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Research Progress of Single-Cell Transcriptome Sequencing Biotechnology

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Abstract. Traditional bulk transcriptome sequencing can only obtain the average expression signal of a cell population, cannot resolve cellular heterogeneity within tissues, and has difficulty revealing the mechanisms of dynamic changes in cell states and interactions with the microenvironment. Single-cell RNA sequencing (scRNA-seq) technology can resolve whole-transcriptome expression profiles at single-cell resolution, breaking through the technical bottleneck of population averaging, and has become a core disruptive biotechnology in the life sciences. This paper systematically sorts out the technical principles, mainstream experimental platforms, and microfluidic supporting technologies of scRNA-seq, and summarizes the core bioinformatics analysis methods and tool platforms such as single-cell data preprocessing, cellular heterogeneity resolution, and differentiation trajectory reconstruction; it focuses on the application progress of this technology in the construction of the human cell atlas, the resolution of the tumor microenvironment, the tracking of development and differentiation, cell communication analysis, and disease biomarker screening; it summarizes frontier technology directions such as single-cell multi-omics integration, spatial transcriptomics fusion, and CRISPR functional screening, and analyzes the current bottleneck problems of cost, throughput, and data analysis. Finally, combined with the trend of technological iteration, it looks forward to the future development directions of high throughput, low cost, multi-dimensional integration, and clinical translation, aiming to provide a systematic reference for the basic research and biomedical applications of single-cell sequencing technology.

Keywords: Single-cell transcriptome sequencing, scRNA-seq, cellular heterogeneity, microfluidics, multi-omics integration

1. Introduction

The tissues and organs of multicellular organisms are composed of a large number of cells with different functions, states, and origins, and cellular heterogeneity is a core biological feature of tissue development, homeostasis maintenance, and disease occurrence. Traditional bulk RNA-seq takes tissues or cell populations as samples, and what it obtains is the average gene expression value of tens of thousands of cells, which masks the specific expression characteristics of rare cells and cell subpopulations, cannot track the temporal changes of dynamic cell processes, and makes it difficult to resolve the molecular mechanisms behind cellular heterogeneity [1].

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In 2009, Tang et al. achieved whole-transcriptome sequencing at the single-cell level for the first time, formally opening the era of single-cell transcriptome research [1]. After more than ten years of technological iteration, scRNA-seq has developed from a low-throughput, high-cost laboratory technology into a high-throughput, standardized mature biotechnology; cell throughput has risen from a single cell at the beginning to millions of cells per experiment, detection sensitivity and data quality have improved substantially, and costs have dropped significantly. This technology can resolve gene expression profiles at single-cell resolution, precisely identify cell subpopulations, track dynamic cell evolution, and resolve intercellular interactions; it has completely changed the research paradigm of the life sciences and pushed developmental biology, tumor biology, immunology, regenerative medicine, and other fields to achieve a series of breakthroughs [2].

This paper systematically sorts out the research progress of single-cell transcriptome sequencing technology from five dimensions—technical principles, experimental systems, bioinformatics analysis, biological applications, and frontier expansion—summarizes the technical advantages and existing bottlenecks, looks forward to future development directions, and provides a systematic technical reference for research in related fields.

2. Principles and experimental system of single-cell transcriptome sequencing technology

2.1. Basic principles and complete workflow of scRNA-seq technology

The core idea of scRNA-seq is: isolate the mRNA in a single cell, reverse-transcribe it into cDNA and amplify it, then obtain the gene expression profile of each cell through high-throughput sequencing, and finally use the "cell × gene" expression matrix as the starting point of all downstream analyses [3]. The complete experimental workflow can be summarized as five consecutive steps (Figure 1a).

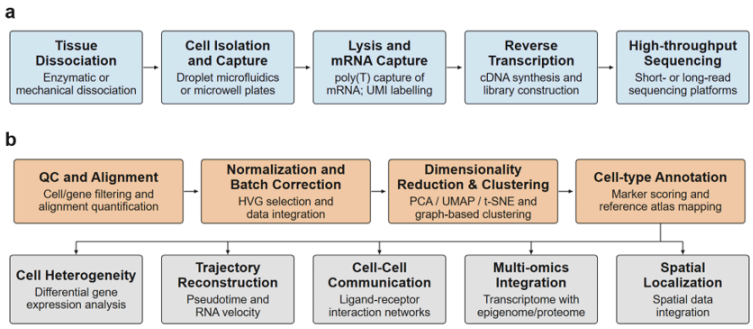


Figure 1. Experimental workflow (a) and bioinformatics analysis framework (b) of single-cell RNA sequencing

