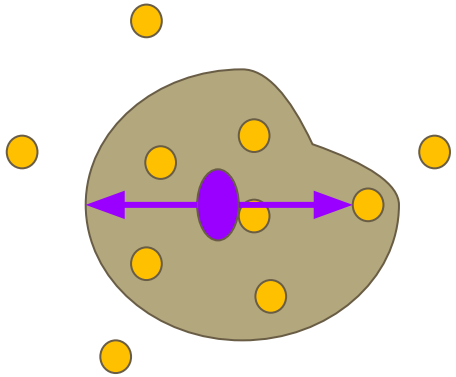
*diagram definitely not to scale

Most methods start with finding the nuclei

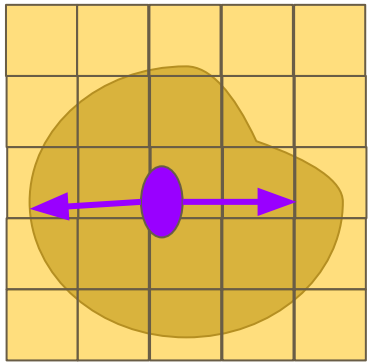
*Example methods: Mesmer, **STARDist**, Cellpose

Where is the nuclei?

Imaging-based
(ex 10X Xenium)



Sequencing-based
(ex. 10X Visium)



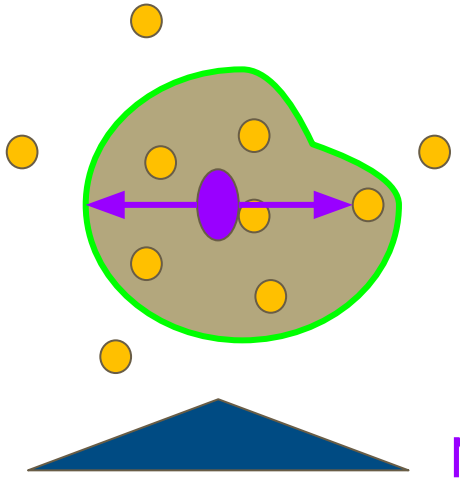
Nuclear staining + expansion

*diagram definitely not to scale

If available, membrane staining to find boundaries

Where is the cell boundary?

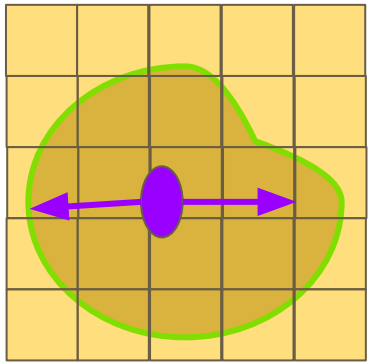
Imaging-based
(ex 10X Xenium)



Membrane staining

Intensity density

Sequencing-based
(ex. 10X Visium)



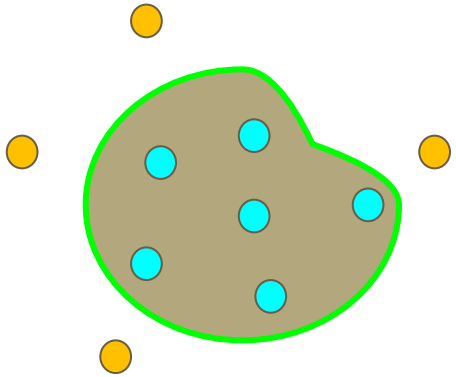
Nuclear staining + expansion

*diagram definitely not to scale

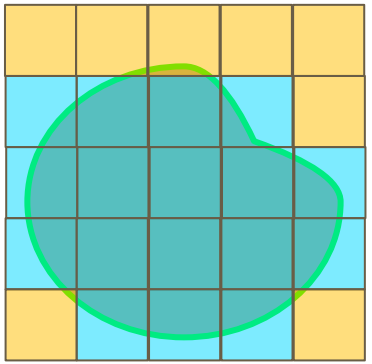
Definition of a cell is the sum of counts in a region

Now group the counts into “cells”

Imaging-based
(ex 10X Xenium)



Sequencing-based
(ex. 10X Visium)

