Computational Molecular Biology 2025, Vol.15, No.4, 183-192
http://bioscipublisher.com/index.php/cmb

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# Computational Frameworks for Spatial Transcriptomics in Tumor Microenvironment

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Computational Molecular Biology, 2025, Vol.15, No.4 doi: 10.5376/cmb.2025.15.0018
Received: 26 May, 2025
Accepted: 08 Jul., 2025
Published: 29 Jul., 2025



**Preferred citation for this article**:
Li J.H., 2025, Computational frameworks for spatial transcriptomics in tumor microenvironment, Computational Molecular Biology, 15(4): 183-192 (doi: 10.5376/cmb.2025.15.0018)

**Abstract** The spatial heterogeneity of the tumor microenvironment (TME) has a significant impact on tumor progression and treatment response. The rise of spatial transcriptomics technology has provided a new perspective for the study of TME, but its high-dimensional data characteristics pose challenges to the analytical methods. This paper constructs a computational modeling framework for TME spatial transcriptome data, integrating graph theory and spatial statistical methods to mine spatial patterns and cellular communication networks in tissues. We systematically expounded the spatial heterogeneity of the tumor microenvironment, the mainstream spatial transcriptome techniques and data characteristics, and proposed corresponding algorithms to identify cell subpopulations, cell communications and differential gene patterns in space. Through the case of spatial transcriptome of breast cancer, we verified the effectiveness of this framework and revealed the significant differences in molecular characteristics and immune microenvironment between the core and margin of the tumor. Studies have shown that computational models of spatial transcriptomics can deeply analyze the structure and function of the tumor microenvironment, providing new support for precision medicine.

**Keywords** Tumor microenvironment; Spatial transcriptomics; Spatial heterogeneity; Computational modeling; Multi-omics integration; Precision medicine

## 1 Introduction

Tumor cells do not exist in isolation. They are surrounded by a whole "small world" - immune cells, fibroblasts, blood vessels, and various extracellular substances are all active within it. This complex environment is called the tumor microenvironment. It is not like a fixed background but more like a participant: constant "dialogue" between cells and between cells and the matrix, and the result will affect whether the tumor grows slowly, spreads rapidly, or becomes sluggish to treatment. Sometimes, the development of a tumor does not entirely depend on the cancer cells themselves; rather, it is the attitudes of these "neighbors" around that determine its fate (He et al., 2025). In recent years, immunotherapy has once again drawn people's attention to the role of this microenvironment - it is not always helpful; more often than not, it interferes with the immune response in a "suppressive" manner, and thus has become an indispensable key link in research and treatment (Cao et al., 2023).

Previous studies on gene expression, such as single-cell RNA sequencing, although they could clearly see what was happening inside the cells, could not see the "position" of these cells in the tissue. Sometimes, knowing "who said what" is not enough; one also needs to know "where they said it". The emergence of spatial transcriptomics has precisely filled this gap - simultaneously measuring gene expression on tissue sections and marking spatial positions, just like drawing a map of gene activity (Li et al., 2022). In the past few years, this technology has developed rapidly and can be seen in various fields, from neuroscience to tumor research. Especially in the study of tumors, it enables us to more clearly observe the molecular differences in different regions, understand the complexity within tumors, and also provides new clues for individualized treatment (Huang et al., 2024).

This research mainly focuses on the spatial transcriptome data of the tumor microenvironment, aiming to understand the hidden spatial differences and biological patterns through computational modeling. We attempt to establish a systematic analytical framework, using graph theory and spatial statistics methods to reconstruct the organizational structure, while integrating multi-omics information into the model to make the results closer to the

real biological processes. The overall design is not merely a stacking of algorithms, but rather aims to strike a balance between methods and biological significance. The article begins with the spatial characteristics of the tumor microenvironment and then transitions to the features and modeling ideas of spatial transcriptome data. Next, the algorithm framework, recognition strategy, and the practical application of the model in breast cancer data will be introduced. Finally, reflect on the current challenges and put forward prospects for the future development direction.

## 2 The Complexity and Spatial Heterogeneity of the Tumor Microenvironment

### 2.1 Spatial distribution characteristics of cell types, signaling pathways and molecular networks

In solid tumors, cells are not randomly scattered. Cancer cells often cluster together, while immune cells prefer to "guard" at the edges and sometimes even form structures similar to lymph nodes (Di Mauro et al., 2024). If we look at the molecular level, different regions also have their own rhythms - certain signaling pathways, such as growth-promoting or inflammation-related signals, will have spatial differences in strength. The tumor center is often a place where proliferation is active, and related genes are frequently expressed there. But as soon as it reaches the edge, the signals of the immune response take the upper hand (Figure 1) (Du et al., 2023). In this way, the interior of the tumor presents a distinct "topographic" feature - cell types, signaling pathways, and molecular networks are interwoven at different positions, jointly forming its complex and hierarchical spatial structure.

### 2.2 The interaction between immune cells, fibroblasts and the vascular system in the microenvironment

In the tumor microenvironment, various cells and blood vessels are like a complex web, none of which can do without the other. Fibroblasts are the most "worried". They secrete cytokines and adjust the extracellular matrix, restricting the movement of immune cells. Sometimes, they even "build walls" to prevent T cells from getting close (Mao et al., 2021). However, immune cells are not the passive party. The chemokines and growth factors they release can in turn stimulate fibroblasts and, incidentally, promote angiogenesis. The uneven distribution of blood vessels makes the situation even more complicated - some areas have abundant oxygen while others lack it, and as a result, the performance of cells varies greatly (Kim et al., 2022). All these seemingly chaotic interactions actually jointly maintain the delicate and unstable balance of the tumor microenvironment.

### 2.3 The role of spatial heterogeneity in tumorigenesis and drug resistance mechanisms

A tumor is not a single entity but more like an ecosystem composed of different "terrains". The microenvironments at different locations vary greatly, with different levels of oxygen, nutrients, and cell types, which subject tumor cells to distinct survival pressures. Some subclones are more adapted in certain regions and gradually gain the upper hand, driving the tumor to evolve in new directions (Wang et al., 2024). However, this spatial disparity also brings trouble - drugs often fail to penetrate deep into the body, and the peripheral areas are frequently surrounded by fibroblasts and immunosuppressive cells, allowing cancer cells to take the opportunity to hide. The result is that the few cells that survive by chance will gradually develop drug resistance (Wu et al., 2025). Often, treatment failure and recurrence are not merely issues of drug efficacy, but rather the covert effect of this spatial heterogeneity.

## 3 Spatial Transcriptomics Technology and Its Data Characteristics

### 3.1 Review of mainstream spatial transcriptomics techniques (such as visium, slide-seq, MERFISH, etc.)

There are currently several mainstream approaches to spatial transcriptomics, each with its own "temperament". Like 10x Visium and Slide-seq, they belong to the sequencing category, while MERFISH follows the imaging route. Visium is an array of spatial barcodes spread all over a slide to capture the transcripts in tissue sections. Slide-seq is finer. It marks positions with micron-sized beads, and the differences of individual cells can almost be seen (Rademacher et al., 2024). MERFISH is different. It relies on multiple rounds of fluorescence in situ hybridization to directly count RNA in tissues, capable of testing hundreds or even thousands of genes at a time (Liu et al., 2022). Some people care more about resolution, while others value gene coverage - from tens of micrometers to subcellular, from the entire transcriptome to specific gene sets, different technologies have their own trade-offs.