The complete scRNA-seq experimental workflow mainly includes six core steps: ① tissue sample dissociation and single-cell suspension preparation, dispersing solid tissue into single living cells through enzymatic digestion, mechanical pipetting, and other means to ensure cell activity and integrity; ② single-cell capture and lysis, isolating single cells through microfluidics, plate sorting, and other means and completing cell lysis to release intracellular mRNA; ③ mRNA capture and reverse transcription, capturing the polyA tail of mRNA with oligo-dT primers and reverse-transcribing the first-strand cDNA; ④ cDNA amplification and library construction, amplifying cDNA through PCR or in vitro transcription, detecting the concentration and fragment distribution of amplification products, fragmenting, adaptor-ligating, and purifying the cDNA, and constructing

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a library that meets sequencing requirements; ⑤ high-throughput sequencing and bioinformatics analysis, obtaining sequence data through second-generation sequencing platforms and carrying out downstream functional analysis after alignment, quantification, and quality control [4].

2.2. Classification and comparison of mainstream scRNA-seq technology platforms

According to the differences in single-cell isolation and reaction systems, mainstream scRNA-seq technologies can be divided into two major categories, droplet microfluidic type and plate type, and the two categories differ significantly in cell throughput, detection sensitivity, cost, and applicable scenarios.

Droplet microfluidic technologies use water-in-oil microdroplets as single reaction units, wrapping single cells and reaction reagents in nanoliter droplets to complete lysis, reverse transcription, and other reactions; representative technologies include the 10x Genomics Chromium system and Drop-seq. The core advantage of this type of technology is extremely high cell throughput—a single experiment can capture thousands to tens of thousands of cells, suitable for heterogeneity analysis of large cell populations—but transcript detection sensitivity is relatively low, and it is mainly 3' end sequencing, making it difficult to cover full-length transcripts [3, 5].

Plate-type technologies use 96-well or 384-well plates as reaction carriers, distributing single cells into wells through fluorescence-activated cell sorting or micromanipulation to independently complete the full set of reactions; representative technologies include Smart-seq2 and CEL-seq2. The advantages of this type of technology are high transcript coverage and high detection sensitivity, and it can obtain full-length transcriptome information, suitable for in-depth analysis of a small number of cells, but cell throughput is low and the cost per sample is high, making it difficult to support large-scale population cell analysis [6, 7].

The performance comparison of the four mainstream technology platforms is shown in Table 1.

Table 1. Performance comparison of mainstream single-cell transcriptome sequencing technology platforms

Technology platform	Single-cell isolation method	Cell throughput (single run)	Transcript coverage characteristics	Detection sensitivity	Cost per sample	Core advantages	Applicable scenarios
10x Genomics Chromium	Droplet microfluidics	1000~100000 cells	3' end sequencing, UMI quantification	Medium	Low	Ultra-high throughput, easy operation, high degree of standardizatio n	Large-scale cell clustering, tissue heterogeneity resolution, cell atlas construction
Drop-seq	Droplet microfluidics	1000~10000 cells	3' end sequencing	Relatively low	Extremely low	Extremely low cost, high throughput	Large-scale primary screening, large-sample population analysis
Smart-seq2	FACS / plate	96~384 cells	Full-length transcripts	High	High	Full-length transcripts, high sensitivity, complete gene coverage	In-depth analysis of small numbers of cells, alternative splicing analysis, allele-specific expression
CEL-seq2	Plate sorting	96~384 cells	3' end sequencing, in vitro transcription amplification	Medium-high	Medium-high	Good reproducibility , accurate quantification	Medium-throughput expression quantification, comparative sample analysis

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Table note: Table 1 compares the core technical parameters and applicable scenarios of the four mainstream scRNA-seq platforms, among which the 10x Genomics platform is currently the most widely used commercialized technology system.

2.3. Supporting mechanisms of microfluidics in single-cell sequencing

Microfluidics is the core supporting technology for high-throughput, low-cost, standardized single-cell sequencing. Through micron-scale channels it precisely manipulates microliter and nanoliter fluids to achieve operations such as single-cell isolation, reaction encapsulation, and reagent mixing, fundamentally improving the technical performance and application potential of scRNA-seq.