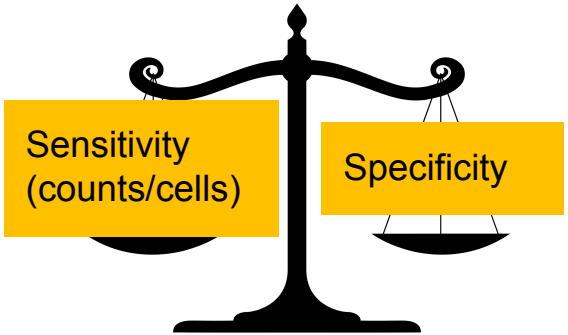


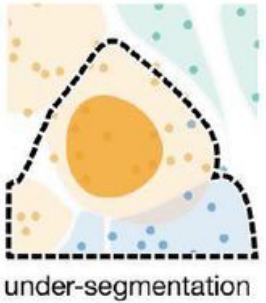
Various methods for the cell boundary + grouping process of cell segmentation [often tissue and method dependent]

*diagram definitely not to scale

“Bad” cell segmentation can limit further analysis

Balance the number of cells with the quality of the cells (no overlapping or empty cells)





Increases in false negatives and false positives

Heidari et al. bioRxiv 2025.03.14.643160; doi: https://doi.org/10.1101/2025.03.14.643160

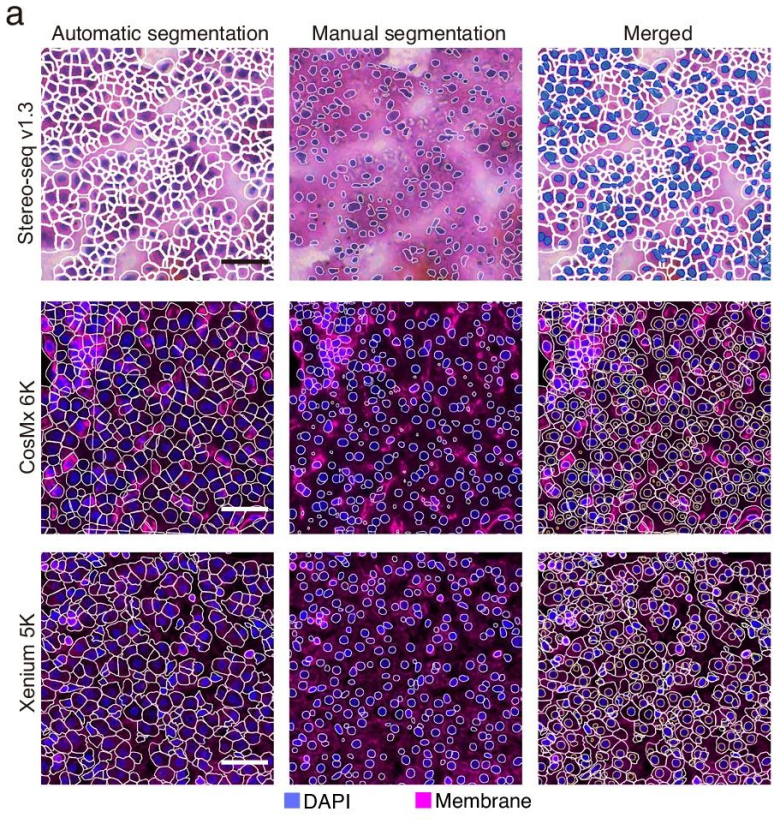
Benchmark: Cell Segmentation

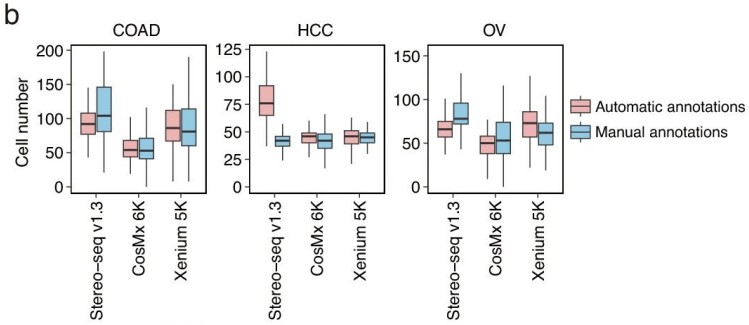
Article | Open access | Published: 17 October 2025