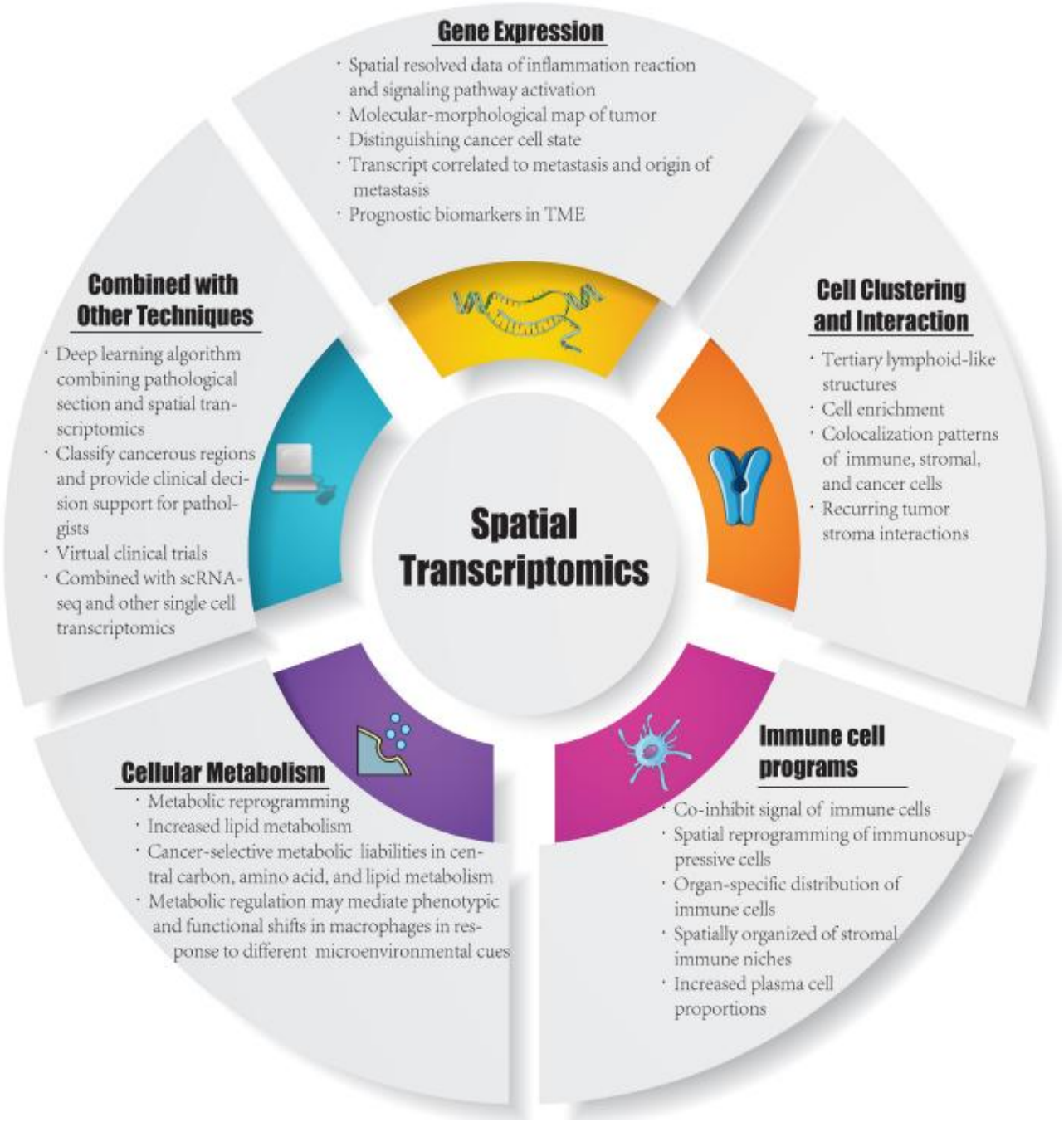


Figure 1 Inspirations in spatial transcriptomics. TME: tumor microenvironment; ScRNA-seq: single cell RNA sequencing (Adopted from Du et al., 2023)

### 3.2 Multi-dimensional characteristics of spatial data: spatial resolution, gene coverage and noise sources

The data of spatial transcriptomes is quite "picky", with various differences piling up layer upon layer. Distinguishing the first is a problem - some technologies can see subcellular details, while others can only cover a few cells. The larger the capture point, the more signals will be mixed together. When it comes to gene coverage, sequencing methods can scan tens of thousands of genes, but imaging methods usually only focus on a few hundred pre-selected targets. Noise is even more troublesome. During sequencing, there is often background RNA doping or insufficient sensitivity to miss signals. During imaging, autofluorescence and localization errors may also occur. The high data dimension and the small sample size, when combined, make the analysis tricky. To extract reliable information from it, various noise reduction and correction methods have to be relied upon to support the process (Shan et al., 2022; Wang et al., 2022).