The supporting role of microfluidics for single-cell sequencing is mainly reflected in three aspects: ① high-throughput precise single-cell isolation; through hydrodynamic focusing, droplet generation, and other means, tens of thousands to hundreds of thousands of water-in-oil droplets can be rapidly generated, each droplet encapsulating a single cell and reaction reagents, achieving large-scale parallel isolation of single cells, with a cell capture efficiency of over 80% [5]; ② ultra-low reaction volume and cost control; the reaction system is reduced from the traditional microliter scale to the nanoliter scale, greatly reducing reagent consumption and per-sample cost, making high-throughput single-cell sequencing economically feasible; ③ integrated reaction workflow; multiple steps such as cell capture, lysis, and reverse transcription can be integrated on a microfluidic chip, reducing manual operation errors and improving experimental stability and standardization [5]. The most commercially successful 10x Genomics platform is precisely based on microfluidic droplet technology, achieving large-scale, standardized application of single-cell sequencing and promoting the popularization of this technology.

3. Bioinformatics analysis system and core algorithms of single-cell transcriptomics

Single-cell sequencing data have the characteristics of high dimensionality, high noise, high heterogeneity, and large data volume; traditional bulk transcriptome analysis methods cannot be directly applied, and a complete bioinformatics analysis system from preprocessing and quality control to functional resolution has now taken shape.

3.1. Preprocessing system for single-cell sequencing data

Data preprocessing is the foundation that determines the reliability of downstream analysis, usually including alignment and quantification, quality control filtering, normalization, and batch correction; the overall analysis pipeline is shown in Figure 1(b). Data preprocessing is the basic step that guarantees the reliability of analysis results, and its core goal is to remove technical noise and retain real biological signals. It mainly includes four major steps: ① raw sequence alignment and quantification, aligning sequencing reads to the reference genome, correcting amplification bias through UMIs (unique molecular identifiers), and precisely quantifying the expression level of each gene; commonly used tools include STAR and Kallisto; ② cell-level quality control, filtering low-quality cells, including abnormal cells with too few/too many genes, apoptotic cells with an excessively high proportion of mitochondrial genes, and doublets; commonly used tools include Scrublet; ③ expression matrix standardization and normalization, eliminating the expression bias caused by differences in sequencing depth; ④ batch effect correction, eliminating technical differences between different batches and different samples through algorithms such as Harmony and Seurat Integration while retaining real biological differences [8].

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3.2. Core analysis methods for resolving cellular heterogeneity

Resolving cellular heterogeneity is the most core analysis content of single-cell transcriptomics, achieving precise identification of cell subpopulations through dimensionality reduction, clustering, and annotation. First, dimensionality reduction algorithms map high-dimensional gene expression data to a low-dimensional space: principal component analysis (PCA) is a linear dimensionality reduction method used for initial data compression; t-SNE and UMAP are nonlinear dimensionality reduction methods that can visually display cell clustering structures in two/three-dimensional space, among which UMAP better preserves the global structure and continuous relationships of cell populations and is currently the mainstream visualization method [9].

After dimensionality reduction, clustering algorithms identify cell subpopulations; the mainstream algorithms are the Louvain and Leiden algorithms, which perform community detection based on expression similarity between cells to obtain different cell groups. After clustering, cell subpopulations are identified for cell type through marker gene matching and automatic annotation tools (such as SingleR), clarifying the cell identity and functional characteristics of each group.

3.3. Dynamic analysis technologies for cell differentiation trajectories

Single-cell transcriptomics can capture transient intermediate states in the processes of cell differentiation, activation, and reprogramming, and reconstruct the dynamic evolution process of cells through trajectory inference technology. The most classic method is pseudotime analysis, represented by the Monocle tool, which constructs cell evolution paths based on expression similarity between cells, arranges cells on a virtual time axis, and resolves the dynamic changes of gene expression during differentiation [10].

RNA velocity analysis is a dynamic analysis technology developed in recent years; by analyzing the spliced/unspliced ratio of mRNA, it infers the direction and rate of changes in cell transcription state and predicts the future evolution trend of cells. It does not rely on prior differentiation path assumptions and can resolve more complex dynamic cell processes. In addition, lineage tracing technology combined with barcode labeling can track the lineage differentiation relationships of cells over the long term and resolve the real lineage paths of in vivo development.

3.4. Comparison of mainstream single-cell analysis platforms

Currently, mainstream single-cell analysis tool platforms are represented by Seurat and Scanpy; both have complete functional coverage and mature ecosystems, supporting the data analysis work of the vast majority of single-cell studies.

