

Table S1. Statistical test of the simulation results with 10% permutation

Datasets	Methods1	Methods2	Pval_AC	Pval_NMI	Pval_ARI
151507	<i>K</i> -means + PCA	DSSC	4.24633E-13	3.28874E-14	2.0376E-13
151507	SC3	DSSC	5.18832E-14	5.36234E-17	4.54032E-15
151507	Seurat	DSSC	4.66238E-15	5.61941E-13	1.68655E-14
151507	BayesSpace	DSSC	0.082742145	0.119675253	0.120332566
151507	Giotto	DSSC	4.66252E-08	1.77105E-07	3.495E-07
151507	spaGCN	DSSC	0.00126916	0.000167417	0.003788902
151507	stLearn	DSSC	9.9485E-11	4.79135E-12	9.39773E-12
osmFish	<i>K</i> -means + PCA	DSSC	1.35883E-10	2.36585E-10	4.95313E-11
osmFish	SC3	DSSC	3.02622E-14	8.97591E-15	9.08673E-16
osmFish	Seurat	DSSC	2.43221E-09	9.52464E-11	7.35759E-10
osmFish	BayesSpace	DSSC	1.60168E-08	3.67412E-08	4.25781E-09
osmFish	Giotto	DSSC	1.12121E-07	9.31088E-07	4.97187E-07
osmFish	spaGCN	DSSC	1.5628E-05	3.05188E-06	2.46248E-05
osmFish	stLearn	DSSC	1.8695E-10	1.86878E-10	5.91744E-10

Table S2. Statistical test of the simulation results with 15% permutation

Datasets	Methods	Methods	Pval_AC	Pval_NMI	Pval_ARI
151507	K-means + PCA	DSSC	2.43431E-09	1.89334E-12	1.40263E-10
151507	SC3	DSSC	1.77821E-11	2.25215E-17	2.07945E-12
151507	Seurat	DSSC	1.46316E-10	1.51041E-11	7.82944E-11
151507	BayesSpace	DSSC	0.283311661	0.322865463	0.345060618
151507	Giotto	DSSC	8.76033E-05	3.53207E-06	8.19187E-05
151507	spaGCN	DSSC	0.024437147	0.000562175	0.012315758
151507	stLearn	DSSC	7.54573E-08	3.32349E-10	1.06938E-08
osmFish	K-means + PCA	DSSC	2.14806E-10	8.87443E-10	5.06215E-10
osmFish	SC3	DSSC	1.1814E-14	7.26523E-17	6.14065E-15
osmFish	Seurat	DSSC	1.89863E-08	3.04302E-10	8.1556E-09
osmFish	BayesSpace	DSSC	7.83113E-08	1.97529E-05	5.59584E-07
osmFish	Giotto	DSSC	1.31499E-09	3.46474E-10	1.24645E-09
osmFish	spaGCN	DSSC	4.02776E-05	1.06192E-05	3.74999E-05
osmFish	stLearn	DSSC	7.13647E-10	3.29909E-10	3.02557E-09

Table S3. Statistical test of the simulation results with 20% permutation

Datasets	Methods	Methods	Pval_AC	Pval_NMI	Pval_ARI
151507	<i>K</i> -means + PCA	DSSC	6.89168E-14	1.04625E-13	3.67873E-15
151507	SC3	DSSC	1.78392E-15	2.81701E-18	7.01654E-16
151507	Seurat	DSSC	2.62682E-13	1.39264E-11	1.23138E-13
151507	BayesSpace	DSSC	0.10972168	0.277267626	0.214369712
151507	Giotto	DSSC	3.68703E-07	1.20838E-07	9.85883E-07
151507	spaGCN	DSSC	0.004347438	0.003294814	0.008378494
151507	stLearn	DSSC	3.32357E-10	1.7915E-10	6.27605E-11
osmFish	<i>K</i> -means + PCA	DSSC	3.99819E-09	2.84669E-08	3.19154E-09
osmFish	SC3	DSSC	9.24393E-13	3.27959E-15	7.3741E-14
osmFish	Seurat	DSSC	4.12662E-08	7.63717E-09	3.81263E-08
osmFish	BayesSpace	DSSC	6.05709E-08	0.000359375	6.56227E-06
osmFish	Giotto	DSSC	1.39367E-07	1.7724E-07	9.13902E-08
osmFish	spaGCN	DSSC	1.19089E-05	1.21578E-05	2.37321E-05
osmFish	stLearn	DSSC	1.26497E-07	5.1673E-08	2.28827E-07

Table S4. Statistical test of the spatialLIBD data results.