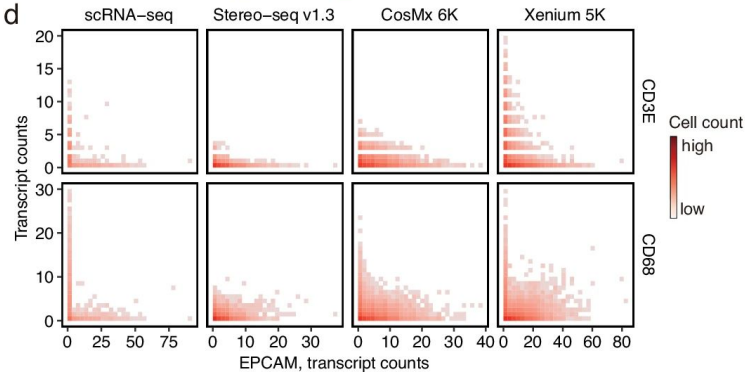
Systematic benchmarking of high-throughput subcellular spatial transcriptomics platforms across human tumors

Pengfei Ren, Rui Zhang, Yunfeng Wang, Peng Zhang, Ce Luo, Suyan Wang, Xiaohong Li, Zongxu Zhang, Yanping Zhao, Yufeng He, Haorui Zhang, Yufeng Li, Zhidong Gao, Xiuping Zhang, Yahui Zhao, Zhihua Liu, Yuanguang Meng ✉, Zhe Zhang ✉ & Zexian Zeng ✉

Nature Communications **16**, Article number: 9232 (2025) | Cite this article







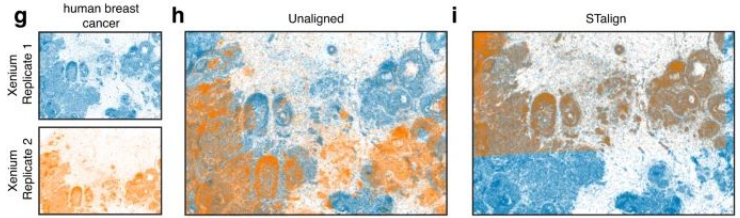
Unclear cell segmentation → unclear cell typing → unclear DEGs and downstream analysis!

Challenges #2: How to Integrate and Align Multiple Samples?

Alignment vs Integration of Slices

Alignment

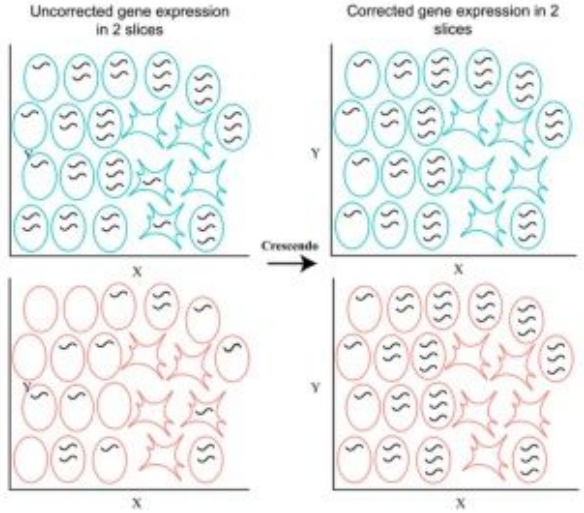
"Merging" the slices together (ex. mapping coordinates to stack)



Clifton, K., Anant, M., Aihara, G. et al. STalign. Nat Commun 14, 8123 (2023). https://doi.org/10.1038/s41467-023-43915-7

Integration

"Combining" the data together (ex. batch correction)



Millard, N., Chen, J.H., Palshikar, M.G. et al. Crescendo. Genome Biol 26, 36 (2025). https://doi.org/10.1186/s13059-025-03479-9