Seurat is developed based on the R language and is currently the most widely used single-cell analysis platform. Its functions cover the whole pipeline of data preprocessing, dimensionality reduction clustering, cell annotation, trajectory analysis, cell communication, multi-omics integration, etc., with powerful visualization functions and a rich plugin ecosystem, suitable for all kinds of analysis needs from basic to advanced, but its memory usage is high and its efficiency in processing ultra-large datasets is relatively low [8].

Scanpy is developed based on the Python language, with an efficient architecture and low memory usage; it can easily handle large-scale datasets of millions of cells, with fast analysis speed and strong scalability. Its functional coverage is basically comparable to Seurat, suitable for high-throughput, large-sample single-cell data analysis, and it is currently the mainstream tool for large-scale cell atlas research [11].

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4. Biological application progress of single-cell transcriptome technology

With the unique advantage of single-cell resolution, single-cell transcriptome technology has been widely applied in various fields of the life sciences and has promoted a series of major biological discoveries. The following elaborates from four core application directions.

4.1. Resolving tissue cellular heterogeneity

4.1.1. Human Cell Atlas construction research

The Human Cell Atlas (HCA) project is a major international cooperation project in the life sciences, aiming to draw single-cell transcriptome atlases of all tissues and all cell types of the human body and systematically resolve the molecular characteristics and classification system of human cells [12]. Based on scRNA-seq technology, cell atlases of dozens of human tissues such as lung, liver, brain, gut, and blood have been completed, and more than a hundred new cell types and cell states have been identified, refreshing the understanding of human cell composition.

The team of Guo Guoji in China took the lead in drawing a Chinese human cell atlas spanning multiple systems, systematically resolving the cell characteristics and expression differences of the Chinese population and providing a reference baseline of the Chinese population for disease research and precision medicine [13]. Cell atlas research has not only established a reference database of human cells, but also discovered a large number of rare cell subpopulations, such as pulmonary epithelial ionocytes and intestinal tuft cells, providing a brand-new perspective for understanding tissue physiological functions and disease pathogenesis.

4.1.2. Tumor microenvironment and immune heterogeneity resolution

Tumor tissue has a high degree of cellular heterogeneity; it contains not only tumor cell subpopulations of different genotypes, but also stromal cells such as immune cells, stromal cells, and endothelial cells, which together constitute the complex tumor microenvironment (TME). scRNA-seq can systematically resolve the cell composition and state characteristics of the tumor microenvironment and is a core technology of tumor immunology research.

Studies have identified various immunosuppressive cell subpopulations through scRNA-seq, such as tumor-associated macrophages (TAMs), exhausted CD8+ T cells, and regulatory T cells, and resolved the formation mechanism of the immunosuppressive tumor microenvironment [14]. At the same time, this technology can reveal the intratumor heterogeneity of tumor cells, identify tumor subclones with different metastatic potentials and different drug resistance, and resolve the molecular mechanisms of tumor progression, metastasis, and drug resistance. In addition, single-cell research has also discovered cell markers of immunotherapy response and resistance, providing an important basis for precise stratification of immunotherapy and the development of new immune targets.

4.2. Tracking cell development, differentiation, and reprogramming mechanisms

4.2.1. Stem cell differentiation and embryonic development mechanism research

Embryonic development and stem cell differentiation are highly dynamic cell fate regulation processes, and traditional bulk technologies have difficulty capturing the transient intermediate states and regulatory networks during differentiation. scRNA-seq can sample at different time

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points, reconstruct the continuous dynamic process of development, and resolve the molecular mechanisms of cell fate determination.

Studies have drawn the cell atlas of human post-implantation embryonic development through single-cell transcriptomics, resolved the gene regulatory network of three-germ-layer differentiation, and identified multiple key developmental regulators. In the study of hematopoietic stem cell differentiation, scRNA-seq revealed the heterogeneity of hematopoietic stem and progenitor cells, discovered intermediate-state cells of directed differentiation, reconstructed the complete trajectory of hematopoietic differentiation, and revised the traditional stepwise differentiation model [15]. In addition, this technology is also widely applied in cell reprogramming research, resolving the barriers and key regulatory factors of the reprogramming process and promoting the development of regenerative medicine technology.

4.2.2. Immune cell activation, polarization, and dynamic regulation

Immune cell activation and polarization are highly dynamic functional regulation processes; under different stimulation conditions they can differentiate into subpopulations with different functions, playing roles such as immune defense and inflammatory regulation. scRNA-seq can precisely capture the dynamic state changes during immune cell activation and resolve the molecular regulatory mechanisms of polarization.

In T cell activation research, single-cell technology revealed the continuous state changes during the activation of naive T cells and identified the differentiation paths and key transcription factors of effector T cells and memory T cells. In macrophage polarization research, the continuous dynamic process of M1/M2 polarization was resolved, and various intermediate polarization states were discovered, breaking through the understanding of the traditional binary polarization model. Related research has provided an important theoretical basis for the immunoregulatory treatment of infectious diseases and autoimmune diseases.

4.3. Cell communication and microenvironment interaction mechanism research

Intercellular signal communication is a core regulatory mechanism of tissue homeostasis maintenance and disease occurrence and development, and traditional technologies have difficulty resolving intercellular interactions at the single-cell level. Based on scRNA-seq data, the signal communication relationships between different cell subpopulations can be systematically inferred through the expression patterns of ligand-receptor pairs; representative tools include CellPhoneDB and NicheNet [16].

In tumor microenvironment research, cell communication analysis revealed the mechanism by which tumor cells recruit and polarize immune cells through chemokine and growth factor signals to construct an immunosuppressive microenvironment; at the same time, it resolved the interaction network between stromal cells and tumor cells and identified multiple key signaling pathways that promote tumor progression. In tissue development research, cell communication analysis revealed the regulatory role of the interaction between epithelial cells and stromal cells in organ morphogenesis. This technology provides a brand-new research means for resolving the cell regulatory networks of complex microenvironments.

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4.4. Disease biomarker screening and pathogenic mechanism research applications

Single-cell transcriptome technology can precisely identify disease-specific cell subpopulations and molecular markers, providing brand-new targets for disease diagnosis, prognosis assessment, and targeted therapy. In tumor research, by comparing the cell composition of tumor tissue and normal tissue, tumor-specific cell subpopulations and characteristic genes were identified, which can serve as markers for early diagnosis and prognosis assessment [14].

In research on autoimmune diseases, neurodegenerative diseases, etc., scRNA-seq resolved the abnormal cell states and gene expression dysregulation under disease conditions and revealed the cellular and molecular mechanisms of disease occurrence. For example, in rheumatoid arthritis research, pathogenic fibroblast subpopulations were identified and the mechanism by which they mediate joint damage was resolved, providing new targets for disease-targeted therapy. In addition, single-cell technology is also widely applied in drug mechanism research and drug target screening, promoting the development of precision medicine.

5. Single-cell multi-omics integration technology and frontier development

5.1. Single-cell multi-omics integration technology system

Single-cell multi-omics technology can simultaneously detect information at multiple omics levels such as transcriptome, genome, epigenome, and proteome in a single cell, fully resolving the regulatory network of cell states. Currently mature technology systems include: ① transcriptome + epigenome, such as scRNA-seq combined with scATAC-seq, simultaneously detecting gene expression and chromatin accessibility to resolve the epigenetic regulatory mechanisms of gene expression; ② transcriptome + proteome, such as CITE-seq technology, detecting dozens to hundreds of surface proteins while performing single-cell transcriptome sequencing, more precisely defining cell functional states [17]; ③ transcriptome + genome, simultaneously detecting somatic mutations and gene expression to resolve the association between genotype and phenotype of tumor cells [18].

5.2. Bioinformatics analysis strategies for multi-omics integration

Multi-omics data have the characteristics of strong heterogeneity, high dimensionality, and large scale, and data integration analysis is the key to technology application. Currently mainstream analysis strategies include: ① anchoring integration, using the transcriptome as the anchor to map other omics data to the transcriptome space for joint multi-omics analysis; ② weighted integration, assigning different weights to different omics data through algorithms to construct an integrated cell feature matrix; ③ joint multi-omics clustering, performing cell clustering based on multi-omics features together to identify finer cell states. Multi-omics integration analysis tools such as Seurat v5 and MultiVI have been developed, supporting the systematic resolution of multi-dimensional single-cell data [19, 20].

