A comprehensive benchmarking for spatially resolved transcriptomics clustering methods across variable technologies, organs, and replicates

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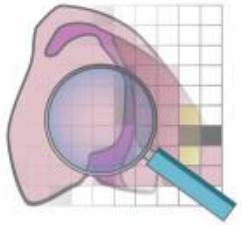
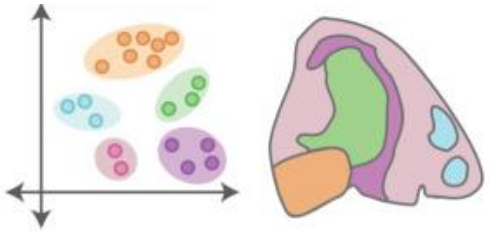
Introduction

Spatial Clustering: A Key Step in Data Analysis

SRT data

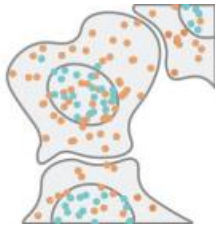


spatial clustering



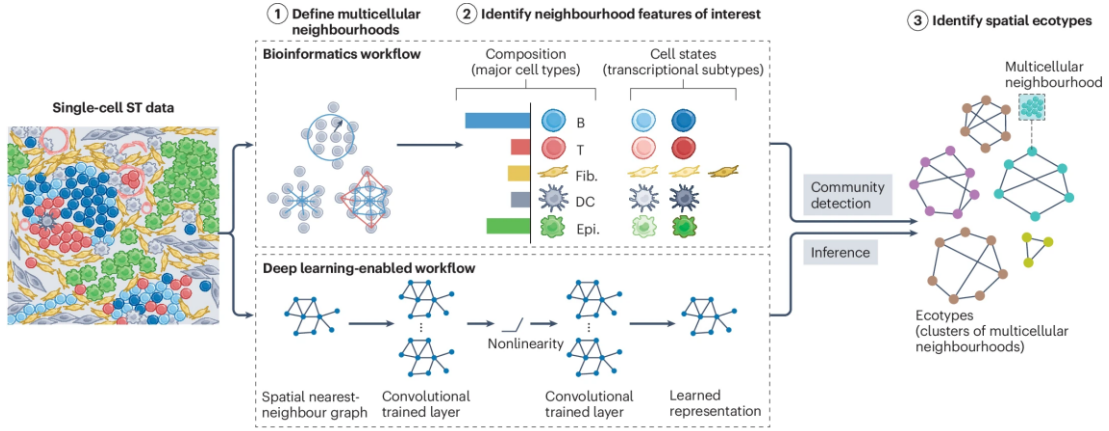
spatially variable genes

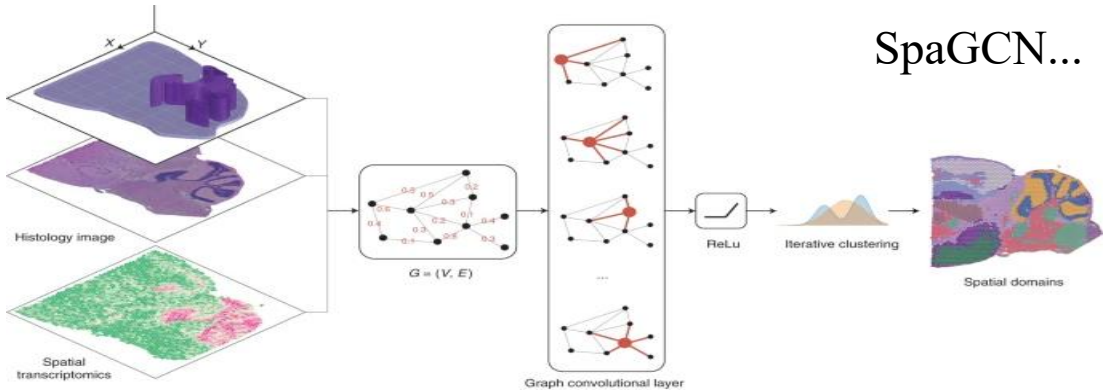
subcellular



cell cell communication

Numerous Spatial Clustering Methods Are Available





SpaGCN...