Alignment and Integration of Slices

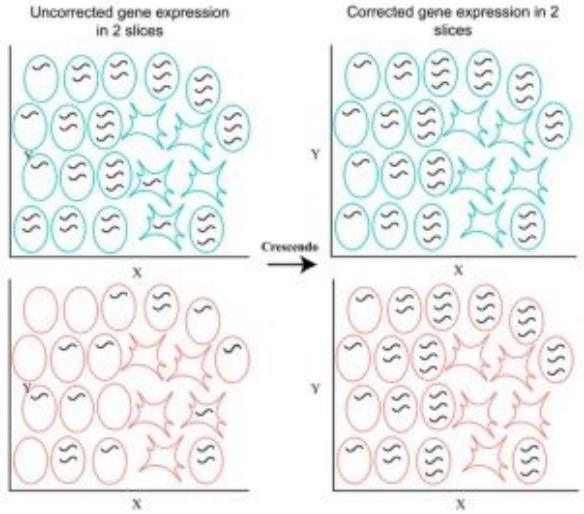
Alignment

"Merging" the slices together (ex. mapping coordinates to stack)

Integration

"Combining" the data together (ex. batch correction)

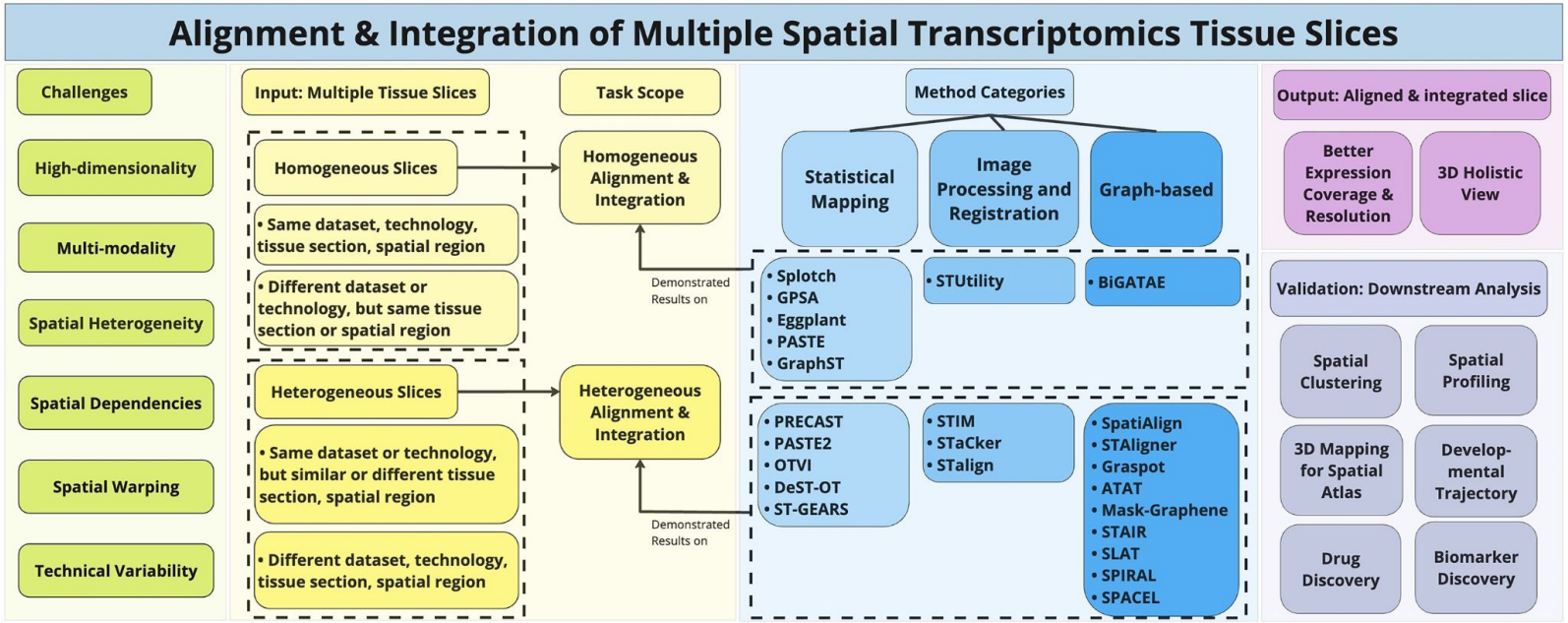
These methods can work together!