5.3. Fusion technology of spatial transcriptomics and single-cell sequencing

Traditional scRNA-seq requires dissociating tissue into single-cell suspensions, which loses the spatial position information of cells and makes it difficult to resolve the spatial distribution characteristics of cells and the spatial structure of the microenvironment. Spatial transcriptomics technology can detect the gene expression profile of tissue sections while retaining spatial positions,

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but its resolution has not yet reached the single-cell level. The fusion of the two can achieve complementary advantages: mapping the cell type annotations of single-cell transcriptomics to spatial transcriptomics sections to resolve the spatial distribution patterns of cell types; analyzing the spatial heterogeneity of the cell microenvironment combined with spatial position information to resolve the regulatory role of spatial signal gradients on cell functions [21].

Spatial-single-cell fusion technology has been widely applied in fields such as oncology and neuroscience, resolving key scientific questions such as the spatial structure of the tumor microenvironment, the spatial distribution of immune cells, and cell interactions at tumor boundaries; it is currently a frontier hotspot direction of single-cell technology.

5.4. CRISPR perturbation combined with single-cell sequencing functional screening technology

The combination of CRISPR gene editing technology and single-cell transcriptomics enables high-throughput gene functional screening; the representative technology is Perturb-seq. This technology introduces a CRISPR sgRNA library into a cell population, where each cell expresses a different sgRNA to achieve single-gene perturbation, and reads out each cell's perturbation type and gene expression profile simultaneously through single-cell sequencing, screening the functions of hundreds to thousands of genes in one go and resolving gene regulatory networks [22].

This technology breaks through the bottlenecks of low throughput and single endpoints of traditional gene screening; it can resolve the whole-transcriptome effects of gene perturbation at single-cell resolution and systematically construct gene regulatory networks, and it is a disruptive technology for functional genomics research. It has now been widely applied in research fields such as tumor drug resistance mechanisms, immune regulatory networks, and developmental regulatory pathways.

5.5. Existing difficulties and limitations of multi-omics technology

Although single-cell multi-omics technology is developing rapidly, many technical bottlenecks still exist: ① high technical complexity and expensive experimental costs; the technical difficulty of simultaneous multi-omics detection is large, reagent and sequencing costs are far higher than single omics, making large-scale application difficult; ② data integration is difficult; the heterogeneity of different omics data is strong, unified integration analysis standards and methods are lacking, and the interpretability of analysis results needs to be improved; ③ relatively low throughput; the cell throughput of most multi-omics technologies is lower than pure transcriptome sequencing, making it difficult to support large-scale population analysis; ④ technical stability and reproducibility need to be improved; the experimental workflow is complex, and batch effects and technical noise are more significant [17, 18].

6. Conclusion and outlook

Through transcriptome resolution at single-cell resolution, single-cell transcriptome sequencing technology has completely broken through the averaging limitation of traditional population sequencing and has become a core fundamental technology in the life sciences. After more than ten years of iteration, this technology has formed a complete experimental system and bioinformatics analysis system, and mainstream platforms can meet research needs of different throughputs and different precisions; it has achieved a series of breakthroughs in fields such as human cell atlas

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construction, tumor microenvironment resolution, development and differentiation tracking, cell communication analysis, and disease biomarker screening, promoting a paradigm revolution in the life sciences. On this basis, frontier technologies such as single-cell multi-omics integration, spatial transcriptomics fusion, and CRISPR functional screening are expanding rapidly, further improving the dimensions and depth of single-cell research.

But current technologies still have many bottlenecks: first, the cost of single-cell sequencing is still relatively high, restricting large-scale clinical application and large-sample research; second, detection sensitivity is still insufficient, and the detection reliability of low-abundance transcripts needs to be improved; third, data analysis complexity is high, standardized analysis pipelines are lacking, and result interpretation highly depends on researchers' experience; fourth, frontier technologies such as multi-omics and spatial single-cell are not mature enough, with high technical barriers and difficulty of popularization.

In the future, single-cell transcriptome technology will develop in three major directions: first, upgrading of technical performance, developing a new generation of single-cell sequencing technology with higher throughput, lower cost, and higher sensitivity to promote technology popularization and clinical translation; second, multi-dimensional integration, promoting the deep integration of single-cell transcriptomics with spatial omics, epigenomics, proteomics, and genomics to achieve multi-dimensional resolution of cell states; third, clinical translation applications, developing standardized, automated clinical single-cell detection technologies to achieve clinical implementation in fields such as early disease diagnosis, prognosis assessment, precision medication, and cell therapy. With the continuous iteration of technology, single-cell sequencing technology will further promote breakthroughs in basic life science research and the development of precision medicine, showing broader application prospects.

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