How to select the appropriate clustering method for different technologies, organs, and replicates?

Gulati et al. (2025) *Nat Rev Mol Cell Biol*.
Hu et al. (2021) *Nat Methods*.
Dries et al. (2021) Genome Res.

Highlights

- A **comprehensive benchmarking** of **14** spatial clustering methods using **~600** datasets across ten technologies and eight organs.
- We provided practical **recommendations for method selection** across technologies, organs, and biological replicates, involving either cell type clustering or spatial domain identification.
- We systematically assessed the influence of data characteristics and spatial patterns on clustering accuracy and offered the optimal **preprocessing pipeline** for spatial clustering methods.

Public Datasets

10× GENOMICS · GEO Gene Expression Omnibus · SPATIAL research · CZ CELL×GENE DISCOVER · SPASCER · STOmicsDB · DB · Mendeley Data

Simulated Datasets

SRTsim · scCube

Brain Datasets from 10 Technologies

Sequencing-based SRT
- ST
- 10× Visium
- Slide-seq
- Stereo-seq
- Visium HD

Imaging-based SRT
- MERFISH
- seqFISH+
- STARmap
- CosMx
- Xenium

10× Visium Datasets from 8 Organs

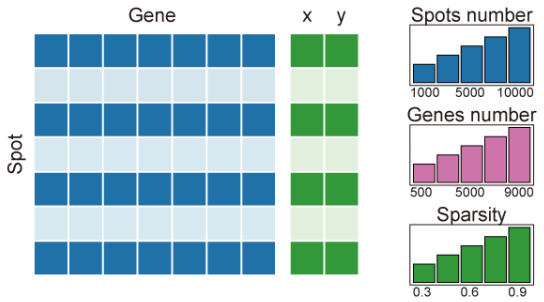
Spatial Clustering Methods

BASS	PRECAST
Banksy	SEDR
BayesSpace	SpaceFlow
CCST	SpatialMGCN
CellCharter	SpaGCN
DeepST	STAGATE
GraphST	stLearn

Preprocessing steps

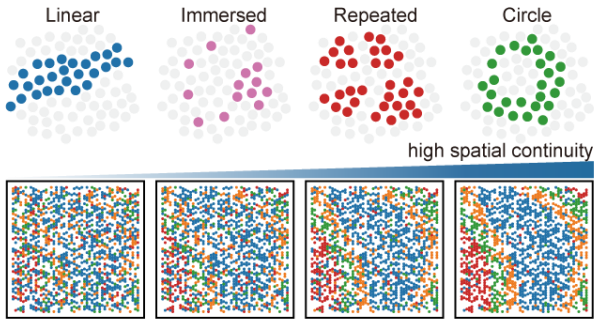
Normalization · Log transformation
Genes selection · Standardization...

Evaluation on datasets from variable technologies varing in spatial resolution



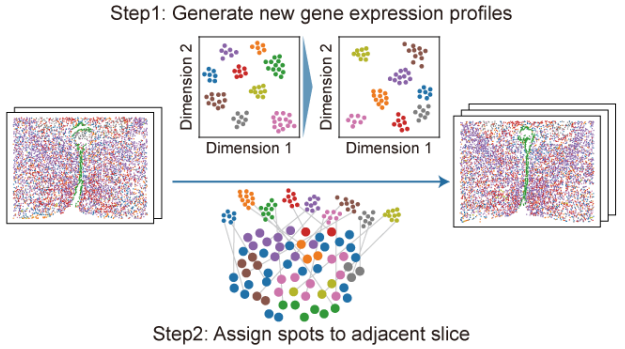
High Spatial Resolution (MERFISH): BASS...
Low Spatial Resolution (10× Visium): STAGATE...

Evaluation on datasets from variable organs varing in spatial continuity



High Spatial Continuity (Brain): STAGATE...
Low Spatial Continuity (Liver): PRECAST...

