1 Supplementary Notes

1.1 Comparison with other spatial domain identification methods parameter settings

We quantitatively compared STMVGAE with other methods on different datasets, including the non-spatial method SCANPY [1], and the spatial methods stlearn [2], SEDR [3], SpaGCN [4], DeepST [5], STAGATE [6] and STAMaker [7]. The parameter settings of these methods are as follows:

- SCANPY: First, we used the same data preprocessing method as STMVGAE to preprocess gene expression (log-transformed, normalized and selecting the top 3,000 HVGs). PCA dimensionality reduction was then used to reduce the gene expression data to 30 PCs. Finally, we used the *scanpy.pp.neighbor()* function default parameters provided by the SCNAPY package [1] to calculate neighbors, and the *scanpy.tl.louvain()* function is used to allocate spots. Additionally, the resolution parameter was tuned manually to ensure the number of clustering is equal to the ground truth.
- stlearn: We chose default parameters for stlearn on the DLPFC dataset. Specifically, the *stLearn.SME.SME_normalized()* function was performed on the raw gene expression of all genes with the parameter use_data="raw" and weights="physical_distance". Then the first 30 PCs of the SME normalized matrix were used for clustering. We did not use stlearn for training on the melanoma dataset because it does not support training without histology images.
- SEDR: SEDR can be trained on all datasets, and we retain all its default parameters except for empirically selecting the number of neighbors on different datasets to ensure reasonable results. We perform the same strategy on each dataset, looking for the number of neighbors that gives the best results between 6 and 12 neighbors. We set n in the *SEDR.graph_construction()* function to 10 on the DLPFC dataset and to 12 on all other datasets.
- SpaGCN: We use its recommended parameters for SpaGCN in all datasets.
- DeepST: We retain all the default parameters of DeepST and set k in the *deepen._get_graph()* function to 12. Additional, We tested the results on the melanoma dataset with DeepST set up without using histological images.
- STAGATE: STAGATE builds the graph by looking for neighbors within a radius, so the parameter r in the *STAGATE.Cal_Spatial_Net()* function changes in each dataset. We used the same rules as SEDR to select r. In DLPFC, we used the recommended parameter r set to 150, r in the BCDC data set to 350, r in the melanoma data set to 2, and r in the BRCA data set to 300.
- STAMaker: Recommended parameters are used in STAMaker, and neighbor selection is consistent with STAGATE. We set n to randomly initialize the model in STAMaker to 5.

1.2 Evaluation metrics of clustering

ARI. The adjusted Rand index (ARI) is a measure of the similarity between two clusterings, and it is an external evaluation index. We introduce ARI to calculate the similarity between the results obtained by STMVGAE spot assignment and manual annotation. The calculation of ARI must first calculate the values of the contingency table. The contingency table contains the following four parts: TF is the count of spot pairs classified into the same cluster in both the true and predicted clustering. TN is the count of spot pairs classified into different clusters in both the true and predicted clustering. FN is the count of spot pairs classified into the same cluster in the true clustering but into different clusters in the predicted clustering. FP is the count of spot pairs classified into different clusters in the true clustering but into the same cluster in the predicted clustering. The value range of ARI is between [-1,1]. Generally, the closer the ARI value is to 1, the better the result. The closer the ARI value is to 0, the clustering result is the same as the random clustering result. The calculation method

of ARI is based on paired samples. It considers the combination of samples of the same category in different clusters in two clustering results and compares it with random situations. ARI is computed as:

$$ARI = \frac{TP + TN - E}{TP + TN + FP + FN - E} \tag{1}$$

The expected value of the index, denoted as E, represents the value that would be obtained if the clustering were entirely random. It is calculated as follows:

$$E = \frac{(TP + FP) \times (TP + FN) + (FN + TN) \times (FP + TN)}{TP + TN + FP + FN} \tag{2}$$

NMI. Normalized Mutual Information (NMI) is an indicator used to evaluate the performance of clustering algorithms. It measures the similarity between two clustering results. The NMI value ranges between [0,1]. The closer the value is to 1, the more similar the two clustering results are, while the closer the value is to 0, the less similar they are. P represents the spatial domain clustering result and T represents the ground truth clustering labels. Their entropies are denoted as $H(P)$ and $H(T)$ respectively. NMI has been widely used to evaluate the performance of spatial domain identification in spatial transcriptomic data analysis. The calculation formula for NMI is as follows:

$$NMI = \frac{MI(P, T)}{\sqrt{H(P)H(T)}} \tag{3}$$

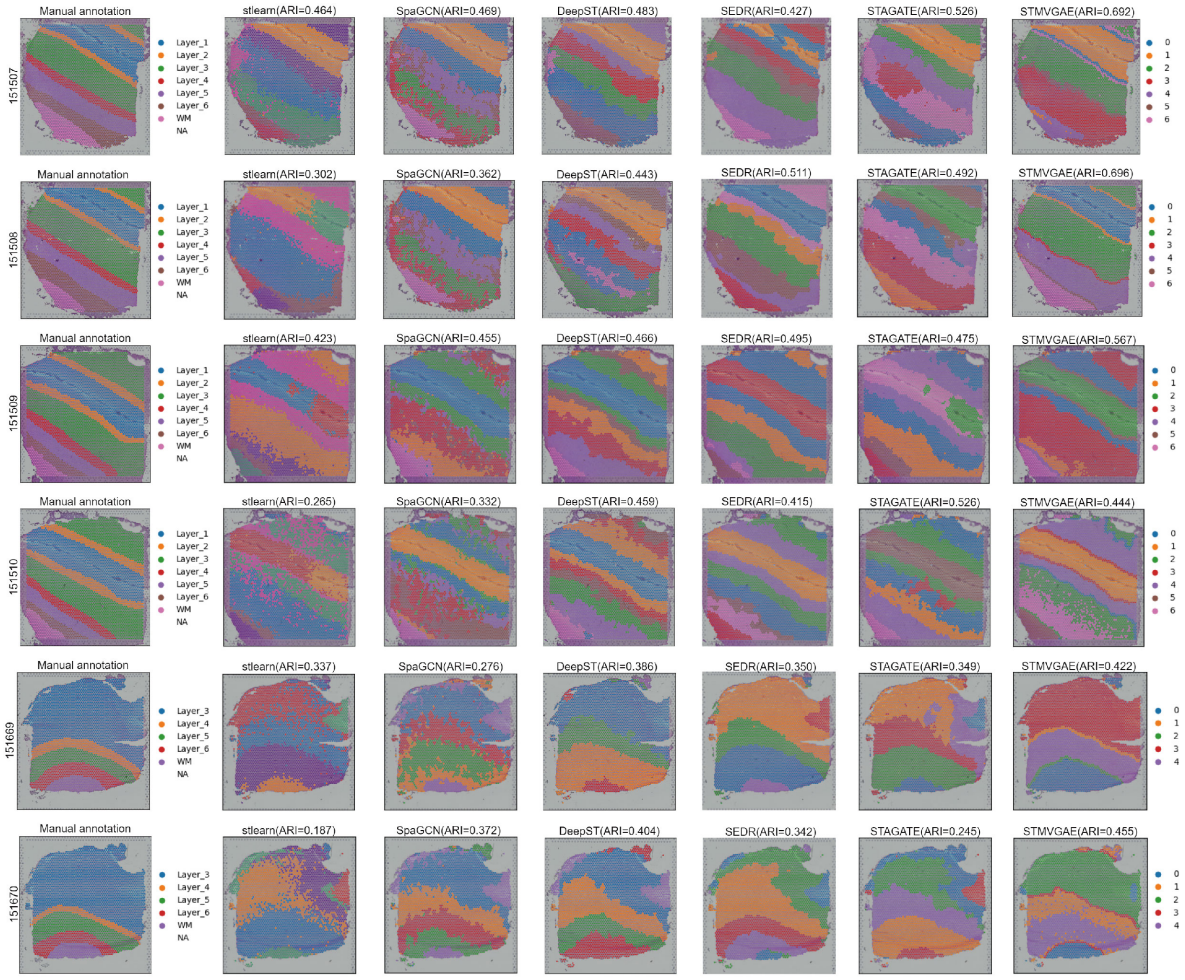
HS. In unsupervised clustering, Homogeneity Score (HS) is a metric used to evaluate clustering results, which measures whether the samples in each cluster belong to the same category [6]. The value of HS ranges from 0 to 1. The closer the value is to 1, the better the clustering result is, that is, each cluster contains samples of the same category. $H(C)$ is the entropy of the true class, which represents the uncertainty of the class distribution of the samples in the data set; $H(C|K)$ is the conditional category entropy of a given clustering result, which represents the uncertainty of the category distribution of the sample when the clustering result is known. The calculation formula for HS is as follows:

$$HS = 1 - \frac{H(C|K)}{H(C)} \tag{4}$$

Purity. In unsupervised clustering, Purity is a metric used to evaluate clustering results, which measures whether the samples contained in each cluster belong to the same category. The value range of Purity is between 0 and 1. The closer the value is to 1, the better the clustering result is, that is, each cluster contains samples of the same category. N is the total number of samples in the dataset, k represents the index of the cluster, j represents the index of the real category, c_k represents the sample set in cluster k, and t_j represents the sample set in real category j. The $|c_k \cap t_j|$ in the formula represents the size of the intersection of samples in cluster k and samples in true category j. The calculation formula for Purity is as follows:

$$Purity = \frac{1}{N} \sum_k max_j |c_k \cap t_j| \tag{5}$$

2 Supplementary Figures



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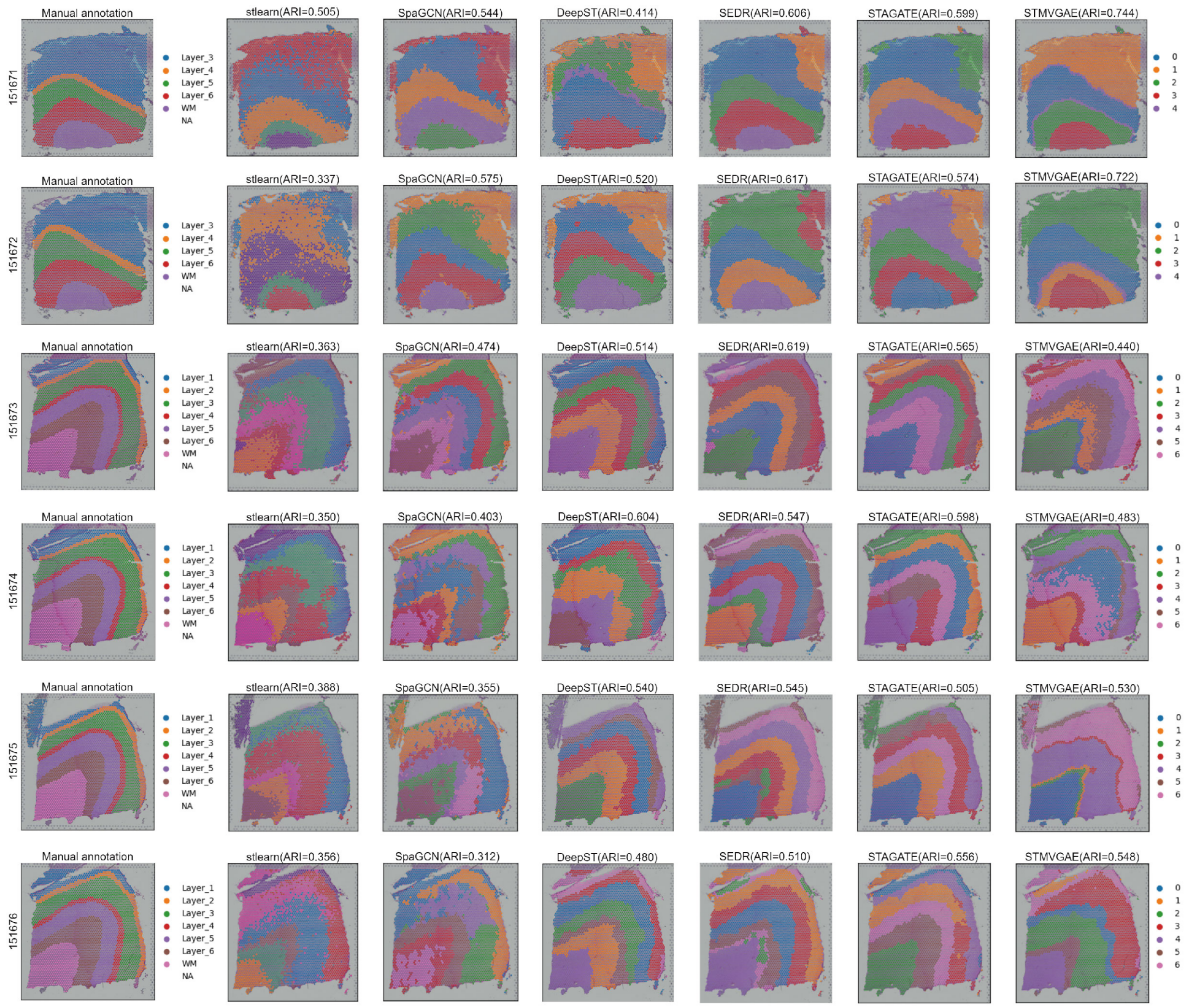
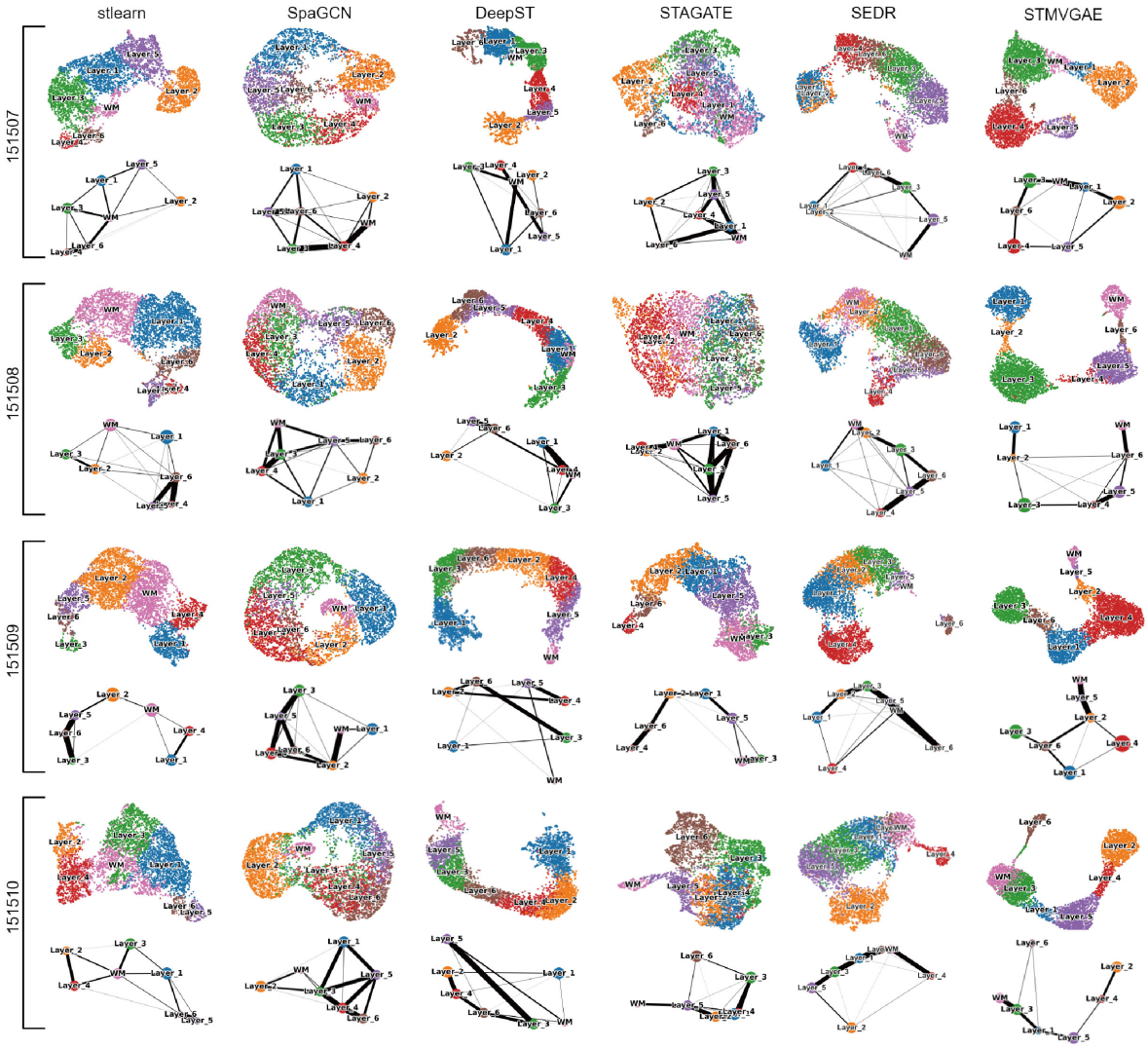


Fig. S1.Comparison of spatial domains identification by clustering assignments via STMVGAE, STAGATE, SEDR, DeepST, stlearn, and manual annotation in all 12 slices of the DLPFC dataset.



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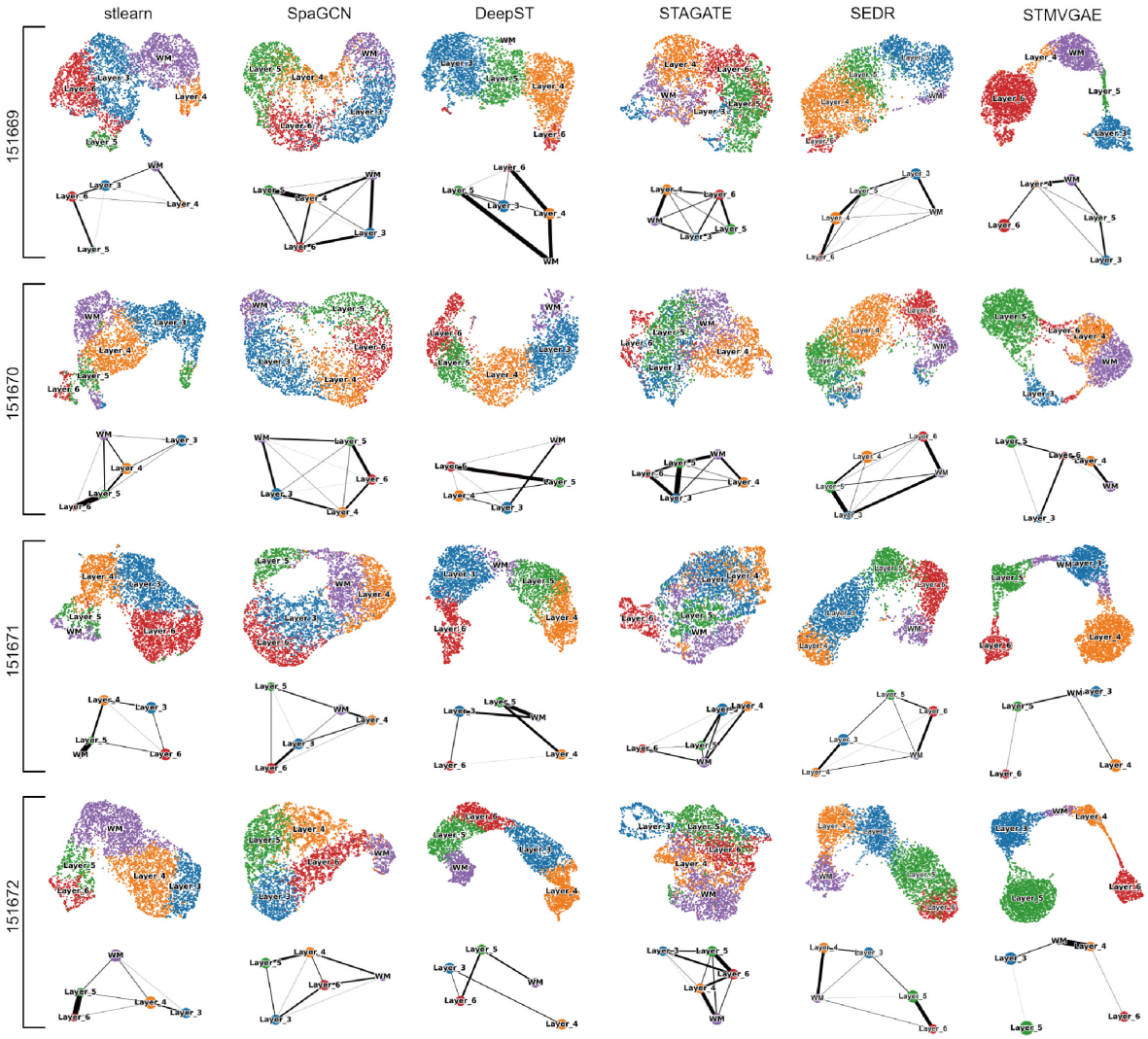

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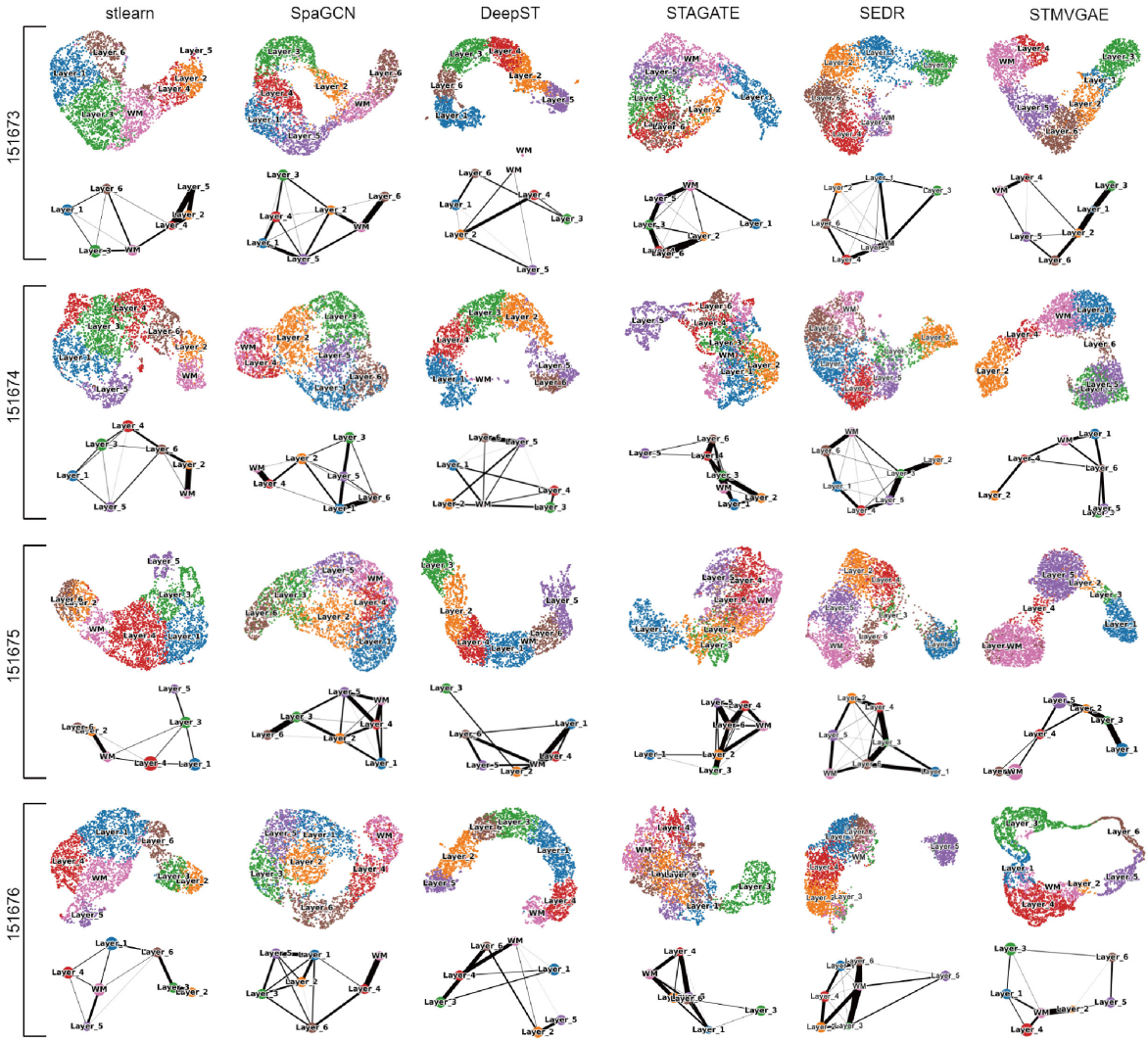