Clifton, K., Anant, M., Aihara, G. et al. STalign. Nat Commun 14, 8123 (2023). https://doi.org/10.1038/s41467-023-43915-7

Millard, N., Chen, J.H., Palshikar, M.G. et al. Crescendo. Genome Biol 26, 36 (2025). https://doi.org/10.1186/s13059-025-03479-9

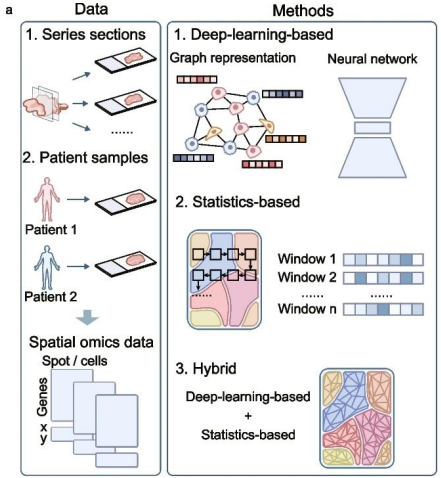
Various Alignment and Integration Methods



Muiz Khan, Suzan Arslanturk, Sorin Draghici, A comprehensive review of spatial transcriptomics data alignment and integration, Nucleic Acids Research, Volume 53, Issue 12, 8 July 2025, gkaf536, https://doi.org/10.1093/nar/gkaf536

A number of methods are available with different underlying statistics/assumptions but often the "best" method will ***depend on tissue type***

Benchmark: Alignment and Integration

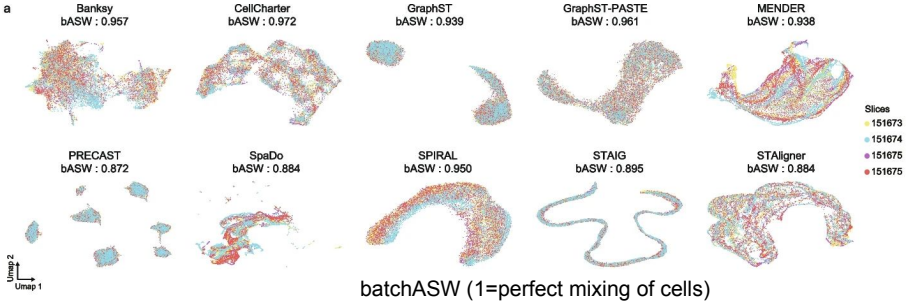


How well the integration performs will affect the downstream analysis (are you getting the "like"/similar cells together?)

Data	Slices number	Total number of cells/spots	Cells/Spots	Genes
DLPFC S1	4	17985	4496	33538
DLPFC S2	4	15101	3775	33538
DLPFC S3	4	14243	3561	33538
MERFISH Preoptic	5	28317	5863	155
MERFISH Brain S2	2	40484	20242	374
MERFISH Brain S3	2	35347	17674	374
MERFISH Brain S4	3	31508	10503	374
MERFISH Brain S5	3	29371	9790	374
MERFISH Brain S6	3	25907	8636	374
MERFISH Brain S7	3	32792	10931	374

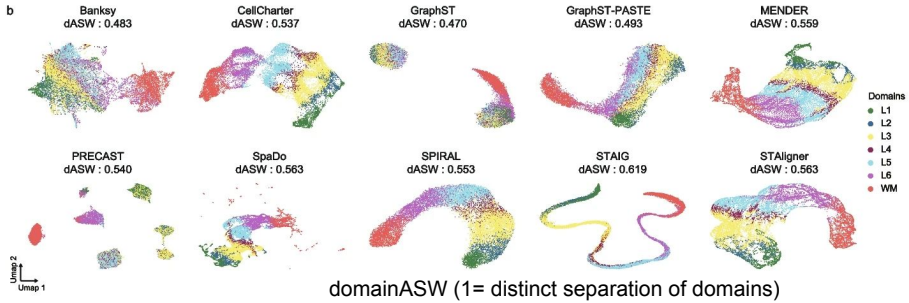
Data	Slices number	Total number of cells/spots	Cells/Spots	Genes
MERFISH Brain S8	3	38338	12779	374
MERFISH Brain S9	3	33868	11289	374
MERFISH Brain S10	3	28105	9368	374
MERFISH Brain S11	3	28955	9652	374
MERFISH Brain S12	2	33241	16621	374
Large-scale dataset	33	161384	4890	254
BaristaSeq	3	5257	1752	79
STARMap	3	3190	1063	166
TNBC	40	197678	4942	36