Method1	Method2	Pval_AC	Pval_NMI	Pval_ARI
BayesSpace	DSSC	0.003729	0.116339	0.008479
spaGCN	DSSC	2.29E-05	1.96E-06	0.000432
stlearn	DSSC	2.2E-07	4.66E-07	8.03E-06
Seurat	DSSC	9.73E-08	8.64E-08	7.82E-06
K-means+PCA	DSSC	1.98E-08	2.99E-08	2.69E-06
SC3	DSSC	2.3E-05	7.28E-08	2.43E-06
Giotto	DSSC	3.43E-06	8.69E-08	7.66E-06

Table S5. Statistical test of the 10xMBAD data results.

Methods1	Methods2	Pval-Silhouette
spaGCN	DSSC	0.211242658
BayesSpace	DSSC	0.476883153
spatialPCA	DSSC	0.002673712
stlearn	DSSC	0.000343514
Seurat	DSSC	0.032782218
K-means+PCA	DSSC	0.028644015
SC3	DSSC	0.043526239
Giotto	DSSC	0.003877227

Table S6. Statistical test of the K (in *k*NN) tuning results.

<i>k</i> NN1	<i>k</i> NN2	pval_AC	pval_NMI	pval_ARI
10	0	0.00036644	7.1717E-07	6.872E-05
20	0	0.00045065	1.5229E-06	0.0002041
40	0	0.00070832	1.444E-06	0.00040561
80	0	0.00032282	3.6124E-06	0.00053503
160	0	0.00083513	7.4927E-06	0.00044418

Table S7. Statistical test of the Gamma (clustering loss) tuning results.

Gamma1	Gamma2	pval_AC	pval_NMI	pval_ARI
0.001	0	0.32270604	0.26621917	0.30667892
0.01	0	0.01047752	0.00072134	0.00151559
0.1	0	0.57586793	0.62270734	0.15635105
1	0	0.94119189	0.92822479	0.8616268
10	0	0.99289988	0.99766288	0.98608235

Table S8. Statistical test of the constraint number tuning results.

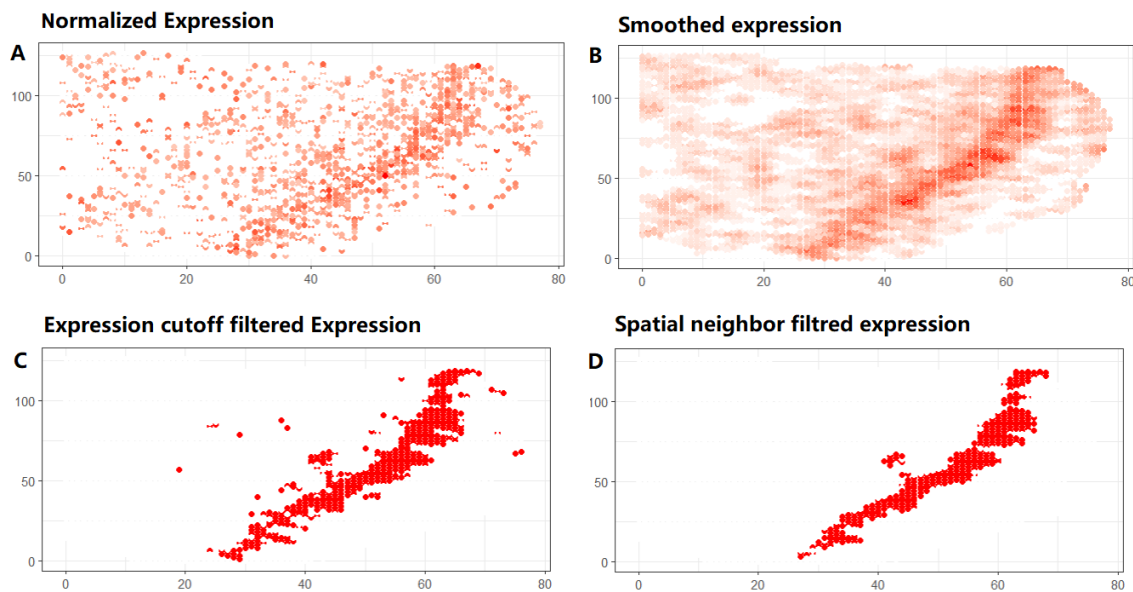
Constraints1	Constraints2	pval_AC	pval_NMI	pval_ARI
2000	0	0.07327009	0.07467783	0.06374234
4000	0	0.01695955	0.00296233	0.02178978
6000	0	0.01707602	0.02126256	0.01863524

Supplementary Notes

Select marker genes as constraints

For the experiments on the SpatialLIBD dataset, we use the marker genes reported from the original paper of this dataset. Users can add other marker genes according to their prior knowledge or the aim of the study. Before using the marker genes, we suggest checking the spatial dependency and the filtered smoothed expression pattern of the genes (Codes are provided in the R script “make_links_from_Markers.R”). The figures below show a good marker and a bad marker, respectively.

1. A good marker expresses in a single region or continuous regions (or only has low expression in a region, such as ENC1 in WM). Here is an example of PCP4:



2. A poor marker expresses in multiple regions. Here is an example of MALAT1:

