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Emerging Insights into the Principles and Advantages of Single-Cell and Spatial Transcriptomics

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Abstract: Single-cell transcriptomics is a high-throughput technology capable of analyzing gene expression at the individual cell level. Spatial transcriptomics, on the other hand, is a technique that simultaneously captures both gene expression profiles and the spatial location information of cells. While single-cell transcriptomics enables sequencing of the transcriptome at a single-cell resolution, spatial transcriptomics provides the added dimension of spatial context alongside gene expression data. These two approaches−single-cell transcriptomics and spatial transcriptomics−are complementary, and their integration can facilitate a more comprehensive and in-depth investigation of biological questions.

Keywords: Single-cell transcriptome sequencing; High-throughput analysis; Spatial transcriptome sequencing; Spatial location information; Cell transcriptome.

1. Introduction

Single-cell RNA sequencing (scRNA-seq) is a powerful technique that enables the analysis of gene expression at the resolution of individual cells. By isolating, labeling, sorting, lysing, reverse transcribing, and sequencing cells from a tissue, scRNA-seq constructs high-resolution gene expression profiles at the single-cell level. This allows for the identification of distinct cell types, functional states, subpopulation heterogeneity, and dynamic transitions [1]. Additionally, scRNA-seq can detect transcript isoforms, such as alternative splicing events, offering critical insights into gene regulatory mechanisms. It is particularly well-suited for identifying rare or novel cell types that are difficult to capture using traditional bulk RNA-sequencing methods. However, scRNA-seq has certain limitations. For instance, spatial information is lost during cell dissociation, making it difficult to reconstruct the original tissue context. Furthermore, the technique is costly, prone to high levels of technical noise, and requires complex computational analysis [2], posing significant challenges for experimental design and data interpretation.

To address the lack of spatial context in scRNA-seq, spatial transcriptomics (ST) technologies have emerged. These methods preserve the tissue architecture while fixing, permeabilizing, and sequencing tissue sections. Through spatial barcoding or probe-based strategies, ST maps RNA molecules to their spatial locations, thereby enabling transcriptome-wide spatial gene expression profiling. ST reveals how cells are organized within the tissue microenvironment, their interactions, and functional states. While ST retains spatial information, its spatial resolution is limited−each capture spot may represent transcripts from multiple cells−making it challenging to resolve expression patterns at true single-cell resolution. In addition, ST generally exhibits lower sensitivity for detecting low-abundance transcripts [3]. Data integration and analysis in ST also remain nontrivial, particularly when aligning and fusing ST data with other modalities such as scRNA-seq.

Despite their individual limitations, both scRNA-seq and ST rely on high-throughput mRNA sequencing to generate large-scale gene expression datasets. When combined with computational tools such as clustering analysis and pseudotime inference, these methods can reveal cellular developmental trajectories and state transitions [4]. Integrating scRNA-seq with ST has become a powerful strategy for dissecting complex tissue architectures and functions. By aligning datasets, performing spatial mapping, and applying joint modeling approaches, researchers can localize single-cell subpopulations within tissues and annotate their functions with high resolution and biological interpretability.

For example, in tumor microenvironment studies, scRNA-seq can identify cellular subtypes−including tumor cells and immune cells such as T cells and macrophages − and characterize the expression of immune checkpoint genes (e.g., PD-1, CTLA-4) [5]. ST can then determine the spatial distribution of these cells, such as whether immune cells cluster at tumor margins or infiltrate the tumor core, thereby revealing immune infiltration patterns. The integration of both modalities enables the construction of detailed tumor microenvironment maps and uncovers signaling networks between cells, as well as their associations with therapeutic responses. For instance, Keren et al. [6] (2018) found that T cells near the tumor core in breast cancer exhibited a more pronounced exhausted phenotype, suggesting that spatial location significantly influences immune function. Similarly, Moncada et al. [7] (2020) observed that the spatial proximity between fibroblasts and tumor cells in pancreatic cancer may promote the formation of the tumor microenvironment.

In neuroscience, the joint application of scRNA-seq and ST has been instrumental in mapping neuronal spatial organization and functional partitioning in the cerebral cortex. For example, ST can first delineate regional expression landscapes, and scRNA-seq can then annotate neuronal subtypes, allowing for precise correlation between functional anatomy and molecular identity [8]. These studies demonstrate that integrating scRNA-seq and ST not only provides complementary spatial resolution but also offers a more comprehensive perspective on tissue function, cellular interactions, and disease mechanisms.

Looking ahead, continued advances in multimodal integration−particularly the combination of scRNA-seq, ST, proteomics, and epigenomics, coupled with AI-driven high-dimensional data analytics−are expected to enable a more systematic understanding of spatial-molecular mechanisms in

biological systems. This progress holds great promise for supporting precision medicine and elucidating the molecular basis of disease.

2. ScRNA-seq (Single-cell RNA sequencing)

2.1. Classification of scRNA-seq Technologies

Since its first application in 2009 for whole-transcriptome mRNA profiling of individual cells, single-cell RNA sequencing (scRNA-seq) has evolved into a diverse array of sub-technologies. These can be broadly categorized into two classes based on the transcript coverage they achieve. The first category includes full-length transcript sequencing technologies, such as Smart-seq2, MATQ-seq (Multiple Annealing and Tailing-based Quantitative sequencing), and SUPeR-seq (Single-cell Universal Poly(A)-independent RNA sequencing). These methods offer high sensitivity and accuracy, enabling comprehensive analysis of transcript isoforms, including alternative splicing and allele-specific expression. They are thus well-suited for studies requiring detailed transcript-level resolution. However, they typically suffer from low cellular throughput and relatively high costs [9].

The second category comprises 3′ - or 5′ -end counting methods, such as Drop-seq, Seq-Well, Chromium (10x Genomics), and STRT-seq (Single-cell Tagged Reverse Transcription sequencing). These techniques are designed for high-throughput, cost-effective profiling by capturing transcript termini and incorporating unique molecular identifiers (UMIs) to quantify gene expression levels. While advantageous for large-scale studies due to their scalability and affordability, these approaches are limited in their ability to resolve transcript isoforms and generally exhibit reduced sensitivity to low-abundance transcripts [10].

2.2. Workflow of scRNA-seq

In practical applications, scRNA-seq workflows require integration with various cell isolation strategies. These include manual micromanipulation (e.g., mouth pipetting), laser capture microdissection (LCM), and fluorescence-activated cell sorting (FACS), as well as more advanced droplet-based and microfluidic technologies [11]. Droplet-based platforms, such as Drop-seq, encapsulate single cells with barcoded beads within nanoliter-scale droplets to achieve parallelized separation and indexing. In contrast, microfluidic platforms like Fluidigm C1 utilize microchannels and valve systems for precise control over single-cell capture and processing [12]. These two approaches differ significantly in their underlying principles, instrumentation, and throughput characteristics. Therefore, the selection of an appropriate method should be carefully determined based on experimental goals, sample types, and the required scale of analysis.

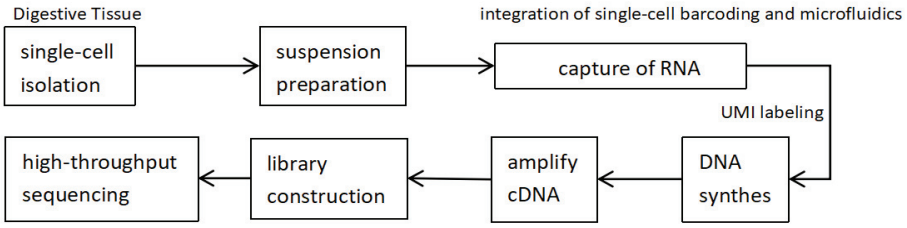

Figure 1. Single-cell transcriptome sequencing workflow

The scRNA-seq workflow is relatively straightforward and efficient: First, tissue is dissociated to isolate single cells and prepare a cell suspension, ensuring cell integrity and viability while minimizing cell death and aggregation. Next, single cells are combined with barcoded beads or microfluidic chips, where they are lysed within a confined reaction space to capture mRNA, which is then reverse transcribed into complementary DNA (cDNA). Unique molecular identifiers (UMIs) are incorporated during this process to distinguish individual molecules. The cDNA is then amplified to generate a sequencing library, with a focus on ensuring uniform amplification and accuracy to avoid batch effects. Finally, the library undergoes high-throughput sequencing, allowing for the acquisition of gene expression data for each individual cell [13].

2.3. Comparison of Key Technologies

Comparison Aspect	10X Genomics (10X Single-Cell Genomics)	Drop-seq (High-Throughput Sequencing)
Technical Principle	Based on Chromium system, microfluidic encapsulation	Droplet-based, relies on lab-built system
Cell Throughput	High, 500–20,000 cells/sample	Lower, 1,000–10,000 cells/sample
Capture Efficiency	~65%, 75% valid reads, high barcode efficiency	~30%, low barcode efficiency, more noise
Sensitivity	High, suitable for low-expression genes	Lower, limited for low-abundance transcripts
Doublet Rate	Low, ~0.9%/1,000 cells	Higher, limited by Poisson distribution
Cost	Higher, ~$0.87/cell	Lower, ~$0.44–0.47/cell, open-source
Operational Ease	Commercial platform, user-friendly	Requires self-assembly, complex, needs optimization
Application Scenarios	Ideal for complex samples, high-throughput studies, e.g., tumor microenvironment	Suitable for budget-limited exploratory research or tech development

Figure 2. Comparison of 10X Genomics and Drop-seq

In the scRNA-seq sequencing workflow, sequencing depth and quality are critical considerations. Taking 10X Genomics' Chromium and Drop-seq as examples, both are droplet-based technologies that perform 3' end sequencing, yet they differ significantly in performance and application. The 10X Genomics Chromium system, leveraging a commercial platform, utilizes microfluidic chips for efficient single-cell encapsulation. This system supports a cell throughput of tens of thousands (500 to 20,000 cells/sample), with a capture efficiency of up to 65% and an effective read ratio of approximately 75%. It offers higher sensitivity for detecting low-expressed genes, a low doublet rate (0.9%/1000 cells), but comes at a higher cost ($0.87 per cell). It is thus well-suited for complex sample studies requiring high-throughput, high-quality data, such as tumor microenvironment analyses [14]. In contrast, Drop-seq relies on a laboratory-built system, with lower throughput (about 1,000 to 10,000 cells), a capture efficiency and effective read ratio of approximately 30%, and insufficient sensitivity for low-abundance transcripts. Additionally, it has a higher doublet rate, but is more cost-effective (～$0.44–0.47 per cell) and offers an open-source workflow, making it ideal for exploratory studies or technical development with budget constraints [15]. Overall, 10X Genomics excels in data quality and ease of use, while Drop-seq offers advantages in cost-effectiveness and flexibility. The choice between these technologies should be based on research objectives, sample characteristics, and budget considerations. The flexible integration of these technologies and workflows enables scRNA-seq to provide deep insights into cellular heterogeneity, functional differences, and developmental dynamics, thus opening new perspectives in biological research.

3. ST (Spatial Transcriptomics)

3.1. Historical Development of Spatial Transcriptomics (ST)

The development of Spatial Transcriptomics (ST) dates back to the 1970s, when techniques such as in situ hybridization (ISH) and laser capture microdissection (LCM) were primarily used to visualize and capture gene expression in spatial contexts [15]. Although these early methods had some spatial resolution, they were limited by low throughput and could only detect a small number of known genes, representing the earliest form of ST. It was not until 2010 that ST achieved high-throughput, high-resolution, and high-diversity gene expression detection in spatial contexts [16]. Researchers generally categorize ST into two main approaches. The first category involves large-scale next-generation sequencing (NGS)-based technologies, where positional information is encoded onto transcripts prior to sequencing. These methods include microarray slides with positional barcodes to capture mRNA from tissues for ST, slides with random barcode beads to capture mRNA for Slide-seq, and the GeoMX Digital Spatial Profiler (DSP), which uses ultraviolet light to release photocleavable gene tags from regions of interest (ROIs) for sequencing and quantification [3]. The second category is imaging-based techniques, typically employing specific probes to amplify and sequence transcripts within tissues. These methods include in situ hybridization (ISH), which uses fluorescent probes for continuous hybridization and imaging of transcripts within tissues, and in situ sequencing (ISS), which enables direct sequencing within tissues [17].

3.2. Workflow of ST

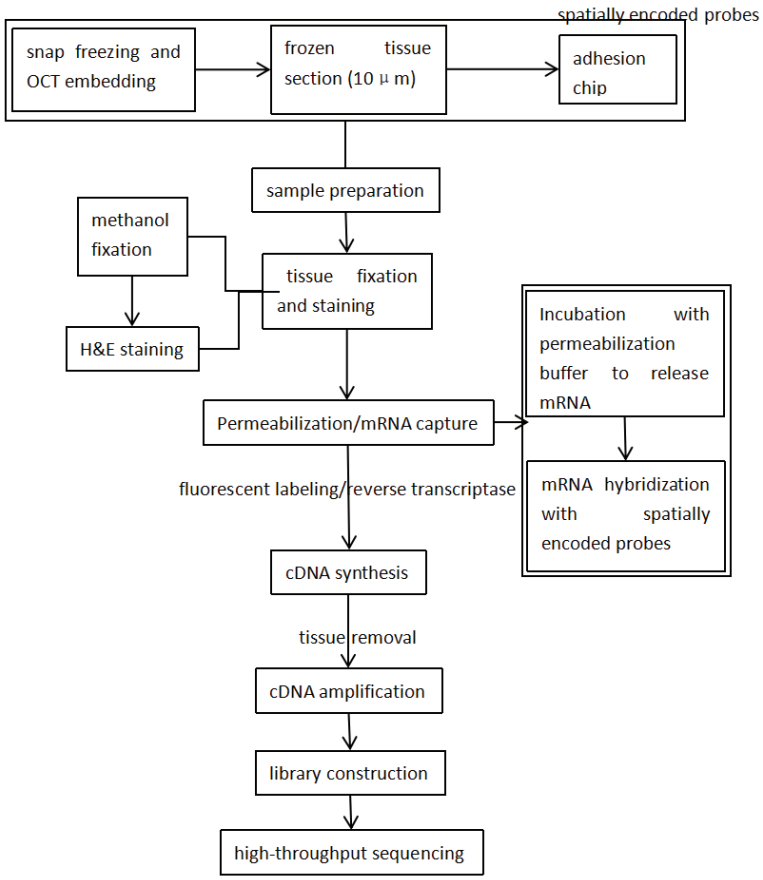

Figure 3. Spatial transcriptomics sequencing workflow

The basic workflow of Spatial Transcriptomics (ST) can be divided into several key steps. The first step involves sample preparation, where fresh tissue samples are rapidly frozen and embedded in OCT (optimal cutting temperature) compound. The samples are then sectioned into approximately 10 μm thick slices using a cryostat and placed onto a chip containing spatially encoded probes. The next step is fixation and staining, where the chip is fixed with methanol for a certain period, followed by H&E (hematoxylin and eosin) staining to observe tissue structure and morphology. Subsequently, the chip is incubated in a permeabilization solution to release mRNA from the cells, which binds to the corresponding probes. Fluorescently labeled reverse transcriptase is then used for cDNA synthesis. The following step involves incubating the chip in a tissue removal solution to remove residual tissue and OCT. The cDNA is then amplified using PCR. Finally, the corresponding sequencing library is prepared, typically through steps including end-repair, A-tailing, adapter ligation, and indexing. The library is then sequenced using a high-throughput sequencing platform [3].

4. Conclusion

4.1. Advantages and Applications of scRNA-seq

Both scRNA-seq and ST have their respective advantages and limitations, and their applications have evolved accordingly. For scRNA-seq, its high resolution allows for the avoidance of the homogenization effect of mixed samples in traditional sequencing methods, preserving the original and authentic gene expression information of each individual cell, thereby reflecting the cell's functions and characteristics. In addition, scRNA-seq enables high-throughput sequencing, which allows for the simultaneous analysis of thousands to tens of thousands of single cells, thereby enhancing sequencing efficiency and coverage. This facilitates the discovery of rare cells and novel cell subtypes [18]. Its high flexibility is another key advantage, making it adaptable to various sample types such as blood, tissue, and cultured cells. It can also be combined with other omics data, such as genomics, epigenomics, and proteomics, to conduct multi-layered analyses [19]. These advantages enable scRNA-seq to be applied in various biological fields, such as large-scale cell atlas construction: by sequencing single cells from specific tissues or organs, it is possible to obtain comprehensive transcriptomic maps, perform cell type clustering and annotation, and analyze the functions and interactions of different cell subtypes, revealing the structure and mechanisms of complex biological systems. It is also useful in neuroscience research: by sequencing individual neurons or neural stem cells in the nervous system, it is possible to obtain complex neuronal transcriptomic maps, classify neuronal subtypes based on gene expression patterns, understand the functions and interconnections of different neuron types, and explore issues related to neural system development, plasticity, and disease. Additionally, this technology can be applied in developmental biology, stem cell development and differentiation studies, and cancer research, among others.

4.2. Strengths and Applications of ST Technology

The primary advantage of ST lies in its spatial capabilities, such as the ability to measure the expression of all genes across complete tissue sections, rather than being limited to a small number of pre-selected genes. It can also analyze different tissue types and sample conditions, including fresh frozen samples and fixed samples. Most importantly, it retains the spatial information of cells in their original location, rather than isolating or disrupting cells before sequencing. ST is mainly used to study the spatiotemporal dynamics of gene expression in multicellular organisms [20], revealing molecular mechanisms involved in processes such as development, differentiation, and signaling, as well as the interactions between neurons and glial cells in the nervous system. It can also be used to explore potential molecular mechanisms underlying neurodegenerative diseases and other neurological disorders.

4.3. Synergy Between scRNA-seq and ST

The advantage of scRNA-seq is its ability to analyze both intracellular and intercellular heterogeneity, identify new cell types and states, explore the trajectories of cell development and differentiation, and understand transcriptional dynamics and gene regulatory relationships. On the other hand, ST combines gene expression data with the morphological features of tissue sections, revealing the heterogeneity and functions of cells in the spatial dimension. In the research process, we can leverage the complementary strengths of both technologies to achieve enhanced transcriptional insights.

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