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Mini-review

Computational modeling for deciphering tissue microenvironment heterogeneity from spatially resolved transcriptomics

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ABSTRACT

Spatial transcriptomics techniques, while measuring gene expression, retain spatial location information, aiding in situ studies of organismal tissue architecture and the progression of pathological processes. These techniques generate vast amounts of omics data, necessitating the development of computational methods to reveal the underlying tissue microenvironment heterogeneity. The main directions in spatial transcriptomics data analysis are spatial domain detection and spatial deconvolution, which can identify spatial functional regions and parse the distribution of cell types in spatial transcriptomics data by integrating single-cell transcriptomics data. In these two research directions, many computational methods have been successively proposed. This article will categorize them into three types: machine learning-based methods, probabilistic models-based methods, and deep learning-based methods. It will list and discuss the representative algorithms of each type along with their advantages and disadvantages and describe the datasets and evaluation metrics used to assess these computational methods, facilitating researchers in selecting suitable computational methods according to their research needs. Finally, combining the latest technological developments and the advantages and disadvantages of current algorithms, this article will look forward to the future directions of computational method development.

1. Introduction

Studying the functionality of organismal tissue structures and the progression of pathological processes often requires analyzing gene expression within spatial context [1–3]. Recently developed spatial transcriptomics technologies, capable of capturing gene expression profiles while preserving their spatial location information, are facilitating advancements in research on tissue structure [4] and pathological development [5]. Spatial transcriptomics techniques are mainly categorized into two types based on their sequencing approach: sequencing-based spatial transcriptomics and imaging-based spatial transcriptomics. Sequencing-based spatial transcriptomics employ high-throughput sequencing to capture gene expression profiles at various spatial locations. These techniques are characterized by their ability to capture genome-wide expression profiles but lack single-cell resolution, with each spatial unit region containing multiple cells. Representative sequencing technologies include Spatial Transcriptomics (ST) [6], 10x Visium, and high-resolution technologies like Slide-seq [7], Slide-seqV2 [8], Stereo-seq [9], Seq-Scope [10], which can achieve near single-cell resolution. Imaging-based spatial transcriptomics technologies capture the expression profiles of targeted genes in each cell through fluorescent imaging. These techniques are characterized by their single-cell resolution but are unable to capture genome-wide expression profiles, focusing instead on specific target genes. Representative technologies in this category include SeqFISH [11], SeqFISH+ [12], MERFISH [13], STARmap [14], and others. These spatial transcriptomics technologies generate a vast amount of data, necessitating the development of computational methods to analyze these data and reveal the underlying biological significance [15].

Spatial transcriptomics typically measures gene expression and

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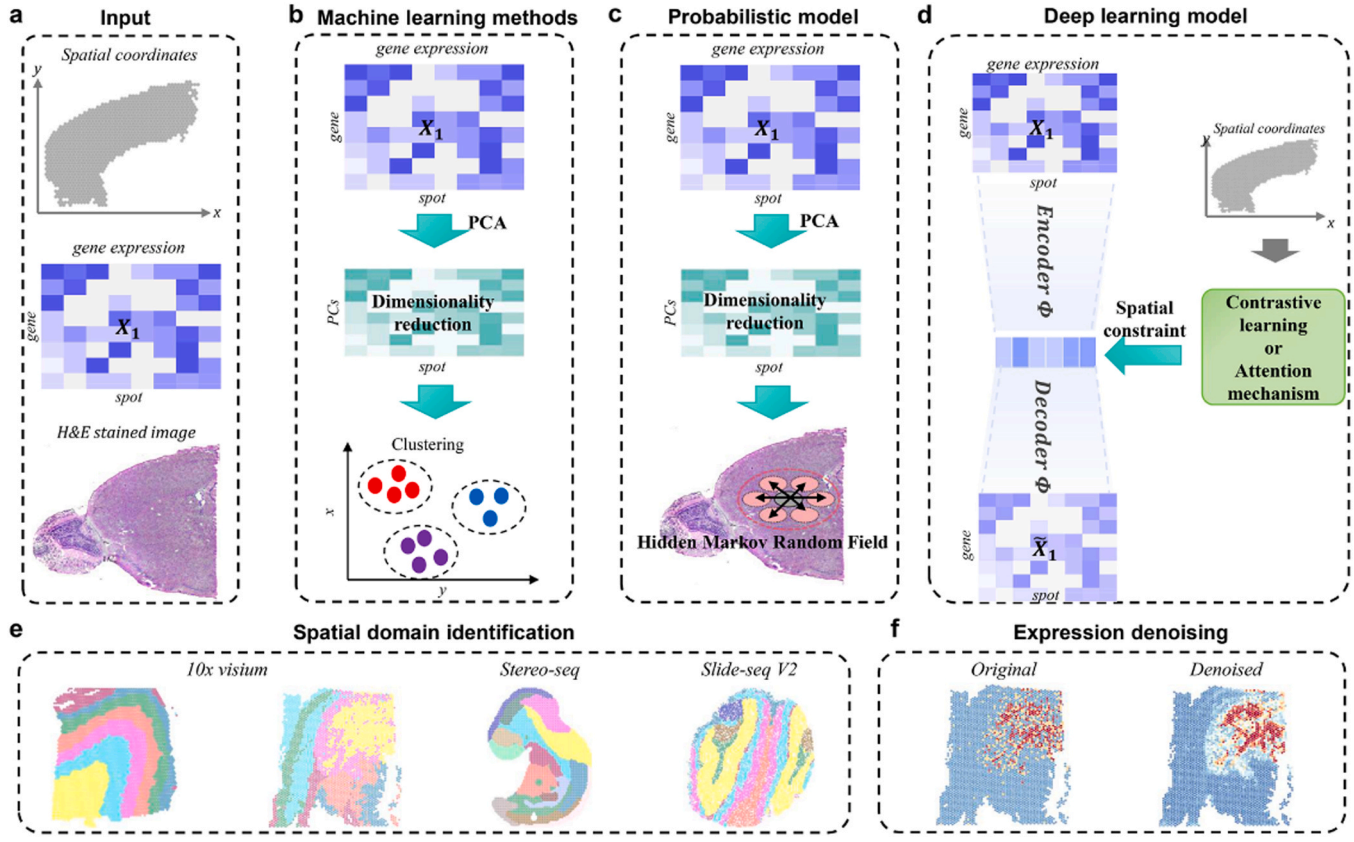

Fig. 1. A summary of spatial domain identification methods. Published tools for spatial domain identification can be divided into three categories according to their main strategy: machine learning-based, probabilistic model-based, and deep learning model-based.

spatial coordinates for each spatial unit region (spot), and some methods also provide H&E stained images, such as 10x Visium. Based on the obtained data, spatial transcriptomics data analysis primarily focuses on two directions: spatial domain identification, which divides slices into different functional areas based on the expression differences and spatial locations of spots, and spatial deconvolution, which uses annotated single-cell transcriptomics datasets as references to infer the distribution of cell types in each spot. Computational methods for spatial domain identification can be broadly categorized into three types based on their model: machine learning methods, such as Seurat [16], SCANPY [17] and NSF [18]; probabilistic model-based methods, such as Hidden Markov Random Field (HMRF) [19], BayesSpace [20], SpatialPCA [21]; and deep learning methods, such as SpaGCN [22], STAGATE [23], GraphST [24] (Fig. 1). Computational methods for spatial deconvolution can also be divided into three categories: machine learning methods, such as SPOTlight [25], SpatialDWLS [26] and SpiceMix [27]; probabilistic model-based methods, such as RCTD [28], Stereoscope [29], Cell2location [30]; and deep learning methods, such as Tangram [31] (Fig. 3). These computational methods, from different perspectives, parse spatial transcriptomics data, providing information support for downstream analysis.

This article will review the existing computational methods for spatial domain identification and spatial deconvolution, analyzing the characteristics of each method, and the corresponding downstream analyses, to facilitate researchers in selecting appropriate methods based on the characteristics and needs of their data.

2. Spatial domain detection methods

A classical problem in the analysis of spatial transcriptomics data is the identification of spatial domains, which involves dividing spatial transcriptomic slices into distinct regions based on the expression differences and spatial locations of each spot. Typically, each spatial domain exhibits a unique pattern of gene expression and a certain degree of spatial continuity, fulfilling different biological functions. Therefore, segmenting spatial transcriptomic slices into spatial domains facilitates the study of differences between regions and their biological significance.

Early methods for spatial domain identification, such as Seurat [16] and SCANPY [17], did not leverage spatial information and clustered spots based solely on differences in gene expression to identify spatial domains. The input data for these methods were limited to gene expression count matrices. The conventional processing workflow includes: 1) selecting 2000 highly variable genes, i.e., genes with significant expression differences across spots, as these genes typically contain more information useful for dividing spatial domains; 2) normalizing, usually by log-transforming the count matrix; 3) using Principal Component Analysis (PCA) for dimensionality reduction, selecting principal components with significant variance to reduce noise impact on subsequent analyses; 4) constructing a neighborhood network, typically using the k-nearest neighbors algorithm to build a network of spots; 5) clustering spots on the neighborhood network using methods such as Leiden [32] or Louvain [33], with the resulting clusters representing identified spatial domains (Fig. 1b). These methods apply single cell data clustering computational approaches directly to spatial transcriptomics, failing to utilize the unique spatial information of spatial transcriptomics. The identified spatial domains are usually more discrete and exhibit poorer spatial continuity.

Another category of spatial domain identification methods is based on probabilistic approaches. These methods model gene expression and spatial coordinates through probabilistic models to infer the spatial domains. This category includes methods like Hidden Markov Random Field (HMRF) [19], BayesSpace [20], and SpatialPCA [21]. The HMRF method models both gene expression and spatial neighbors, initially

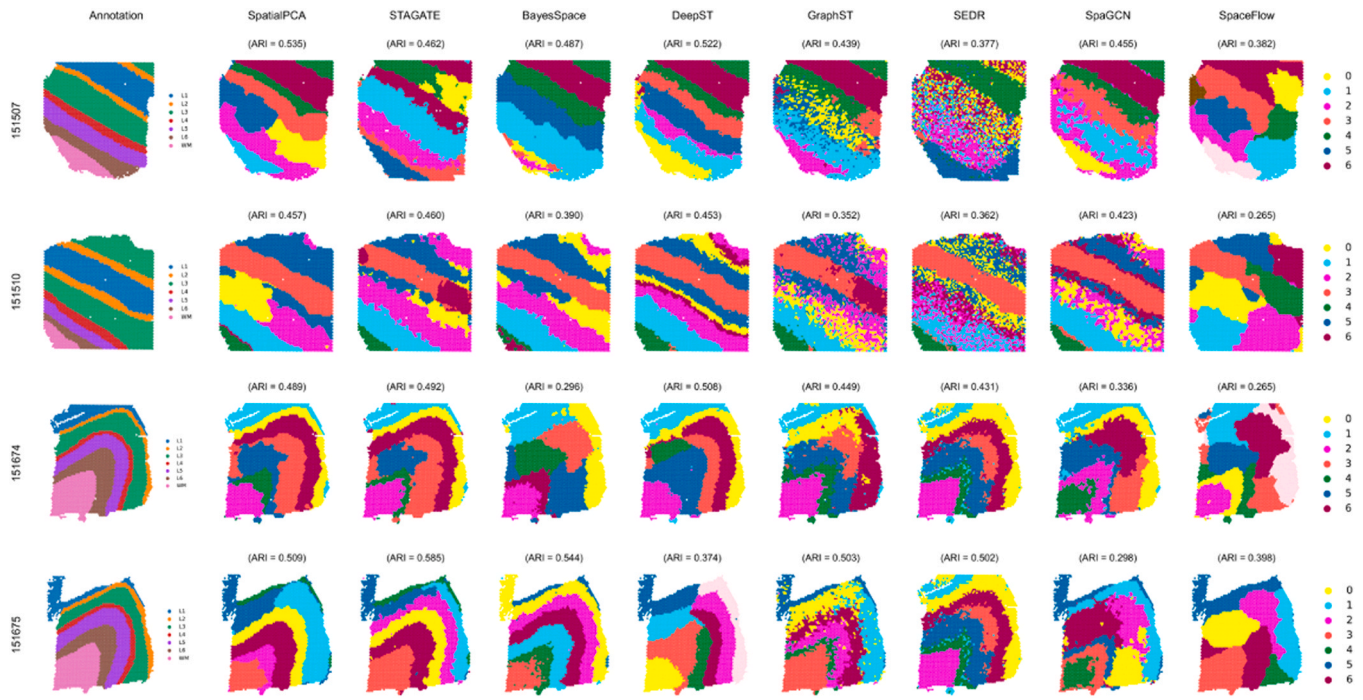

Fig. 2. Comparative performance of different representative methods on spatial domain identification. The manual annotation of 10x Visium DLPFC slice 151507, 151510, 151674 and 151675 on the spatial coordinates. Annotations include L1-L6 (Layer 1-Layer 6) and WM (white matter). Each method's spatial domain is color-coded for clarity, with the ARI value shown above.

applied for spatial domain identification in low-resolution fluorescent imaging spatial transcriptomics data, and later adapted for high-throughput spatial transcriptomics after selecting spatially variable genes (SVG). BayesSpace uses a Bayesian framework to model gene expression and spatial neighbors, modeling the expression matrix with a low-dimensional representation and using spatial priors to encourage spots with spatial neighbors to belong to the same spatial domain, thus achieving spatial domain identification (Fig. 1c). Additionally, BayesSpace implements high resolution spatial transcriptomics data, inferring sub-spot level gene expression through Bayesian statistics. SpatialPCA infers a low-dimensional representation of spatial transcriptomics expression data through probabilistic principal component analysis, while modeling the spatial correlations between spots with a kernel matrix, ensuring the inferred low-dimensional representation includes spatial information. SpatialPCA can also reconstruct high-resolution spatial transcriptomics maps and adjust the resolution as needed. These probabilistic models generally identify spatial domains with higher accuracy and better spatial continuity, though they tend to require longer computation times due to the complexity of probabilistic modeling.

Another approach to spatial domain identification involves deep learning models, which often use neural networks of various architectures to model spatial transcriptomics data. By designing various loss functions to train the neural network, these methods learn representations of spatial transcriptomics data and integrate various clustering methods to identify spatial domains (Fig. 1d). Examples of such methods include SpaGCN [22], STAGATE [23], and GraphST [24]. SpaGCN performs PCA dimensionality reduction on the gene expression matrix in spatial transcriptomics data, then combines spatial coordinates for graph convolution to learn a low-dimensional representation of the data, identifying spatial domains through an unsupervised deep iterative clustering method. STAGATE uses autoencoders to model gene expression data in spatial transcriptomics, feeding the learned latent space combined with spatial information into a self-attention mechanism-based graph convolutional neural network to learn a low-dimensional representation. This representation is then used to construct a neighborhood network, with spatial domains identified using clustering methods such as Leiden or Louvain. GraphST models spatial transcriptomics data using a self-supervised contrastive learning framework, constructing a spatial neighbor graph with spatial information, and using a graph convolutional network as an encoder to iteratively learn low-dimensional representations of gene expression and spatial neighbors, achieving spatial domain identification through enhanced contrastive learning. Additionally, GraphST can be applied to other spatial transcriptomics data analysis applications, including spatial deconvolution, and removing batch effects across multiple slices. These methods utilize deep learning technology to build neural network structures based on the characteristics of spatial transcriptomics data, integrating spatial information with graph convolution modules. Deep learning models can fit more complex data structures, learn data representations more accurately, and incorporate spatial information, resulting in high accuracy and good spatial continuity in identified spatial domains. Benefiting from optimizations in deep learning frameworks for GPU computation, these models also compute quickly.

The performance of spatial domain identification algorithms is primarily evaluated from two aspects: the effectiveness of clustering and spatial continuity. The effectiveness of clustering can be assessed with appropriate metrics depending on the application scenario. When gold standard annotations are available, quantitative metrics can be used to evaluate the clustering effectiveness of spatial domains. When the number of spatial domains matches the number of gold standard annotation categories, the Adjusted Rand Index (ARI) is commonly used to assess clustering effectiveness [34], such as in many algorithmic articles evaluating spatial domains with the dorsolateral prefrontal cortex (DLPFC) dataset [35] (Fig. 2). When gold standard annotations are coarse, meaning the number of spatial domains exceeds the number of annotation categories, clustering purity is often used to assess clustering effectiveness, as in the BayesSpace article evaluating spatial domains with the invasive ductal carcinoma (IDC) dataset [20]. In the absence of gold standard annotations, when there are roughly corresponding anatomical annotations, the match between identified spatial domains and anatomical annotations can be observed, combined with marker

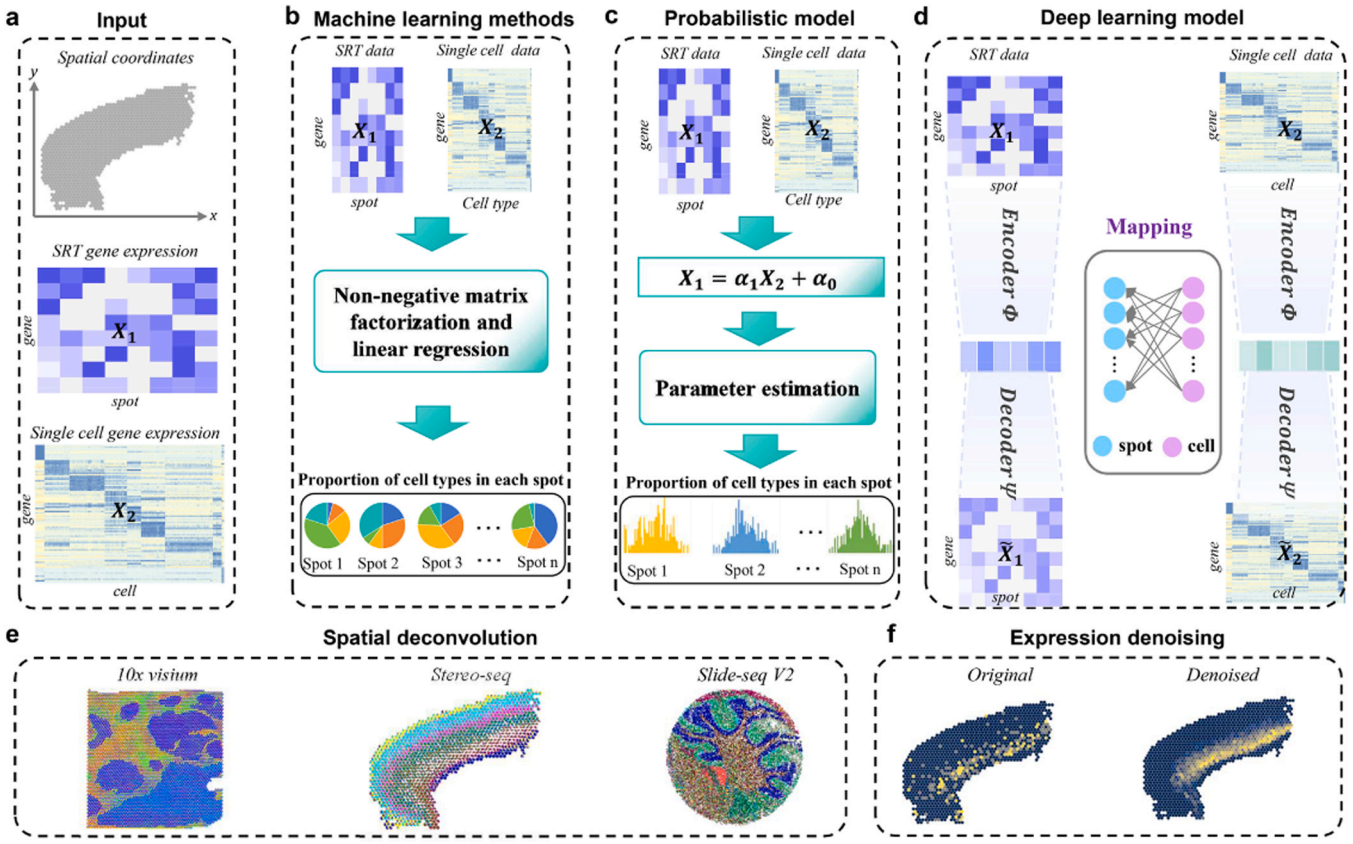

Fig. 3. A summary of spatial deconvolution methods. Published tools for spatial deconvolution can be divided into three categories according to their main strategy: machine learning-based, probabilistic model-based, and deep learning model-based.

genes for each category, to evaluate the spatial domains identified by various methods, such as the mouse brain coronal and sagittal sections in the Allen Brain Atlas. Spatial continuity is primarily quantified from two aspects: spatial autocorrelation, measured by calculating Moran's I [36–38] and Geary's C statistics—the higher these statistics, the better the spatial autocorrelation of the spatial domains—and the consistency of spatial domains within local areas, measured by calculating the Local Inverse Simpson Index (LISI) [39]. A lower LISI indicates that the spots in a local area belong to a consistent spatial domain, while a higher LISI suggests that the spots in a local area belong to multiple different spatial domains.

Note that, spatial domains should not be excessively smoothed; instead, they must effectively balance spatial coherence and expression variability within neighborhoods. Overuse of spatial constraints can overwhelm expression differences, resulting in overly smoothed spatial domains and the loss of relevant biological significance. Conversely, insufficient spatial constraints can lead to poor identification of spatial domains due to high dropout rates in SRT data. This not only results in inaccurate identification but also causes excessive dispersion of spatial domains, which compromises biological interpretability. Therefore, the key to identifying spatial domains lies in the flexible and efficient use of spatial information. The assessment of spatial smoothness should only be considered one aspect of evaluating spatial domain performance, primarily applicable to SRT data with well-defined spatial continuity, such as brain structures.

3. Spatial deconvolution methods

For a more precise study of the biological functions of tissue slice, it's essential to understand the spatial distribution of cell types within each spot, given that spots measured by sequencing-based spatial transcriptomics techniques often contain multiple cells. This necessity has led to the development of spatial deconvolution methods [27], which involve mapping annotated single-cell transcriptomics data onto spatial transcriptomics to solve for the distribution of cell types within each spot.

The initial spatial deconvolution methods were typically based on machine learning models such as matrix decomposition and regression analysis (Fig. 3b). These methods, which include SPOTlight [25] and SpatialDWLS [26], did not utilize spatial information and solved for the cell type distribution within each spot using inputs like the spatial transcriptomics gene expression matrix, single-cell transcriptomics gene expression matrix, and annotations of cell types from single-cell transcriptomics. SPOTlight uses non-negative matrix factorization and non-negative least squares regression, starting with factor matrix initialization using marker genes for each cell type, followed by non-negative matrix factorization of the single-cell gene expression matrix, and finally applying non-negative least squares regression to determine the cell type distribution within each spot. SpatialDWLS uses the damped weighted least squares method, starting with cell type enrichment within each spot using Parametric Analysis of Gene set Enrichment (PAGE), followed by solving for the cell type distribution using marker genes for each cell type. These methods adapt machine learning models for spatial deconvolution data characteristics but are linear methods that provide limited fitting to complex gene expression data and do not utilize spatial information, resulting in more discrete cell type distributions with poorer spatial continuity.

Another category of spatial deconvolution methods is based on probabilistic models (Fig. 3c). These methods, including RCTD [28], Stereoscope [29], and Cell2location [30], use probabilistic models to model spatial transcriptomics and single-cell gene expression data, solving for the cell type distribution within each spot through

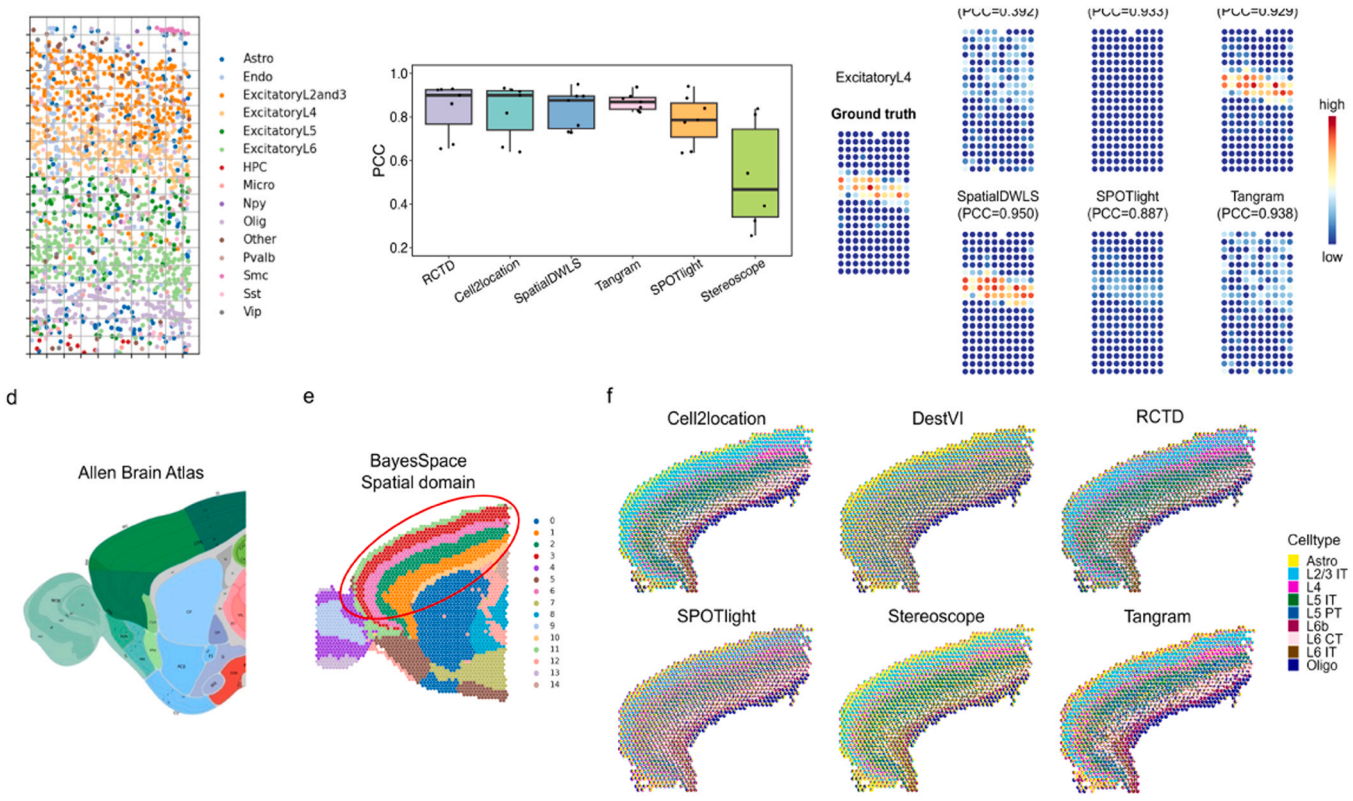

Fig. 4. Comparative performance of different representative deconvolution methods on simulated datasets and 10x Visium mouse cortex. (a-c) We grided a MERFISH mouse visual cortex dataset including 1549 cells of 15 cell types to generate 189 synthetic spots with each containing 1–18 cells [57], and utilize a scRNA-seq dataset profiled by Smart-seq [58] with corresponding cell types as reference for deconvolution. Pearson correlation coefficient (PCC) is systematically used to evaluate the performance of different representative deconvolution methods. (d-f) We focused on a layer-structured cortex region profiled from 10x Visium mouse brain sagittal anterior slice and used a well annotated SMART-Seq2 datasets of 9 cell types as reference for resolving the cell type distribution [58].

probabilistic inference. RCTD models expression data using the Poisson distribution, assuming each spot's gene expression as a linear sum of gene expressions from various cell types, with an error term to fit platform effects. Stereoscope assumes gene expression data from spatial and single-cell transcriptomics follow a negative binomial distribution, using maximum a posteriori estimation (MAP) for solving. Cell2location implements spatial deconvolution within a Bayesian framework, estimating characteristic expression profiles for each reference cell type from single-cell data using negative binomial regression, then deconvolving each spot in spatial transcriptomics according to these profiles. Integrated with the scvi-tools [40] framework, Cell2location uses variational inference and GPU acceleration, ensuring high computational efficiency. These methods offer robust performance due to their probabilistic approach and ability to model complex expression data patterns effectively.

A further class of spatial deconvolution methods relies on deep learning models, designed around the characteristics of spatial deconvolution data (Fig. 3d). These models, such as Tangram [31], incorporate cell type distributions as parameters, designing various loss functions and employing gradient descent for solving the cell type distribution within each spot. Tangram optimally places cells from single-cell data into spatial transcriptomics spots, starting with random cell placement, maximizing a target function based on spatial correlation across genes between single-cell and spatial transcriptomics data until convergence. Tangram extends the application of spatial deconvolution in several ways, including expanding target gene-based spatial transcriptomics to whole-genome scope, correcting low-quality spatial expression profiles, mapping various cell types measured by single-cell sequencing to space, deconvolving low-resolution spatial transcriptomics to single-cell resolution, and resolving spatial chromatin accessibility patterns at single-cell resolution through multi-omics data. Benefiting from the versatility of deep learning model frameworks, these algorithms offer strong scalability and the ability to extend into numerous applications. Deep learning models provide better fitting for complex gene expression patterns, with high computational efficiency thanks to GPU acceleration.

The performance of spatial deconvolution algorithms has been independently evaluated [41,42], establishing a mature evaluation system (Fig. 4). Performance is mainly assessed using simulated data, either by partitioning single-cell resolution spatial transcriptomics (e.g., SeqFISH+ [12], STARmap [14]) into grids to create pseudo-spots or by randomly mixing cells from single-cell data without considering spatial coordinates to simulate spots. Performance metrics include Pearson Correlation Coefficient (PCC), Structural Similarity Index (SSIM), Root Mean Square Error (RMSE), and Jensen–Shannon Divergence (JSD), measuring similarity and differences between predictions and gold standards. Higher similarity metric values indicate better performance, while lower difference metric values suggest improved algorithm performance. An Accuracy Score (AS) can be derived from these four metrics to provide a comprehensive performance evaluation of spatial deconvolution algorithms.

4. Advanced analysis of spatial transcriptomics data

4.1. Spatially variable genes identification

Genes exhibit spatial expression patterns that reflect intrinsic cell type-specific programs and extrinsic effects stemming from cell-cell communication or the tissue microenvironment. The identification of spatially variable genes and spatial functional regions/cell types from spatially resolved transcriptomics (SRT) data can provide detailed insights into gene-phenotype associations within tissues. For detecting

Table 1
Comparing the capabilities of different representative methods.

	STAGATE	GraphST	Cell2location	RCTD	SPARK	SpatialDE	PASTE	STAligner
Platforms	Python	Python	Python	R	R	Python	Python	Python
Spatial representations	√	√	√	x	x	x	x	√
Dimensionality reduction	√	√	√	x	x	x	x	x
Images processing	x	√	x	x	x	x	x	√
Spatial domains	√	√	x	x	x	x	x	√
Cell type Deconvolution	x	√	√	√	x	x	x	x
Batch effect removal	x	x	x	x	x	x	x	√
3D alignment	x	x	x	x	x	x	√	√
Data integration	x	√	x	x	x	x	√	√
Identifying SVG	x	x	x	x	√	√	x	x

genes that demonstrate spatial trends in their expression, SPARK [43] identifies expression trends by applying generalized spatial linear models using various Gaussian and periodic kernel functions. Building upon a robust covariance test framework, SPARK-X [44] facilitates rapid and effective detection of spatially expressed genes in large spatial transcriptomic studies. This approach offers precise type I error control and high power while achieving substantial computational efficiency. SpatialDE [45] employs Gaussian process regression to identify those with spatial patterns. C-SIDE [46] employs a parametric model with predefined covariates, such as spatial location or the cellular microenvironment, to identify cell type-specific differentially expressed genes in spatial transcriptomics. Besides detecting genes with spatially distinct expression patterns, a primary objective of SRT data analysis is to identify spatial domains or cell types characterized by consistent gene expression. Belayer [47] models the expression of each gene as a piecewise linear function of spatial location, accurately identifying tissue layers and biologically significant spatially varying genes. SpaGCN [22] integrates RGB pixel data from tissue images with spatial coordinates to calibrate spatial expression graph weights, employing graph convolution and iterative clustering to identify spatial domains and spatially differentially expressed genes or metagenes.

4.2. Multi-slices spatial transcriptomics integration

Most complex biological regulatory activities occur in three dimensions (3D). Thus, to gain deeper insights into biological processes, it is essential to extend beyond individual two-dimensional (2D) slices. In recent years, several methodologies have been developed to integrate multi-slice spatially resolved transcriptomics (SRT) data, enhancing the effectiveness of downstream analyses [48]. For instance, PASTE computes pairwise alignments of slices using fused Gromov-Wasserstein optimal transport, while also generating a gene expression matrix for a central slice to represent the integrated data from multiple slices. PASTE2 [49] extends this approach by utilizing partial fused Gromov-Wasserstein optimal transport to align partially overlapping SRT slices. GPSA [50] employs a deep Gaussian process to reconstruct the tissue's 3D structure, offering valuable insights into the interplay between gene expression and spatial organization. STAligner [51] integrates a graph attention autoencoder with mutual nearest neighbors (MNN) to mitigate batch effects in the latent space and improve the integration process, enabling the reconstruction of 3D tissue structures. STitch3D [52], a recently developed data integration method, simultaneously tackles spatial domain identification and cell-type deconvolution tasks.

5. Discussions

Spatial transcriptomics sequencing technologies, which measure gene expression while preserving spatial information, are instrumental in studying tissue structure functionality and the progression of pathologies. Spatial transcriptomics data possess unique characteristics; for example, sequencing-based spatial transcriptomics reveal that each spot contains multiple cells, while imaging-based techniques may only detect target genes. Given these characteristics, developing computational methods to parse the biological significance embedded in spatial transcriptomics data is essential. The current main directions in spatial transcriptomics data analysis are spatial domain identification, spatial deconvolution, identifying spatially variable genes and integrating different spatial transcriptomics slices. Numerous algorithms have been developed in these directions, classified according to their mathematical models into machine learning, probabilistic models, and deep learning, with representative methods described for each category (Table 1). This classification helps elucidate how to evaluate algorithm performance, including datasets and evaluation metrics, assisting researchers in selecting suitable algorithms for their studies.

Spatial sequencing technology has expanded from spatial transcriptomics to spatial multi-omics, such as spatial-CITE-seq [53], which measures both the transcriptome and proteome in space, and spatial-ATAC-RNA-seq [54], which simultaneously measures the transcriptome and chromatin accessibility in space. Spatial domain identification methods can extend to multimodal applications, integrating spatial multi-omics data to delineate spatial domains with unique multi-omics patterns. Spatial multi-omics data can also be combined with single-cell multi-omics data for spatial deconvolution, integrating information from multiple omics to resolve the cell type distribution more precisely in each spot. Recently published spatial deconvolution works, like SONAR [55] and CARD [56], model spatial information, whereas most spatial deconvolution studies have not utilized spatial information. As spatial information is a critical component of spatial transcriptomics, incorporating it cleverly into models remains a crucial avenue for algorithmic improvement.

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CRediT authorship contribution statement

Chuanchao Zhang: Funding acquisition. **Lequn Wang:** Visualization, Methodology. **Qianqian Shi:** Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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