Fig. S2.UMAP visualization and PAGA trajectory inference by STMVGAE, SEDR, STAGATE, DeepST, SpaGCN, and stlearn embeddings respectively.

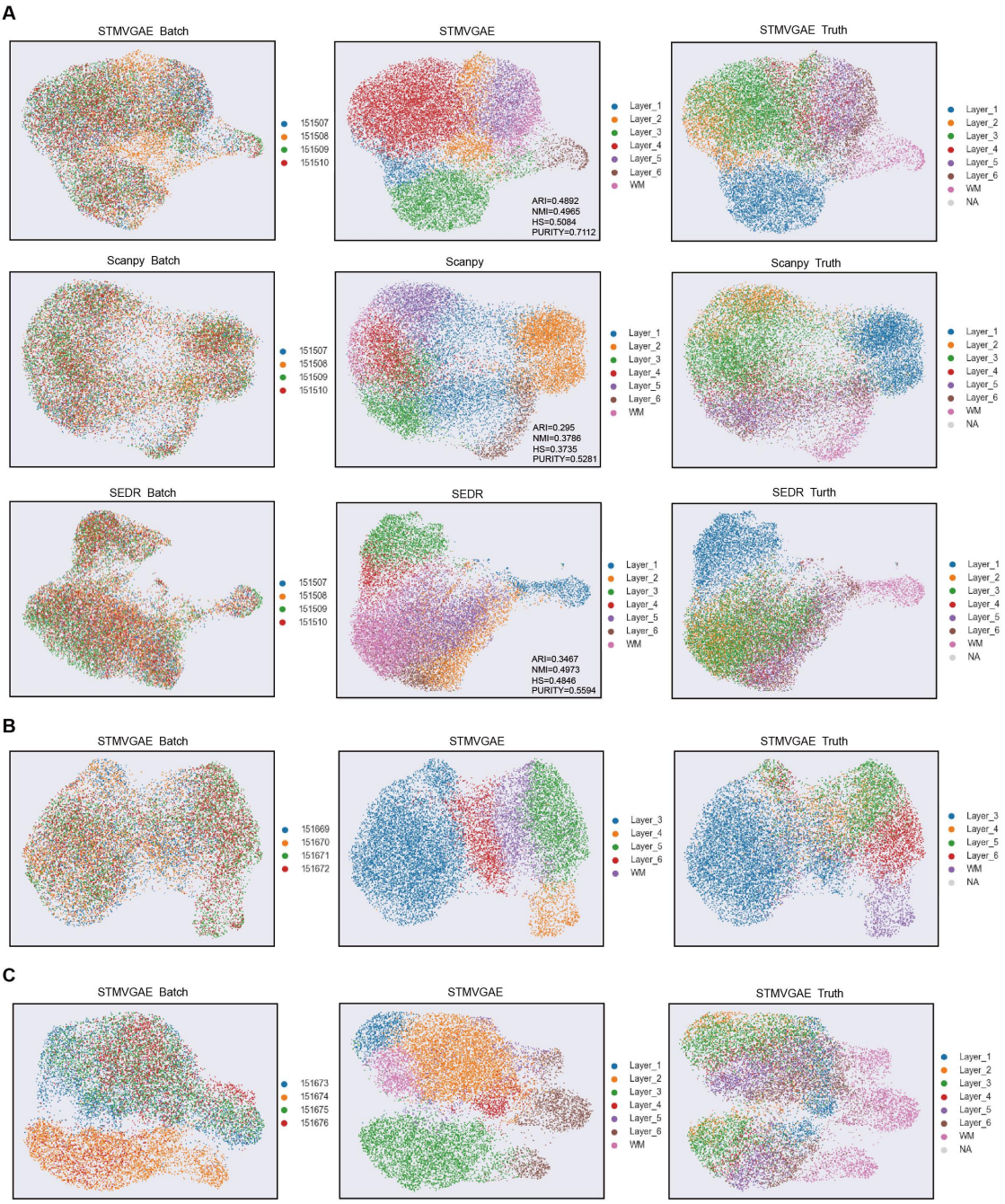

Fig. S3.A UMAP visiulization of batch integration algorithms on 151507,151508,151509,151510 slices in DLPFC datasets. Each row represents the use of STMVGAE, Scanpy, and SEDR methods for batch integration, and each column represents batches, identification spatial domains, and ground truth labels, respectively. B STMVGAE performs batch integration on 151669,151670,151671,151672 slices in the DLPFC dataset. C STMVGAE performs batch integration on 151673,151674,151675,151676 slices in the DLPFC dataset.

3 Supplementary Table

Table S1.Overview of comparative spatial domain identification methods.

Method	Methodology	Input Data	Downstream tasks	Link
SCANPY	Non-spatial method	Gene expression data	Spatial domain identification Visualization Trajectory inference	https://scanpy.readthedocs.io/
stlearn	Deep neural network	Gene expression data Histology information	Spatial domain identification Visualization Trajectory inference	https://github.com/Biomedical MachineLearning/stLearn
SEDR	Variational graph autoencoders	Spatial location data Gene expression data	Spatial domain identification Visualization Trajectory inference Denosing Batch integration	https://github.com/HzFu/SEDR/
SpaGCN	Graph convolutional networks	Spatial location data Gene expression data Histology information	Spatial domain identification Visualization Trajectory inference SVGs identification	https://github.com/jianhuupenn/SpaGCN/
DeepST	Variational graph autoencoders	Spatial location data Gene expression data Histology information	Spatial domain identification Visualization Trajectory inference Batch integration	https://github.com/JiangBioLab/DeepST/
STAGATE	Graph attention autoencoders	Spatial location data Gene expression data	Spatial domain identification Visualization Trajectory inference Denosing	https://github.com/zhanglabtools/STAGATE/

Table S2.Summary of the datasets.

Platform	Tissue	Section	Number of domains	Spots	Genes
10X Visium	Human dorsolateral prefrontal cortex (DLPFC)	151507	7	4226	33538
		151508	7	4384	
		151509	7	4789	
		151510	7	4634	
		151669	5	3661	
		151670	5	3498	
		151671	5	4110	
		151672	5	4015	
		151673	7	3639	
		151674	7	3673	
		151675	7	3592	
		151676	7	3460	
	Human breast cancer: ductal carcinoma in situ	\	2	2518	17943
	Human breast cancer	\	20	3798	36601
Spatialresearch	Melanoma cancer	\	5	293	16148
Stereo-seq	Mouse olfactory bulb	\	\	19109	27106

Slice	$A^{(1)}+A^{(2)}$				$A^{(1)}+A^{(3)}$				$A^{(1)}+A^{(4)}$				$A^{(2)}+A^{(3)}$				$A^{(2)}+A^{(4)}$				$A^{(3)}+A^{(4)}$			
	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur
151507	0.549	0.662	0.664	0.685	0.692	0.712	0.763	0.860	0.548	0.644	0.658	0.737	0.561	0.677	0.675	0.698	0.501	0.648	0.673	0.754	0.567	0.698	0.710	0.750
151508	0.594	0.657	0.681	0.813	0.696	0.703	0.724	0.821	0.666	0.689	0.739	0.841	0.582	0.620	0.640	0.801	0.503	0.604	0.606	0.691	0.573	0.664	0.654	0.686
151509	0.421	0.585	0.573	0.672	0.567	0.644	0.636	0.783	0.421	0.588	0.581	0.704	0.504	0.637	0.609	0.699	0.411	0.567	0.560	0.673	0.604	0.653	0.643	0.773
151510	0.557	0.651	0.610	0.737	0.444	0.562	0.532	0.660	0.403	0.559	0.530	0.653	0.548	0.651	0.607	0.719	0.496	0.648	0.613	0.734	0.410	0.560	0.544	0.648
151669	0.400	0.523	0.513	0.775	0.422	0.570	0.530	0.739	0.415	0.562	0.527	0.776	0.201	0.405	0.386	0.670	0.375	0.492	0.499	0.769	0.342	0.512	0.467	0.701
151670	0.386	0.509	0.468	0.722	0.455	0.559	0.527	0.758	0.337	0.475	0.433	0.693	0.324	0.486	0.433	0.650	0.314	0.455	0.412	0.688	0.246	0.414	0.376	0.601
151671	0.770	0.724	0.711	0.866	0.744	0.707	0.720	0.895	0.784	0.751	0.741	0.894	0.706	0.688	0.665	0.833	0.746	0.703	0.773	0.921	0.698	0.708	0.688	0.833
151672	0.670	0.697	0.814	0.925	0.722	0.724	0.740	0.851	0.640	0.658	0.690	0.811	0.700	0.713	0.762	0.902	0.686	0.701	0.710	0.805	0.617	0.654	0.692	0.831
151673	0.446	0.624	0.670	0.731	0.440	0.618	0.639	0.708	0.464	0.638	0.659	0.739	0.430	0.618	0.647	0.744	0.496	0.645	0.699	0.804	0.499	0.647	0.656	0.749
151674	0.454	0.584	0.591	0.689	0.483	0.608	0.655	0.801	0.427	0.607	0.610	0.667	0.458	0.544	0.552	0.725	0.420	0.554	0.584	0.743	0.466	0.588	0.599	0.711
151675	0.504	0.628	0.656	0.764	0.530	0.601	0.641	0.817	0.538	0.621	0.674	0.839	0.479	0.629	0.697	0.815	0.486	0.601	0.641	0.764	0.528	0.652	0.665	0.767
151676	0.444	0.601	0.641	0.750	0.548	0.643	0.667	0.773	0.513	0.602	0.632	0.758	0.469	0.634	0.664	0.762	0.488	0.611	0.652	0.780	0.477	0.632	0.642	0.715
Average	0.516	0.620	0.633	0.761	0.562	0.638	0.648	0.789	0.513	0.616	0.623	0.759	0.497	0.608	0.611	0.751	0.494	0.602	0.618	0.761	0.502	0.615	0.611	0.730

Table S3.STMVGAE performs graph combination test results on 12 slices of the DLPFC dataset. STMVGAE integrates the results of four different graphs in a free combination manner to calculate ARI, NMI, HS, and Pur (Purity) respectively. $A^{(1)}$, $A^{(2)}$, $A^{(3)}$, and $A^{(4)}$ represent Radius_balltree, Radius_kdtree, KNN_balltree, and KNN_kdtree respectively. The best result is underlined.

Slice	$A^{(1)}+A^{(2)}+A^{(3)}$				$A^{(1)}+A^{(2)}+A^{(4)}$				$A^{(1)}+A^{(3)}+A^{(4)}$				$A^{(2)}+A^{(3)}+A^{(4)}$				$A^{(1)}+A^{(2)}+A^{(3)}+A^{(4)}$			
	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur	ARI	NMI	HS	Pur
151507	0.618	0.708	0.827	0.913	0.581	0.699	0.710	0.761	0.688	0.729	0.773	0.866	0.569	0.700	0.715	0.771	0.583	0.704	0.719	0.776
151508	0.660	0.700	0.721	0.824	0.625	0.674	0.696	0.814	0.705	0.723	0.753	0.839	0.597	0.681	0.724	0.847	0.676	0.708	0.730	0.821
151509	0.570	0.657	0.641	0.776	0.428	0.612	0.601	0.693	0.570	0.646	0.630	0.747	0.496	0.643	0.618	0.696	0.574	0.656	0.644	0.777
151510	0.501	0.652	0.635	0.717	0.438	0.634	0.603	0.680	0.411	0.588	0.563	0.678	0.421	0.622	0.593	0.681	0.433	0.635	0.614	0.686
151669	0.353	0.543	0.506	0.746	0.417	0.551	0.515	0.773	0.374	0.558	0.534	0.739	0.384	0.498	0.495	0.767	0.298	0.513	0.481	0.727
151670	0.335	0.516	0.459	0.673	0.423	0.537	0.493	0.741	0.481	0.534	0.507	0.764	0.379	0.509	0.462	0.685	0.443	0.512	0.470	0.743
151671	0.745	0.723	0.748	0.910	0.826	0.740	0.751	0.890	0.790	0.751	0.742	0.896	0.729	0.701	0.785	0.929	0.798	0.751	0.735	0.882
151672	0.726	0.718	0.755	0.848	0.704	0.705	0.748	0.827	0.725	0.723	0.742	0.855	0.709	0.712	0.728	0.815	0.718	0.717	0.736	0.850
151673	0.500	0.654	0.659	0.694	0.543	0.675	0.694	0.788	0.532	0.672	0.687	0.741	0.498	0.645	0.648	0.738	0.512	0.665	0.665	0.702
151676	0.490	0.592	0.625	0.749	0.422	0.587	0.590	0.662	0.477	0.628	0.620	0.672	0.460	0.592	0.625	0.742	0.463	0.607	0.613	0.693
151675	0.528	0.662	0.691	0.779	0.507	0.624	0.652	0.768	0.462	0.616	0.655	0.760	0.473	0.621	0.680	0.801	0.531	0.661	0.693	0.788
151676	0.442	0.616	0.635	0.708	0.471	0.643	0.679	0.728	0.445	0.616	0.644	0.721	0.479	0.647	0.687	0.730	0.476	0.642	0.654	0.691
Average	0.539	0.645	0.658	0.778	0.532	0.640	0.644	0.760	0.555	0.649	0.654	0.773	0.516	0.631	0.647	0.767	0.542	0.648	0.646	0.761

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