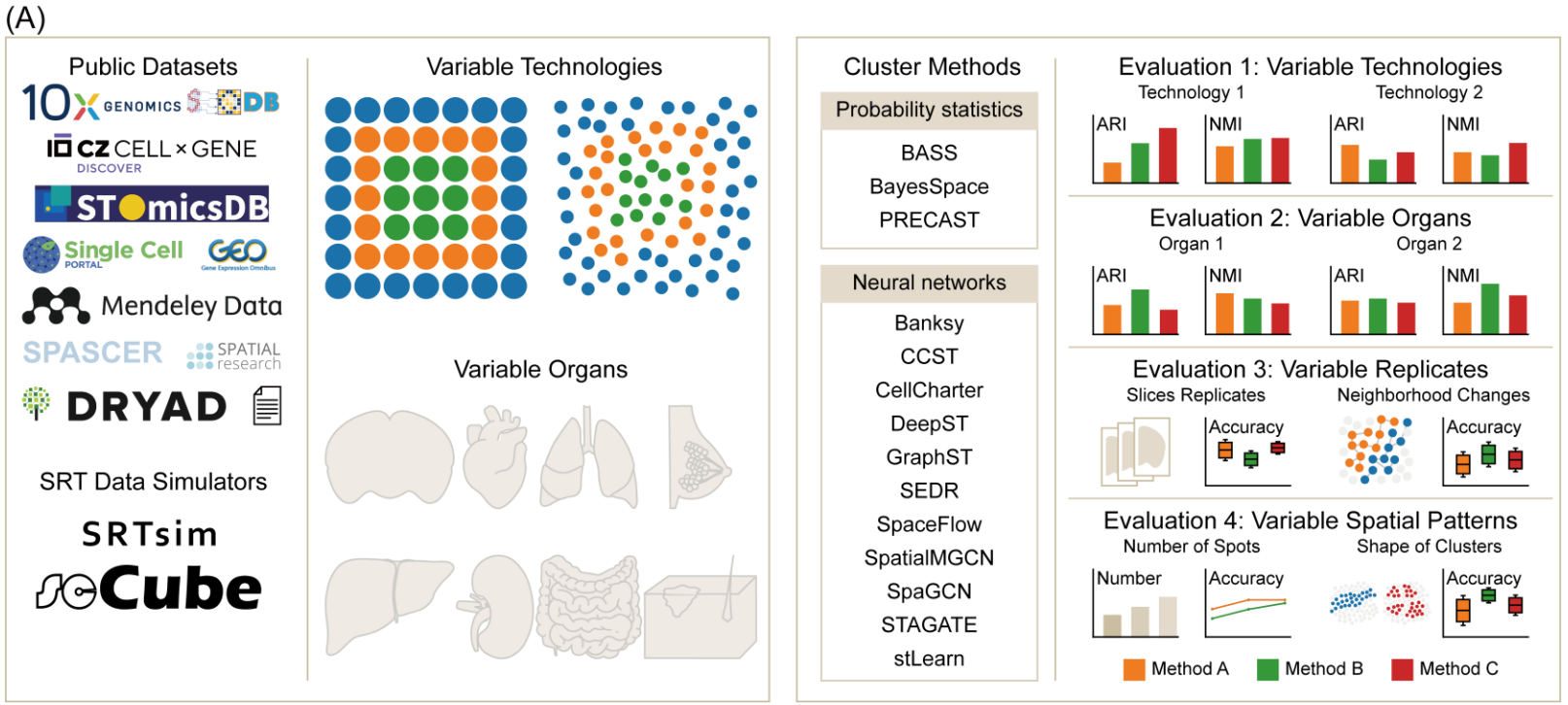
Evaluation on datasets with replicates



Datasets with Replicates: DeepST...

Overview of benchmarking

Figure 1. Overview of datasets and methods for benchmarking.