### 3.3 Key issues in data preprocessing and quality control

Before starting to analyze the spatial transcriptome data, the raw data must be "cleaned up" first; otherwise, no matter how ingenious the model is later, it will be in vain. First, align the captured points with the tissue image. Points that are not within the tissue range or areas with strange signals should be removed. Those points with too few detected genes and high noise levels had better not be retained either. Next, normalization is needed to bring the sequencing depths at different positions back to the same level. The quality control step is more like a physical examination. Usually, it checks how many genes can be detected at each point to judge the overall quality. If a capture point is mixed with multiple cells, single-cell sequencing data still needs to be used to "split" and infer the proportion of each cell. When merging data from different batches or slices, it is also important not to forget to correct the batch effect. Only when these basic tasks are in place can the subsequent analysis be considered solid (Zeira et al., 2022; Hamel et al., 2023).

## 4 Computational Modeling Framework for Spatial Transcriptome Data

### 4.1 Model construction idea: from high-dimensional expression matrices to spatial structure reconstruction

When conducting spatial transcriptional modeling, the key is not only to analyze gene expression, but also to link these expressions with their "coordinates" in the tissue. In other words, each spatial point not only has a string of high-dimensional genetic data, but also has its own location and neighbors. Researchers usually first perform dimensionality reduction, compressing those redundant signals into more core features, and then consider how these points are spatially adjacent. Some people might regard these points as nodes on a graph, with edges representing proximity relationships, and then use algorithms to find regions that have similar expression patterns and are geographically close (Figure 2) (Dong and Zhang, 2021). In this way, the internal structure of the organization, especially the spatial pattern in the tumor microenvironment, can be gradually reconstructed (Lei et al., 2024). The entire process is like restoring a shuffled biological "map".

### 4.2 Model algorithms based on graph theory and spatial statistics

When analyzing spatial transcriptome data, there are roughly two common approaches: one relies on graph theory, and the other leans towards spatial statistics. The former likes to view data as a network graph - each spatial position is a node, and points that are close to each other are connected to form edges. In this way, various graph algorithms can be used to identify which regions have aggregated or associated patterns. Some studies simply apply graph convolutional networks to extract more complex features on this structure (Hu et al., 2021). The approach of spatial statistics is somewhat different. It places more emphasis on measuring "correlation" - for instance, using the Moran index to observe the spatial autocorrelation of gene expression, or employing Gaussian processes to depict the trend of expression varying with position (Lin et al., 2022). Both methods have their own strengths. If the structural sense of the graph and the quantitative ability of statistics can be combined, it is often possible to depict the spatial picture of the tumor microenvironment more realistically.

### 4.3 Comprehensive modeling method integrating multi-omics information

To truly understand the tumor microenvironment, relying solely on the spatial transcriptome is not enough. The current approach is more inclined towards "jigsaw puzzle" modeling, blending different omics data together. For instance, some people would use the results of single-cell RNA sequencing to label the cell types of spatial data, or use deconvolution methods to embed high-resolution cell information back into the spatial matrix. Some people prefer to view spatial transcriptome and proteome or imaging data together - such as comparing the protein map of multiple immunofluorescence with the distribution of gene expression for analysis, thereby verifying some key signals (Yang et al., 2025). Although this approach is complex, it can make up for the blind spots of a single data point. The fusion of multi-omics can reveal the structure and function of tumors from different levels, and also make the model more stable and more explanatory (Zhang et al., 2025).

## 5 Pattern Recognition and Functional Analysis of Spatial transcriptome Data

### 5.1 Cell subpopulation identification and spatial clustering algorithm

When conducting pattern recognition, researchers usually start by identifying cell subpopulations and then look at how they are spatially distributed. The most common approach is unsupervised clustering, which divides spatial

locations into different clusters based on the similarity of gene expression. Most of these clusters represent a group of cells with similar molecular characteristics. However, merely looking at the expression is not enough. Only by taking into account the spatial proximity relationship can these clusters be more "coherent" in their organizational structure. Sometimes researchers also change their approach, using specific marker genes to identify cell types and then map their distribution. If the data resolution is not high and a single point contains multiple cells, the data from single-cell sequencing can still be used as a reference to infer who is in each point. In this way, the cellular composition and spatial pattern in the tumor microenvironment can be gradually restored (Saqib et al., 2023; Zhang et al., 2024).

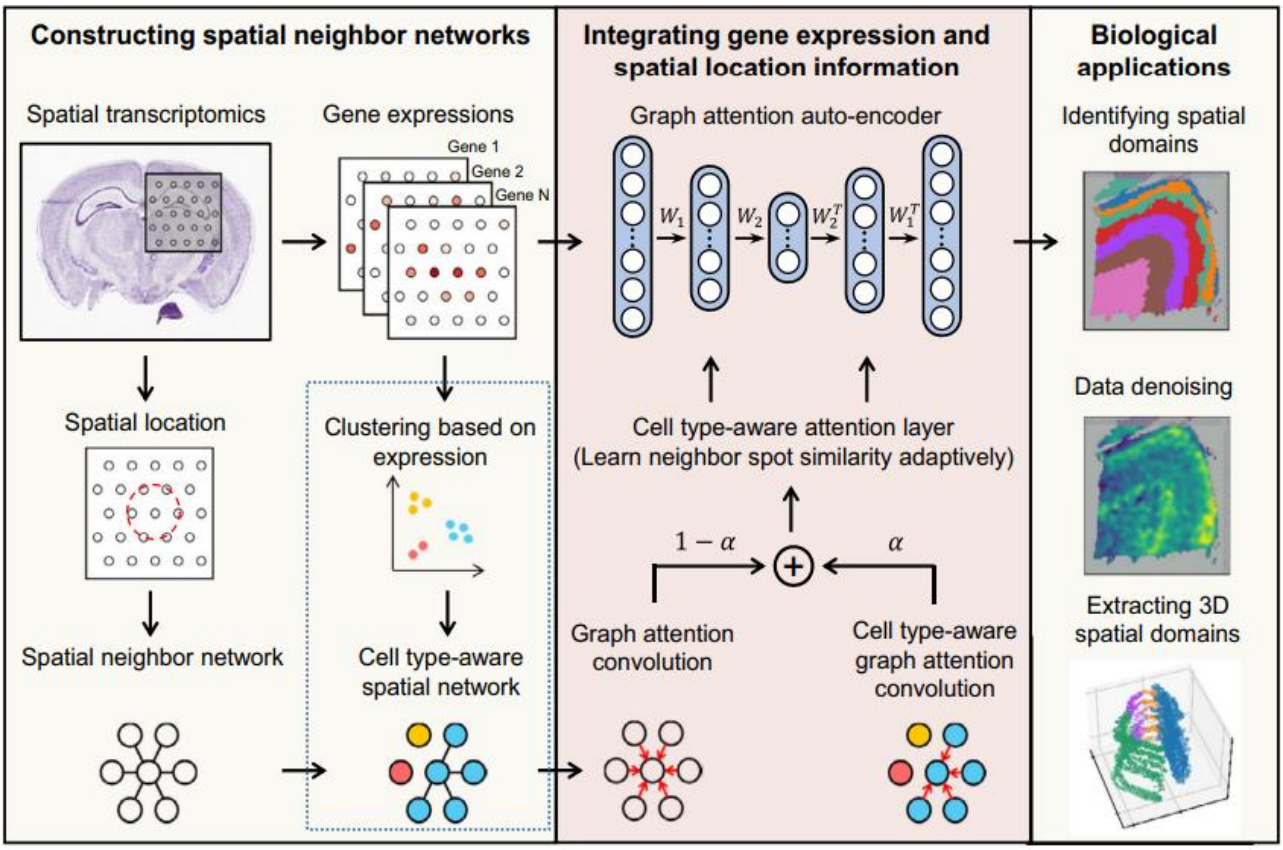


Figure 2 Overview of STAGATE (Adopted from Dong and Zhang, 2021)

Image caption: STAGATE first constructs a spatial neighbor network (SNN) based on a pre-defined radius, and another optional one in the dashed box for 10x Visium data by pruning it according to the pre-clustering of gene expressions to better characterize the spatial similarity at the boundary of spatial domains. STAGATE further learns low-dimensional latent representations with both spatial information and gene expressions via a graph attention auto-encoder. The input of the auto-encoder is the normalized expression matrix, and the graph attention layer is adopted in the middle of the encoder and decoder. The output of STAGATE can be applied for identifying spatial domains, data denoising, and extracting 3D spatial domains (Adopted from Dong and Zhang, 2021)

### 5.2 Inference of intercellular communication networks and modeling of signal paths

In the tumor microenvironment, cells do not fight on their own; they "speak" through the cooperation of receptors and ligands. The data of the spatial transcriptome makes it possible to capture this kind of communication. Usually, researchers observe adjacent cells: if one cell expresses a certain ligand and the adjacent cell happens to highly express the corresponding receptor, then there is a high probability that signal transmission is taking place between the two (Chowdhury et al., 2021). By aggregating hundreds or even thousands of such relationships, a communication network can be drawn - nodes represent different cell types or clusters, and the connections are their communication channels. By comparing these relationships with the known signaling pathways, it can be seen which pathways are more "talkative" in specific tumor environments, that is, the most notable part in regulatory and intervention research (Liu et al., 2024).

### 5.3 Spatial dependence differential gene expression analysis and functional annotation

The purpose of spatial differential gene analysis is actually to see which genes are "abundant and scarce" in tissues. Some genes are particularly active at the edge of the tumor, but almost silent in the core region. Some genes also change bit by bit along the spatial direction, as if there is a gradient (Liang et al., 2024). Such differences often suggest the biological conditions of different regions - for instance, a strong immune response in one area and insufficient oxygen in another. Researchers usually take these gene sets for functional annotation or pathway analysis to see which biological processes they are related to (Li et al., 2025). In this way, the spatial distribution of genes is no longer just a graph but becomes a clue that helps us understand the roles played by each "section" of the tumor microenvironment.

## 6 Challenges and Frontiers in Tumor Microenvironment Modeling

### 6.1 Solutions to data sparsity and high noise problems

The data of spatial transcriptomes always seem a bit "rough" - with many null values and high noise, which is an unavoidable problem when doing modeling. Some people will use smoothing or interpolation methods to make the information between adjacent points "borrow force" from each other, fill in those zero values, and extract the main signal at the same time (Lü et al., 2024). Some people trust statistical modeling more. They first assume that the noise follows certain patterns, such as characterizing it with a negative binomial distribution, and then distinguish the technical error from the true expression (Tian et al., 2024). If conditions permit, integrating data from multiple sets of experiments or different omics can also make the signal more stable. Although these methods cannot completely eliminate noise, they can make the results seem more reliable to some extent and lay a relatively solid foundation for subsequent analysis.

### 6.2 Interpretability of the model and difficulties in biological validation

There is a long-standing and difficult problem with spatial transcription modeling - the model is too smart for people to understand. Especially for deep learning models, they can capture various complex spatial patterns, but no one can clearly explain how the results come about or which genes play a major role (Chitra et al., 2025). What's more troublesome is that those seemingly interesting discoveries in the model, such as new cell interactions or expression hotspots, cannot be verified in experiments with just a turn of the head. It is time-consuming and laborious to conduct staining imaging and functional analysis, and many predictions were eventually put on hold. The result is that the credibility of the model has been compromised and its practical application has also been delayed. The key to the future may not lie in more complex algorithms, but in enabling models to "speak human language" while finding faster and more reliable experimental verification methods (Zhao et al., 2024).

### 6.3 The latest progress of artificial intelligence and deep learning in spatial modeling

Artificial intelligence is gradually venturing into the field of spatial transcription modeling, especially deep learning, which has been gaining momentum in recent years. Models like graph neural networks are now being used to analyze spatial gene expression maps, leveraging the adjacency information between cells to identify more subtle aggregation structures (Li et al., 2023). Some people have combined convolutional neural networks with histological images, hoping to capture more spatial features beyond gene expression. A bolder approach is to use generative models, such as variational autoencoders or Gans, to "create" spatial data for completing, simulating or verifying hypotheses (Hu et al., 2024). The advantage of AI is that it can automatically extract complex relationships from high-dimensional data, which is difficult for traditional algorithms to achieve. However, it also has a vulnerable side - it is prone to overfitting and has poor interpretability. No matter how powerful a model is, if it is not clear what it is looking at, its biological significance becomes a question mark.

## 7 Case Study: Modeling of Breast Cancer Microenvironment Based on Spatial Transcriptome Data

### 7.1 Data sources and experimental design (such as 10x genomics visium platform)

This case used a breast cancer tissue section, and the data was from the Visium platform of 10x Genomics. At the beginning of the experiment, fresh frozen tissue sections were spread on slides with spatial barcodes, and H&E

staining was performed simultaneously to align the morphological structure. Then, the mrnas captured on the slides were sequenced, with each spatial point corresponding to a gene expression profile. The final result is data on thousands of spatial locations, ranging from the core of the tumor all the way to the surrounding microenvironment (Chew et al., 2021). The advantage of such a set of data lies in that it can not only examine gene expression but also preserve the spatial layout of the tissue, providing a relatively complete foundation for subsequent model analysis and biological interpretation (Janesick et al., 2022).

### 7.2 Modeling methods and parameter optimization

This analysis employed a model that combines spatial clustering and graph convolutional networks, mainly aiming to examine the molecular characteristics of different regions in breast cancer tissues. Let's start with clustering. Not only do we group by gene expression, but also impose spatial location constraints to make the results appear continuous on the tissue sections rather than a bunch of scattered points. Next, treat each captured point as a node, connect adjacent points into edges, and form a spatial adjacency graph. Then, let GCN learn features on this graph and gradually delineate different regions (Hu et al., 2021). After the model started running, we repeatedly adjusted the number of clusters and parameters until the result stabilized. Finally, the slices were divided into several sections, each carrying its own "fingerprint" of gene expression, clearly revealing the spatial hierarchy of the tumor tissue (Long et al., 2023).

### 7.3 Biological interpretation and clinical significance of model results

Judging from the model results, this breast cancer tissue is not a homogeneous pile of cells. The gene expression in the central region is the most active, especially those related to the cell cycle and proliferation, indicating that tumor cells proliferate rapidly here. But as you go further out, the situation changes - a large number of immune-related signals appear in the marginal area, with T-cell markers and inflammatory factors all piled up densely there (An et al., 2024). This distribution can also explain some common clinical phenomena. For instance, areas with more immune cells are more responsive to immunotherapy, while regions with severe fibrosis are prone to drug resistance because drugs are less likely to penetrate (Wu et al., 2025). Overall, such spatial differences make it clearer to see the complexity of the breast cancer microenvironment and also remind people that treatment should not be a one-size-fits-all approach.

## 8 Future Outlook and Conclusions

Future spatial omics research seems to be increasingly "greedy" - not only looking at RNA, but also wanting to simultaneously observe protein, metabolic and even epigenetic information. Nowadays, some new spatial multi-omics techniques are attempting to detect multi-layer molecular signals on the same slice, making the picture of the tumor microenvironment more three-dimensional. Meanwhile, multimodal data at the single-cell level is also being "landed" in space. Researchers hope to remap information such as the transcriptome, epigenetics, and proteins to tissue locations to see exactly what the relationship is between gene regulation and spatial structure. When these data can truly be integrated, the cell networks and signal gradients in tumors may become clearer. Perhaps only then will precision oncology truly enter a stage where it can "see the details clearly".

The value of spatial transcriptional modeling lies more in its ability to enable us to "see" the differences within tumors. In fact, each patient's tumor is different. In some areas, the immune system is active, while in others, it is almost silent. Piecing together these spatial features into a map is like drawing a map for the tumor. Doctors can determine from this which areas may respond well to immunotherapy and which areas, due to fibrosis or immune deficiency, may require other means of assistance. Spatial analysis does not end here. It can also help people identify the most aggressive or drug-resistant "danger zones", providing targets for radiotherapy or local medication. When these results are integrated with clinical data, perhaps a more individualized decision-making approach can be formed, making treatment no longer a one-size-fits-all approach but truly "tailored to local conditions".

Overall, the modeling of spatial transcriptomics has provided us with a new way to observe the tumor microenvironment in more detail. As mentioned earlier in the article, this field has taken a considerable step forward, from spatial heterogeneity to data characteristics, and then to specific modeling methods and cases. But

then again, there are quite a few problems. The model calculation is getting faster and faster, but its interpretability often fails to keep up. The volume of data is huge, but the results do not always translate into clear biological significance. What needs to be done in the future is probably not only to speed up the algorithm, but also to make the model's inference more "transparent" and form a virtuous cycle with experimental verification. At the same time, only by truly integrating spatial omics with fields such as immunology and pharmacology can new research ideas be opened up. Technology will eventually mature, but the process of understanding the complexity of living things is likely to take a little longer.

## Acknowledgments

I would like to express my heartfelt thanks to all the teachers who have provided guidance for this study.

## Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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