How well did the samples integrate?



batchASW (1=perfect mixing of cells)

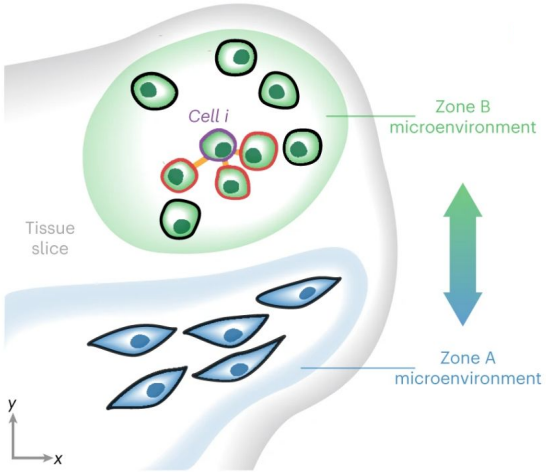
How well were distinct spatial regions separated?



domainASW (1= distinct separation of domains)

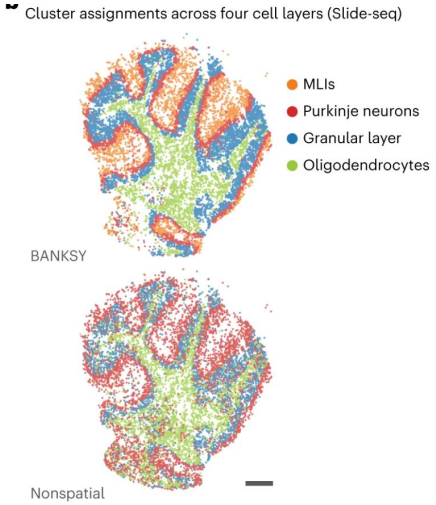
Dong, K., Gao, Y., Zou, Q. et al. Benchmarking multi-slice integration and downstream applications in spatial transcriptomics data analysis. Genome Biol 26, 318 (2025). https://doi.org/10.1186/s13059-025-03796-z