(B)

Technology	Slices	Spatial Resolution	Spots per Slice	Genes Detected	Sparsity	Total Spots
Sequencing-based						
ST	35	200 μm – 100 μm	458	23371	0.822	16024
10× Visium	151	100 μm – 55 μm	2446	32080	0.919	369357
Slide-seq	102	10 μm	24569	23484	0.986	2506054
Stereo-seq	5	0.5 μm – 0.22 μm	72449	25077	0.970	362247
Visium HD	6	2 μm	268419	18572	0.957	1610513
Imaging-based						
seqFISH+	14	≤ 0.1 μm	211	9961	0.819	2956
STARmap	5	0.2 ~ 0.3 μm	1065	508	0.727	5327
MERFISH	41	≤ 0.1 μm	4882	182	0.631	200163
CosMx	6	≤ 0.1 μm	51700	957	0.779	310199
Xenium	3	≤ 0.1 μm	143211	291	0.751	429634

Evaluation 1: Performance comparison across technologies

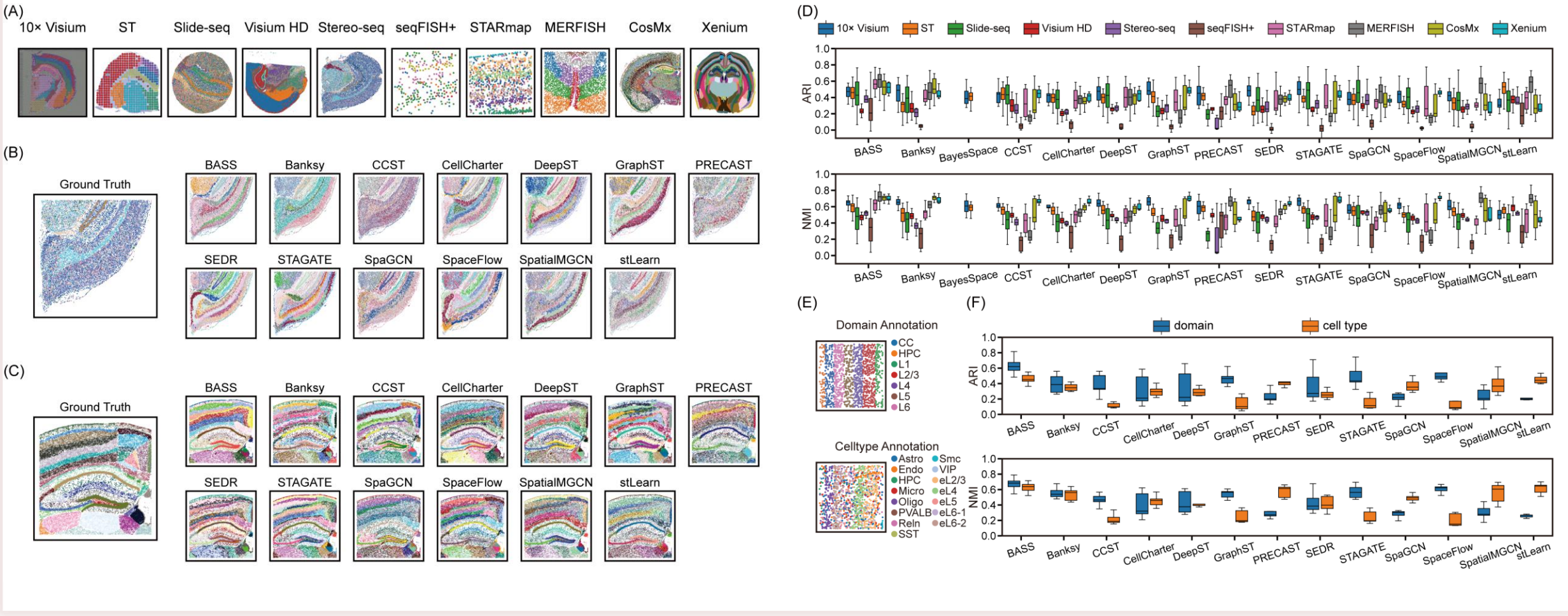


Figure 2. Performance comparison with SRT datasets across technologies.

Evaluation 2: Performance comparison across organs

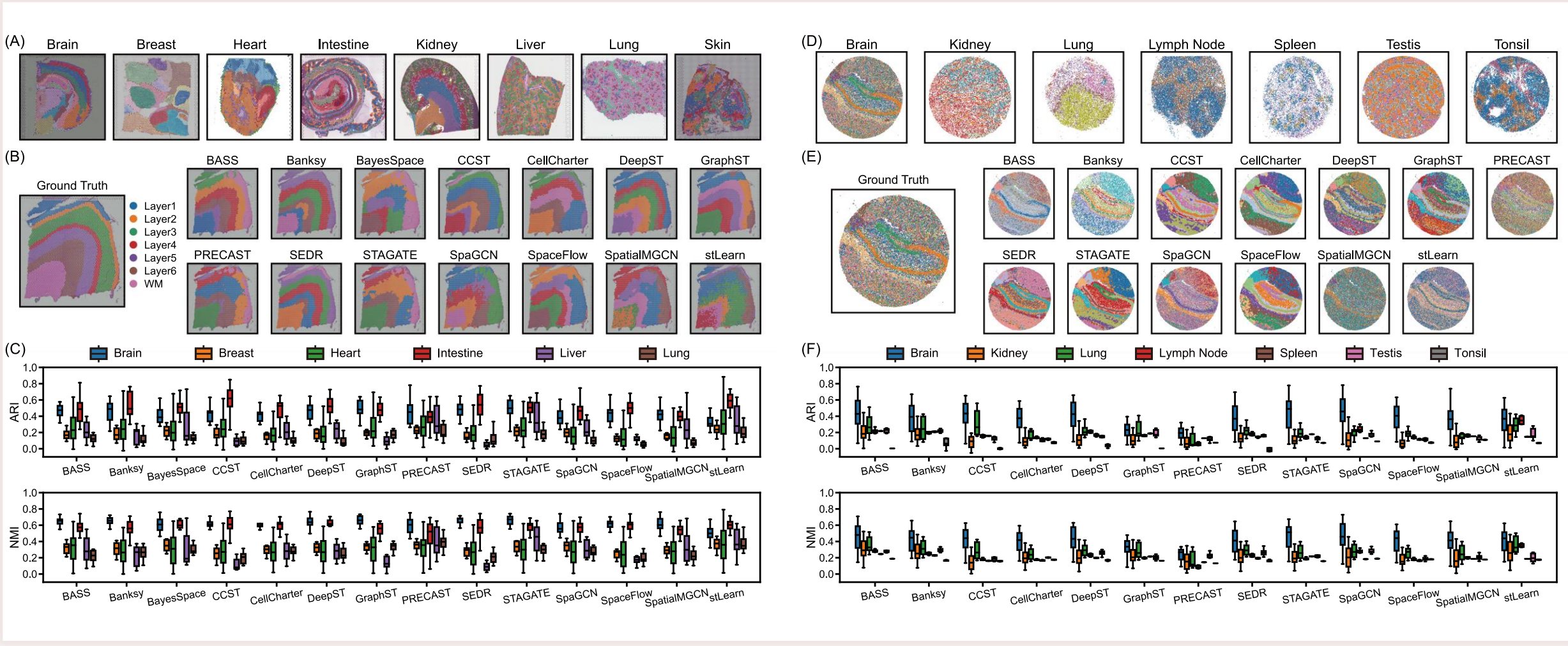


Figure 3. Performance comparison with SRT datasets across organs.