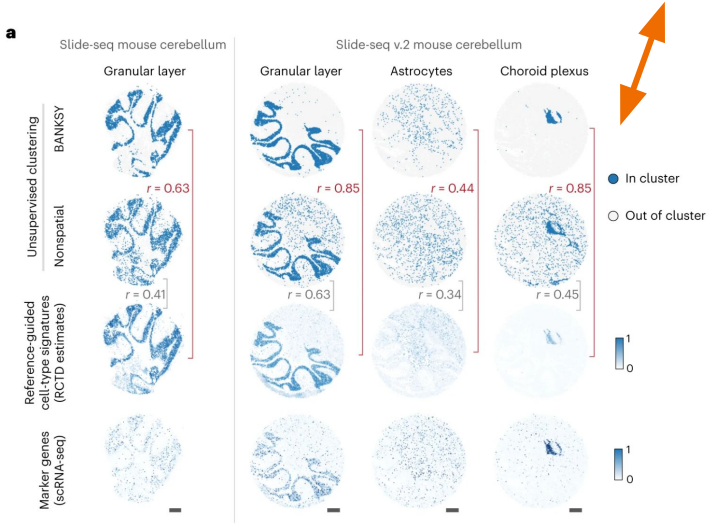
Spatial Clustering Method: BANKSY



Cells live in unique microenvironments

→ Including the spatial information can aid clustering and cell typing

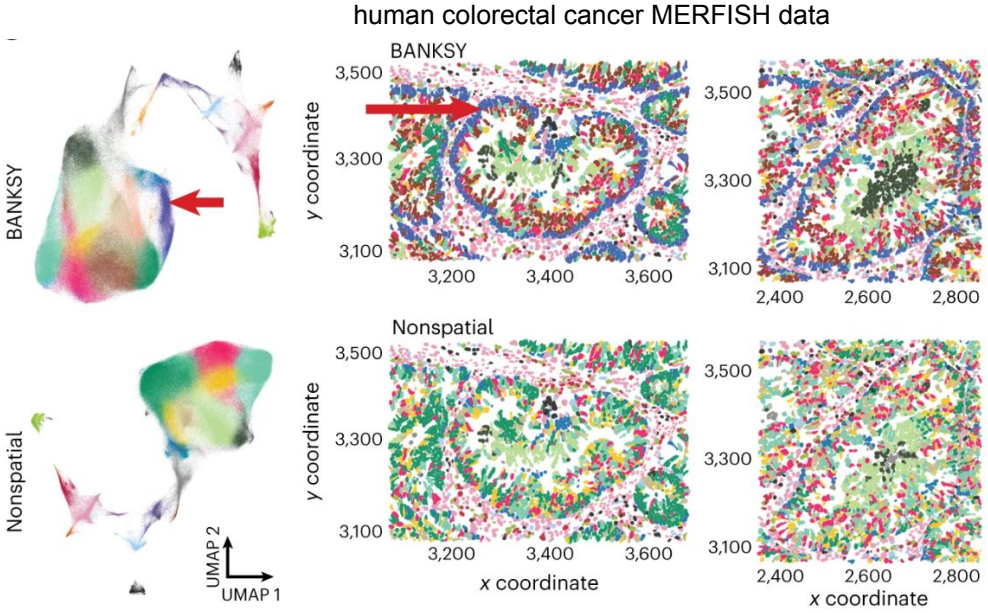




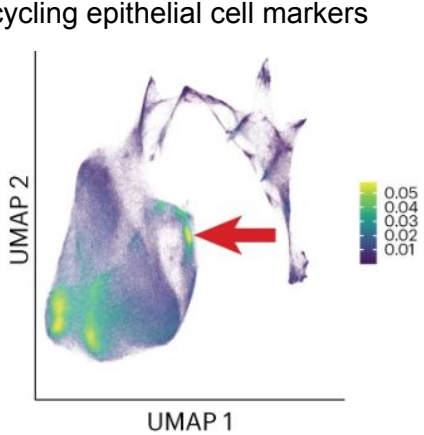
Singhal, V., Chou, N., Lee, J. et al. BANKSY unifies cell typing and tissue domain segmentation for scalable spatial omics data analysis. Nat Genet 56, 431–441 (2024). https://doi.org/10.1038/s41588-024-01664-3

Spatial Clustering Method: BANKSY

*Other methods are available and will perform better/worse depending on the tissue type!



Better clustering*
→ clearer marker gene expression
→ clearer cell typing
→ clearer discoveries on tissue-wide gene expression patterns



Singhal, V., Chou, N., Lee, J. et al. BANKSY unifies cell typing and tissue domain segmentation for scalable spatial omics data analysis. Nat Genet 56, 431–441 (2024). https://doi.org/10.1038/s41588-024-01664-3

Take-home Messages

- There are many methods for spatial transcriptomics–***find the method that works for your experimental design, (budget), and data quality needs***
  - Methods differ by detection method, number of genes, sensitivity
- Cell segmentation is a very important quality check!
  - After cell segmentation steps, if you can get the single-cell feature x count matrix, the analysis is very similar to the scRNAseq analysis methods (just add some spatial plots!)
- Integration and clustering can be challenging and those methods are still being developed and benchmarked
- ***Both the experimental and bioinformatic methods vary depending on the sample tissues so you might need to try multiple methods!***
  - Discuss with your biologist friends and histology experts (understanding tissue structure and cell types can be confusing!)

It is an expanding field so have fun and be patient as you learn new methods!