Evaluation 3: Performance comparison across replicates

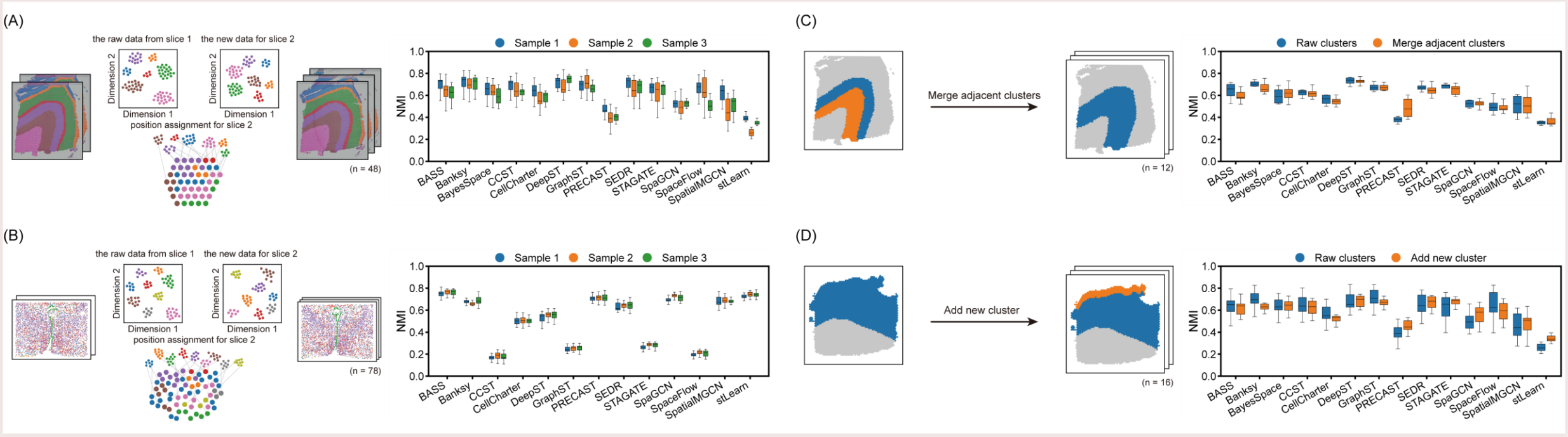


Figure 4. Performance comparison with SRT datasets across biological replicates.

(A) Simulation of variable replicates based on DLPFC 10× Visium datasets. (B) Simulation of variable replicates based on the hypothalamus MERFISH dataset. (C) Design of simulation for neighborhood-changing replicates by merging adjacent clusters. (D) Design of simulation for neighborhood-changing replicates by adding new clusters. Box plots show NMI scores on simulated datasets. The box represents the interquartile range, the horizontal line inside the box indicates the median, and the whiskers extend to 1.5 × interquartile range.

Evaluation 4:Performance comparison across spatial patterns

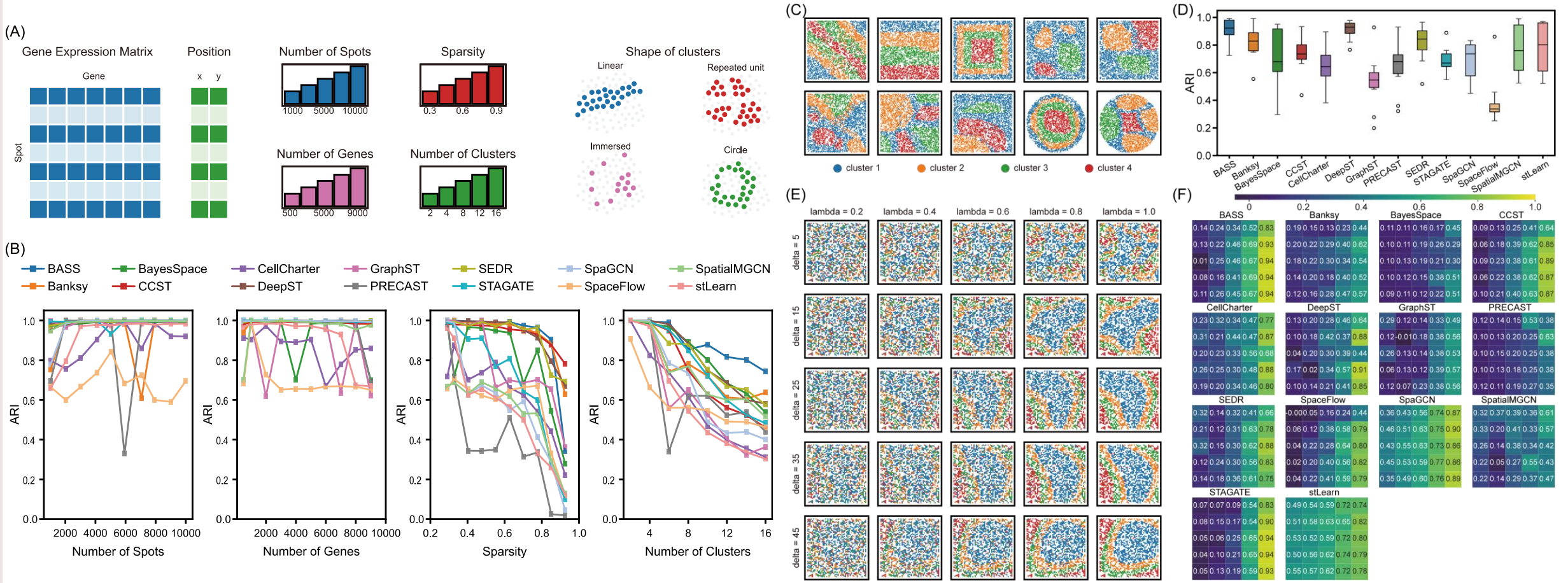


Figure 5. Performance comparison with SRT datasets across spatial patterns.

Summary

- We conducted a comprehensive benchmark of 14 spatial clustering methods across diverse SRT datasets covering multiple technologies, organs, and biological replicates and provided recommendations for method selection.
- Performance variability was largely driven by intrinsic dataset characteristics and methodological design choices, with gene expression sparsity and cluster distribution emerging as critical determinants.
- Preprocessing strategies also exerted variations in performance, and we identified the optimal pipeline for each method.
- Github: https://github.com/ZJUFanLab/SRTBenchmark

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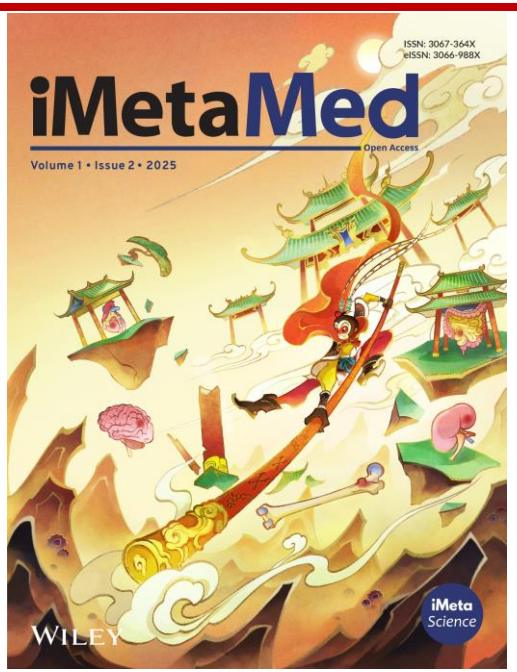

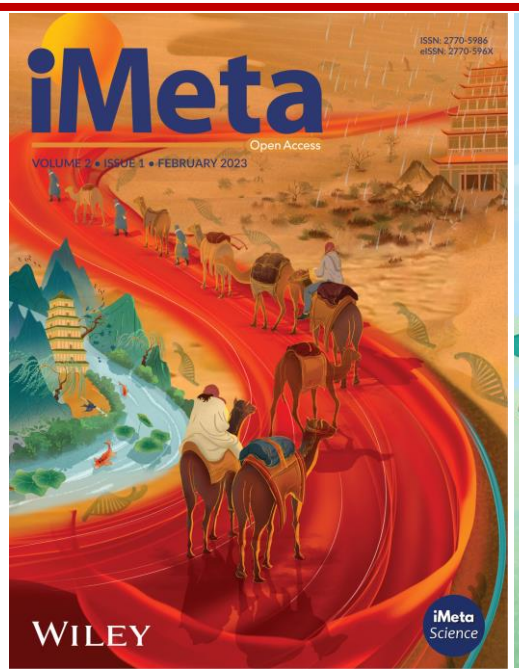

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